Focus on Microscopy

Faster, Smaller, More Economical Imaging Tools at SPIE 2007: The Effect of Nanotech

by Barbara Foster

Nanotechnology is having a big effect on imaging equipment. In semiconductor parlance, it is driving a strong "lab to fab" transition: Equipment once found only in the research and development laboratory is now finding its way into production. While the more sophisticated laboratory versions retain their role for high precision and applicability to multiple applications, the production versions are becoming faster, smaller, sleeker, and more economical.

This trend was highly visible at the recent International Society for Optical Engineering (SPIE) meeting, held August 26–30, 2007, in San Diego, CA. SPIE is an interdisciplinary society dedicated to the science and applications of light, and this particular meeting focused on new ways of investigating nanotechnology. This article highlights some of the new technologies on display during the SPIE exhibit that embody this trend.

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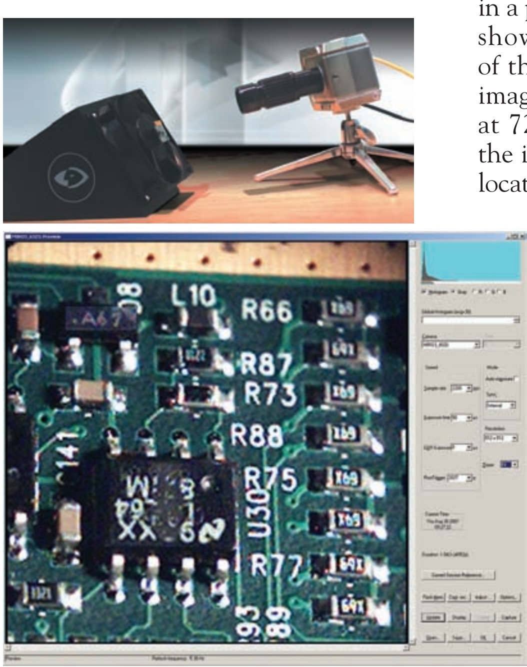

Figure 1 The Miro camera provides long working distances at high magnification coupled with fast action. a) Camera setup illustrating long working distance; b) image of parts taken at that distance, rotating at 7200 rpm. (Image courtesy of ***Vision Research****.)*

Cameras lead the way, from 60 to 2000 fps

Collecting low-light-level images at high speed has long been a challenge for camera manufacturers. Historically, shutters on the cameras have been left open to collect images from dim samples over a period of time and integrate the resulting data. By its very nature, however, this process is slow, and longer integration times also result in the collection of more stray light and electronic noise. Two companies exhibiting at SPIE have solved this historical challenge.

Figure 1a illustrates an uncomplicated setup for a Miro camera (**Vision Research**, Wayne NJ) as it might work in a production environment. Figure1*b* shows the highly magnified image of the electronic circuit board being imaged. The circuit board is rotating at 7200 revolutions per minute, yet the image is clear. Also, the camera is located at a much greater distance than would historically be expected for this level of magnification. These cameras can also operate in dim lighting, despite their fast acquisition times.

The new FocusScope SV200-i (**Photron**, San Diego, CA) features the ability to acquire rapidly, even at low light levels. It mates the newest in complementary metal oxide semiconductor (CMOS) imaging sensors with a Generation III image intensifier to produce a camera capable of collecting 512 × 512 pixel images up to 2000 frames per second, even under low-light-level conditions. The intensifier works well over the full visual spectrum from 280 to 720 nm, and provides a 20,000-fold light boost. Fast image collection is only part of the story, however. The second half is the challenge of transferring a quarter of a megabyte of data that is being collected 2000 times per second. **Photron's** solution is 2.6 GB of onboard recording memory, enabling a recording time of 8.2 sec for 1000 fps.

New ways to image surface texture, profiles, and roughness

In the crowded and surging field of atomic force microscopy (AFM), **Veeco** (Woodbury, NY) is still the recognized leader. Built initially on the 1998 acquisition of **Digital Instruments**, **Veeco** later expanded its SPM group to include technologies from **Park Scientific** and **Topometrix** through the subsequent acquisition of **ThermoMicroscope**. **Veeco** retained all three engineering groups and, until recently, three engineering philosophies. At SPIE, **Veeco** applications scientist Dr. Thomas Müller pointed out that the company has now combined these three groups. He cited the new Innova SPM as the first instrument to emerge from newly combined efforts. This SPM was specifically designed to be more affordable and easier to use, while still offering a full range of AFM and STM (scanning tunneling microscopy) techniques including magnetic force, electrostatic, conductive, thermal, voltage imaging, and nanolithography.

