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Lecture - 1
Materials Characterization
Fundamentals of Optical microscopy

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Hello everyone! Welcome to this material characterization course. In this course, we will see the optical and scanning electron microscopy and its various principles and techniques.

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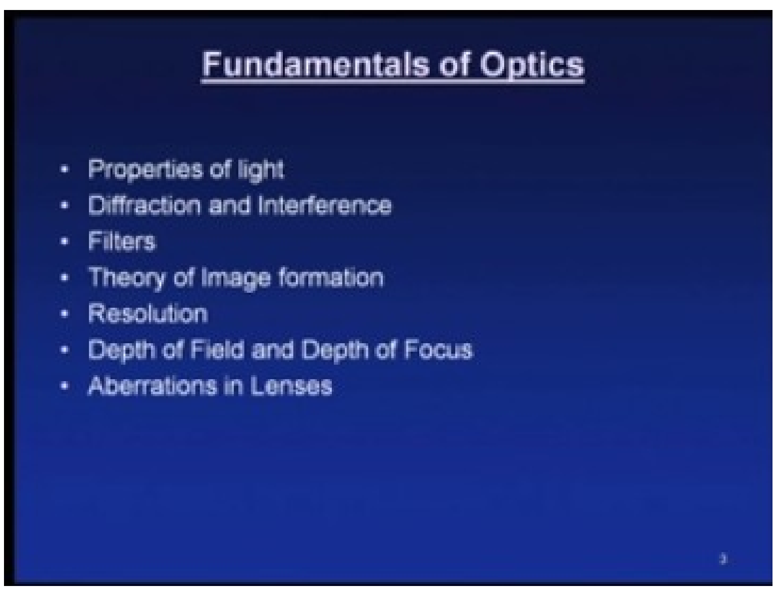

Let me introduce. These are the topics which I will be covering as far as the fundamentals of the optics is concerned. First we will review properties of light and then we will discuss diffraction and interference in brief, and we will also see the filters; various filters which are being used in the optical microscopy. And then we will concentrate on theory of image formation, resolution and depth of field and depth of focus; and most importantly the abrasions in the lenses and how to overcome these abrasions in order to obtain a good image.

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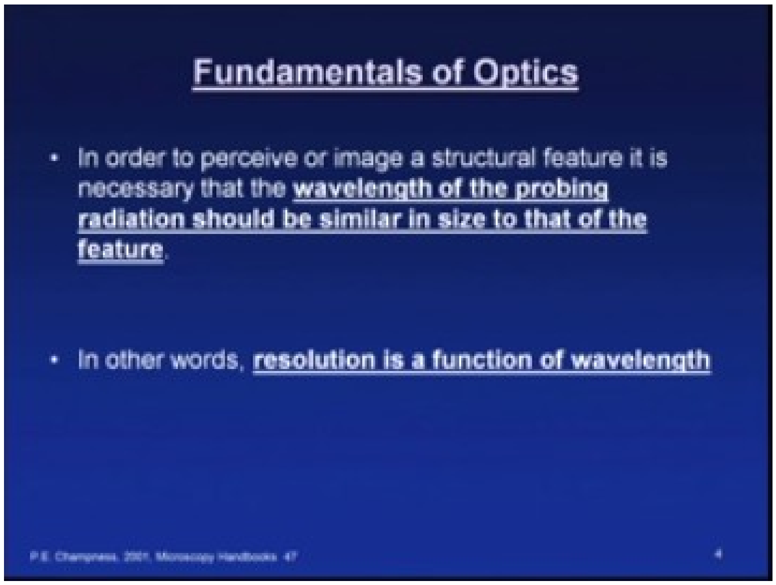

So the first and foremost important thing which you have to understand in order to perceive a or image a can structural feature, it is necessary that the wavelength of the probing radiation should be similar in size to that of the feature. This is a fundamental requirement for any electromagnetic probing radiations. That means you have to make sure that the structural feature which we are interested is necessary that the wavelength of the probing radiation also should be of the same order, and this is what is stated, in other words, resolution is a function of wavelength.

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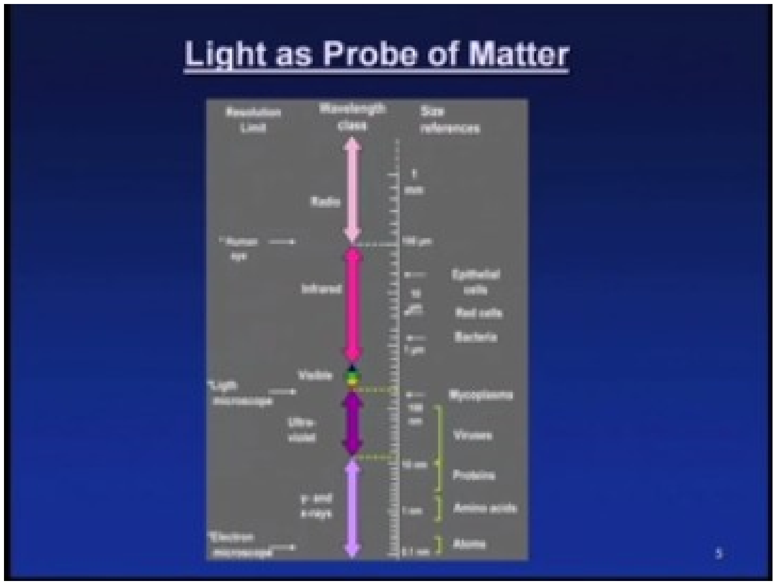

Let us look at this slide when we choose light as in a probing of a matter or light as a probe of a matter, what are the things we have to keep in mind. If you look at this is a complete electromagnetic radiation spectrum is given and what you see is the; left hand side is the resolution limit. And then you have the electromagnetic radiation classification and then the size references is given. So we can appropriately choose the kind of size we are interested or structural features we are interested to resolve.

And then we are supposed to choose the appropriate electromagnetic radiation. For example, if you look at this evenly the blue radiates blue light which is which is far higher compared to the some of the biological trait cells and so on. So you can clearly see that the structural features all the way up to the atoms you have the corresponding electromagnetic radiation to choose. So this is the fundamental requirement before we choose the kind of microscopes which we are interested in for the micro structural characterization.

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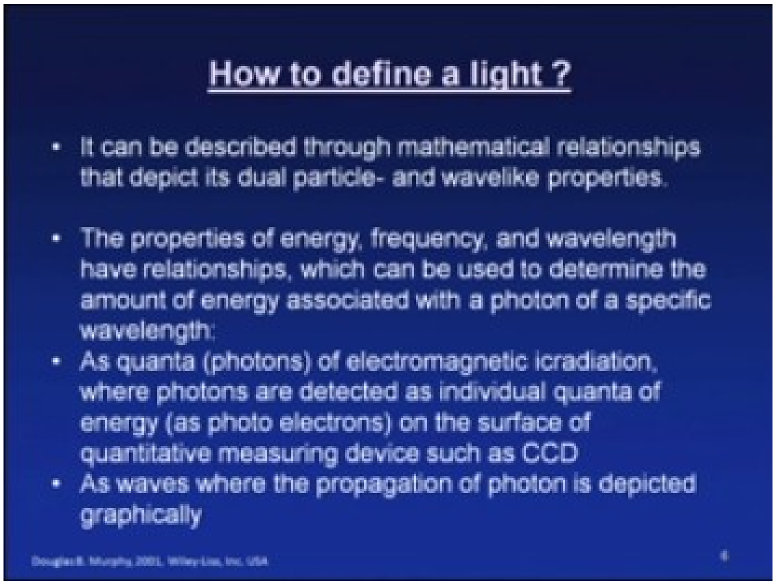

So how to define alight this is very always a very tricky situation: how to define a light? Because we all know that the electromagnetic radiation has a dual nature. That is the light will behave as a particle as well as a wave. so but it can be described through mathematical relationships that depict its dual per particle and wave-like properties, i.e. the properties of energy, frequency and wavelength have the relationship. What are those relationships?

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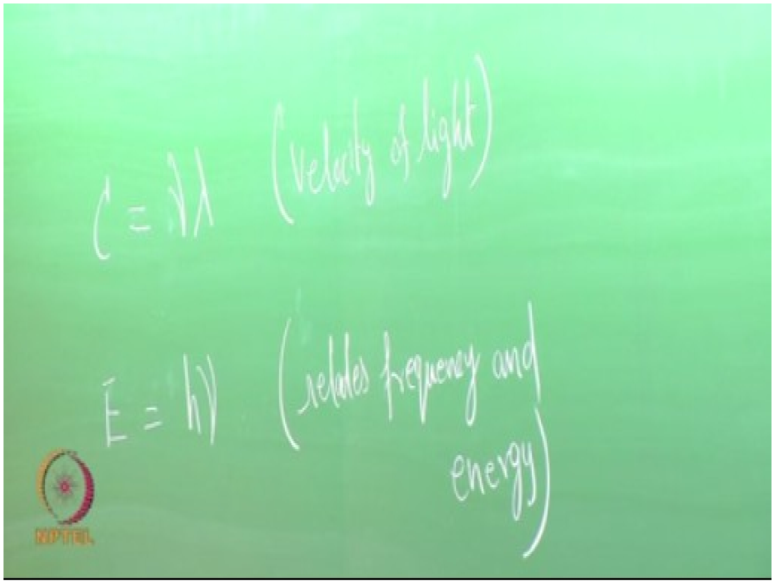

So you have something called C = ν λ. And this is; this is describes this equation describes the velocity of light and then you have the another equation e = hν, relates frequency and energy. This equation is important because we are going to see that when the light passes through the glasses, it changes its velocity. And it will be useful to choose the kind of radiation we are going to look at the structural features. For example, in a biological samples the kind of appropriate radiation has to be chosen based upon the energy. And third, you have, which relates energy and the wavelength.

