Techniques of Material Characterization
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Module 01: Introduction to Microscopy and Basics of Optical Microscopy
Lecture-04
Introduction, Types and Image Formation in Optical Microscopy

Welcome everyone to this NPTEL course on techniques of materials characterization, and we are going to module one that is introduction to microscopy and basics of optical microscope. And today's lecture will focus on introduction types and image formation in optical microscopy.

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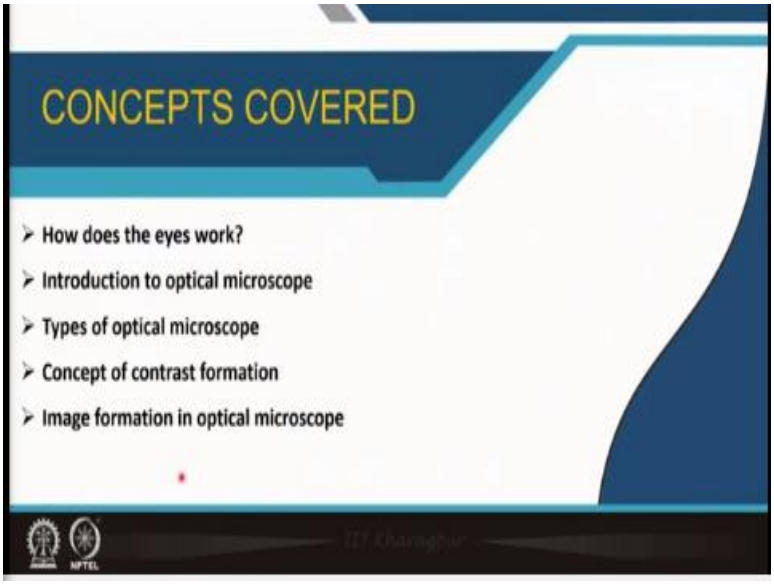

So, it will cover first thing is how does our eyes work. So, this is you may wonder why we are talking about eyes but we will come to know about it later. And then we will go to the introduction to optical microscope, types of optical microscope, concept of contrast formation in any kind of microscopy and image formation in an optical microscope.

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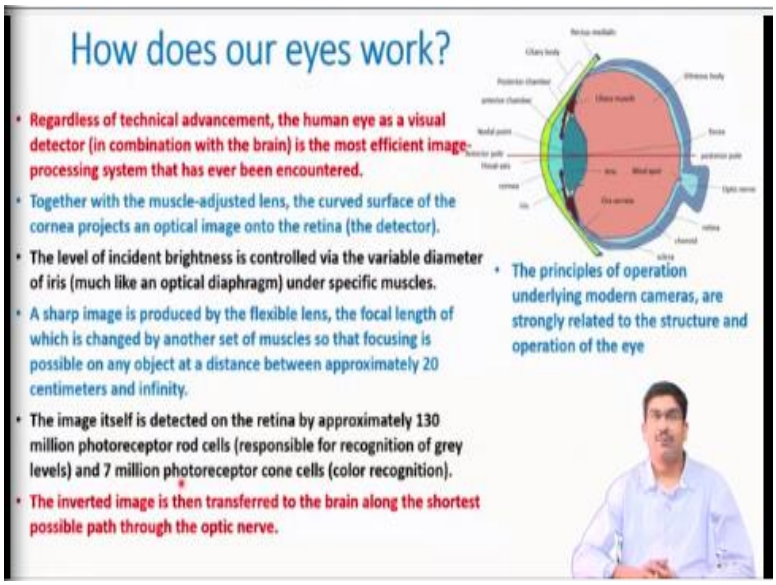

So, the first thing we must understand before we progress with any kind of microscopy, in particular optical microscopy. That we have to understand how our human eye basically works. So, our human eye works in the same principle as an possibly an optical microscope work out. For that matter any kind of phones, digital phones these days digital cameras these days either in your cell phone or the digital camera that you use to capture some photographs.

Or an SLR camera if you are a photography enthusiast, all of this basically works in the same principle our human eye works. And by still date whatever human beings whatever visual or optical based systems, whatever optical based gadget they are ahead to come up with none of them can match the efficiency or intricacy of our human eye. So, human eye is so complex a system. That is because our human eye has this retina here which works like an detector.

And then we have some muscle adjusted lenses here which basically works like lens just like in a regular camera or an optical microscope, this is also a lens but muscle driven lens. So, in this using this lens you can have a very much variable focal length something and you can able to focus some objects which are as close as possibly something like 20cm all the way up to infinity, you can able to focus this.

Then we have this iris here and that works like a what we will discuss later is like an aperture. And that aperture is controls the brightness or the amount of light that is entered through this.

And then we have if we talk about this retina that is a detector, then this detector itself as around 130 million photoreceptor rod cells. And this rod cells are basically recognize the grey levels or grey patterns and then around 7 million photoreceptor cone cells which are basically for colour recognition.

So, all together we have nearly 140 million cells and if you compare the cells as equivalent to the pixels in what you have in your digital camera. Then you can say that our human eye has a resolution in terms of pixels of around 140 megapixels, something like that. It is a different thing that this pixel that cells are much bigger than the pixels that you possibly have in your detector, even if you use an SLR camera.

Another important thing is that our human eye since it uses only one single lens, what it produced is an real inverted image and then that inverted image goes to the human brain through this optic axis. And how that inverted image becomes again got inverted and again it becomes the upright image, it is not fully clear yet, so that you must remember about a human eye. So, we are here to understand how the image forms with our own human eye, our own light based microscope wonderful organ that we have.

