Cellular Bioengineering

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Atomic Force Microscopy

I. Introduction

Atomic force microscopy (AFM) has been a major breakthrough for the sciences [3]. Used primarily as an imaging tool, AFM gives scientists the ability to visualize surfaces at an atomic scale [3], with up to 10 picometer resolution [2]. AFM has been enormously helpful in the field of biology in particular, since the technique can be used with samples in vacuum, ambient air, and aqueous environments [5]. This flexibility allows imaging of samples under more physiological conditions [3] than techniques like electron microscopy for instance, where imaging must be performed in vacuum [5]. Furthermore, the sample does not need to be stained or undergo other forms of harmful treatment so that it isn't sacrificed in the process and can potentially be used again for further analysis [3].

AFM has been used to image live cells and DNA [3], as well as collagen and other proteins at the nanoscale (**Figure 1**) [5]. AFM's imaging capabilities have also helped scientists find new structures, like the cell membrane's fusion pores [3]. Furthermore, AFM can be used for a wealth of other applications, including measuring the elasticity and viscosity of biomaterials, determining the charge on samples surfaces, and quantifying the forces involved in receptor-ligand binding and other binding at nanonewton resolution [5].

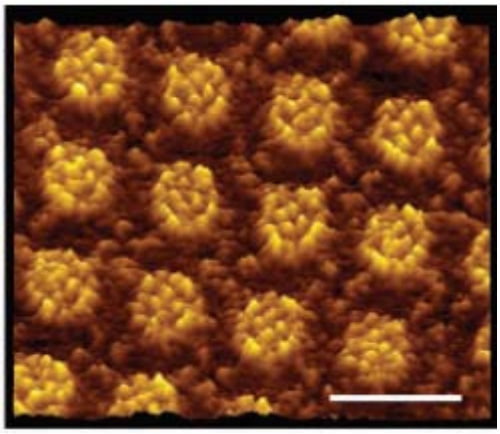

Figure 1. AFM image of the membrane protein aquaporin-Z. Scale bar, 10 nm [3]

II. Atomic Force Microscopy

A. How AFM Works

AFM is a member of the scanning probe microscopy (SPM) family, an important group of imaging techniques that is quickly becoming as important as optical and electron microscopy [3]. The concept behind SPM in general is to have a highly sensitive and stable tip mounted on some spring-like device that serves as a cantilever [3]. Various forces between the tip and the sample produce a measurable deflection in the cantilever when scanning over the sample's surface [3].

In AFM, the deflection is measured using the optical lever system [2], which will be explained later. The limiting factor on resolution with SPM is the size of the probe tip and the distance between the tip and the surface since these factors affect the strength of the forces between the two [3]. Higher resolution is achieved when the tip is closer to the sample but at the same time, the risk of damaging the surface is increased when the tip is making contact [3].

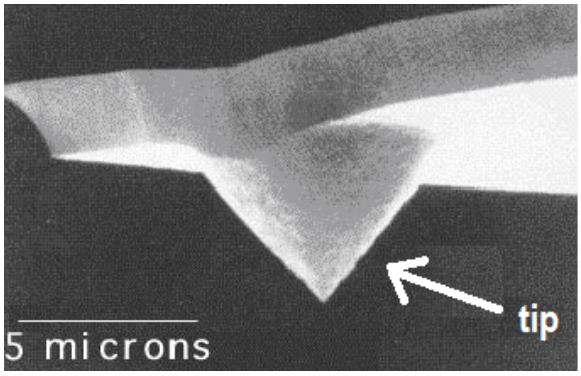

Figure 2. EM image of AFM tip on cantilever [1]

With AFM specifically, the sharp probe tip is generally made of Si or Si_3N_4 [5]. The tip can be conical, tetrahedral, or pyramidal, with the conical tips having diameters of 5 nm and the latter tips having diameters between 10 nm and 50 nm [1]. Conical tips are generally sharper and so are better suited to feeling out steep features, but are also more liable to break than the other tip geometries which are used more frequently [1]. The tip is attached to the end of a cantilever (**Figure 2**) [7], typically constructed from silicon oxynitride, a ceramic, and coated with gold or some other reflective material [2]. AFM cantilevers are generally about 100 μm long [2]. With the small deflections detected, the cantilever obeys Hooke's Law,

$$F = -kx$$

where F is force, k is the spring constant, and x is the deflection [1], so the forces at work can easily be calculated from the measured deflection. Cantilevers used in AFM vary greatly in flexibility, with spring constants starting from 0.1 N/m [2] up to 100 N/m [5]. Stiffer cantilevers are used in ambient conditions whereas more flexible cantilevers are used in aqueous environments [5]. The spring constant is related to the resonant frequency Ω of the cantilever by the following equation,

$$\Omega = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

where m is mass [2]. m is very small since the cantilevers are on the micro-scale, so AFM cantilever resonant frequencies are fairly high [2], generally ranging from 100 to 400 kHz [4]. High resonant frequency is important in order for the system to be quickly responsive to changes in the sample surface's topography [2].

