Atomic Force Microscope

Microcantilevers Learning Module
Instructor Guide

Notes to Instructor

Atomic Force Microscope is a primary knowledge (PK) topic for the Microcantilevers Learning Module. Like the Chemical Sensor Array PK, this PK illustrates an application of microcantilevers; therefore, the participants should already have basic knowledge on the operation of microcantilevers and their modes of operation. Such knowledge can be acquired in the SCME "How Does a Cantilever Work?(http://bit.ly/2jHEMZe)" PK which is part of the Microcantilevers Learning Module.

This Atomic Force Microscope (AFM) PK covers some of the applications of an AFM, the different types of AFMs, and how they work.

The Microcantilevers Learning Module consists of the following:
• *Book 1*
• Learning Module Map for Instructors
• Microcantilever Knowledge Probe
• Microcantilever Applications Overview (PK)
• Chemical Sensor Arrays (PK)
• **Atomic Force Microscopes (PK)**
• *Book 2*
• How Does a Cantilever Work? (PK)
• Microcantilever Model Activity: Resonant Frequency vs. Mass (SCME Kit Available)
• Microcantilevers Terminology and Research Activity
• Final Assessment

See the SCME YouTube (https://www.youtube.com/user/SCME2012/videos) channel for supporting instructional videos.

Description and Estimated Time to Complete

An Atomic Force Microscope (AFM) is a high resolution imaging tool used to view and map the surfaces of micro-size samples, creating detailed images of the micro and nano-size object surfaces. An AFM uses a microcantilever with a nano-sized probe tip to scan a surface and provide a three-dimensional (3D), color topographical image. In this unit you study the applications of AFMs in micro and nanotechnologies, the different types of AFMs and how they work.

It is recommended that you review the SCME Microcantilever unit on "How Does a Cantilever Work?(http://bit.ly/2jHEMZe) " prior to completing this unit.

Estimated Time to Complete
Allow approximately 40 minutes reading through this unit.

Introduction

An Atomic Force Microscope (AFM) is a high resolution scanning and imaging tool that can depict molecules and individual atoms on a sample surface *(Figure 1.)*. Using short-range forces existing between the surface and a probe tip, an AFM, in a sense, *feels* the surface. Its output is a 3D image of the surface's topography. *Figure 1* is an AFM image of individual silicon atoms on a crystalline surface (can you tell it is showing the (111) plane of the crystal wafer surface?).

Figure 1. "An atomic force microscope image by German physicist Franz Giessibl shows dozens of silicon atoms. Scientists have debated whether the light and dark crescents - or wing-shaped features seen on the atoms represent orbitals - the paths of electrons orbiting the atoms."[1] *[Printed with permission: F. J. Giessibl et al., Science 289, 422 (2000)" <Observing the 'Wings' of Atoms>*
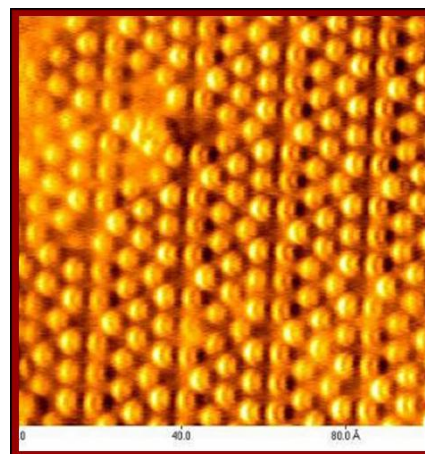
In addition to imaging, AFMs are also used to measure the surface elasticity, as well as measure intermolecular forces between the sample and the probe tip. They are used to reveal defects on the surface of materials by showing where atoms are missing[1] and measure the forces required to move individual atoms on a surface allowing for the manipulation of atoms necessary to build structures from the bottom up, atom by atom.[2] These characteristics allow for AFM applications in fields ranging from material development to medical research.

The following abstract from "Atomic Force Microscopy to Study Intermolecular Forces and Bonds Associated with Bacteria[3]," illustrates the value of the AFM in the field of bacterial adhesion:

"Atomic force microscopy (AFM) operates on a very different principle than other forms of microscopy, such as optical microscopy or electron microscopy. The key component of an AFM is a cantilever that bends in response to forces that it experiences as it touches another surface. Forces

as small as a few picoNewtons can be detected and probed with AFM. AFM has become very useful in biological sciences because it can be used on living cells that are immersed in water. AFM is particularly useful when the cantilever is modified with chemical groups (e.g. amine or carboxylic groups), small beads (e.g. glass or latex), or even a bacterium."

This unit explores many of the applications of AFMs as well as the various operating modes and the processes by which they obtain their measurements. This unit also illustrates how a microcantilever is used to obtain measurements and images.

Objectives

- State at least four specific applications of atomic force microscopes.
- Explain the operational theory of two types of AFMs.

By the end of this unit, you should be able to answer the following questions:
    a. How is a microcantilever used in an AFM?
    b. What are the differences between the contact mode, tapping mode, and non-contact mode of an AFM?
    c. What are the advantages and disadvantages of each operating mode?
   d. What are four AFM applications in least two different fields?

