# Taking the biomolecular pulse

*Researchers are using atomic force microscopy to study drug interactions, cell biology, and disease mechanisms.*

BY JOHN T. THORNTON

For more than two decades, atomic force microscopy (AFM) has provided researchers with the ability to investigate surface structures at nanometer-to-subangstrom resolution in ambient and liquid environments. Indeed, AFM has contributed to groundbreaking research in the investigation of DNA, proteins, and cells in biological studies; structure and component distribution in polymer science; piconewton force interactions and surfactant behavior in colloid science; and physical and mechanical properties and fabrication variables in the materials sciences. Pharmaceutical research often consists of a combination of these scientific branches, making it a particularly viable field for the application of AFM. The ability of AFM to provide high-resolution 3-D surface structure makes it a powerful complement to other common analytical techniques currently available (*1*).

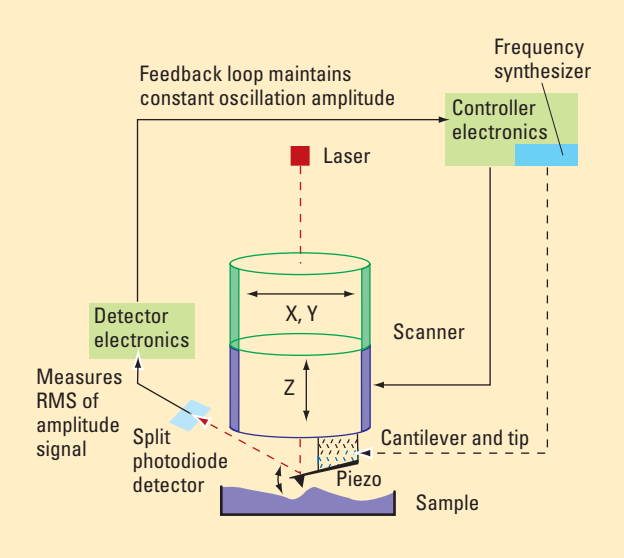


**Figure 1. Atomic force microscopy.** The schematic shows the feedback loop for tapping-mode operation.

## History and methods

AFM is the most commonly used form of the scanning probe microscopy (SPM) family of techniques. SPM began with the development of the scanning tunneling microscope (STM) in 1982 by researchers at IBM in Zurich, a development that earned its inventors the Nobel Prize in Physics in 1986. However, the STM can only be applied to conductive or semiconductive specimens. To broaden this type of microscopy to the study of insulators, the atomic force microscope was developed in a collaboration between IBM and Stanford University in 1986.

In AFM, a sharp tip on the end of a flexible cantilever is scanned across a sample surface, maintaining a small but constant force. The tips typically have an end radius of 5–10 nm, although it can vary depending on tip type, and a piezoelectric tube scanner scans the tip in a raster pattern with respect to the sample (Figure 1). The cantilever deflection or oscillation amplitude is monitored by reflecting a laser off the back of the cantilever onto a split-photodiode detector. The two most commonly used modes of operation are contact-mode AFM and tapping-mode AFM.

In contact-mode AFM, a constant cantilever deflection is maintained by a feedback loop that moves the scanner vertically at each lateral data point to form the topographic image. By maintaining a constant deflection during scanning, a constant vertical force of 0.1–100 nN is maintained between the tip and sample. Although contact-mode AFM has proven useful for a wide range of applications, it sometimes has difficulty on relatively soft samples.

Tapping-mode AFM consists of oscillating the cantilever at its resonance frequency (typically ~300 kHz) and scanning across the surface with a constant, damped amplitude. The feedback loop maintains a constant root-mean-square amplitude by moving the scanner vertically during scanning, which correspondingly maintains a constant applied force. The image is formed from the vertical ($z$) movement of the scanner at every $x$, $y$ data point. The advantage of tapping-mode AFM is that it typically operates with a lower vertical force than that possible with contact mode, and it eliminates the lateral shear forces that can damage some samples. Thus, tapping mode has become the preferred technique for imaging soft, fragile, adhesive, and particulate surfaces.

Although SPM was used initially to produce high-resolution topographic images, several SPM techniques have been developed to study the physical and materials properties of sample surfaces. These techniques are commonly used to investigate variations in friction, adhesion, elasticity, hardness, carrier concentration, conductivity, and temperature distribution. Fundamental force studies are also conducted to study adhesive, attractive, and repulsive interactions between specimens.

