Basics of Fluorescence Spectroscopy
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Lecture – 09

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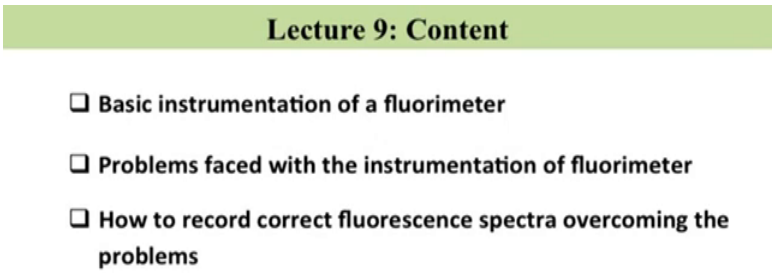

Welcome back. In this lecture, I will go to discuss the basic instrumentation of the basic instrumentation of fluorimeter. So, fluorimeter is the instrument where will going to measure the emission spectra fluorescence spectra of a sample.

So, till the last lecture, what we have discussed the different properties of the fluorescence and different parameters of the fluorescence. And out of these different parameters what we have seen is the first being to the intensity so that should be able to measure in the fluorimeter. The second one is the quantum yield; I should be able to measure this quantum yield by the fluorimeter meter. The third one is the fluorescence lifetime which is not possible to measure with the standard fluorimeter meter we need a time wizard fluorimeter. And the fourth one is emission maxima which can be readily measurable using a fluorimeter.

And we will also going to see how to measure this emission spectra, how to measure the excitation spectra and will going to see that whether this relationship holds good; that

what I said in one of the property that the excitation spectra is same as the absorption spectra. So, let us start one by one because this is a lot of job to do in this lecture today.

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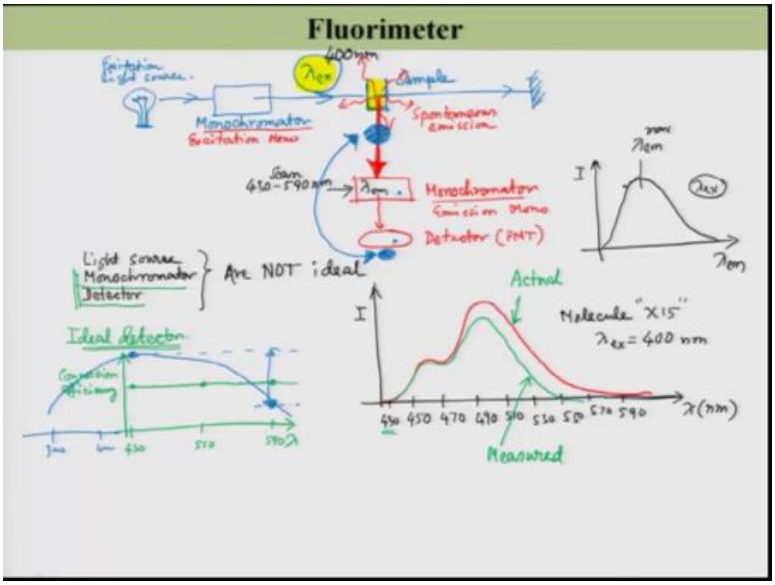

So, first let me start with this basic design of the fluorimeter. So, here is the basic design. I am going to have this light source because you have to excite your sample so you need some light source. And that light source right should be able to generate your light of choice.

So, this light source let me draw over here as I was drawing for this absorption spectrophotometer. And I have these (Refer Time: 02:13) in normal tungsten clamp. Normal tungsten lamp is ok if the excitation wavelength we want is more than 450 nanometer or so. So, it is ok normal tungsten lamp. However, you need to select your excitation wavelength precisely is not it. That means, if you want to excite at 450 nanometer or 500 nanometer or 550 nanometer; that means, you need that wavelength selector which I already discussed during the discussion of the spectrophotometer.

