Atomic force microscopy

Measuring intermolecular interaction forces

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General concept and defining characteristics of AFM

Scanned-proximity probe microscopes provide very high resolution images of various sample properties

The atomic force microscope is one of about two dozen types of scanned-proximity probe microscopes. All of these microscopes work by measuring a local property - such as height, optical absorption, or magnetism - with a probe or "tip" placed very close to the sample. The small probe-sample separation (on the order of the instrument's resolution) makes it possible to take measurements over a small area. To acquire an image the microscope raster-scans the probe over the sample while measuring the local property in question. The resulting image resembles an image on a television screen in that both consist of many rows or lines of information placed one above the other.

Unlike traditional microscopes, scanned-probe systems do not use lenses, so the size of the probe rather than diffraction effects generally limit their resolution.

The atomic force microscope measures topography with a force probe

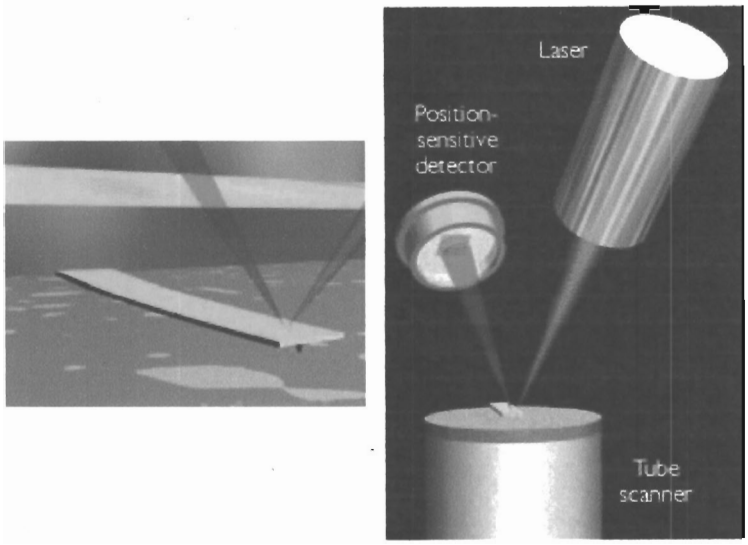


Figure 1. Concept of AFM and the optical lever: (left) a cantilever touching a sample; (right) the optical lever. Scale drawing; the tube scanner measures 24 mm in diameter, while the cantilever is 100 μm long.

AFM (figure 1) operates by measuring attractive or repulsive forces between a tip and the sample (Binnig et al., 1986). In its repulsive "contact" mode, the instrument lightly touches a tip at the end of a leaf spring or "cantilever" to the sample. As a raster-scan drags the tip over the sample, some sort of detection apparatus measures the vertical deflection of the cantilever, which indicates the local sample height. Thus, in contact mode the AFM measures hard-sphere repulsion forces between the tip and sample.

In noncontact mode, the AFM derives topographic images from measurements of attractive forces; the tip does not touch the sample (Albrecht et al., 1991). Because it does not allow the imaging of samples under water, I have not used the attractive mode.

AFMs can achieve a resolution of 10 pm, and unlike electron microscopes, can image samples in air and under liquids.

In principle, AFM resembles the record player as well as the stylus profilometer. However, AFM incorporates a number of refinements that enable it to achieve atomic-scale resolution:

- Sensitive detection
- Flexible cantilevers
- Sharp tips
- High-resolution tip-sample positioning
- Force feedback

I describe these refinements below.

Laser beam deflection offers a convenient and sensitive method of measuring cantilever deflection

AFMs can generally measure the vertical deflection of the cantilever with picometer resolution. To achieve this most AFMs today use the optical lever, a device that achieves resolution comparable to an interferometer while remaining inexpensive and easy to use (Meyer et al., 1988; Alexander et al., 1989).

