Analytical Technologies in Biotechnology

Dr. Ashwani K. Sharma
Department of Biotechnology
Indian Institute of Technology, Roorkee

Module – 1
Microscopy
Lecture - 1
Basic concepts in microscopy 1

Dear students, welcome to this course on analytical technologies in biotechnologies. It is quite evident that advances in technology has been vital in the progress of science. In life sciences, advances in analytical techniques have played a crucial role in understanding of biological functions at the molecular and the cellular label. Biotechnology is an interdisciplinary science that involves applications of chemical and physical techniques, in the field of life sciences.

The recent progress in the field of the biology has been made possible by the application of the many chemical techniques as well as the principles of physics that have helped unrevealed of many mysteries of biology. Now, let us understand this, let us take some examples to understand the crucial rule of technology in biotechnology. Now, cell is the basic unit of life, biology started with the discovery of cell. Now, cell is so small that it cannot be seen with unaided eyes.

The cellular world was unknown and unseen till a Dutch man named Antonnie Van Leeuwenhoek for the first time observed a tiny cell with simple glass lens. Later, advances in the field of microscopy led to the detail study of cells, sub cellular organelles, microorganisms, macromolecular assemblies and so on, with radioisotopes, later fluorescence labeling a bimolecular could be traced, localized, and relative distribution as a function of time could be analyzed through various advanced macroscopic techniques.

New advances like confocal microscopy have enabled three dimensional observation of whole cell or a thick specimen. Likewise, the field of molecular biology was born with a double helix model of Watson and Crick. Till 1940, it was hard to accept DNA as genetic material, but with Watson and Crick model, a part of the data from x-ray diffraction analysis was used to un reveal this structure. This model is good to provide

convincing explanation of how genetic information is stored, retrieved and translated in a living organism.

Now, new advance techniques for analyzing DNA, RNA and proteins have come up. Modern recombinant DNA technologies allows an individual gene or a segment of DNA to be cloned, sequenced and expressed in specific systems technique known as polymer reaction, it has allowed the selective application of specific segments of DNA for further use.

The technique is being used extensively in the field of molecular biology. Medical science and forensic science recently advanced automated DNA sequencing. Methods facilitated the determination of complete genome sequences of many organisms including humans, zebra, and fish and so on. Now, these genomic sequences or information is being unraveling everyday allowed new sets into the field of science.

Many powerful analytical techniques are being used for isolation and characterization of proteins. These include chromatography, electrophoresis, and centrifugation, spectroscopy techniques. Now, chromatography methods could be used for purification of proteins to homogeneity on the basis of charge size, hydro phobicity and affinity. Molecular weight and sub unit composition of proteins at very low concentration can be analyzed.

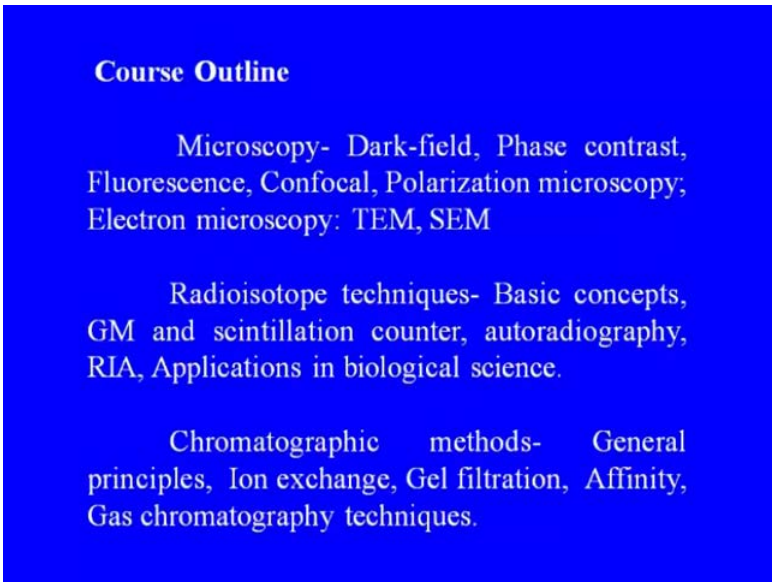
This electrophoresis method could be used for this purpose; two d electrophoresis could be used for resolving large amount of large mixture of proteins. It can be further analyzed by mass spectrometry. Ultra centrifugation could be used for separating various cell components on the basis of size and density. Now, sedimentation behavior of purified by molecules could be derived or determined by analytical ultra-set fuse noble techniques. In the field of this, spectroscopy have led to the understanding of interaction between metal and radiation and facilitated the characterization by bio molecules. Many of the techniques have come up. They include UV, visible, IR spectroscopy.

Three dimensional structures of proteins and DNA are being determined at atomic resolution. Using x-ray crystallography and NMR for x-ray crystallography, purified protein or material needs to crystallize where structure of protein in solution can be determined by NMR. 3 D structures are keys for understanding structure function relationship. Hence, immensely, they have immensely helped in the field of drug

designing against specific targets. For example, methotrexate is used in anti-cancer drugs.

A very sensitive new technique called mass spectrometry allows precise determination of mass of intact protein and derives peptide from them. It is being widely used in proteomics. Many new advances and science seems to be out of science fiction and enabling a complete view of life. This course will focus on theory and practical applications of some of the important analytical techniques used in different areas of biotechnology. We will try to cover most of the topics, most of the content, but not all. Now, all the illustrations used in this course are schematic diagrams and should be remembered that they have been used only for understanding the concepts.

(Refer Slide Time: 07:48)

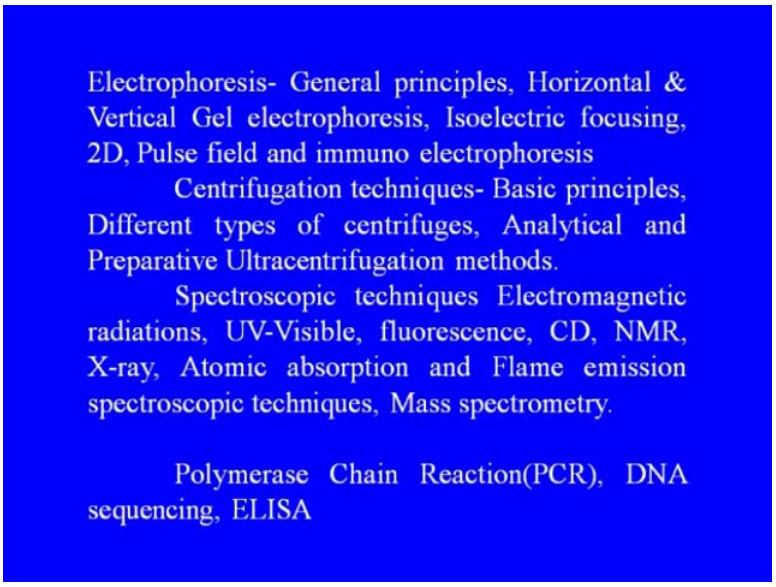


The course is outlined here. The topics, which are going to be covered in this course, are as follows. Number 1 microscopy, in, microscopy we are going to cover basic concepts in microscopy. Then specific techniques like dark field, phase contrast, fluorescence and focal, polarization microscopy and light microscopy, then electron microscopy transmission and scanning electron microscopy techniques.

Radioisotope techniques will cover basic concepts, the Geiger molar scintillation counters, autoradiography, radio immune-acid and applications in biological sciences. Also, the safety issues will be discussed in chromatography method, the general

principles and basic concepts. Then specific techniques, ion exchange, gel filtration, affinity and gas chromatography techniques will be discussed.

(Refer Slide Time: 08:54)



In electrophoresis, the basic principles and different types of electrophoresis techniques like iso electric focusing, two d, pulse field and immune electrophoresis will be discussed. In centrifugation techniques, basic principles, different types of centrifuges, analytical and preparative ultra centrifugation methods will be discussed. Then, in spectroscopy techniques, we will be dealing with basic concepts. Then UV visible, fluorescence, 3 D, NMR, x-ray and atomic absorption and flame emission spectroscopy. Also, here mass spectrometry will be discussed. Other techniques which are going to be discussed, which are advance techniques are polymeric chain reaction, advance DNA sequence and Elisa.

So, we will start with our f irst topic that is microscopy. Now, I think all of you must have used a very simple lens for magnifying a particular object. Now, the microscopy is the science where microscopes are used for being objects that are not visible to unaided eye and other terms that objects are too small to be seen by unaided eyes. The word microscopy comes from Greek roots where micro means small and scope means is to view. So, microscopy means to view small objects.

