Design for Biosecurity

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Lecture 17

AFM for Bio-sensing : Part 2

Welcome back! Today is the seventeenth lecture of the fourth week, and in our last session, we discussed the fundamental configuration of AFM. We explored how the AFM tip can be modified in various ways depending on the substrate and sample under investigation. Now, let's move on to today's lecture, which is Lecture 17, focusing on AFM for biosensing, specifically Part 2 of this topic.

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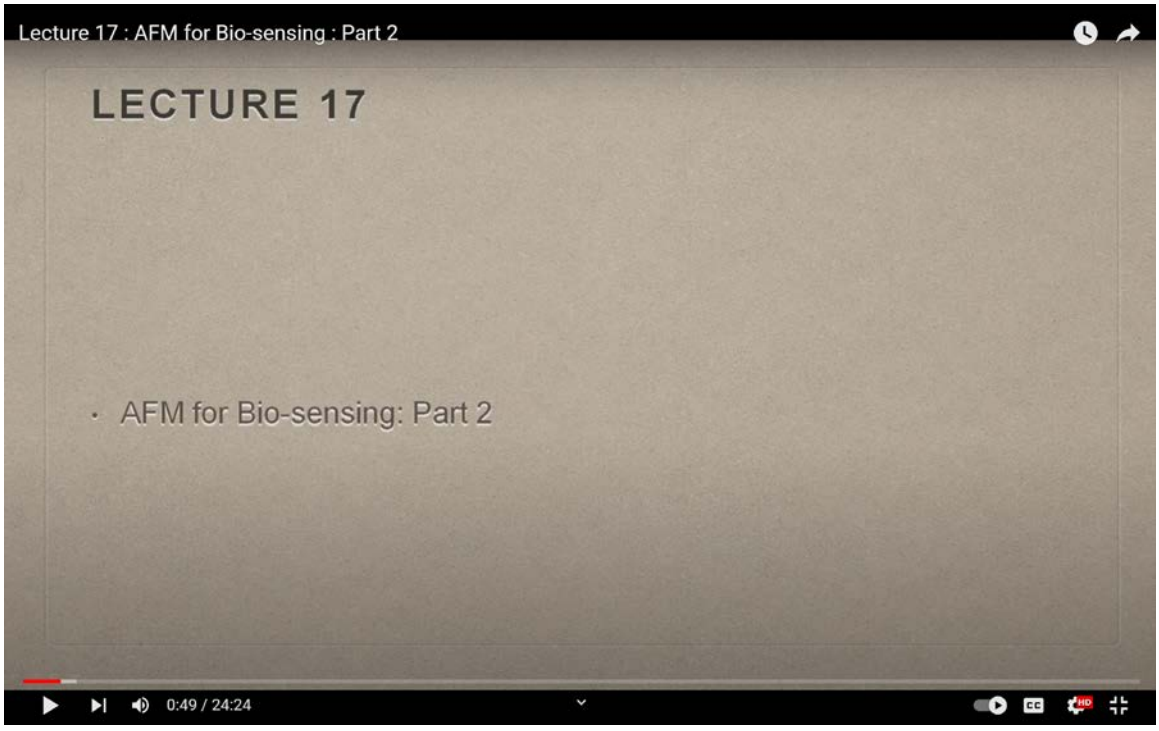

To begin, we will examine the first configuration, which is the type of tip-sample interaction. There are three primary categories of interactions that can occur between the tip and the sample:

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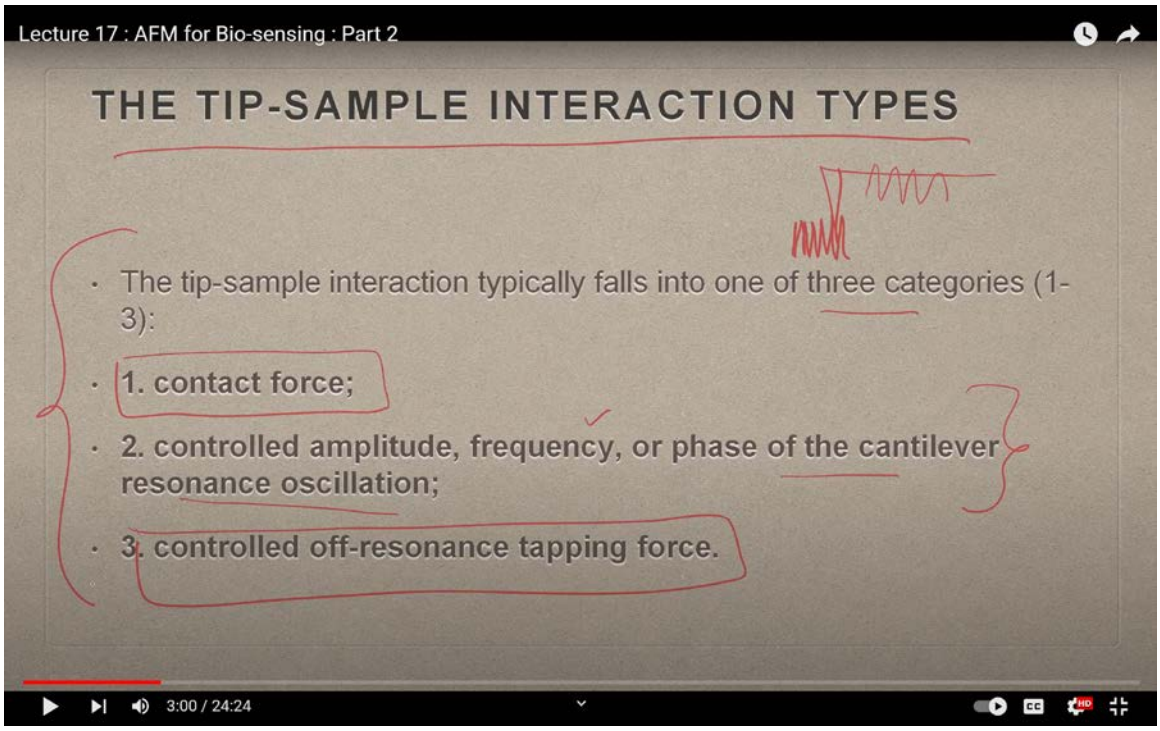

