TappingMode Atomic Force Microscopy Operation in Fluid

By: M. Wright*, I. Revenko

Introduction

Many of the biological samples suitable for imaging with atomic force microscopy (AFM) are subject to artifacts if they are dried. This problem is not unique to AFM imaging. It has long been a concern in electron microscopy and methods have been developed to minimize its effect with that imaging technique (namely fixation, critical point drying, and environmental EM). The solution to this problem when using AFM is much simpler. Rather than drying the sample at all, one can image quite successfully with AFM in fluid.

Biological samples can be imaged with AFM in either of two imaging modes: contact mode or TappingMode. For AFM studies, biological materials are often both delicate and tenuously immobilized on a surface, even more so in fluid than in air. As a result, the vertical and shear forces exerted on the sample via the

tip in contact mode can damage the sample by compressing, tearing, or removing it from the surface. Imaging with TappingMode, particularly in fluid, presents the advantage of greatly reducing shear forces (see related application note, "TappingMode Imaging: Applications and Technology" Lit. code: AN04), allowing delicate samples to be imaged more easily (see "TappingMode AFM Imaging in Fluids for the Study of Colloidal Particle Adsorption", Lit. code: AN09).

Applications of fluid TappingMode AFM in biology are constantly growing and the data obtained with this technique are improving, especially in terms of resolution. Even dynamic processes can be observed almost as they would occur *in-vivo*. The list of studies in which biologists have used AFM includes: high resolution imaging of molecules (proteins, lipids, sugars, etc.); imaging of living cells (virus, yeast, bacteria, neurons, macrophages, and endothelial cells as seen in Figure 1); monitoring the action of drugs or other compounds on cells, and tissues; visualizing changes in the molecular conformation of proteins; studying molecular interactions or kinetics of polymerization of biomolecules (e.g., collagen[1]); and recording crystal growth (e.g., insulin[2]).

Areas of applications are not limited: biochemistry, cell biology, molecular biology, biomaterials and material sciences, neuroscience, and many more can be studied with AFM.
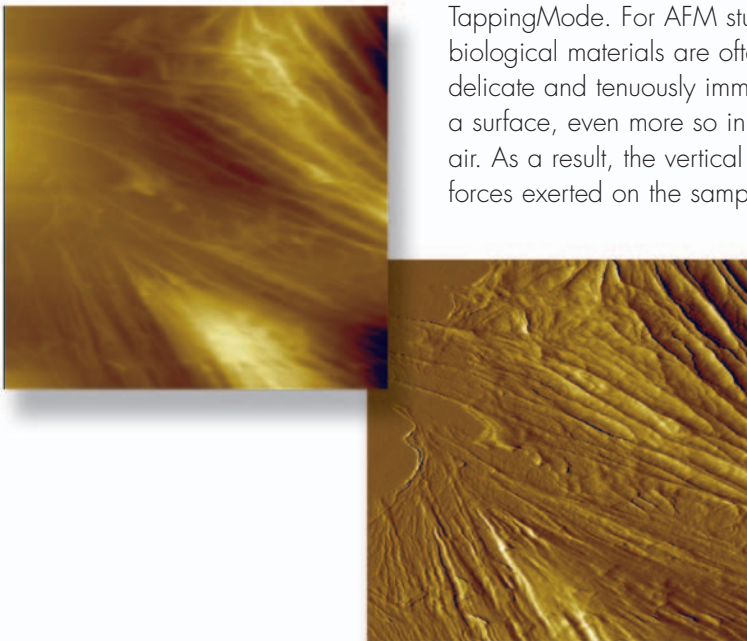

Figure 1. Height (left) and amplitude (right) images of living endothelial cells in culture imaged in TappingMode in liquid. 50μm scan.

Figure 2. Action of PLA2 on a DMPC bilayer deposited on mica. 1μm x 0.5μm scans.

Figure 2 is an example of a real-time enzyme activity study. The succession of images corresponds to the degradation of a dipalmitoylphosphatidylcholine Langmuir-Blodgett bilayer by phospholipase A2[3]. In this experiment it is possible to follow the time course of the hydrolysis of the lipid film.

