Indian Institute of technology Madras
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NPTEL
NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING

Lecture-4
Materials Characterization
Fundamentals of Optical microscopy

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Hello welcome back! In the last class, we have just seen the concepts of depth of focus and depth of field, and then we moved on to the concept of contrast, and then we looked at the meaning of the very definition of the contrast. And then we just started discussing about the lens defects so we will again start look back that the classification of the lens defect.

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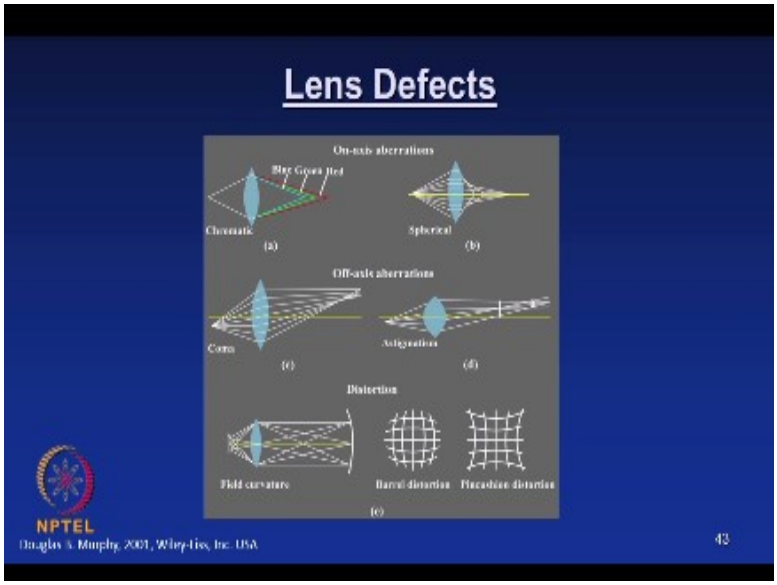

If you look at it I just mentioned lens defects are basically of two types one is on-axis aberrations other one is off axis aberrations. And then we have a distortion, which is also going to impair the quality of the images. So I started off describing the, the first defect and very important defect: a spherical aberration yesterday.

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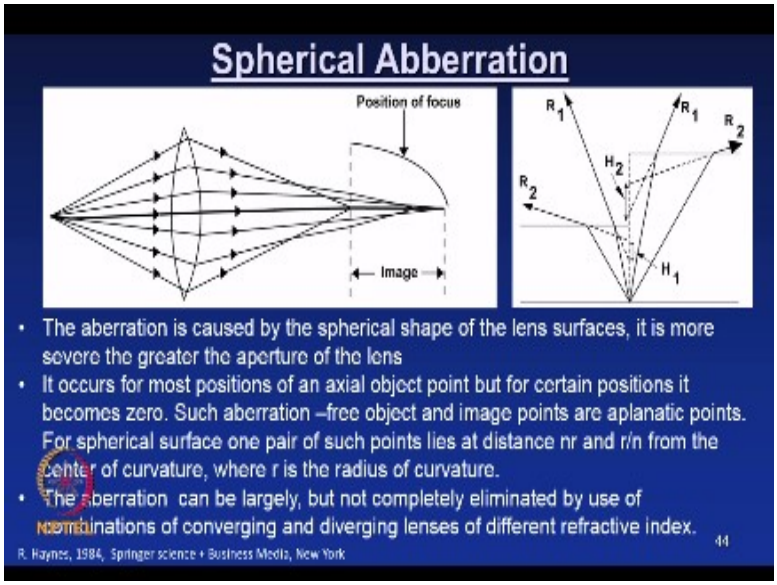

So let us look back and look at this defect again. I mentioned that the, the spherical aberration is a very difficult aberration to eliminate for any lens, because the aberrations causes because of the spherical nature of the lens. So let us look at the review the remarks again once again it is still worth it : the aberration is caused by the spherical shape of the lens surfaces it is more severe the greater the aperture of the lens. And it occurs for the most positions of an axial object point but for certain positions it becomes zero. Such aberration free object and image points or aplanatic points. The for the spherical surface one pair of such points lies at a distance nr and r/n from the center of the curvature.

Where R is the radius of curvature. As I mentioned yesterday this parameter aplanatic points is used to fabricate high power objective lenses. So this aberration can be largely but not

completely eliminated by use of combinations of converging and diverging lenses of different refractive index. We will see that in detail when we complete all the definitions of the defects and then we will see how it can be overcome by the combinations of different lenses which have different optical characteristics. So the one the image which is shown in the right hand side here what I have just tried to bring to your attention is.

What happens to this spherical aberration when you view the specimen with the cover slip. Yesterday we have seen that the numerical aperture and, and it is a light grasping power of an objective lens with oil immersion for a dry lens as well as immersion lens as well as a bare sample, a sample with cover slip. Similarly if you look at this image what you have seen is normally this cover-slip on the specimen is kept at a specific thickness about 0.17mm. And if you do not have this a designed 0.17 mm distance of a cover slip or if you have an arbitrary length or thickness of the cover slip.

Then what happens to this spherical aberration that is what is being illustrated here; and if you look at carefully this the rays which is emanating from the specimen surfaces just get diffracted or rather I would say refracted from this cover slip in this manner. And then if you trace this the refracted ray and then these two rays are differing with the distance of H_1. You can see that if you trace this ray and it falls here and if you trace this ray or two it falls here and these two rays are emanating from the surface of the length H_1. On the other hand if you if you just allow the rays to pass through a cross slip which is of arbitrary length than the 0.17 mm. Then you can see that the refracted ray goes like this that is R_1 and R_2.

And if you trace their path optical path they differ in the range of H_2 which is much higher than the H_1. Obviously the quality of the image will be much affected because of this optical path difference between the standard cover slip thicknesses versus an arbitrary cover slip thickness. So now we will move on to the next defect called astigmatism and curvature of field.

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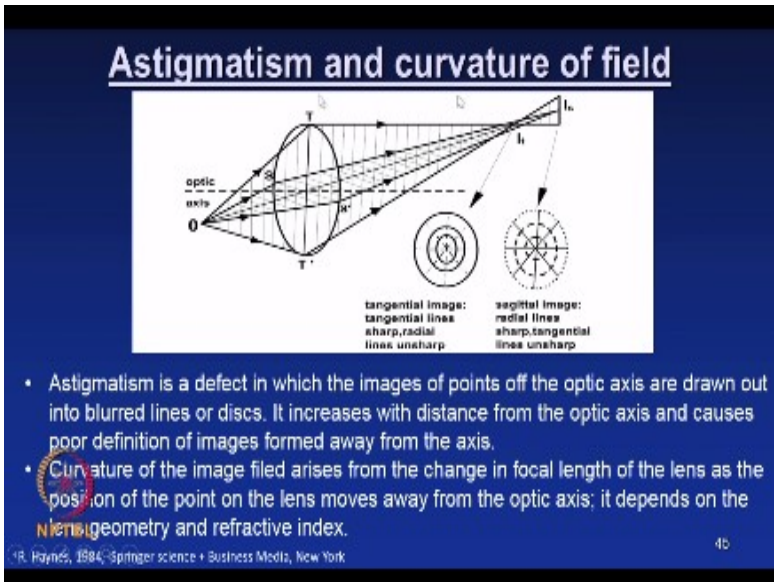

Look at this image carefully as I mentioned this is off axis aberration. And if you look at the schematic you have this optical axis. And then you have this lens here; and there are two different planes are defined here that is the tangential plane T, T' and then and sagittal plane S, S'. And you see that if you let me read the remarks first and then we will come back to the description of the, the effect of this image quality effect of this defect on the image quality we will discuss after going through this remarks. Astigmatism is a defect in which the images of points of, of the optic axis are drawn out into blurred lines or discs.

