

## SEM and AFM: Complementary Techniques for High Resolution Surface Investigations

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There are a wide range of analytical techniques which may be used for materials characterization depending on the type of information needed. For high resolution surface investigations, two commonly used techniques are Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM)—Figures 1 and 2, respectively. Each of these techniques resolves surface structure down to the nanometer scale. However, the image

formation mechanisms are quite different, resulting in different types of information about the surface structure. The occurrence of the SEM and AFM side-by-side is becoming more common in today's analytical laboratories. This article will compare and contrast the two techniques with respect to specific types of surface measurements, and demonstrate how these analytical techniques provide information which is complementary in nature.

### History

The first SEM was constructed in 1938 by von Ardenne by rastering the electron beam of a Transmission Electron Microscope (TEM) to essentially form a Scanning Transmission Electron Microscope (STEM) (1, 2). In 1942, Zworkin et. al. developed the first SEM for bulk samples. This configuration contains many of the basic principles of today's SEMs (2, 3). Cambridge Scientific Instruments

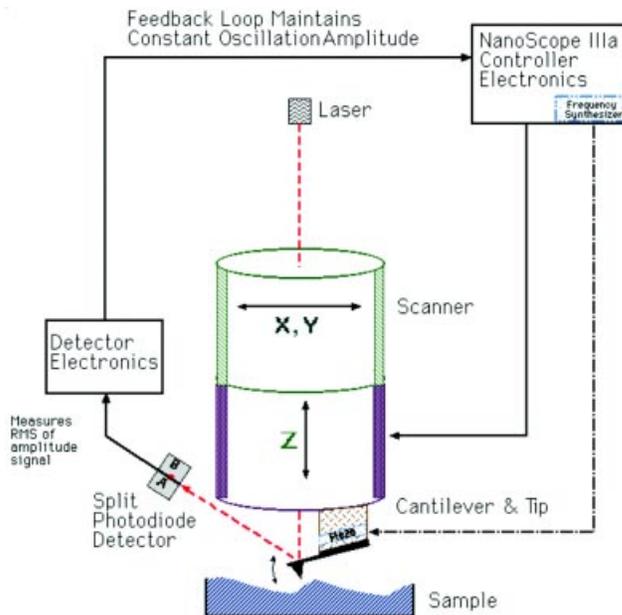


Figure 1. Schematic of the major components of an AFM showing the feedback loop for TappingMode™ operation.

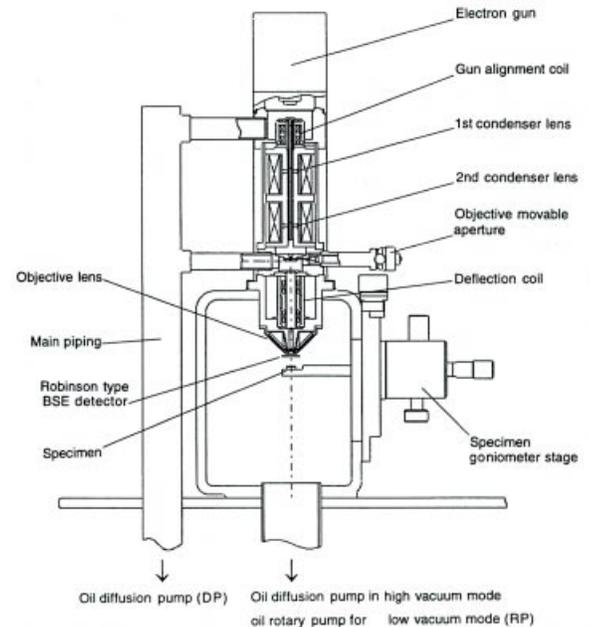


Figure 2. Schematic of the primary components of a typical SEM

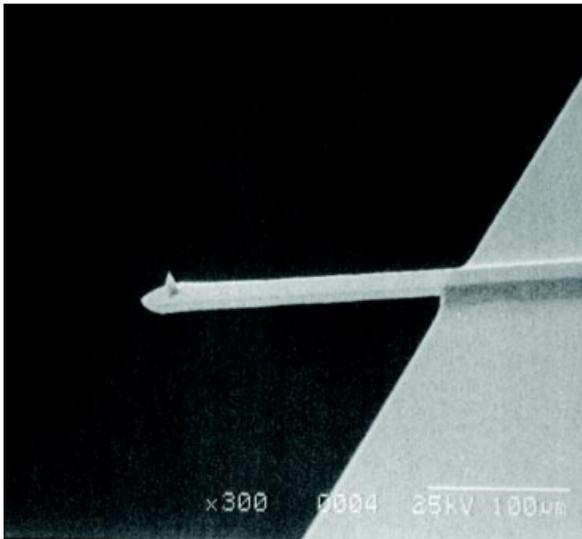


Figure 3. SEM image of an integrated single crystal silicon cantilever and tip which has an end radius of 2 to 10nm. Tips for AFM are typically made of silicon or silicon nitride. Bar=100 $\mu$ m.

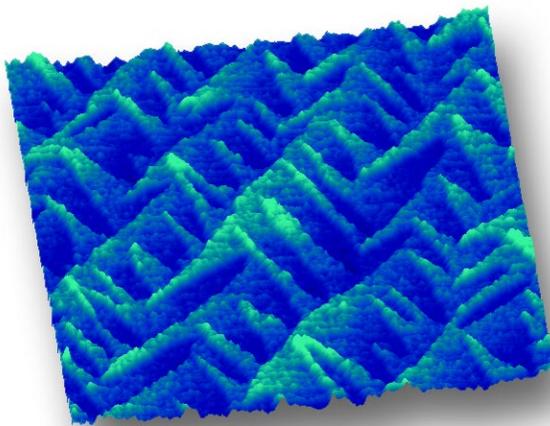


Figure 4: TappingMode AFM image of 1.4 $\text{\AA}$  monoatomic steps on epitaxial silicon deposited on (100) Si. 1 $\mu$ m scan.

produced the first commercial instrument in 1965. A number of improvements have occurred since this time, resulting in an increase in resolution from 50nm in 1942 to  $\sim$ 0.7nm today. Besides the development of morphological imaging, the SEM has been developed to detect signals which are used to determine compositional information, such as X-rays, backscattered electrons, cathodoluminescence, Auger electrons, and specimen current.

