Nanobiophotonics: Touching Our Daily Life
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Lecture No. 08
Introduction to Fluorescence

Right, quick questions. Which one do you think is brighter? A fluorescent marker or a run of the mill ordinary yellow pen marker? Which one is bright and which one do you use? Both of them could be used as a highlighter. Highlighter in the sense that you read a passage in a book or in a journal paper and you find that passage that particular line, that particular sentence, that particular paragraph to be important and you mark them. You mark them, you label them, you label them either with this, this is a fluorescent ah highlighter or you label with a normal marker sketch pen yellow color. Both of these can be utilized as a marker, as a highlighter, as a labeler right. You of course, use it in drawing and painting, but you utilize it most of us who are not artistically inclined utilize it to highlight a particular passage in a book or in some journal notes or any any anything of importance.

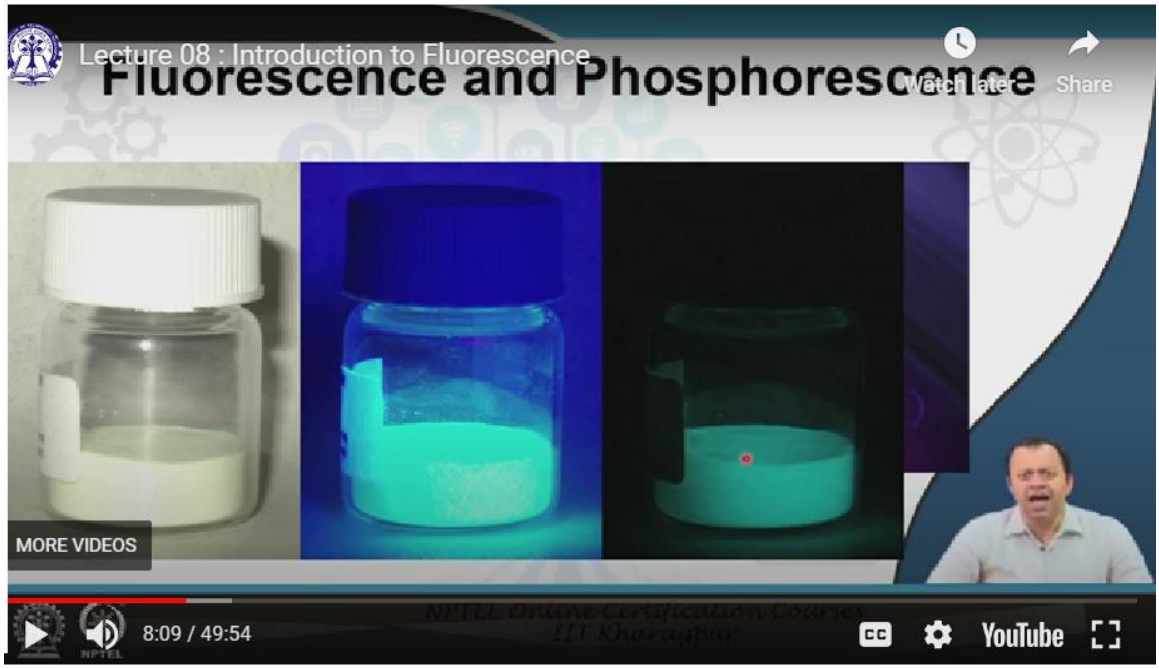
Which one of them do you think is brighter? Which one is more preferred and what is the difference between the brightness of this versus the brightness of this? Think about it and return back to me after you have figured out. One more question when ah it is the middle of the night you have you have no source of light. If you open up that passage which you have highlighted with either of them, if you open up a book which contains a passage which has been marked which has been labeled by either of these at night when there is no external light source. Do you think they glow the passage marked by either this or this glow in the dark? Think about it and come back to me.

So, welcome we were discussing nano bio photonics touching our daily life. Today is the lecture 8 of chapter 2 where I will be introducing you to fluorescence. Now which one do you think is a more prominent process prominent in the sense is reflection more prominent or emission more prominent prominent in the sense which one will give you more intensity greater brightness. If you paint the wall of your house with white color will it be bright or if you cover the entire wall with white bulbs LEDs will that be brighter right that is precisely the difference between these two pens. This yellow pen is is a fluorescent labeler this one is a sketch pen the process generated by this or the dye or the color that is coming out of this is reflective it reflects white light.

Whereas, in this particular case if what they claim in the label is true they are emitting light. This ink emits light emission by definition is always an active process and thereby with all circumstances kept similar is a far appropriate and brighter process depending on

your definition of appropriateness. So, let us now discuss this process of fluorescence where instead of light being reflected light is being emitted instead of the direction of the photon merely changed i.e reflection we generate we create we give birth to a new type of photons with different frequency different wavelength different color and different energy that is what we call as the process of fluorescence. Now what exactly is this process of creation of photons what are the process by which photons could be created photons could be generated fluorescence and phosphorescence are some very very common ways processes methods in which a photon can be created.

