Optical Methods for Solid and Fluid Mechanics
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Lecture - 09
Lab Demo I: Optical Microscope

Welcome to the lab session for microscope demonstration. In this session, we will learn different parts of microscope and after that we will give a demonstration on how to visualize Brownian motion of colloidal particles using three different optical microscopy technique that is bright field, dark field and fluorescent. So, now we will know the different parts of the microscope. **(Video Starts Here: 00:40).**

This is a DMi8 Leica inverted microscope starting from the top. This is the transmitted light arm which we can push it back to have a better access to the staging area where we keep our sample for visualization. Coming down this is the condenser there comes the staging area which is fully motorized and controlled by this joystick then comes this objective lens turret then inside this there is filter cube turret this is the focus knob and this is the eyepiece.

In this objective lens turret there are currently 6 objective lenses which are mounted of magnification 5x, 10x, 20x, 40x, 63x and 100x. The objective lens look something like this. Now in order to see the filter cube turret we have to open this part and if we will rotate this we can see a filter cube. This shiny thing which you are seeing is a rhodamine filter cube and this filter cubes are highly sensitive to the light.

So, you are not supposed to allow them to be exposed to light for longer duration and this is the knob and the camera of this microscope is the on the other side of this microscope and in aligning with this knob if we will close this knob then we can visualize the sample in this eyepiece and if we will open this knob then the light will go to the camera and we can visualize the sample in the system.

And this is the joystick which controls the movement of this stage which is motorized. This is the x movement, y moment and this is the g moment which is used to focus the sample and here you can see four different buttons which are the speed controls for this movement along

the three direction. **(Video Ends Here: 03:28)**. Now we will move towards making a sample for the demonstration of Brownian motion. **(Video Starts Here: 03:40).**

We will prepare the sample using a Di water and red fluorescent polystyrene particle of 1.1 micro meter size. First, we will take around 1 milliliter of Di water and we will put this Di water in this tube which is of size again one milliliter then we will take around one micro liter of this red aqueous fluorescent particle solution and we will mix this with one milliliter of Di water.

Now in order to homogenize this mixture we will put it in vortex shaker for around 30 seconds. So, now the required sample is ready and we already have prepared a visualization chamber using two parafilm stripes and a square cover slip connected to them on a microscopic glass slide. So, this observation chamber is open from left and right side from where we will inject this solution.

Now we will take around 70 microliter of this prepared solution and we will inject this in this observation chamber while injecting this we have to be cautious that no air should be there inside this chamber otherwise there will be evaporation of the sample and which will not give any Brownian motion. So, now after injecting the solution we will cover the two opened site using UV curable glue.

And we already have prepared a sample which contains the solution of the fluorescent particles inside and which is covered from all the side. So, here there is no evaporation there is no chance of evaporation of the solution. Now we will use this sample and visualize the Brownian motion in the microscope using three different techniques. **(Video Ends Here: 06:30)**.

(Video Starts Here: 06:35) This is the chamber which we prepared earlier we will keep it on the stage of the microscope and we will keep the copper slip side downwards as this is a inverted microscope. So, after keeping the slide we will turn this knob on so that we can visualize this the sample using the camera on the screen and the first thing to do is to focus on the plane where you can visualize the Brownian particles.

And in order in order to focus we will first find the border of the parafilm which is a straight line. Now you can see this is not in focus. Now we will adjust the g axis such that we can see a sharp line of the parafilm stripes. So, now you can see a sharp line of this parafilm stripes which indicates that now this is focused. Now we will move to the center of the observation chamber to visualize the Brownian particles.

Now we will increase the magnification from 5x to 63x gradually 5 to 10x then 20x then 40x then 63x. Now here we can see the Brownian particles again we will adjust the g axis a little bit. Now you can see the Brownian particles undergoing Brownian motion and here we have different modes in addition to the bright field, dark field and fluorescent. We have already recorded the videos of this Brownian motion and we will show them now.

So, this is the bright field video which we just witnessed. In bright field microscopy the particle absorbs the light and creates a contrast from the background by creating a dark image in a bright background this is the concept of bright field microscopy and here you can see the Brownian particles the colloidal particles undergoing Brownian motion using bright field microscopy.

The dark images are the Brownian particles and their background is bright that is why it is bright field microscopy and this is the Brownian motion video using dark field microscopy in which the particles are reflecting the light by creating a contrast. The particles are creating a contrast from the background by reflecting the light and they create a bright image in a dark background that is why this is known as dark field microscopy.

And this is as you can see this is the Brownian motion is more clear than the bright field video and now we will move towards the third technique which is fluorescence microscopy. Here you can see that here the again the white dots are the Brownian particles. In fluorescent microscopy this is the white light source which we will turn on and we will allow the white light to pass through the dichroic mirror which we saw earlier in the filter cube turret.

For this particles we are using the rhodamine filter cube which consists of a dichroic mirror. So, this dichroic mirror will separate the 542 wavelength light from this white light source and it will allow it to reach the staging area where the particle will receive this 542 nanometer

light and the particle since these are fluorescent particles the particles will excite and it will emit a wavelength of lower energy that is around 612 nanometer of light.

And again that 612 nanometer light will pass through this dichroic mirror and reach the camera by which we can see the motion of this particle. So, in this demonstration we saw how to visualize Brownian motion of colloidal particles using bright field, dark field and fluorescent microscopic technique. **(Video Ends Here: 11:57)**. Thank you for watching.