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Imaging Methods in Atomic Force Microscopy

Davide Ricci and Pier Carlo Braga

1. Introduction

One can easily distinguish between two general modes of operation of the atomic force microscope (AFM) depending on absence or presence in the instrumentation of an additional device that forces the cantilever to oscillate in the proximity of its resonant frequency. The first case is usually called static mode, or DC mode, because it records the static deflection of the cantilever, whereas the second takes a variety of names (some patented) among which we may point out the resonant or AC mode. In this case, the feedback loop will try to keep at a set value not the deflection but the amplitude of the oscillation of the cantilever while scanning the surface. To do this, additional electronics are necessary in the detection circuit, such as a lock-in or a phase-locked loop amplifier, and also in the cantilever holder to induce the oscillatory excitation.

From a physical point of view, one can make a distinction between the two modes depending on the sign of the forces involved in the interaction between tip and sample, that is, by whether the forces there are attractive or repulsive ***(1)***. In **Fig. 1**, an idealized plot of the forces between tip and sample is shown, highlighting where typical imaging modes operate. In the following we briefly describe the DC and AC modes of operation relevant to the kind of samples that usually are investigated in the biomedical field.

2. DC Modes

2.1. Contact Mode

Also called constant force mode, the contact mode is the most direct AFM mode, where the tip is brought in contact with the surface and the cantilever deflection is kept constant during scanning by the feedback loop. Image contrast depends on the applied force, which again depends on the cantilever spring

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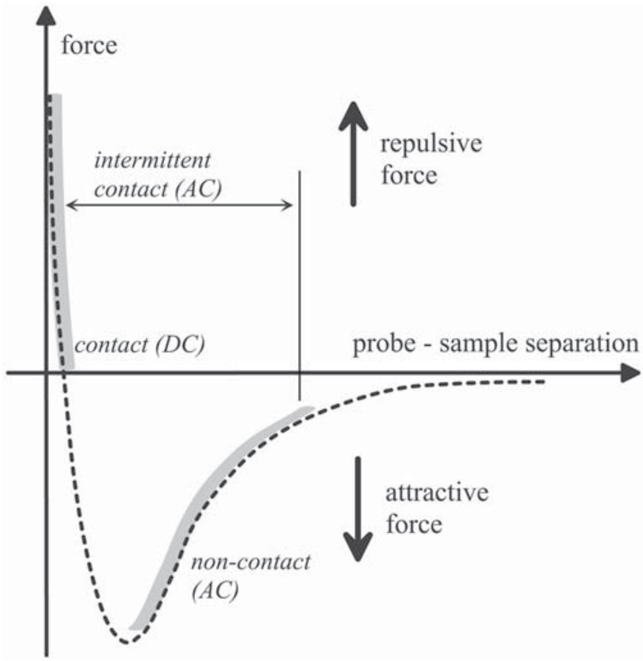

Fig. 1. Idealized plot of the forces between tip and sample, highlighting where typical imaging modes are operative.

Fig. 2. In contact mode, the tip follows directly the topography of the surface while it is scanned.

constant (**Fig. 2**). Softer cantilevers are used for softer samples. It can be used easily also in liquids, allowing a considerable reduction of capillary forces between tip and sample and, hence, damage to the surface (**Fig. 3;** refs. ***2,3***). Because the tip is permanently in contact with the surface while scanning, a considerable shear force can be generated, causing damage to the sample, especially on very soft specimens like biomolecules or living cells ***(4)***.

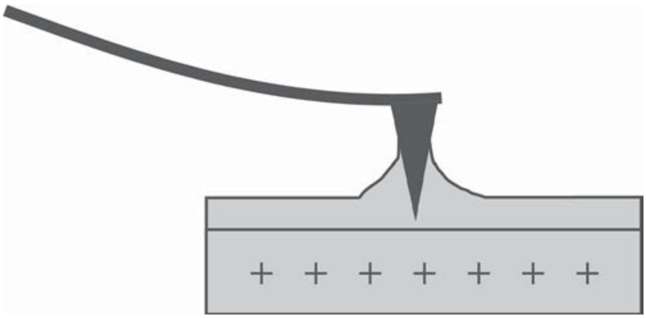

Fig. 3. In contact mode, capillary forces caused by a thin water layer and electrostatic forces can considerably increase the total force between sample and tip.

