Nanobiophotonics: Touching Our Daily Life
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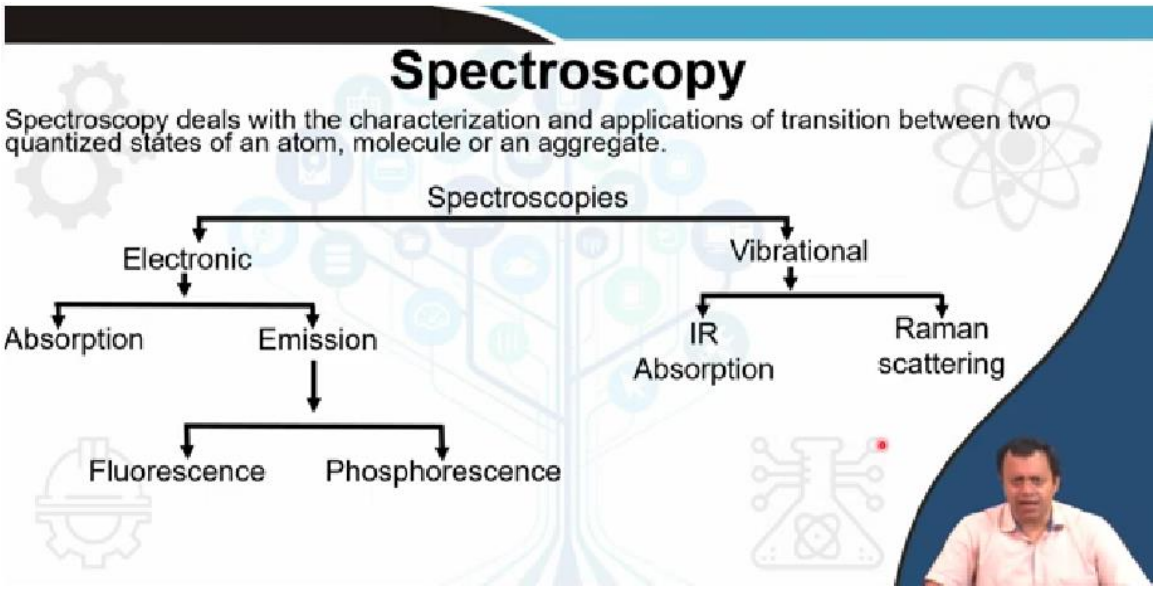
Lecture No. 17
Light-matter interactions in molecules (Basic of Spectroscopy)

Hello and welcome. We will continue our discussion on the fundamentals of Biospectroscopy. In today's lecture, we are going to discuss upon the light matter interaction in molecules, the very basic of spectroscopy. In our previous class, in our previous lecture of this module, we have seen that biological matter reacts interacts very well with light. They perform specific functions of chain reactions usually takes place and that can be exploited that can be utilized in order to understand what is going on and also detect classify specific chemical species whether they are present or not. Remember a particular chemical species absorbs a particular frequency and performs a particular function.



A molecule absorbs 500 nanometer frequency and performs photosynthesis. So, all three are linked to one another. So, like a puzzle if you find a particular frequency 500 nanometer is absorbed and this particular biochemical reaction is taking place, can you not therefore say that species number molecule number A is present. So, what you have done you have identified you have detected right.

On the other hand say molecule A is present you are shining 500 nanometer of light and, but the photosynthesis is not taking place. Then can you not claim that maybe molecule A has been compromised, maybe molecule A has modified itself, maybe there is something wrong in that area where molecule A is present and thereby this particular chemical process is not happening. So, can we now identify look and modify what chemical where chemical

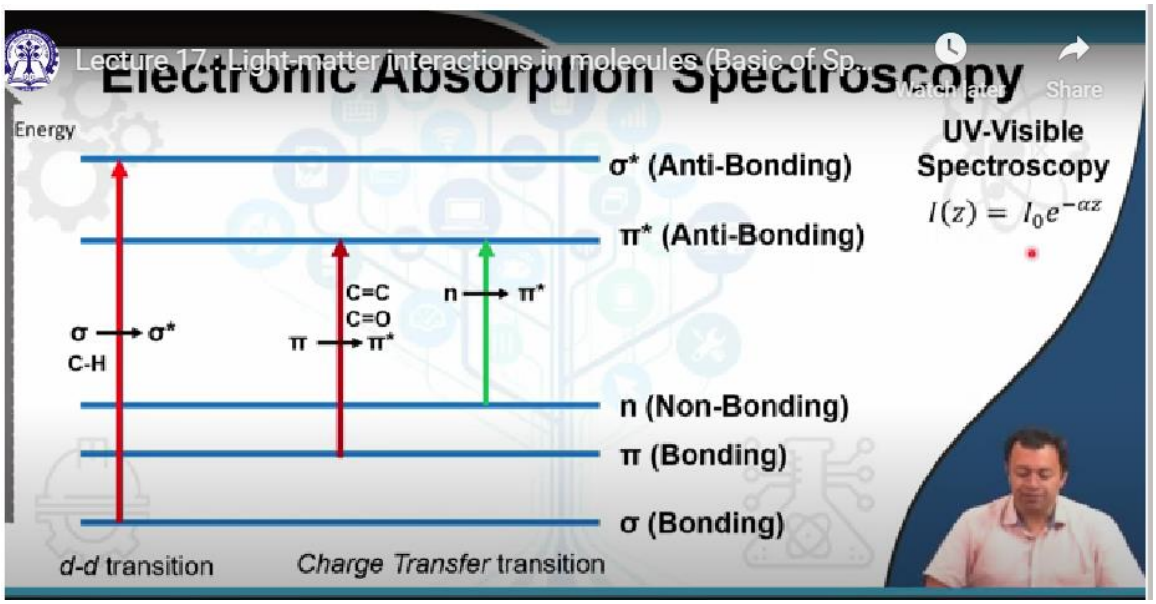
A is think about it. So, anyways all these things can be identified using the field I would use the term field of spectroscopy it is a technology it is a field. Thus far it has mostly been dealt with dealt by the chemists, chemistry people have been dealing with spectroscopy there are various types of spectroscopy nuclear magnetic resonance mass spectroscopy etcetera.