And you can see that you know, the wavelength electromagnetic radiation to have the shorter wavelength will have a higher energy in this whole electromagnetic spectrum. So at least these three mathematical relationships explains the dual nature of the light. And then in we should normally in an optics we always use the electron or a light as a wave and it is propagation is always represented in terms of a wave nature. And then where to use a particle where to use a wave we have to choose in appropriate situation.

For example, when the light comes and interacts with an matter, at that point it is considered as a quanta that is a photons. Even for the when we realize that, when we record this your images in an a quantitative measuring device like a CCD camera, where it is quantized okay. So that means at that situation it is called a counter that is what it is written in the slide.

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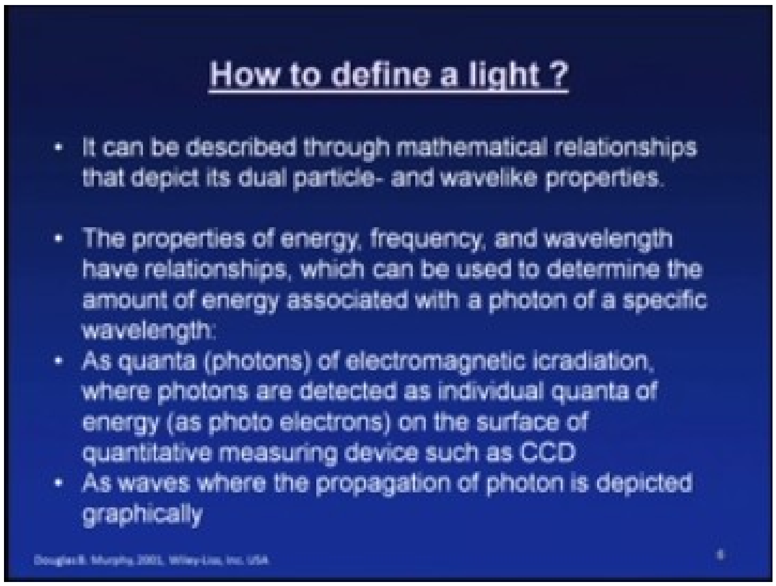

As a quanta or photons of electromagnetic radiation, where the photons are detected as individual quanta of energy as photoelectrons on the surface of the quantitative measuring device such as CCD. So at this point of time this is considered as a quanta or a particle in nature, where the interaction point that interface it is considered a quanta. But all the other properties like when it when you consider the light as a propagate in a propagation mode and ; or the photon propagates then it is depicted like a wave as mentioned as shown here.

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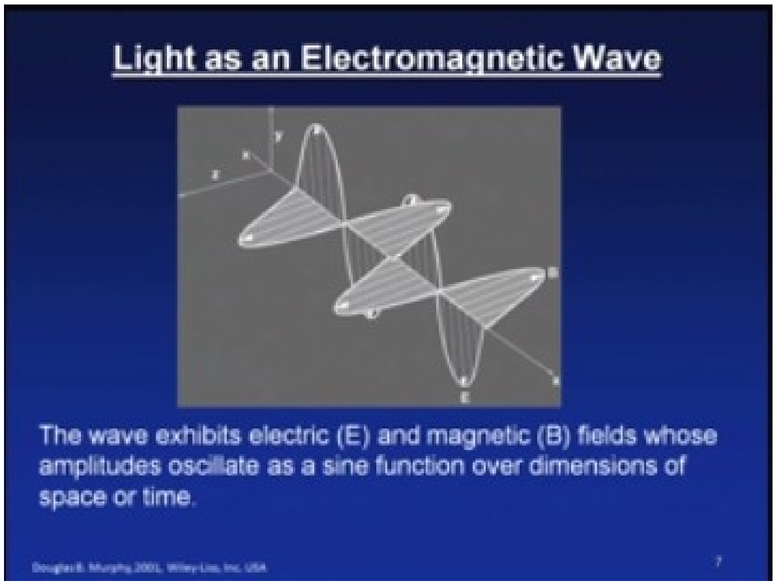

So what you see is a schematic of electromagnetic wave which exhibits an electric and magnetic fields whose amplitude oscillate as a sine function over dimension of space and time. So these two fields are mutually perpendicular to each other and this is how the light is defined when you consider it's propagation to a one medium to other medium or when it propagates from one electromagnetic lens to the other electromagnetic lens, this is how it has been considered. So this is another important introduction for the light.

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And we always talk about in a characterization of materials the quality of light. And let us see what are the quality of light and it is classification. If you look at the slide we have an animation you can see that it could be a monochromatic or a polychromatic, or it could be linearly polarized or non linearly polarized or non polarized; it could be a coherent or non coherent; it could be collimated or divergent. So all these types of the different quality of light can be defined in this manner. But for our clarification I have just made some animation: a monochromatic light will is supposed to be like this if the propagation is like this on the other hand if you look at the polychromatic it propagates in this fashion.

And what is linearly polarized it is linear polarized and non-polarized will have at all the directions the waves will propagate in all directions. And when we talk about a coherent light we should have all the waves in the same phase and non coherent light will have a different phase like this. A collimated beam is supposed to propagate in the same direction like this and a divergent beam will spread like this.

So you get by looking at all the schematics you get some idea about what do we mean by the quality of light because in most of the circumstances we use this term a monochromatic light or a coherent beam or polarized light in the coming lecture. So this will give you a kind of an idea what you mean by the quality of light.

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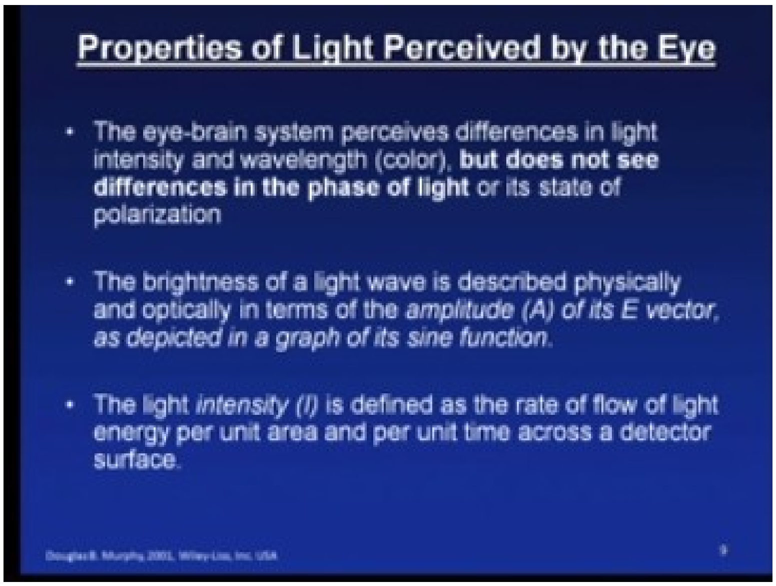

Then we if you start looking at the properties of light perceived by the eye the basic understanding goes like this: the eye brain system perceives differences in light intensity and wave, but does not see the difference differences in the face of light or it is state of polarization. And second point is the brightness of the light wave is described physically and optically in terms of amplitude of the electric vector what we have just seen in the earlier slide as sine function. The light intensity is defined as the rate of flow of light energy per unit area and per unit time across a detector surface.

So in order to understand the image formation in the microscope we must consider it from the point of view of wave theory and then geometrical optics. The wave theory relates the information of resolution of microscope and quality of image and so on and the geometrical optics relates information regarding magnification and type and positions of the image. So if you look at this the basic laws of the light or electromagnetic radiation the first one always we considered is when the speed of the light waves is reduced their frequency and it is unaltered. But the wavelength is shortened.

Consequently, when the ray of light passes from one isotropic medium to another it is refracted that is bent through the angle which is determined by the Snell's law. We all would have heard this fundamentals fundamental law of light where when the light propagates from one isotropic medium to the other it bends or refracts, that is first law. The another law which is very important related to the reflection is : when the light is reflected at the surface it obeys another simple law: this is the incident and the reflected rays make equal angles with normal to the reflect reflecting surface and are coplanar in with nature. Or what it means is the angle of incidence is equal to angle of reflection.

This is one of the important law of reflection when you consider when you say a light is reflected. So this point these three these two laws are very fundamental laws to define the properties of light.

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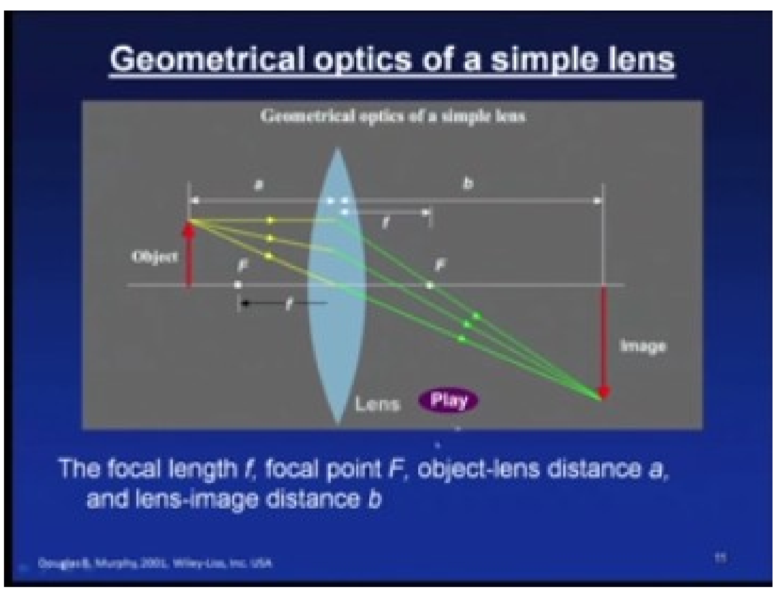

So now let us look at the geometrical optics of a simple lens. You have the glass lengths and this is an object which you have here: red in color. And then the light is passing through the lens and then it get focused on the other end where the image is formed. And the distance between the center of the lens and this two point front there are two points in front of the lens one is at the front and known as the back so these two points denoted as f are called principal focal point and the distance between the object and the lens is a and the distance between the lens and the image is b.

