Optical Methods for Solid and Fluid Mechanics
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Lecture - 13
Light Matter Interaction II (Lab Demostration)

Hello and welcome. So, today we have another lab session and as a follow up I would like to discuss some of the operating principles that we are going to; we will see in the lab. The previous session we saw imaging at the macro scale with a very commonly used setup of a DSLR camera and a lens combination. Today's slab demonstration is going to be different. We are going to image at the micron scale.

Now you might ask really is it required for fluid mechanics work to image at that scale? The answer is yes if your object of interest is let us say a microfluidic device or a lab on a chip device. So, in microfluidic systems you will encounter channels; fluid flow channels which are of the micron scale 100 microns, 500 microns or even less and you often are required to see the fluid flow through them.

Especially in the field of biology microfluidics is often a go to tool for imaging flow around cells, flow around or movement of cells. In that particular in such a case when the object becomes very small you have to use an appropriate optical technique which often is the optical microscope. Now in the lab you will see an inverted microscope.

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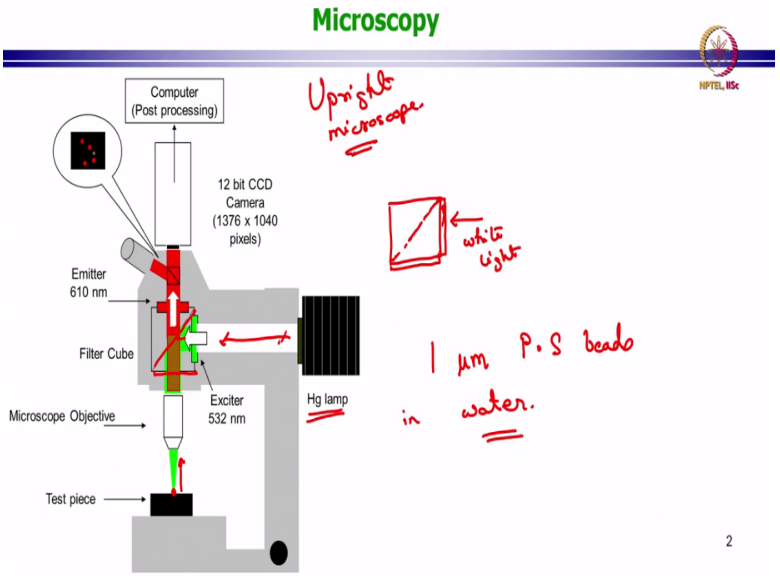

Here I have drawn an upright microscope. Now the upright and the inverted microscopes are different only in the way the lenses and the optical system is placed with respect to the to the object, but the operating principles are more or less the same. So, that is why for completeness I wanted to show you how an upright microscope looks like and what I am going to show you here is the principle of setting up the optical pathway for let us say a fluorescent imaging.

So, what we have here is some sort of a lamp this is nowadays you also have LED lamps and other lamps. In the olden days when I was a student we used to use mercury arc clamps. So, that is why it says a (()) (02:23) and it produces some sort of a white light or a basically light electromagnetic radiation in the visible spectrum over a large wavelength range and the light approximately looks white some sort of a white light is drawn here.

So, this goes from my lamp which need not be just the mercury arc lamps it could be other lamps LED etcetera and then it goes and here what we have is an optical it is a filter cube. Now the filter cube I will come back to why the use of the filter cube but the idea behind a filter cube is rather simple. You have a box in which you put a filter where your illuminating light is coming in.

Then here you will have some sort of a mirror usually not a 100 percent mirror and then you will have another optical filter right here. So, what we want to do is some sort of white light is entering my system or rather a light visible light with a lot of different wavelengths is entering the system and in fluorescent imaging you usually want to illuminate the object with only a very specific wavelength.

So, in this case we want to use let us say green light to illuminate. So, what we can do is we can put a filter which will allow green dominated light to go into and it will cut out all the light. So, you see white light enters and becomes green here then it goes hits this mirror. This mirror is such that it reflects the green light. So, this green light goes through my lens and here whatever the object of interest is it hits that.

So, this green light comes and hits my object then this object emits red dominator red light and this red light travels back through this path. Now as it travels back we have a filter cube sorry the dichroic mirror in the filter cube which lets this red light pass. So, this mirror has

been specially designed such that when a green light hits it reflects, but when the red light comes it just lets it through or red dominated light through.

And then finally we have one more an optical element here which cleans out the light such that you only have wavelengths that are close to the emitting wavelength back into my camera. So, this finally once this light enters you have these are some of the cameras we used to use. So, we had a 12 bit CCD camera in our lab when I was a student and this light goes and is recorded by this particular camera.

But you can have other type of cameras here or some other type of sensor also here to record this particular event. So, this in this type of microscopy this is one of the possible modes. The other possible mode is you do not have any of this you just have this light come in and go through. In this case you can have a reflected bright light microscopy in the upright case whereas for inverted microscopes you can have white light go and just strike this object and just pass through.

So, in that case you can have you can and the camera can be below the object and it can record transmitted light. So, in that case you can have a transmitted bright field imaging. So, in the lab what Praveen and Abhinit are going to show you is imaging of one micron polystyrene particles which I am just going to write it as PS beads in water. There is a reason we have chosen this kind of a bead. Now these particles are such that they have already been processed to be fluorescent.

