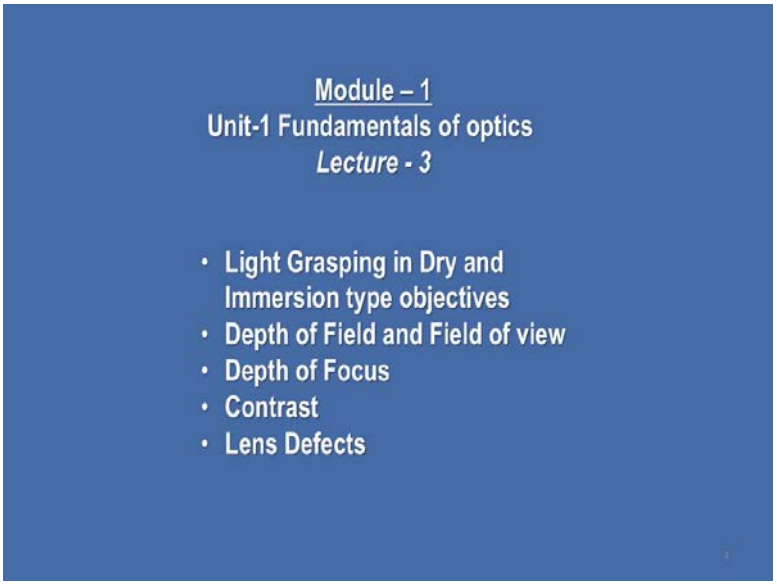
Fundamentals of optical and scanning electron microscopy

Dr. S. Sankaran
Department of Metallurgical and Materials Engineering
Indian Institute of Technology, Madras

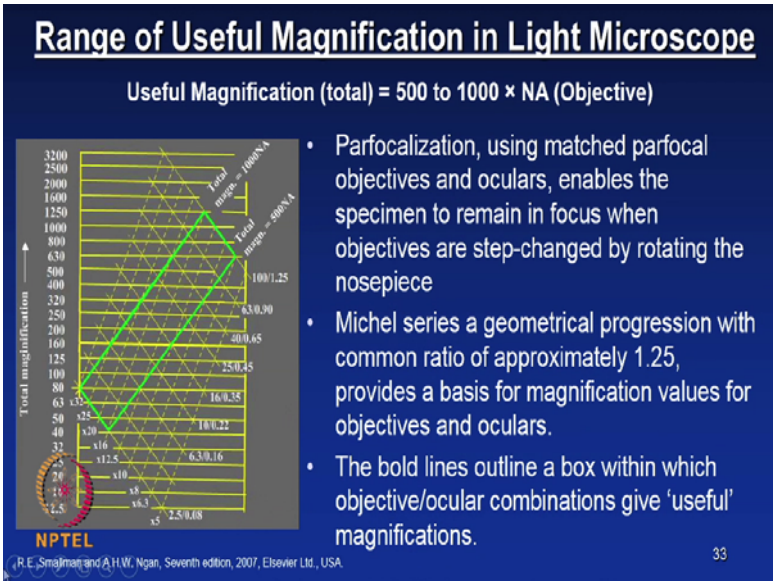
Module – 01
Unit- 1 Fundamentals of optics
Lecture – 03
Light grasping in dry and immersion type objectives
Depth of field and field of view
Depth of focus
Contrast
Lens defects

(Refer Slide Time: 00:11)



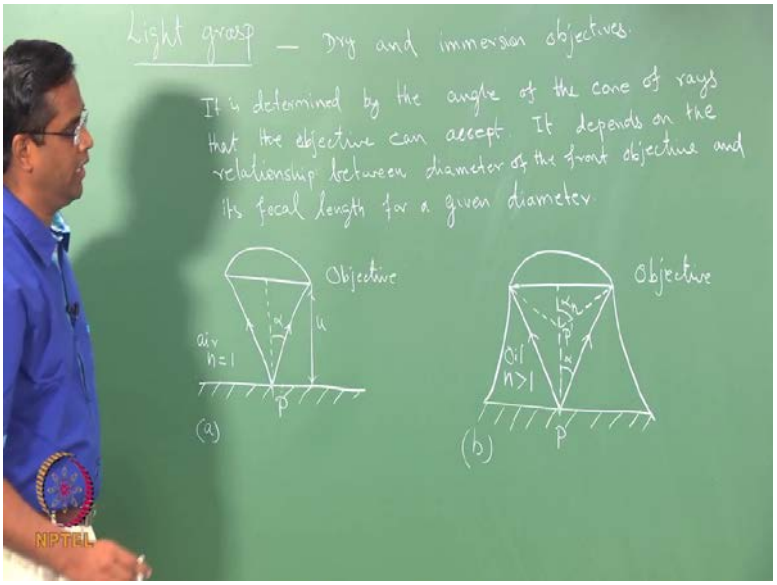
Hello, welcome back. In the last class, we were reviewing the resolution in numerical aperture and then spatial resolution and little bit about magnification and then empty magnification all such parameters. Today we will continue in those lines, and you will just look at the slide which we talked about in last class.