The optical lever (figure 1) operates by reflecting a laser beam off the cantilever. Angular deflection of the cantilever causes a twofold larger angular deflection of the laser beam. The reflected laser beam strikes a position-sensitive photodetector consisting of two side-by-side photodiodes. The difference between the two photodiode signals indicates the position of the laser spot on the detector and thus the angular deflection of the cantilever.

Because the cantilever-to-detector distance generally measures thousands of times the length of the cantilever, the optical lever greatly magnifies motions of the tip. Because of this ~2000-fold magnification optical lever detection can theoretically obtain a noise level of 10^{-14} m/Hz$^{1/2}$ Putman et al., 1992). For measuring cantilever deflection, to date only the relatively cumbersome techniques of interferometry and tunneling detection have approached this value.

AFM cantilevers have high flexibility

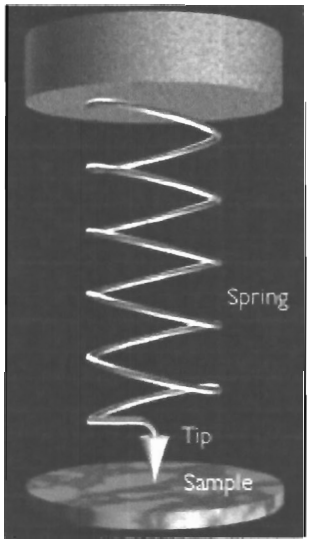


Figure 2. Schematic illustration of the meaning of "spring constant" as applied to cantilevers. Visualizing the cantilever as a coil spring, its spring constant k directly affects the downward force exerted on the sample.

A high flexibility stylus exerts lower downward forces on the sample, resulting in less distortion and damage while scanning. For this reason AFM cantilevers generally have spring constants of about 0.1 N/m (figure 2). As Paul Hansma points out, a Slinky is about ten times stiffer (1 N/m).

It would take a very long time to image a surface by dragging a Slinky over it (in the configuration of figure 2), because a Slinky cannot respond quickly as it passes over features. That is, a Slinky has a low resonant frequency, but an AFM cantilever should have a high resonant frequency.

The equation for the resonant frequency of a spring:

$$\text{resonant frequency} = \frac{1}{2\pi}\sqrt{\frac{\text{spring constant}}{\text{mass}}}$$

shows that a cantilever can have both low spring constant and high resonant frequency if it has a small mass. Therefore AFM cantilevers tend to be very small. Commercial vendors manufacture almost all AFM cantilevers by microlithography processes similar to those used to make computer chips. The Park Scientific Instruments cantilevers in figure 3 measure 100 µm in length and consist of silicon oxynitride with a thin coating of gold for reflectivity.

Micromachining techniques produce inexpensive, reasonably sharp tips

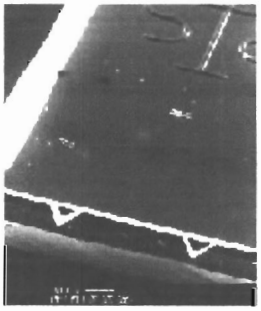


Figure 3. Electron micrograph of two 100 µm long V-shaped cantilevers (by Jean-Paul Revel, Caltech; cantilevers from Park Scientific Instruments, Sunnyvale, CA).

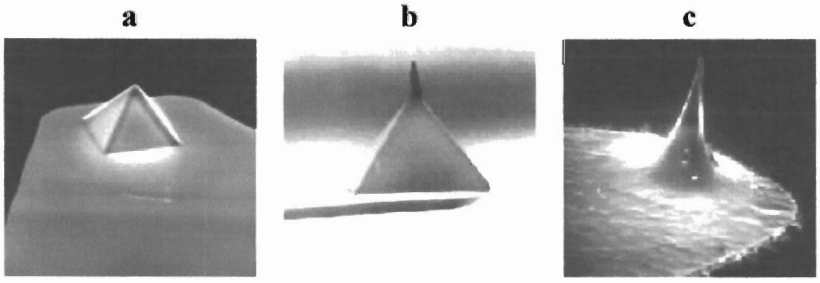


Figure 4. Three common types of AFM tip. (a) normal tip (3 µm tall); (b) supertip; (c) Ultralever (also 3 µm tall). Electron micrographs by Jean-Paul Revel, Caltech. Tips from Park Scientific Instruments; supertip made by Jean-Paul Revel.

