

Electronic System for Cancer Diagnosis
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Lecture – 51
Demonstration of Cleanroom Equipments:
Introduction to Equipments: Stereo Microscope, Metallurgical Microscope,
Inverted Microscope

Hello everyone, welcome to the course on Electronic Systems for Cancer Diagnosis. Today I am here in the clean room facility detailing about the type of microscope the choice of microscope which are required while you work in an environment where you do micro fabrication integrate biology and micro machining techniques.

So, let us start with the cell and tissue culture techniques which was detailed to you by Professor Hardik in one of the course modules. So, what do you do when you have to extract cell or tissue when you want to study cell culture and why do you want to study cell culture samples? It becomes very important to diagnose any disease. It is a gold standard to extract samples or tissue from the diseased area and the mode of extraction could be either it could be a biopsy or it could be micropipette aspiration it could be through micro needles. And all of these are to extract unit cell or tissue samples from the area which is affected or suspected to have disease.

And once these samples are extracted, you study these samples. So, cell culture or tissue culture this is where the term comes. So, once the samples are extracted, you have to keep them keep the cells or tissues alive until you finish your study and you do the testing diagnosis and all of that can be if the prediction can be given only when you have live samples with you. And once these are in your hand so, what is the next step a clinician or what is the next step in a diagnostic procedure?

So, once the samples are there, you culture them. So, to perform any kind of studies the method is called you know to keep the cells alive. This is cell culture media. So, the media it could be PBS which is the Phosphate Buffered Solution or it could be any other solution like DMMA where you put the samples and then you provide them a medium to survive. You create an environment where they could grow provide enough nutrition for them to you know provide enough nutrition for them to take in grow and that is how we analyze different stages of the growth process and see if they are invading.

When it comes to cancer cells, the study says that these cells are invading as they grow; they rapidly invade the other areas of your tissues. So, all these studies the live cell imaging all of this has to be performed and hence we have the culture media which keeps them alive. And once they are alive and how do you live? Minute and micro features the structural features are on a micro nanometer scale. So, the naked eyes cannot decipher these features and we cannot understand them the way you know this different staging.

So, how do we do what techniques. So, we have come up with the technology like the microscope which facilitates us with imaging microscopic features. And that is when use of microscopes in a clean room facility like this integrating biology and engineering, micro fabrication. All of this we need microscopes and that is why today we learn what is the choice of microscope we need, how do we use them and it also there are different features So, how do you understand the features of these microscope and then use it in the right application.

So, now coming to biological samples. So, it is always understood that the cells so, you always have these cells in a medium and then you put them in 35 mm cell culture you know petri dish. So, 35 mm 100 mm that different size of dishes where the these tissues and cells are cultured. So, this culture media has to be studied. So, what is the choice of microscope you use? When it comes to is when you want to understand the type of microscope, you should understand the features. When we have these petri dish it is always understood that these cells have a tendency to settle at the bottom of the petri dish and then, what happens in there in a buffered solution which is highly turbid.

So, in order to analyze this so, we have to think when it comes to microscopy. So, you shine light or it could be any other mechanism, but there would be a lot of interference due to the turbid medium. So, you need to you know a look at them from the bottom where are the cells you have a better focus, you could see, you could better understand; better study the medium when you are studying from the beneath were from the from below the petri dish. So, you need to understand the microscopy technique where you know the objective lens say the lens could be at the bottom and then you shine light from the top.

So, that is where when inverted microscopy comes into picture. More details about inverted microscopy will be explained by me in future. Now so now, that we have

studied when it comes to cells and tissues you have something called as an inverted microscopy which you would use to study them. And then we have already discussed micro sensors which are fabricated on silicon wafers. They are not visible to naked eye; I am sure the fabrication technique and how these sensors look has been shown to you by Professor Hardik.

So, now how do you see them, how do you understand once you perform the fabrication how do you understand if the features which have come which I have been engraved on your wafer are very much you know they comply with your design or your requirements. So, you need to characterize it, you need to understand the design process, understand the procedures, read the features, try to understand if the entire feature in the design or the ma does it comply with the mass design. All of this can be done when you keep them under a microscope. Because the tendency is to magnify something what then the human eyes cannot you see or we cannot magnify features.

So, you need an external device like the lens which could magnify and then show us what could be the features that are formed on our wafer. So, when it comes to silicon wafer or devices like this which are opaque and does not allow light to you know, flow through them. So, there they have these are opaque structures when it comes to wafers.

