Fabrication Techniques for Mems-based Sensors: Clinical Perspective

Prof. Hardik J Pandya
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore

Lecture – 43
Introduction to Equipments: Metallurgical Microscope

Welcome, in the previous module we started covering the basics of microscopy and how it is relevant to a course or a field in micro in fields of micro fabrication. We went through what are the different types of microscopes like optical electron microscopy. And then within an optical microscope what types of microscopes are available likes; stereo metallurgical inverted. I went up went over the details of each of these in a very brief manner. Then we went on to cover what the stereo move microscope is and how it looks like how what is it is utility and all.

Now, today we will be looking at a much more conventional microscope, this is called the Metallurgical Microscope; this is called the Metallurgical Microscope. We will look into it shortly the actual equipment, before we do that. When I told about when we discussed about the stereo microscope, we discussed we understood that stereo microscope works on the principle of reflection of light. But the metallurgical microscope works on the principle of transmission of light. So, we will have a sample and primarily works on the principle of transmission of light, but it can work on both reflection and transmission because, of its unique construction which we will go into. So, now let us actually look at the microscope and then understand what it is.

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So, we have the metallurgical microscope here. It is a very as you can see even first in first instance itself what you can make out is that; this is a much more complex microscopic arrangement as compared to the stereo microscope that we had seen last time. Before I go into the details of what are the different knobs features available on the microscope, basic thing let me introduce first. So, this microscope has both bottom illumination and top illumination.

So, there is a top light source here this black knob that is there at the back of the microscope, is the light source for the top illumination ok. And, there is an arrangement at the bottom where you have a bottom light source. Let me just switch on the bottom light source for you, see now you can see a light glowing here this is the bottom light source. And if I switch on the top light source, I think you might not be able to see it properly, but there is a top light source also. So, if you see you see look at my hand this is light coming from top also. So, these are the two illuminations that are there in the metallurgical microscope.

Now, why are they important? When we saw the stereo microscope the illumination was coming from top. So, what would happen in the stereo microscope that illumination that is coming from the top or the light source that is coming from the top falls on the sample and it gets reflected. And it is picked up further by the eyepiece, but in this microscope you have both options. Let us say so same like what we saw before we have another

sample holder also here, but this sample order is slightly more complex. It is engineered and machined for fine adjustments. So, it is there is a transparent platform there is kept you can keep. If your sampled is transparent let us say if your sample is transparent like a glass slide or a Petri dish or a tissue culture dish. Then you can keep it on top of this and your bottom illumination can actually pass through your sample.

So, that is what that is based on transmission optical optics, it will work on transmission optics. So, the bottom light will pass through after passing through certain filters and aperture adjustment equipment, it will pass through your sample and which will be picked up by your objective and your eyepiece. Now let me get back to a few basic concepts about microscopy the main that the two main optics arrangements for or an optical base microscope are the objective and the eyepiece ok. I hope you can see the objective arrangement here, so this is a circular this is an equipment that moves in a circular fashion. Let me switch off the light source for some time. So, this piece of part that is part of the microscope which is which we can which we see I am rotating it now. This is called the Turret, this turret can house multiple objectives.

Objective is basically your core magnification part in a microscope. The objective consists of a lens of a particular magnification which is connected to your eyepiece, as I as we have discussed before the eyepiece which you can see now here. So, the eyepiece is from where you see the samples the eyepiece provides 10 x magnification usually most of them. And this eyepiece also provides 10 x magnification if you look at the whatever is written on the side, I think you might not be able to see, but this is a this has 10 x magnification. So, the light either transmitted or reflected right, so the metallurgical microscope can work on both transmission mode and reflection mode. If we are using if we are using bottom illumination it will work on transmission mode.

If we are using top illumination like I showed, it will work on reflection mode. And the reflected or transmitted light from the sample is first captured by the objective. Here in this microscope or in any standard metallurgical microscope the turret will provide for having multiple objectives and connected to your eyepiece. So, eyepiece is fixed with 10 x magnification so let us see. So, you see there is a red band here on that this is one objective this is a one lens. If you look at the lens you can see that it is written LM plan 5x slash 0.15 and WD 10.8, WD is called Working Distance.

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See if you can see this 5x 0.15 WD 10.8, WD is called Working Distance it is like that is a measure of how close to the sample that you can get or how far away from the sample is it useful to use that lens; it means both. And 5x tells you what is the magnification that this lens can provide so this is the smallest usually this is the smallest objective lens that is provided on a microscope it will either be 5 x, 3 x, or 4 x. So, what happens so this 5x lens will provide 5x magnification, eyepiece already has 10 x magnification. So, effectively 5 into 10 you will be seeing your sample at 50x magnification that is the overall principle.

Now, suppose I adjust the turret, you can adjust the turret as you see the turret will move and gets logged on to the next objective that is there. Once it gets logged on you will can hear a click sound I do not know I am not sure whether you can hear it, but there is a click sound. And the next objective comes into position so, each objective is usually marked with a color because, as you use over time you will just look at the color and you will know that this is this magnification and this is another magnification. So, as we had seen the 5x has red color.

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So, this one the next eyepiece is 10 x magnification I think you can see that also written 10 x WD 12.2. So, that 10 x magnification eyepiece is yellow in color. Next one what we have is 20 x magnification you can see the next objective that is coming into position. It will keep coming into position because this is where you keep your sample. So, this is 20 x magnification so once you put 20 x, the 20 x 20 x into 10 x. So, it will it has it will give 200 x effective magnification.