(Refer Slide Time: 00:43)



The slide shows that the range of useful magnification in the light microscope. As I mentioned, this is a plot of the combination of objective and ocular lenses where the green box indicates the combination of these two gives the useful magnification. So, in order to emphasize the light gathering power of an objective, this eventually defines the quality of the image. We will now have little bit of understanding or a discussion on the light grasping power of the objective lens. You have two types of objective lens; well one is a dry, and other is an immersion objective lens. Both of them will have a different medium. The dry objective lens will have an air as a medium, where the immersion objective will have a normally an oil, where the refractive index of that oil will be much higher than the air. So, let us see how this is going to help us in grasping the light towards the objective lens, which is eventually going to improve the quality.

(Refer Slide Time: 02:27)



So, let us look at the schematic, which I am going to draw about the light grasp of the objective lens. So, a kind of I have written a definition for the light grasp of an objective lens. It is determined by the angle of cone of rays that the objective can accept. And it also depends on the relationship between diameter of the front objective and its focal length for a given diameter. So that means, your light grasping power of an objective lens is fixed for a given diameter, but it has the relationship between diameter of the front objective lens and its focal length.

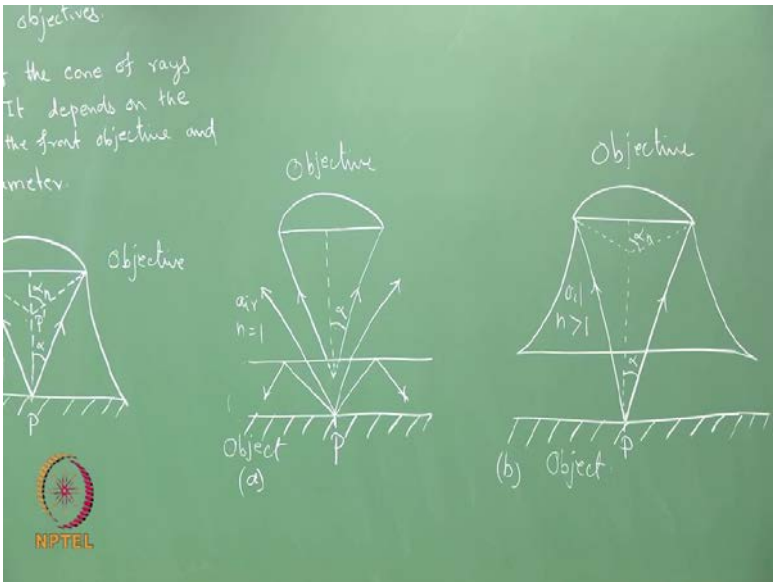
So, let us draw some schematic, which illustrates this concept. Let the surface be the object surface and we will concentrate the point P from where the reflection takes place. And then let us assume this, it is an objective lens. Let it be a center P. This is objective lens. So, this is a dry lens. The medium is air, where refractive index is equal to 1. So, now we will see that the angle of cone accepted by this aperture is alpha and the distance is u. Let us consider this image as ‘a’. So, this is a dry objective lens; this is the object surface and from where the light is reflected, and this is the objective lens. This is the cone it is actually a cross section of a three-dimensional cone and this is the angle, and this is the angle which by refer here is here, this one.

Now, we will draw a similar schematic for an immersion objective. So, now object

surface that would be the point P, and you have similar objective. Let the rays coming from this object, which is getting collected by these objectives also similar. Let us mark the angle alpha - collection angle alpha. Now, we are going to introduce the medium; this is a medium between the objective lens and then object. Let us assume this as an oil, which has that the refractive index greater than 1, and because of this, see up until this point these two are same, the once you introduce the medium between the objective lens and then object, then what happens to the collection angle.

So, let us considered this as P dash, so what I have drawn here is now because of this medium and its refractive index is greater than 1 that is air, your collection angle appears to be at P prime rather than P. So, you have the new collection angle alpha n. So, this makes the objective lens to collect lot more rays compared to the dry objective lens. This is something similar to the water which is water is filled in a pool, you can see that the things which are lying at the bottom will appear much closer than the actual distance, the similar effect you will get. So, you get more clarity here. The point, which I am trying to make here is, once you fill the gap between the objective lens and object with a medium, which is having higher refractive index, the light grasping power will be more.

(Refer Slide Time: 12:06)

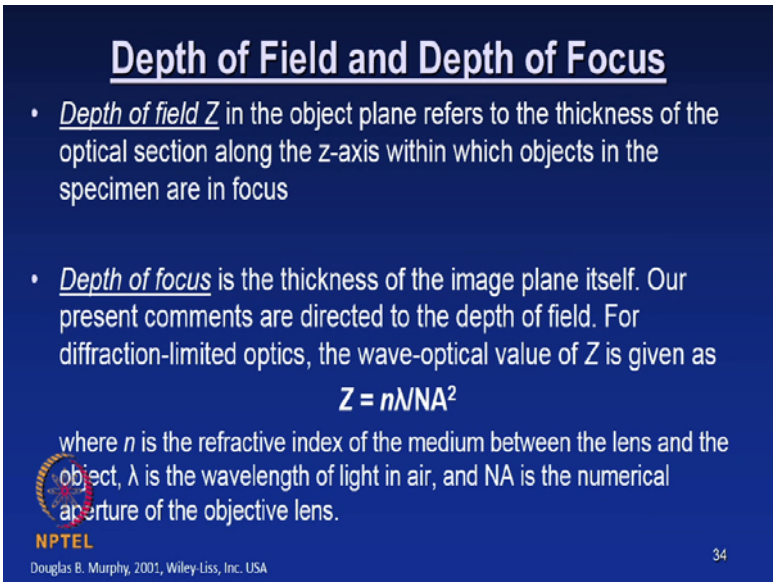


And similar thing you can we can draw for the objects with cover slip. So, similar, this is

