Characterization of Construction Materials
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Lecture – 36
Types of optical microscopy - Part 1

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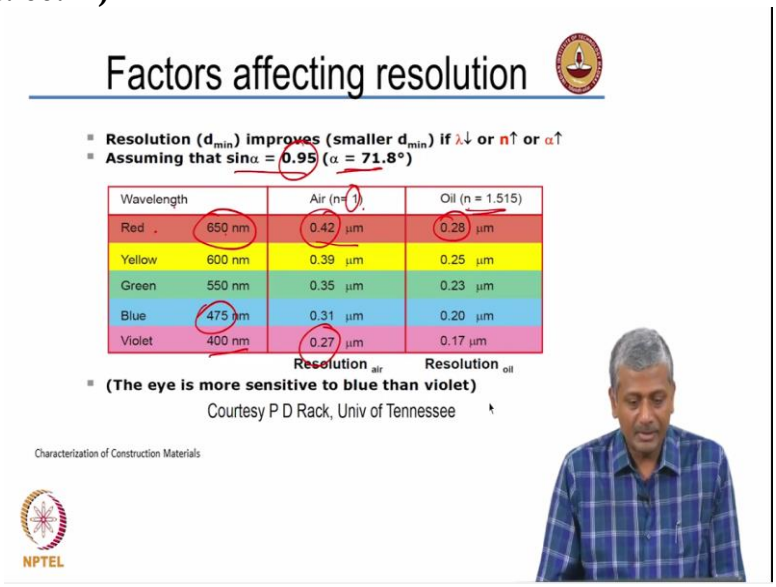

Hello everybody. We will continue our discussion on optical microscopy today. We learnt in the last session as to what happens when we change the numerical aperture of the lenses, and how you actually can improve the resolution by introducing materials of high refractive indices like oil in-between the sample or specimen and the lens and you could clearly see that with the use of oil, or with the use of different wavelengths of the light, we could enhance the resolution by as much as 50%.

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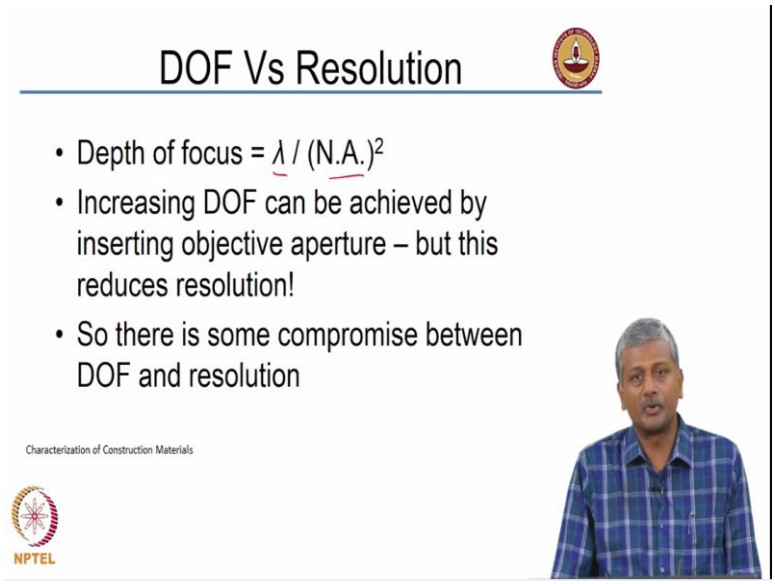

And we also talked briefly about the fact that if you want resolution, you may have to compromise on depth of focus. There is a bit of compromise between the depth of focus and resolution. So, increasing the depth of focus can be achieved by increasing the objective aperture. That means you are letting in more amount of light, but we learnt earlier that increasing the aperture width will lead to decrease in the resolution.

So, one has to work around this problem by ensuring that when depth of field is required for certain type of applications, you will have to have settings in your microscope which corresponds to improve the depth of field. But when you need resolution, primarily for images when you need resolution, you might want to actually reduce the aperture size and choose the settings in such a way that you improve the resolution.

