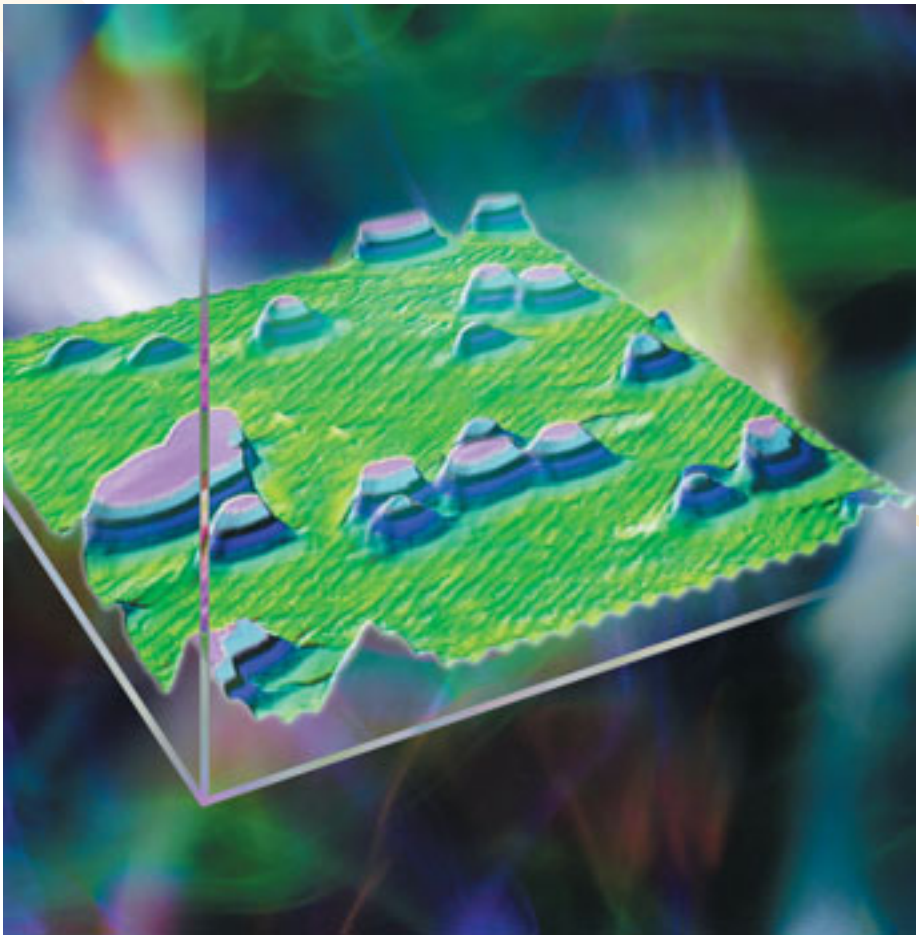
Shedding light on NSOM

Years ago, NSOM had a slow start. Now, scientists are taking full advantage of its technological edge over other scanning probe techniques.

Cheryl M. Harris



It took patience and hard work. Now, finally, researchers are seeing some sunshine break through what was once a cloudy beginning for near-field scanning optical microscopy (NSOM). Paul Barbara of the University of Texas–Austin (UT–Austin) is among a group of analytical chemists who have a deep respect for this tool and see it as a technique ripe with potential, ranging from chemistry to physics.

NSOM, or SNOM, is steadily finding an important place in analytical chemistry, and company representatives are enthusiastic about its future in nanotechnology. Experts describe it as a bridge between atomic force microscopy (AFM) and optical microscopy. "The great thing about NSOM is that it gives you topographic information online with optics. This is something people could never do in the past," says Aaron Lewis of Nanonics, who in the mid-1980s led a group of researchers at Cornell University that published the first papers on the applications of NSOM.

