Fundamental 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 - 02
Resolution- continuation
Image brightness
Useful and empty magnification

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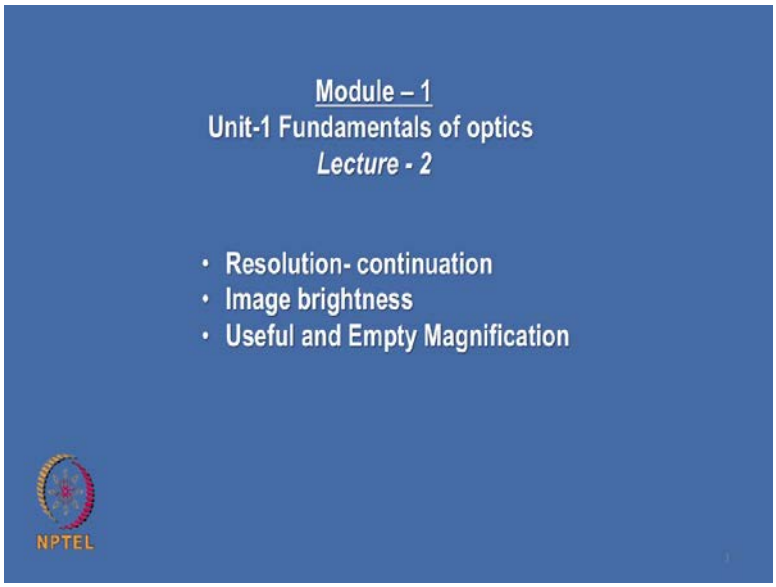

Hello. Welcome back to this Fundamentals of Optical and Scanning Microscopic course. In the last class, we just started reviewing all the fundamental principles and we started looking at the basic rules of light and then, some of the basic definitions of diffraction, refraction, reflection and so on. We also discussed about some of the image formation rules and then, we would like to continue from there and if you look at the criterion for the image formation and this is how it looks.

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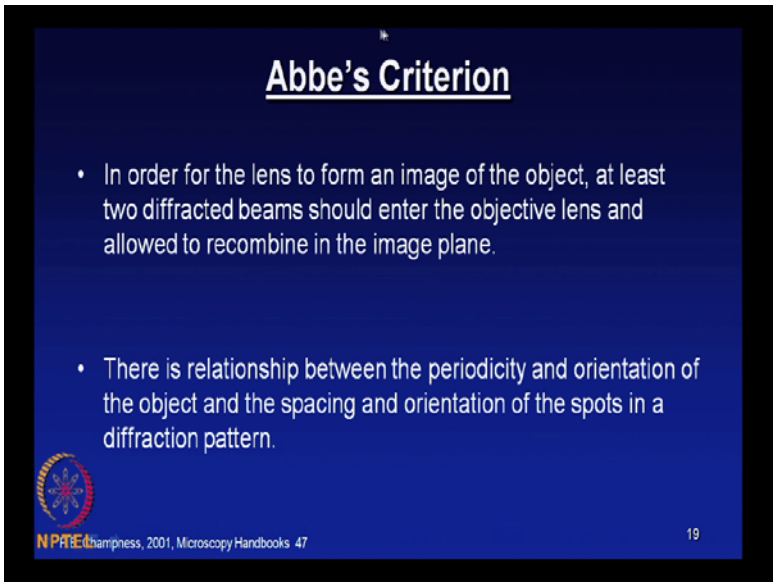

It is Abbe's criterion. In order for the lens to form an image of the object, at least two diffracted beams should enter the objective lens and allowed to recombine in the image plane.

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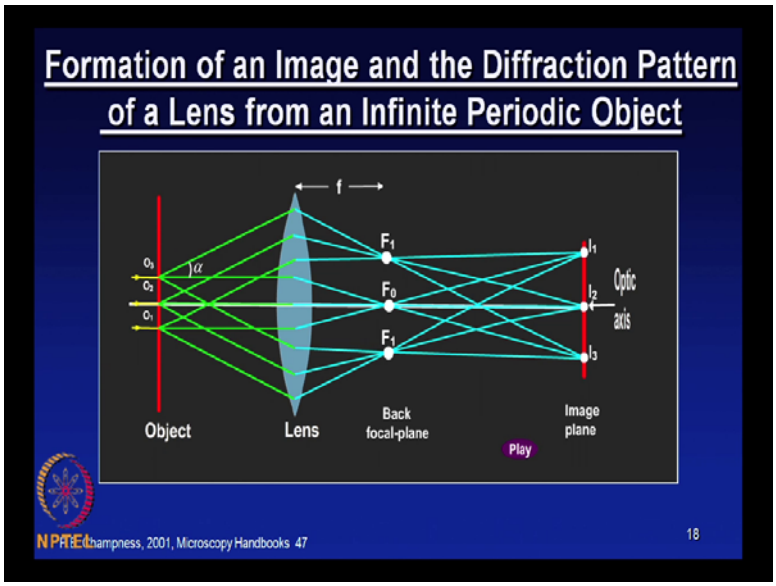

So, if you go back and see the image animation, what I showed in the last class. It is very much clear that the rays which is coming from this periodic object, the orifices from O1, O2, O3, they are all passing through this glass lens, and then you can see that at least two diffracted beams are converging into this image plane to form an image. In this case you have

about 1, 2, 3, but the Abbe's criterion states that at least two diffracted beams will recombine to form an image. So that is valid.

Another important point is, there is a relationship between the periodicity and orientation of the object and the spacing and the orientation of the spots in the diffraction pattern. Let us go back and see again. I just mentioned in the last class the diffraction pattern is forming in the back focal plane, and this is a transmitted spot f naught and f 1 and the f 1 dash they are diffracted spot. So, to prove this statement that is the relationship between the periodicity and orientation of the object and the spacing and the orientation of the spots in a diffraction pattern, we will now take up example like this.

