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Lecture-3
Materials Characterization

Fundamentals of Optical microscopy

Dr. S. Sankaran
Associate Professor
Department of Metallurgical and Materials Engineering
IIT Madras
Email: ssankaran@iitm.ac.in

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

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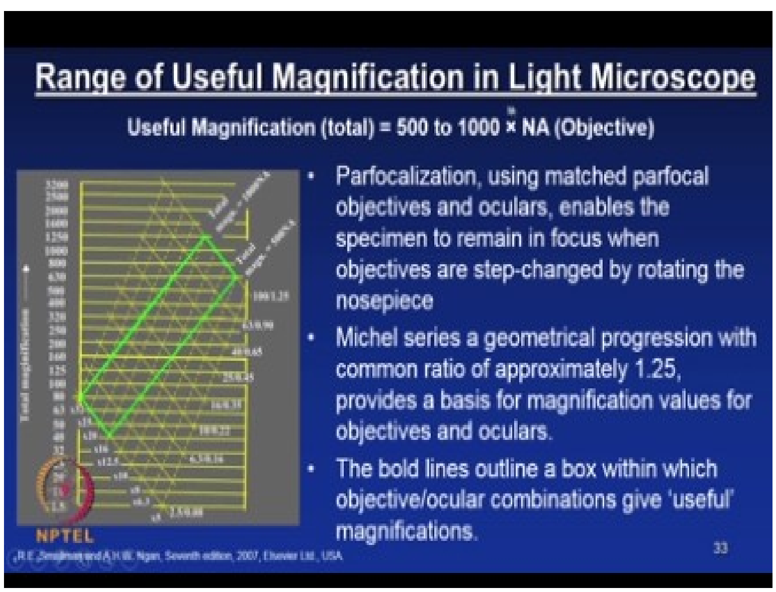

The slide shows that the range of useful magnification in the light microscope as I mentioned this is a plot of the combination of objective and ocular lenses, where the green box indicates the combination of these two gives the useful magnification. So in order to emphasize the light-gathering power of an objective which eventually defines the quality of the image, we will now have a 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 dry and other is an immersion objective lens both of them will have a different medium that dry objective lens will have an air as a medium; where the immersion objective will have normally an oil where the refractive index of the 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. So let us look at the schematic which I am going to draw about the light grasp of the objective lens.

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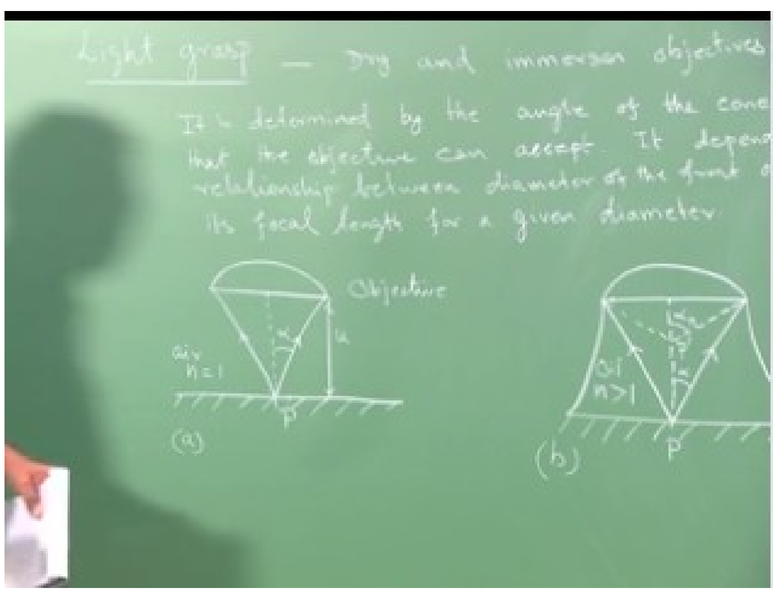

So I am kind of written a definition for the light grasp of an objective lens. It is determined by the angle of cone of rays, that the objective can accept; and it also depends on; depends on the relationship, between diameter of the front objective and, and its focal length for a given diameter. So that means your light grasping power of objective lenses is fixed for a given diameter but it has the relationship between diameter of the front objective lens and it is its focal length. So let us draw some schematic which illustrates this concept. Let this surface be the object surface and we will concentrate the point P from where the, the reflection takes place and then let us assume this is an objective lens.

Let it be center P. this is objective lens. So this is a dry lens so the medium is air where refractive index equal to 1. So now we will see that the angle of cone accepted by this aperture is α and the distance is u. Let us consider this image A. So this is a dry objective lens this is the object surface and from where they write this reflected and this is the objective lens. This is the cone. It is actually across-section of a three-dimensional cone and this is the angle. And this is the angle which I refer here is here this one. So now we will draw a similar schematic for a immersion of objectives.

