Focus on microscopy: Convergence of technologies and companies drive new directions in microscopy

Barbara Foster

Polymers, biotechnology, and thermal analysis dominated the microscopy scene at the 1999 Pittsburgh Conference (Pittcon®). The good news for consumers is that manufacturers are responding to requests for better pricing by introducing lower-cost, entry-level instruments. Environmental scanning electron microscopes (ESEMs) and atomic force microscopes (AFMs) to Raman confocal microscopes are now available at prices that have been more traditional for midrange confocals (the $130–$175K range). Also, the bridge between more traditional analytical techniques and microscopy is stronger than ever: A number of new instruments coupled microscopy with various forms of spectroscopy, gravimetry, thermal, and electrochemical analyses. Quietly and subtly, microscopy is becoming part of the analytical routine as chemists are realizing the importance of being able to watch a process dynamically and microscopists are realizing the added value of these analytical techniques from the chemical laboratory.

Growth against a backdrop of mergers and acquisitions

Thermo Electron's (Waltham, MA) reach extended into microscopy last year, first with the acquisition of **Park Scientific**, then **Topometrix**. The new company, **ThermoMicroscopes** (Sunnyvale, CA), now balances **Park's** high end, academically driven research scanning probe microscopes (SPMs) derived from AutoProbe (**Biomeda Corp.**, Foster City, CA) with **Topo's** more pragmatic Explorer group (the new ExplorerPolymer system is discussed below). In another **Thermo** effort, **Noran** (Middleton, WI) recently acquired long-time business ally, **Kevex**. Together, the **ThermoSpectra** companies now encompass all nonlight microscopies ranging from AFMs to X-ray fluorescence (XRF), energy dispersive and wavelength dispersive spectroscopies (EDX and WDS), and confocal microscopy.

In related areas, **EDAX** (Mahwah, NJ) recently acquired **TexSEM** (Salt Lake City, UT), a market leader in electron backscatter diffraction systems for the characterization of polycrystalline materials. The integration of electron backscatter diffraction with X-ray microanalysis permits the simultaneous mapping of chemistry and crystallography. The combined technology has important applications in semiconductors, superconductors, primary metals (formability, strength, toughness, and corrosion resistance) and geology and mining (phase identification).

The analytical side of electron microscopy drove other acquisitions as well. Some time ago, **Philips** (Mahwah, NJ), a long-time front-runner in both scanning and transmission electron microscopy (SEM and TEM), acquired **ElectroScan** and its low-vapor-pressure, environmental EM capability. This past year found **Philips** lifted to new levels of column technology when it was acquired by **FEI** (Hillsboro, OR). Reflecting a trend for more turnkey systems, **R.J. Lee** acquired **Topcon** and, under the experienced leadership of **Topcon's** Mike McCarthy, has turned its personal SEM and EDS technology into applications-oriented systems targeted at specific markets such as gunshot residue (GSR) and metals characterization, especially wear debris and processing cleanliness.

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Polymers: the new target for expanding microscopy

Polymers are nothing new for atomic force industry leader **Digital Instruments** (**DI**, Santa Barbara, CA). **DI** has been aggressively tracking this market for the past several years, coming up with one new application after another. The backboard in its booth was proof of its success, providing a veritable education in polymer morphology ranging from the nanostructure of a commercial packaging film to polyester urethane block copolymer spin coated onto silicon wafer to the morphology of polyolefins and polyurethane and the lamellar structure of polyethylene (see *Figure 1*). Not to be outdone by other newly announced thermal analysis SPM competitors (see below), **Digital** also showed thermal phase transitions of mezomorphic polymers. Reflecting the movement of this technology into biomedical arenas, there were also panels of biological applications. In the midst of all the images was the new Dimension™ 3100 AFM, with top-down optics for easy viewing of both probe and sample, computer-controlled illumination for faster focusing and zooming to find the feature of interest, and video image capture.

As seen with many of the applications discussed below, polymers are also a prime target for the integration of microscopy, spectroscopy, and other analytical techniques.

