Techniques of Materials Characterization
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Lecture – 6
Bright Field and Dark Field Modes

Welcome everyone to this NPTEL online certification course on techniques of materials characterization and this is module 2 that is second week and in the first week we have learnt about various general concepts of microscopy and which is covering optical microscopy, electron microscopy, its very general concepts and there we studied about resolution, then magnification, depth of field, depth of focus.

All these basic general concepts about microscopy, any kind of microscopy. And we also learnt about aberrations, various type of aberration, their source, their prevention in again optical and electron microscopy and so on and so forth. Then we moved on to optical microscopy and there we learned how image forms in an optical microscope and in general optical microscope, general source of image formation.

And we also studied about various components of an optical microscope like objective lenses, light source, condenser lenses, then the image acquisition and all those. Now in the second module we have various modes of optical microscope that is what we will be learning, we will be studying and the first part of it will be covering bright field and dark field modes.

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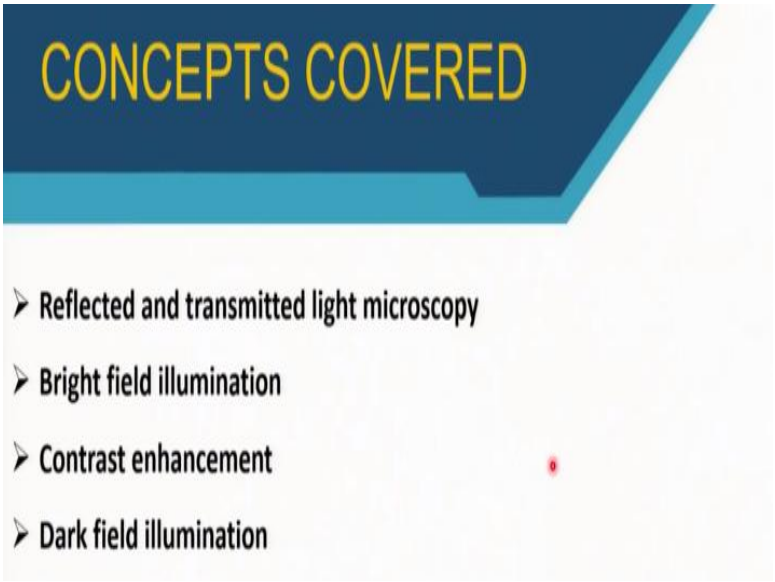

So in bright field and dark field modes, first we will learn about reflected and transmitted light microscopes. Then we will go to bright field illumination and then we will study about contrast enhancement and finally we will end up this lecture with dark field illumination.

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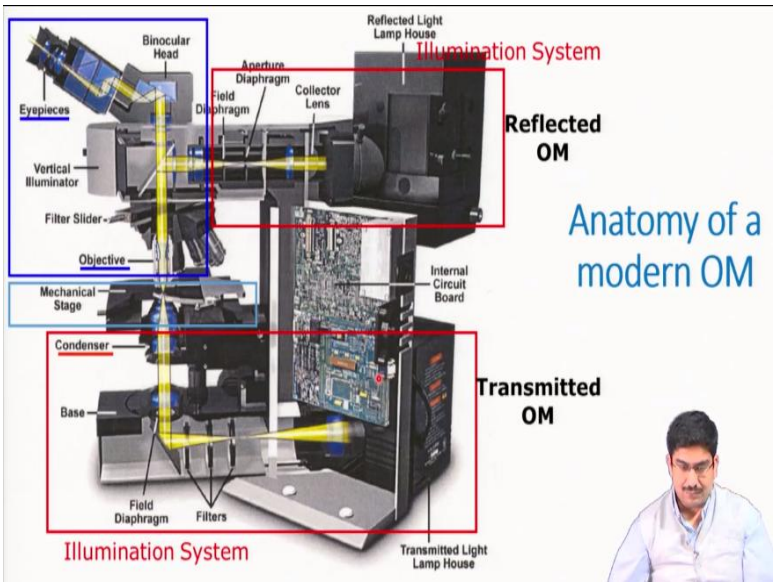

So now where from this all of these things comes, the bright field, dark field, reflected, transmitted and various other modes that we will be learning in the process. So that is because if you look at the anatomy of any modern optical microscope which is not less than I would say not less than cost you something like 20 lakhs or more, so that kind of microscope if you look at the anatomy you will see that it is very complex.

It has different types. So, in the illumination itself you will see that you can have illumination of this sort, another source of illumination of this sort and if you have a special mode called fluorescence then another third source of illumination. Then you will have different types of objective lenses, condenser lenses and within the objective lens and condenser lens you will have various other types of attachments.

So these attachments, these different sources of lights and all, these all things together make the optical microscope such a diverse technique and gives rise to at least I would say 5 different modes generally used. There are many other modes available but at least for our purpose the kind of materials that we study material scientists, biologist, for us at least 5 different modes are very important to know.

