Fabrication Techniques for Mems - based Sensors: Clinical Perspective

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Lecture – 44
Introduction to Equipments: Inverted Microscope

Welcome. In the previous few modules, we have introduced you to microscopy in general. We have told you what is optical microscopy, what is electron microscopy and showed you why an optical microscope would be used in a typical lab setup and how and why electron microscope is a much more specialized requirement that would need a dedicated facility. Then, we went on to see the principles of optical microscopy and first we saw about this 3 D stereo microscope, then and understood its working, what exactly it is used for and what are the how to operate it and we saw few samples and sensors with the 3 D stereo microscope.

Then, we went on to see metallurgical microscope. What are the different types of illuminations available and we saw few samples with it. So, today we will be seeing the next type of microscope, that I was we were continuously talking to you about, which is the Inverted Microscope. So, if the name suggest, it is called inverted microscope. Why is it inverted? If you closely, if you would have closely observed, in the other two microscopes, which we which we showed you the objective.

So, let me recap. The main components of a microscope are: the eyepiece, through which we see the sample, the objective lens arrangement, which actually captures the image on and the collects the light from the sample either the transmitted light or the reflected light and you have the sample holder stage where we keep the sample. So, in the last two, previous two microscopes which we have seen, the objective arrangement was actually above the sample and the eyepiece was connected to the objective. And, the illumination was from either from bottom or from top, but the objective which is the lens, was always above the sample.

In the inverted microscope the situation is different. Here, the objective with the turret, as I had told you before, what is turret? Turret is the circular mechanism, where you can keep multiple objectives of different magnifications. The turret with that various objectives in the inverted microscope is kept below and the illumination is from top. So,

this is the arrangement has been inverted, that is why it is called inverted microscope. Hope this is clear to you now. Now, what is the utility of an inverted microscope? If you look into the literature or if you talk to people working in the research field, they will immediately tell you that the inverted microscope is used for biological applications. They are primarily used in a biology lab or a lab that works in the interface of medicine and engineering.

To look at life cells, tissues, dead tissues, growing cell cultures and various other scenarios, why is the main question. If you look into a biology, bit more closer, if you grow cells in a dish, what the cells will do is, the cells will go and attach on the bottom of the dish. This is their inherent property. It is called cell attachment and matrix formation. So, cells will go and attached to the bottom of the dish and stay there. And the dish can be of any its height. It can be a small 5 mm height dish or it could be even be a flask. There are different types of flask that are available. They are called t 25 flask, t 75 flask etcetera, depending on their sizes.

In all these, we can actually culture cells, but then even here the cells will go and settle at the bottom and even actually attach the bottom surface of the vessel or the dish in which sphere culturing the cells. So, if you keep the objective on top and illuminate from bottom which is the conventional method where we saw in the metallurgical microscope, the light and the light has to pass through multiple layers of material. So, usually the cells will not be grown as such. Cells will be grown in a medium, either in a medium called DMEM which is Dulbecco Minimum Essential Medium or PBS, which is a Phosphate Buffered Saline, which is a buffer solution to keep the cells alive.

If we are having the objective at the top, it will have to focus, it might end up focusing on the surface of the liquid, at it might not go to the cell that is attached to the bottom because there are layers of liquid or content before it reaches the cells. So, I think, I hope it is clear. So, objective is on top, light can either be from bottom or from top, if we are capturing light from the top the objective has to focus at multiple planes. And, we will not be sure whether it is actually focusing on the cell layer or any layer above it because, at the end of the day we are talking about optics.

Now, what is so special in the inverted microscope? In the inverted microscope the cells will be like this, again it will be at the bottom. There might be solutions at the top, but

then your objective will be bottom. So, your objective will always find your cells as the first layer that it has to image. This way you will be able to focus the cells much more easier and with surety. Another important thing is, cells you might be have studied about cells and or in the 2 D images and you might have thought it is a flat thing or a flat surface, but actually all cells are 3 D systems and they are like a sphere.