Applications of the Atomic Force Microscope (AFM)

The number of industries that use AFM technology, the materials being probed, and the devices being characterized are on a rapid rise. Micro and nanotechnology developments have been instrumental in increasing this number because of the small-surface dimensions requiring characterization in micro and nano applications. The AFM assists this technology with its ability to resolve down to molecules and atoms on a surface as well as its ability to manipulate and move atoms and molecules on a surface.

The AFM is an important tool for micro and nanotechnologies. As seen in Figure 1, the image of silicon atoms, the AFM provides a 3D image of angstrom (Å) sized particles on a surface, which in this case are silicon atoms. The darker spacing between silicon atoms in the AFM image is approximately 3 Å.[1]

An AFM also has the ability to observe, measure, and manipulate atoms on a surface into nano-size structures by building from the bottom up, one atom at a time. *Figure 2* shows the construction of a circular quantum corral made by manipulating iron atoms on a copper surface. Starting with the upper left image 1, you can see the surface atoms being repositioned to create the final circular image or corral in square 4.[4] These images were actually produced by a Scanning Tunneling Microscope or STM which is similar in operation to an AFM, but only works on conductive materials. *(More on that later.)*

Figure 2. Images created by and courtesy of IBM Corporation.
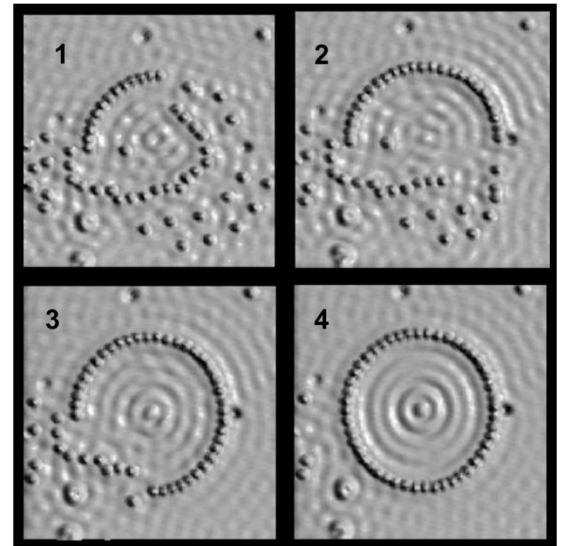
The AFM is capable of scanning a variety of materials (conductive and non-conductive) and sample areas, creating single scan images up to 150 x 150 microns square[2] with a height range of several nanometers to several micrometers.

For the most part, AFMs are used for small-scale applications such as the following:

- o Identify defects on and within a surface
- o Identify individual atoms on surfaces
- o Measure force-distance relationships on a pico-Newton scale.
- o Create topographical images of surfaces like in *Figure 3*.
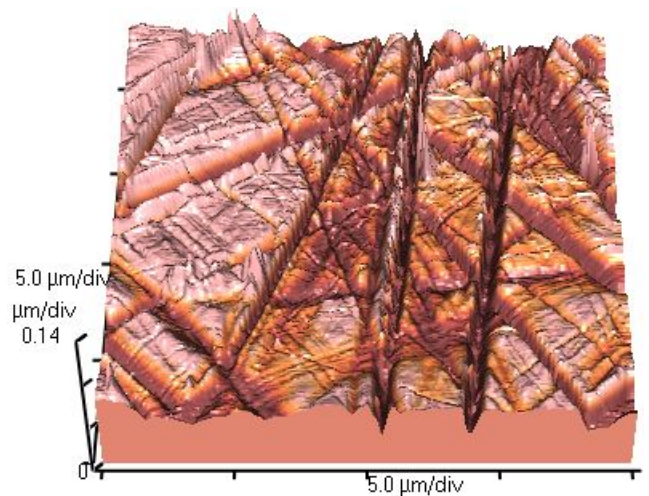
Figure 3: AFM image of a glass surface. The scanned size (x,y,z) is 20 μm x 20 μm x 420 nm. Image is Public Domain (Wikipedia)

More recently, AFM applications have expanded beyond semiconductor materials and devices:

- o The study biological organisms and membranes
- o To investigate coating composite materials
- o To observe, measure and manipulate nanoscale structures[5]
- o To observe thin film surfaces and measure small and deep structures (including optically transparent materials)

Pharmaceutical Applications[6]

One of the areas in which AFMs have proven to be particularly suitable is pharmaceutical applications (i.e., drug research, drug delivery systems). Current research already includes "surface characterization of tablets and their coatings, growth of crystals as a function of manufacturing parameters (concentration, temperature, pH…), and size and form of drug delivery vehicles"[6]. AFMs are also being used to study how specific drugs or drug dosages affect cells or target molecules, and, because AFMs can be used in aqueous conditions, such studies include the ability to do time-lapse experiments in liquid environments.

