Fundamentals of optical and scanning electron microscopy
Dr. S. Sankaran
Department of Metallurgical and Materials Engineering
Indian Institute of Technology, Madras

Module – 01
Unit-1 Fundamentals of optics
Lecture - 01
Properties of light
Diffraction and interference
Theory of image formation
Magnification
Resolution

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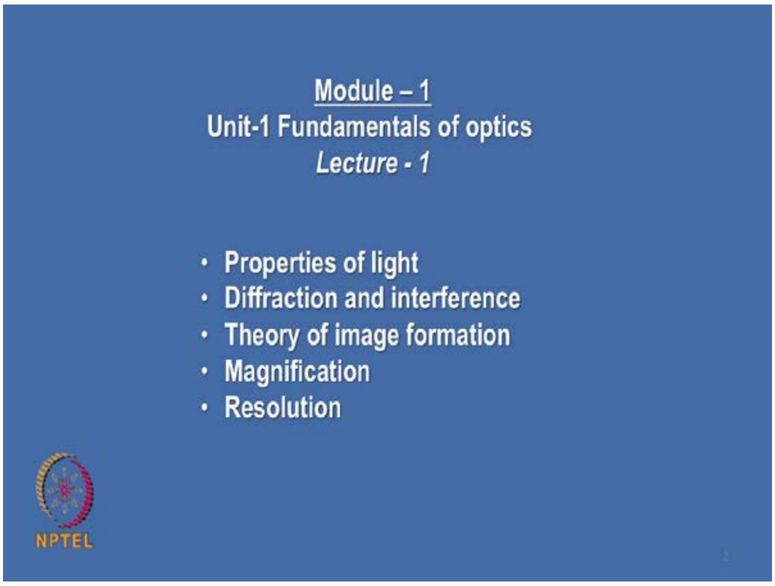

Hello everyone. Welcome to this Material Characterization course. In this course, we will see the optical and scanning electron microscopy and its various principles and techniques.

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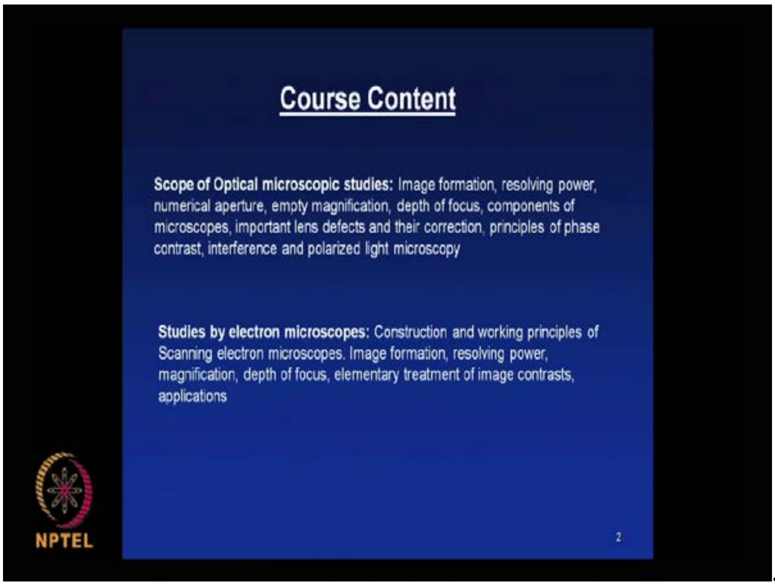

Let me introduce the syllabus of this course. We will be just dealing with this image formation, resolving power, numerical aperture, empty magnification, depth of focus, and component of microscopes, important lens defects and their corrections and then, we will also look at the variance of this optical microscopy, namely phase contrast interference and polarized light microscopy.

This particular syllabus I will be covering in 10 hours of lectures and then, I will introduce electron microscopes namely scanning electron microscopes and we will be covering construction and working principles and then, image formation, resolving power magnification and then, elementary treatment of image contrasts and then few applications.

So, before we really start this syllabus, we will first review most of the fundamentals which you might have already gone through or must have come across in your course or school days which will be useful.

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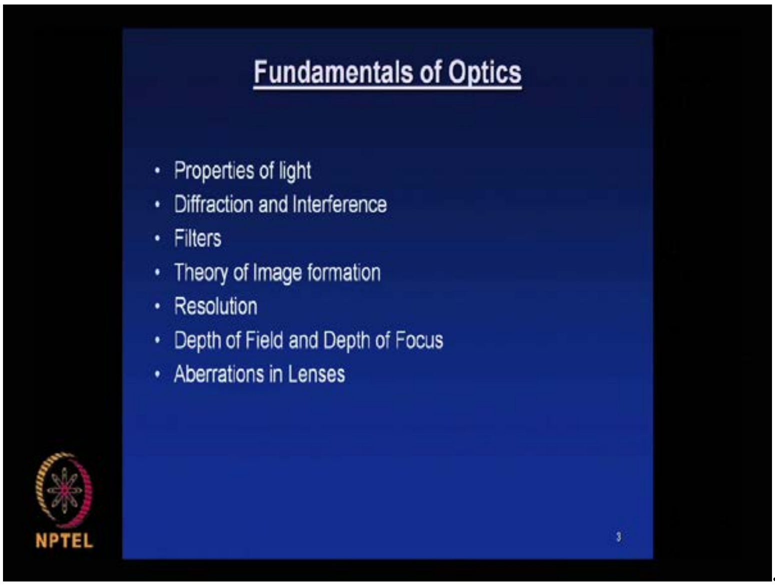

So, when we use these terms and you will be comfortable in dealing with most of the concepts which we deal with and these are the topics which I will be covering as far as the fundamentals of the optics is concerned. First we will review properties of light and then, we will discuss diffraction and interference in brief and then, we will also see the filters, various filters which are being used in the optical microscopy. Then, we will concentrate on theory of image formation, resolution and depth of field and depth of focus and most importantly the aberrations in lenses and how to overcome these aberrations in order to obtain a good image.

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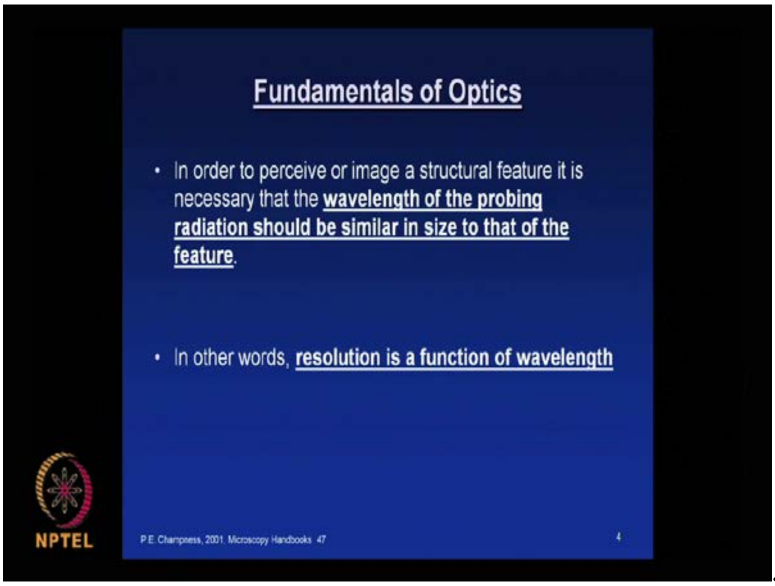