Most users purchase AFM cantilevers with their attached tips from commercial vendors, who manufacture the tips with a variety of microlithographic techniques.

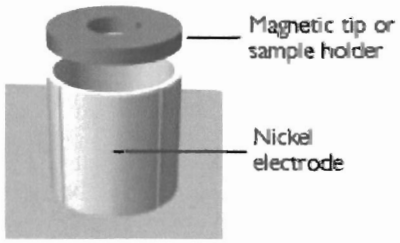
A close enough inspection of any AFM tip reveals that it is rounded off. Therefore force microscopists generally evaluate tips by determining their "end radius." In combination

with tip-sample interaction effects, this end radius generally limits the resolution of AFM. As such, the development of sharper tips is currently a major concern.

Force microscopists generally use one of three types of tip. The "normal tip" (figure 4a; Albrecht et al., 1990) is a 3 μm tall pyramid with ~30 nm end radius. The electron-beam-deposited (EBD) tip or "supertip" (figure 4b; Keller and Chih-Chung, 1992) improves on this with an electron-beam-induced deposit of carbonaceous material made by pointing a normal tip straight into the electron beam of a scanning electron microscope. Especially if the user first contaminates the cantilever with paraffin oil, a supertip will form upon stopping the raster of the electron beam at the apex of the tip for several minutes. The supertip offers a higher aspect ratio (it is long and thin, good for probing pits and crevices) and sometimes a better end radius than the normal tip. Finally, Park Scientific Instruments offers the "Ultralever" (figure 4c), based on an improved microlithography process. Ultralevers offers a moderately high aspect ratio and on occasion a ~10 nm end radius.

Tube piezoceramics position the tip or sample with high resolution

Figure 5. Exploded view of a tube scanner. Applying a voltage to one of the four outer quadrants causes that quadrant to expand and the scanner to tilt away from it (XY movement). A corresponding negative voltage applied to the opposite quadrant doubles the XY range while preventing vertical motion. Applying a voltage to the inner electrode causes the entire tube to expand or contract (Z movement).



Piezoelectric ceramics are a class of materials that expand or contract when in the presence of a voltage gradient or, conversely, create a voltage gradient when forced to expand or contract (Gallego-Juárez, 1989). Piezoceramics make it possible to create three-dimensional positioning devices of arbitrarily high precision. Most scanned-probe microscopes use tube-shaped piezoceramics because they combine a simple one-piece construction with high stability and large scan range. Four electrodes cover the outer surface of the tube, while a single electrode covers the inner surface. Application of voltages to one or more of the electrodes causes the tube to bend or stretch, moving the sample in three dimensions (figure 5).

AFMs use feedback to regulate the force on the sample

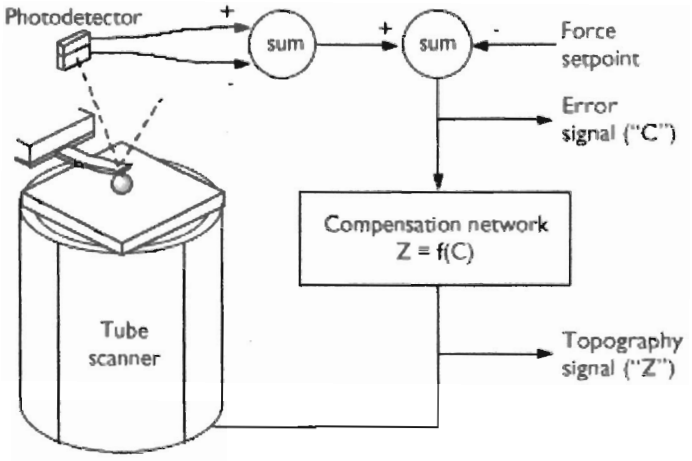


Figure 6. The AFM feedback loop. A compensation network (which in my AFM is a computer program) monitors the cantilever deflection and keeps it constant by adjusting the height of the sample (or cantilever).

