Basics of Fluorescence Spectroscopy
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Lecture – 38

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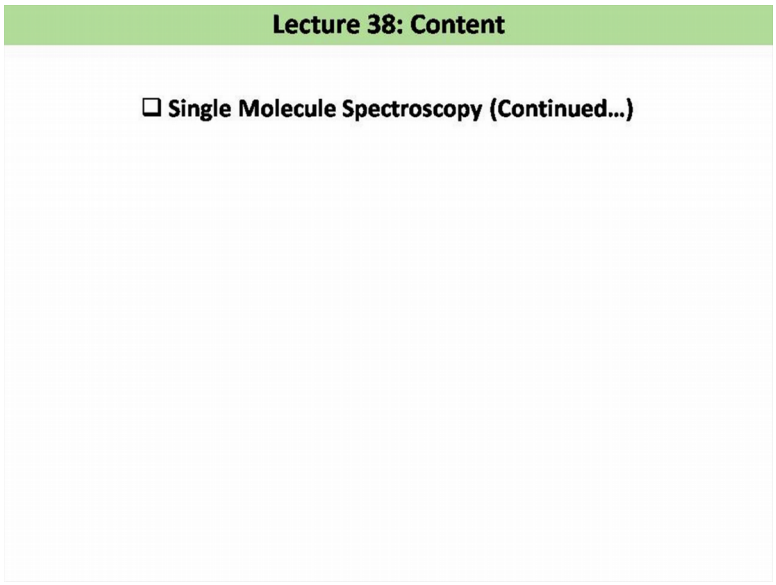

So, in the last lecture we have discussed about the importance of single molecule fluorescence or single molecule spectroscopy itself, many such cases where the ensemble average picture is not good enough to tell about the system exactly. So, in such case the single molecule spectroscopy are very much efficient to comment what is going on.

So, if we are now convinced that single molecule spectroscopy is one of the important topic to discuss right. We have to proceed to the most important part of the single molecule spectroscopy. How is it possible to see a single molecule? Right it is not easy.

So, let me point out few important parameters, which should be improved to observe the fluorescence from a single molecule, right.

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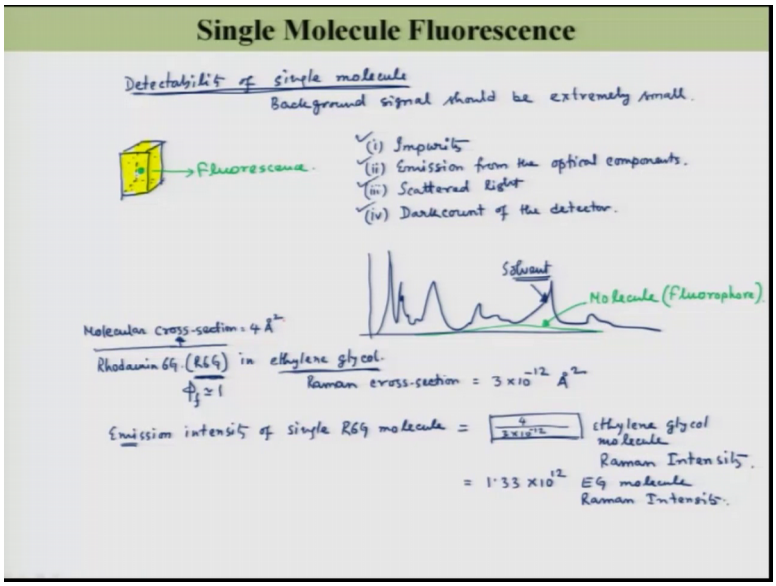

So, here is my detectability of single molecule is a issue and most importantly right I need to ensure that the signal the photon number of photon coming out from a single molecule should be more compare to the background signal. In other words the background should be very very low.

So, the background signal should be extremely small right. Let us say you have this sample cell and you want to take a single molecule inside the sample cell right and sample cell is filled with some other some solvent right is filled with some solvent. And you have a single molecule inside the solvent cell right inside the sample cell and what is your intention? Your intention is to see the fluorescence coming out of the sample cell that is your intention whatever the fluorescence you will going to measure this fluorescence you will going to take this sample in your normal fluorimeter, and then you will excite and then at the right angle geometry you will going to record.

There could be several sources for this background let us say for example, impurity. If you have some impurity present right and those impurity will also emit a broad emission broad right broad type of emission or in that fluorescence wavelength region right then because you only have only one single molecule and impurity level if it is more if in this sample volume, you have 10 impure molecule right because the concentration of the solvent is huge right because it is the pure solvent.

So, in this case the impurity has to be very very low right the and in this particular case when you have only one molecule then there should not be any impurity. Otherwise its impurity may affect the detectability of the photons coming out of your desired fluorophore the fluorophore under investigation.