Lewis still recalls a grant administrator at British Petroleum who in 1982 wrote him a letter that all but dismissed NSOM when the technique was still in its infancy. "The research you describe certainly meets the 'fundamental' criterion," wrote the administrator, "but I have had some difficulty in imagining an outcome of sufficient magnitude to justify an involvement." With that, the administrator told Lewis's group that the research didn't qualify for funding. "Many people in those days were not even taught that near-field optics could, in fact, be anything," recalls Lewis, who now teaches applied physics at Hebrew University in Jerusalem. "Now with all this nanotechnology revolution, people are looking deeper and deeper into how you look at light, how you concentrate light, how you analyze light, [and] how you manipulate light in very small domains."

Researchers and company representatives recall that during the early to mid-1990s, when NSOM instruments started appearing in laboratories, the technique presented many challenges

to scientists, and some instruments didn't work at all, creating a downturn in the market. Although NSOM is still viewed as an academic rather than industrial technique, experts say its ability to obtain optical resolutions of <50 nm, in addition to providing topographical information, presents exciting possibilities in the biological field, materials sciences, and telecommunications.

"NSOM [is] the only technique for directly mapping optical and spectroscopic properties below the diffraction limit of light," says Barbara. "But it is a challenging technique, which, in general, is more difficult to achieve at the same level of investment [in] time and training than the more routine techniques like AFM."

Operating an NSOM takes patience and lots of training, add researchers. Just speaking loudly can make an NSOM tip crash into a surface. "Controlling that tip is the greatest challenge we face," says Barbara. "Remember, NSOM is all 'nano'. There's nothing in NSOM that isn't a … nanotechnology problem."

Today, researchers and company representatives admit that compared with AFM, NSOM isn't as popular. An NSOM system can cost $100,000–$250,000, depending on options, and can operate in conditions ranging from ambient to ultrahigh vacuum and liquid helium temperatures. NSOM tips can cost about $100 each, say company representatives. An AFM instrument, on the other hand, can be purchased for under $100,000, and tips can be bought for as little as a few dollars. Still, add researchers, the investment in NSOM is well worth it. And, as for as the NSOM market, says Klaus Weishaupt of WITec, "it's coming back."

In this Product Review, *Analytical Chemistry* examines the NSOM market and its future. Table 1 lists some examples of selected features of commercial machines. Interested readers should contact companies for further information.

The NSOM market

NSOM allows one to work with optics beyond the diffraction limit that normally restricts the resolution of conventional microscopy. By passing light through an aperture that's smaller than its wavelength, NSOM can obtain spectroscopic measurements of chemical properties with nanometer lateral resolution.

Defining the limits of far-field imaging dates back to the early 1870s, when E. Abbe came up with a criterion to resolve two objects in a light microscope. Using $d > \lambda/(2\sin\theta)$—in which d is the distance between two objects, λ represents the wavelength of the incident light, and 2θ describes the angle through which the light is collected—Abbe had calculated that ~200 nm was the best resolution that could be achieved with optical light. But in the late 1920s, E. H. Synge introduced the idea of having a small aperture to image a surface with subwavelength resolution using optical light. Three decades later, in 1956, mathematician J. A. O'Keefe proposed the concept of NSOM without knowing about Synge's earlier papers. In 1972, E. A. Ash and G. Nicholls demonstrated Synge's concept, and in 1984, Lewis's group and a team led by D. W. Pohl at IBM in Germany published the first papers on near-field imaging in the optical regime. By the mid-1990s, TopoMetrix, now owned by Veeco Metrology Group, was selling the first commercial NSOM systems.

Although NSOM has its limitations compared with AFM, which is more widely used in imaging live cells and analyzing samples immersed in liquids, NSOM's applications in correlating spatial resolution and topography are still impressive, say researchers. Lewis adds that NSOM systems on the market can analyze samples immersed in liquids depending on the types of probes used. Barbara says that NSOM can measure properties of a thin film that's 50 or 100 nm thick by looking at the interface between the thin film and what's underneath, not what's between the tip and the thin film. "The easiest thing to study is the thing on top, [and] there are a thousand techniques for doing AFM," he says. "The hard part is to look at the properties of that thin film or, even harder, to look at the interface underneath that you can't touch. That's called the 'embedded' interface, and that's what NSOM is good for [studying]."

