# Optical Spectroscopy and Microscopy
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## Lecture – 20
## Fundamentals of Optical Measurements and Instrumentation

Hello and welcome to the lecture series on optical spectroscopy and microscopy. We have seen so far how does the light interact with the matter. We looked at it in 3 different pictures, classical treatment of electromagnetic radiation, quantum mechanical treatment of the matter and their interaction, and the phenomenological approach of Einstein and then we jumped into fully quantum mechanical treatment of the entire thing which is the light as well as the matter, and from thereon we said, there is a prediction of spontaneous emission.

Then I said that spontaneous emission is nothing but their fluorescence as predicted by Einstein too. Now upon reaching there I said we will look at the properties of this fluorescence. One property that we saw is that it is isotropic in nature in terms of emission probability when it comes to the direction of emission, right, which is if I am going to ask how is the intensity or the photon numbers distributed as a function of space about the fluorophore, they are equal in all 4 pi directions.

So, that is why we say that it is isotropic, I mean there is no special direction in which you see higher intensity compared to the other. However, there is one important difference that I was trying to point out which is if you go in with a polarized light that is to say that we have seen this before if the incoming incident photons themselves have a defined polarization, we took it as the polarization along the x direction that is the last slide of the previous lecture.

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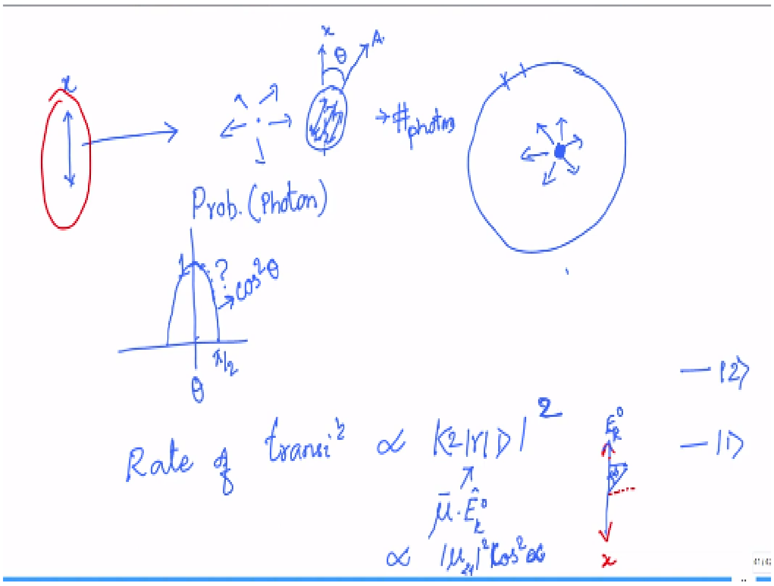

We took the polarization along the x direction right. Once you see that the incident polarization is along the x direction and the light is traveling along the z direction, what we can try to ask now is that once the molecule is emitting its fluorescence, I can ask for the polarization of the emitted photons okay. How do I do that? You can imagine a polarization filter, again the same things that we have seen right at the beginning of this course. These are the polarizers and the each polarizer has crystal axis.

So if you have to measure the photon intensity right here okay, the number of photons if you are going to measure after this polarization filter as a function of the orientation of this crystal axis with respect to the initial polarization that the polarization of the incident light, right, so that is theta okay. So if you do that then what we see is that there is a preferential emission of the fluorescent photons along a particular direction okay.

In a special case, I can think of that they will be along the direction of the incident light radiation itself and then as you go away from that incident polarization direction that is our theta here, then the photon's polarization falls down. I mean the intensity of the photons that you get for different theta okay, this is not polarization, this is the probability of me finding a photon with that particular theta okay. So let us see. I am going to rewrite this little bit so that if this probability of me finding a photon whose polarization is an angle theta from my incident light radiation okay.