So like this, this is the discs two discs we are talking about. It increases with the distance from the optic axis and causes poor definition of images formed away from the axis. So as the distance increases from the optic axis the image quality also will go back. So let us now come back to this schematic again so you have this, the line passing through this T, T' form an image TIT that is a tangential image. And then the rays which are passing through S, S' plane form an image I S. So you can see that in a tangential image the tangential lines are sharp and then radial lines are unsharp. On the other hand if you look at the sagittal image that is this. This S, S' plane image the radical sorry the radial lines are sharp and tangential lines are unsharp.

So the circle of least confusion lies between these two images and the correction is done once these two circles are brought together; but still the image will be lying on the curvature of the surface. You can appreciate that the, the tangential image lie in a sagittal plane; and the sagittal image lie in a tangential plane. So this is a very nice schematic to appreciate the defect of astigmatism; and we will see the curvature of field the curvature of the image field arises from the change in focal length of the lens as the position of the point on the lens moves away from the optic axis. And it depends on the lens geometry and refractive index. We will also see when we look at the correction of these objective lenses how this curvature of the image is also being taken care.

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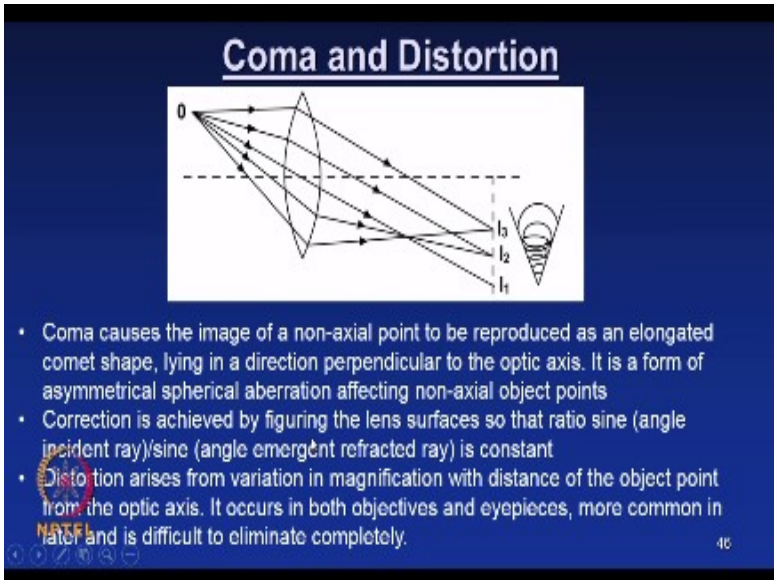

The next defect is coma and distortion, and before we look into the description let us look at the preliminary remarks. Coma causes the image of an non axial point to be reproduced as an elongated comet shape lying in a direction perpendicular to the optic axis. It is a form of asymmetrical spherical aberration affecting non axial object points. You can see that your non axial points are appearing as I_1, I_2, I_3. And then this forms an elongated comet shape perpendicular to the optic axis like this. That causes an image distortion and it is a kind of

asymmetrical spherical aberration okay. The correction is achieved by figuring the lens surfaces so that the ratio of sine angle of incident / sine angle of emergent refracted ray is constant.

So we will discuss this again when we talk about a correction of lenses and the distortion which we have seen in the introductory slide of the lens defects is arising, because of variation in the magnification with the distance of the object point from the optic axis beams. The magnification is varying with the distance of the object from the optic axis that is from this optic axis as you move from the optic axis the magnification changes. And it occurs in both objectives and eyepieces and more common in later and it is difficult to eliminate completely. So you have to live with some defects.

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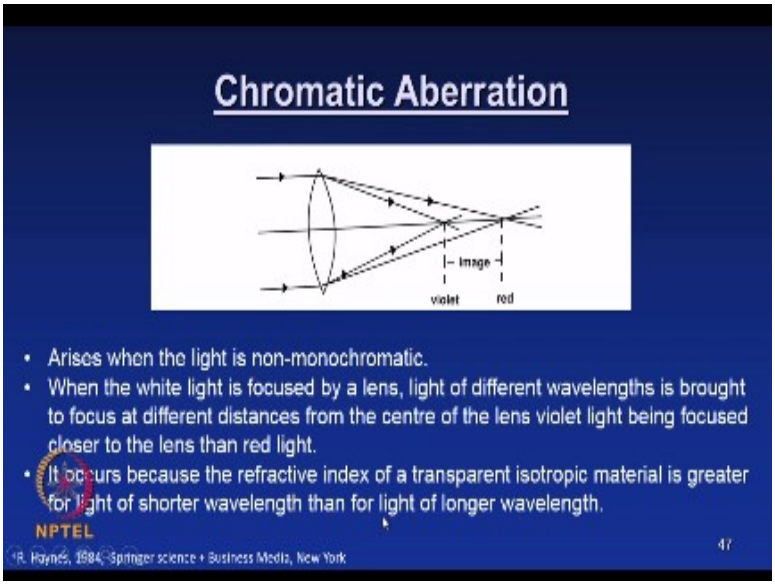

The next aberration which we are going to talk about is chromatic aberration. It is it is very different from what we have discussed the previous ones arises when the light is non monochromatic. Whatever the defects which we had discussed before you talk about coma or astigmatism or spherical aberration, they, they occur when we use monochromatic radiation. And this one the arises when the light is non monochromatic; you have to remember this, this very important point. So let us look at the remarks : when the white light is focused by a lens light of

different wavelengths is brought to focus at different distances from the center of the lens violet light being focused closer to the lens than the red light.

So we are talking about the visible spectrum that means your violet light will have a different wavelength compared to the red light. So they are all being focused at different, different distances and at in dot it occurs because the refractive index of a transparent isotropic material is greater for light of shorter wavelength than for the light of longer wavelengths. This you already know and so the, the effect of this abrasion is like if you have an image then, the periphery of your image is filled with a different color. That means every color will focus at a different, different focal point. So you will see that the periphery of your image we filled with a color fringes it will appear like a color fringes.

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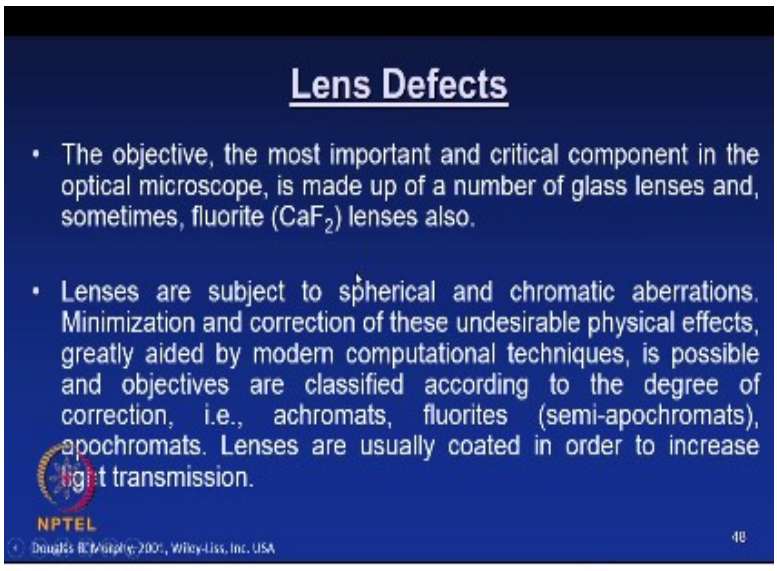

We will see how to correct this. So we will now summarize this lens defects. And we will see how they are correct characterized or corrected based upon the different degree of Corrections. So the objective the most important and critical component in the optical microscope is made up of number of glass lenses and sometimes fluorite lenses also. Lenses are subjected to spiracle command chromatic aberrations minimization and correction of this undesirable physical effects

greatly aided by modern computational techniques are possible. And objectives are classified according to the degree of correction that is achromats, florites. They are also called semi apochromats like that. Lenses are usually coated in order to increase the light transmission now let us see some of the typical characteristic of objective lenses is tabled here.