A microscope is an optical instrument that uses a lens or a combination of lens to produce a magnified image of an object; too small to be seen with the naked eye. There

are 3 branches of microscopy. These are optical or light microscopy, electron microscopy and scanning pro microscopy. Now, the first two microscopy techniques that is light and electron microscopy involves various phenomenons like reflection, refraction and diffraction of electromagnetic radiation or electron beams to generate an image.

Scanning pro microscopy, the third one for example, atomic force microscopy involves the interaction of a scanning pro with the surface of an object. Now, first let us little bit get the glimpse of the history in very brief of the microscopy. The first microscope to be developed was the optical microscope. The first detailed account of the interior construction of living tissue, the stoned use of microscope did not appear until 1644. The greatest contribution came from Antonnie Van Leeuwenhoek in year 1676, who for the first time discovered the microorganisms using his simple lens.

Now, before this Robert Hooke described the compound microscope in his book micrographier published in 1665, the most famous microscopic observation was his study of thin slices of cork. Later on, after much late, together with Carl Zeiss in 1877, almost like 200 years defined the physical law that determined resolving distance of an objective, this is objective lens we should say. That is known as ebb's law. In 1893 august, Koehler developed a key technique for simple illumination or we can say sample illumination called Koehler illumination, which is central to modern light microscopy.

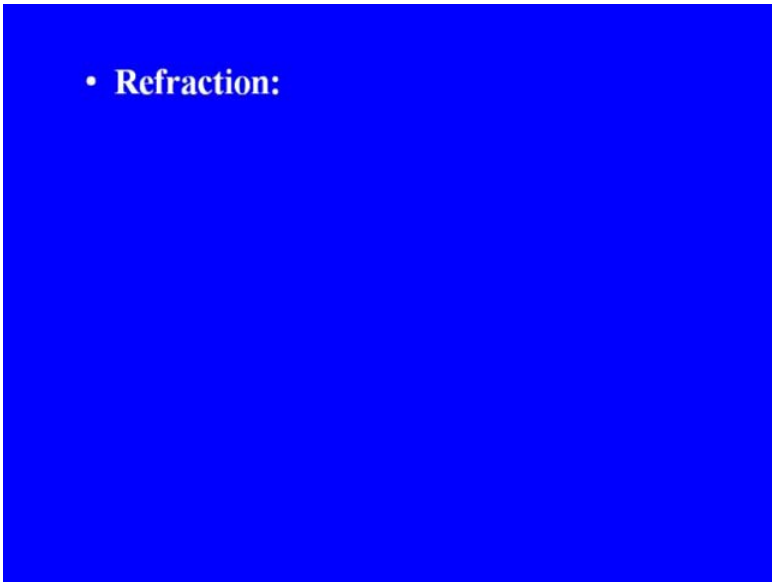
Now, development of first transmission electron microscope was started by in 1931. It was followed by the development of scanning electron microscope in 1935 by Max Knoll where he got first scanning electron microscopy images. Later, Matt in 1937 did pioneering work on physical principles of scanning electron microscope and the beam specimen interactions. In 1953 to 55, friends, harnick and George nomarski contributed towards the development of phase contrast and differential interference contrast illumination, which allowed imaging of transparent samples.

The 1980s saw the development of first scanning pro microscope and from then lot of advancement has taken place in the field of microscopy. Now, let us start with the microscopy. Let us discuss some of the terms, which are related to microscopy. The first term we are going to discuss is absorption.

(Refer Slide Time: 14:18)

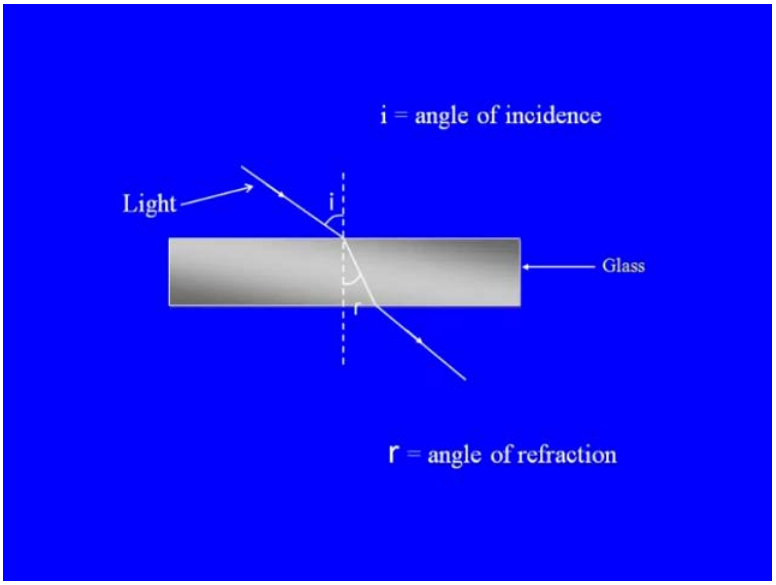
I think everybody understands about absorption. When the light passes through any object, the intensity is reduced depending upon the color absorbed. Thus, the selective absorption of white light produces a colored light. So, any time a light passes through certain object or specimen, which is certain absorption, takes places, which reduces the intensity of the transmitted light.

(Refer Slide Time: 14:51)



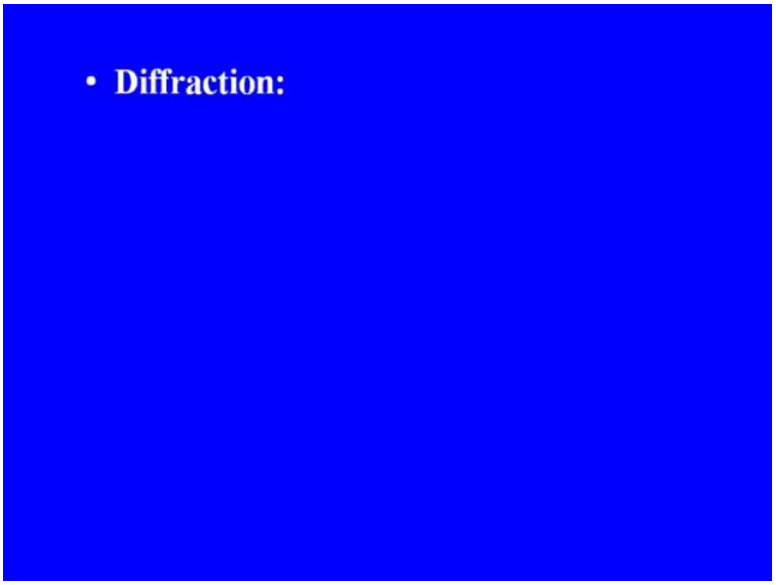
Then, second term is refraction. I think refraction is turning or bending of any wave, such as light wave or sound wave passes from one medium in to another medium from different optical density or refractive index like here shown.

(Refer Slide Time: 15:11)



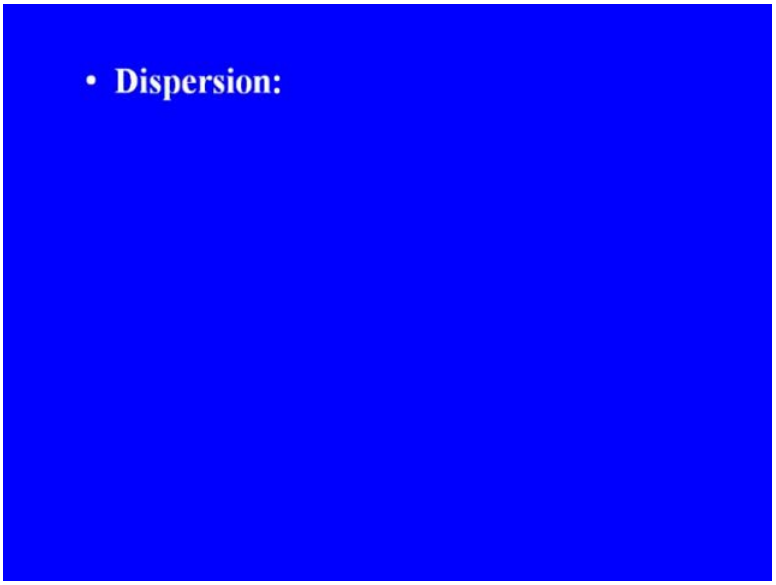
When light passes from air to glass and back into air, there is bending of light. You can see the angle of incidence and angle of refractions are different. I think everybody must have seen this phenomenon when they have seen object dipped in water.