with a specimen with the cover slip. What I have drawn here is it is a transparent glass cover on the specimen; most of the transmission optical microscope, this cover slip is a part of the microscopic system, and even if you look at the light getting power for a specimen, which is covered with cover slip. This is the light gathering angle. Now, we will compare this with again with an oil immersion objective, what happens?

Now, let us compare these two. So, this is an object with a cover slip, and you see that with the light which is emanating from this object before it get collected into this objective lens, it undergoes reflection as well as refraction. And only the rays, which are subtending this angle coming within this angle, get collected into the objective. And if you look at the same thing with an oil immersion, so almost all the rays which are coming from this region, it is get collected. The similar effect what you see here will be felt here, so your light grasping power is enhanced. So, what we have now seen is an object with a dry objective, an object with immersion objective, and object with a cover slip, an object with the cover slip plus immersion. So, these 4 figures clearly indicates that the light gathering power of an objective lens can be enhanced through some of the medium which has got higher refractive index than the air, so that is the point.

(Refer Slide Time: 18:31)



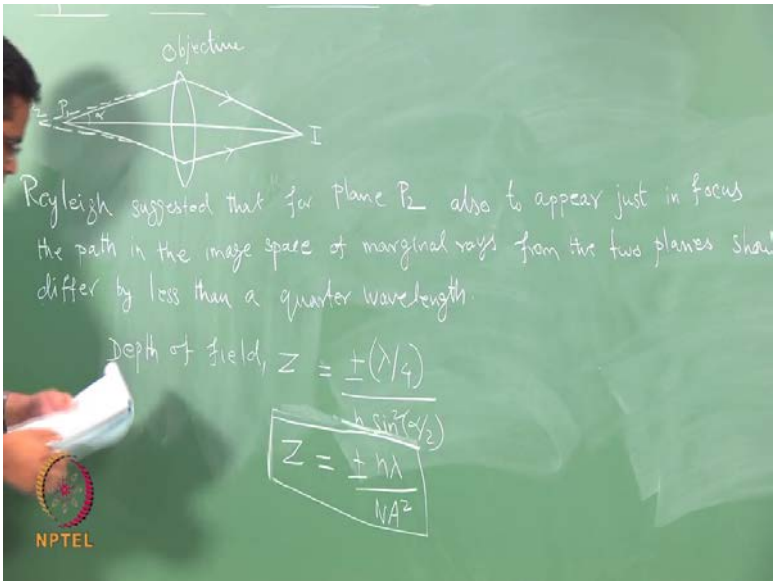
So, now we will move on to the next important parameters or concept in microscope,

depth of field and a depth of focus. So, let us look at the preliminary definitions.

The depth of field Z in the object plane refers to the thickness of the optical section along the z-axis within which objects in the specimen are in focus. On the other hand, depth of focus is the thickness of the image plane itself. So, for diffraction limited optics, the wave-optical value of z that is the depth focus is given as Z equal to n lambda divided by numerical aperture square. Where n is refractive index of the medium between the lens and the object, lambda is a wavelength of the light in the air, and NA is numerical aperture of the objective lens.

So, what you have to remember is a depth of field is related to the object, and depth of focus is related to the image. It is the distance with which you move the object still you see the images in focus that distance is called depth of field. And similarly you have the thickness of the image within which you will have the total image in focus is called depth of focus. We will understand this mathematical relation with few more schematic.

(Refer Slide Time: 20:29)

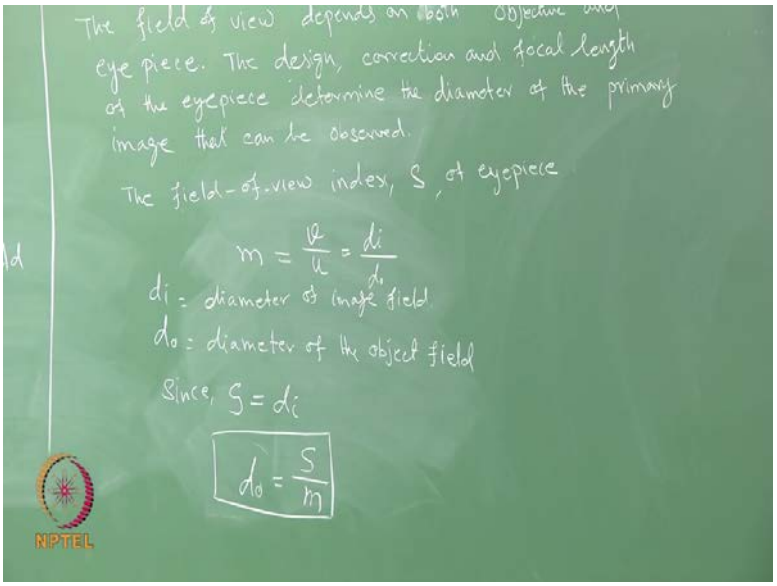


We will also look at the other concept to field of view that is, what is the region, you will be able to view through your eyepiece or objective lens that depends upon some of the basic quantities. So, let us first look at the depth of field. Let me draw a simple ray

