11th INTERNATIONAL CONFERENCE ON ADVANCED VIBRATIONAL SPECTROSCOPY

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ABSTRACT BOOK

PLENARY SPEAKERS

Watching dynamics of single bonds

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plasmon, single molecule, optomechanics, SERS, nanocavity, picocavity

Picocavities are atomic configuration that trap light to the atomic scale in ultralow-volume plasmonic cavities with $V < 1\text{nm}^3$. By cascaded optical coupling, we can efficiently couple light in and out of these, enabling us to watch individual molecules and bonds vibrating [1-5]. It is also possible to watch redox and catalysis at the single molecule scale [6-9]. We are now starting to understand the properties of picocavities in greater detail, which will be the subject of this talk. In particular, how they form, the forces that they generate through optomechanical interactions, and how they can be used to track the atomic environment inside nm-scale gaps and their chemistry are all becoming clearer. We show now that picocavities are produced by light [8]. Plasmonically-focussed irradiation directly influences the Au-Au bonding force at surfaces, but also depends on the molecular environment at the metal surface. This is of direct relevance for catalysis, molecular electronics, and many nano-devices. We demonstrate that optically stabilizing a single surface metal adatom near molecules of interest resolves dynamic metal-molecule interactions at sub-atomic precision. Coordination bonds with nearby metal atoms are shown to chemically perturb the surface molecules. Real-time trajectories of perturbing adatom and all organic atoms involved can be reconstructed with sub-A precision revealing anomalous diffusion of single atoms [10]. We also show that sub-ms 'flare' events [11] originate from the same forces. Visualising how single molecules interact with their immediate environment in real-time at ambient conditions provides an invaluable tool for developing chemistries and devices, as well as accessing the quantum vibrations of single bonds. For instance we are now able to watch pH at the single-molecule level [9]. Strong coupling at this scale opens up a wide variety of new possibilities, forcing the development of new optomechanical theory at this scale [12].

EU, EPSRC

[1] Nature Materials 18, 668 (2019); Extreme nanophotonics from ultrathin metallic gaps [2] Nature 535, 127 (2016); Single-molecule strong coupling at room temperature in plasmonic nanocavities [3] Science 354, 726 (2016); Single-molecule optomechanics in picocavities [4] Nature Comm 10, 1049 (2019); Quantum electrodynamics at 300K coupling a single vibrating molecule with a plasmonic nanocavity [5] Nature Electronics 3, 687 (2020); Real-Time In-Situ Optical Tracking of Oxygen Vacancy Migration in Memristors [6] Nature Comm 8, 994 (2017); Plasmonic tunnel junctions for single-molecule redox chemistry [7] Nature Comm 11:5905 (2020); Optical probes of molecules as nano-mechanical switches [8] under review, Nature Mat (2021); Optical suppression of energy barriers in single molecule-metal binding [9] under review, Adv.Science (2021); Tracking Interfacial Single-molecule pH and Binding Dynamics via Yoctoliter Vibrational Spectroscopy [10] under review, Nature Comm (2021); Resolving Sub-Angstrom Ambient Motion through Reconstructions from Vibrational Spectra [11] Nature Comm 11, 682 (2020); Flickering nm-scale disorder in a crystal lattice. [12] under review, Nature Nano (2021); Softening molecular bonds through the giant optomechanical spring effect in plasmonic nanocavities

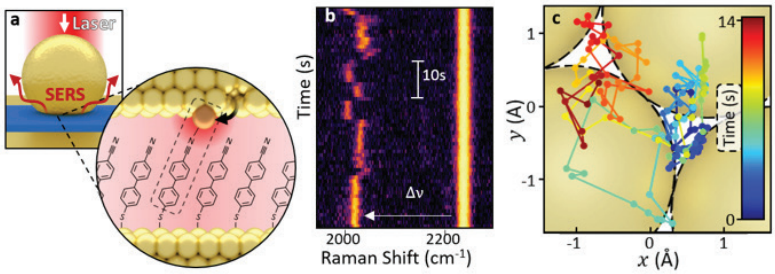

Fig. 1 a) Picocavities generated in nanoparticle-on-mirror (NPoM) plasmonic cavity. b) Real-time SERS spectra of an individual molecule in a picocavity showing shifted CN line, and c) reconstructed position of N atom in 3D space, to 10pm resolution.

Mid-infrared Photothermal Microscopy

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spectroscopy, imaging, photothermal

The recently developed and rapid-growing mid-infrared photothermal IR (MIP) microscopy not only overcomes the diffraction limit in direct IR imaging [1-3]. In MIP microscopy, a visible beam probes the thermal effects induced by an intensity-modulated infrared beam (see Figure 1). MIP signals can be measured in scanning mode or in wide-field manner through an interferometric scattering microscope, a phase microscope, or an interferometric objective. In wide-field MIP, focal area matching of the IR beam with the visible beam and camera-based paralleled detection offer a high imaging speed. Meanwhile, thermal confinement after nanosecond IR pulse excitation offers sub-micron spatial resolution. MIP microscopy has find important applications in life science and materials science.

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[1] Yeran Bai, Jiaze Yin, Ji-Xin Cheng*, "Bond-Selective Imaging by Optically Sensing the Mid-Infrared Photothermal Effect", Science Advances, review, 2021, 7: eabg1559 [2] Yeran Bai, Delong Zhang, Yimin Huang, Lu Lan, Kerry Maize, Ali Shakouri*, Ji-Xin Cheng*, Ultrafast Chemical Imaging by Widefield Photothermal Sensing of Infrared Absorption, Science Advances, 2019, 5, eaav7127 [3] Delong Zhang, Chen Li, Chi Zhang, Mikhail N. Slipchenko, Gregory Eakins, Ji-Xin Cheng*, "Depth-resolved mid-infrared photothermal imaging of living cells and organism with sub-micron spatial resolution", Science Advances, 2016, 2: e1600521.

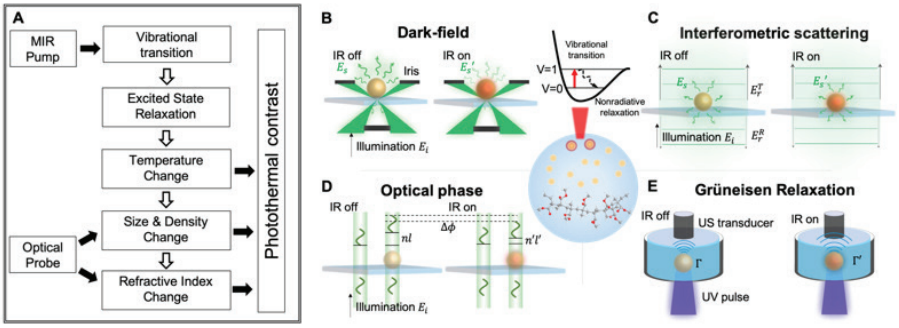

Fig.1. Schematic of mid-infrared photothermal effects and detection mechanisms. (A) Block diagram shows the IR absorption induced photothermal process. After the sample absorb the incident IR photons with specific vibration frequency, the energy quickly dissipated in the form of heat, leading to local temperature increase. Depending on the IR pulse duration and sample thermal property, the thermal expansion and pressure wave emission may occur. Subsequently, the optical property such as refractive index is changed as a result of decreased density and changed polarizability. These modulated physical properties could be detected by optical or acoustical methods, as illustrated in (B-E). Adopted from reference 1.

Material Science and Catalysis
Infrared spectroscopy in analysis of the surfaces of porous materials

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IR spectroscopy has evolved from the originally inexpensive and non-destructive tool used to characterize substances into a modern investigation technique with new features, such as operando spectroscopy, IR imaging, and 2D correlation spectroscopy. During the lecture, I would like to present application of various infrared techniques to study properties of macro and microscopic surfaces in environmental and life sciences. FTIR imaging may be used for drug delivery studies. In presented here studies, modified ATR unit was used to determine the tablet swelling in different media, even in aqueous solutions1. In addition to measuring the drug delivery rate, the influence of undissolved drug particles on swelling capacity of the tablet, and the form of released drug was investigated. Infrared imaging analysis of bone tissue proved to be a valuable tool in the field of osteoporosis, allowing the establishment of important material properties contributing to the bone strength and led to proposal that factors other than bone turnover alone, i.e. collagen crosslink ratio, are responsible for the observed changes in osteoporotic bones2. For microporous materials diffusion in narrow channels may have great impact on the reactivity of catalyst. The concept of optical isotherms was first introduced for photonic crystals3 but may be expanded to crystals of any type or even amorphous phases. Optical isotherms, i.e. dependence of the change in absorption spectra on the amount of adsorbed species, may provide information on the interaction with specific adsorption centers, pore geometry or even the presence of surface barriers4. Operando spectroscopy is an analytical methodology coupling spectroscopic characterization of materials undergoing reaction with measurement of catalytic activity and selectivity5. Such experiments produce information about the reaction mechanism, active site involvement, and competition of reactants for the active site. Specific examples of the role of coordinatively unsaturated metal sites in MOF in electrochemical reaction of hydroxymethylfurtural oxidation will be presented6. Two-dimensional correlation spectroscopy (2D-COS) is especially useful for determination of the reaction mechanisms7. Special set of mathematical rules allows determination of the sequence of events, identification of various inter- and intramolecular interactions, and even enabling correlations between spectra from different techniques. This spectroscopy will be presented based on the example of structure and mechanistic relevance of Ni2+–NO adduct in a model HC SCR reaction over the NiZSM 5 catalyst8.

[1] F.D. Zahoor, K. Mader, P. Timmins , J. Brown, C. Sammon, Mol. Pharmaceutics 2020, 17, 1090-1099. [2] E.P. Paschalis, E. Shane, G. Lyritis, G. Skarantavos, R. Mendelsohn, A.L. Boskey, J. Bone Miner Res. 2004;19:2000–2004. [3] M. Hinterholzinger, A. Ranft, J. M. Feckl, B. Rühle, T. Bein and B. V. Lotsch, J. Mater. Chem., 2012, 22, 10356. [4] Ch. Chmelik, A.Varma, L. Heinke, D. B. Shah, J. Kärger, F. Kremer, U. Wilczok, and W. Schmidt, Chem. Mater., 2007, 19, 6012-6019. [5] B. M. Weckhuysen, Phys. Chem. Chem. Phys., 2003, 5, 4351. [6] N. Heidary, D. Chartrand, A. Guiet, and N. Kornienko, Chem. Sci., 2021, 12, 7324-7333. [7] I. Noda and Y. Ozaki, Two-Dimensional Correlation Spectroscopy - Applications in Vibrational and Optical Spectroscopy, 2004, John Wiley & Sons Ltd. ISBN 978-0-471-62391-5. [8] P. Pietrzyk, K. Góra-Marek, T. Mazur, B. Mozgawa, M. Radoń, M. Chiesa, Z. Zhao, Z. Sojka, J. Catal., 2021, 394, 206-219.Financial support from National Science Centre grant no 2020/37/B/ST5/01258 is acknowledged.

Polarized Infrared Spectroscopy of Collagen from nano-scale fibrils to tissues and scaffolds

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polarization contrast Infrared imaging, collagen, FTIR with FPA, O-PTIR, nanoFTIR

Collagen is ancient, ubiquitous, and critically important to the structure and function of many life forms. In the human body, more than 20 types of collagen are found in skin, bone, tendon and cartilage, and organs, comprising some 30 to 40% of protein in the human body. Layers of collagen underpin and infiltrate critical organs; scaffolds of collagenous fiber networks define the organization of the heart. Vibrational spectroscopy has been used for decades to study collagen in mammalian tissues. The hierarchical structure and radial symmetry of collagen, from the triple helical molecule, through nanoscale fibrils to micron scale fibers and intact tissues, render it a suitable target for study with polarized IR light. While many changes in the spectral profiles appear under polarized Infrared (IR) light, the absorption bands are naturally broad due to tissue heterogeneity. Analysis of nanoscale fibrils aids in the understanding of normal tissue function, in the evaluation of disorder in damaged collagen and in scar tissue, and in the creation of viable artificial collagen scaffolds for repair. This presentation will be an overview of samples, methods and analysis of such spectra obtained with polarized Far Field (FF) FTIR imaging with a Focal Plane Array detector,[1,2] with the relatively new method of FF Optical Photothermal IR (O-PTIR),[1] and with nano-FTIR spectroscopy[1] based on scattering-type scanning near-field optical microscopy (s-SNOM).

SG Baldwin, L Kreplak (Dalhousie U), K Gsell, SP Veres (St. Mary's U) provided tendon; L Bozec (U Toronto) provided collagen scaffolds; Ian Dixon (UManitoba) provided post-infarct rat heart. nanoFTIR: Advanced Light Source resources, U.S. DOE Office of Science User Facility contract # DE-AC02-05CH11231. O-PTIR: M Kansiz, E Dillon (Photothermal Spectroscopy Corp.) Funding: NSERC, CIHR, UManitoba

[1] G Bakir, BE Girouard, R Wiens, S Mastel, E Dillon, M Kansiz, KM Gough; "Orientation Matters: Polarization Dependent IR Spectroscopy of Collagen from Intact Tendon Down to the Single Fibril Level" 2020 Molecules 25 (18), 4295-4308 [2] R Wiens, CR Findlay, SG Baldwin, L Kreplak, JM Lee, SP Veres, KM Gough; "High spatial resolution (1.1 micron and 20 nm) FTIR polarization contrast imaging reveals pre-rupture disorder in damaged tendon" Faraday Discuss., 2016, 187, 555–573

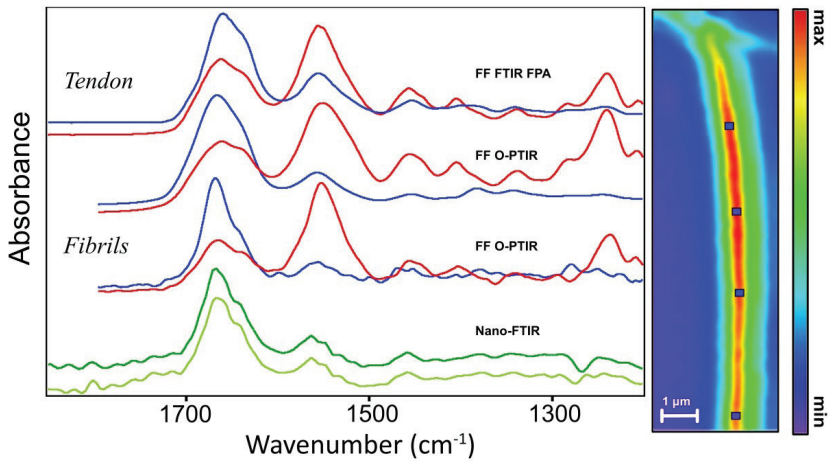

Figure 1. Left: Polarized IR spectra of oriented tendon and fibrils; Right: O-PTIR image of fibril

Challenges in the application of one and two-photon excited SERS to bioorganic samples

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Surface enhanced Raman scattering (SERS) has become the basis of a whole range of spectroanalytical approaches. SERS in the absence of labels, tags or reporter molecules enables a wide variety of investigations of the interaction of biomolecules with nanostructures. The most important points in the discussion of label-free SERS are naturally related to the availability of the plasmonic nanostructures at the points of probing. This necessitates consideration of localization, optical properties, and the potential to interact with the molecules in a biological system, of the plasmonic nanostructures. Another central issue is concerned with the type of information obtained in SERS experiments with cells and tissues, keeping in mind the restrictive condition of a plasmonic nanostructure to be 'nearby'. If a sample is excited with laser light, different physical processes can be used quasi-simultaneously for its optical and spectroscopic characterization. Non-linear excitation offers several advantages over one-photon excitation, particularly for the studies of biological objects, mainly related to its lower-energy excitation and the strong confinement of the excitation volumes. Surface enhanced hyper Raman scattering (SEHRS) is the spontaneous, two-photon excited Raman scattering that occurs for molecules residing in high local optical fields of plasmonic nanostructures.[1] SEHRS can give complementary spectroscopic information resulting from different selection rules and a stronger enhancement due to the non-linearity in excitation. During excitation of the incoherent hyper-Raman scattering, also other nonlinear, coherent optical signals can be obtained from a sample. The local fields of plasmonic nanostructures can also enhance the second harmonic radiation.[2] In this talk, examples will be given of the wealth of vibrational spectroscopic information that can be obtained by SERS and SEHRS, and work will be discussed that has contributed to understanding both effects and that provides directions for bioanalytical spectroscopy.

Funding by ERC grant 259432 MULTIBIOPHOT is gratefully acknowledged.

[1] Madzharova, F.; Heiner, Z.; Kneipp, J., Chemical Society Reviews 2017, 46, 3980-3999 [2] Madzharova, F.; Nodar, Á; Živanović, V.; Huang, M.R.S.; Koch, C.T.; Esteban, R; Aizpurua, J.; Kneipp, J., Advanced Functional Materials, 2019, 29, 1904289 [3] Spedalieri, C.; Szekeres, G.P.; Werner, S.; Guttmann, P.; Kneipp, J., Nanoscale, 2020, 13, 968-979

In situ Raman Probing of Interfacial Structures and Reaction Intermediates using Core-Shell Nanoparticles

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Surface-enhanced Raman spectroscopy; core-shell nanostructure; intermediate

Surface-enhanced Raman spectroscopy (SERS) can provide fingerprint structural information of molecules with ultrahigh surface sensitivity. However, only a few metals (like Au, Ag, and Cu) with particular nanostructures can generate strong SERS effects. Such material and morphology limitations have greatly hindered the applications of SERS. To overcome the long-standing material limitation of SERS, our group developed various methodologies, including the "borrowing" strategy, shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS), and the SHINERS-satellite strategy, through the fabrication of different core-shell nanostructures. Using these strategies, the molecular configuration of water at different electrochemical interfaces and its evolution with the electrochemical environment have been probed. Furthermore, the dynamic processes of important catalytic reactions occurring on model single crystal surfaces, practical nanocatalysts, or even single-atom catalysts, such as the oxygen reduction reaction, the hydrogen evolution reaction, and electrooxidations, etc., have been systematically investigated. Direct spectroscopic evidence has been obtained for the key intermediates (*OH, *OOH, *O2-, etc.) during reactions, which is combined with theoretical simulation to uncover the reaction mechanisms or structure-activity relationships at a molecular level. The concept of shell-isolated nanoparticle-enhancement is being applied to other spectroscopies, such as fluorescence, IR, SHG, SFG, and tip-enhanced spectroscopies to improve the sensitivity or spatial resolution. Such advanced techniques also have great potentials for the in-situ study of reactions/catalysis, at single atoms or a single molecule.

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[1] J. F. Li, Y. F. Huang, Y. Ding, Z. L. Yang, S. B. Li, X. S. Zhou, F. R. Fan, W. Zhang, Z. Y. Zhou, D. Y. Wu, B. Ren, Z. L. Wang, Z. Q. Tian, Nature 2010, 464, 392-395. [2] C. Y. Li, J. B. Le, Y. H. Wang, S. Chen, Z. L. Yang, J. F. Li, J. Cheng, Z. Q. Tian, Nat. Mater. 2019, 18, 697-701. [3] J. C. Dong, X. G. Zhang, V. Briega-Martos, X. Jin, J. Yang, S. Chen, Z. L. Yang, D. Y. Wu, J. M. Feliu, C. T. Williams, Z. Q. Tian, J. F. Li, Nat. Energy 2019, 4, 60-67. [4] C. Y. Li, S. Duan, B. Y. Wen, S. B. Li, M. Kathiresan, L. Q. Xie, S. Chen, J. R. Anema, B. W. Mao, Y. Luo, Z. Q. Tian, J. F. Li, Nat. Nanotechnol. 2020, 15, 922-926.

Infrared vibrational imaging through nondegenerate two-photon absorption

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Mid-infrared imaging, infrared detection, tomographic imaging

Mid-infrared (MIR) microspectroscopy suffers from several technical challenges that have held back a broader implementation of the technique in the biomedical sciences. One of these challenges is the detection of MIR light by suitable cameras. MIR cameras suffer from thermal noise and often need to be cooled to cryogenic temperatures to improve performance. The cooled cameras that are commonly used in MIR microspectrocopy have low pixel densities (128x128), which limit high definition sampling over larger fields of view. [1] The combination of low affordability, cryogenic cooling and low pixel density makes the camera a limiting factor in the practical application of MIR microscopy for biomedical imaging. MIR camera technology stands in stark contrast to Si-based cameras, which are much more affordable, do not rely on cryogenic cooling and feature high pixel densities. Although the qualities of Si-based cameras would benefit MIR microscopy enormously, its high-energy bandgap renders Si inherently blind to MIR light. We have overcome this fundamental hurdle through the process of non-degenerate two-photon absorption (NTA) with a second NIR gate pulse, enabling direct detection of MIR light by a silicon sensor. This new MIR detection approach overcomes a key limitation in MIR microscopy and represents a step toward a more practical implementation of MIR imaging in the biomedical sciences. In NTA, a MIR photon of energy ħω_MIR is co-incident with a NIR photon of energy ħω_NIR on a semiconducting photodetector, such that the photon combined energy exceeds the band gap energy of the semiconductor. This mechanism has been shown to enable efficient MIR detection with single-pixel photodiodes based on direct bandgap semiconductors such as GaAs and GaN.[2-4] We have expanded the NTA method to wide-field imaging with cameras, including off-the-shelf Si CCD cameras, CMOS cameras and InGaAs cameras. For instance, using a front-illuminated CCD camera (1392x1040 pixels), we have captured MIR by using only a ~5 fJ of MIR pulse energy per pixel, gated by similarly low energies of the NIR pulse.[5] In addition, using the fs NIR pulse as a temporal gate, we have performed 3D tomographic imaging, see Figure 1.[6] By using a faster InGaAs camera, this approach has allowed us to record volumetric MIR images in less than 20 ms/volume, as well as chemically selective videography of mixing processes at 500 fps.[7] With subsequent improvements, NTA will enable MIR imaging based on quantum cascade lasers (QCLs), making it possible to perform high-speed MIR microscopy at high pixel density.

National Institutes of Health, R01-GM132506 and R21-GM141774

[1] Yeh, K., et al., Fast Infrared Chemical Imaging with a Quantum Cascade Laser. Analytical Chemistry, 2015. 87(1): p. 485-493. [2] Cirloganu, C.M., et al., Extremely nondegenerate two-photon absorption in direct-gap semiconductors [Invited]. Optics Express, 2011. 19(23): p. 22951-22960. [3] Fishman, D.A., et al., Sensitive mid-infrared detection in wide-bandgap semiconductors using extreme non-degenerate two-photon absorption. Nature Photonics, 2011. 5(9): p. 561-565. [4] Pattanaik, H.S., et al., Three-dimensional IR imaging with uncooled GaN photodiodes using nondegenerate two-photon absorption. Optics Express, 2016. 24(2): p. 1196-1205. [5] Knez, D., et al., Infrared chemical imaging through non-degenerate two-photon absorption in silicon-based cameras. Light: Science & Applications, 2020. 9(1): p. 125. [6] Potma, E.O., et al., Rapid chemically selective 3D imaging in the mid-infrared. Optica, 2021. 8(7): p. 995-1002. [7] Potma, E.O., et al., High-speed 2D and 3D mid-IR imaging with an InGaAs camera. ArXiv, 2021: p. 2107.00720.

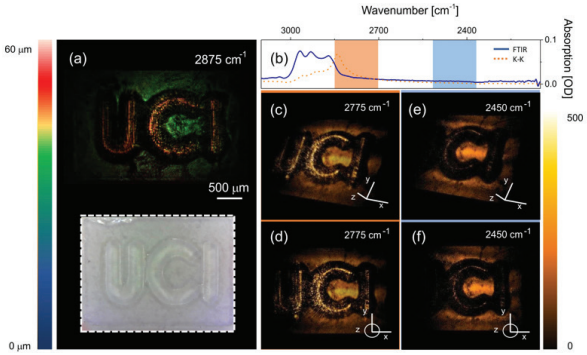

Figure 1. 3D imaging of a resin structure manufactured through projection-based photolithography technique. (a) 3D reconstruction of resin structure. (b) FTIR absorption spectrum of the resin (blue line) and real part of the refractive index obtained through a Kramer-Kronig transformation (orange dotted line). Rectangles represent Gaussian pulse width of ~150 cm-1. (c) and (d) 3D imaging at 2775 cm-1, (e) and (f) 3D imaging at 2450 cm-1. Structure height is ~50 mm. Images have been corrected for non-spectroscopic, spectral NTA efficiency variations (see Supplementary Figure S2). Total image acquisition time is 1 s.

Aqueous Nanoscale Interfaces: Hydrophobicity and Confinement

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Vibrational spectroscopy, Sum frequency scattering, Water, Confinement, Droplets

Water is the most important liquid for life. It is intimately linked to our well-being. Without water, cell membranes cannot function. Charges and charged groups cannot be dissolved, self-assembly cannot occur, and proteins cannot fold. Apart from the intimate link with life, water also shapes the earth and our climate. Our landscape is formed by slow eroding/dissolving processes of rocks in river and sea water; aerosols and rain drops provide a means of transport of water. Because of the complexity of liquid water and aqueous interfaces, the relationship between the unique properties of water and its molecular structure has not been solved. This is especially true for nanoscale interfaces, on which the molecular level structure of water is hard to access. In this presentation I will introduce nonlinear optical light scattering and imaging techniques that were developed recently and demonstrate how they can be used to access the molecular structure of bulk and interfacial water. Focusing on vibrational sum frequency scattering I will finish with unraveling the structure of water in confinement (water droplets) and in contact with purely hydrophobic substances. Our findings demonstrate the importance of length scale, hydrophobicity and confinement on the interfacial water structure as well as on the electrostatic interfacial environment.

Study on intermolecular interaction of polymers using vibrational spectroscopy

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THz spectroscopy, Low-frequency Raman spectroscopy, Imaging, Intermolecular interactions, polymer

Intermolecular interactions in polymers have a significant effect on their crystal structure and miscibility. Therefore, vibrational spectroscopy can be used to study the intermolecular interactions of polymers to obtain very important information. Spectra in the low-frequency range obtained by FIR/THz spectroscopy and low-frequency Raman spectroscopy provide information on the higher-order structure of polymers and intermolecular hydrogen bonding in the lamellar structure of semi-crystalline polymers, because the spectra in the low-frequency range show modes sensitive to the long-range structure of molecules, lattice expansion, weak interactions, and intermolecular vibrations. It is also possible to visualize the changes in intermolecular interactions by ATR-FTIR imaging in polymer blend systems. Here, it will be present some examples of studies on biodegradable polyesters using terahertz spectroscopy and low-frequency Raman spectroscopy, as well as polymer blend phase separation and crystallization using ATR-FTIR imaging.

[1] C. Funaki, S. Yamamoto, H. Hoshina, Polymer, 137, 245(2018) [2] C. Funaki, T. Toyouchi, H. Hoshina, Y. Ozaki, H. Sato, Applied spectroscopy, 71, 1537(2017) [3] Y. Yamamoto, H. Hoshina, H. Sato, Macromolecules, 54, 1052(2021) [4] H. Lu, H. Sato, and S. G. Kazarian, Macromolecules, 53, 9074(2020) [5] H. Lu, H. Sato, and S. G. Kazarian, Applied spectroscopy, in press (2021)

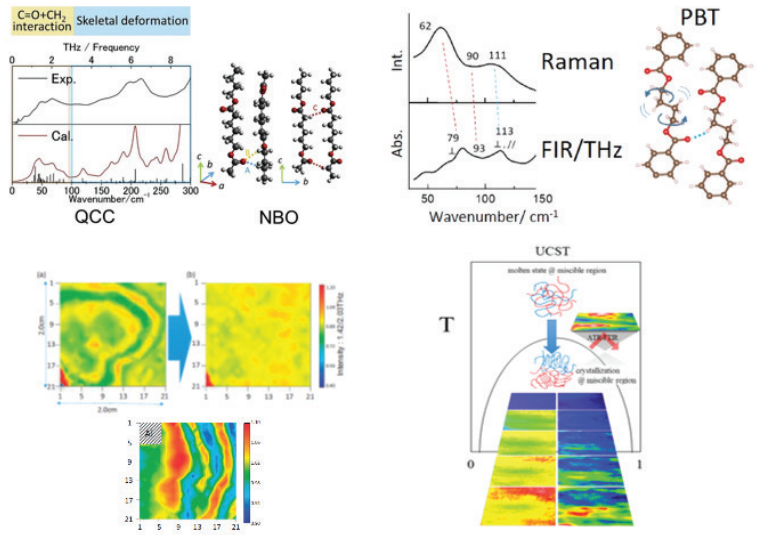

Figure 1.

Spectroscopy goes Viral

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COVID-19, saliva, Hepatitis B, Hepatitis C, malaria, SARS-CoV-2 vrions, Raman spectrosocpy

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented need for diagnostic testing that is critical in controlling the spread of COVID-19. The talk will focus initially on the development of ATR-FTIR spectroscopy to detect hepatitis B and C in serum[1] and the application to malaria diagnosis.[2] ATR-FTIR spectroscopy has a number of disadvantages when it comes to high throughput screening. First, the sample has to be dried onto the internal reflection element (IRE) prior to the measurement to maximize the absorbance. Secondly, the residue must be cleaned off the IRE, which increases the potential of transmission through aerosols and via surfaces, posing a danger to the operator. In pursuit of a cheaper approach to high throughput screening, we propose utilizing infrared reflective slides and a modified reflection accessory to obtain high quality spectra of saliva.[3] Hitherto, most infrared diagnostics have focused on blood components and only few studies have investigated less invasively collected samples such as saliva. Initially, purified virion particles were characterized with Raman spectroscopy, synchrotron infrared (IR) and AFM-IR (Figure 1). A data set comprising 171 transflection infrared spectra from 29 subjects testing positive for SARS-CoV-2 by RT-qPCR and 28 testing negative, was modeled using Monte Carlo Double Cross Validation with 50 randomized test and model sets. The testing sensitivity was 93^% (27/29) with a specificity of 82^% (23/28) that included positive samples on the limit of detection for RT-qPCR.[3] Herein, we demonstrate a proof-of-concept high throughput infrared COVID-19 test that is rapid, inexpensive, portable and utilizes sample self-collection thus minimizing the risk to healthcare workers and ideally suited to mass screening.

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[1] Roy, S.; Perez-Guaita, D.; Bowden, S.; Heraud, P.; Wood, B. R. Clinical Spectroscopy 2019, 1, 100001. [2] Heraud, P.; Chatchawal, P.; Wongwattanakul, M.; Tippayawat, P.; Doerig, C.; Jearanaikoon, P.; Perez-Guaita, D.; Wood, B. R. Malar J 2019, 18, 348. [3] Wood, B. R.; Kochan, K.; Bedolla, D. E.; Salazar-Quiroz, N.; Grimley, S.; Perez-Guaita, D.; Baker, M. J.; Vongsvivut, J.; Tobin, M.; Bambery, K.; Christensen, D.; Pasricha, S.; Eden, A. K.; McLean, A.; Roy, S.; Roberts, J.; Druce, J.; Williamson, D. A.; McAuley, J.; Catton, M., et al. Angew Chem Int Ed Engl 2021.

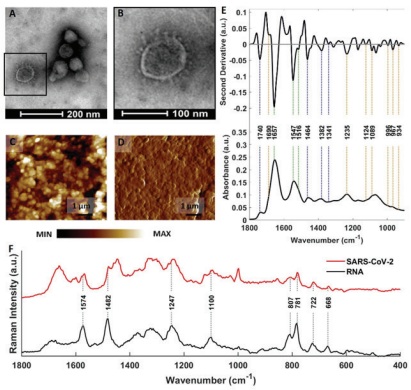

Figure 1. AFM, TEM, synchrotron FTIR and Raman characterization of SARS-CoV-2 virus. A) TEM image of SARS-CoV-2 sample with single virion marked by black square. B) Magnification of the area marked by the black square in (A), showing a single SARS-CoV-2 particle with its characteristic morphological appearance. C) AFM height and D) AFM deflection images of SARS-CoV-2 sample, demonstrating multiple round structures. E) Synchrotron FTIR spectrum and its 2nd derivative transform with the most prominent bands marked. The bands are color-coded as follows: lipids (blue), proteins (green) and nucleic acids (orange). F) Raman spectrum (532 nm) of SARS-CoV-2 virions (red) compared to spectrum of purified RNA (black) with labelled bands.

PERSPECTIVE LECTURES

TERS in the atomistic near-field: pico-optics/photonics/science

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Tip-enhanced Raman spectro-microscopy (TERSM) carried out in the atomistic near-field (ANF) reaches the Å-scale in spatial resolution – a fundamental limit set by the atomic granularity of matter. TERSM benefits from the advantages of universality and chemical selectivity, and beyond structure, the optical images capture dynamics: motions of atoms, charge densities, and intramolecular currents. Seeing normal modes of vibration inside a molecule, imaging electrostatic potential energy surfaces on single molecules, measuring asymmetric intramolecular current and seeing ion-selective atom-resolved images of 2D solids, are among the published examples. Dwelling on the simplest example of seeing an isolated atom – a nitrogen atom adsorbed on copper – it is straightforward to inference that light can be focused down to the size of an atom on the apex of a plasmonic tip. This can be quantified as the time harmonic electric field due to oscillating charge confined on the tip atom, to establish the principles of Gaussian pico-optics. Beyond optics, it is possible to rigorously establish that the photon-plasmon can be confined on a single atom. This is most directly demonstrated through the optical Schrödinger equation, which leads to quantization of the dielectric, and naturally unifies light and matter states, namely the dual nature of the quasi-particle plasmon. In this regard, TERSM in the ANF can be equally-well regarded as near-field optical microscopy or near-field electron microscopy executed with waveguided plasmons, which explains the TERSM resolution that rivals aberration corrected electron microscopy. The blurring of the distinction between light and matter gives continuity to the physical and chemical mechanisms of TERS, provides a natural language to discuss quantum plasmonics and to lay down the foundations of pico-photonics. The applications of these tools herald the post-nano era of pico-science, with demonstrated single molecule devices2 and circuits3 as elementary examples of things to come.

[1] Lee, J., Crampton, K. T., Tallarida, N., Apkarian, V. A. Nature, 568, 78 (2019). [2] Lee, J., Tallarida, N., Chen, X., Jensen, L., Apkarian, V. A., Science Advances, 4, eaat5472 (2018). [3] Crampton, K. T., Lee, J., Apkarian, V. A., ACS Nano, 13, 6363 (2019).

Infrared Spectroscopy: Past, Present (and Future?)

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Almost fifty years ago, Herbert Laitinen, the editor of Analytical Chemistry, wrote an editorial on the seven phases of an analytical instrument, which he proposed that these phases are analogous to Shakepeare's seven ages of man. Laitinen wrote that infrared spectrometry is an analytical technique in its seventh age, ready to be replaced by other instruments of greater sensitivity, speed, resolution, etc. How wrong he was! When this editorial had been published, computer-controlled, laser-fringe-referenced, mid-infrared FT-IR spectrometers incorporating a TGS detector had been on the market for about four years. Arguably, this was probably too short a time for FT-IR to have the impact that it would have over the next 40 years, but in any event, it can be argued that FT-IR spectrometry is now in its sixth age. Is it now time to evaluate whether FT-IR spectrometers have entered their seventh age, and are ready to be replaced by quantum cascade lasers and other such instruments? In this talk I will attempt to delineate those applications where FT-IR spectrometers are still the instrument of choice for acquiring mid-IR spectra and to suggest applications where alternative techniques are more appropriate.

Advanced Vibrational Spectroscopy with HAMAND (Hypothetical Addition Multivariate Analysis with Numerical Differentiation)

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chemometrics, multivariate curve resolution, HAMAND

We discuss the possibility of advanced vibrational spectroscopy with HAMAND [1]. It is capable of quantifying the contributions of known target spectra in an observed complex overlapping spectrum. It can also be used to eliminate known spectral components to make hidden minor components visible. HAMAND is a numerical version of the well-known standard addition method that determines the content of a known target spectrum in the observed. The four steps of HAMAND is schematically shown in Figure 1. (1) A number of model spectra are generated numerically by adding to the observed the target spectrum multiplied with hypothetical addition coefficients. (2) Generated hypothetical spectra and the observed are subject to differentiation; either of 0^{th}, 1^{st} and 2^{nd} order derivative is used depending on noise and background characteristics. (3) They are then subject to a two-component multivariate curve resolution analysis to yield two spectral components W1 and W2 with intensity profiles H1 and H2. W1 is independent of the hypothetical addition coefficient (H1 is constant) and corresponds to the residual spectra other than the target. W2 is linearly dependent on the hypothetical addition coefficient and corresponds to the target spectrum itself. From the intensity profile H2, we obtain a calibration curve to determine the amount of the target spectrum already contained in the observed. (4) The observed spectrum (black) is decomposed into the target spectrum (green) and the remaining (red). A few examples of HAMND analyses will be shown to demonstrate its power when used with vibrational spectroscopy

[1] Ando, M. and Hamaguchi, H. 2015. Journal of the Spectroscopical Society of Japan, 64, 280-284.

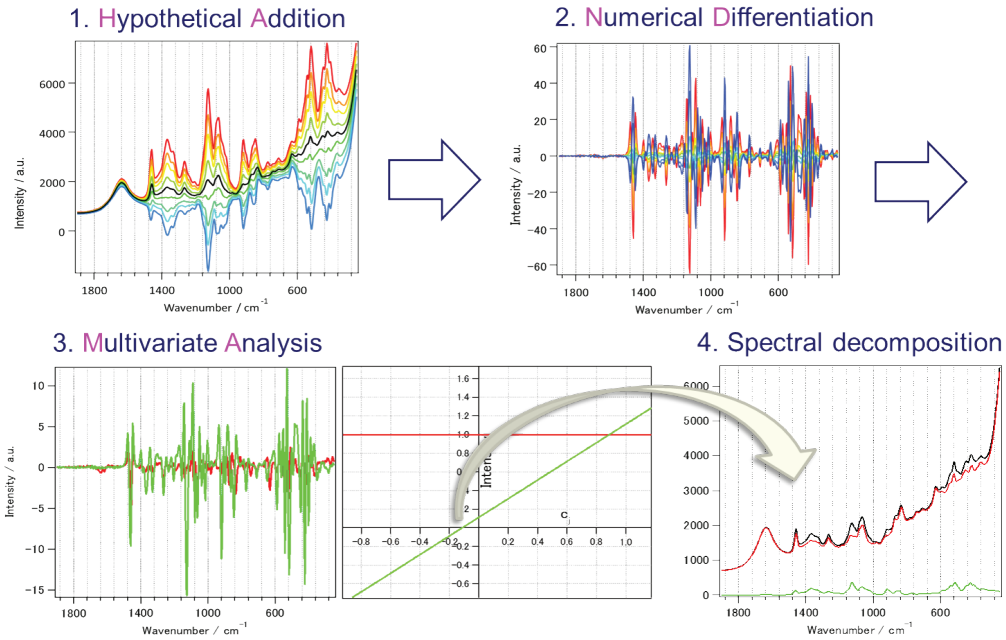

Figure 1. Four steps of HAMAND.

Application of 2D Correlation Vibrational Spectroscopy for the Development of Sustainable Materials

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Bioplastics, Sustainable, 2D correlation, Vibrational spectroscopy,

Plastics are wonderful modern materials, which are light, versatile, clean and relatively inexpensive to produce. With such exceptional convenience and utility, this class of nondegradable materials also comes with a serious penalty, namely the global accumulation of waste in the environment. Bioplascits, derived from natural biological sources instead of petroleum, is gaining increasing interest as a class of new generation of commodity materials to address the issue. Among representative bioplastics, polyhydroxyalkanoates (PHAs) are a class of aliphatic polyesters produced by the bacterial fermentation of vegetable oils and sugars. They are totally biodegradable even without oxygen by the action of common bacterial enzymes. However, properties of PHAs vary dramatically based on the specific molecular architecture of the polymer chain, and only a few PHAs have the desirable attribute necessary for practical applications. Vibrational spectroscopy had played an important role in pinpointing the structural design of truly useful commodity bioplastics for a broad range of applications. Such new and improved forms of PHAs are now commercially produced in a large industrial quantity from ordinary vegetable oils, aiming at the cost-competitive replacement of a number of existing petroleum-based plastics. Two-dimensional correlation spectroscopy (2D-COS) utilizing infrared or Raman probes is a versatile technique well-suited for the in-depth analysis of bioplastics exhibiting spectral variations to various physicochemical parameters, such as temperature, compositions, and nonuniform spatial distributions. This perspective presentation describes how vibrational spectroscopy coupled with the 2D-COS techniques has been utilized in the design and understanding of a new class of bioplastics.

1.1. Plasmonics

Geometric and electronic redox of single-molecule junctions probed with EC-TERS and plasmon-supported break-junction experiments

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electrochemical tip-enhanced Raman spectroscopy (EC-TERS), plasmon-supported break-junction experiments (PBJ), single-molecule junctions, redox switching

Gathering information about the geometric and electronic redox properties of molecules trapped in single-molecule junctions is highly desirable to advance or understanding of – and to ultimately design and control – efficient molecular electronics devices or (physiological) electron transfer systems in general. In my talk, I will highlight our recent methodological advances with operando nearfield Raman spectroscopy and plasmon-supported break-junction experiments. These approaches allow us to gain correlated chemical, topographic and electronic molecular-level information about, for example, adsorption geometry, chemical interaction and conversion and molecular conductance with extreme spatial resolution under reaction conditions.

[1] Aragonés, A. C.; Domke, K. F.: Nearfield trapping increases lifetime of single-molecule junction by one order of magnitude. Cell Reports Physical Science 2, 100389 (2021)

Fractal Metastructures for Infrared Plasmonics

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metastructures, plasmonics, infrared, nanoIR, EELS

Metallic nanostructures that exhibit tunable plasmon resonances on a broad spectral range are of particular interest for a variety of optical processes where the excitation and/or the emission could be enhanced. These multiple rsonances could be used for enhanced spectroscopies, nonlinear optical effects and plasmon-mediated chemical reactions. We explore here the properties of fractal metamaterials that have resonances spanning in the mid-infrared range that can be exploited for a variety of applications including sensing applications through surface-enhanced infrared absorption. Electrodynamic modelling is also key to reveal optical modes and how they hybridize along the different fractal generations. These calculations can be supported by nano-infrared measurements revealing how the field couples to the structure at the distinct resonance wavelengths. In this talk, we present several metastructures based on fractal geometries that exhibit multiple localized surface plasmon resonances across the Visible to the mid-infrared spectral regions.[1-4]

[1] Carving Plasmon Modes in Silver Sierpiński Fractals, ACS Photonics, 2019, 6, 2974. [2] Probing mid-infrared plasmon resonances in extended radial fractal structures, Opt. Lett., 2019, 44, 3865 [3] Advancements in fractal plasmonics: structures, optical properties, and applications, Analyst, 2019, 144, 13. [4] Exploiting Anisotropy of Plasmonic Nanostructures with Polarization Modulation Infrared Linear Dichroism Microscopy (μPM-IRLD), Adv. Opt. Mater., 2018, 6, 1701336.

Figure:

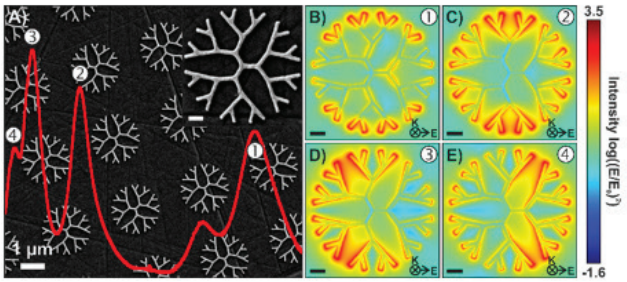

Figure caption:

A) SEM image of fourth-order three branched dendritic fractals. Overlaid on the image is the infrared absorption spectrum of the structure. B-E) Finite difference time domain calculations of the electric field distribution at wavelengths corresponding to the numbered resonances.

Charge Transfer Effect for SERS

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Surface-enhanced Raman scattering (SERS), charge transfer (CT), surface plasmon resonance (SPR), semiconductor, metal-semiconductor nanocomposite

Surface-enhanced Raman scattering (SERS) has the advantages of high sensitivity, high selectivity, high efficiency, and fast and nondestructive detection, and it is a very powerful analytical method for the fingerprint detection of various trace chemical and biological materials. An electromagnetic (EM) and a charge-transfer (CT) enhancement have been generally accepted for SERS enhancement mechanism. With the rapid development of nanotechnology, SERS-active substrates are becoming increasingly diverse, ranging from the metal substrate that was originally studied to the semiconductor substrate that was extensively investigated. For a semiconductor-based SERS substrate, the main contribution of the improvement of SERS activity is mainly due to the change of CT caused by surface plasmon resonance (SPR). We have recently reported SPR induced CT effect for SERS activity of Ag@CuxOS yolk-shell nanostructures [1] and the photo-induced CT enhancement for SERS in a SiO2-Ag-reduced graphene oxide system [2] were explored. The effect of the carrier density in Ag/ITO@PS systems was also explored by 2D correlation analysis, which provides a new method to control SPR by controlling the carrier [3]. In this presentation, results of CT effect for SERS activity on matal-semiconductor nanocomposites will be presented to demonstrate the potential, utility and versatility of semiconductor-based SERS.

No. NRF-2018R1A2A3074587, No. NRF-2020K2A9A2A06036299 and No. NRF-2020R1A4A1016093

[1] B. Han, N. Ma, S. Guo, J. Yu, L. Xiao, Y. Park, E. Park, S. Jin, L. Chen, Y. M. Jung, J. Phys. Chem. C 2020, 124, 16616-16623. [2] S. Guo, S. Jin, E. Park, L. Chen, Z. Mao, Y. M. Jung, ACS Appl. Mater. Interfaces 2021, 13, 5699-5705 [3] B. Han, S, Jin, Q. Chu, Y. Jin, X. Xue, S. Guo, Y. Park, L. Chen, Y. M. Jung, Nanoscale, 2020, 12, 24357–24361

Tip-enhanced Raman spectroscopy for studying 2D materials

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MoS_2, tip-enhanced Raman spectroscopy, EC-TERS, hydrogen evolution reaction

Two dimensional materials hold great promises in electrical, optical, and chemical application due to their extraordinary optical and electronic properties. These properties are significantly influenced by the local defects usually on the nanometer scale or less [1]. However, the understanding of these unique optical and electronic properties and their correlation with defects is still very limited. The main reason is that they are so small that their signals are usually submerged in the strong signals of surrounding pristine materials. Tip-enhanced Raman Spectroscopy (TERS) can obtain not only the topological but also vibrational information of a sample at the sub-10 nm or even sub-molecule spatial resolution[2]. Recently, we demonstrated that TERS can spatially observe the different Raman features of edges of mono- and bi-layer MoS_2 and investigate the edge related modified lattice and electronic length at 10 nm resolution in ambient[3]. We found a new defect-induced Raman peak (396 cm^{-1}) in multilayer MoS_2 which is enhanced by double resonance Raman scattering (DRRS). It showed a unique electron-phonon interaction of defects. We further developed a method to determine the edge types (zigzag and armchair) by performing Raman imaging over the edges of 2D materials. Then we revealed the evolution of the active sites of MoS_2 during the hydrogen evolution reaction by EC-TERS, which can rationally control the reaction by the electrode potential and characterize the reaction at the nanometer spatial resolution[4] on AFM–based EC-TERS. We observed totally different spectral evolutions of the inactive (basal plane) and active edge sites during the electrocatalytic process. The power of TERS demonstrated in MoS_2 can be extended to other 2D materials [5], which may guide the defect engineering for desired properties.

Financial support from NSFC (Grant Nos. 22021001, 21633005 and 21790354) and the MOST of China (Grant No. 2016YFA0200601).

[1] Lin Z. et al., Defect engineering of two-dimensional transition metal dichalcogenides. 2D Mater. (2016); 3, 022002 [2] Zhong J.H. et al., Probing the electronic and catalytic properties of a bimetallic surface with 3 nm resolution. Nat Nanotechnol (2017); 12, 132 [3] Huang T.X. et al., Probing the edge-related properties of atomically thin MoS_2 at nanoscale. Nat Commun (2019); 10, 5544 [4] Wang X. et al., Tip-enhanced Raman spectroscopy for surfaces and interfaces. Chem Soc Rev (2017); 46, 4020 [5] Wu S.S. et al., Photo-induced exfoliation of monolayer transition metal dichalcogenide semiconductors. 2D Mater. (2019); 6, 045052

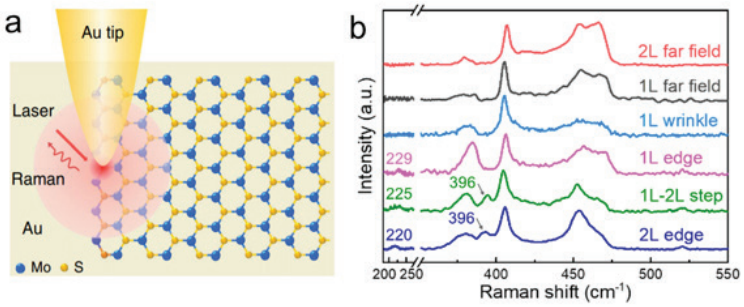

Figure 1. (a) Schematical diagram of AFM-TERS experiment; (b) Raman spectra of four 1D defects and the basal plane in MoS_2.

Mid-infrared lab-on-a-chip for highly-sensitive plasmonic sensing of proteins

Borislav Hinkov*[1], Florian Pilat[2], Laurin Lux[3], Patrícia Lustoza Souza[4], Andreas Schwaighofer [3], Benedikt Schwarz[1], Hermann Detz[5], Aaron M. Andrews[1], Bernhard Lendl[3], Gottfried Strasser[1], Gottfried Strasser[1]

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[2]Institute of Solid State Electronics and Center for Micro- and Nanostructures, TU Wien, Vienna, Austria, Institute of Solid State Physics, Graz University of Technology, Graz, Austria
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[4]Institute of Solid State Electronics and Center for Micro- and Nanostructures, TU Wien, Vienna, Austria, LabSem-CETUC, Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, Brazil
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Lab-on-a-chip, Liquid sensing, protein sensing, plasmonics

The recent advances in high-performance mid-IR quantum cascade devices [1], i.e. quantum cascade laser (QCLs) and detectors (QCDs), enabled a whole new field of liquid spectroscopy by realizing the so-called QCLDs which monolithically integrate both: a coherent light source (QCL) and a high-performance detector (QCD) on the same chip. Connecting them with a dielectric-loaded plasmonic waveguide [1], opens the pathway to highly-sensitive on-chip measurements of liquids, because the optical mode mainly travels outside in the surrounding medium (>> 90%). This combines two important features for liquid spectroscopy: 1.) the high power of mid-IR QCLs allows to penetrate 10s – 100s of micrometers of liquid films, e.g. in H2O, as compared to a few micrometers only with a globar, and 2.) fully monolithically integrated lab-on-a-chip QCLD configurations, which emit and detect mid-IR light at the identical wavelength. In this work we realize such a mid-IR QCLD device. As a proof-of-concept and to show its performance we analyze two different scenarios: 1.) determination of the water content in the solvent isopropyl alcohol (IPA) at l ~ 1630 – 1660 cm-1 up to ~30% (Fig. 1b.) of H2O and measured in a custom made 60-µl microfluidic cell (Fig. 1a.) and 2.) measurement of the absorbance vs concentration curve of the protein bovine serum albumin (BSA) in D2O at 1597 cm-1, for concentrations between ~4 mg/ml up to ~86.5 mg/ml (Fig. 1c.). In this experiment, the entire sensor chip is submerged into the solution. Next, we want to monitor the change of secondary structure of BSA when exposed to increasing temperatures, monitored with our QCLD sensor and compared to similar experiments with other configurations [2].

Financial support from the Austrian Science Fund (FWF) (M2485-N34) is greatly acknowledged.

[1] B. Schwarz et al., "Monolithically integrated mid-infrared lab-on-a-chip using plasmonics and quantum cascade structures", Nat. Commun. 5, 4085 (2014). [2] A. Schwaighofer, et al. External cavity-quantum cascade laser infrared spectroscopy for secondary structure analysis of proteins at low concentrations. Sci. Rep. 6, 33556, (2016).

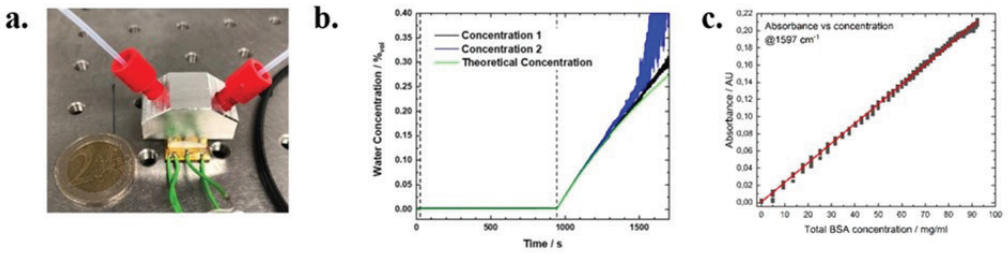

Figure 1: a. Custom made Al fluid-cell (~60 µl) b. Time-dependent water concentration measured in the microfluidic cell. c. Absorbance vs conc. measurement of BSA in D2O, submerging the whole sensor.

Metasurface-Enhanced IR Spectroscopy Augmented by Artificial Intelligence for Resolving Dynamics between Major Biomolecules

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infrared spectroscopy, biosensors, plasmonics, artificial intelligence, metasurfaces

In this work, we present a powerful plasmonic biosensor, which leverages deep learning and multiresonant infrared metasurfaces to provide universal applicability for biomolecular system studies [1]. The ability to detect and monitor biomolecules is crucial for understanding a multitude of biological processes in health and disease. However, these processes are complex as they involve interactions between four major classes of biomolecules, i.e. proteins, nucleic acids, carbohydrates and lipids. Remarkably, our biosensor is capable of differentiating in a label-free, nondestructive and real-time manner between the biomolecules (Fig. 1a). This achievement is demonstrated by using a liposome-based nanoparticle assay featuring simultaneously multiple analytes from the different classes, whose dynamic interactions are effectively resolved by our biosensor (Fig. 1b). The new method relies on a broadband multiresonant metasurface composed of nanoplasmonic antennas tuned to provide three resonances across the mid-IR range, which contains the characteristic spectral fingerprints of all biomolecules. The nanoplasmonic design is based on grating order-coupled nanogap (GONG) arrays [2], which generate strong near-field intensity enhancements up to 50,000 for achieving ultra-high sensitivity. By integrating the metasurface in a microfluidic device, water-dissolved bio-analytes are introduced in a controlled flow while continuously collecting metasurface-enhanced absorption signals. Importantly, due to the real-time format of our broadband optofluidic method, a vast amount of spectrotemporal data is generated, which motivates its coupling with deep learning. This allows to accurately and dynamically discriminate between more biomolecules than we could with our previous approach, in which we used a dual-resonant metasurface [3] coupled to machine learning-based data analysis [4]. Augmenting surface-enhanced IR absorption (SEIRA) spectroscopy with deep learning expands the capabilities to new horizons where experiment complexity and training data wealth go hand in hand [5]. We believe that our pioneering deep learning-augmented metasurface-based biosensor will open up exciting perspectives to study inherently complex biological processes by unraveling molecular mechanisms such as intercellular communication via exosomes encapsulating proteins and nucleic acids, or the interaction of nucleic acids/polysaccharides with proteins in gene regulation and neurodegeneration.

[1] A. John-Herpin, D. Kavungal, L. von Mücke, and H. Altug, Adv. Mater. 33(14), 2006054 (2021). [2] A. John-Herpin, A. Tittl, and H. Altug, "Quantifying the limits of detection of surface-enhanced infrared spectroscopy with grating order-coupled nanogap antennas", ACS Photonics 5(10), 4117 (2018). [3] D. Rodrigo, A. Tittl, A. John-Herpin, O. Limaj, H. Altug, ACS Photonics 5(12), 4903 (2018). [4] D. Rodrigo, A. Tittl, N. Ait-Bouziad, A. John-Herpin, O. Limaj, C. Kelly, D. Yoo, N. J. Wittenberg, S.-H. Oh, H. A. Lashuel, and H. Altug, Nat. Commun. 9(1), 2160 (2018). [5] A. Tittl, A. John-Herpin, A. Leitis, E. R. Arvelo, H. Altug, Angew. Chem. Int. 58(42), 14810 (2019).

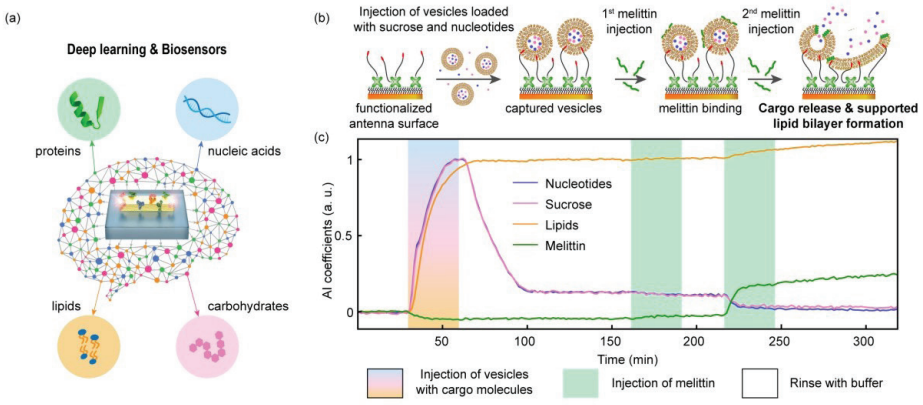

Figure 1. (a) Mid-IR metasurfaces coupled to AI for label-free biosensing. (b) Schematics of biomolecular events unfolding on the metasurface. (c) AI-predicted coefficients of dynamic bio-experiment.

Excitation conditions for surface-enhanced hyper Raman scattering with gold nanospheres and nanorods

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finite-difference time-domain (FDTD), gold nanoparticles, gold nanorods, electromagnetic enhancement, surface-enhanced hyper Raman scattering (SEHRS)

Hyper Raman scattering (HRS), as two photon analogue of spontaneous Raman scattering has a disadvantage of having a very low cross section. This can be overcome by employing the process of surface-enhanced hyper Raman scattering (SEHRS) that improves the weak HRS by chemical and electromagnetic enhancement. As shown by us and others, gold nanoparticles can be used as plasmonic structures for SEHRS (e.g., [1]) Here, we present our findings on the optimum conditions for achieving high electromagnetic enhancements of surface-enhanced hyper Raman scattering (SEHRS). [2] The generation of enhanced local field enhancement depends on the plasmonic properties of the nanoparticles and, therefore, on the excitation conditions. We carried out finite-difference time-domain (FDTD) simulations for the enhancement of the excitation light as well as for the HRS light for excitations in the wavelength range from the visible to the short-wave infrared (SWIR) for dimers of gold nanospheres and nanorods. The results show that the non-linear contribution of the intensity enhancement of the excitation field dominates the overall enhancement for visible excitation wavelengths. Nevertheless, for excitation wavelengths above 1000 nm, the influence of the enhancement of the HRS fields is high. By additional simulations of the absorbance spectra and in experiments, we showed that this is due to the resonance of the HRS photons with plasmonic modes of the gold spheres and nanorods. These findings help us developing more optimized plasmonic systems for two-photon based sensing applications in the SWIR range.

[1] Madzharova, F., et al., The Journal of Physical Chemistry C, 2018. 122(5): p. 2931-2940. [2] Dusa, A., Madzarova, F., Kneipp, J., Frontiers in Chemistry, 2021., in press

Super-resolution Surface-Enhanced Raman Scattering microscopy via polarization contrast

Oleksii Ilchenko[1], Denys Slobodianiuk[2], Yuriy Pilgun[3], Andrii Kutsyk[2], Kaiyu Wu [4], Lasse Højlund Eklund Thamdrup[1], Anja Boisen[1]

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polarized Raman, super resolution, SERS, hot spot, Raman microscopy

It is known, that polarized Raman microscopy can provide material orientation contrast when a sample is mapped multiple times using different combinations of incident laser polarization and analyzer orientation [1]. However, the number of polarization channels used for Raman polarization contrast visualization is usually limited to horizontal/vertical (HV) and horizontal/horizontal (HH) configurations [2]. Here, we expand the number of channels in polarized Raman microscopy up to twenty. Notably, all twenty channels can be registered simultaneously due to the unique optical design of the utilized polarized Raman microscope (THOR, Lightnovo ApS [3]). Twenty polarized Raman maps consisting of 200x200 pixels are simultaneously collected in less than 35 min with an individual pixel registration time of 0.05 s/pixel. The demonstrated polarized Raman imaging capabilities comply perfectly with the requirements of super-resolution reconstruction microscopy techniques such as super-resolution fluorescence polarization microscopy [4]. Here, fluorescent dipoles provide polarization contrast used for image reconstruction via modified algorithms initially developed for STochastic Optical Reconstruction Microscopy (STORM) [5]. In Fig. 1 (A), we demonstrate four of the twenty simultaneously collected polarized Raman maps of 10μM trans-1,2-bis(4-pyridyl)ethylene (BPE) in ethanol solution deposited onto a SERS substrate consisting of high-density Au-capped nanopillars [6]. Map dimensions were 5x5μm2, step size 25 nm; measuring conditions: laser wavelength 785 nm, laser power 70 uW, microscope objective 100x, NA=0.75, registration time 0.05 sec per point. The obtained polarized Raman maps clearly demonstrate polarization contrast which is expected to be present for plasmonic hot spots created by a dimer of Au-capped nanopillars [7]. Fig.1 (B) represents the localization of hot spots (red dots) as the result of applying a modified STORM algorithm to the twenty discrete polarized Raman maps. The reconstructed data demonstrate multiple hot spots being resolved with a resolution way below the diffraction limit at 785 nm laser excitation. We consider these results a very promising step towards far-field super-resolution Raman microscopy. Nevertheless, more solid confirmation of super-resolution accuracy is still required. Therefore, future work should include fabrication of non-stochastic plasmonic structures with spatially well-defined hot spots that allow for probing and evaluating the achievable super-resolution accuracy associated with polarized SERS imaging.

This work was financially supported by DTU Discovery grant (33216 E-1), the IDUN Center of Excellence (grant no. DNRF122) funded by the Danish National Research Foundation and the Villum Foundation (Grant No. 9301) and Lightnovo ApS.

[1] T. Schmid, N. Schäfer, S. Levcenko, T. Rissom, D. Abou-Ras. Sci. Rep. 2015, 5, 1–7. [2] U. Ramabadran, B. Roughani. Mater. Sci. Eng. B Solid-State Mater. Adv. Technol. 2018, 230, 31–42. [3] O. Ilchenko, Yu. Pilgun et al. Nature Communications, 2019, vol. 10, 5555. [4] N. Hafi et all. Nature Methods, 2014, volume 11, pages 579–584. [5] Holden S. et al. Nature Methods, 2011, volume 8, pages 279–280. [6] M. S. Schmidt, J. Hübner, A. Boisen, Adv. Mater. 2012, 24, OP11. [7] Martin Šubr, Marek Procházka. Nanomaterials. 2018 Jun; 8(6): 418.

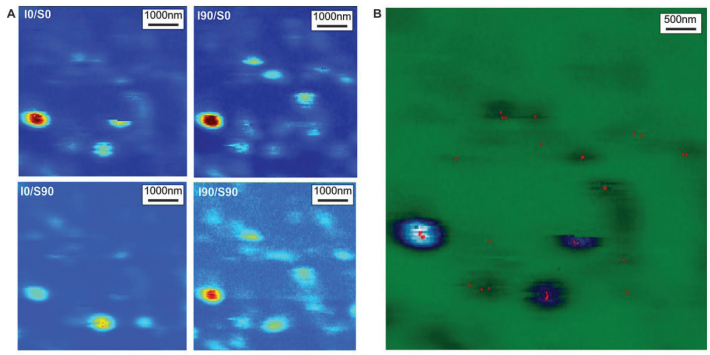

Figure 1. Super-resolution Surface Enhanced Raman Scattering microscopy via polarization contrast. (A) four polarized Raman maps of 10 μM BPE. (B) The localization of hot spots (red dots).

Galvanic vs. electrochemical fabrication of the plasmonic layer: SERS study of yellow natural compounds

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SERS spectroscopy, plasmonic materials, galvanic deposition, yellow natural compounds,

Many different approaches can be applied for the fabrication of quality SERS substrate in terms of repeatability of the preparation and value of the enhancement factor. Methods for fabrication of plasmonic nanostructured surfaces differ in time, financial and technical demands. Thus, it is important to balance the time consumed for preparation and gained quality of the signal amplification. In this study, we aim to compare electrochemical and galvanic preparation of SERS-active substrate with regard to the optimal method for analysing colorful natural compounds. Moreover, SERS studies of natural compounds and biologically active substances often become complicated due to strong fluorescence background when exposed to visible wavelengths favored in Raman spectroscopy. In this study, plasmonic surfaces (Au, Ag, Cu) were obtained using two wet fabrication methods: i) spontaneous galvanic deposition on Al carrier1 and ii) electrochemical deposition on Pt target. We use three natural compounds containing various functional groups with different affinity to the surface to compare both approaches. The model analytes are riboflavin (vitamin B2), berberine (alkaloid), and myricetin (flavonoid). Data were collected using the FT-Raman spectrometer (1064 nm), dispersive Raman (780 nm and 532 nm), and complemented with infrared absorption data. The most suitable experimental conditions (a combination of the analyte, plasmonic substrate, and excitation wavelength), as well as the limit of detection of analytes (down to the concentrations 10^{-9} mol/l) on individual substrates, were obtained. Significant fluorescence of mentioned yellow compounds was reduced by a combination of non-covalent physisorption on the plasmonic surface and the use of infrared excitation. We show that electrochemically prepared layers can attain higher SERS enhancement and lower detection limit, however, their preparation lacks simplicity and requires more precautions and attention when compared to galvanic deposition, which stands out with reasonably high Raman signal amplification along with the time-sparing procedure and mass production potential.

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References 1 O. Volochanskyi, M. Švecová, V. Bartůněk, V. Prokopec: Coll. Surf. A, 616 (2021), 126310

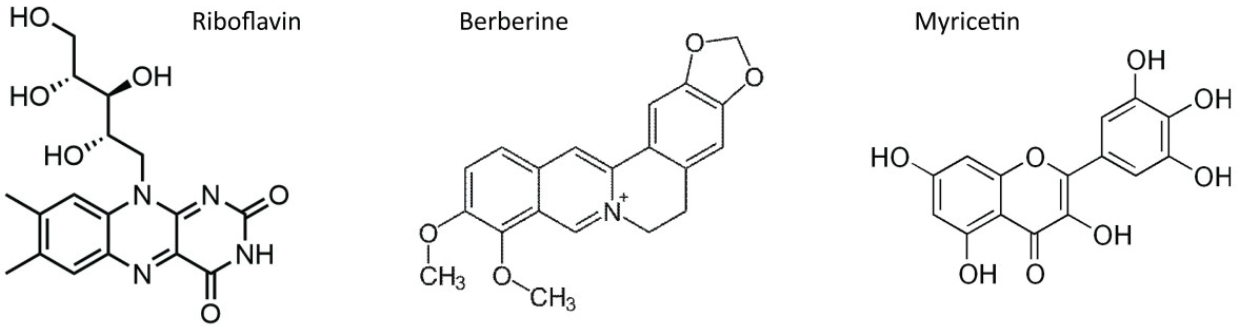

Figure 1. Analytes' structures

Theoretical Investigation of Near-field Probes

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Plasmonic, Nanoantenna, TERS, Scattering

Due to its high spatial resoluion and high sensitivity tip-enhanced Raman spectroscopy (TERS) became an important tool for analyzing nanomaterials. However the specific enhancement of a particular tip is difficult to predict. This issue can be addressed by investigating the tips' plasmonic properties. Existing numerical studies consider the optical responses of the plasmonic tips but mostly deal with simplified and smooth conical tips [1]. Metallic tips determine the performance of probe based near-field optics experiments. Although the importance of tips has been highlighted continuously, there are only few investigations of rough tips. Most models consider smooth tips with symmetric and regular-shaped plasmonic structures or even just a simple sphere as active element. Interestingly, rough tips produced by metal evaporation and subsequent annealing show reliable enhancement and are comparatively easy to produce. For such tips a more elaborate model for the estimation of the far-field and near-field optical response is required [2]. Here we will present a general approach towards modelling the interaction of light with a 3-dimensional plasmonic near-field probe. Using finite element modelling (FEM) based electromagnetic calculations to model the far- and near-field optical response of plasmonic probes, allows to systematically increase the complexity of the tip and obtain realistic results for arbitrary shapes, sizes, and distributions of metal nanoparticles. First, we determine the size distribution of the silver nanoparticles on the plasmonic tips was determined and then based on that we reinvestigate silver dimer structures were investigated. Based on the dimer case, our model we expanded to a 3D plasmonic tip by parametrizing a scanning electron microscopy (SEM) image of a real tip. The near-field investigations will clearly demonstrate the dominant influence of the edge particle on the field enhancement. Last but not least a far-field scattering pattern of a plasmonic probes that indicates peculiarities for specific illumination-collection geometries will be presented.

[1] S. Trautmann, et al., Nanoscale, 10, 9830, (2018) [2] A. Taguchi, et al., Nanoscale, 7, 17424, (2015).

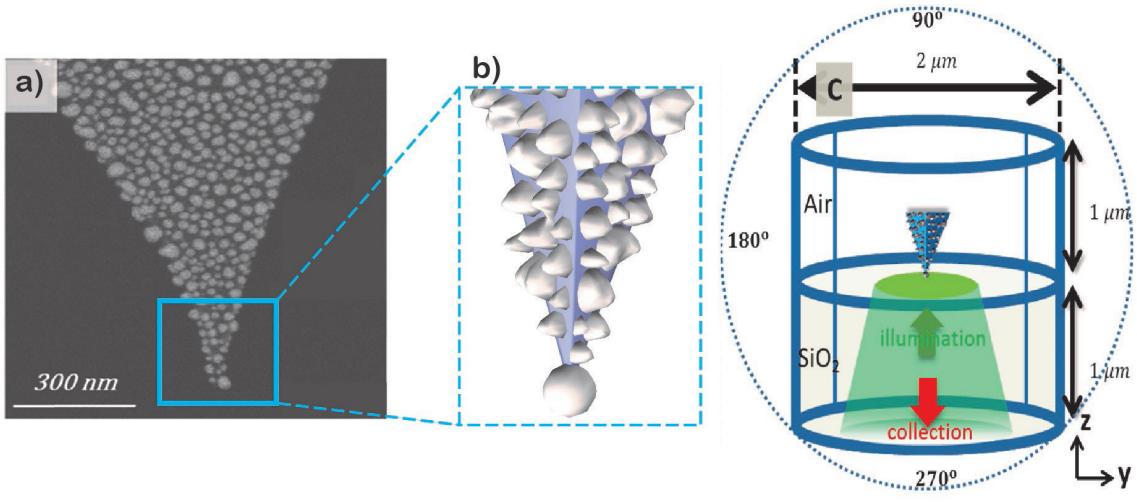

Figure 1. a) SEM image of a "silver-evaporated-on-Si" tip. b) parametrized 3D shape tip modelled in COMSOL Multiphysics for FEM calculations. c) Geometric description of the model used.

Wavelength-Scanned Surface-Enhanced Raman Excitation Spectroscopy – from macroscopic measurements to microscopic research of single gold oligomers

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Wavelength-Scanned SERS, Enhancement profile, BPE, Raman microscopy, Dark-field microscopy

Surface-enhanced Raman spectroscopy is an ultra-sensitive technique that allows the detection of chemical molecules at the level of single molecules. The Raman signal enhancement is closely related to the spectral characteristics of metallic nanostructures and the wavelength of the excitation laser beam. Appropriate tuning of the energy of plasmonic resonances to the laser beam can result in additional enhancement of the Raman signal significantly. This paper presents a study showing the wavelength dependence of the excitation beam on the energy of plasmon resonances in the SERS technique. Spherical nanoparticle oligomers systems and monomers of gold nanorods were used in this study. Gold nanoparticles were coated with trans-1,2-(4-pyridyl)ethylene (BPE) molecule and stabilizing substance – silica shell or linear polymer polyvinylpyrrolidone (PVP). Plasmon resonance bands from oligomers of spherical nanoparticles and monomers of nanorods were excited with a wavelength range of 600-850 nm. The enhancement profiles for the colloidal nanoparticles showed shifts toward longer wavelengths relative to the extinction maximum of the colloidal systems. Microscopic research will include the measurement of Raman spectra of single nanoparticle systems placed on substrates. This part of the study will be correlated with scanning electron microscopy and dark field microscopy to determine the correlation between the dark-field scattering and the enhancement profile.

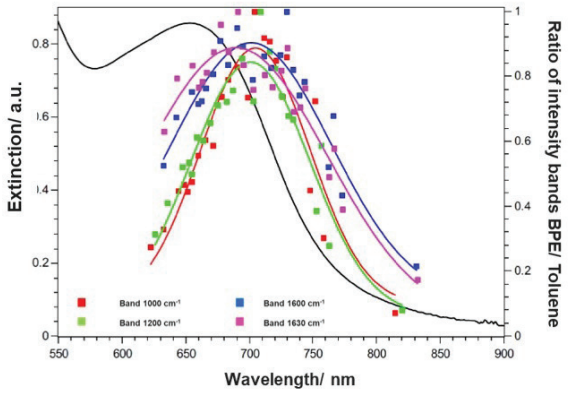

Figure 1. Enhancement profiles for gold oligomers consisting of 32 nm diameter spherical gold nanoparticles coated with BPE and PVP.

Ag-TiO2 nanoplatforms as substrates for Photo-induced Enhanced Raman Spectroscopy. An effect of plasmonic features on PIERS efficacy and lifetime.

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Photo-induced Enhanced Raman Spectroscopy (PIERS), Surface-enhanced Raman Spectroscopy (SERS), Ag-TiO2 nanoplatforms, Localized surface plasmon resonance (LSPR)

Surface-enhanced Raman spectroscopy (SERS) is considered as one of the most powerful analytical technique for identification and quantification different types of biological and chemical analytes. The use of specially prepared substrates such as rough metallic surfaces or metallic nanoparticles (MNPs) enhances the Raman scattering process by molecules adsorbed to this surface, even by a factor of 10^{14} which enables the detection of selected molecules even at ultra-low concentration. Photo-induced enhanced Raman spectroscopy (PIERS) goes one step further compared to the conventional SERS. Suitable substrates with noble metal nanoparticles deposited on the semiconductor provide additional electromagnetic enhancement (EM) due to UV photoactivation resulting in charge transfer from the semiconductor to MNPs. The main goal of our research was to investigate the relationship between the morphology, plasmonic properties of AgNPs photocatalytically grown on 22 ± 1 nm thick TiO_2 coating (Ag-TiO_2) and the amplification of the Raman signal due to the PIERS effect. Their ability to induce surface enhancement was evaluated by us previously [1]. Nanoplatforms were prepared in a two-step procedure. First a thin coating of TiO_2 were obtained on silicon wafers using the sol-gel method, and then AgNPs were photo-grown from solution of $AgNO_3$ under UV illumination at λ=365 nm. Ultimately, three different Ag-TiO_2 substrates were fabricated with maxima of local surface plasmon resonance (LSPR) at 370 nm and 415 nm. The enhancement of the Raman signal was probed with 4-mercaptobenzoic acid with illumination of the substrates by UV light at 254 nm and 365 nm. We evaluated Raman signals in terms of time exposure and its decay. Both samples showed a PIERS response by increasing Raman intensity up to 10 times in comparison to the corresponding SERS spectra and the accumulation of hot electrons in Ag-TiO_2 substrates was observed up to 24 h.

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[1] E. Wiercigroch, A. Kisielewska, A. Blat, A. Wislocka, I. Piwoński, K. Malek, Photocatalytical decoration of thin titania coatings with silver nanostructures provides a robust and reproducible SERS signal, Journal of Raman Spectroscopy, 2019, 50, 1649-1660.

Towards long-wave infrared lab-on-chip sensors using plasmonic and quantum cascade technology

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Plasmonic, Long wave infrared, Lab on chip sensors, Dielectric loaded surface plasmon polariton waveguide, Quantum Cascade Technology

Many molecules of interest in chemistry and life science have their fingerprint absorptions in the mid-infrared (mid-IR), some of them extending to the Long-Wave Infrared (LWIR, 8-12 µm), including e.g. ammonia, glucose and TATP. Noteworthy, significant progress in the monolithic integration of fast and compact mid-IR sources, i.e. (QCLs) and detectors (QCDs), have accelerated the development of a new generation of Photonic Integrated Circuits (PICs). This opens the way to lab-on-chip spectroscopy [1], which also addresses the LWIR spectral region. In this context, Dielectric-Loaded Surface Plasmon Polariton Waveguides (DLSPPWs) have been used as key technology, since they are an effective and simple solution for on-chip guiding of light while simultaneously offering a platform for chemical analysis and sensing. However, the principal challenge towards the realization of LWIR PICs, is to attain low-loss on-chip waveguiding, as most of the commonly used mid-IR materials absorb in this spectral region. In this work, we address this problem, and design, simulate, fabricate and experimentally characterize a hybrid semiconductor-metal plasmonic scheme based on a Germanium (Ge) stripe on top of a gold layer. We optimize the cross-sectional geometrical factors together with numerical simulations aiming at long range propagation as an important figure of merit. Next, we fabricate such optimized Ge ridges on a gold layer evaporated on Silicon substrate for different Ge stripe widths. Measurements of the attenuation and lateral mode profile of the supported mode were performed including using the cut-back method for extrapolation of the coupling losses at a wavelength of interest of 9.12 µm. We used an end-fire coupling characterization setup provided with an EC-QCL which covered the wavelength range between 5.66 and 11.24 µm. The measured plasmonic modes show low loss propagation (<20 dB/mm) for the entire supported mid-IR spectrum of the EC-QCL, being in good agreement with the simulations. The insertion loss measured trough the cut-back technique was found around 12 dB/mm at 9.12 µm, corresponding to a propagation length of 340 µm. The lateral mode profile shows excellent agreement between measurements and simulations. As for on-chip optical spectroscopy applications only distances of a few hundreds of microns need to be addressed, these results suggest germanium loaded plasmonic waveguides as an attractive solution for LWIR plasmon-photonics for on chip detection of species in the surrounding medium as well as chip-level integrated photonics.

[1] B. Schwarz et al., "Monolithically integrated mid-infrared lab-on-a-chip using plasmonics and quantum cascade structures," Nature Communications, vol. 5, no. 1, Art. no. 1, Jun. 2014, doi: 10.1038/ncomms5085

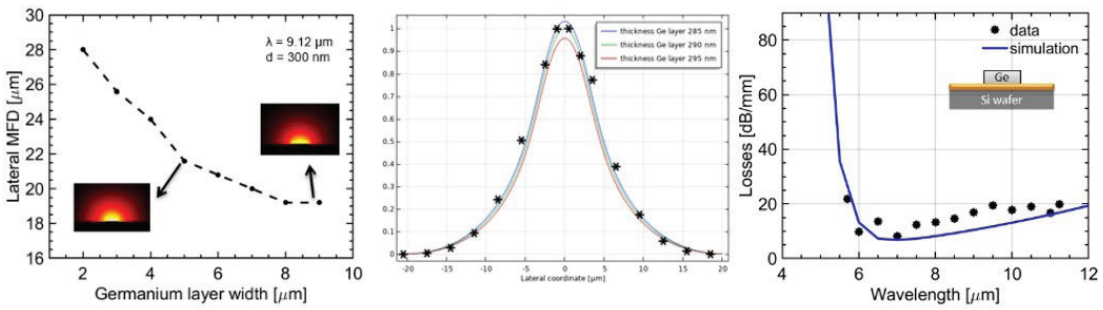

Figure 1: (Left) Lateral mode field diameter vs Germanium layer width. (Centre) Overlap integrals of the simulated (solid lines) and measured (stars) profiles. (Right) Spectral bandwidth of the waveguides and sketch of the cross-section.

1.2. Non-Linear Vibrational Spectroscopy

Fourier-domain stimulated Raman scattering tomography enables label-free volumetric deep tissue imaging with subcellular resolution

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label-free 3D chemical imaging, optical beating technique, stimulated Raman scattering tomography, volumetric deep tissue imaging

We present a novel Fourier-domain stimulated Raman scattering tomography (SRST) which is enabled by using optical beating technique (OBT) associated with non-diffracting Bessel beams to achieve deeper penetration for label-free volumetric chemical imaging with subcellular resolution. In SRST, the Gaussian pump beam is modulated with a spatial light modulator (SLM) to convert to Bessel beam with optical beating, which is overlapped with the Bessel Stokes beam in the sample; By electronically varying the optical beating frequency of the Bessel pump beam through SLM, the depth-resolved SRS signals about the volumetric tissue are encoded in the spatial frequency domain and thus, the depth-resolved SRST can be rapidly retrieved in Fourier domain by implementing inverse fast Fourier transform without a need of mechanical depth-scan for 3D SRS imaging. We demonstrate that SRST imaging using Bessel beams as the pump and Stokes excitation beams with inherent self-reconstructing properties of propagation provides at least 2-fold improvement in imaging depth in highly scattering polymer beads phantom as compared to conventional point-scan SRS microscopy with Gaussian excitation beams. We have also proved the capability of SRST for label-free volumetric deeper molecular imaging on a variety of imaging targets (e.g., Raman-active crystals, plant cells, and biological tissue). We anticipate that the generality of z-scan-free optical sectioning ability of Bessel beam-OBT in SRST can be readily extended to practically any other optical imaging modalities for volumetric 3D deep tissue imaging in biological and biomedical systems.

This work was supported in part by the Academic Research Fund (AcRF) -Tier 1 (WBS R-397-000-326-114, WBS R-397-000-334-114, WBS R-397-000-371-114, and WBS R-397-000-378-114) from Ministry of Education (MOE), and the National Medical Research Council (NMRC) (OFIRG20nov-0113; NMRC/TCR/016-NNI/2016), Singapore

[1] Li Gong, Shulang Lin, and Zhiwei Huang, Stimulated Raman Scattering Tomography Enables Label-Free Volumetric Deep Tissue Imaging, Laser & Photonics Review 2021 (in press)

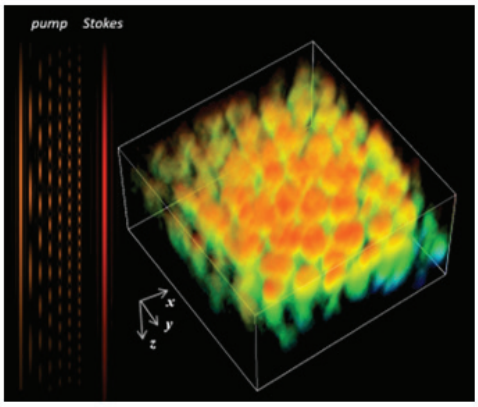

Figure 1. Fouier-domain stimulated Raman scattering tomography technique enables label-free deep tissue 3D imaging of the porcine adipose tissue at submicron resolution.

Multicolor imaging with stimulated Raman scattering

Yasuyuki Ozeki[1]

[1]The University of Tokyo

Stimulated Raman scattering, Multicolor imaging, Wavelength-tunable laser, Imaging flow cytometry, Raman probes

Stimulated Raman scattering (SRS) is now regarded as a powerful modality of molecular-vibrational imaging because SRS provides highly sensitive Raman signal when detected with two-color synchronized picosecond pulses [1,2]. To enhance the power of SRS imaging, we developed an SRS microscope with a high-speed wavelength-tunable laser source, which is capable of video-rate SRS imaging with frame-by-frame wavenumber tunability with a bandwidth of 300 cm-1 [3,4]. This system allows for hyperspectral or multicolor imaging by tuning the wavenumber continuously or discretely, respectively, which is found useful for various vibrational imaging applications including label-free multicolor imaging of animal tissue [3,5], microalgal cells [6], and plants [7]. The imaging speed has been recently upgraded by developing an ultrafast wavelength-switchable pulse source, which realized four-color SRS imaging of cells in a high-speed flow at 20 mm/s on a microfluidic chip [8]. This SRS imaging flow cytometer allows for acquiring SRS image of > 10,000 cells within a few minutes, and hence will be useful for analyzing a heterogeneous population of cells in a label-free manner.Our high-speed SRS imaging system is also useful for multicolor imaging with Raman probes [9], which is paving the way to supermultiplex imaging and imaging of small biomolecules. Recently we have developed functional Raman probes that realize multiplex sensing of enzyme activities [10] and photoswitching of Raman signal [11]. We also discuss how the sensitivity of SRS can be further enhanced by utilizing the quantum nature of light [12].

The work presented here was supported by JST CREST, JSPS KAKENHI, ImPACT Program, and Q-LEAP.

[1] Y. Ozeki et al., Opt. Express 17, 3651-3658 (2009). [2] Y. Ozeki, Chin. Opt. Lett. 18, 12170 (2020). [3] Y. Ozeki et al., Nature Photon. 6, 845-851 (2012). [4] Y. Ozeki et al., IEEE J. Sel. Top. Quantum Electron. 25, 7100211 (2019). [5] S. Satoh et al., Pathology Int. 64, 518-526 (2014). [6] Y. Wakisaka et al., Nature Microbiol. 1, 16124 (2016). [7] T. Iino et al., Analyst 146, 1234-1238 (2021). [8] Y. Suzuki et al., Proc. Natl. Acad. Sci. U.S.A. 116, 15842-15848 (2019). [9] J. Shou et al., Photonics West 11252-45 (2020) [10] H. Fujioka et al., J. Am. Chem. Soc. 142, 20701-20707 (2020). [11] J. Shou and Y. Ozeki, Opt. Lett. 46, 2176-2179 (2021). [12] Y. Ozeki et al., J. Opt. Soc. Am. B 37, 3288-3295 (2020).

Effects of Elevated Temperatures on the Silica Surface Charge Revealed by Interfacial Water SFG Signal

Md Shafiul Azam[1], Canyu Cai[2], Julianne Gibbs[3], Eric Tyrode[4], Dennis Hore[1]

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SFG, Interfacial water, silica/water, temperature, hydrogen bonding

Surface charge on silica governs many environmental and geophysical processes such as dissolution, rock weathering, erosion, and pollutant transport happen on the silica surface. Molecular interactions at the confined environment of silica/water interface regulate the properties of silica-based catalysts, chemical sensors, DNA microarrays, drug delivery platforms, and microfluidics, etc. Although temperature has been long known as one of the important factors influencing all the above applications, identifying the temperature-dependent structure of silica/water is still not well-explored primarily because of the difficulties associated with assessing the surface charge and the thermodynamic parameters governing the silanol deprotonation from potentiometric titration due to the temperature-dependent dissolution of colloidal silica. Studying the interfacial phenomena on a planar silica surface requires surface specific spectroscopic techniques such as vibrational sum frequency generation (SFG) spectroscopy. Herein, we employ SFG to monitor the water O–H stretching band over a temperature range of 10–75 °C to account for the increase in surface potential from the deprotonation of the surface silanol groups. The water signal increases with temperature up to 60°C and then drops as revealed by SFG. We explained this behavior at the silica surface as a balance between increasing surface charge density and a decreasing contribution of water molecules aligned by the surface charge. Using our model that accounts for two different types of silanol sites, we fit our data to calculate the changes in enthalpy and entropy for deprotonation at each site – more acidic (pKa – 5.7) and less acidic (pKa ≈ 8.7) silanol groups. The presentation will discuss this first ever experimental determination of these thermodynamic parameters for hydrated silanol groups at the silica surface, critical to a wide range of geochemical, materials, and technological applications.

Broadband Impulsive Stimulated Raman Scattering based on a Chirped Detection

Giovanni Batiganani[1]

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Nonlinear Raman Spectroscopy, Time Domain Spectroscopy, Impulsive Vibrational Scattering

Recent advances in ultrafast time-domain Raman spectroscopy -namely the advent of impulsive stimulated Raman scattering (ISRS)- introduced a potential method for real time monitoring vibrational oscillations in photo-excited systems: by combining a pump and a probe pulse for stimulating and then probing Raman coherences, ISRS can directly access atomic motions and molecular properties both on the ground or on excited electronic states. During the last decade, the great potential of ISRS has been exploited for studying a broad range of phenomena, at the intersection between Physics and Biochemistry [1–4]. Critically, at odd with frequency-domain Raman approaches, ISRS typically requires long acquisition times since the system response has to be measured scanning a sequence of time delays between pump and probe pulses. As a consequence, ISRS (1) is ineffective for probing irreversible processes, such as phase transition, non-reversible chemical reactions or phenomena accompanied by sample damaging, and (2) can be affected by instabilities or by beams dealignments occurring during a time-delay scan and hence it typically suffers from lower signal-to-noise ratios with respect to frequency domain approaches. For such reasons, developing novel experimental approaches able to improve the ISRS sensitivity is a key issue to actualize the potential of this method, beneficial to the physical chemistry community. In this talk, it will be introduced a novel experimental scheme for the realization of Chirped based Impulsive Stimulated Raman Spectroscopy (CISRS) [5], based on introducing a chirp in the probe pulse for recording the time-domain Raman information without scanning the pump and probe delay: since different probe wavelengths interact with sample at different time delays, the evolution of the stimulated vibrational coherences is encoded in the probe spectrum. We will present an experimental realization on how to use such introduced scheme to measure Raman spectra, ensuring acquisition times 2 orders of magnitude faster, with comparable or better temporal and spectral resolutions.

[1] Kuramochi, H. et al. Probing the early stages of photoreception in photoactive yellow protein with ultrafast time-domain Raman spectroscopy. Nat. Chem. 9, 660 (2017). [2] Schnedermann, C. et al. Vibronic Dynamics of the Ultrafast all-trans to 13-cis Photoisomerization of Retinal in Channelrhodopsin-1. J. Am. Chem. Soc. 138, 4757–4762 (2016). [3] Batignani, G. et al. Probing femtosecond lattice displacement upon photo-carrier generation in lead halide perovskite. Nat. Commun. 9, 1–5 (2018). [4] Fumero, G. et al. Two-Dimensional Impulsively Stimulated Resonant Raman Spectroscopy of Molecular Excited States. Phys. Rev. X 10, 011051 (2020). [5] Batignani, G., Ferrante, C., Fumero, G. & Scopigno, T. Broadband Impulsive Stimulated Raman Scattering Based on a Chirped Detection. J. Phys. Chem. Lett. 10, 7789–7796.

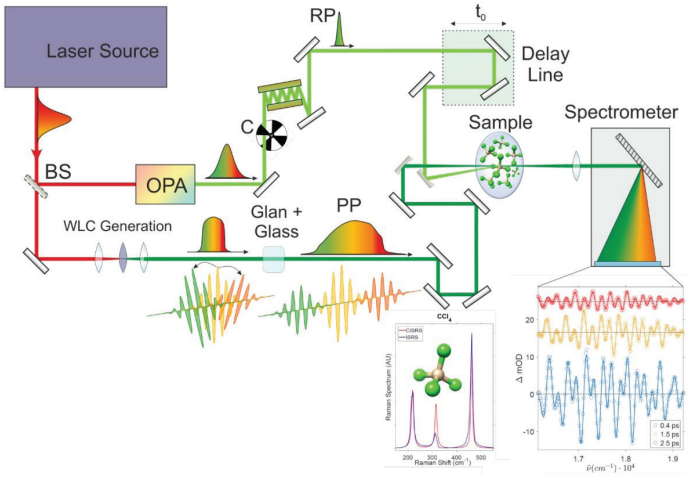

Figure 1.

Studying the Sensing Activities of Organic Nanoparticles and their Interfacial Structure at Air/ Aqueous Interface using Nonlinear Vibrational Spectroscopy

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Nonlinear vibrational spectroscopy, Organic nanoparticles, Carbon dots, Sensing, Interfacial structure

The perpetually increasing demand for compressive sensing of multi-analytes has led to the exploration of new sensing systems. Recently, organic nanoparticles (ONPs) and carbon dots (CDs) have shown tremendous potential towards sensing and regulating the quantification of heavy metal ions in the aqueous medium. [1-3] CD is one of the most promising classes of carbon nanomaterials, used in various scientific fields due to their properties like biocompatibility, low cytotoxicity, tunable fluorescence emission, and excitation, and great aqueous solubility, etc. [1-3] ONPs play a significant role in sensing because of their effortless production, sensitive response, enhanced photostability, and possibility for structural modifications to achieve tunable molecular scaffolds. [3, 4] To probe the interfacial molecular activities, nonlinear vibrational spectroscopy has been developed as a potential spectroscopic tool to examine the molecular structures under different chemical environments at the interfacial region having submonolayer sensitivity. [5-8] In the present work, we have used sum-frequency generation (SFG) vibrational spectroscopy to probe the interfacial structure of ONPs and CDs and their selective binding activities with specific ions at the air-aqueous interface. The selective sensing of ONPs and CDs for different ions is also probed by using fluorescence spectroscopy to extract the bulk signature of both the materials in the aqueous solution. For the case of SFG spectra, the CH- and OH-stretch region from 2800 to 3750 cm^{-1} are probed to observe the interfacial activities of the materials. Interestingly, it is found that the structure of CDs is susceptible in the presence of silver (Ag (I)) ions in the aqueous solution at the air/ aqueous interface. A concentration-dependent approach of SFG studies of Ag (I) ions in the aqueous medium given the opportunity to extract the evidence of CDs binding with the Ag (I) ions. However, we did not observe any substantial alteration of interfacial activities during the case of ONPs in the CH- and OH-stretch spectral region in the presence of cesium ions, indicating a dominant presence of hydrophilic interactions of the ONPs within the aqueous solution. We will present a detailed discussion about the interfacial structural perturbation of both the materials and the interfacial water structure during the process of binding/sensing.

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[1] X. Sun and Y. Lei, Trends Anal. Chem. 89, 163-180 (2017). [2] K. K. Chan, S. H. K. Yap, and K.-T. Yong, Nano-micro Lett. 10, 1-46 (2018). [3] G. Kaur, H. Kaur, A. Singh, M. Chaudhary, N. Kaur, N. Singh, and K. C. Jena, Chem. Asian J. 15, 2160–2165 (2020). [4] A. Kaur, G. Kaur, A. Singh, N. Singh, and N. Kaur, ACS Sustain. Chem. Eng. 4, 94-101 (2016). [5] H. Kaur, D. Tomar, H. Kaur, B. Rana, S. Chaudhary, and K. C. Jena, in Advances in Spectroscopy: Molecules to Materials (Springer, 2019), pp. 39-55. [6] D. Tomar, B. Rana, and K. C. Jena, J. Chem. Phys. 152, 114707 (2020). [7] R. Singh, V. Thorat, H. Kaur, I. Sodhi, S. K. Samal, K. C. Jena, and A. T. Sangamwar, Mol. Pharm. 18 1604-1621 (2021). [8] K. C. Jena, P. A. Covert, and D. K. Hore, J. Phys. Chem. Lett. 2, 1056-1061 (2011).

Elucidating the Effects of Surfactant Competition for Interfaces on the Hydrogen Bonding Network of Water by SFG

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Water, SFG, Chi 3, PFAS,

Perfluorinated alkyl substances (PFAS) are a global environmental concern. Generally, PFAS accumulate in soil, and groundwater. From there PFAS enter plants and animals through ingestion and are linked to a plethora of health issues. In this work, we probe the fundamental environmentally relevant surface properties of ammonium perfluorooctanoate (APFO) as a model PFAS for how these materials accumulate in soil and groundwater using sum frequency generation vibrational spectroscopy (SFG). We also investigate the effect of competition for an interface between PFAS and sodium dodecyl sulfate (SDS) as a model for groundwater. Finally, we probe these influences on the hydrogen bonded network of water to understand the environmentally relevant chemistry which occurs. First, the SFG response from water is diminished when APFO is at the interface versus SDS. This is attributed to the greater hydrophobicity of APFO than SDS, reducing the interactions between the surfactant/surface and interfacial water molecules. Second, we observe the diffuse layer of water molecules is most perturbed when there is a mixture of surfactants competing for the interface, followed by the case of only APFO at the interface, and is least perturbed when SDS is at the interface. This is likely resulting from a surfactant layer of differing hydrophobicities, yielding nonuniform ordering of water molecules which directly interact with the surfactant layer. We simulate the third order response, termed the Chi 3 effect for the water SDS interface to deduce its impact on our findings. We conclude the loss of SFG response in the diffuse layer is predominately from a loss in Chi 3 response since the thickness of the diffuse layer is diminished when the net ordering is diminished. Lastly, the introduction of competition for the interface between SDS and APFO yields a different orientation and net ordering of the SDS molecule compared to the case of only SDS at the interface. The dipole orientation of the methyl group for SDS changes ~10° from the surface normal as a result of the presence of APFO and SDS at the interface. Also, the SFG lineshape for an APFO:SDS resembles a highly disordered monolayer. These results support the calculated ordering factor of SDS at the interface which decreases 4-fold. These findings provide understanding of the accumulation process of PFAS in water/mineral interfaces found throughout the environment.

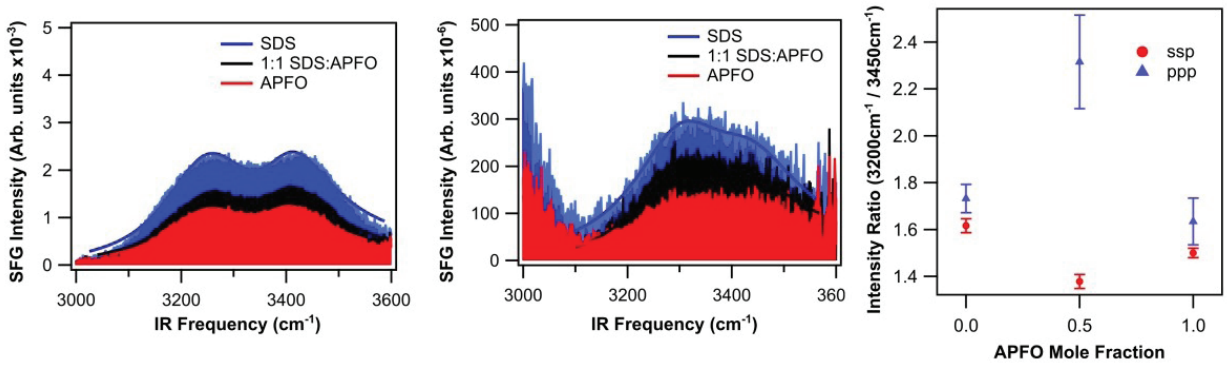

Figure 1. SFG spectra of water in ssp (left) and ppp (middle). Fitted peak intensity ratios of diffuse layer (~3200cm-1/~3300cm-1 in ppp) and stern layer (~3450cm-1) versus surfactant composition (right)

Studying the Evaporation Process at Air/Aqueous Interface using Interferometry and Nonlinear Vibrational Spectroscopy

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Evaporation, Sum Frequency Generation Vibration spectroscopy, Hydrogen Bonding, air/water interface, Ions

Evaporation of water molecules is a topic of fundamental research, as it plays an imperative role in various physical and biochemical processes [1]. Water evaporation is the process in which a single water molecule breaks its hydrogen bonding association with other water molecules located at the air/water interface and liberate to the gas-phase region. The air/water interface consists of a dynamic population of water species with the diverse possibilities of making mono- to tetra hydrogen bonds (HBs) with the neighboring water molecules. There have been some theoretical approaches existing in the literature to probe the role of hydrogen bonding in the evaporation process using molecular dynamics (MD) simulations [2]. Keeping this motivation in mind, we wanted to probe the evaporation process of the water molecules at the air/aqueous interface using interferometry and nonlinear vibrational spectroscopic tools [1, 3, 4]. In 1888, Franz Hofmeister had reported a series of anions ranked according to their propensity to salt out macromolecules from their aqueous solutions, known as Hofmeister series of anions [5]. The ions in this series have been characterized as kosmotropic (structure makers) and chaotropic (structure breakers). The sum frequency generation vibrational spectra are recorded in the OH-stretch region at the air/salt-water interface in the presence of various ions. The observed spectral features from the SFG spectroscopy are compared with the data collected from the interferometry to extract new molecular level understanding on evaporation of water molecules at the air/aqueous interface. We will give a detailed analysis of our findings in the conference.

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[1] A. M. J. Edwards, P. S. Atkinson, C. S. Cheung, H. Liang, D. J. Fairhurst, and F. F. Ouali, Phys. Rev. Lett. 121, 184501 (2018) [2] P. E. Mason, J. Phys. Chem. A 115, 6054 (2011) [3] K. C. Jena, P. A. Covert, and D. K. Hore, J. Phys. Chem. Lett. 2, 1056 (2011) [4] D. Tomar, B. Rana, and K. C. Jena, J. Chem. Phys.152, 114707 (2020) [5] F. Arch. Hofmeister, Exp. Pathol. Pharmakol 24, 247 (1888)

Infrared-visible sum-frequency generation super-resolution microscopy of phonon polaritons in SiC nanostructures

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Sum frequency generation, Nonlinear microscopy, Nanophotonics

Sum-frequency generation (SFG) allows the study of surfaces and inversion broken systems. In a new approach we implemented a wide field sum-frequency microscope combining an infrared free electron laser (IR FEL) as excitation source with visible upconversion. The IR FEL provides a powerful, narrow band, and tunable light source [1]. By direct imaging of the SFG light with a microscope in a wide field scheme without scanning the sample or the focus [2], we achieve a spatial resolution well beyond the infrared diffraction limit. As examples of the applicability of our new microscopy approach we show phonon polaritons in SiC nanopillars and rods. The tunable IR light source allows an investigation of the different polariton resonances (~ 900 cm^{-1}). Due to the high spatial resolution, we are able to investigate the evolution of phonon polariton resonances from the individual nanopillar to nanopillar arrays and the modal structure inside of SiC nanorods.

[1] Schöllkopf et al., Proc. of SPIE (2015) [2] Kiessling et al., ACS Photonics (2019)

Comparative study of excitation profiles in Surface-enhanced 'linear' Raman and 'nonlinear' Raman spectroscopy

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CARS, nonlinear Raman, surface-enhanced Raman, SECARS, localized surface plasmon resonance

The Raman scattering from molecules is enhanced by several orders of magnitude in surface-enhanced Raman scattering (SERS) via the excitation of a localized surface plasmon resonance (LSPR) of the nanoantenna [1]. The less frequently employed 'nonlinear' variant of SERS called surface-enhanced coherent anti-Stokes Raman scattering (SECARS) predicts a significant additional Raman signal enhancement since CARS is a four-wave mixing process [2]. Such signal enhancement opens the possibility of detecting a single molecule by SECARS in a time-resolved experiment [3]. However, the maximum enhancement of SECARS by optimizing pump and Stokes wavelengths with respect to the LSPR of the nanoantenna has not yet been fully exploited in an experiment. To this end, we aim to experimentally compare the SERS and SECARS excitation profiles. The theoretically expected SERS and SECARS excitation profiles are shown in the Figure. In this study, the laser excitation wavelengths are tuned across the LSPR. The maximum SERS intensity is blue-shifted with respect to lmax in the LSPR spectrum. However, the theoretically predicted maximum SECARS intensity should occur around lmax. The experimental demonstration of this prediction for SECARS would help in designing appropriate nanoantenna and in choosing proper CARS excitation wavelengths in order to exploit maximum SECARS signal enhancement.

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[1] S. Schlücker, Angew. Chem. Int. Ed. 2014, 53, 4756. DOI: 10.1002/anie.201205748. [2] C. Steuwe, C. F. Kaminski, J. J. Baumberg, S. Mahajan, Nano Lett. 2011, 11, 5339. DOI: 10.1021/nl202875w. [3] Yampolsky, S., Fishman, D., Dey, S. et al., Nature Photon. 2014 ,8, 650–656. DOI: https://doi.org/10.1038/nphoton.2014.143

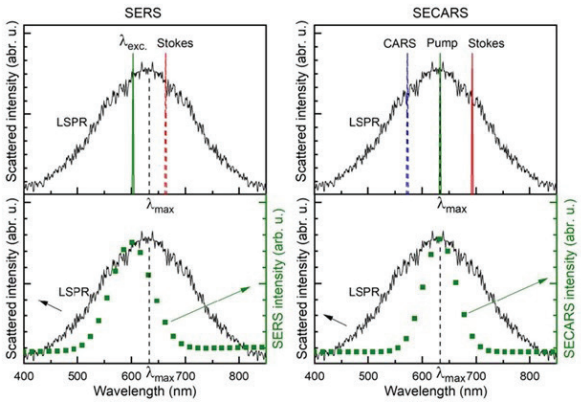

Figure 1. Theoretically expected SERS versus SECARS excitation profiles (bottom, green); Spectral positions of excitation beams and scattered signals (top panels).

Stimulated Raman scattering for biomedical imaging

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Stimulated Raman scattering, Biomedical, Imaging

Stimulated Raman scattering (SRS) imaging have garnered significant interest in biomedical sciences as non-invasive, three-dimensional and real-time imaging technique. Researchers had demonstrated that SRS offers a unique chemical imaging capability and broad biological and medical applications including label-free DNA imaging, lipid quantification, and cancer diagnosis [1-2]. In SRS, two coherent near-infrared picosecond pulsed lasers called Pump and Stokes are used to excite the sample, and the difference in their frequencies is set to match the vibrational frequency of the molecule of interest. Nowadays, the available SRS systems are often described as powerful and easy handling tools, but their efficiency and responsiveness still not automatically guaranteed [3]. Defining optimal conditions and adapting the SRS imaging parameters remains the key element to achieve a high resolved three-dimensional visualization of a complex and heterogeneous biological tissues. In the present work, we highlight the main critical parameters and sensitive components of the SRS configuration to ensure a correct functioning and also to familiarize scientists or ordinary users with the possible anomalies to be encountered. This will help to get more control and ability to guess the probable disturbing elements to fix for optimum SRS performance. To illustrate this, imaging of biological sample was performed by using an SRS set up, composed of an OPO (Picoemerald, APE, Berlin, Germany) pumped by an internal frequency doubled mode locked picosecond Nd:VAN Laser and emitting a train of pulses of 7 ps at 1064 nm at high repetition rate of 80 MHz. The Nd:VAN Laser serves also as Stokes beam. The OPO provides a tunable signal wavelength used as pump beam varying from 720 to 990 nm with typical pulse width of 5 ps and spectral bandwidth of 0.3 nm.

[1] Cheng, J. X., & Xie, X. S. (2015). Vibrational spectroscopic imaging of living systems: An emerging platform for biology and medicine. Science, 350(6264), aaa8870. [2] Cui, S., Zhang, S., & Yue, S. (2018). Raman spectroscopy and imaging for cancer diagnosis. Journal of healthcare engineering, 2018. [3] Ramachandran, P. V., Mutlu, A. S., & Wang, M. C. (2015). Label-free biomedical imaging of lipids by stimulated Raman scattering microscopy. Current protocols in molecular biology, 109, 30.3.1–30.3.17. https://doi.org/10.1002/0471142727.mb3003s109

Exploring the Protein-Protein Associations and their Modulation in Presence of Tungsten Disulphide using Sum Frequency Generation Vibrational Spectroscopy

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Protein-Protein Interactions, Tungsten Disulfide, Nanomaterial, Interface, Sum Frequency Generation Vibrational Spectroscopy

Protein-protein interactions (PPIs) are ubiquitously enrolled in various cellular processes and are also explored rigorously in the field of therapeutics, bio-molecular isolation, and designing next-generation bio-molecular sensors with unprecedented capacities [1]. Numerous techniques have been developed for carrying out PPI characterizations with higher sensitivity, selectivity, and rapid identification of the process [2]. However, these properties are highly governed by the type of an immobilization matrix that is utilized for bio-molecular binding across the hydrophilic-hydrophobic region of the sensor design [3]. Transition metal dichalcogenides (TMDs) with their distinctive properties, have projected tremendous applications in the field of optoelectrical materials, energy storage devices, sensing, etc. Tungsten disulfide (WS_2, a TMD) possesses superior electronic as well as optical properties with higher electron carrier mobility, which makes it an effective material for carrying out label-free sensing applications [4,5]. Utilizing WS_2 based sensing devices with biomolecular interfacing requires a detailed investigation of TMD contribution in conducting protein-protein associations at the molecular-scale. In the present work, we have probed the PPIs in presence of WS_2 QDs at the air/aqueous interface via sum frequency generation vibration spectroscopy (SFG-VS) [5-8]. Interactions among ferritin-antiferritin protein molecules have been analyzed to understand the basis of bio-molecular interactions with WS_2. From our observations, it shows a distinct correlation among the highly ordered hydrophobic moieties of the protein molecules and the corresponding changes in the water molecular bonding around the complex macromolecules in presence of the WS_2 QDs. The quantitative assessments of the present finding conclusively indicates that the strength of biomolecular recognition could fine tune the surrounding water molecular structure along with an increase in the alkyl group ordering at the air-water interface in presence of WS_2 nanomaterial. Further details of our study would be presented during the virtual conference.

Authors are grateful for the research support from the Indian Institute of Technology Ropar (IIT Ropar) and CSIR-CSIO Chandigarh.

[1] Jonsson, U. Fagerstam, L. Ivarsson, B. Johnsson, B. Karlsson, R. Lundh, K. Lofas, S. Persson, B. Roos, H. Ronnberg, I. and Sjolander, S. 1991. Biotechniques, 11(5), 620-627. [2] Lowery, T.J. Garcia, S. Chavez, L. Ruiz, E.J. Wu, T. Brotin, T. Dutasta, J.P. King, D.S. Schultz, P.G. Pines, A. and Wemmer, D.E. 2006. Chem. Bio. Chem, 7(1), 65-73. [3] Turner, A. P. 2013. Biosensors: sense and sensibility. Chemical Society Reviews, 42(8), 3184-3196. [4] Cahen, D. Kahn, A. and Umbach, E. 2005. Materials Today, 8(7), 32-41. [5] Xiao, M. Wei, S. Chen, J. Tian, J. Brooks, C. L. Marsh, E. N. G. Chen, Z. 2019. J. Am. Chem. Soc. 2019, 141, 9980-9988. [6] Kaur, H. Tomar, D. Kaur, H. Rana, B. Chaudhary, S. Jena, K. C. In Advances in Spectroscopy: Molecules to Materials; Springer, Singapore, 2019. [7] Tomar, D. Rana, B. Jena, K. C. 2020. J. Chem. Phys., 152, 114707. [8] Kaur, G. Kaur, H. Singh, A. Chaudhary, M. Kaur, N. Singh, N. & Jena, K. C. 2020. Chemistry–An Asian Journal, 15(14), 2160-2165.

Investigating the Polyelectrolyte Structure with the Presence of Lithium-ion Based Solutes at Air/Aqueous Interface by Sum Frequency Generation Vibrational Spectroscopy

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Polyelectrolyte, sum frequency generation vibrational spectroscopy, interface, Device application

The polyelectrolyte nature has shown significant applications in a variety of fields. The impulse of polyelectrolyte's radical has outshined industries ranging from biomedical treatment to energy-related areas. Methoxyethoxyethoxyphosphazene (MEEP) has demonstrated a tremendously promising candidate as a polyelectrolyte in the formation of water-soluble pre-hydrogel hydrophilic in nature as well as a solid polymer electrolyte for various battery applications such as lithium-ion batteries and organic solar cells [1-4]. In our present study, we have employed sum frequency generation vibrational spectroscopy (SFG-VS) to study the interaction of the completely hydrolyzed MEEP with various lithium anions-based salts at the air/aqueous interface [5-7]. SFG-VS is a potent spectroscopic tool based on second-order non-linear optical process which is inherently surface sensitive and chemically active. It has the potential to investigate the molecular structure and composition of the molecules present at various interfaces, which can be accessible by light. We have explored the OH-stretch region from the range of 3000 to 3800 cm^{-1} to monitor the interfacial water structure. Whereas the structural conformation of the alkyl chains of the polymer depicts the CH-stretch region from 2800-3000 cm^{-1}. From our experimental findings, it is observed that the presence of MEEP has an important role on the hydrogen bonding environment of the interfacial water molecules. The interplay of the polymer-ion interaction impacts the ordering of water molecules profoundly, as confirmed by the air/aqueous interfaces of different conductive lithium salts in the aqueous polyelectrolyte solutions. We will give a detailed analysis of our findings on the interfacial structure of the MEEP and its impact on interfacial water structure as a function of polymer concentration with the presence of various lithium-ion-based solutes in the aqueous polyelectrolyte solution.

