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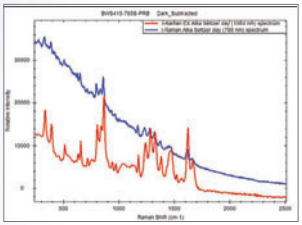

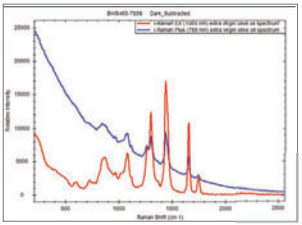

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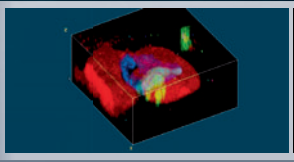
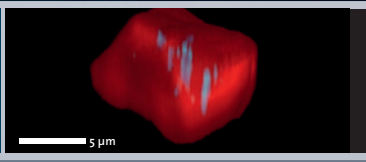


3D Raman image of a fluid inclusion in garnet



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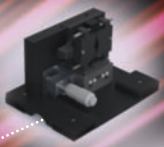
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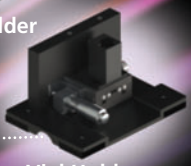
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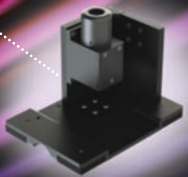
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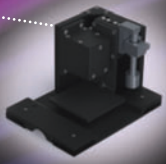
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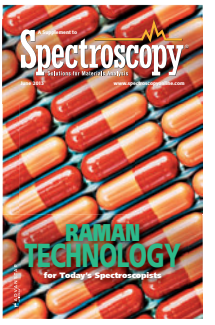

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Discrimination of Bacterial Growth Media Using Portable Raman Spectroscopy with Background Fluorescence Subtraction

We investigated the feasibility of identifying different types of bacterial growth medium using a handheld Raman instrument with a proprietary baseline correction algorithm. Our data suggest that this instrument is capable of differentiating multiple types of growth medium and readily detects the change in the Raman signal when bacteria are present in the growth medium. These findings support the notion that a handheld Raman spectrometer may be used to distinguish highly-fluorescent biological samples without the use of surface-enhanced Raman scattering.

Jessica A. Randall and Mathew G. Lyman

In the field of forensic sciences, handheld Raman spectrometers are used by the military and first responders for the rapid identification of unknown substances (1–3). These unknown materials include toxic industrial chemicals (TICs), toxic industrial materials (TIMs), chemical warfare agents, explosives, narcotics, pharmaceutical compounds, and plastics. However, Raman spectroscopy has historically been problematic for the identification of biological samples, such as bacteria, growth media, and tissues. Sample fluorescence typically overwhelms Raman scattering by several orders of magnitude, resulting in a high background and poor spectral matching (4).

Two general methodologies have been used to detect weak Raman signals from biological organisms and materials: surface-enhanced Raman scattering (SERS) and fluorescence background subtraction (also known as *baseline correction*). SERS is a technique that boosts the signal of Raman-active molecules when measured on customized metal surfaces,

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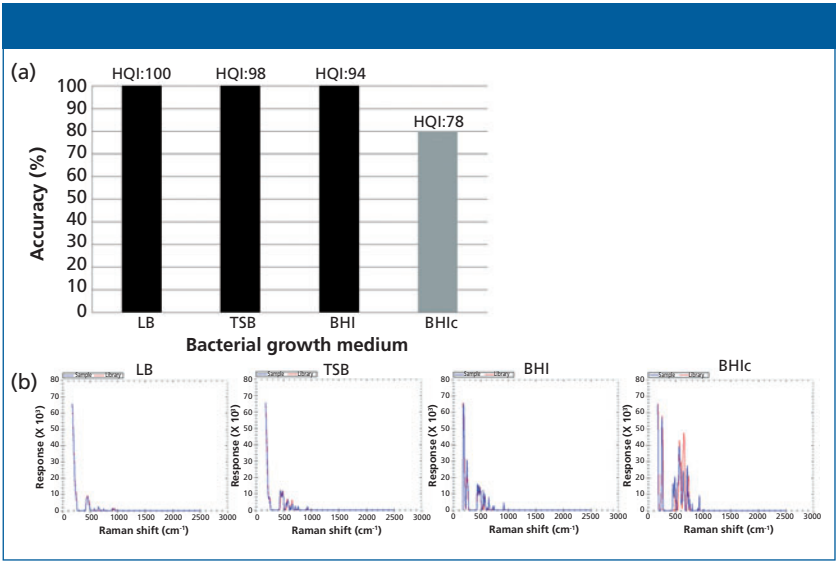

Figure 1: (a) Measuring the accuracy of the portable spectrometer on four types of bacterial growth medium: LB, TSB, BHI, and BHIc. Scans were recorded as a "match" if the instrument correctly identified the medium as the top match with an HQI value ≥ 90. Scans that exceeded this average value are shown in black, while scans below this threshold value are show in grey. (b) Representative overlays of the baseline-corrected sample spectrum (blue line) and the library spectrum (red line) are shown for each of the four types of growth medium.

typically gold, silver, and copper (5,6). The increase in Raman signal on these metals is often dramatic, usually on the order of 10^4–10^6, but it can be as high as 10^{11} (7,8). Therefore, increasing the Raman signal over background fluorescence allows the Raman spectra to be discretely visible. In fact, a number of publications have shown that different types of bacteria and growth media have unique SERS spectra, thereby establishing SERS as a potential pathogen diagnostic tool despite the intrinsic fluorescence of the sample (9–22).

Fluorescence background subtraction is a second option for "teasing out" a weak Raman signal from fluorescent biological samples. In contrast to SERS, fluorescence background subtraction does not increase Raman signal during acquisition; it differentiates it computationally by deducting the background fluorescence from the Raman signal post-acquisition. Several computational methods have been developed for background subtraction from Raman spectra, including background estimation using computational geometry (23), principal component analysis (24), wavelet transformation (25–30), polynomial fitting (4,31–36), frequency-domain filtering (37), and first-order and second-order differentiation (38,39).

In this study, we investigated the feasibility of fluorescence background subtraction using a handheld spectrometer to differentiate several bacterial growth media, specifically in the presence and absence of bacteria. As SERS is often not practical for first responders and military personnel (because of time constraints, limited dexterity in personal protective equipment, and ease of use), we tested a portable Raman spectrometer that uti-

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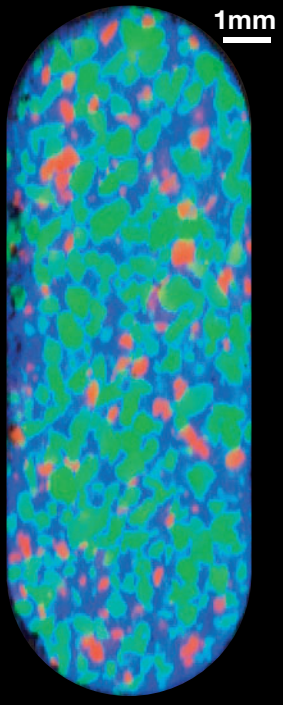

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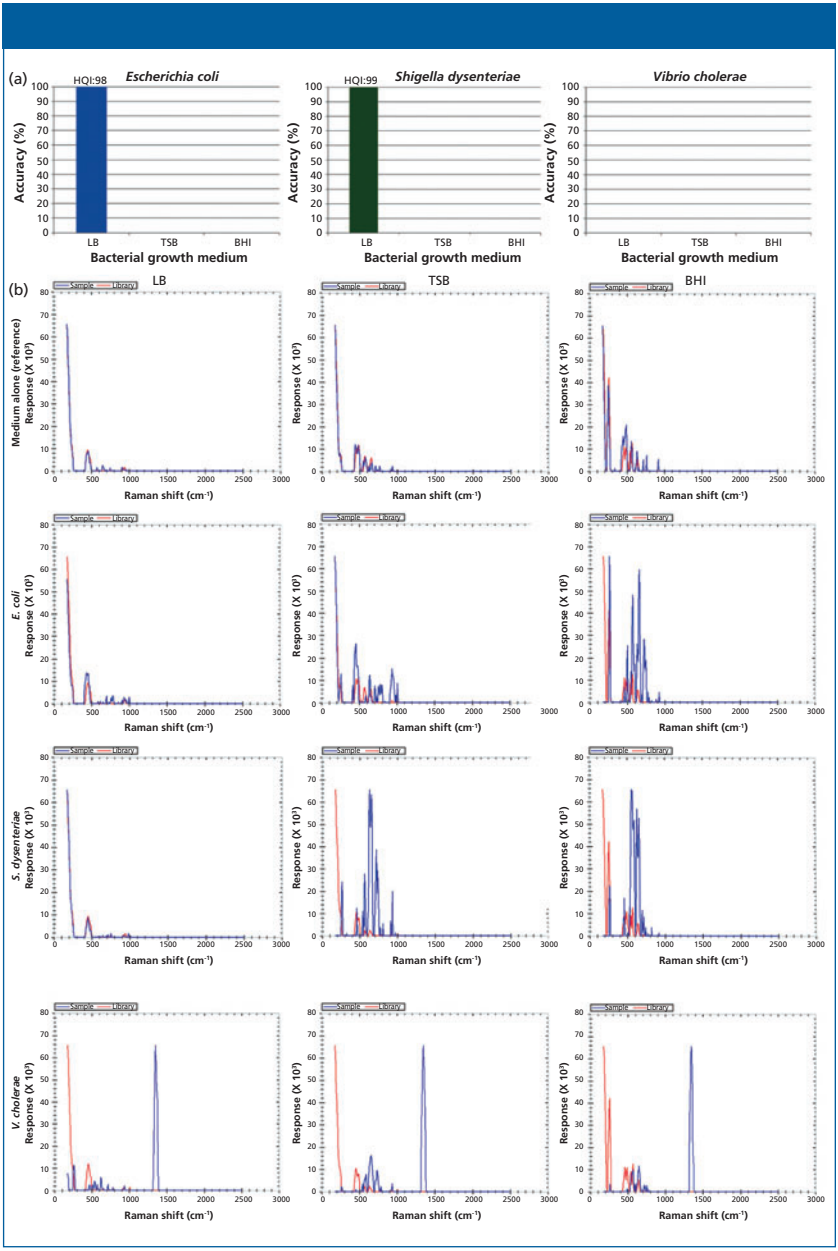

Figure 2: (a) Culturing *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae* in LB, TSB, or BHI markedly decreased the accuracy rate of the portable spectrometer to match to the growth medium spectra in the library. (b) Representative overlays of the baseline-corrected sample spectrum (blue line) and the library spectrum (red line) are shown for each of the three types of growth medium in the presence of *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae*. The medium alone scan (reference) is unadulterated growth medium matched to itself.

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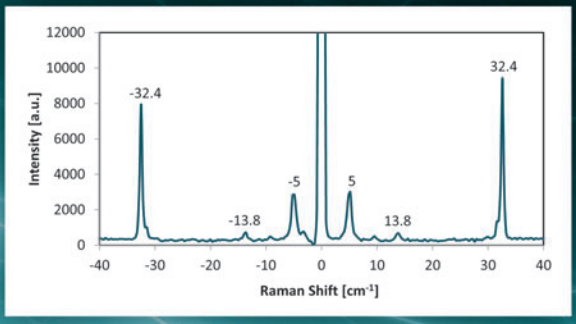

Raman spectrum of MoS_2 flakes measured at 633 nm with BragGrate™ Notch filters and single stage spectrometer

