Experimental Nanobiotechnology

Prof. P. Gopinath

Department of Biosciences and Bioengineering,

Indian Institute of Technology Roorkee

Lecture 11: Atomic Force Microscopy

Hello everyone, today we are going to learn atomic force microscopy. In today's lecture, we will be learning about scanning probe microscopy. Under the scanning probe microscopy, we will be learning about the scanning tunneling microscopy and atomic force microscopy in detail. At the end of the lecture, we will also have a practical demonstration to understand atomic force microscopy in detail. Let us see what is scanning probe microscopy.

Scanning probe microscopy is a high-resolution microscopy technique used to study surfaces at the atomic and nanometer scale. By using scanning probe microscopy, we can obtain high-resolution images, quantitative data, and perform surface-specific analysis. We can use any kind of sample, which is the advantage of scanning probe microscopy. Under the scanning probe microscopy there are several types of microscopy, and in today's lecture we are going to learn

about scanning tunneling microscopy and atomic force microscopy in detail. Let us see what is scanning tunneling microscope. The scanning tunneling microscope (STM) was invented in 1981, and the STM was the first instrument to image surfaces at the atomic level. It was a breakthrough in the field of nanotechnology, and for this research

In 1986, these two scientists got the Nobel Prize. Let us see the working principle of scanning tunneling microscopy. Scanning tunneling microscope relied on the tunneling effect. That means this microscope is mainly working on the basis of tunneling current. It is going to measure the tunneling current between the sample and tip.

The principle of this STM is When you apply the small voltage, electrons can tunnel through a vacuum gap between the sharp metallic tip and a conductive sample. And due to the overlapping of the respective electron clouds, the tunneling current is produced.

The tunneling current will be measured and that will give the final image. So this red color is your tip and the blue color is the sample.

The STM is going to measure the tunneling current between the tip and sample. The change in the distance between the tip and surface while scanning changes the tunneling current and that helps in determining the topography of the sample. For example, we are having a sample like this and when the tip approaches here, the tunneling current is different and when the tip approaches here, the tunneling current will be different.

So, it is going to measure the tunneling current and it is going to give the final image. But one major drawback or challenge in the STM is that it requires a conductive sample and a vacuum environment, which is one of the problems in using STM. So that is the reason atomic force microscopy was developed to overcome the limitations of STM. The first atomic force microscope was built by the same two scientists along with Professor Calvin.

And the advantage of this AFM is it can be useful for studying even non-conductive materials like polymers, ceramics, and biological samples so that is the advantage, and here we don't need any vacuum condition, which is another advantage so this overcomes the drawbacks of STM let us learn about AFM in more detail

AFM provides a 3D topographical map of a sample by measuring the interaction forces between a sharp probe tip and the sample surface. So, this is going to measure the interaction forces between the tip and the sample. So, that is why it is called AFM. It measures the interaction force.

That is why it is called an atomic force microscope. Whereas, in the case of STM, it measures the tunneling current. So, it is called a scanning tunneling microscope. This AFM can determine surface features with sub-nanometer resolution. That is the advantage of this AFM.

And this AFM mainly depends on mechanical interaction. As I told earlier, it mainly depends on the mechanical interaction such as Van der waals or electrostatic forces between the probe tip and the sample surface. Let us see the parts of AFM. The first one is the cantilever surface. The cantilever is a thin, flexible material with a low force constant

and the tip is mounted on the cantilever, which interacts with the sample. And we have a laser. The laser will be deflected off the cantilever upon probe-sample interaction. And we have the photodetector, which captures the reflected laser to quantify cantilever bending or oscillation. And we have a sample stage, which holds the sample.

And we have a piezoelectric scanner which can move the sample. And we have the feedback control system which adjusts the tip height to maintain the laser position. Before we learn about the working principle of AFM, let us see some details about the cantilever. The cantilever and the tip are usually made of silicon or silicon nitride. The cantilever has a very low force constant for enhanced force sensitivity.

And the tip is typically conical or pyramidal to ensure sharpness. The tip radius ranges from 1 to 10 nanometers, which is critical for high-resolution imaging. Let us see the working principle of AFM. The sample surface is scanned by a sharp probe tip mounted on a cantilever to measure the forces between the tip and the sample. The forces between the tip and the sample surface cause the cantilever to deflect.

So once it is deflecting, the deflection is monitored using a laser beam reflected from the cantilever onto a photodetector. And we have a feedback loop system which can adjust the cantilever height to maintain a constant interaction force or deflection. And this movement is recorded to generate a 3D map of the sample surface. Let us understand the working principle in detail.

When sample and probe are present at distance, attractive forces occurs and probe deflects towards the surface. As the distance between sample and probe decreases, repulsive forces occur and probe deflects away from the surface. From this picture, you can understand when tip is in contact with the surface, repulsive regime occurs. And when the tip is pulled toward the surface, attractive regime occurs. And when the tip is far from the surface, there is no deflection.

