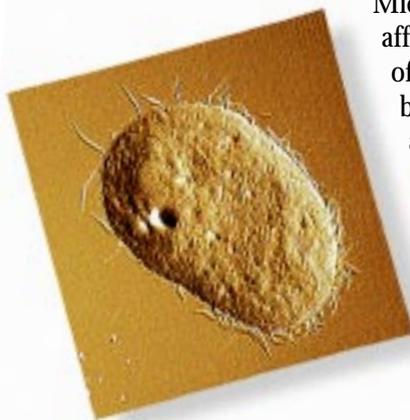


Probing Nano-Scale Forces *with the Atomic Force Microscope*

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The ability of the atomic force microscope (AFM) to create three-dimensional micrographs with resolution down to the nanometer and Angstrom scales has made it an essential tool for imaging surfaces in applications ranging from semiconductor processing to cell biology. In addition to this topographical imaging, however, the AFM can also probe nanomechanical and other fundamental properties of sample surfaces, including their local adhesive or elastic (compliance) properties.



Microscopic adhesion affects a huge variety of events, from the behavior of paints and glues, ceramics and composite materials, to DNA replication and the action of drugs in the human body. Elastic proper-

Figure 2: Typical 3-D surface topographic image produced by the AFM. Sample shown is a *Tetrahymena* Protozoan. 50 μ m scan courtesy E. Henderson, BioForce Labs and Iowa State University.

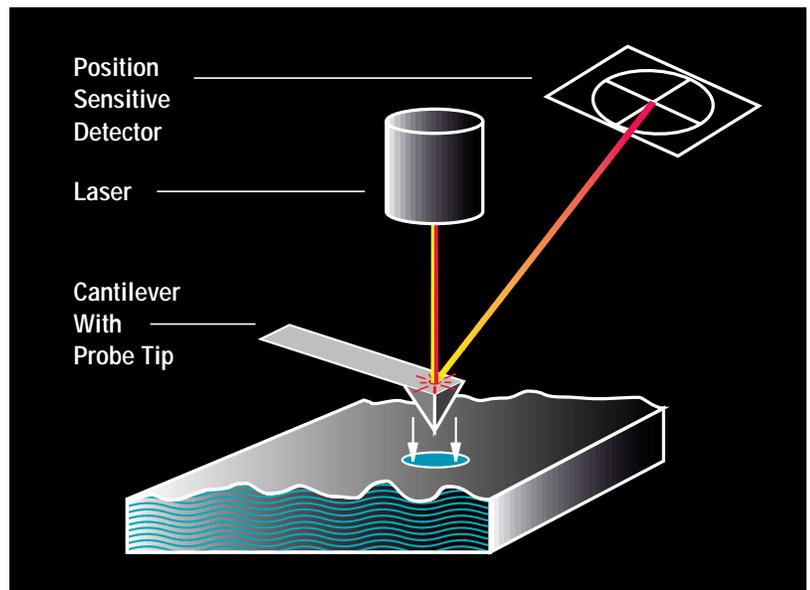


Figure 1: Schematic of an atomic force microscope (AFM) showing the force sensing cantilever.

ties are similarly important, often affecting the structural and dynamic behavior of systems from composite materials to blood cells. AFM offers a new tool to study these important parameters on the micron to nanometer scale using a technique that measures forces on the AFM probe tip as it approaches and retracts from a surface. This application note describes measurement and construction of “force curves” and some of the important applications for this new technology.

Basic AFM Operation

A key element of the AFM is its microscopic force sensor, or cantilever (Figure 1). The cantilever is usually formed by one or more beams of silicon or silicon nitride that is 100 to 500 μ m long and about 0.5 to 5 μ m thick. Mounted on the end of the cantilever is a sharp tip that is used to sense a force between the sample and tip. For normal topographic imaging, the probe tip is brought into continuous or intermittent contact with the sample and



Figure 3: Digital Instruments MultiMode™ AFM, optimized for high resolution force measurements and topographic imaging.

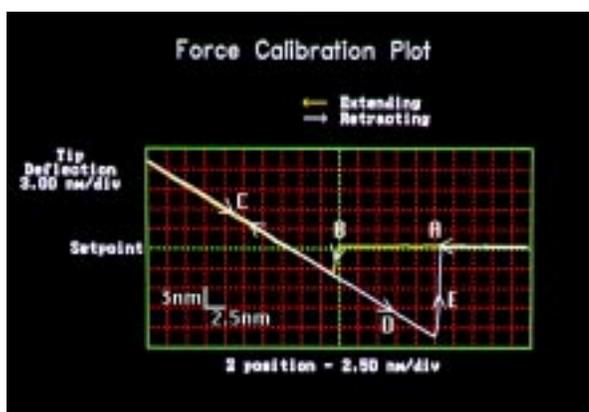


Figure 4-1: AFM Force Measurement (“force curve”) as displayed on Digital Instruments NanoScope® SPM system. The force curve plots the deflection of the force-sensing cantilever as the tip is extended toward the sample (yellow) and retracted from the sample (white). The labeled points on the curve (A-E) are illustrated in Fig. 4-2 (opposite).

raster-scanned over the surface. Fine-motion piezoelectric scanners are used to generate the precision motion needed to generate topographic images and force measurements. A piezoelectric scanner is a device that moves by a microscopic amount when a voltage is applied across its electrodes. Depending on the AFM design, scanners are used to translate either the sample under the cantilever or the cantilever over the sample. Piezoelectric scanners for AFMs usually can translate in three directions (x , y , and z axes) and come in different sizes to allow maximum scan ranges of 0.5 to 125 μm in the x and y axes and several microns in the vertical (z) axis. A well-built scanner can generate stable motion on a scale below 1 Angstrom.

By scanning the AFM cantilever over a sample surface (or scanning a sample under the cantilever) and recording the deflection of the cantilever, the local height of the sample is measured. Three-dimensional topographical maps of the surface are then constructed by plotting the local sample height versus horizontal probe tip position (a summary article on the basics of AFM is available on request from Digital Instruments). Other imaging techniques are also used including measuring the change in amplitude or phase of an oscillating cantilever, using TappingMode™, for example.

Force Curve Measurements

In addition to these topographic measurements, the AFM can also provide much more information. The AFM can also record the amount of force felt by the cantilever as the probe tip is brought close to — and even indented into — a sample surface and then pulled away. This technique can be used to measure the long range attractive or repulsive forces between the probe tip and the sample surface, elucidating local chemical and mechanical properties like adhesion and elasticity, and even thickness of adsorbed molecular layers or bond rupture lengths.

To help examine the basics of AFM force measurements, Figure 4-1 shows a typical “force-versus-distance” curve or force curve, for short, as generated by a NanoScope III AFM system (generation and analysis of these curves is discussed in more detail in the sidebar, “Details of Force Measurements”). Force curves typically show the deflection of the free end of the AFM cantilever as the fixed end of the cantilever is brought vertically towards and then away from the sample surface. Experimentally, this is done by applying a triangle-wave voltage pattern to the electrodes for the z -axis scanner. This causes the scanner to expand and then contract in the vertical direction, generating relative motion between the cantilever and sample. The deflection of

the free end of the cantilever is measured and plotted at many points as the z -axis scanner extends the cantilever towards the surface and then retracts it again. By controlling the amplitude and frequency of the triangle-wave voltage pattern, the researcher can vary the distance and speed that the AFM cantilever tip travels during the force measurement.