The presence of a feedback loop is one of the subtler differences between AFMs and older stylus-based instruments such as record players and stylus profilometers. The AFM not only measures the force on the sample but also regulates it, allowing acquisition of images at very low forces.

The feedback loop (figure 6) consists of the tube scanner that controls the height of the entire sample; the cantilever and optical lever, which measures the local height of the sample; and a feedback circuit that attempts to keep the cantilever deflection constant by adjusting the voltage applied to the scanner.

One point of interest: the faster the feedback loop can correct deviations of the cantilever deflection, the faster the AFM can acquire images; therefore, a well-constructed feedback loop is essential to microscope performance. AFM feedback loops tend to have a bandwidth of about 10 kHz, resulting in image acquisition times of about one minute.

Alternative imaging modes

AFMs have two standard imaging modes

Almost all AFMs can measure sample topography in two ways: by recording the feedback output ("Z") or the cantilever deflection ("error"; see figure 6). The sum of these two signals always yields the actual topography, but given a well-adjusted feedback loop, the error signal should be negligible. As described below, AFMs may have alternative imaging modes in addition to these standard modes.

Optical lever AFMs can measure the friction between tip and sample

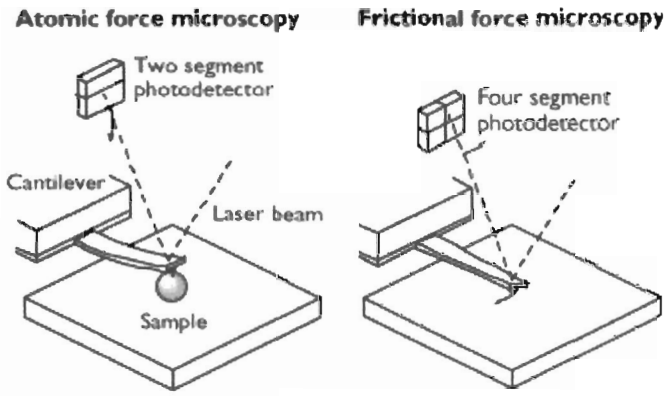


Figure 7. While topographic imaging uses the up-and-down deflection of the cantilever, friction imaging uses torsional deflection.

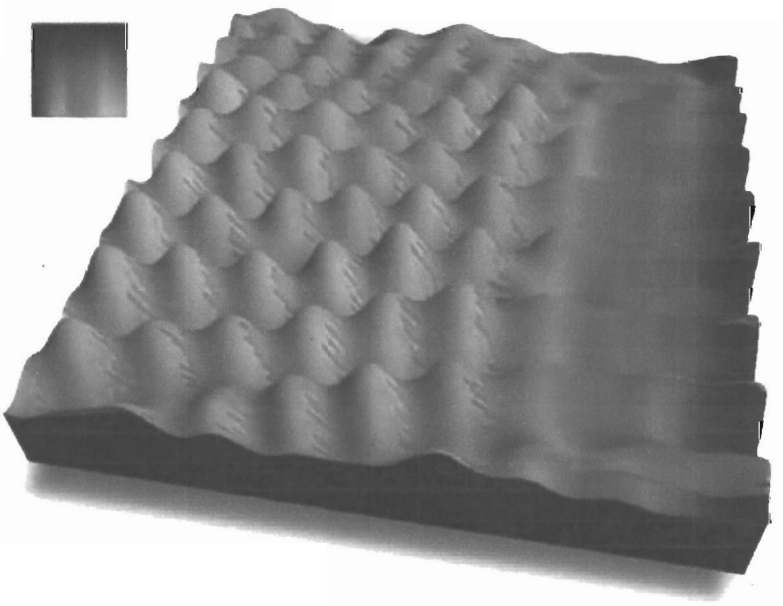


Figure 8. 2.5 x 2.5 nm simultaneous topographic and friction image of highly oriented pyrolytic graphic (HOPG). The bumps represent the topographic atomic corrugation, while the coloring reflects the lateral forces on the tip. The scan direction was right to left.