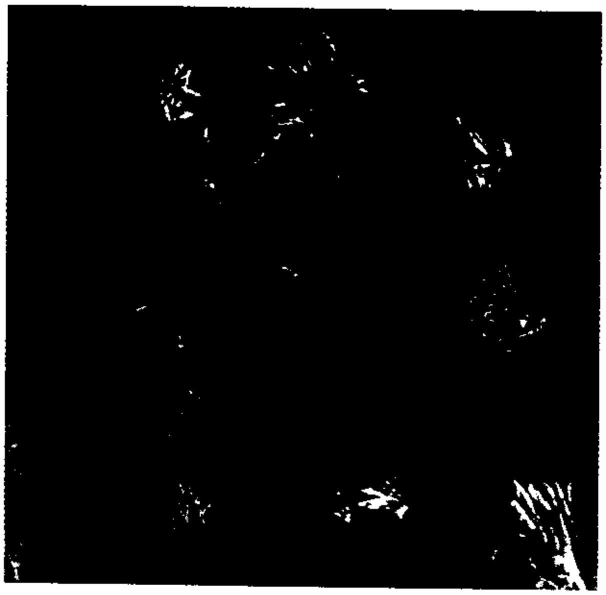

Figure 1 *Lamellar aggregated embedded in amorphous polymer (commercial tape of linear low density polyethylene [LLDPE]). Phase image, 5-µm scan.*

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b

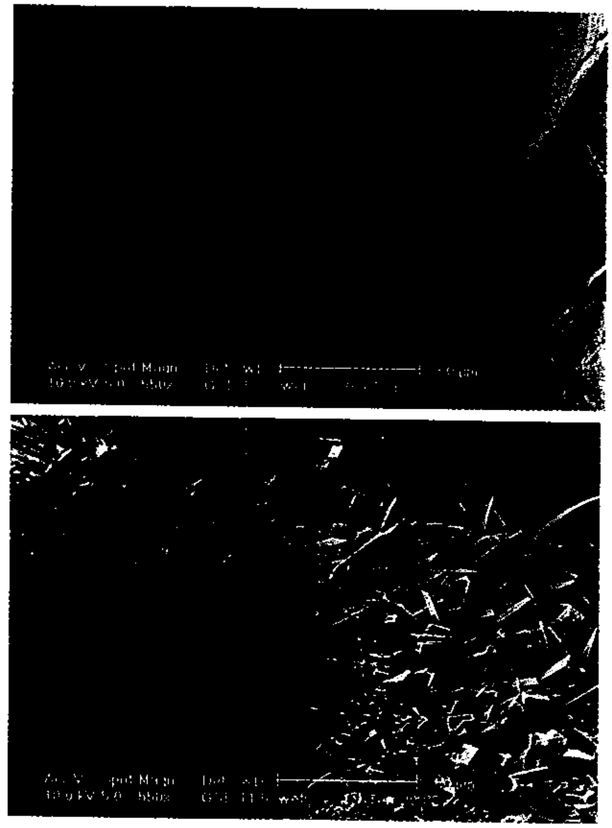

Figure 2 *Oxide growth around a small sulfur inclusion in a piece of iron at 860 °C in a pure (oxygen) atmosphere. (Images courtesy of* ***Philips.****)*

Thermal analysis converges with ESEM and SPM

Philips' new XL30 ESEM-W combines flexibility with low price. It offers a variety of imaging (secondary and/or backscattered) in a variety of modes (high vacuum, low vacuum, and ESEM) with a variety of guns (W, LaB6, and field emission). Its unique secondary electron detector works in low-vacuum mode with stated Kelvin specifications. Most importantly, the XL30 has a new hot stage that allows it to reach temperatures of 1500 °C. For the first time, researchers can conduct in situ, real-time dynamic imaging of high-temperature experiments run in inert oxidizing, and reducing gas environments (*Figure 2*). Together, the ESEM/hot stage combination provides dynamic characterization for materials and materials processes such as thin films, superconductors, ceramics, composites, and specialty metal alloys.

