Techniques of Material Characterization
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Module 01: Introduction to Microscopy and Basics of Optical Microscopy
Lecture-05
Components of Optical Microscope

Welcome everyone to this NPTEL online certification course on techniques of materials characterization and we are in the 5th lecture of module 1. And today we will be discussing on components of optical microscope. We have covered yet the basics general concepts of microscopy and then some basic theories resolution, magnification, aberrations and so on. And then we have also covered introduction about optical microscope and how image forms on an optical microscope.

And today we will be discussing on hardware part of an optical microscope powered which are the elements, which are the components of any optical microscope.

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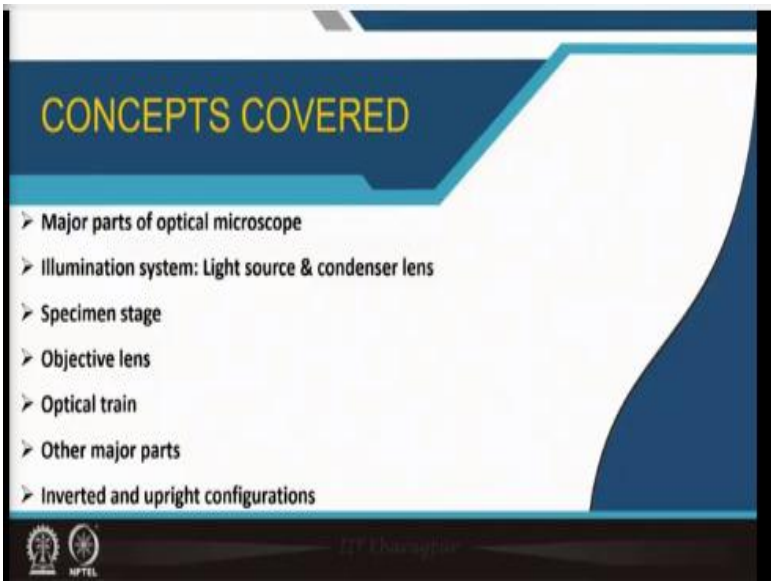

So, these are the contents that will be covered today and it will have something of this few things. Major parts of any optical microscope then the first thing will be illumination system, there we will discuss about light source and condenser lenses, specimen stage, objective lenses,

optical train, few other major parts. And finally we will be discussing about two important configurations of any optical microscope.

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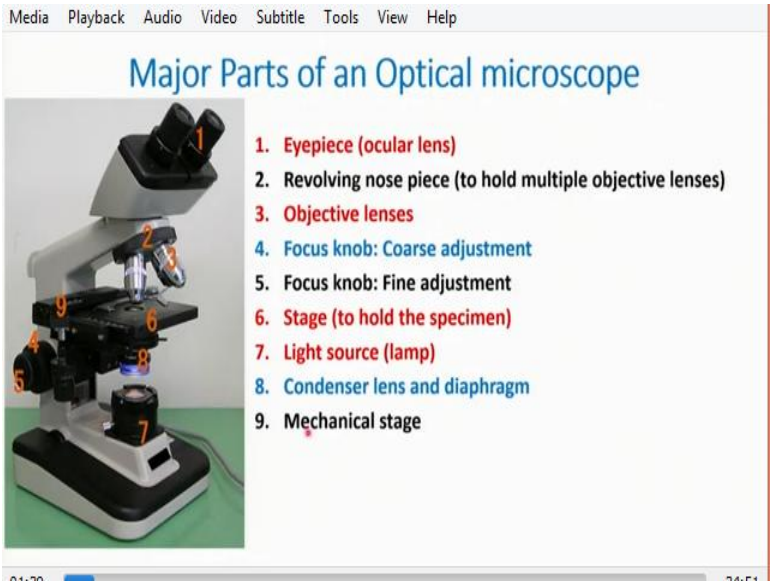

So, these are the major parts of any optical microscope, the first thing you will notice and this is the very simple type of optical microscope. There are many very complex microscopes which you have seen in the last lecture that few research microscopes which can be very, very complex today also we will be showing them. But this is a very simple design and simple geometry of any optical microscope but most of these components are there even if it is a very complex research microscope.

So, the first thing you will notice is this eyepiece through which people will you will be able to see the specimens after the magnification. Second thing you will see is this one, this is the objective lenses and this one is the objective lens turret this holds the objective lenses revolving nosepiece. And third thing is the objective lenses here then you will be seeing the focusing knobs then by which you will be basically controlling the movement of the specimen state.

So, there are 2 major focus knobs you can see first one is at for coarse adjustment that is usually the bigger one and then you will have a smaller one that is a fine adjustment. Next is the stage over which the specimen will be kept and this is in the transmission geometry that means the specimens are transparent the light source is from the bottom and the objective is from the top.

So, there is a hole we will notice we will discuss about the stage and objective lenses in details in a little while, but this is what this position of the stage here. And then of course then you will have the light source and then there will be a condenser lens and condenser aperture system that will basically direct take lights from the source. And then they put in focus the light and make that light fall on the specimen here.

And this entire assembly will be kept under a mechanical stage or all this frame is for holding all these different parts of an optical microscope.