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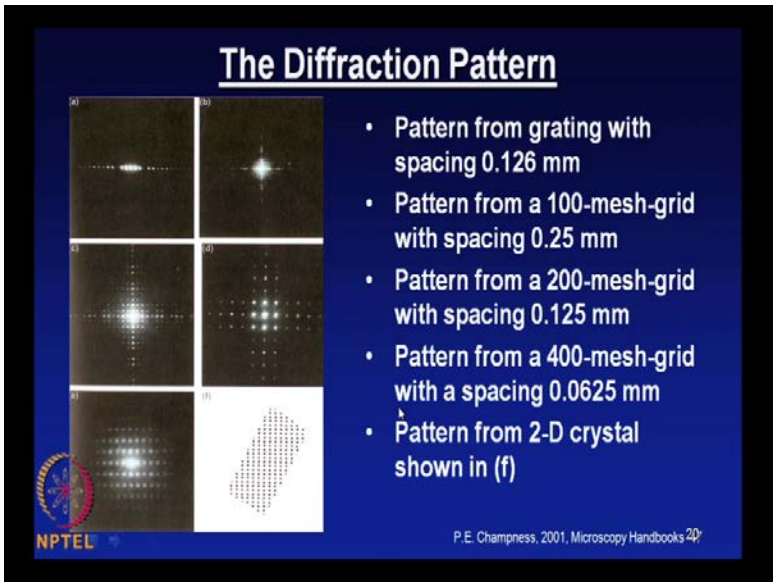

Look at this very interesting Diffraction Pattern; this is an optical diffraction pattern. The pattern a, pattern b, pattern c, pattern d and pattern e, and then this image f look at them carefully. The pattern a, is from the grating, a grating with the spacing of 0.126 mm. This grating is in the form of vertical lines along the length of this image. So you see that diffraction pattern appears perpendicular to that orientation. That is very important information. Suppose, if I have the grating ruling between this horizontal lines, then I will get the diffraction in the vertical direction. That is very important.

So, if you look at the b, c and d, they are pattern coming from 100-mesh-grid with the spacing of 0.25 mm. And then, c is coming from a mesh of 200 grid with spacing of 0.125

mm, and the pattern d is from 400-mesh-grid with a spacing of 0.0625 mm, and the pattern e from 2d crystal shown in f. So, what is that we are trying to understand from this? Look at these numbers very carefully. Of course, the pattern one clearly demonstrates the depending upon the orientation of the objects your diffraction pattern is going to appear. But if you look at the pattern b, c and d, you can see that there is a clear cut relationship between the spacing of the mesh-grid with the spacing in the diffraction pattern, there is a relationship. I hope you will be able to appreciate this. You can see that as the spacing of the grid decreases, the spacing in the diffraction pattern increases. You can clearly see that.

So, just from this optical diffraction pattern it is clearly understood that there is a relationship between periodicity and orientation of the object, and the spacing and orientation of the spots in the diffraction pattern. This is just to prove that how much the diffraction pattern is important as an introduction. So, you will see that how we will exploit this to understand and most of our microstructural and crystallographic data in the due course of this time. So, as an introduction you should know the importance of this diffraction pattern that is why I brought this information.

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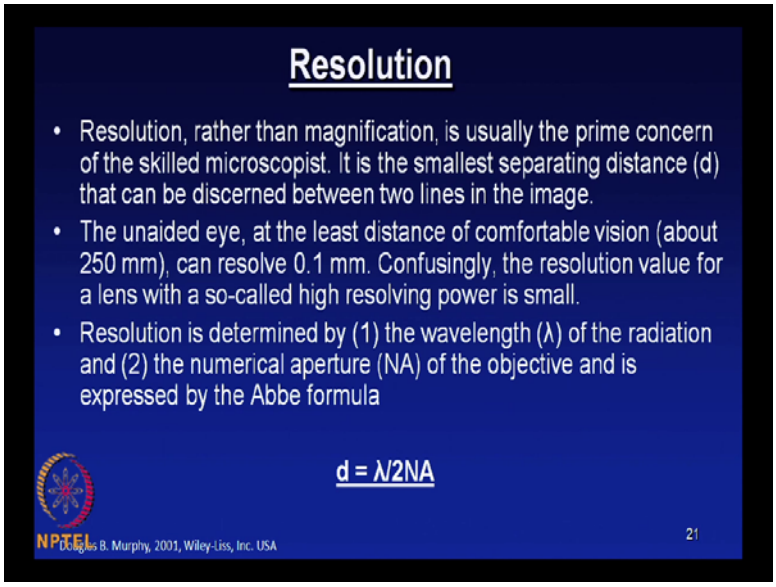

Now, we will concentrate on what is this resolution. Let us look at the introductory remarks. Resolution, rather than magnification is usually the prime concern of a skilled microscopist. It is the smallest separating distance d that can be discerned between two lines in the image. So you have to be very careful you should not confuse resolution with magnification. We

have seen some of the aspects of this magnification in the compound lens microscopy. In the last class, we derived a set of equations you can refer to that. But we will also emphasize or will make little more a discussion in the due course about this magnification. But you have to be very careful resolution is not magnification. Resolution is the smallest separating distance d that can be discerned between two lines in the image.

The unaided eye, at the least distance of comfortable vision about 250 mm that can resolve 0.1 mm. Resolution is determined by 1 wavelength lambda of the radiation and the numerical aperture NA of the objective lens and it is expressed by the Abbes formula d equals lambda divided by two times numerical aperture. This is very important, basic relations. And we will now see what is the theoretical information or theoretical definition for this resolution.