Stefan Kämmer of Veeco says that NSOM is mostly used in materials science, such as polymer films, crystalline films, single molecules, and nanoparticles. David Adams of Columbia University, who is also a former postdoc from Barbara's research group, uses NSOM for single-molecule spectroscopy and to study thin films of nanostructured materials. "It's the forefront of experimentation. That's why we're doing it."

Other researchers are pushing NSOM as a viable technique in analyzing biological samples, something in which AFM already has a head start. Imaging cells with NSOM continues to be a challenge for two reasons, says Barbara. "One is that you need a sensitive distance regulation mechanism for the tip in the sample that works underwater," he says. "And the other [reason] is it needs to be effective and responsive enough that it can keep the tip from crashing into the cell, because if the tip really pushes too hard on the cell, it will puncture the cell."

Nevertheless, some researchers are determined to see just how far NSOM can go. Robert Dunn's group at the University of Kansas, for example, has analyzed the structure and dynamics of membranes and membrane-bound protein channels with NSOM. Richard Saykally's team at the University of California–Berkeley is investigating the use of NSOM for samples immersed in liquids, and at UT–Austin, research under Barbara and, independently, under David Vanden Bout, explores the electronic and optical properties of organic thin films using NSOM. Vanden Bout's group is

Although **NSOM** has its **limitations** compared **with AFM,** NSOM's **applications** in **correlating** spatial **resolution** and topography **are** impressive.

Table 1. Selected NSOM instruments.[1]

Product	AlphaSNOM	NSOM/SPM 100, 2000 and 2000 LT Systems
Company	WITec GmbH Hoervelsinger Weg 6 89081 Ulm Germany +49 731 140 70 0 www.witec.de	Nanonics Imaging, Ltd. Manhat Technology Park, Malcha Jerusalem, Israel 91487 866-220-6828 www.nanonics.co.il or www.nanonicsimaging.com
Price (U.S.D.)	~210,000	~100,000–250,000
Modes	Transmission, fluorescence, collection (optional: reflection)	Reflection, transmission, collection, fluorescence, Scattering NSOM and Shadow NSOM modes[2] (optional: AFM and STM modes)
Feedback operation	Optical beam deflection (also for AFM) with automatic approach	Optical beam-bounce or non-optical
Probes	Microfabricated cantilever sensors	Optical fibers with or without cantilevers or microfabricated cantilevers; nanopipets or dual-wire, thermal-couple pipettes
Aperture size (nm)	~80	50 (aperture probes); 10–300 (nanoparticle probes); 20–250 (Shadow NSOM probes)
Optical resolution (nm)	<100 (25 demonstrated)	≥50
Optical beam delivery	Fiber coupling with single-mode fiber between laser and microscope	Coupling between laser and microscope with single or multimode fibers; illumination through lens of an upright or inverted microscope for nonaperture-based modes of operation; dual-channel illumination with the lens; cantilevered fiber probe tip exposed to the optical axis
Scanner Scan range ($x \times y \times z$ µm)	100 × 100 × 20 (optional 200 × 200 × 20)	100 × 100 × 100
Scanning method	Scan platform with integrated capacitive sensors and closed-loop hardware linearization	Piezo-based tip and/or sample scanning with closed-loop option
Position accuracy (nm)	<2 in x–y; <0.5 in z	<1 in x–y; <0.3 z
Sample positioning (mm)	20 × 20 travel on x–y translation stage	>6 with scanning piezo elements (no x–y rough-stage needed)
Sample size range	120 mm lateral (maximum), 25 mm height (larger sample size optional)	>10 cm lateral (maximum), >10 cm height
Detector	PMT, APD and spectrometer (optional)	PMT, APD, InGaAs APD, CCD, and CCD/spectrometer
Special features	Combined instrument (SNOM, AFM, confocal microscope) with unique microfabricated cantilever SNOM sensors, supports all standard AFM modes	Free optical access from top and bottom of the scan head; full turnkey systems provided; modular solutions available with user's optical microscopes and/or SPM controllers; fits on any optical microscope upright or inverted with or without confocal attachments; z range = 90 µm for integration with optical sectioning techniques; scanner thickness <7 mm; full environmental enclosures for controlled atmosphere, including vacuum operation and gas and liquid chemical delivery with cantilevered nanopipets and optical illumination; imaging of deep trenches >400 µm and side walls

