Basics of Fluorescence Spectroscopy
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Lecture – 11

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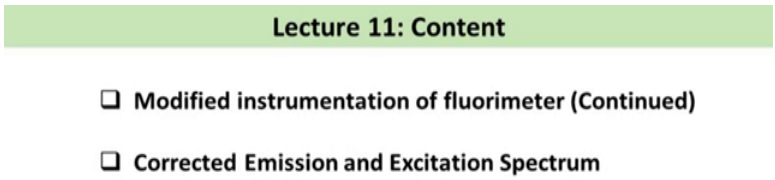

Welcome back after the break. So, I was discussing about this quantum counter.

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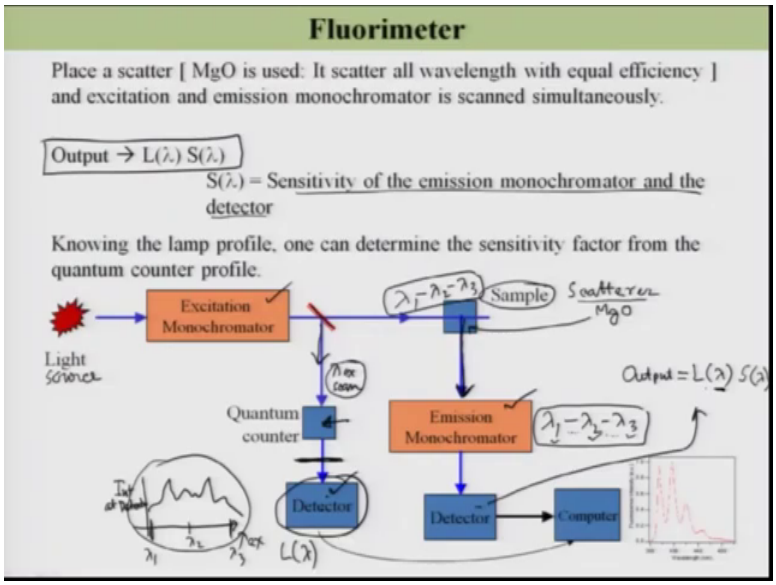

So, in this quantum counter my aim is to; right, my aim is to get the corrected emission spectra, right and then I was discussing about this; the measurement of the total emission coming out from a substance where the inner filter effect is highest right the complete in the filter effect. So, no excitation light is passing through the sample right. So, whole excitation light is being absorbed by the sample the concentration is like so high and that is the reason for that and we will going to collect the total emission spectra coming out of the sample. As discussing if you now scan this lambda excitation and detect the emission are coming out of the sample here. Please note that I do not have any monochromator over here because I am just interested in the total intensity and the total intensity is coming out to this detector.

So, with that the detector efficiency is same for different wavelength or not it does not make any sense in this case. So, I do not need to correct the detector response for in this case right. So, if I now take if I now measure this emission total emission coming out of this quantum counter where there is a function of lambda excitation what I will going to get is this pattern, right and this is nothing but the spectra of the lamp as I showed over here.

So, let us name it as L lambda, let us name it as L lambda. So, this is the lamp profile; lamp profile.

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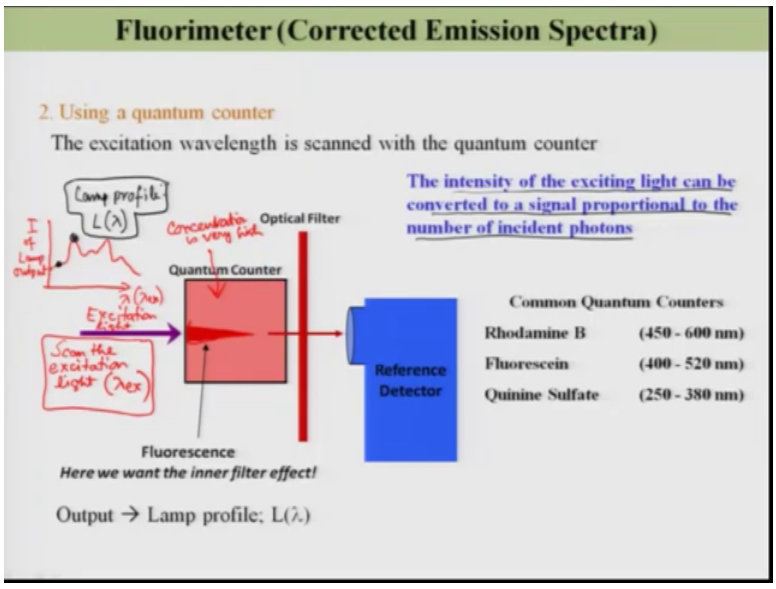