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[1] Nazri G, MacArthur D M, and Ogara J F, Chem Mater, 1(1989) 370. [2] Anna Lee S H, Jackson A M S, Hess A, Fei S T, Purse S M, Basham J, Grimes C A, Horn M W, Allcock H R, and Mallouk T E, J Phy Chem C, 114(2010) 15234. [3] Jankowsky S, Hiller M M, Fromm O, Winter M, Weimhofer H D, Electrochim Acta, 155(2015) 364. [4] Kaur H, Rana B, Tomar D, Kaur S, and Jena K C, In Modern Techniques of Spectroscopy: Basics, Instrumentation, and Applications, Springer, Singapore 13(2021), 3. [5] Scatena L F, Brown M G, Richmond G L, Science, 292 (2001) 908. [6] Covert P A, Jena K C, Hore D K, J Phys Chem Lett, 5(2014) 143. [7] Tomar D, Rana B, and Jena K C, J Chem Phys, 152(2020) 114707.

Coherent anti-Stokes Raman spectroscopy of single and multi-layer graphene

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Graphene, Coherent Anti-Stokes Raman Scattering, Non-linear Raman, imaging, microscopy

Since its isolation in 2004, graphene has been in focus of intense research activities in view of its potential to enable radically new devices. Spontaneous Raman spectroscopy is largely applied for the characterization and the imaging of graphene. In this talk, our recent results of Raman spectroscopy performed with ultrafast laser excitation are presented [1]. More specifically, the existence and nature of non-linear Raman processes (Coherent anti-Stokes Raman Scattering) with respect to spontaneous Raman will be discussed, and how it can be exploited for efficient graphene imaging. Due to gapless nature of graphene, interfering electronic and phononic transitions concur to generate its optical response, preventing to retrieve spectral profiles analogous to those of spontaneous Raman. Here we show Coherent anti-Stokes Raman Scattering response of the G-phonon in single and multi-layer graphene. The nonlinear signal is dominated by a vibrationally non-resonant background, obscuring the Raman lineshape. We demonstrate that the vibrationally resonant coherent anti-Stokes Raman Scattering peak can be measured by reducing the temporal overlap of the laser pulses, suppressing the vibrationally non-resonant background. The spectral response of the system is modelled taking into account the electronically resonant light-matter interaction. Moreover, we show how the interference of electronic and phononic process in coherent anti-Stokes Raman Scattering can enhance the image contrast of graphene preserving the vibrational sensitivity.

We acknowledge funding from the EU Graphene and Quantum Flagship, ERC grant Hetero2D and GSYNCOR, EPSRC grants EP/L016087/1, EP/K01711X/1, EP/K017144/1, and EP/N010345/1.

[1] Virga, A., Ferrante, C., Batignani, G. et al. Coherent anti-Stokes Raman spectroscopy of single and multi-layer graphene. Nat Commun 10, 3658 (2019)

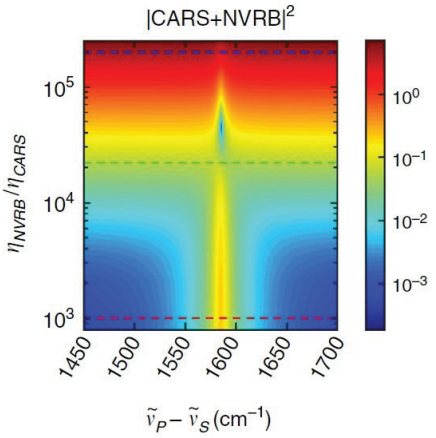

Figure 1. The simulated non-linear Raman response of graphene in electronically resonant condition changing the relative weights between the vibrational signal and vibrationally non-resonant background.

Lipid droplets as a hallmark of inflammation of eosinophil cell line (EoL-1) studied by Raman, CARS and fluorescence microscopy

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Raman imaging, CARS, fluorescence, eosinophils, lipid droplets

Eosinophils (EOS) belong to the granulocytes, type of white blood cells characterized by the presence of granules spread in their cytoplasm. They are involved in the initiation and propagation of inflammatory responses in cells.1 Accumulation of LDs in EOS can be observed in many inflammatory condition such as asthma or allergies, which are linked to the regulation of immune responses involved in controlling and biosynthesis of inflammatory mediators. LDs are involved in membrane trafficking, cell signaling, regulation of lipid metabolism, controlling of synthesis and secretion of inflammatory mediators. Due to short life span of EOS the eosinophilic cell line (EoL-1) is commonly used as an in vitro model for testing their behavior including insight into the pathways of inflammatory responses. Under normal condition EOS are cytologically similar to myeloblast, but in response to stimulation with suitable factors EOS differentiate into mature eosinophils1,2. The common feature of EOS and EoL-1 cell line is fact that under the activation a number of lipid droplets (LDs) increases significantly in both group of cells.1,2 Recently we have reported the biological, morphological and spectroscopic characterization of EoL-1 and EOS cells under stimulation with phorbolmyristate acetate (PMA).3 Here we present the results of studies focused on EoL-1 cells response to different proinflammatory agents such as interleukin 1-β TNF, LPS and butyric acid, which may cause EoL-1 differentiation into eosinophils as well as LDs formation. Results obtained by means of Raman imaging revealed differences in biochemical composition of cells upon activation including change of unsaturation level of LDs found in EoL-1. In turn, fluorescence and CARS microscopies easily differentiated the number of LDs observed in cells as the result of response on applied stimulation and provided quantitative information about the number of newly formed LDs.

This work was supported by National Science Center (DEC- 2016/22/M/ST4/00150).

[1] Mayumi M., "EoL-1, a human eosinophilic cell line, Leuk&Lymp, 7, 243-250 (1992). [2] Olzmann J.A. and Carvalho P. "Dynamics and functions of lipid droplets", Nature Rev Mol Cell Biol, 20 137-155 (2019) [3] Rygula A. et al "Raman imaging highlights biochemical heterogeneity of human eosinophils versus human eosinophilic leukaemia cell line", British Journal of Heamatology, 186(5), 685-94, (2019)

Phospholipid-glycosaminoglycan interactions observed by using vibrational sum-frequency generation spectroscopy

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Vibrational sum-frequency generation, Phospholipid monolayer, Glycosaminoglycan, Chiral spectroscopy

Glycosaminoglycans are ubiquitous at biological barriers, and have been observed to contribute to medical conditions, i.e., atherosclerosis or Alzheimer's disease by interacting with lipids.[1] Here, we present the first vibrational sum-frequency generation (VSFG) spectra of chondroitin sulfate (CS) interacting with dipalmitoyl phosphatidylcholine (DPPC) at the air/liquid interface. The spectra were collected at 100 kHz laser repetition rate, which has recently been shown to facilitate the study of biomolecular structures by allowing for short acquisition times.[2-4] VSFG spectra were recorded of the samples in the spectral regions of 1050-1450 cm^{-1}, 2750-3180 cm^{-1}, and 3200-3825 cm^{-1}, covering characteristic bands in the fingerprint-, C-H stretching-, and O-H stretching regions. We observed the reorientation of the lipid head groups and the realignment of the head-group-bound water molecules when the CS molecules interacted with the DPPC monolayer in the presence of Ca^{2+} ions, while at the same time, the tail groups of the DPPC molecules remained mostly in the same conformational order. The spectra recorded in the 3200-3825 cm^{-1} region in chiral (spp) polarization combination point towards a chiral secondary structural motive in the CS chains, which is most probably a helical coil. These observations were made at a physiologically relevant Ca^{2+} concentration (2.8 mM) and a CS concentration below 200 nM, which exemplifies the relevance of state-of-the-art VSFG technology in studying biomolecular interactions at model physiological barriers.

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[1] G. Siegel et al., Advances in Colloid and Interface Science (2016), 232, 25-35. [2] F. Yesudas et al., Journal of Chemical Physics (2018), 148, 104702. [3] F. Yesudas et al., Analytical and Bioanalytical Chemistry (2019), 411, 4861-4871. [4] Z. Heiner et al., Optics Express (2019), 27, 15289.

1.3. Near-field Vibrational Spectroscopy

Tip-enhanced Raman studies of abnormal protein aggregation – advantages of measurements in liquid

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Tip-enhanced Raman Specroscopy (TERS), Amyloids, TERS in liquid

Despite a plethora of scientific efforts, neurodegenerative disorders remain incurable, mainly because the abnormal protein (amyloids) aggregation phenomenon at the heart of these diseases remains unclear. This knowledge is necessary in order to be able to develop effective therapeutic strategies aimed at inhibiting protein self-assembly. Tip-enhanced Raman spectroscopy (TERS), which combines nanometric resolution of scanning probe microscopy and chemical selectivity of Raman spectroscopy, is a very efficient tool in detailed studies of the molecular structure and composition of each individual amyloid forms, arising during abnormal aggregation: oligomers, protofibrills and fibrills. Our results confirmed, that AFM-TERS allows the hyperspectral mapping of amyloids, enabling localisation of the distribution of the β-sheet conformation in individual aggregates. This work gained knowledge about each microscopic step of the protein transformation and molecular/structural modifications associated with aggregation [1]. However, our previous experiments were performed mainly under ambient conditions, and the amide I band in TER spectra acquired in air is absent mainly due to plasmon-induced dissociation of the peptide backbone. It has also been clearly established already that dehydration had a destructive influence on the protein secondary structure. Therefore, given that polypeptides may lose their conformational properties upon drying, they should be studied under native conditions, which are crucial for their biochemical activity. Additionally, the electron and radical scavenging properties or/and the high heat capacity of liquids prevent the decomposition of samples, which is one of the main limitations of the TERS methodology. Therefore, we have optimized TERS in liquid for amyloid studies. The acquired data was more informative than the spectra collected in ambient conditions, and allowed detailed investigation into secondary structure including a detection of antiparallel β-sheet rearrangement in oligomers and protofibrils, and parallel in fibrils [2]. A further advantage of such an approach is the possibility of introducing extrinsic factors during the measurement process, such as adding anti-aggregation drugs or changing the solution conditions.

National Science Centre, Poland under the "OPUS 19" project (Reg. No. UMO-2020/37/B/ST4/02990)

[1] E. Lipiec, J. Kaderli, J. Kobierski, R. Riek, K. Skirlińska-Nosek, K. Sofińska, M. Szymoński, Renato Zenobi, Nanoscale hyperspectral imaging of amyloid secondary structures in liquid, Angewandte Chemie International Edition, 133 (2021) 4595-4600 [2] E. Lipiec, D. Perez-Guaita, J. Kaderli, B. R. Wood, R. Zenobi, Direct Nano-Spectroscopic Verification of the Amyloid Aggregation Pathway, Angewandte Chemie International Edition, 57, (2018) 8519-8524

Near-field Vibrational Spectroscopy Vibrational exciton and polaron nano-imaging: a molecular ruler to image structure, coupling, and disorder in functional molecular materials

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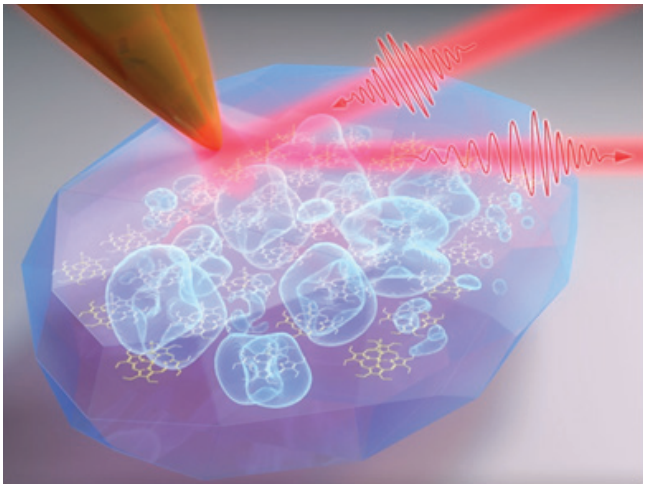
Properties and functions of molecular materials often emerge from intermolecular interactions and associated nanoscale structure and morphology. However, defects and disorder give rise to confinement and many-body localization of the associated wavefunction, disturbing the performance of, e.g., molecular electronic materials. Similarly, in organic-inorganic perovskites, while the soft lattice gives rise to polaron formation believed to be responsible for their extraordinary photovoltaic performance, it simultaneously facilitates a high degree of spatial heterogeneity in the optoelectronic response. However, conventional microscopy techniques lack sensitivity to the low-energy scales of intermolecular interactions, carrier-phonon coupling, and polaron formation leaving a missing link between material structure and observed heterogeneity in the electronic or photonic response. We address this outstanding problem in several novel combinations of spatio-spectral and spatio-temporal infrared nano-imaging. Through probing vibrational exciton formation as a molecular ruler, we resolve the evolution of defects in growth of competing amorphous and crystalline phases in porphyrin model organic electronic materials [1]. Similarly, imaging intramolecular and intermolecular vibrational coupling in polymers [2] and molecular monolayer [3], we spatially resolve the molecular level origin of multiscale morphologies. In the extension to probing both electronic and lattice degrees of freedom, in organic-inorganic perovskites we image the elementary processes of heterogeneous cation-lattice coupling [4], and associated both vibrational and polaron dynamics in ultrafast visible/IR nano-imaging. These nm-ps resolved space-time movies provides for a direct view how the low-energy charge-phonon interactions leads to optoelectronic heterogeneity. As a perspective we show how electronic, vibrational, and polaron quantum state nano-spectroscopy in the low energy landscape of molecular matter provides for functional imaging as a new tool to guide the molecular device fabrication with improved performance.

[1] E. A. Muller, T. P. Gray, Z. Zhou, X. Cheng, O. Khatib, H. A. Bechtel, and M. B. Raschke, "Vibrational exciton nanoimaging of phases and domains in porphyrin nanocrystals", PNAS 117, 7030 (2020). [2] T. P. Gray, J. Nishida, S. C. Johnson, and M. B. Raschke, "2D Vibrational exciton nanoimaging of domain formation in self-assembled monolayers", Nano Lett. 21, 5754 (2021). [3] S. Dönges, P. R. Cline, S. Zeltmann, J. Nishida, B. Metzger, A. Minor, J. Eaves, and M. B. Raschke, "Multidimensional nano-imaging of structure, coupling, and disorder in molecular materials", Nano Lett. ASAP. [4] J. Nishida, A. H. Alfaifi, T. G. Gray, S. E. Shaheen, and M. B. Raschke, "Heterogeneous cation-lattice interaction and dynamics in triple-cation perovskites revealed by infrared nanoscopy", ACS Energy Letters. 5, 1636 (2020).

Vibrational exciton nano-imaging

Vibrational exciton nano-imaging probing vibrational wavefunction delocalization as intrinsic molecular ruler and quantum sensor of intermolecular coupling and disorder on molecular length scales.



Probing liquid interfaces and sub-surface materials with broadband infrared nanospectroscopy

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nanospectroscopy, synchrotron infrared, interfaces, nano-FTIR, graphene

The interface between solid and liquid phases plays a fundamental role in the function of natural and engineered systems, including biological, geological, and electrochemical materials. A full understanding of the compositional, structural, and functional interactions in these systems requires simultaneous nanoscale spatial resolution, wide fields of view, and chemical identification. Synchrotron infrared nano-spectroscopy (SINS), which combines the broad bandwidth, high brightness, and spatial coherence of synchrotron infrared light with scattering-scanning near-field optical microscopy (s-SNOM), enables sensitive vibrational spectroscopy, spanning the entire mid-and far-infrared regions with nanometer spatial resolution [1,2]. This powerful combination provides a new form of broadband spatio-spectral analysis of nanoscale, mesoscale, and surface phenomena that were previously difficult to study with IR techniques. Although SINS and s-SNOM in general have been broadly applied to varieties of soft and hard materials, liquid system applications have been hindered because of the strong absorption of IR light by the liquid and by the strong damping of the oscillatory motion of the AFM cantilever in close proximity to the liquid. Here, we use graphene as an ultra-thin IR transparent "coverslip" that serves as an impermeable liquid barrier, allowing ambient conditions on the probe side and liquid conditions on the other side. We have demonstrated the device by exploring graphene-water, graphene–propylene carbonate (PC) (Fig. 1b), and graphene-ionic salt solution interfaces [3] and explore other applications of this novel configuration.;

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[1] Bechtel, H. A.; Muller, E. A., Olmon, R. L.; Martin, M. C.; Raschke, M. B. 2014. PNAS, 111, 7191-7196. [2] Khatib, O.; Bechtel, H. A.; Martin, M. C.; Raschke, M. B., Carr. G. L. 2018. ACS Photonics, 5, 2773-2779. [3] Lu, Y.-H.; Larson, J. M.; Baskin, A.; Zhao, X.; Ashby, P.D.; Prendergast, D.; Bechtel, H. A.; Kostecki, R.; Salmeron, M. 2019. Nano Lett., 19, 5388-5393.

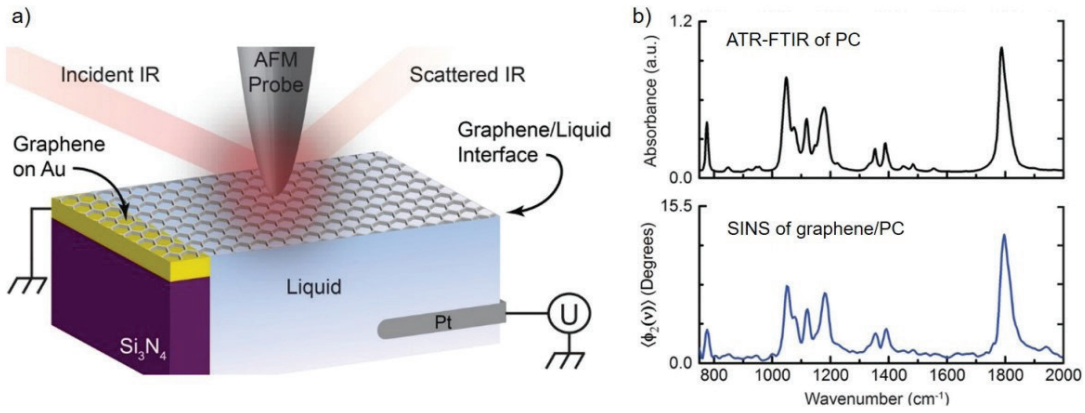

Figure 1. (a) Schematic of a graphene-capped liquid cell. (b) (top) Attenuated total reflectance (ATR) FTIR spectrum of liquid propylene carbonate (PC). (bottom) SINS spectrum of the graphene-PC interface.

Investigation of explosive nanoparticles with TERS

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tip-enhanced Raman scattering (TERS), nanodiamonds, core-shell, explosive

Due to their material properties and chemical inertness, nanodiamonds are promising substrates for a variety of electrical, biological and mechanical applications. Using the starting material hexolite, a mixture of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and TNT (2,4,6-trinitrotoluene), extremely small diamonds with a diameter of less than 4 nm can be produced via the Spray Flash Evaporation (SFE) process. It has been reported that the size distribution of the nanodiamonds is generally influenced by the structure and composition of the explosive precursors.[1,2] In order to control nanodiamond growth, a precise knowledge of the structures of the hexolite particles is of great importance. We present a detailed study of hexolite mixtures containing different RDX:TNT mass ratios. The samples were investigated with atomic force microscopy (AFM), conventional Raman and tip-enhanced Raman scattering (TERS). While the far-field Raman spectra provide information about the composition of the bulk material, the near-field technique enables the characterization of the surface of single hexolite nanoparticles.[3] In the experiments, standard Raman spectra of hexolite (RDX:TNT, 60:40) showed contributions either from RDX or from a mixture of RDX and TNT. Pure TNT spectra were not detected. In contrast, in more than two thirds of the TERS spectra collected on the surfaces of hexolite nanoparticles only TNT was detected. In Figure 1 selected TERS spectra acquired on a hexolite particle (RDX:TNT, 60:40) are shown. The highlighted vibrational modes, NO_2 for TNT (red) and ring breathing for RDX (green), enable a clear distinction of the components.[4] From the combined far- and near-field results it was concluded that the hexolite crystals had a RDX core surrounded by a TNT shell. When the RDX amount in the hexolite was increased (RDX:TNT, 80:20), a core-shell structure was no longer present. Instead, the spectra indicated that heterogeneous structures were formed in which the RDX core was not completely covered with TNT. Based on the experimental results, a building mechanism of the hexolite particles in the SFE process could be postulated.

[1] Risse B., Spitzer D., Hassler D., Preparation of nanoparticles by flash evaporation. Patent, 2013; WO 2013/11767 A1. [2] Pichot V., Risse B., Schnell F., Mary J., Spitzer D., Sci. Rep. 3, (2013), 1-6. [3] T. Deckert-Gaudig, A. Taguchi, S. Kawata, V. Deckert, Chem. Soc. Rev. 2017; 46: 4077-4110. [4] T. Deckert-Gaudig, V. Pichot, D. Spitzer, V. Deckert, Chem. Phys. Chem. 18, (2017), 175-178.

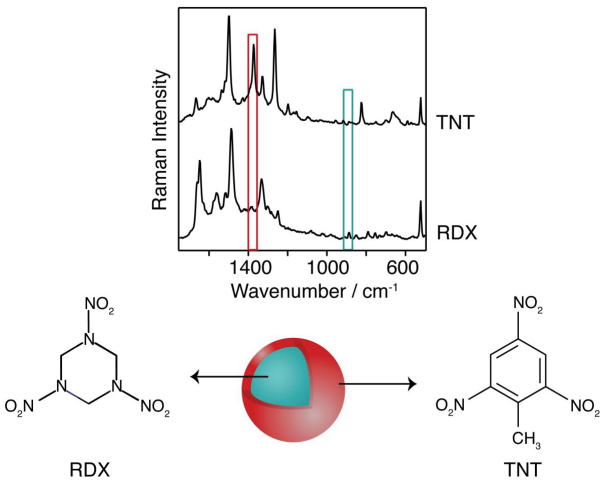

Figure 1.

Nano-FTIR applications for biomaterials and medical research

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nano-FTIR, nanoscale analytics, biomaterials, biomedical

Nano-FTIR has become a key technology to study the chemical composition of organic materials at the nanoscale. This AFM-based technology exploits the strong confinement of light at the end of a sharp, metallic AFM tip to generate a nanoscale optical hotspot at the sample surface. The use of broadband or tunable IR sources combined with a Michelson interferometer enables optical spectroscopy with < 10 nanometer precision, as well as nanoscale mapping of the sample chemical composition. With tremendous sensitivity compared to classical vibrational spectroscopy techniques, nano-FTIR allows chemical identification of organic nanomaterials based on their spectroscopic fingerprint. It further reveals encoded information such as secondary structure in proteins or local chain orientation within highly oriented samples. Various applications of nanoFTIR on different biomaterials with impact on biomedical research will be presented.

Inside core-corona block copolymer micelles: Revealing the local efficiency of core-crosslinking reactions

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Tip enhanced Raman spectroscopy (TERS), Polymer nanoparticles, Diels-Alder reaction, nanomechanical properties

Block copolymers are considered as multi-variable carrier materials for the formulation of functional nanoscale objects [1], in particular for the design of drug delivery systems. The encapsulation of small functional molecules into e.g. spherical and wormlike core-shell micelles bears multiple of challenges, and thus, demands for systems with controllable property-function relations. Understanding of these property-function relations is essential for the design and the improvement of these carrier systems. Beyond an averaging ensemble characterization, gathering information on the nanoscale is important, e.g., in regard to study interfacial influences on the encapsulation process. Investigations of property-function relations require tools, which provide chemical specificity, structural information and also insights into mechanical properties. This can be accomplished by Tip-enhanced Raman Spectroscopy (TERS) investigations which are supplemented with quantitative Atomic Force Microscopy (AFM). Here, we demonstrate the potential of TERS to characterize sliced polymer nanoparticles and fractured block copolymer micelles [2], and pair information from both techniques to study the influence of the core-corona interface on the crosslinking process. Recently, micelles with crosslinked units such as the core have received high attention, due to their higher stability. Here we focus on spherical micelles with a crosslinked core. Core crosslinking is initiated by a Diels Alder reaction with bis-malemide crosslinking reagents (Figure 1) [3]. TERS reveals the local deviations in the crosslinking efficiency by identifying the Diels-Alder product and the retro-Diels-Alder educts, and clearly shows, an inhomogeneous transition for the interfacial core-shell transition region. This TERS study is further supported by mapping the nanomechanical properties across the core-corona interface, which imply that the structure of the interfacial region has impact on the core-crosslinking process. In conclusion this approach enables the investigation of the efficiency of common reactions carried out in confined spaces, which is often altered compared to the corresponding free space reaction.

[1] Schacher, F.H., Rupar P. A. and Manners I.; 2012, Angew. Chem., Int. Ed. 51, 7898 [2] Höppener, C., Schacher, F.H. and Deckert V.; 2020, Small, 16, 1907418 [3] Elter, J.K. et al.; 2018, J. Polymer Chem., 9, 2247

A novel single use sample carrier for bottom illuminated liquid AFM-IR measurements

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AFM-IR, Nanoscale Spectroscopy, IR spectroscopy

Infrared spectroscopy is one of the most important spectroscopic techniques, when it comes to chemical identification. However, the measurement in solvents such as water is difficult due to the high absorption but plays an important role characterizing biological systems (proteins, microorganisms and cells) [1–3]. In addition, the spatial resolution of IR spectroscopy is limited. These limitations are no longer profound with AFM-IR which is a hybrid technique combining atomic force microscopy and mid-infrared spectroscopy. Pulses of a tuned EC-QCL source cause local short-lived photo thermal expansion, which excites the resonant oscillation of the AFM cantilever in contact with the sample. The oscillation amplitude is directly proportional to the absorption and absorption spectra of the sample is generated. AFM-IR measurements in liquids have been demonstrated by several groups [4–6]. All these approaches have in common, that the sample is placed on a high-refractive index prism and a bottom illumination attenuated total reflection (ATR) setup is used. Here the incident beam interacts with the sample via an evanescent field localized on the prism surface, where the sample sits. This approach reduces the large background otherwise caused by absorption of water. Given the requirements for infrared transparency and high refractive index, typically ATR crystal materials ZnSe, ZnS and Ge are used. Using this approach, detection and spectroscopy of few nanometers of polymer as well as amyloid fibrils has been demonstrated 4,5. Nevertheless, as these crystal materials are somewhat exotic – they have little use outside the IR spectroscopy community – literature on their functionalization, sample preparation and nano-structuring is sparse. It would be preferable to replace them with Si where protocols are readily available. Furthermore, the size of prisms adds additional complications to sample preparation: they are simply too large for many tools. Finally, a rather mundane drawback of ATR prisms is their cost. We present a novel approach to liquid AFM-IR measurements that replaces "exotic" prism-materials with a custom flat Si substrate. We not only demonstrate the feasibility of this technique for bottom illumination AFM-IR (air and liquid) but also show how modern rapid prototyping technologies enable the evolution of commercial AFM-IR instrumentations to accept these new substrates. Virtually any established protocol for Si surface functionalization can be applied to this sample carrier and, finally, the low unit cost enables rapid iteration of experiments.

[1] Ruggeri, F.; Marcott, C.; Dinarelli, S.; Longo, G.; Girasole, M.; Dietler, G.; Knowles, T.; Ruggeri, F. S.; Marcott, C.; Dinarelli, S.; Longo, G.; Girasole, M.; Dietler, G.; Knowles, T. P. J. Identification of Oxidative Stress in Red Blood Cells with Nanoscale Chemical Resolution by Infrared Nanospectroscopy. Int. J. Mol. Sci. 2018, 19 (9), 2582. https://doi.org/10.3390/ijms19092582. [2] Akhgar, C. K.; Ramer, G.; Żbik, M.; Trajnerowicz, A.; Pawluczyk, J.; Schwaighofer, A.; Lendl, B. The next Generation of IR Spectroscopy: EC-QCL Based Mid-IR Transmission Spectroscopy of Proteins with Balanced Detection. Anal. Chem. 2020, acs.analchem.0c01406. https://doi.org/10.1021/acs.analchem.0c01406. [3] Baker, M. J.; Trevisan, J.; Bassan, P.; Bhargava, R.; Butler, H. J.; Dorling, K. M.; Fielden, P. R.; Fogarty, S. W.; Fullwood, N. J.; Heys, K. a; Hughes, C.; Lasch, P.; Martin-Hirsch, P. L.; Obinaju, B.; Sockalingum, G. D.; Sulé-Suso, J.; Strong, R. J.; Walsh, M. J.; Wood, B. R.; Gardner, P.

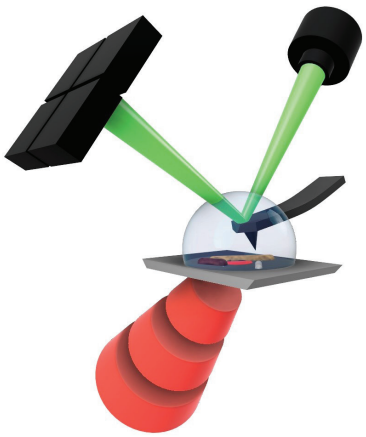

Figure 1. Schematic setup of bottom-illuminated liquid AFM-IR on a novel sample carrier

Prediction of Glaucoma Severity using Raman spectroscopic analysis of human serum

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Glaucoma, Biomarkers, Diagnostics, Raman Spectroscopy, Multivariate Analysis

Glaucoma and macular degeneration are amongst the leading causes of irreversible blindness worldwide, particularly in elderly people worldwide [3,5]. While glaucoma inflicts damage to the optic nerve, macular degeneration involves the deterioration of the centre of the retina [5]. Both conditions are mostly asymptomatic in their early stages, implying that they can be challenging to detect and can gradually progress unnoticed until the disease is advanced and severe damage has been done, resulting in blindness [3,5]. Clinicians depend massively on regular functional and structural examinations, at least until the damage becomes irreversible [1]. There is therefore high demand for fast and reliable diagnostic methods of detecting this fast-growing ocular disease [1,4,5]. The identification of key biomarkers of ocular disease has led to massive improvement in clinical diagnosis and to better understand the pathophysiology of the disease, hence improving therapeutic targets and drug development research lines [2,4]. As an alternative to the costly and labour-intensive techniques of mass spectroscopy and chromatography, this study employs Raman spectroscopy to analyse the low molecular weight fraction of centrifugally filtered serum of patients which have been clinically screened for glaucoma severity and macular pigment degeneration. The spectral profile indicates varying contributions of glycogen and urea. Multivariate models based on Partial least squares regression analysis are constructed to explore the predictive capacity of the technique.

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[1] Barbosa-Breda J, Himmelreich U, Ghesquière B, Rocha-Sousa A, Stalmans I: Clinical Metabolomics and Glaucoma. Ophthalmic Res 2018; 59:1-6. doi: 10.1159/000479158 [2] Pan, CW., Ke, C., Chen, Q. et al. Differential metabolic markers associated with primary open-angle glaucoma and cataract in human aqueous humour. BMC Ophthalmol 20, 183 (2020). https://doi.org/10.1186/s12886-020-01452-7 [3] Rosenberg LF. Glaucoma: early detection and therapy for prevention of vision loss. Am Fam Physician. 1995;52(8):2289-2304. [4] Vohra, R., Dalgaard, L.M., Vibæk, J., Langbøl-l, M.A., Bergersen, L.H., Olsen, N.V., Hassel, B., Chaudhry, F.A. and Kolko, M. (2019), Potential metabolic markers in glaucoma and their regulation in response to hypoxia. Acta Ophthalmol, 97: 567-576. https://doi.org/10.1111/aos.14021 [5] Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. JAMA. 2014;311(18):1901-1911. doi:10.1001/jama.2014.3192

Plasmonic hot spots enable spectroscopic exploration of the local conformational transitions induced by DNA double strand breaks

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SERS, DNA damage, double strand breaks, Atomic force microscopy, bleomycin

Double-strand breaks (DSBs) are detrimental DNA lesions that could lead to cell death, translocations, and cancer-driving mutations. The repair process of such DNA damage is critical to the maintenance of genomic integrity in all forms of life. However, mainly due to the insufficient sensitivity of conventional analytical techniques, detection of the DNA local structural modifications induced by the formation of DNA damage has not been fully explored yet. In this presentation we report on application of a surface enhanced Raman spectroscopy (SERS) for monitoring DNA local structural modifications induced by DSBs. It is known that SERS allows for enhanced Raman scattering by molecules located at nanostructured surfaces such as metallic nanoparticles. In this work we used gold nanoparticles stabilized with cysteamine. We exposed DNA to anticancer drug bleomycin (BLM), a damaging agent that has been proved to induce DNA double-strand breaks. Furthermore, we applied high resolution Atomic Force Microscopy (AFM) for evaluation of the DNA cleavage. Quantitative analysis of the number of breaks per DNA plasmid molecule has been performed by FiberApp software. The schematic representation of our research concept is presented in Figure 1. Analysing the SERS spectra acquired for DNA treated with bleomycin in comparison to control (untreated) one, we identified several bands associated with the DNA backbone and nucleobase vibrations, that changed their intensity and spectral positions, such as: i) changes of the spectral shape of the band at 1024 cm-1 (partial shift towards high wavenumbers) attributed to DNA conformational modifications, ii) decrease of the band intensity at 734 cm-1, which corresponds to interruptions within C5'-O-P-O-C3' phosphodiester bonds. The observed spectral changes correlate with the number of DSBs calculated based on AFM topography images.

National Science Centre, Poland under the "OPUS 16" project(Reg. No. UMO-2018/31/B/ST4/02292)

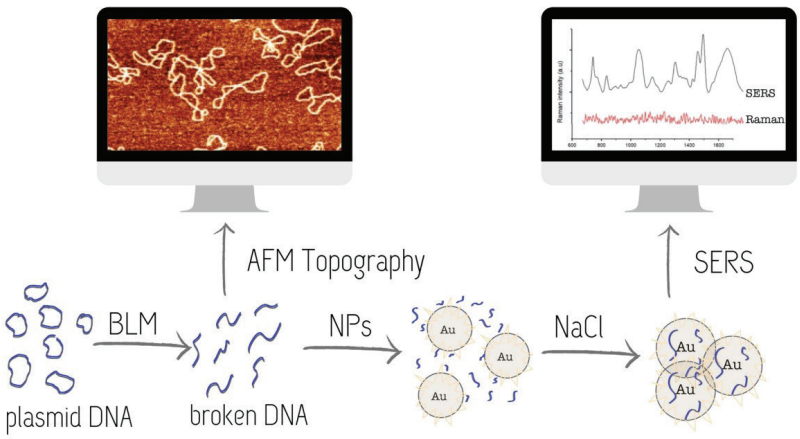

Figure 1. A schematic representation of the measurements.

Polymer analysis and nanoscale IR: a new chapter in a century long story

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PTIR, AFM-IR, Polymer Nanoscale IR Imaging

The story of infrared (IR) spectroscopy is in a way also the story of polymer science, with first experiments being reported in the 1930s.[1] The advent of FTIR in the 1960s brought about a tremendous improvement in the signal-to-noise ratio and measurement times which firmly established IR spectroscopy as one of the standard polymer characterization techniques.[2] In the last century, synthetic polymers have become ubiquitous in day-to-day life due to their advantages over traditional materials, such as lower price, lighter weight, versatility, and durability. This success led to large amounts of polymer waste being released into nature every year where they persist for decades or even centuries.[3] Polymer recycling can be used to both reduce the production of new polymers and the percentage of already existing polymers that become waste. In contrast to recycling of inorganic materials polymer recycling poses some additional challenges: there is a limit to the amount of recycling cycles a polymer can go through before it becomes waste, and the presence of contaminants can lead to undesirable blends.[4,5] While some of the crucial parameters in recycling (e.g. composition, purity) are routinely monitored with FTIR, the key to further improvements to the recycling process lies at the nanoscale. This scale is inaccessible to conventional IR techniques that are diffraction limited to the micrometre range. This is where new, scanning probe-based nanoscale IR techniques like AFM-IR make a difference. AFM-IR, also known as PTIR, combines atomic force microscopy (AFM) and infrared (IR) spectroscopy to overcome the diffraction limit and provide IR spectra at spatial resolutions in the 10 nm range. Since the signals obtained through PTIR and FTIR are both proportional to the wavelength-dependent absorption coefficient of the sample, the long-established spectra-structure correlations of FTIR can also be applied in the interpretation of PTIR spectra. Hence, PTIR is able to rely on almost a century worth of experience in polymer IR spectroscopy to address the challenges of polymer recycling. However, like many nanoscale analysis techniques, PTIR suffers a trade-off between representative sampling and high resolution. Care needs to be taken to not only record pretty images, but also provide relevant data. In this work, we introduce a protocol to specifically address the issue of going from unknown polymer samples to nanoscale chemical distribution maps and demonstrate this approach on industrially relevant polymers (blends and recycled blends).

Part of the COMET Centre CHASE funded by BMVIT, BMDW, Upper Austria, Vienna and the FFG.

[1] R. Stair, W.W. Coblentz, J. Res. Natl. Bur. Stand. 15 (1935) 295. [2] J.L. Koenig, in: Spectrosc. NMR Fluoresc. FT-IR, Springer, Berlin, Heidelberg, 1984, pp. 87–154. [3] A. Chamas, H. Moon, J. Zheng, Y. Qiu, T. Tabassum, J.H. Jang, M. Abu-Omar, S.L. Scott, S. Suh, ACS Sustain. Chem. Eng. 8 (2020) 3494–3511. [4] N. Singh, D. Hui, R. Singh, I.P.S. Ahuja, L. Feo, F. Fraternali, Compos. Part B Eng. 115 (2017) 409–422. [5] J.N. Hahladakis, E. Iacovidou, Sci. Total Environ. 630 (2018) 1394–1400.

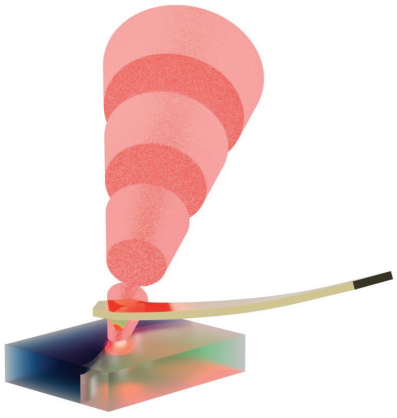

Figure 1. Schematic representation of a PTIR set-up and a polymer sample

HOW TO INVESTIGATE ISOLATED AMYLOID FIBRILS AT THE NANOSCALE: IR NANOSPECTROSCOPY CHALLENGES AND PROSPECTS

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Amyloid fibrils, nanoinfrared spectroscopy, AFM-IR, protein aggregation

Alzheimer's disease (AD) is the most prevalent form of dementia and characterized by fibrillar amyloid deposits in extra neuronal spaces. These amyloid plaques are composed essentially of the amyloid β peptide (Aβ). Aβ is also associated to early onset AD and cerebral amyloid angiopathy due to point mutations in the peptide. This polymorphism of Aβ is apparently reflected in the adopted structures, intrinsically related to its toxicity. Therefore, a better knowledge of aggregated structures of the peptide and its variants is really important for the understanding of AD and the associated pathologies. The commonly used techniques to perform structural analysis with a high-resolution (X-ray diffraction or Nuclear Magnetic Resonnance) on those aggregated forms are not suitable, due to their transitory and/or insoluble states. Infrared spectroscopy is therefore an exquisite tool to study aggregated species, because it is possible to measure quickly protein structure even the insoluble part. Nevertheless, in attenuated total reflection Fourier transform infrared (ATR-FTIR), it is quite difficult to discriminate the different aggregated structures present in complex mixture during aggregation. The recent coupling of infrared spectroscopy with atomic force microscopy (called AFM-IR) [1] overcomes the weak spatial resolution of the usual infrared micro-spectroscopy and achieve a resolution around ten nanometers, which fits well with the size of amyloid fibrils [2]. The AFM-IR allows us recording spectrum on the different aggregated amyloid species (oligomers, isolated fibrils or amorphous aggregates) localized thanks to the height image (morphological description) obtained during AFM measurements [3]. In the future, this capability to investigate the structure and the shape of aggregated species may improve the detection of biomarkers characteristic of Alzheimer's disease and lead to a better understanding of the polymorphism of amyloids proteins. During the presentation, a detailed description of the configuration used for AFM-IR measurements will be given as well as the reasons that bring us to choose one instead of the other. Results on different isolated amyloid fibrils with a diameter of < 10 nm will be shown and discussed based on their structures and orientation.

[1] Dazzi, A.; Prater, C. B., AFM-IR: Technology and Applications in Nanoscale Infrared Spectroscopy and Chemical Imaging. Chemical Reviews 2017, 117 (7), 5146-5173; [2] Waeytens J., Mathurin J., Deniset-Besseau A., Arluison V., Bousset L., Rezaie H., Raussens V., Dazzi A., Probing Amyloid Fibrils Secondary Structures by Infrared Nanospectroscopy: Experimental and Theoretical Considerations, Analyst 2021, 146, 132-145
[3] Waeytens, J.; Van Hemelryck, V.; Deniset-Besseau, A.; Ruysschaert, J.-M.; Dazzi, A.; Raussens, V., Characterization by Nano-Infrared Spectroscopy of Individual Aggregated Species of Amyloid Proteins. Molecules 2020, 25 (12), 2899.

1.4. Time-Resolved Spectroscopy

An isolated water droplet in the aqueous solution of a supramolecular tetrahedral cage as observed by THz spectroscopy

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supramolecular, encapsulation, THz spectroscopy, ab initio molecular dynamics, confined water

Water under nanoconfinement at ambient conditions has exhibited low-dimensional ice formation and liquid–solid phase transitions, but with structural and dynamical signatures that map onto known regions of water's phase diagram. Using terahertz (THz) absorption spectroscopy and ab initio molecular dynamics, we have investigated the ambient water confined in a supramolecular tetrahedral assembly, and determined that a dynamically distinct network of 9 ± 1 water molecules is present within the nanocavity of the host. The low-frequency absorption spectrum and theoretical analysis of the water in the $Ga4L6^{12-}$ host demonstrate that the structure and dynamics of the encapsulated droplet is distinct from any known phase of water. A further inference is that the release of the highly unusual encapsulated water droplet creates a strong thermodynamic driver for the high-affinity binding of guests in aqueous solution for the $Ga4L61^{2-}$ supramolecular construct.

Time-Resolved Spectroscopy
Time-domain Stimulated Resonance Raman goes 2D

T. Scopigno[1]

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Stimulated Raman, two-dimensional vibrational spectroscopy

We bring together some of the key advantages of vibrational and electronic multidimensional spectroscopies in a 2D-Raman scheme to study the vibronic correlations during excited-state dynamics in a selective manner, an open problem in ultrafast science which cannot be tackled by lower order techniques.

Two-dimensional impulsively stimulated resonant Raman spectroscopy of molecular excited-states. G. Fumero, C. Schnedermann, G. Batignani, T. Wende, M. Liebel, G. Bassolino, C. Ferrante, S. Mukamel, P. Kukura, T. Scopigno. Physical Review X, 10, 011051, (2020)

This project has received funding from the European Union's Horizon 2020 research and innovation programme Graphene Flagship under grant agreement No 881603 and from PRIN 2017 Project 201795SBA3 – HARVEST

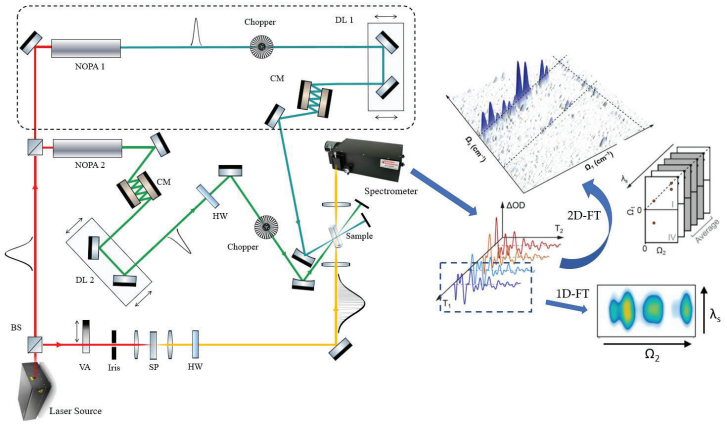

Figure: Multidimensional Stimulated Raman scheme allowing time domain detection of vibronic correlations within selected excited states.

Microsecond Resolved Infrared Spectroelectrochemistry using Dual Frequency Comb IR Lasers

Ian Burgess[1]

[1]University of Saskatchewan, Canada

Dual frequency comb spectroscopy, time resolved spectroelectrochemistry, attenuated total reflectance surface enhanced infrared absorption spectroscopy (ATR-SEIRAS), RC constant

A dual infrared frequency comb spectrometer with heterodyne detection has been used to perform time resolved electrochemical attenuated total reflectance surface enhanced infrared absorption spectroscopy (ATR-SEIRAS). The measurement of the potential dependent desorption of a monolayer of a pyridine derivative (4-dimethylaminopyridine, DMAP) with time resolution as high as 4 µs was achieved without the use of step-scan interferometry. An analysis of the detection limit of the method as a function of both time resolution and measurement co-additions is provided and compared to step-scan experiments of an equivalent system. Dual frequency comb spectroscopy is shown to be highly amenable to time-resolved ATR-SEIRAS. Microsecond resolved spectra can be obtained with high spectral resolution and fractional monolayer detection limits in a total experimental duration that is two orders of magnitude less than the equivalent step-scan experiment.

[1] Erick Lins, Stuart Read, Bipinlal Unni, Scott M. Rosendahl and Ian J. Burgess, Microsecond Resolved Infrared Spectroelectrochemistry Using Dual Frequency Comb IR Lasers. Analytical Chemistry. 92, 2019, 6241-6244.

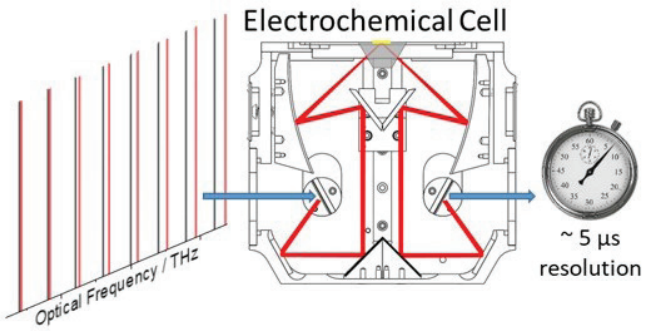

Figure 1. Microsecond resolved spectroelectrochemistry

Non-repetitive protein dynamics on microsecond to second time-scales monitored by mid-infrared dual comb spectroscopy (DCS)

Markus Mangold[1], Florian Eigenmann[1], Raphael Horvath[1], Carsten Kötting [2], Klaus Gerwert[2]

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Time-resolved infrared, G-proteins, Frequency comb, Quantum cascade laser

Dual-comb spectroscopy has recently been introduced to protein dynamics as a tool that enables single-shot measurements with microsecond time resolution. First results have been validated with the recording of transients of the (well-repeatable) photoactivated proton pump bacteriorhodopsin with DCS and stepScan FTIR [1]. In this presentation, we showcase how dual-comb spectroscopy (DCS) can be used to monitor irreversible protein dynamics on a microsecond to second time-scale by applying DCS to the large field of G-proteins [2]. First, we compare the hydrolysis of caged GTPs and their reaction with Gαi monitored by rapid-scan FTIR and DCS. We find a very good agreement between the two techniques. Then, we measure the regulator of G-protein signaling (RGS) catalyzed reaction of Gαi. At room temperature, this reaction elapses too fast for observation with rapid-scan FTIR. In the DCS measurement, the reaction is well resolved and a time constant of 90 milliseconds can be assigned. Interestingly, we further find indication of an intermediate state with a lifetime of only 86 microseconds. In conclusion, we observe good data quality of infrared kinetics of G-proteins with 4 µs time resolution. This is three orders of magnitude faster than any previously reported FTIR measurements of G-proteins. With this development, we pave the way for time-resolved infrared studies of non-repeatable protein reactions, including GTPases and ATPases.

[1] J. L. Klocke, M. Mangold, P. Allmendinger, A. Hugi, M. Geiser, P. Jouy, J. Faist, T. Kottke, Anal. Chem. 2018, 90, 17, 10494–10500. Single-Shot Sub-microsecond Mid-infrared Spectroscopy on Protein Reactions with Quantum Cascade Laser Frequency Combs [2] M. J. Norahan, R. Horvath, N. Woitzik, P. Jouy, F. Eigenmann, K. Gerwert, C. Kötting ACS Anal. Chem. 2021, 93, 17, 6779–6783 Microsecond resolved infrared spectroscopy on non-repetitive protein reactions by applying caged-compounds and quantum cascade laser frequency combs

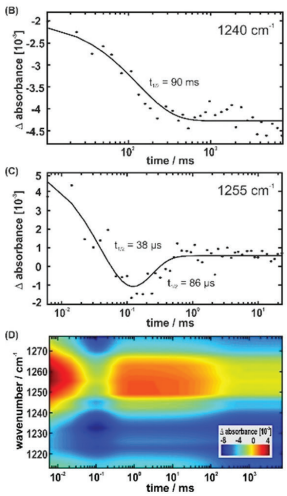

Figure 1. Kinetics and 3D-plot of the absorbance changes observed during GTPase reaction of RGS-catalyzed Gαi [2].

A Quantum Cascade Laser Setup Drastically Reduces Sample Consumption for Studying Irreversible Reactions of Biomolecules in H2O

Tilman Kottke*[1], Jessica Klocke[1]

[1]Physical and Biophysical Chemistry, Bielefeld University, Germany

Time-resolved infrared spectroscopy, Quantum cascade laser, Biomolecule in water, Method development, Irreversible reaction

Time-resolved infrared spectroscopy requires in general a high sample consumption for a systematic variation of probe frequency, interferogram position or time point of acquisition and the necessary extensive averaging. This requirement limits its application to irreversible reactions of biomolecules, which are often available in small quantities. We developed a setup using a quantum cascade laser (QCL) as a probe light source to record time-resolved difference spectra from 20 nanoseconds to 1 second of irreversible photoreactions in H_2O. The combination of the focused QCL with a pressure-tolerant flow cell and a micrometer stage orthogonal to the flow allowed us to drastically reduce the sample consumption to a few microliters for a complete dataset. Moreover, the approach prevents any signal drift at long time ranges because of the very low flow rate of only 15 nL min_{-1}. We investigated the irreversible photoreduction of the cofactor flavin mononucleotide (FMN) in H_2O, which is a common reaction taking place in biological photoreceptors. Kinetics were recorded for a broad tuning range covering 1490-1740 cm^{-1}, which includes signals of relevant carbonyl stretches and the region of the OH bend of water. A continuous dataset in the spectral dimension was generated by applying a fit with a sum of Lorentzians. Subsequent global analysis allowed us to resolve reference spectra of the transient species and the kinetics of the photoreaction [1]. The results are compared to previous studies using flash photolysis [2] and step-scan FTIR spectroscopy [3]. Our approach strongly facilitates the spectroscopic access to irreversible reactions of, for example, photoreceptors and photoenzymes.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) via a Heisenberg fellowship (KO3580/4-2) and grant KO3580/5-1 to T. K.

[1] J. L. Klocke and T. Kottke (2020) Phys. Chem. Chem. Phys. 22, 26459-26467. [2] C. B. Martin, M. L. Tsao, C. M. Hadad and M. S. Platz (2002) J. Am. Chem. Soc. 124, 7226–7234. [3] C. Thöing, A. Pfeifer, S. Kakorin and T. Kottke (2013) Phys. Chem. Chem. Phys. 15, 5916-5926.

1.5. Emerging Techniques

Emerging Techniques
Raman imaging of intracellular structures with improved image contrast

Katsumasa Fujita

Osaka University

Raman microscopy, slit scanning, resonant Raman scattering, multicellular imaging

Raman spectroscopy has become an attractive tool for scientists because of its ability to provide label-free material analysis. Raman spectra reflect the molecular and lattice vibrations of a sample, providing rich information about its composition and environment. However, due to the small cross-sectional area of Raman scattering, it has been challenging to use Raman scattering for imaging biological samples under physiological conditions. We have developed a Raman imaging technique that allows us to utilize the powerful analytical capabilities of Raman spectroscopy for the investigation of intracellular molecules. By applying the spatial multiplexing technique using line illumination and slit detection, we can measure hundreds of Raman spectra using a two-dimensional sensor with a large number of pixels, which dramatically reduces the acquisition time of hyperspectral images [1-3]. The detection slit reduces the background signals from out-of-focus planes, enabling high-contrast 3D imaging of cell and tissue samples. We have applied this Raman imaging technique to observe molecular dynamics in cellular events such as apoptosis, cell division, and cell differentiation. Using 532 nm light for illumination, we monitored mitochondrial and hepatic functions with high contrast in cells via the resonance Raman effect of heme proteins [4]. Recently, we utilized the 3D spatial resolution by slit confocal detection to visualize the structures of multicellular systems, such as spheroids and animal tissues.

[1] Hamada et al., J. Biomed. Opt., 13, 044027 (2012). [2] Okada et al., Proc. Natl. Acad. Sci. USA, 109, 28 (2012). [3] Palonpon et al., Nat. Protoc., 8, 677-692 (2013). [4] Morimoto et al., Analyst, 144, 2540 (2019).

JS MIRAI (JPMJMI20G3), JST COI-NEXT (JPMJPF2009)

Title: Multi-molecular Super Resolution SRS imaging for Studying Metabolic Dynamics

Understanding the dynamics of metabolism in a multicellular organism is essential to unraveling the mechanistic basis of many biological processes. Stimulated Raman scattering (SRS) can generate chemical specific imaging with high resolution, deep penetration of depth, multiplex, chemical selectivity, 3D volumetric and quantitative capability. In the present work, we developed a new method that combines deuterium oxide probing and hyperspectral SRS imaging to visualize metabolic dynamics in living organisms. Within the broad vibrational spectra, we visualized more than 20 different molecules including lipids subtypes-, protein-, and DNA-specific Raman profiles and develop hyperspectral detection methods to obtain various macromolecular multiplex imaging. We further developed deconvolution algorithm to enhance the spatial resolution to generate super resolution SRS hyperspectral images for visualizing subcellular distribution of various molecules. This technology platform is non-invasive, universal applicable, and it can be adapted into a broad range of biological studies such as neurodegeneration, aging, homeostasis, tumor progression, etc. We applied this method to study the diet regulated metabolic dynamics in animals during aging processes, the quantitative lipid and protein turnover rate, the intra-cellular metabolic heterogeneity.

Are plasmonic and Raman resonances synergistic? An example of stimulated Raman signal of liquid water.

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[2]Department of Molecular Physics, Faculty of Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland
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SERS, surface enhanced - femtosecond stimulated Raman scattering, liquid water vibrations, Resonance Raman, Raman gain factor

Recently, we have observed for the first time surface-enhanced (SE) signal of water in an aqueous dispersion of silver nanoparticles in spontaneous (SERS) and femtosecond stimulated Raman (SE-FSRS) processes. [1] We evaluated the enhancement factors as $(3.6 \pm 0.6) \cdot 10^6$ for SE-FSRS and $(4.8 \pm 0.8) \cdot 10^6$ for SERS, for the same excitation wavelength (515 nm). These values are one order of magnitude lower than the maximum enhanced factor obtained earlier by Shin et al. ($EF < 5.41 \cdot 10^7$) for water in a nanomeniscus in a solid silver substrate. [2] What is more important, as we have almost free choice of the wavelength of Raman pump in our FSRS setup [3], we could check whether the observed plasmonic enhancement is synergistic with the Raman resonance enhancement of water signal. For the Raman resonance, we have chosen water absorption mode at around 755 nm, attributed to the combinational overtone of the symmetric and antisymmetric OH stretching modes: av_1+bv_3, where $a + b = 4$ (see the Figure). [4] The plasmonic resonance for the studied dispersion of silver nanoprisms spans over most of the VIS-NIR range (see AgNPs dispersion "blue" in the Figure). Thus, we selected three Raman pump wavelengths, all in the plasmonic resonance with silver nanoprism and with off-resonance (515 nm), pre-resonance (715 nm) and resonance (755 nm) conditions with the water absorption band. We observed strong Raman resonance and pre-resonance signal enhancements of the fundamental OH stretching mode of water. However, plasmonic and Raman resonances appear to be additive rather than multiplicative. This result is essential for evaluating additional enhancement of Raman signals with the use of both plasmonic and Raman resonances mechanisms.

NCN grants: 2017/25/N/ST4/01125, UMO-2015/17/B/ST4/04035, FNP grant: POIR.04.04.00-00-16ED/18-00.

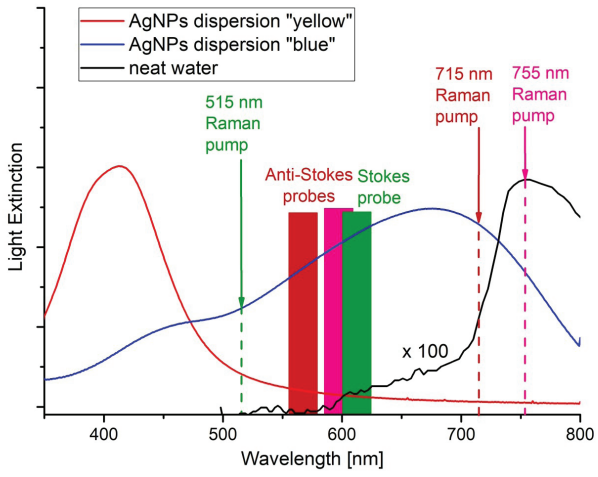

Figure 1. Extinction spectra of pure water and dispersion of two types of AgNPs with marked wavelength of Raman pumps used in this work together with spectral ranges of Raman scattered light

Harnessing visible light for IR imaging

Gianluca Grenci[1], Anna Paterova[2], Sivakumar M. Maniam[1], Hongzhi Yang[2], Mona Suryana [1], Jegan Vishnuwardhana Shanmugar[1], Desmond Toa Zi Siang[2], Leonid Krivitsky[2]

[1]Mechanobiology Institute, National University of Singapore, 117411 Singapore

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IR spectroscopy, Correlated photons, Spontaneous Parametric Down Conversion

In this contribution, we demonstrate an approach to circumvent the need for IR sources and detectors in hyper spectral wide-field IR microscopy by employing quantum optical phenomena. Our method is based on the concept of the nonlinear interference of correlated photons, also known as induced coherence [1,2]. Two photons are generated in a nonlinear crystal via spontaneous parametric down-conversion (SPDC) with one photon (signal) in the visible range, and the correlated photon (idler) in the IR range [3,4]. The crystal is put into an interferometer in which the interference pattern of the detected visible photons carries information about the IR photons, which are the ones interacting with the sample. Information about the sample properties in the IR range is inferred from the measurements of visible range photons using standard visible light components. We demonstrate the chemical mapping of a patterned sample, in which different areas have distinctive IR spectroscopic fingerprints a 20 µm thick layer of SU-8 photoresist was UV-exposed with a pattern of rectangles (figure 1a-b). The exposed and non-exposed SU-8 present a characteristic inversion of the relative intensities of spectral absorption at around 2.9 (figure 1c) µm and 3.2 µm (figure 1d) [5,6]. The technique here demonstrated provides a wide field of view, fast readout, and negligible heat delivered to the sample, which makes it highly relevant to material and biological applications.

[1] X. Y. Zou et al., Induced coherence and indistinguishability in optical interference. Phys. Rev. Lett. 67, 318–321 (1991). [2] L. J. Wang et al. Induced coherence without induced emission. Phys. Rev. A 44, 4614–4622 (1991). [3] C. K. Hong, L. Mandel, Theory of parametric frequency down conversion of light. Phys. Rev. A 31, 2409–2418 (1985). [4] D. N. Klyshko, Photons and Nonlinear Optics (CRC Press, Boca Raton, FLA, 1988). [5] Mitri, E. et al, SU-8 bonding protocol for the fabrication of microfluidic devices dedicated to FTIR microspectroscopy of live cells (2014) Lab on a Chip, 14 (1), pp. 210-218. [6] Paterova, A.V. et al., Hyperspectral infrared microscopy with visible light (2020) Science Advances, 6 (44), art. no. abd0460.

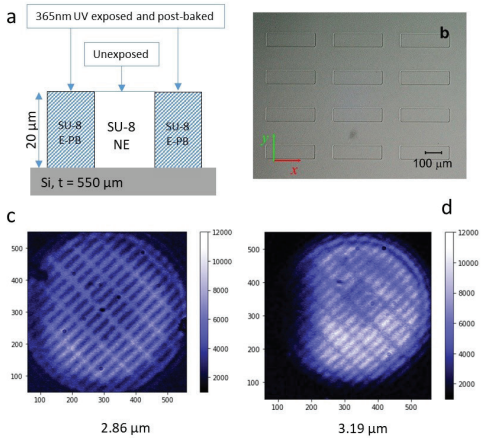

Figure 1. 1a-b scheme of the sample used for the proof of principle and optical image; 1c-d IR absorprtion maps at 2 wavelengths acquired with our optical system.

Broadband Mid-Infrared Sensor employing a Quantum Cascade Laser and a Quantum Cascade Detector for Milk Protein Analysis

Alicja Dąbrowska[1], Mauro David[2], Andreas Schwaighofer[3], Stephan Freitag[3], Aaron Maxwell Andrews [2], Gottfried Strasser[2], Borislav Hinkov[2], Bernhard Lendl*[3]

[1]Technische Universität Wien, Austria

[2]Institute of Solid State Electronics & Center for Micro- and Nanostructures, Technische Universität Wien, Austria

[3]Institute of Chemical Technologies and Analytics, Technische Universität Wien, Austria

quantum cascade detector, quantum cascade laser, mid-infrared spectroscopy, liquid-phase sensor, milk protein analysis

Recent advances of quantum cascade technologies in the mid-IR region enable new spectroscopic sensing schemes. Novel laser light sources, such as external cavity quantum cascade lasers (EC-QCLs) are gaining more attention for qualitative and quantitative analysis of liquids as they can outperform conventional FTIR spectrometers in terms of sensitivity [1]. Accompanied by developments in QCLs, there has also been progress in quantum engineering-based detectors. Quantum cascade detectors (QCDs) offer room-temperature operation, fast response time, low noise and high potential for integration. In contrast to MCT detectors, widely employed in FTIR spectrometers and laser-based spectroscopy setups, QCDs operate in a wide power range of the incident radiation without saturation effects while maintaining excellent linearity. Hence, no additional optical components, i.e. filters, have to be used to reduce the laser beam intensity to remain in the linear range of the detector [1]. Even though QCDs offer many favorable properties for spectroscopic applications, only a few examples of their application were shown so far and their use predominantly focuses on gas-phase analysis. [2,3] In the presented work, we combine a broadly tunable QCL and a QCD for broadband liquid-phase spectroscopy for sensitive and selective detection of bovine milk proteins. A thermoelectrically-cooled EC-QCL (Daylight Solutions) tunable from 1730 to 1470 cm^{-1} was incorporated in the setup. A temperature-stabilized transmission flow cell (d=12.5 µm) was used for sample handling. For signal detection, a ridge QCD was used and operated at room-temperature. The spectral response of the QCD overlaps well with the tuning range of the laser, allowing detection of the two most prominent absorption bands of proteins (amide I and II). Broadband IR spectra casein, β-lactoglobulin and α-lactalbumin dissolved in buffer solution at multiple concentrations ranging from 0.25–15 gL^{-1} were recorded. A comparison to FTIR spectra shows excellent agreement. The RMS noise level obtained by the setup was of 0.067 mAU and the limit of detection for milk protein sensing was at 0.09 gL^{-1}. In summary, this is the first demonstration of the use of a QCD in combination with an EC-QCL for broadband IR spectroscopy of liquid samples. We showcased that a spectrometer fully based on quantum cascade technologies for mid-IR light generation and detection can be successfully used for milk protein analysis, achieving similar performance as a high-end FTIR spectrometer, while maintaining much greater compactness.

Horizon 2020 Framework Programme (780240)

[1] C. K. Akhgar, G. Ramer, M. Żbik, A. Trajnerowicz, J. Pawluczyk, A. Schwaighofer, and B. Lendl, "The next Generation of IR Spectroscopy: EC-QCL based mid-IR Transmission Spectroscopy of Proteins with Balanced Detection," Anal. Chem. 92, 9901–9907 (2020). [2] B. Schwarz, P. Reininger, D. Ristanić, H. Detz, A. M. Andrews, W. Schrenk, and G. Strasser, "Monolithically integrated mid-infrared lab-on-a-chip using plasmonics and quantum cascade structures," Nat. Commun. 5, 1–7 (2014). [3] R. Szedlak, A. Harrer, M. Holzbauer, B. Schwarz, J. P. Waclawek, D. Macfarland, T. Zederbauer, H. Detz, A. M. Andrews, W. Schrenk, B. Lendl, and G. Strasser, "Remote Sensing with Commutable Monolithic Laser and Detector," ACS Photonics 3, 1794–1798 (2016)

Horizon 2020 Framework Programme (780240)

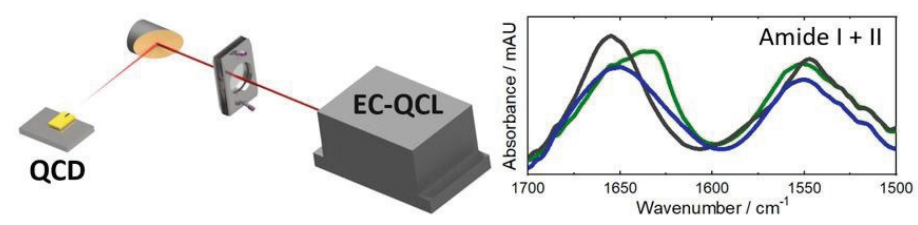

Figure 1. EC-QCL-QCD protein sensor

Quantitative Authentication of Extra Virgin Olive Oil by Time-Gated Raman Spectroscopy

Amuthachelvi Daniel[1], Mari Tenhunen[1], Miia Mikkonen[1]

[1]Timegate Instruments Oy, Finland

Extra virgin olive oil, Time-gate Raman Spectroscopy, Adulteration, Quantitative analysis

Globally food adulteration is a great challenge affecting almost all food commodities. An example for food adulteration is the adulteration of olive oil. Often olive oil is diluted with cheap vegetable or seed oils or low-grade oils or refined oils. The genuineness or purity of Virgin olive oil impacts the consumers and adulteration may lead to health risks as well. In this study we have explored the efficacy of time-gated Raman spectroscopy for testing the purity of olive oil. Binary mixtures of extra virgin olive oil with sunflower oil and rapeseed oil of ratios 10%, 20%, 30%, 40% and 50% were measured and calibrated. Further a cheap olive oil was tested for its purity. Partial Least Squares (PLS) regression models were developed, and these interesting results would be discussed.

High-resolution FTIR-spectrometry of tritium-substituted water vapor using a custom-built optical cell with in-situ synthesis reactor

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Tritium, water vapor, isotopologues, high-resolution, FTIR

Although spectroscopic data are available for most molecules, data of radioactive isotopologues are sparse. Radiation safety and related restrictions to small sample amounts renders spectroscopy of radioactive gases technical challenging. We present an improved setup for the synthesis and high-resolution FTIR-measurement of tritiated water vapor ($HT^{16}O$, $DT^{16}O$ and $T_2^{16}O$). A custom optical cell complying with the technical challenges from the radioactive hydrogen isotope tritium has been built. It has been filled with a 0.15 mbar l tritium-deuterium mixture corresponding to a activity of 10 GBq. The synthesis of the molecular hydrogen to water has been performed by use of the redox-reaction with copper-oxide. The absorption signal is maximized by confining the gas in a thin polished silver light-pipe. The measurements have been recorded on a Bruker 125HR Fourier-transform infrared spectrometer applying a resolution of 0.0075 cm^{-1} at room temperature. The measured spectrum covers four HTO, five DTO and five T_2O transition bands in the range of 3600-5400 cm^{-1} with signal-to-noise ratios up to 140. This cell design can be used to perform optical spectroscopy on hazardous available gases with a high resolution. The obtained spectroscopic data will be useful to test and improve theoretical models of molecular systems and may have an importance for monitoring of nuclear fusion processes.

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Study of macromolecular orientation in tissue samples using the Four-Polarization Method in FT-IR Imaging: experimental aspects

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Molecular orientation, Collagen, Linear Polarization, FT-IR Imaging, Herman's function

Fourier-Transform infrared spectroscopy (FT-IR) is currently widely researched in the context of its biomedical applications such as machine learning supported cancer detection or Alzheimer's disease early diagnosis. It is a consequence of the richness of biochemical information provided by spectra combined with spatial structure offered by an imaging modality. Such wealth of information might be further enriched by the control of radiation polarization status. Utilization of polarized light introduces another condition on absorption by molecular vibrations – it requires parallel alignment of electric field vector and transition dipole moment, therefore, making absorbance become orientation-dependent and allows determination of transition moment directions. Molecular orientation studies giving an orientation angle by means of FT-IR were so far only done for relatively simple systems, like polymer samples, with a well-defined molecular axis, whereas the field of tissue sample analysis was still largely unexplored. Pushing the limits for understanding of biological systems led to the development of macromolecular orientation information extraction. We applied the Four-Polarization method to fibrotic and collagen-rich pancreatic tissue samples, perfectly suitable for molecular orientation study [1]. The cylindrical shape of collagen fibers, along with the main molecular chain consisting mainly of Amide bonds, makes it relatively easy to conclude on fibers direction using Amide I, Amide II and Amide III bonds vibrations. However, care must be taken not to include scattering contribution. After application of the Four-Polarization method, Herman's in-plane Orientation Function and Azimuthal Angle (transition dipole moment change direction) were determined. Results achieved in this research were stunning as this kind of information was never before extracted from such a complex system. Herman's function for Amide III revealed well-oriented fibrotic regions reaching significantly higher values than randomly oriented epithelial tissue. Moreover, Azimuthal Angle results for Amide II and Amide III showed almost parallel character – similar to the main axis of cylindrically shaped fibers. However, the biggest achievement was the sensitivity of the method allowing to confirm perpendicular character for Amide I directions. This shows, that it is possible and beneficial to retrieve molecular orientation information even in complicated systems of tissue samples, with a variety of potential applications ranging from biology to material science.

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[1] P. Koziol, D. Liberda, W. M. Kwiatek, and T. P. Wrobel, "Macromolecular Orientation in Biological Tissues Using a Four-Polarization Method in FT-IR Imaging," Anal. Chem., vol. 92, pp. 13313–13318, 2020, doi: 10.1021/acs.analchem.0c02591.

Impact of interference on spectral quality, histopathologic classification and orientation studies using transmission/transflection infrared imaging data

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infrared imaging, histopathology, interference, cancer, electric field standing wave

Fourier Transform Infrared Imaging (FT-IR) combined with machine learning algorithms has a great potential in biopsies histological recognition. However, there are few aspects needing improvement to introduce the method to the clinic. FT-IR imaging offers the possibility of sample measurement in two modes: transmission and transflection. Many optical effects have influence over acquired data to different degrees, depending on the mode. Spectra obtained with transmission mode are obscured primarily by scattering contribution while transflection is dominated by interference effects (so called Electric Field Standing Wave effect). There has been a discussion about the magnitude of the latter effect and we have shown that in biological tissues it is not expected to be large based on theoretical calculations[1]. Later, we have investigated the influence of the optical parameters of the substrate (low-e and gold) to show, that even this will affect the interference effect[2]. Finally, we have recently used the flat film of paraffin embedding to quantify the effect with and without paraffin on a human biopsy sample in both measurement modes (Figure 1)[3]. We have also investigated the histological classification capability using all cases. Finally, we present orientation studies of macromolecules in a human biopsy measured in transmission mode[4].

Grant No. Homing/2016-2/20; Project No. MRPO.05.01.00-12-013/15; Grant No. 2018/31/D/ST4/01833;

[1] Wrobel TP, Wajnchold B, Byrne HJ, Baranska M. Electric field standing wave effects in FT-IR transflection spectra of biological tissue sections: Simulated models of experimental variability. Vib Spectrosc 2013;69:84–92. doi:10.1016/j.vibspec.2013.09.008. [2] DeVetter BM, Kenkel S, Mittal S, Bhargava R, Wrobel TP. Characterization of the structure of low-e substrates and consequences for IR transflection measurements. Vib Spectrosc 2017;91:119–127. doi:10.1016/j.vibspec.2016.09.001. [3] Liberda D, Koziol P, Raczkowska MK, Kwiatek WM, Wrobel TP. Influence of interference effects on the spectral quality and histological classification by FT-IR imaging in transflection geometry. Analyst 2021;146:646–654. doi:10.1039/d0an01565b. [4] Koziol P, Liberda D, Kwiatek WM, Wrobel TP. Macromolecular Orientation in Biological Tissues Using a Four- Polarization Method in FT-IR Imaging. Anal Chem 2020;92:13313–13318. doi:10.1021/acs.analchem.0c02591.

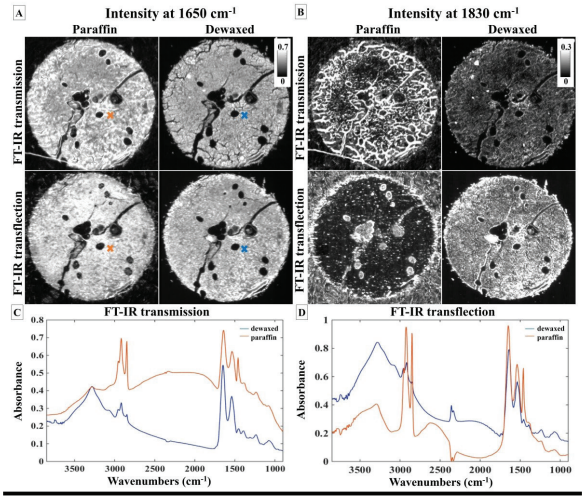

Figure 1. Images of single tissue core measured in transmission and transflection modes with and without paraffin. From [3].

Bovine Milk Fatty Acid Profiling by Attenuated Total Reflection Infrared Spectroscopy and Rapid Lipid Separation

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Mid-infrared spectroscopy, Attenuated total reflection, Bovine Milk, Fatty Acids, Partial least Squares

We report a novel mid-infrared (IR) based approach for predicting the fatty acid content in bovine milk. First, milk fat was separated according to a rapid, solvent-free two-step centrifugation method. The hereby obtained lipid fraction possesses a representative fatty acid composition for whole milk samples. Mid-IR absorbance spectra of the pure lipid fractions were recorded with attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy and compared to transmission spectra of whole milk. The information content regarding absorbances originating from triglycerides was significantly higher in pure lipid spectra, due to lack of overlapping bands from other major milk components and a larger accessible spectral range. Multivariate calibration equations, based on partial least squares (PLS) were calculated in order to relate ATR absorbance spectra to GC/MS reference values. Particularly good predictions were achieved for fatty acid sum parameters such as saturated fatty acids, monounsaturated fatty acids, unsaturated fatty acids, medium chain fatty acids, long chain fatty acids as well as for certain individual fatty acids. Based on a set of 45 milk samples, the obtained PLS figures of merit were significantly better than those reported in literature using whole milk transmission spectra and larger datasets. Based on the PLS selectivity ratio (SR), the applied wavenumber range was individually selected for each prediction parameter. The validity of the models was verified by assigning all spectral regions with high SR, indicating important information, to absorbance bands arising from fatty acids. Covariation structures between individual fatty acids and total fat content, a common challenge in milk fatty acid profiling were inherently eliminated with the applied approach. Correlations between individual fatty acid concentrations were mostly small, indicating that the predicted concentrations resulted mainly from corresponding IR absorption instead of correlations to other fatty acids. The combination of solvent-free lipid separation and ATR-FTIR spectroscopy bears several clear advantages over conventional milk fatty acid profiling methods, revealing high potential for future, high-throughput applications.

COMET Center CHASE (project No 868615)

Applicability of the Real-Time FT-IR technique to determine the degree of conversion of dental compositions

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photopolymerization, Real-Time FTIR technique, photocurable dental resins, photoinitiator

Photopolymerization is an environmentally-friendly, non-destructive, safe and solvent-free method. Moreover it guarantees low energy consumption. Therefore the photopolymerization is used in many scientific disciplines, including dentistry for production photocurable dental materials. The use of photochemically initiated polymerization to obtain dental composites enables the use of unique and innovative features of this method. The most important are: short curing time of the composition (up to a few seconds); carrying out the reaction at room temperature; low energy consumption, spatial resolution (polymerization only in the exposed areas). Nevertheless, many different factors, such as the selection of appropriate monomers, initiators, inorganic fillers, curing time, type of radiation and light power, influence the quality of the composite obtained [1-2]. In this work, influence of different factors on properties of standard dental light-cure composite resins were studied. The effect of the concentration of amines as co-initiators on the final conversion of dental composites was investigated. Studies were also carried out on the effect of the weight ratio of the two standard monomers: Bisphenol A-glycidyl methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) used in the production of dental fillings. Finally, studies on the effect of the type of amines such as ethyl 4-(dimethylamino) benzoate (EDB), bis(hydroxyethyl) methylamine (MDA), 2-(phenylamino) acetic acid (NPG), triethanoloamine (ETA) and triethylamine (EThA) on the properties of final dental fillings (Figure 1) were investigated. To determine the conversion rate of monomers, the Real-time Fourier transform (real time-FT-IR) method was used. The equipment was composed of the Thermo Scientific i10 Nicolet™ spectrometer with an appropriate horizontal adapter adjusted for real time measurements of photopolymerization processes of samples with a thickness of 1.4 mm. It was then shown that the best composite consists of: CQ / EDB (in molar ratio 1: 2); BisGMA / TEGDMA (in weight ratio 7: 3).

This work was financed by the National Science Centre as a research project no.2019/33/N/ST5/03015 "Development and evaluation of physicochemical and mechanical properties of photocurable polymer composites with reduction polymerization shrinkage under visible light" under the "PRELUDIUM" program.

[1] Porto, I. C. C. de M., Soares, L. E. S., Martin, A. A., Cavalli, V., & Liporoni, P. C. S. Influence of the photoinitiator system and light photoactivation units on the degree of conversion of dental composites. Brazilian Oral Research, 2010, 24(4), 475–481. [2] Topa, M, Ortyl, J. Moving Towards a Finer Way of Light-Cured Resin-Based Restorative Dental Materials: Recent Advances in Photoinitiating Systems Based on Iodonium Salts. Materials, 2020, 13, 4093.

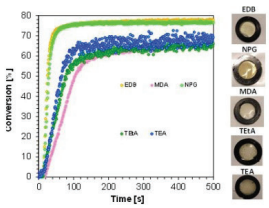

Fig.1. Polymerization profiles (methacrylate function conversion) for bisphenol A-glycidyl methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) in a weight ratio of 7:3 and initiating

Raman microspectroscopic characterization of an oil-degrading bacterium in the biofilm

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Raman imaging, wax ester, triacylglycerol, oil droplet

Petroleum pollution in the sea caused by oil spills is a serious environmental problem. Typically, surfactant-based dispersants are used to clean spilled oil. However, the long-term environmental effects of such methods are still unclear. Bacterial bioremediation is a more environmentally friendly alternative. Among several oil-degrading bacteria, Alcanivorax borkumensis, which degrades alkanes, has attracted much attention. Despite recent studies using standard biological and chemical methods [1-2], the mechanisms of oil uptake and degradation remain unclear. Herein, using confocal Raman microspectroscopy, we characterize chemical compositions of A. borkumensis cells in the biofilm at different times during the oil degradation process to shed light on the biochemical process. In this study, we cultured A. borkumensis in a minimal medium supplemented with hexadecane for a few days. As the time after inoculation increased, we observed oil droplets covered more densely with bacteria (biofilm formation). To characterize the biofilm, we pipetted out 20 µL of emulsion from the liquid culture and transferred it to a glass bottom dish. Then, we acquired 632.8 nm-excited Raman spectra at many locations around the droplets at 24 h intervals, using 30 s exposure time and 3 mW laser power. We found two shape phenotypes of A. borkumensis cells: a typical rod shape and a spherical shape. The representative Raman spectra measured at days 1 and 2 (Fig. 1) show that spherical cells accumulate the decomposition products of hexadecane such as saturated wax esters and unsaturated;triacyclglycerols. In contrast, it appears that the normal rod cells uniformly take up hexadecane and metabolize it. Our approach holds promise for obtaining new insight into the mechanisms of various microbial processes, including physical/chemical factors playing vital roles in oil degradation.

[1] Abbasi, A., G. D. Bothun & A. Bose (2018) Attachment of Alcanivorax borkumensis to Hexadecane-In-Artificial Sea Water Emulsion Droplets. Langmuir, 34, 5352-5357. [2] Godfrin, M. P., M. Sihlabela, A. Bose & A. Tripathi (2018) Behavior of Marine Bacteria in Clean Environment and Oil Spill Conditions. Langmuir, 34, 9047-9053.

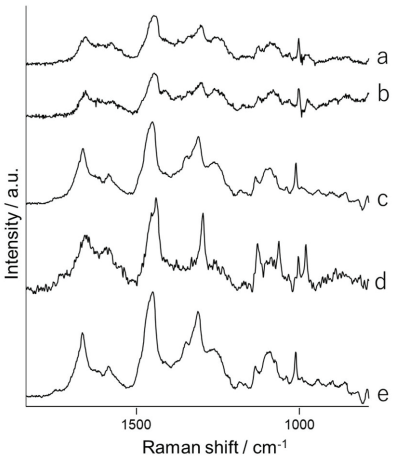

Figure 1. Representative Raman spectra of the A. borkumensis biofilm on days 1 (a) and 2 (b), and of spherical cells on days 1 (c,d) and 2 (e).

1.6. Multimodal spectroscopic imaging

Spectroscopic multi-contrast imaging for an in-vivo or near in-vivo diagnosis, monitoring and therapy of diseases

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Linear Raman Spectroscopy, Nonlinear Raman Spectroscopy, Multimodal Imaging cancer, neurodegenerative disease

Due to an aging society an increase of cancer or neurodegenerative diseases is observed representing unsolved medical needs with respect to early diagnosis and therapy. Thus, in tumor surgery, there is e.g. a great need for new technologies that are able to localize the tumor exactly in order to remove it as complete as possible and that allow for a reliable tumor typing and grading in order to initiate an individual therapy plan tailored to the patient as quickly as possible. Thus, new diagnostic approaches, which can be applied intraoperatively, i.e. in-vivo or near in-vivo (e.g. as frozen section analysis approach) are required. Raman spectroscopy plays a key role in the implementation of these ambitious goals. Here we will highlight our recent efforts in translating Raman spectroscopy towards routine clinical applications by researching and developing compact clinically usable automated Raman spectroscopic instrumentation and their combination with other spectroscopic / optical modalities, to provide a multimodal approach with high TRL levels. We will start with novel multimodal spectroscopic instrumentation (like e.g. innovative Raman fiber probes, clinically usable non-linear multimodal microscopes or endospectroscopic probes etc.) for precise surgical guidance and intraoperative histopathological examination of tissue. Besides innovative photonic technologies, the presentation will also introduce innovative image evaluation algorithms for the translation of multimodal images into quantitative diagnostic markers. We will show that the presented multimodal approaches can be combined with laser tissue ablation for tissue specific laser surgery and for therapy monitoring. In addition, we report on the application of non-resonant Raman spectroscopy for an early diagnosis of neurodegenerative diseases directly in the fundus of the eye, which can be seen as a window to the brain.

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Classifying single cell type and behavior by multimodal quantitative phase and Raman analysis

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Multimodal Imaging, Raman analysis, Single-cell analysis, Immunology, Quantitative phase imaging

In order to understand the nature and behavior of single cells, the best methods can provide a highly detailed examination of cell components and functions. Using sequencing and/or powerful molecular analysis methods such as mass spectrometry, we can obtain quite comprehensive snapshots of the cellular molecules, or a transcription profile that reflects the cell activity and can be used to tease out important signaling pathways. However, the more information gathered about a system, the more the measurement intrudes on the system. In the case of single cell sequencing, the measurement in fact destroys the cell. Maximizing cellular information while minimizing intrusion is then what we should aim for in an ideal cell assay. Recent advances in several distinct fields in microscopy are now raising the bar for what is possible in terms of evaluating single cell characteristics and behavior. In our lab, we combine quantitative phase imaging that can elucidate the morphological features of the cell, with spontaneous Raman scattering imaging that provides a spatio-temporal map of the cell's molecular components. We showed that both quantitative phase imaging and Raman imaging can provide individual cell signatures that are sufficiently informative to allow classification of the cell phenotype and immunological activation state using only the label-free metrics derived from the images [1,2]. Since each cell varies in morphology and specific distribution of components, it is not obvious how a meaningful classification can be achieved merely by inspection of an individual cell. We therefore also exploit computational classification with training data that can automatically determine which cell features are related to variables of interest. For example, taking both quantitative phase and Raman measurements of cells exposed to LPS allows the derivation of a "feature vector" that is intrinsically characteristic of the cellular response to LPS. This can then be used to determine the activation state of a single cell, as well as infer information about the molecular sources of the immune response itself. Similarly, we can assess different cell phenotypes, determine the origin of different cell populations extracted from mice, and examine the effects of inhibitory reagents on the immune response. This talk will give an overview of these methods and recent applications we are developing.

We acknowledge funding from the Japan Society for the Promotion of Science (JSPS) Core to Core program, WPI program, and the Uehara Foundation.

[1] N. Pavillon, A. J. Hobro, S. Akira, and N. I. Smith. "Noninvasive detection of macrophage activation with single-cell resolution through machine learning," Proc. Natl. Acad. Sci. USA 115(12), pp. E2676–E2685 (2018). [2] N. Pavillon and N. I. Smith. "Immune cell type, cell activation, and single cell heterogeneity revealed by label-free optical methods," Sci. Rep. 9, p. 17,054 (2019).

Correlative Raman microscopy, SEM and EDS - How to combine spectral maps from different techniques and evaluate them

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Instrument development, Method development, Correlative microscopy, Machine Learning

The correlation of different microscopic techniques has seen increased interest in recent years due to the possibility of combining the strengths of multiple techniques. In addition to the practical challenges with regard to sample preparation, instruments design and the need for operators experienced in multiple techniques, unique data treatment challenges arise when combining data sets with different resolutions and contrast mechanism. Two key questions arise. How can a SEM image with a pixel resolution of 30 nm, an EDS mapping with a pixel resolution of 100 nm and a Raman mapping with a pixel resolution of 1 µm that are distorted against each other (different contrast mechanism) be combined into a single map? How can we evaluate the resulting map that consist of Raman bands, EDS-elemental concentrations and SEM contrast values? We want to address these questions (on the example of Raman-SEM-EDS), but please note that these approaches generalizes to other combinations. Using specific examples we will not only talk about correlating and combining the data sets, but also point out the benefits of doing so. To give a brief example a larger area mapping of a meteorite is shown in the figure below. In this we used a simple approach of interpolating everything to the resolution of the lowest resolution technique (Raman) after alignment of the maps and images. We then reduced the giant dataset significantly by compressing the EDS and Raman spectra into the significant band intensity. This made it possible to train and run a machine learning algorithm (random forest) on the whole data set for the classification shown on the right in the figure. The benefit of the combined data are more certain interpretations for all phase and some phases are only identifiable by one of the two spectroscopic techniques such as FeS (Raman) or Hydroxyapatite (EDS). This means that the spectral mapping showing all phases simultaneously would not have been possible using the separated data sets. A weakness of this approach is that the higher resolutions of the SEM/EDS were sacrificed in the data treatment and that the reduction of the spectra by band intensities for the machine learning algorithm is rather crude compared to EDS-quantifications or CLS-fits for Raman. Further approaches that may be used to conserve the SEM resolution for phase boundaries and shapes, alternatives for compressing the spectral datasets (which will be necessary in most cases) and possibilities to incorporate microscope image segmentations to aid in the identification of phases will be discussed.

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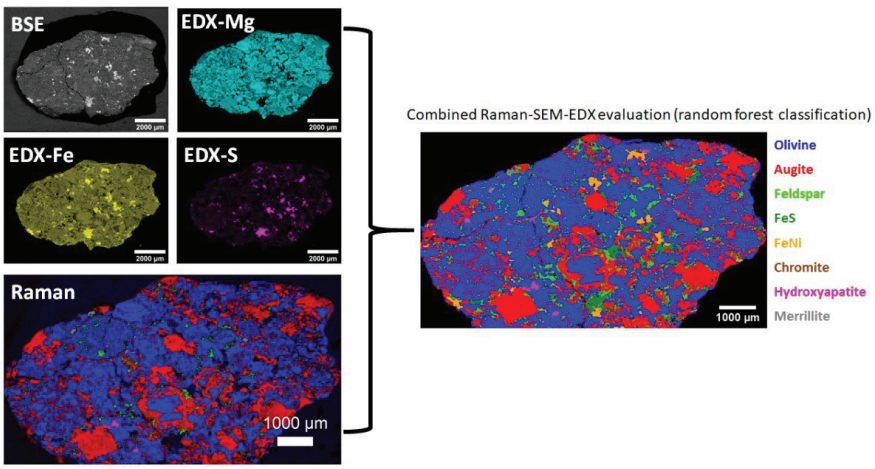

Figure 1. Chelyabinsk-Meteorite

2D FTIR-XRF spectroscopy – a new data analysis protocol for fostering correlative tissue studies by infrared and X-ray radiation

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FTIR microscopy, XRF spectroscopy, Brain Tissue, Imaging, Data Analysis

Correlative Fourier Transform Infra-Red (FTIR) and X-Ray Fluorescence (XRF) microscopy studies of thin biological samples have recently evolved as complementary methods for biochemical fingerprinting of animal/human tissue [1]. This is due to the fact that the combined FTIR-XRF experiments provide complementary multilevel biochemical information ranging from chemical elements to organic moieties [2,3]. This is seen particularly important for tracking neurodegeneration mechanisms, i.e., in Alzheimer/Parkinson disease, in the brain where mishandling of trace metals (Fe, Cu, Zn) seems to be often associated with ongoing damage to molecular components via, among others, oxidative/reductive stress neurotoxicity [4,5]. Despite substantial progress in state-of-the-art detection and data analysis methods, correlative FTIR-XRF experiments have never benefited from in-depth and "quantitative" analysis of observed biochemical correlations between chemical elements and molecular components. We here propose for the first time a novel data analysis pipeline, utilizing the idea of 2D correlation spectroscopy. We utilized combined FTIR-XRF mapping experiments on brain samples mounted onto polypropylene membranes, commercially known as Ultralene. Thanks to the powerful data pretreatment, by the recently developed Multiple Linear Regression Multi-Reference (MLR-MR) [6] algorithm and advanced image processing, artifact-free 2D FTIR-XRF spectra could be obtained by mitigating spectral fringing and substrate-related variability in the data. The proposed method will be shown as a new mean for enhanced identification of distinct histological areas in a sample bringing together spectro-chemical information involving molecular arrangements and chemical elements. Moreover, the applicability of the method in the clinical arena will also be demonstrated by showing its ability to co-localize/correlate metal/nonmetal anomalies with those of molecular arrangement. Taken together, this method opens up for new measures for in-situ unravelling hidden complex biochemical correlations in a biological sample. This step seems crucial for developing new strategies for facilitating the research on the interaction of metals/nonmetals with organic components, which is vital for enhancing our understanding of diseases with abnormalities in metal handling pathways, in particular.

This work was financed by the National Science Centre Poland, grant number DEC-2013/09/B/ NZ4/02539.

[1] Petibois, C. 3D Quantitative Chemical Imaging of Tissues by Spectromics. Trends Biotechnol. 35, 1194–1207 (2017). [2] Surowka, A. D. et al. Combined in-situ imaging of structural organization and elemental composition of substantia nigra neurons in the elderly. Talanta 161, 368–376 (2016). [3 Leskovjan, A. C., Lanzirotti, A. & Miller, L. M. Amyloid plaques in PSAPP mice bind less metal than plaques in human Alzheimer's disease. Neuroimage 47, 1215–1220 (2009). [4] Leskovjan, A. C. et al. Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease. Neuroimage 55, 32–38 (2011). [5] Summers, K. L. et al. A Multimodal Spectroscopic Imaging Method To Characterize the Metal and Macromolecular Content of Proteinaceous Aggregates ('Amyloid Plaques'). Biochemistry 56, 4107–4116 (2017). [6] Surowka, A. D. et al. Model-based correction algorithm for Fourier Transform infrared microscopy measurements of complex tissue-substrate systems. Anal. Chim. Acta 1103,

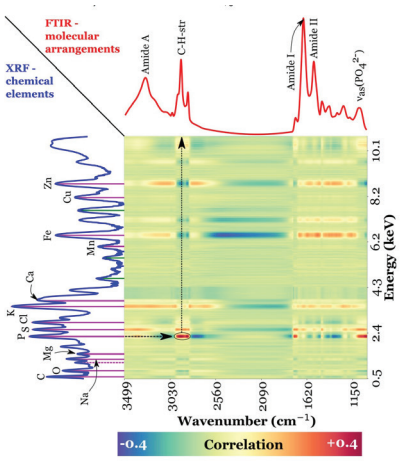

Figure 1. An FTIR-XRF correlogram obtained for the whole coronal brain tissue sample. Positive (dark orange) peaks correspond to the high correlation of the two FTIR/XRF spectral components.

The Benefits of Correlative Microscopy for High Resolution Structural and Chemical Imaging of Materials

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[1]WITec GmbH

Raman-AFM-SNOM-Particle Analysis

The characterization of composite materials greatly benefits the combination of different analytical methods. The interconnection of data from separate methods can deliver the comprehensive understanding often thought. When using different analysis techniques on one and the same sample, the measurement workflow can be accelerated by combining several analytical methods in one instrument. In this contribution, we present a new microscope solution along with a novel operating concept that combines 3D confocal Raman imaging with other nano-analytical techniques such as epifluorescence, profilometry, AFM, or SNOM. This combination of analytical techniques allows for: the identification of cell components in biomedical studies [1, 2], the characterization of phase separations in polymer blends and their wetting behavior on various substrates [3-5], or the visualization of optical properties of materials with resolution below the diffraction limit [6]. The aim of this contribution is to describe and highlight the unique features of such combined scientific instruments, based on examples from composite materials. The opportunity of automated particle analysis with the ParticleScoutTM and their chemical identification using TrueMatchTM software will be highlighted together with the new software features of WITecProjectPlusTM.;

[1] H. Abramczyk, J. Surmacki, M. Kopeć, A. K. Olejnik, A. Kaufman-Szymczykc and K. Fabianowska-Majewskac, Analyst 141(2016) 5646. [2] K. Czamara, K. Majzner, A. Selmi, M. Baranska, Y. Ozaki and A. Kaczor, Sci Rep. 7 (2017) 40889 [3] U. Schmidt, S. Hild, W. Ibach, O. Hollricher, Macromol. Symp. 230 (2005) 133. [4] A. S. de Leon, A. del Campo, M. Fernández-García, J. R. Hernandez, and A. M. Bonilla, ACS Appl. Mater. Interfaces 5 (2013) 3943. [5] U. Schmidt, F. Vargas, M. Kress, T. Dieing, K. Weishaupt, and O. Hollricher, Nanotech. Vol. 4 (2007) 48. [6] K. Bulat, A. Rygula, E. Szafraniec, Y. Ozaki, and M. Baranska, J. Biophotonics 6-7 (2016) 928.

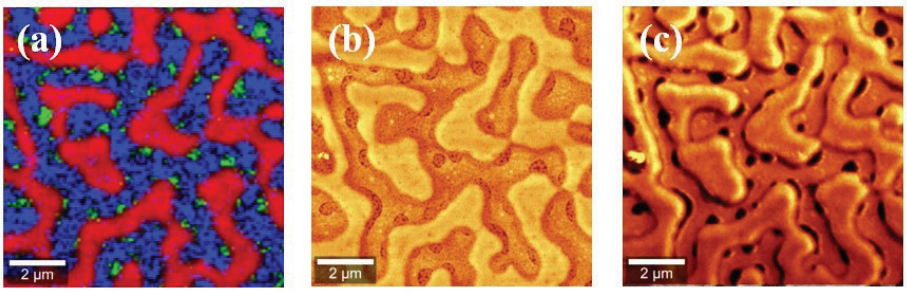

Figure 1. Correlative Raman (a), AFM-Phase image (b), and SNOM (c) images of the same sample area of a three-component polymer blend consisting of poly-styrene (red), acrylate (green), and a mixed ph

nano-FTIR correlation nanoscopy of functional nanostructures

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nano-FTIR, nanoscopy, near-field

Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is a scanning probe approach to optical microscopy and spectroscopy bypassing the ubiquitous diffraction limit of light to achieve a spatial resolution below 20 nanometers. s-SNOM employs the strong confinement of light at the apex of a sharp metallic AFM tip to create a nanoscale optical hot-spot. Analyzing the scattered light from the tip enables the extraction of the optical properties (dielectric function) of the sample directly below the tip and yields nanoscale resolved images simultaneous to topography [1]. In addition, the technology has been advanced to enable Fourier-Transform Infrared Spectroscopy on the nanoscale (nano-FTIR) [2] using broadband radiation from the visible spectral range to THz frequencies. Recently, the combined analysis of complex nanoscale material systems by correlating near-field optical data with information obtained by other SPM-based measurement methodologies has gained significant interest. For example, the material-characteristic nano-FTIR spectra of a phase-separated PS/LDPE polymer blend verifies sharp material interfaces by measuring a lineprofile across a ca. 1 µm m sized LDPE island. Near-field reflection/absorption imaging at 1500cm-1 of the ca. 50nm thin film allows to selectively highlight the distribution of PS in the blend and simultaneously map the mechanical properties like adhesion of the different materials [3,4]. To achieve comprehensive characterization of a nanostructures conductivity properties the near-field optical response of semiconducting samples like graphene (2D) or functional SRAM devices (3D) in different frequency ranges (mid-IR & THz) are correlated to e.g. Kelvin Probe Force Microscopy (KPFM) measurements. Thus, neaspec s-SNOM systems represent an ideal platform to characterize advanced material systems by different near-field and multi-modal AFM methods at the nanoscale.

[1] F. Keilmann, R. Hillenbrand, Phil. Trans. R. Soc. Lond. A 362, 787 (2004). [2] F. Huth, et al., Nano Lett. 12, 3973 (2012). [3] B. Pollard, et al., Beilstein J. of Nanotechn. 7, 605 (2016). [4] I. Amenabar, et al., Nature Commun. 8, 14402 (2017).

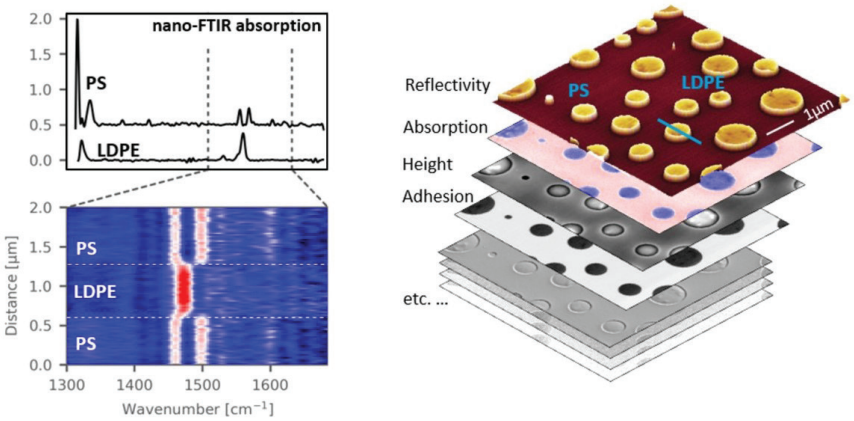

Figure 1. Near-field correlation nanoscopy of a thin PS/LDPE polymer film, highlighting the phase separation of the materials by nano-FTIR measurements as well as the different mechanical properties of t

Raman2imzML: Fitting Raman imaging data into the standard mass spectrometry imaging format.

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Raman Imaging, Mass spectrometry Imaging, Multimodal Imaging, imzML, Data converter

Multimodal imaging combining mass spectrometry imaging (MSI) with Raman imaging is becoming a popular multidisciplinary analytical method used by a growing number of researchers. Computational tools that can operate simultaneously with datasets from both techniques are in high demand. Raman2imzML is an open-source data converter that fits Raman imaging data into the imzML, the standard file format created and adopted by the MSI community. As both techniques generate spectra localized in the pixels of an image, the raw data share multiple characteristics which allow using the same format for MSI and Raman imaging data. Thanks to Raman2imzML we converted Raman datasets to imzML files and visualized Raman images using the open-source software designed for MSI applications. This facilitates introducing Raman imaging datasets to the MSI community, allowing easier collaborations and knowledge exchange. Moreover, using the same data format could be useful for trending research fields such as data/image co-registration and multimodal analysis for compound identification.

MINECO BES-2016-076483, URV 2017PMF-PIPF-60, MICIN RTI2018-096061-B-I00 and AGAUR 2018 BP 00188

[1] Iakab, S.A., Sementé, L., García-Altares, M. et al. Raman2imzML converts Raman imaging data into the standard mass spectrometry imaging format. BMC Bioinformatics 21, 448 (2020). https://doi.org/10.1186/s12859-020-03789-8

Raman and FT-IR imaging in the search of biochemical markers of endothelial progenitor and brain endothelial cells

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Raman imaging, FT-IR imaging, chemometric analysis, Endothelial progenitor cells

Endothelial progenitor cells (EPCs), mainly derived from bone marrow, are the precursors of vascular endothelial cells. Since their first isolation by Asahara in 1997[1], the research on their biological characteristic and therapeutic effects has bloomed mainly due to their potential in forming vessels and differentiating into functional endothelial cells (ECs). Circulating endothelial progenitor cells (CEPCs) are homed to the damaged endothelium and in consequence form a new layer.[2] The potential of migration and interaction of progenitor cells with endothelium in the blood-brain barrier indicated their role as carriers for drug (ex. therapeutic protein encoded inside the cell).[3] However, the conventionally used imaging techniques are not specific enough to investigate these interactions. Therefore, the aim of this work is to propose FTIR and Raman spectroscopic imaging as techniques assessing biochemical characterization of early endothelial progenitor cells (EPCs) and brain endothelial cells (BECs) and their changes upon co-culturing. In this experiment the cultures of immortalized early endothelial progenitor cell line established from aorta–gonad–mesonephros (AGM) of murine embryo[4] and brain endothelial cells were deposited on CaF2 windows and then examined with high resolution Raman and FTIR imaging. Spectroscopic methods assisted with a chemometric analysis enabled the comparison and differentiation of the investigated cells. k-Means cluster analysis of Raman images of single cells discriminated subcellular compartments indicating the presence of lipid droplets with saturated fatty acids and an increased content of cytochromes in endoplasmic reticulum in EPCs. Fast FTIR imaging confirmed these observations. Principal component analysis on Raman and FTIR spectra clearly separated both cultures with variance of ca. 30%. Next, the mixture of investigated cells were examined. The comparative and PCA analysis of the co-cultured cells indicated that brain endothelium changed its phenotype towards the progenitor cells.

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[1] T. Asahara et al. "Isolation of putative progenitor endothelial cells for angiogenesis". Science (80-.). 1997. 275(5302): 964–967. [2] F. Kou et al."Endothelial progenitor cells as the target for cardiovascular disease prediction, personalized prevention, and treatments: progressing beyond the state-of-the-art". EPMA J. EPMA Journal, 2020. 11(4): 629–643. [3] L. Heller et al. "Secretion of proteins and antibody fragments from transiently transfected endothelial progenitor cells". J. Cell. Mol. Med. 2020. 24(15): [4] G. Collet et al. "Endothelial precursor cell-based therapy to target the pathologic angiogenesis and compensate tumor hypoxia". Cancer Lett. Elsevier Ireland Ltd, 2016. 370(2): 345–357.

Raman and fluorescence microscopy based characterisation of lipid droplets in endothelial cells during inflammation induced by LPS and TNF-?

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Endothelial inflammation, Lipid droplets, Proinflammatory factors, Raman spectroscopy, Fluorescence microscopy

Endothelium covers the innermost layer of blood and lymphatic vessels and fulfils many functions maintaining cardiovascular homeostasis, for example regulates the vessel diameter, takes part in the immune system functioning and regulates the endothelial inflammation.[1] The endothelial inflammation is known to have a huge contribution to the development of life-style diseases including atherosclerosis, type 2 diabetes, arterial hypertension or cancers. [2] The inflammation can be induced by many substances such as lipopolysaccharides (LPS) and tumor necrosis -α (TNF-α). They are both proinflammatory factors, but they have different origin, properties and signaling pathways: TNF-α is a cytokine, while LPS is a derivative from the cell wall of Gram negative bacteria, and it is an endotoxin.[3], [4] In the endothelium stimulated with both: LPS and TNF-α the lipid droplets (LDs) formation was negligible, and their content was quickly metabolized as evidenced by the effect of atglistatin, the inhibitor of ATGL. In the presence of atglistatin, LPS and TNF-α induced the LDs formation was studied by Raman spectroscopy to gain information about their chemical composition and level of unsaturation, and by fluorescence microscopy to localize their distribution in the tissue. The results uncovered that the LDs stimulated by TNF-α in the presence of atglistatin were found in the aorta in large numbers, they have high level of unsaturation, but also have negligible content of cholesterols and phospholipids. The microscopic images of LDs showed that they are secreted near cell nuclei, and have irregular distribution within the tissue. In summary, in this work we analyze the changes in the chemical composition of the LDs in LPS or TNF-α induced endothelial cells inflammation in;en face;aorta, which rely on different effect of these factors on the endothelial inflammation. This leads to conclusion that the LDs can have divergent results depending on the type of the proinflammatory factor.

This work was supported by the National Science Centre, Poland, SONATINA1 No.: DEC-2017/24/C/ST4/00075 and supported by the Foundation for Polish Science (FNP START2019 program).

[1] Aird W.C.; Laubichler M.D.; Endothelial biomedicine; Chapter 2 –Introductory Essay: Evolution, comparative biology, and development; Cambridge University Press; 2007; 23-28 [2] Sessa W.C; Pober J.S; Evolving functions of endothelial cells in inflammation; Nature Reviews 7; 2007; 803-815 [3] Pober J.S.; Endothelial biomedicine; Chapter 31 –Tumor necrosis factor; Cambridge University Press; 2007; 261-265 [4] Kaur J.; Kubes P.; Endothelial biomedicine; Chapter 46 – Endothelium: A critical detector of lipopolysaccharides; Cambridge University Press; 2007; 410-418

Multimodal imaging of silicified sorghum leaves

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FTIR, FTIR, Imaging, Plant tissues

The cell wall is a complex composite structure made of polysaccharide, polyphenols, proteins, and minerals whose composition and arrangement are crucial to the tissue function. Although the visualization of the microscopic features of plant tissues is a prerequisite to understand these complex structures, information on their composition, structure, and interactions at the molecular level is the basis for our understanding of how they are formed and of their specific physiological role. The role taken by organic cell wall constituents in the deposition of silica, accumulating to form phytolith structures in the tissue, is not well-understood and is studied with the help of spectroscopic tools (1, 2). Infrared (IR) and Raman microspectroscopy are commonly used to study the cell wall because the spectra provide information on molecular composition and structure of the organic cell wall constituents including cellulose, phenolic compounds, and proteins. The resulting chemical images show the distribution of these constituents in the plant tissue and the differences in the composition between plant organs. We used a multimodal imaging approach to visualize the chemical composition of sorghum leaf cross-sections freshly cut and after paraffin embedding. We used bright-field microscopy and fluorescence microscopy to visualize the plant structures and tissues, and then combined Raman and IR microspectroscopy to obtain the chemical distribution of organic components. Multivariate methods such as principal component analysis (PCA), vertex component analysis (VCA) and hierarchical cluster analysis (HCA) were applied to the data sets to create images and to evaluate the ability of these methods to show the distribution of different compounds of the cell walls. We complemented the vibrational spectroscopic maps with scanning electron microscopy (SEM), which provided great detail of the plant tissues and tissue boundaries, as well as localization of subcellular structures. SEM coupled with energy dispersive X-ray spectroscopy (EDX) enabled the visualization and mapping of the elemental composition, including the silica. We correlated the chemical information of organic components with the distribution of inorganic components such silica and observed co-localization of lignin and silica in the plant epidermis (3).

We acknowledged provision of beam time by HZB-BESSY and thank L. Puskar (BESSY II, Berlin) for her help at the IR beamline. We acknowledge B. Zimmermann and A. Kohler for providing a MATLAB script for EMSC and P. Lasch for providing the CytoSpec software. Financial support provided from DFG (GSC 1013 SALSA) is also gratefully acknowledged.

[1] V. M. R. Zancajo et al., Spectroscopic Discrimination of Sorghum Silica Phytoliths. Frontiers in plant science 10, (2019). [2] V. M. R. Zancajo et al., FTIR Nanospectroscopy Shows Molecular Structures of Plant Biominerals and Cell Walls. Analytical chemistry, (2020). [3] V. M. R. D. Zancajo, S.; Elbaum, R; Kneipp, J., In preparation. (2021).

Multimodal spectroscopic analysis of margins during breast conserving surgery combining total internal eflection imaging and Raman spectroscopy

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intraoperative analysis, total internal reflection, Raman Spectrosocpy, breast cancer, Waveguide

Breast cancer is one of the most common cancer among women. In Europe it was estimate 355,000 new cases in 2020 1. The standard practice is surgical removal of the tumour. Currently, the completeness of cancer excision is confirmed post-operatively via histopathology. For ~20% of patients, histopathology analysis detects inadequate surgical margins and patients require additional surgery to remove the residual cancer 2. The number of re-excision surgeries could be drastically reduced if surgeons could check the tumour margins during the surgery, whilst the patient is in the operating room. Thus far, no technologies are available to determine the status of the margins intraoperatively; they either lack diagnosis speed, accuracy, or spatial resolution (small ductal cell carcinoma in-situ account for 80% of positive margins) 3,4,5. Raman spectroscopy (RS) can analyse fresh breast tissue without requiring any tissue preparation steps, detecting tumour with >95% sensitivity/specificity. However, RS is too slow. Integration of RS with confocal auto-fluorescence imaging reduced analysis times to ~20-25 minutes/side and demonstrated detection of both invasive and DCIS 5. However, this long analysis time is still a barrier for adoption. We developed a new technique based on auto-fluorescence spectral imaging and Raman spectroscopy using total internal reflection 6. Firstly, we investigated the auto-fluorescence spectra of breast tissues at two excitation wavelengths: 405 nm and 365 nm. The latter led to optimal discrimination between breast tissue structures based on fluorescence emission spectra, therefore the 365 nm wavelength was used to build a light emitting diode (LED) powered microscope. The bespoke unit performed auto-fluorescence imaging on a simple waveguide basis and it was paired with Raman spectroscopy to perform quantitative analysis of the BCS margins. The methods developed allowed efficient screening of small tissue samples as well as fresh wide local excisions, delivering the analysis of the entire cruciate surface of BCS specimens (5.1×7.6 cm^2) in less than 45 minutes, also providing information regarding the location of the tumour in the specimen. Full automation of the process will allow for the measurement of BCS specimens within intraoperative time scale (20 minutes). Moreover, the successful analysis of human wide local excisions as well the accuracy, speed and contained costs of the instrumentations makes the device suitable for the translation into clinical environments.