As with TappingMode in air, important information can be obtained pertaining to the physical characteristics of the material using PhaseImaging. Phase information can be recorded simultaneously with topographic data in TappingMode on Digital Instruments MultiMode®, BioScope, and Dimension Series systems. PhaseImaging is a powerful technique that can provide information on changes in viscoelasticity, friction, adhesion or hardness across a sample surface (see "PhaseImaging: Beyond Topography", Lit. code: AN11).

Acoustic vs. Magnetic Actuated Drive

Two drive mechanisms for TappingMode in fluid are available to the user on the MultiMode AFM. The conventional method of driving the cantilever by acoustic excitation has been joined by a magnetic actuated drive. The Dimension and BioScope systems both use the acoustic excitation method exclusively.

Acoustically driven oscillations of the cantilever in liquid on the MultiMode AFM occur by excitation of a piezoelectric ceramic element in the cantilever holder (indirect drive). The acoustic waves induced in the fluid medium cause the cantilever to oscillate. The Magnetic Actuated Drive (MAD) mechanism uses an electromagnet in the fluid cell to create a magnetic field to drive specialized probes (Figure 3). These probes are coated with a magnetic film (Co or Co/Cr) on the backside (only) to preserve the tip sharpness. It is somewhat easier to identify the resonant frequency of the cantilever when working with the magnetic drive, as the tune shows mainly only the resonant frequency oscillation of the probe (Figure 4). However, the magnetic coating on the backside of the cantilever can lead to the possibility of contamination of sensitive samples with soluble rare earth and transition metal ions. Also, the electromagnet of the magnetic drive fluid cell may cause undesirable heating of the sample, which could induce lateral drift in the system during scanning. Also worth noting is that the o-ring seal option is only available on the acoustic TappingMode cell. The MAD fluid cell depends on capillary forces to keep the liquid at the surface of the sample. Studies on lipid bilayers (Figure 5) and DNA molecules (Figure 6) show that the acoustic and magnetic actuated drives are comparable in their ability to image soft samples with minimum perturbation[4]. Both achieve high quality resolution with comparable signal-to-noise ratios, making the magnetic drive equal in every significant way to the more commonly used acoustic drive. Since performance is similar, the user is ultimately left to make their choice depending on factors of ease-of-use, the few minor disadvantages of MAD mode, and cost.

Performing Acoustic TappingMode in fluid

The first step to successful imaging in fluid is selecting the appropriate probe. This choice is largely
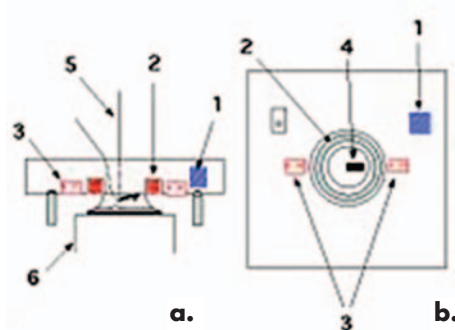

Figure 3. Lateral and top views of the fluid cell for magnetic TappingMode: 1) piezo element, 2) electromagnet, 3) permanent magnets, 4) cantilever, and 5) laser beam.
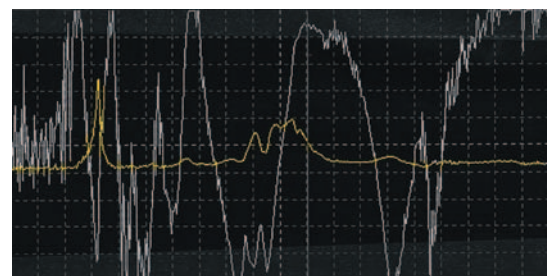