Figure 4 is an AFM image of a drug-eluting stent surface. A drug-eluting stent is an *in vivo* device, inserted into a peripheral or coronary artery during an angioplasty. The surface drug is slowly released preventing scar tissue from forming around the stent, a common occurrence after angioplasty.
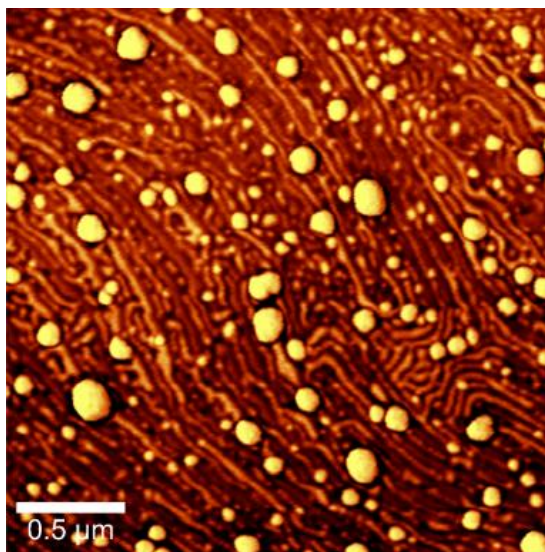

Figure 4: High-resolution AFM phase image of a drug eluting stent surface showing the polymer substrate structure (red) with the embedded drug particle (yellow). Image courtesy of WITec, Focus (http://www.witec.de)

Food Research[7]

To better understand how the food we eat affects our bodies, we need to understand the structure and function of what we eat. As food breaks down within our bodies, it releases nutrients that react with our body's cells and molecules. AFMs have proven to be useful as "one of a number of microscopic techniques for probing changes in chemical and physical structure of starch during plant development and growth"[7]. They are also being used to study fat digestion by looking at the oil-water interface of oil droplets to possibly determine its "biological function in terms of fat digestion and absorption through lipolysis"[7].

Polymer Science and Engineering

Polymers are used worldwide in a variety of industries and today, their use is "larger than the aluminum, copper and steel industries combined".[8] Some specific applications of polymers include the following:

- Agriculture polymers are used for mulch and for improving soil aeration.
- In the medical field polymers are used to build artificial heart valves and blood vessels.
- In the consumer industry their applications go on and on and on – plastic containers, garbage bags, car parts, pipe and tubing, flooring, playground equipment and swimming pools – just to name a few.[8]

AFMs allow us to better understand the properties of polymers to more effectively tailor them for unique properties, to be less expensive, and to be more durable and more sustainable than other materials. AFMs allow for the visualization of polymers at the sub-micron and sub-nanometer scales. At this scale we can study the molecular level forces and the mechanical, thermal and electrical properties of materials. This information can lead to new polymers that are more economical to manufacture, more sustainable and that may even be biodegradable.[9]

A Google search of "AFM images – Polymer" yields many examples of AFM images of polymer applications.

An AFM vs. Other Microscopes Used to View and Measure Sub-micron Structures

There are several types of small scale imaging devices used to look at sub-micron structures. One such device is an optical microscope that typically has trouble resolving structures less than 200nm. The most common tool used for sub-micron inspection is the Scanning Electron Microscope, commonly called a SEM. SEMs include small desktop models with 14nm resolution to large industrial scale automated SEMs with EDS (Energy Dispersive Spectroscopy) and FIB (Focused Ion Beam) capabilities having a minimum resolution of about 4nm. AFM's can resolve down to sub-nanometer resolution as seen in the previous images of the individual silicon atoms shown in Fig. 1.

Figure 1. "An atomic force microscope image by German physicist Franz Giessibl shows dozens of silicon atoms. Scientists have debated whether the light and dark crescents - or wing-shaped features seen on the atoms represent orbitals - the paths of electrons orbiting the atoms."[1] *[Printed with permission: F. J. Giessibl et al., Science 289, 422 (2000)" <Observing the 'Wings' of Atoms>*
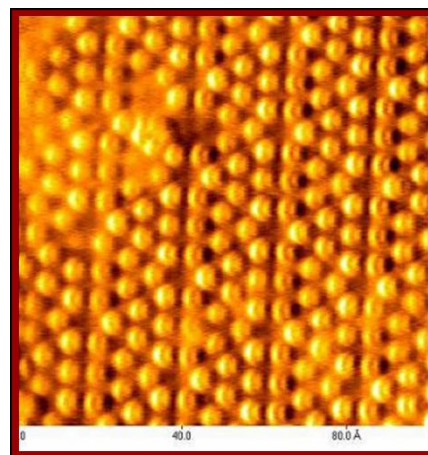
A close cousin to the AFM is the Scanning Tunneling Microscope (STM). Both microscopes (AFM and STM) are similar in their methods of operation, but the AFM has the added advantage of being able to scan non-conductive surfaces such as polymers, glass, ceramics, composites and biological samples. Because of its method of operation, the STM is limited to conducting or semiconducting surfaces.[4] *[Note: Figure 2 of the quantum corral was actually from a STM. The material being manipulated was conductive - iron atoms on a conducting copper surface.]*

Atomic Force Microscopes are frequently compared and contrasted with Scanning Electron Microscopes (SEM), which produce larger, two-dimensional images. Typically, AFMs take several minutes to scan and measure a sample while automated production SEMs can move to a sample location, acquire an image and measure it in less than five seconds.