LEO (Thornwood, NY) also announced its Variable Pressure Secondary Electron (VPSE) detection system. The system is targeted at nonconducting, outgassing, or beam-sensitive specimens such as the polymer tube shown in *Figure 3*. For high throughput, the system can be switched from one operating mode (secondary electron, backscatter, VPSE, or EDX) to another without opening the chamber.

JEOL (Peabody, MA), a well-known leader in electron microscopy, announced a research level, multi-environment scanning probe microscope equipped with a true hot/cold stage (130K–800K/–143 °C to 1073 °C). The SPM pulls a vacuum of approx. 10^{-6} torr to prevent the formation of ice crystals, which would damage to the piezoelectric actuator. It also has self-contained antivibration technology with separate isolation systems for the turbo pump and the microscope stage. Although new in the U.S. this year, this SPM offers all modes of SPM imaging; has a proven and successful track record in Japan; and is one of the new midrange-priced instruments, even with the hot/cold stage and antivibration system.

The **ThermoMicroscopes** ExplorerPolymer combines three different technologies: imaging, microthermoanalysis, and pulsed force (AFM) microscopy for topographical, adhesion, and thickness information to measure real performance properties. Several interesting examples highlighting the booth included thermal and topographic images of light-emitting diodes (LEDs), a PVC/TB blend, the microdifferential thermal analysis of a multilayered packaging film, and the adhesion study shown in *Figure 4*.

Microscopy and spectroscopy: once strangers, now allies

It was a typical sociological phenomenon: attend any meeting that drew both spectroscopists and mi-

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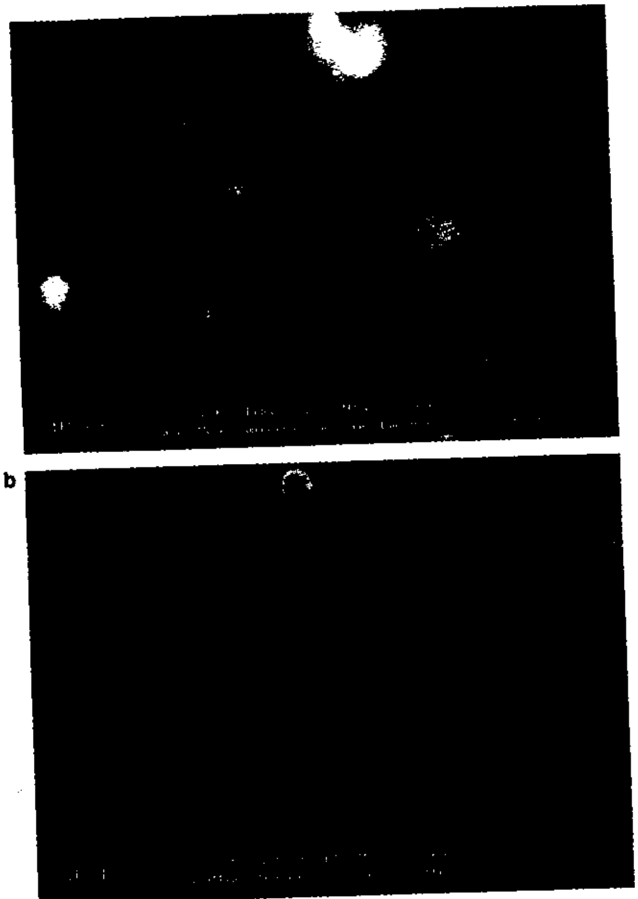

Figure 3 *Two comparative images of a paint surface at ultrahigh magnification (30,000×). a) Imaged with conventional backscattered electron detector. b) Improved image, acquired with LEO's new VPSE detector. pressure: 65 Pa (0.5 torr).*

