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Lecture-2
Materials Characterization
Fundamentals of Optical microscopy

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Hello welcome back to this fundamental of optical and scanning electron microscopy 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 soon. And 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, the criterion for the image formation and this is how it looks.

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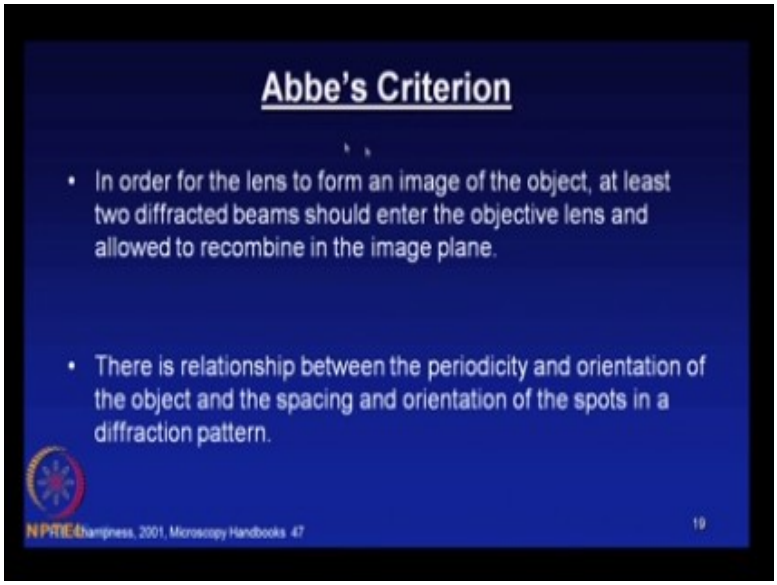

It is Abbe's criterion, in order for the lens to form an image of the object at least two diffracted beam should enter the objective lens and allowed to recombine in the image plane. So if you go back and see the image animation what we what I showed in the last class it is very much clear that the rays which is coming from this periodic object the orifice from O1, O2, O3.

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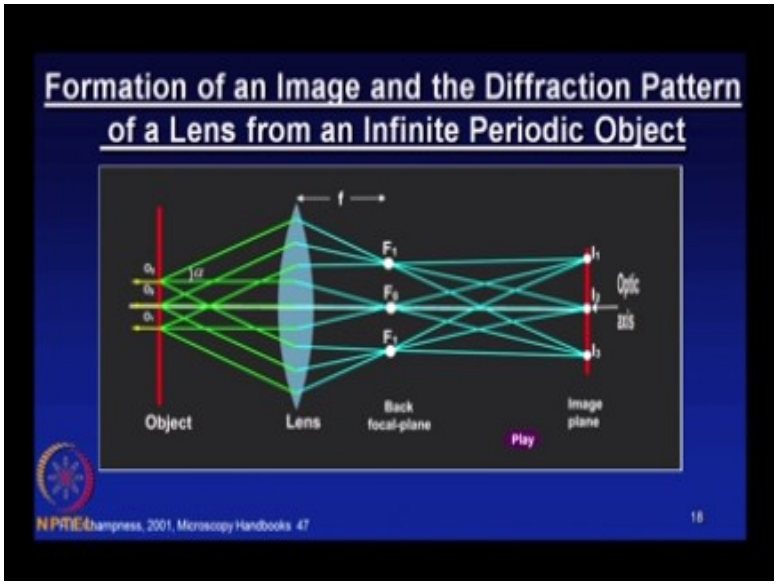

They are all passing through this glass lens and then you can see that at least two diffracted beam are converging into this image plane to form an image. In this case you have got one two three; but the Abbe's criterions states that at least two diffracted beams will recombine to form an image. So that is valid the other important point is,

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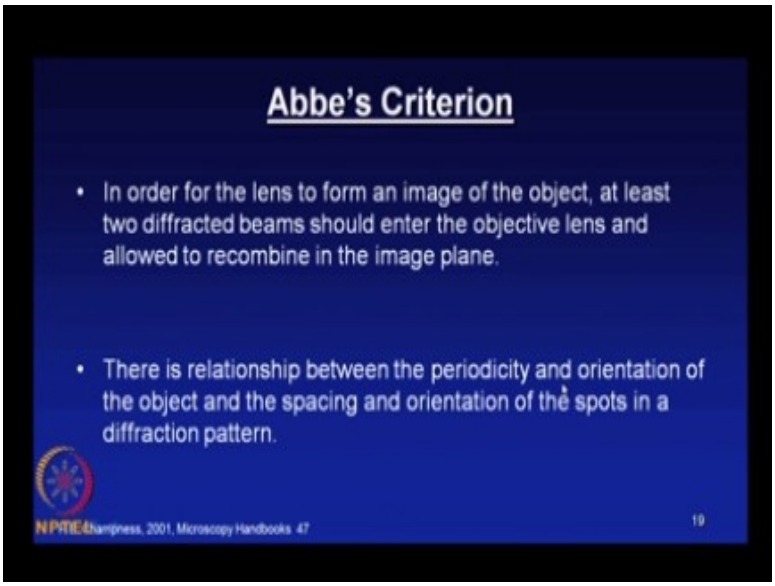

There is a relationship between the periodicity and orientation of the object and the spacing and the orientation of the spots in the diffraction pattern. Let us go back and see again. I just mentioned in the last class the diffraction pattern is forming in the back focal plane and this is a transmitted spot F_o and F_1 and F'_1 ; they are diffracted spot. So to prove this exact I mean the statement that is the relationship between the periodicity and orientation of the object and the spacing and the orientation of the spots in the diffraction pattern. We will now take up an example like this look at this very interesting diffraction pattern.