UK Engineering and Physical Sciences Research Council (grant number EP/L025620/1).

[1] Eu Science Hub, https://ec.europa.eu/jrc/en/news/2020-cancer-incidence-and-mortality-eu-27-countries [2] Jeevan R, Cromwell DA, Trivella M, Lawrence G, Kearins O, Pereira J, et al. Reoperation rates after breast conserving surgery for breast cancer among women in England: retrospective study of hospital episode statistics. BMJ. 2012;345:e4505. [3] Phipps J, Gorpas D, Unger J, Darrow M, Bold R, Marcu L. Automated detection of breast cancer in resected specimens with fluorescence lifetime imaging. Phys Med Bio 2017;63. [4] Zhang J, Rector J, Lin JQ, Young JH, Sans M, Katta N, et al. Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. Sci Transl Med 2017;9:3968. [5] Shipp DW, Rakha EA, Koloydenko AA, Macmillan RD, Ellis IO, Notingher I. Intra-operative spectroscopic assessment of surgical margins during breast conserving surgery. Breast Cancer Res 2018;20: 69. [6] M. G. Lizio, Z. Liao, D. W. Shipp, R. Boitor, R. Mihai, J. S. Sharp, M

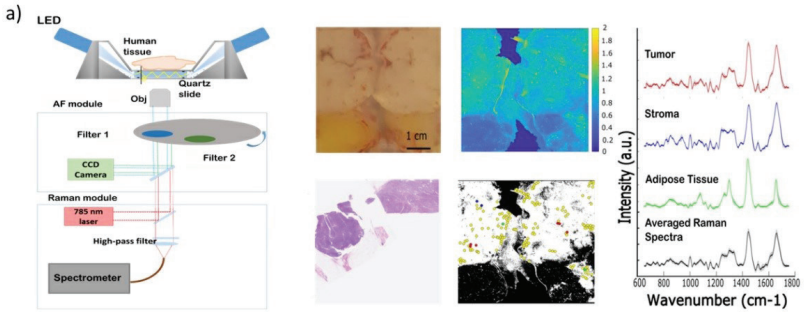

Figure 1. a) schematic representation of the instrumentation. b) Wide-field image of a breast wide local excision (top left), ratiometric image collected using the waveguide imaging unit (top centre), H&E staining (bottom left), threshold image generated by the algorithm based on ratiometric threshold value of T=1 (bottom centre) and Raman spectra acquired in the area isolated by the threshold algorithm (right panel).

1.7. Instrumental Development

Normal-to-Cancer Transition in Human Cells - Insights from Quasi-elastic Neutron Scattering

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Norma-to-cancer transformation, breast cancer, prostate cancer, osteosarcoma, neutron scattering techniques

Cancer is a worldwide health problem and the second leading cause of death globally. Despite its undisputable interest for elucidating cancer initiation, progression and metastasis, allowing to develop improved chemotherapeutic strategies, normal-to-cancer (NTC) transition is still a poorly understood process. Unravelling cellular water behaviour, at the molecular level, is crucial for determining how the biochemical and mechanical properties of cells are affected by this NTC transformation. The aim of the present study was to probe changes in the dynamical activity of intracellular water between healthy and cancerous human cells, as an innovative approach for unveiling particular features of malignancy and identify specific reporters of cancer. Following previous successful studies on intracellular water [1,2], quasi-elastic neutron scattering was applied to study distinct human cell lines: androgen-unresponsive prostate cancer, triple-negative breast carcinoma and osteosarcoma [3]. Cancer cells displayed a significantly higher plasticity relative to their healthy counterparts. Prostate cancer cells showed the highest plasticity when compared to triple-negative breast cancer and osteosarcoma, the latter being remarkably less flexible. Furthermore, the data suggested dynamical differences between the different types of intracellular water, in normal and cancerous cells: the dynamics of hydration water (organised water surrounding biomolecules) remained virtually unaffected, while cytoplasmic water (particularly the rotational motions) was found to undergo significant changes upon normal-to-cancer transition. This increased flexibility associated to malignancy is supposed to allow neoplastic cells to grow uncontrollably and to contribute to their invasiveness and metastatic ability. The results obtained along this study can potentially help to understand the variations in cellular dynamics underlying carcinogenesis, malignant progression and tumour metastasis, with a high impact on human health.

Portuguese FCT (UIDB/00070/2020). STFC ISIS (OSIRIS/RB1910015, DOI: 10.5286/ISIS.E.RB1910015)

[1] M.P.M. Marques et al., PCCP 19 (2017) 2702. DOI: 10.1039/C6CP05198G [2] M.P.M. Marques et al., Int.Rev.Phys.Chem. 39 (2020) 67. DOI: 10.1080/0144235X.2020.1700083 [3] M.P.M. Marques et al., Struc.Dyn. 7 (2020) 054701. DOI: 10.1063/4.0000021

Very high pressure studies with infrared sources: for static to dynamic approaches.

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High pressure, Visible and infrared lasers, Concommittant infrared and Raman dynamic studies

Infrared absorption spectroscopy in combination with Diamond Anvil Cells (DAC) is a powerful non-invasive approach to characterize the structural, chemical and electronic properties of ;materials under high pressure. New opportunities for exploring matter under extreme high pressure have been opened thanks to two technical novel developments for DAC's: novel form for the diamond anvil tip, the t-DAC, enabling reproducibly reaching pressure; up to at least 600 GPa [1], and a piezzo-drive of the force on the piston generating a few ms compression ramp [2]. An home-made long working distance microscope, dedicated to very high pressure studies, and using the ;synchrotron IR radiation, was developed for measurements on a few micron sized samples. It was successfully used to demonstrate that hydrogen turns into a molecular metallic state at 427 GPa, and deuterium behaves the same at higher pressure [3,4]. A second experimental bench, for two purposes, has been developed and is now completed: the first capability is to extend the infrared measurements above 500 GPa, i.e with 2-3 µm sized samples, for transmission and reflection studies. Some materials, like methane, very reluctant to metallization, could then be investigated. The second capability is to perform time resolved (milli- to micro-seconds time scale); measurements adapted for the use of the d-DAC, in order to disclose the chemical mechanism of; material transformation. In static approach, the pressure can be increased step by step, and the continuous closure of the gaps by IR transmission, concomitantly with the IR reflection are followed using a combination of supercontinuum visible and infrared laser sources (energy range covered 0.4 to 4 microns). The reflectivity for such small sample is a key to follow the metallization mechanism, and the superconductive character of the newly formed material [5]. In dynamic approach, pressure can also be generated in a dynamic DAC or d-DAC within few ms time scale. Recording the sequence of vibrational fingerprints of the chemical change under this pressure ramp could help to understand the mechanism underlying the chemical transformation under pressure, and/or the formation of new materials created under dynamic compression. We have developed a complementary dispersion set up, using four prisms as dispersive elements, and an infrared camera (640x512 pixels) to record the time evolved spectra during fast compression. Performances and examples will be shown during the presentation.

[1] Dewaele, A., Loubeyre, P., Occelli, F. et al. Nat Comm. 9, 2913 (2018). https://doi.org/10.1038/s41467-018-05294-2 [2] Jenei Zs. et al., Rev. Sci. Instrum. 90, 065114 (2019); https://doi.org/10.1063/1.5098993 [3] P. Loubeyre, F. Occelli and P. Dumas Nature 577, 631–635 (2020). https://doi.org/10.1038/s41586-019-1927-3 [4] P. Loubeyre, F. Occelli and P. Dumas submitted [5] J.-P. Carbotte, E.-J. Nicol, and T. Timusk, Phys. Rev. Lett. 121, 047002(2018)

An ultra-low cost, synchronisation free, versatile light source for targeted CARS multiphoton imaging.

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Coherent Anti - Stokes Raman Scattering (CARS), Microscopy, Optical Parametric Amplifier (OPA), Label free imaging, Secaond Harmonic Generation (SHG), Two Photon Excited Auto - Fluorescence (TPEAF)

An optical parametric amplifier (OPA) which uses a simple and cheap continuous wave (CW) laser diode source as a seed and a picosecond laser at Ytterbium wavelengths as pump is presented. We demonstrate that it is capable of performing targeted Coherent Anti-Stokes Raman Scattering (CARS) imaging with excellent spectral resolution (approximately 10 cm-1) while offering a limited tunability of approximately 50 cm-1 by temperature tuning the laser diode wavelength. It is also shown that Second Harmonic Generation (SHG) and Two Photon Excited Auto-Fluorescence images can be simultaneously acquired by using this light source. Images of various biological samples and standards that were acquired by deploying this OPA are shown.

[1] T. Steinle, V. Kumar, M. Floess, A. Steinmann, M. Marangoni, C. Koch, C. Wege, G. Cerullo, and H. Giessen, "Synchronization-free all-solid-state laser system for stimulated Raman scattering microscopy," Light: Sci. Appl.5(10), e16149 (2016). [2] Duanyang Xu, Sijing Liang, Lin Xu, Konstantinos N. Bourdakos, Peter Johnson, James Read, Jonathan H. V. Price, Sumeet Mahajan, and David J. Richardson, "Widely-tunable synchronisation-free picosecond laser source for multimodal CARS, SHG, and two-photon microscopy," Biomed. Opt. Express 12, 1010-1019 (2021).

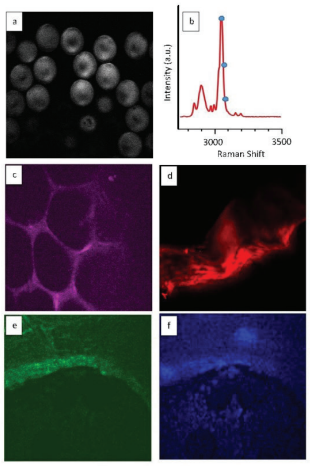

Figure 1. CARS images of a) of polystyrene beads and c) cell membranes, b) red curve Raman spectrum, blue dots CARS signal and SHG images of d) collagen e) bone, f) TPEAF image of bone marrow.

Current state and future of the IRIS THz/Infrared beamline at BESSY II

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infrared spectroscopy, synchrotron, FTIR, near-field, Raman

The IRIS THz/Infrared beamline at the BESSY II synchrotron was inaugurated in December 2001 [1]. Synchrotron infrared radiation, which is some orders of magnitude brighter than standard thermal broadband sources, allows to obtain unique data about molecular structure, composition, and interactions of a biological, inorganic, or synthesized materials, which are not available for tabletop devices. Here we give an overview over current end-stations and facilities available at the IRIS beamline and describe the current beamline upgrade, which includes the implementation of scattering-type near-field optical microscopy in the Far- and Mid- IR regions; the upgrade of the IR-microscopy station and its coupling with a Raman spectrometer. The new multimodal microscopy station will allow the characterization of materials at different scales from micrometer to nanometer range, to simultaneously acquire complementary IR and Raman information, and to carry out polarization dependent studies. Performance and capabilities of the IRIS end-stations are evaluated at different stages of the modernization inter alia by spectroscopic imaging of different types of composite materials. As an example, different building blocks in the cell walls of plants, such as cellulose and its orientation and interaction in situ are investigated. Cellulose is hierarchically structured at the whole range from micro- to nanometer scale, has defined orientation and angle with respect to the long axis of the cells in plants, which makes it an ideal reference sample to validate and develop new multi-scale vibrational microspectroscopic approaches [2]. First spectroscopic results on the characterization of plant cell walls in situ will be presented.

The authors gratefully acknowledge the financial support from the Federal Ministry of Education and Research of Germany (project SyMS).

[1] Schade, U.; Röseler, A.; Korte, E. H.; Bartl, F.; Hofmann, K. P.; Noll, T.; Peatman, W. B. New Infrared Spectroscopic Beamline at BESSY II. Review of Scientific Instruments 2002, 73 (3), 1568–1570. [2] Zancajo, V. M. R.; Lindtner, T.; Eisele, M.; Huber, A. J.; Elbaum, R.; Kneipp, J. FTIR Nano-spectroscopy Shows Molecular Structures of Plant Biominerals and Cell Walls. Anal. Chem. 2020, 92 (20), 13694–13701.

Solaris Advanced InfraRed (SOLAIR) beamline conceptual design

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synchrotron radiation, IR imaging, biomedical samples

The Solaris Advanced InfraRed beamline (SOLAIR) is currently under construction. The large radiation extraction from a bending magnet will allow to collect a very wide wavelength range (0.4-500 μm), covering the near (NIR), mid (MIR) and the far (FIR) infrared spectral range. The extraction of infrared radiation from synchrotron radiation will be achieved using, a flat and slotted mirror (M1), which will be located inside the dipole chamber located at the bending magnet in storage ring. Figure 1 shows the calculated distribution of infrared radiation intensity for two wavelengths: 10 μm (1000 cm^{-1}) and 200 μm (50 cm^{-1}) after M1. The presentation will showcase the current status of the project along with the expected IR beam parameters. It will also highlight microscopic techniques planned to be used at the SOLAIR beamline with their potential applications.

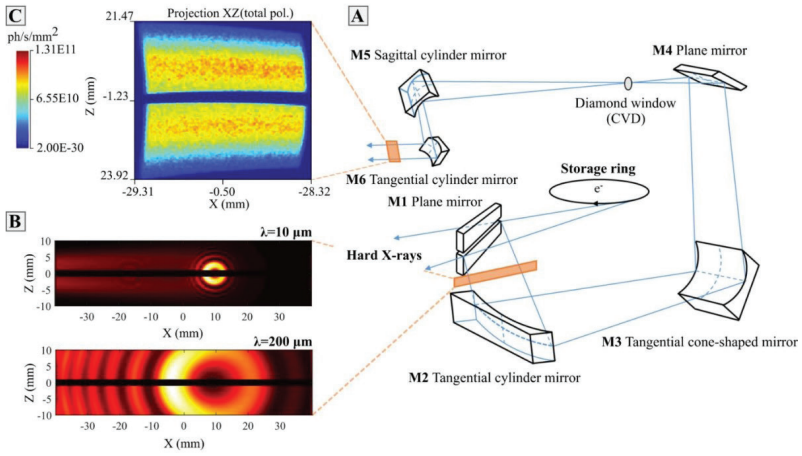

Figure 1. Diagram of the optical system of M1 to M6 mirrors (from synchrotron.uj.edu.pl/en_GB/linie-badawcze/solair)

Distortion and correction of ATR-spectrum of high refractive index material

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ATR, high refractive index, peak distortion, simulation

The last decades have seen a significant increase in the use of attenuated total reflection (ATR) infrared spectroscopy due to its experimental convenience and nondestructive nature. Nevertheless, when it comes to some samples with high refractive index, like black carbon, black rubber, graphene and so on, the spectrum will appear extremely distorted. Therefore, it is important to develop methods to correct for these artefacts and this work aims at making a contribution to these efforts. Our approach starts with Snell's law, combines Fresnel's equations and the Kramers-Kronig (KK) transform to obtain the complex relationship between optical constants. With calculating and from the absorption spectrum, a model for solid and liquid mixture infrared absorption spectrum is established. Furthermore, the influence of the complex refractive index of the mixture on the spectrum through calculation and simulation is obtained. The spectrum is distorted due to the interaction of the real and imagine parts. Accompanying the tilt of the baseline, the positive absorption peak will be a pseudo red shift and a concave part appears on the left side of the peak first, then the negative peak becomes more obvious, the entire absorption spectrum will be overall inverted and the actual blue shift is visible at the end. This is consistent with experimental attenuated total reflectance (ATR) infrared spectroscopy results. We show that our method provides credible peak position for further analysis and research.

Prof. Dr.-Ing. Johannes Kiefer Anja Lampe PD Dr. Thomas Mayerhöfer

[1] Mayerhofer, T. G.; Pahlow, S.; Popp, J., The Bouguer-Beer-Lambert Law: Shining Light on the Obscure. Chemphyschem 2020, 21 (18), 2029-2046. [2] Milosevic, M., Internal reflection and ATR spectroscopy. John Wiley & Sons: 2012; Vol. 176. [3]Bertie, J. E.; Zhang, S. L., Infrared intensities of liquids. IX. The Kramers–Kronig transform, and its approximation by the finite Hilbert transform via fast Fourier transforms. Canadian Journal of Chemistry 1992, 70 (2), 520-531. [4]Tek, G.; Hamm, P., A Correction Scheme for Fano Line Shapes in Two-Dimensional Infrared Spectroscopy. J Phys Chem Lett 2020, 11 (15), 6185-6190

Figure 1. Distorted spectrum

Investigating glue samples with the Arrow consumable ATR solution

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ATR, SIRE

Here we describe the development of a low cost, consumable ATR technology based on a silicon internal reflection element. Industries concerned about cross-contamination between samples, the use of corrosive chemicals, or samples that are difficult to clean can benefit from the consumable nature of this technology. It also enables experiments to be run in duplicate;over multiple days, freeing the equipment for other use in the meantime. To illustrate the benefits of this technology, we have taken a case study of packaging adhesives. In industry, these chemical systems are usually probed using transmission experiments, although care must be taken to ensure consistent pathlength. ATR would eliminate this concern;due to its fixed pathlength, however the difficulty of removing the cured adhesive rules out conventional ATR. Arrow eliminates the requirement for cleaning, opening the door to new application areas. The case study in this talk is based around the reaction of polyols and isocyanate to form polyurethane lamination adhesives commonly used by the food industry. It is critical to ensure complete reaction of the isocyanate as unreacted isocyanates could migrate through packaging and react with water in the food to form carcinogens. FTIR spectroscopy can be used to monitor the N=C=O vibration at 2270 cm-1 making it an ideal technique for this analysis.

Principles and Techniques of Quantitative Raman Imaging for Crystal Orientation Analysis (qRICO)

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polarized Raman microscopy, Orientation mapping, Orientation mapping, grain structure, crystal orientation

Polycrystalline matter is ubiquitous in nature (rocks, sand, ice, bones) and industry (metals, ceramics, semiconductors, drugs). The ability to map the shape and orientation of grains in 2D, 3D, and 4D (as a function of time) is of great interest within many scientific disciplines. During the last 20 years, methods based on electron diffraction (like EBSD) and X-ray diffraction (3DXRD, DCT, and others) have emerged and become a critical part of materials science and geoscience. According to Web of Science, the number of publications based on orientation mapping is beyond 1500 per year. Nevertheless, due to technical limitations for EBSD and issues with access to 3DXRD/DCT, we believe this represents only a tiny fraction of the potential use. In the present study, we demonstrate quantitative Raman Imaging for Crystal Orientation analysis (qRICO) by means of Polarized Raman Microscopy (PRM). While it is known that; PRM is sensitive to orientation changes [1], an actual orientation map has to our knowledge never been presented before. Using a novel concept of ambiguity-free orientation determination data analysis and simultaneous registration of multiple Raman scattering spectra obtained at different polarizations, we obtain 2D and 3D quantitative orientation mapping of multigrain materials [2]. It has been demonstrated that at least nine polarized channels are necessary to reach orientation determination accuracy around 1 degree for polycrystalline Si [2]. Here we present optimized optomechnical design of qRICO technology where twenty polarized Raman channels can be simultaneously acquired (Figure 1A). With improved design orientation mapping, an accuracy of less than 0.4 degrees was achieved on polycrystalline Si solar cell sample which was confirmed by EBSD reference measurements (Figure 1B, 1C). First results for Si, a pharmaceutical tablet and sapphire reveal favorable specifications: sub-micrometre resolution, fast data acquisition, and a high orientation resolution. A volumetric orientation map of polycrystalline sapphire non-destructively obtained by qRICO technology is shown in Figure 1D. qRICO applies to all Raman active materials of any crystal symmetry. Sample preparation is not required. Notably, qRICO has the potential to bring orientation-mapping experiments into conventional optical laboratories on a broad scale.

This work was financially supported by DTU Discovery grant (33216 E-1), the IDUN Center of Excellence (grant no. DNRF122) funded by the Danish National Research Foundation and the Villum Foundation (Grant No. 9301) and Lightnovo ApS.

[1] Schmid, T., Schäfer, N., Levcenko, S., Rissom, T. & Abou-Ras, D. Orientation-distribution mapping of polycrystalline materials by Raman microspectroscopy. Sci. Rep. 5, 1–7 (2015). [2] Ilchenko, O., Pilgun Y., et al. Fast and quantitative 2D and 3D orientation mapping using Raman microscopy. Nat. Commun. 10, (2019).

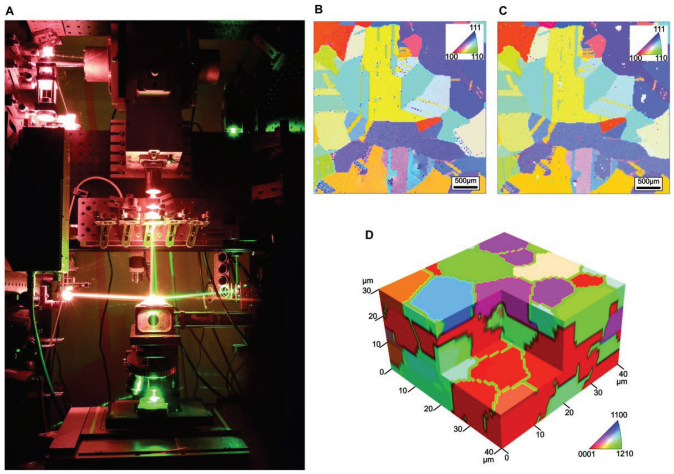

Figure 1. A) Photograph of qRICO. B,C) Orientation map of poly-Si solar cell obtained by qRICO (B) and by electron microscope EBSD (C) of the same area. D) Volumetric qRICO map of poly sapphire.

Single-photon lock-in detection for single-molecule time-resolved coherent anti-Stokes Raman spectroscopy

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Single-photon detection, Coherent anti-Stokes Raman scattering, Non-linear vibrational spectroscopy, Time-resolved spectroscopy, Plasmonics

Non-linear optical experiments require elaborate experimental setups to detect extremely low signal intensities over ultrashort timescales. Typically, the linear response dominates over nonlinear processes leading to poor signal-to-noise. Here, we present a new single-photon lock-in detection technique [1] to remove dark counts and isolate <1 count/s signal from time-resolved coherent anti-Stokes Raman scattering (tr-CARS) [2] of 1-100 molecules in a plasmonic nanocavity (Fig.1a) [3] to measure their ultrafast vibrational lifetime. Using a field-programmable gate array (FPGA) board, we record the arrival time of individual photons at a single-photon detector with picosecond time resolution [4]. At the same time, reference signals such as the laser pulse frequency and the modulation of the lasers are monitored. This allows recovery of the precise signal modulation during each period, implementing lock-in detection for each individual photon. With this setup, the laser pulse shape is recreated (Fig.1b) and 98% of dark counts and stray light are filtered out, thus increasing the signal-to-noise of low count rate experiments by > 60. Moreover, the recovered laser modulation (Fig.1c) allows identification of the nonlinear CARS signal (1 count/s) within a large background (here 300 counts/s). To demonstrate the potential of this technique for spectroscopic applications, the vibrational lifetime of biphenyl-4-thiol molecules in a plasmonic nanocavity is measured (Fig.1d). This newly established method enables tr-CARS experiments at the single-photon level to push towards investigating single molecules [5]. Due to the high flexibility for reference frequencies from Hz to MHz, this concept can be applied universally to all single-photon experiments, drastically increasing their signal-to-noise ratio. The additional time resolution is particularly advantageous for new applications in the areas of quantum correlation, time-of-flight spectroscopy and scanning near-field optical microscopy.

[1] Jakob, L. A. et al. Single-photon lock-in detection by picosecond time stamping, submitted (2021) [2] Yampolsky, S. et al. Seeing a single molecule vibrate through time-resolved coherent anti-Stokes Raman scattering. Nat. Photonics 8, 650–656 (2014). [3] Baumberg, J. J., Aizpurua, J., Mikkelsen, M. H. & Smith, D. R. Extreme nanophotonics from ultrathin metallic gaps. Nat. Mater. 18, 668–678 (2019). [4] Bayer, E. & Traxler, M. A High-Resolution (<10ps RMS) 48-Channel Time-to-Digital Converter (TDC) Implementedin a Field Programmable Gate Array (FPGA). IEEE Trans. Nucl. Sci. 58, 1574–1552 (2011). [5] Benz, F. et al. Single-molecule optomechanics in "picocavities". Science 354, 726–729 (2016).

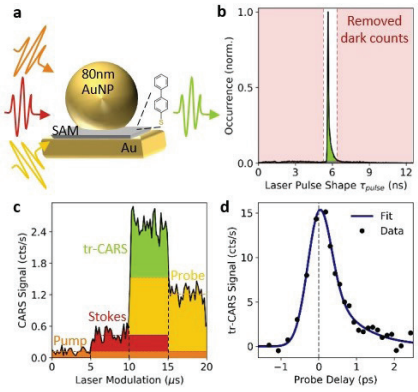

Figure 1. (a) tr-CARS with molecules in a plasmonic nanocavity. (b) Pulse shape of the 80 MHz laser. (c) Laser modulation (50 kHz). (d) Scan of probe beam delay time measuring the vibrational lifetime.

Effects of circular polarization of the excitation light on Raman spectra of diesel

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Polarization, Fluorescence, Diesel., Circular polarization, Linear polarization

The Raman Spectroscopy analysis is based on inciding a laser beam of a certain frequency (which can vary from ultra-violet to near-infrared) on the sample whose characteristics are to be analyzed [1]. The incident light used is commonly linearly polarized [2][3][4]. In this work, excitation light beams with linear polarization (LP) and circular polarization (CP) are used in a NIR (near infrared) Raman system to study their effects on diesel spectrum. The ratio of spectrum intensities varies according to the state of polarization used. The principal changes in the spectrum are presented as a reduction of fluorescence from the 300 cm-1 to 1300 cm-1 and an increase in peak intensities around 1600 cm-1. These characteristics differ from those already reported in the specialized literature, so this new methodology will be of great help for future analyses of liquid samples.

The authors would like to thank CONACyT for the support provided through a postgraduate scholarship.

[1] J.R. Ferraro, K. Nakamoto, Introductory Raman Spectroscopy., Elsevier Science, 1994. [2] S. Crankshaw, M. Moewe, L.C. Chuang, R. Chen, C. Chang-Hasnain, Polarized Raman modes of a single wurtzite GaAs needle, in: Opt. InfoBase Conf. Pap., 2009. https://doi.org/10.1364/cleo.2009.cthcc7. [3] T. Lee, J.H. Kim, Y.J. Choi, J.G. Park, H. Rho, Polarized Raman studies of single GaN nanowire and GaN/AlN hetero-nanowire structures, Thin Solid Films. 671 (2019) 147–151. https://doi.org/10.1016/j.tsf.2018.12.043. [4] G. Zhou, X. Gu, W. Xie, T. Gao, J. Peng, X.S. Wu, Polarized Raman Scattering Studies of Hexagonal YMnO3 Single Crystal, IEEE Trans. Magn. 51 (2015). https://doi.org/10.1109/TMAG.2015.2438154.

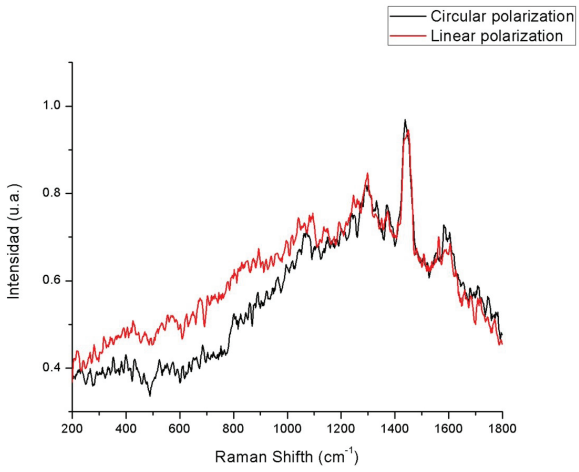

Figure 1. Raman spectra of diesel.

SRS Microscopy Optimization for Biomedical Purposes

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Stimulated Raman Scattering, Microscopy, Biomedical imaging

Nowadays, Stimulated Raman Scattering (SRS), under the name of SRS microscopy, is widely used in chemistry, biology, and biomedicine as a part of modern microscopic techniques. In contrast to Spontaneous Raman Scattering, SRS technique is based on the well-known phenomenon of stimulated emission, which is responsible for amplification of the Raman signal. It provides much better sensitivity and higher signal intensity, which translates into greater contrast of obtained images and significantly reduced data acquisition time.[1] The most popular homemade, as well as commercial SRS microscopy setups, are based on modern pulsed laser sources that offer two synchronized output beams, one tunable (Pump) and the second one at the fixed wavelength (Stokes). Both beams are temporally and spatially overlapped and send to the specimen. The wavelengths of both laser beams are selected so that the energy difference between them, related to the Raman shift, is equal to the energy of a particular molecular vibration of a studied sample.[2] One of the beams (Stokes in most cases) is put under the modulation using an acousto-optic modulator (AOM) or an electro-optic modulator (EOM) and a very weak SRS signal is retrieved with lock-in amplifier techniques. The sample is spatially scanned with overlapped beams (using two moving mirrors) or with a motorized stage without beam movement. Typically, especially in the case of biomedical purposes, inverted microscopes with two objective lenses are in use with SRS microscopy, where the first lens sends beams to the sample and the second one collects the light and sends it to the detector.[3] The second lens, which is used to gather the signal, is usually expensive and of very high quality. Moreover, the need to precisely modify the standard inverted microscope to assembly both lenses significantly complicates the construction of the system. This study is aimed to modify the signal collecting optics in a typical SRS microscopy system by removing the signal gathering objective lens and replacing it directly with the detector in a thoughtful manner. It is planned to investigate how such a modification of the system and simplification of its design will affect the quality and the speed of the SRS microscopy. The possibility of using the signal detector directly without the need to mount additional collecting optics would greatly reduce the complexity of the design of the system, at the same time making it more cost-effective and easier to use.

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[1] Zhang C, Zhang D, Cheng J-X. Coherent Raman Scattering Microscopy in Biology and Medicine. Annu. Rev. Biomed. Eng. 2015; 17:415–445 [2] Christian W. Freudiger, Wei Min, Brian G. Saar, Sijia Lu, Gary R. Holtom, Chengwei He, Jason C. Tsai, Jing X. Kang, X. Sunney Xie, Label-Free Biomedical Imaging with High Sensitivity by Stimulated Raman Scattering Microscopy. 2008; 322:1857-1861 [3] Liao CS, Cheng J-X. In Situ and In Vivo Molecular Analysis by Coherent Raman Scattering Microscopy. Annu. Rev. Anal. Chem. 2016; 9:69–93.

New analytical method that collaborates with Micro-FTIR and Micro-Raman

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FTIR, Raman, Microspectroscopy, Micro plastics

It is widely known that micro-FTIR and micro-Raman are effective tool for characterization of small samples such as foreign materials, multilayer film, micro plastic and so on. However combining these complementary methods is so important, it has been so difficult to align and measure the same position between the micro-FTIR and the micro-Raman. Therefore, we have developed a method for aligning the measurement positions of the two or more microscopes with high accuracy and a new analysis method for analysing both data on a common software. The 'IQ Frame' (Figure 1), which is a holder, developed here roughly moves the stage using the coordinate information stored in the measurement data, and then fine-tunes it using the visible observation image to obtain a perfectly matched position with the two microscopes. As a results, we can measure the same place with micrometer order of accuracy. We also developed functions such as superimposed chemical images of different analytical methods and particle size analysis by chemical image (Figure 2). Here, we report the proposal and applications of a new analytical method that combines micro-FTIR and micro-Raman using these methods

Instrumental Development
Ondax THz Raman probe. Flexible system for high sensitivity and high resolution Raman studies of low frequency vibrations.

Józef Dresner 1, Vitalii Ivanov 2

1Eurotek International, 2 Instytut Fizyki PAN

low-frequency, high-resolution, frequency stabilized laser, micro-Raman, THz Raman, Raman fingerprints

High resolution Raman spectroscopy, where frequencies well below 100 cm^{-1} can be observed becomes a standard tool in all fields of basic and industrial research. In our communication we demonstrate such high performance Raman system in a form of complete, integrated optical probe – Coherent Ondax THz Raman probe. The probe connects via FC/APC fibers with high power frequency stabilized laser and feeds the optical signal to virtually any spectrograph/ camera combination equipped with fiber input. The probe itself can be optically interfaced with free-space beam optics or with the microscope stand to form a true micro-Raman system. This ease of integration allows to add high resolution Raman functionality to other analytical and research equipment – luminescence, IR absorption or X-ray - for multi modal material characterization. In our presentation we show examples of THz probe applications. Using micro-Raman setup we show high sensitivity, spectral and spatial resolution in measurement of CdS nanocrystals, shown in Fig.1. below. Remarkably high S/N ratio is achieved in tens of ms exposure time. We show spectra of bulk material like sulfur showing fingerprints of < 10 cm^{-1}. The details of the experimental configurations will be given.

James Carriere and Matthias Schulze, "THz-Raman – A New Analytical Tool", PhotonicsViews 4/2020

Ondax, a Coherent, Inc. Company for providing THz Raman probe system KawaSka Sp. z o. o. for providing the DM2000 microscope

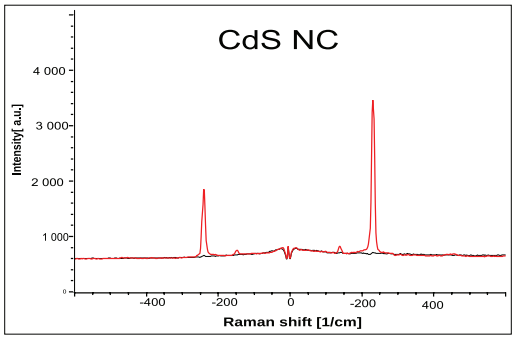

Fig. 1. Micro – Raman spectrum of CdS nanocrystals (NC) in Na-Li-B-Si glass. Approximate average NC concentration is 7 x 10^8 / cm^3. Laser wave-length: 808 nm.
(Red) – spectrum of NC captured by the laser spot. (Black) – laser spot moved ca 20 microns away.

1.8.
Sensing

Sensing
Empowering Surface-Enhanced IR Spectroscopy with Broadband Metasurfaces and Artificial Intelligence

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Nanophotonics, Metasurfaces, Plasmonics, Surface Enhanced Infrared Spectroscopy, Biosensors, Microfluidics, Lab-on-a-Chip, Artificial Intelligence

Metasurfaces have recently emerged as a breakthrough platform for manipulating light at the nanoscale with the use of engineered dielectric and plasmonic nanostructures. They can be tailored to operate over a broad spectrum ranging from terahertz to middle-infrared (Mid-IR) and ultraviolet. Mid-IR metasurfaces are particularly important for the wavelengths between 3 to 20 μm because this spectral range includes atmospheric windows and fundamental absorption bands of chemicals and biomolecules. Infrared (IR) absorption spectroscopy, which accesses these absorption bands is a powerful techniques as it enables label-free and chemically-specific biomolecule detection. Nevertheless, conventional IR spectroscopy suffers from several limitations such as low signal levels, difficulty to operate in aqueous environment and bulky and expensive instrumentation. In this talk I will present some of our recent work with plasmonic and dielectric Mid-IR metasurfaces for realizing novel Mid-IR biosensors through surface enhanced infrared absorption spectroscopy (SEIRA) and incorporation of Mid-IR dielectric metasurfaces with phase change materials for programmable phase control [1-4]. With metallic nanoantennas our lab has been developing ultrasensitive SEIRA based biosensors [5-10]. By integration of Mid-IR plasmonics with microfluidics our sensors can operate in aqueous environment for real-time monitoring of bimolecular interactions and conformational changes [7-10]. Most recently, we introduced a deep learning-augmented Mid-IR nanoplasmonic metasurface, which provides a universal platform to study dynamic biological processes involving analytes from all four major biomolecular classes simultaneously [1]. The optofluidic device incorporating multiresonant plasmonic metasurface and microfluidics enables the collection of a vast amount of spectrotemporal data, which is then used to construct a deep neural network for accurate analyte discrimination. The capabilities of the new method are demonstrated by monitoring dynamically of a multistep bioassay containing sucrose- and nucleotides-loaded liposomes interacting with a small, lipid membrane-perforating peptide. With low-loss dielectric metasurfaces, we harness their high-Q resonances for implementing compact Mid-IR biosensors. In one of the device schemes we utilized large-area imaging with a 2D array of pixelated high-Q metasurface to detect Mid-IR molecular fingerprints without the need for spectrometry and frequency scanning [2]. The broadband metasurface is designed to provide strong near-field enhancement, where the resonance positions of individual metapixels are linearly varied over a target Mid-IR range. With this one-to-one mapping between spectral and spatial information, enhanced absorption signatures of analytes are sampled at multiple spectral points and translated into a 2D barcode-like spatial absorption map for imaging-based detection. In another device scheme, we expanded the operational spectral range of dielectric metasurfaces for spectroscopy by using angle scanning and polarization degree of freedom together [3]. The system provided an impressively wide spectral coverage from 1100 cm-1 to 1800 cm-1 at 1.4 cm-1 spectral resolution.

[1] John-Herpin et al. Advanced Materials (2021) [2] Tittl et al. Science (2018) [3] Leitis et al. Science Advances (2019) [4] Leitis et al. Advanced Functional Materials (2020) [5] Adato et al. Proceedings of National Academy of Sciences (PNAS) Vol. 106, 19227 (2009) [6] Hui et al. Nature Materials Vol.11, pp. 69-75 (2012) [7] Adato and Altug. Nature Communications Vol.4, pp. 2154 (2013) [8] Limaj et al. Nano Letters Vol. 16, pp. 1502-1508 (2016) [9] Etezadi et al. Light Science and Applications Vol.6 e17029 (2017) [10] Etezadi et al. ACS Sensors (2018)

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Metal nanostructures for surface enhanced vibrational spectroscopy: from spheres to anisotropic nanoparticles

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metal nanoparticles, Surface enhanced Raman spectroscopy, Surface enhanced infrared absorption spectroscopy, gold nanospheres, gold nanostars, silver nanoflowers,hybrid substrate

The interest on surface enhanced vibrational spectroscopies (SEVS) such as surface enhanced Raman spectroscopy (SERS) and surface enhanced infrared absorption spectroscopy (SEIRAS) has increased in response to the challenges of single molecule analysis. To date, most SERS/SEIRA signal-enhancing materials are based on metallic nanostructures, i.e. especially noble metals with different geometries including nanospheres, and anisotropic and complex-shaped nanostructures such as nanostars, among others [1]. The materials domain has been gradually extended to transition and semiconductor materials including single-element semiconductors such as graphene. In this communication, different metal nanostructures for both SERS and SEIRAS are presented, ranging from spherical gold nanoparticles [2] to multi-branched gold nanostars [3], which provide a high density of so-called "hot spots" at the surface. A silver nanoflower-coated paper [4] is shown as dual substrate for both SERS and ambient pressure mass spectrometry analysis. In addition, hybrid substrates comprising metal and metal oxide nanostructures [5] are also discussed, which provide the substrate with photocatalytic activity.

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[1] López-Lorente, A.I. Anal. Chim. Acta 2021, 1168, 338474. [2] López-Lorente, A.I.; Wang, P.; Mizaikoff, B. Microchim. Acta 2017, 184, 453-462. [3] Schwenk, N.; Mizaikoff, B.; Cárdenas, S.; López-Lorente, A.I. Analyst 2018, 143, 5103-5111. [4] Díaz-Liñán, M.C.; García-Valverde, M.T.; López-Lorente, A.I.; Cárdenas, S.; Lucena, R. Anal. Bioanal. Chem. 2020, 412, 3547-3557. [5] López-Lorente, A.I.; Picca, R.A.; Izquierdo, J.; Kranz, C.; Mizaikoff, B.; Di Franco C.; Cárdenas, S.; Cioffi, N.; Palazzo, G.; Valentini, A. Microchim. Acta 2018, 185, 153.

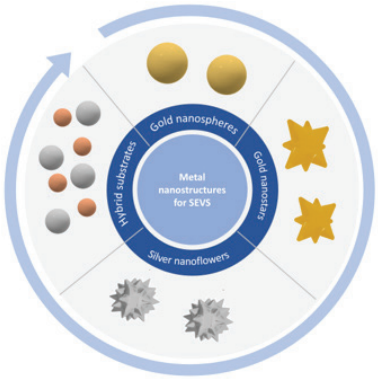

Figure 1. Examples of metal nanostructures with different geomerties used in SEVS, namely gold nanospheres, gold nanostars, silver nanoflowers and hybrid substrates.

Quantum dots for surface-enhanced infrared spectroscopy: an multivariate approach

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Quantum dots (QDs), SEIRA, Multivariate Analysis

Quantum dots (QDs) are semiconductor nanocrystals extensively applied as opto-electronic devices, sensors and, as fluorescent probes for (bio)molecular species. Copper and silver chalcogenides (sulphide, selenide and telluride) are examples of I-VI-based QDs, which have low toxicity, excellent colloidal stability and hold great promise for several applications such as photovoltaics, lighting and displays, and biomedical imaging. In this work, we investigated the potential suface-enhanced Infrared absorption (SEIRA) effect of two different I-VI-based QDs, Cu2-xSe and Ag2Se in aqueous suspensions amplifying the IR signature of a variety of dye molecules and introduced an approach based on principal component analysis (PCA) and Euclidian distances to determine a novel parameter termed 'multivariate enhancement factor' (MEF) [1], for calculating the enhancement effect across the entire IR spectrum rather than at a single wavelength. For this purpose, attenuated total reflection (ATR) of aqueous dye vs. dye/QD solutions spectra were recorded in the spectral range of 4000-600 cm-1 until total evaporation of the liquid at an Si ATR waveguide surface. Principal Component Analysis (PCA) studies evidenced how the enhancement effect by Ag2Se–MSA QDs may indeed occur, and corroborates that chemometrics tools not only aid in data analysis, but may also improve the understanding and knowledge on SEIRA systems. In addition, results evidenced that MEF may be more suitable for multivariate studies performed using design of experiments (DoE) to optimize the experimental conditions for SEIRA studies.

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[1] C. F. Pereira, I. M. A. Viegas, I. Souza Sobrinha, G. Pereira, G. A. de L. Pereira, P. Krebs and B. Mizaikoff, J. Mater. Chem. C, , DOI:10.1039/d0tc02653k

Mid-Infrared Arthroscopy: The Road to Real-Time In Vivo Cartilage Condition Assessment

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mid-infrared sensor technology, cascade laser spectroscopy, mid-infrared arthroscopy, cartilage condition assessment, in vivo IR sensors

The recent technological advances in mid-infrared (3-15 µm; MIR) laser technology and spectroscopy, and especially cascade laser spectroscopy (CSL) based on quantum and interband cascade devices has evolved into a state-of-the-art tool for the selective and sensitive quantification of trace analytes in liquid, solid, and gaseous state in a wide variety of sensing scenarios. High output power, narrow linewidths, single-mode operation, low power consumption, broad tunability and compact dimensions are just some of the most outstanding features of such light sources.Since their introduction in the mid 1990ies, quantum cascade lasers (QCL) and interband cascade lasers (ICL) have rapidly matured into the probably most relevant contemporary MIR laser light sources. In this presentation, we will discuss state-of-the-art sensing platforms that benefit from cascade lasers combined with miniaturized photonic platforms providing direct access to molecule-specific information. Indeed, point-of-care diagnostics in clinical settings are becoming more prevalent taking advantage of the fact that MIR detection schemes do not require reagents or labeled constituents, and therefore facilitate localized on-site analysis close to real-time.With increasing progress towards highly miniaturized MIR optical components, evidently devices useful in medical/clinical applications may directly capitalize on advantageous features of integrated MIR photonics. For sure, in-vivo applications enabling direct sensing and/or condition monitoring within living organisms are considered the 'holy grail'. We will highlight the utility of MIR catheter technology for analyzing cartilage damage in-vivo during arthroscopic surgery, and discuss potential and challenges when translating this technology into clinical practice.

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[1] D. Warnecke et al., Degeneration Alters the Biomechanical Properties and Structural Composition of Meniscal Tissue, Osteoarthritis and Cartilage 28, 1482-1491, 2020. [2] I. Vasilikos et al., Infrared Attenuated Total Reflection Spectroscopic Surface Analysis of Bovine-tail Intervertebral Discs after UV-light-activated Riboflavin-induced Collagen Crosslinking, Journal of Biophotonics 13, e202000110: 1-7, 2020. [3] Á. I. López-Lorente et al., Surface Analysis of Sheep Menisci after Meniscectomy via Infrared Attenuated Total Reflection Spectroscopy, Journal of Biophotonics 8, 1-10, 2019. [4] J. Haas et al., iBEAM: Substrate-Integrated Hollow Waveguides for Efficient Laser Beam Combining, Optics Express 27, 23059-2306, 2019. [5] P. Wang et al., Analysis of Human Menisci Degeneration via Infrared Attenuated Total Reflection Spectroscopy, Analyst 143, 5112-5119, 2018.

Searching for the chemically stable reporter for SERS-based pH nanosensor – from rhodanine to 5-(4-dimethylaminobenzylidene)rhodanine to avoid plasmon-induced dimerization

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SERS nanosenors, pH sensing, Raman reporter stability, plasmon-induced surface reaction, colloidal Ag nanoparticles

Molecular specificity and extraordinary sensitivity offered by surface-enhanced Raman scattering (SERS) spectroscopy opened the route to expand its applications to quantification of "Raman silent" species. Such detection is achieved using SERS-active nanoparticles modified with Raman reporter molecule which changes its structure and/or surface orientation upon the interaction with a small molecule or ion, providing recognizable and concentration-dependent changes in the SERS signal of the reporter. Within this frame, SERS-based nanosensors were developed for localized pH measurement. Nevertheless, to further improve their performance, key issues concerning the stability of both plasmonic component (nanoparticle aggregation [1]) and Raman reporter (chemical transformation [2]), considerably affecting the reliability of the pH measurements, need to be addressed. Here, we focus on the chemical stability of the Raman reporter. Our initial choice was the system comprising Ag nanoparticles (AgNPs) functionalized with rhodanine (2-thioxo-4-thiazolidinone; Rh), whose different tautomeric forms, dependent on the pH of the surroundings, were identified with SERS spectroscopy [3]. However, we detected temporal changes of the SERS signal for Rh attached to AgNPs, which we attribute to the plasmon-induced dimerization. Interestingly, we observed the SERS signature of the product of Knoevenagel condensation of Rh even for the 10-5 M concentration, which lies in a range for which the surface dimerization was previously reported to be less feasible [4]. Moreover, a plasmon-assisted transformation of Rh obscures the pH response of the system, as the formation of the dimer was favored under acidic conditions, while alkaline pH triggered instability of the dimer. However, the use of 5-(4-dimethylaminobenzylidene)rhodanine (DBRh) as Raman reporter, with the position for condensation of two Rh molecules already blocked, provided pH-sensitive but stable (under given pH conditions) SERS response for AgNPs based nanosensors. Density functional theory (DFT) calculations were employed to assist the interpretation of the experimental SERS spectrum. They suggested the pH-dependent strong electronic conjugations within the DBRh molecule. We demonstrated that nanosensors developed here with the DBRh reporter are capable of monitoring pH in a broad range (2.0-8.0), producing robust SERS response. Our results for Rh molecules emphasize how important is verification of temporal evolution of SERS signal to avoid a false readout of local pH, due to the light-driven surface reactions.

Agata Królikowska, grant "Iuventus Plus" no. IP2011 027371, (Polish Ministry of Science and Higher Education)

[1] X.S. Zheng, C. Zong C, X. Wang, B. Ren, "Cell-penetrating peptide conjugated SERS nanosensor for in situ intracellular pH imaging of single living cells during cell cycle", Anal Chem. 2019; 91, 8383 [2] A. Michota, J. Bukowska, „Surface-enhanced Raman scattering (SERS) of 4?mercaptobenzoic acid on silver and gold substrates", J. Raman Spectrosc., 2003, 34, 21 [3] K. M. Marzec, B. Gawel, W. Lasocha, L. M. Proniewicz, K. Malek, „Interaction between rhodanine and silver species on a nanocolloidal surface and in the solid state," J. Raman Spectrosc., 2010, 41, 543 [4] N. Kumar, S. Thomas, R. Rao, N. Maiti, R. J. Kshirsagar, "Plasmon-Induced Dimerization of Thiazolidine-2,4-dione on Silver Nanoparticles: Revealed by Surface-Enhanced Raman Scattering Study", J. Phys. Chem. A, 2019, 123, 9770

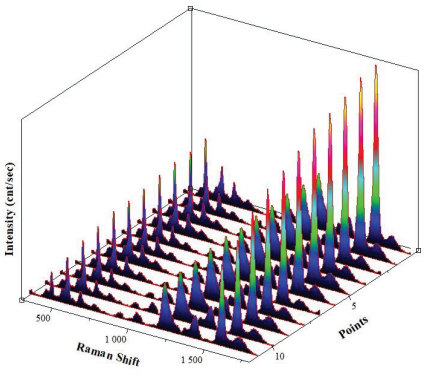

Figure 1. Temporal evolution of SERS signal for 10-5 M Rh adsorbed on AgNPs at pH=5.82 (532 nm excitation line, spectra collected each minute after initial adsorption)

Multispectral fiber sensing for biomedical applications

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Multispectral, Fiber-optics, Spectroscopy, Biomedical, Sensing

Optical spectroscopy is a powerful analytical technique that enables to investigate sample properties at the molecular level in real-time. Its ability of label-free composition analysis makes spectroscopy the best tool for rapid chemical and structural analysis of bio tissues for medical applications in vivo. Biological samples are very complex substances which composition analysis requires to combine several spectroscopic techniques. Fiber-optics and probes on its basis provide compact, flexible, and cost-effective solution to merge different optical modalities in one tool. Multispectral fiber probes enable to use several spectroscopy methods for tissue analysis at the same point. This spatial synchronization of the analysis is critical for heterogeneous biotissue samples. Recent advances in optical fiber manufacturing significantly expand the wavelength range of the analysis from 0,3-2µm range towards middle IR (chalcogenide and AgCl:AgBr Polycrystalline PIR fibers together cover the 1-16 µm range). Therefore, modern fiber spectroscopy seamlessly covers entire wavelength range UV-Vis-MIR to use for biomedical diagnostics. Here we would like to present the latest results on the development of multispectral fiber sensing probes for biomedical applications. We were able to fuse with fiber-optics all 4 key spectroscopic methods (Fluorescence, NIR, MIR, and Raman) in one compact fiber probe. Optical diagnostics of the clinical samples in preliminary studies has shown that a combination of fluorescence with diffuse reflectance NIR or attenuated total reflectance (ATR) IR spectroscopy results in much better accuracy of the tumor margin detection than each of the individual methods separately. This synergy is explained by the capability of different light modalities to deliver complementary chemical information. Also, superficial ATR analysis act at a single-cell level because of the few micrometers penetration depth, while UV-Vis fluorescence and NIR light can penetrate up to several millimeters. Raman fiber-optic spectroscopy enables the combination of this technique with fluorescence spectroscopy from the definition because both are excited by the laser light. Instead of just subtracting the fluorescence background from the Raman spectra we are using its information to enhance the sensitivity and specificity of the analysis. This concept, combined with advanced chemometrics analysis, enables the development of the customized spectral fiber sensors based only on several wavelengths – leading to their simple design, small size, high speed and cost savings.

[1] Bogomolov et al., Synergy Effect of Combining Fluorescence and Mid Infrared Fiber Spectroscopy for Kidney Tumor Diagnostics, Sensors, 17, 2548, 2017; Hocotz et al., Synergy Effect of Combined Near and Mid-Infrared Fibre Spectroscopy for Diagnostics of Abdominal Cancer, Sensors, 20, 6706, 2020; Bogomolov et al., Fiber Probe for Simultaneous Mid-Infrared and Fluorescence Spectroscopic Analysis, Anal. Chem., 93, 6013, 2021.

Occlusion induced bonding state of water molecules decrease in the uppermost stratum corneum determined by in vivo confocal Raman microspectroscopy

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confocal Raman microspectroscopy, stratum corneum, occlusion, skin barrier function, in vivo

Occlusion of the skin with cosmetic formulations is always associated with swelling of the stratum corneum, i.e. with an increase of the stratum corneum thickness due to water accumulation, which is manifested only at intermediate stratum corneum depths [1]. Using in vivo confocal Raman microspectroscopy, we have demonstrated an additional effect of skin occlusion – transformation of water molecules towards a lower bonding state in the uppermost stratum corneum depths [2]. We have analyzed the broad water-related Raman band at ?3000–3700 cm-1 after the skin treatment with cosmetic oils and calculated the depth profiles of water concentrations depending on the strength of their hydrogen bonds with the surrounding molecules in the stratum corneum. We found a significant increase of unbound and weakly bound water and a decrease of strongly bound water at the uppermost stratum corneum depths, although the overall water concentration only decreased slightly after oil treatment. This give rise to the considerable weakening of the hydrogen bonding state of water molecules in the uppermost stratum corneum depths. Taking obtained results into consideration, we assume that water transports intercellularly and intracellularly in the intermediate swelling region and only intercellularly in the uppermost non-swelling region of the stratum corneum. Some cosmetic oils, which do not induce any swelling effect and do not occlude from the conventional viewpoint, induce transformation of water molecules towards lower-bonding state in the uppermost stratum corneum depths and, thus, manifest occlusive properties.

[1] C.S. Choe, J. Schleusener, J. Lademann, M.E. Darvin. Keratin-water-NMF interaction as a three layer model in the human stratum corneum using in vivo confocal Raman microscopy. Scientific Reports. 7(1): 15900, 2017. [2] C.S. Choe, J. Schleusener, S.H. Choe, J.S. Ri, J. Lademann, M.E. Darvin. Stratum corneum occlusion induces water transformation towards lower bonding state: a molecular level in vivo study by Raman microscopy. International Journal of Cosmetic Science, 42: 482-493, 2020.

SERS immunoassays for detection of inflammation factors in biosamples

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immunoassay, immunoSERS microscopy, sandwich-type sensors, inflammatory markers

Immunoassay is one of the most common method for the analysis of biochemical targets such as specific proteins, nuclei acids, hormones or drugs. It has been routinely employed in many areas of clinical diagnostics and life-science research. Surface enhanced Raman spectroscopy (SERS) is increasingly considered as an ultrasensitive and rapid assay readout in the immunoassay techniques. The combination of SERS with immunological methods based on specific reaction between antigen and its antibody gave a new research tool for protein detection even at ultra-low concentrations. The aim of our work was to construct two type of SERS sensor for the sensitive detection of inflammation markers in tissues and body fluids. Immuno-SERS (iSERS) microscopy overcomes limitations of fluorescent labels since generated SERS signals of Raman reporters are highly photostable and offer simultaneous quantification of few biomarkers due to a small width of Raman bands. In our work we demonstrate the application of this new method for the simultaneously localization of two antigen present in mice artery- smooth muscle cells (SMCs) and platelet endothelial cell adhesion molecule (PECAM), which play an important role in the development of atherosclerosis plaque. Indirect iSERS staining yields results comparable to IF staining, demonstrating possible employment of iSERS in research carried on tissues and paving the way for future multiplexed imaging experiments. Another essential direction of our work was to design a diagnostic test for the quantitative detection of inflammatory mediators in biofluids. The use of two metallic substrates and two Raman reporters in a sandwich-type sensor enabled a double gain potential with the signal readout excluding false outcomes. In this design, we included various metal-metal interactions (Ag-Ag, Ag-Au, Au-Au) as well as the method of antibody binding to the capture substrate. The best analytical parameters were obtained for coupling hexagonal gold solid substrates with the gold nanospheres.;

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Detection of the molecular alterations of hemoglobin structures inside living red blood cells and isolated proteins

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Resonance Raman spectroscopy, Hemoglobin, Red blood cells, Isolated proteins

Hemoglobin (Hb) is a key heme protein found in red blood cells (RBCs) responsible for oxygen transport and maintenance of acid-base balance.[1] Majority of Hb-related studies are carried out on isolated proteins dispersed in buffer solution. Meanwhile, the Hb adducts encapsulated inside RBCs and the free Hb molecules exhibit biologically relevant differences, such as changes in activity or ligand affinity.[2,3] Resonance Raman (RR) spectroscopy is an extremely sensitive tool to detect alterations in living RBCs even on a single cell level and within Hb' heme active site.[4,5] Herein, we employed UV–Vis absorption and RR spectroscopies for the comprehensive comparison of the heme active site properties of various Hb adducts formed inside living RBCs and prepared as isolated proteins.[6,7] We characterize the basic Hb adducts including oxyhemoglobin (oxyHb), deoxyhemoglobin (deoxyHb), methemoglobin (metHb), as well as HbCN and its isotopic analogues $Hb^{13}CN$, $HbC^{15}N$ and $Hb^{13}C^{15}N$. Presented here results reveal slight variations in the geometry of porphyrin vinyl groups between free and encapsulated Hbs. The spectra of deoxyHb and metHb enclosed in RBCs showed that the vinyl groups adopt more out-of-plane conformation while in oxyHb more in-plane configurations, as compared to the isolated proteins. The modes associated with the Fe-O-O fragment remained unchanged between the free and encapsulated proteins. In case of HbCN adducts, experiments with isotopic analogues confirmed the same spectral pattern of the ν(Fe-CN) modes in both, the isolated and the RBC encapsulated Hb molecules. However, small differences in wavenumbers of δ (Fe–CN) modes indicate variation in the degree of distortion of the Fe-C≡N fragment. In summary, RR studies proved to be useful in detecting even subtle molecular alterations and provided insight into the properties of Hb adducts. Altogether, these studies highlight importance of isolated protein based approaches and their translatability into functional living cells.

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1. M. L. Kenneth Kaushansky, M. M. L. Josef Prchal, L. B. Oliver Press and M. Caligiuri, Williams [1] Hematology, McGraw-Hill Education, New York, 9th edn., 2015. [2] V. Jeney, G. Balla and J. Balla, Front. Physiol., 2014, 5, 379. [3] D. B. Kim-Shapiro, A. N. Schechter and M. T. Gladwin, Arterioscler. Thromb. Vasc. Biol., 2006, 26, 697–705. [4] J. Dybas, P. Berkowicz, B. Proniewski, K. Dziedzic-Kocurek, J. Stanek, M. Baranska, S. Chlopicki and K. M. Marzec, Analyst, 2018, 143, 4335–4346. [5] K. M. Marzec, J. Dybas, S. Chlopicki and M. Baranska, J. Phys. Chem. B, 2016, 120, 12249–12260. [6] J. Dybas, T. Chiura, K. M. Marzec and P. J. Mak, J. Phys. Chem. B, 2021, 125, 3556–3565. [7] J. Dybas, M. J. Bokamper, K. M. Marzec and P. J. Mak, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 2020, 239, 118530.

SERS switch on/off mediated by adions

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SERS-active sites, chloride activation, adion-specific adsorption

The selective SERS detection of molecular components in complex matrices such as biofluids is currently a challenging task and needs a better understanding of molecular interactions at the metal interface. In this consideration, we showed recently an approach for the selective SERS detection of cationic and anionic species from their mixture. Our results indicate that the chemisorption to the metal surface is mediated by adsorbed ions (adions) such as Cl^-, Br^-, I^- and Ag^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Al^{3+} for cationic and anionic species, respectively [1-3]. Consider a very simple example: 10^{-8} M Nile Blue (NB) cationic dye in a solution of citrate capped silver nanoparticles (cit-AgNPs), showing a blank spectrum (Figure 1). The addition of 0.1 mM $Ca(NO_3)_2$ leads to the appearance of the SERS spectrum of citrate surfactant. Then, the addition of 1 mM Cl^- turns off the spectrum of citrate, and instead the SERS spectrum of NB is switched-on from this mixture [1]. According to the proposed adion-specific adsorption model [3], Ca^{2+} adions mediate the chemisorption of anionic species (i.e. citrate), whereas Cl^- adions facilitate the chemisorption of cationic molecules (i.e. NB). In order to exclude the aggregation of colloidal cit-AgNPs as the reason for the selective SERS switch-on of anionic and cationic analytes, we analyzed the SERS spectrum of a salicylic acid/Cresyl Violet mixture on two solid substrates. We prepared a solid substrate by first mixing the AgNPs with Ca^{2+}. A second substrate was obtained by activating the AgNPs with Cl^-, before drop-casting them. Notably, by adding a drop of anionic/cationic analyte mixture on each substrate, the Ca^{2+} activated substrate only turned-on the SERS spectrum of salicylic acid, whereas the Cl^- activated substrate showed the SERS spectrum of only Cresyl Violet. To highlight potential applications of this SERS switch on/off approach, we analyzed urine samples from patients with microalbuminuria. We show that by activating the AgNPs with Ca^{2+} adions, we are able to detect anionic purine metabolites, whereas the addition of I^- ions leads to the turn-off of purine metabolites spectrum and instead SERS bands of albumin at µg/ml concentration are observed. [4] We hope that the proposed adion-specific adsorption model, after validation in other laboratories, will contribute to better predictability in SERS analysis of complex matrices.

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[1] A. Stefancu, S.D. Iancu, V. Moisoiu, N. Leopold, Specific and selective SERS active sites generation on silver nanoparticles by cationic and anionic adatoms, Romanian Reports in Physics, 70 (2018). [2] N. Leopold, A. Stefancu, K. Herman, I.S. Tódor, S.D. Iancu, V. Moisoiu, L.F. Leopold, The role of adatoms in chloride-activated colloidal silver nanoparticles for surface-enhanced Raman scattering enhancement, Beilstein Journal of Nanotechnology, 9 (2018) 2236-2247. [3] S.D. Iancu, A. Stefancu, V. Moisoiu, L.F. Leopold, N. Leopold, The role of Ag+, Ca2+, Pb2+ and Al3+ adions in the SERS turn-on effect of anionic analytes, Beilstein Journal of Nanotechnology, 10 (2019) 2338-2345. [4] A. Stefancu, V. Moisoiu, C. Bocsa, Z. Bálint, D.T. Cosma, I.A. Veresiu, V. Chiş, N. Leopold, F. Elec, SERS-based quantification of albuminuria in the normal-to-mildly increased range, Analyst, 143 (2018) 5372-5379.

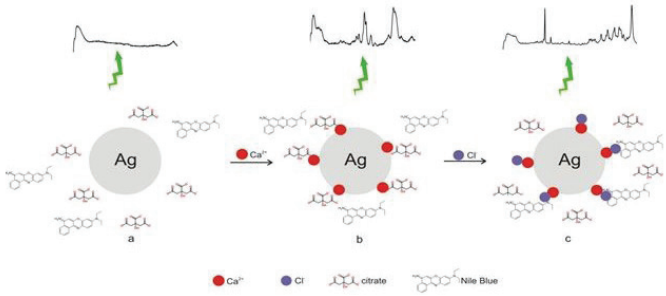

Figure 1. Sequential SERS detection of citrate and Nile Blue by activating the AgNPs with Ca2+ and Cl- adions [1].

Raman reporters as a tool to evaluate promyeloblastic cells differentiation stage- optimization of imaging conditions.

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cell silent region, Raman reporters, myeloid differentiation

Biochemical processes, also associated with pathology development, are recognized to be associated with cellular organelles dysfunctions. For many years, studies on the single-organelle level were possible using fluorescence probes consisting of targeting moiety and reporting part that allows for signal detection. However, certain disadvantages, such as dyes photobleaching, cytotoxicity, or broad emission bands, triggered the development of organelle-specific probes for different spectroscopic techniques. Here, we focus on Raman spectroscopy that offers narrow spectral bands, sensitivity, and a possibility to maintain physiological conditions during measurements. Of particular interest is the spectroscopic region 1800-2800 cm^{-1}, which displays no cell-specific vibrational bands, in opposition to deuterated or triple bond-containing compounds. Application of appropriately designed probes with intense bands in the cell silent region prevents possible overlapping of Raman signals and give better image contrast. The literature describes novel so-called Raman reporters based on triphenylphosphonium targeting moiety, called MitoBady (Fig. 1A) for mitochondria detection, thymine analogues 5-Ethynyl-2'-deoxyuridine (EdU) to study DNA replication and deuterated amino acids, fatty acids, etc.[1] However, every external compound introduced to the cellular system might affect its function, thus their cytotoxicity effect on cell homeostasis and appropriate Raman imaging conditions need to be assessed. The relevance of this approach could be additionally enhanced by the rapid development of non-linear Raman-based techniques, that enables for a significant increase of signal intensity with simultaneously reduced integration time. High sensitivity, in combination with the specificity of Raman probes might provide powerful diagnostic method for the early detection of multiple diseases. Lymphocytes and myeloid lineages, originated from hematopoietic stem cells, are produced in bone marrow and developed towards well-functioning blood cells under specific conditions. In these studies, in vitro model of maturation towards neutrophil like cells was investigated using human leukemia cell line Hl-60. Optimization of Raman imaging condition using MitoBady (Fig. 1B) and EdU allowed following changes associated with mitochondria and nucleus by detecting Raman signal of a living cell at 2120 and 2220 cm^{-1}. The ultimate goal was to use Raman reporters to identify granulocytic differentiation pathologies during acute promyelocytic leukemia development.

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[1] Adamczyk, A.; Matuszyk, E.; Radwan, B.; Rocchetti, S.; Chlopicki, S.; Baranska, M. Toward Raman Subcellular Imaging of Endothelial Dysfunction. J. Med. Chem. 2021. https://doi.org/10.1021/acs.jmedchem.1c00051.

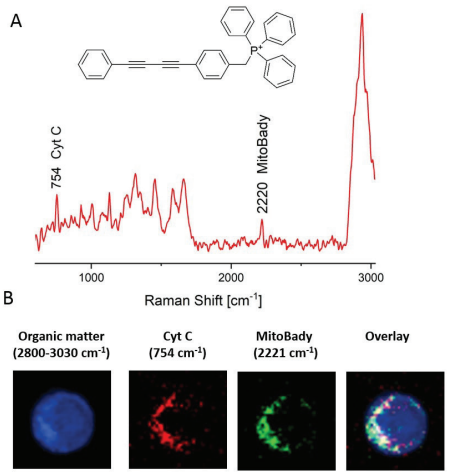

Figure 1. A- MitoBady structure and Raman spectrum of HL-60 cells mitochondria B- Distribution image of organic matter, cytochrome C, Mitbady, and images overlay.

Optimisation of Spectral Pre-processing using High-performance Computing for Disease Detection

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High performance computing, Machine learning, Raman Spectroscopy, Pre-processing, Disease Diagnostics

Pre-processing of biological spectroscopic data is an essential step within data analysis, allowing the removal of undesirable spectral contributions that could mask biological information. This is crucial to allow further analysis via techniques such as machine learning. However, due to the specificity of pre-processing to the sample type and the vast number of potential combinations of pre-processing steps, optimisation of pre-processing via a manual 'trial and error' format is time intensive. Here we present an application of high-performance computing (HPC) for optimisation of data pre-processing with direct improvements on previously achieved results.HPC has been employed to test over 2.4 million pre-processing permutations, evaluated on their ability to detect colorectal cancer (CRC) via machine learning. Multiple variables within the pre-processing procedure are investigated including the effect of varying the pre-processing step order, application of extended multiplicative scatter correction (EMSC), binning, smoothing, baseline correction methods (e.g. rolling circle filter and polynomial) and different normalisation methods. The effect of tuning parameters within each step was also investigated.Optimising via HPC enables improved performance in diagnostic abilities, with sensitivity increasing by 14.6%, specificity increasing by 6.9%, positive predictive value (PPV) increasing by 3.4%, and negative predictive value increasing by 2.4% when compared to a standard pre-processing optimisation.This study demonstrates how HPC can be used to improve classifier performance for CRC detection, and further utilisation for rapid optimisation in data analysis.

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An Innovative Attenuated Total Reflection Sensor Concept for Cascade Laser Infrared Spectroscopy

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IR-ATR Spectroscopy, (Quantum) Cascade Laser, Waveguides, Chemical Sensing

Mid-infrared (MIR) sensors based on attenuated total reflection (ATR) spectroscopy provide robust and sensitive chemical sensing platforms for studying a wide range of solid and liquid compounds in a variety of different scenarios, e.g., environmental monitoring. Combining the advantages of MIR sensors with laser spectroscopy facilitates portable field-deployable measurement systems that lend themselves to miniaturization. If sufficiently robust, such sensing devices may be operated at real-world conditions without demanding for complex and time-consuming sampling, transport, storage, and sample preparation procedures. Herein, we present an innovative IR-ATR sensing concept based on the novel and exceedingly robust combination of a hollow waveguide-based direct optical coupling concept with an ATR waveguide serving as the evanescent field transducer. The benefits of this coupling concept are a permanent optical alignment via substrate-integrated hollow waveguide (iHWG) 1–3 light guiding structures, the innerness against shock and vibration, and the temperature stability. These characteristics and the versatility of the optical concept render this approach suitable for a wide range of in-field sensing scenarios including also clinical monitoring settings. As a functional proof-of-principal for the developed advanced IR-ATR sensor design, a comparison with conventional horizontal ATR assemblies will be discussed along with the investigation of several exemplary liquid phase samples.

[1] Wilk, A. et al. Substrate-integrated hollow waveguides: A new level of integration in mid-infrared gas sensing. Anal. Chem. 85, 11205–11210 (2013). [2] Tütüncü, E., Nägele, M., Fuchs, P., Fischer, M. & Mizaikoff, B. IHWG-ICL: Methane Sensing with Substrate-Integrated Hollow Waveguides Directly Coupled to Interband Cascade Lasers. ACS Sensors 1, 847–851 (2016). [3] José Gomes Da Silva, I. et al. Sensing hydrocarbons with interband cascade lasers and substrate-integrated hollow waveguides. Analyst 141, 4432–4437 (2016).

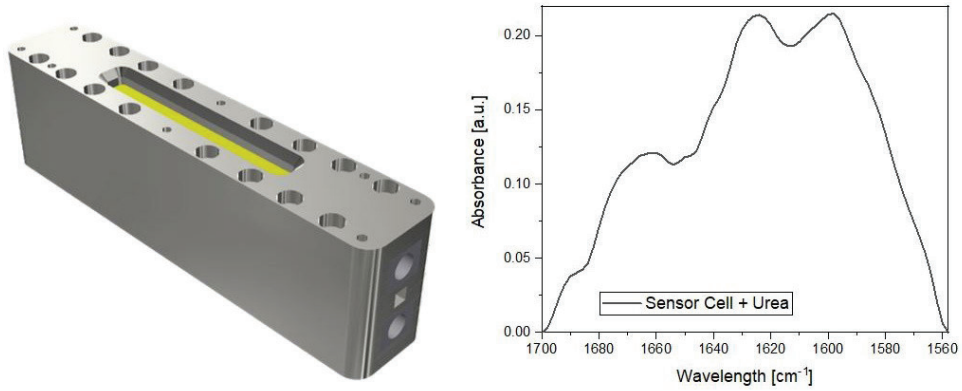

Figure 1. Developed novel sensor cell, schematically depicted as a 3D model and a corresponding IR spectra.

Development of a micromolar sensitivity dipstick mid-IR ATR sensor for phosphate in water

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Liquid sensing, Mid-IR spectroscopy, Mesoporous materials, Functional coating, Phosphate

Phosphate contamination due to agricultural runoff and urban wastewater is one of the main causes of eutrophication and thus loss of water quality. To combat eutrophication caused by the accumulation of phosphates, it is critical to monitor trace amounts of phosphate in water. While conventional mid-infrared (MIR) spectroscopy, is able to detect phosphate in a complex matrix due to its chemical selectivity, its sensitivity is limited due to the high absorption of water bands overlapping with phosphate spectral features. Thus, until now, no IR sensing scheme has been able to measure trace amounts in the low mg/L (µM) range. To remove the water interference, we use mesoporous materials for the preconcentration of analytes in the probed area of an attenuated total reflection (ATR) crystal. This sensing scheme has already been employed to detect small concentrations of organic contaminants in water [1]. For phosphate detection, we apply mesoporous titania. Titania has a high chemical resistivity and a low cut-off wavenumber at 850 cm-1. Here, we exploit the high affinity of titania towards phosphate, which typically forms bidentate surface complexes or electrostatically adsorbed phosphate species[2]. In this work, we demonstrate a phosphate sensor comprising of a Ge ATR unit (45° incidence angle). It is passivated with a 20 nm layer of sputtered TiO2 and coated with a 250 nm layer of ordered mesoporous titania with a pore size (approximated by the d-spacing) of 3.5 nm for the quantification of trace amounts of phosphate (monobasic phosphate) down to micromolar concentrations. The ordered mesoporous titania layer was prepared by a simple, reproducible sol-gel soft templating process adapted from Faustini et al.[3]. The high reproducibility of the mesoporous layer was assessed by using SA-PXRD and GI-SAXS to confirm the ordered mesoporous structure, and profilometry to determine the layer thickness. Calibration of the phosphate sensor was performed using a concentration series of aqueous solutions of monobasic phosphate. Solutions were applied in stepped concentration sequences due to the single use nature of the titania enrichment layer. The baseline corrected absorption spectra were then integrated between 1200 and 1000 cm-1 and the band areas fitted with Langmuir adsorption functions that serve as calibration function. Exceptionally high sensitivity especially below 50 mg/L was observed and a low limit of detection of 0.1 mg/L (determined as 3x standard deviation) was determined.

H2020 project Hydroptics (No.871529) IMEC for providing Ge samples

[1] Baumgartner, B., et al., In Situ IR Spectroscopy of Mesoporous Silica Films for Monitoring Adsorption Processes and Trace Analysis. ACS Applied Nano Materials, 2018. 1(12): p. 7083-7091. [2] Gong, W., A real time in situ ATR-FTIR spectroscopic study of linear phosphate adsorption on titania surfaces. International Journal of Mineral Processing, 2001. 63(3): p. 147-165. [3] Faustini, M., et al., Ultraporous nanocrystalline TiO2-based films: synthesis, patterning and application as anti-reflective, self-cleaning, superhydrophilic coatings. Nanoscale, 2015. 7(46): p. 19419-25.

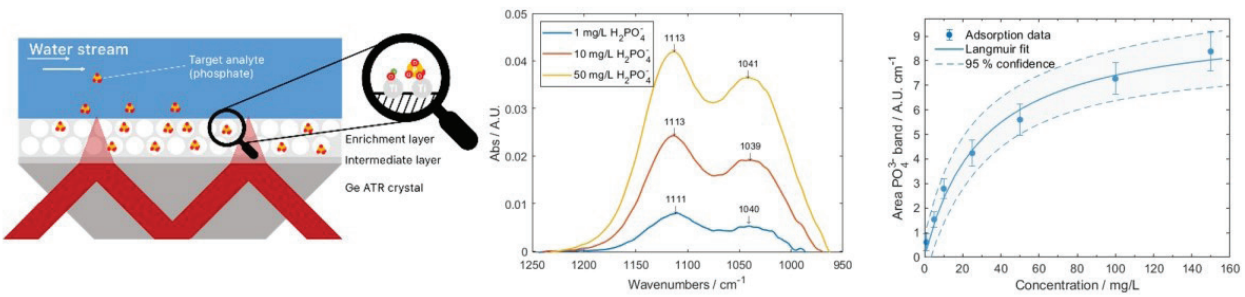

Figure 1. Left: Schematic mechanism of the phosphate enrichment using a mesoporous titania layer. Middle: ATR-IR spectra of enriched phosphates with different concentrations. Right: Adsorption profile of the

A Mesoporous Zirconia Coating for Sensing Applications using ATR-FTIR Spectroscopy

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Mid-IR spectroscopy, Mesoporous materials, Sensing, Enrichment layer, Functional coating

Mid-infrared attenuated total reflection (ATR) spectroscopy is a powerful tool for in situ monitoring of various processes. Mesoporous silica is an extensively studied material and has already been applied in sensing schemes due to its high surface area and tunable surface chemistry1. However, the poor chemical stability in aqueous solutions at pH values higher than 8 as well as the strong absorption below 1250 cm^{-1} limits the range of applications. Therefore, a mesoporous zirconia coating on ATR crystals was developed to circumvent these problems. The synthesis, surface modification and characterization of ordered mesoporous zirconia films on Si-wafers and Si-ATR crystals are presented. Cubic films with a thickness of 237 nm were obtained. The surface of the zirconia coating was modified using trichloro(phenyl)silane and applied in sensing schemes using aromatic and aliphatic nitriles in aqueous solution as organic pollutants. X-ray diffraction (XRD) studies confirm a high thermal stability of the mesoporous structure up to 900°C and for 24 h at 500°C. The mesoporous zirconia coating shows a high chemical resistance when keeping it in alkaline solution with a pH-value of 12 for 72 h. The success of surface modification is confirmed by FTIR spectroscopy and contact angle measurements. Traces of benzonitrile and valeronitrile in water are used as model analytes to evaluate the enrichment performance of the film. The experimental results are fitted using Freundlich isotherms and enrichment factors of 162 and 26 are calculated for 10 mg L^{-1} benzonitrile and 25 mg L^{-1} valeronitrile in water, respectively. Limits of detection of 1 mg L^{-1} for benzonitrile and 11 mg L^{-1} for valeronitrile are obtained. After the enrichment the sensing layer was completely regenerated by flushing with water. The high thermal and chemical stability of this coating allows the application in fields other than sensing like catalysis with the possibility of in situ monitoring using FTIR spectroscopy.

H2020 project Hydroptics (No. 871529) for funding imec for providing Si crystals

[1] Baumgartner, B.; Hayden, J.; Schwaighofer, A.; Lendl, B., In Situ IR Spectroscopy of Mesoporous Silica Films for Monitoring Adsorption Processes and Trace Analysis. Acs Appl Nano Mater 2018, 1 (12), 7083-7091.

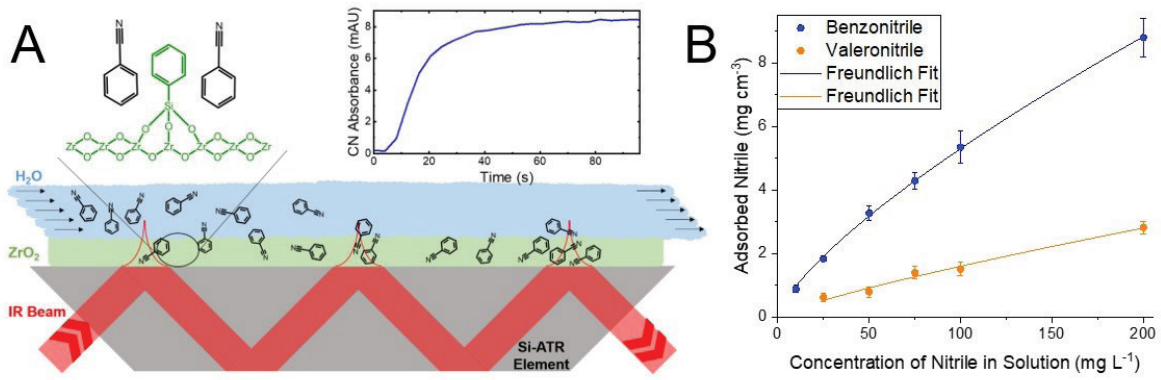

Figure 1. (A) Enrichment of an aqueous benzonitrile solution using a phenylsilylated ZrO2-coating on a Si-ATR element. (B) Freundlich adsorption isotherms for the enrichment of two analyte solutions.

Highly sensitive detection of cell endogenous hydrogen sulfide based on in-situ dynamic reaction of Ag NPs

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Hydrogen sulfide, In-situ dynamic reaction, Electrochemical solid-state phase transformation, Microelectrode, Surface enhanced Raman spectroscopy

The performance of nanoscale sensors can often provide insights into the extremely subtle differences between biomolecules. Meanwhile, several innovative signal transduction pathways of biosensing systems have attracted a lot of attention, but most of these are always driven by a single mechanism[1]. Herein, we demonstrate a biosensor for in situ quantification of active sulfide in the cell that utilizes the electrochemical solid-state phase transformation mechanism of the self-assembled silver nanoparticles[2], which is immobilized on the surface of the gold microelectrode through Raman signal molecules (4-aminothiophenol, 4-ATP). Taking the detection of cell endogenous hydrogen sulfide (H_2S) as an example, Ag NPs could first slowly react with local H_2S. Under the occurrence of the electrochemical oxidation reaction, a large number of silver ions (Ag+) are released to quickly react with H_2S. Subsequently, the execution of the reduction reaction brings the complex complexes to the electrode surface. The electrochemical solid-phase transfer mechanism has a fast reaction rate, and the shift of the redox peak of Ag NPs and the strength of the Raman spectrum of 4-ATP can quickly and accurately quantitatively analyze the cell endogenous H_2S with two channels, and the detection limit can reach nanomolar level. Going beyond conventional biosensing, the elaborate dynamic coupling of multiple mechanisms in a micro/nanoscale interface may open a new approach for future highly sensitive biosensing.

[1] H Zhou, G Ran, JF Masson, C Wang, Y Zhao, Q Song, Analytical Chemistry 2018, 90(5), 3374-3381 [2] H Zhou, R Yu, G Ran, S Moussa, Q Song, J Mauzeroll, JF Masson, Sensors and Actuators B: Chemical 2020, 319, 128315

SURFACE-ENHANCED RAMAN SPECTRAL DETECTION OF BILIRUBIN AND TEMPORALLY SYNCHRONIZED MONITORING OF ITS PHOTOCHEMICAL TRANSFORMATIONS BY SERS AND ELECTRONIC ABSORPTION SPECTROSCOPY

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Ag nanosponges, Bilirubin, Biliverdin, Photochemical transformations, SERS (Surface-enhancement Raman scatteting) spectroscopy

Surface-enhanced Raman scattering (SERS)-active systems [1] based on macroscopic Ag nanosponge aggregates as well as the conditions of SERS spectral measurements were optimized for selective and sensitive detection of a biomedically important, amphiphilic bile pigment [2] bilirubin (BR) in alkaline aqueous solutions and in its solutions in a selected water miscible solvent, namely dimethylsulfoxide (DMSO) and/or water-immiscible solvent, namely CH_2Cl_2. Ag nanosponges assembled from Ag nanoparticles (AgNPs) by using HCl as a pre-aggregation agent were found to be the optimal SERS-active systems for a reliable detection of BR in all the above-mentioned solvents [3]. The protonated form of adsorbed BR has been detected upon BR incorporation into; Ag nanosponges, and its marker bands have been established by SERS spectral probing at excitation wavelengthsin the 445-780 nm range. The sensitivity of SERS spectral detection was evaluated in terms of the concentration values of SERS spectral detection limits (SERS LODs) of BR incorporated into Ag nanosponges. The SERS LOD for BR incorporated from its alkaline aqueous solution is 1×10^{-8} M (at 532 nm excitation), for BR incorporated from its solution in DMSO, its value is also 1×10^{-8} M (at 532 and/or 633 nm excitations), and for incorporation from the solution of BR in CH_2Cl_2, its value is 1×10^{-7} M (at 780 nm excitation) and 1×10^{-6} M (at 532 nm excitation). The dianionic form of adsorbed; BR has been detected in Ag nanosponges into which BR has been incorporated from alkaline aqueous solutions using NaCl as the pre-aggregation agent with SERS LODs of 1×10^{-6} M (both at 532 nm excitation). Furthermore, the ex-situ SERS spectral monitoring based on incorporation of the reaction mixture components into Ag nanosponges and temporally synchronized with in-situ monitoring by electronic absorption spectroscopy has been employed for following the progress and outcome of daylight-induced photochemical transformations of BR in CH_2Cl_2 and in the alkaline aqueous solution. Biliverdin (BV) has been detected as the final product of the daylight induced photochemical transformation of BR in CH_2Cl_2 simultaneously by both methods. In contrast, while only some indices of BV formation during the more complex daylight-induced photochemical transformations of BR in the alkaline aqueous solution have been obtained by electronic absorption spectral measurements, BV has been clearly detected as an intermediate of this reaction by observation of SERS spectral marker bands of the protonated form of BV.

This work was supported by the Charles University Grant Agency (GAUK) 1100120.

[1] Procházka, M. Surface-Enhanced Raman Spectroscopy-Bioanalytical, Biomolecular and Medical Applications; Springer International Publishing: Switzerland, 2016. [2] Matouš, B. Základy Lékařské Chemie a Biochemie; Galén: Praha, 2010. [3] Sutrová, V.; Šloufová, I.; Nevoralová, M.; Vlčková, B. J. Raman Spectrosc. 2015, 46 (6), 559–565.

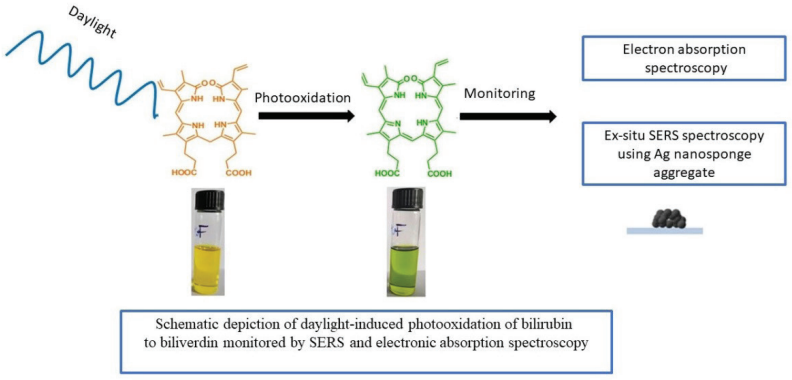

Figure 1. Scheme of the experiment

Development of sensor for lactate using SERS spectral changes triggered by enzyme reaction

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SERS, sensor for lactate, enzyme reaction, spectral change

Lactate is formed from the anaerobic respiration of the cell.[1] A high concentration of lactate in blood indicated extreme conditions such as extensive physical exercise, cancer and cardiogenic shocks.[1, 2] Thus, the development of a rapid, sensitive, and reliable sensor for lactate quantification is important for monitoring medical care. Surface-enhanced Raman scattering (SERS) is a versatile ananlycial method with many advantages such as high sensitivity, selectivity, and non-destructive.[3] Our group has previously reported that enzyme reactions affect changes of charge transfer and electron density of conjugated molecules and change SERS spectra.[4] By introducing this result, we can detect lactate with high sensitivity and high selectivity. In this study, SERS reporter (4-mercaptobenzoic acid, 4MBA)-functionalized silver nanoparticles combined with lactate oxidase were fabricated. This proposed SERS sensor was characterized by using UV-Vis spectrometer, zeta potential and dynamic light scattering. The SERS spectrum of 4MBA was changed with the lactate concentration, and the detection limit was 10 nM. Details on the result will be discussed in the presentation.

[1] L. Rassaei, W. Olthuis, S. Tsujimura, E.J. Sudhölter, A. van den Berg, Lactate biosensors: current status and outlook, Analytical and bioanalytical chemistry, 406 (2014) 123-137. [2] S. Azzouzi, L. Rotariu, A.M. Benito, W.K. Maser, M.B. Ali, C. Bala, A novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide for sensitive detection of L-lactate tumor biomarker, Biosensors and Bioelectronics, 69 (2015) 280-286. [3] C. Song, S. Guo, S. Jin, L. Chen, Y.M. Jung, Biomarkers Determination Based on Surface-Enhanced Raman Scattering, Chemosensors, 8 (2020) 118. [4] Z. Yu, L. Chen, Y. Park, Q. Cong, X. Han, B. Zhao, Y.M. Jung, The mechanism of an enzymatic reaction-induced SERS transformation for the study of enzyme–molecule interfacial interactions, Physical Chemistry Chemical Physics, 18 (2016) 31787-31795.

Advanced Mid-Infrared Light Sources Combined with Substrate-Integrated Hollow Waveguides for Respiratory Gas Analysis

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advanced MIR light sources, iHWG, respiratory gas analysis

Respiratory gas analysis has become an important field of research, as it has been shown that volatile organic compounds (VOCs) present in the human respiratory tract may serve as biomarkers for various diseases [1]. Exhaled breath analysis can therefore be used as a method of diagnosis or to monitor the progress of therapy, which is convenient for the patient as breath sampling is non-invasive and therefore not painful [2]. Currently, the most common techniques for the detection of VOCs in exhaled breath include gas chromatography coupled with mass spectrometry (GC-MS), selective ion flow tube mass spectrometry (SIFT-MS), and proton transfer reaction mass spectrometry (PTR-MS). These techniques are powerful, but require highly skilled operators, are time consuming and costly. As an alternative, infrared laser absorption spectroscopy (IRLAS) has made substantial progress due to recent advancements and availability of novel mid-infrared laser sources such as quantum cascade lasers (QCL) and interband cascade lasers (ICL). With a high optical output power at low spectral linewidth and the option of tunability operated at room temperature these light sources are viable alternatives vs. conventional broadband emitters [3,4]. The fundamental vibrational, rovibraional, and rotational transitions in the mid-infrared (MIR) regime, which are characterized by high absorption coefficients in combination with the high spectral power density of MIR laser sources have led to absorption-based sensing concepts enabling highly sensitive molecular analysis down to ppt concentrations [5]. Recent studies have shown that a combined IRLAS set-up utilizing structures termed substrate-integrated hollow waveguides (iHWGs) developed by our research team and collaborators, have led to a wide range of gas sensing concepts based on iHWGs providing an optimal ratio of optical path length vs. gas cell volume [6–8]. However, there is also a need for small yet low-cost MIR light sources. Hence, iHWGs were alternatively combined with advanced non-laser MIR light sources aiming at compact yet cheap gas sensors for the analysis of selected VOCs in exhaled breath.

[1] Miekisch, W.; Schubert, J.K.; Noeldge-Schomburg, G.F.E. Diagnostic potential of breath analysis - Focus on volatile organic compounds. Clin. Chim. Acta 2004, 347, 25–39, doi:10.1016/j.cccn.2004.04.023. [2]Buszewski, B.; Kęsy, M.; Ligor, T.; Amann, A. Human exhaled air analytics: biomarkers of diseases. Biomed. Chromatogr. 2007, 21, 553–566, doi:10.1002/bmc.835. [3] Li, J.S.; Chen, W.; Fischer, H. Quantum cascade laser spectrometry techniques: A new trend in atmospheric chemistry. Appl. Spectrosc. Rev. 2013, 48, 523–559, doi:10.1080/05704928.2012.757232. [4] Selvaraj, R.; Vasa, N.J.; Nagendra, S.M.S.; Mizaikoff, B. Advances in Mid-Infrared Spectroscopy-Based Sensing Techniques for Exhaled Breath Diagnostics. Molecules 2020, 25, 2227, doi:10.3390/molecules25092227. [5] Ventrillard, I.; Gorrotxategi-Carbajo, P.; Romanini, D. Part per trillion nitric oxide measurement by optical feedback cavity-enhanced absorption spectroscopy in the mid-infrared. Appl. Phys. B Lasers Opt. 2017, 123,

1.9. Computational Spectroscopy/ /Quantum Approach

Title Vibrational signatures of biomolecules from anharmonic computations

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Anharmonicity, IR, Raman, VCD, ROA, VPT2, DFT

Advanced spectroscopic experiments are widely applied for understanding the biomolecules structure-function relationships, allowing a direct detection of different 3D-conformational schemes via microwave (MW) measurements or indirect analysis through 'fingerprint' vibrational features in infrared (IR), Raman, Resonance Raman, UV-vis or fluorescence spectra, including also their chiral counterparts. However, the conformational flexibility and great variety of possible interactions results that these experimental spectroscopic studies are extremely difficult to interpret. In this talk I will present the status and perspectives of the ongoing project aiming to bridge the gap between sophisticated experimental techniques and often over-simplified analysis. In this context, inclusion of anharmonic effects on both line positions and intensities of vibrational (IR, Raman, VCD, ROA..) spectra is paving a way toward significantly improved level of accuracy and understanding of state-of-the-art contemporary spectroscopic results. In this framework, computational protocol is developed through a multistep strategy, starting from isolated molecules, oligomers and weakly-bonded complexes/clusters. Extension to larger and more complex systems relies on reduced dimensionality approaches and effective schemes to select transitions of interest. Such procedures will be discussed focusing on the protein and DNA building blocks.

[1] V. Barone, S. Alessandrini, M. Biczysko, J. R. Cheeseman, D. C. Clary, A. B. McCoy, R. DiRisio, F. Neese, M. Melosso, C. Puzzarini "Computational molecular spectroscopy" Nature Reviews Methods Primers 1, 38, 2021 [2] M. Biczysko, J. Bloino, C. Puzzarini, Wires Comp. Mol. Sci., 8 (2018) e1349. [3] J. Bloino, A. Baiardi, M. Biczysko Int. J. Quant. Chem. 116 (2016) 1543

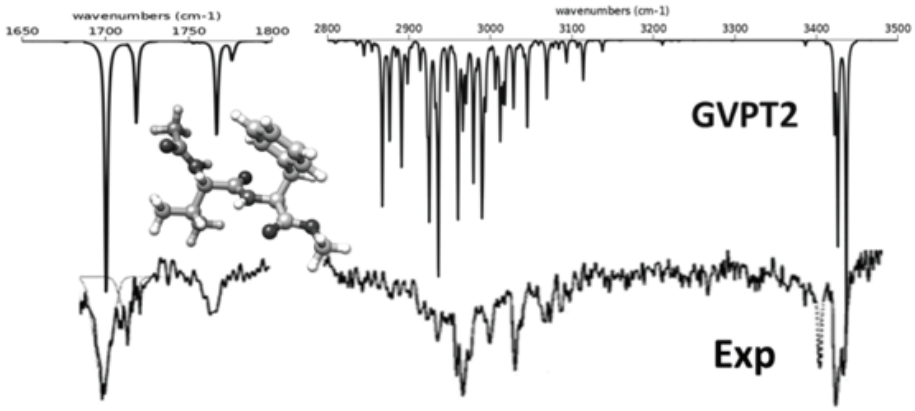

Figure 1. Experimental and fully anharmonic (GVPT2) IR spectrum of Ac-Val-Phe-OMe dipeptide

Quantum Chemical Approaches in Molecular Spectroscopy of the Condensed Phase – Advances, Challenges, and Perspectives

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Vibrational spectroscopy, Raman spectroscopy, Quantum chemical approach, Near-infrared spectroscopy, low-frequency vibrational spectroscopy

In this conference we report advances, challenges, and perspectives of quantum chemical approaches in molecular spectroscopy of the condensed phase. Recently, quantum chemical approaches play crucial roles in the studies of band assignments, molecular and electronic structures, and vibrational and electronic transitions. The quantum chemical calculations also provide important information about anharmonicity, vibrational coupling, vibrational potentials, and so on. The combination of spectroscopic approaches and quantum chemical calculations is a powerful tool for spectroscopy, in general. Thus, our studies, which are concerned with various spectroscopy and quantum chemical approaches, should have strong implications in the wider spectroscopy community. This presentation covers vibrational spectroscopy like IR, NIR, FIR/terahertz and Raman spectroscopy and electronic spectroscopy particularly FUV spectroscopy. As quantum chemical approaches, we discuss several anharmonic approaches such as vibrational self-consistent field (VSCF) and the combination of periodic harmonic calculations with anharmonic corrections based on finite models, grid-based techniques like the Numerov approach, the Cartesian coordinate tensor transfer (CCT) method, Symmetry-Adapted Cluster Configuration-Interaction (SAC-CI), and the ZINDO (Semi-empirical calculations at Zerner's Intermediate Neglect of Differential Overlap). One can use anharmonic approaches and grid-based approaches for both infrared (IR) and near-infrared (NIR) spectroscopy, while CCT methods are employed for Raman, Raman optical activity (ROA), FIR/Terahertz and low-frequency Raman spectroscopy. Therefore, this presentation overviews cross relations between molecular spectroscopy and quantum chemical approaches, and provides various kinds of close-reality advanced spectral simulation for condensed phases. We select various applications such as those to basic molecules, polymers, biological materials. nanomaterials, and ionic liquids.

Quantum mechanical calculation of NIR spectra – from basic molecules to biomolecules

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near infrared (NIR) spectroscopy, vibrational spectroscopy, quantum chemical calculation, overtones and combination bands

Near-infrared (NIR; 12,500-4000 cm-1; 800-2500 nm) spectroscopy has distinguished itself over the last few decades as one of the most successful analytical techniques throughout academia and industry [1]. While NIR spectroscopy is primarily viewed as an analytical instrument, it has contributed significantly to the field of physical chemistry, for example through a better understanding of the anharmonic nature of molecular vibrations or intermolecular interactions [2]. In all of these areas, the inherent complexity of NIR spectra has often been a major obstacle. Staggering number of superimposed peaks and anharmonic effects create complex patterns of spectral relationships that in many cases make it difficult to understand and directly interpret NIR spectra [3]. In thriving analytical applications of NIR spectroscopy, multi-variate analysis gives NIR spectroscopy the analytical performance but lacks in providing answers on the origins of the spectral variability nor its relationships with the molecular background [4]. In contrast to mid-infrared (MIR) and Raman techniques, NIR spectroscopy has been hindered in forming practically accessible synergy with computational chemistry [5]. In recent few years, however, advances in the tools of computational chemistry have created an opportunity to make a step beyond this barrier [1,5,6]. The presentation summarizes our most recent accomplishments in the emerging field. We present our results of quantum mechanical simulation of NIR spectra of a variety of compounds, which are significant from the point of view of physiochemical and analytical spectroscopy. The examples range from basic molecules (alcohols, nitriles, carboxylic acids) to complex molecules with importance to biophysical science (fatty acids, nucleobases) and analytical chemistry (natural drugs, polyphenols, food adulterants). A remarkable potential arises from the growing applicability of anharmonic computations to solving the problems, which arise in both basic and analytical NIR spectroscopy. Highly convoluted, overlapping nature of NIR spectra can be successfully dissected in theoretical spectra. The elucidated rich information stemming from numerous NIR bands can subsequently be used to improve basic understanding of NIR spectroscopy as well as to advance its applications.

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[1] Ozaki, Y.; Huck, C.W.; Bec?, K.B. Near-IR spectroscopy and its applications. In: Molecular and laser spectroscopy. Advances and applications. Gupta, V.P. Ed.; San Diego, Calif.: Elsevier, 2018, p. 11–38. [2] Czarnecki, M.A.; Bec?, K.B.; Grabska, J.; Hofer, T.S.; Ozaki, Y. Overview of application of NIR spectroscopy to physical chemistry. In: Near-infrared spectroscopy, Ozaki, Y., Huck, C.W., Tsuchikawa, S., Engelsen, S.B., Eds.; Springer: Singapore, 2021, p. 297-330. [3] Grabska, J.; Ishigaki, M.; Bec?, K.B.; Wójcik, M.J.; Ozaki, Y. J. Phys. Chem. A 2017, 121, 3437–3451. [4] Bec?, K.B.; Grabska, J.; Kirchler, C.G.; Huck, C.W. J. Mol. Liq. 2018, 268, 895–902. [5] Bec?, K.B.; Grabska, J.; Hofer, T.S. Introduction to quantum vibrational spectroscopy. In: Near-infrared spectroscopy, Ozaki, Y., Huck, C.W., Tsuchikawa, S., Engelsen, S.B., Eds.; Springer: Singapore, 2021, p. 83-110. [6] Bec?, K.B.; Grabska, J.; Huck, C.W.; Spectrochim. Acta A 2021, 254, 119625.

Molecular Structure, second and third-order nonlinear optical properties of SynthesizedDichlorobis(pyridine-3-carbaldehyde)Zinc(II) complex by density functional theory and experimental Methods

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DFT, Hirshfeld surface, LDT, SHG, THG

S. Chitrambalama, Sunila Abrahamb,and I. Hubert Joea*, a Department of Physics, University of Kerala, Kariavattom, Thiruvananthapuram-695 015, Kerala, India bResearch and postgraduate Department of Physics, Research centre, University of Kerala, Christian College, Chengannur-689122, Kerala, India. Coordination complexes have gained significant attention in materials science because of their versatility of structure and diversity in applications, such as sensors, frequency-conversion technology, electrical conductivity, luminescence and nonlinear optics [1]. Single crystals of the coordination complex Dichlorobis(pyridine-3-carbaldehyde)Zinc(II) have been grown. The crystallinity of the synthesized complex is confirmed using single crystal/powder X-ray diffraction and 1H proton NMR spectra. The computation of structural geometry, vibrational wavenumbers, UV-Visible spectrum and energy of natural bond orbital interactions are at the B3LYP level of 6-311++G(d,p) basis set. The theoretically predicted structural properties and experimental results are comparable.FTIR and FT Raman spectra of the complex are recorded[Fig.1] and analyzed. The C-H stretching wavenumber indicates the presence of intermolecular C-H&hellip &pi and C-H&hellip Cl hydrogen bonding interaction and is substantiated by NBO analysis. The frontier molecular orbital energy calculations show the charge transfer interactions within the molecule, proving its reactivity and stability. Hirshfeld surface mapand fingerprint plot analysis confirm that the C-H&hellip O and C-H&hellip Cl hydrogen bonds stabilize the complex. Laser-induced surface damage threshold yields the surface resistance of the crystal. Characterization of the second and third-order nonlinear optical properties uses Kurtz-Perry powder diffraction and Z-scan technique. The open-aperture Z-scan result shows that the compound exhibits the reverse saturation absorption behaviour, indicating the use of this material as a potential candidate for optical power limiting applications[2]. Keywords: DFT; Hirshfeld surface; SHG; THG *Corresponding author: e-mail: hubertjoe@gmail.com; Conclusions The structural geometry and NBO analysis reveal that the C-H&hellip Cl and C-H&hellip O inter-and intramolecular hydrogen bonds contribute to the structural stabilization of the P3CZC complex. The HOMO-LUMO energy gap is found to be 4.3349 eV. Hirshfeld surface analysis confirms the strongest hydrogen bonding interaction with C-H&hellip O. The The multiple shot LDT value is 3.22 GW cm-2. The complex exhibits good optical limiting behaviour at 532 nm.

[1] M. Zhao, J. Tan, J. Su, J. Zhang, S. Zhang, J. Wu, Y. Tian, Syntheses, crystal structures and third-order nonlinear optical properties of two series of Zn(II) complexes using the thiophene-based terpyridine ligands, Dyes Pigments.130 (2016) 216-225.http://dx.doi.org/10.1016/j.dyepig.2016.03.005. [2] S. Valligatla, K. K. Haldar, A. Patra, N. R. Desai, Nonlinear optical switching and optical limiting in colloidal CdSe quantum dots investigated by nanosecond Z-scan measurement, Opt. Laser Technol. 84, (2016) 87–93.http://dx.doi.org/10.1016/j.optlastec.2016.05.009.

Are ions reflected in water IR signature?

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water hydrogen bond network, IR spectroscopy, ions, multivariate curve resolution with alternating least squares, kosmotropes and chaotropes

Hydrogen bond (HB) network formed between water (H_2O) molecules in liquid phase displays exceptional structural and dynamic features resulting in unique physicochemical properties. Owing to two HB donating (-OH) and two HB accepting centers (free electron pairs on O-atom), one H_2O molecule can be simultaneously engaged in four HBs. Despite being scrutinized for decades, the average occupancy of HB centers remains ambiguous and the employment of different experimental methods participates in the discrepancy enhancement [1, 2]. Properties of water HB network become additionally complicated with the presence of ions in water solution. The traditional division of ions on kosmotropes and chaotropes, making HB network between water molecules stronger or weaker, respectively, is questioned by MD simulations [3], whereas experimental study based on steady-state IR spectra of aqueous solutions of various salts implies opposite conclusions [4]. In addition to the finding that the presence of monoatomic ions in aqueous solutions can be detected from signals of (anti-)symmetric stretching of water molecules ($nu_{(a)s}OH$) [5], water signature originated from the combination of water bending (deltaHOH) and libration of water molecules (nu_L) revealed high sensitivity on water surroundings as well [4]. In this regard, we have examined aqueous solutions of sodium (Na^+) and potassium (K^+) salts of chloride (Cl^-), bromide (Br^-), iodide (I^-), nitrate (NO_3^-), nitrite (NO_2^-) and acetate (CH_3COO^-) of equal ionic strengths (I = 1 M) by IR spectroscopy in transmission regime, in the temperature range 25 – 70°C. In order to examine the impact of pH on the signals of water, IR spectra of NaOH and KOH (I = 1 M) were acquired as well. The signatures of different ions entangled in the combination band are unraveled by multivariate analysis of IR spectra (Fig. 1) [6]. Fig. 1. a) Temperature-dependent IR spectra of KBr in H_2O solution (solid lines) and spectral profiles of low- and high-temperature components (dotted lines) in temperature range 25 – 70°C; b) relative concentrations of low- and high-temperature components with the intersection point at T_i = 43.8°C.

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[1] Y. Maréchal, The Hydrogen Bond and the Water Molecule, First Ed., Elsevier Science, Amsterdam, 2007, pp. 215–248. [2] F. Cipcigan, V. Sokhan, G. Martyna, J. Crain, Sci Rep. 8 (2018) 1718. [3] H. I. Okur, J. Hladílkova,? K. B. Rembert, Y. Cho, J. Heyda, J. Dzubiella, P. S. Cremer, P. Jungwirth, J. Phys. Chem. B 121 (2017) 1997–2014. [4] P. K. Verma, A. Kundu, M. S. Puretz, C. Dhoonmoon, O. S. Chegwidden, C. H. Londergan, M. Cho, J. Phys. Chem. B 122 (2018) 2587–2599. [5] F. Rauh and B. Mizaikof, Appl. Spectrosc. 70 (2016) 1214–1227. [6] P. Maleš, Z. Brkljača, I. Crnolatac, D. Bakarić, Colloids Surf. B 201 (2021) 111645.

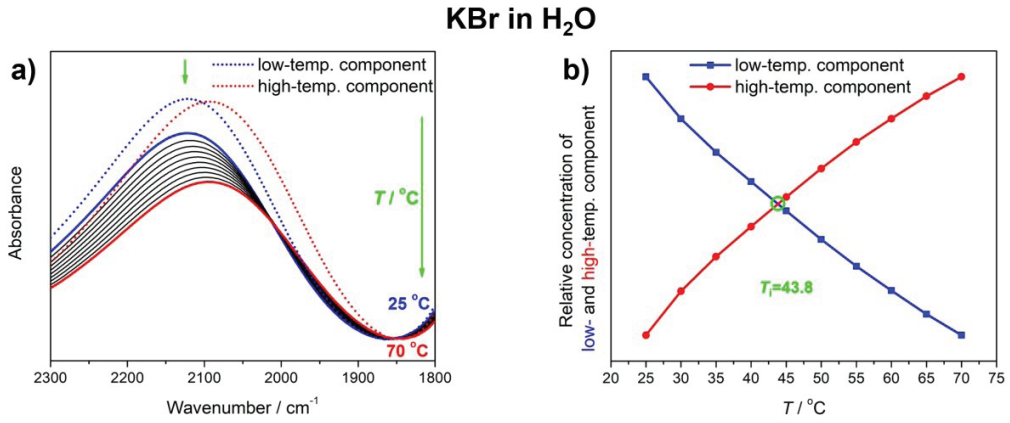

Figure 1. Described in text

Ultraviolet Resonance Raman spectroscopy of Anthracene: Experiment and Theory

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UVRR, resonance Raman, Raman Spectroscopy, chemical calculations, polyaromatic hydrocarbon

Ultraviolet resonance Raman scattering is a highly sensitive and selective label-free vibrational spectroscopic technique with a broad range of applications from polyaromatic hydrocarbons to biomolecular systems (peptides/proteins and nucleic acids) and catalysts [1,2]. The interpretation of experimental UVRR spectra is not as straightforward as in purely vibrational Raman scattering (Placzek approximation) due to the involvement of higher-lying electronic states and vibronic coupling. This necessitates the comparison with theoretical UVRR spectra computed by electronic structure calculations. Anthracene is an ideal model system for such a comparison between experiment and theory since it is rigid, symmetric and of moderate size [3]. Even with a computationally rather cheap approach of a double-ζ basis set (SNSD) and the B3LYP functional, a good qualitative agreement close to the resonance condition is achieved by taking into account Herzberg-Teller contributions including Duschinsky effects, bulk solvent effects and anharmonic contributions to frequencies. With this we can show that within the framework of TD-DFT a general and robust approach for the analysis and interpretation of resonance Raman spectra of medium- to large-size molecules is available.

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[1] Zakeri B, Niebling S, Martinéz AG, Sokkar P, Sanchez-Garcia E, Schmuck C, Schlücker S Phys. Chem. Chem. Phys. 2018, 20, 1817. [2] Jin S, Feng Z, Fan F, Li C Catalysis Letters 2015, 145, 468. [3] Dierksen M, Grimme S J. Chem. Phys. 2004, 120, 3544.

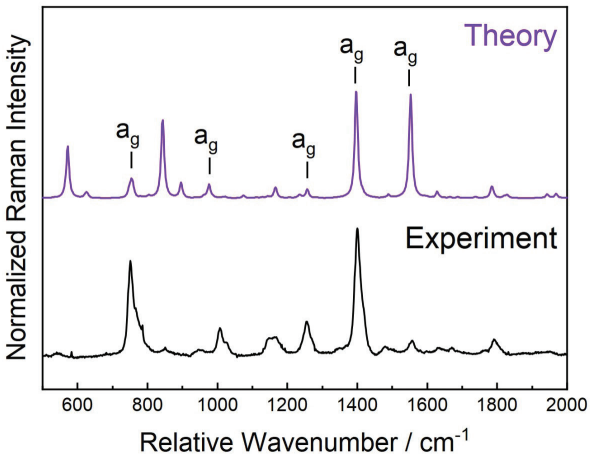

Figure 1. Calculated (violet, top) and experimental (black, bottom) ultraviolet Resonance Raman spectra of anthracene in acetonitrile. Lorentzian broadening with half-widths at half-maximum of 5 cm-1 has been a

How much anharmonicity is in vibrational spectra of CH3I, CD3I, CHCl3, CDCl3, CHBr3 and CDBr3?

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Vibrational intensities, Anharmonicity, Mid-infrared (MIR), Near-infrared (NIR), Raman spectroscopy

This work provides a new quantitative information on vibrational spectra of liquid CH_3I, CD_3I, $CHCl_3$, $CDCl_3$, $CHBr_3$ and $CDBr_3$. Deconvolution of the mid-infrared (MIR) and near-infrared (NIR) spectra provided the parameters of fundamentals, overtones and combination bands. Simultaneously, the anharmonic vibrational spectra were calculated by generalized second-order vibrational perturbation theory (GVPT2). The normalization of ATR-IR spectra permitted for direct comparison of the intensities from ATR-IR and NIR transmission spectra. For the first time, we estimated the experimental and theoretical contributions from the anharmonic vibrations (overtones and combination bands) to MIR spectra of all studied compounds. Besides, we estimated the contributions from the MIR and NIR ranges to the total vibrational intensity. It is of note that the theoretical contributions from MIR and NIR regions are very close to the experimental ones. It results from accurate theoretical prediction of the intensities of the fundamental bands, which contribute to more than 90% of total intensity. On the other hand, the intensities of the overtones and combination bands are predicted less satisfactory, which is reflected from the NIR spectra. The MIR spectra of all the compounds revealed the presence of Fermi resonances, which increase the intensity of the non-fundamental transitions and are responsible for differences in contributions from individual vibrational modes between different compounds.