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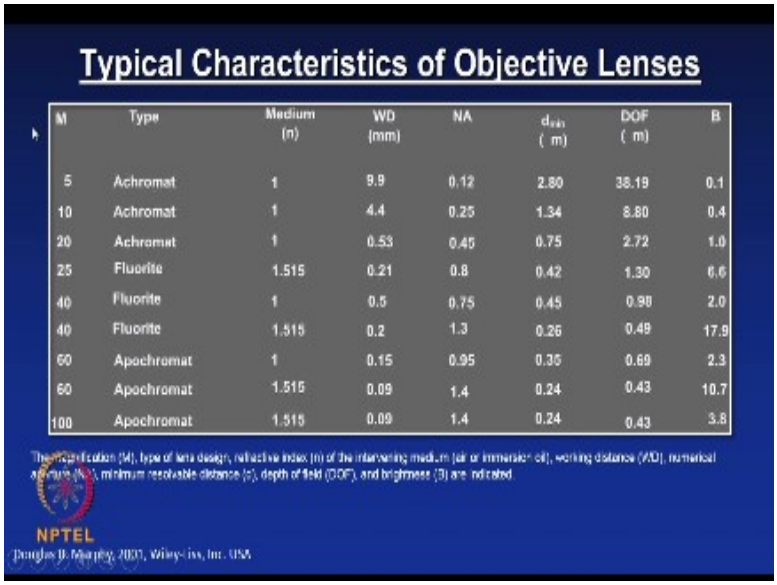

Look at this table carefully as I mentioned depending upon the degree of corrections they are being classified. And you see that M is a magnification and this is the type of objectives and you have the medium and this is the working distance W is W_d is our working distance in millimeter. This is numerical aperture $D_{minimum}$ is the minimum resorbable distance. This is depth of focus in meters and B stands for brightness. So you see that depending as the magnification increases and then how this values are, are changing okay. And then you can also see that how the refractive indexes also influence the other parameters especially depth of focus and minimum resolvable distance and so on.

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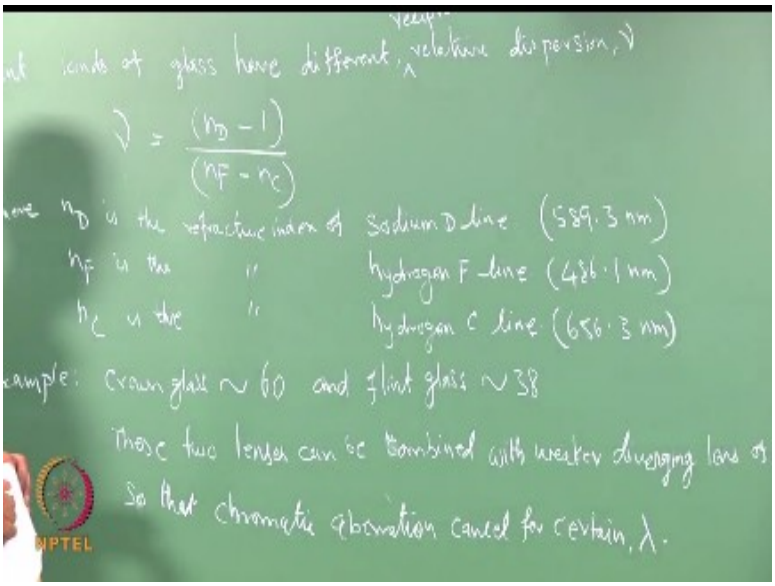

So it gives it gives you a broad idea about what kind of Corrections we can make our we can take up. And then probably I just show you some of the correction which is made on a blackboard. So let us try to give one example how this correction is being made, let us write like this : different kinds of glass have different relative dispersion, dispersion ν. So let us impact it as reciprocal, reciprocal relative dispersion ν is defined as $n_d - 1/n_f - n_C$. Where n_d is refractive index of sodium D line, and n_f is the refractive index of hydrogen F line, n_C n_C is the refractive index of hydrogen C line. You can also note down this values 589.39 nanometers 486.1 nanometer 656.3 nanometers.

So I am just giving you this because you should know how this correction is made and what the basis these are all spectral value. And then different kind of glass will have a different reciprocal relative dispersion μ which is defined by this formula. And for example you can take you can take example a crown glass will have around ~ 60 ν value of 60 and the Flint glass will have the value of ~ 38. So consequently these two lenses can be combined. We will write these two lenses that can combined with weaker diverging lens of Flint glass. So that chromatic aberration cancels for certain λ.

So this is one case study how this correction is done in the case of chromatic aberration. There is a parameter called a reciprocal relative dispersion ν and this value for characteristic of different lenses. So for the crown, crown glass it is 60; for a Flint glass it is 38. So these two can be combined because with the weaker diverging lens of a Flint glass, and that will correct the aberration chromatic aberration for certain λ. So we can can also draw some schematic how that character doublet will look like so,

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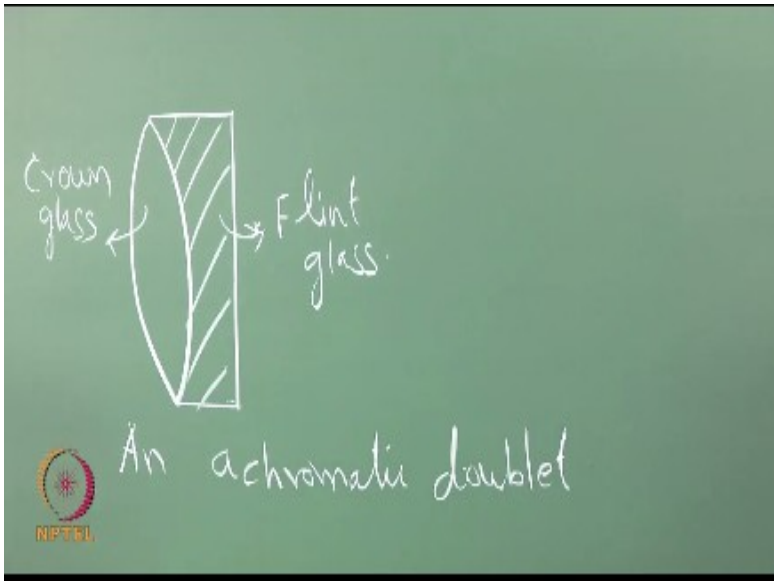

So you have this lens and then so this is a crown glass and this is flint glass. This is an achromatic doublet so I just give, give an example how this Corrections are being made it gives you an idea. So similarly all those listed in that table follow certain procedures to take care of the kind of correction which required are the degree of Corrections which is required. And based on which the subjective lenses are classified. So now we will move on to the next item.