diagram for a glass lens. So, this is an optic axis when you have rays coming from the object and it forms an image I. And this is our alpha, you know that now, this is objective. So, let us say point P 1. Now let me draw another point P 2. Let me write few lines. What I am trying to emphasize here is, so this is I just said that your depth of field is the object plane. The distance with which you can move the objects still you get the image under the complete focus.

Rayleigh suggested that let us assume this is a this ray coming from P 1 plane and this is from P 2 plane; he suggested that for a plane P 2 also appearing just focus the path in the image space of the marginal rays - these two rays, the path the optical path of these two marginal rays from these two planes P 1 and P 2 should differ by less than a quarter wavelength. If this path difference is within a quarter wavelengths then the image will be in the focus. So, for that he has written given expression a depth of field, since we call it is Z let us put it here is equal to plus or minus lambda by 4 divided by n sine square alpha by 2 which is nothing but Z equal to plus or minus n lambda divided by numerical aperture square. So this is what I have just shown in the slide. So, it is given from the Rayleigh's explanation for the depth of field.

(Refer Slide Time: 27:11)

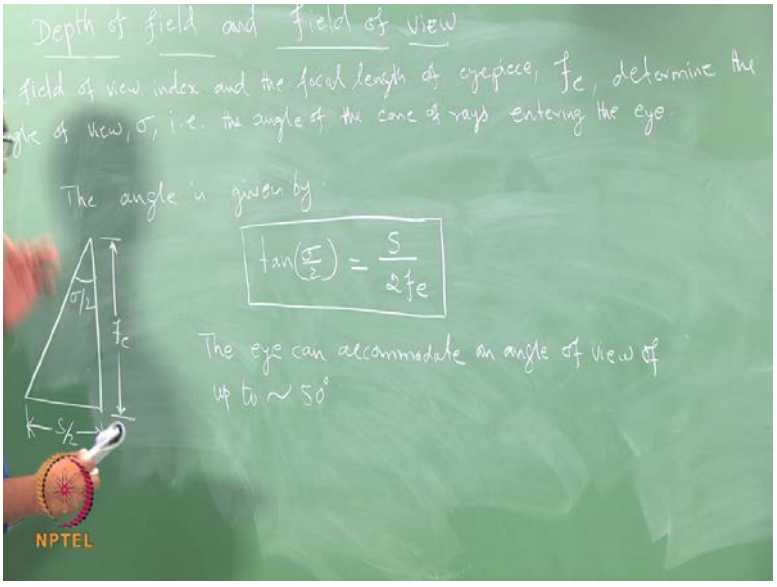


So, now let us look at what is field-of-view. Let me write some few lines. The field of

view depends on both objective as well as eyepiece. The design, correction and focal length of the eyepiece determine diameter of the primary image that can be observed. So, let us look at this sentence again. The field of view depends upon both objective and eyepiece. The design, correction; what is correction? We will talk about lengths defects just after this. And then we have to correct that defects and then you have to choose a focal length and that depends upon these design correction and focal length various eyepiece are possible, similarly objectives, various objective lenses are also possible, so that is what it is, so that time you will appreciate this term. So, these three designs, correction and focal length of the eyepiece determine the diameter of the primary image that can be observed.

So, we can write a simple expression the field of view index S of eyepiece, we can relate with this a magnification. This is true for a simple ray diagram, where di is a diameter of image field, and d naught equal to diameter of the object field. And since your field of view index S is equal to di we can write d naught is equal to S by m, this is magnification - m is magnification, S is field-of-view of index, and d naught is the diameter of the object field. So, what it means? The lower the magnification of the objective, the greater becomes the field-of-view.

(Refer Slide Time: 32:14)

