# THz-Raman – A New Analytical Tool

## Extending Raman spectroscopy to other spectral regions – modality for microscopes, vials and well plates

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THz-Raman is a new analytical technique that uses optical sensing to obtain quantitative information about macroscopic motion and structure in samples, including phase changes, ligand binding of proteins, and biopolymer conformational data. This technique is now packaged for easy integration into optical microscopes and spectrometers for research use, as well as in stand-alone well plate readers for use in high-throughput drug discovery and related applications.

Optical interrogation of samples, often involving lasers, is key to several types of automated and high throughput analyses in fields such as drug discovery, flow cytometry for blood assays, and DNA sequencing. Most of these life sciences applications entail determining the molecular (chemical) composition of the target, often by using laser-induced fluorescence to do spatial mapping.

The current world health backdrop where we are running out of non-resistant antibiotics was already creating pressure to conduct drug discovery faster and more intensely than ever. But the immediate threat of the Covid-19 pandemic has naturally driven researchers to push that much harder in all aspects of life science research, in order to speed up fundamental understanding of the virus, develop therapeutic drugs to mitigate the effects of infection,, and, at some point, to create a vaccine to stop this pandemic. Any and all current analytical methods are being used in this urgent worldwide multidisciplinary effort.

A completely new type of modality has recently been added to the analytical arsenal. Specifically, THz-Raman is yet another optical technique that can be harnessed for life sciences applications, with all the usual benefits inherent to optical methods, including automation, speed, microscopic resolution and so forth. But unlike other optical techniques, this new modality maps the local physical state or phase of a sample rather than just its composition: the degree of crystallinity, the amount of different drug polymorphs, distinguishing between polymer conformations, whether two materials are co-crystallizing, and whether a protein and ligand are binding or not, etc. Moreover, because it can be seamlessly integrated with 'conventional' Raman, it provides a unique means to simultaneously interrogate both the local chemical and structural composition information.

## Raman vibrational spectroscopy

Every chemical, i.e., every molecular species, has a unique arrangement of atoms and interatomic bonds. The vibrational energy states of these bonds correspond to photon energies of infrared light. So each molecular species has a unique pattern of resonance peaks – a chemical signature – in its infrared

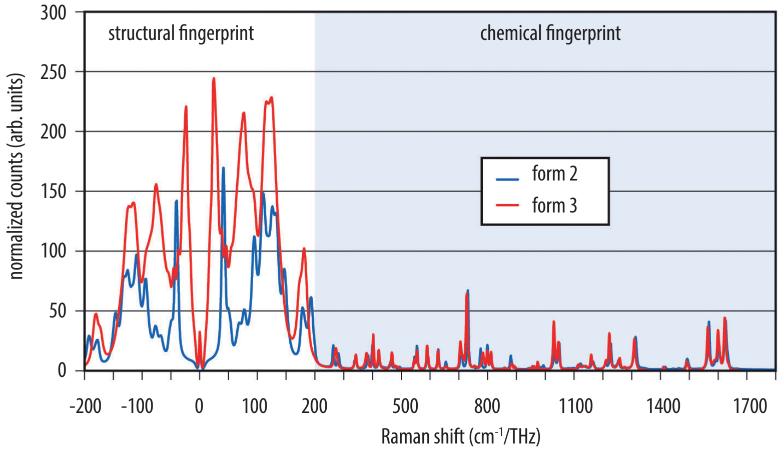

**Fig. 1** A THz-Raman spectrum of two forms (polymorphs) of a pharmaceutical compound. Conventional Raman provides chemical fingerprint data (blue section), where differentiation of form is difficult to detect. Extending Raman to the low frequency (THz) domain (white section) provides unique structural fingerprint information with dramatically higher magnitude and highly differentiating characteristics.

spectrum. This is the reason that infrared absorption, usually in the form of Fourier transform infraRed (FTIR), is one of the most common analytical tools in chemistry and biochemistry research labs. But infrared light is limited to larger vibrational energies that correspond to submolecular bonds. To probe the crystal phonon modes or detect some of the other conformational changes related to changes in the structural properties of these materials, the required frequencies extend into the terahertz range which are much more difficult to produce and detect, requiring special sample preparation and suffering from poor signal to noise. Fortunately, a once obscure technique called Raman spectroscopy provides an alternative route to the same data that avoids most of these limitations.