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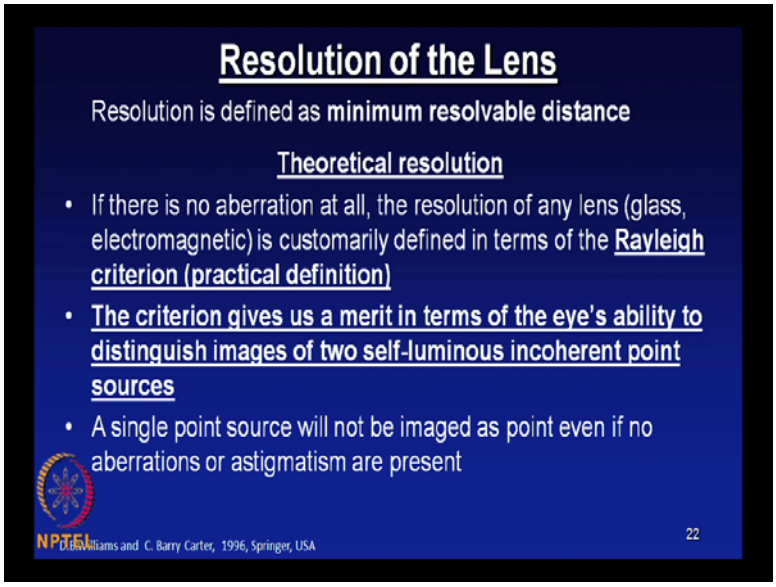

Resolution is defined as the minimum resolvable distance. And if you look at the theoretical resolution, if there is no aberration at all, the resolution of any lens whether it is a glass or electromagnetic is customarily defined in terms of the Rayleigh criterion which is a practical definition. We will now look at what is this Rayleigh criterion.

The criterion gives us a merit in terms of the eye's ability to distinguish images of two self-luminous incoherent point sources. To understand this statement, we will now look at some of the simple schematic and then animation. Because, single point source will not be imaged as a point even if no aberrations or astigmatism are present. Let us look at this.

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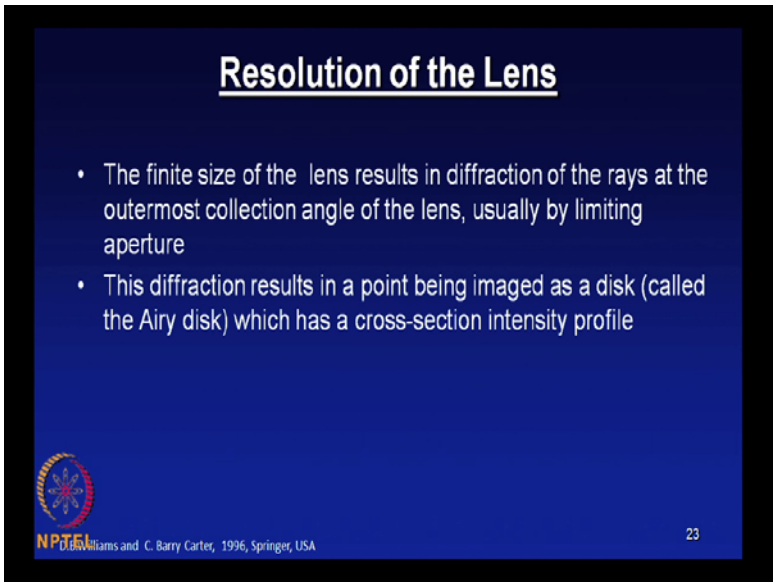

Before we see the animation let us look at two more points. The finite size of the lens results in the diffraction of the rays at the outermost collection angle of the lens, usually by the limiting aperture. This diffraction results in a point being imaged as a disk called the Airy disk which has the cross-section intensity profile.

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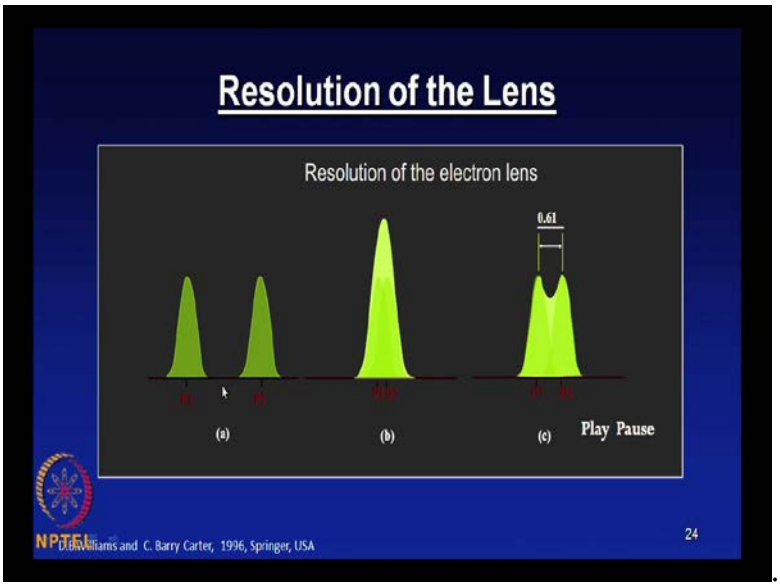

Let us look at this schematic and the animation. So, the individual point P1 is a self-luminous point source and this particular point source is called Airy disk and this is a cross-section profile, intensity profile. Let us look at this point P1 and P2 they are two self-luminous point

sources. And then, as you have witnessed when these two point sources merged together, you see that there is an increase in the intensity and the amplitude. However, our interest is to find out what is the distance at which our eye will be able to identify these two self-luminous point sources as an individual image that is what the Rayleigh criterion is trying to state. So if you look at this image point c. I will play this animation again for the clarity, I want you to look at this animation little more carefully to appreciate what is this Rayleigh resolution we are talking about.

So, in the first case it is merging together and the second case it is approaching, and then it stops. So, to understand this we can consider like this you look at the point source P1. The maxima of this P1 it is overlapping with the minima of the second source. If you look at the center point, it exactly come and overlaps with the minima of the second source. And this is the distance. This is actually the distance what we defined earlier as an airy disk. This is a disk and this is the radius of the airy disk that is fixed 0.61 times lambda by beta. If you look at this where is this dip will occur, it is approximately had about 80 percent of the maximum intensity of the source P1. So, this is the distance basically as I mentioned this is the Airy disk and with this distance, I will be able to distinguish these two point sources as an individual image.