Similar measurements can be made with oscillating probe systems like TappingMode and non-contact AFM. This sort of work is just beginning for oscillating probe systems, but measurements of cantilever amplitude and/or phase versus separation can provide more information about the details of magnetic and electric fields over surfaces and also provide information about viscoelastic properties of sample surfaces.

Anatomy of a Force Curve

Several points along a typical force curve are shown schematically in Figure 4-2. The cantilever starts (point A) not touching the surface. In this region, if the cantilever feels a long-range attractive (or repulsive) force it will deflect downwards (or upwards) before making contact with the surface. In the case shown, there is minimal long-range force, so this “non-contact” part of the force curve shows no deflection. As the probe tip is brought very close to the surface, it may jump into

contact (B) if it feels sufficient attractive force from the sample. Once the tip is in contact with the surface, cantilever deflection will increase (C) as the fixed end of the cantilever is brought closer to the sample. If the cantilever is sufficiently stiff, the probe tip may indent into the surface at this point. In this case, the slope or shape of the contact part of the force curve (C) can provide information about the elasticity of the sample surface.

After loading the cantilever to a desired force value, the process is reversed. As the cantilever is withdrawn, adhesion or bonds formed during contact with the surface may cause the cantilever to adhere to the sample (section D) some distance past the initial contact point on the approach curve (B). A key measurement of the AFM force curve is the point (E) at which the adhesion is broken and the cantilever comes free from the surface. This can be used to measure the rupture force required to break the bond or adhesion.

Beginnings — Controlling Surface Adhesion

One of the first uses of force measurements was to improve the quality of AFM images by monitoring and minimizing the attractive forces between the tip and sample. Large adhesive forces can reduce imaging resolution, damage the sample and probe, and/or create unwanted artifacts.

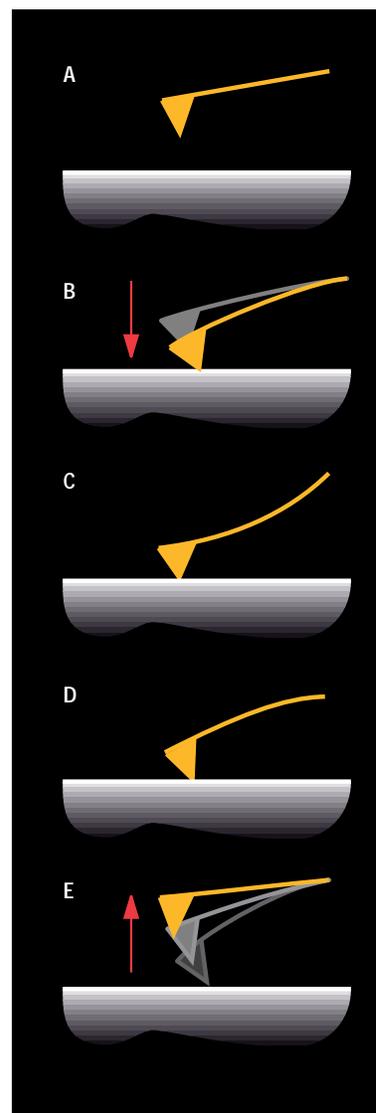


Figure 4-2: AFM Force Measurement (“force curve”) of cantilever-sample interaction shown schematically at several points along the force curve shown in Fig. 4-1 (opposite), including: A) the approach (“non-contact” region), B) jump to contact, C) contact, D) adhesion, and E) pulloff.

Details of Force Measurements

This section discusses in more detail the process used to make AFM force measurements. As described above, force measurements are made by recording the deflection of the free end of the AFM cantilever as the fixed end of the cantilever is extended towards and retracted from the sample. AFMs typically measure cantilever deflections by bouncing a laser beam off the free end of the cantilever (Figure 1). Deflections of the cantilever cause the reflected laser beam to change its angle. The motion of the reflected laser beam is detected by a multiple segment photodiode known as a Position Sensitive Detector (PSD). The vertical sensitivity of an AFM measurement depends on the length of the cantilever—for the same vertical deflection shorter cantilevers produce a larger angular change than longer cantilevers.

The basic “force curve” as measured by an AFM is actually a curve showing “PSD voltage versus scanner position.” The PSD voltage can be converted into a cantilever deflection distance by a simple calibration procedure: The AFM cantilever tip is brought in contact with a hard surface and then the cantilever or sample is moved by a known amount using the z -axis scanner. The AFM system records how much PSD voltage is generated for a known deflection, and this conversion factor is used to convert future PSD measurements into cantilever deflections.

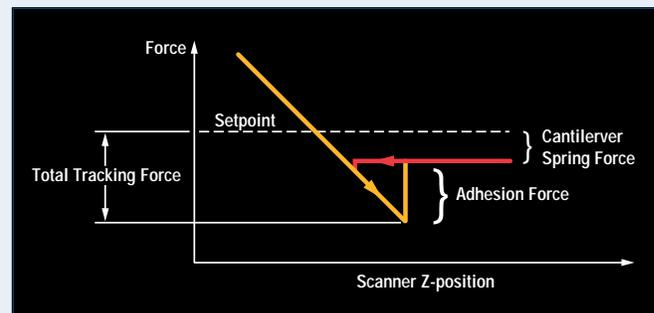
Calculating Forces

The force sensed by the AFM probe is calculated by multiplying the deflection of the cantilever by the cantilever’s spring constant. For example, if a cantilever with a spring constant of 1 Newton per meter is deflected by 1nm, the spring force exerted by the cantilever is 1nN. The spring constant of AFM cantilevers are determined both by the geometric properties (lengths, widths, and thickness), and by the elastic modulus of the cantilever material. Cantilevers are available with a variety of spring constants from roughly 0.01 to 100 Newtons/meter (or nanoNewtons/nanometer), providing a range of measurement capabilities for various surfaces. Because cantilever spring constants can vary from cantilever to cantilever, several methods have been developed to measure (90-95) or calculate (96-98) the cantilever spring constant.

Setpoint and Tracking Forces

The majority of this article discusses using force curves to measure forces between surfaces under study or probing the mechanical properties of sample surfaces. Another common use of force curves is to set the tracking force applied to the sample by the AFM tip during imaging. AFMs usually work by maintaining a constant deflection of the cantilever or, for oscillating cantilever techniques, a constant amplitude. The tracking force is adjusted by changing the “setpoint” deflection or amplitude. Force curves provide a graphic way of seeing how much force is exerted by a given deflection or amplitude setpoint (see Figure). For contact

mode AFM, the total amount of force exerted on a sample comes from two sources—the cantilever spring force *plus* any adhesion between the tip and sample. These two sources are indicated in the figure. The spring force results from bending the cantilever from its equilibrium (zero-force) position. The “setpoint” shown in the figure is adjusted by the user depending on the amount of cantilever spring force desired. Any adhesion forces between the tip and sample will add to the selected spring force, resulting in a “total tracking force,” as shown. Force curves are useful for adjusting the tracking force because they allow the user to visualize the amounts of cantilever spring force and adhesion, and then to select an appropriate setpoint. In practice, the setpoint is usually selected to minimize the cantilever spring force. Sometimes, however, it is possible to choose a setpoint that results in a “negative” spring force. In this case, the cantilever bends downwards toward the sample and its spring force opposes the adhesive force. “Negative” spring forces can produce the lowest total tracking forces, but imaging can also be unstable as the cantilever may unexpectedly spring off the sample surface.