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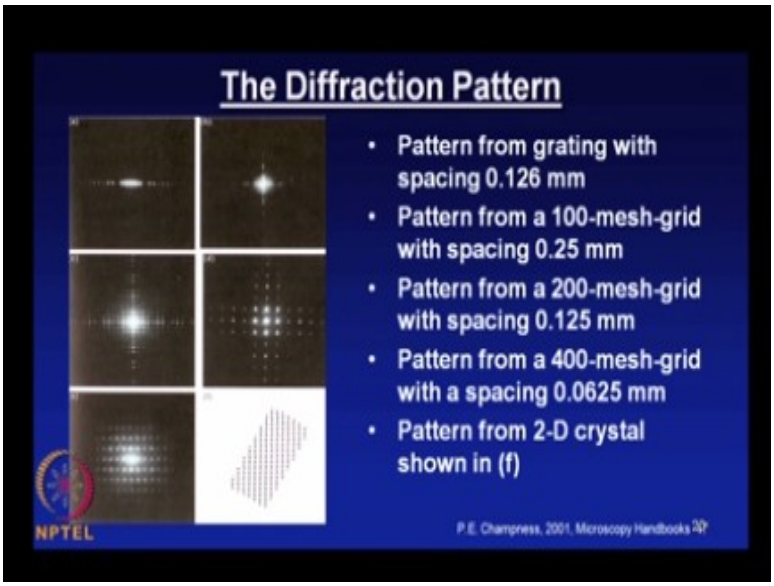

This is an optical diffraction pattern the pattern A, pattern B, pattern C, pattern D, and pattern E and then this image F. Look at them carefully the pattern A is from the, the grating, a grating with the spacing of 0.126 mm. This grating is in the form of vertical lines along the length of this image. So you see that diffraction pattern appears perpendicular to that orientation; okay. 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 okay. That is very important so if you look at the B, C, and D.

They are from pattern coming from a hundred 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 a 400 mesh grid with the spacing of 0.0625 mm. And the pattern E from 2d crystals shown in F. So what is that we are trying to understand from this? Look at this number is very carefully of course the pattern A 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 that is a clear-cut relationship between the spacing of the mesh grid with the spacing in the diffraction pattern.

That is a relationship. I hope you will be able to appreciate this you can see that as the spacing in 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 micro structural 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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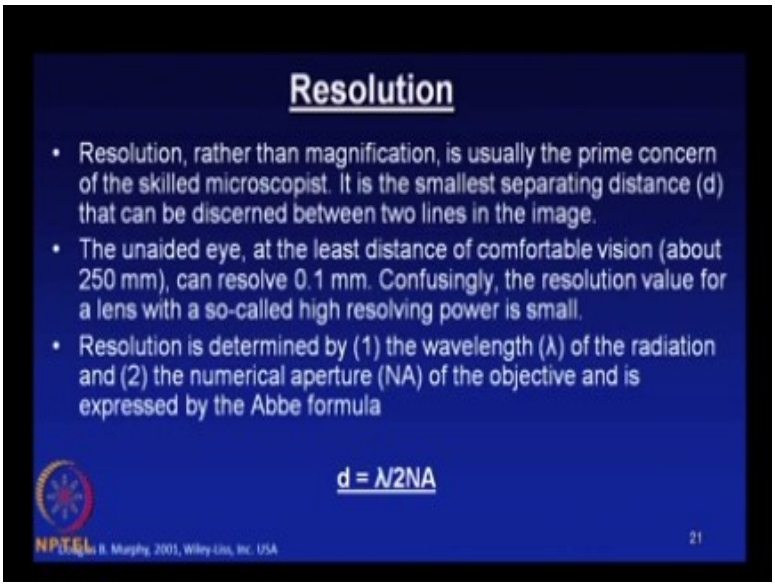

Now we will concentrate on what is this resolution. Let us look at the introductory remarks resolution rather than magnification is usually the prime concern of a skilled microscopist. It is the smallest separating distance D that can be discerned between two lines in the image. So you have to be very 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. The solution is the smallest separating distance D that can be discerned between the two lines in the image. The unaided eye at the least distance of comfortable vision about 250 mm. That can resolve 0.1mm. Resolution is determined by one wavelength λ of the radiation and the numerical aperture NA of the objective lens. And it is expressed by the Abbe's formula D = λ/2*(numerical aperture). This is very important basic relations.

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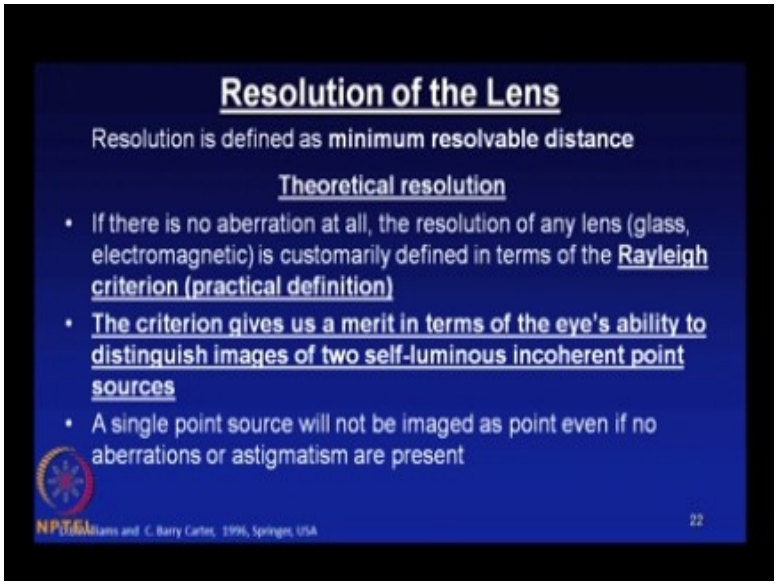

And we will now see what is the theoretical information or theoretical definition for this resolution. The resolution is defined as the minimum resolvable distance, and if you look at the theoretical resolution. If there is no aberration at all there resolution of any lens whether it is a glass or electromagnetic 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 okay, because yes single point source will not be imaged as the point even if no aberrations are astigmatism are present.