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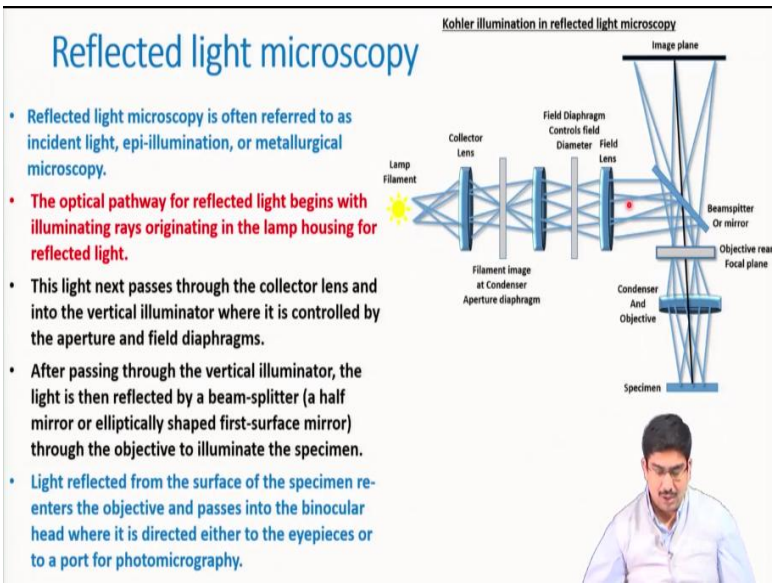

So, first thing we must know is that two different types of optical microscope purely depending on how the light source where exactly the source is and where exactly how the image are formed purely like that. So, the first one can be reflected light microscopy. As the name suggests this reflected light microscope this one optical microscope purely based on reflection.

Reflection of light here and this reflected light microscopy is also known as sometimes it is called incident light microscope, epi-illumination or often times we used to hear this as metallurgical microscope, although it is a very much, I would say very much underestimation of the capability of reflected light microscopes. They are used for various other purposes other than studying the metallurgical systems.

So, what happens here? As I said it all depends on the where exactly the source of illumination is. So, basically it starts with here. You have some kind of a lamp, we have studied about various type of lamps. Imagine that it is one of them tungsten lamp, pure simple tungsten lamp or maybe an LED lamp. So, it starts with here and then we have also studied about Kohler illumination.

And in Kohler illumination you have this collector lenses and you have various type of field diaphragm, control diaphragm and so on. So all of these collector lens has, we discussed in Kohler illumination collector lens basically forms a magnified image of the filament which is now used as a virtual source and then all of these different lenses their purpose is to make the light the way you want, whether a parallel ray of light or sometimes to play around with the intensity and so on.

You have different type of apertures, diaphragms and so. So, light basically comes from this end and then it encounters a beam splitter or a mirror. So, what reflected light microscope is done is that basically the source of the light and the objective lens both of them stays in the same side of the specimen. So, specimen stays at the bottom and then you have this condenser lens basically and the purpose of the condenser lens is exactly the same.

The condenser lens basically it illuminates the specimen. The light passes through here, this one illuminates the entire specimen and then from here itself the light gets reflected and it travels back. And when it travels back it basically goes through the objective lens. So, the condenser lens and objective lens is pretty much stays within the same configuration, within the same structure both of them remains here.

And there is a beam splitter or a mirror which is basically a half mirror and purpose of that mirror is to basically this light which is coming from here this Kohler illumination it transfers that light to the condenser lens and when the reflected light comes that does not undergo any kind of deviation from its path. This beam splitter does not work for them.

So, this purpose of the beam splitter is basically it divides the optical pathway of the light which is coming the source light and the reflected light the source of signal. This

source of illumination and the source of image formation these two are separated by this beam splitter here. So, this is basically how the reflected light microscopes they work okay.

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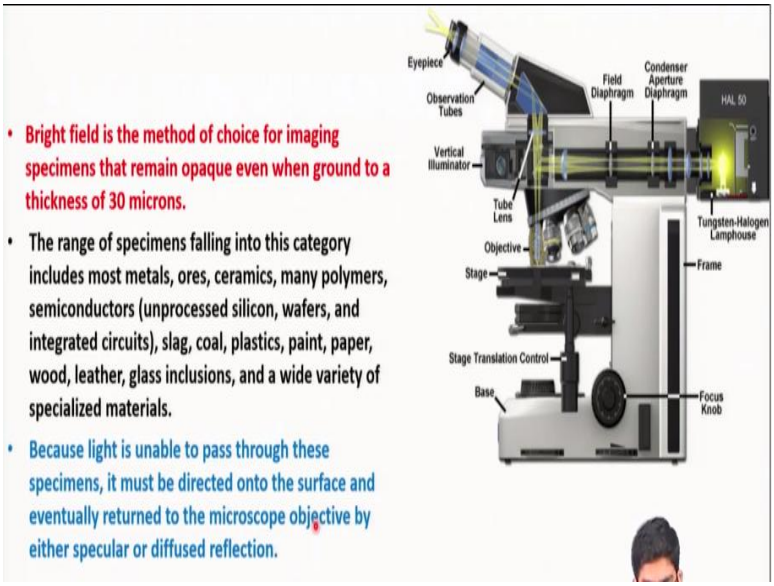

And this is what is called also sometimes bright filled microscopy, but this reflected light can be transmitted light for other modes as well but most often we used to see that in a bright field microscopy. So, this reflected light microscopy most often works with opaque specimen. Now opaque specimen means what we will learn in the next section when we learned about the electron microscope is that if you reduce the thickness of any material, so the materials which are opaque to electron beam.

