STRaman™ Technology: Raman for See Through Material Identification

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Introduction

A new Raman system design is presented that expands the applicability of Raman to See Through diffusely scattering media such as opaque packaging materials, as well as to measure the Raman spectrum and identify thermolabile, photolabile, or heterogeneous samples.

Traditional Raman fiber optics probes employ a focused design similar to confocal microscopes. The excitation light emerging from the laser fiber is collimated and then focused on the sample by a focusing optics, and the same optics also collimates the Raman signal beam scattered by the sample with another lens focusing it to a signal collection fiber. The sample is usually placed at the focal plane of the focusing lens. In this way excitation power density and consequently the Raman signal density is maximized at the sampled volume, and only signal from this tightly focused volume is collected by the collection fiber. This confocal design has the advantage of maximum throughput, and can be used to measure samples inside transparent containers in the same way a confocal microscope does optical sectioning. However the confocal approach loses its effectiveness when the container diffuses the light strongly, as the light can no longer be focused inside the container. The Raman signal for materials inside diffusely scattering containers is weak, and is often accompanied by strong features from the container itself.

Spatially Offset Raman Spectroscopy (SORS)[1] intentionally offsets the excitation beam and collection beam, and can be used effectively to collect Raman signal generated underneath diffusive top layers while largely avoiding their signature from overwhelming that of the sample. However, probes of such design cannot be easily used in a confocal arrangement and are generally very inefficient for direct sample measurements.

The See Through STRaman Analyzer developed by B&W Tek illuminates and collects the Raman scattered light from a large sample area. This greatly increases the effective sampling depth, allowing the measurement of material inside visually opaque containers as the relative intensity of the signal from the deeper layers increases even as the overall signal is lower. The larger sampling area has the additional advantage of preventing sample damage by reducing the power density, as well as improving measurement accuracy by eliminating heterogeneous effect. With its high throughput design, the See Through analyzer provides identity of common chemicals through various non-transparent packaging materials in seconds. For conventional confocal Raman measurements, a range of accessories can be used to take advantage of the system's high throughput design allowing for standoff, contact, or microscopy Raman analysis. This flexibility plus its portability makes the i-Raman® Pro ST Analyzer an ideal tool for a variety of applications, from incoming material identification to QC to demanding research.

Following are some examples illustrating the benefits of the STRaman™ technology. All spectra are acquired on an i-Raman Pro ST spectrometer equipped with a 450 mW 785 nm laser (B&W Tek), and the intensity axis corrected using the procedure developed at National Institute of Standards and Technology.[2]

i-Raman Pro ST See Through measurements

Through Diffusely Scattering Plastic Containers

Many solid chemicals purchased in small quantities are delivered in white plastic containers, often made of titanium dioxide filled polyethylene. The filler material scatters light and makes the plastic appear opaque. Laser light cannot be focused inside such containers due to the strong scattering, precluding the use of conventional Raman backscatter measurement designs. Using the STRaman technology, both 785 nm laser and the Raman scattered light can penetrate the plastic wall through a diffuse scattering mechanism, and its large effective sampling depth allows such materials to be interrogated and identified.

Shown in figure 1 is the measurement of sodium benzoate through a white plastic bottle. Using the STRaman configuration, strong contribution from the sodium benzoate is evident on top of the container signature as in 1(a), while the spectrum acquired using a traditional configuration is dominated by the container material, with only a small contribution of the content material as in 1(b). Subtracting properly scaled (b) from (a) results in 1(c), which is in close agreement with the spectrum of pure sodium benzoate shown in 1(d).

The STID software uses a proprietary algorithm to isolate the content signature from the container and is capable of identifying even very weak Raman scatterers such as metal oxides inside strong scattering package material.

