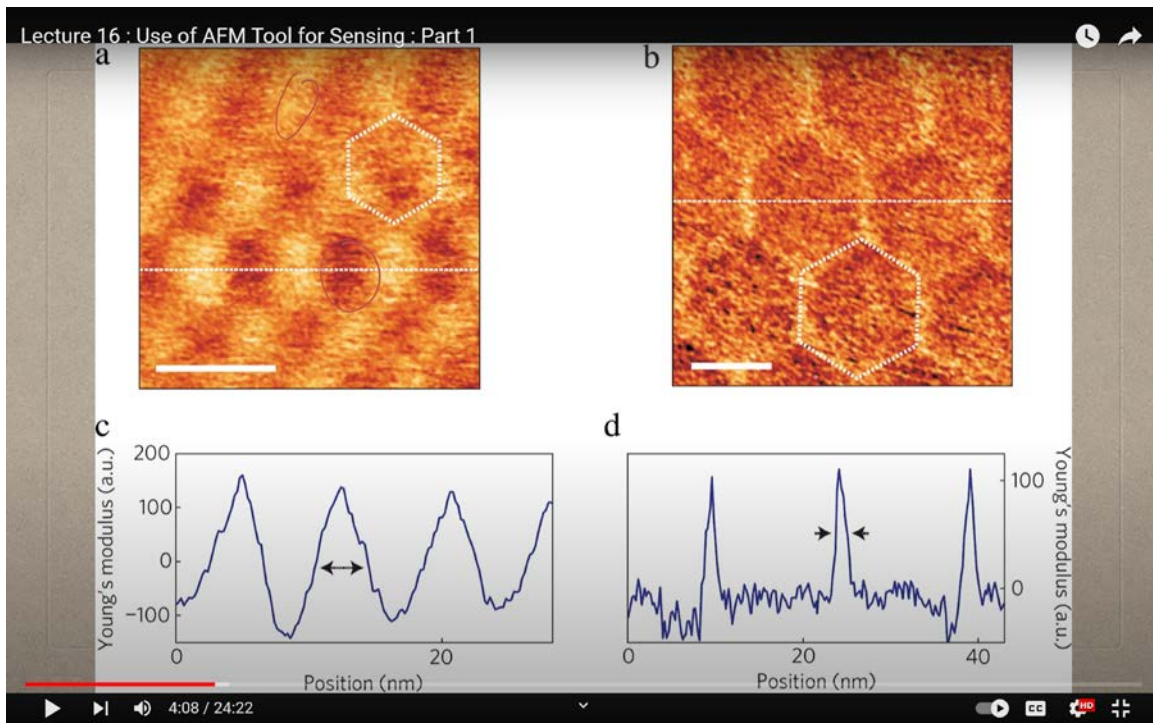


Design for Biosecurity
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Lecture 16
Use of AFM Tool for Sensing : Part 1

Welcome back to the fourth week of this course. So far, we've discussed some of the historical contexts of this century, especially in light of significant bioterrorism threats. Last week, we delved into one of the earliest sensors, the quartz crystal microbalance. From this point onward, I would like to emphasize the importance of modern instrumentation and detection tools, which are currently being employed and will likely continue to evolve into combinations of multiple tools in the future.

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What do I mean by this? For instance, consider when you're examining something under a microscope. You may be able to gather morphological information, meaning the structural

details of your sample. However, this method alone doesn't provide chemical information. To obtain that, you'd need a different set of tools. Now, imagine your sample is limited in quantity. In such a case, you're always at risk of losing critical information if you have to divide it between different methods, using one part for microscopy, another for spectroscopy, and yet another for analyzing its physical properties. This can quickly become cumbersome. And in the context of bioterrorism or similar agents, these samples tend to be present in very trace amounts, making the process even more challenging.

Therefore, the techniques we use must adhere to certain principles. Firstly, they should preferably be non-invasive to avoid altering or destroying the sample. Secondly, you should aim to have a detection system where a single sample can be analyzed for various parameters, morphological, chemical, and physical, without needing to break the system apart or use multiple samples. This is crucial because once you break the system, you not only lose out on cohesive data but also expose yourself to the risk of contamination. What you'll come to appreciate in this week's discussions is how modern biosensing and detection techniques, particularly those coupled with electrochemical systems, are becoming more modular and integrated. These advancements allow you to perform multiple analyses within the same system, making it a much more efficient process.