This work was supported by the National Science Center Poland, Grant 2017/27/B/ST4/00948. Calculations have been carried out in Wroclaw Centre for Networking and Supercomputing (http:/www. wcss.pl), under Grant no. 163. We are very grateful Dr Krzysztof Bec´ (University of Innsbruck) for help during the theoretical calculations.

How to remove systematic errors from experimental IR spectra

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Dispersion analysis, Smart Error Sum, 2D-correlation analysis, Electric field standing wave effect, Transflection spectra

Transflection spectra are often hampered by reflectance values above unity due to changing reference conditions/ spectra. A naive way to remove these obvious systematic errors is to divide the spectrum by its highest reflectance value.[1] Although such spectra can then be quantitatively evaluated by advanced processing methods like dispersion analysis, wavenumber dependent errors still taint the results. Recently, we found a way to enable quantitative spectrum evaluation with automatic correction of such errors.[2,3] Our proposed method, called the smart error sum, is based on a smart version of the conventional sum of least squares. Instead of forcing the fit to agree with the experimental spectrum at all costs (which would obviously be a bad idea for, e.g., reflectance values beyond unity), this smart error sum focuses on preserving correlations that exist within the experimental spectra and is thus able to remove systematic error to a large degree. Correspondingly, the smart error sum is related to 2D correlation spectroscopy (2D-COS), pioneered by Isao Noda.[4] Our basic idea, which consists of the use of hybrid maps and their symmetry properties, allows, in contrast to 2D-COS, a fully quantitative description of the similarity of two spectral series,[2] or, in the limiting case, even of two spectra,[3] i.e. an experimental and a simulated one. This quantitative description is implemented in the smart error sum and in the two-trace two-dimensional (2T2D) smart error sum. We demonstrate the capabilities for removing systematic errors by spectral fitting with dispersion analysis, a much more advanced progenitor of band fitting, using the smart error sum on experimental transflection spectra of PMMA layers on gold substrates. In addition to corrected spectra and accurate optical constants, we obtain further meaningful parameters like layer thicknesses and oscillator parameters, despite of the well-known strong interference ("electric field standing wave") effects, which additionally complicate quantitative spectral evaluation in such systems.

[1] T. G. Mayerhöfer, S. Pahlow, U. Hübner, and J. Popp. Analyst. 2018. 143(13): 3164-3175. [2] T. G. Mayerhöfer, M. Richard-Lacroix, S. Pahlow, U. Hübner, J. Popp. 2021, in preparation. [3] T. G. Mayerhöfer, M. Richard-Lacroix, S. Pahlow, U. Hübner, J. Popp. 2021, in preparation.
[4] I. Noda and Y. Ozaki, Two-Dimensional Correlation Spectroscopy: Applications in Vibrational and Optical Spectroscopy (Wiley, 2005).

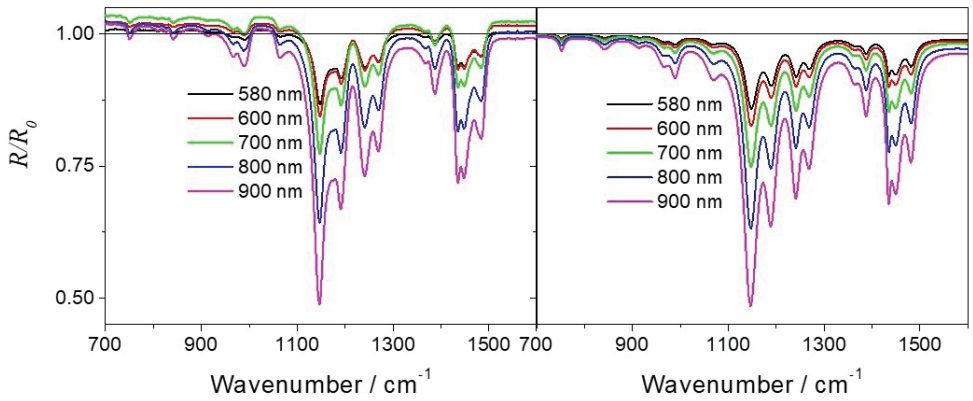

Figure 1. Uncorrected (left) vs. corrected infrared transflection spectra of PMMA layers on gold

Molecular Structure, Vibrational Spectra and NLO activity of 3-D Supramolecular organically templated chained chlorocadmate crystal [H2pip]Cd2Cl6(H2O)2] using Density Functional Theory

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Density Functional Theory, Optimized Geometry, Fourier Transform Infrared and Raman Spectroscopy, Natural Bond Orbital Analysis, Optical Limiter

The crystals of Piperazinedi-ium tetrakis (μ2-chloro)-diaqua-dichloro-di-cadmium(ii) (PTCD) were grown by slow evaporation from saturated aqueous solutions[1] of piperazine (C4H10N2), cadmium carbonate (CdCO3) and hydrochloric acid (HCl) in the stoichiometric ratio 1:2:6. Piperazine is an organic compound with a molecular formula of $C_4H_{10}N_2$ that consists of a six-membered ring containing two nitrogen atoms at opposite positions in the ring. It is used as a building block for pharmaceuticals [2].The organically template piperazine with chlorocadmate complex is found to have increased hyperpolarizability values. The most stable molecular structure (Figure 1) is optimized by DFT/ B3LYP method with LAN2DZ basis set in the Gaussian 09 program package [3]. The intra and inter molecular interactions, donor-acceptor energy relations are studied using the NBO 5.0 program [4].The charge transfer from the metal atom to the amine group of the molecule is evident from the natural bond orbital analysis. The hydrogen bonded intermolecular interactions such as N-H..Cl, O-H...Cl, C-H...Cl and charge delocalization give stability to the molecule. The theoretical and experimental FT-IR (Fig.2) and FT-Raman (Fig.3) spectra of PTCD molecule have been analyzed using density functional theory by considering its monomer as well as dimer form. The vibrational wave numbers are calculated and assigned on the basis of potential energy distribution using MOLVIB software [5]. The N-H stretching vibrations are observed as weak bands at 3177 and 3355cm-1 in Raman spectrum. The N6-H17 and N38-H49 symmetric stretching bands are red shifted by 53 cm-1 from that of pure piperazine due to the N6-H17...O21 and N38-H49...O30 inter molecular hydrogen bonding. Hirshfeld surface analysis provides additional insight in to the intermolecular interaction through close contacts. The 3D Hirshfeld surfaces and 2D finger print plots are generated using the Crystal Explorer 3.1. Some quantitative information about the individual contribution of the intermolecular interaction in the asymmetric unit are provided by 2D fingerprint maps. The open-aperture Z-scan analysis was carried out to study the non-linear absorption behaviour of the crystal. Numerically estimated two-photon absorption coefficient β value 8.7001 x 10-5 m/W of the molecule suggests its potential application as an optical limiter. The Photoluminescence spectrum with excitation wavelength 300 nm indicates the use of PTCD as a violet light emitting material for use in light emitting diodes and other photonic applications.

The author Nimmy L. John is thankful to The University of Kerala for the Junior Research Fellowship

[1] J. Yu, X. Wang, L. Ye, Q. Hou, Q. Yang, J. Xu, Organically templated chained chlorocadmates and cadmium-chloro thiocyanates, CrystEngComm. 11 (2009) 1037–1045. [2] E. Yi, E. Jeong, J. Joo, H. Kwon, Anti-angiogenic and anti-tumour apoptotic activities of SJ-8002, a new piperazine derivative, Int. J. Oncol. 25 (2004) 365–372. [3] M.J. Frisch, et al, Gaussian 09, Revision C 02. Gaussian, Inc., Wallingford CT (2009). [4] F.W. E. Glendening, J. Badenhoop, A. Reed, J. Carpenter, J. Bohmann, C. Morales, Theor. Chem. The University of Wisconsin, Madison (2001). [5] T. Sundius, J. Mol. Struct.218 (1990) 321-326.

An FTIR chemical biomarker accurately predicts neurodegenerative disease class in the absence of overt symptoms

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Fourier Transform infrared microspectrometry, neurodegeneration, Spectral phenotyping, Biomarker

Although some neurodegenerative diseases can be identified by visible features relatively late in disease progression, we currently lack methods to predict who has developed disease before the onset of symptoms, when onset will occur, or the outcome of therapeutics. New biomarkers are needed. Here we describe spectral phenotyping, a new kind of biomarker that makes disease predictions based on chemical rather than biological endpoints in cells. Spectral phenotyping uses Fourier transform infrared (FTIR) spectromicroscopy to produce an absorbance signature as a rapid physiological indicator of disease state. FTIR spectromicroscopy has over the past been used in differential diagnoses of manifest disease. Here, we report that the unique FTIR chemical signature accurately predicts disease class in mouse with high probability in the absence of brain pathology. In human cells, the FTIR biomarker accurately predicts neurodegenerative disease class using fibroblasts as surrogate cells

IR beamline at Berkekely Las ALS

Spectral fingerprint of polymers from overtones and combination bands in mid- and near-infrared regions and its analytical application

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nera-infared (NIR) spectroscopy, mid-infrared (MIR) spectroscopy, overtones and combination bands, microplastics, polymers

Recently, anharmonic calculations have become practically feasible in predicting overtones and combination bands of reasonably complex molecules [1,2]. These vibrational transitions are conventionally linked with near-infrared (NIR; 12,500-4000 cm^{-1}; 800-2500 nm) spectral region. Low absorption index of these bands grants NIR spectroscopy its valued non-destructive character of analysis. By contrast, weak in intensity overtones and combination bands are often not considered as meaningful source of information in in mid-infrared (MIR; 4000-400 cm^{1}; 2500-25000 nm) spectroscopy, where the focus is hijacked by intense and easier in analysis fundamental bands. However, the weak overtones and combination bands can form a superior source of information in the scenarios, where the accessibility to fundamental peaks is limited; e.g. because of the saturation effect. With the anharmonic quantum chemical calculations, these weak bands can be accurately reproduced and reliably interpreted. This way, these bands gain the structural specificity, for which vibrational spectroscopy is commonly valued. This gain is particularly evident in the MIR region typically free from the presence of fundamental bands, i.e. at ca. 2700-1500 cm^{-1} (Fig. 1) [3]. Here, we present the potential for integrating these "forgotten bands" in MIR spectra in the detection and identification of microplastic pollutants by MIR hyperspectral imaging technique. In the configuration suitable for straightforward analysis, the reliance on the fundamental bands leads to inferior performance resulting from detector saturation. In contrast, the spectral information carried by the non-fundamental peaks is easily accessible, as their moderate intensity prevents any distortion of the spectral lineshape. Higher image contrast is further supplemented by more reliable "fingerprinting" based on the overtones and combination bands present in hyperspectral images. This leads to more accurate detection and identification of microplastic particles. The results are presented for nine polymers; acrylonitrile butadiene styrene (ABS), ethylene-vinyl acetate (EVAC), polycarbonate (PC), polyethylene terephthalate (PET), polylactide or polylactic acid (PLA), polymethylmethacrylate (PMMA), polyoxymethylene (POM), polystyrene (PS) and polyvinylchloride (PVC).

This work was supported by the Austrian Science Fund (FWF), M2729-N28.

[1] Bec?, K.B.; Grabska, J.; Hofer, T.S. Introduction to quantum vibrational spectroscopy. In: Near-infrared spectroscopy, Ozaki, Y., Huck, C.W., Tsuchikawa, S., Engelsen, S.B., Eds.; Springer: Singapore, 2021, p. 83. [2] Bec?, K.B.; Huck, C.W.; Front. Chem. 2019, 7, 48. [3] Beć, K.B.; Grabska, J.; Ozaki, Y.; Hawranek, J.P.; Huck, C.W. J. Phys. Chem. A 2017, 121, 1412.

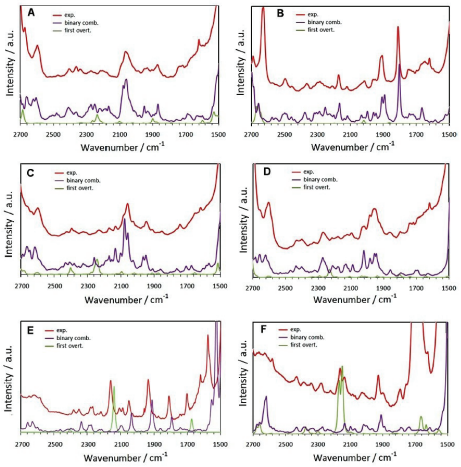

Figure 1. The detailed assignments of the MIR region of six aliphatic ethers populated by non-fundamental bands (ca. 2700-1500 cm-1) available from GVPT2//B2PLYP/SNST calculations [3].

Theoretical Approach to Study Water Structure at the Solid-Water Interface

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Many processes at the macro scale get governed by the influence of water interaction at the solid-water interface, which in turn depends on the nature of the material used for the solid surface [1]. An interfacial insight into the water structure can aid certain macroscopic processes like waste water treatment, corrosion etc. and technological appliances like biosensors and electrochemical devices. Hydrophobicity of the surface plays a pivotal role in modifying the interfacial water structure controlling the surface phenomenon [2]. In the present study, the solid-water interface is investigated by using molecular dynamics (MD) Simulation to probe the water structure at the solid-aqueous interface. Contact angle and density variation of water molecules at the interface are the targeted properties of interest. The surface behaviour is simulated using a pseudo continuum model approach [3]. According to contact angle analysis, the contact angle is very susceptible to the hydrophobic attributes of the surface until it is less than 10o. Below this value, contact angle varies sluggishly on tuning the surface hydrophobicity/hydrophilicity. In a similar manner, water density variation perpendicular to the surface for varying hydrophobic characters is investigated in details. Water density curve is classified in two regimes to predict the processes involved at the water/solid interfaces. One is the non-wetting regime whose water density curve is similar to the air-water interface whereas the other identified as the wetting regime in which a sigmoidal water density curves oscillates around the bulk water density value (~1 gm/cm3). We observed a transition between these two regimes at a contact angle ~130°. We will give a detailed analysis of our results on water contact angle and density variation curve for interfacial water on varying nature of solid surface. This analysis can be used to pick out surface materials with desirable hydrophobic traits depending on the purpose such as preventing rusting and corrosion or selecting a material which will act as a good biosensor.

[1] Shalaka Dewan, Vincenzo Carnevale, Arindam Bankura, Ali Eftekhari-Bafrooei, Giacomo Fiorin, Michael L. Klein and Eric Borguet Langmuir 2014, 30, 8056–8065. [2] Travis G. Trudeau, Kailash C. Jena and Dennis K. Hore; J. Phys. Chem. C 2009, 113, 20002–20008. [3] Meysam Makaremi, Myung S. Jhon, Meagan S. Mauter and Lorenz T. Biegler; J. Phys. Chem. C 2016, 120, 11528–11534. [4] Bo Shi and Vijay K. Dhira; J. Chem. Phys. 2009, 130, 034705. [5] Jianwei Wang, Andrey G. Kalinichev, R. James Kirkpatrickand Randall T. Cygan; J. Phys. Chem. B 2005, 109, 15893-15905.

1.10. Chemometrics, Artificial Intelligence and Machine Learning

Experimental Designs for Spectroscopic Calibration: Measuring Uncertainty from Various Sources

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chemometrics, experimental design, inverted nested design, hierarchical data, nested factor

Typically, a number of different sources conribute to the total uncertainty of an analysis result. The question is then: how to allocate experimental resources efficiently? As a rule of thumb, it is best to concentrate the experimental effort on the largest sources of uncertainty. These sources of variation in biological systems as well as production processes as well as along the analytical procedure often lead to a hierarchical data structure, they are so-called nested factors. We present sampling schemes for estimating the variance which do not require exponentially growing sample numbers for nested data. These staggered and inverted nested designs have been known since the 1960s [1] but only nowadays the computational resources to analyze such data have become readily available. As an example, we discuss the sampling plan and design of experiments for chemical reference analysis for calibration of NIR spectra in the context of quality control during cacao fermentation. The experimental design was set up primarily to allow estimation of the biochemical variation in the analyte content between cocoa beans of the same fermentation but also to monitor the random uncertainty introduced by the various steps in the analytical process. While we discuss these experimental design principles with the example of quantitative reference data for regression/calibration, they apply just the same to classification.

Project "CocoaChain" (IGF 169 EN/3) was financially supported by the AIF (Arbeitskreis industrielle Forschung) and FEI (Forschungskreis der Ernährungsindustrie).

[1] Bainbridge T.R.: Staggered nested designs for estimating Variance components, Industrial Quality Control (1965), 22, 1, 12-20.

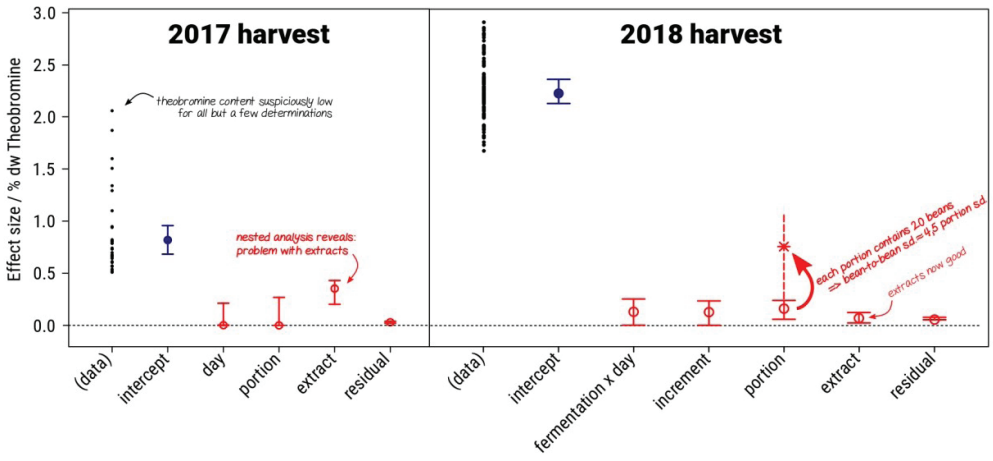

Figure 1. Contributing factors to total uncertainty in reference analyses of the theobromine content of cacao beans

Assessment of Oocyte Maturation using resonance Raman Spectroscopy based on the mitochondrial respiratory activity and lipid metabolism

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Raman spectroscopy, Resonance Raman spectroscopy, Mouse oocyte

Oocyte quality is a very important factor affecting the success rate of infertility treatment in humans and a novel technology to assess oocyte quality is required worldwide. The observation of morphological features, such as the shape of the spindle and the polar body, under a microscope is currently the only nondestructive means of determining the optimal fertilization period. Therefore, a novel evaluation technique is required to assess oocyte quality based on in situ molecular information. In this study, we aimed to develop a method to determine the degree of oocyte maturation in metaphase II in situ based on the balance between mitochondrial respiratory activity and lipid metabolism using resonance Raman spectroscopy. Raman spectra were obtained from living oocytes at Phases I, II, III, and IV after the injection of hCG hormon using a 532-nm excitation. Some distinct peaks from proteins, lipids, DNA/RNA, and especially cytochromes were observed. Principal component analysis (PCA) was carried out by selecting the most contralateral group of immature and overmature oocytes. Among the combinations of score plots of principal components (PCs) in two dimensions, PC 2 vs PC 3 showed the best pattern of distinguishing between immature and overmature oocytes. Combined with the detailed examination of their loading plots, the relative concentration of lipids increased at Phase IV and the decrease in the cytochrome c peak intensities for Phase IV can be interpreted as the deviation of cytochrome c from the reduction state. The multi curve resolution (MCR) results provided a quantitative basis for the concentration variation in in ovo components associated with oocyte maturation, which was consistent with the PCA results. A new index for measuring the oocyte quality in situ was propose by defining the proportion of the peak intensities (I_{750} / I_{1438} and I_{1127} / I_{1438}), represented by the Raman peak intensities due to reduced cytochrome c (750 and 1127 cm^{-1}) and lipids (1438 cm^{-1}). The new product index reasonably represents the variation of oocyte quality in the course of maturation. In vitro fertilization was carried out for laser-irradiated oocytes, and embryonic development was observed for five days. Comparing the developmental rate with and without laser irradiation showed that the laser had no significant effect on the rate. These results indicate that resonance Raman spectroscopy can be used to noninvasively evaluate the oocyte quality in situ based on respiratory activity and lipid metabolism.

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Comparability of Raman Spectroscopic Configurations: A Large Scale Cross-Laboratory Study

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Raman spectroscopy, data science, chemometrics, data comparability, setup dependence

Raman spectroscopy is a spectroscopic method that can solve large number of unmet needs in real-world scenarios such as clinical diagnostics [1]. For such real-world applications data driven models are generated, that should ideally be usable to predict newly acquired data. If the new acquired data is measured with another Raman spectroscopic setup or other measurement related parameters change, the predictive performance is often lower than the predictive performance estimated on the training dataset [2-4]. Therefore, it is important that the data is consistent, reproducible and comparable. To investigate these properties, the COST (European Cooperation in Science and Technology) action 'Raman4clinics' including working groups from 15 institutes in Europe performed a ring trial (see Figure 1). The consortium checked the compatibility of Raman spectra acquired with 35 Raman spectroscopic devices with different configurations [5]. The ring trial allowed for various instrumental configurations ranging from highly confocal setups to fiber-optic based systems with different excitation wavelengths. The setup dependence of the Raman spectra was quantified based on peak shifts, intensity variations, peak width changes, and noise levels. We could conclude that the whole Raman community needs to work on data standardization to improve inter-laboratory studies that would be needed if Raman spectroscopy should be applied in application scenarios like I clinics.

The work of the' Raman4Clinics' consortium and funding by COST for the Action BM1401 'Raman4Clinics' are highly acknowledged. The funding by the BMBF for the project LPI-BT1 (FKZ 13N15466) is gratefully acknowledged. The work is related to the NFDI4Chem project (441958208) funded by the German Research Foundation (DFG).

[1] Bocklitz, T. W., Guo, S., Ryabchykov, O., Vogler, N. & Popp, J. Raman based molecular imaging and analytics: a magic bullet for biomedical applications!? Analytical Chemistry 88, 133–151 (2016). [2] Guo, S., Heinke, R., Stöckel, S., Rösch, P., Popp, J. & Bocklitz, T. Model transfer for Raman spectroscopy based bacterial classification. Journal of Raman Spectroscopy 49, 627–637 (2018). [3] Guo, S., Kohler, A., Zimmermann, B., Heinke, R., Stöckel, S., Rösch, P., Popp, J. & Bocklitz, T. W. EMSC Based Model Transfer for Raman Spectroscopy in Biological Applications. Analytical Chemistry 90, 9787–9795 (2018). [4] Guo, S., Heinke, R., Stöckel, S., Rösch, P., Bocklitz, T. & Popp, J. Towards an Improvement of Model Transferability for Raman Spectroscopy in Biological Applications. Vibrational Spectroscopy 91, 111–118 (2017). [5] Guo, S., Beleites, C., Neugebauer, U., Abalde-Cela, S., Afseth, N. K., Alsamad, F., Anand, S., Araujo-Andrade, C., Askrabic, S., Avci, E., Baia, M., Baranska, M., Baria, E., Batista de Carvalho, L. A. E., de Bettignies, P., Bonifacio, A., Bonnier, F., Brauchle, E. M., Byrne, H. J., Chourpa, I., Cicchi, R., Cuisinier, F., Culha, M., Dahms, M., David, C., Duponchel, L., Duraipandian, S., El-Mashtoly, S. F., Ellis, D. I., Eppe, G., Falgayrac, G., Gamulin, O., Gardner, B., Gardner, P., Gerwert, K., Giamarellos-Bourboulis, E. J., Gizurarson, S., Gnyba, M., Goodacre, R., Grysan, P., Guntinas-Lichius, O., Helgadottir, H., Grosev, V. M., Kendall, C., Kiselev, R., Kölbach, M., Krafft, C., Krishnamoorthy, S., Kubryck, P., Lendl, B., Loza-Alvarez, P., Lyng, F. M., Machill, S., Malherbe, C., Marro, M., Marques, M. P. M., Matuszyk, E., Morasso, C. F., Moreau, M., Muhamadali, H., Mussi, V., Notingher, I., Pacia, M. Z., Pavone, F. S., Penel, G., Petersen, D., Piot, O., Rau, J. V., Richter, M., Rybarczyk, M. K., Salehi, H., Schenke-Layland, K., Schlücker, S., Schosserer, M., Schütze, K., Sergo, V., Sinjab, F., Smulko, J., Sockalingum, G. D., Stiebing, C., Stone, N., Untereiner, V., Vanna, R., Wieland, K., Popp, J. & Bocklitz, T. Comparability of Raman Spectroscopic Configurations: A Large Scale Cross-Laboratory Study. Anal. Chem. (2020). doi:10.1021/acs.analchem.0c02696

Fig.1. The Raman4Clinics ring trial including the comparison of different Raman spectroscopic devices is visualized conceptionally

Chemometrics, Artificial Intelligence and Machine Learning
The Class Imbalance Problem: AdaBoost to the Rescue?

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Machine Learning, Chemometrics

In tissue biopsy analysis we are often presented with a mismatch in the number of spectra from different cell types. This class imbalance causes problems when building machine learning chemometric models to identify cancerous regions of tissue. In this presentation we will highlight the discrepancies in Random Forests classification outcome, as a function of class imbalance. We will then explore strategies to mitigate this, including the potential of Adaptive Boosting as an alternative classification algorithm.

Chemometrics, Artificial Inelligence and Machine Learning
Model-based pre-processing and deep learning for correcting scatter effects in highly scatter-distorted infrared spectra of cells and tissues

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Extended multiplicative signal correction, Descatter Autoencoder

Scatter distortions can severely distort infrared spectra. Especially, in infrared microscopy of cells and tissues strong effects have been encountered [1]. Extended multiplicative signal correction (EMSC) has been introduced in the 90ies as a model-based chemometric method for correcting scatter effects such as baselines and multiplicative effects. We have during the recent years developed a framework for retrieving pure absorbance spectra from highly scatter-distorted infrared spectra of cells and tissues by incorporating Mie theory and other electromagnetic models in the EMSC framework. The Mie Extinction Extended Multiplicative Signal Correction (ME-EMSC) algorithm is the state-of-the-art pre-processing technique [2] and can recover pure absorbance spectra from highly scatter distorted spectra [3]. The MIE-EMSC algorithm is computationally expensive, and the correction of large infrared images could require hours of computations. We have trained a deep convolutional Descatter Autoencoder on ME-EMSC corrected spectra for correction of hyperspectral infrared images of cells and tissues [4]. In terms of speed, robustness and noise-levels, the Descattering Autoencoder outperformed the ME-EMSC algorithm which makes the Descattering Autoencoder particularly appropriate for correcting hyperspectral maps. The speed advantage of the Descattering Autoencoder could allow to preprocess the images in near real-time, and thereby making it feasible to use such images in medical applications.

[1] Mohlenhoff, B., Romeo, M., Diem, M., & Wood, B. R. Mie-type scattering and non-Beer-Lambert absorption behavior of human cells in infrared microspectroscopy. Biophysical journal, 88(5), 3635-3640, 2005. [2] Solheim, J. H., Gunko, E., Petersen, D., Großerüschkamp, F., Gerwert, K., & Kohler, A. An open-source code for Mie extinction extended multiplicative signal correction for infrared microscopy spectra of cells and tissues. Journal of biophotonics, 12(8), e201800415, 2019. [3] Sirovica S., Solheim J.H., Skoda M.W., Hirschmugl C.J., Mattson E.C., Aboualizadeh E., Guo Y., Chen X., Kohler A., Romanyk D.L. Origin of micro-scale heterogeneity in polymerisation of photo-activated resin composites. J Nature Communications 11(1):1-10, 2020. [4] Magnussen, E.A., Solheim J.H., Blazhko U., Tafintseva V., Tøndel K., Liland K.H., Dzurendova S., Shapaval V., Sandt C., Borondics F., & Kohler A. Deep convolutional neural network recovers pure absorbance spectra from highly scatter-distorted spectra of cells. Journal of Biophotonics 2020:e202000204, 2020.

Machine learning-assisted single-cell Raman microspectroscopy for in situ prokaryotic classification in complex microbial populations

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[3]The University of Tokyo

Raman microspectroscopy, Random forest algorithm, Microorganisms, Species classification

Gaining access to enormous uncultivated microorganisms (known as microbial dark matter) in a variety of Earth environments requires accurate, nondestructive classification and molecular understanding of the microorganisms at the single-cell level, which are not feasible with the current genomics approaches. In this work, we demonstrate a combined approach of random forest machine learning and less-invasive, single-cell Raman microspectroscopy for accurate classification of microbial-ecologically relevant prokaryotes (three bacterial and three archaeal species from different phyla). The classification accuracy we achieve is 98.8±1.9% 1.9% among the six species and 98.4% for three species in a mixed population that mimics an environmental sample. Random forest allows for readily visualizing the important features (wavenumbers) that contribute to the classification. We reveal that in addition to protein and DNA/RNA abundances, the presence of carotenoids and structure of membrane lipids play key roles in distinguishing the prokaryotic species. We also find unique Raman markers for an ammonia-oxidizing archaeon. Our approach is designed to be relatively easy to use for non-spectroscopists and thus offers microbiologists a new single-cell tool for shedding light on microbial dark matter.

Deciphering biochemical heterogeneity by Raman spectroscopy and Trajectory inference.

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Raman spectroscopy, Trajectory inference, Cell Heterogeneity, Cancer, Adipocyte

A myriad of chemometric methods associated with Raman spectroscopy has been successfully applied to biological samples studies. These methods correspond to classification or regression algorithms allowing to discriminate cells, tissues or biofluids according to their physiopathological state. However many biological processes, such as cell differentiation or cancer development cannot be sufficiently described by a discrete classification system. In order to capture transition between cell states, branching differentiation process or unsynchronized changes, we require appropriate models capable of accounting for the continuous and dynamic nature of such a biological processes. To address this issue a class of specific methods known as trajectory inference (TI)[1] can interpret scRNA-seq data as a snapshot of a continous process, offering the possibility to reconstruct the relationships and hierarchies hidden in a dataset[2]. It is thus possible to visualize, from a transcriptomic point of view, the different paths that a cell can take during a continuous process. But, so far, these tools are reduced to scRNA-seq. Here, we demonstrate how Raman spectroscopy can be used in association with TI to visualize and resolve a branching and/or asynchronous process. More specifically we show the interest to use the Partition-based graph abstraction algorithm (PAGA)[3] paired with a recent graph-based dimensionality reduction algorithm called Uniform Manifold Approximation and Projection (UMAP)[4]. These tools have the ability to encode the multidimensional topology of a data set in a preserving data relationships 2D representation. This demonstration was led on a toy dataset and on real Raman dataset. The toy-dataset is meant to mimic a developmental biffurcating process, based only on spectral variations. The real Raman dataset was collected during the differentiation process of 3T3-L1 fibroblast-like cells into adipocytes. This cellular model is widely used to investigate different mechanisms involving fat cells, more specifically during cancer progrssion. However, heterogeneous conversion of fibroblasts into mature adipocytes is a major drawback of this cell culture model. The obtained results show that TI tools are capable as in a similar way to scRNA-seq to highlight trajectories or biffurcations only based on spectral consideration. This new approach will pave the way for a better comprehension of cell heterogeneity and may reveal new molecular states and subpopulation-specific responses to external perturbations, such as cancer cells.

[1] Saelens, W., Cannoodt, R., Todorov, H. et al.A comparison of single-cell trajectory inference methods. Nat Biotechnol 37, 547–554 (2019). https://doi.org/10.1038/s41587-019-0071-9 [2] Wu, F., Fan, J., He, Y. et al.Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. Nat Commun 12, 2540 (2021). https://doi.org/10.1038/s41467-021-22801-0 [3] Wolf, F.A., Hamey, F.K., Plass, M. et al. PAGA: graph abstraction reconciles clustering with trajectory inference through a topology preserving map of single cells. Genome Biol 20, 59 (2019). [4] McInnes, L, Healy, J, UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction, ArXiv e-prints 1802.03426, 2018

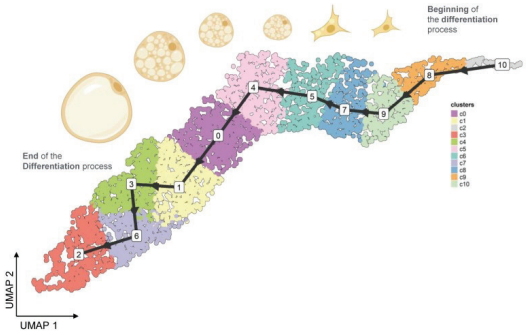

Figure 1. Trajectory inference methods allows cells to be reordered along one or more trajectories. Here the differentiation process of 3t3l1 could be reconstructed using Raman spectroscopy.

The importance of choosing the right protocol for leukemia cell studies using Raman imaging

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glutaraldehyde fixation, Raman imaging, T-ALL, PBMCs,

Leukemias are a very heterogeneous group of blood cancers. Different types of leukemia can be distinguished depending on specific mutations causing malignancy. Razing correct diagnosis is crucial in order to apply the appropriate treatment. Nowadays, diagnostic tests are based mainly on the assessment of the morphology of bone marrow cells. It is also believed that Raman spectroscopy could be an efficient technique supporting the rapid diagnosis of leukemia due to high chemical sensitivity. In routine clinical practice, blood or bone marrow cells collected from patients are subjected to multiple external factors that influence its quality and resulting from the collection, transportation and storage. Therefore, analysis of living cells is not always possible and studies of fixed cells are necessary. Due to the high sensitivity of Raman spectroscopy, sample preparation can affect the results obtained [1–3] and the protocol of preparation should be chosen with care and optimized, particularly in the context of clinical practice [4]. In our studies, we tested the influence of GA fixation [5] at different concentrations (0.1%, 0.5% and 2.5% GA) on the molecular structure of leukemia cells (T cell acute lymphoblastic leukemia (T-ALL)) and normal peripheral blood mononuclear cells (PBMCs) with the use of Raman micro-imaging. The aim of our studies was to identify the most optimal concentration of GA in order to detect spectral markers related to oncogenesis in the future. Results of our studies show a change in the protein secondary structure manifested by the increase of the intensity of the band at 1041 cm-1, characteristic for phenylalanine [5,6] in spectra of cells fixed with higher GA concentration. GA at a concentration of 0.5% was showed to be the most optimal in fixing both normal and cancer cells. We also tested the chemical stability of fixed cells within 11 days of storage. 0.1% concentration of GA was found to be insufficient to preserve the molecular structure of PBMCs over time. In order to store cells and ensure their high survival rate, banking in the vapor phase of the liquid nitrogen is used. In our studies, we tested the influence of preculturing of cells for 72h after thawing. In the case of cells fixed with 0.5% GA, no significant impact was detected on spectral profiles of T-ALL cells. Our results show that chemical procedures applied in the preparation of cells may affect Raman spectra and influence the identification of spectroscopic markers characteristic for different subtypes of leukemia.

The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

[1] A.J. Hobro, N.I. Smith, Vib. Spectrosc. 91 (2017) 31–45. https://doi.org/10.1016/j.vibspec.2016.10.012. [2] A.D. Meade, C. Clarke, F. Draux, G.D. Sockalingum, M. Manfait, F.M. Lyng, H.J. Byrne, Anal. Bioanal. Chem. 396 (2010) 1781–1791. https://doi.org/10.1007/s00216-009-3411-7. [3] E. Gazi, J. Dwyer, N.P. Lockyer, J. Miyan, P. Gardner, C. Hart, M. Brown, N.W. Clarke, Biopolymers. 77 (2005) 18–30. https://doi.org/10.1002/bip.20167. [4] N. Chaudhary, T.N. Que Nguyen, A. Maguire, C. Wynne, A.D. Meade, Anal. Methods. (2021) 1019–1032. https://doi.org/10.1039/d0ay02040k. [5] E. Bik, A. Dorosz, L. Mateuszuk, M. Baranska, K. Majzner, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 240 (2020) 118460. https://doi.org/10.1016/j.saa.2020.118460. [6] B. Hernández, F. Pflüger, S.G. Kruglik, M. Ghomi, J. Raman Spectrosc. 44 (2013) 827–833. https://doi.org/10.1002/jrs.4290.

Temperature perturbation as a tool to enhance near-infrared spectroscopic discrimination of geographical origins of agricultural products

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Near-infrared (NIR), discriminant analysis, geographical origins, two-trace two-dimensional correlation analysis (2T2D), temperature perturbation

To improve accuracy for near-infrared (NIR) spectroscopic discrimination of geographical origins of agricultural products, a strategy of combining temperature-induced NIR spectra and two-trace two-dimensional correlation (2T2D) analysis has been attempted. 2T2D correlation analysis was employed to sensitively recognize dissimilar temperature-driven spectral feature, which would be more sample-to-sample discriminant. For the study, 3 different agricultural products (red pepper, bracken, garlic) were prepared including 50 imported and 50 domestic samples for each product. Then, diffuse reflectance NIR spectra of the samples were collected at collected at 20 and 40°C. Initially, principal component analysis (PCA) was separately performed using the spectra collected 20 and 40°C, the two-group discriminability of each case in a subsequent principal component (PC) domain was evaluated. Next, 2T2D analysis was performed using the temperature-varied spectra at both temperatures. The resulting 2T2D slice spectra were used for PCA to evaluate two-group discriminability in the corresponding PC domain compared to those of constant-temperature measurements.;

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Application of multivariate statistics and 2D infrared correlation spectroscopy in the investigation of drying process of propolis

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propolis, 2D correlation spectroscopy, multivariate statistics

Propolis is a substance known as the bee glue, which is a generic name that refers to the resinous substance accumulated by the bees from various types of plants. Propolis functionality includes: sealing holes and cracks in the reconstruction of the beehive, smoothing the inner surface of the beehive, retaining the hive's internal temperature, preventing weathering and invasion by predators, hardening the cell wall and contribution to an aseptic internal environment. In the apiculuture, propolis and its extracts have numerous applications in treating various diseases due to its antiseptic, anti-inflammatory, antioxidant, antibacterial, antimycotic, antifungal, antiulcer and anticancer properties. Fourier-transform infrared spectroscopy (FT-IR) and two-dimensional infrared (2D IR) correlation spectroscopy were applied to analyze the process of drying propolis samples. 2D IR correlation analysis not only resolves overlapping peaks in the infrared spectrum, but also probes the sequential order of spectral intensity changes during the measurements. Obtained results suggest that 2D infrared correlation spectroscopy is a direct and effective technique for the analysis of the components of propolis and their changes in time. Standard for 2D infrared correlation spectroscopy analysis of synchronous and asynchronous spectra in conjunction with multivariate statistical analysis provides better insight into the drying process of propolis.

[1] da Silva, C., Prasniewski, A., Calegari, M.A. et al. Determination of Total Phenolic Compounds and Antioxidant Activity of Ethanolic Extracts of Propolis Using ATR–FT-IR Spectroscopy and Chemometrics. Food Anal. Methods 11, 2013–2021 (2018) [2] Noda I. Recent advancement in the field of two-dimensional correlation spectroscopy Journal of Molecular Structure, 883 pp. 2-26 (2008) [3] Geitner R., Fritzsch R., Popp J., Bocklitz T.W. corr2D: Implementation of Two-Dimensional Correlation Analysis in R. Journal of Statistical Software, 90(3), 1-33. (2019)

How many components do ideal binary liquid mixtures have?

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Beer Approximation, 2D-Correlation Spectroscopy, Principal component analysis, Multivariate Curve Resolution

It is believed that for ideal mixtures Beer's approximation is valid,[1] i.e. the absorbance of the mixture is assumed to be linearly dependent on the spectra of the pure components weighted by their volume fractions. Accordingly, if the spectra series would be investigated by 2D-correlation spectroscopy (2D-COS), the asynchronous map would be the null spectrum. Principal component analysis (PCA) would show as many principal components as there are chemical components in the mixture, apart from those that consist of noise. The number of these components would allow to apply multiple curve resolution (MCR), which would reveal any complexes that form in the mixture. For ideal mixtures the expectation is that the number of different species of formed complexes equals zero. We investigated (quasi-)ideal mixtures of Benzene-Toluene, Benzene-Cyclohexane and Benzene-Carbon tetrachloride. For none of these systems, the asynchronous map equals the null spectrum. In each case, PCA determines at least 3 components and MCR provides spectra for these third components which simply consist of linear combinations of the two other components. The latter results are not meaningful, since in each case a complex would have a lower symmetry than its components and new vibrations should become IR-active. If the spectra of mixtures are forward calculated using the Lorentz-Lorenz relation,[2] the so-calculated asynchronous maps and the first three principal components as well as the third component spectra are similar to those obtained experimentally. However, band shifts and intensity changes are more complex in the experimental spectra, revealing that infrared spectra are sensitive to short-range order. Accordingly, they are influenced by the 3D-structure of the molecules and the anisotropy of their frequency-dependent complex polarizability. As a consequence, the results of conventional chemometric tools that are based on Beer's approximation like PCA and MCR must be interpreted with care.

[1] Z. Xiong, F. Pfeifer, and H. W. Siesler. "Evaluating the Molecular Interaction of Organic Liquid Mixtures Using Near-Infrared Spectroscopy". Appl. Spectrosc. 2016. 70(4): 635-644. [2] T. G. Mayerhöfer and J. Popp. "Beyond Beer's Law: Spectral Mixing Rules". Appl. Spectrosc. 2020. 74(10): 1287-1294. [3] T. G. Mayerhöfer, O. Ilchenko, A. Kutsyk, J. Popp. „Beyond Beer's law: Ideal binary liquid mixtures". 2021, in preparation.

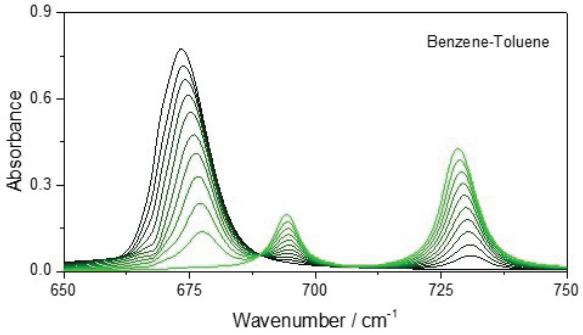

Figure 1. IR absorbance spectra of mixtures of Benzene and Toluene

Optimal pre-processing of Raman spectra for discrimination purposes through genetic algorithms

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[2]Department of Chemistry, University of Torino, Italy
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Raman spectroscopy, Genetic algorithm, Variance components, Likelihood ratio, Forensic applications

Despite the ease of visualization, spectroscopic data requires a lot of effort to turn the raw data into more meaningful information that better fits for the purpose of data analysis. This applies to appropriately tailored preparation of the signals, called preprocessing, whose aim is to remove unwanted variation and deteriorating effects (noise or baseline drift) and standardize the signals through denoising, smoothing, baseline correction, and normalization operations [1]. Preprocessing is considered a critical step since it may damage even the most promising statistical models whenever improperly conducted, but conversely it guarantees success when adequately chosen. Establishing the appropriate sequence of the applied methods for spectra correction as well as suitable assortment of their parameters are tightly dependent on the goals of further analyses. These goals are mostly addressed to discrimination or classification of the samples, which is easy when the distances between the group means are much wider than those among the data within the groups, i.e. the variation of the group means (B) is greater than the variation of the data in the groups (W), averaged over all groups. This also applies in forensics when evidence materials are compared to conclude whether they may have originated from the same source or not. Aim of the present research was to verify if the B/W ratio parameter can represent an objective measure of the goodness of the preprocessing strategies, when the goal is to study the samples similarities to establish if they may be members of the same class. B/W was estimated from regularized MANOVA [2], where the regularization process makes it feasible for finding the latent variables from highly multivariate data along which the classes are maximally separated. Searching for optimal preprocessing methods to yield the highest B/W was conducted either through the grid search process, where B/W is computed for each preprocessing strategy, or executed using less time-consuming heuristic alternative such as genetic algorithms [3]. The adequacy of the procedure for finding the optimal preprocessing strategy was successfully evaluated in the studies aiming at the comparison of Raman spectra from blood traces to establish if they are of the same age and Raman spectra from car paints to conclude if they may come from the same car. The solutions were delivered by the likelihood ratio models, which are an adequate tool for forensic investigations whether the samples share common membership [4,5].

We would like to thank Prof. Gianmario Martra for his invaluable contribution to the research.

[1] J. Engel et al. Anal. Chim. Acta, 899 (2015) 1–12. [2] J. Engel, L. Blanchet, B. Bloemen, L. Heuvel, U. Engelke, R. Wevers, L. Buydens, Anal. Chim. Acta, 899 (2015) 1–12. [3] R. Leardi, J. Chromatogr. A, 1158 (2007) 226–233. [4] G. Zadora, A. Martyna, D. Ramos, C. Aitken, Evidential Value of Multivariate Physicochemical Data, Wiley, 2014. [5] A. Martyna, A. Menżyk, A. Damin, A. Michalska, G. Martra, E. Alladio, G. Zadora, Chemometr. Intell. Lab. Syst., 202 (2020) 104029

Using Raman spectra as digital fingerprints for biomedicines

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vaccine, biomedicine, classification, standardisation, quality control

Raman spectroscopy has proven its value in the identification of chemical compounds with applications in the quality control of pharmaceutical products. This non-destructive, rapid and cost-efficient method bears equal potential for biomedicines like vaccines or therapeutic allergens. These are complex biochemical formulations with multiple components and often inherent variability. However, biomedicines also introduce new challenges to spectroscopic methods in terms of experimental setup and standardisation: liquid formulations typically show strong signals from water and excipients. Turbid formulations as can be found in vaccines show temporal variations due to sedimentation over spectral acquisition times that are required to ensure adequate signal to noise ratios. Therapeutic allergen products are derived from biological sources with inherent variability with excipients contributing to the spectral signal. The often subtle differences of interest between product spectra further urge the need for careful, standardised and transparent analytical procedures in spectral pre-processing and subsequent training and validation of classification models. These requirements guided the choice of R as an OpenSource data analysis platform for our approach. We will present our findings on vaccines [1] and therapeutic allergen products showing that these products can be distinguished by spectroscopic methods with high sensitivity and specificity despite experimental and analytical challenges. This encourages the refinement of the method aiming at the definition of product specific spectroscopic fingerprints. Rapid methods for the identification and quality control of biomedicines as presented here are particularly relevant for urgently needed medicines as shortages may lead to a rising circulation of fraudulent products. While currently COVID-19 vaccines are a paramount example of use we will conclude with an outlook of further application fields.

[1] C. Kamp, B. Becker, W. Matheis, V. Öppling, I. Bekeredjian-Ding, How to draw the line – Raman spectroscopy as a tool for the assessment of biomedicines, Biol. Chem. Epub ahead of print, 2021

Cocrystal Formation between Ibuprofen and Nicotinamide Revealed Using THz and IR Spectroscopy with Multivariate Analysis

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cocrystal, multivariate analysis, terahertz spectroscopy, IR spectroscopy, MCR-ALS

Introduction: Even if the candidates possess superior pharmacological properties, the compounds deviate from the pharmaceutical development because of their poor water solubility. Cocrystals are a molecular complex of active pharmaceutical ingredients (APIs) with a highly water-soluble cocrystal former (coformer). Crystallization can be used to enhance the solubility of APIs. However, since a cocrystal is constructed by molecules with two or more components, its formation process is complicated. In this study, we used Terahertz (THz) and infrared (IR) spectroscopy to characterize cocrystallization between ibuprofen (IBF) and nicotinamide (NCT). Multivariate curve resolution with alternating least squares (MCR-ALS) was applied to time-dependent THz and IR spectra to identify the intermolecular interactions between these cocrystallizing species. Materials and Methods: Racemic IBF as an API, and NCT as a coformer were used. An equimolecular physical mixture (PM) of IBF and NCT was prepared by mixing the two compounds. 200 mg tablets were formed by pressing PM using a tableting machine. Three tablets were kept in a container. The relative humidity (RH) inside the container was held at 0, 30 or 75%RH, and the container was stored in an oven at 60 &ordm C. The stored tablets were measured regularly by THz and IR spectroscopy. Results and Discussion: IR spectra analysis by MCR-ALS: The reaction rate of the intermolecular N-H···O hydrogen bond at 0% RH stagnated at 63%. Eight N-H···O hydrogen bonds originating from NCT dimers, and four N-H···O hydrogen bonds between NCT and IBF, were present in one unit cell. The formation of N-H···O hydrogen bonds seems to be stopped at around 66% completion, because the formation of hydrogen bonds between NCT and IBF did not proceed under low humidity conditions. THz spectra analysis by MCR-ALS Under all humidity conditions, about half of the reactions proceeded until the first sampling point after the start of storage. However, after 50% completion, the reaction rate increased with increasing humidity. It is likely that enantiomeric differences affect the reaction rate of racemic IBF with NCT. Thus, the following reaction process can be proposed. R-IBF, which seems to interact more strongly with NCT, forms the initial R-IBFNCT cocrystal. Conclusion The analytical results revealed cocrystal formation through a two-step reaction, in which the steps were dominated by thermal energy and water vapor, respectively. It is inferred that the activation energy of cocrystal formation was significantly reduced by the presence of water.

Overfitting One-Dimensional Convolutional Neural Networks for Raman Spectral Identification

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Machine Learning for Chemometrics, One-Dimensional Convolutional Neural Network for Pattern Recognition, Deep Learning and AI, Vibrational Spectroscopy, Analytical Methods for Raman Spectroscopy

Dedicated handheld spectrometers have been adopted by first responders and law enforcement agencies for in situ identification of unknown substances. Real-time spectral matching process is a pixel-by-pixel comparing of the unknown spectra with reference data. In fact, the success rate of this process using a miniaturized portable Raman spectrometer relies mainly on the variety of reference data carried on the memory. This is a hurdle in miniaturizing and affordability of the current handheld spectrometers due to limited memory and computational power. In this study, we aim to mitigate this issue by utilizing the power of one-dimensional Convolutional Neural Networks (1DCNN) trained on millions of Raman spectra augmented from standard available reference databases. Specifically, an intentionally overfitted 1DCNN model can be substituted with the reference database of handheld spectrometers to alleviate the memory size and increase the identification process speed and accuracy. Our experimental results revealed that 1DCNN could identify one pure unknown Raman instance from thousands of classes with a high accuracy without the requirement of any data preprocessing.

A mystery of an outer potential well of the lowest Rydberg state of CdAr complex

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van der Waals complex, molecular beam, neural network, genetic algorithm, laser-induced fluorescence

It has long been known that the $E^3\Sigma^+_1(6^3S_1)$, the lowest Rydberg state potential of CdAr van der Waals complex, possesses a double-well structure [1]. However, until now the shallow outer well (E_{out}) has not been fully characterized (Fig. 1a). Hitherto, the well depth, the bond length and parameters of a potential barrier between the two wells have been only estimated [2]. However, the necessary spectroscopic information is encoded in laser-induced fluorescence (LIF) excitation spectra obtained experimentally. In the experiment, CdAr complexes are cooled in a supersonic beam to their lowest ground-state vibrational levels and then excited from the ground state in optical-optical double resonance (OODR) process (Fig. 1b). Next, LIF excitation spectra of the $E^3\Sigma^+_1 \leftarrow B^3\Sigma^+_1(5^3P_1)(\upsilon''=0\text{-}7)$ transitions consisting of both vibrational bound ← bound and free ← bound parts are recorded (Fig. 1c). Having had the intermediate $B^3\Sigma^+_1$ -state potential well-characterized, the information on the E_{out}'s bond length is obtained from the former, whereas the potential barrier shape is modelled from the latter (Fig. 1e). Spectroscopic information from both parts of experimental data are not directly available, though. Complete characterization of the $E^3\Sigma^+_1$ -state outer well requires sophisticated tools to reconstruct the respective parts of the potential. Thus, in our studies we introduced genetic algorithm and neural network methods (Fig.1d). Fig. 1. General idea of the presented study. a) The main goal of the experiment – complete characterization of the $E^3\Sigma^+_1(6^3S_1)$ – state outer well in CdAr. b) The experiment – laser spectroscopy of the molecules in a molecular beam. c) LIF excitation spectra of the $E^3\Sigma^+_1 \leftarrow B^3$ Σ$^+_1(5^3P_1)$ ($\upsilon'' = 0\text{-}7$) transitions consisting of free ← bound (black line) and a bound ← bound parts (red line). d) Genetic algorithm as a method for obtaining spectroscopic parameters of Eout from LIF spectra. e) Ab-initio calculated $E^3\Sigma^+_1$ - state [3] (for the sake of clarity, the Eout well depth is multiplied by 3).

[1] E. Czuchaj, M. Krośnicki, H. Stoll, Theor. Chem. Acc. 105 (2001) 219. [2] T. Urbańczyk, J. Koperski, Chem. Phys. Lett. 640 (2015) 82.
[3] M. Krośnicki, A. Kędziorski, T. Urbańczyk, J. Koperski, Phys. Rev. A 99 (2019), 052510.

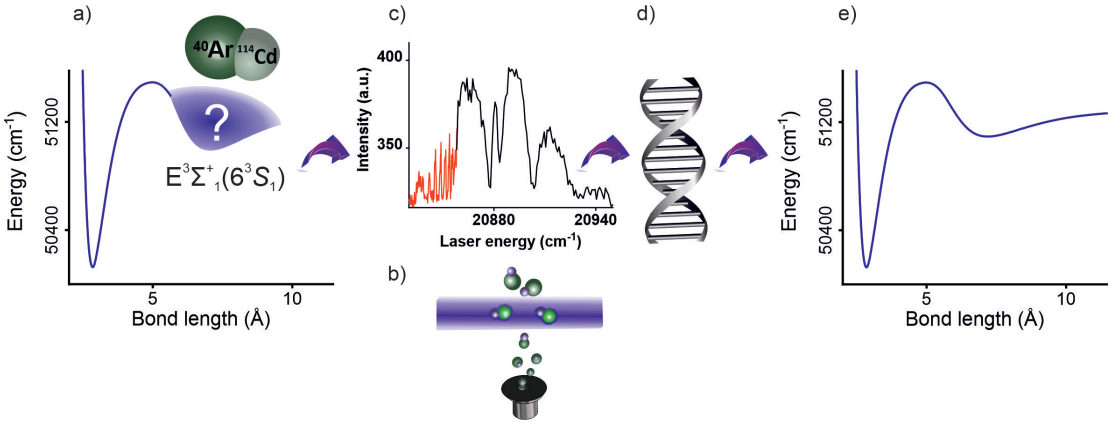

Figure 1. General idea of the presented study. a) The main goal of the experiment – complete characterization of the E3Σ+1(63S1) – state outer well in CdAr. b) The experiment – laser spectroscopy of the molecules in a molecular beam. c) LIF excitation spectra of the $E^3\Sigma^+1 \leftarrow B^3\Sigma^+1(5^3P_1)(\upsilon''=0\text{-}7)$ transitions consisting of free ← bound (black line) and a bound ← bound parts (red line). d) Genetic algorithm as a method for obtaining spectroscopic parameters of Eout from LIF spectra. e) Ab-initio calculated $E^3\Sigma^+1$ – state [3] (for the sake of clarity, the Eout well depth is multiplied by 3).

FT-IR imaging of tissues: a comparison of spectral regions on accuracy of tissue structures classification

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FT-IR imaging, tissue classification, machine learning, histology

Fourier-transform infrared spectroscopy in combination with machine learning and chemometrics is an intensively developed, powerful tool for investigation of tissue biochemical composition with simultaneous microscopic visualization. Tissue classification using high wavenumber region of the IR spectrum has high potential in clinical application, due to ability of using inexpensive glass slides. Therefore, the potential of two mid-infrared spectral regions: "fingerprint" (1900-800 cm^{-1}) and high wavenumbers (4000-2700 cm^{-1}) was explored in pancreatic tissue structures prediction with random forest classification. The effect of spatial resolution was also examined (high and standard definition images). The same number of metrics (112) was generated for two mid-infrared regions based on band maximum value, band integration and center of band gravity. Classification at the pixel level with fivefold cross-validation for 29 patients was done to separate three structures/classes: lobules, fibers and blood. For high wavenumber data sets, model properly classified even small fibers, at the same time "fingerprint" data sets provides better classification results for blood cells. True Positive Rate (TPR) values of HD model for high wavenumber region were: 95.5% for lobules, 94.5% for fibers and 24.3% for blood. For "fingerprint" region: 97.3, 95.5 and 74.3%, respectively. The data exploration showed that model with "fingerprint" region gave good prediction results for all classes, independently from imaging definition. At the same time, high wavenumber region had higher ability to predict small tissue structures, even separate single blood cells, what was clearly visible on classification images. In Figure H&E E image of analyzed biopsy, HD image for amide A band (3282 cm-1) and classifications results of fivefold cross-validation for HD and SD predicted images are showed*. *Note: Based on "Spatial sampling effect on data structure and Random Forest classification of tissue types in High Definition and Standard Definition FT-IR imaging" by P. Liberda, K. Kosowska, P. Kozioł, T.P. Wróbel 2021, Chemom. Intell. Lab. Syst. (in review). Acknowledgements: This research was supported the National Science Centre, Poland (Grant No. 2018/31/D/ST4/01833). This research was performed using equipment purchased in the frame of the project co-funded by the Malopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project no. MRPO.05.01.00-12-013/15.

This research was supported the National Science Centre, Poland: Grant No. 2018/31/D/ST4/01833

[1] M. Fanous, A. Keikhosravi, A. Kajdacsy-Balla, K.W. Eliceiri, G. Popescu, Quantitative phase imaging of stromal prognostic markers in pancreatic ductal adenocarcinoma, Biomed. Opt. Express. 11 (2020) 1354. https://doi.org/10.1364/boe.383242. [2] S. Mittal, R. Bhargava, A comparison of mid-infrared spectral regions on accuracy of tissue classification, Analyst. 144 (2019) 2635–2642. https://doi.org/10.1039/c8an01782d [3] P. Koziol, M.K. Raczkowska, J. Skibinska, S. Urbaniak-Wasik, C. Paluszkiewicz, W. Kwiatek, T.P. Wrobel, Comparison of spectral and spatial denoising techniques in the context of High Definition FT-IR imaging hyperspectral data, Sci. Rep. 8 (2018) 1–11. https://doi.org/10.1038/s41598-018-32713-7.

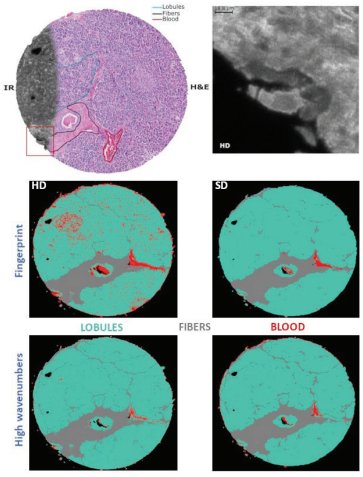

Fig. 1 Classification results

Optimization of discrimination models based on IR chemical imaging with different systems and configurations

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FT-IR spectroscopy, QCL, data preprocessing, interference effect, Random Forest

Fourier Transform Infrared Imaging (FT-IR) supported by machine learning algorithms is a great tool in tissue types and cancerous patient classification. Nevertheless, a method needs; to meet a few conditions to be applicable in the clinic. The most basic condition results from histopathological and histological classification models prediction abilities and robustness. The second one is connected with measurement time – a system equipped with a quantum cascade laser (QCL) allows to measure only chosen frequencies which influences measurement time reduction. However, biochemical information content about measured tissue is lower than in the case of FT-IR system which can influence classification result. In our research, we optimized and translated the esophagus biopsies classification model from FT-IR modality to QCL modality with very high AUC values (>0.95) [1]. To be applicable in the clinic, the technique should be also relatively inexpensive in everyday workflow. Sample can be measured with IR imaging in two modes: transmission and transflection. Spectra obtained with transmission mode are of better quality but sample carriers that are transparent for IR radiation are very expensive (20-80$ per piece). In the case of transflection mode, inexpensive low-e slides (2$ per piece) are applicable but additional spectral distortion coming from interference occurs in the spectra which also can influence classification result. In order to investigate the degree of that effect in a quantitative manner, we measured with FT-IR imaging pancreas Tissue Micro Array (TMA) embedded in paraffin and after deparaffinization in both measurement modes [2]. Results of this research have shown that even though in transflection mode larger variability coming from measurement is present, machine learning algorithms allow creating very promising histological classification models for all sample and measurement configurations. Finally, spectra collected with FT-IR imaging are not devoid of distortions coming from noise, scattering or thickness. Consequently, to build robust models which provide relevant classification results, methods applied in each preprocessing step: denoising, baseline correction, and normalization need to be optimized. Results of our research show that the biggest impact on the classification of prostate cell lines using IR spectra has the baseline correction step [3]. Taking into account the above it is clear that data preprocessing and classification models creation need to be optimized for a specific system, modality, and biopsy type.

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[1] Liberda, D., Hermes, M., Koziol, P., Stone, N. & Wrobel, T. P. Translation of an esophagus histopathological FT-IR imaging model to a fast quantum cascade laser modality. J. Biophotonics 1–9 (2020) doi:10.1002/jbio.202000122. [2] Liberda, D., Koziol, P., Raczkowska, M. K., Kwiatek, W. M. & Wrobel, T. P. Influence of interference effects on the spectral quality and histological classification by FT-IR imaging in transflection geometry. Analyst (2020) doi:10.1039/d0an01565b. [3] Liberda, D.; Pięta, E.; Pogoda, K.; Piergies, N.; Roman, M.; Koziol, P.; Wrobel, T.P.; Paluszkiewicz, C.; Kwiatek, W. M. The Impact of Preprocessing Methods for a Successful Prostate Cell Lines Discrimination Using Partial Least Squares. (2021).

A Multiple Model Strategy for Improved Fentanyl Quantification Using Infrared Absorption Spectroscopy

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Drug checking, Fentanyl, Chemometrics, Machine learning, Infrared absorption spectroscopy

Fentanyl has continued to be a primary contributor to overdose deaths amid the current opioid epidemic and is being increasingly found in the illicit drug supply [1,2]. Its accurate quantification is therefore of interest to people who use drugs [3]. Six machine learning models were evaluated in their ability to predict fentanyl mixtures using infrared absorption spectroscopic data, including partial least squares regression, principal component regression, random forest regression, AdaBoost regression, gradient boosting regression, and an integrative voting regressor model. Models were compared in their performance on training data and displayed no significant differences in their relative predictive abilities through the use of a CV-ANOVA one- way test. Models were then compared based on their relative performance in predicting samples collected at a point-of-care drug checking service. The integrative voting model had the best overall performance while including an inherent strategy for indicating model suitability through analyzing the level of agreement between the individual predictions of the base regressor models. This use of multiple models is proposed as a strategy for more robust fentanyl prediction in a drug checking service utilizing IR absorption spectroscopy.

This project was funded by a grant from the Health Canada Substance Use and Addictions Program.

[1] Escalating BC's response to the overdose emergency, Tech. rep., BC Ministry of Mental Health and Addictions (February 2019). [2] BC Coroners Service, Illicit drug toxicity type of drug data, Tech. rep., Ministry of Public Safety & Soliciter General, Victoria, BC (2020). [3] M. Karamouzian, K. Papamihali, B. Graham, A. Crabtree, C. Mill, K. Margot, Y. Sara, J. A. Buxton, Known fentanyl use among clients of harm reduction sites in British Columbia, Canada, Int. J. Drug Policy 77 (2020) 102665.

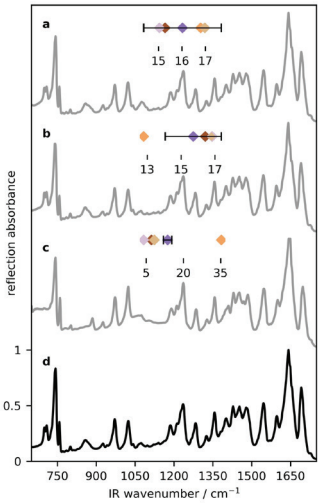

Figure 1. Multi-model predictions

Fentanyl Detection and Quantification using Portable Raman Spectroscopy in Community Drug Checking

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Drug checking, Raman spectroscopy, partial least squares regression, fentanyl, harm reduction

In North America there is an ongoing overdose crisis that has largely been marked by the introduction of potent synthetic opioids, such as fentanyl, into the illicit drug market, raising questions of how to improve safer supply initiatives.[1] Within this context, several community-based drug checking projects have begun exploring the use of portable technologies as an overdose prevention measure.[2,3] Here, we develop a model, using a portable Raman spectrometer and the statistical method of partial least squares regression, to quantify fentanyl in both powder binary mixtures and more complex ternary mixtures. The model is then applied to samples collected over a two-year period while operating a drug checking service. As an unpredictable drug supply will always pose a risk for quantification with portable drug checking technologies, we implement check steps that guide the harm reduction decisions and conversations surrounding quantitative results. This work presents an important step in the use of portable Raman spectrometers for quantitative work and focuses its assessment on drug checking and the overdose crisis.

This project was funded by a grant from the Health Canada Substance Use and Addictions Program.

[1] T. Kerr, J. Epidemiol. Community Health 2019, 73, 377. [2] M. K. Laing, K. W. Tupper, N. Fairbairn, Int. J. Drug Policy 2018, 62, 59.
[3] J. J. Palamar, A. Salomone, M. J. Barratt, Curr. Opin. Psychiatry 2020, 33, 301.

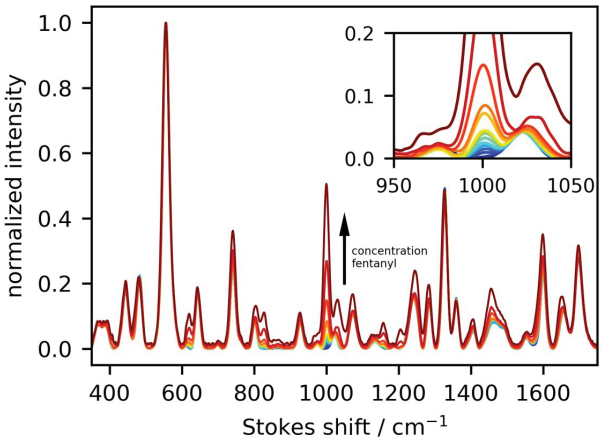

Figure 1. Fentanyl mixtures

Classification of individual grass pollen grains embedded in paraffin using FTIR microspectroscopy and non-negative matrix factorization

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FTIR microspectroscopy, chemometrics, machine learning, classification, pollen

Grass pollen grains from different species have similar morphologies, so the common identification using light microscopy is difficult. Fourier-transform infrared (FTIR) spectroscopy is more suitable and shows promising results. Nevertheless, the acquisition of reliable FTIR spectra of single pollen grains is challenging due to a strong contribution from Mie scattering that occurs by measurements of the micron-sized particles[1]. A promising approach for the measurement of reliable spectra is the embedding of the pollen grains in paraffin, which on the one hand reduces the scattering artifacts but on the other hand adds the spectral contribution from the paraffin[2]. Here, grass pollen grains from five different species of the Poaceae family were embedded in paraffin, and their single-grain spectra were obtained by FTIR microspectroscopy. Using non-negative matrix factorization (NMF), the different spectral contributions can be separated from one another, which enables the analysis of the paraffin and the pollen signals individually. By using the pollen spectra contribution, classification regarding the different pollen species is possible using partial least square-discriminant analysis (PLS-DA) and machine learning approaches, including artificial neural networks and random forest analysis[3]. The NMF-based decomposition of the spectra shows the great potential of FTIR microspectroscopy for automated identification and characterization of single pollen grains and the analysis of micron-sized particles in general.

[1] P. Bassan P. Gardner. Moss D, editor. Cambridge: Royal Soc Chemistry; 2011. 260-276 p. [2] B. Zimmerman, V. Tafintseva, M. Bagcioglu, M. Hoegh Berdahl, A. Kohler. Anal Chem. 2016; 88(1):803-11. [3] Diehn S, Zimmermann B, Tafintseva V, Ba?c?o?lu M, Kohler A, Ohlson M, Fjehlheim S., Kneipp, J. Anal Bioanal Chem. 2020 ;412(24):6459-74

Fentanyl Quantification using Portable Infrared Absorption Spectroscopy. A Framework for Community Drug Checking

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infrared spectroscopy, fentanyl, drug checking, chemometrics

Infrared absorption spectroscopy has been used as part of a multi-technology approach implemented in a community drug checking project in Victoria, BC, Canada. As most synthetic opioids are either binary mixtures of fentanyl and caffeine, or ternary mixtures of fentanyl with caffeine and a sugar alcohol, standards consisting of such mixtures have been prepared and were used to train a partial least squares regression model to quantify the fentanyl concentration in the mixture. The model was then used to evaluate the fentanyl concentration of samples brought to the service by people who use drugs. This demonstrates that portable infrared absorption spectroscopy can be used not only to identify components in drug mixtures, but also to determine concentrations of fentanyl in complex mixtures as seen in illicit opioids.

SERS and PLS - an ideal marriage?

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Partial Least Squares Regression, Leave-One-Out cross-validation, SERS, DNA damage, Double Strand Breaks

Double Strand Breaks (DSBs) are the most dangerous type of DNA damage. Unrepaired DSBs might be responsible for cell death, translocations, or mutations that cause cancer. [1] Therefore, their repair is the key mechanism maintaining genomic integrity in all life forms. Current knowledge on the mentioned mechanisms is still incomplete due to the insufficient sensitivity of classical analytical methods. [2] In order to increase the strength of classical molecular spectroscopy to track local modifications of DNA chemical structure during induction of damage and its repair, the Surface- enhanced Raman Spectroscopy and multivariate statistical analysis were used. Before the multivariate statistical analysis, the SERS spectra of DNA plasmid treated with bleomycin were baseline corrected, smoothed and normalized in the fingerprint spectral range -unique for biological molecules. Partial Least Squares Regression (PLSR) was performed on the averaged spectra for each corresponding number of DSBs induced by bleomycin molar concentration with Leave-One-Out cross-validation. In order to determine the optimal number of Latent Variables (LVs) of the model, the weight randomization test was performed [3]. The strong correlation between the predicted and observed number of DSBs is prominent for the model. All distinct features present in the PLSR β plots were assigned according to the literature. To determine the level of contribution of specific band in discrimination, the Variable Importance in Projection (VIP) scores were considered. This approach allows to indicate the specific peak assignment during the analysis. Results suggested a strong correlation between the predicted and observed response, therefore the changes in DNA conformation caused by the interaction with bleomycin can be forecasted. Raman bands related to the ring breathing vibrations of adenine and cytosine may suggest that bleomycin induces not only DNA breaks but also ring modifications [4]. The important contribution is also related to the DNA conformation markers, suggesting conformational changes induced single and double strand breaks. Furthermore, the symmetric PO2 stretches have also the significant impact in the analysis suggesting the DNA conformation changes and oxidation of nucleic acids [5]. This research shows that SERS studies of DNA treated by bleomycin are sensitive enough to discriminate the local changes in the DNA conformation, that cannot be detected with conventional spectroscopic tools due to the lack of sufficient sensitivity.

National Science Centre, Poland: the "OPUS 16" project (Reg. No. UMO-2018/31/B/ST4/02292)

[1] P.S. Jackson et al., Nature 2009, 1071 [2] K. Sofińska et al., Molecules 2020, 25, 3 [3] Tran, T et al., Journal of Chemometrics. 2017, 31, 5
[4] M. Banyay et al., Biophys. Chem. 2003, 104 [5] E. Benedetti et al., Appl. Spectrosc. 1997, 51, 6

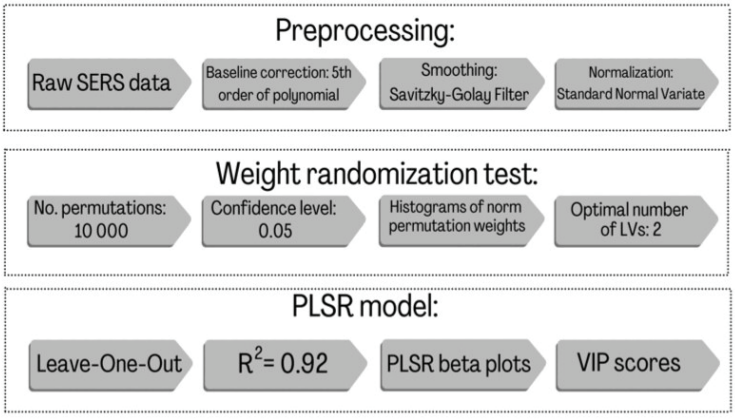

Figure 1. Scheme of the analysis performed on the raw SERS spectra in order to build the PLSR model.

1.11. Photothermal and Photoacousting imaging

Combining infrared microspectroscopy and neurobiology for understanding Alzheimer's disease.

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O-PTIR, SNOM, Amyloid aggregtion

Alzheimer's disease affects the lives of millions of people worldwide, already costing about 1 % of the global economy. Alzheimer's disease is characterized by the formation of protein amyloid aggregates, so-called amyloid oligomers. These oligomers are characterized by β-sheet structures and are thought to be neurotoxic. However, the secondary structure of amyloid oligomers is polymorphic and the structure that contributes most to the neurotoxicity is unknown. This lack of knowledge exists mainly because it is incredibly challenging to characterize the secondary structure of amyloid proteins directly in cells. To investigate molecular changes in proteins directly in cells, we used synchrotron-based infrared microspectroscopy, a label-free and non-destructive technique available for in situ molecular imaging. Specifically, we evaluated the formation of β-sheet structures in cells that simultaneously express human amyloid proteins, models of p amyloid aggregation related to Alzheimer's disease.

Single-Molecule Infrared Absorption Nanospectroscopy

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[1]Wageningen University

Infrared Nanospectroscopy, Photothermal Spectroscopy, Single-Molecule, Protein Science

Biological processes rely on a wide class of biomolecules that have nanoscale physical dimensions and whose function emerges from a correlation between their chemical and structural properties. A fundamental objective of modern analytical methods in physics, chemistry and biology is the comprehension of how the physical-chemical state and heterogeneity of biomolecules determine their role in cellular function and disease. Here, we show the development and application of photothermal infrared absorption nanospectroscopy (AFM-IR) as a real breakthrough for the analysis of heterogeneous biomolecules and their interactions from the single molecule [1-3] to the single cell and organism scale [4]. As a major advance in the field, we demonstrate the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power and short pulse infrared nanospectroscopy (ORS-nanoIR) [1-3]. Pushing the sensitivity of AFM-IR to its current limit, we prove the determination of the secondary structure of single proteins molecules and amyloid self-assemblies, which are involved in the onset of neurodegenerative disorders, with similar accuracy as obtained in bulk by FTIR [1-2]. Our approach further enables the unravelling of the molecular interaction fingerprint of amyloid species with a small molecule capable to prevent the disease in animal models of neurodegeneration [1]. Finally, we recently exploited this unprecedented sensitivity to unravel the surface properties of artificial model membranes [5] and the structure of functional protein self-assemblies as promising candidates for the development of novel class of biocompatible and sustainable biomaterials in bioscience [6-9]. Overall, our aim is to expand the capabilities of nanoscale vibrational spectroscopy to shed light on the structure-activity relationship of biomolecules for applications in nano-medicine, materials science and biotechnology.

[1] Ruggeri, Nature Comm., 2021. [2] Ruggeri, Nature Comm., 2020. [3] Adamcik, Ruggeri, Advanced Science, 2021. [4] Otzen, Small Methods, 2021. [5] Marchesi, Advanced Functional Materials, 2020. [6] Ramer*, Ruggeri*, ACS Nano, 2018. [7] Shen, Ruggeri, Nature Nanotechnology, 2020. [8] Qamar*, Wang*, Randle*, Ruggeri*, Cell, 2018. [9] Ruggeri, Nature Comm., 2015.

Review of Life Science Applications using submicron O-PTIR and Simultaneous Raman microscopy – A new paradigm in Vibrational Spectroscopy

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O-PTIR, Raman, Cells, Bacteria, Tissues

The recent advent of Optical Photothermal IR (O-PTIR) spectroscopy, has enabled for the first time, true submicron infrared microscopy in far-field reflection mode, generating "FTIR transmission-like" spectral quality, without spectral artefacts and distortions such as Mie Scattering associated with traditional FTIR or other emerging QCL based IR microscopy systems. Furthermore, it is now possible to combine O-PTIR with Raman for correlative IR & Raman microscopy. Photothermal spectroscopy is not new and has been exploited for decades with techniques such as PhotoAcoustic Spectroscopy (PAS) and AFM-IR (nano-IR). Where O-PTIR differs to is that it uses an optical (green laser) probe for detection, being analogous to the microphone in PAS and the AFM tip in AFM-IR. The use of this optical probe is the key enabling breakthrough in O-PTIR allowing for non-contact measurements, providing for advantages in capabilities relative to traditional FTIR/QCL microscopy but also in instrument architecture, thus enabling the first combined (correlative) IR and Raman (IR+Raman) platform that provides for simultaneous IR and Raman spectral information at the same time, from the same spot with the same submicron spatial resolution. These unique and exciting synergistic capabilities are now spawning interest in life science applications [1-2]. A broad range of life science applications, which are otherwise impossible with traditional FTIR/QCL microscopy, will be presented, ranging from live cell imaging in water, to ultra-high resolution images of breast tissue calcifications, amyloid aggregates in neurons (neurites and dendritic spines), individual collagen fibrils with polarized IR and individual isotopically labelled bacterial cells and more.

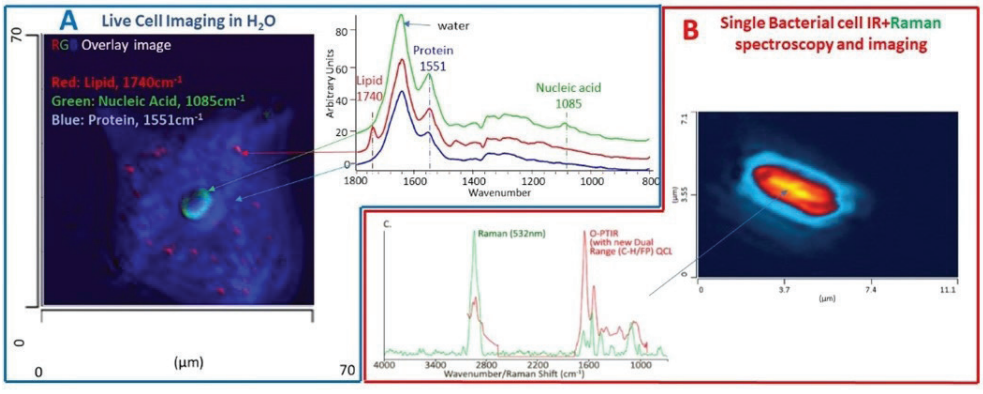

Figure 1. A) Live cell imaging in H2O B) O-PTIR image at 1650cm-1 of a single E.Coli bacterial cell, with simultaeous submicron IR+Raman spectra collected from the centre of the cell.

A novel approach to characterize a drug-loaded nanoparticle system by means of IR nanospectroscopy

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metal nanoparticle mono-layer, drug's adsorption, infrared nanospectroscopy, erlotinib

For years, the development of drug delivery systems is one of the directions to improve cancer therapy [1]. The use of vehicles that deliver the drugs to a specific target increases the treatment efficiency by controlling the drug accumulation inside the cancer cells. The main advantage of this strategy is to reduce the drug dose and prevent the bothersome side effects for the patient [2]. Nevertheless, there are still open questions concerning how the drug connects to the nanocarrier and how its conformation undergoes changes during this connection. It is extremely important since the drug conformation determines its biological activity [3]. The metal nanoparticles (NPs) allow performing the deep monitoring of how the drug adsorbs on their surface and estimate the stability of the formed conjugates [3,4,5]. In this study, atomic force microscopy in combination with infrared spectroscopy (AFM-IR) was applied for the first time to elucidate very locally how erlotinib (drug introduced in the non-small cell lung cancer therapy) interacts with the 15-nm silver and gold NPs deposited on solid substrates in the form of monolayers [6]. The two ways of the drug immobilization on these metal monolayers have been used. In the first approach, erlotinib was deposited dropwise on the silver and gold NPs, respectively. The obtained results show clearly how this therapeutic agent attaches to the applied NPs. What is more important, the recorded tapping AFM-IR maps show a unique visualization of the erlotinib distribution on this metal NP monolayers. The second approach was the deposition of the erlotinib molecules on the silver NP monolayer under flow conditions using quartz crystal microbalance (QCM) (the schematic view, Fig. 1). The next step was the characterization of this erlotinib/AgNPs/QCM sensor system employing the AFM-IR technique. Such procedure allowed for recording spectral data and disclosure of the obtained extreme signal enhancement for an ultra-thin single monolayer of erlotinib molecules adsorbs on the metal NP monolayer by AFM-IR. With this protocol, it is possible to determine the correlation between the mass of the adsorbed drug and coverage of the silver NPs, the binding energy of the formed drug molecules/nanoparticles connection, and the molecules/nanoparticles interaction. This ensures complex information about the designed drug-loaded nanocarrier system.

This work was supported by the National Science Centre Poland (Grant No. 2016/21/D/ST4/02178 to N.P.) and the French Government and the French Embassy in Poland. This research was partially supported by the Małopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007-2013, project No. MRPO.05.01.00-12-013/15 and the Paris Ile-de-France Region – DIM Matériaux anciens et patrimoniaux.

[1] Han Bae Y., Park K., J. Controlled Release 2011, 153, 198. [2] Brigger I., Dubernet C., Couvreur P., Adv. Drug Deliver. Rev. 2012, 64, 24. [3]Piergies N., Oćwieja M., Paluszkiewicz C., Kwiatek W.M., Spectrochimica Acta A 2020, 228, 1. [4]Piergies N., Ocwieja M., Paluszkiewicz C., Kwiatek W.M., J. Raman Spectrosc. 2018, 49, 1265. [5]Piergies N., Oćwieja M., Paluszkiewicz C., Kwiatek W.M., Appl. Surf. Sci. 2021, 537, 147897. [6]Piergies N., Dazzi A., Deniset-Besseau A., Mathurin J., Oćwieja M., Paluszkiewicz C., Kwiatek W.M., Nano Res. 2020, 13, 1020.

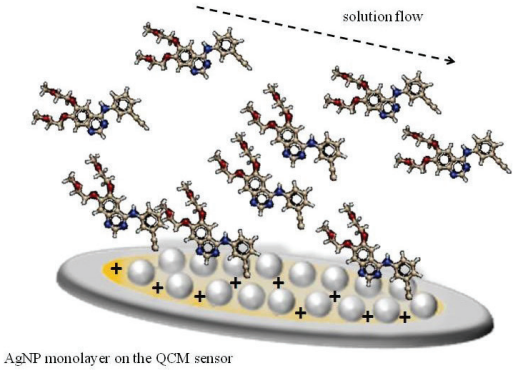

Figure 1. The experimental scheme of the erlotinib molecules deposition on the AgNP monolayer/QCM sensor system.

Surface sensitive photothermal AFM-IR – Probing nanoscale chemistry at the surfaces

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Photothermal AFM-IR, Surface Sensitivity, nanoscale IR spectroscopy and imaging, , nanoIR

One method of nanoscale infrared spectroscopy and imaging, atomic force microscope based infrared spectroscopy (AFM-IR) directly detects IR radiation absorbed by the sample using the AFM probe tip to sense thermal expansion. This thermal expansion depends primarily on the absorption coefficient of the sample and is largely independent of other optical properties of the AFM tip and the sample. Over the last years, we have developed two major improvements in the photothermal AFM-IR technique and introduced (1) resonance enhanced version of AFM-IR [1] and (2) Tapping photothermal-based AFM-IR spectroscopy and imaging [2]. Recently, building on our knowledge in photothermal AFM-IR, we have invented the surface sensitive AFM-IR mode. This mode allows to chemically analyze sample surfaces with a high degree of surface sensitivity by measuring IR spectra and/or images of the top of the sample surfaces. This presentation will describe the underlying technology of the new surface sensitive AFM-IR mode. Additionally, we will discuss the differences between traditional resonance enhanced AFM-IR vs surface sensitive AFM-IR and will also highlight numerous applications.

[1] Alexandre Dazzi et al, Chemical Reviews, Vol. 117 (7), 2017. [2] Jérémie Mathurin et al, Analyst, Vol. 143, 2018.

Analysis of fixed and live single cells using optical photothermal infrared with concomitant Raman spectroscopy

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OPTIR, Photothermal, infrared spectroscopy, Raman, Live cells, Breast cancer, Pancreatic cancer

In this presentation I will discuss the first use of a completely optically based photothermal method (O-PTIR) for obtaining infrared spectra of both fixed and living cells using a quantum cascade laser (QCL) and optical parametric oscillator (OPO) laser as excitation sources. The use of the two lasers enables the full biologically important range to be covered meaning that all biologically relevant vibrations can be analysed at sub-micron spatial resolution. In addition, infrared data acquisition is combined with concomitant Raman spectra from exactly the same excitation location, meaning the full vibrational profile of the cell can be obtained. The pancreatic cancer cell line MIA-PaCa-2 and the breast cancer cell line MDA-MB-231, are used as model cells to demonstrate the capabilities of the new instrumentation. These combined modalities can be used to analyze subcellular structures in both fixed and, more importantly, live cells under aqueous conditions. The problem of water absorption is alliviated by using an inverted geometry whereby the cells are attached to the underside of an infrared transparent plate. The visible probe beam is essentially unhindered by the water below the cell meaning that good spectra can still be onbained. and We also show that protein secondary structure and lipid rich bodies can be identified on the sub-micron scale in the aqueous environment.[1]

This work was supported by the North West Centre of Advanced Drug Delivery (NoWCADD), a collaborative partnership between the Division of Pharmacy and Optometry, University of Manchester and AstraZeneca.

[1] A. Spadea, J. Denbigh, M.J. Lawrence, M. Kansiz and P. Gardner, Analysis of fixed and live single cells using optical photothermal infrared with concomitant Raman spectroscopy, Anal Chem 2021, 93, (8), 3938–3950

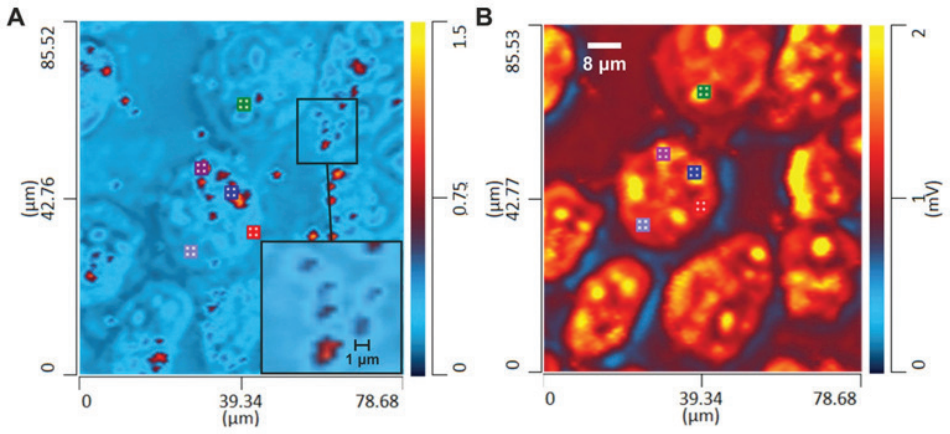

Figure 1. Representative single-frequency image of MIA PaCa-2 live cells in PBS buffer at (A) 2929 cm–1 and (B) 1550 cm–1.

Do we really need sub-micron resolution to analyze single cell molecular features through vibrational spectroscopy?

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FTIR microspectroscopy, O-PTIR, AFM-IR, single cell, malaria

Malaria is one of the major life-threatening diseases to afflict humanity with an estimated 228 million cases worldwide in 2018. There exists no approved Malaria vaccine on the market yet, partly due to the complexity of the parasite life cycle and the vast repertoire of polymorphic proteins they express during different stages of development. In this work, we have tested two emerging spectroscopic approaches: Optical Photothermal Infrared (O-PTIR) spectroscopy and Atomic Force Microscopy combined with infrared spectroscopy (AFM-IR) in contrast to the more traditional Fourier Transform InfraRed (FTIR) microspectroscopy which has emerged recently as a new promising tool to provide label-free analysis of cell and tissue sections. Examples of chemical spatial distributions of selected bands and spectra for Plasmodium falciparum infected RBCs collected with the three modalities are presented and compared together with advantages and limitations of each method. Based on these results, it appears that O-PTIR and AFM-IR techniques can be explored as powerful tools for the analysis of cells and ipso facto, these methods can help in better understanding complex processes occurring within heterogeneous objects such as infected RBC due to their superior spatial resolution in comparison with traditional approaches for infrared spectroscopic characterization.

2*f* zero-crossing locked Interferometric Cavity-Assisted Photothermal Spectroscopy system for NO sensing

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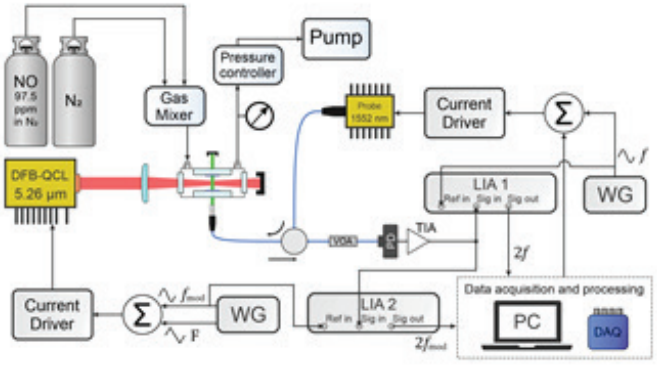
Photothermal Spectroscopy (PTS) is an ultra-sensitive technique capable of measuring thermal-induced effects on a gas sample as a consequence of photon absorption and molecular non-radiative relaxation. The absorbed energy is released in the form of heat, spatially confined to the interaction volume, i.e. the focal volume of the excitation source. In interferometric cavity-assisted photothermal spectroscopy (ICAPS) a Fabry-Pérot Interferometer (FPI) and a probe laser are used as optical transducer to detect refractive index variations, induced by gas thermal expansion [1]. The FPI reflectivity depends on the phase of the electromagnetic wave travelling between the planar mirrors. Any refractive index change – which affects the wave phase – will induce a detectable change in the reflected intensity. Spatially overlapping the probe beam and excitation beam allows sensitive probing of gas thermal expansion. The highest sensitivity is achieved when the probe laser wavelength is locked to the inflection point of the resonance profile. At the inflection point, the slope of the reflectivity curve is maximized, while the 2nd derivative of the curve reaches a zero. An approach to achieve this operational mode, which allows to compensate for any drifts, consists in 2*f* zero-crossing locking: the probe wavelength is dithered at a frequency f, while the reflected intensity signal is fed to a lock-in amplifier (LIA) and is demodulated at the 2nd harmonic. The demodulated signal corresponds to the 2nd derivative of the resonance profile. A LabView-based PID controller acts on the probe current to keep the in-phase component of the LIA signal at the zero-crossing. In this work we made use of a DFB-QCL emitting at 1900 cm^{-1} to target the NO absorption line. The QCL current was modulated with a triangular ramp to scan across the absorption line. A modulation frequency fmod was superimposed for 2*f*-WMS detection approach. A 2 mm air-spaced FPI with mirror reflectivities R=98.9% (finesse of ~230) was used. A NIR diode laser (1552 nm) was fibre-coupled to an optical circulator: the emitted radiation was collimated by a GRIN-lens, while the reflected intensity was measured by a transimpedance amplified (TIA) photodetector (PD). The PD signal was fed to both LIA 1, for 2*f* zero-crossing locking, and LIA 2 to retrieve the 2*f*-ICAPS signal. Preliminary results of the ICAPS-sensor for NO detection are shown in Fig. 1(b).

[1]J. P. Waclawek, H. Moser, and B. Lendl, 'Balanced-detection interferometric cavity-assisted photothermal spectroscopy employing an all-fiber-coupled probe laser configuration', Opt. Express, vol. 29, no. 5, pp. 7794–7808, Mar. 2021, doi: 10.1364/OE.416536.

a)



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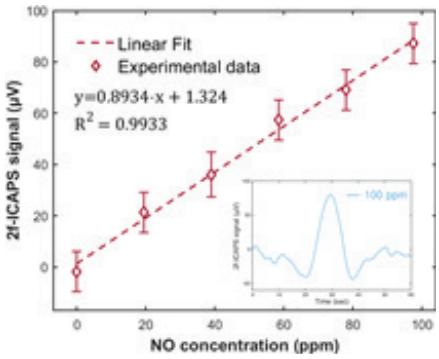

Figure 1. (a) Schematic of the experimental setup. DAQ – data-acquisition card; VOA – variable optical attenuator; WG – waveform generator. (b) Calibration curve of ICAPS sensor for NO detection. In the inset, the 2f-ICAPS signal acquired during a spectral scan of the NO absorption line.

12CO2 and 13CO2 detection at 2291 cm-1 by interferometric cavity-assisted photothermal spectroscopy (ICAPS)

Stefan Lindner[1], Davide Pinto[1], Harald Moser[1], Bernhard Lendl[1]

[1]Technische Universität Wien

Photothermal spectroscopy, Carbon dioxide sensing, Isotope sensing, Laser spectroscopy

Interferometric cavity-assisted photothermal spectroscopy (ICAPS) is a novel, sensitive technique for trace gas analysis requiring only a sample volumes in the sub; cm3 range1,2. Its working principle lies in the detection of a periodic refractive index change of a gaseous sample as result of modulated analyte excitation by means of an excitation laser. For this purpose, a distributed feedback quantum cascade laser (DFB-QCL) of 100 mW power is used. The gaseous sample is introduced between the mirrors of a Fabry-Perot (FP) cavity which serves as sensitive means to detect the induced refraction index changes. A fiber coupled NIR probe laser (EMCORE, 1550 nm telecom laser, 50 mW) shining perpendicular into the cavity using a GRIN lens and intersecting the QCL beam is used to probe the induced refractive index change. For doing so, the wavelength of the probe laser is set at an inflection point of the periodic transmission function of the employed cavity (Finesse F = 20, Reflectivity 80 %, 1 mm spacing). The back reflected light is collected by the GRIN lens and guided via fiber to a NIR photodetector via an optical circulator. To record a spectrum, the emitted wavelength of the QCL excitation laser is tuned by applying a saw tooth current ramp of 5 mHz which is superposed by periodic sinusoidal current modulation of 113 Hz to be able to apply the 2f-wavelength modulation technique3. The absolute height of the periodic cavity reflection function as well as its slope in the inflection points were used to lock the probe laser to one of its points of inflection. This is crucial to keep sensitivity and linearity of the ICAPS sensor at its highest possible values and to avoid drifts. Technical strategies for practical implementation of these laser-cavity-locking techniques, continuous and discontinuous in time, are presented. Results in terms of noise and long-term stability are presented as well as spectra of CO2 at a wavenumber of 2290 to 2291.5 cm-1, where well-separated absorption lines, emerging from C=O rotational transitions and asymmetric C=O stretching modes of 12CO2 and 13CO2 are located. A limit of detection of 4 ppmV has been achieved. These results introduce ICAPS being capable of performing isotope resolved sensing of carbon dioxide.

We acknowledge financial support by the Austrian research funding association (FFG) under the scope of the research project ATMO-SENSE (FFG 861581)

[1] J.P. Waclawek, V.C. Bauer, H. Moser, B. Lendl. "2f-wavelength modulation Fabry-Perot photothermal interferometry". Opt. Express. 2016. 10.1364/OE.24.028958. [2] J.P. Waclawek, H. Moser, B. Lendl. "Balanced-detection interferometric cavity-assisted photothermal spectroscopy employing an all-fiber-coupled probe laser configuration". Opt. Express. 2021. 29(5): 7794. 10.1364/oe.416536. [3] S. Schilt, L. Thévenaz. "Wavelength modulation photoacoustic spectroscopy: Theoretical description and experimental results". Infrared Phys. Technol. 2006. 48(2): 154–162. 10.1016/j.infrared.2005.09.001.

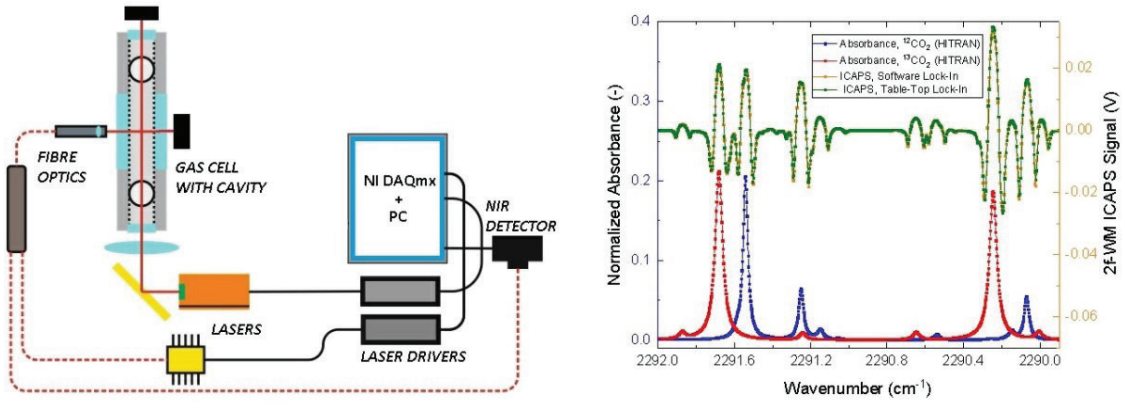

Figure 1. Left: ICAPS Setup Sketch Right Recorded 2f -WM ICAPS spectra of CO2 (250 mbar, 10000ppmV) compared to HITRAN reference absorbance spectra of 12CO2 and 13CO2.

Pump-probe photothermal spectroscopy Mach-Zehnder interferometer employing an external cavity quantum cascade laser for liquid phase sensing

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Photothermal Spectroscopy, Liquid Sensing, Mach-Zehnder Interferometer, Quantum Cascade Laser, Analytical Chemistry

Over the last decade, infrared spectroscopy has rapidly adopted quantum cascade lasers (QCL) as light sources. The favorable properties of these sources (high power, broad tunability across the mid-IR, high brilliance) have opened up many new possibilities for mid-IR spectroscopy and enabled novel spectroscopic sensing schemes widely used in several scientific fields.[1] Still, these sources are often used in conventional direct absorption schemes, that rely on detecting minute difference in intensity and thus do not fully benefit from QCLs' high power. In contrast, photothermal spectroscopy (PTS) is an indirect detection approach where optical absorption signal is directly proportional to the laser emission intensity, increasing the power, means signal-to-noise ratios increase and the sensitivity is enhanced. The PTS signal comes from a photoinduced change in the sample thermal state probed as the consequent sample refractive index change (Δn). The challenge lies in detecting the smallest Δn. In our latest generation liquid PTS IR sensor, this is achieved using a Mach-Zehnder Interferometer (MZI). Our approach is optimized for sensitivity and long-term stability using a pump-probe configuration and balanced detection. MZIs sense sub-nm phase shifts between their two arms. We place sample and reference channels, respectively, in the two arms of the interferometer. Pumping the sample with a chopped QCL induces a temperature gradient and a resulting Δn that leads to a relative phase shift between both branches. The phase shift is proportional to IR absorption. Our design consists of an MZI using a HeNe probe laser and an external cavity (EC)-QCL tunable from 1730 to 1565 cm^{-1} pump laser. Three components are key for performance: both the flow cell and the opto-mechanic components are actively temperature stabilized (22.5°C). The MZI is held in its quadrature point (=equal intensity in both outputs) using a PID controlled piezo electric transducer (PZT) mounted directly on a mirror in one arm of the MZI. This ensures linearity of the PTS signal and highest IR sensitivity and reduces signal drifts (environmental, thermal). Moreover, a balanced-detection scheme has been adopted in order to reject probe laser common mode noise. In this presentation, we introduce our design and demonstrate its sensitivity for IR sensing. The system is bench marked against commercial FTIR spectrometers. It is shown to be in excellent agreement with regards to band shapes, positions and relative intensities and to compare favorably in terms of sensitivity.

[1] Schwaighofer, A.; Akhgar, C. K.; Lendl, B. Broadband Laser-Based Mid-IR Spectroscopy for Analysis of Proteins and Monitoring of Enzyme Activity. Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 2021, 253, 119563. https://doi.org/10.1016/j.saa.2021.119563.

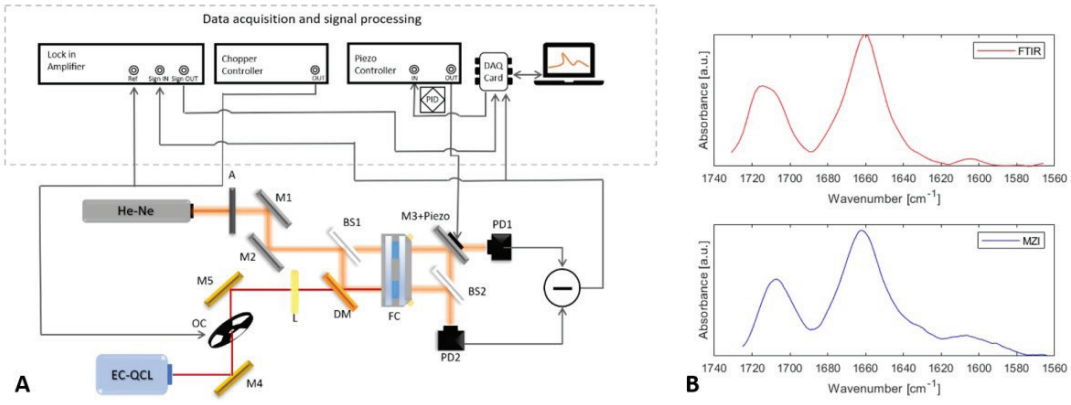

Figure 1. (A) Experimental setup employing a He-Ne as a probe laser, an EC-QCL as a pump laser for liquid sensing using a PTS-MZI (B) IR spectra of caffeine dissolved in ethanol FTIR – MZI comparison

1.12. Brillouin spectroscopy

Brillouin spectroscopy for the evaluation of viscoelasticity in biological systems

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Biophotonics, inelastic light scattering, cells, tissues, viscoelasticity

In the domain of light scattering techniques, Brillouin spectroscopy has emerged as a microscale tool for evaluating the viscoelastic properties of biological systems, owing to recent advances in instrumentation and analysis (Palombo & Fioretto, Chem. Rev., 119, 7833–7847, 2020). The mechanical properties of living systems are key to their physiological function, and changes are implicated in diseases ranging from atherosclerosis to osteoarthritis. Brillouin microscopy has opened a new spectral range at Gigahertz frequency shifts whereby acoustic wave propagation in matter provides the contrast mechanism to investigate biomechanics and viscosity on a microscale. To establish the biological significance of Brillouin resonances in biological systems, our work has focused on model systems in the form of bioderived hydrogels that enable to tune viscoelasticity and water content by concentration change. This work forms the bases for understanding the biophysics of biological samples, ranging from fibrous collagen and elastin to cartilage and bone up to pre-cancerous epithelial tissue and Alzheimer's mouse brain, for quantitative analysis of biomechanics and mechanobiology.

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Brillouin Spectroscopy of Chemically Tunable 2D Layered Oxides

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Brillouin scattering, 2D materials, acoustic phonons

Two-dimensional layered materials offer significant promise for applications ranging from energy storage to optoelectronics. Of these, molybdenum trioxide (MoO3) and vanadium pentoxide (V2O5) are unique metal oxides with possible application as electrochromic materials. They change in color from transparent white to dark blue or orange to grey, respectively, with reversible intercalation of metals. Additionally, the vibrational and mechanical properties of these layered oxides can be directly controlled through intercalation. Here, we use Brillouin spectroscopy to map the entire angular dispersion curves of multiple acoustic phonon branches of 2D layered MoO3 and V2O5 - directly probing the effects of phonon quantum confinement in a 2D layered material. Since acoustic phonons dictate elasticity, we determine the complete elastic stiffness tensor and the thickness of each material to within less than a monolayer. This work establishes methodology to extract precise elastic constants from complex Brillouin scattering of 2D materials. It takes advantage of phonon confinement to capture the complete elastic response with minimal different scattering geometries. Finally, we demonstrate how the intercalation of metallic Sn, Co, and Cu can chemically tune the quantized acoustic phonons and elasticity of 2D layered metal oxides.

Brillouin and Raman micro-Spectroscopy: an emerging technique for simultaneous chemo-mechanical investigation of human bone and cartilage tissues properties

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Brillouin Spectroscopy, Raman Spectroscopy, bone research, biomedical application, orthopedic disease diagnosis

Brillouin and Raman micro-Spectroscopy (BRamS) is an imaging scattering technique that allows the simultaneous investigation of the soft matter chemo-mechanical properties with a micrometric resolution [1]. Due to its unique characteristics of being contact-less, not-destructive, and not requiring the use of external labels, it has gained increasing consensus in the field of biomedical sciences and medical diagnosis. New findings have been obtained into the study of both cellular and tissue-phantom chemo-mechanics, in the investigation of microbial biofilms growing on metallic surfaces and in the characterization of whole-tissue pathological processes, such as Alzheimer's disease, Barrett's oesophagus and corneal keratoconus [2,3]. Moreover, Brillouin and Raman micro-Spectroscopy has been applied by our group to the description of the chemo-mechanical properties of human bone and cartilage tissues in both physiological and pathological conditions [4]. Human lamellar bone and cartilage are biological systems characterized by a stringent structure-function relation. In particular, bone exhibits a hierarchical architecture with several different levels of organization from the macroscale down to the nanoscale, whilst the articular cartilage shows a peculiar bearing-like structure, specifically designed to allocate mechanical stresses throughout the joint interface, preventing bone friction during motion [5,6]. Several orthopaedic diseases derive from the impairment of whole-tissue performance, due to the failure of these tissues to maintain the correct arrangement of their constituents starting from the microscale. Here, we present some results obtained by Brillouin and Raman micro-Spectroscopy in the investigation of bone-material properties, with particular focus on the characterization of cortical and trabecular bone organization in the human femur, and the first attempt to apply this knowledge to the detection of hip osteoarthritis.

[1] Scarponi, F. et al. (2017). High-Performance Versatile Setup for Simultaneous Brillouin-Raman spectroscopy: A probe of tissue micromechanics. Science Advances, 6 (44). [2] Palombo, F. & Fioretto, D. (2019). Brillouin Light Scattering: Applications in Biomedical Sciences. Chem Rev. 119 (13). 7833-7847. [3] Bailey, M. et al. (2020). Viscoelastic properties of biopolymer hydrogels determined by Brillouin microspectroscopy. Phys. Rev. X, 7. [4] Cardinali, M.A., et al. (2020) Mechano-chemistry of human femoral diaphysis revealed by correlative Brillouin–Raman microspectroscopy. Scient. Reports, 10. [5] Reznikov N. et al. (2018). Fractal-like hierarchical organization of bone begins at the nanoscale. Science Vol. 360 (Issue 6388). [6] Fox, S. (2009). The basic science of articular cartilage: structure, composition, and function. Sports Health; 1(6), 461-468.

Exploring a hidden structural-dynamics landscape in living cells via angle-resolved spontaneous Brillouin Light Scattering imaging

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Brillouin microspectroscopy, mechanical anisotropy

Spontaneous Brillouin light scattering (BLS), as applied to studying biologically relevant samples, measures the velocity of spontaneous GHz acoustic phonon vibrations, which can in turn be used to calculate the microscopic elastic constant of a material. Recent advances in imaging spectrometer designs have opened the door for fast and efficient measurement of BLS spectra, which can be combined with a confocal microscope for Brillouin Microscopy. The beauty of Brillouin light scattering, is not only that it is all-optical, label-free and allows one to measure inside a sample, but also that it happens in almost all directions. Namely, light impinging on a sample with a given wave vector will not only be scattered directly backwards, but also at different angles. In Brillouin microscopy, one typically collects light from many of these angles and feeds them to a spectrometer via e.g. a fiber, to obtain the effective BLS modulus at one small volume, that can be scanned through a sample. However, each of the different collection angles corresponds to a different scattering wave vector and thus probes the elastic modulus in a different direction. I will talk about an experimental setup we have developed that makes use of this, and is able to map in 3D with high spatial resolution the elastic modulus associated with the first three diagonal stiffness tensor components in living biological cells. For each position a spectral projection is recorded, in typically <300ms, which contains all the information necessary to extract the different elastic constants, and can thus be used also for studying dynamic processes. We have applied this to study numerous cellular contexts revealing an astoundingly rich structural-dynamic landscape. I will focus on two of these here: mammalian cell nuclei and plant cell walls. In the former we can identify states of chromatin condensation, chromatin territories, and a potential mechanical signaling pathway between extracellular nuclear cues to the molecular scale. In the latter we can identify structural phase transitions that can account for the observed asymmetric cell growth via directional "loosening" of cell walls, and an increased transverse modulus of outer cell walls that may serve for protection from environmental perturbations and/or a structural purpose. Our results suggest that the BLS-measured properties are not only relevant via proxy to a quasi-static stiffness, but in of themselves essential for understanding dynamic biological processes on the microscopic scale.

Brillouin microscopy studies on phase separated protein droplets

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Brillouin microscopy, Phase separation

Membraneless organelles, created by reversible phase separation, are crucial for intracellular organization and are involved, for example, in metabolic control and DNA repair. These phase-separated compartments can sometimes also undergo an irreversible solidification, which has been associated with severe neurodegenerative diseases. This phenomenon has been mostly studied qualitatively and indirectly, and a direct quantitative determination ofthe bulk material properties during the transition from liquid to solid is still missing. Current techniques to study phase-separated compartments are limited to probe either liquids or solids and are therefore unable to capture a complete picture of this phenomenon. Here, we use Brillouin microscopy to investigate phase-separated FUS protein droplets in vitro. Brillouin microscopy is an emerging technique which allows the measurement of optomechanical properties with opticalresolution in a non-invasive manner employing (spontaneous) Brillouin scattering. This type of non-elastic lightscattering occurs when light is scattered by (thermally excited) soundwaves in the sample. Quantification of the Brillouin frequency shift gives direct access to the longitudinal modulus, refractive index and local mass density, while the linewidth is linked to the viscosity. We followed the solidification of FUS protein droplets over time in a tightly controlled environment and monitored the changes in Brillouin shift and linewidth. Furthermore, we tuned the intermolecular interactions in the protein condensates by applying different buffer conditions and investigated how they are displayed in Brillouin frequency shift and linewidth.

Brillouin Microscopy to Explore the Viscoelastic Behaviour of Tissue-mimicking Hydrogels

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Biophotonics, Light scattering, Tissue phantoms, Viscoelasticity

Brillouin microscopy (BM) is an all-optical technique, providing information on micromechanics through the scattering of light from acoustic waves, or phonons. Here, it has been used to measure the micromechanics of tissue-mimicking hydrogels over a wide range of hydration levels, thus giving a unique insight into the viscoelastic properties of tissue phantoms spanning from the highly hydrated to those of very low hydration. As simple models for a host of biological systems, the hydrogels demonstrate the effect of hydration on collagen-based systems and set the basis for Brillouin imaging in mechanobiology and biomechanics. Denatured collagen hydrogels were studied over a range of concentrations, and the storage and loss moduli were determined from the Brillouin spectra. Most striking was the observation of a glass-like transition as a function of water content (measured at room temperature), denoted by a sigmoidal evolution of the Brillouin frequency shift and a maximum in linewidth. This corresponds to a dramatic slowdown of the structural relaxation process, ubiquitous to colloidal systems and, for the first time here, observed with BM in biologically relevant systems. Correlative techniques such as compressive testing, ultrasound elastography and Raman spectroscopy were also applied to provide further insight into the behaviour of the hydrogels. These findings provide a comprehensive description of biopolymer hydrogels at varying hydration levels, giving a unique description of the viscoelastic properties across a wide range of physical states, from the highly hydrated to the solid-like phase, and the transition between the two. Results show that the phase transition properties of these gels depend not only on water content but crucially on the chemical nature of the solute, in this case the hydrophobicity of collagen molecules.