Now instead of taking your sample for the emission measurement let me take some others material in this sample position what; what is the material is a scattering like is scatterer. So, scatterer that that scatterer I will choose the material in such a way that the scattering efficiency is equal for all the wavelength and the substance for that is m g o emission oxide. So, I take this scatterer inside my sample chamber. So, it is just a scattering just a Rayleigh's scattering right just a Rayleigh scattering.

So, when this excitation light will fall on this scatterer this scattering will takes place in all the directions almost and the scattering intensity will also come in this direction right scattering will also come in this direction. That means, if I said that wavelength of excitation as lambda 1 I only get the signal in the detector I only get the signal in the detector when the emission monochromator will also set at lambda 1. That means, in this case I will scan this part this wavelength region that is my lambda excitation let me write here lambda 1, lambda 2, and lambda 3. So, I will scan it lambda 1, 2 lambda 2, lambda 3, these region and simultaneously I also have to set this value over here lambda 2 lambda 3. That means synchronous change of wavelength of the excitation monochromator as well as the emission monochromator.

So, in this case what you will get you should get you these spectra again. So, in this detector what you should get L lambda. But please note here that these detector at a time measuring only 1 lambda some time lambda 1 sometime lambda some lambda between here then lambda 2 then some lambda between here then lambda 3. That means, the detector efficiency or monochromator efficiency is coming into the picture is not it. So, this efficiency is coming into the picture. That means, this observed signal right observed signal I just write output we will not going to be equal to L lambda, but it will be equal to L lambda multiplied by the sensitivity of the monochromator and detector together. So, let us name it as S lambda I have written over here. So, output is L lambda into S lambda, S lambda is the sensitivity of the emission monochromator and the detector.

So, what you got you got L lambda S lambda from here you got only L lambda from here. So, you just divide L lambda S lambda by L lambda you will get the S lambda and using that that S lambda is nothing but your correction file, but that correction file you will be able to generate what is the what is the range of this lambda that range of this lambda will be guided by the absorption spectra of the molecule you have taken in this quantum counter absorption spectra of the molecule we have taken inside this quantum

counter. So, for different region of this lambda you should use different molecule is for example, over here rhodamine b is generally used for these wavelength region. Now if you want this wavelength region you need a fluorescein if you want the UV region you need quinine sulfate and so on and so forth there will several quantum counters available.

So you understand hopefully that how to correct the emission spectra and whenever you will go in to if you leave this fluorescence spectroscopy ever in your life. So, please remember that we should report the corrected emission spectra not just simple emission spectra simple emission spectrum means without that correction file without that correction file, but we should report the corrected emission spectra.

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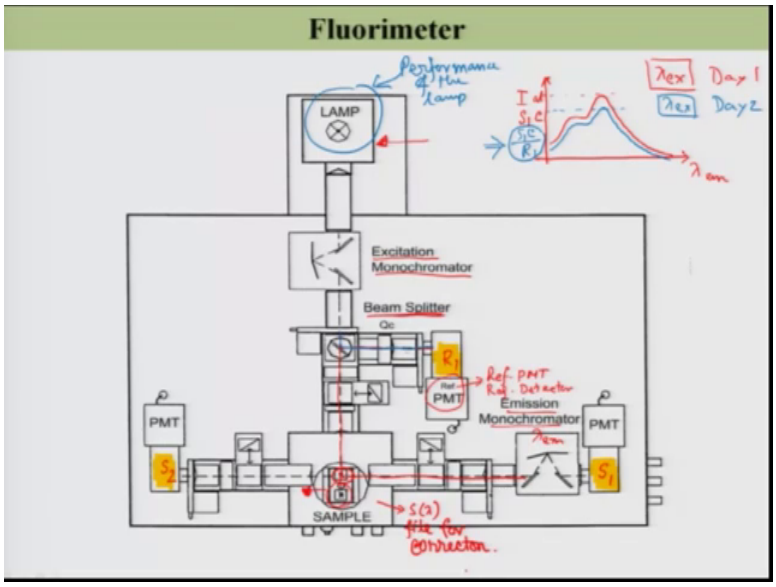

Let me show you the modern fluorimeter this is the modern fluorimeter, I have this lamp let me change the color, yes. So, here is your excitation light source lamp here is your excitation monochromator; now you know what is the role of that. Now this light is coming like over here over here over here, here is your sample or you can put your quantum counter over here and measure the S lambda file once you will measure this S lambda and that is that is fixed for this instrument you do not have to measure it every day you measure it and that is stored it for your future purpose and that is it.

