Design for Biosecurity

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Lecture 19

Applications of Raman

Welcome back to the 19th lecture in this series. In our previous class, we delved into a comparative analysis of Raman and IR spectroscopy. Let's take a moment to revisit some of the key points we discussed. Both Raman and IR spectroscopy fall under the category of vibrational spectroscopy, serving as signatures of atomic vibrations. However, the fundamental difference lies in their mechanisms. In IR spectroscopy, light is absorbed by the atom, leading to vibrations and a consequent change in the dipole moment. On the other hand, in Raman spectroscopy, the light is scattered, inducing vibrations that result in a change in the molecule's polarizability.

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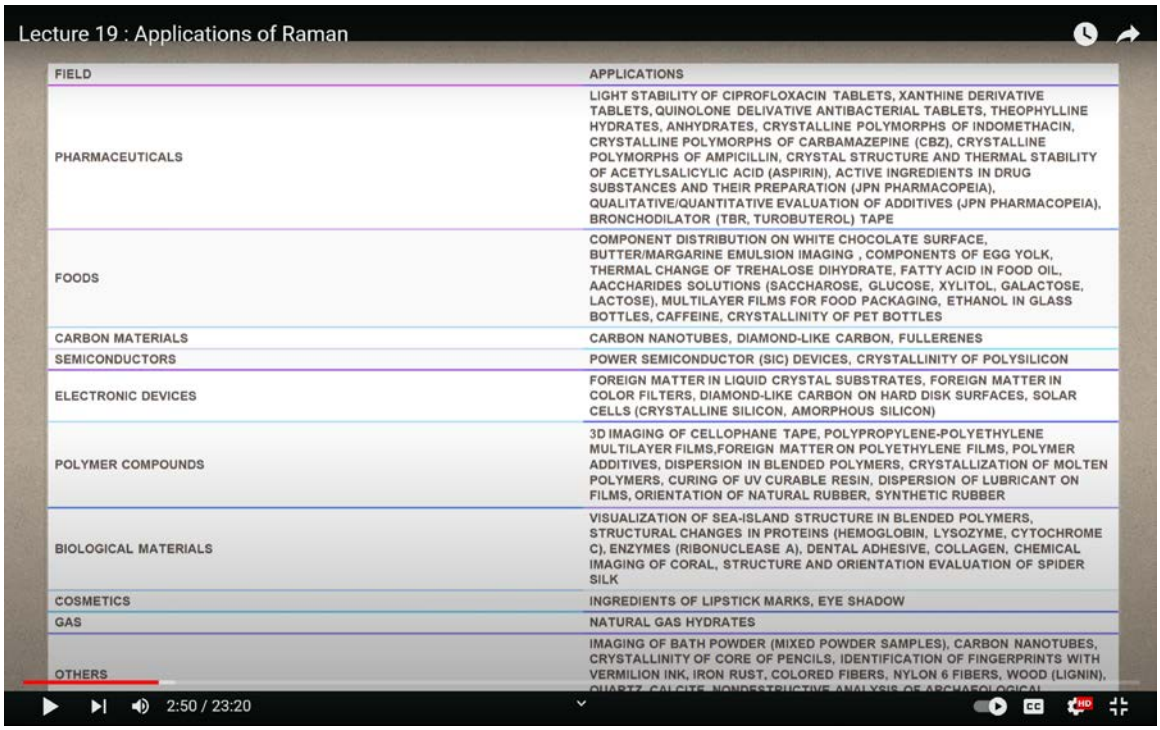

We also noted that IR spectroscopy is particularly effective for analyzing heteronuclear functional groups, while Raman spectroscopy is more suitable for homonuclear groups. These techniques are not just complementary; they are indispensable when used together to gain a comprehensive understanding of a material's properties. To truly grasp the intricacies of any substance, one must be familiar with both tools, their applications, and their limitations.

When these techniques are combined with Atomic Force Microscopy (AFM), the potential for analysis expands even further. You can map the distribution of molecular structures on a surface, observe changes in electrical properties, and study alterations in oxidation-reduction potential, topics we'll explore in our upcoming classes.

