# Microscopy and Spectroscopy Open New Vistas in the Emerging World of Nanotechnology: Part 2

by Barbara Foster

Microscopy and spectroscopy will both provide valuable avenues to imaging and analyzing the nanoworld, as described in Part 1 of this article in the November 2003 issue. However, this new realm is also demanding new approaches to sample preparation and instrumentation as well as fundamental changes in the analytical process itself.

## *New approaches to sample preparation*

Science in the U.S. has tended to be highly compartmentalized: Biologists study cells, chemists study reactions, and physicists study fundamental processes. Biotechnology and drug discovery have started to reverse that trend, and nanotechnology has picked up the banner, demanding more interdisciplinary thought and experimental approaches.

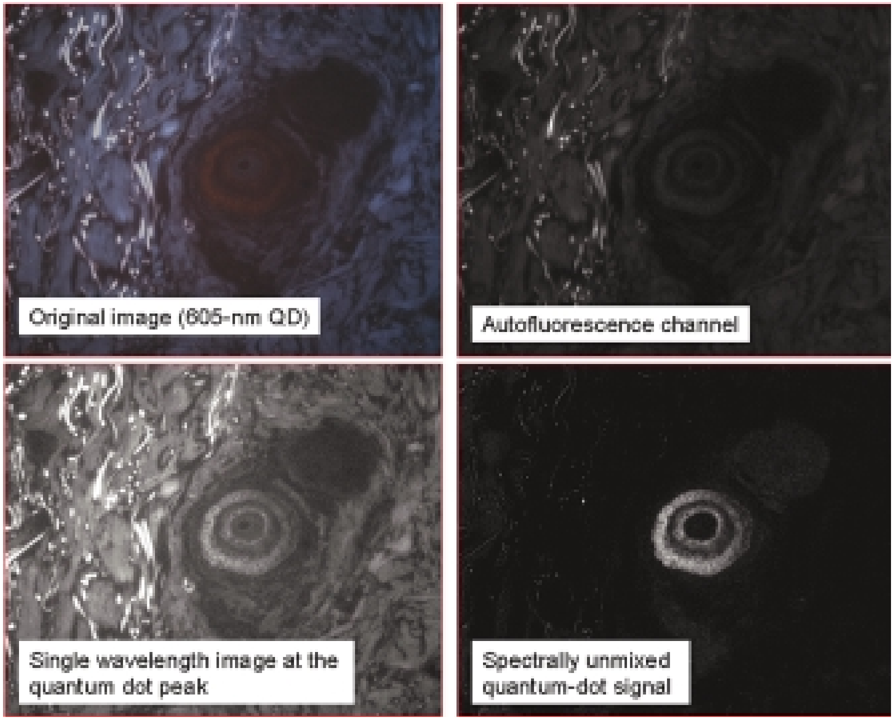


*Figure 1* *Quantum dots—new semiconductor nanocrystals that produce multiple fluorescence emissions from single fluorescence excitations in an extremely photosensitive yet stable fluorescence tag. (Image courtesy of CRI, Inc., Woburn, MA; sample courtesy of Dr. Thomas Grogan.)*

Some of the revolution is taking place at the sampling step. For example, TSI (Amherst, MA) has developed nanometer aerosol samplers for the size selection and sampling of airborne nanoparticles, while Zymark (Hopkinton, MA) has begun to use microelectromechanical (MEMs)-based microflow meters to control nanodispensing for fluids in drug discovery experiments.

Other revolutions are taking place in sample preparation. Nanoprobes (Yaphank, NY) has long been recognized for its ability to tag proteins, liposomes, and DNA with gold. Clemente (Madison, CT) has engineered 35-nm magnetic particles tagged with the widely used fluorochrome, fluoro isothiocyanate (FITC), for intercellular tracking and locating intracellular compartments and vesicles.

Quantum dots (Quantum Dot Corp. [QDC], Hayward, CA) are one of the newest and most exciting entries in the sample preparation arena. Like conventional organic probes and dyes, these semiconductor nanocrystals bind to intracellular structures. However, they are extremely photosensitive and stable, promising to revolutionize the world of fluorescence microscopy. An added advantage is that one excitation wavelength produces multiple fluorescence responses. The top right image in *Figure 1* displays unwanted autofluorescence in a tissue. The top and bottom left images show two quantum dot (QD) images: the top left at the relatively dim 605-nm QD signal, and the bottom left at a much brighter 640-nm peak. Even when using a narrow-band filter centered precisely on this QD emission, autofluorescence still obscures critical information. A viable alternative is using a liquid crystal tunable filter to isolate the fluorescence at a series of wavelengths, then feeding the information into a computer program to mathematically unmix the spectra. As seen in the lower right, the autofluorescence can be isolated and removed, leaving the clear, clean QD signal.

Quantum dots are also affecting the business front by creating a number of strategic alliances, particularly with companies involved in spectral discrimination. Omega Filters (Brattleboro, VT) is working with QDC to develop new filter sets to fit conventional microscopes, and Optical Insights (Sante Fe, NM) is customizing its MicroImager for simultaneous two- or four-channel QD detection.