So, once you will excite your sample that sample fluorescence can be collected like this way right then you have this emission monochromator and you have PMT, but you notice here, here another thing is present here they use a beam splitter and have taken

one part of this light and feed it to the reference PMT decision, can you read it is a reference PMT or we call this a reference detector right reference detector.

Let us now name the detectors let us name it as S 1 let us name it as R 1 how to name it here R 1 and it also has another detector over here for some other purpose forget about that list name it as S 2. So, here is my S 2 here is my S 1 and here is my R 1. So, now, now when you will going to measure the end and is also clear at this point that to get the corrected emission spectra you already have generated the correction file you already have generated the correction file correction file is lambda file; I just explained. So, you already have this S lambda file for correction suppose. Now you are measuring emission spectra you put your sample over here and now you are recording your sample.

So, what you can do you can measure the intensity at S 1 plotted versus lambda emission that lambda emission will be set by this emission monochromator right you set it what is the lambda emission. So, you plot it over here like this way and you will get something like this, but this is not correct you know. So, better you plot S 1 into correction file and let us name it as S 1 C.

So, in the computer attached with this, so correction file is already present and what you can do you can set you can instruct the computer like that whenever you will measure the intensity at a particular lambda mission you multiply with the correction file. So, you will immediately get the corrected intensity at each wavelength, so better not to plot S 1 by lambda emission, but S on C by lambda emission. So, let me delete here. So, let us plot a S 1 in to C. So, this is a multiplied by the corrected file a S 1 C.

Now, for example, this is for a particular lambda excitation is not it. So, lambda excitation for this lambda excitation right for this lambda excitation you will get this emission spectra and this is your intensity you are very happy and for a particular concentration, next a when will come and you start measuring it you have used S 1 C, next a different color, next will come you have used the same lambda excitation. So, this is the day one this is day one next you come you use the same lambda excitation day 2 same lambda excitation same S 1 C by R 1 you are plotting versus lambda emission. So, you should get the same craft same; same and dependence same fluorescence spectra, but what a probably it is possible that he will turn up end up with sorry instead of these what you end up with this emission spectra, what is the reason?

So, then emission intensity are different now. So, for the day one emission intensity is this much and for day 2 emission intensity this much that is there is a problem right. So, the problem is that the performance of this lamp is not constant the performance of the lamp is the problematic situation over here. So, yesterday probably the lamp was much brighter than today. So, the excitation light intensity is decreased today. So, that is why in day 2 you get will intensity because fluorescence intensity is directly proportional to the excitation light intensity. So, how to tackle this right there will be this fluctuation and we cannot avoid that right for a real instrument. So, that is what; what you have this differentiate R 1 A, some part of this excitation light just a very little amount of excitation light is taken in the other direction and measure the; what is the excitation light intensity is it not.

So, instead of doing the S 1 C if you now do S 1 C by R 1 then whenever you will measure with this condition. Obviously, the concentration of your molecule will be has to be same. So, in this condition every time you will be going to get the exact emission spectra including the intensity not only the shape, but also the intensity although initially I said the intensity quantum will is better than intensity, but sometime you also need the intensity right for everyday life. So, while measuring the emission spectra make sure that you are following this convention S 1 C by R 1.

Let us come to this, another measurement which is excitation spectra I briefly mentioned earlier that excitation spectra is nothing but you keep the emission wavelength fixed that is your detected emission wavelength and scan the excitation wavelength and plot the intensity of emission at that detected wavelength versus the lambda excitation wavelength and what we will going to get must be very similar to the absorption spectra that is one of the property I said right idea let us see how will going to get this. So, in this case what you have to do is you need to fix right this let me go to the next page.

