1

How the Atomic Force Microscope Works

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1. Introduction

Microscopes have always been one of the essential instruments for research in the biomedical field. Radiation-based microscopes (such as the light microscope and the electron microscope) have become trustworthy companions in the laboratory and have contributed greatly to our scientific knowledge. However, although digital techniques in recent years have still enhanced their performance, the limits of their inherent capabilities have been progressively reached.

The advent of scanning probe microscopes and especially of the atomic force microscope (AFM; **ref. *1***) has opened new perspectives in the investigation of biomedical specimens and induces to look again with rejuvenated excitement at what we can learn by "looking" at our samples. Novices are at first mesmerized by two features: the name of the instrument and the colorful 3D computer visualization of surfaces. One later learns that quite often it is not possible to obtain the "atomic" resolution that one hoped to achieve ***(2–4)*** but that nevertheless images do contain details not observable with any other instrument. The tri-dimensional mapping of the surface gains scientific relevance when one realizes that it is not just fancy surface reconstruction but that true topographic data with vertical resolution down to the subnanometer range is readily available. Moreover, when simplified sample preparation and the possibility of investigating specimens in liquid environment become apparent, one becomes convinced that he or she must find a way to apply AFM to his or her own research.

2. Performance Range of AFM

AFM images show significant information about surface features with unprecedented clarity. The AFM can examine any sufficiently rigid surface

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either in air or with the specimen immersed in a liquid. Recently developed instruments can allow temperature control of the sample, can be equipped with a closed chamber for environmental control, and can be mounted on an inverted microscope for simultaneous imaging through advanced optical techniques.

The field of view can vary from the atomic and molecular scale up to sizes larger than 125 μm so that data can be compared with other information obtained with lower resolution techniques. The AFM can also examine rough surfaces because its vertical range can be up to 8–10 μm. Large samples can be fitted directly in the microscope without cutting. With stand-alone instruments, any area on flat or nearly flat specimens can be investigated. In addition to its superior resolution with respect to optical microscopes, the AFM has these key advantages with respect to electron microscopes. Compared with the scanning electron microscope (SEM), the AFM provides superior topographic contrast, in addition to direct measurements of surface features providing quantitative height information.

Because the sample need not be electrically conductive, no metallic coating of the sample is required. Hence, no dehydration of the sample is necessary as with SEM, and samples may be imaged in their hydrated state. This eliminates the shrinkage of biofilm associated with imaging using SEM, yielding a non-destructive technique. The resolution of AFM is higher than that of environmental SEM, where hydrated images can also be obtained and extracellular polymeric substances may not be imaged.

Compared with transmission electron microscopes, 3D AFM images are obtained without expensive sample preparation and yield far more complete information than the 2D profiles available from cross-sectioned samples.

In the following subheadings we will give a brief outline of how the AFM works followed by a description of the parts that can be added to the basic instrument. Our overview makes no pretense to completeness but aims at simplicity. For a more thorough description of the physical principles involved in the operation of these instruments, we refer you to the specialized literature.

3. The Microscope

In **Fig. 1**, a schematic diagram of an AFM is shown ***(1,5)***. In principle, AFM can bring to mind the record player, but it incorporates a number of refinements that enable it to achieve atomic-scale resolution, such as very sharp tips, flexible cantilevers, a sensitive deflection sensor, and high-resolution tip–sample positioning.

3.1. The Tip and Cantilever

The tip, which is mounted at the end of a small cantilever, is the heart of the instrument because it is brought in closest contact with the sample and gives

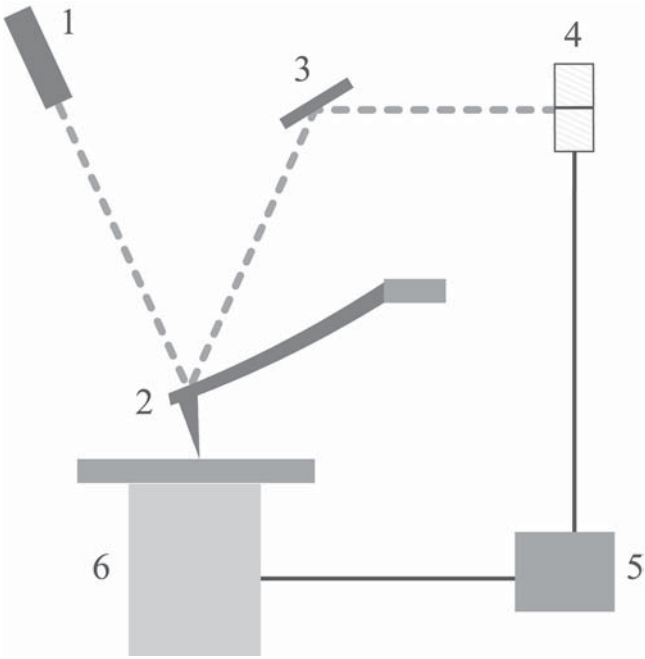

Fig. 1. Schematic diagram of a scanned-sample AFM. In the case of scanned probe, it is the tip that is scanned instead of the sample. *1*, Laser diode; *2,* cantilever; *3,* mirror; *4,* position-sensitive photodetector; *5,* electronics; and *6,* scanner with sample.

