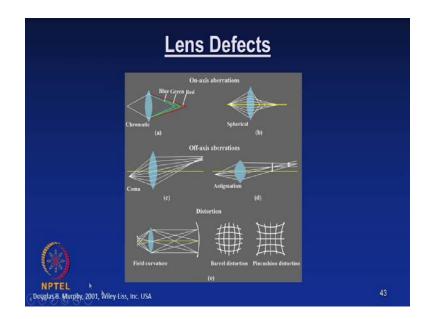
### Fundamentals of optical and scanning electron microscopy Dr. S. Sankaran Department of Metallurgical and Materials Engineering Indian Institute of Technology, Madras

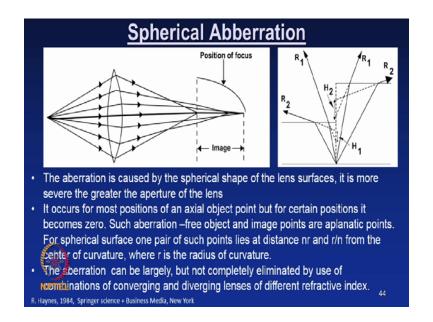
Module – 01 Unit-1 Fundamentals of optics – Continuation Lecture – 04 Lens defects-continuation Filters Light or optical microscope- introduction

Hello, welcome back. In the last class we have just seen the concepts of Depth of Focus and Depth of Field, 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.

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So, we will again start look back that the classification of the lens defects. If you look at it, I just mention lens defects are basically of two types; one is on axis aberrations, other one is off axis aberrations. Then we have a distortion which is also going to impair the quality of the images.



So I started of describing the first defect and very important defect a Spherical Abberration yesterday. So let us look back, and look at this defect again. I mention that the spherical abberration is a very difficult aberration to eliminate for any lens because, this aberration causes because of the spherical nature of the lens.

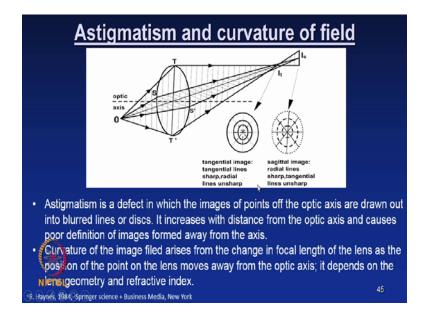
So let us look at the review the remarks again once again, it is still worth it. The aberration is caused by the spherical shape of the lens surfaces, it is more severe the greater the aperture of the lens. And it occurs for the most positions of an axial object point but for certain positions it becomes zero. Such aberration -free object and image points are aplanatic points; For the spherical surface one pair of such points lies at distance nr and r by 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 a 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 details when we complete all the definitions of the defects, and then we will see how it can over combine the combinations of lenses which have different optical characteristics. So, the image which is shown in the right hand side here, what I have just try 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 it is or 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 the 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 0.17 mm. 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 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. Then if you trace this 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 R 2 it falls here, and these two rays are emanating from the surface of the length H 1.

On the other hand if you just allow the rays to pause through a across 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 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 their standard cover slip thickness verses 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. There are two different planes are defined here, that is the tangential plane T, T dash and then sagittal plane S, S dash.

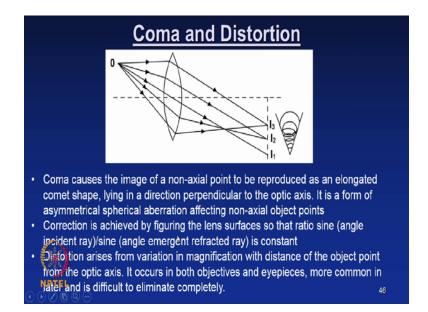
Let me read the remarks first and then we will come back to the description of the effect of this image quality, defect on the image quality we will discuss after going through this remarks. Astigmatism is a defect in which the images of points off the optic axis are drawn out into blurred lines or discs. So like this, this is the discs, two discs we are talking about. It increases with 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, what the line passing through this T T prime form an image I t, that is a tangential image and then the rays which are passing through S S prime 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 of you look at the sagittal image that is this S S prime plane image, 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 tangential image lie in a sagittal plane and the sagittal image lie in a tangential plane.

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 this subjective lenses how this curvature of the images also been taken care.

