Basics of Fluorescence Spectroscopy
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Lecture – 27

Welcome to the lecture number 27. In the last lecture we have started with the fluorescence anisotropy.

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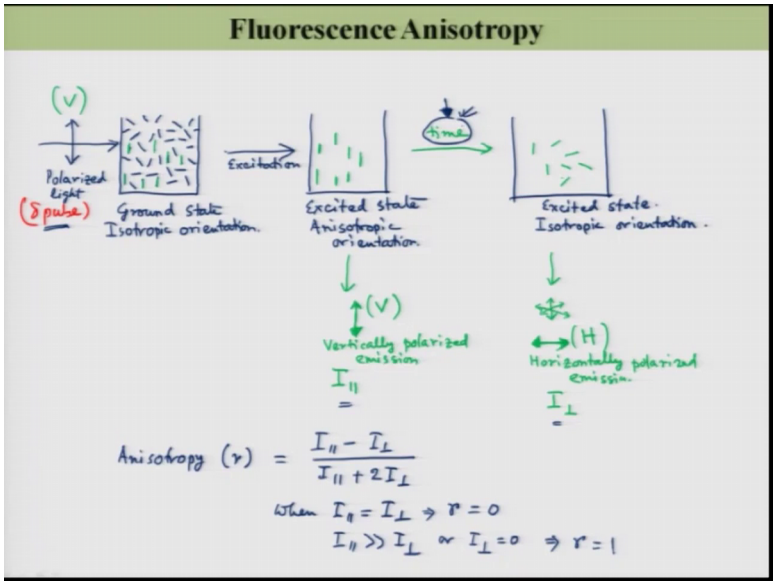

And we said we actually was doing the excitation of the molecule using a polarized light let me draw it once again. So, here is my sample, cell containing molecules oriented randomly that I showed you like these are the transition moment of this molecules, molecular frame may be different, but this is just I am showing the transition moment the duration of the transition moment. So, they are oriented randomly, see like this, this, several different types of orientations are possible that is why this system these condition is called the isotropic orientation of this molecules in the solution.

Now, if I use a polarized light, the polarization the direction of this electric field of this light this a plain polarised light is oscillating only in this direction, only in this direction and this is the direction of the propagation of light then right, so this is my polarized light and I said that only those molecule whose transition moment is oriented along the polarization of this light those molecule will be preferentially excited. So, let us now

show you. So, this is my ground state ground state. So, I write the isotropic orientation of the molecules now with this excitation and let us consider that this excitation is delta pulse excitation right.

So, I just impose another condition it will be clear to you delta pulse; that means, these electric field is present only for a short time this is the pulse excitation like we have seen in case of t c s p c or in the up conversion. So, immediately after excitation because you know excitation takes very less amount of time. So, after excitation the system will absorb the energy and the molecule will go to the excited state those molecules whose transition moments are parallel right to the electric field of the plain polarised light; that means, this guy and this one, this one, this one. So, let me draw over here. So, one will be here another would be here, another is here, another is here and then let us say this one. So, another would be here let us say this one another would be here and where let us say this one. So, another would be here. So, this will be the molecules and now promoted to the excited state.

So, here I can write this is my excited state and this is the anisotropic situation anisotropic orientation. So, this is my excitation right. Now this molecule when will come back from the excited state to the ground state it will emit radiation right; obviously, it has some radioactive nonradioactive pathway this consider the radioactive pathway, so it will emit the photon and the polarization of this photon in the early time will be along that particular direction in that direction the molecules are oriented that means the transition moments of the molecules are oriented in the excited state. So, the emission will also be in the same direction. So, in the early time the emission whatever you will get the electric field of those emitted light will be like this, but as time goes allow sometime these molecules will reorient them self because of the random motion right. So, this molecule will reorient themselves and let us say after sometime these molecule is like this, this one is like that, this one is like this, this one is like this 1 2 3 4 5 7 like this. So, now the molecules are like that.

So, you have in this case the contribution of this vertical emission is reduced because only these 2 molecules are now present in this case and you also got this type of emission you also got this type of emission and so on. In other words I can tell you that there will be some contribution in the horizontally polarised emission. So, in this case only vertically polarised emission, but as time goes the contribution towards the horizontally

polarised emission will increase. So, here I write vertically polarised emission. So, this intensity vertically polarised emission intensity if I referred to as I parallel, parallel with respect to the excitation, excitation is also vertical emission is also vertical, so this is parallel and if this is the horizontally polarised emission. So, this is horizontal and this can be referred as I perpendicular.

