Basics of Fluorescence Spectroscopy
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Lecture – 04

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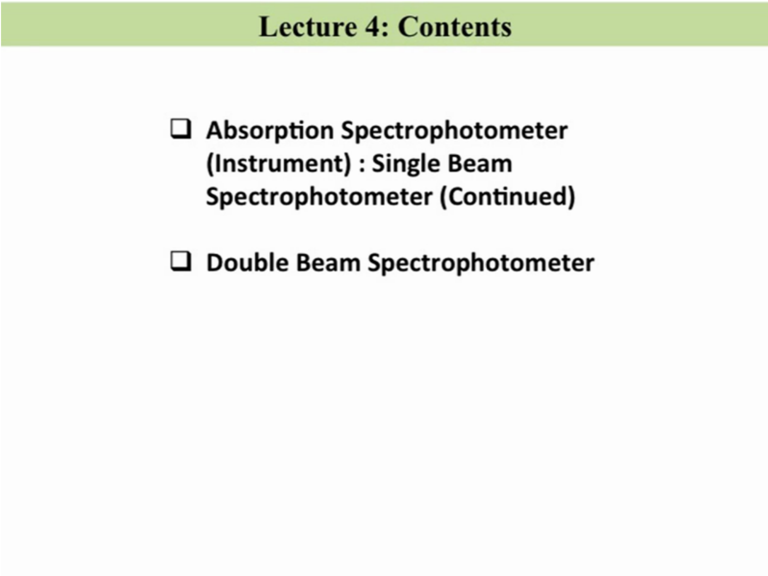

Welcome to the fourth lecture of the course Basics of Fluorescence Spectroscopy. In the last class, we are discussing about the instrumentation of absorption spectrophotometer the device which is used a preferred to measure the absorption spectra of the sample.

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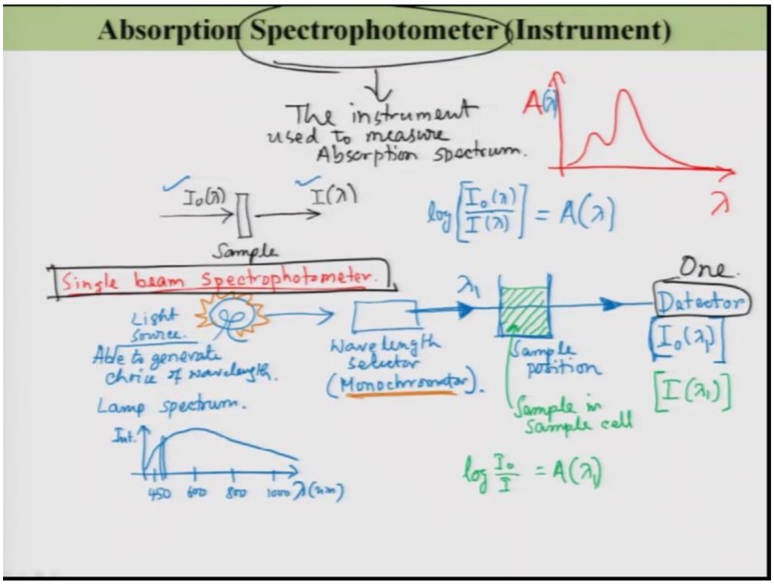

And we have started with the single (Refer Time: 00:37) spectrophotometer as I was doing in this particular place and as you remember I said that my aim is to measure this I 0 I which is a function of lambda that is it and it once I know the I 0 by I, I can simply take this ratio I 0 by I and then I will take this log of that which is a function of lambda right and with this what I will going to get is A; obviously, is the function of lambda I will just simply brought a function of lambda versus lambda I will get that from spectra. So, my aim is to determine that I 0 and I as a further particular value of lambda particular value of light particular color of light.

So, I have started with this normal lamp tungsten lamp in this case and in this tungsten lamp. So, this is; obviously, glowing and as I mentioned last day that the wavelength of light emitted by this tungsten lab is really white starting from 450 to 1000. Now if you use a general lab you can go to the e v region e v is a deuterium lamp you will get e v region also. So, depending on the choice of your wavelength you will choose your own lamp and now; obviously, you do not want the all the lights to come and interact with sample because you want the wavelength a dependence of the attenuation of the incident light I 0 2 I which depends on the wavelength. So, you use a device which is called the monochromator which is nothing, but the wavelength selector and just as you have seen in that high school that prism can divide at the component of life height light to this spectrum.