When light interacts with most molecules and material samples (solid, liquid or gas) a small amount of the light is scattered at different frequencies, leaving the molecules in a different final energy state. Conservation of energy means that the scattered light can be at a longer wavelength (Stokes-shifted) or shorter wavelength (anti-Stokes) depending on whether the sample is left in a higher excited state or lower excited state. This is called the Raman effect. Whereas direct absorption requires infrared frequencies (wavelengths) to change the vibrational state, in Raman the signal is shifted relative to the original light source by an amount corresponding to that change in vibrational energy state. If the excitation light source is monochromatic, the Raman-scattered signal can be dispersed to reveal a frequency spectrum of sharp vibrational peaks in a frequency band called the chemical fingerprint region.

Compared to FTIR, Raman has the advantage that it can be performed using visible light or NIR light, enabling non-contact sampling through glass windows, microscope optics and using standard silicon CCD-based detectors. However, Raman scattering is a second order effect and relatively weak and therefore requires a laser source to provide measurable signal intensity. At the same time, laser light scattered by the sample (Rayleigh scatter) and by the system optics is orders of magnitude more intense than the Raman signal and creates a noise background that must be selectively blocked. This limited the early adoption of Raman. But over the last twenty to thirty years, advances in solid-state and diode lasers, holographic gel-based filters, and scientific grade cameras have all combined to eliminate the need for inefficient cumbersome equipment such as scanning monochromators, and eventually enabled the development of compact self-contained Raman spectrometers and Raman microscopes.

## THz-Raman

What about extending Raman to look at other types of resonances (i.e., different spectral regions) in a sample? With larger molecules like polymers and proteins, macroscopic motion of the large molecules or crystal lattices can occur at sample-specific frequencies, particularly in the 0.15 – 6 THz energy range corresponding to 5 – 200 $\text{cm}^{-1}$ Raman shifts. Spectral data here can reveal tremendous details about the local intermolecular environment: the amount of crystallinity vs. amorphous material, the amount of liquid phase, the coiling and uncoiling of proteins and other polymers, and the binding of proteins, etc. Recently, scientists have been studying samples in this under-exploited part of the spectrum. However, THz is much more difficult radiation to generate, detect and manipulate. The sources are complex and inefficient, often based on ultrafast lasers. And the detectors are equally complex. As a result, THz spectroscopy and imaging has struggled to translate from a research subject to an analytical technique with a few very small niche exceptions.

In theory, low frequency Raman, i.e., Raman with THz shifts, could easily get this same data. But in practice, as the Raman shift is decreased and the intense

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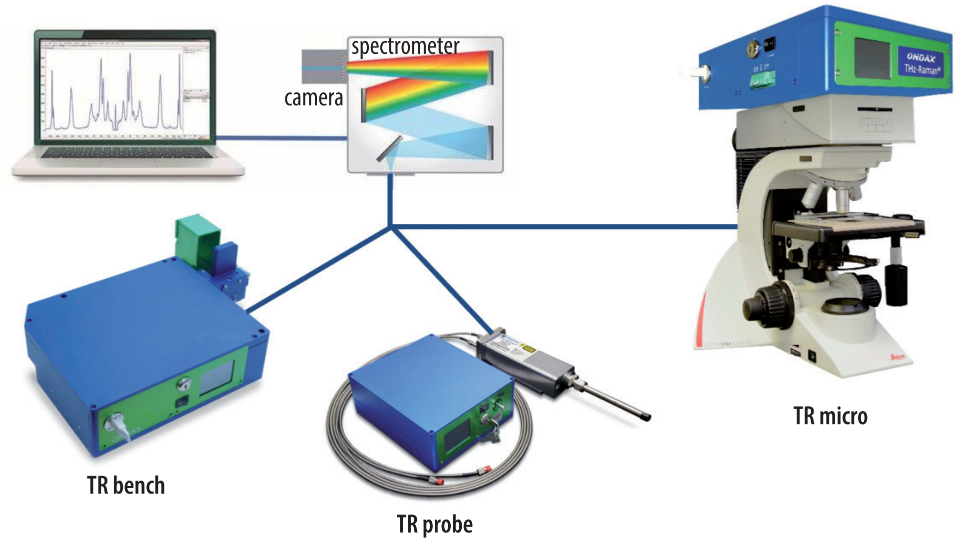