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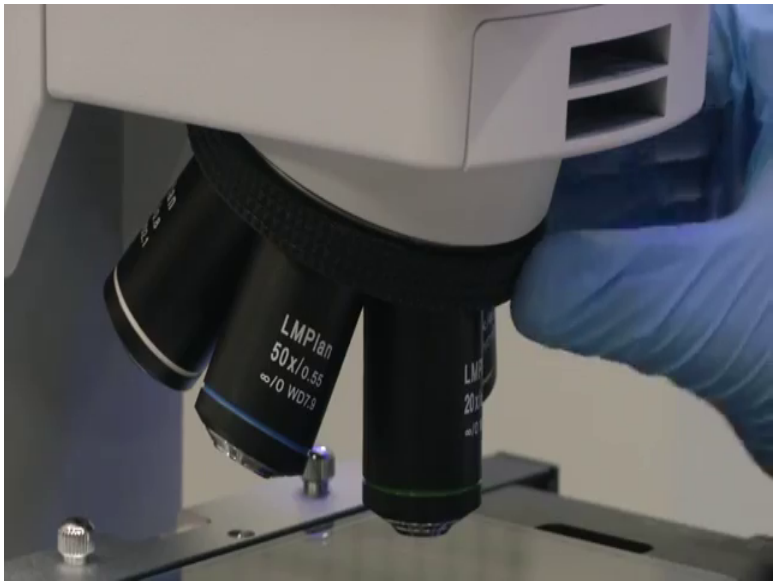

Then we have two more which is, 50 x here blue one and 100 x which is 0.8.

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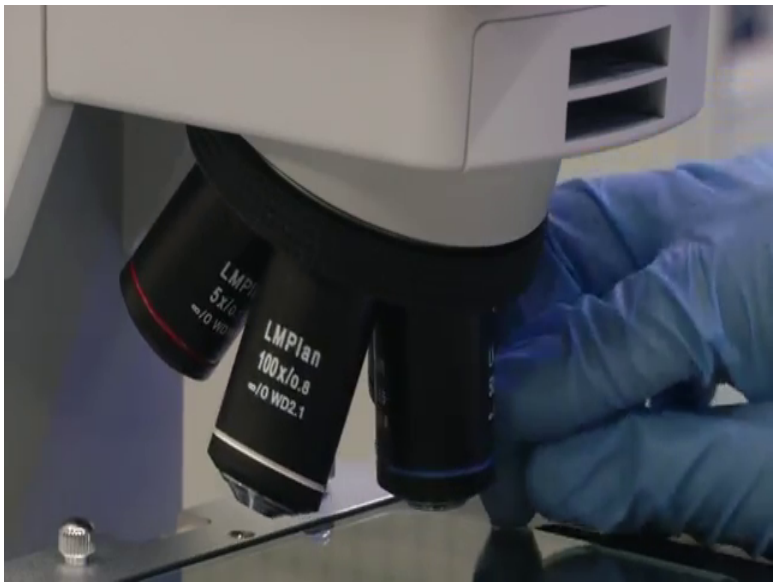

See if you see the working distance for each of these lenses is coming down as you come down. This is because, with a 100 x magnification you have to be very close to your sample for you to use that lens. So, that working distance will automatically come down that is why here WD is 2.1 and the magnification provided is 100 x.

So, let me recap so we have this turret which causes all the objective lenses that are used by the microscope. You can have multiple options and usually a turret will give for at least 5 what you called slots to insert your different objectives. So, here in our lab we have inserted 5x which is red in color, 10 x which is yellow band in color, 20 x which is green in color, 50 x which is blue in color, and 100 x which is white in color. These colors are only for your easy use during your day to day research life, you need not go and read each lens what is the magnification. So, once we fix once we decide what is the magnification that we need we will keep the objective lens in that magnification.

Insert our sample on the sample holder and then look at it through the eyepiece. Now the sample holder I think you can see the sample hold we had seen the stereo microscope before. Compared to the stereo microscope this sampled holder is a much more complex equipment. So, the just below the sample holder there is a knob where my blue gloves are I think you can see it.

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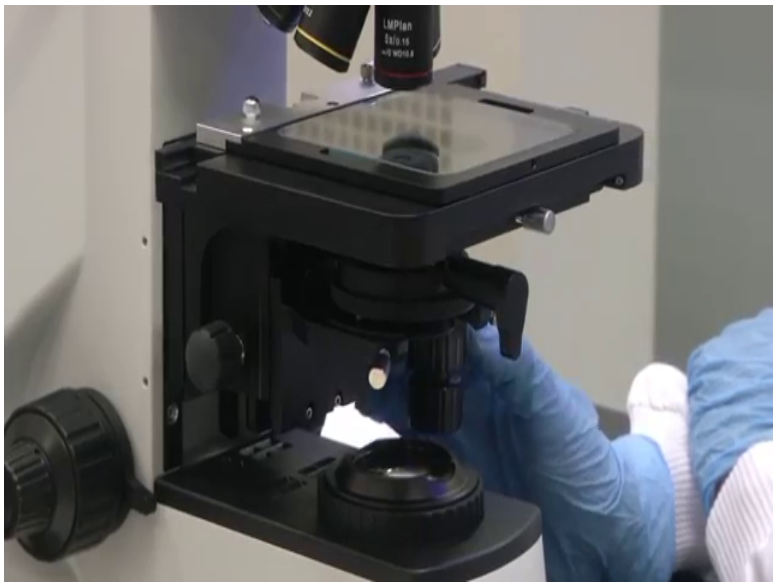

So, this knob is use for x y movement of your stage this is called Stage, the sample holder in a metallurgical microscope is called a Stage. So, you can actually if you adjust the knobs here you can actually move the stage I think you can see we will be able to see the stage moving. Now I am adjusting the x values so the stage is moving in x direction and I am addressing the y, y value now and it is moving in the y direction.

So, the illumination will be provided from bottom or top. So, at the at that point you cannot put your sample in the entire sample holder because that only one particular part of the sample will come under the view of our object of your objective or eyepiece. For that matter it is very essential that you are you should be a given the option to move your sampled around; that is how this x y staging or movement becomes useful. Next we have a few knobs that are below the stage, these are used for aperture adjustment.