Force vs. Separation

For some measurements it is also desirable to replace the “force-versus-scanner-position” curve with a “force-versus-separation” curve. In the presence of long-range forces, the free end of the cantilever will bend towards or away from the sample surface during the measurement. In this case, the motion of the end of the cantilever will no longer match the motion of the z -axis scanner. But “force-versus scanner position” curves can easily be converted to “force-versus-separation” plots by simply correcting the recorded scanner position by the measured deflection of the cantilever. For example, if the scanner moves 10nm toward a surface, and the cantilever tip is attracted to the surface and also deflects 2nm towards the surface, the actual tip-sample separation is reduced by 12 nm. Force-versus-separation curves are often more useful for comparing measurements with theory because they show directly the dependence of the force on distance between the tip and sample. In fact, Dr. Derek Chan of the University of Melbourne has written analysis software for AFM force measurements. (99) The software graphically compares force-versus-separation curves to predictions for Van der Waals, electrostatic, and other forces, and it can extract such properties as sample surface charge.

In 1989, Weisenhorn and Hansma of the University of California at Santa Barbara (1) noted that the typical adhesive force between a standard silicon nitride AFM tip and a mica surface imaged in ambient air was about 100 nanoNewtons ($nN = 10^{-9}$ Newtons); however, they found that this force could be reduced by a factor of 100 (to about 1nN) by simply immersing the sample in water (Fig 5). The reason for the large adhesion in ambient air is that most samples in air are covered by a thin layer of water and other condensed contaminants. These contaminants often form a capillary bridge between the tip and sample, generating large adhesive forces. When the entire cantilever is completely submerged in liquid, these capillary forces largely disappear.

Mate and others used the onset of the capillary force to their advantage. By recording the distance between the onset of capillary force and the point when the AFM tip contacts the surface, they could measure the thickness of the adsorbed molecular layers, down to a thickness of 2nm. (2)

Since these discoveries, many refinements have been made. Weihs and others (3-5) noticed that the adhesive force between the AFM tip and sample decreased with tip radius. Sharper tips yield lower adhesive forces and, therefore, lower imaging forces. Force measurements were also used to demonstrate similarly reduced capillary forces for samples in vacuum (6) and

in reduced humidity environments. (5, 7)

In 1990, Hartmann (8) calculated that attractive Van der Waals forces could be substantially reduced by operating in appropriate solvents, especially ethanol. Weisenhorn and others (9-11) confirmed with AFM force measurements that tip-sample adhesion was dramatically reduced in ethanol as well as other solvents. Even small additions of ethanol to water reduced adhesive forces up to a factor of 100 over pure water; the effect was probably enhanced by ethanol's ability to remove some organic contaminants from the sample surface. Thundat (12) also noticed reduced adhesion between the AFM tip and sample by first treating the tip with ozone or ultraviolet light to remove organic contamination. The combination of these and other improvements can reduce tip-sample forces during AFM imaging to less than 0.1nN. AFM users now routinely use force curve measurements to check tip-surface adhesion to ensure that the tip and sample are free of contamination that could hinder AFM imaging.

However, controlling surface contaminants may only be half the story. Alley (13) recently observed improved image quality on silicon surfaces by treating the AFM tip with a silane monolayer using octadecyltrichlorosilane (OTS). Even in ambient air, Alley noted adhesive forces of less than 1nN,

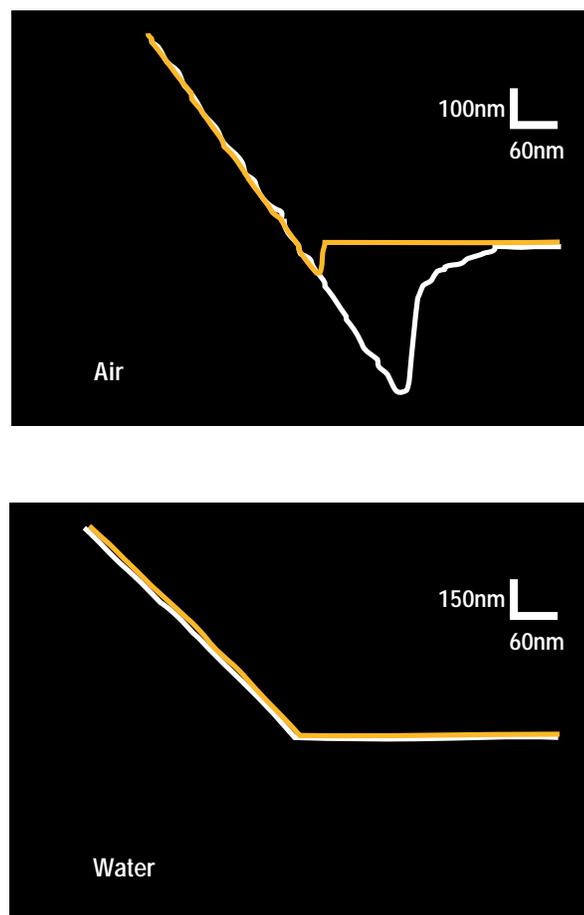


Figure 5: Measurement of the forces between an AFM tip and a mica surface in air and water. The measurement in air shows a large adhesive force due to capillary forces from the liquid contaminant layer on the surface. Once the whole cantilever probe is immersed in water, capillary forces are mostly eliminated and adhesion is substantially diminished. From A.L. Weisenhorn, reference (1).

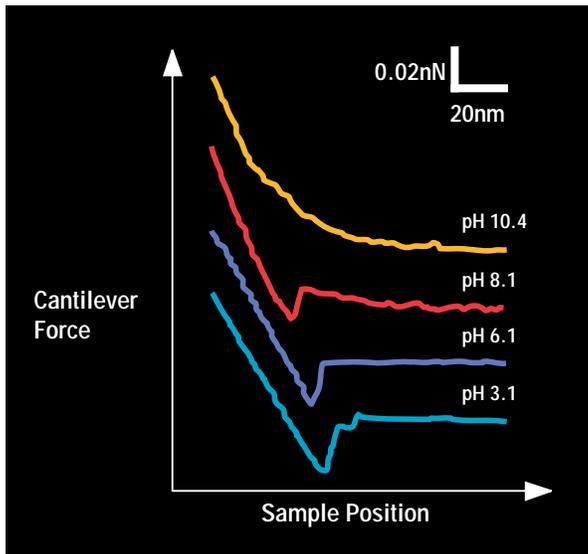


Figure 6: Force measurements from a NanoScope AFM system of the tip-sample forces between a silicon nitride cantilever and a mica surface under varying pH. Notice that at low pH there is a very strong attraction between the tip and sample. At high pH, the situation is reversed where long-range repulsion dominates. At an intermediate pH, the overall tip-sample forces can be minimized. Reprinted by permission, reference (27).

again about two orders of magnitude less than for an untreated tip. By reducing the force that the AFM applies to the sample surface, all of these discoveries have helped improve AFM image quality.

