Design for Biosecurity

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Lecture 18

AFM and Recap Raman and IR Spectroscopy

Welcome back to Lecture 18, where we will delve deeper into Atomic Force Microscopy (AFM) and revisit key concepts of Raman and Infrared (IR) Spectroscopy. As we explore these topics, we'll revisit the bioelectrochemical and optical sensors, along with the fascinating interactions between light and matter. I believe it's essential to provide a comprehensive recap so you can truly appreciate the remarkable capabilities of these advanced spectroscopic tools. Over the past few lectures, I have emphasized the trend towards multimodal instrumentation, platforms that integrate optical, electrochemical, spectroscopic, electronic, and mechanical features of molecules into a single, cohesive system.

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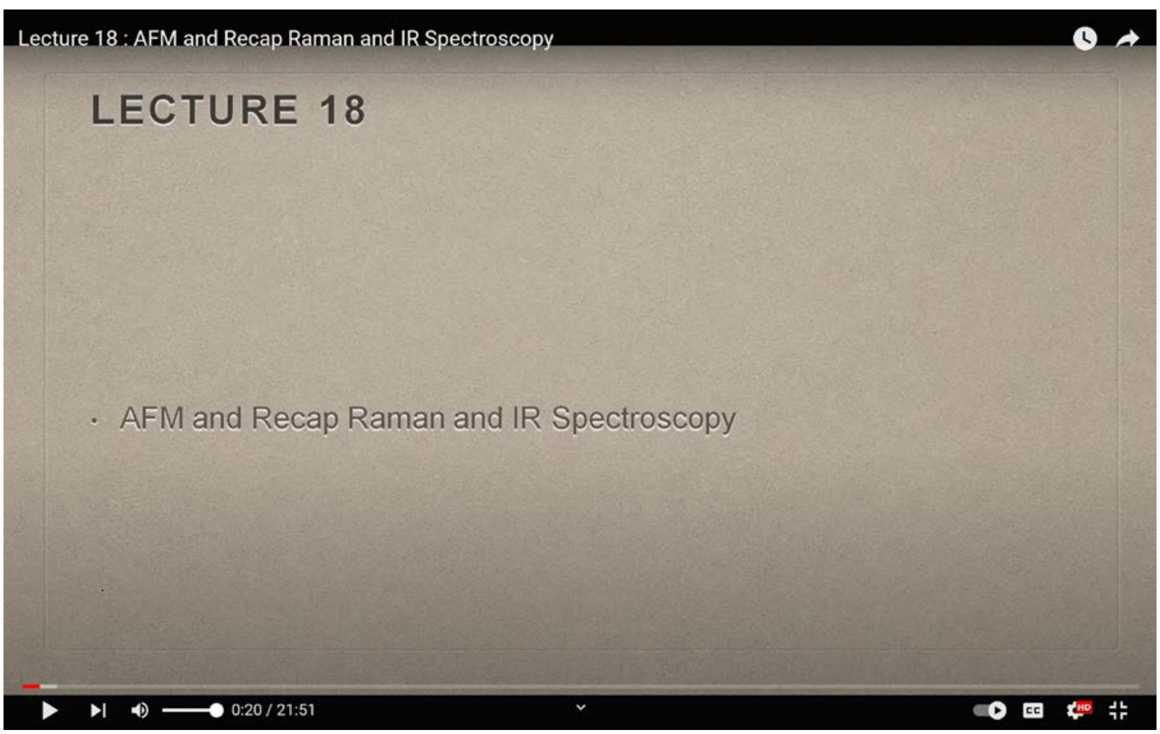

In our previous session, we concluded with a discussion on tapping mode AFM and its application in IR spectroscopy. We explored how photothermal expansion, triggered by sample absorption, leads to an increase in amplitude. This phenomenon is particularly useful in understanding the sensitivity of biological membranes and conducting chemical imaging. Today, we will transition to a discussion on contact resonance AFM.

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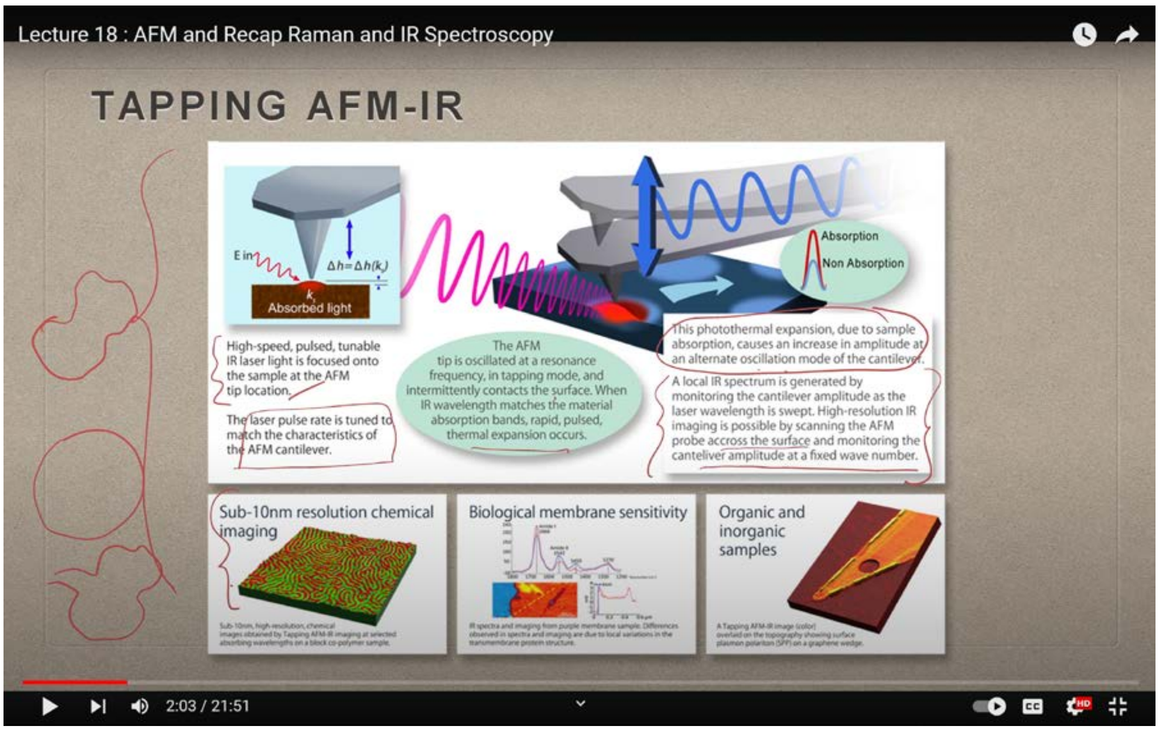

