Characterization of Construction Materials
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Lecture - 35
Optical and Scanning Microscopy-Features and Function- Part 2

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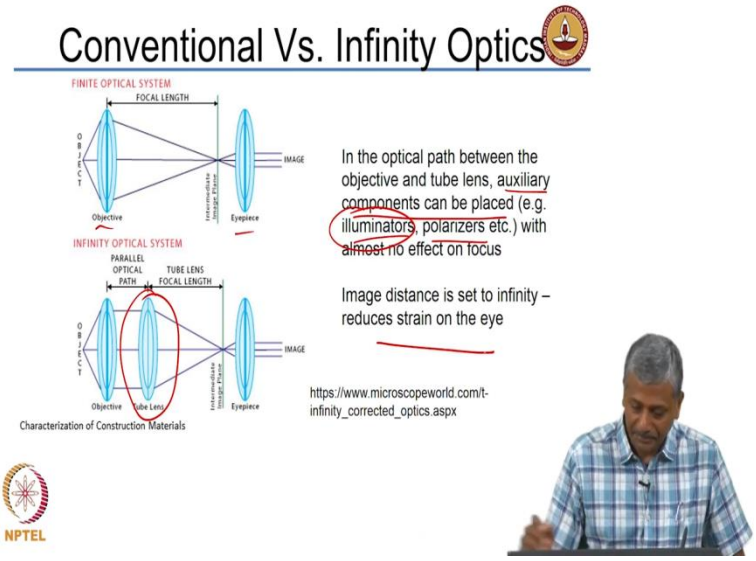

So, again this is a ray diagram description of the conventional versus infinity optics. So, again you have your objective and the eyepiece in the case of a regular conventional system and an infinity optical system, you have the intermediate lenses called tube lens, which leads to a condition where you are actually able to focus on something in infinity. So, again the advantage of this flexible tube system is that you can also place auxiliary components like illuminators, which can give additional light for instance, and polarizers. So, in the path that is there between the objective and the eyepiece lens or in the tube path, you can actually place additional components. That is the advantage of having this kind of modern microscope system, which has this tube inside, where you can actually place illuminators which are providing additional light or polarizers.

Now polarization of light is a property - since light is vibrating all directions as a particle, a polarizer tends to cut off the vibration in several other directions and makes light vibrate only in a specific direction. And with this polarized light, you can do a lot, which we will see later, in

terms of microscopy of thin section images of rocks and other components. So again, image distance id set to infinity which reduces the strain on the eye and that is a big advantage of the modern microscopy systems.

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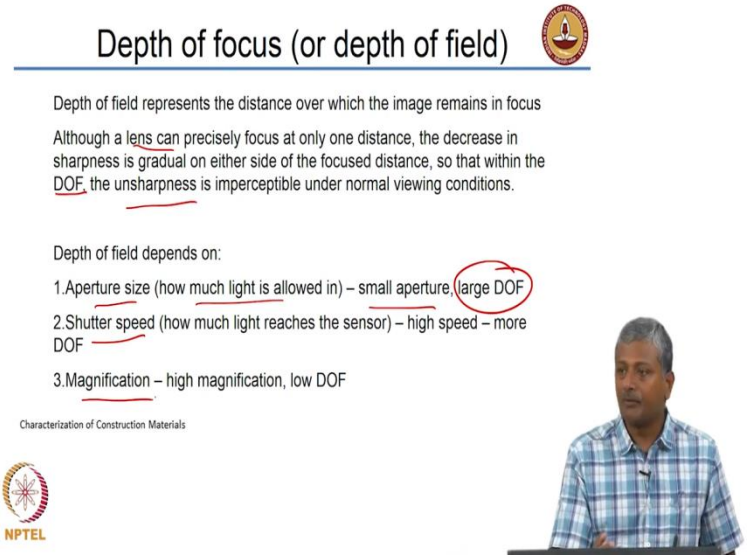

Now let us look at some concepts which relate to microscopy. Before we actually get into the process of understanding how a reflected light or a transparent light microscope actually behaves. There are certain terms that we talk about here which will be quite commonly available in all your cameras too.