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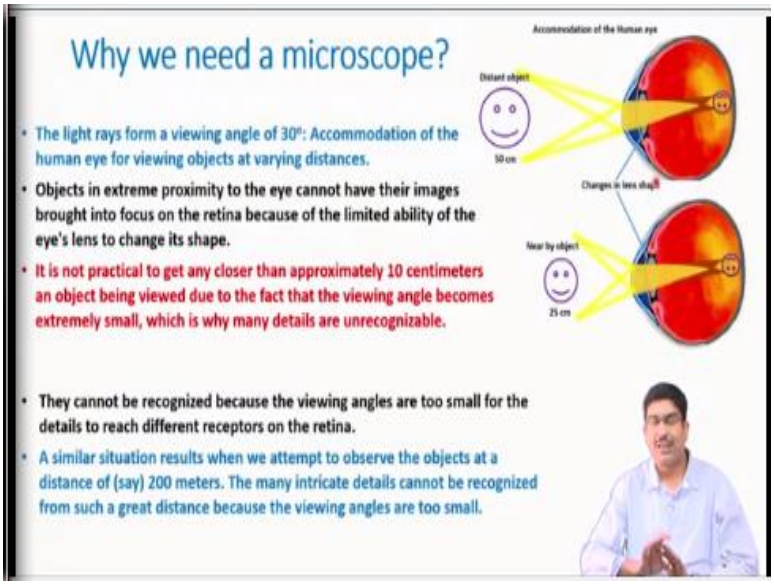

Now if our human eye is so good, why do we need a microscope in the first place? And that is because what we suffer is a problem called viewing angle or the field of view. So, what does this

human eye does is that let us say if you imagine you have an object at around 50cm away from your human eye, you are very you have this muscle adjusted lenses and they will very much adjust.

So, that this one now lies within the focal length will change and this object, they will try to bring it close to something like F just outside the focal length of our human eye. And then I will be able to see it and I will be able to project it on this retina that is this image plane that is very good. So, I will be able to see an image of this and then in that process, there will be a magnification of at least there will be the same, same size of the image will be projected here.

The problem is that when I bring this very close to my human eye around 20 to 25 cm, I will not be able to do this in anymore, I will not be able to focus this object of focus this image on my retina. That is the limit how close you can be and if you are possibly all of us have done this, that you try to bring this finger and you try to bring close to your eyes and try to see that when are you able to see this, you try to just take it away.

And you will see that how nicely it gets focused in all the places except when you bring this very close to your eye, so that is the limit. On other hand side if you put it very, very far off let us say 200 meters or 300 meters, 500 meters you put the object still you will be able to see this, it will be focused. Problem is if you bring it too close, or if you bring it too far off the viewing angle, the angle with which this object makes with your human eye or on our retina.

This angle will be very smart if you bring it close or if you take it too far away. So, there is an optimum range within which the viewing angle or the field of view will be enough for you to see the details of this object that is the problem. So, that means if something is very, very far off from us we in order to see that intricate details of that object, we have to use some tool. Same thing if something is very, very close to our eye, we will not be able to magnify or we will not be able to see the details.

In order to so that is exactly why we need a telescope in order to see the stars and that is exactly why we need a microscope to see the very fine objects which we are not able to see just because

of this problem of field, viewing angle gets very small and the field of view gets restricted. So, in one case stars, you are able to see the star but you will be able to see what is there inside the star. For that you need this telescope, same thing you are able to see possibly and if you just imagine a biological sample, if you possibly able to see a drop of blood, what do you do not?

You will not be able to see is that the cells within that blood because the same problem will not be able to magnify, you will not be able to see or not the viewing angle or the field of view will be restricted so much. So, that is why you need to have a microscope in the first place otherwise our human is perfect, very nice.

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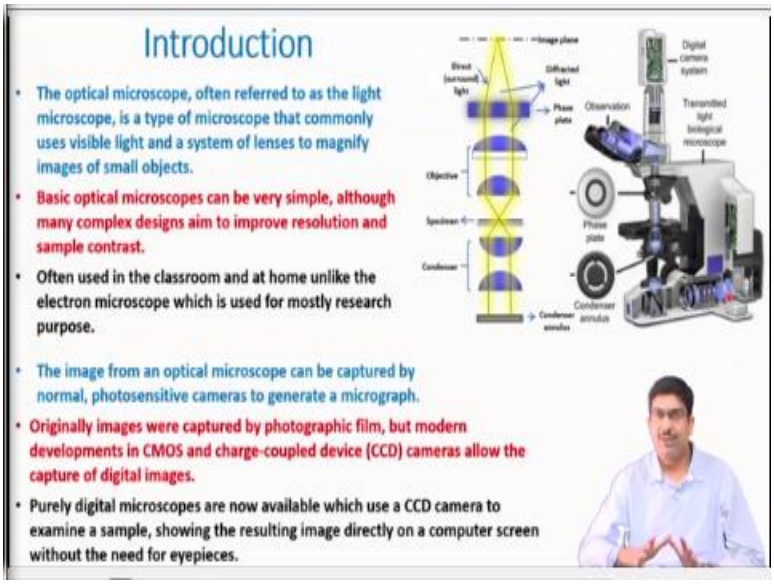

So, the optical microscopes sometimes also referred to as the light microscope. And this is because it generally uses light, visible light as a source of signal. And remember what I said about source of signal and then detection signal and the way of detection. So, in this case the source of signal is basically light, that is why it is called a light microscope. And in this truer sense, the optical microscopes are very, very simple.

We will see that those kinds of microscopes optical microscopes are known as simple microscope, the best example is possibly a magnifying glass. It works in the same principle as a human eye, you have an object, you have a lens and then you sort of magnify it. And then we form this image in our human eye that is it. And such kind of simple microscopes we often used

in classrooms, you have must have seen this microscope simple, very simple microscopes and home and so on optical microscopes.

