Biophotonics
Professor. Basudev Lahiri
Department of E and ECE
Indian Institute of Technology, Kharagpur
Lecture No-10
Introduction to Fluorescence

Do you use one of these, it is a highlighter, it is a highlighter pen you know something to mark passages or lines from your textbook or any other material. So, that area, that place, that line, that paragraphs become highlighted. You must have used it, if you have not used it you must have seen other people using it. These are available very cheaply I got it for around 15 Indian rupees which is less than a dollar US. We mostly, most of us know what this is, this is a highlighter as the name suggest.

Compared to this how different is this, this is a yellow sketch pen I borrowed it from my daughters' vast array of colored pencils and this can also produce yellow ink you can also mark it, mark your passages in your textbook lines, paragraphs, etc. with this yellow pen. But what exactly is the difference between the highlighter here and the yellow sketch pen that is here, think about it you can pause the video a bit and then think your answer and return and we can discuss this.

So, many of you might have thought that this one is brighter this is a fluorescent highlighter, fluorescent marker this is brighter and this is not. But what if I increase the intensity of the yellow paint, the yellow ink of this normal sketch pen, will it still be as bright as this or this is going to be more brighter, this highlighter, this fluorescent marker. This fluorescent highlighter is always going to be brighter than this yellow pen, think about it.

Well, if you think that the marker, the highlighter, the fluorescent maker is always going to be brighter then perhaps you are right. But why exactly is that, why would the fluorescent marker always going to be brighter, well it is the same reason a yellow light bulb is always going to be brighter than a yellow paint. A light bulb emits light, a yellow paint reflects light, there is always going to be difference between emission and reflection.

What do you ask, well emission in an active process some amount of energy is getting converted and the light that is getting emitted is a process of energy conversion. It might be some amount of light is getting absorbed, some amount of photons are getting absorbed and that is reconverted to a different wavelength and another sets of photon coming out or another different phenomenon is happening.

But emission is always some amount of energy is associated, some amount of energy is created, some amount of energy is converted. Reflection on the other hand is simply passive phenomena, a bunch of photon goes little bit is absorbed, little bit is transmitted and its the remaining portion is reflected, remember the equation 1 = T+R+A.

So technically in reflection, no new energy or nothing actually is getting converted it is simply the light is getting reflected the wave front changes its direction that is what the basic difference between reflection and emission is. This is therefore, an active device, this emits light, this on the other hand simply reflects light. Do me a favor, if any one of you have this marker you must have or you can buy it very cheaply available just mark some pages in your textbook or any blank page and then try to see if this glows in the dark.

Do you think this will glow or this will show something without the absence of light, think about it do this experiment and you will see what I am talking about. Now, imagine the exact same phenomena when it comes to microscopy, you all know what microscopes are. We take glass slides put some amount of sample and put it under the stage there are lenses through which we observe what is the material, what is the sample present in the glass slide in a magnified format.

Now, think about reflective microscopy, light is coming it is heating the sample and then through your lens it is getting magnified and you are observing it ten times, twenty times, hundred times magnified, reflected microscopy light is getting from the sample and it is getting magnified by the lens etc.

Now, instead of reflection think about emission, where when light is hitting the sample instead of the sample reflecting light it is emitting light, instead of a yellow paint you have yellow light bulbs around. the sample starts emitting light. Which sample do you think will be brighter, will be clearer, will be more proper?

Welcome to the course of Biophotonics, and today we are going to discuss about fluorescence, introduction to fluorescence precisely. Now, what exactly is this fluorescence, fluorescence is part of this emission or luminescence as we have discussed in previous class.

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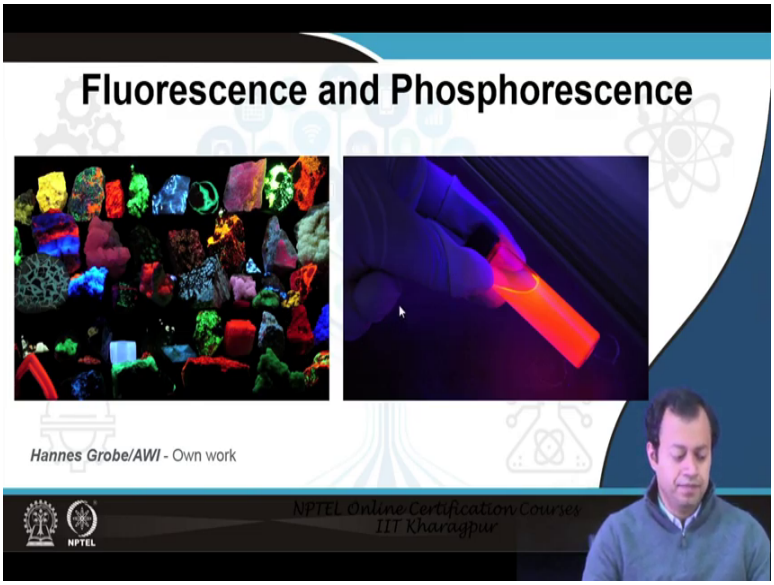

Now, you all know that there are certain materials that glow or when they are subjected to say ultraviolet or light, they emit a specific color of wavelength which is characteristic feature we have seen this in numerous movies, numerous shows.

You can just see them yourself several materials if you have a UV light bulb these are also available and put some kind of salt, some kind of fluorite material under this UV light bulb you will see that they have a distinct glow, a different glow than they were in visible light. And this happens whenever that UV light is present, if you switch off this light, if you switch off this UV light it does not glow anymore this entire process can very well be said as fluorescence, it might not be there are exceptions.

But for today's class, let us consider the fact that they might be fluorescence. I will tell you why it might not also be. Do you possess one of those radium watches, the watches that glows in the dark, you know this process of phosphorescence.