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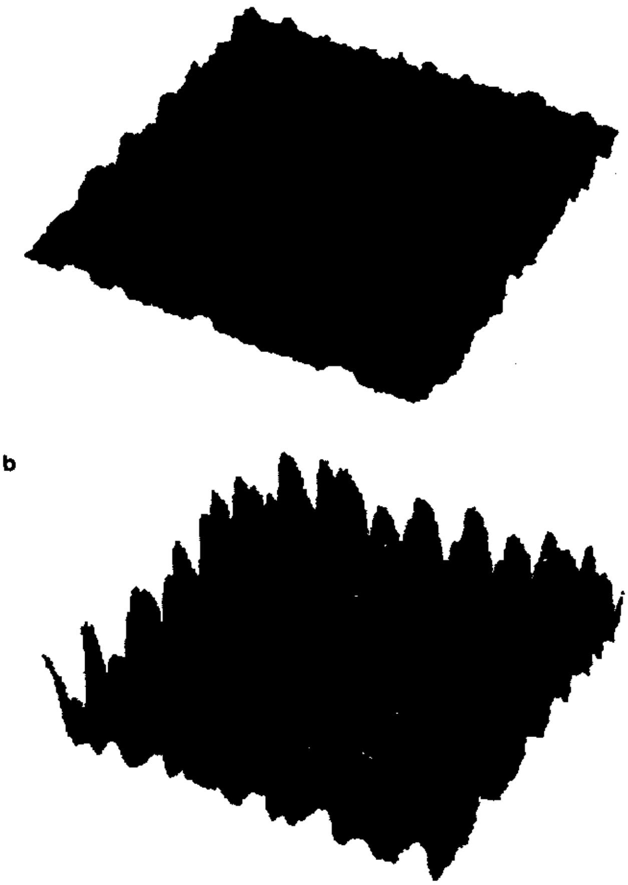

Figure 4 *Effects of temperature on adhesion of a PMMA blend as the polymer is heated above its glass transition state. a) Adhesive below Tg, b) adhesive above Tg. (Images courtesy of* ***ThermoMicroscopes.****)*

croscopists and they would segment themselves to separate sides of the room. No more. **SpectraTech's** (Shelton, CT) new Continuum will make both groups feel comfortable. It looks like a microscope, does differential interference contrast (DIC) like a microscope, but it also does FTIR for chemical analysis, in one, integrated system. Fully upgradeable from a basic microscope to a totally automated system with chemical imaging, the Continuum saves time and eliminates errors by providing continuous viewing of the sample even during IR data collection. Its new ReFlex™ automated aperture focuses on areas of interest and eliminates interference from the surrounding area.

SensIR (Danbury, CT) is a new name in the industry but is built on a long-standing foundation. Previously known as **ASI**, then **ASI Applied Systems, SensIR** will derive considerable benefit from FTIR veterans Don Sting and John Reffner. The company's mission statement is "Making FTIR Useful™," and its new DuraScope™ attenuated total reflection (ATR) system promises to fulfill that mission. DuraScope integrates ATR with a video microscope. ATR spectroscopy is a versatile and powerful technique that requires no sample preparation for most applications. A sensing window is brought into direct contact with the sample and the resulting spectrum produces both quantitative and qualitative IR analysis. Because of potential damage to the window, the technique has been limited to soft or chemically compatible materials. In other ATR systems, as the quality of the window degraded, the system lost its accuracy and the required time-consuming recalibration. **SensIR** has overcome both problems with a new diamond composite ATR front element, expanding application of this technology to intractable solids, a wider range of powders, and liquids. The addition of an imaging and video system completes the system and allows the user to watch the sample as spectra are collected.

Expanding UV and infrared analysis with Raman confocal

The *Spectroscopy of Polymers*[1] discusses both the importance of Raman spectroscopy and the reasons

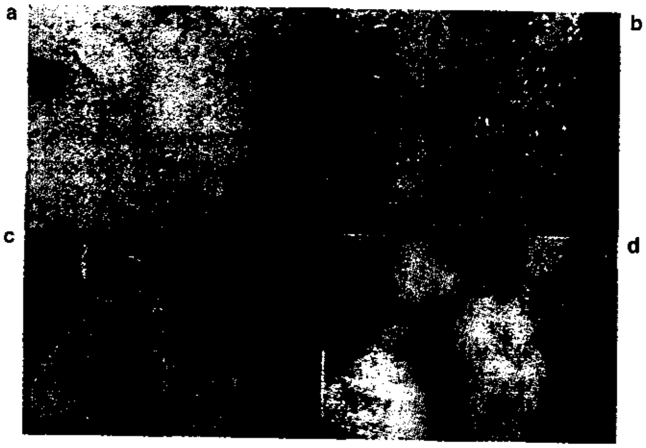

Figure 5 *One hundred percent spray-dried amorphous lactose during humidity ramping: a) 0% RH, amorphous structure; b) 50% RH, glassy structure; c) 60% RH, solution; and d) 90% RH, new crystal formation of α-lactose monohydrate. (Images courtesy of* **Surface Measurement Systems, Ltd.***)*