So, the first and fore most important thing which we have to understand in order to perceive or image a structural feature, it is necessary that the wavelength of the probing radiation should be similar in size to that of the feature. This is the fundamental requirement for any electromagnetic probing 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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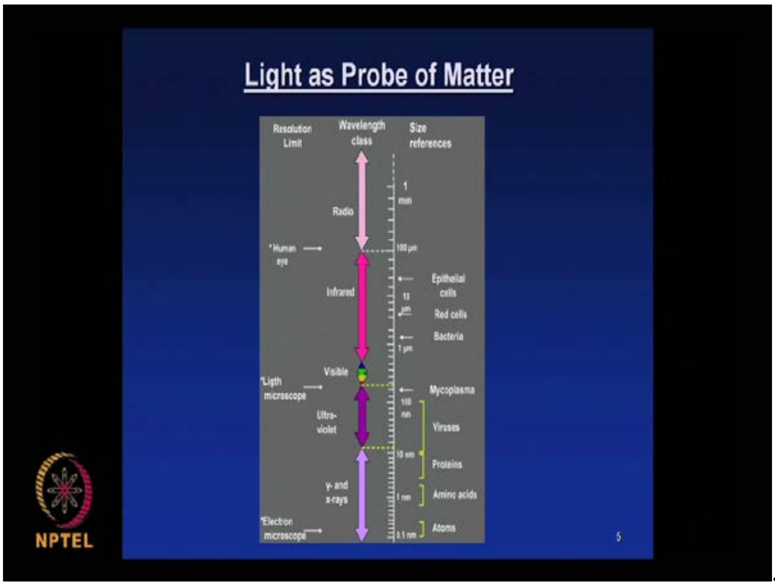

Let us look at this slide. When we choose a light as a probing of matter or light as a probe of matter, what all things we have to keep in mind? If you look at this, this is a complete electromagnetic radiation spectrum must give and what you see in the left hand side is the resolution limit and then, 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 a structural feature we are interested to resolve and then, we are supposed to choose the appropriate electromagnetic radiation.

For example, if you look at this, even the blue radiate, blue light which is far higher compared to some of the biological trade 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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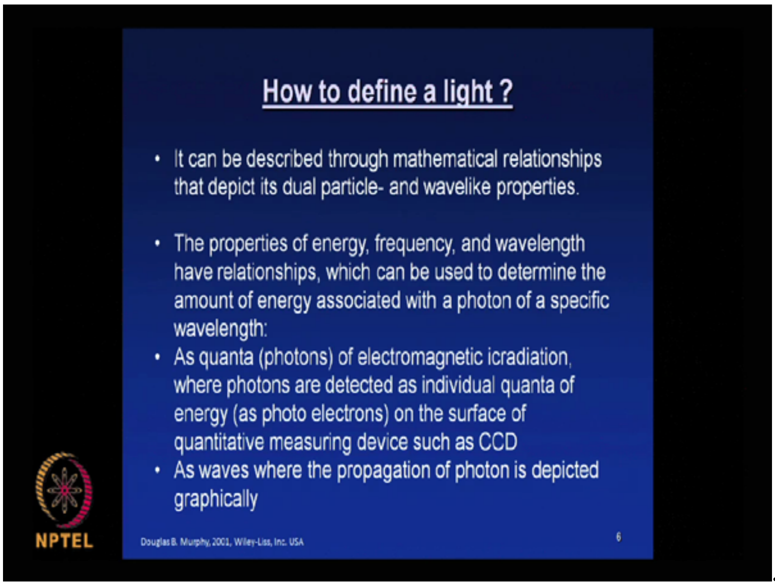

So, how to define a light? This is very always a very tricky situation how to define a light because we all know that the electromagnetic radiation has a dual nature, that is a light will behave as particle as well as wave, but it can be described through, a mathematical relationships that depicts its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

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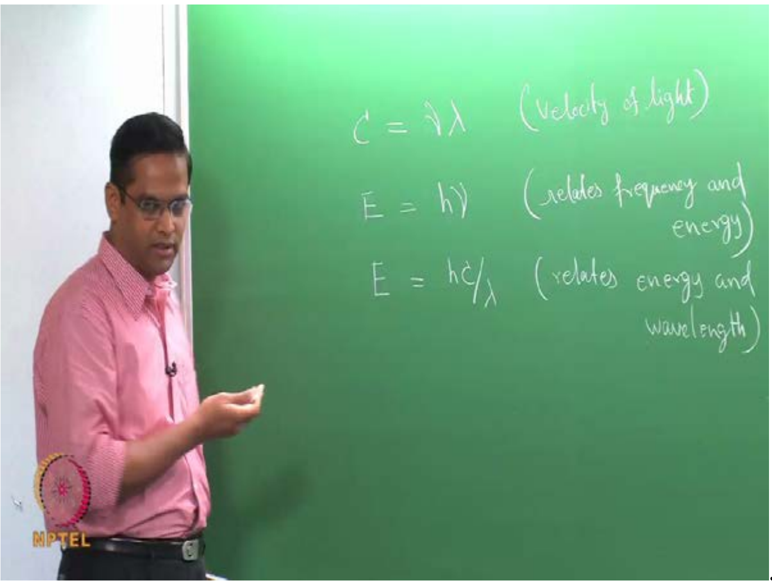

So, you have something called C equal nu lambda and this equation describes the velocity of light and then, you have another equation E is equal to H nu relates frequency and energy. This equation is important because, we are going to see there the light process through the glasses. It changes. It is velocity and it will be useful to choose the kind of radiation. We are going to look at this structural feature.

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 wave length and you can see that you know the wavelength electromagnetic radiations which will have the shorter wavelength will have a higher energy in this whole electromagnetic spectrum.

So, at least these three mathematical relationships explain the dual nature of the light and then, we should norm in optics. We always use the electron or a light as a wave and its propagation always represents in terms of a wave nature and then, where to use a particle, where to use a wave, we have to choose inappropriate situation. For example, when a light comes and interacts with a matter at that point, it is considered as a quanta that is photons even for when we realized that when we record your images in a quantitative measuring device like CCD camera where it is quantized, so that means at that situation it is called a quanta.

That is what it is written in the slide as quanta are photons of electromagnetic radiation, where the photons are detected as individual quanta of energy as photo electrons on the surface of the quantitative measuring device such as CCD. So, at this point of thing this is considered as quanta are a particle in nature where the interaction point that interface is considered quanta, but all the other properties like when you consider the light in a propagation mode or the photon propagates, then it is depicted like a wave as mentioned, as showed here.

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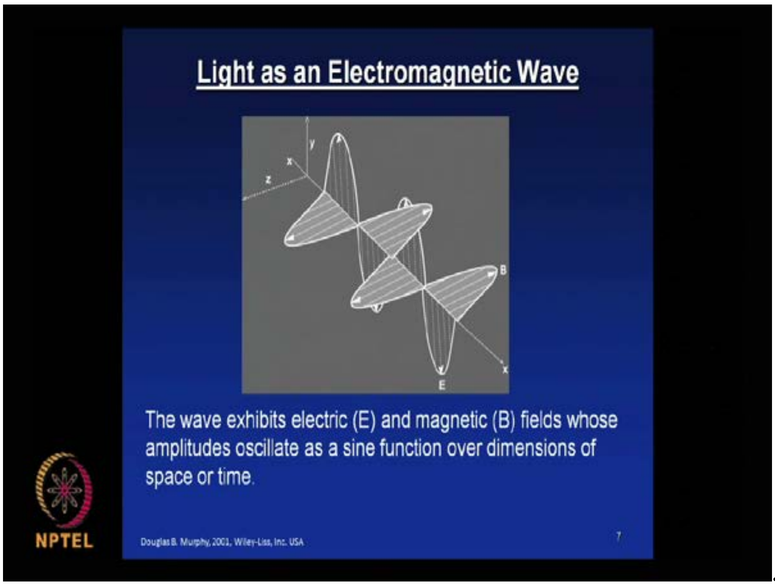

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So, what you see is a schematic of electromagnetic wave which exhibits an electric and magnetic fields whose amplitudes oscillate as a sine function over dimension of space and time. So, these two fields are mutually perpendicular to each other and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

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So, this is another important introduction for the light and we always talk about in characterization of materials, the quality of light and let us see the quality of light and its 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 coherent or non-coherent; it could be collimated or divergent. So, all these types of the different quality of light can be found in this manner, but for our clarification we have just made some animation.