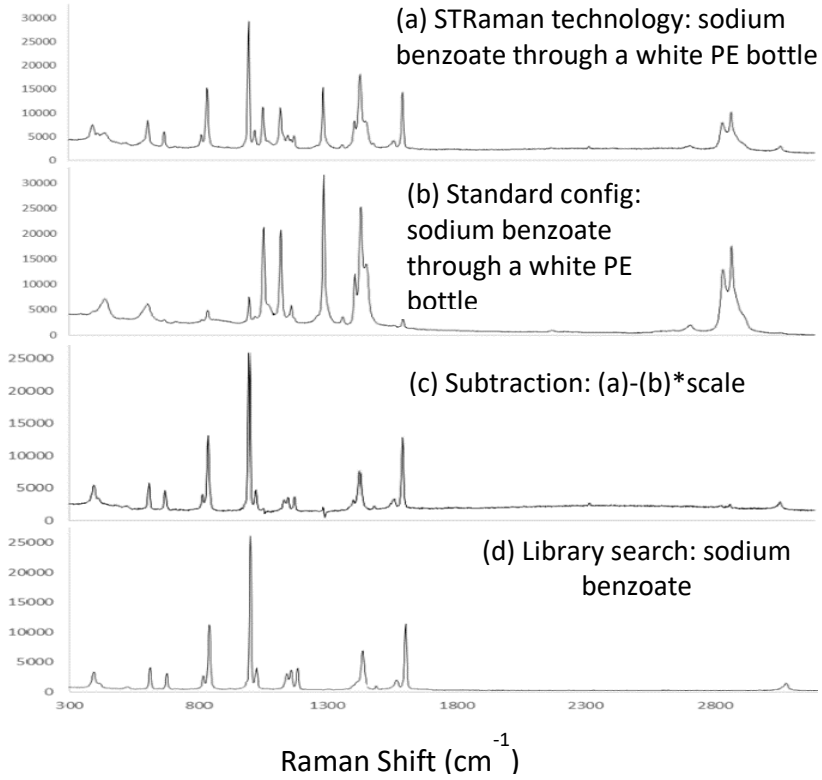

Figure 1. STRaman for identification of sodium benzoate through a white polyethylene bottle: (a) Spectrum measured through the bottle using the STRaman technology; (b) spectrum measured with a standard Raman configuration; (c) the result of scaled subtraction of (b) from (a); and (d) pure spectrum of sodim benzoate.

Through paper envelopes

Paper is made of mostly cellulose fibers that strongly scatter light. Raman spectra of paper products are often accompanied by strong fluorescence. Using the STRaman technology, D-(+) glucose is readily identified when measured through a manila envelope, as shown in figure 2(a). With a regular confocal Raman configuration, only the signature of cellulose is observable on top of a fluorescence background, as in 2(b)

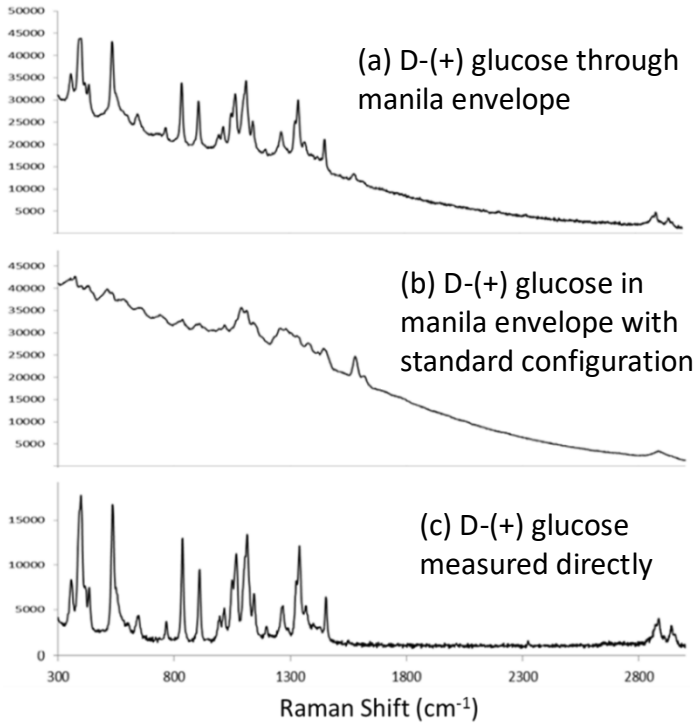

Figure 2. STRaman for identification of D-(+) glucose through a manila envelope. (a) Spectrum measured through the envelope using the STRaman technology; (b) spectrum measured in envelope with a standard Raman configuration; (c) spectrum of D-(+) glucose measured directly with a standard Raman configuration (no packaging materials).

