

**Indian Institute of technology Madras  
Presents**

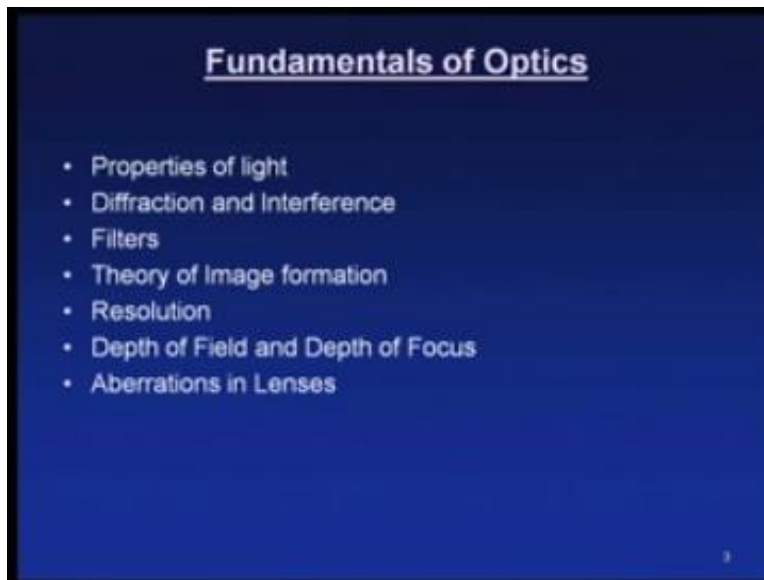
**NPTEL  
NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING**

**Lecture - 1  
Materials Characterization  
Fundamentals of Optical microscopy**

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

Hello 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.

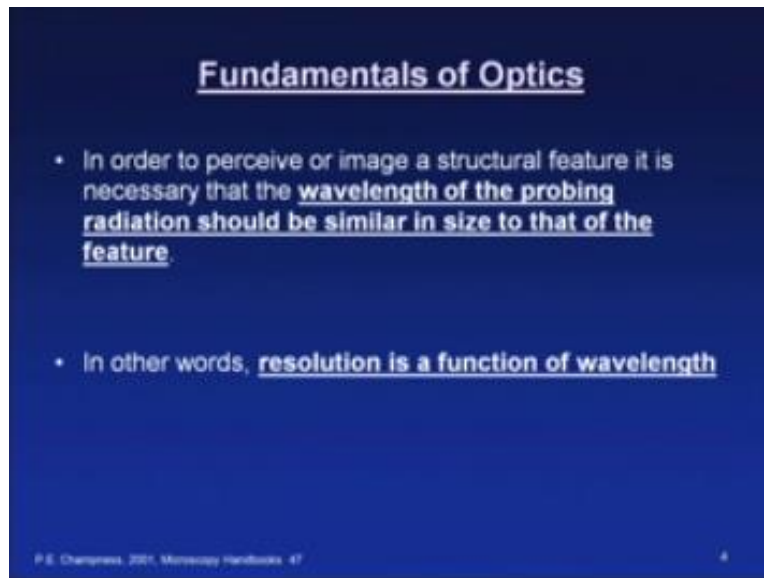
(Refer Slide Time: 00:32)



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

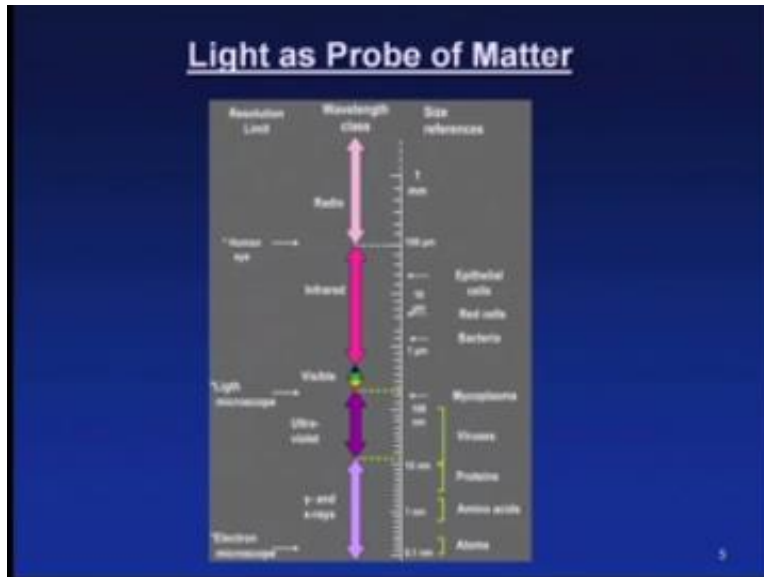
optical microscopy and then we will concentrate on theory of image formation resolution and depth of field and depth of focus and most importantly the aberrations in the lenses and how to overcome these aberrations in order to obtain a good image.

(Refer Slide Time: 01:08)



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.

(Refer Slide Time: 02:15)



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.

(Refer Slide Time: 03: 51)

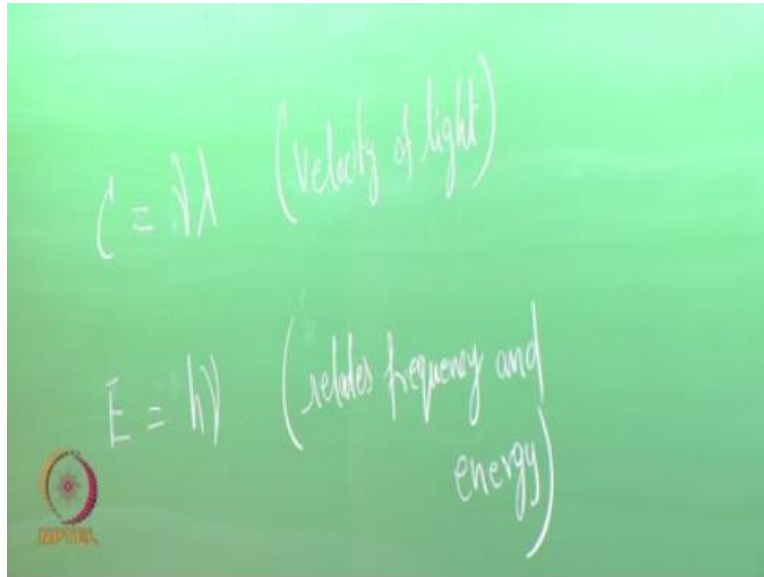
How to define a light ?

- It can be described through mathematical relationships that depict its dual particle- and wavelike properties.
- The properties of energy, frequency, and wavelength have relationships, which can be used to determine the amount of energy associated with a photon of a specific wavelength:
- As quanta (photons) of electromagnetic irradiation, where photons are detected as individual quanta of energy (as photo electrons) on the surface of quantitative measuring device such as CCD
- As waves where the propagation of photon is depicted graphically

Copyright © Murdoch, 2011, Wiley Ltd, Inc. USA

So how to define 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 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 that is the properties of energy frequency and wavelength have the relationship what are those relationships.