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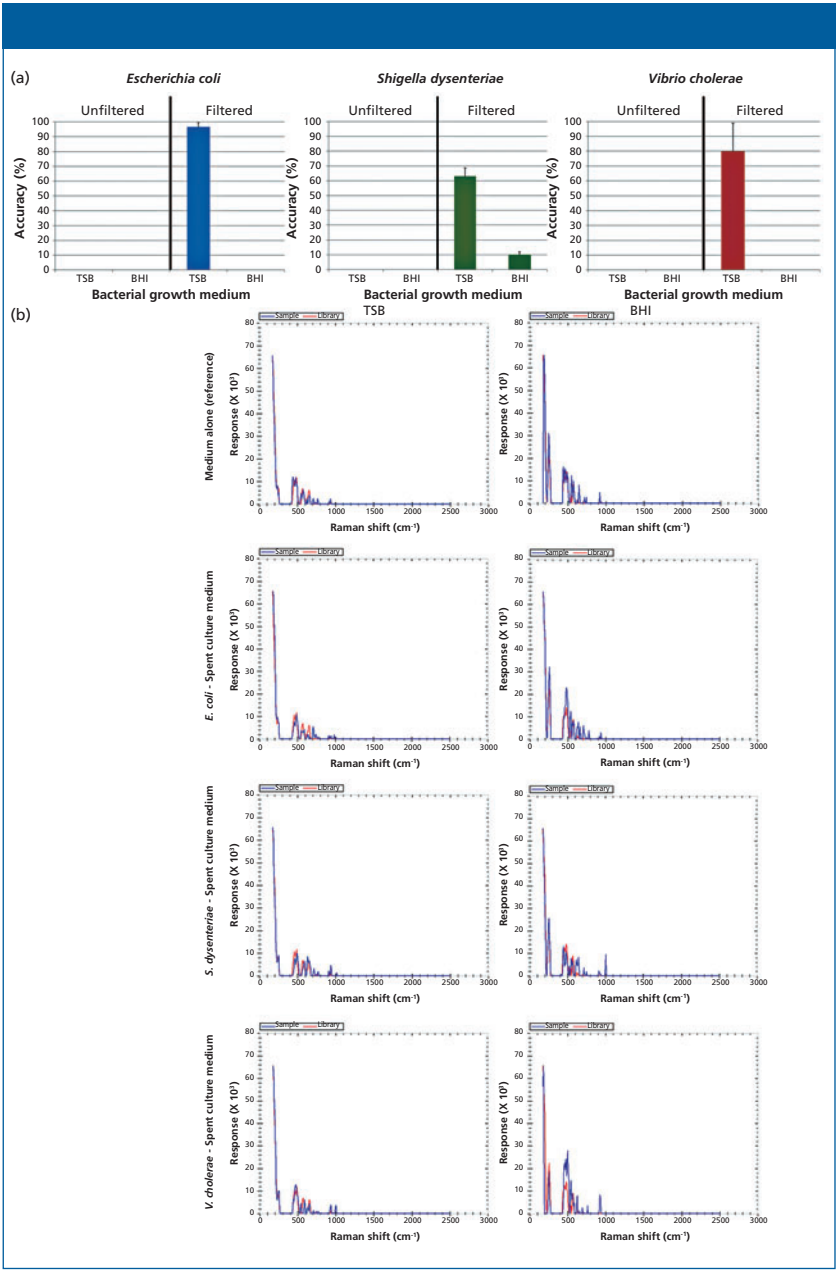

Figure 3: (a) Removing bacteria from culture medium partially restores the ability of the handheld spectrometer to match to the growth medium spectra in the library. (b) Representative overlays of the baseline-corrected spent culture medium (blue line) and the library spectrum (red line) are shown for TSB and BHI after *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae* have been filtered from the culture.

lizes a proprietary algorithm to subtract background fluorescence. This baseline correction capability may allow for the interrogation of biologic materials that would otherwise "swamp" traditional handheld Raman instruments used in forensic analyses.

Experimental Conditions

Handheld Raman Spectrometers

A NanoRam handheld spectrometer was obtained from B&W Tek, Inc. The operation presets were as follows: excitation wavelength = 785 nm, laser power = 90% (270 mW), number of hits displayed = 3, and spectral range = 176–2900 cm^{-1}. For sample analysis, the spectrometer was fitted with the vial adaptor for 4-mL glass vials.

Bacterial Growth Media

Four types of bacterial growth media were analyzed for this study: LB-Miller broth, tryptic soy broth (TSB), brain heart infusion (BHI) broth, and brain heart infusion-cysteine (BHIc) broth. All base media were purchased from Becton Dickinson and prepared according to the manufacturer's recommendations. BHIc medium required additional supplementation as reported previously (40). Briefly, 1 L of BHI medium (37 g/L) containing cysteine (1 g/L) was sterilized following the manufacturer's recommendations. The following supplements were then prepared and added before use: β-nicotinamide adenine dinucleotide (β-NAD) (1 mg/mL — dissolve 200 mg β-NAD in 200 mL of deionized water, filter sterilize using a 0.2-µm filter, store at 4 °C), heme-histidine (1 mg/mL — add 200 mg of L-histidine, 200 mg of hemin-HCl and 4 mL of 1 N NaOH to 196 mL of deionized water; microwave briefly to dissolve; filter sterilize; store at 4 °C away from light), and glucose (25 mL, 20% w/v). All BHIc supplements were purchased from Sigma-Aldrich.

Raman Spectroscopy of Bacterial Growth Media

For Raman spectroscopy of bacterial media, 1 mL of LB-Miller broth, TSB, BHI, and BHIc media were transferred to separate 4-mL screw-cap glass vials (Fisher). Each medium sample was then added to a "User Defined Library." For the handheld spectrometer, a unique Raman spectrum for each medium was captured and added to the library. After the user library was established, each medium vial was subsequently analyzed an additional 10 times ($n = 10$) to determine if the spectrometer could distinguish between the different growth media. The accuracy rate and hit quality index (HQI) value (that is, confidence score) for the 10 iterations were then recorded.

Raman Spectroscopy of Bacterial Cultures

Overnight cultures were set up in each medium with three different organisms: *Escherichia coli* C3000 (obtained from Oklahoma State University), *Shigella dysenteriae* (BEI number NR-520), and *Vibrio cholerae* (BEI number NR-144). A loopful of frozen glycerol stock was used to inoculate 5 mL of each medium in a 50-mL vented flask (Cellstar) and cultured for 18 h at 37 °C with shaking. Then, 1 mL of each sample was analyzed ($n = 10$) and matched against the User Defined Library. Scans were recorded as a "match" if the instrument correctly identified the medium as the top match and an HQI ≥ 90. If there were no hits, or the instrument did not correctly identify the compound, it was recorded as a "no-match." The accuracy rate and HQI value (that is, confi-

dence score) for the 10 iterations were then recorded. The bacterial titer for each culture was calculated by performing serial 10-fold dilution and spotted onto tryptic soy agar (TSA) culture plates (20 µL/spot) in triplicate for each sample and dilution. Plates were incubated under aerobic conditions at 37 °C and the colony forming units (cfu) were calculated. The titers for *Escherichia coli* in LB, TSB, and BHI were 5×10^8, 9.5×10^8, and 4.5×10^8 cfu/mL, respectively. The titers for *Shigella dysenteriae* in LB, TSB, and BHI were 3×10^6, 1×10^8, and 8×10^7 cfu/mL, respectively. The titers for *Vibrio cholerae* in LB, TSB, and BHI were 9.0×10^7, 1.3×10^8, and 2.5×10^8 cfu/mL, respectively.

Raman Spectroscopy of Filtered Bacterial Cultures (Spent Culture Medium)

Overnight cultures were started in LB, BHI, and TSB media with *Escherichia coli* C3000, *Shigella dysenteriae*, and *Vibrio cholerae* as described previously. The following day, bacteria were removed from the cultures using a 50-mL Millipore sterile disposable vacuum filtration unit with a 0.22-µm PES membrane. The resulting filtered media samples were then analyzed ($n = 10$) by the handheld Raman instrument in replicate experiments (three separate cultures).

Data Analysis

All data scans were downloaded from the handheld Raman instrument into NanoRam ID Client v3.10 software. In ID Client, each overlay (sample scan and library match) was reviewed and a representative scan was chosen for each data set. Screen shots of the data scans were then captured and imported into Photoshop CS5 software (Adobe) where they were cropped and organized into figures. All graphing was performed in Microsoft Excel software. A notable limitation of the handheld instrument is displaying a library spectrum with a sample spectrum that has a low HQI value (does not match). By default, the instrument displays the sample spectrum overlaid with the library spectrum that has the highest HQI value (the best match). Therefore, to have the instrument overlay a library spectrum with a dissimilar sample spectrum (for the purpose of demonstration in the figure), we created some user libraries with only a single medium entry (for example, unadulterated BHI was entered into a user library and used to match bacterial cultures propagated in BHI; even though the Raman spectra were dramatically different, the instrument would overlay the library spectrum of BHI alone with the sample spectrum of BHI and the bacteria).

Results

To investigate the utility of using a handheld Raman spectrometer for identifying bacterial medium (Figure 1), we tested four different types of growth medium with increasing complexity: LB-Miller Broth (LB), Tryptic Soy Broth (TSB), Brain-Heart Infusion Broth (BHI), and a complex derivative of BHI used for culturing *Francisella tularensis* (BHIc). The ingredients for each medium are listed in Table I.

The accuracy rate (%) was determined by interrogating 10 sample replicates for each type of medium. Scans were recorded as a "match" if the instrument correctly identified the medium as the top match in the library, and the HQI value was ≥ 90. The HQI value indicates how well the unknown scan matches to the library scan; an HQI of 100 indicates an identical match while lower values indicate the dissimilarity between scans

(41). As shown in Figure 1a, the NanoRam spectrometer was able to match LB, TSB, and BHI media with 100% accuracy with HQI values ≥ 94. BHIc, the most complex medium in the study, matched with 80% accuracy with an average HQI value of 78. Figure 1b shows a representative spectrum from each medium analyzed. Scans for LB, TSB, and BHI were nearly identical to the library scans (blue tightly fit to red). However, the spectrum for the BHIc medium was more variable compared to the library spectrum (red visible with no blue), indicating that the match was of lower quality. These findings suggest that the NanoRam is likely able to match to a variety of bacterial growth media using its proprietary background subtraction algorithm. However, analyzing highly-complex medium such as BHIc results in lower HQI values.

After determining that the instrument was able to distinguish between different types of growth medium, we investigated whether culturing three types of enteric bacteria (*Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae*) dramatically affected the Raman spectra compared to culture medium alone. We also assessed whether these three types of bacteria could be visually distinguished from one another by their subtracted Raman spectra (as a proof-of-principle). *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae* were cultured in LB, TSB, and BHI; these cultures were then analyzed by the NanoRam spectrometer to compare spectra of broth alone and broth containing bacteria. Interestingly, both the *Escherichia coli* and *Shigella dysenteriae* cultures in LB matched, with 100% accuracy, to the library scans of LB medium alone (Figure 2a). A similar finding was observed by Premasiri and colleagues (18), who noted that "media containing extracts from other micro-organisms such as yeast extract" showed a similar appearance to SERS spectra of bacterial cells, an observation consistent with culturing these bacteria in LB (a yeast extract base). By contrast, spectra from cultures of *Escherichia coli*, *Shigella dysenteriae*, and *Vibrio cholerae* in TSB and BHI were notably different than those of medium alone, and did not match the library spectra (Figure 2a). Figure 2b shows the Raman spectra of LB, TSB, and BHI alone (reference scans, first row) compared to the three enteric bacteria cultured in these media (rows 2–4). Note that the red library scan, for each of the representative Raman plots, was from pure medium alone (LB, TSB, or BHI); the blue sample scan represents the change in the Raman spectrum from the bacterial culture relative to medium alone. Although *Escherichia coli* and *Shigella dysenteriae* cultured in LB had similar scans relative to LB alone, all three bacteria showed unique background-subtracted Raman spectra in TSB and BHI, suggesting that it may be feasible to distinguish between multiple types of bacteria cultured in a variety of growth media using a handheld Raman spectrometer without using SERS.

Next, we investigated whether the robust Raman peaks observed in the bacterial cultures from Figure 2 were caused exclusively by the presence of the bacteria, or whether the Raman-active components in the growth medium had also changed during bacterial growth. We hypothesized that if the dramatic changes in Raman spectra were due to bacteria alone, filtering out these bacteria from the spent culture medium would restore the ability of the handheld Raman spectrometer to match to the "medium alone" library scans, specifically for TSB and BHI (bacterial

Table I: Individual components of bacterial growth media analyzed in this study

LB-Miller Broth (per L)	Tryptic Soy Broth (per L)	Brain Heart Infusion Broth (per L)	BHIc Broth (per L)
Trytone (10 g)	Pancreatic digest of Casein (17 g)	Calf brains, infusion from 200 g (7.7g)	Calf brains, infusion from 200 g (7.7g
Yeast extract (5 g)	Papaic digest of soybean (3g)	Beef heart, infusion from 250 g (9.8 g)	Beef heart, infusion from 250 g (9.8 g)
Sodium chloride (10 g)	Dextrose (2.5 g)	Proteose peptone (10 g)	Proteose peptone (10 g)
	Sodium chloride (5 g)	Dextrose (2 g)	Dextrose (2 g)
	Dipotassium phosphate (2.5 g)	Sodium chloride (5 g)	Sodium chloride (5 g)
		Disodium phosphate (2.5 g)	Disodium phosphate (2.5 g)
			Cysteine (1 g)
			β-Nicotinamide adenine dinucleotide (10 mL, 1 mg/mL)
			Heme-histidine (10 mL, 1 mg/mL)
			Glucose (25 mL, 20% w/v)

cultures that had a 0% match rate for all three bacteria). To test this hypothesis, overnight bacterial cultures were grown to saturation. Cultures were then filtered through a 0.22-µm membrane to remove bacteria; spent culture media were then analyzed using the handheld Raman spectrometer (Figure 3a). Filtering out the bacteria largely restored the ability of the instrument to match TSB spent culture medium to the original TSB library scan (with an HQI value ≥ 90), whereas the BHI spent culture medium did not match to the library scan at the original HQI threshold. However, most of the BHI scans had HQI values ranging from 75 to 89, suggesting that the BHI spent culture medium was similar to unadulterated BHI. Representative scans also supported the notion that the spent culture medium had minor differences in their Raman spectra compared to the original growth medium, although these differences were enough to drop the HQI value below our threshold for a match (Figure 3b). These data suggest that the bacteria in the culture medium are primarily responsible for the dramatic change in the background-subtracted Raman spectra compared to medium alone.