So, generally in photography or let us say microscopy also there are few things that you look at like photography enthusiasts among you might know there is aperture there is expo exposure time the there is something called the F number. that is like that measures the focal length at which you are capturing the images. Similar concepts apply to microscopy as well

So, depending on how much aperture you want. So, what is aperture? Apertures refers to the what you call your hole the diameter of the optical light collecting mechanism. So, if you so this is there is a knob here below which with which you can adjust the aperture of

your objective with which objective that collects the light. So, with this you can adjust it and you can also adjust contrast through a ring face ring that is provided below. So, you can adjust the contrast and work with it. So, there are lot of options provided on the microscope like this and you need to overtime when you use it you will get to know the exact utility of each of these options.

Now, let us say let me get in to the basic knobs first; so as I showed you before this is the bottom light source. So, here you see a switch here so this is for switching on the bottom light source. So, let me switch it on it is just a rotation, I rotate it and the light source comes into picture I think you can see the light, see my hands are glowing let me switch it off now so it is switched off. Similarly on the other side you might not be able to see there is another knob with which you switch on the top illumination.

So, I have switched on the top illumination see my gloves and the top illumination is on. Now just like in the stereo microscope we have two knobs here these two knobs you can see just follow my gloves two knobs here this top is used for coarse adjustment of your focusing. So, as we had seen before the stage we can move x in x and y direction, but to focus you need to move your stage in the z direction. So, that your sample comes into focus with respect to the respective objective that you have used in the turret.

So, you can have coarse adjustment and once you know that your sample is reasonably in focus you need to make it sharper right. To make it sharper you have fine adjustment here this is used for fine adjustment of your focusing. We will see a sample shortly and know how this is done. Then this is the eyepiece this is the eyepiece this eyepiece you can move around like this so that it will suit your eye which what is the shape of your eye accordingly you can adjust your eyepiece. And then this eyepiece has 10 x magnification as you can see here 10 x is written here and whatever is seen by the eyepiece is also captured by this camera.

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This is the camera so this is the focusing eyepiece for the camera and this is actually the Sony camera. This camera is connected via USB into a system. So, whatever we are seeing we can actually capture it through a software that comes along with the microscope. So, if you see the screen if you see the screen.

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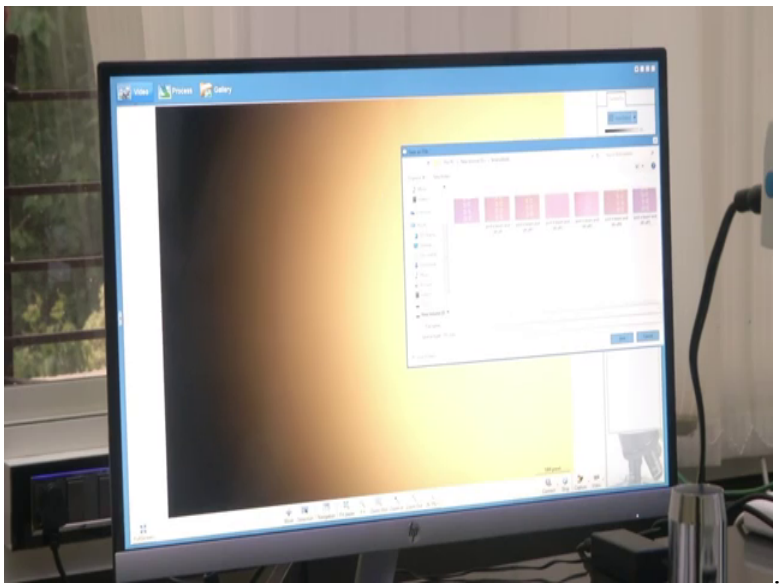

You have a software provided.

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If you see the screen you have a software provided to capture the images, is a very basic software you just see the sample under your view. There is a capture option here this is just click it capture to a file and then just capture it. And you can save it as an image like this in your desktop or in your drive. And you can you have options to can do wide balancing for your image and you can change your arch red green blue saturation levels in the image. And you can adjust the resolution at which you want to capture your images and like auto exposure you can do. So, that it adjusts for adjusts for that also so many things are there.

Let me take it back to the default values. So, I have done white balancing so this is the software you can which you use to capture the images. Now as les let us get back to the microscope get back to the microscope. So, as you had seen this is the light source for the bottom top illumination, this black color square that is seen at the backside that is a light source for the top illumination. So, there are few knobs here see so there is this is again there is a piece here.

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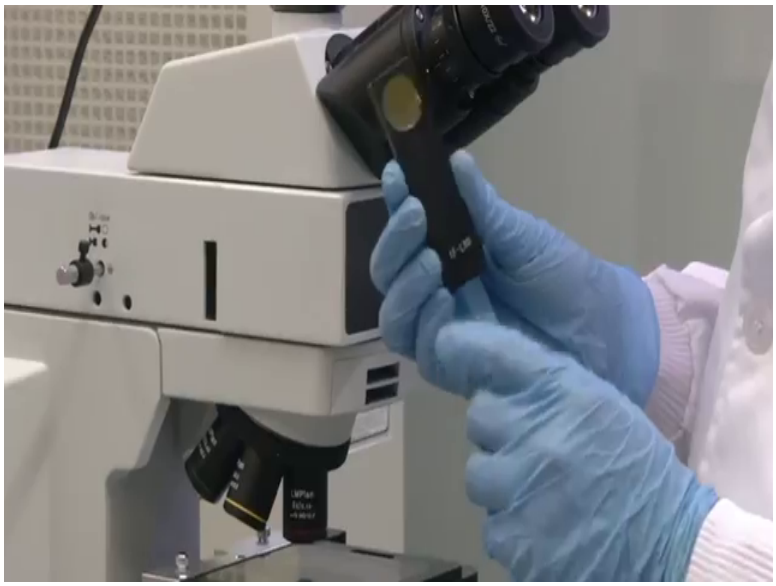

I hope you can see s o this is for adjusting your aperture again how much of your source light actually falls on your sample. So, if you want to adjust that you can actually. So, what that that is not basically why this used for that is also a good utility, but more importantly this can be used for putting filters like color based filters like red filter, the

green filter, blue filter, etcetera. Which you can put which you can put some filters are there.