1. Contact Force:

The first type is the contact force. Imagine you're positioning the tip, and it makes contact with the surface. The key question here is: What is the force of this contact? How hard is the tip pressing against the surface? This force determines how the substrate behaves, whether it compresses, deforms, or remains unchanged. The nature of this contact force is crucial for understanding the properties of the substrate.

2. Controlled Amplitude, Frequency, or Phase of Cantilever Resonance Oscillation:

The second interaction involves the controlled amplitude, frequency, or phase of the cantilever's resonance oscillation. To illustrate, picture the cantilever with its tip as it makes contact with the substrate. The cantilever starts to vibrate, creating a resonance frequency. For instance, if the cantilever is tapping the surface at a specific frequency, this frequency might shift upon contact. This interaction is fundamental to understanding how the substrate influences the cantilever's oscillations.

3. Controlled Resonance Tapping Force:

The third category involves controlling the resonance tapping force. Here, you introduce a tapping force and observe how it changes as the tip moves across the surface. This interaction provides insight into the mechanical properties of the surface.

These three interactions are the core principles of AFM. Let's break them down further with some visual aids.

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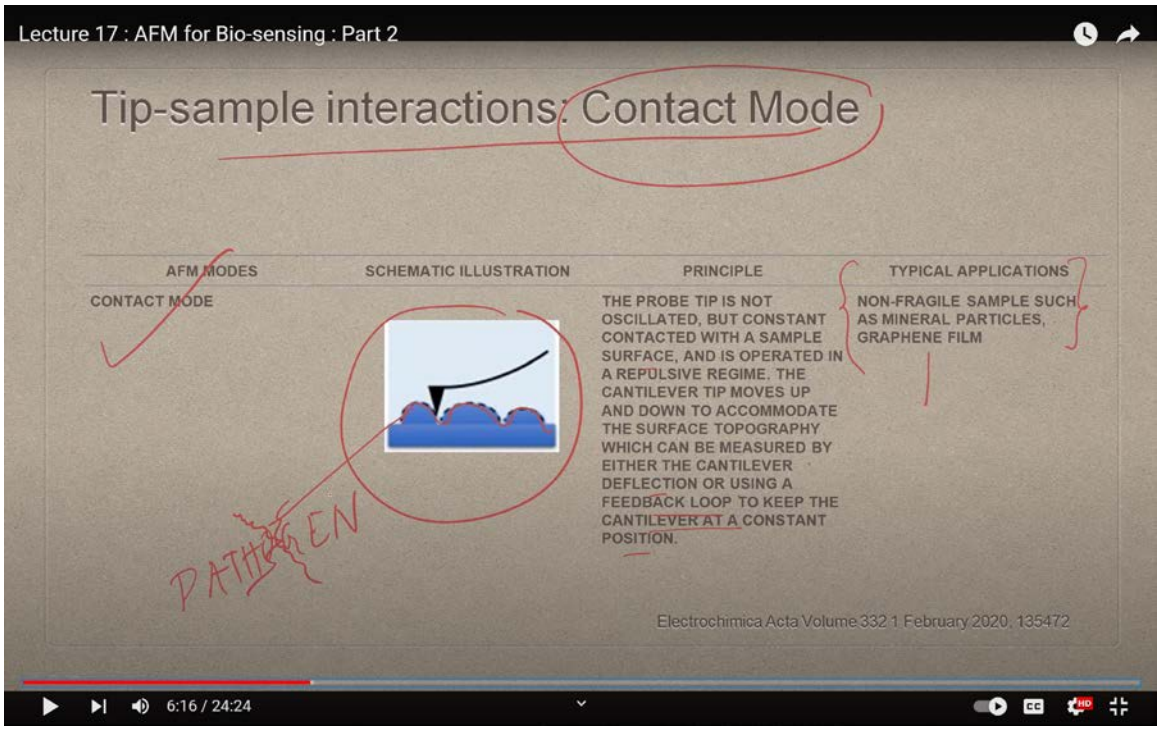

Contact Mode:

In contact mode, the principle is straightforward. The tip of the cantilever is not oscillating; instead, it remains in constant contact with the sample surface and operates in a repulsive regime. As the cantilever moves up and down, it accommodates the surface topography. This movement can be measured either by observing the cantilever's deflection or by using a feedback loop to maintain the cantilever's position at a constant level. However, it's essential to be cautious because continuous contact can wear down or even break the tip.

In typical applications, non-fragile samples, such as mineral particles and graphene films, are robust enough to withstand this mode, but care must still be taken.