So one most important term is called Depth of focus or depth of field. Depth of focus or depth of field is the distance over which the image remains in focus. Now, you can test this quite easily, when you take your camera, and you are trying to take the image of your friend on the other side, you will see that there are objects in front of your friend and behind your friend, which are also captured quite easily in your camera. Now, if I am trying to focus on a particular plane, or the person sitting right in front, if I'm trying to focus on the person here, the image of the persons at the back becomes more and more unclear as I move away from the person. But there is a distance up to which I can still focus on the other objects. Although I am trying to focus on one particular location, there is a distance in front and back which are still visible to me quite clearly. That basically is the depth of focus or depth of field.

So, the lens actually can precisely focus only at one distance because it has a particular focal length. But the decrease in sharpness or the unsharpness is gradual on both sides of the image. So there will be a lot of other components that you actually capture in your photograph, or look at with your eye, which will still remain in focus, although you are looking at only one particular plane. So, within the depth of field, that is, within the depth over which you can see images, the unsharpness is imperceptible. That means all the other components that are within the depth of field can also be seen quite clearly in the image or in your eye. So that is the idea of a depth of field.

Now, what does this depth of field depend on? Technically, it depends on the aperture size. So in your camera, you have a component called aperture. Aperture is basically the window or opening that lets in light. So, how much light is allowed in is governed by the size of the aperture. When you have a smaller aperture, you have a high depth of field. But what is the disadvantage of a smaller aperture? You let in very less light when you have a small aperture, so, other aspects may get compromised. For example, if you want very large amount of illumination in your image that will not come, but you will get sufficiently large depth of field.

Shutter speed dictates how much light actually gets to the sensor. If you have a high speed, you get more depth of field. And magnification is very important - with a high magnification, you actually get a low depth of field. So please remember every time, this is a common misconception that if I want to see objects more clearly I want to increase my magnification, so I can see it more clearly. But more magnification generally tends to increase the level of confusion that is there in the image. And especially when you are trying to view images which are over a certain range, in the same picture, then increasing magnification will reduce the depth over which the entire image will be in focus. So that is a very important aspect for you to understand. Magnification generally is only to ensure that you can see a magnified view of an object at a particular location. But you cannot expect the same degree of sharpness on either side of the image when you increase the magnification. So generally, higher magnification implies a lower depth of field, and later I will introduce you to one more term for resolution. When you increase the magnification, we generally tend to reduce the resolution also.

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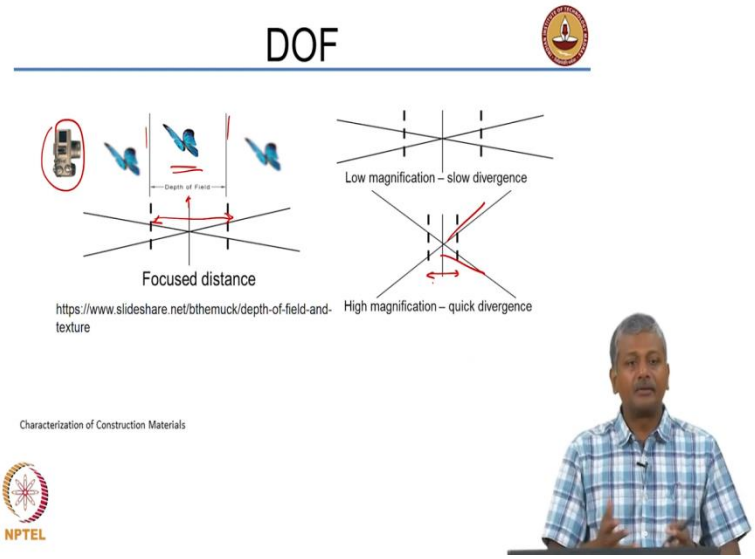

So again, this is just an example of the depth of field. So here there is a camera which is trying to focus on a butterfly and there is a distance before and after the butterfly, that is basically the depth of field of the camera. So essentially your focus distance is at this location, but then all the aspects in front and back up to a certain distance is still clear to the camera.

Now if you increase the magnification, what is going to happen is these light rays are going to diverge much more from the focal plane. So if there is a greater divergence, it reduces the distance over which you can actually observe the image clearly. So, with a low magnification you get slow divergence, that means you have a greater depth of field, and with a high magnification you get quick divergence which reduces the depth of field at high magnifications.