Measuring Fundamental Forces

Concern with the fundamental interactions between surfaces extends across physics, chemistry, materials science and a variety of other disciplines.

With a force sensitivity on the order of a few piconewtons ($\text{pN} = 10^{-12} \text{ N}$), AFMs are excellent tools for probing these fundamental force interactions. (14-18) For example, AFM has been used to probe the nature of attractive Van der Waals and attractive/repulsive electrostatic forces between such systems as metal probes and insulating mica surfaces (19, 20), and insulating probes on insulating and conducting samples with such materials as silicon nitride, diamond, alumina, mica, glass and graphite. (4, 9, 21, 22)

With an eye towards studies of adhesion, friction and wear, Binggeli and Mate (23) have used AFM to study the formation or suppression of capillary condensation on hydrophilic silicon, amorphous carbon, and lubricated SiO_2 surfaces. Torii has also studied variations in capillary adhesion on glass and rubber samples caused by material differences and variations in surface topography. (24) Radmacher et al. have even measured the forces between an AFM tip and individual protein molecules. (25)

Measurements in Liquids

As discussed above, force measurements in water revealed the benefits of AFM imaging in this environment due to the lower tip-sample forces. Some of the most interesting force measurements have also been performed with samples under

liquids where the environment can be quickly changed to adjust the concentration of various chemical components.

In liquids, electrostatic forces between dissolved ions and other charged groups play an important role in determining the forces sensed by an AFM cantilever. Butt, Jaschke and Ducker (26) have recently written an excellent review of AFM force measurements in liquid environments. The liquid environment has become an important stage for fundamental force measurement because researchers can control many of the details of the probe surface force interaction by adjusting properties of the liquid. Experimentally, Butt and others (21, 27, 28) have shown, for example, that electrostatic tip-sample forces depend strongly on pH and salt concentration (Figure 6). In fact, Butt showed that it is often possible to adjust the pH or salt concentration such that the attractive Van der Waals forces are effectively negated by repulsive electrostatic forces. This has been an important discovery because it can allow tuning of the liquid environment to minimize adhesive tip-sample forces that can damage the sample during imaging.

Lin et al. (29) have also measured force profiles in liquid at various pH values to determine the surface charge of an Si_3N_4 AFM tip and, as a result, the isoelectric point, the pH (6.0 in their experiment) at which the

AFM tip was electrically neutral. Further details of surface chemistry and tip-sample interactions in fluid for Si_3N_4 AFM tips have been determined by Senden and Drummond. (30) Drummond and Senden have also recently published a method for determining the radius of a Si_3N_4 tip by measuring electric double layer forces in liquid. (31) The liquid environment can also be used to prepare surfaces or solutions that contain more complex molecules with more detailed force interactions. For example, Torii and coworkers have compared adhesive forces between the AFM tip and a silica surface in a variety of organic and inorganic solutions. (32, 33)

Quantized Adhesion

Butt's balancing of attractive and repulsive forces has also been used to quantify the adhesion between a silicon nitride tip and a glass surface. (34, 35) Hoh noticed that when he adjusted the overall tip-sample adhesion to around 1nN, repeated measurements of the adhesive force varied in discrete units of around 0.01nN. More recent work by Cleveland et al. (36) suggests that these discrete interactions may be caused by the ordered layers of water molecules that form on the sample surface, creating periodic force "steps" for the probe tip. By analyzing the quantization of forces, the strength of single binding events can be determined.

Colloidal Forces (Top Work from Down Under)

Australia has been an important source of recent work on AFM force measurements involving colloids. William Ducker and co-workers have pioneered the attachment of colloidal spheres (particles on the micron scale) to AFM cantilevers and then measuring forces between the sphere and a sample of interest (Figure 7). This is an extremely interesting technique because, as Ducker writes, "The forces between colloidal particles dominate the behavior of a great variety of materials, including paints, paper, soils, clays and, in some circumstances, biological cells." (37) Colloid forces also play a critical role in the formation of ceramics and composite materials.

Colloidal spheres of glass, silica, polystyrene, various metals and other materials are readily available, allowing use of probes of known size and shape such that normalized force curves can be calculated and quantified. These quantitative force measurements have allowed detailed studies of such phenomena as surface charging in liquids, polymer-solid interactions, and hydrophobic interactions, to name a few.

Using colloidal probes, studies have been made of interaction forces between silica, (37) gold, (38) titanium dioxide, (39, 40) zirconia, (41) and polystyrene and p-type silicon (42). Biggs

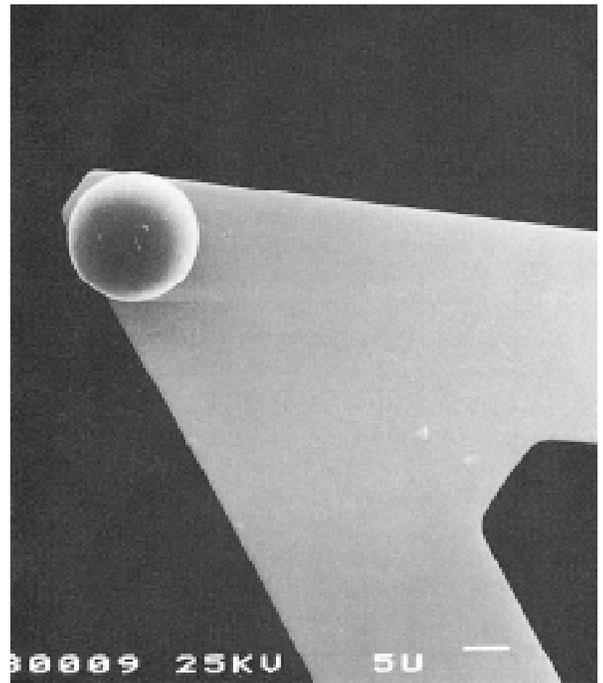


Figure 7: The AFM can measure forces on colloidal particles. In this case, a silica bead was glued to an AFM cantilever to probe the force between the bead and water droplets or air bubbles. Reprinted by permission, reference (44).

and Healy (41, 43) have measured steric and bridging forces between colloidal zirconia and polymer chains of polyacrylic acid. Steric forces are forces associated with the entropic arrangement or ordering of atoms and molecules; these forces can dominate the structure of slurries used in making ceramics. Biggs and Healy used the AFM to move the zirconia probe in and out of the polymer layer at a rate similar to those expected for Brownian collisions in a ceramic slurry and to measure the effect of pH on this