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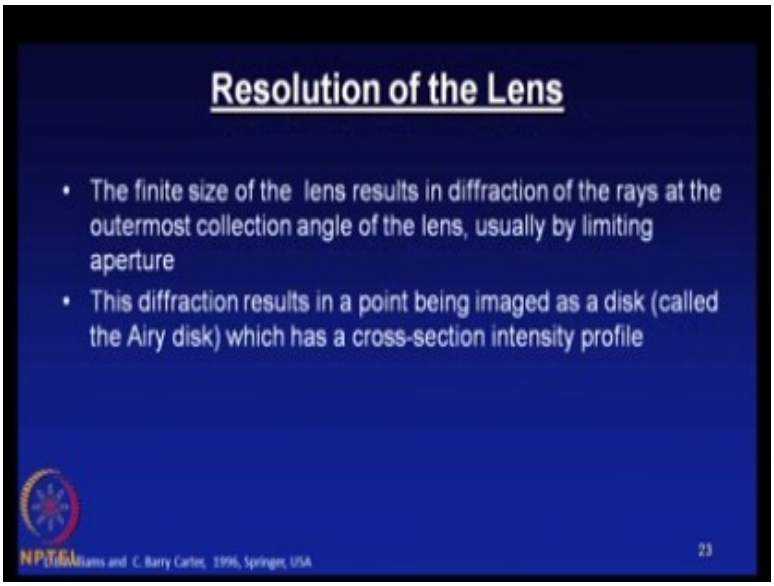

Let us look at this before we see the animation let us look at two more points the finite size of the lens results in the diffraction of the rays. At the outermost collection angle of the lens usually by the limiting aperture. This diffraction results in your point being image as a disk called the airy disk. This has the cross-section intensity profile.

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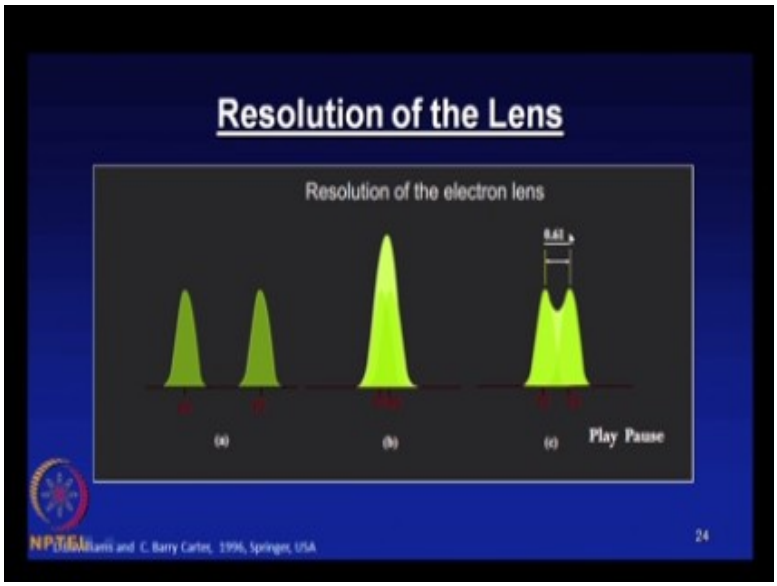

Let us look at this schematic and the animation, so the individual point P1 it is a self luminous point source and this particular point source is called added disk, and this is the cross section profile intensity profile. And let us look at this point P1 and P2 they are two self luminous point sources. And then as you have witnessed when these two point sources merge together you see that there is an increase in the intensity and the amplitude. However our interest is to find out to what is the distance at which our eye will be able to identify these two self-luminous point sources as a 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 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, the second source. If you look at the center point it exactly come and hold up with the minima of the second source. Here; 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 aired disk. That is fixed 0.61 * λ / β and if you look at this where is this dip will

occur it is approximately at about eighty percent of the maximum intensity of the source p 1, so this is the distance basically as I mentioned this is if the airy disk and with this distance our eye will be able to distinguish these two point sources as an individual image.

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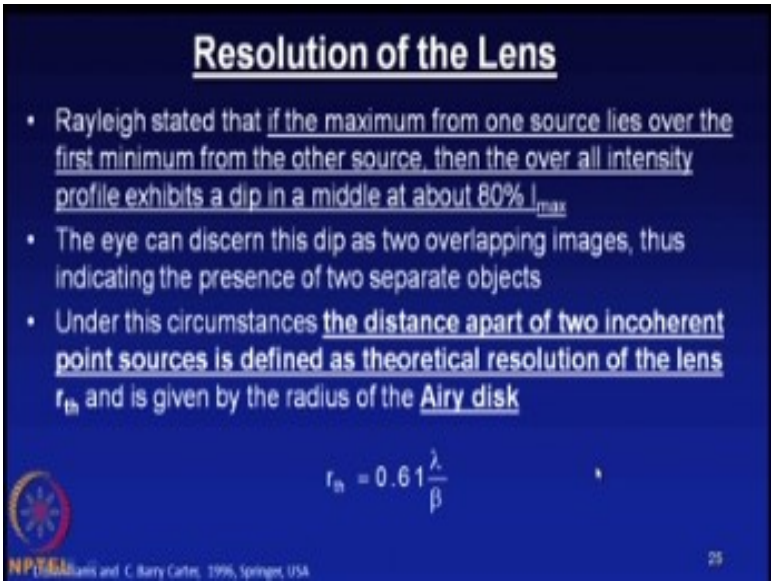

So let us now summarize this : Rayleigh stated that if the maximum from one source lies over the first minimum from the other source then the overall intensity profile exhibits a dip in a middle at about eighty percent of I_{max} ; the eye can discern this dip as two overlapping images thus indicating the presence of two separate objects, under these 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 discs are $r_{th} = 0.61 * \lambda / \beta$ where λ is a wavelength of the radiation beta is the semi aperture angle so I hope you have some idea about the Rayleigh criterion now.

