Basics of Fluorescence Spectroscopy
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Lecture – 29

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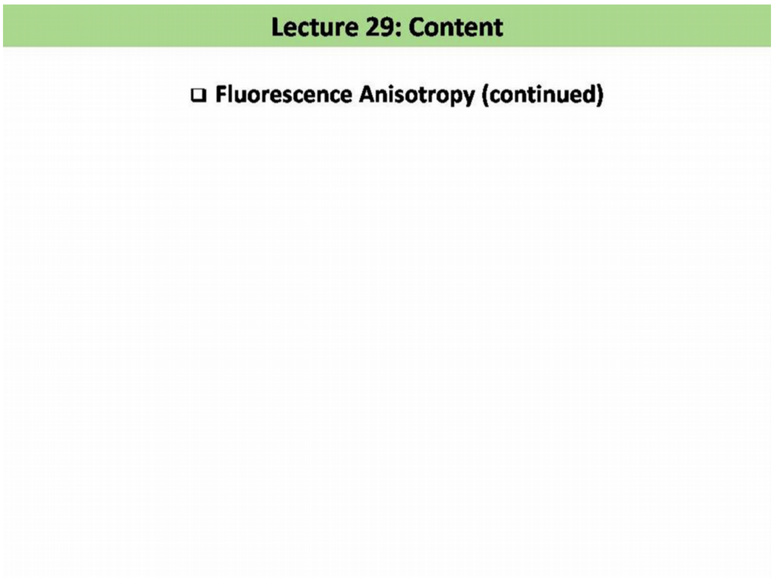

Welcome to the lecture number 29. Let us continue our discussion on fluorescence anisotropy and till date what we have done is to find out what will be the time dependence of the intensity of fluorescence when collected in the parallel direction; that means, I parallel as a function of t that we have seen in the in the last class let us write it once again.

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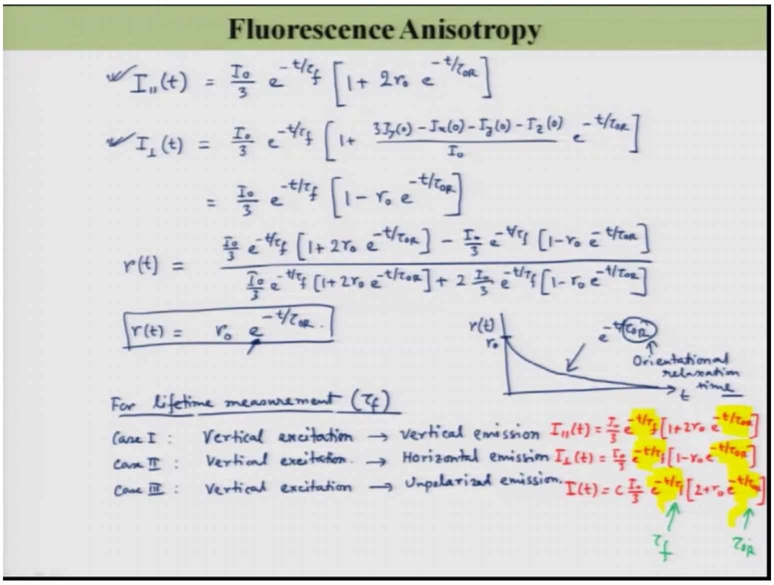

So, what we have seen? That I parallel t is equal to I 0 by 3 e to the power minus t by tau f into 1 plus twice r 0 e to the power minus t by tau over. Similarly we can also calculate that the same procedure can be used to evaluate the I perpendicular t and if you do so I will just write 2 steps over here you will get I 0 by 3 e to the power minus t by tau f and immediately you will get 1 plus 3 I y 0 minus I x 0 minus I y 0 minus I z 0 divided by I 0 e to the power minus t by tau over. So, this is I 0 by 3 e to the power minus t by tau f from here it will be minus so into 1 minus r 0 e to the power minus t by tau over, good. So, what I got? I got 2 equation 1 is for I parallel t and another is for I perpendicular t.