The development of the AFM was preceded by the development of the Scanning Tunneling Microscope (STM) in 1981 at IBM Zurich Research Laboratory by Binnig and Rohrer (4). Its ability to view the atomic lattice of a sample surface earned the inventors the Nobel Prize in Physics in 1986. Although the STM provides subangstrom resolution in all three dimensions, it is limited to conductive and semiconductive samples. To image insulators as well as conductors, the Atomic Force Microscope (AFM) was developed in 1986 (5), and the first commercial AFMs were produced in 1989 by Digital Instruments.

AFM provides three-dimensional surface topography at nanometer lateral and subangstrom vertical resolution on insulators and conductors. From this beginning, the field of Scanning Probe Microscopy (SPM) was born which consists of a family of techniques that involves scanning a sharp tip across the sample surface while monitoring the tip-sample interaction to form a high resolution image. Although the AFM has become the most commonly

used form of SPM, many other SPM techniques have been developed which provide information on differences in friction, adhesion, elasticity, hardness, electric fields, magnetic fields, carrier concentration, temperature distribution, spreading resistance, and conductivity.

## Imaging Mechanisms

### Scanning Electron Microscopy

The operation of the SEM consists of applying a voltage between a conductive sample and filament, resulting in electron emission from the filament to the sample. This occurs in a vacuum environment ranging from  $10^{-4}$  to  $10^{-10}$  Torr. The electrons are guided to the sample by a series of electromagnetic lenses in the electron column. A schematic of a typical SEM is shown in Figure 2. The resolution and depth of field of the image are determined by the beam current and the final spot size, which are adjusted with one or more condenser lenses and the final, probe-forming objective lenses. The lenses are also used to shape the beam to minimize the effects of spherical aberration, chromatic aberration, diffraction, and astigmatism.

The electrons interact with the sample within a few nanometers to several microns of the surface, depending on beam parameters and sample type. Electrons are emitted from the sample primarily as either backscattered electrons or secondary electrons. Secondary electrons are the most common signal used for investigations of surface morphology. They are produced as a result of interac-

tions between the beam electrons and weakly bound electrons in the conduction band of the sample. Some energy from the beam electrons is transferred to the conduction band electrons in the sample, providing enough energy for their escape from the sample surface as secondary electrons. Secondary electrons are low energy electrons ( $<50\text{eV}$ ), so only those formed within the first few nanometers of the sample surface have enough energy to escape and be detected. High energy beam electrons which are scattered back out of the sample (backscattered electrons) can also form secondary electrons when they leave the surface. Since these electrons travel farther into the sample than the secondary electrons, they can emerge from the sample at a much larger distance away from the impact of the incident beam which makes their spatial distribution larger. Once these electrons escape from the sample surface, they are typically detected by an Everhart-Thornley scintillator-photomultiplier detector. The SEM image formed is the result of the intensity of the secondary electron emission from the sample at each  $x,y$  data point during the rastering of the electron beam across the surface.

### Atomic Force Microscopy

AFM consists of scanning a sharp tip on the end of a flexible cantilever across a sample surface while maintaining a small, constant force. An integrated silicon tip and cantilever can be seen in Figure 3. The tips typically have an end radius of 2nm to 20nm, depending on tip type. The scanning motion is

conducted by a piezoelectric tube scanner which scans the tip in a raster pattern with respect to the sample (or scans to the sample with respect to the tip). The tip-sample interaction is monitored by reflecting a laser off the back of the cantilever into a split photodiode detector. By detecting the difference in the photodetector output voltages, changes in the cantilever deflection or oscillation amplitude are determined. A schematic of this can be seen in Figure 1.

The two most commonly used modes of operation are contact mode AFM and TappingMode™ AFM, which are conducted in air or liquid environments. Contact mode AFM consists of scanning the probe across a sample surface while monitoring the change in cantilever deflection with the split photodiode detector. A feedback loop maintains a constant cantilever deflection by vertically moving the scanner to maintain a constant photodetector difference signal. The distance the scanner moves vertically at each  $x,y$  data point is stored by the computer to form the topographic image of the sample surface. This feedback loop maintains a constant force during imaging, which typically ranges between 0.1 to 100nN.

TappingMode AFM consists of oscillating the cantilever at its resonance frequency (typically  $\sim 300\text{kHz}$ ) and lightly “tapping” on the surface during scanning. The laser deflection method is used to detect the root-mean-

