The Evolution of Scanning Probe Microscopes for Biological Imaging

Application Note

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Introduction:

Approximately twenty years ago, Gerd Binnig and Heinrich Rohrer published images from their new invention, the scanning tunneling microscope (STM), which was the first scanning probe microscope (SPM) ever developed [Binnig, 1982]. Their new instrument was able to resolve atomic structures by raster scanning a conductive tip, mounted on a ceramic piezoelectric element, over a conductive sample. The incredible resolution of this new microscope was met with great enthusiasm within the international scientific community and laboratories throughout the world began building similar instruments. The implications of a high-resolution imaging tool that could operate under ambient conditions were not lost on biologists, who quickly teamed with physicists to develop new STM techniques to apply to biological samples. Although STM images of biological molecules did appear in the literature, they were difficult to reproduce. This frustrated initial attempts to utilized STM in some biological applications. Nevertheless, STM was responsible for establishing a fresh, new focus on microscopy, and research groups throughout the world would embrace the next generation of SPM instruments, which they envisioned would have a greater impact biological research. In 1986, the next generation SPM was invented [Binnig, 1986] and called the atomic force microscope (AFM) and the first AFM finally became commercially available in 1989. A number of review articles and books offer a comprehensive review of AFM instrumentation and the biological applications of AFM [Bustamante, 1996; Hansma, 1997; Morris, 1999; Watanabe, 2000; Bonnell, 2000]. These articles offer valuable information about how AFM technology has been applied to applications in the life sciences, while the current article focuses on the evolution of AFM into a valuable tool for biological research in the twenty-first century. One might consider AFM simply as just a minor extension of STM, but there are several critical differences between AFM and STM. For example, instead of relying on an electronic probe at the end of a ceramic piezoelectric element to map electrical potentials, AFM utilizes a flexible cantilever that has an extremely sharp tip to map the physical contours of samples. The flexible cantilever is rastered over the sample surface while data points are acquired in the X, Y and Z directions. A small laser, which is focused on the back of the cantilever, is reflected onto a sensitive diode, so as the AFM tip is scanned over the sample's surface, changes in the sample's height, can be detected. A feedback loop applies a voltage to a ceramic piezoelectric element, which moves the AFM cantilever up or down in order to precisely maintain the laser in a fixed position in the Z axis. The compensating voltage from the Z axis feedback loop is used by the AFM, along with the X and Y data points, to plot an image of the sample's topography.

Atomic force microscopy results are often considered easier to obtain than results from STM experiments but, unlike STM, AFM is able to image even non-conductive or insulating materials. Consequently, as scientists quickly embraced this new technology AFM images of proteins [Quist, 1995; Radmacher, 1994; Hallett, 1995], DNA [Bustamante, 1992; Thundat, 1992; Hansma, 1992; Lindsay, 1992], and living cells [Henderson, 1992; Radmacher, 1992], began to appear in the literature.

Future Biological Research:

A remarkable characteristic of biological processes is their scale. All living organisms, regardless of size, are organized down to the molecular scale. Genetically encoded instructions, thousands of different gene-based products, various small molecules and metabolic reactants, intermediates and products are all arranged and manipulated by living systems within very small volumes. With a tremendous amount of genomic sequence information in hand, new biological initiatives are now focusing on understanding of the roles that genes and their coded proteins play in the structural and functional organization cells. Unraveling these complex systems will require sensitive experimental techniques that can identify, localize, and quantify the molecular interactions that occur between thousands of discreet molecules and molecular systems. New and improved instrumentation will be required in order to meet these new research challenges. Scanning probe microscopy, specifically AFM, can assume a prominent role in the post-genomics era by providing, among other things, a new approach to molecular screening and high resolution imaging under physiological conditions. Of course, AFM is not limited to just biological applications; many materials science and chemistry applications are also enabled by AFM-based methods.