Second let us say emission from the optical components, when you excite because in the fluorimeter you have several optical component with coatings and this and that right and they also sometime emits little bit of different colours of light. So, that also has to be taken care, because you are going to detect the photons from a single molecule. So, remember that.

Number 3 is scattered light right. So, if there is any scattered light like you are doing this experiment in this room you have covered, but the covering was not good enough. So, there is some leakage from this side that side this side. So, some scattered light will be there that will going to destroy your measurement; because the number of photon coming out from a single molecule is really small right if some lights are coming from here and there then you will not be able to measure right.

And number 4, I can think of all these different issues number 4 is the dark count of the detector that I already informed mentioned that the dark count of a detector is because of the thermal electrons right. If that is at this level and the count or this photo electrons originating from few photons which is coming from the single molecule is over here, then you would not be able to see right because of this high level of the dark count. So, you have to lower this dark count in a minimum over zero value, and then little bit will be visible right. So, this is another issue.

Now, you consider that you have a sophisticated instrumentation laboratory. Sophisticated purification facility so, that impurity taken care emission from tactical component taken care scattered light taken care and dark count of the detector is also taken care; but still you will not be able to detect this emission from this single molecule, the reason is the intrinsic Raman's scattering of the solvent. So, this is most important thing.

If someone will tell you or someone will ask you what is the Raman's scattering of a particular solvent. We usually mention the strongest bit right. So, the Raman sheet for water is about 3400 way of number right I will get a big over there, but if you really

because of the presence of several vibrational and rotational state although the intensity are small right, but still the Raman's scattering could be present the throughout this emission wavelength.

And now if you consider that how many solvent molecules are present inside the solvent cell and having a particular cross section of Raman's scattering. If you calculate then what you will going to see is that the Raman's scattering cross section is much much let me do such kind of a calculation over here.

So, what I want to say is that even you take care of all this different problem, but because of this Raman's scattering is represented like this way, like this and your fluorescence will be somewhere here. So, you would not be able to see this fluorescence in presence of this Raman's scattering. This fluorescence is coming from your molecule right that means, your fluorophore, and this Raman's scattering is coming from your solvent this is important. Let me give you one specific example then this will be more clear.

Let us say that I have taken one molecule this is called Rhodamine 6G let me represent as R6G, in some solvent. Let us take the solvent as ethylene glycol if I have to represent this emission characteristic of this Rhodamine 6G, what information I should give you? I should give you the information of this fluorescence quantum yield.

So, let us say this phi f let us assume that this is equal to one; that means; the red constant of this non radiative pathway is 0. So, only that radiative pathway is available for such for this particular molecule Rhodamine 6G my assumption.

Now, I will also tell you the one thing that is the absorption cross section of this molecule is that is my also assumption the absorption cross section of this Rhodamine 6G molecule is same to the molecular cross section right that means, the number of photons which will excite this molecule right will be 100 percent right. If there are incoming 100 photons all 100 photons will be absorbed by this right and the fluorescence quantum yield is one. So, there is no loss at all.

Now, this Raman cross section of this ethylene glycol, Raman cross section of this ethylene glycol is 3 into 10 to the power minus 12 angstrom square. So, let me write here the emission intensity of single Rhodamine 6G molecule, should be equal to some number of ethylene glycol molecule Raman intensity.

Now, can I find out this number I said that the absorption cross section of Rhodamine 6G is same as the molecular cross section of the Rhodamine 6G, but here this Raman cross section of this ethylene glycol is really small right it means that if I know this absorption cross section or molecule cross section of this Rhodamine 6G right, then the number of molecule which will interact with Rhodamine 6G right that I will get a idea about that how many such kind of photons is actually going incoming right incoming photon, is actually going to create my intensity.

So, if the Rhodamine 6G molecular cross section is let us say 4 angstrom square, it means that emission intensity of single Rhodamine 6G should be equal to this 4 divided by this right. So, here should be 4 divided by 3 into 10 to the power minus 12, now this is number right. So, these many molecules have ethylene glycol, because here for the Raman cross section is really tiny 10 to the power minus 12 angstrom square. So, in emission intensity from one Rhodamine 6G molecule should be equal to 1.33 into 10 to the power 12. Ethylene glycol let me right as EG ethylene glycol molecule Raman intensity that is for sure.

That means one number of photon coming out of a single Rhodamine 6G molecule means is equal to the Raman scattering or those photons of the inelastic scattering of the similar wavelength let us say of 1.33 into 10 to the power 12, number of ethylene glycol molecule they are same they are same.

So, now I will take one condition that is our ensemble average condition usually we prepare a solution of micro molar right. So, let me take this condition.