Now, in this 19th lecture, we'll shift our focus to the practical applications of Raman spectroscopy, a remarkably powerful tool in modern analytical chemistry. For instance, in the pharmaceutical industry, Raman spectroscopy is employed to assess the light stability of drugs such as ciprofloxacin tablets, xanthine derivatives, quinone derivatives, and antibacterial tablets. It's also used to analyze the properties of theophylline hydrates, anhydrides, crystalline polymers, and many other compounds.

Similarly, in the food industry, Raman spectroscopy finds applications in the analysis of chocolate surfaces, emulsion imaging, components of egg yolk, thermal changes in tree hollows, dehydration processes, fatty acids and food oils, lactose, and multi-layer films used in packaging.

In the realm of carbon materials, Raman spectroscopy is invaluable for identifying various carbon allotropes, assessing the crystallinity of polysilicon in electronic devices, and detecting foreign matter in liquid crystal substrates. It also allows for the analysis of dopants within these materials. For polymers, this technique enables 3D imaging of cellophane tape, polypropylene, and the study of sea island structures in blended polymers.

Moreover, Raman spectroscopy is crucial in studying structural changes in proteins such as hemoglobin, lysozyme, cytochrome, enzyme ribonuclease A, and dental adhesion

collagen. It’s also used for chemical imaging of coral structures and the evaluation of the orientation of spider silk and mulberry silk, providing exceptionally detailed visualizations.

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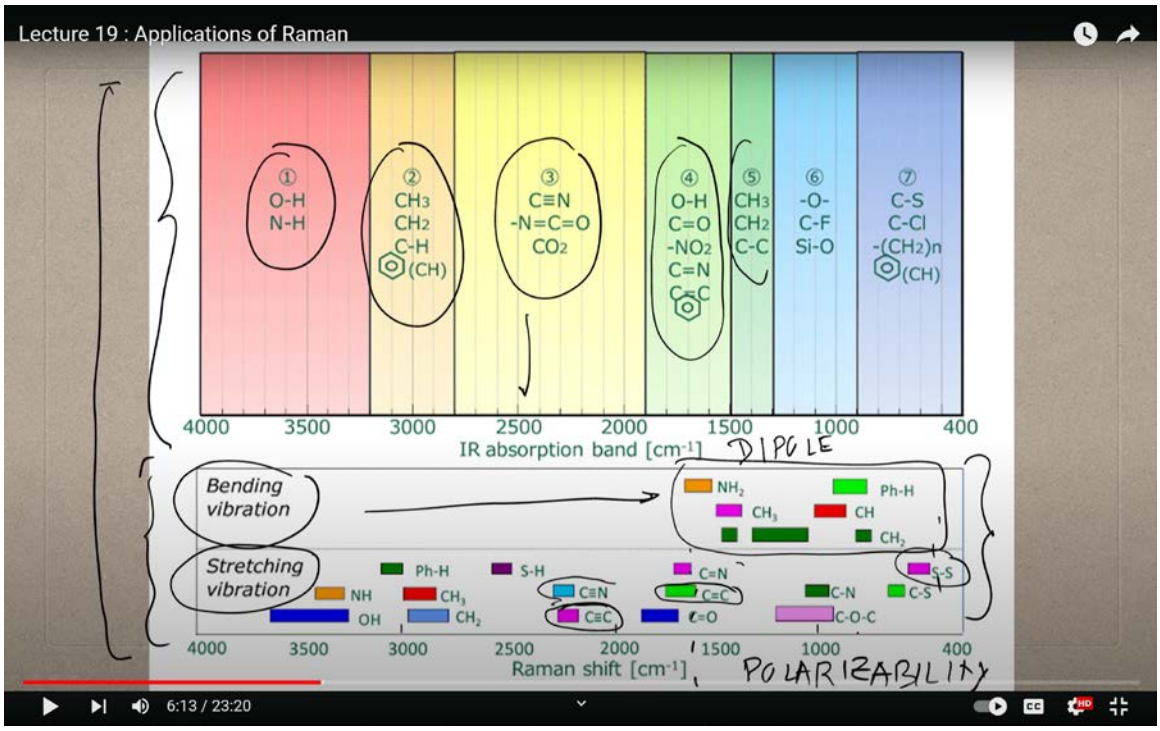

In the cosmetics industry, Raman spectroscopy can detect the components of various makeup products. Additionally, it has applications in analyzing natural gas hydrates and other gases. The scope of Raman spectroscopy extends far beyond what we can cover in this course, but it’s essential to grasp the foundational principles of this technique.