Imagine a scenario where you have a surface, and a beam is directed at it, reflecting off as expected. As soon as the cantilever tip of the AFM moves, there is a noticeable shift in frequency, a key characteristic of contact resonance AFM (CR-AFM), which is a dynamic contact technique.

Recall the different AFM modes we've previously discussed: contact, non-contact, and intermittent contact modes. It's crucial to distinguish between them. In this context, we focus on the contact mode, where the vibrational behavior of the cantilever is monitored while the tip remains in continuous contact with the sample. A notable observation is that an increase in the sample's stiffness typically results in a higher contact resonance frequency. To illustrate, consider the difference between jumping on a soft surface like sand versus a hard surface like concrete. On sand, the impact is absorbed, causing little harm, but on concrete, the reverse force is significantly stronger, leading to potential injury. Similarly, in AFM, a stiffer sample causes a higher contact resonance frequency, much like how a harder surface produces a stronger reaction to force.

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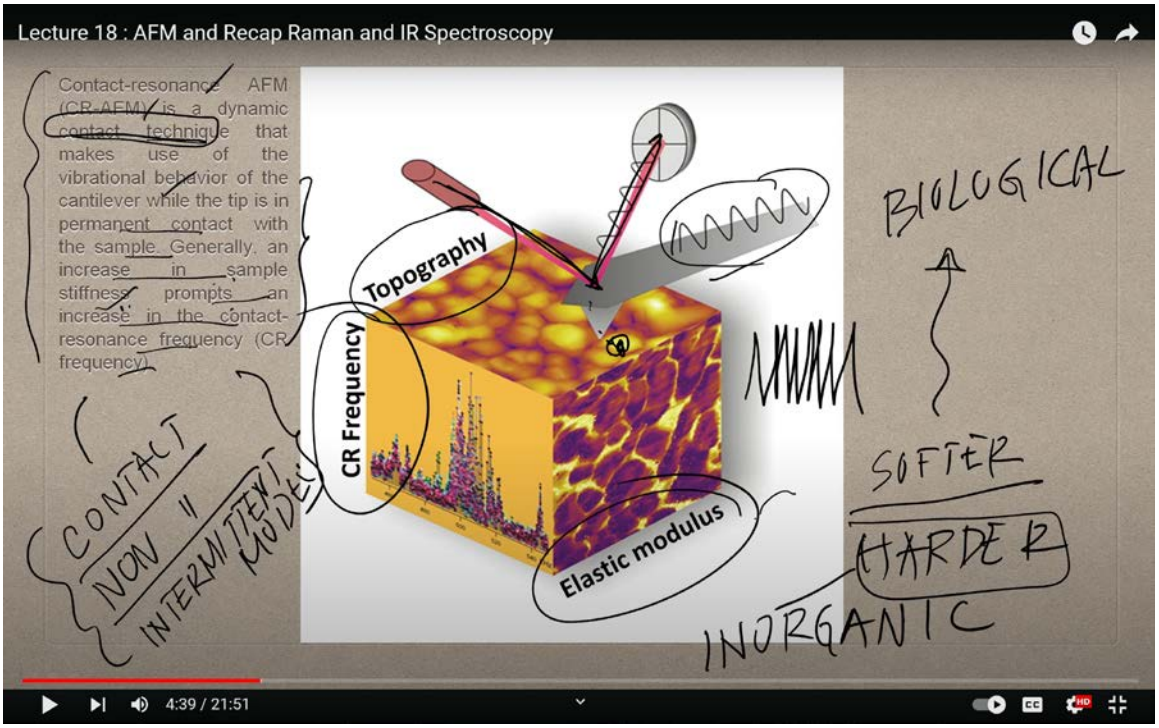

Understanding the distinction between soft and hard surfaces is crucial, especially when we consider biological materials, which typically represent softer surfaces, and inorganic systems, which tend to be harder. Depending on the type of sample you're analyzing, you need to make an informed judgment. This judgment allows you to develop the topography, as well as determine the contact resonance (C-R) frequency and elastic modulus of the specific sample.

