Biophotonics
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Light-Matter Intersection in Molecules
(Basic of Spectroscopy)

Hello, and welcome to the biophotonics course, thus far, we have done our revision on what biophotonics is, what the nature of light is both as a wave and a particle, and then we have discussed the basic of matter. Afterwards we have discussed the interaction of light with matter. Now, since biophotonics is dealing with biological matter, it is but natural that our natural progression from our previous chapters previous modules to this one is going to be interaction of light with biological matter.

Now, I have overall in general divided the biological matter into three different parts, molecules, cells and tissues, you will understand immediately why this decision was made, but at the same time, you can intuitively think that molecules cells and tissues or a combination of this creates combines or makes all biological matter per se. So, in today's class, we are going to discuss the light matter interaction with molecules.

In subsequent classes we will be discussing specifically about light matter interaction, lights interaction with cells and then lights interaction with tissues. But for today's class, let us discuss light matter interaction in molecules, which basically is spectroscopy. So, let us make a start.

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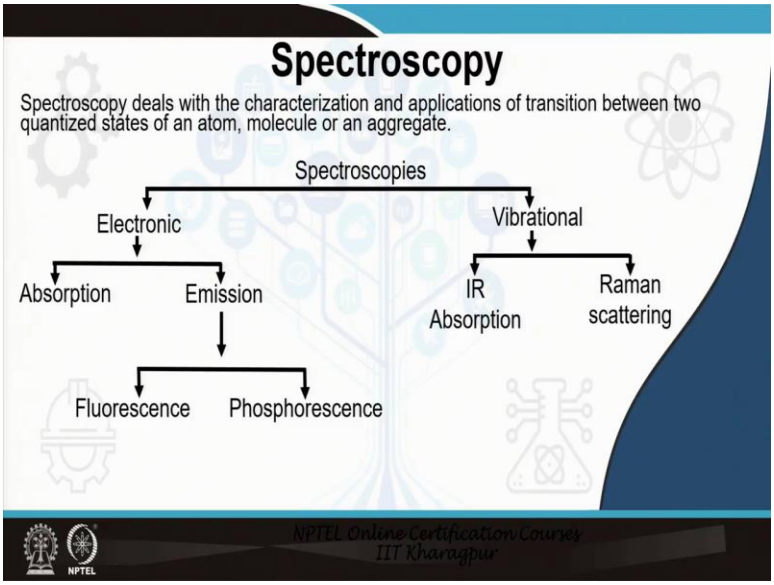

Spectroscopy is the branch of science which mostly deals with characterization and application of transition between two quantized states of an atom molecule or aggregate. Now, what does that actually mean? It simply means, that molecules or part of molecules absorb light. Molecules or part of molecules, what do I mean by part of molecules I means electrons, electrons are parts of atoms electrons are parts of molecules.

Here when I am talking about part of molecule I specifically mean the electron cloud enveloping the overall molecule or the complex molecule as such conjugate molecule as such. And upon absorption of light, they modify they change, there is some kind of change that occurs they move from one quantized, one discrete energy level to another specific quantized discrete energy level. Remember, I cannot stretch this enough, I cannot repeat this enough that the energy levels in atoms in molecules are discrete, they have quantized specific values, they are not continuous.

So, upon excitement by light, either the whole molecule or part of the molecule gets energized, absorbs that photon, absorbs that light and move to a separate energy level. That energy level and this present non-excited energy level has a gap. We look, we identify, we detect molecules by sending these kinds of light trying to see where they have moved, every molecule have their characteristic feature, every molecule have their characteristic discrete quanta energy levels, which they move upon absorption of specific wavelength of light, specific photons.

And if we can identify that specific wavelength of light has taken a specific molecule to a specific level. We back calculate that this is a characteristic feature, this is a fingerprint, this is a signature of a specific molecule. Now, let me ask you a question. All of you must have read in new stories, magazines, televisions, newspapers as such that every few months, NASA or European Space Agency from the space-based telescope found exo-planet, a planet which revolves around a star which is other from our sun, hence the name exo-planet.

And several of these exo-planets are similar in their environment to that of earth. And they are fifty light years, hundred light years, five hundred light years away? How do we know this? How do we actually know that planet which is revolving around the distant sun in either a separate galaxy altogether or in a far-fetched corner of our galaxy, several miles, several kilometers away from us, has water or has environment, which is matching to that of Earth.