Evolution of the AFM:

Imaging with AFM: Contact Mode

As discussed above, AFM operates by monitoring the position of a very sharp tip, on the end of a flexible microcantilever, as it is scanned over a sample surface. Early AFMs operated only in contact mode; wherein an AFM tip [Binnig, 1986] is in physical contact with the sample at all times. In contact mode, the tip-sample interaction is maintained at a nearly constant force by ceramic piezoelectric elements housed within the AFM scanner. The pizeoelectric elements moves the cantilever up and down as it scans over the sample. The motion of the scanner at each data point is then plotted to generate a profile of the sample's topography. Physical interactions between the tip of the AFM cantilever and the sample create a significant amount of lateral force, so contact mode imaging of biological samples requires that the samples be firmly affixed to an atomically flat substrate. DNA molecules can be immobilized to an atomically flat mica surface by introducing divalent cations, such as magnesium, cobalt, or nickel, to the mounting media [Bustamante, 1992; Thundat, 1992]. Alternatively, by reacting aminosilane reagents, such as aminopropyltriethoxysilane (APTES), with mica surfaces, DNA can also be immobilized for AFM imaging [Lindsay, 1992; Lyubchenko, 1992]. The details of techniques for immobilizing biomolecules to surfaces is an ongoing challenge that is beyond the scope of this paper. There are numerous review articles and book chapters that serve as excellent sources of information for the interested reader [Bustamante, 1996; Hansma, 1997; Morris, 1999; Watanabe, 2000; Bonnell, 2000]. Many very soft biological samples are not compatible with contact mode AFM because contact with the AFM tip and large lateral forces can deform or even damage the sample's surface. Consequently, many contact mode images for soft samples are not high resolution. The advent of AC mode AFM, which can operate in either a non-contact regime or an intermittent contact regime, provides a viable solution for soft biological samples.

Imaging with AFM: Dynamic or AC AFM

Dynamic, or AC AFM, is especially useful for imaging soft, delicate biological samples. In AC AFM, the AFM cantilever is driven to oscillate up and down at the cantilever's resonant frequency by an externally applied source so the AFM tip and the sample interact predominately in the vertical axis. Variations in cantilever oscillation, amplitude, frequency and phase are resolved by the AFM and plotted in order to determine the proximity of the AFM tip to the sample's surface and to generate other images of the sample [Lanz, 1994; Han, 1996]. Negligible lateral forces are encountered in AC AFM, so it is a more gentle technique than contact mode AFM and the severe sample degradation effects that are often observed in contact mode often do not occur.

In AC AFM, as the sharp tip of the AFM cantilever is brought proximal to the surface of a sample, the tip and the sample interact through van der Waals and other short range forces, so the sample causes a slight dampening of cantilever's oscillation amplitude. The AFM system monitors the amplitude and the oscillation frequency of the cantilever and keeps the distance between the AFM tip and the sample constant by a feedback circuit that moves the AFM scanner head up and down [Hansma, 1994]. The motion of the scanner at each data point is used to generate an image of the sample's topography. Interactions between the AFM tip and the sample cause perturbations in the frequency of the oscillating cantilever, which are directly related to the mechanical properties of the sample. The spatial variations in this information are collected at various data points simultaneously, and then compared and processed. A change in the cantilever's oscillation amplitude at each data point is used to generate an amplitude image of the sample. The contrast of the phase image results from the phase lag, or differences in phase between the measured AC input frequency and the output frequency, which is the frequency at which the cantilever oscillates after it interacts with a sample. Phase images are very useful because they are due to variations in the sample's mechanical properties. In addition, fine morphological features can often be extracted from the amplitude and the phase images.

Depending on the oscillation amplitude and the forces that result from tip-sample interactions, AC AFM can operate in either non-contact or intermittent contact regimes. In the intermittent contact regime, the AFM cantilever is oscillated at relatively small amplitudes over the sample, so interactions between the tip and the sample tend to dampen the oscillation amplitude significantly. Consequently, each bottom-most point of the AFM tip's down cycle puts the tip-sample interaction forces in the repulsive region. In the non-contact regime, the AFM cantilever oscillates more freely; it is less encumbered by tip-sample interactions at a relatively larger amplitude and each bottom-most point of the AFM tip's down cycle puts the tip-sample interaction forces in the attractive region.

