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Applications of Force Volume Imaging With *the NanoScope® Atomic Force Microscope*

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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 intermolecular forces between the tip (or particle of your choice) and a surface. This is done by recording the cantilever deflection as the tip approaches a surface and then plotting a force curve as a function of distance between the tip and the surface. 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 automated on NanoScope AFMs and is referred to as "Force Volume Imaging".

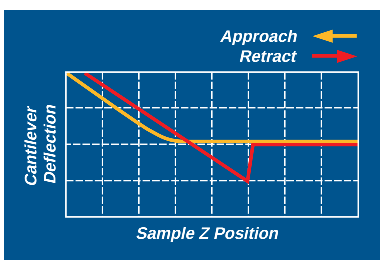


Figure 1A: Standard force curve with both the extending and retracting portion displayed.

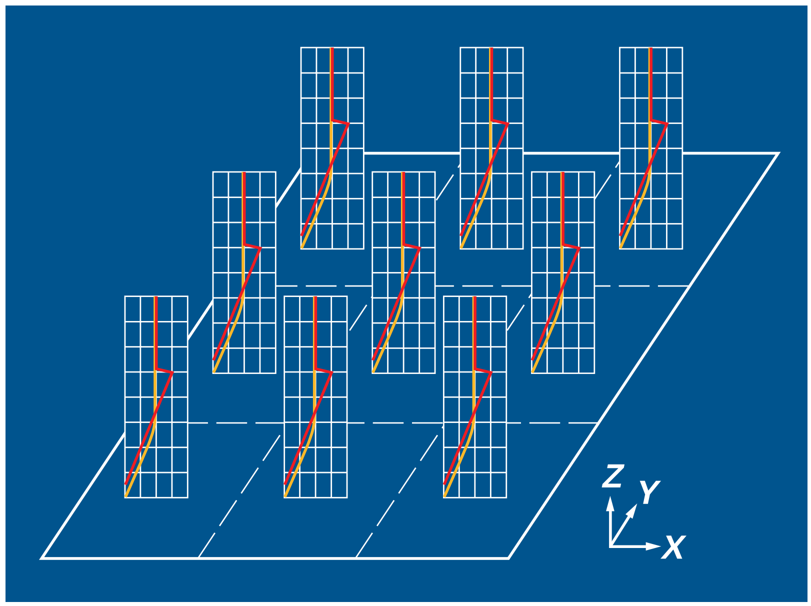


Figure 1B: A force volume data set – an array of regularly spaced force curves yields three-dimensional force information. All the force curves are identical in the figure to simplify the diagram. Of course, in a force volume data set, the force curves will vary with x-y position.

How it Works

A simple force curve records the force felt by the tip 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 and is generated by ramping the *z* piezo as the tip scans across the area

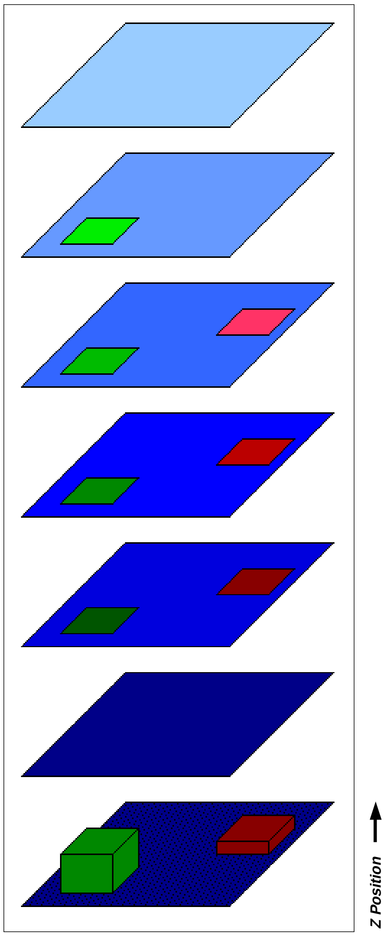


Figure 2: Horizontal slices through a force volume data set show the distribution of deflection values at each z position. In the figure, darker regions correspond to larger deflections. The top slice has a uniform distribution of the lowest force because the tip is not experiencing any force due to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

(Figure 1B). 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. The value at a point (*x,y,z*) in the volume is the deflection (force) of the cantilever at that position in space.