So that is going to have a special I mean some kind of a distribution something like that centered around theta equal to 0 in a special case okay. So if you have this preferential orientation of this emitted photon’s polarization, then can we think about modeling this? Can you think about understanding where it originates and how can we explain this using whatever we know so far okay. For that, I said we can go back to what we know so far which is the expression for the rate of transitions, right.

Rate of transition now explicitly we wrote from our time-dependent perturbation theory is proportional to the matrix element square and then from second quantization analysis, we also got something similar here right, where it is modulus square of the matrix element, we call it as r in here. So I continue with that same notation, 2 and 1, 2 being the excited state and 1 being the lower energy ground state okay. Now not just this, apart from this proportionality we had few other terms right.

So let us go back, it comes from here, so you see that there is h omega k that dome and then you have this E0 k alright. It is an unit vector E0 is an unit vector pointing along the direction of the radiation field okay that is what we said and then we went ahead and quantized the radiation field itself right, but the key point is E0 is the unit vector pointing along the radiation field. So now, what you can actually see is that, we go back, so now when you do that naturally that is going to have this whole rate will have a directional dependence.

What it attributes is that this r per se it is going to have as a dot product of okay, this r will be a dot product of dipole moment vector, we will talk about this a little later in the course, but this is essentially the dipole moment because of the transition and this is something that we wrote in the time dependent part as e dot r. So there it comes that the mu, the transition dipole moment, times that vector that is the scalar product of that, right. So basically, what you are looking at is this okay.

So now since this is a scalar product, we know that there is a directional dependence, meaning if the incident light radiations e field that is right now polarized, the polarization direction is along the x axis and if our molecule were to be oriented at an angle phi or theta or alpha from that x axis, then the rate of transition is going to have very strong dependence because you are going to take, so if you are representing the unit Ek0 not along this axis and then the transition dipole moment were to be some angle alpha in here in general.

So then, what this does is it actually rewriting the probability of excitation or the rate of transition as directly proportional to the R vector or the rest of it, we do not have to write the R but since you are expanding we can actually write it as 2 mu R, why do not we write it as simply modulus 21 square, since it is a unit vector we can take, we do not have to worry about the Ek0, but what we need to worry about is the orientation factor which is cos square in here I represented it as alpha, let us stick to that, it is alpha okay.

So when it is completely perfectly oriented right, so we take if the incident polarization that is along the x axis okay. Now the molecules transition dipole moment if it is completely oriented, then the entire dipole moment is taken into contribute towards the rate transition but as it moves away and it reaches a point where it is orthogonal to the initial incident polarization itself, then the phi will hardly has any interaction with the molecule at all that is what is predicted by this cos alpha term because what you are taking is essentially a projection of one vector onto the other.

A scalar projection of one vector onto the other and that gives you the relationship that is the rate of transition go as mu 21 modulus square cos square alpha alright. So now, that is good. So we see that molecules oriented at different angles with respect to the incident polarization have different probability of excitation and that is exactly why you have this distribution coming into picture. So we know this, now can we actually predict what the distribution is going to look like? That is pretty simple because it is cos square alpha.

So this probability, since we know this probability goes as cos square alpha, now we know the answer to this question of how does the probability of me finding a photon whose polarization is theta from its R and alpha in my case from the initial incident polarization, now we have the answer right. So when it is completely aligned, the probability is 1 and as we move away, the probability falls down and then it reaches 0 right, and this function it reaches zero at 90 degrees alright. So, we can write it as pi by 2.

So now this goes as cos square theta okay. It starts from 1 and then reaches 0 at pi by 2. Same thing is the symmetric right, so same thing for the negative axis too. So we know this distribution okay. Now from this, can we predict an experimentally observable quantity that is easy to measure in a regular laboratory setting, what that would be?

