

Focus on Microscopy: AFM's New Nanotomography Expands 3-D Imaging

by Barbara Foster

Atomic force microscopy (AFM) is well known for its ability to image and measure surface properties. Whether these properties are electrical, magnetic, physical, or chemical characterization, the images of these properties are typically represented as 3-D images. A device called the NTegra NanoTome™ (NT-MDT, Zelenograd, Russia) (Figure 1) integrates an AFM into an ultramicrotome to extend these surface investigations into the real world of 3-D, opening new possibilities for imaging biological ultrastructure as well as nanostructures and polymer domains, inclusions, and voids.¹



Figure 1 NTegra NanoTome.

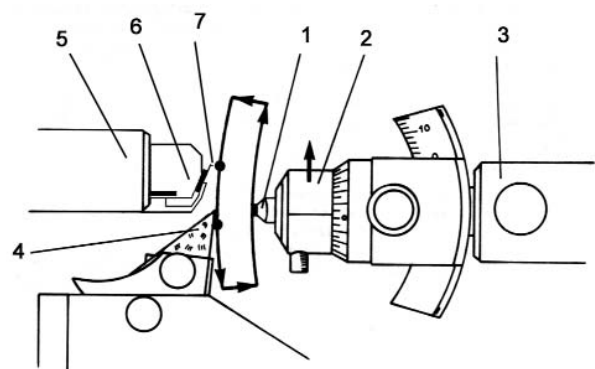


Figure 2 Nanotomography diagram of AFM/knife interface (image courtesy of NT-MDT). 1) Sample, 2) sample holder, 3) ultramicrotome arm, 4) ultramicrotome knife, 5) AFM/SPM scanner, 6) holder for AFM/SPM cantilever or probe, 7) AFM/SPM probe.

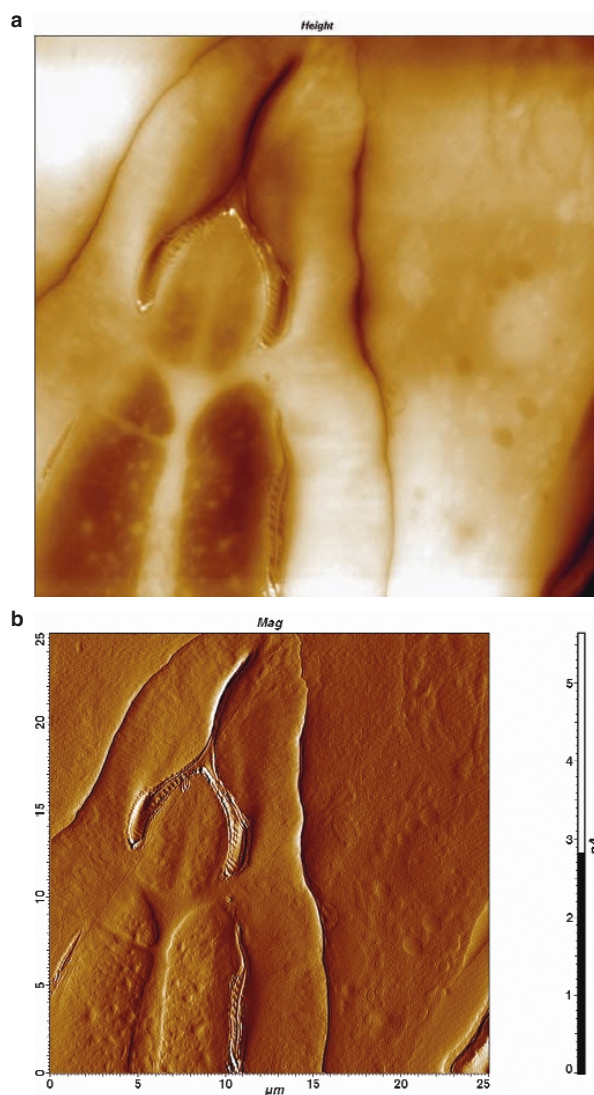


Figure 3 Cross-section of *C. elegans* imaged using a) conventional tomography mode, and b) feedback mode, measuring local variation in elasticity. Scan size: $25\ \mu\text{m} \times 25\ \mu\text{m}$. (Sample courtesy of Dr. Martin Mueller and Dr. Nadejda Matsko, ETH, Zurich, Switzerland.² All sample images courtesy of Dr. Anton Efimov, NT-MDT.)