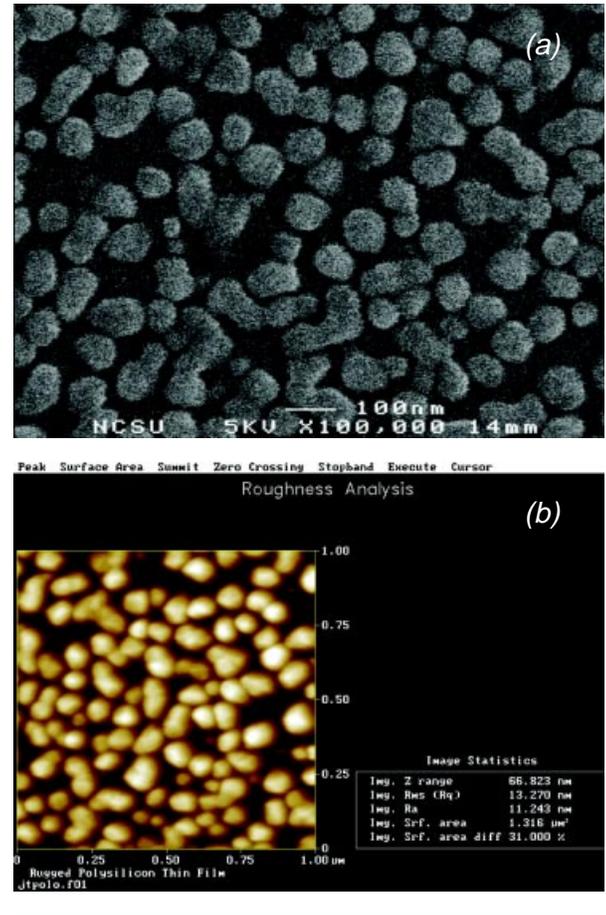
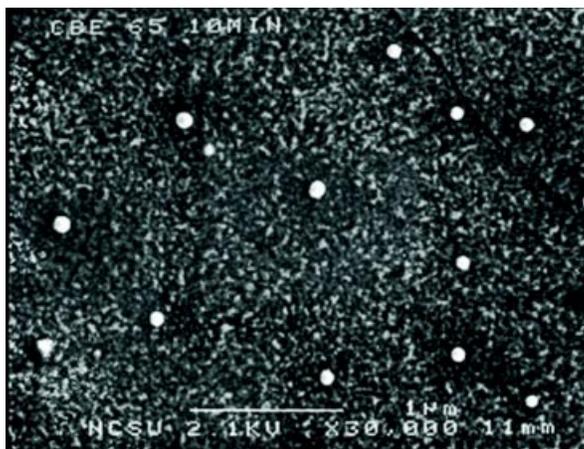
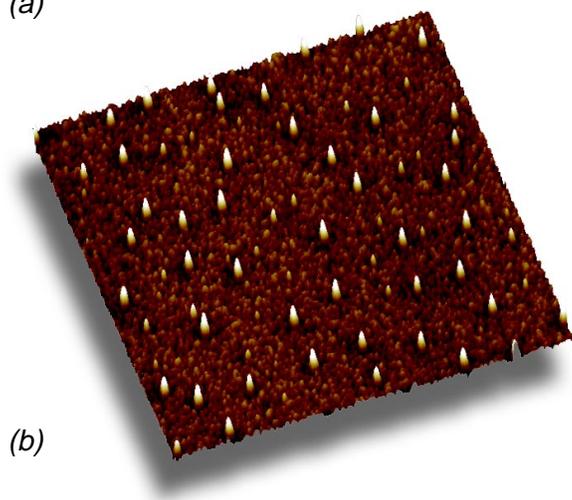


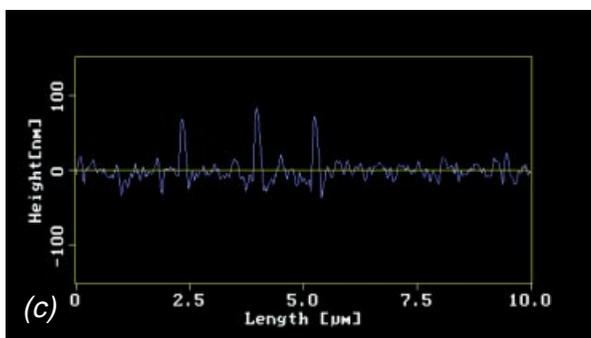
Figure 5: (a) SEM image of rugged polysilicon thin film. 100,000x, Bar=0.1 $\mu\text{m}$ ; (b) TappingMode AFM image of the same with roughness measurement. 1 $\mu\text{m}$  scan.



(a)



(b)



(c)

Figure 6. (a) SEM image of partially GaP-covered Si after chemical beam epitaxy deposition for 10 minutes. 30,000x, Bar=1µm; (b) AFM image of the same sample as in figure 6a showing the presence of nodules during the growth of GaP by chemical beam epitaxy. 10µm scan; (c) Cross-sectional measurement with AFM across the image in Figure 6b showing 3 nodules which have a height of approximately 70nm. (16)

square (RMS) amplitude of cantilever oscillation. A feedback loop maintains a constant oscillation amplitude by moving the scanner vertically at every x,y data point. Recording this movement forms the topographical image. The advantage of TappingMode with respect to contact mode is that it eliminates the lateral, shear forces present in contact mode. This enables TappingMode to image soft, fragile, and adhesive surfaces without damaging them, which can be a drawback of contact mode AFM.

### Comparison of Techniques

There are a number of different ways to compare and contrast these two techniques with respect to each other. Although investigations that use both SEM and AFM to characterize a material are common, there are just a few studies that directly discuss the complementary nature of the techniques (6-13). A comparison of these techniques will be conducted with respect to 3 factors: (1) Surface Structure, (2) Composition, and (3) Environment. The comparisons are presented for typical equipment configurations and operating procedures.

### Surface Structure

Although both SEM and AFM are similar in lateral resolution, there are situations in which one technique can provide a more complete representation of the sample surface, depending on the information desired. One principle difference is in how the two techniques process vertical changes in topography. Below we

will discuss measurements of different vertical scales of topography, beginning with very smooth surfaces and working up to very rough surfaces to determine how the surface topography affects the ability of each technique to perform the measurement.

### Atomically Smooth Surfaces

Atomically smooth surfaces can occur either naturally, such as on mineral surfaces, or by processing, such as polishing and epitaxial growth on semiconductor, data storage, and optical surfaces. A TappingMode AFM image of an epitaxial silicon surface is shown in Figure 4. Note that, unlike SEM, the AFM can measure in all three dimensions (x, y, and z) with a single scan. Since the AFM has a vertical resolution of  $<0.5\text{\AA}$ , it can resolve the  $1.4\text{\AA}$  monoatomic silicon steps on the surface as well as calculate an RMS roughness of  $0.7\text{\AA}$  (14). On a sample this smooth, the SEM has difficulty resolving these features due to the subtle variations in height.