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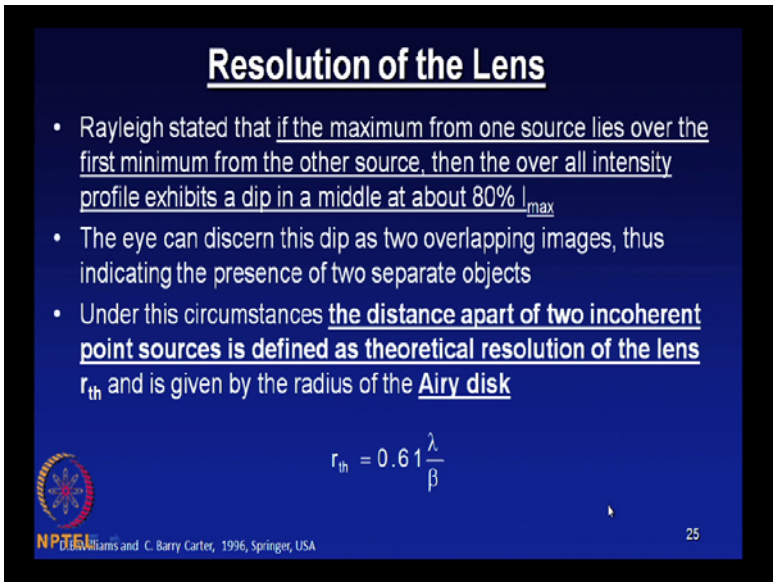

So let us now summarize this, Rayleigh stated that if the maximum from one source lies over the first minimum from the other source, then the overall intensity profile exhibits a dip in a

middle at about 80 percent of I max. The eye can discern this dip as a two overlapping images, thus indicating the presence of two separate objects. Under this circumstances, the distance apart of the two incoherent point sources is defined as theoretical resolution of the lens r th and it is given by the radius of the Airy disk. Rth is equal to 0.61 times lambda by beta, where lambda is a wavelength of the radiation, beta is the semi-aperture angle. So, I hope you have some idea about with the Rayleigh criterion now.

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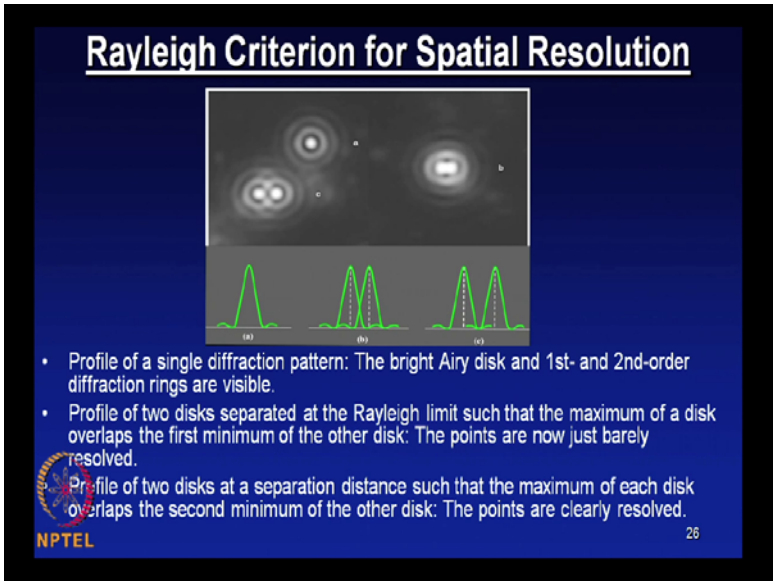

Now, let us take some of the examples of some images which display that Rayleigh criterion for a spatial resolution. Look at this image carefully. This is again an intensity profile for the point source a, you can see that it has got zero order, first order, second order and so on. It is a self-luminous point source of light. And then if you look at the b, then you have these two points overlap corresponding to this intensity profile match. And then point c, you have these two points just touching each other correspond to intensity profile c. So, the profile of the two disks separated at the Rayleigh limit such that the maximum of the disk overlap with the first minimum of the other disk.

So this is what is shown in the image b. It is two points are barely resolved, but if you look at the intensity profile c, two disks at a separation distance such that the maximum of each disk overlaps the second minimum of the other disk. Like, we have seen in the previous schematic. You can see that the minima of the second source are matching with the first

maxima, and then the points are clearly resolved. So that clearly tells that Rayleigh criterion for a spatial resolution. I hope you will get this.

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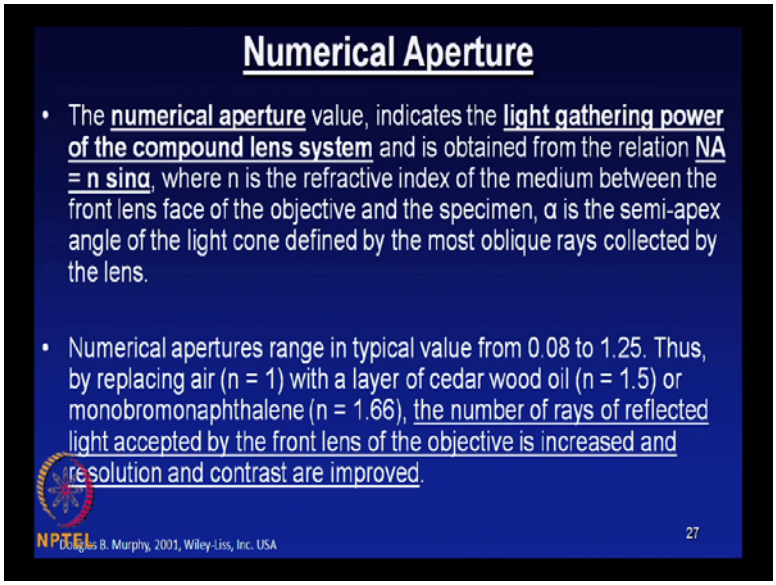

Now, we will move on to the other important parameter called Numerical Aperture. We have seen that in the very definition of the resolution, this d equals to the inversely proportional to this numerical aperture that is what we have seen. In order to understand what the numerical aperture is, first we will just look at the introduction remarks.