And the light rays are traveling in a particular fashion, right? So these the light which is passing through the center of the lens is un-deviated, but the rays which are passing through the periphery of the lens this region are deviated, right? So, these are the properties which are governed by the some set of rules.

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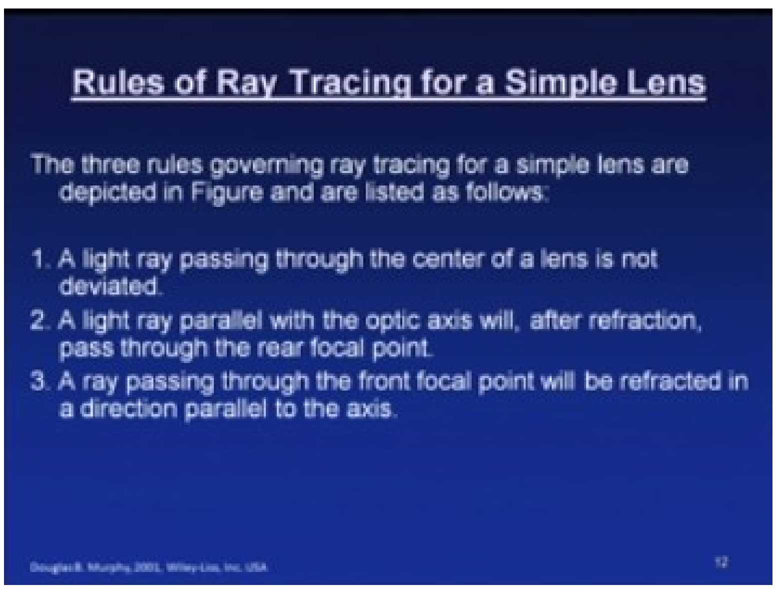

Why it is so we have some set of rules called rules of ray-tracing for a simple lens these rules are an assumption on the Snell's law. Okay! This lens law is not strictly followed there is some assumption like Sin I / sin R = μ we say it is some assumption based on then they as sin I / sin R = I / R. We will not get into the details but I just want to mention that these rules are based on some assumption of the Snell's law and let us see what are these ray's rule. There are three primary rules governing the ray tracing for a simple lens and are; and we will show in the figure also. We will first see what are the rules : a light ray passing through the center of a lens is not deviated; a light ray parallel with the optic axis will after refraction pass through the rear focal point.

A ray passing through the front focal point will be refracted in a direction parallel to the axis. So let us see whether these three rules are indeed the case.

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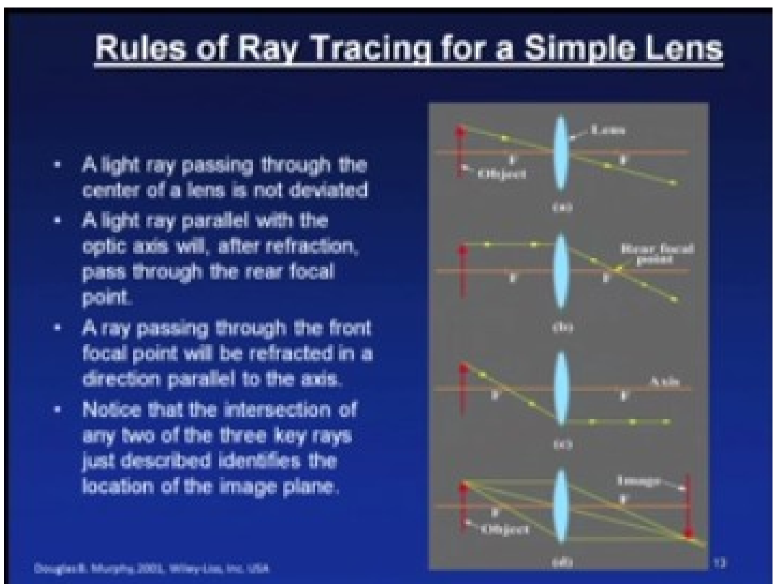

So if you look at this schematic the first image (a) shows a light ray passing through the center of the lens is not deviated, that is the first rule. A light ray parallel with the optic axis will after refraction pass through the rear focal point. So this is the rear focal point. A ray passing through the front focal point will be refracted in a direction parallel to the axis so this is what it is it is it is parallel to this axis. And notice that the intersection of any two of the three ray just described identifies the location of the image plane: so this is the image plane where these two diffracted beam interacts and that describes where the intersection point is shown is some image here. We will now see what are the conditions to form an image.

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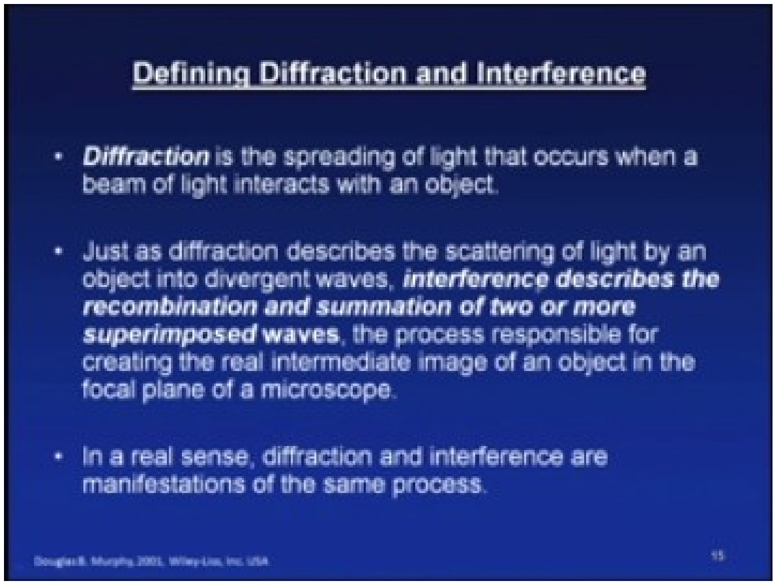

If you look at this another ray diagram which clearly shows the image formation.

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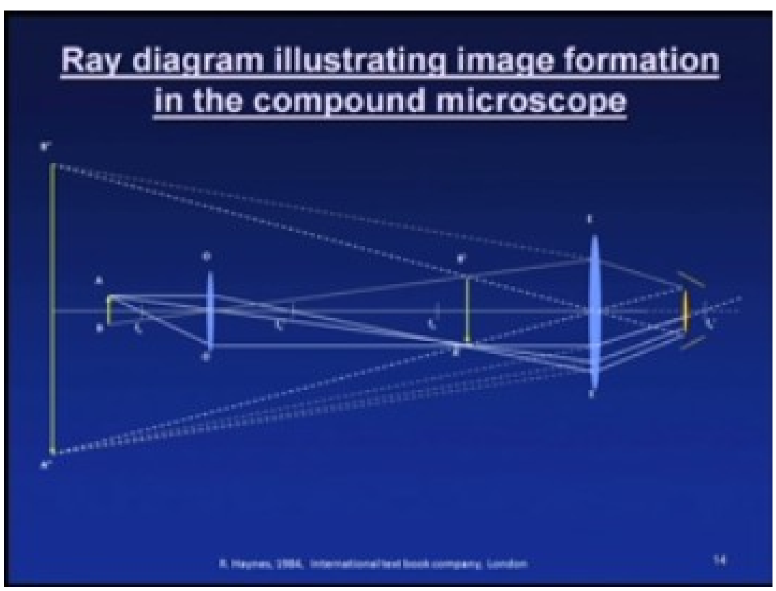

Let us look at that the details of this image here is an object a b and then you have this objective lens and then you have the image formation a` b`; this is an inverted image and then you have another lens, here it is called eyepiece basically. What why I am bringing this slide because most of the microscopes they use a compound lens you have two types of microscopes you are whether you have a simple microscope or a compound lens microscope. In a simple microscope you will have only one set of links where you will have the reflection type and you have another microscopes you have an objective as well as eyepiece.

So to just mention these two what is a compound microscope to illustrate that concept I brought the slide. So you now look at this for an objective the image forms here and for the eyepiece this image is projected as a` b` in this case and this is how your this is a I which is perceiving the virtual image here a`` and b`` is a virtual image and you are a` and b` is the real image of course the virtual image is not inverted again whatever the objective produces here it is just magnifying to this length.

So it also kind of explains the magnification how the magnification takes place. We will see how we can understand this magnification.