Discussion and Conclusion

We investigated whether a handheld Raman instrument that subtracts background fluorescence was able to identify

increasingly complex bacterial growth medium, with and without bacteria. We found that the instrument was effective in identifying several types of traditional growth medium at an HQI value of ≥ 90, but was less accurate with complex specialty mediums, such as BHIc. In addition, culturing bacteria in these media resulted in markedly different Raman spectra compared to media alone, suggesting that it may be feasible to distinguish between multiple types of bacteria cultured in a variety of growth media if an on-board reference library is generated first. We also noted variability in the intensity and position of peaks in replicate spectra for each condition, suggesting that if a reference library were to be generated, each library spectrum would need to be an average of multiple scans (ideally).

Two recent studies have discussed the use of SERS to differentiate bacterial growth medium and different species of bacteria (that is, by boosting the Raman signal during acquisition) (16,18). We show in this study that growth medium and bacteria could also be differentiated using a fluorescence background subtraction algorithm post-acquisition. This is particularly relevant for first responders and military personnel who use handheld Raman spectrometers to identify chemicals (TICs), toxic industrial materials (TIMs), chemical warfare agents, explosives, and narcotics, but historically struggle with "bio" materials because of their high background fluorescence. Therefore, future studies will build upon these initial findings and investigate the ability of handheld Raman spectrometers to correctly identify a multitude of crude bacterial cultures, purified bacterial cells (washed), as well as dried spores.

References

(1) H. Markert, J. Rings, N. Campbell, and K. Grates, N. F. S. T. Center, Ed. (National Institute of Justice, Largo, Florida, 2011), pp. 1–8.

(2) W. Smith, P. White, C. Rodger, and G. Dent, in *Handbook of Raman Spectroscopy: From the Research Laboratory to the Process Line*, I.R. Lewis and H. Edwards, Eds. (Marcel Dekker, New York, New York, 2001).

(3) V. Otieno-Alego and N. Speers, in *Infrared and Raman Spectroscopy in Forensic Science*, J.M. Chalmers, H. Edwards, and M. Hargreaves, Eds. (John Wiley & Sons Ltd, West Sussex, UK, 2012).

(4) C.A. Lieber and A. Mahadevan-Jansen, *Appl. Spectrosc.* **57,** 1363 (2003).

(5) M. Culha, B. Cullum, N. Lavrik, and C. Klutse, *J. Nanotechnol.* **2012,** 15 (2012).

(6) B. Sharma, R. Frontiera, A. Henry, E. Ringe, and R. Van Duyne, *Mater. Today* **15,** 16 (2012).

(7) K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari, and M.S. Feld, *Chem. Rev.* **99,** 2957 (1999).

(8) K. Kneipp, H. Kneipp, I. Itzkan, R.R. Dasari, and M.S. Feld, *Curr. Sci.* **77,** (1999).

(9) H. Chu, Y. Huang, and Y. Zhao, *Appl. Spectrosc.* **62,** 922 (2008).

(10) J.A. Guicheteau, K. Gonser, and S.D. Christesen, A.P.G. National Research Council Associate at the U.S. Army ECBC Research Lab, Ed. (U.S. Government, Edgewood, 2004).

(11) R.M. Jarvis, A. Brooker, and R. Goodacre, *Anal. Chem.* **76,** 5198 (2004).

(12) R.M. Jarvis and R. Goodacre, *Anal Chem.* **76,** 40 (2004).

(13) M. Kahraman, M.M. Yazici, F. Sahin, and M. Culha, *J. Biomed. Opt.* **12,** 054015 (2007).
(14) M. Kahraman, M.M. Yazici, F. Sahin, and M. Culha, *Langmuir* **24,** 894 (2008).
(15) T.T. Liu et al., *PLoS One* **4,** e5470 (2009).
(16) N.E. Marotta and L.A. Bottomley, *Appl. Spectrosc.* **64,** 601 (2010).
(17) I.S. Patel, W.R. Premasiri, D.T. Moir, and L.D. Ziegler, *J. Raman Spectrosc.* **39,** 1660 (2008).
(18) W.R. Premasiri, Y. Gebregziabher, and L.D. Ziegler, *Appl. Spectrosc.* **65,** 493 (2011).
(19) W.R. Premasiri et al., *J. Phys. Chem. B* **109,** 312 (2005).
(20) W.R. Premasiri, D.T. Moir, M.S. Klempner, and L.D. Ziegler, Eds., *New Approaches in Biomedical Spectroscopy,* (Oxford University Press, New York, 2007).
(21) A. Sengupta, M.L. Laucks, and E.J. Davis, *Appl. Spectrosc.* **59,** 1016 (2005).
(22) L. Zeiri, B.V. Bronk, Y. Shabtai, J. Eichler, and S. Efrima, *Appl. Spectrosc.* **58,** 33 (2004).
(23) N. Kourkoumelis, A. Polymeros, and M. Tzaphlidou, *Spectrosc. Int. J.* **27,** 311 (2012).
(24) T. Hasegawa, J. Nishijo, and J. Umemura, *Chem. Phys. Lett.* **317,** 642 (2000).
(25) T.T. Cai, D. Zhang, and D. Ben-Amotz, *Appl. Spectrosc.* **55,** 1124 (2001).
(26) C. Camerlingo, F. Zenone, G.M. Gaeta, R. Riccio, and M. Lepore, *Meas. Sci. Technol.* **17,** 298 (2006).
(27) Y. Hu et al., *Chemom. Intell. Lab. Syst.* **85,** 94 (2007).
(28) J. Li, L.P. Choo-Smith, Z. Tang, and M.G. Sowa, *J. Raman Spectrosc.* **42,** 580 (2011).
(29) P.M. Ramos and I. Ruisánchez, *J. Raman Spectrosc.* **36,** 848 (2005).
(30) Z. Zhang et al., *J. Raman Spectrosc.* **41,** 659 (2010).
(31) B.D. Beier and A.J. Berger, *Analyst* **134,** 1198 (2009).
(32) A. Jirasek, G. Schulze, M.M. Yu, M.W. Blades, and R.F. Turner, *Appl. Spectrosc.* **58,** 1488 (2004).
(33) M.N. Leger, A.G. Ryder, *Appl. Spectrosc.* **60,** 182 (2006).
(34) V. Mazet, C. Carteret, D. Brie, J. Idier, and B. Humbert, *Chemom. Intell. Lab. Syst.* **76,** 121 (2005).
(35) T.J. Vickers, R.E. Wambles, and C.K. Mann, *Appl. Spectrosc.* **55,** 389 (2001).
(36) J. Zhao, H. Lui, D.I. McLean, and H. Zeng, *Appl. Spectrosc.* **61,** 1225 (2007).
(37) P.A. Mosier-Boss, S.H. Lieberman, and R. Newbery, *Appl. Spectrosc.* **49,** 630 (1995).
(38) A. O'Grady, A.C. Dennis, D. Denvir, J.J. McGarvey, and S.E. Bell, *Anal. Chem.* **73,** 2058 (2001).
(39) D. Zhang and D. Ben-Amotz, *Appl. Spectrosc.* **54,** 1379 (2000).
(40) P. Mc Gann et al., *J. Microbiol Methods* **80,** 164 (2010).
(41) J. Kauffman, J.D. Rodriguez, and L.F. Buhse, *Am. Pharm. Rev.* **14,** (2011).

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Advantages of 1064-nm Portable Raman for Counterfeit Pharmaceuticals Authentication

The counterfeiting of pharmaceuticals is a global issue that is expanding each year. The severity of drugs counterfeited has increased from lifestyle drugs to include lifesaving (such as anticancer) types in recent times. The miniaturization of instrumentation has led to out-of-laboratory analysis for product authentication. Raman spectroscopy is ideal for pharmaceutical identifications and has been proven to detect counterfeit drugs. Portable instruments are available with dispersive technology and laser excitation at 785 nm for remote analysis. This study compares a modern portable Raman instrument equipped with a 1064-nm laser with one using a 785-nm laser. It is shown that authentic tablets are distinguished from the counterfeit tablets nondestructively, without the need to remove tablet coating.

N.W. Broad, C. Dentinger, and J. Pasmore

Counterfeit medications are a problem globally, both in developing countries and developed nations. It is estimated that more than 10% of the global market is made up of counterfeits, with that number varying widely based on geographic region. Recently, there have been more reports of counterfeit lifesaving (for example, anticancer) types of drugs, not just the lifestyle drugs that made up much of the counterfeit market in the past (1,2). Fast, easy, and reliable methods to identify counterfeit drugs are crucial to reducing this problem. Raman spectroscopy is sensitive to molecular vibrations and as such is highly specific to the material that is measured. This makes Raman spectroscopy ideal for pharmaceutical identifications, and it has been proven to detect counterfeit drugs (3–6). The development of miniaturized portable Raman instruments in recent years has led to out-of-laboratory analysis for product authentication, which makes counterfeit identification practical at many different points in the supply chain and allows

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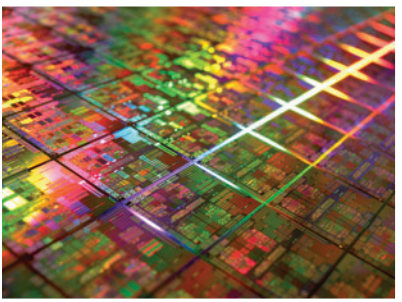

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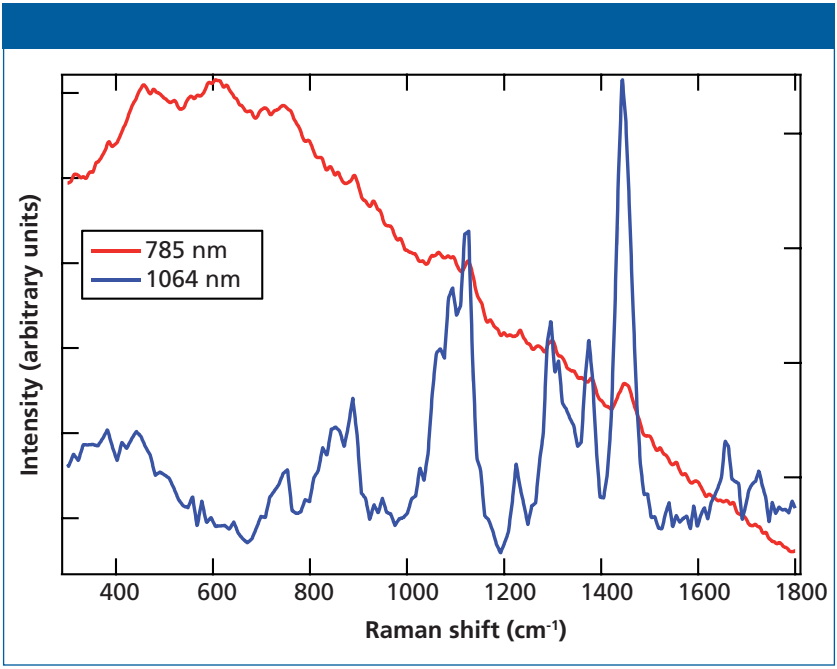

Figure 1: Genuine orlistat (Alli) Raman spectra collected with a 785-nm excitation instrument and a 1064-nm excitation instrument.

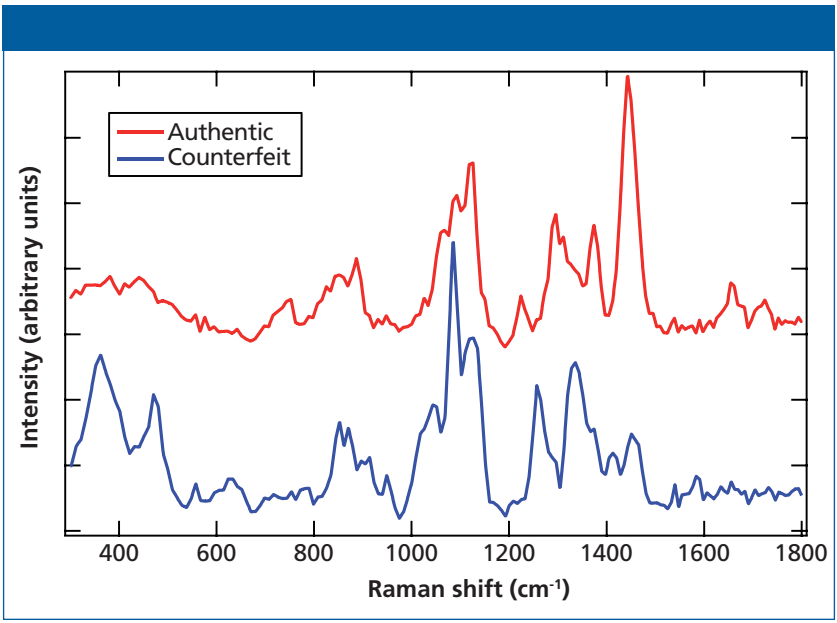

Figure 2: Authentic and counterfeit orlistat (Alli) capsule blend scanned using 1064-nm excitation, viewed with offset intensity.

