Editor's Page

Micro Raman at Pittcon® 2012

by Barbara Foster

Micro Raman was big news at Pittcon® 2012. While the hardware has stabilized, there were intriguing new developments in the science and its applications.

Bridging the microscopy/spectroscopy chasm

Historically, atomic force microscopy (AFM) has been the gateway for Raman microscopy, but the emphasis at Pittcon was on the optical. Major manufacturers—from micro Raman industry leaders **Renishaw** (www.renishaw.com) and **Horiba** (www.horiba.com) to **Bruker Optics** (www.brukeroptics.com), **Jasco** (www.jascoinc.com), **Kaiser Optical Systems** (www.kosi.com), and **Thermo Fisher Scientific** (www.thermofisher.com)—all featured fully integrated systems, typically centered on **Olympus** BX research-level microscopes (www.olympusamerica.com). (**WITec** [www.witec.de] and **CRAIC Technologies** [www.microspectra.com], which follow similar formats, elected not to exhibit at Pittcon this year.)

While similar in design, each company features something slightly different, making comparison shopping with your own sample especially important. For example, **Jasco** offers proprietary vertical scanning (SPRintS), which keeps the beam perpendicular to the sample throughout the scan to remove any pin-cushioning aberration, and a special Dual Spatial Filter (DSF); this removes not only fluorescence, which can often mask the Raman signal, but background Raman as well. *Figure 1a* shows a nanodiamond in the center of a groove in a polymer matrix. Figure 1*b* shows the typical Raman spectrum, with both fluorescence, which obscures the baseline, and a high background signal from the polymer matrix. The DSF (1c) effectively removes both, revealing a clean, distinct peak for the nanodiamond.

Renishaw joined the 3-D revolution with the new StreamLine HR™. The new software makes 3-D images using Raman band intensity or more complex parameters such as principal components derived from chemometric analysis. The user can rapidly review the data by rotating and zooming in on the volume in real-time, with control over colors and transparency.

In *Figure 2*, for example, a ruby's chromium R2 photoluminescence band was used to reveal internal stress. First, the surface was indented. Next, using moveable plane slices within the volume, spectra were displayed from three different perspectives—in this case, two planes perpendicular to the surface across the center of the indent, and a third in a plane below the indent. Data like these help materials scientists understand deformation and fracture mechanisms.

Micro Raman for the nonresearch user

Micro Raman is not just for the research lab, however. **B&W Tek** (www.bwtek.com) introduced the new BAC151A Video Microscope Sampling System (*Figure 3*). The new system is compatible with all **B&W Tek** Raman probes, featuring XY control with coarse and fine adjustments as well as

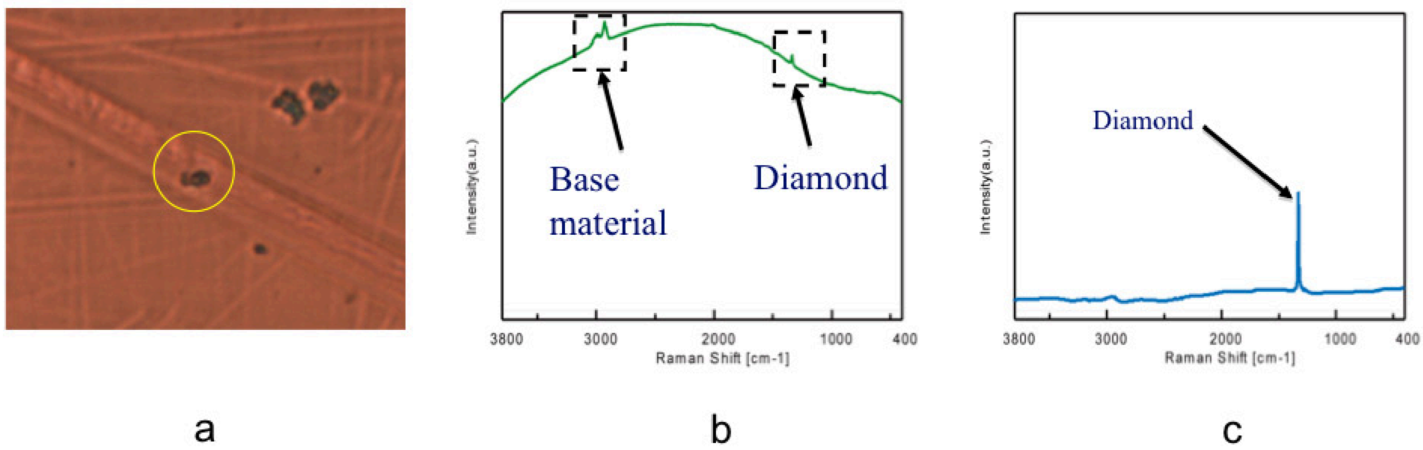

Figure 1 Nanodiamond in polymer matrix: a) 1 µm diamond (center speck) in polymer matrix (yellow circle), b) Raman spectrum without DSF, and c) Raman spectrum with DSF revealing clean spectrum of diamond without interference of fluorescence background or Raman signal from the matrix.

