Biophotonics
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Lecture – 17
Light Matter Intersection in Molecules
(Basic of Spectroscopy)

Welcome back, let us continue our discussion on interaction of light with molecules, biological molecules specifically and that is basic of spectroscopy.

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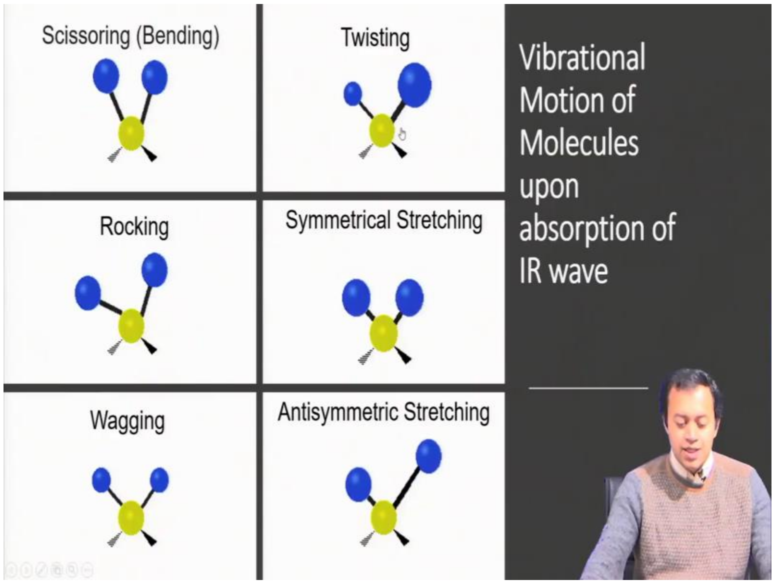

Scissoring (Bending)
Twisting
Rocking
Symmetrical Stretching
Wagging
Antisymmetric Stretching

Vibrational Motion of Molecules upon absorption of IR wave

The second part is the molecular vibration. Now, see these beautiful pictures, these are the different the ways a molecule will vibrate upon getting or upon excitement by light not just any light, it usually is infrared light. Now, infrared is associated with heat and that will open up a whole new can of worms if I try to discuss thermal radiation. Infrared is associated with heat which is correct yes and no infrared allows.

So, the wavelength of infrared or the frequency or the energy of infrared allows for the molecules to vibrate, the vibration overall gets dissipated in terms of heat and thereby we several times associate infrared directly with heat. So, understand this infrared light falls on to a material usually organic material, the molecules absorb these infrared molecules not electron not electron, I cannot emphasize it more molecules absorb this light they start moving in these various different ways scissoring rocking, wagging, symmetric stretching, anti-symmetric stretching etc.

And each of this each of this is because of specific wavelength of infrared being absorbed and upon absorption, they do not emit most of them do not emit they dissipate the heat thermally or non-radioactively. As a result the temperature increases and hence we say that infrared is associated with temperature which is not technically wrong infrared is associated with temperature.

But when we are talking about thermal radiation, remember any wavelength has the capacity to produce heat. Think about those vast and tremendous stars, think about our sun, what is the temperature at the core or the corona of our sun and what are the waves that are being coming out of the sun is do sun only emit infrared, do you not see any other terahertz, gamma rays, x rays, cosmic rays, all of these things coming out.

So, technically any wave can produce thermal radiation or thermal raise basically is conversion of heat into electromagnetic radiation electromagnetic spectrum. When it comes to measuring organic compounds in earth, it is usually, infrared give rise to agitation in molecules, these molecules dissipate heat etc. But thermal radiation for all intents and purpose can come from other wavelengths of light as well.

So, make no mistake, for low temperature we discuss in earth measurement of organic compounds, we stick to infrared waves, because infrared waves upon falling into organic material vibrates the molecule the molecule vibrates like this and they dissipate their energy non-radioactively thermally. Each of these, each of these individual vibrations are quantized or discrete and take specific energy, specific photon has specific quanta.