Understanding these basics will equip you to explore the vast array of applications that Raman spectroscopy offers in various fields. In our next session, we’ll continue to delve deeper into the fascinating world of spectroscopy. Thank you for your attention.

This is something I really wanted you to focus on in the last class. Although I touched on it briefly, I didn’t dive into the details, but I’m bringing it up again because it’s crucial. Always remember that in IR spectroscopy, there is a change in the dipole moment, while in Raman spectroscopy, there is a change in polarizability. This distinction is key. When you examine the compounds, you’ll notice that Raman spectroscopy gives better signatures

for homonuclear complexes. In contrast, in IR spectroscopy, certain zones are more distinct. It's important that you mentally map out this particular slide. You'll see that bending vibrations are localized within a specific zone, whereas stretching vibrations are spread across the entire spectrum.

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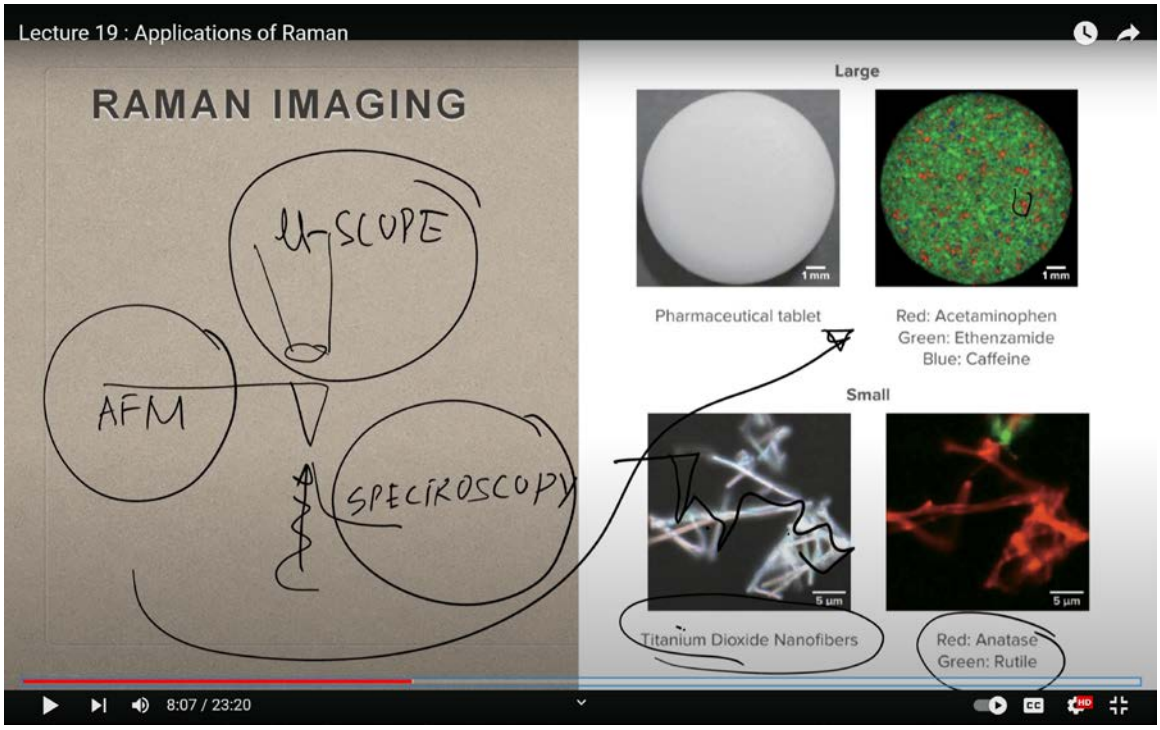

Now, let's move on to an exciting topic: coupling Raman spectroscopy with imaging tools. Imagine you have a sample that you suspect contains a unique chemical signature. How do you analyze it? One approach is to use a combination of microscopy, AFM (Atomic Force Microscopy), and spectroscopy. When you integrate these techniques, you achieve a multimodal resolution. For instance, the red spots you observe might represent anatase crystals or titanium dioxide nanofibers. This combination allows you to visualize not just the presence of these materials but also their arrangement and geometry, potentially even in three dimensions.