[1] M. Bailey et al.. (2020). Viscoelastic properties of biopolymer hydrogels determined by Brillouin spectroscopy: A probe of tissue micromechanics. Science advances, 6(44), eabc1937.

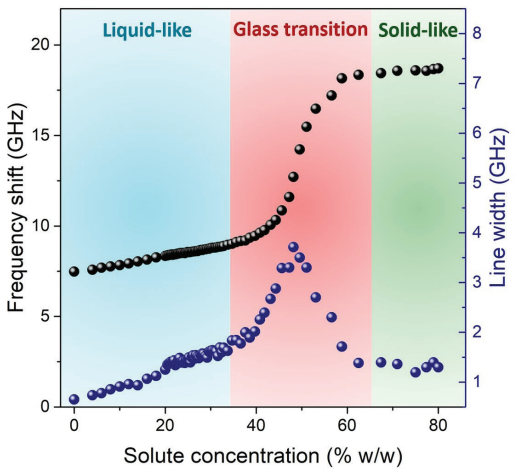

Figure 1. Glass-like transition as a function of concentration, observed through the evolution of Brillouin frequency shift and linewidth.

Charge Transfer Mechanism in SiO2-Ag-rGO System

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Defect Concentration, Charge Transfer, SiO2-Ag-rGO, Resonance Raman Scattering, SERS Activity

In this study, to study the influence of the disorder caused by doping effects on material's optical properties, a highly active SERS substrate, SiO_2-Ag-reduced graphene oxide (rGO) was prepared. For this SiO_2-Ag-rGO substrate, Ag nanoparticles (NPs) and SiO_2 were in turn decorated on the surface of rGO. The introduction of SiO_2 can not only control the plasma effect of Ag NP, but more importantly, the Si atoms in the structure can also control the defects concentration of GO. The influence of this rGO defects on the charge transfer in the system was studied by analyzing spectral changes in the SERS spectrum of probe molecule, 4-mercaptobenzoic acid (4-MBA). Defects in the crystal structure causes the rGO to enter a metastable state, which facilitates charge separation and transfer in the SERS system. In addition, the defects of the crystal structure can also affect the energy level position, therefore, control of the defect concentration can realize the charge transfer resonant coupling in system. The formation of this charge transfer resonance coupling in the system is mainly demonstrated by the sensitive response of molecular signals in the SERS spectra to excitation light with different energies. This research not only obtained the ultra-high activity SERS substrate of carbon-based materials, but also used SERS to study the influence of crystal structure on the optical properties of carbon-based systems.

1.13 Chiroptical spectroscopy

"Watching" a Molecular Twist in a Protein by Raman Optical Activity

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vibrational optical activity, cofactor, photoreceptor, microbial rhodopsin

Light-absorbing chromophores in photoreceptors contain a π-electron system and are intrinsically planar molecules. However, within a protein environment these cofactors often become nonplanar and chiral in a manner that is widely believed to be functionally important. When the same chromophore is out-of-plane distorted in opposite directions in different members of a protein family, such conformers become a set of enantiomers. In techniques using chiral optical spectroscopy like Raman optical activity (ROA), such proteins are expected to show opposite signs in their spectra. Here we use two microbial rhodopsins, Gloeobacter rhodopsin (GR) and sodium ion pump rhodopsin (NaR) to test this idea. As shown in the figure, the crystal structure of GR demonstrates that its protonated Schiff base NH moiety forms a hydrogen bond with a water molecule. In contrast, the Schiff base NH group forms a hydrogen bond with an aspartate near the chromophore in NaR. This ionic hydrogen bond makes the retinal NH moiety to point toward the aspartate, leading to an oppositely out-of-plane distorted chromophore in NaR compared to that of GR. As shown in the figure, the observed ROA spectra of GR and NaR were opposite in signs. This observation provides the first experimental evidence that the twist direction of the retinal chromophore indeed determines a sign of the ROA spectrum. Furthermore, we disrupted a hydrogen bond responsible for the distortion of the retinal in NaR and showed that the sign of the ROA signals of this non-functional mutant is flipped. The reported ROA spectra are monosignate, a property that has been seen for a variety of photoreceptors, which we attribute to an energetically favorable gradual curvature of the chromophore.

[1] Matsuo, J., Kikukawa, T., Fujisawa, T., Hoff, W. D., Unno, M. J. Phys. Chem. Lett. 11, 8579-8584 (2020)

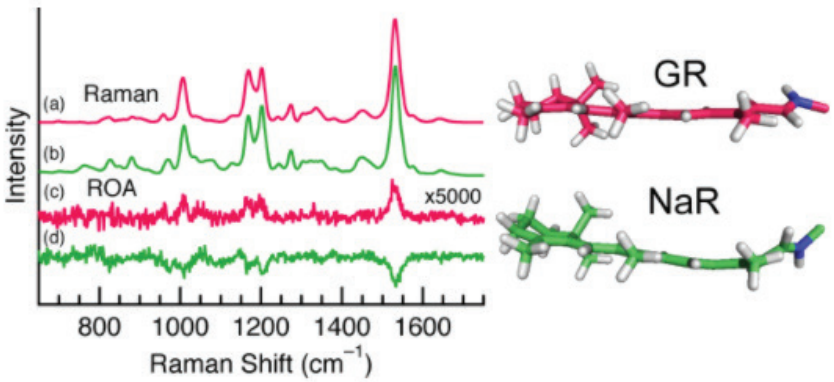

Figure 1. Raman and ROA spectra of GR and NaR. Structures of the retinal chromophore are also shown.

Interplay of the ECD and RROA induced chirality: towards understanding the solvent and substituent effects on naphthalene diimide spectra

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chirality, NDI, substituent and solvent effect, intra- and intermolecular interactions, DFT calculations

Naphthalene diimide (NDI) substituted by chiral BINAM group induce chirality transfer to an achiral solvent in the Raman Optical Activity (ROA) spectra.[1] According to the Single Electronic State (SES) theory,[2] the Resonance ROA (RROA) spectrum should be monosignate and directed oppositely to the sign of the Electronic Circular Dichroism (ECD) band in resonance with the excitation line. Our experiments were inconsistent with the SES theory: the monosigned RROA bands' sign was the same as the appropriate ECD band. Wu et al.[3] have explained the RROA phenomenon by an intertwining of the ROA and ECD effects and demonstrated how the shape of the ECD band is responsible for the mono- or bisigned pattern of the RROA spectrum. The interplay between the RROA and ECD spectra needs a scrupulous interpretation of the ECD spectra of solute dissolved in various solvents. Here, we present such an interpretation for the atropisomeric naphthalene diimide (aNDI) derivatives dissolved in different solvents by means of DFT calculation. It is usually believed that the ECD spectra are only slightly solvent dependent and that the implicit solvent models sufficiently reproduce the experimental data. However, for some aNDIs which are quite rigid, the longest wavelength (LWB) ECD band ranges with solvent within ca. 100 nm. The band is due to the HOMO → LUMO transition, i.e., from the electron-rich substituted 1,1′-binaphthyl moiety to the adjacent electron-poor NDI system. The LWB position and sign varies not only with solvent but also with π-electron donor-acceptor properties of the BINAM substituent, its bulkiness, and ability for change of conformation, e.g., due to the formation of intermolecular hydrogen bonds with the solvent. All these factors influence the final RROA spectra. Here, the changes of the LWB ECD have been interpreted by means of the DFT calculations combined with hybrid explicit?implicit solvation models in which the closest molecular environment was simulated by the explicit presence of several solvent molecules in the first solvation sphere, while the solvent bulk was mimicked by using the implicit PCM approach.

National Science Centre in Poland Grant No. UMO-2017/25/B/ST5/02267 and Świerk Computing Centre.

[1] E. Machalska, G. Zajac, M. Baranska, D. Kaczorek, R. Kawęcki, P. F. J. Lipiński, J. E. Rode, J. Cz. Dobrowolski, On Raman optical activity sign-switching between the ground and excited states leading to an unusual resonance ROA induced chirality, Chem. Science 2021, 12, 911. [2] L. A. Nafie, Theory of resonance Raman optical activity: the single electronic state limit, Chem. Phys. 1996, 205, 309. [3] A. T. Wu, G. Li, J. Kapitan, J. Kessler, Y. Xu and P. Bo?r, Two Spectroscopies in One: Interference of Circular Dichroism and Raman Optical Activity, Angew. Chem. Int. Ed. 2020, 59, 21895.

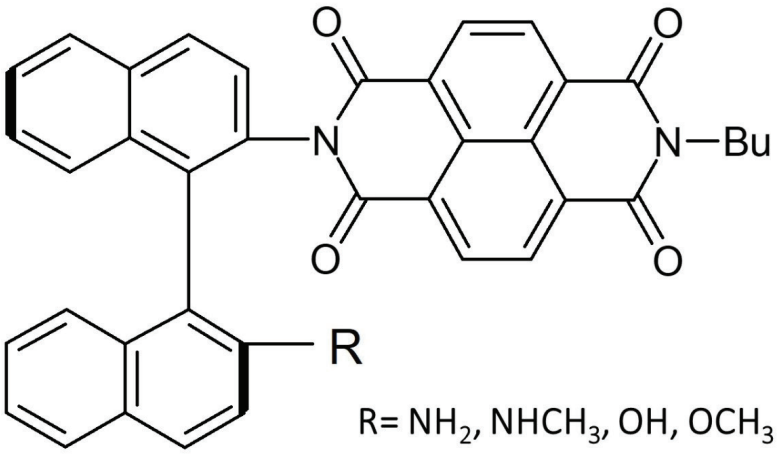

Figure 1. (S)-naphthalene diimide (aNDI) derivatives

From cirrhosis to cancer – A spectroscopic study

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hepatocellular carcinoma, cirrhosis, spectroscopy, blood plasma

Hepatic cirrhosis develops mostly on the basis of hepatitis B/C infection. Nonetheless, regular alcohol consumption and high-fat diet have been contributing to gradual increase in cirrhosis cases within today's developed population. Unfortunately, many cirrhosis patients are prone to develop hepatocellular carcinoma (HCC). Despite the use of highly sophisticated diagnostic procedures, including specialized imaging techniques, HCC is usually diagnosed too late to save the patient. This makes HCC one of the most lethal cancers worldwide, not to mention its incidence nearly matching the death rate. As proven many times in the past years, specific pathology-induced changes may be detected throughout the body before any disease starts to manifest physically. These changes appear on the molecular level and, thus, may be easily inspected via the analysis of blood and its derivatives. The most suitable technique for the analysis of blood-based samples is vibrational spectroscopy. It offers a fast, indestructible analysis of small sample volumes yielding highly reliable results. For the study of hepatic cirrhosis and its progression to HCC, we measured the blood plasma of affected individuals by infrared and Raman spectroscopy, the performance of which was enhanced by chiroptical techniques, namely electronic circular dichroism and Raman optical activity. While standard vibrational spectroscopy showed mainly concentration changes, chiroptical spectroscopy provided an insight into structural alterations of several groups of biomolecules (proteins, lipids etc.). Based on the subsequent statistical processing of the acquired data, which resulted in a highly accurate discrimination of the two studied groups, it may be stated that spectroscopy; of blood plasma is more than suitable for the study of pathological processes underlying the conversion between cirrhosis and hepatocellular carcinoma.

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True and false resonance Raman optical activity: a case of Vitamin B12 derivatives

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chirality, Raman optical activity, circular dichroism, vitamin B12

Vitamin B12 is a chiral organometallic molecule possessing number of functional groups available to chemical modifications, thereby it is possible to combine its native structure with various biologically active molecules Consequently, cobalamin was applied as a delivery tool into bacterial or mammalian cells and used to alter the bioavailability of proteins or anti-cancer drugs. Vitamin B12 derivatives have all relatively strong circular dichroism (CD) close to the ROA excitation laser wavelength (532 nm), which makes it possible to observe also their resonance Raman optical activity (RROA) [1]. RROA promises to be a useful tool for biomolecular detection, but its measurement is difficult. Only the recently reported effect of electronic CD on the spectra makes reliable RROA measurement of chiral color compounds possible [2]. This is demonstrated on a series of vitamin B12 analogues. We use the fact that the CD-ROA effect is proportional to concentration and in the measured data competes with molecular RROA. Apart of "true" RROA, vitamin B12 thus induces CD-ROA signals in the solvent and in its own vibrational bands (false RROA). We believe that this phenomenon has been overlooked or misinterpreted in earlier works. The interpretation of the spectra is supported by computational modeling and density functional theory. The molecules are chemically similar but exhibit a surprising variability in terms of their spectroscopic behavior (Figure 1).

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[1] E. Machalska, G. Zajac, A. Gruca, F. Zobi, M. Baranska, A. Kaczor, J. Phys. Chem. Lett. 2020, 11, 5037–5043. [2] T. Wu, G. Li, J. Kapitán, J. Kessler, Y. Xu, P. Bouř, Angew. Chem. Int. Ed. 2020, 59, 21895–21898.

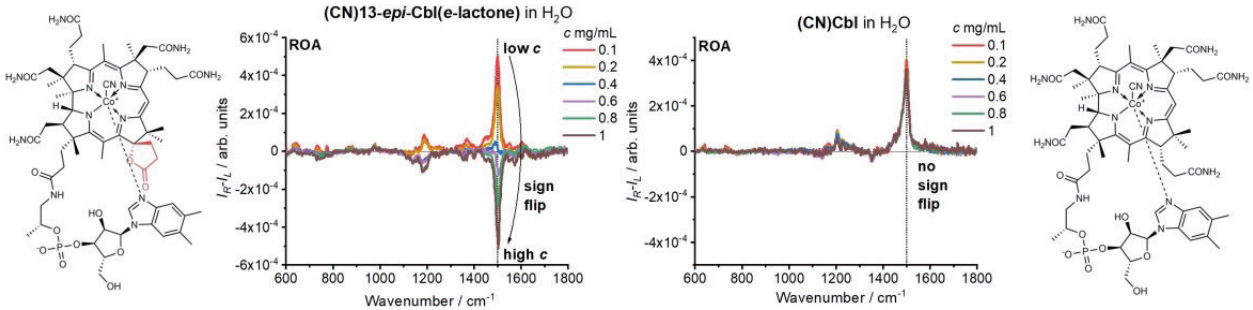

Figure 1. Chemical structures and concentration dependent ROA spectra of (CN)13-epi-Cbl(e-lactone) and (CN)Cbl.

Raman optical activity in extended spectral range

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Raman optical activity, molecular dynamics, density functional theory, spectra simulations, molecular interactions

Spectroscopy of vibrational Raman optical activity (ROA) is established as a powerful tool to study molecular structures and interactions of chiral molecules in solution. Usually, molecular transitions only within a relatively narrow frequency interval are analyzed. We analyzed a broader range of vibrational frequencies (40–4000 cm^{-1}) measured by a custom-built double-grating VIS-ROA spectrometer (SCP, backscattering, 532 nm excitation wavelength), that makes possible simultaneous recording of low-energy intermolecular interactions observed within 40–150 cm^{-1}, the high-energy CH stretching fundamental transitions (2800–3100 cm^{-1}), but also a set of almost two-orders weaker overtone and combination bands, observed for the first time. Strong ROA bands of small molecules like 2-chloropropionitrile or α-pinene have been observed within 40–150 cm^{-1}, and based on combined molecular dynamics and density functional theory simulations assigned to solute-solute interactions.[1] Assignment of combinational and overtone ROA bands was based on perturbation and variational anharmonic approaches and verified by absorption and vibrational circular dichroism (VCD) spectra.[2,3] First results also suggest that the simulations are usable for spectral interpretations, although the accuracy is lower than for the fundamental modes. Rather unexplored ROA CH stretching region (2800–3100 cm^{-1}) proved to be useful and sensitive to the conformational analysis of flexible saccharides.[4] The spectral bands in this region cannot be simply simulated using the harmonic approximation, which significantly overestimates the calculated values of the wavenumbers and is not always able to describe at least the basic shape of the experimental spectrum. The recorded spectra thus provide a unique test for various computational approaches beyond the harmonic approximation. Spectra recorded in the extended spectral range also require accurate intensity calibration to be comparable with ab initio simulations. This of course requires that the correct radiometric quantities are used, taking into account, for example, the dependence of the Raman scattering intensity on the scattered radiation wavenumber. The developed methodology will also be presented on spectra of camphor recorded in different solvents.

The work was supported by the Palacký University (IGA PRrF 2021_002).

[1] P. Michal et al., J. Phys. Chem. B, 123 (9), 2147-2156 (2019). [2] J. Bloino, B. Malgorzatas, V. Barone, J. Phys. Chem. A, 119 (49), 11862-11874 (2015). [3] P. Daněček, P. Bouř, J. Comput. Chem., 28, 1617-1624 (2007). [4] V. Palivec et al., ChemPhysChem, 21, 1272 (2020).

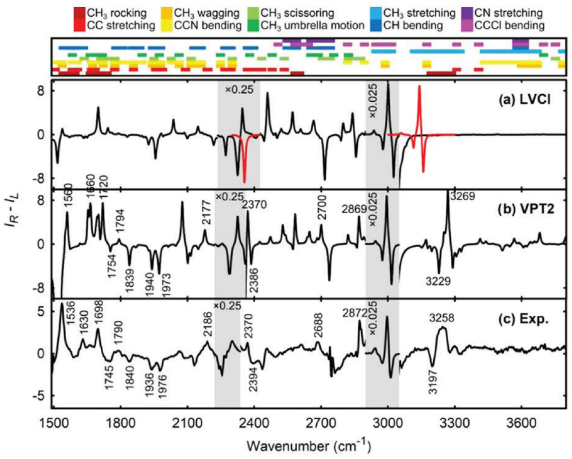

Figure 1. ROA spectra of 2-chloropropionitrile as calculated using vibrational configuration interaction (a), vibrational second-order perturbation calculus (b), and experiment (c).

Solid-state Vibrational Circular Dichroism Spectra of New Chiral Dihydrobenzo[de]isoquinolinones

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chirality, VCD, crystal structure, DFT calculations, substituent effect

Vibrational Circular Dichroism (VCD) spectroscopy supported by quantum mechanical calculations is a powerful tool for assigning absolute configuration, monitoring structural changes in chiral molecules, conformational analysis, and studying of intermolecular interaction. However, VCD is ca. 1000 times less sensitive than IR and the high sample concentration is desired for measurements. But, for many hardly soluble compounds this requirement is difficult to be satisfied. In such a case, the solid-state VCD spectra measurements can help to overcome the problem. The solid state VCD can also be helpful in studies of systems with the C=O group for which the ν(C=O) VCD band in solution is weak or very weak.[1] However, both experiments and simulations of the solid-state chiroptical spectra are challenging. Indeed, the possible artefacts may be due to the light scattering on crystallites and there is still lack of routine algorithms for simulation of the VCD spectra of crystals. A few years ago, we studied the VCD spectra of substituted chiral isoindolinones[2] containing the amide HN-C=O moiety. We showed that the ν(C=O) VCD bands were strong and firmly depended on the substituent.[2] Here, we present similar behaviour in chiral dihydrobenzo[de] isoquinolinones (naphthalenoamides, NA)[3] based on their X-ray data and the experimental and calculated solid-state IR and VCD spectra. The studied spectra exhibit the ν(C=O) band pattern which distinctly changes with the substituent. Even more, for some derivatives the sequence of band signs is exchanged. To interpret these patterns, it was necessary to solve the crystal structures and to calculate the spectra based on molecular clusters cut out from the crystals. We show that the crystal packing symmetry and the number of molecules in the crystal cell are substituent dependent. In Me derivative, the H-bond dimers are present while in the remaining compounds, the intermolecular H-bond chains are formed. The simulations based on the crystal fragments quite well reproduced the experimental solid-state IR and VCD spectra.

National Science Centre in Poland Grant No. UMO-2017/25/B/ST5/02267 and Świerk Computing Centre

[1] V. P. Nicu, E. Debie, W. Herrebout, B. van der Veken, P. Bultinck, E. J. Baerends: Chirality 2009, 21, E287; J. Kong, L. A. Joyce, J. Liu, T. M. Jarrell, J. C. Culberson, E. C. Sherer: Chirality 2017, 29, 854; R. F. Sprenger, S. S. Thomasi, A. G. Ferreira, Q. B. Cass, J. M. Batista Junior: Org. Biomol. Chem. 2016, 14, 3369; [2] J. E. Rode, K. Lyczko, M. Jawiczuk, R. Kawęcki, W. Stańczyk, A. Jaglińska, J. Cz. Dobrowolski: ChemPhysChem 2018, 19, 2411; [3] D. Kaczorek, R. Kawęcki: Tetrahedron Lett. 2020, 26, 152034.

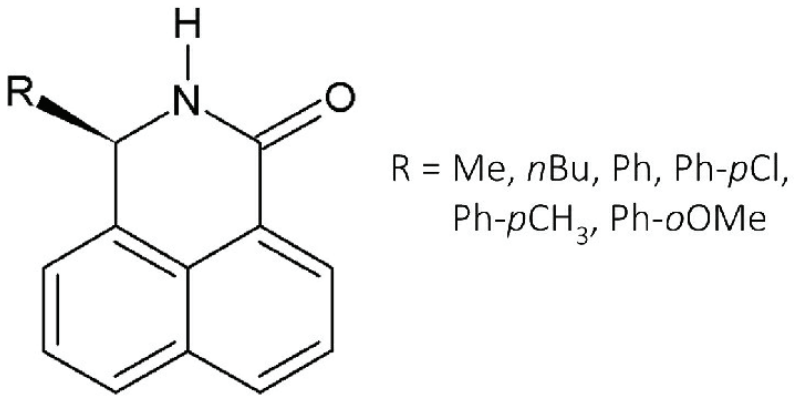

Figure 1. Molecular structure of NA derivatives

Chiroptical spectroscopy as a sensitive tool to study various biomolecules

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Proteins, Carotenoids, Supramolecular Chirality, Circular Dichroism, Raman Optical Activity

Chiroptical spectroscopy methods, i.e., Electronic Circular Dichroism (ECD) and Raman Optical Activity (ROA), together are a set of sensitive tools to study the chirality, structure, and function of important biomolecules such as proteins [1], carotenoids [2] and carbohydrates [3], both at the molecular and supramolecular level. The possibility of testing aqueous solutions by means of ECD and ROA allows to monitor structural changes of biomolecules in their natural environment, which may be related to changes taking place, e.g., in cells under the influence of developing dysfunction. ROA spectra contain more structural information than ECD ones. For example, ROA spectra of proteins provide information on secondary and tertiary structures of polypeptide backbones, backbone hydration, and side chain conformations, as well as on structural elements present in unfolded states [1]. In some cases, when the ECD absorption band of a sample coincides with the excitation used in ROA measurements, it is possible to register a strongly enhanced resonance ROA signal (RROA), what is of importance in studies of low concentrated solutions [2]. Here, we present the ECD study of the Cas9 protein and its ribonucleoprotein (RNP) complex belonging to the CRISP/Cas system known as "molecular scissors" for a precise and effective genome editing, recently rewarded by the Nobel Prize in Chemistry in 2020 [4]. Thus, we show that ECD spectroscopy can be used for verification of the Cas9 ability to bind specific gRNA and for identification of a successfully formed RNP complex. The experiments present the potential of the ECD in structural studies of a various Cas proteins and their interactions with specific gRNAs, in an undoubtedly non-destructive manner. The second aspect of this work is the chirality induction process in supramolecular systems built from biomolecules naturally occurring in nature. As an example, we show the ECD and RROA studies on the aggregation process of achiral carotenoid such as canthaxanthin (CAX). CAX molecules in neutral organic-water environment built non-optically active aggregates (H type), which do not expose chirality at supramolecular level, what is confirmed by the zero signal of ECD and ROA obtained for measured systems. The reverse situation is observed when the aggregation process of CAX molecules takes place in chiral environment, i.e., in water solution of glycosaminoglycans like heparin and hyaluronic acid. Then, the intense chiral induced ECD and RROA signals are registered, typical only for the carotenoid self-assembly (J aggregate).

NCN OPUS 15 no. 2018/29/B/ST4/00335 NCN PhD scholarship ETIUDA 7 no. 2019/32/T/ST4/00230

[1] E. W. Blanch, L. Hecht, L. D. Barron, Methods 2003, 29, 196–209, DOI: 10.1016/S1046-2023(02)00310-9. [2] M. Dudek, E. Machalska, T. Oleszkiewicz, E. Grzebelus, R. Baranski, P. Szcześniak, J. Mlynarski, G. Zajac, A. Kaczor, M. Baranska, Angew. Chemie - Int. Ed. 2019, 58, DOI: 10.1002/anie.201901441. [3] M. Dudek, G. Zajac, E. Szafraniec, E. Wiercigroch, S. Tott, K. Malek, A. Kaczor, M. Baranska, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2019, 206, 597–612, DOI: 10.1016/j.saa.2018.08.017. [4] M. Halat, M. Klimek-Chodacka, J. Orleanska, M. Baranska, R. Baranski, Int. J. Mol. Sci. 2021, 22, DOI 10.3390/ijms22062937.

Raman and ROA study of structural and optical isomers of carenes

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carenes, ROA spectroscopy, DFT calculation

Carenes, i.e. 2-carene, 3-carene and 4-carene are broadly distributed in nature. They are present in essential oils derived from Conifer species, Alpina species, blackcurrant and many other plants. Members of carenes family exhibit different biological activity. For example (+)-3-carene is known from its antibacterial, antifungal and anti-inflammatory activity. The experimental and calculated Raman and Raman optical activity (ROA) spectra of 2- and 3-carene were reported in literature [1-3]. However, to the best of our knowledge both experimental and calculated Raman and ROA spectra of two isomers of 4-carene have not been shown in the literature so far. Thus, in our work were experimentally measured Raman and ROA spectra of (1S,3R)-cis-4-carene and (1S,3S)-trans-4-carene. No doubt, the Raman and ROA spectra of even not complicated molecules can be complex and can posses many details. In that context, quantum-chemical calculations are useful for better description of these spectra. Therefore, both Raman and ROA theoretical spectra were calculated at the B3LYP/aug-cc-pVTZ. The theoretical data are in good agreement with the experiment. As a result experimental and theoretical Raman and ROA spectra of four members of carene family (Figure 1) were compared. Spectra of all structural and optical isomers show characteristic features helpful for identification of selected compound. For example the strongest band in the experimental Raman spectra of all investigated molecules connected with the stretching vibration of C=C bond in the ring occurs at 1690, 1669, 1643 and 1639 cm-1 for 3-carene, 2-carene, cis-4-carene and trans-4-carene, respectively. The strongest bands in the ROA spectra are present at 790, 908, 960 and 968 cm-1 for 3-carene, 2-carene, cis-4-carene and trans-4-carene, respectively. The knowledge about characteristic bands for individual enantiomers is valuable in assessment of the content of these enantiomers in the mixture. Thus, the comparison of signs of observed bands in the ROA spectra of commercially available pine oil samples show the presence predominantly or solely of a bicyclic monoterpene (+)-3-carene in the sample. These data are consistent with the literature study which confirms the existence only the dextrorotatory form of 3-carene in pine oil samples.

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[1] Chruszcz-Lipska K., Blanch E.W. (2014). Optical Spectroscopy and Computational Methods in Biology and Medicine. Chapter 4, 61-81, Springer Dordrecht Heidelberg New York London. [2] Barron LD., Clark B.P. (1979) JCS Perkin II, 1171-1175. [3] Prasad L. Polavarapu, Cody L. Covington, Katarzyna Chruszcz-Lipska,Grzegorz Zajac, Malgorzata Baranska (2017) Journal of Raman Spectroscopy, Vol. 48, Issue 2, 305-313.

Chiral Lanthanide Complexes with L- and D-Alanine – structural and VCD spectroscopy studies

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chiral lanthanide complexes, alanine, crystal structure, VCD spectroscopy, DFT calculations

A whole series of $[Ln(H_2O)_4(Ala)]_2^{6+}$ dimeric cationic lanthanide complexes (but radioactive Pm) with L- and D-alanine enantiomers was synthesized.[1] Their crystal structures were determined at 100 and 292 K using single-crystal X-ray diffraction. In all complexes, the metal ion is coordinated by four water molecules and the four alanine ligands form bridges between the two $[Ln(H_2O)_4]^{3+}$ moieties. Two different types of dimers (I and II) are formed depend on the measurement temperature and the lanthanide type. Between the dimer centers, the alanine molecules behave as bridging (μ_2-O,O'-) and chelating bridging (μ_2-O,O,O'-) ligands. The first type of bridge is present in dimer I, while both bridging forms can be observed in dimer II. Dimer I was observed for heavy lanthanide complexes (Dy-Lu) at 100 K and for all lanthanides at room temperature. Dimer II was found only for the light lanthanides ("La–Nd") measured at 100 K. The IR and VCD spectra of all L- and D-alanine $[Ln(H_2O)_4(Ala)_2]_2^{6+}$ complexes were registered in the 1750-1250 cm^1 range as KBr pellets. All IR spectra are similar and the VCD spectra display satisfactory mirror-image pattern. Several IR band positions and intensities reveal correlations with the Ln–O1 distances exemplifying the lanthanide contraction effect. The v(C=O) VCD bands in the solid state are the strongest in the spectra and split into two bands. They exhibit spectral pattern varying irregularly with the lanthanide. Moreover, the positions of the two v(C=O) VCD bands correlate with the number of 4*f* Ln electrons revealing the lanthanide contraction effect observed for the first time in the VCD spectra. For the selected structures, the experimental data were interpreted by means of the DFT calculations. The molecular geometry in crystals was well reproduced by the structures with the highest-spin of the Ln ions. The simulated IR and VCD spectra strongly depended on the Ln electron configuration. The best overall agreement was reached for the Lu complex, which is the only system with the fully filled *f*-shell.

Institute of Nuclear Chemistry and Technology (Statutory Research) and Świerk Computing Centre

[1] K. Lyczko, J. E. Rode, J. Cz. Dobrowolski: Molecules 2020, 25, 2729.

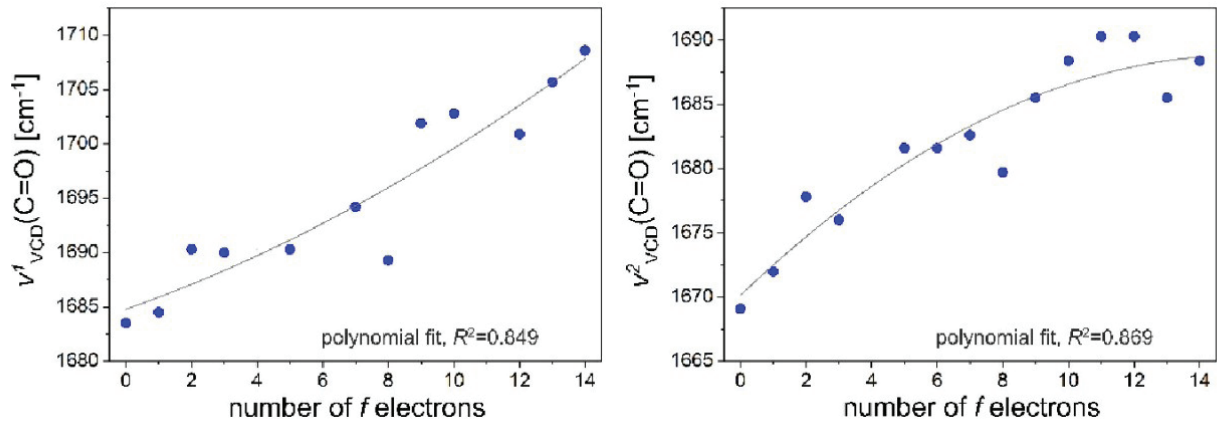

Figure 1. Variation of the v(C=O) VCD band positions with the number of 4*f* electrons in the dimeric Ln alanine complexes

Resonance Raman optical activity as a method suitable for revealing subtle structural detail – study on truncated vitamin B12 analogues

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vitamin B12, cobinamide, resonance Raman spectroscopy, resonance ROA, electronic circular dichroism

Raman optical activity (ROA) is a sensitive tool for chirality detection, which measures the intensity difference in Raman scattering of right and left circularly polarized light deriving from optically active molecules. The power of ROA lies in its ability to reveal structural details of biomolecules, e.g., proteins, amino acids and carbohydrates. However, the application of non-resonance ROA effect is limited by its low sensitivity, therefore the resonance methods enhancing the intensity of chiroptical signal are in demand [1]. In the present study, we explored the potential of resonance ROA to investigate the structure of vitamin B_{12} and its truncated derivatives modified within the nucleotide loop. Vitamin B_{12} can be characterized by a structural element known as a corrin macrocycle which incorporates a low-spin Co^{3+} ion. In the cobalamin species the dimethylbenzimidazole base (Dmb) is attached to one of the corrin's acetamide side chains via a nucleotide loop. Whereas, in case of its truncated analogs, such as cobinamide, this loop terminates before the phosphate group, leaving the open coordination side. As truncated cobalamins are chiral and possess sufficiently high rotational strength in the range of ROA excitation (532 nm), we were able to record their spectra in the resonance condition at relatively low concentration (~10^{-5}M) [2]. In contrast to UV-Vis, ECD and resonance Raman spectra, RROA spectra showed several distinct spectral features allowing to distinguish the studied compounds. The study revealed that the chemical modification within the nucleotide loop, i.e., the attachment of the phosphate group at the *f*-side chain of cobinamide, that in principle should only slightly affect the corrin macrocycle conformation, can be successfully detected by resonance ROA spectroscopy (Figure 1). Moreover, the improved capacity of RROA method is based here on the excitation of molecules via more than two electronic states giving rise to the bisignate RROA spectrum, significantly distinct than a parent Raman spectrum.

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NIH (R01 GM133184), NCN (UMO-2019/33/N/ST4/01986), FNP (POIR.04.04.00–00–4232/17–00)

[1] A.L. Nafie, Vibrational Optical Activity: Principles and Applications; John Wiley & Sons, 2011 [2] E. Machalska, G. Zajac, M. Halat, A. J. Wierzba, D. Gryko, M. Baranska, Molecules 2020, 25(19), 4386

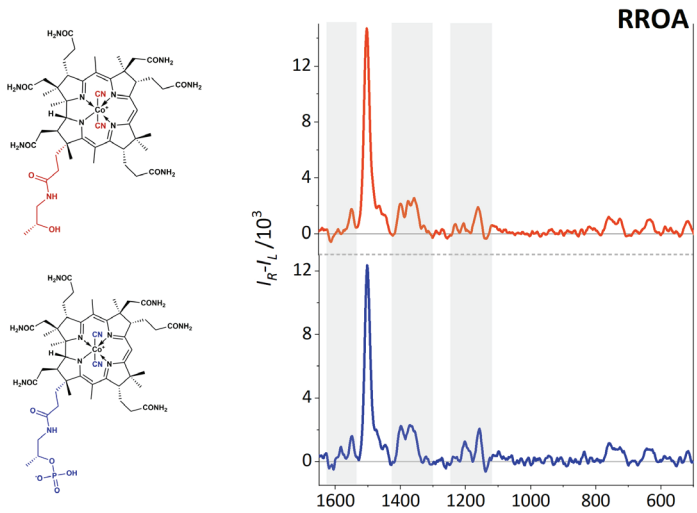

Figure 1. Resonance ROA spectra of studied compounds modified within the nucleotide loop.

2.1. Analytical Techniques in Industry

Analytical techniques in industry
On-line process monitoring using vibrational spectroscopy

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process monitoring, on-line analysis, Raman spectroscopy, infrared spectroscopy, multivariate analysis, pre-processing

Vibrational spectroscopic techniques are being used increasingly for on-line process monitoring in the chemical-using industries. Chemical processes often contain multiple components, and therefore, multivariate analysis is usually required to extract the information of interest from spectra. A number of factors contribute to vibrational spectra including the chemical composition, temperature, and physical properties such as particle size. Hence, spectral pre-processing is required to remove the effects of properties that are undesirable while retaining the information of interest. The use of vibrational spectroscopic techniques, in conjunction with multivariate analysis, for on-line process monitoring will be illustrated using two examples. In the first example, the use of Raman spectroscopy for in situ monitoring of styrene polymerisation is described. Construction of accurate partial least squares (PLS) calibration models requires use of pre-processing algorithms to remove the effects of light scattering from spectra. In this example, a novel dual calibration algorithm was used, which gave more accurate and robust prediction of extent of conversion. In the second example, the use of attenuated total reflectance (ATR) mid infrared (MIR) spectroscopy for the monitoring of solute concentration during cooling crystallisation is presented. The effects of temperature on the spectra were removed using loading space standardisation (LSS). It was shown that more accurate prediction of solute concentration was obtained using spectra pre-processed using LSS than with a global PLS model. These examples illustrate that vibrational spectroscopic techniques, in conjunction with multivariate analysis, can be used for on-line monitoring of a wide range of processes in the chemical-using industries.

EU FP7 and the Engineering and Physical Sciences Research Council, UK.

[1] 'Quantitative spectroscopic analysis of heterogeneous mixtures: the correction of multiplicative effects caused by variations in physical properties of samples', J.-W. Jin, Z.-P. Chen, L.-M Li, R. Steponavicius, S.N. Thennadil, J. Yang and R.-Q. Yu, Analytical Chemistry, 2012, 84, 320 – 326.
[2] 'Correction of temperature-induced spectral variations by loading space standardisation', Z.-P. Chen, J. Morris and E. Martin, Analytical Chemistry, 2005, 77, 1376 – 1384.

In vivo quantitative analysis of skin penetration of topically applied products by Raman spectroscopy

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It is common "knowledge" that the skin is the barrier between the body and the outside world. The total skin surface area of the adult body is about 2 m^2. It prevents uncontrolled loss of water and keeps chemicals and microorganisms out. However, until now the ability to actually measure and quantify how well this barrier works has eluded us. Current methods are indirect, have reproducibility issues, are invasive and/or use conditions that are far from actual in vivo conditions. They leave a lot to wish for. This poses a problem for topical product development in personal care industry and pharmaceutical industry. The absence of a reliable method which non-invasively determines the penetration and permeation of a topically applied product, also creates a hurdle for so-called bioequivalence studies, which are required to bring a generic pharmaceutical product. This presentation will demonstrate how *in vivo* confocal Raman spectroscopy is filling that gap.

Perspective on near-infrared spectroscopy – new avenues for established analytical technique

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Near-infrared (NIR) spectroscopy, NIRS, Miniaturized spectroscopy, Handheld spectrometers, Analytical applications

Near-infrared (NIR) spectroscopy nowadays is a mature and wide-spread analytical technique, valued for rapidness, flexibility and high-throughput capacity. It bears almost unparalleled practical value in diverse applications throughout analytical chemistry labs, various industries, agriculture, forestry, subjects involved in environmental monitoring and food safety institutions. Despite gaining an ultimate value in several areas of application, certain limitations of this technique have been apparent that somewhat limited its further spread. However, recent few years have seen fundamental advancements in NIR spectroscopy, which decisively changed the potential for applications and our understanding of NIR spectra. Dynamic development of miniaturized spectrometers enabled a new array of applications. On-site capable NIR spectrometers and unmanned aerial vehicle (UAV; i.e. drone)-mounted sensors are currently revolutionizing the applications of this technique. Development of ultra-miniaturized NIR spectrometers towards on-chip sensor will enable implementing these instruments in wearables and may ultimately place NIR spectroscopy as part of everybody's daily life. On the other hand, advancements in methods for spectral data-analysis and interpretation increase ones' understanding of the subtle spectral features, and enable better optimization of the analytical procedures. Notable advances were made at the foundations of NIR spectroscopy, e.g. with the ability to theoretically predict absorption bands by quantum chemical simulations. This made a leap in our understanding of physical chemistry manifested by overtones and combination bands. The advancements in basic science remarkably pushed the limits of interpretability of NIR spectra. Further, they enabled understanding of the instrumental difference in analytical applications and agile designing of the analysis towards specific compound. Accompanied by a continuous refinement of established approaches, these factors decisively changed the potential for future applications of NIR spectroscopy. Combined advancements in fundamental research and technology bring a new perspective onto NIR spectroscopy seen not only as an analytical technique but also a powerful tool in basic science. During this talk the audience's attention will be pointed to the thriving potential of NIR spectroscopy and current developments that set up a new horizon for this potent technique.

[1] Huck, C.W.; Phytochem. Lett. 2017, 20, 491. [2] Beć, K.B.; Grabska, J.; Siesler, H.W.; Huck, C.W., NIR News 2020, 31(3–4), 28. [3] Beć, K.B.; Grabska, J.; Huck, C.W.; Chem. Eur. J. 2021, 27, 1514. [4] Beć, K.B.; Huck, C.W.; Front. Chem. 2019, 7, 48. [5] Beć, K.B.; Grabska, J.; Huck, C.W.; Spectrochim. Acta A 2021, 254, 119625.

MONITORING CELL CULTURE MEDIA BY TIME-GATED RAMAN SPECTROSCOPY - A burgeoning analytical tool

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[1]Timegate Instruments Oy

Time-gated Raman spectroscopy, Cell culture media, Monitoring amino acid

Real-time monitoring and control of metabolites in a cell culture process mitigates the risks associated with the production of biotherapeutic proteins. Many of these cell culture media generate highly fluorescent molecular species which pose a great challenge to be monitored. This predicament is resolved by Timegate Instruments' patented technology for recording Raman spectra before the advent of fluorescence. In this study, we have tested the potential of this technology to quantify phenylalanine, an amino acid, spiked in the cell culture media. A statistical model was developed and shows promising results to quantify phenylalanine which would be discussed in detail

We thank Prof. Simo Saarakkala, Physics and Technology, Faculty of Medicine, University of Oulu.

Monitoring of physical blowing agent concentration in a foam injection molding process by near-infrared spectroscopy

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Near infrared spectroscopy, Foam injection molding process, Online monitoring

The foam injection molding (FIM) process is an environmentally benign polymer processing technique because of the reduction of polymeric materials. This process provides the inside of polymeric parts the microcellular structure, which causes various functions: lightweight, thermal insulation, etc.[1] The FIM process produces the microcellular foams with a physical blowing agent (PBA), such as nitrogen (N_2) and carbon dioxide (CO_2). PBA is usually pressurized and dissolved into the molten polymer in the FIM machine. One of the most important issues for conducting the stable production of the polymer foam is to control the PBA concentration in the molten polymer. However, there was no measurement technique for controlling the concentration in a feedback manner. We have developed the new NIR sensor probe to measure in-line the PBA concentration in the FIM machine. The new NIR sensor probes with high-pressure resistance of 120 MPa and heat resistance of 230°C can be installed at the tip of the barrel of the FIM machine. The probes were connected to FT-NIR (FIR1000L, Yokogawa Electric Corporation) with the optical fibers. The transmission NIR spectrum was acquired with 4 cm^{-1} spectral resolution in the wavenumber range between 10000 and 4000 cm^{-1} and optical length of 8 mm. Isotactic polypropylene (iPP) foam was produced by a 35-ton clamping force electric injection molding machine (J35AD-AD30H, Japan Steel Work, Ltd.) with CO_2 as PBA. The FIM process was conducted with the resin temperature of 210°C and the CO_2 gas delivery pressure changing from 1 MPa to 5 MPa. NIR spectra were measured during the FIM operation, and the change in the spectral band at 4952 cm^{-1} [2] was confirmed according to the increase of the gas delivery pressure. The NIR spectrum measured without CO_2 was subtracted from each NIR spectrum, and the baseline correction was conducted with basepoints at 5022 and 4875 cm^{-1}. The obtained absorbance (ACO_2) increased in proportion to the gas delivery pressure. The averaged CO_2 concentration in molten iPP at each gas delivery pressure condition was calculated from the weight ratio of total amounts of consumed gas and polymer during 100 shots. The calibration line was obtained by comparing ACO_2 and the averaged concentration, as shown in Figure. This relationship was used for investigating the change in CO_2 concentration depending various operating conditions of FIM process.

This research was supported by the GAP fund program of Kyoto University.

[1] Xu, J., Microcellular Injection Molding. John Wiley and Sons Ltd, 2010.[2] Guadagno, T and Kazarian, S.G. The Journal of Physical Chemistry B. 108, 13995 (2004)

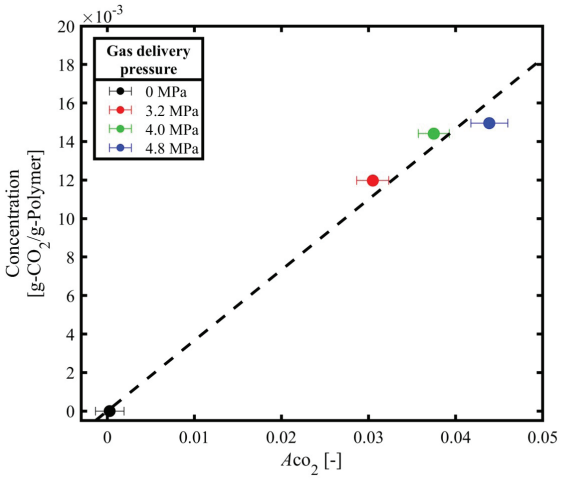

Figure 1. Relationship between the averaged CO2 concentration during the FIM process and the absorbance at 4952 cm-1 (ACO2) after the baseline correction

InCIMa4: a platform to characterize environmentally friendly materials connecting research and SMEs

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FTIR microtomography, green materials, analytical techniques SMEs, tannin derivatives

InCIMa (Intelligent Characterization of intelligent Materials) is a project funded by the European Regional Development Fund and Interreg V-A Italy-Austria 2014-2020 [1]. InCIMa established an Italy-Austria cross-border platform connecting laboratories for synthesis and characterization of smart materials at nano, micro and macro scale by the use of spectroscopic techniques with a wide radiation range from far infrared to hard X-rays, using synchrotron radiation and conventional sources. Indeed, InCIMa assumes that functional behavior of materials relies on their morpho-chemical properties at the meso-scale, therefore the acquired knowledge will guide the choice of the synthesis parameters. In particular, the study was focused on the morpho-chemical characterization of tannin-furanic rigid foams, bio-based copolymers of tannin plant extract and furfuryl alcohol, promising candidates to replace synthetic insulation foams such as polyurethanes and phenolics in eco-sustainable buildings thanks to their functional properties, such as lightness of the material and fire resistance. Despite their relevance as environmental-friendly alternatives to petroleum derivatives, many aspects on the spatial heterogeneity of the foam, i.e. whether the foam constituents prevalently polymerase in spatially segregated blocks or distribute almost homogenously in the foam volume, remain still unclear. To address this question, we integrated UV-Vis Raman spectromicroscopy [2], X-ray and FTIR microtomography (μ-CT) and imaging. The results of these studies will be here presented, highlighting the role of FTIR μ-CT (see Fig. 1). The integrated approach of InCIMa and its paradigm applies on the characterization of any type of functional material, also of industrial relevance and the platform has progressed nowadays for industrial research in the framework of the project InCIMa4 (InCIMa for Science and SMEs) [3]. InCIMa4SMEs has the specific objective of connecting the industry with research centres associated to the platform to solve issues that the R& labs from the SMEs cannot address otherwise. Examples of industrial activities conducted within the framework of the project will be also presented, and will reveal indeed how circular economy and bio-based industries are looking for state-of-the-art analytical tools for optimizing their products and making the future greener.

InCIMa and InCIMa4 projects are funded by European Regional Development Fund and Interreg V-A Italy-Austria 2014-2020.

[1] Project website: http://www.elettra.eu/Prj/InCIMa/ [2] Tondi, G.; Cefarin, N.; Sepperer, T.; D'Amico, F.; Berger, R.J.F.; Musso, M.; Birarda, G.; Reyer, A.; Schnabel, T.; Vaccari, L. Understanding the Polymerization of Polyfurfuryl Alcohol: Ring Opening and Diels-Alder Reactions. Polymers 2019, 11, 2126. [3] Project website: https://www.incima4.eu/it/homepage/

Time-gated Raman spectroscopy as mineral analysis tool in multi-sensor Ancorelog drill core logger

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Time-gated Raman spectroscopy, Drill core analysis, Ancorelog, Raman mineralogy, multi-sensor analysis

Modern mining industry undergoes extensive drilling campaigns during exploration, mining, processing and recycling of raw materials. There is a growing demand for more precise and automated digital processes to reduce the needed time, cost and labour of ore exploration and handling. A precise and analytical drill core logging process utilising multi-sensor approach could provide fast and timely method for mineralogy tracking. Most of the conventional spectroscopic methods in drill core analysis reveal their elemental information. This information can be used to create models and predict the mineralogy, but it does suffer from the inability to detect the molecular bonds and autonomously build precise predictions. Elemental information requires background information on the sample mineralogy to be able to build the models and it can be disturbed by similar mineral consistencies. Raman spectroscopy is a good support for data fusion with elemental spectroscopy such as XRF and LIBS, because it can indicate the molecular bonds instead of elements [1]. However, the conventional Raman spectrometers have limited amount of minerals they can distinguish, because the fluorescence of the sample can lower the detection sensitivity or sometimes overflow the signal entirely. This problem is common especially in minerals. A solution for reducing the elevated signal background comes in the form of time-gated Raman spectroscopy [2]. This technology uses short laser pulses to excite the sample and measures the scattered radiation in short time-window before the slower timescale phenomena, fluorescence, has time to manifest. In many cases, this can eliminate the fluorescence background and bring on to view spectral features that are commonly hidden or weak using traditional Raman techniques. In this study, timegated PicoRaman M2 spectrometer, utilizing 532 nm pulsed excitation laser, is integrated to a multi-sensor Ancorelog drill core logger solution as a mineral identification tool. The measurement utilises an auto-focus probe developed at VTT with ability to set the system in focus autonomously during measurements. In addition, a mineral identification software is developed for automated identification of minerals using Raman spectroscopy. The solution utilizes reference spectra from the RRUFF online database of minerals [3]. A classification model correlates the measured spectra with reference spectra, which are selected using expert knowledge of the sample mineralogy. The correlation utilizes a dot product between the spectra as a metric.

T-REX: Timegated Raman for Exploration project. Funded by the EIT RawMaterials, Horizon 2020

[1] Uusitalo, Sanna, et al. "Online analysis of minerals from sulfide ore using near?infrared [1] Raman spectroscopy." Journal of Raman Spectroscopy 51.6 (2020): 978-988. [2] Kögler, Martin, and Bryan Heilala. "Time-gated Raman spectroscopy–a review." Measurement Science and Technology 32.1 (2020): 012002 [3] Lafuente B, Downs R T, Yang H, Stone N (2015) The power of databases: the RRUFF project. In: Highlights in Mineralogical Crystallography, T Armbruster and R M Danisi, eds. Berlin, Germany, W. De Gruyter, pp 1-30

Raman spectroscopy as a tool to estimate particle size distribution in pharmaceutical products

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particle size, pharmaceutical products, Raman spectrosocpy

In the recent years, Raman spectroscopy become one of the more promising techniques with increasing application potential in the pharmaceutical industry. Due to its development and improved instrumental versatility, this technique gained high enough precision to be used in determination, identification and quantification of drug substances, including both, active pharmaceutical ingredients (API) and excipients. Raman spectroscopy in pharmaceutical analysis is widely used in many fields, e.g., in identification of raw materials [1], quantitative determination of active substances in different formulation [2, 3], supporting polymorphic screenings, supporting chemical development process [4]. In such studies, Raman imaging emerges as particularly beneficial allowing for simultaneous acquisition of chemical information and spatial distribution of the sample content. Herein, we would like to focus on estimation of the particle size distribution in pharmaceutical products using Raman imaging. Our methodology was firstly validated using polystyrene microsphere size standards (7 and 30 µm m). Acquired data allowed to prepare integration Raman images which reflects semi-quantitatively concentration of the each excipient as well as k-means cluster analysis (KMC) images which help to identify and differentiate API from excipients. Subsequently, KMC images were used to estimate particle size distribution with high precision and accuracy (with the coefficient of variation up to 15.7%). Another approach to obtain particle size distribution is to apply ParticeScout (Witec, Ulm, Germany) – the Raman imaging tool that can identify, quantify, classify and analyze particles in a sample. Such approach greatly decrease amount of time needed to collect size distribution data. Importantly, such measurements can be performed on both, final products as well as on each step of the product design. Currently, there is no other replacement for Raman spectroscopy in this field, as any other analytical technique does not allow for simultaneous acquisition of chemical information and particle size distribution in non-label and non-destructive manner.

[1] M. de Veij, P. Vandenabeele, T. De Beer, J. P. Remon and L. Moens, J. Raman Spectrosc. 2009, 40, 297–307 [2] A. Kuriyama, Y. Ozaki, AAPS PharmSciTech, 2014, 15, 375-387 [3] J. I. S. da Silva de Jesus , R. Löbenberg, N. A. Bou-Chacra, J Pharm Pharm Sci, 2020, 23, 24-46 [4] S. Šašić, Anal Chim Acta 2008, 611, 73-79

Tensiometry and FTIR study of the synergy in mixed SDS:DDAO surfactant solutions at varying pH

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Mixed surfactants, Surface Tension, Synergism, ATR-FTIR, Sodium Dodecyl Sulfate, Dimethyldodecylamine Oxide, pH

The interactions between a model anionic and amphoteric surfactant pair in aqueous solution are examined as a function of composition, at floating and fixed pH, employing a combination of tensiometry, regular solution theory analysis, and FTIR spectroscopy. An extensive series of pure and mixed ratios of sodium dodecyl sulfate (SDS) and N,Ndimethyldodecylamine N-oxide (DDAO), ranging from 0.0016 to 100 mM, yielding 77 data points below and above the critical micelle concentrations (CMC), is investigated. Compared to either pure surfactant solutions, the CMC of mixed SDS:DDAO solutions is found to decrease by up to 20-fold, and the surface tension (γ) at CMC down to ~23 mN/m. At all concentrations, the most prominent effects are observed at equimolar SDS:DDAO ratios. Further, the pH of mixed micellar solutions is found to increase with respect to the pure surfactant solutions (from ~7 up to ~9.5), which is attributed to the enhanced protonation of DDAO in the presence of SDS, and supported by FTIR frequency shifts of isolated O-H stretching vibrations. Vibrational responses from CH2 stretching of the methylene tails, and the S-O stretching modes for the sulfate headgroups indicate strong lateral interaction and enhanced packing between SDS and DDAO. From regular solution theory analysis of tensiometry data, the molecular interaction parameters are found to have a larger magnitude (i.e., more negative) at the interface as compared to within micelles. At fixed solution pH, a decrease from pH 9.5 to 7.5 results in minimal changes in both interfacial and micellar parameters, indicating the intrinsic origin of these pairwise interactions. Overall, our findings demonstrate a pronounced synergistic interaction between SDS and DDAO, arising from diminished electrostatic and steric repulsions in, respectively, SDS and DDAO, accompanied by enhanced lateral surfactant packing.

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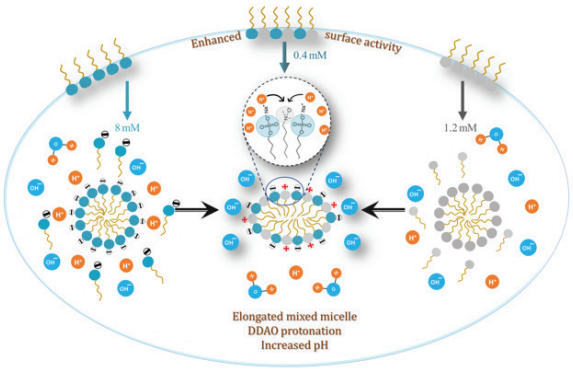

Figure1: Schematic representation of the synergistic association of SDS and DDAO in solution, underpinning the CMC and interaction parameter changes, surfactant protonation and micelle elongation.

Photochemistry studies of the intelligent luminescent molecular sensors for monitoring of polymerization processes

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The fluorescence spectroscopy is an important analytical technique that has been widely used in a variety of applications, such as biomedicine, biology, and science, which makes it unique thanks to extraordinary sensitivity and selectivity, short delay time, and the fact that it is neither invasive nor destructive so that it can be used for the in-situ measurements. A fascinating and essential application of luminescent sensors is the research of testing polymeric materials by fluorescence spectroscopy. There is a high demand for a quick and reliable method of polymerization progress monitoring applied directly in production lines. Fluorescence Probe Technology is the answer to this type of need because it is based on fluorescent probes as molecular sensors and quanta of light for information transfer between the probe molecules and the monitoring system. In the FPT method, changes in luminescence characteristics of appropriate molecular sensors caused by changes of polarity or microviscosity of the medium in which the probe is dissolved are monitored in real-time. The changes in the probe response usually correlate very well with the changes of other parameters occurring in the system. For example, during the polymerization of monomers, the probe molecules interact with the monomer and polymer molecules present in the probe vicinity. With the polymerization progress, the degree of solvation of the excited probe molecules changes, which causes the change of their energy at the moment of light emission, and consequently, a change of fluorescence characteristics. Hence, the quanta of light emitted by the probe molecules carry information about the changes in the reacting system. In most of the monomers, when a fluorescent probe is dissolved in a monomer and the monomer is polymerized, the system polarity decreases because monomers are usually more polar than the corresponding polymers. Consequently, the fluorescence spectrum of the probe shifts towards shorter wavelengths, while the shift magnitude is proportional to the extent of the change that has occurred in the system. The main objective of this research is synthesis, characterization and investigations of compounds that exhibit luminescence strongly dependent on changes in their environment during polymerization processes.

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New fluorescent molecular sensors for monitoring of free-radical photopolymerization processes using fluorescence spectroscopy

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Study of molecular environment of small molecules by fluorescence methods becomes more and more important in many areas of life sciences and chemical technologies, such as medicine and biology for the study of components of leaving cells and tissues, determination of DNA sequences and antibodies, as well as for the study of polymers and polymerization processes. Intensive developments and applications of fluorescent molecular sensors, called probes, in polymer chemistry, started in the eighties of the XX century, when advancements in the construction of appropriate rapid scan fluorimeters and automatic data acquisition systems using microcomputers accelerated that growth. Theoretically, every chemical or physical process and parameter, where changes of physicochemical properties of the system studied occur, can be monitored using appropriate molecular sensors. However, depending on the system type, different probes are required. Not all luminescing compounds are suitable for application as probes. Only some fluorophores are sensitive to changes in the physicochemical properties of their environment. The probes applicable for polymeric materials processes usually respond to changes in polarity and/or microviscosity occurring in the polymerizing system. Nevertheless, there are no versatile probes suitable for every polymerization type. The probes that work well in systems polymerized by free radical polymerization usually are not good enough for the systems polymerized by cationic polymerization. Therefore, careful design of the probe structure usually is required for specific applications. For example, to date, only several fluorescent probes suitable for monitoring cationic polymerization have been developed. Other types of polymerization, such as thiol-ene addition polymerization, or hybrid polymerization processes have never been monitored by the FPT method, because of the lack of suitable probes. There are several requirements that fluorescent probes must meet to be useful for monitoring polymerization reactions. The probe should have a high absorption coefficient, high fluorescence quantum yield and large Stokes shift to avoid fluorescence self-absorption. In addition, if the probe is to be used for the following photoinduced polymerizations the absorption of the probe should not interfere with the absorption of the photoinitiator and the probe should be photochemically stable under curing conditions (irradiation wavelength and irradiation time). We present in this work new fluorescent probes which can be used to follow photopolymerization processes in the full conversion range of monomers. The aim of the work is to study the influence of the structure of the probes on their sensing properties. The properties of the new probes have been compared with those of the classical probes.

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Additional, special thanks to the project manager – dr hab. inż. Joanna Ortyl, prof PK

New bi-component photoinitiating system for initiation the photopolymerization processes

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Photopolymerisation is an energy-efficient and environmentally friendly method of producing polymeric protective coatings. It is used in photo-curable solvent-free paints, varnishes, inks, dental fillings and other materials.[1] Two types of photochemically initiated polymerisation are most commonly used in industry: radical and cationic photopolymerisation. These types of photopolymerisation differ in their mechanism and in the type of monomers and initiators. The basis of widely used radical photopolymerisation systems are acrylate and methacrylate monomers, which polymerise according to a radical mechanism. However, an important drawback of radical polymerizing compounds is the common unfavorable phenomenon of oxygen inhibition, caused by the presence of atmospheric oxygen during the polymerization process.[2] Thiol-ene photopolymerisation follows a radical mechanism. Typical advantages of the thiol-ene reaction include its high rate, lack of sensitivity to the presence of oxygen and almost 100% conversion of monomers. A great advantage of the described photopolymerisation process is the great variety of monomers that can be polymerised by this mechanism. This makes it possible to select monomers in such a way as to obtain a product with the desired properties. The main stage of the research was to check the usefulness of the developed initiator system based on biphenyl derivatives during photopolymerisation. The cationic and radical photopolymerizations were monitored in the FT-IR spectrum and the disappearance of different bands originating from functional groups in monomers, respectively, was observed.

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Additional, special thanks to the project manager - dr hab inż. Joanna Ortyl, prof. PK

[1] Z. Chen, Y. Zhang, B. Chisholm, D. Webster, J. Polym. Sci. A – Polym. Chem., 2008, 46, 4344-4351 [2] M. Topa, E. Hola, M. Galek, F. Petko, M. Pilch, R. Popielarz, F. Morlet-Savary, B. Graff, J. Lalevée, J. Ortyl, Polym. Chem., 2020, 11, 5261-5278

2.2. Biomedical Applications

Highlighting the clinical utility of a novel spectroscopic liquid biopsy for triage of patients with suspected brain cancer

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Brain tumours are extremely difficult to detect early through the current diagnostic pathway, as many patients present with non-specific symptoms – such as headaches and migraines – meaning they visit their primary care general practitioner multiple times before ultimately being diagnosed in the emergency department. Diagnostic delays often impact on quality of life and survival of affected patients; hence it is crucial to strive for an earlier diagnosis. A simple and rapid blood test that can identify which patients are most likely to have a brain tumour at an earlier stage based on their clinical assessment in primary care, would enable faster brain imaging referrals for those who need it most. Here we report the results of the second phase of our clinical feasibility study of brain tumour early diagnosis (BRAIN-ED2). Blood serum samples have been prospectively collected from 605 patients, either with generic non-specific symptoms that may be indicative of a brain tumour, or those with a new brain tumour diagnosis, within the primary care setting in NHS Lothian, Scotland. Patient samples were analysed using the Dxcover liquid biopsy test, which utilises novel technology based upon infrared spectroscopy combined with our diagnostic algorithm, to predict the presence of intercranial disease. Our liquid biopsy approach has reported a receiver operating characteristic curve with an area under the curve (AUC) value of 0.8013. When tuned for sensitivity, the optimum algorithm achieves an 90% sensitivity with 55% specificity. When tuned for a higher specificity, the model with 90% specificity yields a sensitivity of 44%. In a symptomatic population of 10,000 patients, approximately 9,900 patients would not have a brain tumour, and by utilising the specificity-tuned algorithm we would save an estimated 4,356 unnecessary brain scans. For the 100 patients with a brain tumour, the sensitivity-tuned model could diagnose 90% of these patients faster than standard care. Integrating our blood test into existing diagnostic pathways would lower the threshold for suspecting a brain tumour and permit more effective triage of patients for medical imaging, expediting assessment for 'at risk' patients whilst excluding a brain tumour diagnosis in others.

On self-assembling intracellular Raman reporters and their clinical potential

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Abstract: Raman spectroscopy has received considerable attention for in vivo imaging of biochemical constituents present in cells and tissues without jeopardizing the internal structure and altering their function. The innate advantages of label-free imaging, minimum sample preparation, non-invasiveness, and higher spatial resolution when compared to magnetic resonance imaging (MRI) and computed tomography (CT) has led to its use in complex situations such as surgical margin assessment and brain surgery requiring real-time feedback. Despite these unique features, its widespread use for objective cancer detection has been limited by the spectral congestion among overlapping features emanating from the myriad constituents of the cellular and tissue matrices. Hence, development of targeted Raman scattering agents for sensing of biomarkers characteristic of tumor progression is highly desired. In this talk, I will discuss the design of intracellular self-assembly of Raman reporters into larger nanoassemblies that increases the concentration of drugs locally enhancing the Raman signal. We draw inspiration from recent progress in the design of tumor retention of imaging agents or anticancer drugs, which focus on the rational engineering of probes that undergo a tumor-specific enzymatic reaction preventing them from being pumped out of the tumor cell. Our work shows the utility of the self-assembling Raman probes for in vitro detection of furin-overexpressing colorectal carcinoma cells and, crucially, for in vivo delineation of such tumors in mice.

Investigation of the chemical composition of microcalcifications in breast cancer using simultaneously Optical Photothermal Infrared (O-PTIR) and Raman spectroscopy

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Microcalcifications, Breast cancer, O-PTIR spectroscopy, Raman spectroscopy

Breast cancer is the second most common cause of cancer death in the UK, in women. Despite the improvement of diagnostic techniques, it remains a significant public health issue. Approximately 11,500 women die each year in the UK despite a high-quality screening programme[1]. According National Health Service (NHS) data in England, in 2017-18, 2.5 million women were invited for a mammography; 18000 cancer cases were detected of which 14000 were invasive[2]. A better understanding of breast cancer could improve the survival rate, treatment response and patient wellbeing. In this context, the study of the microcalcifications is relevant as they appear to be a unique early marker for breast disease. Microcalcification in breast tissue has a range of different morphologies and many varying compositions. Two types of calcifications are found: type I, composed of calcium oxalate dihydrate ($CaC2O4{\bullet}2H_2O$), and type II, more complex, made of Hydroxyapatite (Hap) ($Ca_{10}(PO_4)_6(OH)_2$) with different types of carbonate ion substitution in the crystal lattice. We investigated the chemical composition of microcalcifications using a range of different imaging techniques. Amongst them, vibrational spectroscopy such as Raman and infrared (IR) spectroscopy which can be used to identify specific features of calcified tissues, in particular phosphate and carbonate bands[3,4]. O-PTIR spectroscopy with a high resolution (0.5 µm) was used to evaluate the microcalcifications and their microenvironment in detail. We applied this new technique for analysing breast tissue sections containing microcalcifications, as illustrated in Figure 1. Different single IR images were acquired at 1656, 1044 and 872 cm^{-1} for Amide I, phosphate and carbonate bands, respectively. The results show the spatial distribution of these different components and highlight a combination of proteins and carbonated Hap within the microcalcifications. Our study shows that O-PTIR spectroscopy is a rapid and highly sensitive technique for gaining more spectral information about microcalcification in breast tissue.

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[1] www.cancerresearchuk.org [2] M. Richard, « independent review of National Screening Programmes in England », 2019 [3] M. Kerssens et al., Analyst, vol. 135, no. 12, pp. 3156-3161, 2010. [4] Haka et al., Cancer Res., vol. 62, no. 18, pp. 5375–5380, 2002.

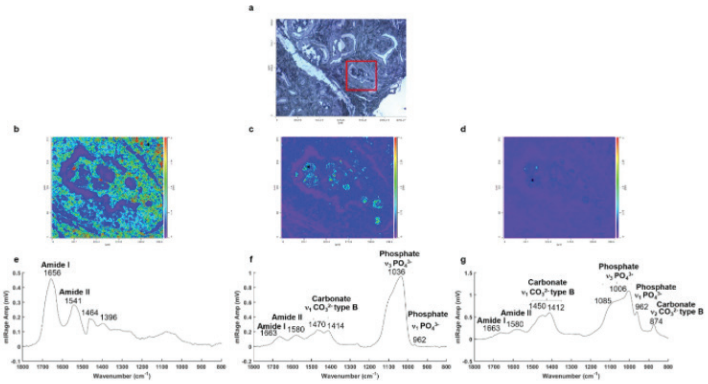

Figure 1. (a) Visible image of a breast tissue section containing microcalcifications (red square), X10 objective. Series of discrete frequency IR images at (b) 1656 cm-1, (c) 1044 cm-1 and 872 cm-1. For each IR image, (d-f) a representative O-PTIR spectrum was collected (black square) in the fingerprint region between 1800 and 800 cm-1

Quantitative Analysis of Human Blood Serum using Vibrational Spectroscopy.

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Analysis of bodily fluids using vibrational spectroscopy has attracted increasing attention in recent years. In particular, infrared spectroscopic screening of blood products, particularly blood serum, for disease diagnostics has been advanced considerably, attracting commercial interests. However, analyses requiring quantification of endogenous constituents or exogenous agents in blood are less well advanced. Recent advances towards this end are reviewed, focussing on infrared and Raman spectroscopic analyses of human blood serum. The importance of spectroscopic analysis in the native aqueous environment is highlighted, and the relative merits of infrared absorption versus Raman spectroscopy are considered, in this context. It is argued that Raman spectroscopic analysis is more suitable to quantitative analysis in liquid samples, and superior performance for quantification of high and low molecular weight components, is demonstrated. Applications for quantitation of viral loads, and therapeutic drug monitoring are also discussed.

Breast Cancer Detection Using Infrared Spectral Pathology from H&E Stained Tissue on Glass Slides

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Infrared, Hyperspectral Imaging, Pathology, Breast Cancer, Glass slides, H&E, Cancer Screening

Infrared spectral pathology has gained significant attention in the last few years, since it has been demonstrated to be able to readily identify cancerous tissue in biopsy samples. The Infrared technique, however, normally requires tissue sections to be mounted on infrared transparent slides. Unfortunately, these slides are both expensive and particularly frangible. In addition, mounting samples on specialist slides is an additional step in the sample preparation workflow, which ideally should be avoided. Applying infrared imaging directly to the H&E stained tissue on the glass slides that are normally used by pathologists, could help the infrared imaging technique be incorporated into current cancer diagnosis work flow and lower the total cost of detection [1,2]. The disadvantage of using glass slides is that the spectral range available is restricted to just the high wavenumber region (2500-3600 cm^{-1}) In this work a study has been conducted on 120 breast tissues biopsy cores from different patients, to demonstrate that with the limited spectral information, breast cancer can be identified from the H&E glass slides. A four-class histological Adboost classification model has been constructed. Optimisation of the classification threshold was carried out to reduce the number of false negatives. Using a threshold of 0.1 the cancerous cores could be detected with an accuracy of 95.8%. This was incorporated into a simple traffic light system that could be used as a prescreening tool. (Figure 1). This work thus helps remove one of the barriers to successful translation of infrared spectral pathology into the clinic.

This work was supported in part by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868). The FTIR microscope was funded by a generous donation from the Williamson Trust, UK.

[1] P. Bassan, J. Mellor, J. Shapiro, K.J. Williams, M.P. Lisanti, P. Gardner, Transmission FT-IR chemical imaging on glass substrates: Applications in infrared spectral histopathology, Anal. Chem. (2014) 86 1648-1653 [2] M.J. Pilling, A. Henderson, J.H. Shanks, M.D. Brown, N.W. Clarke, P. Gardner, Infrared spectral histopathology using haematoxylin and eosin (H&E) stained glass slides: a major step forward towards clinical translation, Analyst. (2017), 142, 1258–1268. [3] Jiayi Tang, Daniela Kurfürstová, Peter Gardner, Breast Cancer Detection Using Infrared Spectral Pathology from H&E Stained Tissue on Glass Slides, Clinical Spectroscopy, 2021, 3, 100008

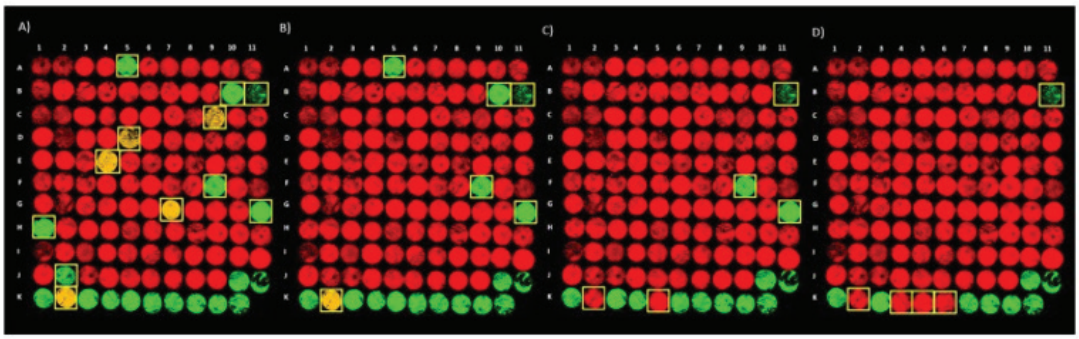

Figure 1. False colour images of 120 grade II breast cancer cores using traffic light system with a threshold of A) 0.5, B) 0.3, C) 0.2, D) 0.1, where red represents cancerous, green shows normal cores and amber represents cores that could not be classified as either cancerous or normal and therefore should be looked at by a pathologist.

Biomedical Applications (CLIRSPEC Biomedical spectroscopy session)
Raman spectroscopy for early detection of cervical pre-cancer using minimally invasive exfoliated cell samples

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Raman spectroscopy, spectral cytopathology, exfoliated cells, pre-cancer, screening, early detection, cervical cancer

There is an unmet clinical need for new methods to aid clinicians in the early detection of cervical cancer and pre-cancer. Current gold standard methods include HPV testing, cytopathology and histopathology but these methods are limited in terms of subjectivity, cost and time. Spectroscopic methods such as Raman spectroscopy can provide a rapid, label-free and non-destructive measurement of the biochemical fingerprint of cells, tissues and biofluids. Many studies have focused on 'spectral histopathology' but there has not been as much attention on 'spectral cytopathology'. This talk will present our recent studies on spectral cytopathology which have addressed many of the challenges of working with exfoliated cell samples and have shown promising results for identification of cervical pre-cancer.

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Biochemical and nanomechanical characteristic of human normal and cancer tissues and cells of digestive tract in oxidative and protective conditions

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Raman Spectroscopy, gastrointestinal cancer, Raman bioimaging, oxidative stress, antioxidant treatment

Colon cancer is one of the most commonly detected pathology and is the third world wild cause of death in the US and Europe and ranks second with regard to the incidence of malignant tumors in population. Gastric cancer is the fifth most common cancer and the third most common cause of cancer death globally. We will show that noninvasive Raman imaging of non-fixed and not stained human colon and stomach tissues based on vibrational properties of noncancerous and cancerous tissues can enabling effectively the differentiation of tumor tissue form the healthy one. The main goals of this work was to evaluate the biochemical characteristic of colon and stomach cancer and to show the clinical merits of Raman imaging and spectroscopy analysis. The tissues measurements were complemented by studies of human colon and stomach normal and cancer cells including cells in oxidative stress conditions. The protective effect of β-carotene, vitamin C and vitamin E on the oxidative stress injury of human normal colon and stomach cells will be also discussed. We will show that the treatment with β-carotene, vitamin C and vitamin E altered the level of ROS based on intensities of Raman peaks typical for lipids, proteins and nucleic acids. Presented study confirmed the antioxidant properties of natural compounds against ROS by using spectroscopic label-free Raman techniques. The biochemistry properties of human digestive tract were completed also by AFM studies for colon and stomach normal and cancer cells including ROS-treated samples. The influence of antioxidants on nanomechanical characteristic of human colon and stomach samples will be shown.

The project was funded through The National Science Centre Poland Grant UMO-2017/25/B/ST4/01788.

[1] B. Brozek-Pluska, K. Beton, Oxidative stress induced by tBHP in human normal colon cells by label free Raman spectroscopy and imaging. The protective role of natural antioxidants in the form of β-carotene, RSC Advances, 2021, accepted for publication. [2] B. Brozek-Pluska, A. Jarota, R. Kania, H. Abramczyk, Zinc Phthalocyanine Photochemistry by Raman Imaging, Fluorescence Spectroscopy and Femtosecond Spectroscopy in Normal and Cancerous Human Colon Tissues and Single Cells, Molecules, 2020, 25, 2688. [3] B. Brozek-Pluska, Statistics assisted analysis of Raman spectra and imaging of human colon cell lines – Label free, spectroscopic diagnostics of colorectal cancer, J. Mol. Struc., 2020, 1218, 128524. [4] Beata Brozek-Pluska, Adam Dziki, Halina Abramczyk, Virtual spectral histopathology of colon cancer – biomedical applications of Raman spectroscopy and imaging, J. Mol. Liq, 2020, 303, 112676. [5] B. Brozek-Pluska, K. Miazek, J. Musiał, R. Kordek, Label-free diagnostics and cancer surge

MCR-ALS assisted identification of spectral markers for objective discrimination of breast cancer from mammary epithelial cells by Raman microspectroscopy

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MCR-ALS, Raman microspectroscopy, breast cancer, Spectral marker, chemometrics

Background: Histopathology is generally considered a 'Gold Standard' in diagnosis of cancers. But it is invasive and time consuming. Moreover, it may not be easy to perform biopsy in many situations. Therefore cytology-based non-invasive diagnosis using cells extracted from body fluids such as blood, urine etc is recommended. Cytodiagnosis of cancers is normally done by staining and observing cellular morphology under the optical microscope. However, conventional cytology suffers from low sensitivity and specificity. This makes definitive diagnosis difficult. There is a need to develop alternative technologies for reliable cytodiagnosis. Raman microspectroscopy (RM) is a rapid, non-invasive, label-free method that provides rich chemical information. Application of RM in cytology is anticipated to solve indeterminate cases. However, screening of spectral markers unaffected by excitation wavelength is necessary for universal adoption of this technique. Methods: In this study, Raman spectra were obtained from 30 independent human mammary epithelial cells (HMEpC) and breast cancer cells (MCF-7) cells with two popular excitation wavelengths, 633 and 532 nm. Both conventional univariate approach and various multivariate statistical methods including, principal component analysis (PCA), linear discriminant analysis (LDA), support vector machine (SVM) and multivariate curve resolution (MCR-ALS) were utilized to either classify or model pure component Raman spectra. Results: LDA and SVM provided high classification accuracy between MCF-7 and HMEpC cells. To obtain molecular basis, MCR-ALS analysis of 633 nm and 532 nm excited RM data was performed. We identified physically meaningful pure biomolecular spectra comprising DNA, proteins and 3 independent lipids. Change of excitation did not affect the proposed model. Their relative abundances, especially that of unsaturated lipids, were markedly different and served as potential spectral markers (Figure 1). Conclusion: To understand key molecular changes in cancer cells and develop a robust clinical diagnostic method, MCR-ALS assisted RM for discrimination based on pure molecular composition has been studied. We successfully identified Raman spectral markers and propose to use unsaturated lipids as spectral hallmark to discriminate breast cancer cells. RM, as an adjunct to morphology, has the potential to become an excellent cytodiagnostic tool that can both accurately and objectively discriminate cancer from normal cells.

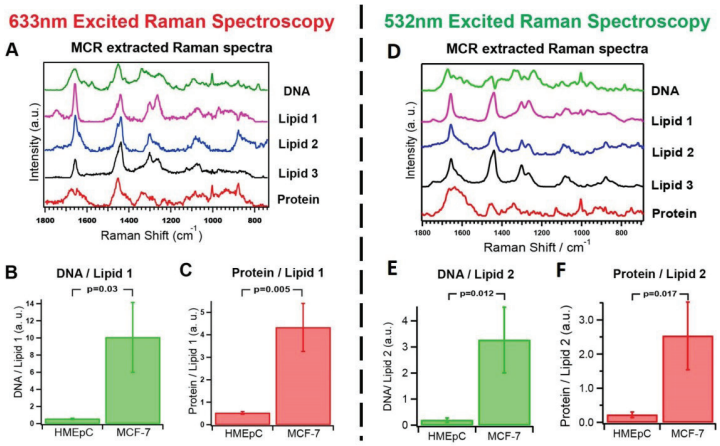

Figure 1. Results of MCR-ALS analysis from 633nm excited Raman spectroscopy (A-C) and 532nm excited Raman spectroscopy (D-F).

Soft polyacrylamide hydrogels as a cell culture matrix for mechanical and spectroscopic study of glioblastoma development

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Raman spectroscopy, polyacrylamide hydrogels, glioblastoma, mechanomarkers, biomarkers

The increasing number of studies that assess broadly defined cells response to the substrate stiffness on which they grow, indicate that mechanical environment might be an important determinant of cancer development. Cells grown on soft polyacrylamide (PAA) hydrogels have been proven to be more physiologically relevant as a model system for glioblastoma studies than conventional cultures on glass and gave improvements in several studies of cell viability, growth, motility and invasion [1]. Observations of cell area, stiffness, proliferation or motility changes in the response to extracellular matrix (ECM) stiffness confirm the need for further investigations of this phenomena, but do not allow to assess the molecular mechanism of glioma development. Raman spectroscopy with its capability of reporting biochemical changes in the cell state without introducing any label can help to identify spectroscopic markers associated with ECM changes and in the perspective, develop new therapeutic interventions by targeting specific molecules in glioma cells. In this research project, we aimed to demonstrate the advantage of soft PAA hydrogels mimicking ECM as support for spectroscopic analysis of live LN18 glioma cells. In contrary to tissues studies, we could follow the biochemical response of single cells to controlled changes of their environment. Figure shows the Raman spectra of the same glioblastoma cells growing on PAA substrates of different stiffness (0.3 kPa, 5 kPa, 30 kPA and CaF2 surface). Our initial analysis shows that there is a significant effect of the substrate stiffness propagated to the cells chemical composition that can be detected using Raman spectroscopy.

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[1] Pogoda, Katarzyna, et al. "Soft substrates containing hyaluronan mimic the effects of increased stiffness on morphology, motility, and proliferation of glioma cells." Biomacromolecules 18.10 (2017): 3040-3051.

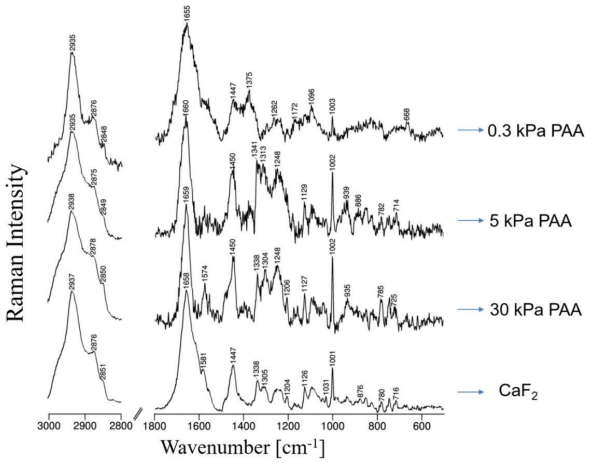

Figure 1. The Raman spectra of the LN18 glioblastoma cells grown on polyacrylamide substrates of different stiffness (0.3 kPa, 5 kPa, 30 kPA and CaF2 surface).

Monitoring of microbes causing urinary tract infections using Raman spectroscopy

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Raman spectroscopy, bacteria, urinary tract infections

Identification and characterization of pathogens in a rapid and reliable way is the crucial first step in the classification of microbial infections. Thus, we can envision following wishful scenario in clinical practice – a sick person enters a hospital emergency ward with what appears to be an infection. Then, the doctor on duty collects a specimen (e.g. urine sample) from this sick person. Consequently, in an ideal case, doctor could quicky and simply analyze the sample in the examination room. This analysis should provide him with enough information for both qualitative and quantitative conclusive identification on pathogen. In this scenario a point-of-care (POC) instrument which quickly identifies the pathogens in minutes is required. Thus, the clinician could prescribe tailored antibiotics. This can greatly improve management of the infection and even save lives. To this end, we believe that Raman based techniques – namely Raman tweezers – could also contribute and play important role in fast microbiological diagnostic for quick POC testing of body fluids and for early and accurate infection management. Optical trapping represents an elegant approach to conduct Raman microspectroscopy measurements on individual bacterial cells. With the optical trap, living cells flowing in a liquid can be immobilized using the forces generated by tightly focused laser beam and consequently analyzed. Thus, Raman tweezers can form the basis for a unique system for identification of pathogens without time demanding culturing step. The main limitations of Raman spectroscopy include the absence of a large database of microbial Raman fingerprints that would allow for quick identification of microbes. Therefore, we have prepared a database from 254 microbial strains grown on agar plates. Our results show that Raman spectroscopy can reliably differ among various microbial species causing urinary tract infections – overall accuracy for individual spectra was 95.9%. Raman spectroscopy could significantly speed up the diagnostic process – it could allow identification and characterisation of pathogens directly from liquid human specimen and at POC could serve as a quick screening method (a few minutes) for detection of obligatory pathogens in urine.

Accurate and highly stable indicators of protein synthesis through sparse classification

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Spontaneous Raman, Live macrophage cell, Linear classification, Protein synthesis, Immune activation

Raman spectroscopy is able to retrieve large amount of information regarding the molecular content of live samples in a non-invasive way through optical means. We employ here this technique to measure single-cell spectra from macrophage-like cells (Raw264) and study their activation state induced by lipopolysaccharide (LPS), a molecule simulating the presence of Gram-negative bacteria.We previously developed a system allowing the measurement of large amounts of cells (typically in the thousands per class) and showed that it is possible to generate highly accurate models based on spectroscopic data to account for the minute changes that occur during immune activation within relatively homogenous cell populations [1]. We also showed that such a method is able to study the cellular heterogeneity between functionally different macrophage cells [2]. We study here the performance and stability of different algorithms to derive linear classification models. In particular, we compare the widely used PCA/LDA approach with regularized logistic regression (Lasso), which automatically selects the Raman bands most relevant for classification, and show that Lasso can provide more accurate and stable models, with very high temporal stability [3]. Measurements taken up to a year after the data used to generate the models can still provide highly accurate classification for the prediction of single-cell immune activation state (see Fig. 1A). Furthermore, we show that PCA, a widely used method for exploratory analysis and dimensionality reduction, can have a detrimental effect to accuracy and stability when generating classification models. We then study the molecular indicators most relevant to classification in the context of a well-understood biological process. Namely, we study the molecular changes occurring during protein synthesis by comparing LPS-stimulated cells, which are expected to produce various pro-inflammatory cytokines, with cells in which protein synthesis has been blocked by cycloheximide (CHX), which inhibits the translation of messenger RNA into proteins. We show that the automatic feature selection that occurs with Lasso leads to the selection of side bands for the classification, while the Raman region with large signal (CH stretching, etc.) are mostly left out from the model (see Fig. 1B). We hypothesize that these regions are representative of too many different molecules to allow accurate classification, while the side bands are representative of mRNA accumulation and amino-acids depletion, consistent with a blockage of protein synthesis.

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[1] N. Pavillon, A. J. Hobro, S. Akira, and N. I. Smith. "Noninvasive detection of macrophage activation with single-cell resolution through machine learning," Proc. Natl. Acad. Sci. USA 115(12), pp. E2676–E2685 (2018). [2] N. Pavillon and N. I. Smith. "Immune cell type, cell activation, and single cell heterogeneity revealed by label-free optical methods," Sci. Rep. 9, p. 17,054 (2019). [3] N. Pavillon, and N. I. Smith, "Deriving accurate molecular indicators of protein synthesis through Raman-based sparse classification," Analyst (accepted) (2021), DOI: 10.1039/D1AN00412C.

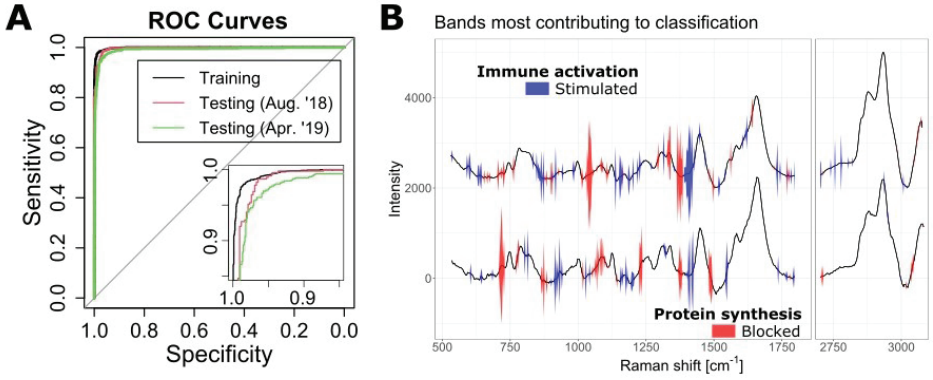

Figure 1. (A) ROC curves of the detection of immune activation, showing high stability on test data and (B) Most representative bands (in color) for classification based on sparse models.

Developing a way of detecting COVID-19 from saliva

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Raman spectroscopy, Label-free, Viruses, Biofluid analysis, Diagnostics

Context: To date, there are two principal ways of detecting SARS-CoV-2: PCR tests from nasopharyngeal swabs or saliva, and blood tests for antibodies against the virus. Both methods require tailored biochemical reagents which can be costly, are often back-ordered and limited in supply. Moreover, these analyses often require hours for a diagnosis, which could lead to viral transmission through asymptomatic individuals in airports, schools, hospitals and workplaces. Primers and antibodies must potentially be re-adapted for new viral strains which can be a challenge in pandemic management as new variants of concerns (VOCs) emerge. Approach: We aimed to address the limitations of current SARS-CoV-2 screening strategies by using laser-based Raman spectroscopy (RS) to detect the intrinsic biomolecular signature of saliva and use its fingerprint as an indication of COVID-19 infection. Results: We took 550 saliva samples from a COVID testing clinic, 37 of which were COVID positive by PCR (Figure 1 and Table 1). 74 samples (1:1 ratio between COVID-positive and negative) were imaged using a Renishaw InVia Raman microscope ensuring matching in terms of age group, sex, smoking habits and symptoms (asymptomatic, respiratory, non-respiratory). 30 spectra were taken from each sample. We are currently taking measurements from 30 additional positive samples from hospitalized patients (including samples from the same patient collected on different days). We are developing classification models using SVM (support vector matrix) machine learning and MILES (multiple instance learning via embedded instance selection) to generate models based on both individual spectra and average spectra from each saliva sample. We will determine whether we can classify samples based on COVID status and how factors such as age group, sex, smoking habits affect the Raman signal of saliva. We are replicating this using a low-cost, custom built system (Fig 2). Conclusion: The simplicity and rapidity of the new test would allow improved pandemic control through real-time on-site testing. Remote regions; increased accessibility would allow medics to determine whether these populations are disproportionally affected and to rapidly identify outbreaks in remote regions. Further impacts: The new system will be adaptable to other biofluids e.g. blood, urine and tears, and the detection of other diseases, e.g. seasonal influenza which kills 500,000 people each year, measles which is one of the most infectious human viruses, and the early stages of cancer.

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[1] Stellrecht, K. A. The drift in molecular testing for influenza: Mutations affecting assay performance. J. Clin. Microbiol. 56, 1–8 (2018). [2] Centre for Disease Control and Prevention. SARS-CoV-2 Variant Classifications and Definitions. COVID-19 Data and Surveillance https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html (2021). [3] Chen, Y., Bi, J., Wang, J. Z. Multiple-Instance Learning via Embedded Instance Selection. IEEE Transactions on Pattern Analysis. 28,1931-1947 (2006).

Figure 1: Saliva biobank

Table 1: Clinical characteristics of the cohorts

	COVID-19 -	COVID-19 +
	513	37
Age range, *n* (%)		
0-20	58 (11)	6 (16)
21-40	255 (50)	15 (41)
41-60	135 (26)	12 (35)
61-80	60 (12)	3 (8)
81+	1 (0)	0 (0)
Not reported	4 (1)	1 (3)
Sex at birth, *n* (%)		
Female	225 (44)	15 (41)
Male	206 (40)	17 (46)
Prefer not to say	82 (16)	5 (13)
Symptoms, *n* (%)		
Respiratory symptoms	187 (36)	20 (54)
Non-respiratory symptoms	30 (6)	5 (14)
None	279 (54)	8 (22)
Not reported	17 (3)	4 (11)
Disease, *n* (%)		
Other disease	124 (24)	6 (16)
None	385 (76)	31 (84)
Nicotine consumption, *n* (%)		
Smoking	96 (19)	4 (11)
Vaping	32 (6)	0 (0)
Alcohol consumption, *n* (%)	323 (63)	18 (49)

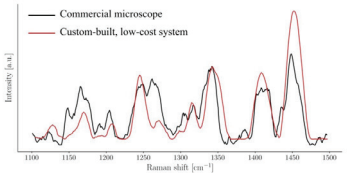

Figure 1.

Can the Diagnostics of Colorectal Polyps Benefit from Using In Vivo Raman Spectroscopy?

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Colorectal carcinoma, Raman spectroscopy, in vivo, fibre-optic

Although colorectal carcinoma still represents one of the leading causes of cancer death in Western countries [1], the implementation of population-wide screening procedures in the last decades has led not only to a decrease in its mortality, but also its incidence in many states. Screening procedures are able to detect and diagnose precancerous lesions, adenomatous polyps, before they become malignant [2]. The last-year pandemic of COVID-19 and following protective measures reduced the amount of elective and preventive care by 75% compared to previous years, consequently clinicians can expect a surge of patients diagnosed with late stage cancer due to this neglect [3], and what is more, an elevation of colorectal cancer mortality and incidence rates to values long unseen. It would therefore be very beneficial to make the diagnostic process as efficient as possible. The means to make the diagnostic procedure of colorectal adenomatous polyps faster and less stressful for the patient, might be spectroscopic methods, namely, Raman spectroscopy, as it can determine the changes in the biochemical composition of the proliferating cancerous tissue before the clinical symptoms of colorectal carcinoma occur. Employing this method may enable fast stratification of polyps found during screening colonoscopy without the need of biopsy and lengthy histopathological analysis while preserving or even improving the diagnostic accuracy. Using a custom-made fibre-optic microprobe, we were able to collect Raman spectra of healthy, precancerous and cancerous sites of the patient colon and rectum, during routine colonoscopic procedures. Associating the spectral data, any of which took only 12 seconds to collect, with patient clinical data, such as histopathological diagnosis, enabled us to determine the spectral differences between healthy tissue and precancerous or malignant lesions. The spectra of healthy tissue indicated a higher relative content of proteins in alpha-helical conformation, collagen and lipids. Moreover, the spectra of premalignant and malignant lesions suggested a decreased content of carotenoids compared to normal colorectal tissue.

Supported by grant NU20-09-00229 provided by the MOH of the Czech Republic and SUR A2_FCHI_2021_006.

[1] Sung, H., et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 202. [2] Akkoca, A.N., et al., TNM and Modified Dukes staging along with the demographic characteristics of patients with colorectal carcinoma. Int J Clin Exp Med, 2014. 7(9): p. 2828–2835. [3] Patt, D., et al., Impact of COVID-19 on Cancer Care: How the Pandemic Is Delaying Cancer Diagnosis and Treatment for American Seniors. JCO Clinical Cancer Informatics, 2020(4): p. 1059–1071.

Raman signature of pancreatic cancer

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pancreatic cancer, Raman spectroscopy, Hierarchical Cluster Analysis (HCA), Raman mapping

Pancreatic cancer is one of the most aggressive and lethal malignant neoplasms that caused 432,242 new deaths in 2018 (GLOBOCAN 2018 estimates) and it is the seventh leading cause of cancer-related deaths in the world [1]. Despite much improvements in diagnosis and management, still 5-year survival rates are below 10%. Further studies of the molecular nature of the pancreatic cancer are needed to improve those statistics. Routine histopathologic examination of pancreatic cancer slides, due to the neoplasms morphological complexity, is sometimes challenging. The most demanding and crucial information that should be delivered by pathologists, are detailed margins of the neoplasms invasion. No wildly used and unique immunohistochemical staining is available to distinguish benign pancreatic and cancerous glands. Another issue, is the histological type of the cancer and differentiating the pancreatic cancer with large ampulla of Vater tumors [2]. Finding new methods to help pathologists better assess the tumor is of a great manner. The Raman spectroscopy seems to be a promising tool. Therefore, we have applied Raman spectroscopic mapping for molecular characterization of pancreatic cancer tissue sections. Noncancerous tissue fragments were also investigated for comparison. Two step Hierarchical Cluster Analysis (HCA) was applied to reduce the amount of data collected and extract Raman marker bands of pancreatic cancer. The first step consisted of an initial clustering, which allowed an extraction of all the spectra from the area of interest – cancerous glands. In the second step the heterogeneity of the spectra acquired from glandular cells was analysed. These spectra were used as an input to the further HCA analysis to present the spectral differences across this dataset. The typical Raman maps treated with HCA, acquired in cancerous and noncancerous areas of pancreatic tissue are presented in Fig. 1. The areas of map collection are marked on the routine hematoxylin-eozin (H&E) slide image. The averaged spectra of each cluster are also demonstrated. Significant differences between the spectra of noncancerous (red) and cancerous (green) cells are observed for the bands at 1304 cm^{-1} and 1440 cm^{-1}, corresponding mainly to methyl and methylene deformational motions in proteins and lipids respectively.

This research was supported by NCN under the "OPUS 16" project (Reg. No. UMO-2018/31/B/ST4/02292).

[1] Rawla P, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. World J Oncol. 2019, 10(1):10-27 [2] Zimmermann, C., Wolk, S., Aust, D.E. et al. The pathohistological subtype strongly predicts survival in patients with ampullary carcinoma. Sci Rep. 2019, 9(1): 12676

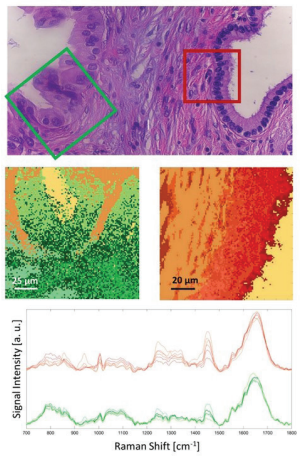

Figure 1. The hematoxylin-eozin slide image of pancreatic tissue with two Raman maps treated with HCA acquired in cancerous (green frame) and noncancerous areas (red frame), with averaged spectra.

Detection of changes in the hemoglobin structure inside intact erythrocytes with use of vibrational spectroscopy

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red blood cells, hemoglobin, tertiary and quaternary protein structure alterations, Fourier–Transform Infrared spectroscopy, Raman Spectroscopy

Apart from classical methods of red blood cells (RBCs) analysis, vibrational spectroscopy techniques, including Raman spectroscopy (RS) and Fourier–Transform Infrared spectroscopy (FTIR) are useful tools to detect changes in the structure of hemoglobin (Hb) that may influence the performance of RBCs as oxygen carriers. In our recent works [1-3] performed on the RBCs isolated from humans and different animal models, we were able to show the usefulness of FTIR and RS in the investigation of changes in the secondary structure of Hb, followed by alterations in its tertiary and quaternary structures. Spectroscopic results were backed-up with classical methods of biological investigation, what allowed us to link the impact of disturbances in Hb with other alterations in RBC function. We have shown that RBC storage resulted in a gradual increase in irreversible changes in the secondary and quaternary structures of Hb, with subsequent impairment of the T ↔ R transition. This was associated with the presence of newly discovered altered quaternary structure of Hb-– R'- which is irreversibly settled in the relaxed form inside functional human RBCs. FTIR analysis showed that on the level of secondary structure, disordered protein organization involved increased formation of β-sheets and a decrease in α-helices, related to the aggregation process stabilized by strong intermolecular hydrogen bonding. These alterations were followed by changes in the quaternary structure revealed by BGA, UV-Vis and RS as a time-dependent increase in oxyHb [1]. In a study comprising ApoE/LDLR–/– mouse model of atherosclerosis and ageing, FTIR-ATR was used to investigate Hb structure in the intact RBCs. Results showed significant changes in unordered structures, namely α-helices and turns, related to advanced age and progression of atherosclerosis [2]. In a murine C57BL-based model of accelerated ageing using D-galactose, spectroscopically– derived biochemical profile of intact RBCs revealed some differences in the secondary structure of cytosol, however, natural aging induced stronger changes in the secondary structures of the proteins of the RBCs' interior. Taken together, the results of our multidisciplinary studies show wide possibilities of application of vibrational spectroscopy in combination with other spectroscopic and non-spectroscopic techniques to analyze the capacity of RBCs to perform their functions. The development of spectroscopic research in the field of RBC analysis may contribute to the improvement of transfusion medicine and treatment of circulation-related disease.

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[1] Szczesny-Malysiak, E. et al. Irreversible alterations in the hemoglobin structure affect oxygen binding in human packed red blood cells. Biochim. Biophys. Acta – Mol. Cell Res. 1867 (11),118803 (2020). [2] Dybas, J. et al. Age-related and atherosclerosis-related erythropathy in ApoE/LDLR-/- mice. Biochim. Biophys. acta. Mol. basis Dis. 1866 (12), 165972 (2020). [3] Blat, A. et al. Spectroscopic Signature of Red Blood Cells in the D-galactose-Induced Accelerated Aging Model. Int. J. Mol. Sci. 22(5), 2660 (2021)

Simultaneous use of near-infrared and laser induced breakdown spectroscopy to identify gall bladder cancer by direct raw bile juices analysis

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Near-infrared spectroscopy, Laser-induced breakdown spectroscopy, Bile juice, Gallbladder cancer, Disease diagnosis

Near-infrared (NIR) and laser-induced breakdown spectroscopy (LIBS) were cooperatively combined to enhance the discrimination of gall bladder (GB) cancer against other GB diseases (GB polyp and GB cancer) by directly measuring raw bile juices. Since element contents as well as metabolite compositions of bile juices would vary according to the pathological conditions of GB patients, the use of both complementary spectroscopic information would be synergetic to secure better discrimination of GB cancer. The ratio of major NIR peak differed and absorbance of NIR protein peak was higher in the case of GB cancer. Also, the ratios of Na and K peak areas (ANa/AK) and the Mg and Na peak areas (AMg/ANa) in the LIBS spectra of GB cancer samples were fairly different from those of remained GB diseases. Nonetheless, clear separation of GB cancer was hardly realized when each spectroscopic method was separately used. Meanwhile, when the 2nd principal components of the NIR spectra and the ANa/AK values were combined, the discrimination of GB cancer samples became clear without interferences of the remained samples. The incorporation of NIR spectral features and LIBS elemental information (ANa/AK) of the bile components was cooperative to improve the discrimination.

In vitro cell culture environment investigation with Raman microspectroscopy: Comparison of 2D and 3D cell culture models.

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Raman microspectroscopy, Mesenchymal Stem Cell, Differentiation, 3D hydrogel, Chondrocyte

Stem cell technology has attracted considerable attention, due to its potential to extend the recovery capability of the human body following many diseases and injuries which are not treatable by current medical procedures. Over the recent decades, it has become clear that the use of more realistic in vitro cell culture models has a huge impact on the gene expression behaviour and stemness properties of stem cells (Zschenker et al. 2012), such as morphology, changes in cell metabolism and signalling. The evolution from 2D to 3D in vitro tissue and cell culture models has been an topic of increasing discussion, and significant effort have been devoted to the production of substrates that aim to mimic the natural cellular growth environment. Numerous studies have been published on the different cell-substrate interactions (Enam & Jin 2015; Rizwan et al. 2019) and many different materials have been developed for 3D hydrogels substrates, and investigated. The use of biomimetic hydrogels for 3D cell culture also preserves the deposition of extracellular matrix (ECM) surrounding chondrocytes, whereas cells cultured as monolayers tend to lose their original phenotypic and functional characteristics. (Vonk et al. 2010) Collagen I rat-tail gels, well-documented substrates, have been evaluated for the spectroscopic study of cells and demonstrated suitable, due to the minimum background contributions to the spectra across the fingerprint region. (Bonnier et al. 2015; Bonnier, Knief, et al. 2010; Bonnier, Meade, et al. 2010). In this study a suitable 3D gel model is developed to facilitate the preservation of the natural cell growth and differentiation of stem cells.The differentiation process of MSCs into chondrogenic and osteogenic lineages is carried out onto the optimized substrate, and the conventional 2D culture will be compared with the more realistic 3D in vitro cell culture models at time intervals of 7, 14, 21 and 28 days. Raman Micro Spectroscopy (RMS), a powerful analytical tool, which provides detailed label free biochemical fingerprint information in a non-invasive way, for analysis of cells, tissues and body fluids is explored to monitor the process of MSCs differentiation into chondrocytes in vitro. Specific chemical composition differences between the two bioenvironments are investigated, providing an in situ holistic molecular picture of cellular events governing the differentiation and the formation of extracellular matrix (ECM).

Figure 1.

Characterization of cariogenic streptococci-derived biofilms using FR-IR and Raman spectroscopic imaging

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bacterial polysaccharides, FT-IR microspectroscopy, Raman spectroscopy, biofilms, dental caries

Streptococci bacteria are well known for their caries forming ability. They cause tooth damage, pain and might lead to other, more severe diseases. Usually dental caries is associated with poor oral hygiene but not only. The commonness of the dental caries is indicated by bacterial colony durability, arising from the special structures that they create– bacterial biofilms. These structures consist of a mixture of polysaccharides, proteins and bacteria [1]. However, it is stated that the most essential components in terms of biofilm formation are α-D-glucans [2]. The chemical composition determines the stability and other characteristics of bacterial biofilm, and therefore its analysis is crucial and may lead to the development of new methods of treatment and prevention of dental caries. The streptococcal strains (Streptococcus mutans CAPM 6067, S. sobrinus CAPM 6070, S. sobrinus/downei CCUG 21020, S. sanguis ATCC 10556, S. sobrinus DSMZ 20381 and a mixture of strains) were cultured on an aluminium coated slides where they formed a biofilm. The samples were measured using FT-IR and Raman spectroscopic imaging. The strains showed differences in saccharide (including D-glucans), protein and lipid composition. The highest amount of (1→3), (1→6)-α-D-glucan was produced by S. sobrinus DSMZ 20381 and S. sobrinus CAPM 6070, which might contribute to higher caries forming potential of these strains. The distribution of Amide I and (1→3)-α-D-glucan varied between the samples indicating different degree of dental caries progression. The mixture of strains is known to be the most virulent and the most durable in a dental plaque formation. The highest similarities to the mixture of strains were detected in S. mutans CAPM 6067, S. sanguis ATCC 10556, and S. sanguis/downei CCUG 21020 samples, however they differed in terms of the saccharide content, which might contribute to the lower carcinogenicity of these strains. FT-IR and Raman spectroscopies have proved to be useful in determining the chemical composition of streptococcal biofilms, and thus could be used to evaluate the virulence of specific strains in further studies.

[1] H. C. Flemming and J. Wingender, Nature Reviews Microbiology, 8, (2010), 623–633. [2] M. Matsumoto-nakano, Japanese Dental Science Review, 54, (2018), 22–29.

Spectroscopic and microscopic evaluation of gelation temperature effect on the curdlan matrix: molecular structure and properties

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biopolymers, 1,3-β-D-glucan, biomaterials, vibrational spectroscopy

1,3-β-D-glucan (curdlan) is a well-known natural polymer consisting of D-glucose monomers linked by glycosidic bonds. It is water-insoluble microbial exopolysaccharide produced by *Alcaligenes faecalis* var. *myxogenes* 10C3 strain characterised by its unique gel forming properties. Curdlan is widely applied in food, medicine and pharmaceutical industry, as it creates flavourless, non-toxic biopolymers associated with helical structures and properties, depending on the temperature of production. The aim of the study was to determine characteristics of curdlan polymer matrices gelled at two different temperatures with spectroscopic and microscopic techniques. The polysaccharide films with thickness of 82± 9.6 µm were produced by identical protocols, spreading 8% (w/v) curdlan (Wako Pure Chemicals Industries, Japan) in distilled water, followed by 20-min thermal gelation at 80°C or 90°C. Matrices were subjected to structure analysis with spectroscopic techniques: ATR FT-IR, Raman and X-ray photoelectron spectroscopy (XPS). Additional information from microscopy was obtained with use of atomic force microscopy (AFM) and scanning electron microscopy (SEM). Curdlan matrix gelled at 80°C is characterised by more densely packed fibres with randomly distributed smaller sized pores (30–70 nm vs. 50–70 nm) in comparison with the matrix gelled at 90°C. Second-order derivative of FT-IR spectra showed bands attributed to α-helices in 80°C sample, absent in 90°C, thus consisting of single- or triple-helical structures. Helical forming polysaccharides are prone to aggregate, creating a structure of greater complexity and denser package, which was subsequently confirmed with XPS. AFM data presented the surface of 80°C with lower roughness and spherical-like pores, also confirmed with SEM. Curdlan polymer gelled at 80°C has a distinctly different structure than the matrix gelled at 90°C, which is less densely cross-linked. Cooperative use of spectroscopic and microscopic methods provides thorough structural information on curdlan matrices gelled at different temperatures, possibly changing properties important for biomedical applications, e.g., entrapment of drugs or production of biomaterials for tissue regeneration. Presented approach facilitate the optimization, modification, and design of manufacturing processes of biomaterials with desired characteristics.

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[1] Emanuele A et al. Time – Resolved study of network self?organization from a biopolymeric solution. Biopolymers 31, 859–868, 1991 Funami T et al. A rheological study on the effects of heating rate and dispersing method on the gelling characteristics of curdlan aqueous dispersions. Food Hydrocoll 14, 509–518, 2000 [2] Mangolim CS et al. Use of FT-IR, FT-Raman and thermal analysis to evaluate the gel formation of curdlan produced by Agrobacterium sp. IFO 13140 and determination of its rheological properties with food applicability. Food Chem. 232, 369–378, 2017 McIntosh M et al. Curdlan and other bacterial (1 → 3)-β-d-glucans. Appl Microbio. Biotechnol 68, 163–173, 2005 [3] Prado BM et al. Differentiation of Carbohydrate Gums and Mixtures Using Fourier Transform Infrared Spectroscopy and Chemometrics. J Agric Food Chem 53, 2823–2829, 2005 [4] Sun Y et al. Preparation and characterization of novel curdlan/chitosan blending membranes for antibacterial applications. Carbohydr Polym 84, 952–959, 2011

Optimisation and development of a Raman needle probe towards diagnosis of Lymphoma

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Raman spectroscopy, fibre optic, diagnosis, cancer, Raman probes

Lymphoma is cancer of the lymphatic system; a network of tissues and organs that help to rid the body of toxins and waste, and a critical component of the body's immune system. Encompassed within this definition are both primary and secondary cancer types, wherein the cancerous cells either originate within the tissue of lymph nodes, or metastasize from another site in the body, respectively. Additionally, lymph node metastasis is an important prognostic indicator for a variety of cancer types. Incorporating the chemical specificity of Raman spectroscopy into the existing diagnostic pathway through the development of a fibre optic Raman needle probe has the potential to facilitate the rapid, highly accurate assessment of suspicious lymph node lesions. A probe previously developed within the group for the discrimination of malignant and benign lymph node tissue achieved extremely promising results of 85% sensitivity and 77% specificity. Whilst these results represent a highly powerful diagnostic tool, increasing these values will improve diagnostic ability, enabling earlier and more accurate diagnoses if incorporated into the diagnostic pathway. One method of doing this is to improve the quality of the data collected with the probe, and to maximise the collection efficiency of the Raman system. Raman scattering is an inherently weak effect, and due to anatomical restraints and compatibility with existing diagnostic protocol probes must adhere to strict dimension parameters. Therefore maximising the signal collected within these constraints is critically important to a successful probe design. Two other factors known to complicate discrimination are the presence of background signals generated within the silica fibres and the presence of etaloning within the measured spectra. Etaloning is an oscillatory optical effect that creates artefacts within the data, caused by the reflectance and subsequent interference of photons within the photosensitive region of the CCD. The work presented in this abstract is a series of experiments investigating the effect parameters such as laser wavelength, fibre core diameter and filter position have on the quality of data; in terms of signal to noise ratio, levels of etaloning and background signals. The characterisation of the influence of various design parameters on data quality will allow for the selection of optimal parameters to maximise diagnostic potential. Thus the results of these experiments will be combined into a 'final design' fibre optic probe which should provide an improvement in diagnostic capability.