We have obviously not sent a probe, we have obviously not sent spacecraft that far, the farthest spacecraft, probably voyager had gone just outside the edge of our solar system. And that took a tremendous engineering feat. So, how do we recognize or how do we understand or how do we detect the presence of say water or oxygen or carbon dioxide in a planet, which is thousands of miles away without sending any rocket or any spaceships per se.

We do it using spectroscopy, we detect the light that is coming from that planet, we detect, we analyze the light that is being reflected by that planet. Yes, it is incredibly feeble, it is incredibly feeble. Thus that far away and usually the light reflected by the planet will be merged with so many different lights coming from other salacious, other luminous bodies. But overall, we now have the capacity to analyze light coming from that far from a specific small area very, very small areas compared to the surrounding to compare to the sun, it is revolving or compared to the galaxy or compared to the constellation where it is located.

Not only we filter out that light, we analyze it and then make a claim that this light reveals that the area from where the light has reflected or the area from where light is being emitted, contains elements, contains molecules that could give rise to life. That is the advancement that we have done thus far. And a significant portion of that is because of spectroscopy. Spectroscopy analyzes lights coming out of molecules and thereby identify them not just molecules, atoms, conjugate complex molecules and thereby you identify the matter itself.

You identify the molecules, you identify the bonds and you identify the matter themselves. So, broadly speaking, spectroscopy can be divided into two separate categories electronic and vibrational. Electronic as the name suggests is where the electrons specifically absorb that particular photon that particular wave that particular light goes to an excited state and we see the manifestation of this excitement.

The manifestation is either absorption, this light has been absorbed by the electron it moves to an upper level. So, you are sending say ten different wavelengths, ten different bunch of lights, ten different frequencies, one of them is missing one of them is not being transmitted. So, you understand that this particular photon has been absorbed and we can back calculate we understand that the electron probably minds my word, probably have absorbed this specific photon to go to a higher energy level.

That is given by the absorption part of electronic spectroscopy, the other being emission, where upon absorption of a particular photon, upon going to an excited state subjected to absorption by a photon, the electron returns back to its original position and emits either the same photon or a different photon with a different energy. We have discussed at length about that particular emission process in our previous class where we discussed fluorescence a bit of phosphorescence.

Usually in biological matter we mostly look for fluorescence. It is not that phosphorescence in biological matter is completely absent. Some inset, some beetles, some jellyfish, some aquatic plant might have phosphorescence but usually for all intent and purpose when we are trying to detect diseases in human bodies, it is usually we look for fluorescence. And since fluorescence was that important, I took a specific class, **thirty** minutes class dealing just with fluorescence.

It does not mean that the other part of spectroscopy is not important, but for a biological point of view, I had my bias towards fluorescence and hence, I taught that earlier. So, again, electronics part of spectroscopy deals with electrons absorbing energy, electrons absorbing light and either going to upper level or returning back from upper energy level to lower energy level emitting a photon.

So, you can understand that this absorption electronic spectroscopy is complimentary to the emission, the emission part will only happen when the light has been absorbed. So, absorption and emission are complimentary to one another be absolutely clear about this thing, they are not two completely separate things, one give rise to another without any absorption, you will not be having any emission per se.

So, today we are going to discuss a bit about this electronic absorption part, this part has already been covered, I asked you if you have missed that video, go back rewind and you will see that at previous video just end of, just before the biology part just before module three, I believe end of module two there we have discussed flow sensor phosphorescence. The second most important part of spectroscopy is the vibrational spectroscopy.

Vibrational spectroscopy deals with molecules, deals with atoms, electronic deals with electrons, vibration deals with molecules or atoms specifically molecules. Here upon excitement by a specific wavelength of light, upon excitement by a specific photon, a photon with a specific

frequency a specific energy the molecule, the complex molecule that it has formed, the conjugate molecule it has formed, it vibrates.

It vibrates, previously it absorbs goes back to original excited states and returns back electron. This one is a molecule; a molecule has a vibrational moment it vibrates and upon excitement upon subjected to some kind of light wave the vibration changes or vibration modifies or vibration goes to a different level. Now make no mistake, even when I am talking about vibration, it is moving up down, it is vibrating fast or slow it is vibrating different we will see I have beautiful pictures for that gifs.