So, when they are put under micro optical microscopy if you supply green light to them they actually appear red. Now you will often have monochrome cameras in the lab so the particles will actually not appear red they will actually appear white. So, unlike the demonstration in the previous class the DSLR camera was actually a color camera. In this particular case what you will have is a monochrome camera. **(Video Starts Here: 07:12).**

So, this is the first of the set of images that Praveen and Abhinith have taken and I am just going to play this video for you once. So, you can see these particles are slowly moving and this is your water. So, this background right here this is your water and all these are one micron polystyrene particles. These particles are such that they are neutrally buoyant in

water, there is no flow, but you can see that they are moving. This movement is caused by Brownian motion.

So, we will see that when it comes to creating flow fields from images of tracer particles the Brownian motion is one of the things that we have to watch out for and when so this after this imaging he changes the setup and goes in for a slightly different imaging setup, the microscope is still the same all he changes is a is a lens on the system and once after the lens he also changes some small other parameters in the optical train.

And then you end up having this image of the same system. So, this is still the one micron polystyrene particle in water, but now being image through a different lens the camera remains the same. So, you can see I hope you can notice the big difference. Here this is bright field transmission light microscopy and now you have dark field imaging and in this case this dark is now your water.

So, the water does not interact much and hence there is no signal there. All you have a signals from these particles that are moving. You can see some particles are nicely small and round whereas some other for example here you see this huge red circle. This is because this particle is far away from the focal plane and it has created this kind of a large structure and its also very much dimmer than the particles which are in focus.

So, this is a particle which is in focus in the focal planes so it appears nice small and round whereas all the particles which are moving away from the focal plane they start to have all these bright rings around them. This also reflects one of the differences between imaging and the microscale. In the third imaging he introduces a filter cube and he also shows the filter cube to you and this is fluorescent microscopy and I am just going to play this video again.

You see the same type of motion, but this is now fluorescent imaging. These are red fluorescent particles. So, there is a filter cube in the path of the optical of the light just like I showed you in the first slide right here there is a filter cube and then Praveen shows you that usage of that filter cube. Now the way the reason these are important is because you see there was very small changes that were made to the optical path.

But that totally changes the type of image that you receive of the same object. Let us analyze some of these images using image J. So, what I have done is I have taken these images and then I have analyzed it using image J software. So, what I did is I opened this image in image J then I drew this particular line in this particular case here right here and then I plotted the signal at this location using that one of the tools there.

So, now you can see this signal, you can see that this is sort of the average, this is the background. So, the background is quite high the gray value is around 160 and the peak here occurs at 220 which corresponds to this particle peak here and there is another one here which actually corresponds to one of the darker. So, this is also a particle, but it is slightly dark.

And this is just the way the particle is interacting with the light and the glass slide system. So, some particles are close to the glass slide some are far away from the glass slide and this particle light interaction changes the particle from white to black in certain cases, but approximately you have a particle is basically either a hill or a valley like this and this is your overall signal this is your background.

So, you can see that your difference here is about 40 is your difference about 60 and here your difference is slightly lesser this is maybe 100. So, this difference is about 50. So, you have in both the cases the particles are very easily visible, but the signal has your background has a high signal value 160 here and the particle peak is around 60 units higher than your background. Let us see what happens with the other image.

So, this is your dark field image. So, when I take a dark field image in this case I have taken a small line here and then I have again done the same thing. Now you see this is your background, this is your black background. So, in the dark field image I have taken a cut here and you can see that this is your background value and this is your particle peak. Now this background is black so the value that you see here is very small which is what you would expect your intensity value is very small.

And the particle center is almost saturated, it is almost close to 255 it is not exactly, but now this difference of your signal to the background is very high. So, this is close to some 250 or 220 so greater than 200 units is your signal and you can see you have a flat top right here in

the dark field image and you have this sharp increase for the particle. Let us do same thing with the fluorescent image and let us see what we get.

So, here in the fluorescent image I have again taken a cut and I have plotted the signal profile, this the background is still dark. So, you can see this is a very small value over here maybe some 10 or 20 or something like that and then your signal now has a large value where it is probably greater than so the signal has a value that is much greater than 100 over your baseline.

So, your particle is very clearly visible so your signal-to-noise ratio in the three cases are very different. So, just to repeat in your first case in the bright field imaging your base or your signal baseline was somewhere around 160 and the difference of the particle peak was somewhere around 50 or 60 units whereas in the same system you now suddenly have this in the dark field imaging you have this clear difference of 200 units in the signal over the baseline.

And similarly in fluorescence you also have a very large number here greater than 100 and your background is very well suppressed and black. You will also see that the three types of curves are different there is a reason for that and this has a flat top, this does not have a flat top and here obviously you also had hills and valleys both were representing particles. So, how the signal behaves in the three imaging cases is very different.

I am unable to go into too much depths about why the signals are the way they are, but you can theoretically predict this kind of a curve that is going to be out of the scope of this particular course maybe a course in optics can deal with it. So, I will not go into that but this shows you the difference or the benefits of imaging and how that affects very, very strongly image quality in different cases. We had the same object.

We had a same light source more or less in the three different cases, but the images were very, very different. So, with that I hope you enjoyed this lab session and it gave you quite a bit of experience with respect to how microscopy images can be very different from the microscopic images. **(Video Ends Here: 16:45)** and we will start off with flow visualization in the next class. So, thank you very much.