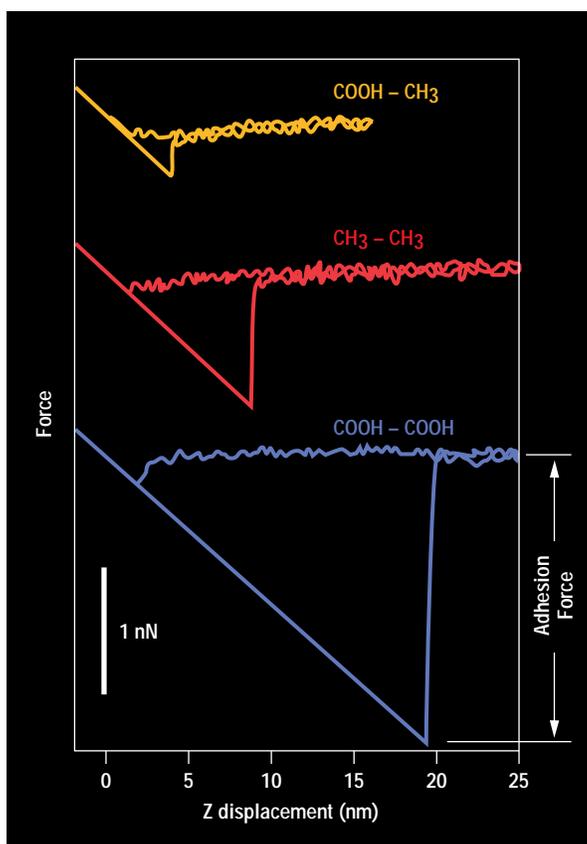
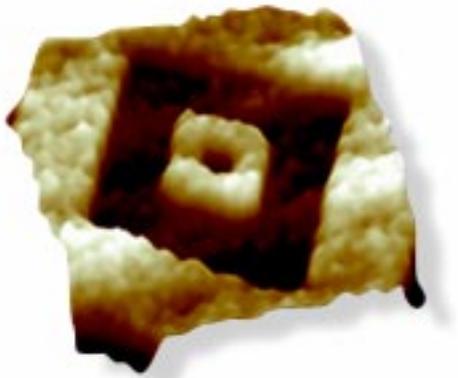


Figure 8: (Top) AFM friction (chemical force) measurement of patterned organic monolayers on gold as scanned with tip coated with similar monolayer. The difference in friction is due to different functional groups terminating the patterned regions of the sample. 50 μ m scan.

(Bottom) Force curves from a NanoScope MultiMode AFM on patterned regions for different tip and sample functional group terminations. Reprinted by permission, references (55, 56).

steric force. Butt has even measured the forces on a colloidal glass particle as it enters an air bubble or water drop. (44)

Chemical Sensitivity

The above discussion illustrates that AFM force measurements are extremely sensitive to tip and surface properties. Extending this work to the chemical realm, Burnham and co-workers (45) used AFM force measurements to distinguish two similar samples, each covered with a single chemical monolayer — the only difference being that one surface was terminated with CH_3 groups, the other with CF_3 . O'Shea (46) also observed changes in frictional forces between the AFM tip and surface that were dependent on the presence or absence of a single molecular layer. Olsson (47) studied methylated silicon samples with a tungsten tip and found he could identify 100nm-sized methylated patches on the silicon by observing variations in the capillary forces between the tip and sample.

Additionally, Ishino and co-workers have recently used the AFM to detect the charge polarity of various functional monolayers deposited on gold. (48, 49) For this work, they held the gold sample surface under electrochemical control and measured the AFM tip-sample force while adjusting the potential on the gold surface.

Similar measurements of electrostatic charge density on biological membranes have been performed by Butt (see Biological Applications, below). Electric double layer forces have also been studied on self-assembled monolayers (hexadecyltrimethylammonium halides), deposited on colloidal silica spheres. (50, 51)

Chemical sensing has been taken one step further by binding sensing molecules to the AFM tip or to colloidal spheres attached to the AFM cantilever. Surface chemistry techniques already used — for example in liquid chromatography — offer many model preparations for creating AFM probes with chemically modified surfaces. Molecules bound to the AFM probe can then be used as chemical sensors to detect forces between molecules on the tip and target molecules on the surface. Ducker and others have used this technique to create hydrophobic AFM colloidal probes to study the nature of the hydrophobic force. (52, 53) When octadecyltrichlorosilane (OTS) was bound to AFM tips, Nakagawa et al. (54) found that the AFM could discriminate between monolayers of 4 different alkyltrichlorosilanes ($\text{CH}_3(\text{CH}_2)_n\text{SiCl}_2$ with $n = 1, 8, 13, 17$) deposited on silicon. Charles Lieber and his team at Harvard bound functionalized thiol monolayers to gold-coated AFM cantilever tips and also to gold-coated target samples. By varying the functional group

terminating the monolayers on the tip and sample, they were able to study the adhesion and friction between combinations of acid ($-\text{COOH}$) and methyl ($-\text{CH}_3$) functional groups (55, 56) (Figure 8).

Biological Applications

The life sciences are a natural arena for the study of nano-scale force interactions. Countless biological processes — DNA replication, protein synthesis, drug interaction, and many others — are largely governed by intermolecular forces. And with its sensitivity down to tens of piconewtons, the AFM is an excellent tool for probing these interactions. In addition, the AFM can make nanomechanical measurements (like elasticity) on biological specimens, offering insight into subjects like cellular and even protein dynamics. Recently, for example, Dammer and co-workers reported in *Science* that they had used the AFM to measure the binding strength of cell adhesion proteoglycans (molecules containing a mixture of proteins and sugars) from a marine sponge (Figure 9). (57) They measured the force between a single pair of proteoglycan adhesion molecules to be as high as 400pN, strong enough to hold the weight of 1600 cells. They reasoned that this high binding force per molecule and the 1000 proteoglycan bonds per cell are responsible for the substantial structural integrity of the marine sponge.

Binding Forces

As with the chemically-modified probes described above, biological researchers are finding growing applications for AFM probes that have been tailored to sense a specific biological reaction. For example, Florin, Moy and Gaub recently measured the binding forces of individual ligand-receptor pairs (58-60) (Ligands are molecules that bind to a specific site on another molecule, usually a protein. A receptor is a protein that receives and binds a ligand molecule and then produces some response). These measurements were accomplished by modifying an AFM probe tip so that its outer surface was coated with avidin receptor molecules (Figure 10). Gaub's team used agarose beads coated with various forms of biotin, a ligand that binds to avidin. By blocking most of the adhesive force with free avidin molecules in solution, the group was able to reduce the total adhesion to a point at which they were able to see the rupture of a finite number of bonds. By analyzing the variation in the adhesive forces, the researchers determined individual receptor-ligand binding forces to be 160pN for biotin-avidin and 85pN for iminobiotin-avidin. They were also able to calculate the effective rupture length of the biotin-avidin bond.