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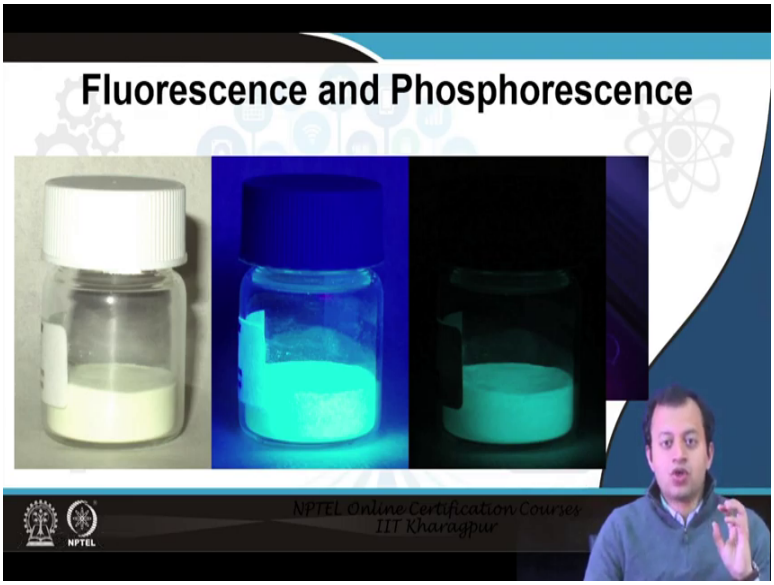

You shine light on it and you switch off the light completely but you still see the material glowing. Previously we used to have these radium watches where if you look at your watch in the middle of the night, pitch black you can still see it glowing, some of the clocks also glow. So, what is the major difference between phosphorus and fluorescence, in fluorescence you will always need a constant supply of light. I asked you to look at the marker here in the absence of light, tell me if you see it glow, my guess is it does not glow. That is because, it is fluorescent.

Fluorescent requires a constant supply of light all the time and that light gets converted into another form of wavelength of light, another frequency and that is what is getting emitted. You switch off the input, output gets automatically switched.

However not in case of phosphorus, phosphorescence takes that light, it works with it and it keeps on radiating that light, it keeps on emitting that light, this phosphorescence long after the original source of light, the input have ceased to exist long after the light has been switched off completely that is the fundamental difference between fluorescence and phosphorus. Now, let us understand little bit about the history of it and why it has been able to, this fluorescence have been able to revolutionize biology or life sciences in general for good.

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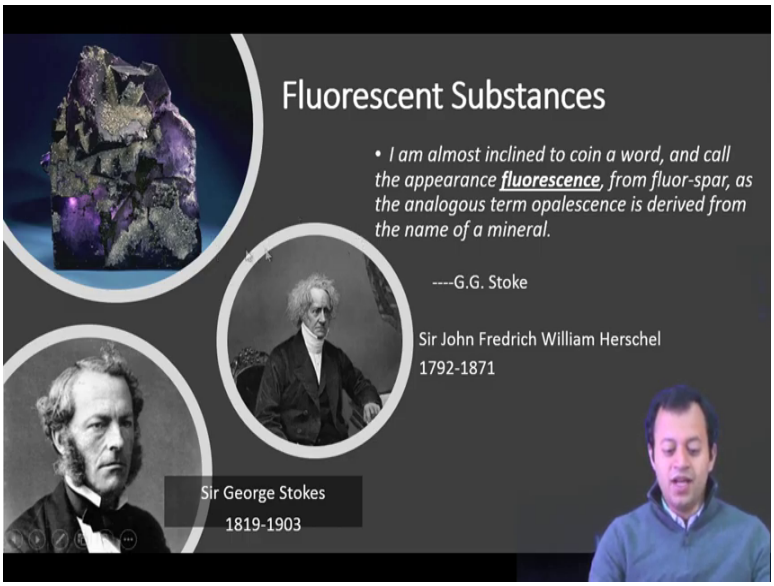

So, it all started with this gentlemen, Sir John Fredrich Herschel in 1792 to 1817. This gentleman, British gentlemen was working on quinine, you know quinine is present, this kind of molecule was first extracted from certain barks in Latin America, South America that is and they help in making drugs, anti-malarial drugs. Nowadays, obviously it has changed, quinine is still used in tonic water.

So this gentlemen Sir Herschel, was looking at emission of water that has quinine mixed onto it and he found out that when you are subjecting it to blue light this solution water mixed with quinine it gives a what he called a celestial blue emission. He reported it but he was unable to explain it properly. But that was the beginning of a certain experiment in this regard. Then came Sir George Stokes, Sir George Stokes was looking at a mineral that time they used to call it fluor-spar, these days we call it calcium fluoride or fluoride basically, calcium with fluorine CaF2.

And he was looking at the emission of it and he was mostly dismayed that is has a very small amount of glow, people want to have light for a longer period of time he would say that after hitting this material with some amount of light an additional, a different kind of light is getting reflected or not reflected per say is emitted. And he coined the term basically, fluorescence as compared to opalescence previously people have opals, opals are semi-precious material that emits different amounts of light and hence used in jewellery and opalescence was a nice term

previously used, phosphorus has been discovered and phosphorus glows in dark so phosphorus was used.

So, Sir George Stokes thought that since the mineral is fluor-spar, fluorite and since it emits a light albeit for a very, very small duration as compared to opals or any other semi-precious materials or phosphorus, let us name it fluorescence, let me call it fluorescence this happens to be Sir George Stokes. Another curious, another curious observation that I made is if you look closely Sir George Stokes hairline look very much similar to mine. So, you do not know what I will discover in future. Yes, I could see you roll your eyes. What professor (())(12:56) does not want his students to roll their eyes upon his jokes. So, anyways.