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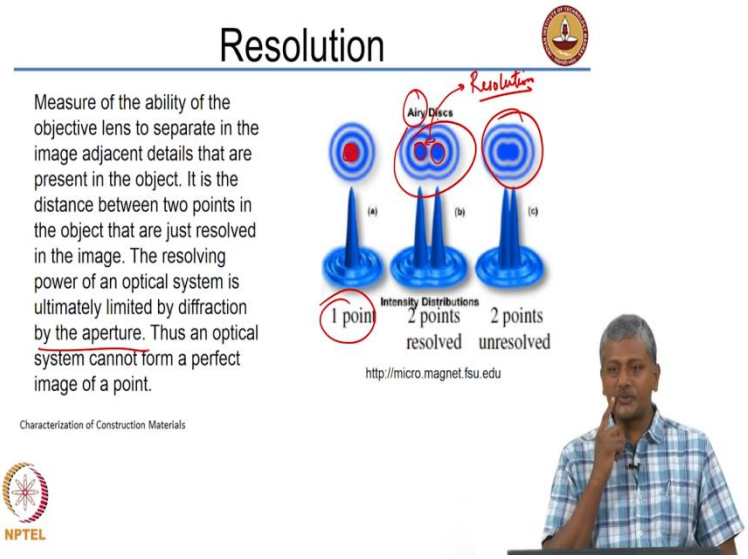

So, the other important aspect as far as microscopy or even regular photography is concerned is resolution. Now very often we confuse resolution with magnification, but I said earlier that magnification is only to produce a larger view of the object. But resolution is actually the clarity of the image that you can actually observe.

Now what is this clarity? Generally what happens is, when your microscope tends to focus on one particular point, the light that is collected by the microscope is through an aperture. That is, the microscope collects the light coming from the specimen through an aperture. So there is a direct beam of light which is coming from the specimen into the aperture, but because of the width of the aperture being small, this beam is also getting bent and diffracted, because of which any one point that you observe in a microscope will not be seen as a point but seen as a disc. You will see a central location in this point which is very dark, but because of diffraction you will see these gratings or the halo will also be seen around that one point.

What will happen is, if you have another point close to that point which is trying to be imaged, the disc of confusion around that point or the airy disc as it is called will be converged. So, what will happen is, if those 2 points are very close together, the confusion around those 2 points will be so large that you will not be able to distinguish them as 2 points but as 1 point. That is what is happening in this case (2 points unresolved). In this case (2 points resolved), what has happened is these 2 points are just far apart. So, their airy discs or the discs of confusion

around those points are almost getting separated. This minimum distance that can be resolved by a microscope is called resolution.

So, resolution is the ability to resolve 2 points in an image which are lying next to each other. So, what is a good image, which has a smaller resolution or a higher resolution? Obviously higher resolution, but what it means in terms of the distance is, smaller distance between points. Higher resolution implies that your image is able to distinguish between points that are located very close by.

Now, I can easily start thinking why don't I just increase the magnification which will increase the difference in distance between the 2 points? The problem there is, when I increase the magnification, I also increase this confusion around, the amount of diffraction grating or the diffraction halo can lead to more confusion when I simply increase the magnification. So, increasing magnification will not give you a very high resolution. But I said earlier that you have an aperture through which you are allowing this light. Now, what will the aperture do with respect to resolution, if I have a small aperture or a large aperture, what will happen? Anyway, we will come across that point a little bit later.

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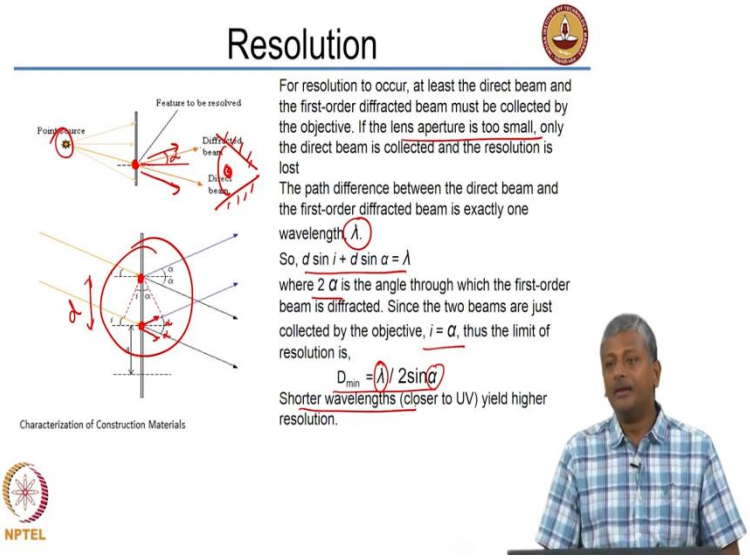