So now object surface; let it be the point P and you have similar objective .Let the rays coming from this object which is the getting collected by this objective is also similar. Let us mark the angle α collection angle α. Now we are going to introduce the medium: this is a medium between the objective lens and then object. Let us assume this is in oil which is that the refractive index greater than 1, and because of this, say up until this point these two are same. The once you introduce the medium between the objective lens and the object then what happens to the collection angle.

So let us consider this as P'. So what I have drawn here is now because of this medium and its refractive index is greater than one, that is air; your collection angle appears to be at P' rather than P. So you have a new collection angle α_M . So this makes the objective lens to collect lot more ray's compared to the dry objective lens. This is something similar to the, 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, the point which I am trying to make here is once you fill the gap between the objective lens and object within a medium which is having higher refractive index, the light grasping power will be more and similar thing you can we can draw for the objects with cover slip. So similar this is with a specimen with a 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;

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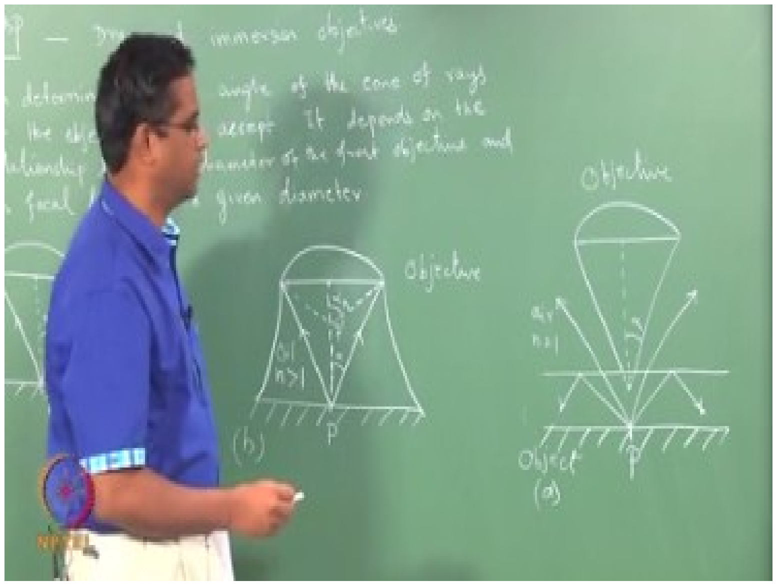

a part of the microscopic system and even if you look at the light-gathering power for a specimen which is covered with cover slip this is the light gathering angle. So now what will we will compare this with again with the oil immersion objective what happens.

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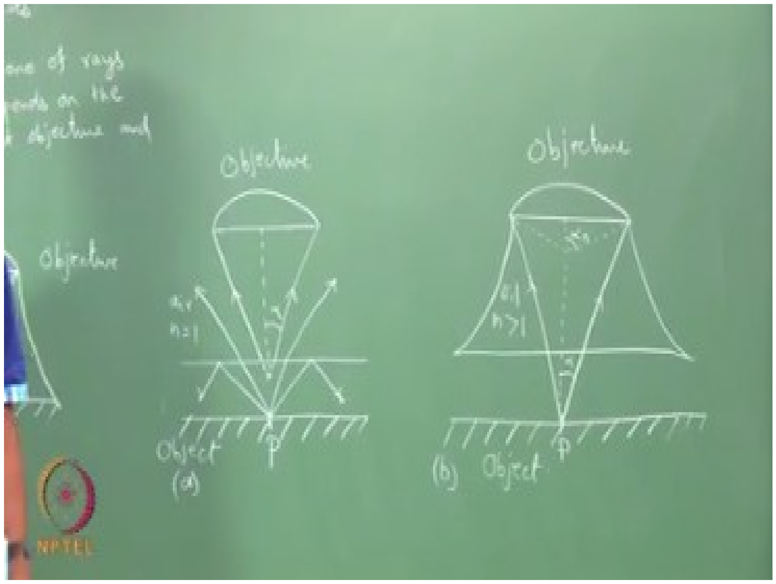

So now let us compare these two. So this is an object within a cover slip and you see that with the light which is emanating from this object before it get collected into this objective lens it undergoes reflection as well as refraction. And only the rays which is subtending this angle coming within 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 airy region is get collected the similar effect what you see here will be felt here.

So you are light grasping power is enhanced. So what we have now seen is an object with a dry objective, an object with an immersion objective and object with a cover slip an object with the cover slip plus the immersion so these four 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. So now we will move on to the next important parameters.

