Basics of Fluorescence Spectroscopy
Prof. Pratik Sen
Department of Chemistry
Indian Institute of Technology, Kanpur

Lecture – 17

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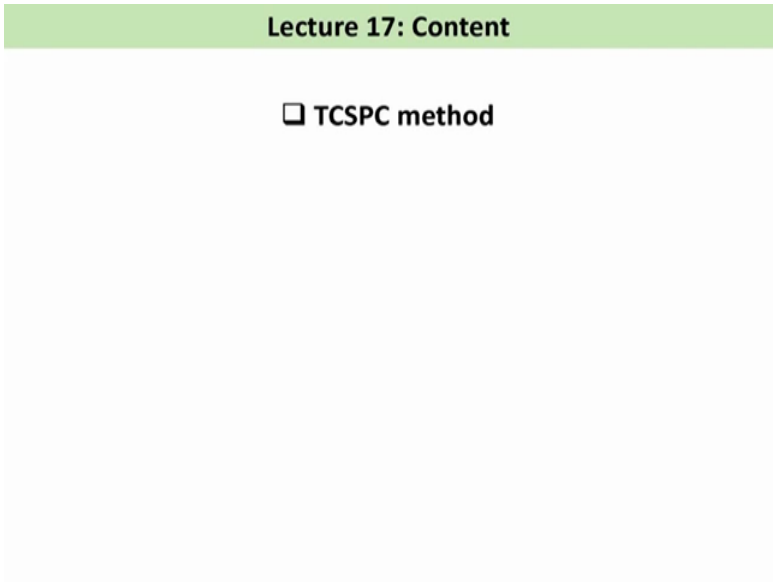

Today what we will going to discuss is the time domain measurement of the lifetime of the fluorescence lifetime; and the last day what we are discussing about three different methods for the time domain lifetime measurements.

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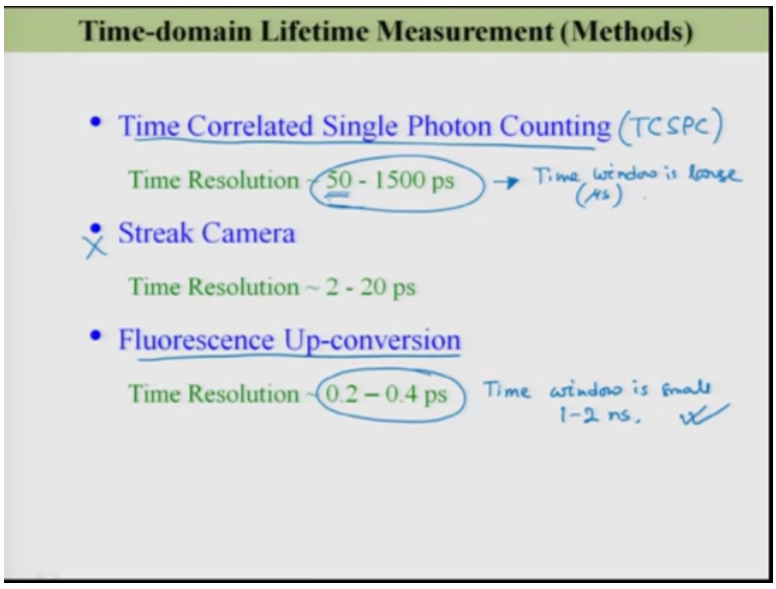

The first one is time correlated single photon counting method in one word this is known as TCSPC. And the other method which I will going to discuss maybe not today maybe on the next class is the fluorescence up-conversion method, and I will not going to discuss this streak camera because probably time will not permit me to do so.

So, just let us compare this time correlative single photon counting setup and the fluorescence up-conversion setup. Each setup has its own benefit and also each setup has some limitation of it. For example, this time correlated single photon counting method the time resolution are the IRF is not very high depending on system to system this can go to 50 picosecond to 1500 picoseconds also. However, this fluorescence up-conversion system as you can see this time resolution can go down to 0.2 to 0.4 picosecond. So, in that sense the fluorescence of conversion setup is superior to time correlated single photon counting setup. However, in the TCSPC setup the advantage is that it has a very big time window.