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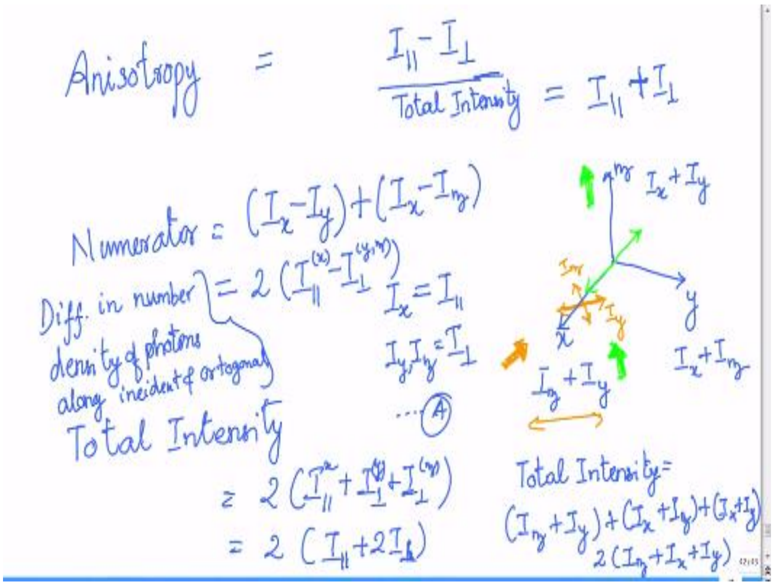

Anisotropy $= \frac{I_{\parallel} - I_{\perp}}{\text{Total Intensity}}$ $= I_{\parallel} + I_{\perp}$

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

Diff. in number density of photons along incident & ortogonal $= 2(I_{\parallel}^{(x)} - I_{\perp}^{(y,z)})$

$I_x = I_{\parallel}$

$I_y, I_z = I_{\perp}$

$\cdots$ Ⓐ

Total Intensity

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

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

Total Intensity=

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

$2(I_z + I_x + I_y)$

What that would be is something defined as a quantity called as anisotropy, right okay. What would be anisotropy? Anisotropy is basically if I am measuring the intensity alright that is coming parallel to my incident light radiation. The intensity when I am marking, I am actually marking the intensity of the emitted fluorescence okay I parallel minus I perpendicular okay.

So this is the emitted intensity in a perpendicular direction, so this difference, I parallel minus I perpendicular tells me how different the polarization is because you see that if you have a lot of photons coming in I parallel that means that they are all aligned to the incident light radiation, and if you have lot of photons along the I perpendicular, then it tells you that the emission is preferentially along the perpendicular direction. So what I am going to do is I am going to take the difference between these two as a measure of this distribution, but the intensity themselves can be very different, right.

Somebody may be emitting a lot and somebody may be emitting less. So if I were to measure the absolute number of photons that are having a polarization along the parallel axis while for the other photons which are at a 90 degree to the incident polarization, then that may not be truly reflective because this number is directly proportional to the emitted fluorescence photons themselves. To normalize that, I have to divide by the total intensity okay. Now at this place, it is very tempting to write it as I parallel + I perpendicular because that is all the directions that we know of anyway.

So we can actually write it as I parallel and perpendicular and they turns out that is not completely true, in order to determine the total intensity we need to understand that we are here talking about a molecule in space and then the fluorescent photons are isotropic in all 4 pi solid angle, which means you are not only looking at the plane that is containing the incident thing but also things going around or in other words imagine we have a reference frame alright and we have x, y, and z and what we are actually doing is that we are actually trying to measure the intensities of the emitted photon.

So if there is a molecule sitting here and it is going to emit the photon and I said that the molecule is sitting at the center and then we are put in the reference frame as x, y, and z. Then what we are asking is what are all the photons that are coming in the direction x, y, and z under polarizations with respect to them right? So we can think of suppose if we are looking at photons coming along the x axis. Now, these polarizations can be of 2 kinds, we can ask about the light photons whose polarization is along the z axis plus the polarization along the y axis okay.

