

**Fabrication Techniques for Mems-based Sensors: Clinical Perspective**  
**Prof. Hardik J Pandya**  
**Department of Electronic Systems Engineering**  
**Indian Institute of Science, Bangalore**

**Lecture – 42**  
**Biomedical and Electronic Engineering Systems Lab**

Welcome, today we will be covering microscopy that is a very fundamental part of characterizing any device or sensor that we will fabricate. So, when you look at microscopy there are fundamentally two basic types of microscopy, optical and electron.

For a normal basic level lab setup you usually have a optical microscopy arrangement in inside your lab. Electron base microscope is are both very costly and consumes lot of space to put in a lab. So, usually they are they have they are provided a dedicated facility. And so right now in for the purpose of this course we will not be covering electron microscopy we will be looking at optical microscopy.

So, within optical microscopy usually in lab we will have basically 3 different types of microscopes, one is stereomicroscope, another one is metallurgical microscope and the third one is called inverted microscope. We will be going through the basics of how to operate and why what is the use of each of this microscopes in detail in the duration of this course.

So, primarily as you as I have as have set it is basically optical microscopy. So, it works on the principle of either transmission of light or reflection of light. In the 3 microscopes that I have mentioned the metallurgical microscope and the inverted microscope works on the transmission of light. And metallurgical microscope as a name suggests it is metallurgical, so something to do with opaque bodies and metals or metal based or something that is derived from the soil like, silicon is also derived from the soil silicon wafers.

For the purposes of this course silicon wafer is more relevant and generally for metallurgical microscopes are used to view opaque samples. And inverted microscopes are primarily used to see biological specimen usually tissue culture dishes, slides, glass slides, blood samples for all these things inverted microscopes are used.

So, to begin with we will not going to both of this microscopes metallurgical and inverted we will start off with a much more basic, but very profound microscope called the stereo microscope. So, as you can see the stereo microscope is here I will be showing it now. I am sitting in front of the stereo microscope in this lab so, there are two ways in which an optical microscope can be used, I have told its either transmission based or reflections based.

A stereo microscope works on the principle of reflection of light and it is used to analyze samples like dissecting specimen, looking at the general features that are present on your sensor at a slightly macro scale. It does not go into that much detail like 100 x magnification, 200 x magnification and all. But it gives a good idea of how this how the final sensor or the device is, as also gives your depth perception and final details which cannot necessarily design from your like normal vision.

So, how it works? So, it sends there will be a there will be usually be a light source and you will be keeping your sample on a sample holder or a platform that is flat. The microscopes by themselves should be placed on top of something called an anti vibration table. This is because when you go into very high magnifications like 100 x or 50 x even small perturbations on your table will cause your sample to vibrate and you will not be able to capture your images or view the microscopic images with that accuracy or its sharpness.

So, the sharpness we will get affected. That is why it is very important that you use microscopes on a vibration free anti vibration table. So, we are also using in our lab microscopes on top of a anti vibration table. Now, we were discussing about stereo microscopes. So, there will be a sample you will be placing it on a sample holder, then there will be a light source the light source can also be of different types, when I show you the microscope and it is different knobs I will show you the details about the light source. So, the light source will emit light or shine light on top of the sample and this light will get reflected back.

This reflected light is captured by two optical parts basically here the eyepiece. So, the any of any microscope will have an eyepiece that is where that is through where you will view your samples.

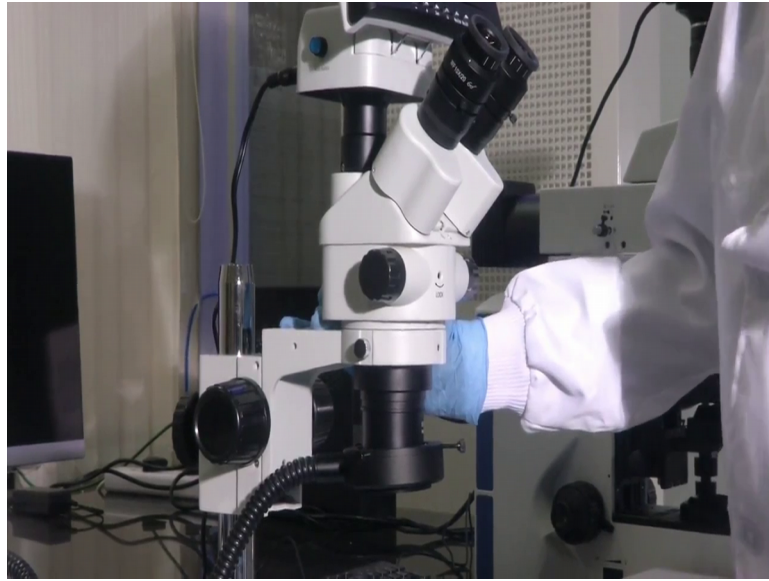
So, the eyepiece takes in this two optical parts and a slight difference. This slight difference causes 3D perception. Just like our eye works, so our eyes are usually referred to as binocular vision so it just because we capture light independently from using two objectives. So, our eye is an objective, so we capture light reflected from my a body from two independent paths, because of this these two independent paths we will get depth perception.