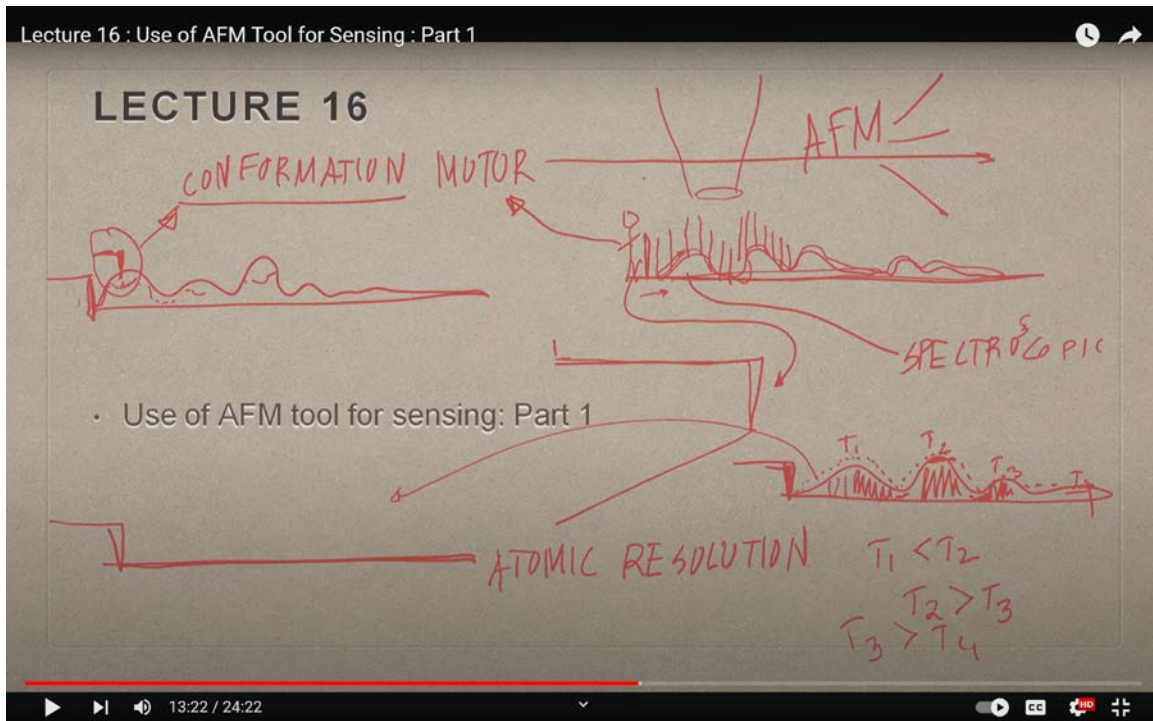
Now, moving forward, this is Lecture 16, and we'll begin by diving into the fundamentals of Atomic Force Microscopy (AFM), a tool that has gained immense popularity in the field of biosensing.

What exactly is AFM? As the name suggests, atomic force microscopy allows us to observe things at the atomic level. But how does it work? You may have encountered AFM images before, pictures showing darker and lighter patches on a substrate. These images represent the variations in surface topography and help researchers deduce patterns at the atomic scale.

Before we delve into the technical aspects of AFM, let's think about it conceptually. Imagine you have a sample, let's not worry about its exact size or scale for now. Suppose this sample consists of various compounds, distributed across its surface in an uneven manner. Now, visualize yourself walking down a street with a cane, much like a visually

impaired person would. As you walk, your cane taps the ground at different points, sometimes hitting a raised area, sometimes descending into a dip, and so on. In essence, your cane is "feeling" the terrain in front of you, helping you navigate your path.

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This analogy is very close to how an AFM operates. The AFM tip behaves like the cane, scanning the surface of a sample, detecting the tiniest changes in topography, and providing us with a detailed map of the atomic landscape.

Let's break this down conceptually.

Imagine, instead of using a simple stick, we now have a highly sophisticated tip. This tip, unlike the stick, operates at atomic resolution. Now, envision yourself walking, but instead of moving on your own, you're replaced by a motor that controls your movement. This motor is now maneuvering the tip along the surface. So, as the tip moves, it traces the contours of the surface, just as you would with the stick.