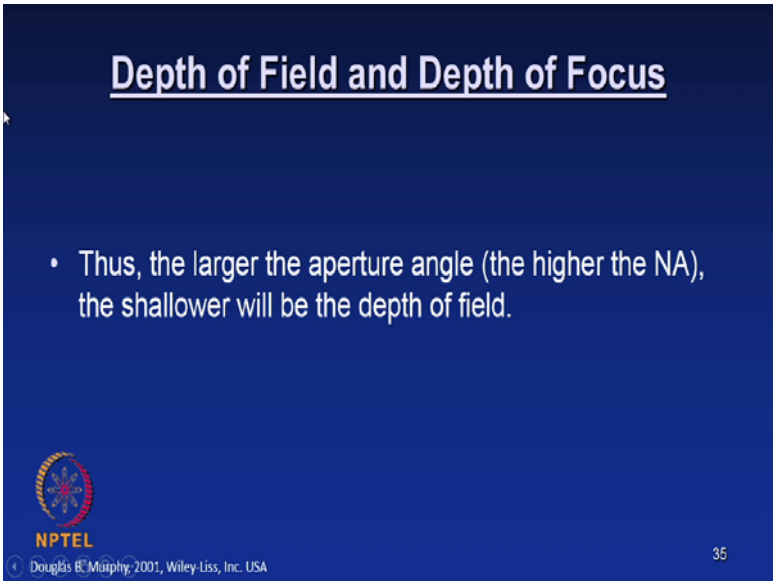


And we will also write one more expression. The field of view index and the focal length of eyepiece that is f e, determine angle of angle of view, sigma that is the angle of cone of rays entering the eye. So, ultimately we are interested in this, the field of view index and the focal length of the eyepiece f e, determine the angle of view sigma that is the angle of cone of rays that is entering into your eye when you look at the microscope.

So, this angle is given by if you consider this as the cone section and this is your sigma by 2, this is an angle of view here it is sigma. The sigma and the alpha is the same since I have taken from different references, they have used different symbol. So, here it is alpha, the correction angles alpha here, it is refereed a sigma both are same. And this distances is a f e that is focal length of the eyepiece; and this is S by 2, this is a field of view index. Since we are consider only in the half of the cone section, so this is S by 2. So, based on this we can the angle you can write tan sigma by 2 equals S divided by 2 times f e. So, this is we can write the eye can accommodate an angle of view of up to approximately 50 degree.

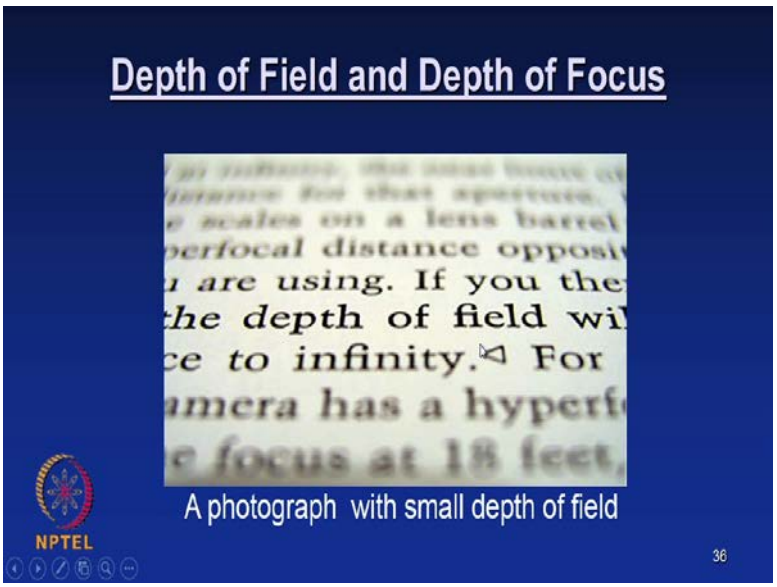
So, we just looked at the light grasping power of an objective lens and similarly we also where interested in the field of view up to which you can just see that object of the interest and that is determined by the eyepiece characteristics that is the design, correction, focal length and so on. And then it is specifically, it can be considered through this formula so that is another aspect of the field of view.

(Refer Slide Time: 37:02)



Let us now look at the come back to this depth of field and depth of focus. So, the larger the aperture angle that means, the higher the numerical aperture, the shallower will be the depth of field.

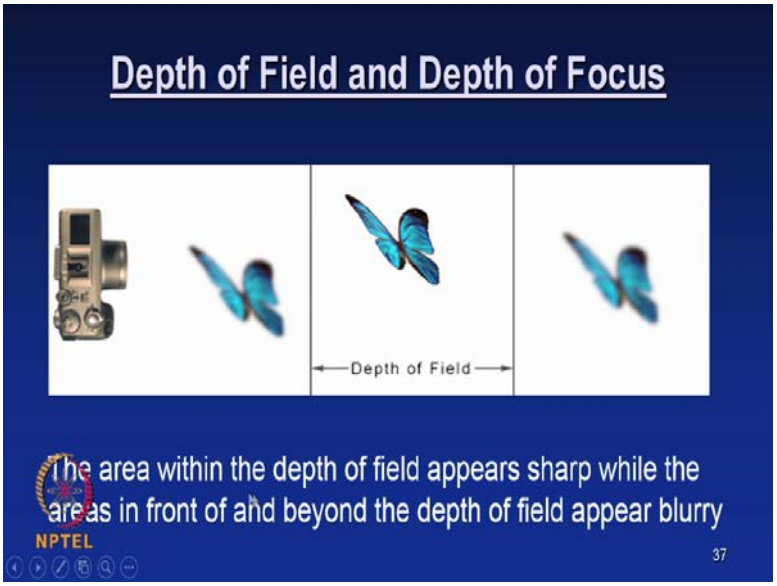
(Refer Slide Time: 37:17)



And you can see this example a photograph with the small depth of field. So, when you