Time window is large we can go to microsecond no problem, but in this case the time window is small typically one go to 1 to 2 nanosecond time window for this; that means, you cannot measure the fluorescence intensity for a longer time period in this case although the time resolution is high. However, in this case time resolution is like a 50 picosecond, but we can measure for a longer time period right. So, time window is very large ok.

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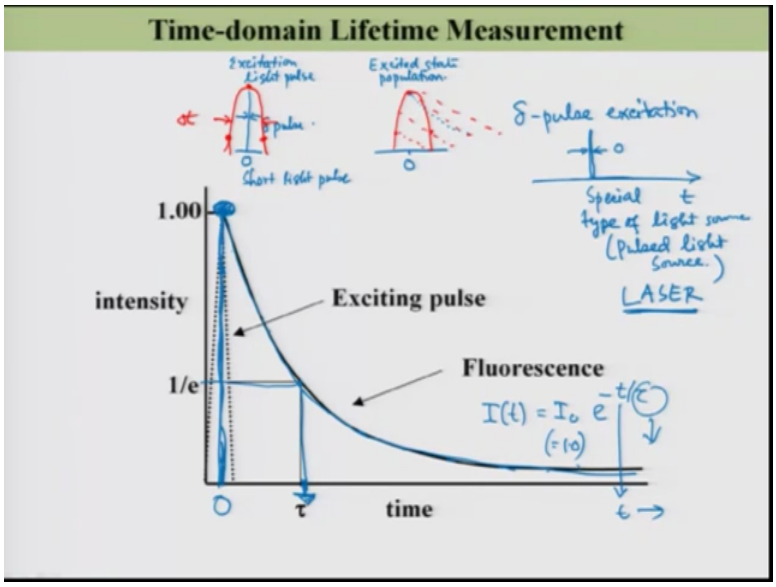

Let us start the TCSPC setup and as I said that for the time domain lifetime measurement. So, that it is TCSPC or whether it is up-conversion what we usually do we usually excite the sample with an ultra short light pulse right. So, light is present for very short time and ideally the light is present so short time that this light pulse is treated as a delta pulse right. So, delta pulse excitation; it means if I plot the intensity of excitation light as a function of time then if this is the time axis.

So, the light will come like this way and then there is no light and this width is 0. So, this is the my delta pulse this is a ideal situation, but in reality it depends on the quality of the laser or the property of the laser, because I want to generate the light pulse as short as we can and that cannot be generated by normal light source that is what we have discussed like a filament bulb or the general lamp or dodge ram lamp those things we have already discussed.

So, we need a special type of light source to generate such kind of short light pulse right what is that special type of equipment we need is a laser. So, special type of light source we called this pulsed light source, and laser is very famous for that we will going to discuss the little bit about this. So, once we excite this sample with this delta pulse light source as I have done here. So, in no time this is my zero, in no time the molecules will go to the excited state.

On the other hand, if you use a broad light pulse for the excitation, what will happen just briefly I am drawing over here, suppose you are exciting a delta pulse for the excitation? So, this is my 0 time. So, the population this is the excitation light pulse and this is the excited state molecule population excited state population of those molecules you are exciting at the 0 time, the population will be like this maximum and then this population will decay. On the other hand if you use such kind of light pulse in this case this is not a delta pulse right has some width delta t.

So, in this case this part of your light pulse will excite some molecule. So, the population is something like this at time t equal to 0 here the intensity is maximum. So, I will get this maximum intensity. So, and at this time the intensity again small. So, I will get the intensity small. So, the excitation right the excited state population will take such kind of ship and each of them will decay and the decay will be distorted that we are going to discuss later also.

So, as you have understand that what I need I need a very short light pulse like this, short light pulse and once you excite you got this excite state population, whatever the population I have just normalized to 1. And then I will monitor how the population will decay right how the population will decay like this. And that is it if I know this then I will be able to calculate the fluorescence lifetime. Because I know fluorescence lifetime we already have seen that flow for fluorescence lifetime the dependence of this intensity I is the function of t is equal to I 0 which is initial intensity in this case is equal to 1 for this particular case into e to the power minus t by tau.