When using Contact Resonance Atomic Force Microscopy (CR-AFM), we probe the local elasticity of a surface. This technique involves observing the shift in one of the eigenmode resonance frequencies of the AFM probe when it comes into contact with the surface. The resonance frequencies of the AFM cantilever adjust based on the elastic stiffness at the point of contact between the probe and the sample. If we examine this process, you can see the frequency changes directly. The schematic of the CR-AFM measurement setup, with mechanical modulation from the base, reveals the CR-AFM frequency spectrum. This spectrum highlights the shift in resonance frequency in the first two eigenmodes as the probe transitions from out of contact to contact with a silicon surface (Si). These two eigenmodes are observed clearly in the air as the cantilever hits the surface repeatedly.

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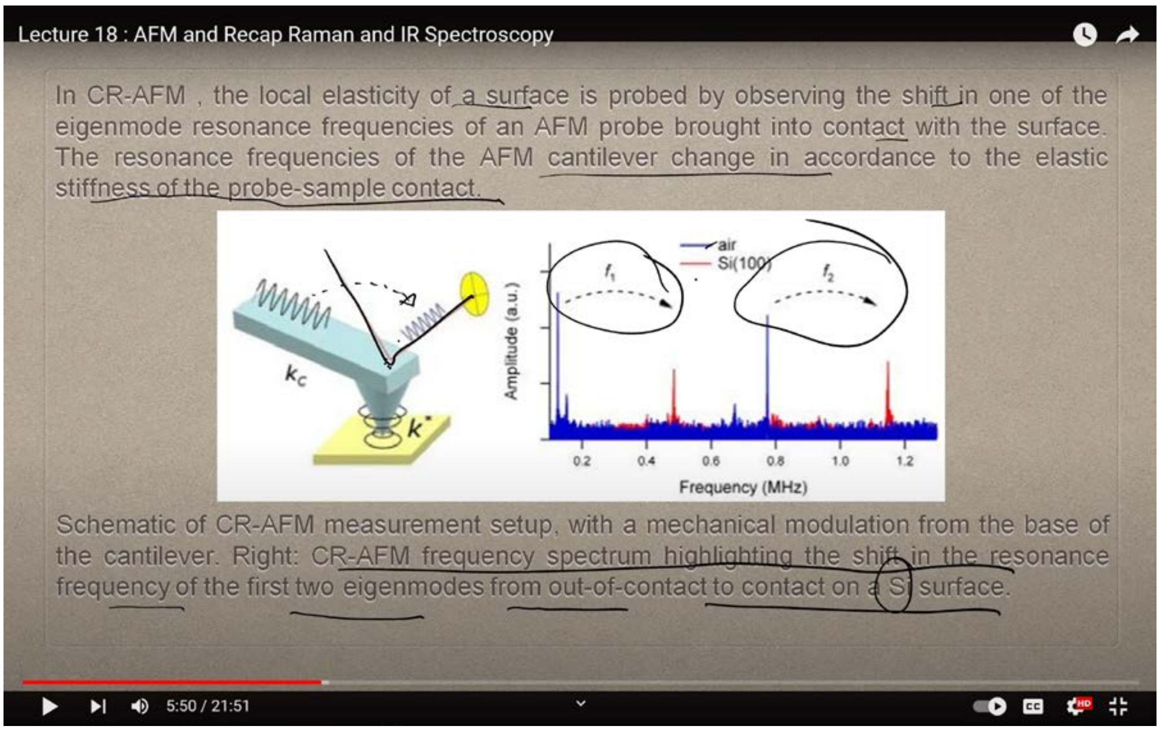

When discussing CR-AFM based on cantilever dynamics, the measured contact resonance frequencies are converted into contact stiffness. This contact stiffness is then used to determine the elastic modulus of the sample, applying appropriate contact mechanics models. However, this process does present challenges, particularly when dealing with unknown geometrical parameters, tip shape, contact radius, varying surface mechanics across different materials, adhesion, and viscoelasticity. Additionally, there is often a need for an analytical contact mechanics model to account for various sample geometries. The analysis of contact mechanics can be performed using either an analytical model or numerical calculation in cases where an analytical solution is unavailable.