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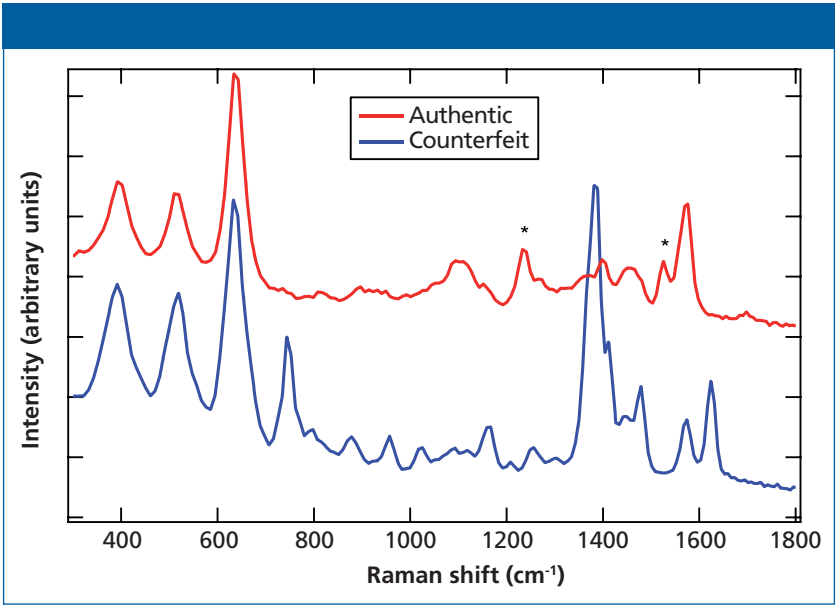

Figure 3: Offset spectra of an authentic 50-mg sildenafil citrate (Viagra) tablet and a counterfeit tablet measured with 1064-nm excitation.

for faster detection and shut down of counterfeiting operations.

This study compares Raman spectra measured from two different portable Raman spectrometers: one with the more traditionally used 785-nm excitation and the other with the more recently developed 1064-nm excitation. It looks at the specificity of the Raman spectra from the different excitation wavelengths as well as the ability to measure nondestructively through tablet coatings and original medication packaging with no sample preparation.

Experimental

Samples were analyzed with two different instruments. Measurements obtained with 1064-nm laser excitation were done using a Rigaku Xantus-2 portable Raman instrument equipped with a cooled InGaAs detector. Measurements with the 785-nm laser excitation were performed using a portable Raman instrument equipped with a charge-coupled device (CCD) detector. For both instruments, a laser power of 300 mW was used.

Samples were measured in a variety of physical shapes: capsules, powders, tablets, and original vials containing powders. Samples that were capsules or powders were transferred to borosilicate vials and measured through glass vials using each instrument's specific vial holder. Tablets and powders in original vials were measured as is with each instrument's on-board point-and-shoot tip. Samples of authentic and counterfeit products used were: Alli (orlistat), Viagra (sildenafil citrate), and Duphaston (dydrogesterone). Authentic products were purchased from bona-fide pharmacies; counterfeits originated in Asia.

Raman data were reviewed post calibration from 300 to 1800 cm^{-1} using

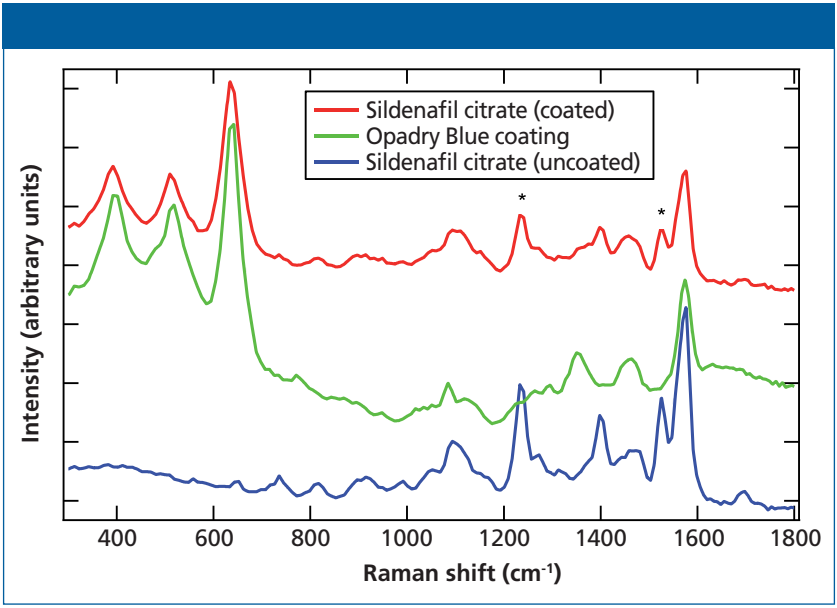

Figure 4: Sildenafil citrate coated and uncoated tablets (100 mg) and Opadry Blue coating material, offset spectra at 1064 nm.

Thermo Omnic software (version 8.2), or with Igor Pro software (version 6.22A).

Results and Discussion

Comparison of 785-nm and 1064-nm Excitation

To obtain the best possible Raman spectra for the genuine samples, each of the authentic orlistat, sildenafil citrate, and dydrogesterone samples were measured with both the 785-nm and 1064-nm excitation instruments. Figure 1 shows the spectra for a genuine capsule of the weight loss medication orlistat collected with both 785-nm and 1064-nm excitation. The spectra are shown in the optimal comparative working range of 300–1800 cm^{-1}. The spectrum collected with 785-nm excitation shows significant fluorescence as observed in the broad *y*-axis curvature. This fluorescence makes it difficult to see the sharper Raman peaks that are present on top of this curvature, and very significantly reduces the dynamic range available for the Raman signal. For many samples, fluorescence may be reduced by moving to a longer excitation wavelength. This is seen with the orlistat sample measured with 1064-nm excitation. The fluorescence is greatly reduced by moving to the 1064-nm excitation, thereby improving the Raman spectral quality and specificity, which makes the genuine sample easier to identify. Similar results were seen when authentic sildenafil citrate and dydrogesterone samples were measured with both the 785-nm and 1064-nm excitation instruments (data not shown).

1064-nm Excitation for Distinguishing Authentic and Counterfeit Pharmaceuticals

The significance of the improved Raman spectral quality obtained with

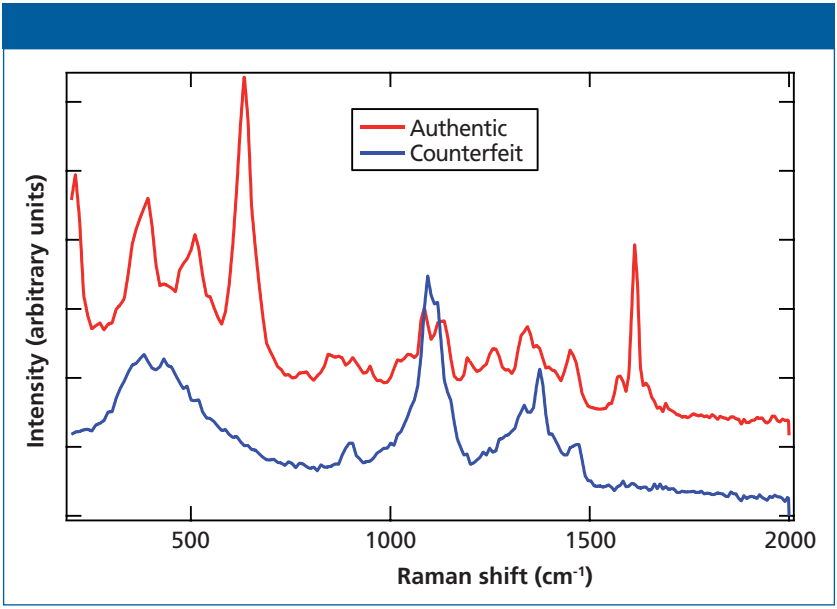

Figure 5: Authentic and counterfeit dydrogesterone, offset spectra measured with 1064-nm excitation.

the 1064 nm excitation is evident by the comparison of the authentic product with the counterfeit. Authentic orlistat (60 mg) spectral data were overlaid directly with the counterfeit spectral data. The differences are clearly visible between the authentic and counterfeit samples measured with 1064-nm excitation, as shown in Figure 2. These differences can be seen without the need for further data treatment or chemometrics (such as principal components analysis).

Authentic and counterfeit versions of sildenafil citrate, which is used for the treatment of erectile dysfunction and pulmonary arterial hypertension, were also measured with 1064-nm excitation. These spectra are shown in Figure 3. The counterfeit and authentic versions of the sildenafil citrate are also easily distinguishable by sight. The differences between the genuine and the counterfeit can be seen at many different frequencies. In fact, the primary peaks that are similar between the two spectra are the triplet at low frequency which is characteristic of TiO_2, a common ingredient in tablet coatings.

To better understand the peaks that are present in the genuine sildenafil citrate spectrum measured with 1064-nm excitation, three different spectra were compared. The spectra of the coating for sildenafil citrate (Opadry Blue), an authentic 100-mg sildenafil citrate tablet measured through the coating, and an authentic 100-mg sildenafil citrate tablet with the coating mechanically removed are shown in Figure 4. Peaks specific to the Opadry Blue coating were seen around 399, 516, 635 (TiO_2), 1460, and 1573 cm^{-1}. Sharp, specific peaks for the active pharmaceutical ingredient (API), sildenafil citrate, were therefore confirmed at 1234 and 1525 cm^{-1} (7,8).

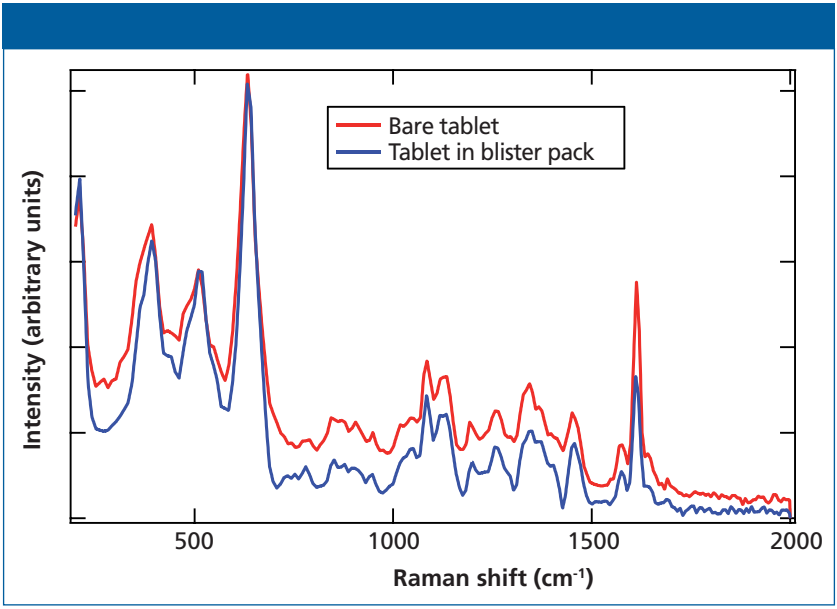

Figure 6: Overlay of dydrogesterone bare tablet and tablet scanned through blister pack with the packaging spectrum subtracted, measured with 1064-nm excitation.

These peaks are labeled with an asterisk in Figures 3 and 4. Of obvious interest with any counterfeit pharmaceutical is whether or not the API is present and in the proper amount. After identifying which peaks are caused by the sildenafil citrate, one can look back at Figure 3 where the peaks that are from the API are marked with an asterisk. Even by just looking at the results, it appears that the API is not present or at least is not present in as high a concentration in the counterfeit as in the genuine tablet.

A final comparison analysis for authentic and counterfeit samples was tested with the 10-mg dydrogesterone tablet. Dydrogesterone is used for the treatment of a wide variety of gynecological conditions related to progesterone deficiency. The genuine and counterfeit versions of dydrogesterone are shown in Figure 5. The differences between these two spectra are quite marked. The authentic type has a white Opadry coating, of which the counterfeit is devoid; the counterfeit shows no telltale sign of TiO_2. Further analysis of the counterfeit dydrogesterone was done by comparing the measured spectrum to the high-resolution Aldrich Raman spectral library. This gave a strong match of the counterfeit tablet bulk contents to cellulose powder. The analysis of the Raman spectra for both the sildenafil citrate and dydrogesterone suggest the well-known fact that counterfeits are a significant health risk because many do not contain the API at all or not in the appropriate dosage amount.

