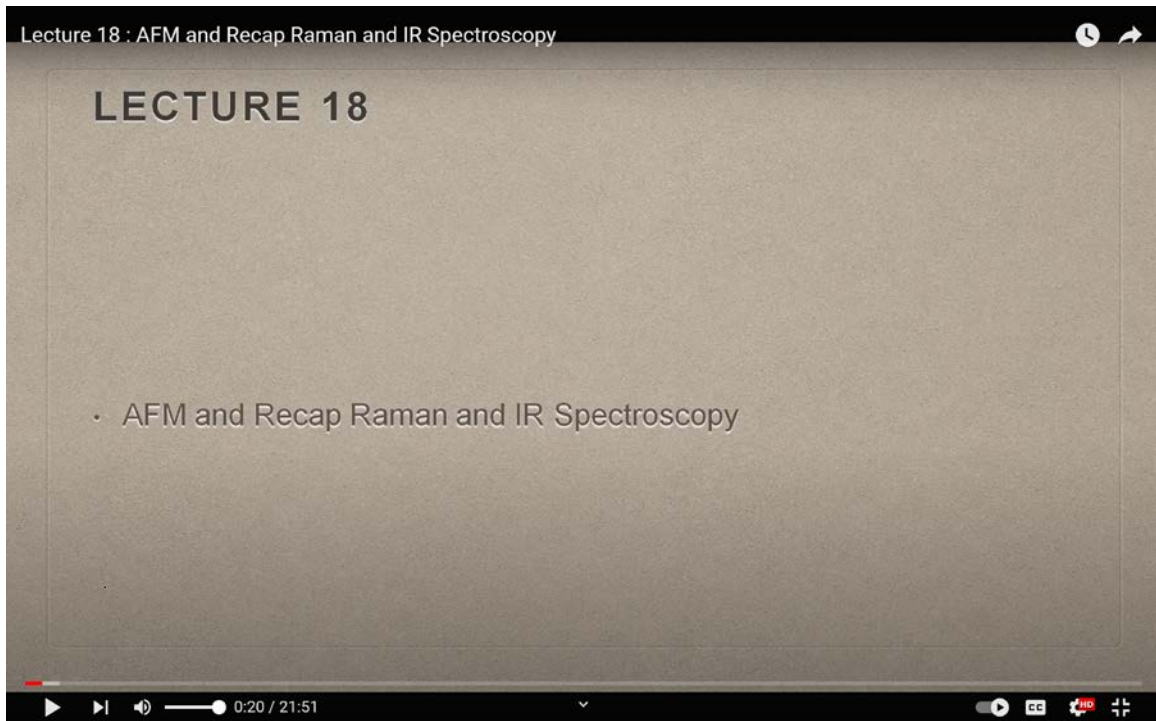


**Design for Biosecurity**  
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**Indian Institute of Technology, Kanpur**  
**Lecture 18**  
**AFM and Recap Raman and IR Spectroscopy**

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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

## TAPPING AFM-IR

The diagram illustrates the Tapping AFM-IR mechanism. It shows an AFM tip oscillating at a resonance frequency and intermittently contacting the surface. When the IR wavelength matches the material absorption bands, rapid, pulsed, thermal expansion occurs. This photothermal expansion, due to sample absorption, causes an increase in amplitude at an alternate oscillation mode of the cantilever. A local IR spectrum is generated by monitoring the cantilever amplitude as the laser wavelength is swept. High-resolution IR imaging is possible by scanning the AFM probe across the surface and monitoring the cantilever amplitude at a fixed wave number.

High-speed, pulsed, tunable IR laser light is focused onto the sample at the AFM tip location. The laser pulse rate is tuned to match the characteristics of the AFM cantilever.

The AFM tip is oscillated at a resonance frequency, in tapping mode, and intermittently contacts the surface. When IR wavelength matches the material absorption bands, rapid, pulsed, thermal expansion occurs.

This photothermal expansion, due to sample absorption, causes an increase in amplitude at an alternate oscillation mode of the cantilever. A local IR spectrum is generated by monitoring the cantilever amplitude as the laser wavelength is swept. High-resolution IR imaging is possible by scanning the AFM probe across the surface and monitoring the cantilever amplitude at a fixed wave number.