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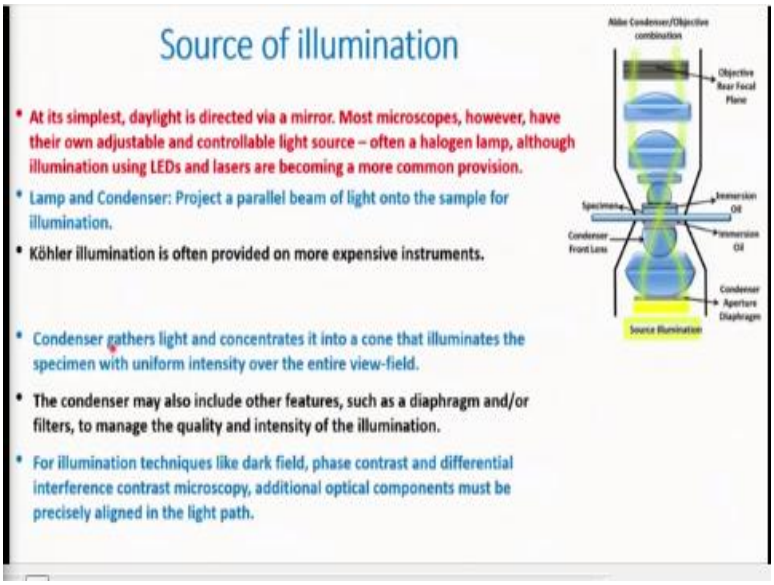

So, the first thing let us discuss is about the illumination source. So, if yesterday in the last class we have discussed about the various type of microscopes, simple microscope, compound microscope and then research microscopes all of them. So, in the simple or in the compound microscopes, that simple microscope we have discussed, that magnifying glass is a very good example of it.

And then compound microscope is the typical one that you have possibly seen in your +2 in the biology experiments. Both of them they physically use the daylight and that daylight is then through a mirror the daylight is made to fall on the specimen, this is what a very simple

geometry. But most of the research microscopes that we use these days these are all have their adjustable and controllable light source, we no longer depend on the daylight as a light source.

And most often these light sources are halogen lamps or sometimes LED'S these days mostly it comes as a LED or even laser lights. So, each of these has their own advantages and disadvantages that we will discuss. But basically these are the very common light sources that we use. And imagine remember one thing that the source of illumination contains two components not only one and one is the light source and second one is the condenser lens system.

So, the light source is here this is a source of illumination and then you have the condenser lens system. And as I discussed earlier, whenever you think about a lens there should be an aperture diaphragm or they should mean aperture simply. So, this condenser lens and condenser aperture collects the light from here and make it fall on the specimen. So, all together you can imagine that this is the source, this total condenser lens and the light source this together works as a source of illumination.

There is a special type of illumination we will discuss about that that is called Kohler illumination and this is provided mostly for research microscopes. So, condenser as I said basically gathers the light from here and then make it fall and condenser also controls the amount of light and also the condenser aperture also control the size of the spot in the way the numerical aperture basically and the resolution is controlled by the condenser lens to from extent.

This is most general light illumination system and this one is true for most of the microscope most modes of the microscope. So, we will be discussing possibly in next classes, we will be discussing about various modes of light, various modes of optical microscopes. The basic one is of course the bright field microscope, which we were discussing in the last class. There are other modes which are called contrast enhancement mode like dark fill mode, phase contrast mode, interference contrast mode, polarized light microscopy etcetera.

In those cases there used to be some additional components which are put in the before the light reaches to the specimen. So, the in the source of illumination in that system itself there are some

additional components that are put because that is a requirement for that particular mode. So, we will discuss all of this there plus this condenser may also have different type of filters at times we you want to restrict the kind of light you make want to put on the specimen. So, various filters, various apertures all of this are part of this condenser lenses.

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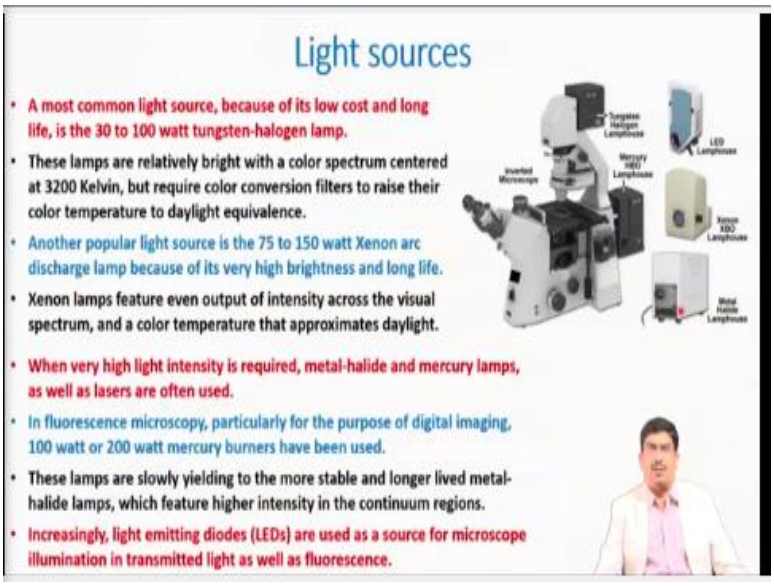

So, first thing let us discuss about the light sources and most common light source which used to be use in good old days was this simple 30 to 100 watt tungsten halogen lamp. And this is pretty much the same halogen; same tungsten wire kind of lamp and they are this thermionic effect will happen that will produce the light resistant setting will be there. And it will produce the light.