The movement they attach to the surface of the dish, they form like a cuboid like this with the bottom flat. If you try to image it from the top, you will actually see a projection of this spherical structure only on your image, but let us say you image from the bottom, when the image from the bottom what happens already, the cell is spread out like this with a spherical surface on top. But, if your image from the bottom, you can actually see the interface itself, which you cannot get when you are imaging from the top. That is why, we have we are going for inverted microscope.

This has to be very clear to you because, at any point people will ask you why different different types of microscopes are there? What is their utility? Why are using this we can use that only know. You should be in a sound position to tell them that, no, this is my application and this is why I need to go for this microscopy and this is the size of the object that I am looking for. So, I need to go for objective of this magnification. Before you would go and do your experiments or before and go and do your imaging, you should be very clear what you are looking at. So, that you do not end up making mistakes, which are very silly and avoidable. So, you should do your background work properly.

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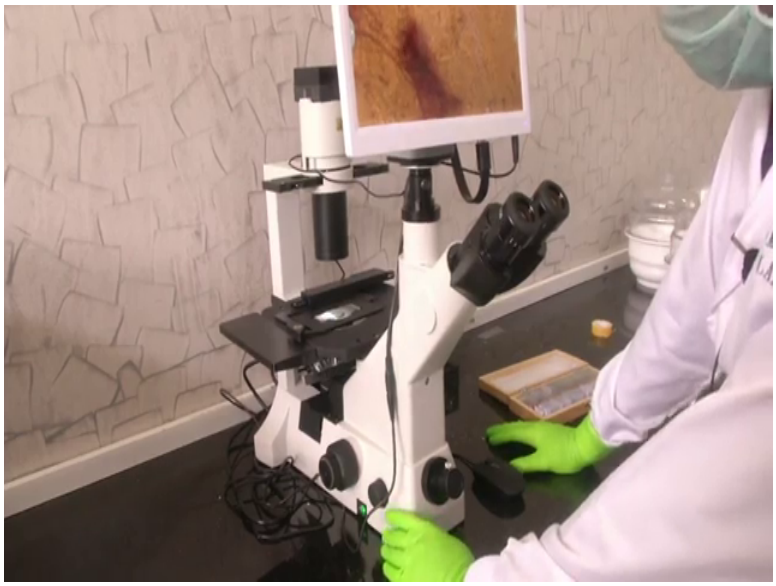

So, as I told, we have an inverted microscope here with us. It is a modern inverted microscope, which has been recently purchased. So, it is all the modern facilities in it. So, this is the overall image, overall structure of how the inverted microscope looks like, let us run you through each part of it. So, this now, we will focus it.

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So, this is the eyepiece, which is present in almost all microscopes. You can adjust with your eye size and adjust the eye, eye piece accordingly like this and then see through the eyepiece.

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So, the eyepiece in the (Refer time: 07:52) by itself it has a 10 X magnification. If you see, it is by default getting giving you 10 X magnification. So, eyepiece it is the common component of any microscope system. Now, let us let us look at the core part of the inverted microscope, which is the turret objective array. So, let us see we will focus there.

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So, they here you see, this is the turret. So, it has different objective lenses. If you remember correctly, I had run you through the different color codes that are there for

objectives. So, if you see, you may might not be able to see the print, but you might be able to see the color, yellow color there. I think now you will be able to see the turret moving very clearly with the different objective lenses coming into focus.

So, as and when each objective comes into focus you will hear a click sound. So, this if you see if you notice, this is the main difference. In the in the previous microscopes which we saw the turret was here on top.

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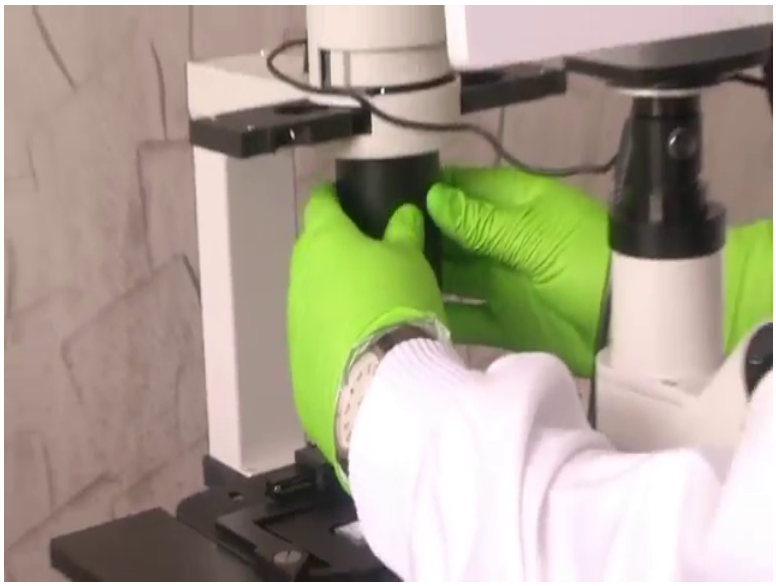