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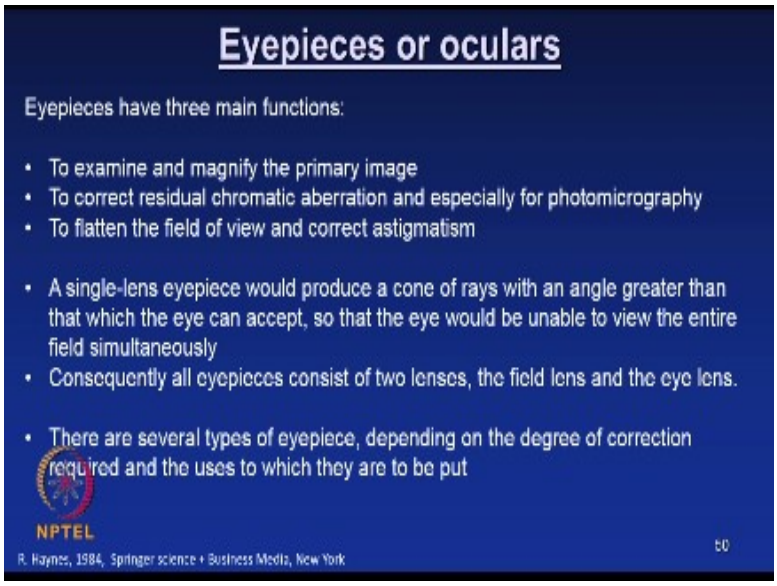

That is eyepieces and oculars I just want to mention the important functions of these eyepieces we have talked a lot about objective lenses, because they are very critical and important as I mentioned in the last slide. So let us have some idea about what this eyepieces are, are doing in a microscope. They are also called oculars and eye pieces have three main functions and they are to examine and magnify the primary image. And they are also to correct the residual chromatic aberration and especially for photomicrography. And they are to flatten the field of view with just which we have seen that is a problem and correct astigmatism.

So these are the three primary functions of an eye pieces. A single lens eyepiece would produce a cone of rays with an angle greater than that which the eye can accept. So that the eye would be unable to view the entire field simultaneously consequently, all eyepieces consists of two lenses the field lens and the eye lens. There are several types of eyepiece depending upon the degree of correction required and the uses to which they are to be put in. So similar to objective lenses you have in eyepieces also had different types based upon the degree of Corrections. And as you know that depending upon the kind of sophistication one, one require to build a microscope, the combination of an objective and eyepiece are selected ; if you recall that table which we have shown that you know the kind of useful magnification which produces the combination of these

two pictures. Now you will get an idea how the quality of a microscope is decided and how these two lenses where objectives and eyepieces are being selected okay. So now we will move on to some other important parts of microscope.

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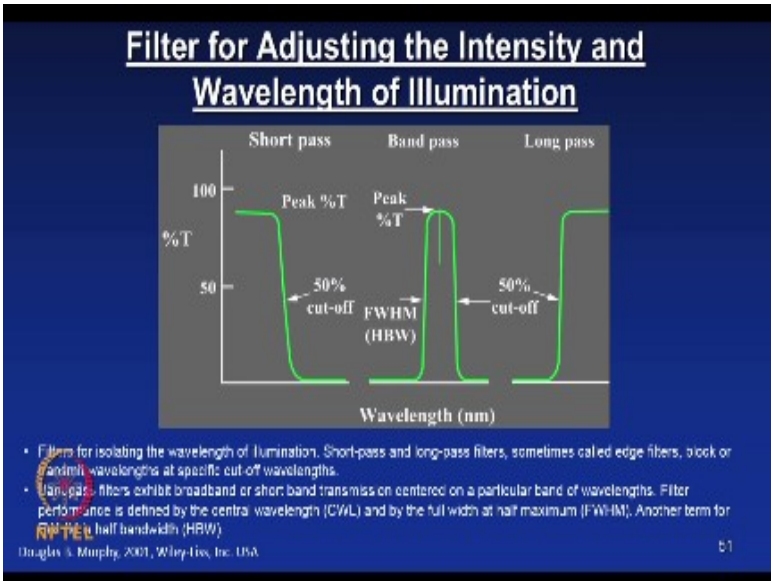

I would like to talk about this few filters for adjusting the intensity and wavelength of illumination. Look at this slide and for getting a full brightness illumination is also an very important aspect of it and if you most of you. You will see that in some of the microscopes you will have lot of color filters just after the illumination source I am going to show you. And you should know what these filters are doing; so this is about that. So look at this plot this is percentage transmission versus wavelength plot: you have a short pass, a band pass, a long pass. The name itself tells that the filters for isolating the wavelength of illumination short pass long pass filter sometimes called edge filters, block or transmitted wavelengths at a specific cutoff wavelength.

You can see that it is 50% cut off it is a peak transmission and the band pass filters exhibit broadband or a short band transmission centered on the particular band of wavelengths here ; you can see that. And this filter performance is defined by the central wavelength and by the full

width at half maximum this is full width at half maximum. So another term for full width half maximum is half bandwidth.

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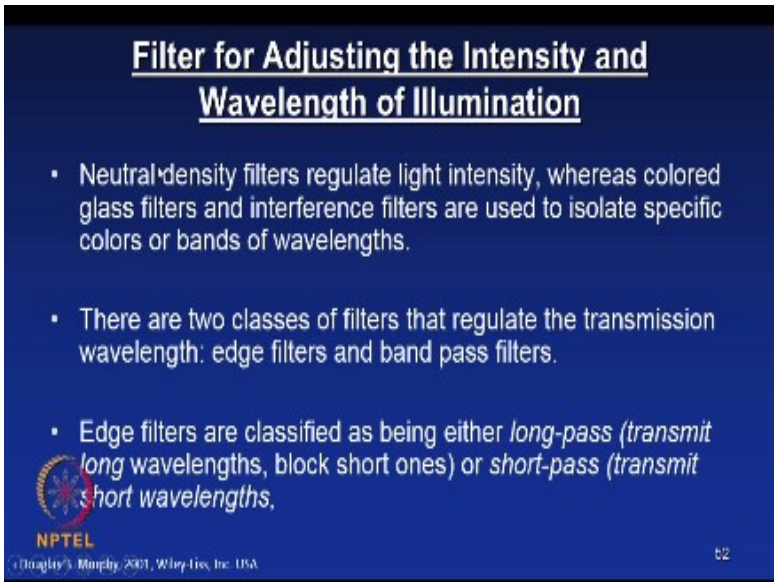

So let us look at some more remotes on these filters neutral density filters regulate light intensity whereas colored glass filters and interference filters are used to isolate specific colors are bands of wavelengths. There are two classes of filters that regulate the transmission wave ength edge filters and band pass filters. Edge filters are classified as being either long pass that transmit long wavelengths and block short ones or short pass which transmit a shorter wavelengths. So I think this is a kind of introduction to this kind what are the filters and what are their primary functions are.