So, it is basically the same kind of light or same kind of light bulb that used to be used good old days in our household purpose. So, it is very typical a variation of that is typically used for this purpose. And these lights are pretty bright of course and colour spectrum is usually the temperature of the colour is around 3200 K that is what the measure of the colour in terms of how much or largely speaking how much it is closed in like daylight.

That is the measure of that is that like what is the temperature of the colour or rather if you take a tungsten filament and heat it up at what temperature that kind of colour comes up basically simply or in crude language this is what it is the colour. So, but the problem with this one is the halogen lamps and possibly you have noticed this how bright and yellowish light it generates,

that light is not very close to the daylight equivalence or it is daylight equivalence is very poor that is why you need to use some kind of a filters for this.

And this daylight equivalence is very important for any kind of microscopy because our eyes are sensitive; our eyes are very much used to of seeing things under daylight. So, the light source that is used if that is close to the daylight, what the light temperature of daylight that is better for our eyes that is the point. So, that is what the problem with these tungsten halogen lamps. The next source of this is the xenon arc discharge lamp 75 to 150 watt xenon arc discharge lamps and this produce a very high brightness and very long life.

Also this xenon arc lamp that temperature of that light that is produced is very close to the daylight. So, this is why xenon arc lamps are a much better option for these optical microscopes. The problem is this bright intensity is ok more or less ok if you go for a brightfield microscope, but if you go for a contrast enhancement techniques and then like dark field like interference contrast.

In that case you do something with this light itself and you chop up most of the light intensity. So you need even higher intensity for those contrast enhancement modes and then you go for something like metal halide lamps or mercury lamps as well as sometimes lasers are also used. Lasers are used for some very special purpose confocal laser microscopy or state microscopy and so on, those modes the laser light is used that has it is own advantages.

Then another mode is there, that is called fluorescence microscopy, we will we will discuss briefly about that fluorescence microscopy. And fluorescence microscopy basically for that you need a very special type of light because they are the phenomenon with which this entire microscopy works fluorescence is very really sensitive to the light wavelength of the light. So, a very special type of lights are needed for fluorescence microscopy.

So, in general the microscopes which has a fluorescence mode typically used to have two different light sources. So, one is used for brightfield, dark field and all other modes and one is typically used for fluorescence mode that is what it shown in this image as well. And these days

increasingly people are using LED's for both fluorescence and brightfield of it, LED's has it is own advantage, it is bright, the control is very high.

The kind of wavelength of the light that you can generate the intensity that you can generate, the colour that you can generate all of this can be very nicely controlled by an LED light source.

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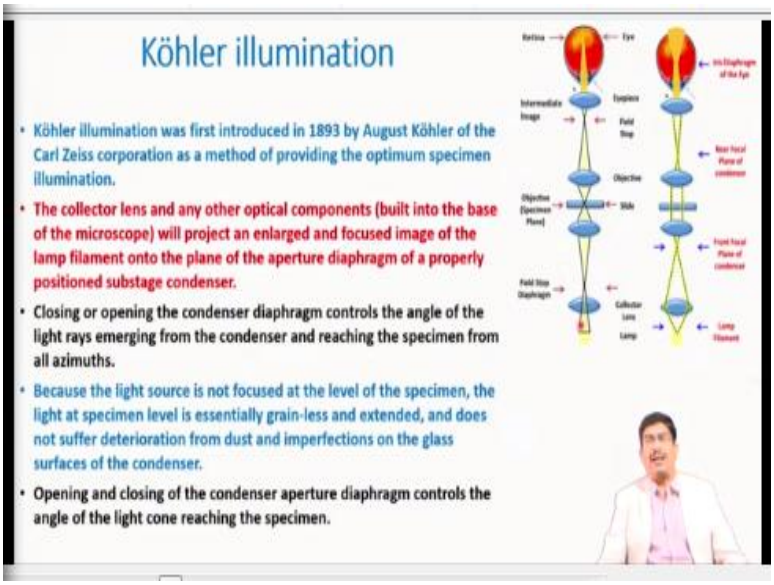

So, modern microscopes many of them are coming with LED lights. So, now what about the Kohler illumination? So, Kohler illumination is something that was discovered by a person called August Kohler, he was working in Carl Zeiss and it is a proprietary method used by Carl Zeiss for a very long time. And in this case what you do is that in between the light source on in between the lamp and the condenser lens there is another lens that is used, that is called a collector lens.

So, this collector lens what it does is that, it captures the light coming from the lamp and make it that light to focus at a point before it reaches to the condenser lens. So, basically what it does it creates a virtual image of this light source and that base works that virtual source basically works as a source for this condenser lens for that for all practical purpose this is the source for this condenser lens and upward.

So, the advantage that happens in this case is that the brightness you can be controlled and can be further magnified from the lamp the brightness can be further magnified by using this collector lens here. Also the second thing is that the light that passes through from the condenser lens if it directly falls directly condenser lens directly takes it from this lamp and makes it fall on the objective lens that light will be very much gray kind.

So, because it suffers scattering of whatever medium is there in between. Since it is first collected by the collector lamp. So, this and this is usually works like a virtual source that grayness problem, scattering problem will not be that much in this case. So, this is what is called the Kohler illumination and this can be used both for transmitted mode and reflection mode. Also but this is of course this comes with it is own cost and that is why this is only used for a very high end research microscopes not all time you will get this Kohler illumination in this case.