(Refer Slide Time: 04:43)



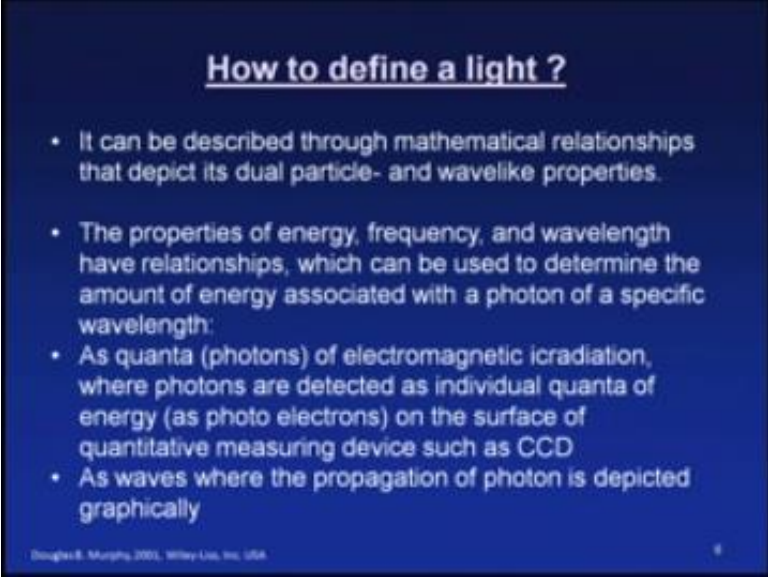
So you have something called  $C = v \lambda$  and this is this is describes this equation describes the velocity of light and then you have the another equation  $e$  is called  $h \nu$  relates frequency and energy this equation is important because we are going to see that when the light process through 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 inappropriate situation.

For example when the light comes and interacts within a 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.

(Refer Slide Time: 07:57)



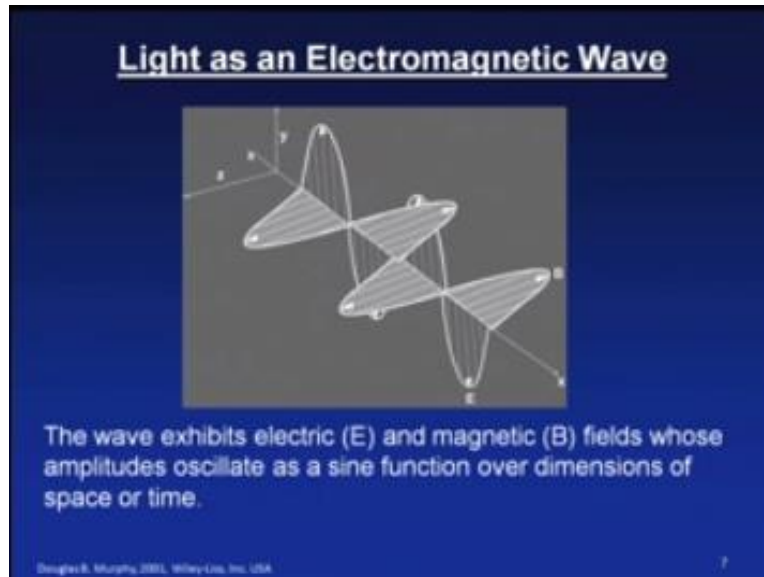
**How to define a light ?**

- It can be described through mathematical relationships that depict its dual particle- and wavelike properties.
- The properties of energy, frequency, and wavelength have relationships, which can be used to determine the amount of energy associated with a photon of a specific wavelength:
- As quanta (photons) of electromagnetic irradiation, where photons are detected as individual quanta of energy (as photo electrons) on the surface of quantitative measuring device such as CCD
- As waves where the propagation of photon is depicted graphically

Copyright ©, Murphy, 2001, Wiley-Liss, Inc. USA

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.

(Refer Slide Time: 08:46)



As mentioned as shown here 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 is 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.

(Refer Slide Time: 09:36)



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.

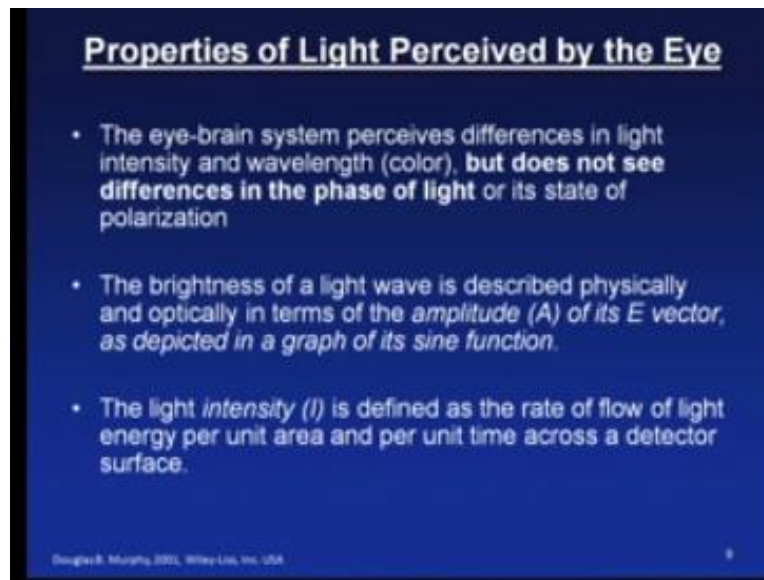
And what is linearly polarized it is linear polarized and non-polarized will have at all the directions the waves will propagate in all directions and when we talk about a coherent light we should have all the waves in the same phase and non coherent light will have a different phase like this a collimated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

(Refer Slide Time: 11:57)



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 are 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.