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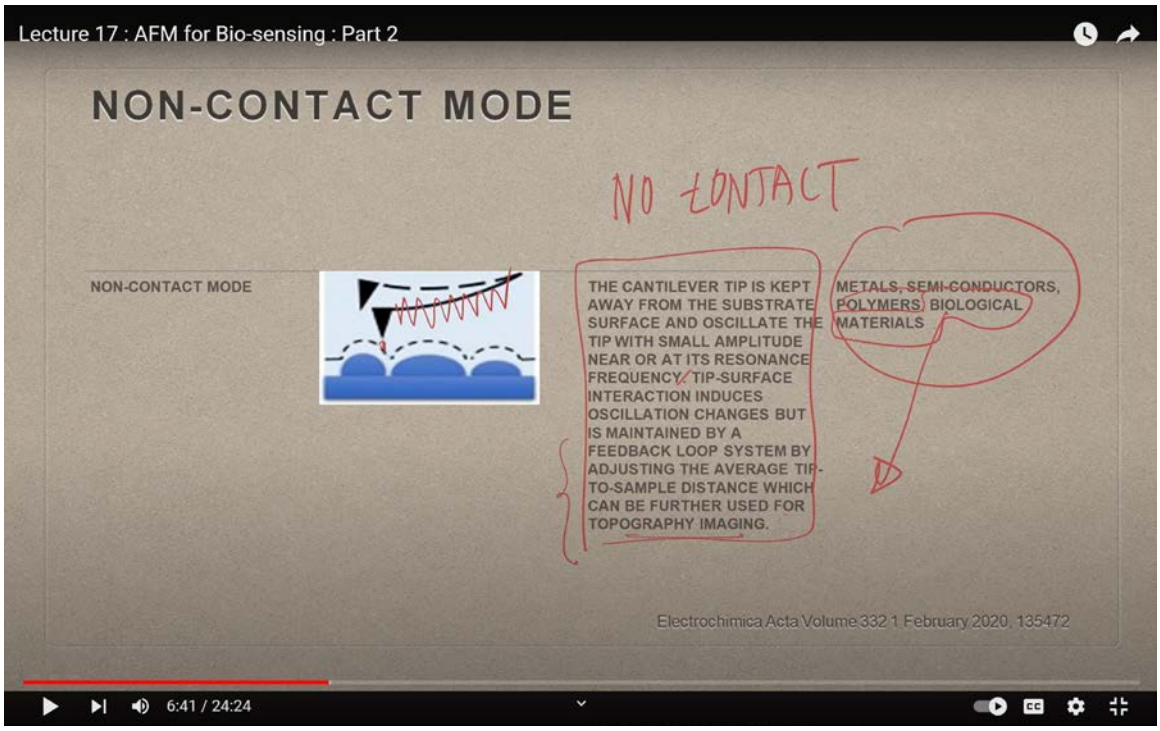

Non-Contact Mode:

The next mode is the non-contact mode, which is more delicate and nuanced. In this mode, the cantilever tip is kept at a distance from the substrate, it does not actually touch the surface. Instead, the tip oscillates with a small amplitude near, or at, its resonance frequency. The interaction between the tip and the surface causes changes in this oscillation, but a feedback loop system maintains the average distance between the tip and the sample. This mode is particularly useful for topography imaging, as it allows you to maintain a consistent, finite distance from the surface, ensuring that the tip does not make contact.

So, in non-contact mode, the cantilever tip remains away from the substrate, oscillating at a small amplitude near its resonance frequency. This mode avoids direct contact with the

sample, and the oscillation is carefully controlled by adjusting the average tip-to-sample distance, making it an ideal technique for delicate topographical studies.

So, in this process, you're oscillating the tip, and the interaction between the tip and the surface induces changes in the oscillation. However, these oscillations are controlled by a feedback loop, which maintains stability. The frequency of these oscillations is constantly being monitored by the detector. This second mode of operation is commonly used for examining metal-semiconductor polymers and biological materials. This non-contact mode is particularly significant in our context, especially when dealing with biological samples.

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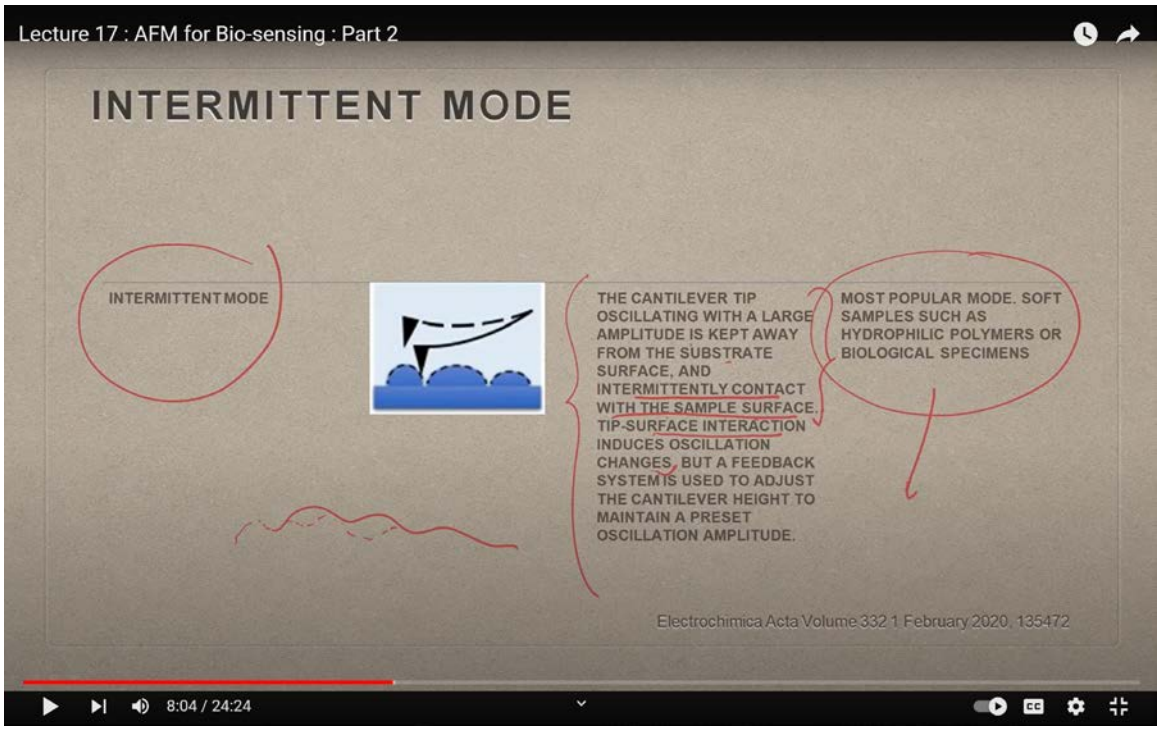