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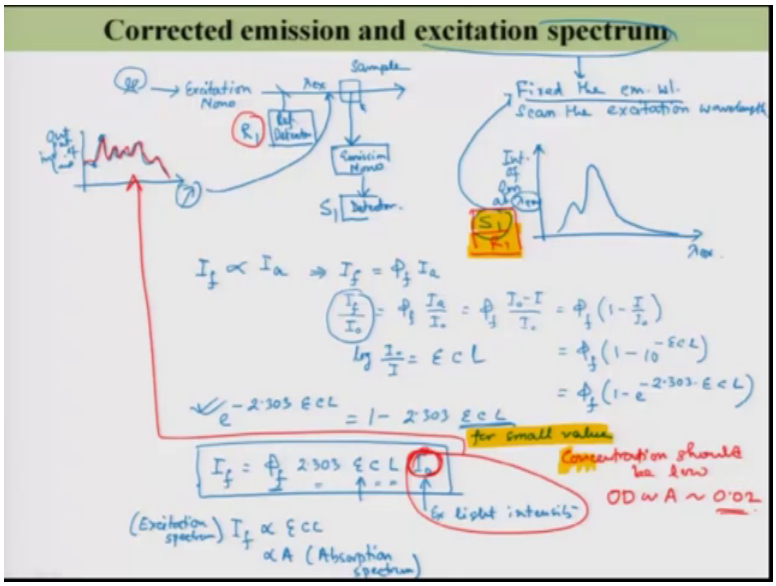

So, in this case, what you need to do is your excitation mono your sample emission is coming here is your emission mono and detector as I said I already have a reference channel over here this is my reference detector and I named it as I named this detector as S 1, I named it as R 1 the simplified version of that complicated fluorimeter. So, in this case for the excitation spectra also here I have written for this for this excitation spectra what you need to do you fixed the emission wavelength right at your choice and then scan the excitation wavelength. And then what will go in to plot is the intensity of emission right at that desired wavelength where you have chose it for you have fixed it intensity of emission at lambda emission that lambda emission is at your choice.

So, whatever you have fixed over here and versus lambda excitation that will going to scan in the emission monochromator. So, here is your lambda excitation. So, in this case what will going to see you will going to see the some spectra like that and I said that this spectra is same as a absorption spectra of the molecule.

So either you can plot S 1 the here either you can plot this S 1 if you plot S 1 what will happen let us see if I just simply plot S 1 then the emission intensity coming out of this sample is a function of concentration; concentration remain same. So, there is no concentration dependent and these also function of excitation light intensity and if you remember I said that lamp is not ideal. That means the output intensity of different wavelength coming out of the excitation lamp here is my lamp you know that is my lamp

coming out of this lamp is not constant is not same is not a horizontal line, but on the other hand this looks something like this.

So, this output intensity of lamp versus wavelength this is nothing but this excited lambda excitation later on right is like this right. So, when the lambda excitation is over here; obviously, the fluorescence intensity will be small when the lambda excitation is like this then intense fluorescence intensive will be more. Obviously, it also depends on the absorption spectra that let me show.

So, I said that intensity of fluorescence is proportional to the intensity of excitation light right and the concentration if concentration remains same then this is just proportional to the intensity of the excitation light; that means, the intensity of absorption. So, I f is proportional to I a and what is the proportionality constant that is the quantum yield right how much what is the rate of radiative transition over the total rate constant over the rate of absorption right over the rate of absorption if quantum yield is more. I will get one over here if the quantum yield is one it is just directly proportional that of whatever the molecule you have excited and that everything will coming back to the ground state by emission of radiation if it is not then some other factor will come.

So, simply this is going to be I f is equal to phi f into I a. So, let me let me divide both side by I 0 I f divided by I 0 is equal to phi f I a divided by I 0. So, this I a by I 0 is nothing but I 0 minus I by I 0. So, this is equal to phi f to I 0 minus I by I 0. So, this is equal to phi f 1 minus I by I 0 and what I know log I 0 by I equal to epsilon C L I also know log I 0 by I is equal to epsilon C L spot length right is a concentration C is a concentration.

So, here I can write this equal to phi f 1 minus right E 2 10 to the power minus epsilon C L 10 to the power minus epsilon C L, I can write that. So, I can; let me write it once again over here. Now I will changes the; from 10 to the power to E to the power. So, these can be written as phi f 1 minus E to the power minus 2.303 epsilon C L E to the power minus 2.303 epsilon C L. So, E to the power minus 2.303 epsilon C L I can write it as 1 minus 2.303 epsilon C L provided that epsilon C L the total quantity is very small right. So, I can write E to the power minus 2.303 epsilon C L is equal to 1 minus 2.303 epsilon C L provided this quantity is small quantity right provided this quantity is a small quantity.