So again, just to look at the physics of resolution. So for resolution to occur, in terms of light passing through the object or passing through a slit, resolution of the point requires that both the direct beam that is coming from the point and the first order diffracted beam, for example, here

this is the point that you are trying to image (labeled as 'Feature to be resolved' in figure), that is the light source (labeled as Point source) So, the direct beam and the first order diffracted beam, if you are viewing that point with your eye. The first order diffracted beam and direct beam should both be collected by the lens for you to be able to resolve that there is this point here.

On the other hand when you have 2 points which need to be distinguished, the same concept will apply. Your lens system should be able to capture both the direct and the first order diffracted beam. But if the lens aperture is too small, only the direct beam is collected and the resolution will be lost. So, in the previous slide I asked you a question, what will happen with the aperture width is small or large? When your aperture width is small, you are only going to be collecting the direct beam and not the diffracted beam and because of which your resolution will get lost.

The path difference between the direct beam and the first-order diffracted beam is exactly one wavelength λ. So, again you can work that out based on simple physics, that the diffracted beam is out of phase from the direct beam by just exactly one wavelength. So, if you apply simple geometry to this situation, you will actually get λ as:

$$\lambda = d \sin i + d \sin \alpha$$

where 'd' is the distance between the 2 points, or in other words, 'd' is the resolution minimum distance between 2 points that can be resolved in an image.

And based on Snell's law, we can equate 'i' with 'α' (i=α).

2α is the angle through which the first order beam is diffracted. What do you mean by that? So, this is the main beam (direct beam) and that is the diffracted beam (Refer figure in slide). So, this is α and that is α, so 2α is the change in path or change in the direction of the beam when it undergoes the first-order diffraction. Please remember the concept of diffraction, there are several orders of diffraction that can actually happen, based upon the number of times the light gets bent around the edges. So, again 2α is the angle through which the first-order beam is diffracted, since i= α, you get the resolution, D_{min} as:

$$D_{min} = \lambda / (2 \sin \alpha)$$

So, your resolution is dependent directly on wavelength (λ) and inversely on the half angle subtended by the beam on the lens (α is the half angle). So, if your lens is here, α is nothing but the half angle. So, α and λ are the two parameters that will govern the resolution.

Now, one thing that you come across here is that, the shorter wavelengths, which are closer to violet or ultraviolet, will lead to higher resolution. So if you were to perform microscopy using visible light, and you put in a blue filter, which absorbs red and green and transmits only blue, you will probably get only the smaller wavelengths to be used for your microscopy, which may increase the resolution of your technique. So wavelength becoming small implies resolution becomes greater (better).

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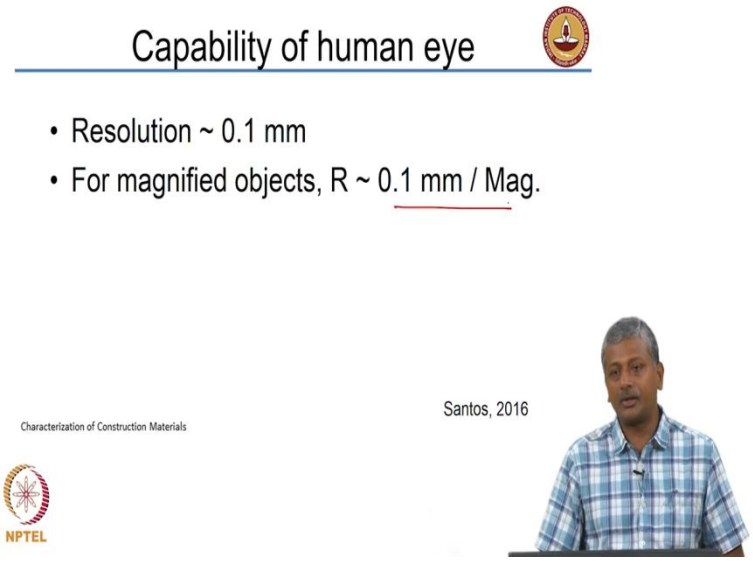