There will be these states, these vibrational states are also discrete, also quantized. So, if we have specific level and if we have back calculated that upon absorption of photon of energy E1 molecule A moves to state B and if we observe this manifestation that E1 is either missing or a molecule is either in position B, we can back calculate that it has to be molecule A. Molecule A absorbs E1and goes to B.

So, we are identifying the molecule, we are identifying that this planet might have water, this planet might have oxygen, this planet might have carbon dioxide. A significant part of that vibrational spectroscopy is IR spectroscopy IR absorption, where infrared light is absorbed. I will be discussing a bit about infrared today. And then the very famous Raman scattering, Raman spectroscopy based on Raman effect given by Sir CV Raman one of the first Nobel laureates of India, in physics, the other being Rabindranath Tagore.

But let us discuss Raman scattering, we will be discussing electronic absorption, infrared absorption and Raman scattering these three based spectroscopies in today's class. Because the emission part has already been covered in your previous class. So, let us start with electronic absorption.

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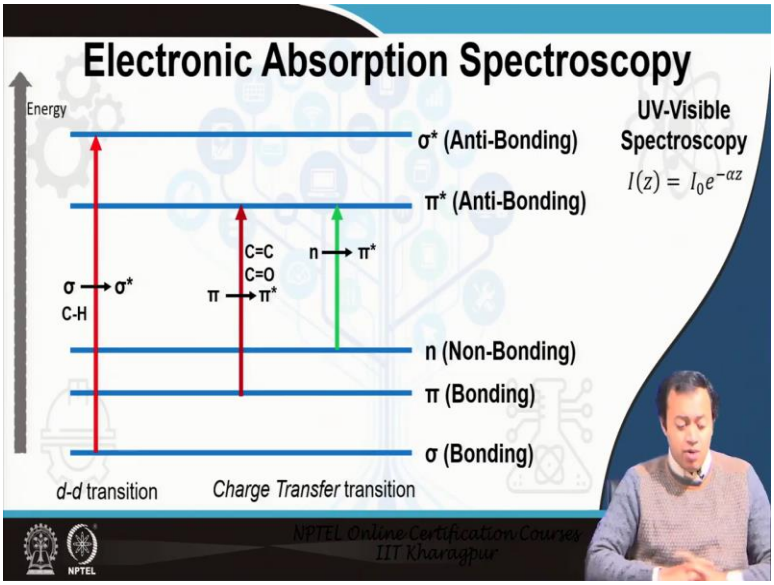

Electronic absorption spectroscopy is also called UV visible spectroscopy or as you cool kid will say UV vis spectroscopy. I think several of you already have seen a UV vis spectrometer. UV vis spectrometer is quite common to have in colleges and universities and labs. So, UV vis spectroscopy deals mostly with the transition of electron in a molecule moving from lower level to higher level, lower energy level, lower energy state to higher energy state.

It mostly follows the Beer's law or the Beer Lambert law. Remember we discussed about this a bit in our initial interaction of light with matter where αis the absorption coefficient, Z is the thickness $I_0$is the intensity of light, when before light is entering a particular material and this is light is entering a material in up till Z direction, this is the length basically that has been covered.

So, there are several transitions that can happen electronically electrons can move from several different layers, several different levels beg your pardon not layers levels, lower level to higher level. But usually when it comes to electronic absorption spectroscopy UV visible spectroscopy, we mostly deal with these kinds of transition. We deal with electron present at a σthis bonding molecular level bonding energy level to σ*anti-bonding level, πto π*bonding level or a non-bonding to a π* bonding level.

These are the three most common transition of electrons from one energy level in a molecule to the other energy level in the same molecule. Remember the same term will be important this will

come important, this will attach itself heavily when it talks about charge transfer. But first let us understand this. Remember we discussed a bit about σ bonding S bonding. There is bonding and anti-bonding orbitals, bonding where the attractive forces prevail, there is the energy has whether it was reduced or increased, can you tell me?