But here we will specify our self to bio spectroscopy. For us for us spectroscopy will be characterization and application of transition between two quantized state of an atom molecule or an aggregate. Meaning spectroscopy will deal with either the absorption or scattering of light by atoms molecules combination of atom of molecules large number more number of molecules macromolecules or some component of molecules. What do I mean by component I it can be sub atomic particle electrons etcetera or of a large macromolecule a particular set of molecule absorb a light and goes to a next higher level these level are quantized these level not continuous these level are quantized. And this transition between two different quantized state two different quantized state is something that we are going to characterize and apply it for our understanding identification detection.

For us this is not an exhaustive list for us nanobio photonics people spectroscopy can be roughly divided into electronic and vibrational right. Again please please be aware I do not want any chemist to come back and say that you have not discussed nuclear magnetic resonance of mass spectroscopy etcetera etcetera those are not part of ah nanobio photonics you can make them ah there is nothing ah stopping you, but overall overall we will be dealing with visible and infrared one spectroscopy right and there can be divided into simply electronic and vibrational this also make the thing ah simple. Remember I discussed about bio spectroscopy not just spectroscopy as a field. So, spectroscopy for us I am dividing it into electronic and vibrational part. In electronic part as you know electron is absorbing the light going from one level to another level and finally, emitting the photon.

Electron is absorbing photon a particular frequency of photon higher energy higher frequency ah sorry higher energy yes higher energy higher frequency lower wavelength going up and then after some time the electron returns back to its original state ground state emitting a particular photon out depending on the emission you can divide it into fluorescence and phosphorescence. We have discussed what fluorescence is we have also discussed where phosphorescence is we will mostly target fluorescence while spectroscopy fluorescence spectroscopy is considered. So, this is simply straightforward. Another thing is vibrational here mostly the molecules absorb light molecules absorb light and their ah molecular motion vibrational motion changes. It can be infrared absorption or it can be Raman scattering we will be discussing both of them in somewhat detail in in in in this class ah.

One is absorption and one is scattering. Ah One in a particular case light is consumed absorbed one in particular case light is scattered out light hits the molecule and it scatters away. So, scattering versus absorption remember absorption and scattering are analogous processes. So, these spectroscopic methods are also analogous to one another we will discuss that. So, let us discuss about the electronic absorption spectroscopy you know UV visible spectroscopy a particular intensity of light ah travelling through a material in z direction with I naught is the maximum intensity at z equal to 0 ah performs this Beer Lambert's law which states that alpha is the absorption coefficient z is the distance ah exponential towards this and this is the overall intensity can be converted into power etcetera Beer Lambert's law of what happens to the intensity of light energy of light power of light all of that thing can be derived from this particular equation.



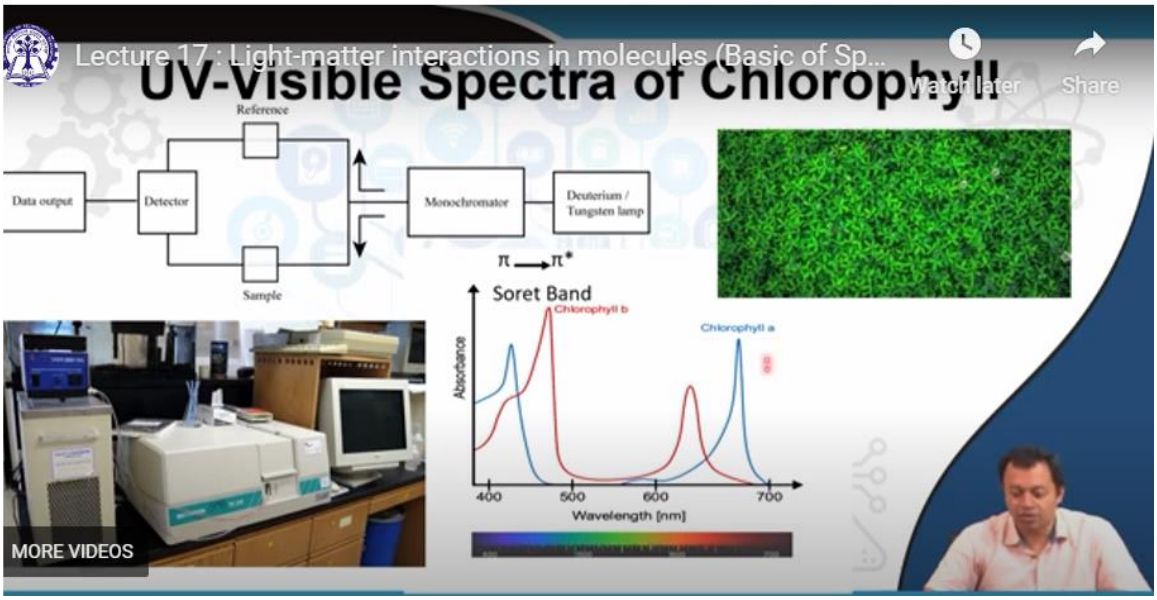
So, where exactly where exactly is the light going well depending on the frequency of the light and usually this is high frequency light because electrons are vibrating at a very high frequency if they needs to be vibrated further more you have to give them very high energy and this energy is usually available in ultraviolet light or ah visible ah blue or visible violet high energy very high energy light. And this results in electrons going from ah their bonding state to an anti bonding state from their bonding state to anti bonding state. We have discussed about bonding and anti bonding remember bonding is where low energy molecules have come together the attractive forces have one and they have bonded where as high energy molecules are repelling each other molecules are repelling each other the repulsion forces are born and they have you know started ah repulsion among each other. So, you can have ah sigma to sigma star bonding. So, molecule which have formed s or sigma bonding you remember the electronic configuration s p etcetera the sigma bonding you can sense a particular ah frequency of light high energy and the electrons at sigma bonding can go to sigma star anti bonding.