Now, in terms of the human eye itself, the resolution which we are capable of with the eye lens is 0.1 millimeter. I do not know. Have you ever tried viewing objects that are apart by just 0.1 millimeter? Well, maybe if you try very hard you could. 1 millimeter we can easily see, Anything smaller than 1 millimeter, we do not really know how to define that with our eye itself. But the capability of the human eye is 0.1 millimeter.

If you are magnifying the object, the human eye can actually resolve to 0.1 divided by the magnification (R ~ 0.1mm/ Mag.), so if I magnify the object 10 times, my resolution power becomes 0.01 millimeter. So those are some things that I can see for myself, but for a lens obviously, the resolving power will depend on the kind of structure that your lens system has, the

aperture, the amount of light and all the other aspects that come within the optical microscopy realm.

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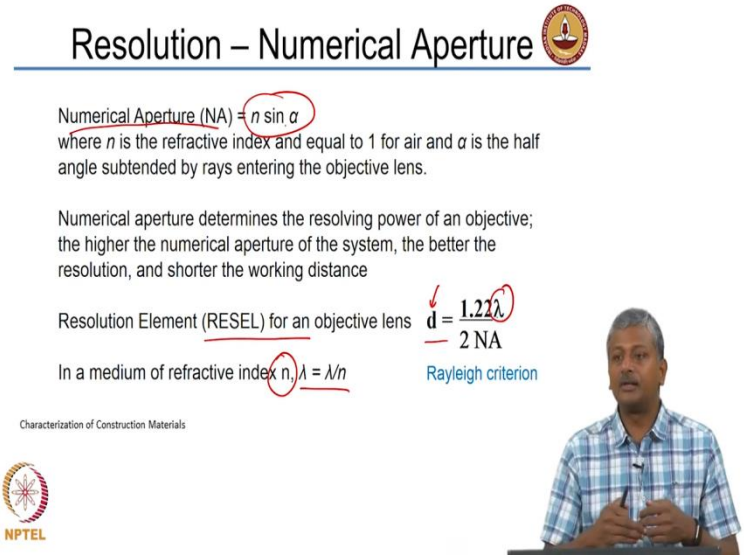

So one of the important aspects as far as resolution is concerned, is the numerical aperture (NA) of a lens, which is nothing but,

$$NA = n \sin \alpha$$

where α is half angle subtended by the rays entering the lens.

n is the refractive index.

So, if you take a particular lens, if you observe any microscope, the lens that is provided with the microscope they will have some symbol called NA. That is nothing but numerical aperture of that lens and that is nothing but NA= n sinα.

Now, in most cases we use simple lenses which are air lenses, because we are observing through air, so, n = 1. Now, if you want to increase the numerical aperture, you may want to use an oil immersion lens, for example, you have a droplet of oil placed between the lens and the object to be imaged. And that will increase the n and because the n is increased more, the angle α will become wider and wider.

For n = 1, let us say this is α that is getting into the lens. If instead of air, I put in oil in-between, oil will tend to have a greater refraction, it will bend that light more and more and you will get a wider beam α, into the lens. That means you are increasing your n and that will also increase your α. In other words in such oil immersion lenses, what will happen to the resolution

is, resolution will be better, you will be able to resolve better because your sin α will become greater. That means if you go back to the previous equation here ($D_{min} = \lambda / (2 \sin \alpha)$), since sin α is greater, you will actually end up having a much better resolution.

So, numerical aperture defines the resolving power of an objective lens. The higher the numerical aperture, the better is the resolution, and shorter is the working distance. Now what is this concept called working distance? Working distance is the distance between object and objective lens. So, if on your microscope platform you keep your specimen or the object and you have the objective lens on top, that distance is called the working distance. So, if you lower the platform, what will happen is the working distance increases.

As the working distance increases, what will happen to α? So, at a closer working distance, it is very wide, as you increase the working distance, α becomes smaller and smaller. That means at closer working distances you will get better resolution, and when the object goes far away from the objective lens you get poorer resolution.