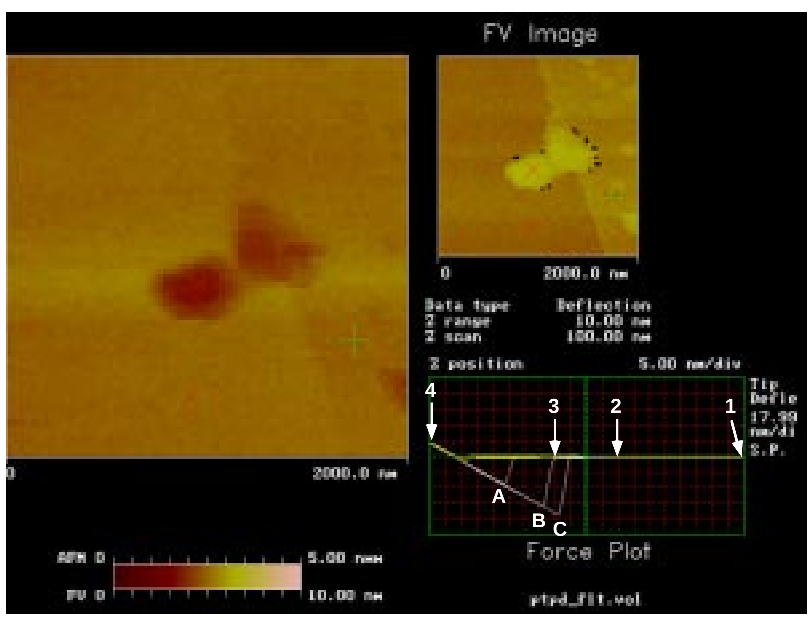
In this application note we only discuss the use of contact mode, (i.e., DC measurements of deflection) force curves. However, volumes can also be produced from any available imaging mode such as TappingMode™ or Phase Imaging. Force volumes can be collected in any environment in which the microscope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

When the tip comes into contact with the surface during a force curve, 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.

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 the potential energy between surfaces.

The 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 the standard complement of NanoScope off-line analysis software.

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 a number of reasons. Under ambient conditions the presence of a capillary bridge between the tip and the fluid (“contamination”) layer on the sample surface 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). In principle, 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. However, several investigators have attempted this and it remains an extremely challenging experimental problem.

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 anisotropic

Figure 3: *The force volume interface for the NanoScope software. The image is of a glass/platinum boundary. The large window on the left displays the height image, determined by the type of information in Channel 1 of the microscope. In contact mode, this could be a deflection image or a height image. Other imaging modes (TappingMode, friction, 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.*

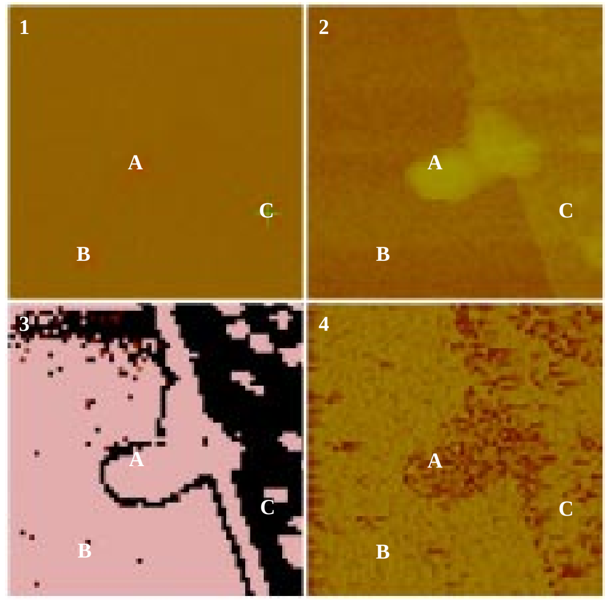
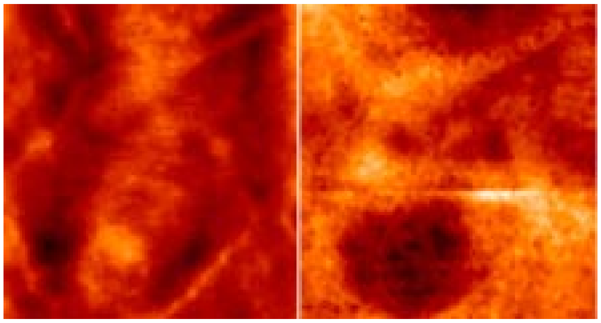


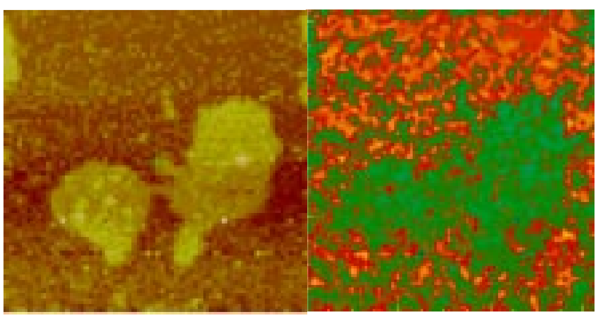
Figure 4: Horizontal slices taken from the force volume data set in Figure 3. Slices are 2µm square and are taken at the Z positions labeled in Figure 3. The distribution of adhesion forces is shown, i.e., slices from the retracting portion of the force curves. The labels (A,B,C) and numbering (1-4)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

Figure 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.



(a) (b)

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, a relative trigger was set so that the tip would indent the cells to 100nm. 30µm scan.



(a) (b)

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.

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 solution 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.

Summary

Force volume imaging is a powerful feature 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 can be decoupled from topographic data to offer new insight into material and surface properties. Force volume imaging capability requires no new hardware, it is standard in all NanoScope III software v4.2 and higher, and can be applied in all imaging environments. Force volume imaging will likely become a standard tool for studying nanoscale forces and interactions.

Suggested Readings

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