This usually happens in electrons of the methyl group or C H well methyl is one type of C H C H bond molecules they form sigma bond very strong usually and they can have ah a particular frequency allows them to go from ah the lowest energy state to the higher energy state. Then you have pi bonding pi bonding the p orbitals they combine together to form pi bonding a particular frequency of light can be given and the electron consumes that particular photon goes from lower energy state bonding is always always lower energy state ah attractive forces have one several forces have cancelled each other they have one and it goes from pi star anti bonding. Remember sigma is very strongly bonded ground state you need very high energy to break this pi is something ah moderately strong. So, ah a moderate amount of energy results in electrons transitioning from ground level to higher level ah instead of saying ground level of pi to pi star I am saying ah instead of saying ground level to higher level I am saying pi to pi star anti bonding level ah this is basically carbon carbon double bond carbon oxygen double bond and they are pretty pretty common in in any any biological material this is also pretty common, but you require very high an amount of energy UV and what not and this can be formed using normal visible light. Then also non bonding non bonding is that part which forms between bonding and anti bonding non bonding is the neutral ground where the two molecules have come together they do not know if they are attractive forces or repulsive forces have one they are neutral to one another they have not yet ah married or divorced they are meeting for the first time or sometime.

So, it is a neutral you might have asked what happens when attractive and ah repulsive forces cancels each other well the result is nothing no bond still forms, but there is a possibility of forming bond bond will only form only form when attractive forces have one if attractive forces have not one bond will not form in anti bonding case it will never form in non bonding case it has not formed, but there is a possibility it might form in future job we met right. So, that is also a higher energy level your energy reduces when bonding has happened. So, higher energy level non bonding non bonding to ah pi star though not very common as compared to this is also available. There is also d to d transition happens here this is sigma to sigma star pi to pi star n to pi star there is d to d transition d orbitals you know these d orbitals are available in elements of higher atomic number in the periodic table I think 16 17 or above ah no further below further transitional metals d ah d orbital is occupied or partially occupied. So, d d transition is also there where little bit less amount of energy can also help you ah break the bond.

And then there is also charge transfer transition meaning there is a particular molecule you have shine light the electron has gone to a higher state and when the light is removed instead of returning to its own parent molecule it has moved to another molecule which is nearby photochemical reaction happens like that and that is charge transfer transition. So,

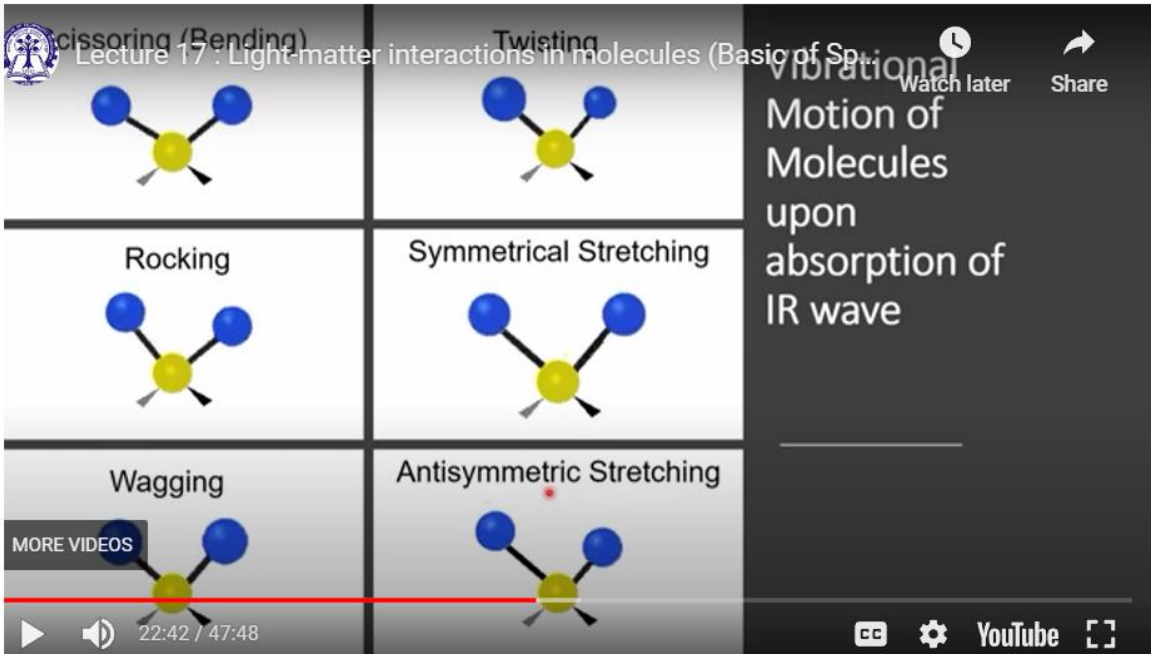
the charge in this case charge means electron, electron has gone to a higher level, but instead of coming returning back to its parent nucleus it has returned to another nucleus which has a slightly higher energy than the parent nucleus and thereby it has formed ah well it has it has gone to another atom of the large complicated molecule. Obviously, the result is for complications. So, these values are fixed yeah these values are fixed in natural material artificially you can create band gaps etcetera, but overall these values are fixed. So, if you have given a particular set of energy since the band gaps are quantized since the bands are quantized a particular set of energy is being given and you are seeing that a particular energy particular frequency is absorbed resulting in another biochemical reaction perhaps you can be certain that CH bond is present in this in this biological matter or C double bond C is available in this biological matter or C double bond O is available in this biological matter you can identify the unknown material and that gives rise to UV visible spectrum.