So, in general for an objective lens, you have what is known as a Resolution Element (RESEL) which is:

$$d = \frac{1.22\lambda}{2\ NA}$$

If you are trying to have a different medium in between your objective lens and the sample, of a different refractive index 'n', then your λ effectively becomes (λ/n). Again what will that do? It will reduce λ in the Rayleigh criterion($d = \frac{1.22\lambda}{2\ NA}$), so it will improve the Resolution Element. If λ gets lower, 'd' also gets lower in terms of resolution, that means you have a better resolution.

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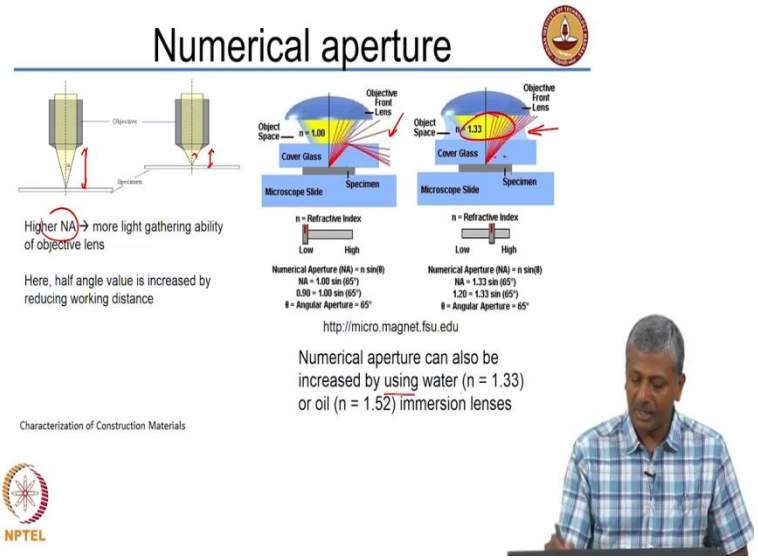

Again, this is just an example of what I was talking about. You have a larger working distance, you have a smaller angle α. For a shorter working distance you have a greater angle α. So there is more light gathering ability when you are working distances smaller. Also with a higher numerical aperture, your lens is able to gather more and more light.

Now, this is what I was talking about in terms of using an air lens or using an oil immersion lens. So, if you have an oil droplet in between your lens and the specimen location, obviously you cannot put oil directly on the specimen, so you have a cover glass with which you cover the specimen and then you put the droplet of oil between the objective lens and the cover glass. So, what will happen is the light that is coming from the specimen will tend to bend a lot more when it goes through the oil. So that will increase the angle α which increases the resolution. So numerical aperture can be increased by using water lenses also because water you know has a refractive index of 1.33. But oil is better because it has an even greater refractive index of 1.52.

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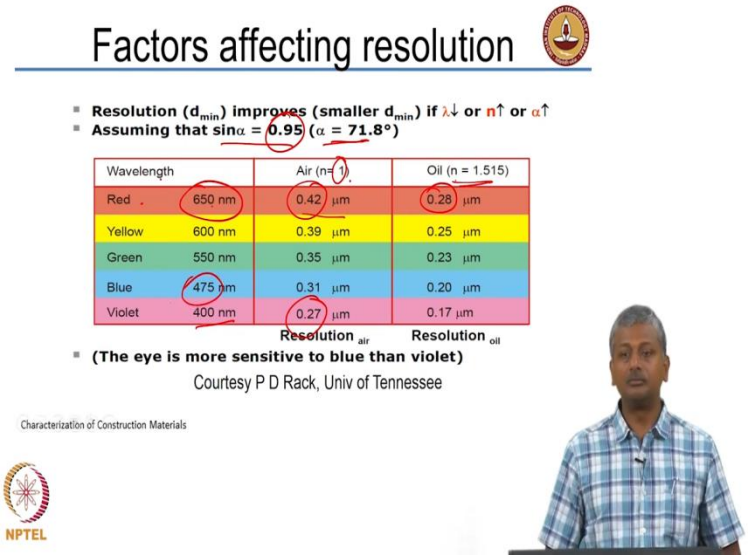

So again, just to give you some numbers here. So resolution obviously improves with smaller wavelength. It also improves with higher Numerical Aperture (NA) or higher refractive index of the lens. So, here assuming that sin α = 0.95 that means you have a fixed working distance which produces α = 71.8°. So, your wavelength of red for instance, which has λ = 650 nm, in air, that means there is only air between the objective lens and the sample, that means n = 1 your resolution will be 0.42 μm. But when you have oil immersion lens of 1.51 or 1.52 as the 'n' value, that improves your resolution 0.28 μm. So 30% improvement in resolution has been obtained by putting oil in-between.