Currently, what you're seeing is a two-dimensional view. But with the power of advanced imaging techniques like EFM (Electrostatic Force Microscopy), you can delve deeper,

probing the sample to understand its full three-dimensional structure. This is the beauty of combining these techniques, you achieve a remarkable resolution and gain a comprehensive understanding of the sample's architecture.

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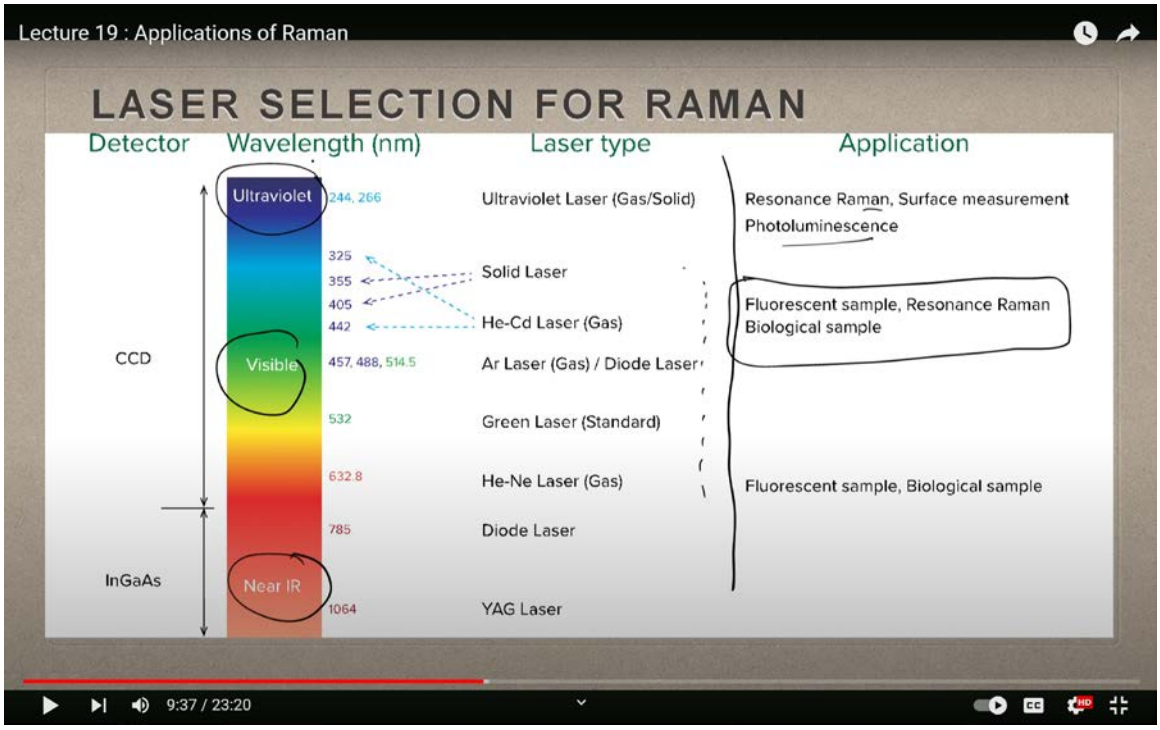

Now, for those of you interested in biosensing, another critical aspect is the selection of the laser for Raman spectroscopy. This decision is vital because not every laser will work for every application. Consider the type of sample you're working with. Fluorescent samples, resonance tumors, and biological specimens require specific lasers. For example, ultraviolet lasers are suited for resonance, surface measurements, photoluminescence, and fluorescence in biological samples.

When selecting a laser, you must be extremely cautious, as each type of laser, whether visible, ultraviolet, or another, comes with its own set of features, advantages, and disadvantages. High-energy lasers in the ultraviolet zone have specific pros and cons that must be considered. The selection of the appropriate laser is not only crucial for Raman spectroscopy but also for IR spectroscopy.