When you are collecting light from the x axis, that is the 2 possible polarizations that you can think of. Similarly for the y axis, you can think of as Ix and Iz. For the z axis you are looking from the top, then again you have Ix + Iy okay. Imagine I told you that it has a equal probability of emitting the photon in all this direction, but what we are actually measuring is what is the polarization angle of the photons right.

So that now if you think about this that all the photons that are having the polarization along the z axis when you are detecting the photon from this direction, from the x direction here, so then what you are asking is how many of them have the polarization that is along this, like this or like this okay. So now this, we call it as Iz and those photons that are having the polarization parallel to the y axis we call it as Iy and like that we actually estimate okay. So for Iy and Iz, so I am going to just to clearly represent this.

What I am going to do is I am going to redraw this diagram so that. Now, we are detecting some photons and that aspect I am going to represent by orange or brick red. So from this angle or this axis, we are actually detecting our fluorescence okay. Now when we do that, then the photons or whatever I am looking at I am going to collapse them to having the polarization because my polarizer is going to be put such that either it is aligned towards x axis or in a mutually perpendicular direction which is the y axis.

So I call this as my Iz and this as my Iy, right, that is what we have written here and we can do that for all the 3 different directions. Now since we have accounted for 3-dimensional localization of the fluorophore and a uniform emission along all the directions, now the total intensity then would be simply the sum of all of this and if you do that and going back to the same, so the total intensity is sum of all of this irrespective of it is isotropic or not, but the point is we will get to that in a moment.

So the sum of all of this, we add which is Iz + Iy + Ix + Iz + Ix + Iy, good. So now when we write it, we can see that this can be written as 2 times Iz + Ix + Iy. Now, the photons that are having polarization along the y or x right, so let us take a step back. If you are going to think about a molecule here and then now my incident light radiation is going to come in such that, so let us say that the light is traveling along the z axis, so from bottom all the way up okay, it is traveling along this axis.

The incident light I am going to represent it through the green arrows so that is actually traveling along this axis okay. It is going up like this okay. So since it is going up like this to be consistent with our initial statement about the polarization, so the polarization of the photons or the incident light are along this axis like this okay, this is my incident light's polarization. So now, everybody whose polarization is aligned to Ix, we would like to call that as I parallel in our experimental setting.

While all those guys which are aligned to y and z, really the yz plane is actually perpendicular plane to that of the x axis, so what we have is that Iy, Iz would be our I perpendicular alright. So now if you use this information that Ix = I parallel and Iy, Iz = I perpendicular and really you can see that for defining the perpendicular direction you do not really need to choose Ix and you choose to call one as y and other as z, so you could have called the other way around too, which means Iy would be equal to that of the Iz.

We could say that using this in this relationship okay, so we can call it as some A in the total intensity. Now, we can write the total intensity as equals 2 times Ix becomes I parallel + Iy and Iz are I perpendicular, so you have one I perpendicular for y axis and then another I perpendicular for z or in other words you have 2 times I parallel + 2I perpendicular, alright. So we need to do a similar kind of analysis for writing the numerator in terms of Ix and Iy. So, we want to know how do we write it?

So what we see is that the I parallel, so this I parallel minus I perpendicular right we have it when we detect the light along the z axis or the y axis right, the guys who were traveling along or getting emitted along the x axis, they will because remember they are actually propagating in the x axis, which means the polarization vector then would be either along the z or the y are in the yz plane in general, right. I mean it will not be, you will not have any parallel component there which means the anisotropy you would be defining in these two terms.

So, I mean you will have Ix - Iz so that is one term okay, the numerator here. We will have 2 terms, one corresponding to anisotropy that we are measuring when we are actually looking at from the top, I mean in this scheme of things when you are looking from the z-axis, then all the intensity of all the photons whose polarization is along the x axis and traveling in the z on top as well as the bottom, we collect it and call it as Ix and that would be your I parallel while the photons which have their the field that is along the y, it is 90 degree with respect to the parallel, so we call it as Iy.