The AFM's notable advantage is in its three-dimensional measurement capability. Both SEM and AFM's can produce 3D images through software enhancements, however, AFM's data is directly given in terms of x, y and z while SEMs image data is in the form of 2D (x,y) data – several SEM images acquired at different focal planes and/or angles can be used to construct a 3D image as is also done with optical microscopy. *Figure 5* is an AFM image of red blood cells after having been treated with an antibiotic peptide. *Figure 6* is a SEM image of three types of blood cells- red blood cell, platelet and leukocyte.

Figure 5. AFM image of red bloods Cells treated with antibiotic peptide[10]. *[Printing with permission-Dr Luciano Paulino Silva. Embrapa Recursos Genéticos e Biotecnologia (Brazil)[9]]*
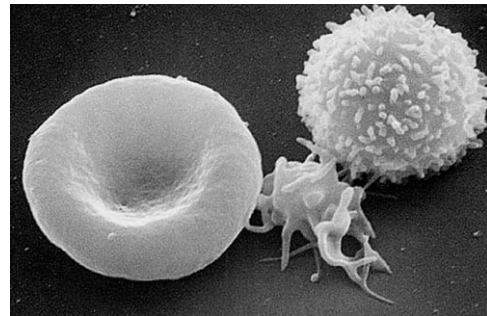
Figure 6: Scanning electron micrograph of blood cells. From left to right: human erythrocyte, thrombocyte (platelet), leukocyte. *(Image is public domain)*

Another important advantage of an AFM over a SEM is the AFM's ability to image a sample in ambient atmosphere or in a liquid. A SEM usually requires that the sample be in a high vacuum environment adding a significant amount to the cost of the equipment. The minimum resolution needed, cost, and application (sample material) usually dictate which tool is best for the job.

To view more AFM images, visit the Gallery at Asylum Research. (http://www.asylumresearch.com/Gallery/Gallery.shtml)

AFM Theory of Operation

The Atomic Force Microscope works by moving a micro-cantilevered tip or probe over the surface of a small sample. As the sample moves in the x-y direction, the probe tip, attached to the free end of the cantilever, moves in the z-direction due to atomic force interactions between the probe tip and the sample surface. Since the microcantilevers are normally fabricated from silicon or nitride, and due to their very thin construction, there is ample amount of flexibility (*the cantilever's spring or k constant*) that allows the cantilever to flex as the probe tip moves across the sample surface. The spring constant is directly proportional to the thickness of the cantilever, which is typically on the order of one micron (micrometer).

The probe's tip can have a radius as large as 2 microns (2000 nm) to as small as 2 nanometers[11], much smaller than the tip of a sewing needle. The smaller the tip radius and the longer the probe, the better the image resolution. The tip is typically made of a ceramic or semiconductor material (e.g., monolithic silicon) and manufactured using micromachining processes. *Figure 7* shows two SEM images of a micromachined AFM tip and cantilever. Using the scales at the bottom, you should be able to estimate the width of the cantilever, the width of the probe tip, and the length of the probe. Some AFM tips add a carbon nanotube to the tip to make it even thinner and longer.
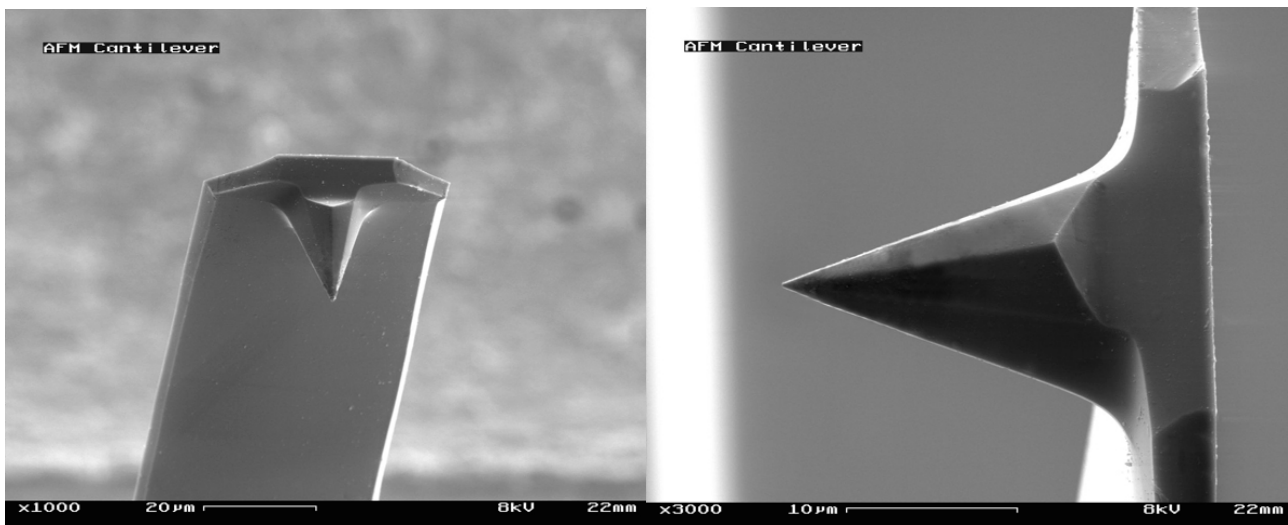
Figure 7. These SEM images were modified by SCME from original images found on Wikipedia – Atomic Force Microscope. These images are available to use through CC BY-SA 3.0.