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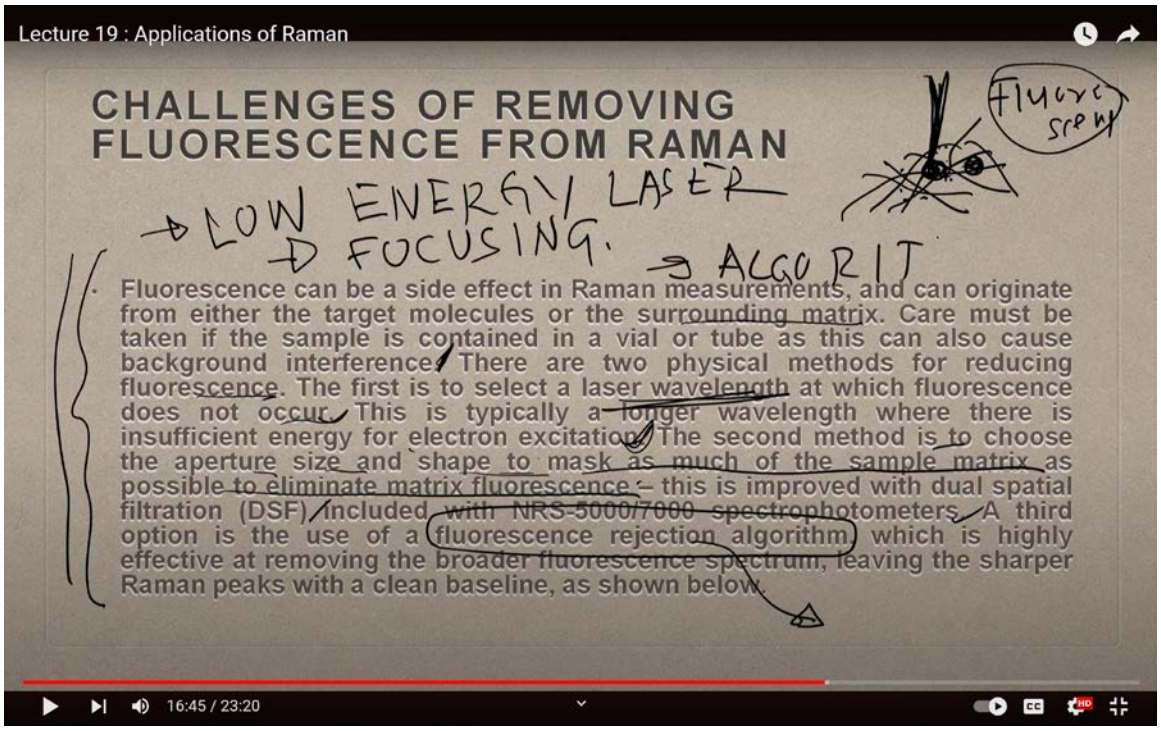

However, one of the major challenges in Raman spectroscopy is the problem of fluorescence interference. This is a common issue that can make the Raman signal noisy and deter users from choosing this technique. But don't be discouraged, there are clever ways to minimize or eliminate fluorescence. Understanding how fluorescence can affect Raman measurements is essential. It can originate from either the target molecule or the surrounding matrix, and even the vial or tube holding the sample can cause background interference. Therefore, careful consideration and technique are required to avoid these issues.

There are two physical methods to reduce fluorescence, which we'll explore further. These strategies are crucial for ensuring that you can obtain clear and accurate results without the interference of unwanted fluorescence.

The first approach to mitigating fluorescence in Raman spectroscopy is to select a laser wavelength at which fluorescence does not occur. This is why it's crucial to understand which lasers are least likely to induce fluorescence. Typically, a longer wavelength laser,

which lacks sufficient energy for electron excitation, is ideal. Remember, if you use a laser with a longer wavelength, the energy won't be enough to cause electrons to jump to higher energy levels. However, if you use high-intensity UV lasers, which are shorter in wavelength and therefore higher in frequency, they carry a lot of energy. As a result, you must be cautious about the type of laser you choose, as shorter wavelengths with higher frequencies have the potential to excite electrons and induce fluorescence.

The second method involves carefully selecting the aperture size and shape to mask as much of the sample matrix as possible, thereby eliminating matrix fluorescence. This is a fascinating technique because it doesn't involve changing the laser itself. Instead, it's akin to adjusting the settings on a camera. By minimizing the aperture and focusing on a specific point, you can reduce noise from the surrounding matrix and focus solely on the desired sample. As someone with experience in microscopy, I can tell you that this technique is tricky and requires a lot of practice. Mastery comes only with time, as controlling the aperture and getting the perfect image demands precision and familiarity with the equipment. It's not a skill that can be acquired overnight.

