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APPLICATION NOTE

by Barbara Foster

Focus on Microscopy: Pittcon® Gives New Eyes to Chemistry

Pittcon® is not normally thought of as an imaging show, but this year's exhibit floor proved that assumption false. Novel new light microscopies, desktop scanning electron microscopes (SEMs) designed for nonmicroscopists, and a proliferation of Raman imaging brought a wave of new vision to the chemistry laboratory.

Handheld, midrange, and automated technologies drive light microscopy

Heldhand technology, new to many analytical techniques including X-ray fluorescence (XRF) and Raman, has long been a staple in microscopy, with the two industry leaders, **KEYENCE** (Woodcliff Lake, NJ) and **Hirox-USA** (River Edge, NJ) both present on the show floor. The **KEYENCE** family features both a color laser scanning microscope and the new handheld digital microscope, the VHX, featuring a 54-million-pixel, 3-CCD (charge-coupled device) camera. Both the **KEYENCE** and **Hirox** systems offer unique, real-time solutions to dynamic, high-resolution 3-D imaging in applications ranging from circuitry to medical devices.

Motic (Richmond, BC), a well-known provider of laboratory-level conventional microscopes, announced two new systems at Pittcon: the BA 300 for routine transmitted light observations and the BA 300 Pol, a competent laboratory-level polarized light system. These robust, easy-to-use systems are ideal for routine analysis, for laboratories just getting into microscopy, and for introductory research.

Figure 1 **Micros**, *a new entrant in the midrange microscopy market, caught attention with the Rose Bling, a limited-edition, hand-painted microscope inlaid with Swarovski crystals.*

In addition to **Motic**, this midrange technology was well represented by a new player in the field, **Micros** (Vienna, Austria). The product line displayed in its booth was amazingly complete for a new entrant, with instruments ranging from multiple-head teaching microscopes to upright and inverted compound microscopes, all refreshingly named after flowers. And, for the laboratory that has everything, **Micros** has limited edition, hand-painted microscopes, including the Rose Bling, inlaid with Swarovski crystals (*Figure 1*).

At the other end of the spectrum is **TILL Photonics's** (Gräfelfing, Germany) iMIC, soon to become part of **Agilent's** (Santa Clara, CA) growing family of tools for nanotechnology. Billed as a "fully motorized imaging platform," the elegantly simple architecture seen in *Figure 2* harkens back to **Zeiss's** (Thornwood, NY) historical Axiomat. Built on a similar multifloor optical bench concept, the octagonal iMIC offers unparalleled flexibility for choice of illumination, optics, detectors, and even laser scanning.

Objectives are the mastermind of any light microscope, and **TILL Photonics's** engineering handles them masterfully. Each objective is placed on a cantilever where piezo-controlled mounts uniquely and automatically center and focus it. For beam splitting, critical to optimum control of fluorescence techniques, a beam multiplexer expands beam direction from the normal three to up to eight different directions. All of these components are under computer control and can be programmed for individual user profiles. Similar to the adjustable seat and steering wheel customization on a luxury sedan, the profile code can be keyed in to change the settings on the microscope from user 1 to user 2, etc.

Two of the more sophisticated applications for iMIC include FRET (Förster or fluorescence resonance energy transfer, depending on whether the interacting molecules are fluorescing), which characterizes molecular dynamics such as protein conformational changes, and protein–protein or protein–DNA interactions, and TIRF (total internal reflection fluorescence) used to image activity at the cell surface such as cell binding, cell adhesion, and secretion of neurotransmitters.

Microscope illumination continues to evolve. **Lumencor** (Beaverton, OR) presented the latest in that area: a compact, novel design that pumps the illumination from light-emitting diodes (LEDs) into a phosphor-filled tube or light pipe. The refractive index of the tube is designed so that the only exit for the illumination is through a hollow lumen in the center. The result: 10–20 fold increase in power over a broad range of narrow bands, with no spectral drift and very low out-of-band noise. The system was recently retrofitted by Dr. Simon C. Watkins, Director of the Center for Biological Imaging at the University of Pittsburgh, retrofitted to the **Olympus** (Melville, NY) DSU disk scanning confocal and the **Olympus** IX81.