So, if you can identify say carbon dioxide has I am just saying, I am giving the example symmetric stretching by absorbing 4.4 micrometer wavelength of infrared light. You have got a bunch of light from somewhere any spectrometer and you are saying that the molecule is symmetrically stretching and 4.4 is missing. What kind of conclusion can you bring into, can you detect the molecule, think about it.

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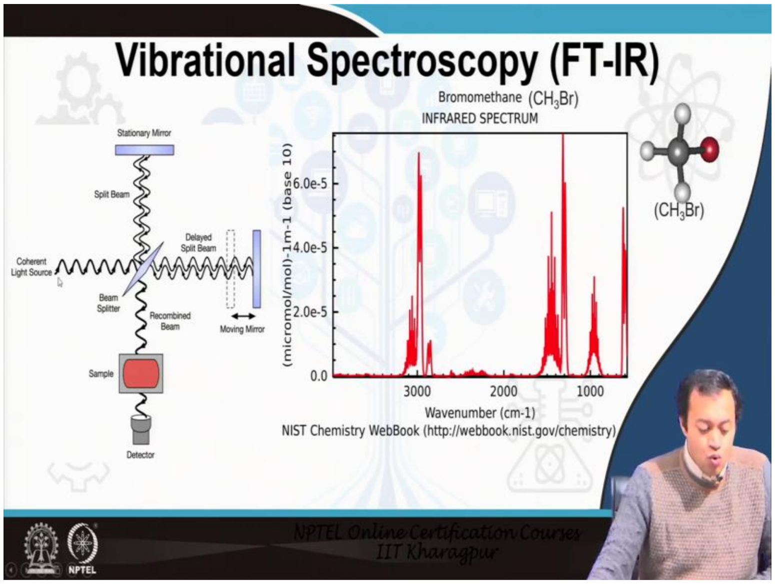

Vibrational Spectroscopy (FT-IR)

NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

The primary way in which we can look into vibrational spectroscopy, infrared vibrational spectroscopy is called FT-IR. FT-IR Fourier transform infrared spectroscopy has become tremendously popular again an FT-IR equipment is quite common these days to be seen in laboratories I am sure if you have not seen it in your particular college in your particular Institute, your particular University, you might have heard you must have heard of infrared spectroscopy, FT-IR, etc.

So, what does FT-IR does? Remember in UVV spectroscopy you used a monochromator, monochromator give monochrome one light one wavelength, here, we do not give one single light to illuminate the sample, what do we do, we send a bunch of light from a coherent light source. So, several different wavelengths of light are made to pass through and they fall into a beam splitter, beam splitter as the name suggests, splits the beam. One part goes into a mirror which is stationary, one part goes into a mirror which is moveable.

So, you send a b c d e f g h wavelength of light onto a beam splitter that by passes that bifurcates that divides I do not think divide is the right term, you might feel that half of it goes this way half of it goes that way. It does not happen like that, it doubles it goes in two different direction one to a mirror, which is fixed in nature, another to a mirror, which is movable. The light from each of these mirrors stationary mirror and the moving mirror, then is reflected back again at the beam splitter.

The light from the stationary mirror as well as from the moving mirror returns back to the beam splitter where they interfere, where they interfere either constructively or destructively. Whatever happens whatever the final result is, falls on to the sample and is being detected. So, understand this, we send bunch of light one to a stationary mirror, one to a movable mirror, they are then made to interfere some of them will interfere constructively, some of them will be moved to, some of them will be interfering destructively.

Then, either similar or slightly different combination of the frequencies are made to pass through or go through the same process however, this time the mirror is moved. However, this time the mirror is moved as a result, there will be a slight difference in the interference pattern from previous. Previously you had this position light coming from here and light coming from here or interfering.

