Basics of Fluorescence Spectroscopy
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Lecture – 26

Welcome back to the lecture number 26. So, today we will be going to discuss a new topic called Fluorescence Anisotropy. So, before discussing this fluorescence anisotropy let us see what is called the polarization of light.

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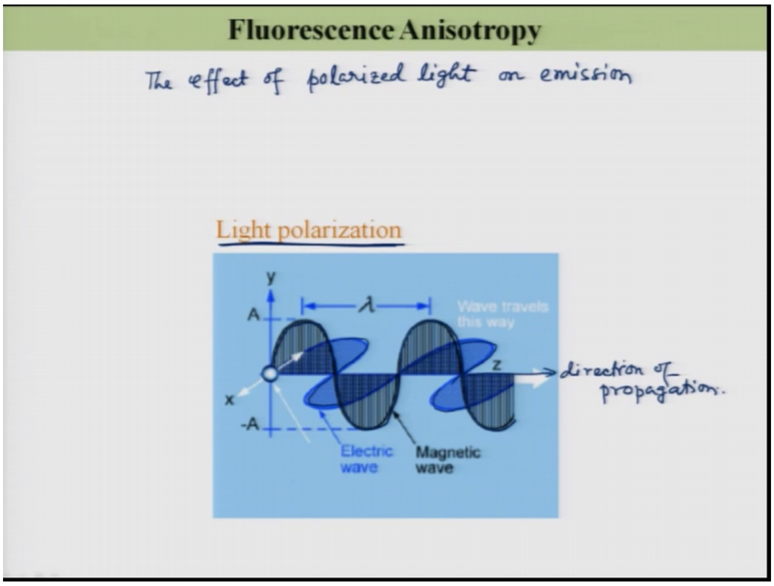

Fluorescence Anisotropy

The effect of polarized light on emission

Light polarization

So, this fluorescence anisotropy is the effect of polarized light on emission. So, this is called the fluorescence anisotropy. So, what is light polarization? We know that light is a transverse electromagnetic wave here I have the electric field oscillating right with a characteristics frequency of this light that is depending on the wavelength of the light and I also have this magnetic field also oscillating like this way and both are oscillating perpendicular to the direction of the propagation here is my direction of propagation, direction of propagation.

Now, if you see the normal lights right the normal lights, then that normal light can be represented as the electric field oscillating in all the direction, right.

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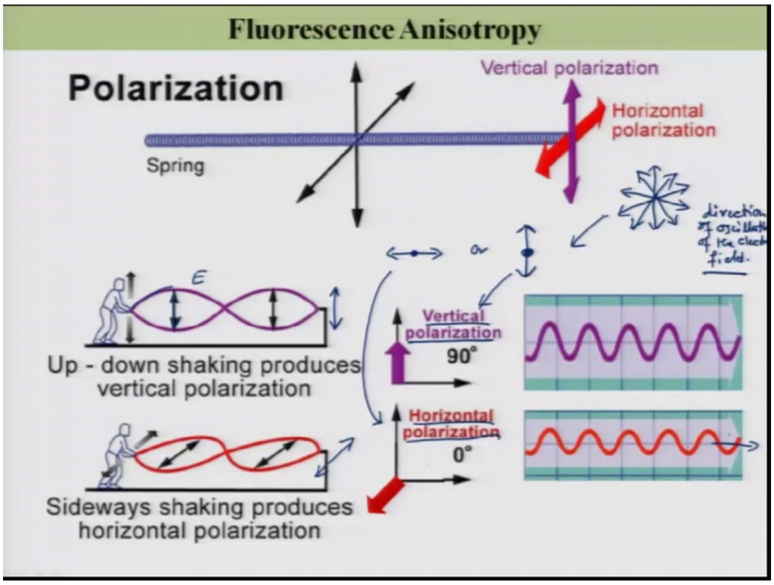

Fluorescence Anisotropy

Polarization

Up - down shaking produces vertical polarization

Sideways shaking produces horizontal polarization

So, in this case you see here like this; this is a kind of spring and I am actually oscillating this spring in the vertical direction here in this vertical direction in this direction I am oscillating this spring what you can do you can also oscillate this spring in the horizontal direction like that way. So, here the spring is oscillating like this way. So, depending on your wish you can oscillate this spring either in the vertical or horizontal or any direction in between them.