On the other hand, there are far more complex optical microscopes which are used for something like research purpose. And then there are something like electron microscopes which are exclusively used for research purpose only. And how we capture images in these optical microscopes? Generally in good old days there used to be camera, photosensitive camera which takes this image these days everything is done using a CCD or charge coupled device or even better if you have a CMOS kind of detector.

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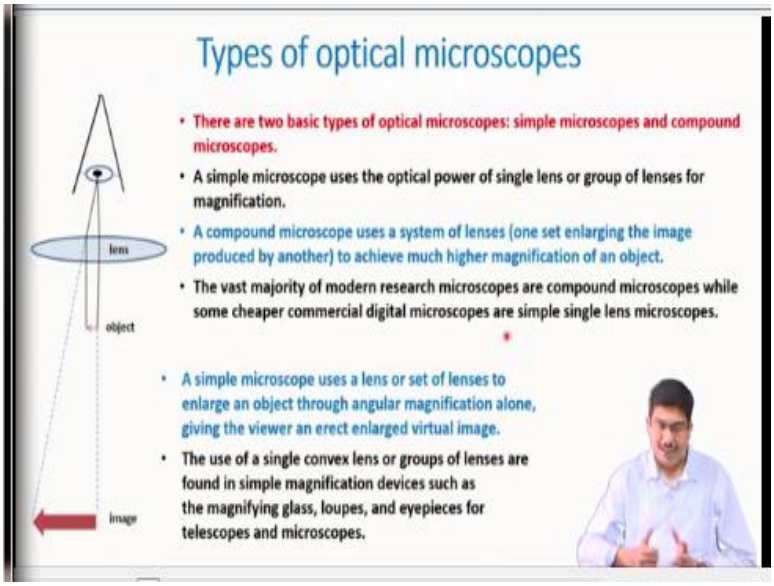

So, these are the ways the images are formed. And as I just said now say there are 2 basic types of optical microscopes. One you can call a simple microscope and another one is a compound microscope. Simple microscope, as the name suggests you just have usually one single lens like a magnifying glass or you can have multiple lens also, if you want to just improve the magnification you tend to have a little bit more complex system and bit more complex lens system more than one lens.

Compound microscopes, on the other hand will have a complex geometry, it will always have more than one lens a combination of lenses for something like. And the way they are working is the magnification produced by one will be further magnified by another lens that you have. So,

the object, the image formed by one lens system will work like a object for the next lens, so that is how compound microscopes basically use. And they normally they contains as I said more than one set of lenses.

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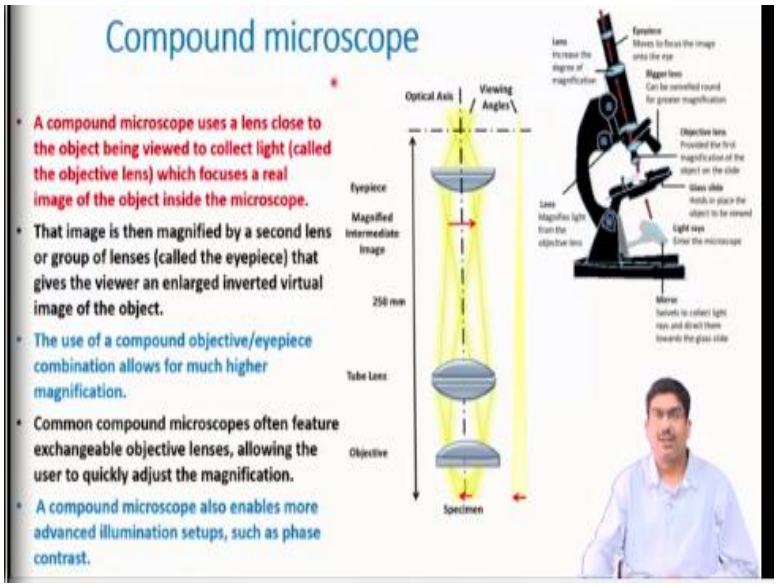

So, compound microscopes, again this is possibly what we have seen in your school, in +2, this is a fine example of that compound microscope where you have one set of lens which is called usually the objective lenses. And objective lenses, what does it do? It basically so you have a lens you have a source, usually as the mirror that and if you use the light as sunlight, that is what we generally do our good old days, that is how compound microscopes used to be.

So, the light falls here and it passes through the specimen, transparent specimen again. And this objective collects this light and does one set of magnification and then you have another set of magnification in this which is called eyepiece. And through that 2 stage of magnification after that it reaches to our eye and we are able to see a magnified image of this whatever the sample is here.

So, that is what is a compound microscope, it is kind of a usually this kind of at least in this one there is a 2 stage magnification. And in later we will see that in case of when we discussed about empty magnification you understand that there is no point magnifying it further any further than

1000X around 1000X. So, and you can very safely do that just by using 2 different lenses, that is it.

And generally what we have is again these objective lenses since in an optical microscope you have very fixed focal lens for your different types of objective lenses. And already I said that in a microscope like this the object and the image both of these are fixed basically you are seeing it here and the object is placed here. So, object distance and image distance cannot be changed because of the microscope configuration.

The best way to change the magnification here is by changing the object lens focal length, so that is how it is done.

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Research microscopes as I said these are examples of 2 research microscopes which are there in my own lab research lab and we very regularly use them. And this is far I would say far more simple, simpler ones. And there are much more complex microscopes are available in the market, we also have them, these are the 2 simple one. That is why I wanted to tell you this is possibly much simpler than even this, this one that we have.

Please note down the different configurations they have, 2 different types one is called inverted, one is called upright we will discuss about that later.

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So, before that we must know about this, what is contrast in an image? This we possibly have heard many times in terms of just a digital photography or in terms of many other things that contrast. What exactly is a contrast when you would consider an image? So, look at this images here, in one side possibly what you see is the intensity is decreasing, the overall intensity of these images is decreasing, this is or you can call that something to do with the brightness.