Two technologies have evolved for dynamically driving, or oscillating, AFM cantilevers for AC imaging. Invented in 1987, acoustic AC (AAC) mode AFM is currently utilized in a wide assortment of AFM brands and platforms. It was first commercialized by Digital Instruments and is often referred to as "tapping mode" AFM. In AAC mode AFM, a voltage is applied to a piezoelectric actuator (PZT) that is contained within the AFM cantilever holder. The PZT generates high frequency sound waves, which cause the cantilever to oscillate at its resonant frequency. As the tip of the AFM cantilever oscillates, it is brought proximal to a sample surface by the scanner, so surface forces, such as van der Waals interactions, between the sample and the AFM tip cause a dampening of the cantilever's oscillation amplitude. This reduction in oscillation amplitude is utilized by the AFM to construct a representation of a sample's surface topography [Hansma, 1994].

One drawback to AAC mode AFM is that the acoustic wave excites a multitude of mechanical resonances. In AAC mode, the acoustic wave causes not only the AFM cantilever to vibrate, but also the cantilever holder and the fluid surrounding the sample and the cantilever. The actual response is a combination of the intrinsic resonances of the cantilever and the sample. This results in a complicated signal and a noisy background that limits the smallest amplitude of oscillation that can be used with AAC mode. A great advantage of operating AFM in

fluid is a relative lack of adhesion between the AFM and sample, but because of the interfering disturbances caused by the acoustic waves in AAC mode, a “forest of peaks” occurs in liquid. It is often quite difficult to find a suitably sensitive resonance peak at which to oscillate the cantilever within the “forest of peaks”.

Contact between the AFM tip and the sample is most often considered to be in the intermittent regime in AAC mode, but the another type of AC AFM mode, MAC Mode (magnetic AC mode). Magnetic AC mode has obvious advantages over AAC or “tapping mode” and it is far superior as a liquid imaging mode, at least in part because it lacks disturbances from acoustic waves, so it provides a much cleaner AFM signal in liquids, which results in superior AFM images. This is especially important for meeting the challenges that face biological imaging.

In MAC Mode, an AFM cantilever that has been coated with a paramagnetic material is precisely excited to oscillate at resonance by an external magnetic field. The magnetic field is generated by applying an ac voltage to a small coil that is wrapped around a ferrite core that is placed either directly above or below the AFM cantilever, in either the AFM scanner or in the sample plate. Directly and precisely oscillating the AFM cantilever means that, in MAC Mode the fluid medium, the cantilever holder, and other components are left unperturbed when the cantilever is oscillated. The result is a very clean resonance signal, so there is no “forest of peaks”, making it easier to choose the correct frequency to oscillate the cantilever. Another result of directly driving the AFM cantilever, and only the cantilever is it is possible to image with small oscillation amplitudes. The energy transferred to a sample by an oscillating AFM cantilever varies as the square of the oscillation amplitude, so a consequence of using smaller amplitudes is that MAC Mode is a much gentler imaging mode than AAC mode. In fact, MAC Mode is thousands of times gentler than AAC mode. Since it is an extremely gentle imaging mode, sample deformations are not an issue and image contrast can be extremely good in MAC Mode. This has benefits for extremely soft, sensitive samples like, for example, biological samples. MAC Mode is a proprietary imaging method that was developed by Molecular Imaging. It is now commercialized and offered solely by Agilent Technologies.

Sample Scanning vs. Cantilever Scanning

Commercial scanning probe microscopes can typically be divided further into two additional categories, sample scanning or device scanning instruments. Sample scanning keeps the cantilever stationary and moves the sample beneath the AFM cantilever during imaging. Consequently, the piezoelectric mechanisms that generate and control motion are associated with the sample plate. This can lead to issues because the area around or beneath the sample can become severely limited, creating problems for the adaptation of additional accessories to the AFM. For example, placing a heating-cooling stage directly on the piezoelectric scanning mechanism underneath is often problematic. Another complication exists when imaging in a liquid environment; since any leakage of liquid from the sample cell poses a significant risk of damage to the piezoelectric scanning mechanism underneath. This problem can be serious because a high bias voltage is required to operate the scanning mechanism.