(Refer Slide Time: 15:34)



Third term is diffraction. Now, diffraction is the change in the direction and the intensities of group of waves after passing by an obstacle or through an aperture, whose size is approximately the same as the wavelength of the wave. Now, for in simple terms, light rays bend around edges or apertures. The new wave fronts are generated at sharp edges. So, diffraction is an important term with relation to resolution as we will discuss later on.

(Refer Slide Time: 16:12)

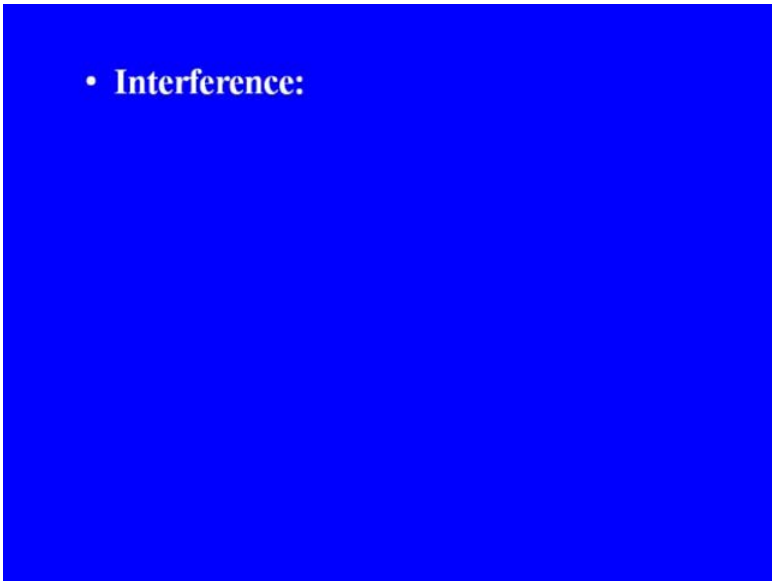


Next term is dispersion. Dispersion is a phenomenon, in which separation of light into its constituent wavelength occurs from entering a transparent medium. For example, white light consist consists of more than one wavelength. They would be separated or they would distribute when they pass through prism or certain other medium.

(Refer Slide Time: 16:39)

Next term is very important. Magnification, as we use lens or microscopes for magnification. Now, magnification is the process of enlarging an object only in appearance and not in physical size. So, when we are looking at the magnified image, it appears to be large. It is not really large as such.

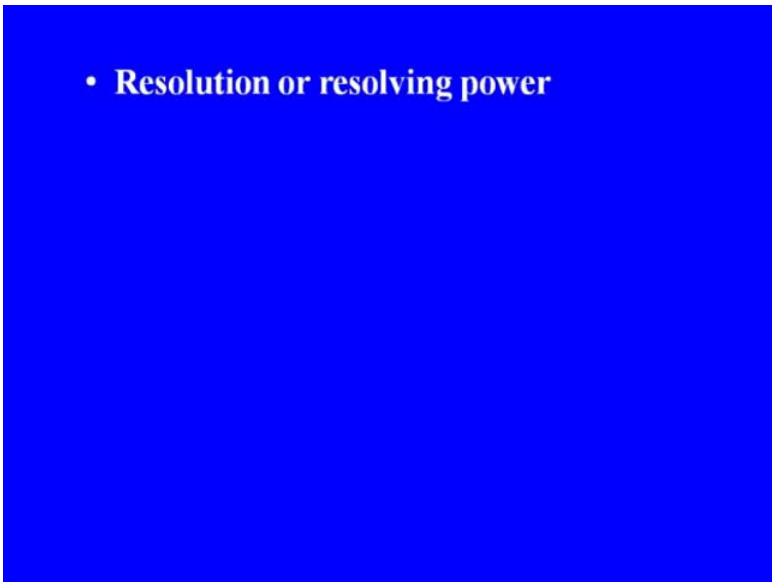
(Refer Slide Time: 17:03)



Next term is interference. Now, interference is the variation of wave amplitude that occurs when waves of the same or different frequency come together. There could be

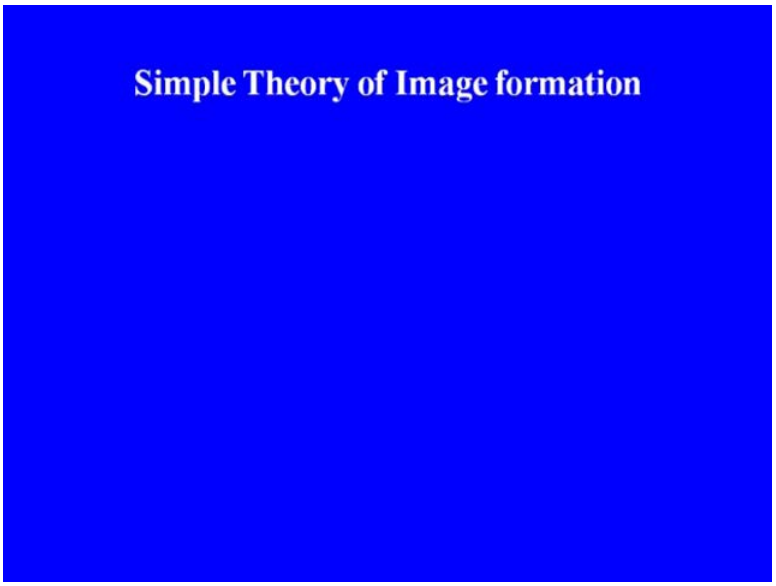
constructive interference or there could be destructive interference. I think we will discuss these in detail later on.

(Refer Slide Time: 17:27)



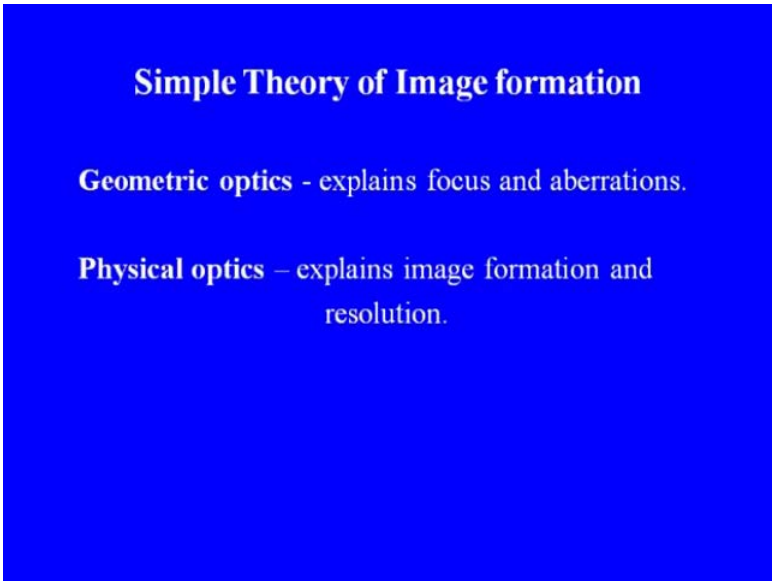
The next very important term is resolution or resolving power of a lens. Now, resolving power is defined as the distance separating 2 point objects within the specimen that can just be distinguished from one another in the image. This is also referred to as minimum resolvable distance. If you can distinguish 2 objects separated by a particular distance, it will be called the resolving power or minimum resolvable distance. These are few terms, which require a mention here.

(Refer Slide Time: 18:05)



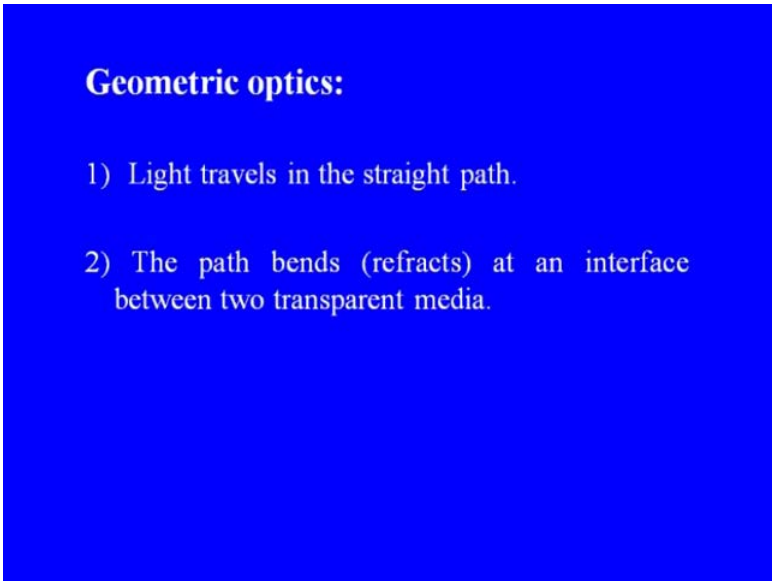
Let us get into the theory of image formation by a lens. This is important in microscopy. Let us understand this. Now, theory of image formation by lens can be defined or discussed in terms of either geometric optics or physical optics.