So, if I fit this measured fluorescence intensity as a function of time with such kind of equation, then simply I will get this value, because this t is my variable t is over here t. So, I will get this tau or I can say that when the population decay is about 63 percent a then I will get whatever the time I can denote it as my tau fluorescence lifetime.

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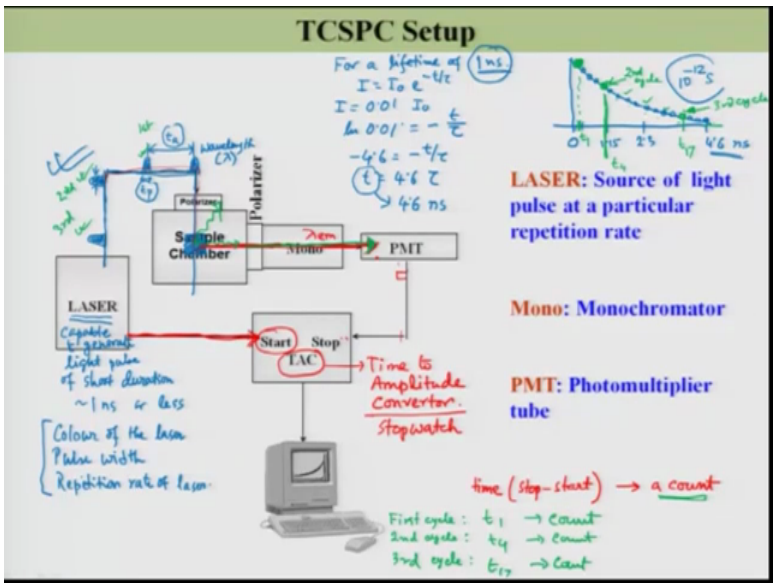

Let us go to this TCSPC setup the set up by which we can determine this lifetime. As I said earlier that if the life time is very long long in the sense in hours right then each second you can measure the fluorescence intensity by your normal flora meter so no problem. However, the life time you know using the order of nanosecond that we have discussed; that means, through let us say for such kind of nanosecond life time, if we start with intensity 1 then that intensity will going to be 0 in about 5 into tau, so 5 nanoseconds.

So, that simply we can calculate over here for a lifetime of 1 nanosecond right. So, my equation is I equal to I 0 e square minus t by tau, this tau is 1 nanosecond let us say I say that when I is equal to 0.001 times I 0 then I said that intensity will completely decayed; because this is exponential function it will be 0 where of the infinite time ideally mathematically, but for us when it is like just 1 percent I said that the intensity is 0 right finished. So, in this case what I can do I can do l n 0 point l and 0.00 so 1 percent I said na. So, let me write 01.

So, l n 0 1 is equal to minus t by tau. So, this is be equal to minus 4.6 equal to minus t by tau. So, t will be equal to 4.6 time of tau; that means, when the lifetime is about 1 nanosecond the time window declared to see the complete decay is about 4.6 nanosecond and if this time window is 4.6 nanosecond that is 0, and here is 4.6 here is 2.3 here is 1.15 like that and if you want to see this complete intensity decay right you need to get several points over here right several several points over here. That means, you need to measure the intensity of fluorescence right at right every point 1 nanosecond or point 0 1 nanosecond or 0.001 nanosecond something like that, which cannot be done right using your standard flora meter it was if it is hour, but it is in nanosecond.

So, I need to measure the fluorescence intensity at every picosecond also; that means 10 to the power minus 12 second that cannot be done so easily. So, what we should do? We should use some setup like this way just I will briefly explain you this setup here I use a laser over here as you can see here, so the property of the laser is such that it will generate the ultra short light pulse.

So, this is capable to generate light pulse of short duration. Now how short there is the question mark right. If the total time window let us say for this 1 nanosecond the total time window is 4.6 nanosecond and if your light pulse is 10 nanosecond. So obviously, we will not be able to see anything, it is simply like that using a meter scale you cannot measure a millimeter. Like if your wristwatch does not have a second needle right you cannot measure 1 second or 2 second using your wristwatch very to that if your excitation light pulse is much broader than this time window then you will not be able to measure that. So, your excitation light pulse has to be shorter than this.