Z-axis to focus the laser on the desired plane to maximize the Raman signal. An optional dual laser configuration offers input for two different lasers through a single port. The integrated camera facilitates precise Raman sampling through BWSpec™, permitting both laser beam tracking and image capture. **Enwave** (www.enwaveopt.com) takes a similar approach with the µSense line, which uses fiber optics and video to connect Raman to laboratory-based stands.

BaySpec (www.bayspec.com) retains full capabilities in its upper end offering, Nomadic, but introduced the clever new MovingLab, which integrates a rotatable PC into the microscope head, making it especially useful for forensics labs and field work.

The **DeltaNu** ExamineR™, developed by its **Intevac Vision Systems** group (www.intevac.com), uses a free-space design to create a unique modular system that mounts atop virtually any microscope. The system comes in two wavelength variations—532 nm and 785 nm—and offers two software options: NuSpec™ for instrument control, data acquisition, and library development; and NuImage™ for Raman mapping. An additional attachment performs Raman analyses on liquids.

*Figure 3 **B&W TEK's** BAC151A Video Microscope Sampling System uses a standard C-mount head and fiber optic Raman feed to attach to any microscope stand.*

Micro Raman for students

BioTools (www.btools.com) announced the new BioRAMAN™ at Pittcon (*Figure 4*). This shoe-box-sized micro Raman system features interchangeable objectives (40× and 60×) for micro work and UV cuvettes for solution work, an ideal combination for college labs or small start-ups interested in using micro Raman to investigate the biomolecular structure of proteins and similar materials. Dr. Rina Dukor also explained that their unique new design has improved their signal-to-noise ratio twofold.

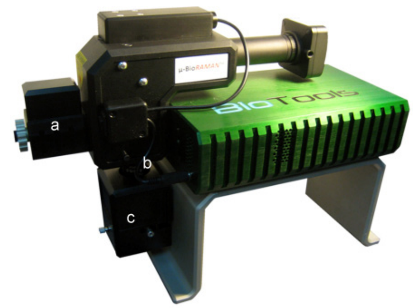

*Figure 4 The new shoe-box-sized BioRAMAN from **BioTools** offers the flexibility of both a microscope and a cuvette system, and features sample holders for a) liquids, b) microscopy samples, and c) solids.*

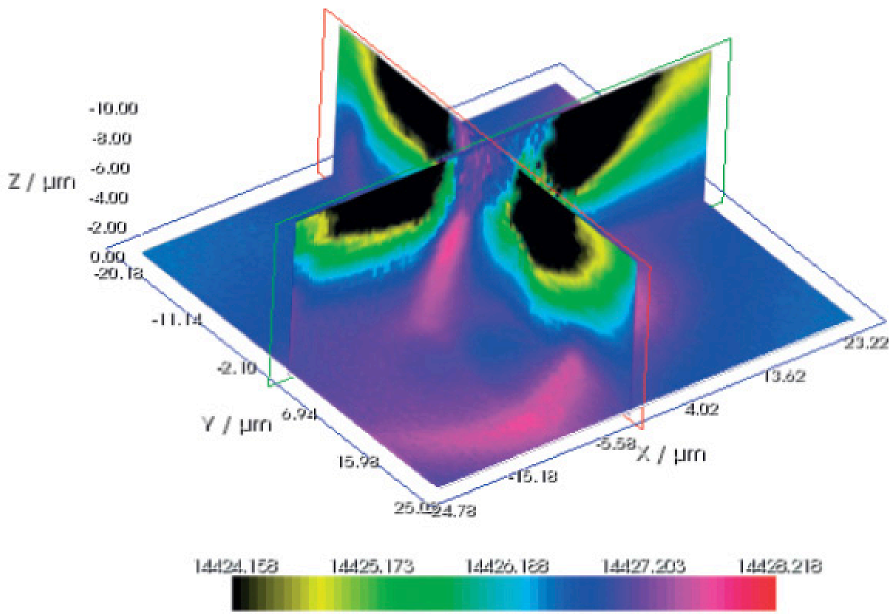

*Figure 2 3-D imaging of stress within a ruby using **Renishaw's** new StreamLineHR.*