The numerical aperture value indicates the light gathering power of the compound lens system and it is obtained from the relation Numerical Aperture equal to n sign alpha, where n is the refractive index of the medium between the front lens space of the objective and the specimen, and alpha is the semi-apex angle of the light cone defined by the most oblique rays collected by the lens. In order to understand this, the light gathering capability of the objective lens I would like to draw some schematic on the board.

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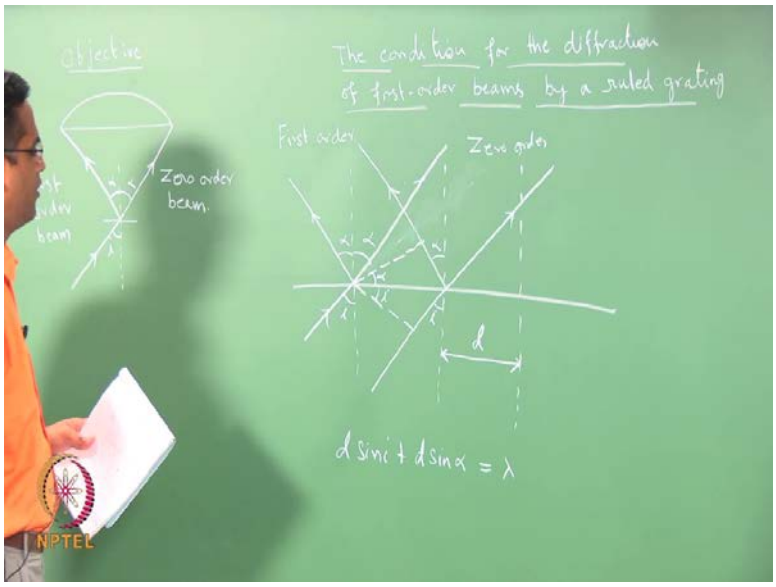

So let us now see, first I will draw the objective lens because objective lens only collects the rays coming out of first order, second order and so on. So, let us assume this and objective lens let us like. So this is an angle alpha and this is I, this angle of incidence and angle of reflection. So, this is a first order. Let us consider this as a first order beam and this as zero-order beam. This is a collection of first order and the zero-order beam by the objective lens like this, and this is how the angles are defined. We will now write, we will kind of draw another schematic; the condition for the diffraction of these two.

So let us write the condition for, the diffraction of first order by a ruled grating. The object is ruled grating and then we will see what the condition is for the diffraction, which is further being collected by the objective lens. So let us consider this as the ruled grating and then, I have a set of ray is coming and this also draw the zeroth order. Draw this much clear. So this is a zero order case and this is first order case. That is not the angles alpha and then this is angle i alpha, this is angle i. Let us assume this the distance between the rulings or the grating is d.

Now, let us look at the path difference between this zero order rays and the first order rays. For that let us draw. So this is alpha and this is i. We can write the path difference between this zero and the first order rays from the successive ruling. This is also ruling is exactly one wave length. That means we are going to write d i sin i plus d sin alpha is equal to lambda.

So, the path difference between the zero and the first order diffracted from this ruling with spacing d is exactly equal to lambda.

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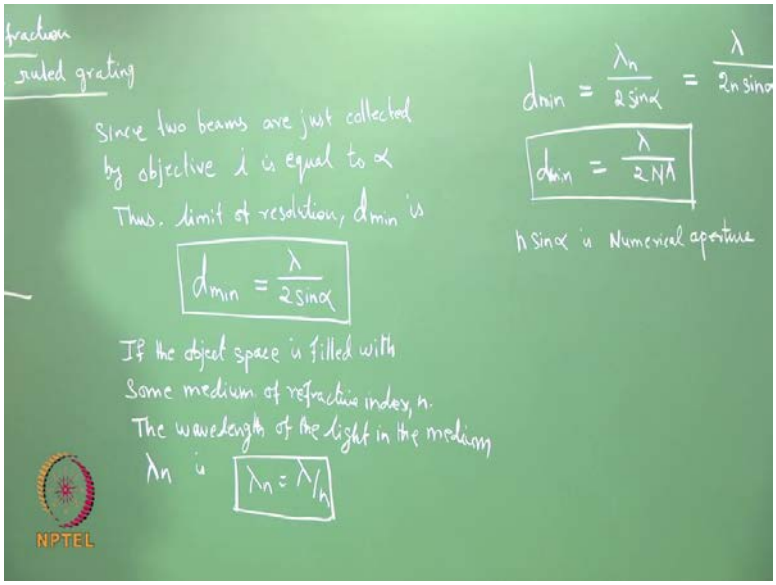

We will write, since two beams are just collected by objective i equal to alpha. So, now we can write the limit of resolution, that is d minimum is equal to lambda by 2 sin alpha. So this is one expression from this Ray diagram you can write. And we can also now say if the objective lenses filled with some medium of refractive index n, then the wave length of the light in the medium lambda n is, we can write that lambda n. So now, we can write d minimum is equal to lambda n by 2 sin alpha which is equal to lambda by 2 n sin alpha which is equal to lambda by 2 NA. So, this is d minimum and lambda by 2 NA. Where, n sin alpha is called Numerical aperture.

So, now you will appreciate the resolution definition we just stated before we get from this path difference ray diagram, a small derivation. And then, now we will see the effect of the numerical aperture on the resolution.