Now, I can immediately write the form of r t right this is my anisotropy which is also time dependent. So, for this r t what I will do? I will put this expression for this I parallel t minus I perpendicular t divided by I parallel plus twice I perpendicular. In this case I am taking the G value equal to 1, but if there is any g value I can take, but the form right will be like this only right. Now let me write this 2 form, so here will be my I 0 by 3 e to the power minus t by tau f 1 plus 2 r 0 e to the power minus t by tau over minus this I parallel and minus I perpendicular. So, I 0 by 3 e to the power minus t by tau f 1 minus r 0 e to the power minus t by tau over divided by this is straight forward nothing special just you have to substitute this expression for I parallel and I perpendicular and you just need to solve it simplify it and looks this looks like, but if you simplify and which you can do later what you will going to see is a very simple form of this which is nothing but r 0 into e to the power minus t by tau over.

So, you see this simplification right I will get like this way. So, ultimately what I got r t is r 0 e to the power minus t by tau over; that means, the value of r will start from r 0 and when the t is very large eventually the value of r will going to be equal to 0. So, the value of r which is anisotropy will start from r 0 and will decay to 0 and that decay will be exponential in nature. You see here I have this e to the power t over here e to the power. So, if I now plot r t as a function of t. So, this will be r 0 and it will be something like this right. So, this will be something like this and the corresponding time constant right. So, you feet it with e to the power minus t by tau over, so you will get this orientational relaxation time. Now if you are interested on this anisotropy that how this is the molecule actually reorient then you can do this kind of prescription and you can get this tau over value.

Now, as I told you that you can earlier during our discussion on TCSPC setup and the fluorescence up conversion up conversion setup. I told you that this in both the setup there are 2 few 2 components optical elements was there and I told you that I will explain those optical elements later on and now this is the time to discuss about that. Now consider that you have a setup and either TCSPC or up conversion and you want to measure the lifetime right. So, what will be your way to do that, either you will excite vertically and emission is collected vertically or you excite vertically emission is horizontal or you excite vertically and the emission is unpolarised right you do not select, you do not put the analyser. So, let me write this.

So, for lifetime measurement I could have 3 different situation like here I want to measure tau f not tau over right please note that. So, the 3 cases case 1 I have vertical excitation and vertical emission this is nothing but I parallel t I already showed you right. I can have vertical excitation horizontal emission. Case III, I can have vertical excitation and unpolarised emission. In the first case you already have seen what is the first case? This is nothing, but I parallel t. So, I can simply write the expression of I parallel t over here for the case 1, for the second 1 this is nothing, but I perpendicular t. So, let me write the expression and for the third one I have not derived, but it can be derived without any problem and let me also write that expression right. So, here this is nothing, but I parallel t which is equal to I 0 by 3 e to the power minus t by tau f to 1 plus 2 r 0 e to the power minus t by tau over. This one is nothing but I perpendicular t, so this is I 0 by 3 e to the power minus t by tau f to 1 minus r 0 e to the power minus t by tau over and this is for the unpolarised. So, I do not try it I parallel or perpendicular I just write I t this will going to be equal to some constant will be there then I 0 by 3 e to the power minus t by tau f into 2 plus r 0 e to the power minus t by tau over.

Now, you see in all these 3 cases right this 3 cases are possible case. So, in all this 3 cases what you can see is that you wanted to measure the tau f, but you land up with the measurement of such kind of complex see here, here tau f is present here you have this tau f here you have this tau over here you have this tau f you have the tau over and here also this tau f and this tau over, but you wanted to measure only the tau f end up with measuring a mixture of tau f and tau over and you will never get the fluorescence lifetime that is defined as the tau f, but what is the prescription now for the measurement of fluorescence lifetime allow. So, that will going to discuss now.