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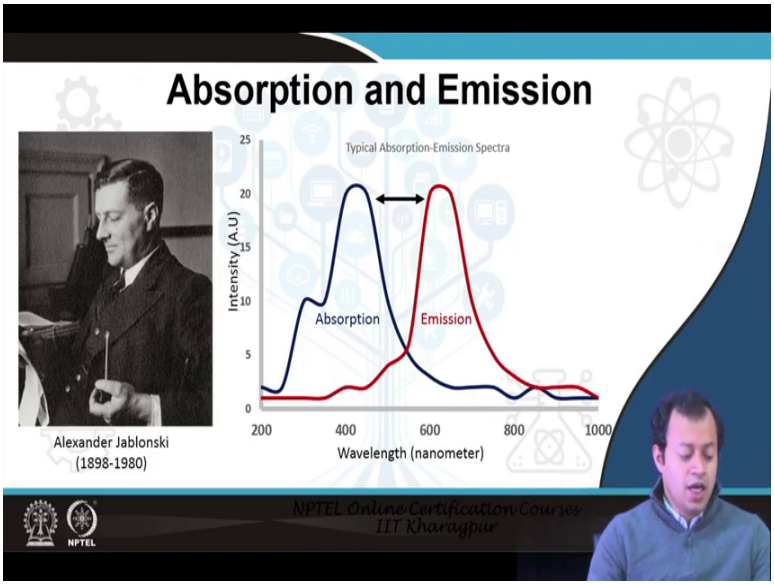

Cut to the nineteenth century, this gentlemen Alexander Jablonski born in Ukraine, Russian federation later joined the newly formed country of Poland started working on the luminescent spectra of several different materials. Just read a bit about professor Jablonski you will find that he is a fascinating personality, a musician above all a soldier. After joining the Polish republic, newly formed he joined the police militia and fought against, I think Germany in World War 1, got medal of honor retuned to academia started teaching, started taking PHD students, started doing research.

World War 2 broke he again joined the army, the polish army fought against first the Nazis, the Nazis captured him put into jail he got tortured, he was released by the Germans after the, after

the Soviets took over and the Soviets put him back to jail again where he got tortured. And then the Soviets released him, well he just brushed everything off and join university of Warsaw and continue the same research which we had left before World War 2.

And this gentleman explained the entire phenomena of the difference between absorption and emission in these kinds of fluorescence material the term fluorophore all of these new descriptions have significant contribution by professor Jablonski. He figured out or he was able to explain in a proper manner that, why we are seeing this difference, a material absorbs light at a specific frequency, it emits light at a different frequency there is always this difference between absorption and emission.

Number two, almost always a majority of the time the absorption happens at a higher frequency, at a higher energy. The emission on the other hand occurs at a lower frequency, a lower energy there is a difference in the energy levels between absorption and emission. And why exactly this is happening, what exactly is going on internally in the atomic scale for material that shows this process of fluorescence, this entire process is called fluorescence where certain material absorbs light and then emit light at a different frequency. It absorbs light at a higher frequency; it emits light at a lower frequency this entire process is called fluorescence.

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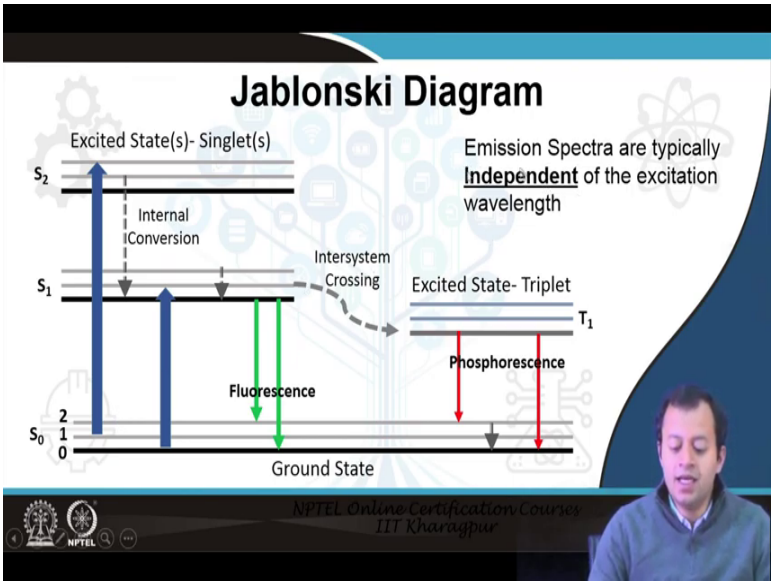

And this gentlemen Jablonski, professor Jablonski explained it using this very famous Jablonski diagram. No fluorescence topic is complete without fully understanding what Jablonski diagrams

are. So, let us try to understand this, this forms the fundamental of any fluorescence studies and you have to, have to understand it. So, like I said previously that for many molecular material, organic material, conjugated material they have energy bands, the energy bands are actually quite thick and each energy band contains this large number orbitals joined together they have sub bands in between they have orbitals in between.

So, the ground state itself contains not one but many. Similarly, the excited states also contain not one but many orbitals overlap to one another they are very close in frequency among each other, they are very close in energy level among each other but they are still distinct, they are similar but not same. Understand the difference between similarity and same, they are similar but not same, so they are joined together.

Whenever you send some amount of so at the very, at the very beginning let us start. At the very beginning in the ground state most of the electrons are occupied, then you subject it to some kind of absorption, you send some amount of light, some amount of photon and the electrons in the ground state moves up to the excited states. Electron absorbs this photon; electron eat this photon and moves up to the excited state. There is no requirement that the electron will have to move to the first excited state it can go to the second excited state or it can go to further and further.

But, the fact of matter remains that these are pi bonding, pi bonding have the delocalized molecules these are actually carbon based or conjugated molecules. So, it might have higher energy levels S2, S3, S4, S5 but they are very, very unstable. The electrons can actually go to any other level but the higher it goes, the higher energy level it goes more unstable it becomes and it tends to return to either the stable or the stablest or the moderately stable level which happens to be S1.

This is the stablest S_0, S1 is also moderately stable S2, S3, S4 are incredibly unstable. Remember the molecules are, I discussed in last class the molecules in conjugated materials they are strongly bound but they are very weekly bound with respect to other molecules Van der Waal forces. So, if you supply more and more energy, they melt organic compounds melt, they have low melting point.

So anyways, even if there is an electron which goes to higher level S2, S3, S4 by internal conversion it quickly falls to the nearest semi stable mode which happens to be the S1 which is

to be the next excited there are several excited states it falls on to the nearest or the lowest excited state and this happen in internal conversion. And this process, this entire process where electron goes to very, very high energy level and then drops back to the original or the fast energy level happens at a rate of 10 to the power minus 12 to 10 to the power minus 15 seconds, you know picosecond, auto second, femtosecond scale.