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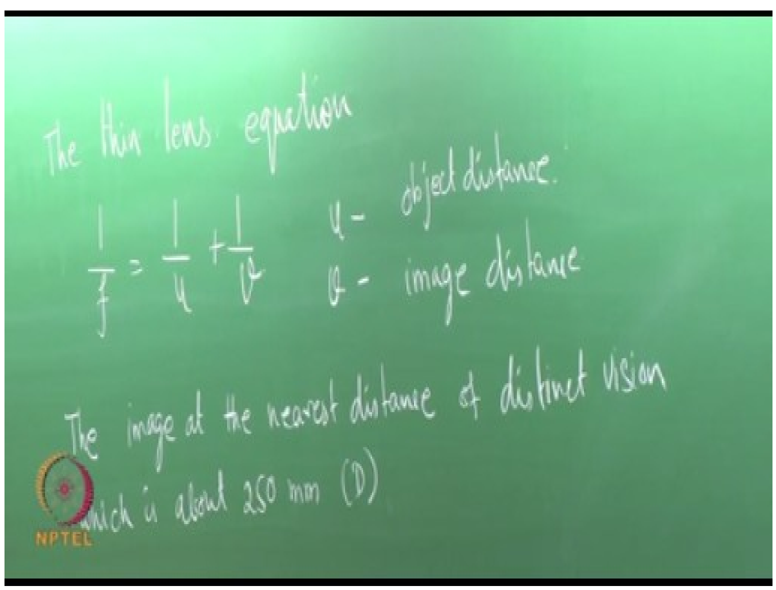

So we will write the popular lens equation, i.e., 1 / f = 1 / u + 1 / v so here instead of a and b, I have used U and V which is very common notation in the optics. So u: object distance and v is image distance. So we should also know that the image at nearest distance of a distinct vision which is about 250 mm or 25 cm this is normally do network denoted as D capital D this is what we this is the minimum nearest distance with which

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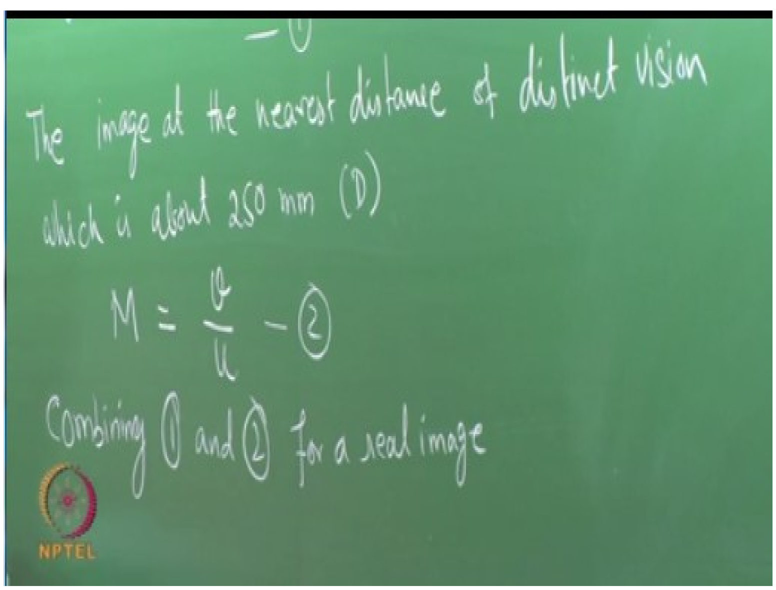

we are able to see any of the object with our normal vision. Which is 20 250 mm. so normally we write magnification is v/ u. So this is the magnification given by any of any lens and combining this suppose if you combine these two i.e., let us call this as equation 1 and this is 2. For a real image which in this case is an objective or our D.

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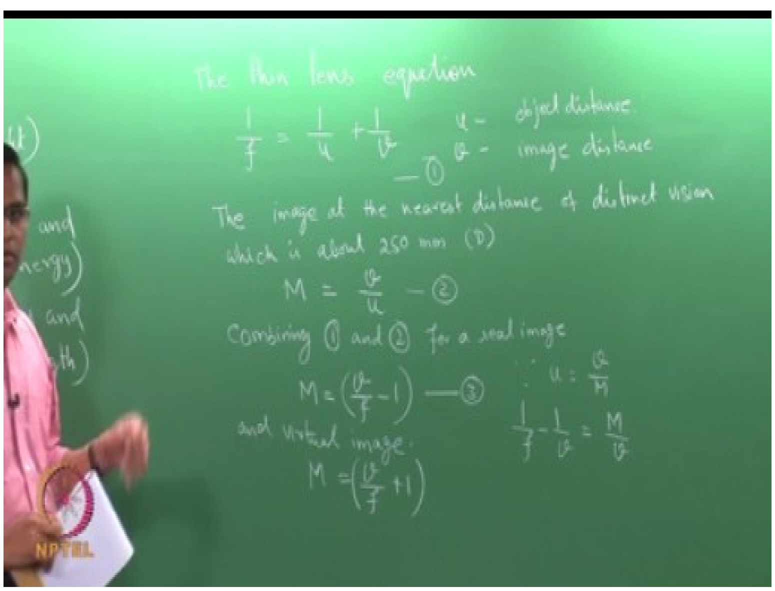

So you put $u = v / m$ and then substitute this in this equation $1 / f - 1 / v = M / v$ then with this we can write $M = v / f - 1$ this is equation 3. And for virtual image $m = v / f + 1$ the sign changes because if you look at the image if you look at this image for objective it is in one direction. Now we are talking about eyepiece.

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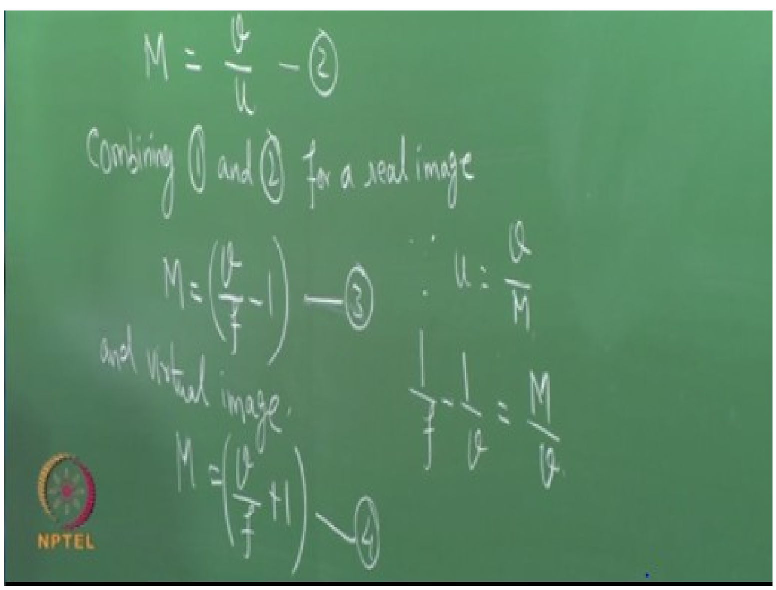

So this is in the opposite direction so that is why the sign changes here. So this is for the virtual image which our eye sees this is equation 4. So now we can write for objective lens.

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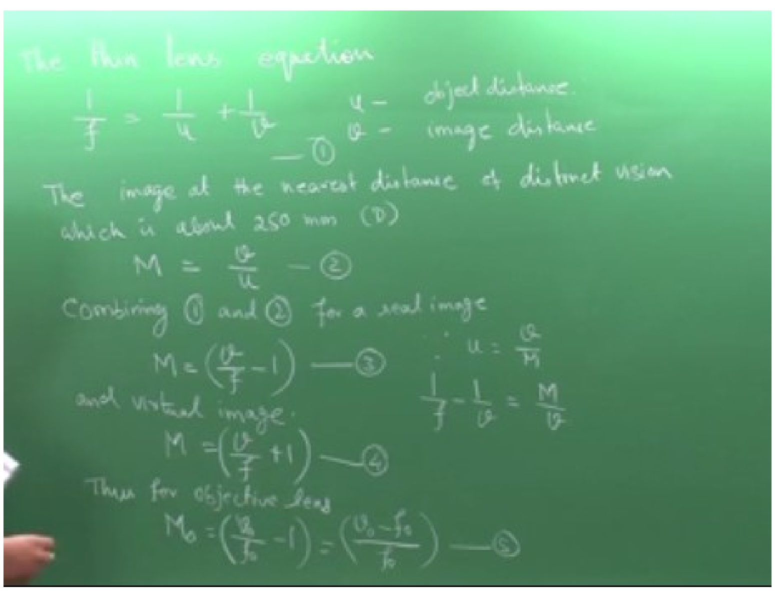

So this is for objective lens magnification; the subscript o describes objective lens for the magnification and similarly we can write for eyepiece.

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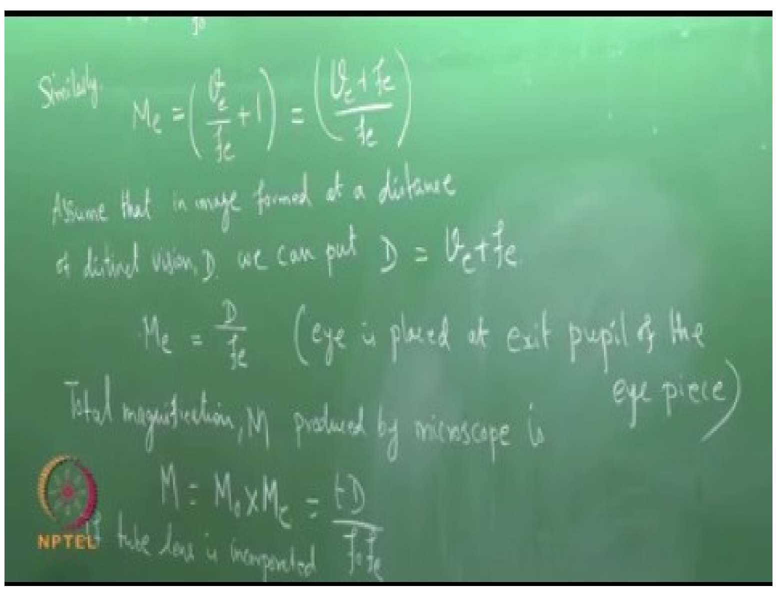

But before that and most of the microscopes use this optical tube so that the length of the optical tube is also taken into consideration which is considered T so from the ray diagram we can show that this is $T = V- F_0 V - F$ so we can write objective magnification like T – F, similarly the magnification of the eyepiece can be written like this. And here we assume that the image formed at a distance of distinct vision i.e., D.