SPM: scanning probe microscopy
[1]Some companies offer multiple instruments. Contact the vendors for their full product lines.
[2]Scattering and Shadow NSOM: incorporates on-line AFM, Raman spectroscopy, and photoluminescence.

also researching fluorescence lifetime and polarization imaging with NSOM.

Weishaupt estimates that about 95% of NSOM instruments go to universities or national laboratories. Barbara and Adams note that university researchers tend to buy commercial NSOMs and then modify them. Most of the systems bought by industry are used to examine computer data storage systems, such as whether optical rather than magnetic data storage can be implemented in computer hard drives, says Weishaupt. IBM, for example, has shown an interest in NSOM, he says. NSOM systems can also analyze conjugated polymers found in light-emitting flat panel displays for computers and phones, adds Barbara.

Putting NSOM in its place

NSOM systems fall into two categories: aperture NSOM, which incorporates an optical fiber tip with a small opening at the end to illuminate the sample; and apertureless NSOM, which uses, for example, a solid metal probe instead, such as the one found in AFMs. "To go from an AFM to an apertureless NSOM is to, literally, merely put an AFM on top of an optical microscope and add some detectors," says Barbara.

When it comes to aperture and apertureless NSOM, says Barbara, "there are advantages to both; otherwise, both wouldn't exist." With the aperture technique, light irradiates a specific part of the sample to reveal the topography. The resolution range for aperture NSOM is ~30–100 nm, which is controlled by the size of the smallest aperture one can effectively make, says Barbara. He adds that his group typically collects 10 × 10 μm images. With apertureless NSOM, however, a larger area of the sample is illuminated, he says.

Experts say that in principle, the apertureless method offers higher spatial resolution, which could be <30 nm under favorable conditions. Lewis adds that designing an apertureless NSOM system is a complicated process, and questions still remain about how exactly it works.

Another probing method for NSOM has a cantilever with a hole in it for the light source or a long, tapered optical fiber cantilevered at the end, which is also called a "bent tip" probe. Adams says tips and cantilevers as NSOM probes are still experimental and that one method isn't better than the other. Most researchers using NSOM still don't fully understand why aperture and apertureless methods work the way they do, explains Barbara. "We've always taken the very [analytical] chemist perspective," he says. "What do you want to learn? What kinds of materials do you want to look at? Then, let's talk about how NSOM can solve that problem for you or not." For organic or thin-film analysis, the aperture approach has many advantages, but for thinner film work, such as studying monolayers, the apertureless technique has some clear advantages, adds Barbara.

Tips on NSOM

Most aperture NSOM systems use an optical fiber covered by an opaque material, usually a metal, to probe the sample. Everything is covered but the small aperture at the tip, where the light is emitted.

NSOMs typically use photomultiplier tubes (PMTs) and avalanche photodiode detectors (APDs) to collect light. Picking a detector depends on the intensity of the detected signal one wants, says Margit Walter of Omicron. For example, she says, a PMT is good for most standard applications in reflection and transmission modes, but if one is interested in the light emission of weakly fluorescent or luminescent samples, such as single molecules, then an APD is the best choice. Barbara adds that APDs are also more sensitive and less noisy. Other options include CCDs for spectroscopy.