So, it is very, very difficult to look and actually measure it. After that once it has fallen into the next excited level, the lowest excited level S1 it can move inside the S1 band, it can move there are several different orbitals within the S1 it can move within this using internal conversion, which I told you vibration photonic vibration or basically heat. And from there it jumps back to the original position giving away a photon which we called as fluorescence.

Now understand the fundamental difference, electronics engineers when they talk about electronic jumping from valance band to conduction band, they do not care which part of conduction band or which part of valance band it is going from. In case of organic molecules, in case of molecular materials which part of organic, which part of valence band or which part of conduction band is quite important their because valence band conduction bands are quite thick, they contain several different molecular levels, molecular orbitals and it can jump from either stage 1 or stage 2 or stage 0 to all of these different stages.

Now, electron moving inside a particular band does not require photon to be emitted. It is like as I said, moving within room 1 to room 2 of ground floor or room 1 to room 2 of first floor or moving from lane 1 or lane 2 of a highway, it does not require much of an effort and simply by shaking it off, simply by vibration the electron can move.

But if an electron needs to change the entire highway, if the electron needs to move to a different highway or if the electron moves to go from first floor to second floor it has to do some amount of hard work and that is by emitting a photon and that is done by the process of fluorescence.

So, therefore there is a mismatch there is a gap, there is a difference. The photon that is absorbed takes it to a different level, the photon that is emitted comes from a different level, different sub level. Mark my word, different sub level, different sub level of the same energy band. And this fluorescence happens at a rate of around 10 nanoseconds, 10 into 10 to the power minus 9 second.

And this process, this entire process where there is a mismatch between energy absorbed and energy emitted because of the internal conversion because of having several different sub-bands within a single band is what is the reason of the gap between absorption and emission and the entire process is fluorescence. There is another process and that is phosphorescence. The fundamental difference as I said, in phosphorescence and fluorescence; fluorescence is a fast process it requires constant supply of energy, phosphorescence on the other hand does not require a constant supply of energy.

Why, because well here it gets interesting it gets little bit of quantum mechanics to come in. The energy band here, the S1 energy band under certain circumstances can split, can split it is called spin orbital coupling, do not worry I will not discuss too much detail onto it because this is not a quantum physics course this is a biophotonics course. But understand this, spin orbital coupling happens, what the spin orbital coupling happens, it means that there are three different fundamental forces or fundamental let use the term factors affecting this energy level.

There is this magnetic dipole, there is the spin of electrons and then there is this electrostatic potential that can break one particular bond, one particular energy level into two or three different, further different sub-bands. They can be at higher energy level, lower energy level but splitting, the energy splitting, the energy bands can split under certain circumstances. The spin, last time remember from previous class you can rewind and go to previous lectures.

Usually what happens, the electron here at ground state and the electron at S1 are paired i.e if the spin of electron at ground state is up the spin at excited state is down and these two are paired with one another.

Even if the electron returns to the original position, they are anti-parallel to one another, they are anti-parallel to one and according to Pauli's exclusion principle this is accepted two electrons can stay in the same, around same molecular orbital with anti-parallel spins. However, due to inter system crossing, due to inter system crossing may be some kind of vibration, may be energy split what happens is that when electron moves from this level to this level its spin reverses.

So, now at the excited triplet state the spin is this, at the ground speed the spin is this, when the excited state molecule returns to the original state it is violating the spin rule, it is violating the spin rule and this is not accepted. Since, this is not accepted it require, it basically the electron is disallowed from returning home, unless it changes its behavior it is not welcome back in home and it takes huge amount of time to change and return to the original case.

Because of that you have send energy, this has absorbed energy it has returned back but since its spin has been turned upside down it is having difficulty in returning. It takes all those time to emit energy, somehow shake, vibrate emit energy whatever it can to return to ground state. This is a fast process, this is a slow process; this is fluorescence, this is phosphorescence.

Hence, you see your radium clocks, radium watches glowing at night because they have been excited by light in the daytime and they are, they are emitting light for a long, long period of time with a very feeble, the brightness is much less as compared to the fluorescence, comparatively part by part one electron produces less, high energy of photon in fluorescence as compared to phosphorus. And so, this is the highest the absorb photon, then comes the fluorescence photon that energy is slightly less and phosphorous is even less, of the same type of material if possible.

However, in real life you also see the same material giving you a combination of fluorescence and phosphorus that is also there. So, the fundamental thing you have to understand here that emission spectra are typically independent of excitation wavelength. What does that mean, meaning as long as this hurdle between S_0 to S1 is over you will get a fluorescence.

Because no matter what energy have you submitted or what energy you have supplied if you are not breaking the system, if you are not completely destroying the system it does not matter if it has gone to S2, S3 or S4 because from S2 to S1 or S3 to S1 or S100 to S1 is very, very rapid very, very fast because these are incredibly unstable energy levels, electron does not like to stay there at all as soon as electron gets there if it can without destroying the system if it can returns back at a femtosecond level.

And then once it has a breathing space, once it can breathe it can come back to S1 a semi stable level it within some amount of nanosecond emit the light. So, as long as you have covered the

distance, the minimum distance the threshold distance of S0 to S1 the ground state to first excited state if does not matter it is independent of the excitation wavelength.

So, this energy, this energy, this absorption energy as long as it is above this threshold frequency does not matter you will keep seeing the same fluorescence because transition from S2 to S1 to S3 to S1 does not cause fluorescence they are highly unstable, light can simply electron can simply shake and jump back to the next level.