A monochromatic light is opposed to be like the propagation is like this, and the other hand if you look at the polychromatic, it propagates in this fashion. What is linearly polarized? It is linear polarized and non-polarized will have all the directions. The waves will propagate in all directions and when we talk about the coherent light, it should have all the waves in the same phase.

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

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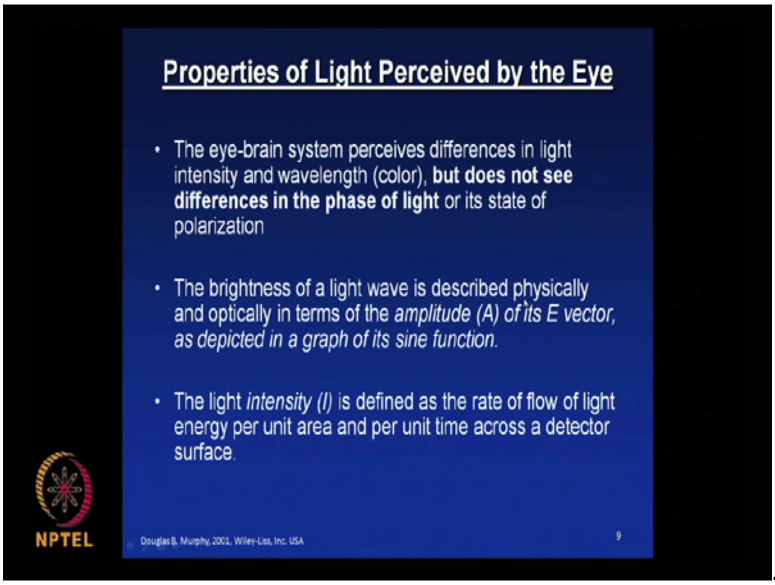

Then, if you start looking at the properties of light perceived by the eye, the basic understanding goes like this. The eye, brain system perceives differences in light intensity and wave, but does not see the differences in the phase of light or its state of polarization. The second point is the brightness 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 a sine function, the light intensity is defined as the rate of flow of light energy per unit area and per unit time across the detector surface.

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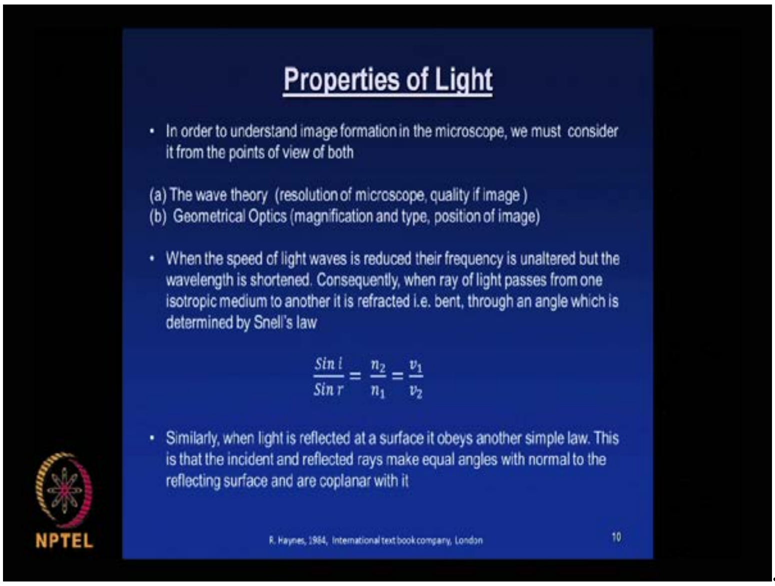

So, in order to understand the image formation in the microscope, we must consider it from the point of view of wave theory and then, geometrical optics. The wave theory relates the information of resolution of microscope and quality of image and so on and the geometrical optics relates the information regarding magnification and type and positions of the image.

So, if you look at this, the basic loss of the light or electromagnetic radiation, the first one always we consider as when the speed of the light waves reduce their frequency and it is unaltered, but the wavelength is shortened consequently when the ray of light passes from one isotropic medium to the another. It is refracted that is bent through the angle which is determined by the Snell's law. We all would have heard this fundamental law of light, where when the light propagates from one isotropic medium to the other, it bits or refracts. That is first law.

This is another law which is very important related to the reflection is when the light is reflected at the surface, it obeys another simple law. The incident and reflected rays make equal angles with normal to 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 laws of reflection when you consider when you say a light is reflected. So, this point, these two laws are very fundamental laws to define the properties of light.

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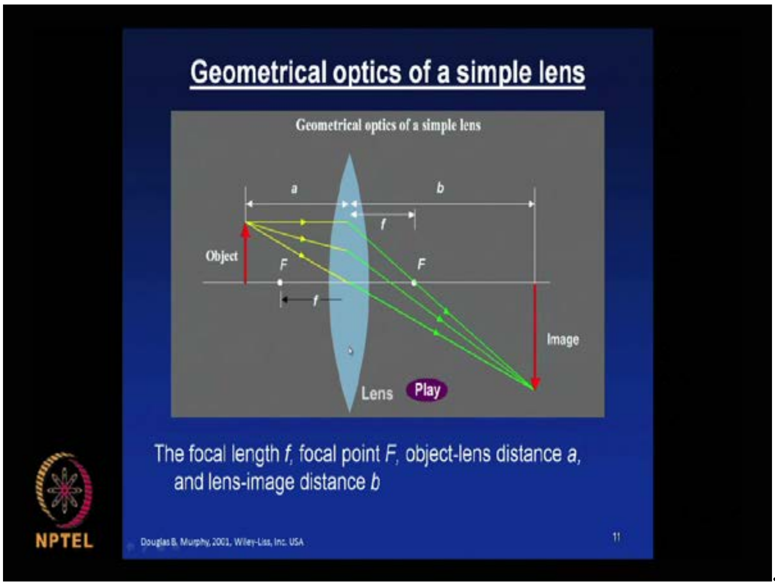

So, now let us look at the geometrical optics of a simple lens. You have the glass lens and this is in object which you have here red in color and then, the light is passing through the lens and then, it get focused on the other end where the image is formed and that the distance between the center of the lens and these two points front there are two points in front of the lens. One is in the front and one is at the back.

So, these two points denoted as F are called principle focal point and the distance between the object and the lens and the distance between the lens and image is b, and the light rays are traveling in a particular fashion, right. So, the light which is passing through the center of the lens is not deviated, but the rays which are passing through the periphery of the lens in this region are deviated, right. So, these are the properties which are governed by the few set of rules. Why it is?