### Thin Films

On most thin films, the SEM and AFM produce a similar representation of the sample surface. A common application of surface investigations of thin films consists of determining changes in morphology with variations of deposition parameters, such as temperature, pressure, time, etc. Figure 5 shows SEM and AFM images of a polysilicon thin film at approximately the same lateral magnification. The two images show similar surface structure, however, they differ in the other types of information that can be

acquired on this sample. The three-dimensional nature of the AFM can be used to calculate changes in roughness and surface area variations due to differences in deposition parameters. For the SEM, a large area view of the variations in surface structure can be acquired all at once (such as several mm's), whereas a  $100\mu\text{m} \times 100\mu\text{m}$  area is typically the largest area viewed by an AFM. These images are an example of "rugged" polysilicon films which are used as capacitors in memory devices. By making these films rough, the surface area is increased which makes it possible to hold more charge without increasing the lateral dimensions of the capacitors on the chips. By adjusting the deposition parameters and using the AFM to analyze the surface area of the films, the deposition parameters needed to produce a film with the maximum surface area were determined (15).

Another example of the difference between the two techniques is in interpreting subtle differences in height. In the SEM image, changes in slope can result in an increase in electron emission from the sample surface, producing a higher intensity in the image. However, it can sometimes be difficult to determine whether the feature is sloping up or down. For instance, in the SEM image in Figure 6a it is very difficult to determine whether the small round structures are bumps or pits, even when tilting the sample stage in the SEM. The only other option would be to cleave the sample through one of these features and look at the sample in cross-section, which would be

tedious and time consuming. Since the AFM data contains the height information, determining whether a feature is a bump or pit is straightforward. As can be seen in Figures 6b and 6c, the features on this sample are bumps. This information was used in the study of the growth mechanisms of GaP on Si during chemical beam epitaxy deposition (16). Determination of whether these features were small bumps or depressions would have changed how the deposition process was altered to produce an epitaxial GaP film.

#### High Aspect Ratio Structures

Semiconductor processing commonly requires measurements of high aspect ratio structures such as trenches and via holes. In a SEM, these structures are typically measured in cross section by cleaving the wafer and imaging the sample on end to obtain the dimensions of the structure. A common example of this is seen in Figure 7a. In contrast, the AFM image of a trench or via is made by scanning over the sample surface. The ability of the AFM to measure these structures nondestructively makes it possible for the wafer to be returned to the production line after the measurement is acquired. An AFM image of vias in photoresist is shown in Figure 7b. To image some higher aspect ratio structures, the proper tip shape is needed for the AFM to scan narrow openings and steep sidewalls. Although the SEM measurement is destructive to the sample, the ability to image the undercuts of these lines is a useful application that AFMs are not typically designed to perform.

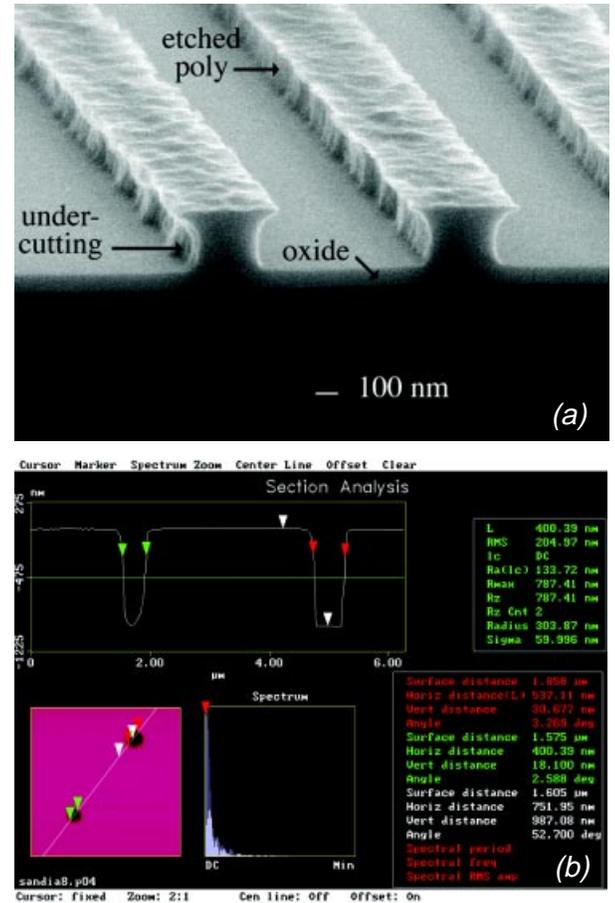


Figure 7. (a) Cross-sectional SEM image of polysilicon lines which shows undercutting due to reactive ion etching. Scale bar=100nm; (b) Cross-sectional measurement of developed and incompletely developed vias in photoresist acquired by TappingMode AFM. In order to image the high aspect ratio structures on the sample, a silicon tip machined with a focused ion beam was used to scan the vias.  $6.2\mu\text{m}$  scan.