Coated Tablets

Many oral medications are in the form of coated tablets. This may make it difficult to measure using traditional Raman systems which do not have a deep penetration depth. As shown in figure 3(b), the drug's Raman signature is almost completely masked by the coating (the peaks between 600 and 1400 cm^{-1} are signatures of sucrose, which is part of the coating). Using the STRaman technology, however, the Raman signature from the drug dominates, as shown in 3(a), which is comparable to the pure drug spectrum in 3(c).

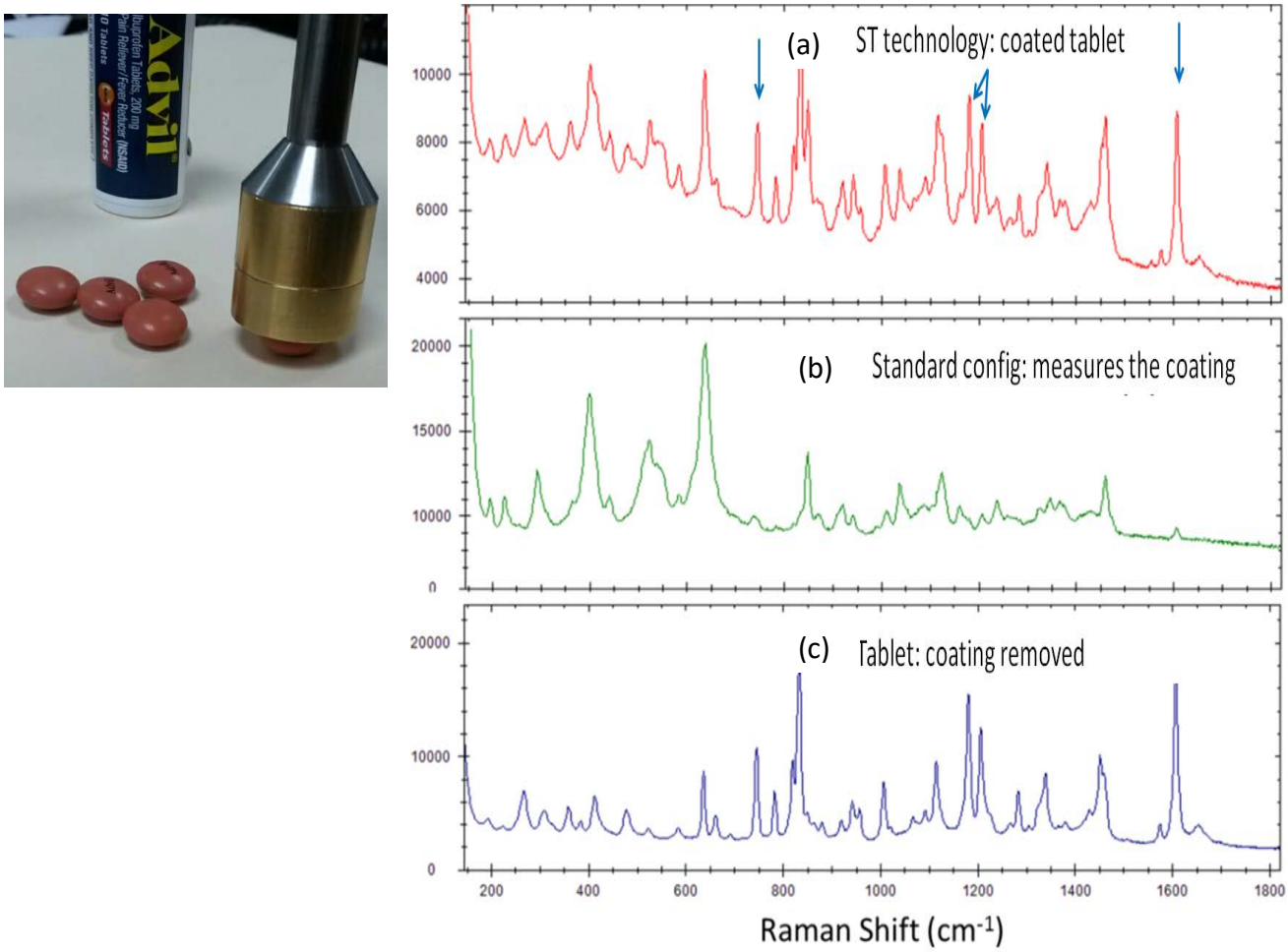

Figure 3. STRaman for measurement of coated Advil tablets. (a) Coated tablet spectrum measured using the STRaman technology; (b) Coated tablet spectrum measured with a standard Raman configuration; (c) spectrum of the tablet with the coating stripped measured with a standard configuration.