If you consider the light the normal light source and this is the electric field of this light source right here is my electric filed then the oscillation of this electric filed could be at any direction either in the vertical or horizontal or any direction between the vertical and the horizontal. So, the electric filed here is either in this direction or in this direction or in this direction like all the possible direction is possible. So, this is the direction of the oscillation of electric field direction of oscillation of the electric field, but if you can do something that the electric filed is only either oscillating only in this direction or like in this direction right what is the direction of propagation of air light like out of this plane right out of this plane this is the direction of propagation of the light.

So, then you will get a different type of light and that is called the polarized light in the first case you will get the vertical polarization vertically polarized light. So, electric field is oscillating here the direction of the propagation and in this case you will get horizontal polarization or 0 degree. So, electric field is oscillating in the horizontal direction towards the horizontal and here this is your direction of propagation, but you note that in both the cases these oscillation of electric field is perpendicular to the direction of propagation, but in this case this is vertically oscillating and in this case it is horizontally oscillating.

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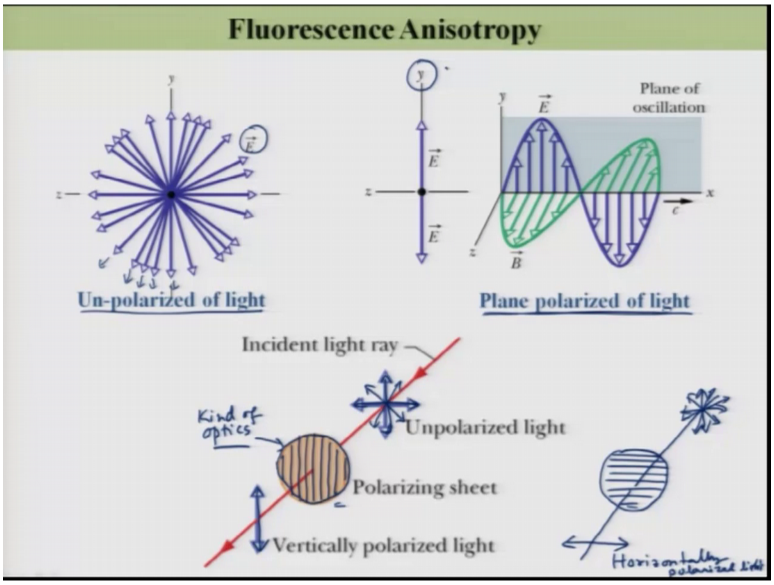

Fluorescence Anisotropy

So, as I said for this un-polarised light here the direction of oscillation could be anything like this is my electric field as I said. So, it could be in this direction it could be in this direction and all the directions are allowed or possible.

However for a plane polarized light where it is oscillating only in this direction here it is only oscillating in this y direction you see. So, then this electric field is only oscillating along this x y plane, but it is not oscillating along any other plane right. So, this is called the plane polarized light. So, this is special type of light form this normal light normal light is un-polarised light and this light is a plane polarized light or some time we call also called as a polarized light it is possible to convert the un-polarised light to the plane polarized light by using some kind of optical device is called the polarizer.

So, if you use a polarizer is a optical element right. So, it is a kind of optics then if you pass this un-polarised light it will become the plane polarized light see after passing through it will become plane polarized light and depending on the orientation of this polarizer. So, if it is oriented like this way you see this; this is the direction some directions which is written in this polarizer if it is oriented like this way then what you get you will get this kind of polarization, but if you change the orientation of the polarizer if it is now oriented like this way then when a un-polarised light will pass through.

So, how I denote the un-polarised light by putting this arrow in all the directions that is how will going to indicate this is un-polarised light, but when this light will pass through this polarizer. Now this polarizer is oriented in a different fashion compare to this one compared to this one then the light which will come through is like this again it is a plane polarised light, but it is not vertically polarized light this is the horizontally polarized light. So, in this case this is a horizontally polarized light with this thing.