Suppose you close your one eye and try to see you will not be able to feel the depth or how far objectives away from you, you will only be able to see a 2D image. It is because your having two eyes that you are able to see a depth and perspective view comes only because you are having two eyes and that is called binocular vision. There are many organisms that I only one eye and they are evolved in such a way. So, that is disc that is a discussion for a later date. So, the stereo microscope uses a similar method.

So, the light reflects from the sample and it is captured through two distinct optical paths to the eyepiece. So, when we so we get light just like we get light on to our eyes we get light onto the eyepiece from two a optical paths, because of this and because of the extra magnification that a microscope provides you will be able to see your sensors or your devices at in a 3D perception view and also in a zoomed in magnified manner. This is the basic working of a stereo microscope.

And this is how it differs from normal metallurgical or inverted microscopes or it is called compound microscope, the normal microscopes, they work on the concept of transmission of light, we will be covering that shortly. So, let us first look at how the stereo microscope looks like, I will run you through what are the different knobs what are the different parts of the microscope.

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So, this is the stereo microscope that I was talking about. So, this is when you look at it looks like a when you; if you have seen other compound and complex microscopes when you look at the stereo microscope we will be attracted by its simplicity. So, let me run you through what are the different parts of it.

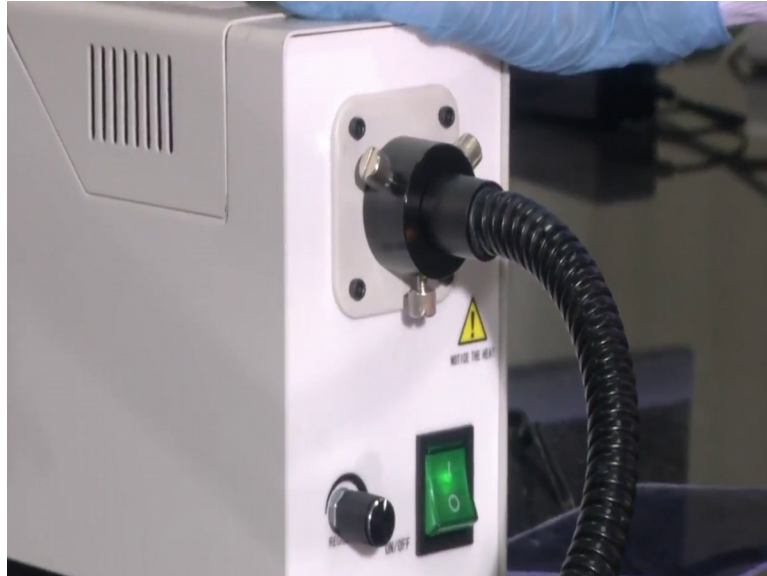
So, this is the sample holder platform that I was talking about, this is where you will keep the sample it is a very flat surface and this surface itself should sit on an anti vibration table preferably. So, there are two clips that you can see these are for a holding the sampling place. As I told before this is for microscopic macroscopic view of the samples, just to get an idea of overall how the sample looks like; it is slightly better than how our normal vision can see. So, the sample holders are enough to keep the sample in place. When you see the other microscopes you will see much more complex arrangement to keep the sample in place.

So, these two clamps we will helps him keep the sample place this is a sample holder. So, these are these two knobs as you can see these two are the eyepieces. So, these eyepieces by themselves have a particular magnification so, that is called 10 x. I am not sure if you can see this, but in the eyepiece it will be written so, at the eyepiece itself has a magnification of 10 x and it is through this eyepiece that we have to view.

Now, coming to the black color attachment that you see here, this is a major light source for the microscope. So, this light source it will shine light onto the sample. Let me just

switch on the light and see how this comes up. So, now, you can see the light intensity has gone. So, if you just see it, so there is a wire attached cable attached from the light source that this is where the light is getting emitted, but the source is not here.

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So, there is a cable attached it goes to this unit, this is the light source, it is this unit that generates the light source of a part. So, you will see the it is just a light source why do you need this much big occupant. This is because, it will it is engineered to produce light of particular frequencies only so that you can have a quantification of what you are seeing, because if you put visible light at all frequencies on your sample you are not sure it is it generally does not matter in microscopy, but then you need a complex arrangement. So, that you are light sources of good fidelity or clarity clean signal should be there has a light source so, this is one type of light source that we have.