From this animation, you can understand the working principle of AFM. You can see here when the tip scans the sample, you can see the laser deflection. That can be detected by the detector and you will get the final topography of your sample. So the working principle of AFM is similar to this animation. It is going to scan the sample and it is going to give the final topography of a sample and

Let us see what are the various forces involved in AFM. The AFM primarily follows Hooke's law for the interpretation of the deflection.

Hooke's law is $F = -k\Delta x$

Where, Δx is the cantilever deflection and K is the cantilever spring constant.

The various forces involved are the Van der Waals forces, electrostatic forces and capillary forces and these Van der Waals forces are weak short range forces arising from induced dipole interaction, and these are dominant in non-contact mode. So, the modes of AFM I will explain later. And next one is electrostatic forces due to charge difference between the tip and sample. The electrostatic forces play an important role. And the third one is capillary forces, due to a thin layer of water or other liquid on the sample surface in ambient conditions.

Let us see the difference between AFM and STM.The main advantage of AFM is we can use non-conducting samples and also we can do the analysis. In air and liquid, we can also use the liquid sample. That is the advantage of this AFM. And in AFM, we have three modes. That is tapping mode, contact, and non-contact mode.

So these are the advantages of AFM when compared to STM. Let us see the various modes of AFM. AFM has three modes. The first one is contact mode. And the second one is non-contact mode.

And the third one is tapping mode. Let us see the contact mode in detail. In contact mode, the AFM tip stays in continuous physical contact with the sample surface while scanning. The cantilever deflection is proportional to the force between the tip and sample, and it operates in the repulsive force regime of Van der Waals forces. The surface topography is determined by the tip's vertical movement.

You can see here the tip is in continuous physical contact with the sample surface. So you get very high-resolution images, and the application is we can use it for imaging hard surfaces or rigid samples, and we get high-resolution measurements of surface topography. As I mentioned earlier, the advantage is high spatial resolution, and we can get direct measurement of surface properties but the drawback is if your sample is soft, then it can damage your sample, or if the sample is very hard, then it can damage the tip.

Let us see the non-contact mode in detail. In non-contact mode, the tip oscillates at a small distance, typically between 1 to 10 nanometers from the sample surface, without making physical contact. You can see here the tip is not touching the sample; it is above the sample.

It operates in the attractive force regime of the Van der Waals forces. The cantilever oscillates near its resonance frequency. The tip-sample distance is constantly adjusted to maintain consistent oscillation. The applications are that we can use it for imaging soft or delicate samples like polymers or biomolecules. We can also use it for working in environments such as vacuum or low-humidity conditions.

The advantages are that it minimizes sample or tip damage. It is also suitable for imaging sensitive surfaces. The main disadvantage is lower resolution compared to other modes. The next mode is tapping mode. In the tapping mode, the tip oscillates close to the sample surface, intermittently tapping the surface.

You can see here the tip is not touching these samples. But it oscillates close to the sample surface. In this way, it can achieve high resolution. In this mode, it alternates between the repulsive and attractive force regimes during each oscillation. The cantilever oscillates at or near its resonance frequency, but with a larger amplitude than in non-contact mode.

And the tip height is adjusted to maintain a constant oscillation amplitude. The applications are mainly useful for imaging soft and sticky samples like biological samples, polymeric samples, or for studying surfaces with heterogeneous mechanical properties. The advantages are that it minimizes sample damage as well as tip wear, and it provides high-resolution imaging comparable to contact mode.

The disadvantage is that it requires careful optimization of oscillation parameters. Let us see the substrate properties for AFM samples. The substrate must be atomically flat to ensure that it does not interfere with accurate imaging and measurement of the sample surface topography. So, depending on your sample, you have to select the right substrate.

For example, we can use mica for biomolecules, thin films, and nanoparticles, and most labs use glass for various samples. It is very easy to prepare samples for AFM using glass, and depending on your application, we can even use a silicon substrate if you are working with semiconductor materials. Let us see the applications of AFM. In material science, AFM is useful for mapping 3D surfaces and measuring hardness.

In the case of nanotechnology, we can use it for imaging nanoparticles and verifying the patterns and structures. In the semiconductor industry, it will be useful for detecting defects and irregularities in semiconductor wafers. In biological science, it will be useful for understanding proteins, DNA, and measuring molecular interactions. Let us see how we can use this AFM for imaging biological samples. So, this is the AFM image of baby hamster kidney cells, which are BHK21 cells, and this is untreated.

In the untreated sample, you can see these are long cells, and these are nanoparticle-treated cells. The nanoparticle that is inducing cell death is called apoptosis. You can see that in the early stage of cell death, the cells start shrinking. In the late stage, the cells become completely rounded and shrink. And this is the untreated one.

By using the AFM, we can also measure the surface roughness. You can see here, in the untreated sample, the surface is smooth. The cell is intact, so the surface is smooth. Once the cells undergo cell death, that is called apoptosis, the cell membrane is shrinking or blebbing. Due to that, you can see that the surface roughness increases.