We can put $D = V_c + F_c$. Then objective we use this optical tube length, in image we use this distinctive vision D which is 250 nanometers or sorry, 250 mm that is 25 centimeters, so if we can substitute this into this magnification of eye piece then it is D/F_e. So since this is appropriate because your eye is placed in the microscope at the exit pupil of the of eye piece, so now we can write the total magnification for a compound lens microscope is that is tD/F.

So this is coming from these two equations and if we can include the tube length also, so M total magnification is equal to objective magnification times eye piece magnification times this optical tube length. So you get an idea of why we use or my most of the optical microscopy uses a compound lens, and it gives an idea how the magnification is perceived in a compound lens microscopes.

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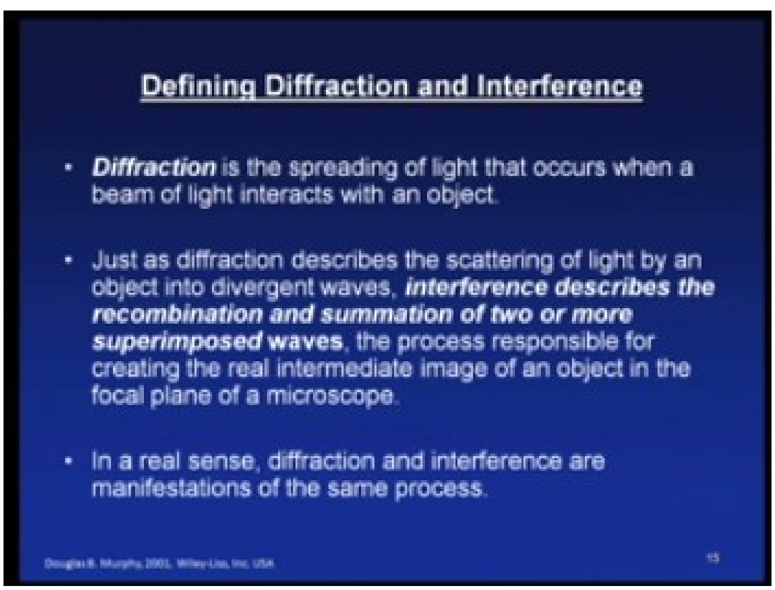

So let us look at the another concept called diffraction, so if you look at this first introduction lines of a diffraction is the spreading of light that occurs when the beam of light interacts with an object. So it is a very general statement for the to define a diffraction; we will slowly get into the core concept of diffraction. So before you get into the idea of diffraction let me just talk about some of the general things.

All the electromagnetic interaction with matters is generally called scattering. So your reflection a refraction, diffraction everything is a part of a scattering phenomena, and then we have just looked at the refraction Snell's law as well as the when you call reflection and similarly diffraction also has a set of rules which we which will define in few minutes. So first you have some clear clarity on this all this phenomena is part of a scattering.

So you have a Snell's law to or to bending of the light is defined by the Snell's law and then you have a law for reflection and similarly we can see something specific rules for a diffraction also. So before we get into this let me also dry some draw try to draw some schematic.

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So what I am trying to draw is when the light passes through a edge like this, it produces a shadow here this is because of diffraction this is one of the manifestation of a diffraction. I am not trying to define a diffraction here but just the effect of diffraction then these things will help you to understand the concept much more easily. So this is a when the beam of light comes and it exit to an edge like this and then you have the shadow.

Let me draw this, you write diffraction of light at an inch. This is shadow which causes the diffuse light, I will also draw some more schematic which clearly shows this effect, okay. What is that I am trying to draw and tell you. So when the light passes through a slit like this and then as I said earlier like here it causes some shadow effect here at the edges, and when you narrow down this gap slip then what happens and this is your intensity versus distance plot for this event.

And then now you narrow down the slit then it produces a subsidiary Maxima, sorry like this and this is your other intensity profile for this gap and this is for the edge. So suppose if I reduce this little more further then I will produce one more maxima and like this a dark and a bright fringes will come. So this is the a simple as simple one can do with any of the slit or even you take a very curtain with a torch light or laser light and you can see this effect. So this is one simple example where you can see the effect of diffraction and then, we will now see that why we see this and how this is important this is every image formation the diffraction is very important because of the diffraction will be the image is form you can see the image and then what are the governing principles.

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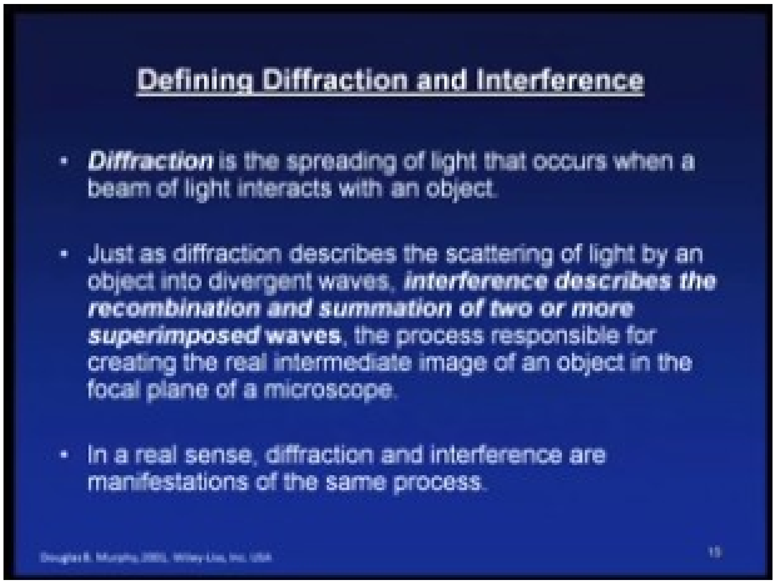

So you just see that just as diffraction describes the scattering of light by an object into a divergent wave and there is something called interference which describes the recombination and summation of two or more superimposed waves. The process that is the diffraction sorry, the interference process responsible for creating a real intermediate image of an object in the focal plane of the Microscope.

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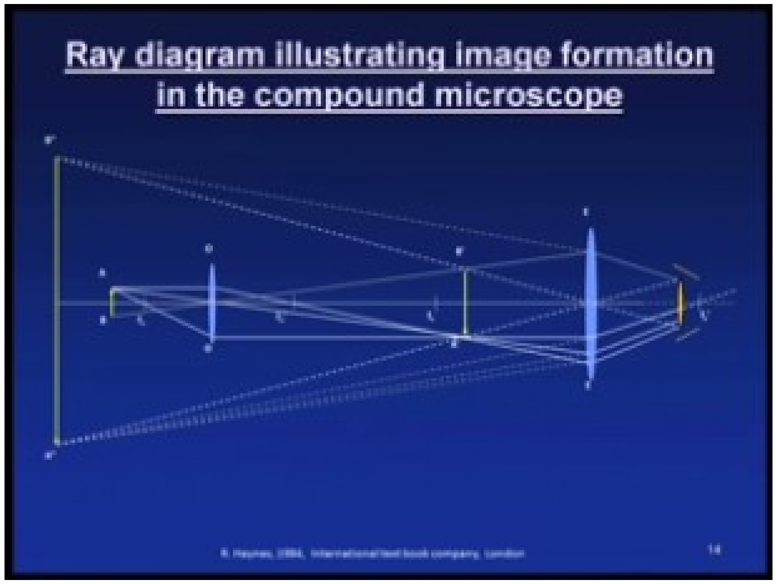

So let us go back what we talked about this is the image we are seeing this is an intermediate image which we described here and for that we say that the interference is important. So in a real sense diffraction and interference are manifestations of the same process, so I hope this would have given you some kind of an introduction to the concept of diffraction.

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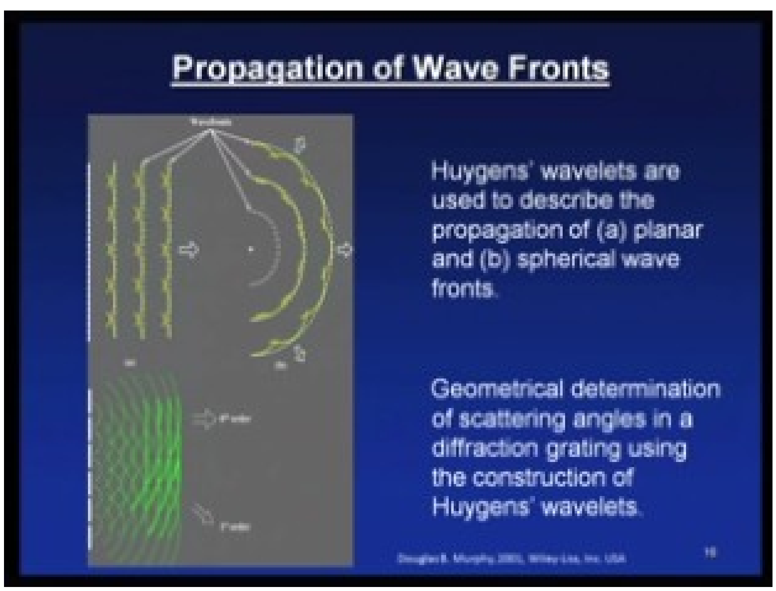

We will see a much more detailed information about the diffraction as we go along if you look at this how the wave propagates there is a schematic you can see that this is described by Huygens wavelets are used to describe the propagation of a planar and spherical fronts. So you can see that this schematic where it shows the wavelets are propagating like this and then you have the schematic (b) describes the geometrical determination of scattering angles in a diffraction grating using the construction of Huygens wavelets. So this is how the interference is taking place and then you have this zero order and the first order. So this is how the wave propagation is described in a optics.