rise to the image though its force interactions with the surface. When the first AFM was made, a very small diamond fragment was carefully glued to one end of a tiny piece of gold foil. Today, the tip–cantilever assembly typically is fabricated from silicon or silicon nitride and, using technology similar to that applied to integrated circuit fabrication, allows a good uniformity of characteristics and reproducibility of results ***(6,7)***. The essential parameters are the sharpness of the apex, measured by the radius of curvature, and the aspect ratio of the whole tip (**Fig. 2**).

Although it would seem that sharper tips should yield more detailed images, this may not occur with all samples: in fact, quite often, so-called "atomic resolution" on crystals is obtained best with standard silicon nitride tips. In general, one can choose among one of three types of tip. The standard tip is usually a 3-μm tall pyramid with approx 30-nm end radius. The electron-beam-deposited tip or "super tip" improves on this with an electron-beam–induced deposit of material at the apex of the tip, offering a higher aspect ratio and end radius than the normal tip, albeit with the drawback of fragility. Finally, tips made from silicon (either polysilicon or single crystal) through improved

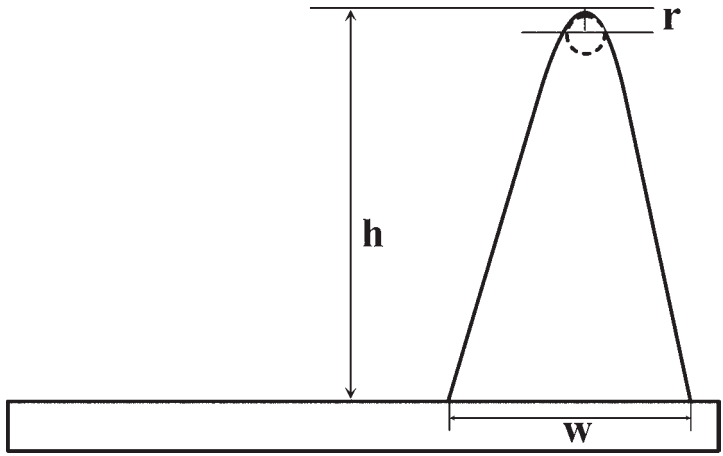

Fig. 2. The essential parameters in a tip are the radius of curvature (*r*) and the aspect ratio (ratio of *h* to *w*).

microlithographic techniques have a higher aspect ratio and small apex radius of curvature, maintaining reproducibility and durability ***(8)***.

The cantilever carrying the tip is attached to a small glass "chip" that allows easy handling and positioning in the instrument. There are essentially two designs for cantilevers, the "V" shaped and the single-arm kind (**Fig. 3**), which have different torsional properties. The length, width, and thickness of the beam(s) determine the mechanical properties of the cantilever and have to be chosen depending on mode of operation needed and on the sample to be investigated. Cantilevers are essentially classified by their force (or spring) constant and resonance frequency: soft and low-resonance frequency cantilevers are more suitable for imaging in contact and resonance mode in liquid, whereas stiff and high-resonance frequency cantilevers are more appropriate for resonance mode in air ***(9)***.

3.2. Deflection Sensor

AFMs can generally measure the vertical deflection of the cantilever with picometer resolution. To achieve this, most AFMs today use the optical lever or beam-bounce method, a device that achieves resolution comparable to an interferometer while remaining inexpensive and easy to use.

In this system, a laser beam is reflected from the backside of the cantilever (often coated by a thin metal layer to make a mirror) onto a position-sensitive