Figure 4. Frequency tune for a silicon nitride cantilever coated with a Co/Cr film and driven with a magnetic field.
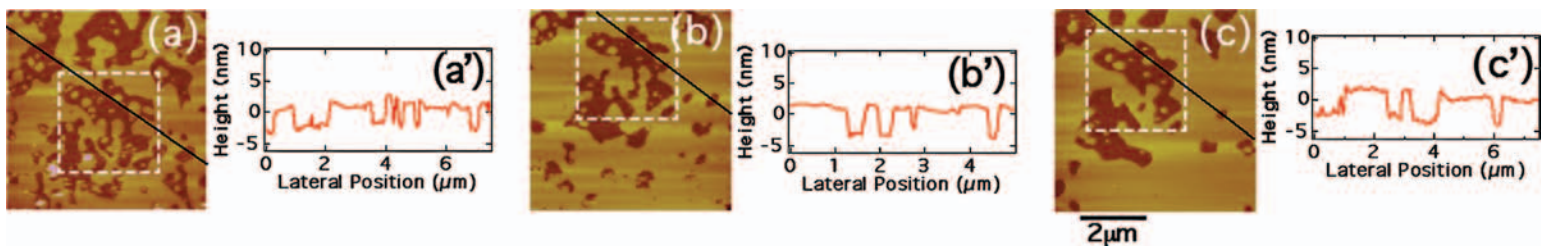
Figure 5. Phosphatidylserine bilayers imaged in TappingMode in liquid: (a) and (c) were obtained with the magnetic drive mode and (b) was obtained with the acoustic drive mode. 6μm scans with Z range = 12nm. The dashed square outlines a characteristic feature visible during the whole experiment[4].

dependent on the sample characteristics (hardness, roughness, etc.). The preferred probes for TappingMode in fluid are the silicon nitride cantilevers. Generally, the short, narrow cantilever of the "NP" series or the shorter of the two cantilevers on the "OTR-4" chip is suggested (see "Choosing AFM probes for Biological Applications", Lit. code: AN44).

For users of the MultiMode AFM, another decision to be made is whether or not to use an o-ring when operating in fluid. The use of an o-ring is recommended when fluid exchange in the cell is desired or when evaporation is an issue (e.g., working with heated fluids or solvents). Otherwise, capillary forces are strong enough to ensure that the fluid remains in between the substrate and the fluid cell and does not overflow onto the scanner. A small amount of fluid should be used in that case (typically ~100μL), which also presents the advantage of limiting thermal drift problems.

Select a peak using the frequency tune menu. Empirical experience shows us that relatively low frequencies of oscillation provide the best conditions for acquisition of images. We suggest using a frequency of about 8kHz for the silicon nitride probes. In any case, apply a drive voltage to the cantilever of about 500mV. Choose a fairly defined, tall peak from those that appear in the amplitude line on the sweep. Offset slightly to the left side

of the peak. If there are no peaks in the expected range, the peaks are poorly defined, or you require an unreasonably high drive amplitude to get large enough peaks, you may be using the wrong cantilever, a defective cantilever, or you may simply be too far from the surface. The TappingMode response of the BioScope and Dimension systems are especially sensitive to tip-sample distance.

Upon returning to the main imaging controls, check the RMS amplitude of the probe oscillation. The optimal RMS value depends greatly on the sample being studied. A suggested value would be about 0.8V for TappingMode imaging of soft samples (and even less for fragile ones like living cells).

The "Force Calibration" function may also be used after engaging to ensure that the tip is in fact engaged properly and that a minimal force is being used on the sample. Use caution, however, because this procedure can damage the tip if it is brought too close to the surface. Refer to the Force Calibration section of your microscope manual for details of this procedure.

It is important to keep in mind that the adjustments are more subtle than in air and it is often preferable to actually type the numbers than to use the arrow keys to change the setpoint and the gains (see "Guidelines for Fluid Operation with a MultiMode AFM", Support Note #PN 013-290-000).
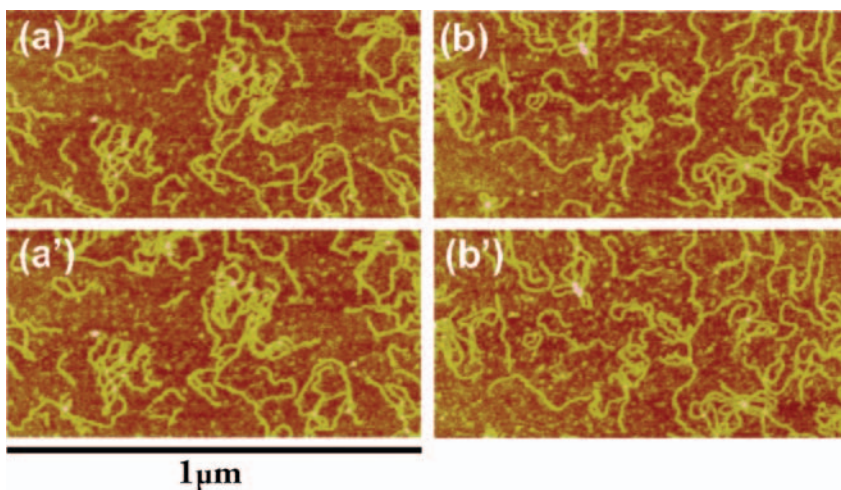