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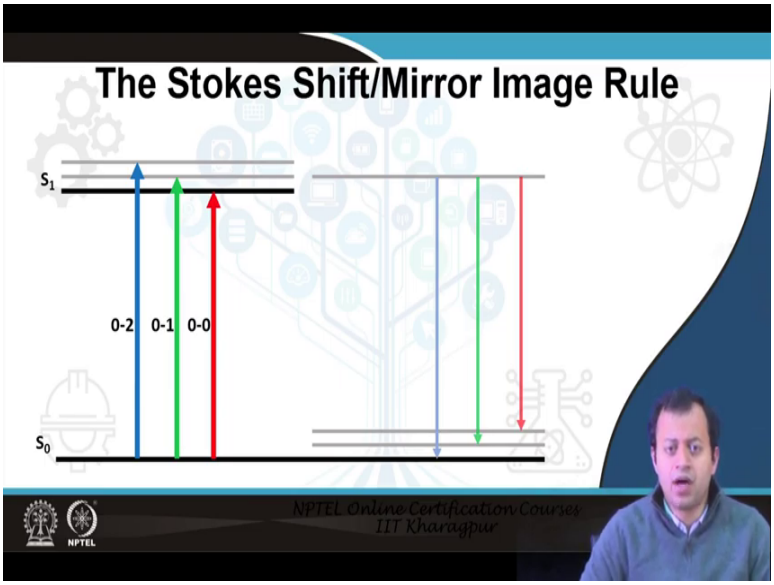

Now, stoke gave reason for this mirror image rule sometimes it is used, sometimes it is also not used but what is actually means is that because of the Frank Condon principle that we discussed last time, since the nucleus are much heavier, and remember the previous lecture nucleus takes huge amount of time to move, electrons can jump at a very smaller period of time the energy level, the sub band splitting, the sub-band arrangement of energy level at higher the nearest excited level and that of the ground states are mirror image to one another.

It is assumed, it is expected and although it is a rule it states that the way this has moved up from zero level to second level, zero level to first level, from zero level to zero level it to can return similarly, in a similar fashion. So, moral of the story what it says is that the shape of the spectra in the absorption will be the shape of the spectra in emission, there will be a gap, there is going to be a gap because the electron that has moved here has come this side and then but there is going to be a mirror symmetry, a symmetrical image between emission and absorption.

The mirror image rule is several times violated it's not always the case that the arrangement of energy levels at this case and this case will be same well the reason for this is they say that the arrangement of this sub-level sub-molecular these molecular orbitals in an energy band is mostly due to the boundary condition put by nucleus.

And even if you have excited the electrons since the nucleus is not moving per say according to Frank Condon principle at a rapid rate the energy the configuration of the energy level is also not changing remember Frank Condon principle I said previously. So, there is a mirror image taking place although there is regular exceptions that we see to that.

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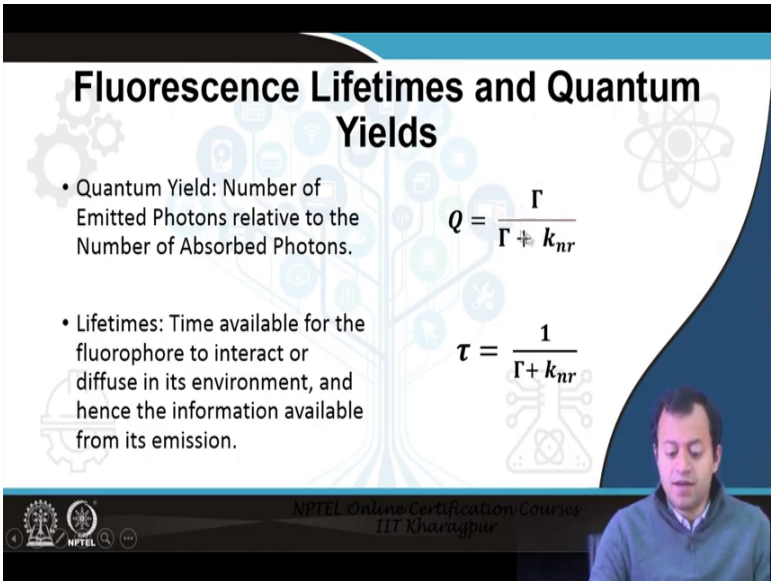

So, several other factors that you need to understand when it comes to fluorescence first is quantum yield and lifetime. Quantum yield basically tell you that emitted photons with respect to number of absorbed photons.

So, it is basically a term for efficiency. Yield, how much photon you have absorbed and how much photon you have emitted. So, this is the formula gamma is the emissivity or emitting rate and k_{nr} is the non-radiative radiation this is the amount of photon, this is the electrons shaking and coming from lower and higher energy level to lower energy level by phonon or vibration or just by shaking themselves.

So, how much of non-radiative transition and happened and how much of gamma I_{photon} has come up. Obviously at the best case this non radiative transition is almost zero. We see this in laser, remember in laser if you know a little bit background of laser do not worry if you do not know I will be discussing about laser anyways, the same photon that is doing the spontaneous emission is being reflected.

So, its amplifying so there is no difference in photon energy in a laser. But this is not laser this is organic compounds; this is fluorescence or fluorescent materials are also known as Fluro force. So, this is the formula there is always a term which is the non radiative emission and that reduces overall the quantum yield. There is also lifetime the time available for the fluorophore to interact. How much time the emission photon will be available and that is given by this particular formula. These are some of the factors that you need to know these are very self-intuitive just reading it you will be able to understand.

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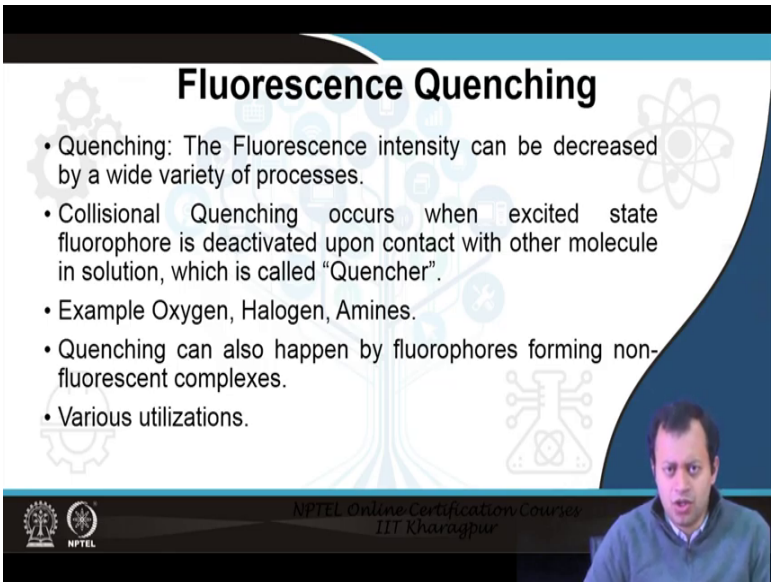

Quenching is an important phenomenon in fluorescence. So, nothing is perfect life is of course not perfect. You will not have a fluorophore just by itself, you usually have a fluorophore fluorescent material in an environment where it is surrounded by several other molecules, several other materials, like in a solution, you have different type like quinine present in water, like a rhodamine dye present in some kind of a methylene blue or these kinds of dye present in some kind of solution.