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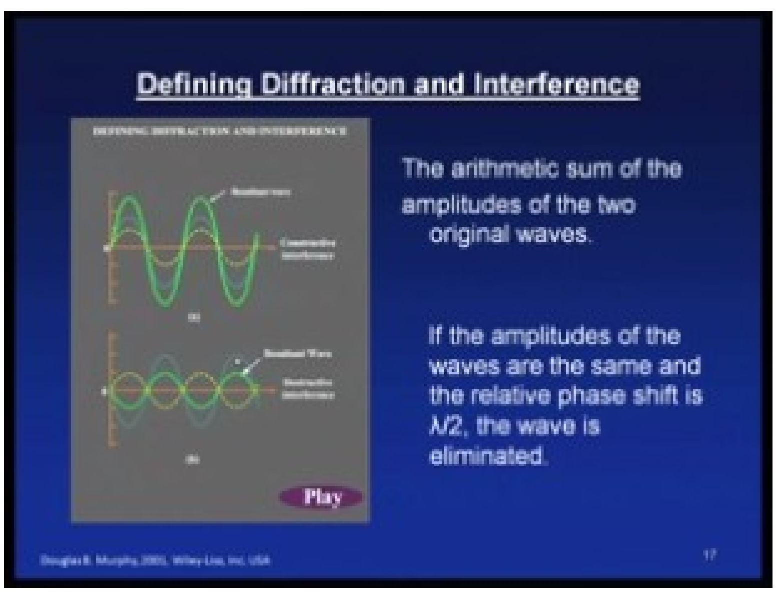

So here is an important slide where we are going to define the diffraction interference, so first I would like you to look at this schematic, so where the event (a) describes the constructive interference that is the arithmetic sum of amplitudes of the two original waves so you have the resultant wave depicted as a green line. So this is called constructive interference and then you have the (b) which describes the destructive interference you can see that the resultant wave has much lower amplitude compared to the constructive interference, okay.

If the amplitudes of the waves are the same and the relative phase shift is $\lambda/2$ as you can see that the wave is eliminated. So this is another important rule one should keep in mind when you think about diffraction though the constructive and destructive interference play an important role in order to perceive the diffraction phenomena, so let us move on to next concept.

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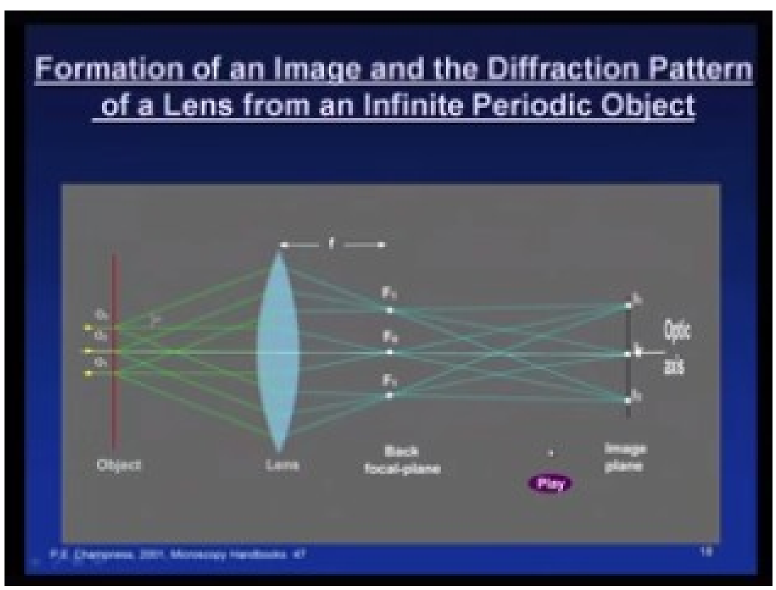

Now I take you to the image formation involving diffraction how the images perceived in a lens through a lens from an infinite periodic object, first you just observe this schematic then we will discuss that; so you have the object where you have it is a periodic object with Q1, Q2, Q3 with equal distance and you have the light rays pass through this lens. Remember all this rays tracing this trajectories if we can look at the rules of the rays they will all follow the same thing that is the light which is passing to the center of the lens will go through undeviated.

The rays which is passing through the peripheral of the lens will deviate and then it will go to this image plane. And what I would like you to just concentrate on this look at this each ray and then where it goes; and then you have something called principle focal point like I mentioned in the earlier slides, and I have not shown the other focal point in this the other side of the lens so this is a principal focal point. It is a back focal plane this plane is called back focal plane F_0 is a principal focal point and f_1 and this supposed to be f_1' there are other points. These points are nothing but a diffraction pattern. This each focal point is called a diffraction pattern and this plane is called a back focal plane.

So now you remember, your diffraction pattern forms in the back focal plane in the ray diagram and then what is the significance you see that all the diffracted ray converges here from the different different source and then again it diverges and then recombine in the image plane. So the important point to remember here is suppose if you if you look at only any one of this diffracted spot since the rays which is coming out of this diffracted spot go to the different location in the image plane that is also important, so that means you should have; what it means is each diffracted spot in a back focal plane contains some information about the image. This spot will have some information about the image and this spot will have some other information about the image.

So it means the before the image formation your diffraction takes place and each diffracted spot carries some information about the image so this is what very important and then if you look at this image plane also you see at least two diffracted beings recombine to form this image. You can see any one of this spot in the image plane it contains at least two diffracted spot before it forms an image. So that clearly shows that the image formation involves diffraction in first and then the recombination of the diffraction beam in the image plane.

So I will just play this one more time just observe so light comes and I will stop here then again I will start so it all forms first the diffraction pattern or they are all converge in the back focal plane at a particular point this particular plane is called a back focal plane and the all the patterns diffraction patterns or all this sorry, all these focused points are called diffraction pattern and from there you have the rays converging again in an image plane to form the final image. So this is another important aspect of how to perceive this diffraction pattern.

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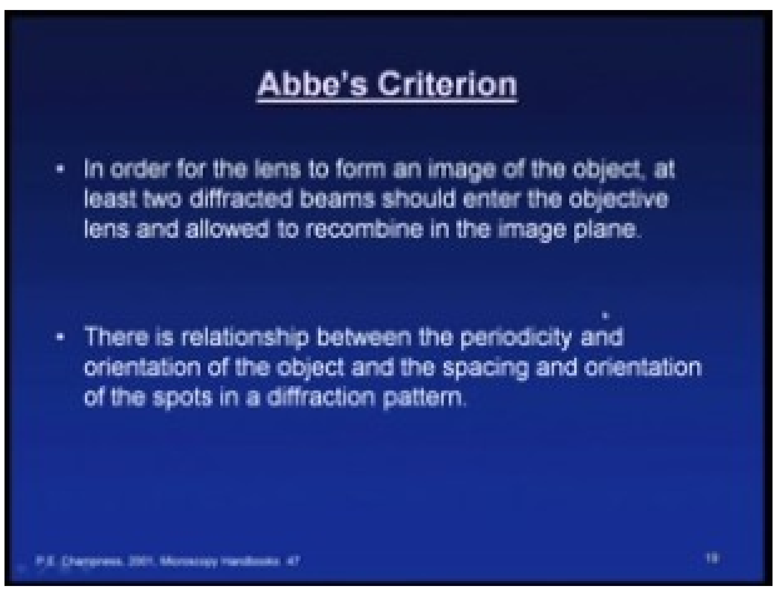

And then we will now look at the criterion now for the image formation so whatever just we have seen in the previous slide we will just see whether this obeys that Abbe's criterion in order for the lens to form an image of an object at least two diffracted beams should enter the objective lens and allowed to recombine in the image plane, so you can just go back and see at least two diffracted beams you can just count one two and then three here you have three so it was says at least two so definitely it obeys that criterion for the image formation.

And most importantly 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. So the combination of two diffracted beam in an imaging plane is in a criterion defined by Abbe and then how this diffraction pattern is going to be useful that is what we are going to see in the next and you look at this statement again carefully there is a relationship between the periodicity and the orientation of the object and the spacing and the orientation of the spots in the diffraction pattern.

This particular concept will be useful in the electron microscopy also we will see it when we deal with that and now we will see one example to prove this concept in a diffraction pattern.