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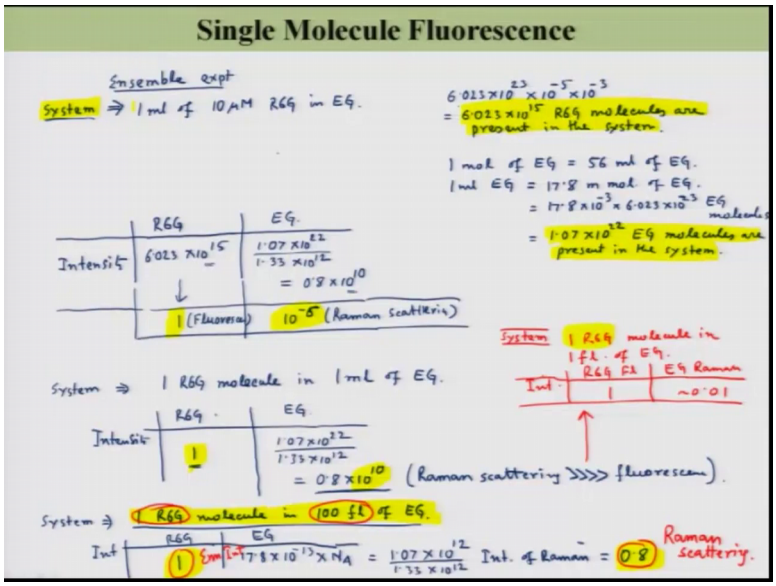

That for the ensemble experiment, you have let us say 1 m l of 10 micro molar Rhodamine 6G in ethylene glycol, this is my system right. So, your system is this right. So, I can readily calculate how many Rhodamine 6G molecules are present, how many ethylene glycol molecules are present right in the system. So, let me right one by one.

So, Avogadro number 10 micro molar and 1 m l 10 to the power minus 3 right this equal to 6.023 into 10 to the power 15 right R 6G molecules right are present in the system how many number of ethylene glycol. So, I know this one mole of ethylene glycol is equal to 56 m l of ethylene glycol that I know. So, 1 m l ethylene glycol is equal to just simply this 17.8 milli mole of ethylene glycol right. So, this equal to 17.8 into 10 to the power minus 3, 6.023 10 to the power minus no no not minus 23 EG molecules, if you do that this will be equal to 1.07 to 10 to the power 22 EG molecules in the molecules are present in the system.

Look at this two number one is this one. So, I will just highlight this one is this one. So, this many of Rhodamine 6G molecules are present in the system and these many of ethylene glycol molecules are present in the system, what is your system your system is this one. So, this is your system.

Now, if I consider that one Rhodamine 6G molecule right will the fluorescence quantum yield is one that is why I have already assumed. So, we will give one photon right or one emission intensity in a unity emission intensity right. So, 6.023 10 to the power 15

Rhodamine 6G will give the emission intensity. So, now, I will write a intensity right coming out of Rhodamine 6G will be 6.023 to 10 to the power 15.

Now, the intensity from EG that I should calculate here. So, in this case I have this number 1.07 10 to the power 22 EG molecules are present. So, 1.07 to 10 to the power 22 molecules are present, and I have seen in my previous see here emission intensity of single Rhodamine 6G molecule means 1.33 to 10 to the power 12 EG molecule.

So, I should divide these number by these number 1.33 10 to the power 12; that means, it will be comparable to one Rhodamine 6G molecule right. So, these divided by 1.33 into 10 to the power 12, then this will going to be 0.8 into 10 to the power 10.

So, as you can see here if this is the intensity of emission from the Rhodamine 6G which is 10 to the power 15, and the intensity of Raman's scattering from this ethylene glycol is 10 to the power 10, which is much much smaller right compare to this intensity of Rhodamine 6G.

Now, if I consider the for such a case in my this system right that is 10 micro molar Rhodamine 6G in ethylene glycol and I have taken 1 m l of such solution then I can simply convert these as I can write this as 1. So, this emission intensity will be roughly right roughly 10 to the power of minus 6, 10 to the power minus 6. So, this intensity is because of the fluorescence and this is because of the Raman's scattering. So, which is negligible this is one this is 10 to the power minus 6. So, this is negligible.

Now, my interest is not this ensemble average picture my interest is 1 Rhodamine 6G molecule that is what I was discussing here right. 1 Rhodamine 6G I have taken in 1 m l solution. So, next I will change my system to the single molecular level now my system is one Rhodamine 6G molecule in 1 m l of ethylene glycol right. So, now, this is my system.

