Applications of Force Volume Imaging
with Atomic Force Microscopes

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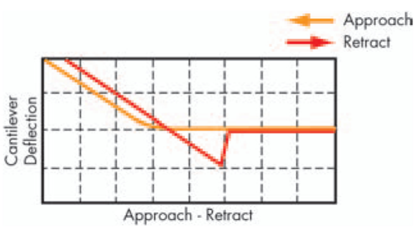


Figure 1a. Standard force curve with both the extending and retracting portion displayed.

Any small particle that approaches a surface experiences a number of forces before and after contact with the surface. Using an atomic force microscope (AFM) tip, or a small particle attached to an AFM cantilever, the AFM can be used to probe these forces between the tip (or particle of your choice) and a surface. This is done by recording the cantilever deflection as the tip approaches, contacts, and retracts from a surface, then plotting a force curve as a function of the approach-retract travel distance. These types of force measurements can contain information about the electrostatic, chemical, and magnetic properties of surfaces and materials. Because most surfaces being examined are not homogeneous, it is often of interest to collect an array of force curves. Such an array produces information about the lateral distribution of different surface and/or material properties. For example, using a charged tip to probe a surface with patches of charge allows one to localize and characterize the patches on the surface. Previously, obtaining distributions of force curves over an area required manual setting of the x and y offset parameters to each new position in the array. This was tedious and resulted in sparse force sampling of the area. Now collecting this array of data is highly automated on Veeco Instruments AFMs and is referred to as "Force Volume Imaging".

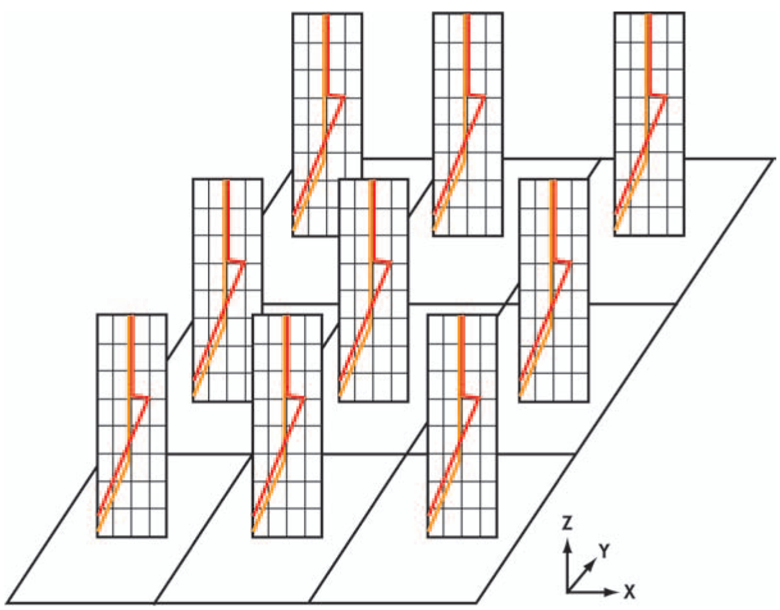


Figure 1b. A force volume data set – an array of regularly spaced force curves yields three-dimensional force information. In a force volume data set, the force curves will vary with x-y position.

How it Works

A simple force curve records the force felt by the tip or the particle attached to the cantilever as it approaches and retracts from a point on the sample surface (Figure 1a). A force volume contains an array of force curves over the entire sample area. Each force curve is measured at a unique x-y position in the area, and force curves from an array of x-y points are combined into a three-dimensional array, or "volume," of force data (Figure 1b).

The value at a point (x,y,z) in the volume is the deflection (force) on the cantilever at that position in space.

In this application note we discuss the use of contact mode force curves (i.e., DC measurements of deflection). However, volumes can also be produced from any available imaging mode such as TappingMode or phase imaging. Force volumes can be collected in any environment in which the microscope normally operates, including air and liquids.

When the tip comes into contact with the surface during a force curve cycle, the point of contact provides the topography of the sample at that x-y position. This can be used to produce a low (lateral) resolution height image of the surface, which is extremely useful when attempting to uncouple the interaction force data from topographic information. Since the tip is not dragged across the surface of the sample while obtaining the data, lateral forces, which might otherwise damage the sample, are very small.