i-Raman Pro ST Provides Decreased Power Density

Dark material absorbs light and can be a challenge to analyze with Raman without damaging the sample. Because the STRaman system spreads the laser power over a larger sampling area, it can be successfully used in such cases with minimal sample heating and damage. This feature of the technology expands the capability of Raman to measuring sensitive samples such as photolabile or thermolabile material, and tissues. As an example, the spectrum of gun powder shown in Figure 4 obtained with the STRaman technology at full power, shows Raman peaks of the gun powder component sulfur at 217.6 cm^{-1} and 471.5 cm^{-1}, and nitrate at 1049.3 cm^{-1}.

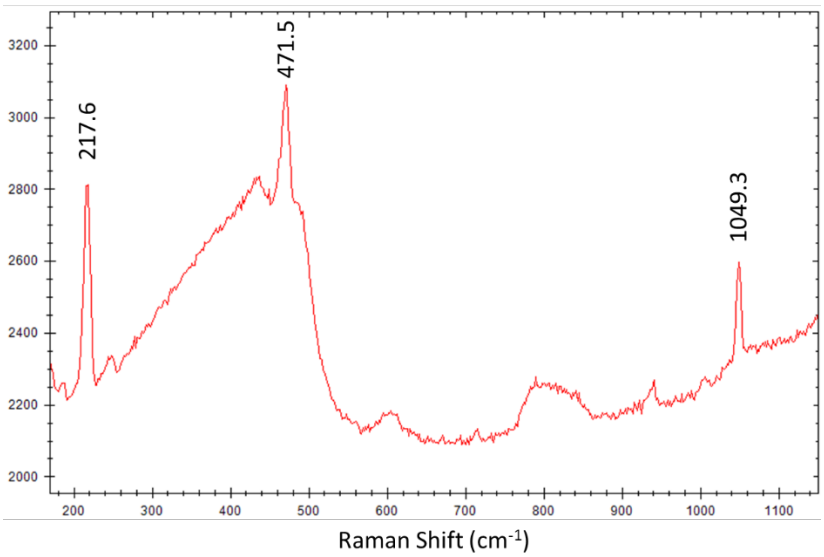

Figure 4: i-Raman Pro ST spectrum of gun powder with main peaks of sulfur and nitrate marked.

The deep penetration and low power density as a result of the large sampling area make the STRaman technology suitable for examining mammal tissue *in vivo*. Figure 5 shows the spectra of human tibia and muscle tissue examined transcutaneously. As expected, the phosphate signature is prominent in the tibia spectrum measured with STRaman while absent in the leg tissue. Other differences that can be seen include stronger peaks in the tissue spectrum arising from the amide III, the CH_2 wagging, the amide I,[3] and the C=O stretching bands.[4]