So, 1 Rhodamine 6G molecule will going to give you one intensity. So, if I now write this intensity Rhodamine 6G, what I will going to get is just one over here, and the intensity of Raman's scattering by this ethylene glycol that depends on the how many ethylene glycol molecules are present that number I have already have determined over here; so 1.07, 10 to the power 22.

So, in this case the intensity of these scattering will be 1.07 to 10 to the power 22, and this cross section right that number which I have already calculated one Rhodamine 6G is proportional to this much. So, I have to divide this with 1.33 into 10 to the power 12 basically this number is same and it has to be equal to same because it depends on the how much ethylene glycol you have taken. In this case one m l in this case also 1 m l, but here you had taken one micro molar solution of Rhodamine 6 G. So, number of Rhodamine 6G molecule was very large right 10 to the power of 15, but here is only one. So, here that Raman's scattering is 0.8 into 10 to the power 10.

Now, you see this is 10 to the power 10 and this is one. So, fluorescence intensity is negligible will compare to the Raman's scattering. So, here Raman scattering is much more than the fluorescence.

Let me take my third system as, one Rhodamine 6G molecule in 100 femtoliter of ethylene glycol right. If it is 100 femtoliter, so then intensity R 6G this is one because I have only one and here ethylene glycol then this number will change. So, in this case the number is 17.8 into because I have one femtoliter. So, the 17.8 into 10 to the power minus 13 right into Avogadro number. So, you should be proportional to this. So, then this will be into Avogadro number means this will be 1.07 into 10 to the power 12 molecules of EG, then I will divide this by 1.33 into 10 to the power 12 right. So, then this will going to be intensity now this no longer be molecule. So, then this will be going to be intensity of Raman scattering. So, this is going to be 0.8.

So, you see here for such case when one Rhodamine not here one Rhodamine 6G molecule in 100 femtoliter of ethylene glycol, I got the fluorescence is one I got the Raman is 0.8 right. So, this is the catch. So, for the ensemble I had 1 fluorescence 10 to the power minus 6 Raman. So, no problem, but when I took one Rhodamine 6G in 1 m l ethylene glycol, what I got one fluorescence means 10 to the power 10 Raman and it is problematic right. You cannot see 10 to the power one fluorescence in front of 10 to the power 10 Raman, but when you have taken one Rhodamine 6G in 100 femtoliter of EG you see the emission intensity is one. So, this is your emission intensity and 0.8 is your Raman scattering.

Now, if you further decrease from 100 femtoliter to one femtoliter, then these values will be 0.008 right 0.008. So, if you further decrease the volume of the sample; that means,

my next system and which is my system for future studies is 1 R 6G molecule in one femtoliter of ethylene glycol. In this case intensity R 6G fluorescence and EG Raman will be if it is one then it will be roughly 0.01.

So, this is a good situation for me to monitor the fluorescence intensity coming out from a single Rhodamine 6G molecules. So, the catch here is I have to combine this one Rhodamine 6G molecule in one femtoliter it is not1 m l it is a very very tiny volume. So, that is the catch and if you can achieve it you will be able to see the single molecule as I have described till now.

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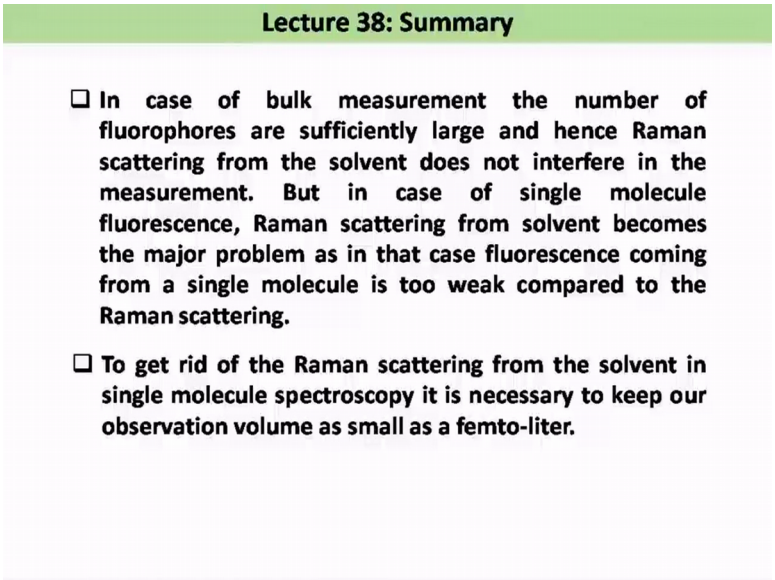

And in the next class what I will going to do, I will going to show you how to achieve such kind of small observation volume. And this is a key right if you cannot achieve such kind of small observation volume, you would not be able to see the fluorescence from a single molecule. So, let me finish here and we will continue our discussion on the next day.

Thank you very much.