Let me explain this further with a simple sketch: Picture a surface with different elevations. As the tip moves under the influence of the motor, it will record the height variations at each point. It will say, "Here, the height is like this," and "Here, it's a bit lower," and so on. For example, at one point, let's call it T1, the height is slightly lower. At the next point, T2, the height is higher. Then at T3, it dips again. By moving the tip across the surface, you obtain a detailed height profile, showing you precisely how the surface varies at the atomic level. This is one of the primary applications of Atomic Force Microscopy (AFM): generating surface profiles.

Now, imagine the surface is perfectly flat, there would be no variation in the recorded profile, just a straight line with no contrast changes. However, if the surface has peaks and valleys, these will show up as fluctuations in the profile. You could also visualize this by looking at the corresponding depth profile, lighter areas might indicate deeper regions, while darker areas represent higher peaks. If you analyze these patterns and notice repetition, you might even deduce some level of order. This order could be correlated to a specific chemical structure, which is when you know you're hitting the bull's eye. This technique of AFM allows us to drill down to that precise level.

But there's another scenario to consider. Imagine the surface you're scanning isn't rigid. Think of it like sitting on a sofa. When you sit down, the sofa compresses beneath you, but when you get up, it returns to its original shape. Similarly, certain surfaces will flex or deform when the AFM tip presses against them.

As the tip moves across such a flexible surface, it might push down further than expected. When this happens, two types of information can be gathered. Let's say the tip presses against a surface that's like the palm of your hand. If you press on your palm, it will collapse slightly, forming a dent. The same thing happens with the AFM tip. As it hits the surface, it causes a vibration because of the interaction with the material. You can feel this vibration as a response to the surface's properties.

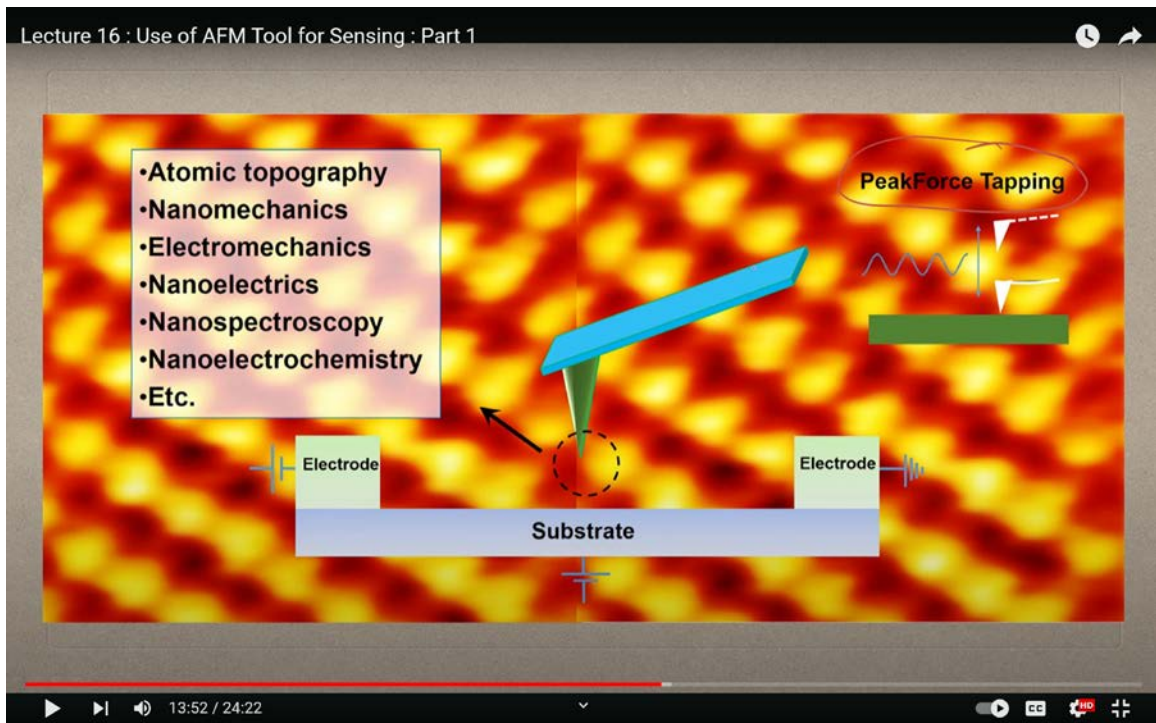
Alternatively, instead of just waiting for the surface to react, we can actively induce vibrations in the AFM tip itself, generating a "tapping" motion. This tapping occurs at a known frequency, and as the tip touches the surface, any change in this frequency provides

insight into the surface characteristics. For example, if the surface is particularly hard, the tapping frequency will shift differently compared to a softer surface. So, by monitoring these changes in tapping frequency, AFM can reveal the distinct physical properties of the surface, whether it's rigid, flexible, or somewhere in between.