And here also the in the collector lamp, so additional one more control you have is that you can use an aperture here in the collector lamps and there you can sort of control that how much what will be the size of this source itself. So, in that way you can again control the size of this beam here after it passes through the condenser lens. So, you can somehow control on the resolution that you can achieve here.

Also through the collector lamps by changing the collector lamps you can either make a beam to focus by this condenser lens, it can be either focused on the objective or you can make a parallel beam to pass through the objective whatever is your pass through this specimen, whatever is your purpose you can basically change the way the lights are falling on the specimen just by using this collector lens and condenser lens together. So, this is the advantage of Kohler illumination.

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So, condenser lens system is the second one as I said after the light source, the purpose of the condenser lens is that the lights are the light rays are organized and precisely guided through the instrument that is what the condenser lens does. And this is the most important controllable variable for a light source. So, there are many variables of course but this is one of the most important variables for achieving very high quality images with adequate resolution.

So, all this resolution of all we are talking about is controlled by this numerical aperture of this condenser lenses. So, they basically controls the beam size and in turn that beam size will control the resolution of the optical microscopes. And in this case it also controls of course the brightness of the beam and usually the kind of laser source as I said the the kind of light sources that we use.

There is no problem with any kind of numerical aperture you use. There is no problem with brightfield mode, so long as you are in brightfield mode intensity usually is not a problem. The moment you go for a other kinds of contrast enhancing mode then the brightness is a big question and there the condenser lenses are very, very important situation, this. Of course the condenser lens also has not only a lens, the aperture.

And that aperture basically controls the semi angle and in turn, that aperture controls the numerical aperture. That numerical aperture of this condenser lens system and in turn the

numerical aperture also can also control the field of view for this condenser lens system here. If you notice this one as the numerical aperture is going down, the field of view the beam that is coming out of it is also smaller and smaller. That means you are using a much more small aperture here. So, intensity can also be controlled while controlling the numerical aperture, so you have to be very careful about this**.**

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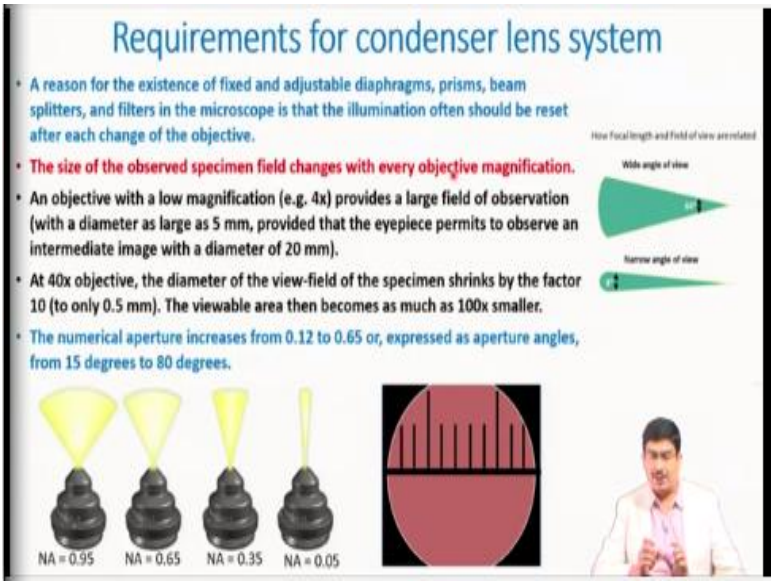

The next thing is the requirements for condenser lens system and here there is a relationship that exists between the condenser lens and the objective lens. And that relationship is because the size of the field of view for an objective lens is changes with every objective magnification. And that means if you are looking at something at say at 4x magnification and the diameter is around 5 millimeter.

Then if you increase this magnification by a factor of 10, if you are going for 40x objective magnification, then what will happen your field of view will reduce by 10 times. So, field of view will be 0.5 mm and then this means there is almost 100 times decrease in the area of this viewable area. So, the condenser lens also has should respond to this by changing it is numerical aperture.

And this is something similar problem like the one we discussed in terms of empty of empty magnification. So, if you are increasing the magnification then your resolution also should

increase. And as we already said the resolution is controlled by the numerical aperture of this condenser lenses. So, condenser lenses should be producing a beam with as narrow as possible of something of high numerical aperture in order to get a better resolution when you are going for a higher magnification.

So, they should respond to this magnification as well and that in some sense economical as well. Because if you are going to higher magnification by changing the objective magnification, then you do not need to illuminate the larger area as well, a small area will be good enough for because your viewing field of view is also reducing. So, this is the kind of change that the condenser lens system should produce, if you are changing the object magnification as well.