Depending on the operating mode of the AFM (static or dynamic), the probe tip is either gently touching the sample surface (static mode) or oscillating above the sample surface (dynamic mode). As the tip interacts with the sample, it experiences attractive and repulsive forces, forces that attract and repel it at the sample's surface. Such forces can include electrostatic, electromagnetic, solvation, capillary, and van der Waals (London) forces. In fact, van der Waals forces always exist between the probe tip and the surface.[12] As the distance between the tip and surface varies, so do the forces. These variations in van der Waals forces are interpreted by the AFM to measure surface topography. Any of these repulsive and attractive forces between the tip and the surface cause the

microcantilever to deflect up or down (static mode), or they can change the amplitude and phase of an oscillating cantilever (dynamic mode).[13] Any and all of this data can be used by an AFM to map out surface topology.

An important component of all AFMs is the z-feedback loop or z-servo. This feedback loop allows the system to measure the interactions between the probe tip and the sample surface. These interactions are measured by monitoring the displacements of the probe end of the cantilever. The primary components of this loop are the input or desired set point, the proportional-integral-differential (PID) feedback controller (compares the set point to the actual value), the actuator (device that drives the cantilever/probe), the sensor (senses changes at the probe tip) and the convertor (converts the data from the sensor to a readable output and sends it to the PID for comparison).

Figure 8 is a block diagram that illustrates the servo loop that maintains a constant tip to surface distance. In the dynamic mode, the tip doesn't actually physically touch (hence, damage) the surface but maintains a constant distance above it, usually just a few nanometers. In the contact mode, the tip "gently" touches the surface, but the "touch" is usually non-damaging due to the attractive and repulsive forces on the surface being held constant by the servo loop to prevent damage of the surface and the probe tip.
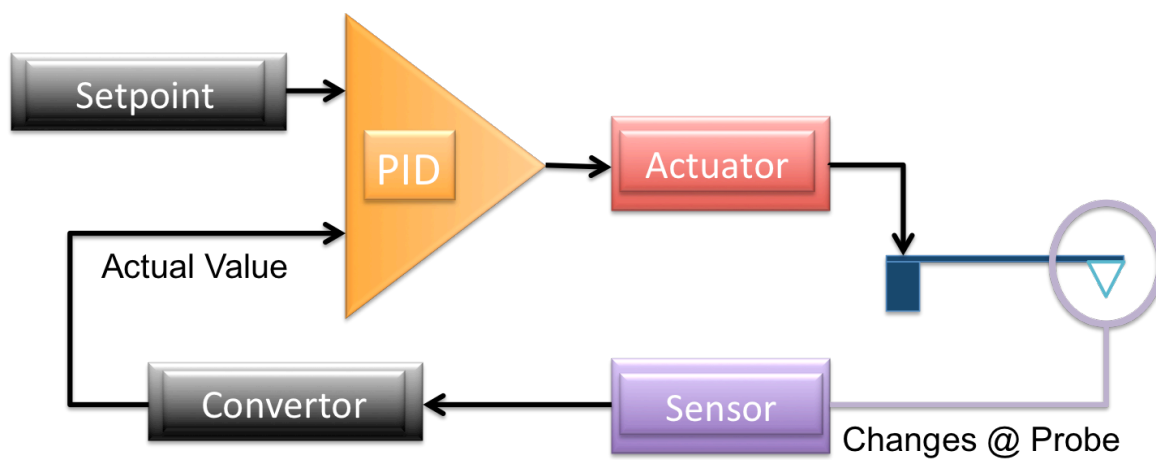

Figure 8. Block diagram of AFM servo loop

Operating Modes of the AFM

Before discussing the different operating modes, let's discuss a couple of characteristics of AFM probes to consider in the design of an AFM for a specific operating mode. In the design an AFM, one must consider the stiffness of the cantilever and the tip shape. "Stiffness or springiness" is how flexible the cantilever is, how much will it bend under varying forces and how well will it oscillate. This is property is determined by the spring constant also know as Young's modulus. Tip shape is important because the profile acquired by the AFM is a convolution of the tip shape and the sample's topography.

Hooke's Law

When designing for flexibility (stiffness), Hooke's Law comes into play. Hooke's Law is the law of elasticity. It is a principle of physics that states that the force needed to extend or compress a spring by some distance is proportional to that distance.

$$F = -kx$$

Where F is the force on a spring (cantilever in this case), k is the spring constant, and x is the displacement of the spring due to the force F.

For a cantilever, the spring constant k is:

$$k = \frac{Et^3 w}{4l^3}$$

where t is the thickness of the cantilever, w is the width, l is the cantilever length and E is the bulk modulus of elasticity (also known as the Young's modulus, a material property which represents the "springiness" of the material). The spring constant is a function of the material and the shape of the spring. Take a few minutes to study this formula and to identify how the characteristics of a cantilever (dimensions and material) affect its spring constant.