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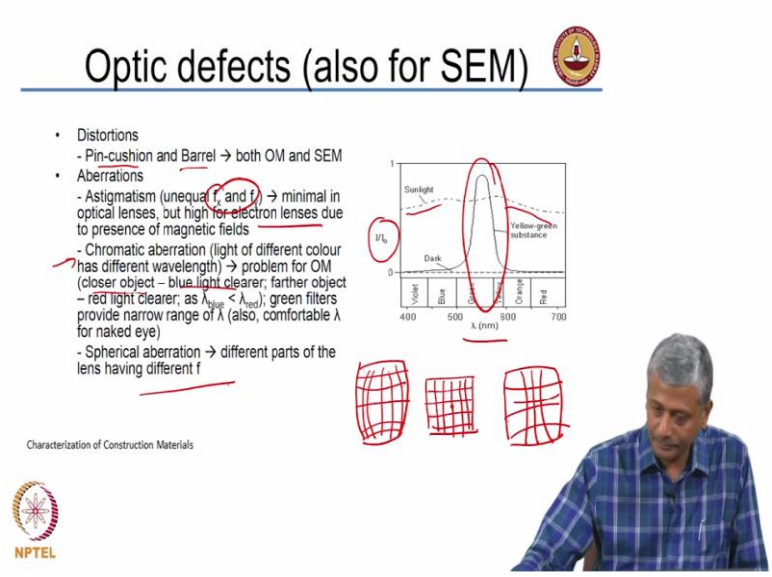

So moving on, just like our eye lenses have defects, optical lenses as well as scanning electron lenses can also have defects. Now, what are these defects? One popular defect is the pin-cushion and barrel defect. Now what does this mean? If you take a cushion and if you prick the centre of the cushion with a pin, so if this cushion were marked by these vertical and horizontal lines, if you are going to be pricking the centre of the cushion with a pin, what will happen? The inner lines will come closer and the outer lines will go far apart. So, what will happen is your inner lines will get closer and the outer lines will get far apart when you are trying to prick the centre of the cushion with a pin.

Similarly, the other thing is the barrel distortion. In the case of a barrel distortion, you will get a barrel type image. So what will happen here is the outer lines will get closer and the inner lines are far apart. So that is a barrel distortion, it just describes what happens when you actually prick the cushion with pin in the centre. So it sort of curves some part of the image in such a way, that it is no longer clear or flat. So that is called a pin-cushion distortion and a barrel distortion.

Apart from this you can have other problems which are very common to eye lenses also, for example, astigmatism. This is a very common problem with eye lenses. What does this astigmatism mean? It means that your focal lengths in the X and the Y-directions are not equal. So when we are observing an object in the plane, if this is your Y, and that is X, we are able to observe the shape and size and height of objects very clearly, because both the foci are at the

same location. But if your X and Y foci are different, you will end up seeing images which are distorted. For example, you might see people longer or shorter than they actually are, depends on the extent of the astigmatism that you may have. So again, in optical lenses this astigmatism is not that big a problem, of course in eye lenses it is a problem sometimes because of the muscular effects that you may have, especially in old age people always get astigmatism because the muscles that control the lens become weaker. So they always have to get some lenses to correct for the astigmatism. But in electron lenses, astigmatism can be significantly large because in electron lenses the electron beam is controlled with the action of magnetic field. So whenever you have a lot of controlled magnetic field, there is always a chance of increasing the level of astigmatism.

The other is, in optical microscopy, you may have a chromatic aberration. So what happens is, since light is composed of many different wavelengths, you may observe different objects clearer with different types of wavelengths. For example, in closer objects a blue light may yield to be clearer with respect to the image. For farther objects, the red light will be clearer because there is a difference in the wavelengths of blue and red light. So, green filters are typically used to ensure that you cut down the visible light into a very narrow wavelength.

So what happens when you put a green filter, for example, if this is your relative intensity versus wavelength (Refer to graph in slide), in sunlight, we have all possible wavelengths ranging from violet to red. But when you put this through a green filter, it cuts off all the other effective wavelengths and maximizes the green wavelength range. So you get a very narrow band of wavelengths and that improves the imaging quality, so you do not get this chromatic aberration. You often hear of this problem in people also called red-green color blindness. That often happens, that is because of a chromosomal effect. But essentially, it leads to the kind of effects that we are talking about here - chromatic aberration.