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ATR-FTIR screening of liver grafts at the operating room

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ATR-FTIR, LIVER TRANSPLANTATION, LIPIDOMICS, STEATOSIS, CHEMOMETRICS

Liver transplantation has become the therapy of choice for patients with irreversible end-stage liver diseases, and is a lifesaving procedure for patients with acute liver failure. The great success of this surgical procedure has led to increasing demand for transplantable organs and the shortage of donor grafts to meet the demand which led surgeons to address the use of sub-optimal grafts. One of the main reasons for rejecting a liver for transplantation is the accumulation of an excess of fat, and many asymptomatic healthy donors, as well many marginal donors display a variable degree of steatosis. Visual inspection of the liver of the cadaveric donor does not provide a precise and accurate estimation of the degree of steatosis and so, it is qualitatively and semi-quantitatively evaluated by histological analysis under light microscopy of an extemporaneous biopsy, after organ extraction and transportation to the transplantation scenario. Thus, there is an unmet need for transplantation surgeons to have access to non-invasive analytical tools enabling a fast, "in situ" quantitative and reproducible grading of liver steatosis before liver extraction from donors. We have developed and assessed an ATR-FTIR spectroscopic method compatible with the requirements of an operation room, for the rapid evaluation of the lipid contents in human livers. Human liver biopsies obtained from organs intended for transplantation were analyzed by expert pathologists, ATR-FTIR spectroscopy (n=67), lipid bio-chemical analysis (n=20), and lipidomic profiling (n=20). Comparative analysis of multi-source data showed strong correlations between ATR-FTIR, clinical information and lipid profiles. Results show that ATR-FTIR accurately describes the major lipid components of the liver, and allows a consistent quantification of TAGs in liver biopsies. Preliminary results also included the comparison between ATR-FTIR spectra collected from the parenchyma and through the barrier that represents the Glisson's capsule –a thin layer of connective tissue composed primarily by ;type I collagen and, to a lesser extent, type III collagen-, as well as between fresh tissue and after long-term storage in a biobank facility. Glisson's capsule did not represent a major drawback for the liver lipid content estimation by ATR-FTIR. The strategy and equipment used seem suitable and compatible with the surgery scenario limitations and may represent a future valuable tool for liver transplantation surgeons.

Effect of ionising radiation on prostate cancer cells studied by vibrational spectroscopy

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prostate cancer, vibrational spectroscopy, ionising radiation, biological cell response, chemometrics

Prostate cancer is one of the top ranked cancers in men worldwide, in terms of both the total number of cases and deaths [1]. Radiation therapy (or "radiotherapy") is one of the most important elements of cancer treatment, using ionising radiation to destroy tumours by inducing cell damage beyond a repair threshold. Since biological response involves many complex pathways and depends substantially on cell type and irradiation procedure, there is still a strong need for detailed investigations of cell response mechanisms induced by various types of radiation. Nowadays, biochemical methods are used very often to detect radiation damage and biological response to irradiation. However, most of these methods provide only selected chemical information at the single cell level. Therefore, the importance of the application of various alternative, label-free and information rich techniques is evident. Vibrational spectroscopy is well known for its uniqueness as a non–destructive tool used for the identification of biomolecules in single cells [2,3]. As a sensitive tool it can be applied to follow chemical changes in cancer cells after irradiation [4]. The number of spectroscopic reports focusing on cell radiosensitivity, organelle-specific response to irradiation, and stages of the effect of radiation exposure on cells is strictly limited. Thus, in our study, we aim to shed new light on radiation induced biochemical changes in radioresistant prostate cancer cells at the subcellular level. Studies were performed on the main components (cell nucleus, cytoplasm, lipid droplets) of cells of the prostate PC-3 cell line, using various vibrational spectroscopy methods (Raman mapping, FT-IR imaging, AFM-IR) to analyse component-specific response of radioresistant cancer cells to high (10 Gy to 50 Gy) and clinical (up to 10 Gy) doses of X-ray irradiation [5-9]. It seems vitally important to assess subcellular response in order to provide systematic high-resolution information to develop a rigorous theory of ionising radiation action at the cellular and molecular level. Additionally, Raman spectroscopy was successfully applied to differentiate between two stages of ionising radiation effect on living matter, i.e. physicochemical damage and biological response to irradiation. Spectroscopic studies were supported by biochemical methods and followed by chemometric analysis (mainly PLS regression). The research has been performed to better understand the response of radioresistant cancer cells to ionising radiation and to improve current radiotherapy treatment.

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[1] H. Sung, et al., CA-Cancer J. Clin. 0 (2021) 1–41. [2] I.W. Schie and T. Huser, Methods and applications of Raman microspectroscopy to single-cell analysis, in: Appl. Spectrosc., SAGE PublicationsSage UK: London, England, 2013: pp. 813–828. [3] J. Doherty, et al., Appl. Spectrosc. Rev. 52 (2017) 560–587. [4] Q. Matthews, et al., Phys. Med. Biol. 56 (2011) 6839–6855. [5] M. Roman, et al., Sci. Rep. 9 (2019) 8715. [6] M. Roman, et al., Nanotechnology. 30 (2019) 425502. [7] M. Roman, et al., J. Biophotonics. 13 (2020) e202000252. [8] M. Roman, et al., BBA - Mol. Cell Biol. Lipids. 1865 (2020) 158753. [9] M. Roman, et al., Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 255 (2021) 119653.

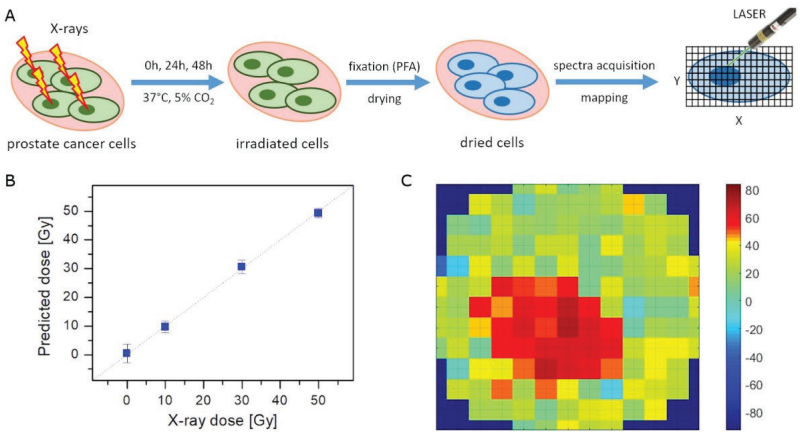

Figure 1. Schematic layout of the performed experiments (A), PLSR model for cells fixed 48 h after irradiation (B), Raman map of predicted X-ray dose for the 48 h timepoint and X-ray dose of 30 Gy ©

Spectroscopic markers of bleomycin induced DNA breaks in living cells

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Raman spectroscopy, Cell Culture, DNA damage, Hierarchical Cluster Analysis, Hyperspectral imaging

DNA sequence errors and structural changes of double helix are common phenomena and can occur either spontaneously or due to various physicochemical processes. These changes can cause many pathological phenomena like mutations, carcinogenesis, or even the cell death. In this presentation we report on structural changes in chromatin of the HeLa tumor cell line incubated with bleomycin (BLM), a chemical compound that induces single and double strand breaks of DNA (SSBs and DSBs, respectively). To assess these conformation changes a Raman micro-spectroscopy technique was used [1]. It allowed the hyperspectral imaging with sub-micrometer resolution, demonstrating spatial distribution of the DNA conformational transition spectral markers: shifts of phosphate stretching motions in the DNA backbone. An important part of this work is the statistical approach to the acquired spectroscopic data. To reduce data dimensionality and extract the most important information (marker bands of DNA damage and repair) form the acquired data, a multivariate data analysis was applied. Figure 1 demonstrates the results of a Hierarchical Cluster Analysis (HCA) treatment of the Raman map acquired from HeLa cell incubated with 150 μM of BLM. This analysis allowed detection of BLM induced changes in the chemical structure of chromatin located in the nuclei and nucleolus.

This work is supported by the National Science Centre, Poland under the "OPUS 16" project.

[1] C. Petibois, Imaging methods for elemental, chemical, molecular, and morphological analyses of single cells. Anal. Bioanal. Chem. 397, 2051–2065 (2010). [2] M. Hedegaard et al., Spectral unmixing and clustering algorithms for assessment of single cells by Raman microscopic imaging. Theor. Chem. Acc. 130, 1249–1260 (2011). [3] K. Sofińska, N. Wilkosz, M. Szymonski, E. Lipiec, Molecular Spectroscopic Markers of DNA Damage. Molecules 25, 561 (2020).

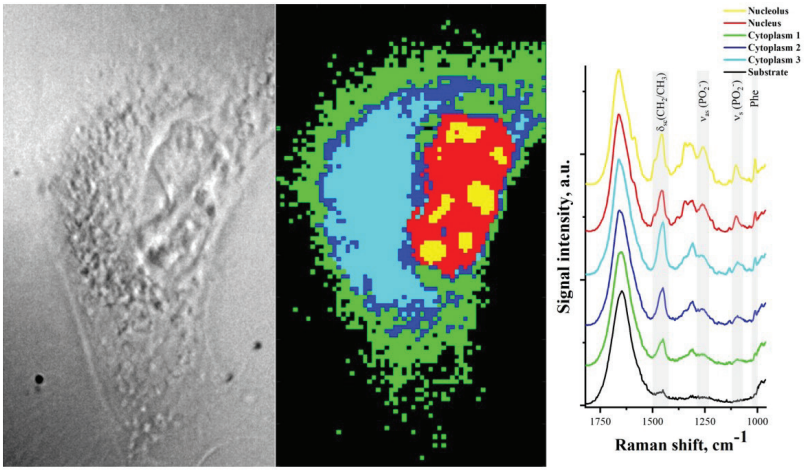

Figure 1. HCA of a single HeLa cell

Vibrational spectroscopy as a valuable tool for investigation of biochemical markers of glioblastoma multiforme invasiveness

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glioblastoma multiforme (GBM), animal model of GBM, Fourier transform infrared (FTIR) microspectroscopy

The methods of vibrational spectroscopy are often used for the assessment of biochemical changes accompanying pathological processes occurring in biological tissues. What is more, the existing literature evidence indicates that both the Raman microscopy and Fourier transform infrared (FTIR) microspectroscopy may be used as potential tools for clinical diagnostics of different diseases, including cancers [1,2]. One of the most aggressive brain tumor is glioblastoma multiforme (GBM) originated from glial cells which, together with neurons, form the nervous tissue. GBM accounts for about 16% of all brain tumors and its high morbidity is demonstrated by the poor recovery outcome – the median of patients survival is 14-16 months from the diagnosis. The etiology of glioblastoma is not fully understood and therefore many investigations are focused on getting the better knowledge about its nature and on new treatment strategies [3]. In our study, FTIR microspectroscopy was utilized to examine anomalies in the distribution, accumulation and structure of biomolecules appearing in the rat brain as a result of GBM development. For this purpose, selected three human GBM cell lines were implanted into the brains of Wistar rats. 21 days later, the animals were euthanized, they brains were removed from the skulls an cut into 12-micrometer thick slices in cryomicrotome. The sections including the areas of implantation were subjected to the topographic and quantitative biochemical analysis focused on the determination of the differences between the developed lesion and the normal brain tissue. The biochemical information obtained with the FTIR microspectroscopy were stated with data concerning the accumulation and distribution of light elements from SR-XRF study and the results of microscopic evaluation what allowed us to indicate potential biomolecular markers of carcinogenesis process. According to our study, accumulation of saturated fatty acids and compounds containing carbonyl groups differ significantly within the tumor mass and its surroundings what may be used for objective differentiation between normal and tumor-changed brain tissue.

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[1] Su K. Y., Lee W. L. Fourier Transform Infrared Spectroscopy as a Cancer Screening and Diagnostic Tool: A Review and Prospects. Cancers (Basel). 2020 Jan 1;12(1):115. doi: 10.3390/cancers12010115 [2] Auner G. W., Koya S. K., Huang C., Broadbent B., Trexler M., Auner Z., Elias A., Mehne K. C., Brusatori M. A. Applications of Raman spectroscopy in cancer diagnosis. Cancer Metastasis Rev. 2018 Dec;37(4):691-717. doi: 10.1007/s10555-018-9770-9 [3] Czapski, B., Baluszek, S., Herold-Mende, C., Kaminska, B. Clinical and immunological correlates of long term survival in glioblastoma. Contemp. Oncol. 2018:22(1A):81–85 doi: 10.5114/wo.2018.73893

Using astrophysics to improve breast cancer detection

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Raman spectroscopy, Breast cancer, Monte Carlo, Microcalcifications, Computational

Breast cancer is one of the most common cancers worldwide, with over 50,000 women diagnosed every year in the UK alone. The gold standard for breast cancer diagnosis is currently a mammogram followed by a needle biopsy and histopathology, which is often insufficient for early diagnosis, and unpleasant for the patient. Moreover, 80\% of these biopsies return benign results. Utilising Raman spectroscopy before a biopsy could ensure only those patients who need a needle biopsy receive one, thus reducing NHS costs and patient stress. Raman spectroscopy has been shown to be useful for label-free identification of molecules in tissue. Deep Raman techniques can be exploited to detect microcalcifications that are present in breast tissue. Chemically distinct calcifications are present in different pathological types of breast tissue, and can act as a biomarker for breast cancer. These microcalcifications fall into two categories: type 1 (benign) and type 2 (malignant or benign). Therefore, by determining which type of calcification is present, a needle biopsy can be recommended or not. There is also potential for this to become a minimally invasive method of detecting breast cancer. Tissue is a highly scattering medium which poses a problem for recovery of a Raman signal at depth in breast tissue, which is what this method would require. Tissue phantoms of Intralipid and India ink, or chicken breasts and pork chops, are often used to imitate the optical environment when carrying out experiments. A greater understanding of the light transport processes can be gained by using the Monte Carlo method to randomly sample the volume and obtain probabilistic information about the system. This will allow a variety of probe designs to be investigated and an optimum geometry between the laser and the detector to be developed. The code that we have developed is derived from an astrophysical code; it has been adapted for biological systems with complex morphologies and optically thick media. I will present our work to validate our code and derive estimates for scattering coefficients in Intralipid. Additionally, I will discuss our efforts to model microcalcifications and how to maximise their detected signal by exploring different probe geometries in simulations.

Biochemical and nanomechanical analysis of human enteron cells supplemented with antioxidant and exposed to ROS by Raman imaging and AFM

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colon cancer, Raman spectroscopy, colon cells, oxidative stress, bioimaging

In recognition of importance of cancer in Poland and in the World to public health we conducted the research on medical diagnostics of cancer by Raman spectroscopy and imaging and AFM and on influence of reactive oxygen species (ROS) on cancer transformation based on nanomechanical and biochemical properties of human cells of gastrointestinal tract. Spectroscopic and microscopic methods allow the fast, precise and unambiguous differentiation of healthy and cancerous biological samples. Moreover, a very important advantage of Raman spectroscopy is ability to identify many individual components of biological samples in one measurement which helps in their differentiation. Based on Raman spectra, cell structures, such as the nucleus, mitochondria or cell membranes can also be visualized. Tumor transformation is associated with activation of proto-oncogenes and/or inactivation of suppressor genes or abnormal cell differentiation. More and more data indicate that one of the most important factors responsible for the induction of tumor transformation are ROS. At the same time, ROS production is a natural part of oxygen metabolism. The balance between the production of ROS and the efficiency of antioxidant systems prevent oxidative stress and subsequent damage to important macromolecules such as DNA, proteins and lipids. The research carried out proves that label-free Raman spectroscopy may play an important role in clinical diagnostics differentiation of normal and cancerous colon cells and may be a source of intraoperative information supporting histopathological analysis. Statistically assisted analysis of Raman spectra and AFM data such as: stiffness, Young modulus shows that normal and cancerous human cells can be distinguished based on their unique vibrational and nanomechanical properties.

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[1] B. Brozek-Pluska, K. Beton, Oxidative stress induced by tBHP in human normal colon cells by label free Raman spectroscopy and imaging. The protective role of natural antioxidants in the form of β-carotene, RSC Advances, 2021, accepted for publication. [2] B. Brozek-Pluska, Statistics assisted analysis of Raman spectra and imaging of human colon cell lines – Label free, spectroscopic diagnostics of colorectal cancer, J. Mol. Struc., 2020, 1218, 128524. [3] B. Brozek-Pluska, J. Musial, R. Kordek, H. Abramczyk, Analysis of human colon by Raman spectroscopy and imaging-elucidation of biochemical changes in carcinogenesis, International Journal of Molecular Sciences, 2019, 20(14), 3398. [4] B. Brozek-Pluska, J. Musial, R. Kordek, H. Abramczyk, Analysis of human colon by Raman spectroscopy and imaging-elucidation of biochemical changes in carcinogenesis, International Journal of Molecular Sciences, 2019, 20(14), 3398.

Spectroscopic and electrochemical investigation of stainless steel in simulated physiological conditions: effects of tryptophan

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Raman spectroscopy (RS), Fourier transform infrared absorption spectroscopy (FTIR), corrosion, tryptophan

Stainless steel (316L) has been broadly used as a metallic biomaterial, for example as a significant component of biomedical treatments, for orthopedic, dental, and cardiovascular implants [1]. However, when metallic implants are in contact with body fluids (which contain inorganic and organic molecules, i.e. proteins), different chemical reactions arise on the metallic surface and can induce the corrosion of these materials [2]. The corrosion process of an implant is influenced by various factors such as the presence of chloride ions, surface topography, pH, etc.[3]. The passive film which is formed on the metal surface can be broken down due to mechanical impact such as that which takes place at the sliding interface of the implant [3]. The oxide layer violation leads to the release of toxic and potentially carcinogenic metallic species into the surrounding tissues and provides a biological reaction [4]. One of the effective methods for improving corrosion resistivity of implants is the surface modification of biomaterials, for example by organic molecules [5]. The use of amino acids as corrosion inhibitors has many advantages, they are i.e. nontoxic, biogenic, and play important role in various physiological processes, as well as in biofilm formation on implants [6,7]. In our studies, the corrosion process of the stainless steel under various controlled conditions (polishing procedure, time up to 24 h, temperature (37 °C), pH (7.4; 3)) in simulated body fluids – PBS solution (phosphate buffer saline) with and without the addition of different tryptophan concentrations was investigated. Complementary spectroscopic methods, Fourier transform infrared absorption spectroscopy (FTIR) and Raman spectroscopy (RS) have been used to describe the adsorption process of tryptophan onto the corroded metallic surface. Spectroscopic studies were supported by electrochemical methods like potentiodynamic polarization experiments to simulated the pitting corrosion process and measure the corrosion parameters. Figure 1 presents the results obtained for corroded stainless steel with the presence of tryptophan (10^{-2} M, 24 h). Amino acid changed the corrosion behaviour of stainless steel in PBS solution (pH 3). The interaction between the indole ring of tryptophan (bands at 757 cm^{-1}, 1010 cm^{-1}, 1550 cm^{-1}, RS spectrum) which adopted tilted orientation on the corroded surface of steel was observed.

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[1] M. Prakasam, J. Locs, K. Salma-Ancane, D. Loca, A. Largeteau, L. Berzina-Cimdina, J. Funct. Biomater. 8(4), 44 (2017) [2] M. Talha, Y. Ma, P. Kumar, Y. Lin, A. Singh, Colloids Surf. B 176, 494 (2019) [3] M. F. Ulum, W. Caesarendra, R. Alavi, H. Hermawan, Coatings 9(5), 282 (2019) [4] Y. S. Hedberg, I. O. Wallinder, Biointerphases 11, 018901 (2016) [5] M. Talha, Y. Ma, Y. Lin, A. Singh, W. Liu, X. Kong New J. Chem. 43, 1943 (2019) [6] Y. Liu, D. Zhu, D. Pierre, J. L. Gilbert Acta Biomater. 97, 565 (2019) [7] D. Święch, C. Paluszkiewicz, N. Piergies, E. Pięta, K. Kollbek, W. M. Kwiatek Materials 13(20), 4482 (2020).

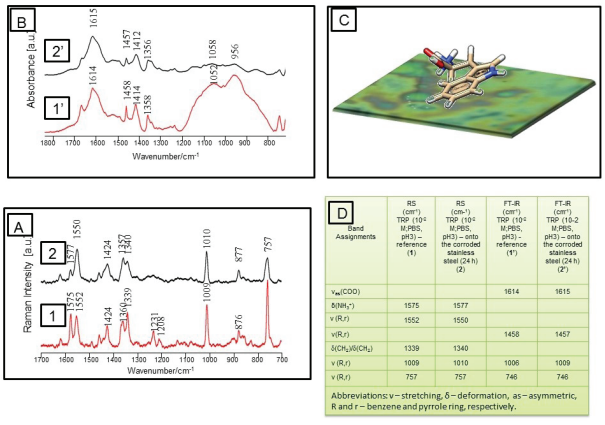

Figure 1. RS (A) and FT-IR (B) spectra of Trp – reference (1;1'), and Trp adsorbed onto the corroded steel (2,2'). (C) the suggested orientation of Trp onto 316L. (D) table – the proposed band

Spectral Histopathology – A Rising Field for Breast Cancer Diagnosis

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Spectral histopathology, Raman, FTIR, Breast Cancer, Invasive Cancer vs Benign Lesions

Breast cancer is the most common cancer worldwide, affecting females and males with an incidence and mortality of 47.8 and 13.6 per 100 000, respectively[1]. Histopathology is the gold-standard methodology for cancer diagnosis. Formalin-fixed paraffin-embedded (FFPE) samples, stained with haematoxylin and eosin, are observed by experienced anatomopathologists. Immunohistochemical (IHC) analysis of the biospecimen gives some insights regarding the tumour's metabolism and biochemical features however, it is estimated that 20% of IHC testing worldwide are inaccurate[2]. Interpretation of the biospecimens determines the treatment choice regarding patients' prognosis. Nevertheless, due to tumours morphological heterogenicity, a high subjectivity is associated with this practice[3-5] hence, misidentification or misinterpretation may lead to diagnostic errors, compromising adequate treatment and therefore the prognosis. Raman and FTIR microspectroscopies provide accurate chemical information with high sensitivity and specificity, offering the spatial distribution of the biochemical components within a sample, an add-on tool to answer the mentioned clinical needs. Vibrational spectroscopy, especially Raman, is widely applied to fresh tissue samples, with the purpose of being used in situ during surgeries, aiming to achieve adequate surgical margins. Studies on FFPE samples, on the other hand, are not as common, sparse research being found. In this work, Raman and FTIR microspectroscopy techniques were used to study FFPE samples of invasive human breast cancer and benign breast lesions. Two contiguous sections were prepared: one with 10 um thickness for Raman and FTIR analysis and another with 3 um for standard histopathology, for correlation purposes. Nine tissue sections were chemically dewaxed prior to spectral analysis, after which digital dewaxing[6] was still necessary. Digital dewaxing was performed using ICA/PLS for the FTIR data and NNLS for the Raman data. Data was then statistically analysed by PCA. Invasive cancer vs normal structures were clearly distinguished in the first 7 PCs, by the two techniques. The vibrational bands identified as discriminant were mainly assigned to imbalances in the protein, lipid and DNA/RNA content. A quantitative and objective spectral profile was obtained for the two sample categories (healthy and invasive cancer), proving Raman and FTIR to have good potential to assist histopathology. The next step of this study consists of creating classification algorithms for the subsequent diagnosis of undiagnosed samples.

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[1] Globocan. Estimated age-standardized incidence and mortality rates (World) in 2020, worldwide, both sexes, all ages Accessed on 12.05.2021 [2] Tang, P.; Bui, M.M.; Peng, Y. Practical Anatomic Pathology, Y., P., P., T., Eds. Springer, Cham: Switzerland, 2019; https://doi.org/10.1007/978-3-030-16518-5_7pp. 173-192. [3] Brunye, T.T.; Mercan, E.; Weaver, D.L.; Elmore, J.G. J Biomed Inform 2017, 66, 171-179, doi:10.1016/j.jbi.2017.01.004. [4] Elmore, J.G.; Nelson, H.D.; Pepe, M.S.; Longton, G.M.; Tosteson, A.N.; Geller, B.; Onega, T.; Carney, P.A.; Jackson, S.L.; Allison, K.H., et al. Ann Intern Med 2016, 164, 649-655, doi:10.7326/M15-0964. [5] Elmore, J.G.; Longton, G.M.; Carney, P.A.; Geller, B.M.; Onega, T.; Tosteson, A.N.; Nelson, H.D.; Pepe, M.S.; Allison, K.H.; Schnitt, S.J., et al. JAMA 2015, 313, 1122-1132, doi:10.1001/jama.2015.1405. [6] Tfayli, A.; Gobinet, C.; Vrabie, V.; Huez, R.; Manfait, M.; Piot, O. Applied Spectroscopy 2009, 63, 564-570

Generation of lipid droplets in the heart after administration of iron oxide nanoparticles in a D-mannitol coating

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lipid droplets (LDs), FTIR, heart

Lipid droplets (LDs) are spherical organelles consisting of a core of fatty origin, the outer single phospholipid layer as well as surface proteins. The core of the mature LDs is mainly composed of inert fats such as triacylglycerols, cholesterol esters and diacylglycerols. Proteins are also an important component of LDs associated with fat metabolism and their task is to participate in the biogenesis of LDs, their stabilization and lipolysis [1]. The maintaining of the proper lipid homeostasis of the heart muscle depends on the processes that require coordinated action between LDs and mitochondria. These processes are prevent the intracellular accumulation of toxic or bioactive lipids but also to ensure the efficient supply of lipids for the conversion of ATP. The processes occurring within the heart involving LDs have still not been fully understood. The two main hypothesis suggest that fats accumulated in the form of LDs may be either a marker of increased accumulation of lipids which finally leads to the heart disorders, or they can prevent lipid toxicity by occupying toxic lipids such as cholesterol or ceramide [2-3]. The aim of our study was to evaluate the biochemical changes occurring in the heart muscle after administration of D-mannitol coated superparamagnetic iron (III) oxide nanoparticles (M-IONPs). Ten male Wistar rats at the age of 60 days, which were randomly divided into two equal groups, were used for investigation. The biochemical analysis of 12 μm thick sections of the heart muscle placed on MirrIR slides was performed using an FTIR microscope Thermo Scientific Nicolet iN10MX and the measurements were carried out in transflection mode. The collected absorption spectra stated the basis of the semi-quantitative biomolecular analysis. For every area 40 spectra were selected and then averaged. To verify statistical significance of differences between animals subjected to M-IONPs and normal rats the non-parametric U Mann-Whitney statistical test for independent groups was used. Its results showed the existence of statistically relevant differences between animals in the intensity of bands/massifs occurring at the wavenumber: (I)3000-2800cm^{-1}, (II)1484-1358cm^{-1}, (III)1484-1428cm^{-1}, (IV)1428-1358cm^{-1} and (V)1141-995cm^{-1} which are characteristic for lipids, cholesterol esters, triglycerides, proteins and compounds containing phosphate groups. The observed anomalies seem to suggest that the administration of even low concentration of M-IONPs may induce the formation and/or accumulation of LDs in the ventricular areas of the heart muscle.

[1] I. Jedrzejowska. Krople lipidowe: nowe spojrzenie na strukturę, biogenezę i funkcje. Postępy Biologii Komórki 37.3 (2010), s. 641–655 (in polish). [2] H. Wang et al. Analysis of lipid droplets in cardiac muscle. Methods in cel biology 116 (2013), s. 129–149. [3] I. J Goldberg et al. Deciphering the role of lipid droplets in cardiovascular disease: A report from the 2017 National Heart, Lung, and Blood Institute Workshop. Circulation 138.3 (2018), s. 305–315.

Can diet induce biochemical changes in the brain?

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Raman microspectroscopy, obesity, high – fat diet, brain tissue

Obesity is a big and growing problem in modern societies, hence the answer to the question of whether diet affects the brain biochemical changes seems so important [1,2]. The effect of the high-fat diet in the animal model was studied. The features observed for rats fed with high-fat diet were compared with the reference group. The brain tissue of seven to eight weeks old, Sprague Dawley rats, after 2-3 weeks of high fat diet, were investigated. No fixation method was used to prepare the samples, the brain slices were kept in an artificial cerebrospinal fluid during Raman measurements. The spectra were obtained using a Renishaw inVia spectrometer equipped with a Leica microscope and water immersive objective (60× NA=0.75), exciting samples with the 785 nm HP NIR diode laser. Each sample was measured three times to verify its homogeneity. The principal component analysis (PCA) (Unscrambler X software) was used to reveal differences between two investigated groups of rats, the research and reference group. The statistical analysis of PCA was performed in the entire spectral range and in selected smaller marker ranges. The group of rats exposed to the development of obesity showed much greater variation of Raman spectra. The analysis show that a high-fat diet has a significant impact on the molecular structure and lipid ordering of the studied brain area. The reason for the diversification is, apart from some lipid remodeling, also the differences in amino acid composition e.g. Trp, His, Asp, Glu and changes in protein structure. The cause of the modifications that appeared in the tissue, apart from some lipid remodeling, are also differences in the composition of amino acids, eg Trp, His, Asp, Glu and changes in the structure of proteins.

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[1] Hurt, R.T.; Kulisek, C.; Buchanan, L.A.; McClave, S. A.; 2010, Gastroenterol Hepatol , 6, 780-792. [2] Katz. D.L., 2014, Nature, 508, S57.

PCA-supported FTIR analysis of tissues taken from the site of mechanical brain damage

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Principal component analysis, Traumatic brain injury, Fourier transform infrared microspectroscopy, Raman spectroscopy, Astrogliosis

Traumatic brain injury (TBI), meaning functional or structural damage which appear as a result of the application of the external physical force, constitutes the main cause of death and disability of individuals and a great socioeconomic problem. To search for the new therapeutic strategies for TBI, better knowledge about posttraumatic pathological changes occurring in the brain is necessary. The Fourier transform infrared microspectroscopy was used in this study for topographic and semiquantitative biochemical analysis of brain tissue taken from rats subjected to mechanically induced injury. The recorded IR spectra were processed with the use of principal component analysis (PCA) to verify whether the spectral differences observed between unharmed and injured brain tissue allow for objective differentiation of the site of injury from the surrounding tissue. The chemical mapping of the injury site revealed that significantly decreased accumulation of lipids as well as compounds containing phosphate and carbonyl groups and the elevated levels of proteins and cholesterol/cholesterol esters are characteristic for the injured brain tissue. PCA showed, moreover, that IR spectra from the site of the injury and the surrounding cortex differed enough to form the separate groups in the space of the first two principal components. In turn, the analysis of the Raman depth profiles from the place of injury indicated that the observed differences are the result of both changes in biochemical composition of the tissue and of the decreased thickness of the slice within the glial scar.

The molecular changes appearing in the hippocampal formation as a result of repetitive electrical stimulation: FTIR microspectroscopy study

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kindling model of epilepsy, Fourier transform infrared microspectrocopy, biochemical analysis, transauricural electroshocks

The animal kindling models of seizures have been widely used to investigate pathomechanisms related to the occurrence of the temporal lobe epilepsy (TLE) and the processes underlying drug resistance in TLE [1]. TLE is the most widespread form of epilepsy and it is often characterized by hippocampal sclerosis [2,3]. The hippocampal formation is known as epileptogenic and highly susceptible to structural and functional damages and for this reason most research based on animal models of seizures are focused on this area of the brain [4,5]. The purpose of our study was the analysis of biomolecular anomalies appearing in the rat hippocampal formation in the electrical kindling model of seizures in which repetitive transauricular electroshocks were used for electrical brain stimulation. Fourier transform infrared microspectroscopy was applied for the topographic and quantitative analysis of main biological macromolecules such as proteins, lipids, compounds containing phosphate and carbonyl groups and cholesterol. The examination of accumulation and distribution of these biomolecules was performed using chemical mapping of their main absorption bands or ratios and for the statistical analysis of differences between animal groups the U-Mann Whitney test was applied. What is more, the data from behavioral observations describing the follow-up seizures (the cumulative intensity and time of seizures) were processed with cluster analysis what allowed to divide electrically stimulated animals into subgroups. The results obtained in the study confirmed that the repetitive electrical stimulation influenced the composition and distribution of the macromolecules in the cellular layers of hippocampal formation. The observed changes included the anomalies in protein secondary structure, which manifested as increased relative level of proteins with β-sheet secondary structure, and in the distribution of saturated fatty acids, cholesterol/cholesterol esters and compounds containing phosphate and carbonyl groups. The observed anomalies may testify about the occurrence of the processes associated with the oxidative stress.

[1] Löscher W. Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs, Neurochem Res. 2017 Jul;42(7):1873-1888. [2] Al Sufiani F, Ang LC. Neuropathology of Temporal Lobe Epilepsy, Epilepsy Res Treat. 2012. [3] Miller-Delaney SF, Bryan K, Das S, et al. Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. Brain: a Journal of Neurology. 2015;138(Pt 3):616-631. [4] Wang Y, Zhou D, Wang B, Li H, Chai H, Zhou Q, Zhang S, Stefan H. A kindling model of pharmacoresistant temporal lobe epilepsy in Sprague-Dawley rats induced by Coriaria lactone and its possible mechanism. Epilepsia. 2003; 44(4):475-488. [5] Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. Neurosci Biobehav Rev. 2013; 37(10):2887-2899.

Raman spectroscopy investigations of hydrated hydroxypropyl cellulose mixtures with low soluble salicylic acid: molecular interactions and the water binding structure

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Hydroxypropyl cellulose, Salicylic acid, Raman spectroscopy, Hydration, Non-freezing water

Hydroxypropyl cellulose (HPC) is a non-ionic, water-soluble cellulose ether derivative (Figure 1). It is widely explored as a drug delivery carrier for oral drug delivery systems in pharmacy. Accordingly, the influence of conformations and its interactions with drugs or excipients is of scientific and practical interest of many research groups [1,2]. As for the other hydrophilic polysaccharides, it is believed that HPC and water interactions are related to hydrogen bonding. However, it was demonstrated [1,3] that hydrogen bond bound water is only one of the various physical states in the polymer, because formation of "nanocavities" have also a great ability in changing the polymer mechanical and chemical properties. For that reason Raman spectroscopy was used to investigate the water-binding structure and molecular interactions in hydrated hydroxypropyl cellulose type MF [4,5]. To examine the influence of drug solubility, the matrices composed of low soluble salicylic acid were also studied. The most significant changes were observed in the asymmetrical CH stretching vibrations of methylene and also in symmetrical CH stretching vibrations of CH_3 (3200-2700 cm^{-1} range). This type of research may contribute to better understanding of both highly and poorly soluble drug release from tablet formulations and their dissolution behaviour.

[1] Talik P, Hubicka U. J Therm Anal Calorim. 2017; 132(1):445-451. [2] Talik P, Piotrowska J, Hubicka U. AAPS PharmSciTech 2019;20:187. [3] Liu GL, Yao KD. Polymer 2001;42:3943-7. [4] Paudel A, Raijada D, Rantanen J, Adv Drug Deliv Rev., 2015, 89, 3-20. [5] Talik P, Moskal P, Proniewicz LM, Wesełucha-Birczyńska A. J. Mol. Struct. 1210, 2020, 128062

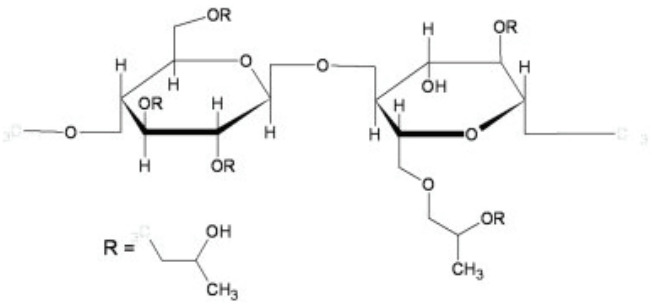

Figure 1. Structure of HPC

Multi-wavelength Raman spectroscopic studies of human RBCs in the course of P. falciparum malaria

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malaria, Plasmodium falciparum, RBC, Raman microspectroscopy, PCA

Malaria is one of the most dangerous infectious diseases in the world, most fatal cases are recorded when the parasite is P. falciparum. The development of Raman microspectroscopy and Raman resonance techniques made it possible to gain an insight into the structural properties of hemoglobin also in pathological states. The structure of the heme chromophore, due to the polarization of this molecule, allows the enhancement of vibrational modes and registration of the scattering phenomenon at the level of a single cell. In addition, significant changes also occur in the lipid composition of the erythrocyte cell membrane during the intra-erythrocyte phase of the life cycle of the malaria parasite P. falciparum. Raman spectra were recorded on blood samples obtained directly from patients, hospitalized in the University Hospital, infected with P. falciparum. The control group consisted of healthy volunteers. In the Raman spectroscopy studies, three excitation laser lines were used: 442 nm, 514.5 nm and 785 nm. The structure of the heme chromophore allowed the selective enhancement of some vibrational modes, using the phenomenon of resonance, which makes it possible to indicate its features and pathological changes occurring at the level of individual erythrocyte. The use of the principal component analysis (PCA) method allowed to recognize changes taking place in infected erythrocyte compared to a healthy one. The PCA method made it possible to visualize and systematize the data, providing information about the similarities and differences in the molecular structure of infected P. falciparum cells during hospitalization. Pointing to the significant characteristics of the Raman spectrum, the intense *v*4 band from the heme ring vibrations at 1353 cm-1 (deoxy-Hb form) characterizes the first days of hospitalization. Bands from the malaria pigment, hemozoin, were observed in the Raman spectra, eg., at 1586 cm-1 (*v*37), which indicate activity of malaria parasites in the blood cell. A band at 787 cm-1 from vibrations of the Plasmodium DNA vibrations is also observed. In addition, other bands appeared to indicate the presence of free amino acids. Changes in the secondary structure of hemoglobin during hospitalization were also observed. The conducted research and analyzes indicate that Raman spectroscopy may be an additional diagnostic method for malaria.

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Raman Microspectroscopy study of Epilepsy

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Raman microspectroscopy, brain tissue, epilepsy, PCA

Epilepsy is a neurological disorder characterized by recurrent und unpredictable seizures or brief absence seizures. The well-validated genetic animal model of absence epilepsy is WAG/Rij rats which share epileptic symptoms with human patients suffering from absence seizures [1]. Epilepsy is a disease that involves multiple brain regions. For our study two different brain structures were chosen: the dorsal lateral geniculate nucleus (DLG) and the somatosensory cortex (Sc), widely investigated as a model of an absence epilepsy [2-3]. Raman spectroscopy was employed as a noninvasive diagnostic tool which can detect small changes in the tissue at the molecular level at early stages of a disease. The brain tissue of young rats (epilepsy model from WAG / Rij strain and Wistar rat control group) was examined before the appearance of seizures. No fixation method was used for sample preparation, fresh sections of the brain were kept in an artificial cerebrospinal fluid during the measurements by Raman microspectroscopy. The spectra were obtained using a Renishaw inVia spectrometer equipped with a;Leica microscope and the 785 nm HP NIR diode laser. A StreamLine fast Raman imaging technique with a water immersive objective was used. Each brain sample was measured three times in different spots to verify its homogeneity. The Principal Component Analysis (PCA) was used to reveal subtle differences in the Raman spectra of the brain tissue of epileptic rats compared to the control group. The studies have shown that epileptic seizures stimulate the variations in the molecular organization of membrane lipids, which have potential to influence the structures in connection with functions of membrane proteins [4]. Analysis of the amide I band of WAG / Rij rats with epilepsy and the Wistar control group may indicate the expression of GFAP in the brain structures of Sc and DLG. These results are in line with epilepsy-related lesion studies indicating that GFAP appears in various epilepsy pathologies in certain parts of the brain [5-6]. Although brain of young rats is poor in protein, they play very important roles in neurological system and apparently in the pathogenesis of epilepsy.

[1] K. Sarkisova, G. van Luijtelaar, Progress in Neuro-Psychopharmacology & Biological Psychiatry, 2011, 35: 854–76 [2] L. Chrobok, K. Palus, M. H. Lewandowski, Neuropharmacology, 2016, 103: 236–246 [3] T. Mizuno, A. Hayashi, K. Tashibu, S. Maraishi, K. Kawauchi, Y. Ozaki, Neuroscience Letters, 1992, 141: 47-52 [4] A. Weselucha-Birczynska, J. Sacharz, J. Zieba-Palus, M. H. Lewandowski, R. Kowalski, K. Palus, L. Chrobok, M. Birczynska, A. Sozanska, Vibrational Spectroscopy, 2016, 85, 48-54 [5] L. Martinian, K. Boer, J. Middeldorp, E. M. Hol, S. M. Sisodiya, W. Squier, E. Aronica, M. Thom, Neuropathology and Applied Neurobiology, 2009, 35: 394–405 [6] M. Dutuit, M.Didier-Bazes, M. Vergnes, M. Mutin, A. Conjard, H. Akaoka, M.-F. Belin, M. Touret, Glia, 2000, 32: 15–24

Extended wavelength detection of low-level malaria parasitemia in whole blood using electronic and near-infrared spectroscopy

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Malaria, NIR spectroscopy, UV -VIS spectroscopy, Parasitemia, Machine learning

The scourge of malaria infection continues to strike hardest against pregnant women and children in Africa and South East Asia. For global elimination, testing methods which are ultra-sensitive to low level parasitemia should be urgently developed. Mid – infrared (MIR) spectroscopy is label free, faster and an order of magnitude cheaper than conventional testing for detecting low level parasitemia. The technique, however, is limited by the preparation of blood samples, which requires drying and often methanol fixation. This often leads to false interpretation and loss of important chemical markers needed for a holistic assessment of malaria infected blood. In this study, we used a novel approach to diagnosis malaria infection by combining both electronic UV – Vis spectroscopy, where absorption coefficient of water is non-extinct and near infrared spectroscopy (NIR), which offers the advantage of better sample penetration to detect and quantify low level (1-0.000001%) ring staged malaria infected whole blood under physiological conditions. Electronic and NIR spectra (200-2500 nm) were acquired from both RBCs and whole blood spiked samples using a Cary 5000 UV-VIS-NIR spectrometer. Classification and quantification models based on different wavelength regions were built with different machine learning methods. The results show that for all machine learning approaches, extended wavelength range (EWR) is optimal for classifying and quantifying malaria infection. PLS-R analysis of spectra of samples from whole blood spiked with malaria parasite enabled quantification of up to 0.001% parasitemia for both lower wavelength range (LWR) and higher wavelength range (HWR) models (R2 = 0.081 and 0.711 respectively). EWR models however, achieved a higher sensitivity for quantifying; parasitemia, 0.000001 % (R2 = 0.855). Similarly, SVM (multiclass classification) performed best with the EWR with a precision accuracy of 100 % and 92% for the RBCs and WB samples respectively demonstrating the potential of accurately diagnosing malaria infected patients without the need for excessive drying and other sample preparation steps associated with existing MIR approach.

Australian Red Cross Blood service. Mr. Finlay Shanks

[1] WHO. World malaria report 2020: 20 years of global progress and challenges. (2020). [2] Adegoke, J. A., Kochan, K., Heraud, P. & Wood, B. R. A Near-Infrared "Matchbox Size" Spectrometer to Detect and Quantify Malaria Parasitemia. Anal Chem 93, 5451-5458, doi:10.1021/acs.analchem.0c05103 (2021). [3] Kochan, K. et al. EXPRESS: Infrared Spectroscopy of Blood. Appl Spectrosc, 3702820985856, doi:10.1177/0003702820985856 (2020).

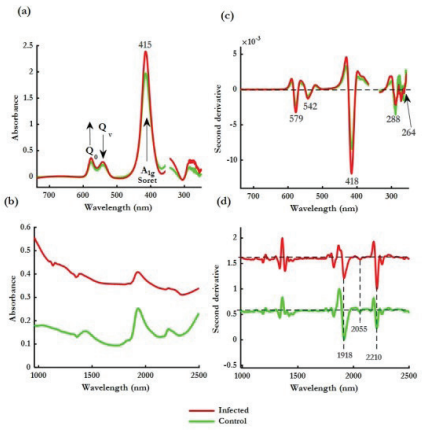

Figure 1. Raw and second der. Spec.

Disintegration of Insulin Amyloid Fibrils Monitored with Atomic Force Microscopy and Surface-Enhanced Raman Spectroscopy

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Amyloid fibril, Insulin, Atomic force microscopy, Surface-enhanced Raman spectroscopy

Insulin amyloid fibrils are characterized by the conversion of the insulin native secondary structure into a β-sheet rich conformation [1]. In vitro, several external factors such as organic compounds [2] or pH value [3] could influence the insulin amyloid fibrils growth or dissociation. Our study is focusing on the irreversible disassembly of amyloid fibrils initiated by a pH value change. We will demonstrate the effect of pH 4-7 solutions on the insulin amyloid fibril morphology and conformation. The generated structures are analyzed by atomic force microscopy (AFM) and surface-enhanced Raman spectroscopy (SERS). Mature fibrils grown in solution at pH 2.5 exhibit a long and intertwined morphology as shown in figure 1A. Amide I bands at 1668-1676 cm-1 (red bar) in figure 1B indicates the presence of β-sheet structures. A step-wise increase of the pH value of the solution induces the dissociation of the fibrils into spherical aggregates. The acquired surface-enhanced Raman spectrum data support the formation of less-ordered structures by changes of the amide I band position. The results demonstrate that no special reagents are required to destroy insulin fibrils, but a pH change is sufficient to dissolve the highly ordered molecular arrangement.

[1] D. Kurouski et al., Analyst (2015); 140, 4967-80. [2] T. Deckert-Gaudig, V. Deckert, Sci. Rep. (2016); 6, 39622. [3] E.Y. Darussalam, O. Peterfi, T. Deckert-Gaudig et al., Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2021); 256, 119672.

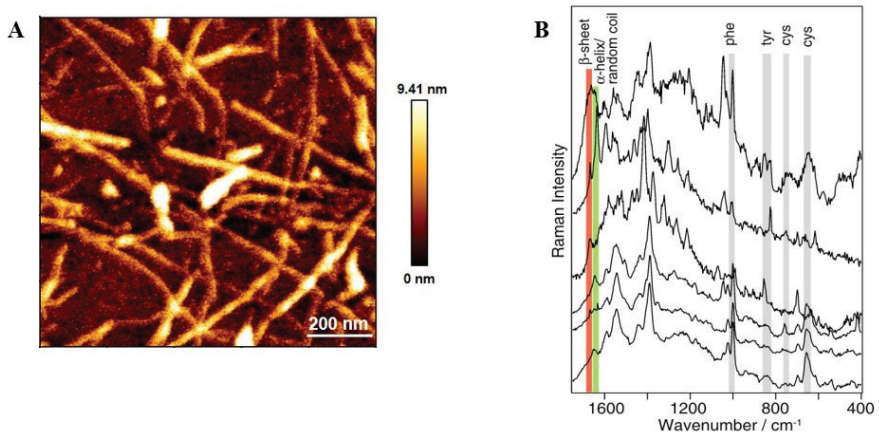

Figure 1. (A) AFM (B) SERS

Probing liposomes of different composition as enzyme metabolites by surface-enhanced Raman scattering

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surface-enhanced Raman scattering(SERS), gold nanoparticles, liposomes, ceramide, sphingomyelin

Lipids are a very important class of biomolecules and involved in many different biological functions. Having roles in cellular signaling, energy storage and as structural components of cellular membranes, they are metabolized and synthesized in the cell. As substrates of enzymes, they could act as convenient probes to monitor enzyme action in complex environments, specifically near membranes, including enzyme inhibition by drugs or in metabolic disorders. We characterize liposomes of different composition in situ and in vivo in a label-free fashion by surface-enhanced Raman scattering (SERS), in our aim to study important lipases in the physiological context and further understand biochemical process of lipid storage disorder based on this spectroscopy. We prepare liposome models composed of specific lipids that play important roles in metabolic diseases. Spectra obtained from lipid structures of different composition and their interactions with metal nanostructures are compared and discussed based on their SERS spectrum, extending initial work with such models [1] to different lipid molecules. As will be shown, the spectra reveal an influence of the content of specific lipids and preparation methods. The data are being used to build an optimized liposome model for studies of enzyme function at membranes in living cells [2].

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[1] V. Živanović et al. J. Phys. Chem. Lett., 2018, 9, 6767–6772 [2] V. Živanović et al. ACS Nano, 2019, 13, 9363-9375

Discrimination of normal brain tissue from dysplastic tissue in focal cortical dysplasia using Raman spectroscopy

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focal cortical dysplasia, raman spectroscopy, optical spectroscopy

Focal cortical dysplasias (FCD), characterized by cortical dyslamination, or abnormal cells such dysmorphic neurons and balloon cells, are the most common cause of refractory focal epilepsy in the pediatric population. Only surgery can remove FCD lesions to cure focal epilepsy, but surgical success depends on the ability to resect the lesion completely while minimizing damage to perilesional normal tissues. While the epicenter of some FCDs can be seen on MRI, abnormal cortex can extend beyond radiological signal abnormalities. Therefore, it remains extremely challenging to remove FCD lesions completely, which explains the 40-60% chance of seizure recurrence after surgery. Thus, better methods of delineating FCD lesions and their borders are needed to improve post-surgical seizure outcomes. Raman spectroscopy employs incident radiation to induce vibrations in the molecules of a sample and the scattered radiation is used to characterize the sample. This technique is fast and sensitive to subtle biochemical changes occurring at the molecular level. The goal of this in vitro prospective study is to use Raman spectroscopy to discriminate between normal brain tissue and dysplastic tissue using formalin fixed paraffin preserved (FFPP) specimens of focal cortical dysplasia patients. Haematoxylin and Eosin stained sections of biopsy specimens from 30 children with focal epilepsy were acquired and assessed by a pediatric neuropathologist. Raman map points were recorded from a parallel unstained and deparaffinized tissue section that targeted the abnormal structures characterizing FCD: dysmorphic neurons, balloon cells and cortical dyslamination. Significant spectral differences were observed between the dysplastic tissue regions and normal regions in the cortex. Indeed, FCD tissues exhibit significantly increased spectral at 1302 cm–1 and 1660 cm–1 peaks, indicating a higher quantity of lipid components and abnormal stretching mode of protein, and C=C stretch vibrations of non-saturated fatty acid chains in lipids in the dysplastic tissues, respectively. In addition, the fingerprint region contains bands from proteins, lipids, nucleic acids and other biomolecules including those assigned to the amino acids tyrosine, phenylalanine, tryptophan. Although further research is needed to identify the underlying mechanisms between the different molecular interactions, these findings suggest the potential spectral fingerprint of Raman spectroscopy as an aid to histopathological diagnosis of focal cortical dysplasia.

Disposable substrate for malaria screening using ATR-FTIR spectroscopy

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infrared substrates, malaria diagnosis, Attenuated Total Reflection Fourier transform infrared (ATR-FTIR) spectroscopy, Partial Least-Squares Regression (PLS-R)

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is a convenient alternative to transmission spectroscopy for fluid specimens because it yields a constant and reproducible effective optical path length and thus good sample-to-sample precision[1]. However, after each measurement the ATR crystal or internal reflection element (IRE) requires cleaning, making continuous collection of data difficult. As an alternative, an inexpensive coverslip was tested in an effort to reduce the sample preparation time by enabling multiple samples to be collectively dried under the same temperature and conditions. This provides a time and reproducibility advantage compared to directly depositing the biofluid onto the IRE where each sample has to be independently dried. Since the coverslip is made up of high-grade glass, the unprocessed spectrum of the coverslip depicts a pronounced absorption band around 877 cm^{-1}, which is attributed to a Si-O-Si bond and a band at 773 cm^{-1} from the Si-C bond in glass. However, the ATR spectra recorded of the sample on the coverslip did not show any significant spectral contributions from the coverslip. To compare the coverslip approach with the direct deposition method we used malarial infected red blood cells as a model system. Partial least squares regression (PLS-R) was performed to ascertain the effectiveness in quantifying malaria parasitemia by comparing the coverslip approach and the direct method. A negative regression band at 1213 cm^{-1} assigned to the C-O stretching vibration from the propionate group of hemozoin, which is a prime marker for quantifying late stage parasitemia (Figure 1). The model produced low root mean square error of cross-validation (RMSECV) value (0.177) and a high R^2value (0.985) indicating that this method is a suitable alternative to the conventional direct deposition approach. Hence, the proposed disposable coverslip shows promise as a substrate for high throughput blood screening for parasitic infections, with improved performance compared to direct deposition onto the IRE.

[1] Perez-Guaita, D., Marzec, K.M., Hudson, A., Evans, C., Chernenko, T., Matthaäus, C., Miljkovic, M., Diem, M., Heraud, P., Richards, J.S. and Andrew, D., 2018. Parasites under the spotlight: applications of vibrational spectroscopy to malaria research. Chemical reviews, 118(11), pp.5330-5358.

Spectroscopic analysis of biofluids – hidden diagnostic method for pancreatic cancer?

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vibrational spectroscopy, chiroptical spectroscopy, pancreatic cancer, blood plasma, statistical processing

Humanity fears countless diseases, but most of all cancer. Though pancreatic cancer does not stand out by its incidence, the mortality rate is one of the highest. The increasing trend of the 5-year survival rate is auspicious, yet 9% (ref.[1]) is still a tragic number. One of the reasons behind those unpromising outcomes is the lack of a suitable diagnostic procedure enabling early diagnosis. The diagnosis of pancreatic cancer is usually confirmed by a series of imaging examinations, blood tests and biopsy. However, due to the mostly asymptomatic onset, the final verdict often comes too late. Treatment in the form of surgical resection is possible only if there are no metastases, which are characteristic for this aggressive form of neoplasm. Pancreatic cancer may be usually found in close connection with another civilization disease, diabetes mellitus. Despite the fact that diabetes is a risk factor for many other diseases, pancreatic cancer may occur in 74% of cases within a 24-month window following the diabetes diagnosis[2]. This fact may be useful for the development of a screening of at-risk patients. Spectroscopic techniques in general are able to provide a fast and non-destructive analysis of various samples; are reagent free and need little or no sample processing. In the field of medical research, the spectroscopic analysis of biofluids and tissues is extensively studied[3]. There are numerous advantages of using biofluids, such as blood plasma, mainly its availability and ease of handling. Blood circulating around perfused tumour tissue carries altered molecules that might be targeted with spectroscopic methods. Vibrational techniques represented by infrared and Raman spectroscopy coupled with chiroptical spectroscopy, namely, electronic circular dichroism and Raman optical activity[4], enabled us to describe blood plasma samples of pancreatic cancer patients and diabetic individuals from different perspectives. Subtle biochemical changes including alterations in protein composition and structure, or differences in the concentration of other significant biomolecules were observed between the studied groups. To find hidden specific trends among the large amount of variables acquired, multivariate statistical methods were used, reducing the dimensionality of the input data. Multiple approaches, including principal component analysis and linear discriminant analysis, provided high values of clinically relevant performance characteristics (sensitivity, specificity), thereby declaring great prospects for the spectroscopic screening of pancreatic cancer.

Supported from the grants of Specific university research No. A1_FCHI_2021_003 and A2_FCHI_2021_009.

[1] World Cancer Report: Cancer Research for Cancer Prevention. Wild C. P., Weiderpass E., Stewart B. W., Eds. International Agency for Research on Cancer: Lyon, France, 2020. [2] Pannala R., et al., Gastroenterology 2008, 134 (4), 981–987. [3] Baker M. J., et al., Chem. Soc. Rev. 2016, 45 (7), 1803–1818. [4] Tatarkovič M., et al., Anal. Bioanal. Chem. 2015, 407 (5), 1335–1342.

Spectroscopic view on neurodegeneration – Raman spectroscopy and Raman optical activity in the diagnostics of Alzheimer's disease

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Alzheimer's disease, Raman spectroscopy, Raman optical activity, fluorescence, carotenoids

Thanks to advantageous properties, such as sensitivity to the spatial structure of molecules, non-destructiveness, simple sample preparation and the possibility of analysis in an aqueous environment, Raman spectroscopy is widespread in the field of biological applications, including medical diagnostics. Within the presented research focused on the diagnostics of Alzheimer's disease (AD), we also employed its advanced polarized version, Raman optical activity (ROA), which allowed to capture information about the spatial structure of the biomolecules contained in blood plasma in a more detailed manner. The currently clinically utilized methods for AD diagnostics lack sensitivity to;the early stages, are cost- and time-demanding, sparsely available and/or highly invasive; therefore, the development of a simple blood-based spectroscopic test would be an important breakthrough in AD diagnostics. With;protein misfolding being the fundamental feature or possibly even the cause of the disease, Raman spectroscopy and especially ROA appear to be suitable tools for that task. Within this study, both Raman and ROA spectra of blood plasma samples of patients suffering from various stages of AD, vascular dementia, and age-matched control subjects were acquired simultaneously on a ROA spectrometer using 532-nm excitation. Unfortunately, the utilized excitation leads to undesired fluorescence of blood plasma, causing significant loss of spectral information or, in some cases, completely overlapping the measured Raman spectrum. Therefore, we have developed a methodology to reduce the fluorescence background and concomitantly achieve a much higher signal-to-noise ratio in ROA spectra[1]. In the resulting spectra, the most apparent differences between the studied groups were found in the intensities of the bands assigned to carotenoids, in which resonance enhancement occurs due to the utilized excitation wavelength. With AD progression, the intensity of these bands decreased, which is presumably a general sign of increasing oxidative stress in the diseased organism. To also evaluate less pronounced spectral differences, the acquired data were subjected to statistical analysis utilizing a combination of principal component analysis and linear discriminant analysis. The created statistical models were able to distinguish controls and patients with AD as well as vascular dementia with the performance characteristics meeting the requirements for a clinically relevant biomarker (i.e. sensitivity and specificity of 85% and 75%, respectively).

Supported from the grants of Specific university research No. A2_FCHI_2021_009 and A1_FCHI_2021_003.

[1] Tatarkovič M., et al.: Anal. Bioanal. Chem., 2015, 407(5), p. 1335–1342.

Evaluation of the biomolecular composition of the various types of human muscles tissues.

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FTIR, muscles, muscle fibers

The main aim of the experiment was to check whether there are differences between different types of muscles: type I and type II, and whether the pathogenic changes affect the biomolecular change of the patient's fibers. This characteristic was measured on samples recognized as myopathic and compared with the control group. The samples designed to the elemental analysis were prepared and diagnosed at the Department of Pathomorfology of Collegium Medicum Jagiellonian University. The tissue material came from a surgical biopsies and has been prepared by shock freezing in liquid C3H8. For each specimen two adjacent tissue slices were cut into 8 micrometers on the cryo-microtome and placed on the microscope slide and Silicon Nitride Window (membrane thickness: 200 nm, window size: 2x2 mm), respectively. The experiment was carried out at the B22 beamline, DIAMOND Light Source using the FTIR microscope Hyperion 3000 (Bruker Optics, Germany, Ettlingen) with high sensitivity 50× 50 µm MCT detector. There were 128 and 256 scans respectively per sample and background, collected with 4 cm-1 spectral resolution and with aperture size 5×5 µm2 (the single sample point measurement took about 15 seconds). Preliminary studies show differences in bimolecular composition both between the diseased fibers and the control group, as well as comparisons between fiber types. There are the differences in amid I and II as well as lipids content – it seems that it is higher level for control group muscle comparing with myopathic.

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[1] Milenkovic M. S., Kojic S., Stojanovic D., Skeletal Muscle: From Pharmacology to Clinical Practice, 155-170, (2015). [2] J. Talbot, Skeletal muscle fiber type: using insights from muscle development biology to dissect for susceptibility and resistant to muscle disease, Wiley Interdiscio Rev Biol. (2016), 5(4), 518-534. [3] M. Barańska (Editor), (2014), Optical Spectroscopy and Computational Methods in Biology and Medicine.

Raman studies of activated lymphocytes in the infectious mononucleosis

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Raman spectroscopy, Mononucleosis

Viral diseases pose a significant threat to human health and life. In our research using the Raman spectroscopy method, we have attempted to recognize some characteristic features of the course of infectious mononucleosis, reflected in the pattern of lymphocyte activation [1]. Naïve lymphocytes become activated upon contact with the virus. It begins to take on a new function, which leads to a change in the composition of the cell and the distribution of its relevant components. A spectroscopic marker of this process is the appearance of a 520 cm-1 band, indicating the formation of immunoglobulins. Blood samples were taken from patients diagnosed with infectious mononucleosis at the University Hospital in Krakow. The control group consisted of healthy volunteers. For Raman microspectroscopy measurements, a Renishaw InVia Raman spectrometer combined with a Leica optical microscope was employed, and an argon laser beam with an excitation wavelength of 514.5 nm was used. The mapping technique was used in the measurements. The analysis of the results was carried out using the PCA statistical method. In acute mononucleosis, the lymphocyte cell remains activated throughout the course of the disease. The analyzed entire activated surface of the lymphocyte cells is characterized mainly by the vibrations of the disulfide bridge. There is also a band of approximately 780 cm-1 (nucleic acids, DNA, T), as well at position 1573 cm-1 (Asp), in addition also 1666 cm-1(amide I, â-sheet conformation). [2] These bands indicate the readiness of the lymphocytes to form a clone of identical cells. [3] Signals from amino acids like Asp also indicate the formation of the γ chain of a ligand- receptor complex [2], [4]; The polarization properties of the disulfide vibration were also analyzed, because they may indicate symmetries of molecular groups, which consequently enables the differentiation of pathological systems. [2].

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[1] M. Pietruszewska, G. Biesiada, J. Czepiel, M. Birczyńska, P. Moskal, M. Kozicki, E.Hola, A. Garlicki, A. Weselucha-Birczynska, Lymphocytes studied by Raman microspectroscopy, in Lymphocytes, IntechOpen 2019 (ISBN: 978-1-78984-920-2). [2] H. Abramczyk, B. Brozek-Pluska, M. Kopec, J. Mol. Liq., 259 (2018) 102–111. [3] M. Pietruszewska, G. Biesiada, J. Czepiel, M. Birczyńska-Zych, P. Moskal, A. Garlicki, A. Wesełucha-Birczyńska, J. Mol. Struci., 1229 (2021) 129837 (1-9). [4] O.J. Hamming, L. Kang, A. Svensson, J.L. Karlsen, H. Rahbek-Nielsen, S.R. Paludan, S. A. Hjorth, K. Bondensgaard, R. Hartmann, J. Biol. Chem., 287 (2012) 9454–9460.

Effect of Nb-doping on dissolution process of hydroxyapatite: FTIR studies.

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biomaterials, hydroxyapatite, niobium, fourier-transform infrared spectroscopy

Hydroxyapatite is a major inorganic components of the hard tissues and because of that, it has been widely researched over the last few decades. As it exhibits biocompatibility, osteoconductivity and bioactivity, it is widely used in impantology and tissue engineering [1], among others. Since different applications require specific properties of hydroxyapatite, many different methods are used to synthesize it. Of these, the easiest and one of the most popular are mechanochemical [2] and precipitation [3] methods. Another way of influencing the properties of the obtained material is doping and in the case of hydroxyapatite, the use of niobium is promising. Its use affects the physico-chemical properties (e.g., crystallite size and degree of crystallinity) of hydroxyapatite, which improves its biological properties, and the presence of niobium itself e.g. improves the anti-inflammatory properties of the material [4]. In this work, we present the results of ATR-FTIR analysis of niobium-doped hydroxyapatite of general composition 77·CaO-(23-x)·P2O5-x·Nb2O5 where x = 0.00; 1.15; 2.30; 4.60 (which corresponds to 0, 5, 10 and 20 mol % of phosphorous, respectively), prepared via simple mechanochemical (MS) and precipitation (PR) methods. Subsequently, to simulate interactions under biological conditions, the materials were dissolved in phosphate-buffered saline (pH 7.4) at 37°C for 4 weeks. The results of, both qualitative and quantitative, spectroscopy analyses show that the effect of niobium content on pristine biomaterials is subtle, but strongly influences their dissolution behavior. Maxima of the spectral lines related to PO4 3- and CO3 2- vibrational modes gently change positions with increasing Nb content. This indicates that niobium is inbuilt into the materials structure. Additionally, its influence is strongly manifested in the structural changes resulting from the dissolution of the material. In this case, regardless of the synthesis technique, the presence of niobium slows down this phenomenon. Furthermore, in the case of MS samples, niobium also promotes a more stable structure of hydroxyapatite at the expense of the structure of tricalcium phosphate. It is also worth noting that all spectra show bands correlated with carbonate groups, which increase with an increase in the content of Nb. Those bands are more intense for biomaterials prepared by mechanochemical method than those obtained by precipitation and in all cases the B-type substituted carbonates dominate.

[1] W. Habraken, et al. Materials Today 19 (2) (2016) 69-87. [2] S. Adzila, et al. Indian Journal of Chemistry 52A (2013) 1570-1575. [3] S. Ramesh, et al. Journal of Ceramic Processing Research 14 (4) (2013) 448-452. [4] S. Saranya, M. Prema Rani, Materials Today: Proceedings (2021).

Biomedical Applications
Hydration of Extracellular Matrices Assessed by Low Frequency THz-TDS

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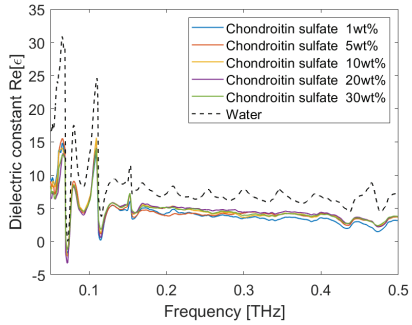
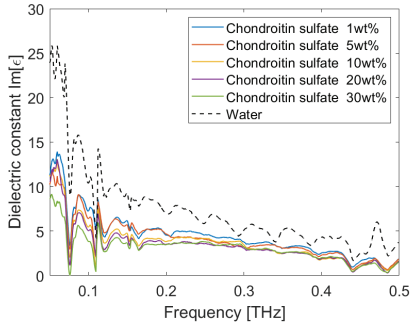
Time resolved spectroscopy and dynamics, THz-TDS, Cartilage, Dielectric relaxation, Hydration

1. Introduction: In recent years, regenerative medicine has been used as a treatment for knee osteoarthritis. Cartilage consists of chondrocytes and 65-80% water, extracellular matrix 12-22% collagen, 4-7% proteoglycans and the like. To evaluate the hydration state of regenerated cartilage, the permittivity of water in the aqueous solution was calculated using the formula (Bruggeman). Hydration evaluation of aqueous solution in the previous research is pure water and water. It has been done using the permittivity of the whole solution. Using the permittivity of the water in the aqueous solution and the whole aqueous solution, the study aims accurate estimation of hydration of cartilage matrix aqueous solution.
2. Measurements: The Terahertz time domain spectroscopy (THz-TDS) experimental system and analysis: A THz-TDS spectrometer, IRS 2700NP, advanced infrared, was applied with a whole range of measurements from 0.01 to 10 THz. The sample was held by the window material. The material used is PTX, which has high terahertz wave transmittance. It was also a spacer made of SUS340 with 40 μm thickness. The sample thickness is regulated by sandwiching the sheet between the window materials. The obtained time domain waveform was decomposed into a frequency spectrum and a phase, dividing the measurements of each sample by reference. Calculation of water permittivity using Bruggeman's equation: Induction of water contained inside from the permittivity of the sample, the electricity rate was calculated. In the imaginary part, when the bound water changes to hydrated water, it drops sharply. Because 0.5THZ depends on hydration, and chondroichin is dominated by relaxtion. The complex permittivity is determined by slow relaxation: It can be decomposed into sums. The first term is slow. Relaxing and aggregated molicules of water molecule clusters correspond to mitigation. The second term is fast relaxation. Free water orientation relaxation or away from hydrogen bonds. It is thought to be the relaxation of water molecules. Dielectric relaxation time γα and γβ are fixed, and the time relaxation intensity $\varepsilon\alpha$, $\varepsilon\beta$, $\varepsilon\infty$ is fixed.
3. Results: Measurement of chondroitin sulfate aqueous solution: Chondroitin sulfate aqueous solution (1wt%, 5wt%, 10wt, 20wt%, 30wt%) were measured. Refer to Fig.1 and Fig.2. $\varepsilon\alpha$ decreases as the concentration of condoroitin sulfate increases. The reduction of free water in the aqueous solution and the total amount of water represents a decrease. $\varepsilon\beta$ depends on temperature. A device-relaxing fitting of the permittivity of complex of water in aqueous solution using Bruggman's equation was performed. Rich $\varepsilon\alpha$, w decreased as the degree increased. This is because that high concentration reduces the total amount of water. The decrease in $\varepsilon\beta$, w is the sum of the dielectric relaxation strength used as the fitting condition. Since it was fixed, it increased to compensate for the decrease in $\varepsilon\beta$, w. It is thought that this is the cause





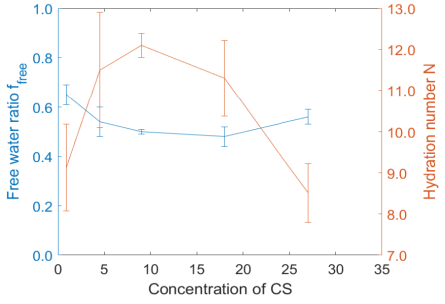


Fig.1 Real part of Chondroitin sulfate, Fig. 2 Imaginary part of Chondroitin sulfate, Fig.3 Hydration of mixtures
Number of the session: 2-2. Biomedical Applications Name and surname: Seiji Nishizawa Date of sending the abstract: May 17, 2021

Study of endogenous hyaluronic acid in human dermis by in vivo confocal Raman spectroscopy

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Hyaluronic acid, human dermis, confocal Raman spectroscopy, immunohistochemistry

Hyaluronic acid (HA) is an important component present in the skin whose main characteristic is to contribute to the skin hydration process, in addition to acting on its maintenance, reducing the aspect of aging1-2. In this study, we analyzed the dermis of 10 study participants, divided into two large groups - 20-30 years and 50-65 years – using the techniques of confocal Raman spectroscopy (CRS) and immunohistochemistry (IHC). For this, the Rivers Diagnostic equipment (Model 3510 Skin Composition Analyzer) was used, coupled to a 785 nm excitation laser, with a fixed power between 25 ± 2 mW. The laser light was centered on the surface of the skin with a microscope objective (40x) located under the quartz window of the equipment. The spectral range from 400 to 1800 cm-1 was analyzed, in a depth that varied between 100 to 110 µm. To validate the results obtained by the CRS, all participants had their skin evaluated by the IHC technique, in order to quantify the CD44 marker for the presence of HA in the dermis. By evaluating the average Raman spectra of the dermis and the spectrum of pure HA, it was possible to identify several marker peaks for HA in the skin. Among these, the peak of 1104 cm-1 was chosen due to its variation between the groups, which made it possible to show a higher concentration of HA in the dermis region for the 20-30 years group (1.878 a.u.) compared to the 50-65 years group (1,166 a.u.) (Figure 1A and 1B). The results obtained were statistically significant by Student's T Test a value of $p < 0.05$. Significant correlation has been found bettwen CRS and IHC results. Confocal Raman spectroscopy was able to provide important information regarding the concentration of HA, enabling an effective, fast and non invasive procedures, being an important tool in the study of the constituents of human skin.

grant 2020/00928-7, 2021/02667-9, São Paulo Research Foundation (FAPESP). CNPq 310375/2017-7

[1] Coleman, S. R., & Grover, R. (2006). The anatomy of the aging face: volume loss and changes in 3-dimensional topography. Aesthetic surgery journal, 26(1S), S4–S9. https://doi.org/10.1016/j.asj.2005.09.012 [2] Weindl, G., Schaller, M., Schäfer-Korting, M., & Korting, H. C. (2004). Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin pharmacology and physiology, 17(5), 207–213. https://doi.org/10.1159/000080213

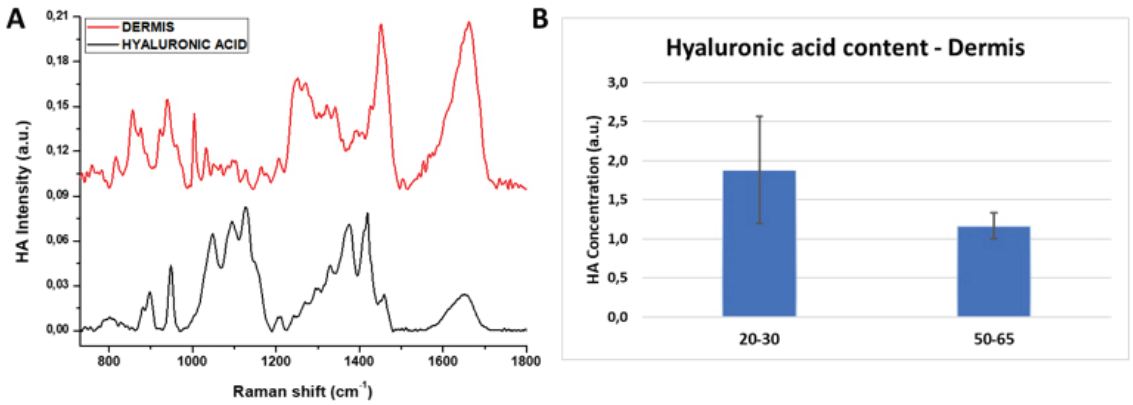

Figure 1. A: Raman spectra of the dermis compared to the HA spectrum; B: Concentration of HA on the dermis.

In vivo study of deep hydration by confocal Raman spectroscopy

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Confocal Raman Spectroscopy, Hydration, Skin, Water, in vivo, Moisturizing

Water is a crucial component of healthy skin, responsible for maintaining its proper structural and mechanical properties Moisturizers are important elements of basic skin care, aiming to prevent dehydration, flaccidity and decreased elasticity, resulting from aging-related loss of water.1-2 Skin hydration is determined by the water content present at the epidermis, as commonly described, but also at the dermis. Water in skin is present as free or bonded water. Moisturizing effect is the most common benefit claimed by cosmetic products, but the focus is on changes observed at the skin surface. This study aimed to determine, in vivo, changes in the free and bounded water content within the skin dermis upon product application. The study was submitted and approved by the Ethic committee under number 3.825.279 and 11 volunteers, aged between 30 to 40 years were selected under previous evaluation by a specialist physician and signing of a consent form. Confocal Raman System (Rivers Diagnostics® – Model 3510 Skin Composition Analyzer) coupled to a 671 nm excitation energy laser was used. Epidermis and dermis were analyzed (0-110µm) with depth steps between 4 to 10µm. Skin was treated with four investigational formulations (P1, P2, P3) vs. placebo formula P4 for 30 consecutive days on four different selected areas of the participants forearms. An additional area was selected for control (CTR). Measurements were performed after seven days washout before applying any product to the skin and then after 15 and 30 days of continuum use. The three peaks evaluated in the study were 3277, 3458 and 3604 cm-1, corresponding to DDAA-OH (strongly bound water), DA-OH (weakly bound water) and Free-water3, respectively (Figure 1). The data process involved the average and standard deviation analysis, statistical analysis of peaks intensity variations by subtraction the data and by spectra deconvolution. After performing cluster analysis and considering the groups variations, it was found that the product P1 has shown the best performance to increase the water content of all water types at dermis after 15 and 30 days of continuum use.

CNPq 310375/2017-7

[1] Verdier-Sévrain, S. and Bonté, F. (2007), Skin hydration: a review on its molecular mechanisms. Journal of Cosmetic Dermatology, 6: 75-82. [2] Choi, J.W., Kwon, S.H., Huh, C.H., Park, K.C. and Youn, S.W. (2013), The influences of skin visco-elasticity, hydration level and aging on the formation of wrinkles: a comprehensive and objective approach. Skin Res Technol, 19: e349-e355. [3] Choe, C.; Lademann, J.; Darvin, M. E. Depth profiles of hydrogen bound water molecule types and their relation to lipid and protein interaction in the human stratum corneum in vivo. The Analyst, v. 141, p. 6329-6337, 2016

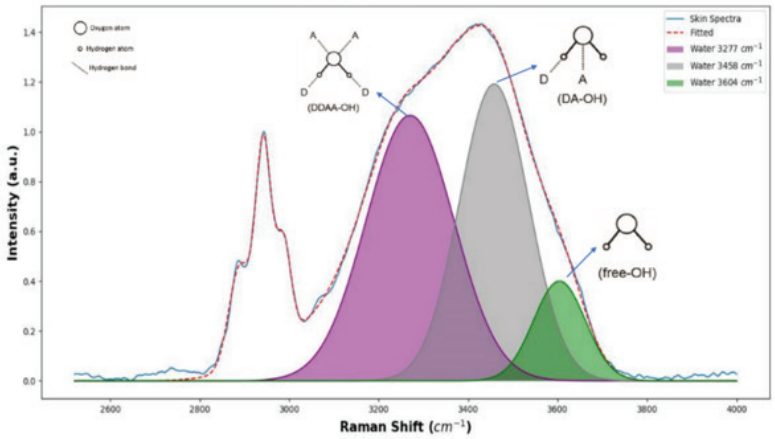

Figure 1. Deconvolution of the OH region (3000 to 3800 cm-1). Peaks evaluated: 3277 (DDAA-OH, double donor-double acceptor), 3458 (DA-OH, single donor-single acceptor) and 3604 cm-1 (free-OH).

Biofluid Vibrational Spectroscopy and The Prospect of a Novel Pancreatic Cancer Biomarker for Early Diagnosis

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Vibrational spectroscopy, biofluid, pancreatic cancer

Pancreatic cancer is the seventh leading cause of cancer related deaths globally in both men and women, with this projected to rise to the second leading cause by 2030. As of yet, there are no reliable biological biomarkers for pancreatic cancer that aid in early diagnosis of the disease due to its complex nature and patient-to-patient heterogeneity. There remains scope to identify a reliable pancreatic cancer biomarker that is highly sensitive, highly specific, non-invasive and can differentiate between dysplasia and cancer. Vibrational spectroscopy has emerged in recent years as a novel approach to differentiate between healthy and diseased tissue. Proof of concept studies with cells and tissues have established that vibrational spectroscopy has the potential to be used in clinical environments to aid in early diagnosis of many different cancers including those of the breast and brain. This project is investigating vibrational spectroscopy as a single marker platform diagnostic for pancreatic cancer using biofluid samples. Preliminary data from this work have demonstrated encouraging diagnostic performance with spectral measurements and machine learning approaches. Future requirements for development in this space will be discussed.

Biochemical and morphological alterations of leukocytes recoved from different cryopreservation methods

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PBMC, Cryopreservation, Atomic force microscopy, Raman Spectroscopy

Collection and cryopreservation of viable peripheral blood mononuclear cells (PBMC) is crucial in immunotyping and functional studies. Established methods employ rate-controlled freezing (RCF) and snap freezing (SF). We aimed to compare both methods employing the classical cell viability analysis combined with assessment of cell biochemistry and morphology in nano-scale, applying Raman spectroscopy (RS) and atomic force microscopy (AFM), respectively. PBMC isolated from healthy donors, after addition of cryoprotectant (DMSO), underwent SF and RCF. In SF method (SF 1), based on optimized method of snap freezing, cells were directly placed -196C. In two RCF methods (RCF 1 and RCF 2) cells were first slowly brought to -80C. Viability and cell count were assessed prior freezing and after thawing. Furthermore, thawed cells underwent RS and AFM measurements. In total, 30 PBMC's were imaged per each sample and cryopreservation approach. Non-contact mode (AC) using standard force modulation probes was applied, while for RS measurements 532 nm excitation wavelength was used. Cell count and viability decreased after freezing. SF 1 had highest drop in both: fraction of live cells ($p< 0,05$ vs. RCF 1 and vs. RCF 2) and number of recovered cells ($p< 0,05$ vs. RCF 1 and vs. RCF 2). Based on Raman spectra from post thawed PBMCs, Principal Component Analysis grouped SF 1 and RCF 1 together with separate RCF 2 group. This was reflected in lipid/protein ratio, with significant difference between RCF 2 vs SF 1 ($p< 0,001$) and no significant difference between RCF 1 and SF 1 ($p>0,05$). Based on AFM imaging height and diameter of PBMC post thawing was greater 1.9 ($p< 0,05$) and 1.7 times ($p< 0,01$), respectively, in RCF 1 compared to SF 1, and 2.2 ($p< 0,01$) and 1.8 times ($p<0,0001$), respectively, in RCF 2 compared to SF 1. Vesicles (size range: 100-600 nm) formed on the cell membrane were present in all methods, and were smaller in RCF 2 vs. RCF 1 ($p< 0,05$). Interestingly, cell membrane cavities (size range: 100-650 nm) were only present on PBMC's in RCF 1 and RCF 2 (smaller in RCF1 $p< 0,05$). The results suggest that optimal cell survival and yield requires cells on one hand to preserve high protein/lipid ratio and on the other to induce controlled relaxation of cell membrane. Hence, maintaining spherical shape, reducing size of vesicles and forming larger cavities corelated with better recovery results. Increase in lipid content and cell membrane blebbing, proven indicators of high oxidative stress and apoptosis, may be responsible for lowest cell recovery and viability in SF 1.

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[1]M. Kaczmarska, et al. Nanomedicine: NBM 2020, 28,102221 [2] P.43217; PCT/PL2020/050093.

Investigating the Effects of Fluid Resuscitation on Red Blood Cells using Raman Tweezers spectroscopy

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Raman spectroscopy, Red blood cell, Optical tweezers, Haemaccel

Investigating the response of red blood cells on the clinical administration of resuscitation fluids has been always an important issue for clinicians while dealing with trauma patients. Probing of biochemical modifications of cells under externally induced (chemical and physical means) conditions are getting interest using vibrational spectroscopy methods. Raman spectroscopy has been found to be highly suitable for the characterization of biological/clinical samples due to minimal interference of vibrational frequencies from aqueous environment. Raman spectroscopy analysis of live single cell under physiological condition is difficult due to Brownian motion of micron sized cells. The use of traditional cell fixation techniques on plane substrates can often result in loss of cellular integrity. This disadvantage can overcome with the aid of optical tweezers, which involves the capture of the cell of interest in the medium using a tightly focused laser beam. The combination of Raman spectroscopy along with optical tweezers (trap) known as "Raman Tweezers" can facilitate biochemical examination of single live blood cells under different external factors. Haemaccel, 3.5% colloidal infusion solution of polygeline with electrolytes, is a colloidal intravenous fluid, which is usually given as a short term fluid replacement after trauma. Haemaccel infusion works by replenishing the body's fluid and electrolytes. The impact of this resuscitation fluid on human red blood cells has been least explored via spectroscopic modalities. The present study investigates the impact of Haemaccel on red blood cells for the first time using Raman Tweezers spectroscopy. All these studies were compared with the red blood cells in blood plasma as control. The present study has shown an adverse impact in hemoglobin oxygenation of the red blood cells upon the use of haemaccel. Spectral assignments belonging to methine deformation region, spin marker region and pyrrole deformation region have been investigated, which demonstrated the possibility of higher hemoglobin deoxygenation in haemaccel. The variations in spectral signatures were evident in view of the display of effective discrimination of cells in haemaccel with respect to plasma after performing principle component analysis.

Authors are thankful to DBT and VGST, Govt. of Karnataka for providing the financial support for the fellows working in the project. Authors also like to acknowledge Vittal Shenoy and the staffs in Blood Bank, Kasturba Medical College, Manipal for their support during this research work.

2.3. Drug Delivery and Monitoring

Combining Pharmacokinetics and Vibrational Spectroscopy: MCR-ALS Hard soft modelling of drug uptake in vitro using tailored kinetic constraints

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Chemometrics, Drug Uptake, MCR-ALS

Raman and Infrared spectroscopy are label free techniques very suitable for the investigation of drug uptake in pharmacokinetics. However, the high complexity of the vibrational spectra makes the identification of spectral patterns associated with the drug and subsequent cellular responses difficult. Indeed, multivariate methods that relate spectral features to the inoculation time do not normally take into account the kinetics involved, and important theoretical information which could assist in the elucidation of the bands is excluded. Here, we propose the integration of those complex kinetic equations in the modelling process. For this, Multivariate Curve Resolution-Alternate Least Squares (MCR-ALS) was applied using tailored kinetic constrains based on a system of ordinary differential equations, which represented the drug uptake and subsequent cellular responses. On each iteration of the MCR-ALS, the concentration and reference spectra of drugs and responses, as well as the different kinetic constants is recalculated. The methodology was firstly evaluated with artificial datasets that simulated Raman and IR spectra from cells inoculated with a DNA binding drug across the cytoplasm, nucleus, and nucleolus. Finally, IR and Raman spectra from A549 inoculated with doxorubicin was used to assess the advantages and challenges of the method. Results evidenced that the model is very dependant on the system of equations used, and the complexity of equations is limited by the quality of dataset (e.g. the temporal resolution). On the other hand, the use of tailored kinetic constrains in the MCR-ALS allowed for a more comprehensive modelling of the system, enabling the elucidation of not only the kinetic profiles and spectral features of the drug binding and cellular responses, but also the computation of the kinetic constants.

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Low frequency Raman spectroscopy for monitoring drug solubilisation in milk-based formulations during digestion

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low frequency Raman spectroscopy, drug digestion, principle component analysis

The bioavailability of poorly water soluble lipophilic drugs is often limited by drug dissolution. Lipid based vehicles have been proposed as an approach to maintain solubilisation during digestion.[1] One approach is the co-administration of milk to enhance bioavailability in poorly water soluble drugs. In situ assessment of the dissolution of poorly soluble drugs in a complex media such as milk or formula is desirable. Low frequency (or low wavenumber) Raman (LFR) spectroscopy (10-300 cm^{-1}) gives information often associated with intermolecular vibrations, making this technique particularly sensitive to detecting different crystal packing arrangements (solid state form) of pharmaceutics. [2]The relatively rapid acquisition times makes this technique an appropriate candidate for continuous monitoring of processes such as drug digestion. Low frequency Raman spectroscopy was used to continuously monitor the digestion of a series of pharmaceuticals in complex media such as milk, infant formula and buffer. The resulting spectra were qualitatively assessed with principal component analysis where the dissolution of the drug with digestion was observed and associated with the loss of crystalline drug features. Low frequency Raman gave similar results to those obtained via synchrotron small angle x-ray scattering3-4 making it a viable technique for drug dissolution processes in complex media.

[1] Boyd, B. J.; Salim, M.; Clulow, A. J.; Ramirez, G.; Pham, A. C.; Hawley, A., The impact of digestion is essential to the understanding of milk as a drug delivery system for poorly water soluble drugs. Journal of Controlled Release 2018, 292, 13-17. [2] Bērziņš, K.; Fraser-Miller, S. J.; Gordon, K. C., Recent Advances in Low-Frequency Raman Spectroscopy for Pharmaceutical Applications. International Journal of Pharmaceutics 2020, 120034. [3] Salim, M.; Fraser-Miller, S. J.; Sutton, J. J.; Bērziņš, K. r.; Hawley, A.; Clulow, A. J.; Beilles, S. p.; Gordon, K. C.; Boyd, B. J., Application of Low-Frequency Raman Scattering Spectroscopy to Probe in Situ Drug Solubilization in Milk during Digestion. The journal of physical chemistry letters 2019, 10 (9), 2258-2263. [4] Salim, M.; Fraser-Miller, S. J.; Bērziņš, K. r.; Sutton, J. J.; Ramirez, G.; Clulow, A. J.; Hawley, A.; Beilles, S. p.; Gordon, K. C.; Boyd, B. J., Low-Frequency Raman Scattering Spectroscopy as an Accessible Approach to Understand Drug Solubilization in Milk-Based Formulations during Digestion. Molecular pharmaceutics 2020, 17 (3), 885-899.

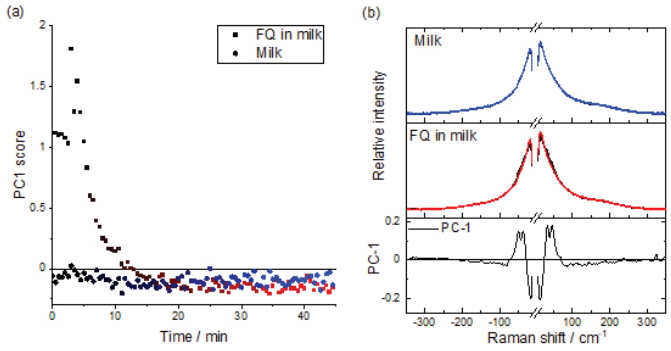

Figure 1. Comparision of LFR spectra collected during the digestion process of milk (blue) and milk + ferroquine (FQ) (red). (a) Plot of the first principle component (PC1) score value versus time and (b) the associated PC1 loadings in comparsion with spectra from the two runs.

How Ionophore-Valinomycin Enters and Transports K+ Across a Model Bilayer Lipid Membrane

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valinomycin, PM IRRAS, biomimetic membrane, supported bilayer, ion transport

Valinomycin, a cyclic peptide, was incorporated into a biomimetic lipid membranes either tethered (tBLM) to or floating at the surface of a gold (111) electrode. Electrochemical Impedance Spectroscopy (EIS) was used to study the ionophore properties of the peptide and polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) was employed to determine the conformation and orientation of valinomycin in the membrane. The combination of these two techniques provided unique information about the ionophore mechanism where valinomycin transports ions across the membrane by creating a complex with potassium ions and forming an ion pair with a counter anion. The ion pair resides within the hydrophobic fragment of the membrane and adopts a small angle of ~22o with respect to the surface normal. This study provides new insights explaining the valinomycin ion transport mechanism in model biological membranes.

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Towards Raman spectroscopy- based high-content profiling of endothelial cytotoxicity

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Raman imaging, fluorescence, cytotoxicity, endothelium, drug-cell interactions

Adverse effects of various drugs on the vascular endothelium are related to several mechanisms of cytotoxicity. The endothelium is the layer of cells that lines the blood vessels and its condition determines the homeostasis of the cardiovascular system. Pharmacological treatment with various drugs may contribute to endothelial dysfunction, which in turn may lead to the formation or progression of cardiovascular diseases. Endothelial toxicity was not shown for a number of drugs until the introduction stage. In fact, many clinically-used drugs induced hypertension or cardiovascular side effects, including the heart failure that in some cases might be also linked to endothelial toxicity, as in the case of doxorubicin (1, 2). Lack of knowledge on endothelial mechanisms that are responsible for their actions, makes it difficult to reduce these side effects of patients' pharmacotherapy. Therefore, the better profiling of drugs at the stage of preclinical research requires the development of new methodologies that could quickly and unambiguously demonstrate whether or not a given drug or drug candidate, has toxic effects on the endothelium and which mechanisms are involved. In the present study, we aimed to identify a unique Raman signatures of given mechanisms of endothelial cytotoxicity, whereas fluorescence evaluation or analysis of biochemical changes were used to validate our findings as regards the presence of selected mechanisms of cytotoxicity in cultured vascular endothelial cells. Therefore, we analyzed the pharmacological response of endothelial cells to selected drugs or chemical compounds, known to induce cytotoxicity based on mechanisms related to oxidative stress (3), inflammation, endoplasmic reticulum stress (4) or phospholipidosis (5). Spectroscopic markers defined based on listed above in vitro model systems concerned inter alia a decreased intensity of marker bands for DNA, Phe and cytochrome, increased content of unsaturated lipids, changes in secondary structure of proteins observed in amide III region, and increased content of choline-containing lipids in perinuclear area, respectively. In turn, the spectroscopic signature of drug-induced mechanism of DNA damage was manifested itself not only by an overall decreased signal of DNA but also changes in DNA conformations were observed. Our results will provide the development of a diagnostic panel that will enable quick identification of these mechanisms, in the case of studies on drugs and substances of unknown action that show signs of endothelial cytotoxicity.

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[1] T. Wojcik et al., Comparative endothelial profiling of doxorubicin and daunorubicin in cultured endothelial cells Toxicology In Vitro, 2015, 29(3), 512-521 [2] T. Wojcik et al., Detrimental effects of chemotherapeutics and other drugs on the endothelium: A call for endothelial toxicity profiling. Pharmacol Rep 2015;67(4):811-7. [3] E.Bik et al., Menadione-induced endothelial inflammation detected by Raman spectroscopy, BBA - Molecular Cell Research, 2021, 1868(2), [4] E. Bik et al., Tunicamycin induced endoplasmatic reticulum changes in endothelial cells investigated in vitro by confocal Raman imaging, Analyst, 2019, 144, 6561-6569, [5] E. Bik et al., Chloroquine-induced accumulation of autophagosomes and lipids in the endothelium, International Journal of Molecular Sciences, 2021, 22(5), 2401.

The fate of targeted SERS probes: Will they make it to the nucleus?

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surface-enhanced Raman scattering, gold nanoparticles, nuclear targeting, nuclear localization sequence, bioanalytics

Understanding how nanomaterials can cross intracellular barriers is of high interest in nanomedicine both for drug delivery and diagnostics. Nanoparticles modified with specific peptides have been observed to cross intracellular barriers, specifically the membrane of endolysosomal vesicles and the nucleus.[1] The composition of the biomolecule corona surrounding the nanoparticles determines their path within the cell.[2] In this study, gold nanoparticles were modified with different nuclear localization sequences (NLS) to be applied as probes for surface-enhanced Raman scattering (SERS) in order to better understand the effect of the NLS on the fate of the nanoparticles in the cell. Furthermore, ultrastructural methods are utilized to study the intracellular path of the particlesfrom uptake, over endosomal escape to nuclear translocation. The size and 3D morphology of nanoparticle aggregates must be characterized to understand these dynamic endo/lysosomal processes and the interaction with the biological matrix.[3] Transmission electron micrographs show that nuclear localization strongly depends on the selected incubation conditions. NLS-functionalized particles are processed differently in endosomes compared to unmodified particlesand show an enhanced particle uptake as evidenced by three-dimensional X-ray tomography as well as by quantification using laser-ablation inductively-coupled plasma mass spectrometry. SERS gives molecular information about the immediate biochemical surroundings of the NLS-functionalized;nanoparticles. The spectra show specific spectral features for particles in endosomes, in the cytosol or in the nucleus.For samples where endosomal escape was observed by the ultrastructural data, the SERS spectra show bands assigned to vibrations of nucleic acids of the nuclear matrix and thus clearly differ from those with unmodified gold nanoparticles. Our findings have implications for understanding interactions at the nano-bio interface and the application of nuclear targeting nanostructures in;medical theranostics, biotechnology and bioanalytics.

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[1] Feldherr, C.; Lanford, R.; Akin, D., Proc. Natl. Acad. Sci. U.S.A. 1992, 89, 11002. [2] Szekeres, G. P.; Werner, S.; Guttmann, P.; Spedalieri, C.; Drescher, D.; Živanović, V.; Montes-Bayón, M.; Bettmer, J.; Kneipp, J., Nanoscale 2020, 12 (33), 17450-17461. [3] Drescher, D.; Büchner, T.; Guttmann, P.; Werner, S.; Schneider, G.; Kneipp, J., Nanoscale Adv. 2019, 1 (8), 2937-2945.

Monitoring of structural changes in the corneal layers caused by transcorneal transfer of cannabidiol formulations

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Cornea, Transcorneal drug delivery, Effect of CBD and transcorneal enhancers, Vibrational spectroscopy and microscopy techniques, Multivariate statistical methods

The cornea, consisting of five layers, occupies about 20% of the eyeball's s surface. Its unique structure together with proteins and enzymes in tears creates a natural barrier responsible for the low permeation of lipo- or hydrophilic drugs. Hence, the increase of ophthalmic drug bioavailability becomes increasingly important. A description of the micro? and nano-level of the phenomena occurring during the transcorneal permeation is necessary for drug delivery improvements. At this time, preparations with cannabinoids help to treat minor injuries, eczema, chronic pain, or epilepsy. However, due to their positive effect on the human body, they could be used to treat glaucoma (increased intraocular pressure caused by the accumulation of aqueous humor in the eye) in the future. In this study, we focused on characterizing corneal epithelium (CEP) and endothelium (CEN) layers and monitoring their structural changes caused by the permeation of specific transcorneal enhancers with the addition of cannabidiol (CBD) used as a model cannabinoid. We used pig corneas instead of human corneas because of their biological similarity. The freshly isolated and disinfected corneas were attached to the chambers suitable for transcorneal transport filled with the balanced salt solution (BSS) tempered at 34°C. The corneas were treated by formulations with CBD (2.5% w/w) for 24 hours. The topography of CEP and CEN layers was studied by Atomic Force Microscopy. The effect of CBD on the CEP and CEN layers was observed by the Attenuated Total Reflection technique and FT-Raman microscopy mapping (excitation wavelength at 1064 nm) using multivariate statistical methods namely Principal Component Analysis, Partial Least Square Regression, and Linear Discriminant Analysis. The depth profiling spectral measurement by confocal Raman microscope (excitation wavelength at 785 nm) brought information about the distribution of CBD in the corneal layers. Moreover, by analysis of BSS using ultra-high-performance liquid chromatography with UV and MS detectors (UHPLC?UV/MS) has been shown, that CBD was permeated through the corneas in the order of tens of ng/mL. Combining the classical vibrational spectroscopy and microscopy techniques, we obtained a detailed description of the phenomena occurring during transcorneal transport that can lead to the preparation of more suitable formulations (drops, ointments) for the treatment of ocular diseases.

TN01000048 (National Center of Competence) and Eye Tissue Bank-Royal Vinohrady University Hospital

Spectroscopy–based assessment of drug induced phospholipidosis in endothelium

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phospholipidosis, Raman imaging, endothelium

Phospholipidosis can be defined as a lysosomal storage disorder which leads to abnormal accumulation of lipids and lamellar bodies formation which can be result of cationic amphiphilic drugs (CADs) administration. Phospholipidosis in endothelium can be triggered by CADs of various therapeutics actions, e.g. antipsychotic, antidepressant, antiarrhythmic and antimalarial [1]. It is already known that CADs may exhibit side effects on endothelium leading to an increased cardiovascular risk [2]. Raman imaging is a method of choice to study the biochemical composition at subcellular level when expected changes are lipid-related. It enables the analysis of both lipids' content and composition, their distribution, and at the same time the drugs (phospholipidosis-inducers) accumulation in the cells. This method is sensitive to an identification of various lipidic groups and allows for recognition of main cell' organelles based on their unique spectroscopic "signatures" [3]. Raman and fluorescence microscopies were used to monitor the process of lipids formation, their distribution and other biochemical alterations (i.e. autophagosomes formation) caused by selected drugs belonging to CADs group such as fluoxetine, clozapine, chloroquine and amiodarone in HMEC-1 (human microvascular endothelial cell-1) cell line. In this work, we propose comprehensive methodologies combining vibrational spectroscopy and fluorescent staining together to better understand and detect phospholipidosis and therefore explain the increased cardiovascular risk in e.g. psychiatric patients treated with antipsychotic or antidepressive drugs belonging to CADs group [4].

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[1] Anderson N, Borlak J, FEBS Letters (2006); 580; 5533–5540. [2] Wu SZ, Liang X, Geng J, Zhang MB, Xie N, Su XY, World Journal of Clinical Cases (2019); 24, 4377–4383. [3] Majzner K, Chlopicki S, Baranska M, Journal of Biophotonics (2016); 9, 396–405. [4] Ungvari Z, Tarantini S, Yabluchanskiy A, Csiszar A, Frontiers in Genetics (2019); 10, 898–905.

Surface characterization of Vitamin A-functionalized polymer-based nanoparticles with atomic force microscopy and surface-enhanced Raman spectroscopy

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Polymer-based nanoparticle, AFM, SERS, Drug delivery

Gene carriers are of high interest in medical applications and block copolymers have been identified as suitable building blocks to encapsulate the genetic material. Here, P(MMA-stat-DMAEMA)-b-P(PEGMA-stat-PEGMA-RA) is formulated into nanoparticles (NPs) and the incorporated Retinoic acid (RA) acts as a targeting ligand. The formulated Vitamin A-functionalized NPs are characterized by surface enhanced Raman spectroscopy (SERS) and in addition, Atomic force microscopy (AFM), primarily with the aim to demonstrate that the RA is located in the PPEGMA shell region, and thus, enables NP interaction with the targeted cells. In particular, SERS was used to provide chemical information. Due to its surface sensitivity, SERS enables particularly detecting RA in areas of the polymer nanoparticle close to the surface. AFM investigations confirmed the spherical shapes of these NPs as well as the diameter of about 100 nm, which is smaller than examined by dynamic light scattering because of drying artifacts. Therefore, SERS experiments were carried out in an aqueous environment. A comparison of Raman spectra of NP, RA-NP and RA revealed that bands at 1575 cm-1, 1198 cm-1, 1162 cm-1 and 1000 cm-1 could only be detected for the NP with retinoyl groups using the same excitation power. In conclusion, the SERS investigations demonstrated the presence of RA in the RA positive samples. Furthermore, the comparison with pure RA demonstrated that RA is covalently bound to the block copolymer. Considering the pre-resonance Raman (PRR) effect of the retinoyl groups result in a depth information of 2 to 8 nm for the Raman measurements.[1] Consequently, the signatures of the RA detected in the SERS experiments confirm that retinoyl functionalities are located in the outer PPEGMA shell of the block copolymer NP, and hence, potentially provide the desired drug activity.

[1] R. Bohme, M. Mkandawire, U. Krause-Buchholz, P. Rosch, G. Rodel, J. Popp, V. Deckert, Characterizing cytochrome c states--TERS studies of whole mitochondria, Chem Commun (Camb), 47 (2011) 11453-11455.

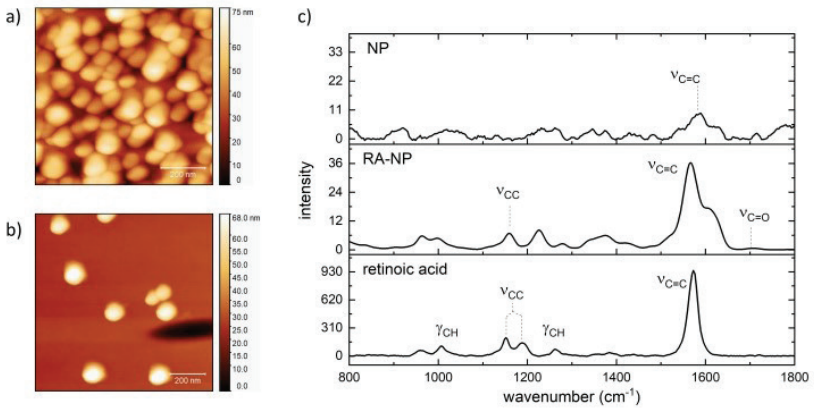

Figure 1. Topography of a) RA-NP and b)diluted RA-NP, c) SERS spectra of NP, RA-NP and RA

Combining Raman imaging and MCR-ALS analysis for monitoring retinol permeation in human skin

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Transcutaneous permeation, Raman imaging, MCR-ALS analysis, Human skin, Retinol

Many exogenous molecules penetrate the skin barrier poorly and require optimized formulations to ensure their efficiency. Monitoring the penetration of these molecules through skin layers using conventional techniques requires usually sample labelling or long pre-analytical preparation. However, Raman spectroscopy is a label-free and non-destructive method that gives access to molecular information and spatial distribution of tracked actives in skin. The aim of our study was to prove the interest of Raman imaging coupled with Multivariate Curve Resolution Alternating Least Square (MCR-ALS) analysis in monitoring retinol penetration into frozen and living excised human skin. After topical treatment of skin samples by free or encapsulated retinol, Raman images were recorded on thin transverse sections. MCR-ALS method was then applied for image processing and heat maps were constructed in order to compare the retinol permeation and distribution through skin layers. In a first time, our results allowed the identification of specific spectroscopic markers that are relevant for retinol monitoring in skin layers. Then, by matching these markers and MCR-ALS estimated spectra, we have shown retinol transepidermal permeation. The encapsulated form of retinol has shown more efficient penetration in the skin than the free form. Finally, retinol distribution in skin sections was illustrated and showed a storage reservoir in the Stratum Corneum. These results were validated on both human skin models used in this study. This study shows a proof of concept for the evaluation of retinol penetration in skin using Raman imaging coupled with MCR-ALS. This concept needs to be evaluated on more subjects to include inter-individual variability but also other factors affecting skin permeation (age, sex, pH, "..." po pH, ...). Our study can be extended to other actives.

Ritonavir induced cytotoxicity of human endothelial cells revealed by Raman imaging

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Raman imaging, fluorescence, endothelial cells, ritonavir, chemometric methods

Ritonavir (RIV) is a HIV protease inhibitor widely used in antiviral therapy of HIV-infected patients. The main mechanism of its antiretroviral properties is based on protease inhibition, what next impairs virus replication cycle. RIV is also considered for the treatment of several types of cancer[1]. Despite its effectiveness of RIV in antiviral therapy many metabolic side effects and premature cardiovascular diseases associated with this therapy were reported. The mechanisms of these complications are not clear and fully understood. In this study, we investigated in vitro the effect of RIV (1, 5, 10, 25 and 50 μM) on human endothelial cell culture (HMEC-1). By using viability test (MTT) and fluorescence staining, HMEC-1 cells treated for 24h with RIV at 25 and 50 μM concentrations showed a significant decrease in cell viability and an increased cytotoxicity in a dose-dependent fashion. However, Raman imaging results revealed a decreased lipid unsaturation in perinuclear area and nuclear DNA damage together with mitochondrial cytochrome and DNA impairment. Observed changes were dose dependent and detected in all studied RIV-treated cells. In contrast, for 1-10 μM RIV a very limited effect on endothelial dysfunction and apoptosis, as assessed by MTT and number of cell analyses, was observed. Multivariate statistical methods such as principal component analysis (PCA) and partial least squares (PLS) were used to show that the Raman spectra collected from RIV-treated cells exhibit information about biochemical changes associated with the RIV concentration. Endothelial mitochondrial DNA damage caused by RIV is a known effect[2], however our results indicate that RIV at concentrations close to clinical plasma levels can cause endothelial dysfunction and cell impairs via a few possible active cytotoxic mechanisms such as mitochondrial dysfunction, DNA damage and oxidative stress. All together can be associated with HIV protease inhibitor–mediated endothelial injury and can lead to cardiovascular complications. The use of cell component analyses reflecting changes in mitochondrial, nuclear and cytoplasm manifested by nucleic acid, protein and lipid signals provide the basis for further studies to better understand endothelial cytotoxicity mechanisms induced by RIV.

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[1] Eatemadi A. et al., Role of protease and protease inhibitors in cancer pathogenesis and treatment. Biomed Pharmacother. 2017;221–231.
[2] Zhong DS et al., HIV protease inhibitor ritonavir induces cytotoxicity of human endothelial cells. Arterioscler Thromb Vasc Biol. 2002 Oct 1;22(10):1560-6

2.4. Life Sciences, Environmental Sci. and Geoscience

Metabolic raman imaging in cancer research

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redox state of cytochrome c, optical biopsy, Raman spectroscopy and imaging, brain and breast cancer, cytochrome c

To monitor redox state changes and biological mechanisms occurring in mitochondrial cytochromes in cancers novel methods are required. We used Raman spectroscopy and Raman imaging to monitor changes in the redox state of the mitochondrial cytochromes in ex vivo human brain and breast tissues at 532 nm, 633 nm, 785 nm. We identified the oncogenic processes that characterize human infiltrating ductal carcinoma (IDC) and human brain tumors: gliomas; astrocytoma and medulloblastoma based on the quantification of cytochrome redox status by exploiting the resonance – enhancement effect of Raman scattering. We visualized localization of cytochromes by Raman imaging in the breast and brain tissues and analyzed cytochrome c vibrations at 750, 1126, 1337 and 1584 cm-1 as a function of malignancy grade. We found that the concentration of reduced cytochrome c becomes abnormally high in human brain tumors and breast cancers and correlates with the grade of cancer. We showed that Raman imaging provides insight into the biology of brain gliomas and breast ductal invasive cancer, which can be used for noninvasive grading, and diagnosis.

This work was supported by the National Science Centre of Poland (Narodowe Centrum Nauki, UMO-2019/33/B/ST4/01961).

[1] Abramczyk, H.; kopeć M. Brozek-Pluska, B., Surmacki, J. Błaszczyk M., Radek M., Cancers 2021, 13(5), 960; [2] Abramczyk, H.; Brozek-Pluska, B.; Jarota, A.; Surmacki, J.; Imiela, A.; Kopec, M. A look into the use of Raman spectroscopy for brain and breast cancer diagnostics: linear and non-linear optics in cancer research as a gateway to tumor cell identity. Expert Rev. Mol. Diagn. 2020, 20, 99-115. [3] Abramczyk, H.; Imiela, A.; Brozek-Pluska, B.; Kopec, M. Advances in Raman imaging combined with AFM and fluorescence microscopy are beneficial for oncology and cancer research. Nanomed. 2019, 14, 1873–1888. [4] Kopec, M.; Imiela, A.; Abramczyk, H. Monitoring glycosylation metabolism in brain and breast cancer by Raman imaging. Sci. Rep. 2019, 9, 1-13. [5] Abramczyk, H.; Imiela, A.; Śliwińska, A. Novel strategies of Raman imaging for exploring cancer lipid reprogramming. J. Mol. Liq. 2019, 274, 52–59. [6] Polis, B.; Imiela, A.; Polis, L.; Abramczyk, H. Raman spectroscopy for medulloblastoma. Childs Nerv. Syst. J. Int. Soc. Pediatr. Neurosurg. 2018, 34, 2425–2430. [7] Abramczyk, H.; Imiela, A. The biochemical, nano-mechanical and chemometric signatures of brain cancer. Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 2018, 188, 8–19. [8] Imiela, A.; Polis, B.; Polis, L.; Abramczyk, H. Novel strategies of Raman imaging for brain tumor research. Oncotarget 2017, 8, 85290–85310

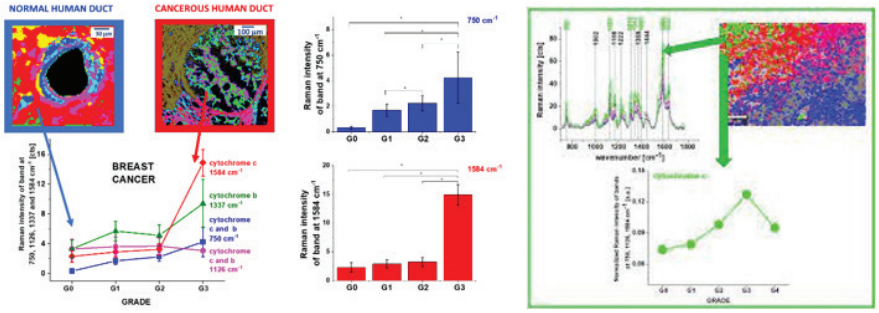

Figure 1. Raman intensities of the bands at 750, 1126, 1337 and 1584 cm-1 as a function of grade for (A) breast normal (G0) and cancer (invasive ductal cancer) human tissue (G1, G2, G3) (average +/- SD, number of patients n=39), (B) brain normal (G0) and brain tumor human tissue (G1, G2, G3, G4) (average +/- SD, number of patients n=42)

FTIR for quantitative analysis of bioactive substances in food products

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mid-infrared, antioxidants, bioactive, food

Determination of product quality and authenticity are major issues for the food industry. Given the inherent complexity of food products, most instrumental techniques employed for such analyses (i.e., chromatographic methods) are time demanding, expensive and labor intensive. Therefore, there has been an increasing interest in simpler and faster analytical methods for the evaluation of food products, with emphasis on spectroscopic techniques. These methods have been extensively employed recently because they require minimal or no sample preparation, provide rapid and on-line analysis, and are non-destructive (Franca & Nollet, 2017). Among evaluated food quality parameters, fiber content, antioxidant potential and concentration of bioactive substances are of particular interest due to well established health benefits associated with these compounds. Thus, in this study, we present an overview of the application of Fourier Transform Infrared – FTIR, for the evaluation and quantification of food quality attributes. Evaluated food products include chocolates and exotic fruit peel flours. In general, chemometric models based on FTIR data were able to provide satisfactory predictions (R2 over 0.9, low RMSEP and RMSEC values) of several parameters including antioxidant activity, soluble and insoluble fiber content, amines and phenolics (cyanidin-3-O-glucoside, ellagic acid and others).