But now here, what do we have? We have the light source. See, the light source is coming from top.

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This is the arrangement that generates the light source, this black color box here. So, in the previous designs, we had the light source from bottom and in the metallurgical microscope which we saw there was a light source from top and from bottom, but in all the microscopes the turret was on top. Here, we have the turret below. This is the core difference of the inverted microscope when compared to any other microscope.

Now, the other knobs in the microscope are common. So, if you see, as usual there will be a focusing knob for coarse and fine adjustment, coarse and fine adjustment of the focusing.

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So, this bigger knob is for the coarse adjustment, the smaller knob is for the fine adjustment. We have the intensity adjustment through this. This will adjust the intensity of the light source, this knob and power on off switch will be there and adjust coarse adjustments. Then, we have the sample stage which is also part of any microscope.

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This is a sample stage. We can keep our sample stage on this and the sample stage also comes with stage holders, that are specifically made for different types of sample holders.

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So, there is a stage holder for putting this type class glass (Refer time: 10:37). You can put the stage holder like this, then the slide will stay there without slipping. So, this is the sample holder which we discuss right now. So, the slides, usually biological specimen are, if you take a blood, if you have gone to hospitals, if you take blood, what in your old days olden times what they used to do? They used to come prick your finger with a needle and then ask you to put it swipe it on a glass slide like this. This is a blank glass slide.

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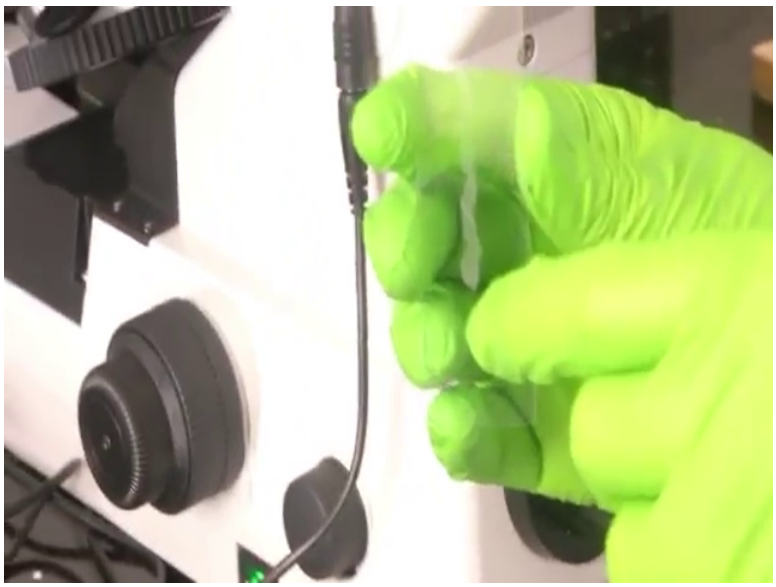

Now, the methods have improved, but this is the standard glass slide size which used to collect samples.

So, once we have the glass slide with the sample for the cultured cells we can put it on the sample holder like this. So, this portion, if you see, is that size is for the glass slide.