Now spherical aberration is what we most commonly have when we wear glasses. What happens is, different parts of the lens have different focal lengths because of which the eye is not able to perfectly focus on one particular location. So, several microscopes can also have the same sort of a problem. So this can be corrected in an electron lens by adjusting the magnetic field to

ensure that you are able to converge all the rays on to a single point. In an optical lens, it is quite difficult so, what you have to do is add another lens to it. For example, you wear a glass, you are basically adding one more lens to your eyes, and that helps you actually focus much better. So spherical corrections, we know that we do it for short sightedness, or long sightedness - myopia or hypermetropia. All that is because of the fact that your shape or curvature of the eye in the spherical sense, is not perfect and that is the reason for differences in the way that we focus on certain objects. So, these defects could be there for optical microscopy as well as scanning electron microscopy. So, when you get images, please understand that they are not just the function of the type of specimen preparation or the user's perspective, it is because there are several other possibilities of errors also creeping into the imaging technique.

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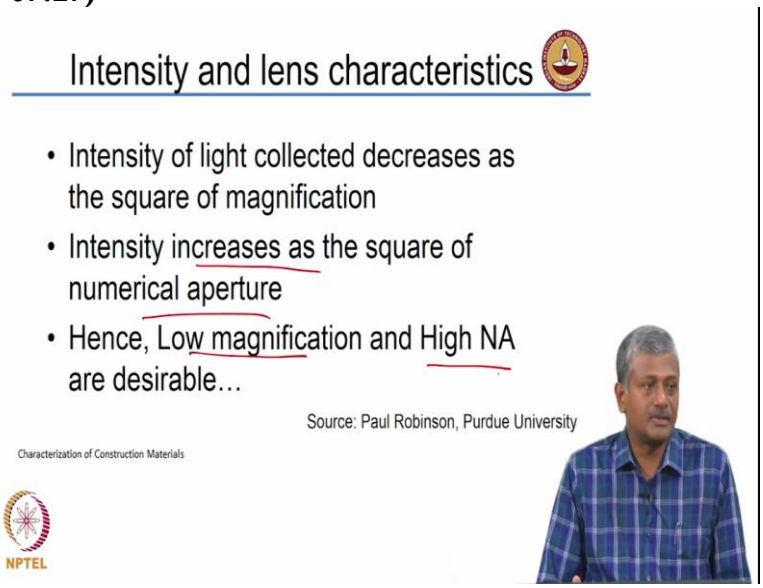

So, what you have to also understand is the intensity of the light that is collected can decrease with the square of the magnification and further intensity increases as the square of the numerical aperture. Obviously, if you are increasing the aperture, you are increasing the width over which the light is getting collected, so you collect more and more light. So intensity increases with the width of the numerical aperture (as a square function) and decreases with the square of the magnification. So generally what you want for good quality imaging is low magnification and high numerical aperture. So if you want to produce high quality images and increase the resolution, low magnification and high numerical aperture are desirable

However, in some cases you may want to get additional features of an object and look at things closer, in which case you cannot avoid getting a higher magnification, you need that sometimes. But you have to understand that when you have high magnification, you will not be getting the best of resolutions as well as intensities.

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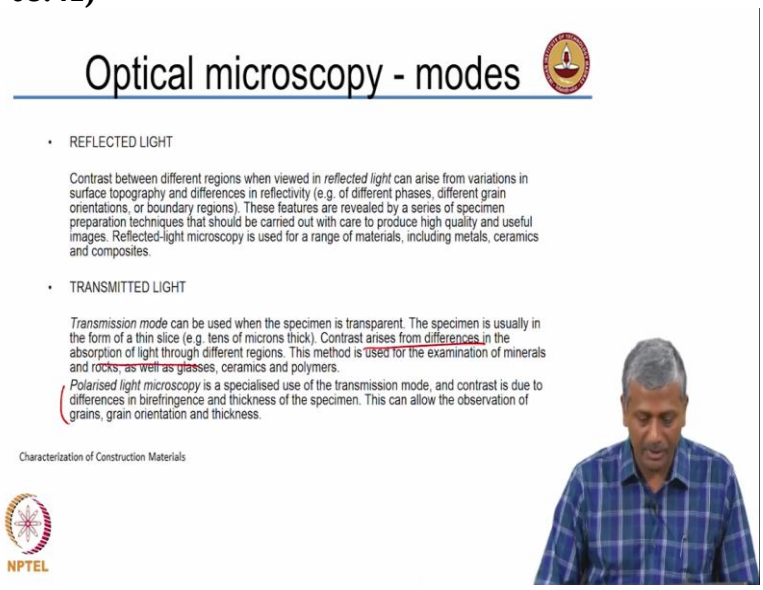