There is another light source which is LED based. As you can see, so this is the LED based light source just like this black circular knob that is attached you can attach this white color knob also. This is the LED strip if we can attach it here and it will produce LED light. Just like here, so this is the power on off for the light source there is a power cable which you connect here you will switch it on or off and then you can control the intensity of your light like this. Similar arrangement is there for the currently connected light source. If you see the source ok, I think now you can see the source this is the power on off button for the source this is where the life finally, enters this tube.

This is like an optical waveguide; it will take this light take it inside and bring it to the microscope then we have this knob here. So, if you adjust this knob it will adjust the intensity of light that is falling on the sample. This we can use to adjust the contrast brightness and all at which you want to view your sample. So, it can go up to maximum very good brightness and you can control it to the bare minimum, it is almost like there is no light. So, this is how you can adjust the intensity of a sample.

The same arrangement is there for the led based light source also. So, light source is a very important component of the microscope that is why I am let us speaking little extra about it. So, this is the light source. So, that part is covered now, we will keep the sample here light will fall on it. So, as I told this light will get reflected it will get reflected it will enter the optical path and come through to the eyepiece, clear. So, whatever the eyepiece is seen let us just get focused back to the microscope. So, whatever the eyepiece is seeing same image is captured by a camera that is placed here.

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Here a camera is place if you can see my hand, I have a blue gloves put up. So, you I think you can see my hand here. So, camera is placed here so, this is the camera and cameras on off switch is here and we have attached display on to the camera. So, this is a display to the camera, whatever we are seeing through the eyepiece we will be able to see through the display and in the display you can separately adjust the brightness contrast and so that we can see the image property.

Now, next main thing the main thing for you to capture image of a device is to first put the image in focus. So, to adjust the focusing there are knobs provided here for focusing when you adjust these knobs the compound setup consisting of a light source eyepiece camera it moves up and down. While do you do this I will I will be showing shortly you will be able to focus your sample.

Now, there is another knob provided that is there only for a stereo microscope is back side there is a another knob if this knob we have to use very carefully, I think you can see it this knob if you loosen it you can actually rotate your whole set up like this. And you have to be very careful while handling microscope these are usually very expensive equipment and you have to take extreme care while handling them and now it is locked into position ok, these at the major knobs.

Now, another knob is at what magnification do you want to see it; at what magnification? Because this knob only takes care of the focusing correct. So, there is another knob here this black knob that is used for what is the magnification that you need, this IPs will give you 10 x magnification on top of that how much magnification do we need.

So, the options available on this microscope are one which is the default from the eyepiece whatever magnification you are getting that you will get, then there is 1.5, 2, 3, 4, 4.5 say up to 4.5 magnification you can get. So, for you to focus your sample we have to play with both this magnification and this movement so that your device comes into focus.

Now, let us actually see device so, let us take a device now. We have several sensors fabricated in our lab with us we will show you how we see one of the sensors. So, this is a wafer holder this are used for getting a smaller wafers like 3 inch wafers and all, and then let us take one device. So, we were thinking, so I showed you the wafer holder where we are carrying small wafers now I have taken one sensor from that and I have kept it on the sample holder as you can see sensor is there. So, it is a series of like electrode structures or you can say micro heater arrangements with their respective of pads.

So, as you can see if you can see some flower kind of arrangements matrix type on the sensor there is what you will be seeing. Now, but you will not be you are not able to see any details right on this so; this is where the stereo microscopes effects come into

picture. So, always you have to handle your wafers preferably with that tweezer and if you are wearing gloves it is still ok, but please never handle your micro sensors with your bare hands because, it will contaminate the sensor and all your effort will go waste.

Now, so, we have adjusted the what you called focusing and the magnification to such in such a way that you will be able to see one sensor arrangement, it is an array of sensors in the wafer one sensor array, you can see in the display so, let us see another one.

So, as you can see at the middle there is some disturbance. So, there is because which is coded with the sensing material. So, these sensors are mainly for something like gas sensing applications so, for the gas sensing applications at the main core area of the sensor you will put some sensing material that will react to the gas in question and change its properties, this change in property of the sensing material is then used to detect that particular gas.

So, and this big squares that you see on this monitor these are the pads from which you take out the signal then you have further signal conditioning circuits and data acquisition circuits. So, this sensor we will actually so, this winding path that you are saying is the electrode you can say for the sensor and the sensing material is put on top of that sensor.

The sensing material will then react I hope you can see it see in through the camera display, the sensing material will react to the gas, changes properties which is picked up by this network of the metal and then that is again captured through the pads which are shown here. So, let us see another sensor, it is an array of similar sensors or with some sensitive material put here.