Lee and co-workers (61) have used a technique similar to that of Florin to directly measure interaction forces between

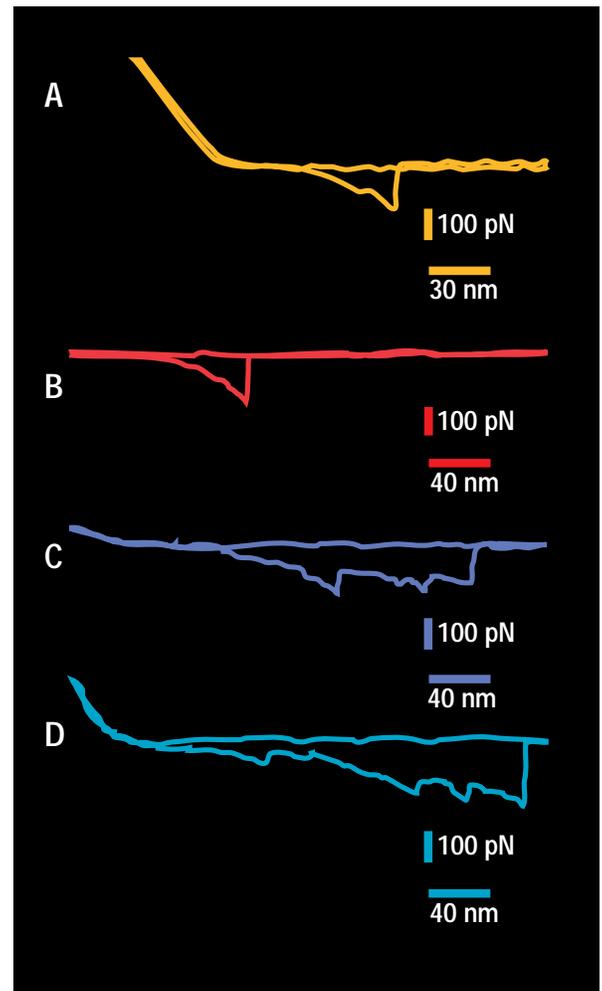
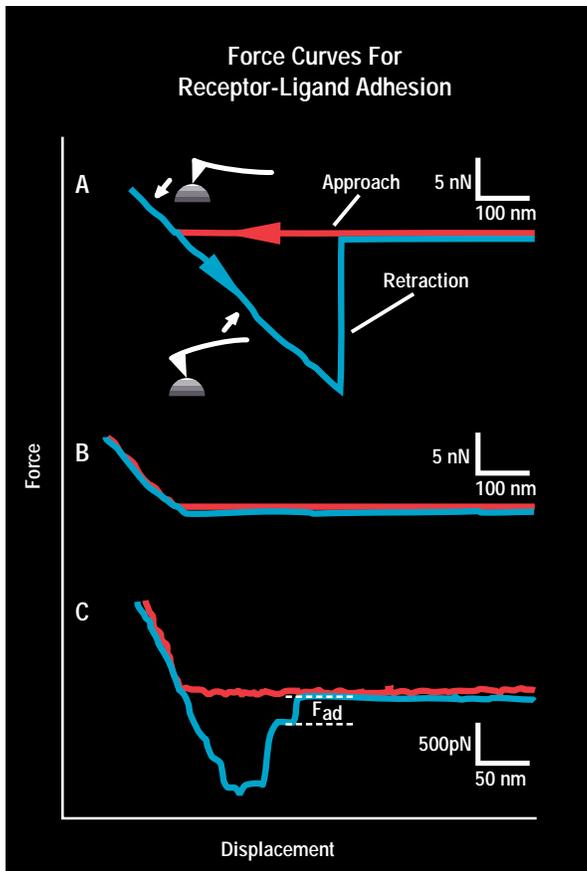
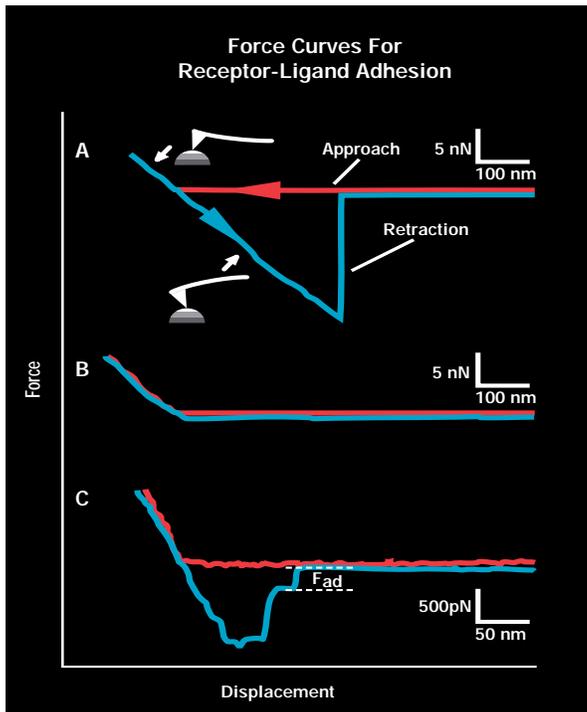


Figure 9: Typical AFM force curves for adhesion proteoglycan (AP) molecules on both the AFM tip and on a mica substrate. The retracting part of the force curves shows discrete jumps as AP-AP bonds are broken. Reprinted by permission, reference (57). Note that the NanoScope MultiMode AFM can resolve picoNewton-scale forces.



complementary chains of single-stranded DNA. Single-stranded DNA, twenty bases long, was bound to a sample surface and also to a spherical probe attached to the AFM cantilever. The DNA strands on the spherical probe contained a sequence that was complementary to that of strands on the sample. Lee observed a distribution of binding forces with peaks at three distinct values: 1.52, 1.11, and 0.52 nN. The researchers attributed the distribution of binding forces to the formation and then rupture of 20, 16, or 12 base pairs.

Boland and Ratner (62) at the University of Washington recently measured the binding forces between various bare DNA nucleotide bases. They created self-assembled monolayers of DNA bases on a gold sample substrate and also on a gold-coated AFM tip. Binding forces due to hydrogen bonding were only observed when the tip and sample were coated with complementary bases, for example adenine (A) on the tip and thymine (T) on the surface.

Tethered Molecular Sensors

Schindler and colleagues at the University of Linz, Austria have developed another novel technique for measuring binding forces and foresee a technique for imaging binding sites with the AFM. Schindler's team has created a technique they call sensor force microscopy (63, 64) in which a single biological molecule (the sensor) is attached to the AFM tip on the end of a molecular tether (Figure 11). The molecular tether is an elastic linker molecule a few nanometers long based on polyethylene glycol (PEG) molecules. The PEG tether gives the sensor molecule the freedom to orient itself properly to bind to its target on the surface. When a binding event takes place, the AFM detects the additional force required to break the molecular adhesion. Schindler's group has used this technique to record binding forces between human serum albumin (HSA) and its associated antibody (Ab_{HSA}), with measurements averaging 470 pN.

Figure 10: (Top) Schematic of the avidin-functionalized AFM tip and the biotinylated agarose bead, shown here partially blocked with avidin.

(Bottom) Cantilever deflection curves on approach and retraction of an avidin tip on a biotinylated agarose bead (A) before and (B) after blockage with an excess of free avidin (200 μ g/ml). (C) Magnification of a force scan on a biotinylated agarose bead approximately 95% blocked with free avidin. F_{ad} is the measured unbinding force plotted. Reprinted by permission, reference (58).