All of you might have seen this UV visible spectroscopy in your college or nearby ah some research lab UV visible spectro spectrometer is very very common what it does it has a simple lamp a tungsten lamp that allows large number of molecule a large number of wavelengths beg your pardon large number of wavelengths to pass through monochromator is a filter light filter mono means single chromator is color meaning it only allows a particular frequency to pass through it is a filter lamp produces thousands of wavelengths of light monochromator filters blocks all the light say it produces all the 7 colors of it only allows visible green or visible blue to pass through it everything else is blocked and then it is passed through this particular chamber one chamber you put a sample the sample in this particular case can be leaf any biological matter per say another is a reference sample where technically empty or specific things can also be put the light coming out of the monochromator is divided into 2 part we can do this using something we call beam splitter look it up beam splitter can divide light into 2 different parts and this

reference goes into the detector this is the reference that this is a particular frequency that goes through with complete vacuum chamber or anything anything specific and the detector produces a particular ah graph how does that produce these are usually photo detectors ah optoelectronic material when light falls on to them some kind of electric current is passed through you know this it happens in semiconductors all the time and this electric current is mapped with respect to the wavelength of light yeah that when light is falling in there directly into the detector versus light is falling through the sample there will be a modification with respect to reference and this modification this modification allows us to understand if a particular frequency is absorbed by the sample or not example is where we when we put leaves or chlorophyll in it we see ah the absorption is happening with respect to reference when there is no chlorophyll no leaf a particular frequency is getting absorbed when green light blue light etcetera. So, monochromator can also change. So, you at one instance you only allow 400 next instance you allow ah 450 next instance you allow 550 to go through and all of these things references one at a time goes inside the detector and the detector gives rise to a particular absorption when there is no sample per say compared to that compared to that when the material is put inside when when when the sample is put inside the sample box we see few of the wavelengths that has previously passed without any absorption are now hugely absorbed and it is missing in the detector. So, the detector gives a huge huge absorption peak chlorophyll a and chlorophyll b this is an example of the UV visible spectra chlorophyll chlorophyll a and chlorophyll b is are part of ah chlorophyll high school ah biology just read it up chlorophyll contains these two part performs different functions and you can see what are the colors in the visible spectrum that is mostly absorbed by chlorophyll 400 to 500 nanometer is very much absorbed around 650 to 700 are very much absorbed this is the region 500 to 600 nanometer is not absorbed is not absorbed if it is not absorbed that means, it is reflected what color is reflected when you look at leaves this color right and as as the leaves get older or die I either chlorophyll their chlorophyll reduces or dies out have you seen the color of the leaves changes to this particular frequency meaning they have started reflecting this meaning they are no longer absorbing this particular frequency meaning chlorophyll absorbs this particular frequency this particular frequency is not absorbed can you say chlorophyll is not present and if chlorophyll is not present maybe the leaf is not going to perform a particular function like photosynthesis and perhaps it is dying I am just giving an example think about it. So, this pi to pi star this this this band is the pi to pi star ah C double bond C ah C double bond O formation and this was first identified by the French scientist Sore the T is silent I think, but if you know French correct me how to pronounce it and this is the pi star bond ah tell me which which ah electron it would be sigma to sigma or n to pi is this lower energy or higher energy is this lower energy frequency lower energy or higher energy and if this is whatever it is what band or what particular electrons ah do you think are absorbing think about it.

So, UV visible spectrum is very very simple you have a monochromator is a light filter at one time it allows one frequency to pass through tungsten lamp produce thousands of frequencies consider seven different frequencies for the timing V i B g Y O R it allows at one time 400 nanometer then 450 depending on how you have calibrated it depending on how sophisticated your monochromator is depending how much money you have spent. So, 400, 410, 420, 430 like that or 400, 450, 500 or 400, 500, 600, 700 allows one particular frequency to come through at a time that is passed through reference empty material versus sample empty material will show no refer no absorption detector will give you a flat line or something similar near about flat line sample maybe one light is in pass through one particular light is pass through zero absorption one particular frequency of light one particular wavelength of light is passed through zero absorption another frequency of light is completely absorbed detected do not get it. So, it it it near about 90 percent absorption at that particular frequency it shows and you can simply map and this absorption versus wavelength wavelength versus absorption or transmission of reflection or particular chemical species is called spectra spectra analysis of spectra creation of spectra is spectroscopy yeah.



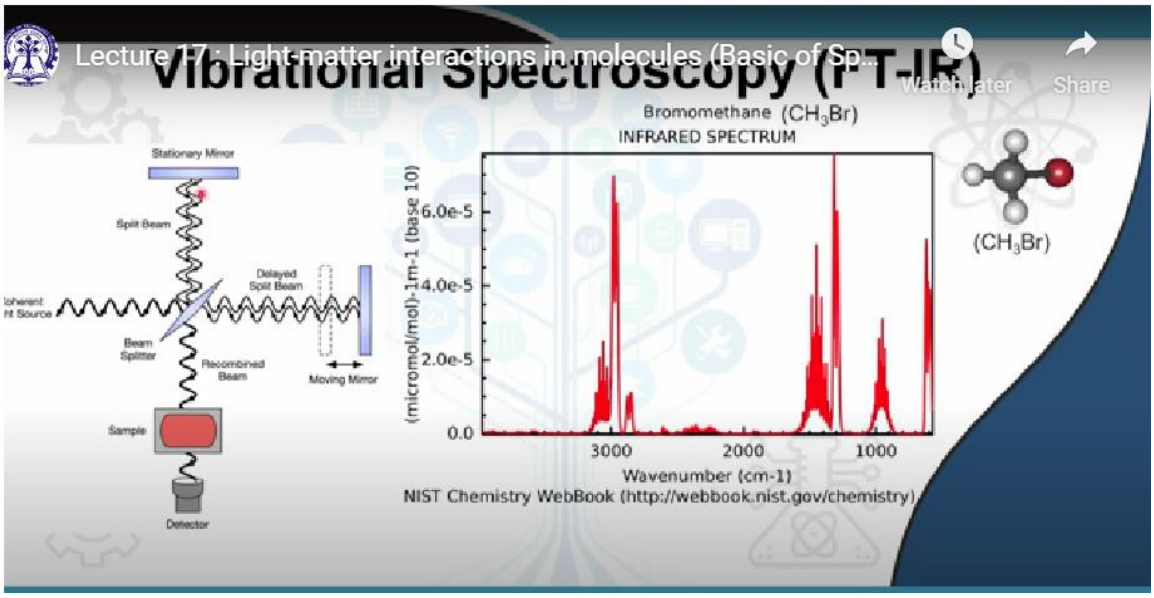
So, this is UV visible spectra then comes infrared spectroscopy infrared spectroscopy deals with vibration of molecules in the previous case we have electrons absorbing electrons absorbing and going from lower level to higher level electrons vibrated higher frequency in order to vibrate them further you need far higher energy of light UV or visible hence UV visible spectroscopy, but in this particular case you need infrared light infrared is always associated almost always associated with heat because molecules absorb this and molecules perform their dance these are the different types of way in which molecules can