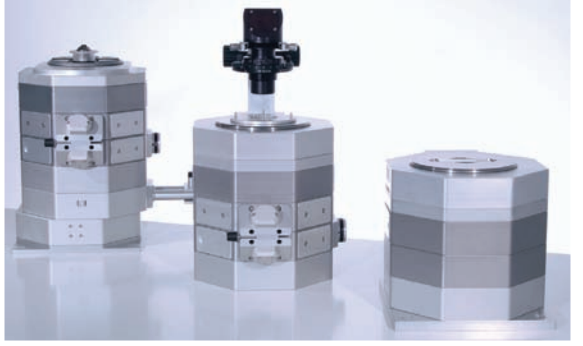

Figure 2 ***TILL Photonics's*** *flexible, automated, space-age iMIC offers multiple ports for cameras and light sources and programmable user profiles. From left to right: a) DiChrotome configuration for dual-emission fluorescence and FRET experiments, b) more traditional inverted microscopy configuration with conventional condenser and transmitted light illumination, and c) core module for fluorescence and light microscopy. (Image courtesy of* **Agilent** *and* ***TILL Photonics****.)*

Hybrid light microscope/SEMs—simple enough for a 10-year-old

The concept of a desktop SEM is not new. Topcon/ISI sold many of them throughout the 1980s and '90s. However, there has been a considerable gap in time between that pioneering effort and the new crop of desktop SEMs sprouting on the Pittcon floor. **Hitachi** (Pleasanton, CA) began the new trend about a year ago, with the introduction of the T1000. **FEI** (Hillsboro, OR) quickly followed suit with the much-discussed Phenom, and was shortly joined by **Evex's** (Princeton, NJ) Mini-SEM. The latest entry, NeoScope, a joint venture between **JEOL** (Peabody, MA) and **Nikon USA** (Melville, NY), made its debut at Pittcon.

As shown in *Figure 3*, these new instruments are designed to fill the magnification gap between light microscopy (typical maximum magnification: 1000×) and research-level electron-beam instruments (20,000×) by integrating light microscopy for navigation with electron microscopy for higher magnification and depth of field. All the entrants in this new market stressed that these instruments are most useful for the 80% of applications that require 20,000× magnification or less.

This new family of hybrids is already beginning to segment itself. Both the T1000 and Mini-SEM offer a combination of imaging with elemental mapping and, therefore, require some knowledge of specimen preparation and electron-beam mechanics. However, both the Phenom and NeoScope were designed for more routine use by inexperienced or nonmicroscopy-oriented staff. With variable voltages and high- and low-vacuum modes, the NeoScope can handle a wide variety of conductive and nonconductive samples, delivering a magnification range from 10× (optical microscopy) to 20,000× (electron microscopy). As a testament to its ease-of-use, the NeoScope will be sold by **Nikon's** optical microscopy sales force rather than **JEOL's** electron microscopy group. **FEI's** Phea

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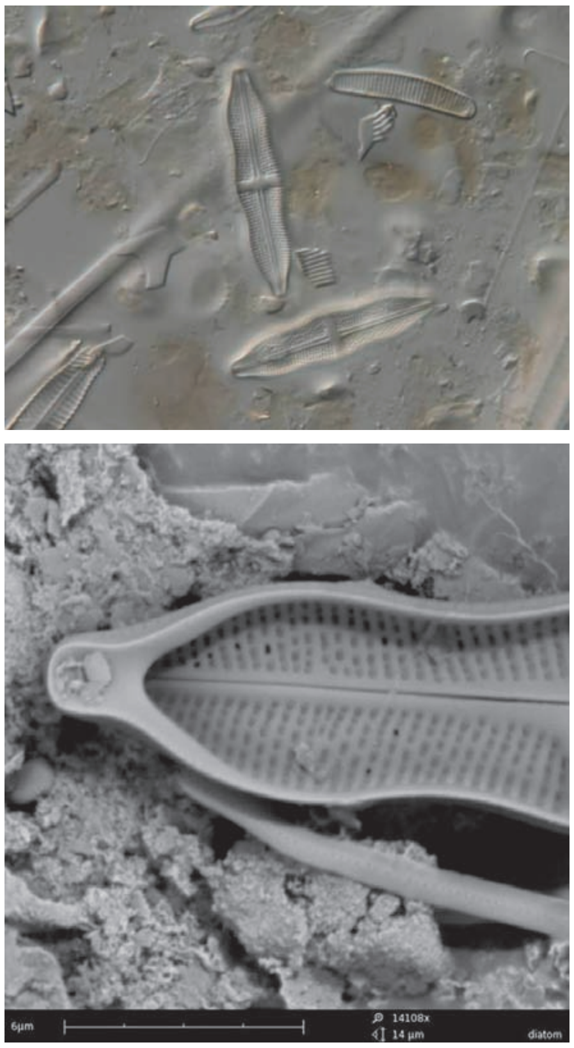

Figure 3 a) Light microscopy image of a typical diatom (Stauroneis legumen), 1000×; and b) electron microscopy image of the same diatom at 14,000×. (Image courtesy of **FEI**.)

nom sports a fast-loading sample holder (only 20 sec from loading to image, compared to 5–10 min with conventional SEM) and a touchscreen control interface that always presents both the optical and electron microscopy images for "Never Lost" navigation: A touch of the optical image directs the Phenom to navigate to that area of interest. Focus and contrast are automatically sensed and adjusted to fit the sample. Dr. Steven Berger, **FEI's** business develop manager in charge of the Phenom project, who gave a presentation at Pittcon in the New Technology section, indicated that **FEI** tested the interface with a group of 10-year-old students. When the students were taking pictures successfully with only a few minutes of training, **FEI** decided that the microscope was ready for use by anyone.