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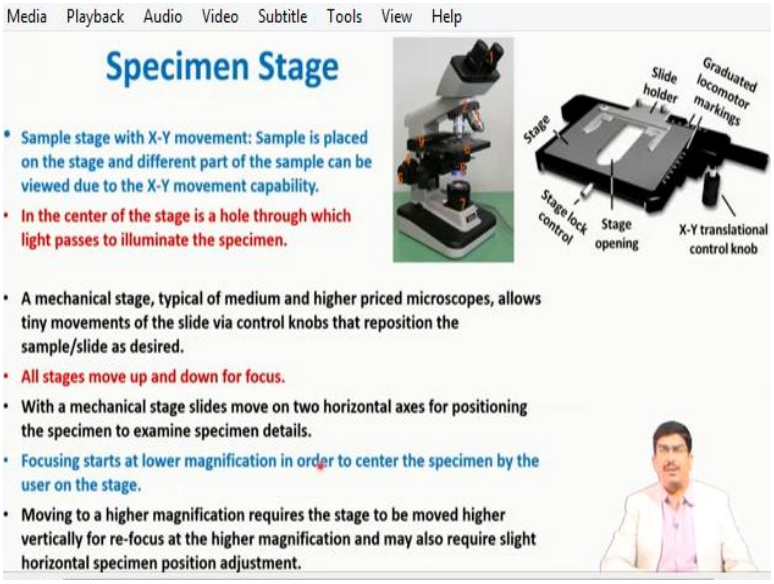

The next thing to discuss is the specimen stage. So, specimen stage basically as the name says suggest that it is there to hold the specimen, the specimen that you are or object that you are going to see. So, this specimen stage primary purpose of this is to produce the support to any specimen stage and it should produce an X and Y movement in horizontal and horizontal in this plane, it should produce an Y movement as well as an X movement.

So, that you can change your field of view, you can see different parts of the object specimen and this is usually done by this translation come the control knobs here. This is shown for a manual mode but there are specimen stages available which are motorized completely motorized. And in

these cases, the X and Y movements are can be controlled using software and there is a big implication for that if you want to do something called montage.

If you want to take sequential images and which is similar to something like a panorama image, which you do in your cell phone. If you want to do similar things in case of an optical microscope, you need to have a motorized stage where X and Y moments are completely controlled by a motor completely controlled by a software. So that one kind of it is a special kind of stage that is there.

The another purpose of the stage is basically it helps in focusing. So, as I said the object distance this basically here you have this image distance and object distance focal length is fixed. So, now to bring this object into the focus of this objective lens, the only way you can do that is by changing this is by making this specimen stage to move vertically. And that is how you can bring the object into the focus of this objective lens.

That is another purpose of this specimen they usually have a hole in the middle if you are looking them if you are using a transmission mode. So, it is usually for a transparent specimen. So, you need this hole here, so that the light can passes through from the bottom. And if it is in a reflected mode, of course you do not need this kind of a hole but there are other kinds of geometry which we will be discussing called inverted microscopes.

There also you need this hole here. So, you generally start the focusing at a lower magnification and to say just to center the specimen and then you slowly go up at higher and higher magnification and there you take. So, first you do the focusing by changing the coarse now. And then slowly as you are increasing the magnification you are changing you will be doing this focusing by this finer knob, so that is what is the purpose of a specimen stage.

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And the next thing to encounter in the optical microscope is the objective lenses. And objective lens is perhaps the most important component in any optical microscope. And that is possibly the most costliest part as well just because of the optical your cost can be a very much it can be an order of magnitude reduced if you are changing the objective lenses. If you are taking just low magnification objective there usually used to be a set of 5 magnifications.

If you are taking only the smaller ones smaller magnification once cost will be something. And if you are going for the higher magnification objective lenses the cost will be completely different, huge difference will happen, that is how much this objective lenses are important. And that is because these objective lenses are responsible for primary image formation. And also these are the first components that lights are encountering after they are coming out of the specimen.

So, first the condenser lens produce this light, that falls on the object to falls on the specimen and from there this lights are coming and encountering this objective lenses that is the first components, and it also controls the magnification that is why it is very, very important as well. And this objective lenses are all mounted, so as I said they are usually there used to be 5 such objective lenses with different, different magnification basically different, different focal lengths and different, different numerical apertures.

And this one is usually kept into this neck piece into this piece one piece and the this is called this nose piece, this is called a turret and this turret objective lens turret it by moving this in some it is manually moving you have to use your hands to move one objective lens and bring another one. And in some cases with this also used to be motorized, you can just simply use a software to change your objective lenses.

And one important thing you can notice here that this objective lenses, this is at 4x and this is the 100x objective lens. So, you can see the difference in their length the difference in their height that this one produced from something like 4x all the way if you are going to 100x. And that is because these objective lenses another purpose these are. So, this is not a one single lens here, this is something like much more than one lens.

So, in wherein a condenser lens usually use or eyepiece lens uses some kind of 2 to 8 lenses. And in objective lenses it is almost like 10 to 15 number of lenses are used in making this entire objective lens.

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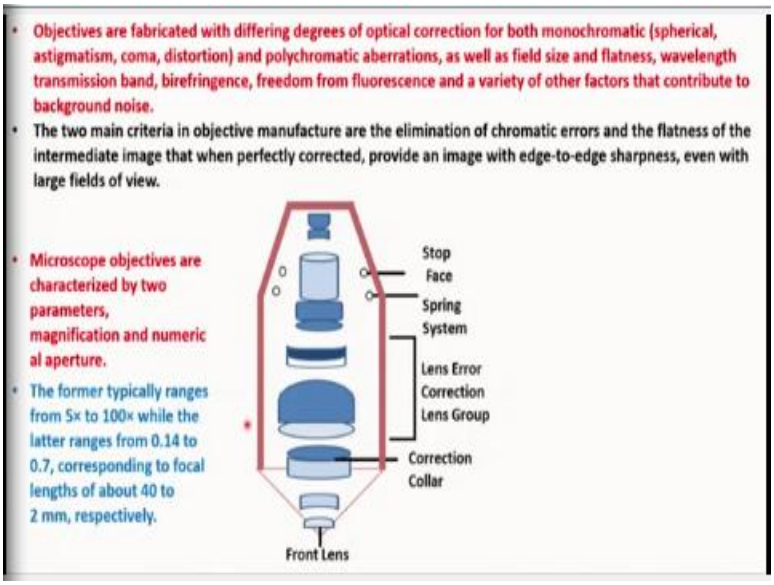