(Refer Slide Time: 18:27)



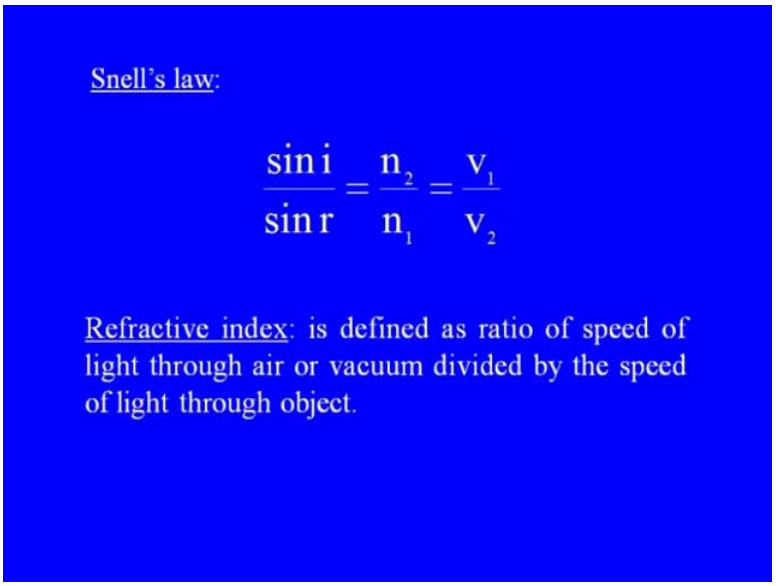
Let us now, geometric optics explains primarily focused and aberrations, whereas physical optics explains image formation in terms of contrast and other things and resolution. Let us take both of these things one by one.

(Refer Slide Time: 18:48)



Let us first discuss geometric optics. The 2 rules of geometric optics are that light travels in the straight path that is the first rule. Second rule is that path bends or the light path bends or we can say refracts, as we have discussed earlier, at an interface between the 2 transparent mediums.

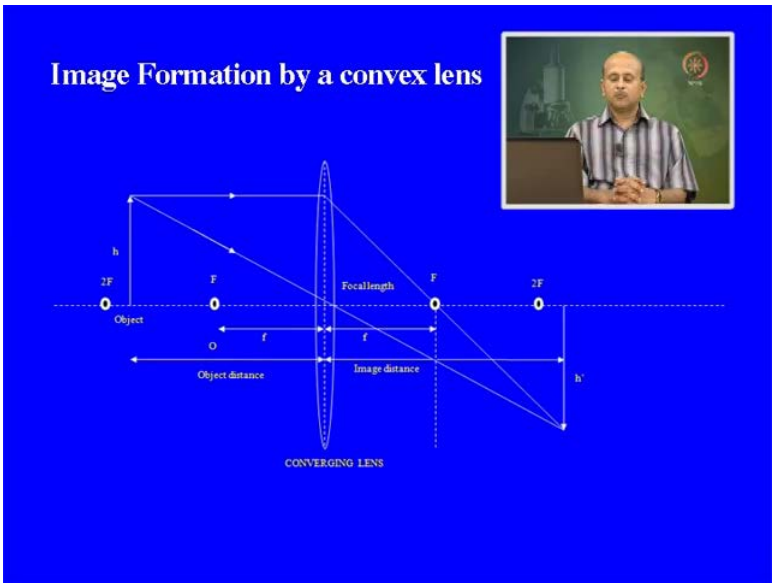
(Refer Slide Time: 19:24)



Now, where you can see the relationship between the angle of incidence and the angle of refraction can be given by, explained by Snell's law where sin i upon sin r equals n 2 upon n 1. Now, n 2 and n 1 are refractive indices of the medium and i and r are the angle of incident and refraction.

Refractive index is defined as the ratio of speed of light through air or vacuum divided by the speed light through object. So, this Snell's law explains the relationship between the angle of incidence and the angle of refraction as light passes from one medium into the other. Now, let us see how the image formation takes place by the lens.

(Refer Slide Time: 20:07)



Here, what we have shown is a convex lens. A lens is called could be of two types mainly one is converging lens, which is convex or double convex lens. Other is diverging lens or concave or double concave lens. Now, let us first discuss about the converging lens. Then we will see the diverging lens.

Converging lens are thinner at the edges and thicker at the center. As you can see here, this is a converging lens. The surface here both is convex on both sides. Now, this could be various combinations can be put in here, which could be Plano convex or Plano concave and other combination can come in. Now, let us discuss in detail this figure here. If you see here there are few things, which need to be seen one is that focal length.

Now, this lens is a biconvex or double convex lens. This lens here will converge the light rays, which are falling parallel to it. Now, the f or the focal point is the point where the lens will focus all the light, which is falling parallel rays, which are falling on it. Now, the length from the lens the point where the light is focused is called the focal length. The plane at the focus focal point is called the focal plane.

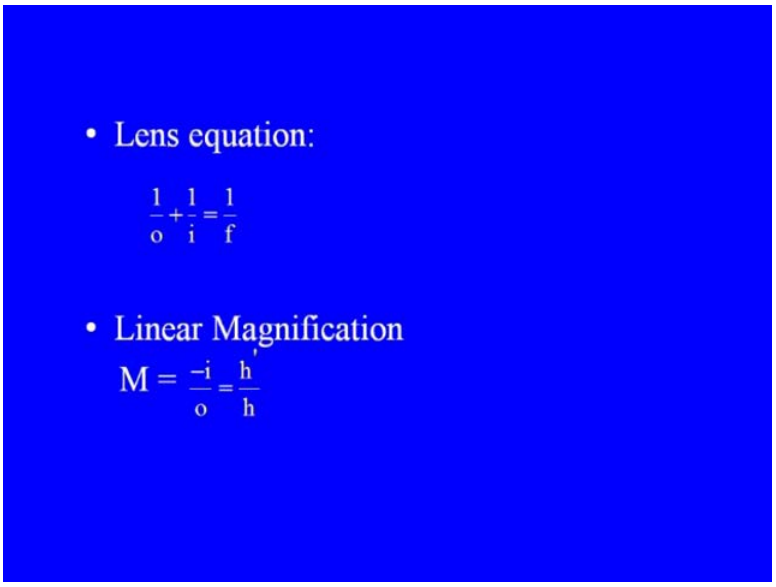
Now, there are other things to be seen. If you see here object has been placed beyond one focal length and you can see that an image is being formed, which is inverted image that is upside down. Now, on the left, object is placed. The distance where a distance between the lens and a point where an object is placed is called the object distance and where the object is placed is called the object plane. Likewise, on the right side place where the

image is being formed is called the image plane. The distance between the lenses, the center of the lens to the point where the image is being formed is called the image distance.

Now, as we will see later on the relationship between the position of the object and the image formation. Here, what we see is a converging lens forms a real inverted image on the opposite side of the lens that is where the object is on the left side. The image is being formed on the other side. When we say it is a real image, it means that the rays or the light rays are converging at that point.

For example, if you put a piece of paper or a screen, the image could be seen there could be recorded there. So, that is what a real image is inverted means if you can see that the arrow has been inverted that is it is upside down. That is what inverted image is called. This image has been formed when you have placed the object beyond a single focal length. You will see how the relationship is between the object position and the image. Now, this whole thing could be explained. You can say the image distance, object distance and focal length can be related by a simple Gaussian form of lens equation.