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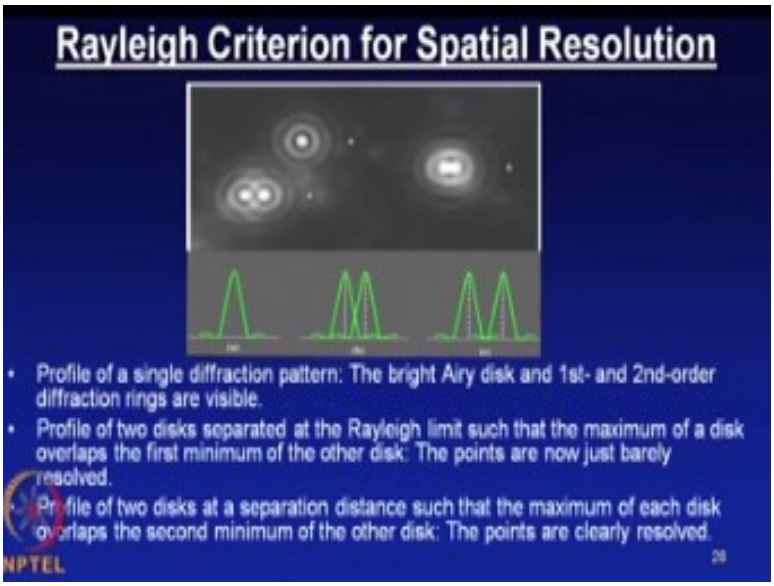

We will now let us take some of the examples of some images which displays that the Rayleigh criterion for a spatial resolution. Look at this image carefully : this is again a intensity profile for the point source A you can see that it has got zero order first order second order and so on it is a self 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 discs separated at the Rayleigh limit such that the maximum of the disc overlap with the first minimum of the other disc; so this is what shown in the image(b): it is two points or barely resolved. But if you look at the intensity profile see two disks at the separation distance such that the maximum of each disk overlaps the second minimum of the other disk like me we have seen in the previous schematic you can see that the minima of the second source is matching with the first Maxima then the points are clearly resolved. So that clearly tells that the Rayleigh criterion for a spatial resolution I hope you will get this.

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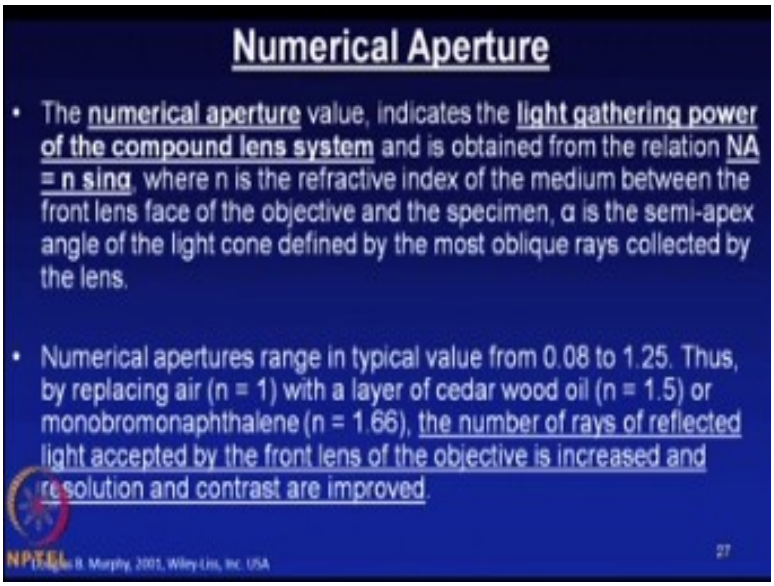

Now we will move on to the other important parameter called numerical aperture. We have seen that in the very definition of the resolution : this d equal to the inversely proportional to this numerical aperture that is what we have seen so in order to understand this what is the numerical aperture first 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 = n sin α where n is the refractive index of the medium between the front lines phase of the objective and this specimen and α is the semi apex angle of the light cone defined by the most oblique rays collected by the lens. So in order to understand this light-gathering capability of the objective lens, I would like to draw some schematic on the board.

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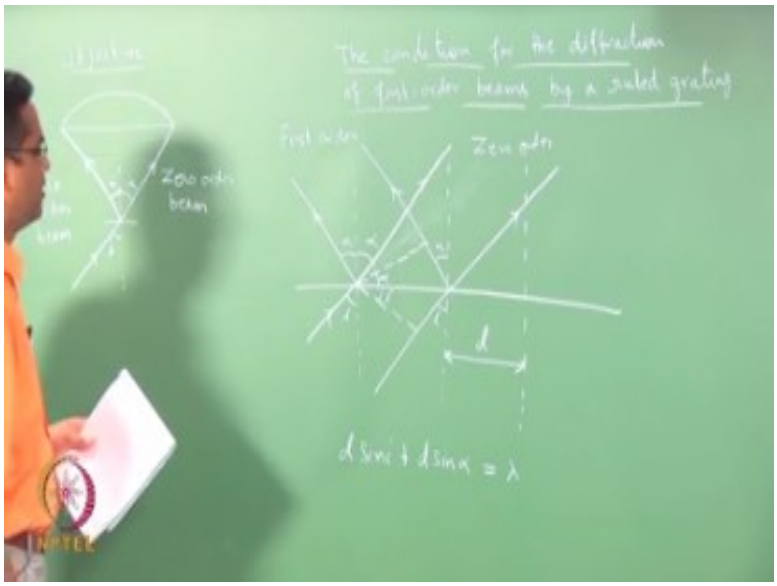

So let us now see. First I will draw the objective lens because objective lens only collects the Rays coming out of first order second order and so on, so let us assume this an objective lens ; let us like. So this is a angle α and this is eye this is angle of incidence and angle of reflection so this is a first order. Let us consider this as a first order beam and this is zero order beam. So this is a collection of first order and zero order beam by the objective lens like this and this is how the angles are defined okay.