And that is because another purpose of the objective lenses is to correct the aberrations, various kinds of aberrations that we talked in the last classes. It is responsible for mostly for monochromatic aberrations like spherical aberration, astigmatism, coma distortion etc. sometimes it is also it can correct the polychromatic or chromatic aberrations. So, it contains

multiple lenses in combination and all of those lenses together they works and they forms this objective lens.

So, if you are going to higher and higher magnification, it becomes important that you achieve higher and higher resolution. Otherwise there is no point going to we have already discussed empty magnification, there is no point going to higher magnification unless you improve the resolution. And we discussed in the aberration case the improving resolution means you have to reduce the aberrations.

So, diffraction related resolution you cannot do much with it. Whereas aberration related resolutions that you can try to rectify and that way you can increase the resolution of your objective of your image if you are going to higher and higher magnification. And of course the primary purpose of using this objective lens is that to provide an image with an edge to edge sharpness.

That means to reduce edge to edge sharpness means to reduce the distortion and to reduce the aberrations as well. So, all together these are the couple of kind of some few lenses that you can see lots of lenses with different focal lens, different shapes and all, all together they makes this objective lens. And that is why this 100x magnification objective lens usually used to be real big much bigger than something like 4x magnification, the very minimal magnification.

Because this one has to do all the correction, aberration corrections in a much more stringent way than this objective lens. And of course each of these objective lenses are characterized by two parameters, mostly the magnification and numerical aperture. The magnification changes usually from 5x to 100x, meaning 5x to 100x an objective lens plus a fixed 10x magnification in the eyepiece, all together they gave something like 50xto 1000x magnification.

And the numerical aperture usually used to be 0.14, 2.7 but of course there are many different types of objective lenses are available with different, different numerical apertures.

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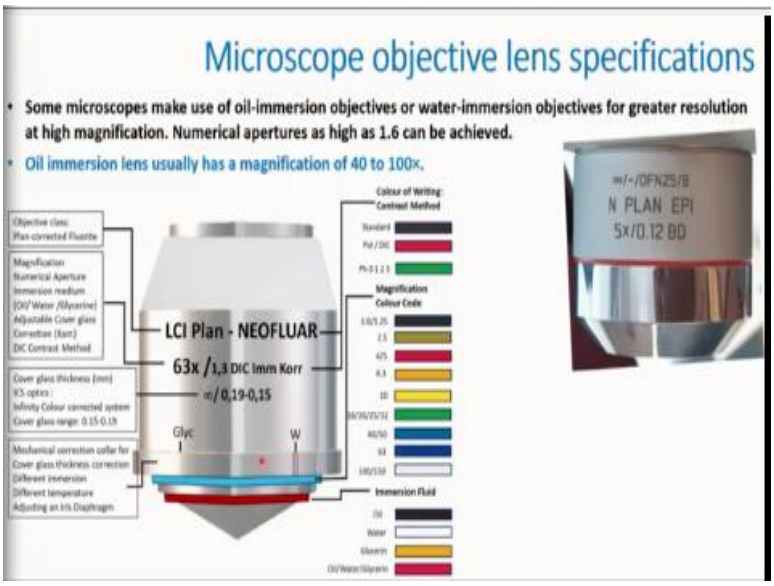

So, if you look at any of this objective lens, microscope objective lens you will be able to see this kind of couple of things written over this magnitude of these objective lenses. So, this is a real objective lens over which different things are written and there is also some kind of a colour coding given here all of this has a meaning. So, for example, what it does is that? The first one this colour coding basically one of the colour coding the meaning of it is that the magnification which magnification it is?

In case it is not written here or if you are having difficulty because often you are moving it this all of this used to be in fixed in this turret. And if you are not able to look at the behind one it is very difficult to see at least you can be able to see the colour and make out that what magnification it is. Then this one the other colour that shows that what kind of an immersion fluid you are using because that will control your numerical aperture as well.

And plus there are many other things written post of this is like what kind of a aberration correction this objective lens is doing.

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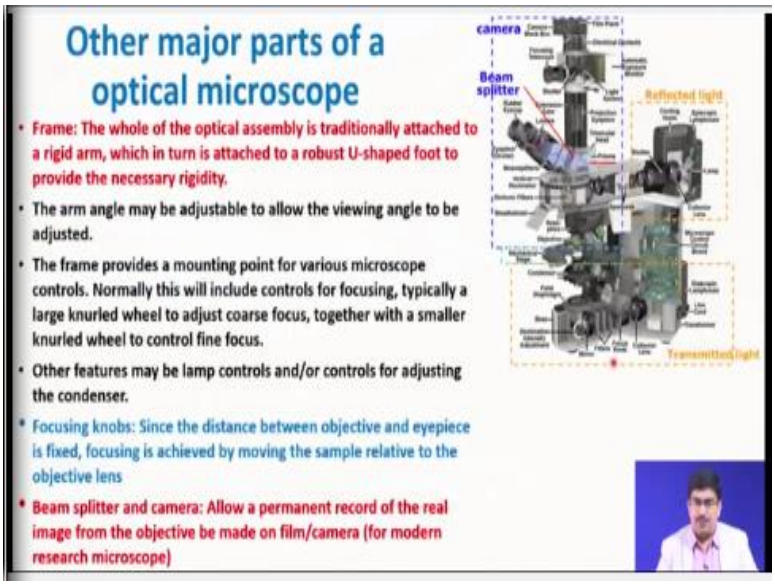