So, when we are dealing with such device, what is the choice of microscope? So, what we use in the lab here in order to characterize the device functionality or you know study the features which are formed on our minute sensors. So, we have the metallurgical microscope. And when it comes to metallurgical microscope, it is mainly designed to study features of structures like the wafers or any other metal coatings which needs to be studied. So, in that case we the choice for an optimal choice could be a metallurgical microscope. And then one of the basic type of microscope which could be a which are mostly available in most of the lab laboratories laboratory facilities is the stereo microscope.

How different I am sure everybody has heard about compound microscope. So, when you are studying your intermediate or you all we always used a microscope with general features, you have used thin slices, you have taken onion and then cut them to thin slices and kept it under the compound microscope and then understood the features of the

sample. So, all of that requires slicing and then understanding the features so, the compound microscope has a high magnification power.

But the same hand, when you compare a compound under the stereomicroscope what we are using the stereomicroscope what we would be using is has less magnification, but the feature of a stereomicroscope is it could give a depth per se depth perception of the object what you are looking at. So, this 3D image or the depth perception ability of a stereomicroscope has been leveraged for our case where we have these microfluidic devices or a chip or in some cases even the entire tissue which has been dissected huge samples on a macro scale can be studied using a stereo microscope.

So, all they all of these would have listed the metallurgical microscope, stereo microscope and the inverted microscope. All of these are optic based that is based on transmission and reflectance of light either the light flows through the sample. And then reflects back the reflected light is used to capture and then image is formed based on the reflected light that is based on the reflection principle. And when it comes to transmission principle, it is the light which has transmitted through your sample and that the transmitted light is captured to form your image.

So, based on the image processing it could be transmitted or reflected based optical methods of imaging. And there are other methods like the electron based which are highly sophisticated and which are not really required for us. So, now, that we have these three devices; let us get into the details of these devices the mechanisms and how do you operate these devices and how the features could be deciphered using these microscopes. So, let us get a hands on of these devices.

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So, this is a stereo microscope what I was talking about any microscope. The basic parts like this is the eyepiece, this is the arm and the base and here where the sample sits. So, the sample holder, these are the sample holder and this here this is the light source and then this is the turret. Turret is also called as the rotatable nose piece which could be used to increase the magnification of the objective the magnification power.

So, let us see the when you are talking about a microscope and what is the power what is the magnification power of the microscope. So, one thing which you have to remember is the eyepiece lens has some power and then the objective lens also has some power. So, the when you multiply the power of the eyepiece with the objective lens power that is when you can call your the multiplication the result is the entire magnification what your microscope can provide. In this case, the stereo microscope would we have has the display attached to it and then there is a camera.

So, this is optional you could for a better view you could always have a camera with a display which is easy when you are; when you are trying to manipulate or trying to study. Now as you can see there is 2 eye piece this enables us to give to decipher, the depth. It gives us the depth profile of the 3D image and this is the light source what you can see. The light source is connected to the optical waveguide and here is the source. This is the halogen source. The light source what is here could be replaced with other source

depending on the; depending on the type of experiment what you are carrying out the type of wavelength that is required for these samples.

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And then let us see we also have another light source and alternative to the current light source which is connected to the device and this is the LED based light source. The LED strip along the source illuminates the sample which is under examination and here the intensity of the light source could be varied using the knob. This is one additional feature which has come with a microscope what we have.

So, now let me start off let me turn on and show you. So, slightly varied the light intensity and you could see light and if in case I have my sample on this base here which is a sample holder, light shines through this and what is reflected by the sample is captured and an image is formed what you see through your display and you could also see it through the eyepiece. Now how do we adjust, how do we adjust the magnification? So, as so there are different knobs which are provided on the microscope what we had here.

So, with the eyepiece which could be moved accordingly and then matched so that it fits properly to your eyes and then these 2 knobs here are to adjust your focus. So, it just below the display here is your eyepiece through which you can look into that sample and there is also a facility to adjust these and just these eyepiece. So, that it can sit properly according to your the dimensions of your eye. So, this is an additional feature which