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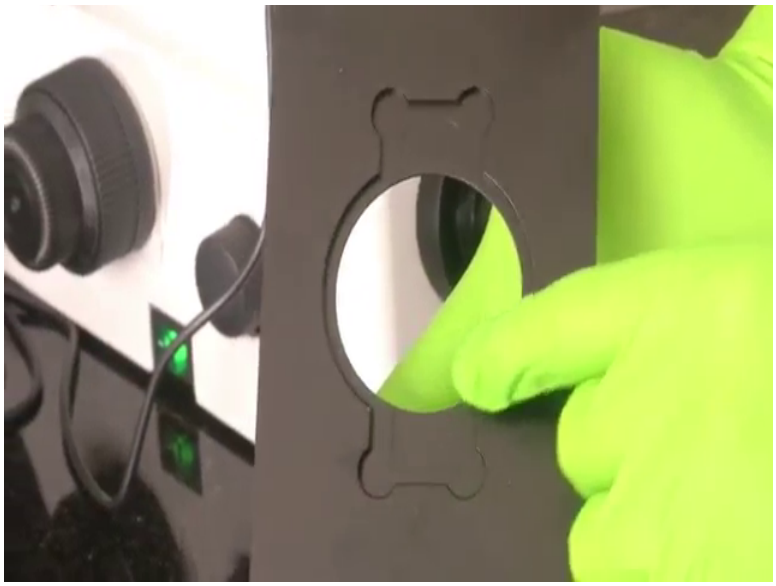

Now, if I remove the glass slide, you see a circular portion right, hm. So, this you can use for 100 mm dishes. So, that 100 mm dishes can be placed in this, in the circular portion it will come and sit and lock there. Like this sample holder, there are other sample holders also. Let us have a look at those. So, just like this glass slide there are bigger glass slides, we are not having the such glass slides with us right now.

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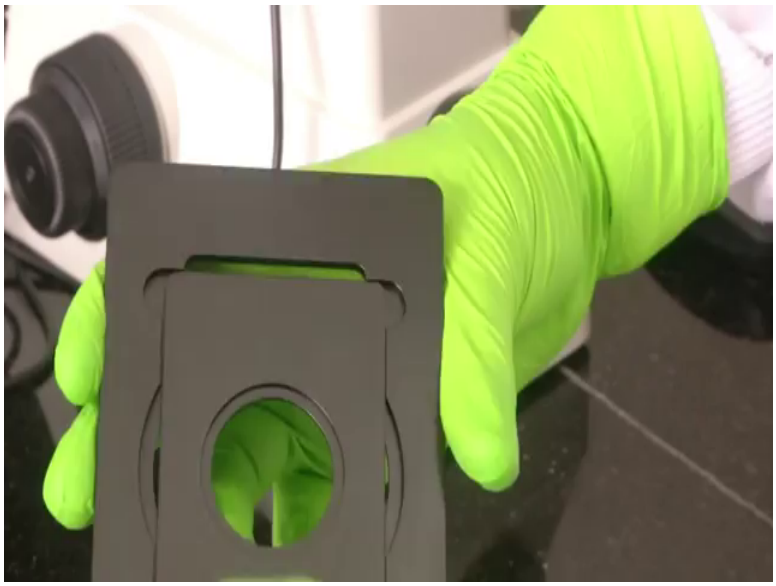

If you see, this is another sample holder. This has this much size glass slide. So, you can put that glass slide here. Big glass slides, if you want exam you know, that you can put it here. It will get locked and it will sit here. Now there is an add on to this, where you can add a small edition to this, you added like this, then it will become smaller and you can add, what is called a 35 mm tissue culture dish on this. Let me just show you a 35 mm tissue culture dish.

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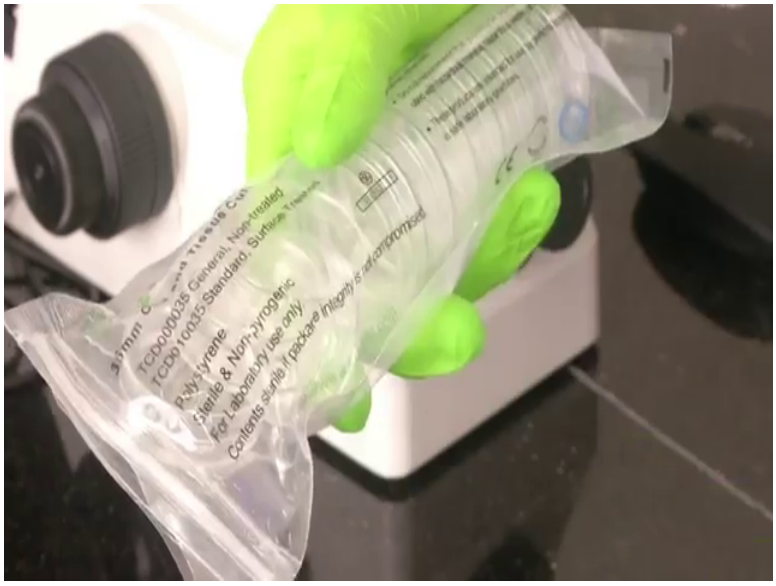

