Basics of Fluorescence Spectroscopy
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Lecture – 16

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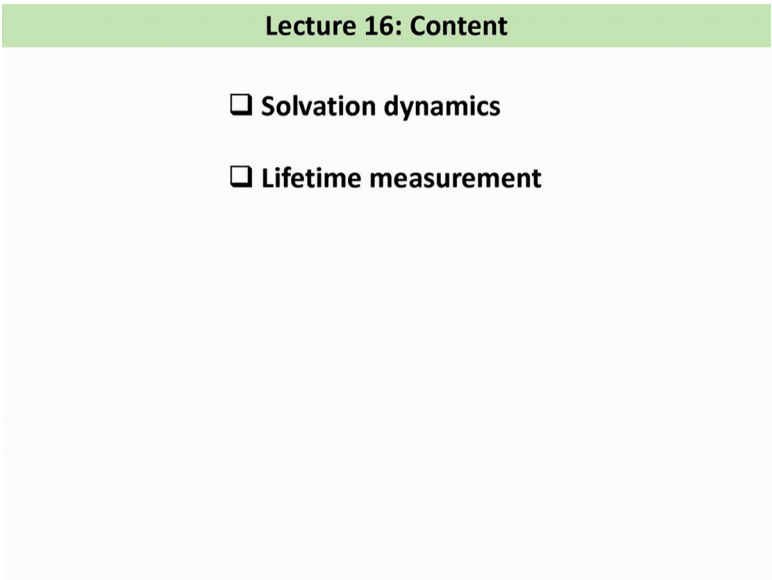

Welcome back to the lecture number 16 of this course. So, in the last lecture we are discussing about the Solvation Dynamics. So, when a molecule is being excited from the ground state to the excited state and there is a change in the dipole moment, let us consider the dipole moment has been increased during the excitation; that means, mu g is less than mu e.

In that case the solvent molecule will solvate the excited state dipole moment of the species in the ground state the solvation structure by the solvent molecules was different than in the excited state in that case, because the solvent reorientation is not instantaneous then I would get a different solvated state as the function of time that is what I was discussing last day.

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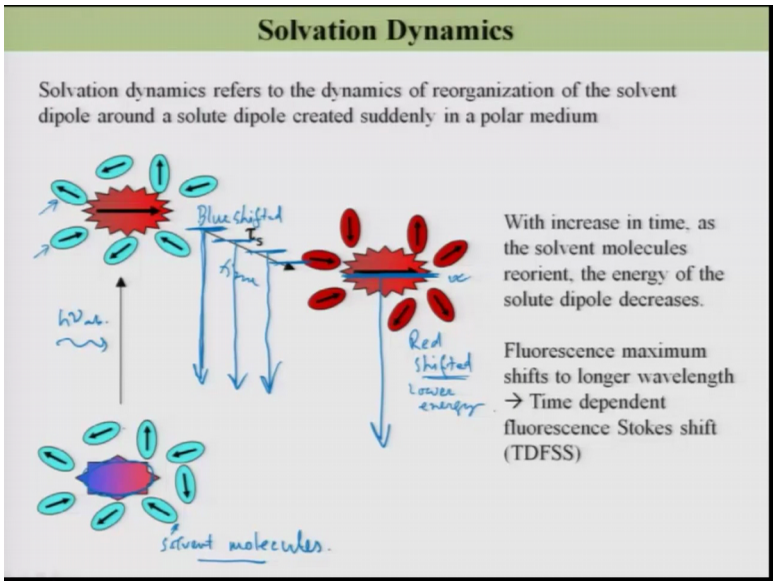

Suppose this is my solvent dipole. So, this is my solute over here and this is the random orientation of the solvent molecule these are the solvent molecules. So, after excitation right this is my excitation h nu absorption of this molecule. So, I will be going to create a dipole like that one.

So, just after the excitation this solvent molecules right these are the solvent molecules they will have the similar orientation that of in the ground state, but as time goes here is my time axis here as time goes. So, this solvent molecule will reorient themselves and at each step in between these right that emission spectra will come. So, when the solvation structure is such that the energy of these excited state dipole is over here till the emission will come from here when the energy is like this then the emission will come from this state when energies like that then emission is like this and when it is fully solvated at this state. So, then the energy will come from this state.