Because a single molecule can be bound to the end of the tether, Schindler's group is optimistic that this technique will be successful in creating entire images of binding sites by scanning the sensor molecule across the surface to detect binding events. The high sensitivity of the AFM's force detection and the growing experience in measuring biological binding forces holds great promise for the study of such topics as DNA replication, DNA/protein interactions, cell immune response and drug development and testing.

Charge Density on Surfaces

Another application of AFM force measurements is to image or quantify electrical surface charge. The dynamics of many biological systems depends on the electrical properties of the sample surface. Butt, for example, used the force on the AFM probe to estimate the surface charge of the purple membrane of *Halobacteria hohobium*. (65) Leng and Williams have made similar measurements in air on test samples including red blood cells. (66)

Biomechanical Measurements

In addition to measuring binding forces and electrostatic forces, the AFM can also probe the micromechanical properties of biological samples. Specifically, the AFM can observe the

elasticity and, in fact, the viscosity of samples ranging from live cells and membranes to bone and cartilage.

In 1992, Tao and co-workers first studied the microelastic properties of hydrated cow tibia in comparison to standards like rubber and stainless steel. (67) Weisenhorn continued in this vein, using force curves to measure the elasticity of cartilage and living cells. (68, 69) In addition to measuring the cells' mechanical properties, Weisenhorn et al. were also able to predict the maximum probe forces necessary for high-resolution AFM imaging of cells.

Schoenenberger and Hoh have used AFM force measurements to determine the stiffness of a monolayer of living Madin-Darby canine kidney (MDCK) cells. (70) The authors were also able to record the increasing stiffness of these cells over time during gluteraldehyde fixation (Figure 12). Radmacher and coworkers have recently made quantitative measurements of viscoelasticity of human platelets and other soft samples using similar AFM techniques. (71, 72) By making stiffness or adhesion measurements at many points on a sample, the AFM can also create entire images of surface elasticity or surface adhesion using force modulation (73-77) or related techniques. (78-80) (An application note on the force modulation technique is available from Digital Instruments.)

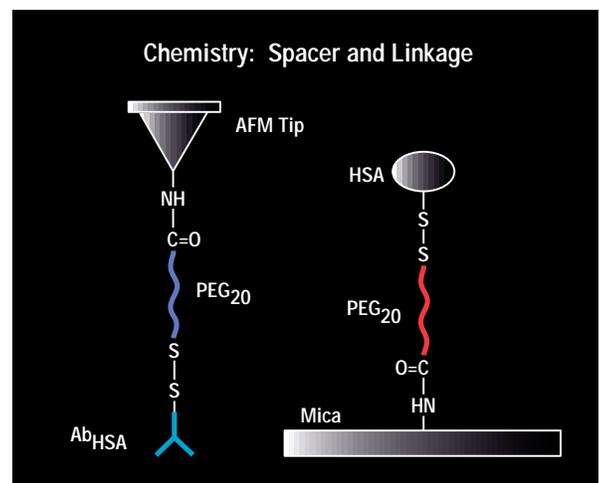
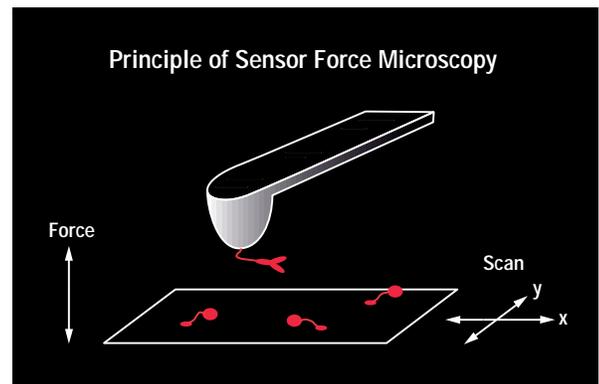


Figure 11: (Top) Schematic of "sensor force microscopy." A biological sensor molecule, in this case Ab_{HSA} , is attached to an AFM tip with a molecular tether, the PEG_{20} molecule. The sensor molecule is scanned across the surface while force measurements detect whether a binding event occurs when a target molecule is encountered. (Bottom) Details of chemical link of sensor and target molecules. Reprinted by permission, reference (63, 64).

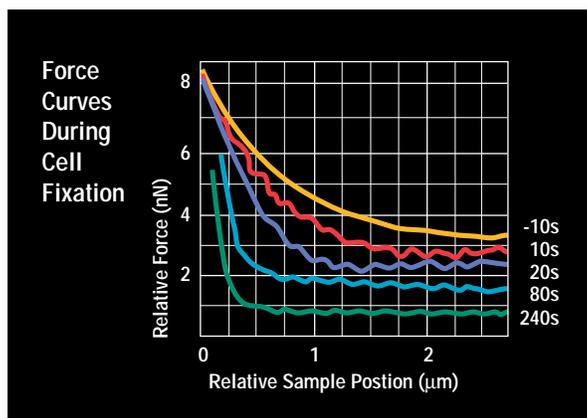
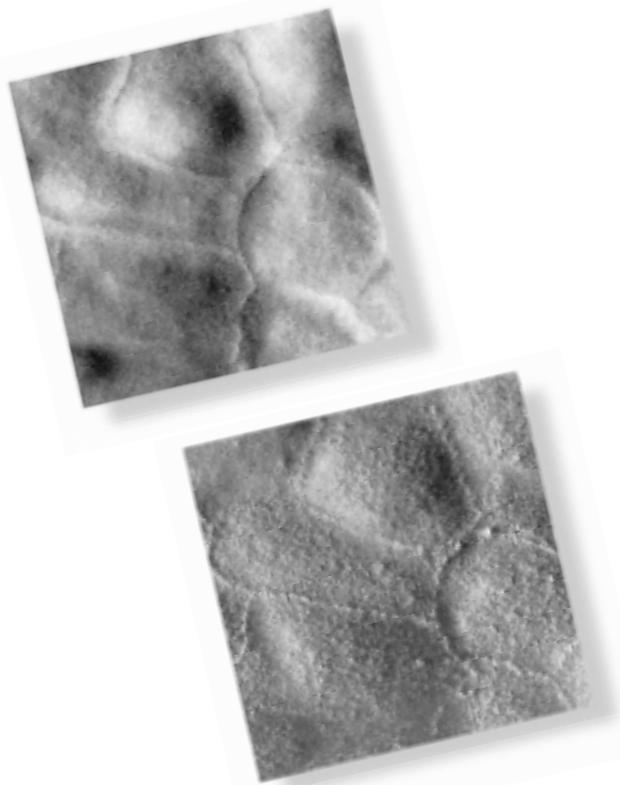


Figure 12: (A) and (B) show the surface of an MDCK monolayer before (A) and after (B) glutaraldehyde fixation. The surface (B) is markedly rougher. The graph shows sequential AFM force measurements on the cell monolayer during fixation. The force curves become increasingly steep over time, illustrating the mechanical stiffening of the monolayer during fixation. This work was done on a NanoScope MultiMode AFM. Reprinted by permission, reference (70).