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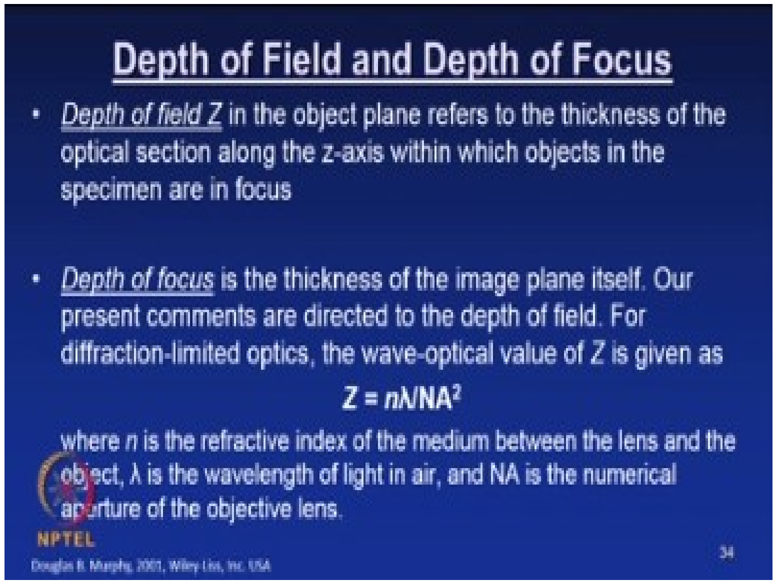

Our concept in microscope: depth of field and depth of focus. So let us look at the preliminary definitions the depth of field is that in the object plane refers to the thickness of the optical section along the z-axis within which objects in the specimen are in focus. On the other hand depth of focus is the thickness of the image plane itself. So for a diffraction-limited optics the wave optical value of Zm that is depth of focus is given as $Z = n\lambda / (NA)^2$.

Where n is refractive index of the medium between the lens and the object; λ is the wavelength of the length 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 the mathematical relation with few more schematic.

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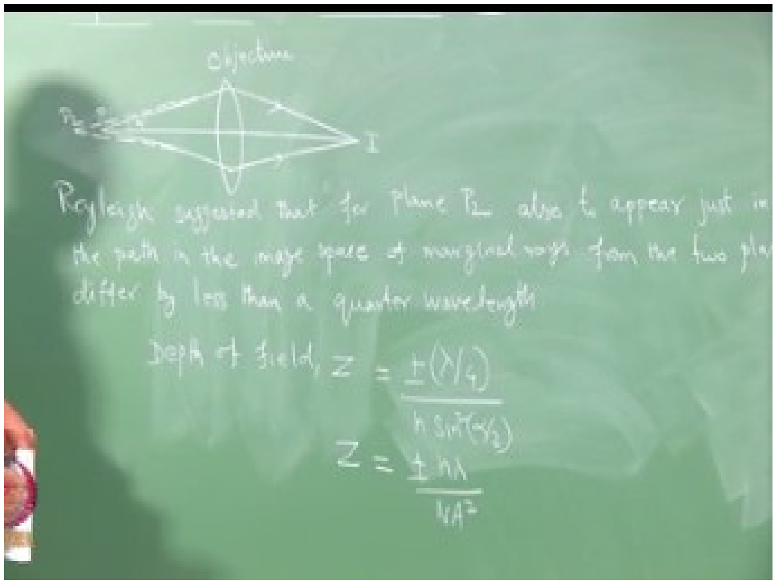

We will also look at the other concept: field of view that is what is the region you will be able to view through your eyepiece or objective lens; that depends upon some of the basic quantities. So let us first look at the depth of field. Let me draw a simple ray diagram for a glass lens. So this is optic axis, then you have rays coming from the object and it forms an image I; and this is our alpha you know that now this is injective.

So let us say point p1. Now let me draw another point p2, let me write few lines. So what I am trying to emphasize here is, so this is the I just said that your depth of field is the object plane so the distance with which you can move the object; still you get the image under the complete focus. So Rayleigh suggested that let us assume this is this ray coming from p1 plane and this is from p2 plane, he suggested that for a plane p 2 also appear in 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 the two planes from these two planes p1 and p2 should differ by less than a quarter wavelength.

So if this path def difference is within a quarter wavelength then the image will be in the focus so for that he has written given expression a depth of field since we call it a Z let us put it here $= +\_\lambda / 4 n \sin^2 (\alpha / 2)$ just nothing but $Z = n \lambda / NA^2$. So this is what I have just shown in the slide so you just given from the Rayleigh's explanation for the depth of field. 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.