Now, that is comparing air and oil, but what if you compare different wavelengths of light. We know that red is the largest wavelength, violet is the smallest wavelength, and look at the comparison here in resolution, 0.42 μm for red light and 0.27 μm for violet light, just by using just air. So we are reducing 30%, the distance between the 2 objects, by simply using violet light instead of red light. So if you are using blue or violet filters, your resolution is going to get enhanced, you will be able to image objects which are much closer than otherwise. So with this table, you can actually appreciate the effect of the wavelength and the type of the lens which is simply going to be used in the system. All this is at a fixed working distance, which fixes your angle. The working distance fixes the angle α at 71.8°.

Eye is more sensitive to blue than violet, because violet goes slightly on the border of the visible range, so the eye is not able to make out violet light as easily as blue light. So, we may not be able to use violet filters for instance for imaging where we have to look at the microscope with the eye, you may want to go for blue imaging. So, you will not get the full benefit of using violet in that case.

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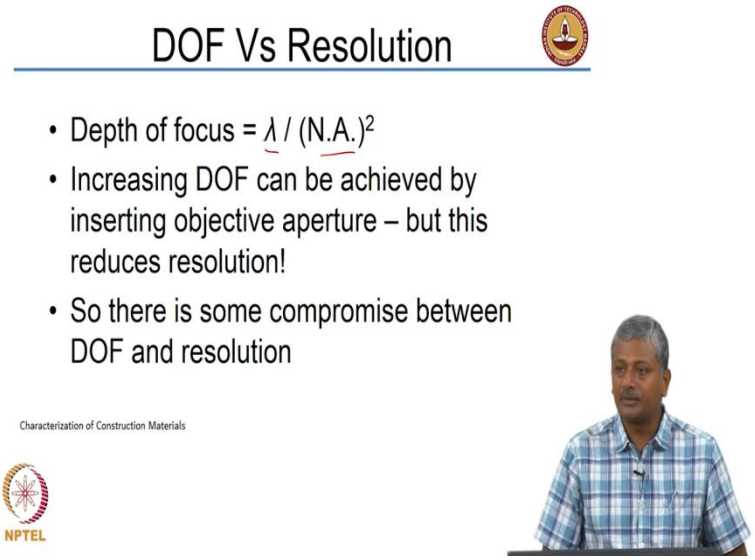

So, depth of field is something that we talked about earlier. Resolution is something that we have talked about just now. How are these 2 related? The depth of field or depth of focus in terms of the lens' ability is given by:

$$Depth\ of\ focus = \frac{\lambda}{(N.A.)^2}$$

Now please remember, for resolution to be good, the D that you get should be small. The depth of field on the other hand for it to be good, the value of the depth of field that you get should be large. If a depth of field is large, we see that, depth of field is good. So, in this case, look at how the depth of field is related to the wavelength and numerical aperture. Now, if you reduce the wavelength, what will happen to the depth of field? It will reduce. If you increase the numerical aperture what will happen to the depth of field? Again it will reduce. So, there is some compromise between depth of field and resolution. So, increasing depth of field can be achieved by inserting objective aperture. We talked about the fact earlier that when you have an aperture, you can increase the depth of field. But because you have an aperture now, you are only

collecting the direct beam, and that is limiting your resolution. So, there will be some tradeoff between depth of field and resolution.

Again, you can try this out with simple cameras itself. You try to look at the quality of images with different aperture sizes. If you have an SLR camera, you can do all those adjustments, not in a mobile camera, probably you do not have that thing. So you can actually adjust the aperture sizes and try to take different pictures and see for yourself how it affects the sharpness of the objects before and after the focal plane, that is your depth of field and the clarity of the image itself, which is basically your resolution. So with that we will stop for this lecture and in the next talk we will continue with our understanding of optical microscopy. Thank you.