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The next defect is Coma and Distortion. Before we look into the description, let us look at the preliminary remarks. Coma causes the image of a 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 in distortion

and it is a kind of asymmetrical spherical aberration. The correction is achieved by figuring the lens surfaces so that the ratio of sine angle of incident divided by sine angle of emergent refracted ray is constant. 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, variation in the magnification with the distance of the object point from the optic axis. The magnification is varying with the distance of the object from the topic 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 are 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 very different from what we have discussed in the previous once; arises when the light is non-monochromatic. Whatever the defects which we have discussed before, you talk about coma or astigmatism or spherical aberration they occur when we use monochromatic radiation. And this one arises when the light is non-monochromatic; you have to remember this is 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 a focus at different distances from the centre of the lens violet light being focused closer to the lens than red light. So we are talking about the visible spectrum that means your violet light will have a different wavelength compare to the red light so they are all being focused at different distances. And it occurs because the refractive index of a transparent isotropic material is greater for light of shorter wavelength than for the light of longer wavelength. This you already know.

The effect of this aberration is like, if you have an image the periphery of your images filled with a different color. That means, every color will focus at a different focal point so you will see that the peripheral of your image where filled with a color fringes, it will appear like a color fringes. We will see how to correct this.

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# Lens Defects

- The objective, the most important and critical component in the optical microscope, is made up of a number of glass lenses and, sometimes, fluorite (CaF<sub>2</sub>) lenses also.
- Lenses are subject to spherical and chromatic aberrations. Minimization and correction of these undesirable physical effects, greatly aided by modern computational techniques, is possible and objectives are classified according to the degree of correction, i.e., achromats, fluorites (semi-apochromats), apochromats. Lenses are usually coated in order to increase light transmission.

Douglas 6: Multiphy, 2001, Wiley-Liss, Inc. USA

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So, we will now summarize this lens defects and we will see how they are 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 subject to spherical and chromatic aberrations. Minimization and correction of these undesirable physical effects greatly aided by modern computational techniques, is possible and objectives are classified according to the degree of correction that is; achromats, fluorites they are also called semi-apochromats, apochromats like that. Lenses are usually coated in order to increase the light transmission.

м	Туре	Medium (n)	WD (mm)	NA	d <sub>min</sub> (m)	DOF (m)	В
5	Achromat		9.9	0.12	2.80	38.19	0.1
10	Achromat		4.4	0.25	1.34	8.80	0.4
20	Achromat		0.53	0.45	0.75	2.72	1.(
25	Fluorite	1.515	0.21	0.8	0.42	1.30	6.6
40	Fluorite		0.5	0.75	0.45	0.98	2.0
40	Fluorite	1.515	0.2	1.3	0.26	0.49	17.
60	Apochromat		0.15	0.95	0.35	0.69	2.3
60	Apochromat	1.515	0.09	1.4	0.24	0.43	10.
100	Apochromat	1.515	0.09	1.4	0.24	0.43	3.

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Now, let us see some of the typical characteristic of objective lenses is stabled here. Look at this stable 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 a type of objectives and you have the Medium and this is the Working Distance, WD so working distance in millimeter, this is Numerical Aperture, D minimum is the minimum resolvable distance, this is Depth of Focus in meters and B stands for Brightness.

So you see that as the magnification increases and then how these values are changing. Then you can also see that how the refractive index also influence the other parameters, especially depth of focus and minimum resolvable distance and so on. It gives you a broad idea about what kind of corrections you can make or we can take up. And then probably I will just show you some of the correction which is made on a black board.

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 mu. In fact, it is reciprocal relative dispersion mu is defined as, n D minus 1 divided by n F minus 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 is the refractive index of hydrogen C line. Then also note down this values 589.3 nanometers, 486.1 nanometers, 656.3 nanometers.

So, I am just giving you this because you should know this correction made and what is the basic, these are all spectral values. And then different kind of glass will have different reciprocal relative dispersion mu which is defined by this formula. For example, you can take example a crown glass will have around mus value of 60, and a flint glass will have the value of 38. So consequently these two lenses can be combined we will write, these two lenses can be combined with weaker diverging lens of flint glass so that chromatic aberration cancel for certain lambda.

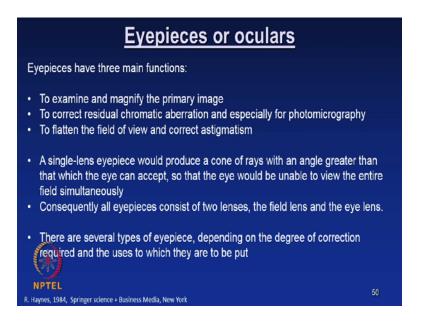
This is one case study, how this correction is done in the case of chromatic aberration. There is a parameter called reciprocal relative dispersion mu and this value for characteristic of different lenses, so for the crown glass it is 60 for a flint glass it is 38; so these two can be combined, because with a weaker diverging lens of a flint glass and that will correct the aberration chromatic aberration for certain lambda.