2.2. Deflection or Error Mode

In same cases, especially on rough and relatively rigid samples, the error signal (i.e., the difference between the set point and the effective deflection of the cantilever that occurs during scanning as a result of the finite time response of the feedback loop) is used to record images. By turning down on purpose the feedback gain, the cantilever will press harder on asperities and less on depressions, giving rise to images that contain high-frequency information otherwise not visible ***(5)***. This method has been extensively used to image submembrane features in living cells. The same method is also often used to record high-resolution images on crystals.

2.3. Lateral Force Microscopy

In this case (a variation of standard contact mode), while scanning the sample not only the vertical deflection of the cantilever but also the lateral deflection (torsion) is measured by the photodetector assembly, which in this case will have four photodiodes instead of two (**Fig. 4**). The degree of torsion of the cantilever supporting the probe is a relative measure of surface friction caused by the lateral force exerted on the scanning probe ***(6)***. This method has been used to discriminate between areas of the sample that have the same height (i.e., that are on a same plane) but that present different frictional properties because of absorbates.

3. AC Modes

All AC modes require setting the cantilever in oscillation using an additional driving signal. This can be accomplished by driving the cantilever with a piezoelectric motor (acoustic mode) or, as developed more recently, by directly driving by external coils a probe coated with a magnetic layer (magnetic mode).

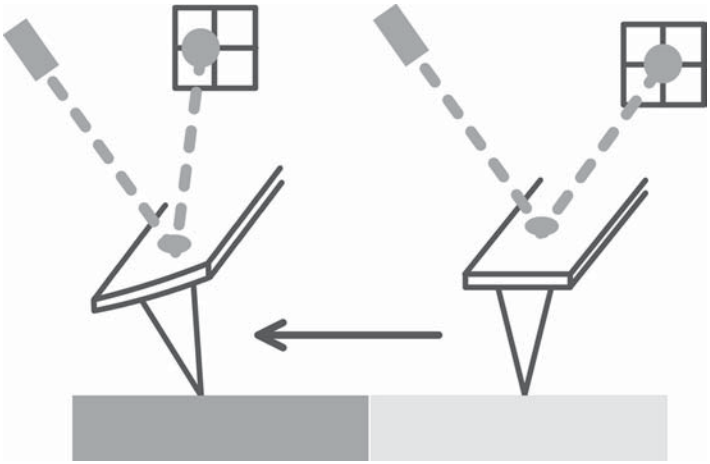

Fig. 4. Using a four-section photodetector, it is possible to measure also the torsion of the cantilever during contact mode AFM scanning. The torsion of the cantilever reflects changes in the surface chemical composition.

This second method is giving interesting results, especially in liquid, as it allows better control of the oscillation dynamics and has inherently less noise *(7,8)*.

3.1. Noncontact Mode

An oscillating probe is brought into proximity of (but without touching) the surface of the sample and senses the van der Waals attractive forces that induce a frequency shift in the resonant frequency of a stiff cantilever (**Fig. 5**; **ref.** ***9***). Images are taken by keeping a constant frequency shift during scanning, and usually this is performed by monitoring the amplitude of the cantilever oscillation at a fixed frequency and feeding the corresponding value to the feedback loop exactly as for the DC modes. The tip–sample interactions are very small in noncontact mode, and good vertical resolution can be achieved, whereas lateral resolution is lower than in other operating modes. The greatest drawback is that it cannot be used in liquid environment, only on dry samples. Also, even on dry samples, if a thick contamination or water layer is present the tip can sometimes be trapped, not having sufficient energy to detach from the sample because of the small amplitude of oscillation.