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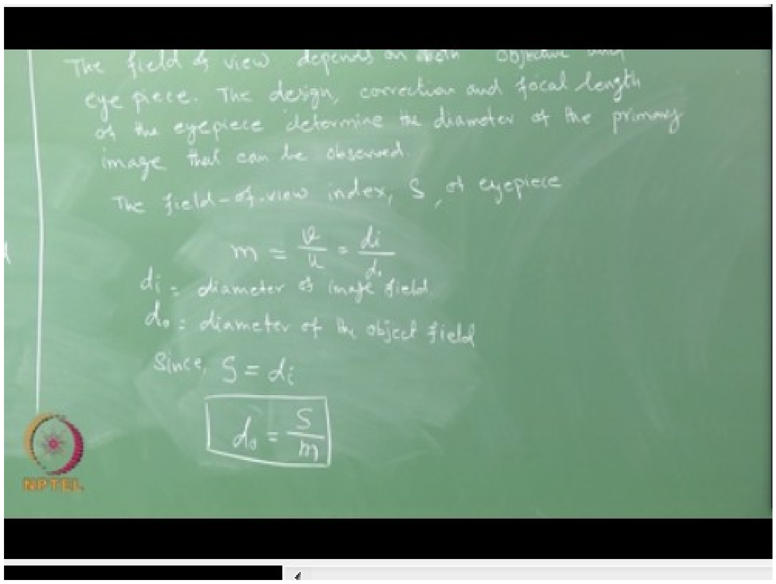

The design, corrections and focal length of the eyepiece determine the diameter of the primary image that can be observed. So let us look at this sentence again the field of view depends upon both objective and eyepiece the design, correction; what is correction? We will talk about a lens defects just after this and then we have to correct that defects; we have to choose a focal length and that depends upon these design correction and focal length various eye pieces are possible similarly objectives various eye objective lenses are also possible.

So that is what it is. So, that time you will appreciate this term. So these three design 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; if we can relate with this a magnification this is true for a simple ray diagram where D_i is a diameter of image field and D_o equal to diameter of the object field and since your field of view index s = Di we can write D_o is equal toS/m ; m,this is magnification.

S is the field of view of index and Do is a diameter of the object field. So what did what it means the lower the magnification of the objective the greater becomes the field of view and we will also write one more expression.

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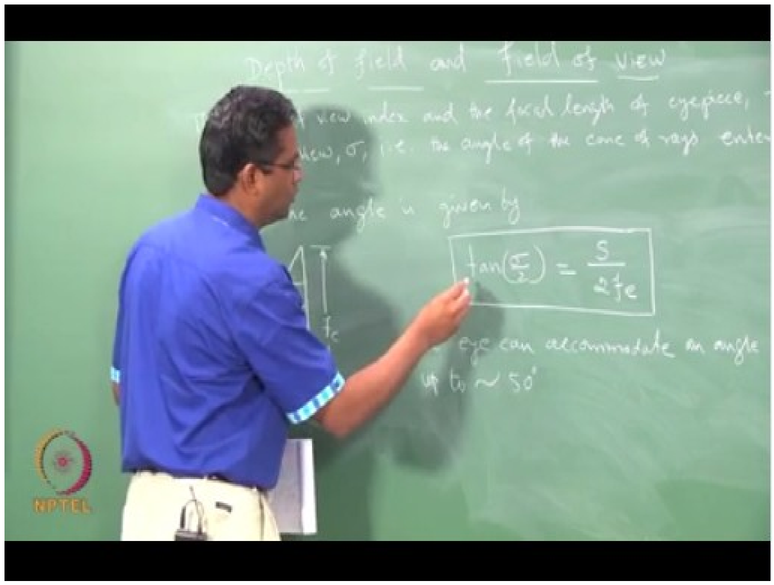

The field of view index and the focal length of eyepiece, that is f_e determine angle of view sigma; that is the angle of cone of rays entering the eye. So ultimately we are interested in this the field of view index and the focal length of the eyepiece Fe determine the angle of view sigma that is the angle of cone of rays that is entering into a eye when you look at the microscope.

So this angle is given by given; by if you consider this as the cone's section and this is your $\sigma/2$; this is an angle of view here it's Sigma. The sigma and alpha is the same since I have taken from different references they have used a different symbol so here it is alpha the collection angles alpha here it's referred as Sigma both are same and this distance is fe that is focal length of the IPS and this is s/ 2 this is a field of view index.

Since we are considering only in the off of the cone section so this is s/2 so based on this we can the angle you can write $\tan \sigma/2 = s/2f_e$. So this is the we can write the eye can accommodate and angle of view of up to approximately 50º. We just looked at the line tracking power of the objective lens and similarly we also were 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 a design, correction, focal length and so on and then it is specifically it can be considered through this formula.