1064-nm Raman Measurements Through Original Packaging

It is essential to understand the competency of Raman to identify samples

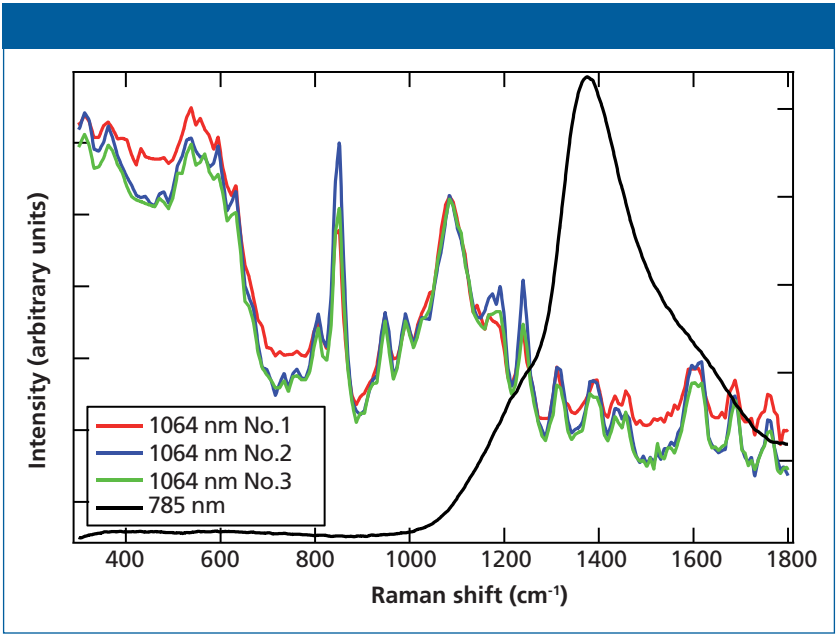

Figure 7: Amoxicillin analyzed through a glass vial with both 785-nm excitation and 1064-nm excitation instruments. Three replicate measurements were done with 1064-nm excitation.

through packaging, such as glass and plastic, so as to not expose the operator to a potent drug or contaminate the medication and to rapidly determine authenticity. Genuine dydrogesterone tablets were scanned either as a bare tablet or through the original blister packaging. Figure 6 shows the spectra of the two tablets measured with 1064-nm excitation. One was measured after removing it from the packaging and the second was measured through the blister pack. The spectrum measured through the blister pack used the Xantus-2 software to subtract a "user background" consisting of the Raman signature of an area of blister pack containing no product. It can be seen in the figure that these two spectra are nearly identical and that the genuine dydrogesterone could be identified through the blister pack.

An additional example of a non-destructive measurement through original packing was performed using amoxicillin powder packaged in a glass vial for injection. This is particularly relevant because of an alarming increase in incidents of counterfeited vial-containing-drug pharmaceutical preparations, for example, bevacizumab (Avastin) counterfeits were uncovered in the United Kingdom and the United States (1). Figure 7 shows spectra obtained for 1 g of amoxicillin in a vial for injection measured with both 785-nm and 1064-nm excitation instruments. From the spectra, it is clear that highly specific Raman peaks of the amoxicillin can be seen with the 1064-nm excitation instrument, but

the 785-nm instrument only shows a broad fluorescent or optical phonon peak from the glass. Figure 7 also shows three replicate measurements of the amoxicillin sample collected on two days to assess the spectral reproducibility of the amoxicillin measured in the original packaging. As can be seen by the close overlap of the 1064-nm spectra, the reproducibility of measurements even through packaging is quite good.

Conclusions

Portable Raman spectroscopy, enabled by miniaturized instruments, continues to be at the forefront of the anticounterfeiting strategy. This study compares Raman spectra collected on a portable instrument with 1064-nm excitation to those collected on a 785-nm excitation instrument. In the cases shown, the 1064-nm excitation has a clear advantage in terms of reduced fluorescence and enhanced specificity, allowing for clear differences to be seen between Raman spectra from authentic and counterfeit medications. In addition to identifying the authentic medication, Raman spectroscopy can probe what is present (or not present, as is often the case with the API) in the counterfeit pharmaceuticals. Finally, it has been demonstrated that the 1064-nm excitation will allow for nondestructive Raman measurements of pharmaceuticals through their original packaging, which is essential for wide-scale authentication.

References

(1) U.S. Food and Drug Administration, "U.S. Food and Drug Administration. Avastin (bevacizumab): Counterfeit Product - FDA Issues Letters to 19 Medical Practices," Accessed online February 14, 2012: http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm291968.htm.

(2) World Heath Organization, "Medicines: spurious/falsely-labelled/World Health Organization," Accessed online May 1, 2012: www.who.int/mediacentre/factsheets/fs275/en/index.html.

(3) F.M. Fernandez, M.D. Green, and P.N. Newton., *Ind. Eng. Chem. Res.* **47,** 585–590 (2008).

(4) M. De Veij, P. Vandenabeele, K.A. Hall, F.M. Fernandez, M.D. Green, N.J. White, A.M. Dondorp, P.N. Newton, and L. Moens, *J. Raman Spec.* **38,** 181–187 (2007).

(5) M. Hajjou, Y. Qin, S. Bradby, D. Bempong, and P. Lukulay, *J. Pharma. Biomed. Anal.* **74,** 47–55 (2013).

(6) M.R. Witkowski, *Am. Pharma. Rev.* **8,** 55–60 (2005).

(7) M. De Veij, A. Denker, P. Vandenabeele, D. de Kaste, and L. Moens, *J. Pharm. Biomed. Anal.* **46,** 303–309 (2008).

(8) M. De Veij, P. Vandenabeele, and L. Moens, *Spectrosc. Eur.* **20,** 7–10 (2008).

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Classical or Transmission Raman, SERS or TERS: Which Raman Spectroscopy Technique Is Right for You?

Raman spectroscopy has found its place as an important analytical tool after years of exclusion as an "academic" technique. The intense instrumental and software developments made easier — or simply possible — the use of different types of Raman spectroscopy. However, as is usual in a relatively new field, Raman spectroscopy — or more exactly, Raman spectroscopies — suffer from some misunderstandings, common (and sometimes false) beliefs, and "fashion" attitudes that make the choice of an appropriate technique and the required parameters of the system difficult. This article re-explains and demystifies some definitions and opinions concerning Raman spectroscopy from two distinct sides: academic and industrial. Different Raman techniques are compared with respect to their main field of applications, possibilities, advantages, difficulties, and limits.

Renata Lewandowska

Some time ago I read on the packaging of facial cream that the product was tested using Raman spectroscopy. My first thought was "Great, finally Raman has found its place in industry." The second thought was that the cosmetic laboratory found it worth mentioning on the box. As a Raman spectroscopist, I'm obviously happy to see the technique rise to fame as it is truly useful and information-rich, and, thus, well-worthy of wider interest. However, between the high-end research, the analytical market, and the marketing, the knowledge about Raman spectroscopy and technique — popularized too quickly sometimes — can be confusing or lost. I see this clearly in my job as a Raman applications scientist, which

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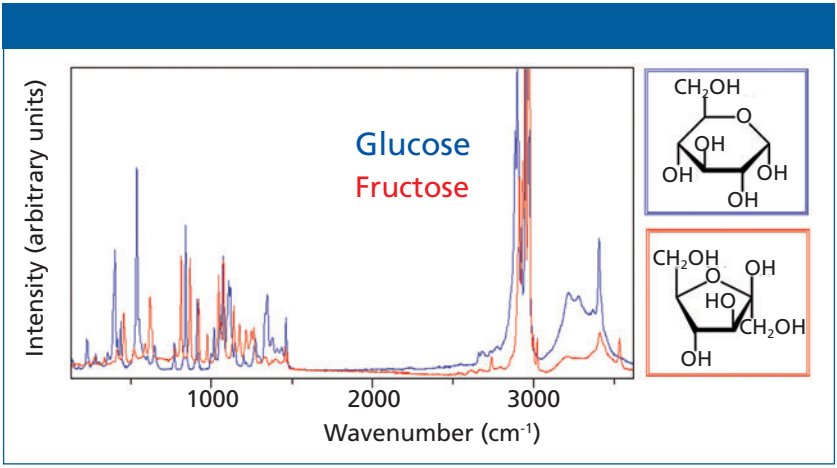

Figure 1: Comparison of Raman spectra of two isomers (which have the same chemical formula but different molecular structure): glucose and fructose.

offers an interesting view on changing Raman applications, technology, and user profiles. Today's Raman spectroscopy market offers systems ranging from easy-to-use handheld systems dedicated to one application, through automated analytical systems to multipurpose high-end research systems. The intense instrumental and software developments have made easier, or simply possible, the use of different types of Raman spectroscopy, like the plasmon enhancement techniques used in surface-enhanced Raman spectroscopy (SERS) or tip-enhanced Raman spectroscopy (TERS), spatially offset Raman spectroscopy (SORS), transmission Raman, and Raman optical activity (ROA), to name just a few, which made their way independently from classical Raman spectroscopy to become new techniques on their own.

In this contribution, addressed to nonexpert Raman users, I will review some Raman-related techniques. I've focused on those I have personal experience with to show their advantages and limitations.

Classical Raman Microspectroscopy

Raman spectroscopy is a universal and versatile method for analysis of the atomic structure and chemical properties of matter by analysis of the interaction between the light and molecular and crystalline vibrations, similarly to near-infrared (NIR) absorption spectroscopy (1).

Raman spectroscopy has numerous advantages. The spectra allow a differentiation between even chemically similar species or different polytypes and their analysis can be quite direct (Figure 1). The confocal setup of modern Raman spectrometers gives lateral and axial spatial resolution on a submicrometer level. It does not require any sample preparation and is nondestructive — an important advantage over other techniques, particularly when the integrity of the sample is an issue. Obviously, laser power should be taken into consideration because burning may happen at high power, but this is a problem related more to the sample itself than to Raman spectroscopy.

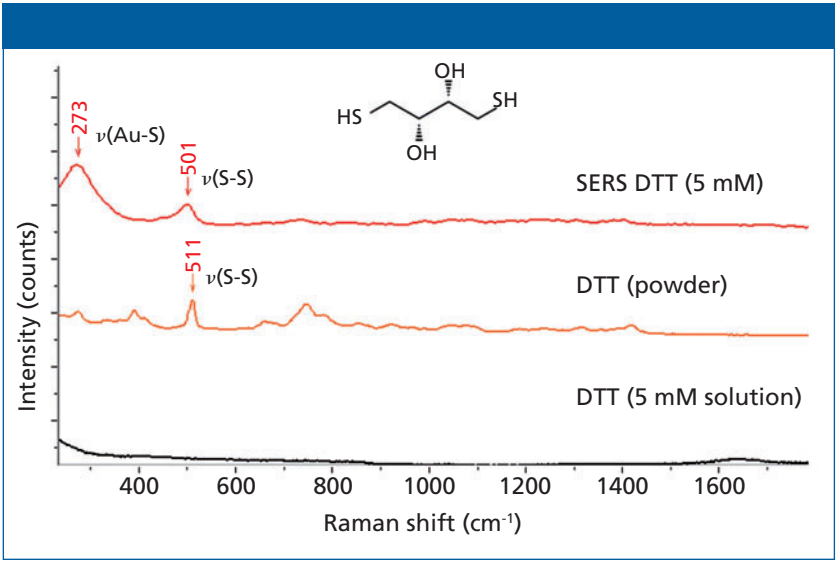

Figure 2: Example of Raman and SERS measurements of a solution of 5 mM DTT, compared to the Raman spectrum of the powder. No Raman signal is observed for the solution because of its low concentration and low Raman efficiency, however the same concentration can be detected using the SERS technique.

Raman spectroscopy is not considered very efficient, meaning low sensitivity and long acquisition times. Indeed, compared to a fluorescence signal, for example, Raman does not look very impressive, even if some good Raman scatterers can give a signal in a few milliseconds or less. However, the advantage of Raman spectroscopy over other techniques is that the information is intrinsic to the material and, thus, label-free. Fluorescence itself can be a problem because it may appear in a Raman spectrum. Usually much more intense, it can mask the Raman signal. It can be intrinsic to the sample coming from light diffused in porous or powder samples or from color centers; it can also be related to the preparation of the sample, such as fluorescent markers, resins used to fix the sample, or organic solvents. Methods exist to avoid or reduce it, however some precaution should be taken during the preparation of the sample.

Who Is It For?