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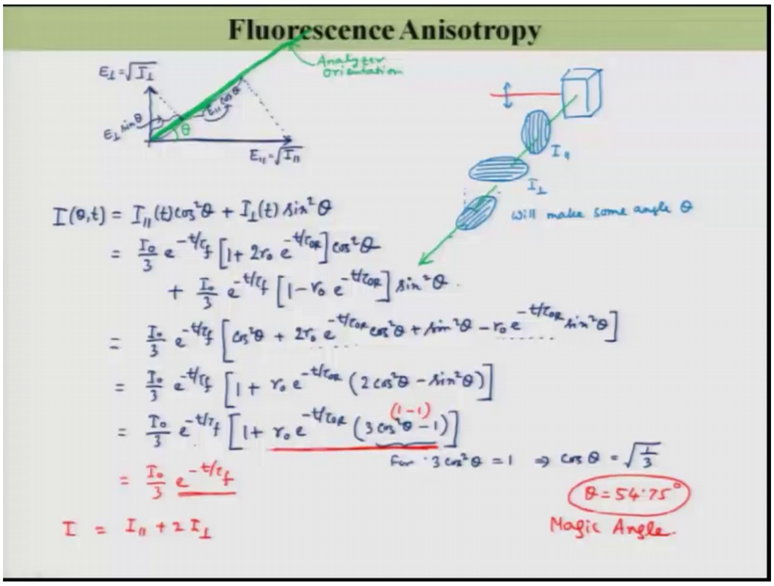

Now, let me consider that these 2 this is my I perpendicular and this is my I parallel right let I am consider it like that way right here and better I write the electric field instead of intensity which is the square of this intensity electric field. So, I write this E parallel equal to square root of I parallel and E perpendicular is equal to square root of I perpendicular. So, right now what you are doing in other cases, either I was measuring this I parallel t or I was measuring this I perpendicular t or I was measuring everything right that was the thing what I said, but is it possible that I measure something between I parallel and I perpendicular why not, because you remember that when I used my let us say this is my sample cell and you are exciting your sample like this way and emission is getting collected at the right angle and you had your polariser over here.

Now, when the polariser is oriented like this way I assume that this is I parallel when I rotate this polariser like this way this is I perpendicular right this is I parallel because the this is like this and this is I perpendicular and if I rotate the polariser like this way then it will make some angle theta is not parallel not perpendicular. So, it is will make some angle let us say theta from the vertical excitation right from the parallel component. So, from here if this is this axes then this may be theta right that is what I will going to draw over here. So, now, if you collect the emission then this emission will be collected at that particular angle theta. So, that is what I am going to write. Let us assume that you put your that analyser in such a way that the light having such kind of polarisation is only passing through right this analyser. So, let me extend it over here. So, this is the position of my analyser this analyser orientation and I said that this is oriented at an angle theta from the parallel component.

Now, I can simply do some trigonometry over here. So, the component of E parallel which will projected on this angle theta I can calculate it I will just write draw a normal over here right, if this is I E parallel then this will be E parallel cos theta. So, this will be my E parallel cosine theta and if this is E perpendicular then I will they are normal. So, then this part, from here to here this part will be E perpendicular sin theta. So, let me and this is right. Now my life is simple let me write it. So, I just simply write over here if it is E parallel sin theta; that means, the intensity is the square of that when it E parallel cos theta then its intensity is just square of that. So, now, I can write it like this way you see I is a function of t right, no I is not only function of t it is also function of theta now. So, I will, here I is a function of theta and t is equal to I parallel t cosine square theta plus I perpendicular t sin square theta.

So, now I will put the expression for this I parallel t and I perpendicular t into it and then I will try to simplify this equation and let us see what happen right. So, now, I will put this expression first one is I 0 by 3 e to the power minus t by tau f to 1 plus 2 r 0 e to the power minus t by tau over multiplied by cosine square theta and I 0 by 3 e to the power minus t by tau f here 1 minus r 0 e to the power minus t by tau over to sin square theta. This will be equal to I 0 by 3 e to the power minus t by tau f I will take this common and then I will write it once again.

