Optical Spectroscopy and Microscopy
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Module No # 05
Lecture No # 21
Fundamentals of optical measurements and instrumentation

Hello and welcome to the lecture series on the optical spectroscopy and microscopy course and we have seen so far we were currently looking at the anisotropy if you followed it through all along right now we are at the lecture where we are actually looking at the fluorescent anisotropy okay. And I have in the last lecture told you how do you actually defined an anisotropy in terms of the intensity of the light that you are measuring in an equipment.

And the only the difference here is that before the detector you have a polarizer sitting and whose crystal access is either align to the incident polarization direction or orthogonal to the incident polarization direction and we call it as I parallel and I perpendicular to this 2 different directions intensity obtained in these 2 directions. And then we define the anisotropy I parallel minus I perpendicular divided by I parallel plus 2 I perpendicular.

At that point I told you that we can go beyond and then actually model what would be number what would be the behavior of this anisotropy and what parameters affect this anisotropy. If you understand little bit more about the ensample nature of the fluorescence's itself okay. We had 1 molecule and then excited that molecule with incident light radiation whose polarization is along the X direction okay the light is travelling in the Z.

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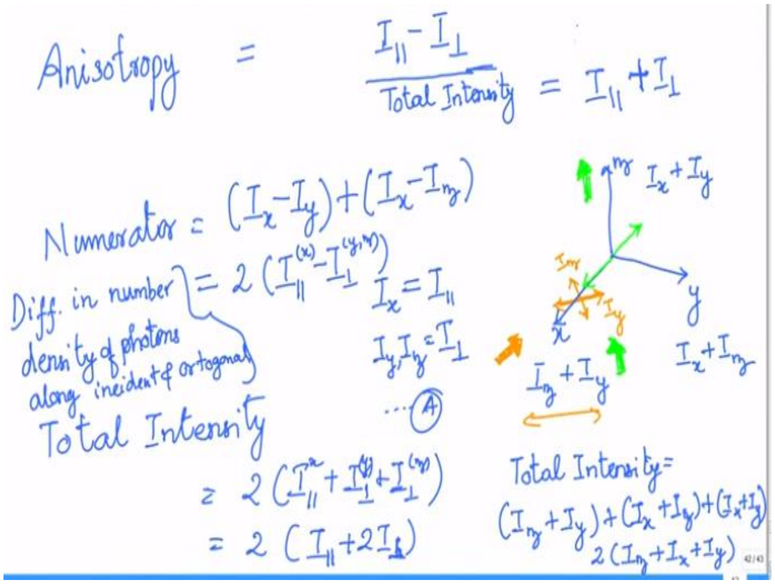

Anisotropy = $\frac{I_{\parallel} - I_{\perp}}{\text{Total Intensity}}$ = $I_{\parallel} + I_{\perp}$

Numerator = $(I_x - I_y) + (I_x - I_z)$

$= 2\left(I_{\parallel}^{(x)} - I_{\perp}^{(y,z)}\right)$

Diff. in number density of photons along incident & ortogonal

$I_x = I_{\parallel}$

$I_y, I_z = I_{\perp}$

… Ⓐ

Total Intensity

$= 2\left(I_{\parallel}^{(x)} + I_{\perp}^{(y)} + I_{\perp}^{(z)}\right)$

$= 2\left(I_{\parallel} + 2I_{\perp}\right)$

Total Intensity =

$(I_z + I_y) + (I_x + I_y) + (I_x + I_z)$

$2(I_z + I_x + I_y)$

In my previous diagram we I showed it as coming from bottom going all way up. So that is the Z axis and now the polarization was along the X axis okay.

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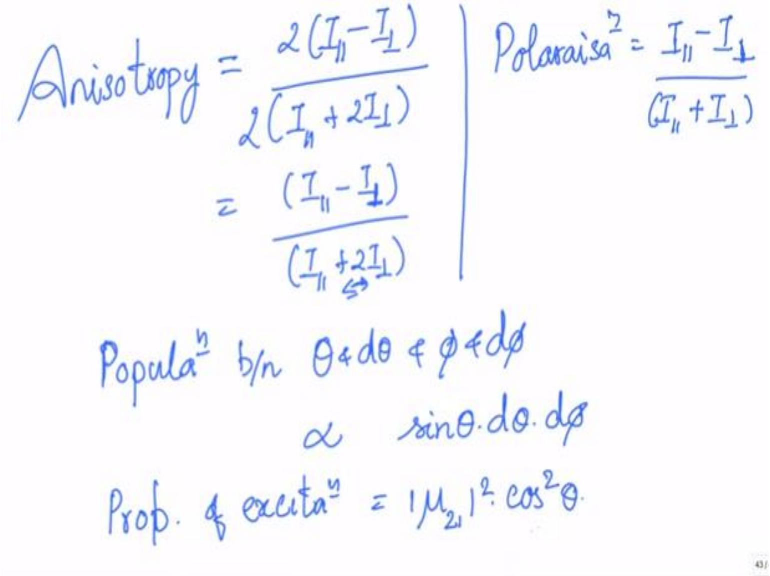