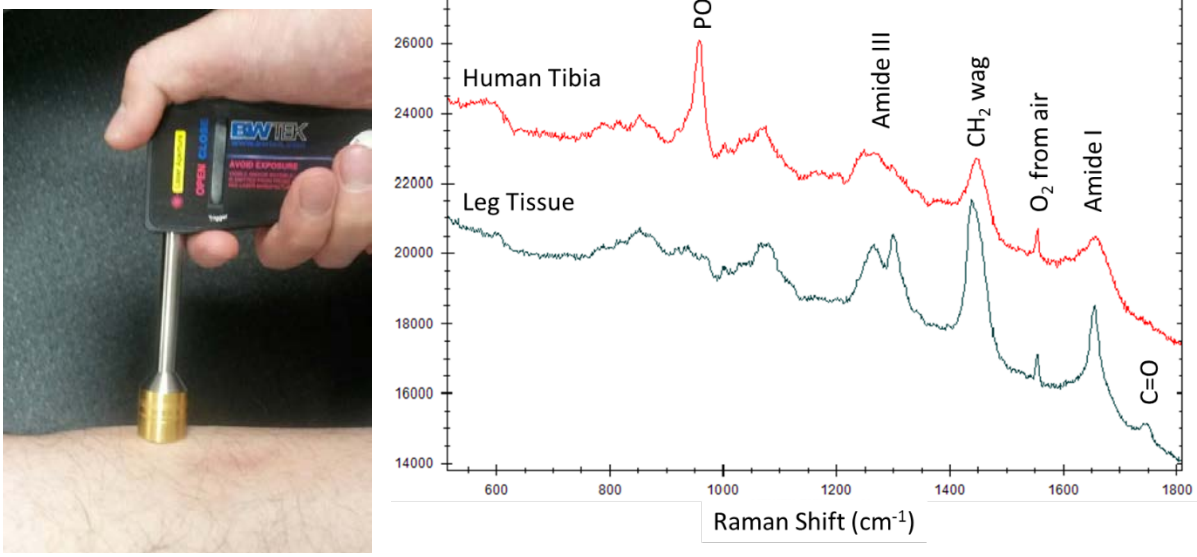

Figure 5: Transcutaneously collected STRaman spectra of human tibia and leg tissue.

STRaman With Larger Sampling Area for Heterogeneous Samples

Representative sampling of heterogeneous material to obtain bulk properties can be difficult for Raman spectroscopy which conventially has a small sampling area on the order of about 100 microns. This can make the mesaurement results vary from one sampling point to another. A larger sampling area is needed to get more consistent measurements of typical products with multiple components, including excipients and one or more active ingredients. When the ingredients are not well mixed on a scale smaller than the sampling area, collection of spectra at different points on the sample can produce widely varying results.

The difference in results from a conventional Raman measurements with spot size of ~ 100 microns vs. the larger sampling area of several mm with the STRaman probe can be seen in the spectra of an Excedrin® Migraine tablet overlaid in figure 6 (a). The 15 spectra (baseline-subtracted and intensity normalized) were collected over randomly chosen positions using a standard configuration, which has a sampling area of roughly 0.3 mm in diameter. The signatures of the 3 active ingredients, namely acetaminophen, aspirin, and caffeine can be detected in all the spectra, though their relative intensities vary greatly reflecting the sample heterogeneity. This makes it hard to reliably identify the product. This can be demonstrated by taking a number of such spectra, computing their average, and using it to represent the product in a spectral library. For each measurement we search the library and obtain a hit quality index (HQI) against the Excedrin Migraine reference spectrum, and get a distribution of results reflecting the sample heterogeneity at this small scale. Figure 6 (b) shows a histogram of 43 such measurements. Using a passing threshold of 95, 18 of the sample spectra result in false negatives. The average and standard deviation of the 43 HQI values are 93.4 and 6.1, respectively.

Figures 6 (c) and 6 (d) show the corresponding results of 17 measurements obtained with the STRaman configuration which has larger sampling area, and thus each measurement is more representative of the bulk tablet properties. With the average and standard deviation of the HQI values at 99.89 and 0.07, respectively, the reproducibility of the spectrum and therefore the identification is greatly improved, with no false negative results in the 17 measurements.