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[1] A.S. Franca, L. Nollet Spectroscopic methods in food analysis (1st ed.), CRC Press, Boca Raton (2017).

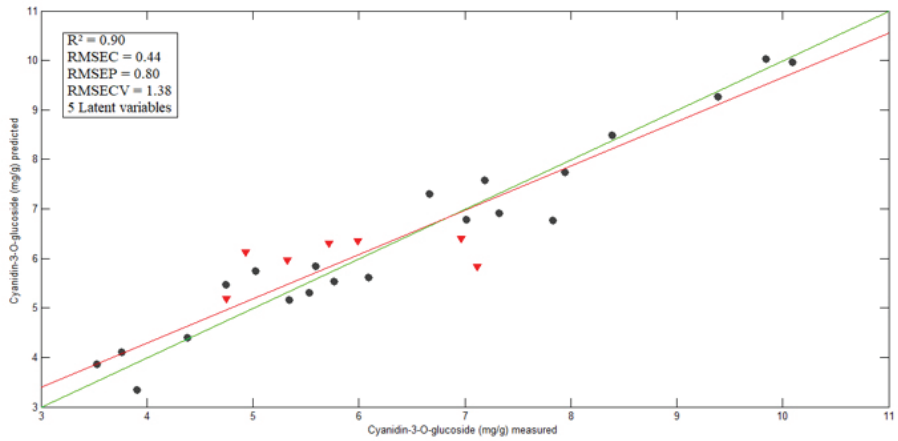

Figure 1. Predicted (FTIR) vs. measured (HPLC_ values of cyanidin-3-O-glucoside concentration in jabuticaba peel flours.

Anthropogenic effects on the Carbon biogeochemical cycle studied at the SISSI-Bio Beamline @ Elettra

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FTIR, Ecology, Microplastics, Cigarettes, Bioremediation

In the last century, the anthropogenic effects on the main biogeochemical cycles have massively increased. From the atmosphere, to the ground and the sea, the effects of human activities had left traces that will be visible for ages. These phenomena have been thoroughly reported and studied and vibrational spectroscopies can help shine some light on them. In the last few years, at the SISSI-Bio Beamline at Elettra Synchrotron light source, the collaborations and the users proposing experiments related to the environmental sciences grew substantially. In this contribution, I will present the recent activities and experiments carried out, that aimed at understanding both the damage and some remediation processes that the modern civilization done, and it is doing, to the environment. As a leitmotif, I will use the carbon cycle and how specific steps in it can be hindered or facilitated. I will present some results testifying how plastic pollution can reach the furthest regions of the globe, like Antarctica,(1) or how cigarette filters can harm foraminifera in the Mediterranean Sea,(2) and the repercussions on their respective ecosystems. Then I will focus more on remediation processes that can be developed to clean up the contaminated areas, and on the development of green energy sources or the increase of the carbon storage,(3,4) exploiting bio-sequestration processes. Infrared spectroscopy and imaging demonstrated to be key tools to provide a full chemical characterization of the samples, being the issue the identification of foreign particles within organism or the determination of the effect of these pollutants to the animal or cell. They also allowed following biochemical processes within single cells and bacterial colonies without the need of any label, monitoring the spectral variations happening upon changing the environmental conditions. The aim of the results presented in this contribution is to show that damage to even the smallest organism, like, for example, the pollution of in invertebrate in Antarctica, can have consequences on the bigger cycle, and make the whole system to fall in pieces.

[1] Bergami E, Rota E, Caruso T, Birarda G, Vaccari L, Corsi I. Plastics everywhere: first evidence of polystyrene fragments inside the common Antarctic collembolan Cryptopygus antarcticus: Microplastics in Antarctic collembola. Biol Lett. 2020;16(6). [2] Caridi F, Sabbatini A, Birarda G, Costanzi E, Giudici G De, Galeazzi R, et al. Cigarette butts, a threat for marine environments?: Lessons from benthic foraminifera (Protista). Mar Environ Res [Internet]. 2020;162(May):105150. Available from: https://doi.org/10.1016/j.marenvres.2020.105150 [3] Medas D, De Giudici G, Pusceddu C, Casu MA, Birarda G, Vaccari L, et al. Impact of Zn excess on biomineralization processes in Juncus acutus grown in mine polluted sites. J Hazard Mater. 2017; [4] Fanesi A, Wagner H, Birarda G, Vaccari L, Wilhelm C. Quantitative macromolecular patterns in phyto-plankton communities resolved at the taxonomical level by single-cell Synchrotron FTIR-spectroscopy. 2019;1–14.

532 nm–excited hyper-Raman spectroscopy of amino acids and bovine serum albumin

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hyper-Raman spectroscopy, biomolecules, protein, amino acid, resonance effect

Hyper-Raman (HR) spectroscopy reveals unique information about molecular structures, because of its different selection rule, compared with that of the infrared (IR) and Raman spectroscopy. This paper reports an application of HR spectroscopy to amino acids. The purpose is to obtain basic information on the spectral features for future application to the proteins. In the HR spectra of the amino acids, the bands assignable to the vibrations of the COO^- group were observed commonly. While the peak position of the other HR bands is mostly identical between the HR and Raman spectra, the intensity pattern is not. We discuss the similarities and dissimilarities between the two spectra of each amino acid and give possible assignments to each HR band by comparing it with those for the corresponding Raman band. Also, the HR spectroscopy is applied to a globular protein, bovine serum albumin (BSA). It is shown that the HR spectroscopy brings the information of the aromatic amino acids, namely Trp and Tyr. The selective detection of these groups is explained in terms of the resonance effect of the excitation beam (532 nm) to the electronic absorption around 266 nm (L^a of Trp, L_b of Tyr). The assignment of the HR bands is given by analogy with the 266 nm-excited UV resonance Raman spectra of these groups.

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[1] 532 nm-excited Hyper-Raman spectroscopy of globular protein and aromatic amino acids, Wen, Hiramatsu, J. Raman Spectrosc., 2020, 51, 274-278. [2] 532 nm–excited hyper-Raman spectroscopy of amino acids, Wen, Yu, Thirumalaivasan, Hiramatsu, J. Raman Spectroscopy, 2021, 52, 641-654.

Breakthrough Approach of QCL IRM Hyperspectral Imaging and 2D-COS of mAbs in Solution for Developability Assessment

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Therapeutic protein, CQA's, developability, comparability, ProteinMentor platform technology

Real-time Hyperspectral images obtained using a state-of-the-art Quantum Cascade Laser transmission microscope has proven useful for comparative analysis of an array of monoclonal full-length antibodies under thermal stress. 2D-COS analysis allowed for the determination of aggregation, deamidation and stability proteins under thermal stress. Quantum Cascade Lasers (QCL) enable enhanced SNR ensuring the quality of the protein spectra independent of size or post-translational modification under a range of concentrations. Deamidation is a phenomenon that occurs spontaneously in proteins, while in solution, when subject to heat stress, low or high pH conditions. The mechanism of deamidation is known. More importantly, the introduction of the new charge in the protein sequence due to a deamidation event can lead to reduced efficacy and safety due to loss of target affinity and/or specificity, decreased stability, aggregation and at times even truncation of the therapeutic protein. Our approach provides unprecedented molecular information crucial towards developability assessment. We have evaluated several clinical monoclonal antibodies under varying conditions and compared the results of our platform technology with other analytical tools to explore the potential for orthogonality. The samples were generously supplied by: Liz Culyba from Verseau Therapeutics. She provided clinical samples that were generated using the INN sequence using IgG4 as the scaffold. Our motivation is to help ensure safety and efficacy of therapeutic proteins for patients.

Resonance Raman studies of a non-canonical heme oxygenase from Mycobacterium tuberculosis

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resonance Raman, MhuD, Mycobacterium tuberculosis, heme oxygenase

Mycobacterium heme utilization degrader (MhuD) is a non-canonical heme oxygenase enzyme that plays a crucial role in the heme uptake pathway in Mycobacterium tuberculosis [1]. MhuD differs from canonical heme oxygenase enzymes by its heme degradation products, releasing mycobilins and ferrous iron without generating carbon monoxide [2]. Interestingly, MhuD can bind one or two heme molecules in the same active site. Monoheme MhuD is enzymatically active, and diheme was proposed to be an inactive form. Gaining insight into the structure-function correlation of MhuD can enable the exploitation of its differences from that of canonical heme oxygenase enzymes in the development of new antitubercular drugs and treatment strategies [3]. The aim of this work is to use resonance Raman (rR) spectroscopy to determine differences in the active site environment between mono- and diheme MhuD. Resonance Raman spectroscopy can provide information about oxidation, spin, and coordination states of heme iron, as well as the disposition of heme peripheral groups and deformations of the heme macrocycle [4]. Most importantly, it can also effectively monitor the strength of the linkage between heme iron and endogenous and exogenous ligands, including the elusive reactive intermediates [4]. The heme pocket environment and structural features of MhuD samples were probed by resonance Raman spectroscopy in the ferric, ferrous states, and ligated forms. Interestingly, our data show that the presence of the second heme in the heme pocket does not affect the structure of the His-ligated heme. Furthermore, our studies involving isotopically labeled hemes show that the binding of the second heme molecule caused the displacement of the originally His-bound heme into heme moiety, followed by the binding of the new incoming heme to the proximal His residue. Finally, our spectroscopic data, in conjunction with the enzymatic assays, clearly indicate that both mono- and diheme MhuD are enzymatically active.

[1] Chim, N.; Iniguez, A.; Nguyen, T. Q.; Goulding, C. W. J. Mol. Biol. 2010, 395, 595-609. [2] Wilks, A.; Heinzl, G. Arch. Biochem. Biophys. 2014, 544, 87-95. [3] McLean, K. J.; Munro, A. W. Drug Discovery Today 2017, 22, 566-575. [4] Spiro, T. G.; Soldatova, A. V.; Balakrishnan, G. Coord. Chem. Rev. 2013, 257, 511-527.

Quantitative and qualitative characterization of extracellular vesicles by IR spectroscopy

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extracellular vesicles, protein-to-lipid ratio, protein quantification, lipid quantification, ATR-IR

Background: Extracellular vesicles (EVs) are in the focus of scientific interest: it was recognized that cells emit nano- and micro-sized structures bounded by a phospholipid bilayer, which play a significant role in intercellular communication between cells. Infrared (IR) spectroscopy, completed with standardized measurement conditions and data processing procedures, was recently introduced to characterize EVs. Aim: Since IR spectroscopy provides information about proteins and lipids and/or other EV components simultaneously, a single assay quantification protocol for both proteins and lipids (phospholipids) might be feasible. Methods: EVs (microvesicles) formed isolated from erythrocyte and platelet concentrates were examined by IR spectroscopy. The used ATR FT-IR technique requires small sample amount (~3µl) without any sample preparation and enables the measurement of aqueous suspensions with low concentration. Results: The integrated area of the amide I band proved to be proportional to the protein quantity in the EV samples (up to 1 mg/ml), regardless of its secondary structure. Our results based on a calibration with bovine serum albumin was further affirmed also by multivariate modelling on raw spectra using Partial Least Squares regression1. Lipids are essential molecular components of EVs, but at the moment only limited knowledge about their quantification is available2. To extend the possibilities of IR spectroscopy, an effort has been made to elaborate an adequate lipid calibration, by using reference vesicles of bovine serum albumin and synthetic lipids. Conclusion: A fast, label-free method based on the area of selected IR bands might gain an important role in EV research. Compact ATR-FTIR instruments are already available and the proposed spectral analysis protocols can be readily automated.

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[1] Szentirmai, V.; Wacha, A.; Németh, C.; Kitka, D.; Rácz, A.; Héberger, K.; Mihály, J.; Varga, Z. Reagent-Free Total Protein Quantification of Intact Extracellular Vesicles by Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy. Anal Bioanal Chem 2020, 412 (19), 4619–4628. https://doi.org/10.1007/s00216-020-02711-8. [2] Visnovitz, T.; Osteikoetxea, X.; Sódar, B. W.; Mihály, J.; Lőrincz, P.; Vukman, K. V.; Tóth, E. Á.; Koncz, A.; Székács, I.; Horváth, R.; Varga, Z.; Buzás, E. I. An Improved 96 Well Plate Format Lipid Quantification Assay for Standardisation of Experiments with Extracellular Vesicles. Journal of Extracellular Vesicles 2019, 8 (1), 1565263. https://doi.org/10.1080/20013078.2019.1565263.

An insight into murine primary adipocytes and matured preadipocytes studied with Raman imaging

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primary adipocytes, matured preadipocytes, adipose tissue, Raman imaging, lipids

Today, obesity and other diseases causally linked with the development of cardiovascular disorders i.e. atherosclerosis, have become global problems. Besides preventing them by promoting a healthy lifestyle through the benefits of a balanced diet and regular exercises, one of the best ways for scientists to combat adipose tissue-related diseases is to better understand adipose tissue (AT) physiology. A growing interest in the role of AT under physiological conditions and upon disease development has led to increasing demand for its representative in vitro models, in particular, suitable for suitable for mechanism studies and drug testing. Both, energy-storing white adipocytes and thermogenic brown and beige adipocytes secrete a variety of effectors, including lipids, exosomes, inflammatory cytokines, and peptide hormones that act in both paracrine and endocrine capacities to impact local and systemic metabolic responses. Besides adipocytes, AT is comprised of inter alia endothelial cells, blood cells, fibroblasts, preadipocytes, and several types of immune cells. These non-adipocyte cell types are commonly referred to as the AT stromal vascular fraction (SVF) [1]. Under the complex treatment, the fraction of SVF cells differentiates to a mature adipocyte cells [2]. Due to significant Raman cross-section for lipids, Raman spectroscopy is a perfect tool to study AT. [3,4] In this work, a Raman imaging system (WITec Alpha, Ulm, Germany) with a 532 nm excitation was used to study isolated murine primary adipocytes and differentiated SVF cells derived from epididymal (eWAT) and interscapular (iBAT) adipose tissue of C57Bl/6 mice. The work aimed to compare phenotype of both types of cells and their response to inflammatory/protective factors i.e. TNF-α fatty acids, and carotenoids. The results show, among others, tremendous differences in morphology and lipid distribution in primary and mature SVF-derived adipocytes (Fig. 1). Moreover, the comparison of Raman spectra of both types of cells revealed some differences manifested by i.e. variable level of lipid unsaturation, that correspond to the type of AT, and for SVF cells changes during maturation. Our work demonstrates the applicability of Raman spectroscopy to evaluate and determine the alterations in in vitro models of adipocytes that may serve as a future methodology to test the effect of i.e. fat-burning drugs in obesity-reducing therapies.

National Science Centre Poland (NCN, OPUS17, 2019/33/B/ST4/0087).

[1] A. Chait et al. Front. Cardiovasc. Med. 2020, 7, 22. [2] K. Kwiecien et al. Sci Rep. 2020, 10, 13702. [3] K. Czamara et al. Int. J. Mol. Sci. 2020, 21, 4838 [4] A. Bar et al. J Am Heart Assoc. 2020, 9, e016929.

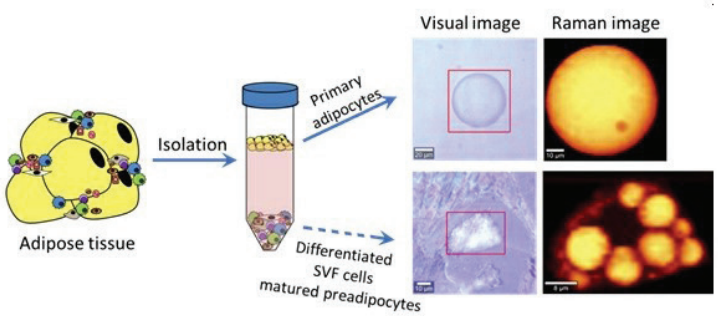

Figure 1. Schematic diagram of AT cells preparation and measurement with use of Raman imaging.

Rac1 regulates biochemical, nanomechanical and nanostructural aspects of TNF-?-induced inflammatory response in vascular endothelium in aorta

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endothelium, lipid droplets, Raman imaging, atomic force microscopy, inflammation

Endothelial inflammation is recognized as a critical condition in the development of cardiovascular disorders. Changes in the biochemical (formation of lipid droplets determined Raman and fluorescence imaging), nanomechanical (augmented cortical stiffness determined by using AFM) and nanostructural properties (AFM and SEM imaging) of TNF-α-activated-activated endothelial cells (ECs) in the isolated blood vessels were ascribed to the development of vascular inflammation, as evidenced by overexpression by ICAM-1, but the mechanical insight linking these parameters to each other was missing. Using the combination of techniques: Raman spectroscopy, fluorescence imaging, atomic force (AFM) and scanning electron microscopy (SEM) we demonstrated that formation of lipid droplets (LDs), polymerization of F-actin, alterations in cortical stiffness and nanostructural properties were Rac-1-dependant, and partially reversible by the inhibition of Rac-1. Raman imaging uncovered that the reservoir of LDs included mainly LDs rich in highly unsaturated lipids and negligible content of cholesterols and phospholipids. Furthermore, it was possible to distinguish LDs localized in endothelium and smooth muscle cells (SMCs). In conclusion, this work demonstrated that Rac-1 activation is the mechanism crosslinking biochemical, nanomechanical and nanostructural alterations of TNF-induced endothelial cell inflammation. In particular, we revealed a significant role of Rac-1 in the regulation of the formation of high-unsaturated LDs. Our results suggest Rac-1 as a central pathway in the regulation of biochemical, nanomechanical and nanostructural aspects of vascular inflammation. This work was supported by the National Science Centre, Poland, SONATINA1 No.: DEC-2017/24/C/ST4/00075.

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From single neoplastic cells to advanced cancer: detection and characterization of pulmonary metastasis of breast cancer by FTIR spectral histopathology

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FT-IR spectroscopy, IR imaging, Pulmonary metastasis of breast cancer, Mouse model, Chemometrics

Breast cancer is the most common neoplastic disease in women. Surprisingly, the most frequent cause of death is not the development of primary tumor itself, but metastasis resulting from it. However, the recognition at an early stage is still challenging due to the small size of the lesions or the presence of single neoplastic cells. Despite the advances in technology, commonly used imaging techniques not always are able to visualize the early, subtle changes induced by the neoplastic process. Fourier transform infrared (FTIR) spectroscopy is a technique which gives not only information about the morphology of the tissue, but also provides insight into the chemical composition of the studied samples reflecting their overall biochemical profile. Moreover, combined with the imaging system, allows for display the spatial distribution of macromolecules even at the subcellular level. Therefore, this technique is called spectral histopathology (SHP) and can be used as a diagnostic tool. Cancer cells which metastasize from primary site and circulates in the blood already lead to changes in the structure and organization of proteins as in other molecules through the cell signaling. Therefore, it is important to search for sensitive techniques which allows to identify the early stages of neoplastic progression, which may help in the fastest and more efficient diagnosis in the future. The aspect of metastasis was studied with the use of 4T1 orthotropic mice model. It very well reconstructs breast cancer and its metastasis to lungs, where metastases are naturally formed. This allows to investigate stages of the metastasis process form pre metastatic niche to advance tumor. The undertaken investigation allowed for the characterization of developed metastases in lung tissue, showed the potential in the detection of early neoplastic changes and presented their biochemical diversity.[1] In addition, it was possible to define changes in the lung parenchyma that occur before the colonization of the first cancer cells as well as changes in protein reorientation, which cannot be defined by classical histological techniques which currently are the gold standard in the assessment of neoplastic changes.[2,3] FTIR imaging is an attractive and innovative alternative compared to conventional methods currently used in oncological diagnostics. Moreover, it is a promising tool for exploring the issues related to the development of the premetastatic niche and for better understanding the mechanism of the metastatic process.

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[1] Chrabaszcz K et al. Label-free FTIR spectroscopy detects and visualizes the early stage of pulmonary micrometastasis seeded from breast carcinoma. Biochim Biophys Acta - Mol Basis Dis. 2018;1864(11):3574-3584. [2] Chrabaszcz K et al. Tracking extracellular matrix remodeling in lungs induced by breast cancer metastasis. Fourier transform infrared spectroscopic studies. Molecules. 2020;25(1). [3] Chrabaszcz K et al. Fourier transform infrared polarization contrast imaging recognizes proteins degradation in lungs upon metastasis from breast cancer. Cancers (Basel). 2021;13(2):1-16.

ATR-FTIR and Raman spectroscopic imaging in evaluation of stem cells-induced bone formation on the hydroxyapatite-based scaffold.

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vibrational spectroscopy, stem cells, biomaterials, chitosan, hydroxyapatite

Tissue engineering products, such as implants designed to fill bone deficits are expected to be highly biocompatible, bioactive and surgically handy. This properties can be achieved by creating scaffold from mixed compounds of different characteristics. In this research the ceramic-based biomaterial consisting of chitosan, β-1,3-D-glucan and hydroxyapatite (HA) was seeded with mesenchymal stem cells. The aim of the study was to investigate biomaterial surface after 20 days of incubation at 37 °C with spectroscopic imaging: macro ATR-FTIR and Raman. Two different stem cells were used: adipose-derived stem cells (ADSCs) and bone marrow-derived stem cells (BMDSCs), after differentiation to osteoblasts induced by osteogenic culture medium. Simultaneously, a control sample was also incubated in identical conditions. Spectroscopic approaches were chosen according to their non-invasiveness and cost-effectiveness in describing molecular structures of newly formed organic matter concurrently with inorganic and organic compounds of biomaterial. The application of the macro ATR FT-IR and Raman spectroscopic imaging allowed the identification of changes resulting from culturing ADSCs and BMDSCs on a chitosan/β-1,3-D-glucan/hydroxyapatite composite surface. Spectral data delivered information on active protein and lipid production which is desired at early stages of bone formation. FT-IR absorbance spectra allows determining state and integrity of collagen, by imaging 1660/1690 cm^{-1} (mature/immature) subbands absorbance ratio, since collagens are constituting 90% of extra cellular matrix (ECM). ADSCs on ceramic-based material synthesised greater amount of quickly maturing collagen I with following greater ECM mineralisation [1]. Raman spectroscopy extended evaluation on micro-level detection on hydroxyapatite deposition and alteration [2]. Crystalline form of biomaterial's HA was replaced by newly formed, amorphous HA, deposited in niches of biomaterial. Acquired results were additionally confirmed with confocal microscope analysis, by specific immunofluorescent staining of collagen I and osteocalcin, confirming ongoing ECM formation processes from beginning through other phases. Vibrational spectroscopy and imaging were effective in evaluation of tissue engineering product during early stages of bone formation in vitro, reducing the cost and time of the experiments, confirming that designed biomaterials has great biomedical potential as an implantable scaffold.

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[1] G. Kalisz et all; Materials Science & Engineering C 119 (2021) 111634; [2] G. Kalisz et all; Int. J. Mol. Sci. 2021, 22, 485

Influence of the measurement mode on the results of spectral and biochemical analysis carried out with FTIR microspectroscopy

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FTIR microspectroscopy, transmission mode, transfection mode, biological samples

FTIR microspectroscopy is a powerful tool in the analysis of biological samples. The technique is a combination of optical microscopy, providing high spatial resolution, and infrared spectroscopy, which allows obtaining biochemical information about the test sample. Due to this features FTIR microspectroscopy attracts great interest in biological sciences. FTIR microspectroscopy measurements are usually performed in the transmission mode, in which IR radiation penetrates a thin sample with a micrometer thickness placed on an IR-transparent substrate. Calcium fluoride or barium fluoride slides are commonly used sample carriers for measurement in the transmission mode. Their main drawback is high price and fragility 1. These obstacles made the transmission-reflection (transflection) mode gain popularity. In this mode, the sample is placed on an IR reflecting substrate and IR radiation passes through the sample twice, which increases the optical path travelled by the beam and enhances the sensitivity of the method. The carriers, which are routinely used for transflective measurements, are low-emission silver-coated glass slides that main advantages are low cost and high durability 1. However, research conducted in recent years suggests that the spectral information obtained in the transflective mode may be distorted due to the electric field standing wave (EFSW) phenomenon 2–5. There are few research on the impact of the EFSW effect on the spectra and chemical maps obtained in the transflection mode 1–7. Moreover, existing studies has focused mainly on theoretical considerations and computer simulations 3–5, tissue-like materials 2,7 or cell lines 2. Tissue experiments were limited to specimens previously fixed in formalin and paraffin-embedded 1. However, it is known that the spectra of samples prepared with organic reagents (even after removal of paraffin) show some anomalies compared to non-fixed ones 8. The main objective of the presented research is to compare the results of FTIR analysis of unfixed biological samples performed with transflection and transmission mode. To achieve this goal the samples of brain cross-sections obtained from normal Wistar rats as well as once implanted with U87MG glioblastoma multiforme (GBM) cell line will be analysed. The measurement mode influence on the results of statistical analysis of biochemical changes in GBM affected brain will be assessed. Moreover, the principal component analysis will be applied to evaluate the differences in the FTIR spectra obtained with the transflection and transmission modes.

[1] M. J. Pilling, P. Bassan and P. Gardner, Analyst, 2015, 140, 2383–2392. [2] J. Filik, M. D. Frogley, J. K. Pijanka, K. Wehbe and G. Cinque, Analyst, 2012, 137, 853–861. [3] B. J. Davis, P. S. Carney and R. Bhargava, Anal. Chem., 2010, 82, 3487–3499. [4] T. G. Mayerhöfer and J. Popp, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc., 2018, 191, 283–289. [5] T. P. Wrobel, B. Wajnchold, H. J. Byrne and M. Baranska, Vib. Spectrosc., 2013, 69, 84–92. [6] E. Staniszewska-Slezak, A. Rygula, K. Malek and M. Baranska, Analyst, 2015, 140, 2412–2421. [7] H. Brooke, B. V. Bronk, J. N. McCutcheon, S. L. Morgan and M. L. Myrick, Appl. Spectrosc., 2009, 63, 1293–1302. [8] E. Ó Faoláin, M. B. Hunter, J. M. Byrne, P. Kelehan, M. McNamara, H. J. Byrne and F. M. Lyng, Vib. Spectrosc., 2005, 38, 121–127.

Hydration and illumination scheme effects on photoactive proteins studied by NIR spectroscopy, quantum chemical calculations, time-resolved FTIR

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rapid-scan FTIR, Photosystem II, step-scan FTIR, Orange Carotenoid Protein, protein hydration

Photoactive proteins are widely studied. Not only they possess a chemical/biological relavance, but their applications to several fields (biohybrid photosynthesis, optogenetics etc) is well-established. In understanding their working mechanism, light-induced IR difference spectroscopy, especially when time-resolved, occupies a peculiar relevance and it is considered an almost mandatory technique[1,2]. Nevertheless, little attention has been paid to date to two key factors, namely the hydration state of the protein and the kind of illumination used (e.g. single laser flash vs continuous illumination). We have studied these factors in 3 different photoactive proteins: photosystem II (PSII, the water splitting enzyme), the bacterial reaction centers (RC), and the Orange Carotenoid protein (OCP). In herbicide-treated PSII, where a simple, reversible charge separationcan take place, through rapid-scan FTIR we found that shining 20 flashes instead of a single one induce a much stabler, light-adapted charge separated state, which recombines with a 3-fold slower kinetics. Double-difference IR spectra suggest that small, localized conformational changes are responsible for this effect[3]. In herbicide-reated RC, again a simple, reversible charge separation can take place. Using 20 s of continuous illumination, a much long-lived charge separated state. Again, IR double difference spectra suggest that not only this should be ascribed to localized conformational change, but also to movement of internal H2O molecules, as confirmed by experiments in D2O and ^{18}H2O. In RC the effect was studied at two hydration level, corresponding to 14% and 74% of relative humidity. The hydration level and the strength of water-protein H-bonds was checked trough specific bands in the NIR region. Rapid-scan/step-scan FTIR spectra suggest that low hydration mitigate this light-adaptation phenomenon. Nevertheless, under identical conditions (e.g. after 1 laser flash) dehydration shorten the charge recombination reaction. Time-res. IR underlines that all spectral components decay with the same kinetics[4]. The study was finally extended to OCP. In this protein, dehydration can block or modify the photocycle. Interstingly, when OCP is alsmost dry, a kind of slow, dark-activation, similar to light activation, can take place[5]. The study was completed by time-resolved UV-Vis experiments for more precise kinetics measurements and by calculations to better understand these light adaptation and hydration phenomena and to attribute vibrational bands in time-resolved FTIR difference spectra[6].

[1] A Mezzetti in RSC Specialistic Periodical Reports: Photochemistry, A Albini SProtti eds, 2020, pp 159-195 [2] Skopintsev et al. Nature (2020) 583 314-318 [3] G. Sipka, M. Magyar, A. Mezzetti et al. Plant Cell (2021) in press https://doi.org/10.1093/plcell/koab008 [4] M. Malerrari, A. Mezzetti, F. Francia. G. Venturoli, Biochim Biophys Acta Bioenerg. (2013) 1827 328-339 [5] S Leccese, A Wilson, R. Spezia, D Kirilovsky, A. Mezzetti in preparation [6] A. Mezzetti, M. Alexandre et al. J. Phys Chem B (2019) 123 3259-3266

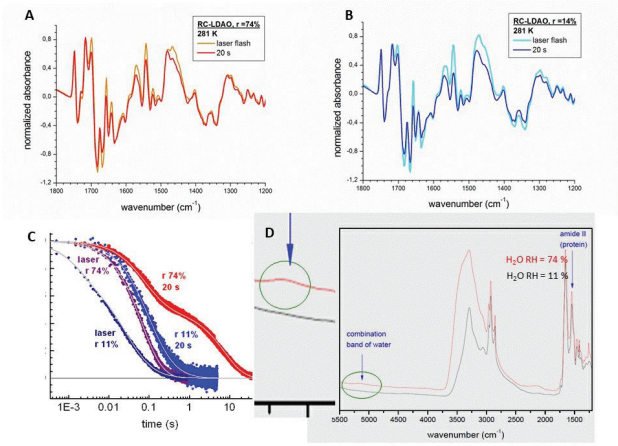

Figure 1. (A) Time-res. FTIR spectra at 7 ms after 1 flash and after 20 s lamp at RH=74%; (B) the same as in A, but at RH=11%; (C) kinetics at the 2 RHs and with laser/lamp; (D) FTIR/FTNIR of RC at the 2 RHs

Quantum cascade laser-based IR spectroscopy for highly sensitive analysis of proteins

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Infrared spectroscopy, Quantum cascade laser, protein secondary structure, external perturbation

Mid-IR spectroscopy is capable to provide both qualitative and quantitative information on proteins in a fast, non-destructive and label-free manner by probing the strong, fundamental vibrations of molecules. In protein analysis determination of the secondary structure (α-helix, β-sheet, random coil, etc. ...) of a given protein is the most relevant qualitative information accessible by mid-IR spectroscopy. Protein analysis in aqueous solutions by conventional Fourier transform infrared (FTIR) spectroscopy is limited due to the strong water absorption overlapping with the information-rich protein amide I band. Consequently, only short path-lengths (<10 µm) can be used for measurements as otherwise all light would be absorbed by the aqueous sample matrix. The significant progress made in mid-IR lasers has recently changed this situation. High spectral power densities and broad tuning ranges, as made possible by modern external cavity quantum cascade lasers (EC-QCL), now allow for pathlengths of 30 µm and more, even for analysis of the amide I band. We present a broadband EC-QCL based IR transmission setup with balanced detection covering the amide I+II bands for highly sensitive protein sensing that outperforms commercially available IR spectrometers by almost an order of magnitude. Furthermore, we report on application of laser-based IR spectroscopy on monitoring protein conformational changes after external perturbation (chemical, temperature, pH).

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[1] C.K. Akhgar, G. Ramer, M. Żbik, A. Trajnerowicz, J. Pawluczyk, A. Schwaighofer, B. Lendl, The Next Generation of IR Spectroscopy: EC-QCL-Based Mid-IR Transmission Spectroscopy of Proteins with Balanced Detection, Analytical Chemistry 92 (2020) 9901-9907. [2] A. Schwaighofer, M.R. Alcaraz, L. Lux, B. Lendl, pH titration of β-lactoglobulin monitored by laser-based Mid-IR transmission spectroscopy coupled to chemometric analysis, Spectrochim. Acta, Part A 226 (2020) 117636.

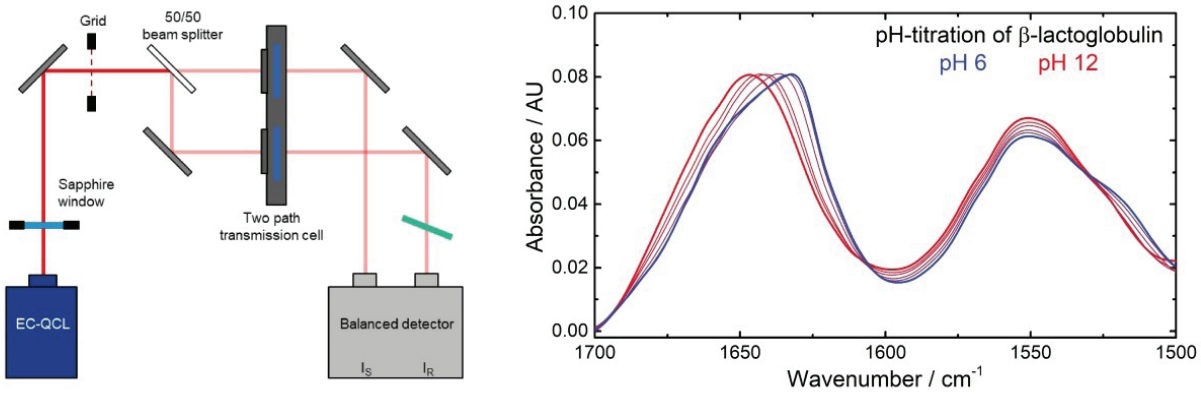

Figure 1. (left) Schematic of balanced EC-QCL IR transmission setup. (right) Laser-based IR spectra of pH titration of β-lactoglobulin between pH 6 and pH 12.

Towards Rapid Virus Detection on Protective Face Masks

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Infrared Spectroscopy, Virus, Chemometry, Masks

Fast and reliable detection of SARS-CoV-2 is of particular importance in order to contain and control the spread of the virus via targeted measures. The staff in clinics / hospitals, in nursing homes, and in first-aid scenarios where daily exposure during occupational activities represents an exceedingly high risk of infection requires fast and reliable virus detection. There is currently no label-free rapid test format that directly provides short-term exposure results (i.e., during a period of several hours) similar to e.g., monitoring of workplace safety using dosimetry for chemicals exposure. In this study, a radically new strategy has been investigated mitigating exposure to SARS-CoV-2 using infrared spectroscopic techniques. There are literally tons of samples being produced worldwide that have been neglected to date – used protective face masks and the (virus) particles they retain at the outside upon inhalation, and at the inside upon exhalation! Hence, we demonstrate the analysis of worn face masks at both sides via infrared spectroscopic signatures augmented by multivariate data evaluation/classification schemes. Recently, our research team has demonstrated that infrared spectroscopic analysis directly-on-filter (IR-DoF) is a promising technique for particle identification and quantification. In this novel approach, the same concept was applied for analyzing virus particles captured within protective facemask materials. [1-3] The feasibility of this test format was demonstrated using infrared attenuated total reflection (IR-ATR) as well as external reflectance infrared spectroscopy for test species such as virus-like particles without any further sample preparation. The obtained results indeed confirmed that direct virus classification on a mask is possible and promises a rapid test format facilitating interventions in hazardous exposure scenarios.

We are grateful for the support of this project by the Ministerium für Wissenschaft, Forschung und Kunst (MKW) Baden-Württemberg, Germany under the Program "Special Measures against the SARS-CoV-2 Pandemic".

[1] R. Stach, T. Barone, E. Cauda, P. Krebs, B. Pejcic, S. Daboss, B. Mizaikoff, Direct infrared spectroscopy for the size-independent identification and quantification of respirable particles relative mass in mine dusts, Analytical and Bioanalytical Chemistry, 412, 3499–3508, 2020. [2] R. Stach, T. Barone, E. Cauda, B. Mizaikoff, A Novel Calibration Method for the Quantification of Respirable Particles for Mining Scenarios via Fourier Transform Infrared Spectroscopy, Applied Spectroscopy, 75 , 3, 2021. [3] T. Barone, T. Lee, E. Cauda, A. Mazzella, R. Stach, B. Mizaikoff, Segregation of Respirable Dust for Chemical and Toxicological Analyses, Archives of Environmental and Occupational Health,76, 3, 2020

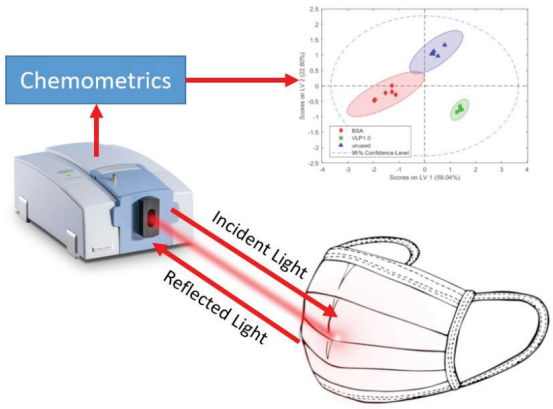

Figure 1. Experimental set-up for directly analyzing used protective face masks.

Infrared micro-spectroscopic characterization of the hygroscopically-active awn of Avena sterilis

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hygroscopic movement, infrared micro-spectroscopy, cell wall

Efficient seed dispersal is crucial for plants, as this step ensures passing of DNA to future generations. For annual plants, such as the wild oat (Avena sterilis), successful growth of new populations therefore has to ensures survival of the specie each year. Many diverse mechanisms of seed dispersal have evolved. This includes strategies that use external cues like wind, animals or water streams. The seed dispersal unit of Avena sterilis shows a unique mechanism that relies on changes in the humidity and can be triggered by rain or the diurnal cycle. The reversible twisting and bending of two awns propagate the unit over the ground and finally even transport the seeds to a safe germination place by drilling the unit into the topsoil. Strikingly, the awns that provide the self-propelled movement consist of dead tissue only. Thereby, the whole motion is the result of the hierarchical structure of the plant cell walls and their composition. Here, we present the characterization of the hygroscopically-active tissue of Avena sterilis and the cellular constituents by infrared micro-spectroscopy. Multivariate analysis, including principal component analysis enabled the characterization of native and chemically altered samples. Our previous work revealed that different parts of the awn react differently to changes in the humidity, both on the macroscopic and at the cellular level [1]. Here, different regions of 10 µm cross sections of the Avena sterilis awn were mounted on CaF_2 slides and studied with infrared micro-spectroscopy. The analysis of the spectra showed that the cell wall composition also depends on the position within the awn. Those compositional differences were distinct for different regions of the awn and involved variation in major cell wall components, including aromatic compounds. This is especially interesting, as such aromatics were shown to play an important role in the water-tissue interaction of plants [2]. Our approach opens the way to understanding the chemistry of functional plant tissues in micrometer scale.

[1] Lindtner et al., manuscript submitted [2] Abraham, Y., Dong, Y., Aharoni, A., & Elbaum, R. (2018). Mapping of cell wall aromatic moieties and their effect on hygroscopic movement in the awns of stork's bill. Cellulose, 25(7), 3827–3841.

Micro-Raman study of the light harvesting pigments in Chlamydomonas reinhardtii under salinity stress

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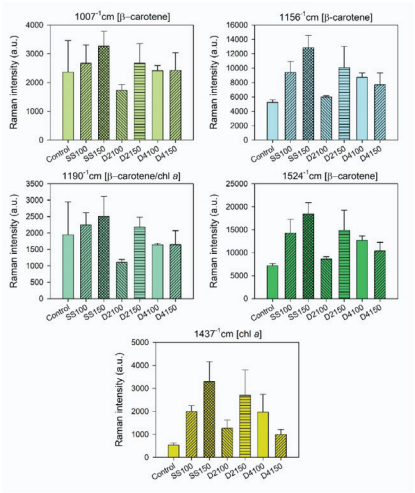
Chlamydomonas reinhardtii, micro-Raman spectroscopy, salt stress, carotenoid, nutraceutical

Together with chlorophylls, carotenoids pay a key role in light harvesting for photosynthesis in the unicellular green microalga Chlamydomonas reinhardtii. Carotenoids also protect the cells against photooxidative damage [1]. Imposing microalgal cells to abiotic stresses such as nutrient depletion, high intensity light or salinity stress has been shown to result in accumulation of carotenoids, which can be further harvested to yield high-value carotenoids with broad applications in food, feed, cosmetic or nutraceutical industry [2-4]. We have used single-cell micro-Raman spectroscopy to study the response of chlorophylls and carotenoids in C. reinhardtii cells grown in single-stage and two-stage salt stress at the end-log and the stationary growth phase. This permitted a non-invasive assay to explore the heterogeneities with respect to the pigment content of carotenoids (carotenes and xanthophylls) accumulated on exposure to salt stress. Raman spectra were analyzed to study possible carotenoid degradation under salt stress, and the role of the mode of cultivation in carotenoid content. Possibility of stress driven adaptive growth was studied from Pearson's correlation coefficients, which gave the inter-relationship between chlorophylls and carotenoids as a function of mode of salt stress and age of the culture. Finally, the role of carotenoids in regulating the autophagic pathway was also analyzed.

Debjani Bagchi would like to thank GSBTM, Gandhinagar, India, for financial support for this project.

[1] Zuluaga, Marisol et al. Carotenoids from microalgae to block oxidative stress. BioImpacts: BI vol. 7,1 (2017): 1-3. [2] Gong Mengyue, Bassi Amarjeet, Carotenoids from microalgae: A review of recent developments, Biotechnology Advances (2016) [3] Minhas Amritpreet K. et. al., A Review on the Assessment of Stress Conditions for Simultaneous Production of Microalgal Lipids and Carotenoids, Frontiers in Microbiology 7 (2016) 546 [4] Koen Goiris, Willem Van Colen, Isabel Wilches, Fabián León-Tamariz, Luc De Cooman, Koenraad Muylaert, Impact of nutrient stress on antioxidant production in three species of microalgae, Algal Research, 7 (2015), Pages 51-57



Fugure 1. Raman intensities obtained for beta carotene and chlorophyll peaks on the sixth day of growth for control culture without salt stress, single stage salt stress and two stage salt stress

Raman 18O-Labeling and Two-Dimensional Correlation Spectroscopy for Accessing the Metabolic Activity of Bacteria

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Raman microspectroscopy, 18O-labeling, single bacterial cells, 2D-Raman correlation spectroscopy

The need to identify metabolically active bacteria in their natural habitat is of great concern in clinical diagnosis and environmental microbiology, since microbial communities are essential for the function of most ecosystems including eukaryotes [1, 2]. Raman stable isotope labeling with ^{2}H, ^{13}C, or ^{15}N has been used to study the metabolic activity of bacteria [3, 4]. Active cells take up labeled substrates and incorporate the isotope into newly synthesized biomolecules like proteins, nucleic acids and lipids. As a result, the vibrational frequency of the labeled functional group shifts to lower frequency. Owing to this red-shift, metabolically active cells can be differentiated from non-active cells [1-4]. In this study, a new approach termed Raman ^{18}O-labeling was developed. It is the combination of Raman microspectroscopy and ^{18}O-labeling to monitor the metabolic activity of bacteria. Here, the time-dependent; ^{18}O-uptake in proteins and nucleic acids has been investigated at excitation wavelengths of 532 nm and 244 nm. 2D correlation analysis identified small spectral changes resulting from ^{18}O incorporation and revealed the growth strategy of E. coli cells; this information is not visible to the naked eye in the Raman spectrum. Hence, we foresee wide application potential of Raman-^{18}O labeling combined with 2D correlation analysis to obtain deeper insights into biochemical processes of bacteria at a single cell level. This novel approach may open up new opportunities to provide a crucial link between the metabolic activity and the in-situ functions of phosphate solubilizing bacteria, since stable phosphate isotopes are not commercially available.;

Financial support provided by the Deutsche Forschungsgemeinschaft (DFG) via the CRC 1076 AquaDiva.

[1] Azemtsop Matanfack, G., et al., Imaging the invisible – Bioorthogonal Raman probes for imaging of cells and tissues. Journal of Biophotonics, 2020. 13(9): p. e202000129. [2] Wang, Y., et al., Single cell stable isotope probing in microbiology using Raman microspectroscopy. Curr. Opin. Biotechnol., 2016. 41: p. 34-42. [3] Taubert, M., et al., Tracking active groundwater microbes with D2O labelling to understand their ecosystem function. Environ. Microbiol., 2018. 20(1): p. 369-384. [4] Song, Y., et al., Raman-Deuterium Isotope Probing for in-situ identification of antimicrobial resistant bacteria in Thames River. Scientific Reports, 2017. 7(1): p. 16648.

Macro ATR-FTIR imaging of fungal decomposition of complex organic matter in soil

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Macro ATR-FTIR spectroscopic imaging, Microfluidic soil chips, Fungal decomposition

A grand challenge for mankind is to fight climate change, which involves both reducing and reverting CO_2 emissions. Soils store more C than the atmosphere and biosphere combined, and it is microorganisms that govern whether the C compounds remain in soil, or if they are decomposed and released to the atmosphere as CO_2. The microbial influence on C cycling range from the way they decompose soil organic matter (SOM) to their contributions on the formation of soil aggregates that are particularly important for physical C stabilization in soils. However, the relationship between the microbial activity, SOM properties and physicochemical microenvironment, including complexity of soil structure is not well understood. Therefore, the aim of this work has been to take use of an analytical approach for studying the influence of pore space architecture on fungal SOM decomposition and dynamics by integrating two novel tools in soil sciences – microfluidic chips, which mimic soil structure, and infrared (IR) spectroscopic imaging, which provides detailed information about chemical properties of materials within these chips. We have used microstructures in the chips to simulate different levels of complexity of soil pore space. The hypothesis is that the more complex the chip structures – the less decomposition of SOM will be observed, as more of it will be 'hidden' from its decomposers within hard-to-reach spaces. For the IR spectroscopic imaging, macro attenuated total reflection (ATR) accessory has been used. In this mode, an ATR element of high refractive index is put in contact with the microchip and total internal reflection signal at the boundary between the element and the sample is recorded with an imaging focal plane array (FPA) detector. With the recorded IR spectra serving as fingerprints for identifying molecules, spatially and temporally resolved observation of chemistry and chemical changes of a SOM substrate initially filling the microchip structures and undergoing decomposition by subsequently inoculated fungal cultures can be made. Our pilot data suggests feasibility of the approach for analysis of complex substrates such as lignin. We observe chemical changes and overall concentration decrease of lignin in the chips inoculated with lignin decomposing fungus Bjerkandera adusta. We are further exploring the potential of the set up to analyze decomposition of other types of substrates, such as proteins or SOM extracts from real soils, and in chip designs simulating soil structures of different complexity.

We acknowledge financial support from SSF (Sweden) and Royal physiographic society of Lund.

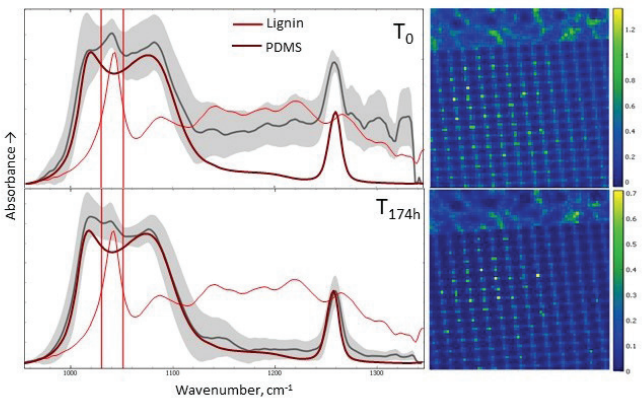

Figure 1. IR spectra and chemical images of lignin in microfluidic chips before (T0) and after (T174 = 174 h after inoculation) overgoing fungal decomposition. The size of the chemical images are 4x4 mm.

Raman spectroscopic differentiation between human peripheral blood mononuclear cells and molecular characterization of naïve and activated T lymphocytes

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T cells, B cells, activated T cells, confocal Raman imaging, carotenoids

Human peripheral blood mononuclear cells (PBMCs) are a heterogeneous population of cells that includes T, B lymphocytes, natural killer cells, monocytes, and dendritic cells. Lymphocytes represent the most numerous cell population within PBMCs (70-90%) [1] and are characterized by very similar morphological features such as, size around 8-10 µm, large nucleus surrounded by thin layer of cytoplasm containing subcellular structures [2]. To become fully functional effector cells naïve T lymphocytes have to be activated by specific antigens binding to T-cell receptors (TCR) on its surface. Activation requires also co-stimulating interactions of other surface proteins and cytokines. Activating signal involves a series of biochemical intracellular events leading to production subtype specific effector proteins and accelerated proliferation [3]. Activation of T cells plays essential role in adaptive immunity function as different subtypes act as initiators, effectors or regulators of the response. The total number of lymphocytes and their percentage in the blood can be a marker for the diagnosis of several human diseases. Currently, cytometric methods are widely used to distinguish subtypes of leukocyte and quantify their number. These techniques use cell immunophenotyping, which is limited by the number of fluorochrome-labeled antibodies that can be applied simultaneously. In our studies we applied a label-free Raman spectroscopy imaging method for molecular characterization and discrimination of PBMCs and activated T cells. We have defined spectra biomarkers detected and visualised on the sub-cellular level characteristic for carotenoids, nucleic acids as well as proteins and lipids fractions for this purpose. We have shown that although the presence of carotenoids depends on the individual donor variability it is a reliable marker for distinguishing T cells among PBMCs and for tracking of T cells activation. The accumulation of carotenoids exclusively in lymphocytes T was clearly evidenced in the Raman spectra and supported by quantitative analysis carried out with the application of HPLC method. The reliable recognition was possible only after detailed analysis of the average spectra and application of PCA and PLS methods.

This work was supported by „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project carried out within the Team-Net program of the Foundation for Polish Science co-financed by the EU under the ERDF

[1] Murphy K (2012) Janeway's Immunobiology, 8th ed. Garland Science, Taylor & Francis Group, New York [2] Young NA, Al-Saleem T (2008) CHAPTER 24 - Lymph Nodes: Cytomorphology and Flow Cytometry. In: Bibbo M, Wilbur DBT-CC (Third E (eds). W.B. Saunders, Edinburgh, pp 671–711 [3] D. G. Waller, A. P. Sampson (2018) Rheumatoid arthritis, other inflammatory arthritides and osteoarthritis, Medical Pharmacology and Therapeutics (Fifth Edition), Elsevier

The investigation of SARS-CoV-2 using AFM-TERS

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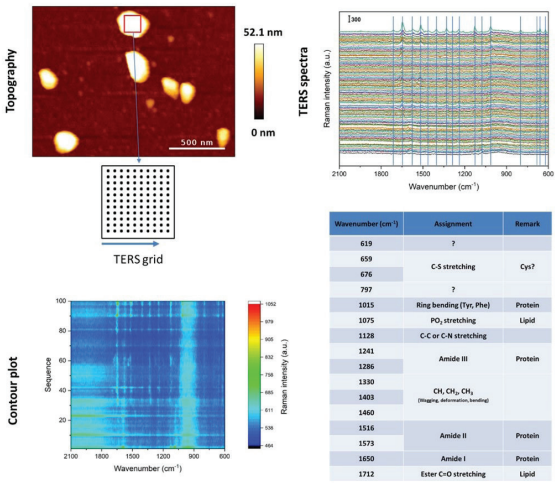
AFM-TERS, SARS-CoV-2

The outbreak of SARS-CoV-2 since 2019 clearly showed the need for a fast and reliable direct virus diagnosis [1]. Currently, PCR and antibody tests are broadly used to screen positive cases. While Raman (or vibrational spectroscopy in general) spectroscopy is unlikely to replace those techniques, it can potentially provide insight in structural compositions and variations of pathogens in an early stage when staining procedures still have to be optimized. Raman spectroscopy has been broadly used in the applications of life science with its intrinsic advantages to acquire chemical information of biosamples. Furthermore, the low Raman scattering cross-section and optical diffraction limit of conventional Raman spectroscopy has been overcome by tip-enhanced Raman spectroscopy (TERS). With the combination of scanning probe microscopy and plasmon techniques, nanoscale investigations of biosamples have been realized by TERS [2]. In this work, we will present our pristine investigations of SARS-CoV-2 virus using AFM-TERS. Benefited of the combination of AFM and Raman spectroscopy, the topography and chemical information of SARS-CoV-2 virions could be acquired simultaneously by TERS in ambient conditions. Fig. 1 presents the TERS spectra of a selected virion. From the assignment, it is known that this virion has a dimension of around 200 nm and its surface is enriched in proteins and lipids. In contrast, epithelial cells are protected by protein-enriched microplicae on which lipid signals are difficult to be detected (data not presented here). In short, it is believed that TERS is able to distinguish virions from the background of oral epithelial cells where virions reproduce and will be a promising technique in the investigation of SARS-CoV-2.

Thanks to the financial supports of Leibniz InfectoOptics SAS-2015-HKI-LWC and DFG CRC-1375-NOA-C2.

[1] B. D. Kevadiya, et al., Diagnostics for SARS-CoV-2 infections. Nat. Mater. 2021, 20, 593–605. [2] T. Deckert-Gaudig, et al., Tip-enhanced Raman spectroscopy – from early developments to recent advances. Chem. Soc. Rev., 2017,46, 4077-4110.



Wavenumber (cm⁻¹)	Assignment	Remark
619	?	
659	C-S stretching	Cys?
676		
797	?	
1015	Ring bending (Tyr, Phe)	Protein
1075	PO_2 stretching	Lipid
1128	C-C or C-N stretching	
1241	Amide III	Protein
1286		
1330	CH, CH_2, CH_3 (Wagging, deformation, bending)	
1403		
1460		
1516	Amide II	Protein
1573		
1650	Amide I	Protein
1712	Ester C=O stretching	Lipid

Figure 1. TERS spectra of a SARS-CoV-2 virion

A spectroscopic and microscopic model of oxidative damage in erythrocytes caused by a chemically-induced oxidative stress

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vibrational spectroscopy, erythrocytes, oxidative stress

Oxidative stress has been supposed to play an important role in various pathological processes in organism. Understanding and correlation of changes induced by oxidative stress in red blood cells (RBCs) could broaden the knowledge about oxidative mechanisms in the aging process and many diseases. It is assumed that the first signs of oxidative stress in erythrocytes occur in membranes, but a final effect depends on the chemical nature of stress factors. We investigated this hypohotesis and performed in vitro studies to induce controlled oxidative damage of RBCs. For this purpose, we chose two different chemical agents – hydrogen peroxide, which is permeable through membranes, and t-butyl peroxide, which penetrates into the inner hydrophobic part of the membrane lipid bilayer. We applied vibrational spectroscopies with the combination of other analytical and microscopic techniques to analyze biochemical and morphological alterations in RBCs membranes and cytoplasm due to oxidative stress. Such an approach allowed us to deduce that the used chemical agents exhibited a slightly different effect on RBCs depending on their structure and chemical features. Similarities mainly embraced changes in morphological parameters, i.e. we observed that a majority of erythrocytes exposed to the oxidative agents exhibited a smaller diameter and an increased height compared to control. From the chemical point of view, the chemically-induced oxidative stress caused a decrease in lipid unsaturation and the level of phospholipids in membranes. On the other hand, a detailed analysis of FTIR and Raman spectra of whole erythrocytes showed that the organic peroxide led to extensive lipid peroxidation and stronger changes in RBCs than H2O2. In addition, t-butyl peroxide induced significant changes in the secondary structures of the cytoplasmic proteins associated with their aggregation.

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[1] A. Blat, et al. Anal. Chem. 2019, 91, 9867-9874 [2] A. Blat, et.al. Int. J. Mol. Sci. 2021, 22 (5), 2660 [3] J. Dybas, et al. Biochim Biophys Acta Mol Basis Dis. 1866 (12), 165972

IDH-mutated transgenic cell lines as in vitro models of leukemia investigated and characterized by means of Raman imaging

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IDH mutations, HEL cell line, Raman imaging, chemometrics

Genetic abnormalities in myeloid or lymphoid progenitors underlie the development of leukemia. They can lead to disruption of cell differentiation and promotion of rapid uncontrolled proliferation of malignant cells. Specific gene mutations that characterize different types of leukemia are responsible for their different phenotypes and sensitivity for a treatment. New in vitro models are now being searched for drug screening and functional analysis. Genes, which encode isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are frequently mutated in different leukemia types [1] and, therefore investigation and modeling of transgenic cell lines with IDH1 and IDH2 gene rearrangements are of high interest in studies focused on leukemia development. The aim of our study was to investigate the suitability of Raman imaging for the identification of metabolic changes associated with IDH1 and IDH2 mutations in a HEL cells based in vitro model [2]. The first mutant isogenic line was related with IDH1 gene, causing a replacement of arginine with histidine at the residue 132 (IDH1/R132H). The second studied mutant cell line was obtained by gene rearrangement of IDH2 that alters a single arginine residue at position 140 by substitution of glutamine (IDH2/R140Q). Indirectly, IDH1 and IDH2 gene rearrangements influence DNA methylation and thus epigenetic regulation of many genes affecting differentiation, proliferation, metabolism and phenotype of leukemia cells. Control samples constituted the cells with a wild-type (WT) sequence of IDH1 and IDH2 genes. Empty pMIG viral vector and not transfected HEL cells served as additional controls for comparison. Mutant and control cells; were imaged with the use of confocal Raman system WITec Alpha 300. At least 50 cells per group were imaged and analyzed. Raman images of each measured cell were subjected to k-means cluster analysis in order to obtain averaged spectra of different cellular compartments. To reveal metabolic changes caused by IDH mutations, principal components analysis (PCA) and partial least squares regression (PLS) chemometric methods were applied. It was observed that IDH1 and IDH2 gene rearrangements caused subtle but significant changes in metabolism of IDH1/R132H and IDH2/R140Q in compare to parental HEL cell line and IDH WT cells. Observed differences were primarily related to the protein and nucleic acids composition of cells.

The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

[1] H. Yang, D. Ye, K-L. Guan, Y. Xiong, IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives, Clin Cancer Res, 2012, DOI: 10.1158/1078-0432.CCR-12-1773. [2] P. Martin and T. Papayannopoulou, HEL cells: a new human erythroleukemia cell line with spontaneous and induced globin expression, Science, 1982, DOI: 10.1126/science.6177045

Valorization of Plastic Waste via Advanced Separation and Processing

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Plastic recycling, Plastic sorting, Infrared spectroscopy, Quantum cascade laser, Machine learning

The plastic waste crisis is among one of the planet's most pressing environmental problems, impacting wildlife, food production, drinking water, and many other critically important aspects of life. To combat this issue, a multidisciplinary approach, spanning the gamut from chemistry to engineering to computational science, is necessary. This work aims to utilize high-throughput multi-modal sensor recognition and autonomous sorting to advance mixed plastic waste sorting technologies and further valorize recovered plastic. Specifically, Attenuated Total Reflectance–Fourier Transform Infrared spectroscopy is utilized to register molecular fingerprints of household plastics products and generate a reliable database of infrared spectra. This library is then used for the purpose of machine learning training to autonomously classify plastic samples. Furthermore, this work leverages the tunability of a quantum cascade laser to investigate molecular signatures in the mid-infrared spectral region. This quantum cascade lasing technique uniquely combines the selectivity of infrared spectroscopy with the sensitivity of a stand-off microcantilever detection setup. The overarching goal of these three complementary research tasks is to help reduce plastic waste streams that wind up in landfills and surrounding environments, as well as potentially being integrated into existing technologies at existing materials recovery facilities and other industries.

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The application of FT-IR spectroscopic imaging in the evaluation of pathogenesis of Agrobacterium tumefaciens in cultivated dicotyledons

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Agrobacterium tumefaciens, FT-IR imaging, crown gall disease, tumor tissue, alkaloids

To Agrobacterium tumefaciens species belong soil, Gram-negative bacteria, causing crown gall deformations on stems and roots of many varieties of dicotyledons and some gymnosperms. A. tumefaciens attacks mainly wounded plants, recognising them by compounds released at the places of injury, such as sugars and phenols. After infection the pathogen transforms hosts' cells, forcing their excessive division, which leads to the formation of tumour tissue [1]. Transformation of plant cells results from the ability of these bacteria to transfer a fragment of their own DNA to plant cells and to integrate it with the genetic material of the host plant. The source of the transferred DNA (so-called T-DNA) is the Ti plasmid. Expression of genes encoded by T-DNA in a plant cell leads to the production of enzymes that catalyze the synthesis of plant hormones – auxins and gibberellins, responsible for tissue (callus) growth. Infected plant tissue produces opins – low molecular weight compounds that can only be metabolized by A. tumefaciens cells, serving them as a source of carbon and nitrogen, and thus providing a selective advantage for this bacterial species [2]. One of main factors determining pathogenic interactions between Agrobacterium and plants is a conservative element of outer membrane lipopolysaccharide's – lipid A. These bacteria are able to synthesize and insert into their lipid A so called very long chain hydroxy fatty acids (VLCFA) [3]. The presence of VLCFA makes bacteria's outer membrane more condensed and more resistant to changing environmental conditions. However, any changes in lipid A fatty acid composition may cause alterations in Agrobacterium virulence. In this study, the ability of mutants in the gene cluster acpXL-msbB, a region responsible for VLCFA synthesis was analyzed, to conduct the transformation of plant cells and their virulent capabilities. For that purpose, we monitored the development of the infection and tumor growth using FT-IR microspectroscopy. The FT-IR spectroscopic imaging ;was used to analyse irreversible anatomical and metabolic changes under the influence of bacterial infection both by wild type and by the mutants. Moreover, the spectral analyses allowed to examine representative alkaloids known as signalling compounds participating in plants antimicrobial reaction.

This work was supported by National Science Centre within the OPUS project (2018/31/B/NZ9/01755).

[1] Ziemienowicz A., Acta Biochim. Pol. (2001) 48, 623-635 [2] Pitzschke A, Hirtt H, EMBO J. (2010) 29 1021-1032 [3] Silipo A., et. al, Glycobiology (2004) 14, 805–815

Evaluation of gamma irradiated and dyed sheep wool fibre structure changes by FTIR spectroscopy and HPLC

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FTIR spectroscopy, HPLC, sheep wool felt fibres, gamma irradiation, Congo Red

Introduction Sheep wool is more often regarded as a promising biosorbent. However, the sorption capacity depends on several factors and can be enhanced by relevant chemical or physical treatment. Congo red (CR) is one of the environmental pollutants and thus was used to assess the irradiation-induced structural changes related to sorption. The aim of this study was to evaluate the irradiation-induced sorption capacity and fibre structural changes. Materials and Methods Sheep wool felt (Folder Kids Ltd., Latvia) was irradiated by TBI 4850–150 tote box type gamma irradiator (Scandinavian Clinics Estonia OÜ) to absorbed doses 50 and 100 kGy. Wool samples (10 mg) were dyed with CR of 5-200 µM under static conditions in 30 mM phosphate buffer (pH=7.03) as adsorption media. Supernatants were analysed by HPLC and data fitted to different theoretical adsorption models by NLLS method built in IBM SPSS (v.21). Wool fibres were crushed, suspended in 0.8 ml of dH2O, 5 µl of suspension dried on a 384 well silicon microplate. FTIR spectra were recorded on Vertex 70 coupled with HTS-XT (Bruker, Germany) in range of 4000 – 600 cm-1. Results HPLC results show that adsorption of CR on wool felt samples is best described by the Langmuir adsorption model. Gained isotherms indicate slight changes in CR saturation capacities and interaction constants. CR adsorption capacity of non-irradiated wool sample was higher (5.6 µmol/g) than irradiated by 100 kGy (4.7 µmol/g) and 50 kGy (4.3 µmol/g). Thus, showing the influence of gamma irradiation on the structure and availability of the CR adsorption sites. FTIR absorption spectra clearly show changes in the proportion between Amide I (1650 cm-1) and Amide II (1539 cm-1) after sorption of CR. This indicates the wool keratin secondary structure changes. The 2nd derivative spectra of Amide I shows decrease of absorption intensity at 1650 cm-1 compared to non-dyed wool indicating destabilization of keratin α-structures. Slight increase of 1695 and 1669 cm-1 show an increase in β-sheets and more disordered conformations of wool keratin. Conclusions FTIR spectroscopy and HPLC data showed that: 1) the sorption capacity of CR of sheep wool felt fibres can be changed by gamma irradiation, 2) irradiation and dye sorption induced changes in wool fibres structure can be clearly detected by the; 2nd derivative FTIR spectra, Gamma irradiation induced structural changes in sheep wool keratin via CR, leads to further studies of gaseous component sorption.

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In the search of a Raman probe to detect mitochondrial dysfunction in endothelial cells.

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endothelial dysfunction, mitochondrial dysfunction, cytochrome c, MitoBADY, Raman spectroscopy

The endothelium, which forms an active biologic interface between the blood and all other tissues, is a spatially distributed organ, which plays a significant role in many physiological functions, i.e. control of vasomotor tone, regulation of inflammatory response. [1-2] Endothelial dysfunction contributes to the development of various cardiovascular diseases and the key players in pathogenesis are among others vascular inflammation and oxidative stress. [3] The latter is very closely related to the mitochondria dysfunction. Mitochondria are highly dynamic organelles that play a remarkably diverse set of cellular function. Most prominent of these is the production of ATP, but mitochondria also take part in the regulation of apoptosis, reactive oxygen species synthesis, inflammation. [2] Raman spectroscopy (RS) is one of the few label-free and non-invasive methods that can provide chemical information from living specimens. In addition to the methodology based on label-free measurements, a new approach using molecular reporters, that give a Raman signal in the silent region in the spectra of cells, seems very promising. [4,5]. It is reported that in a label-free RS variant mitochondrial dysfunction can be detected by checking the cytochrome redox state, a reliable indicator of mitochondrial activity. [5] On the other hand, Raman reporters can be used to enhanced sensitivity and selectivity of Raman analysis of cells. One of the first selective Raman reporter accumulating in mitochondria was MitoBADY, applied previously to HeLa tumor cells. [6] Our research was focused on selecting the appropriate conditions for the use of MitoBADY, both in live and fixed endothelial cells. The experiments aimed to develop a protocol that could be applied to subsequent Raman reporters. As a marker to detect mitochondria cytochrome c was used. We applied MitoBADY at various concentration and incubation time on endothelial cells. Moreover, the influence of the mitochondria decoupler (FCCP) on the potential of the mitochondrial membrane was tested. We came to conclusion that with the higher concentration and longer incubation time the selectivity of MitoBADY decreases. Nonetheless, the colocalization with lipids was observed. The optimal condition to detect MitoBADY in live and fixed cells was concentration of 100 nM and 30min incubation time, and concentration of 400 nM and 60 min incubation time, respectively. The effect of FCCP was observed at very low concentration (0.7 µM), characterized by reduced intensity of the cytochrome bands, but this research is in progress.

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[1] W. C. Aird, "Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms," Circ. Res., vol. 100, no. 2, pp. 158–173, 2007. [2] A. Szewczyk et al., "Mitochondrial mechanisms of endothelial dysfunction," Pharmacological Reports, vol. 67, no. 4. Elsevier B.V., pp. 704–710, Aug. 29, 2015. [3] A. Adamczyk et al., "Toward Raman Subcellular Imaging of Endothelial Dysfunction," J. Med. Chem., vol. 64, pp. 4396–4409, Apr. 2021. [4] E. Matuszyk et al., "Multiplex Raman imaging of organelles in endothelial cells," Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 255, p. 119658, Mar. 2021. [5] T. Morimoto et al., "Using redox-sensitive mitochondrial cytochrome Raman bands for label-free detection of mitochondrial dysfunction," Analyst, vol. 144, no. 8, pp. 2531–2540, 2019. [6] H. Yamakoshi et al., "A sensitive and specific Raman probe based on bisarylbutadiyne for live cell imaging of mitochondria," Bioorganic Med. Chem. Lett., vol. 25, no.3, pp. 664–667,2015

EdU-labelling: Raman-based click-free detection of endothelial cell proliferation

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Raman imaging, Endothelial dysfunction, Molecular Raman reporters, Endothelial cell Proliferation, 5-ethynyl-2'-deoxyuridine (EdU)

Endothelial dysfunction (ED) has been linked to the development of many lifestyle diseases[1] and the phenotype of ED is often linked to altered capacity of endothelial regeneration unable to repair vascular dysfunction. Therefore, new methods to study endothelial cell proliferation are desirable. Although Raman spectroscopy allows label-free detection of key biological compounds i.e. proteins, lipids and nucleic acids, the use of Raman reporters improves sensitivity and selectivity of subcellular organelles imaging and tracking specific molecules [2,3]. One example of this is the detection of alkyne-tagged μM) (EdU). EdU is a thymidine analogue that incorporates into new DNA during replication. EdU subsequent detection, after its Cu-catalysed cycloadditionreaction "Click Chemistry" with a fluorescent azide, has been used in fluorescence imaging to follow DNA synthesis of proliferating cells [4,5]. Due to the alkyne tag, EdU could be detected in the Raman spectra of cells as it gives a Raman band at ca. 2122 cm^{-1} in the "silent region" where there are no interferences in the signal from other biological compounds[6]. The aim of this study is to investigate the changes in the proliferation of endothelial cells using EdU as an indicator of DNA replication. We studied the effects of cycloheximide (CHX), a protein synthesis inhibitor, [7] on EdU-tagged HMEC-1 cells. Raman imaging of control cells incubated with EdU for 24h showed a very clear distribution of the alkyne band at ca. 2122 cm^{-1} in cell nuclei, whereas CHX pre-treated cells, incubated with EdU for the same period, showed a significantly lower EdU signal. It was clear that cell proliferation was inhibited after CHX treatment and this effect could be detected using Raman EdU-labelling approach, which was not detectable using label-free Raman imaging. When fluorescent detection of Alexa Fluor® 488-tagged EdU was used as a reference method, the intensity of the signal from Alexa Fluor as well as the percentage of EdU positive cells were also decreased in CHX pre-treated cells. The Raman imaging results are in agreement with the fluorescence method, furthermore, the Raman-based approach omits cell permeabilization step and allows EdU detection without the need of the fluorescent dye. In conclusion, the results of this study show the feasibility of click-free detection of EdU-labelled DNA in endothelial cells using Raman spectroscopy and this method will be further used and optimized to detect endothelial proliferation using Raman-based approach in the isolated vessel ex vivo.

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[1] Rajendran P, Rengarajan T, Thangavel J, et al (2013) The vascular endothelium and human diseases. Int J Biol Sci 9:1057–1069. [2] Baranska M, Kaczor A, Malek K, et al (2015) Raman microscopy as a novel tool to detect endothelial dysfunction. Pharmacol Reports 67:736–743. [3] Adamczyk A, Matuszyk E, Radwan B, et al (2021) Toward Raman Subcellular Imaging of Endothelial Dysfunction. J Med Chem 64:4396–4409. [4] Ishizuka T, Liu HS, Ito K, Xu Y (2016) Fluorescence imaging of chromosomal DNA using click chemistry. Sci Rep 6:33217. [5] Basile DP, Friedrich JL, Spahic J, et al (2011) Impaired endothelial proliferation and mesenchymal transition contribute to vascular rarefaction following acute kidney injury. Am J Physiol Physiol 300:F721–F733. [6] Yamakoshi H, Dodo K, Okada M, et al (2011) Imaging of EdU, an Alkyne-Tagged Cell Proliferation Probe, by Raman Microscopy. J Am Chem Soc 133:6102–6105. [7] Henriksson S, Groth P, Gustafsson N, Helleday T (2018) Cell Cycle 17:568–579.

Conformation of hemoglobin in red blood cells with a change in the partial pressure of oxygen

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Raman spectroscopy, red blood cells, hemoglobin, heme conformation, oxygen saturation

The conformational and structure changes of heme side-chains of human hemoglobin (Hb) in red blood cells and intact hemoglobin at various oxygen saturation. Using Raman spectroscopy in the range 1000-3000 cm^{-1} it is shown that the conformation of heme and globin in Hb changes with a change in pO_2 and is different for Hb in the cell and Hb in solution. In RBC, the heme conformation changes in the range from 0-15 mm Hg, in the range from 15-60 mm Hg and in the range from 70-120 mm Hg. The conformation of heme, which characterizes the affinity for ligands, decreases and changes similarly for heme Hb in the erythrocyte and Hb in solution, while the changes in the conformation of heme, which characterize the ability of Hb to form complexes with NO, are different. It was found that changes in the heme conformation are accompanied by differences in the conformation of Hb globin both in the cell and in solution. In the cell, with an increase in pO_2, changes in the conformation of globin Hb are caused by an increase in the contribution of vibrations of the H-methine groups of amino acids The dependence of the changes in the conformation of heme in the Hb solution is different. We showed modification in the hemoglobin vibration bands in the CH^2CH_3 stretching bands in the 2800-3100 cm^{-1} region of the symmetric / asymmetric CH, stretch, and symmetric / asymmetric CH_3, stretch of histidine (2850, 2860, 2900 cm^{-1}) and lysine amino acids (2880, 2860 cm^{-1}). In solution, during pO_2 increase, changes in the conformation of globin Hb are caused by a reversible decrease in the contribution of symmetric vibrations of CH-methylene groups of amino acids and the contribution of vibrations of H-methine groups of amino acids Hb. The reported study was funded by RFBR according to the research project Nº 20-34-90073.

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Evaluation of the spectroscopic profile of acute myeloid leukemias in clinical samples and in vitro models

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Raman imaging, acute myeloid leukemia, MOLM14, KG1, chemometrics

Leukemia is a type of blood cancer, characterized by the presence of malignant cells in blood and bone marrow. In case of acute leukemias, depending on progenitor cell line in which cancer transformation occurred, we can distinguish between lymphoblastic and myeloid leukemias[1]. Acute myeloid leukemia (AML) is related to the increased pathological proliferation of immature cells derived from the myeloid line of hematopoiesis. This malignancy mainly affects adults and therefore its incidence increases with age. AML is highly heterogenous disease and several recurrent genetic abnormalities that contribute to this malignancy development have been identified. Raman imaging is considered a promising diagnostic method[2] in the characterization of leukemias because of its sensitivity and high spatial resolution, which enables imaging of subcellular structures and explore the content and distribution of chemical components. This method is non-destructive and does not need earlier sample preparation, which makes it easily applicable in clinical practice. In our studies, we used Raman imaging to characterize leukemia cells derived from model AML cell lines and clinical samples. The goal of the studies was to identify spectral markers characteristic for AML leukemia cells in comparison to normal mononuclear cells (fraction of leukocytes without lymphocytes, n=3). Clinical samples were blasts isolated from patients who suffered from acute myeloid leukemia (type M5 according to FAB classification, with genetic rearrangement MLL (KMT2A-MLLT3), n=2). In addition, two different cell lines were chosen as in vitro models of AML leukemia: – MOLM-14 and KG1 (n=2). MOLM-14 cell line represents AML FAB M5a. Whereas KG1 cell line originates from cells collected from men with erythroleukemia. Cells were measured with Raman spectrometer Witec Alpha 300 conjugated with a confocal microscope. For Raman imaging measurements, we used two different laser lines with excitation wavelengths of 532 nm and 633 nm. In order to distinguish examined cells and to explore spectral differences between them, we used chemometric methods (k-means cluster analysis (KMCA) and principal component analysis (PCA)). As a result, we mainly observed differences in Raman signal derived from nucleic acids in normal mononuclear cells as compared to cancer cells. Additionally, we detected the change of protein-lipids profile of malignant cells.

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[1] K. Kaushansky et al., Williams Hematology ninth edition. McGraw-Hill Education, 2006. [2] A. C. De Luca, S. Managò, G. Zito; Raman microscopy based sensing of leukemia cells; Optics and Laser Technology 108 (2018) 7–16

Characterisation and sub-classification of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) by Raman spectroscopy

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Raman imaging, acute B lymphoblastic leukemia (B-ALL), fusion genes, B cells, chemometrics

B cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common pediatric malignancy that originates from abnormal B cell lymphoid progenitors. This is a very heterogenous disease resulting from several types of molecular abnormalities including copy number alterations, point mutations and gene fusions. The latter frequently define particular subtypes of ALL since they influence the prognosis and the effectiveness of the therapy [1]. Therefore, rapid and sensitive methods for identifying molecular subtype of ALL are more than desirable. Raman spectroscopy (RS) has a chance of becoming a valuable tool for this purpose since it delivers spatial resolution at the subcellular level and provides detailed information about biochemical composition [2, 3]. In this work, Raman imaging and chemometrics were used to characterize and differentiate healthy B cells and leukemic blasts isolated from marrow aspirate of patients suffering from B-ALL. Samples of selected leukemia molecular subtypes (BCR-ABL1 (N=3), TCF3-PBX1 (N=4), TEL-AML1 (N=4)) and B cells isolated from healthy donors (N=5) were measured by Raman microscopy with excitation at 532 nm and 633 nm. Single cell Raman imaging was analysed by k-means cluster method and the obtained mean spectra were successfully classified by principal component analysis (PCA). The results indicated that normal B cells differ significantly from studied leukemic cells. The PCA loading plots revealed that the spectroscopic fingerprint of B lymphocytes is dominated by nucleic acids and proteins signal (795, 1096, 1378 and 1492 cm^{-1}), and it is changed for abnormal cells. B cell and leukemic blasts fingerprint spectral analysis was performed using partial least squares method. The algorithm was trained to recognize B cells among leukemic cells and delivered successful results with high accuracy. Even though B cells differ from BCP-ALL cells, we could not clearly separate spectra of all the particular BCP-ALL subtypes which were studied. The clustering trend was observed in the case of BCR-ABL1 and TCF3-PBX1 cells. These results demonstrate the potential of RS in combination with chemometric analysis for the efficient diagnosis of leukemia in clinical practice. However, to differentiate individual leukemia subtypes, more advanced data mining methods are required. Moreover, patient database need to be enlarged in order to exclude individual variability in each group of leukemia subtype.

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[1] Larson, R.A. Acute lymphoblastic leukemia. In Williams Hematology; McGraw-Hill Education, 2016; pp. 1505–1526 ISBN 978-0-07-183301-1 [2] Managò, S.; Valente, C.; Mirabelli, P.; De Luca, A.C. Discrimination and classification of acute lymphoblastic leukemia cells by Raman spectroscopy. Opt. Sensors 2015 2015, 9506, 95060Z, doi:10.1117/12.2179486 [3] Hassoun, M.; Köse, N.; Kiselev, R.; Kirchberger-Tolstik, T.; Schie, I.W.; Krafft, C.; Popp, J. Quantitation of acute monocytic leukemia cells spiked in control monocytes using surface-enhanced Raman spectroscopy. Anal. Methods 2018, 10, 2785–2791

Spectroscopic characterization of Philadelphia chromosome-positive leukemias

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acute lymphoblastic leukemia, Philadelphia chromosome, Raman imaging, chemometrics

In Poland, 4,184 cases of malady and 3,089 deaths from leukemia were registered in 2018, constituting 2.7% of the total number of deaths from cancer [1]. Early diagnosis and classification of leukemia are expected to increase patients' survival rate. Classification of leukemia is obtained primarily through the morphological and immunophenotypic analysis of cell samples from bone marrow or peripheral blood. However, these methods are expensive and time-consuming. Raman spectroscopy is increasingly applied as a sensitive diagnostic tool which does not require the use of markers. De Luca et al. demonstrated that Raman imaging distinguishes healthy cells from and leukemic lymphoblasts [2]. The Philadelphia chromosome (Ph) is the most common cytogenetic abnormality in adults with acute lymphoblastic leukemia (ALL), accounting for 20-30% of cases. However, it occurs only in 3-5% of pediatric patients [3]. The Ph chromosome results from a reciprocal translocation between chromosomes 9 and 22 (t [9,22] [q34; q11]) that leads to gene fusion BCR-ABL1. The aim of this study was to optimize a methodology aiming identification of the specific biochemical features of Ph-positive cells using Raman spectroscopy and chemometric methods including cluster analysis (CA) and principal components analysis (PCA). Two different ALL cell lines harboring BCR-ABL1 fusion (BV-173, SUP-B15, n=3) as well as blasts collected from patients suffering from Ph-positive ALL (n=3) were analyzed with respect to normal B cells. Two laser lines (532 nm, 633 nm) were used in order to obtain complete information on cell metabolism. Our results show the possible dissection of Ph+ cancer cells from control cells, mainly based on the bands from the base oscillations in nucleic acids (eg. 785 cm^{-1}, 1380 cm^{-1}). Moreover, it was possible to distinguish cell lines BV173 and SUP-B15 that represent different subtypes of leukemia on the basis of spectroscopic differences in the lipid and hemoprotein classes of Raman spectra. The spectral distinction was based mainly on changes in the intensity of bands characteristic for lipids (eg. 1658 cm^{-1}, 1460 cm^{-1}, 1265 cm^{-1}). Our studies show the promising potential of Raman spectroscopy, which can be further develop as a diagnostic tool.

The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

[1] World Health Organization [2] A. C. De Luca, S. Managò, G. Zito; Raman microscopy based sensing of leukemia cells; Optics and Laser Technology 108 (2018) 7–16 [3] Philadelphia chromosome-positive acute lymphoblastic leukemia in childhood, Korean J Pediatr. 2011 Mar; 54(3): 106–110.

Analysis of the molecular structure of the porphyrin system inside "green erythrocytes" using molecular spectroscopy techniques

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Sulfhemoglobinemia, red blood cell, Raman spectroscopy, UV-Vis absorption spectroscopy

Sulfhemoglobinemia is a pathological condition manifested by the excessive presence of sulfhemoglobin (SHb) in the blood. It's the hemoglobin (Hb) derivative containing bounded sulphur atom which cannot be converted back to the functional Hb state [1]. However, despite extensive studies, the exact mechanism of SHb formation is still unclear [2,3]. The modified porphyrin ring absorbs light at 623 nm what causes greenish coloration of blood (and formation of so-called "green erythrocytes"). In this form, Hb is unable to bind oxygen what leads to anaemia and cyanosis [4]. Sulfhemoglobinemia is undoubtedly connected with modification of the porphyrin ring, especially caused by oxidation of the iron ion to the ferric or ferryl states[4,5]. Most of the described sulfhemoglobinemia cases were caused by overdose or continuous consumption of drugs such as phenacetin, dapsone, benzocaine, hydroxylamines what is particularly dangerous in children. In order to precisely describe molecular features of SHb formed inside functional erythrocytes the set of complementary molecular spectroscopy techniques was applied including UV-Vis absorption spectroscopy, electronic circular dichroism (ECD), Fourier-transform infrared (FTIR) and Raman spectroscopy. Vibrational spectroscopy techniques proved to be useful in determination of biochemical profile of red blood cells (RBCs) and its changes in different pathologies [6,7]. Obtained results proved slight variations in geometry and conformation of the SHb adduct formed as isolated protein compared with SHb inside RBCs. Depending on the way of sulfhemoglobinemia induction, e.g., temperature, concentration of sodium sulfide and presence or absence of hydrogen peroxide (H_2O), the amount and type of formed SHb adducts varied. Oxidation stress caused by H_2O_2 and subsequent addition of Na_2S lead to Hb oxidation, what was accelerated by heating to 37 °C. Higher concentrations of Na_2S caused extensive protein aggregation and Heinz bodies formation. Ferric and ferryl forms of Hb formed after exposition to H_2O_2 accelerated formation of SHb, however too high concentration led to hemolysis and Hb degradation. However, in each case, SHb with sulphur atom bounded to the protein part seemed to dominate over the other types of SHb forms with sulphur atom bounded to the iron ion – ferrous-SH and ferric-SH. In summary, presented results shed new light on formation of SHb adducts, however, more extensive studies are required to elucidate, e.g., how modification of the Hb globin part affects porphyrin ring preventing from oxygen binding.

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[1] A. C. Mot, EPR detection of sulfanyl radical during sulfhemoglobin formation –Influence of catalase. Free Radical Biology and Medicine. 2019. [2] C. L. Biancoa, Investigations on the role of hemoglobin in sulfide metabolism by intact human red blood cells. Biochemical Pharmacology. 149 (2018) 163-173, 2018. [3] M. R. Filipovic, Chemical Biology of H2S Signaling through Persulfidation. Chemical Reviews. [4] K. Steven C. C., Hematologic Syndromes: Hemolysis, Methemoglobinemia and Sulfhemoglobinemia. Critical Care Toxicology. 2016. [5] G. Campagna, A Case of Sulfhemoglobinemia Secondary to a Urinary Trackt Infection. CLINICAL AND LABORATORY OBSERVATIONS, J Pediatr Hematol Oncol. 2019. [6] A.Blat, T.Stepanenko, Spectroscopic Signature of Red Blood Cells in a D-Galactose-Induced Accelerated Aging Model, International Journal of Molecular Sciences 22(5):2660, 2021 [7] J. Dybas, Age–related and atherosclerosis–related erythropathy in ApoE/LDLR-/- mice", BBA – Mol. Basis Dis. 1866 (2020) 165972

Can Raman imaging classify abnormal B-lymphoblast? Spectroscopic studies of MLL-rearranged, Philadelphia chromosome- positive and JAK2-mutated B-cell precursor acute lymphoblastic leukemias

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acute lymphoblastic leukemia, Raman imaging, chemometrics

Acute lymphoblastic leukemia (ALL) results from impaired maturation and differentiation of lymphoid progenitors that invade bone marrow, peripheral blood and extramedullary sites. Several genetic alterations have been already found as drivers of ALL, uncovering the genetic heterogeneity of the disease. The presence of specific molecular aberrations including MLL gene rearrangements, BCR-ABL1 gene fusion (Philadelphia chromosome), and JAK2 mutations affect the response to conventional chemotherapy. Next to classical diagnostic techniques like immunophenotyping or karyotyping, Raman spectroscopy is considered as a new promising tool, which can support the diagnosis of leukemia in clinical practice. Raman spectroscopy provides many advantages in biological studies of cancer cells, including those resulting from insignificant water bands intensity, high spatial resolution, photostability, sample non- destructiveness, high sensitivity and possibility to obtain full information of all the chemical components at once.[1] Therefore, in our studies, we used confocal Raman imaging in combination with advanced chemometric analysis to classify pathological B-lymphoblast representing different molecular subtypes of ALL. We performed Raman measurements of ALL cell lines with Philadelphia chromosome (Ph+, SUP-B15 cell line), harboring JAK2 point mutation (MHH-CALL4 cell line) and MLL rearrangement (RS4 11, SEM-K2 cell lines) with the use of two laser excitation wavelengths 532 nm and 633 nm in order to fully explore biochemical diversity of the cell lines studied. To obtain enough statistics, a minimum of 50 cells were measured from each sample and the experiment was repeated a minimum of three times. Our results show that in vitro models of three different subtypes of B-ALL leukemia representing B-lymphoblast with Ph+, MLL, or JAK2 gene mutations display characteristic spectroscopic features of biochemical cell state. Based on our studies, well discrimination can be observed between leukemia cells and normal blood lymphocytes, mainly based on the Raman signals characteristic for nucleic acids (eg. 790 cm^{-1} band). This overall picture demonstrates the diagnostic potential of Raman spectroscopy in combination with chemometric analysis (k-means analysis, principal component analysis, partial least squares regression) for the diagnosis of subtypes of leukemia in clinical practice. Moreover, detailed characterization of cellular metabolism is a key point for further Raman-based studies of already existing and new chemotherapeutic agents' influence and effectiveness.

The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

[1] Managò, S.; Zito, G.; De Luca, A. C., Raman Microscopy Based Sensing of Leukemia Cells: A Review. Opt. Laser Technol. 2018, 108, 7–16. https://doi.org/https://doi.org/10.1016/j.optlastec.2018.06.034.

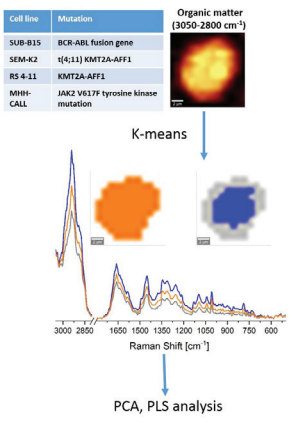

Figure 1. Analysis scheme of a single cell: Upper part: integration image at 3050-2800 cm-1 range, bottom part: a false colour image of whole-cell, cytoplasm and nucleus class and Raman spectra (532nm)

Carotenoid aggregates in liposomes are chiral and stable at liposome phase transition: ECD and ROA study

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Carotenoid aggregation, Liposomes, Chiroptical Spectroscopy, Electronic Circular Dichroism, Raman Optical Activity

Carotenoids are rather unstable compounds that undergo degradation, oxidation and/or isomerization in the presence of light and/or temperature[1]. However, their incorporation into biological membranes stabilize carotenoid structures. A simple model of biomembranes are liposomes, vesicles filled with aqua solutions and surrounded by a lipid bilayer. The presence of carotenoids in membranes, also in liposomes, change their properties including affecting the value of the phase transition temperature of lipids, at which lipids undergo from a gel to liquid crystalline state[2]. The aim of this study was to investigate aggregates of a-carotene in DPPC liposomes and stability of their structures above liposome phase transition temperature using Electronic Circular Dichroism (ECD) and Raman Optical Activity (ROA). Comparing ECD (not shown) as well as Raman and ROA spectra (Fig. 1) it can be concluded that the signature of a-carotene aggregates at different temperatures retain their profile, relative intensities and band wavenumbers. Characteristic bands for carotenoids are related to the C=C stretching vibrations at about 1530 cm^{-1}, C-C stretching vibrations at 1120-1200 cm^{-1}; bending C-CH_3 vibrations in the plane of the polyene chain at 1000 cm^{-1} and the HOOP (hydrogen-out-of-plane) band observed at 960 cm^{-1}. The HOOP signal is significantly more intense in ROA compared to Raman spectra, showing that a-carotene monomers adopt in aggregates a tilted-chain form. We hypothesize that this is the reason for significant stabilization of a-carotene assemblies in liposomes making them stable even when considerable reorganization of liposomes at the phase transition temperature occurs.

This work was supported by the National Science Centre Poland (project 2017/25/B/ST4/00854)

[1] G.Britton, FASEB J., 1995, 9, 1551-1558. [2] A. V. Popova, A. S. Andreeva, Carotenoid–Lipid Interactions, w: Aleš Iglič, Julia Genova, Advances in Planar Lipid Bilayers and Liposomes, 2013, 17, 215-236.

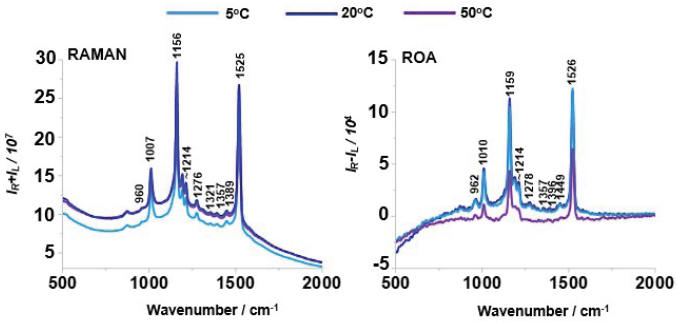

Figure 1. Raman and ROA spectra of DPPC liposomes containing 1% a-carotene in different temperatures: 5oC, 20°C and 50°C.

Raman portable device to distinguish conventional and transgene cotton seed genotyes

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Pattern recognation, Agricultural, Portable device

The use of Raman spectroscopy [1] combined with multivariate pattern recognition techniques have shown to be promising for investigating genetically modified organisms (GMO) [2]. In Brazil, cotton is grown in humid tropical conditions which demands the used of large amounts of phytosanitary chemicals [3]. Genetic improvement can be carried out to produce species tolerant to herbicides, resistant to fungi and insects, or even provide greater productivity and better quality. Even with these advantages, it is necessary to manage and limit the contact of transgenic species with the native ones, avoiding possible contamination and/or possibility of extinction of conventional species. The conventional identification of the presence of GMOs is based on complex DNA-based analysis, which could be laborious, expensive, and time-consuming. In this context the present work intends develop a new methodology using a portable Raman device and partial least squares discriminant analysis (PLS-DA) to distinguish conventional and transgenic cotton seed genotypes. Two genotypes of cotton seeds, Gossypium L., one transgenic and one conventional variety, were analyzed. Due to the high fluorescence in Raman profiles, 20 seeds (10 conventional and 10 genetically modified) were sectioned in the longitudinal direction on two opposite sides for spectral acquisition (Figure 1). Four spectra were obtained for each seed, with two replicates for each face of the seed. Each side of the seed was represented by the average of the two spectra. The data matrix was composed of 20 spectra from each genotype variety. The spectral measurements were acquired using the portable Raman spectrometer Metrohm Mira Ra DS, with a spectral range from 400 to 2300 cm-1, spectral resolution from 8 to 10 cm-1. The equipment has a single 785 nm (± 0.5 nm) laser and laser power of approximately 100 mW and USB 2.0 interface with data transmission power supply with USB cable. The Raman methodology developed in this work are very promising for discriminate between conventional and transgenic cotton seeds. Adequate values ??of sensitivity and specificity, and low classification errors was reached (0.0%). The use of portable Raman spectrometer demonstrated that this method is capable of providing individual information about the seeds and the equipment can be used for in field analysis.

The authors would like to thank the Brazilian Embrapa (SEG 20.20.00.120.00.00 and 30.19.00.135.00.00), Brazilian agencies CNPq and CAPES for scholarships support for this work, PROPESQ/UEPB (1.06.04.00-6-398/2017-1) for the funds granted for the research and NUQAAPE (Advanced Analytical Chemistry Nucleus of the State of Pernambuco) .

[1] R. Baranski, M. Baranska, Discrimination between nongenetically modified (Non-GM) and GM plant tissue expressing cysteine-rich polypeptide using FT-raman spectroscopy, J Agric Food Chem . 2008 Jun 25;56(12):4491-6. [2] W. Xu, X. Liu, L. Xie Y. Ying, Comparison of fourier transform near-infrared, visible near-infrared, mid-infrared, and Raman spectroscopy as non-invasive tools for transgenic rice discrimination, Transactions of the ASABE 57(2014) 141-150. [3] A. Nega, Review on Concepts in Biological Control of Plant Pathogens, Journal of Biol., Agric. and Health. 4 (2014) 33-55.

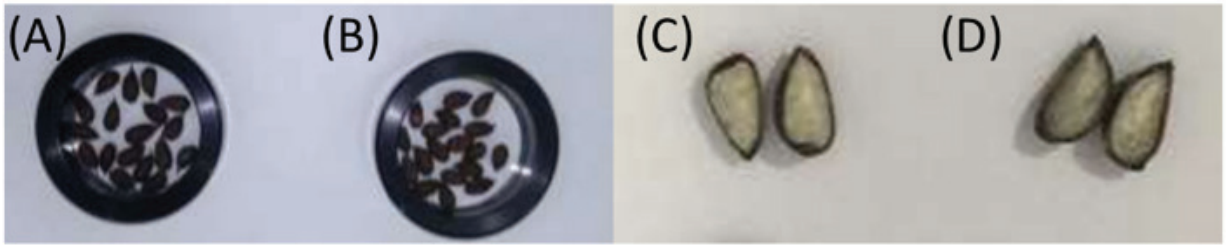

Figure 1. Cotton seeds genotypes from (A and C) conventional and (B and D) modified genetically used modified used for Raman analysis.

DFT study of carotenoid Raman spectra: dependence on the conjugation length, structure of the end/side groups and isomer type

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Density functional theory, Carotenoids, Antioxidants, Raman shift

Carotenoids are involved in numerous vitally important biochemical processes in plants and in a human body. The carotenoid properties, including the bioavailability, the provitamin A activity and antioxidant characteristics, depend on both the chemical structure and the isomeric composition. Thus, it is important to distinguish various carotenoids as well as various isomers of carotenoids in biological tissues, food, food additives and carotenoid-containing cosmetics. Lycopene, α-, β-, γ-carotenes are among the most important carotenoids for humans. These carotenoids have the same chemical formulae C40H56 but different structures, including the molecule symmetry, the conjugation length, the number of stable conformations and the number of cis-isomers. Raman spectroscopy is a highly sensitive technique to analyze carotenoids due to the resonance enhancement of their Raman bands under the excitation in the blue spectral range. One of the promising directions in this field is in vivo analysis of carotenoids in human skin [1]. DFT studies were shown to be very effective for analysis of vibrational spectra of organic molecules, especially in the case of substances with low photo-, thermal and oxidation stability like carotenoids. In this work, we present DFT analysis of Raman spectra of four carotenoids: lycopene, α-, β-, γ-carotenes, as well as study of the spectra of polyenes of various lengths and with various end and side groups. We showed that the peak positions, profiles and intensities of the Raman bands depend greatly on the conjugation length, structure of the end/side groups and type of the isomer. An increase in the conjugation length results in the low-wavenumber shifts of the most intense peaks in the region of the C-C and C=C vibrations with simultaneous increase in the intensities. Vibrations of both end and side groups affect the region of the C-C and C=C stretching vibrations. The ionone rings have a greater influence on this region than the lycopene-type end groups. The C-C and C=C stretching bands shift and split in the spectra of carotenoid cis-isomers. The isomer spectra change non-monotonously with variation in the position of the cis double bond [2]. Our DFT results proved that Raman spectroscopy is a powerful tool to analyze the carotenoid chemical and isomer compositions. The results of this study can be also applied for interpreting the Raman spectra of polyacetylene and polyene sequences in thermo- or UV degraded polyvinylchloride.

[1] M.E. Darvin, M.C. Meinke, W. Sterry, J. Lademann. Optical methods for non-invasive determination of carotenoids in human and animal skin. Journal of Biomedical Optics, 18(6): 061230-(1-9), 2013. [2] V.S. Novikov, V.V. Kuzmin, S.M. Kuznetsov, M.E. Darvin, J. Lademann, E.A. Sagitova, L.Yu. Ustynyuk, K.A. Prokhorov, G.Yu. Nikolaeva. DFT study of Raman spectra of polyenes and ß-carotene: dependence on length of polyene chain and isomer type. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 255: 119668, 2021.

Aggregation determination of bacterial amyloid signaling motifs using FT-Raman spectroscopy

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FT-Raman, Amyloids, Aggregation

Amyloids are fibrillar protein aggregates commonly associated with severe neurodegenerative diseases. However They can perform many physiological functions across almost all domains of life. In this study we aimed to characterize amyloid properties of fungal and bacterial amyloid signaling motifs (BASS). BASS motifs occur specifically in filamentous bacteria such as Actinobacteria, Cyanobacteria and Euryarchaeota, where they might play a role in an apoptosis processes [1]. It was found recently that BASS motifs are capable to form fibrils in vitro, yet the mechanism of fibril assembly is not fully understood and may be highly affected by environmental conditions. We showed Fourier-Transform Raman (FT-Raman) spectroscopy can provide insight into structure of amyloid fibers formed by BASS motifs. Secondary structure investigations have been carried out on the dataset of BASS sequences detected by a novel machine learning grammatical model [2]. Peptides were synthesized with >95% purity and lyophilized. Peptides were dissolved in the initial solvent (NaOH) and next diluted by adding the phosphate buffered saline to adjust the mixed solution to pH 7.4. FR-Raman measurements were performed on golden plates. We confirmed the aggregation of BASS peptides based on the presence of an intensive and narrow Amide I band at 1670 cm-1 associated with beta structures. Furthermore analysis of Amide III band revealed that analyzed peptides possessed beta solenoid conformations reported before for the HET-s and PrPSc prion motifs. Obtained results were supported by ATR-FTIR and AFM measurements, as well as Congo Red assays.

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[1] Dyrka W, Coustou V, Daskalov A, Lends A, Bardin T, Berbon M, Kauffmann B, Blancard C, Salin B, Loquet A, Saupe SJ. Identification of NLR-associated Amyloid Signaling Motifs in Bacterial Genomes. J Mol Biol. Nov 432, 6005 (2020) [2] Dyrka, W., Gąsior-Głogowska, M., Szefczyk, M. et al. Searching for universal model of amyloid signaling motifs using probabilistic context-free grammars. BMC Bioinformatics 22, 222 (2021)

2.5. Archeology and Cultural Heritage

Sub-Surface Molecular Investigation of Cultural Heritage materials with advanced Raman spectroscopy methods

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Raman Spectroscopy, Micro-Spatially Offset Raman Spectroscopy (micro-SORS), Time-gated methods, Non-invasive methods, Heritage Science

Recent advances in Raman spectroscopy methods and techniques are making a major impact on the cultural heritage field. These include Spatially Offset Raman Spectroscopy (SORS) [1], developed in 2005 for biomedical and security applications, and transformed for heritage field in 2015 through its extension to the microscale, micro-SORS. Over the last several years, micro-SORS analytical capability has been explored, confirming its valuable contribution to the non-invasive investigation of compounds located below the surface, for instance, in a hidden painted layer, in a preparation layer or in a substrate (i.e. plaster) [2]. A natural next step for the micro-SORS research in heritage sciences comprise the development of a portable micro-SORS instrument, unlocking thus non-invasive and in-situ analyses. To face this new challenge two prototypes have been demonstrated, based around a modified conventional portable Raman instrumentation. Using these encouraging preliminary results have been recently obtained on a Pinacoteca di Brera painting; a layer sequence has been reconstructed in selected areas, down to the preparation gypsum layer [3]. One of the latest research streams includes the monitoring of the diffusion of an agent into a matrix, which is an important situation in Cultural Heritage, for example, in cases where conservation or decay products diffuse into a plaster or stone. Micro-SORS demonstrated a capability to "observe" the absorption and diffusion processes non-invasively, providing essential information about the penetration depth of a product [4]. The method is effective both in simple situations, where the matrix has constant concentration as well as with more complex samples, where the concentration of the matrix varies with depth. Through a collaboration between CNR-ISPC (Italy), Nottingham University (UK) and the Rutherford Appleton Laboratory (UK), proof-of-concept experiments have been recently carried out coupling micro-SORS with Time-Gated Raman Spectral Multiplexing method. Time-domain methods allow separating Raman signal from fluorescence background using an ultrafast laser pulses and fast temporal gating. The combination of the methods permits to acquire Raman signal from fluorescent subsurface domains, enabling fast molecular Raman mapping of fluorescing and non-fluorescing samples with sub-millimeter depth resolution [5]. In this presentation, a "brushstroke" of these advanced Raman spectroscopy methods will be given, underlining their impact in heritage science.

Pavel Matousek (RAL) Ioan Notingher, Christopher Corden (Nottingham University -UK)

[1] S. Mosca, C. Conti, N. Stone, P. Matousek, Nat. Rev. Methods Primers 2021, 1, 21. [2] C. Conti, A. Botteon, C. Colombo, D. Pinna, M. Realini, P. Matousek, J. Cult. Herit. 2020, 43, 319. [3] A. Botteon, C. Colombo, M. Realini, C. Castiglioni, A. Piccirillo, P. Matousek, C. Conti, J. Raman Spectrosc. 2020, 51, 2016. [4] A. Botteon, J. Yiming, S. Prati, G. Sciutto, M. Realini, C. Colombo, C. Castiglioni, P. Matousek, C. Conti, Talanta 2020, 218, 121078. [5] C. Corden, P. Matousek , C. Conti, I. Notingher, Appl. Spectrosc. 2021, 75, 156.

Cultural Heritage at SISSI-Bio: from paleo-archeology to musical instruments

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Cultural Heritage, Synchrotron Radiation FTIR microscopy, Human evolution, ancient inks, musical instruments

The exploitation of Synchrotron Radiation (SR) for the investigation of samples of historical and artistic importance has been increasing over the past years, and experiments related to Cultural Heritage (CH) have been routinely performed at many beamlines of Elettra (Trieste, Italy). The present contribution focuses on the most recent achievements at SISSI-Bio infrared beamline in CH field, from paleo-archaeology to Roman inks and musical instruments. The brilliance advantage of infrared synchrotron radiation was fundamental for better understanding human evolution. Minute residues on backed lithic tools (Uluzzian technocomplex at Grotta del Cavallo, southern Italy), from ~45,000-40,000 ya, were identified by FTIR microscopy to be a mixture of plant/tree gum and beeswax intentionally mixed with ochre and applied as adhesive. The results allowed confirming the earliest evidence for mechanically delivered projectile weapons in Europe [1]. Instead, the combined SEM/FTIR analysis of wear traces and use-related biogenic residues was the key strategy for the identification of starch-grains on ground stones from Pontic Steppe, dated back ~36,000 ya. FTIRallowed distinguishing characteristic features of modern and ancient starches, discriminating them from environmental contaminants [2,3]. Sometimes prehistoric objects are not only tools but could have had symbolic purposes. This was the case of white-tailed eagle talons worn by Neanderthals in Krapina (HR) around 130,000 ya. FTIR inspection of one of the talons revealed the presence of a collagen fibre, probably a residue of the string of a necklace, and probable traces of pigments, thus confirming the symbolism capabilities of Neanderthals [4]. Moving to a closer time period, a multi-instrumental approach of highly sensitive SR-based techniques was used to provide information on the original composition of a dry black ink powder found in a bronze inkwell of the first century AD [5]. Finally, we will present results on micro- and nano- spectroscopic investigations that has been carried out on cross-sectional samples collected from two of the most important violins produced by Antonio Stradivari, the Toscano (1690) and the San Lorenzo (1718), representative of two different periods of Stradivari's manufacturing [6]. The ability of infrared spectroscopy to unveil secrets of formulations and pigments nature is well-established, but many relevant artistic and historical questions are still open, and demands for multi-technique approaches and innovative strategies.

[1] Sano K., et al., The earliest evidence for mechanically delivered projectile weapons in Europe. Nature Ecology & Evolution, Volume 3, 2019, 1409–1414. [2] Birarda, G., et al., Direct morpho-chemical characterization of elusive plant residues from Aurignacian Pontic Steppe ground stones. https://doi.org/10.1101/2020.07.23.212324 [3] Longo, L., et al, At the origins of starch diet. A multidimensional approach to investigate use-related biogenic residues on stone tools. Environmental Archaeology (in press). [4] Radovčić D., et al., Surface analysis of an eagle talon from Krapina, Scientific Reports, 2020, 10:6329 [5] Sibilia M., et al., A multidisciplinary study unveils the nature of a Roman ink of the I century AD., Scientific Reports, 2021, 11:7231. [6] Fiocco G., et al, Reflection FTIR spectroscopy for the study of historical bowed string instruments: Invasive and non-invasive approaches, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 245, 2021, 118926.

18th Century Chinese artefacts enamelled with European technologies: On-site Raman identification of technology transfer

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Mobile Raman microspectroscopy, Enamels, Masterpieces, Non-invasive analysis, China

The miniaturization and development of the laser sources, optical systems and computer performance in general use for analytical experiments has profoundly changed scientific instrumentation over the past decade. It is possible to analyze the objects on site as well as open-air which revolutionizes the analytical approach to the non-invasive analysis of premium quality objects. Two mobile non-invasive analytical techniques that are particularly suited to the study of coloured materials are X-ray fluorescence spectrometry and Raman microspectroscopy. China has now become the leading factory of the world, but in many cases the artefacts produced remain dependent on non-Chinese technologies. To the best of our knowledge, it was also the same in the past. For instance, the common opinion that 'the Chinese invented porcelain' is true, but it gets more complicated when it comes to the enamels. Realistic enamelling, the creation of a chemically stable, sophisticated glassy decoration, comparable to a painting on a substrate of metal, glass or porcelain, appeared with majolica in Europe during the 16th century. The technological enamelled marvels (watches, clocks, etc.) introduced by the Jesuits at the Japanese and Chinese Courts led to the request of the Asian sovereigns that these objects could also be manufactured "at home". As the technologies used remain inscribed in the objects, alongside the study of the information present in historical documents, the analysis of their enamels makes it possible to compare the evolution of technologies and to identify Chinese objects (and Japanese) made with European ingredients or recipes.[1-3] The very high value or the scarcity of these objects requires non-invasive analyses carried out where the objects are being stored, by mobile techniques. Three colours in particular make it possible to trace the arrival of European recipes/ingredients: blue made of arsenic-based cobalt, yellow (lead pyrochlore solid solution also called Naples Yellow) and pink / red / violet made by gold nanoparticles (Perrot' red and Cassius' purple). The authors warmly thank B. Quette, C. Delery, and V. Mesqui Curators, and the Directors and staff of the Musée des arts décoratifs (UCAD, Paris), the Musée national des arts asiatiques-Guimet (Paris) and the Cité de la Céramique (Sévres) for their help and the possibility of analyzing the objects in their collections.

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[1] Colomban, Ph.; Kirmizi, B.; Zhao, B.; Clais, J.B.; Yang, Y.; Droguet, V. Herit. 2020, 3 (3), 915–940. [2] Colomban, Ph.; Kirmizi, B.; Zhao, B.; Clais, J.B.; Yang, Y.; Droguet, V. Coatings, 2020, 10 (5), 471 [3] Colomban, Ph.; Kirmizi, B.; Simsek Franci, G. Minerals, 2021, submitted.

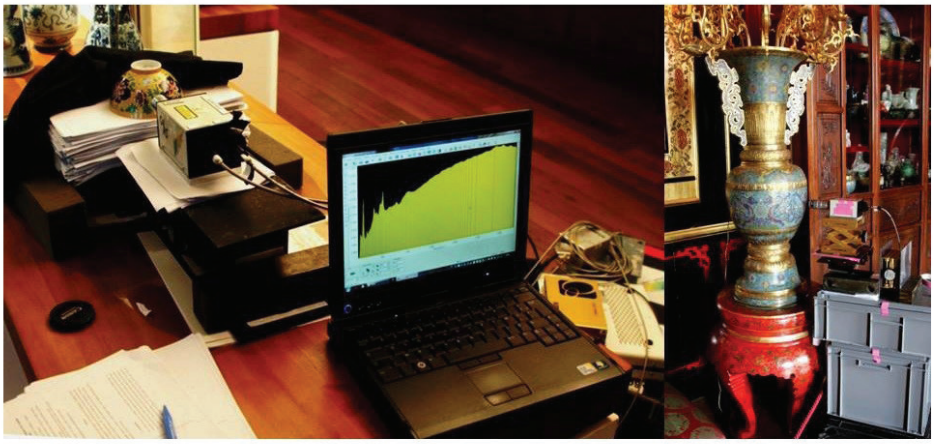

Figure 1. Mobile Raman μanalysis

Micro ATR-FTIR spectroscopic imaging: a technique to discover the conservation history hidden in the stratigraphies painted on terracotta statues

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Micro ATR-FTIR spectroscopic imaging, Paint layers, Metal soaps, Lead white, FT-IR spectroscopy

Micro ATR-FTIR spectroscopic imaging is a powerful analytical technique which can identify and localize compounds down to the microscale. Its applications in heritage science embrace the study of original materials, the investigation of decay processes and the support to up-to-date conservation strategies. Spring et al. have demonstrated the potential of micro-ATR-FTIR spectroscopic imaging to unravel complex chemical information in samples from paintings, including the detection of pigments, binders and lead soaps [1]. The latter are decay products formed by the reaction of lead ions of lead-based pigments and fatty acids of organic binders. Their formation depends on complex causes and humidity is acknowledged to be one of them [2]. Nowadays, research on lead soaps is constantly growing and micro-ATR-FTIR spectroscopic imaging has been used to study lead soaps in several organic matrices, including lipidic and terpenic ones [2]. In our study, micro-ATR-FTIR spectroscopic imaging has been applied to the complex stratigraphies of cold painted terracotta statues (Sacred Mount, Varallo, UNESCO) constantly exposed to humidity and the focus was to: 1) identify IR-active lead-based pigments and lead soaps in complex paint layers; 2) explore the correlation between the exposure of selected paint layers to humidity with the formation and localization of lead soaps [3]. The results showed that different types of lead white pigments (lead carbonate, basic lead carbonate, and a mixture of them) are combined with other pigments and localized in layers made in different periods. All of these lead white pigments reacted with the binder but a different extent of decay is observed in different layers. These differences have been discussed in the light of: i) how the exposure to the same environmental moisture affected the formation and localization of lead soaps in different paint layers as well as ii) hydrophilic layers applied during past restorations act as additional moisture source within the stratigraphy to influence the formation of lead soaps. This innovative application of micro-ATR-FTIR spectroscopic imaging sheds new light on the complex conservation history of these artefacts highlighting the link between the decay of paint layers with the manufacturing technique, the build-up of layers and past restorations. Such results, rising from vibrational spectroscopy and chemical data, unveil the information hidden in materials of cultural heritage allowing a holistic knowledge of the artefacts.