Device scanning instruments are commonly referred to as top-down or cantilever scanning, because the

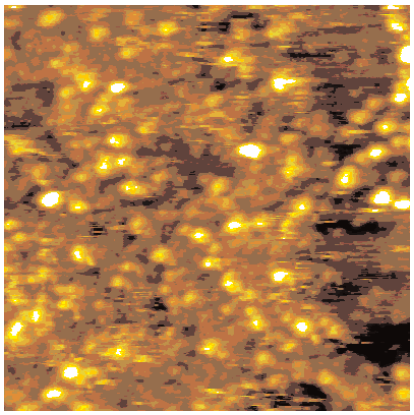

Fig. 1: High-resolution AFM image of S-layer proteins isolated from bacteria and imaged by MAC Mode in liquid.

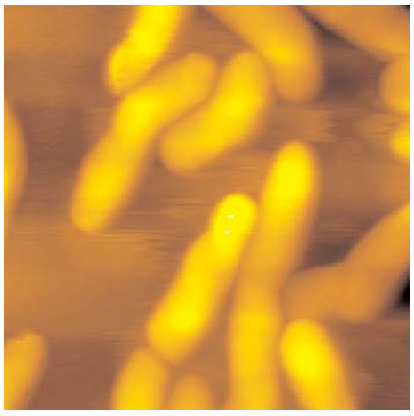

Fig. 2: MAC Mode AFM image of living Rhodopseudomonas palastris bacteria immobilized on a gelatin coated mica surface and imaged in liquid.

AFM cantilever is mounted directly on the AFM scanner, which is translated onto the sample for imaging. This avoids many of the problems associated with sample scanning and can offer significant advantages for combining AFM with other instruments or accessories. For example, the AFM can be combined with an optical microscope. Alternative sample stage configurations, such as heating-cooling devices or additional translatable stages for positioning microarrays, can also be implemented in the open space underneath the sample stage in a top-down scanning AFM. However, most commercial AFMs utilize a cantilever tracking mechanism that is based on laser beam deflection to maintain the laser's position on the cantilever during imaging. Laser tracking systems are often complex and require great precision in assembly. An imperfection in the beam tracking mechanism can cause issues with the position of the cantilever, resulting in defects in the images. These image defects include abnormal bow effects, which can make the image appear concave instead of flat, or waves in the background of the image. These problems have been solved by Agilent with the 5500 AFM, which utilizes a patented beam tracking system that maintains laser spot focus in a fixed position on the cantilever during imaging.

AFM as a Research Tool

As both AFM instrumentation and biological sample preparation matured, imaging a variety of biological molecules and even living cells became relatively more routine undertakings. High-resolution air and liquid MAC Mode AFM images of samples such as bacterial S-layer proteins (shown in figure 1) became relatively commonplace. Since adherent cells do not require complex-immobilization schemes, cell lines that are naturally adherent can be anchored directly to flat glass or mica. A number of laboratories have successfully imaged naturally adherent living mammalian cells in liquid environment. In contrast, in order to image non-adherent living cells in liquid, such as the bacterial cells in figure 2, the cells must first be immobilized to, for example, gelatin- or collagen-treated mica or glass [Doktycz, 2003].

Many life science AFM publications, include a statement in their introductions proclaiming that the AFM is an instrument that is capable of imaging in any environment, including liquids. It is precisely liquid imaging capability at molecular resolution that makes AFM especially attractive for many biological applications. That somewhat unfulfilled proclamation about AFM's liquid imaging capability is becoming a reality today as the AFM takes its rightful place as an imaging tool for investigating real biological problems in both air and, especially, in liquids. For example, restriction mapping of DNA molecules by AFM [Allison, 1996, 1997] has been accomplished by immobilizing a mutant restriction enzyme (EcoRI), that binds to DNA sequences in a sequence specific

Fig. 3: AFM image of a mouse cosmid (35 kb) imaged in air. The dark spots on the DNA indicate individual EcoRI endonuclease molecules that bind to DNA molecules in a site specific manner. This illustrates the usefulness of AFM to study DNA-protein complexes and to map DNA sequences with site specific proteins under ambient conditions.