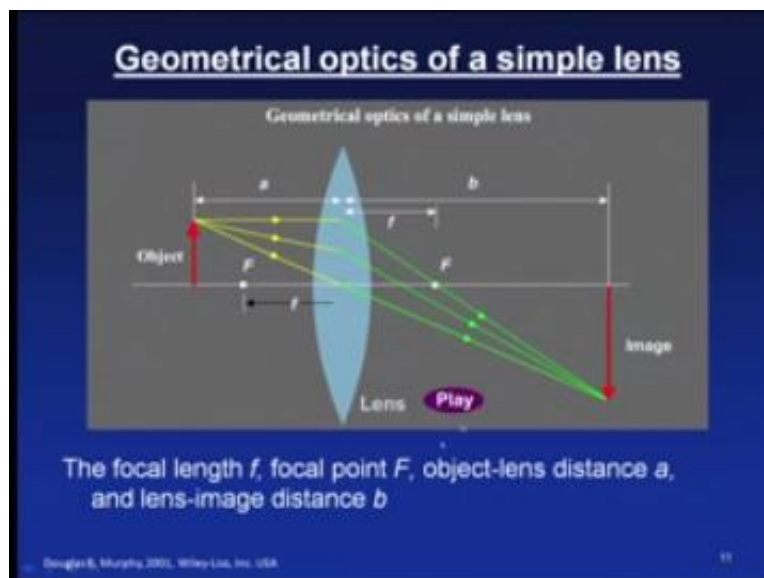
So in order to understand the image formation in the microscope we must consider it from the point of view of wave theory and then geometrical optics the wave theory relates the information of resolution of microscope and quality of image and so on and the geometrical optics relates information regarding magnification and type and positions of the image. So if you look at this the basic loss 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 gets or refract 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.

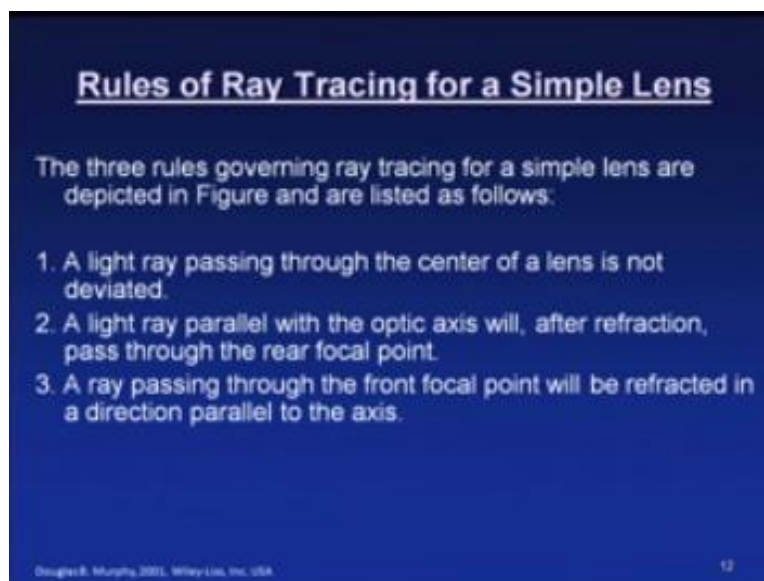
(Refer Slide Time: 15:10)



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 you 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 our 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 undeviated 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.

(Refer Slide Time: 17:10)

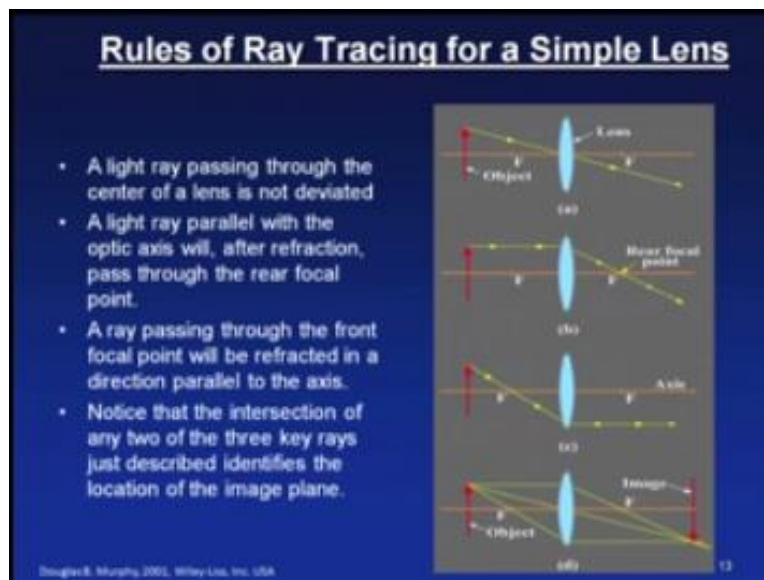


Why it is so we have some set of rules called rules of ray-tracing for a simple lens these rules are an assumption on the Snell's law okay this lens law is not strictly followed there is some assumption like  $\sin I / \sin R = \mu$  we say it is some assumption based on then they as  $\sin I / \sin R = I / R$  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 real focal point.

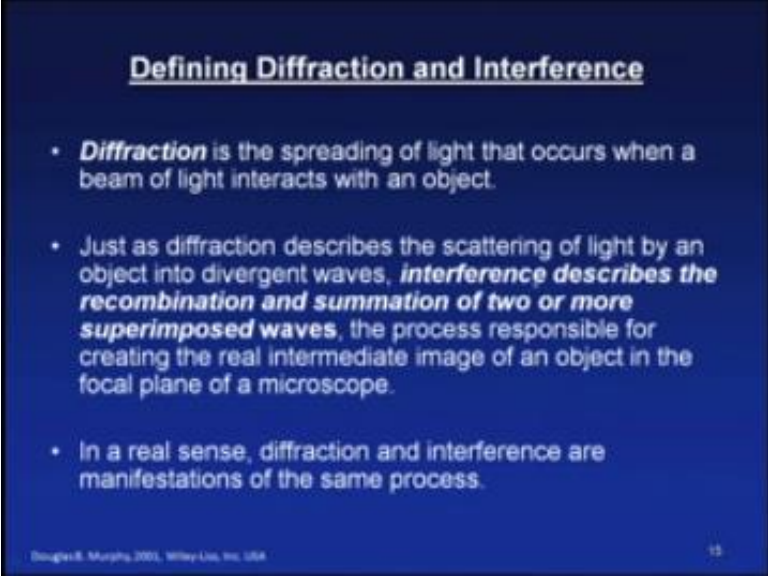
A ray passing through the front focal point will be refracted in a direction parallel to the axis so let us see whether these three rules are indeed the case.

(Refer Slide Time: 18:23)



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 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 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.