So, the condition as I said, it is now less anisotropic and if you allow too much of time then all molecule will randomly reorient themselves, so then it will be again the isotropic. But these isotropic is in the excited state and in that from the excited state from the isotropic condition the molecule will emits fluorescence, molecule will emit photon and those photon like those electric field of those emitted photons they will not going to be oscillating in the particular direction not only vertical, but also horizontal and any other directions also depending on its orientation. So, here I will write excited state, but isotropic orientation.

Now, if I replace these delta pulse excitation with c w excitation delta pulse excitation and c w excitation means when I will use the c w excitation then the condition is the steady state condition; that means, the molecules will keep on going the excitation; by the way let me talk about this time if this time is short, shorter than the lifetime of the molecule in the excited state then such kind of process randomisation is possible, if this time is very slow then fully random full randomization of the orientation of these molecules will not be possible. So, there is some relationship that how much is this time compared to the lifetime of this molecule. If the lifetime of this molecule let us say for example, is write now 1 nanosecond and this time for the reorientation time is 10 nanosecond then the contribution of I perpendicular will be much smaller then I parallel because before the molecule will convert from this vertical position to the horizontal position right later on we will see that this is not horizontal 90 degree it is 54.7 degree, but that will come later. So, that for this reorientation will take more time than the lifetime.

By the time it will reorient the molecules are not in the excited state it will come back to the ground state. So, the emitted photon will be like a vertically polarised light. However, if the reorientation time like this time what I am talking about this is actually reorientation time. If these time is much shorter than the lifetime let us say lifetime is 2 nanosecond and these time this reorientation time is 0.1 nanosecond 100 picosecond then

immediately after you excite in the within 100 picosecond timescale, this is time constant remember. So, that reorientation time in another 100 picosecond also, so all molecules will randomise, but the lifetime is 2 nanosecond. So, you will be able to see that after sometime right after 500 picosecond or 600 picosecond the intensity of this parallel and intensity of this perpendicular they will be same.

But now when we excite with the c w light; that means, we will going to integrate right the response from initial time to final time, so this kind of a steady state situation right. So, in this case the molecule will keep on go on to the excitation state and the excitation state will reactivate right. So, depending on the this reorientation right I will get these 2 values are different, at any way this I parallel will be similar to I perpendicular, but it cannot exceed the I perpendicular because you are exciting with this vertically polarised light. At most the I perpendicular will be equal to I parallel and in the other extreme the I perpendicular will be 0 and I parallel will is the maximum. So, these are the 2 extreme situation I could have.

Suppose you are exciting this molecule right which are rigid they cannot rotate and you can make such kind of molecular system by embedding this molecules in a glass metrics. So, it is not solution anymore it is solid. So, in solid how the molecule will reorient them self this is impossible. So, then you excite this molecule will c w light, you will only going to see the emission in this vertical direction; that means, the fluorescence light which is emitted from the system what is my system molecules embedded in glass matrix; that means, the molecule cannot reorient themselves.

So, in that case the fluorescence will be polarised and that polarization vertical same as the excitation polarization. So, then the I parallel will be maximum and I perpendicular will be 0, and if you have taken this molecule in a solution where the viscosity is very very low there is no restriction then after excitation the molecule will rotate. If the rotation is to too fast let us say for example, it is ultra fast femtosecond then once we excite immediately molecule can rotate, molecule is actually rotating constantly because nobody can stop molecule is rotating not because you are exciting note it down. Once you are exciting some molecules whose transition moment is parallel to the electric field of your excitation light those are excited and immediately they are rotated because that is the inherent phenomena of this molecule. So, in that case the rotation is. So, fast that the

contribution of this parallel and the contribution of this perpendicular they are they will going to be same.

So, whatever I said we can write this in terms of equation like this. So, I can define a term this is called anisotropy denoted as r equal to I parallel minus I perpendicular by I parallel plus twice I perpendicular. So, you see this equation in this equation it says that right when I parallel equal to I perpendicular this quantity right when I parallel equal to I perpendicular this r is going to be equal to 0 and when I parallel is much much greater than I perpendicular or I can say when I perpendicular is 0 right or I can say for those thing I said rigid metrics or for I perpendicular equal to 0 I can write r is equal to 1.