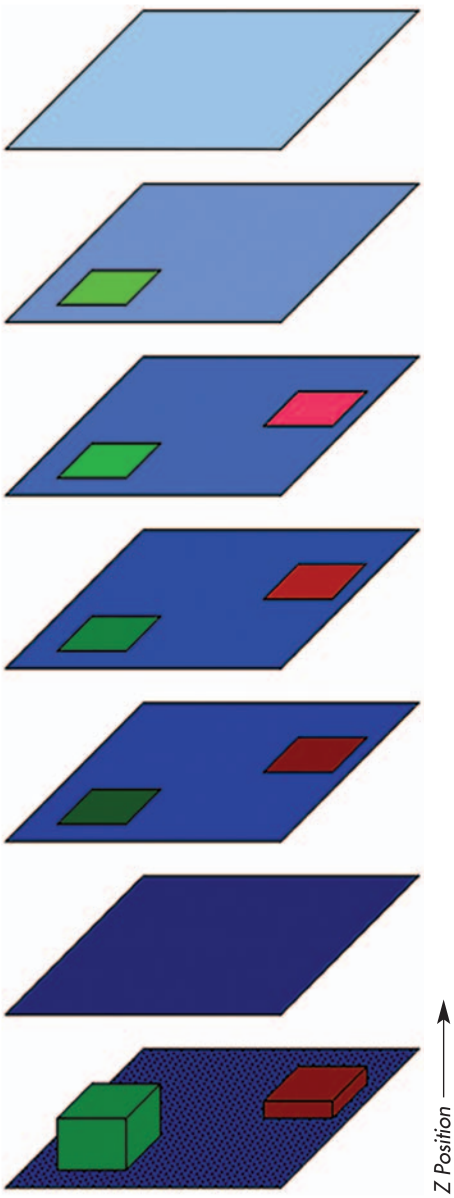


Figure 2. Horizontal slices through a force volume data set show the distribution of deflection values at each z position. In the figure, darker regions correspond to larger deflections. The top slice has a uniform distribution of the lowest force because the tip is not experiencing any force due to the surface. The lowest slice has a uniform high force because at each x-y position, the tip is in contact with the surface.

Applications

Force volumes allow investigation of the spatial distribution of almost any force between tip and sample that varies with the distance between the two. Since force is the derivative of energy with respect to distance, the volume data can be used to infer a potential energy map.

Digital Instruments NanoScope® software provides several methods to investigate the three-dimensional data set generated by force volume imaging. For example, simply clicking on the pixel corresponding to the x-y position of the curve(s) in the array (Figure 1) displays individual and multiple force curves. Horizontal slices through the volume of data can also be displayed, showing the distribution of deflection values at particular z-piezo positions (Figure 2). The graphic interface for force volume imaging is shown in Figure 3. After data collection, the images and curves can be processed with Digital Instruments NanoScope off-line analysis software.