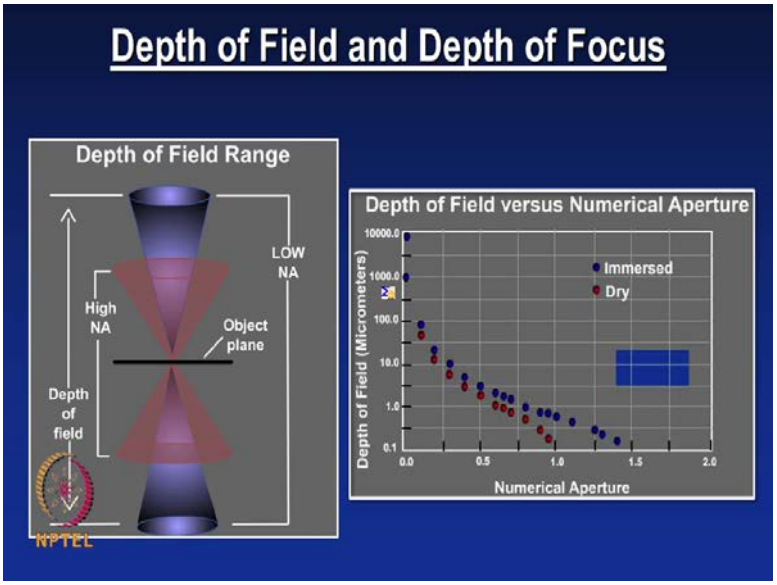
have a shallow depth of field, this is how your image will look like.

(Refer Slide Time: 37:34)



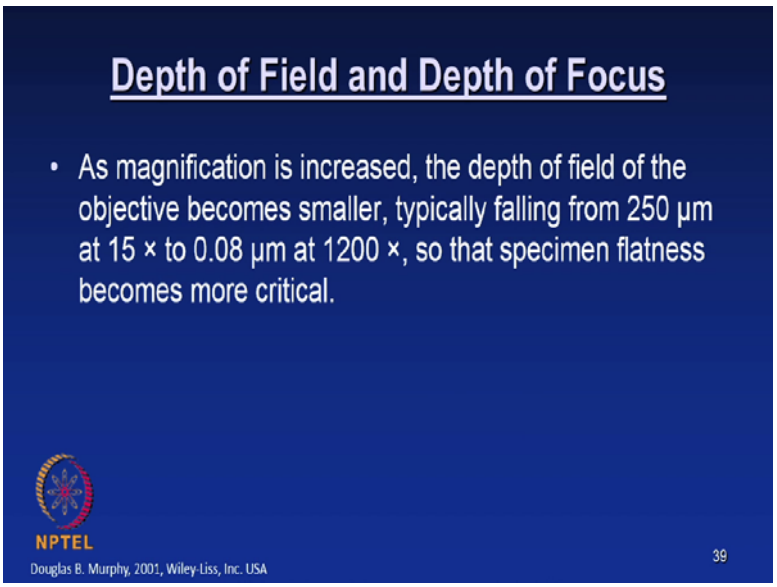
And this another example where you have the area within the depth of field appear sharp, this the butterfly image appear sharp within this depth of field; while the areas in front of and the beyond depth of field appear blurry.

(Refer Slide Time: 37:57)



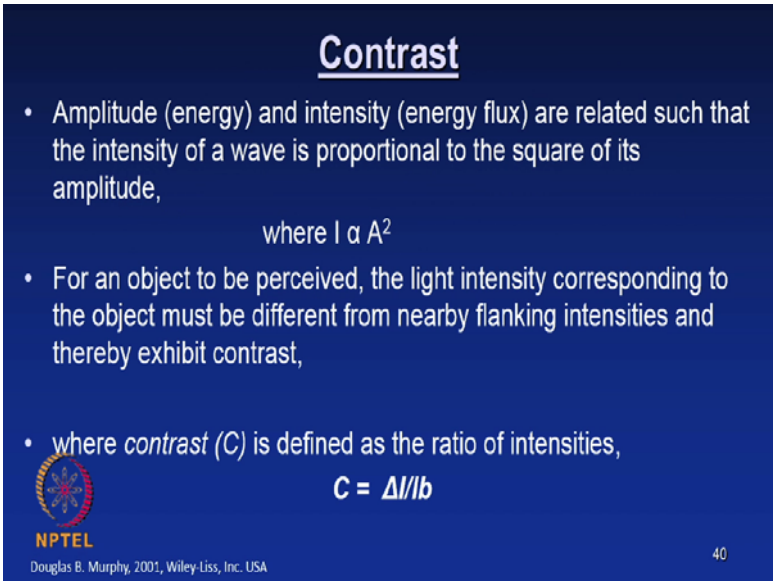
And these two schematics illustrate the depth of field range as the function of numerical aperture. You can see that as we stated earlier. And your higher numerical aperture means a smaller depth of field, lower numerical aperture means higher depth of field. And this plot shows clearly that the depth of field versus numerical aperture as the numerical aperture increases, your depth of field decreases. And these two data points for dry and immersed objectives and obviously, you see that the immersed objective lens performs better as compared to dry objective lens. And now that you know why it is so we have clearly seen on the blackboard with some example, how the immersion aperture enhances the light grasping power of the lens and then eventually the image quality.