This sophisticated method of sensing variations in surface characteristics, whether from height changes, flexibility, or tapping frequency, is one of the reasons AFM is so powerful as a biosensing tool.

As we move forward, it's important to understand that the configuration of the AFM tip can be modified in various ways. This flexibility in tip design offers a significant degree of freedom to the user, allowing them to tailor the AFM tool to suit a wide range of applications. It's crucial to stretch your imagination when considering what AFM can achieve because, in addition to scanning surfaces, the tip interacts directly with atoms, potentially inducing vibrations or other disturbances at the atomic level.

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Now, imagine this scenario: you have a surface, and the AFM tip is tapping it. Simultaneously, you could have a microscope providing a visual representation of the surface, and beneath the setup, a spectroscopic system is analyzing the chemical composition. In this integrated approach, AFM works alongside other technologies, each providing complementary information. While the AFM delivers a surface profile, the microscope reveals finer surface features, and the spectroscope identifies the types of atoms present. By coupling AFM with these other systems, you're able to extract an incredible amount of information from even the tiniest of samples.

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Lecture 16 : Use of AFM Tool for Sensing : Part 1

WHY AFM?

- "Atomic Force Microscopy (AFM) is generally used to capture 3-D topographic images of sample surfaces. Today, it can routinely achieve atomic resolution to identify monatomic steps and ripples in the -direction and resolve lattice orientation and surface defects in the -plane. In addition to traditional contact and tapping modes, novel modes have been developed to stably control ultralow imaging force, thus preserving both the AFM tip and fragile samples. These modes also allow precise local stress to be applied to samples for mechanical, electromechanical, and piezoelectrical studies. Beyond topography and local force control, advanced AFM modes allow researchers to capture maps of modulus, adhesion, deformation, conductivity, surface potential, permittivity, optical absorption and reflection, electrochemistry, etc. This information is generally captured through multimodal imaging at normal or high-speed acquisition rates, which makes correlation among different properties possible. In addition, AFM works not only under ambient conditions but also in controlled environments, including liquid, varied temperature, dry box, vacuum, etc. All these capabilities contained within one single instrument makes AFM a versatile platform for investigating highly multidisciplinary research."

<https://www.sciencedirect.com/science/article/pii/S0030401817303887>

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This is precisely why many modern techniques combine multiple instruments into one platform, as it allows for the maximum extraction of data from a single setup. To give you an example of how this works, consider peak force tapping, a specific AFM mode. Here, you have a substrate and the AFM tip. This technique allows you to explore atomic topography, nanomechanics, electromechanics, nanoelectronics, nanospectroscopy, and even nano-electrochemistry. When these methods are combined, you can analyze

extremely small samples with unprecedented detail, making it a dream setup for detection systems.

Now, let's delve deeper into why Atomic Force Microscopy (AFM) is so valuable. AFM is typically used to capture detailed 3D topographic images of sample surfaces. As you move the tip across the surface, you are effectively reconstructing its three-dimensional geometry. Today, AFM can routinely achieve atomic resolution, allowing us to identify individual atomic steps, observe ripples in the material, and resolve lattice orientations or surface defects. This capability provides researchers with incredibly precise information.

Moreover, beyond traditional AFM modes like contact and tapping, new advanced modes have been developed. These novel modes allow for ultra-low imaging forces, preserving both the AFM tip and delicate samples. This opens the door to studying mechanical properties, electromechanical behaviors, and piezoelectric effects with extreme accuracy.

So, what does this mean? Essentially, by using the AFM tip in tapping mode, you can determine whether a surface deforms under pressure. If the surface does deform, it alters the vibration frequency of the tip, and from this change, you can infer the mechanical properties of the substrate. This concept builds on the idea I introduced earlier, by tapping the surface and observing the response, you can gather information about the material's mechanical behavior.

But that's not all. Beyond just topography and local force measurements, advanced AFM modes enable researchers to capture images that reveal a wealth of additional properties, such as modulus, adhesion, deformation, conductivity, surface potential, permittivity, optical absorption, and reflection, as well as electrochemical characteristics. These properties are usually captured through multimodal imaging at either normal or high-speed acquisition rates. This makes it possible to correlate various physical, chemical, and mechanical properties of the sample in a highly integrated manner. Thus, AFM is an incredibly versatile tool that, when coupled with other techniques, allows researchers to unlock a wide range of insights from even the smallest of samples.