When they are quite thick the same material when you thin them down up to a level, of course it depends on the signal also, the energy of the signal that also it depends on. But if you thicken them reasonably well, then they become electron transparent. But this does not happen for most of these materials we know, even if you thin them down to a reasonable limit which we can possibly achieve just by from a bottom top-down approach.

If we keep on polishing them by various method, we just bring their thickness down, even if we go down all the way up to 30 microns we would not be able to make them transparent to light. So that kind of materials which are quite opaque even at that thickness those for them this reflected light microscopy is the only way to do it.

So the range of materials what you can imagine you can think that most of the metals, ores, minerals, ceramics, polymers many of the polymers, semiconductors, slag, coal, plastics, paint, paper, wood, leather glass inclusions and a wide variety of specialized materials. All of these materials whichever are opaque you cannot make them light transparent.

All the way if you thin them down up to that level 30 micron or even below for them your only choice is this reflected light microscopies okay. And that is because purely this light is always unable to pass through these specimens. So that is why you have to take advantage of reflection or reflected light.

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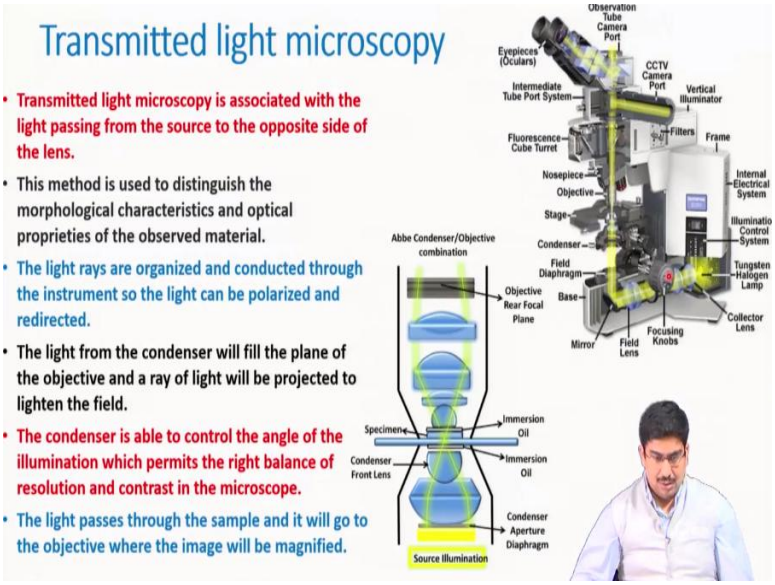

So of course, if you can make in something or if some material is inherently transparent to light, then you can go for another mode which is the transmitted light microscopy. So transmitted light microscopy as the name suggest of course it transmits light. And when it transmits lights then you will have something of this kind of a configuration where the source and then the condenser lens and the objective lens will stay on either side of the specimen.

So that is where it is different from reflected light microscopy. In reflected light microscopy condenser lens and objective lens, so condenser lens is a part of the illumination system, objective lens is a part of the image formation system. Both of them stays in the same side in reflected light microscopy, whereas in transmitted light microscopy these two stays in two different side, either side of this specimen.

So that is the basic difference between these two configurations. And here pretty much the same thing you have a light source and you can produce Kohler illumination and dot Kohler illumination. After Kohler illumination you can have a mirror, again a beam splitter and that will direct these lights through the condenser lens on the specimen. Now, this light will then pass through this specimen and it will reach to the objective lens.

And finally, through the objective lens it will go to either the eyepiece or camera whatever you have there. And this is what we pretty much have discussed and this is how the transmitted light microscopes works. Now transmitted light microscopy as I already said that list of materials that are opaque and that can be only seen under reflected light microscopy, other than them most of the materials you can see them transmitted light.

And most important example of this is biological systems. Biological systems mean cells, tissues and bacteria and all other things that you can think of. They are mostly transparent to light and they are seen in this transmitted light microscopy or in this transmitted mode.

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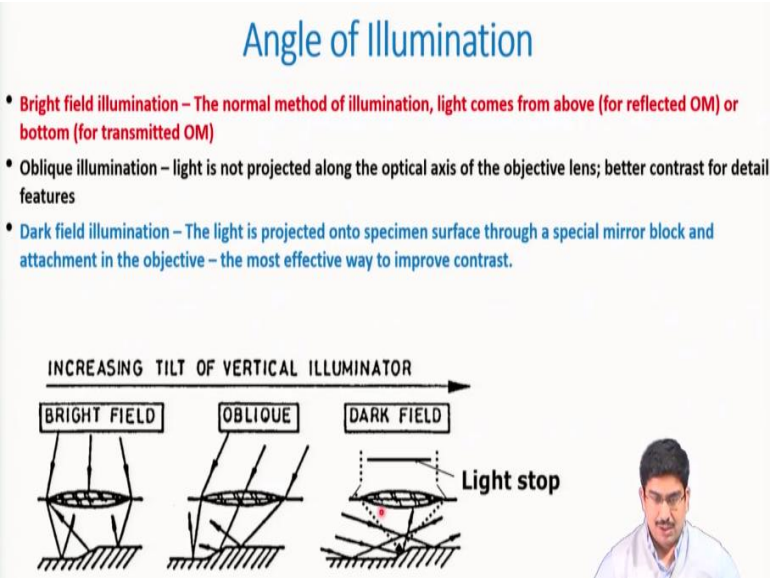