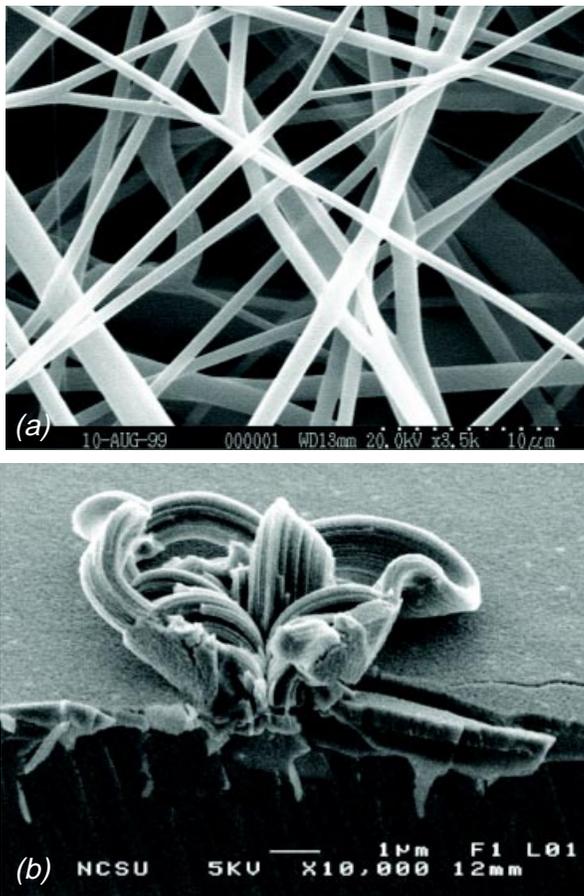


Figure 8. (a) SEM image of a non-woven textile sample of polyethylene oxide fibers. The large depth of field of the SEM makes it possible to image fibers which are 10's of  $\mu\text{m}$ 's below the upper layer of fibers. Bar= $10\mu\text{m}$ ; (b) SEM image of  $\text{Y}_2\text{O}_3$  crystal. Bar= $1\mu\text{m}$ .

### Rough Surfaces

One of the key advantages of the SEM with respect to other types of microscopy is its large depth of field. This ability makes it possible to image very rough surfaces with millimeters of vertical information within a single image. A SEM image of non-woven polyethylene oxide fibers can be seen in Figure 8a. The depth of field and small beam size makes it possible to image the fibers far below the top layer. This ability also makes it possible to measure very rough surfaces over larger lateral areas as well. Although the AFM can measure vertical surface variations below  $0.5\text{\AA}$ , its ability to measure a tall structure comes from how far the scanner can move vertically. Standard scanners typically have 5 to  $6\mu\text{m}$  of vertical range, however, in some configurations the vertical range approaches  $10\mu\text{m}$  or larger. For scanning areas that have heights of greater than 5 to  $10\mu\text{m}$ 's of variation, the SEM would be better suited for the analysis.

Another example of a complex three-dimensional surface structure which shows how the SEM and AFM can complement each other can be seen in Figure 8b. The convoluted three dimensional  $\text{Y}_2\text{O}_3$  oxide crystal shown growing out of a relatively flat  $\text{Y}_2\text{O}_3$  thin film on a Si substrate is easily imaged in the SEM (Figure 8b). Although the AFM would have problems imaging the obtuse angles and enclosed areas of this surface, the roughness of the  $\text{Y}_2\text{O}_3$  film can be measured whereas in the SEM image the surface roughness is not evident. Therefore, the two techniques together

give a more complete "picture" of the sample.

### Composition

Both SEM and SPM provide compositional information through a variety of techniques. SEM is the only one of the two techniques which provides elemental analysis, however, both SEM and AFM are associated with techniques which can provide compositional information through analyzing materials and physical properties of the sample. Some of the most common of these methods are described below.

### SEM

Along with the secondary electron emission which is used to form a morphological image of the surface in the SEM, a number of other signals are emitted as a result of the electron beam impinging on the surface, as shown in Figure 9. Each of these signals carries information about the sample which provide clues to its composition.

Two of the most commonly used signals for investigating composition are x-rays and backscattered electrons. X-ray signals are commonly used to provide elemental analysis by the attachment of an Energy-Dispersive Spectrometer (EDS) or Wavelength-Dispersive Spectrometer (WDS) to the SEM system. X-ray emission results from inelastic scattering between the beam electrons and the electrons of the sample atoms. This interaction results in the ejection of an inner shell electron from the atom,

creating a vacancy that is filled by an outer shell electron. This jump from an outer to inner shell results in a change in energy that produces either a x-ray or Auger electron. The emitted x-ray has energy equal to this change. The x-rays are then detected by either a lithium-drifted silicon detector for an EDS system, or a gas proportional counter detector for a WDS system. A typical x-ray spectrum collected with an EDS system is shown in Figure 10.

Backscattered electrons are the result of beam electrons being scattered back out of the sample. In this case, the incident beam electrons undergo a number of scattering events within the specimen in which very little energy is lost, allowing these electrons to go much deeper into the sample than secondary electrons and still emerge from the sample surface to be detected. The percentage of beam electrons that become backscattered electrons has been found to be dependent on the atomic number of the material, which makes it a useful signal for analyzing the material composition. Once these electrons escape from the surface they are detected by either the Everhart-Thornley detector or a solid state detector. An example of a backscattered image of a PbSn alloy is shown in Figure 11.

#### AFM/SPM

Although an AFM does not provide elemental analysis, it can supply compositional information by differentiating materials based on physical properties, such as stiffness, elasticity, compliance, friction, adhesion, magnetic and

electrostatic fields, carrier concentration, temperature distribution, spreading resistance, and conductivity. Many of these techniques consist of looking simultaneously at another signal while performing standard AFM imaging. One of the most common techniques for mapping differences in materials properties is PhaseImaging™. PhaseImaging is conducted during TappingMode AFM operation by monitoring the phase lag between the oscillating drive signal used to drive the cantilever and the oscillating detection signal from the photodiode detector. This signal will indicate differences in viscoelasticity and/or adhesion across the imaged area. This technique is commonly applied to mapping the distribution of polymers in a heterogeneous system, or mapping the distribution of filler, such as silica or carbon black, in a polymer matrix. An example of PhaseImaging on a polyethylene film is shown in Figure 12. Other ways to get similar information are by Force Modulation AFM, which maps differences in elasticity across the sample surface, and Lateral Force Microscopy (LFM), which maps differences in friction across the sample surface.