So, the value of r ranges from 1 to 0, by looking at this value of r I can comment on the rigidity of the system, if r is coming to be very high it is very difficult to tell that r is 1 because it is not, but still I am telling. So, anyway if the r value is let us say 0.4 it means that the reorientation is not that feasible, but if r is very small 0.001, 0.005 that means, that your orientation is very fast. So, qualitatively I can comment and if you recall your store cons entity by integration ship right this reorientation depends on the size of the system, if size is small reorientation is fast size is large reorientation is slow or and so on right. So, this r can be directly related to the size of the micro molecule which I will come later. Let us continue in this direction.

Now, question is how I will going to measure this r right that is my question. Let us try to draw some experimental setup by which we will going to measure this r value. So, here is my excitation.

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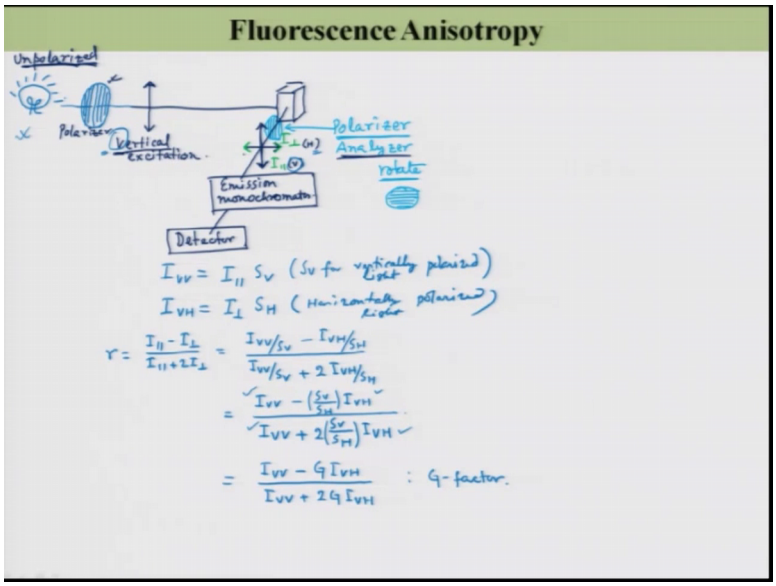

So, this is my vertical excitation and here is your sample looks good and emission is collected at the right angle and I have emission monochromator and then I have detector where you can measure the signal when this is vertically excited and then you will have 2 type of emission - one is like this vertical another will be horizontal right. So, I referred this as I parallel just notation and this one is I perpendicular. Now when it will pass through; obviously, this emission right will have some colour depends on the colour of the light right. So, that is why you have this emission monochromator we can choose the colour right and then it will be directed by this director. So, when it will be pass through this emission monochromator and will be detected by the detector in detector I should get 2 value for this I parallel I will get one value for I perpendicular I will get another value the question how will going to select.

I have to put then that optical element right that optical device which is called the polariser right. So, for the generation of this vertical excitation light because my light source is not polarised light right, this is my light source, this is unpolarised light. So, once you use that kind of device like is called polariser I will not tell you the details of this how does it work, I will just show you the use of it, so this is my polariser. To make this vertical excitation light from this unpolarised light source you have use this polariser, now this fluorescence again is not polarised anymore because of the reorientation under the constant excitation. So, both vertical and horizontal component

and in between all those things everything is there, but we are interested only in the vertical and horizontal which is refereed as I parallel and I perpendicular.

So, to select whether I will take the I parallel or I perpendicular I also need to use this one right. So, let me draw this over here let us say here I have this polariser, but generally it is called not polariser, same, same thing this one and this one they are exactly same thing, but as your using this to analyse the emitted light, so it is called the analyser. Now if you put this analyser in this direction you will get vertically polarised light coming out of this other lights will be blocked, if you rotate it rotate to like this then the horizontal polarised light will come pass through other lights will be blocked. So, in that case you will be able to measure I parallel and I perpendicular independently by setting the orientation of this analyser which is nothing, but a polariser.