Now, you have this position or this position, where the mirror has moved where the mirror has moved one is kept fixed, but the other has moved there by different combination of waves at different time function to the movement of the mirror, either construct interfere either construct either interfere constructively beg your pardon or interfere destructively different types of modulation takes place, different types of interference takes place.

And you finally use a computer back calculate for each individual wave what is happening it is it is not very counter intuitive. It is counter intuitive. It is not very intuitive. Previously we sent one light, get it back analyze it, it has been absorbed or returning back to me. Another wavelength of light, it goes comes back we analyze them part by part one by one and that is usually what happens in UV spectrometer.

Here vibrational spectroscopy, we are sending bunch of light, we are sending bunch of light at one position, the same combination, interfere constructively at another position when the mirror has moved. Similar waves same waves interfere destructively. We go through all of them with the help of a computer. Finally, back calculates each, the property of each single wave each single photon each single wavelength when it falls into the sample, what does that do?

Using a computer using a moving mirror and most importantly, using a Fourier-transform the name Fourier comes from here Fourier transform give us the output of the sample’s absorption with respect to the movement of the mirror. Now, since we have done Fourier transform, it will inverse it Fourier transform is available in high school mathematics textbook I am not going to tell you what Fourier transform means, Fourier transform involves from one domain to another domain.

So, the movement of mirror is in centimeter yeah, Fourier transforms inverse it and you give it give it get it in centimeter inverse or wave number, wave number can immediately be calculated or return converted into wavelength frequency energy whatsoever. So, this is an example of Bromo methane, where most of the absorption has happened at 3000 or 1500-500 centimeter inverse as an exercise try to calculate what it will be in wavelength very easy to calculate.

All of these absorption ranges all of these absorption peak shows some kind of vibrational movement, some kind of vibrational spectra and we can identify particular molecule by identifying these specific spectra, this specific absorption peak, these are fingerprints these are signature just like we have fingerprints or signatures, which identifies you, these are the specific absorption that a molecule perform or molecule exhibit or molecule displays upon excitement by light.

So, no matter what Bromo methane will always show absorption at around 3000 centimeter inverse wave number. This represents a specific vibrational moment a specific vibrational stage of the molecule Bromo methane, and there are a combination of several other all those anti-symmetric stretch symmetric stretch, you can identify each one of them by this particular peaks, each one of them corresponds to those vibrational moment and when you have a library, a database of all of these.

You can then put any unknown sample here, send bunch of light get the spectra, identify the spectra with match the spectra with your library with your database and thereby identify the molecule whatever light I am getting from an exo-planet, I analyze it using this and if it matches these particular spectra, I say it is Bromo methane. Obviously, in the very beginning to make the spectra I start with known samples, I know this is salt, this is NaCl dense and bunch of light.

Thereby I create my spectra in the first place, then I take a blind sample and unknown sample anything, I put it in my sample box, I put it in FT-IR and see which of the these kinds of absorption spectra is matching with which of my library database. That is, your identification. Read a bit about FT-IR I am not going through full detail like this is this this processes are

Michelson interferometer with a moving mirror with respect to stationary mirror. I regularly perform FT-IR for detection of oral cancer tissues and in next classes we will be discussing that in detail, but read a bit about FT-IR spectroscopy.

