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Focus on Microscopy: Spectroscopy + Microscopy + ...? What's Next?

by Barbara Foster

Thomas Friedman, author of the recent book, *The World is Flat*,[1] identified convergence of technologies as a key driving force shaping today's world. At one extreme, he cites the globalization resulting from Google, ebay, and e-mail; on the other, the latest generation of copy machine that scans an image, saves to the computer, prints, faxes, and e-mails. The result: "A whole new degree of freedom to the way we work, especially work of an intellectual nature." Although his observations center on forces driving macro events on the global scale, his observations apply equally well to the analytical laboratory and, particularly, to profound changes happening in microscopy.

Analytical chemists saw this trend emerge in the late 1970s with the integration of gas chromatography and mass spectroscopy, forming the GC-MS, now the GC-LC-MS-ICP-. . . *ad infinitum*. Several similar microscopy amalgamations have been reported in this column,[2–5] but the NTegra Spectra from **NT-MDT** (Zelenograd, Russia, distributed by **NanoTech America,** Allen, TX) (*Figure 1*) provides a glimpse of the next big trend.

The system, which debuted at the American Chemical Society Annual meeting,[6] offers researchers seven fully integrated modalities. At the core are light microscopy, fluorescence, and confocal laser scanning microscopy (CLSM). On the nanolevel, it incorporates nearfield scanning optical microscopy (NSOM) and a full range of atomic force/scanning probe (AFM/SPM) microscopies, and finally, from the world of chemistry, dual-channel fluorescence-Raman spectroscopy. When fitted with nanosilver-coated AFM tips, it can image with nanometer resolution, then provide the tip-enhanced Raman spectrum (TERS) of a single molecule.

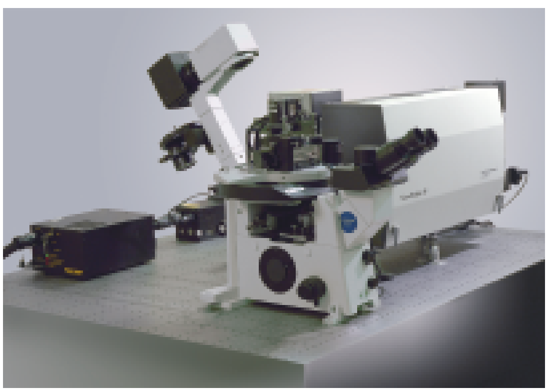

Figure 1 NTegra Spectra.

Who needs this power?

Microscopy/Marketing & Education (Allen, TX) recently asked a select group of microscopists whether this technology provided enough of a solution that they would consider purchasing one for their laboratories. Of the over 205 respondents, 18% indicated interest in an integrated system of this level.

What do they perceive this technology will offer them that others did not? Beyond correlative microscopies, they are looking for complementary chemical information important to studies as diverse as proteins and nanocomposites. For proteins, they see it as a potential tool to study aggregate structure and to characterize membrane proteins in 2-D matrices and in situ protein interactions. For the pharmaceutical industry, they perceive it as a way to identify behavior in a single cell or identify unknown impurities in pharmaceutical products. In the nanoworld, it is seen as a way to image and chemically characterize nanotubes, surface phases, and magnetic domains.

Integrated software and automation

As a long-time manufacturer of advanced instrumentation, **NT-MDT** understands the importance of simplicity. Although the NTegra Spectra is powerful, the system is easy to use. Windows-based NOVA software (**NT-MDT**) forms the universal platform to control the entire system, from image acquisition to archiving, image processing, and measurement to reporting.

The software also controls most of the system settings. The user can adjust the pinhole diameter for the laser scanning confocal, insert or remove polarization optics, select AFM parameters, or choose the single or combination lasers for Raman and/or fluorescence spectroscopy, all at the click of a mouse. As shown in Figure 1, the system is sleek and unencumbered.

Enhanced optical and AFM stability

A triangular AFM base is the hallmark of the NTegra line. This shape, coupled with titanium construction, produces a uniquely stable platform that is nonmagnetic and matched in thermal expansion to the piezo, which drives the AFM tip. For additional stability, which is especially important for lengthy experiments, the objective for optical microscopy is mounted directly into the AFM base. The result is a system that is mechanically solid, with unusually low noise, even for small area scans.

