Techniques of Material Characterization
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Lecture-02
Topic-Image Formation, Resolution, Magnification, Depth of Field and Depth of Focus

Welcome everyone to this NPTEL course on techniques of materials characterization. And we are continuing with module 1 that is introduction to microscopy and basics of optical microscopy. And today's lecture we will discuss about image formation, resolution, magnification, depth of field and depth of focus that is some general concepts of microscopy. And these are broadly the topics that we will be discussing.

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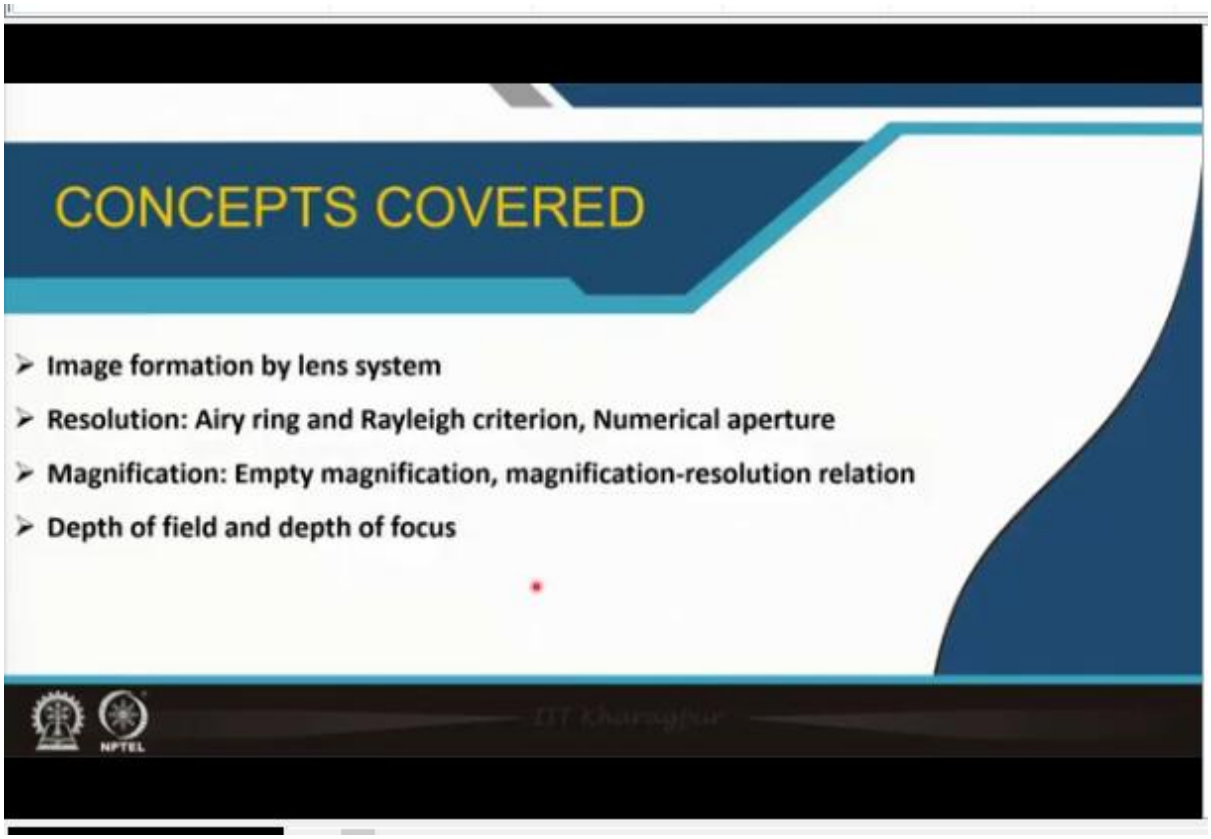

Image formation by lens system and then resolution and the resolution, we will discuss about airy ring, Rayleigh criterion, numerical aperture, magnification, there we will be discussing about empty magnification, magnification resolution relation. And then we will be finally we will discuss about depth of field and depth of focus.

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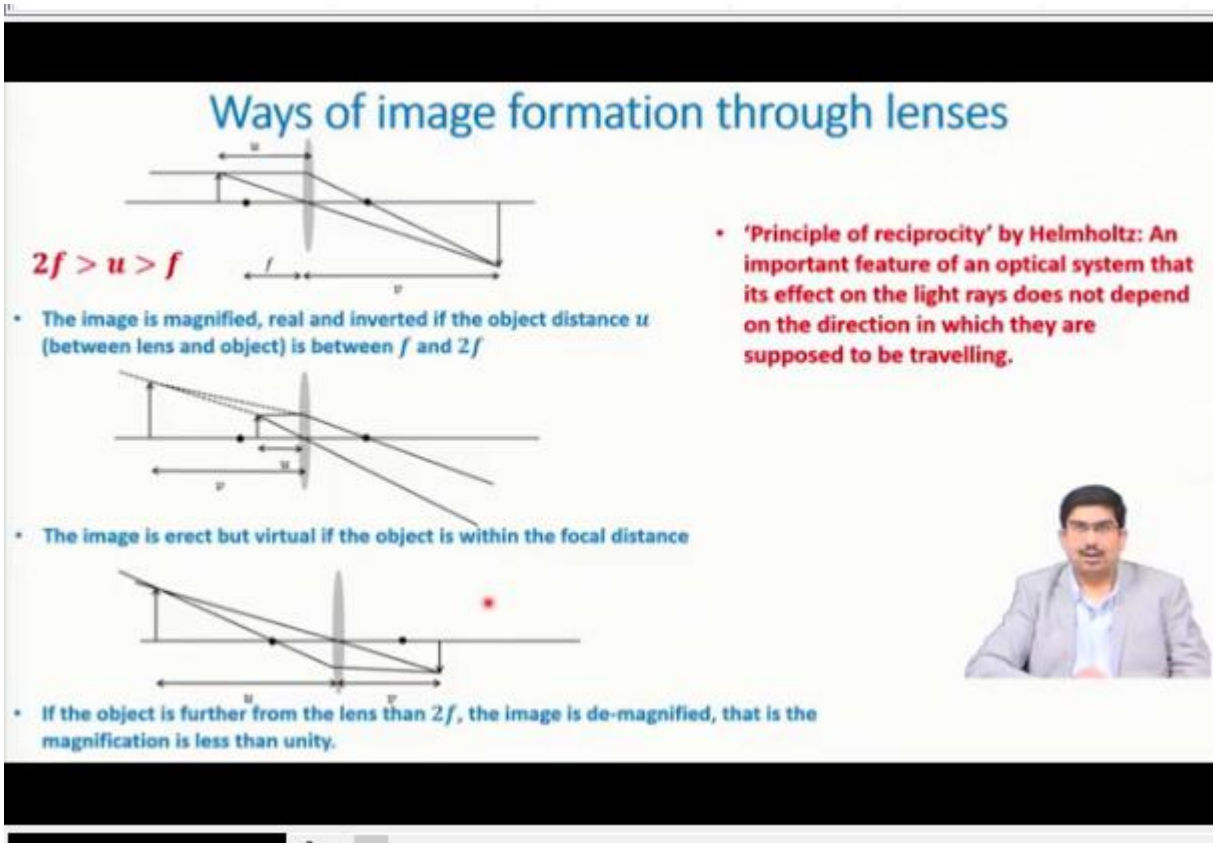

So, let us discuss about the basics of image formation through lenses and this is something that possibly all of you know from your class to physics and the first conditions that will happen here you have a lens and in the lens, if your object is in the one side and other side the image will form. So, in order to fulfill this, the object should be placed within a distance of f and to 2f, where f is the focal length of this lens.

And the object will be magnified and the image will be magnified and the image will be real, but it will be inverted if this condition is satisfied. There is another condition when you that is the object distance is shorter than f that means the object is placed within the focal length of the lens. In that case, the image will be erect, but it will be virtual image. So, there will not be any real image forming.

And then the third condition that will happen is that when the object is our object distance is greater than 2f, then what will happen is that you will still have an image formed on the other side, that image will be demagnified, that will be a real image inverted image, but it will be a demagnified image. So, from if you compare these 2, the first one first condition and the second condition, there is a very important principle that comes out of this.

And that is called Helmholtz principle of reciprocity, that is this one and this one is exactly the same, if you see them from 2 different sides, that means if you consider this as your object, then and this is your image, then this one is exactly same as this. So, basically, the principle of reciprocity says that, in any kind of lens based system, the effect on the light rays, it is said in terms of light, but it is also applicable.

For any kind of lens based system, that the effect of the lens system on the light rays does not depend on the direction in which the lights are traveling. So, it is basically the same. Now, if you discuss or if you think from a microscopy point of view, then this condition and this condition these 2 are not suitable for microscopy, because in this case, the image that forms out of this object is not real.

And in order to see something the purpose of the microscopy is basically to make something as magnified, so that it will help our naked eye to see is very small, that is the basic definition of microscopy. So, from that point of view, neither this condition nor this condition is suitable for microscopy here the image is real, but it is demagnified and in this case the image is not real at all, so will not be able to see.

So, the only condition which is very useful for us is this one, that is when object distance is within f and 2f and little later you will find out that for getting the maximum magnification basically the u is almost close to f that means the object is placed almost at the focal length of this lens system.