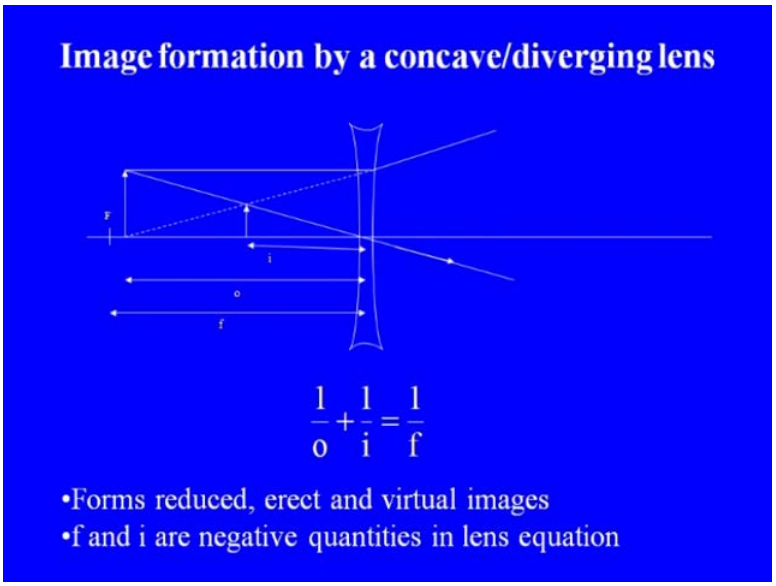
(Refer Slide Time: 24:03)



It is 1 upon o plus 1 upon i equals 1 upon f, where o is object distance, i is image distance and f is focal length of the lens. Linear magnification, which is the magnification obtained is given by the either by i upon o. Now, here i will be negative. Negative sign indicates that an inverted image is being formed. So, it can be governed by

the ratio of height of the image to the height of the object that can also be used for determining the magnification. Now, let us, little bit will be discussing more of the converging lens. Let us see how the image formation takes places in a concave lens or a diverging lens.

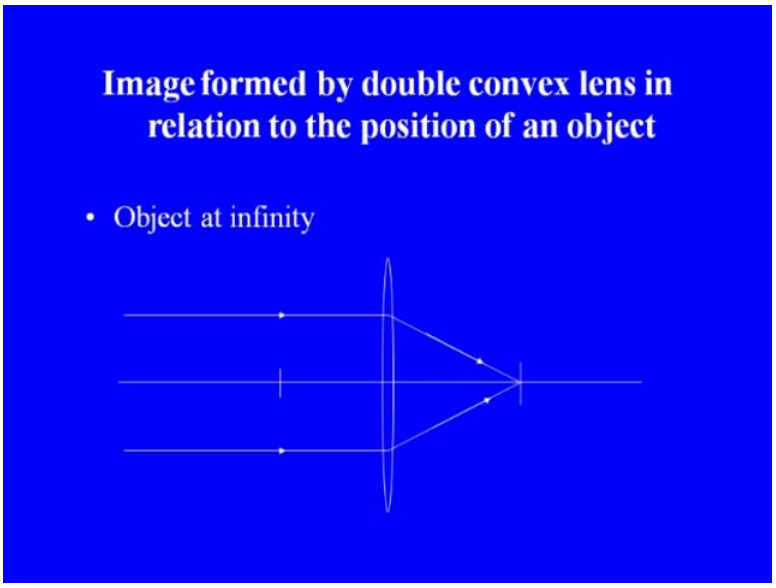
(Refer Slide Time: 24:59)



As you can see here like I said the concave or diverging lenses are thicker at the edges and thinner at the center. Now, interestingly here, the rays seem to be diverging rather than converging. As you can see here that the object wherever you place on the left hand side of the place, it always forms a reduced and erect and virtual image. Virtual image because it does not form a real image. The virtual image is formed on the same side of lens on the side where the object is placed.

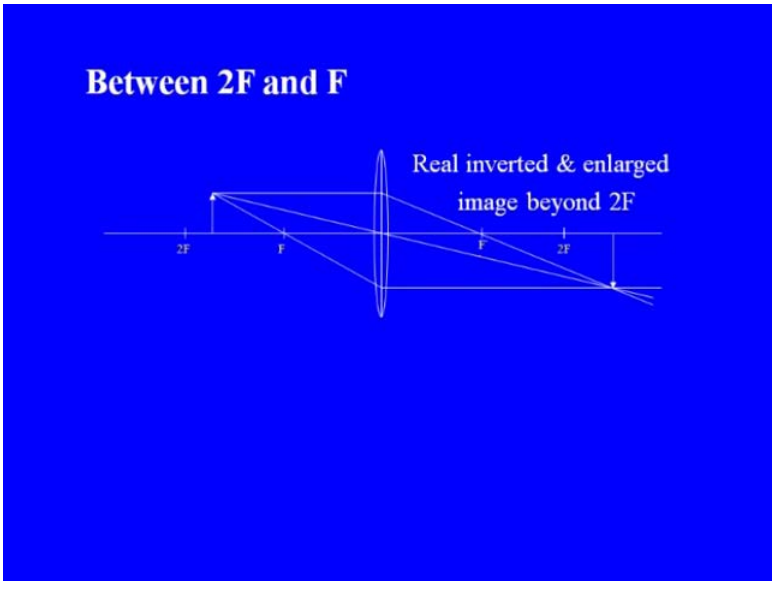
As you can see, this object is placed. Image is also being formed on the left hand side. Now, here both f and i are negative quantities in lens equation. Image distances on the left hand side and the focal point in terms of that, if you draw backwards the diverging rays from the lens, it will focus, it will come to the focus. That is why the f and i are negative quantities in the lens equation. Now, let us little bit go into as we are discussing the focus here, let us little bit go into the relationship between the image formed by double convex lens in terms of position of that particular object.

(Refer Slide Time: 26:21)



Now, first situation could be or first option could be that object placed at infinity. When object is placed at infinity, then parallel rays of light will hit the lens or will fall on the lens. Now, if you could recall the parallel rays of light when they are focused onto the lens or when it will fall onto the lens, they will focus on the focal point. That is what the focal point is because the lens focuses this light rays on that point. So, if object is placed on infinity, then the image will be formed at the focal point. That is principle focal point. Now, let us bring the object little close.

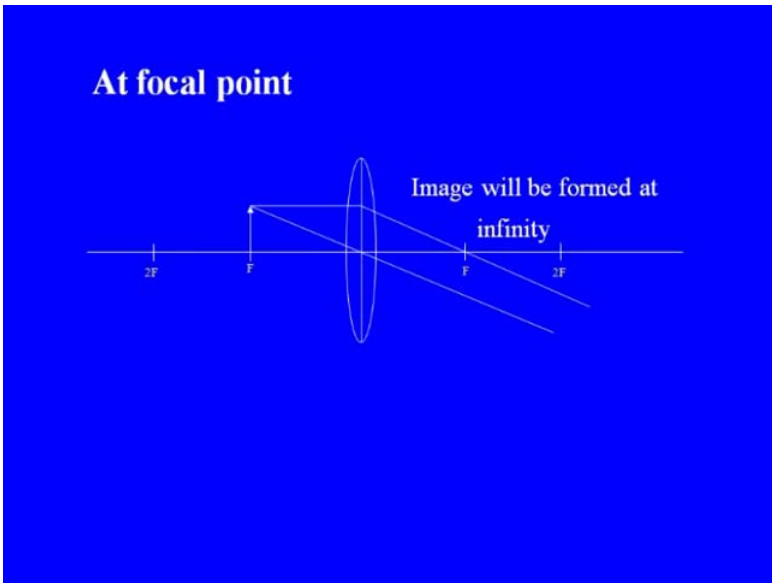
(Refer Slide Time: 27:17)



When you bring the object beyond 2F, then the image is formed, which is inverted, diminished and real. Image is formed between the 2F and F. Here, 2F is 2 focal length and F is 1 length. So, as you can see as you have brought the object closer to the lens that is beyond 2 focal lengths, then the image is formed between F and 2F. It is inverted and diminished. Here, diminished means of smaller size that is the size of the object is bigger than the size of the image.

The third scenario can be that as you bring the object much closer between 2F and F that is between 2focal length and 1 focal length, and then real inverted and enlarged image is formed. Remember, last time it was reduced image or diminished image was formed. Here, it is an enlarged image. Now, enlarged image is formed beyond 2F. As you are bringing the object is closer, then the image is being formed at the farthest point at the other side of the lens. So, in case where you have put the object between F and 2F, the images you formed are farther from 2 focal length. It is inverted, real and enlarged. It is magnified image as we can say.

