Optical Spectroscopy and Microscopy
Prof. Balaji Jayaprakash
Centre for Neuroscience
Indian Institute of Science - Bangalore

Lecture – 57
Fundamentals of Optical Measurements and Instrumentation

Hello and welcome to the next lab session on the optical spectroscopy and microscopy course, we saw so far about how to align the laser, how to get the laser light into the fiber optic device or a fiber actually, in an optical fiber. Now, in during that time I had actually told you that we would replace that fiber optic with florescent solution to with the hope of showing you a small home-built fluorescence spectrometer right, laser induced fluorescence spectrometer.

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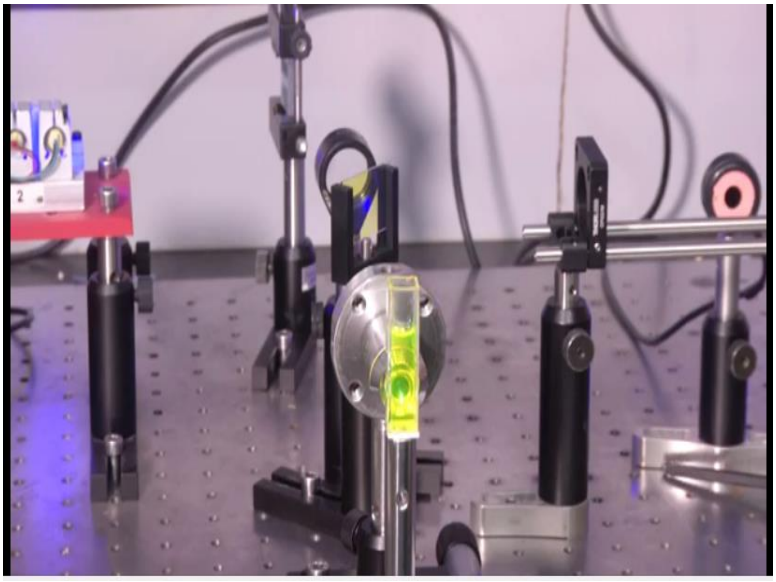

And let us go ahead and see how we actually do that now, just a quick recap; the light source we have; 3 light sources, 3 laser diodes we are operating only the 1; 470 nanometre and that blue light is coming through here hitting this mirror, hitting this mirror and one extra part that we have kept here which is the neutral density filter actually, it reduces the intensity so that it is in a range that it is good enough for us to actually detect and see things.

And this until here there is no change at all and even here there is not much change, what we have is a 2 lens system to actually expand the beam alright, so we have seen this in the morning except now, in the morning what we had was that another lens focusing onto an

optical fiber. Now, we have taken of that lens instead we have a new optical element coming here now, this new optical element is a dichroic mirror, okay.

In the dichroic mirror again, we have seen it in the class, so it has a special property we also talked about it in the morning when we were talking about the laser source there alright, where I told you that the first laser, this mirrors have a special property of transmitting this colour while reflecting this laser colour and this laser colour. Similarly, now I have here used a dichroic which will, which is going to reflect the blue laser that 470 nanometre laser.

And that laser light can be focused using a lens, we will come to that in a minute but it is going to reflect the blue laser and transmit the red shifted right, the stroke shifted green fluorescence alright.

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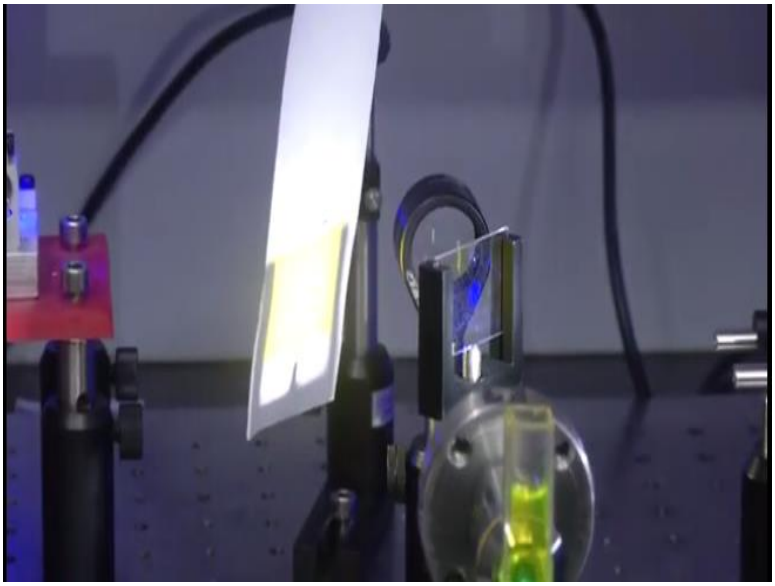