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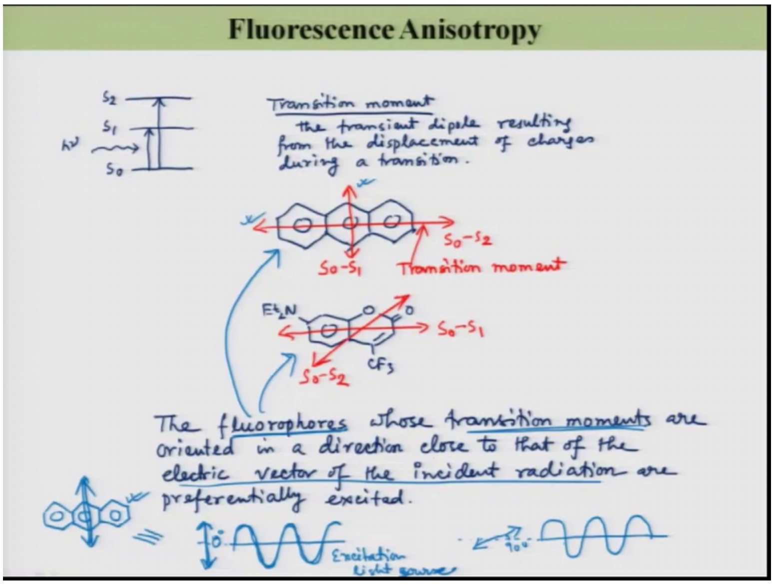

Fluorescence Anisotropy

Transition moment
the transient dipole resulting from the displacement of charges during a transition.

The fluorophores whose transition moments are oriented in a direction close to that of the electric vector of the incident radiation are preferentially excited.

Now, let us see what will be the fate of excitation when will use a plane polarized light compared to the un-polarised light before that I also have to tell you one important thing which is known as transition moment. Let me draw one molecule, let us say this is the S 0 state of this molecule ground state this type of diagram we have seen many times in the early part of this course this is my S 2 and when you excite the molecule from S 0 to S 1 state or S 0 to S 2 state right that excitation is possible only when right, it is allowed transition as well as the available energy is will match with the energy difference between the 2 states right that what we have discussed.

Now, along with this transition there must be some change in the electronic distribution in this molecule and in this case this electronic distribution or in the molecule can be designated as the transition moment of this molecule of this excitation. Let us see if I write this as transition moment then this transition moment is defined as the transient dipole resulting from the displacement of charges during a transition.

So, in most of the cases this transition moment is represented by vector quantity in this molecular frame let me show you this molecular frame of anthracene over here. So, just I am drawing the anthracene molecule right. So, I can excite this anthracene either from S 0 to S state or S 0 to S 2 state now for this S 0 to S 1 state and S 0 to S 2 state there will; obviously, there will be a change in the electron density of this molecule from this HOMO to LUMO or HOMO to LUMO plus one HOMO to LUMO plus 2.

So, see here this is the anthracene molecule and for this anthracene molecule for the S 0 S 1 transition or S 0 S 2 transition; that means, the change of this from HOMO to LUMO the electron is going either from HOMO to LUMO for this is one state or HOMO to LUMO plus one depending on the situation so; that means, what I wanted to say is the there is a change in the electron density; that means, there is a change in the charges distribution of the charge. So, that can be represented as a vector that in which direction the charges are changing. So, in this molecular frame and if you plot that what you will going to see is you see a line associated with the transition let us say this is that my S 0 S 2 transition. So, the transition moment is along this molecular frame. So, this is my transition moment and this is along this direction of this anthracene molecule if you excite to from S 0 to S 1. So, that S 0 S 1 transition is along this direction in this molecular frame I take a different molecule and this will be different.

Let s say I will take a acumen in let us take I have taken thin acumen in this case the direction of this transition moment are different for the S 0 S 1 is over like this way this is S 0 S 1 transition moment for S 0 S 2 it is in like this way this is S 0 S 2 transition moment. So, depending on the molecule the direction is different and if you use a polarized light plane polarized light right; that means, the electric field is oscillating in a particular direction right if this is a propagation of the light electric field is oscillating like this not all the directions are allowed directions right. So, only in one direction it could be like this.