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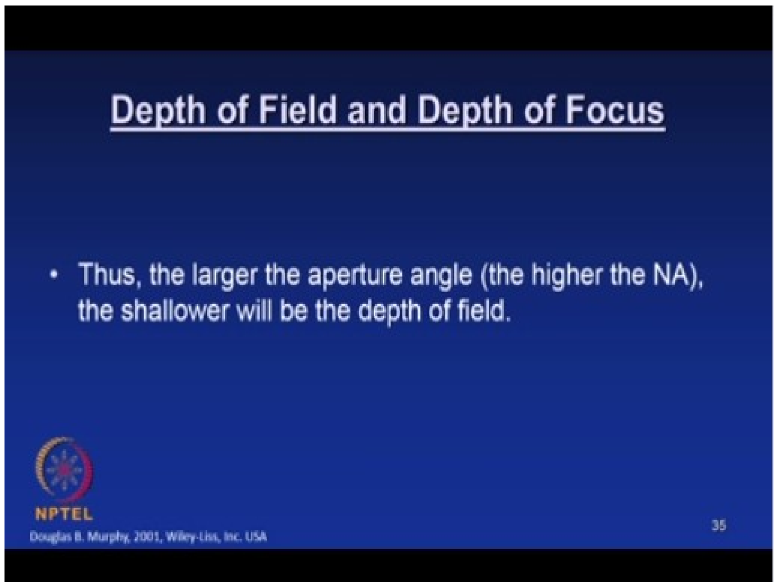

So that is the another aspect of the field of view. Let us now look at the come back to this depth of field and depth of focus, so the larger the aperture angle that means the higher the numerical shallower will be the depth of field. And you can see this example a photograph

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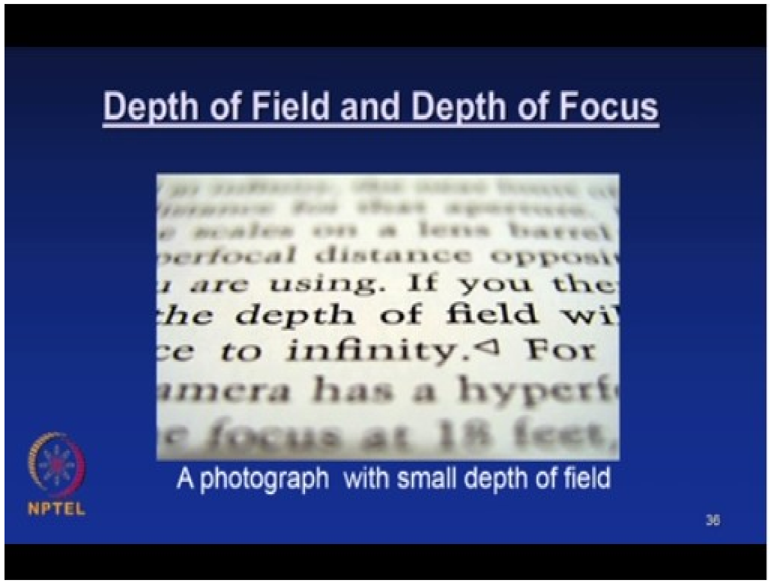

With a small depth of field. So when you have a shallow depth of field this is how your image will look like.

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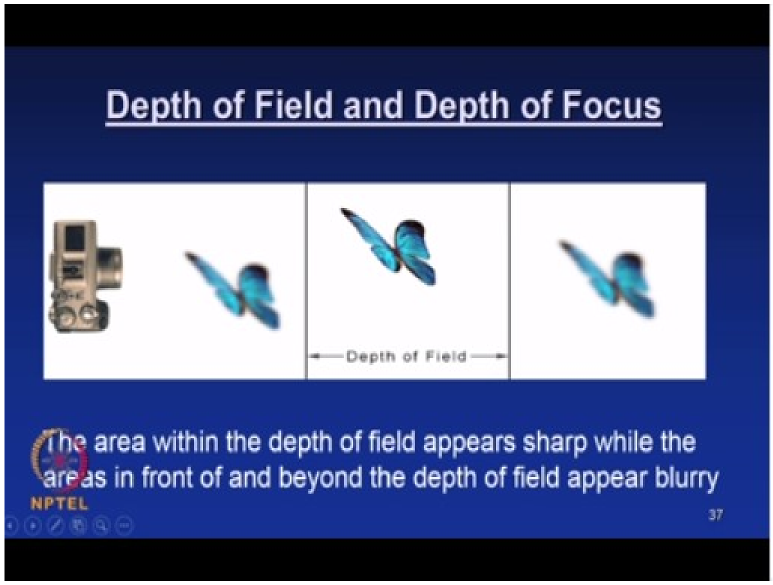

And this is another example where you have the area within the depth of field appeared sharp this the butterfly image appear sharp within this depth of field well the areas in front and the beyond depth of field appear blurry.