Think of it this way: when you're handling a delicate pathogen, for example, you don't want the tip to make direct contact with the pathogen’s surface. If that happens, there’s a real risk that the tip might pierce the surface, potentially damaging the pathogen and leading to contamination, something we definitely want to avoid. So, in such cases, non-contact mode becomes the preferred choice. Here, the tip stays free from direct interaction with the

sample while still providing a wealth of information. As a researcher, it's important to understand the capabilities of each probe and choose the one that best suits your needs.

Now, let's move on to the third mode: intermittent mode. This mode lies somewhere between contact and non-contact. In this configuration, the cantilever tip oscillates with a larger amplitude than in non-contact mode, and while it mostly stays away from the surface, it intermittently makes contact with the sample. The interaction between the tip and the surface again induces changes in oscillation, but the system has a feedback mechanism that adjusts the height of the cantilever to maintain a preset oscillation amplitude. This intermittent mode is especially useful for softer samples, such as hydrophilic polymers and biological specimens.

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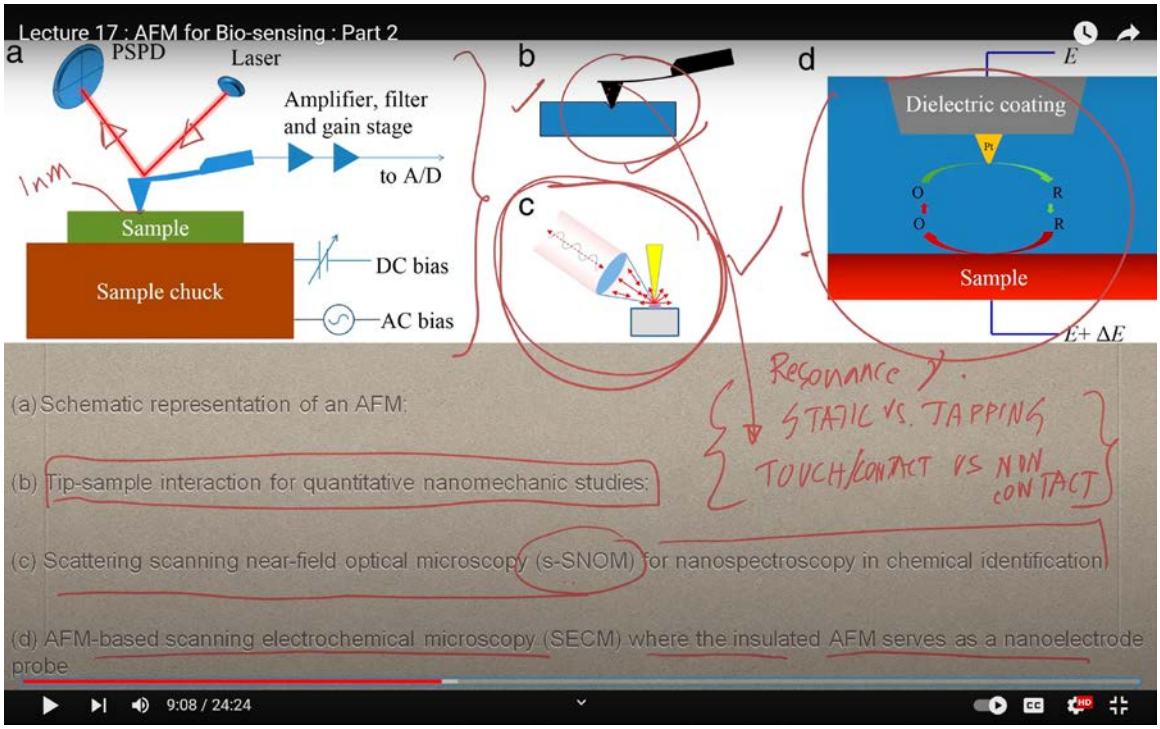

What makes this mode so fascinating is that it allows us to detect surface deformities in the sample. Do you remember how, in the first lecture, we discussed detecting surface irregularities? Well, intermittent mode helps us do just that, while maintaining a relatively stable distance between the tip and the substrate, it periodically makes contact, picking up

those deformities. The feedback loop continually adjusts the cantilever's height to ensure that the oscillation amplitude remains consistent. This is a crucial feature for maintaining the integrity of delicate samples.

So, these are the three primary modes of AFM, contact, non-contact, and intermittent. Both intermittent and non-contact modes are typically preferred for soft matter and biological samples due to their gentle handling of the material.