So, brightness of this I have just artificially take that image captured by me, of course, so copyright issues. So, these images I just reduced the brightness of these images, so brightness is lowest here and it is increasing all the way up there. Then in another set of image I take the same image and I what I increased is something like a contrast. So, this is done entirely by software, so I increase the contrast.

And when I increase the contrast what it does is that, it brings this difference making it even bigger, larger and larger, this one. So, the difference between different types of colours in this case, simplest way difference between different types of colours are becoming more and more prominent. So, if you look at this somewhere like this ground and the sky, the difference between them here the colour difference between them here is much more here, good or bad, I am not going to that, the difference is much higher, so this is what the contrast.

So, contrast you can define, in case of a digital image you can define contrast is the difference in luminescence or colour can be both. Contrast is basically the difference and the difference can be whatever. So, difference in luminescence or colour between that makes an object distinguishable compared to the rest of the other objects or compared to the background. So, the point is our human visual system, our human eye is more sensitive to contrast them absolute luminescence.

So, if I just reduce the brightness here, it is not so difficult for you to look at this. But if we increase the contrast, if it does not have enough contrast that is a problem for us, we will not be able to see that image if we do not have enough contrast. If it is not having enough luminescence brightness, we can still adjust our human eye to sort of allow more amount of light to come in and to increase the brightness.

But this contrast enhancement cannot be possible just by changing the lens system that is it. So, there are many possible definitions of contrast and one which is useful for us it is a average contrast is basically the luminescence difference versus the average luminescence. So, if you look at this one between the white and the black. So, these are 2 extreme cases where this maximum contrast we are able to see between those 2 regions, so that is how a contrast is defined.

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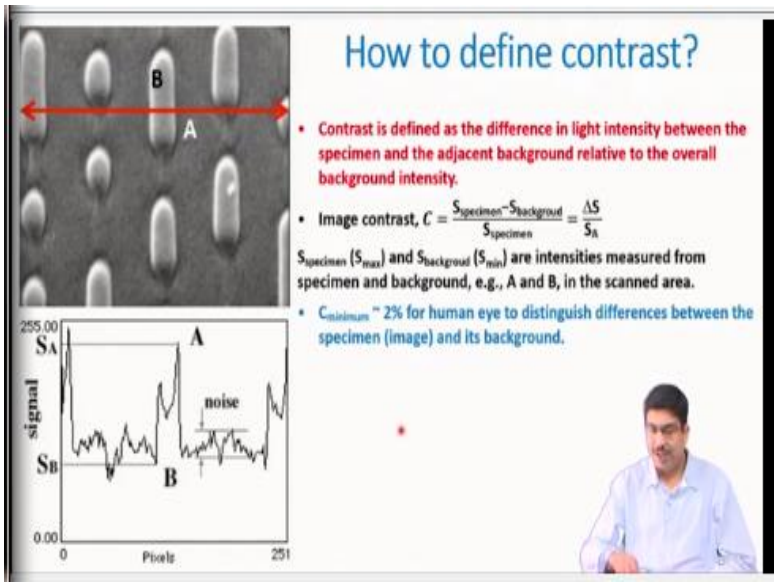

Again how to define the contrast in when you have a real image, real let us say optical or any microscopy image? So, look at this image and if we take an intensity distribution along this line, we will see certain objects here appears much more intense than certain other. So, in this side, it is 0 to 255, this is basically the grayscale values. And that same grayscale value I possibly discussed when we were discussing in pixels and digital images.

There I said that every pixel they are in a digital image, they are characterized by one of the values from 0 to 255, 0 means absolute dark, 255 absolutely white. So, if I take this intensity distribution in wherever I hit this brighter one, I have like higher intensity or it is a different one. So, I can define the contrast now in this way that the image contrast (C) = $(S_{specimen}-S_{background})/S_{specimen}$.

So, this is the intensities that are measured at different, places. The point is our human eye just like we have a resolution in terms of the distance. I said that our human eye cannot detect anything less than 0.2 mm; we have a resolution in terms of contrast as well. And that contrast is usually around 2%, so anything if you do not have contrast difference of 2% or more, we will not be able to detect anything here.

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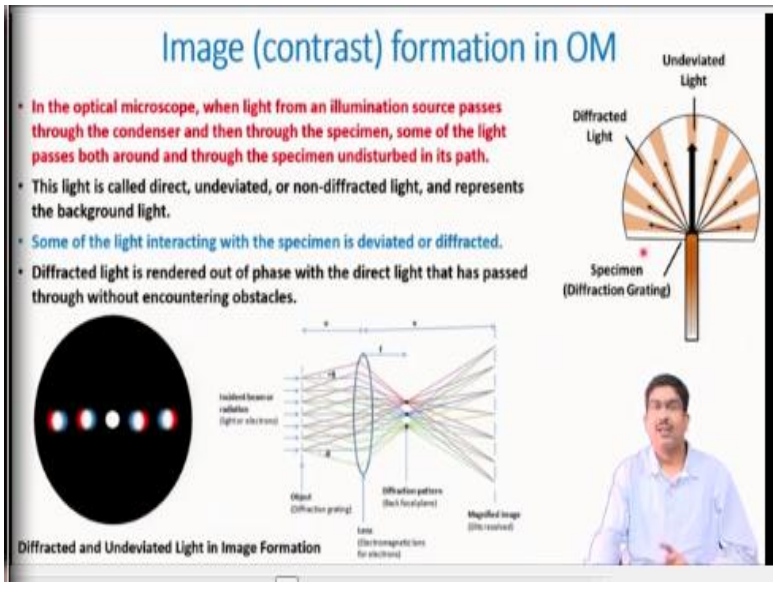

Now let us discuss how the image formation happens in an optical microscope. So, in the optical microscope, what happens is that when the light passes through, let us say that this is an specimen which is kept here, the light falls over it. And some part of the light or maximum part

of the light basically goes through without getting any kind of deviation from it is actual path and that is called an undeviated or direct light.