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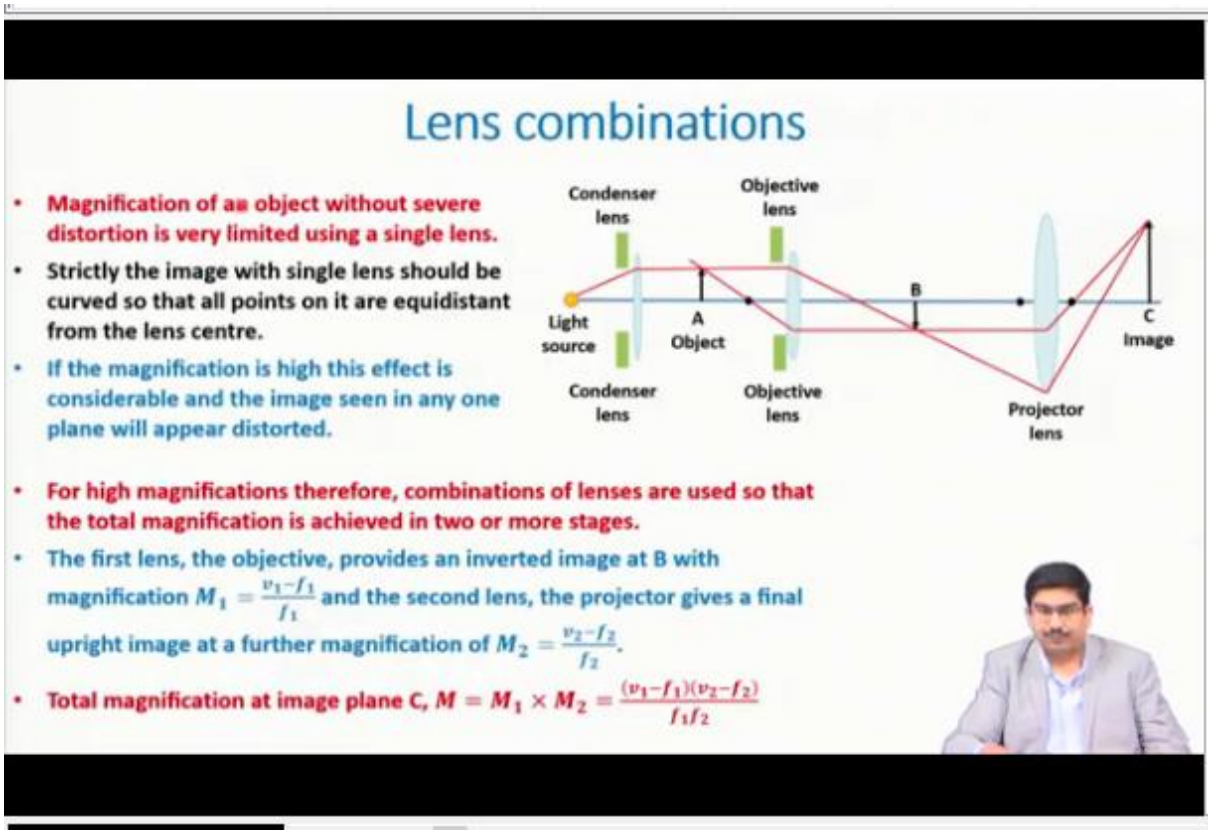

So, let us see that lens combination. So, from one single lens what will happen, we will get a magnification of an object, but that magnification will be limited. If we now want more magnification from any lens system we have to add some other more number of lenses and in those cases the magnification will just be multiplied. So, ideally with one single lens just by changing the focal length, we will be able to get any magnification we want.

But what will happen is that there is some other problems appearing that is called aberration. We will discuss about aberration problems in little later. But using one single lens is not an advantages situation. So, then for getting higher and higher magnification, it is much wiser to use more than one lens. And in most of the general conditions and most of the imaging systems, most of the microscopes, we tend to use 3 different lenses.

The first lens is called condenser lens and basically the condenser lens source collects lights from the source and puts it on the object. And then we have something called objective lens which picks up the signal from this object. And finally, we have a projector lens and that projector lens forms the final image here and it is basically the magnification gets multiplied between this objective lens and the projector lens.

Then, now if we consider just this objective lens and projector lens, basically the first objective lens gives a magnification, which is equivalent to this M that is a magnification. And from there we will get if we use something called thin lens equation, I am not going to do that, but this is the amount of magnification that we will be able to produce. And the second lens that is a projector lens, it will further magnify by another amount $(v_2 - f_2) / f_2$.

So, all together to total magnification in the image plane that will come as $(v_1 - f_1)(v_2 - f_2) / f_1 f_2$. So, here you will see that just by changing the focal length, keeping even your object and image to be same or almost stationary, just by changing the magnification you can basically increase the magnified or certain object to any extent.

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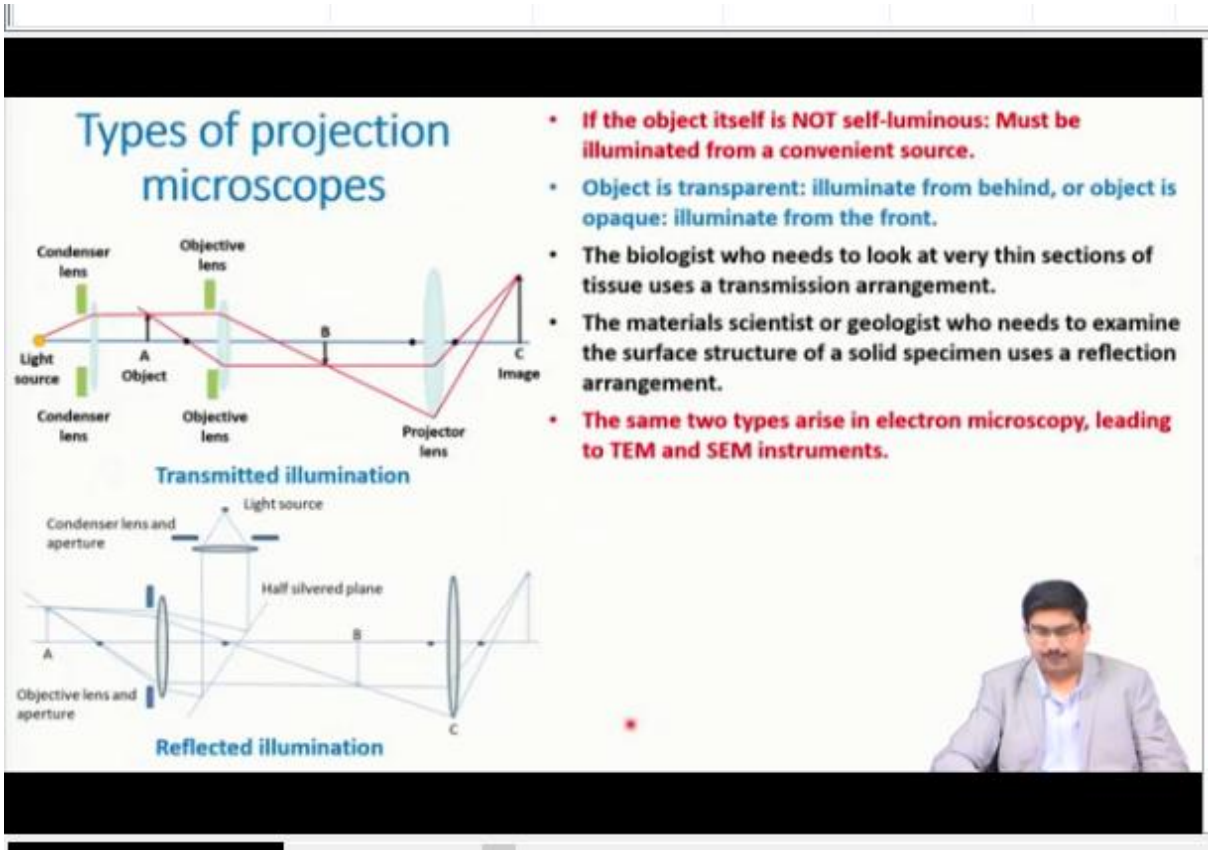

So, until up to now, we were considering that all these ways of image formation we were considering that basically the object is almost self luminous. So, then that is not typically the case for most conditions, the objects are never used to be self luminous and they must be illuminated by a convenient source, that is something like a light source. And this itself makes 2 very important divisions in the microscope systems in any kind of imaging system.

In one type what we have is a transparent object that means the light source stays at the back of the object and that is in the other side, this light passes through the object and then falls on the objective lenses. So, this is one type of illumination or one type of microscopy system. And typically this is used in case of the biologist's polymer scientist, who has something like if I am talking about this.

Just at this moment, I restrict myself within objects or optical microscopes. So, this kind of optical microscopes transmission mode, that is what we call is typically used by the biologists, because they have transparent specimens and also some kind of polymers also comes in a transparent mode, but most often, what we used to have is called reflection mode. And that is typically used by material scientists, geologists and all who has opaque specimens.

So, opaque specimen basically, light cannot pass through them and that is why it is a little more complicated design is there. In this case, what happens you have the same light source condenser lens everything is same, but you have some kind of a mirror which puts the light on this first it is putting the light on the object and then the light gets reflected and that reflected light is used for imaging.

So, the objective lens and the projector lens everything remains here, but more or less the light source condenser lens and objective lenses everything remains in the same side. One important point you must remember and most of my students get confused by this one is that this is a schematic and here it is appears that as if the reflected that light used for illumination and light that passes through the object of falling or going through the same direction the same optical path, but actually when I show you a real microscope, you immediately imagine that these 2 paths optical paths are different.

So, these 2 are not the same line or they are not traveling following the same path, the light used for elimination and the light use for imaging. These 2 are separate following. They are going through the 2 separate paths, so do not get confused there.

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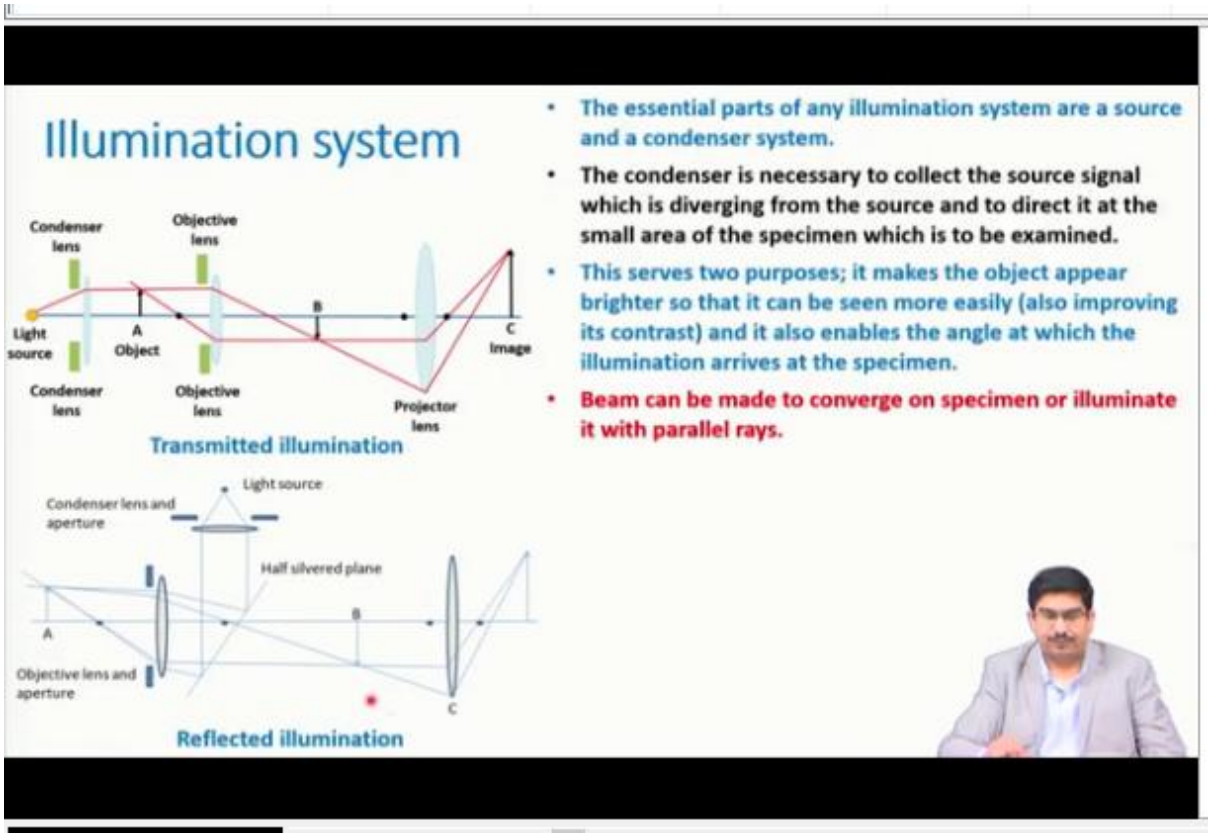

So, again, if we look at the illumination systems the illumination system typically they contains a light source and a condenser system and basically the condenser system is our condenser lens or condenser system and typically what it contains is a lens plus an aperture. So, any kind of lens you see basically condenser lens and objective lens that comes with an aperture system. So, together they formed this condenser system and objective lens system.