So, now if I have un-polarised light and if I have a polarized light for the excitation what is the difference to show this difference let me take a sample having these kind of fluorophore before that let me write one sentence over here right which just see this sentence and I will explain this later on in more detail. So, here I am writing the fluorophores whose transition moments are oriented in a direction close to that of the electric vector of the incident radiation are preferentially excited let me explain this. So, here I said the fluorophores see here this fluorophores means either this or that or some other whose transition moments are oriented in a direction close to that of electric vector of the incident radiation. So, here the transition moment which transition moment when I am exciting it from S 0 to S 1 then I have to talk about this transition moment if I am exciting from S 0 to S 2 then I have to talk about this transition moment.

So, for a particular excitation either from S 0 to S zone or S 0 S 2 lets freeze that for the time being. So, let us say consider that I am exciting from S 0 to S 1. So, for this molecule for this anthracene molecule what I will write for this anthracene molecule; this is my transition moment and now let me replace this molecule only with this transition moment. So, this replace with only this; that means, this is my anthracene molecule and this is my transition moment which transition moment S 0 S 1 transition moment and if I draw this molecule I can tell that the molecule is oriented like this way.

Now if the molecule is oriented like this way and if the electric field of the incoming radiation right incoming light is along oscillating along this way then the excitation is more favourable than if the molecule the transition moment is oriented like this way and the excitation is like vertical. So, here you see this, this is ninety degree and here it is 0 degree 0 degree. So, that is what I am telling here the fluorophore whose transition moments are oriented in the direction close to that of the electric vector of the incident radiation. So, here the orientation the of the transition moment is close to that of electric field this electric vector of the incoming light. So, this is my excitation light source excitation light source.

So, in this case I have this oscillation right this electric field oscillation is very close to the orientation of this transition moment in this case it is not now let us take a situation that I have many many molecules in solution.

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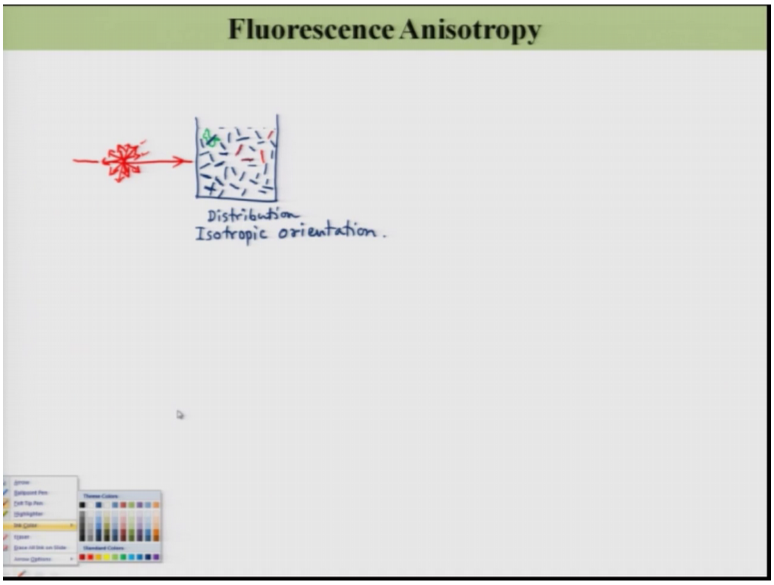

Fluorescence Anisotropy

Let me draw one sample chamber over here and here you have taken say for lots of this fluorophores in a particular solvent now the distribution of this fluorophores within this in this solvent will be random and the relative orientation will also be random right. So, that is in one word called isotropic ally oriented right what I want to say is that this is in the ground state right. So, the some molecule will oriented like this way whenever I am drawing it like this way I am drawing is transition moment as I have already explained the shape of the molecule could be like this the shape of the molecule could be like this, but its transition moment is along this. So, I am just writing the transition moment. So, here I am most worried about how the transition moment is oriented in this solution.