(Refer Slide Time: 39:14)



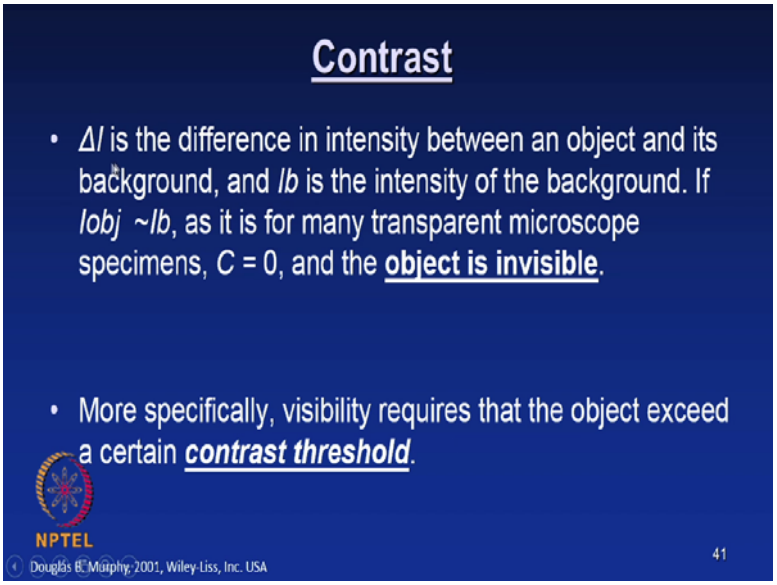
So, now so this is the same thing as the magnification is increased, the depth of field of the objective becomes smaller, and typically falling from 250 micrometer at 15 x to 0.08 micrometer at 1200 x, it is a kind of a range; so that specimen flatness becomes more critical. So, that is why you see that a very shallow depth of field, once you cross this range.

(Refer Slide Time: 39:50)



Now, we will go to the next concept called contrast. See most of the optical micrograph or for instance you take any a micrograph, people are interested in the contrast, best contrast and the quality. So, let us understand the term contrast. What is contrast? Let us look at the preliminary remarks. Amplitude (energy) and intensity which is energy flux are related such that the intensity of the wave is proportional to the square of its amplitude. That is I read it again, amplitude, which is energy and the intensity which is energy flux are related such that the intensity of the wave is proportional to the square of its amplitude, I propositional to A square. For an object to be perceived, the light intensity corresponding to the object must be different from the nearby flanking intensities and thereby exhibit contrast. The contrast C is defined as the ratio of intensities that is C is equal to delta I by Ib.

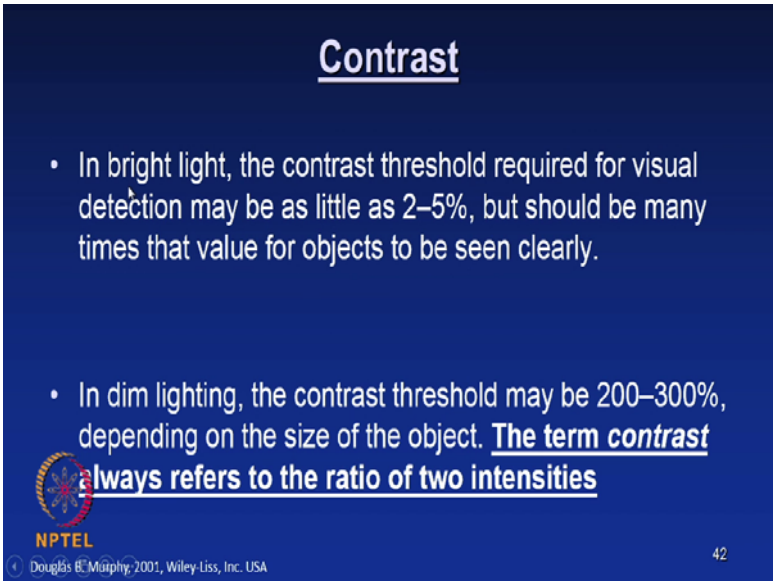
(Refer Slide Time: 41:23)



Where delta I is a difference in the intensity between an object and its background, and Ib is the intensity of the background. If I objective is equal to I background as it is for many transparent microscope specimens, C is equal to 0, and the object is invisible. More specifically, the visibility requires that the object exceed a certain contrast threshold.

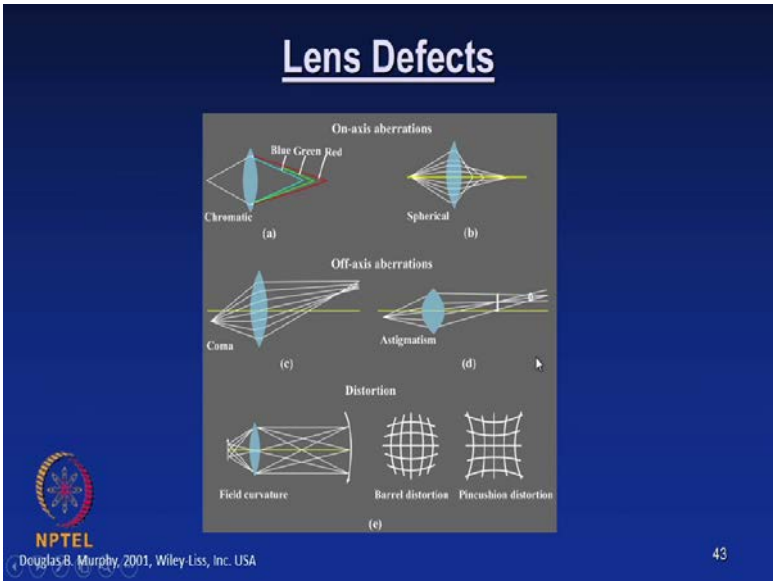
So, what we are interested in any micrograph as I mentioned, we are interested in the features which we are looking for. And to examine that feature you need to look at them much more clearly for that what you normally see in any micrograph is three colors - a bright, a gray, and a black. So, out of these three, your object will be perceived as individual entities, and the entities which you are looking at are much more clear only when this the contrast is good. So, what is that?