So in the reflected light microscope now you have another kind of difference or another kind of difference you can create it just by the way a specimen gets illuminated. So, you can change the angle of illumination basically and this will give you two different modes

of any optical microscopes. The bright field illumination, now the bright field illumination is a normal method of illumination.

Here light comes from the above for a reflected optical microscope or bottom whatever it is, but basically you are working with a direct light. Here the light either it passes through the specimen or it gets reflected but through the objective lens you basically allow only the direct beam. You just allow the direct beam to form the image and of course then you have diffracted beams.

And those diffracted beams will go and modify the contrast and will create the contrast that is basically the point. So, here you are allowing the direct beam. Then what you can have another extreme is of course the dark field mode. In the dark field mode what you do, we will come to this dark field mode in a little while. So, in dark field mode what you do is that you do not allow the direct beam to pass through this objective lenses.

You do not use the direct beam to form the light. So, do you how do you do it? Basically sometimes, we will discuss about that, you basically change the angle of illumination, the way light is falling on the specimen you just change that angle. And if you change that angle what you will be having is that instead of direct now direct beam also become oblique to the specimen.

Instead of direct beam now you will make the diffracted beams of scattered beams to go to that will be 90^{o} or perpendicular to the specimen and that will travel through the objective lenses. So that is what just by changing this angle of illumination you can define or you can change which of these lights will go through or pass through the objective length.

So, this also can give you another very important difference in the optical microscope, two different modes by bright field and darker. We will discuss There are more differences to it, we will discuss about that. And remember that both in reflected light microscope and in transmitted light microscope, you can have this bright field or dark field.

So bright field, dark field are two modes and reflected and transmitted light microscope those are two different modes that depends on how this configuration of an optical microscope is, which side is the condenser, whether the reflection is used for image formation or whether light transmits. So, it is completely based on the configuration of the microscope.

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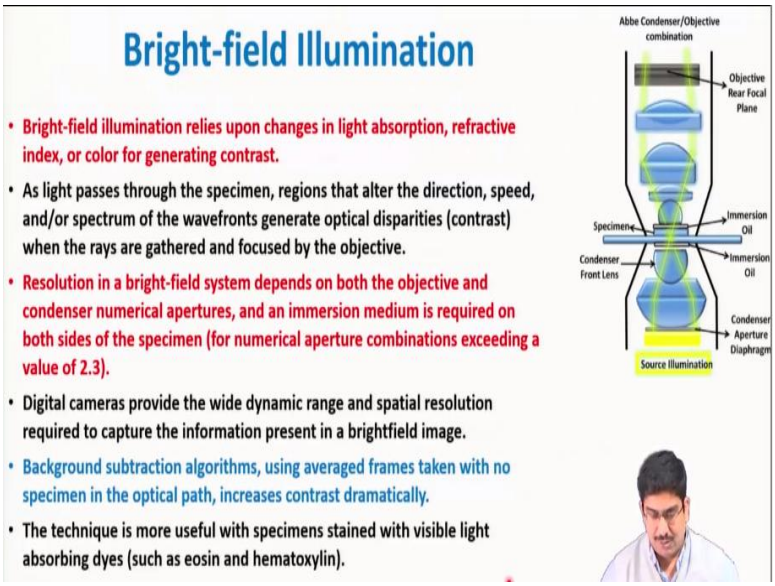

So let us first start about bright-field illumination and bright-field illumination as I said it is easier to understand. Because this is what we were discussing when we were discussing about image formation in an optical microscope, we were basically discussing about bright-field illumination only. So bright-field illumination of course depends on the change in light, so as I said direct light is used here.

So since we are using direct light, the contrast generation here depends on the change in absorption, refractive index, color all of these things is basically creating the contrast. It will change the intensity of the direct beam here depending on your specimen. So, different regions of your specimen if they have difference in light absorption or light scattering, then of course the direct beam when it reaches to your objective lens or to the image formation system then you will get a contrast depending on that.

And as the light passes through the specimen of course you will have different type this light there will be a change in the direction, speed, a spectrum of wavefronts which will give you the contrast. In the bright-field mode of course both the objective and condenser numerical apertures that will dictate your resolution. We have already

discussed about this how resolution is dictated by the numerical aperture if you remember.

The diffraction related resolution is controlled by the numerical aperture of both condenser lens as well as the objective lens, both of these. And condenser lens basically creates the beam, so the beam size is very important there in controlling the resolution and that is depending on the numerical aperture of the condenser length. Similarly objective lens numerical aperture is also important for forming the final image.