Commercial AFM units are now equipped to control the sample environment. Imaging at elevated temperatures has made it possible to study thermal phase transitions under ambient atmosphere or inert gas. Images can also be obtained for samples in fluid, working at elevated temperatures such as 37 °C. Atmospheric hoods are also commonly used in AFM to control humidity or to conduct experiments under specific atmospheric conditions.

## Drug interactions

AFM has been used to study drug interactions with a variety of biological specimens,

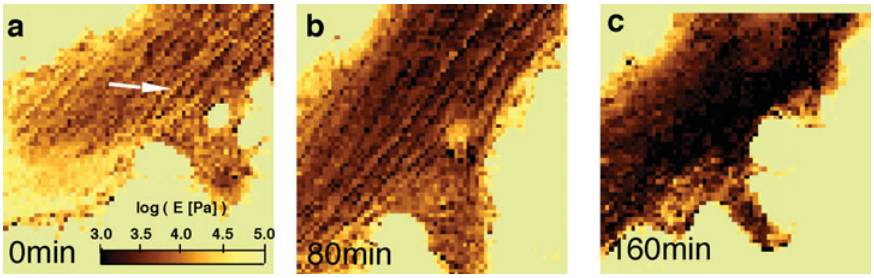


**Figure 2. Mapping elasticity.** AFM measures the effect of cytochalasin B on the elastic modulus of the 3T3 fibroblast over time. Before addition of the drug (a), the actin filaments (arrow), which are stiffer than the rest of the cell, are seen in the force volume image. After adding cytochalasin B to the fluid chamber with the cells (b, c), the disaggregation of the actin network produces a decrease in elasticity, which is indicated by the darker areas in the images. (Images courtesy of Christian Rotsch and Manfred Radmacher of Universität München, Germany.)

and interactions between biological specimens. For example, AFM-based immunological studies have investigated antibody–antigen binding interactions, and drug–DNA complexes have been studied with AFM to determine DNA ligand mode-of-binding. This is important because nucleic acid ligands are commonly used as anticancer drugs and in the treatment of genetic diseases. However, determining whether these ligands bind to DNA by intercalation within major and minor grooves, by "nonclassical" modes, or by a combination of these modes can often be difficult and labor-intensive.

Lawrence Bottomley and colleagues at the Georgia Institute of Technology (Atlanta) used AFM to study a drug's DNA-binding mode, affinity, and exclusion number by comparing the length of DNA fragments that have and have not been exposed to the drug (*2*). If intercalative binding occurs, the DNA strand lengthens by unwinding. Furthermore, the degree of elongation is related to the binding affinity and the site-exclusion number.

When exposed to ethidium bromide, a well-characterized intercalator, AFM showed that the DNA strand increased in length from 3300 nm to 5250 nm. Similarly, when DNA was exposed to daunomycin, an anticancer drug used to treat leukemia, AFM intercalative binding studies showed that the DNA strands lengthened from 3300 nm to 4670 nm. This technique has also been successfully applied to new drugs in which the mode of binding was unclear. Exposure of 2,5-bis(4-amidinophenyl), a new drug for the treatment of *Pneumocystis carinii* pneumonia, did not produce lengthening of the DNA strands, indicating that the drug binds by nonintercalative modes (*3*).

## Cell analysis

Although many AFM studies have concentrated on interactions at the molecular level, responses of living cells have also been visualized. Because cells can be imaged in a physiological solution at 37 °C, it is possible to monitor the interaction between a metabolically active cell and a chemical or biological additive.

Christian Rotsch and Manfred Radmacher at Universität München (Germany) conducted "force mapping" studies on 3T3 and NRK fibroblast cells to visualize the effects of various drugs (*4*). Force mapping traces a lateral array of force curves (force vs tip-elevation plots) across a sample surface that produces a force-interaction image. Collecting force curves consists of recording the change in the cantilever deflection as it moves vertically toward the sample until it touches the surface, and then pulls away from the surface and retracts back to its starting position. Force curves can map repulsive, attractive, and adhesive interactions in the piconewton-to-nanonewton range.

In this study, force volume was used to map elasticity changes over time due to the interaction with F-actin disabling drugs. Actin filaments form a network that provides mechanical stability to the cell. F-actin disabling drugs were added to the cell medium, and the disaggregation of the actin filaments was studied directly by observing the change in the elasticity of the cells. In AFM, a decrease in elasticity reduces the degree of cantilever deflection when the tip is in contact with the cell.