**Fig. 2** A cost-effective way to support THz-Raman applications is to package the technology as an add-on module for existing Raman spectrometers and microscopes.

Rayleigh wavelength is approached, the blocking characteristics of the filters attenuate the signals and even weak broadband amplified spontaneous emission (ASE) from the laser means that there is a steep non-linear increase in the background noise. This limited most Raman systems from capturing <200 $cm^{-1}$ Raman signals (also known as the low-frequency or low-wavenumber region) with conventional Raman technology. Low-frequency Raman therefore required a quantum leap in wavelength blocking and discrimination efficiency, i.e., filters with much sharper cut-off characteristics and narrower bandwidths.

Fortunately, a new type of volume holographic optic based on photo-sensitive glass (rather than gels) has solved this problem. These filters are used to clean up the laser output in terms of its spectral spread, and then efficiently filter the signal to eliminate the Rayleigh scattered laser light. As a result, instruments based on these optics can now provide excellent signal to noise in the 5 – 200 $cm^{-1}$ region of the spectrum, now nicknamed the 'structural fingerprint' region – see Fig. 1.

## Turnkey spectrometers, modules and microscopes

To support and grow the nascent field of THz-Raman requires not just novel optics, but turnkey instruments that incorporate those optics. However, since most applications for Raman will benefit from having both chemical and structural information, a particularly cost-effective way to package THz-Raman for these applications is in the form of turnkey modules that add plug-and-play THz functionality to existing Raman spectrometers – see Fig. 2. These modules all include a wavelength stabilized laser, high performance spectral cleanup filters, and ultra-narrowband notch filters to block only the Rayleigh line of the scattered spectrum. They are available in benchtop and microscope module formats; or by decoupling the laser from the filter head, a more compact probe format. To enable testing of multiple sample types, various optional sample interface accessories can be easily attached and interchanged, including a vial holder to support typical analytical applications, a contact/immersion probe tip, a non-contact optic, and a large area illumination adapter. Each module interfaces to an existing spectrometer via a simple fiber connection