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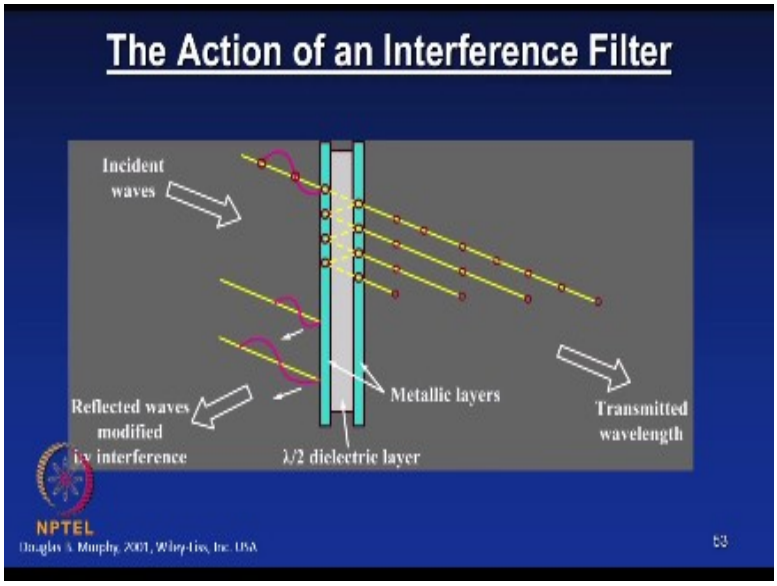

We will now move onto another important filter : an interference filter look at this slide and what you are seeing is, is an action of an interference filter. We will be using this filter in one of the variants of the optical microscope called differential interference contrast microscope. So let us look at this function of this interference filter you have the incident wave coming here, and some of the muscle rays are reflected or modified by interference and some of them are transmitted. And we have to know how it is done correctly so you see that the two metallic layers are coated on the dielectric material in such a way that their optical path length is $\lambda /2$. So when the wave incident wave which comes and enters this filter perpendicular to the face and only those wavelengths will be allowed to pass through and then rest of them will be reflected back. Since all the transmitted waves are in the face, they will be allowed to constructively interfere and then and becomes a transmitted wave. So this is the function of these interference filter we will see the the usage of this filter much more detail when we actually take up the, the variant of this microscope in the coming lectures.

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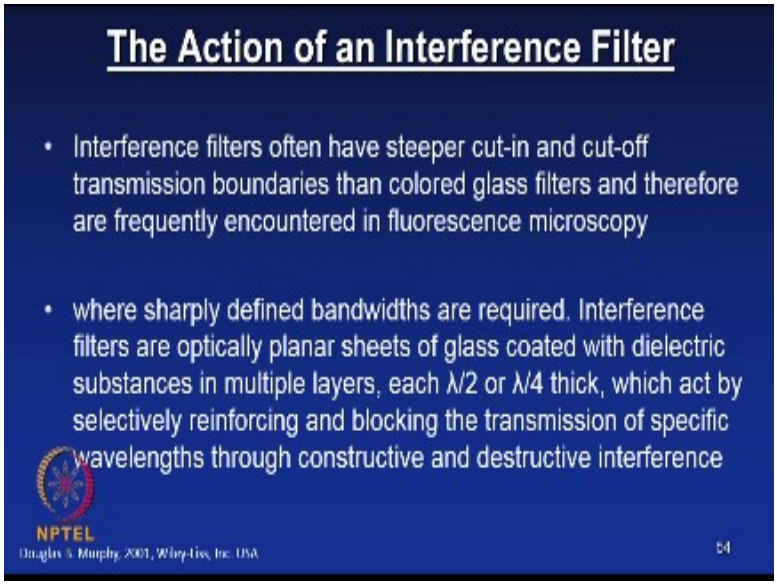

Let us see few more remarks on these filters interference filters often have a steeper cutting and cut off transmission boundaries than the colored glass filters. And therefore are frequently encountered in the fluorescence microscope. Where sharply defined bandwidths are required interference filters are optically planar sheets of glass coated with dielectric substances in multiple layers. Each it could be λ /2 or λ /4 ,thick which act as selectively reinforcing and blocking the transmission of specific wavelengths through constructive and destructive interference. This is what just I mentioned so this is about the interference filter we will now look at another important parameter called optical path length.

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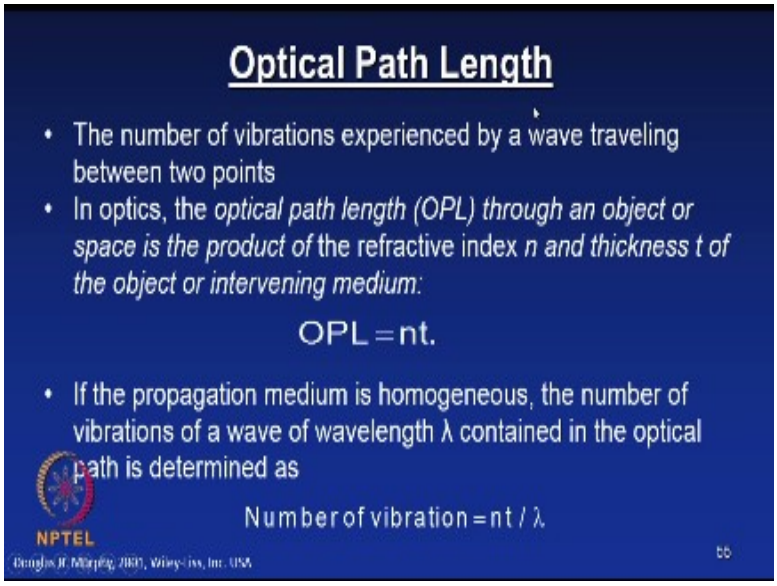

We will be using this concept in one of the air again another variant of the optical microscopy. So let us see what is this optical path length? The number of vibrations experienced by a wave travelling between two points in optics the optical path length OPL through an object or space is the product of refractive index n and the thickness T of the object or intervening medium. So OPL = n * t. That is optical path length is a product of thickness and the refractive index of the medium. If the propagation medium is homogeneous the number of vibrations of a wave of a wavelength λ contained in the optical path is determined as Number of vibration= n * t/ λ.

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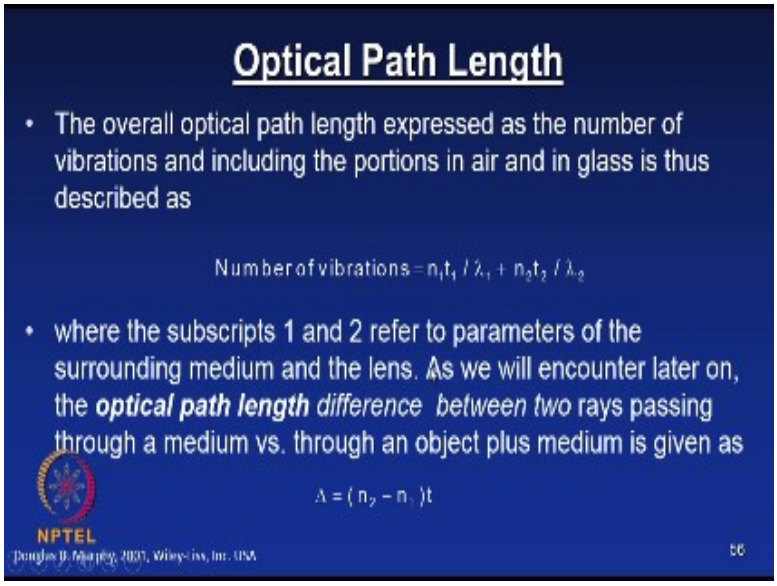

The overall optical path length expressed as the number of vibrations and including the portions in air and in glass is thus described as Number of vibrations = $(n_1 t_1 / \lambda_1) + (n_2 t_2 / \lambda_2)$. Where the subscripts one and two refer to the parameters of the surrounding medium and the lens, as we will encounter later on the optical path length difference between the two rays passing through a medium versus through an object plus medium is given by $\Delta = (n_2 - n_1)*T$. So as I mentioned we will be using this parameter in one of the variant of the optical microscope which I will be discussing it that is why I have introduced this concept.