Polarized light emerging as an important Raman and IR adjunct

Horiba's technical guru, David Tuschel, stressed the importance of keeping the microscope fully functional while asking, "What else is possible?" and found that using polarized light opened opportunities for new crystallographic investigations that complement X-ray diffraction studies. In his recent molecular spectroscopy article,[1] David explains that Raman scattering depends not only on molecular mass and bond strength, but also crystal symmetry and orientation. As a result, it is highly susceptible to the direction and polarization of both the incident and resulting scattered illumination. Using the conventional polarizer on a microscope to control the incident illumination and the analyzer to control the polarization direction of the scattered light enables collection of highly informative micro Raman spectra. As he points out in the abstract, these "polarization/orientation (P/O) micro-Raman [spectra] can be used to identify vibrational modes, determine crystal structure, distinguish allotropes and polymorphs, differentiate single from polycrystalline materials, and determine orientation of the crystal and degree of disorder, all on a micrometer scale." He goes on to describe application to ionic solids, covalent solids, and molecular crystals and for the characterization of minerals, integrated electronics, photonic devices, microelectromechanical systems (MEMs), photovoltaics, field effect transistors (FETs), and the newly emerging organic semiconductors including light-emitting diodes (OLEDs).

Anasys (www.anasysinstruments.com), well known for its "AFM+" (AFM+thermal analysis, AFM+IR), have now added Pol to study molecular orientation. A nanosecond pulse of polarized IR radiation causes the AFM probe to resonate. Measuring the amplitude of the IR absorbance directly under the probe as a function of polarization angle delivers direct measurement of molecular orientation. Using the AFM as a platform allows the user to measure and visualize molecular orientation with nano scale spatial resolution.

Traditionally, micro Raman has been most extensively used in the materials sciences. However, **BioTools** has taken the combination of the Pol-Raman to a different level, especially targeted at the life sciences. The ChiralRAMAN2X™ measures Raman Optical Activity (ROA), defined as the difference in Raman intensity for the right (R) minus left (L) circularly polarized light incident on or scattered by chiral molecules. The ChiralRAMAN2X integrates a Raman spectrometer with an inverted microscope and is especially helpful in characterizing biomolecular structures and behavior of proteins, nucleic acids, carbohydrates, and viruses under realistic, aqueous conditions.

Micro Raman moves into health care and high throughput

Renishaw debuted the RA800 series, a new automated platform for health care, developed with partner **Renishaw Diagnostics** (www.renishawdiagnostics.com) in Glasgow, Scotland. One variant is already in use with **Renishaw Diagnostics Ltd. (RDL)**, where it forms part of **RDL's** RenDx™ RUO Multiplex Assay System, developed as a tool for research into infectious diseases. The accompanying SP-1000 robotic sample processor contains several different modules including a washing station with unique integrated magnetic heater/shaker. The SP-1000 is thermally controlled and can process 46 samples and controls in under 2.5 hr. All RenDx assays use novel patented technology based on surface enhanced resonance Raman spectroscopy (SERS).

SERS has been a topic of some discussion over the past several years. Until recently, these active substrates have been difficult to manufacture. However, in addition to **Renishaw's** offerings, the Pittcon floor saw a dramatic expansion in SERS activities, notably from companies like **Thermo Fisher Scientific**, offering both silver and gold substrates, and **Real-Time Analyzers** (www.rta.biz), which targets the high-throughput arena with its SERS 96-well plates, capillaries, and vials.

Summary

From research stand to student "shoe box," the microscopy/spectroscopy chasm is definitely shrinking as micro Raman becomes part of the analytical mainstream in both the materials and life sciences. Optical micro Raman has become a mainstay and is now an important correlative technique to AFM-Raman, giving "eyes to chemistry" from the nano scale to the micro scale.

Reference

1. Tuschel, D. Molecular spectroscopy workbench: Raman crystallography in theory and in practice. *Spectroscopy* Mar **2012**, *27*(3); www.spectroscopyonline.com.

Barbara Foster is President and Chief Strategic Consultant, ***The Microscopy & Imaging Place, Inc.*** *("****The MIP****"), McKinney, TX, U.S.A., and Consulting Editor, American Laboratory/Labcompare; tel.: 972-924-5310; e-mail: bfoster@the-mip.com; www.MicroscopyMarket.com.* *Ms. Foster extends her thanks to all of the product managers, technical, and senior staff who so generously provided their time for interviews at Pittcon.*