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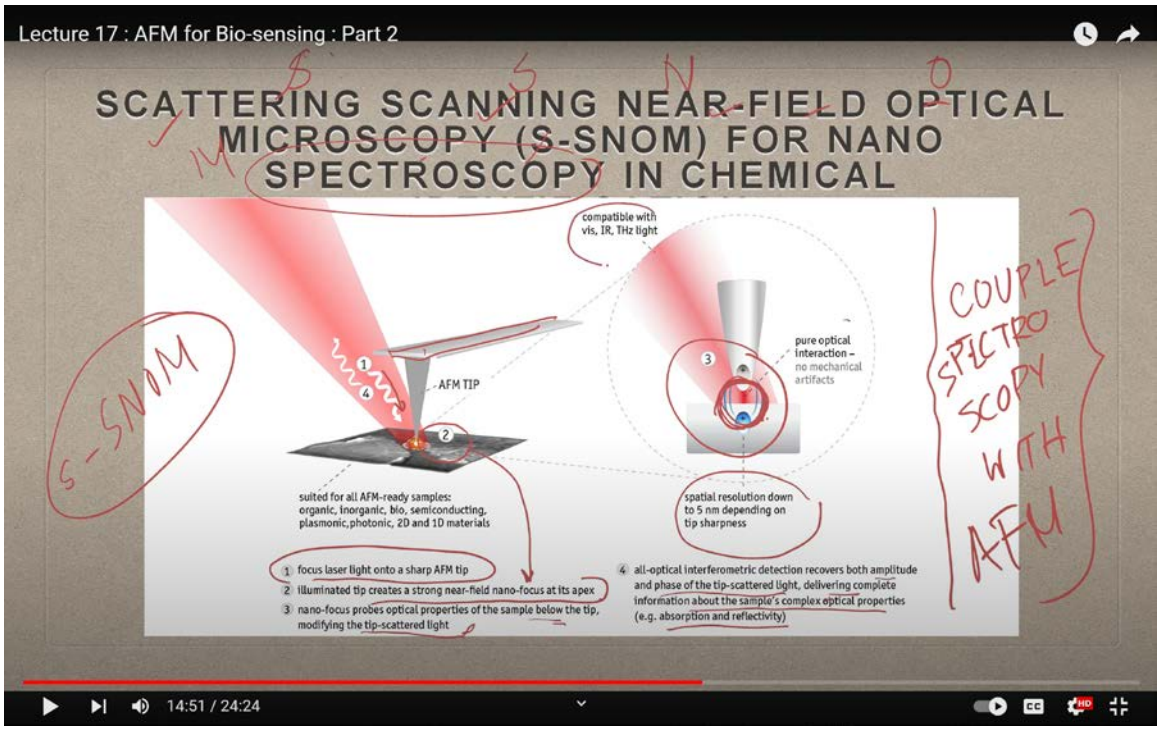

Now, moving on to the next topic, which is Scattering Scanning Near-Field Optical Microscopy (SNOM) or nano-spectroscopy for chemical identification, let's refer to this as part C. In short, you may encounter the term s-SNOM in the literature, with the lowercase "s" referring to scattering. This technique is particularly valuable for nano-spectroscopy, allowing us to identify chemical compositions at the nanoscale.

Before we dive deeper into SNOM, I want to highlight something important. Likely in the next week's lecture, I'll provide a tutorial on the interaction between light and matter. This is critical because, when you're working with a probe that interacts with a substrate, there

is a high likelihood of disturbing atoms on the surface, which in turn creates atomic vibrations. Understanding how light interacts with these atomic disturbances is key to making the most of SNOM and other advanced microscopy techniques.

When you shine a laser on a sample, atomic vibrations are created, which are fundamental to the interaction of light with matter. This is the underlying principle behind any form of light-based spectroscopy. Now, imagine if we combine spectroscopy with AFM. How would that work? Let's break it down.

First, you have the AFM tip in place, and you direct a laser beam onto the sample. Step one involves focusing the laser light onto the sharp AFM tip, this is your incoming laser beam. The goal is to illuminate the tip, creating a strong near-field nano-focus at its apex. So, what happens next? This nano-focus interacts with the sample's optical properties just below the tip, altering the scattered light produced by the tip.

When discussing scattered light, a few key things occur. Let's say you have a sample with a light beam directed at it. Some of this light will reflect, some will scatter, and some will pass through the sample. These interactions depend heavily on the atomic structure of the sample. Light that passes through gaps in the atomic structure is refracted, while light hitting atoms directly may reflect or scatter in various directions. What's fascinating is that all of these interactions, whether the light reflects, scatters, refracts, or even passes through, provide valuable spectroscopic data. Not a single piece of information is wasted.

In addition to scattering and reflection, some light might also be absorbed by the material, and sometimes this absorbed light induces vibrations within the atoms themselves. So, to summarize: when you shine light onto a sample, several things happen. Part of the light is reflected, part is scattered, part is refracted and passes through, some of it is absorbed, and some of it creates atomic vibrations. All of these phenomena, reflection, scattering, refraction, absorption, and atomic vibrations, can be harnessed for spectroscopic analysis.

Now, if we could couple this process with AFM, it opens up a whole new dimension of possibilities for analyzing material properties. This brings us to point four, where the interaction between the nano-focused light and the sample below the AFM tip modifies the

scattered light from the tip. What's exciting is that this scattered light can be measured and quantified, providing insights into the sample's optical properties.

Through optical interferometric detection, we can recover both the amplitude and phase of the tip-scattered light, allowing us to obtain a complete picture of the sample's complex optical characteristics, including its absorption and reflectivity. The spatial resolution achieved can be as fine as 5 nanometers, depending on the sharpness of the AFM tip.