Figure 6. DNA step ladder molecules imaged in TappingMode in liquid. (a) and (b) were obtained using acoustic driven TappingMode. (a') and (b') were obtained using the magnetic driven tapping mode. Z range = 10nm.[4]

A General Procedure for Protein Imaging

This procedure was designed for the reproducible imaging of the globular protein lysozyme. It may also make a good starting point for imaging other globular proteins and has already shown some success for visualizing collagen molecules.

The protein was obtained from Sigma and imaged under potassium phosphate buffer (pH=4). Details of sample preparation can be highly variable, depending upon the molecule under investigation. A suggested concentration would be 1µg/ml and the pH of the buffer solution generally should be below the isoelectric point of the protein. This creates an overall positive charge on the protein, enabling it to adsorb easily to the negatively charged mica.

An 50µl aliquot of the protein solution is incubated on freshly cleaved mica for twenty minutes. The mica was then gently rinsed and imaged with the same (pH=4) ptassium phosphate buffer solution.

The tip should be engaged on a relatively small scan size (0 to 5nm). A force curve is then taken to ensure the tip is in good contact with the mica substrate. A typical curve more or less identical to that on plain mica should be observed.

From here, increase the scan size and check the scope trace to ensure that the tip is properly tracking the sample surface. It is often necessary to decrease the amplitude setpoint slightly from its engage value. Set the desired scan size and optimize the image using the gains.

Application to Cells in Culture

A few key points are important for imaging cells. If the tip is too sharp, it may poke through the cell membranes and tear them. NP and DNP tips are therefore recommended since they are more blunt than the sharpened series of tips. A small RMS amplitude is necessary (0.3 to 0.5V) and it is important while imaging to always control the setpoint in order to minimize the vertical forces. Depending on the cells, it may also be necessary to scan very slowly (0.3Hz). If the cells are moving underneath the tip, some coating on the petri dish may be helpful: collagen, polylysine, Cell Tak or other cell adhesion coatings.

Summary

The integration of TappingMode imaging techniques into a fluid environment makes this a powerful tool for studying biological samples. When operating in fluid, a range of applications becomes available to the user. The acoustically driven fluid TappingMode is currently the most widely employed mode of AFM in biology, used for a variety of samples in several different areas of research. With this technique, researchers have successfully imaged a diverse range of samples without artifacts caused by drying or excessive force. Every sample has unique challenges and requirements but, with practice, there are few limits to the range of samples that can be investigated.

References

1. Hansma, P.K. et al, (1994) "Tapping mode atomic force microscopy in liquids." Appl. Phys Lett. 64 (13): 1738-1740.

2. Putman, C.A.J. et al, (1994) "Tapping mode atomic force microscopy in liquid." Appl. Phys. Lett. 64 (18): 2454-2456.

3. Grandbois, M., Clause-Schaumann, H., and Gaub, H., "Atomic force microscope imaging of phospholipid bilayer degradation by phospholipase A2. Biophysical J., 1998, 74, 2398-2404.

4. Revenko, I. and Proksch, R., (2000) "Magnetic and acoustic tapping mode microscopy of liquid phase phospholipid bilayers and DNA molecules". J. Appl. Phys. 87(1): 526-533.

5. Radmacher, M. et al, (1994) "Imaging adhesion forces and elasticity of lysozyme adsorbed on mica with the atomic force microscope". Langmuir. 10: 3809-3814.

* University of Waterloo, Ontario, Canada

Solutions for a nanoscale world.