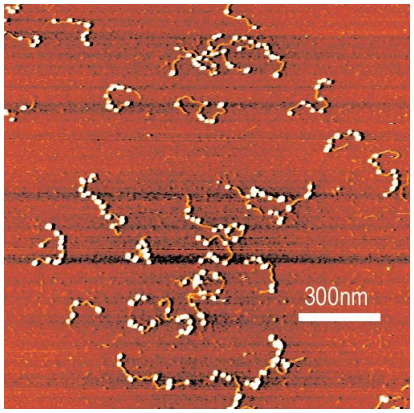

Fig. 4: MAC Mode AFM image of chromatin, which is a DNA–protein complex. The proteins, called nucleosomes, package the DNA. This sample was imaged under ambient conditions using a flow through liquid cell. The liquid cell was later used to change the ionic strength of the imaging buffer, causing the DNA to dissociate from the nucleosomes (not shown).

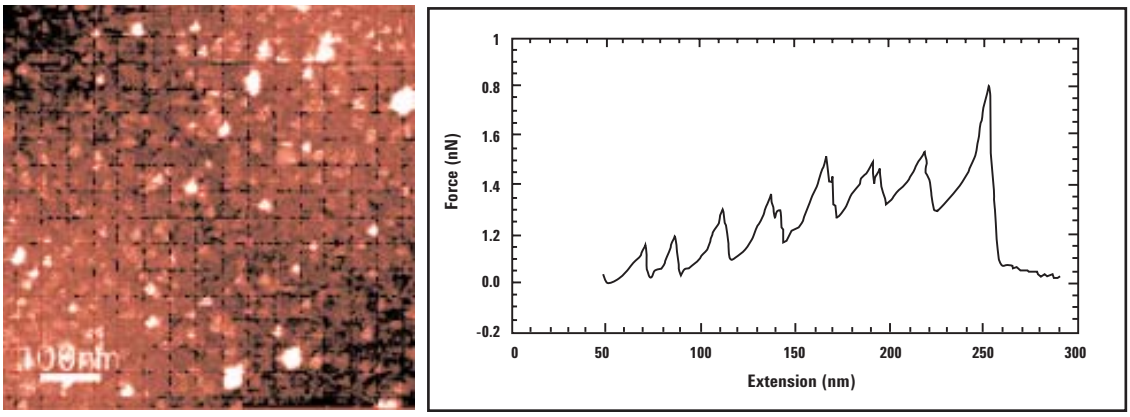

Fig. 5: Left: An AFM image of titin molecules. Right: A saw tooth pattern force-distance curve resulting from the rupture of individual domains of a single titin molecule. The titin molecule was immobilized to a solid surface and then extended between an AFM tip and that surface. Each maxima corresponds to the force required to rupture an individual domain. The distances between the peaks in the curve correspond to the length of each domain.

manner, to the tip of an AFM cantilever. The restriction enzyme was modified so that it did not cleave the DNA. The enzyme-modified AFM tip was used image and map EcoRI binding sites on cosmid size (30-50 kb) DNA samples. Images of this experiment, taken in air, are shown in figure 3. The ability to image DNA in liquid environments [Hansma 1996; Thompson, 1996] has led to a number of dynamic studies. By limiting the concentration of nucleotide triphosphate (NTP), RNA transcription was dynamically imaged. In this experiment, mica was chemically treated to immobilize DNA. RNA polymerase was added and, as the polymerase molecule moved along a DNA molecule, AFM images of the resulting RNA transcription product became discernible [Kasas, 1997]. Another dynamic study performed in liquid demonstrated that changes in protein shape that occur with enzymatic activity can also be observed by AFM [Radmacher, 1994]. An especially elegant study from the laboratory of Stuart Lindsay [Wang, 2003] illustrated that dynamic changes in protein-DNA complexes can be monitored with AFM. Chromatin (a nucleosome-DNA complex) was immobilized. The mica had been treated with an amino silane and glutaraldehyde to covalently attach the chromatin's nucleosomes, but not its DNA, directly to the mica. Consequently, the DNA was immobilized to the substrate only indirectly, through noncovalent interactions with the nucleosomes. The chromatin was imaged in MAC Mode (figure 4) and a flow-through liquid cell was utilized to change the salt concentration of the buffer during imaging. At low NaCl concentrations, intact nucleosome-DNA complexes were observed in the AFM images, but at progressively higher salt concentrations the DNA was released from its interactions with the nucleosomes, so that only the nucleosomes were visible. Dynamic studies like this are not confined to protein-DNA interactions. Structural changes in mammalian cells, such as the destruction of an actin network in fibroblasts by cytochalasin B, have also been recorded with AFM [Radmacher, 2000].