vibrate this is not an exhaustive lips they are several several other way molecules vibrate I call them the dance of the molecules and all of these frequencies all of these frequencies all of these frequencies are quantized they are very very specific all of these frequencies can be given a particular number 400 nanometer per micron 5 micron 5.2 micron 8 micron 9 micron 10 micron 12.

2 micron they are very very specific a particular molecules say you can consider it as carbon dioxide carbon and 2 oxygens say it can be hydrogen water as well H2O, but say if it is carbon dioxide there are anything anything they will absorb a specific specific frequency of light they will absorb a specific specific frequency of light and and they will vibrate at a specific frequency these two are one and one only carbon dioxide will not twist unless you provide a specific frequency it will not twist. So, it is very well called as signature or fingerprint like your signature your fingerprint is unique quite similarly molecular vibration is also specific very very unique particular frequency of light getting absorbed allowing it to make a vibrational movement in a specific way symmetric stretching will happen at a particular frequency asymmetric stretching will happen at a particular frequency for the single single molecule all molecules are same in this particular case look at the color code all molecules are same in the particular case they are all molecule they all absorb different frequencies of light and thereby vibrate differently these vibrations are tied with the frequency of light they are absorbing and nothing will allow them to change it frequency a results in vibration a frequency b results in vibration b frequency c will result in vibration c no exception no exception whatsoever think about it in I think I have discussed this before, but for those of you are new you keep on hearing in news all the time every few months that NASA has discovered a particular planet few galaxy away or few light years away that has earth like atmosphere you know exoplanets exoplanet has been discovered by Hubble telescope NASA's Hubble telescope in space that has that is like a twin of earth or that has atmosphere matching that of earth or that has water in that planet that is rotating around a particular star few light year away from us how do they know how do they know have they sent a probe that far away they have not sent that far away the highest thing Voyager I think has just cross our solar system man made object that has crossed solar system is a satellite or it is a space craft called Voyager to the best of my knowledge they have not sent that far away space craft. So, how are they identifying it they are analyzing the light that is reflected from that planet Hubble telescope is measuring the light that is reflected from that exoplanet analyzing which are the wavelengths that are missing for over a year if a particular wavelength is missing that means, a particular wavelength is being absorbed by a particular molecule there is fast space between that galaxy that planet and us there is nothing in between that could absorb there is not water vapor or anything in between us yeah it is vacuum it is space that is the point vacuum means it has nothing. So, the light is coming light is reflected from that planet is absorbed is is is detected by Hubble telescope they are measuring it they are

identifying it and trying to see what are the molecules that absorbs that particular frequency water absorbed 3.

3 micrometer as I said. So, whatever light that is getting reflected out of that planet out of that exoplanet so far away they are analyzed and if they continuously for a year see 3.3 micrometer is missing that means, the atmosphere of the planet has absorbed 3.3 micrometer what molecule absorbs 3.3 micrometer there is only one molecule H 2. So, you can identify far away planet without even going that that place contains water.



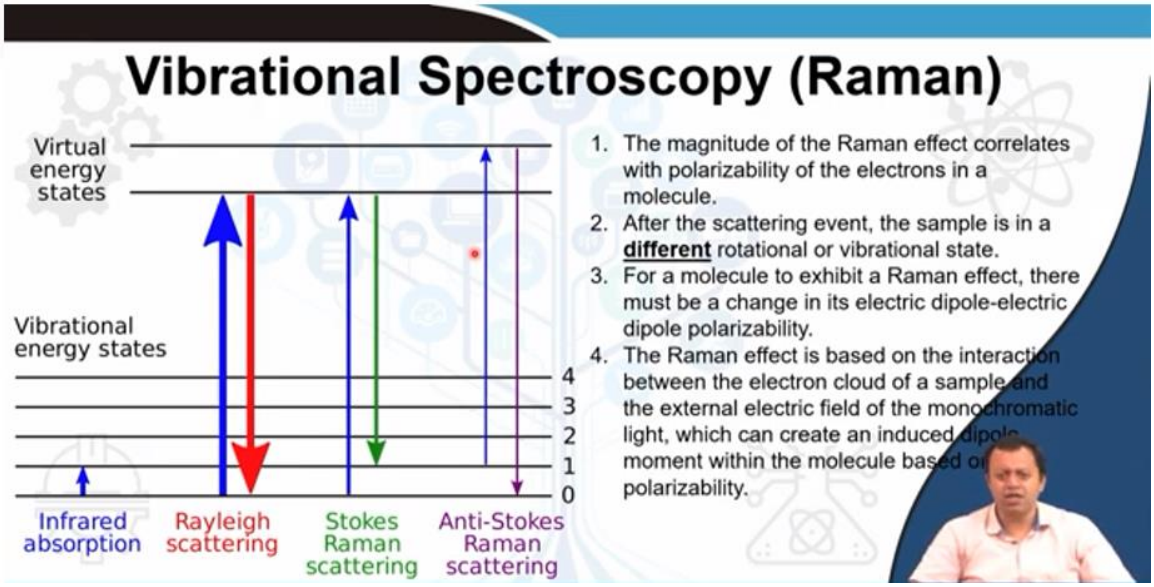
So, if you can use it in telescope can you not use it in microscope and that is basically what vibrational spectroscopy infrared spectroscopy to a better sophisticated version of vibrational spectroscopy is Fourier transform infrared spectroscopy. In Fourier transform infrared spectroscopy you have two mirrors one is a stationary mirror one is a moving mirror the moving mirror moves few centimeters a coherent light source is sent which sends bunch of frequency of light bunch of frequency light not one at a time like the previous case this is the beam splitter I was talking about the bunch of frequencies falls into the stationary mirror some falls onto the moving mirror they again return back they goes and then return back they goes and return back mirror at the end of the day you have to reflect. So, at the beam splitter these two beams which has previously been splitted will recombine and will pass through sample some of it will be absorbed some of it will be reflected some of it will be transmitted it will detect those light which is absorbed the mirror is then moved a little bit and the same process is done. So, depending on the movement of the mirror depending on the movement of the mirror this light that is returning back combining with the stationary mirror will have constructive or destructive interference. So, you have sent a bunch of light another bunch of light they are combining this combination will depend on how far the light has travelled how far this light has travelled depends on the position of the mirror if the mirror is closed mirror is close the