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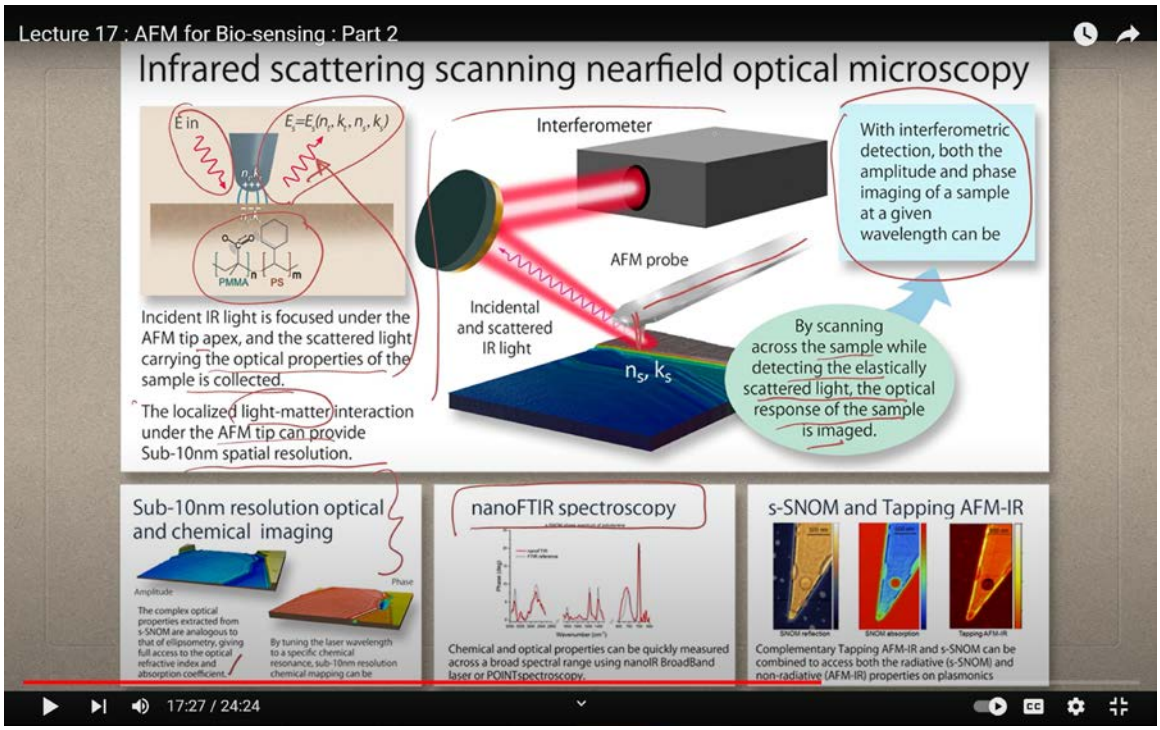

This technique is highly versatile and compatible with different light sources, such as infrared (IR), Raman, and other forms of light. In fact, as we delve further into this, we'll talk about infrared scattering in particular. If you refer back to a previous image, you'll recall seeing the scattering process in action. This is precisely what we're focusing on now, the behavior and significance of that scattering.

Looking at an example, you have an incident infrared beam that is focused on the AFM tip's apex. The light scattered by this interaction carries vital information about the sample's optical properties, which can be collected and analyzed. To make things clearer,

the compounds being studied can reveal specific optical behaviors that help us better understand their characteristics.

The localized interaction of light and matter under the AFM tip allows for highly precise spatial resolution. For example, if you are scanning a surface composed of various compounds or functional groups, the AFM tip can map out exactly where each group is located. It can identify the presence of hydroxyl groups, amine groups, imidazole rings, sulfur atoms, and so on across the sample. Essentially, as the tip moves over the sample, it provides a detailed chemical map.

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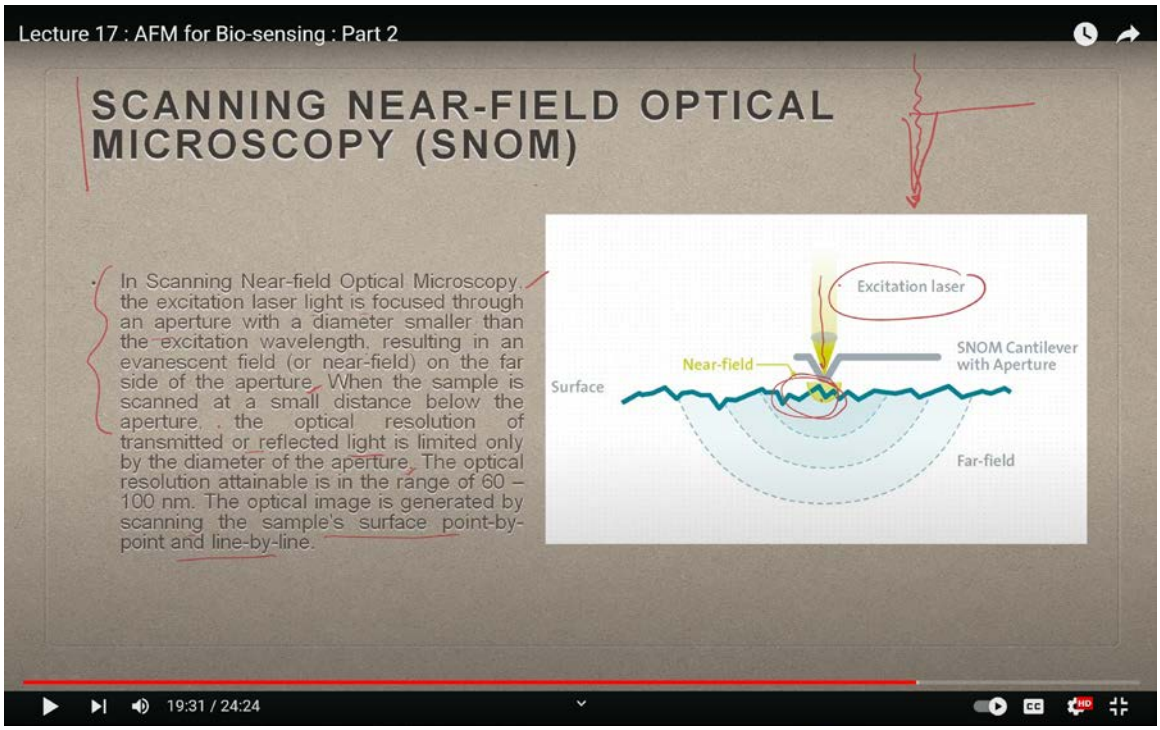