So that means, you need the monochromator over here, the monochromator will be placed just after the spectral lamp. So, here is my monochromator, here is my excitation light source. And with this monochromator will going to choose the desired wavelength and is going to excite your sample, here is your sample, here is your sample going to excite sample. And now often excitation the molecule will go to the excited state: it some light will be used to excite, some will pass through. So, you need to block this residual

excitation light by some metal block or something like this. So, the molecule will go to the excited state and then molecule will come back eventually. Eventually the molecular will come back from the excited state to the ground sate and it will limit the radiation. This is that k are the radioactive pathway is responsible for the emission of the photon, you remember.

So, the properties of these emitted photons are such that they are direction less. So, the m jet photon could go either in this direction, these direction also, this direction also, here and here, all the direction, that the photon will be limited in all the direction. So, this is the spontaneous emission, right. Probably we have heard of stimulated emission which is directed, but let us not discuss all these final details over here. Just to tell you that this is spontaneous emission and this is direction less. So, this is my spontaneous emission. So, I can detect this emitted photon either by putting something over here or putting something or this direction or putting something over this direction and so on and so forth.

But to avoid some scattering we usually put our detector in the right angle geometry. And this emitted photon is being detected with the perpendicular direction of this excitation. Now this is not tapered single color it also like a band; the fluorescence is also like a band. So, to measure the intensity of the constituent wavelengths I need to disperse this light. Similarly is a monochromator, so I must have the monochromator over here where the constituent wavelength of this emitted photons emitted light are dispersed so that I can measure the intensity of each wavelength individually, is not it.

So, this is monochromator, similar device but we need two over here: one is here; this is the excitation monochromator that is used to choose the excitation light. So, we more precisely write this as excitation mono just in short form and this we write as emission mono emission mono.

So, after passing through this monochromator this will be dispersed and will be able to get the individual wavelengths depending on the slit width obviously. Now, this is guided to the detector. So, I will going to have this detector over, here this is my detector similar to that detector I discussed in spectrophotometer which in general it is like PMT which detect the intensity just by converting the photon to the photoelectron and then

measure this either current or the voltage across the resistance; so just determining the intensity of individual wavelength, that is created in the monochromator.

So, under a particular excitation light over here, if you use a particular light to excite the system over here, they say the light you used is lambda e x, right for exciting this sample. So, what I will going to do I will scan this lambda emission and plot the intensity versus lambda emission. So, here what I will be going to do is I will make a plot over here, this is like this lambda emission. So, lambda mission: what lambda mission that will be selected over here. You choose whatever you want, you say that lambda emission is 511 nanometer. So, then you will measure at 511 nanometer the intensity.

Then you change it to 512 nanometer; then will measure the 512 nanometer the emission intensity. Then you will use a 513 nanometer; then we will measure 513 nanometer. So, like that you will measure the intensity as a function of emission wavelength. And that is nothing but your emission spectra and that maxima we generally called lambda emission maxima. For that wavelength of excitation is lambda excitation. For this lambda excitation wavelength this is my emission spectra and this is my emission maxima. And you know this a as per kasha's rule that this emission spectra will not going to depend on this lambda excitation wavelength. So, even you change little bit here and there this length excitation this emission spectra will remain as it is.

So, this is a very simple instrumentation for my fluorimeter, however, I would like to point out several problem with this: the first problem being that this none of these device in this fluorimeter or idea; that means, the light source the monochromator and detector, only three device I have talked about right, but they are not ideal what I wanted to say is. First let me say that clearly the light source monochromator and detector they are not ideal; are not ideal why I want to discuss these things. Let us start with the detector part let say- let me draw a an emission spectra of particular compound and that emission spectra is let us assume that somebody's given you the dimension spectra which is the correct emission spectra of that particular molecule. And let me draw for a molecule x the emission spectra.

So, this is the axis. So, let me use red color to draw that, nice emission spectra. And let me write few wavelengths over here let us say for this is for molecule x 15 just I named it like that way; any name any name does not matter. And let me write it like these way-

this is 450 nanometer, this is 470, 490, 510, 530 550, 570, 590; this is 430, so this is the intensity versus lambda so this is the emission spectra. And imagine that this is the actual emission spectra of the system.