less distance if the mirror is far different distance depending on the distance phase will change remember phase depending on the distance of the mirror the phase will change depending on the phase when it will have either constructive or destructive interference with the stationary mirrors beam combining you do it bunch of times several number of times by moving the mirror you will get different wavelengths falling onto the sample different wavelengths falling into the sample of different intensity depending on how much it has interfered how much constructive and destructive interference have taken place and how much amount of absorbing the sample is how much absorption the sample have had the detector absorbs detector gets the transmitted sometimes if you put the detector here it can also get the reflected light and figure out 1 is equal to a plus t plus r the absorbed frequency the absorption peaks are given at different wavelengths and you identify the molecule according to their characteristic frequencies that they absorb.

Now this why you call it Fourier transform this movement of mirror the movement of mirror is in centimeter right the mirror moves few centimeter at a time resulting in different distance being travelled by the this beam number 2 this beam splitter divided into beam 1 and beam 2 beam 2 travels different distances depending on the movement of the mirror far away close that results in change of phase, phase combining with this beam 1 results in specific amount of interference of destruction constructive or destructive interference resulting in this form. We convert the movement of the mirror in Fourier transform. So, we get some sort of a frequency domain, but Fourier transform results in formation of centimeter movement into centimeter inverse centimeter inverse is wave number as you know centimeter inverse is wave number directly related to wavelength you can simply convert it and thereby you can show wavelength with respect to absorption and these are specific specific for specific molecules. Chemists have been utilizing it to identify molecules we are now utilizing it to identify biomolecules biomolecules mostly organic and thereby try to see a particular frequency or a particular absorption is there or not thereby overall trying to see in a large cell or large tissue the presence or absence of a specific specific molecule think about it every molecule absorbs a particular frequency and that is the signature no other molecule if water absorbs 3.3 no other molecule will absorb 3.

3 not all peaks of any other molecule will match that of a water molecule H 2 O has a specific signature like you have a specific signature like you have a specific thumbprint no other person has specific thumbprint or your retinal scan or something like that similarly all 10 fingers 1 or 2 might match somehow some coincidentally, but all 10 fingers all 10 fingerprints of yours is not possible to have with another human being similarly all the different motions of H 2 O is fixed no other molecule will have the same other vibrational frequencies same other absorption ranges so you can very well identify now suppose you have thereby divided a normal cell cellular components with particular frequencies that it

absorbs now a particular cell has come to it you have put it into the sample and you have measured it you are seeing a different frequency being absorbed can you thereby say that a foreign body has invaded that cell remember all these things this light combining etcetera happens in few seconds yeah less than few seconds because these are all light so thereby can you detect the presence or absence of a foreign particle foreign body pathogen antigen in the tissue or cellular structure think about it can it thereby be utilized for detection of disease communicable disease where something external has come so that is vibrational spectroscopy similarly another thing that is