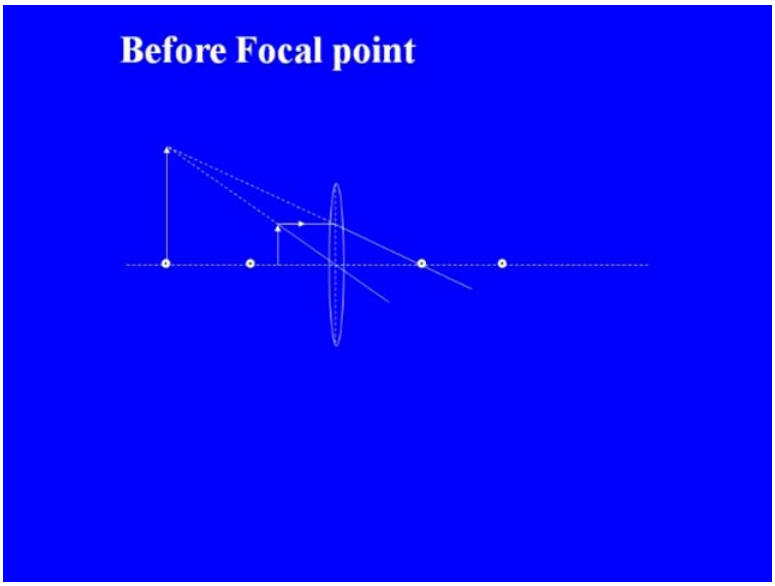
(Refer Slide Time: 28:53)



Now, if you bring it further closer like at focal point, and then what happens is that image is essentially not formed. We can say that image will be formed, but at infinity. This is because as the rays leave the lens, they are the parallel rays, which are focused at infinity. So, this will be scenario if object is placed at focal point. The last scenario is

that you can bring the object further closer to lens that is before the focal point or less than 1 focal length.

(Refer Slide Time: 29:29)



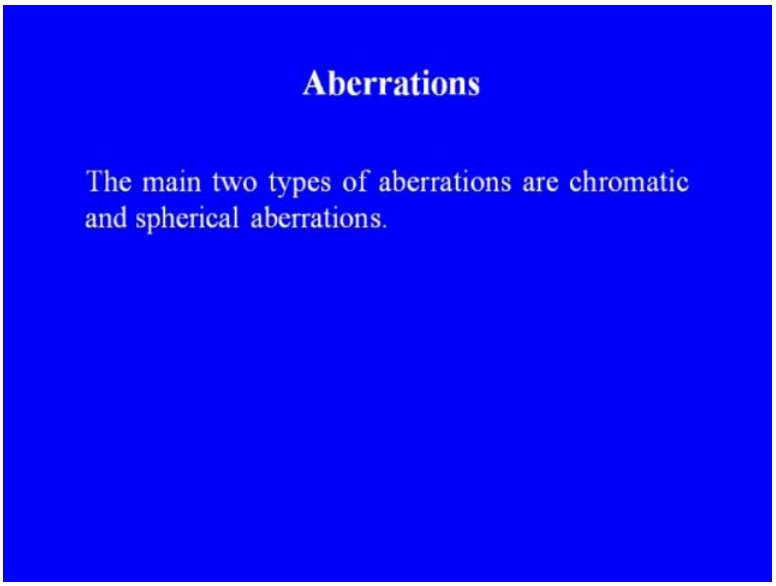
In this situation, what will happen is rather than a real image is being formed, a virtual upright and enlarged image will be formed. As we say virtual image, then this image is formed at the same side as the lens as the object is placed. So, if the object is placed at a distance shorter than focal length, then virtual upright image is formed. It is not inverted. Remember, when we say magnification, it means negative y upon o. Here, negative and negative will become positive. So, that is difference here indicating that it is a upright image.

So, what we have seen in here is if you see, if we have to infer from here that as the object is farther from lens, the image is formed closer to the lens on the other side. As you bring the object very close to the lens, the image is formed at the farthest and or much further from the lens on the image plane side or on the right hand side. Now, if you bring the object too close, then the virtual image is formed, which is upright. So, that is the main point here, which needs to be considered. This we have discussed is one part. How image is formed and object is placed at different places on the left hand side of the lens or one side of the lens.

Now, let us go into the other part of the geometric optics that is aberrations. Aberrations are when lenses fail to bring rays or all rays on the given point on the object to a unique

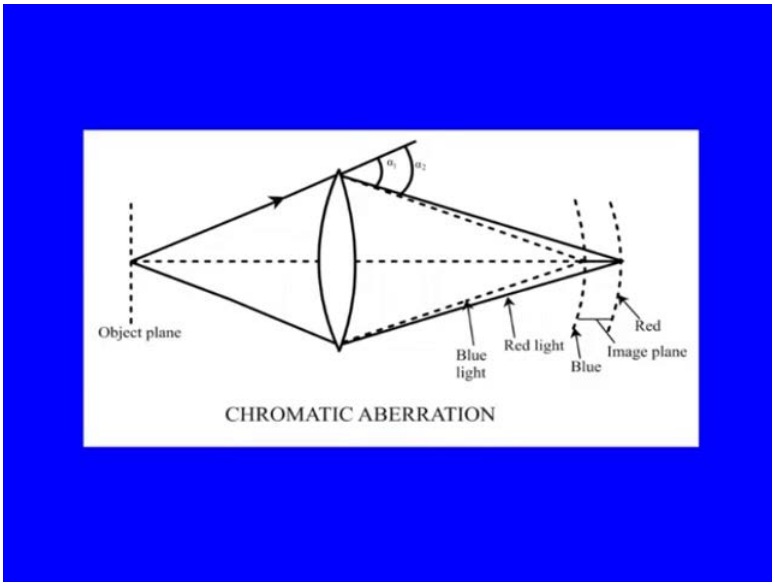
focus. That is called aberrations. Now, when there is an aberration, then blurred or not so clear images are formed. This is because all rays are not focused at the same point and you do not get sharp image.

(Refer Slide Time: 31:34)



There are 2 main types of aberrations here. 1 is called the chromatic aberration and another is called spherical aberration. Let us see what the 2 kinds of aberrations are. Now, chromatic aberration occurs because index of refraction of any substance depends upon the wavelength. The position of the focal point is dependent on wavelength. So, what happens is that supposing a white light containing a different wavelength is focused on the lens, and then they will focus on different point. They will not be focused on the same point.

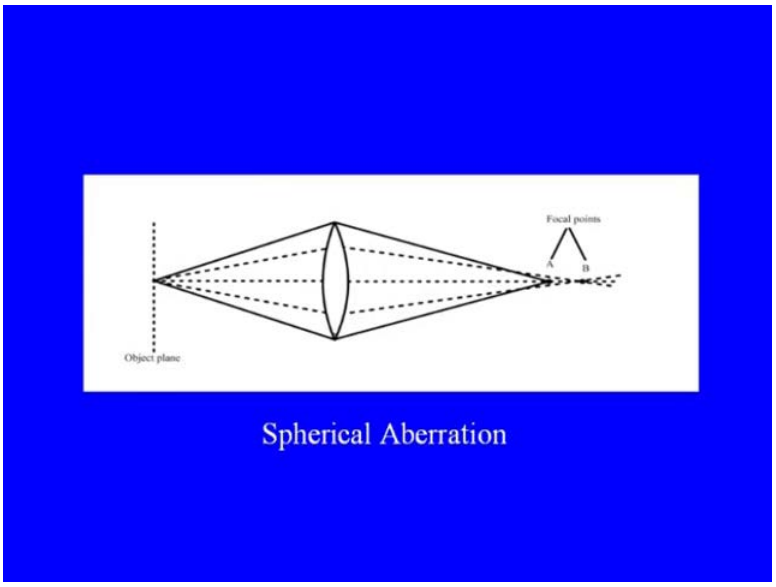
(Refer Slide Time: 32:18)



For example, blue light will be focused closer to the lens like it will have a smaller focal length as compared to the higher wavelength light. For example, we can have red or green. So, these lights are not focusing at the same point. What you get is blurred image with different color separation.

Now, this chromatic aberration is kind of problem, but it has been corrected by using a lens system consisting of several types of glasses or lenses for which the relationship been the index of refraction and wavelength balances to make the refractive index independent of wavelength. So, what you get is you get a clear and a sharp image, the corrective lens system. These are called achromatic or apo chromatic lenses.

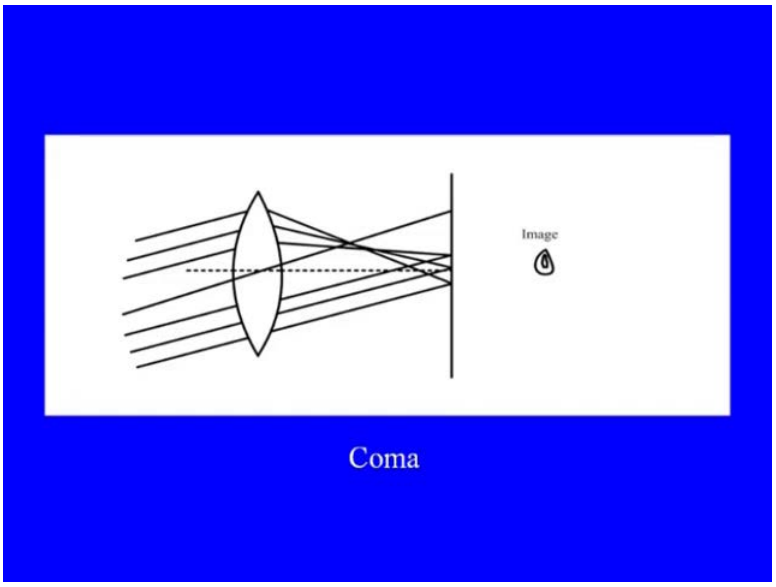
(Refer Slide Time: 33:12)



The next aberration is physical aberration. Spherical aberration as we call it. It is also called principle point imaging aberration. Now, here it results from that the fact that all rays from a single point do not pass through the same unique point or same image point. This is because they are passing from different parts of the lens. As you can see here, the rays passing from the different parts of lens are being focused at different places like 1.