So, you have taken this sample in your this sample holder and so your sample is over here and you use a particular excitation wavelength of your choice let us say this excitation wavelength. And in that supply it is also known that lambda excitation is equal to 400 nanometers, so let us you have used the 400 nanometer light to excite your sample. And obviously, this sample will be excited and you will get the emission. And now you scan this lambda emission from 430 to 590 nanometer. So, scan between 430 to 590 nanometer and you will get the different wavelengths coming to the detector which will going to plot it over here.

If you do not do anything and you just go and purchase a fluorimeter and do this experiment and simply plot it, I will show you how the plot will look like. Just let me change the color let me take this green. So, if you plot it what you will going to see is like this. If you do not do anything and just simply plot you will going to see it like this. That means, this is your measured one and this is your actual one; they are not same that is there is some problem in the measurement, but when I discussed this nice simple fluorimeter it seems that everything was alright. But here things are different because it is not matching with the reported one is the actual one what is the problem. And that is over here that the detector and the monochromator they are not ideal.

What I want to say is following: suppose I have this detector so light intensity fall on the detector and it will generate the voltage. So, if the intensity is high voltage will be high, if the intensity is low voltage will be low. But if the same intensity of different color will fall on the detector it must give the same intensity same voltage. That means, that if I plot the response of the detector versus the wavelength that should be a horizontal line in case of ideal detector.

So, for ideal detector the conversion if we generally called it conversion efficiency or quantum efficiency; so there is just how efficiently the detector can detect. So, just let me write this conversion efficiency versus wavelength that should be horizontal straight line, is not it. So, the efficiency of the detector to convert; a given intensity of light at 430 nanometer is same as 510 nanometer is same as 590 nanometer. So, here is my 430 as I

have written over here, here is my 430 let us say, here is my 590 let us say and here is my 510. So, the conversion efficiency for this light is this much, this light is this much, this light is this much and it is really constant all of are this wavelength. So, this is the ideal behavior of the director.

But, if you really see the real carve of this conversion efficiency of a detector that looks completely different than this, it looks something like this in this region. And if I extend it over here is 400, 300 then it is something like this. It means that when the light of 430 nanometer falls upon the on the photocathode of this TMT detector so the conversion efficiency is more than when a 590 nanometer light falls on this photo cathode. So, the efficiency is different. The difference is this much; the efficiency itself is different. That means, if the intensity of the incoming 430 nanometer light and incoming 590 nanometer light they are same still the detected signal by the detector will be different. The detector will tell that for have 430 nanometer light intensity is more than the 590 nanometer light, because that conversion efficiency is different. That means, I have to do something over here, I have to do something over here, I have to do some corrections over here. And how will going to do this correction we have to discuss.

Again, let us take this monochromator. So, monochromator pass the light, but that throughput of this monochromator is also wavelength dependent. The throughput of the monochromator is also wavelength dependent. So, for some light the throughput is more, for some light the throughput is less. That means, thus the true spectra over here over this region just up the after the sample. And the detected one they are different. So, I have to do something to correct it. This spectra here and the spectra here, because this monochromator and this detector they actually distort the spectra. So, I have to do something for the correction.

So, will going to discuss how will going to correct it. Now let us see this light source, the ideal light source.