So, when you measure these emission spectra of this particular solute molecule; that means, you are monitoring the excited state of the species. So, you will get the contribution from this state will be more, but the contribution of all these states will be there; that means, it is the time integration of all these different emission spectra over time.

So, if you have some way to get the emission spectra of the solute molecule as a function of time then at very early time right what you will get you will get the emission spectra

which is originating from this particular state and at the later time it we will get the emission spectra which is originating from this particular state. Obviously, this state emission spectra will be more blue shifted more blue shifted for this will be the blue shifted and this will be the red shifted. That means, this emission spectra will be of higher energy and this emission spectra will be of lower energy just to show you this I have one small video over here.

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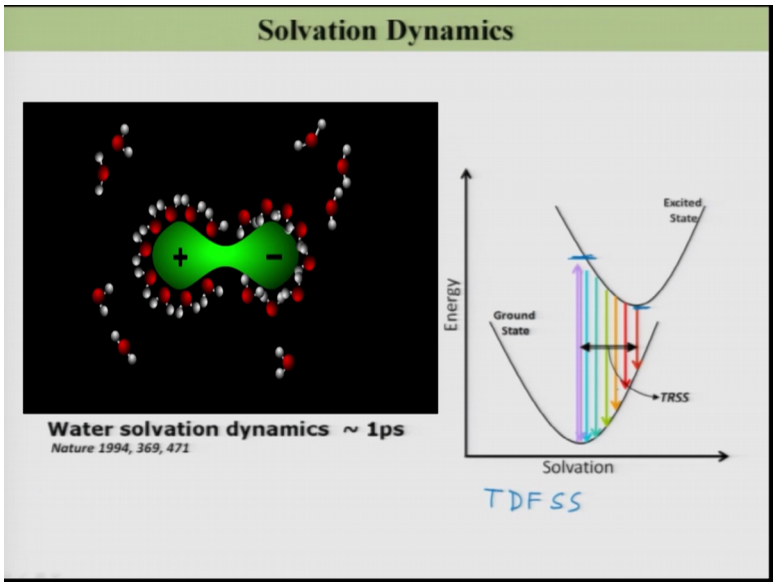

So, let us say this is your solute molecule and after the photo excitation that dipole has been created instantaneously.

Now, if you see that orientations of the solvent molecules are random right, but when the dipole has been created in this case as you can see over here. So, as the dipole will created these solvent molecules will be reorient themselves to stabilize the species right now if we just look at this energy; energy of this excited state that during this solvation the energy will keep on decrease and decrease and decrease and at this finally, it will be solvated fully.

So, the emission spectra will start from here and then it will end over here; that means, I will get a time dependent emission spectra; that means, if this is known as time dependent fluorescence stokes shift.

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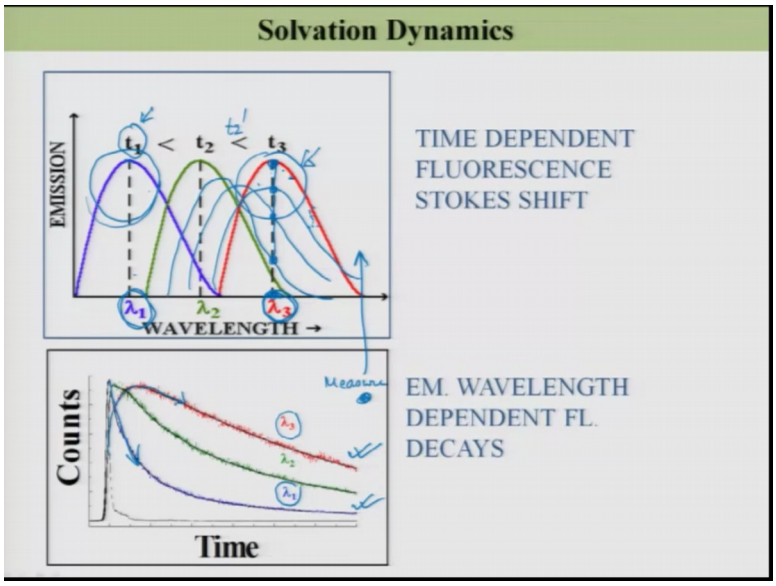