Tip Shape

Tip shape is critical when probing high aspect ratio structures (tall and thin, or deep and narrow, for example). It is important to understand that the topography the AFM produces is a convolution of the tip shape and the actual surface topography one is trying to measure. You can see in *Figures 9 and 10* how this works. *Figure 9* shows what the shape of the sample (obstacle) will look like after the scan using a relatively sharp tip. In *Figure 10* where the obstacle is a high aspect ratio channel, the sharp tip has a problem imaging the deep, narrow groove of the channel. Instead of the desired rectangular shape measurement of the actual structure, the resulting image is a depiction of the tip and more of a triangular shape. When a nanometer diameter carbon nanotube is used as the tip, the instrument response (tip shape) is less and the subsequent profile of the sample is more accurate and closely follows the real profile of the sample being measured.
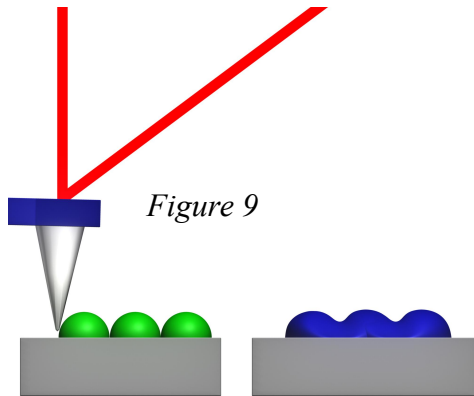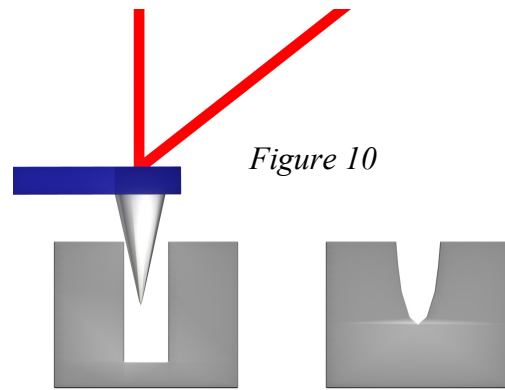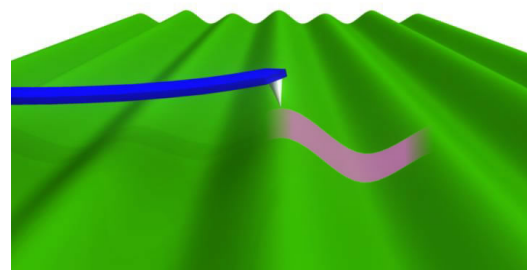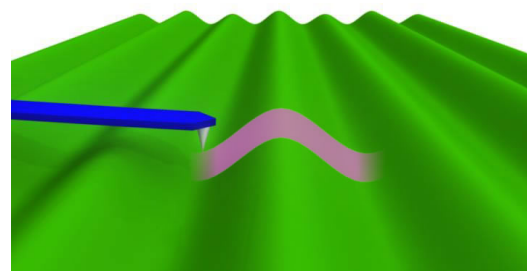
Figure 9

Figure 10

"Due to the nature of AFM probes, they cannot normally measure steep walls or overhangs. Specially made cantilevers and AFMs can be used to modulate the probe sideways as well as up and down (as with dynamic contact and non-contact modes) to measure sidewalls, at the cost of more expensive cantilevers, lower lateral resolution and additional artifacts[14]."

Contact Mode (Static Mode)

In the contact mode, the cantilever's tip gently "moves" along the sample's surface, maintaining a constant "distance" between the tip and the surface. This distance is determined by the interaction of the attractive and deflective (repulsive) forces at the sample's surface. As the tip is drawn closer and closer to the surface by the close-range attractive forces, increasing repulsive forces take over, pushing the tip away from the surface. These force interactions provide feedback to the servo system that maintains a constant tip to surface distance of around 10 angstroms. As the sample moves in the x-y directions directly under the tip, the movement of the tip up or down in the z-direction is used to define the topography of the sample's surface and create an image.

In *Figures 11 and 12*, the pink ribbon indicates the result of the interacting forces between the surface and the probe tip. As previously mentioned, the servo system monitors these forces and maintains a constant distance above the surface (pink-band), however slight it may be. However, the strong attractive forces at the surface *can cause* the tip to "snap" or "stick" to the surface making momentary contact if the overall force at the surface is not maintained as repulsive. In order to keep the interaction of these repulsive and attractive forces low while maintaining a large enough distance to prevent damaging the sample surface, the spring constant (k) of contact mode cantilevers are relatively low.