And in contrast to that certain part of the light basically gets deviated and what we call them as a diffracted light. So, they gets deviated from their original path**,** so that is called diffracted light and this happens whenever you have an object in the light path. So, this is the 2 kinds of light, so the moment the light falls on a specimen these 2 kinds of lights are produced and these are real things.

So, if you see it here, this is an image how this image this is formed and how we capture? I will be explained possibly little later. But this is coming out of the diffract or directing tool or direct light and this other spots are coming out of this diffracted light. So, this is a real thing and you can able to see it just by changing your image position, which I said if you keep your image position somewhere over here, you will get this diffraction pattern this kind of pattern.

If you keep it here, you will be able to get it image. So, this is very important in electron microscope, we will discuss it that time as well. And usually these diffracted lights are out of phase; this has phase difference with the undeviated or direct light. There are some kind of some amount of phase difference always happens between them.

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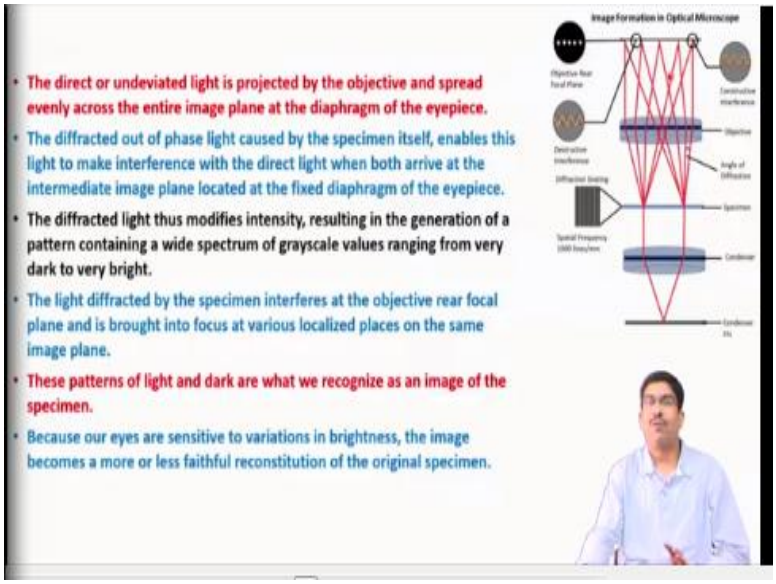

Now what happens is when the light, direct light passes through the specimen and it is collected, it passes through the objective lens and it is collected on this specimen or on this image plane, they forms the background there, background light. So, that undeviated light carries most of the intensity and they are all spread nicely around here on the image plane and form the background there. What happens is that diffracted lights since they are some way out of phase with or they have a phase difference phase shift whatever you call.

They have a phase difference with the diffractive or direct light. So, they will go and produce a kind of an interference on this image plane. That means, imagine that you have 2 different points in this object and those 2 different points are creating these direct light and diffracted lights. So, both of them are working like diffraction patterns or diffraction something that is causing these diffraction diffracted lights, we generally call it diffraction grating, we will discuss about that.

So, this diffracted lights from possibly from one source one of these sources if we consider them point sources, one of the source the diffracted light interacts with the direct light of the other one and vice versa. And same similarly all the points over this specimen they works as a point source and they create this direct light and diffracted light and they interfere basically on the image plane.

Because of this interference, what happens is that there is a difference happens in the intensity of the light some places the intensity gets modified based on what is the phase relation, how the diffracted lights are interfering with the direct light and so on and so forth. All together there is a contrast that is generated on this image plane basically. There is a difference happens at every plane and that directly corresponds to this specimen exactly the same way the diffraction or diffracted beam is generated, direct beam, diffracted beam is generated on the specimen.

Follow exactly the same pattern there is this intensity gets modified on this image plane. And that is how you get this image which is you can think of that this is a direct representation of this specimen. This is how the image forms in case of an optical microscope or for that matter in case of any kind of lens based system, this is how the image from. This is how the contrast forms without this we will not be able to see.

If you do not have the diffracted beam then all we will be seeing here is direct beam. That means we are as if we are seeing the source here, we are not able to see anything like this specimen here. So, that is how we are seeing this contrast and since our human eyes very sensitive to brightness to this contrast. The image that we see is become more or less is a reconstruction or a restructuring of this original specimen here that is how the image form.

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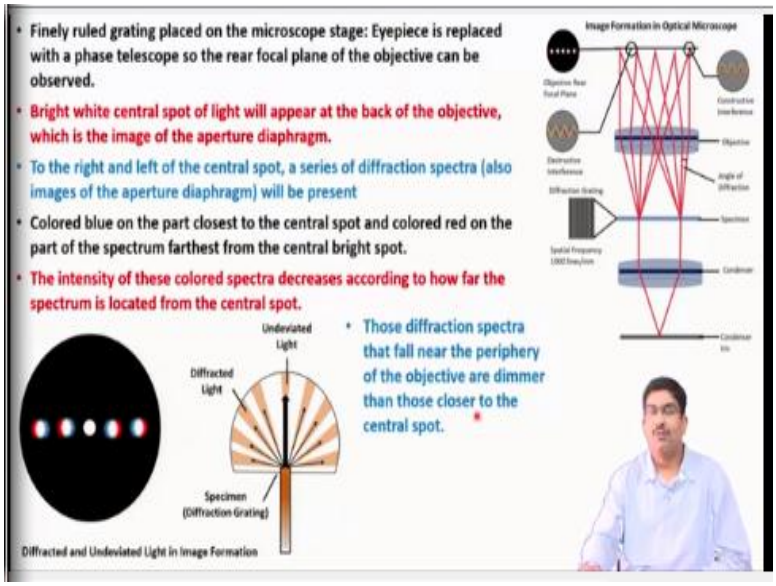

Now, in order to really verify that, this is the mechanism of image formation, what we can do? We can do a simple experiment and we can bring something called a diffraction grating, which is nothing but an specimen having a very regular pattern onto it, very regular openings onto it diffraction, that is called diffraction grating. So, this diffraction grating when we brought here, then what we will be getting is this kind.