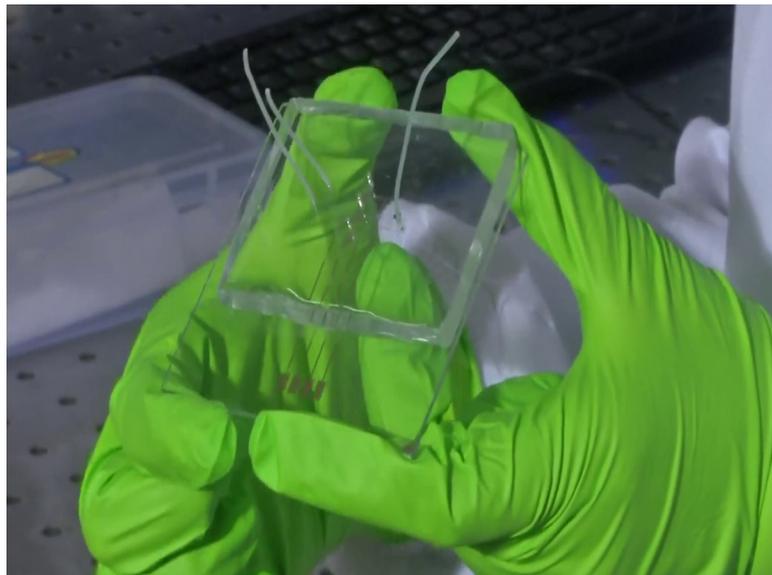
comes below the eyepiece. Once this adjustment is made so, your sample is sitting on the desk and now how do we adjust a magnification, there is one knob here to adjust your focus.

So, the knob here can be used to adjust focus; let me directly put the sample. So, the knobs here can be used to adjust. So, now, that we do not have a sample; let me show you while I keep a sample, then we could adjust the features. And this here is provided to this is their different steps 1.8 0.7 . This is the like other features like I said, this is the objective in order to vary or objective there different numbers which you should multiply say the eye piece here is written as 10 X and then you set this to 1 on this knob.

So, what is the entire magnification now the setup will be able to give you is 20 X into 1 which is 20 X a 10 X or 10 X into 1 that is the entire magnification that the device can provide. So, this eye piece magnification is fixed; however, you have a facility like this thing could be rotated and you can alter the magnification that the objective good provide. This the power of this lens the eyepiece lens is remains fixed. So, let us say we keep it at 1 and then we have the objective fixed and the light source fixed.

Now, let us say I get my sample; for now let us use the sample which has been fabricated in our lab.

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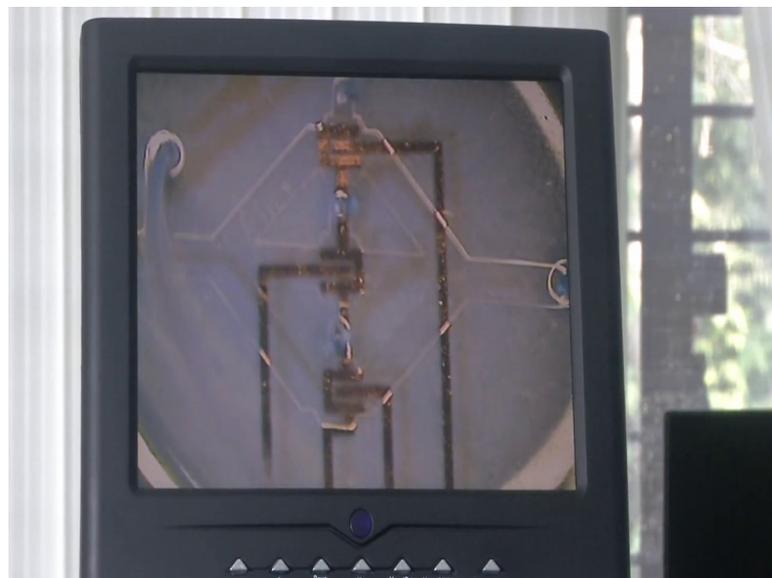


Let me just show you the sample. So, this is a sample what I have. So, if you recall, there are these my channels. Their channels in a hexagon shape, we had done we had used the same mold. This the silicon wafer mold on which we had put PDMS and the similar features were molded onto PDMS and I had already shown you these micro tubings, this the tubing through which you could flow this could be an inlet and this could be the outlet tube through which you flow the fluid.

So, there these nanometer or micrometer channels which are formed on the glass plate. So, the glass slide already has these electrodes, gold electrodes which were etched on the glass slide. On top of that, you have this PDMS mold with features on it and then you could see the punch through which your tubing sits. So, let us say you are trying to study if the features of this device have been obtained like the way you have designed it. So, let us say I want to understand this device.

Now, that now this is a this more or less you know is a 3D. Now you want to decipher what is the depth, what are the features which are formed on this. So, let us study how this looks under a stereomicroscope. So now, I have placed this device in the sample slot.