So, this device is simple like that it based on the gratings and you can select the wavelength of light of your choice right you using this monochromator. So, if you choose 500 nanometer light to come from this monochromator only 500 nanometer light; obviously, with some bandwidth will come out of this device if you want it is no not 500, but 600 nanometer. So, 600 nanometer light will come out of this device with some bandwidth nevertheless. So, with this light right what you got is I 0; that means, you need to measure the intensity of this light right as desired.

So, this is my sample position right the sample position sample position and I incident this light of this particular lambda of your choice and pass through right and here is my detector which detect the intensity of this light. Now if I do not have any sample in this position; that means it is the blank one then the signal intensity detected at this detector will be I 0. So, I can get the value of I 0 easily for this particular lambda right. So, I 0 for this particular lambda let me name it as lambda 1. So, lambda 1 correct and here is like this I 0 for this lambda 1 now let me add sample right over here. So, now, I add this sample over here. So, I have added sample in sample cell right then the intensity right of this light of wavelength lambda 1 will no longer be I 0 because some of the light will being absorbed by the system.

So, let us that intensity is I for this lambda one. So, simply we will get I 0 and I once you will get I 0 and I will be able to calculate A. So, just to take log I 0 by I which is equal to A, for this particular wavelength lambda 1. So, we looks very simple looks very very much fine, but there is some problem with such kind of setup. First of all that lamp what I showed over here this particular listed the tungsten filament lamp so the output of the lamp for this particular wavelength lambda 1 if I now may plot the output intensity of this lambda 1 from this tungsten filament lamp as a function of time if it is like this. So, just change the color yes here so the intensity versus time of the lamp output.

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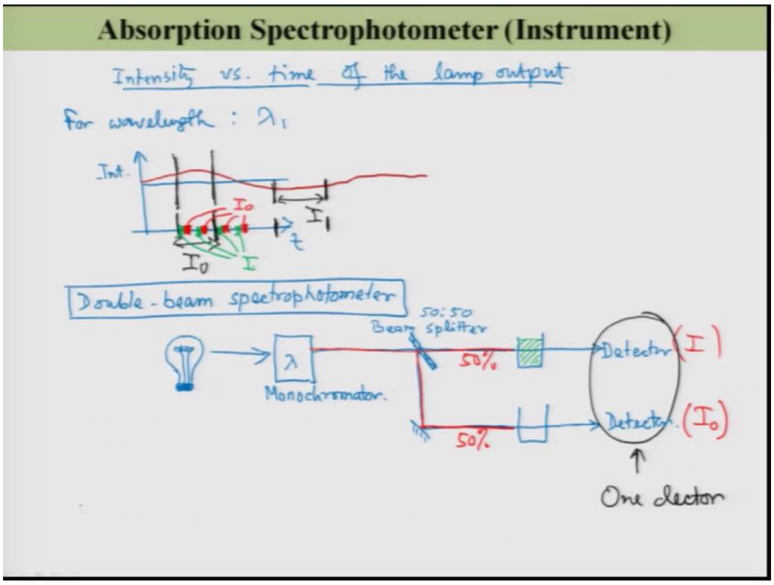

So, the wavelength; obviously, that wavelength I have already selected lambda 1. So, this for lambda 1 wavelength for wavelength lambda 1, I am going to plot the intensity versus time this time is my real time right. So, intensity if remain constant then there is no problem with my previous setup; however, this is this is not like that the real intensity profile along this time axis is something like this is something like this the intensity starts from here may increase something decrease like that way now consider you are measuring I 0 at this time, you are measuring I 0s at this real time window here you are measuring I 0 and you are measuring I when the lamp output intensity is little low here you are measuring I in this real time window.

That means, even the sample will not going to absorb a light of lambda 1 still here you will see that there is some observance or o d for this particular sample; that means, this is the error in the measurement and these error in the measurement comes from the fluctuation of the intensity of the spectral lamp right. So, we need to take care of that otherwise the measurements will be erroneous and very much erroneous.

So, people have developed this particular setup is known as a single beam spectrophotometer e this is a very primitive set up for the spectrophotometric measurement and. So, where shall I write here you know I have written? So, single beam spectrophotometer because there is only one beam; however, this particular problem of this spectrometer what is the problem I say that is the intensity actually depends actually is a function of time is there the intensity is not stable right. So, although there is no change in the intensity supposed to be, but because of the intensity of the spectral lamp is changing. So, that could lead to generation of some absorbance of the sample itself.