So, here is vertical excitation and here is a horizontal emission right, this is a vertical excitation this is my horizontal emission and here is my vertical emission. So, in the detector what will going to measure is this 2 intensities either I parallel or I perpendicular. Now as you know as I already have discussed right when the particular intensity of light; that means, the particular flask photon flask is arriving to the detector through the monochromator the monochromator has its specific though put which is obviously, orient dependent that we have discussed earlier that is what all those emission collected emission spectra all those things comes into picture. So, when a particular intensity is coming through emission monochromator and then being detected at the detector that value will be different right there will be some proportionality constant I parallel if it is 1000; obviously, in detector you will not going to get 1000 it should be multiplied with some factor those factors are coming from the through put of the emission monochromator and the detection efficiency of the detector right some, but that should be proportional.

So, in this case let me write the intensity which is detected at the detector for this I parallel light as I parallel multiplied by that factor sensitivity factor I named it as S V which is equal to your measured quantity at the detector correct. So, let me name it as I what I shall name? Let me name it as I VV because here is my V and here is my v. So, that is why I name it as vv, so I vv.

Now, when you will set the analyser for the detection of the I perpendicular; obviously, that light intensity will be I perpendicular and that has to be multiplied with that sensitivity of the monochromator and the detector. So, I will write I perpendicular multiplied by the sensitivity of the monochromator and the detector for the horizontally polarised light this was for vertically polarised light, this is S V for vertically polarised polarised light, but for horizontally polarised light this sensitivity is as not same as the vertically polarised light they are different. So, here this sensitivity is for horizontally polarised light right, that is it, and I name it as I or shall VH here is my vertical and here is my horizontal. So, I am done. So, when I will write this I parallel minus I perpendicular what I have to write? I have to write in terms of I VV and I VH right. So, I have already defined this anisotropy r.

So, now r will be written as I parallel minus I perpendicular by I parallel plus twice I perpendicular is not it, but in the detector we were not measuring this in detector you are measuring something else you are measuring I VV, I VH that is related to I parallel when it I parallel is multiplied by S V and that is related to I perpendicular when the I perpendicular multiplied by S H. So, I will write this as I VV by S V minus I VH by S H right here I parallel equal to I VV divided by S V divided by I VV by S V plus 2 I VH by S H. Now if I multiply both the numerator and denominator by S V what will happen? Simply it will be I VV minus S V by S H into I VH divided by I VV plus 2 S V by S H into I VH, as so you see here this is your measured quantity right this I VV this I VV is your measured quantity I VH is a measured quantity.

So, what you got that I VV has to be subtracted with I VH when multiplied by with this factor. So, this S V and S H this is fixed for a particular monochromator and detector right and this is a ratio, ratio of 2 constant so I can replace this ratio of 2 constant with one constant. So, let me write this as another constant. I VV minus G I VH divided by I VV plus 2 G I VH; that means, whatever will going to measure in the detector you have to multiply with a factor whatever the value of I VH you will measure in the detector you have to multiply with a factor. So, this is known as G factor. We have to multiply with this G factor then only you will be able to calculate what is the value of anisotropy otherwise you will not be able to calculate that. Now my question is how I will going to determine what is the value of G right, that is my that is what we will going to do now.

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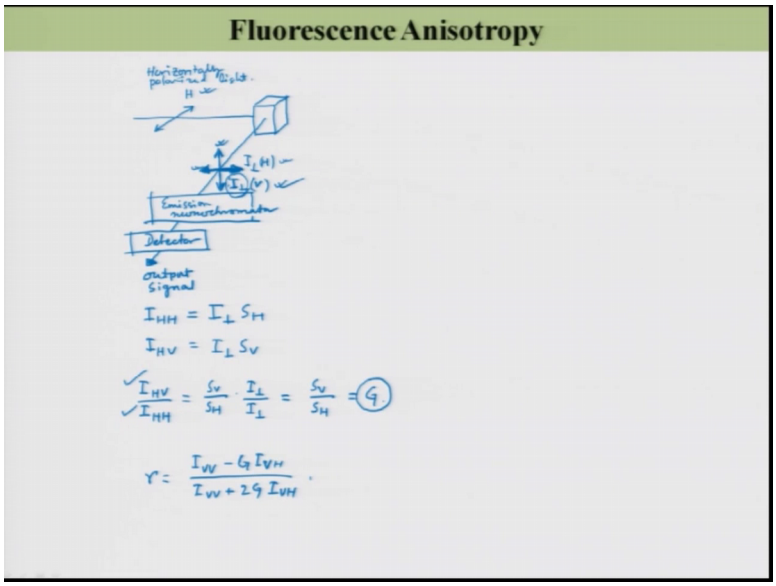