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These are the microfluidic channels which we have already seen in the PDMS molding and below that there are these inter digitated electrodes. The electrodes are sitting just beneath the channels. So, this is the inlet and this is the outlet. See has no fluid through

this inlet and the fluid flows through these or it could be any kind of fluid say you are trying to understand the impedance property of some fluid which is flowing through this.

And then you have these electrodes and then you flow them through these electrodes and then you capture. See you have another solution which you will flow through the other tube which would alter its you know physical parameters and then its resistance change can be observed using these electrode pads which are here. So, this first this is how you could understand the features or any other depth perception using a stereo microscope.

Now, that it has been adjusted, you could also vary the intensity of the light if required and you could adjust the entire setup, you can move them up and down there are different knobs maybe you could explore the different knobs. Say I hold this and there is this something called as a work the working distance with the distance between your objective lens and the sample. So, you have to maintain the minimum distance which has been mentioned on your on the microscope what you would be using.

So, here there is a specification of the working distance say I want to change my working distance I would like to move the scope. So, there is another knob which is provided here; this is how you can alter your working distance. So, let us say I fix it to this. So, this is how you can change your distance and then you could alter your magnification using this knob and you could do the tuning focusing using this.

Now, that we have studied how a stereomicroscope can be used in a laboratory. Now let us look into the other microscope. Let us get into how a metallurgical microscope works.

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So, this is the inverted metallurgical microscope cut.

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Start. Now, let us discuss more about a metallurgical microscope; this what I am pointing to is a metallurgical microscope. As you can see, it looks more sophisticated than the stereo microscope what we had discussed. Hence, let us know how to operate each of these. So, like the stereo microscope, they are also provided with the eyepiece here; the eyepiece lens can be adjusted this way. Just to fix a fixed right value look into it gives a proper fit it is more like a comfort feature which could help you fix them tightly properly to your eyes and then see through the samples.

So, this is the eyepiece and this again has it has both transmission reflectance here. What I mean is their lights or the two light sources there is light source at the bottom and they also have a light source at the top. Based on your application, you could use either or both of these light sources and here this is the stage the sample stage where the sample sits the stage could be moved in X Y and Z direction.

So, there is a knob at the right which I am operating for it to move in the Y plane. And on some and similar basis like you can see I am moving it in the X plane X Y and you could also move this entire stage in the Z direction. So, there is another knob on this side which

could help you move the stage in the Z direction. Now that we have this stage and then you could place your sample on this and what is this is the rotating nose piece or the turret. Here this is more sophisticated unlike the stereo microscope, we just had no because a magnification power when it comes to a stereo microscope is not too high.

But when you see here, the metallurgical microscope has 1, 2, 3, 4 and 5 objective lens like you can see there are 5 objective lens which is sitting on the turret. So, the entire setup can give you 5 different magnification powers says it. So, here say the eye piece like I mentioned, the power of the eyepiece here which is 10 X like I mentioned the entire setup could give you a magnification which is a combination of the power what the eyepiece can provide and then your objective lens can provide.

Now, the eyepiece here just like the previous one is written 10 X. So, this can give you 10 X magnification which is fixed here as well and you could change the magnification here. So, let us see. Now this is focusing on a 10 X. So, it is 10 into 10, 100 X magnification can be provided with this. If you can notice each of the objective lens each objective lens has been provided with a different color strip for easy identification. And then you have the 20 X and as it sits there is a clip which ensures the objective is sitting properly and then now the turret is holding a 10 X objective lens.

So, the entire system could offer you a 10 into 10 100 X magnification for the sample which you are viewing. And another thing you have to observe is while I move the turret, the objective lens while it moves and it gets it fixes and then there is a click sound just to ensure that is sitting in the right position. So, now, I have fixed it to 20 X. So, the net objective power could be 20 X into 10 that is like 200 X.

If you could see the amount of magnification was stereo microscope could kill was max 10 X into 1, it could go up to 80 X. But when it comes to the metallurgical microscope there is 100 X 200 X; if 500 X, the magnification level what this microscope could provide is really high. Let us say I have fixed it to 50 X magnification; if you heard the click sound that ensures the proper seating.

So, now as you can see there is 50 500 X let us see. So, there is 100 X 100 into 10. So, 1000 X is the magnification what the metallurgical microscope can provide and each of this objective lens the nose which is standing here is each of them have the magnification power which is mentioned and there is also a working distance on each of them. Working

distance is there is some amount of distance which has to be maintained between your sample which is on your stage and the objective lens which is capturing the reflected or the transmitted light image.