for its not being better known. As the author points out, Raman's high sensitivity for nonpolar chemical groups makes it useful for the analysis of chemical composition and structure. It is also powerful in analyzing solution behavior: Water absorbs strongly in the IR, making moisture a problem for more conventional IR analysis, but it is a weak Raman scatterer, making Raman an ideal tool for observing the conformational changes that occur on "dissolution or melting, or [when] undergoing transformation with changes in the pH, ionic strength, or salt content of [a] solution."

Renishaw (Shaumburg, IL) unveiled a new UV Raman confocal. Keeping to the trend of many integrated instruments shown at Pittcon, it is fully upgradeable, and the entry-level system has been priced comparably to a conventional confocal. Working with Neil Lewis and Ira Levin at the National Institutes of Health (NIH), **Renishaw** has improved the spectrometer component. The wavelength of the incoming light is now isolated by a liquid crystal tunable filter (LCTF), which can be used in either Raman or regular fluorescence imaging. With about one-third the bandwidth of a conventional filter, it can be tuned to specific wavelengths electronically rather than mechanically, offering both increased speed and accuracy of optical positioning. An impromptu interview with booth visitor Valentin Shelyaskov of **World Precision Laboratories** (Hong Kong) pointed out the importance of the new filter technology: His research looks at water in zeolytes, requiring the magnification of a compound microscope to identify the area of interest. Raman confocal provided the solution but also generated a secondary problem: Zeolytes tend to autofluoresce. The LCTF provided the solution by isolating the individual spectral peaks and avoiding the autofluorescence.

The winner for integration of laboratory automation with microscopy was mounted on this **Renishaw** instrument: a special stage with a 2.5-in. rotating disk from **Lab Connections** (Marlborough, MA). This system can spot aliquots from HPLC or GC in evenly spaced dots around the edges of the disk. The disk can then be put under the microscope for visual and Raman analysis. As the turntable rotates, the system takes Raman spectra at each point, and then compares results to the library of spectra for identification.

Two other Raman systems were on view at Pittcon. LabRam Infinity, a confocal Raman from **Instruments SA** (Edison, NJ), boasted new software. Designed specifically for nonspecialist users in quality control and analytical laboratories, the software automates analytical tasks and even changes lasers to make repetitive measurements more reproducible. **ChemiCon** (Pittsburgh, PA) introduced the Falcon Raman chemical imaging microscope. Falcon's Duet Vision Technology™ (DVT) provides simultaneous imaging and spectroscopy, and also features the high spectral and spatial resolution of LCTF technology. The integrated detector requires only one calibration procedure during system setup. **ChemiCon** recently

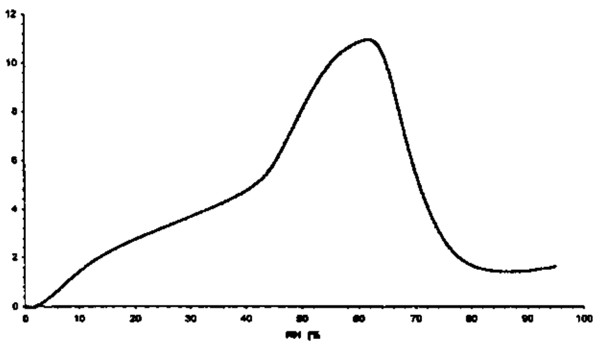

Figure 6 *Gravimetric plot showing transitions related to Figures 5a–d.*

received a $3 million NIST (Gaithersburg, MD) award through the Advanced Technology Program (ATP) for a joint venture with **II-VI Inc.** (Saxonburg, PA) to develop an instrument for automated inspection of structural defects in semiconductors, yet another step toward automated imaging and analysis.