So, just to demonstrate that let us do this which is I have here a white light source right, pretty much a white light, it is a; you can see that white spot on the card here right, it is pretty white. Now, what I am going to do is; I am going to actually move it and then shine it on the dichroic, when I do that first thing you notice is that the colour of the light that is actually transmitted right that is pretty orange alright.

So, the orange colour gets transmitted, so the white light, so it is all white right, it is only from here, the transmitted light is orange, so in the shadow of the dichroic you see that it is orange in colour okay. On the other hand, if you actually look at the reflection okay, the way

I do the reflection is that now, what I have done is that I have yeah, so now you can take a look at the blue light; the card here now, the reflection is blue right.

This is exactly what we want, we actually want the blue excitation light to be reflected onto this pathway while the fluorescence; the yellow, green fluorescence to be transmitted.

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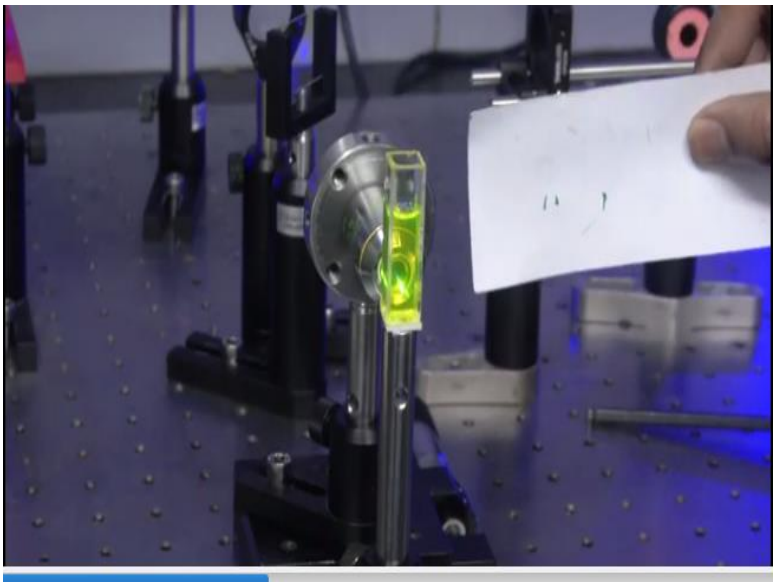

So, now for us to generate the fluorescence okay what we need is that this light coming out right so, the nature of the light that is coming out is collimated right, because we have not put any lens here, so it is a parallel beam of light that is entering into this objective okay. This is an objective lens and we have seen this in the class right, in order to know about the objective lens what we need is; we need to look at these scriptures that are written here okay.

And unfortunately, you have to take it from me; it is 10x 0.25 numerical aperture and air objective. So, what is going to happen is that it is going to start focusing the light, so what we saw is that the thin beam of light going backward and forward. A few things to notice there; one is that the thin beam is not a spot but it has a length, when you are seeing it from the other direction right, in the z direction right.

The direction orthogonal to the propagation if you see, you see that the thin beams stays in for over a longer period of time and that is our Rayleigh range, we have seen in the class 2. Now, what we have created is the fluorescence coming out from the fluorescein solution which is excited by this objective lens, the same objective lens collects backs this fluorescence we should and then becomes collimated travels through this dichroic.