So, there is some amount of distance which has to be maintained that distance can be looked upon on this which is mentioned on this nose. So, always ensure you do not just use a different magnification, but also vary the working distance according to the specification which is mentioned here, only then you would properly be easily you could identify the fine features which you are trying to study or understand.

Now let us see this is the bottom light source and this is 800 X this is a knob which is provided for the bottom light source and there is another this like I mentioned earlier there is another light source at the bottom. So, you have 2 light source for this microscope and there is a camera and in this case we have attached it to a system here. So, it has this port which goes to your USB port which goes to the system and directly we could view the images what we could see in the eye piece are directly imaged through the PC here.

Now, let us take a opaque sample; opaque sample in essence the wafer designs. Earlier we had to match we had to view the 3D image of the device. Hence we use the microfluidic device and then the depth perception you could understand how the depth perception could be deciphered using a stereo microscope. So now, when we talk, how do we understand the importance of this? So, say I am using a metallurgical microscope. You should not be using a tissue sample while you know using this. So, this is dedicatedly devised and designed to study an opaque sample.

So, let us say I will let us take one of the devices which has been fabricated. Put it under this throw a shoe a shine light and adjust the magnification thoroughly focus it and see how the features could be viewed through the monitor using this microscope like I mentioned about the light source. So, this is throwing light from the bottom if you could see just vary the intensity to the maximum and this is the light source which falls on the sample.

So, there is an aperture through which the light actually falls here and there is another there is another light source for each of this which falls from the top. So, the intensity is very low if you can see this is the light which is shining from the top and each of this it

flows through each of these objective lenses. So, there is a small led shining light from the top onto the sample for better image capturing. So, another utility what the metallurgical microscope has is a slot here

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So, this is to adjust your aperture and another function what the slot could provide here is filter. You could filter light so that based on application different wavelength, the ray or the required wavelength could be used and then the that particular wavelength of light is actually the which flows onto your sample. So, let us say I let us see the others which can be used along with this.

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This can be integrated. So, this is one such lens. It is more bluish in order to filter light of one particular wavelength. You could use these type of lens based on the application based on the requirements. This again goes on the light source which is at the bottom, you could change the more or less looks like the plain glass. So, the which can be put on this source and let me show you the other filters.

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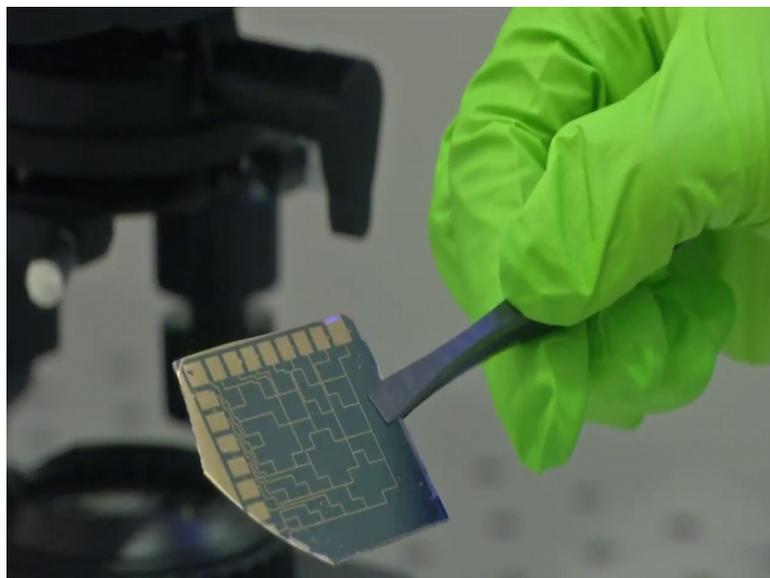


So, this is another filter which reads less than or equal to 480 nanometer. This is nothing, but the wavelength the effective wavelength for filtration. So, this is one such filter and there are many other filters which could be adjusted in your slot.

Let us say this one, this is another and you could see the wavelength on the filter offers 520 to 570 nanometer. So, depending on your application, the amount the light source what you want to flow through your sample all of that decides on the choice of filter what you can use. Now let us say let us see how a silicon wafer can be placed on your sample holder, the stage what we set up and let us see how we could focus it and then view it on our system.

Now that we have seen how the different filters can be used. Let me put back this slot. So, it goes in here and then you could use this utility in order to control your aperture that is the amount the focus area and you could also use it for filtration; filter the light. So, like I mentioned we were studying how the metallurgical device can be operated, what are its functionality, what features can be studied and what is the purpose of this microscope.