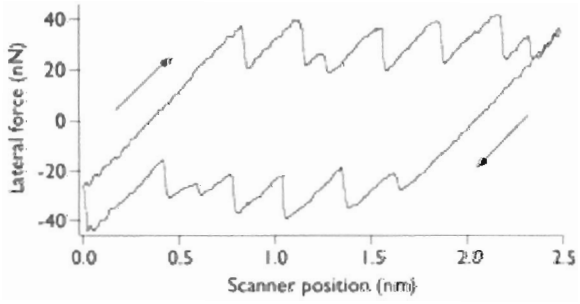


Figure 9. Cross-sectional profile of friction data from above image showing stick-slip behavior.

If the scanner moves the sample perpendicular to the long axis of the cantilever (figure 7), friction between the tip and sample causes the cantilever to twist. A photodetector position-sensitive in two dimensions can distinguish the resulting left-and-right motion of the reflected laser beam from the up-and-down motion caused by topographic variations (Meyer and Amer, 1990).

Therefore, AFMs can measure tip-sample friction while imaging sample topography. Besides serving as an indicator of sample properties, friction (or "lateral force," or "lateral deflection") measurements provide valuable information about the tip-sample interaction.

Figure 8 shows a simultaneous friction and topography image of graphite atoms in which I have plotted the topography image as a three-dimensional projection colored by the friction data. Each bump represents one carbon atom. As the tip moves from right to left, it bumps into an atom and gets stuck behind it. The scanner continues to move and lateral force builds up until the tip slips past the atom and sticks behind the next one. This "stick-slip" behavior creates a characteristic sawtooth waveform in the friction image (figure 9).

AFMs can measure sample elasticity

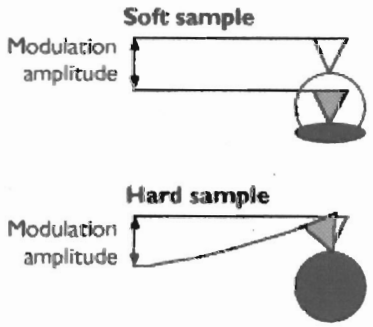


Figure 10. AFMs can image sample elasticity by pressing the tip into the sample and measuring the resulting cantilever deflection.

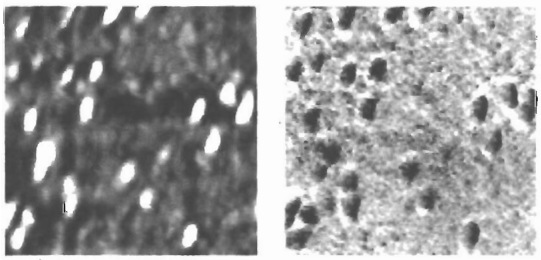


Figure 11. 1 x 1 μm simultaneous topography (left) and elasticity (right) images of bovine serum albumen on silicon (sample prepared by Sie-Ting Wong of Abbott Laboratories).

AFM can also image the softness of a sample by pressing the cantilever into it at each point in a scan. The scanner raises the sample or lowers the cantilever by a preset amount, the "modulation amplitude" (usually 1-10 nm). In response, the cantilever deflects an amount dependent on the softness of the sample: the harder the sample, the more the

cantilever deflects (figure 10).

Figure 11 shows an image of bovine serum albumen (BSA) on silicon. A number of bumps appear in the topography image, each presumably corresponding to a single BSA molecule. The elasticity image reveals that each of the bumps is soft relative to the silicon substrate, a reasonable result for protein molecules.