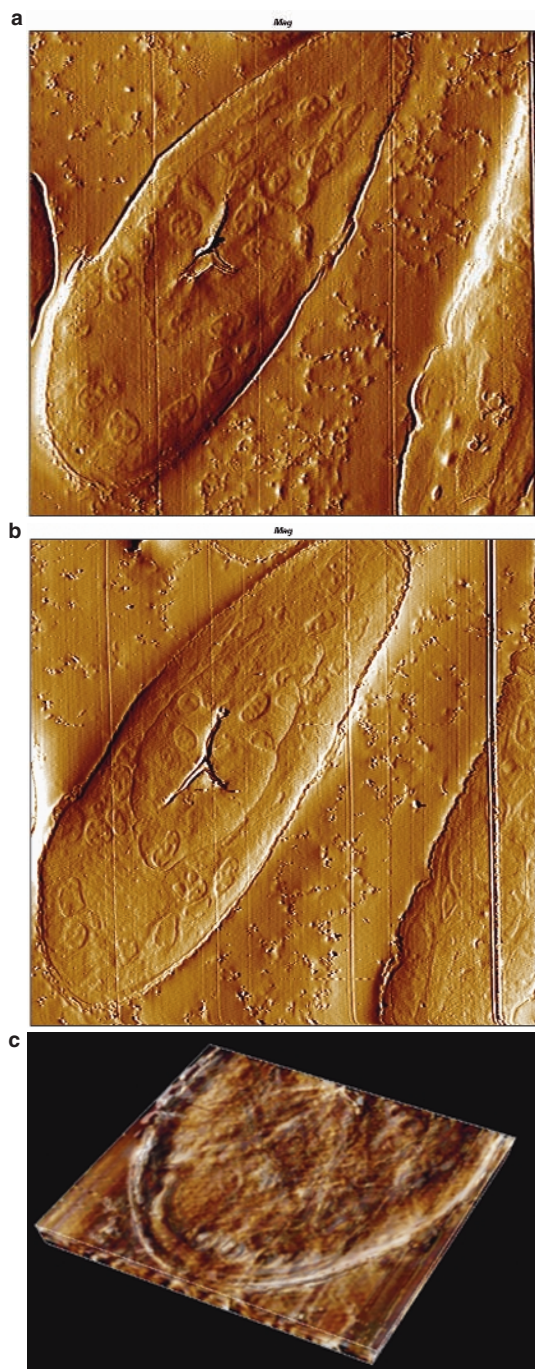


Figure 4 The nematode, *C. elegans*, is a well-known biological model. *a)* and *b)* Sequential single sections, phase imaging (each image is $10\ \mu\text{m} \times 20\ \mu\text{m}$). *c)* 3-D reconstruction made from a stack of seven sequential block face images, sectioning interval of 200 nm each. (Sample courtesy of Dr. Martin Mueller and Dr. Nadejda Matsko, **ETH**.)

System operation

The basic approach underlying the new technology is conventional: The microtome then slices the AFM images. As with any tomographic approach, the process is repeated until the necessary number of individual images is collected to adequately represent the 3-D structures of interest.

What makes the NanoTome distinctive is its integration. As shown in *Figure 2*, the AFM images directly from the block face after each slice, eliminating typical artifacts such as stretching, tearing, and wrinkling inherent in imaging individual slices. Additionally, imaging from the block face ensures accurate slice-to-slice alignment in the 3-D reconstruction, minimizing the tedious and complex alignment processing involved when working from individual slices instead of the block face.

The NanoTome is based on the NTegra microscope (**NT-MDT**), a next-generation design whose open architecture permits both extensive modularity and flexibility in the base instrument and facile integration with other devices such as the ultramicrotome and Raman spectrometers. A slight modification of the standard scanner allows use of the routine stereo viewing system. Coupled with the use of a 35° diamond knife, it makes installation quick and easy. The manufacturer recently entered into a strategic agreement with **Leica** (Bannockburn, IL) to retrofit its UC6.

Looking beyond topography for ultra-structure

Because the diamond knife leaves an ultrasmooth surface, the commonly used AFM topography mode reveals little information (*Figure 3a*). However, the use of techniques that elicit other physical responses, such as local differences in elasticity, uncovers hidden details (*Figure 3b*). Animations illustrating various imaging modes are available at www.nt-america.com.