Most NSOM optical fibers are clad in aluminum because the metal's "skin depth" in the visible region is the smallest of all metals, says Kämmer. This means the aluminum coating allows the smallest amount of light to pass through. Researchers have also experimented with silver and gold, but the choice is mostly determined by the wavelength of light used in the experiment, say experts. Aluminum does form an oxide, so fibers are coated quickly under a vacuum, adds Kämmer. It's important to cover the fiber quickly; otherwise, huge aluminum oxide grains can form and decrease the reflectivity of the coating, which can ruin resolution.

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The NSOM approach

To map a sample, NSOM tips need to be brought close to or in contact with a surface with force feedback. The common types of force feedback in NSOM are the shear-force and standard normal-force feedback used in AFM—contact and tapping modes. In contact mode, the nanoscopic tip comes into contact with the sample's surface; in tapping mode, the tip is attached to a cantilever and is vibrated above the surface, hitting the surface at the end of each oscillation. Shear force occurs when the tip is moved horizontally above the sample surface and experiences a constant force.

A cantilever turns out to be a good approach for contact and tapping modes, and using a tuning fork works well for shear force, says Barbara. Of the three techniques, contact and tapping modes tend to be destructive to an aperture-type probe, he says. "[But] that doesn't mean someone can't try to achieve it, and certainly there are people who do," adds Barbara. Shear-force mode, in Barbara's opinion, is the most protective for an aperture probe and the best method to analyze biological samples. "But you really have to work on that tuning fork and tip arrangement to get it just right."

NSOM has been used with many imaging techniques, including transmission, reflection, collection, fluorescence, polarization, and time-resolved spectroscopies. Experts say that transmission mode is mostly used in NSOM. Kämmer says that this mode allows researchers to easily image transparent samples. "I would say that fluorescence transmission mode is the most popular mode in [NSOM] today."

What to look for

Position accuracy is also very important in NSOM, say experts. There's a difference between "coarse" positioning of the sample and "fine" positioning of the scanner, says Walter.

The range of the coarse positioning gives one the maximum area of the sample studied with an NSOM system without preparing it in a different way, and coarse positioning is needed to place the tip into focus of the collection optics, she explains. This means the sample area one

Table 1. Selected NSOM instruments (continued).[1]

Product	TwinSNOM	Aurora-3 NSOM
Company	Omicron Associates 12100 Singletree La., Ste. 199 Eden Prairie, MN 55344 952-746-1316 www.omicron.de	Veeco Instruments 112 Robin Hill Rd. Santa Barbara, CA 93117 800-873-9750 www.veeco.com
Price (U.S.D.)	~130,000–170,000	~130,000–260,000
Modes	Transmission, reflection, collection, fluorescence, polarization, spectroscopy (other modes available upon request)	Transmission, reflection (optional: fluorescence, polarization, collection, CCD spectroscopy)
Feedback operation	Shear-force	Noncontact lateral shear-force
Probes	Straight; pulled; microfabricated fiber optic tips	Straight; pulled optical fiber probes
Aperture size (nm)	~50	50–80
Optical resolution (nm)	~50	50–80 (30–40 possible)
Optical beam delivery	Laser light coupled in and out with single mode and/or multimode optical fibers using standard fiber connectors or splices; laser light focused to the sample using lenses and/or light paths of microscopes (upright and inverted); open architecture allows easy access to the space around the sample for additional optics	Fiber coupling with single-mode fiber and standard industrial connectors between laser and microscope
Scanner **Scan range ($x \times y \times z$ µm)** **Scanning method**	$100 \times 100 \times 20$ In situ linearized and calibrated scanner	$30 \times 30 \times 5$ Scanned platform with integrated closed-loop hardware linearization
Position accuracy (nm)	<1 in x–y; <0.1 in z	<0.3 in x–y; <1 in z
Sample positioning (mm)	30×30 travel on x–y translation stage	3×3 travel on x–y translation stage
Sample size range	>15 mm lateral (maximum)	Transmission: 0.15 mm (thick) × 25 mm × 25 mm for coverslips and 1 mm (thick) × 25 mm × 75 mm for slides; reflection: x, y dimensions same as transmission, but solid substrates <1.0 mm allowed
Detector	PMT (more sensitive or different wavelength detectors available)	Dual-color CCD; PMT, multi-alkali (optional: APD and CCD for spectroscopy)
Special features	Conventional microscopy in reflection and transmission; optional laser scanning confocal microscope system with full and professional capabilities; optional needle-sensor AFM modular design, allowing easy upgrades and customized solutions; coarse-positioning readout for accurate repositioning of the sample available; may be customized	AFM compatibility; optional packages for nanolithography and optical CCD spectroscopy; open optical path and modular design for upgrade or integration with existing optical components; open software option (Visual Basic); NSOM probes available for high-resolution, high-throughout, UV, or premounted tuning forks for customer-supplied optical fibers