Now all of us we have gone through these ah type of naturally occurring corals or materials which normally look ordinary nothing extraordinary about them, but when they are illuminated using an ultraviolet light they tend to glow they tend to glow remember the when I talk about glow I am talking about the process of emission a bulb glows the gas light the cooker that you use the gas cooker that you use at your home to make food that glows yeah your wall usually will not glow your sketch pen will not usually glow several materials do not glow normally under natural light, but if you put them under ultraviolet light having an ultraviolet lamp you will see several such minerals corals naturally occurring materials starts glowing several artificial materials are also glowing in the under the illumination by ultraviolet light we have seen it in discotheques magic shows fairs movie theaters etcetera where there is a glowing light which shows you direction at night or at low visibility conditions . So, the gloviness can come from one of two different processes either of two different processes one being fluorescence and one being phosphorescence. The fundamental difference between fluorescence and phosphorescence is in fluorescence an external energy source is required all the time mark my word emphasis on all the time in fluorescence you shine a high a particular material a fluorescence material material that can fluoresce material that can emit light using a high energy photon ultraviolet common example the ultraviolet allows the ultraviolet photons allows the electron in the ground state of that material to go to higher state after some time it returns back to its ground state the electron will return back to its lower level will return back to its original low energy state in the process it will it will lose some of the original energy that it has consumed from the ultraviolet photon into heat and vibration and another photon is emitted which is of a lower frequency which is of a lower energy higher wavelength than the original ultraviolet photon visible light is in ultraviolet visible light is lower in energy than ultraviolet. So, usually usually these materials after consuming ultraviolet light emit in the visible spectrum that we can see this is the process of fluorescence you remove the ultraviolet source you remove the ultraviolet external source external photon no absorption takes place electrons remains at ground position and nothing happens these do not do not glow. So, it is an ordinary process.

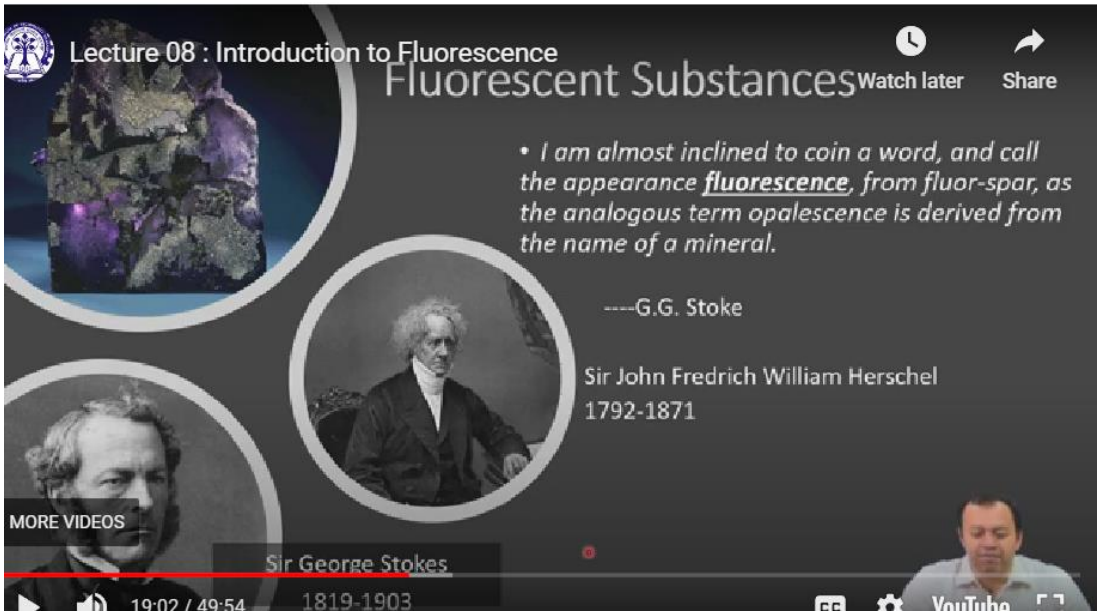


Phosphorescence is the case phosphorescence is the process in which initially it is exactly the same ultraviolet or any high energy light comes any high energy photon comes the electron absorbs goes from lower level to upper level, but stays in the upper level for a far far far longer time than the previous case of fluorescence. This electron which is at an excited state which at a higher energy state will stay there even when the ultraviolet electrons has been removed they keep on glowing and it will take far more greater time for this electron which has gone to the higher level to return back to the ground state and thereby it keeps on emitting a very very very faint glow even when there is no source of light nearby even though the input is removed even though it is pitch dark that is the process of phosphorescence. Phosphorescence glows in dark fluorescence glows in light fluorescence can glow in ultraviolet light phosphorescence can glow in no light. In today's class we are going to see the actual differences and what exactly is happening. You must have used fluorescence and phosphorescence in your daily life this is a fluorescence marker.

Similarly if you have radium watches ah those those those those watches ah they glow in the night there is a pale you know ghostly color ghostly emission coming out of it which is not very bright you cannot illuminate your entire house with it, but in phosphorescence a small small amount of light is present which is enough for you to look at the clock hands to know what time it is. So, phosphorescence these days are commercially not these days from a long period has been used mostly in watches or in ah night lamp etcetera. So, let us try to understand what exactly is fluorescence and why this fluorescence have been seminal in revolutionizing biology. We will go into biology pretty soon perhaps in the next ah set of lectures, but we need to understand how photonic technologies how light waste