And for that what we basically need to see as I just now showed this back focal plane of objective lens, this is what we have to see. We do not need to see somewhere out of the real image basically formed somewhere over here. Remember, there are another set of lens that is eyepiece lens, which forms the image final image. Instead of that if we just try to see this back focal plane of the objective lens, we will be able to see this kind of a something called diffraction pattern.

We try to see the diffraction pattern and then we will able to realize these entire phenomena of image formation. So, if we see this with through this diffraction grating, what we will see is basically the central spot, this one. And if we do not have the diffraction grating, we will be just seeing this direct light because there is no diffracted light nothing. So, if we try to see here, it will be just like this light passing through without any specimen.

So, we will just see this central spot, the central spot is as I said forming because of the direct light and it is usually called the zeroth order beam. And then what we will be seeing on the either side of them and they appears exactly there used to be 2 in the exact same distance to spot such spots and these are forming from this diffracted beams. And if you notice there that deflected beams, they have slightly colour, they have a colour effect.

And the bluish one is nearer to the central spot, whereas the reddish one is further away from the central spot that is first observation. Second observation is that the intensity of the spots diffractive spots that intensity goes down as we are going further from this central spot that is another observation. So, 2 observations can be made from here that blue lights or bluish hollow bluish tinge whatever you say this is closest to this.

Or these are closure for every diffraction pattern, these are closer to the central spot and intensity is going down for the diffracted beam, intensity is going down for if you have this more and more for this diffracted beams**.**

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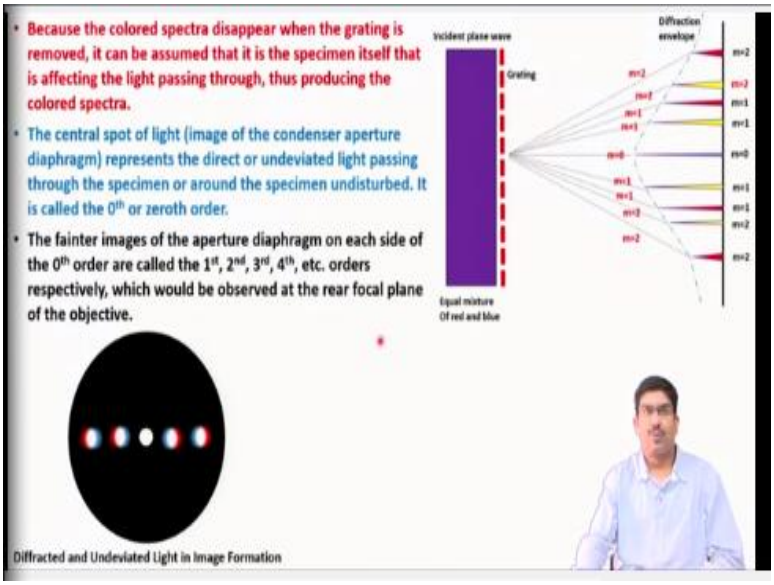

So, why this is happening? As I said this is a diffraction grating that you have the central beam or the undeviated direct beam that is passing through that is called the zeroth order beam. And the other ones are basically called the first one is called a first order diffracted beam, then you have the second order diffracted beam and so on and so forth. That depends on the order of diffraction; we are not going into that when we discuss about the diffraction phenomena.

Mostly perspective electron microscopy and X-ray diffraction then we will discuss that time we will realize what is this first order diffraction, second order diffraction and so on. Just remember now, that when the diffraction phenomena happens there is a direct beam. And then there are multiple diffracted beams forms and some of them are called first order diffracted beams and then second order refractive beam and so on and so forth.

So, if grating is removed as I said we will just be having this central spot and that is the zeroth order diffracted beam.

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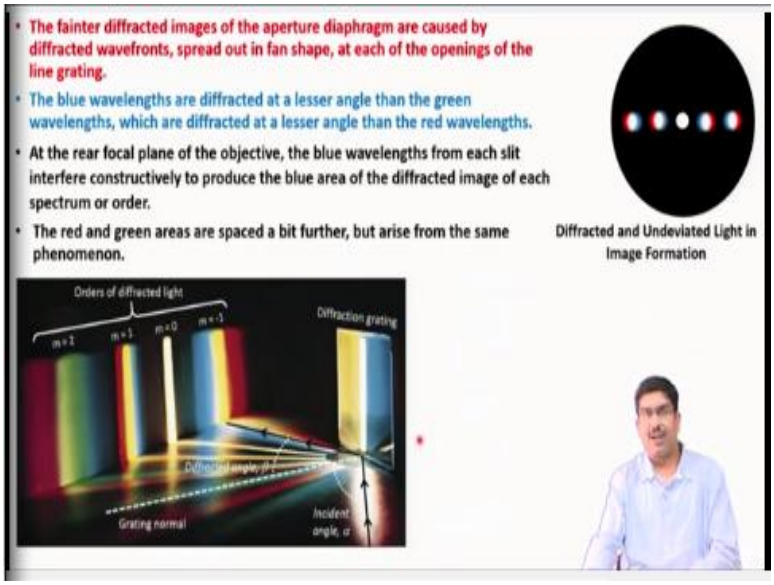

Now what happens is that when the diffraction grating is placed there and the diffraction phenomena happens through this diffraction grating one, single one of them. Depending on the wavelength, so this diffraction phenomena is very, very sensitive to the wavelength. And the wavelength in a white light what we are considering the direct light which is white light and that white light is now that is divided between these various colours depending on their wavelength.