So, as I said the transition moment can orient like this way there is no restriction. So, that transition moment could also take this orientation it could also take this. So, I will going to have a random orientation of the transition moment throughout this solvent. So, there is no restriction of that and; obviously, some like this. So, all this different orientations are possible it means that the orientation is isotropic the orientation is isotropic. So, I will just write here isotropic orientation now consider you are exciting your sample with a un-polarised light right with un-polarised light and I am drawing this un-polarised light like this way which is understandable to you. So, this is the excitation light and this is un-polarised that can be described by all this different arrows over here. So, this is un-polarised light; that means, I have the electric field oscillating in all the possible direction which all of them are perpendicular to the direction of propagation of the light.

So, one of these orientation we will find its counterpart in the un-polarised light this one lest say this one this one will find this one is very close the orientations is very close this guy will find that this one the orientation is very close for this guy will find that this one orientation is very close for this one it will find that orientation of this one is very close. So, all this molecules will eventually excited from the ground state to the excited state as all this transition moment we will see we will find the electric field which is oriented along it during the excitation. So, eventually all probably all the molecule will be excited from the ground state to the excited state this is extreme situation I am talking about right.

Now, consider that you do not have this un-polarised light whether, but you have this polarised light. So, if we have this polarised light let me erase it.

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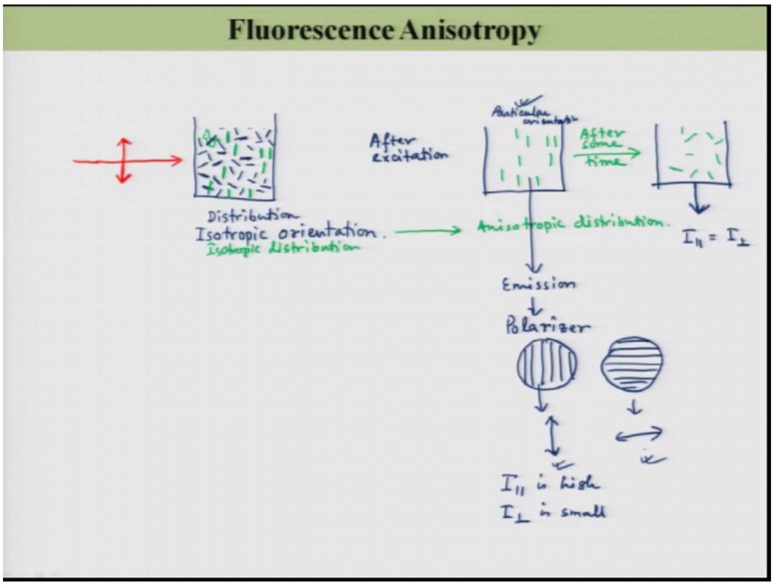
Fluorescence Anisotropy

Now if I have this polarised light then the light polarisation is means the electric field is oscillating only along a particular direction. So, it is a plane polarization like. So, now, consider my electric field is oscillating along this vertical direction it means that out of all these different molecules which are present in the solution not everything will going to be excited because the condition I said the orientation of the transition moment which is similar to the electric field of the excitation light source those will be preferentially excited right. So, in this case the; you see here this guy will be excited this will be excited this will be excited here this one will be excited here probably it will be excited these one this one this guy and that is it probably here this one. So, out of all these molecule only this green things are excited. So, I have changed the colour to the green.

Now, if I draw the fate of excitation after excitation means right after excitation. So, after excitation the situation is like that. So, you will have one molecule like this, this, this, this, this, this, this one this one this one this one right because all those molecules which have which are the transition moment or oriented along the electric field of the incoming light. So, those are excited and the excited situation is like that. So, you see the excited state it looks like that all the molecules are oriented in a similar fashion right. So, it is no longer a and isotropic distribution this is now anisotropic distribution.