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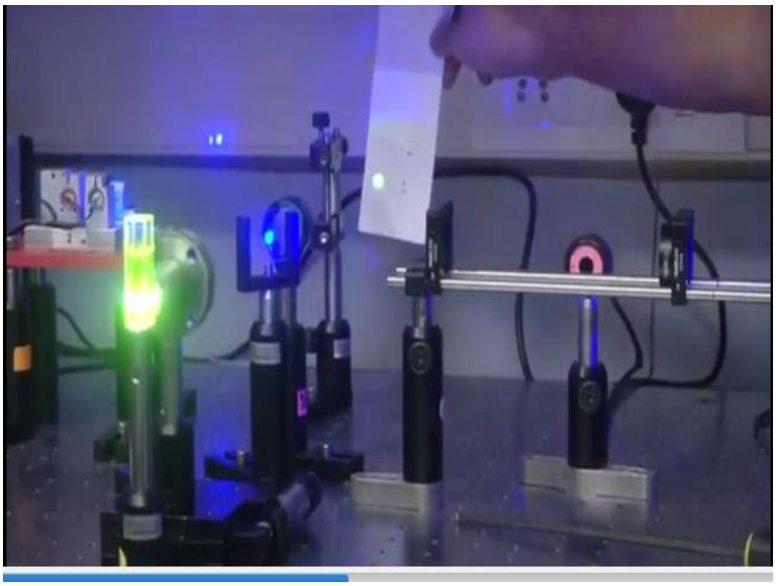

Remember, this dichroic transmits the greenish yellow light, right and it transmits the greenish yellow light, you have a lens here, this is the same lens that we had it in the morning here alright, now that lens is about 35mm lens. So, if you actually look at, you place a beam we expect I mean, going to increase the power laser intensity and if you actually place a card you can actually start to see that the beam is now, the fluorescence beam is actually showing, it is in a green.

In fact, if you do it there that is actually the back we are getting the image of the back aperture of the objective because this is a phase objective you can actually see the phase ring, so I am actually moving it, so now this is the actual focus of the fluorescent solution, this is the image of the fluorescent solution, I go back; as I go back what I am actually seeing is the image of the back aperture of the objective.

Now, that back aperture is uniformly illuminated we have seen such kind of illumination before right, in the course we were talking about kohler illumination, it is the principle is exactly the same, so if you add to image this onto to your sample, you get a uniform illumination except in the kohler elimination instead of fluorescence, you have incandescent lamp.

Here, what we have is a fluorescent sample generating enough fluorescence illuminating the back aperture of the objective, okay you can see nicely coming in focus okay good. So, now that is good, just the colour of it tells you it is fluorescent but then we are; when I am; when

we were talking about the spectrum, we are actually looking for spectrometer, we are actually looking for a spectrum right.

A spectrum where in different intensities come in at different spatial distances, right as a function of spatial distance, you see the wavelength being different. So, now in order to do that there are quite a few things that you can do; one is by putting in a prism and then disperse it or using a grating and we are going to use grating and this is a special kind of a grating, it is called as a reflection grating.

So, unlike a transmission grating where you will see the grating structure in the transmission, what you will see here is that in reflection, the diffraction pattern, so let me introduce the grating and then show you how it is really works as a grating.

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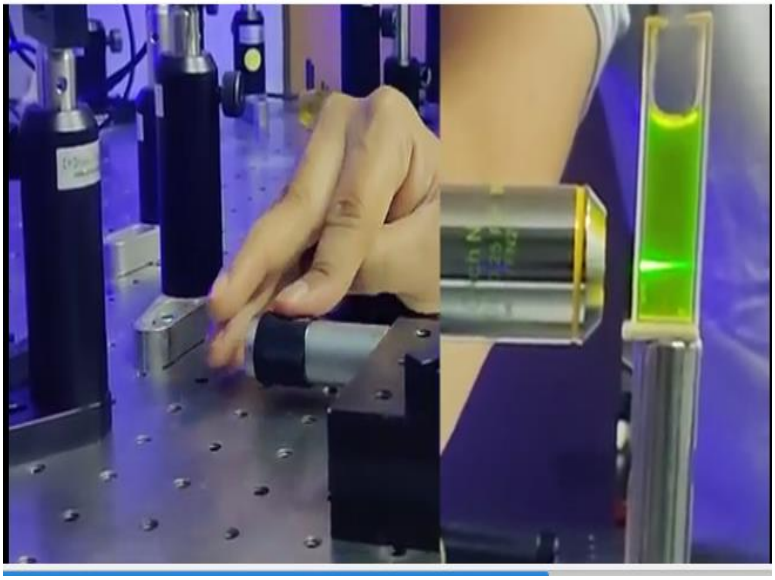