AFM operates under a variety of conditions, including ambient environments, controlled settings like liquids, varying temperatures, dry boxes, and even vacuums. These capabilities make AFM a remarkably versatile instrument, perfect for conducting highly interdisciplinary research. This is precisely what I've been trying to convey to you: modern AFM is an incredibly robust tool.

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Lecture 16 : Use of AFM Tool for Sensing : Part 1

BASIC FORMS OF AFM

Cantilever
SURFACE-PROFILING/

- In its most basic form, AFM involves scanning a sharp probe across a sample surface while capturing information about surface properties. An AFM probe is composed of a substrate, a cantilever, and a sharp tip. The substrate is a rectangular chip with sides measuring on the millimeter scale and serves as the connection point for the probe. The cantilever protrudes from the substrate, can be triangular, rectangular, or other forms, and measures in micrometers. The tip, the part that interacts with the sample surface and provides high resolution, is at the end of the cantilever and measures in nanometers. The AFM probe is capable of high-resolution imaging due to a combination of efficient piezoelectric actuation that is used to precisely drive the tip or the sample; the use of a sharp tip with optimal mechanical properties; and a fast feedback control loop for maintaining the user-defined tip-sample interaction, the so-called setpoint. The piezoelectric actuators together with the feedback loop allow for tip or sample movement control at a picometer scale. AFM probes with different chemical, geometrical, and mechanical properties are commercially available, each of which may provide different information depending on their interaction with the sample. Probes with nominal end tip curvature radii of 1 and 2 nm are now also commercially available.

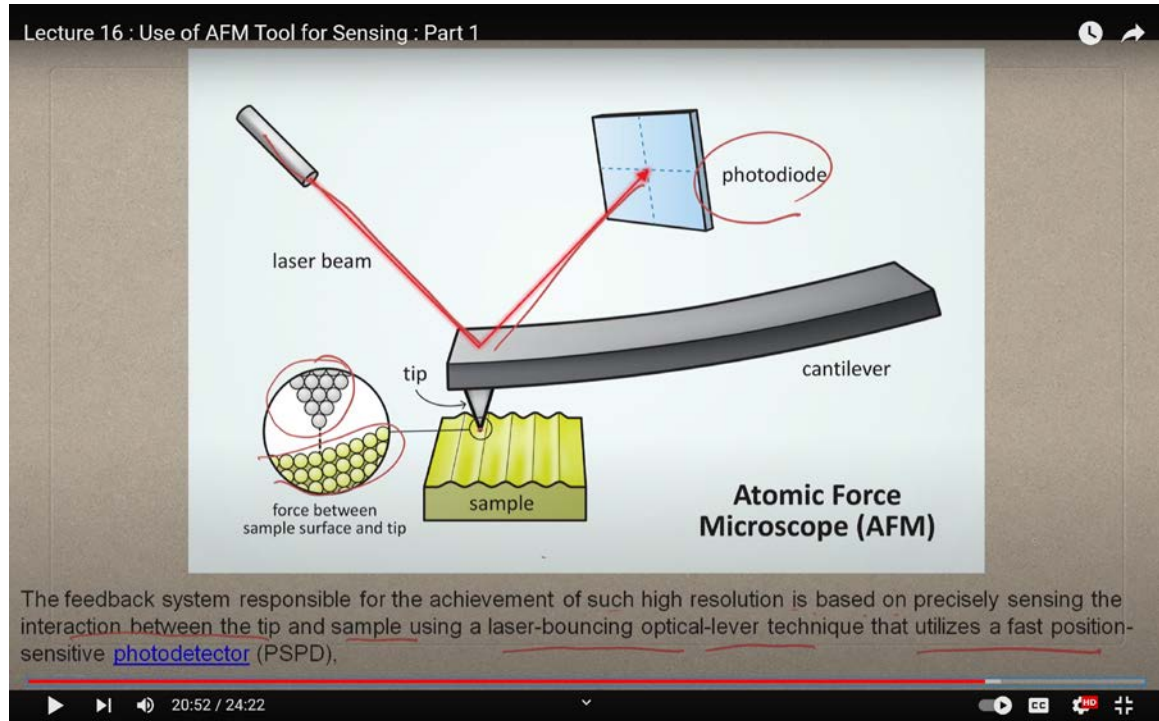
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Let's break down the basics of AFM. In its simplest form, AFM involves scanning a sharp probe across the surface of a sample, while simultaneously capturing detailed information about the sample's surface properties. Originally, AFM was mainly used for surface profiling, creating detailed scans of the surface structure. The AFM probe itself consists of three key components: the substrate, the cantilever, and the sharp tip.