Materials Science Applications

So far we have focused on mechanical and adhesive measurements on biological samples. It should be no surprise that the chemistry, physics and materials science communities are also very interested in the nano-scale mechanical properties of a variety of polymers, metals, semiconductors and insulators. Measurement of magnetic and electric forces over industrial samples like magnetic hard disks or doped semiconductors is already a rapidly maturing field. In addition, measurements of adhesion, elasticity and even plastic deformation are becoming more common.

Nano-scale Mechanics

One of the main application areas of AFM force measurements in materials science is the study of mechanical properties of nanometer scale thin films and clusters. As microstructures such as integrated circuits continue to shrink, extrapolation of the mechanical behavior of thin films from known properties of the bulk materials becomes increasingly inaccurate. Continuing demand for faster computers and larger capacity memory and storage devices places increasing importance on understanding nano-scale mechanics of metals and other commonly used materials. With its fine scale resolution and force measurement capabilities, AFM is becoming an essential tool for

studying these nano-scale mechanics. An extensive discussion of quantitative measurements of thin film mechanical properties using the AFM is presented in the recent review by Hues, Colton, Meyer, and Güntherodt. (81)

Polymers and Organic Films

Polymers and organic films are often ideal samples for AFM force studies since they are usually soft enough to be easily indented by the AFM tip forces. In one study, Mizes and co-workers used AFM force measurements to perform nanoindentation and adhesion studies on doped and undoped polycarbonate surfaces. (82) Using force curves to map local adhesion, they identified areas where adhesion varied independent of the surface topography of the samples. Aimé and others have recently described a macroscopic theory to allow interpretation of AFM force measurements on polymer surfaces. (83)

Organic films have also been studied by several groups. For example, Radmacher and others used the force modulation technique to study elasticity and viscosity of Langmuir-Blodgett (LB) films. (74) Overney et al. used the AFM to study elasticity, wear and frictional properties of organic thin films including LB films and mixtures of fluorohydrocarbons. (84) Elastic properties of fullerene (buckyball) films have also been studied with the AFM by Juai et

al. (85) Juai's team also used AFM tip for molecular transportation by moving fullerene molecules from the original deposited film to another substrate.

Thin Films, Clusters and Single Crystals

One way to study mechanical properties of metal thin films is to prepare unsupported metal films that are thin enough to be bent by the forces of the AFM cantilever. Torii and co-workers used this novel technique to measure elasticity of very thin aluminum samples. (86) The shape of the contact region of the AFM force curve was used to calculate sample elasticity. Torii calculated the elastic modulus of the aluminum in the thin film by first calibrating the spring constant of the AFM cantilever and then measuring the change in slope of the force curve for the aluminum test sample.

Elastic measurements of thin metal films on solid substrates can also be made. Chromium, molybdenum, and tungsten thin films were studied by Hues et al. (87), whereas Schaefer and colleagues measured the elastic modulus of nanometer-scale gold clusters.

Indentation and Plastic Deformation

Plastic deformation results when a material's elastic limit is exceeded and a permanent deformation is caused. Plastic

properties are usually studied through a technique called indentation, which refers to applying a known force to a sample with the AFM probe tip (or other probe) and then observing the size of the plastic indentation left by the probe. A force curve showing the process of plastic indentation on a magnetic recording tape is shown in Figure 13.

Indentation and, in fact, micromachining of copper and nickel surfaces has been performed by Sumomogi et al. (88) Bhushan and coworkers have even used the AFM with a specially prepared diamond tip to perform indentation studies on single crystal silicon. (89) They used loading forces from 60 to 100 μ N, creating indentations as small as 1nm deep (Figure 14).

Summary

Atomic Force Microscopy has made its mark on a wide variety of applications as a topographic measurement and mapping tool. Now AFM force measurements are providing information on atomic- and molecular-scale interactions as well as nano-scale adhesive and elastic response. These measurements are beginning to revolutionize the way we quantitatively observe and, indeed, think about our chemical, biological and physical world. The vast majority of AFM force measurements — including nearly all of those

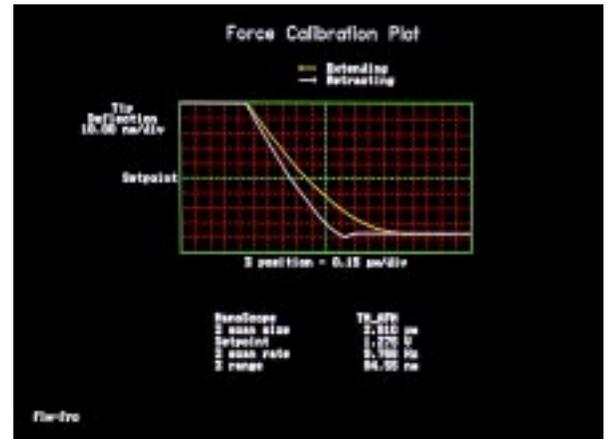


Figure 13: NanoScope force curve showing plastic indentation on a magnetic tape sample. The graph shows two curves, one as the tip advances towards and then indents into the surface (yellow), and another (white) as the tip withdraws from the surface. The separation between these two curves shows that material has been plastically displaced by the tip. Courtesy J. Elings, Digital Instruments.

described here — have been performed with NanoScope systems and we are actively adding functionality to make these measurements increasingly accurate, repeatable and easier to perform. As described in this article, much has already been accomplished in a variety of applications. With this technology still in its relative infancy, there is much more exciting work yet to come.

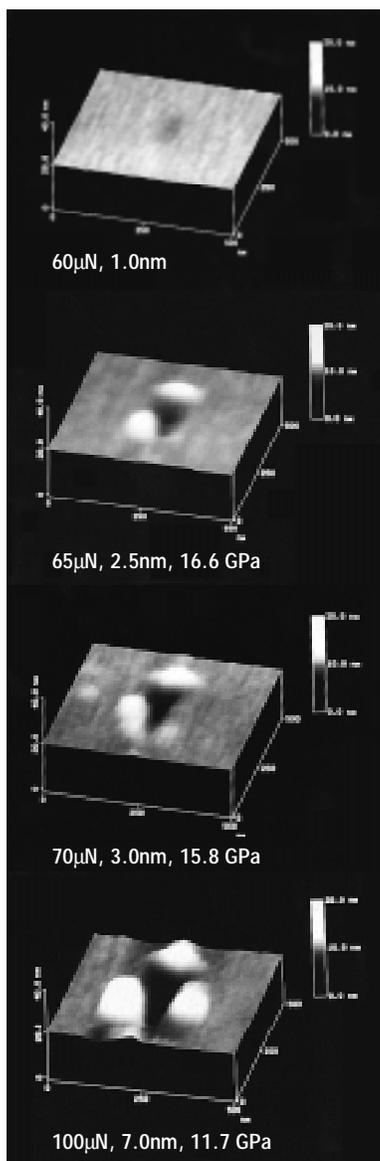


Figure 14: NanoScope AFM images of silicon 111 surfaces after indentation with a diamond AFM tip with loads of 60, 65, 70, and 100 μ N. The smallest load produced an indentation only 1nm deep, the largest load created an indentation about 7nm deep. Reprinted by permission, reference (89).

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