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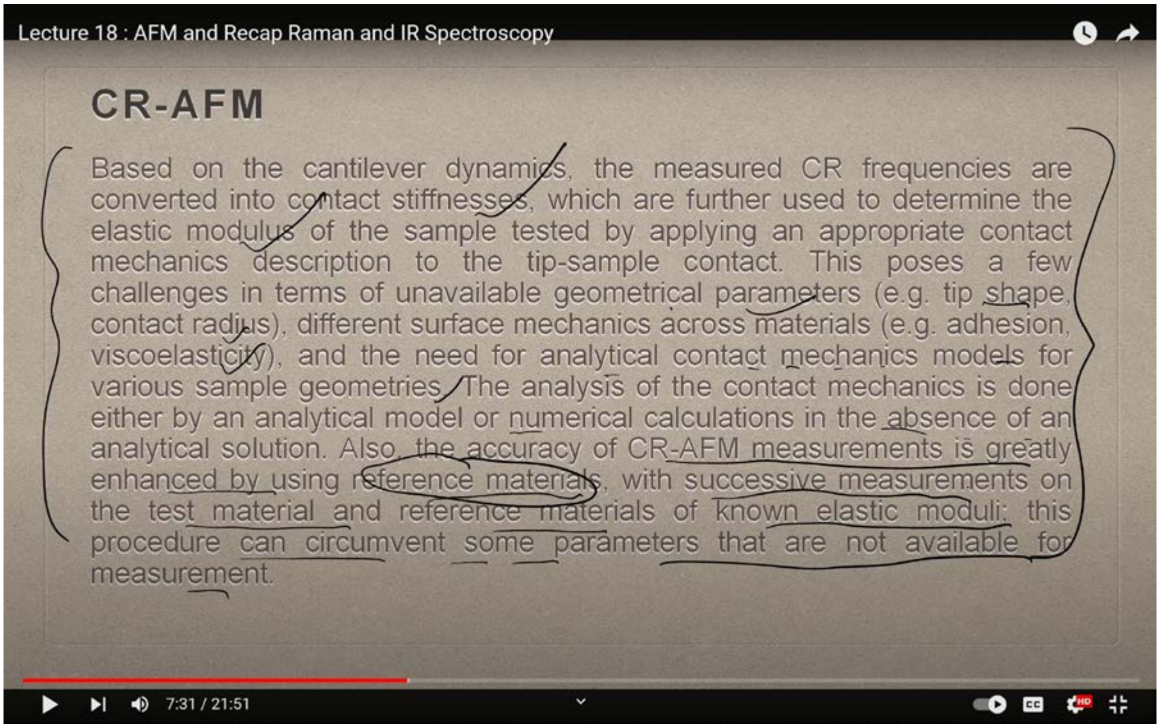

The accuracy of CR-AFM measurements can be significantly enhanced by using reference materials and conducting successive measurements of both the test material and a reference material with known elastic moduli. This approach helps overcome some of the challenges posed by unavailable measurement parameters. A practical method is to use a well-characterized system as a reference frame. Once you have established a known system as your reference, you can conduct successive measurements and, in doing so, detect and characterize new materials in subsequent experiments.

From here, we will transition to the second part of today's lecture, which is a recap of Raman and IR spectroscopy. These two techniques are frequently coupled with AFM systems for multimodal imaging. Let's revisit the basics of Raman and IR spectroscopy. Before diving into these techniques, it's essential to grasp a few fundamental concepts regarding the interaction of light with matter. Consider a piece of matter composed of atoms.

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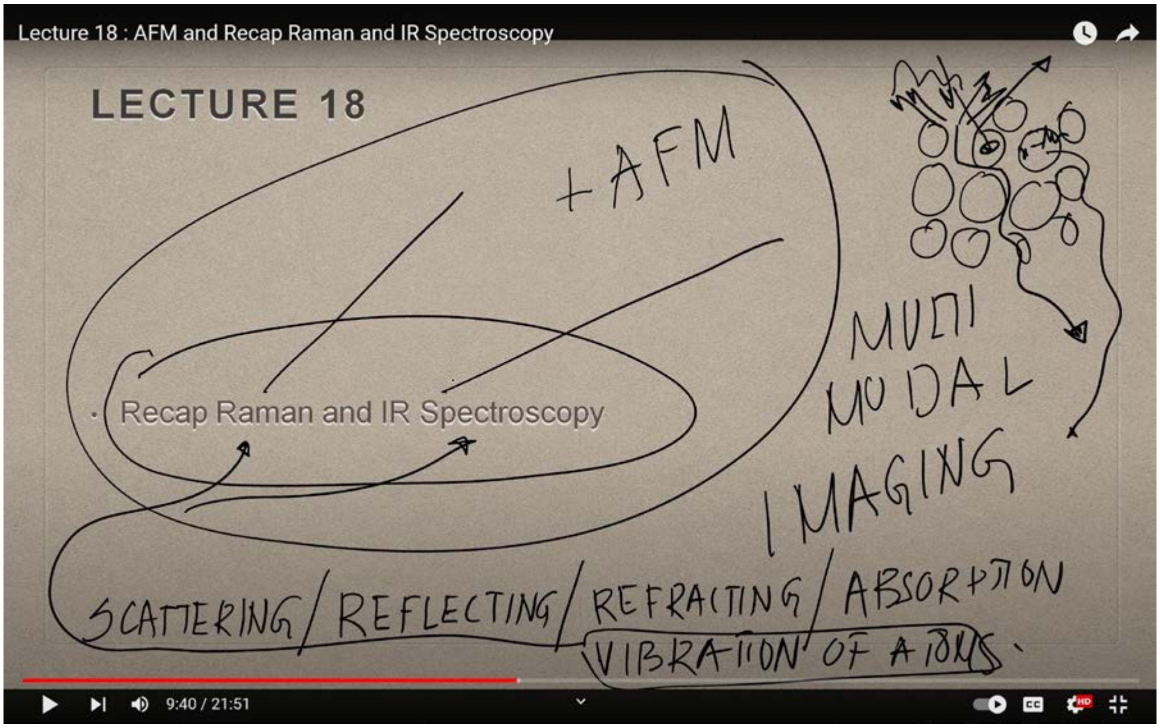

When light strikes this matter, various phenomena occur: some light bends and passes through, some is reflected, some is scattered, and some is absorbed. We observe scattering, reflection, refraction, absorption, or even the induction of vibrations within the atoms. These vibrations are the foundation of the spectroscopic techniques known as Infrared (IR) and Raman spectroscopy. Both fall under the category of vibrational spectroscopy, focusing on capturing the unique vibrational modes of atoms within a material and assigning these vibrations a specific chemical nature.