Now, consider the setup: you have the AFM tip, and alongside it, an interferometer is in place to capture both the incident and scattered IR light. Through interferometric detection, both the amplitude and phase images of a sample at a given wavelength can be obtained. As the AFM tip scans across the sample, elastically scattered light is detected, allowing the optical response of the sample to be imaged. Remarkably, this setup enables sub-10 nanometer resolution optical chemical imaging. This is achieved through nano-FTIR

spectroscopy, which simultaneously detects the chemical composition of the compound under investigation.

This entire system, where AFM is coupled with this optical detection method, allows for incredibly precise chemical and spatial analysis. Now, let’s consider an alternative perspective. Previously, we discussed the scattering process (indicated by the "S" in SNOM for Scattering Scanning Near-Field Optical Microscopy). But what about SNOM without the scattering? Let’s explore that.

In Scanning Near-Field Optical Microscopy (SNOM), there is another interesting configuration involving the cantilever. Imagine a scenario where you have a cantilever tip, and instead of the laser being reflected, it passes through the tip. This creates a near-field interaction. The excitation laser, in this case, is focused through an aperture with a diameter smaller than the wavelength of the laser. This setup results in the generation of an evanescent or near-field on the opposite side of the aperture.

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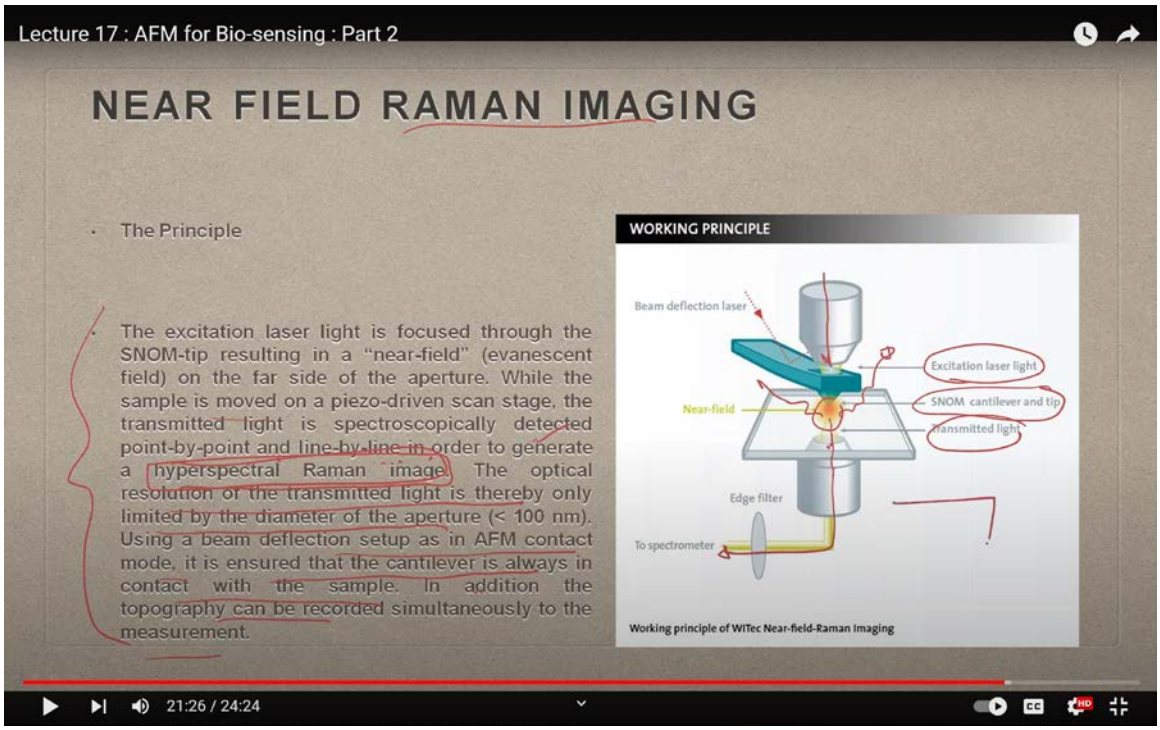

To clarify, in SNOM, the excitation laser is focused through an aperture smaller than the excitation wavelength, producing a near-field on the far side of the aperture. As the sample is scanned just beneath this aperture, the optical resolution of the transmitted or reflected light is determined solely by the size of the aperture. The optical resolution achievable ranges from 60 to 100 nanometers, and the resulting optical image is produced by scanning the sample point by point and line by line. This advanced form of SNOM, which relies on transmitted light rather than scattered light, represents a different but equally powerful technique.