So, stock shift is being changed because of the solvation and the if I now plot this emission spectra as a function of time what you will see that at time t 1 which is the laser time the smaller time just after the excitation this emission is like this way as time goes the emission will shift to the longer wavelength.

Now, as I said the steady state emission spectra of this molecule is nothing but the time integrated spectra of all these different-different times. So, the steady state will be somewhere here where the contribution of this one will be very small because it exists only for a very short time right, but the contribution of this guy will be large because this is my equilibrium structure equilibrium solvation structure of this solute dipole.

On the other hand, if you see that at time t equal to 0, this species has not formed because it took some time to form this stabilised structure. So, at time t equal to 0 these species has not formed. So, if I now take the intensity versus time at different-different wavelengths then the intensity versus time plot of different wavelengths should behave differently what I wanted to say is that let us take this particular wavelength lambda 3 over here in case of lambda 3. So, if you start measuring the fluorescence intensity after the photo excitation. So, if you start measuring the fluorescence intensity after photo excitation at time t equal to 0, there is no fluorescence intensity as you can see over here the value is 0.

But as time goes that fluorescence intensity will increase. So, if I now draw some other emission spectra here. So, let us say time t; t 2 prime that may looks something like this right. So, here at time t 2 prime we got small emission intensity over here as time goes this spectra will shift like this way this way and this emission intensity will increase from here to here from here to here from here to here and so on and so forth. That means, if I plot the emission intensity of lambda 3 as a function of time I must be able to see first increase in the fluorescence intensity then; obviously, the fluorescence intensity will decrease because of the lifetime of this molecule.

On the other hand if you see this lambda 1 wavelength right; lambda 1 wavelength then just after the excitation thus emission intensity is maximum. So, it will start from this maximum value right instantaneously and then it will decay as per its own lifetime; that means 1 over k r plus k n r as well as this intensity will decrease because of the shift in the emission spectrum.

So, if I now plot just see here that I have plotted it. So, this is in case of lambda 3 you see that emission spectra was less over here and the emission spectra increased like this way and then it will decrease. So, then decreases just because of its own lifetime fluorescence lifetime on the other hand if you see this lambda 1 you see from the beginning is started decreasing you started decreasing like this way.

So, from the measured lifetime; lifetime means these, these, these, these graphs from measure these graphs this graphs is nothing but fluorescence intensity versus time for different-different emission wavelength I can get back this from here I can measure this guy I can measure this guy and I can convert this to here and this process is known as the formation or construction of time result emission spectra let us see how we can do that.

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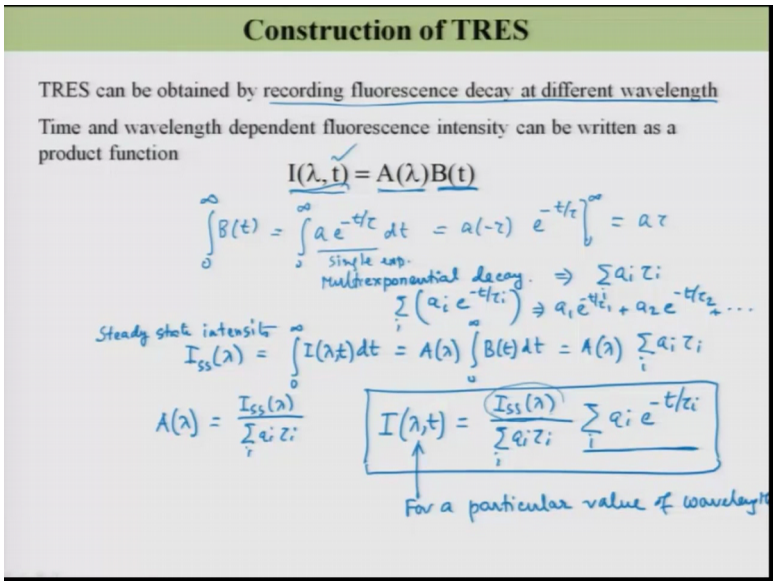