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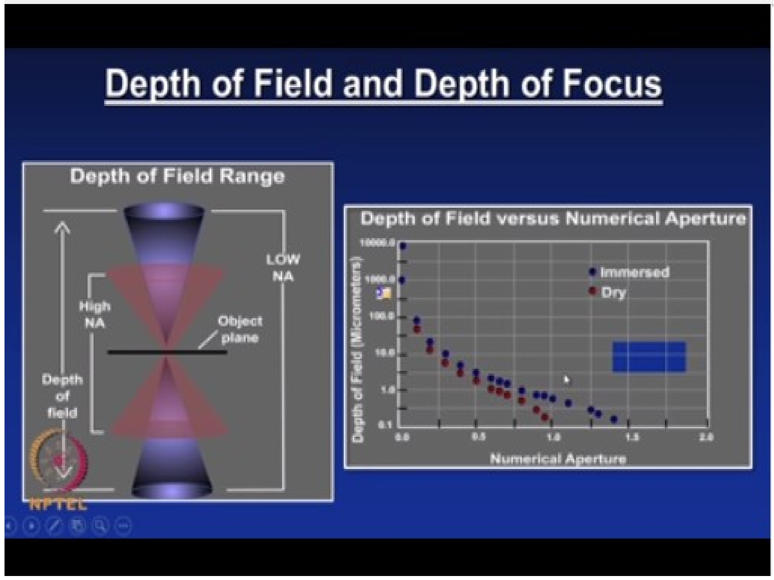

And these two graphs of sorry schematics illustrates the depth of field range as a function of numerical aperture you can see that as we stated earlier and your higher numerical aperture means lower depth of field lower numerical aperture means higher depth of field; and this plot shows clearly that the depth of field versus numerical apertures. 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 that lenses and then eventually the image quality.

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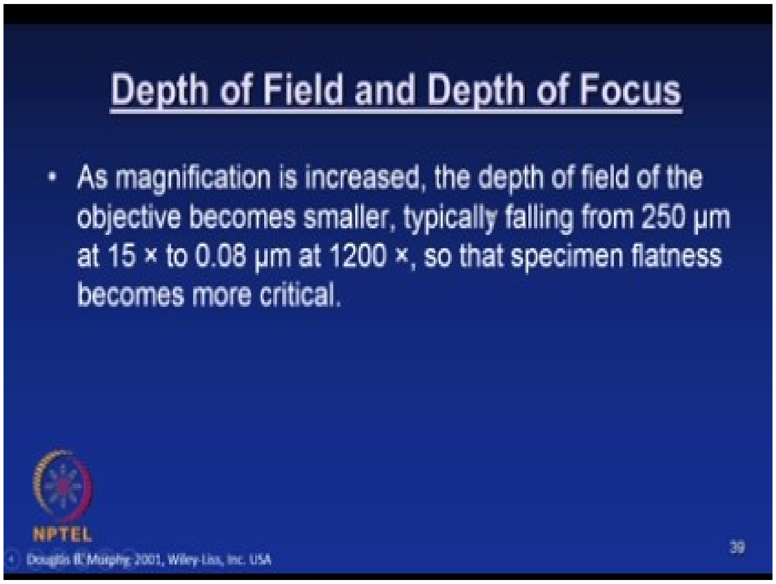

So now so this is the same thing as the magnification is increased the depth of field of the objective becomes smaller and typically falling from 250 micrometer at 15 X to 0.08 micrometer at 12, 000 X. It is a kind of range the specimen flatness becomes more critical. So that is why you see that a very you know shallow depth of field once you cross this range.

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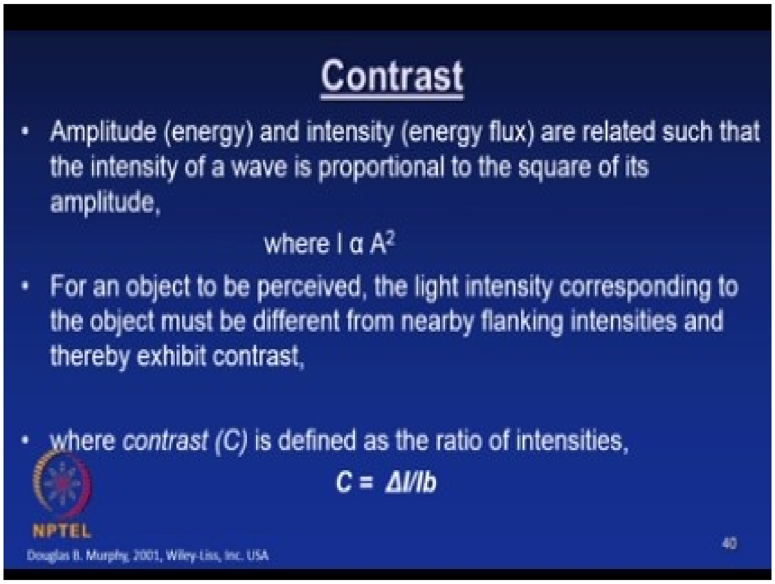