Optimizing weak Raman signals

Raman scattering is derived from very weak vibrational processes. A simple thought experiment puts Raman into context. The world around us is filled with plants that we see as green because of the light reflected from chlorophyll in the leaves. However, mixed in with that

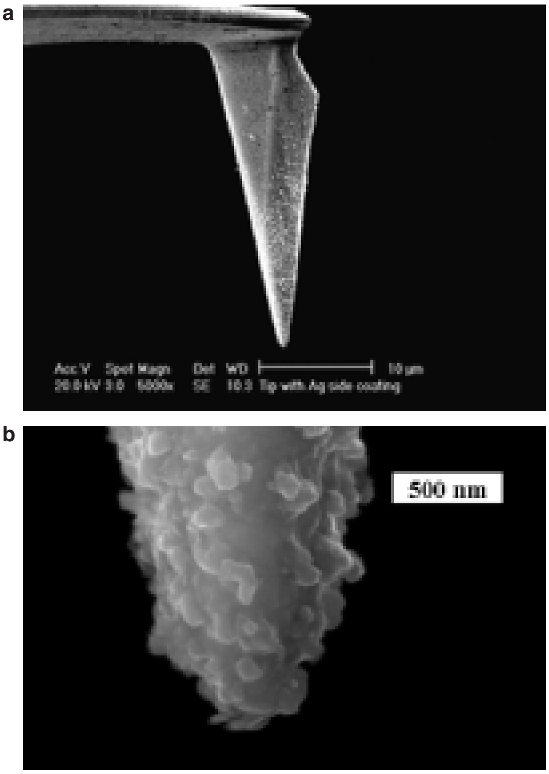

Figure 2 Scanning electron microscope (SEM) image of the special TERS cantilever tip with electrochemically deposited silver nanoparticles protected with polymer matrix. (SEM image courtesy of Dr. Joachim Loos and Prof. Bert de With, TU Eindhoven, Dept of Chemical Engineering & Chemistry, Eindhoven, The Netherlands.) a) tip. b) close-up showing silver nanoparticles.

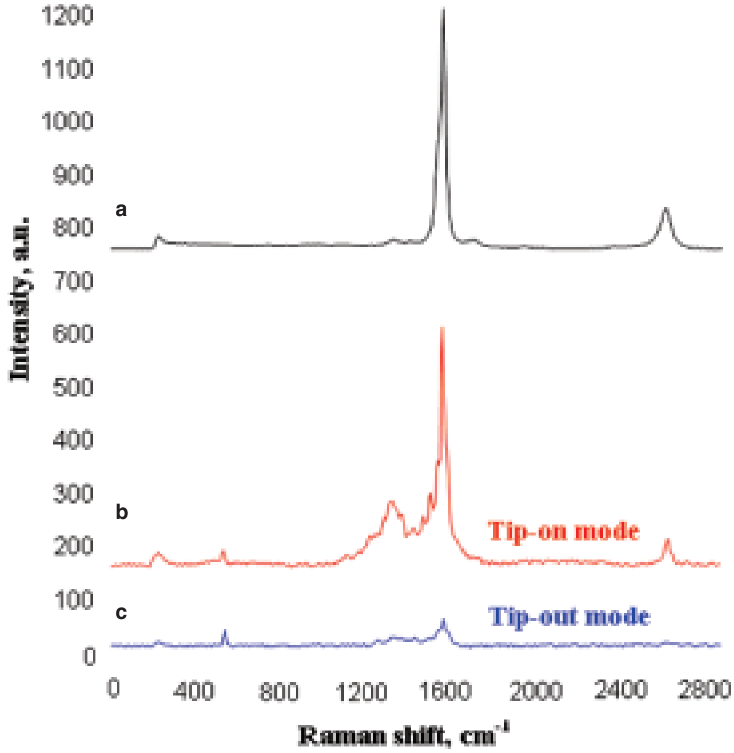

Figure 3 Comparison of Raman spectra. a) Bulk sample of single-walled carbon nanotubes (SWCNT). b) TERS of single nanotube. c) Single nanotube. (Data courtesy of Dr. Joachim Loos.)

green signal is a much weaker red fluorescence. The fluorescence is approximately 1/10,000th the intensity of the reflected light. In comparison, Raman is on the order of 1/1000th–1/10,000th the intensity of the typical fluorescence signal, making it exceptionally difficult to capture. The NTegra Spectra uses two technologies to optimize the Raman signal: a choice of lasers and a dual-channel spectrometer.