Packet of 35 mm dish tissue culture dishes so, if you look at so, these, so, tissue culture, I think we you have already learnt in the class or if you have not, it is a very critical process. You have to protect it from contamination, if you want to have your experiments, it is experimental research to be validated by others.

So, if you see, these are met with very high standards, this tissue culture dishes. So, if you see, it is written 35 mm Cell and Tissue Culture Dishes TCD00035 General, Non treated, Standard, Surface treated. So, ours is actually, standard surface treated tissue culture dish. So, this is (Refer time: 13:09) made up of what; polystyrene, Sterile and Non pyrogenic. What is pyrogenic? Pyrogenic means, it will not catch fire. So, you can there some safety provisions that are in introduced; sterile means, these dishes have been sterilized such that there are no foreign substances like bacteria, fungi or anything in these dishes. They are perfectly sterile, there is no contamination.

You can happily go ahead and culture yourselves in these dishes. Then, what they are telling; for laboratory use only, contents sterile if package integrity is not compromised. What they are telling is, we can guarantee you sterility or cleanliness only (Refer time: 13:47) as the packaging that we have given to you is not opened. After that, it is your responsibility to make sure of contamination. This is why again the need for a clean room environment comes in. That is why, we are maintaining there is environment in a very clean manner as also personal hygiene, personal hygiene is not necessarily for personal life also, even in your professional life you need to maintain this hygiene and you need to follow the lab protocols properly so that your work fundamentally, if you do all these things your work will progress faster. Otherwise, your pro work will get affected by unforeseen issues like contamination.

Contamination is a very serious problem that is where we have to take these precautions. So, they have provided some warnings in this. Let us just run through these warnings. So, what are the warnings they provided? What they have written is, should this product be used with infectious or otherwise hazardous materials, those materials should be rendered harmless by sterilization or other suitable treatment prior to product disposal. See, they are taking care of downstream processes also or at least they are warning us about downstream processes, if at all we forget. What is downstream process? You will be very excited to do your experiments, you will go, you will make the dishes, you will put the cells, you will grow cells, you might even be working with very contagious

viruses like HIV virus, Influenza virus or the new Nipah virus you do not know. You might be working with so many contagious viruses.

But, you will be more interested while you do the experiment to see the results of your experimental plan. Once the experiment is done, you might not have that much enthusiasm to dispose whatever you have done, but that is part of your good lab practices. Your work should not get affected. Accordingly, it is your own responsibility to make sure that other peoples work are also not affected and the health of the members in the lab as well as people who are associated with the lab are also not affected. For that it is very important that, you have set protocols within your lab to dispose of these infectious agents in the adequate manner, that they do not affect others. So, that is why they have told, should this product be used with infectious or otherwise hazardous materials, those materials should be rendered harmless by sterilization; that means, they should be killed basically or other suitable treatment.

For sterilization, what we do in our lab is, we have bleach chemical. Once our experiment is done, we add the bleach on the dish. This bleach is a very hypotonic solution. It will kill the cells or whatever virus we are working with so that we can dispose it, priority product disposal. Let us look at other warning. Do not recommend that you are recycled products that have been used with hazardous material, hazardous waste or bio, biohazard items. So, they are telling, you just use it and throw it, because, you should not recycle it, because, it will affect both your experimental results as well as the safety, bio safety of your lab.