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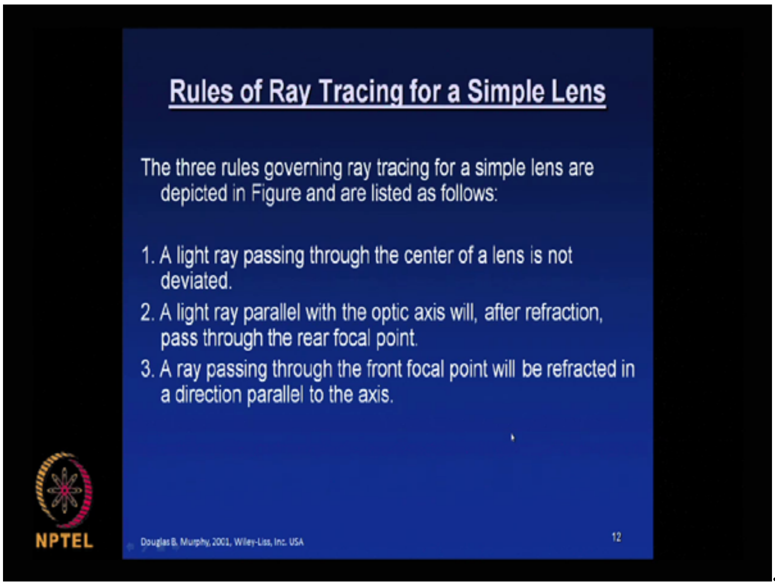

So, we have some set of rules called rules of ray tracing for a simple lens. These rules are an assumption on this Snell's law. Snell’s law is not strictly followed. There is some assumption like sine I by sine R is equal to mu. We say it is some assumption based on the sine I by sine R is equal to I by R. We will not get into the details, but I just want to mention that these rules are based on some assumptions of this Snell's law and let us see what these rules are. There are three primary rules governing the ray tracing for a simple lens and we will show in the figure also.

We will first see what the rules are. 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 and 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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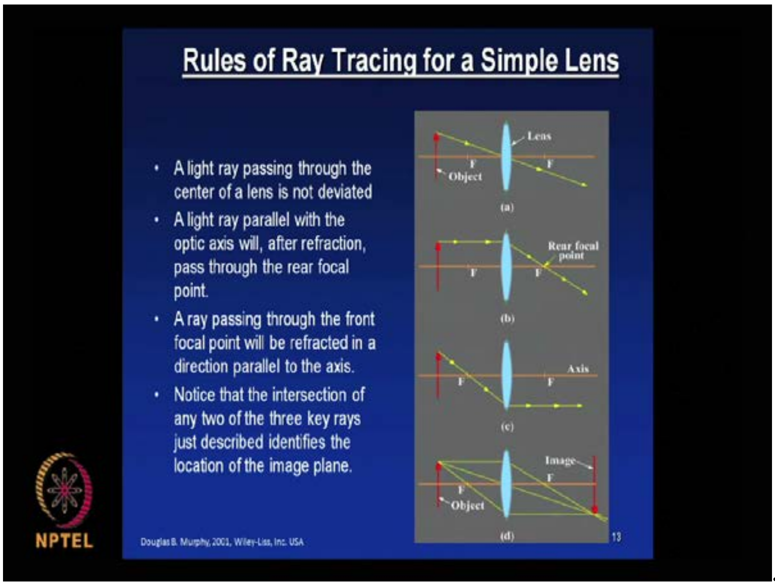

So, if you look at this schematic, the first image shows a light ray passing through the center of the lens is not deviated. That is the first rule. A light ray parallel with the optic axis will after refraction pass through the rear focal point. So, this is the real focal point. A ray passing through the front focal point will be refracted in a direction parallel to the axis. So, this is what it is parallel to this axis and notice that the intersection of any two of the three rays 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 in image here. We will now see what the conditions are to form an image.

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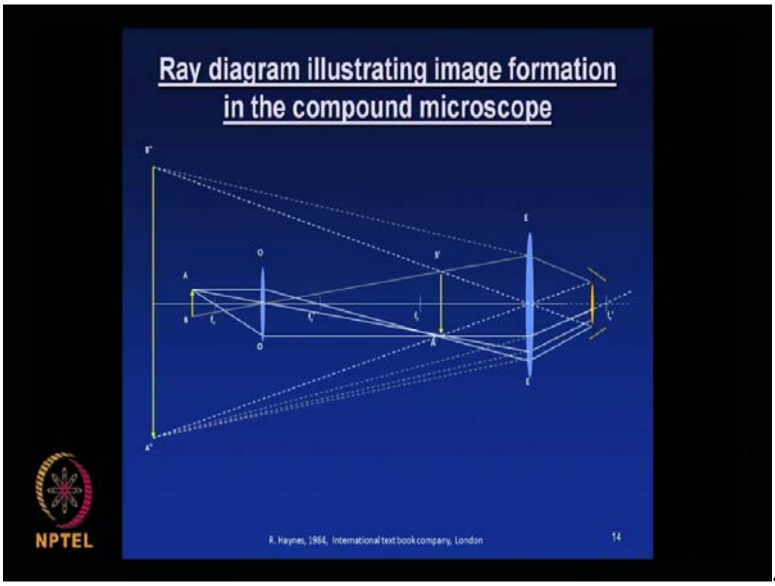

If you look at this another ray diagram which clearly shows the image formation. Let us look at the details of this image here is an object AB and then, you have this objective lens and then, you have the image formation A dash B dash. This is an inverted image and then, you have another lens here it is called eye piece basically. Why I am bringing this slide because most of the micro scopes they use, the compound lens you have two types of micro scopes. We have either a simple microscope or a compound lens microscope. In a simple microscope, you will have only one set of lens where you will have the reflection type and you have another microscope, you have an objective as well as eye piece. So, do just mention these two.

What is a compound microscope? To illustrate that concept, I brought this slide. So, you now look at this for an objective, the image forms here and for the eye piece this image is projected as A dash B dash in this case and this is which is perceiving the virtual image. Here A double dash and B double dash is a virtual image and you are A dash and B dash is the real image. Of course, the virtual image which is not inverted again whatever the objective produces here, it is just magnifying to this length. So, it also kind of explains the magnification as how the magnification takes place. We will see how we can understand this magnification.

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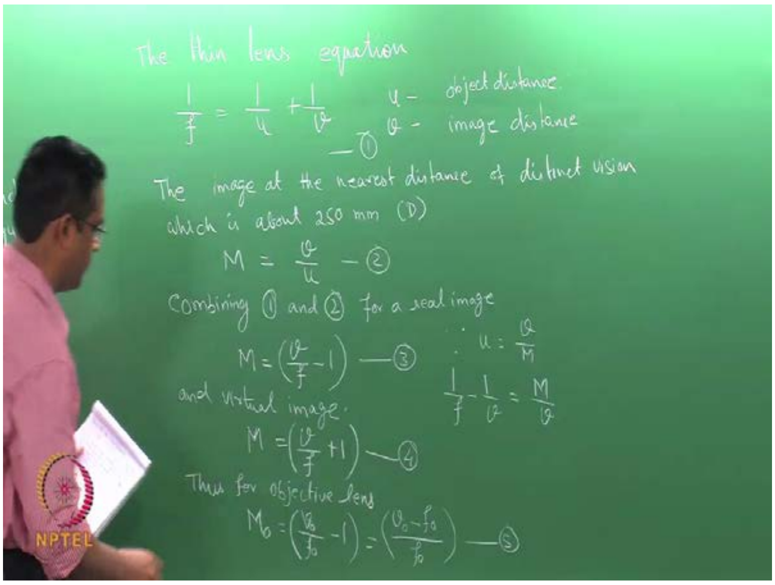

So, we will write the popular lens equation, that is 1 by f equal to 1 by u plus 1 by v. So, here instead of A and B, I have used u and v which is very common in notation in the optics. So, u object distance and v is image distance. So, we should also know that the image at nearest distance of a distinct vision which is about 250 mm or 25 centimeter, this is normally denoted as D, capital D. This is the minimum nearest distance with which we are able to see any of the objects with our normal vision which is 250 mm. So, normally we write magnification is v by u.