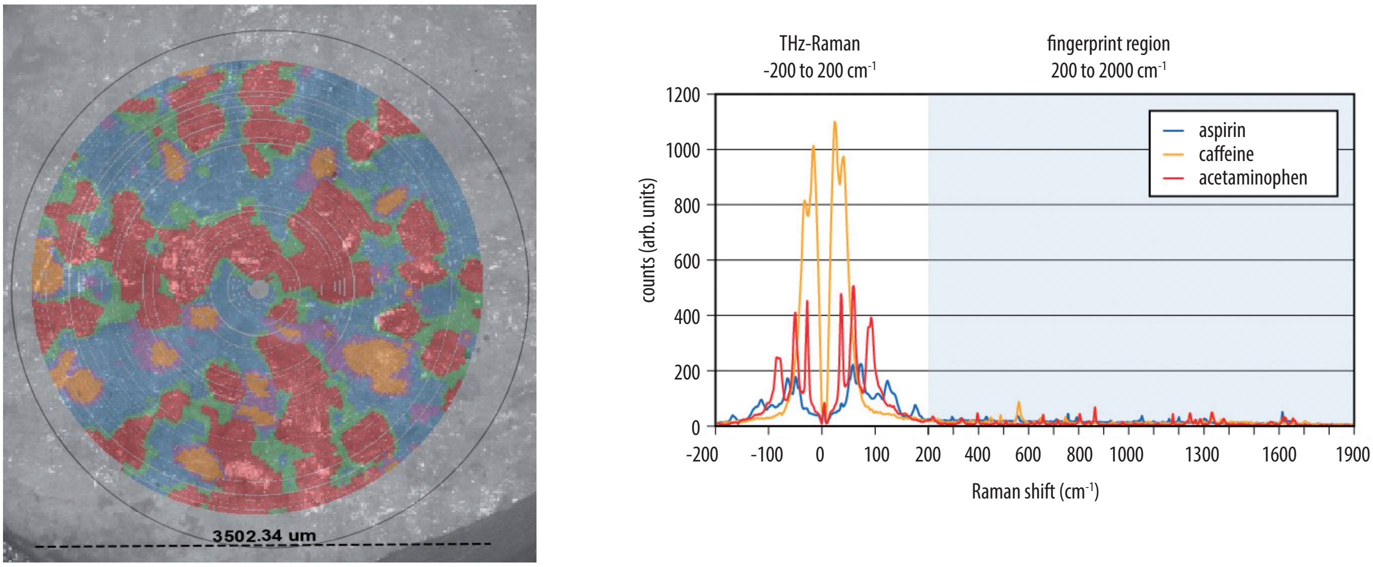

**Fig. 3** Rapid tablet mapping image of an excedrin tablet placed in a well, based on >27 k spectral data points collected over a 3.5 mm diameter region of the tablet in nine minutes. The colored Raman map showing the various components is overlaid on the polarized light microscopy image for easier data visualization. The corresponding spectra to the right illustrate the strong signals found in the structural fingerprint region.

that is already common for most of the spectrometer installed base, making them a simple solution to upgrade the performance of Raman instruments in the field. Alternatively, any of these modules are also available integrated with its own stand-alone spectrometer as a stand-alone instrument.

## Raman well plate readers

At this time, a particularly important area for virtually any analytical instrumentation is supporting high throughput drug development. As the rapidly developing worldwide Covid-19 pandemic has shown, the need to quickly evaluate the efficacy of new and existing drug candidates is greater than ever. To enable massive parallel analysis, much drug discovery work is performed in well plates. Here, the combination of conventional and THz Raman with its optical microscope compatibility can deliver fast, sensitive and reliable measurements of both chemical composition and molecular structure.

Coherent supports these applications with a completely self-contained specialty Raman instrument called the THz-Raman Well-Plate System (TR-WPS). This automatically captures the entire Raman spectrum from -400 $cm^{-1}$ to +2100 $cm^{-1}$ for common well-plate formats; it is configured to accept standard well-plate sizes from 6 to 1536 wells. The software and user-selected objectives mean that intrawell mapping is also possible with a spatial resolution on the order of several microns – see Fig. 3. Moreover, the TR-WPS utilizes a linearly polarized illumination source to also provide polarized microscopy images of the wells that can be spatially overlaid with the Raman data for direct visual comparison of spectral features. (Polarized microscopy is a proven method of imaging polycrystalline and mixed phase samples that highlights the discontinuities and phase boundaries.)

The TR-WPS is thus ideal for screening well plates for chemical make-up as well as for new optical measurements for the identification of polymorphic compounds and co-crystals, or for quantifying the degree of crystallinity in pharmaceuticals, or for following conformational changes in biopolymers, e.g., tertiary and quaternary protein conformations, as well as for the binding of proteins and ligands, to name just a few applications.

## Summary

In conclusion, a new analytic modality, THz-Raman, enables fast non-contact and quantitative assessment of structural information about samples. Now packaged with conventional Raman in a well-plate reader, THz-Raman brings a new dimension by which researchers can rapidly and automatically analyze their samples.

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