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ach romatic doublet

We can also draw some schematic how that doublet will look like. So, you have this lens, and then this is a crown glass and this is a flint glass, this is an Achromatic Doublet. So, I just give gave you one example how this correction are been made it gives you an idea. Similarly, all those listed in that table follows certain procedures to take care of the kind of correction which is required or the degree of corrections which is required, and based on which the subjective lenses are classified.

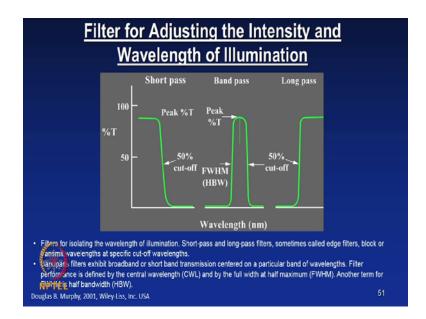
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So, now we will move on to the next item that is Eyepieces and Oculars. I just what to mention the important functions of this eyepiece. We have talked a lot about objective lenses because, they are very critical and important as I mentioned in the last slide. So, let us have some idea about what this eyepieces are doing in a microscope they are also called oculars. Eyepieces have three main functions; and they are to examine and magnify the primary image, and they are also to correct residual chromatic aberration and especially for photomicrography, and they are to flatten the field of view just which we have seen that is a problems and correct astigmatism.

So, these are the three primary functions of eyepieces. 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 consist 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 have different types based upon the degree of correction. And as we know that depending upon the kind of sophistication one requires to build a microscope the combination of an objective and a eyepiece or ocular being selected. If you recall that table which we have shown that the kind of useful magnification which produces the combination of these two (Refer Time: 29:01). Now you will get an idea how a quality of a microscope is decided and how these two lenses where objectives and eyepieces are being selected.

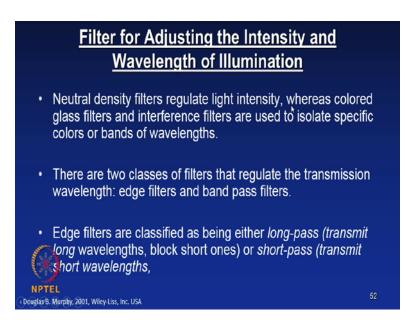


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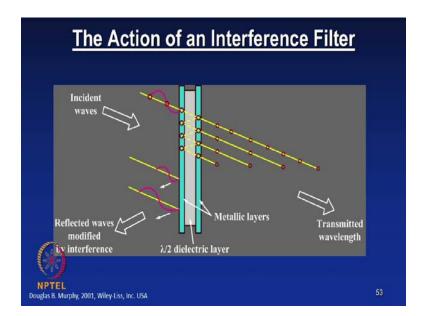
So now, we move on to some other important parts of microscope. I would like to talk about this few Filters for Adjusting the Intensity and Wavelength of Illumination. Look at the slide and for getting a full brightness illumination is also a very important aspect of it. And most of you will see that 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 are these filters 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 shortpass, long-pass filters sometimes called edge filters, block or transmit wavelengths at specific cut-off wavelengths. You can see that it is 50 percent cut-off, it is peak transmission. And the band-pass filters exhibit broadband or a short band transmission centered on the particular band of wavelength 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 band width.

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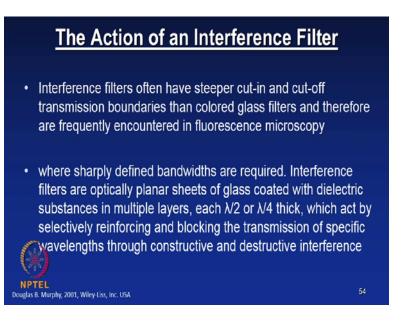


So let us look at some more remarks on these filters. Neutral density filters regulate light intensity, whereas colored glass filters and interference filters are used to isolate specific colors or bands of wavelengths. There are two classes of filters that regulate the transmission wavelength - edge 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 shorter wavelengths. So, I think this is a kind of introduction to this, what are the filters and what their primary functions are.



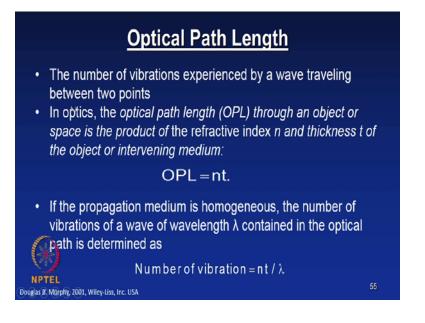
We will now move on to another important filter, An Interference Filter. Look at the slide, and what you are seeing is an action of an interference filter will be using this filter in one of the variants of the optical microscope called Differential Interference Contrast Microscope. So let us look at functions of this interference filter. You have the incident wave coming here, and some of them are waves are reflected are modified by interference and some of them are transmitted. And we have to know how it is done correctly.