Veterans in the field are answering the need for unique sample preparation as well. Mr. Ted Pella, President of Ted Pella, Inc. (Redding, CA), reports that nanotechnology is having a "revitalizing effect on electron microscopy" as well as expanding the market for atomic force microscope (AFM) accessories. Dr. Charles Garber, President of Structure Probe, Inc. (West Chester, PA), recounts new demands for structureless, featureless surfaces, driving more microscopists to use osmium coaters and SiN supports for their electron microscopy samples. Also, as new facilities gear up for nanotechnology, new laboratories are opening, fueling a substantial increase in overall business. Mr. John Arnott, President of Ladd Inc. (Williston, VT), has seen a rise in ultrasmall, custom apertures for electron microscopy and satellite xenon ion propulsion systems.

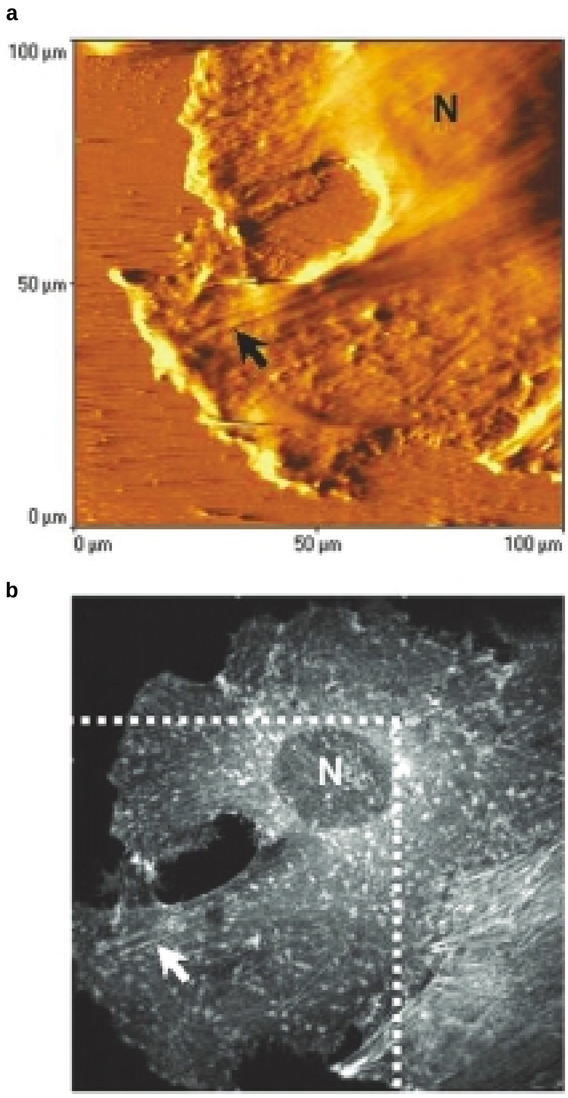


*Figure 2 a) AFM image taken of illustration shown by arrow in (b) confocal image. (Image courtesy of Bio-Rad Cell Sciences Div., Hemel Hempstead, U.K.)*

## *Microscopes within microscopes and hyphenated technologies*

According to Mr. Alain Deibold of Sematech (Austin, TX), synergy is the key to success in this new world. The nanoworld demands interdisciplinary thinking and correlative microscopy.

What microscopies can be combined? According to Dr. Andrew Dixon of Bio-Rad Laboratories (Hercules, CA), sometimes it's just a matter of "let's try it and see what happens."[1] Application note #35 describes a confocal–AFM experiment (see *Figure 2*). The confocal component of the system was used to find a specific area of interest. The AFM was then used to zero in on tiny structures (microtubules, in this case, on the order of 100 nm) that could not be imaged by the first technique.

Many of the atomic force companies have integrated AFM with optical microscopy. Novascan (Ames, IA) touts true Koehler illumination in both transmitted and reflected light modes with simultaneous AFM use. Still other instruments are microscopes within a microscope. In an elegantly simple application, Accurion (Menlo Park, CA) offers a special AFM objective to retrofit existing light microscopes. Kleindeik Nanotechnik (Reutinglen, Germany) produces an AFM that fits within a scanning electron microscope (SEM), opening the door to rapid conventional SEM scanning across the surface of a material, then 3-D AFM examination of a feature or region of interest.

Some instruments are true hybrids, frequently the result of joint ventures. New Raman confocal–NSOMs (near-field scanning optical microscopies) are now available through the partnership between Renishaw (Hoffman Estates, IL) and Nanonics (Jerusalem, IL). The integrated light microscope focuses laser light onto the sample and collects the scattered light, a Raman spectrometer acquires the chemical fingerprint, and an AFM maps the high-resolution topography and improves the Raman's spatial resolution.[2] Symphotic TII (Camarillo, CA), in a collaboration between Tokyo Instrument, Inc. (Tokyo, Japan) and NT-MDT (Moscow, Russia), offers a similar integration of light, SPM, Raman, and confocal microscopy.