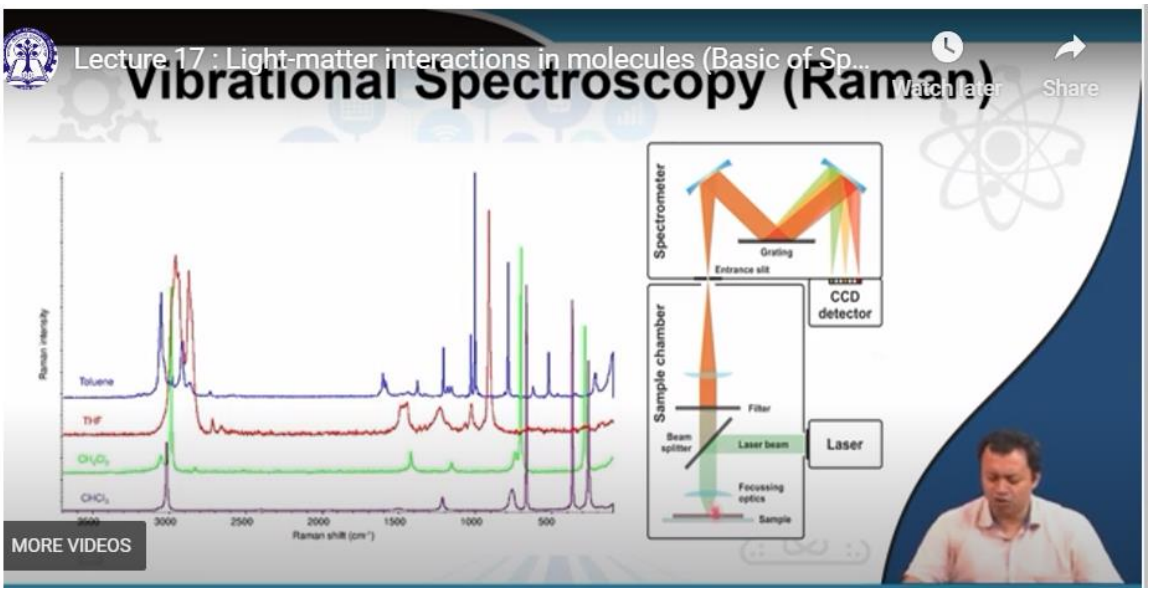


available for us to utilize is Raman spectroscopy so in Raman spectroscopy what actually happens is they if there is a molecule surrounding if a molecule surrounded by its electron cloud an intense amount of photon hits it suppose it is not getting absorbed suppose it is not getting absorbed it might so happen that it is not getting absorbed but still the electric field is affecting the electrons the electron cloud the electron cloud still has some amount of a function they have moved they have moved from one area to another area resulting in change of the polarizability of the molecule resulting in change rearrangement of the electron cloud surrounding the molecule you have collected back the scattered photon the photon is not absorbed it simply scattered however the scattered photon is not of the same frequency as that not of the same energy same frequency same wavelength as the incoming photon it has either absorbed some amount of either it has imparted some amount of its energy to the molecule or it has absorbed some amount of its energy it has taken some amount of energy from the molecule both are possible. So the Raman effect is based on the interaction between the electron cloud of the sample and the external electric field of the photon monochromatic light which can create an induced dipole moment changing the molecular polarizability for a molecule to exhibit Raman effect there must be a change in its electric dipole electric polarizability the photon will not simply be consumed so this is

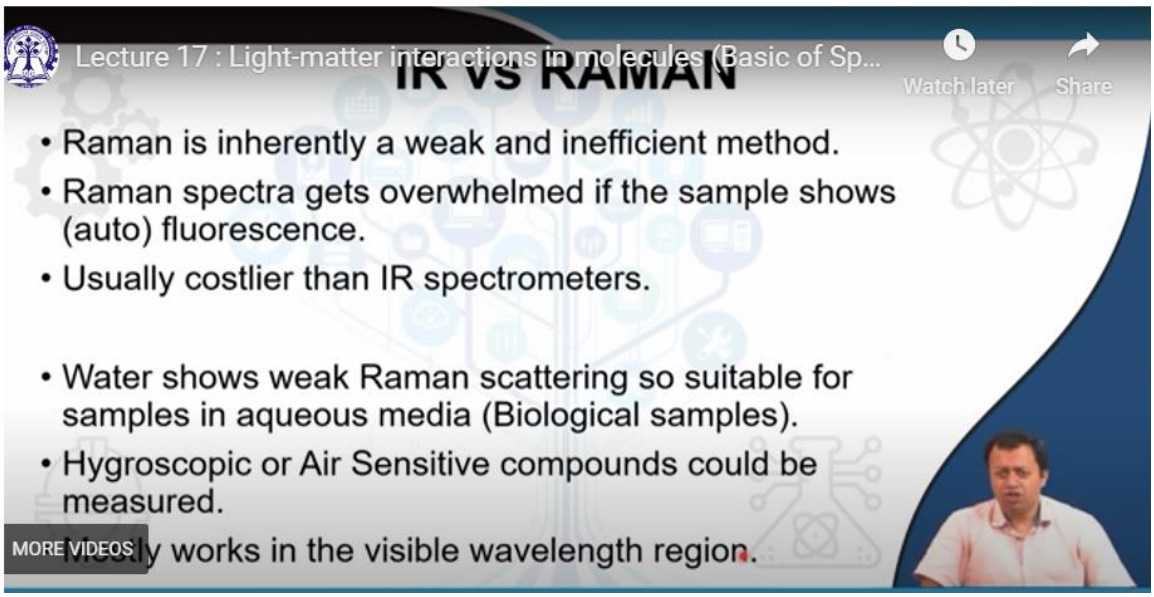
infrared absorption the light is consumed the photon is consumed by either the electron or the molecule resulting in either emission of a particular frequency or emission of heat or vibration or something like that Raman is scattering remember scattering and absorption are analogous to one another the result more or less is same result more or less is same, but scattering and absorption are fundamentally different property. So what happens is three of the possible things is possible are available when Raman spectroscopy is taking place the molecule absorb light goes to a very high energy state because you have given compare to infrared very very high energy and these are virtual states very unstable immediately the electron or the molecule return back and you get the exact photon back to elastic scattering the interaction of light with the matter is negligible you can simply state that input is equal to output that is Rayleigh scattering. Then there are two other scattering stokes and anti stokes in which if the molecule was at a specific energy level subjected to intense light it you have subjected to your intrinsic intense laser light the electron cloud has affected they have rearranged they have not necessarily rearranged they well they have not necessarily absorbed they have rearranged themselves and the molecule which was at a ground state goes to a virtual excited state and has returned back, but it has not returned back to the exact ground state it originally was. If it has returned to a state which is of a higher energy than what it originally was this much energy is lost and this is the gap between energy state 1 and energy state nil ground state versus 1 energy state and that is reflected in the scattered photon that you are measuring input light it has done something to the molecule the molecule has gone up returned back, but not exactly to the ground position slightly above the ground position.