So, the condenser lens this serves 2 different purpose is that is it makes the object appear brighter, that means, it falls it makes it focuses the light on the object. So, that the object can be seen and the enough contrast can be generated from the object and also at times if you need you can also change the angle of this illumination that beam with which you are illuminating this object you can change that angle and plus you can either make it focus on the object or you can even make it parallel beam. So, these are the purposes of illumination system.

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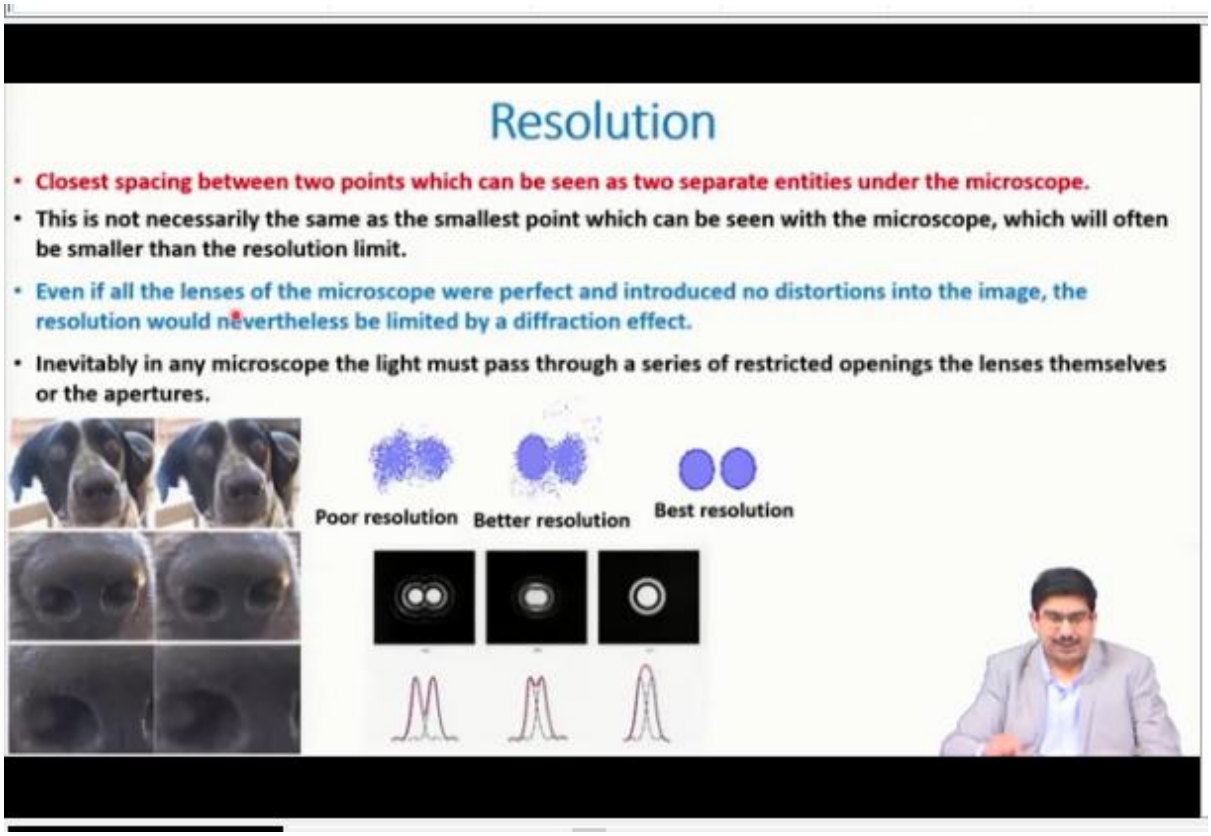

With this now we are moving towards another very important point and another very important topic for any kind of microscopy system, does not matter whether you have an optical microscope or you have an electron microscope, this is the most fundamental concept of any kind of microscopy system. The first one is the resolution. So, what does we mean resolution?

And this is what possibly everyone knows, if you go and buy your phone and you are using a camera or phone or any kind of digital camera, the first thing you will be possibly asking what is the resolution of that, 16 MPa if you go for a very costly phone possibly it can offer even higher resolution. So, what exactly resolution means? Now resolution, the way you can define it is something like this.

It is a closest spacing between 2 points, which can be seen as 2 separate entities under the microscope. So, if you would like now, if you just see this entity, so, what I am seeing first is 2 different spots and these 2 different spots possibly I can see them very distinctly. If I just neglect all other spots and just I focus on the central spot, these 2 central spots I am possibly seeing here separately.

But in this case, I am not able to see those 2 spots separately, they are somehow overlapping and I am not in this intermediate regions, I am not able to see them separately and in this is an extreme case where basically these 2 spots are completely overlap and I am just seeing 1 single spot. So, the resolution of this system through which this sports are made basically.

So, the resolution of this system is defined by this distance, the distance between these 2 spots when they are seen separately. So, in microscopic term, this is what is the resolution basically. One thing critical point that you need to understand is that resolution basically is not exactly the same as the smallest point or smallest feature that can be seen under the microscope, which will sometimes be often smaller than the resolution limit.

That is dependent what kind of a smaller feature you will be able to see that depends on the magnification, but whether you are able to see that smallest feature with sharply and clearly that is dictated by the resolution, we will come to that when we discuss about resolution and magnification and then we will go to this point more. Just here you can see that these are the 2 points if you consider these 2 points here, this is possibly you can call it a poor resolution.

Because these 2 points are many of the features are basically they are sort of overlapping with each other. This is somehow better one of them at least you are able to see them as one single spot and this is of course the best resolution when you are able to see both of them as separate spots. And again, you can see the same thing if you take this poor dog and try to take their images This is what taken in a digital camera, no optical or no microscopy system I am going.

And then if you zoom a tap in one case, you are seeing that you are able to see this nose and this features very sharply another case you are not able to do that. So, you can very safely say that this is having a better resolution then this image. So, to begin with, to start with, if I take these 2 images and if I zoom it up the image with a better resolution, I can able to magnify it much better and much further and even at a very high magnification I can able to differentiate between various features here**.**

Now point is if we consider in a microscopy systems, then even if the microscopy system is very, very perfectly we will understand this, the importance of this when we will discuss about something called aberration. So, even if all the lenses, all parts of microscopes are

perfect and they introduce no distortion into the image, still the resolution of any microscopy system or resolution for any kind of optical system even for your cell phone camera or a digital camera, that is limited by something called diffraction effect.

And this diffraction effect, what it does is that when light or any kind of electromagnetic ray for that matter, it also happens to the electrons or x rays everything. So, what happens is when this light that passes through a series of restricted openings, then they sort of forms instead of one single spot, they actually form certain level of cones.

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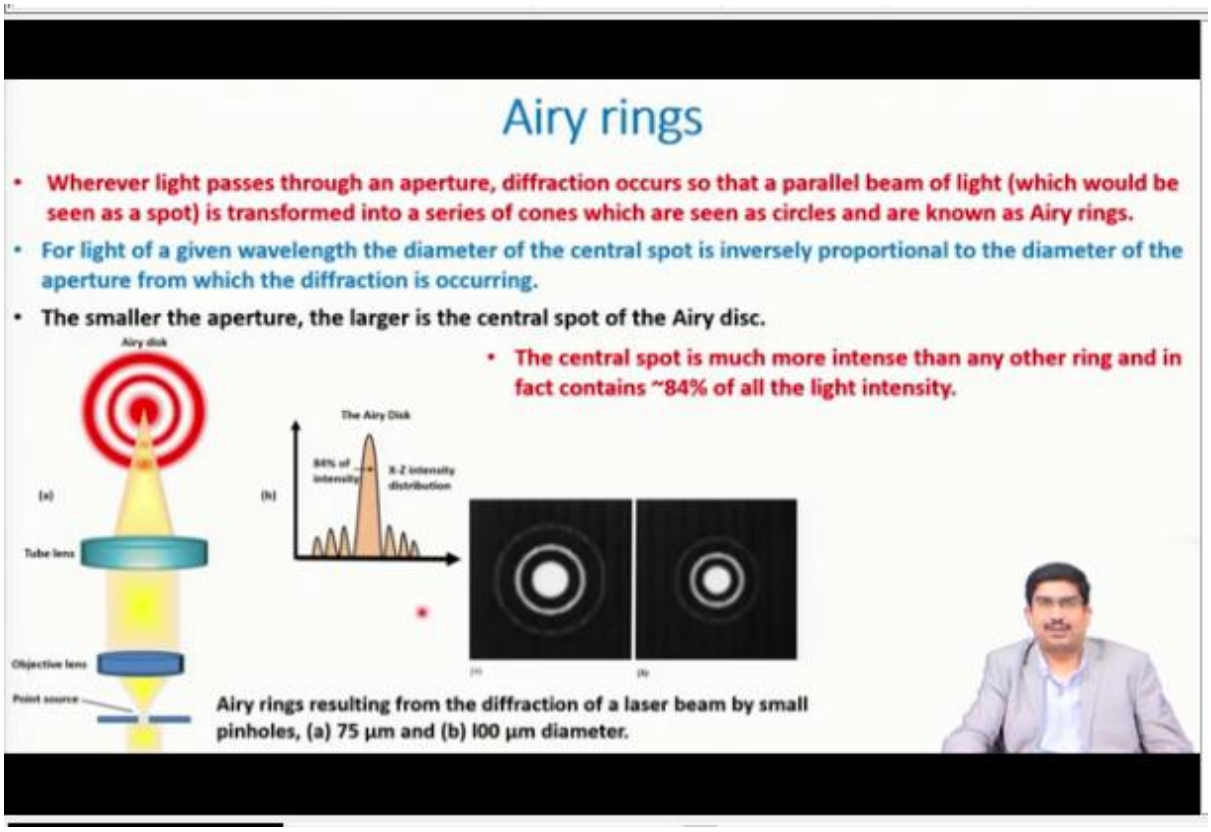

And that is what is shown here that, when the light passes through any small opening and in this case, the small opening you can consider that as the apertures that is used along with this either condenser lens system or objective lens system, the apertures which are used that can you can consider them as this very small opening. So, when this parallel beam of light which is otherwise should be seen as a central justice spot.