Anisotropy = $\frac{2(I_{\parallel} - I_{\perp})}{2(I_{\parallel} + 2I_{\perp})}$

$= \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + 2I_{\perp})}$

Polaraisaⁿ = $\frac{I_{\parallel} - I_{\perp}}{(I_{\parallel} + I_{\perp})}$

Populaⁿ b/n θ & $d\theta$ & ϕ & $d\phi$

$\propto \sin\theta \cdot d\theta \cdot d\phi$

Prob. of excitaⁿ = $|\mu_{21}|^2 \cdot \cos^2\theta$.

So now what we can actually do is we can actually take a glassy state of a fluorescence solution. Now what does it mean imagine you take a fluorescence solution fluorescence sodium salt is a one such example and then you prepared it in a lab. So it is solution existing there now you freeze it when you freeze quite a few things are going to happen you are going to freeze all the molecular motions in time right at that moment in time when the solution was frozen whatever the orientation of the individual fluorescence sodium salt you have captured that in your frozen them right.

Which means if you actually take a small sample and ask for the probability of finding a molecule oriented in a particular direction; you will see that is the oriented in all possible directions right. That is I mean there is nothing initially to actually bias the orientation of the molecules towards one direction or the other since there is nothing to bias they are equally distributed all over the place.

Now in that situation we are introducing a incident right whose polarization is along the X axis which means what you are going to do is even though the molecule to start with or oriented equally what you are going to do is you are going to excite preferentially the molecules who are oriented along the X direction okay. They actually flip there are molecules I mean if they flip then they will everybody can actually get excited but now that is exactly why what you are doing is we are taking a glassy state whether you are frozen the molecule.

So they do not have the opportunity to tumble or turn around so as a result each of this molecules dipole moment the transition dipole moment oriented in 1 particular direction in space and if that direction happens to be direction of the incident polarization you have maximum and we know as you deviate from this angle your probability of excitation goes down it reaches the 0 if it is completely orthogonal.

So if you in such a state you are actually exciting the molecule then what you do is that you have a may you have selected the population right if you draw here in this graph in this reference frame the probability of excitation and you will see all those molecules that are oriented along this axis right I mean will have maximum probability of oxidation and then so you would have they see the orientation wise they are actually good nice solid sphere.

If you were to represent the number density of the function of different angles but here because of the preferential selection what you have done is you have actually selectively excited those molecules which are having in this angle and in this manner so you can actually see that it is going to follow a shape something like that. Now because they are actually preferentially getting excited you have a state now whose emission polarization of the emitted light will have some preference in the direction because in this case what I am going to assume here I will elaborate on this little later.

I am going to assume that the absorption dipole moment and the emission dipole moment are aligned with each other there is no difference in their floor of what we are looking at. So in that case what you would see is that the florescent emission will have a preferential direction too that is they will be dominated by the perpendicular fluorophores along this direction. However the flurophores I mean it is not to say that you will not have any other I mean you will not you cannot say that there not be any source and photons that are having polarization at an orientation that is different from the that of the incident one right.

Because you have some probability of exciting them which means there have some probability of seeing the emission too. So now we can actually estimate the probability because it is a glassy state you have fixed it so now to get that what we do is that we are going to make an estimate about the population okay we are going to look at the population of the molecules between angle theta and d theta small change I mean small volume element we are defining theta and d theta and Phi and d Phi.

Now I would normalize this that to the probability of one right so it is a units sphere we can think of that as a units sphere and then I am going to ask how many of the molecules are going to be having the orientation between theta and d theta and Phi and d Phi right because r is 1. So now for this what I need to do is need to integrate all those population I mean all those population between these angles.

So now that is very simple because this is just if you think of in terms of the volume element then this is sin theta d theta and d Phi r being 1 so we ignore them so there is no need to integrate over that our thing under. Now the point here is that this is the number of molecules that are oriented along this is the distribution of the molecule that are oriented along the angle between the angle theta and theta d theta and the Phi d phi but each of them has a definite probability of getting excited they are not getting excited we are not interested in them because they are going to emit the fluorescent photons.

And if they get excited they emitted photon will have a polarization similar to that of the orientation of the molecule which means I mean since we assume that the emission dipolar moment is same as that of the absorption dipole moment. So to get my probability of excitation are what we need to do is that we have taken it we have written it down before which is Mu t Mu to 1 modulus square Cos square alpha right and then alpha here would be my theta in this nomenclature so I can write it as modulus to 1 square Cos square theta that is my probability of excitation.