AFMs operate much like a phonograph needle, hovering above or hopping across the surface being imaged. Clearly, electronic noise is a disruptive factor. Until recently, many SPM companies vacillated between two engineering approaches: open-loop (nonfeedback) systems, which provide lower noise thresholds, or closed-loop systems, which provide greater control of the AFM probe. The Innova features a high-

Figure 2 Mini Ram, a portable Raman system, will soon be available as an accessory for light microscopes. (Image courtesy of **B&W Tek***.)*

resolution, closed-loop scan that provides one-step zoom without distortion and exceptionally low noise. As an added feature, the closed-loop function can be deactivated dynamically, even during a scan. It has also been designed to provide fast and easy tip and sample exchange and is fitted with top-down optics with electronic zoom to locate tip position with respect to the sample. Its new SPM-lab software allows data from partially acquired images to be analyzed on the fly at any time during the acquisition process without interrupting the scan.

Scanning white light interferometry (SWLI) has long been a staple for optical profiling, and was well-represented on the SPIE show floor by the two giants in the industry (**Veeco**, selling the Wyko line, and **Zygo**, Middlefield, CT) as well as by more recent entry **4D Technologies** (Tucson, AZ). **Veeco** has automated its NT9100 to form the new NT9800, a major step toward the production line. An internal laser reference, unique to the company, provides self-calibration and compensates for system drift due to environmental or mechanical instabilities. Automated measurement sequences (recipes) are also part of the software, as well as datalogging and pass–fail criteria for real-time process feedback and statistical process control (SPC). The key application for the NT9100 is imaging microelectromechanical devices (MEMs) and dynamic MEMs.

The **PhaseView** (San Diego, CA) system takes a slightly different approach to optical profiling and represents the new category of simpler, less expensive instruments. Comprising a series of projection lenses built into a special adapter, it takes two or more images at different focal planes. Proprietary algorithms then convert the differences in intensity between the planes into phase information. The resulting data can be used to create 3-D images, 2-D profiles, step height, waviness parameters, and a wide variety of roughness characteristics including Rz, Rq, Rsk, Rku, Rv, and Rt. Like the SWLIs, it can be used to characterize and image MEMS, the topography of electric circuits, and fracture versus smooth metal or ceramic finishes. Because the images are collected rapidly, the system is less sensitive to vibration. PhaseView is available in a standalone version as well as a module that can be added to an existing light microscope via the conventional C-mount camera port.

The microscopy/spectroscopy convergence continues

Integrating AFM with Raman spectroscopy has been a major trend over the past several years. **B&W Tek** (Newark, DE) promises to extend this chemical fingerprinting capability to light microscopy (LM) in the near future, using its elegant, portable MiniRam system (*Figure 2*). To achieve this small footprint and economic pricing, the company designed its own low-cost diode lasers, thermoelectric cooling, and sensitive CCD sensor. As shown in Figure 2, there is a slim, onboard computer as well as USB communication to a laptop or desktop. The result: a lightweight, portable spectrometer with a spectral range from 175 to 3100 cm^{-1}, at less than one-tenth the cost of a typical laboratory instrument. While large laboratory spectrometers typically have spectral resolution on the order of 0.1 cm^{-1}, the MiniRam's spectral resolution is 5 cm^{-1}, very reasonable for less demanding, routine analyses. A slightly larger desktop version of this system has already been retrofitted to a confocal microscope, and **B&W** is investigating a similar configuration for the MiniRam.

LM gets a nanoboost in thin film measurement and imaging nanotubes

Whenever technology moves into a new realm, someone announces that light microscopy is dead, and the move to nanotechnology was no different. **Nano-Lane** (distributed in the U.S. through **Micro Photonics**, Irvine, CA) has announced a new substrate to take LM into this next technology revolution. Sounding like something from a Dr. Seuss story, their Sarfus Surfs are contrast-enhancing supports onto which samples are deposited. Precise control of the deposited layers on the Surf substrate produces unique control of the reflection properties of polarized light impinging on the Surf. The result is increased axial sensitivity by a factor of 100.

Figure 3 illustrates the ability of a Surf to enhance contrast and resolution. Figure 3*a* shows the image of double-walled car-

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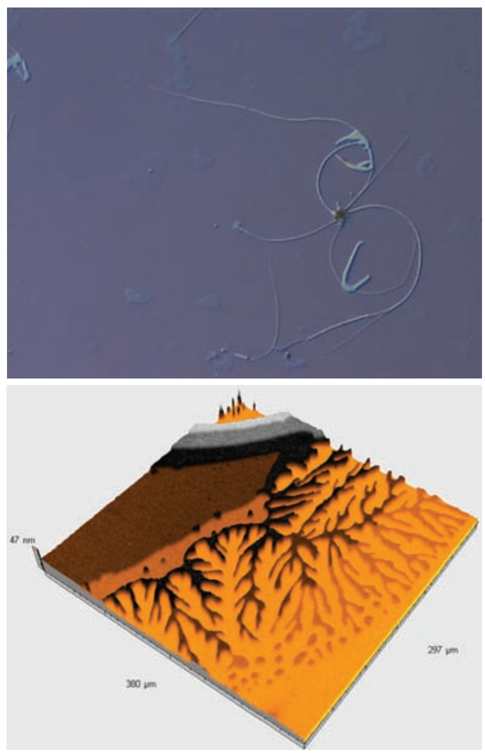