If there is no diffraction effect, it should be seen as a spot you do not have any sample here, just the light source and that is passing to the condenser system and then you have the objective lenses or objective system here and finally, you are checking it out. So, if there is

no diffraction effect, there will be only a central spot, but what will happen this diffraction then what it will do is that it will make that parallel beam that small central spot that will be divided by many, many cones of concentric cones.

And if you take see them in the image plane basically you are taking a section of this concentric planes which are three dimensional, you will be seeing some concentric circles, the central spot will be there and that central spot will be the brightest, it will contain around 84% of all the intensity and then you will be seeing some other concentric rings and the intensity of those concentric rings will continuously decrease here and do not think that this is just a concept. It is really true.

So, these are the 2 airy rings. So, these are called the airy rings the other thing, so, the central spot and then all these concentric circles, these are formed by diffraction and they are called airy rings. So, the same thing is shown here experimentally and these are the airy rings. This is a central spot and then you have some airy rings here. Now, what happens is that, the smaller the aperture, the larger will be the central spot in this airy disc that is what again; it is related to the diffraction effect similarly.

So, that means, when you take it something like with 75μm of aperture or small pinhole, that is what and here you use a laser beam that is why you are able to see this airy rings basically you are imaging that. So, this one is much smaller aperture and much bigger central spot, whereas, this is done with 100μm diameter and that is why this is a much smaller one. So, basically if you want to reduce this diffraction effect, one point immediately you can understand that I should or we should keep the aperture as large as possible and you will understand why.

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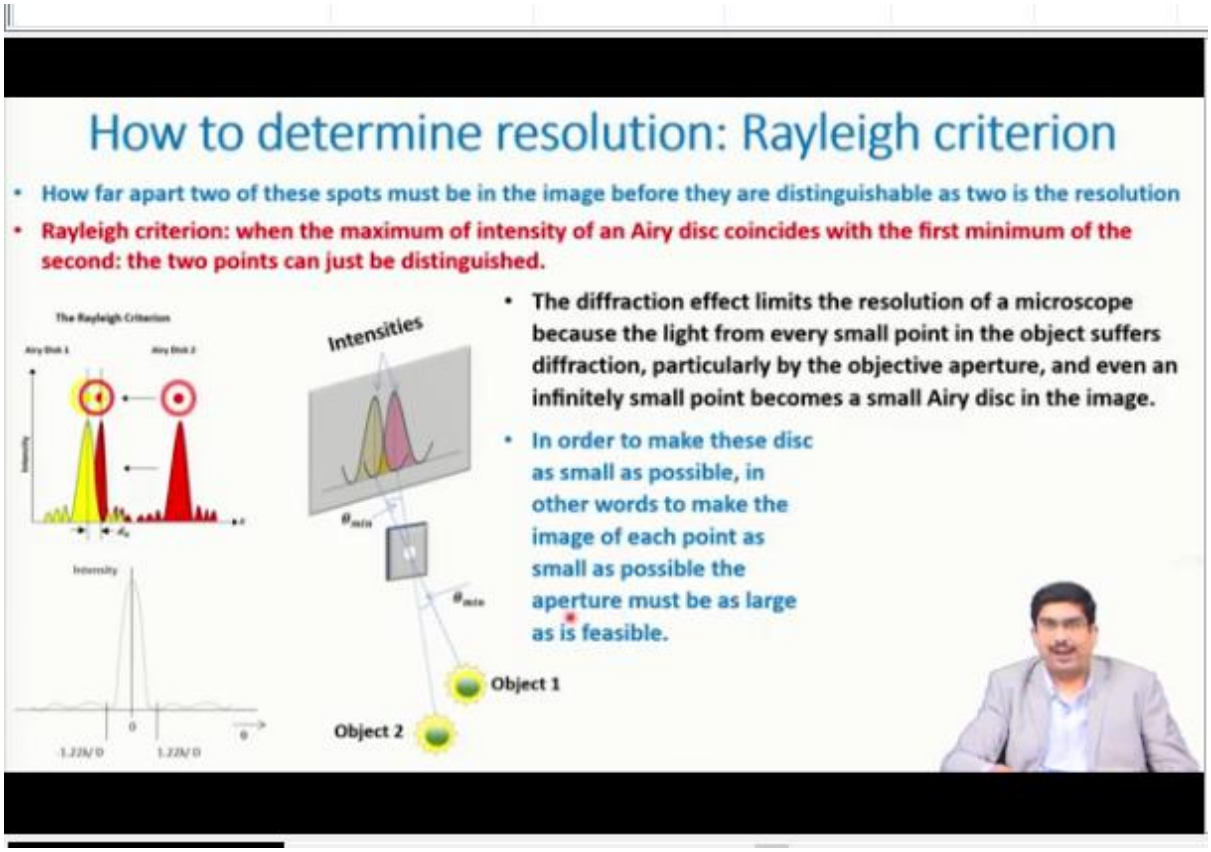

Now, because the question is now, how to determine this resolution and this is where comes a person who has given the criterion lord Rayleigh that is known as the Rayleigh's criteria. So, the question is how far apart 2 of these spots must be in the image before they can be distinguished as 2 separate and that is what we have defined resolution in that time that 2 different spots should be distinguishable the minimum distance between them.

And what is that minimum distance that is what lord rarely has said that when the maximum intensity of airy disc. So, you have 2 different airy rings forming here. So, like you can imagine that you have 2 different objects and 2 different objects mean 2 different sources. And that is going through a small pinhole same pinhole and they will be forming their individual airy rings here.

So, there will be a small, there will be the maximum intensity and then there will be the next maximum diffraction ring, the airy rings intensity, so these 2 intensities. So, what will be the maximum or what will be the minimum distance between these 2 intensities here? So, what Rayleigh says that when the intensity maximum of one airy ring coincides with the first minimum of the second one.

That means this is the condition the first one the first maximum has to coincide with the minimum, first minimum of the second one that is exactly what it has happened here. The first maximum is coinciding with the first minimum of the second one and that is the limit up to which you are able to see, you will be able to see these 2 spots, you will be able to distinguish them if these 2 spots are anywhere nearer than this distance then you will not be able to see them.

The condition will be something similar to this, they will start overlapping and if you bring them even closer that is what is shown here also, if you bring the even closer they will just overlap and form like one single spot. So, this is what exactly is what Rayleigh's criterion happened. Now, how is it important for an image formation? Now, the diffraction effect basically what happens is that in your object you can imagine that every point depending on the again the beam size, the beam that the condenser lens is forming.

And the beam that light beam or electron beam that is falling on your object and from the object again if we can imagine it gets reflected or transmitted whatever, then every point on this object depending on the beam size every object point can be considered as a source. So, these 2 sources you can imagine that they are basically forming on the objective plane. And this is the distance between these 2 object points, minimum distance that will define their resolution.

So, that is the importance of diffraction in microscopy. In microscopy, those 2 object points the distance between those 2 object points and the diffraction effect finally limits the resolution, what should be the minimum distance between these 2 object points, so, that they can be seen in the image as 2 separate distinguished, 2 separate points all together.

So, of course, as I said that in order to make this disc as small as possible, that means, in order to increase this images, I mean, in order to increase the resolution in the final image, the aperture must be as large as possible, because, just now, I discussed that this size of this airy rings or size of the central spot that means, the size that this the grid of this maximum that is depending on the size of the aperture.

The larger, the size of the aperture, the smaller this width of this maximum and that will be better resolution, because then there will be better chances you can bring them much closer to

each other and still they will fulfill the Rayleigh's criteria, they will not overlap. So, that is how Rayleigh's criterion is relevant or the resolution the concept of resolution is relevant for image formation.

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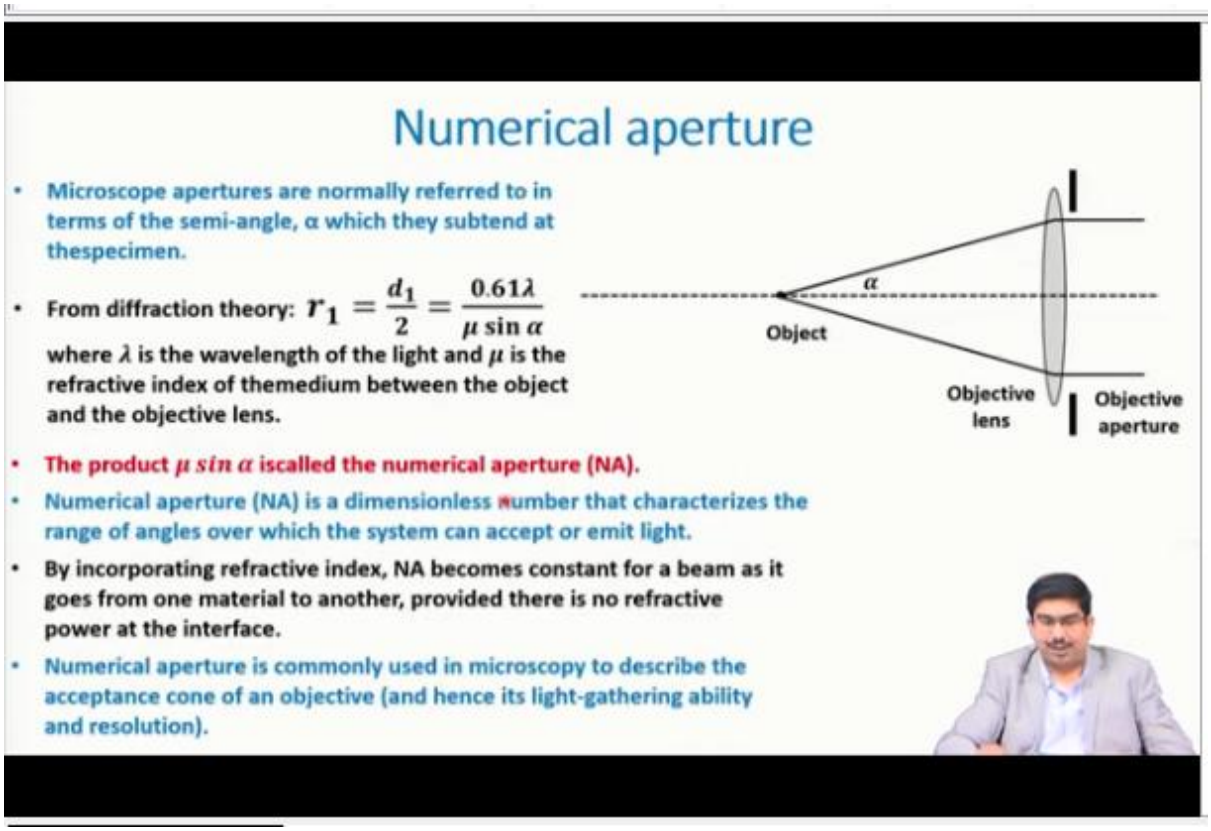

Now, we are coming to another concept that is called numerical apertures. So, just now, I told that these apertures are basically controlling the resolution. And microscope apertures are typically they are characterized by something called their numerical aperture. And that is related to this alpha that is the angle semi angle that they basically subtend at the specimen. That that is how or that is what is the characteristics of any kind of aperture systems.