So, this is the magnification given by any lens and combining this, suppose if you combine these two that is let us call this as equation 1 and this is 2 for a real image which in this case, it is an objective for Re. So, you put u equal to v by m and then, substitute this in this equation 1 by f minus 1 by v is equal to m by v. Then, with this we can write m is equal v by f minus 1. This is for equation 3 and for virtual image m equal to v by f plus 1 the sine changes because if you look at the image for an objective, it is in one direction.

Now, we are talking about eye piece. So, this is in the opposite direction. So, that is why the sine changes here. So, this is for the virtual image which our eye sees. This is equation 4. So, now you can write for objective lens. So, this is for objective lens magnification. The sub script O describes objective lens for the magnification.

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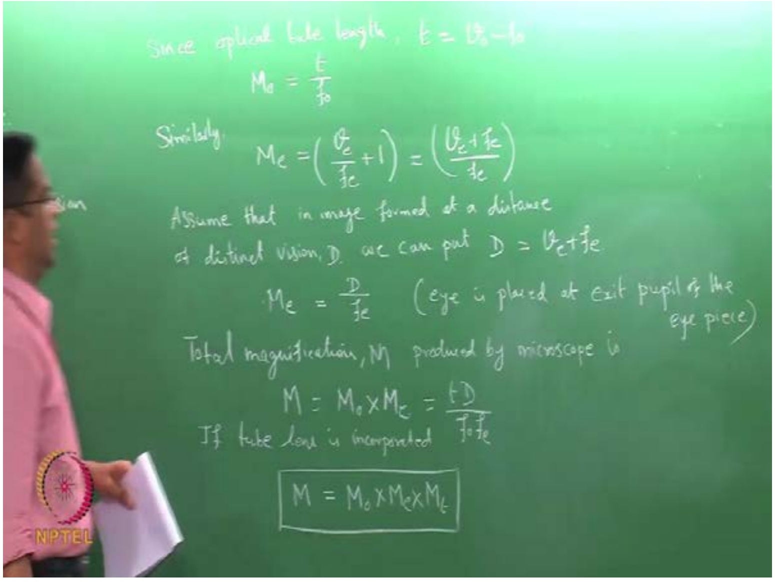

Similarly, we can write for eye piece, but before that and most of the microscope use these optical tubes, so that the length of the optical tube is also taken into consideration which is considered t. So, from the ray diagram we can show that this is t is equal to v minus f naught v minus f. So, we can write objective magnification like t minus f. Similarly, the magnification of the p can be written like this, and here we assume that the image formed at a distance of distinct vision that is D. We can put D is equal to ve plus f e and then, objective we use this is optical tube length in image. We use this as a distinct vision D which is 250 in nanometers is sorry 2250 mm that is 25 centimeters. So, if you can substitute this into this magnification of i p's, then it is D by f e.

So, since this is appropriate because your eye is placed in the microscope at the exit pupil of the eye piece. So, now we can write the total magnification for a compound lens microscope is that is ed by f. So, this is coming from these two equations and if you 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 idea of why we use or why 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 another concept called diffraction. So, if you look at this first introduction lines, diffraction is the spreading of light that occurs when the beam of light interacts with an object. So, it is a very general statement to define diffraction. We will slowly get to 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 which matters is generally called Scattering. So, your reflection, a refraction diffraction everything is a part of a scattering phenomenon and then, we have just looked at refract, Snell's law as well as when do you call reflection and similarly, diffraction also has a set of rules which we will define in in few minutes.

So, first you have some clear clarity on this. All these phenomena are part of scattering. So, you have Snell's law or to bending up the light is defined by Snell's law and then, you have a law for reflection and similarly, we can see something specific rule for diffraction also. So, before we get into this, let me also draw, try to draw some schematic.

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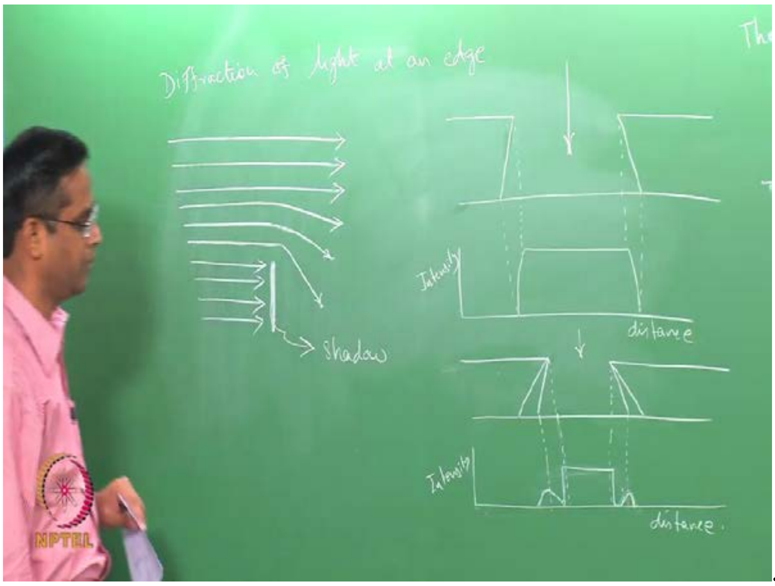

So, what I am trying to draw is when there light passes through an edge like this, it produces a shadow here. This is because of diffraction. This is one of the manifestations of diffraction. I am not trying to define diffraction here, but just the effect of diffraction. Then these things will help you to understand the concept much easily. So, this is when the beam of light comes and it exits through an edge like this and then, you have the shadow. Let me draw this. You write diffraction of light at an edge. This is shadow which causes the defuse light. I will also draw some more schematic which clearly shows this effect.

What is that I am trying to draw and tell you? So, where 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 slipped, then what happens and this is your intensity versus distance plot for this event and then, now you narrow down this slit, then it produces 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 dot and bright fringes will come.

So, this is the simple experiment 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 only, the image is formed. You tend to see the image and then what are the governing principles. So, you just see that just as diffraction describes the scattering of light by an object into divergent waves and there is something called interference which describes the recombination and summation of two or more superimposed waves. The process is diffraction, sorry the interference process responsible for creating a real intermediate image of an object in the focal plane of the microscope.