AFM and biology

Dull tips and tip-sample interaction forces prevent high-resolution imaging of biological structures

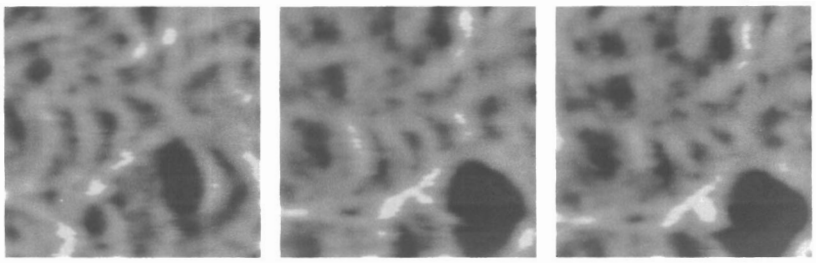


Figure 12. Images 1, 50, and 100 of small collagen fibrils from a sequence of 100 images. Repetitive scanning of the same area progressively detaches the fibrils from the glass substrate, causing distortion in the direction of scanning, left-to-right and top-to-bottom.

The ability of AFM to image at atomic resolution, combined with its ability to image a wide variety of samples under a wide variety of conditions, has created a great deal of interest in applying it to the study of biological structures. Images have appeared in the literature showing DNA, single proteins, structures such as gap junctions, and living cells (for a review see Hoh and Hansma, 1992).

Unfortunately, AFM cannot image all samples at atomic resolution. The end radii of available tips confines atomic resolution to flat, periodic samples such as graphite. In addition, because biological structures are soft, the tip-sample interaction tends to distort or destroy them. Figure 12, for example, shows how forces exerted on small collagen fibrils tend to detach them from the substrate over a period of time, resulting in progressively greater distortion.

A number of companies are attempting to develop sharper tips, primarily by improved microfabrication processes. I have concentrated on investigating the tip-sample interaction with alternative imaging modes.

The meniscus force is the most important influence on the tip-sample

interaction force when imaging in air

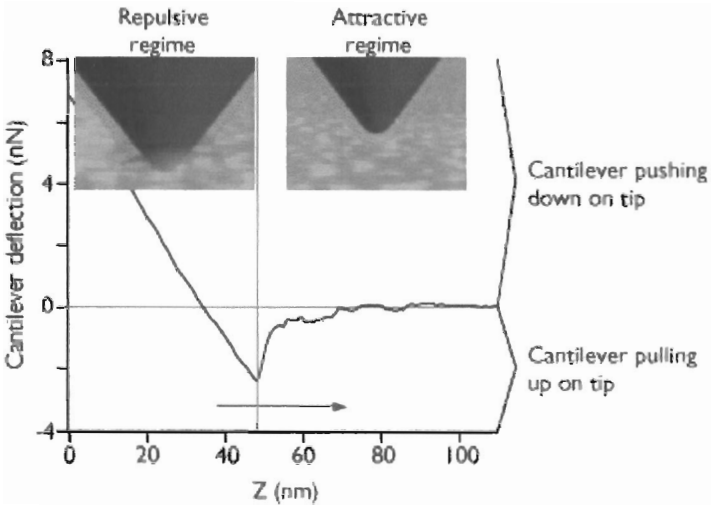


Figure 13. Force curve taken in air. At Z=0 nm the cantilever pushes down on the tip, and tip and sample are in contact. As Z increases, the cantilever exerts less force and then begins to pull up on the tip (negative force). Eventually the cantilever exerts enough force to pull the tip free of the meniscus (2 nN in this case, an unusually low figure). After this point, only attractive forces affect the cantilever deflection.

When imaging in air, a layer of water condensation and other contamination covers both the tip and sample, forming a meniscus that pulls the two together (Weisenhorn et al., 1989).

"Force curves" showing cantilever deflection as the scanner lowers the sample reveal the attractive meniscus force (figure 13): the cantilever has to exert an upward force to pull the tip free of the meniscus. This force equals the attractive force of the meniscus, usually 10-100 nN.

The great strength of the meniscus makes it the most important influence on the tip-sample interaction. Force microscopists often eliminate the meniscus by completely immersing both tip and sample in water.

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