How much this lens and aperture system how much they make the light rays bend and where exactly they focus, that is what. So, from a diffraction theory, again, I am not going in how this is derived, you can go through the suggested reading those books, the relationship that you can find out is the minimum distance that is this our r_1, the minimum distance or the resolution limit, this $r_1 = d_1/2$.

So, d 1 here is the basically the central spot the diameter of the central spot. So, the resolution is related to the diameter of the central spot and you can typically find it out from here that

this criterion when this criterion has to be true, this Rayleigh's criterion to be true, the minimum resolution that or the distance between these 2 intensity or the central spots will be $d_1/2$, basically and from there, you can derive this relationship that the resolution is related to the lambda that is the wavelength of the incoming signal.

And then mu sine alpha, where mu is basically the refractive index of the medium between the object and the object lens and the objective lens. This is the refractive index and alpha just now as I discussed is the semi angle of this lens system. Now the product mu sine alpha is basically called the numerical aperture of this complete lens system here. This is a dimensionless number, the numerical aperture is basically a dimensionless number, and it characterizes the range of angles over which the system can accept or emit lights.

So, it basically deals with the amount of light this aperture system takes up and then how much it is able to diverge or it is able to converge them on the object. So, that is what is basically the numerical aperture. And now, with the introduction of the refractive index, numerical aperture basically becomes constant for any beam as it goes through from one material to another material.

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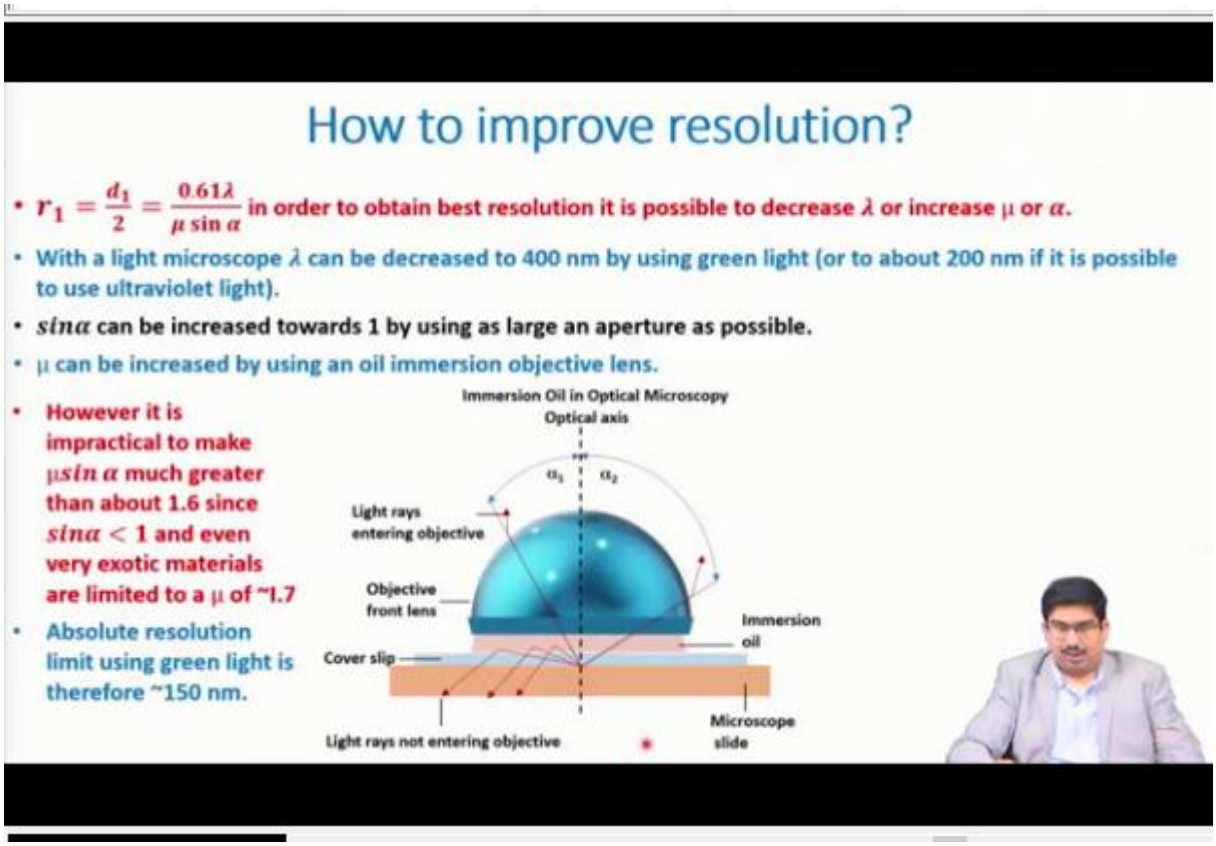

So, how to improve resolution from the concept of numerical aperture and this relationship that just now we have discussed. So, how we can improve resolution of any microscopy system is that is what we want, basically. So, in order to achieve**,** maximum resolution, we have 2 options. Either we can decrease lambda or we can increase mu or alpha here from this.

So, increasing resolution basically means that we want to decrease this just do not get confused here, improving resolution, meaning that r value should decrease. That means 2 points which are even closer together we will be able to see if you want to improve the resolution. So, either we can improve either lambda or decrease lambda or we can increase μ or α.

So, with a light microscope, what we can do is that we can go down at the max 400nm by using green light or around 200nm to use ultraviolet light. And when we discuss about electron microscope, you will see that the λ value for electron microscope is very less, that is another one advantage. That is why electron microscopes are characterized by better resolution.

The second thing is of course, increase mu and alpha and here sine alpha can be increased all the way up to 1 by using as large as aperture as possible. That means, I can increase α and I can take sin α to the maximum of 1, but that means α is 90^{o}, I am not having any aperture that is as good as not having any aperture. So, what will happen is that basically, by changing the numerical aperture $\mu \sin \alpha$, it cannot go anywhere beyond 1.6, add the max I can go is 1.7.

That is what α is max limit is 1 and with very exotic materials, whatever the material I can use, I can use something like instead of air I can use immersion oil, I can go to an numerical aperture of max 1.7 and that will give me an resolution with this system around 200 nm not more than that and with possibly with very good or using ultraviolet rays or something I can go down all the way to 115nm that is what with any kind of optical microscopes, I can go down.

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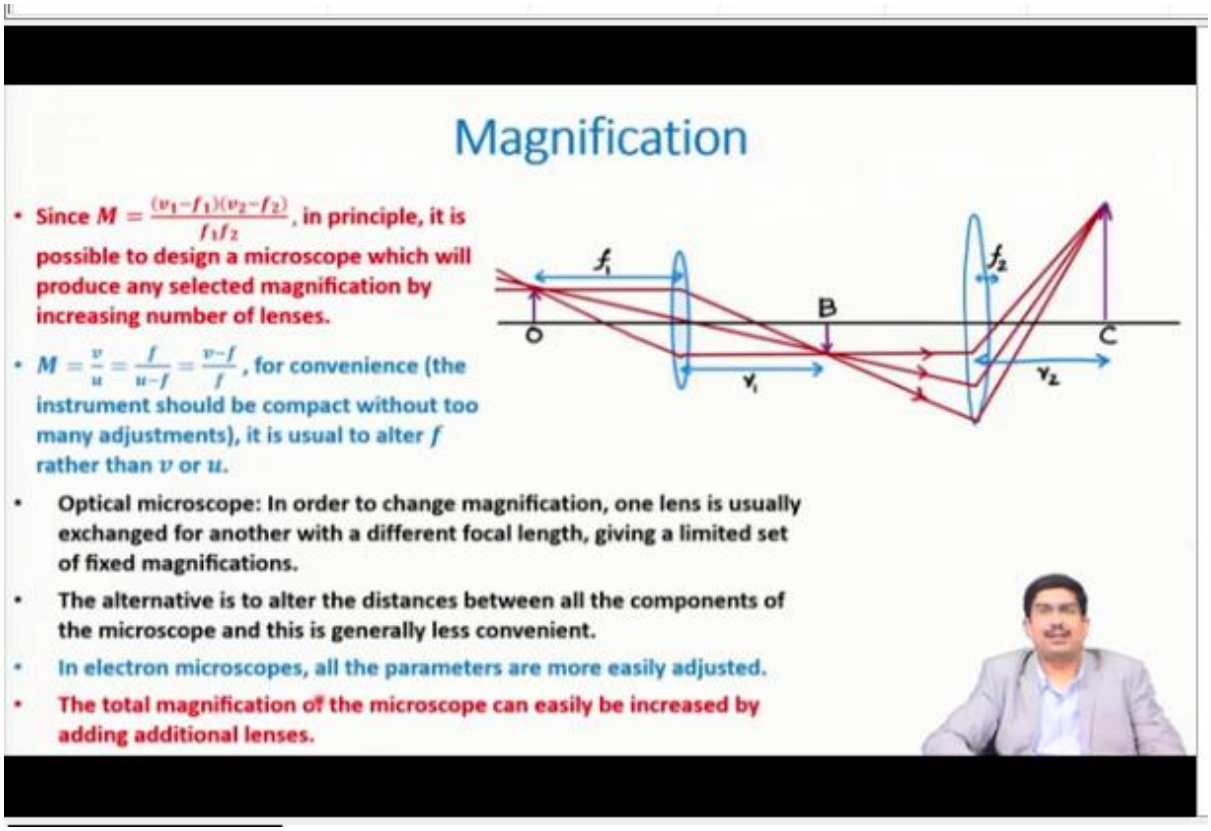

So, the next one is magnification and just now we discussed that in magnification using a lens combination the maximum magnification that we can have is this much. So, what happens is that if you see this magnification which is obviously expressed by the image distance by the object distance and also the magnification you can express it in this way from the thin lens equation.