Some filters will be there which you can insert here then you have one more similar arrangement for multiple filtering here see it is a similar one, but just that it is a smaller radius here if you can see you have we have put a filter here I think you can see a small color difference. So, that is one filter that is put so these filters you have to insert there are places where you can insert these filters into the microscope and they will come into picture. So, suppose you want to do fluorescence imaging so what is fluorescence? Let us say you are studying some sample. So, I will show you a few more a few more filters which we are having I thought because any wave into the filters without we will show you. So, let us see this is like a this is a yellow color filter.

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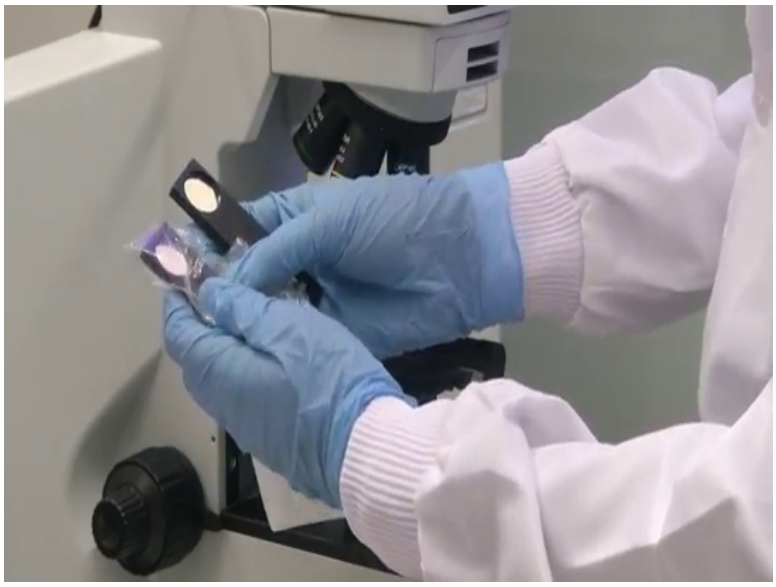

I do not know of through the camera it might come as a different color, but this yellow filter. Likewise we have blue filter, green filter, blue filter. So, this is blue filter, this is green filter if you can see it is written ISB less than or equal to 480 nanometer. This is the wavelength of the light.

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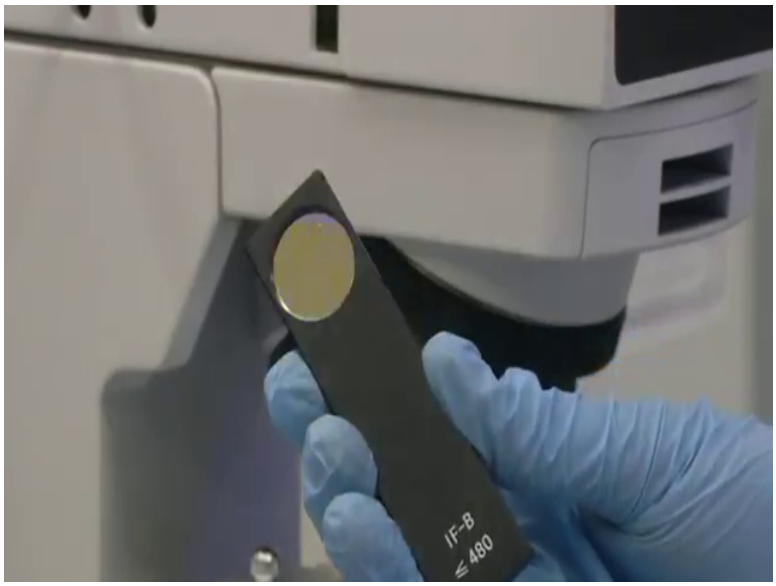

So this is like it is telling you that it will pass anything below 480 nanometer and that is blue light filter. So, as you know if you are aware of optical microscopy and energy related relations. So, basically blue light has more energy than yellow light, yellow light goes closer to the infrared spectrum. Blue light glows goes close to the ultraviolet spectrum and blue light has lower wavelength, yellow light has higher wavelength.

If you know Planck's law the wavelength energy relation is h nu where nu is the frequency or h nu is also equal to h c by lambda. So, as lambda goes up your energy comes down similarly as lambda goes down your energy goes up. Blue light has low lambda 480 nanometer less than 480 nanometer. So, blue light has higher energy yellow light has higher wavelength. So, it has lower energy remember h c by lambda h is Planck's constant c is speed of light lambda is a wavelength of the light. So, here if you see this is green filter again so green falls in between blue and yellow. So, as from your school days you might be studying about VIBGYOR violet, indigo, blue, green, yellow, orange, red, VIBGYOR so this is green 520 to 557 nanometer you can see right.

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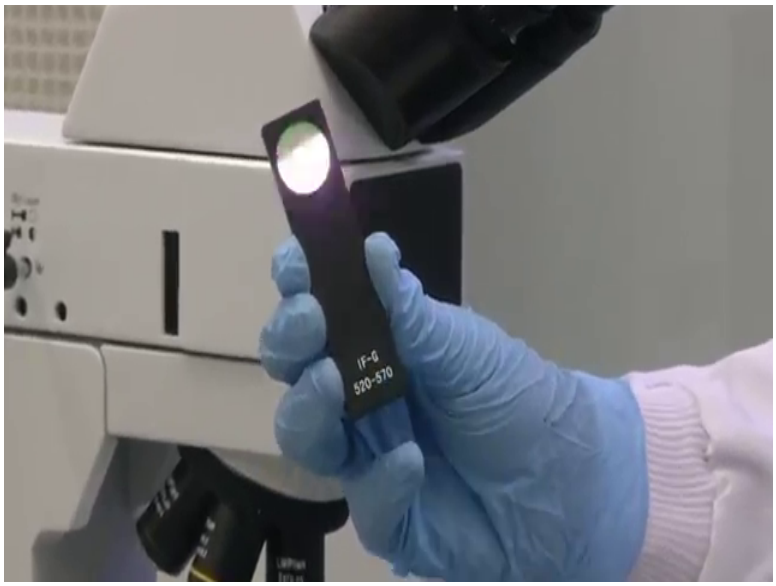

You can see right similarly we have blue, less than 480 nanometer. Likewise you can use these filters insert it into the corresponding insert it into the corresponding slots in your microscope and use it. So, when we tell this we need to discuss about fluorescence, fluorescence microscope. There is a subclass fluorescence microscopy so there is a subclass of microscopy what happens in that. Let us say we have a sample and we add a particular chemical to the sample. What that chemical does is? It goes and interacts with some particular feature of that sample in such a way that after interacting; that particular sub feature of the sample emits a particular light so it starts emitting light.