Other analytical techniques turn to microscopy for a new view

Those analysts involved in gravimetric analysis will find the new DVS µScope from **Surface Measurement Systems Ltd.** (Coopersburg, PA) interesting. DVS (dynamic vapor sorption) measures moisture-induced changes in mass. In the DVS µScope, a video microscope is mounted directly below a flat-bottomed quartz sample pan and is interfaced to a microcomputer that controls the instrument via a digital frame grabber and collects both images and gravimetric information simultaneously (see *Figures 5* and *6*). The system can be run manually or automatically, via software.

For in situ electrochemical analysis, the instrument to try is the Scanning Electrochemical Microscope (SECM) from **CH Instruments** (Cordova, TN). By mapping both topography and electrochemical current, the SECM generates chemical reactivity images of surfaces as well as quantitative measurements of reaction rates. Its applications include corrosion studies, investigation of biological systems (e.g., determining the specific morphology and response of the oxygen generation site in photosynthesis), membranes, and liquid/liquid interfaces. Of special importance for polymer scientists, the SECM can image polymer films as they go from very dry to wet environments. Since it can be used to image and quantify the drug transport mechanism across skin, it also has impact in the biopharmaceutical arena.

EM and related technologies

EDAX showed the new Eagle II µ-Probe, a tabletop, micro-X-Ray fluorescence analyzer that uses sophisticated capillary optics to focus the beam to a 100-µm spot on the sample. It has a healthy 10-mm working distance, uses a charge coupled device (CCD) camera for imaging, and has a motorized stage for scanning across the sample. The high-performance version has a polycapillary lens option that produces an ultrahigh intensity X-ray beam that can be varied from 40 to 100 µm, and a higher precision XYZ stage, which takes advantage of the smaller X-ray spot size. Its spectrometer is also equipped with video magnification capabilities. Both systems use the Phoenix analyzer for digital signal processing. With eight processing time constants, it provides optimum detector resolution, even with X-ray counts as high as 100,000 cps.

PGT (Princeton, NJ) released the Avalon, a combined X-ray microanalysis and digital imaging system that provides an economical upgrade path from older EDS systems to current PC technology and X-ray pulse processing. Avalon can be interfaced to any manufacturer's EDS detector or any of the PGT Prism detectors.

Fischione Instruments (Export, PA) announced four new products at Pittcon: a high angular dark-field STEM, a new SteadyTilt™ specimen holder, a high-temperature specimen holder, and an in situ processing and vacuum transfer system. Both specimen holders are double-tilt devices. The SteadyTilt features a jeweled tilt mechanism that eliminates long-term wear, minimizes backlash, and has high angular repeatability. The high-temperature model tilts about two perpendicular axes and includes a furnace that can reach and maintain temperatures up to 1000 °C. The new detector was initially developed for use on **Philips** TEMs with active STEM coils. By coupling a single crystal scintillator to the photomultiplier tube, the system minimizes the number of optical junctions and increases quantum efficiency to the point that it can detect a single electron. The transfer system is also built for use with TEM. Samples are loaded into a high-temperature, single-tilt specimen holder on the laboratory bench. The system is then evacuated first via an integrated, high-vacuum pumping stack. At this point, the sample can be subjected to oxidation, reduction, or catalysis. Before insertion into the TEM, the stack valve is turned off and disconnected and an integral ion pump turned on. While still under vacuum, the system is attached to the goniometer stage of the TEM, the gate valve is opened, and the specimen is placed in the TEM.

The MTXL ultramicrotome from **RMC/Ventana** (Tucson, AZ) provides nanometer-scale ultrathin sections to micron range-thick sections for FTIR. It features four settable sectioning thicknesses, and an eight-way lighting system with a fully adjustable scan and tile stereomicroscope.