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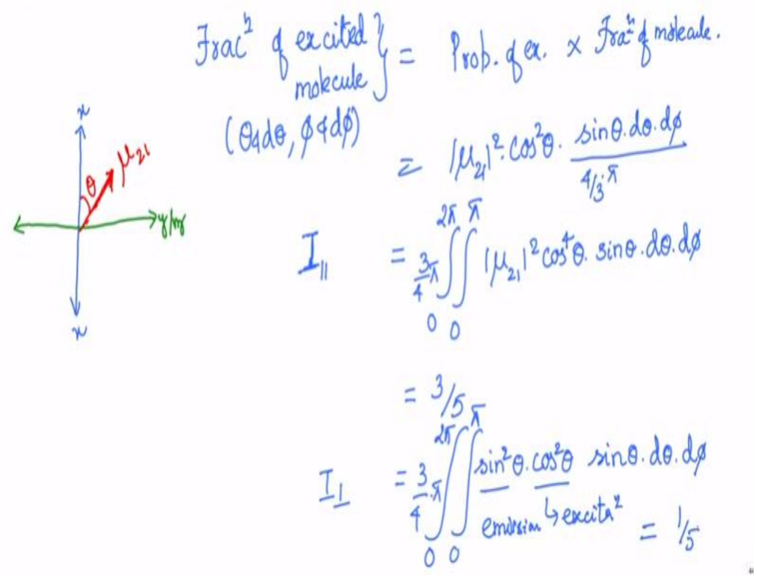

$$\left.\begin{array}{l}\text{Frac}^{n}\text{ of excited}\\ \text{molecule}\end{array}\right\} = \text{Prob. of ex.} \times \text{Frac}^{n}\text{ of molecule.}$$

$(\theta + d\theta, \phi + d\phi)$

$$= |\mu_{21}|^2 \cdot \cos^2\theta \cdot \frac{\sin\theta . d\theta . d\phi}{4/3 \cdot \pi}$$

$$I_{\parallel} = \frac{3}{4\pi}\int_0^{2\pi}\int_0^{\pi} |\mu_{21}|^2 \cos^4\theta . \sin\theta . d\theta . d\phi$$

$$= 3/5$$

$$I_{\perp} = \frac{3}{4 \cdot \pi}\int_0^{2\pi}\int_0^{\pi} \underbrace{\sin^2\theta}_{\text{emission}} . \underbrace{\cos^2\theta}_{\text{excitation}} \sin\theta . d\theta . d\phi = 1/5$$

And what really I am interested in is the total number of molecules that are getting excited are actually really the fraction of the molecules that get that are getting excited with fraction of excited molecule whose orientation is between theta and d theta phi and d phi alright. So now that is easy to get which is basically multiply the probability of a excitation by the fraction of molecules themselves the number right molecules that you will find right.

So now probability of excitation we have written down right which is Mu t 1 square Cos square theta times then it is the fraction right which means basically the number by the total. So the number again we have written down which is basically the integral over this which is sin theta d theta define and divided by the total is the it is a since it is a unit sphere it is the volume of the sphere 4 / 3 Pi r cube r is 1 so it is 4 /3 Pi.

Now of course we need to integrate this over the entire the entire space to get the total intensity that corresponds to I parallel right because Cos square theta you remember I am going to write again is this is my polarization axis of the incident beam X and this is the orientation of the molecule and so let us draw it at orange (()) (12:42) is the direction of Mu to 1 the transition dipole moment X is the polarization vector orientation and theta is our angle between them.

So now what we have done is we have if when theta equal to 0 it is parallel to the; it is aligned along the polarization angle. So we could if we integrate this over space what we would get is the intensity of the light that is parallel to the incident light radiation. So we can introduce right over 0 to 2 Pi we are integrating over dt term d phi right and this is 0 to Pi u to 1 square Cos square theta 3 / 4 Pi sin theta d theta p phi.

Now if we do that we can numerically estimate and to the point of time I will move that integration to the additional lectures where we discussed about the special mathematical there i mean numerical and the computations and mathematical properties separately right. So I am going to give you state the result of this integral that directly you can actually estimate it pretty simple but still if you need to know then we can talk about it at the end but right now it is 3 / 5.

Similarly we can write down an expression for I perpendicular there what you are going to do is that we are going to think about the molecules who are distributed around not the X axis but in this plane okay. So we will call it as you can call it as y rz it could be any of them. So now if you do that then for that we have the number being a exactly the same which as sin theta d theta d phi. However what we will see is that I am sorry I missed one important thing so we are actually calculating the intensity for the intensity this is what it gives you is the fraction of excited molecules and we are actually need to take the emission component one more time we are.