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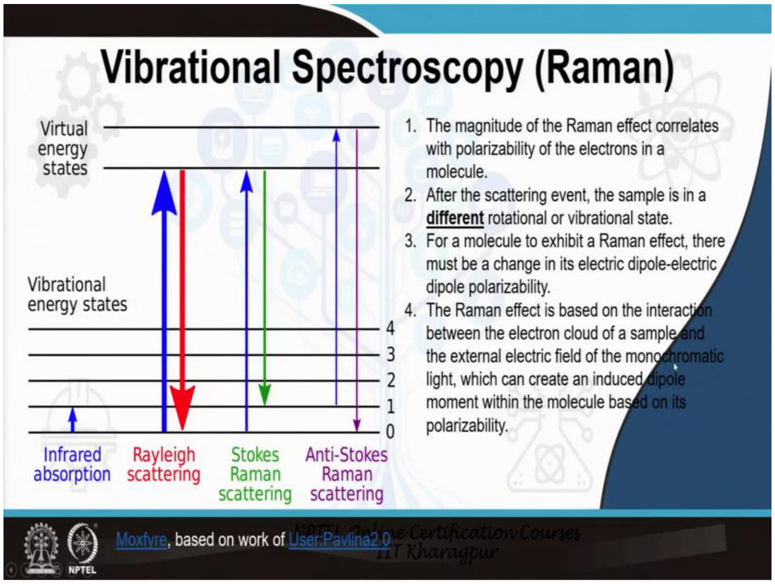

Vibrational Spectroscopy (Raman)

1. The magnitude of the Raman effect correlates with polarizability of the electrons in a molecule.
2. After the scattering event, the sample is in a **different** rotational or vibrational state.
3. For a molecule to exhibit a Raman effect, there must be a change in its electric dipole-electric dipole polarizability.
4. The Raman effect is based on the interaction between the electron cloud of a sample and the external electric field of the monochromatic light, which can create an induced dipole moment within the molecule based on its polarizability.

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Now, comes the overall the very, very famous Raman spectroscopy and this will bring in intense debate I know, so bear with me indulge me in this particular regard. Raman spectroscopy is inelastic, inelastic scattering or inelastic method. What does that mean? Usually, usually, a photon interacts with matter. It either get absorbed, or it either get reflected. So, whatever photon has come, whatever energy has come somehow or the other is utilized.

It is completely up reserved or completely reflected back inelastic scattering is the one that happens when the photon interacting with matter when the photon upon interaction with the matter get scattered. Mark my words, it is called also Raman scattering, a specific case of scattering, when a photon interacts with a matter and gets scattered. The scattered photon is of a different energy then the original photon that has come and interacted with matter.

Now, here is the crux of the matter the photon that is being scattered. So, photon is coming hitting the matter usually molecule and scattering back like billiards like carom. This energy of the scattered photons. So, this is before, this is after, energy of before photon is different from energy of after photon. The crux of the matter remains, the fundamental aspect of the matter remains, the after photon the scattered photon can have an energy both lower than the before photon as well as higher than the before photon.

In fluorescence, you have seen that there is an emission happening and usually the emission is at a lower wavelength, this is not fluorescence do not, do not confuse it with fluorescence, fluorescence is an electronic phenomenon. This is a molecular phenomenon. Raman scattering is a molecular phenomenon it is an inelastic phenomenon where molecules are interacting with light, light interacting with molecule and here there is a possibility that the scattered light that the scattered photon has a higher energy than what it had started than the before photon then the photon it was before getting scattered.

Where is the extra energy coming from? The energy is coming from the system, energy is coming from the matter where else. So, what does that happens? Usually, let me just quickly tell you, in Raman scattering, if the input photon has energy higher than the output photon, output photon is of a lower energy, we call it Stokes scattering. Or if the output photon has higher a energy than the input photon, we call it anti-stokes scattering.

How exactly that happens? Well, you can read it yourself. But I will give you a very, very specific and easy example. So, what happens is that you have a molecule, the molecule has an electron cloud enveloping it. The electron cloud is enveloping it, you have sent a photon to interact with this molecule with an electron cloud surrounding it, the electric field of the photon will interact with the electric field of the electron cloud, the electric field of the photon will interact with the electric field of the electron cloud.

I am not talking about magnetic field, because magnetic field is generally weak and we are not talking about magnetic materials as such. So, again you have a molecule you have electric electron cloud enveloping it and you have a photon which has an electromagnetic field around it, that electric field of the electromagnetic field of the photon is interacting with the electron cloud of the molecule.