So, instead of this single beam spectrophotometer what you can do we can do this double beam spectrophotometer. So, in case of double beam spectrophotometer, this is my double beam spectrophotometer how is going to solve this problem I can easily solve this problem if I measure the I 0 and I simultaneously. So, whenever I am measuring I 0 I am measuring I whenever I am measuring I am measuring I 0 right if I can do that then this fluctuation of this lamp intensity will no longer be a problem right. So, simply what I have will do I will do I will I will do something like this. So, let us say this is my spectral lamp right and this emits light and this light is being selected by the desired wavelength is being selected by this monochromator you can set which lambda you want right and then I will do something where by which these light will be divided into 2 parts.

So, I will use a some optical element over here this optical element is known as beam splitter beam splitter by which this by which this light will be divided into 2 path then I will use a mirror over here and this beam splitter is such that just consider this beam splitter is such that it will split the intensity 50-50. So, 50-50 beam splitter half of the intensity will come in this direction half of the intensity will come in the other direction now you could have your sample sale over here and here nothing right you could have your sample over here and here nothing the color of my sample was just for the consistency here is your sample right and here is nothing.

So, if I now use a detector over here this is one detector this is another detector then in this detector I will going to measure I add this detector I will going to measure I 0 that is it. So, here I will going to measure I and here I will going to measure I 0 and now I can take log I 0 by I l get the A, but here also there is some problem the problem is that the sensitivity of these 2 detector cannot be exactly same if they are not then there will be a problem in the measurement of this I. Even if I said and it is true for example, there these light the intensity of these light is divided in equally in this 2 different path this 50 percent these direction another 50 percent the other direction right still because of these 2 detector. So, take my word granted no 2 detector in the world behaves ideally or exactly similar fashion. So, the performance of this detector always be different than the performance of that detector.

So, in this case probably then I should have I equal to I 0, but you will never get this I equal to I 0; that means, this is the problem of the detector and if time permits and also if it is I found it to require for this course later on I will discuss a little bit about this detector function, but right now let me just limited my discussion by saying just that the 2 detector response will be different they cannot be equal to same in most of the cases.

So, what I have to do I have to somehow make a different design so that these 2 detector will be replaced by one detector. So, what you have seen for this one detector this is only one detector one detector right in this case what you have seen that there is a problem in the fluctuation of the lamp intensity right lamp intensity so now, if you will have come up with varieties type of design. So, I need another page over here. So, the new design what we will going to have is something like this.

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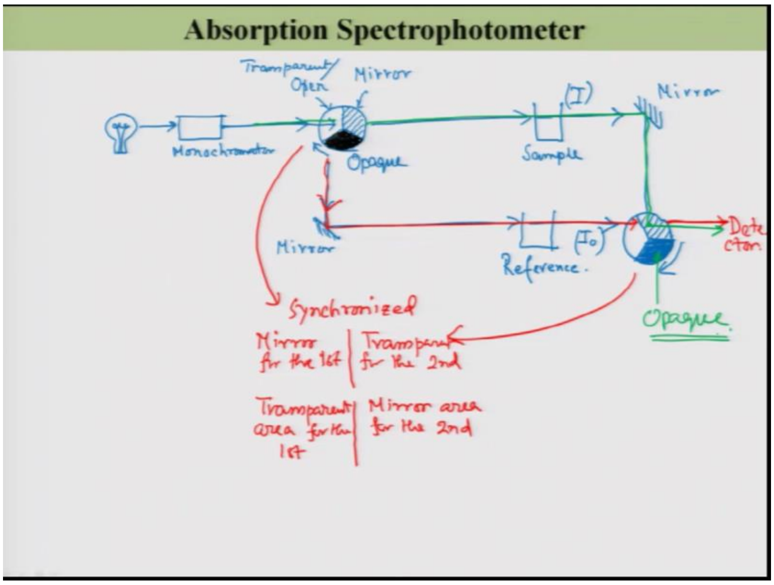

Now, I have this lamp over here now I have wavelength selector and from here instead of that beam splitter let us have a nice arrangement which looks something like this like this and here one part of this is mirrored. So, this is mirror right let me write over here this part is mirror this part is blank. So, nothing is there open. So, this part is open and this part is opaque complete opaque black color this part is completely opaque.