What is fluorescence? Fluorescence is when a substance starts emitting light. So, when it emits light we can capture that so let us say there is something called nuclear staining of cells. So, let us say we have a Petri dish containing low order cells. And then we want to study how the cell's nucleus you all know nucleus is the core part of a cell. We want to know how the nucleus is behaving what we do is there is a chemical or a dye it is called the dapi.

We add that chemical to the cells what this dapi does? It dapi goes into the cell goes into the nucleus binds to the DNA that is there. And once that happen that binding event happens it starts emitting blue light. So, then if you put this filter for a blue light say; let us say, fluorescence microscope and a filter which we use without using any illumination after adding the chemical because this sample itself we will act as a lights light source.

Why? Because this is fluorescence so you add a chemical to the sample, the sample reacts with the chemical in a specific manner and it starts emitting light from specific regions within the sample. Then what happens it emits light that light is captured, when you look at the image you will see light coming out from only where the nucleus is present because that chemical dapi went and interacted only with the nucleus of the cells.

So, in the image you will see dot blue, blue color dots throughout your field of view. And, those blue colored dots will correspond to the nucleus of your cells. This is the basic method in which fluorescence microscopy works. Fluorescence microscopy can also be done with this microscope we will not be getting into that much detail because that is a more biology oriented thing and this is a micro fabrication course, but we thought that you should know about it. So, this microscope, but right now also it is capable of carrying out fluorescence microscopy also.

So, as I told there are filters and there are aperture adjustment equipment like this, we can adjust the aperture like this and then conduct the microscoping. Now we will look at a few samples one or two samples, and see how bottom illumination captures images how top illumination captures images etcetera. We will see a particular sample and how we are seeing the image through the microscope I we have there is something called sample samples basically.

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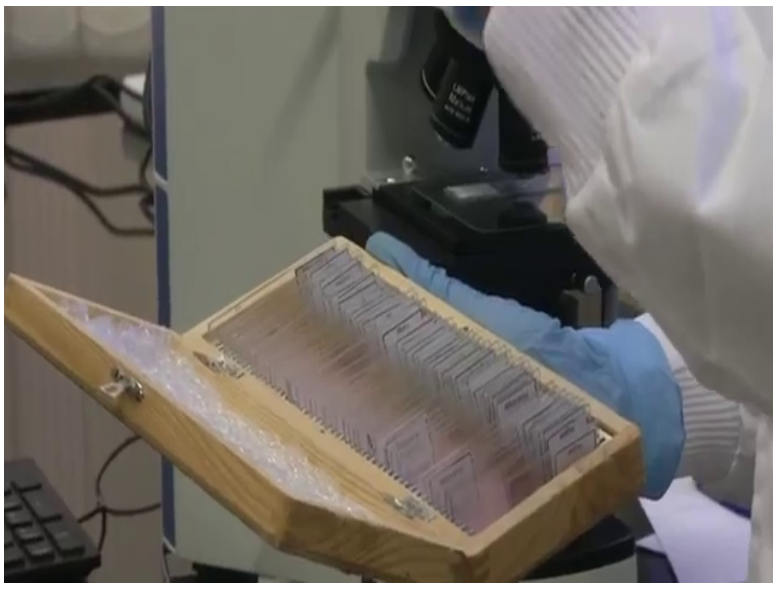

So, these are this is a box that comes this is readily available that you can purchase and this will have representative samples of different cell types. So, these are slides that are in all are inactivated nothing to worry about these are used for us to study. This purely for academic purposes say let us say this is histology of mammalian cells and this is cardiac muscle histology, can you see this.

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Cardiac muscle histology let us say let us see one more I just I will randomly select. We have entamoeba histolytica which is a protozoa.

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Today we let us see I have taken one sample from here and that is inactivated malarial parasite. Because you all of you are aware of malaria disease so, I thought we will show you malarial parasite.

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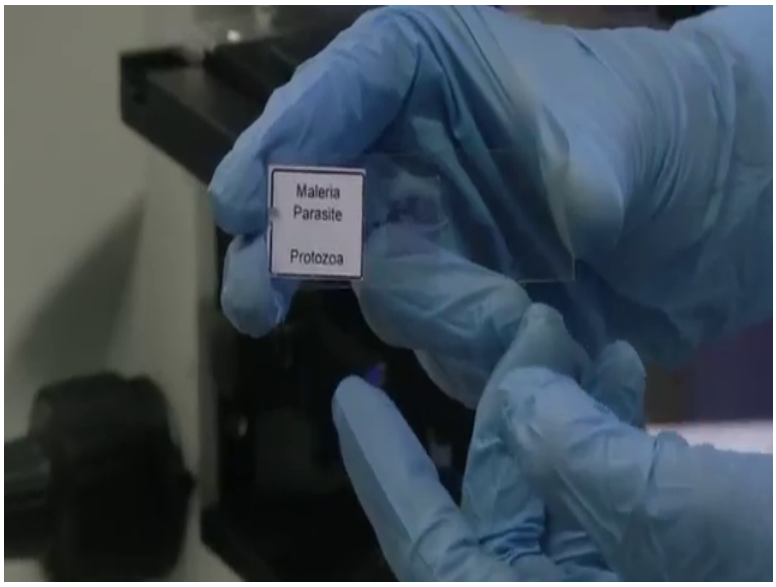

This is basically inactivated nothing to worry about you will not get any disease with this, but you have to always take precautions because you are handling biology related topics ok. So, now once we put the sample we have to first see it through the eyepiece because we have a camera connected to the sample. You can actually see what we are seeing on the slide in the screen, if you look at the screen you can see.