I was discussing about it remember Schrodinger wave equation length increases or length decreases. I am not going to repeat that you should be remembering this by that time by now. Anyway σ bonding where you can look at this energy, the energy has reduced, the electron moves from the lower level to upper level to a σ* anti-bonding. So, a specific photon needs to be injected, a specific photon needs to be absorbed.

Only when this specific photon is absorbed the electron present in this bonding level the σ energy level goes to σ*level. Remember I told you anti-bonding is the place where your molecule starts disassociating, your molecules break down, but you will immediately say that but electrons regularly go to sigma star anti-bonding level and returned back. Well, remember the key is returned back it returned back quite fast in pico second, femtosecond or nanosecond term, maximum fluorescence spectroscopy happens in nanosecond terms.

Meaning if the electron stays for a very long period of time at the sigma star anti-bonding level, well, anti-bonding will happen, if there is anti-bonding, what do you think will happen to the molecule which has bonded? So, sigma to sigma star jump of electron usually happen in CH bond, carbon hydrogen bonds. Which are quite common, but this is also requires huge amount of energy.

σ bonding has the electron very close to very stable very nicely attached to the nucleus to break it to take it, to the sigma star level you need to actually supply huge amount of energy and this energy is usually around 125 nanometer that takes you to deep ultraviolet. Deep ultraviolet where electron starts absorbing any kind of electron present in C and H type of bond starts carbohydrate basically are any C and H are very very common any organic compound basically has CH bond.

They start absorbing and they start going into anti-bonding which by definition is destabilizing or less stabilized then σ bonding. Then you have π to π* bonding. Usually conjugate molecules are

π bonded. So, you will see a lot of π to π*bonding from π bonding to π* anti-bonding level they move upon getting a specific photon upon absorbing a specific photon. And this happens in C =C, C = O.

This is also quite common, there are non-bonding orbitals you understand what non-bonding is we discussed it a bit nonbonding orbital is higher than σ and π but lower than any of the excited states. And you can also make the electron move from non-bonding level to a π*bonding level. This happens usually, when electrons in oxygen molecules move from either C = O to C = C or similar things.

π to π* is one of the commonest transitions that you will see in conjugated biological molecules, but make no mistakes all other are also available in terms of energy σ to σ* is highly energetic, it requires a huge amount of energy to break it, π to π* is moderately. Nonbonding to anti-bonding is less than that other than these two transitions of molecules from one level to another level, there is DD transition molecular orbital D to D transition and charge transfer transition what does that mean?

D to D transition as σ to σ* transition or π to π* transition means D orbital to another D orbital. Now, you understand that for D orbital to exist it has to be elements with slightly higher atomic numbers, you do not get D orbitals in normal organic compound methane, butane, etc do not have particularly correct me if I am wrong D orbitals. Normal carbon and hydrogen, D orbitals are usually present in higher atomic number to the best of my knowledge chemistry will correct me.

It is usually organo-metallic molecules, organic molecules which contain a metal counterpart. Example, hemoglobin, remember hemoglobin is a protein you all know where hemoglobin is present. Hemoglobin contains iron, this iron helps carry oxygen and this oxygen gets transported all over our body. So, here upon attachment or detachment of oxygen to the iron that is connected with the hemoglobin which is an organic protein, which is an organic molecule, you can sometimes see D to D transition.

So, usually materials such as Porphyrin etc I am forgetting it. They contain this organometallic, these are organometallic compounds, organo metallic molecules they contain zinc or manganese

I believe and these are very complex conjugated molecules. So, organic compounds containing a metal group therefore, D orbital and therefore, they have some combine of transition when they are performing a specific function.

Charge transfer transition is also quite interesting, though I would not say it is uncommon, but it is relatively less common as compared to π to π^*. Charge transfer transition is when an electron at a hormone level highest occupied molecular orbital goes to the LUMO level, lowest unoccupied molecular orbital of a different atom, of a different molecule. Remember, all these transition from lower level to upper level is happening in the same molecule.

Even DD transition σ σ^* transition is happening in one single molecule complex it might be but it is one single molecule, one single complicated molecule organometallic molecule where electron is moving from one level to another level charge transfer. Hence, the term transfer, transfer happens when the upon excitement, upon absorption of light a particular electron of a lower level of molecule A moves to the upper level of molecule B.