3.2. Intermittent Contact Mode

The general scheme is similar to that of noncontact mode, but in this case during oscillation the tip is brought into contact with the sample surface so that a dampening of the cantilever oscillation amplitude is induced by the same

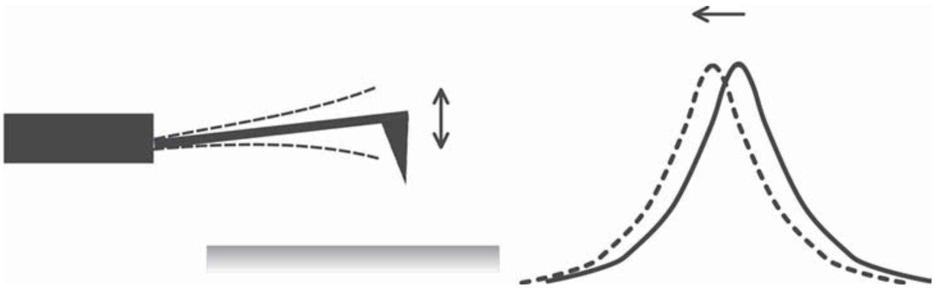

Fig. 5. In noncontact mode of operation, a vibrating tip is brought near the sample surface, sensing the attractive forces. This induces a frequency shift in the resonance peak of the cantilever that is used to operate the feedback.

repulsive forces that are present in contact mode (**Fig. 6**). Usually in intermittent contact the oscillation amplitude of the cantilever is larger than the one used for noncontact. There are several advantages that have made this mode of operation quite popular. The vertical resolution is very good together with lateral resolution, there is less interaction with the sample compared with contact mode (especially lateral forces are greatly reduced), and it can be used in liquid environment ***(10–14)***. This mode of operation is the most generally used for imaging biological samples and is still under constant improvement, thanks to additional features such as Q-control ***(15)*** or magnetically driven tips ***(7,8)***.

3.3. Phase Imaging Mode

If the phase lag of the cantilever oscillation relative to driving signal is recorded in a second acquisition channel during imaging in intermittent contact mode, noteworthy information on local properties, such as stiffness, viscosity, and adhesion, can be detected that are not revealed by other AFM techniques ***(16)***. In fact, it is good practice to always acquire simultaneously both the amplitude and phase signals during intermittent contact operation, as the physical information is entwined and all the data is necessary to interpret the images obtained ***(17–21)***.

3.4. Force Modulation

In this case, a low-frequency oscillation is induced (usually to the sample) and the corresponding cantilever deflection recorded while the tip is kept in contact with the sample (**Fig. 7**). The varying stiffness of surface features will induce a corresponding dampening of the cantilever oscillation, so that local relative visco-elastic properties can be imaged.

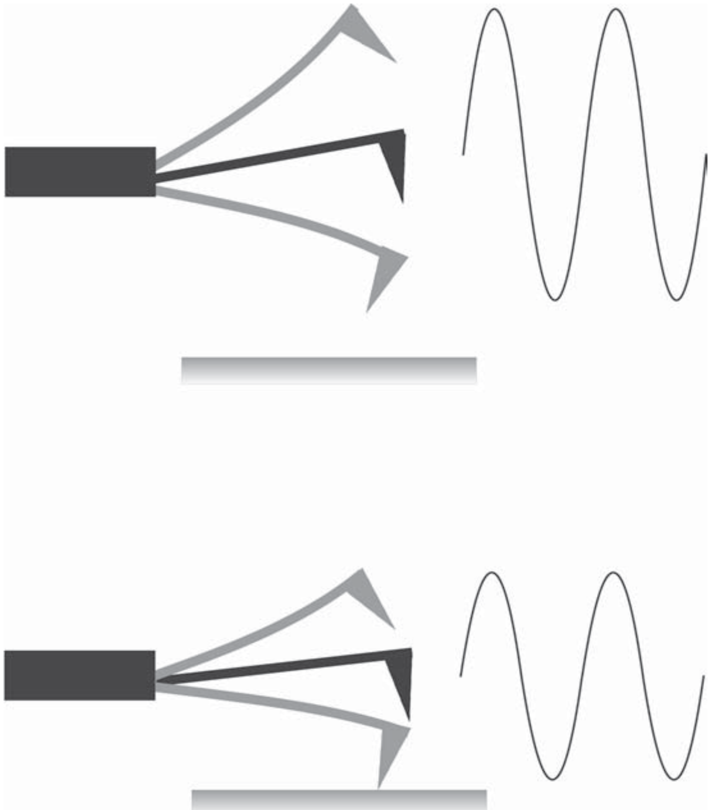

Fig. 6. In intermittent contact mode, the free oscillation of a vibrating cantilever is dampened when the tip touches the sample surface at each cycle. The image is performed keeping constant the oscillation amplitude decrease while scanning.