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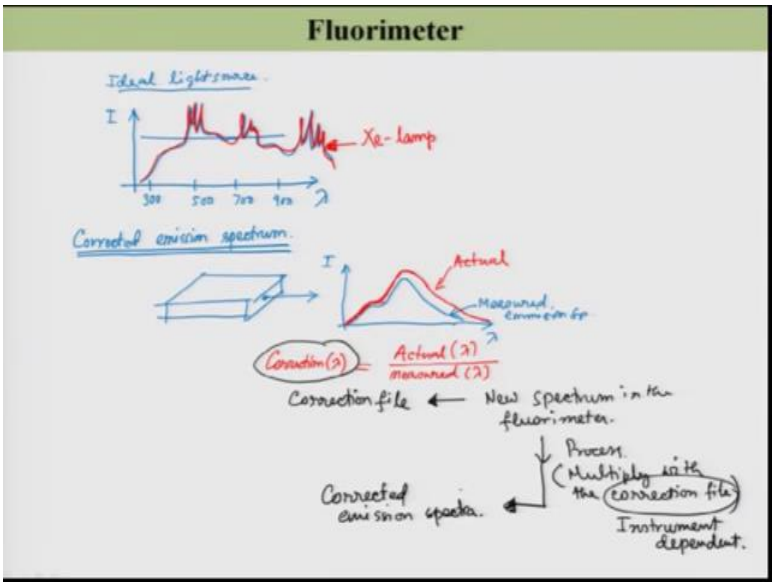

The ideal light source is a source where the intensity of light remains constant over the entire wavelength region. So, if I now plot here the intensity versus wavelength, so I should get something like this for the ideal light source. But for the real light source this looks like something like this. Here is your 300 nanometer, this is your 500 nanometer this obviously 700 nanometer, 900 nanometer. This is the typical of both the color are same. So, let me change the color over here. So, this looks something like this.

So, this is the typical light source for xenon, typical spectrum of a xenon lamp. So, why this words shape is disturbing for us, this is because when we talk about the excitation spectra as I said in that case what you usually do we generally fix the emission wavelength and scan the excitation spectra. So, if the excitation light intensity varies with wavelength then for high for the stronger wave length; for the stronger intensity the fluorescence intensity will be stronger, because intensity depends on concentration as well as excitation light intensity. So, eventually what will going to get is kind of shape. So, what we have to do we have to correct this excitation light spectra for the estimation of the excitation spectrum of a molecule, is not it.

So, let us try to do it stepwise, first let us see how will going to get the corrected emission spectra. So now, my aim is to get the correct ted emission spectrum. So, if you simply measure in one fluorimeter that will not going to be correct emission spectra, you have to do something to get this correct emission spectra. So, let me give you one quick

solution for that. Let us say that you have this fluorimeter in your lab. So, this is your fluorimeter and from here you will get the emission spectra of a known molecule. So, this is the measured emission spectra. So, this is this is a measured emission spectra. And you already know the actual emission spectra of that molecule, but if it is known and you already have that. So, this is your actual emission spectra of this molecule.

So, what you can do simply? You can generate one wave by dividing actual by measured. So, if you do like this way actual by measured what you will get; you will get obviously at particular lambda; so is a lambda, this is a lambda. So, you will get the correction file. You will get this correction which obviously depends on the lambda. So, in this case; at this wavelength let us say here it is very very very is overlapping. So, in this region this value will be near to 1 and in this region this value will be better than 1.

So, what you will get, you will get a correction term as a function of lambda in this case. That means, whenever you will going to measure some spectra you simply have to multiply your spectra with this correction file right. So, in this case what you will going to get is correction file and whenever you will going to measure the new spectrum in the fluorimeter you have to process it; process means multiply with the correction file.

Note that this correction file is specific for your instrument: for my instrument the correction file is different than your instrument, for his instrument, for her instrument. Because, the detector, the monochromator used to develop this instrument is unique so the two detectors are not identical. So, the correction file for my instrument will be different than your instrument.

So, this correction file is instrument dependant. So, once you multiply with this correction file then what you will going to get is the corrected emission spectra. So, will stop here and the more on this correction on the next lecture.

Thank you very much.

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Lecture 9: Summary

- The most basic fluorimeter consists of four major components-
  - Excitation light source
  - Excitation monochromator
  - Emission monochromator
  - Detector
- Conversion efficiency of an ideal detector and throughput of an ideal monochromator should be constant for all the wavelength
- Intensity of an ideal light source should be constant for all the wavelength
- Non-ideality of the components affects the recording of emission and excitation spectra in a fluorimeter
- Determination of correction file for each and every fluorimeter is must to record a correct fluorescence spectra from the fluorimeter