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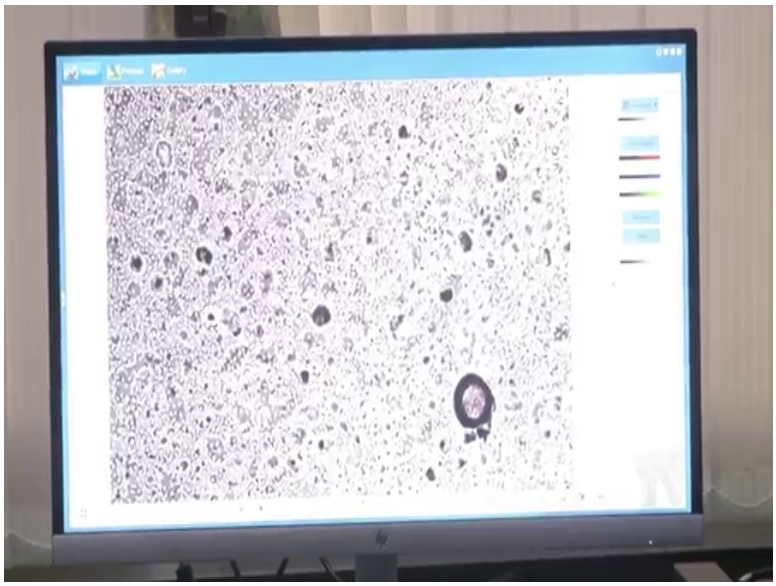

Now, you can actually look at only the screen as I change the different knobs. Let us say now you can see right there are lot of dots and other bigger of pieces; so this is the malarial parasite slide which we are seeing. Now let us say I am moving it in the x direction so as I move it in the x direction this the slide will also move and you can actually see how it is changing, how the view is changing and all you can see. Now let us say I move it in the y direction what will happen see it is going down. So, you can see the different, different, different parts of the cells of the parasite what are there you can see so many things ok.

Now, let us me let me adjust the focusing so if I adjust the focusing right this will go out a focus. Now we will not be able to see much see, now you have to adjust the focusing so that you are able to see what you are intending to see. Yes, now I have used 20 x magnification like that you can use the 10 x 1 so that you will get a much more microscopic view of things.

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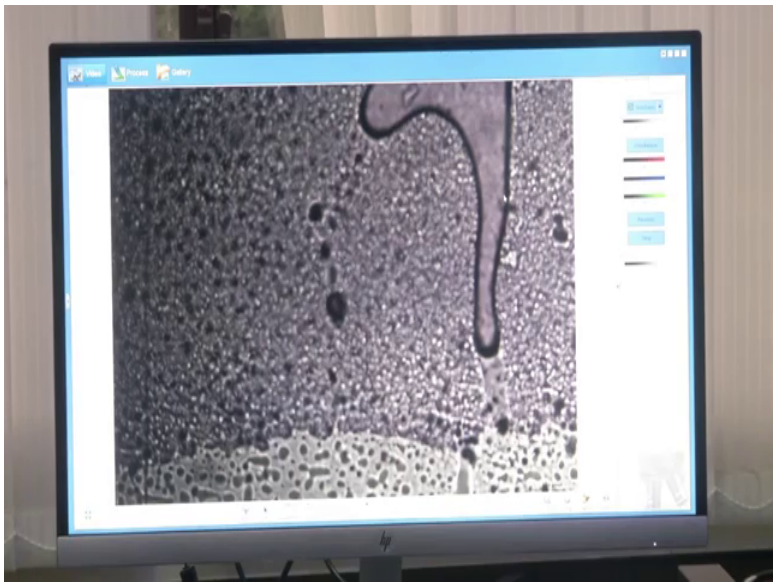

You can see the things changing this is a microscopic view now the first I go to 50 x it will be a totally different view. So, this is a 50 x image of the same.

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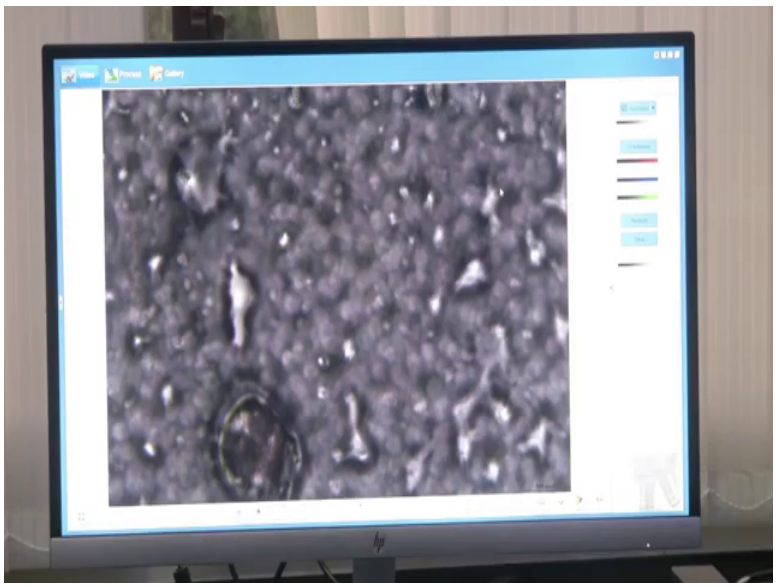

Or now let me take it into focus.