So, as I said here the construction of time resolution spectra and it can be done by recording the fluorescence decay at different wavelengths right and this is my fluorescence decay I lambda I is a function of lambda t because it is also a function of lambda because at different-different wavelength right emission intent emission intensity is different as I showed here this is for lambda 1 this is for lambda 3; that means, it depends on the wavelength right and also it depends on the time that is because of this natural lifetime 1 over k r plus k n r.

So, and here we can say that this I which is a function of lambda and t can be written as a lambda n b t. So, a quantity which depends only on the lambda this is my that stoke shift part and this is my natural lifetime part right this is and a lambda into b t.

So, now if an earlier what I showed you that this b t is in is exponential in nature right. So, if I integrate this b t over 0 to infinity; that means, the full time region then what I will going to see is integration 0 to infinity a e to the power minus t by tau where a is its amplitude it means it may be one it may be hundred it may be thousand depending on the initial fluorescence intensity right. So, is a and e to the power minus t by tau this t is my variable this time and this tau is the life time of the species and d t.

So, if I do this integration what I will going to get is a minus tau e to the power minus t by tau that limit is 0 and infinity which will going to give me a tau. Now if it is not a single exponential function here is my single exponential decay. So, I will get this one if

it is a multi exponential decay. So, for multi exponential multi exponential decay this will going to be sum over i a i tau I, in this case the decay function is sum over i a i e to the power minus t by tau i this is my fluorescence decay right it means a one e to the power minus t by tau 1 plus a 2 e to the power minus t by tau 2 and so on depending on the value of i.

So, now let me write here the steady state intensity is defined let me define as I s s; obviously, it is a function of lambda right. So, that is nothing but integration 0 to infinity I lambda t d t and in this case I have a separated this. So, one is the function of lambda and integration 0 to infinity b t d t and I already got the value of b t d t integration 0 to infinity b t d t. So, this is nothing but a lambda a is a function of lambda and sum over I a i tau i. So, if I want to calculate this a lambda is I can write it as I steady state lambda divided by sum over i a i tau i from this relation.

So, from here what I can write I which is a function of lambda t is equal to I steady state for this particular I am talking about that for this particular lambda right for different-different lambda I have to do that same thing again and again. So, this is equal to sum over i a i tau i into sum over i a i e to the power minus t by tau i. So, I got this equation.

So, in this equation you see that for a particular lambda for a particular value of wavelength what I got I got that how the intensity will vary with time right and in. So, if you have different-different wavelengths. So, for a particular time you can plot the intensity at different-different lambda for a particular time the intensity at different-different lambda; that means different-different emission wavelength. So, that can be calculated from here this is nothing but this lifetime of the molecule and here is my steady state intensity over here and here is again that lifetime component a i and tau i.

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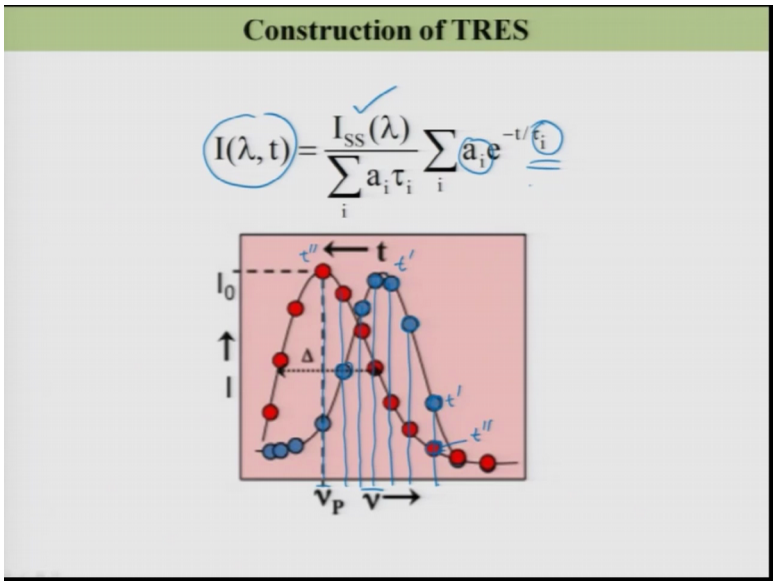