This interaction between light and matter gives rise to one of the most fascinating fields in analytical chemistry: spectroscopy. With this foundation in mind, let's delve into Raman and Infrared (IR) spectroscopy.

Raman spectroscopy, a technique we proudly associate with the pioneering work of C.V. Raman in 1928, is widely used for analyzing molecular structures. It complements infrared spectroscopy, or IR spectroscopy, by offering unique insights into molecular vibrations. Raman spectroscopy is fundamentally based on the Raman effect, first discovered by C.V. Raman, and it hinges on the scattering of light. This scattering includes both elastic (Rayleigh scattering) and inelastic scattering, the latter being the core of Raman spectroscopy.

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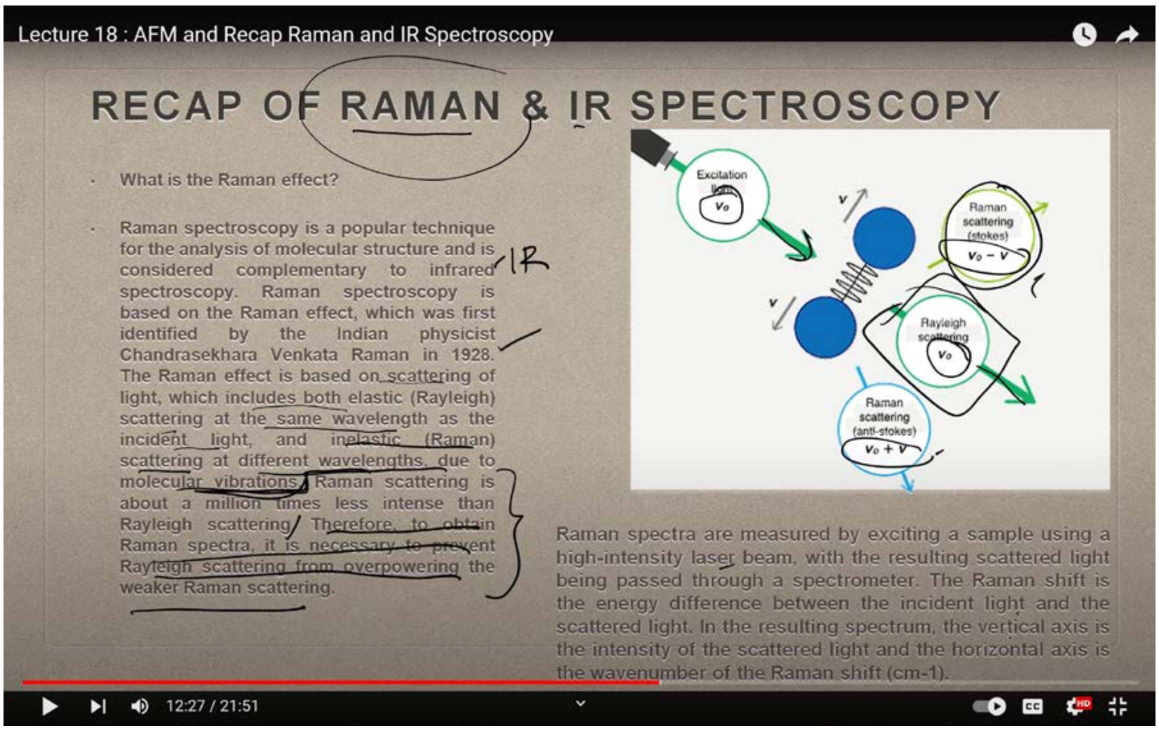

When a light beam interacts with a sample, several things can occur. The light may scatter at the same wavelength as the incident light, known as Rayleigh scattering, denoted as V_0. However, it can also scatter inelastically, resulting in a shift in wavelength due to molecular vibrations, this is known as Raman scattering. Unlike Rayleigh scattering, Raman scattering occurs at wavelengths different from the incident light, giving rise to two distinct types of shifts: Stokes and anti-Stokes.

Raman scattering is inherently much weaker, about a million times less intense, than Rayleigh scattering. This poses a significant challenge because the more pronounced Rayleigh scattering can easily overshadow the weaker Raman signals. To accurately obtain

Raman spectra, it's crucial to suppress the Rayleigh scattering. Raman spectra are typically measured by exciting a sample with a high-intensity laser beam, which causes the light to scatter. The scattered light is then passed through a spectrophotometer.

The Raman shift refers to the energy difference between the incident and scattered light. In the resulting spectrum, the vertical axis represents the intensity of the scattered light, while the horizontal axis indicates the wave number of the Raman shift, measured in cm^{-1}. A classic example is the Raman spectrum of sulfur. Here, you'll observe the Rayleigh scattering as the most prominent feature. Beyond this, the spectrum reveals the Stokes and anti-Stokes Raman shifts.