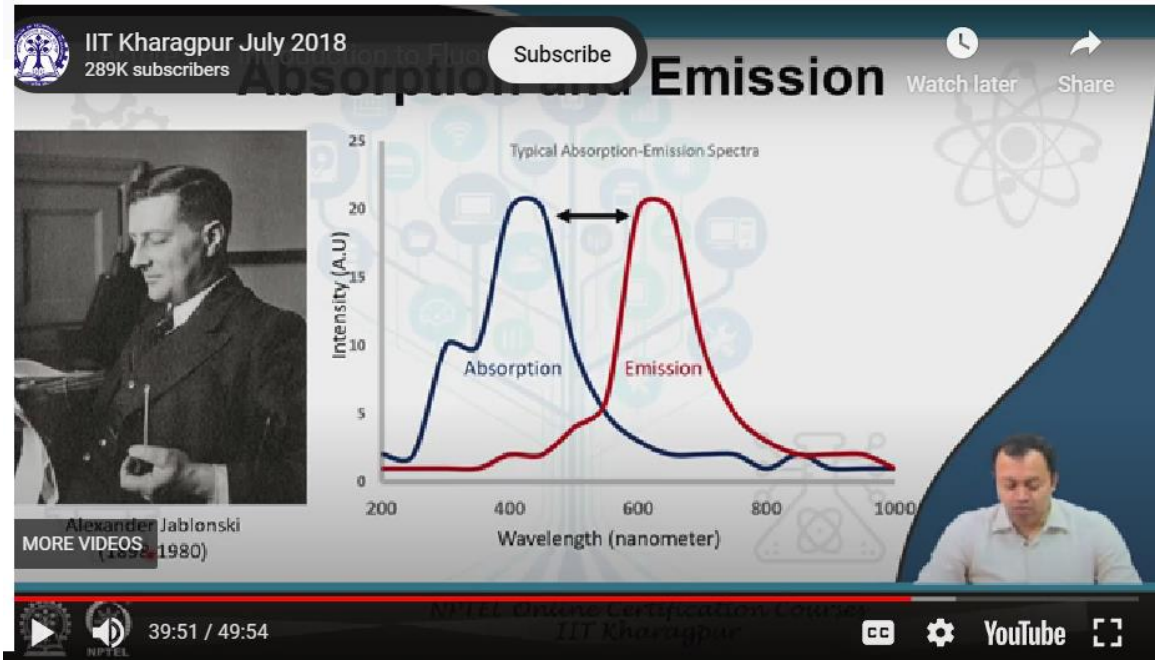
technologies have been able to interact with biological material and thereby revolutionize the field of ah microscopy especially life science microscopy. So, the concept of fluorescence is quite old this Sir John Herschel see the date figured out that there is he was basically drinking in his study with tonic water he had put his very very rich people obviously, who have studies studies basically a living room within a living room which is slightly private where you have ah big old mahogany furnitures with lots of books and a fireplace and painted windows and classical music is being played I am imagining I do not I I have seen it only in movies and museums.

So, this gentleman very rich at that time was sitting in his study drinking tonic water that goes very well with gin I do not know I have heard have been told not by anybody close a distant relative. So, tonic water was present in a glass and the glass was near his window because rich people the window was painted the window was painted with different colors blue green yellow beautiful colored windows and light was coming out moonlight was filtering out from that window a part of moonlight was coming out from the blue portion of the window and striking beam of light sunlight moonlight they sometimes come in beams from from window panes they were striking his drink striking his cup which was also made up of glass and what not ultra rich setting. So, use your imagination go back to 1792-1871 they have just conquered India they have just conquered India properly they have just conquered India and lots of materials from India or other colonies have been to united kingdom. So, there is no no no no dearth of money for ah people like him anyways. So, light was falling on to his cup of tonic water and the tonic water in turn was reflecting a celestial blue light he observed that and he wrote it down that the blue light that is being reflected out of the tonic water cup is of a far far different color or different intensity than the light thus moonlight that was beaming that was the input light that was coming through the window pane and he could not describe it, but he said this phenomena have been observed someone else should take it over and describe what this processes in which the output light is of a completely different color per say than the incoming light.



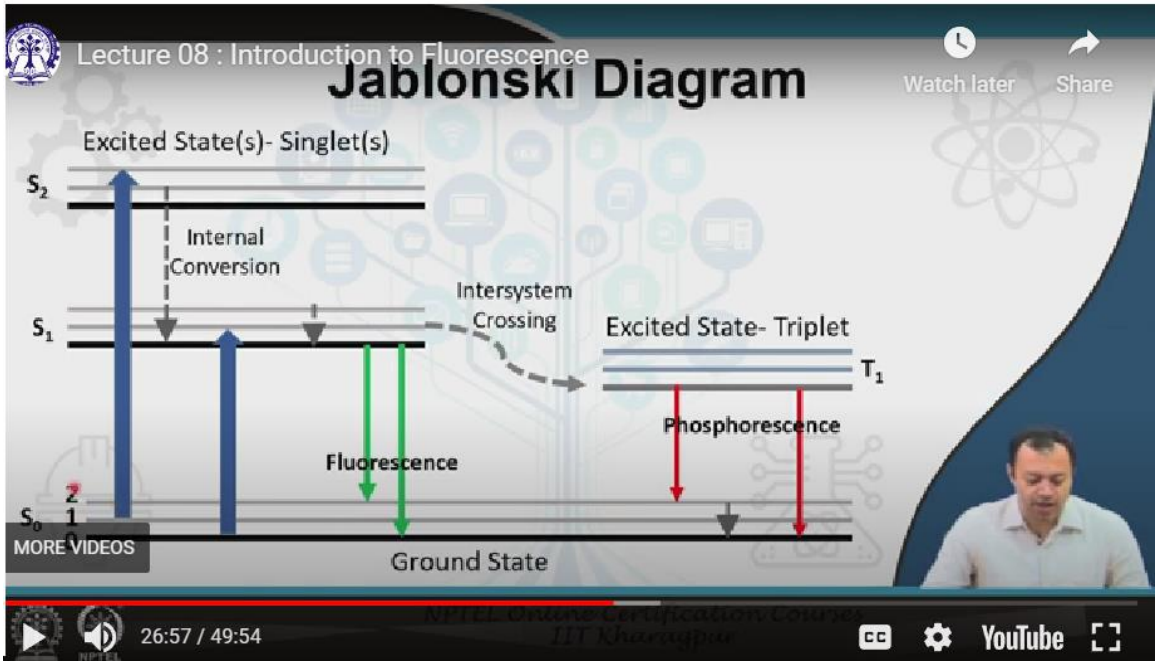
Remember the color what he saw was blue, but they were different shades of blue they were different shades of blue not completely red light was emitting it was blue light was emitting, but he called it celestial which I tend to think celestial means heavenly or pertaining to sky. So, I think it was light blue color which was described light blue sky color right. Next came Sir George Stokes and Sir George Stokes G G Stokes was also geologist and he came across this mineral which they found out from one of the mines in central Africa it was called fluoride and this mineral fluoride now we know it as calcium fluoride they used to call it fluorspar these gentleman at 1819 used to call it fluorspar ah we call it fluoride or calcium fluoride at that time none of these were completely known completely resolved CAF 2 and this gentleman got his hands on to one of those minerals fluorspar mineral and he saw that whenever different color lights usually of the higher energy blue etcetera or violet etcetera falls it generates a heavenly color, but that color stops coming out once the input source is removed and this color is not well this color is significantly different from the precious stones like gems, jewels, rubies, emeralds this glow is significantly different this glow is significantly different than any other type of glue or reflection that they have seen and Sir George Stokes was able to call this that I am almost inclined to coin a word and call the appearance fluorescence from fluorspar this material was fluorspar it emits some specific kind of light and thereby it they call it fluorspar the analogous terms opalescence is derived from the name of the mineral opalescence opals were corals that also emit different type of light this opalescence opal or coral were found in India opal is a Sanskrit name corals also emit light as you saw in the first slide and since the light emission by opals or corals type of corals was called opalescence he decided that this mineral that we have found also emit also glow some sort of light. So, thereby we call it fluorescence fluorescence is the light emitted by the mineral fluorspar, but neither Sir George Stokes or Sir ah John Herschel was able to describe the phenomena as what exactly is making the mineral or the tonic water glow what is it is not