So, if I just show you this plot over here as you can see here at shorter time. So, this is my spectra this is plotted in nu bar. So, so in this case this is my shorter time spectra. So, here you see that these intensities are like these way intensities are like this way for different-different lambda or different-different nu bar these are the corresponding nu bar these are the corresponding nu bar right as shown here.

Now, if I change that time from this time to this time let us say this is tau t prime let us say this is t double prime and I recalculate this thing you see the position that the intensity of all these lambdas are now different. So, it was like here now it become here for different value of t it was for t prime, but this is now for t double prime, but at the same wavelength or same wave number.

So, if I plot this time dependence of this emission spectra then I will get a idea that how the system is getting stabilized or how the whole system is getting solvated by the solvation nevertheless what we need to get this I lambda t is these quantities like this one, these quantities this is know because we already know how to measure the emission spectra in the steady state that is time integrated emission spectra, but I although talked about the lifetime, but I have not talked about that how will going to measure this fluorescence lifetime. So, measurement of fluorescence lifetime is important.

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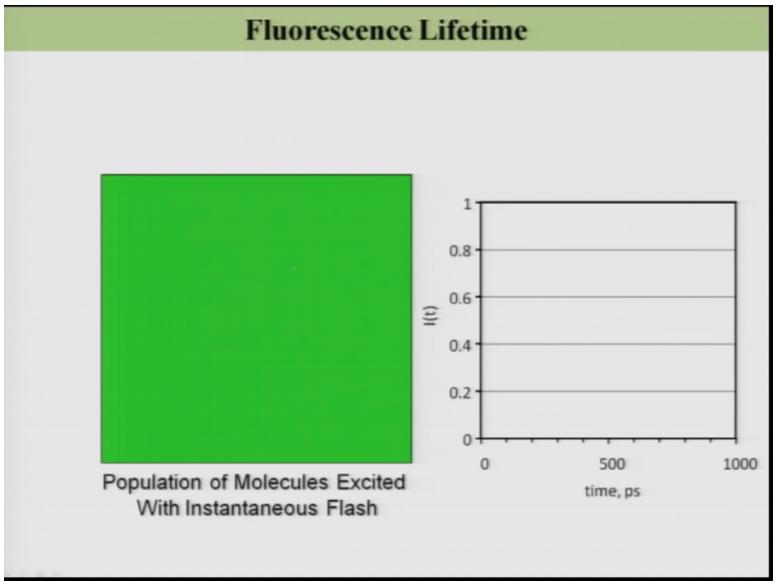

So, let us see that how will going to measure this fluorescence lifetime as I said earlier that fluorescence lifetime of a sample tau it means that the average time the molecule spend in the excited state.

Let us take this molecule right this molecule I have taken in this box and this molecules are excited with an instantaneous flash; that means, the time taken for excitation I have assumed that is infinitely small delta function. So, immediately all the molecules are taken into the excited state ideal situation real situation I will come later. So, ideal situation all the molecules are now in the excited state. So, in this case you can see over here the green colour represent that the molecule are in the molecules are in the excited state.

So, now if you start looking at that what is the fate of this molecule the molecule will come back to the ground state, but it is not like that that all this molecule will come back from the excited state to the ground state all of a sudden all the molecule will come back in at a particular time delay between the excitation right what happens is the that some molecule will come back from the excited state to the ground state at early time some will come at the later time yes.