And in order to improve of course the resolution you can use some other kind of medium, you can go for an oil immersion lenses, oil motion condenser lens, oil immersion objective lens both and that typically we do if we need higher resolution in higher magnification. This also we discussed if you remember that the relationship between resolution and magnification and the relationship between condenser lens and objective lens.

And finally, you use a digital camera. These days every microscope comes with digital cameras and those digital cameras you can get another kind of or another mode of special resolution from the digital cameras as well. What can come very handy in bright-field illumination sometimes you do that is called background subtraction and background subtraction means basically you remove the specimen.

And you take a blank image where so if you remove the specimen as you can understand there will not be any source of this light absorption or scattering, there will not be any such effect in there. So direct light, direct beam will straight away go from condenser lens to the objective lens and you will not be able to see any kind of contrast in your image plane but that is very important.

What you can do is that you can just artificially using certain algorithms using certain software you can basically take some such background image and then subtract it from your original image with the specimen the image that you capture that way sometimes you can play around with the intensity, sometimes you can play around with the contrast between different features.

And that will improve the contrast between features and this is very important. In fact for another mode which we will discuss that is called fluorescence. So bright-field illumination this mode is very effective when you have something like a fluorescence mode. In fluorescence mode, we can bright field transmitted light, fluorescence works in the transmission usually.

So there this bright-field mode and fluorescence it works very good and that time you need to have some visible light absorption dies. We will discuss about that when we discuss about fluorescence microscopy.

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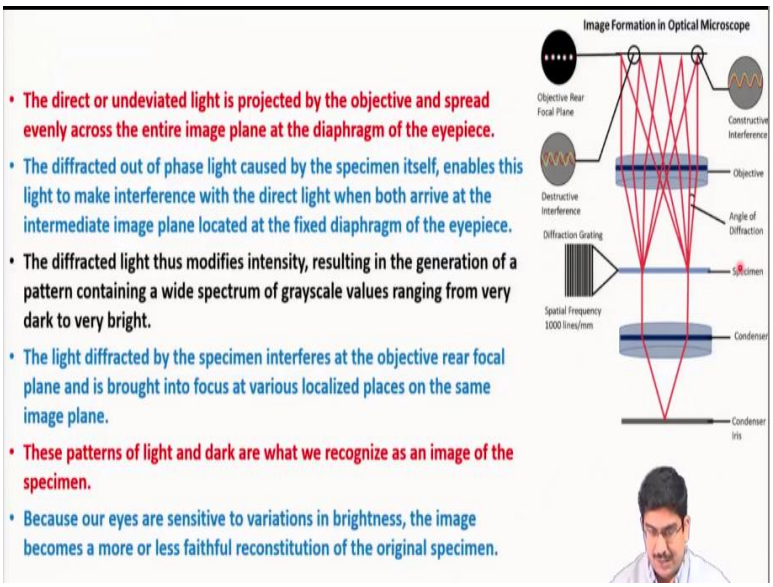

This is pretty much the same slide that we showed how exactly the image forms in an optical microscope with the diffraction grating and so on. So, basically this is same thing as the image forms in a bright-field microscope. So you have this condenser lens, you have the specimen. Specimen works like a diffraction grating and then you have the direct beam which forms through the objective it passes and it forms basically here the background the entire intensity

And then you have the scattered beams or diffracted beams and those diffracted beams goes and modifies the direct beam, intensity of the direction it interferes with the direct beam and it modifies the intensity and this intensity modification is exactly in proportion to the diffraction of the features which are present in this specimen, the features which are causing this diffraction. So ultimately the image that you get here is a true representation of all the features that you have within your specimen.

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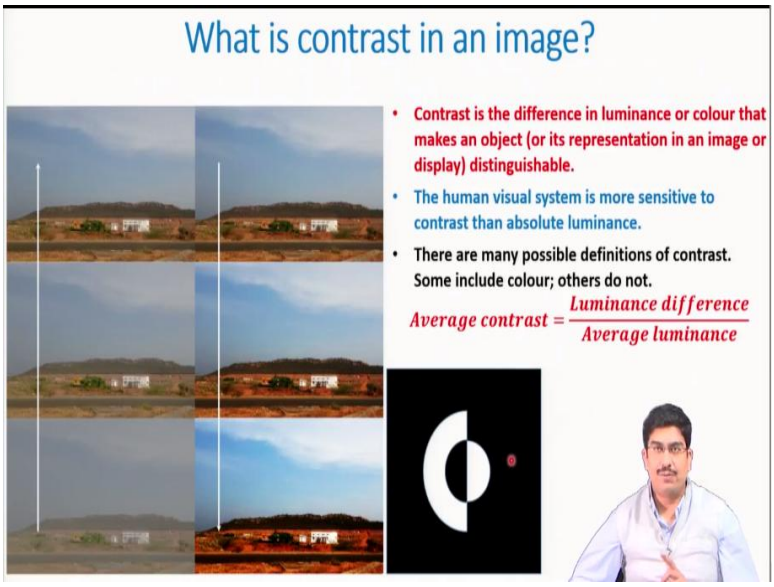

So before we move on to the dark-field image, again we have to recall the contrast because dark-field mode onwards whatever we will discuss dark field, polarized light microscope, interference contrast, fluorescence, phase contrast all these different modes are together called contrast enhancing mode because in this modes what you do by various mechanisms you try to improve contrast or you try to play with the contrast of your image, not artificially, in a true sense.