We can use the AFM to measure the surface roughness as well. Let us see the advantages and limitations of AFM. AFM provides nanoscale resolution up to 0.1 nanometer, and it is a non-destructive technique. The sample does not need to be conductive, unlike other techniques like STM, where we need a conductive sample. Also, in electron microscopy, we need conductive coatings.

AFM can also measure topography, adhesion, hardness, and friction. It requires minimal sample preparation. The sample preparation is very simple and easy. Some of the limitations are slow scan speed, and it can analyze small sample areas, usually less than 100 micrometers. It requires a substrate with an ultra-flat surface.

It provides two-dimensional topographical data. So these are some of the limitations of AFM. Let us see some of the challenges in AFM imaging and how to overcome them. The first one is: if you are facing artifacts in images, it may be due to sample contamination, tip contamination, or improper scanning parameters. So clean the tip properly and ensure there is no contamination on the sample.

And also recheck the scanning parameters. The next problem is sample deformation. It may be due to excessive contact force or inappropriate scanning mode. How can we overcome that? We can use non-contact or tapping mode for sensitive samples, or we can reduce the contact force and use softer cantilevers.

The third problem is noise and drift. If we are facing this noise and drift, it may be due to environmental factors like vibrations, temperature fluctuations, or it may be due to electronic noise. How to overcome that? We can provide vibration isolation for the instrument.

And if you are getting poor resolution, it may be due to an incorrect tip. Choose the appropriate tip. And the last one is adhesion problems. It may be due to higher humidity. So reduce the humidity or use anti-adhesion coatings on the tip.

I hope you got the overall idea about AFM. Let us go to the lab and learn this technique in more detail. In this demonstration, we are going to perform atomic force microscopy on a sample of BSA nanoparticles which we synthesized earlier in one of the previous lab demonstrations. This is an atomic force microscope from Bruker.

Here we have two sets of controllers. This one is the stage control unit, which controls the stage movement along the X and Y axis. The other one is the Nanoscale feedback controller, which controls the vertical movement of the cantilever. The microscope operations are controlled by software. Coming to the atomic force microscope, the machine is enclosed within a vibration-free chamber

which reduces noise and improves the accuracy of the measurements. This is the stage where we mount our sample in the middle. For the measurement, we have the laser source. The laser is generated from the source. It then goes toward the bottom where the cantilever is attached.

The laser is reflected from the cantilever and moves toward the photodetector. It measures the changes in the tip position relative to the sample surface. This camera is used to monitor the sample and check the cantilever position. The display unit at the top shows the vertical and horizontal coordinates. Here is the holder where the cantilever is placed.

To attach the cantilever, first carefully remove the laser assembly. The cantilever is then attached to the bottom of the laser assembly. There are multiple types of tips, depending on the AFM mode. This is the RTSP 300 tip, which we will use for tapping mode. You can see what a cantilever looks like.

There is also the SCANASYST tip from Bruker, which is a versatile tip used for high resolution imaging of almost all samples. The SCM-PIT probe is a multifunctional electrical AFM probe. There are various types of probes available depending on the specific applications. Let us proceed to microscopy. First, open the software.

You can see there are multiple modes of AFM. We will be using tapping mode today. In the setup, select the type of probe that is installed in the machine. The cantilever will automatically come into focus. The small rectangular structure is the cantilever, and the tip is attached at the very end of the cantilever.

Now we will align the laser on top of the cantilever. Similarly, align the laser position at the center of the detector. Now let us load the sample into the machine. This is a BSA nanoparticle sample that has been drop-cast on a clean glass slide. Place the slide at the center of the stage and start the process.

The sample will move to the center position. Then, using the software, first bring the sample surface into focus. Once the sample surface is in focus, move the cantilever down until the tip is in focus. When both these steps are done, proceed with the scan. You can see that the sample is being scanned.

Then select the desired area to magnify it and take a scan. The raised portions in the scan are visible as bright areas, while the deeper areas are represented in dark colors. This is a 3D map of the sample surface that has been captured using atomic force microscopy. The left panel is the 2D height sensor image. This 2D image represents the height profile of the nanoparticles distributed on the surface.

The brighter areas correspond to higher elevations, while the darker regions indicate the lower elevations. The nanoparticles appear as distinct dispersed dots, suggesting a uniform distribution of the BSA nanoparticles. However, there may be a few agglomerations visible as larger clusters at the center and near the edges, you can see there are some larger clusters. The right-side panel is a 3D topographical image.

So, this 3D view provides an enhanced visualization of the surface morphology and elevation. The peaks represent the nanoparticles, and the height scale in nanometers indicates their approximate size from the measurement scale bars. The nanoparticles exhibit a well-defined spherical or semi-spherical morphology with a surface reference of around 203 nanometers. Estimated from this topographical profile.

The sharpness and height of the peaks further confirm the presence of nanoparticles on the substrate. As a summary, in today's lecture, we learned about the principle of AFM and various modes of AFM. Also, through practical demonstration, we learned this AFM technique in more detail. Thank you for your kind attention. I will see you in another interesting lecture.