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 the 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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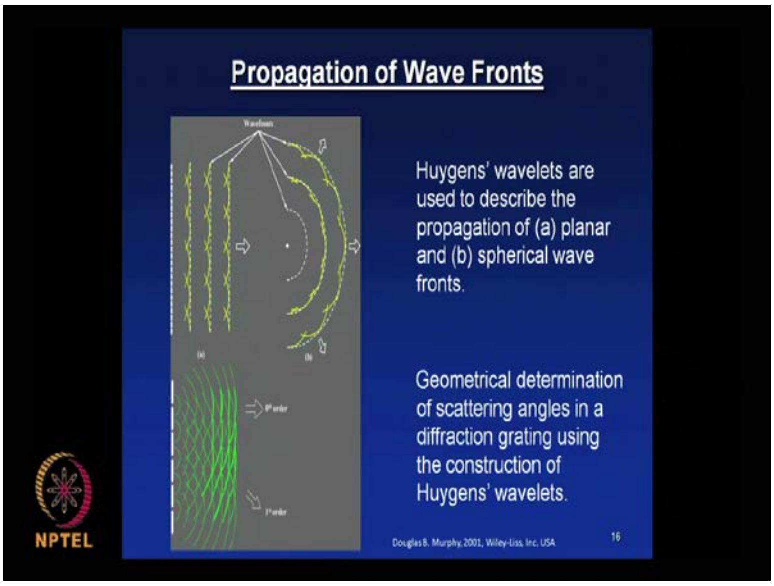

We will see much more detailed information about the diffraction as we go along. If you look at this, how the wave propagates there is a schematic you can see that this is described by Huygens wavelets are used to describe the propagation of a planar and spherical fronts. So, you can see that the schematic 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 optics.

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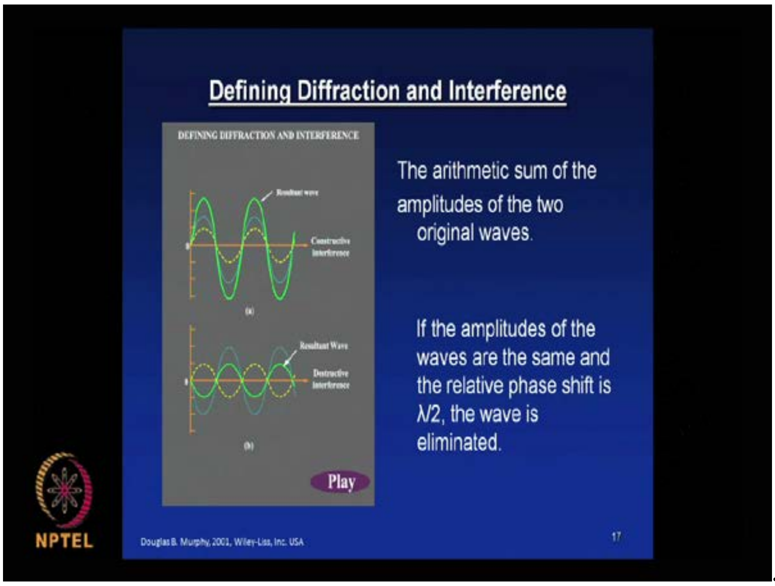

So, here is an important slide where we are going to define the diffraction and interference. So, first I would like you to look at this schematic where the event describes the constructive interference that is the arithmetic sum of amplitudes of the two original waves. So, you have the resultant wave depicted as a green line. So, this is called constructive interference and then, you have b which describes the destructive interference. You can see that the resultant wave has much lower amplitude compared to the constructive interference. If the amplitudes of the waves are the same and the relative phase shift is lambda by 2, as you can see that the wave is eliminated.

So, this is another important rule and 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.

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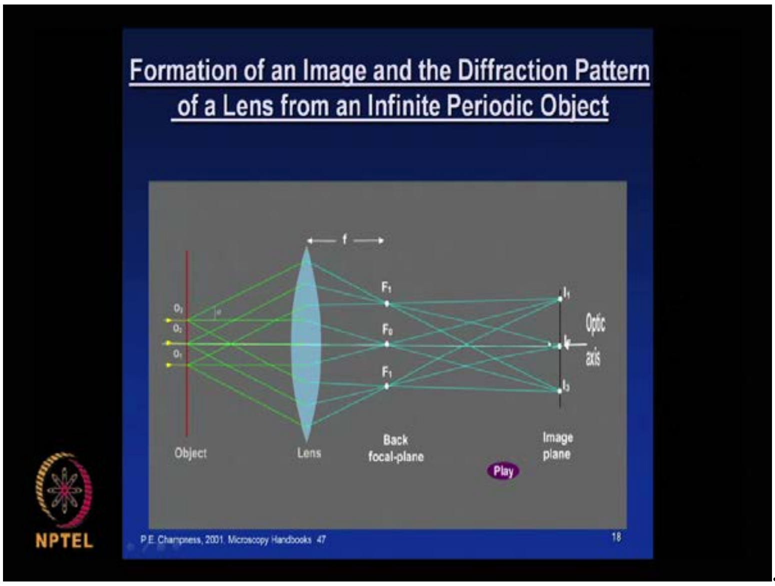

So, let us move on to next concept. Now, I will take you to the image formation involving diffraction. How the images perceived in a lens through a lens from an infinite periodic object. First, you just observe this schematic and then, we will discuss that. So, you have the object where you have it is a periodic object with Q1, Q2, Q3 with equal distance. You have light rays pass through this lens. Remember all these rays tracing this trajectories if you can look at the rules of the rays, they all follow the same thing that is the light which is passing through this center of the lens will go through non-deviated. The ray which is passing through the peripheral of the lens will deviate and then, it will go to this image plane.

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 showed the other focal point in this other side of the lens. So, this is the principle focal point. It is a back focal plane. This is plane is called back focal plane, f naught is a principle focal point and f 1 and this post be F1 dash, they are other points. These points are nothing, but the diffraction pattern. These 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 rays converge here from the 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 look at only 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 before the image formation, your diffraction takes place and each diffracted spot carries some information about the image.

So, this is very important and then, if you look at this image plane also, you see at least two diffracted beams recombined 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 stop here, then again I will start. So, it all forms first diffraction pattern or they are all convergence. The black focal plane at a particular point, this particular plane is called the back focal plane and all the patterns, diffract patterns are 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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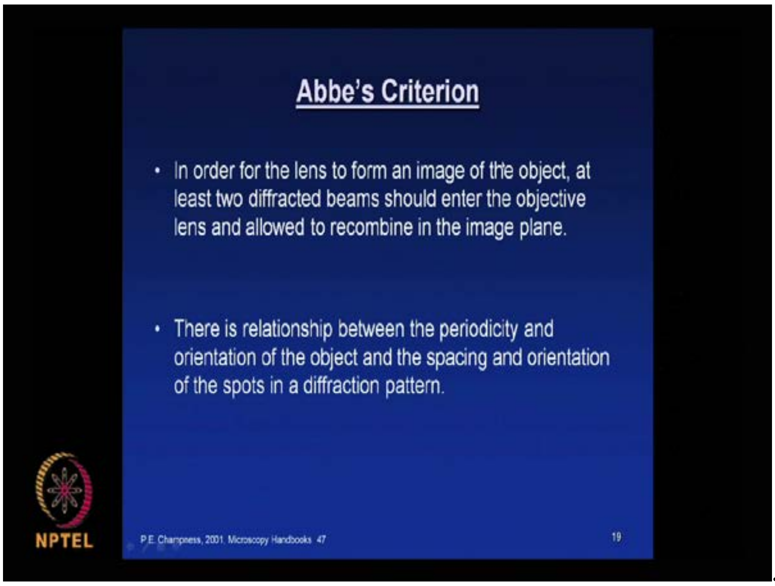