Artificially you can of course do as I did here. I told you and that in this case I just play around like increase the brightness and in this case, I basically play around with the contrast in software artificially completely, but this is just to show you what basically the contrast and brightness is all about. This using this contrast enhancing modes you can basically play around and do and try to do this physically within your sample that is why they are calling contrast enhancement.

So, contrast as we also develop this relationship it is basically the difference, difference between the background and certain features. So if you have two different regions for example if you have this image and you have two different regions, one is dark, one is completely black, one is completely white so the difference between these two is the contrast.

So it is not about the absolute value of this intensity, it is about the difference in intensity between two regions of the image. And in the contrast enhancing method this is what we will be playing around.

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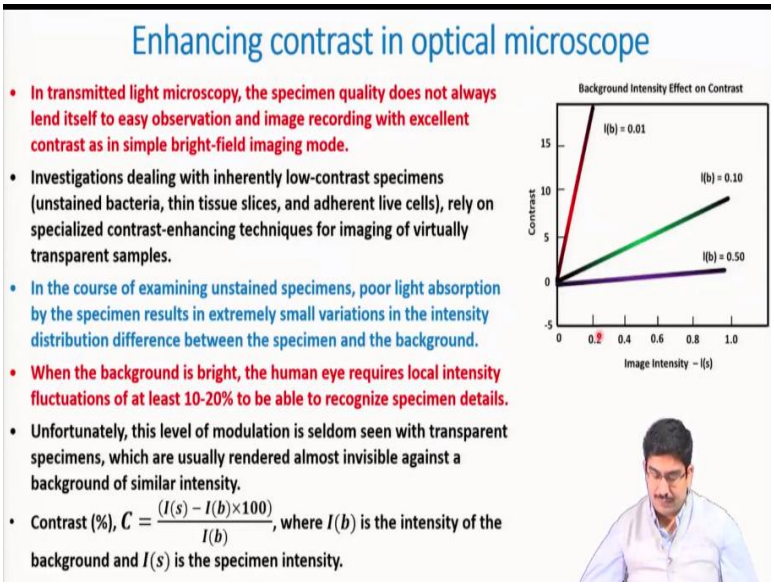

So, this is how we can improve the contrast in an optical microscope. And this is a problem not so much with the reflected light microscope, but with a transmitted light microscope usually the specimens are such that they do not give a good contrast which is so very much possible in a bright-field mode. Bright-field mode typically gives you the wave bright-field mode as I said there the direct light is used.

So, what you are finally dealing with is so huge amount of light intensity. So, this light intensity is available for you to form this contrast and just now I discussed that you can use something like a background correction algorithm which will artificially you can play around and you can try to improve the contrast with that background correction. But this you cannot do in any other kind of modes which inherently for example dark-field mode.

I just now talked and the darkfield mode typically uses the scattered beam or diffracted beam and we have seen it during our discussion in resolution and our discussion in that image formation we have seen that the direct beam carries almost 84% of intensity, whereas even the first diffracted beam carries much less than this only hardly 10-12% and then it decreases even further.

So basically, in all these contrast formation modes for example dark-field mode you are using a very source of image formation the signal that you are getting from your specimen for image formation itself is very weak. So, you cannot do much with it. It does really give you contrast as such. One typical example I used to tell in my classes to my students that imagine you have 100.

And some feature has a 100 intensity, some feature has a 95 intensity, then the contrast is very less okay and again so if now you imagine that something has 10 intensity and 9 intensity, now the contrast the absolute relative difference is increasing here. So that kind of an analogy you can bring and you can imagine that that is what you do to enhance the contrast in this contrast enhancing methods.

And in transmitted as I said transmitted light microscopy the kind of samples that you use as I said biological sample where inherently the contrast that you get from different features is very low. So, you must use some kind of a contrast enhancing method there and for example and there is a very important point to here just like we have a resolution in our eye, I already discussed with you that the resolution, spatial resolution is around 0.2 mm.

So if anything is smaller than 0.2 mm, our eye which is also an optical system that resolution we cannot detect anything. Similarly, we have a resolution, for our human eye we have a resolution in terms of contrast and that resolution is around 10 to 20%. So if two features they have a difference in intensity below this 10% we will not be able to detect them properly okay.

So, we need to have the contrast difference between two features within this minimum 10% or maximum 20%. We need to have this difference to able to see them as two different features, right. So, we can see this relationship now here how to increase the contrast or how this contrast enhancing modes basically works out. So, if you see here this contrast, in this side there is a contrast, in this side there is an image intensity.

So, if your image intensity if your background and contrast as just now we defined the contrast is basically defined is like this, so you have this specimen intensity minus in terms of percent of course, specimen intensity minus the background intensity and

divided by the background intensity. So if your background intensity is very low, then for the same amount of image intensity you can get a very high contrast.