Now, let us consider that that same experimental setup we will going to use like you see here, this is the same way like this same experimental setup as I have shown over here we will going to use let us write it over here once again. So, I am just writing a part of that not the whole setup because it is not necessary. So, here is your q head and this right angle the emission is coming I have 2 different polarisation for the emission and this is coming and here is your monochromator emission, monochromator and then here is your detector. So and here is your output signal. This is obviously, towards this setup this, this, this one this one means difficult here nah because all the mouse pointer is not visible let us say this one is vertical right. So, I can simply write this as V, I am just writing over here this is V and this one is horizontal. So, I am just writing here is H.

Earlier what I have done? I have excited earlier, what I have done vertically excite; vertically polarised light was used for the excitation like this, but now what I going to do is little different I will use like such kind of horizontal polarised light for the excitation. So, this is H. So, horizontally polarised light for the excitation. So, towards this horizontal excitation these vertical these V is I perpendicular simple, it is exciting like this like this excitation this kind of emission perpendicular. Now towards this horizontal light these emission which is horizontal here as I have shown horizontal this horizontal means light is exciting like this way and emission is like this way this is also perpendicular. So, this is also I perpendicular. Although this is horizontal this is vertical towards, towards what? Towards the monochromator and detector towards the

monochromator and detector monochromator for the monochromator detector this one is vertically polarised light, this one is horizontally polarised light, but for this excitation light both are I perpendicular, if both are I perpendicular their intensity has to be same right.

And I already know that for vertically polarised light the that sensitivity factor of the monochromator and detector is S V for the horizontally polarised light is S H and then there several things are easy. So, I can simply write the output signal in this detector and monochromator pair for this I parallel which was this H that should be equal to I sorry I perpendicular which was this H that should be equal to I perpendicular into S H and this guy it should be I perpendicular into S V let me write that. So, I perpendicular into S H what name you want to give? I here H and here H, so I HH, I HH.

For the other one this is I perpendicular, but these I perpendicular look here these I perpendicular is vertical towards the monochromator and detector right. So, the sensitivity factor what I will going to use is S V. So, this equal to I H V is it. So, what I can write? I can write I HV divided by I HH equal to S V by S H into I perpendicular by I perpendicular which is nothing, but S V by S H and that I already have defined as my G value that I already have defined as my G value. So, what you have to do? You have to simply take this 2 intensity I HV and I HH just take the ratio of this 2 you will going to get G once you will going to get G you can readily get this r as I VV minus G I VH divided by I VV plus 2 G I VH as I have already discussed right. So, we finish here and we will continue our discussion on the next day.

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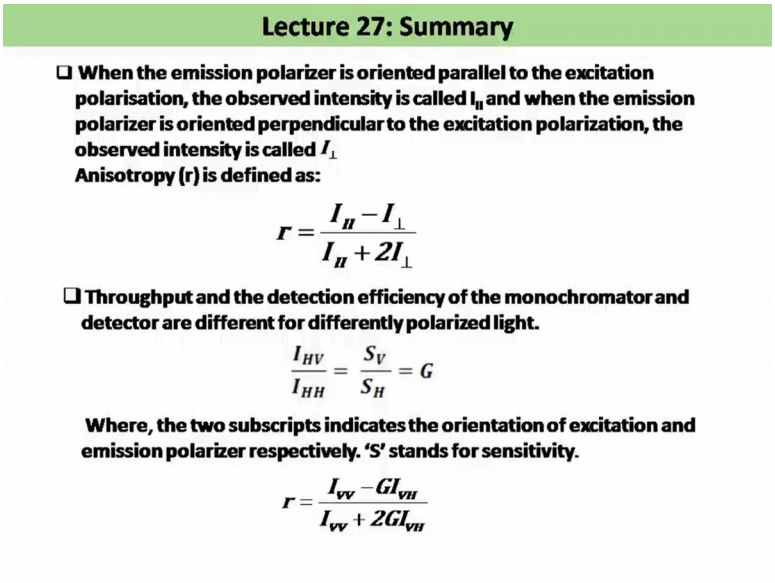

Thank you very much.