Then, we will now look at the criterion for the image formation. So, whatever just we have seen in the previous slide, we will just see whether this obeys that Abbe's criterion. In order for the lens to form an image of an object, at least two diffracted beams should enter the objective lens and allowed to recombine in the image plane. So, you can just go back and see at least two diffracted beams. You can just count 1, 2 and then, 3. Here you have three. So, the law 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 image in plane is criterion defined by Abbey 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 these parts 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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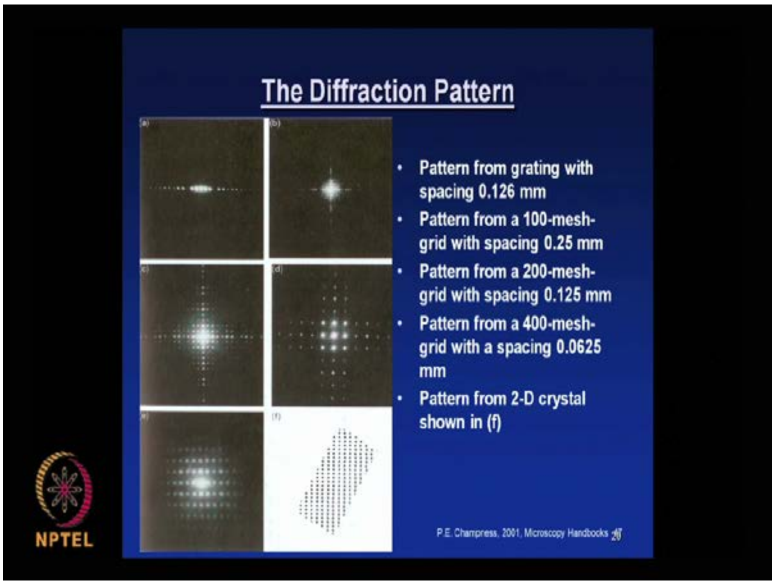

So, what you see here is an electron diffraction pattern and this is been brought just to prove the concept what we just mentioned before. Look at this pattern carefully a b c d e and f and these are all the patterns from gratings with the spacing of point 126 millimeter. That is a and the pattern from 100 mesh grid with the spacing 0.25 mm b and pattern from 200 mesh grid with the spacing 0.125 mm. That is c and this pattern from 400 mesh grid with a spacing of 0.0625 mm. That is pattern d.

So, what is that you are observing from this pattern if you carefully look at it, you will be able to make out and you just have a look at these numbers spacing 0.126, 0.25 and 0.125 and then, 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 these spot increases. So, this is what we have stated in previously. We will go back and see. 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. See 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 patterns, you get two information that is, about the spacing between the objects and the orientation of the objects. So, the diffraction is pattern is a very powerful tool or powerful information it gives about the object what we see. So, we will look at this in much more detail later. I just want to introduce this concept of diffraction as 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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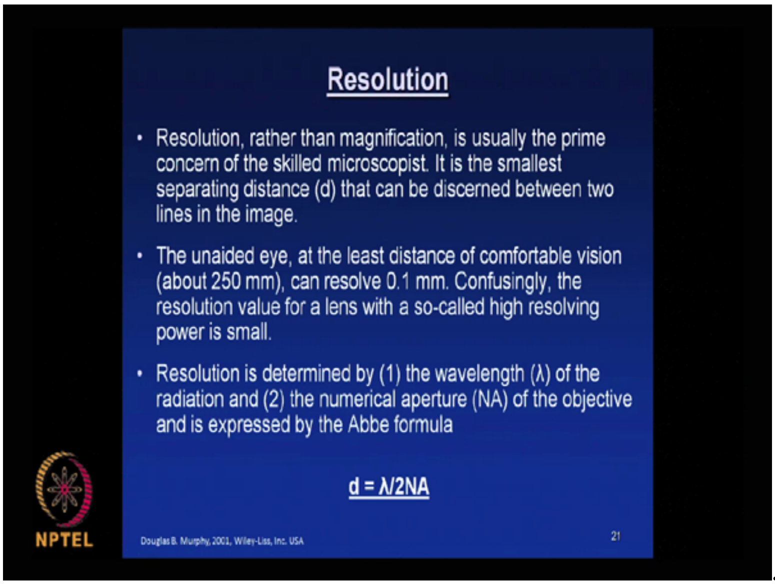

We will now move on to the topic, the concept called Resolution. First let us look at the introduction remarks.

Resolution, rather than magnification, is usually the prime concern of the skilled microscopist. It is the smallest separating distance (d) that can be discerned between two lines in the images. So, you should not confuse this resolution with the magnification. Magnification is very different. We have just seen some of this, but we will also see about the magnification in much more detail. So, do not confuse with the magnification with resolution. It is the smallest separating distance that can be discerned between two lines of the images 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 of the radiation and the numerical aperture of the objective and it is expressed by the formula d equals lambda divided by two times numerical aperture.

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Let us look at what is the resolution. Resolution is defined as minimum resolvable distance and we will first look at the theoretical resolution. If there is no aberration at all, the resolutions 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 in terms of the eyes ability to distinguish images of two self-luminous incoherent 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 point even if no aberrations or astigmatisms are present.

So, now we will see how we can understand this concept with help of some magnify, I mean sorry animation and so on.

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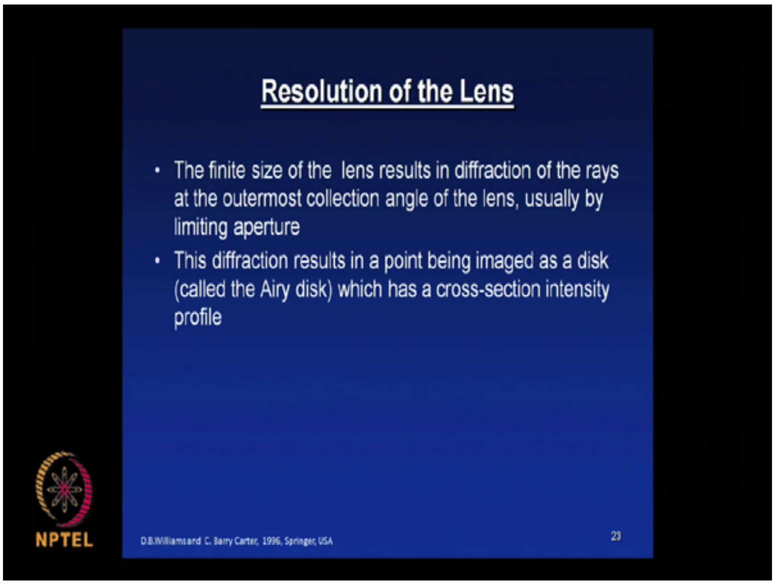

So, the finite size of the lens results in diffraction of the rays at the outermost collection angle of the lens, usually by the limiting aperture. This diffraction results in a point being imaged as a disk which has the cross-section intensity profile.

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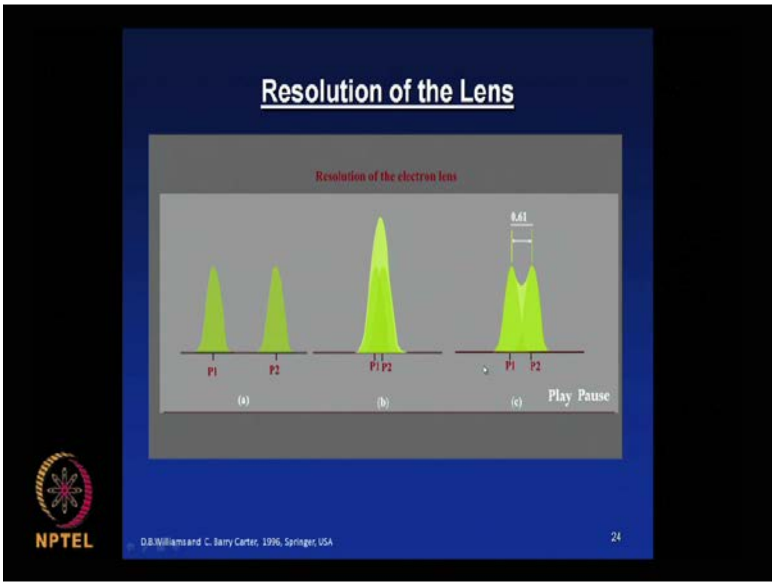

We will see that as an animation, then the understanding will be better and then, we will discuss that look at this. This is a self-luminous point source 1 and this is point source 2, p 1 and p 2. When these two point sources are coming and merging with each other, then it produces higher intensity point source and if you look at the animation c, it just come close and then stops at a point where our icon discerned these two self-luminous points as an individual source which is a fixed number.