AFM Force Spectroscopy

A unique attribute of the scanning probe microscope is its ability to measure the minute forces that are responsible for various molecular interactions. A dynamic force microscopy (DFM) or dynamic force spectroscopy (DFS) experiment can be performed with an AFM by immobilizing one end of a biological polymer, such as DNA or a protein, to the tip of an AFM cantilever and the other end of the biological polymer to a solid surface. When the AFM tip is retracted away from the surface, the intramolecular forces that must be overcome in order to extend the biological polymer can then be measured and the results used to generate a force-distance curve (also referred to as a force-extension curve). Force-distance curves are possible because, the forces involved in the molecular interactions of the biological polymer samples can be obtained by measuring sub-Angstrom deflections as the cantilever is retracted away from the surface. The results can be used to quantify intramolecular forces as small as a few weak van der Waals interactions or even the unbinding force a single hydrogen bond [Hoh, 1992]. In a DFM protein extension experiment, a protein molecule with a complex tertiary structure was absorbed onto a gold coated mica surface. The tip of an AFM cantilever, which was also gold coated to adhere it to the protein, was brought into contact with the immobilized protein molecule and then retracted. A pattern that relates to the extension of the protein was evident from the

force-distance curve. The mechanical proteins titin [Rief, 1997; Marszalek, 1999; Tskhovrebova, 2000] and tenascin [Oberhauser 1998] have been extensively studied in single molecule DFM extension experiments. Each minimum in the force-distance plot for titin in figure 5 indicates force maximum; where an individual titin domain was extended by retracting the AFM cantilever. The distance between each peak minimum corresponds to the length of a structural domain. In this experiment, the force required to extend a titin domain was calculated to range from 150 to 300 pN, with a periodicity between domain extensions of 25 to 28 nm. This correlates well with data from other technologies in which the distance required to unravel a single titin Ig domain was determined to be 31 nm [Rief, 1997]. Force-distance extension experiments have also been reported for polysaccharides [Li, 1999] DNA molecules [Rief, 1999] and various alcohols [Li, 2000].

The extreme sensitivity of AFM can also be exploited to measure molecular forces that involve complementary pairs of molecules. For example, the energy required to rupture hydrogen bonds between complementary strands of DNA was measured by attaching a single-stranded DNA molecule to the tip of an AFM cantilever and immobilizing the complementary strand of DNA to a substrate. After the DNA strands were allowed to anneal, the AFM cantilever was retracted, to pull the DNA strands apart [Lee, 1994a; Boland, 1995], and the energy required for dissociation of the DNA was plotted. Small molecule-receptor interactions have also been measured with AFM. For example, biotin-avidin and biotin-streptavidin unbinding interactions have been quantified by attaching biotin to the tip of an AFM cantilever and immobilizing avidin [Florin, 1994; Ludwig, 1994] or streptavidin [Lee, 1994b] to a substrate. After allowing the biotin and avidin or streptavidin molecules to interact, the AFM cantilever was retracted and the force required to pull them apart was measured in order to determine the unbinding force of the interaction. Another interesting report describes how a eukaryotic cell was attached to the tip of an AFM cantilever and then allowed to interact with another cell that was growing in a Petri dish. The tip of the AFM cantilever was withdrawn away from the Petri dish so that the forc required to break the adhesive interactions between the two cells could be measured [Benoit, 2000]. Adhesive interactions between pairs of molecules have been measured and compared with different AFMs. The results have been found to be reasonably consistent. For example, a gold coated AFM tip and a gold coated substrate were both modified with 11-mercaptoundecanoic acid. The sulfur groups of each mercaptoundecanoic acid molecule bonded to the gold coated AFM tip and to the gold coated substrate. As the AFM tip was withdrawn from the substrate, the force required to break a single hydrogen bond was measured by the AFM and calculated to be 16.6 pN [Han, 1995]. In a similar experiment the rupture force required to break individual hydrogen bonds was measured at 12 pN [Hoh, 1992].