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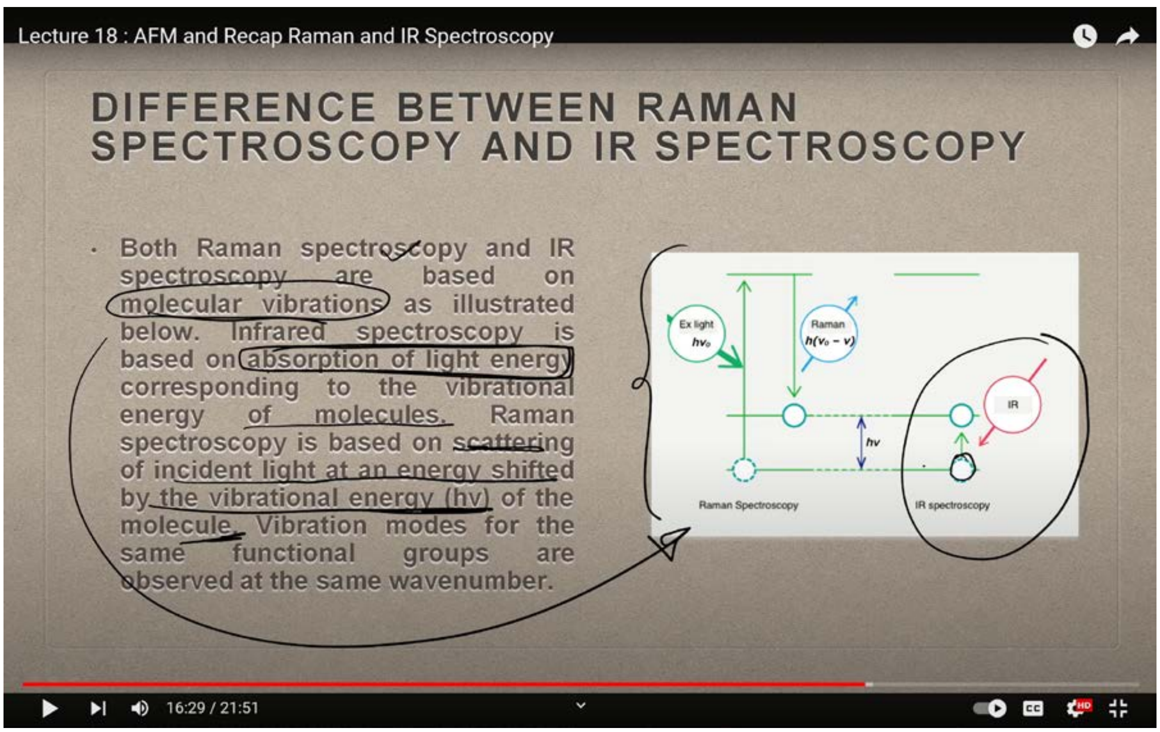

The Raman shift is linked to two different energy transitions. When the scattered light has a wavelength longer than the incident light, it’s termed Stokes scattering. Conversely, when the scattered light has a shorter wavelength, it's known as anti-Stokes scattering. Mathematically, this can be expressed as $V_0 + V$ for Stokes scattering and $V_0 - V$ for anti-Stokes scattering, where V_0 is the frequency of the incident light and V is the vibrational energy of the molecule. This results in a Raman spectrum where the shifts are represented as positive and negative values on either side of the spectrum.

For instance, the Raman spectrum of sulfur, measured with an excitation wavelength of 532 nanometers using a green laser, shows Stokes scattering in the lower wave number region (longer wavelength) and anti-Stokes scattering in the higher wave number region (shorter wavelength). Typically, high-intensity Stokes peaks are favored for analysis, although anti-Stokes peaks are also valuable.

When we report Raman spectra, we typically focus on the higher intensity peaks, but it's important to remember that the lower intensity peaks also play a significant role and are sometimes utilized in analysis. This is a key aspect of understanding how the Raman shift occurs, specifically, the shifts corresponding to $V_0 + V$ and $V_0 - V$, which represent the Stokes and anti-Stokes scattering, respectively.

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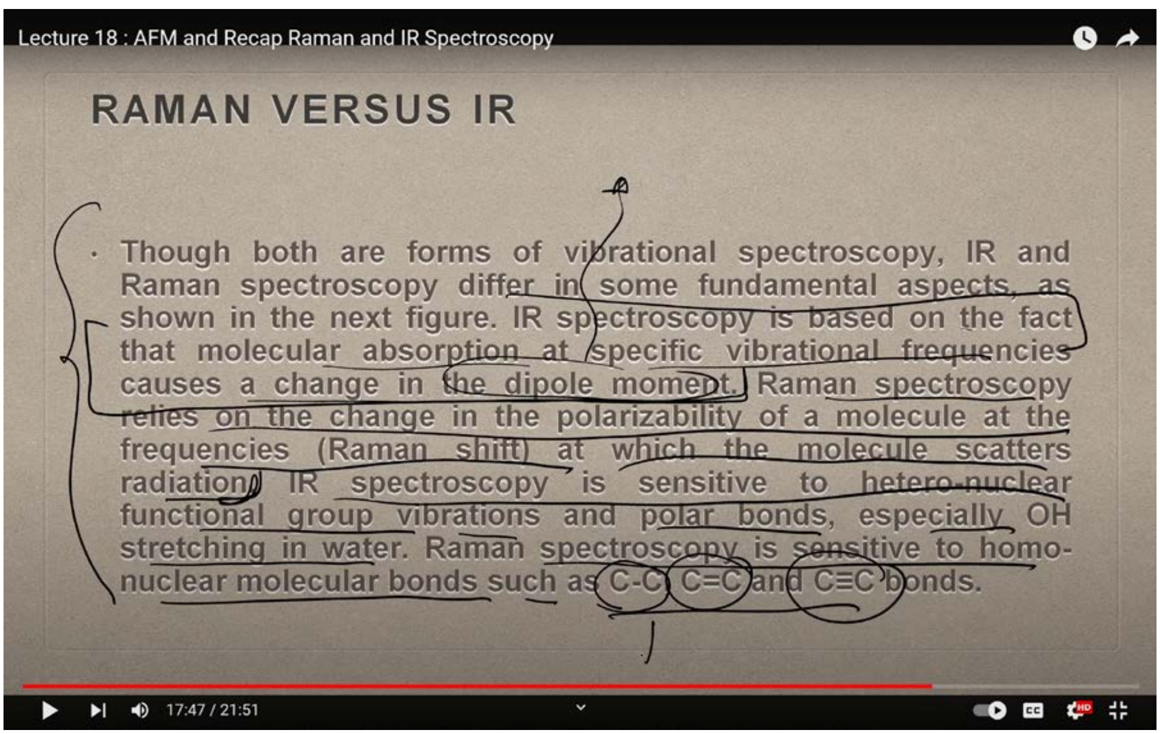