(Refer Slide Time: 19:39)



**Defining Diffraction and Interference**

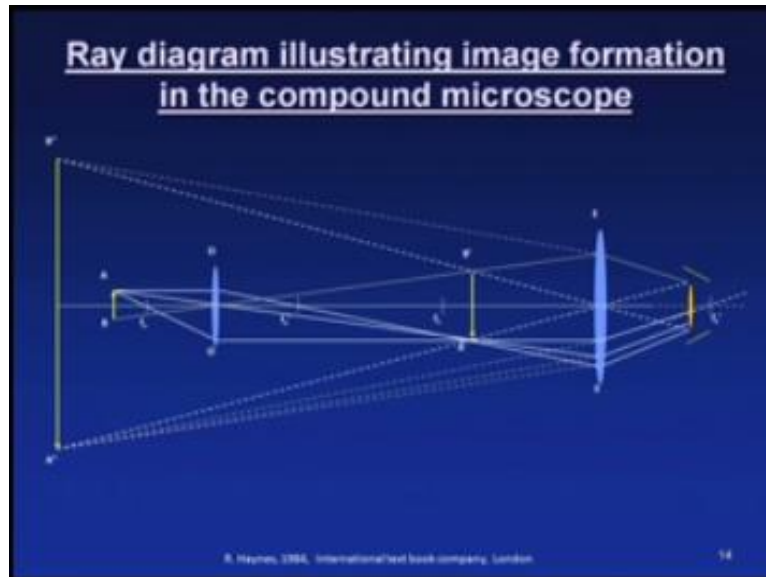
- ***Diffraction*** is the spreading of light that occurs when a beam of light interacts with an object.
- Just as diffraction describes the scattering of light by an object into divergent waves, ***interference describes the recombination and summation of two or more superimposed waves***, the process responsible for creating the real intermediate image of an object in the focal plane of a microscope.
- In a real sense, diffraction and interference are manifestations of the same process.

Douglas B. Murphy, 2001, Wiley-Liss, Inc. USA

15

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

(Refer Slide Time: 19:51)

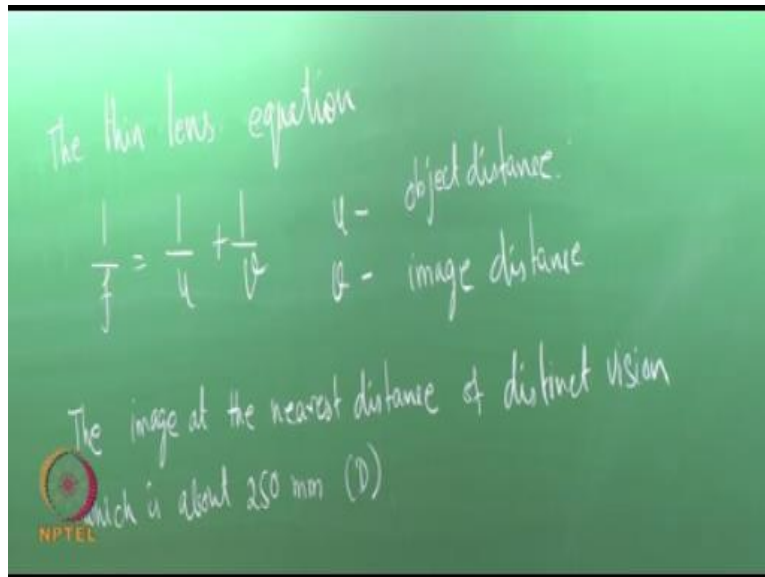


Let us look at that the details of this image here is an object a b and then you have this objective lens and then you have the image formation a` b` this is an inverted image and then you have another lens here it is called eyepiece basically what why I am bringing this slide because most of the microscopes 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.

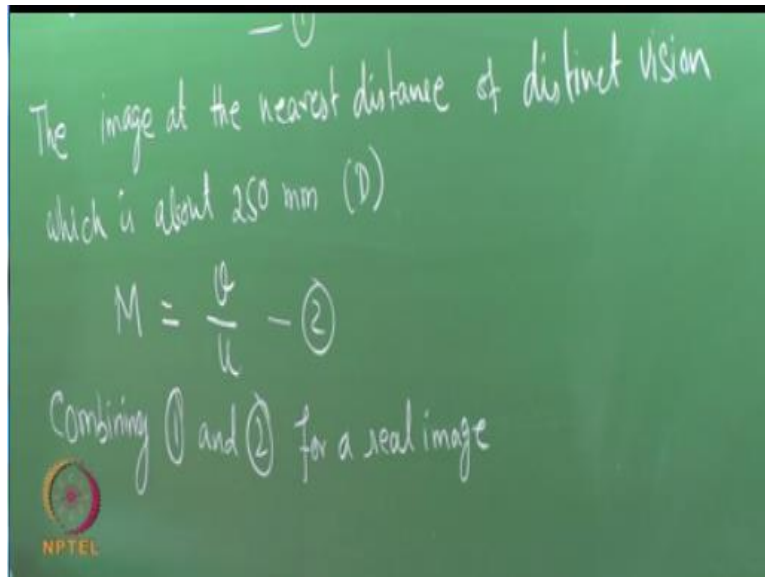
So it also kind of explains the magnification how the magnification takes place we will see how we can understand this magnification.

(Refer Slide Time: 22:10)



So we will write the popular lens equation that is  $1 / f = 1 / u + 1 / v$  so here instead of a and b I have used U and V which is very common notation in the effects 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.

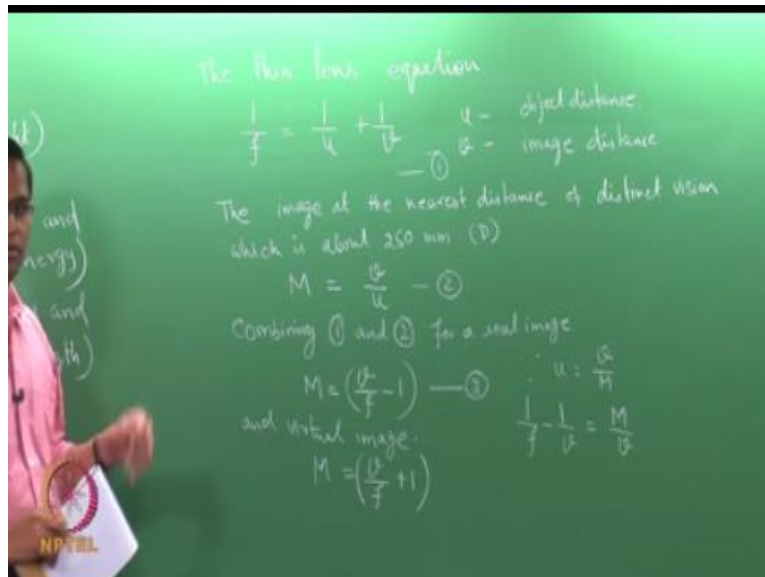
(Refer Slide Time: 24:10)



We are able to see any of the object with our normal vision which is 20 250 mm so normally the right magnification is  $v/u$  so this is the magnification given by any of 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 is an objective Ferrari.



(Refer Slide Time: 25:18)



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  $m = v / f + one$  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.

(Refer Slide Time: 26:32)

$M = \frac{v}{u} \quad \text{--- (2)}$


Combining (1) and (2) for a real image

$M = \left(\frac{v}{f} - 1\right) \quad \text{--- (3)}$

and virtual image.