Then, what is a third one that they are telling? These products are intended for use by persons knowledgeable in safe laboratory practices. See, again that they are telling you should be very well versed, very well aware of safe laboratory practices. Safe laboratory practices, good laboratory practices this you should follow. Whatever small, even, if your experiment is very small, it is there only for 10 seconds, even then you should follow these protocols properly. So, we have taken a slight detour because, I wanted to run you through. See, even in this packaging, these companies are providing as much with much warnings. They are, they are following their own ethics of working and marketing things. We should also follow these good practices, so that whatever work we are doing is sustainable and also help society.

Now, let us get back. Let us open the package and take out the 35 mm dish. So, let us open the packet. So, finally, we have opened it. This is a self ceiling, ceiling arrangement. So, I have open, I am, we are opening the packet. We will take out one dish from it. So, we have taken out one dish.

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So, once we take out one dish, you have to close the packet properly. So, that others are do not get contaminated. That is done; we have to put this back. So, we have one dish now with us. So, this is one dish. See, there is a bottom and this is the top cover. So, now, what we were, where did we start? We told you about this sample holder right, so, there this is for putting bigger glass slides. If you insert this small sample also into it, it will become another sample holder basically, to which we can actually put this 35 mm dish.

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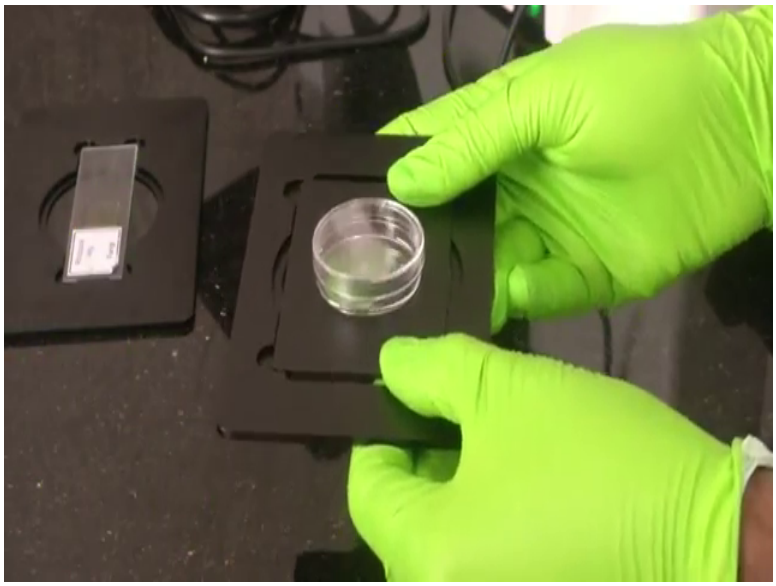

Then it will nicely come and sit there. See, now you can put it and examine your samples properly.

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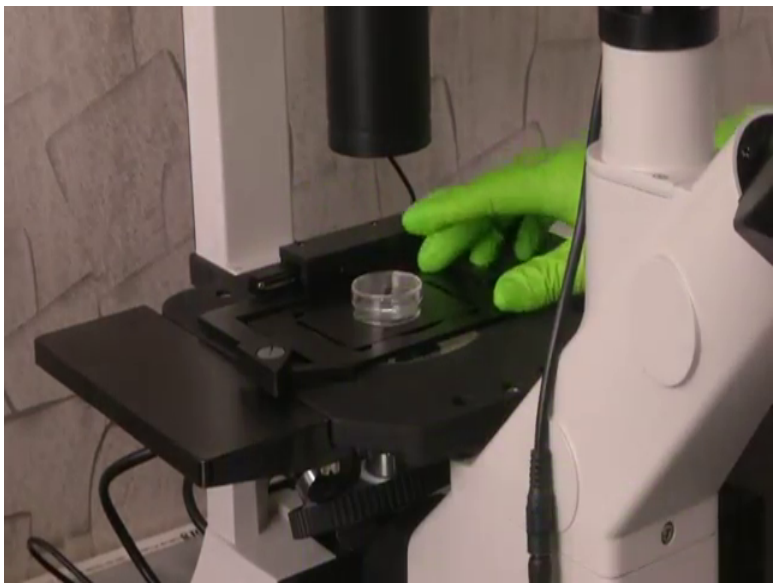

Like this, if you put, we can put this on the sample holder and you can examine the samples properly. This is how the sample holders are important. Once the sample is held in place, just like in the other microscopes, we have the x, y stage adjustment.