So, for like a normal fluorescence lifetime measurement right if the lifetime is about a 10 nanosecond we usually use a light source of either 1 nanosecond or less than that. So, the

light pulse should be about nanosecond 1 nanosecond or less right then I will be able to measure it right. So, these light pulse. So, these are the pulse light. So, pulse light means please note that the light is present for some time, then for the rest of the time light is not present. Again light is present for some time this width is the pulse width of the light and then light is not present for some time again light is present again light is not present, again light is present then light is not present; that means, what I will going to get I will get a series of such kind of pulses and that series of pulses can be defined by its reputation rate.

So, for example, if the time between these two successive pulse is t r then and this pulse width is t p right then by defining these two quantity by defining this t r and this t p I will be able to characterize such kind of light and another quantity also have to defined here that is a wavelength of the light because you need a particular wavelength for the excitation and another property is the wavelength lambda.

So, for a laser what I say that I will fix what is the color of the laser what is the pulse width and what is the repetition rate of the laser. So, these three property I need to look at, now let us see that this laser is coming out of this laser these pulse rates are coming out of this laser and eventually it will excite your sample, your sample is situated in the middle of the sample chamber and then this fluorescence will come out of this right this then the fluorescence will come out of this. So, this fluorescence is not a single wavelength. So, you need to choose your lambda emission your desired lambda emission that will be done by this monochromator and that will be directed to the PMT to measure is intensity.

So, these setup works in a way that when this light these short pulses are going to excite the sample over here, at the same time it will send one signal to a device called TAC, this TAC is time to amplitude converter. So, at the time this laser will going to excite your sample at the same time the laser will send another signal to this device TAC. The details of the TAC I will discuss later, but for now let us take this TAC as a kind of stopwatch. So, once it will send the signal to the TAC the start signal to the TAC, it will start counting the time right and this stopwatch is a very nice stopwatch, it can count the time in the order of picoseconds. So, this laser will send one signal to this TAC. That means, now I have excited my sample please start counting time. So, then this device will start counting the time.

So, this; obviously, at that same time the sample has been excited. Now once you excite the sample several molecule will go from the ground state to the excited state, it depends on the how many photons are present in the each pulse and what is the concentration of your sample and so on. So, you will get a handsome population in the excited state and as I said the last day that this not all this excited state molecule will come back to the ground state at a particular time, but this is a random process; some molecule will come back from the excited state to the ground state at early time some molecule will come back from the excited state to the ground state later time some molecule will come back at the time t equal to tau.

So, the molecule will come back from the excited state to the ground state and I can measure this emission right by this PMT. The condition is that in this case that when one photon from this sample position will reach the PMT, when one photon from this sample position will reach the PMT right then this PMT will convert this photon to the electron and he will generate a electron pulse over here. So, he will generate electron pulse over here that is the current pulse over here and that these current pulse will come and it will stop this stopwatch.

So, now stopwatch will measure the time. So, what stopwatch will measure? Stopwatch will measure my TAC here stopwatch means my TAC. So, this stopwatch will measure the time delay between the start and the stop; that means, you have excited your sample and not immediately the photon is coming out of it may take some time and you will get a photon following on the photocathode of this PMT, and this was converted to the electron and then current pulse will stop it.

So, the delay between excitation and emission plus the path length required to excite and then the whole process required to reach to this stop pulse right. So, this is the difference between the start and the stop. So, in this case what you will going to get? You will get a time which is start or stop minus start and for that particular time you will get a count right we will get a count. You remember that you have excited several several molecules right in your sample chamber some of them will emit early time some of them will emit at the later time.

In this case what I have to ensure that the first emitted photon is not necessarily be the first detected photon by this PMT. Let me explain it in a better way; suppose this is my

decay profile as I showed here. So, these are the probably the first emitted photon is at this time short time, it is not guaranteed that these photon will come along this direction it is possible that this photon will go in that direction because these type of emission is a spontaneous emission is a direction less . So, it can go to any direction right it can go to the upper side lower side left right and all these directions.