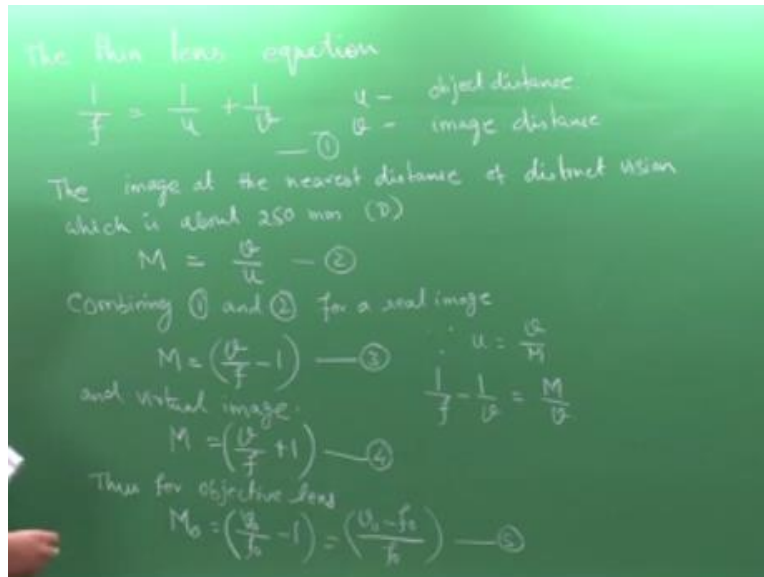
$M = \left(\frac{v}{f} + 1\right) \quad \text{--- (4)}$

$\frac{1}{f} - \frac{1}{v} = \frac{M}{v}$



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.

(Refer Slide Time: 27:11)



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

(Refer Slide Time: 28:07)

Similarly  $M_e = \left(\frac{v_e}{f_e} + 1\right) = \left(\frac{v_e + f_e}{f_e}\right)$

Assume that an image formed at a distance of distinct vision,  $D$ , we can put  $D = v_e + f_e$

$M_e = \frac{D}{f_e}$  (eye is placed at exit pupil of the eye piece)

Total magnification,  $M$  produced by microscope is

$M = M_o \times M_e = \frac{D}{f_e}$

NPTEL

But before that and most of the microscopes use this optical  $Q$  so that the length of the optical cube is also taken into consideration which is considered  $T$  so from the ray diagram we can show that this is  $T = V - F^0V - F$  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 form at a distance of distinct vision that is  $D$ .

We can put  $D = V_c + F_c$  then objective we use this optical cube length, in image we use this distinctive vision  $D$  which is 250 nanometers or so 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  $PD/F$ .

So this is coming from these two equations and if we can include the cube length also so  $M$  total magnification is equal to objective magnification times eye piece magnification times this optical cube 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.

(Refer Slide Time: 33:24)

**Defining Diffraction and Interference**

- ***Diffraction*** is the spreading of light that occurs when a beam of light interacts with an object.
- Just as diffraction describes the scattering of light by an object into divergent waves, ***interference describes the recombination and summation of two or more superimposed waves***, the process responsible for creating the real intermediate image of an object in the focal plane of a microscope.
- In a real sense, diffraction and interference are manifestations of the same process.

Douglas B. Murphy, 2003, Wiley-Liss, Inc. USA

15

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.

(Refer Slide Time: 35:17)



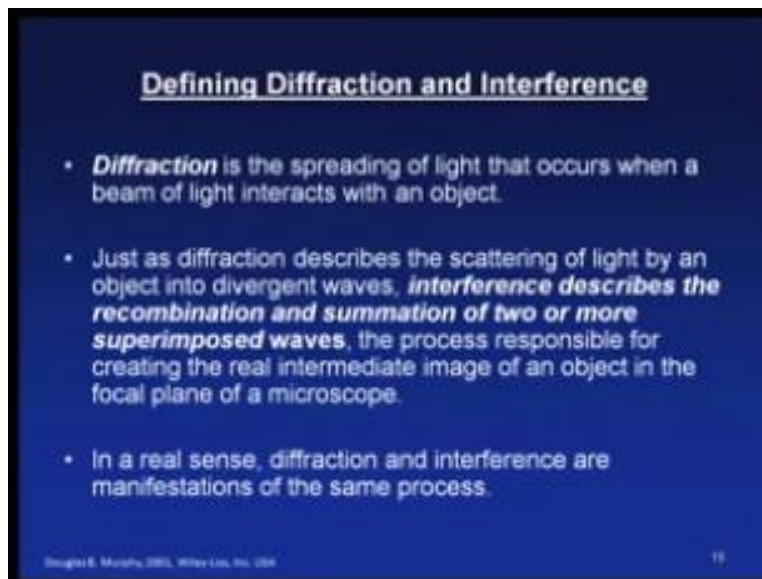
So what I am trying to draw is when the light passes through a edge like this it produces a shadow here this is because of diffraction this is one of the manifestation of a diffraction I am not trying to define a diffraction here but just the effect of diffraction then these things will help you to understand 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.

(Refer Slide Time: 42:54)



**Defining Diffraction and Interference**

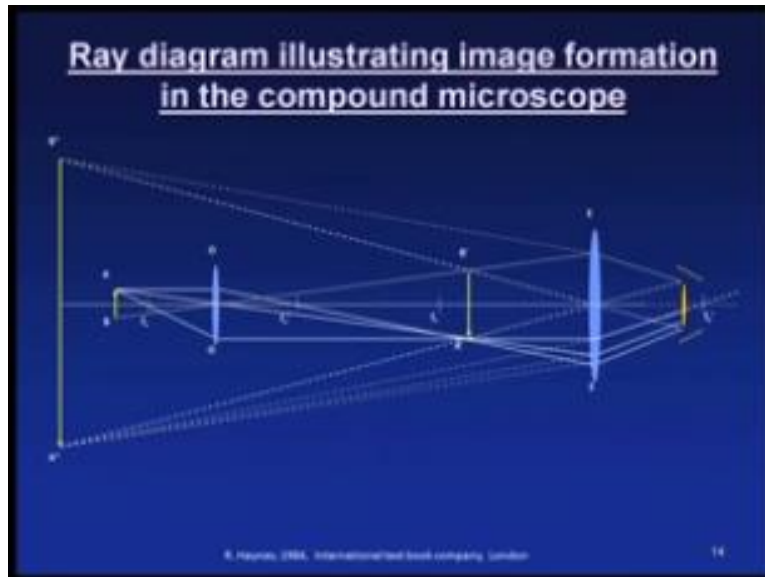
- **Diffraction** is the spreading of light that occurs when a beam of light interacts with an object.
- Just as diffraction describes the scattering of light by an object into divergent waves, **interference describes the recombination and summation of two or more superimposed waves**, the process responsible for creating the real intermediate image of an object in the focal plane of a microscope.
- In a real sense, diffraction and interference are manifestations of the same process.

Copyright ©, March 2005, Wiley Ltd, Inc. USA

19

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 Micra.