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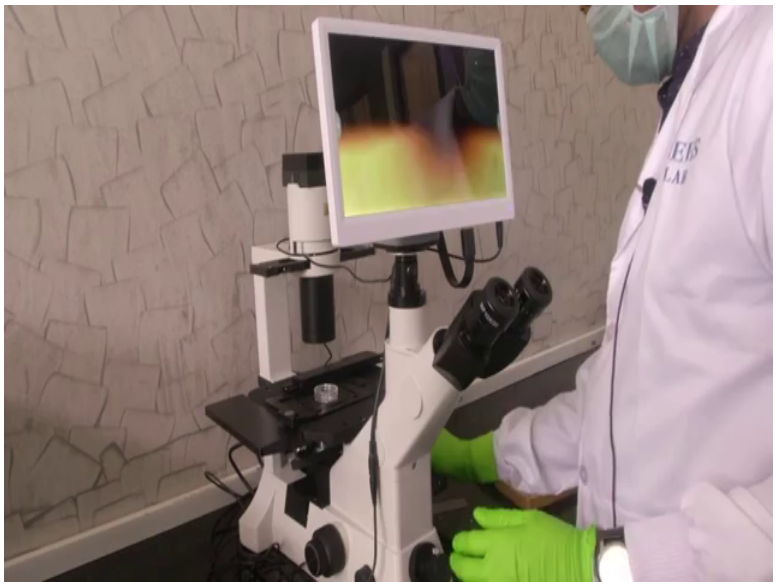

So, if I move, to see, this sample holder is moving. It is moving. It is moving x, y, x direction. It is moving y direction. So, this is how the sample can be moved around. So, these are the details about the sample holder. Now, finally, we will show you with few representative samples, but before we go into that, let us see the camera arrangement. So, as we have seen before also, as we have seen before also, the whatever the eyepiece sees, you can see from your camera also. So, I will just rotate this.

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So, we have HDMI high resolution camera; that is HDMI port also on this. So, you can you can connect, there is a USB cable adopt option for it. So, you can connect a mouse to this and to this camera there we can actually connect display. That is a small OS running on it. So, this display, this kept camera will capture whatever the eyepiece is showing and it will show it on the display. So, this is the display. I hope you can see it. Now, we have a mouse. So, with that mouse we can move around, you can actually capture the images. If you come to the bottom, cc photo, if you click photo, it will capture this image. See, image is captured, image 18 dot jpg captured.

Now, let us look at a sample. So, what we have here is, I have Rhizopus switch fungi. I am having Rhizopus switch fungi. So, let us see the, sample. I am putting it to on the sample holder. Then, I am bringing it under the objective. Now, I will see with it with which magnification it is put, it is currently in 5 x. Let us put it at 10 x, 10 x magnification. Now, that let us adjust the focusing hm. So, now, it is an un come under focus. See, it is very easy to focus because the biological sample is attached to the bottom of the slide, yes. So, these are the fungi. I think, you can see this hairy structure. So, that is the fungi.

So, you might think fungi, you will can pronounce it fungi, fungi. So, there is one pronunciations that, that you that just follow that pronunciation that is all. So, it is a problem for fungus. So, fungi so, this should be clear to you now. So, let us see, now, if you adjust the brightness, adjust the light intensity that is falling on it, it that image will become brighter. If you reduce it, it will become darker. Now, so, this you see a yellow colour hue in this image, right. Let us say, we do not want that, we do not want that, we want that change this image.