Now let us look at what are the different modes in which you can actually do optical microscopy. Two common modes are reflected light mode and transmitted light mode. Obviously, you will choose reflected light when the specimen that you are trying to image is dense and not impregnable with respect to light, so that light cannot impregnate through. In other words, you have an opaque specimen. So, now what happens is you are shining a light onto your opaque specimen and looking at the reflectivity of the light from the surface of the specimen. So different phases, depending upon their characteristics of light absorption and reflection may reflect different amounts of light. And that is what your image is going to capture - the reflection of the light from the surface of the specimen. Depending upon the phase composition some phases may be able to reflect more light than the others. So, that is why it produces a contrast in your reflected light imaging.

In the case of a transmission mode of microscopy, your specimen is either transparent or can be ground to make it almost translucent or even transparent. For example, when you have to observe the surface of rocks or mineralogy of rocks, very often we make thin sections of these rocks and transmit the light through it and then do the imaging. So, this transmitted light imaging

produces very different features. So, here please remember you are talking about light passing through the object. So, depending upon the refractive index of the material, the light will be bent to different degrees, and when light gets bent to different degrees, you will perceive the object in different sorts of colors, depending upon the refractive index, you may or may not be able to capture all the spectra that emanate when the light comes out of the material. So, you need to ensure that you can get phase contrast by having a material which has phases which have different refractive indices. So, light is transmitting through, and so it will rely on the property of the phases to transmit based on the refractive index. So contrast arises from differences in absorption of light through different regions and also the amount of the light that is bent because of the different refractive indices.

We do not have any accessory items to help us identify the mineralogical composition. So, how will you identify a specific mineralogical composition is by comparing a material of several phases with certain known phases. You cannot arrive at a conclusion, for example, later you will see that in scanning electron microscopy, we can get some sort of compositional analysis or elemental analysis by looking at the X-rays that come out of specific features on sample, but the same thing cannot happen in the case of optical microscopy. We are only dealing with how light is passing through or reflected by the different phases. So, while this can tell us some things about the material, but we cannot really do a complete identification unless we have reference patterns available for different plain or pure materials.

Now, in the transmission mode, when I was talking about the refractive indices, one of the most important characteristics is to use what is known as polarised light microscopy. So, light as you know can vibrate in all directions, polarisation means you are causing the light to vibrate only in a specific direction, and when this light that vibrates in a specific direction passes through materials that are isotropic, you do not get any change in the light direction, but when the light passes through anisotropic materials, you may actually get the splitting of this single direction of vibration into two components and then with the use of another polarizer or which is also called the analyzer, you can actually cut off specific wavelengths and then observe the features differently.

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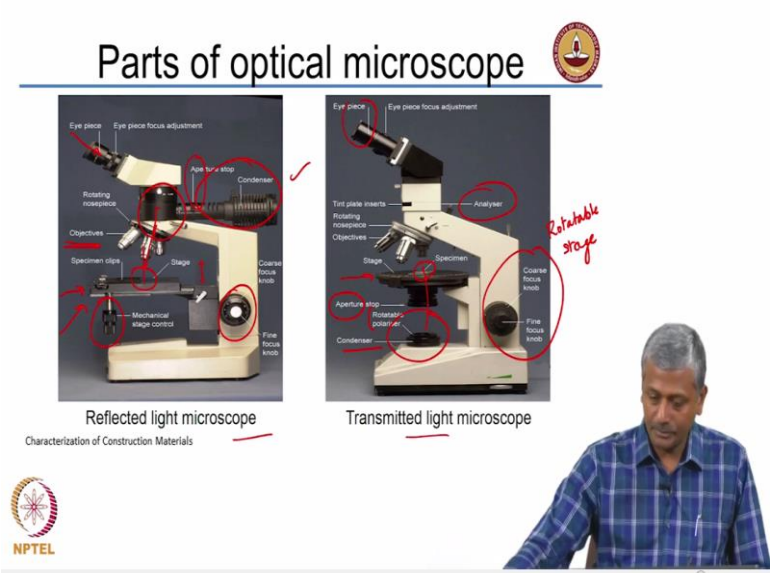