They are passing from the edges, are focused closer as compared to these that are passing through the center. This is problem with lot of senses. Spherical aberrations like chromatic aberrations also have been corrected by an appropriate lens construction both in advance microscopes. Now, other chromatic aberrations and spherical aberrations have been corrected. These are aplanatic lens like achromatic lenses.

(Refer Slide Time: 34:17)



There is one point in imaging aberrations. I just wanted to show here that is coma because it gives a comic shape to the object in the image plane. Symmetry is almost lost here. So, these were two aberrations, which were common. Now, in advance system, these are being corrected with combination of lens systems. So, this was about geometric optics

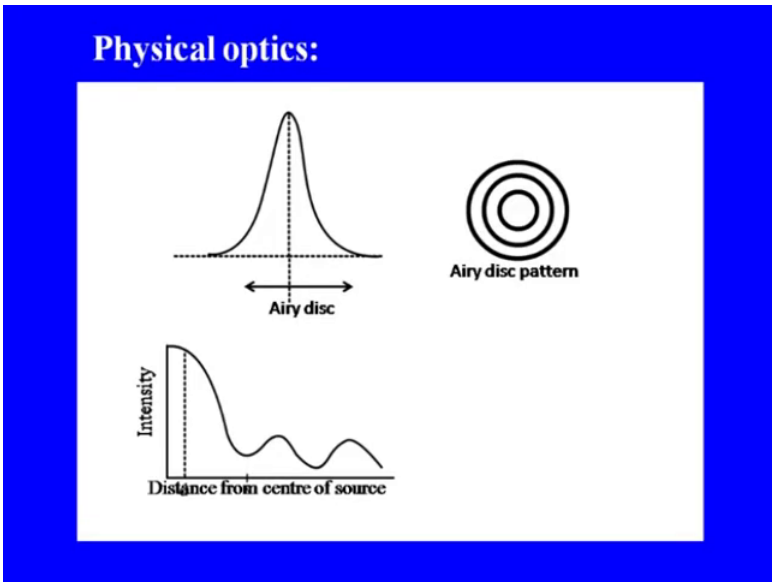
Now, let us go into the physical optics. The physical optics explains image formation and resolution, the two important things, image formation in terms of contrast or sharp images being formed and resolution. Now, here physical optics light is considered as electromagnetic radiation. It is a wave form. Light is diffracted at edges. Apertures can interfere constructively or destructively.

Now, when diffraction occurs or when light passes through a specimen, then diffraction occurs. This is because you can consider that there are edges or there apertures. In them, a lens can also be considered as an aperture and can diffract light. So, what happens is that when an illuminated point in the object plane, it appears as a circle of light surrounded by series of bright concentric rings resulting from constructive interference in the image plane.

Now, the pattern is defined as an airy disc or airy pattern, airy discs. The center light disc and the concentric rings total in totality, it is airy pattern. Now, resolution is determined by the ability to separate different airy discs patterns arising from each points closely

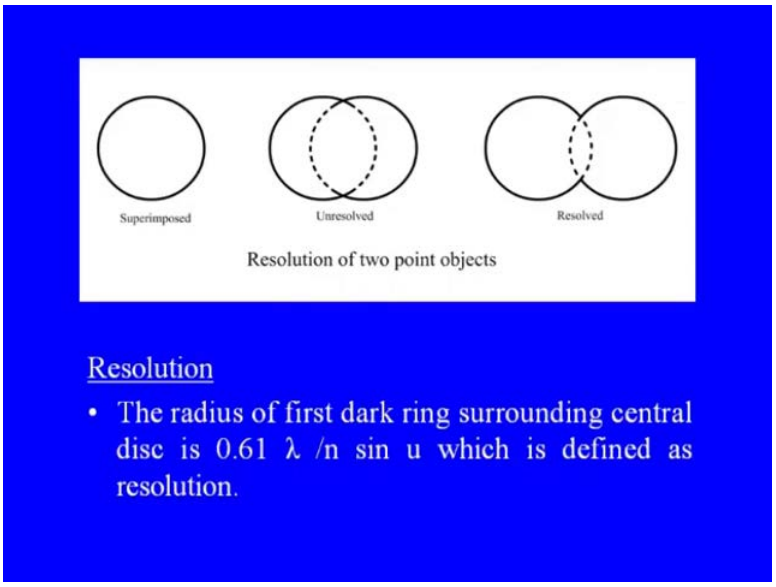
placed. There will be lot of points from where diffraction will occur. Each will give its own pattern. Each pattern is to be separated.

(Refer Slide Time: 36:29)



As you can see here that airy discs, in the sense you have airy disc pattern. Here, there is a center bright light. Then it is surrounded by concentric rings where light and dark rings, which are resultant of destructive and constructive interference. Now, if you consider here, like I was saying, if the 2 airy discs are, if the two points are too close, the airy disc patterns or diffraction patterns overlap, then you will not be able to distinguish them. If 2 airy disc patterns particularly the center light disc can be separated, then the 2 point objects can be resolved.

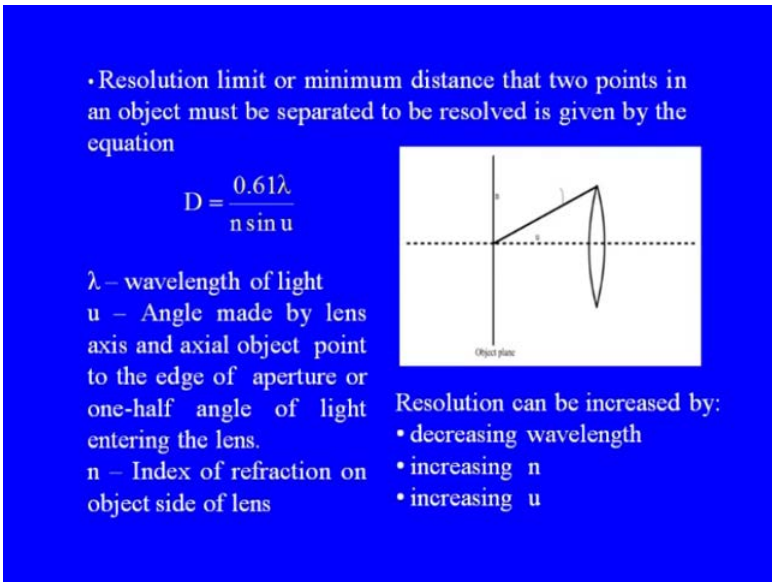
(Refer Slide Time: 37:19)



This could be seen here that in the first case, you can see only one airy disc, which you can see one circle. This is because both patterns are superimposed. The two point objects cannot be resolved. Second one is that they are partly overlapping. Since, the patterns are partly overlapping, it is not completely resolved.

In the third case, you can see that two point objects are completely resolved as there airy patterns or airy disc center light is only partly overlapped, so to get the resolution or to see two point objects as two separate entities. These two diffraction patterns have to be resolved. This had defined resolution in terms that radius of the first dark ring surrounding the central disc has been calculated to be 0.61 lambda upon n sin u, which is defined as resolution. We can see it in detail here.