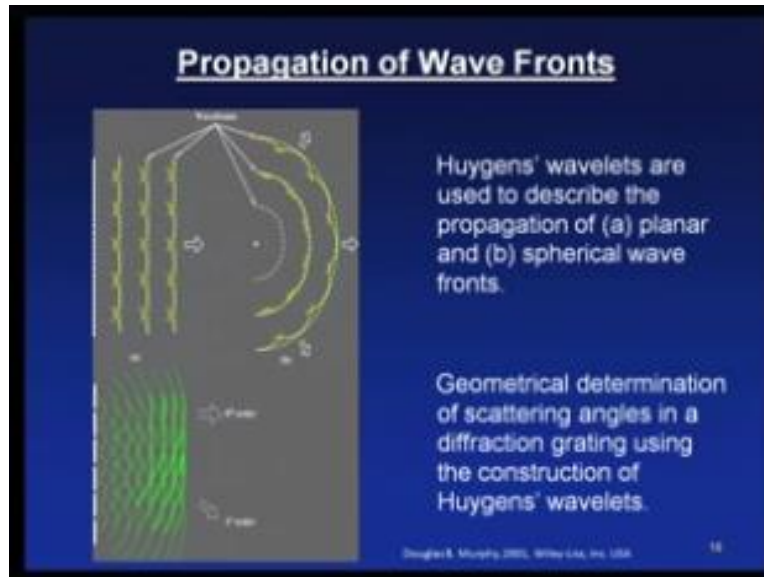
(Refer Slide Time: 43:28)



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.



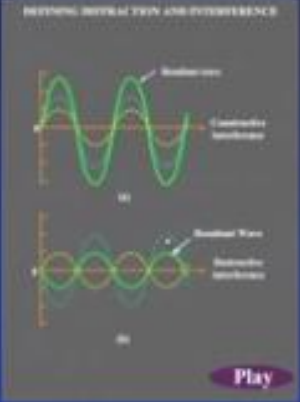
(Refer Slide Time: 44:06)



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 0 order and the first order. So this is how the wave propagation is described in a optics.

(Refer Slide Time: 45:11)

**Defining Diffraction and Interference**



The arithmetic sum of the amplitudes of the two original waves.

If the amplitudes of the waves are the same and the relative phase shift is  $\lambda/2$ , the wave is eliminated.

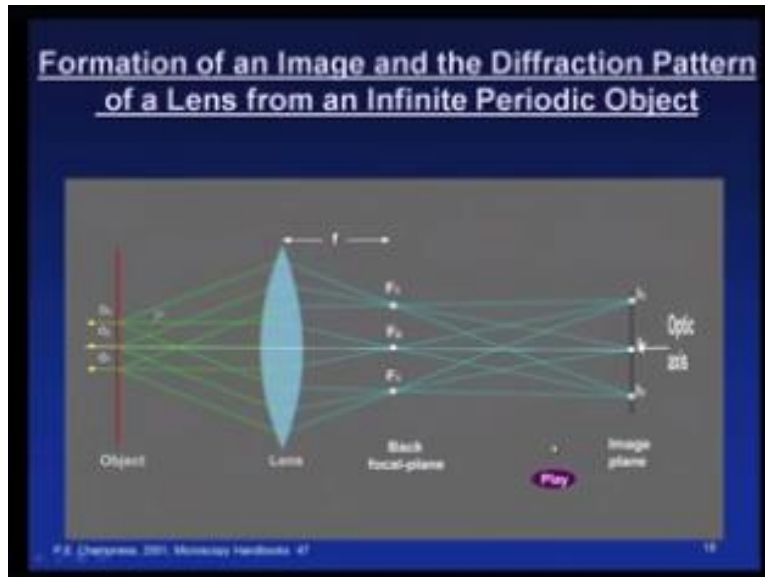
Play

Douglas E. Murphy, 2005, Wiley-Liss, Inc. USA 17

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.

(Refer Slide Time: 46:48)



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  $Q_1$ ,  $Q_2$ ,  $Q_3$  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 un-deviated.

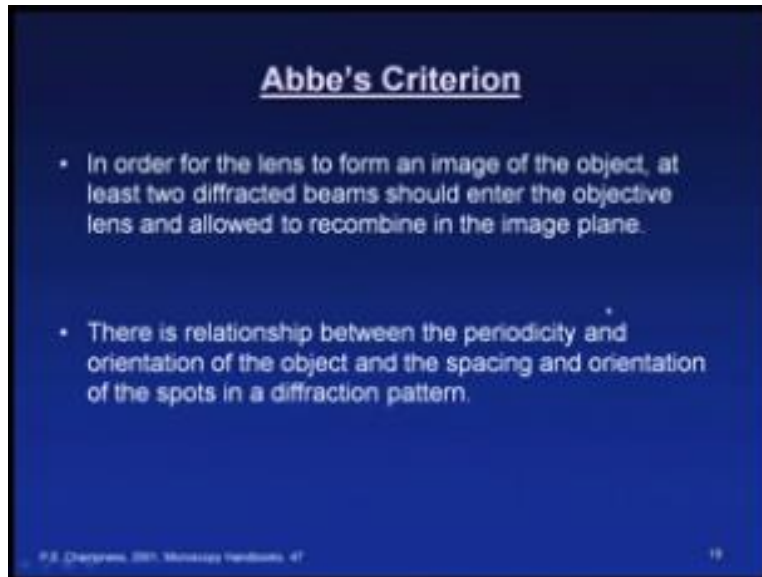
The rays which is passing through the peripheral of the lens will deviate and then it will go to this image plane and what I would like you to just concentrate on this look at this each ray and then where it goes and then you have something called principle focal point like I mentioned in the earlier 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 part 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.

(Refer Slide Time: 52:55)

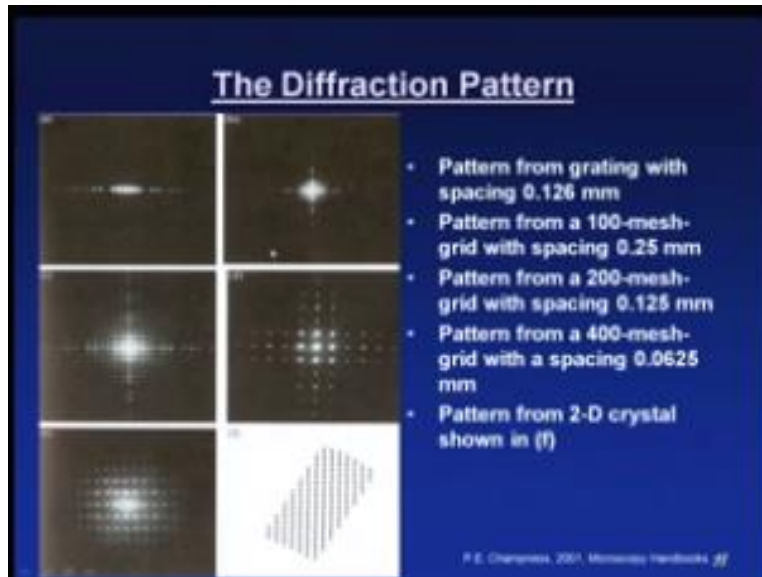


And then we will now look at the criterion now for the image formation so whatever just we have seen in the previous slide we will just see whether this obeys that Abbe's criterion in order for the lens to form an image of an object at least to diffracted beams should enter the objective lens and allowed 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.