So, this will be cosine square theta plus twice r 0 e to the power minus t by tau over cos square theta plus sin square theta and then it will come as a minus r 0 e to the power minus t by tau over and sin square theta. So, I can rewrite this as I 0 by 3 e to the power minus this is important, very important thing I am doing now. So, into here you see cos square theta plus sin square theta. So, I have 1 plus and let me take this here r 0 this part right. So, then let me write this r 0 e to the power minus t by tau over and then this will be 2 cosine square theta minus sin square theta. So, 2 cosine square theta minus sin square theta this means I am almost done. So, I 0 by e to the power minus t by tau f I will keep this part as it is r 0 e to the power minus t by tau over and this can be written as 3 cosine square theta minus 1 I am done.

Now you see for a certain value of theta this 3 cosine square theta minus 1 this value could be 0 right if so what will happen? This is 0 let me write for 3 cosine square theta equal to 1 right I can write here cos theta is equal to root over 1 upon 3 then when the value of 3 cos square theta is equal to 1 then this will be 1 minus 1, this will be 1 minus 1 then this quantity will going to be 0 then this whole quantity will be 0. So, what I will get? I will get this equal to I 0 by 3 e to the power minus t by tau f. See for a particular value of theta when cos theta equal to root over 1 by 3; that means, theta equal to 54.75 degree. When theta equal to 54.75 degree; that means, if the analyser is placed at an angle 54.75 degree from the direction of excitation or from the I parallel like this, this is I parallel, this is I perpendicular like this.

So, in that case what you will going to get is that these term these this part this part vanish this part vanishes and what you will left over with this I 0 by 3 e to the power minus t by tau f; that means, you will be able to measure the lifetime otherwise not. You can do another thing if you just simply take I parallel plus 2 I perpendicular let me write here, I, suppose you have collected the fluorescence transient or fluorescence decay I parallel and I perpendicular and then you do this one - I parallel plus twice I perpendicular just put all these values I mean that expression of I parallel and I perpendicular and simplify it you will also get that tau f term is there.

In this case you have to measure 2 times at in the magic and if you put this angle during measurement then you have to measure only single time result is same right and this angle is a special angle and this angle is known as magic angle.

So, now you understand the role of those things I have drawn in TCSPC setup and up conversion setup those are the polariser or analyser. You have to set the analyser at the magic angle then only, then only you will get the correct lifetime. Now here one point I should now tell you that suppose the lifetime, but sometime you will see that people are measuring without that magic angle polarization; that means, everything is wrong or under some condition that the lifetime measure without analyser without magic angle polarization is ok, it is ok only when the lifetime is very large compared to the rotational time right. So, in case the tau over is much smaller than tau f, much much smaller than tau f then the decay of this part will not going to affect that way that tau f, but if you are interested in the timescale which is comparable with the tau over then your decay will be destroyed right.

For example suppose you have fluorophores lifetime is 1 microsecond. So, you will going to measure this decay from 0 micro second to let us say we are going to measure like 5 microsecond like if it is 1 microsecond, but the rotation of this fluorophore right is typically is in the order of tens of picosecond 50 picosecond, 60 picosecond, 100 picosecond. So, the isotropic situation will be achieved in the solution within let us say 200 picosecond, but 200 picosecond is nothing in front of microsecond. So, the error will be very very small. So, for those kind of long lifetime sample you really do not have to worry about this magic angle polarisation for the measurement of the fluorescence tangent, but if you are in the domain of few tens of picosecond it is must right, otherwise all your data will be wrong.

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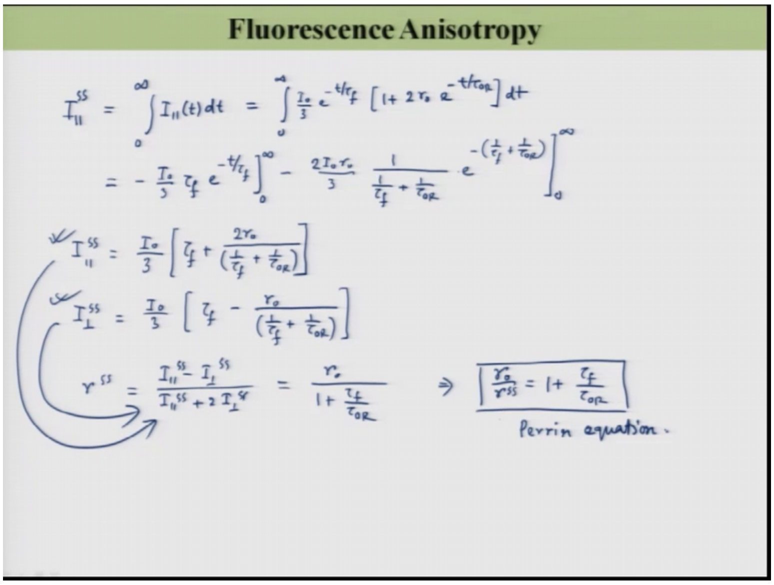