Figure 3 Surf substrates extend light microscopy's imaging in X, Y, and Z. a) Double-walled carbon nanotubes (50 nm diam). b) When the liquid crystal 4-n-octyl-4-cyanobiphenyl (8CB) is deposited on a Surf at its smectic/nematic transitions temperature, the drop spontaneously spreads to form steps of regular heights on the order of tens of nanometers. (Image courtesy of **Micro Photonics***.)*

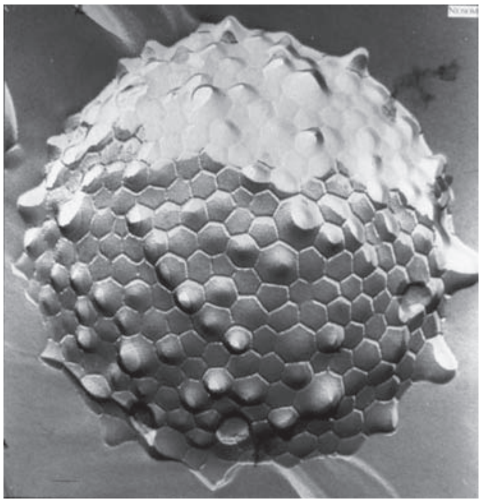

Figure 4 The geodetic structure of a niosome, revealed via freeze-fracture electron microscopy, has a strong impact on surfactant properties. (Image courtesy of Dr. Brigitte Papahadjopoulos-Sternberg.)

bon nanotubes (DWCNT) created using a light microscope fitted with conventional DIC (differential interference contrast) optics. The normal limit of resolution for a light microscope, even under ideal conditions, is approx. 250 nm, but the 50-nm-diam CNTs are clearly visible. In Figure 3*b*, a thin liquid crystal film was imaged on the Surf using a similar DIC setup. The normal depth of field of a light microscope is on the order of 500 nm, yet using DIC and Sarfus calibration standard and software, the system is capable of measuring film thicknesses on the order of tens of nanometers.

Surfs are available in a standard configuration similar to a regular light microscope slide surface, with hydrophobic surfaces, or with customized top layers with varying metals, oxides, fluorides, polymers, or organic substrates. There is also a Locator Surf consisting of a printed 10 × 10 mm grid consisting of a framework of 3 × 3 arrays of 100-µm cells nested within a 20 × 20 array of 400-µm cells, each of which can be addressed via binary notation. Providing precise X-Y positioning of nano-objects, the grid can be used for conventional light microscopy as well as AFM, Raman, X-ray photoelectron spectroscopy (XPS), and selected ion monitoring (SIM) experiments, and is especially important for correlative experiments in which the sample needs to be moved from one instrument to another. The **Nano-Lane** Web site (www.nano-lane.com) provides a wealth of applications.

New services help laboratories access the microworld and nanoworld

As shown in *Figure 4*, freeze-fracture electron microscopy is an excellent tool for accessing the internal structure of biological and material entities. Its use, however, is limited in many laboratories because of the cost of instrumentation and high level of expertise required. Dr. Brigitte Papahadjopoulos-Sternberg has solved that problem. Her new firm, **Nano Analytical Laboratory** (San Francisco, CA), will conduct freeze-fracture electron microscopy from sample preparation to a detailed report, including annotated electron micrographs, in less than three weeks.

Using this technique, Dr. Papahadjopoulos-Sternberg has produced excellent results in characterizing the morphology of drug carriers such as liposomes, niosomes,[1] and cochleate cylinders. She has also been able to image novel structures formed by liposome/DNA complexes used as nonviral vectors for gene delivery and for accessing the hydrophobic interior of biological as well as artificial membranes. During discussions at SPIE, she also pointed out that it is the method of choice for analyzing super-structure transformations of bilayers.

Conclusion

SPIE was a refreshing glimpse of what's next on the microscopy and imaging horizon. Although the Society is driven by scientists involved in fundamental research in light, the practical results are driving down costs and simplifying instrumentation, making imaging tools available to a wider audience.

Reference

1. Sternberg,B.; Moody, M.F.; Yoshioka, T.; Florence, A.T. Geodetic surfactant structure. *Nature* **1995**, 378, 21.

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