So, when I set this optical device one divided into three part one part is mirror part another part is open part and another the third part is the just opaque part right and this device is set in such a way that this is in the rotating condition. Some arrangements some mechanical device has been used to make it rotate and so as you understand whenever this incoming beam will fall on this transparent or open part right this is the transparent or open part transparent or open part it will pass through that is it and now here I have my samples over here sample right and then it will pass through the sample; however, whenever this incoming light from this monochromator will encountered the mirror part because this is a mirror system right this is a circular device. So, it is rotating like this way. So, this is the incoming beam sometime. So, this is let say opaque part this is the mirror part and this is the your transparent part. So, when it touched the mirror part it will be reflected back and when it touch the transparent part it will pass through right.

So, when it touch the mirror part let us say we have made the system in such a way that it will be reflected along these direction and then I have one another set of mirror over here and it will pass through a place where sample is not present; that means, this is my sample and this is my reference right this is my reference. So, that means, this is my I 0 and this is my I. So, this is my I 0 and this is my I.

Now, I have to combine these 2 somehow to get this I and I 0 be detected within the same detector because there was a detector problem I said. So, now, you use another mirror over here somehow. So, these are the mirror let me write for you m i r r o r this is also the mirror. So, you reflect this light right not good in this direction right and then you use another such a device where one part is opaque another part is mirror and another part is transparent right like this and you set it the mutual rotation of these 2 our mirror system the circular mirror system is synchronized in such a way that these 2 rotations are synchronized in such a way that when this one is mirror for the first one; that means, this light will be going to reflector like this it will be transparent area for the second one transparent for the second one right. That means, when this light will be reflected in this direction right hope you will follow here light will be reflected in these direction then these light will pass through this transparent region right and will be detected by this detector now it is detector by the detector. So, we will be able to measure the intensity.

Now, when this will be open or transparent area transparent area for the first system this will be the mirror area for the second one. So, mirror area for the second one; that means, when it is transparent let me take another corner over here to when it is transparent; that means, it will go through this system it will pass through the sample and then will be reflected by this mirror and will be detected by the detector right. So, for this in this case we will be able to measure this I; here these 2 these rotating mirror systems are rotated in high speed. So, that in this graph right, in this graph if you are measuring I 0 the measurement window of in real time the measurement window of I 0 and I are really small and you are repeating this measurement many many many times, for example, earlier for the single beam spectrophotometer these was I said now these was your measuring window measuring window for I 0 is this much and I was like over here right, but in this case what will going to do is going to measure I this is I over here measure I then again you will measure I over here again you will measure I over here again you will measure I over here and just next to it you will going to measure I 0.

So, for example, you will going to measure I 0 at this position another I 0 at this position another time I 0 here again I 0 over here and something like that. So, these are here these are these, these, these, these, these, all are I 0 and this one, this one, this one, this one, this all are I. So, I can measure this I and I 0 in such a way that although there is some drifting in this lamp output they will be compensate with each other because I am measuring in many many many many times over here and if you notice over here I will going to have another region which is this opaque region. So, these also important parts of this opaque region and with these we can actually minimize the error in the measurement of I and I 0 because with this we can actually eliminate the dark count of the detector dark count of the detector.

So, let me stop over here for today and in the next class will go in to discuss little bit of the application of this absorption spectroscopy and then will proceed to the fluorescence spectroscopy feature.

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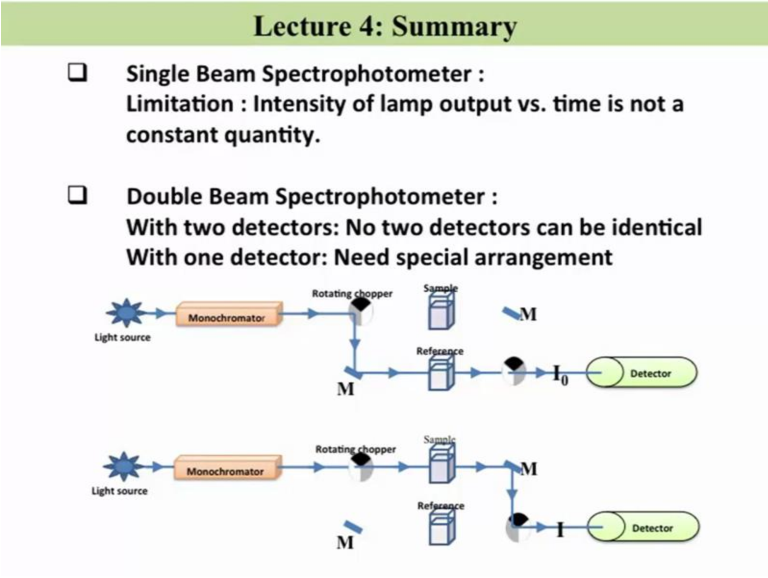

Thank you very much.