So, that diffracted angle is different for different kind of lights, different kind of depending on their wavelength. So, the single white light is now divided into red, blue and what are intermediate colours to extremes are red and blue. Their diffraction angle, the angle by which they get diffracted is different. And this because of the difference in their wavelength the blue one all diffracted less than the red one.

And that happens for all this order of diffractions and that is why the blue spots are always closer to the diffraction diffracted central or direct beam that central spot 0^{th} order beam and the reddish one is always further off from them, that is the reason first of all. So, this is because of this diffraction phenomena.

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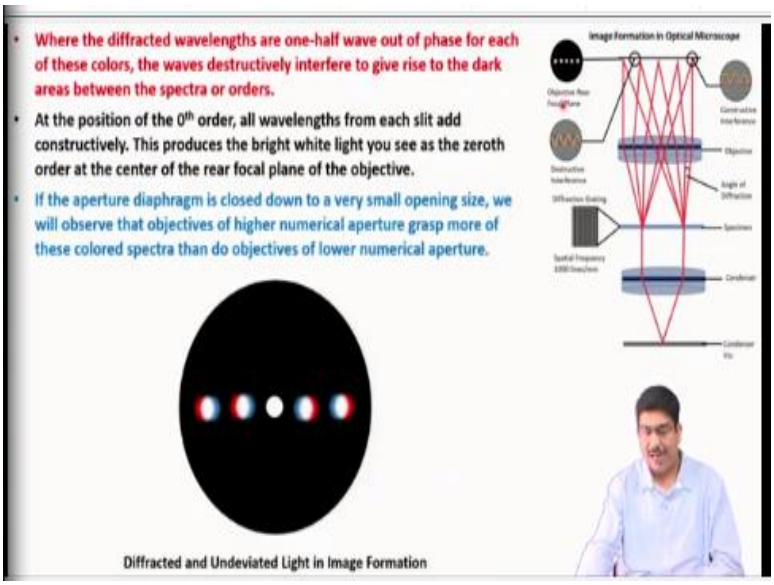

Then the next thing that happens is that when it diffracted wavelengths are exactly out of phase. And if you just able to understand that this is one diffraction, this is happening from one of the diffraction grating and the sample has many, many such diffraction grating. So, this kind of blue and 1^{st} order diffracted beam, 2^{nd} order diffracted beam, direct beam they are forming for each of these diffraction patterns.

And each of the diffraction patterns is generating this difference between red and blue lights. So, in the final object plane here back focal plane of this objective lens, all of these diffracted beams are sort of interfering. They are interfering with the direct beam and forming image they are interfering with themselves as well. And what happens most of them they are having this phase relationship and except for certain direction or certain definite spot certain definite angles, all other places they are exactly destructive interference.

They are exactly out of phase by one half wavelength and they are undergoing destructive interference. So, they are causing this completely black background except for these places where they are constructively interfering and they are forming this nice diffracted pattern. Same thing for direct beam if you consider 0^{th} order beam all in this place the 0^{th} order beams are constructive interference happens for them all other rest of the places they are just destructively interfere, this is one of the phenomena.

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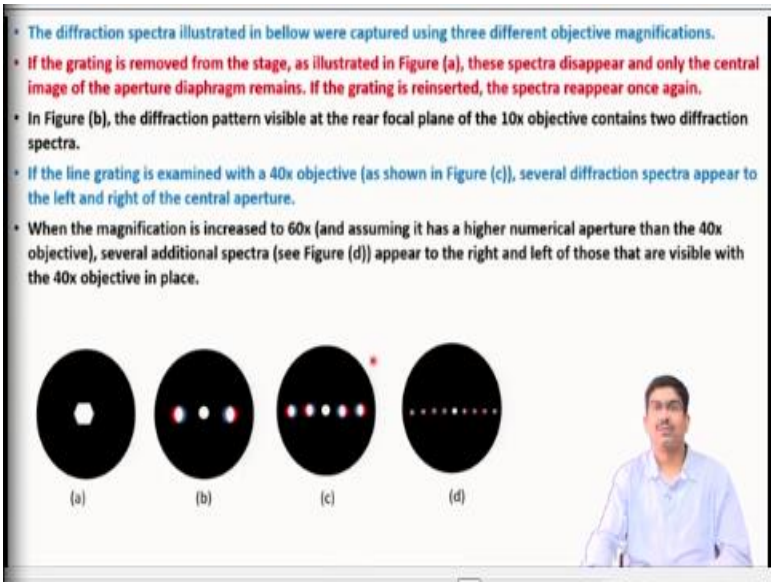

Then another thing that we can discuss about certain features of this entire image formation things. And one of the observations that we can make from this experiment is that the diffraction patterns when we captured them with different kind of magnification. That means when we change the numerical aperture to numerical objective changing magnification means we are bringing different, different objective lenses with different numerical aperture.

Higher the numerical aperture higher will be the magnification basically. So, if we see first of all the central beam when there is no diffraction grating, this specimen is not there, we are just seeing the central spot out there. Then at lower magnification or at a smaller objective lens if we use smaller numerical aperture objective lens is we are used, we will be able to see only 2 of this diffraction spots here.

The moment we increase this magnification or the moment we are going for slower, higher and higher numerical aperture we are able to see capture more and more of this diffraction patterns in this case. So, this means that these things are really real and this is related to the objective lens numerical aperture as well and this is how the diffracted beams are happening.