So, this I have changed from isotropic orientation or isotropic distribution to anisotropic distribution now if you allow sometime right let us allow sometime then after sometime this molecule will reorient because; obviously, there is no force so, that this molecule will remain like that. So, they will constantly go undergo the reorientation. So, after this molecule will reorient themselves then the situation will be again isotropic so, then this probably like this, this, this, this here like that way. So, it is a lost isotropic.

So, you see that number of molecules are same right 1 2 3 4 5 6 7 8 9 here also one 2 there 4 5 6 7 8 9. So, while changing its orientation it will also come back to the ground state right because of its natural lifetime. So, this is like complicated situation. So, here what you will see let me talk about the emission intensity here. So, if you see the emission intensity as a function of time the total emission intensity will decay as its natural lifetime, but if you see the emission after passing through that special optical element what I said as a polarizer this polarizer is if it is oriented like this way it will pass the light which are vertically polarised if it is set like this way then it will pass lights which are horizontally polarised.

So, in this case what will going to see just after the excitation the intensity of light which is polarised vertically that intensity will be more compared to this intensity as time goes the intensity of this one and intensity of this one will be very similar and that is the subject of fluorescence anisotropy right it means that the fluorescence intensity and the vertical direction is more compared to the horizontal direction horizontal direction particular means the polarization of this fluorescence emitted light right. So, the intensity along the vertical direction is more compared to the horizontal direction at the initial time, but; that means, the system is an isotropic because the system is anisotropic all the molecules are oriented like this way that is why all the molecules are oriented like this way here. So, this particular orientation a particular orientation; that means, the emission right is also coming out of a particular direction.

The light which is coming out of this system the electric field of this light is oscillating only in a particular direction; that means, this is a polarised right. So, this is kind of anisotropic distribution anisotropic polarization, but as time goes the molecular will reorient themselves you make it isotropic; that means, the light which will come out from this one from this system after a certain time then here the intensity of the vertical direction and intensity of the horizontal direction they will be similar. So, here I can write in mathematical word here I parallel is high N for this case right for this case and I perpendicular is very small N. However, after it will reorient themselves to you make it isotropic distribution in the in solution in this case I parallel will be equal to I perpendicular right and we will continue our discussion on this topic on the next class.

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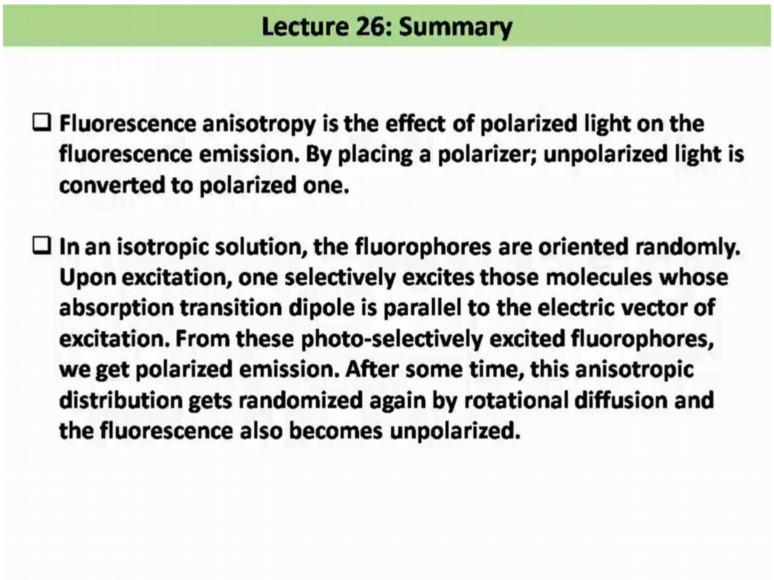

Lecture 26: Summary

☐ **Fluorescence anisotropy is the effect of polarized light on the fluorescence emission. By placing a polarizer; unpolarized light is converted to polarized one.**

☐ **In an isotropic solution, the fluorophores are oriented randomly. Upon excitation, one selectively excites those molecules whose absorption transition dipole is parallel to the electric vector of excitation. From these photo-selectively excited fluorophores, we get polarized emission. After some time, this anisotropic distribution gets randomized again by rotational diffusion and the fluorescence also becomes unpolarized.**

Thank you very much.