**Sub-10nm resolution chemical imaging**

Sub-10nm, high-resolution, chemical images obtained by Tapping AFM-IR imaging at several absorbing wavelengths on a block copolymer sample.

**Biological membrane sensitivity**

IR spectra and imaging from purple membrane samples. Differences observed in spectra and imaging are due to local variations in the transmembrane protein structure.

**Organic and inorganic samples**

A Tapping AFM-IR image (color) overlaid on the topography showing surface plasmon polarization (SPP) on a graphene wedge.

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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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The image is a screenshot of a video lecture titled "Lecture 18 : AFM and Recap Raman and IR Spectroscopy". The video player shows a timestamp of 4:39 / 21:51. The main content is a diagram of an AFM probe tip in contact with a sample surface. The diagram is annotated with handwritten text and circles:

- Topography**: A label pointing to the surface profile of the sample.
- CR Frequency**: A label pointing to the contact resonance frequency spectrum shown as a bar chart below the topography.
- Elastic modulus**: A label pointing to the 3D surface map of the sample.
- CONTACT NOV // INTERMITTENT MODE**: A handwritten note on the left side.
- BIOLOGICAL**: A handwritten word at the top right, with an arrow pointing to a wavy line representing a soft surface.
- SOFTER**: A handwritten word below "BIOLOGICAL".
- HARDER**: A handwritten word in a box below "SOFTER".
- INORGANIC**: A handwritten word at the bottom right, with an arrow pointing to a straight line representing a hard surface.

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.

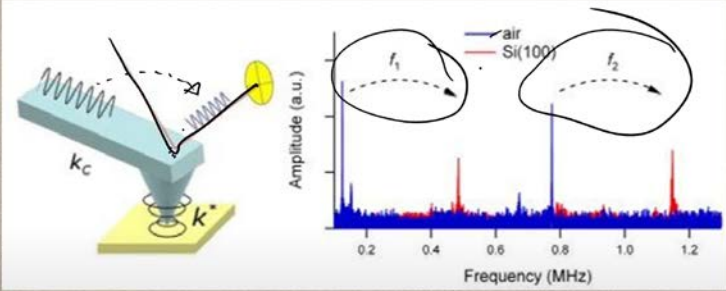
When using Contact Resonance Atomic Force Microscopy (CR-AFM), we probe the local elasticity of a surface. This technique involves observing the shift in one of the eigenmode resonance frequencies of the AFM probe when it comes into contact with the surface. The

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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

In CR-AFM, the local elasticity of a surface is probed by observing the shift in one of the eigenmode resonance frequencies of an AFM probe brought into contact with the surface. The resonance frequencies of the AFM cantilever change in accordance to the elastic stiffness of the probe-sample contact.