HUMO to LUMO transition, but a transfer from one molecule to another molecule charge transfer transition. So, a combination of all of these gives specific energy levels you understand a specific number of photons, a specific amount of light energy, a specific wavelength of light which specific frequency needs to be supplied to breach this particular gap. And every different conjugate molecule however complex it might be having these kinds of energy levels inside their molecules.

So, each specific conjugate molecule has specific energy levels specific energy gap call it band gap I do not mind, specific band gap and if you are seeing, if you are seeing that some amount of energy has been absorbed and that corresponds to the band gap that corresponds to the energy gap between π bond π to π^*. Can you make an attempt, can you make an attempt to identify the molecule, think about it?

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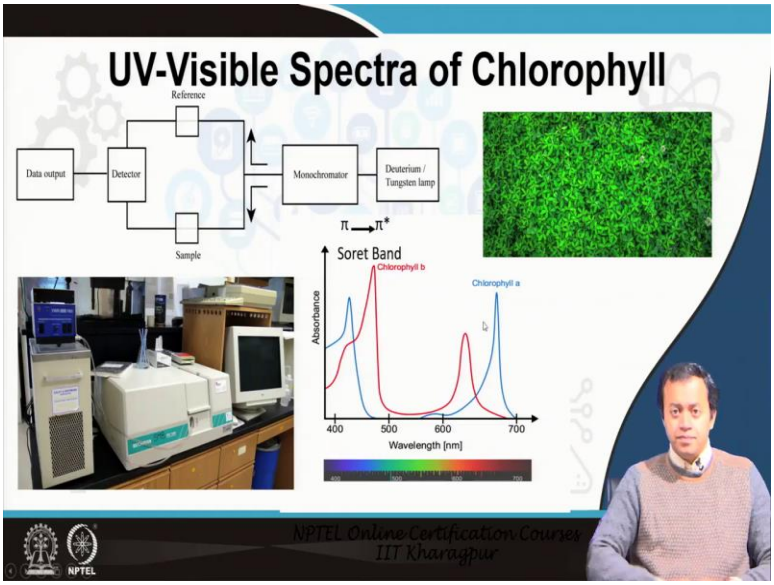

A common example of such UV visual visible spectroscopy is detection of chlorophyll. Several of you might have seen this UV visible spectrometer present somewhere in your lab. How does that work? It has a source, a tungsten lamp, it passes through a monochromator. What does monochromator do? Monochromator provides, it filters basically a light filter, mono means one chromator means color.

So, one color one wavelength it gives one single wavelength of light that is made to bypass, made to pass through two different paths, one is the reference and one is the sample. The reference usually allows the light to completely pass without any hindrance. So, that forms the reference and then you put a sample here any sample which will absorb this specific wavelength of light monochromator one color one wavelength, wavelength means frequency, frequency means energy, one specific energy, one specific series of photons which specific energy.

The sample will absorb the electrons will go to upper level and that how much of this energy is reduced as compared to the reference is being put into the detector and then you do several calculations and you see the data output. A common example is when you try to put chlorophyll. Chlorophyll absorbs there is chlorophyll A and B remember high school biology, I am not going to discuss chlorophyll A and B, may be photosynthesis will come at a later stage. But, chlorophyll has this strong absorption between 400 to 500 nanometers as well as around 600 to 700 nanometers.

Now, this intense absorption around 400 to 500 this is a π to π* movement. This absorption at this particular frequency is characteristic feature of the electron present in the π bonding state moving to π* anti-bonding state. This is the π to π* transition, discovered by gentlemen Soret, or Soret and therefore, it is called Soret Band. I leave it as an exercise for you to figure out what electronic transition happens in a higher energy higher wavelength lower energy level 600 to 700 nanometer.

What do you think is the transition where from which band the electron is moving so that you see an absorption measurement like that? So, this is your UV visible spectroscopy, it is quite common available in several laboratories you put a sample into the chamber, the monochromator passes light through the chamber as well as the reference, the reference without any hindrance allow the energy to pass through, allow the light to pass through.

The sample absorbs we compare reference with respect to sample and get these spectra. Spectra is usually absorption or light intensity with respect to wavelength, the output light intensity with respect to wavelength absorption, transmitting, reflectance are measured using spectra. So, UV visible spectra you understood. Thank you very much. I shall see in next class.