In a follow-up interview, Dr. Berger discussed the expanding market for this technology. "People in a wide range of applications from routine metallurgy to pharmaceutical QA to nanostructure characterization need SEM images. In many cases, they don't own an SEM in their immediate department because of cost, complexity, and the need for a specialist to run the instrument. As a result, they outsource these analyses, either to an internal central service lab or outside facility, at a typical cost of approximately $300/sample. Similarly, current SEM and TEM (transmission electron microscope) owners need a low-cost imaging tool for their routine work. In addition, light microscopists, dealing with smaller and smaller structures, are constantly pushing the limits of magnification." He went on to explain that the Phenom is a good fit for all of these groups and supported his statement with a simple calculation of cost of ownership. Unlike a conventional SEM, there is no need for elaborate facilities or a highly trained operator. Amortizing the cost of both the instrument and its modest service contract over five years and assuming use for 2000 hr per year, the resulting cost of ownership (CoO) per hour is only $10, comparable to the cost of ownership of a sophisticated light microscope and much less than the cost of sending a sample out to a service laboratory.

AFM moves further into the mainstream, often integrating with Raman

Atomic force microscopy (AFM) has long been used for polymer and materials analysis, but the Pittcon floor reflected recent trends toward simpler systems, biological applications, and Raman integration. From the biological perspective, **Agilent** highlighted the industry-leading microscopes from the original Molecular Imaging line, **Agilent's** first microscopy acquisition. On the Raman front, **Park Systems** (Santa Clara, CA) is now integrating with **Horiba-JY** (Edison, NJ) spectrometers, and newcomer **AIST-NT (Advanced, Integrated Scanning Technology for Nanotechnology)** (McKinney, TX) announced that it would be integrating with a variety of Raman spectrometers, with early sales based on **Tokyo Instruments (TII)** (Tokyo, Japan) technology. Following the **Veeco/Asylum** (both of Santa Clara, CA) model, **AIST-NT** is comprised of many of the engineers that made the progressive scanning probe technology for **NT-MDT** (Zelenograd, Moscow, Russia). The company also announced the first of its new line of AFMs, featuring automatic detector centration and probe approach.

WITec (Ulm, Germany) launched its first-ever press conference in fine style, announcing two new automated confocal Raman/AFM systems, the alpha500 (see *Figure 4*) and alpha700. These systems provide a combination of 3-D chemical imaging with high-resolution structural imaging in an automated platform. The stage on the alpha500 has a range of 150 × 100 mm, while the alpha700 handles larger samples, up to 250 × 300 mm. Applications for these new automated tools range from wafer inspection and pharmaceutical screening to thin film analysis, forensics, and tissue screening.

Confocal Raman becomes smaller, faster, and easier to use

Slow scan speed has long been a barrier for Raman imaging. At least four companies on the show floor announced new, fast-scanning technologies, including **WITec**, **Renishaw** (Hoffman Estates, IL), **Horiba**, and **ChemImage** (Pittsburgh, PA). **Horiba's** SWIFT™ reportedly acquires a 50,000-spectrum image and can be partnered with the new DuoScan™, through which the user can match image pixel size to features of interest to optimize mapping speed. **WITec's** Ultrafast Imaging Option, coupled with an EMCCD detector, can complete a 250 × 250 pixel hyperspectral image (spectrum at each point) in less than a minute, one of the key success components to the new automated Alpha series of integrated microscopes discussed above.

Raman acquisition has also become fast enough to track processes and produce movies. **ChemImage** announced its W.H.I.P.™ (Widefield Hyperspectral Imaging Platform) as the

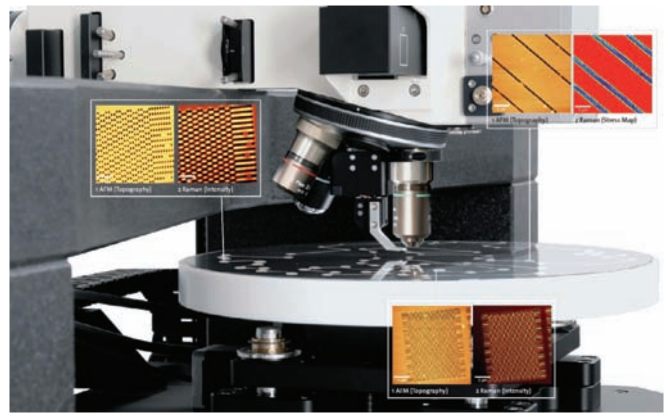

Figure 4 **WITec's** alpha500 integrates conventional light and atomic force microscopy for high-resolution imaging with confocal Raman for chemical fingerprinting, all on an automated platform designed for high-throughput experiments.

power behind both the Falcon II™ and Condor™ chemical imaging systems. "With our W.H.I.P. technology, dynamic processes can be studied to reveal information about structure/activity relationships that we've never seen before," stated David Tuschel, **ChemImage's** Director of Product and Applications development. Based on this technology, the company has entered into a CRADA (Cooperative Research and Development Agreement) with the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS). The focus of this joint effort will be to develop high-speed imaging technology for monitoring chemical and biological contaminants in food.