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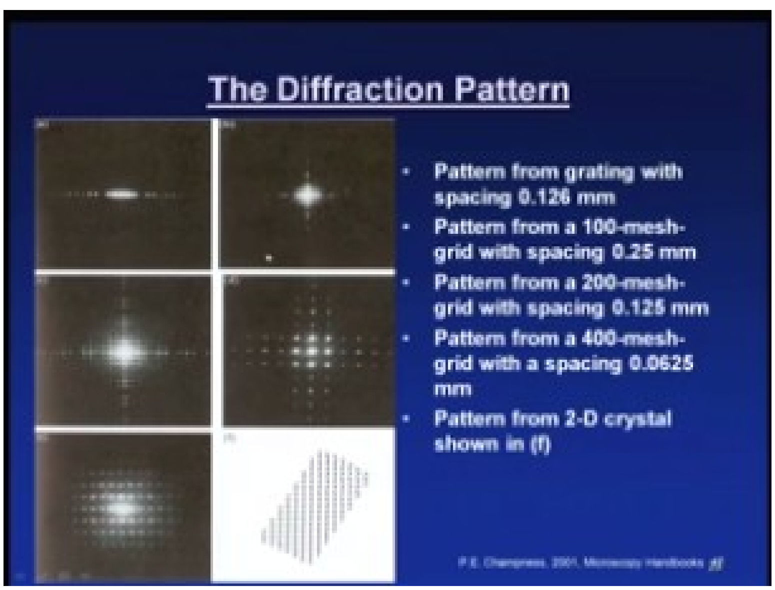

So what you see here is an electron diffraction pattern and this has been brought to just to prove the concept what we just mentioned before. Look at this pattern carefully A,B,C, D, E and f and these are all the pattern from the grading, grating with the spacing of 0.126 millimeter that is a and the pattern from 100 mesh grid with a spacing 0.25 mm b and pattern from a 200 mesh grid with a spacing 0.125 mm that is C and there is a pattern from 400 mesh grid with the spacing of 0.0625 mm that is patterned D. So what is that you are observing from this pattern if you carefully

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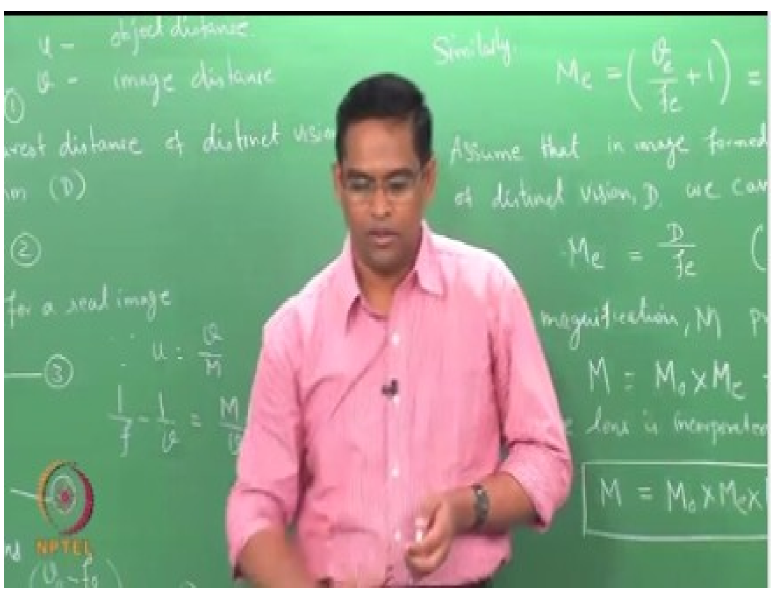

look at it you will be able to make out and you just have a look at this number spacing's 0.126 0.25 and 0.125 and then point 0.06 so what do we observe see as the spacing in the grid decreases and what you see here in the diffraction pattern; the spacing between the spot increases so this is what we have stated in previously we will go back and see.

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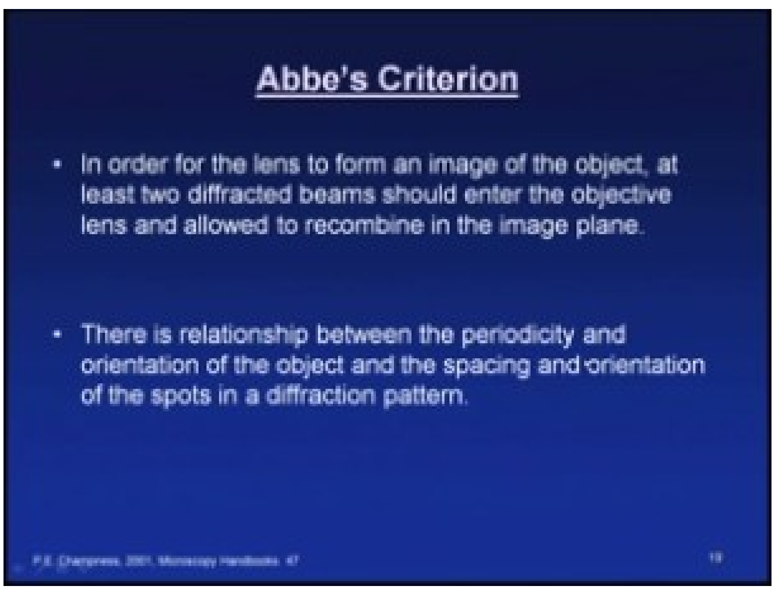

So there is a relationship between periodicity and the orientation of the object and the spacing and the orientation of the spots in a diffraction pattern. So we have just talked about the spacing it is not just the spacing alone but the orientation of the object as well how it is understood from this pattern so you look at this diffraction pattern why it should appear as a horizontal line? Why it appears like a cross so this is again related to the object orientation. So from the diffraction pattern you get the two information about the spacing between the objects or the orientation of the objects.

So the diffraction is pattern is a very powerful tool are powerful information, it gives about the object what we see. So we will look at this in much more detail later just want to introduce. This concept of diffraction how it occurs and what it causes and what information you get from this so at least that information you should know as an introduction.

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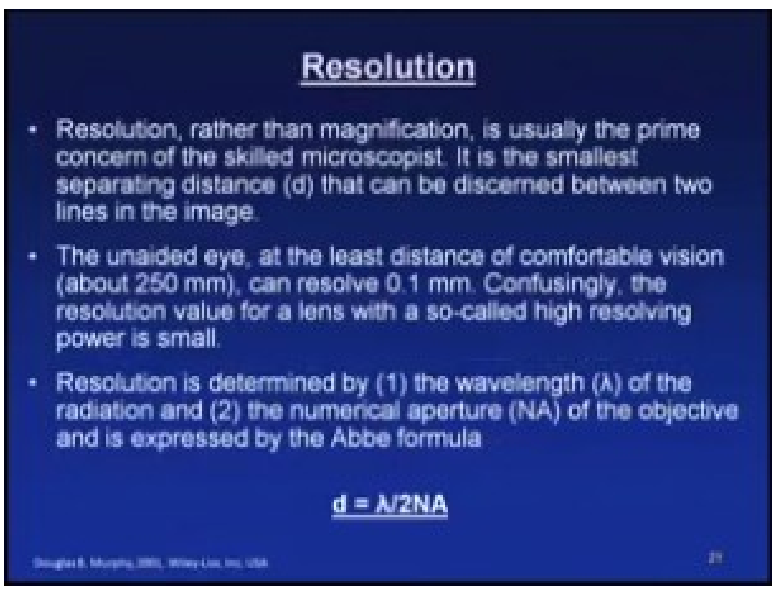

We will now move on to the topic the concept called: resolution. First let us look at the introduction remarks resolution rather than magnification is usually the prime concern of distilled microscopes: it is a smallest separating distance D that can be discerned between two lines in the image. So you should not confuse this resolution with the magnification is very different we have just seen some of this but we will also see about the magnification much more details, so do not confuse with the magnification with the resolution this is it is the smallest separating distance

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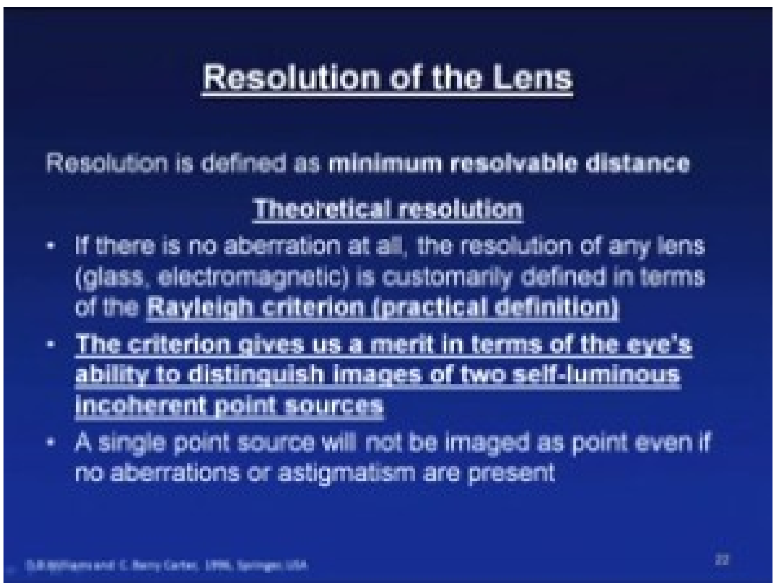

that can be discerned between two lines of the image for an unaided eye at the least distance of the comfortable vision which we talked about 250 mm which can resolve 0.1 mm. And then resolution is determined by the wavelength lambda λ of the radiation and the numerical aperture of the objective and it is expressed by the formula D = λ / (2 *numerical aperture). So let us look at what is the resolution. Resolution is defined as a minimum resolvable distance and we will first look at a theoretical resolution if there is no abrasion at all the resolution of any lens are either it could be a glass or electromagnetic is customarily defined in terms of Rayleigh criterion which is a practical definition the criterion gives us a merit

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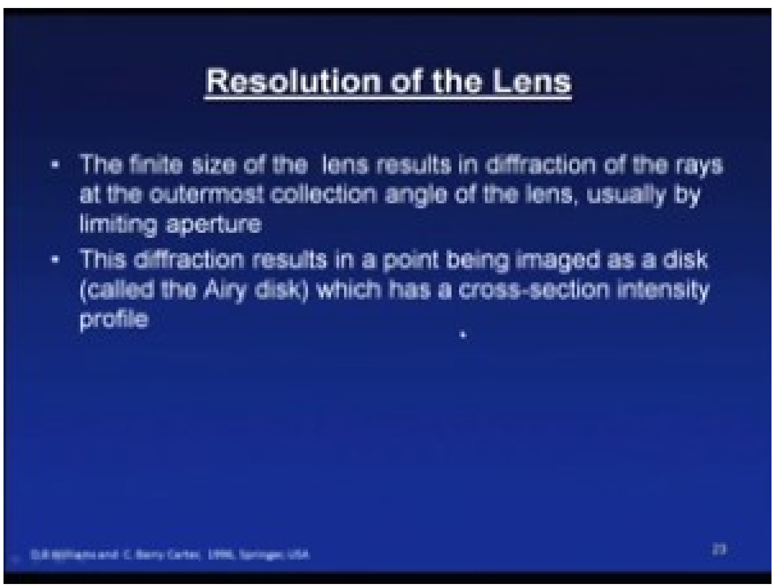

in terms of the eyes ability to distinguish images of two self-luminous in coherent point sources so I will read it again the criterion gives that is a Rayleigh criterion gives as a merit in terms of the eyes ability to distinguish images of two self-luminous incoherent point sources. A single point source will not be imaged as a point even if no abrasions or astigmatism are present.