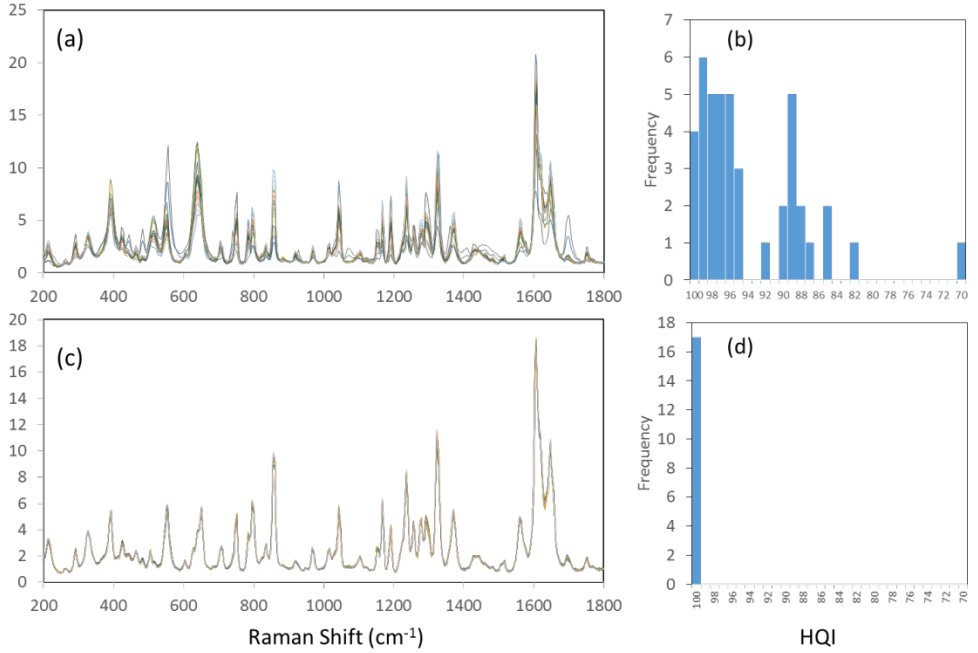

Figure 6: Comparative spectra of Excedrin migraine tablet as measured at various sample positions. (a) overlay of 15 spectra acquired with conventional Raman configuration; (b) histogram of 43 HQI values with conventional Raman configuration. (c) overlay of 15 spectra acquired with STRaman configuration; (d) histogram of 17 HQI values with STRaman configuration.

Samples of large crystalline form can also present problems when measured with a small samplig area. Due to Raman polarization effect, the spectrum of a crystalline sample generally appears different as its orientation varies relative to the direction and polarization of the excitation and collection beams. Figure 7 (a) is an overlay of 15 spectra (baseline-subtracted and intensity normalized) measured over randomly chosen positions on a bag of xylitol using a standard configuration and spot size on the order of 100 microns. The relative intensity of the Raman peaks vary so much that the spectra can easily be mistaken as being of different materials. Figure 7 (b) shows a HQI histogram of 21 such measurements. Using a passing threshold of 95, there are 3 false negatives. The average and standard deviation of the 21 HQI values are 97.6 and 2.7, respectively. Figures 7 (c) and 7 (d) show the corresponding results of 27 measurements of the same sample bag obtained with the STRaman configuration, with the average and standard deviation of the HQI values at 99.98 and 0.011, respectively. With the larger sampling area of this configuration, the reproducibility is greatly improved, and the rate of false negative is reduced to zero.