Let us continue and we could see a nice equation now. What I will do now is the following. I will integrate it, what I will integrate? Integration 0 to infinity I parallel t d t I will do that, I will do this integration. Let me write integration 0 to infinity that is the form of I parallel t, I 0 by 3 e to the power minus t by tau f right. This integration 0 to infinity I parallel t d t this is nothing, but the steady state I parallel. So, I just termed it as I parallel steady state right. So, it is integration for all the time this I parallel t is changing with time, but this it just I have integrate it. So, if you evaluate this integral, what you will get? You will get such kind of form let me write without evaluating which you can check later on if you want otherwise take my word I 0 by 3 tau f e to the power minus t by tau f that limit 0 infinity minus 2 I 0 r 0 by 3 into 1 divided by 1 by this is little lengthy, plus 1 over tau over into e to the power minus 1 over tau f plus 1 over tau over and here limit is again 0 to infinity.

So, if you put this value this limit, so what will get is I 0 by 3 into tau f plus twice r 0 divided by 1 over tau f plus 1 over tau over. So, you will get I parallel steady state as this. Now similarly I can also calculate this I perpendicular steady state. So, this I perpendicular steady state similarly right, similarly we will do is you will get as I 0 by 3 to tau f minus r 0 divided by 1 over tau f plus 1 over tau over and r which is steady state you can measure I already told you how to measure it this r right which is nothing, but I parallel steady state minus I perpendicular steady state divided by I parallel steady state plus to I perpendicular steady state and if you plug in the expression for I parallel and I perpendicular in this equation and simplify you will be surprised to see that after simplification this result will be very simple - 1 plus tau f by tau over. Now I can rearrange it and I can write it as r 0 by r ss is equal to 1 plus tau f by tau over. Very important equation, this equation is known as Perrin equation.

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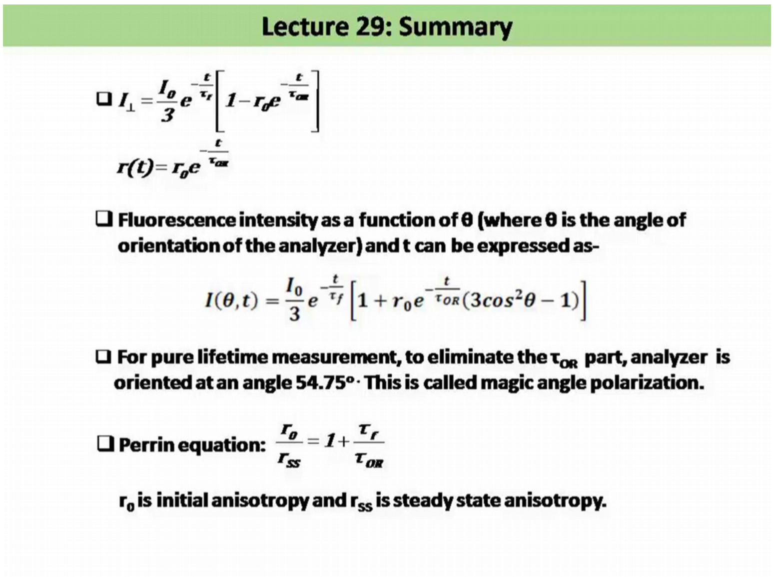

And we will stop here today and we will continue our discussion on the next class.

Thank you.