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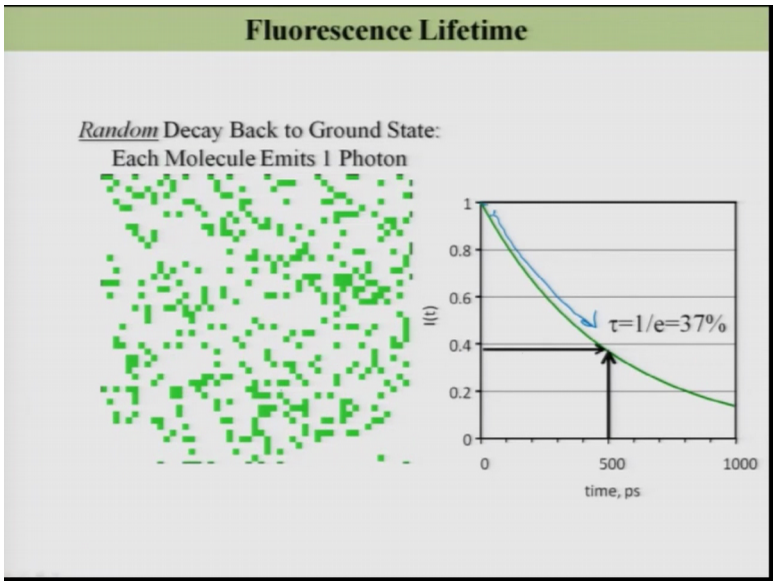

I have tried to show you by this animation as you can see here that this molecule was in the excited now it is becoming the ground state. So, this molecule is in the excited state now it is in the ground state.

So, that green means excited state white means ground state. So, it is a random process random process it means let me run it out again. So, here when the all the molecule was in the excited state the emission intensity was maximum as time goes the emission intensity is decreasing right as the molecules is coming back from the excited state to the ground state and I already have discussed when this value is like 37 percent then we said that this time is my fluorescence lifetime of the sample.

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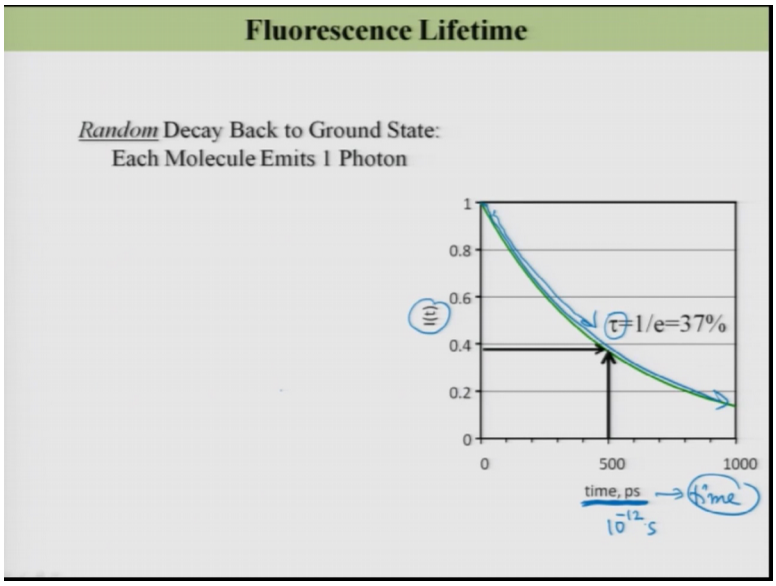

Now, question is that how will going to measure it right how will going to measure this tau or how will going to measure such kind of decay or how will going to measure this intensity versus time if it is if this time if this time is in the time window of hours then no problem I can simply take this molecule in my fluorometer I measure the intensity now I measured intensity after 2 second I measure the intensity after five second and so on and keep on measuring for an hour also and I will get this plot, but the problem is that as I said earlier the lifetime of this molecules are in the order of nanosecond; that means, you need to measure all this time in picosecond time resolution, then because again hundred because again time resolution.