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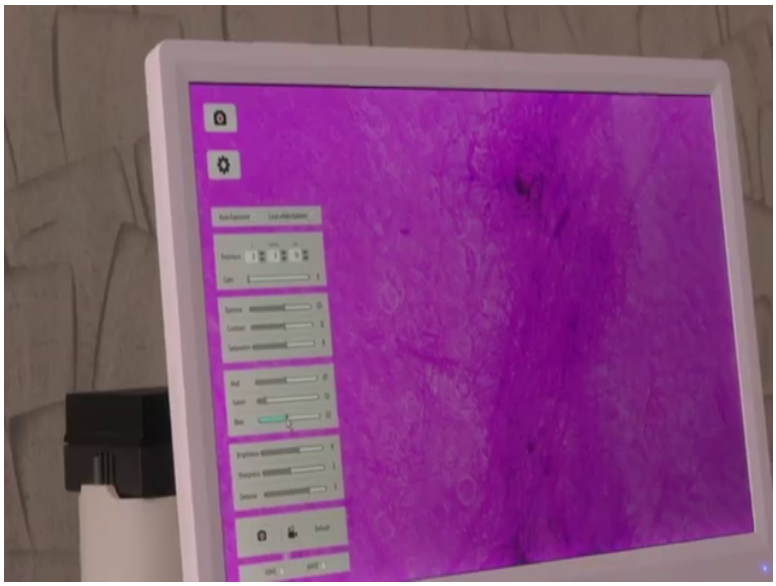

So, just go over them, go to the settings. See, there are this red blue, green blue adjustment, brightness adjustment, sharpness adjustment and all and you can actually put white balance. So, we have removed white balance now. So, it is become black and white, almost black and white in colour. Now, let us see, let us adjust the RGB values. So, the settings is come again. So, let us adjust, let us increase a content of red, see it is becoming red. Increase green, it is blue.

So, it you can change the what in what way you want to capture the image, you can change it, sharpness, brightness all this things you can adjust. Now, you can even take videos with this camera. Why, are see there is an option here, you can either snap this or you can take a video. When, what happens when you take the video? So, you take a video and then you move the sample around and then you stop it. So, the video is captured, video dot avia is captured.

Now, why do we need a video? It is not for moving the sample around then capturing the whole area that is not the use. So, many applications would involve what is called live cell imaging. You can either have yourselves dead on your tissue or the cells might be live and multiplying and you want to study them. When you study them, these cells particularly cancer cells, have a property called migration. What is migration? In normal terms also, what is migration? When people move from one place to another it is called migration. Just like that, cancer cells have a property to migrate from one region to other.

This is how they invade other parts of the body and that is why cancer is a very very (Refer time: 24:56) disease.

Now, suppose we have cells on the tissue cultured dish and we are studying, we do not know whether these cells are cancer cells or not. But, we know that cancer cells have the ability to migrate, then what will people do? People will make the dish and at the bottom of the dish they will make a line, it is called scraping. They will make a line separation and the left side of the line on the right side of the line they will provide a matrix kind of structure allowing for the cells to move, but in the bottom there will not be anything.

Then, these cells will be grown on both sides of this divide, ok. And then it is cells will continuously grow, we will keep it under the microscope and we will press this video capture button. Over time, what we will observe is that, if these cells are cancer cells, these cells we will start moving across this line indicating that these cells are trying to invade from one region to the other. This is a particular assay. So, all this particular protocol based experiments in biology are called assays.

These are lot of assays. There is assays, there are assays called live dead assay. This is used to see how many cells within your dish are alive and how many cells are dead. So, there are definite protocols to find out this number and that protocol together with the set up experiments is called live dead assay. This, whatever I explain just before is called cell migration assay, like that, so many assays are there. If you want to study such migration assays, such real time assays, we have to have live cell imaging.

For live cell imaging, we need to have option in our microscope to capture the video. That is how that video option comes in to use. So, folks, these are the main principles of the inverted microscope. You have understood: what is the difference between an inverted microscope and the normal compound microscope. Why is it so important in a biological applications, how we can take images and what are the different methods for which we need the use of an inverted microscope.

There are other different knobs in this microscope which are not very important. Like, you can switch off that the imaging to your camera and, like this let us say, let us focus there. So, if there is a knob, there is a knob if you cut it out, you can switch of the imaging to the camera, then you can just look at it through the eyepiece. Then, you can even shut out the eyepiece with another switch. So, these are minor ergonomic options

that are provided with different designs. These will vary between different models of inverted microscopes. But, the core idea, the core philosophy of the microscope remains the same. Hope all of you have got a good idea about how the inverted microscope works. It is very important and this is a fundamental equipment in any laboratory that wants to work in the biology or medical field and also work with engineering.

Thank you.