But if your background intensity is very high then for the same level of image intensity. For example if you consider 0.2 of your image intensity, if your background intensity is very low you will get very high contrast for the same level of image intensity. But if your background contrast is very high for getting that kind of a contrast you will need to have a very high image intensity which is not possible and this is what we try to do in the contrast enhancing mode. We basically try to reduce the background intensity.

So, if you reduce the background intensity so for the same amount of image intensity you can have a very high level of contrast. So, most of the contrast enhancement method at least for the dark-field method this is what we try to do. We try to reduce the background intensity that means background intensity as you can understand that if you are using a bright-field method background intensity will automatically be very high. So, we have to go for some other contrast enhancing methods.

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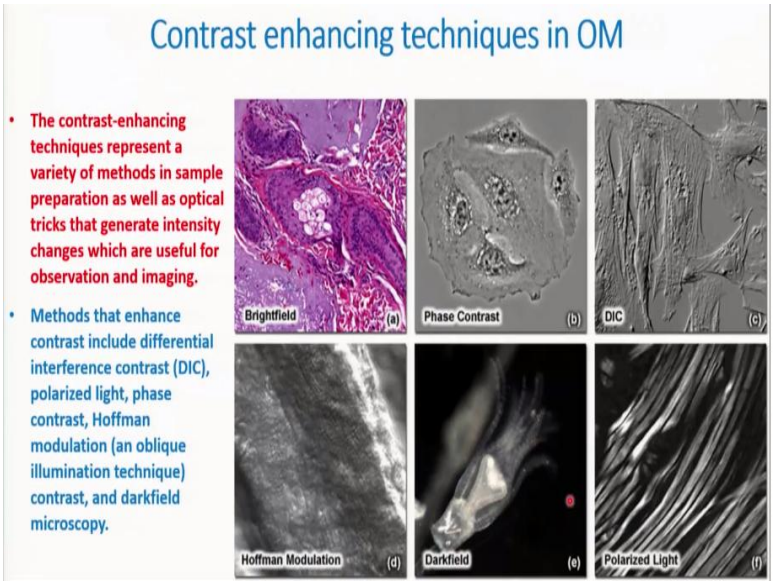

And contrast enhancing methods here we have many different typed of contrast enhancing methods. So this is a bright field and then two of this four, we will discuss 5 five different modes in optical microscope. Out of that these 4 are basically the contrast enhancing methods which we will be discussing. Phase contrast, differential interference contrast, dark field and polarized light. So, these 4 different contrast enhancing methods we will be discussing in subsequent classes.

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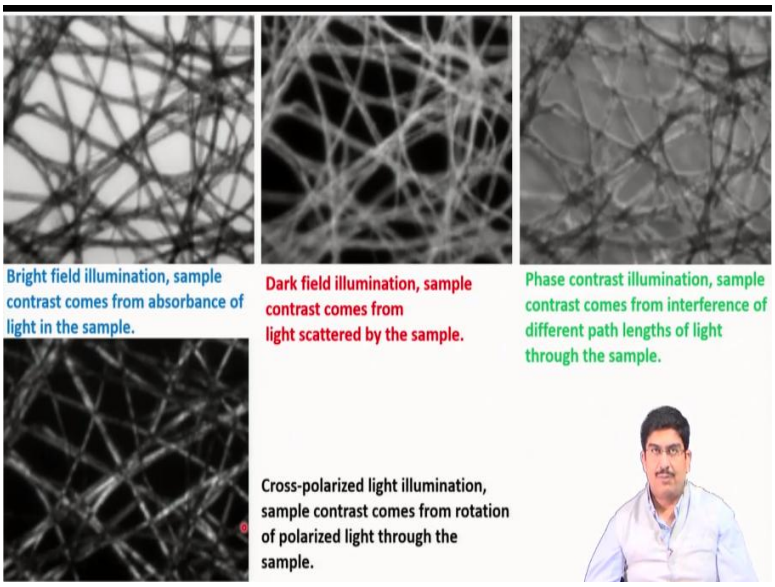

Again, one more nice example is shown here. This is a bright-field illumination here and this is the dark-field illumination. What I did is that I just cut this background so the background that you are seeing here that is cut and the moment you cut that background now these fibers that you are seeing here the fiber becomes much more prominent. So, you remove the background somehow.

You chop up the background and then automatically the contrast between these features will be pretty high. So that is what we try to do in dark-field illumination. Other kind of contrast enhancing methods this is not very effective, there we basically try to change the entire process of image formation, the entire we basically play around the way image forms with the source and with the detection signal, we play around with them and there we again try to increase the difference.

We do not try to reduce the background there. We try to increase the difference by some other methods and that is what we also do in case of phase contrast and polarized light. There are some other differences, but we will come to know about them a little later.