Extensive scientific or technical experience is not required to use and understand Raman spectroscopy, at least at the basic level. Of course, more advanced analyses require more-detailed knowledge about the investigated sample. Most often used in academic research, or industrial development, classical Raman can be adapted to dedicated quality control applications. Because Raman spectroscopy can investigate almost all materials except metals and all states of matter, the applications are numerous. The most common applications involve the analysis of molecular or crystalline structure and chemical composition of materials (polymers, carbon, and semiconductors, to name a few), pharmaceuticals, inorganics and

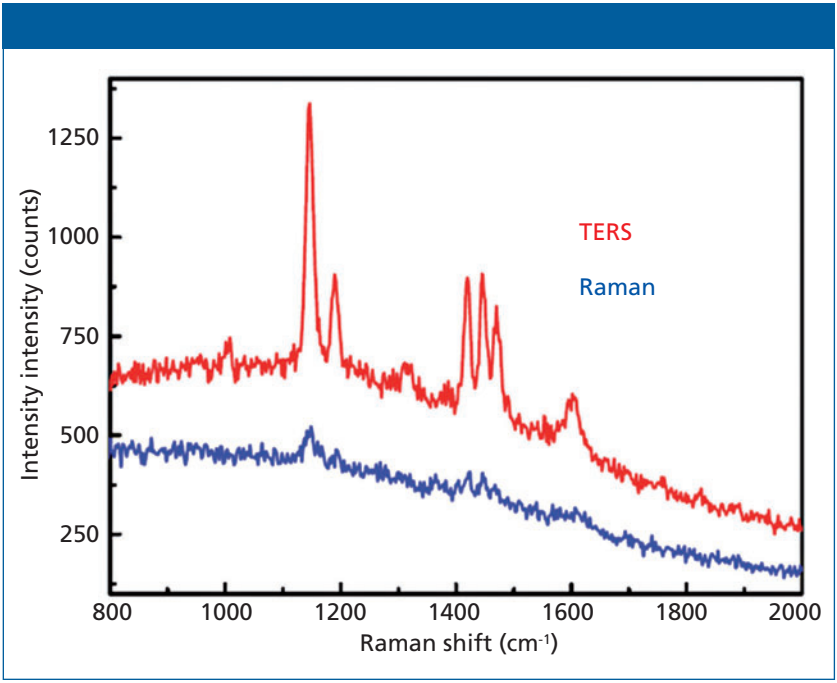

Figure 3: Comparison of Raman and TERS spectra of azobenzene. The integration time was 30 s for the Raman spectrum and 1 s for the TERS spectrum. Courtesy of Marc Chaigneau, Ecole Polytechnique, Paris.

minerals, and biological samples, thus, there are few limitations from this point of view. Corrosion or catalysis studies can be more challenging mainly because of the small volumes, but Raman spectroscopy remains an interesting method, considering the complexity of the investigated structures.

Surface-Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy (SERS) is one of the answers to low Raman signals. Discovered (and rediscovered) in the 1970s, today SERS is a star of spectroscopic conferences and scientific reviews. No wonder, signal enhancement of several orders of magnitude and single molecule detection sound promising. However, looking at the publications and conferences related to SERS, it becomes clear that only a small part of them concerns the application of SERS; a relatively large part relates to the development of the SERS supports (substrates or colloids). So, what is the real SERS situation?

The obvious advantage of SERS is the enhancement of the signal, which sometimes cannot even be measured by classical Raman spectroscopy because of the low concentration of the analyte. SERS inherits, of course, the high chemical selectivity of Raman spectroscopy. SERS spectra and Raman spectra are basically similar, but they are not always exactly the same. There are some reasons for that. The SERS effect is highly localized, therefore, only a signal from part of the molecule, close to the substrate,

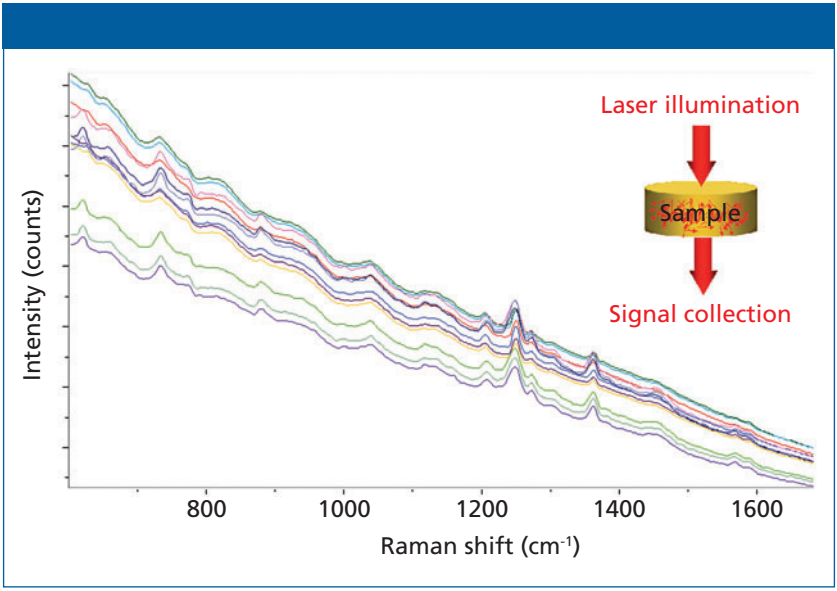

Figure 4: Typical TRS spectra obtained from a pharmaceutical sample.

will be enhanced. When the molecule is adsorbed on the surface, its symmetry might slightly change, and so do the selection rules. Also, because plasmon resonance is wavelength dependent, the different spectral regions of the spectrum may be enhanced differently (Figure 2).

However, the reproducibility of the SERS supports is still the major issue, which probably explains the number of publications related to substrate development. Other issues are the capacity of molecules to attach to the substrate and signal enhancement itself (which, incidentally, has something like 20 various definitions, and, thus, is not a reliable parameter for the comparison of different substrates). The holy grail — substrates that give high enhancement, are reproducible, uniform, and easy and cheap to mass produce — has not been found yet. This is very well summarized in the "SERS uncertainty principle": We either have high enhancement and low reproducibility, essential for very low concentration measurements like single-molecule SERS; or we have relatively good reproducibility with less impressive enhancement, which is still a very interesting option in the case of routine analysis when the classical Raman signal is not strong enough (2).

Who Is It For?

SERS is for those who analyze very small amounts of materials or substances of low Raman efficiency, which could be the case in applications related to pharmaceuticals, life sciences, forensics, or art (2–5). In the case of standard measurements, once optimized, SERS does not require more experience than classical Raman spectroscopy; however, sample preparation is necessary, which can sometimes be time consuming to ensure good adsorption

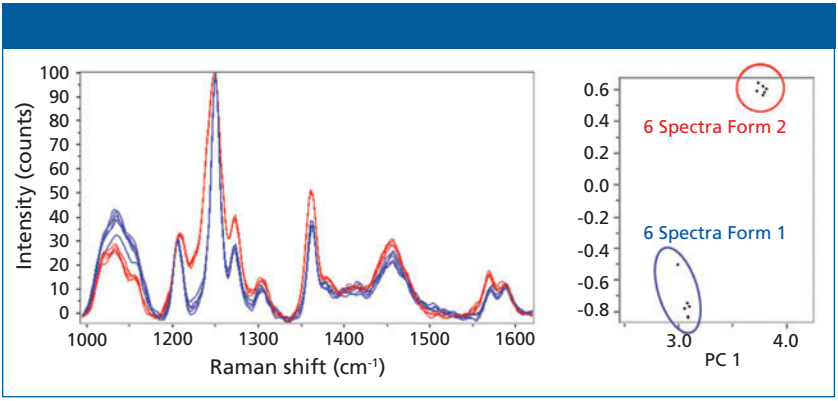

Figure 5: Example of processing TRS spectra: preprocessed spectra from Figure 3 and results of principal component analysis (PCA) decomposition.

of the sample on the SERS substrate. Optimization consists of the choice of an appropriate substrate (with a uniform enhancement on the whole surface some commercially available substrates are reasonably good), a laser wavelength to match the resonance, a suitable concentration range (very low concentrations can suffer from blinking effects), and an operation protocol. There are no particular requirements for the Raman system, which should be obviously adapted to the application. In the case of more extreme measurements (lower concentrations, higher enhancements), specially designed SERS supports can be necessary and tunable lasers can be an interesting option (6).

Tip-Enhanced Raman Spectroscopy

Tip-enhanced Raman spectroscopy (TERS) is based on the same physical phenomenon as SERS. The difference lies in the technical solution. The Raman spectrometer uses a scanning probe microscopy (SPM) system as the sampling device. The SPM system (usually an atomic force microscope) uses a special metallic or metal-coated tip that acts as a plasmonic antenna. Signal is enhanced, as in SERS, but the enhancement is confined to the nanometer-scale area under the tip (Figure 3). The advantage of this technique is not only the enhancement of the Raman signal but also the spatial resolution on the nanometer scale. Therefore, its applications are mainly in the domain of nanomaterials based on semiconductors, carbon or polymers, life sciences (such as cell analysis), and molecule interrogation (in particular, DNA or RNA) (7–11). It is worth remembering that TERS is not the same as colocalized Raman–atomic force microscopy (AFM) measurements, although a TERS system can perform both of them. The latter combines the information on physical properties of the sample with the spatial resolution of SPM (nanometer scale) and the chemical information with the spatial resolution of Raman spectroscopy (hundreds of nanometers scale). Colocalization ensures that these two results come from the same area on the sample. Taking into account that both

SPM and Raman spectroscopy are relatively mature techniques, such analysis is relatively simple.

TERS provides chemical information on the nanometer scale; the special TERS probe tip is key to create the signal enhancement that is necessary to obtain information from the near-field. Although the SPM tips are easily available, few TERS tips are commercially available and all have limitations. Most TERS researchers coat the SPM tips with a noble metal on their own, or produce the full-metal tips by electrochemical etching. Just as the case with SERS, definition of the enhancement factor is still an open question.

Who Is It For?

Today, TERS is not a push-button technique and it requires some experience in Raman spectroscopy, SPM microscopy, and resonance enhancement. A very stable laboratory environment is necessary, including mechanical isolation. Generally speaking, TERS requires significant investment not only in equipment, but also in time. However, when mastered, it brings quite impressive results, as proved by research groups around the world specializing in TERS spectroscopy.

Transmission Raman Spectroscopy

Transmission Raman spectroscopy (TRS) shows an opposite trend to TERS in materials analysis. The latter aims at the nanometer scale, whereas TRS targets the collection of information about the bulk volume of a sample, because it analyses the light that traveled through the entire thickness of the sample. First tests in a forward configuration were done in the late 1960s, but it is only the recent advances in signal detection and availability of small, but powerful lasers and efficient rejection of Rayleigh scattering that made TRS practically possible.

The technique was initially developed for the pharmaceutical industry, for which it is very well adapted (12,13). TRS requires diffusing materials, such as pharmaceutical tablets, and provides information about the whole volume of the sample without a strong influence on the tablet coating, which can be a problem in classical backscattering configuration. It can be used for the analysis of component concentration, and, in particular, content uniformity. It also works for capsules and other materials outside of the pharmaceutical field, which are diffusing, translucent, and require whole sample analysis. The examples of applications can be in the domain of biomaterials (tissues, food), polymers, or even geology. It can be used successfully through the packaging.

Who Is It For?

TRS is a technique for analysts looking for bulk analysis; however, TRS is limited to translucent samples. It is an interesting solution for the routine analysis of samples content (Figure 4). No preparation of the sample is necessary and it does not require any particular experience from the operator. It is often combined with multivariate analysis techniques for data processing because it may bring large datasets of similar spectra (Figure 5). Although it exists as a separate technique, it can be an excellent add-on to the classical Raman system to combine the bulk overall information provided by TRS and more detailed, spatial information given by Raman microspectroscopy.

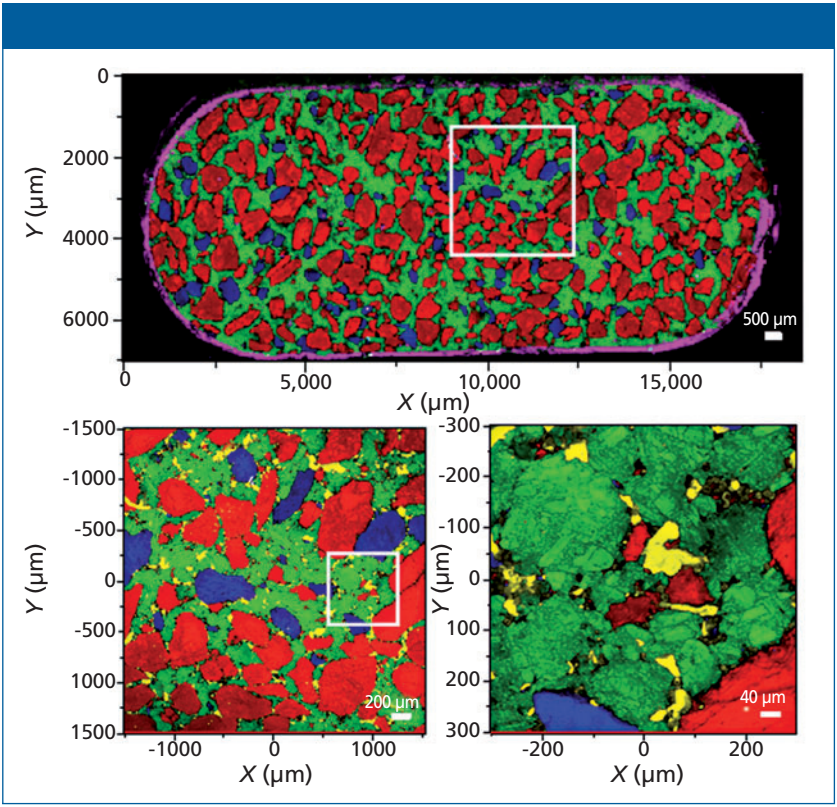

Figure 6: Raman maps of a pharmaceutical tablet on three levels of detail: the whole tablet covering a 7 mm × 18 mm area (upper image), a higher resolution image highlighting a fourth, minor, component (lower left), and a detailed Raman image acquired with 2-µm steps (lower right). Sample constituents: aspirin (red), paracetamol (green), caffeine (blue), and cellulose (yellow); in addition to the tablet coating (pink).

