

THz-Raman[®]: Advancing Efficiency and Reliability in Pharmaceutical Analysis and Quality Control

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Introduction

The pharmaceutical industry faces many challenges in drug development, manufacturing, and quality control, considering the consequences of an inappropriate or ineffective drug being consumed by a patient. One primary concern is that many active pharmaceutical ingredients (APIs) exhibit *polymorphism*, where different forms or molecular structures of a compound can dramatically affect the efficacy, stability and bio-availability of the drug, with potentially devastating effects on patient outcomes. These structural changes are in many cases quite subtle and hard to detect, and may unintentionally occur during various stages of formulation, storage, packaging and handling. Consequently, rapid and reliable identification of polymorphs during the development, manufacturing, and quality assurance process is critical to all pharmaceutical manufacturing companies.

Observing such structural shifts of a compound can be accomplished several ways. *Raman spectroscopy* is used to observe small band shifts in the “fingerprint” region (200-1800 cm^{-1}), however these reflect subtle shifts in functional groups and are often difficult to detect for many polymorphs. *X-ray diffraction (XRD)* techniques yield extremely quantitative and conclusive analysis, but require expensive equipment and destructive off-line testing. *Terahertz (THz) spectroscopy* can easily differentiate structural shifts, as these signals correspond to large scale motions in the molecular and inter-molecular structure, however THz spectroscopy has limited spectroscopic range, is susceptible to the presence of moisture, is comparatively expensive, and can require special sample preparation or temperature changes to boost the signal.

A new Raman-based solution has recently been commercialized that incorporates a new capability to observe *molecular structure*, while concurrently performing the comprehensive, non-destructive chemical compositional analysis already associated with traditional Raman spectroscopy. These new spectroscopy systems extend the range of traditional Raman spectroscopy down into the low frequency (low wavenumber) spectral regime and beyond into the anti-Stokes region, where important structural details - including lattice or polymer structures, crystal orientation, spin waves, and phonon modes - can be clearly discerned. Since these vibrational energies correspond to molecular transitions and vibrations in the 5 cm^{-1} to 200 cm^{-1} range (equivalent to 0.15 to 6.0 THz), the term “**THz-Raman[®]**” is used to describe this new spectral region and the associated instrumentation (Figure 1).

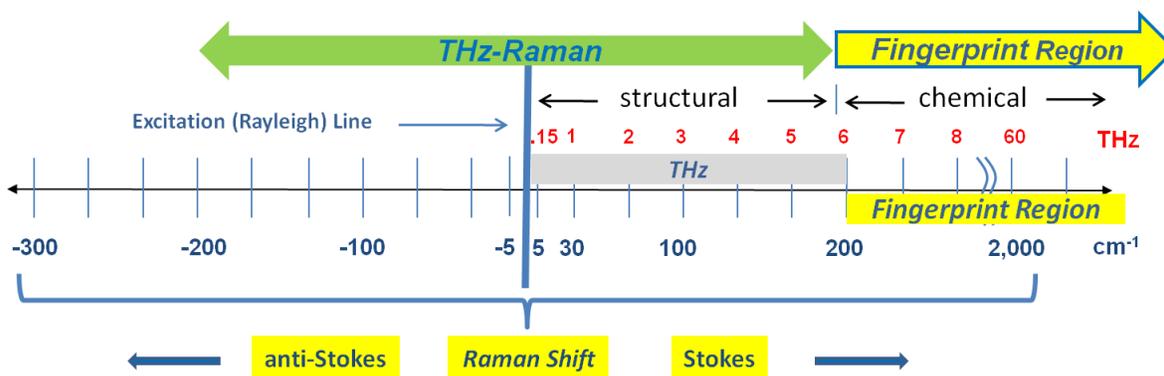


Figure 1. Spectral range of THz-Raman spectroscopy showing both fingerprint and THz-Raman regions.

THz-Raman is rapidly gaining significant interest and application as an important new tool for chemists across the pharmaceutical, industrial, and petro-chemical industries. In addition to providing fast, unambiguous differentiation of polymorphs, THz-Raman can also be used to differentiate synthetic pathways, raw materials, and contaminants, as well as improving counterfeit detection and surety testing. By including both low-

frequency (low wavenumber) and anti-Stokes signals, THz-Raman systems boost overall Raman intensity and improve SNR. Plus, the symmetrical nature of the anti-Stokes signals act as a confirmation of Stokes peaks as well as providing an inherent self-calibration capability, improving overall reliability. THz-Raman systems provide fast, unambiguous differentiation of polymorphs, while preserving the complete Raman “fingerprint region” for chemical identification and analysis.