The way to look at that is we know that the 488 nanometre light right, or 470 nanometre light, this blue light that we are seeing is pretty monochromatic, so now what we are going to do is that we are going to put that onto the grating and then look at the reflection from the grating, there I will show you the 0th order which does not deviate at all, which obeys just like if the grating were to be a plane mirror, wherever the light would have gone that it follows the same path, you will also see in addition to that first order, second order and so on.

This particular grating is specifically glazed and tuned for 500 nanometre light but let us try to see I mean, the first order intensity will be maximum there but let us try to see here anyway how we; how it behaves with the 470 nanometre light okay, from here the light

entering through the objective starts to focus and you can actually see the wider beam towards this end of the objective becoming thinner and becoming, and then later becoming wider again.

Now, how do I know that; it is actually the focus, you can actually move the relative distance between the objective lens that is this and the cuvette, now if it is the focus what you are doing is you are taking it; when you take the objective lens back you are taking that thin beam closer towards that valve alright and okay.

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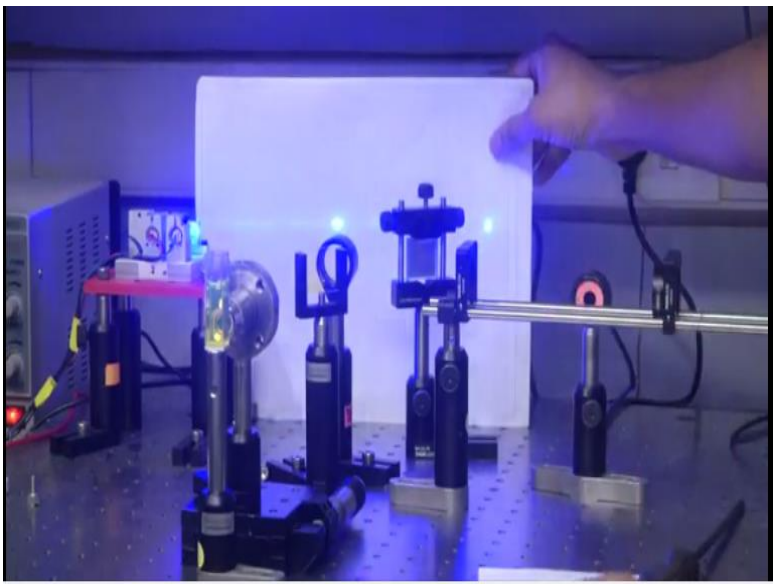

So, now what I have done is; I have placed this grating; diffraction grating, reflection grating on the path of the laser beam usually, if it is a mirror you would expect to see just that spot okay, nothing else however, what you see now is the first orders on both the sides, so you can see that they are slightly more intense than the centre beam and you will see this very nicely and distinctly when we look at not the monochromatic light but like the laser.

But actually, the multi-colour light like a fluorescence when we do that, what we see is that we will be able to separate the fluorescence that is originating from the solution in will be able to separate them into multiple different wavelengths, okay.

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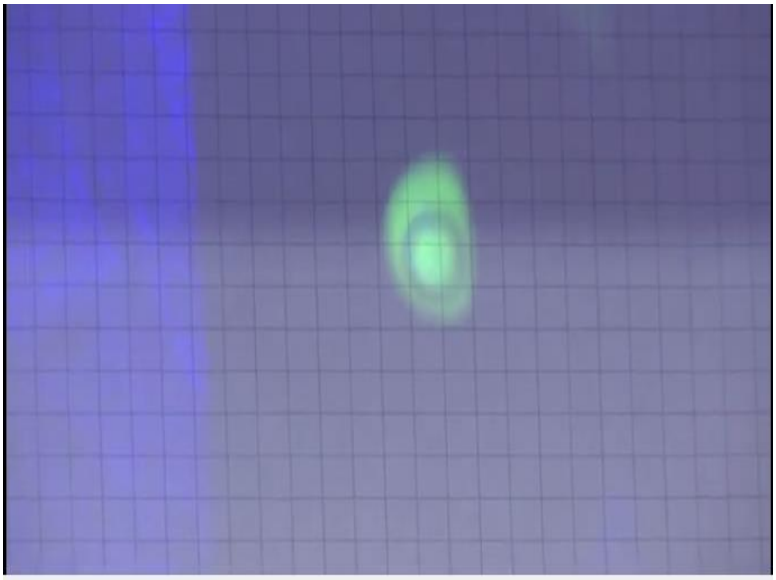