Since the intensity of a Raman signal is inversely proportional to the fourth power of the excitation wavelength, one way to maximize signal is to use short wavelength excitation. However, short wavelength light is often damaging, especially for polymeric or biological samples. Conversely, longer wavelength light penetrates more deeply and is less damaging. To accommodate this tradeoff, the instrument can be configured with up to three different lasers, with laser choice software controlled.

Strong autofluorescence often masks the Raman signal. To avoid this problem, the NTegra Spectra uses a dual-channel detector that splits the two signals into independent channels. One channel receives the fluorescence from the laser scanning confocal image, and the second receives the Raman spectrum, simultaneously, from exactly the same point in the sample.

Finally, the spectrometer has been optimized for both resolution and throughput, offering spectral resolution of 0.03 cm^{-1}, with sufficient throughput for both conventional Raman mapping and single-molecule detection.

TERS and single-molecule spectra

The most recent development for enhancing Raman signal is based on the use of silver nanoparticles deposited on a surface that comes into contact with the sample. In conventional Raman, special silver-coated slides can be used, generating surface-enhanced Raman spectra (SERS). When Raman is combined with AFM, special tips are used (*Figure 2*), generating very specific local augmentation called tip-enhanced Raman spectroscopy (TERS).

As shown in *Figure 3*, the results are dramatic. The top curve in the spectrum is typical of the Raman signal taken from single-walled carbon nanotubes (SWCNT) in bulk. The strength of the signal is derived from the summation of spectra taken from thousands of nanotubes. In comparison, the bottom curve shows the typical signal from one nanotube. Weak and poorly resolved, very little can be learned from this spectrum.

The middle spectrum shows the impact of TERS. Although it was taken from only a single nanotube, it is strong with well-defined peaks. While other Raman systems can produce similarly enhanced results, the NTegra Spectra's potency is derived from the ability to combine TERS with the power of light microscopy, the 3-D imaging of confocal laser scanning, the nanometer resolution of AFM and NSOM, and the availability of other scanning probe modalities such as electrical and magnetic measurements.

Remote diagnostics

An important question to ask when getting involved with this level of instrumentation is, "Does the company offer remote diagnostics *and* technical support?" The answer for the NTegra Spectra is "yes." **NT-MDT** is supported in the U.S. by two technical applications specialists, one in the home office in Allen, TX, and the other in the new applications center in Menlo Park, CA. Additionally, all NTegra microscopes can be accessed electronically for troubleshooting and assistance in problem-solving.

Learning resources

A system this powerful requires competency in many disciplines. What resources are available for learning more about these technologies? For light microscopy, the McCrone Institute (Chicago, IL) and Microscopy/Marketing & Education (MME) are two good resources. MME, which specializes in customized, on-site, hands-on courses, also has consultants for confocal and AFM as well as electronic or digital imaging.

Microscopists are active communicators. They energetically support two key list servers, one sponsored by the Microscopy Society of America,[7] and the other dedicated to confocal microscopy, by SUNY/Buffalo (NY).[8] Although the Raman world does not have as active a communication network, SpectroscopyNOW[9] is a good resource, offering introductory materials and the opportunity to post questions.

An excellent tool for learning AFM is the animations on the **NT-MDT** Web sites.[10] Supported by brief descriptions, they clearly illustrate over 30 different scanning techniques involved in AFM, scanning tunneling microscopy (STM), and NSOM.

For a more global approach to the multiple techniques presented by the NTegra Spectra, the reader should consider the *Comprehensive Desk Reference of Polymer Characterization and Analysis*[11] from the American Chemical Society. In addition to general information on polymer characterization, it contains a chapter on vibrational microscopy (including Raman) and individual chapters on light, confocal, and atomic force microscopies.

Conclusion

The new generation of instrumentation offers exciting opportunities for analyses. While all of this power can be daunting, there are excellent resources available for harnessing it. As with any large project, it is important to take an organized approach and to set realistic expectations. One should not expect to learn everything at once. The conventional wisdom in optical microscopy is that it takes a full year to become proficient. The good news is that these skills can be learned in tandem. The NTegra Spectra will not be for everyone, but for those who need complete imaging and chemical information from very small entities, it heralds the next generation in integrated technologies.

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