[1] Spring M, Ricci C, Peggie DA, Kazarian SG (2008) ATR-FTIR imaging for the analysis of organic materials in paint cross sections: case studies on paint samples from the National Gallery, London. Anal Bioanal Chem 392:37–45 . https://doi.org/10.1007/s00216-008-2092-y [2] Casadio F, Keune K, Noble P, van Loon A, Hendriks E, Centeno S, Osmond G (2019) Metal Soaps in Art. Springer International Publishing, Cham [3] Possenti E, Colombo C, Realini M, Song CL, Kazarian SG (2021) Insight into the effects of moisture and layer build-up on the formation of lead soaps using micro-ATR-FTIR spectroscopic imaging of complex painted stratigraphies. Anal Bioanal Chem 413:455–467. https://doi.org/10.1007/s00216-020-03016-6

Advanced analytical investigation of ancient wall-painting fragments discovered in the Roman Baths from Alburnus Maior

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Roman wall paintings, pigments, FTIR, hyperspectral imaging, X-ray techniques

Several wall-painting fragments discovered in the Roman Baths from the archeological site Alburnus Maior (Roşia Montană, Romania) were analyzed in this study with the aim to investigate the material composition of both plasters and pictorial layers. Dated from the beginning of the 2nd century AD, these rare findings stand among the oldest examples of preserved decorative polychrome painting on plaster excavated so far on the former territory of the Roman province of Dacia. For the study a minimally invasive methodology based on X-ray fluorescence (XRF), X-ray Powder Diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Hyperspectral Imaging (HSI) was used. Results of the carried investigations are expected to highlight the general color palette used in this part of the Roman Empire, provide details on the pictorial technique, choice of materials, as well as possible raw materials sources used by local craftsmen. Chemical and mineralogical information obtained are discussed in the light of the archeological context, as well as of previously studies carried on wall-painting fragments from the same period, excavated from other nearby Roman archeological sites.

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Infrared and X-ray spectroscopies study on ancient rock art from northern Thailand

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ancient rock art, pigment, Pratu Pha, FTIR, SEM-EDS

There are lots of rock art found in many parts of Thailand, especially the north. They represent a piece of important cultural information, moreover, their styles and materials used in the production of arts have been interested. In this work, selected ancient rock art samples from the Pratu Pha Valley site at Lampang province in northern Thailand have been studied on the structure and composition using a scanning electron microscope coupled with an energy dispersive X-ray spectrometer (SEM-EDS) and infrared absorption spectroscopy (IR). It has been found that C, O, Al, Si, Ca, and Fe are present in the pigment of all samples, while Mg, K, S, and Mn have been detected in some samples. The constituents of functional groups corresponding to the wavenumber have been confirmed by the IR spectra. These results indicate the origin of the samples. Some complex chemicals for conservation have been found in these archaeological pictures.

Kasetsart University (Department of Earth Sciences, Faculty of Science) Chiang Mai University (Center of Excellence in Materials Science and Technology) Silpakorn University (Department of Archaeology).

2.6. Forensic and Security Sciences

Is it possible to detect counterfeited edible birds' nests (EBN) products by means of FTIR spectroscopy?

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counterfeited food, edible bird nests, FTIR spectroscopy

Edible birds' nests (EBN) are the part of traditional Chinese cuisine and medicine. They are made by cave-dwelling birds called swiftlets. As they are considered a culinary delicacy, their price can reach over 4000 USD per kilogram. EBN have been scientifically proven to have nutritional, anti-aging and antiviral properties; they also show improvement in bone strength cell division [1,2]. However, recent studies have also highlighted EBN related health risks. High nitrite content was found in EBN collected inside caves, what increases the risk of cancer due to the increased production of carcinogenic nitrosamines [3]. In 2013, to combat this, China imposed a trade ban on EBN with some South East Asian countries (including Malaysia) until healthier harvesting practices were adopted. Only a few producers were able to meet requirement. The low supply drove up the prices of EBN. Some producers, in order to meet demand, allegedly used adulteration techniques to add impurities to increase the weight of the final product. Common adulteration practices include mixing: tremella fungus, karaya gum, fried pork skin, soybean, red seaweed, egg white with EBN. Bleaching is another adulteration process used to ease cleaning, and make feathers invisible. Bleaching, however, introduces semicarbazide to EBN, a recognized food hazard according to the Journal of Food Protection [4]. The need for authentication techniques of EBN is vital to its industry and the health of consumers. As a result, there has been a surge in research over the verification of EBN. In this talk, based on our own experiments performed on authentic and counterfeited EBN, I will try to answer the question whether FTIR spectroscopy can be used as an accurate method of determining the authenticity of EBN.

The authors acknowledge the Singapore Synchrotron Light Source (SSLS) for providing the facility necessary for conducting the research. The Laboratory is a National Research Infrastructure under the National Research Foundation Singapore. Experiments were performed under the IAEA research project F11021: "Enhancing Nuclear Analytical Techniques to Meet the Needs of Forensic Sciences

[1] Matsukawa, N., Matsumoto, M., Bukawa, W., Chiji, H., Nakayama, K., Hara, H., et al. (2011). Bioscience, Biotechnology, and Biochemistry, 75(3), 590–592. [2] Kim KC, Kang KA, Lim CM, Park JH, Jung KS and Hyun JW, J Korean Soc Appl Biol Chem 55:347–354 (2012). [3] Katan, M. B. (2009). American Journal of Clinical Nutrition, 90, 11–12. [4] Xing, Y.-N., Ni, H.-G., Chen, Z.-Y. (2012). Journal of Food Protection, 75(9), 1654–1659.

BREAKING WITH TRENDS IN BLOODSTAINS AGE DETERMINATION: A LIKELIHOOD RATIO-BASED APPROACH

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Bloodstains, Raman spectroscopy, Forensic dating, Likelihood ratio, Comparison problem

A batch of evidence that has gained prominence in courtrooms are bloodstains (BS), often the main driving force behind an investigation process. However, in the era of DNA testing, it gets forgotten that verifying the BS donor is not always the most critical issue. Thus, to increase the evidential value of the trace, it is necessary to demonstrate a unity of time and place, proving that a suspect was on the crime scene in a given time [1]. This can be done by providing the final piece of the forensic puzzle – information about BS deposition time. Despite the nearly century-old research efforts [2, 3], a reliable method for estimating BS age is still missing. Having looked into previous examples of dating studies, it can be concluded that it is the wrong approach for data analysis, which should be blamed for the delayed exploitation of developed methods in forensic practice. According to the universally adopted strategy, most of proposed dating techniques have been guided by a simple principle – they have sought the dependency between some dynamic properties of degrading blood and time, usually through the regression analysis. The aging process, however, is not only a matter of time. No two two degradation pathways of blood deposits are precisely the same; hence, using dating models trained on the very limited datasets might lead to misestimations of BS age, depriving these conventional methods of practical value. Thus, the solution should be sought elsewhere. Perhaps by looking at the dating issue from a different angle.Impediments resulting from aging kinetics' variability could be addressed by substituting a case-suited comparison problem for the conventional dating approach. The key aspect of this concept, discussed during the presentation, is the likelihood ratio-based estimation of the (dis)similarity [4] between the stage of evidence decomposition and sets of reference materials obtained by supervised aging. Meaning that every dating procedure would be constructed on a case-by-case basis to fit the examined traces. To enable this comparison, the information inherent to changes accompanying blood aging also has to be delivered. Within this study, Raman spectroscopy was proposed as a tool for characterizing the state of BS degradation, which once again proved to be a powerful technique in the examination of hemoproteins [5]. We would like to pay our gratitude to our colleague and co-author of this study Prof. Gianmario Martra who passed away in September of 2020.

[1] C. Weyermann, O. Ribaux, Sci Justice, 52 (2012) 68. [2] R.H. Bremmer, K.G. de Bruin, M.J.C. van Gemert et al., Forensic Sci Int, 216 (2012) 1. [3] G. Zadora, A. Menżyk, TrAC, 105 (2018) 137–165. [4] G. Zadora, A. Martyna, D. Ramos, C. Aitken, Evidential Value of Multivariate Physico-chemical Data, Wiley, 2014. [5] C.G. Atkins, K. Buckley, M.W. Blades, R.F.B. Turner, Appl. Spectrosc. 71 (2017) 767–793.

Raman spectroscopy guided analysis of non-diagenetic areas of tooth archaeological samples

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Raman spectrosocopy, archaeology, tooth, diagenesis, elemental analysis

Bones and teeth from buried human remains are often the only tissues that withstand the changes induced by external and environmental stressors over a long period and thus are available for archeological studies. However, these samples are prone to diagenetic changes due to chemical and microbial exposures that may affect scientific analysis. It can be difficult to detect the extent of diagenesis and a methodology to identify and avoid localized areas of diagenesis can save a lot of time and efforts in obtaining biogenic signals from viable areas in archaeological samples. Raman spectroscopy (RS) has emerged as a method of choice for nondestructive and rapid analysis of biological samples in the recent past. Being non-destructive and sensitive to chemical/biochemical perturbations, RS can be a suitable tool to identify viable areas in teeth for subsequent analysis. Raman has already been used to explore caries and stress-signatures in teeth samples. In this study, we explored RS on archaeological teeth (n = 2) to identify diagenetic regions. WITec Alpha 300 confocal microscope was used to obtain Raman maps at 532 nm (63X objective). Raw spectra were subjected to cosmic-ray correction, filtration and area normalization and analyzed through multivariate methods including K means cluster analysis using WITec Project 5.2 software. Average spectra of diagenetic regions were compared with normal teeth. Raman findings were corroborated with white light microscopy and elemental analysis to compare biogenic elements of interest. Enamel was found to be particularly resistant to diagenesis due to hardness and low porosity. Major bands in enamel corresponded to phosphate and carbonates. Dentine regions showed alterations with varying degrees of diagenesis in different areas. Significant changes were observed in the Amide III, Amide I and CH deformation bands compared to enamel and healthy teeth in the regions of diagenesis. The findings indicate the efficacy of RS-guided analytical exploration of archaeological tooth samples. Further studies with complementary techniques can also reveal the indicators of diagenesis and help understand the geobiochemical information of burial grounds as well as prevalent burial customs.

Selective androgen receptor modulators studied by vibrational and chiroptical spectroscopy

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chiroptical spectroscopy, vibrational spectroscopy, selective androgen receptor modulators, SARM, DFT calculations

Selective androgen receptor modulators (SARMs) have begun to attract increased attention in recent years. They have been designed for treatments of various diseases, such as cancer, osteoporosis, Alzheimer's disease and others. The effects of SARMs on the human organism are similar to anabolic steroids, while their side effects are generally considered to be less serious. SARMs have been known for being abused in the professional sport, not to mention a significant increase in their popularity among amateur sportsmen and bodybuilders during the last decade. Many dietary supplements containing SARMs are available on the Internet and they are presented to potential customers as a safe alternative to anabolic steroids for rapid muscle growth and/or fat burning. However, their unsupervised usage may lead to serious health problems including liver damage or heart attack. Another health risk is caused by an uncertain composition of the preparations from suspicious sources, as the manufacturing process in these cases usually does not meet required quality standards. For these reasons, rapid and reliable methods for the analysis of SARMs are called for and vibrational spectroscopy offers a suitable choice. The present work is focused on the analysis and identification of SARMs (for instance ostarine, andarine, ligandrol) in real samples from police seizures by means of Raman and infrared spectroscopies. Detailed knowledge of the 3D structure of compounds with such important physiological effects that SARMs exhibit is very important for a better understanding of their mechanism of transport and biological activity in the human organism. For this purpose, chiroptical spectroscopy, namely Raman optical activity, vibrational circular dichroism and electronic circular dichroism, supported by ab initio density functional theory calculations were applied for the detailed description of stable conformers of selected SARMs in solutions. These results will be further used for studying more complex systems simulating important bioinspired interactions of the studied substances.

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Spectroscopy as a useful tool for identifying changes over time in post-mortem vitreous humor samples.

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ATR FT-IR spectroscopy, vitreous humor, forensic, post-mortem analysis, PCA

Vitreous humor (VH) is an alternative matrix commonly used in forensic analysis mainly due to its high resistance to degradation in post-mortem processes, and thus longer availability for analysis. Currently, the most important forensic application of VH is the identification of the time elapsed since death based on biochemical changes in its composition [1]. Thus, techniques are needed that can effectively detect and identify these changes. An additional benefit for the analysis of forensic-relevant samples would be the use of simple, fast, minimally destructive, and relatively portable techniques. These requirements can be met with Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, which has already proven its potential to detect biochemical changes over time in post-mortem VH samples [2]. The aim of this study was to investigate changes occurring in vitreous humor samples during 30 days of storage at three temperatures of -20, 4 and 20°C. Bovine vitreous humor samples were used. The samples were deposited on microscope slides and, after 24 hours of drying at room temperature, scraped with a scalpel, and measured on a diamond crystal ATR-FTIR spectrometer. In the obtained ATR-FTIR spectra, changes in the intensity and shifts were observed for the amide I band at ~1640 cm-1 and for the bands assigned to COO- groups of free amino acids located at ~1414 and ~1590 cm-1. The described changes occurred fastest at 20°C - from day 18, then at 4°C - from day 24, while at -20 °C slight changes were visible only on the spectra obtained on the 30th day of storage. These results were confirmed by statistical analysis using Principal Component Analysis performed on the second derivatives of the spectra. For temperatures of 20 and 4°C, a clear distinction of spectra into two groups of earlier and longer time points was observed, while for samples stored at -20°C, only the last group of samples obtained on day 30 was distinguished. The obtained results confirmed that ATR-FTIR technique can be successfully used to analyze the biochemical changes occurring in vitreous humor samples over time. It has been shown that storing samples for a month, even at -20 °C, does not protect them from certain changes in proteins and amino acids. Moreover, the enormous potential of ATR-FTIR method to identify post-mortem changes in tissues and the possibility of their correlation with the post-mortem interval should be indicated.

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[1] Zhang J, Wei X, Huang J, Lin H, Deng K, Li Z, Shao Y, Zou D, Chen Y, Huang P, Wang Z (2018) Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectral prediction of postmortem interval from vitreous humor samples. Anal Bioanal Chem 410:7611-7620. [2] Wójtowicz A, Wietecha-Posłuszny R, Snamina M (2020) Contemporary Trends in Drug Analysis of Vitreous Humor: A critical review. TrAC Trends in Analytical Chemistry 129:115935.

Data fusion approaches in the spectroscopic determination of bloodstains age

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Bloodstains, Forensic dating, Infrared spectroscopy, UV-Vis spectroscopy, Data fusion

Despite significant efforts over the past decades, the forensic practitioners' quest to establish a;method for estimating bloodstains (BS) deposition time still ends in failure [1,2]. Whilst analytical methods proposed to date evidently demonstrate that the physicochemical characteristics of BS alter with time, the challenge remains in translating these findings into forensic practice. In the case of blood evidence, information on the temporal dimension may prove helpful in many ways, depending on the staging of the criminal procedure [3]. During the investigative phase, when circumstances of a crime remain unclear, the time of BS formation can establish the timeline of events or verify the relevance of preserved traces. Obviously, prompt response to these questions would require (preferably) an on-site dating tool to deliver reliable answers of high accuracy. A single technique, however, may not ever be capable of providing these levels of resolution. Thus, research should investigate "multiplex" approaches of complementary techniques applied simultaneously. Such an approach might not only benefit from improving accuracy but also enable an in-built cross-checking. In the present study, the potential of non-destructive or low-invasive spectroscopic methods to provide information on blood chemical modifications over time was investigated. Capillary blood was deposited onto non-absorbent substrates and stored under laboratory conditions. The aging of BS was followed for 16 days by measuring attenuated total reflection mid-infrared (ATR-MIR), transflection near-infrared (NIR), and diffuse-transmittance ultraviolet-visible (UV-Vis) spectra. Spectral signatures were processed through multivariate methods, such as partial least squares (PLS) regression, for predicting BS age from spectral information. A crucial part of the study was dedicated to interpreting model coefficients to assess the contribution of different spectral regions in the age prediction. Consequently, it was possible to distinguish two main processes in the spectral evolution of BS. The first one (within the first ca. 24 h), related to dehydration phenomena and mainly reflected in MIR and NIR spectra, was paralleled by the second one, which primarily involved modifications of hemoglobin, observed with UV-Vis spectroscopy. Finally, obtained signatures were also considered together, in a data fusion perspective on two different levels (Fig. 1), building joint prediction models to verify if the "multiplex" approach might offer a more penetrating vision into the process of bloodstains aging.

[1] R.H. Bremmer, K.G. de Bruin, M.J.C. van Gemert, T.G. van Leeuwen, M.C.G. Aalders, Forensic Science International, 216 (2012) 1–11. [2] G. Zadora, A. Menżyk, Trends in Analytical Chemistry, 105 (2018) 137–165. [3] S. S. Kind, Journal of Forensic Science Society, 34 (1994) 155–164.

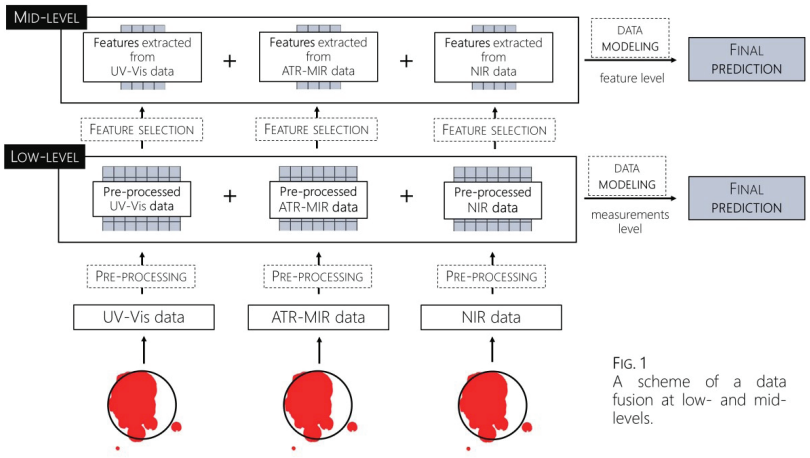

FIG. 1
A scheme of a data fusion at low- and mid-levels.

Fig. 1 A scheme of a data fusion at low- and mid-levels.

Examination of degraded papers by vibrational spectroscopy for forensic purposes

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Paper aging, PCA, Vibrational Spectroscopy

The paper itself is often the subject of forensic analysis in order to make a decision about a questioned document's authenticity. Frequently it is necessary to distinguish between paper of the same type but of a different age. Thus, it is essential to know whether the degradation process of paper influences the possibility of differentiation between paper samples. The ageing process of cellulose is related to different factors: oxidative agents, light moisture, pollution and degree of acidity, both on the cellulose itself and on its environment. As a consequence of the ageing process, oxidized groups (carbonyl and carboxyl groups) are formed on the cellulose chains. Earlier research concerned paper that was aged artificially in the aging chambers at controlled air temperature and humidity. This research presents the results of comparing the processes occurring in paper under two types of conditions, of natural and artificial aging environment. Attention was paid to the rate of changes and their magnitude and mechanism. Samples of 11 types of white paper were artificially aged in a climatic chamber (HCP 153 Memmert) under 95% relative humidity in air at 60°C for various periods of time up to 35 days. The second set of 11 samples were naturally aged in sun light, and in shadow, for various periods of time up to 3 years. The conditioned samples were examined by the use of vibrational spectroscopy methods. Three different spots on each paper card were measured to assure reproducibility of the aging experiment monitored by spectroscopic methods. The possibility of differentiation between aged samples was evaluated. The PCA statistical method and 2D correlation analysis based on the Noda's method was carried out using obtained spectra as an input data for generating the correlation maps. It was found that PCA score plots and pattern of ;2D maps allow to distinguish tested paper samples, identified its components and get insight into paper degradation mechanism.

2.7. Material Science and Catalysis

Raman Study of Organic – Inorganic Hybrid Halide Perovskites

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Raman spectroscopy, Hybrid Halide Perovskite, Bandwidth, Motional narrowing, Phase transition

Hybrid halide perovskite ABX_3 (A: methylammonium CH_3NH_3 = MA, formamidinium $CH(NH_2)_2$ = FA; B: Pb, Sn; X: Br, I) shows high performance as as active materials in solar cells and thermoelectric devices for energy harvesting. These materials have a common crystal structure shown in Fig. 1. In the preious work [1], we reported that the observed Raman bands of $MAPbBr_3$ are assigned to the intramolecular vibrations of MA, lattice vibrations, and an MA–$PbBr_3$ cage vibration, which is called ν_1 band. With increasing temperature the broad ν1 band at ~326 cm^{-1} exhibits motional narrowing originating from fast changes between orientational states of MA in the $PbBr_3$ cage. In this work, we observed Raman spectra of compressed pellets of crystalline powders of $FAPbBr_3$ and $MASnBr_3$ on a Renishaw InVia Raman microscope in the temperature range from 290 to 77 K with the excitation at 532, 632.8, and 830 nm. We will discuss the effect of FA and Sn on the Raman spectra. The broad bands observed at ~310 cm^{-1} for $FAPbBr_3$ at ~350 cm^{-1} for $MASnBr_3$ were assigned to the ν_1 band; other observed bands were assigned to the intramolecular vibrations of ammonium ions or lattice vibrations. The width of each ν_1 band was much larger than those of the intramolecular and lattice vibrations, as was observed for $MAPbBr_3$. With increasing temperature, the width of the ν_1 band of $FAPbBr_3$ increased. The ν_1 band did not exhibit motional narrowing. Onthe other hand, the width of the ν_1 band of $MASnBr_3$ decreased with increasing temperature; the ν_1 band exhibited motional narrowing. Thus, MA takes several orientational states in the $SnBr_3$ cage, although FA does not take several orientational states in $PbBr_3$ cage. The orientational dynamics of FA is probably different from that of MA. A DFT calculation (B3LYP, 6-31G++**) indicated that FA takes a planar structure with C_{2v} symmetry; it has two CNH_2 groups, which take strong interactions with the $PbBr_3$ cage. In the literature, $FAPbBr_3$ shows phase transitions: α phase (cubic, Pm3m, O_h, Z = 1) | 266 K | β phase (tetragonal, P4/mbm, D_{4h}, Z = 2) | 153 K | γ phase (orthorhombic, Pnma, D_{2h}); $MASnBr_3$ shows phase transitions: α phase (cubic, Pm3m, O_h, Z = 1) | 230 K | β phase | 185 K | γ phase. On the other hand, $MAPbBr_3$ shows phase transitions: α phase (cubic, Pm3m, O_h, Z = 1) | 236 K | β phase (tetragonal, I4/mcm, D_{4h}, Z = 4) | 154 K | γ phase (tetragonal, P4/mmm, D_{4h}) | 149 K | δ phase (orthorhombic, Pnma, D_{2h}). Temperature dependences of the widths (full width at half maximum, FWHM) and peak positions were measured. The FWHM and peak position of the ν_1 band of $FAPbBr_3$ showed adrupt changes at T_1 = ~153 K and T_2 = ~266 K K, indicating that phase transitions at T_1 and T_2 are first order. A band observed at ~520 cm^{-1} assigned to the NCN bending vibration was also useful for characterizing the T_1 phase transition for $FAPbBr_3$.

[1] K. Nakada, Y. Matsumoto, Y. Shimoi, K. Yamada, and Y. Furukawa, Molecules, 24 (2019) 626.

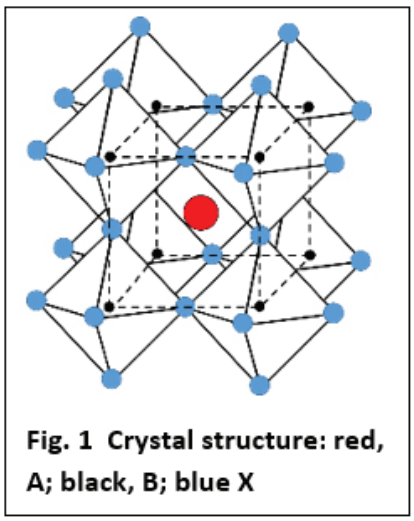

Fig. 1 Crystal structure: red, A; black, B; blue X

Figure 1. Crystal structure: red, A; black, B; blue X

Vibrational Spectra of Zeolite Y as a Function of Ion Exchange

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zeolite, faujasite, ion exchange, cationic sites, lattice dynamics calculations, virational spectroscopy

Zeolite Y is one of the earliest known and most widely used synthetic zeolites. Many experimental investigations verify the valuable ion exchange capability of this zeolite. In this study, we assessed the effects of ion exchange on its vibrational spectra. Due to the complex structure, we applied classical lattice dynamics methods for IR and Raman intensity calculations. Computed spectra of optimized zeolite Y structures with different cations were compared with experimental data. The spectra obtained in this study are in agreement with previous experimental and computational studies on zeolites from the faujasite group. One of the important goals of the work was to use the results obtained from the calculations carried out for various model FAU structures and the theoretical spectra resulting from these calculations for a rather complicated interpretation process of experimental faujasite vibrational spectra. Good concordance of the theoretical and real spectra allows for inference about the correctness of the model structure and the correctness of the assigned bands on the experimental spectra. We anticipate that the calculated infrared and Raman spectra presented here will be useful in future experimental characterizations of ion-exchanged zeolites, regardless of the final application. Detailed analysis of the obtained results allowed identification of the bands in IR and Raman spectra changing both, the intensity and position with the type of extra-framework cation in the structure. Based on the literature data, these bands were assigned to fully symmetrical vibrations of six-membered rings—the so-called ring opening (RO) vibrations. MIR range spectra for the Y zeolite measured after the sorption process shows visible changes in the pseudo-lattice vibrations caused by the ion exchange process. In IR spectrum, the band located at approx. 580 cm^{-1} can be assigned to bending vibrations of Si–O–Al bridges; its position and intensity changes significantly with non-tetrahedral cation and its position relative to the six-membered ring. The Raman spectrum, as more sensitive to changes in the symmetry of the system, is a good indicator of the sorption process. The bands at 503 and 352cm^{-1} undergo changes in position and integral intensity, respectively. Similar observations can be made also in the case of spectra obtained for all analyzed "A", "B" and "C" model structures with various distribution of extra-framework cations in the host structure.

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Molecularly bridged plasmonic nanoparticles aggregates promising as optical organic-inorganic hybrid materials: comprehensive look on self-organization and SERS response

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Plasmonic materials, SERS, noble nanoparticles, molecular linkers, self-organization

Organic-inorganic hybrid materials attract a great attention due to their advantages characteristics of both, organic and inorganic, parts. Materials, where metal nanoparticles (NPs) modified by organic molecules are the building blocks, are promising for photonic and sensing applications. Despite a great attraction of these materials, the complete understanding of the mechanism of NPs self-organization promoted by organic linkers has not been reached yet. Therefore development of plasmonic hybrid materials with high spatial organization of metal cores is still challenging. We present herein the study of Ag, Au nanoparticles self-organization initiated by addition of polyfunctional molecules well-proven in metal-organic frameworks (MOF) design. Different type of organic ligands with various degree of springiness, including rigid tolanes (also called as "organic rods"), more flexible stilbenes and crown-type cyclenes were tested as the molecular bridges. Obtained organic-inorganic aggregates showed intensive surface-enhanced Raman scattering (SERS) response and their spectral patterns turned out to be very sensitive to surface coverage and environment. In-depth study of morphology of the obtained NPs aggregates by TEM, AFM microscopy, UV-Vis spectroscopy and dynamic light scattering supplemented the obtained SERS data. We found that sub- or monolayer surface coverage provides the linking of single NPs which replaced by overall aggregation at multilayer coverage. Two features of SERS response characteristic for linked nanoparticles were revealed: non-monotonic concentration profile of the SERS spectra and highly intensive overtones in conditions of hot spots. Comparative analysis between the NPs aggregates obtained with different molecular bridges showed following trends. i) Increase in the aromaticity and springiness of the organic linker decreases the tendency of NPs to aggregation which is immediate for aliphatic cyclenes and minimized for highly conjugated tolanes. ii) A degree of conjugation of the molecular linker determines the possibility of metal-to-molecule charge transfer, which is established for Ag NPs aggregates containing stilbenes and tolanes.

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Water dynamics in [Cu(H2O)4](ReO4)2 studied by vibrational spectroscopy (IR, RS and neutron scattering methods)

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inelastic neutron scattering (IINS), temperature dependent IR spectroscopy, reorientational dynamics, DFT calculation

We present a complementary investigations of water dynamics in an ionic $[Cu(H_2O)_4](ReO_4)_2$. This compound has one reversible phase transition in the solid state below room temperature. At room temperature, tetraaquacopper(II) rhenate(VII) crystallizes in a triclinic crystal system, within the space group No. 2 = P-1, with one molecule in the unit cell [1]. X-ray single crystal diffraction measurements indicate that the crystal structure does not change significantly at the phase transition. The hydrogen atoms are hardly located in the structure because of the presence of heavy ReO_4^- anions. However, neutron diffraction experiments performed on the powdered sample revealed some subtle changes in the registered patterns. Vibrational–reorientational dynamics of H_2O ligands in the high (I) and low-temperature (II) phases of $[Cu(H_2O)_4](ReO_4)_2$ was probed by a set of complementary spectroscopy techniques such as Fourier transform middle and far-infrared spectroscopy (FT-MIR and FT-FIR), Raman Spectroscopy (RS) and inelastic incoherent Neutron Scattering (IINS) methods [2] in a wide temperature range. Temperature dependent Raman light scattering measurements (RS) (295–95K range) and infrared spectroscopy (295–14K) showed that the bands associated with internal H_2O vibrations modes narrow continuously with temperature decreasing. The dynamics of the H_2O molecules in both phases was investigated by means of band shape analysis performed for IR bands. The temperature dependency of full width at half maximum (FWHM) of the FT-FIR band at 239 cm^{-1} (rocking mode) suggests that the observed phase transition is not connected with a change of the H_2O reorientational dynamics. The H_2O ligands perform fast (t_R»10^{-12}-10^{-13}s) stochastic reorientational motions in the phases I and II with a mean value of activation energy: 6.13 $kJmol^{-1}$. However, a continuous change of bands connected with hydrogen bonds is observed. The neutron spectroscopy is especially sensitive for hydrogen motion. This is due to high incoherent cross section for hydrogen scattering. Moreover, the discussed compound crystalizes in a centrosymmetric structure and hence a mutual exclusion rule applies for IR and RS active vibrations. The lack of symmetry dependent selection rules for neutron scattering causes that all vibrations are active and in principle might be measured. The neutron spectroscopy is particularly useful in the investigation of water containing samples. Using vibrational spectroscopy methods (IINS, IR and RS) all characteristic wavenumbers of the H_2O and ReO_4^- vibrations were detected.

[1] M.B. Varfolomeev, V.N. Khrustalev, A.P. Pisarevskii, N.B. Shamrai, Yu.V. Syrov, Zh. Neorg. Khim. 44, 9 (1999) 1335–1337. [2] I. Natkaniec, D. Chudoba, Ł. Hetmańczyk, V.Yu. Kazimirov, J. Krawczyk, I.L. Sashin, S. Zalewski, Journal of Physics: Conference Series, 554 (2014) 012002.

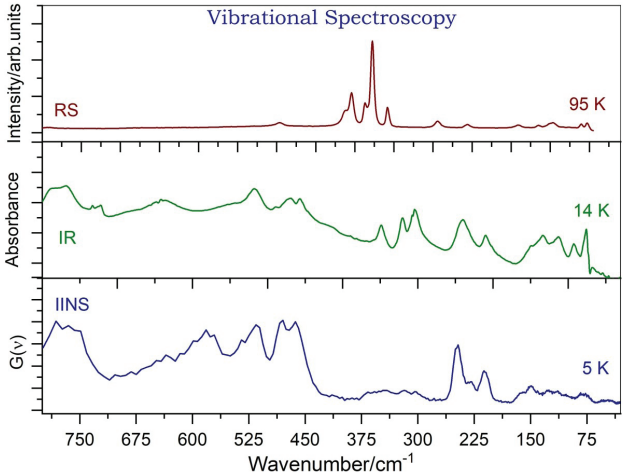

Figure 1. The experimental phonon density of states spectrum G□ of [Cu(H2O)4](ReO4)2 compared to experimental IR and RS spectra in the low wavenumber range (800–30 cm-1).

Infrared spectroscopy studies of water structure in the sub-membrane region of a floating lipid bilayer

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surface enhanced infrared absorption spectroscopy, water structure, floating bilayer lipid membrane

The structure of water in the sub-membrane region of the DPhPC floating bilayer on 1-thio-β-D-glucose-D-glucose (β-Tg) monolayer modified gold nanoparticle film was studied by surface enhanced infrared absorption spectroscopy (SEIRAS). SEIRAS employs surface enhancement of the mean square electric field of the photon which is acting on a few molecular layers above the film of gold nanoparticles. Therefore, it is uniquely suited to probe water molecules in the sub-membrane region and provides unique information concerning the structure of the hydrogen bond network of water surrounding the lipid bilayer. The IR spectra indicated that water with a strong hydrogen network is separating the membrane from the gold surface. This water is more ordered than water in the bulk. When alamethicin, a peptide forming ion channels, is inserted into the membrane the water network is only slightly loosened. The addition of amiloride-an ion channel blocker results in a significant decrease in the amount of water in the sub-membrane region. The remaining water has a significantly distorted hydrogen bonds network. This study provides unique information about the effect of the ion channel on water transport across the bilayer. The electrode potential has a relatively small effect on water structure in the sub-membrane region. However, the IR studies demonstrated that water is less ordered at positive transmembrane potentials. The present results provide significant insight into the nature of hydration of floating lipid bilayer on the gold electrode surface.

Natural Sciences and Engineering Research Council of Canada and Polish National Science Centre

[1] ZhangFei Su, Joanna Juhaniewicz-Debinska, Slawomir Sek and Jacek Lipkowski, Water structure in the sub-membrane region of a floating lipid bilayer without and with an ion-channel and an ion channel blocker, Langmuir, 2020, 36, 409-418.

Investigation of hydrogen bonding of water in hydrate melt

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hydrogen bonding, hydrate melt, water in salt

Title Investigation of hydrogen bonding of water in hydrate melt Authors; Yusuke Morisawa, Nami Ueno, Mutsumi Imawari Yusuke Morisawa, Kindai University, School of Science and Engineering Nami Ueno, Innsbruck Unviersity, Department of Analytical Chemistry and Radiochemistry Mutsumi Imawari, Kindai University, School of Science and Engineering Abstract Hydrate Melt (HM) is a high-concentration lithium salt aqueous solution composed of TFSI and BETI mixed anions. Since it promotes the coordination of lithium ions with strong Lewis acidity to water molecules, it is possible to improve the upper limit of the electromotive force of the water system to 3.0 V or more even though it is a water system electrolyte. It is well known that the molar ratio of HM is LiTFSI: LiBETI: H2O = 0.7: 0.3: 0.2. MD calculations predicted that water did not form a hydrogen bond network in HM. In this study, we investigated aqueous solutions with this unique hydrogen-bonding environment using near-infrared spectroscopy, which makes it easier to observe hydrogen-bond-free species which is difficult to observed in fundamentals. As a result, it was found that a sharp absorption band characteristic of HM was observed on the high frequency side of the peak of the saturated aqueous solution, which was not observed in the Raman spectrum. Aqueous solutions of single and mixed salts of LiTFSI, LiBETI and LiFTS were observed by FT-NIR. Fig. shows the Li (BETI) concentration dependence of the NIR spectrum v1 + v3 region of water in the Li (TFSI / BETI) mixed salt aqueous solutions. The solution with 5.0M Li+ means the nearly saturated aqueous solution of LiTFSI. Adding LiBETI, a higher concentration electrolyte aqueous solution can be prepared. From NIR spectra (a), there are two bands in 7100 and 6850 cm-1. The absorption of 6850 cm-1 is a band due to 2v2 + v3 of water, which is less affected by changes in the hydrogen bond environment.The second derivative spectra (b) revealed that by adding LiBETI, a new sharp peak appeared near 7200 cm-1. On the other hand, the peak of 7100 cm-1 becomes smaller with the concentration increases. The peak around 7200 cm-1 should assigned to component that is particularly large in the mixed salt, and it is considered that the water is coordinated with Li+ as a Lewis base.

[1] Kadu, B. S. Suzuki-Miyaura Cross Coupling Reaction: Recent Advancements in Catalysis and Organic Synthesis. Catal. Sci. Technol. 2021, 11 (4), 1186–1221. [2] Zhao, Y.; Du, L.; Li, H.; Xie, W.; Chen, J. Is the Suzuki-Miyaura Cross-Coupling Reaction in the Presence of Pd Nanoparticles Heterogeneously or Homogeneously Catalyzed? An Interfacial Surface-Enhanced Raman Spectroscopy Study. J. Phys. Chem. Lett. 2019, 10 (6), 1286–1291.

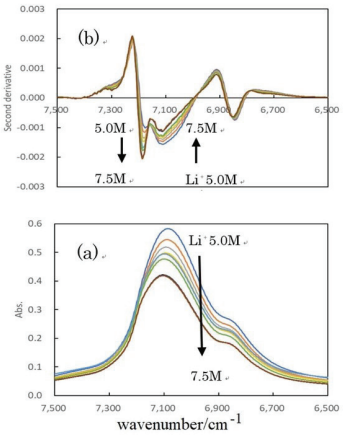

Figure 1. NIR spectra (a) and its second derivative spectra (b) of LI(TFSI/BETI) mixed aqueous solutions in the region of vl and v3 of water.

Symmetry Specific Low-Frequency Vibrational Spectra of Crystals Measured by Two-beam 3CBCRS

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Coherent Raman, 3CBCRS, Crystal, Lithium Niobate, vibrations

After demonstrating the usefulness of polarization-sensitive two-beam 3CBCRS for isotropic samples [1,2], we have applied the method to measure symmetry-specific low-frequency vibrational spectra of two crystals Bismuth Germanate and Lithium Niobate. These belong to cubic and trigonal crystal classes, respectively. The results compared to the measured Raman spectra with the same tabletop nonlinear spectroscopy system. We will explain the technical difficulties detecting coherent Raman spectra in the epi and forward geometry and the identification and measurements of the symmetry-dependent susceptibility tensor components. The developed coherent Raman method opens a new area of material characterization regarding, e.g., phonon and vibrational signatures of crystalline samples.

Contribution from the Crystal Physics Research Group of the Wigner Institute is acknowledged.

[1] Ujj L, Contribution to the development of low-frequency terahertz coherent Raman microspectroscopy and microscopy, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. (2018); 199, 448-454. [2] Ujj L, Olsson T, Schundelmier B, Bestor T, Effective Polarization Suppression in Two-Beam 3-color Broadband Coherent Raman Micro-Spectroscopy (3CBCRS), Vib. Spectrosc. (2020), 108, May, 103056.

Control of Molecular Orientation in Thin Films of Small-Molecule Organic Semiconductors Independent of Substrates

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Organic Semiconductors, Molecular Orientation, Thin Film, MAIRS, X-ray diffraction (XRD)

Rod-shaped molecules represented by pentacene and perylene tetracarboxylic diimide typically form a polycrystalline thin film with the long axis of the molecule oriented perpendicular to the substrate surface, i.e., end-on orientation. The face-on oriented thin film, on the other hand, where the molecular plane is parallel to the substrate, has never been found on an inert substrate such as silicon. As a result, the face-on orientation has long been believed to be generated only on specific surfaces such as graphene. A low-temperature deposition technique is a candidate for obtaining the metastable face-on crystalline film on an inert surface [1,2]. In the present study, the face-on orientation of various organic semiconductors has been realized for the first time, and the uniaxial oriented structure is identified by means of p-polarized multiple-angle incidence resolution spectrometry (pMAIRS) [3] and two-dimensional grazing incidence X-ray diffraction (2D-GIXD). The pMAIRS spectra clearly discriminate the face-on thin film structure from the conventionally known end-on one. The present study demonstrates that the molecular orientation of small-molecule organic semiconductors can be controlled without a template layer.

[1] Shioya, N. et al., Sci. Rep. 2019, 9, 579. [2] Duva, G. et al., J. Phys. Chem. Lett. 2019, 10, 1031. [3] Hasegawa, T.; Shioya, N. Bull. Chem. Soc. Jpn. 2020, 93, 1127.

Raman spectroscopic studies of Polymer Derived Ceramics in the form of protective coatings for SOFCs' interconnects

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Polymer Derived Ceramics, Solid Oxide Fuel Cells, interconnects, Raman Confocal imaging, Protective coatings

Nowadays, with an exponentially increasing amount of people over the world along with a rising demand for the energy, an emphasis is put on the development of the low-emission energy production technologies such as Solid Oxide Fuel Cells (SOFCs). However, despite high electrical efficiency, easiness in manufacturing and low costs of their production, the problem of high-temperature corrosion of SOFCs' interconnects casts a shadow on such a promising idea [1]. Among abundant solutions, the application of protective coatings on the ferritic steels seems to be the most interesting one [1]. Thus, in this work, Polymer Derived Ceramics (PDCs) in the form of SiAlOC glasses' coatings were proposed as a very innovative protection measure. Due to the duality of carbon form (Si-C covalent strong bonds and so "free carbon phase"), the necessary balance between high thermomechanical stability and electrical conductivity is provided, respectively. Moreover, sol-gel method gives an opportunity to form PDCs in numerous shapes i.e. layers [2]. It is crucial to report that vibrational spectroscopy, including especially Raman spectroscopy is the powerful tool when investigating material's structure, but at the same time very underestimated while determining corrosion phenomena and mechanisms of diffusive processes, particularly for the ferritic The main aim of this study was to present the possibilities of Raman spectroscopy as a method of the evaluation of corrosion rate of Crofer 22APU ferritic steel used for SOFCs' interconnects in two systems: uncoated and coated with layers based on SiAlOC glasses. The corrosion resistance of the protective layers was investigated using thermogravimetric analysis within isothermal (500 h) and cyclic (500 1h-cycles) conditions at 800°C in the laboratory air. The application of Raman spectroscopy allowed for the determination of: a) bulk material's (gel and glasses) structure used as reference for coatings – point measurements, b) structure of coating along with coating/steel interface ($MnCr_2O_4$ spinel) – linear depth profiles, c) distribution of oxidation products on the surface and across the multi-layered scale – imaging. The Raman studies supported with additional structural (XRD, FTIR) and microstructural (SEM+EPMA) investigation, revealed the outbreak improvement in the oxidation resistance of the steel due to the application of SiAlOC glasses coatings.

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[1] Tan KH, Rahman HA, Taib H, International Journal of Hydrogen Energy (2019); 44, 30591-30605. [2] Stabler C, Ionescu E, Graczyk-Zajac M, Gonzalo-Juan I, Riedel R, (2018); Journal of the American Ceramic Society 101, 4817-4856.

Applications of Au/TiO2/WO3 ternary composite systems as SERS materials and photocatalysts

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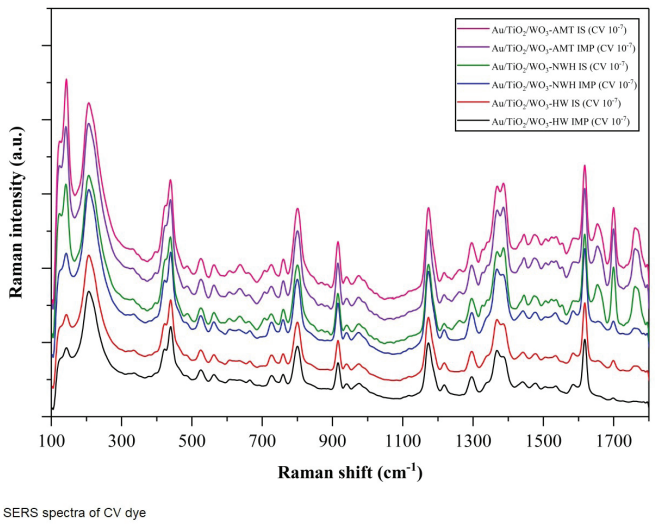
Turkevich reduction, SERS material, Crystal violet detection, Photocatalytic performance, Au/TiO2/WO3 ternary composites

The most commonly used photocatalyst is the well-known commercial TiO_2. Titanium dioxide is an efficient photocatalyst in removing certain pollutants from water [1]. However, it also has a fast charge separation (e-, h+) recombination rate, limiting its photocatalytic efficiency. This so-called undesirable property of the TiO_2 can be overcome by adding another metal oxide (WO_3) to the system or reducing noble metals (Au) upon the semiconductor's surface. These binary composite systems will result in a more efficient photocatalyst by inhibiting the composite systems' recombination rate. Ternary composite systems based on TiO_2, other metal oxides, and noble metal are also employed for water treatment processes but can be applied as sensors or SERS materials as well. We present the synthesis, morpho-structural characterization, and Au/TiO_2/WO_3 ternary composite systems' applicability in this work. Our first goal was to obtain tungsten trioxide semiconductors with different morphologies, then the preparation and characterization of TiO_2/WO_3 composite systems, and last but not least, the synthesis of the six Au/TiO_2/WO_3 composite systems. Our study revealed multiple future applications of the products. Au/TiO_2/WO_3 composite systems were synthesized applying the Turkevich method. WO_3 semiconductors with three different morphologies (rods and sheets; fibers; stars) [2-4] were selected to obtain the products. The reduction of the gold nanoparticles was carried out in situ and by impregnation, resulting in six samples with ≈1 wt. % Au concentration. The as-prepared samples were investigated employing X-ray diffraction, UV-Vis spectroscopy, Transmission Electron Microscopy, and Raman Spectroscopy. Crystal violet dye (CCV= 10^{-7}-10^{-8}M) detection was investigated to establish the composites' sensorial applicability. The photocatalytic efficiency was determined by methyl orange dye (CMO= 125 μM), respectively phenol (CPh=0.5 mM) removal from aqueous solution under UV light irradiation. Ternary composite systems with multiple applications were obtained. The Turkevich method yielded Au nanospheres with particle sizes between 20-40 nm; TEM micrographs and DRS spectra confirmed their presence. The deposition of Au nanoparticles improved the composites' photocatalytic activity, achieving higher efficiency than the commercial TiO_2. The obtained materials were proven to exhibit multiple applicability, such as sensors for oxalic acid, photocatalysts with improved photoactivity, and SERS materials to detect organic pollutants in very low concentrations.

Romanian National Authority for Scientific Research, project number PN-III-P1-1.1-TE-2016-1588.

[1] V.S. Kosera, T.M. Cruz, E.S. Chaves, E.R.L. Tiburtius, Triclosan degradation by heterogeneous photocatalysis using ZnO immobilized in bi-opolymer as catalyst, J. Photochem. Photobiol. A Chem. (2017). doi:10.1016/j.jphotochem.2017.05.014. [2] L. Zhou, J. Zou, M. Yu, P. Lu, J. Wei, Y. Qian, Y. Wang, C. Yu, Green synthesis of hexagonal-shaped WO3· 0.33H2O nanodiscs composed of nanosheets, Cryst. Growth Des. 8 (2008) 3993–3998. doi:10.1021/cg800609n. [3] X. Liu, J. Zhang, T. Yang, X. Guo, S. Wu, S. Wang, Synthesis of Pt nanoparticles functionalized WO3 nanorods and their gas sensing properties, Sensors Actuators B Chem. 156 (2011) 918–923. doi:10.1016/j.snb.2011.03.006. [4] S.K. Biswas, J.O. Baeg, A facile one-step synthesis of single crystalline hierarchical WO3with enhanced activity for photoelectrochemical solar water oxidation, Int. J. Hydrogen Energy. 38 (2013) 3177–3188. doi:10.1016/j.ijhydene.2012.12.114.



SERS spectra of CV dye

Figure 1. SERS spectra of CV dye

(High pressure) Raman study of the phonons / phonon-polaritons of the Cd1-xZnxTe semiconductor mixed crystal

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Forward/backward Raman scattering on Cd1-xZnxTe, phonons phonon-polaritons, ab initio phonon calculations, high pressure, percolation scheme

Owing to the dramatic contrast in the bond physical properties of the end compounds (length: ~6%, ionicity: ~24%), which generates exacerbated vibrational properties, Cd1-xZnxTe is a model system to shed light onto the phonon mode behavior of mixed crystals in general. Moreover Cd1-xZnxTe crystallizes in the high symmetry cubic zincblende structure, leading to an ultimately simple phonon pattern. This is ideal to achieve a clear understanding. Cd1-xZnxTe was long considered to exhibit the classical two-mode behavior (one per bond) in its Raman spectra taken in the longitudinal optic (LO) symmetry at low temperature on epitaxial layers [1] and bulk crystals [2] with moderate Zn content (less than 50 at.%). Such two-mode behavior has been described within the well-known modified-random-element-isodisplacement (MREI) model. One disconcerting feature, though, yet unexplained, is that the Zn-Te and Cd-Te LO modes exhibit comparable Raman intensities in the Zn-dilute (less than 10 at.%); limit [1-3]. Recently, a careful far-infrared reflectivity study done on similar bulk crystals by Kozyrev [4] revealed a multi-mode pattern behind the Zn-Te bond vibrating at high frequency. This deviation from the MREI model was explained within our percolation model developed over the past decades [4], which motivates further Raman insight. In this work, we perform an exhaustive Raman study of Cd1-xZnxTe bulk crystals covering the composition domain. Special attention is awarded to the transverse optic (TO) modes, and notably to their phonon-polariton variants studied by near-forward scattering (schematically operating « in transmission »), with ellipsometry measurement of the dispersion of the refractive index in support. We achieve a quantitative description of the distinct phonon-polariton and LO couplings – mediated via their accompanying dispersive and non-dispersive long range electric fields – as evidenced in the Cd- and Zn-dilute limits, respectively, with ab initio calculations of the underlying TO modes in support. Last, we extend the Raman study to high pressure, i.e., up to the first pressure-induced structural transition (at ~10 GPa), independently identified by high-pressure X-ray diffraction measurements done on the SOLEIL synchrotron. The aim is to check whether the Zn-Te Raman doublet actually closes under pressure, as predicted within our recent percolation-based partition of II-VI and III-V mixed crystals emphasizing a key role of the mesostructure on the pressure dependence of the phonon properties of mixed crystals [5], or not.

[1] D.N. Talwar et al., Phys. Rev. B 48, 17064 (1993) [2] D.J. Olego et al., Phys. Rev. B 33, 3819 (1986) [3] S. Perkowitz et al., Phys. Rev. B 42, 1455 (1990). [4] S.P. Kozyrev, Semiconductors 49, 889 (2015) [5] M.B. Shoker et al., Scientific Reports 10, 19803 (2020).

Oriented photoactive yellow protein films investigated by broadband vibrational sum-frequency generation spectroscopy

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photoactive yellow protein, broadband vibrational sum-frequency generation spectroscopy, polyelectrolyte

The photoactive yellow protein (PYP) is a light-sensitive protein, which, upon light absorption, enters a photochemical cycle, going through conformational changes before returning to the ground state. Each intermediate state possesses a refractive index different from that of the protein's ground state, which makes PYP a good candidate for future integrated optical (IO) and biophotonics applications. For IO purposes, the utilization of protein films as opposed to protein solutions is preferred due to practical reasons [1]. Additionally, orienting the protein molecules maximizes the achievable IO signal at a certain number of molecules, which should be kept at a minimum to save manufacturing costs. To deposit the protein molecules on a substrate, an appropriate support-layer is needed between the protein molecules and the substrate surface that also leads to the formation of an oriented protein film. We created PYP monolayers by adsorbing the protein on charged polyelectrolyte surfaces. We prepared a homogenous polyelectrolyte layer with the layer-by-layer technique using positively charged poly-L-lysine (PLL) and negatively charged poly-L-glutamic acid (PGA). The PYP was then adsorbed to the topmost layer of the multilayer structure, either a PGA or a PLL layer. The structure was then investigated by chiral and achiral broadband vibrational sum-frequency generation spectroscopy (BB-VSFG). BB-VSFG is a label-free and interface specific technique, allowing to study slight structural and orientational changes of PYP molecules with high sensitivity. We investigated PYP monolayers with a 100-kHz BB-VSFG spectrometer [2-3] in multiple polarization combinations, and in three vibrational regions: the amide I, the C-H, and the N-H / O-H stretching regions. By investigating the layers in chiral (spp and psp) polarization combinations, we obtained information about the PYP's secondary structure. We have found that the topmost PGA layer forms a random coil structure, while the topmost PLL layer has an anti-parallel β-sheet structure. Putting PYP on the top of these charged layers, its orientation changes depending on the polyelectrolyte layer's charge. From our measured spectra, we found that PYP maintains its anti-parallel β-sheet structure on both charged surfaces, albeit differently. Our experimental findings were compared to molecular dynamics simulations to understand the small orientational changes of PYP on PLL and PGA layers. Based on our results, PYP monolayer films oriented on polyelectrolyte layers are promising for further IO applications.

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[1] Krekic, S., Zakar, T., Gombos, Z., Valkai, S., Mero, M., Zimányi, L., Heiner, Z., Dér, A. 2020 Front. Plant Sci. 11:547818 [2] Heiner, Z., Petrov, V., Mero, M. 2017 APL Photonics 2, 066102. [3] Heiner, Z., Wang, L., Petrov, V., Mero, M. 2019 Opt. Express 27, 15289-15297

Crystallization and structural investigation of strontium iron-phosphate glasses using Raman and IR spectroscopy

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Raman spectroscopy, FT-IR spectroscopy, DSC analysis, structural studies, glass crystallization

Iron-phosphate glasses have a lower transformation temperature than silicate and excellent chemical durability [1,2]. Because of these properties, the glasses can be used in nuclear waste immobilization. One of the main fission short-lived products is ^{90}Sr [3]. It is a source of β-radiation ^{90}Sr is highly water-soluble and may enter groundwater from the waste [4]. Due to easy absorption and permanent incorporation of Sr into the body, radioactive isotopes of Sr especially ^{90}Sr are particularly dangerous. The effect of strontium on the crystallization behavior of glass is a quite important parameter from the waste immobilization perspective. The subject of studies was (100-x)(30 Fe_2O_3-70P_2O_5)-xSrO mol. % glass, where x=10,20, ...,50., 50. Thermal properties have been identified using DSC analysis. The composition of glasses was checked using the XRF and amorphous character using the XRD technique. Structural investigations have also been carried out using Raman and FT-IR spectroscopy. The glasses were crystalized at crystallization temperatures obtained from DSC analysis. The composition and structure of obtained glass-ceramics were investigated using XRD analysis and Raman spectroscopy. From the DSC curves were determined glass transformation (Tg) and crystallization (Tc) temperatures. Additionally, the heat capacity accompanying the glass transformation (ΔCp) was evaluated. The glass transformation decreases with an increase in the content of SrO up to 30 mol. % and then increase. This suggests structure rebuilding for 30 mol. % content of SrO. Spectroscopic studies of glasses showed that increasing the content of SrO caused gradual depolymerization of the phosphate network. At high SrO content, mainly crystallize Sr-rich phases such as $Sr_2P_2O_7$ and $Sr(PO_3)_2$.

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[1] K. Joseph, M.C. Stennett, N.C. Hyatt, R. Asuvathraman, C.L. Dube, A.S. Gandy, K. V. Govindan Kutty, K. Jolley, P.R. Vasudeva Rao, R. Smith, Iron phosphate glasses: Bulk properties and atomic scale structure, J. Nucl. Mater. 494 (2017) 342–353. [2] X. Yu, D.E. Day, G.J. Long, R.K. Brow, Properties and structure of sodium-iron phosphate glasses, J. Non. Cryst. Solids. 215 (1997) 21–31. doi:10.1016/S0022-3093(97)00022-7. [3] M.I. Ojovan, W.E. Lee, An Introduction to Nuclear Waste Immobilisation, Second Edi, Elsevier, 2014. [4] S.P. Kumar, G. Buvaneswari, Synthesis of apatite phosphates containing Cs+, Sr2+ and RE3+ ions and chemical durability studies, Mater. Res. Bull. 48 (2013) 324–332.

Spectroscopic study of Suzuki-Miyaura cross-coupling reaction

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Raman, SERS, Suzuki-Miyaura cross-coupling reaction, nanoparticles, DFT calculations

The cross-coupling reactions catalyzed by transition metals have been studied intensively for over the last few decades. The importance of cross-coupling reactions has been widely recognized. Its authors, R. Heck, E. Negishi and A. Suzuki, were awarded by the Chemistry Nobel Prize in 2010. These catalyzed reactions opened a new synthetic pathway for products which cannot be easily synthetized. The reaction mechanism has not been fully elucidated yet[1]. The main aim of this study is monitoring of Suzuki-Miyaura cross-coupling reaction (SMCR) between aryl halide and arylboronic acid in the presence of a base on the surface of the plasmonic nanoparticles (PNP) [2] by surface-enhanced Raman spectroscopy (SERS). The reaction mixture was formed by the 4-substituted aryl halides (mainly aryl iodides) together with aryl boronic acids, K2CO3, K2PdCl4 as a catalyst and Ag nanoparticles as a SERS substrate. The expected reaction products, derivates of biphenyl, were identified on the basis of newly formed C-C inter-ring stretching vibration approximately at 1288 cm-1, depending on the substituents, as shown in Figure 1 ((A) SERS spectra SMCR between 4-iodobenzonitrile (IBN) and phenylboronic acid (PBA) with K2CO3 and K2PdCl4 with exc. wavelength 532 nm. B) SERS spectra SMCR between 4-iodobenzonitrile (IBN) and 4-(methylthio)phenylboronic acid (MTPBA) with K2CO3 and K2PdCl4 with exc. wavelength 532 nm.). Not surprisingly, SMCR was not the only ongoing reaction in the system. Therefore, the gas chromatography was applied for further identification of the side reaction products. Moreover, the spectral assignment of the SERS and normal Raman spectra of both reactants and products was supported by density functional theory calculations.

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[1] Z. Chen, Y. Zhang, B. Chisholm, D. Webster, J. Polym. Sci. A – Polym. Chem., 2008, 46, 4344-4351 [2] M. Sangermano, A. Chiolerio, Silver and Gold polymer nanocomposites and electrical properties thereof. In: A. Chiolerio, P. Allia, editors. Nanoparticles Featuring Properties: From Science to Engineering. Research Signpost; Kerala, India, 2012, 85–104 [3] A. Vitale, M. Sangermano, R. Bongiovanni, P. Burtscher, N. Moszner, Materials. 2014, 7, 554–562 [4] S. C. Ligon, R. Liska, J. Stampfl, M. Gurr, R. Mülhaupt, Chem. Rev. 2017, 117, 10212-10290 [5] X. Fernández-Francos, S. Kazarian, X. Ramis, A. Serra, Applied spectroscopy, 2013, 67, 1427-1436 [6] M. Topa, E. Hola, M. Galek, F. Petko, M. Pilch, R. Popielarz, F. Morlet-Savary, B. Graff, J. Lalevée, J. Ortyl, Polym. Chem., 2020, 11, 5261-5278

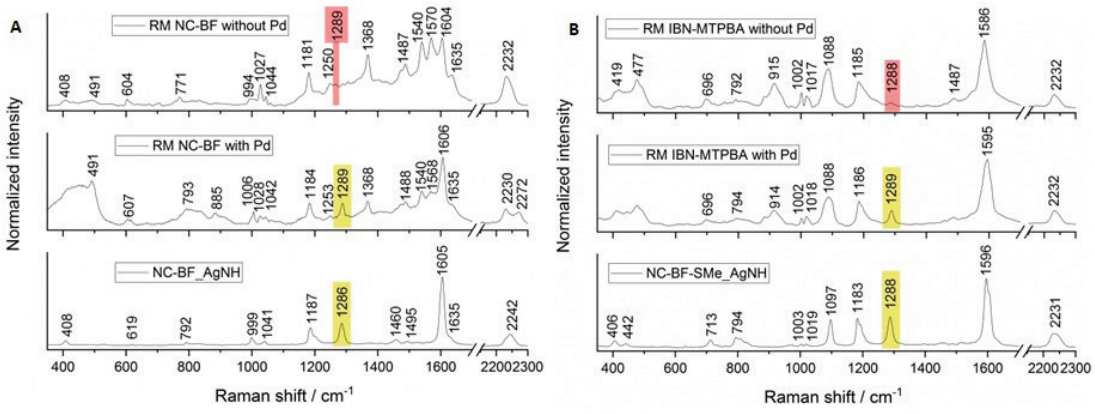

Figure 1. SERS spectra of SMCR

Real-time FT-IR as a non-destructive and non-invasive method for straightforward on-line monitoring of cationic photopolymerization

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Real-time FR-IR, Cationic photopolymerization, Photoinitiators, On-line measurements

Materials based on the cationic photopolymerization of monomers, such as vinyl ethers, epoxides, oxetanes, and glycidyls, exhibit favorable properties as the fast cure, low shrinkage, good adhesion, good electrical properties, and no oxygen inhibition [1]. That the reason for the growing interest in cationic photopolymerization in many fields of industry [2-4]. Hence, the need for an effective technique for monitoring the progress of cationic photopolymerization. FT-IR is one of the most common techniques used to examine the monomers behavior during polymerization [5]. The use of this method in real-time mode provides direct insight into the course of the polymerization on-line and allows to monitor the decrease in monomer concentration and, in some cases, increase in the number of polymeric bonds in time during the polymerization process. This technique makes it possible to precisely determine the degree of monomer conversion. Additionally, it can be used to monitor photoinitiated polymerization. For this purpose, the spectrometer is equipped with a special attachment with a sample holder and light guide that provide irradiation to the sample. Polymerizing compositions are measured on the barium fluoride tablet or between polypropylene foils. This approach allows for simultaneous irradiation of sample and IR spectrum recording with no interference in the photopolymerization process. The real-time FT-IR technique is very convenient in the examination of photoinitiators activity during the polymerization process. Hence, we would like to present the utilization of this method in monitoring polymerization of cationically polymerizable monomers photoinitiated with a new highly efficient Sylanto photoinitiator [6]. Measurements were carried out using an LED light source emitting light at 405nm. During irradiation decrease in the band intensity of such monomers as vinyl ether (~1620cm^{-1}), epoxides (~790cm^{-1}), oxetanes (~980cm^{-1}), and glycidyls (~915cm^{-1}) was monitored in;real-time;mode. Results are presented as time dependence of the monomer conversion degree profiles. Their comparison makes it possible to determine the activity of the used photoinitiator.

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Imaging FT-IR Study on Molecular Structure of Silk Protein and Viscid Droplets for Spider's Capture Thread

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spider silk, capture thread, mapping IR, fiber protein, denaturation

The microscopic imaging FT-IR study on molecular structural analysis of capture thread, including its viscid droplets of oriental golden orb-web spider Nephila clavata, has been performed. Spider's threads, which are fibrous silk proteins, have marvelous properties both in strength and elasticity. It has been reported that the property of a viscid droplet on the capture thread shows strange behavior, such as an in-drop capillary spooling of the thread. These characteristic properties of spider's capture thread seem to encourage and inspire the developments of high-performance new materials involving hybrid fibers. The oriental golden orb-web spider, Nephila clavata, is reared in clean cases individually so as to avoid them from attacking each other. The capture thread of the adult spider's orb-web was naturally collected on a Ca_2 substrate by means of hand-made instruments. For removing the viscid droplets from the fiber thread, distilled water was carefully sprayed. A Mettler-Toledo HS82 microscope hot-stage was employed for temperature control. Measurements of IR mapping spectra were performed by using a Lumos FT-IR spectrometer (Bruker). The spectra were accumulated in the region of 4000–800 cm^{-1} with a 4 cm–1 resolution and an aperture unit of 100 μm square. The obtained spectra of capture threads with and without viscid droplets indicate that the features in the region of 1400-1000cm^{-1} will be useful as marker bands for the degree of the dissolving of viscid droplet; further, the bands at 1395 and 1335 cm^{-1} are attributable to the components of anchoring granules located at the inner side of viscid droplets. By recrystallization and its infrared measurements, the main chemical component of viscid droplets is assignable to glycosylated proline. The spectral changes during increasing temperature of the capture thread show that the band intensity at 1632 cm^{-1} assigned to the β-sheet structure of the amide I band decreased over 60°C, while the feature in the region of 1400–1000 cm^{-1} due to the viscid droplets began to decrease over 160°C. This result suggests that the molecular conformation of the silk fiber protein of the spider's capture thread is denatured at 60°C, whereas the viscid droplets on the capture thread retain their structure. Thus, this study demonstrates that the imaging FT-IR analysis of spider's capture thread is powerful tool to reveal the molecular conformation and chemical property of the silk fiber protein and viscid droplets of spider's thread.

[1] X. Yu, D.E. Day, G.J. Long, R.K. Brow, Properties and structure of sodium-iron phosphate glasses, J. Non. Cryst. Solids. 215 (1997) 21–31. [2] I. Ojovan, W.E. Lee, W.E. Lee, An Introduction to Nuclear Waste Immobilisation, Elsevier Science, 2010. [3] M.R. Majhi, R. Kumar, S.P. Singh, R. Pyare, Physico-chemical properties and characterization of CaO-Fe2O3-P2O5 glass as a bioactive ceramic material, J. Biomim. Biomater. Tissue Eng. 12 (2012) 1–24.

Raman and IR investigation of calcium iron-phosphate glasses

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Raman spectroscopy, FT-IR spectroscopy, Mössbauer spectroscopy, structural studies

The phosphate glasses with a high content of Fe_2O_3 (about 30 mol. %) have good chemical resistance [1,2]. Therefore, can be used in the immobilization of low-level radioactive waste (LLW) incineration ash of high content of CaO and heavy metals. Also, glasses contain mainly P_2O_5, SiO_2, CaO and Na_2O are called bio-glasses witch create chemical bonds with tissues and can be used as bone implants [3]. Additionally, P_2O_5-Fe_2O_3-CaO glasses are non-toxic, bio-chemically, and bio-mechanically compatible. The glasses of composition (100-x)(30 Fe_2O_3-70P_2O_5)-xCaO mol. % where x=10, 20, 40 were the subject of the studies. Glasses were synthesized using the conventional casting method. The batches were melted in an electric laboratory furnace in Al_2O_3 crucibles in an air atmosphere. The melting temperature was 1200°C and glasses were kept at this temperature for 2 h. The melts were vitrified by casting onto steel plates. The structural properties have been determined using experimental methods like FTIR, Raman spectroscopy. Also, mössbauer spectroscopy has been used to determine the quantity of divalent iron. The increasing content of CaO causes an increase in $Fe(II)/Fe_{tot}$ ratio and gradual depolymerization of the iron phosphate glass network. The intensities of bands related to Q^2 structural units decrease with an increasing quantity of CaO in the glass structure. The intensities of bands related to the Q^1 structural unit behave the opposite way. Mössbauer spectroscopy showed that the quantity of divalent iron increase with increasing CaO content.

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[1] Hutchinson G., et al., Use of standard addition to quantify in situ FTIR reaction data. J. Org. Chem., 2021, 86, 2012–2016. [2] Steinbach J. C., A process analytical concept for in-line FTIR monitoring of polysiloxane formation. Polymers, 2020, 12, 2473-2486

FTIR cure process monitoring of radical photopolymerization of acrylate/methacrylate monomers and thiol-ene systems

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Photopolymerization, Radical photoinitiators, FTIR spectroscopy, process analysis and process control

Radical photopolymerization is a complex process consisting of multiple reactions taking place inside the polymerization system. Starting from the decay of the initiator into radicals, through the activation of monomer and chain propagation stage, ending with the termination of created polymers chain, the whole process takes only a few seconds. In a chemical view, rapid and simultaneous functional group transformations occurred during this time, which impacted final product properties. That is why, to ensure reproducible product, a method allowing monitoring of the synthesis process is necessary [1]. Until today, many measurement techniques were used to observe polymerization, such as chromatographic, calorimetric, or spectroscopic methods. Of all of them, FTIR is currently the most popular. It allows for real-time monitoring of complex reactions in the multivariate and non-destructive way of analysis. Moreover, thanks to using different light sources in light wavelength and power, it is an excellent tool for monitoring the photopolymerization process [2]. In our work, we used real-time FT-IR spectroscopy to monitor the radical photopolymerization of acrylate/methacrylate monomers and thiol-ene systems in thick and thin layers. Compositions were initiating with diphenyl 2,4,6 trimethyl – benzoyl phosphine oxide (TPO) under the radiation of 405 nm Vis-LED. The evolution of the double bond of acrylate TMPTA content was continuously monitored by real-time FT-IR spectroscopy (Nicolet iS10, from Thermo Scientific U.S.) at about 1634 cm^{-1}. The photosensitive thiol-ene formulations which contain 1,3,5-triallyl-1,3,5-triazine-2,4,6-trione (TATATO) and MERCAPTO monomers (50%/50% w/w) were deposited on a BaF_2 pellet. The evolution of the thiol (S–H) group content was continuously monitored by real-time FT-IR spectroscopy at approximately 2570 cm^{-1}. FT-IR also followed the double bond conversion of TATATO at about 3083 cm^{-1}.

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Material Science and Catalysis
Efficiency Improvement of Dye-Sensitized Solar Cells by UV-Ozone Treatment of TiO2 Mesoporous Layer

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photovoltaics, solar cells, dye-sensitized solar cell, organic impurities

Organic residues on titanium(IV) oxide may be a significant factor limiting the dye-sensitized solar cells (DSSC) efficiency. To prepare the TiO2 layers, screen printing methods are commonly used. After the deposition of titanium paste, which contains, the samples are sintered at high temperature (up to 600°C). This step eliminates the organic components and leads the nanoparticles to form a mesoporous structure with a large active surface. Here we suggest a further UV-ozone cleaning process, which removes impurities from the surface of TiO_2 before dye sensitizing. Scanning electron microscopy (SEM), Kelvin probe, Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy proved that the amount of organic contamination was successfully reduced. UV-VIS spectrophotometry and spectrofluorometry measurements showed higher adsorption of dye molecules after ozonisation, while the bulk properties of titanium(IV) oxide were not modified. Due to the removal of organic impurities by UV-ozone treatment, the power conversion efficiency (PCE) of the prepared DSSC devices was boosted from 4.59% up to 5.89%. This was mostly caused by the increment in the short circuit current (Isc) from 10.3 mA/cm^2 to 12.3 mA/cm^2 and a slight improvement in the fill factor (FF) from 0.67 to 0.70.

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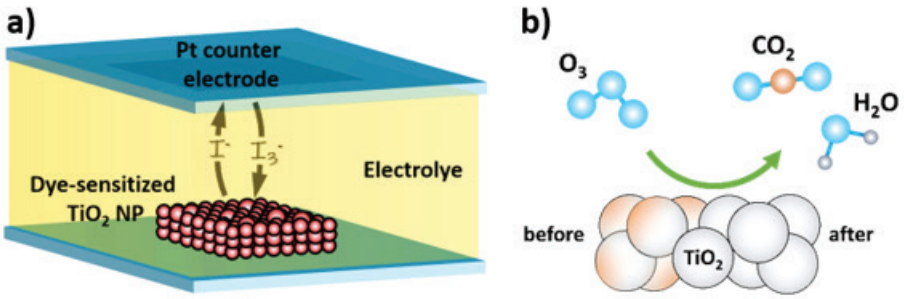

Fig. 1. Schematic representation of a) dye-sensitized solar cell (DSSC) structure and b) principle of UV-ozone cleaning method.

Consider for: poster flash presentation

2.8. Nanomaterials and nanostructures

Tunable thermoplasmonics: sensing phase transitions at the nanoscale

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Tunable thermoplasmonics, plasmon resonance, Raman thermometry, optical heating, glass transition temperature

This work tells us about spectroscopic sensing of the glass transition temperature (*Tg*) of spatially confined PMMA polymers deposited on a plasmonic refractory metasurface using pump-controlled Raman thermometry [1]. The refractory metasurface design represents an array of square-shaped TiN pads on a c-Si (100) substrate, as shown in Fig. 1. The latter functions as a temperature-sensing Raman reporter. This "thermometer" was thoroughly calibrated through both a temperature-dependent anti-Stokes/Stokes ratio and the Raman peak shift by utilizing a macroscopic resistive hot plate. We have theoretically and experimentally investigated the optical heating of a TiN pad under cw laser illumination. FDTD/FEM simulation allows us to detect the temperature increase by 170 K using a 200×200×50 nm^3 TiN pad mounted on a 200 nm high c-Si pillar. We have realized that optical heating can be tuned through extruding c-Si pillars beneath the TiN pad. The longer the c-Si pillar the larger net temperature is achieved. Under cw illumination, a typical behavior of a temperature profile is changed by $r^{-|\gamma|}$($\gamma \leq 1$) that corresponds to a much less confined profile. This enables thicker polymer films to warm up or to reduce the thermal gradients. The spatial resolution in monitoring the *Tg* is defined by the lateral size of the TiN pad. The obtained results pave a route for designing thermoplasmonic metasurfaces able to heat up a sample of interest non-uniformly. In particular, it enables one to create a system of distributed thermal sources generating heat of different magnitudes under the given cw laser illumination. It is important to emphasize that the optical heating is made within a single TiN pad only and, therefore, the rest of the specimen remains intact. Finally, this study will benefit the development of spatially resolved spectroscopic sensing methods for 2D mapping phase transitions of heterogeneous glassy polymers and liquid crystals, polymeric blends and 3D confined polymers.

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[1] Kharintsev S.S., Chernykh E.A., Shelaev A.V., Kazarian S.G., Nanoscale Sensing Vitrification of 3D Confined Glassy Polymers Through Refractory Thermoplasmonics // ACS Photonics 8, 5, 1477-1488 (2021).

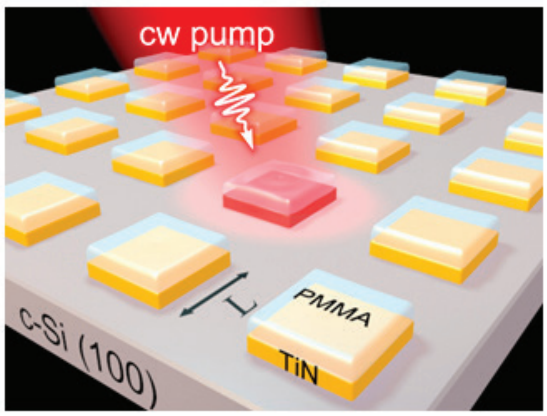

Figure 1. Sketch of a metasurface consisting of square-shaped TiN nanoantennas on a c-Si substrate for sensing the glass transition in a PMMA polymer.

Plasmonic supercrystals for surface-enhanced infrared absorption and surface-enhanced Raman scattering

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SERS, SEIRAS, plasmon, supercrystals,

Plasmonic supercrystals are artificial crystals made out of metal nanoparticles. We recently showed that such crystals have an extremely strong interaction with light, reaching the so-called deep strong regime of light-matter coupling.[1] The electric fields inside plasmonic supercrystals are structured into a collection of nanometer sized hotspots, which makes these materials fascinating environments for enhanced spectroscopy. Particularly exciting is the prospect of combining surface-enhanced Raman scattering (SERS) and surface-enhanced infrared absorption (SEIRAS) using the same substrates. Here we show that plasmonic supercrystals induce near-field enhancement all the way from the visible to the mid infrared [2]. The crystals are highly-ordered three-dimensional (3D) assemblies of gold nanoparticles which we synthesize via the self-assembly at a liquid subphase [3]. Light is confined into regularly arranged hotspots across the entire crystal volume. We probe the near field enhancement through the vibrations of polystyrene ligand molecules. Using SERS with tunable excitation wavelength, we measure pronounced polariton resonances in the near infrared that correlate with the optical absorption of the supercrystals. A polariton resonance leads to a 300-fold increase of the Raman intensity that is uniform across the crystal. The resonance wavelength can be tailored by the crystal thickness and geometry. The resonances of multilayered crystals extend into the mid-infrared enabling SEIRAS of the polystyrene vibrations. The infrared absorption increases by 400% when a polaritonic resonance matches the molecular vibrations. We explain the enhancement with a coupled oscillator model that provides design rules for plasmonic supercrystals with resonant SEIRAS enhancement. Plasmonic supercrystals therefore offer the possibility to combine SERS and SEIRAS on a single substrate.

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[1] Niclas S. Mueller, Yu Okamura, Bruno G.M. Vieira, Sabrina Juergensen, Holger Lange, Eduardo B. Barros, Florian Schulz, Stephanie Reich, Deep strong light-matter coupling in plasmonic nanoparticle crystals, Nature 583, 780-784 (2020). [2] Niclas S. Mueller, Emanuel Pfitzner, Yu Okamura, Georgy Gordeev, Patryk Kusch, Holger Lange, Joachim Heberle, Florian Schulz, Stephanie Reich, Surface-Enhanced Raman Scattering and Surface-Enhanced Infrared Absorption by Plasmon Polaritons in Three-Dimensional Nanoparticle Supercrystals, ACS Nano 15, 5523–5533 (2021). [3] Florian Schulz, Ondřej Pavelka, Felix Lehmkühler, Fabian Westermeier, Yu Okamura, Niclas S. Mueller, Stephanie Reich, Holger Lange, Structural order in plasmonic superlattices, Nature Communications 11, 3821 (2020).

Dynamic Behavior of Surface-Enhanced Raman Scattering substrate of AuNRs interacting with R6G: Implication for Analyses under Wet versus Dry Conditions

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Gold nanorods, SERS, dielectric environment analysis, least projection square, BEM simulation

Massive efforts have been employed to comprehend electromagnetic interactions at hot spots by the dynamic behavior of molecule interaction based on near-field effects of complex electromagnetic interactions that affect deeply the shape of SERS spectrum. We present here a systematic investigation on the impact of the drying process of rhodamine 6G dye (R6G, 10^{-8} mol L^{-1}) solution on SERS substrates prepared by self-assembled AuNRs nanostructures. SERS spectra displayed a strong time-dependence intensity in wet to dry transition states. FEG-SEM images of SERS substrates displayed AuNRs stacking organization that lead to Raman signal improvements due to the formation of a 3D hot spot matrix acting as an excellent trap for target molecules. We observed that the SERS spectra exhibits a characteristic profile of intensities due to different dielectric environmental conditions. This intensity profile provides a pattern that can be recognized by statistical analysis, which in our case corresponded to a SERS response exhibiting a silhouette coefficient of 0.92 using the least-squares projection technique. Changes in the SERS spectra profile from wet to dry state conditions of R6G dye solution can be interpreted by the dynamic behavior of R6G molecules correlated to distinct molecular adsorption and (or) surface distribution of the R6G molecules proving different plasmonic resonances. BEM calculations are in agreement with experimental data and reveal that the SERS enhancement is strongly dependent on the nanoparticle coupling at nanoscale and the dielectric environment.

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[1] Mao, M.; Zhou, B.; Tang, X.; Chen, C.; Ge, M.; Li, P.; Huang, X.; Yang, L.; Liu, J. Natural Deposition Strategy for Interfacial, Self-Assembled, Large-Scale, Densely Packed, Monolayer Film with Ligand-Exchanged Gold Nanorods for In Situ Surface-Enhanced Raman Scattering Drug Detection. Chemistry – A European Journal 2018, 24 (16), 4094–4102.

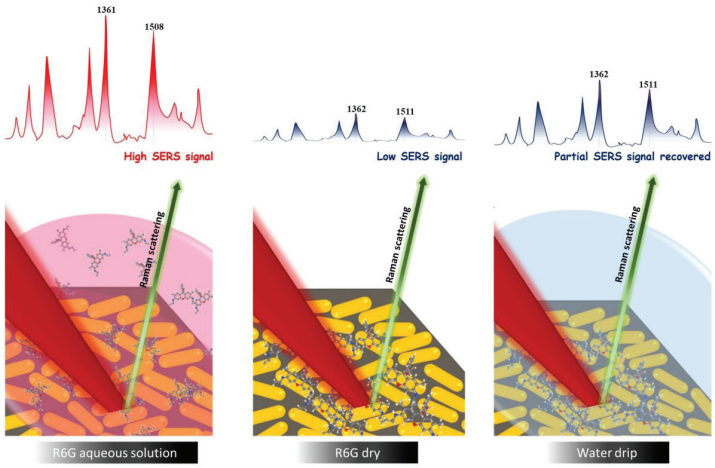

Figure 1. Schematic illustration of enhanced Raman signal under wet and dry analysis conditions

Trapping carrier the spin-locked states in atomic valley by absorption of chiral L-cysteine

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MoS2, Valley Polarisation, helicity resolved spectroscopy, Raman, Chiral molecule

In a chiral or non-centrosymmetric structure, valley is a quantum number which defines the electronic system using energetically degenerate energy bands with non-equivalent local minima (conduction band) or maxima (valence band). The pseudospin polarisation of the system is encoded in the corresponding wavefunction and dipole vector of the optical matrix element. Valley polarization, the selective population of one valley, can be achieved by tuning the incident photon angular momentum. This polarization can be preserved for longer than 1 ns. Finally, the polarisation decays following preferred valley relaxation channels and determines the excitonic valley physics in TMDs [1,2]. In this work, we demonstrate enhanced transitions for valley contrasting spin-momentum locked states in SL MoS_2 coupled with chiral L-Cysteine molecule at ambient condition by helicity-resolved photoluminescence and Raman measurement. Figure 1(a) describes, helicity-resolved steady state photoluminescence for pristine SL-MoS_2 at ambient condition in resonance with A exciton. XD represents defect related transitions. The extent of valley polarisation in pristine SL MoS_2, SL MoS2 when attached with chiral/ achiral molecule (Ch-SL-MoS2/aCh-SL-MoS2) is clarified by considering increment in photoluminescence helicity contrast □=(I+-I-)/(I++I-) as shown in Figure 1(b), where, I+ and I- are the intensities of the receiving signals corresponding to the same or cross circular polarisation. An enhanced contrast (□) is observed in case of Ch-SL-MoS_2 further supported by depolarisation pathways explored by helicity-resolved Raman spectra of the respective samples. The obtained results demonstrate a simple and effective method for entrapping/confining the carrier and tuning optical transitions in a preferred spin singlet state of SL-MoS_2 due to adsorption of chiral L-cysteine (Fig. 1.c). Achiral molecule of similar length cannot induce similar effect. The observed phenomena can be useful for improving the efficiency of light emitting diode devices. Other than this, we propose that the valley selective information can encoded in these chiral spin locked singlet states in by pumping at the A exciton energy and can be extracted when needed – an approach towards future valley based logical device.

S.B. thanks DSKPDF, UGC for financial assistance and Prof. A.K. Sood for constant scientific support

[1] K. F. Mak, K. He, J. Shan & T. F. Heinz, Control of valley polarization in monolayer MoS2 by optical helicity, Nature Nanotech. 2012, 7, 494–498. [2] F. P.-Milton, R. McKenna, L. J. Brennan, C. P. Cullen, L. Guillemeney, N. V. Tepliakov, A. S. Baimuratov, I. D. Rukhlenko, T. S. Perova, G. S. Duesberg, Alexander V. Baranov, Anatoly V. Fedorov, and Yurii K. Gun'ko Induction of Chirality in Two-Dimensional Nanomaterials: Chiral 2D MoS2 Nanostructures, ACS Nano. 2018, 12 (2), 954–964.

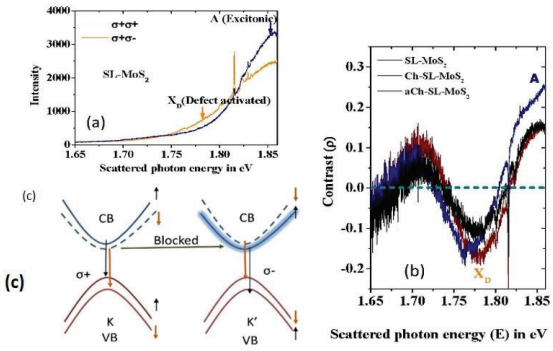

Figure 1. (a) Photoliminescence spectra of pristine SL-MoS2 at ambient temperature (293 K) with incident left circularly polarised light (σ+) excitation of 1.88 eV at ambient condition. Blue line corresponds to same detection configuration (σ+) and oragne line cross detection (σ-) configuration. (b)The helicity contrast spectrum for SL MoS_2 (wine), Ch-Sl-MoS_2 (blue) and aCh-SL-MoS2 (black line). (c) Schematic representation of possible Singlet transition and blockage of intervalley transition in Ch-SL-MoS_2.

Nanoscale Infrared Spectroscopy at SISSI beamline at Elettra Sincrotrone

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Nano-FTIR, nanotubes, DNA, vaccine adjuvants, Infrared

The SISSI (Synchrotron Infrared Source for Spectroscopy and Imaging) beamline of Elettra Sincrotrone Trieste and CNR IOM delivers light over a broad spectral range, from THz to visible. The laboratory represents a valuable tool in several research fields, spanning from biophysics and biology to cultural heritage and solid state physics. Exploiting the high brilliance of synchrotron light, SISSI experimental end stations, SISSI-Bio and SISSI-Mat, offer the possibility to probe matter with diffraction limited lateral resolution of few microns in the mid-IR spectral range. The beamline capabilities have been further upgraded since the recent installation of the nano-FTIR (Neaspec) instrument. Exploiting infrared light of DFG and QCL lasers, as well as of SR, the instrument extends indeed the IR lateral resolution below diffraction-limit, up to 10-20 nm, for both spectroscopy and imaging. We present here three selected nano-FTIR experiments recently performed at SISSI beamline on systems of biophysical interests. We show the experiments performed on DNA adsorbed on clay nanotubes[1]. The data enlighten the capabilities offered by the nano-FTIR in the study of nanocarriers for drug delivery and gene transfer, revealing partial covering of nanotube outer surface by DNA molecules, with concomitant DNA structural modification. Then, a nanoscopic study obtained in the contest of vaccine adjuvants development is also presented, showing model proteins structural modification induced by their adsorption on alumina nanoparticles at different concentrations. Finally, nano-resolved experiments applied to the study of bioinspired materials for wound dressing and tissues scaffolding are presented: the results reveal the presence of nanostructured fiber-like domains in which protein and polymer molecules do not mix. Albeit being only few examples, the presented experiments aim to provide the audience of the conference the flavor of the new capabilities and science that will be achievable by fully exploiting the Nano-FTIR and Nano-imaging techniques.

This work was partly performed in the framework of the Nanoscience Foundry and Fine Analysis (NFFA)

[1] Piccirilli F, Tardani F, D'Arco A, Birarda G, Vaccari L, Sennato S, Casciardi S, Lupi S. Infrared Nanospectroscopy Reveals DNA Structural Modifications upon Immobilization onto Clay Nanotubes. Nanomaterials. 2021; 11(5):1103. https://doi.org/10.3390/nano11051103

Raman scattering obtained from laser excitation of MAPbI3 single crystal

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Perovskite, Raman, Resonant Raman, Single Crystal, Decomposition, Phase Transition

Finding renewable energy sources is of paramount importance to meet the increasing global energy demand whilst minimizing the impact on the environment. The research community has focused on solar energy as it is endlessly available and researchers have ranked the methylammonium lead iodide ($MAPbI_3$) as one of the most promising candidate amongst perovskite solar cells. Despite its high efficiency, the $MAPbI_3$ is sensitive to humidity, light, and temperature, its instability affects primarily on the crystalline structure and eventually leads to degradation [1]. Three crystalline structures are known for this material, orthorhombic, tetragonal, and cubic which exist in different temperatures. Here we report on several processes detected from laser excitation of $MAPbI_3$ single crystal at ambient conditions. A phase transition from tetragonal to cubic phase was induced by excitation of over 15 mW laser power [See Figure 1(B)]. The phases were characterized by LF-Raman and photoluminescence, taken simultaneously with the increase of exciting laser power and the spectral changes were assigned to the structural differences. In addition, Raman stimulation of iodine vapors signal was observed as shown in Figure 1(C) which matches the reported spectrum of iodine vapor in the literature [2]. The vapors were generated from the core of the focus wherein the highest temperature led to degradation. The stimulated Raman phenomenon was enabled due to the unique properties of the $MAPbI_3$ single crystal and revealed viability to use this material for additional applications in other research fields [3].

[1] Ava TT, Al Mamun A, Marsillac S, Namkoong S, A review: thermal stability of methylammonium lead halide based perovskite solar cells. Appl. Sci. (2019); 9, 188. [2] Fouche DG, Chang RK, Observation of resonance Raman scattering below the dissociation limit in I2 vapor, Phys. Rev. Lett. (1972); 29, 536. [3] Ben-Uliel T, Aviv H, Zhou J, Li M, Avadyayev S, Kapon O, Damle V, Yi C, Tischler YR, Raman scattering obtained from laser excitation of MAPbI3 single crystal. Appl. Mater. Today (2020); 19, 100571.

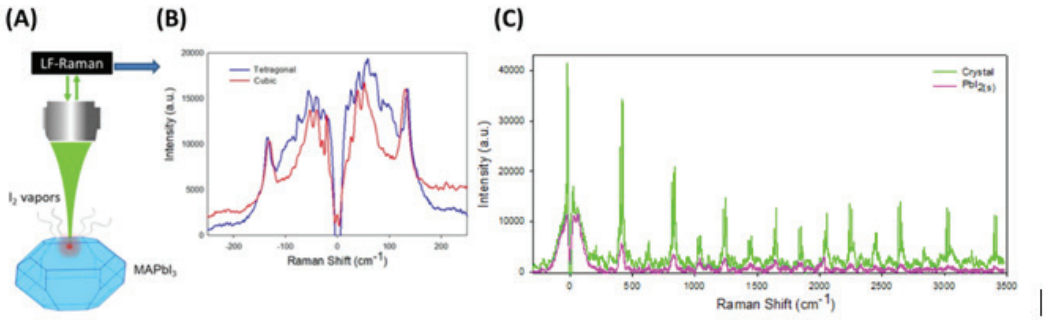

Figure 1. Resonant Raman Detection of trapped iodine vapor in Perovskite single crystal. (A) Schematic representing the processes of phase transition and decomposition. (B) Raman scattering spectra of tetragonal and cubic phases of the crystal. (C) Raman spectra of the Perovskite single crystal exhibiting Resonant Raman scattering from the Perovskite single crystal compared Raman from PbI_2.

UNDERSTANDING INTRACELLULAR INTERACTIONS OF GOLD NANOSTARS

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Gold nanostars, SERS, nanotomography, nanoparticle uptake

Gold nanostars, anisotropic nanostructures with sharp tips, are promising candidates for a wide range of potential applications, such as drug delivery or early disease diagnosis. Their use in surface enhanced Raman scattering (SERS) spectroscopy for label-free detection of biomolecules inside living cells gives relevant information on nanostructure-biomolecule interactions that affect further applications.[1] To better understand the processing of these nanostructures in their cellular environment and to assess the optical information that can be retrieved from them, it is important to combine techniques that provide chemical and ultrastructural information. We present results on the distribution of gold nanostars in cells of three different cell lines together with detailed information from SERS on the structure and composition at their surface and their optical properties. The SERS spectra observed after cellular uptake of gold nanostars indicate a close interaction mainly with protein components in the endolysosomal system. The quality of these interactions is clearly different depending on the cell line. Additionally, the biomolecule-nanostar interactions also show differences with the incubation times, reflecting the different stages of endolysosomal processing of the nanostars.As we found by synthesizing nanostars of different morphology, nanostructures of different spike length interact differently with lipids and hydrophobic components regardless of the cell line they reside in. Furthermore, their optical properties differ, as demonstrated in experiments and FDTD simulations of the electromagnetic enhancement of their nanoaggregates, with higher enhancements found for nanostars of shorter spike length. The processing of the intracellular aggregates that are formed, their properties and their interaction with the biological matrix was also characterized by synchrotron soft X-ray tomography (SXT). Localization, size, morphology, and density of the intracellular gold nanoaggregates vary with cell line and incubation time.[2] The combination of vibrational microspectroscopy and nanoscale tomography to understand the intracellular fate of metallic nanostructures will improve the design of safe and efficient nanoprobes, with implications for further developments as tools in bioanalysis, biotechnology and theranostics.

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[1] C. Spedalieri, G.P. Szekeres, S. Werner, P. Guttmann, J. Kneipp. Intracellular optical probing with gold nanostars. Nanoscale 2021, 13, 968-979 [2] C. Spedalieri, G.P. Szekeres, S. Werner, P. Guttmann, J. Kneipp. Probing the intracellular bio-nano interface in different cell lines with gold nanostars. Nanomaterials 2021, 11(5), 1183

Insights into the physicochemical interactions at the nano-bio interface: A multispectroscopy approach

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surface-enhanced Raman spectroscopy (SERS), surface-enhanced infrared spectroscopy (SEIRA), nanospectroscopy, charge-transfer, gold nanoparticles sensors

This study focuses on exploring the possibility of conventional Raman (RS), infrared (IR), surface-enhanced Raman (SERS) and surface-enhanced infrared (SEIRA) spectroscopies in micro- and nanoscale to describe physicochemical interactions at the metal/anti-breast cancer drug interface (Fig. 1) [1,2]. The influence of two orthogonal polarization modulations in SEIRA nanospectroscopy on the deduction of the bond arrangement in the adsorbed molecule was also taken into account [3]. This was made possible by combining the atomic force microscopy (AFM) with the IR method. Furthermore, the effect of the electrode potential and excitation wavelength on the metal-molecule surface states modeling is also discussed. The performed SERS analysis for drug adsorbed on Ag electrode surface confirmed the selective contribution of the charge-transfer mechanism along with the formation of various metal complexes adsorbed on the substrate [4]. This type of approach, based on specific phenomena occurring at the metal/drug interface, can lead to a far-reaching improvement in the field of biosensing and bioimaging, which can result in a significant improvement in combination anticancer therapy. Accurate characterization of the vibrational structure of the selected drug, as well as the strength and manner of its interaction with the metal surface, are very important to better plan the introduction of the drug/nanosensor conjugate into cancer cells.

The research was performed by the use of the equipment purchased in the frame of the project co-funded by the Małopolska Regional Operational Program Measure 5.1 Krakow Metropolitan Area as an important hub of the European Research Area for 2007–2013 (No. MRPO.05.01.00–12–013/15). This study was also supported by the National Science Centre Poland (No.2017/01/X/ST4/00428).

[1] E. Pięta, C. Paluszkiewicz, M. Oćwieja, W. M. Kwiatek, Appl. Surf. Sci. 404 (2017) 168. [2] E. Pięta, C. Paluszkiewicz, W. M. Kwiatek, Phys. Chem. Chem. Phys. 20 (2018) 27992. [3] E. Pięta, C. Petibois, C. Paluszkiewicz, W. M. Kwiatek, Appl. Surf. Sci. 499 (2020) 143975. [4] E. Pięta, M.R. Lopez-Ramirez, C. Paluszkiewicz, W.M. Kwiatek, under review.

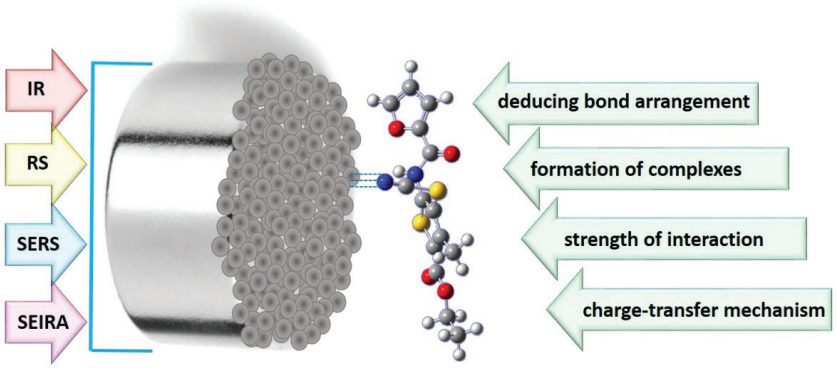

Figure 1. Schematic representation of the performed experiments.

In operando investigation of polyyne formation during pulsed laser ablation in liquid by surface-enhanced Raman spectroscopy

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Polyyne, Raman spectroscopy, Surface enhanced Raman spectroscopy, Pulsed laser ablation in liquid, Carbon nanostructure

Besides the most known allotropic forms of carbon, polyynes are conjugated linear chains made by the alternation of single and triple sp-hybridized carbon bonds[1]. During the last decades, their remarkable predicted properties attracted the interest of material scientists[1]. These properties are deeply connected to polyyne structure, so a deeper control of their synthesis will open the route to future applications. In particular, their vibrational properties have been widely analyzed through Raman spectroscopy[2]. Indeed, sp carbon chains possess a peculiar Raman fingerprint that consists in the collective stretching motion of the CC bonds of the wire. The position and the shape of this mode are strongly influenced by the length and the endgroups of the chain[2]. Surface-enhanced Raman spectroscopy (SERS) is commonly employed when the concentration of polyynes is too low to induce a good Raman signal. The interaction with metal nanoparticles heavily modifies the electronic structure of the chain and produces the appearance of low-frequency bands[2] Among the physical methods used to synthesize polyynes, pulsed laser ablation in liquid is commonly employed[1]. However, the mechanism of the formation of polyynes during the ablation remains unclear[3]. In this framework, we investigated the process of formation and degradation of polyynes during ablation. We performed in operando analysis during ablation in acetonitrile with a 532 nm ns-pulsed laser, using a SERS probe able to detect even very low polyyne concentrations. SERS spectra are continuously collected during the 60 minutes ablation process (Fig. 1a). We found a peculiar evolution of the signal of polyynes (1800-2200 cm^{1}) compared to that of other sp^2 carbon species (1000-1600 cm^{-1}) (Fig. 1b). We suppose that the decrease is mainly due to the degradation of polyynes anchored to the nanoparticles that crosslink with other compounds in solution. We compared the differences in the formation of long (1800-2000 cm^{-1} and short chains (2000-2200 cm^{-1}) during the ablation (Fig. 1b). We noticed that shorter chains grow more as the ablation proceeds, as confirmed by ex-situ chromatographic analyses. We also developed a computational method to deconvolve the overall sp signal and highlight the contribution of the chains with different lengths in the mixture, as a function of ablation time. SERS spectra of a series of size-selected H-capped polyynes (6 to 20 C atoms) were employed. In such a way, we were able to further understand the mechanisms of formation of the different chains during the ablation.

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[1] Casari C.S., Nanoscale (2016), 8(8), 4414–4435 [2] Milani A., Beilstein J. Nanotech. (2015), 6, 480–491 [3] S. Peggiani et al., Phys. Chem. Chem. Phys., 2020, 22, 26312–26321

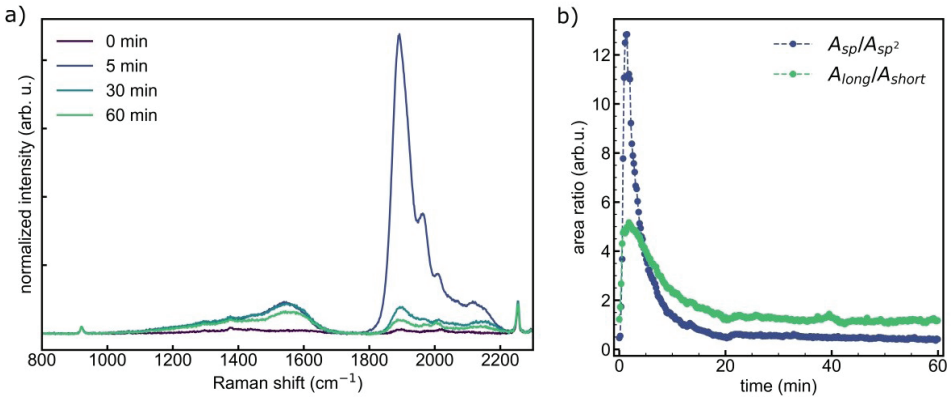

Figure 1. a) SERS spectra at fixed time interval of the ablation (0, 5, 30 and 60 minutes) b) Evolution during the ablation of the ratio of sp vs sp2 areas, and long vs short polyynes.

The Raman study of the carbon nanotubes layers deposited on titanium and steel support.

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Carbon nanomaterials, Multi-walled carbon nanotubes, Multi-wavelength Raman microspectroscopy, Carbon coatings, Simulated body fluid

The extraordinary potential of nanotechnology comes from its ability to control material properties by modifying them in a nanoscale size, what leads to the huge increase in the size to the volume ratio of the structures. Nanotechnology proposed new method to produce materials for the usage, e.g., in the field of medical therapies and diagnostics. Due to the size and surface properties, nanoparticles are able to mimic natural tissues and even they may be involved in biological processes. Among a broad spectrum of nanomaterials, especially carbon nanotubes are of great interest for biomedical applications, because of their biocompatibility, also extraordinary mechanical and electrical properties.[1] The aim of presented study was to analyse and compare the model of partially oxidized carbon nanotubes (MWCNTs) layer obtained by electrophoretic deposition on two types of supports, on titanium and stainless steel plate. The biocompatibility and influence of body environment were tested for both type of materials by their incubation in simulated body fluid (SBF) for 3 weeks. Raman spectroscopy was the method of choice and the spectra were obtained using of several laser wavelengths over the entire visible range. For each measured sample Raman spectra contained the G band about 1600 cm^{-1} (resulted of the in-plane vibrations of the sp^2 carbons), as well as, D1 band in the range of 1315-1360 cm^{-1} which indicates a disorder in the carbon nanostructure. In order to access information about the degree of organisation of studied carbon layers before and after incubation in SBF, the I_{D1} / I_G ratio was calculated (assuming a three band model in the curve fitting procedure). The results indicate that human body environment alternate the molecular structure of CNT layers, I_{D1} / I_G ratio decreased after incubation in SBF, and this characteristic was more pronounced for steel plates. It suggests the decrease of defects which could be connected to the reduction of oxidic groups on the surface of CNT. Dispersion of the G- and D1-band position, that reflects the disorder in the nano-carbonous samples, has also been observed. It turned out that the D1- band shows the greatest dispersion, and it is slightly higher for the CNT layers deposited on titanium compared to the layers on a steel substrate. To sum up, Raman spectroscopy allows to get insight into degree of molecular organisations of carbon nanostructures deposited as a layer on two types of implant plates. The influence of simulated human body environment on the CNT layers, and thus their biocompatibility were evaluated.

This work has been supported by projects: EU POWR.03.02.00-00-I004/16 and NCN PL 2017/25/B/ST8/02602

[1] Zhang, L.; Webster, T.J. Nanotechnology and nanomaterials: Promises for improved tissue regeneration. Nano Today 2009, 4, 66–80, doi:10.1016/j.nantod.2008.10.014.

Investigation of surface adherent polydopamine layers on various substrates by means of near-field infrared microscopy

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polydopamine, adhesive layers, FTIR, scanning near-field infrared microscopy, atomic force microscopy

Polydopamine (PDA) is a synthetic melanin-like polymer material commonly used as a universal adhesive layer for biomedical and material applications. PDA is formed *via* oxidative self-polymerization polymerization of dopamine, which occurs spontaneously in a slightly basic environment forming oligomers and nanoparticles and allows PDA to be deposited on practically any substrate. Thanks to its chemical structure, incorporating functional groups such as catechol, quinone, amine, and imine, the PDA layer may be further modified with molecules carrying nucleophilic groups or with metallic nanoparticles. Nevertheless, the precise chemical structure of PDA, the mechanism of the polymerization, and the effect of the type of substrate on the PDA layer formation are yet to be fully described.[1-3] Within this study, we have employed the techniques of grazing angle attenuated total reflection Fourier-transform infrared microspectroscopy (GAATR-FTIR) and scanning near field infrared microscopy (SNIM) to study the formation and growth of the PDA layer and the dependence on the deposition time and the type of underlying substrate. Seven samples, each with different PDA deposition times, were prepared for three types of substrates – Si/SiO_x, N-TiO_2, and Au. The GAATR-FTIR spectral analysis was followed by principal component analysis and partial least squares regression, to reveal the most time-dependent spectral bands. The identified bands were assigned mainly to the formed PDA and to quinone species, which are intermediate products of the polymerization. The intensity of quinone bands exhibited a maximum at the 5-minutes of PDA deposition time and continually decreased for longer deposition times. On the other hand, the intensity of all bands assigned to PDA increased steadily with increasing polymerization time. For a detailed analysis of the PDA layers, the samples were further studied by SNIM, which is a modern nanoscopic technique that combines the excellent spatial resolution of tapping AFM and chemical sensitivity of FTIR spectroscopy. To acquire SNIM maps of the optical response of PDA on each substrate, we have selected several excitation wavelengths to match the absorption bands of PDA (1600 and 1510 cm^{-1}) and quinone intermediates (1720 cm^{-1}) based on the GAATR-FTIR spectra. As a result, we were able to detect PDA micro- and nanoparticles on all the prepared samples and monitor their distribution and size. With this approach, we aim to contribute to the overall understanding of PDA layers' formation and the role of the substrate type and deposition time.

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[1] Liu Y. et al., Chemical Reviews, 2014, 114(9), p. 5057–5115. [2] Batul R. et al., Biomaterials Science, 2017, 5(7), p. 1204–1229. [3] Zangmeister R.A. et al., Langmuir, 2013, 29(27), p. 8619–8628.

Evaluation of biological response to iron oxide nanoparticles in in vitro cellular models by Raman spectroscopy

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iron oxide nanoparticles, Raman spectroscopy, biomolecular analysis

Superparamagnetic iron oxide nanoparticles (IONPs) are intensively investigated due to their great potential in medical applications, among others the tissue repair, targeted drug delivery, contrast enhancement in MRI and cancer treatment using the phenomenon of local hyperthermia [1,2]. Being an appropriate candidate for biomedical applications results from the unique features of IONPs such as small size, biocompatibility and superparamagnetic properties [3]. However, the successful transfer of these nanomaterials (NMs) from basic research to clinics requires the precise assessment of the relationship between physical and chemical properties of IONPs and their biological response what is a great challenge of nowadays nanotoxicology. The main objective of our study was analysis of biomolecular changes occurring in the human embryonic kidney cells (HEK293T), mouse macrophages (P388D1) and human glioblastoma multiforme cell line (U87MG) as a result of after 24-hour exposure to PEG-coated magnetite NPs having the cores of 5, 10 and 30 nm in diameter. Besides the core size, the influence of IONPs concentration on observed anomalies was verified and for this purpose the cells were exposed to solutions of NMs in the concentrations of 5 and 25 μg Fe/ml. The anomalies in the distribution and structure of biomolecules introduced by IONPs were studied with the use of Raman microscopy with the spatial resolution of 0,5 micrometer. The Raman spectra recorded for treated cells, besides the presence of absorption bands typical for examined cell lines, revealed additional Raman peaks around 1870-2000 cm^{-1}, 2360 cm^{-1}, 3581 cm^{-1} and 3800-4100 cm^{-1} wavenumbers which probably origin from the complexes or adducts consisting of Fe and some organic compounds. Further study on this topic are necessary as they may shed the new light on the mechanisms underlying the impairment in functioning of cells exposed to IONPs.

NJ-O has been partly supported by the EU Project POWR.03.02.00-00-I004/16

[1] Palanisamy S, Wang YM. Superparamagnetic iron oxide nanoparticulate system: synthesis, targeting, drug delivery and therapy in cancer. Dalton Trans. 2019 Jul 2;48(26):9490-9515. doi: 10.1039/c9dt00459a. PMID: 31211303. [2] Zhao S, Yu X, Qian Y, Chen W, Shen J. Multifunctional magnetic iron oxide nanoparticles: an advanced platform for cancer theranostics. Theranostics. 2020 May 15;10(14):6278-6309. doi: 10.7150/thno.42564. PMID: 32483453; PMCID: PMC7255022. [3] Malhotra N, Lee JS, Liman RAD, Ruallo JMS, Villaflores OB, Ger TR, Hsiao CD. Potential Toxicity of Iron Oxide Magnetic Nanoparticles: A Review. Molecules. 2020 Jul 10;25(14):3159. doi: 10.3390/molecules25143159. PMID: 32664325; PMCID: PMC7397295.

Analysing the crystallinity of PLA stereocomplexes and nanoparticles by the means of Raman spectroscopy in solid state and in suspension

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PLA based nanomaterial, crystallinity, raman spectroscopy, dielectrophoretic capturing

It is well known that the physico-chemical features of nanoparticles influence drug encapsulation as well as release kinetics [1,2]. In this study Raman spectroscopy was used to characterize crystallinity of polymer nanomaterials. Usually, it is difficult to change the crystallinity without altering the hydrophilic hydrophobic balance (HHB). However, incorporation of different amounts of racemic 3-ethylglycolide (EtGly) into polylactic acid (PLA) results in nanomaterials of varying crystallinity while keeping the HHB constant [1,2]. Within the CRC Polytarget, the D- and L-form of PLA comprising EtGly were merged to form stereocomplexes [1], from which nanoparticles with different EtGly content were produced under different crystallization conditions [2]. The crystallinity of those stereocomplexes and nanoparticles was characterized by means of Raman spectroscopy in solid state and in suspensions [2]. Spectral changes in the C=O stretching band at around 1760 cm-1 were correlated to crystallinity values obtained by standard methods, like Wide-Angle X-ray Scattering (WAXS) and Differential Scanning Calorimetry (DSC). Using dielectrophoretic capturing [3] nanoparticles with different EtGly content and different times of stereocomplexation before nanoprecipitation were analyzed directly in suspensions. It was shown that only nanoparticles with pure PLA and sufficient time for stereocomplexation showed significant crystallinity. Heating curves of solid nanoparticles in an in-house built heating chamber were used to analyse the temperature-dependence of the spectra using 2D correlation as well as to determine glass transition and melting point of the different materials.

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[1] D. Bandelli, J. Alex, C. Helbing, N. Ueberschaar, H. Görls, P. Bellstedt, C. Weber, K. D. Jandt, and U. S. Schubert, Polym. Chem. 2019, 10, 5440 -5451. [2] K. Scheuer, D. Bandelli, C. Helbing, C. Weber, J. Alex, J. B. Max, A. Hocken, O. Stranik, L. Seiler, F. Gladigau, U. Neugebauer, F. H. Schacher, U. S. Schubert and K. D. Jandt, Macromolecules 2020, 53, 19, 8340–8351 [3] U.-C. Schröder, A. Ramoji, U. Glaser, S. Sachse, C. Leiterer, A. Csaki, U. Hübner, W. Fritzsche, W. Pfister, M. Bauer, J. Popp, and U. Neugebauer, Anal. Chem. 2013, 85, 10717 -10724.