In figure 6, the topography image of avidin molecules, immobilized on a mica surface, is shown. In one experiment, biotin was

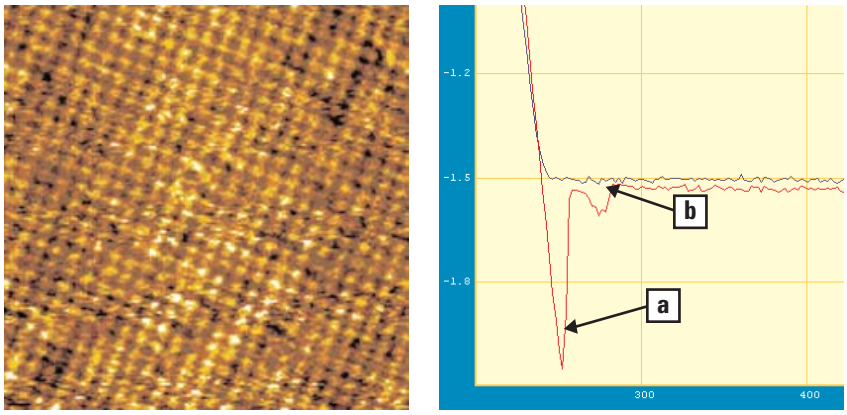

Fig. 6: Left: A MAC Mode AFM image of avidin on a mica surface. By probing the avidin coated surface with the tip of an AFM cantilever that was functionalized with biotin molecules, a force-distance curve (right) for biotin-avidin interactions was generated. The blue line represents approach of the AFM tip to the avidin coated surface. The red line represents withdrawal of the biotinylated AFM tip away from the surface. In the force-distance curve, a peak that represents nonspecific adhesion between the AFM tip and the avidin coated surface was resolved (a). In addition, a peak that corresponds to the force required for a single, specific unbinding event between biotin and avidin was also resolved (b).

attached to the tip of an AFM cantilever by a 30 nm PEG linker and a force-distance curve for biotin-avidin unbinding was generated. As the AFM cantilever was withdrawn away from the avidin on the surface, an initial peak, resulting from adhesion between the AFM tip and avidin, became visible in the force-distance curve. As the biotinylated AFM cantilever was withdrawn further away fromthe surface a second peak can be seen. The second peak is located approximately 30 nm from the initial adhesion peak, and was a result of an unbinding event between the biotin molecule, which was attached to the 30 nm long PEG linker, and an avidin molecule on the surface.

Intramolecular and intermolecular bond extension and rupture forces can be measured with most modern AFMs, but a low coherence laser, which can dramatically reduce background noise in force-distance curves, with a closed loop Z piezoelectric element, to obtain extremely accurate positioning of the AFM tip, is also desirable. A custom scripting capability, in which the AFM operator can program the movement of the AFM cantilever, is another desirable feature. Additionally, user access to programmed applications that allow a predetermined constant force or force at a constant rate to be applied to a sample enables these types of experiments.

Molecular Recognition Force Microscopy (MRFM) is another area where AFM is playing a significant role in current biomolecular research and should continue to be an important part in future research. MRFM combines topography imaging with simultaneous molecular recognition mapping between host-guest or ligand-receptor pairs. In MRFM, a probe molecule is attached to the tip of an AFM cantilever through a short (6-8nm) polyethylene glycol (PEG) tether [Haselgruber, 1995]. As the functionalized AFM cantilever is raster scanned over a sample surface, a topography image of the sample is obtained along with a map of molecular interactions between the probe molecule on the AFM tip and complementary areas on the sample. MRFM was first demonstrated by imaging lysozyme in MAC Mode with an AFM tip that had a PEG linker and an antibody to lysozyme attached. As the antibody and linker-functionalized AFM tip was raster scanned over an immobilized lysozyme molecules,a topography image, that contained molecular recognition information about antibody-antigen interaction, was obtained [Rabb, 1999]. However, the topography image was distorted because of the molecular interactions between lysozyme molecules and the antibody.