So, the fluorescence intensity can be decreased when the fluorophore the material which is emitting this light that molecule, that fluorophore, that substance is interacting with another substance is interacting with another molecule which is not at all fluorophore. A fluorophore molecule in a solution is interacting with a separate molecule which can be organic, which cannot be inorganic and they can collide their simply collision will let the electron fall without any kind of energy emission.

It can also happen that fluorophore makes a compound with this other element, makes a compound with this other material and thereby the entire molecular orbital things the energy levels changes because it is no longer fluorophore, it has converted itself it has formed a compound which is a non-fluorescent complex.

And some of the typical examples of quencher something that attacks or changes or modifies fluorophores are something common as oxygen, halogen and amines. But remember, there is certain examples or utilization of the quenching process. These days with the coronavirus pandemic you know about oximeter, pulse oximeter you are putting it in your finger and you are measuring how much your blood has oxygen level if it is below 80 you should go and see the doctor immediately, they say think about that this is not the same thing but think about that.

If I have a solution blood say, and I want, I have puts amount of fluorophore emitting light and I am constantly supplying oxygen into it with the amount of quenching less and less amount of light is getting emitted from the fluorescence can I make a measurement, an optimization of how much oxygen this molecule is having, this molecule is retaining, can I think in that particular direction, do you think the pulse oximeter uses fluorescence, think about it.

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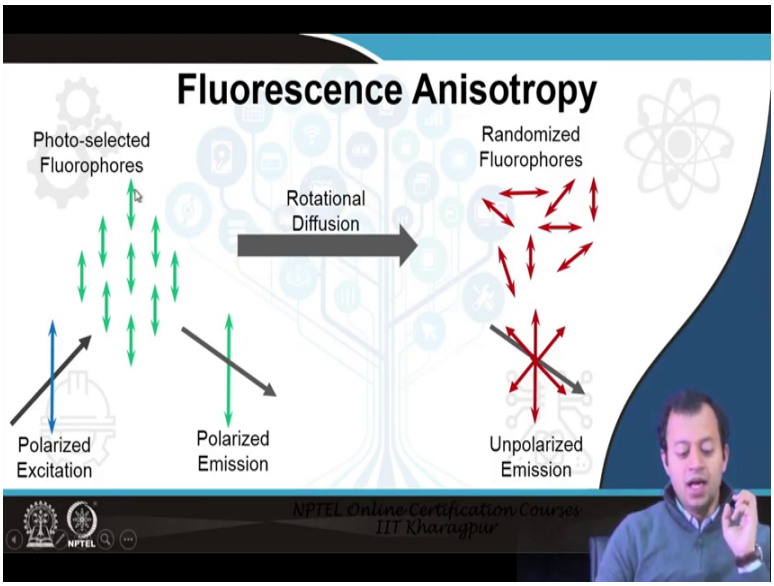

Fluorescence anisotropy is a very important topic. So, fluoro force try to absorb they have a preference for absorbing light which has the electric field antiparallel or electric field vector in the direction of their dipole moment. So, if you have made some kind of a polarized light, if you have sent some kind of polarized light that have put all those fluorophores in a solution, in a particular direction you will see a polarized emission most amount of light is being absorbed and most amount of light is being emitted.

After some time obviously, when the polarization removes the distribution of the fluorophore become randomized that also start reducing your emission this is called rotational diffusion. You can therefore think about the time frame it requires for your fluorophore to get aligned and to disalign how much time it requires. We require all of these information when we are looking for say, protein studies. We need to see how proteins are interacting with one another, are all the protons in similar direction or they are randomly oriented, what about DNA, what about the DNA base pairs are they all aligned together or they are disaligned together.

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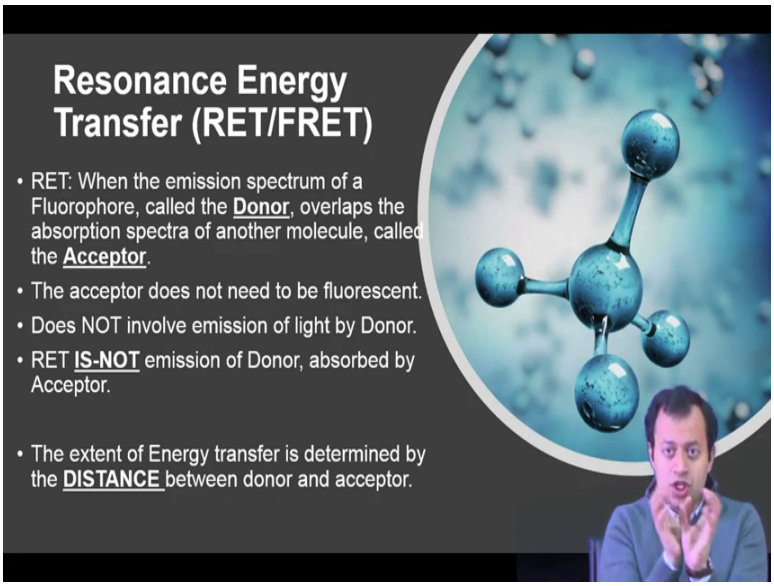

So, another important thing which we are going to discuss in the bio imaging part is the resonance energy transfer, I think medical students have had learnt about this quite a lot that is fluster resonance energy transfer or fluorescence energy resonance transfer. It happens when there are two molecules; one is emitter which we call as donor and one is acceptor. The emission spectra of this matches with the absorption spectra of this.

Two molecules, this molecule emits a particular photon, the emission of this photon matches with the absorption of that specific molecule. Two separate molecules, the emission spectra of this molecule matches with the absorption of another molecule.