On the imaging front

The latest offering from the realm of digital cameras is the **Polaroid** (Cambridge, MA) DMC-le. A megapixel camera, it combines high resolution with true color (36-bit color depth) and a larger imaging area (12.15-mm chip compared to 11-mm/2–3 in. chip). The reconfigured software allows the user to adjust brightness, contrast, and image size as well as insertion of a micron bar and simple annotation. A dual SCSI-2 connector allows multiple digital cameras to be attached to a single computer. The DMC-le has automatic white balance and color correction, but also offers fine tuning via white, gray, or black "eyedroppers." For high resolution and storage flexibility, there are three different resolution settings: 1600 × 1200, 800 × 600, and 400 × 300 pixels. The image can be previewed in either color or black and white at frame rates up to 11 frames per second. For fast, easy focusing, the preview window can be zoomed in up to 4× normal size.

LECO (St. Joseph, MI) announced the new IA32 image analyzer. Built to accommodate users with varying levels of expertise, the analyzer is targeted at materials science applications with analyses ranging from coating thickness, porosity and nodularity, grain sizing, dendrite arm spacing, area fractions, retained austenite, and fiber lengths. There are applications wizards for customizing applications as well as the ability to construct macros for routine analyses.

Omega Filters (Brattleboro, VT) brought out a new family of filters, dubbed the Alpha Series. Using a new proprietary design and deposition technology (patent pending), the company has engineered filters with very steep shoulders and high throughput. The series has both long-pass (400–950 nm) and short-pass (400–1200 nm) filters and is recommended for demanding applications, including Raman and fluorescence. For those end users who have not enjoyed the luxury of fine-tuning their filter sets, there is also a new reprint available from **Omega** on how to choose filters, as well as the new 16-page *Fluorescence Filter Selection Guide for Microscopy* (www.omegafilters.com).

On a more industrial note

Lasentec® (Redmond, WA) demonstrated the PVM© continuous in-process imaging microscope, which can produce quality images in solutions containing up to 60% solids. With a resolution down to 5 μm, the PVM can collect five images/sec and save them to disk. It works with a complementary tool, Focused Beam Reflectance Measurement (FBRM©), to count particles and measure particle geometry (function of shape and dimension). Applications include particle or droplet growth, reduction or stability in polymerization, fluid beds, crystallization, and dispersion.

Microscopy in the palm of the hand

Cole-Parmer™ (Vernon Hills, IL) announced a clever digital microscopic photography system that can operate in two ways: as a handheld field microscope or attached to a conventional microscope via C mount. It uses a 2-in. liquid crystal display for direct viewing, has an optional computer interface kit, and can take 640 × 480 digital photographs at the touch of a button. It works on a rechargeable lithium-ion battery (1.5 hr of operation) and stores images on an editable microdisk that can hold up to 48 images and slips into an adapter to be read by a PC. The standard lens, 30×, is spring loaded and comes with an adapter so that the user can view widefield. Other available lenses range from 1× to 200×.

For the more industrial environment, **Hi-Scope Systems** (Closter, NJ), a pioneer in hand- or stand-held video microscopy, has added an attachment to its modular zoom 3-D rotational microscope. The attachment clips onto an existing **Hi-Scope** microscope and continuously varies the viewing angle from 25° to 55° to allow the user to look under edges, around corners, and into holes. The system integrates the company's patented optics with built-in lighting and the latest in video technology (a 900,000-pixel CCD camera with on-board digital signal processing) to produce high-resolution color images that are perfect for inspection, process control, failure analysis, or image analysis. The live, crisp, three-dimensional images can be viewed on a video monitor or computer screen, or can be easily digitized for analysis transmission or documentation. The new angled viewing head extends the magnification range to 600× (5× continues as the low power), with exceptional depth of field. Applications include inspection of electronic circuit boards, fabrics and fibers, small medical parts and devices, tubing, and powders.

Reference

1. Koenig JL. Spectroscopy of polymers. Washington, DC: ACS, 1992.

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Call for papers

PITTCON 2000 will be held in New Orleans, LA, March 12–17, 2000, focusing on "Science for the 21st Century." The deadline for receipt of abstracts (250 words) is July 28, 1999. The emphasis will be on a balanced program showcasing cutting-edge technologies, recent developments in instrument design, analytical applications, and methodologies. For more information phone 412-825-3220, fax 412-825-3224, or e-mail program@pittcon.org.