In this case you look at this, the maxima of the primary source here overlapping with the minima of the next source. These are two in the same line. The maxima of the primary source is matching with or overlapping with the minima of the next source, and these two distances is 0.61 times of lambda by beta and this particular dip in the intensity occurs at approximately about 80 percent of the maximum intensity. So, this is the physical interpretation of the Rayleigh criterion. So, how you will be able to distinguish two self-luminous light sources which can come close together. As I said that is a fixed number that is 0.165, sorry 0.61 times lambda by beta. The lambda is a wavelength beta is the semi-aperture angle.

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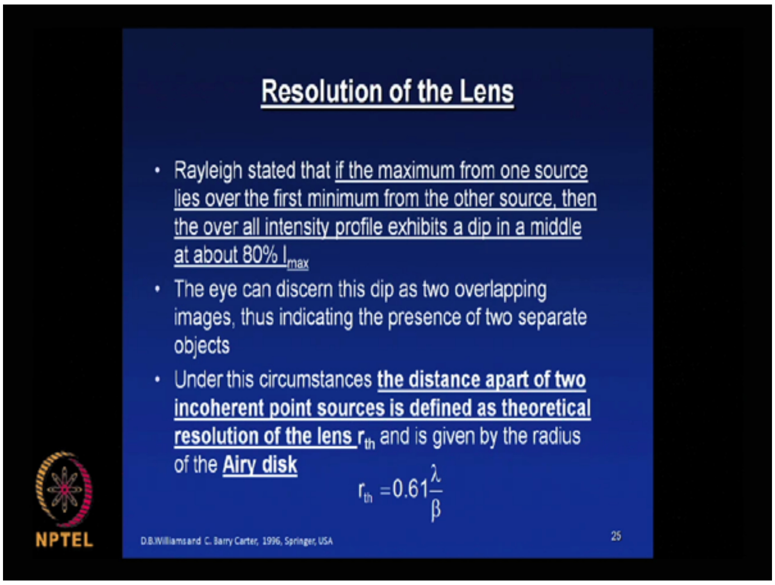

So, Rayleigh stated that if the maximum from one source lies over the first minimum from the other source, then the overall intensity profile exhibits a dip in middle at about

80 percent. That is I max. This is what I just showed you. The eye can discern this dip as two overlapping images, thus indicating the presence of two separate objects. Under this 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 airy disk r th equals 0.61 times lambda by beta. This is what airy disk is. If you look at this, this is the airy disk.

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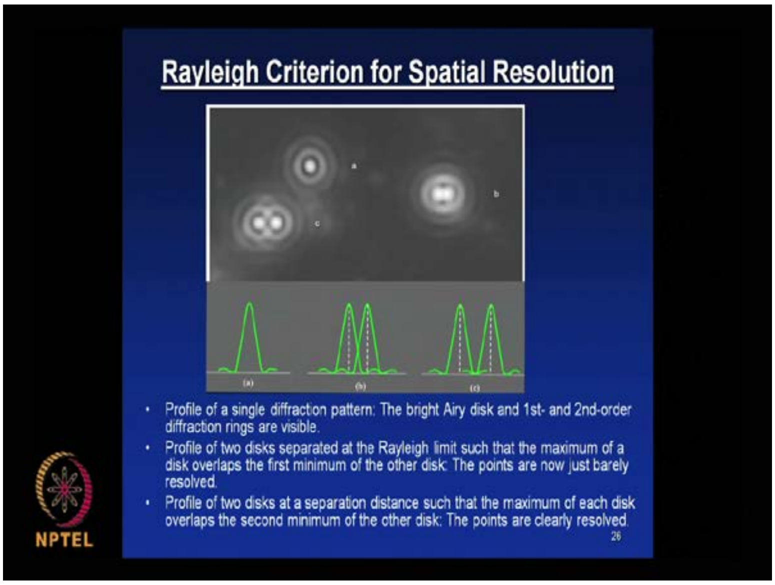

If you look at Rayleigh criterion for a spatial resolution, most of a microscope we use a spatial resolution, what all we talk about is a spatial resolution. So, if you look at this slide, you have a profile of a single diffraction pattern here and the bright airy disk and the first and second order diffraction rings are visible. So, you see that this is a zero order, first order, second order and third order and so on. So, this is a, and profile of the two disks separated at Rayleigh limit, such as that the maximum of the disk 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 you look at 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, when you look at this, the second minimum and the maximum overlaps, then we are able to see these two points. These points are clearly resolved. So, this is another illustration of Rayleigh criterion for the spatial resolution.

Now, we will just look at what numerical aperture is because we use this term in the reso, I mean resolving power or resolution definition.

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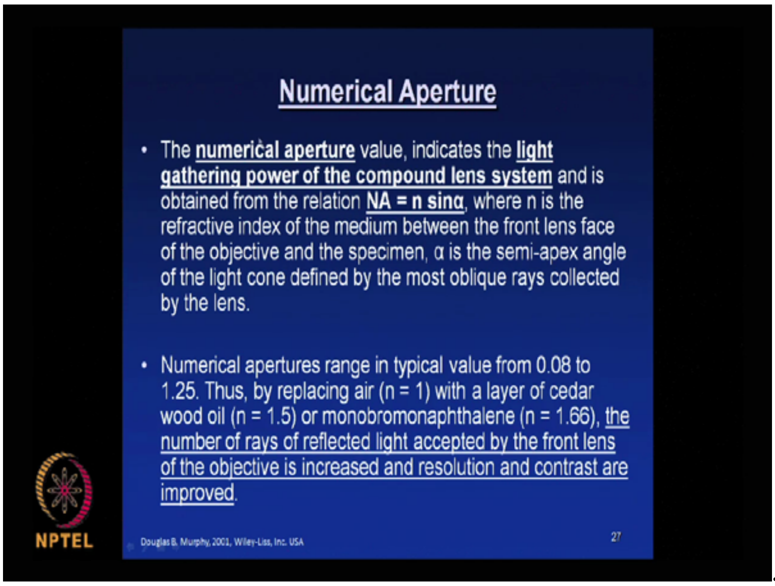

The numerical aperture value indicates the light gathering power of the compound lens system and it is obtained from the relation numerical aperture equals n sine alpha, where n is the refractive index of the medium between the front lens face of the objective and the specimen alpha is the semi-apex angle of the light cone defined by the most oblique rays collected by the lens.

So, numerical apertures range in typical value from 0.08 to 1.25. We can just thus replacing air which is a refracting index of one with the layer of a cedar wood oil, where n is equal to 1.5 or monobromonaphthalene 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 resolution and the contrast are improved. So, this is the typical definition of a numerical aperture. We will see how to derive the basic equation for this in the next class.