So, these are the typical features observed inside optical microscopes. So on the left you have a reflected light microscope and on the right is the transmitted light microscope. So in both these cases you have a stage - specimen stage is available in both these cases. One principal difference that you will see is that, in the transmitted light microscope, you have a rotatable stage and that is there for a reason because in a transmitted light microscope, the rotatable stage is provided to ensure that you are able to do polarised light microscopy, because what happens is, the extinction of the light that passes through the specimen happens at different angles for different types of minerals. So because of that, you should be able to rotate the stage to observe the features much more clearly in the case of transmitted light microscopy.

In a reflected light microscope, your light source is located here - the condenser, which is basically a feature that ensures that you get parallel rays of light. A beam of light goes in a parallel fashion through the aperture and then goes down through the nosepiece, hits the specimen which is sitting on the stage, and then you are able to observe through the objective and through the eyepiece. So the light is coming through the nosepiece. Nosepiece is where the objective lenses are collected, so here through the aperture, the light is coming down, shining on the specimen and the reflection is actually taken in through the objective lenses and observed in the eyepiece. Ofcoure, you can control the stage mechanically and you can control the up and down movement of the stage using this knob here and that is nothing but the ‘focus knob’. So for a specific objective lens, moving the stage up or down is going to change the working distance,

which is the distance between the specimen and the objective lens and that is basically the adjustment of the focus. When you move in larger increments, that is called the coarse focus, and when you move in very small increments, it is called the fine focus. So you need to move the object closer or far away from the objective lens to get a focus on the object.

Now moving on to this transmitted light microscope, obviously, because it is transmitted light, the light source is located at the bottom of the microscope. It goes right through the specimen and then the light is collected by the objective lens, and then you are measuring it or viewing it with the eyepiece. So you can also see there are other features here. There is a rotatable polarizer in this location and there is also an analyzer on the other end. A polarizer basically is a series of slits that is basically cutting off the light in all other directions except one and then the light passes through the specimen, goes through the objective, and when it is going up to the eyepiece, you have the analyzer in place. The analyzer can be made to be parallel to the polarizer, which will allow the same light ray to pass through or perpendicular, which will cut off this light ray that passes through. So you can use that combination to actually view a series of different phases in your object. We'll come back to that in just a minute,

So again, whenever there is light, the light source is a condenser which ensures that there is a parallel beam of light going, there is always an aperture provided, whether it is the transmitted light microscope or the reflected light microscope, you always have an aperture to ensure that you can control the amount of light that is actually getting in. In the case of the reflected microscope your stage is not capable of rotary movement, but you can actually move the stage in the X and Y directions. And then using the focus knob you can move the stage up or down. In the case of transmitted light microscope, your stage is capable of rotary movement and then, you can also move it up and down using the focus knobs - the coarse focus knob and the fine focus knob. The other additional detail in your transmitted light microscope is the presence of polarizer and analyzer, which helps you do polarised light microscopy.

So when you did your biological specimens in school, it must have been most probably with the transmitted light microscope, because biological samples are transparent and they all allow the light to pass through.