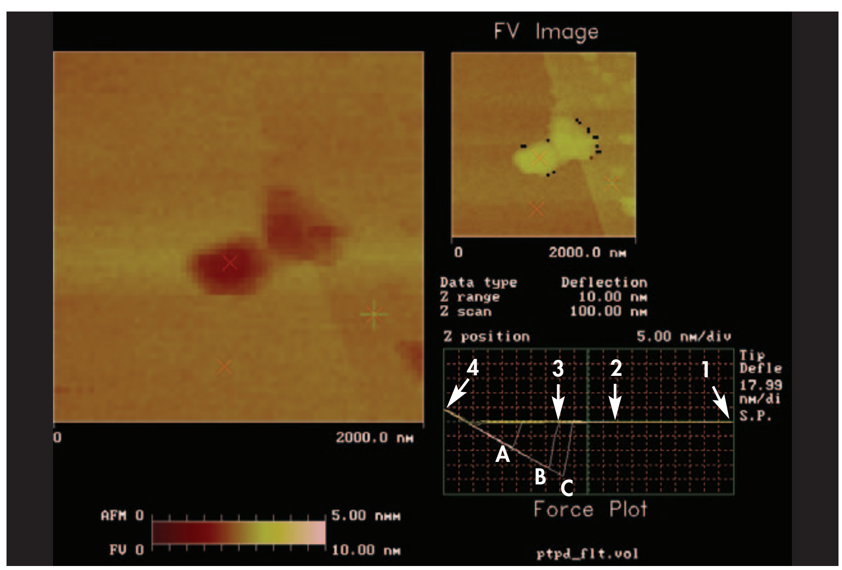


Figure 3. The force volume interface for Digital Instruments NanoScope software. The image is of a glass/platinum boundary. The large window on the left displays the height (or topography) image. Other imaging modes (friction, phase, etc.) can be displayed here. The rectangular window in the lower right displays the force curves as they are collected in real time. The small window in the upper right corner shows a slice through the volume of data. The force curves at particular x-y positions can be displayed by simply clicking on either the height or force volume image. 2μm scan.

Currently most force volume imaging is done with conventional AFM tips. However, modifying tips to confer specific chemical properties or attaching particles/molecules to act as specific probes allows a wide range of nanoscale interactions to be investigated.

Examples of the types of interactions that can be examined follow.

Adhesion Maps

Adhesion between the tip and sample can develop for numerous reasons. Under ambient conditions the presence of a capillary bridge between the tip and the fluid ("contamination") layer on the sample surface is the predominant origin of adhesion. Adhesion maps can be used to investigate the distribution of hydrophilic and hydrophobic regions on a surface which would differ in amount of hydration (and, therefore, adhesion; Figures 4 and 5). It is possible to perform the receptor-ligand type binding experiments that have been described in the literature in an attempt to map the distribution of a receptor or ligand in a cell membrane. A highly sensitive, specialized model AFM, the Digital Instruments MultiMode® PicoForce, has been developed by Veeco Instruments for these experiments (see www.veeco.com for more information).

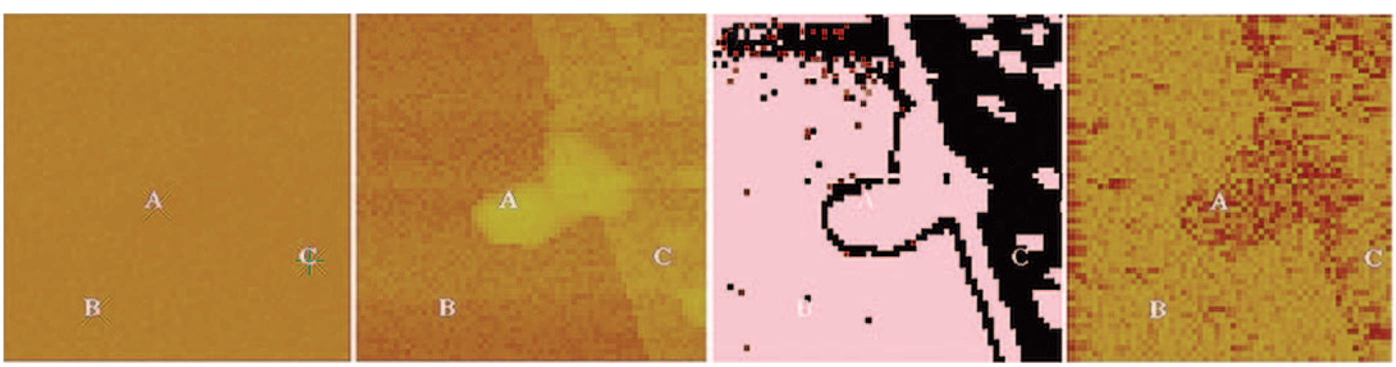
Elasticity Maps

Force curves can also be used to investigate the elastic properties of a material, by measuring the force required to indent or deform the surface. Force volumes can, therefore, be used to produce micro-elasticity maps of the sample that show local variations in surface stiffness (Figure 6). Different fabrication processes and treatments of materials can result in inhomogeneities within the material, resulting in different values of the Young's modulus. Elasticity maps can identify such defects. Similarly, elasticity measurements of biological cells, whose stiffness changes in response to many factors, may identify rearrangements of cytoskeletal elements and other cellular components.