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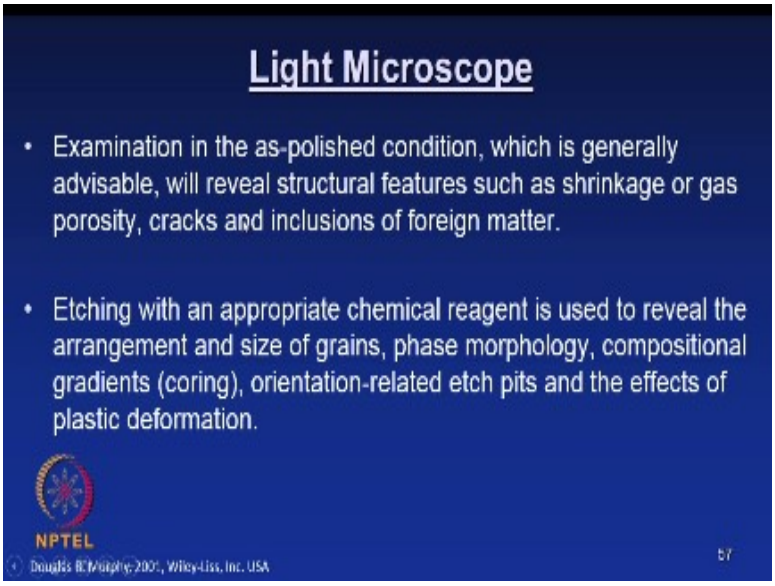

We will now see the general description of light microscope. So what I have done is if you look at all the three classes I have taken some of the cons fundamental concepts which you require to understand before we get into the use this light optical microscope. I hope it will be it was it will be useful to in order to understand the, the functions of different variants of optical microscopes. So now what I am going to do is I am going to just describe what a general light microscope does and I will just disk I will also take you to the lab and then show some of the videos of actual Mic light microscopes which we have in our laboratory.

So let us look at the description of a light microscope. Why do we use this light microscope? So examination in the as-polished condition which is generally advisable will reveal the structure features such as shrinkage or gas porosity cracks inclusion of foreign matter. And for that we need to do something called etching : I will be dealing with it in much more detail when my when I talk about a sample preparation for all this microscopy techniques. However you just look at the initial remarks etching with an appropriate chemical reagent is used to reveal the arrangement and size of grains, phase morphology, compositional gradients, sometimes called coring, orientation related etch pits and the effects of plastic deformation.

These are all only as some of the features which I have just mentioned but in, in reality we will see how, how much we can use this or how effectively we can use this microscopic techniques for various applications in material science and, and so on.

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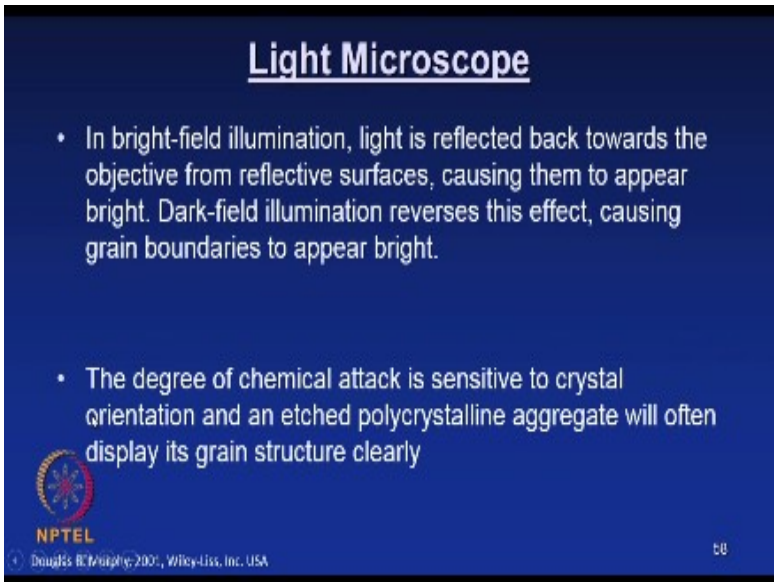

So we have something called a bright-field illumination. Light is reflected back towards the objective from the reflective surfaces causing them to appear bright. And then you also have a dark field illumination reverses this effect and causing the grain boundaries to appear, appear bright. So I will just take up this too I mean the actual microscopic part a when we discuss a specific application, and this is just to give your idea of what kind of method even in a light microscope a basic imaging techniques on his bright field illumination another is dark field illumination. And the image quality is depending upon the degree of chemical attack is sensitive to the crystal orientation and an etched polycrystalline aggregate will often display its grain structure clearly.

So we will also talk about this etching behavior : we will see what is etching and then how it affects the image quality and so on in, in a coming class.

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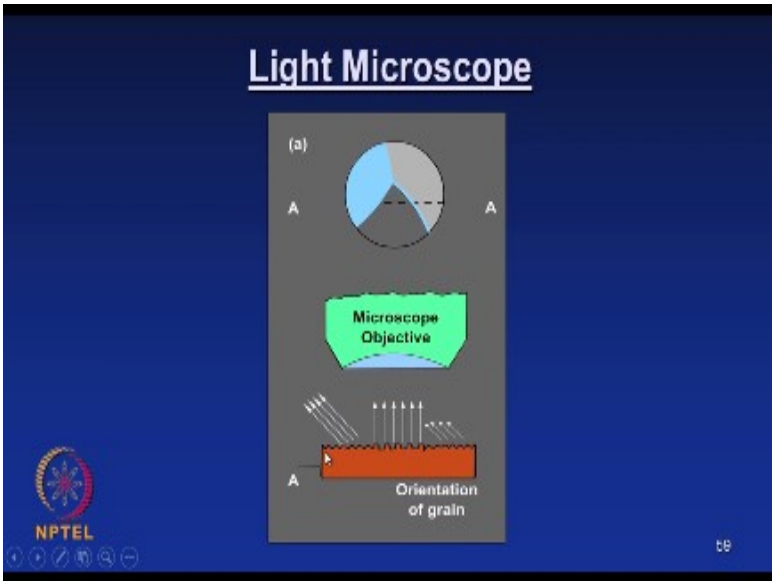

And this is just give you an introduction about this microscope you have an objective you have the specimen here; it is an is a schematic of an etched ; etched surface. And you see that light is being reflected at a different orientation because due to their different orientation of the grain. We will see actually the experiments now you can look at this references.

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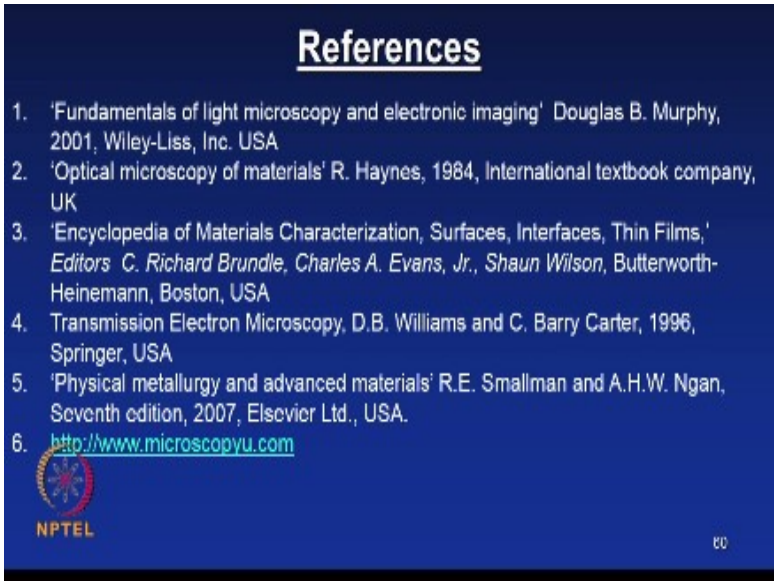

Very important references you can follow for this course one is fundamentals of light microscopy and electronic imaging by Douglas B Murphy 2001 Wiley-Liss international USA. And second important reference optical microscopy of materials by Haynes 1984 international textbook company UK. You can also refer this encyclopedia of material characterization surfaces interfaces thin films by Richard Brundle, Charles evens and Shaun Wilson, Butterworth's and Heinemann, Boston USA. You can also read this transmission electron microscopy by D. B. Williams and Barry Carter, Springer USA for some of the basic concepts of optics.