So, there are other major parts of these objective lenses, for example this frame the mechanical frame that you have which is controlling. So, this is one very complex object optical microscope that you are seeing and there are many, many different parts which are there. And one of them and it has a transmission mode, it has a reflection mode. And this other part, this part is basically responsible for image formation here.

So, you have the frame entire frame which is capturing which is holding this entire microscope here. And then you have like focusing knobs which are basically used to be used to control this specimen stage here. And then you have a beam splitter, which is basically a mirror and that beam splitter controls whether the light will go through this eyepiece or whether the light will go through this path and reach to the camera.

This is called triaxial geometry for this optical microscope when you have both of these and we have either the eyepiece or you have the option of adding a camera using this beam splitter here.

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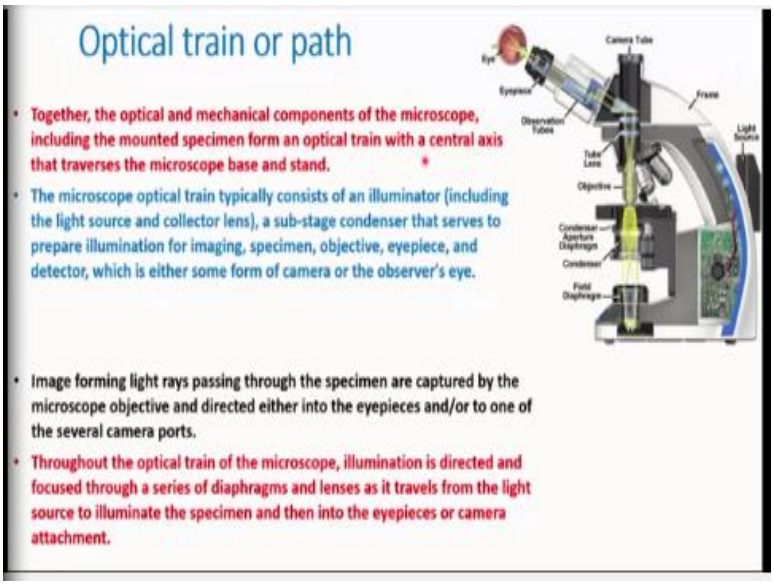

The optical train or optical path is basically the optical and mechanical components which are used in the microscope altogether they are called the optical train or optical path here. So, this typically contains the optical train or optical path of any optical microscope typically contents it starts all the way from the light source and then the condenser lens system. So, I am showing here a complete section of a transparent or a your this is the transmitted light microscope.

And here you have the light source and you have the condenser lenses here and then condenser apertures and from there it reaches to the specimen. And the specimen from there after that it reaches to the objective lens and then it finally goes to the eyepiece. So, these entire optical components all plus these mechanical components which are there all together it is called the optical train or optical path for this.

Now, if you look at here you will be able to see that here you have for transmitted and reflected both of these either you can have 2 different light source altogether or you can have the same light source and then part of the light source you can direct it in the transmitted mode whatever it is. But the optical train is completely different for the reflected light and the transmitted light; they have completely different optical train or optical path here.

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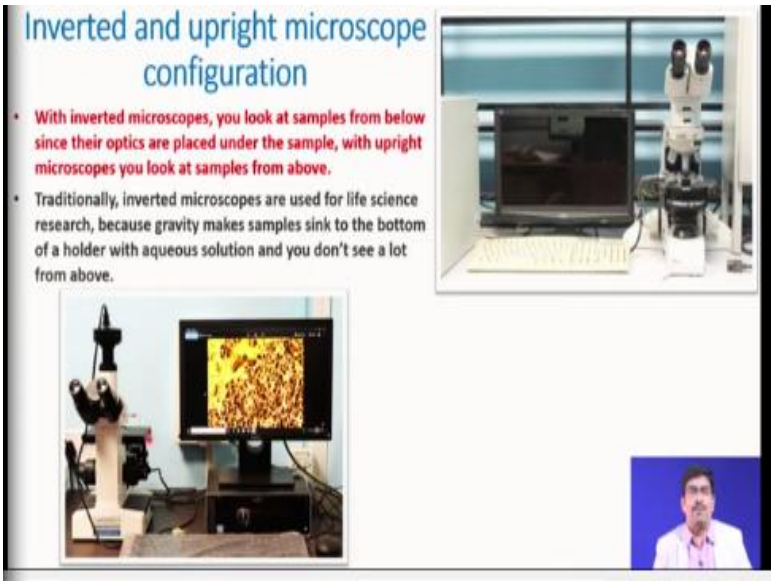

So, now the last thing here is about two very important microscope configuration. These are the typically two kinds of microscope configuration that you will be able to see in most often cases. So, either you will be able to see the microscopes like this in this configuration which is called inverted microscopes. Or in most general cases you will be able to see this kind of microscopes which are known as upright microscopes.