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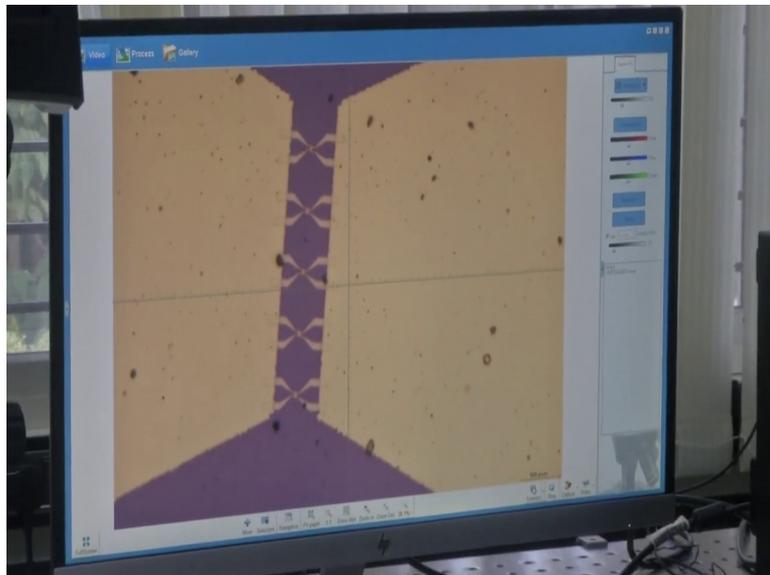


This is one such wafer what I am holding; make sure you hold it with that tweezer. This is a broken wafer, but as this is you know a demonstration purpose more of us study purpose. If you can see each of these are electrode pads which could be seen I mean, they can be visualized just through the naked eyes and the fine lines which are coming out of

these electrode pads are the extension the connections to each of these electrode pads and then there are inter digitated electrodes.

The details for sure has been covered by Professor Hardik. I am sure the electrodes which are on this wafer are 50 nanometer in dimension and these minute structures cannot be which cannot be seen through the naked eyes. Now how do we identify the features of these electrodes which have been; which have been fabricated on this wafer? Now let us say I put a similar one on the metallurgical microscope. I have just put the other part of this on the microscope and then when you focus it, if you can see the one on the monitor.

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In between the fine lines are the electrodes, the other the right and the left side are the electrode pads and then the inter digitated electrodes could be seen which is in the center more like the canal. So, they have nano meters and these cannot be deciphered through the naked or just through the visuals or just by looking at it the features cannot be identified. So, you have coarse tuning and then fine tuning. Now that we are able to look at the features; I will be fine tuning, yes. So, these are the fine lines the electrodes which are in between and the X which forms the connections to the electrode pads.

So, we have seen how a silicon wafer the features the fabrication of fine features of nanometer of which are a few nanometer and dimension which cannot be visual visually assessed can we can use a metallurgical microscope to study the fine features. So, if you

could see if there is some sort of a distortion, all of that could be analyzed just by looking into the image which is formed using this microscope.

So, we have seen how the serum microscope works and how the metallurgical microscope can be used in case of opaque samples like the silicon wafer, the devices which are fabricated in our lab. So, we would be using the biology samples and then using the electrodes silicon wafers with electrodes engraved on them and then you put the sample on top of it to study the electrical thermal, mechanical, phenotyping the different features. So, now you have know now you kind of understood what is the utility of each of this microscope. Another important microscope in the clean room facility here is the inverted microscope.

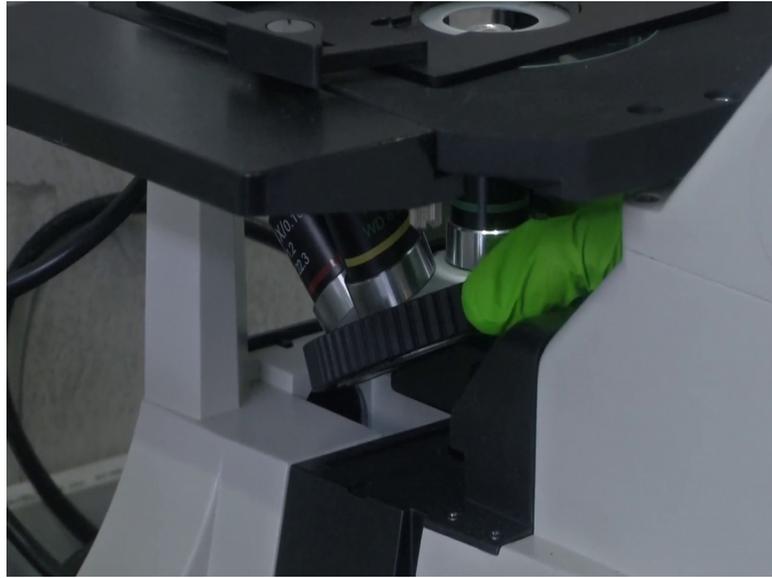
So, let us get into the details of inverted microscope and then you will have an idea of each of the 3 that is the stereo microscope, the metallurgical microscope and the inverted microscope.