What happens then, what happens then is there is a dipole, dipole interaction there is a redistribution mark my word there is a redistribution of the charge there is a redistribution there is a modification on the shape of the electron cloud enveloping the molecules enveloping the nucleus of the various atoms that form the molecule. So, this electric field has modified the electron cloud, the electric field has modified the electron cloud and since there is a modification of the electron cloud has taken place, the molecules will reorient rearrange it themselves from their previous position to achieve stability.

Yeah, that is what happens the electric field of the photon makes permanent change, semi-permanent change, it is not for a infinite period of time, it makes a change in the orientation of the molecule, whatever the molecule previously, these are molecules, these are the energy levels of molecules not necessarily electrons, they look like electronic energy states, but these are actually molecular energy states.

So, the molecule was at this energy after interacting with the photon, it has reoriented itself to go to a different vibrational energy, this vibrational energy happens to be less than the original position. So, the photon that is emitted is of a lesser wavelength. Similarly, here, the molecule was present at energy level one it has interacted with the photon and upon the rearrangement of its electron cloud upon rearrangement of its rotation its vibration, it has returned back to an energy level to a vibrational level, which is of lower energy.

Which is often lower energy than the original state. So, when it has returned back to a lower energy, it has to emit a photon of higher energy. Here it has moved to a higher energy level. So, it has to emit a photon of lower energy here it has moved to a lower energy level the molecule has moved to a lower energy level, it has to for conservation of energy for the total energy of the system to remain same the thermodynamics law etc, etc.

It will emit a photon of higher energy. Anti-stokes is therefore, a photon of higher energy getting scattered. This is the overall science behind it. Now, obviously, this is slightly incomplete, you have to read a bit more about it. But overall, the magnitude of Raman effect depends how much the electrons in the molecules can get polarized, obviously, the electrons that are enveloping the molecule need to modify themselves to the electric field.

If the electric field is unable to modify, then what is the point of any of this? Then they can simply absorb go to an upper state and we will come back to the lower state. Here remember, a modification is happening, where the sample is in a different rotational or vibrational state after the photon has heated the photon has interacted it.

Not all molecules do that, not all molecules do that, very few molecules will rearrange, reorient themselves and come back to a different position than its original position upon interaction with light and this different effect this specific effect this special effect, where certain molecules upon excitement by light do not return do not mark my term, do not return to their original position do not return to their previous ground state is what Raman effect is all about.

Now, you know what Raman effect is? The Raman effect is based on interaction between electron cloud of samples and the external electric field, which can create an induced dipole moment with the molecule based on polarizability. If a molecule exhibits a Raman effect, then there must be a change in electric dipole electric dipole polarizability. The electron or the electron cloud is changing itself it is changing polarizability overall the dipole moment is changing significantly enough upon interaction with the electric field of the photon.

So, much so that it is resulting in the overall molecule to change for the sake of stability for not to break it apart. So, previously, the molecule was like this, you have shown some amount of light it is now this. It will not stay in this position permanently, obviously not. But it will stay in this position for an enough period of time for you to do the measurement, you have shown light the light has returned back a light has scattered you either look at the molecule itself the how the orientation are changed or you look at these photons that are being emitted.

If there is an up-conversion, down-conversion if there is a Stokes scattering or anti-Stokes scattering, if the energy of the output photon is large or more, more or less, you can characterize it with specific molecules that do that only specific molecules will be doing that not every molecule shows Raman effect. Therefore, we call certain Molecules Raman active Raman inactive. Water does not show anything any Raman activity, water does not show any.

So, no matter how much you hate hydrogen and oxygen molecule in a water configuration, they will vibrate and finally returned back to the original position from where they have started, they will not rearrange, reorientate, re-vibrate depending on the difference of the electron cloud because the electron cloud is quite stable quite naturally oriented across the each of their nucleus.