In Figure 2, the effect of cytochalasin B on the elastic modulus of the 3T3 fibroblast can be seen over time. Before addition of the drug, the actin filaments, which are stiffer than the rest of the cell, can be seen in the force volume image (arrow in Figure 2a). After adding cytochalasin B to the fluid chamber with the cells, the disaggregation of the actin network produces a decrease in elasticity, which is indicated by the darker areas in the images. Over the 160-min time frame, the elasticity decreased by a factor of 3.1. The effects of drug concentration on the rate of interaction and the decrease in elasticity were successfully studied for each of the drugs applied.

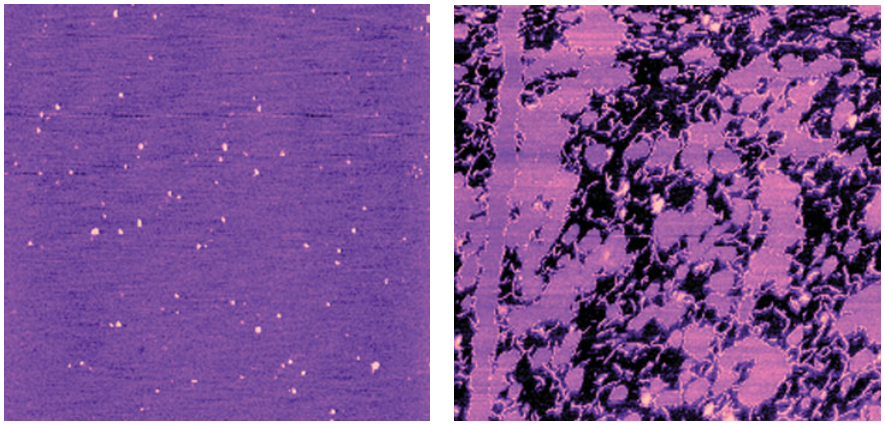

**Figure 3. A look at Alzheimer's.** After introducing the amyloid-β peptides (Aβ1–40) into a solution of brain-lipid-extracted bilayers (left), tapping-mode AFM showed that the peptides were partially inserted into the bilayer surface. Fibril growth was initiated from these sites, resulting in membrane disruption after 15 h (right). (Images courtesy of Christopher Yip at the University of Toronto.)

## Disease mechanisms

AFM is also commonly used to better understand physiological mechanisms associated with disease. For example, AFM has been used to study Parkinson's disease, diabetes, pancreatitis, and cancer. AFM is useful for this type of work because these investigations can be performed in situ.

Christopher Yip and JoAnne McLaurin of the University of Toronto used AFM to study the mechanisms of amyloid-β (Aβ) fibrillogenesis, which plays a role in Alzheimer's disease (*5*). In situ tapping-mode images of total brain lipid bilayers were used to study the role of membrane composition and peptide structure. Brain lipid bilayers were deposited onto mica and imaged in phosphate buffer solution. Comparing images of the bilayers before and after introducing the monomeric Aβ peptides into the buffer solution revealed that Aβ1–40 molecules were partially inserted into the bilayer surface (Figure 3). After 15 h, fibril growth was initiated from these sites, resulting in membrane disruption. To study the specificity of lipid bilayer composition and Aβ sequence, the same experiment was conducted with DMPC (dimyristoylphosphatidylcholine) bilayers and Aβ1–28 peptides.

From these studies, it was determined that the fibril formation occurs in the presence of acidic lipids and that the peptide requires the hydrophobic C-terminal domain, which is critical for anchoring to the lipid to induce fibrillogenesis. Without these critical requirements, the formation of Aβ aggregates disrupts the membrane without evidence of fibril formation.

## Biomed and beyond

AFM has many uses in pharmaceutical research, including the investigation of in situ processes, interaction mechanisms, behavioral properties, and structure–function relationships. Although these examples are just a sampling of the work that has been conducted in drug interaction and disease mechanism studies, they indicate how important the atomic force microscope can be in furthering pharmaceutical sciences. The complementary nature of AFM with other analytical techniques will certainly result in many more applications as the pharmaceutical community further adopts it and as new AFM and SPM techniques are developed.

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**John T. Thornton** is an applications scientist at Digital Instruments, Veeco Metrology Group (Chadds Ford, PA). Send your comments or questions about this article to mdd@acs.org or to the Editorial Office address on page 3. ■