Now we will go to the next concept called contrast seen most of the optical micrograph or for instance you take any micrograph, people are interested in the contrast; best contrast and the quality. So let us understand the term contrast what is contrast let us look at the preliminary remarks: amplitude, energy and intensity which is energy flux or 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 or related such that the intensity of the wave is proportional to the square of its amplitude: $I \propto A^2$ 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. Contrast (C) is defined as a ratio of intensities that is $C = \Delta I / I_b$.

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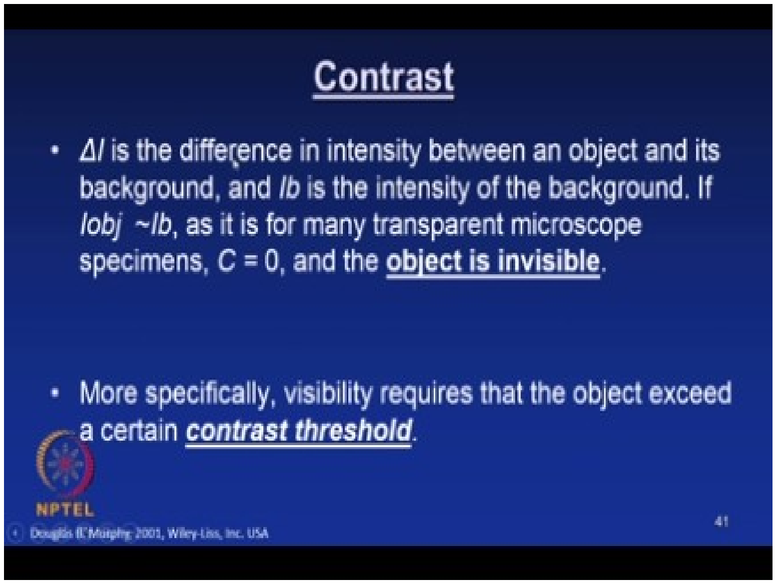

Where Δ I is a difference in the intensity between an object and its background and I_B is the intensity of the background. If $I_{objective}$ is equal to $I_{background}$ as it is for many transparent microscope specimens C=0 and the object is invisible ok. More specifically the visibility requires that the object exceed a certain contrast threshold.

So what we are interested in any micrograph I say mentioned you have a 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 right a bright and grey and black. So out of these, these three your object will be perceived as an, an individual entities.

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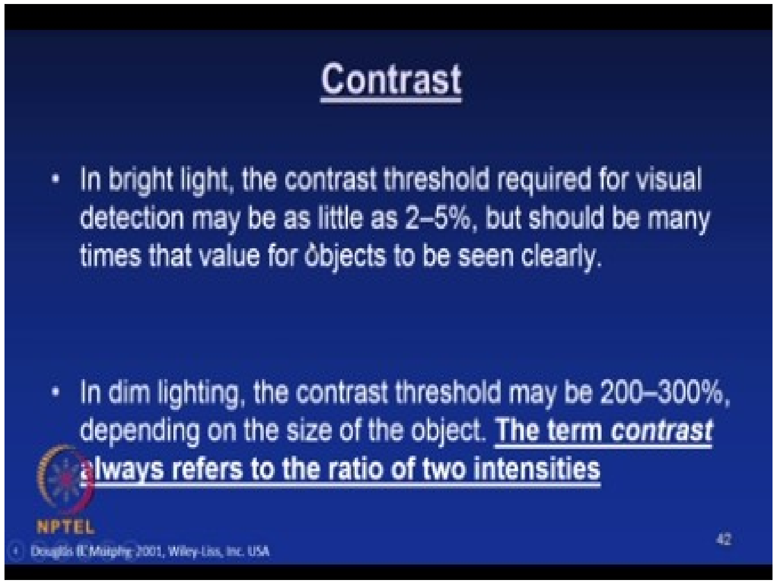

And the entities which you are looking at is mini much more clear only when this the contrast is good. So what is that? In a bright light the contrast threshold required for visual detection may be as little as 2-5% but should be many times that value for object to be seen clearly. In dim lighting the contrasts threshold may be 200-300% depending upon the size of the object. The term contrast always refers to the ratio of two intensities.