That is Raman effect in a nutshell, obviously, both for infrared spectroscopy and Raman spectroscopy, there is a sea of knowledge that requires to be taken into effect if you are looking for to know more about spectroscopy, there are several NPTEL courses on spectroscopy. Go through them there are several beautiful books, we are we need to know this much to understand the final effect the final manifestation.

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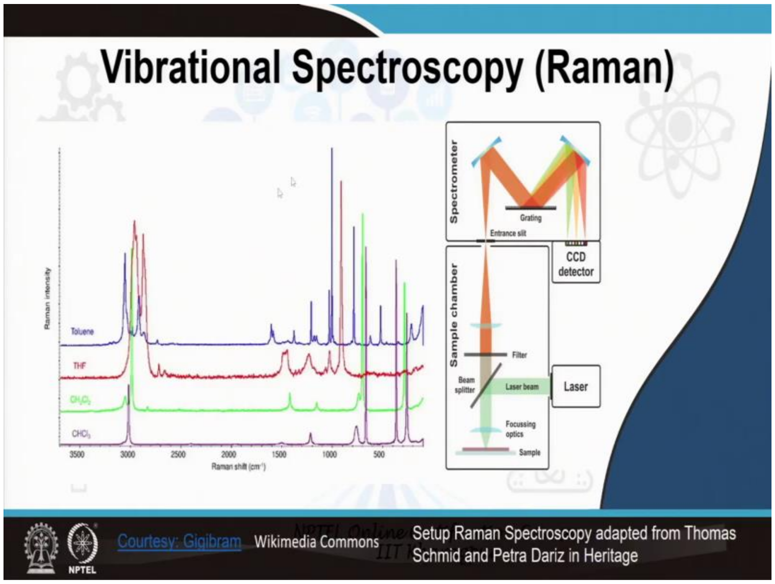

Vibrational Spectroscopy (Raman)

Courtesy: Gigibram Wikimedia Commons

Setup Raman Spectroscopy adapted from Thomas Schmid and Petra Dariz in Heritage

For example, this is the vibrational spectroscopy or Raman spectroscopy of several different molecules, you see where the photons changes where the Raman shift is actually occurring. This is very quickly, I know I am running out of time, how the measurement is done. So, the laser light comes we usually take laser and it hits the particular sample, the light that is hitting the particular sample passes through the beam splitter and filter, we need to take out the Rally scattering Rally scattering is that scattering which is elastic.

Quite intense, that goes through spectrometer that is put into a grating. The grating then filters out all the different wavelengths that has come out of the sample all the different photons that has come out of the sample and then they are filtered out diverged out and they are put into a CCD detector thereby you measure each single photon or it is specific photon each photon with a specific frequency specific wavelength and certain materials will obviously show this Stokes and anti-Stokes you identify this token anti-Stokes you match it again with your library and you identify the material.

Several molecules the same molecule upon going through different phases denaturalization we call it. Show different values of Raman effect or IR effect. Say for example, you have a tissue you are measuring both IR and Raman out of it and it is showing different sorts of absorption different types of Raman shift the same tissue then has undergone some kind of mutation metastasis that happened some kind of abolition have taken place. Something has changed some kind of virus particle have affected some kind of spores have formed.

Over all the molecular arrangement over all the molecules are going to change of that specific tissue. If the molecular arrangements are changing either by addition of a foreign particle, foreign body pathogen virus bacteria, or something internally has happened. Different types of proteins are formed because of mutation, you have smoked quite a lot. So, they are by mutation of setting the mutation is starting to produce carcinoma and metastasizing is happening.

There will be a rearrangement of proteins the proteins will change proteins will denature the proteins will mutate the proteins will modify thereby the molecular arrangement will change if the molecular arrangement will change, you will see a marked difference before and after in the infrared spectra before and after the Raman spectra. Can you therefore with this identify diseases? Think about it.