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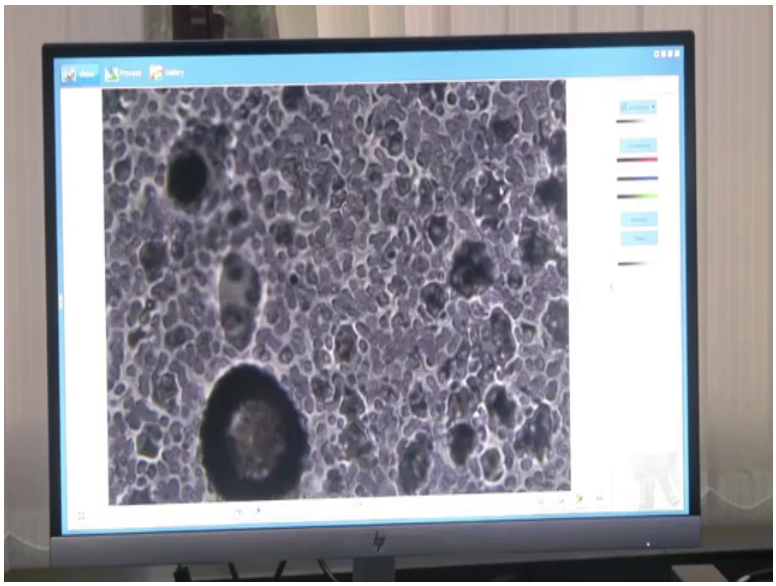

I have kept it into focus now it is in focus, like this you can go in and try this. Now I have used bottom illumination see that the light at the bottom is blinking and top illumination is not used. Suppose I switch on top illumination I can even have both illuminations at the same time. See the images getting brighter I have used top illumination.

So, and you can even have only top illumination let us say I switch off the bottom illumination and you work with only the top illumination. So, that time see the image seen is slightly different because, we have used a blue filter. Let us say I remove the blue filter you will get white color. So, I have removed we had this blue filter kept so let me put it back then you will see again that images become blue.

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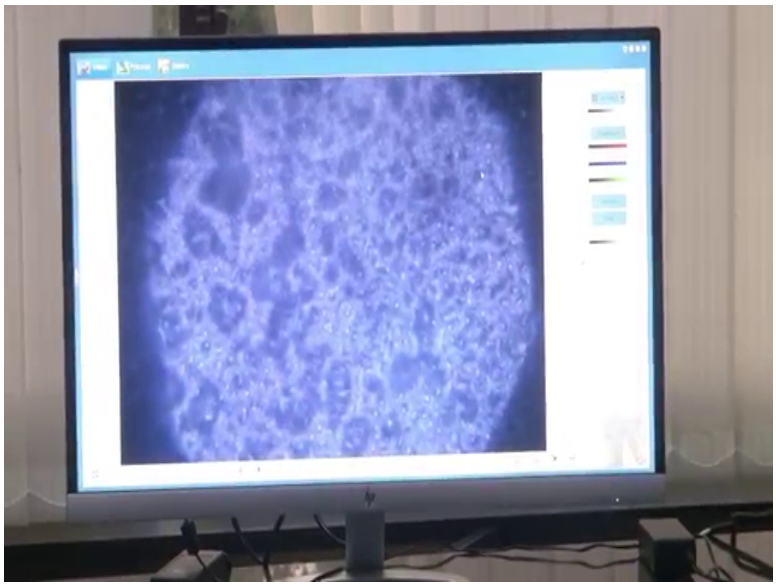

And that too how see you are seeing a circular image right that is because you have put a filter like this circular filter. Then now let us see one more thing so, now you have understood how top illumination whereas, bottom illumination whereas, how we have to focus it, how images will be there, how image will be captured, as I have shown. Now two more things we let us see let us introduce; just like the filter for the top illumination we have filters for the bottom illumination; these are the filters for the bottom illumination. Let us look at the names of it so let us say we have I F 550.

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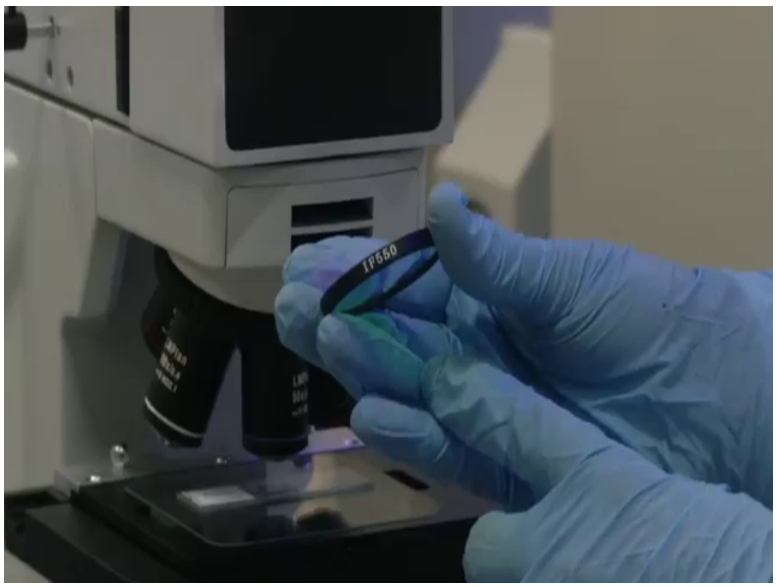

So, Intermediate Frequency 550 so it is like it represents the wavelength at which this light will be filtering and like what light effectively will going will be going to your sample, like that this also has a name called LBD. So, let us put LDB in this then see what happens and then we have once we are putting it in the bottom illumination we have to switch on bottom illumination right.