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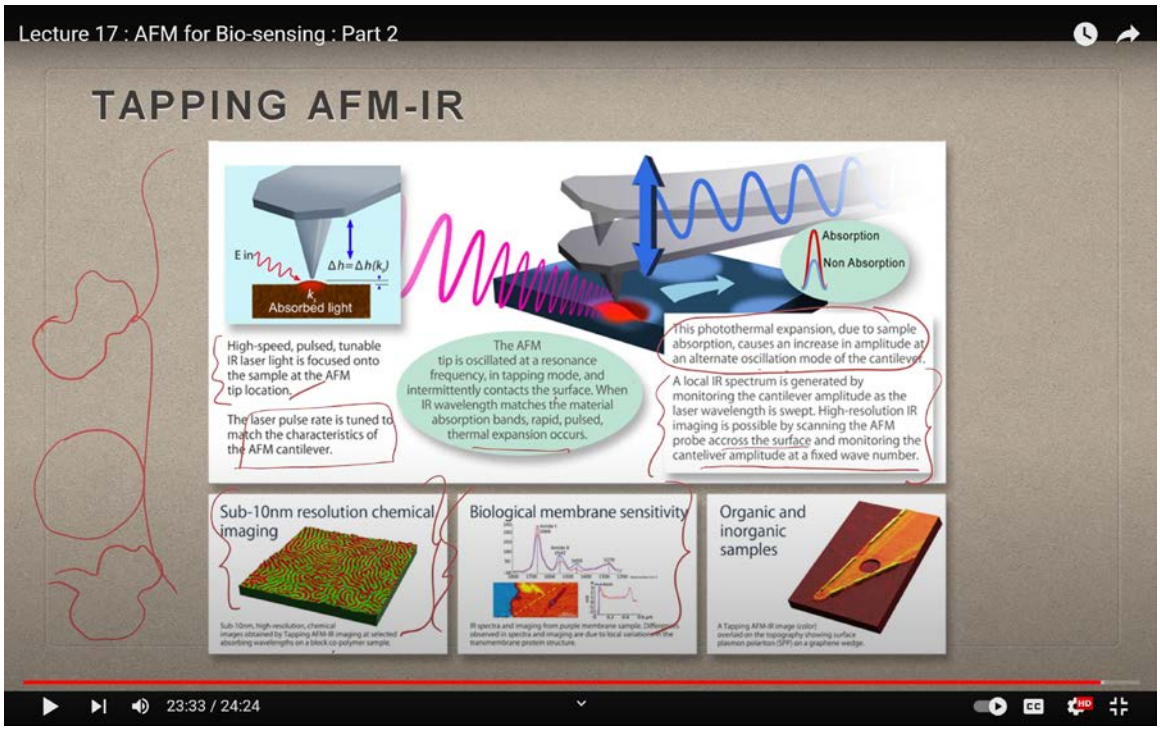

In fact, this approach also opens the door to near-field Raman imaging, which we will discuss further in future lectures. For now, let’s focus on the mechanics: your beam creates the near-field, and as light transmits through the sample, it reaches a spectrophotometer. The SNOM cantilever tip aids in detecting this transmitted light. So, unlike the previously discussed scattered light, here we are examining light that passes directly through the sample. This distinction in technique offers different insights into the material being studied.

The excitation laser light is directed through the SNOM tip, creating both a near field and an evanescent field on the far side of the aperture. As the sample is moved on a piezo-driven scan stage, the transmitted light is detected spectroscopically, point by point and line by line, to produce a hyperspectral Raman image. The optical resolution of this transmitted light is constrained only by the diameter of the aperture, which can be less than 100 nanometers, similar to a beam deflection setup in AFM contact mode. This ensures that the cantilever remains in constant contact with the sample, allowing simultaneous recording of topographical data along with the spectroscopic measurements. This fundamental principle of Raman imaging involves detecting vibrational modes to achieve atomic resolution. We will delve deeper into Raman spectroscopy in subsequent discussions.

The use of a cantilever tip in conjunction with Raman spectroscopy is a powerful technique that allows for combined imaging and spectroscopic analysis within a single setup. An even more advanced technique is tapping AFM IR, which involves tapping the surface while collecting infrared data. This method provides additional, crucial information, particularly when examining surfaces at the microscopic level, such as viral surfaces. Unlike simplified illustrations of cells, which often depict them as uniform shapes, real cells have complex, varied structures that this advanced technique helps to reveal.

Cells exhibit a range of curves and bends, characterized by their softness and flexibility, allowing them to change shape. In this advanced imaging technique, a high-speed, pulse-tunable IR laser light is directed onto the sample at the location of the AFM tip. The laser pulse rate is adjusted to synchronize with the characteristic frequency of the AFM cantilever. In tapping mode, the AFM tip oscillates at its resonance frequency and makes intermittent contact with the surface.

When the IR wavelength aligns with the material's absorption bands, rapid thermal expansion occurs. This photothermal effect leads to an increase in the amplitude of the cantilever's oscillation at an alternate mode. By monitoring the cantilever amplitude as the laser wavelength is varied, a local IR spectrum of the sample is generated. High-resolution IR images are obtained by scanning the AFM probe across the surface while tracking the

cantilever amplitude at a fixed wave number. This technique allows for sub-nanometer resolution in chemical imaging, with applications across biological membranes, organic, and inorganic samples.

This technique's capabilities highlight its power, and in the next class, we will explore how these methods can be advanced even further. Thank you.