(Refer Slide Time: 42:57)



In a bright light, the contrast threshold required for visual detection may be as little as 2 to 5 percent, but should be many times that value for objects to be seen clearly. In dim lighting, the contrast threshold may be 200 to 300 percent depending upon the size of the object. The term contrast always refers to the ratio of two intensities.

(Refer Slide Time: 43:31)

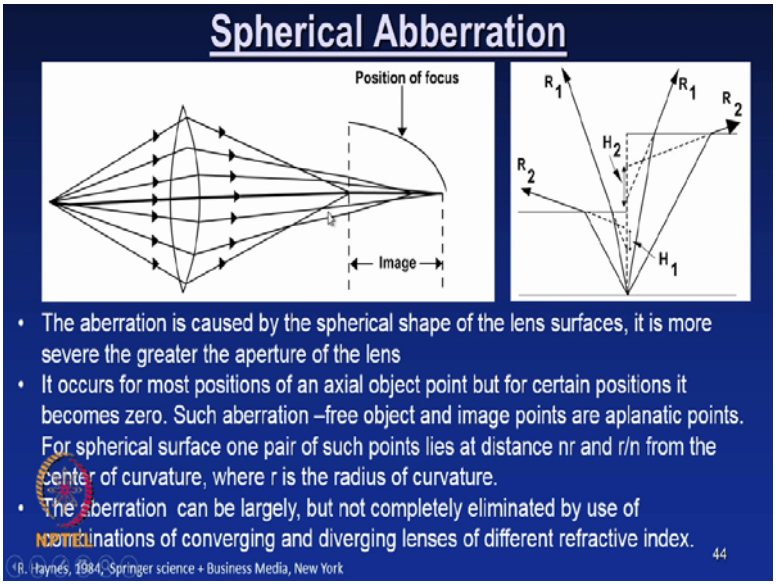


So, before we wind up the contrast, let us recall the definition of the very definition of the contrast in order to perceive that image, you need to have an object should have a particular intensity and its background should have a very different intensity. Unless there is significant difference in intensity between that object and its background, your entity will not be able to be recognized by your eye. So, always the contrast is the ratio of the difference in the intensity of the object and then background to the object intensity of the background. So, it is a ratio, ratio of the two intensities as a contrast. We will talk about this contrast much more when we progress in the image analysis in the latter part of it. And for time being, I just want you to just remember this definition, basic definition.

Now we will talk about a lens defects. All the time we talked about an objective lens, and an eyepiece, and their characteristics, and light gathering power and so on. So, all this light gathering power and then which have the complete control of image quality depending upon the defects of the lens also. So, let us first look at what are the lens defects in general and then we will see in each defect how it can be corrected for a better quality image.

So, let us look at this slide, which summarizes the whole lens defects. The schematic-a is a chromatic aberration, and schematic-b is a spherical aberration. And these two chromatic and spherical aberrations are classified as on-axis aberrations. And schematics-c is a coma and schematic-d is astigmatism, and the coma and astigmatism are classified as off-axis aberrations. And then we have off shoot of these, a distortion which is a feed curvature, and you have a barrel distortion or pincushion distortion. So, we will see one by one and will try to understand what are these defect really means.

(Refer Slide Time: 46:43)



First, look at the spherical aberration. Look at the schematic. What do we see, we see that the rays which are coming close to the optic axis are focused farthest, and rays which are passing through the periphery of the lens are focus in the nearest. And the rays which are passing through in between these two are focused between these two points. So, you have the position of focus ranging from a distance like this which constitutive an image.

So, let us look at the preliminary remarks, the aberration is caused by the spherical shape of the lens surface that is because of this spherical surface, the name itself tells; it is more severe the greater the aperture of the lens. It occurs for most positions of an axial object point but for certain position, it becomes zero. Such aberration - free object and image points are aplanatic points. For a spherical surface, one pair of such points lies at a distance nr and r by n from the center of curvature, where r is the radius of the curvature. And this parameter is used to fabricate the high power lenses that are aplanatic points concept. And this aberration can be largely, but not completely eliminated by use of combinations of converging and diverging lenses of different refractive indexes.

This is one of the important and very difficult defects to be eliminated in the optical lens. So, we will look at this spherical aberration, how it is being eliminated in the due course. And other aberrations, we will see in the next class.