So this tells you the fraction of the excited molecule but what I am interested in is the I parallel which is basically the excited molecules and then of that is the fraction and then I am collecting the fluorescents right which is again a polarization axis I mean polar passing through a polarizer. So now that would be in that this term gets us square there is this Cos square dependence as actually gets squared.

So we have what actually we have is a cost to the power 4 sin theta d theta d phi and then for the perpendicular what we have is that we have excited with Cos square no doubt about it but we are deducting with Y and Z the polarizer. So we are only taking the fraction of the component along this so that fraction is given by sin square theta of the Cos square theta. So now this is because of the excitation selection.

And this is because of the emission and we are looking at the part of the emission right not all of them because we are looking at the; those I mean using the polarizer to ask the question of what fraction of them are actually having the polarization along this axis. So now this we need to integrate between 2 Phi I mean 0 to 2 Phi and 0 to Phi in a similar same way when you do that the value that will get is 1 over Phi.

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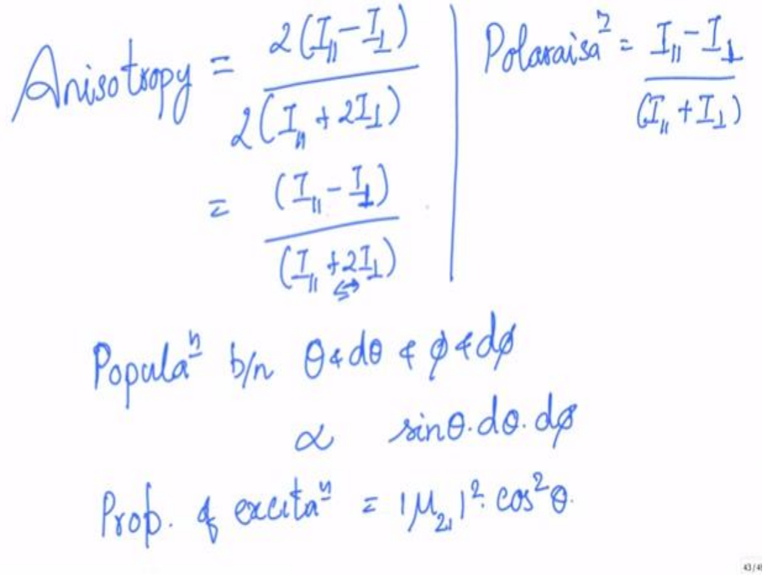

$$\text{Anisotropy} = \frac{2(I_{\parallel} - I_{\perp})}{2(I_{\parallel} + 2I_{\perp})} = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + 2I_{\perp})}$$

$$\text{Polaraisa}^{n} = \frac{I_{\parallel} - I_{\perp}}{(I_{\parallel} + I_{\perp})}$$

$$\text{Popula}^{n} \text{ b/n } \theta + d\theta \;\&\; \phi + d\phi \propto \sin\theta . d\theta . d\phi$$

$$\text{Prob. of excita}^{n} = |\mu_{21}|^2 . \cos^2\theta .$$

And we can put this plug in this values for our anisotropy as I parallel which is 3/5 minus I perpendicular which is 1/5 divided by I parallel +2 times I perpendicular which essentially means we have 0.4 okay. Now this is the beauty of this which is if you take a any solution right any solution you have not given any I mean any bias at all they are actually randomly oriented all over the place.

You just force them in space and time and then you are coming in with a plane polarized light and then asking how is the polarization of the emitted light looks like is it isotropic or is it anisotropic the answer is it is anisotropic to the fraction of 0.4 alright. So now what it tells you is that because of the excitation selection that we have given we have preferential excitation of the molecules we are going to have an anisotropic emission I mean in I mean when you are looking at the polarization of the emitted photons.

Or in other words when you take a isotropic mixture a solution and then let us you have an very fine very high time resolution okay or at time resolution will talk about the time resolved aspect little bit more in detail but you have a times resolution such that effectively you are looking at a glassy state okay. So when will that be we will see right I mean we will see in the next class and we will elaborate on that little bit more but somehow we by looking at the specific time resolution timescales.

If you measure the isotropy they would you would get a number of 0.4 and it is not the; it is not 1 or as you would have actually predicted it should be say here we look at the definition of anisotropy yeah here. So in a even in a complete if you would have predicted the maximum value would be that of anisotropy approaching equal to 1 right and it does not happen it really does not happen even if you freeze them really freeze them okay and you do not did not have any time for the molecule to actually tumble around.

Even then the value that you get is about 0.4 and not really 1 and it is not 0 clearly because you have a preferential excitation. So now what can we learn from a this and then few more things about the properties of anisotropy will see in the next class.