We will now write we will kind of draw another schematic the condition for the diffraction of these two. So let us write condition for the diffraction of first order by a ruled grating. The object is a ruled grating and then we will see what is the condition for the diffraction which is further being collected by the objective lens. So let us consider this as a the rule grating and then I have a set of ray is coming, let us thus also draw the zeroth order. Let me draw this much more clear.
So this is as zero order rays and this is first order rays. Let us mark the angles α and then this is angle i. Let us assume this the distance between this the rules rulings or the grating is d. Now let us look at the path difference between the zero-order rays and the first-order rays for that let us draw, so this is α and this is i; we can write the path difference between this zero and the first

order raised from the successive ruling this is such a ruling is exactly one wavelength ; that means we will write D sine i + D sine α = λ.

So the path difference between zero and the first order diffracted from this ruling with spacing D is exactly equal to λ. We will write; since two beams are just collected by objective i = α. So now we can write the limit of resolution; that is d minimum is: d_{min}= to λ / 2 sine α. So this is one expression from this ray diagram we can write.

And we can also now say if the objective lens is filled with some medium of refractive index n then the wavelength of the light in the medium λn, is we can write that λ; and so now we can write the minimum is equal to λn / 2 sine α which is equal to λ / 2 n sin α which is equal to λ /2 NA, so this is D_{min} . So, λ by 2 NA. Where n sine α is called numerical aperture.

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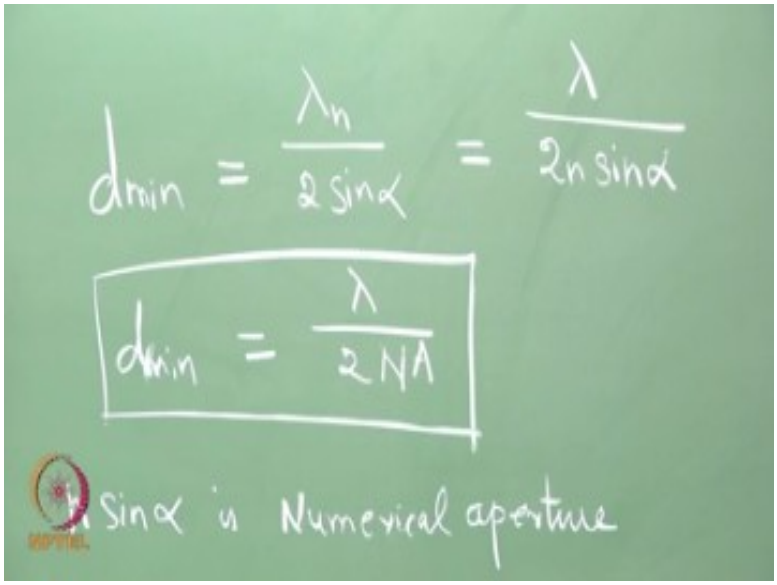

So now you will appreciate the resolution definition we just stated before we get from this a path difference ray diagram a small derivation and then now we will see the effect of the numerical aperture on the resolution we can simply write either increase in numerical aperture that means the light collection ability of the objective lens increases or decrease in λ will produce the same

effect of the on the resolution, so this is we have to remember that is what this mathematical relationship explains either increase in numerical aperture are decreased in λ will influence the resolution power of the objective lens.

Now we can take some example in the well-known electromagnetic spectrum you just take the visible light ranges in wavelength around 650 nanometer at the red end of this spectrum to at 400 nanometers at the blue end ; see we all know this I just want you to give an emphasis that is why I have written this the visible light ranges in the wavelengths from 650 nanometers to that is the red end of the spectrum to 400 nanometers of the blue end.

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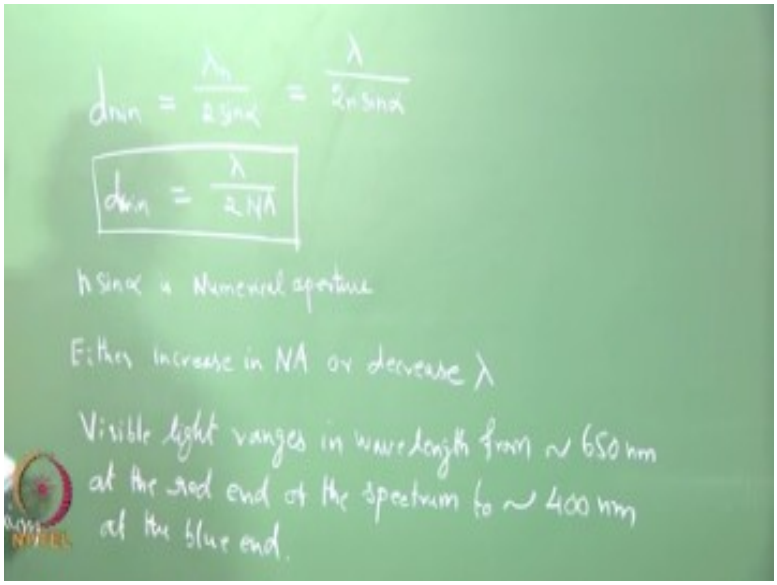

So the limit of if you consider this range the limit of the resolution at the blue end one and a half times better than at the red end.