A piezoelectric tube scanner is used to position the cantilever and the probe tip over the sample [6], since currently no mechanical motor provides adequate precision at the atomic scale for this purpose [1]. (The tube scanner can alternatively be used to move the sample underneath the probe tip [4].) The scanner is generally made from the piezoelectric ceramic, lead zirconium titanate (PZT) [1]. The tube, as a piezoelectric transducer, expands when voltage is applied [6], with the piezoceramic's expansion coefficient ranging from 0.1 nm/Volt to 300 nm/Volt [1]. A voltage is applied to the scanner in response to feedback [2], to maintain a constant cantilever deflection, applied force, or probe height, depending on the mode used [5]. The scanner can move with sub-Angstrom (< 0.1 nm) resolution in the x, y, and z (up and down) direction [7].

An optical lever system is used to measure the deflection of the cantilever (**Figure 3**), which in turn is used to provide the feedback needed to adjust the scanner according to the setting used [2]. The system is very sensitive and can detect sub-Angstrom movement in the cantilever [4].

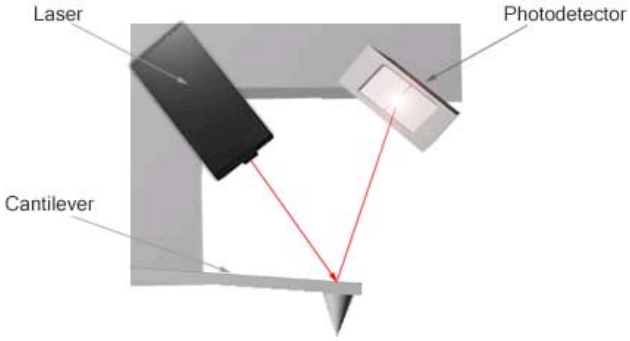

Figure 3. Optical light lever system [6]

A laser beam hits the reflective cantilever [5], which produces a reflected beam that is magnified because of the relative angles at which the components are set up in the AFM [2]. The reflected beam strikes a photodetector, made from two adjacent photodiodes [2]. The difference in intensity between what is detected by each photodiode is translated into a voltage that is fed back to the piezoelectric scanner, which adjusts accordingly [5]. The adjustment that is recorded or the deflection of the cantilever is used then to map out a surface topography [2] in constant height mode [4].

B. Modes of AFM

AFM can operate in contact, non-contact, and tapping mode. Since AFM works essentially by measuring the forces between the tip and the surface [3], it is helpful to look at a force-distance curve (**Figure 4**) to determine the nature of the forces with the different modes. When the tip is far away from the sample surface, the forces between the two are very small because the distance is too great; as the tip comes closer to the sample, attractive Van der Waals forces pull them together as dipoles and induced dipoles interact; when the tip and sample are very close together, electron orbitals of the sample and tip come in contact and repulse each other [4].

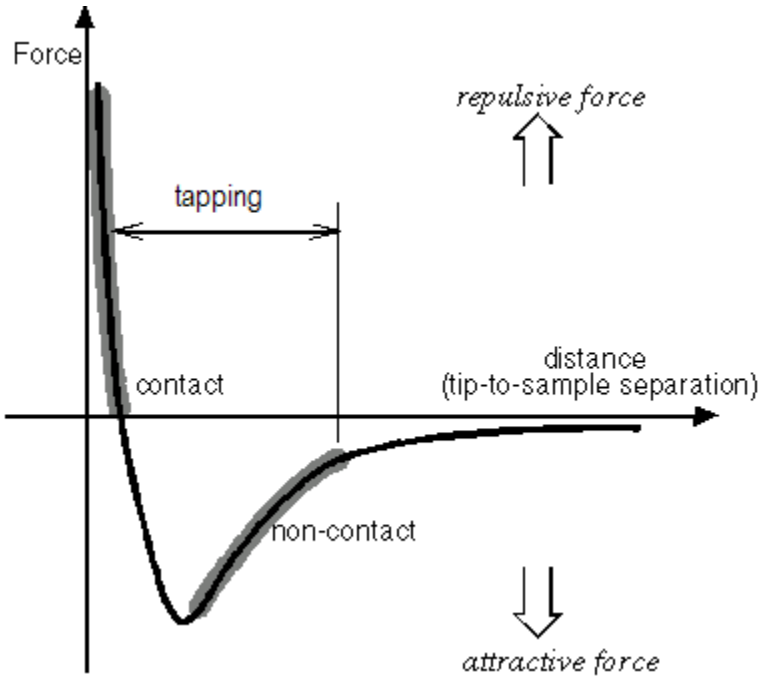

Figure 4. Force versus distance curve with modes outlined [4].