reflection they figured it out immediately it is you can immediately figure out if it is glowing or simply being reflected. So, what exactly is happening inside this mineral that is allowing the material to glow remember glowing is always different from mere reflection everything reflects, but not everything glows.



Cut to the 19th century there was this ultra famous scientist well he became ultra famousah at a later date Alexander Zablonsky spare me some time I will tell you about professor Zablonsky and why his life is fascinating for all of us. Alexander Zablonsky was born in 1898 in Russian occupied territory which is presently at Ukraine presently in news I think the city was Kharkov where he was studying in a college for his ah bachelors degree. He was transcripted in Russian ah military and then in 1910 when after 120 years of foreign occupation the new country of Poland was formed he immediately joined Poland because he was Polish scientist he was Polish ethnically he joined Poland the new country of Poland ah that was formed in 1910 and started his study in I think Warsaw, but as soon as Poland was created in 1910 I think 1912 or 1914 world war one started he joined world war one fought battle got wounded captured by the enemy forces German forces then he was released when the Germans lost or the war finished he got the medal for extreme courage joined the university again to finish his PhD in PhD his PhD doctorate degree in 1930 he finished on these emission spectra of minerals trying to discuss about emission spectra of minerals and then started teaching as a professor of physics in university of Warsaw, but by 1936 talk about ill luck Poland got invaded by Germany again and he joined the Polish army to fight against the Germans soldier professor soldier again went to fight against the world war two against the German army the I think defeated or Germany captured Poland and he got captured by the German forces again in world war two when Germany was retreating Soviet Russia invaded Poland and got hold of him and captured him tortured him and when war finished in 1945 after bombing of Hiroshima and Nagasaki and

complete surrender of Germany he was released from a Soviet prison and guess what he did well he joined his university of Warsaw back again and started teaching he just dusted himself off picked himself from not one, but two world wars he saw creation of a country he saw the destruction or the complete occupation of his country again and then rebuilt and went on to describe the entire process of fluorescence fluorescence and phosphorescence whatsoever we know majority of that was described by professor Alexander Zablonski you usually do not associate a soldier with an academic professor or a professor usually is not a soldier right these two are as far away apparently as far away as possible, but this is one gentleman who was soldier as well as a professor and guess what he was highly talented musician he sang opera and at the age of 14 or 15 he had to decide between singing opera become a professional musician or a professional scientist at age of 14 or 17 he decided to not sing into opera not become a musician, but instead become a scientist think about how many talents a human being can get he had all of that though he did not join music professionally as a professor he used to sing and he used to play the violin like any other virtuoso per say anyways enough of his life I just tell you about these scientists life because they are human beings we tend to reduced scientists into some set of formulas like Einstein is equal to equal to mc square right Newton is some apple falling down, but they are also human beings with their own successes failures triumphs and tragedies you need to humanize the person behind the formula a human being cannot simply be a formula no matter how great that formula is they also have gone through failures and troubles and heartbreaks and wars and famines and torture and what not as given by Alexander Zablonsky. So, Alexander Zablonsky came up with this mathematical calculation or logical understanding in which he told that there are certain materials especially organic materials organic chemicals that can have very thick upper layer and lower layer of excitement for where electrons can exist thick as in these individual energy levels these individual energy levels have subsequent sub bands inside them electrons can go from ground state to the excited state in the excited state they lose some amount of energy they lose some amount of energy in the process of vibration and then finally, return back finally, return back to the ground state to the less energy to the to the to the original state by emitting a photon which they of a frequency of a frequency smaller than what they have of an energy smaller than what they have absorbed. So, thereby the absorption spectrum the frequency of the light the frequency of the photon that it has absorbed versus the frequency of the the light that it has emitted is always different usually it absorbs high frequency high energy low wavelength light and it emits high wavelength low frequency low energy light and this entire thing phosphorescence and fluorescence can be described by what we call as Zablonsky diagram.