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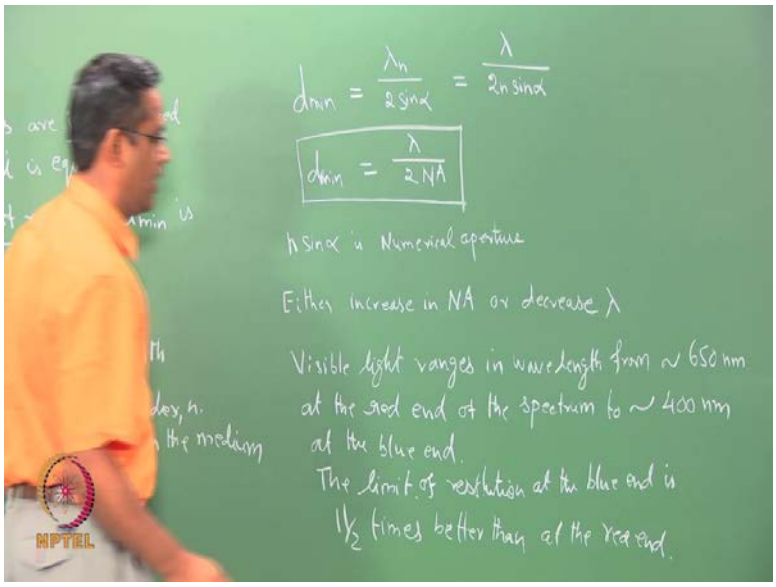

We can simply write either increase in numerical aperture, that means the light correction ability of the objective lens increases. Or decreases in lambda it will produce the same effect on the resolution. This you have to remember. That is what this mathematical relationship explains either increased in numerical aperture or decrease in lambda will influence the resolution power of the objective lens.

Now, we can take some example in a well known electromagnetic spectrum. You would just take the visible light ranges in wavelength around 650 nanometer at the rent red end of the spectrum to at 400 nanometer at the blue end, so we all know this. I just want you to give emphasis that is why I have written this. The visible light ranges in the wavelength from 650 nanometers to that is the red end of the spectrum to a 400 nanometers of the blue end. If you consider this range, the limit of the resolution at the blue end is one and half times better than at the end. Just to give an immediate example from the well known visible spectrum, and you have the limit of resolution at the blue end, that is here 400 nanometers is one and half times better than the red end spectrum which will have the wave length of 650 nanometers. So, I hope you got some idea about this numerical aperture, and then how it affects the resolution.

Now look at this slide. Come back to the slide, this numerical aperture range in the typical value from 0.08 to 1.25. Suppose, this is for the medium air where the refractive index is equal to 1.Suppose, if you replace this with a layer of cedar wood oil which has got the refractive index of 1.5 or monobromonaphthalene which has got a refractive index of 1.66,

the number of rays of the reflected light accepted by the front lens of the objective is increased and the resolution and the contrast are improved. And this is what is shown in this schematic.

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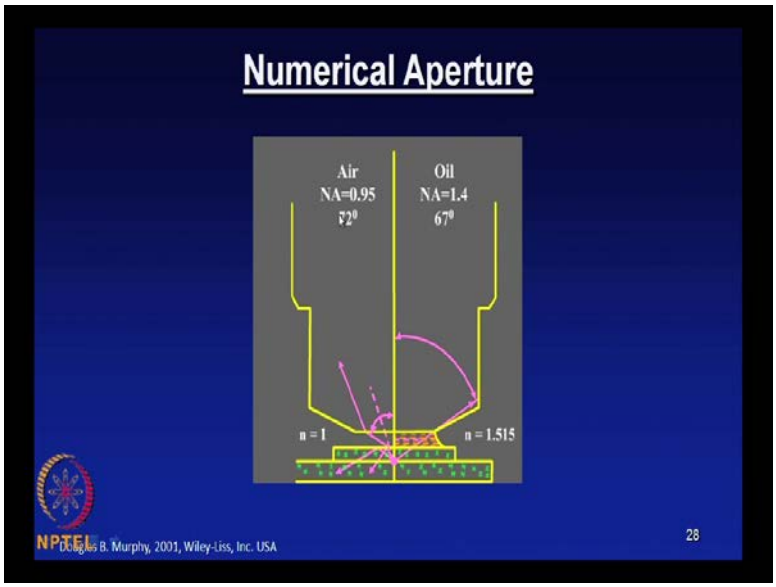

Look at this. This is an objective piece, just to show the difference between the dry lenses that is a dry objective immerse with the medium in oil. So you see that when the refractive index is 1, you can see that the ray is reflected from the specimen, the rays which are trying to enter the objective have some limiting angle. This is roughly about 41 degree and then rest of the rays is reflected back by the internal reflection. On the other hand, if you look at the objective filled with the oil, this the internal reflection is totally avoided, and then the same location from where the rays were reflected back due to the internal reflection they are now accepted by the objective lens because it has got an oil which is having higher refractive index. So, that is clearly shown in this. This 72 degree is acceptance angle, and this is 67 for the oil immersion objective.

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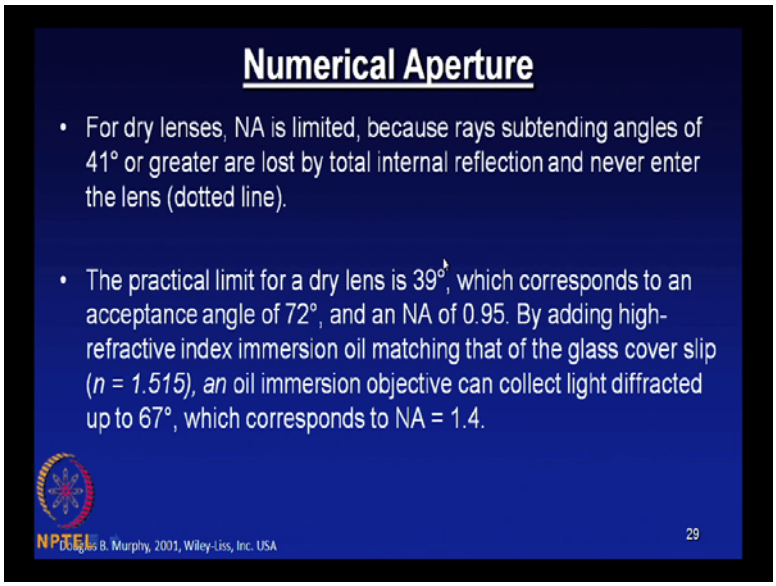

Let us look at few more points on the numerical aperture. Whatever I just stated before, for dry lenses numerical aperture is limited because the rays subtending angles of 41 degrees. This is what I said this is subtending angle here shown. Or greater are lost to internal reflection and never enter the lens. The practical limit for a dry lens is about 39 degrees, which corresponds to an acceptance angle of 72 degree, and numerical aperture value 0.95. By adding high refractive index immersion oil that matching that of the glass cover slip, cover slip is because it is covered with this oil on the objective which is having the refractive index of 1.515 which can collect the light diffracted up to 67 degree, which corresponds to numerical apertures of 1.4.