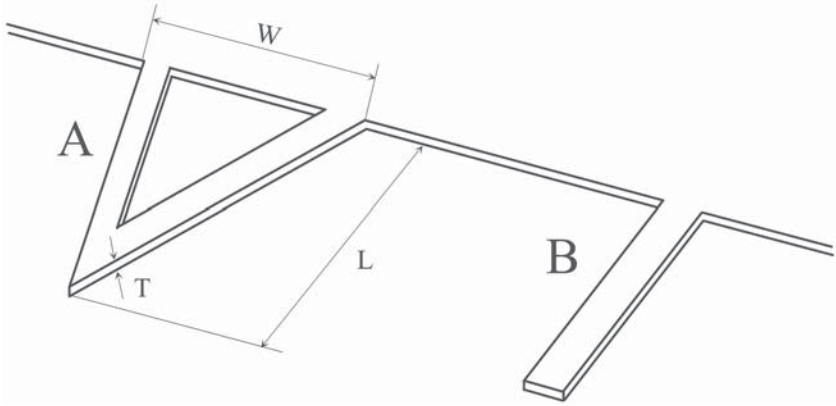

Fig. 3. Triangular **(A)** and single-beam **(B)** cantilevers. The mechanical properties, such as the force constant and resonant frequency, depend on the values of width (*W*), length (*L*), and thickness (*T*).

photodetector consisting of two side-by-side photodiodes. In this arrangement, a small deflection of the cantilever will tilt the reflected beam and change the position of beam on the photodetector. 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 distance between cantilever and detector is generally three orders of magnitude greater than the length of the cantilever (millimeters compared to micrometers), the optical lever greatly magnifies motions of the tip giving rise to an extremely high sensitivity.

3.3. Image Formation

Images are formed by recording the effects of the interaction forces between tip and surface as the cantilever is scanned over the sample. The scanner and the electronic feedback circuit, together with sample, cantilever, and optical lever form a feedback loop set up for the purpose. The presence of a feedback loop is a key difference between AFM and older stylus-based instruments so that AFM not only measures the force on the sample but also controls it, allowing acquisition of images at very low tip-to-sample forces ***(5,10)***.

The scanner is an extremely accurate positioning stage used to move the tip over the sample (or the sample under the tip) to form an image, and generally in modern instruments is made from a piezoelectric tube. The AFM electronics drives the scanner across the first line of the scan and back. It then steps in the

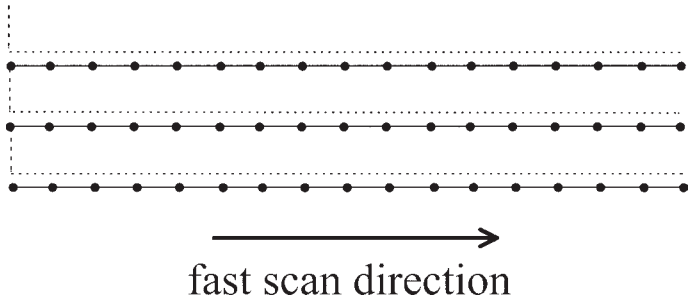

Fig. 4. Raster scan for image acquisition. The AFM electronics drive the scanner across the first line of the scan and back. The scanner then steps in the perpendicular direction to the second scan line, moves across it and back, then to the third line, and so forth.

perpendicular direction to the second scan line, moves across it and back, then to the third line, and so forth (**Fig. 4**).

As the probe is scanned over the surface, a topographic image is obtained storing the vertical control signals sent by the feedback circuit to the scanner moving it up and down to follow the surface morphology while keeping the interaction forces constant. The image data are sampled digitally at equally spaced intervals, generally from 64 up to 2048 points per line. The number of lines is usually chosen to be equal to the number of data points per line, obtaining at the end a square grid of data points each corresponding to the relative x, y, and z coordinates in space of the sample surface ***(11)***.

Usually during scanning data are represented by gray scale images, in which the brightness of points can range from black to white across 256 levels corresponding to the information acquired by the microscope (that can be height, force, phase, and so on).

4. A Variety of Instruments and Options

The first instruments introduced on the market had all very similar features and range of applications: they had scanners with small range, limited optical access, and could accommodate only small samples. Essentially they where built to make very high-resolution imaging on flat samples in a dry environment. As the possibilities of AFM were developed, a wider range of instruments, optimized for specific applications, have been developed. We can now find instruments that are specifically designed for large samples, such as silicon wafers, that have metrological capabilities, utilize scanner close loop operation, are optimized for liquid and electrochemistry operation, and can be

mounted on an inverted microscope for biological investigations. Usually, one single instrument can have different options to extend its capabilities, but to date it is not possible to have an instrument that covers all possible applications with maximum performance. For this reason, it is necessary to have clearly in mind what will be the main features that are desired in an instrument before its purchase, understanding at the same time that a loss of performance in other aspects may be possible.

One can distinguish between two main classes: scanned-sample and scanned-tip microscopes. We give a brief description of the advantages of one system with respect to the other.

4.1. Scanned Sample

This scanned-sample AFM is the first design in which the sample is attached to the scanner and moved under the tip. Depending on how the cantilever holder, laser, and photodetector are assembled, it can easily accommodate an overhead microscope provided that long focal length objectives are used. A clear view of where the tip is landing is usually possible, speeding up the time it takes to get a meaningful image of the sample.