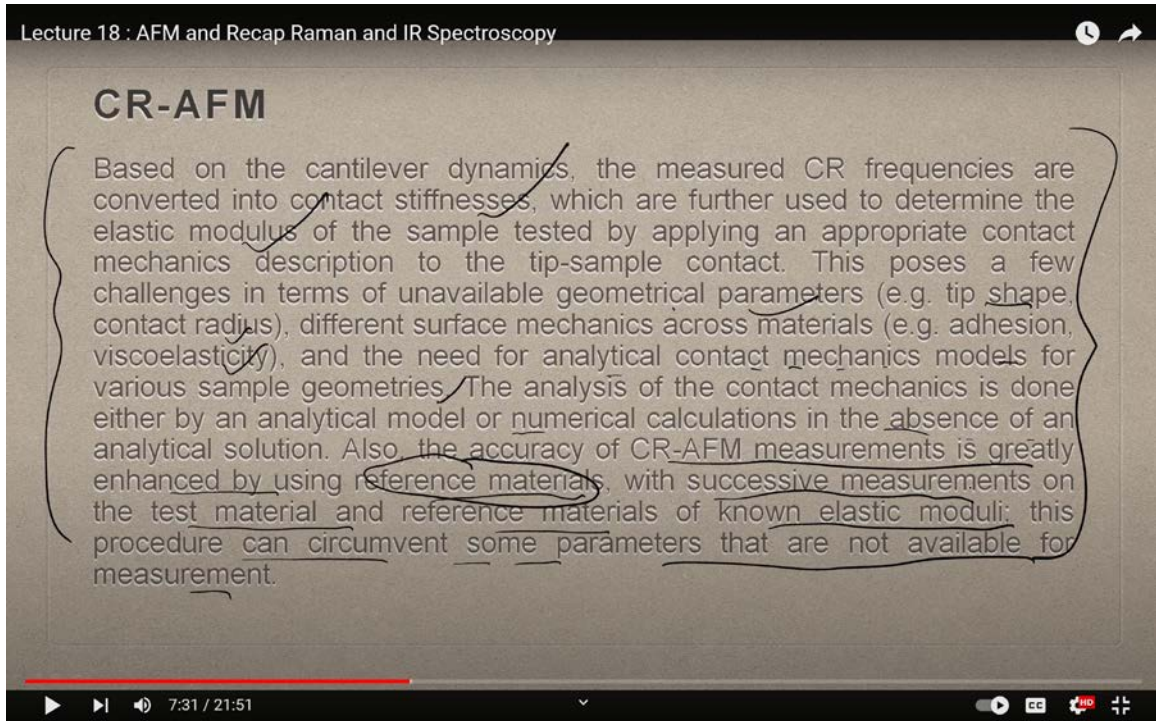
Schematic of CR-AFM measurement setup, with a mechanical modulation from the base of the cantilever. Right: CR-AFM frequency spectrum highlighting the shift in the resonance frequency of the first two eigenmodes from out-of-contact to contact on a Si surface.

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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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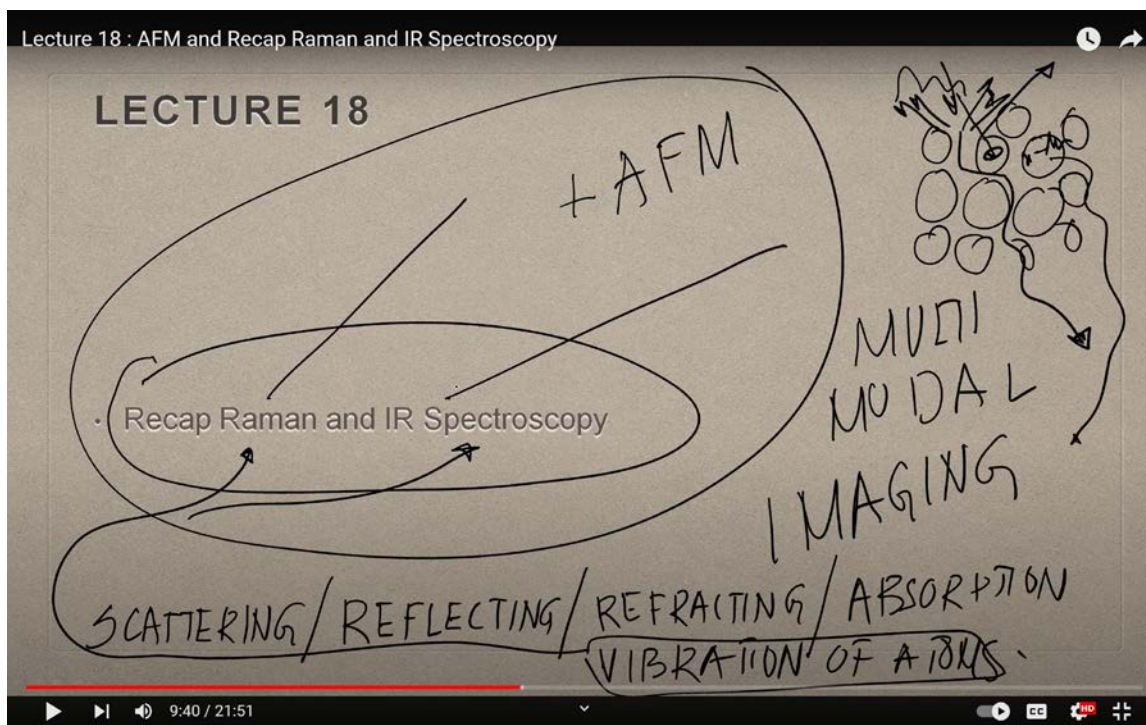
The image shows a video player interface for a lecture. The title bar at the top reads "Lecture 18 : AFM and Recap Raman and IR Spectroscopy". The main content is a slide titled "CR-AFM" with the following text: "Based on the cantilever dynamics, the measured CR frequencies are converted into contact stiffnesses, which are further used to determine the elastic modulus of the sample tested by applying an appropriate contact mechanics description to the tip-sample contact. This poses a few challenges in terms of unavailable geometrical parameters (e.g. tip shape, contact radius), different surface mechanics across materials (e.g. adhesion, viscoelasticity), and the need for analytical contact mechanics models for various sample geometries. The analysis of the contact mechanics is done either by an analytical model or numerical calculations in the absence of an analytical solution. Also, the accuracy of CR-AFM measurements is greatly enhanced by using reference materials, with successive measurements on the test material and reference materials of known elastic moduli; this procedure can circumvent some parameters that are not available for measurement." The video player controls at the bottom show a play button, a progress bar at 7:31 / 21:51, and other standard controls.

