PITTCON® microscopy review

Barbara Foster

The world of imaging is changing, and this year's PITTCON® reflected many of these changes. A search for "microscope" or various types of microscopy on the wonderfully easy-to-use PITTCON reference CD reveals over 100 papers. Many entries combined FTIR or Raman spectroscopy with more conventional confocal or fluorescence microscopy. Atomic force microscopy, especially the new scanning electrochemical variation, also figured prominently. The resulting analyses combined these microscopies with conventional techniques, computational analysis, and new sample preparation to expand the investigative-imaging envelope.

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Two key trends dominated applications: biotechnology and the continued convergence of spectroscopy and microscopy in new approaches for everything from depth profiling of heterogeneous polymers to the molecular structure of membranes and DNA. Here are a few highlights from the papers and the trade show floor.

Presentations of interest

Biotechnology

One of the greatest challenges in genome mapping has been determining the sequence of the molecular snippets produced by chemical cleavage. Schwartz et al.[1] adhered the DNA to doped (derivatized) glass microscope slides, cleaved the molecules, and then used fluorescence microscopy to directly image individual DNA molecules. This process leaves the cleaved segments in place, separated by an easily identified gap, creating a "landmark map of the DNA sequence," according to Schwartz. Using Shotgun Optical Mapping, an extension of this concept, the group mapped whole genomes of several bacteria and parasites using DNA extracted directly from the cells.

Microscopy and imaging techniques also figured strongly in discussions for building[2,3] and analyzing microarrays.[4]

Biosensors

Cells were stained with a calcium-sensitive probe such as Fura-2. When an analyte is bound to a receptor in an organelle or the plasma cell membrane, free intracellular Ca^{++} ions are released. The analytes can be fractionated using capillary electrophoresis (CE), then identified based on retention time and selective interaction with the receptor.[5] The amount of analyte can be quantified by calculating the ratio between the intensity of fluorescence signals from bound versus unbound species, providing an accurate and sensitive biosensor for CE.

Conventional biology

Image Correlation Spectroscopy[6] (ICS) fuses confocal and spectral analysis of live cells and is a likely candidate for expansion to the new 2-photon technology, touted for minimizing photobleaching and phototoxicity. Along similar lines, fluorescence microscopy can be used to image freeze fractured cell surfaces, then combine with time-of-flight scanning ion microspectrometry (TOF-SIMS) to define the biology of cellular membranes at the molecular level.[7]

Polymer and materials analyses

In his presentation on the history of chemical measurement, Kurt Heinrich (NIST, Gaithersburg, MD) acknowledged the importance of integrating chemistry and imaging: "It became increasingly obvious that the properties of materials such as ceramics and alloys depended as much on their microscopic structure as on the overall chemical composition."[8] Apollo I extended its studies to minerals. Environmental and health studies followed shortly after. The laser Raman microprobe was expanded to chemical analyses.

Raman's ability to investigate both specimens containing annoying O–H or N–H functional groups as well as fluorescing specimens is driving its rapid convergence with many types of microscopy. Evidence came through papers citing the use of Raman with conventional polarized light to measure birefringence and crystallinity, as measured by X-ray diffraction[9] and the weathering of heavy petroleum products.[10] Combining Raman with confocal microscopy enables the laboratory to perform depth profiling for materials and biosamples[11] as well as heterogeneous polymers.[12]

Electrochemical imaging is a rapid area of growth in atomic force microscopy. Growing nanowires[13]

with 15 Å to µm diameters for interconnects or sensors is an interesting twist on this technique.

Report from the floor

New microscope line

The BX51 microscope, the flagship of the new **Olympus** (Melville, NY) line, has been designed from the ground up to provide increased application and flexibility. Its seven-position, revolving nosepiece is matched with a newly expanded, eight-position, universal condenser and a six-cube fluorescence turret for customized single- and multiband imaging. The new fluorescence illuminator offers improved optical efficiency, extending low-light-level fluorescence detection and doubling image brightness. For better differentiation of multiple labels and detail visibility, it accepts patented exciter balancers (**Olympus**) for continuously variable excitation bandwidth.

3-D and long working distance at one's fingertips

Hi-Scope (Haworth, NJ), one of the best-kept secrets in the industrial and dermatology arenas, expanded its line of handheld microscopes with new lens systems that offer high magnification (up to 2500× total magnification) coupled with 65-mm working distances. For those who need 3-D, the rotating lenses generate impressively crisp, true, real-time color images.