Now, stay with me this is the ground state called S naught and it has inside it sub bands of 0 1 and 2. So, the entire S 0 is the ground state entire S 0 is ground state and it has sub bands of 0 1 and 2 it has the next excited level the so called conduction band this is valence band or conduction band this is LUMO this is HOMO highest occupied molecular orbital. So, this is HOMO and this is LUMO beg your pardon I am electronics engineer I am more used to saying valence band and conduction band, but anyways this is HOMO highest occupied molecular orbital and this lowest unoccupied molecular orbital S 1 S 1 also has 3 sub bands 3 just for the sake of it and then there are S 2 S 5 S 10 S 12 S 13 S 100 S 1000 does not matter, but these is the lowest level ground level and this is the next highest level highest occupied molecular orbital and lowest unoccupied molecular orbital remember highest occupied and lowest higher highest and lowest are the important terms this is higher than S 2 S 3. So, you have electrons in all of these sub band sub band states. Now, some amount of energy some amount of input light have been given to the electrons present in this it can go to S 2 depending on the energy that you have consumed not necessarily it will go to S 1 it can go to S 2 S 3 S 5 S 10 S 12 S 100 S 1000, but the further up you go S 2 S 3 S 4 etcetera the more unstable it becomes chances are if the electron goes into S 2 or S 3 level it does not stay there it stays there for less than few attosecond or few femtosecond it will return back either directly to S naught or it will return to S 1 and then finally, return to S naught S 2 S 3 S 5 S 6 higher levels are very very unstable electrons do not want to stay there even if you have given it energy momentarily it go up and immediately come down in few attosecond femtosecond 10 to the power minus 18 second 10 to the power minus 14 minus 15 seconds thus far something that our instruments have difficulty measuring we measure their existence theoretically that electron can go to S 2 S 3 S 5, but will not stay there will stay there for less than 1 of 10 to the power minus 18 second and immediately return back to ground second.

So, it is no good, but it might so happen that either you send an electron to S 1 only lowest unoccupied molecular orbital or some electron that has previously gone to some place at a higher S 2 S 3 or S 4 by internal conversion has come back to S 1 this is stabilized most stable state these are highly highly highly unstable state this is moderately stable more directly stable. Electrons that come here will also return to S naught will also return to S naught by spontaneous emission randomly, but they can stay here at a slightly larger time what is the larger time 1 to 10 nanosecond 10 to the power minus 9 second is the transition between this to this right 10 nanosecond we can measure 10 nanosecond we can measure 10 to the power minus 9 second we cannot measure 10 to the power minus 18 second minus 15 second maybe minus 14 is now possible again very unstable highly unstable state electrons will not stay here or stay for very very small period of time very stable this is moderately stable. Now, electron has gone from here to here or it has returned back this 10 nanosecond electron that stays here will finally, return to ground state losing some amount of energy in internal conversion in internal conversion as I said yesterday in terms of vibration in terms of heat navigating the thick very thick HOMO and LUMO part vibrating or navigating through the sub bands there is these sub bands are very close, but they are finite they have an energy of 10 to the power minus 5 to minus 8 electron volt and going through 100 and 1000s of them can result in ah reduction of 0.1 or 0.01 electron volt and so on and so forth here.

So, the light that is emitted the light that is emitted is of a lower frequency lower energy higher wavelength remember wavelength and frequency are opposite to one another high frequency low wavelength low frequency high wavelength energy and frequency are directly proportional $E=h\nu, E=hc/\lambda$. So, energy is directly proportional to frequency inversely proportional to wavelength. So, high energy means high frequency high energy means low wavelength. So, emission is at higher wavelength meaning lower frequency lower energy than the absorption and it returns back to ground state losing some energy losing some energy in the process of navigating through the thick HOMO and LUMO navigating through the internal sub bands. Now, I told you electrons can return back.

So, all electrons in S naught states singlet states are spin paired if this is plus spin this is minus spin and they are together because Pauli's exclusion principle is not being violated they can return back and life is good this has spin 1 this has spin 2 as long as they are spin paired spin opposite to one another they are returning back and life is good life is not a problem. However, under certain materials there exist some intermediate state between S naught and S 1 between HOMO and LUMO there exist some intermediate defect state this state should not have been there, but by some anomaly and that is the term anomaly by some exception that is the term exception certain things has happened nothing is perfect there is a scratch some atom has you know flow flown off some impurity atom has taken

part something something has happened and you have an intermediate state between S 1 and S naught an intermediate state between S 1 and S naught it is intermediate in between S 1 and S naught. So, it has lower energy it has lower energy than S 1 electrons will always prefer the lower energy state it is not preferring S 2 it is not preferring S 3 S 4 S 5 S 6 it is preferring S 1 to reach to S naught it can. So, happen that if there exist an intermediate defect state intermediate defect state lower lower than LUMO its defect it can go there it can cross over from S 1 to this defect state before coming to ground state why because this has a slightly lower energy it is coming in steps it is coming from S 2 to S 1 and S 1 to S naught if by any chance there exist an intermediate state S 0.

5 S 0.5 between S 1 and S naught it simply crosses over and stays in the excited state. While this inter system crossing takes place where electrons from S 1 cross over to this defect state to this anomaly state it may so happen that the spin has reversed. Now, there we have a problem if the spin is same as the native electron that has never received any light and never gone up it cannot return to this position having the exact same spin this spin is not allowed this spin is not allowed in the same energy level this is allowed or this is allowed, but this this here I am trying to see the blue background this is not allowed, but it cannot stay here infinitely this is a higher energy state no matter it is comparatively lower than the excited state, but this is still everything every electron wants to come to the ground state no matter what. So, what to do this is called a excited state a triplet state a triplet state which is spin disallowed a singlet state is spin allowed meaning this is paired spins it can return back to its original position and everything is paired Pauli's exclusion principle is not not violated and it remain as it is whereas, in T 1 the triplet states lower energy state more stable than its one energy state, but still it is an excited state still it is an excited state it cannot remain there forever it cannot remain here it cannot also come back it cannot come back till its spin has been changed back. So, it tries to do so by slowly emitting energy here the electron stays in this singlet state LUMO it stays for 10 nanosecond here it can stay for microsecond millisecond or even one second an electron can exist here for one whole second before coming back and thereby a faint glow keeps on coming out.