Polymorph Detection

Figures 2 and 3 demonstrate the efficiency of THz-Raman for detection of polymorphs. Carbamazepine (CBZ) is an anticonvulsant and mood-stabilizing drug commonly prescribed in the treatment of epilepsy and bipolar disorder. It has four different polymorphic forms that have been well characterized in the literature^{i,ii,iii,iv,v} with form 3 being the active pharmaceutical ingredient. We obtained pure samples of form 2 and form 3 as well as the hydrated form and measured the entire Raman spectra with an Ondax THz-Raman® XLF-CLM system (spectra shown in Figure 2, system shown in Figure 7). Due to the similarities of chemical composition, the fingerprint region signals are extremely similar. However, the differences in molecular structural can be easily seen the THz-Raman region, with signal intensities of up to 4.5 times those in the fingerprint region. Figure 3 shows an expanded view of CBZ polymorphs, including the hydrated form, in the THz-Raman regime. Note the symmetry of anti-Stokes signals across the laser line, which further serve to validate the low-frequency measurements and provide additional signal that can be used to boost the detection sensitivity of the system.

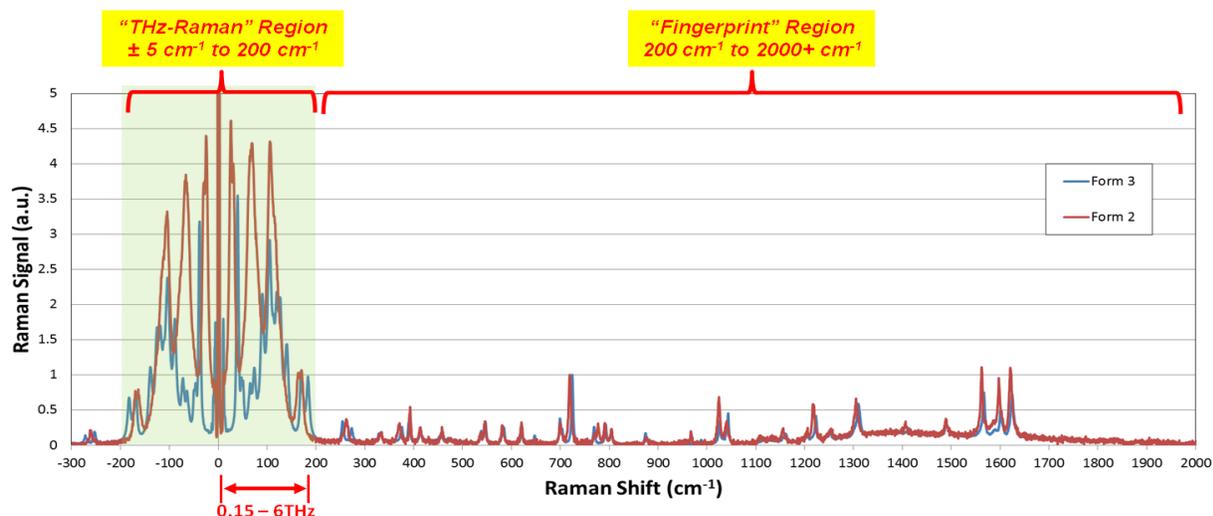


Figure 2: Complete Raman spectra of Carbamazepine Polymorphs Forms 2 and 3. Note much clearer differentiation in the THz-Raman region, as well as peak intensities more than 4x those in the fingerprint region.

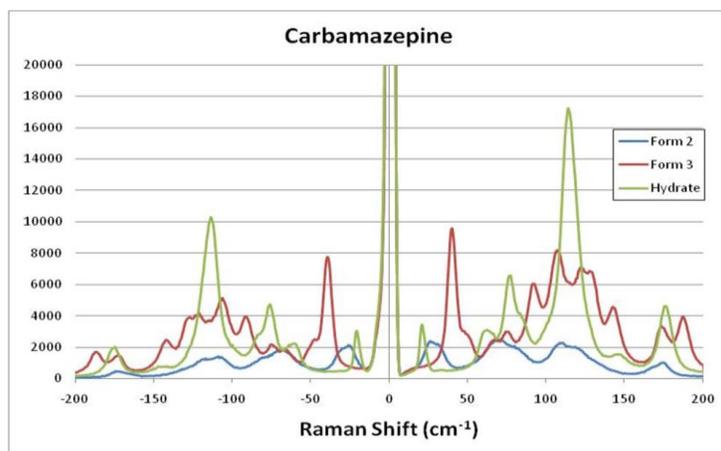


Figure 3. Expanded view of THz-Raman spectra of Carbamazepine polymorphs, including the hydrated form.