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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

## RECAP OF RAMAN & IR SPECTROSCOPY

- What is the Raman effect?
- Raman spectroscopy is a popular technique for the analysis of molecular structure and is considered complementary to infrared spectroscopy. Raman spectroscopy is based on the Raman effect, which was first identified by the Indian physicist Chandrasekhara Venkata Raman in 1928. The Raman effect is based on scattering of light, which includes both elastic (Rayleigh) scattering at the same wavelength as the incident light, and inelastic (Raman) scattering at different wavelengths, due to molecular vibrations. Raman scattering is about a million times less intense than Rayleigh scattering. Therefore, to obtain Raman spectra, it is necessary to prevent Rayleigh scattering from overpowering the weaker Raman scattering.

Raman spectra are measured by exciting a sample using a high-intensity laser beam, with the resulting scattered light being passed through a spectrometer. The Raman shift is the energy difference between the incident light and the scattered light. In the resulting spectrum, the vertical axis is the intensity of the scattered light and the horizontal axis is the wavenumber of the Raman shift ( $\text{cm}^{-1}$ ).

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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  $\text{cm}^{-1}$ . 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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

## DIFFERENCE BETWEEN RAMAN SPECTROSCOPY AND IR SPECTROSCOPY

- Both Raman spectroscopy and IR spectroscopy are based on molecular vibrations as illustrated below. Infrared spectroscopy is based on absorption of light energy corresponding to the vibrational energy of molecules. Raman spectroscopy is based on scattering of incident light at an energy shifted by the vibrational energy ( $h\nu$ ) of the molecule. Vibration modes for the same functional groups are observed at the same wavenumber.

The diagram illustrates the energy levels and transitions for Raman and IR spectroscopy. On the left, Raman Spectroscopy is shown with incident light of energy  $h\nu_0$  and scattered light of energy  $h(\nu_0 - \nu)$ . On the right, IR spectroscopy is shown with absorption of light energy  $h\nu$  corresponding to vibrational energy. The diagram shows that both techniques are based on molecular vibrations, and vibration modes for the same functional groups are observed at the same wavenumber.

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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  $\nu_0 + \nu$  for Stokes scattering and  $\nu_0 - \nu$  for anti-Stokes scattering, where  $\nu_0$  is the frequency of the incident light and  $\nu$  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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

## RAMAN VERSUS IR

- Though both are forms of vibrational spectroscopy, IR and Raman spectroscopy differ in some fundamental aspects, as shown in the next figure. IR spectroscopy is based on the fact that molecular absorption at specific vibrational frequencies causes a change in the dipole moment. Raman spectroscopy relies on the change in the polarizability of a molecule at the frequencies (Raman shift) at which the molecule scatters radiation. IR spectroscopy is sensitive to hetero-nuclear functional group vibrations and polar bonds, especially OH stretching in water. Raman spectroscopy is sensitive to homo-nuclear molecular bonds such as C-C, C=C and C≡C bonds.