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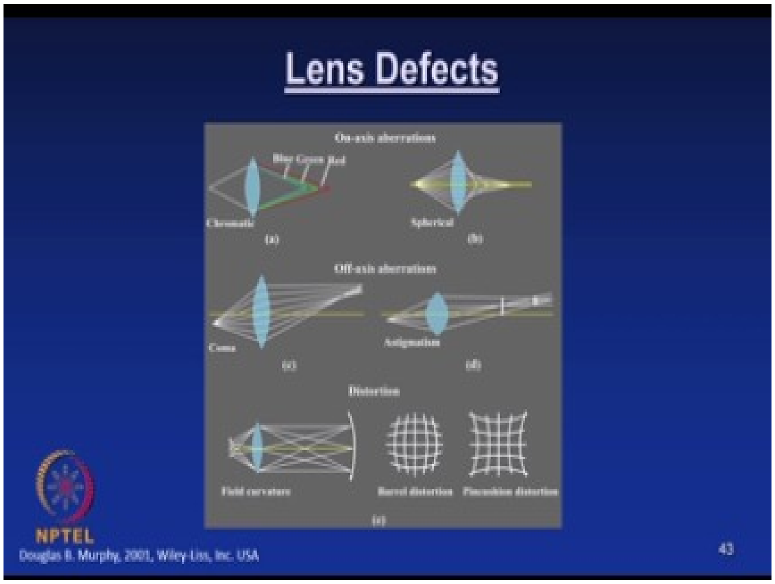

So before we wind up the contrast let us recall the definition of the very definition of the contrast in order to perceive that image you need to have an object should have a particular intensity and its background should have a very different intensity. Unless there is a significant difference in intensity between the object and in a background your entity will not be able to be recognized by your eye.

So always the contrast is the, the ratio of the, the difference in the intensity of the object and then a background to the intensity of the background so it is a ratio, ratio of the two intensities as a contrast. We will talk about this contrasts much more when we progress in the image analysis in the later part of it and for time being I just want you to just remember this definition basic definition.

Now we will talk about 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, which has the complete control of image quality, depending upon the defects of the lens also so let us first look at the what are the lens defects in general, and then we will see a in each defects 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 abrasions are classified as on axis abrasions; okay. And schematic (c) is a coma and schematic (d) is astigmatism and the coma and astigmatism are classified as half axis aberrations. And then we have offshoot of this a distortion which is a field curvature and you have barrel distortion or pin-cushion distortion.

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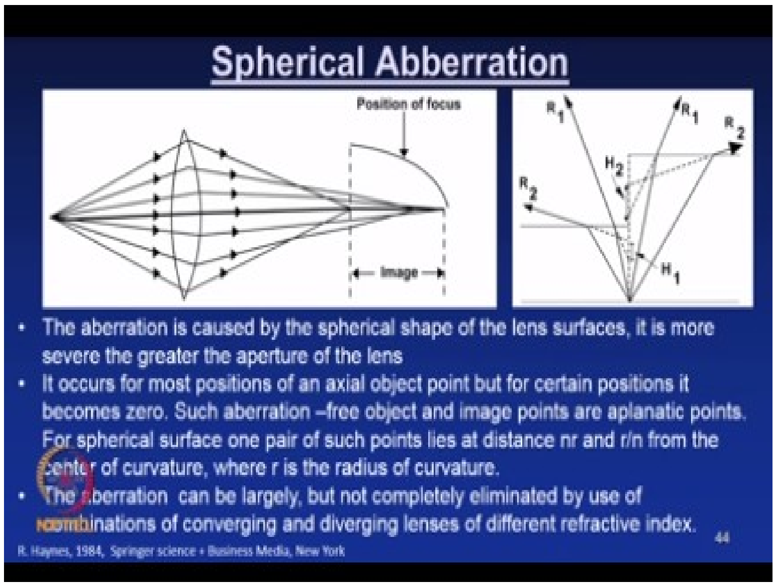

So we will see one by one and we will try to understand what are these defect really means first look at look at the spherical aberration look at this schematic. What do we see? We see that the rays which are coming close to the optic axis are focused the farthest and the rays which are passing through the periphery of the lens are focused in the nearest; and the rays which are passing through in between these two are focused between this two points.

So you have the position of focus ranging from a distance like this which is constitute an image so let us look at the preliminary remarks the aberration is caused by the spherical shape of the plane 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 positions it becomes zero. Such aberration free object and image points are aplanatic points excuse me; aplanatic points. For a spherical surface one pair of such points lies at a distance n_r and r\n the center of curvature where r is the radius of the curvature.

And this parameter is used to fabricate the high-power lenses that is aplanatic points concept and this abrasion 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, to be eliminated in the optical lens. So we will look at this spherical aberrations how it is being eliminated in the due course and other aberrations we will see in the next class.

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