You see that the two metallic layers are coated on the dielectric material in such a way that their optical path length is lambda by 2. So when the incident wave which comes and enters this filter perpendicular to the phase, and only those wavelengths will be allowed to pass through and then rest of them will be reflected back. Since all the transmitted waves or in the phase they will be allowed to constructively interfere and becomes a transmitted wave. So, this is the function of the interference filter. We will see the usage of this filter much more detail when we actually take up the variant of this microscope in the coming lectures.



Let us see few more remarks on these filters. Interference filters often have a steeper cutin and cut-off transmission boundaries than the colored glass filter and therefore are frequently encountered in a fluorescence microscopy.

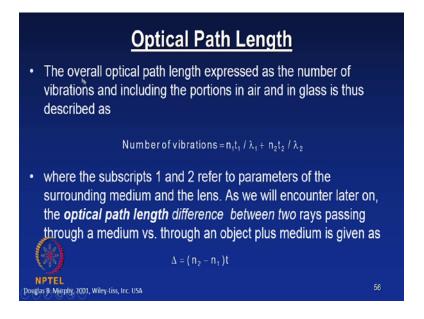
Where, sharply defined bandwidths are required. Interference filters are optically planar sheets of glass coated with dielectric substances in multiple layers, each it could be lambda by 2 or lambda by 4 thick, which act as selectively reinforcing and blocking the transmission of specific wavelengths 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. We will be using this concept in one of the, again another variant of the optical microscopy. so let us see what this optical path length is. The number of vibrations experienced by a wave traveling 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 is equal to n times 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 lambda contained in the optical path is determined as; number of vibration is equal to n times t divided by lambda.

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The overall optical path length expressed as the number of vibrations and including the portions in air and in glass in thus described as, number of vibrations equal to n 1 t 1 divided by lambda 1 plus n 2 t 2 divided by lambda 2.

Where, the subscripts 1 and 2 refer to 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 equal to n 2 minus n 1 times t. So I mentioned we will be using this parameter in one of the variants of the optical microscope which I will be discussing it, that is why I was introduce this concept.

# Light Microscope

- Examination in the as-polished condition, which is generally advisable, will reveal structural features such as shrinkage or gas porosity, cracks and inclusions of foreign matter.
- Etching with an appropriate chemical reagent is used to reveal the arrangement and size of grains, phase morphology, compositional gradients (coring), orientation-related etch pits and the effects of plastic deformation.

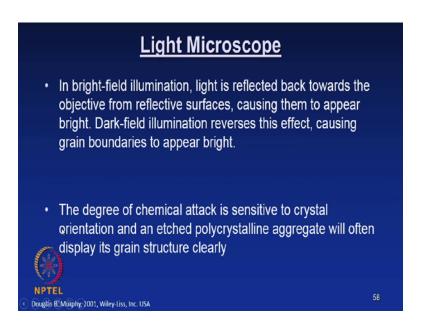
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We will now see the general description of Light Microscope. So what I have done is, if we look at all the three classes I have taken some of the fundamental concepts which you require to understand before we get into the use this light optical microscope. I hope it will be useful in order to understand the function 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 also take you to the lab and then show some of the videos of actual 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 structural features such as shrinkage or gas porosity, cracks and inclusions of foreign matter. And for that you need to something called Etching, I will be dealing with that in much more detail when why when I talk about a sample preparation for all this microscopic techniques. However, you just look at the initial remarks.

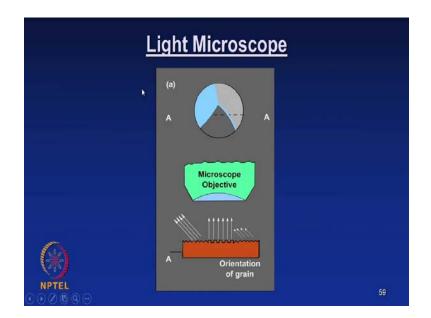
Etching with an appropriate chemical reagent is used to reveal the arrangement and size of grains, phase morphology, compositional gradients sometimes called coring, orientation-related etch pits and the effects of plastic deformation. These are all only a some of the features which I have just mentioned, but in reality we will see how much we can use this or how effectively we can use this microscopic techniques for various application is material science 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 dark appears bright. So I will just take up this two, I mean the actual microscopic part when we discuss a specific application and this is just to give you idea of what kind of method even in a light microscope a basic imaging techniques one is bright field illumination another is a dark field illumination. And the image quality is depending upon the degree of chemical attack is sensitive to crystal orientation and an etched polycrystalline aggregate will often display it is grain structure clearly.