There are also techniques that can be used to investigate long range forces across the imaged area. Magnetic Force Microscopy (MFM) and Electric Force Microscopy (EFM) map the magnetic and electrostatic field gradients, respectively, which extend from the sample surface. These techniques are performed by using either a magnetic or conductive probe to map the attractive and repulsive forces

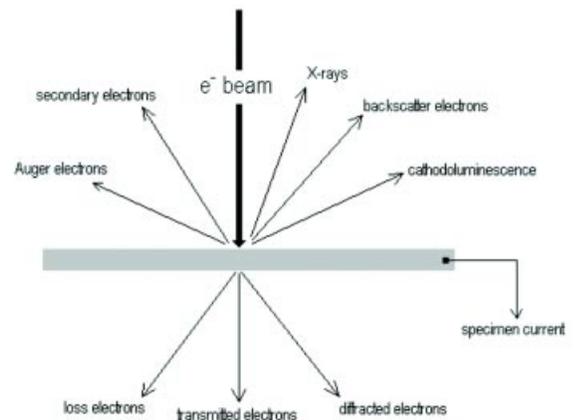


Figure 9. Signals emitted from a sample surface after interaction with an electron beam.

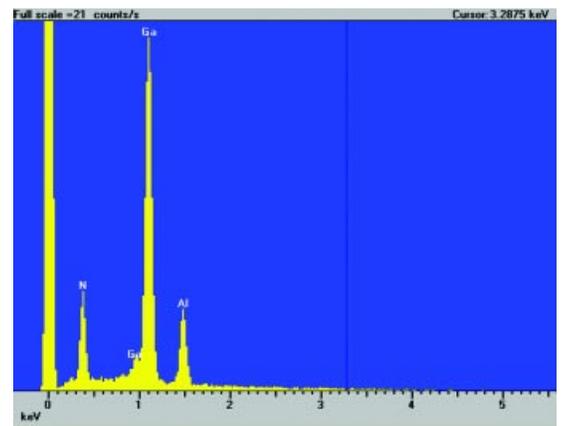


Figure 10. EDS X-ray spectrum of an AlGaIn thin film on SiC substrate showing the presence of N, Ga, and Al.

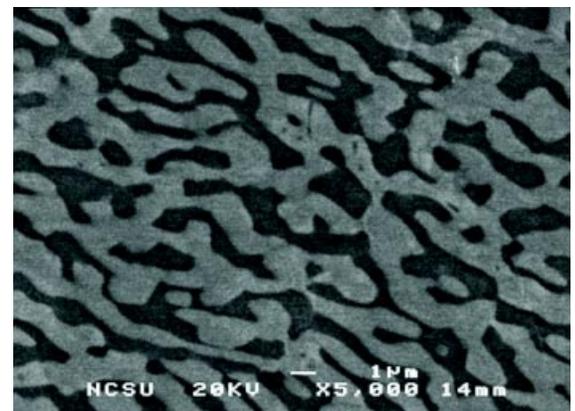


Figure 11. Backscattered SEM image of a PbSn alloy showing contrast based on the atomic number of the two components. The brighter areas are Pb-rich. 5,000x, Scale bar=1μm.

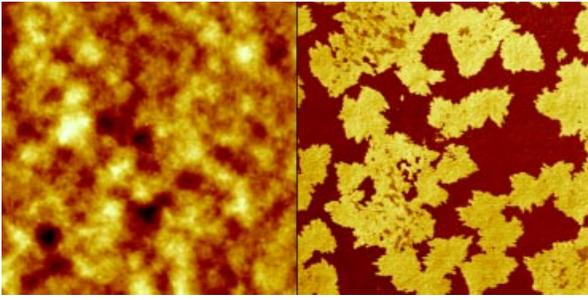


Figure 12. Phase image of two components which are used to form a polyethelene (PE) film. The phase image (right) clearly shows the distribution of the two polymers due to differences in stiffness which is not evident from the topographic image (left).  $2\mu\text{m}$  scan.

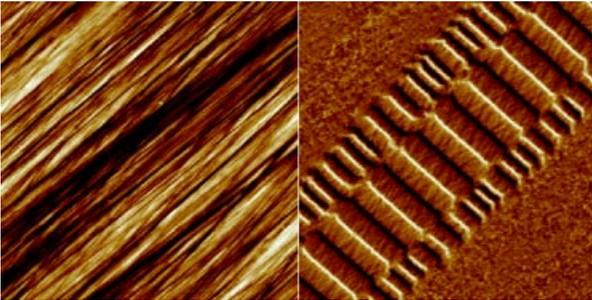


Figure 13. Magnetic Force Microscopy (MFM) image of overwritten tracks on a textured hard disk. The topography (left) was imaged using TappingMode; the magnetic force image of the same area (right) was captured with LiftMode (lift height 35 nm) by mapping shifts in cantilever resonant frequency.  $25\mu\text{m}$  scan. (17)

between the tip and the sample. MFM is commonly used to detect the domain structure of magnetic bits written on magnetic media, to evaluate the performance of magnetic heads, and to investigate the magnetic structure of experimental materials. This is conducted by a routine called LiftMode™ in which a Tapping-Mode topographic image and a magnetic image are acquired over the same area. LiftMode consists of first collecting a line scan in TappingMode of the surface morphology. The tip is then lifted above the surface and a second scan is made over the same line using the saved topographic scan to maintain a constant tip-sample separation. The long-range magnetic forces shift the resonance frequency of the oscillating cantilever, which is detected to produce the magnetic image. An example of bits written on a textured hard disk is shown in Figure 13.

Although the AFM is applied so that it is nondestructive to the sample surface, it can be used to study differences in mechanical properties by performing nanoindentation to investigate hardness differences between materials. This technique uses a diamond tip mounted on a stiff, stainless steel cantilever. A TappingMode AFM image is collected with the probe to determine the area of interest for indentation, the nanoindentation is then made at a specified force, and an image is then collected of the indented area. An example of comparing the difference between diamond like carbon films on a hard disk is shown in Figure 14. In this example, the two films demonstrate a difference in

hardness from indentations made at the same forces, producing different sized indents. Scratching and wear testing may also be conducted with this configuration to investigate adhesion and delamination of films under a small applied force.