As the tip scans the surface, friction or lateral forces between the surface and tip can also cause a twisting or deflection of the tip and a bending of the cantilever. *(See Figure 13)* If measured, this lateral deflection of the tip can provide additional data about the topography of the surface as well as additional data about sample surface characteristics. Lateral Force Microscopy or LFM is enhanced by slope variations of the sample's surface and the friction changes on both sides of the slope (up/down). To capture this data, measurements are taken of the slope of the tip's deflection or the amount of change added to the tip's movement due to increased or decreased friction.[12]
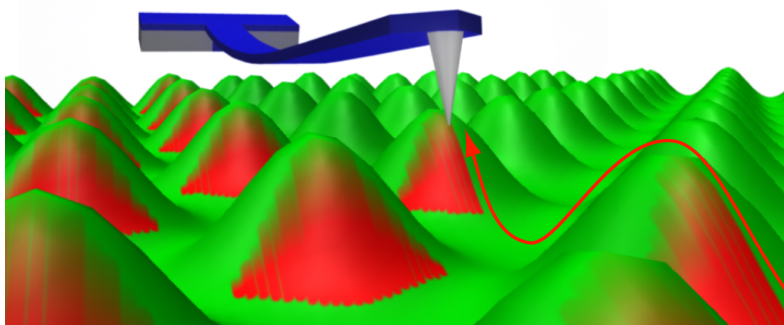
One of the more common methods used to capture the changes in a sample's surface topography (or the z-movement of the tip) is by projecting a laser beam onto the top of the microcantilever (as shown in *Figure 14*) and measuring the angle of the reflected light with an optical detector consisting of position sensitive photodiodes (PSPD). As the tip of the cantilever moves in the z direction due to sample-tip interactive forces, the laser's reflected angle off the top of the cantilever changes. The PSPD measures this change and the data is collected and processed. Hooke's Law is implemented to plot and produce the surface topography using the collected data. The result is an accurate topographical map of the sample's surface features.
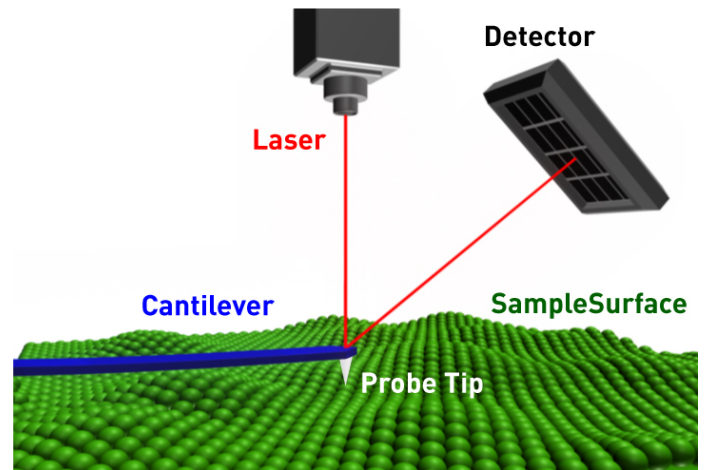
Figure 14. Using a laser and detector to capture probe tip movement on the z-axis.

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Tapping Mode (Contact mode – Dynamic)

The tapping mode is another form of contact mode. In the tapping mode, the cantilever's tip lightly taps the surface as it scans along the sample's surface *(See Figure 15)*. This is similar to a blind person using a tapping cane to create a "picture" of the surface as he navigates his environment. The rate at which the AFM "taps" is based on a resonant frequency of the cantilever that is generated by an external stimulus (e.g., piezoelectric, electromagnetic, thermal). As the oscillating probe tip scans the surface, its amplitude changes. The z-feedback loop (e.g., a reflected and detected laser beam off the cantilever's surface) detects changes in amplitude and adjusts the input signal to minimize these changes. The adjustments needed to the input signal (set point) are used to determine minute changes in the surface topology and to create an image of the surface.
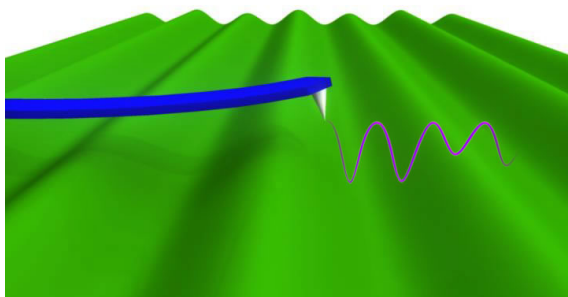

Figure 15. Tapping mode Cantilever oscillates at its resonant frequency. The amplitude changes as the surface changes.

The problem with the contact modes is the damage that can occur to the sample as well as the probe tip. Actual physical contact with the sample surface occurs in both static and dynamic contact modes; however in the tapping mode, the contact time is shorter and the lateral forces are lower than those in the static mode. In either mode, this contact can dent or damage the sample, particularly a soft sample. When the probe tip strikes the surface, even so softly, the tip can also be damaged or become "dull", just like the edge of a knife. This leads to poor resolution and in turn, poor results.

Figure 16 is a SEM image of a probe tip that has been used to image several samples using the tapping mode. You can see particles from the sample's surface remaining on the tip after the scan.

Figure 16. This SEM image of a "used AFM tip" was found on Wikipedia – Atomic Force Microscope and is available to use through CC BY-SA 3.0.
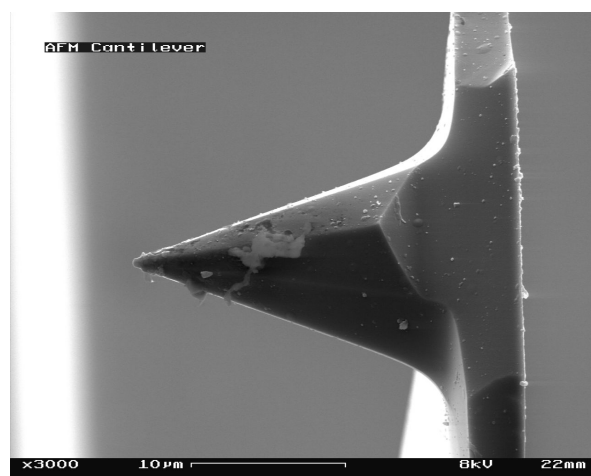
Non-Contact Mode: Oscillating Cantilever (Dynamic Mode)

There are several types of AFMs that use a non-contact mode. In this mode, the tip does not make contact at all with the sample surface.