So, that is called donor an acceptor however here you need to be sure that it does not involve necessarily emission of light by the donor, it is also not emission of donor absorbed by acceptor it simply tells you about the distance between donor and acceptor. Meaning, if molecule a and molecule b, molecule b's emission matches absorption of molecule b. Unless they are close to one another unless, there is a physical interaction between them this process does not occur. So, even in the solution there are molecule a and b if they are not close enough, this resonance energy transfer will not happen.

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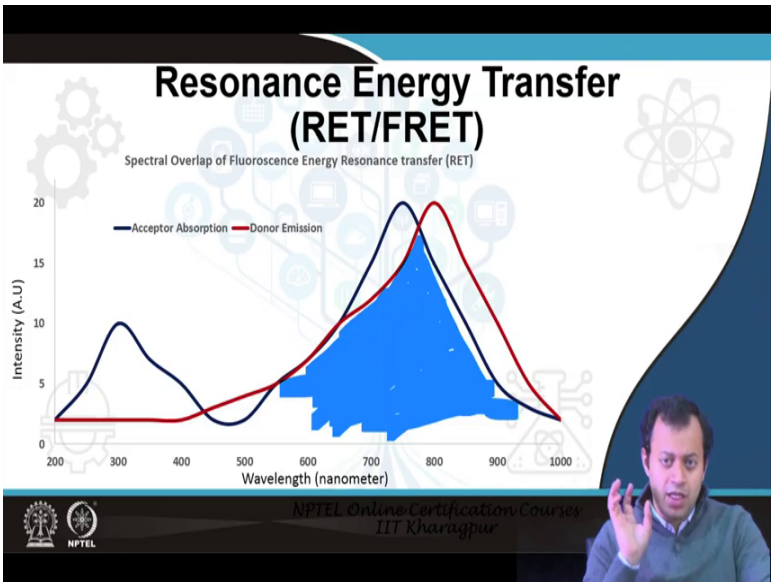

This is the shaded area where the energy transfer can takes place because, this is two separate molecules acceptors absorption and donors emission are near about overlapping. And this process can only happen when absorption and emission molecules, acceptor and donor molecules are physically close to one another, if they are quite separate from one another even in the same environment, even in the same solution it does not take place.

Thereby, we understand inside a cell how close a particular organelle is with say another protein, how close a pathogen is with respect to a cell or with respect to a nucleus or somewhere else or how close protein a is with protein b. If they are closer together, they will make some kind of a complex which we need to think about. So, we will be discussing FRET quite in a bit more detail in subsequent classes, for the time being I just gave you a hint of what this is.

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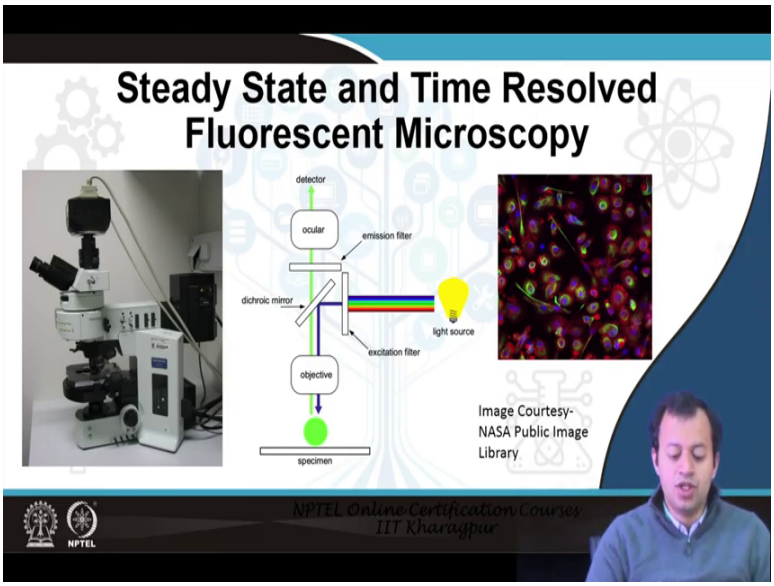

So now, coming back to our original thing why do you use this marker, why do you use this marker, you use this marker to highlight a specific of a certain passage you have a big page, several lines are written here, one particular line, one particular paragraph is important and you have therefore marked it with a, with a marker.

The same thing happens in microscopy you want to understand say, for example, how the nucleus of a specific cell is behaving you therefore use a fluorophore, a very, very small fluorophore that fluorophore get tagged, get attached to the nucleus, chemically we can make certain materials attached to another organic material. So, this fluorophore attaches to the nucleus or any other specific part and then you are shining light, you are shining a specific light which this fluorophore tagged to it absorbs.

Or let me use this term, this fluorophore is attached to my hand and this fluorophore absorbs yellow light. Whenever yellow light, whenever some other light, it does not absorb yellow light it emits yellow light, say it absorb blue light and emit yellow light and with this whenever you put it under microscope, whenever you see a yellow emission coming up you will understand that is coming from the area of the nucleus region. Thereby, you can identify this very beautiful inside the cell even to a single cellular, single molecular level, this is the fluorescence microscopy, this is revolutionized biology.

You simply have a specimen, there is a light source, excitation filters out, the blue light, the high wavelength light, this the material absorbs, the material then emits another different frequency this different frequency is quite different from the illuminating light. Obviously, there is a gap, we have utilized this difference and we have used a filter that can only filter out the emitting light this is not or difficult in normal stereo zoom microscope.

Here we are only filtering out the emitted light and thereby you see this beautiful microphage where more than our these days we use multiple fluorophores. One absorb blue light gives green light, one absorbs green light give red light, etc. and thereby you get a good beautiful picture such as this. To very quickly I will tell you two separate measurements in fluorescence microscopy; one is steady state, one is time reserved. Steady state is under constant illumination, the light source is constantly on and you are measuring it.

And then there come time resolved where the light source and pulses its usually laser and it pulses, it is a dynamic measurement every time you send light you get an image there is a gap then you send light get another light and you match these two images and you see how the cell, cell is a living material how it, what is the dynamic processes how it is slowly changing its like animation one picture after another, picture after another picture and you merge it all it together and you see how the cell has moved, what kind of chemical reaction have had happened.