Now this upright microscopes here these are very traditionally used by material scientist and geologist whereas this inverted microscopes are very often used by biologists and people engaged in life science research. This is also the kind of microscope possibly you will be able to see when you go to a patho lab for your checkup with your blood cells and cell counts and all will be able to see that all of them are working invariably by the inverted type of microscopes.

And there is a reason for that, the primary reason is that the biological samples which are there and they are mostly immersed in an aqueous medium. What happens is that the cells because of the gravity, the cells and samples they tend to sink at the bottom of your holder and when you tend to use aqueous solution that is why if you try to see them in upright then you will not be able to see them, you will not be able to focus them properly.

Whereas if you use this inverted geometry, it should be you will be able to focus them very easily. The main difference that comes here is that the objective the position of the objective lens

with respect to the specimen. So, in this case the light this objective lens is at the bottom of the specimen, the specimen is the top and this side there is a completely empty there is nothing in this side.

So, lights are coming from this direction and then our either from the top direction and objective lens is in this direction. Whereas in the upright geometry light if it is transmitted mode then light is coming from behind from bottom and objective lens is on the top, so this is the point. The objective lens if it is in the top of the specimen, it is an upright geometry and the objective lens, if it is in the bottom of the specimen, then it is an inverted geometry.

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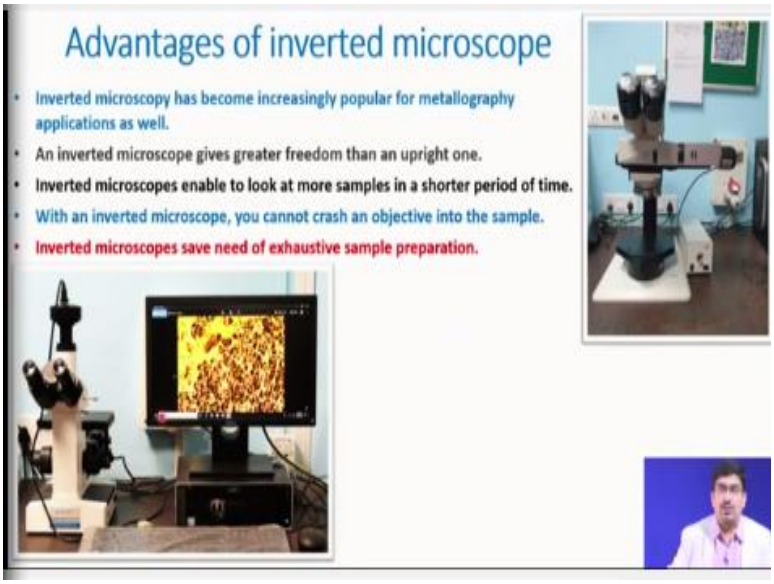

So, that is basically the entire difference. So, the advantages of inverted microscope this is what I am written completely from my personal experience and this may vary from people to people. And this is one of the major advantage of this inverted microscopy is this that this case a higher freedom than an upright microscope. So, if you look at in upright geometry, you will have a restriction in the sample.

So, what the maximum size of the sample that you can use here there is a restriction. So, that is often a problem for using a bigger sample and in this case the biggest any size of the sample you can fit. Because essentially in this direction in this side there is nothing and this is in the reflection mode by the way. So, the problems why this inverted microscopes was not so popular

with material scientist and geologist is because you this problem that you use of using this inverted configuration for generating and reflected type microscope is very difficult.

It was very much designed difficulty was there. So, at the moment and as soon as this design problem is solved and this one is started becoming a very popular one for even if the in the metallography or in the metallurgist material scientists for geologists field. So, because most of this the first and foremost advantage that it offers is that you can have any kind of sample all you need is this direction it should rest on this specimen that is it.

The second one that it gives is basically you can, so in the transmission mode to see the transmission mode anything. As you understand that, the other side also has to be completely flat. So, not only your top surface observation surface needs to be completely flat in order to have very highly focused image in order because you know the depth of focus is very, very small for optical microscope.

And that is why you need to have a very, very flat surface. So, the surface roughness should be as minimum as possible, but that also imply that on the other side of the specimen also needs to be completely flat. So, that means the parallel those two surfaces must be extremely parallel in order to focus entire region of observation. So, this problem also is not there the other surface can be anything, it can be very uneven also does not matter.

Because the sample itself is resting on the specimen stage and in turn does not matter on the other side whether it is parallel or it is not. And of course the third and very important advantage is that this by mistake if you are using a specimen and if you are trying to focus it by moving the specimen stage, there is always a possibility finite possibility that the specimen will hit this objective.

Because as I said the objective lenses are having different, different height. That means, they are of different size, so as you are going higher and higher magnification, you are bringing this specimen closer and closer to your objective lens. So, if you are not very careful, you have every possibility that this specimen will go and hit these objective lenses. In this case of course, that

possibility is very less because in between the objective lens and the specimen there is always a specimen strain. So, in no case during focusing you will be able to or your specimen will hit the objective lens. So, that is another very my big advantage in this inverted geometry.

So, that is why this inverted geometry are becoming very popular, even other than biologists other than life science people involved in life science also with the material scientist and geologists. So with this we are ending this part of the lecture. And then the next lectures we will be talking about various modes of optical microscopes and various contrast enhancing modes and fluorescence mode and so on, thank you.