Then the physical metallurgy and advanced materials by R. E. Smallman and A. H. W. Ngan and publication you can also go through the website http://www.microscopyu.com. So now what I am going to do is I am just going to introduce you,

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To the some of the microscopes what is coming on the screen is a typical metallurgical microscope. So one is there are two basic types of microscopes on as vertical type another inverter type. So what you are now seeing is an inverted optical microscope I will just show some of the main parts which we have talked about like the specimen stage.

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This is an vertical since the vertical microscope it is a specimen stage will be on the top and these are all oculars and eye piece.

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Just we, we have we have now read about quite a bit on this how the eyepiece will appear.

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And this is a CCD camera which is being attached to this microscope and, and these are all some of the polarizing lenses.

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And apertures I will talk about this little later is one of the variants as I mentioned that time we will use this variants I mean apertures.

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And you see that now the illumination is coming from the bottom and then you keep your sample on this light. And what now you are seeing is another vertical simple type microscope this is a standard microscope in any of the metallurgical laboratory you see that ocular,

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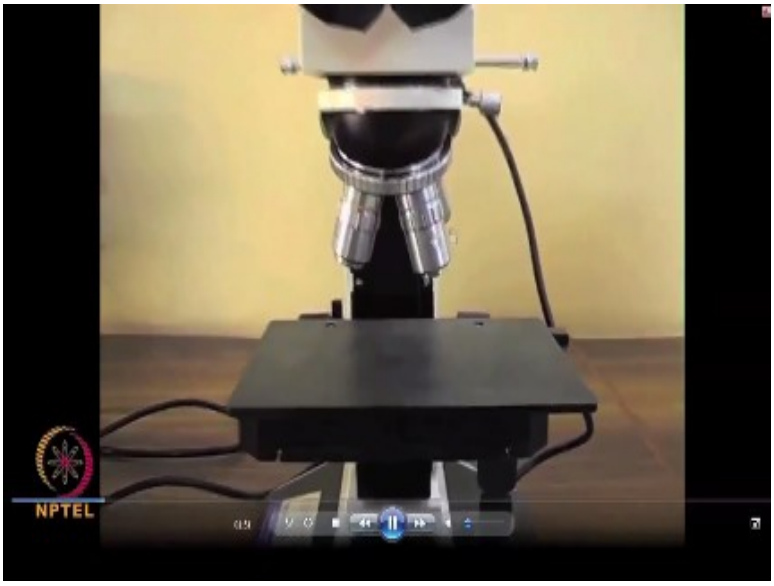

And you see the objectives usually you will have three to five objectives is there.

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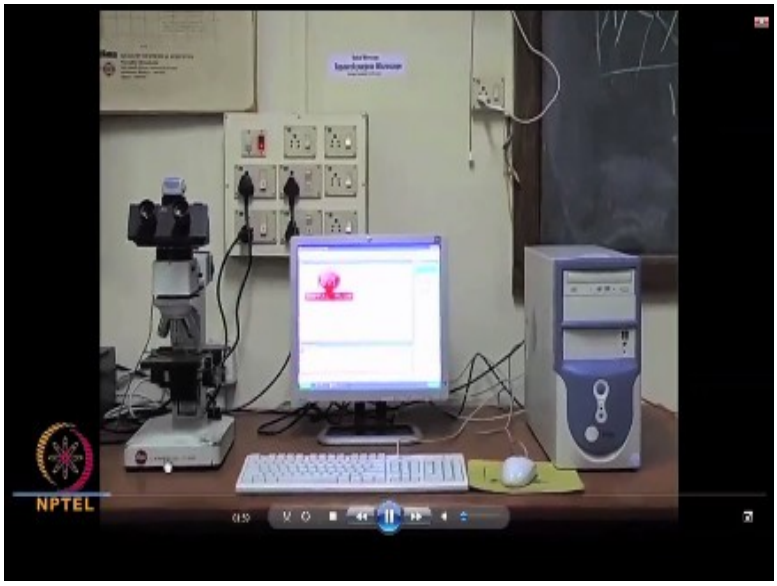

And this is again another microscope which is attached with the image analysis system or it is it is having an interface with the computer. You can clearly see that the objective lenses now as I mentioned. You can also you are also observing that you know some of the letters are written on the objective which is which they talk about the magnification some refractive index and whether it is a oil or a dry all those informations are given on this objectives. Usually it is with it comes with 5 x 10 x 20 x 40x. So and then sometimes 50 x and you can and it, it goes up to 100x also depending upon the microscopic system.

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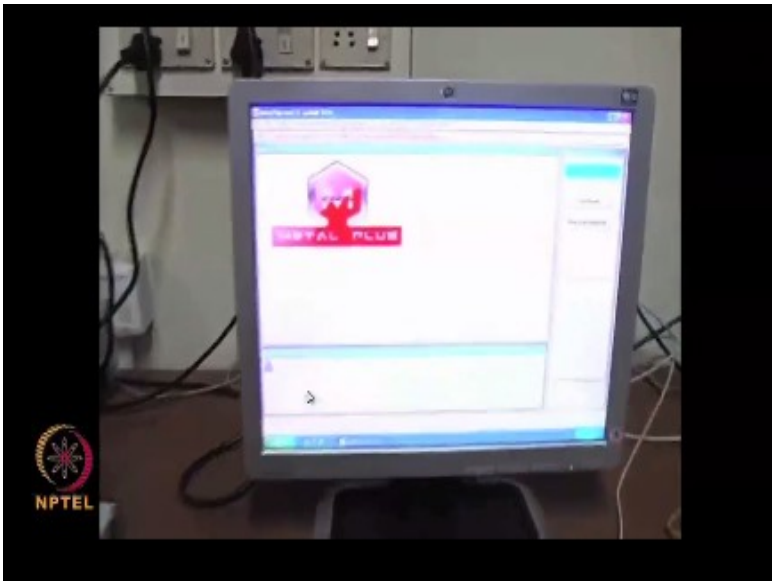

So this is a at the tool which is called a leveling press to make the solid specimen.

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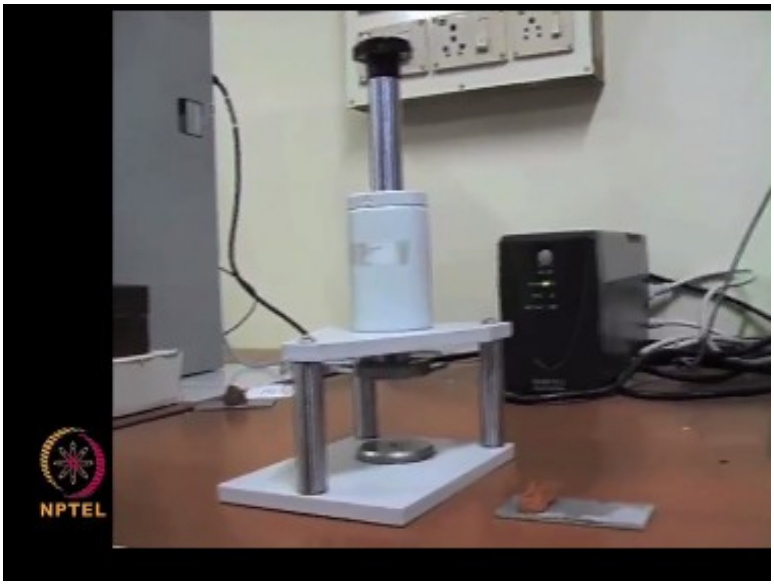

In a same level using this plasticizer,

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So I am just describing this assuming that we will be using only the solid metal piece to examine under the microscope. Not necessarily the case we will see the other materials how it is being viewed in the other type of microscope optical microscope and this is how the solid metal pieces leveled using this leveling press.