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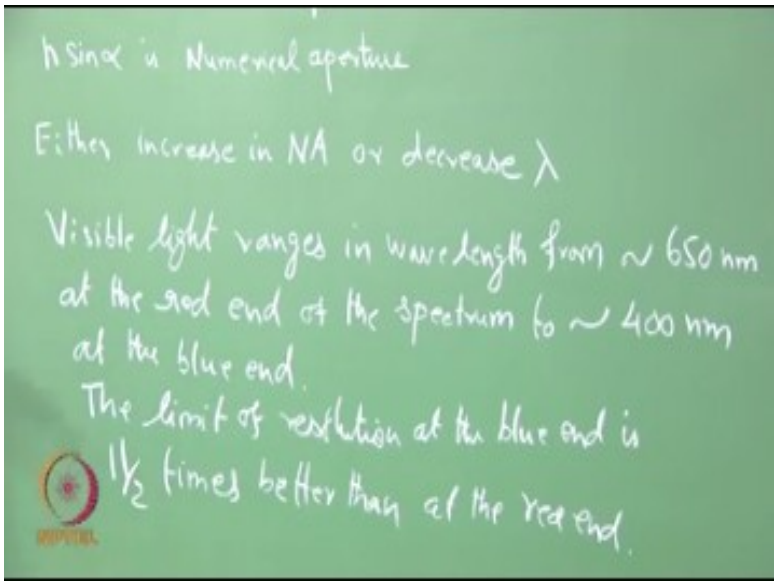

Just to give an immediate example from the well-known visible spectrum and you have the limit of resolution at the blue end that is this here 400 nanometers is one and a half times better than the red end spectrum which will have the wavelength of 650 nanometers. So I hope you got the some idea about this numerical aperture and then how it affects the resolution, so now look at this slide come back to the slide.

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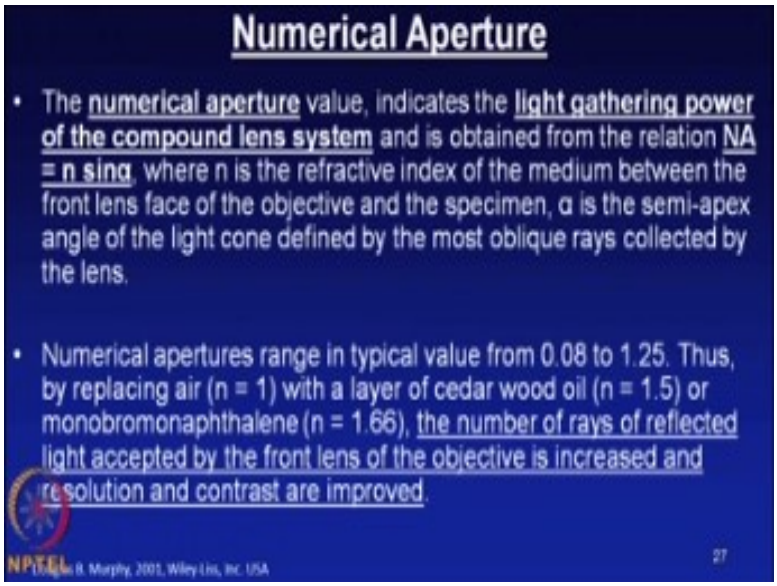

This numerical aperture range in the typical value from 0.08 to 1.25. Suppose this is for this is for the medium air where the refractive in the index is equal to 1. Suppose if you replace this with the layer of cedar wood oil which has got the refractive index of 1.5 or mono bromo naphthalene which has got a refractive index of 1.66 the number of rays of the reflected light accepted by the front lines of the objective is increased, and the resolution and the contrast are improved.

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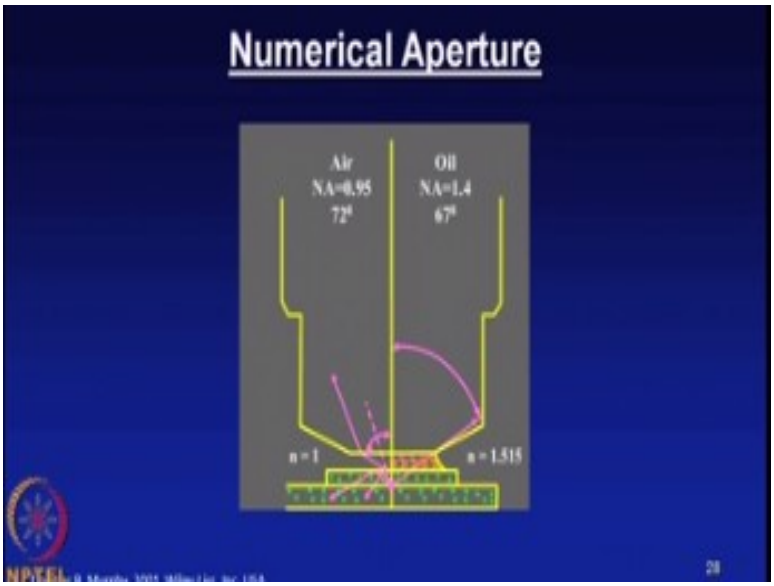

And this is what shown in the schematic look at this, this is an objective piece just to show the difference between a dry lens that is a dry objective with the objective immersed with the medium and oil, so you see that when the refractive index is one ; then you can see that the Ray reflected from the specimen which are the rays which are trying to enter the objective has some limiting angle; this is roughly about 45^0 and then rest of the rays are 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 the where the Rays were reflected back due to the internal reflection they are now accepted by the objective lens because it has an oil which is having higher refractive index. So that is a clearly shown in this and these 72^0 is acceptance angle and this is 67 for the oil immersion objective.