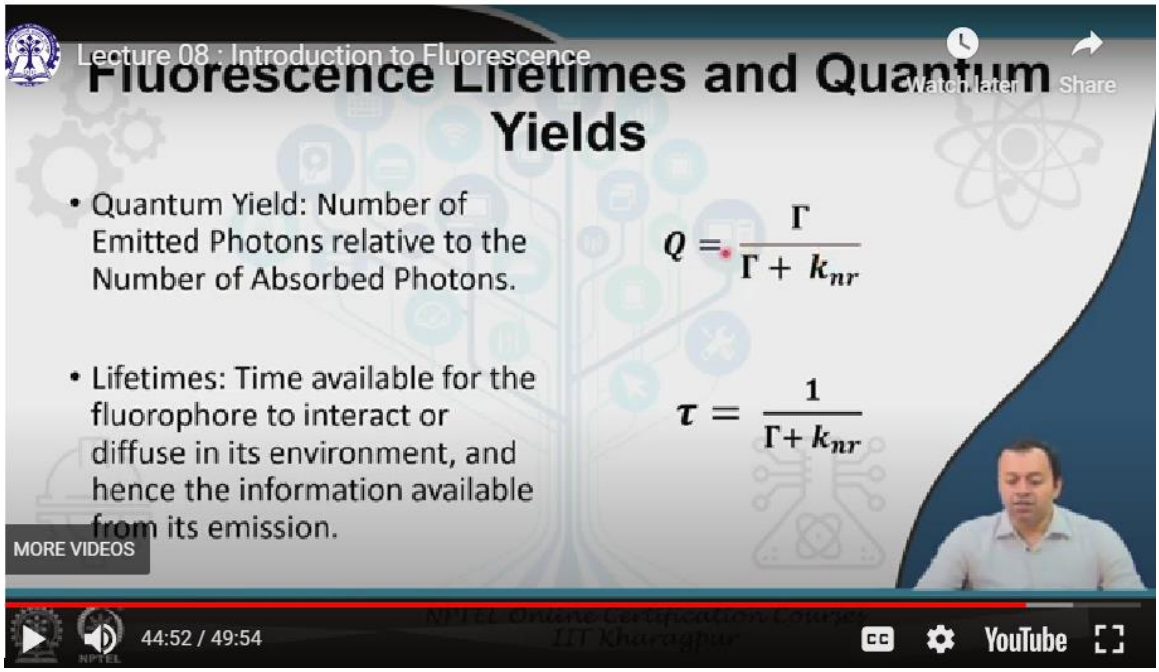
So, fluorescence spin allowed phosphorescence spin not allowed fluorescence electron goes up stays there returns back emit photon phosphorescence electron goes up come slightly down, but now it does not know what to do it cannot return back because spin is violated phosphorescence spin violation without correcting the spin it cannot return it cannot stay higher energy nothing stays there infinitely at a higher energy state it has to come down, but it cannot come down because Pauli's exclusion principle is violated. So, it is a ghost. So, it is a ghost it keeps on losing energy faint energy thinking that its spin will repeat its its spins will oppose get opposite its spin will flip and if and when that happens it can only return back. So, even if your energy source has been removed this

keeps on staying in the triplet state it keeps on staying in the triplet state till it returns back why they are called singlet and triplet state well previously when they are trying to look into the spectra emission spectra of phosphorescence and fluorescence they were observing that singlet states fluorescence give you something similar like this a nice spectra like this a single line right whereas, the triplet state at that time in that particular material what they have looked into in the beginning 1900 well after world war 2 I assume during war what kind of experiment you will do if your university is bombed the Germans bombed out the university of Warsaw I had the fortune to visit Warsaw in Poland it is a medieval city built in I think long long time ago no building not a single building in Warsaw is older than I think 1950s or late 1940s why because the entire city was bombed not a single building in Warsaw was standing erect when world war 2 finished it got attacked occupied and destroyed first by Germans after world war 2 and then the Soviets came in got attacked occupied and destroyed by the Soviets later. So, nothing was left now they have recreated it and it is a beautiful beautiful city, but think about the resilience of human beings every time I look at the nation of Poland I admire them because of their resilience what they have gone through yet they are still saying and nothing probably is better than ah the resilience of professor Zablonski soldier musician scientist this is something he figured out while fighting for his country while getting tortured by enemy soldiers in captivity while playing music while singing opera.

So, fluorescence spin allowed immediately returned back phosphorescence spin disallowed not returning back emission spectra are typically independent of the excitation wavelength now this might cause confusion what does this mean is as long as your input light as long as the energy of the input light is above this band gap above the difference between HOMO and LUMO it does not matter whether you have given energy input energy to S 2 S 3 S 5 S 10 or not 10 and above say 10 well 10 is very high say the electron volt is 5 if you have excitation wavelength of 6 10 12 20 electron volt as long as it is not burning the material through as long as it is not melting the material through you do not care if your initial electron is going to S 2 S 5 S 10 or S 100 because it is those those eigenstates those energy states are invalid they will not remain the electron will not remain here for more than few attoseconds which you are not going to measure anyways and it will return back to S 1 either it will return back directly to S naught, but chances are it will return back to S 1 first and then return to S naught. So, as long as you have covered as long as the threshold as long as the threshold is crossed you are good you are ready to see fluorescence if you have defected it you can say phosphorescence. Phosphorus material or these some kinds of naturally occurring material like corals etcetera which are biological materials with large amount of elements combining together to form molecular materials they are they by definition can have higher tendency to contains defects. So, phosphorescence can happen. So, emission spectra are typically independent of the excitation wavelength this simply means that as long as the band gap that gap between

HOMO and LUMO is breached you do not care whether the electron is at S 2 or S 3 or S 4 because they will not stay at S 2 and S 3 and S 4 they will return to S 1 and will show fluorescence.