So now we will see how we can understand this concept with the help of some I mean sorry animation and so on. So the finite size of the lens results in 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 disc which has the cross-section intensity profile we will see that

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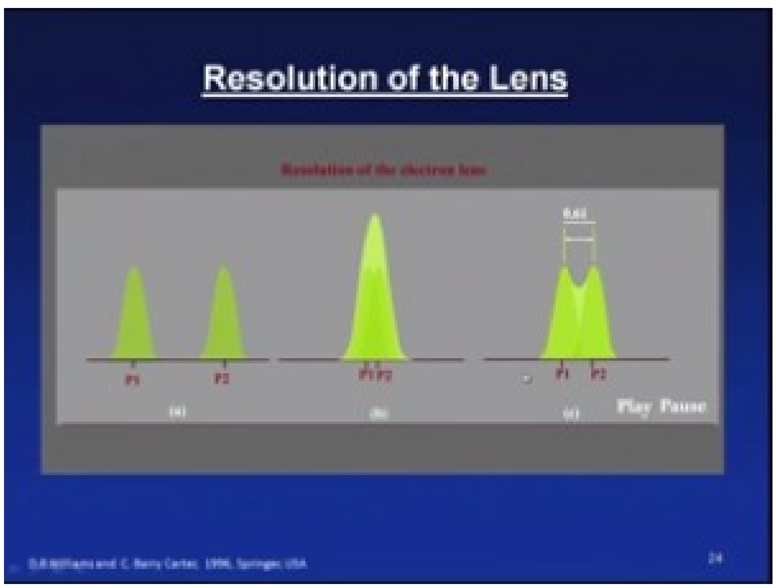

as an animation then the understanding will be better then we will discuss that look at this is a self-luminous point-source 1 and this is point source 2 P1 and P 2 when these two point sources are coming and merging with each other then it produces higher intensity point source and if you look at the animation (c) it just come closest and then stops at a point where our eye can discern this to self-luminous point as an individual sources which is a fixed number in this case you look at this: the Maxima of the primary source here is overlapping with the minima of the next source.

This is these two are in the same line. The Maxima of the primary source is matching with the are overlapping with the minima of the next source; and this two distance is 0.61 times of γ by β and this particular dip in the intensity occurs at approximately about 80% of the maximum intensity so this is the physical interpretation of the Rayleigh criterion so how you will be able to distinguish at two self luminous light sources which are which can come close together as I said that is the fixed number that is 0.165 sorry 0.61 * ʎ / β . ʎ is the wavelength β is the semi aperture angle so rarely stated that if the maximum

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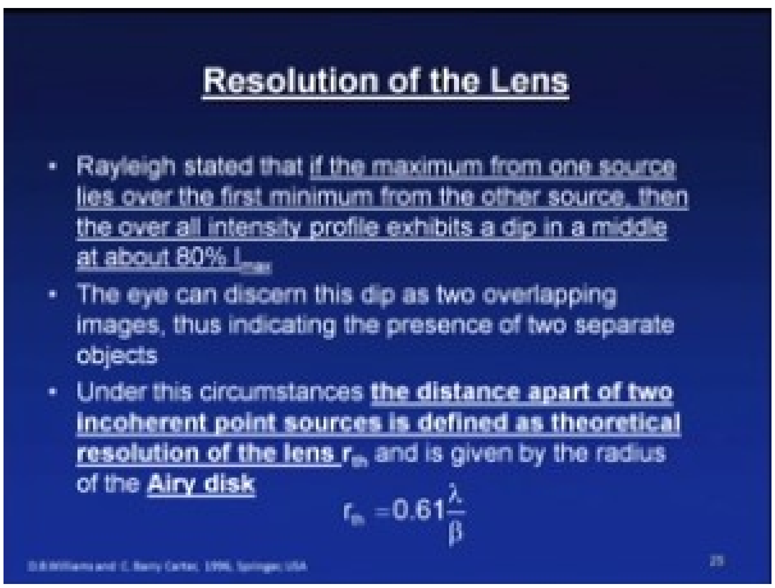

from one source lies over the first minimum from the other source then the overall intensity profile exhibits a dip in a middle at about eighty percent that is I_{MAX} this is what I just showed you the I can discern this dip has two overlapping images thus indicating the presence of two separate objects. Under these circumstances the distance apart of two incoherent point sources is defined as theoretical resolution of the lens that is r_{th} and it is given by the radius of the array disc: r_{th} = 0.61 *ʎ / β.

And this what is this array disc? If you look at this, is the array disc if you look at this is the array disc.

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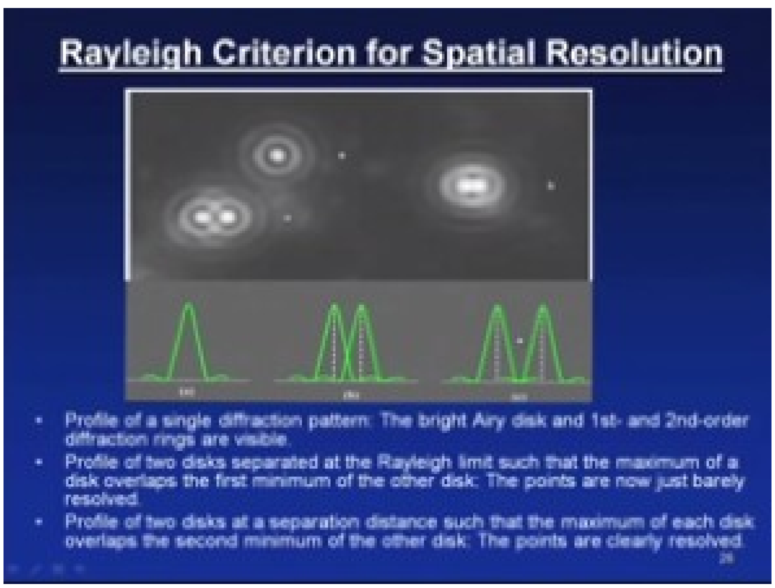

And if you look at the Rayleigh criterion for a spatial resolution most of microscopy we use a spatial resolution or what all we talk about is a spatial resolution. So if you look at this slide you have the a profile of a single diffraction pattern here, and the bright array disc and the first and second order diffraction things are visible. So you see that this is the zero order first order second order third order and so on so this is (a); and profile of the two discs separated at the rally limit such as that the maximum of the disc overlap with the first minimum.

So like here the points are now just barely resolved that is (b) you can see that points are barely resolved; and if look at the (c) the profile of the two disks at a separation distance such as the maximum of each disk overlaps the second minimum of the other so if you when you look at this the second minimum and the maximum overlaps then we are able to see this two points or these points are clearly resolved so this is the another illustration of the valid criterion for the spatial resolution.

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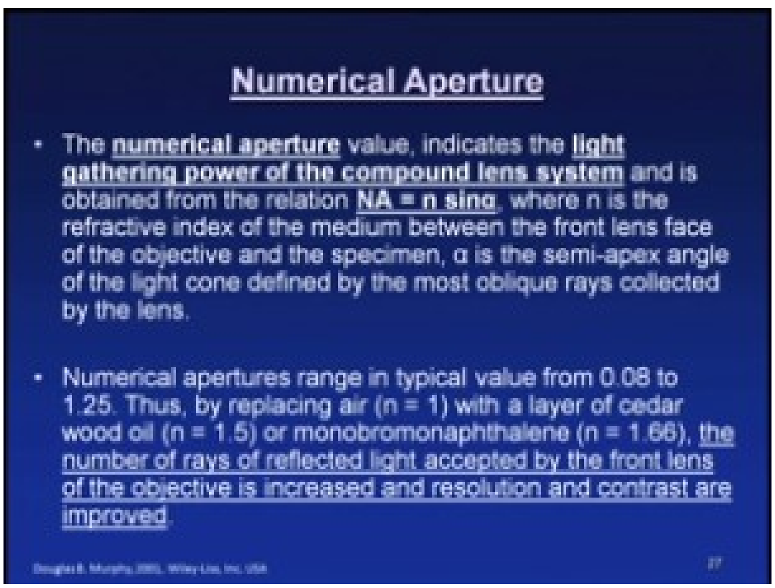

Now we will just look at what is numerical aperture because we use this term in the Reza I mean resolving power resolution definition. The numerical aperture value indicates the light-gathering power of the compound lens system and it is obtained from the relation numerical aperture equals n sin α where n is the refractive index of the medium between the front lens phase of the objective and the specimen alpha is a semi apex angle of the light cone defined by the most oblique rays collected by the lens.

So numerical aperture range in typical value from 0.08 to 1.25 we can just the replacing air which is a refractive index of 1 with the layer of a cedar wood oil where n is equal to 1.5 or mono broke bromo naphthalene which has got n is equal to 1.66 the number of rays of reflected light accepted by the front lens of the objective is increased and the resolution and the contrast are improved. So this is the typical definition of numerical aperture we will see how to derive the basic equation for this in the next class.

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