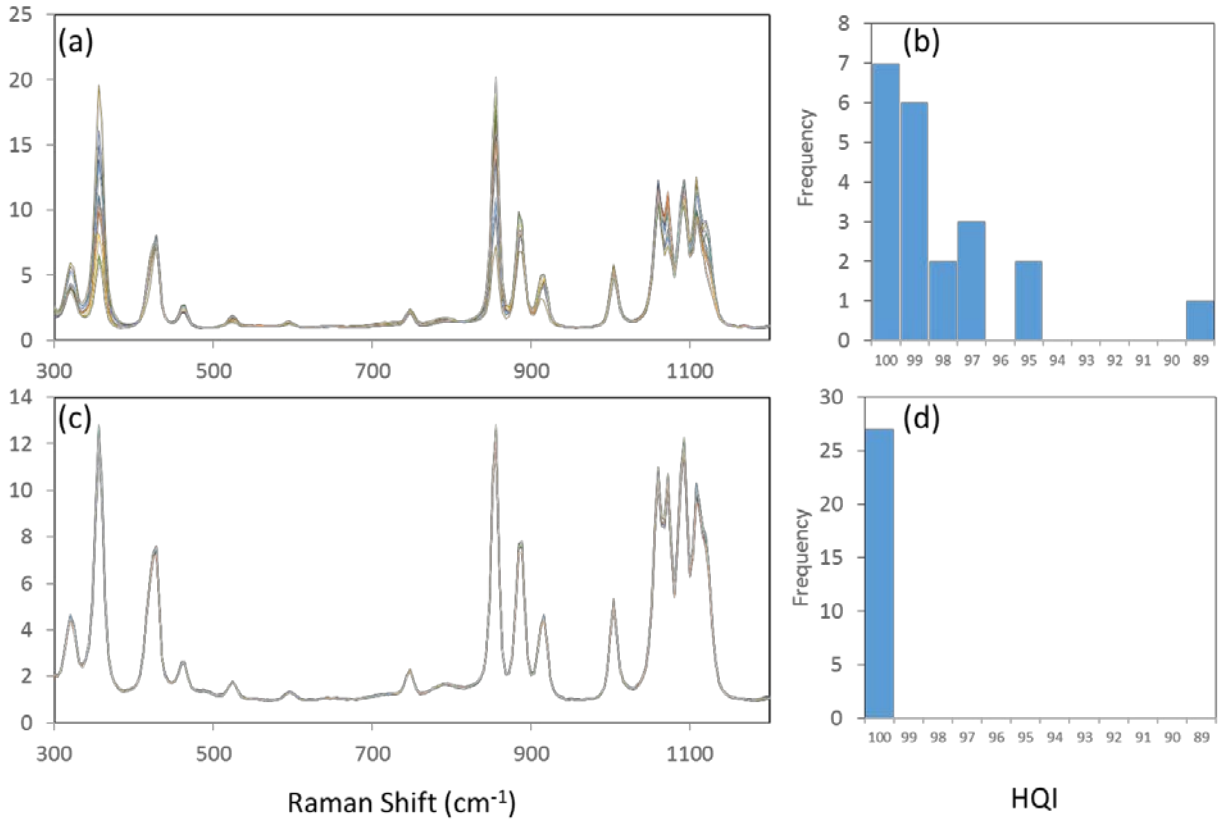

Figure 7: Comparative spectra of crystalline xylitol as measured at various sample positions. (a) overlay of 15 spectra acquired with conventional Raman configuration; (b) histogram of 21 HQI values with conventional Raman configuration. (c) overlay of 15 spectra acquired with STRaman configuration; (d) histogram of 27 HQI values with STRaman configuration.

System Versatility with Sampling Kits

The See Through STRaman system can be equipped with a variety of optimized sampling kits that address specific application needs. These adaptors take advantage of the same high performance hardware and software platforms and can be attached to the ST probe for optimal measurement of different sample forms directly (Focus Adaptor and Surface Regulator). The Focus Adaptor focuses the excitation beam from the ST probe into a spot roughly 100 µm in diameter, with working distance of roughly 15 mm. The Surface Regulator when attached to the Focus Adaptor brings the focus to contact distance.

The Raman spectrum of samples such as liquids in glass bottles is best measured with a traditional confocal probe. For thick glass bottles, or double-layered glass bottles, the STRaman configuration can be used with these specialized sampling kits with working distances optimized such that the laser is focused on the sample deep inside the glass container, with the additional benefit of minimizing the fluorescence interference due to the glass.

The See Through configuration can also be easily adapted to a confocal configuration, allowing standoff, contact, or microscopy Raman analysis.

Summary

The See Through STRaman technology makes it possible to examine and identify chemical species covered by diffusely scattering media. Its large sampling area improves measurement reproducibility for heterogeneous and crystalline samples. The reduced power density allows measurement of photolabile or thermolabile samples. These characteristics greatly expand the utility of Raman spectroscopy.

Additional Resources:
i-Raman® Pro ST Product Information
i-Raman® Pro ST Product Introduction Video
Raman Spectroscopy overview
Portable Raman Comparison chart

References

1. P. Matousek, I. P. Clark, E. R. C. Draper, M. D. Morris, A. E. Goodship, N. Everall, M. Towrie, W. F. Finney, and A. W. Parker, “Subsurface Probing in Diffusely Scattering Media Using Spatially Offset Raman Spectroscopy”. Appl. Spectrosc. 59, 393 (2005).
2. S.J. Choquette, E. S. Etz, W.S. Hurst, D. H. Blackburn, S.D. Leigh. “Relative Intensity Correction of Raman Spectrometers: NIST SRMs 2241 through 2243 for 785 nm, 532 nm, and 488 nm/514.5 nm Excitation”. Appl. Spectrosc. 2007. 61(2): 117-129.
3. P. Matousek, E. R. C. Draper, A. E. Goodship, I. P. Clark, K. L. Ronayne, and A. W. Parker, “Noninvasive Raman Spectroscopy of Human Tissue In Vivo”. Appl. Spectrosc. 60, 758-763 (2006).
4. X.-F. Ling, Y.-Z. Xu, S.-F. Weng, W. H. Li, Xu Zhi, R. M. Hammaker, W. G. Fateley, F. Wang, X.-S. Zhou, R. D. Soloway, J. R. Ferraro, and J.-G. Wu. “Investigation of Normal and Malignant Tissue Samples from the Human Stomach Using Fourier Transform Raman Spectroscopy”. Appl. Spectrosc. 56, 570-573 (2002).