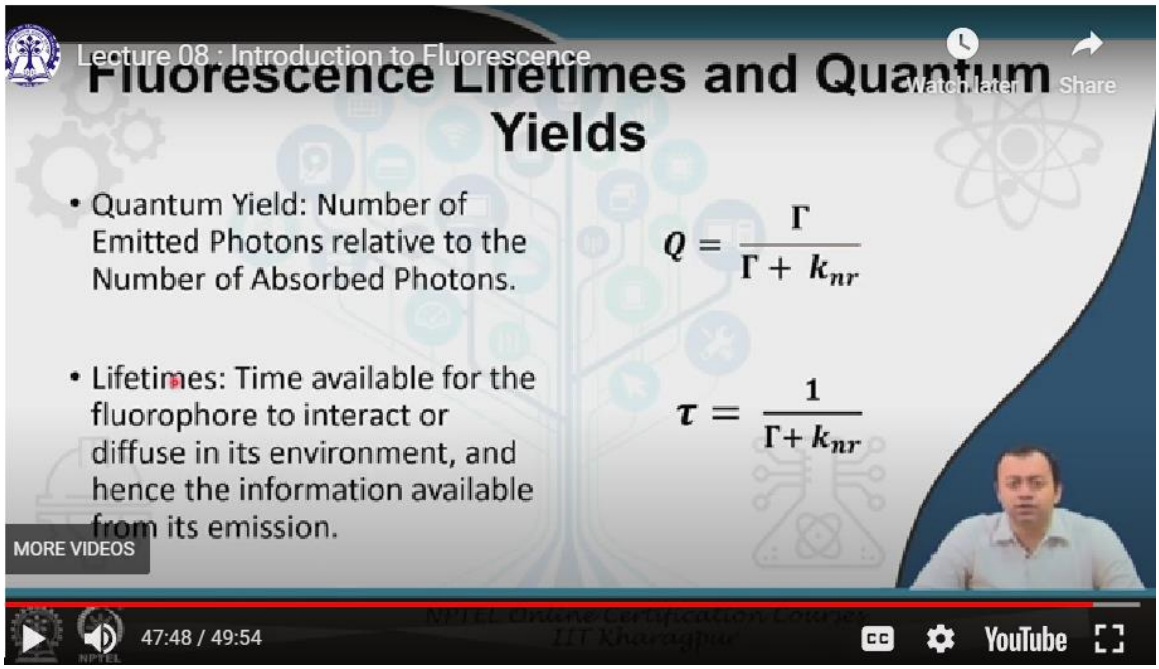
So, an input wavelength of if the threshold is say 5 electron volt fluorescence will happen with 5 electron volt the same fluorescence will happen with 6 electron volt the same fluorescence will happen with 10 electron volt 20 electron volt as long as you are not destroying the material. Now, there are couple of other things that you need to know about fluorescence one is quantum yield and lifetime quantum yield simply means the number of emitted photons related to the number of absorbed photons. So, gamma is the number of emitted photons and k n r is the non radiative transition meaning meaning this is the loss this is the heat. So, quantum yield is efficiency this is the vibration this is the amount of energy amount of photon that has been converted into vibration. So, this is the total versus this is the actual one which is emitted right.



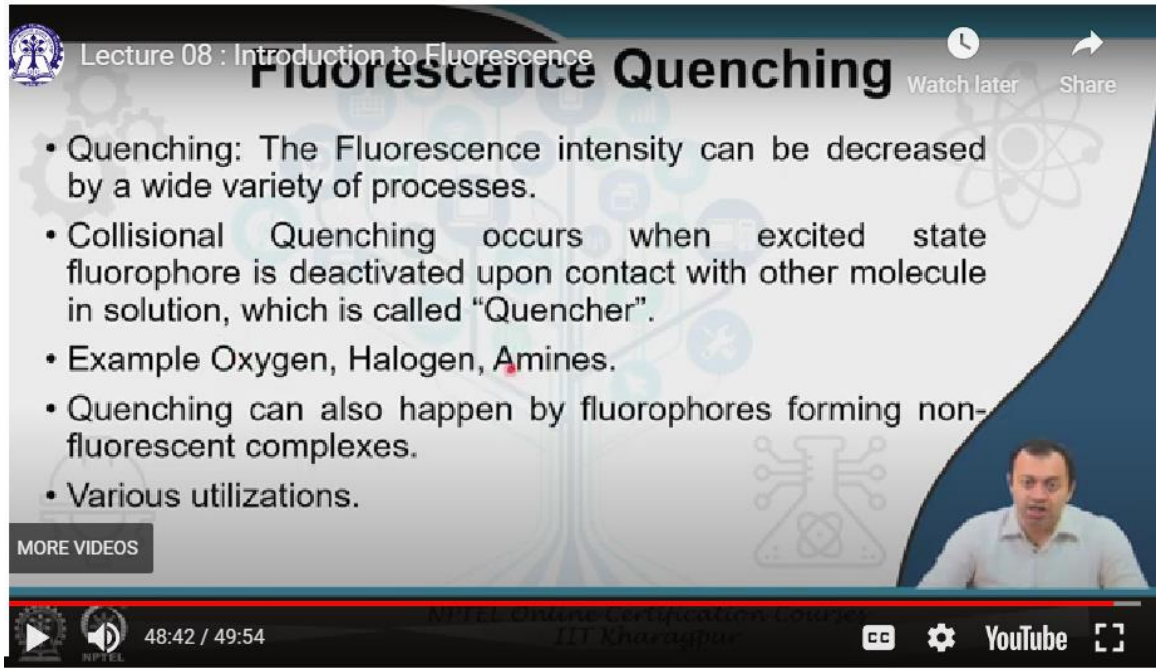
So, we need to reduce this to as close as possible to 0. So, that this Q becomes 1 gamma versus gamma is 1 meaning whatever absorbed has been emitted absorption is equal to emission, but because of the presence of this non radiative transition non radiative transfer this is the vibration this is the energy loss due to navigating within the sub band of HOMO or sub band of LUMO you have efficiency. These days our fluorophores fluorophore is the term you should remember fluorophore are material that fluoresce fluorescent material could be considered as fluorophore, but fluorophore has some other other functions as well fluorophores are mostly from a biology or chemistry point of view materials that that has functions other than simply fluorescing, but for the time being considered fluorophores as fluorescent materials. Nowadays we have fluorescent materials whose efficiency reaches above 90 percent regularly, but they are never 1 or 100 percent right. Similarly how long