## *High throughput*

One of the most exciting branches of nanotechnology centers on combinatorial chemistry. This technique has been used extensively since the early 1990s in biotechnology and drug discovery to generate the high-density arrays of tests on a microscope slide or similar substrate known as biochips or labs-on-a-chip. Interestingly, this approach to high-throughput research and testing is now returning to the chemistry laboratory.

High-throughput analysis involves three key steps: printing the array, imaging the array, and analyzing the data. NanoInk (Chicago, IL) has used scanning probe microscopy as the foundation for a new printing technology called dip pen nanolithography (DPN). The "ink" can be DNA, electrostatic materials, or any specific-binding ligand (*Figure 3a*). The "pen" is a specially modified scanning probe microscope, and the "paper" can be any material or substrate to which the ink can bind. Dot size, spacing, and chemistry are all controllable parameters. Figure 3*b* shows a combinatorial pattern of 16-mercaptohexadecanoic acid (MHA) on gold used for screening template properties for a particle assembly process.[3] The spacing on this particular microarray ranges from 50 to 700 nm. After a passivation process, a solution of polystyrene particles was placed on the patterned substrate, allowed to sit for 1 hr, and then rinsed with distilled water. Microarrays can also use fluorescence as the indicator and derivatives of confocal microscopes as the reader, or gold nanoparticles as the indicator and optical microscopes to detect the image. In this particular nanoexperiment, the surface was scanned with an AFM, revealing that single polystyrene particles bound selectively to the 300-nm spaced dots. Because it reveals information about precise positioning of individual building blocks, this experiment is an important precursor to organizing nanostructures into functional materials.

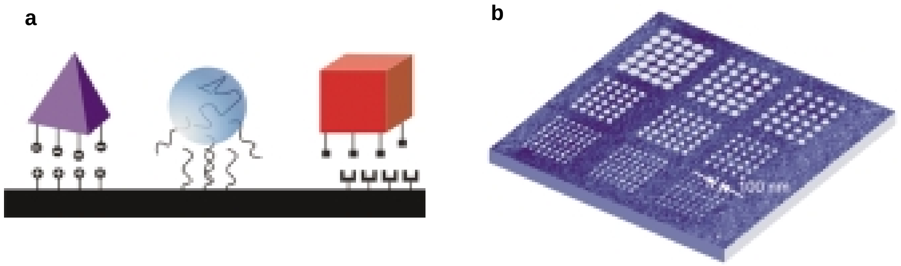


*Figure 3 Combinatorial chemistry offers researchers the opportunity to perform hundreds or thousands of chemical tests in tandem. a) Attachment strategies (left to right): charge-based recognition, macromolecular encoding (i.e., DNA), and specific binding of ligand. b) Combinatorial microarray (MHA on gold) used for determining how individual building blocks position themselves as a precursor to a particle assembly experiment. (Image courtesy of NanoInk.)*

## Valuable resources

A number of key meetings in the microscopy and spectroscopy arena have focus sessions on nanotechnology, including Microscopy & Microanalysis (the annual joint meeting of the Microscopy Society of America and Microbeam Analytical Society), the spring and fall meetings of the Materials Research Society (MRS), and SPIE (International Society for Optical Engineers). Meeting descriptions and links are available at www.MicroscopyEducation.com. NNI (National Nanotechnology Initiative) has an extremely informative Web site (www.nano.gov) that includes experiments for interested hobbyists and children.

## What's next?

Dr. Andrew Whitely of Jobin-Yvon (Edison, NJ) skillfully summarized the scope and potential of this brave new world: "Useful applications for nanotechnology exist in areas as diverse as materials, manufacturing, biomedical, electronics, computing, and transportation. Nanostructured metals and ceramics, for example, could be made into exact shapes without machining. Abrasives, coatings, paints, and composites all could be made stronger and better using nanoparticles. Integrated nanosensors can enable massive amounts of data to be acquired, processed, and shared with minimal size, weight, and power consumption. Less expensive remote and in vivo devices will provide new routes for drug delivery. More durable, rejection-resistant artificial organs can be created. And in automobiles, nanotechnology can lead to wear-resistant tires, improved battery technology, and lightweight composites for increasing fuel efficiency."

Mr. Mike Thompson of FEI (Hillsboro, OR) provided the most astute observation of the new interface between microscopy and the nanoworld. He noted that, in four decades of space exploration, only eight people have walked on the moon and that, as a nation, the U.S. has spent billions on the exploration of outer space. On the opposite side of the spectrum, quietly and without much fanfare, every day tens of thousands of people investigate inner space using microscopy, and, now, related spectroscopy. Clearly, as we move from the microworld into the nanoworld, these techniques will be leading the way into this new frontier.

## References


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3. *Templating. NanoInk appl note.*


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