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



And this is just to give you an introduction about this microscope. You have an objective, you have this specimen here, it is a schematic of an edge surface, and you see that light if being reflected at a different orientation because due to their different orientation of the grain. We will see actually the experiments.

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Now you can look at these references, 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. 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 Evans, and Shaun Wilson, Butterworth-Heinemann, Boston, USA. You can also read this, Transmission Electron Microscopy by B.D. 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, Elsevier publication. You can also go through the website, www dot microscopy u dot com.

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Now, what I am going to do is I am just going to introduce you to the some of the microscopes. What is coming on the screen is a typical a metallurgical microscope. There are two basic types of microscopes; one is vertical type, another one is inverted type. So what you are now seeing is inverted optical microscope. I will just show some of the main parts which we have talked about.



This is the specimen stage. Since your vertical microscope, this is the specimen stage will be on the top.

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And these are oculars.

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An eyepiece, just 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.

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And these are all some of the polarizing lenses and (Refer Time: 44:50) I will talk about this little later is one of the medium size as I mention that time we will use this. I mean (Refer Time: 44:59).

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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.

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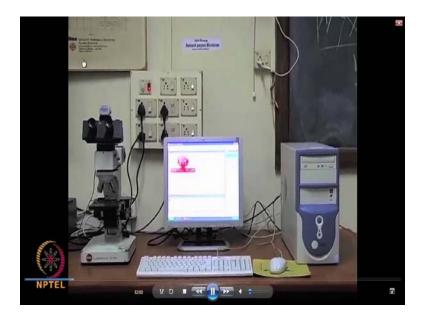


And what now you are seeing is another vertical simple type microscope, a standard microscope. In any of the metallurgical laboratory you see that oculars.

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And you see the objectives. Usually, we will have 3 to 5 objectives it is there.



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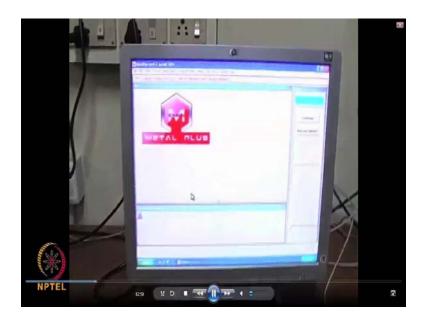
And this is again another microscope which is attached with the image analysis system.

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

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So, this is the tool which is called Leveling Press to make the solid specimen in a same level, using this plasticizer.



So, I am just describing this assuming that we will be using only the solid metal piece to examiner with the microscope, not necessary in the case.

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We will see the other materials how it is being viewed in the other type of optical microscope.

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And this is how the solid metal pieces leveled using this a leveling press, and then it will be placed on this microscope as you can see that. I will get into the details of all this preparations in a separate class.

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I am just introducing how the microscope will look like and how people use it and for those who have not come across this kind of an experience. So you can see that now the specimen is being loaded in the specimen stage and then you choose the appropriate I mean objective lens. You can start with a lowest magnification to highest magnification. You can slowly move from lowest aperture to the highest aperture, and that magnification is multiplication of these two. For example, you have volt 5 x here and then here it is 10, so it is 50. If you choose 50 here it is 500, something like that.

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So, here again for image grabbing you have the CCD camera attached to this.

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Now, what you are now seeing is this another type of microscope called Transmission Optical Microscope, which is very different from what you have just seen before.

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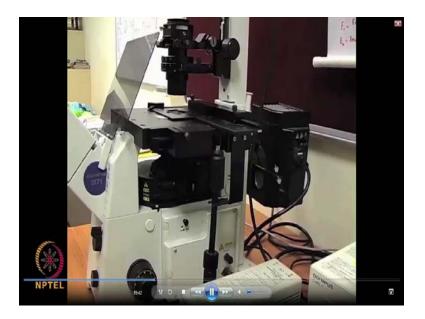


That is one is vertical.



Another is inverted microscope.

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Here, you have two type of illumination attached to it, one is a mercury lamp and another is halogen lamp on the top.



So, I will just describe these microscopic parts so that you will get familiar with what are the important things you need to understand. What is that being shown here it is a polarizer; and you when you do not use that polarizing mode, then it will be a slot for a bright field mode. Then you also have a kind of a condenser aperture lenses in this which is having a different slots. I will talk about it in much more elaborately in the next class.

Thank you.