So, for small value for small value right epsilon C L is nothing but the observance nothing but the observance. So, when the observance is small then only I can write. So, then I can write it like this way I f equal to phi f into 1 minus 1 plus. So, this is nothing but 2.303 epsilon C L and I must have I 0, what is my I 0 over here I f; I f by I 0. So, once I write I f then I must have this I 0. So, I got this final equation see here. So, this is just I have a formulate that how the fluorescence intensity of your desired lambda emission will depends on these different factors this I 0 is that excitation light intensity excitation light intensity this is your molar extinction coefficient right and this is a constant for a constant molecule, because that depends on the observed emission wavelength.

So, this is fixed this is a constant this guy is a constant this is a constant this concentration you are not going to change it. So, it is the constant the path length you are not going to change it. So, it is a constant. So, what I have if the I 0 that excitation light intensity is constant over the excitation wavelength then I f is I am sorry proportional to epsilon right epsilon means are proportional to epsilon I can write proportional to epsilon to C L because C and L are constant itself. So, epsilon sorry, C l so it is proportional to A. So, it is proportional to A and; that means, these this is my excitation spectrum what you are going to plot is I f right this is excitation spectra is proportional to a right. So, this is excitation spectra I will just write here excitation spectrum is same as the absorption spectrum.

So, what I was telling here- if I now plot this S 1 and lambda excitation these spectra will be equal to will be same as absorption spectra provided 2 things I have taken the concentration is low if not then I will not be able to write I will not be able to write this one so concentration was below. And secondly, that this is most important so. Secondly, is this the excitation light intensity should be fixed over this wavelength, but it is not you look here you see here see here how to how to show you over here over here over here, but it is not right is not it is changing is like that is like this it is like this.

So, excitation light intensity I 0 is changing. So, what you have to do you have to then use this reference detector. Now if you do S 1 by R 1; that means, you are normalizing the observed intensity as the S 1 detector with the intensity. That means, see you somehow making this I 0 remain as a constant just by putting this S 1 by R 1. So, this is also please note that whenever you will going to measure this excitation spectra and this

is important because if that if the absorption spectra and excitation spectra are same it means that there is no structure change there is no reaction taking place in the excited state if they are different. That means, there is a change in the structure of the molecule in the excited state.

So, we some we every time need to perform this excitation spectra right and to see whether it is same as the absorption spectra, but it will never match if you do not do it like that way that is S 1 by R 1 you have to do the S 1 by R 1 and also you need to take concentration should be small; small sorry.

So, concentration should be small that is it concentration should be low how low the o d or absorbance should be in the order of 0 point 0 2 something like that then it is applicable. So, today what I have shown you is that corrected emission spectra how to get the corrected emission spectra and then excitation spectrum and I have taken I have introduced this reference detector which is very important in this case and then I showed you that under which condition this excitation spectra is same as the absorption spectra and that is it for today.

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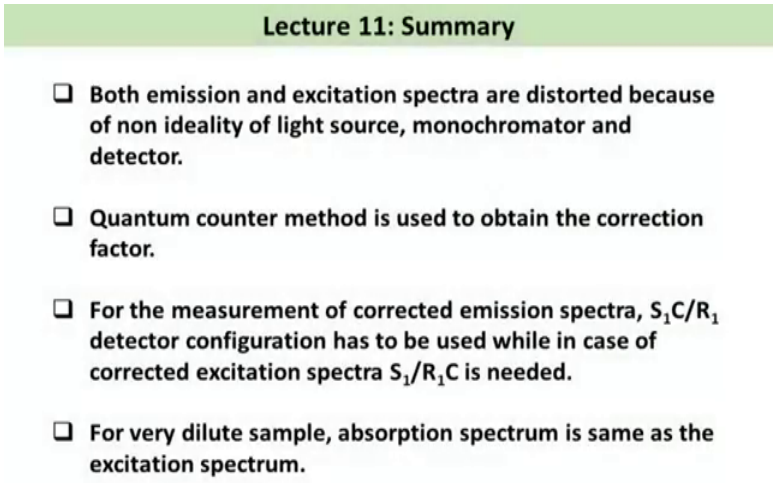

Thank you very much for your attention.