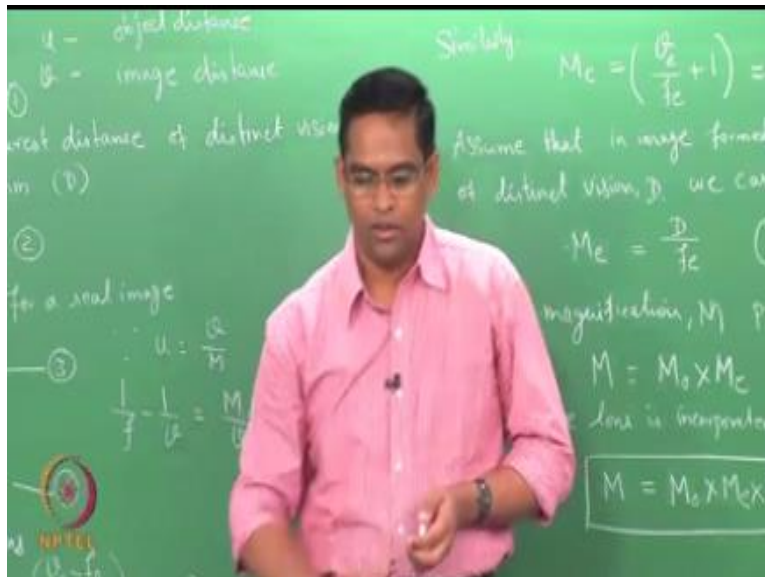
This particular concept will be useful in the electron microscopy also we will see it when we deal with that and now we will see one example to prove this concept in a diffraction pattern.

(Refer Slide Time: 54:51)



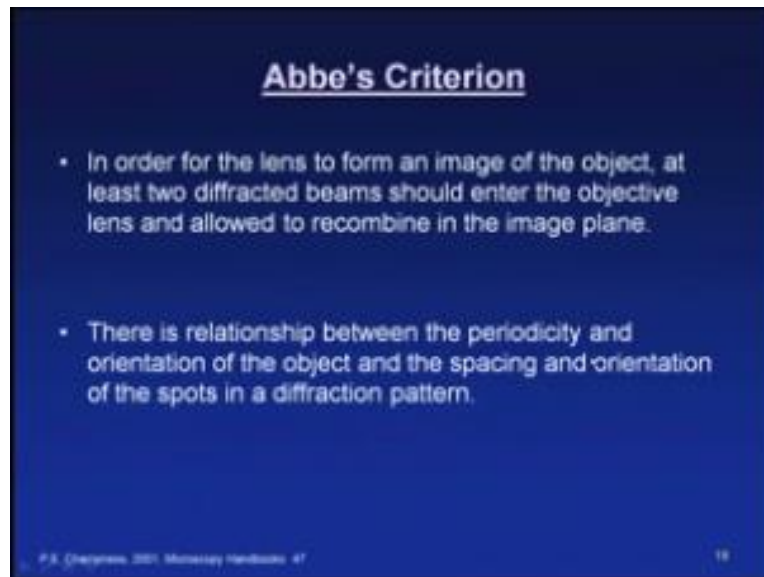
So what you see here is an electron diffraction pattern and this has been brought to just to prove the concept what we just mentioned before look at this pattern carefully A,B,C, D, E and f and these are all the pattern from the grating, grating with the spacing of 0.1 to 6 millimeter that is a and the 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.

(Refer Slide Time: 56:04)



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 0.6 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.

(Refer Slide Time: 56:47)

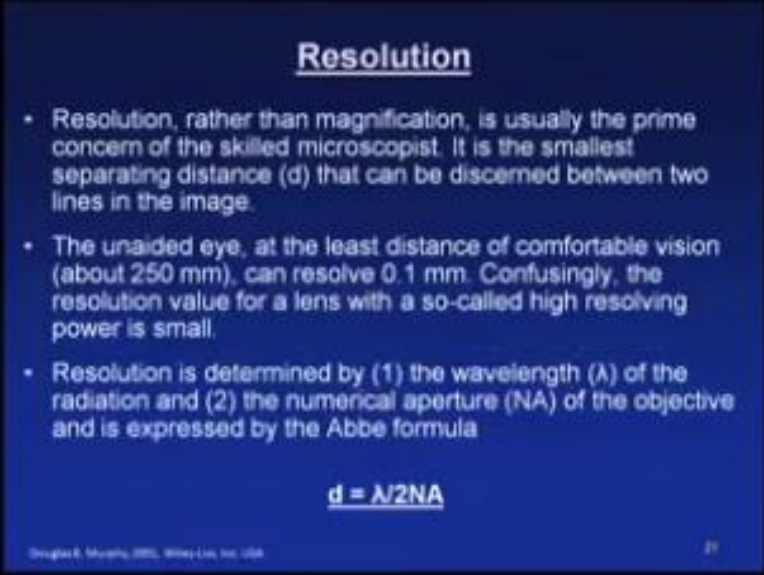


So there is a relationship between periodicity and the orientation of the object and the spacing and the orientation of the spots in a diffraction pattern so we have just talked about the spacing it is not just the spacing alone but the orientation of the object as well how it is understood from this 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.



(Refer Slide Time: 58:10)



**Resolution**

- 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 image.
- The unaided eye, at the least distance of comfortable vision (about 250 mm), can resolve 0.1 mm. Confusingly, the resolution value for a lens with a so-called high resolving power is small.
- Resolution is determined by (1) the wavelength ( $\lambda$ ) of the radiation and (2) the numerical aperture (NA) of the objective and is expressed by the Abbe formula

$$d = \lambda/2NA$$

Copyright © 2010, Wiley-Liss, Inc. USA

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.