So, this single-handedly revolutionized life sciences completely. Now, we are able to observe by fluorescence microscopy single molecule level, molecular level changes happening inside a cellular environment

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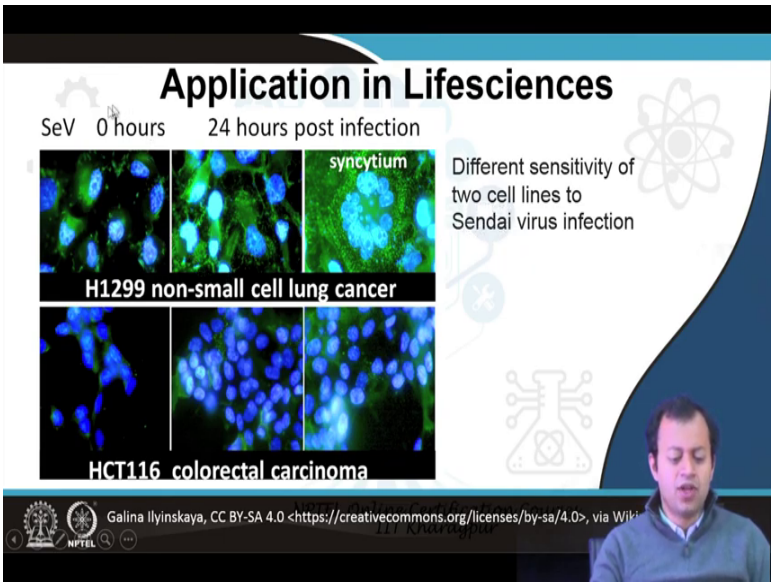

Application in life science see how beautiful this has happened. Sendai virus, this is murine respirovirus that these days is called this mostly affect rodents not helps humans and they are trying to see how the infection rate have changes from 0 hours to 24 hours. The blue part are the antibodies that this Sendai virus generates and the green part is either cell or the cellular material. So, they wanted to see what happens, how exactly the virus replicates, how exactly the virus has changes from 0 to 24 hours in two different you know cells.

One is a lung cancer cell, one is a colorectal carcinoma rodents and all of these static as well as dynamic measurements can be done from 0 to 24 hours at a difference of say 5 minutes, 10 minutes, 15 minutes you can have all these pictures and you can see the rate of infection how the virus is spreading. Can it be used for detection of coronavirus in human nasopharyngeal or oropharyngeal swap, can we utilize this kind of technique to image and understand the spread of coronavirus, what do you think?

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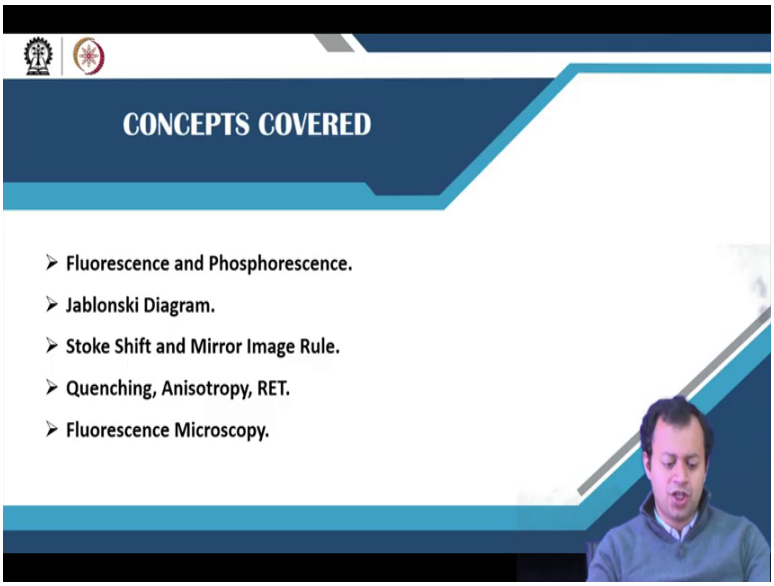

Anyways, that brings us to the end of this particular chapter as well as module number two which was the very basic of light matter interaction. We discussed about fluorescence and phosphorus, Jablonski diagram, quenching. Just go through it.

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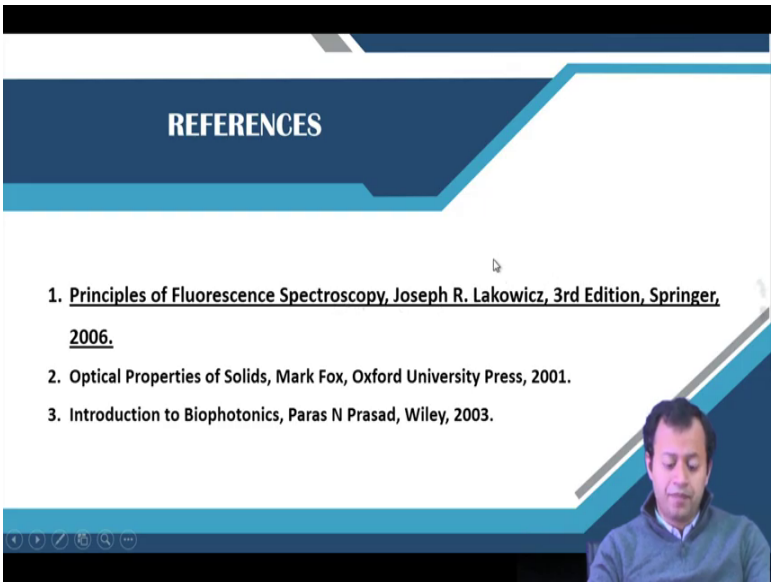

The significant book that I have used in to prepare this lecture is, Principle of Fluorescence Spectroscopy by Professor Lakowicz, this is one of the best books on fluorescence microscopy. If you are interested to know more about fluorescence microscopy read this, if you are more

interested in quantum mechanics, I am not talking more about quantum mechanics you might think it is hand waving.

But remember, quantum mechanics is a vast field in itself so, some of you who are interested to know why the energy splits, why basically singlet states come to triplet states, why the spin moves, I urge you to check for those. And if there is enough demand for me to teach you, take a separate section just for quantum mechanics maybe that will be done. But for the time being I would like to focus on biophotonics which is the main theme of the course after all. So, next class I will be teaching you biology. Thank you very much.