For both microscopists and spectroscopists, taking the step into the other's universe can be intimidating. Two companies, **Thermo Fisher Scientific** (Waltham, MA) and **Horiba-JY**, presented imaging spectroscopy systems that significantly lower that barrier.

Two of **Thermo's** groups showcased imaging systems with "Point and Measure" simplicity. FTIR (Fourier transform infrared) analysis has long been a staple for characterizing materials from solid drug delivery systems to flat-screen displays. According to Dr. Ian Jardine, Vice President of Global Research and Development, **Thermo Nicolet's** new iN10 FTIR microscope, in combination with its OMNIC Picta software, "closes the gap between the simplicity of FTIR spectroscopy and the complexity of infrared microscopy." The iN10 is highly automated and uses machine vision technology to facilitate sample load-

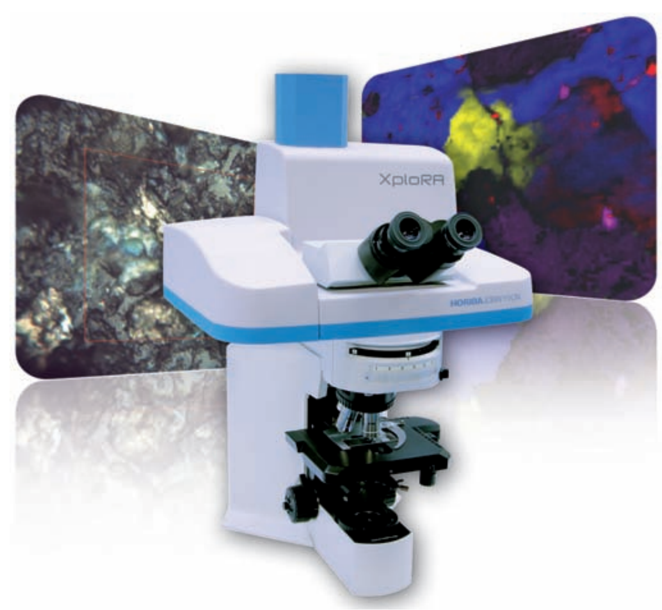

Figure 5 With a dramatically smaller profile and lower price, **Horiba-JY's** XPlorA propels confocal Raman into the mainstream for both microscopists and spectroscopists.

ing, locating, and measurement, while the OMNIC Picta software extracts comprehensive chemical and physical information from the data.

As with its FTIR system, **Thermo Fisher Scientific's** new DXR Raman microscope design is derived from more conventional Raman imaging systems, adding the magnification of a single objective to a Raman spectrometer and producing spatial resolution to 1 µm. Built specifically for nonspecialist users, the DXR incorporates fully integrated, prealigned components for fast and easy installation and configuration, as well as autocalibration to ensure reliable results. The result is an easy-to-use system that optimizes measurement as though there were an expert in the laboratory.

For researchers and scientists needing more extensive microscopy with their Raman, **Horiba-JY's** new XPlorA combines the best of both worlds. Taking advantage of new, smaller lasers, a reduced optical path for its spectrometer (200 mm compared to the typical 150 mm), and proprietary optical design, **Horiba** has miniaturized its technology to fit seamlessly on a research microscope (*Figure 5*). According to the company's technical staff, the performance is comparable to prior instruments. New marketing manager, Ishal Nir, promised that the technology would also put Raman into a much more affordable range, making it more available to a much wider audience. Further research confirmed that this new hybrid Raman/research microscope system will be available for under $100,000. For spectroscopists making this move, microscopy training is available from several sources, including **The McCrone Institute** (Chicago, IL) and **Microscopy/Microscopy Education** (McKinney, TX). For microscopists, **Horiba** provides intensive one-on-one training during installation as well as providing libraries of spectra at no cost. Both approaches should lower the barrier to entry for this exciting cross-over technology.

Chemistry doesn't have to be blind anymore!

Pittcon is normally known for its focus on spectroscopy and chromatography. However, as shown by the technologies reviewed in this article, microscopy and imaging have given eyes to the analyst and researcher. With lower costs, more approachable instrumentation, and the ability to add vision to chemistry, maybe the time has really come for microscopy to be part of the chemistry mainstream.

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