Similarly one more Ix term we have that is when we are measuring it from the y direction alright. So here it will be Ix - Iz and as the argument goes like in the denominator too, Iy and Iz is equal, so essentially we can write this numerator as 2 times I parallel for the x-axis minus I perpendicular for y or z axis. Now substituting this back into the anisotropy expression because that is really how we want to define, which is the difference, numerator is nothing but difference in number density of photons along incident and orthogonal to incident okay. Now this by the total intensity would give us anisotropy.

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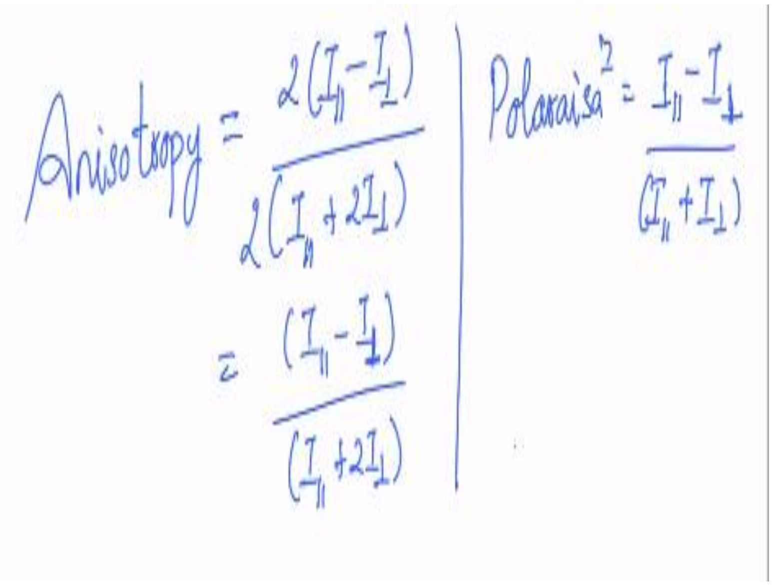

$$\text{Anisotropy} = \frac{2(I_{\parallel} - I_{\perp})}{2(I_{\parallel} + 2I_{\perp})} = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + 2I_{\perp})}$$

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

So we write it as 2 times parallel my I perpendicular the numerator term that we calculated divided by 2 times I parallel + 2I perpendicular giving you the expression I initially stated which as I parallel minus I perpendicular divided by I parallel + 2I perpendicular. Now this is just giving a rationalization, why the anisotropy is defined the way it is defined. There is an equivalent terminology called the polarization that is defined as.

Now, you can also measure that, I mean you can also measure the polarization, but it turns out then when you want to model and predict how these physical observables would behave in response to molecular physical properties like a rotation of motion of the molecule and so on and so forth. The anisotropy being directly corresponding to the observation that has been made because you are accounting for every single photon that is emitted in all 4 pi directions.

The expressions that you obtain for anisotropy are more tractable while for the polarization it gets little cumbersome, particularly when you deal with many number of molecules and so on and so forth. However, it is only the mathematical convenience and the aliens with which the anisotropy allows us to perform, we choose to work with anisotropy, but in principle you could actually use any of them as you can very well see both of them measure more or less the same thing except for the denominator being different with 2 being here.

So now, that is the definition of anisotropy and why we define the anisotropy in the way we are defining it. What we set out at the beginning was to actually say can we predict, how can we predict the behavior of this anisotropy in general right. So I said you can and for that we even went to the extent of writing down the rate of transition and then saying okay now where is this directional dependence coming from.

So we did that, now what we are going to do is in the next class we are going to model the behavior, the anisotropy behavior by explicitly calculating the I parallel and I perpendicular given that we know the rate of transition has a direct bearing, I mean it gets directly affected by the polarization itself okay. Well, thank you. I will see you in the next lecture.