Electrostatic Maps

Electrostatic interactions between two surfaces in liquids have been described in great detail, and excellent theoretical models exist. These models predict the force experienced by a charged particle approaching a charged surface. AFM force curves of a charged tip interacting with a charged surface can be fit to such models, providing information about the electrostatic properties of a surface (Figure 7). Knowledge of the distribution of surface charge density can, for example, offer insight into the adsorption behavior at heterogeneous liquid-solid interferences, as well as help characterize domains in membranes and other biological structures.

Figure 5. Adhesion map of the platinum/glass interface of Figure 3. Lighter pixels represent greater adhesion. The map was constructed by plotting the minimum value of each retracting curve at its x-y position. Note the variation of adhesion across the interface.



1. 2. 3. 4.

Figure 4. Horizontal slices taken from the force volume data set in Figure 3. Slices are 2μm square and are taken at the Z positions 1, 2, 3, 4 labeled in Figure 3. The distribution of adhesion forces is shown, i.e., slices from the retracting portion of the force curves. The labels (A,B,C) and numbering (1-4) are the same as in Figure 3.

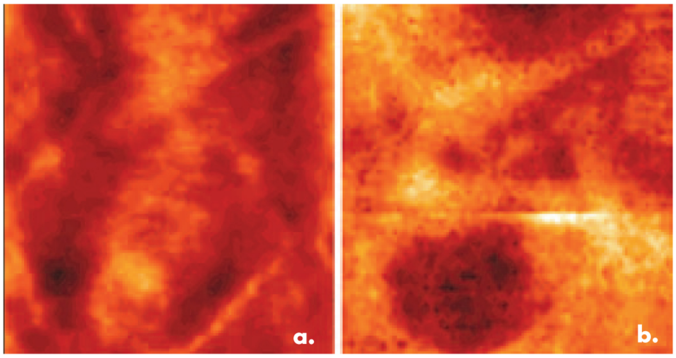


Figure 6. (a) Height image of MDCK cells on glass imaged with force volume. Lighter pixels represent higher topography. (b) Elasticity map constructed from the MDCK force volume. Lighter pixels represent greater stiffness. For this force volume, the software was set so that the tip would indent the cells to 100nm. 30μm scan.

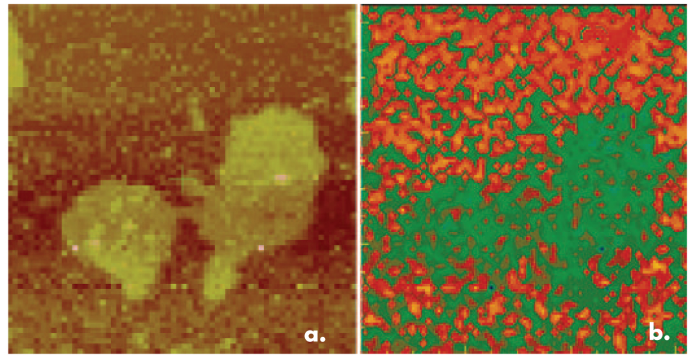


Figure 7. (a) Height image of a bacteriorhodopsin membrane adsorbed to a mica substrate in water imaged in force volume mode. 2.7μm scan. (b) Relative surface charge density map extracted from the fit of the force curve data in the volume to the Gouy-Chapman theory for a sphere approaching a plane.

Summary

Force volume imaging is a powerful AFM technique that can be used to investigate material, adhesive, electrical, magnetic and chemical properties of samples by recording an array of force curves over an entire area. A topographic image is also recorded with minimal damage to the surface. The information in the force volume measurement may be decoupled from topographic data to offer new insight into material and surface properties.

Suggested Readings

1. M. Radmacher, M. Fritz, J.P. Cleveland, D.A. Walters, P.K. Hansma "Imaging Adhesion Forces and Elasticity of Lysozyme Adsorbed on Mica with the Atomic Force Microscope" Langmuir 10, 3809-3814. (1994).
2. E. A-Hassan, W.F. Heinz, M.D. Antonik, N.P. D'Costa, S. Nagaswaran, C.A. Schoenenberger, and J.H. Hoh "Relative Micro-Elastic Mapping of Living Cells by Atomic Force Microscopy" Biophysical Journal 74:3. (1998).
3. C. Rotsch, M. Radmacher "Mapping Local Electrostatic Forces with the Atomic Force Microscope" Langmuir 13:2825-2832. (1997).