Many other common APIs exhibit polymorphs. Figure 4 shows another example of complete Raman spectra for different polymorphs of Indomethacin (IDM), Probucol (PBC) and Acetaminophen (APAP). These confirm the general trend that THz-Raman spectra are both much larger in intensity, and more easily distinguishable than signals in the fingerprint region.

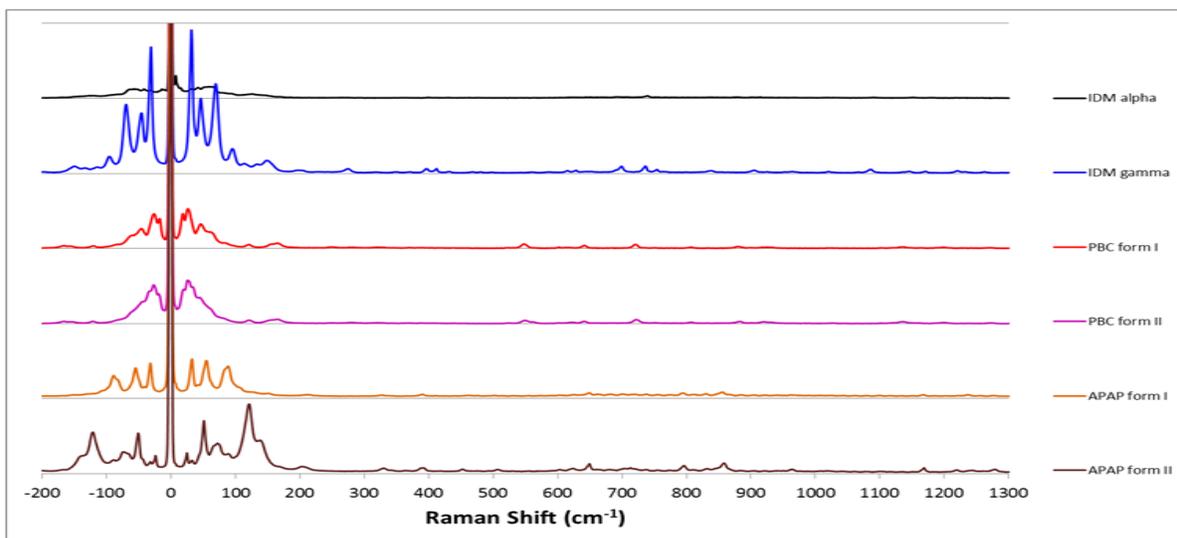


Fig. 4a

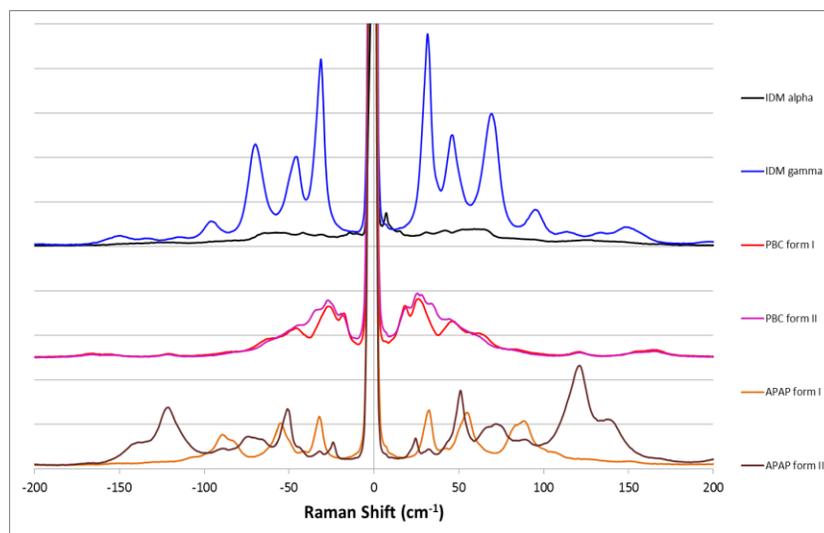


Fig. 4b

Figure 4.: Complete Raman spectra (a) of polymorphs of several APIs: Indomethacin (IDM alpha & gamma), Probucol (PBC forms I & II) and Acetaminophen (APAP forms I and II). Note the stronger intensity (a) and clearer differentiation of peaks in the THz-Raman region (b). (Samples and spectra courtesy Dr. Tatsuo Koide, National Institute of Health Sciences, Division of Drugs, Tokyo, Japan and Dr. Toshiro Fukami, Nihon University, School of Pharmacy, Funabashi, Japan).