Contact Mode

AFM is most commonly operated in contact mode [7]. As the name suggests, in contact mode, the probe tip is always in contact with the sample [5]. The cantilever pushes down on the sample with forces ranging from 10^{-8} to 10^{-6}N [4]. The sample's topography is obtained in either constant height or constant force mode [4]. With constant force mode, which could just as easily be called constant deflection mode, a force is set (on the order of10^{-9} N) at which the scanner should hold the cantilever/probe [5]. The topography is mapped out using the adjustments in the scanner's vertical movement made to maintain this set force [4]. With constant height mode, the probe is held at a set height throughout scanning and the deflection of the cantilever is measured to map out the sample's topography [4].

Contact mode has a number of drawbacks. The constant contact with the sample can damage the sample surface as the probe is drawn across the surface [4]. Furthermore, when the AFM is being operated in atmospheric conditions (i.e. in open air), the tip is probing a contaminant layer over the surface consisting of water vapor and nitrogen that pulls the probe in with capillary forces on the order of 10^{-8} N [4], producing distorted data [5]. When operating the AFM in contact mode, scientists often will place their samples in aqueous environments since the liquid removes the capillary forces and reduces the effect of the Van der Waals forces [5]. (The collective effect of

the contaminant layer is known as the meniscus force [2].) In all the modes, though, it should be noted that the material properties of the surface studied will have an impact on the images produced because of the differing hardness and friction [6].

Non-Contact Mode

In an attempt to prevent the sample damage that sometimes occurs with contact mode, non-contact mode was developed [5]. In non-contact mode, the tip scans 50 to 150 Å over the sample [5], which is close enough to be in the weakly attractive regime of **Figure 4** [5]. Non-contact mode is not as effective as contact mode because the forces being measured are much weaker (on the order of 10^{-12} N); to compensate, the tip is oscillated from 100 to 400kHz, around the cantilever's resonant frequency, so that the forces can be detected by quantifying changes in the cantilever response to the sample's "force gradients" [4]. With non-contact mode, the frequency or amplitude of vibration is maintained at a set level, with the adjustments reflecting the distance between the tip and the sample, yielding the surface topography [4]. In ambient conditions, the meniscus force draws the probe into the contaminant layer, which disrupts the oscillation and can distort the resulting images [5]. This drawing in is referred to "jump-to-contact" [7]. Consequently, non-contact mode is not frequently used [7].

Tapping Mode

With tapping mode, like with non-contact mode, the probe tip is again oscillated around the resonant frequency of the cantilever, except that the tip is allowed to tap the surface on the downswing of its oscillation [5]. As **Figure 4** illustrates, tapping mode goes between the strong repulsive forces experienced upon contact and the weakly attractive forces during liftoff [4]. When the probe contacts the surface, the oscillation is slowed due to the collision and the amplitude changes resulting are then quantified; the adjustment to maintain some set constant vibration amplitude is used to obtain a surface topography, as amplitude increases over dents in the sample and decrease over protrusions [5]. Damage to the surface is negligible, since the frequency of the probe's contact makes the surface viscoelastic so the probe is not making extended contact with the surface [5]. Furthermore, there is no shear force affecting the results since the force is always coming down vertically and then lifting off, unlike in contact mode where in scanning the tip is essentially pulled across the surface [5]. When operating in aqueous environments, the fluid that the sample is immersed in is oscillated to produce the desired

oscillation in the cantilever (generally 5 kHz to 40kHz), which is much more flexible than those used in ambient conditions, with spring constants around 0.1 N/m in contrast to 1 to 100N/m [5].

Figure 5. (a) Contact mode; (b) Non-contact mode; and (c) Tapping mode. [6]

III. Novel Application: Knife-Edged AFM System [8]

AFM, as mentioned earlier, is commonly used in DNA imaging. This technology allows examination of the convoluted structure of chromosomes, which previously, with electron and light microscopy, were only observed on the macro scale. Previously, AFM tips had been used to "dynamically plow" through resist films and semiconductors and in DNA dissection; Saito et al. wanted to go beyond that and design a new probe specifically to dissect chromosomes [8].

The research group microfabricated knife-edged probe tips from silicon nitrate using reactive ion etching. They produced 20 µm long tips, either 4 or 6 µm thick (**Figure 6**), and mounted them on 200 µm long silicon cantilevers. The 4 µm tip had a theoretical spring constant of 13 N/m and a measured resonant frequency of 103.3 kHz in contrast to the 6 µm thick cantilever which had a theoretical spring constant of 45 N/m and measured resonant frequency of 165.4 kHz. Saito et al. compared the performance of these new probes with a conventional pyramid probe which had a spring constant of 42 N/m and a resonant frequency of 300 kHz [8].