So, practically what happens in a real microscope, there is this object and the image is basically fixed. So, this is for our convenience, we cannot change the object distance or image distance in an real microscope, that is very much troublesome. Rather than that what we used to do is we change this lens system that means we change the focal length, we take we go for a completely different lens, we go for a completely different focal length and in that case, what will happen we can play around with a magnification.

So, if you want to increase the magnification of any system or any lens system, it is wise to go for a completely different lens with a different focal length.

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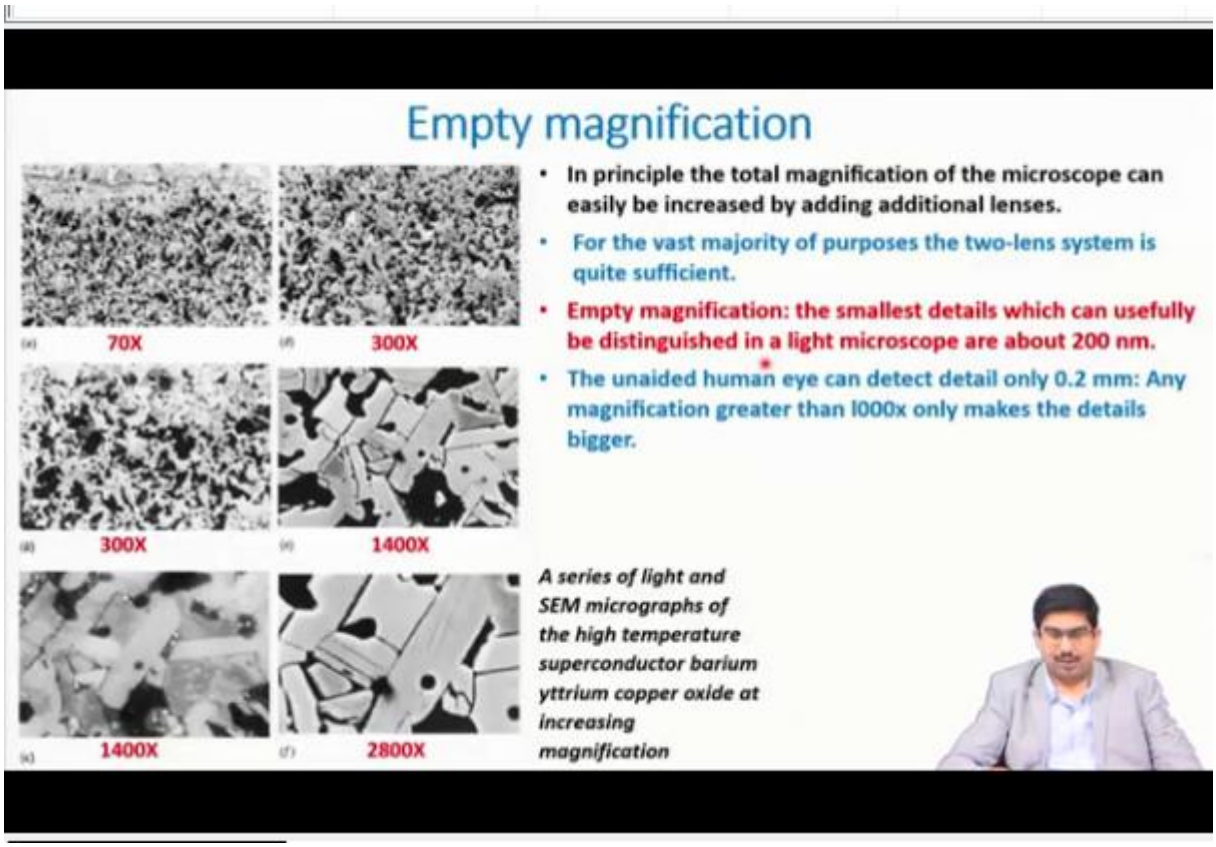

So, now as I said that we can discuss about the relationship between magnification and resolution and then there comes one important concept known as empty magnification. So, what is empty magnification? Just now as I said that the resolution of any light microscope is around 200nm. Similarly, and in the first class, I told that our eye is also kind of a lens system.

And the purpose of the microscope is to bring the images visible to our naked eye. So, that means we have to just match the resolution of our human eye, it has to match, the image that we are seeing, it has to must have a resolution, which matches to our human eye. And that resolution is around 0.2mm is what r if you do not believe me try to take a scale and then put it and close to your eye and try to see you cannot see anything less than this value.

So, that means if I now try to take some image in under an optical microscope, and I want my eye to be able to see the smallest feature, all I need is basically this much of magnification, some features which are 200nm away from each other, I cannot see anything less than this, I have to just zoom it up or magnify at the max 1000X to be able to see it with my naked eye.

So, that means in a optical microscope, there is no purpose or there is no advantage of going anywhere more than 100X magnification. If we try to do that, we can do that by using many, many lens system. But that will only increase the aberrations without really adding anything under resolution. That is exactly what is shown here. So, this side, it is all electron microscopy images of a supercomputer, barium yttrium, copper oxide.

And this side it is all optical microscope images. So, if you go from 70X to 300X, in optical microscope, definitely, you are able to now see many more features within this smaller feature. So, whatever features you are not able to see here, now by zooming in up to 300X, you are able to see those features. But if you zoom it further up to all the way up to 1400X, you are not able to see any new feature within this.

You are just making these same features what you are seeing here bigger and bigger. On the contrary, if you are doing it the same thing on an optical microscope and you are going all the way up to 20800X magnification, you are still able to see features within these features, which you are seeing at lower magnification, you are still able to. So, these features are again still you are able to resolve.

So, that is happening because electron microscope is having much better resolution than optical microscopes. So, the same thing is shown that why and this basically explains that why you should not go for a much higher or much more magnification with more than 1000X magnification in an optical microscope.

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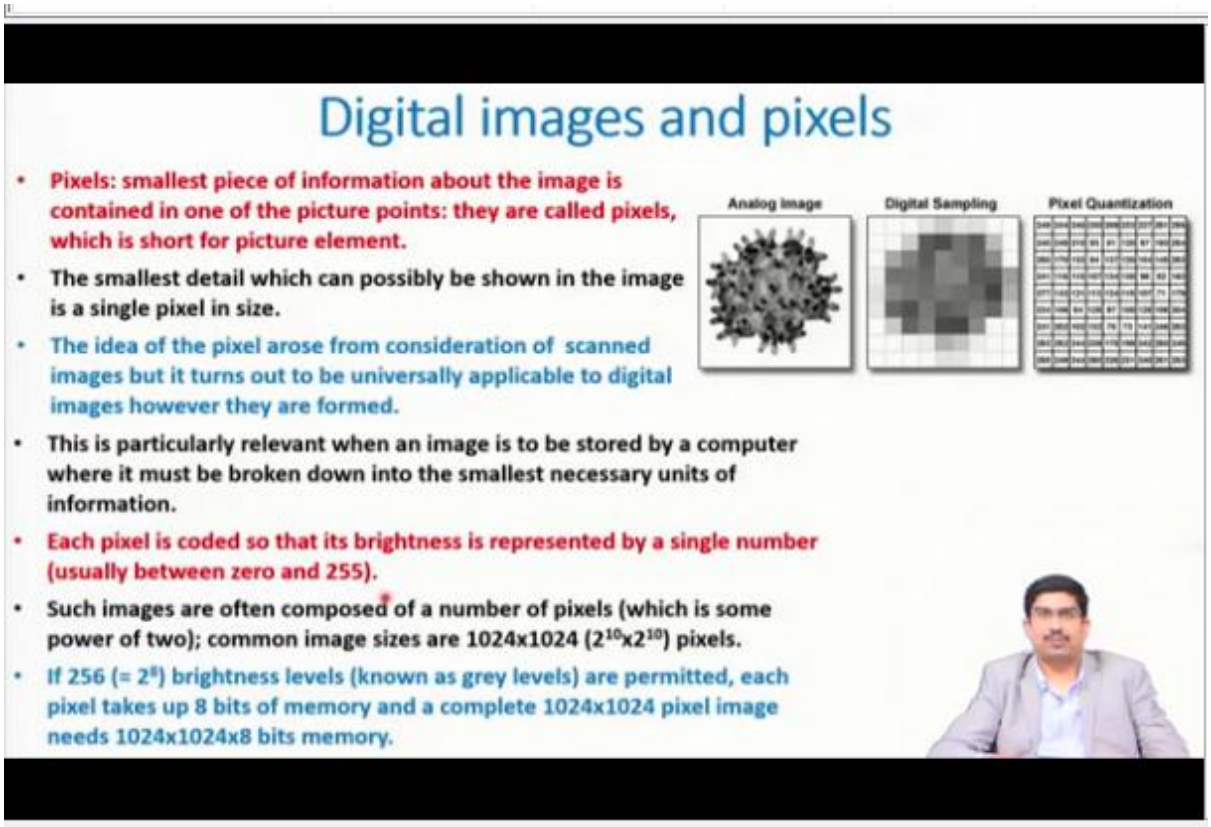

One more important point that we can possibly discuss here is the digital images and pixels. And basically digital images these days all microscopy systems are taking images and as a digital image. So, in a digital image, what do you do, this is an analog system or analog image and you correspondingly you create a digital image, digital sampling. Digital images are basically characterized by something called pixels, which is a short form of picture elements.

So, in this pixels, basically you have the x and y coordinates and then if it is a grayscale image basically. Then and you define a number which is from 0 to 255 and that number is characterized by a particular shade of grayscales. So, 0 is complete black and 255 is complete white, any number within that is a combination of black and white basically and that means it is a grayscale.

So, that is how the digital images are created from the analog signal. So, mostly in microscopy, you get analog signals and from those analog signals, you create these digital images that is through this pixel and if you remember I told that there can be parallel images and there can be scanning images. So, scanning images, the way it is captured inherently this

is almost like a pixel by pixel. So, the digital images that is captured from a scanning process just represent the same scanning or pixel by pixel scanning in a digital form.