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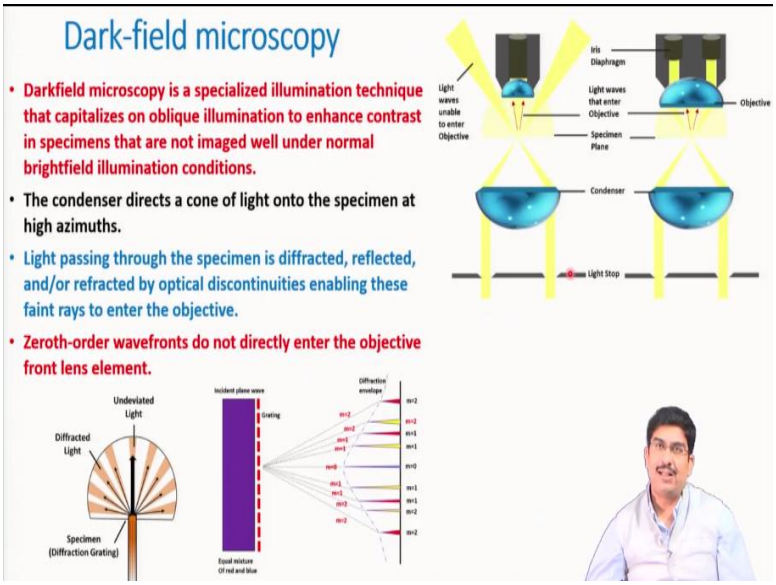

So here of course we will just learn the first one today in this lecture about dark-field microscopy and as I already explained the dark-field microscopy basically when a light encounters or passes through a specimen it forms this direct light and it forms this diffracted light. All of these diffracted lights. And these diffracted lights of course they will have some special kind of relationship and the scattering angle everything is determined, very determined.

So, this is the 0^{th} order beam the direct beam and then you have first order beam, second order beam and so on and so forth. What you do here is that you put an oblique illumination. So, you make sure that only your let us say you choose the first order beam, only the first order beam passes through the objective lens whereas illumination is such that, now this illumination this is the illumination and this is the direct light beam.

So direct beam is still going parallel to the illumination, but now since it is oblique illumination only the diffracted beam is 90^{o} to the specimen and that is why only the diffracted beam is passing through this specimen. Afterwards the image formation is pretty much the exactly the same through the objective lens when the diffracted beam passes through first order diffracted beam.

Now forming instead of direct beam first order diffracted beam is forming the background, other higher order diffracted beams are interfering with it and they are forming the contrast that is it. So that means you are removing the direct beam

completely, you are reducing this background intensity hugely. The total intensity goes down contrast enhances automatically.

Instead of first order beam, you can go for a second order beam, third beam and so on, but obviously with a reduced intensity. Those intensity of those higher order beams are very less, so generally people tend to use the first order beam and for that since as I said you know this angular relationship, you know what kind of an angle oblique illumination you need to use so that you can avoid this direct beam and you can just take up the first order diffracted beam.

And this everything is done through the condenser length. So, this condenser lens here basically the Kohler illumination and all, so this condenser lens changes the entire illumination, the oblique this entire angle and that is how you do it. The rest of the things is pretty much the same. Bright field and dark field you do not introduce any extra attachment either in the condenser lens or in the objective lens, nothing.

Just you have this one light stop which is there extra thing in dark field and if you basically have that, you just apply this light stop and you basically go from bright field to dark field mode.

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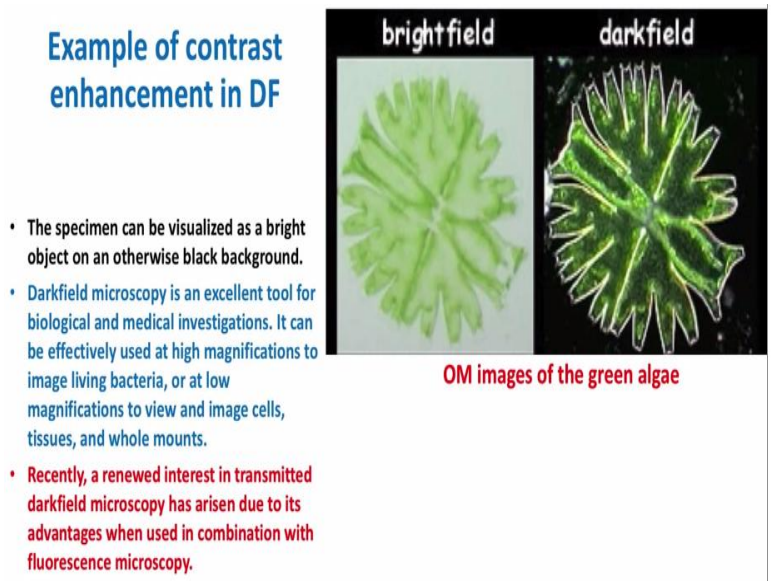

So, there is an example of contrast formation in here. So, this is the bright-field image. Now you can see that basically in the dark field you are removing the direct light and you are removing the complete background. So, generally in dark field we used to see

everything in black. The entire background comes out to be black and that is why the contrast basically enhances much higher, much more of these features here.

So, these features still appear in the same, only thing is that their contrast is now much more enhanced if you imagine these two regions, this dark green versus this light green, here now they are much more prominent. The difference between them is much more prominent. And obviously the ends, these ends you are not able to see quite well in the bright-field mode that you can very well see in the dark-field mode.

Again because of the same reason that now you increase the difference between the light intensity coming from these regions and somewhere in this region. So, this is what you do in dark-field mode. And with this we stop here and we will continue in the next class with some other modes of optical microscopy. Thank you.