Review of Some Typical Raman Spectrometer Options

Today, imaging is one of the most advertised options for spectroscopy software. Is it really worth it? I'm convinced that yes, it is worth it. Beyond the esthetics, hyperspectral imaging is an excellent and quick method to get and analyze overall information about the sample: for example, component identification and distribution (particularly when some of them could be not detected if just a few measurements were made in discrete locations) (Figure 6) or local changes of structure or polarization. Going further, what about fast imaging? With the exception of signal-enhancing electron multiplying charge coupled device (EMCCD) detectors, the techniques of "fast imaging" actually do not improve the Raman signal (which is strictly related to the Raman efficiency of the sample), but they efficiently optimize the hardware and software and the gain of time is real without compromising the quality of the data (too much).

But where there's "imaging," there are "large datasets," and thus the necessity

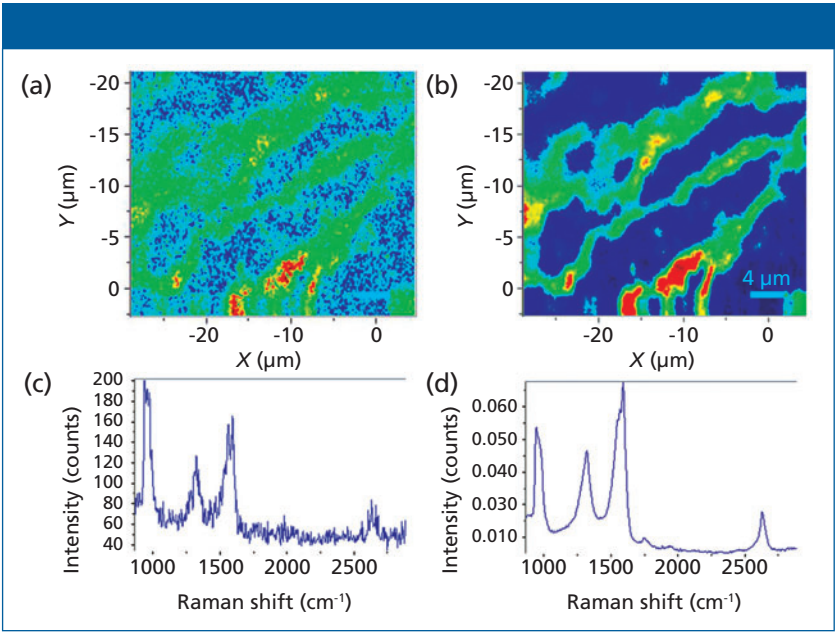

Figure 7: Raman fast imaging of carbon nanotubes (CNT): (a) Raman image after a basic treatment, (b) Raman image after a multivariate processing (multicurve resolution method, MCR), (c) original spectrum, (d) MCR loading.

of efficient data treatment. The classical method of data analysis consists of processing spectrum by spectrum. Today, software packages usually offer a wide range of functionalities for automatic treatment of groups of spectra and hyperspectral images, including baseline correction, smoothing, normalization, or peak fitting. Multivariate analysis, which offers a more holistic attitude to data analysis, has become a standard functionality for spectroscopy data treatment software. Multivariate analysis can easily show the patterns in large data sets, find the pure component spectra (loadings), or simply improve the contrast of images (Figure 7).

A Raman spectrum is strictly independent of laser wavelength, but this parameter must be taken into consideration, as it will influence the Raman efficiency, penetration depth (and thereby, analyzed volume), resonance conditions, and (potential) fluorescence. UV excitations are of interest as a solution to avoid fluorescence, provide possible resonance effects, produce low penetration depths (which indeed, improves the detection and analysis of very thin layers), and high Raman efficiency (which is inversely proportional to the fourth power of the wavelength). This last argument is obviously true, at least from the theoretical point of view. However, the volume analyzed by UV lasers is very small (because of its low penetration depth) and the optics and detectors are typically less efficient in the UV region than in the visible region. The NIR lasers are also used as an ultimate solution to fluorescence issues, which is essentially true because the substances

that give fluorescence in this spectral region are rare. However, in this case, we will struggle with low Raman efficiency and the necessity to use specialized, less-efficient detectors (beyond 1000 nm). Putting all this together, green or blue lasers show the best compromise between Raman efficiency and analyzed volume, and thus the strongest effective Raman signal. In addition, blue lasers, which are relatively new on the market, can be an option to avoid fluorescence because they are on the frontier between UV and visible excitation. The 785 nm laser remains an optimized solution for NIR excitation.

Conclusions

The aim of this contribution was to show the practical aspects of some techniques related to Raman spectroscopy (or spectroscopies). Without a doubt, the latest generation of Raman spectrometers in all their guises (classical, SERS, TERS, TRS, and so on) open up a vast wealth of analytical opportunities, and Raman continues to experience impressive growth as a technique of choice by analysts in many varied fields. The choice of subjects covered here was not exhaustive, but was based on my own experiences and the techniques' relative importance for understanding the advantages (and the difficulties) related to Raman spectroscopy.

References

(1) J.M. Chalmers and P.R. Griffiths, Eds., *Handbook of Vibrational Spectroscopy* (John Wiley & Sons Ltd, Chichester, UK, 2002).

(2) E.C. Le Ru and P.G. Etchegoin, *Principles of Surface-Enhanced Raman Spectrscopy and related plasmonic effects* (Elsevier B.V., Amsterdam, The Netherlands, 2009).

(3) K. Kneipp, M. Moskovits, and H. Kneipp, Eds., *Surface-Enhanced Raman Scattering – Physics and Applications 103* (Springer, Berlin/Heidelberg, 2006).

(4) B. Sharma, R.R. Frontiera, A.-I. Henry, E. Ringe, and R.P. Van Duyne, *Mater. Today* **15,** 16–25 (2012).

(5) D. Cialla, A. März, R. Böhme, F. Theil, K. Weber, M. Schmitt, and J. Popp, *Anal. Bioanal Chem.* **403,** 27–54 (2012).

(6) A.R. Tripp, A.R. Dluhy, and Y. Zhao, *NanoToday* **3,** 31–37, (2008).

(7) E. Bailo and V. Deckert, *Chem. Soc. Rev.* **37,** 921–930 (2008).

(8) A. Hartschuh, *Angew. Chem. Int. Ed.* **47,** 8178–8191 (2008).

(9) J. Stadler, T. Schmidt, and R. Zenobi, *Nanoscale* **4,** 1856–1870 (2012).

(10) E. Bailo and V. Deckert, *Angew. Chem. Int. Ed.* **47,** 1658–1661 (2008).

(11) G. Picardi, M. Chaigneau, and R. Ossikovski, *Chem. Phys. Lett.* **469,** 161–165 (2009).

(12) K. Buckley and P. Matousek, *J. Pharm. Biomed. Anal.* **55,** 645–652 (2011).

(13) J. Johansson, A. Sparén, O. Svensson, S. Folestad, and M. Claybourn, *Appl. Spectrosc.* **61,** 1211–1218 (2007).

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Polymorph Identification and Analysis Using Ultralow-Frequency Raman Spectroscopy

Ultralow-frequency Raman spectroscopy has commonly been cited as an effective technique for polymorph identification because it accesses the lattice vibrations related to the physical structure of the molecule. However, these frequencies have been difficult and expensive to access with traditional Raman spectrometer systems. Recent advances in volume holographic grating (VHG) filter technology enable rapid acquisition of high-quality ultralow-frequency Raman spectra in the 5–200 cm^{-1} region using a compact filter system and single-stage spectrograph, greatly simplifying and reducing the cost of utilizing this technique. We report results using this system with both visible and near infrared (NIR) excitation wavelengths to analyze multiple forms of the polymorphic active pharmaceutical ingredient carbamazepine. Both the intense low-frequency Raman bands and fingerprint region transitions are simultaneously captured and compared, demonstrating the range, ease of use, and efficacy of this technique.

James Carriere, Randy Heyler, and Brian Smith

In many industries the rapid and reliable detection and identification of polymorphs — solid materials that exist in more than one form or crystal structure — is essential to formulation, analysis, quality assurance, and process control. For example, in pharmaceutical development and manufacturing, active pharmaceutical ingredients (APIs) can have significantly different efficacies and bioavailabilities depending on their form. And for polymers, both the physical properties and chemical interactions will be strongly affected by the molecular and intermolecular structure of the polymeric chain. The consequences of inadequate detection of polymorphs in the manufacturing

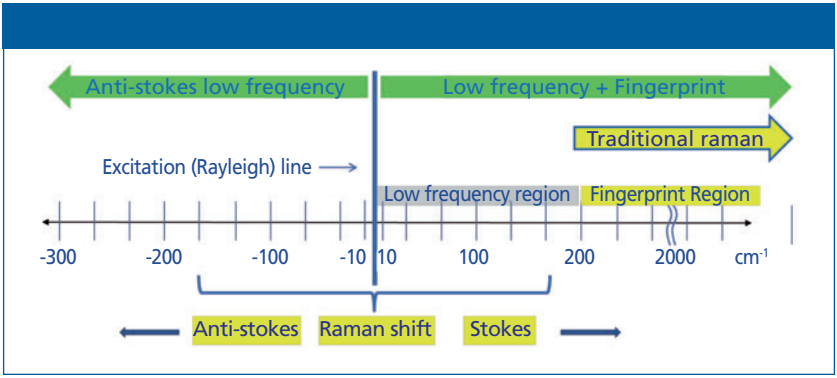

Figure 1: Spectral range of Raman spectroscopy components indicating both fingerprint and low frequency regions.

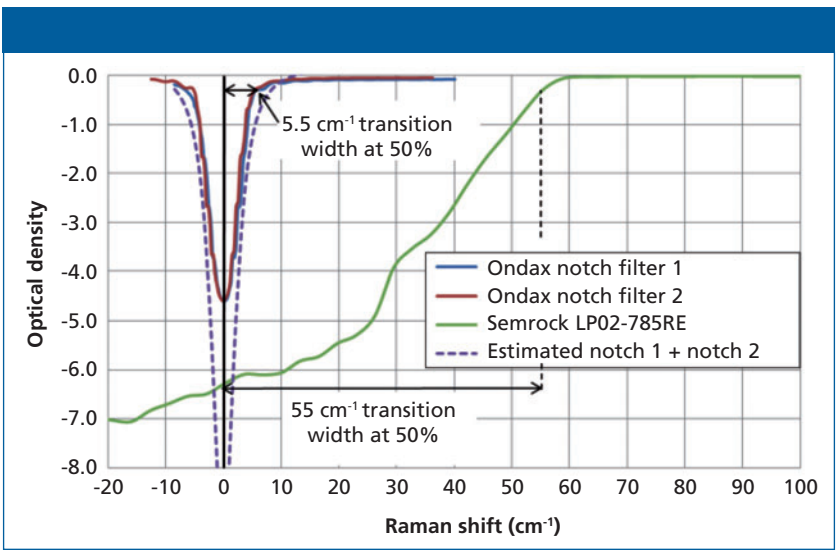

Figure 2: Bandwidth of traditional edge filters vs. VHG notch filters.

process can be both costly and time consuming or even hazardous.

Conventional approaches to polymorph discrimination in the fingerprint region often rely on the analysis of relative peak magnitudes, which can be tricky and unreliable. Ultralow-frequency Raman spectroscopy is an effective alternative technique for polymorph identification because it accesses the lattice vibrations related to the physical structure of the molecule or intermolecular interactions (1). However, these frequencies, which reside extremely close to the Rayleigh excitation wavelength, have been both difficult and expensive to access with traditional Raman spectrometer systems. Rayleigh attenuation is critical to all Raman systems, because the process of Raman scat-

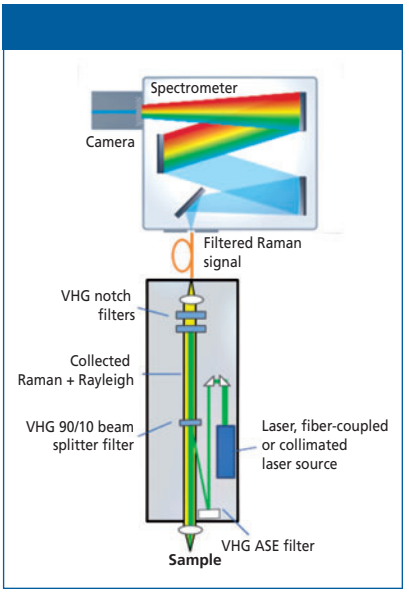

Figure 3: System schematic of the low-frequency Raman spectrometer platform.

tering is extremely inefficient (only about 1×10^{-9} of the incident photons will produce a Raman signal). So, to resolve these extremely weak signals, the excitation wavelength needs to be attenuated with o.d. 8 or better.

Most commercial Raman systems use thin-film edge filters to completely remove the Rayleigh excitation that typically cuts off all signals below about 200 cm^{-1} from the Rayleigh line, which also blocks the entire anti-Stokes region. The use of most commercially available notch filters will allow capture of anti-Stokes signals, but will also block low-frequency signals because of limitations on their transition bandwidth. When detection of ultralow-frequency signals is required, a multistage (or cascaded) monochromator system has been the historical solution for achieving extremely high Rayleigh reduction while preserving the signals that are close to the laser line, but at the cost of greatly reducing the overall Raman signal as well. These systems are quite large, expensive, and require a great deal of expertise to operate, thus limiting their usefulness in a manufacturing environment.