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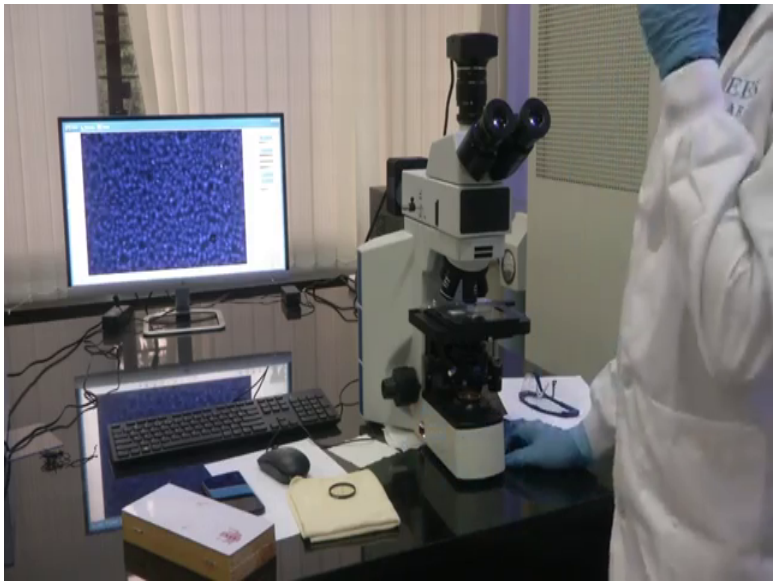

So, we have switched on bottom illumination; this is another light source another method by which we can florescence microscopy which I discussed before. What happens in this will be you excite your sample with a particular light, so it your sample will respond to only a particular wavelength. Let us say you have excite your sample with blue light only then parts of the sampled which will respond to that blue light will start emitting another light back. So, that way you can measure fluorescence microscopy. So, now I put the blue light filter know, now let me remove the filter you will see the difference in the image.

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So, it has become so it is white, now like bright field image.

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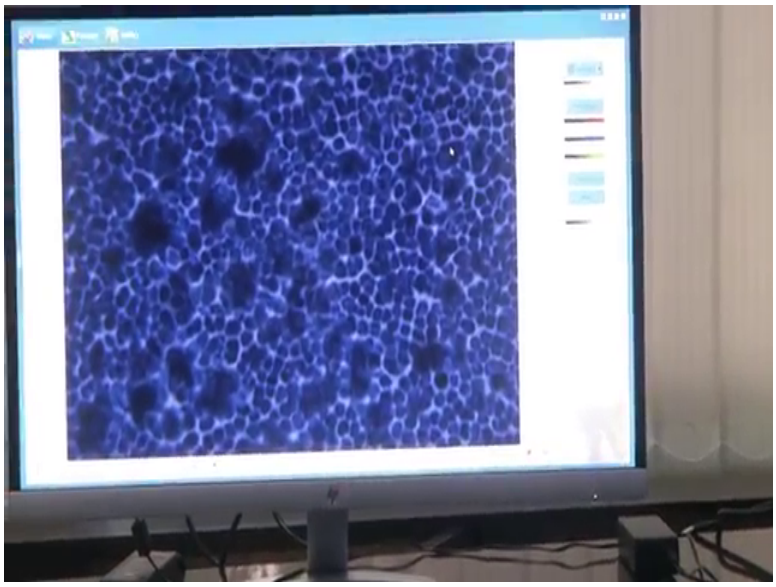

Then let us put the blue light filter; see it has become blue. Now let me put the next filter let us see what happens, this is green light see.

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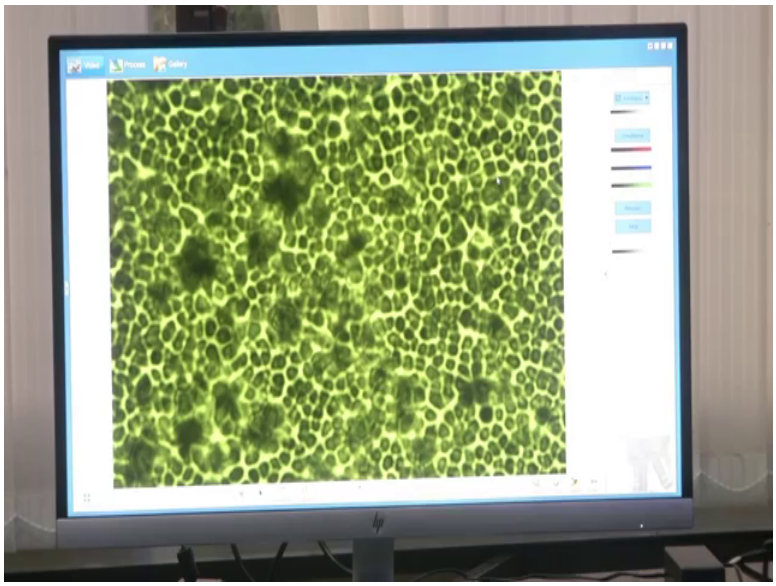

It has become green so this way you can do a lot of things with microscopy. Microscopy in itself is a very broad field, we are just giving a glimpse of it. And, which is only required for you to characterize your sensors and your devices like MEMS devices. So, this is basics about metallurgical microscope.

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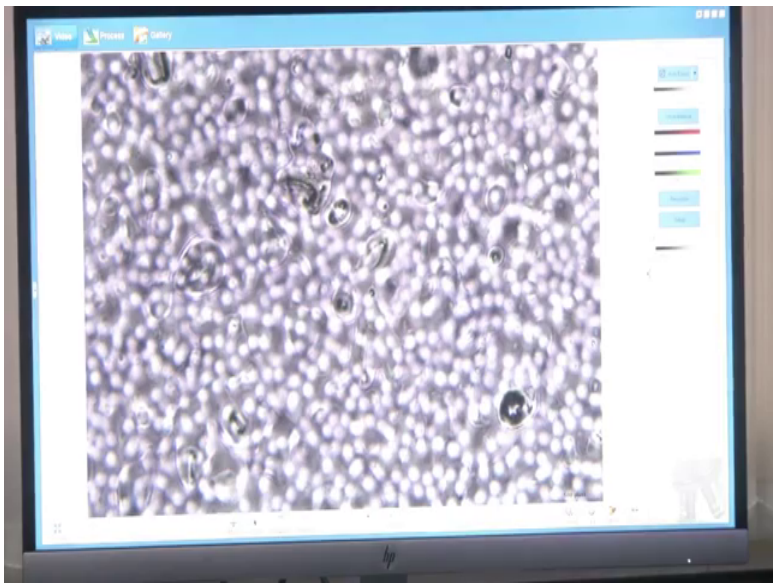

Now, we will be covering the inverted microscope also. So, you get a wholesome understanding about the different types of microscopes that are available.

Thank you.