(Refer Slide Time: 38:29)

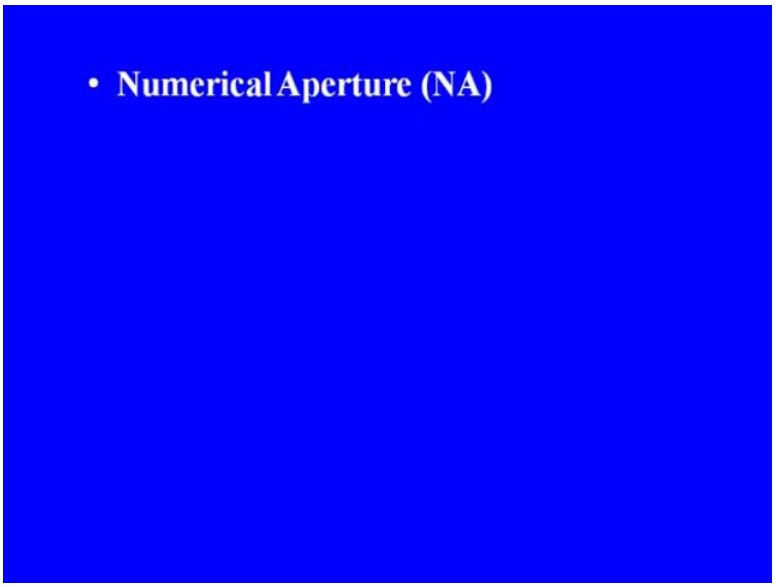


The resolution limit or the minimum distance that two points in an object must be separated to resolve is given by D, which is the minimum distance. It could be resolved by 0.61 lambda by n sin u. Now, here the minimum distance or D, which should be resolved, is limited by the wavelength. So, if you see lambda as the wavelength, u is the angle made by the lens and at axial object. It is the half the angle, you can say of the total cone of light. You can say the aperture or one half angle of the light is entering the lens here.

Then, n is the index of refraction on object side of the lens. Now, most of the time, you will have air as the medium and n will be the index of refraction of the air. It could be lenses where you could have oil immersed lenses or water immersed lenses as you will see. Now, resolution can be increased by decreasing wavelength like here. One has to understand that as D decreases, we say resolution is increasing. So, D has to decrease in order to increase the resolution.

So, if you decrease the wavelength, D will decrease. Hence, the resolution will increase with increasing n that is refractive index. So, when you have oil immersion lenses, the refractive index is higher. So, you can get better resolution and increasing u. As you bring the lens closer to the object, the u will increase. That will certainly increase the amount of light gathered. That will certainly increase. Therefore, it will increase the resolution obtained.

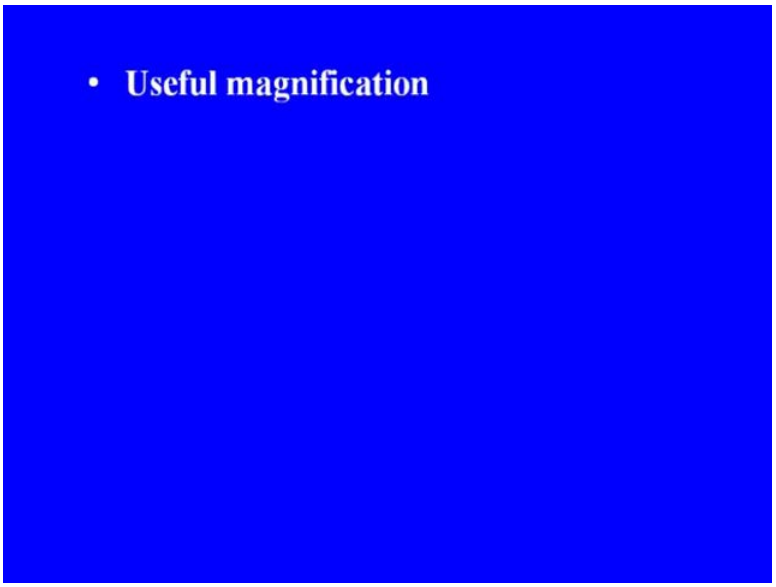
(Refer Slide Time: 40:24)



Now, the denominator that is n sin u is called numerical aperture. Now, this in optics as we say, numerical aperture of an optical system is a dimensionless number. It characterizes the range of angles over which the system can accept or emit light. NA is constant for each lens and is a measure of its light gathering qualities. The maximum possible numerical aperture for an object designed to be used in air is 1. This is because refractive index of air is 1. Practically, it can be made it can be up to 0.5, but you can increase that numerical aperture. For example, glycerin refractive index is 1.33. For immersed oil, it is 1.51. For water, it is 1.33. For glycerin, it is 1.47. For immersed oil, it is 1.57.

Now, for objects immersed in oil, numerical aperture is up to 1.5. So, you can increase the resolution drastically. The wider the angle the lens is capable of receiving light, the greater will be the resolving power. How can we understand this? This could be understood by simple diffraction term. Now, as the object diffracts the light, then higher the order of diffraction that is if two point objects or two points in the specimen are closely spaced, then they will diffract the light at higher angle rather than the two points, which are not so closely placed. So, higher the order of diffraction that is higher the angle of diffraction, the more will be the information contained in it. If the lens can collect higher order of diffraction, then it can provide higher resolution. You can say that the lens has higher resolving power.

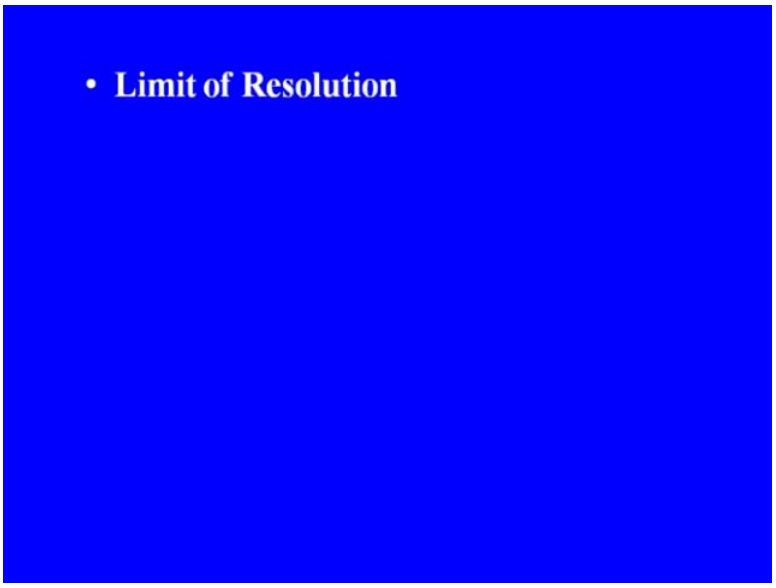
(Refer Slide Time: 42:46)



Now, what is like useful magnification? For example, we can use different lenses with different magnification powers. What is the useful magnification in terms of resolution? Now, here it has been given that useful magnification has been calculated approximately 1000 times NA of the objected lens that is the numerical aperture of the objected lens beyond. This results in empty magnification and the image deteriorates.

So, what we have to see is that you have say objective of 40 x and say other lens that is we will discuss of 5 x or 10 x to certain level. If NA is 1, then 1000 times 1000 x can be the maximum magnification. You can go to get maximum details of the specimen. But, beyond that point, the image will be deteriorated. You will not get any more information. Now, higher NA is achieved by using lens with a short focal length, which allows lens to be placed very close to the specimen and decreasing the wavelength.

(Refer Slide Time: 44:08)



Now, limit of resolution, if you consider all different things, limit of resolution, light microscopy can be determined by substituting minimum possible wavelength and maximum possible NA. Now, that value obtained with the conventional lenses is about 0.2 micrometer, which is sufficient to resolve large cellular organelles. So, that is the limit of light microscopy because you cannot go below a certain wavelength for light microscopy. You could have only; numerical aperture could reach up to certain place.

For example, in oil immersion lens, it could be highest. So, if you substitute maximum value for this, then resolution will go up to little less than 0.2 micrometer. It is quite good for many cellular experiments or to view many cellular organelles. If you compare it with naked eye, the numerical aperture of naked eye is 0.004. The limit of resolution is 0.1 millimeter. So, you cannot see at the best. You can see the objects or the points, which are separated by 0.1millimeter.

Let us go into little bit. As we have seen through the physical optics and geometric optics, let us summarize the two things in geometric optics. What we are discussing is the focus and the aberrations where you have seen that lens focuses the light on a certain point. It is called principle focal point. The object, the image formation takes place in terms of where the object is placed.

Other thing was the aberration. Aberration was that lens fails to focus light at the lights of different wavelength at the same place. It is a chromatic aberration and the spherical

aberration. This is because of construction of lens or because of light passing through different parts of the lens is not focused at the same place. Therefore, the spherical aberration or point imaging aberration occurs. Both have been corrected for in the advanced microscopes.

Resolution is again function of like we have seen the wavelength, the refractive index and the angle at which the light is being collected. So, the n sin u that is numerical aperture is very important here in terms of determining the resolution and in terms of light gathering capabilities of a lens. Today, we will stop here. In the next lecture, we will start with the microscope, which is compound microscope, different parts. We will discuss different parts of compound microscopes and the importance of those constituents.

Thank you very much.