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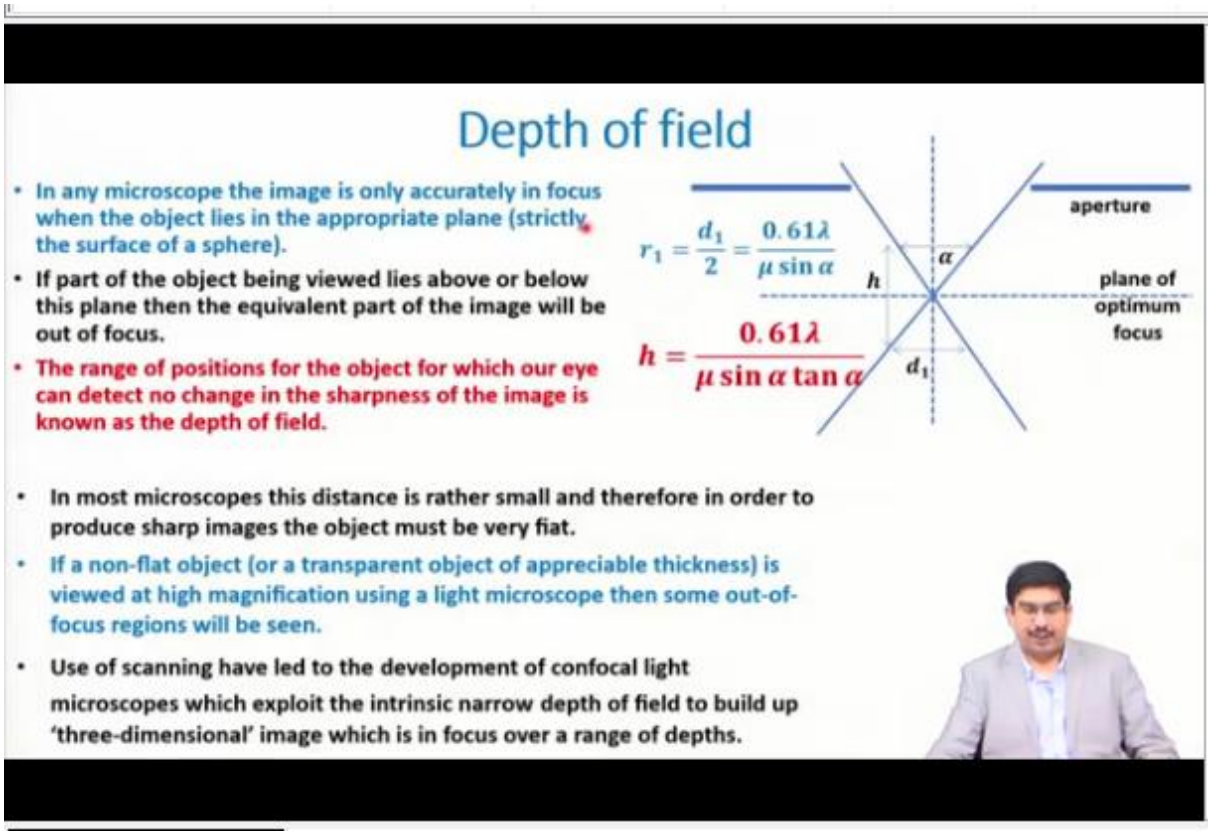

The next concept that we will be discussing about microscopy is depth of field. So, what exactly is depth of field? This is something to do with the object itself. So, this is the range of positions for the object for which our eye cannot detect any change the sharpness or other any change the resolution or any change in the quality of the image and that is called the depth of focus. So and why the concept of depth of focus appears.

That is because in any microscope, the image is only accurately in focus when the object lies at a straight line particularly but the lenses are all having some kind of a curvature. So, that means what is happening is that for object distance is changing corresponding to the lens for every object point the object distance is different for the lens because of this lens curvature and sometimes even the object also is not a flat objects.

So, object also sometimes having this curvature and object also has some surface roughness and so on. So, the object is changes with respect to the lens and correspondingly in the image side also the image distance changes for this object and altogether what happens is that the

object is not focused. So, in focus some regions will be appear out of focus and the depth up to which rather the surface this curvature or the roughness of the object for which this entire region will appear in focus is the depth of field for any kind of lens system.

So, this is again it is related we can calculate or we can imagine this or we can calculate this from the resolution criteria. So, what you can see here, this is the resolution criteria that we have already discussed. Now, if we consider this as this schematic, so, what happens is that between this age up to this distance as we know that resolution = $d_1/2$. So, this distance this spatial resolution up to here to here the object or every point of the object is still within the resolution limit.

So, within this height, we are still within the resolution limit. So, we can very safely say that this is the depth of focus for this particular lens system. And this depth of focus again, I am not going in how it is derived from the geometry you can derive this one as $h = 0.61\lambda/\mu \sin \alpha \tan \alpha$. So, this time α is the only extra factor that is coming when the depth of field expression for depth of field compared to the resolution.

And this is also the reason why before putting any system for microscopy, electron microscopy or particularly in optical microscopy, this is the reason why you need a completely flat surface as flat as possible, because usually this depth of field for any microscopy system is quite low, particularly optical microscopes, the depth of field is very, very low electron microscope, it is still better.

So, optical microscopes, before putting them you need to polish and make the surface as smooth as possible. This is the reason, this is one of the reasons that is why polishing always go on microscopy, you basically do polishing.

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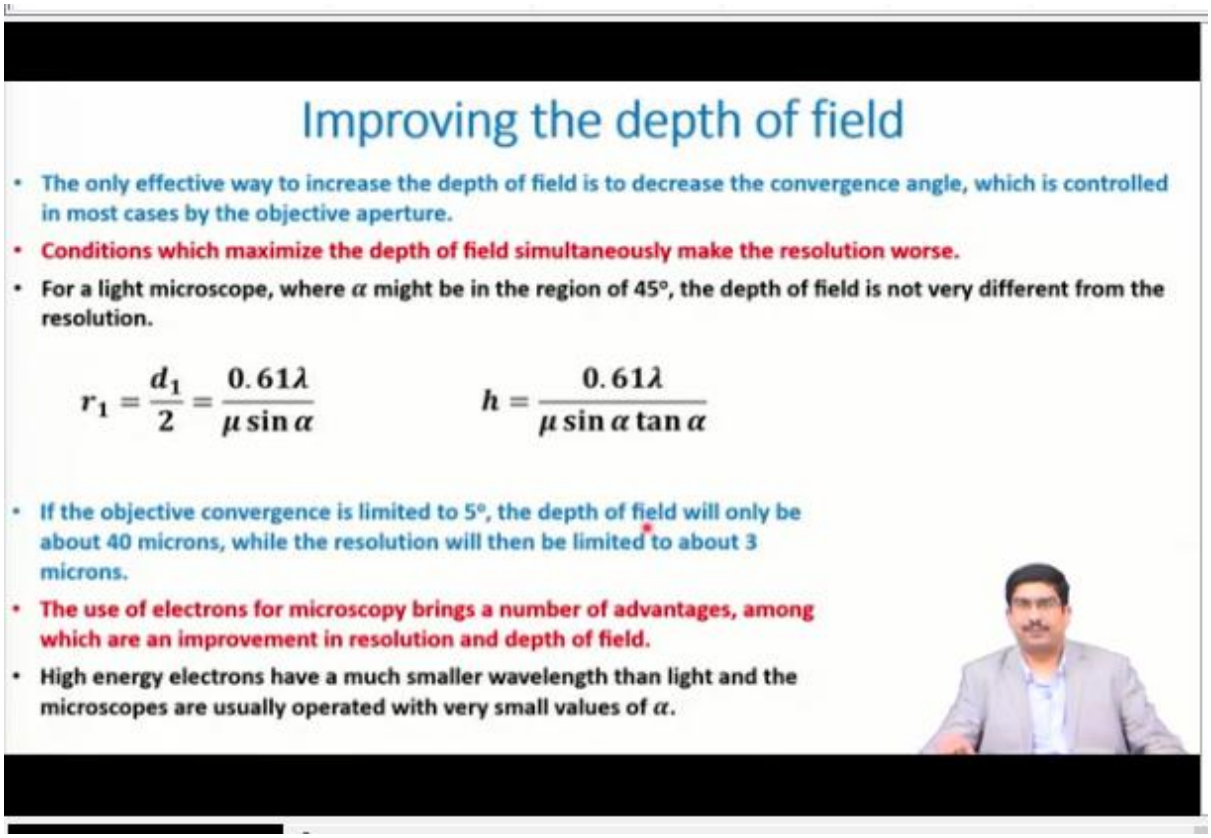

How you can improve the depth of field? You can go here and here also you can see that improving depth of field means I am basically going up, I am trying to increase this h value here, the way I can do improve h is basically I can either increase λ or again I can decrease this μsin α, this entire μsin α, numerical aperture. So, I can decrease the numerical aperture or I can increase the λ value and then I can have a better h value that means a better length.

Problem is the conditions which improve the depth of field is basically making the resolution worse and remember resolution worse means the decreasing the r value means improving resolution. So, the same effect, same in order to increase h I am actually increasing r. That means I am decreasing the resolution, if I am increasing the λ value, if I am decreasing the numerical aperture μsin α.

Then all of them it will improve depth of field, it will increase height, but at the same time it will be decreasing or it will be increasing r that means it will be decreasing resolution. That is the point and that is why it is kind of a trade off in any kind of a microscopy system it is kind of a tradeoff between resolution and this depth of field, if we only consider this sin α or this numerical aperture.

And one of the major point of electron microscopy is that there the resolution is improved by this lambda contribution not so much with the numerical aperture there. That is why in electron microscopes, it is possible to get good resolution and a better depth of field as well.

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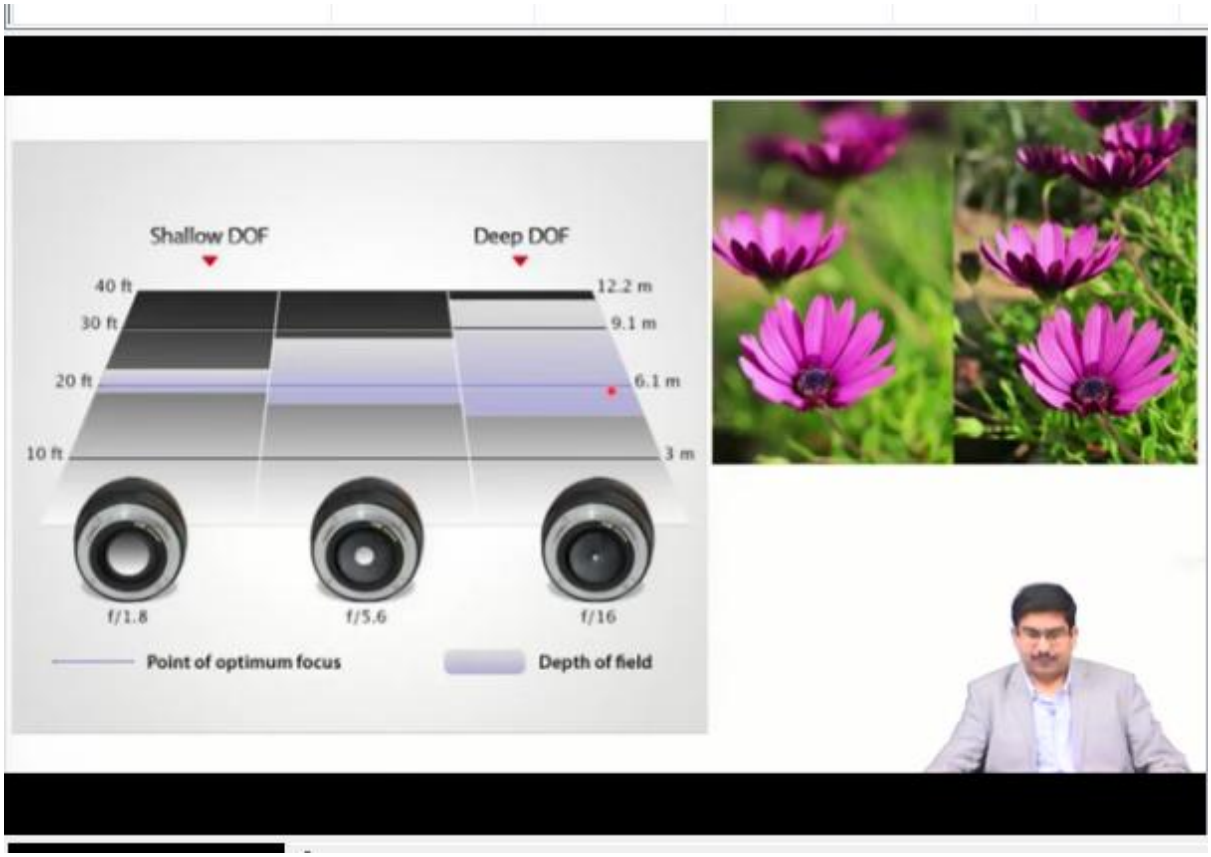