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I am standing here, this microscope is called the inverted microscope. Why is it inverted microscope? A major difference between I mean you could even call it as a unique feature of this microscope and compared to the stereo and metallurgical microscope. That is the turret or the rotating nose piece is here down below.

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Unlike the serial metallurgical microscope, where the turret was on top and then the light source at the bottom. Here the objective the turret which is holding the objective lens is at the bottom this. Why do we have this place in an inverted position or placed below and then the light shows camera at the top?

I already mentioned the significance in the beginning of this lecture that when we are viewing biological samples a cultured cell or tissue sample, the cells always have a tendency to settle at the bottom and on the top is the turbid medium that is the phosphate buffered solution which one such solution for culturing would remain at the top and this creates a turbid medium for us to view. Because there at the bottom it becomes very difficult to light through penetrate and the reflected image capturing the reflected image would not give us the right features.

So, this is how the turret has been located in case of a inverted microscope. A major difference between this and the regular microscope is this the position and why it is called as an inverted microscope. Now that we have seen just like the others it is housed with objective lens of different magnification So, here we have 4 X magnification that is 1 and then another 2 which is 40 X magnification and then 3 20 X magnification and then the 4th one which is 10 X magnification. So, each of this with the required working distance can be set depending on your use.

Now that we have seen how the turret sits in case of an inverted microscope. This is the stage and this is the sample holder. Like the previous one, this stage has knobs. You could move it to the right X axis to the move it to the Y axis. Let me just demonstrate, it could be moved also in the Z axis. This way your stage can be adjusted and then these additional knobs here are for adjusting your focus. Once your magnification is fixed, you could adjust your focus using these knobs which are on put the size and for fine tuning coarse and fine tuning and the light source and the switch.

Now that there are 2 eye piece each of this eyepiece like the previous two also provide 10 X magnification which remains fixed and can be adjusted for proper view. And this is the light source the light source here which is housed and this is the arm supporting the entire structure and the display here the camera connected to the display via the USB port.

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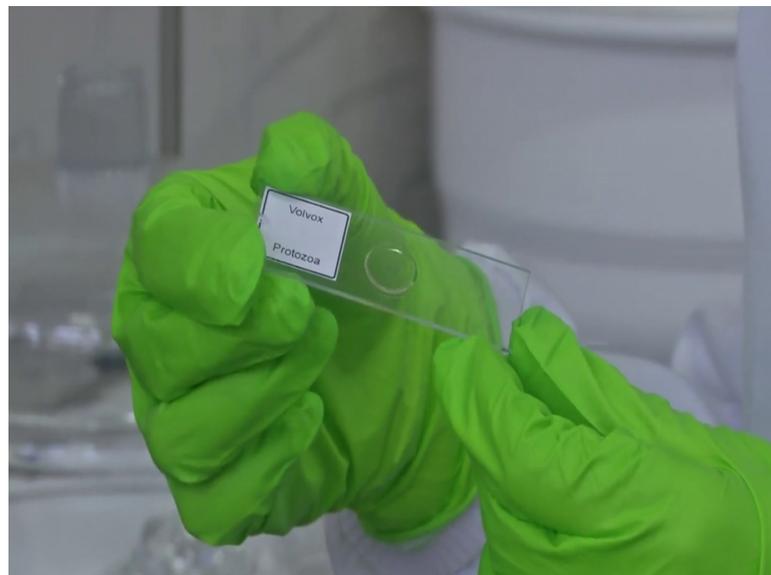
Now, this what I am holding in my hand is where you can house or you can fix your slides. Since we know we are talking about biological samples so, they could be the petri dish which you want to study the live cell imaging under the microscope or it could be samples which are smeared like in the lectures what Professor Hardik has mentioned. You would take biopsy, you could do needle aspiration. You take out these samples smear them cytology the term which is commonly used extraction of the cell samples, smear them on this glass slice and then they are studied under the microscope.