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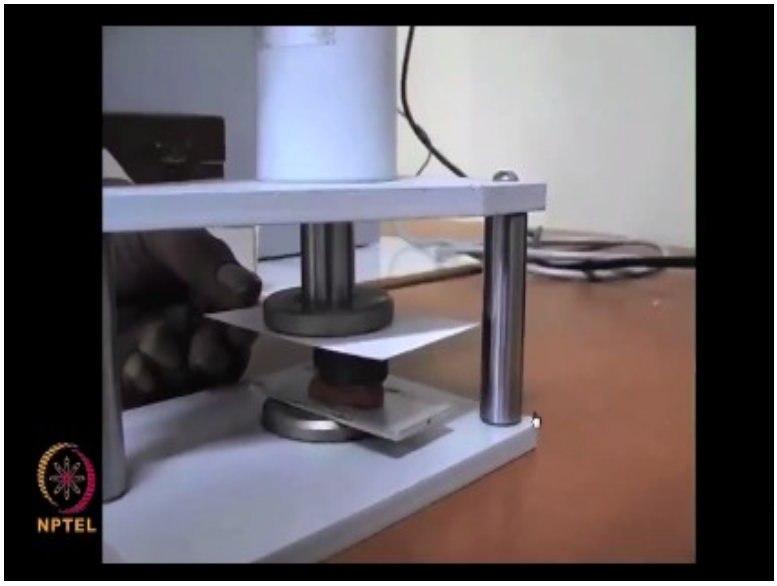

And then it will it will be placed on this microscope as you can see that I will get into the details of all these preparations in a separate class just, just I am just introducing how the microscope will look like. And how people use it and for those who are not come across this kind of am experience.

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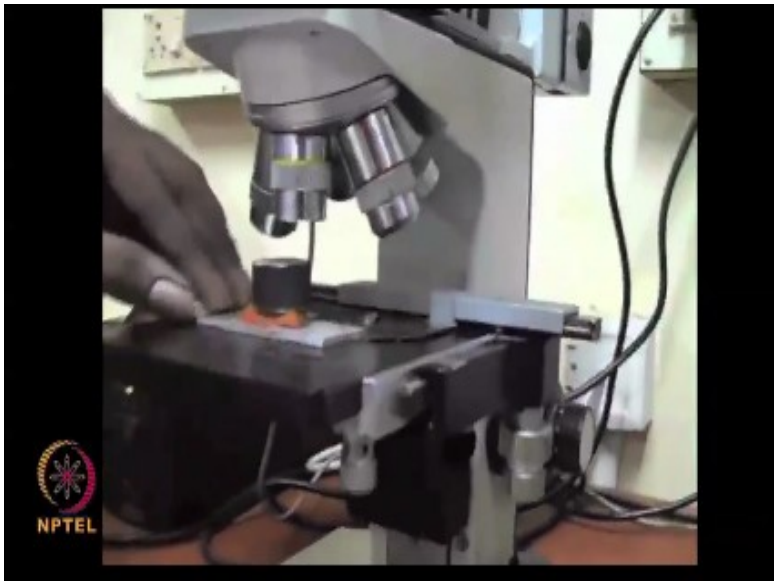

So you can see that now the specimen is being loaded in this stage specimen stage. then you choose the appropriate I mean objective lens. You can start with a lowest magnification to highest mag- you can slowly move from lowest temperature to the highest aperture. And the magnification is multiplication of these two for example you have about 5 X here and then here it is 10 so it is 50 if you choose 50 here it is 500 something like that. So here again for image grabbing,

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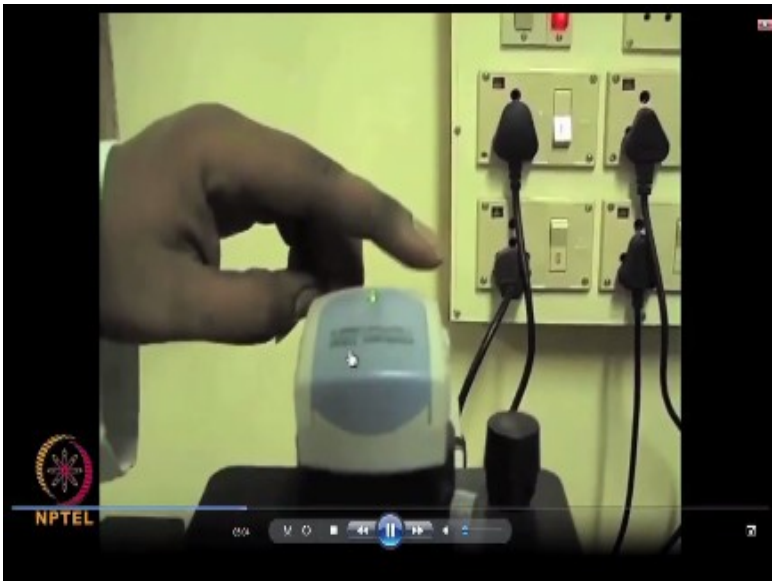

You have the CCD camera attached to this, and now what you are now seeing with another type of microscope is called a transmission optical microscope.

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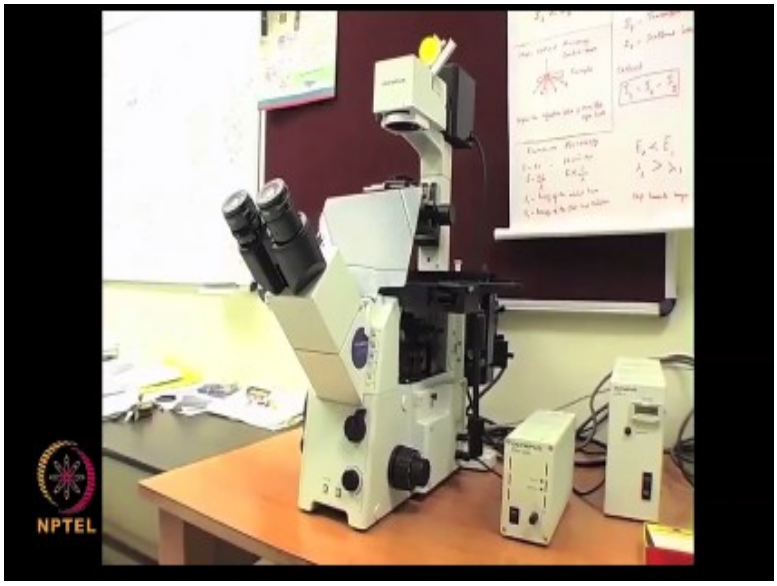

We which is very different from what you have just seen before that is one is vertical another is you know inverted microscope.

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You have here you have two type of illumination attached to it one as mercury lamp and one other is a halogen lamp on the top.

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So I will just describe this microscopic parts so that you will get familiar with what are the important things you need to understand what, what is that being shown here is it is a polarizer, and when you do not use that polarizing mode,

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Then it will be a slot for a bright-field mode and then you also have a kind of a condenser aperture lenses in this which is having a different, different slots. I will talk about it in much more elaborately in the next class. Thank you.

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