So, now let us go ahead and let the beam inside and I am going to move the grating away from the beam, so now we can actually see this fluorescence nicely, alright and in fact, so that is our focus spot okay. Now, what I am going to do is that I am going to place this, the reflection grating we are going to come; jump onto the other side and then place the reflection grating right here.

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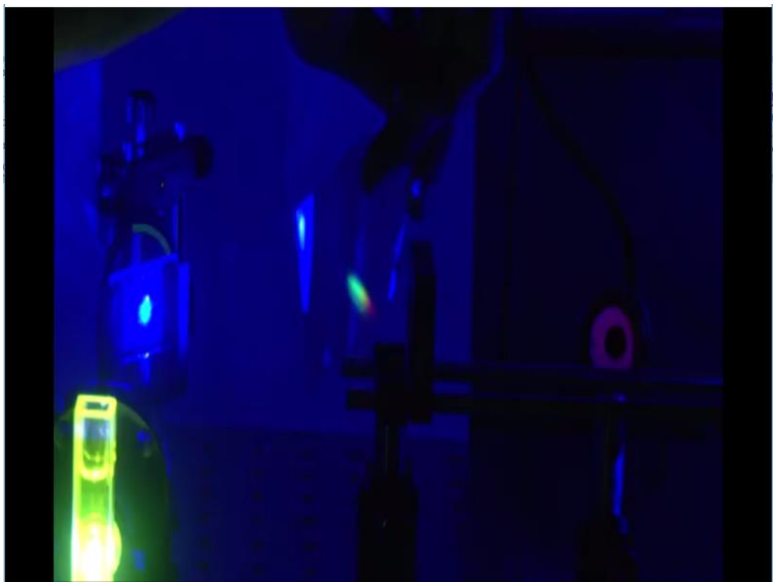

And then show you from the reflection that there is a beautiful spectrum okay so okay, so now we have kept the; so we see the incident beam goes through nicely and then there is a dichroic and we have the fluorescence, now that falling on the grating, now the when you see the grating the first order right, so right there you see that green that is the first order that is focal spot, this is exactly what we saw in this direction.

Now, we do not see it because it is a reflection grating, so it is reflecting there and in fact, now if you see you can actually get the back; the image of the back aperture, right and this is now we; if we remember what happened with the blue light was that displaced in angle was the first order and if you look at the displaced angle, so if you actually look at this now, you see this beautiful spectrum red down the right and left you have the green.

And now, all I am doing is I am just rotating back to get the first order, so you could think of having a wider paper like that if I keep it like this, you can see I am capturing both the first order as well as the 0th order in the same paper, so and then the what you are seeing is the resolved spectrum. Now, this can be captured onto the CCD camera or you can actually put in a slit assembly and move the slit.

And then measure the intensity throughput as a function of space, so imagine this being one of the slit blade and all that you are doing is you are just moving it across and measuring the total intensity, now that should give you the amount of light as a function of different wavelength. So, now that is the simplest spectrometer that you can think of; the emission spectrometer that you can think of laser of the; laser induced fluorescence that you can think of.

Then, what we have actually done is that we have done this in the lab in a completely open setup and be able to show you that it is very simple and easy to follow okay. Now, this is done through 1 photon excitation that is why you see this fluorescence as a big streak in fact, if you see it from this side is nicely on 1 little spot which is wider but on the other side, you can see it as a streak of line.

And later we will see, if we do the same thing in a 2 photon, it is a very, very sharp and localized fluorescence and top of it, you will see that it has some characteristic feature that requires the laser to be pulsed okay, these 2 things we will see in the forthcoming classes alright, thank you.