4. Beyond Topography Using Force Curves

The AFM can provide much more information than taking images of the surface of the sample. The instrument can be used to record the amount of force felt by the cantilever as the probe tip is brought close to a sample surface, eventually indent the surface and then pulled away. By doing this, the long-range attractive or repulsive forces between the probe tip and the sample surface can be studied, local chemical and mechanical properties like adhesion and elasticity may be investigated, and even the bonding forces between molecules may be directly measured ***(22–24)***. By acquiring a series of force curves, one at each point of a square grid, it is possible to acquire a so called force-vs-volume map that will allow the user to compute images representing local mechanical properties of the sample observed.

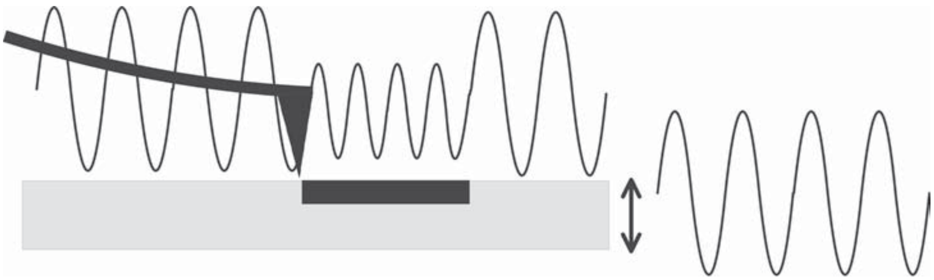

Fig. 7. During force modulation, the tip is kept in contact with the sample and the different local properties of the sample will be reflected in the amplitude of the oscillation induced in the cantilever.

Force curves typically show the deflection of the cantilever as the probe is brought vertically towards and then away from the sample surface using the vertical motion of the scanner driven by a triangular wave (**Fig. 8**). By controlling the amplitude and frequency of the vertical movement of the scanner it is possible to change the distance and speed that the AFM probe travels during the force measurement. Conceptually what happens during a force curve is not much different from what happens between tip and sample during intermittent contact imaging. The differences are in the frequency, much lower for force curves, and the distance of travel of the probe, much smaller in intermittent contact. In a force curve, many data points are acquired during the motion, so that very small forces can be detected and interpreted by fitting the force curve according to theoretical models.

Two details of technique are worth special care when obtaining quantitative data from force-vs-distance curves. The position-sensitive photodetector signal has to be calibrated so to measure accurately the deflection of the cantilever, and after calibration it is essential that the laser alignment is left unchanged. Usually the software of the AFM has a routine for such calibration, performed by taking a force curve on a hard sample and using the scanner's vertical movement as reference (which means that the scanner also has to be accurately calibrated). At this point, the curve we are plotting is not yet a force curve but a calibrated deflection curve. The next step is to convert it to a force curve using the force constant of the cantilever we are using. Manufacturers usually specify this value, but for each cantilever there can be quite large variations, so that for accurate work direct determination becomes necessary. There are different ways to measure the force constant, some requiring external equipment for measuring resonant frequency (such as spectrum analyzers) and others making use of reference cantilevers ***(25,26)***.