In the oscillating cantilever non-contact mode, an external stimulus sets the cantilever to vibrate at its resonant frequency. However, unlike the tapping mode, the probe tip does not "contact" or touch the sample surface. As the tip scans the surface, "van der Waals attractive force between the tip and the sample acts upon the cantilever and causes changes in both the amplitude and the phase of the cantilever vibrations"[14]. These changes are monitored by the system's Z-axis servo system (feedback loop) that makes the necessary adjustments to the input to maintain a constant distance between the tip and the sample surface while maintaining constant amplitude. This is illustrated in *Figure 17*.
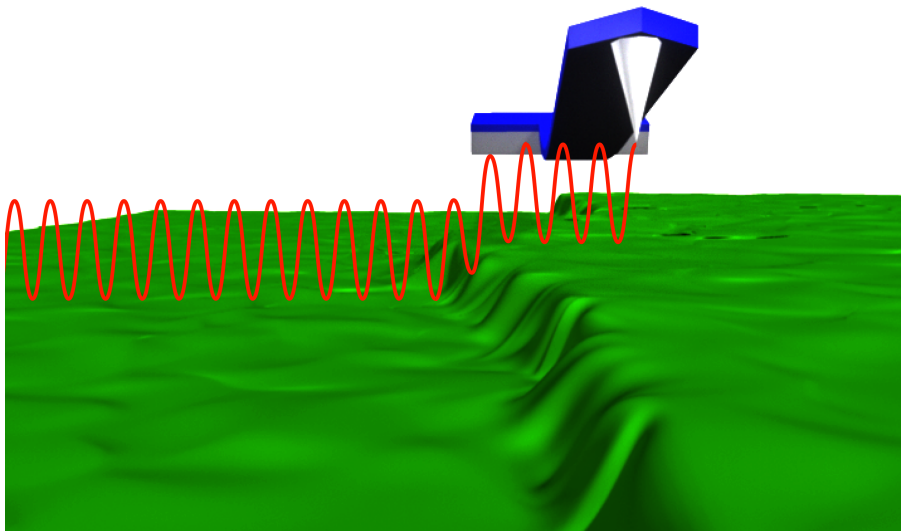
Figure 17. Non-contact tapping mode AFM. Changes in surface topography, causes changes in amplitude of cantilever oscillations. A constant amplitude and constant distance between probe tip and sample surface is maintained by the servo loop.

In this case, since no physical contact is made, neither the sample surface nor the probe tip becomes damaged. This leads to higher resolution images than the contact tapping mode and thus, better results.

Non-Contact Mode: Magnetic Force Microscope (MFM)

Another method of measuring surface topography is magnetic force imaging which uses a magnetic probe for the tip. As the probe moves along the surface, its up and down movements are a reaction to the magnetic forces existing between the magnetic tip and the sample's surface. In addition to the magnetic forces, Van der Waals forces also exist and vary with the distances between the tip and the sample.

In a MFM, images are created by measuring the spatial variation of magnetic forces between a magnetic probe tip and a magnetized sample. The probe tip is coated with a ferromagnetic thin film. The probe tip oscillates over the surface of the sample at the cantilever's resonant frequency without making contact with the sample surface. This non-contact is enabled by the interacting magnetic forces and van der Waals force at the tip and surface. As the probe scans the surface, the amplitude and phase of the tip's frequency change as the surface topography changes. The magnetic force changes with changes in distance between the tip and the surface. During the scan, information is collected on the changes in both forces (van der Waals and magnetic). This information is used to create the surface topology as well as the surface's magnetic property, respectively.

But how does the MFM separate this information to know which force is generating which data? In short, van de Waals is a short range force and the magnetic force is a long range force. By varying the distance between the tip and the sample, the MFM can separate the two forces allowing it to identify the oscillation changes caused by van de Waals and those caused by the magnetic forces.

To learn more about the resonant frequency of microcantilevers *and how this frequency is affected by the cantilever's material, length, width, thickness and mass, complete the activity "Microcantilever's Model Activity" found in the Microcantilevers Learning Module.*

Summary

AFMs are opening up new worlds and new possibilities in energy, medical, manufacturing, and science technologies. We can now see things smaller than we've ever been able to see before. We can study their properties, observe changes due to exposure to external stimuli, and build structures one atom at a time from the bottom up.

The technology of AFMs has improved over the years enabling them to provide higher resolution images, more accurate and consistent data and different types of data. There are several different categories of AFMs in use today allowing for a diverse amount of materials and surfaces to be scanned, and for micro or nano-sized resolution, depending upon the application.

Food for Thought

- How have AFMs affected micro and nanotechnologies?

References *(You may need to copy and paste the URLs to view the links online.)*

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