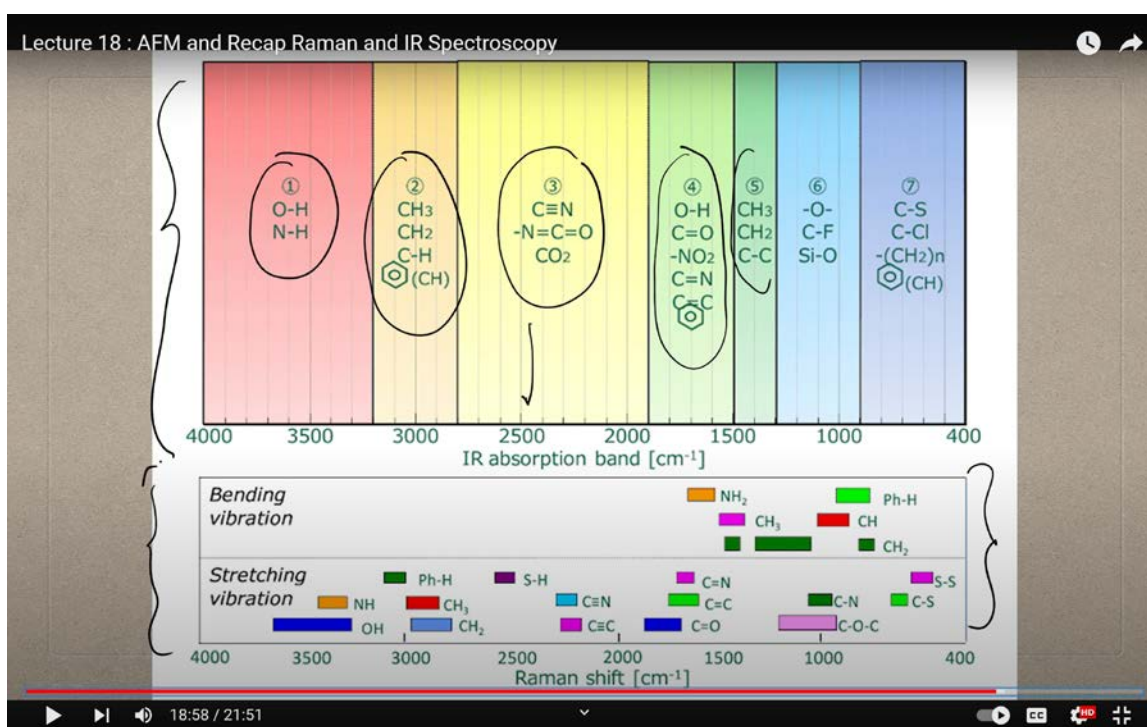
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Now, let's delve into the intriguing differences between Raman spectroscopy and IR spectroscopy. I vividly recall a time when I used to think they were nearly identical, both

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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Lecture 18 : AFM and Recap Raman and IR Spectroscopy

## COMPLIMENTARY RELATIONSHIP OF RAMAN & IR

A comparison of IR transmission and Raman spectra for L-cysteine is shown in Figure. The intensity of the two spectra exhibit mirror symmetry, so IR and Raman spectra are often considered to be "complementary". But they are different in the type of physical phenomenon they can measure. In IR measurements, the spectral intensity depends on the size of the dipole moment for vibration modes for bonds such as C=O and O-H. On the other hand, in Raman spectroscopy, the intensity depends on the degree of polarizability (electron volume) for vibration modes for bonds such as S-S, C-C, and CN.

degree of polarizability

Scroll for details

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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<sub>3</sub>, CH<sub>2</sub>, 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 closing here, and in the next session, we'll delve deeper into the nuances of Raman spectral analysis. Thank you.