To give you a visual example, imagine your sample is surrounded by a matrix. You need to ensure that your laser beam is focused in such a way that it targets only the sample, avoiding the matrix that could cause fluorescence. This issue is particularly common for researchers working with hydrogels and cryogens, as these materials often exhibit inherent autofluorescence. Overcoming this autofluorescence is a significant challenge, and focusing the laser precisely on the biological sample is key. Additionally, you must work quickly to avoid damaging the sample over time. The effectiveness of this method is enhanced when dual spatial filtration (DSF) is used, which is included with the NRS systems of 5000 to 7000 respective parameters.

The third option is to use a fluorescence rejection algorithm. These algorithms are available on the market, and they are highly effective at removing a broad spectrum of fluorescence, leaving a cleaner Raman signal with a sharp peak and a clean baseline. However, there is a potential drawback to using these algorithms: they are often patented and come with preset baseline values. The issue here is that you may not fully know what the algorithm is

filtering out because it’s based on predefined baseline values that may not match your specific sample. Filters are designed to pick up certain values and eliminate anything below a certain threshold, but this could result in the loss of valuable signals that fall outside the algorithm’s preset parameters.

When dealing with unknown samples, this can sometimes become misleading, as you may unintentionally eliminate significant signals. It’s crucial to make an informed choice. To summarize the key points, the options available to you are as follows: First, select a low-energy laser that won’t have enough energy to eject electrons, thereby preventing fluorescence when electrons return to their shells. This is one approach, using a low-energy laser. The second approach is to focus on your target by carefully adjusting the aperture. The third option involves utilizing algorithmic systems. There isn't a single solution to address this, so these two approaches, aperture adjustment and algorithmic correction, offer multimodal application options.

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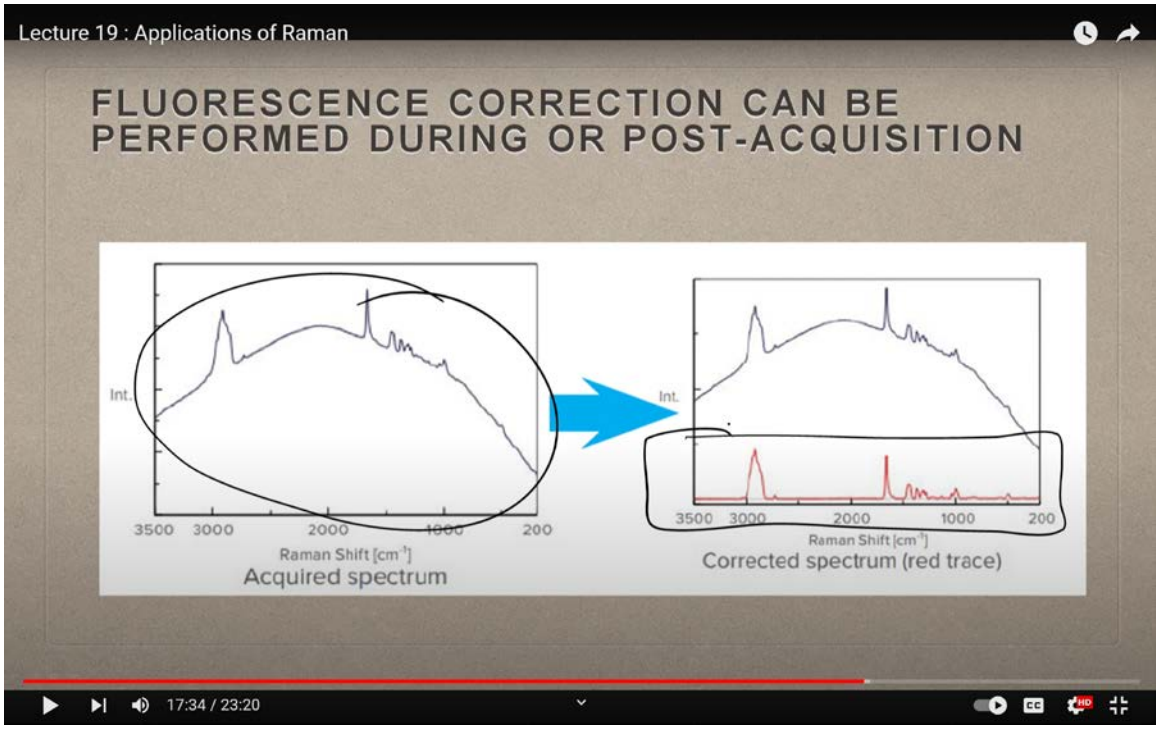