So, that is what you see here I have written the time picosecond over here right is the picosecond over here; that means, you must have a stopwatch right or a machine which can measure the time in or in the picosecond time region right at least in the picosecond time region. So, picosecond is 10 to the power minus 12 second as you know and that kind first stopwatch; obviously, is not available easily in the market. So, we need a special treatment to get such kind of decay.

Basically the idea is just after the excitation all the molecules are in the excited state considering the hundred percent excitation which is not true again, but for these basic course let me consider that on excitation all the molecules are now in the excited state. And if you measure the fluorescence intensity as a function of time then the fluorescence

intensity should decrease right gradually and if you plot that intensity versus time you will get exponential type of curve if it is single exponential it is a bi-exponential then the curve will be little bit distorted and so on and so forth. So, ultimately you will get the intensity versus time plot and from there you will be able to calculate; what is the value of the lifetime tau?

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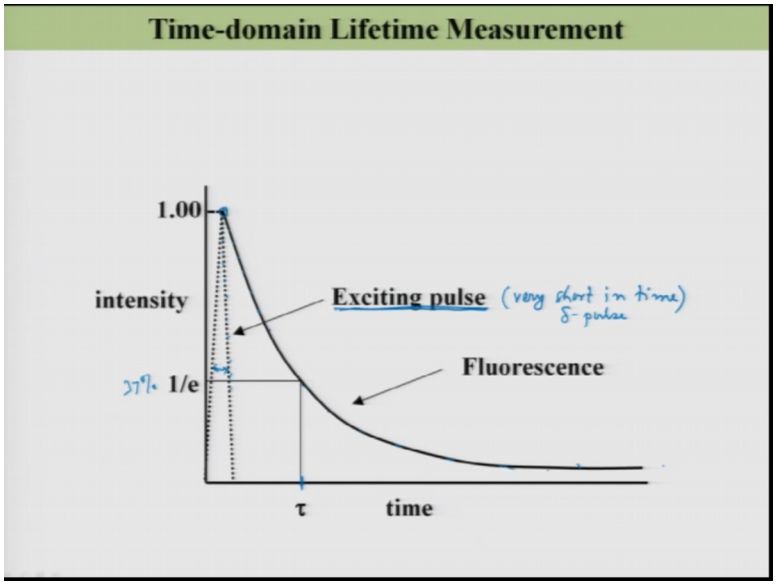

So, now let us discuss how we will going to measure as I said that lifetime can be measured in 2 way: one is the frequency domain method another is the time domain method, but in this course I will discuss only the time domain method.

So, in the time domain method as I said that you will going to excite the molecule with instantaneous pulse that is what I have shown here with this dotted line over here. So, these time; this time is very very small time very very small compared to these decay; that means, that when you are excited this molecule all the molecule will go to the excited state all of a sudden right there is no time depend time required to take this all the molecule from ground state to the excited state that is what I consider here. So, this is known as the excitation pulse. So, this excitation pulse is very short very short in time let us consider that this is a delta pulse; that means the width is 0 delta pulse.

Now, after excitation this emission intensity let us consider one it may be thousand maybe 1 lakh may be 1 crore, but I can always normalize it to one. So, let us say that this emission intensity is one right and then I will monitor how the emission intensity is

decreasing as mentioned and from here I will get or whenever that that remaining part is on 37 percent or so then I will say that this time is my lifetime and I will get all these intensity values as a function of time. So, I need to measure it the question is how I will going to do that.

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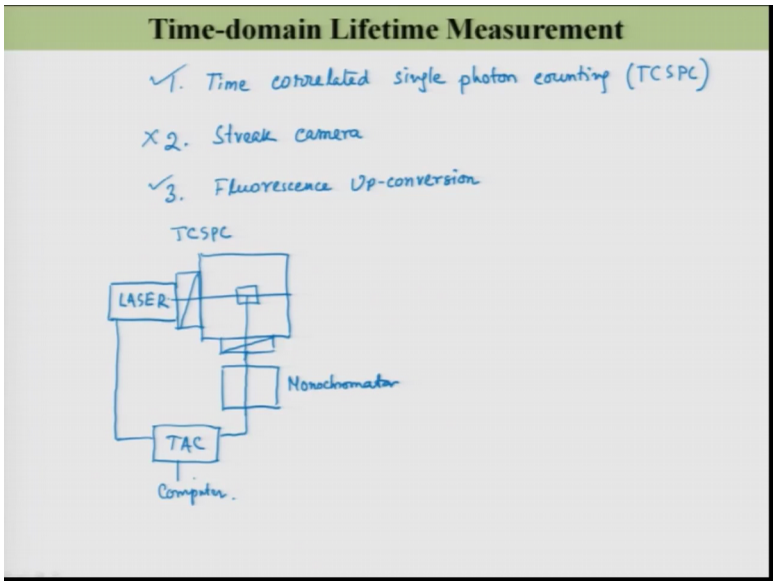