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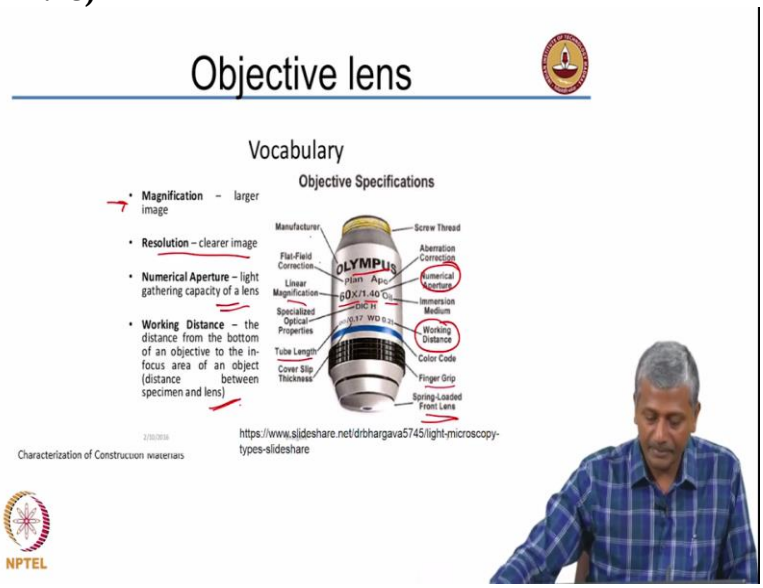

So again, just to give you a closer view of an objective lens, this is what it looks like. Olympus is the name of a company that manufactures microscopes and lenses, they are very well known. The other famous company includes Carl Zeiss which is a very common instrument that you see in many laboratories. So, again manufacturer's name is given, you have a flat-field correction, then you have a linear magnification how much it is capable of, for example, this lens is capable of 60 times magnification (60x). What other features are there? You have tube length which is written as infinity, in this case, what does it mean? That the optics is such that, the image will appear as if it is at infinity, so it will not have a strain on the eye. It then has numerical aperture also written on that as 1.4, and it says it is an oil immersion lens. So the lens itself inside may have a film of oil under the lens, it does not directly touch the specimen, but the film may already be inside. The working distance is also provided and ofcourse there is a finger grip also, in this case, and it is a spring-loaded front lens which is at the top of the objective.

So what is meant by different parameters? Magnification means you are simply enlarging your image and observing the features. Resolution means you are making the image sharper or clearer. Numerical aperture is how much light a lens can gather. Greater half angle means more light can be gathered by the lens. Working distance is the distance from the bottom of the objective to the in-focus area of an object, that is distance between specimen and lens.

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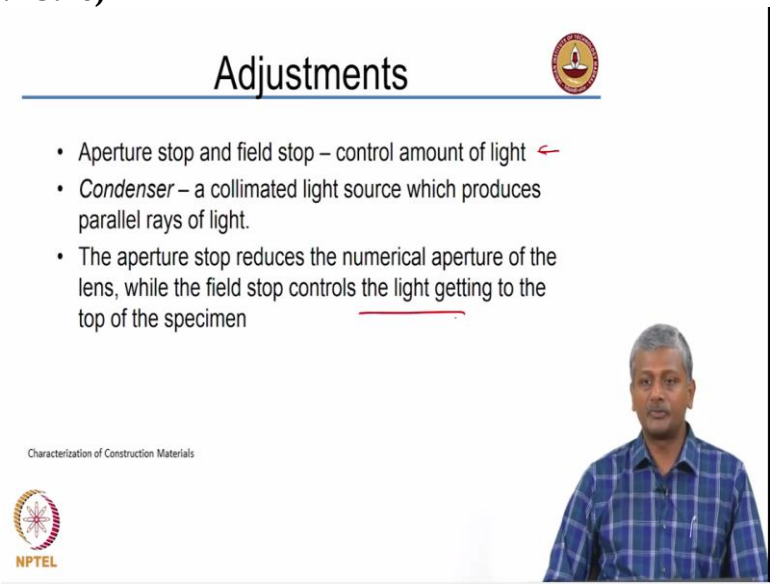

So, the adjustments of the light are provided here. For example, aperture stop and field stop are the ones which control the amount of light that goes on the specimen and can be collected by the objective. A condenser is a collimated light source which produces parallel rays of light. The aperture stop reduces numerical aperture of the lens, please remember when we increase the size of the aperture, the numerical aperture will go down. The field stop controls the light getting to the top of the specimen. So, in this case, if you look at the microscope (Parts of optical microscope slide), you see the aperture stop in this location here and you also have the aperture stop in the case of the transmitted light microscope in that location.