When considering how to perform fluorescence correction post-acquisition, this is what the acquired spectra might look like. For example, if you send your data to a reviewer, they might ask for a baseline correction. This baseline correction is essentially the process of removing all the noise present in the spectra. When you report the results, this clean-up process is vital. In the red trace, you can see how a Raman spectrum is reported, revealing the functional groups present and the types of molecules vibrating on the surface. This is how the spectra should appear after thorough clean-up.

Let's return to a critical slide I've mentioned before. This slide is of utmost importance, which is why I keep revisiting it. Keep this slide and its concepts in mind whenever we discuss the interaction between matter and light. There are five possible outcomes: scattering, reflection, refraction, absorption, and vibration. Absorption, especially if induced by a high-energy laser, can lead to fluorescence, photoluminescence, and other related phenomena because of electron movement.

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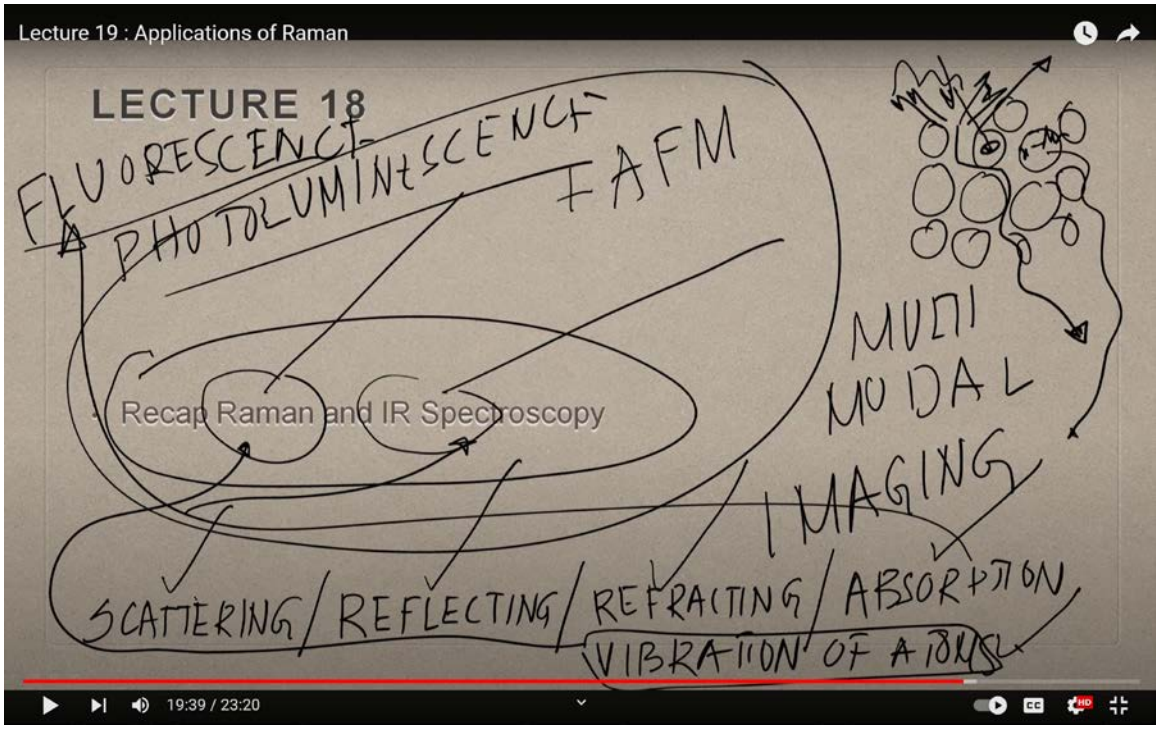

All these concepts are relatively simple if you can close your eyes and visualize what happens when light interacts with a material, how the material or atoms alter scattering. We've discussed SNOM, reflection, and refraction (where light passes through), as well as absorption and atomic vibration. We've talked about Rayleigh scattering, Raman scattering, Stokes and anti-Stokes shifts, where the original wavelength is either increased or decreased. The anti-Stokes shift adds to the energy, while the Stokes shift represents a subtraction, $V_0 - V$.