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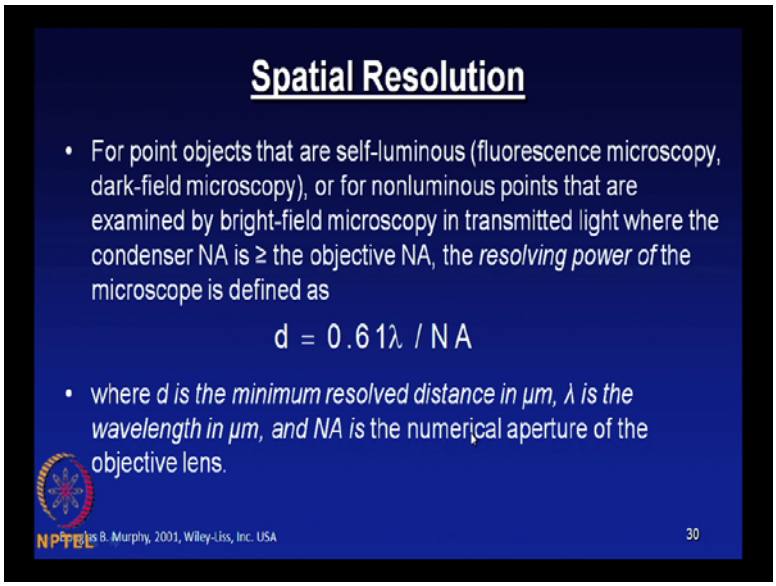

So let us now look at the other terminologies, Spatial Resolution. For all practical purposes we are using only the spatial resolution. Whatever we are looking at is only a spatial resolution in a microscope, and we will see what are the points to remember.

For point objects that are self-luminous whether it is a fluorescence microscopy or a dark field microscopy we will see them in that principles and techniques in the course, or even for non-luminous points that are examined by the bright-field microscopy in a transmitted light where the condenser numerical aperture is greater than or equal to the objective Numerical Aperture. The resolving power of the microscopy is defined as d is equal to 0.61 times lambda by Numerical Aperture. Where, d is the minimum resolved distance in micrometer, lambda is the wavelength in micrometer and NA is the numerical aperture of the objective lens.

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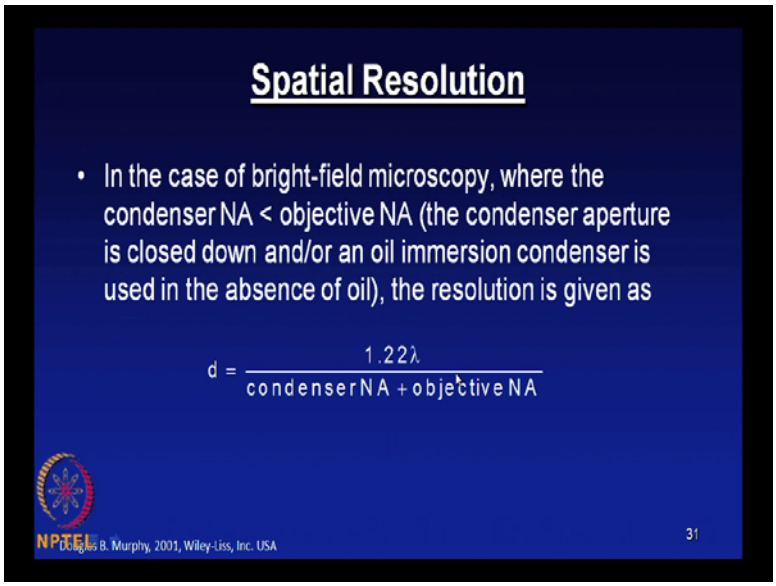

In the case of a bright field microscopy, where the condenser numerical aperture is less than objective numerical aperture that is the condenser aperture is closed down and an oil immersion condenser is used in the absence of oil. The resolution is given as d is equal to 1.22 lambda divided by condenser Numerical Aperture plus objective Numerical Aperture. Please look at this, the number 1.22 here is considered the complete diameter of the Airy disk. Here, we have considered only the radius of the Airy disk. So, do not be confused with this, this is hint. But here it is diameter of the Airy disk is considered, there its radius of the Airy disk is considered. And another important parameter which we talk about in the light microscopy or any microscopy is image brightness is very important.

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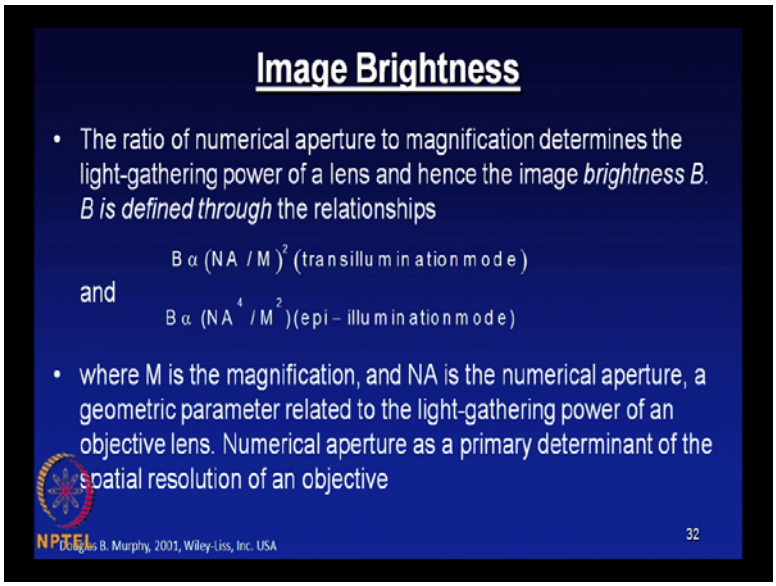

The ratio of numerical aperture to the magnification determines the light gathering power of lens and hence the image brightness B. B is defined through the relationship like this, B is proportional to Numerical Aperture loaded by magnification whole square for thetrans illumination mode. Where, B is proportional to numerical to the power of 4 divided by magnifications square for epi-illumination mode. We will see what are these two illumination modes are when we go to instrumentation details.