Comfortable alternative for light microscopy viewing

Known for decades in industrial circles for its spinning lenticular disk projection microscopes, **Vision Engineering** (**VE**) (New Milford, CT) announced ISIS, a revolutionary replacement for conventional eyepieces. VE began translating its core expertise to stereomicroscope systems several years ago, but ISIS is a major technology breakthrough. In an interesting experiment, the company's staff had conference attendees view a slide through normal eyepieces, then view the same specimen through ISIS. With its ultrahigh, 35-mm eyepoint, there was a noticeable difference in visual comfort and freedom of movement.

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New cameras

Camera technology is the fastest-changing domain within the world of microscopy. Three new systems were announced at PITTCON: the DMC-2 (**Polaroid,** Wayland, MA), SynCool 435 (**Syncroscopy ,** Cambridge, U.K.), and PI-Max (**Princeton Instr uments,** Princeton, NJ).

The DMC-2 debuted at a special breakfast. Faster and less expensive than its predecessor, the DMC has become a strong competitor in the digital microscope color camera race. It offers snap-on ease of installation, 11 frame-per-second preview mode, and new software for working with low-light-level fluorescence.

The SynCool 435 is a cooled three-chip camera that is ideal for applications requiring a quick change from brightfield to faint fluorescence. The cooling system significantly reduces noise, especially for the long integration times necessary for low-light-level situations.

No matter how good the camera and the microscopy, the image may still contain extreme areas of dark and bright. **Syncroscopy' s** Quantage offers a solution. The easy-to-use software goes an extra step to balance the intensity histogram, resulting in better contrast for the lost information without sacrificing scientific content (*Figure 1*).

PI-Max is a high-sensitivity, intensified camera that features fast gating and high quantum efficiency. The high-end system offers a choice of photocathodes tailored to specific applications, as well as a patented Programmable Timing Generator, which controls even complex gate sequences.

Other offerings

Digital Instruments (Santa Barbara, CA) is always pushing the atomic force industry to new heights. Its latest move is to offer new approaches for surface metrology coordinating scanning electron microscopy (SEM) and atomic force microscopy (AFM). This year's booth featured a Webcast featuring Dr. Phillip Russell (North Carolina University, Raleigh, NC) discussing the advantages and ease of integration of this new dual

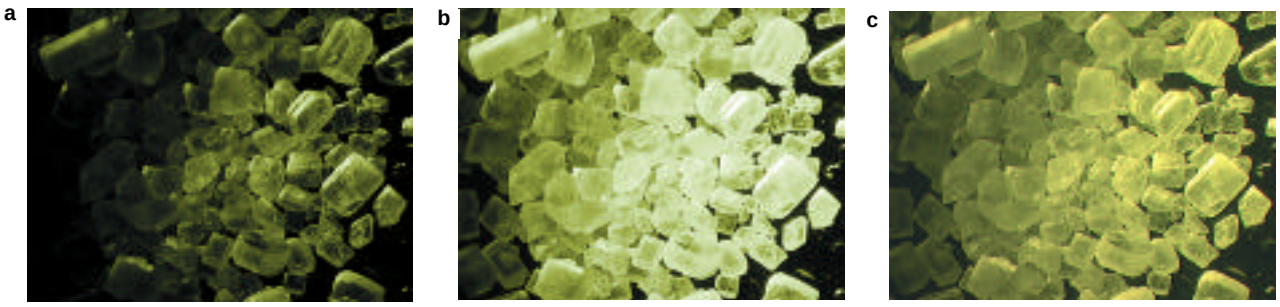

Figure 1 *Quantage software reveals information hidden in a) extremely dark or b) overly bright areas of an image. c) More bal - anced gray scale of a Quantage processed image. (Image courtesy of* ***Syncroscopy****).*