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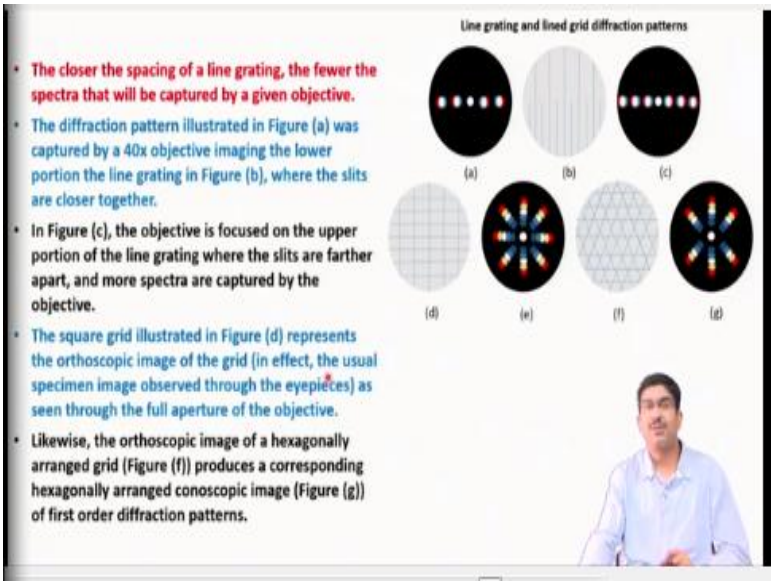

The another point that is diffraction or phenomena on the diffracted beams is related to the specimen features, in order to prove that we can just look at 2 other things. First of all we can check out this kind of a diffraction grating in which there is a difference between the distances of these diffraction gratings from which the diffraction phenomena is happening. And what we can observe is that the closure the spacing of this line grating, so these are called line gratings.

So, the closer the spacing between them the further the diffraction spots are away from each other. So, the distance between these spots is directly related to the distance in the real specimen as well. This will be become more clearer when we discuss about something called reciprocal space or reciprocal lattice. That time we will explain why this happens. But just understand this that this one where we are seeing less number of such spots.

Or in other sense the spots are further away the distance between the spots are higher when we are imaging this part, that is when this lines are closer to each other. On the other hand, when we are capturing these images on the back focal plane of objective lens, then this diffraction spots more number of diffraction spots are there. And the distance between them is also less.

So, here if the distance is more here the distance is less. So, that means the diffracted beam generation direct to this entire phenomena is sensitive to the specimen pattern as well. Second thing is this is also sensitive to the specimen symmetry, the symmetry of these patterns again. So,

if you have this patterns in form of a square or square grid, then what will happen is in the diffracted beam or diffraction this patterns that happens there, there also you will be able to see them in a square pattern.

So, look at this nice square pattern that forms between them on the other hand if you have something like an hexagonal pattern in the specimen, here this also will show you an hexagonal symmetrical pattern. So, these 2 are directly related to the specimen itself.

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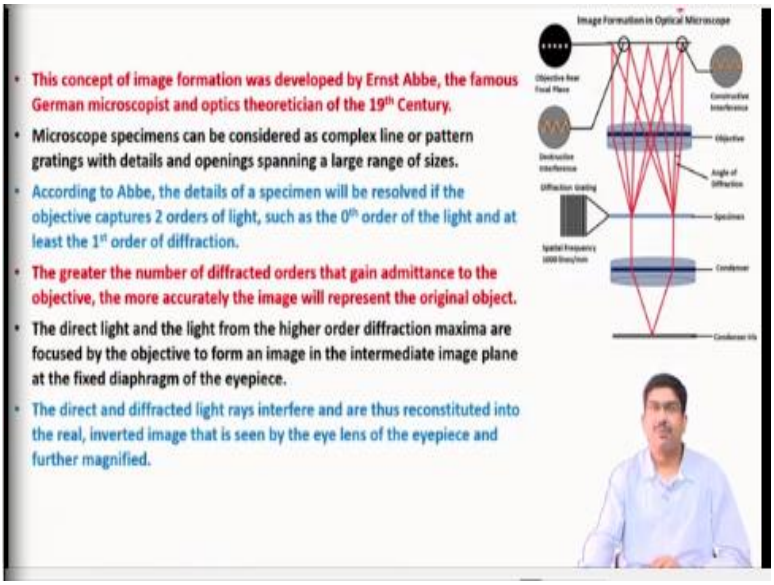

And this is how the image formation happens in the real plane in somewhere over here, in the image plane this is how the image forms in case of an optical microscope. And little later we will come to know that this method of forming the image is called bright field image generally. Now, this entire concept of image formation is basically credited to one person called Ernst Abbe.

And he was a famous German microscopist and he was famous of theoretician this entire phenomena he basically did it with mathematics and mathematical formulas, we are not going into that. But he is credited for this entire theory of optical this image formation diffracted, how this diffracted beam or modifying the intensity and so on. Point is that I said the diffracted beam should interfere with the direct beam and then only the image forms.

Then the point the question immediately comes that how many such diffracted beams I need in order to form a reliable image for our specimen. And according to Abbe the criterion that he has given is that you need the 2 orders of light the 0^{th} order that is the direct beam and at least one diffracted beam. Then preferably the first order diffraction beam because that is the most intense one.

As I just now said the intensity goes down if you are going further apart from the central spot. So, preferably your image formation will happen minimum for one diffracted beam you need to make this interference happen and to form this image, that is the first thing that is what Abbe. The more you get the more the merrier, the more number of diffracted beams you are able to capture, the more interference will happen, the more intensity difference or contrast generation will happen.

And far more better contrast will be produced in the image and it will be much more a better representation of the specimen there. So, this is how the image forms in an optical microscope. And in the later lectures, we will be discussing about the components of the optical microscopes and various modes of optical microscope in the lectures after this. So, we are stopping here and we will be starting again with the next lecture, thank you.