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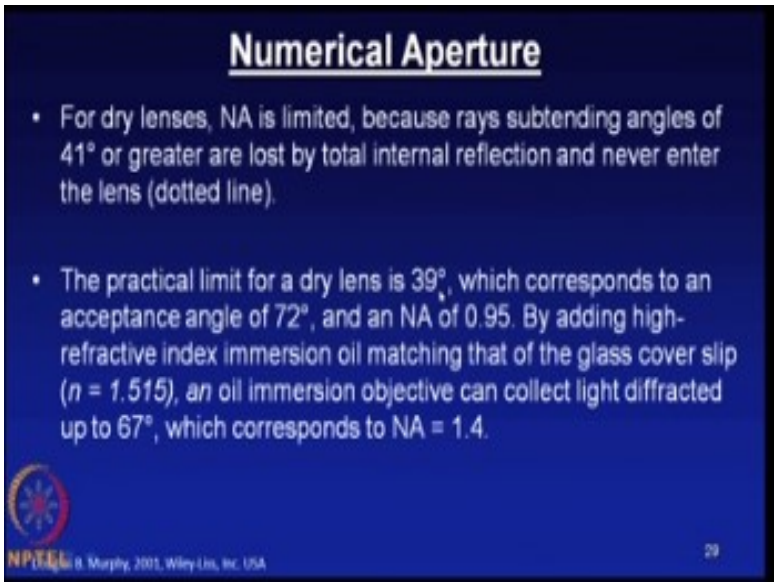

Let us look at a few more points on the numerical aperture, so whatever I just stated before : for dry lenses numerical aperture is limited because, the Rays subtending angles of 41°.

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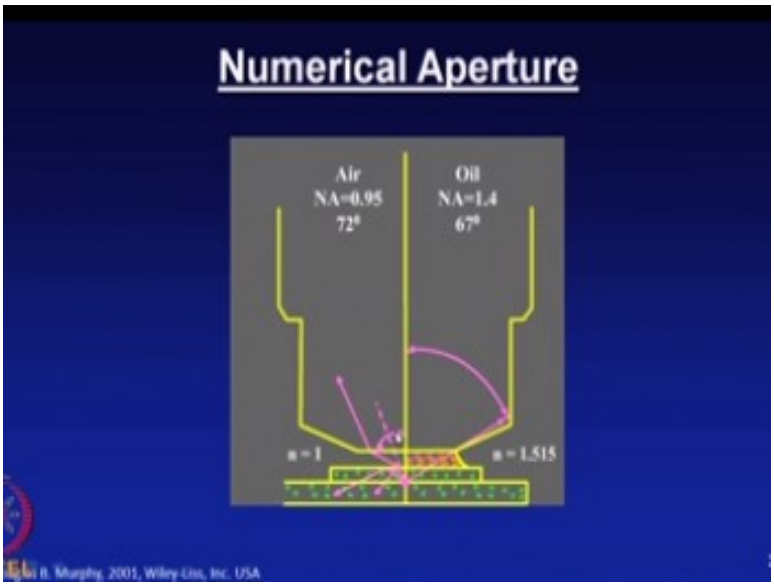

This is what I said this is subtending angle here shown or greater or lost to internal reflection and never enter the lens. The practical limit for a dry lens is about 39° which corresponds to an acceptance angle of 72°, and an numerical aperture value of 0.95. By adding high refractive index immersion I 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 reflected up to 67°, which corresponds to numerical apertures of 1.4?

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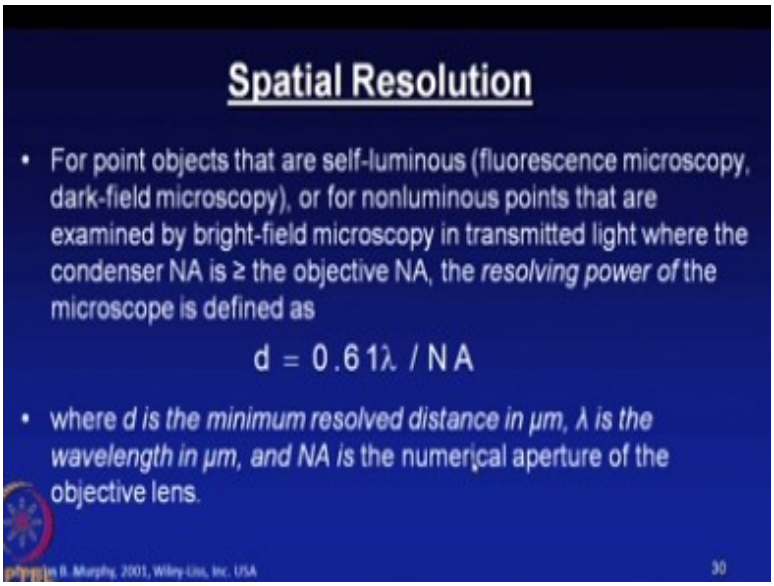

So let us now look at the other terminology spatial resolution. For all practical purposes we are we are using only the spatial resolution. Whatever we are looking at is only a spatial resolution in the microscope, and we will see what the points to remember are. For point objects that are self luminous whether it is a fluorescence microscopy or a Dark field microscopy. We will see them that principles and techniques in due 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 microscope is defined as D = 0.61 * λ /(numerical aperture). Where D is a minimum resolved distance in micrometer λ is the wavelength in micrometer. And NA is the numerical aperture of the objective lens,

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And in the case of bright field microscopy where the condenser numerical aperture is less than objective numerical aperture. That is the condenser aperture is closed down and are an oil immersion conduction is used in the absence of oil the resolution is given as D = 1.22λ / (condenser numerical aperture + objective numerical aperture). Please look at this the number 1.22 here it is considered, a complete diameter of the airy disc here we have considered only the, the radius of the airy disc. So do not get confused with this, this is he but here it is diameter of the airy disk is considered.