I will just show you the corresponding illumination mode when you look at the instrumentation. And this is how the brightness is defined. Where M is the magnification and NA is Numerical Aperture. A geometric parameter related to the light gathering power of an objective lens and numerical aperture as a primary determinant of the spatial resolution of the objective. So, it is very important to note that numerical aperture of an objective lens determines the resolution power. And of course, it has got some range of values we will see that.

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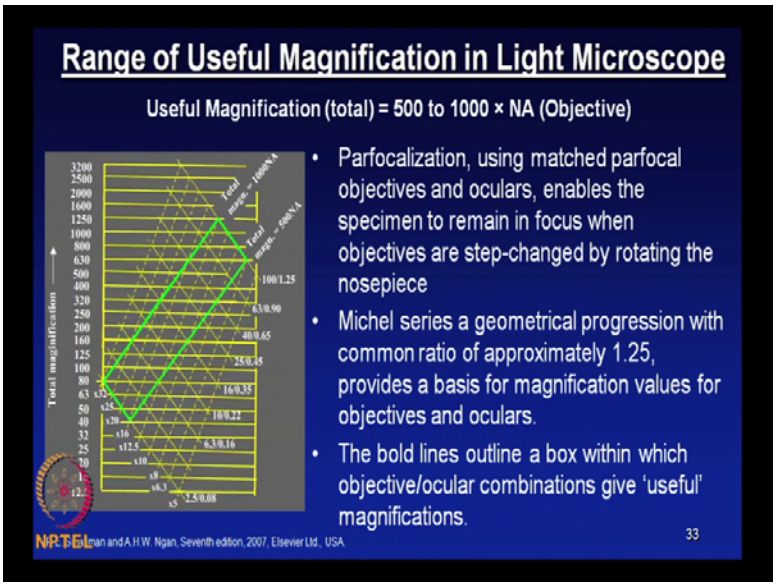

Not that all the values of the numerical aperture is going to be useful, but if we look at actually the useful magnification is total, if you look at it is 500 to 1000 times of the numerical aperture value of any objective. This is a total magnification which is useful. If you look at this slide what is shown here is, the Range of Useful Magnification in Light Microscope. So, we should not think that suppose if you keep on increasing the numerical aperture value or you keep on decreasing or adjusting the other parameters we are going to achieve the magnification that is not true. What is shown here is the total magnification here in this schematic plot, and then these grids show some kind of way of reaching some kind of a progression, geometrical progression of an objective and ocular combinations.

So, we have seen in the beginning. I said most of the microscope we use compound lens. One will have objective as well as object ocular eyepiece. The total magnification we have seen that it is the magnification achieved by the objective times, the magnification achieved by the ocular or the eyepiece. So, these two eyepieces as well as the objective have a range of values. Only that combination will give you the useful magnification. This diagram conveys only that, that is the combination of eyepiece and combination of objective. And the green line shows the useful magnification range. So this is done with parfocal objectives with the combination of a different Ocular lens.

It is Parfocalization, using matched parfocal objectives and oculars enable the specimen to remain in focus when the objectives are step-changed by rotating the nosepiece. It is called

Michel series, a geometrical progression with the common ratio of approximately 1.25, you can see this is approximately 1.25 provides a basis for magnification values for the objectives and oculars. So, the bold lines outline a box within which objective or ocular combinations give the useful magnification. So this is the boundary. So, you take an ocular magnification and then objective magnification. So, within this matrix you find that the total magnification is useful. It is not that all magnification values are useful.

So, now we just come to a situation and we were initially talking about magnification now we are talking about useful magnification and that is anything which is not useful magnification out of this green box is called Empty Magnification. So, we can write a kind of definition for this empty magnification.

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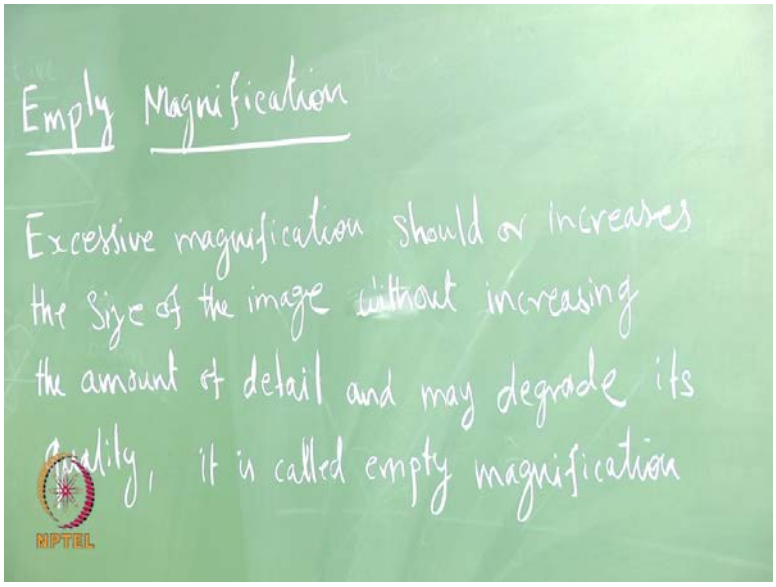

So we write that "Excessive magnification should or increases the size of the image without increasing the amount of detail and it may degrade its image quality", it is called empty magnification. So it is not that we have all the values for the objective numerical aperture is going to be useful, we have the limitation and this is how the empty magnification is defined.

So, we will see few more concepts and parameters in the next class.