Using specially designed hardware and MAC Mode, information about the interactions between specific ligands and receptors can be separated from topography images to generate recognition maps of ligand-receptor and antibody-antigen interactions. The Hinterdorfer group tethered biotin to the tip of an AFM cantilever and immobi-

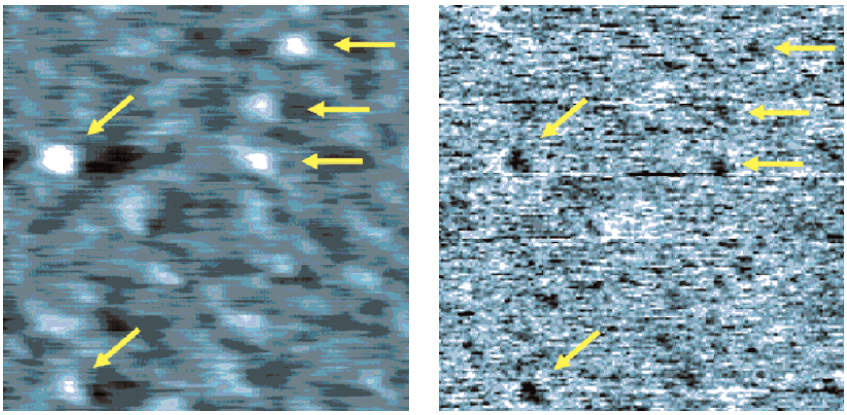

Fig. 7: MAC Mode topography (left) and molecular recognition images (right) of avidin and biotin-avidin interactions obtained simultaneously using PicoTREC and an Agilent 5500 AFM/SPM. A mica surface was treated with avidin molecules and then scanned with an AFM tip that was modified with biotin. In the resulting topography image, avidin molecules can be seen as white areas. In the recognition image, dark areas that correspond to molecular interactions between biotin and avidin can bee seen. The molecular interactions are in the same locations that avidin molecules were identified in the topography image.

lized avidin on a mica substrate. As the biotinylated AFM tip was scanned over the avidin coated substrate, MAC Mode topography images for avidin and recognition images for the biotin-avidin interaction were resolved and plotted simultaneously (figure 7). The hardware that is required to resolve molecular recognition images from MAC Mode topography images, called PicoTREC, is available as an option to Agilent's 5500 series AFM/SPM.

Microarray analysis by AFM

Microarray technology has made its biggest impact in the areas of gene expression profiling and DNA sequence or SNP identification, but other materials besides nucleic acids, including proteins, membranes, cells, and small molecules, can also be arrayed and assayed for activity with microarrays. Unfortunately, convenient labeling and detection methods for materials other than nucleic acids and proteins are not readily available, so called label-less detection schemes would be of benefit to microarray technology. Various biological molecules can be attached to AFM cantilevers, but dynamic force microscopy DFM on large arrays of biological molecules will require advances in hardware and software. Conventional microarrays are routinely created by various means, including, reagent jetting, pin-based spotting, or lithographic techniques. Microarrays are typically composed of individual micron-sized spots of discrete chemical identity organized on glass microscope slides. Hundreds or thousands of these spots can be arranged on a typical microarray. The arrays can be reacted with various assay reagents and, with the aid of specialized instrumentation and software, thousands of specific interactions evaluated. Current microarray technology calls attention to thousands of molecular interactions on the array; it typically does not quanify the forces of interaction between interacting species, nor does it evaluate these interactions at the single molecule level. By combining AFM with microarray technology, the forces of molecular interaction between array elements and assay reagents can be determined. This requires an AFM scanning mechanism in a top-down configuration and also that the AFM cantilever be affixed to the scanning mechanism in order to permit a large enough space under the sample plate to accommodate a translatable stage for aligning individual microarray elements with the AFM cantilever.

Summary:

Scanning probe microscopy has matured to a point where it can assume an important role in life science research in the twenty first century. The field is in a state of rapid growth. Manufacturers of commercial instruments are eager to incorporate promising new ideas from researchers into commercial products. Since speculation about future development of instrumentation is risky, a better approach may be to look at the future of biological research for directions regarding the development of instrumentation. The end of the twentieth century saw the sequencing of the human genome. Early in this century, a significant amount of effort in biological research is being devoted to understanding how the products of genes interact to maintain physiological process, cells and living organisms. It has been indicated in this article this will require methods for identifying, mapping, and measuring a variety of the numerous, weak, single molecule interactions that occur within living cells. How AFM development will be used to respond to these challenges has yet to be determined.

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