So, suppose let us say I adjust the height then this it will go out of focus. So, display, just look at the display so, now, it is going out of focus see you cannot see anything. So, you have to always adjust your size and your magnification to such an extent that you can see it now let me come out, let me zoom out so that we can see more than one sensor. See now you can see more than one sensor at a time. Then now again you have to adjust the height in such a way that you can see it most effectively, see this is now going out of focus we have to bring it back to focus.

How do you know whether it is in focus or not? You look at only some sharp line that is there, you have your ability to make out the sharp line that means, that your images in your



camera is in focus and your objective is in focus, you have to capture images after that. So, now, you can see the matrix of sensors, we can just move around the sensor with your tweezers and then you can see other sensors that are there, hope you are all able to see this. This is a sensor which we have already used for testing so, it has slight amount of what you call rough places because it has already been used. So, you want to show your used sensor so, these parts are where the sensing material has reacted with the gas.

So, this is how you see, you I think now you understood the utility of this microscope. This is not for analyzing the nitty gritty details of your sensor, you just want to see you have designed a sensor, you wanted your electrodes we placed here and after sufficient distance you wanted your pads to be there. And then you wanted your sensing distance to be within this much distance of your electrode.

For you to get that macroscopic design limits that you have set for your device you need to look at this anyway this arrangement you cannot see from your bare eyes. So, you need to use some kind of an equipment, that is where this see now I have focused into the pads, now you can actually see how the pads are, that the pads adjust or nice or not, are there any rough surfaces at the pads adjust, are there any shorts, shorts in the looping that is going on. These things you can see this is where the utility of a stereo microscope comes.

This say, this I am talking from a micro fabrication or a sensor fabrication point of view same rule applies to another application of the stereo microscope which is dissection. Suppose you have taken an animal like a cockroach or a frog and you are dissecting it, instead of seeing it just with your bare eyes if you dissect it under a stereo microscope. You just need to look at this camera screen or through the micro or to the or through the eyepiece and you have to and you can dissect your specimen this is the main utility. So, stereo microscopes evolved like that, so basically for that. This the name basic name is stereo microscope, but when you can see 3D perception through the eyepiece because, this is the 2D image you are not able to see the 3D perceptions. If you look at it through the eyepiece you can actually see a 3D view of this then if this microscope offers that such kind of a view then it is called as 3D stereo microscope.

What we are showing you now here, this is a 3D stereo microscope, before this came into market there are other designs called stereo microscopes, these are used for

dissection. Just now we saw one sensor where which had a gas sensing material and it is pads, now the we have another sensor that is we have kept on the platform. So, this is these are actually micro wells with an electrode or a pad underneath it so, let us see it.

So, if you see the screen you can actually see two wells and this actually a 3D this circular pattern is a opening of the well, that well goes into these pads, these contact pads. And you can actually probe them and see the difference. So, when you see the eyepiece you have to see through the eyepiece and you can see what is actually happening. So, when you look at you through the eyepiece you can actually have a 3D view of the sample.

Now, let us focus on one particular well so, I am going to higher magnification then I am adjusting my sample so that one well comes into view. See now one well has come into view, now let us focus it so, now, I have focused it. So, the circle is the well here you see a part of a square which is the material that is underneath below that well and you can see continuation of that. Because, this well is made of a transparent material you can see this part is actually surface this is a well it goes into the bottom at the bottom we can see this material and materials continuation you can see through the transparent surface.

Like this you can analyze how whether your the radius of your well has become correctly, your pad how much of your pad is actually coming under the well, with whether I need to improve your design, is there any contamination from your this from your fabrication process or is there any contamination from your repeated usage. So, see here there is a lot of rough patches here with this is, because we have used the sensor many times now it has become like we unusable.

So, you are just showing in for academic purposes here. So, these parts have become rough with repeated usage. So, that is why usually sensors we use for 2-3 times only and that is also why this fabrication process is very expensive. So, you have to take extreme care and make sure that you are controls and you your experimental plan is solid. So, that you do not waste the sensor that you put so much cost time effort into fabricating.

So, this is another well you can see again the material that is below, I am just running you through the different wells that are available. See, this is a multiple well three wells have merge together let me just zoom out so that you can see it properly. So, this is two send, two pads are there here and here and there is a bigger well. So, it can accommodate

more sample. So, usually we usually use say, let us say for the sensor we use liquid samples say cells in liquid or some other chemical that is dissolved in another solvent that we can load on this well and see the changes in their properties like impedance, capacitance etcetera so, that is how these sensors will be tested.

Before we test as I have told we have to see whether whatever we have descent has come, for that stereo microscope is the best, especially 3D stereo microscope is the best option that you have. So, in the next module we will see the other microscope which are more conventional microscopes called the metallurgical microscope and then we will see the inverted microscope.