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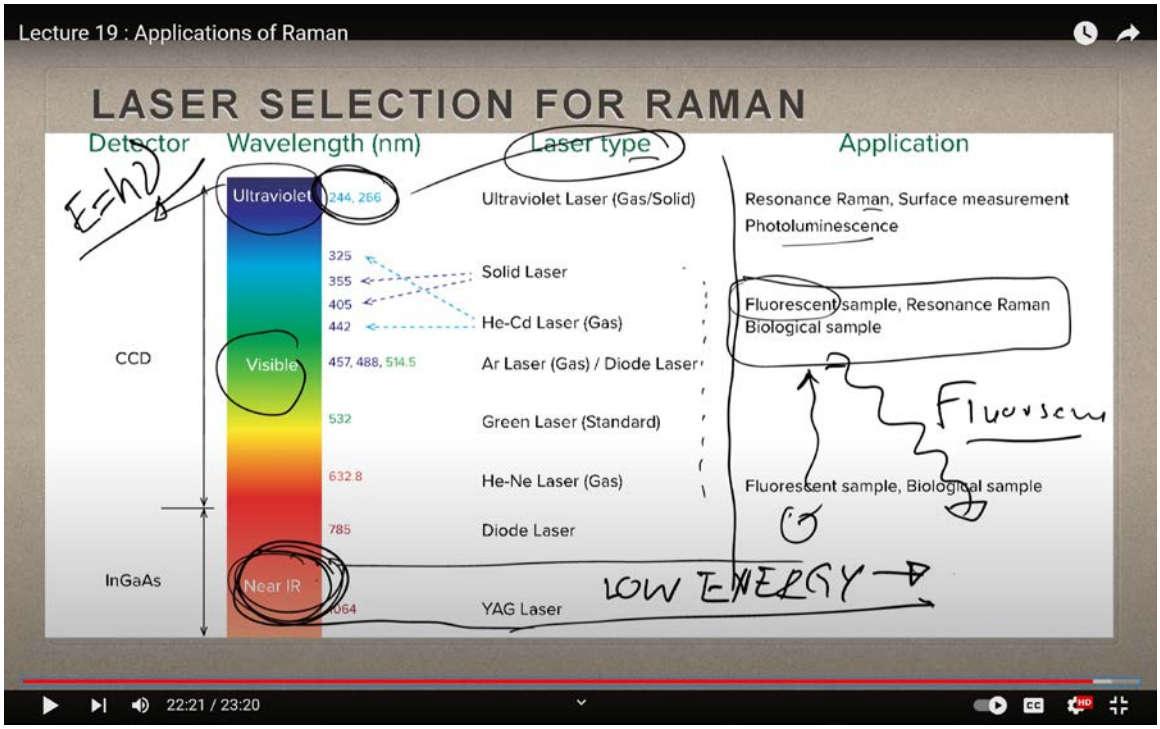

Moving on to the IR spectrum, it's also important to remember the differences between homonuclear and heteronuclear complexes for Raman and IR-based systems, and the degree of polarizability, particularly for Raman spectra. These techniques have numerous applications, such as detecting structural changes in proteins like hemoglobin, lysozyme, cytochrome, and even different types of viruses. They're also used in studying spider silk, mulberry silk, and various other proteins, as well as in detecting a wide range of pharmaceutical agents and food contaminants. Furthermore, these techniques are widely applied to all sorts of polymers and biopolymers.

We've already discussed multimodal imaging by coupling AFM with microscopy and spectroscopic techniques, which allows you to detect the types of atoms on a surface and reconstruct their three-dimensional geometry. We also touched on the importance of laser selection. If you use a low-energy laser, your chances of minimizing fluorescence are higher compared to using a high-energy laser, which can eject electrons. Once an electron is ejected, it follows a path to return to its ground state, emitting fluorescence, phosphorescence, and photoluminescence along the way. Therefore, it's critical to be cautious about the type of laser you choose.

Finally, we discussed the challenges of removing fluorescence from Raman spectra and how this can be achieved by selecting the appropriate laser, adjusting the aperture, or using specific algorithms. We also looked at what the final spectra should look like after these corrections. So, I will close in here and move to the next lecture after this. Thank you.