To visualize it, think of the cantilever as a small arm extending from the substrate, which is a rectangular chip. The cantilever can come in various shapes, triangular, rectangular, or others, and its size is typically measured in micrometers. The critical component is the

sharp tip at the end of the cantilever, which interacts directly with the sample surface. This tip, measured in nanometers, allows for extremely high-resolution imaging.

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Now, the secret to AFM's high-resolution imaging lies in the combination of piezoelectric actuation, a finely tuned feedback control loop, and the precise mechanics of the tip. If you recall from our discussion on quartz crystal microbalance (QCM) and piezoelectric processes, AFM also leverages piezoelectricity to control the exact movements of the tip with nanometer precision. The tip's optimal mechanical properties and the feedback control loop allow the instrument to maintain the user-defined distance between the tip and sample, ensuring accurate imaging.

Imagine you're shining a laser beam onto the surface, this is where a photodiode comes into play. The laser beam reflects off the cantilever, and the photodiode detects this reflection. In this setup, the tip and the surface interact, with the force between them being carefully monitored. This process is facilitated by a feedback system that uses a laser-based optical lever technique. The laser bounces off the cantilever, and a fast, position-sensitive

photodetector (PSPD) picks up on the minute changes in the cantilever's position. This precision is what enables AFM to achieve such incredibly high-resolution imaging.

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Lecture 16 : Use of AFM Tool for Sensing : Part 1

(a) Schematic representation of an AFM;

(b) Tip-sample interaction for quantitative nanomechanic studies;

(c) Scattering scanning near-field optical microscopy (s-SNOM) for nanospectroscopy in chemical identification

(d) AFM-based scanning electrochemical microscopy (SECM) where the insulated AFM serves as a nanoelectrode probe

Resonance
 STATIC VS. TAPPING
 TOUCH/CONTACT VS. NON CONTACT

23:18 / 24:22

Returning to the role of piezoelectric actuators, they work in tandem with the feedback loop to control the movements of the tip or the sample at a picometer scale, an astonishingly tiny level of control. AFM probes, designed with different chemical, geometrical, and mechanical properties, are commercially available. Each type of probe offers unique insights depending on how it interacts with the sample. For example, probes with normal tip curvature radii of 1 to 2 nanometers are available on the market today, which means we can now achieve atomic-level resolutions, down to detecting 10 to 15 atoms at a time, or even greater detail. This is a profound capability that showcases just how powerful and precise AFM technology has become.

So, that is the true power of atomic force microscopy, and this is only the beginning. There's so much more being integrated into it now. For instance, one aspect I haven't mentioned yet involves the laser. When you shine the laser, what if the laser actually passes through

the tip itself? What happens then? This introduces a new mode of operation, which we will explore later.

With that said, let's wrap up this part by highlighting the various configurations available with AFM. The basic setup involves the laser, which you shine onto the surface. There's a photodetector, and, of course, the tip that can reach down to a 1-nanometer scale, now commercially available. This tip moves across the sample, allowing for detailed exploration of surface features. This is the fundamental setup that facilitates tip-sample interactions and enables quantitative nanomechanical studies, which we will delve into in the next session.

Additionally, I want to introduce other techniques that build upon AFM. One of them is the Scattering Scanning Near-Field Optical Microscope (SNOM), a key modification that expands our optical capabilities at the nanoscale. Another technique is the Scanning Electrochemical Microscope (SECM), where the insulated AFM tip functions as a nanoelectric probe.

These techniques represent just the foundational aspects of AFM. Moving forward, we'll discuss different operational modes for the AFM tip. For example, the probe can be either static or tapping. In tapping mode, the tip intermittently touches or comes into close proximity with the surface, creating two key scenarios: contact mode and non-contact mode. Additionally, we can intentionally create known disturbances and then observe how the system responds, especially in terms of resonance. We will explore how these probes resonate at various frequencies and how these resonance frequencies vary with different types of probes.

In the next session, we'll delve into the fascinating modifications that have been made to AFM and how these developments are completely transforming our understanding of molecular detection and structural analysis. Thank you!