Phase and Crystal Monitoring

Two other important applications in pharmaceutical and industrial chemistry are real-time monitoring of phase changes or crystallization. A good demonstration of the benefits of THz-Raman for phase monitoring can be seen in Sulfur, which forms over 30 different allotropes^{vi}. The most common and easiest to produce are forms α , β and λ . A sample of α sulfur was placed on a microscope slide and heated with a hot plate while measuring the Raman spectra with the THz-Raman system as a function of temperature, using 80mW of 785nm laser power on the sample and 10 seconds of total integration time at each temperature setting (Figure 5). When the sample temperature was increased above 95.2°C, the form changed from α to β , then changing again to λ at the melting point of 115.21°C. Note that while there are clearly recognizable changes in both

peak location and magnitude in the THz-Raman region, there is no obvious shift in the position of the peaks in the Raman fingerprint region.

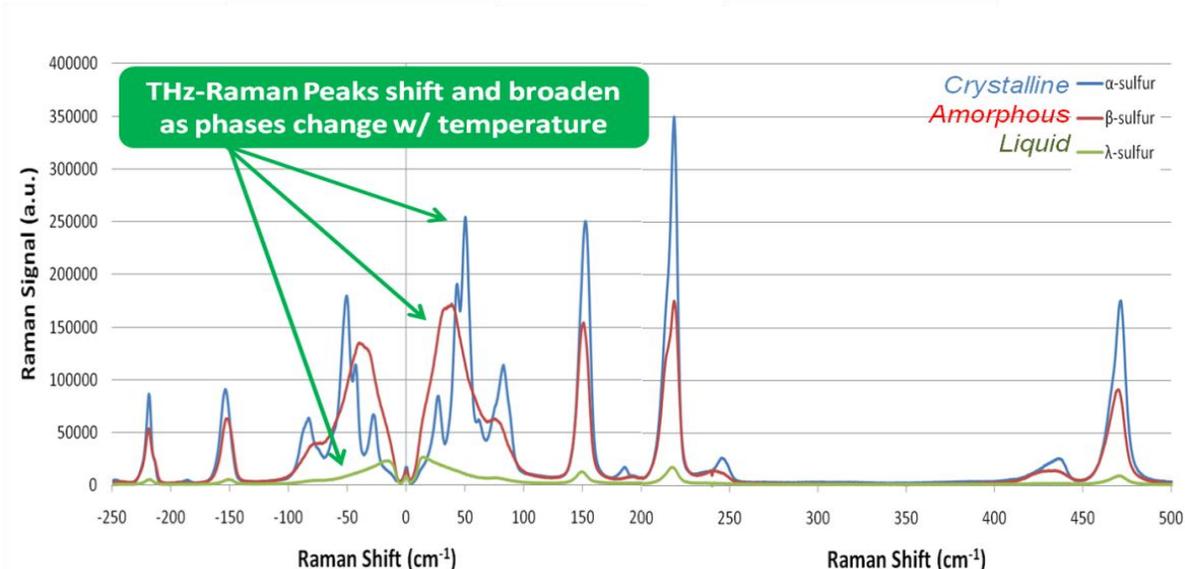


Figure 5: Monitoring phase changes in Sulfur. As the phase shifts from highly crystalline (α phase) to amorphous (β phase) to liquid (λ phase), THz-Raman peaks can be seen to shift and broaden.

Monitoring the presence or formation of cocrystals is also improved using THz-Raman spectra. Figure 6 shows the clearly recognizable peak shifts that occur when cocrystals are formed in a mixture of Caffeine and 2-Benzoic acid.