So, when these particular times photon is along this line, then only it will be detected. Let us consider that you got this particular time photon is emitted and it is along this particular line in this particular line and you got a signal that this count is for time t 1 you got a count; obviously, after that the photon of this one, photon at this time, photon at this time, photon at this time photon at this time all this time will be emitted until it will be reach the 4.6 nanosecond in this particular case, but for this setup we will not going to use all these emitted photon.

So, once I will detect one photon which actually make the stopwatch stop, we finish the experiment and we will wait for the second excitation pulse to come and excite the sample. So, for one turn one light pulse comes in excite the emitted photon is detected which emitted photon? I do not know it could be this one, it could be this one, it could be this one, it could be that one, but in this particular case I said that this is the emitted photon at time t equal to t 1 and that photon has been detected, but this excited state molecules are much more right is not only one. So, other molecule will also emit at the different different times, but I will not going to consider those photons for this particular light pulse. What I will going to do? I will wait for all the excited state to come back to the ground state. So, I have to give enough time for relaxation; that means, all the excited sate molecule will come back to the ground state, then when the situation is like before excitation.

So, the situation is just like the before excitation all the molecules are now in the ground state. So, now, what I will do? I will use the next excitation pulse let us say the next one like here. So, this will come and then again excite. So, again this light pulse will take all the molecules from the ground state to the excited state, and for in that cycle it is not necessarily that these photon will be directed along this path is not necessarily. It could be that these photon this time these photon is directed along this path for the second cycle. So, that this is for the second cycle these photon probably directed along this path of the PMT, and let us say this is the time t 4.

So, for the first cycle I got a count at time t equal to t 1. For the second cycle I got a count at time t equal to t 4 right then again I wait how long I should wait? I wait till all them excited state molecule will come back in that to the ground state, then I use the third pulse right; that means, is my third cycle right this is the first, this is the second pulse, this is the third pulse right like that. So, when the third one will come that is going to be my third cycle.

So, let us say for the third cycle this photon which is emitted at very later time. So, this is my third cycle this is t 7 or maybe t 17. So, these photon which is emitted that very late that is the only photon which is directed to this path which is directed to this path, then in the third cycle I will get a count that time t equal to t 17 right. Now consider you are repeating the same 10 million times; that means, that same cycle, cycle 1, cycle 2, cycle 3 cycle n cycle million times cycle 10 million times and so on.

So, there is always a possibility that in that histogram you will get the photon of all these different times to be accumulated right. So, that is what I was showing over here.

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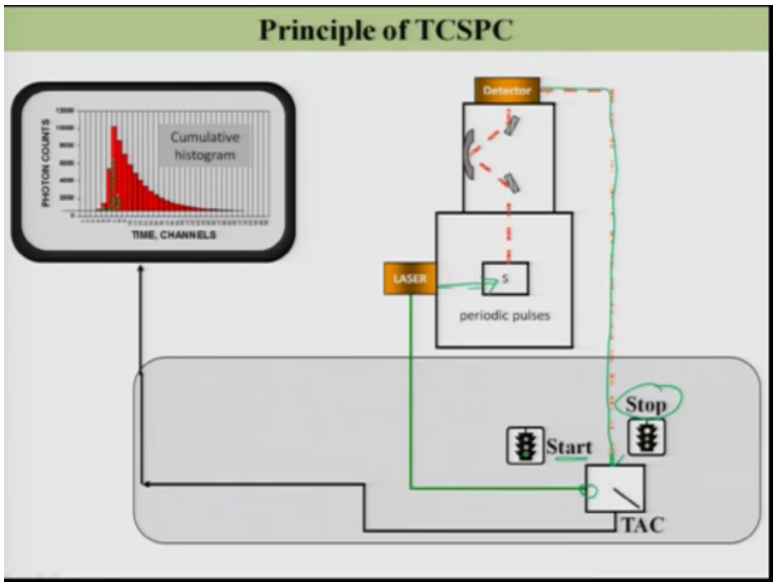

So, here is the laser, laser is sending one signal, here to start the stopwatch and then this is the pulse train it is exciting and then this photon is coming it is not necessarily that the first emitted photon has to be first detected photon, because the spontaneous emission is direction less and we do something over here to reduce the detection ability of the photon.