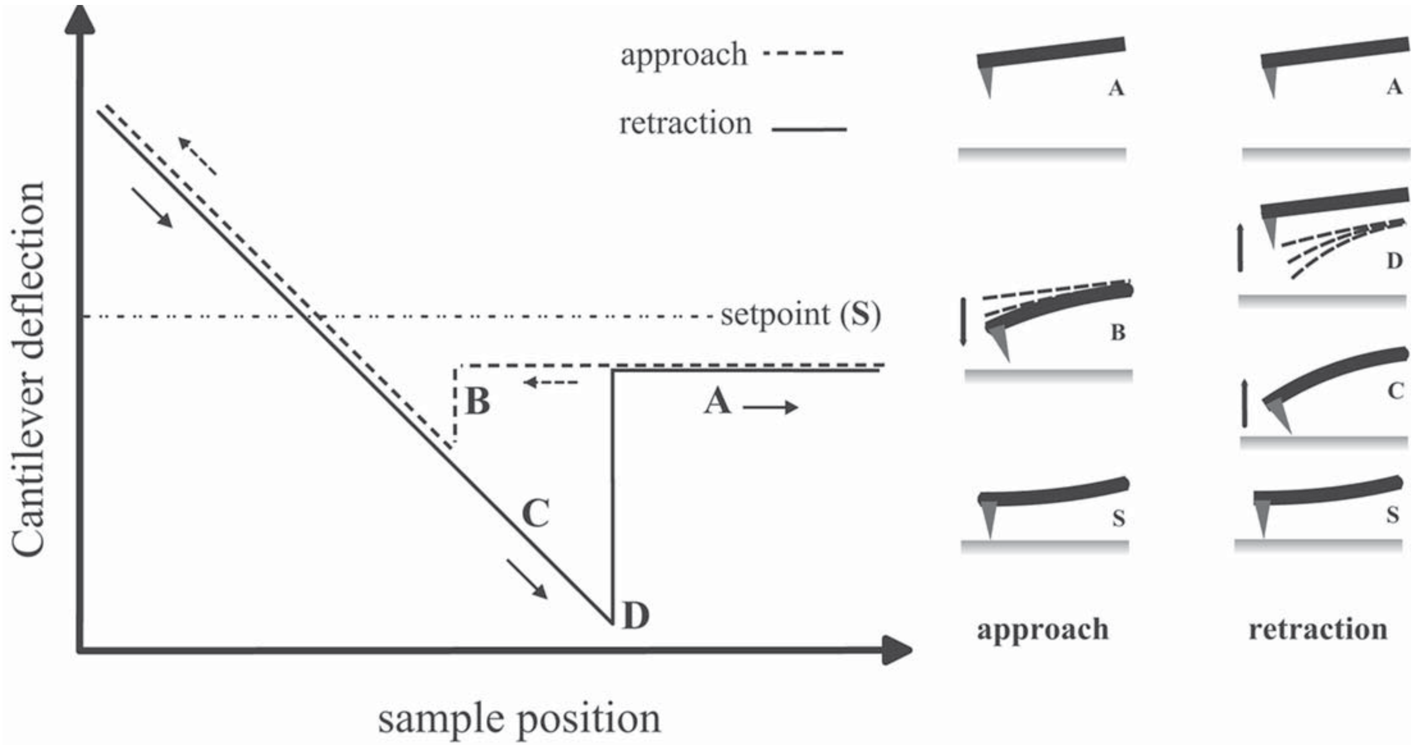

Fig. 8. Idealized force curve and cantilever behavior. From positions A to B, the tip is approaching the surface, and at position B contact is made (if an attractive or repulsive force is active before contact, the portion of the force curve will reflect it). After B, the cantilever bends until it reaches the specified force limit that is to be applied (S). Depending on the relative stiffness of the cantilever with respect to the sample, during this portion of the curve the tip can indent the surface. The tip is then withdrawn towards positions C and D. At position D, under application of the retraction force, the tip detaches from the sample (often referred to as ‘snap off’). Between positions D and A, the cantilever returns to its resting position and is ready for another measurement.

Form the point of view of biomedical applications, interesting experiments can be performed by coating the tip with a ligand and approaching through a force curve a surface where receptor molecules can be found. In this case the portion of the curve before snap off will have a different shape, reflecting the elongation of the bond between ligand and receptor before dissociation: from the shape the curve, it is possible to derive quantitative information on the binding forces ***(27–29)***.

If a force curve is taken at each point of a $N \times N$ grid, it is possible to derive images that are directly correlated to a physical property of the surface of the sample. For example, if the approach portion of each curve after contact is fitted using indentation theory, a map of the sample stiffness can be calculated. This data can be represented by an image in which the level of gray of each pixel, instead of representing the height of the sample, will correspond to the elasticity modulus. Similar images can be calculated for adhesion, binding, electrostatic forces, and so on ***(30,31)***.

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