Recent advances in volume holographic grating (VHG) filter technology (2,3) have enabled the manufacture of exceptionally narrow bandwidth notch filters with very high throughput. This has led to systems that are capable of rapid acquisition of high-quality, ultralow frequency Raman spectra in the 5–200 cm^{-1} region (Figure 1). These systems are based on a stable wavelength laser source, a compact series of VHG filters, and a single-stage spectrograph. Each VHG filter has a notch profile that is designed to diffract only one specific wavelength matching the laser and to transmit all other wavelengths. The ultranarrow transition bandwidth of these filters enables extremely high attenuation of the laser wavelength (> o.d. 4), while maintaining very high transmission of nearby Raman signals beyond ~5 cm^{-1} (Figure 2). This combination of strong Rayleigh attenuation and high broadband transmission enables 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 poly-

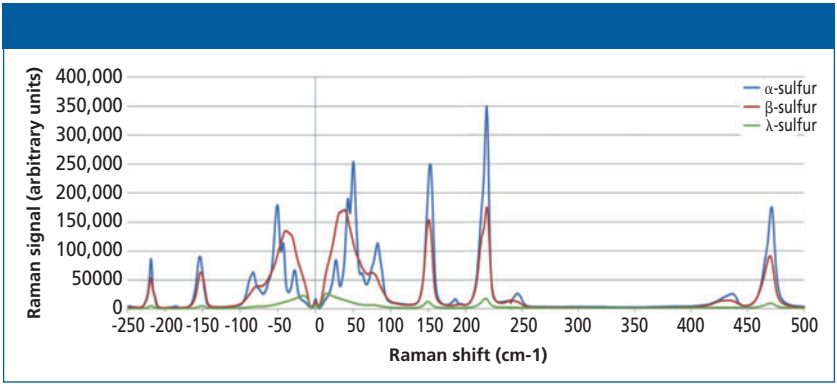

Figure 4: Differentiation of different allotropes of sulfur are easily observed in the low-frequency Raman regime.

morph identification and other applications.

Experimental

Experimental measurements were taken with a custom confocal Raman system (Figure 3), comprising a single-mode 785-nm stabilized diode laser (SureLock LM series, Ondax, Inc.), and a series of ultranarrowband VHG filters that were spectrally matched to the laser output wavelength.

First, two VHG amplified spontaneous emission (ASE) suppression filters (NoiseBlock, Ondax, Inc.) were used to remove any nearby ASE from the laser that tends to be the same order of magnitude or larger than the Raman signals and reduce the signal-to-noise ratio (S/N) in the system. Next, a dichroic 90:10 VHG beamsplitter filter (NoiseBlock, Ondax, Inc.) was used to redirect the laser towards the sample. A 10× objective lens focused the laser onto the sample and collected the backscattered light. The 90:10 beamsplitter then reflected 90% of the Rayleigh scattered light back toward the laser while transmitting the Raman shifted signals. The dichroic nature of the 90:10 beamsplitter resulted in an almost fourfold improvement in collected Raman signal compared to a broadband 50:50 beamsplitter. Finally, two ultranarrowband VHG notch filters (SureBlock, Ondax, Inc.), each having optical density greater than 4.0 were used to further attenuate the collected Rayleigh scattered light while transmitting the Raman signals with an estimated system transmission efficiency of >80%. The entire laser and filter assembly is extremely compact — approximately the size of a ream of notebook paper — and the low power requirements of the laser also make the system operable by battery supply if desired.

It is important to note that the ultranarrow bandwidth of the VHG filters (<0.1 nm) requires the laser to have a very stable wavelength. Nonstabilized 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

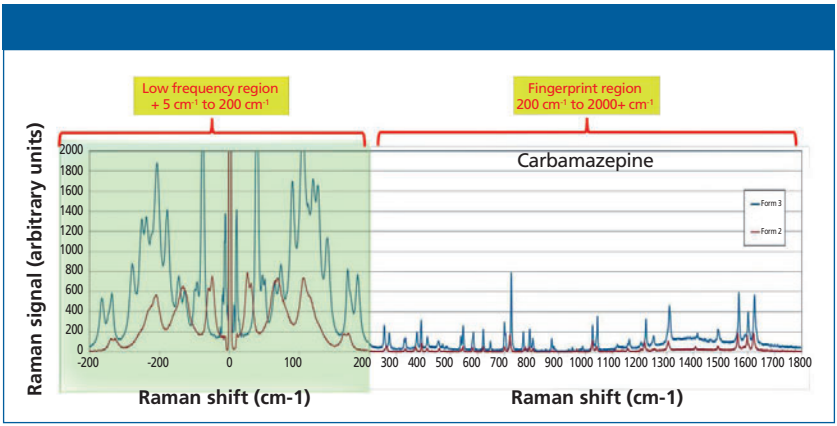

Figure 5: Complete spectra of carbamazepine, showing clearly differentiating low-frequency signals.

Rayleigh suppression or a complete loss of signal in the described configuration.

The filtered signal was focused into a 25-µm core diameter, 0.1-NA step index fiber (HPSC25, ThorLabs), and connected to a high-resolution, high-throughput, single-stage, 0.3-m imaging spectrometer (IsoPlane series, Princeton Instruments). It was equipped with a 1200-line/mm grating and a 1340 × 400 imaging array (Pixis model 400BR with eXcelon, Princeton Instruments) with 20 × 20 µm pixel size and 98% peak quantum efficiency to ensure maximum signal collection and ~1.25 cm^{-1} resolution; appropriately matched for analysis of the 5–200 cm^{-1} frequency range.

Results

Two samples having multiple allotropic or polymorphic forms (different structural forms for the same chemical composition) were investigated for this analysis: sulfur and carbamazepine. Sulfur forms more than 30 different allotropes (4), but 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 the Raman spectra were measured with the described system as a function of temperature with 80 mW of laser power on the sample and 10 s of total integration time at each temperature setting. The hot plate temperature was monitored with a thermocouple. When the sample temperature was increased above 95.2 °C, the form changed from α to β. Further increasing the temperature above the melting point at 115.21 °C resulted in a second form change to λ. The corresponding Raman spectra for each form were captured and plotted in Figure 4. Note that while there is a corresponding change in magnitude of the peaks in the Raman fingerprint region, there is no obvious shift in the position of the peaks. By comparison, the ultralow-frequency region changes dramatically from one form to another in both magnitude and location of the peaks,

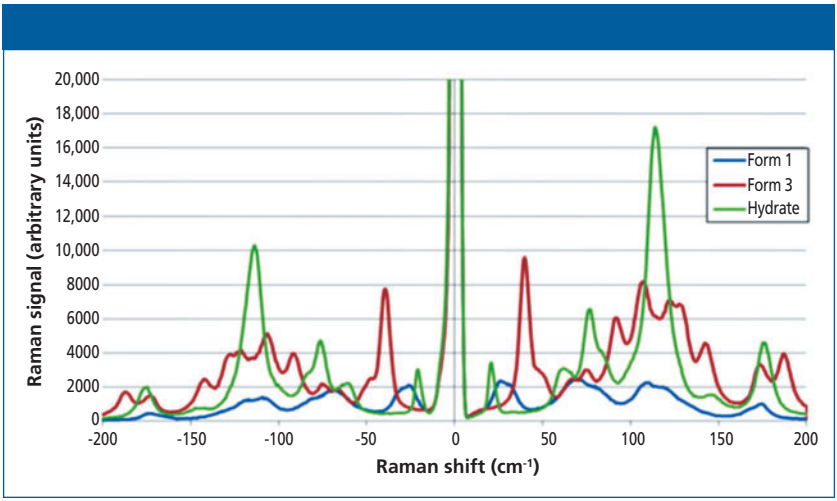

Figure 6: Low frequency and anti-Stokes spectra of carbamazepine.

enabling clear differentiation of the allotropes.

Carbamazepine is an anticonvulsant and mood-stabilizing drug that is commonly prescribed in the treatment of epilepsy and bipolar disorder. It has four different polymorphic forms that have been well characterized in the literature (5–9) with form three being the active pharmaceutical ingredient. We obtained pure samples of both form two and form three, as well as the hydrated form and measured the carbamazepine spectra with the same excitation laser and integration conditions (Figure 5). Because the molecules have the same chemical composition, the fingerprint region signals are quite similar, whereas the different structural forms of the polymorphs clearly present themselves as differences in the low-frequency signals. Figure 6 shows additional details of the various polymorphs in the low-frequency regime, including the anti-Stokes signals, which clearly validate the low-frequency measurements. The anti-Stokes signals can often be used to verify low signals in the 5–500 cm^{-1} region because of the inherent symmetry about the laser line, thus providing additional information that can be used to boost the detection capabilities of the system by removing spurious background noise or signals that break this symmetry.

Discussion

Both the sulfur and carbamazepine data demonstrate the value of low frequency Raman signals for the discrimination of different polymorphic forms. The signals found in this region are much stronger in intensity and more clearly differentiating than those in the fingerprint regions (200–2000 cm^{-1}), making them ideal for rapid detection systems and algorithms by reducing the computational complexity required for form discrimination. And be-

cause many of the various manufacturing and formulation processes (including temperature, humidity, and pressure) can lead to a change in molecular structure, it is essential that the polymorphic constituents are constantly monitored during formulation to ensure purity of the material. This additional information can be used to inform manufacturing process changes and improve overall yields while reducing manufacturing costs. The compact and affordable nature of the system allows it to be easily integrated into locations where Raman spectroscopy is currently used.

Summary and Conclusions

We have shown that low-frequency Raman signals can quickly and easily discriminate between different material polymorphs with a compact, VHG-enabled, low-frequency Raman system. The use of ultranarrow-band notch filters in place of conventional edge filters to reduce the Rayleigh signal enables the collection of a wealth of information about the physical structure of the molecule in addition to the chemical composition. Aside from polymorph identification, there are many other applications in which low-frequency Raman signals can be used to gain important new information about materials and boost system sensitivity, such as explosives trace detection and forensics, polymer and industrial chemical development and manufacturing, cancer detection, and basic material science. This new platform will open up the possibility of using low-frequency Raman for these and a multitude of other industrial and scientific uses in the years ahead.

References

(1) A. Hédoux, L. Paccou, Y. Guinet, J.-F. Willart, and M. Descamps, *Eur. J. Pharm. Sci.* **38,** 156–164 (2009).

(2) C. Moser and F. Havermeyer, *Appl. Phys. B: Lasers Opt.* **95**(3), 597–601 (2009).

(3) C. Moser, et al. "Compact Raman spectrometer system for low frequency spectroscopy," *Proc. SPIE*, **7598,** (2010).

(4) R. Steudel and B. Eckert, *Top. Curr. Chem.* **230,** 1–80. doi:10.1007/b12110. ISBN 978-3-540-40191-9 (2003).

(5) H. Pohlmann, C. Gulde, R. Jahn, and S. Pfeifer, *Pharmazie* **30,** 709–711 (1975).

(6) C. Lefebvre, A.M. Guyot-Hermann, M. Draguet-Brughmans, R. Bouche, and J.C. Guyot, *Drug Dev. Ind. Pharm.* **12,** 1913–1927 (1986).

(7) C. Rustichelli, G. Gamberini, V. Ferioli, M.C. Gamberini, R. Ficarra, and S. Tommasini, *J. Pharm. Biomed. Anal.* **23,** 41–54 (2000).

(8) M. Lang, J.W. Kampf, and A.J. Matzger, *J. Pharm. Sci.* **91,** 1186–1190 (2002).

(9) G.M. Day et al., *J. Phys. Chem. B* **110,** 447-456 (2006).

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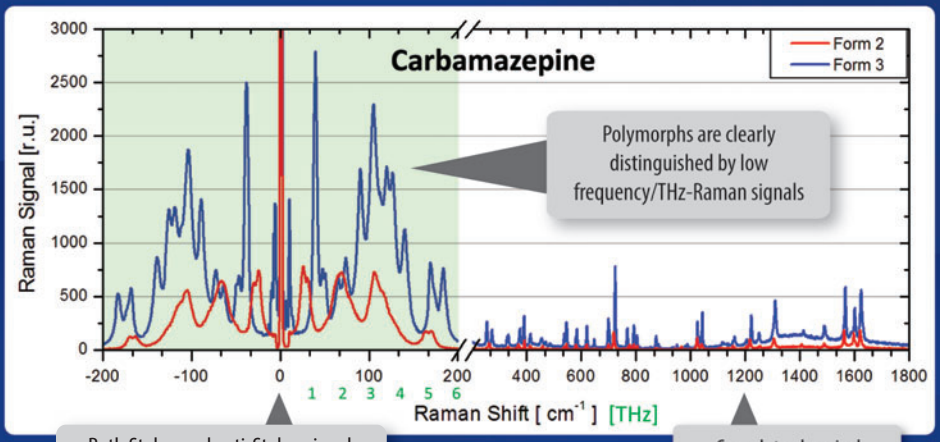

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