In time domain there are 3 different way to measure it the first one let me write is time correlated single photon counting method in short it is known as TCSPC second one is streak camera streak camera also this is very popular. And the third one is fluorescence up conversion method. So, this is also very popular all these methods are popular each method had its own advantage and own disadvantage first let me tell you this about this time correlated single photon counting. And I will not going to discuss this streak camera because it is little complicated. And I do not want to you discuss this in this basic course and then I will discuss about this fluorescence up conversion.

So, let me draw the basic setup of this time correlated single photon counting. So, here what do you need you need a laser why I need laser because I want to create a short pulse the laser has unique property that it can create ultra short light pulse I said that in the in my previous discussion over here I said that very short in time that kind of excitation pulse I need how short pulse you can create for let us say for a light bulb if you turn it on and off very quickly it could be 1 over 10 second. So, light is on only for 1 over 10 second that will; that means, 10 to the power minus 1 second, but I need 10 to the power

minus 12 second I need 10 to the minus 15 second how I will going to get that you cannot make a electrical device by which we can turn on and turn off in show so fast.

So, I need some unique device which can generate such kind of short light pulse and laser is one of is the device which can generate that short light pulse I will discuss very briefly about how you are going to get this ultra short light pulse by laser, but now let me consider 2 light draw that basic setup of the TCSPC system. So, this is my TCSPC and here I have this laser and just after the laser I have something over here then I have my sample; sample is placing over here.

So, when the laser light will come it will excite the molecule and I have again something over here I will discuss what are these things later, because it is not yet the time to discuss the these things and then emission will come from this side I will going to have here my monochromator to choose the emission wavelength then I have a device which is called tack time to amplitude converter which is connected to the computer this device is very very basic TCSPC setup for our discussion and today the time is up. So, I will continue the discussion on the next day.

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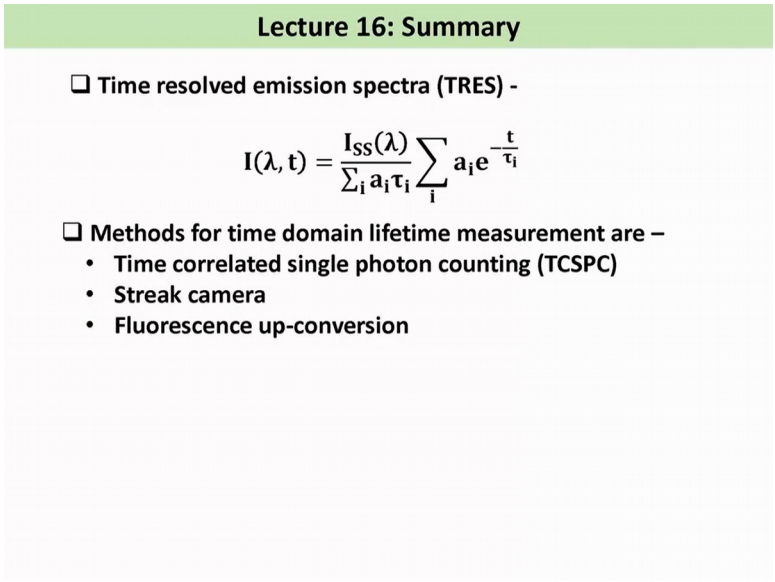

Thank you for your kind attention.