So, this is what is the psychology and then the path cytopathology is studying the diseases that are associated with these alterations in cell or tissue structure. All of this has been covered in the course the regular course by professor.

And now let us see how we could practically use the glass slide and then study them under the microscope. Along with this stage this adapter which could be used on the stage, there is another one here where you could place your petri dish and this is a larger area. It could be a 100 mm petri dish or say if you are using a 35 mm petri dish and then you could use this for proper housing of your dish or slide on this and then fix them on the stage. And all of this are sitting on an anti vibration table.

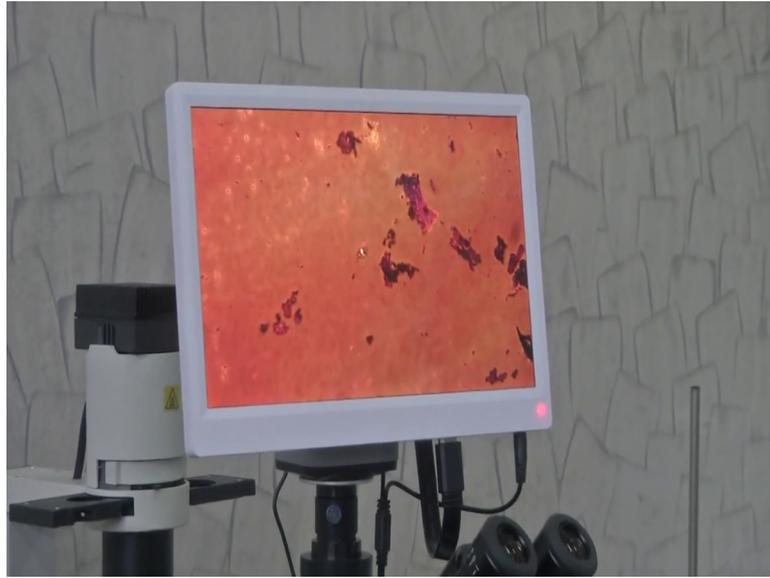
It becomes very important for these microscopes to be vibration. If they are susceptible in the in the readings what you see. They could be easily altered because of vibration or any other changes. In order to ensure nothing else the other parameters do not affect your research or study all of these are mounted on an anti vibration table in the facility here.

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Now that we have these two, let us say I have a glass slide. Let us say this is one of the protozoa slide which is smeared on to the last slide and we would want to study the features of this protozoa with the help of the microscope. Now, let me use this, place my slide on this and then fix it on the stage. So, let us focus them.

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So, these are the features; so, these are the features which can be seen using this slide. Now say I use another one with a different color stain. So, this is the blood cells smear and place this and let us see. So, this is more reddish in color. If I find unit you could see the fine features which are on the slide, like you can see now that now that we can see that there are different samples which can be tested the biological samples for now I have just showed you how a smeared sample on a glass light can be viewed using this inverted microscope. You could also use your tissue culture petri dish the live culturing can be seen using the inverted microscope.

So, there are multiple options which are provided here like you could actually the display allows us to live record features or even captured images which are seen the feature. In this feature of capturing images is also there in the stereo and the metallurgical microscope which are shown. And if I click on this, the image is being captured. If you can see, the image has been captured and you can use it for future reference and there are other setting features.

Like you could see you could adjust the contrast, you could adjust saturation, you could also play with other options the brightness, sharpness, noise reduction and you could even record. So, this here shows a record option which will live record whatever is displayed under your whatever is there under your lens. So, when does that become important? Like I said when you are studying cancer cells which are highly invading and

you want to see the cell growth or you want to understand the different parameters of live cell imaging or when they are when they are in petri dish cell culture techniques, the entire petri dish could be placed in this lot and you could do the recording study them study their properties study their invasive.

So, it allows you to do a lot of research on how they are you know the growth factors would affect with different environment condition. You could also study them with altering their the environment properties. All of that can be studied using the inverted microscope.

So, now that you have seen the utility. You know the right choice which right choice of microscope; if you are using a biology sample to study, then go with an inverted microscope and if you are having an opaque object like silicon wafer or any other metal coated device, then you have to choose the metallurgical microscope and say you just want to study the during the overall physics. The physical properties of the device which has been fabricated get a proper 3D depth perception then you have to go with the stereo microscope. So, this was the intended talk for today.

Thank you.