(Refer Slide Time: 59:03)

**Resolution of the Lens**

Resolution is defined as **minimum resolvable distance**

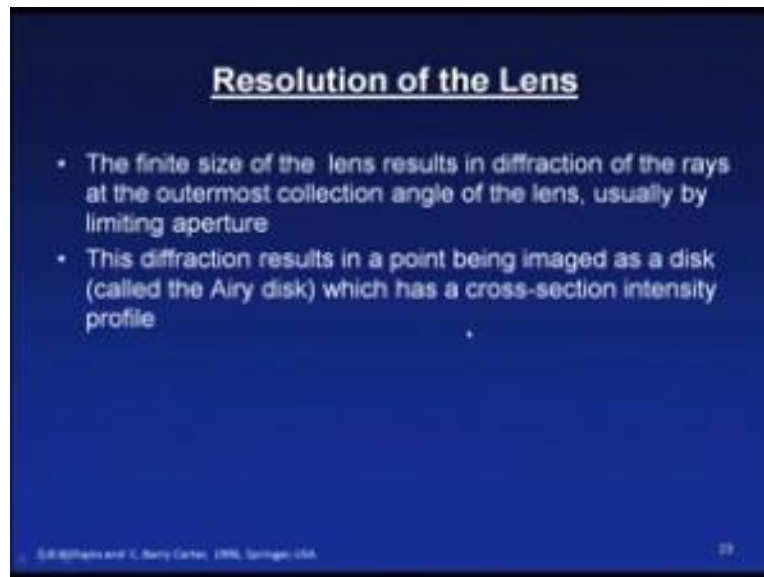
**Theoretical resolution**

- If there is no aberration at all, the resolution of any lens (glass, electromagnetic) is customarily defined in terms of the **Rayleigh criterion (practical definition)**
- **The criterion gives us a merit in terms of the eye's 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 astigmatism are present

© R. Williams and C. Barr (2004), 1996, Springer USA

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 point 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  $\gamma$  divided by 2 times numerical aperture so let us look at what is the 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.

(Refer Slide Time: 1:00:32)



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 in coherent point sources a single point source will not be imaged as a point even if no abrasions are of stigmatism 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 race 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.

(Refer Slide Time: 1:01:43)



As an animation then the understanding will be better then we will discuss that look at this is a self-luminous point-source one 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 see it just come closest and then stops at a point where our I 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  $\gamma$  by  $\beta$  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 0.61 times  $\gamma$  by  $\beta$  a  $\gamma$  is the wavelength  $\beta$  is the semi aperture angle so rarely stated that if the maximum.

(Refer Slide Time: 1:04:05)

### Resolution of the Lens

- Rayleigh stated that if the maximum from one source lies over the first minimum from the other source, then the over all intensity profile exhibits a dip in a middle at about 80%  $I_{max}$
- 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  $r_{th}$  and is given by the radius of the Airy disk

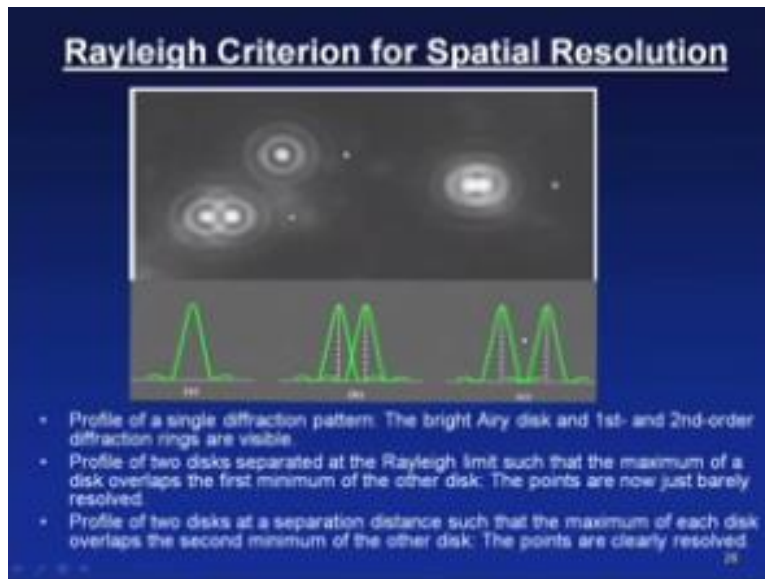
$$r_{th} = 0.61 \frac{\lambda}{\beta}$$

© 2008 Pearson Education, Inc. All rights reserved. 1996, Springer, USA

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 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 in current 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 or  $TH$  equals  $0.61$  times  $\gamma$  by  $\beta$ .

And this what is this added disc if you look at this is the parrot disc if you look at this is the array disc.

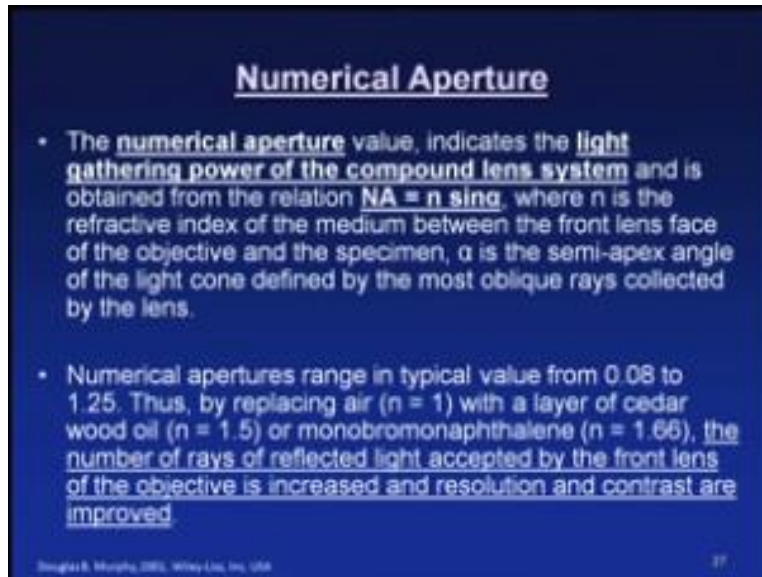
(Refer Slide Time: 1:05:02)



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 Airy disk and the first and second order diffraction things are visible so you see that this is the 0 order first order second order third order and so on so this is a and profile of the two disks separated at the 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 look at the see 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 are these points are clearly resolved so this is the another illustration of the valid criterion for the spatial resolution.

(Refer Slide Time: 1:06:50)



**Numerical Aperture**

- The **numerical aperture** value, indicates the **light gathering power of the compound lens system** and is obtained from the relation  **$NA = n \sin \alpha$** , 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.
- Numerical apertures range in typical value from 0.08 to 1.25. Thus, by replacing air ( $n = 1$ ) with a layer of cedar wood oil ( $n = 1.5$ ) or monobromonaphthalene ( $n = 1.66$ ), **the number of rays of reflected light accepted by the front lens of the objective is increased and resolution and contrast are improved.**

Copyright ©, Murphy, 2005, Wiley-Liss, Inc. 108

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  $n \sin \alpha$  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 stress placing 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 point 6 6 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 you.

**IIT Madras Production**

Funded by

Department of Higher Education

Ministry of Human Resource Development

Government of India

[www.nptel.ac.in](http://www.nptel.ac.in)

Copyrights Reserved