[1]Some companies offer multiple instruments. Contact the vendors for their full product lines.

wants to study should be smaller than the system's coarse range. For coarse positioning, the required accuracy may depend on the sample or on whether one needs to position the NSOM tip using a microscope, says Walter. The minimum steps to do this are usually in the range of 100 nm, she adds. If one wants to position the sample to the exact area again later, position accuracy and the ability to reposition become very important, says Walter.

For fine positioning, she adds, in which a linearized and calibrated scanner is most suitable, the scan must be stable and the accuracy has to be better than a tenth of the expected resolution. If a scanner is not linearized, says Walter, hysteresis may occur. "The scan may look very strange." Walter also recommends that prospective buyers look for an instrument that is mechanically stable and "flexible"—with the ability to add on options, which can be very important for academic researchers.

Kämmer advises that users make sure the instrument's "noise floor", or uncontrolled movement of the tip, is <1 nm. "You want to avoid uncontrolled movement of your tip as much as possible."

Barbara reminds prospective buyers that it's fruitless to look for a single NSOM approach. Instead, buyers should find researchers and companies that have lots of experiences with their types of applications. The wrong thing to do, in Barbara's opinion, is to see NSOM as a technique "in search of an application." Barbara says that first-time users should initially repeat other research work and then elaborate upon that. "I have noticed … that a lot of people who have started with NSOM have come in with maybe being a little overconfident in their ability to take a new technique like NSOM and quickly get it working in their lab," he says. "Don't try to work with your salesperson to design a completely different experiment that's never been done before and try that one first."

The NSOM future

Experts say the final NSOM frontier lies in building better tips. Unlike AFM tips, NSOM tips have a high spring constant, which means that forces generated during the force feedback to map the sample are enough to destroy living cells. Such problems have led researchers to make tips with comparable spring constant for biological applications. "If you're looking for great advances in NSOM, the future lies in nanofabrication strategies for NSOM tips tailored to particular applications, and that includes making tips themselves biologically active and biologically sensitive," says Barbara.

As NSOM's popularity grows, Barbara warns that it's a mistake to try to determine which way of doing NSOM is the best. "This has been one of the things that I think held the field back a little bit," he says. "Some people, who are more the instrument developers than the chemists who actually use it, would like to drive the paradigm of the field by which is the right way to do NSOM. What if the answer is [that] there is no single right way?" Meanwhile, researchers continue to push NSOM's potential. For example, Barbara's group is working on "multi-functional imaging" with NSOM and is attempting to image many chemically and physically relevant parameters simultaneously.

Kämmer and other company representatives believe it's only a matter of time until industry will fully realize the power of NSOM. "Industry simply has to use it more in the future, because nanotechnology is not some esoteric research subject," says Kämmer. "I mean, it is something that's going to affect industry [and] manufacturing processes. And how do you want to characterize products that you make that have properties that change on the nanoscale if you don't have the tools to do that?"

Cheryl M. Harris is an associate editor of Analytical Chemistry.