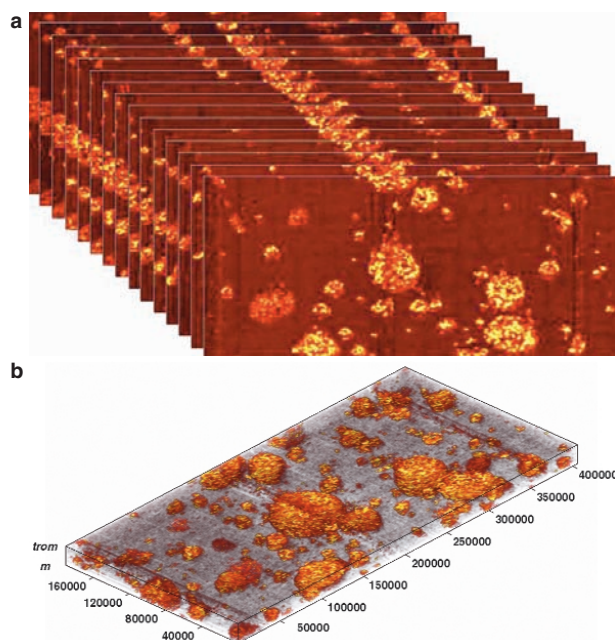


Figure 5 *a)* Fifteen sequential images of polystyrene/high-impact polystyrene (HIPS) blend with silica (hard inclusions). Image size: $40 \times 20\ \mu\text{m}$ with 200 nm between sections. *b)* 3-D reconstruction made from those 15 sections: $40 \times 20 \times 3.0\ \mu\text{m}$. (Sample courtesy of Dr. Aliza Tzur, **Technion**, Israel.)

Standard sample preparation/multiple microscopies

Because the AFM does not limit the ultramicrotome, this technology presents an opportunity for multiple imaging modes that present complementary information. Biological samples are prepared using standard freeze substitution protocols. As a result, the AFM can image from the block face while the slices can be further processed and observed using transmission electron microscopy (TEM). For example, the *C. elegans* used in Figure 4 was prepared by standard freeze substitution. Figure 4a and b illustrate sequential atomic force images taken from the block face, with contrast generated using phase imaging. Figure 4b shows the 3-D reconstruction developed from seven such images. The individually cut sections can also be mounted for further TEM investigation.

Sample preparation for polymers, advanced materials, and new nanomaterials is even easier. In many cases, a block of material can be cut and mounted directly on the microtome. Because the AFM has a variety of imaging modes, 3-D information can be acquired regarding domains, inclusions, voids, distribution of magnetic fields, etc. For instance, Figure 5 clearly illustrates the volumetric distribution of hard silicon clusters embedded in a polystyrene/high-impact polystyrene matrix. For the first time, material scientists can image structures such as spherulites in true 3-D, gaining important information to relate the material's internal structure to its function and behavior.

New questions

The new technology has prompted expected questions, the most fundamental of which is, "How thin a slice can be imaged and what is the XY limit of resolution?" As with any microtomy process, the depth of the cut and therefore the Z resolution is determined by the nature of the material and the features of interest. Typically, slices are on the order of tens of nanometers in thickness. Because the AFM uses nonoptical imaging modalities, the XY resolution is limited only by the AFM technique. For local elasticity, for instance, that distance is on the order of 10 nm.

A second key question centers on the availability of the microtome for other routine activities. Because of the ease of installation, the AFM can be readily attached or detached as needed, leaving the microtome free for other routine uses in the laboratory.

A third question arises from the 3-D reconstructions themselves. Specifically, how are they viewed, and are measurements such as distances in 3-D or volumes available? Using the integrated NOVA™ software, the system presents "aquarium" constructions that can be rotated, cut, and viewed from any angle. Data can also be exported into existing software such as 3-D Constructor® for Image-Pro Plus (Media Cybernetics, Silver Spring, MD) for a variety of measurements including distance in 3-D space, volume, and branch length.

Conclusion

The NanoTome is the latest in a growing arsenal of 3-D imaging tools. Because it is derived from conventional AFM and microtomy techniques, the learning curve is short. However, the resulting benefits are greater than the sum of its parts, opening intriguing new opportunities for 3-D imaging of ultrastructure and the understanding of structure-to-function relationships.

References

1. Efimov AE, Matsko N, Mueller M, Saunin S. 3D reconstruction of internal cell structures with ultramicrotome SPM. Poster presented at the American Society of Cell Biology, Washington, DC, Dec 4–8, 2004.
2. Matsko N, Mueller M. AFM of biological material embedded in epoxy resin. *J Struc Biol* 2004; 146(3):334.

Ms. Foster is President of **Microscopy/Marketing & Education**, 313 S. Jupiter Rd., Ste. 100, Allen, TX 75002, U.S.A.; tel.: 972-954-8011; fax: 972-954-8018; e-mail: bfoster@mme1.com.