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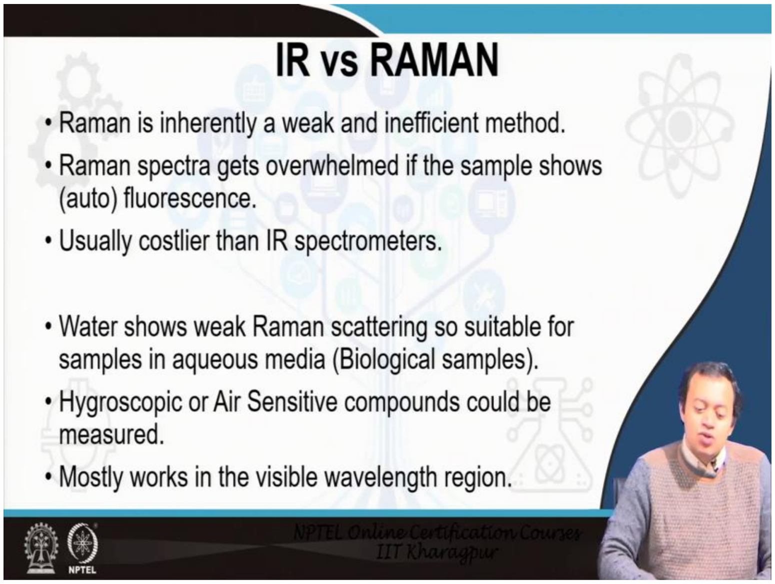

IR vs RAMAN

- Raman is inherently a weak and inefficient method.
- Raman spectra gets overwhelmed if the sample shows (auto) fluorescence.
- Usually costlier than IR spectrometers.
- Water shows weak Raman scattering so suitable for samples in aqueous media (Biological samples).
- Hygroscopic or Air Sensitive compounds could be measured.
- Mostly works in the visible wavelength region.

Now, here I am going to describe something which is bit controversial IR versus Raman. Now, my experience goes that scientists, technicians, academics are very, very passionate about the team they belong. So, there is a group of scientists who are IR specialist. And then there are a group of scientists who are Raman specialist and these two have intense rivalry. Believe you me I am telling this from personal experience.

Every time I go and present some of my results people say oh no, these results could have been done better in Raman every time I show my Raman results another group of scientists tell me the same thing could have done much better in IR. So, it is an intense rivalry between football teams or cricket teams or you know, my favorite actor is this your favorite actor is that we should not meet.

At the end of the day IR and Raman are complimentary to one another, they are providing you different aspects of the same truth. You must have heard of this story of a few blind men try to assert an elephant and each one of them said different things but they touch different parts of the elephant and they gave different information one said that elephant is like a trunk, one said that elephant is like a cylinder, one said that elephant is like a big wall and then they all fought among each other that neither of them are true.

Fact of matter remains they were all looking at the same thing from a different angle. So, IR and Raman are not complimentary. All of you who are listening to this. I mean, this is my request I am begging of you do not fall into this team that IR is better than Raman or Raman is better than a IR depends on what kind of sample you are using. For example, Raman is inherently weak. I know I have already offended several of the Raman people who might be watching it.

Raman is an inherently weak and inefficient method. Only few molecules will show it, only few molecules in a plethora of molecules will show Raman effect. Yes, I know Ramanan resonance is coming up you will tell me in the comment box I know I am I am proving myself for that. I am bracing myself for that. But overall come on think about it compared to you know, photon getting simply absorbed a photon getting scattered with a different frequency getting frequency from the system is slightly rare in my opinion, do not sue me.

So, Raman spectra gets overwhelmed if they sample source auto-fluorescence I will discuss auto-fluorescence. Auto-fluorescence is sometimes when molecules start to flourish all by themselves you know you send some amount of light you try to see what light got scattered, but that light got used in something else fluorescence spectra starts this emission will come up and this auto-fluorescence is a problem.