Scanners with wide x,y, and z range are usually available and closed loop control feedback is more easily implemented in this scheme and often a lower mechanical noise level can be obtained allowing higher ultimate resolution.

There are quite a few drawbacks. First of all, the size and weight of the sample has to be limited because it is sitting on the scanner and may change its behavior. For the same reason, operation in liquid is impaired because liquid cells tend to be small and difficult to seal, and liquid flow or temperature control are more complicated to implement. Notwithstanding these difficulties, excellent results can be obtained on typical biomedical science specimens by ingeniously adapting them to the instruments characteristics.

4.2. Scanned Tip

In the scanned-tip method of operation, the sample stays still and it is the cantilever, attached to the scanner, which is moved across the surface. Although for scanning tunneling microscopes this was one of the first solutions applied, to build a scanned tip AFM requires overcoming some difficulties, essentially related to adapting the beam bounce detection scheme to a moving cantilever. For this reason, it has been only recently that models made according to this design have been marketed, after appropriate technology was developed. The first examples were the so-called “stand-alone” systems, usually an AFM resting on three legs and able to scan the surface of any object under its probe. Later, specialized instruments were developed, capable of being coupled or even integrated into inverted optical microscopes for biological applications.

With respect to the scanned-sample models, scanned-tip instruments can be more easily equipped with temperature-controlled stages, open or closed liquid cells, liquid flow systems, electrochemistry cells, and controlled atmosphere chambers. Concerning limitations, one could say that what is gained on one side is lost on the other. For example, often the overall noise level is higher, limiting ultimate resolution. Large scan areas are more difficult to scan because tracking systems have to be used to keep the laser spot on the back of the cantilever. A top view of samples is obstructed by the scanner assembly: special hollow tubes have been developed recently, but even so on-axis microscopes, which are useful on nontransparent samples, will still have limited resolution and lateral field of view.

5. Loading a Sample in the Microscope

5.1. Imaging Dry Samples

Samples to be imaged in atmospheric environment are often simply glued to a sample holder, usually a metal disk. The disk is then inserted in the AFM, where it is held firmly by a small magnet. An essential point is that the sample has to be firmly adherent to the sample holder; otherwise, very poor imaging will be achieved. For this reason, one has to be careful in the choice of the glue or sticky tape: slow drying glue or thick sticky tape should be avoided. A drawback is that after use in the AFM, the sample is difficult to take off without damage.

Some systems, usually scanned-tip, can accept samples directly, securing them with a metal clip or springs. This method allows sample recovery without damage for further use in other experiments, but it can be less stable and needs special care for high-resolution work.

Sometimes, because of the ease of use of the AFM, one forgets to be careful while handling the sample and either fingerprints or dust from a dirty environment contaminates the sample. It is best to keep a reserved area of the laboratory free from contaminants for the operations of sample and cantilever mounting.

5.2. Imaging in Liquid

One of the main reasons for the success of AFM in biomedical investigations is its ability to scan samples in physiological condition, that is, immersed in liquid solutions ***(12,13)***. Just to make an example, scanned-tip systems can often be directly used to image cells into a standard Petri dish. Each manufacturer has its own design of liquid cells, sometimes different ones depending on the application, and users may decide to make their own to fit specific needs. A few additional things that have to be taken care of when imaging in liquid are

the temperature of the solution (eventually added during imaging; **ref. *14***) and maintenance of the liquid cell and cantilever holder assembly. Because the cantilever is extremely sensitive to temperature changes, it is important to let the system equilibrate before taking images. For example, in the case of contact mode imaging with silicon nitride cantilevers and tips, a large change in time of the signal on the photodetector corresponding to cantilever deflection can be observed in the presence of a temperature change ***(15)***. If temperature is not stable prior to approach of the tip to the sample and one starts taking images, after some time the applied force could be quite different than at the beginning of the imaging session.

Once finished using the microscope for imaging in liquid, it is essential to immediately clean thoroughly all parts that have been in contact with the solution to avoid contamination of future experiments. Usually, it should be possible to disassemble and sonicate all vital parts of the liquid cell and the cantilever holder.

6. Future Developments

The AFM is part of a family of scanning probe microscopes that has a great growth potential. It is a fact that the majority of novel applications and techniques developed in scanning probe microscopes in the last years are related to the life sciences. There is still much room for technical improvement: electronics, scanners, and tips are constantly improving. Scan speed limitations, sample accessibility, and ease of use have been addressed and can be still improved. As more and more biomedical researchers will be involved in the use of AFM, with their experience they will be able contribute in developing an instrument less related to the physical science (its origin) and more tailored to our specific needs.

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