Now, let’s delve into the intriguing differences between Raman spectroscopy and IR spectroscopy. I vividly recall a time when I used to think they were nearly identical, both focusing on molecular vibrations. However, this is not the case, there are crucial distinctions between them that we need to grasp clearly.

Both Raman and IR spectroscopy are indeed based on molecular vibrations, but the mechanisms behind them differ significantly. Infrared (IR) spectroscopy is grounded in the absorption of light energy, which corresponds to the vibrational energy of the molecule. Essentially, when a molecule vibrates, it absorbs light, leading to IR absorption.

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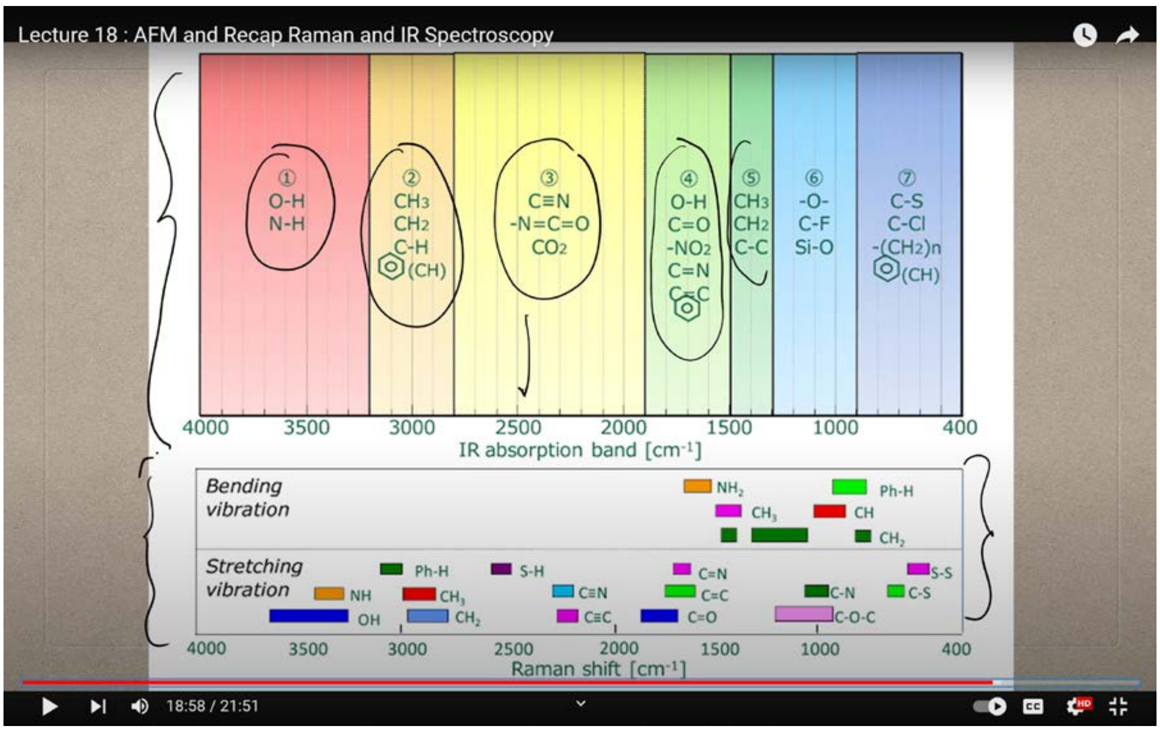

On the other hand, Raman spectroscopy is based on the scattering of incident light, with the energy of the scattered light being shifted by the vibrational energy $h\nu$ of the molecule. To put it simply, in IR spectroscopy, when light hits a molecule, it gets absorbed and causes the molecule to vibrate. In contrast, in Raman spectroscopy, the light is scattered after interacting with the molecule, and this scattering is influenced by the molecular vibrations.

To illustrate, imagine an atom as a ball, if light strikes it in the context of IR spectroscopy, the light is absorbed, and the ball (molecule) vibrates. In Raman spectroscopy, the light is scattered, and the ball still vibrates. These fundamental differences are crucial for understanding the distinct nature of these two spectroscopic techniques.

Moving further into the comparison, while both IR and Raman spectroscopy are forms of vibrational spectroscopy, they differ in several fundamental ways. IR spectroscopy is based on the fact that when molecules absorb light at specific vibrational frequencies, it results in a change in dipole moment. This change in dipole moment is key for detection in IR spectroscopy.