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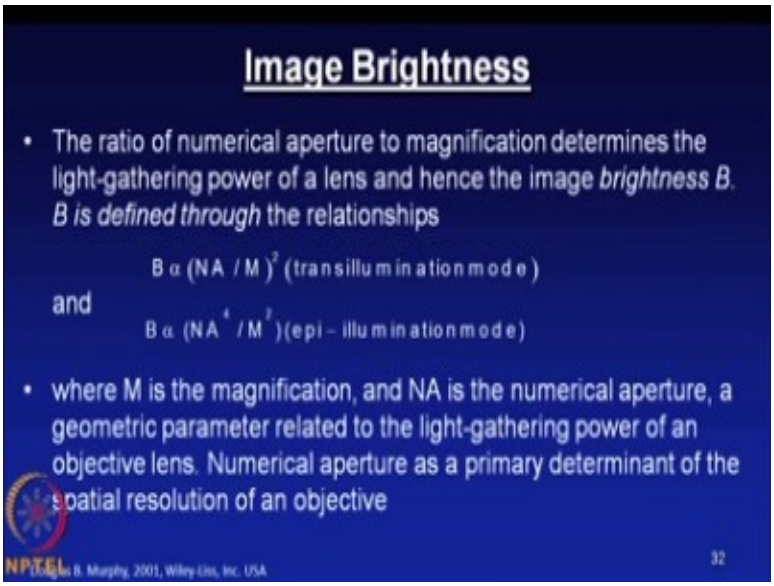

There it is radius of the airy disk is considered. An another important a parameter which we talked about in the light microscopy or any microscopy is an image brightness very important, and the ratio of numerical aperture to the magnification determines the light-gathering power of the lens. And hence the image brightness B, B is defined through the relationship like this B is proportional to (numerical aperture/magnification)^2 for the Trans illumination mode Tran's illumination mode. Where and B is proportional to (numerical aperture)^4 / (magnification)^2 for a epi illumination mode.

We will see what are these two illumination mode when we when we go to the, the instrumentation details; I will just show you the corresponding the illumination mode. When we look at the instrumentation and this is how the brightness is defined and where M is the magnification and the NA is numerical aperture. A geometric parameter related to the light-gathering power of an objective lens a numerical aperture as a primary determinant of the spatial resolution of the objective. So it is very important to note that numerical aperture of objective lens determines the resolution power and of course it has got some range of values.

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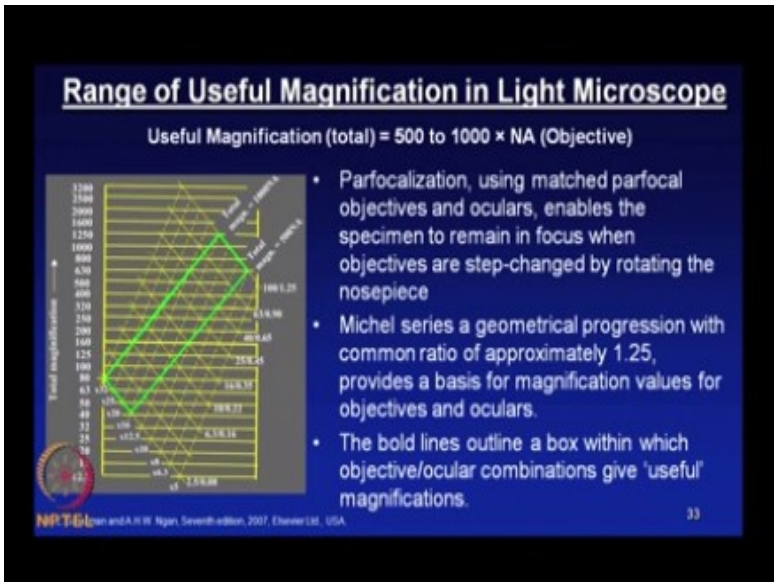

We will see that; not that all the values of the numerical aperture required be useful but if you look at actually the useful magnification is totally if you look at it, it is 500 to 1000 times of the numerical aperture value of any objective. This is a total magnification which is useful okay, and if you look at this slide what is shown here is the range of useful magnification in the light microscope. So we should not think that suppose if you keep on increasing the numerical aperture value or you will keep on decreasing the 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 this grits shows some kind of, the way of reaching some kind of a progression geometrical progression of objective and ocular combinations. So we have seen in the beginning I said most of the microscope we use a compound lens one is one we have objective as well as subject 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 eyepiece as well as the objective has a range of values only that combination will give you the useful magnification okay.

So this diagram conveys only that, that is the combination of eyepiece and combination of objective and the Green Line shows these the useful magnification range. So this is done with per-focal objectives with the combination of different, different ocular lens. So it is perfocalization using matched perfocal objective and oculars enables the specimen to remain in focus when the objectives are stepped changed by rotating the nose piece. It is called Michael series. A geometrical progression with the common ratio of approximately 1.2 for 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. So it is not that all the, you know magnification values are useful. So now we be just come to a situation that is 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.

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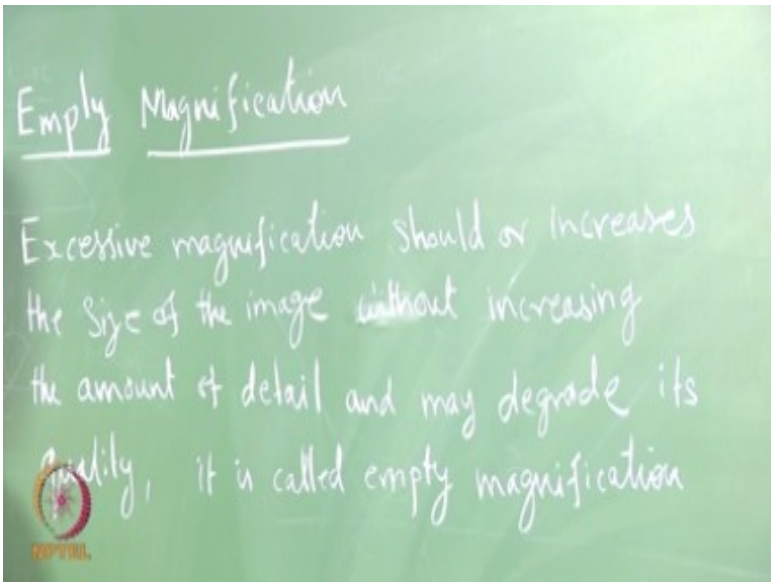

So we can write a kind of a definition for this empty magnification. So we write that excessive magnification should or increases, the size of the image, without increasing the amount of detail and it may degrade also it's image quality it is called empty magnification. So it is not that we have all the values for the objective numerical aperture is going to be useful. So 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.

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