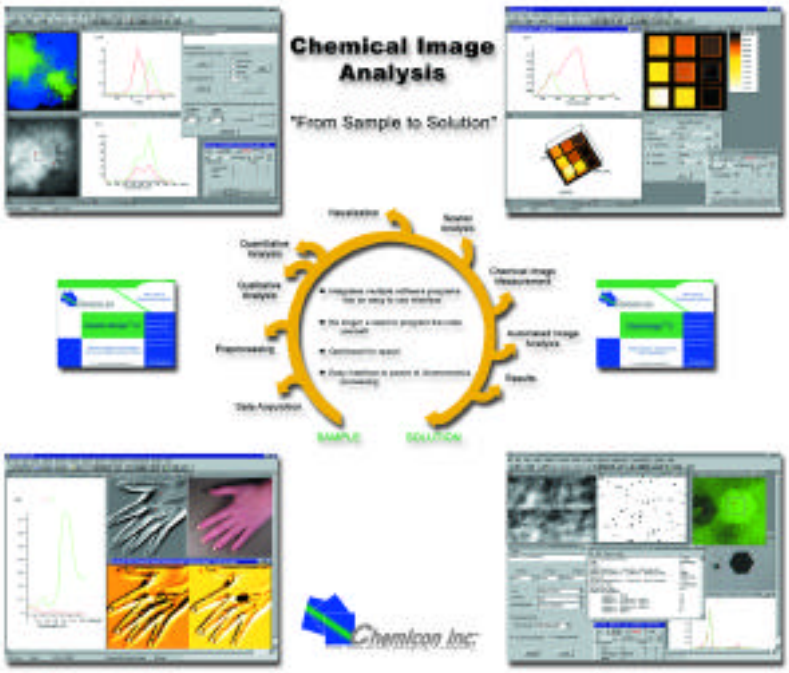

Figure 2 *ChemImage 5.0 software presents simultaneous visualization and manipulation of images and spectra. (Image courtesy of* ***ChemIcon,*** *Pittsburgh, PA.)*

technology (also available as a monograph).[14]

Princeton Gamma-Tec (Princeton, NJ) unveiled the latest software for its new SPIRIT microanalysis system. With SPIRIT, full multisignal data are acquired from an SEM during rapid, video rate scans. All data are collected simultaneously: two SEM signals, total X-ray signals for all elements, and multiple external signals. The result is full-frame X-ray mapping with dynamically controlled magnification and digital panning.

Proliferation of Raman/FTIR

It did not take many visits to Raman and FTIR manufacturers to see that spectrometers are starting to look more and more like conventional microscopes, with **Olympus** being the primary choice. Many of these systems offer spectroscopy combined with a full range of imaging techniques, from brightfield and darkfield to Nomarski/differential interference contrast (DIC) and fluorescence.

The **ThermoElectron Nicolet** family (Madison, WI) offers the most complete range of equipment in this area, from the integrated Continuum to the new Almega dispersive Raman. The Almega is built around a conventional **Olympus** BX stand, coupled to an extensive spectrometer. For larger-than-imaged sample areas, Mosaic™ image tiles individual images. A single interactive window presents views of the video image and spectrum as well as the chemical map and 3-D spectral image. Key competitor **Chromex** (Albany, NY) offers an even more simplified FTIR microscope-in-a-box.

Spectral Dimensions (Olney, MD) is the new kid on the block. However, the company is built on the long-standing experience of veterans such as Neil Lewis, and an expanded product line that includes reliable existing equipment such as InSight IR from industry leader **Spectra-Tech** (Shelton, CT). **Spectral Dimensions'** newly developed NIR (near infrared) Matrix IR system is especially good at separating components of a mixture, and is targeted primarily for pharmaceutical analyses.

SensIR (Danbury, CT) is expanding the applications of its novel TravelIR, the 26-lb mobile stereomicroscope with attenuated total reflectance (ATR) attachment. In one example, a very well-done video demonstrated the instrument at work at a toxic dump cleanup site.

New distribution/products

ChemIcon (Pittsburgh, PA) has formed a strategic alliance with **Bruker Optics** (Billerica, MA) to sell the **ChemIcon** FALCON Raman chemical imaging microscope. **ChemIcon** released two new products: ChemImage 5.0 and CONDOR, which are sold directly to end-users. ChemImage software permits simultaneous visualization and manipulation of images and spectra (*Figure 2*). According to company spokesperson Andrew Wood, "ChemImage reveals subtle features of a material often not observable using conventional Raman microscopy or alternative imaging techniques." CONDOR is a full macrochemical imaging system, configured with visible and NIR absorption as well as visible fluorescence and photoluminescence chemical imaging.

Conclusion

PITTCON has not traditionally been known as an imaging show, but as evidenced by this year's offerings, it provides a wide range of equipment and applications for those crossing the chasm between microscopy and spectroscopy.

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Ms. Foster is President, ***Microscopy / Marketing & Education,*** *125 Paridon St., Ste. 102, Springfield, MA 01118, U.S.A.; tel.: 413-746-6931; fax: 413-746-9311; e-mail: mme@map.com.*