Raman spectroscopy, however, relies on changes in the polarizability of the molecule at the frequency corresponding to the Raman shift, which is when the molecule scatters radiation. IR spectroscopy is particularly sensitive to heteronuclear functional groups, such as those involving polar bonds, especially hydroxyl groups and the stretching vibrations in water.

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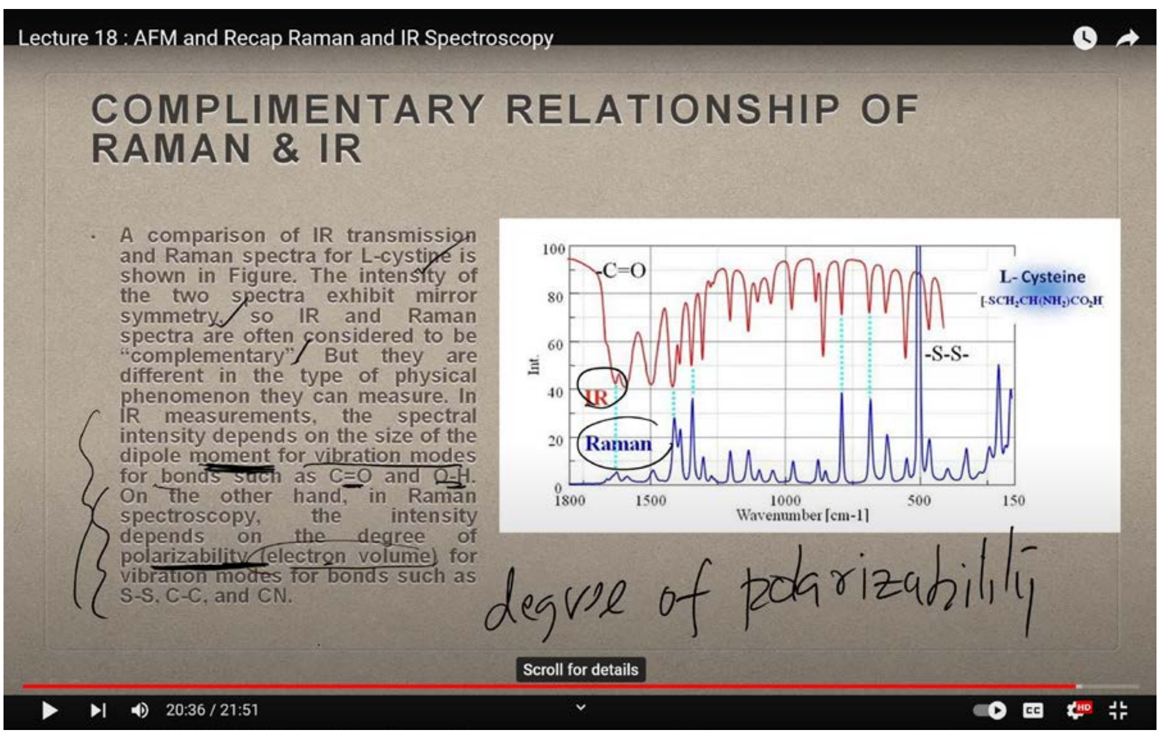

Conversely, Raman spectroscopy is more sensitive to homonuclear molecular bonds, such as carbon-carbon single, double, and triple bonds. For molecular structures with homonuclear bonds, Raman spectroscopy is preferable, whereas for heteronuclear functional groups, IR spectroscopy is generally more suitable.

So, these distinctions between IR and Raman spectroscopy, from their underlying principles to their sensitivity to different molecular bonds, are essential for selecting the appropriate technique based on the specific molecular features you wish to analyze.

You can observe the IR absorption across different regions, highlighting various heteronuclear functional groups such as hydroxyl, nitrogen-hydrogen, CH_3, CH_2, CH, as well as phenolic and carbon-nitrogen bonds. Each functional group has its specific absorption zone, which can be detected using IR spectroscopy. In Raman spectroscopy, the orientation of the functional groups is slightly different, which is crucial when analyzing data from either technique.

To accurately interpret the results from IR or Raman spectroscopy, one must refer to established charts that have been developed over decades of research, trial and error, and precise identification. These charts help explain the molecular reasons behind the specific absorption patterns or polarizability observed in different zones. For example, in IR spectroscopy, certain zones show a characteristic dipole moment, while in Raman spectroscopy, the focus is on polarizability in those specific regions.

What’s particularly interesting is the complementary relationship between Raman and IR spectroscopy. Although the IR spectra (shown in red) and Raman spectra (shown in blue) of a molecule like the amino acid L-cysteine may appear similar at first glance, they are fundamentally different. These differences are critical because IR and Raman spectra, while complementary, measure different physical phenomena.

In IR spectroscopy, the spectral intensity is influenced by the size of the dipole moment, which is significant for vibrational modes in bonds such as carbon-oxygen and oxygen-hydrogen. Conversely, in Raman spectroscopy, the intensity is determined by the degree of polarizability, essentially, the electron volume during vibrational modes in bonds like sulfur-sulfur, carbon-carbon, and carbon-nitrogen. These bonds produce distinctive signatures in Raman spectra.

Understanding these molecular signatures is crucial, especially in the field of bio-detection, where subtle cues from these spectra can provide valuable insights. This knowledge forms the foundation for detecting and analyzing various substances at the molecular level.

We are closeing here, and in the next session, we'll delve deeper into the nuances of Raman spectral analysis. Thank you.