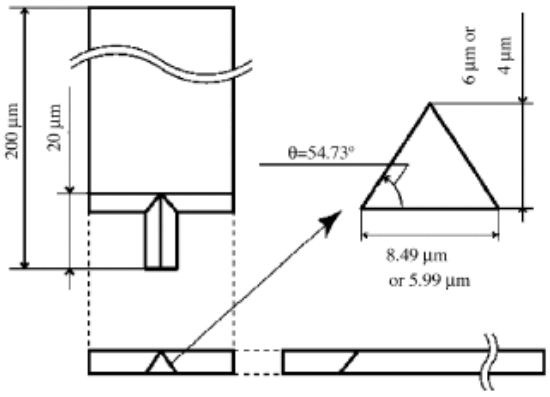

Figure 6. Knife-edged probe design [8]

The probes were used to dissect and image metaphase chromosomes from human lymphocytes mounted on a silicon substrate. Before dissection, the tips were used to image the chromosomes in tapping mode. For the dissection phase of the experiment, with the conventional probe, the AFM unit was operated in contact mode using a set loading force following some line established using a vector scanning program. With the knife-edged probes, the dissection was

performed in tapping mode. The dissection was controlled by referencing the amplitude of the cantilever deflection. After the dissection, the DNA was imaged with a new conventional pyramidal tip in tapping mode, regardless of which probe was actually used to perform the dissection [8].

The dissection with the conventional probe resulted in considerable debris, so some of the chromosomal material was lost. The 4 µm probe did not penetrate chromosome very deeply but did not produce any debris. The sharper 6 µm probe was more successful in performing deeper dissections. The 6 µm probe was also successfully used to nudge the dissected chromosome fragments around (**Figure 7**) [8].

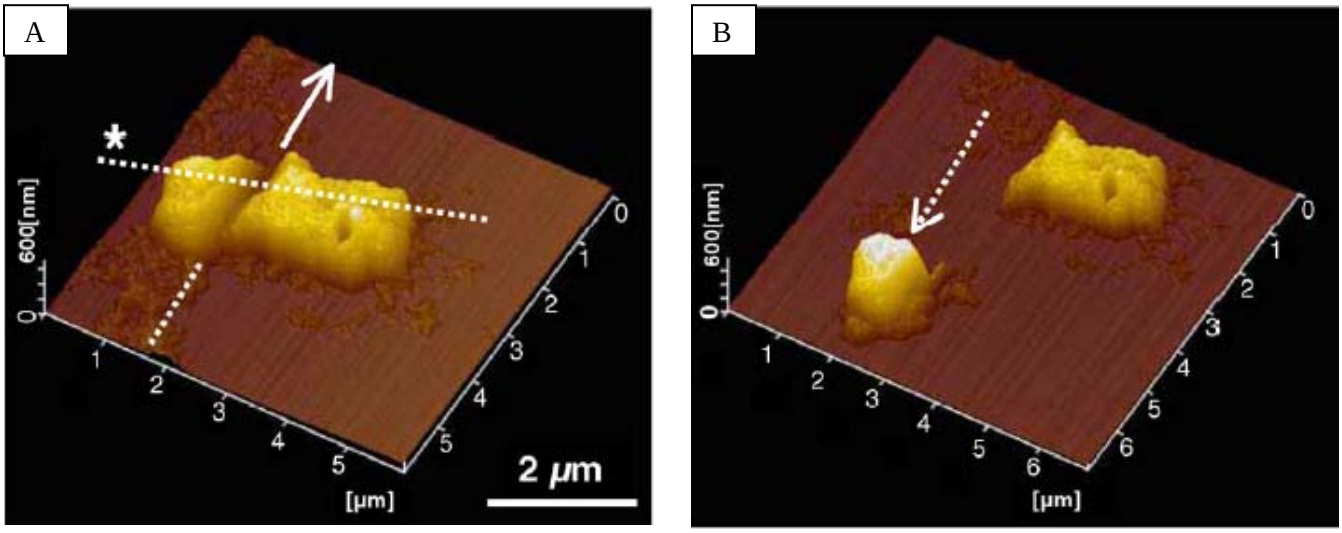

Figure 7. (a) Chromosome dissected with 6 µm probe; (b) dissected fragment moved with probe [8]

IV. Conclusions

AFM is a very useful technique that has numerous biological applications [3]. Since AFM is a relatively new technology, at just over 20 years old, [5] more and more uses for the system are still being developed. As it is, the AFM has quickly established itself as one of the most important tools in the laboratory.

V. References

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