And usually IR spectrometers are costly, you have to accept that any Ramanite who is listening to this he will know how costly IR spectrometer are. Advantages of course, Raman has several advantages, water shows weak Raman scattering, whereas, water absorbs huge amount of infrared light 3.3 is a nightmare 3.3 micrometer, water absorbs it. So, if you are trying to measure biological samples which are present in water, infrared is probably going to give you trouble.

Raman on the other hand, no problem boom just put it you get beautiful, beautiful spectra hygroscopic or ear sensitive compounds, again put in a sealed glass the glass will start absorbing infrared light not so much for Raman. The light will simply pass through and you will see the Raman scattered light beautifully. And Raman mostly works in the visible wavelength region.

It works in the visible wavelength region you can see from your own eye the detection is comparatively easier though in both cases, you use sophisticated computers and image processing tools. Now it is both FT-IR as well as Raman are added with microscopes and these microscopes takes beautiful pictures you focus the light in a specific point and not only you see the morphology you also see the chemical image because light will hit that specific area.

Which are being converged by your microscope lens and information is extracted from that area and then you can through image processing and software, chemically map that image. So, all of these things are happening. These are three of the most common spectroscopies that I discussed, there are a plethora of other which I did not for you to find out I use them because I explained them because these are the most common which you will find in most Institutes, most laboratories most universities.

There are some others which will require which you need to know but we will discuss them in detail later. For example, circular dichroism where left circularly polarized light or right circularly polarized light are absorbed depending on how the molecule is oriented. So, different molecules get oriented differently and therefore they have a preference to absorb a specific polarization of light. Depending on the different types of polarization of light.

You can somehow identify the molecules is that the molecules are well. What is the orientation of the molecule understand the orientation of the molecule, you understand what is the overall shape of the molecule. This is usually for protein studies where protein aggregation or protein conformation takes place, but we will discuss those circular dichroisms at a later stage. For the time being just go through what I said and read a bit more about infrared versus Raman.

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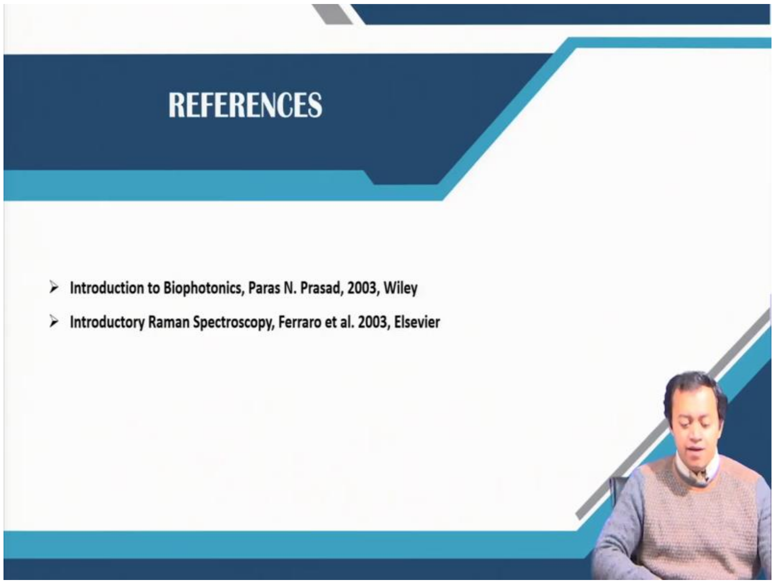
CONCEPTS COVERED

1. Spectroscopy
2. Electronic Absorption Spectroscopy
3. FTIR
4. Raman spectroscopy



REFERENCES

- Introduction to Biophotonics, Paras N. Prasad, 2003, Wiley
- Introductory Raman Spectroscopy, Ferraro et al. 2003, Elsevier

So, these are the concepts that I covered for today. And these are my references introduction to Raman spectroscopy is a very, very good book please, please go through it. I learned a lot from it. I learned my Raman from this particular book. I quite liked it. Thank you very much. I shall see you in next class.