it is the lifetime or time available for the fluorophore to interact and diffuse in the environment is the lifetime of the fluorophore to interact or diffuse in the environment is given by this particular time how long will it emit the time period available for fluorophore to in.

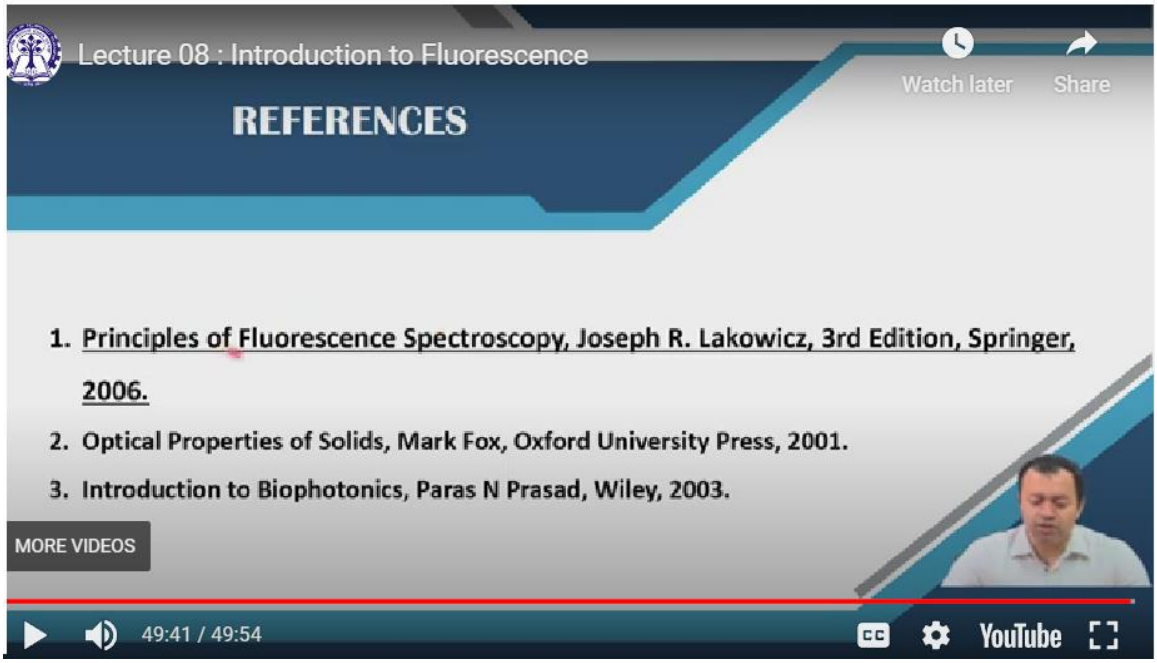
So, what is the total duration of the process what is the total duration of the process you have excited it, it has gone up, it has stayed there and then it has returned back emitting a particular light what exactly is the whole time taken from absorption to emission is lifetime of a fluorophore. These two are very very necessary when we are going to solve some mathematical problems regarding fluorophore or when we design fluorophore etcetera, but remember the electron cannot stay at that S 1 level for more than ah 10 nanosecond 10 nanosecond is the limit. So, lifetime is not 10 nanosecond 10 nanosecond is how long electron will stay in the upper level lifetime is the entire process from absorption to emission time. A part of it is electron staying upstairs electron do take some amount of time to go from lower to up and then staying there and then coming back. So, the lifetime is the entire entire process whereas, 10 nanosecond is the time it stays up.



Do you know how far light can travel in 10 nanosecond 30 meters I am told no sorry beg your pardon I am told one feet which is what 12 inches 30 centimeter not 30 meter beg your pardon 30 centimeter is the distance that light can travel in I think 10 nanosecond. Do the calculation figure it out and you will see how far light goes.



So, anyways fluorescence quenching is something that comes up regularly when we are talking about biological materials that fluorescence intensity can be decreased by a wide variety of processes. You basically prevent the electron from going either up even though energy has been given by having collision by having other materials nearby oxygen halogen amines which forms bond which eat up the extra electron the excited electron or sometimes you simply knock out the electron which is an an excited level from preventing it from coming back to the ground level by ah converting the entire energy into heat because of the presence of oxygen halogens or amines or various other things the excited state fluorophore is deactivated upon contact with other molecules in solution which we call quencher. We utilize it beg your pardon ah I have allergies pollen outside is too much. So, um sometimes my eyes water sometimes my nose. So, this quencher can also be this quenching phenomenon of fluorescence could be also be utilized to detect the particular \ chemical species in a solution.



These are my references please if anyone can please go through this beautiful beautiful book principles of fluorescence spectroscopy it starts from the scratch and take you to the next level and from next two classes we will discuss little bit of biology. Thank you very much.