So, the light that has come out energy has to be similar input energy has to be output energy. So, it has not come back to its original position it has written some amount of extra energy. So, the light that is coming out the scattered light the output light has a mismatch of this particular frequency that is particular energy this is measurement of the electronic state opposite is also true suppose the molecule was at state 1 not ground state slightly higher than that the light has hit it the molecule the electron has changed electron cloud has changed its position ah polarizability has changed after going to a very virtual energy state it has returned back to the original ground state there is still a mismatch, but here the mismatch is positive here the mismatch is negative the photon that is scattered photon that is coming out will get this energy back. So, here in both cases stokes and anti stokes the scattered photon the scattered photon will have either higher energy than the input photon or lower energy than the input photon where is the higher and lower energy coming from is coming from the system either it has given some amount of its energy into the system or it has taken some amount of energy from the system this some amount of energy given or taken is quantized is quantized and it determines the overall energy states how many energy states are available what are their gap what are the differences between them it can be given by this delta E this delta E between input and output Raman spectroscopy is

therefore, very very important in to understand the internal energy levels of a molecule C double bond C versus C single bond C versus C triple bond C will have different energy states internally different molecular energy states internally where it will go and return back and they can be very nicely resolved using Raman spectroscopy. Remember IR spectroscopy is absorption simple absorption light goes comes back low energies thing Raman spectroscopy on the other hand is changing the polarizability it is changing the polarizability of molecule and trying to see scattering scattering and absorption are fundamentally different, but analogous one is analogous to one another.

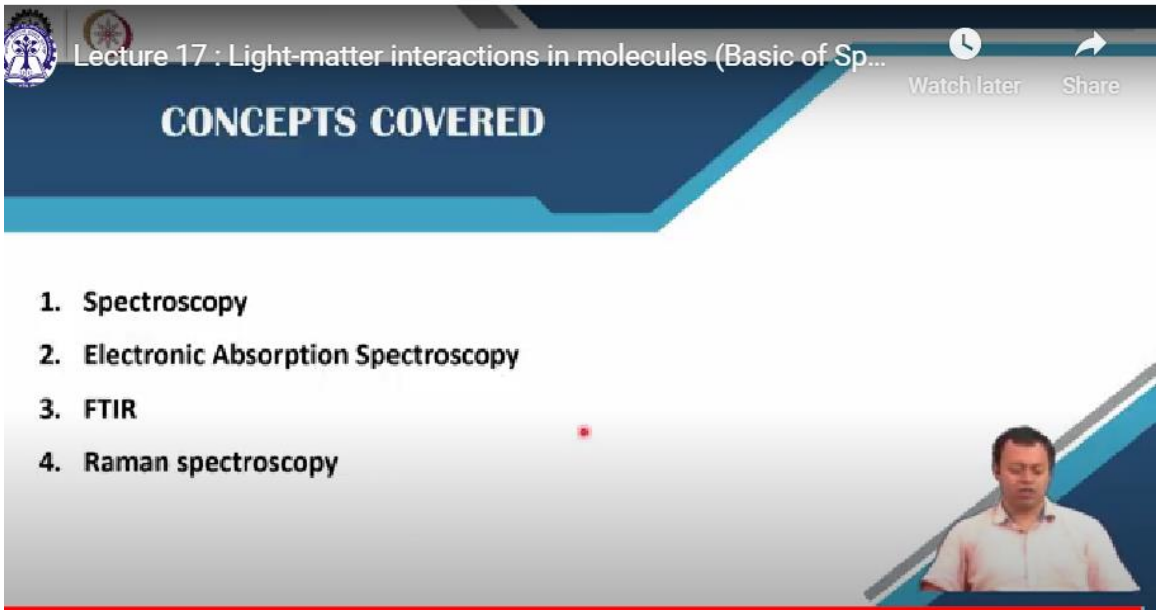


So, in order to understand a material properly you need both infrared as well as Raman spectroscopy. So, this is the particular ah equipment in which a laser beam is made to focus on the sample the sample reflects the light it goes through the beam splitter and then put into a spectrometer the grating breaks down all the frequency of light that is falling on to it into its individual components and thereby we measure whether this light into the output detector is of a different frequency than the laser beam that has been sent whether it is increased frequency or decreased frequency and the gap this gap between this and this can be corroborated with the energy gap of the particular molecule thereby we map the different orbitals different energy levels in which the molecules exist.



There is a huge debate that goes on between which one is better infrared is better or Raman is better please do not go into a debate these are not competitive they are complementary it is futile to say whether absorption is important or scattering is important any material at specific frequency will have all the components combined it will have absorption it will have scattering at a particular frequency absorption is more scattering is less at another particular frequencies of the same material absorption is less scattering is more they are two simple different phenomena it is like saying which is better day or night you need to have both you need to understand both in order to function this is a direct comparison between IR versus Raman, but if anyone ask you I get this question why you have done IR why you have not done Raman why you have done Raman why you have not done IR basically it depends completely down to economics which equipment you have what equipment you have I want to do both infrared and Raman to understand a completely unknown sample, but I do not have it I have only infra red I have I therefore, I did it it depends on your sample what are you trying to figure out at the end of the day you are trying to figure out the vibrational spectra or you are trying to find this scattering spectra what do you want to understand what are the light that is getting absorbed or what are the light that is getting scattered for in order to identify carbon carbon double bond or carbon carbon single bond Raman is better if you are trying to identify a difference between graphite and diamond Raman is better, but if you want to determine simply whether carbon is present or not present in a complicated molecule which is completely unknown I would say infrared is slightly better. At the end of the day everything boils down to economics Raman is very costly you use laser you use detector and it is inherently weak not all molecules will change its polarizability upon you know excitement by ah infra high intensity light also it has several amount of problems with fluorescence etcetera. The biggest advantage for biologist is that ah water does not scatter ah light and show Raman effect.

So, for biological samples it is very easy whereas, infrared is completely absorbed by water. So, most of your light will be absorbed if you are looking at a ah nano scale biological matter your ah environment has to be sanitized or the atmosphere water can absorb infrared light and cause problems, but overall overall Raman and infrared are spectroscopic method that are complementary to each other in order to fully understand a biological phenomena we need both of them. One is not better than the other one is not better than the other because one is based on absorption another is based on scattering absorption or scattering are not ah better than one another they are simply different they are simply analogous than one another you need both for understanding a proper material the optical property of proper material is better understood when you have understood the absorption profile as well as scattering profile same in here as well.



With this I would like to finish I think I took some more time,

but overall these are my references and I will see you in next class. Thank you very much.