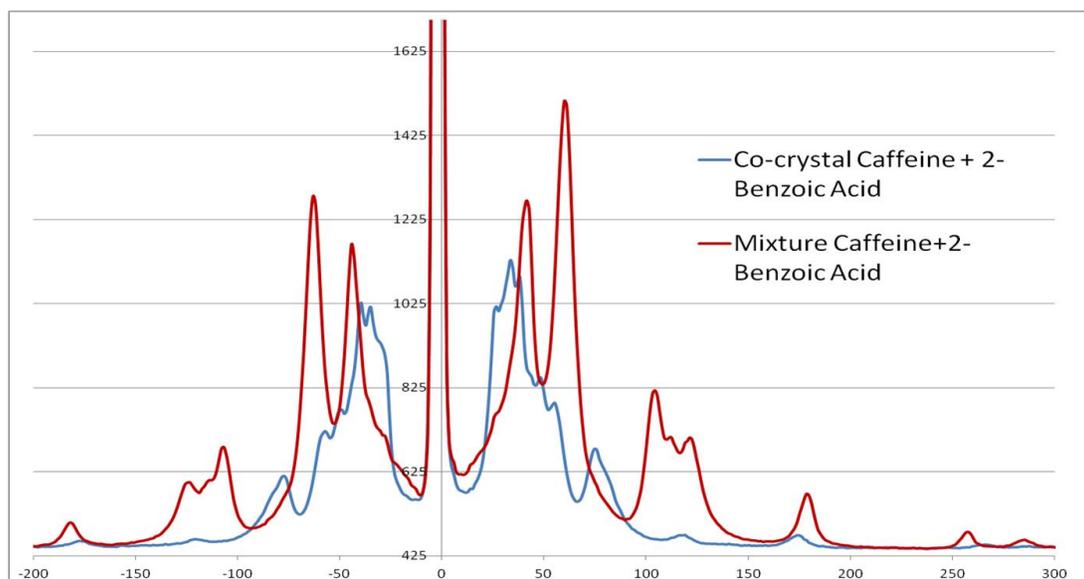


Figure 6: THz-Raman spectra of caffeine and 2-benzoic acid, showing the shift in spectra when cocrystals are formed.).

THz-Raman Instrumentation

The unique, patented Ondax THz-Raman[®] platform used for these measurements utilizes a series of proprietary ultra-narrow-band volume holographic grating (VHG) notch filters, which are able to precisely and comprehensively block just the Rayleigh excitation without compromising any of the Raman scattering signature. By comparison, most commercial Raman systems utilize thin film edge filters which will completely remove both the Rayleigh excitation as well as all anti-Stokes signals, and typically cut off all signals below about 200cm⁻¹. Even the best edge-filter systems will usually block all signals below 50cm⁻¹.

An alternative approach is to use notch filters, which will allow capture of anti-Stokes signals, but do not have sufficiently narrow bandwidth to allow capture of low-frequency signals. Finally, a multi-stage (or cascaded) monochromator system can be used but is large, complex and expensive; furthermore it drastically reduces the overall Raman signal and cannot simultaneously capture anti-Stokes signals.

Recent advances in volume holographic grating (VHG) filter technology have enabled the manufacture of these exceptionally narrow bandwidth notch filters with very high throughput^{vii}. This has led to systems that are capable of rapid acquisition of high-quality, ultra-low frequency Raman spectra in the $\pm 5\text{-}200\text{ cm}^{-1}$ region (Figure 7)^{viii,ix}. These systems are based on a stabilized wavelength diode laser source, a compact series of VHG filters and a single stage spectrograph (Figure 8). Each VHG filter has a notch profile that is designed to diffract only one specific wavelength matching the laser and transmit all other wavelengths. The ultra-narrow transition bandwidth of these filters enables extremely high attenuation of the laser wavelength (system $>OD\ 9$), while maintaining very high transmission of nearby Raman signals beyond $\sim 5\text{ cm}^{-1}$. This combination of strong Rayleigh attenuation and high broadband transmission enable the system to simultaneously capture both the intense low-frequency Raman bands and fingerprint region transitions, greatly simplifying the overall system and reducing the cost, while improving the sensitivity and reliability of using Raman for polymorph identification and other applications. The entire laser and filter assembly is also extremely compact, and the low power requirements of the laser make the system operable by battery supply if desired.