## Environment

One of the primary differences between these two types of microscopy is the environment in which they are performed, i.e., SEM is conducted in a vacuum environment, and AFM is conducted in an ambient or fluid environment. There are several issues which make environment an important issue. First, there is a frequent need in fields such as biology and biomaterials to study hydrated samples. These two techniques compensate for this need by different means: an environmental chamber for a SEM, and a fluid cell for the AFM. Second, the SEM is required to work in a vacuum environment due to the nature of the technique which brings up the issues of vacuum compatibility of the sample, the conductivity of the surface, and vacuum maintenance. To image poorly conductive surfaces without sample charging may require conductive coatings or staining, which may alter or obscure the features of interest; or it may require low voltage operation, or an environmental chamber, which may sacrifice resolution.

For SEM, hydrated samples are addressed by placing a specimen in an environmental chamber with either an electron transparent window or a small aperture for the beam to enter the chamber. The

chamber is typically flushed with an inert gas saturated with water vapor. Common applications are to either investigate hydrated surfaces to preserve their surface structure when hydrated, or to reduce charging on insulating samples. An example of imaging of a pesticide film on skin can be seen in Figure 15. For the electron beam to interact with the surface in this configuration, it must go through an environment of gas and water vapor. One drawback of this configuration is that it will result in an increase in scattering of the electron beam on the way to and from the surface, which may result in the sacrifice of image quality and resolution.

One of the primary attractions to the AFM is its ability to image insulating surfaces at high resolution in fluid. Imaging samples in a hydrated state with an AFM is commonly performed by enclosing the sample and probe in a fluid environment, as shown in Figure 16. Since AFM is not based on conductivity, the image and scanning mechanism is not disturbed by the presence of the fluid. Common applications for AFM investigations in fluid are in the biological sciences, biomaterials, crystal growth, force interaction studies, and for investigating processes *in situ* (Figures 17, 18). The resolution of the image will be determined by the radius of the tip, the applied force, and the noise floor of the instrument. Because of these factors, this configuration allows the study of hydrated specimens at a lateral resolution of 1 to 5nm and a vertical resolution down to 0.5Å without sample damage, as seen in the image of the GroES chaperon (Figure 17). With the appropriate

accessories, AFM can also be used in varied gaseous environments and at elevated temperature. The latter is particularly important for research and development of polymers.

### Further Discussion

One thing to keep in mind when comparing these two techniques is that although SEM and AFM appear very different, they actually share a number of similarities. Both techniques raster a probe across the surface to detect some interaction with the surface to form an image. Both have a lateral resolution which is similar in scale (although under certain conditions AFM is superior). And both techniques have image artifacts that the operator is trained to identify. The SEM has had a much longer time to mature as a technique and to develop an understanding of how to identify and avoid artifacts, but the rapid adoption and implementation of AFM has resulted in a similar understanding of artifacts. This article has avoided discussing such artifacts unless they are relevant to the comparison. Furthermore, by using two techniques which are complementary, one technique will often compensate for the imaging artifact of the other technique.

However, one should be wary of combined systems in which an AFM is placed inside the SEM chamber. One of the true advantages of the AFM is its ability to perform high resolution measurements outside of a vacuum environment. Placing it inside a vacuum environment reduces its flexibility and increases its operating time. Combined

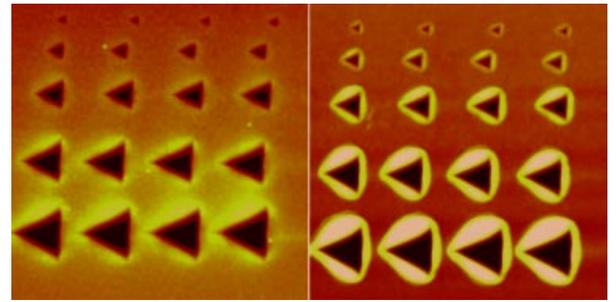


Figure 14: Indentations on two different diamond-like carbon thin films using three different forces (23, 34, and 45 $\mu$ N) with four indents made at each force to compare differences in hardness. 500nm scans.

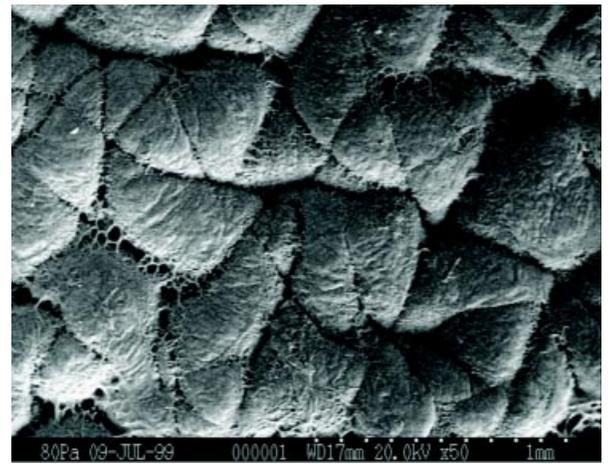


Figure 15. Environmental SEM image of a pesticide film on skin. A hydrated environment was needed in order to maintain the integrity of the pesticide layer and to reduce charging. Bar=1mm.

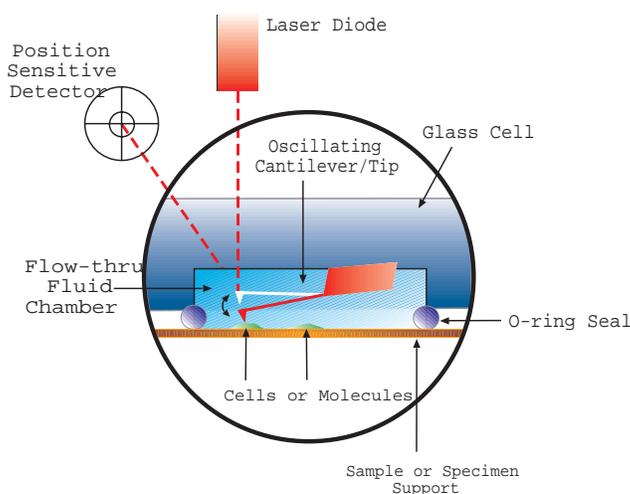


Figure 16. Fluid cell for an AFM which allows imaging in an enclosed, liquid environment.