So, here also again, the same thing is shown the relationship between the numerical aperture and the resolution. So, as you can understand that numerical aperture if I have a smaller numerical aperture, then that means smaller numerical aperture means smaller α value, the semi angle for this any aperture system if I have it smaller, then I can improve even not only resolution I can improve the depth of field here.

That is exactly as shown here, when I am using and this is for any kind of lens, it is not like only optical microscope electronic or any kind of lens based system any kind of digital camera also the same concept. So, if you can see here that when I am using the smallest aperture, the depth of field is much better and you can immediately imagine that this is taken with the r aperture that is why this last one here it is not in focus whereas this is taken with the small aperture this image and that is why all the way everything is in focus. So, this is the depth of focus that we are talking about.

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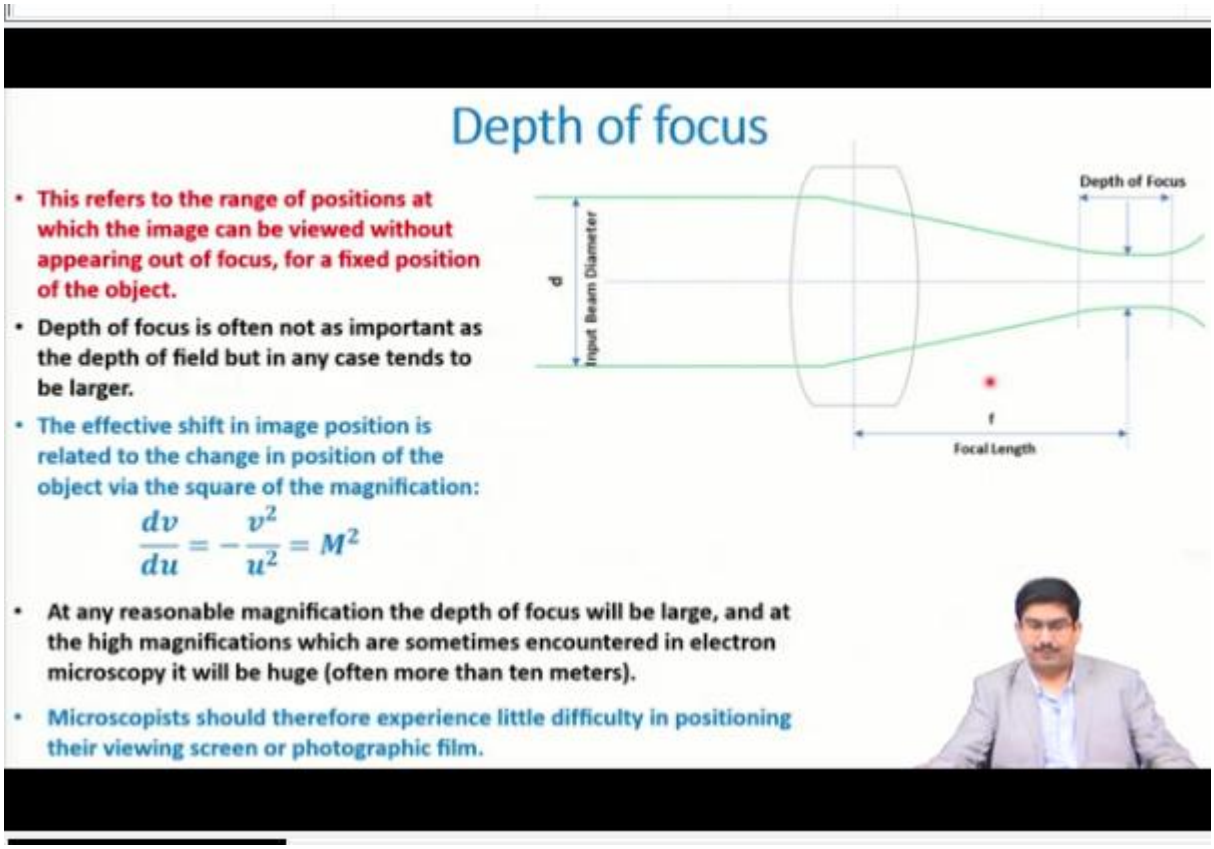

The similar term is depth of focus. So, this is what the depth of field I am sorry. This is what the depth of field and another point is depth of focus. So, depth of focus is something to do with the image side. That means it is dealing with the distance within which the image plane. The image can be move and still appear in focus. For the same object distance without moving the object how much I can move the image plane so that they will still appear in focus.

That is what is basically the depth of focus. So, it is the range of position at which the image can be viewed without appearing out of focus even for a fixed position of the object. And usually the depth of field and depth of focus are related by this relation if we consider that this is the shift in the image position. This basically comes from the same lens equation. So, this is magnification. So, magnification is M = v/u.

Now if you consider the derivative of it that means the shift in the position of object and shift in the position of the image. So, then you are getting that this is related to M^2. So, that is that means basically the depth of focus is M^2depth of field. And that is why even if we get very small depth of field in most of the microscopes, the depth of focus is usually very, very large

and if you go at a very high magnification which is possible in electron microscopes we are sometimes getting very high depth of focus almost in meters.

So, it does not matter exactly where we are keeping our camera for recording the image, we can keep it anywhere possibly, because the depth of focus is in meters. So, wherever the camera stays the image will always appear in focus.

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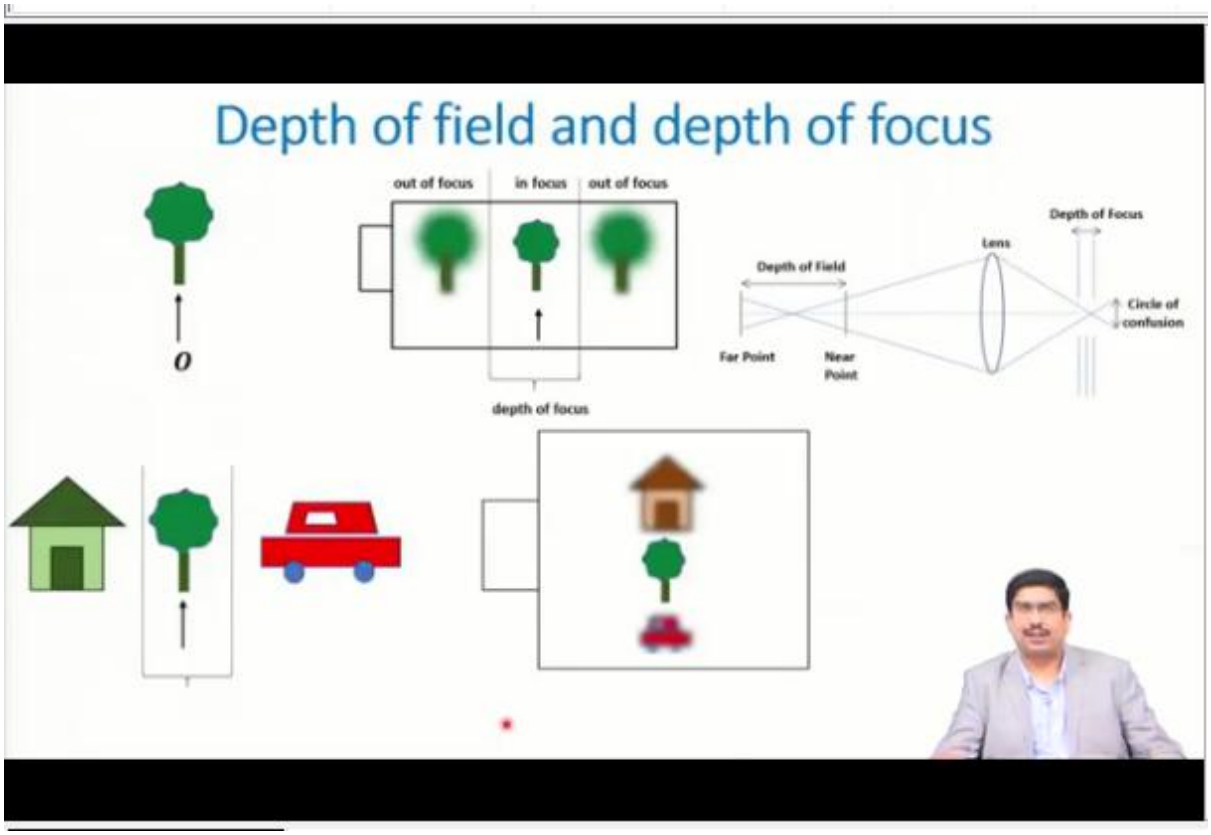

So, that is the importance of depth of focus and here it is again shown through the schematic. So, as I said depth of field as to do something with the object distance whereas depth of focus is dealing with the image distance. So, when you consider depth of focus this is for a fixed object, this is what is your depth of focus in the image side, whereas when you have sort of your object is moving.

This is your different objects at different positions and because of the depth of field only the one of these objects are in focus. That is what the difference between the 2 and here we are now this is the end of second lecture and we will be continue in the next lecture will be continuing with the aberrations, that is again some general concepts of microscopy aberration

of lenses and then we will be moving into the image formation using an optical microscope. Thank you.