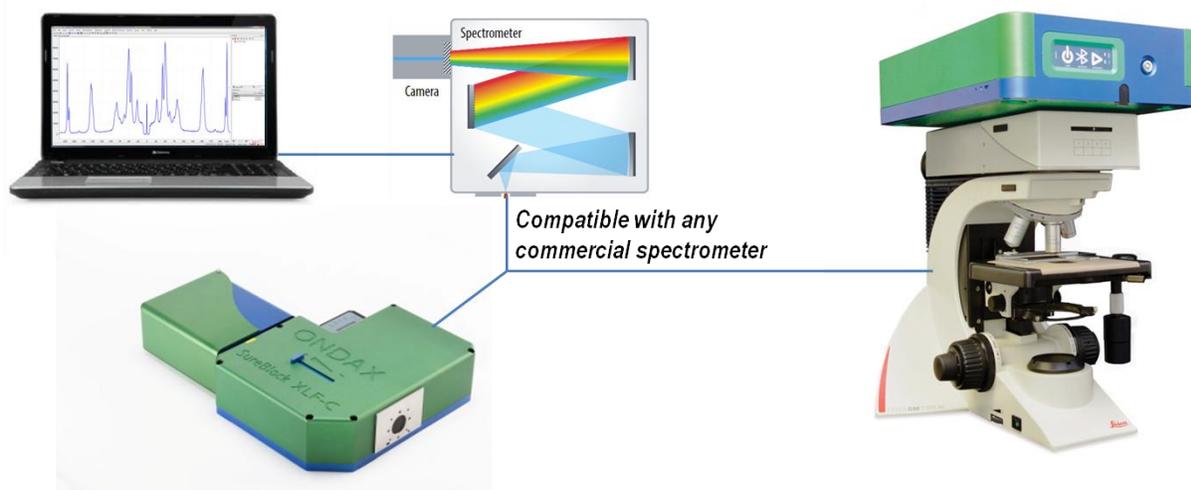


Figure 7. Ondax THz-Raman[®] Spectroscopy systems come in either benchtop or microscope configurations. Benchtop systems come with a convenient vial holder for sample measurements.

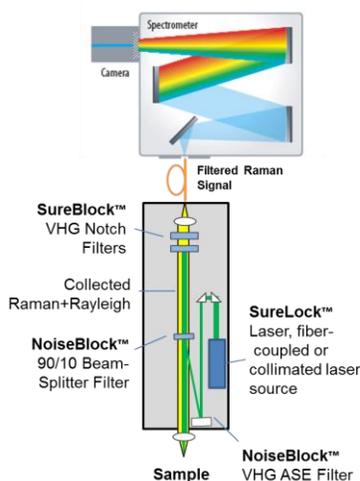


Figure 8. System schematic of a THz-Raman spectrometer platform, showing multiple VHG filters and a wavelength stabilized laser

The ultra-narrow bandwidth of the VHG filters (<0.1nm) also requires the laser to have a very stable wavelength. Non-stabilized diode lasers tend to be highly subject to mode hops which can shift the laser wavelength outside the blocking range of the filters and result in either reduced Rayleigh suppression or a complete loss of signal in the described configuration. Gas lasers and DPSS lasers are normally sufficiently narrow in linewidth and stability to work with VHG filters, however they are limited to visible wavelengths which can cause fluorescence in many materials. Both visible and NIR diode lasers can be stabilized using VHG filters to provide the stable, single-frequency performance required for THz-Raman systems.

Summary and Conclusions

Variations in manufacturing and formulation processes, synthetic pathways, ingredients and environmental conditions (including temperature, pressure, solvents, contaminants, etc.) can lead to changes in molecular structure. THz-Raman makes it possible to actively monitor – *in real time and with a single instrument* – structural changes during formulation, processing, or packaging to ensure the quality and integrity of the material. THz-Raman signals are much stronger in intensity and more clearly differentiating than those in the fingerprint region, plus the addition of anti-Stokes signals also improves overall SNR and the spectral symmetry provides an inherent calibration reference, thereby eliminating concerns about ongoing calibration. And the compact and affordable nature of the system allows it to be easily integrated into existing Raman systems or configured for application-specific uses.

By promising improved speed, simplicity and reliability of polymorph detection, as well as overall chemical analysis, THz-Raman is becoming an important new tool for the pharmaceutical industry to drive manufacturing process improvements, increase overall yields and safety, and reduce manufacturing costs. Besides polymorph identification, there are many other applications where THz-Raman signals can be used to gain important new information about materials and boost system sensitivity, including explosives trace detection and forensics, polymer and industrial chemical development and manufacturing, cancer detection, and basic material science. These new platforms will open up the possibility of using THz-Raman for these and a multitude of other industrial and scientific uses in the years ahead.

Acknowledgements

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