So, most of the photon should be lost somewhere right that I will going to discuss later probably so that a emitted photon one of the emitted photon will reach the detector over here, and this detector will send the stop signal as you can see here and it will measure the time. So, as at different different cycles you will get a different different point at different different time like and this different at different time you will get a count.

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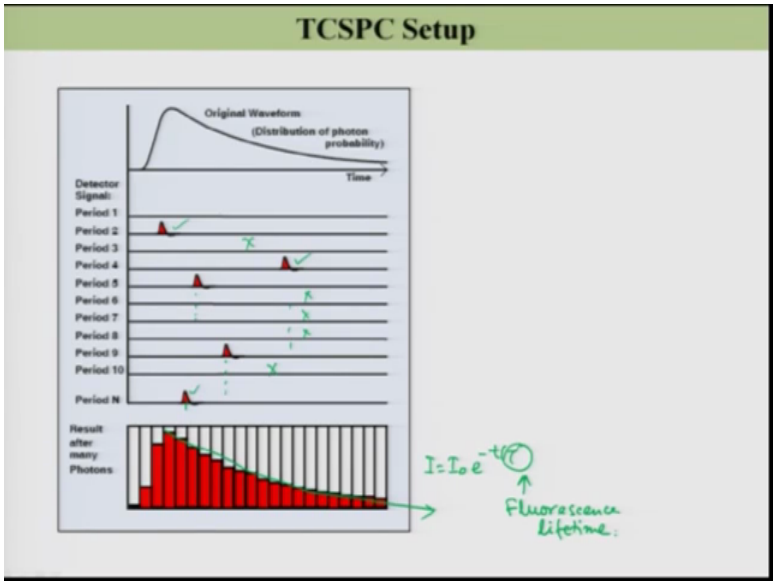

So, ultimately what you can do you can plot a histogram like that as shown here for the period one probably none of the photon has been detected, in period 2 one photon has been detected here nothing, here one photon at this particular time, here one photon at this time, here nothing, here nothing, here nothing here one photon this time, here nothing, here one photon at this time and if you sum it up right for 10 million times then what you will get? You will get such kind of histogram which is nothing but the dependence of fluorescence intensity as a function of time. And now if you feed this using that equation I equal to I 0 to e to the power minus t by tau then you will be able to get the fluorescence lifetime of your sample well.

So, let me finish here because time is up and on the next day what I will going to show you that the basic setup which I have shown here will actually not going to work.

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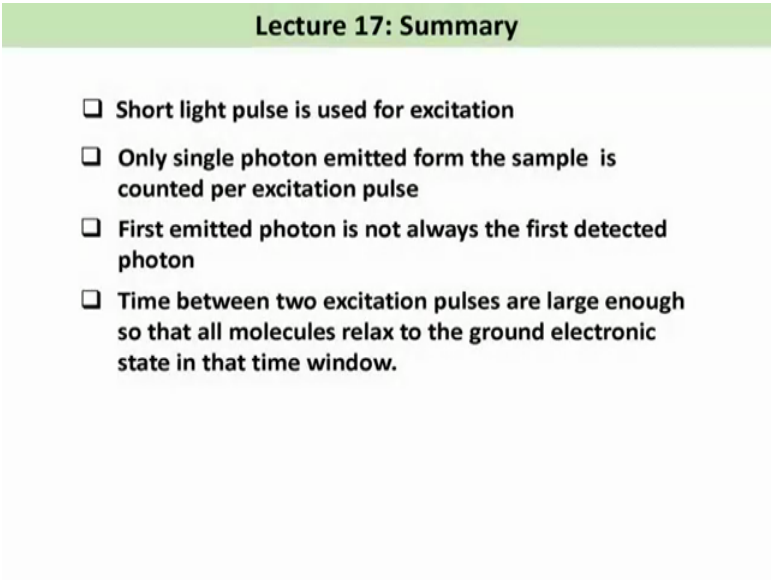

We have to do several modifications on these basic setups to actually get this time because of several problems in this system and those things we will be going to discuss on the next class.

Thank you.