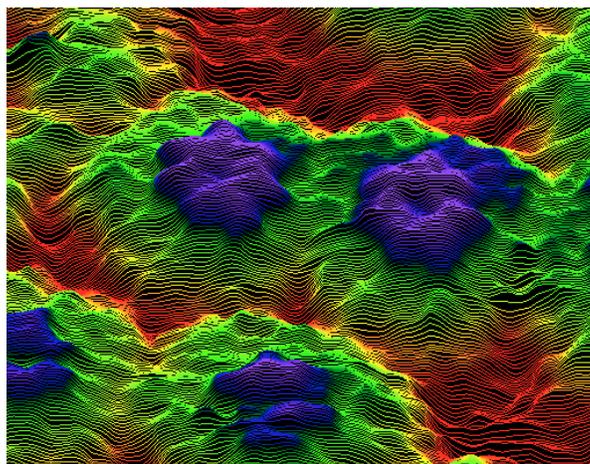


Figure 17. Image of two GroES molecules positioned side-by-side in physiologic fluid, demonstrating  $10\text{\AA}$  lateral resolution and  $1\text{\AA}$  vertical resolution. The entire molecule measures  $84\text{\AA}$  across, and a distinct  $45\text{\AA}$  heptameric "crown" structure protrudes  $8\text{\AA}$  above the remaining GroES surface and surrounds a central depression.  $18\text{nm}$  scan. Image courtesy of Z. Shao, University of Virginia. (18)

AFM/SEM systems often have reduced capabilities and typically compromise the performance of both instruments.

## Summary

SEM and AFM are complementary techniques that provide a more complete representation of a surface when used together than if each were the only technique available. These techniques overlap in their capabilities to provide nanometer scale lateral information. However, they deviate in the fact that the AFM can provide measurements in all three dimensions, including height information with a vertical resolution of  $<0.5\text{\AA}$ , whereas the SEM has the ability to image very rough samples due to its large depth of field and large lateral field of view.

The SEM can provide elemental analysis using X-ray detection, whereas the AFM can provide compositional information based on physical properties. The fact that the two techniques operate in different environments can be a strength when used together since the AFM does not encounter vacuum issues (difficult sample preparation, sample modification, etc.) and may image samples in an enclosed fluid or other environment. The vacuum environment of the SEM makes it possible to conduct a number of techniques that require vacuum, such as X-ray analysis. By having both techniques side-by-side in an analytical facility, the overall scope of analytical capabilities is broadened, adding to the flexibility of the facility.

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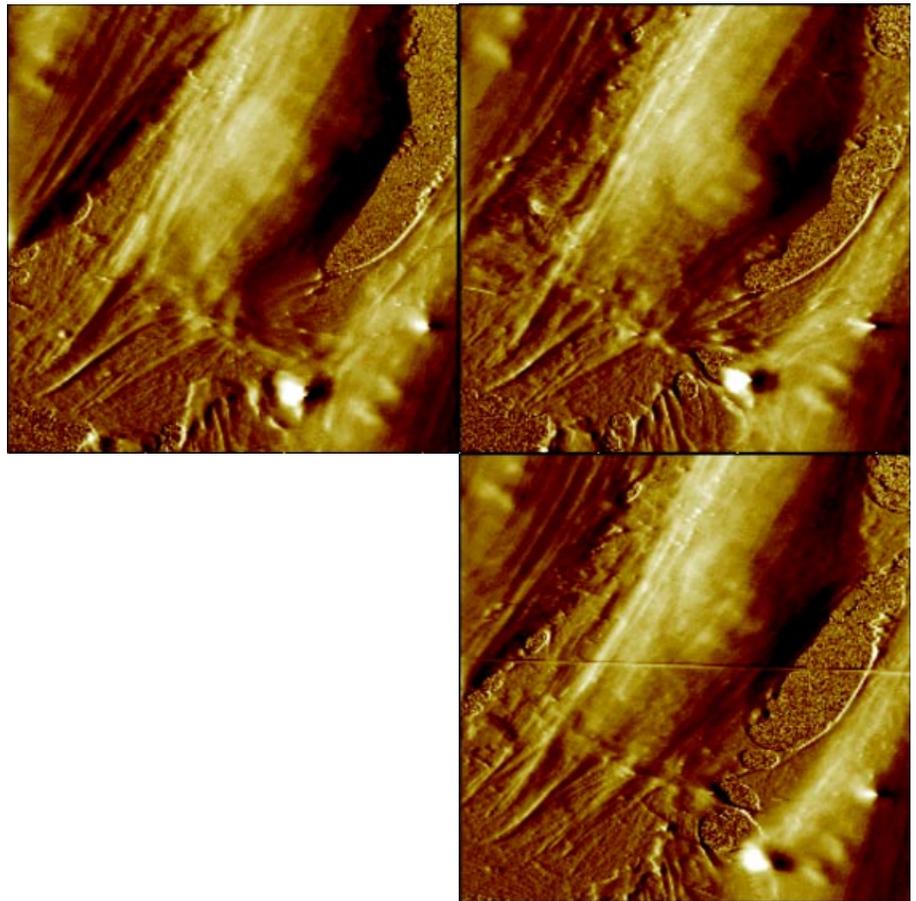


Figure 18. Living human vascular endothelial cells imaged in culture media by TappingMode AFM. These images, collected at 30 minute intervals, reveal the movement of living cells which were incubated with 200ng/ml vascular endothelia growth factor (VEGF). With TappingMode, the nucleus as well as other submembrane structures are visible. These cells appear flatter and more elongated compared to control (untreated) cells. 65 $\mu$ m scans. Sample courtesy of Georges Primbs, Miravant Inc.

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