**Techniques of Material Characterization**
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**Module 01: Introduction to Microscopy and Basics of Optical Microscopy**
**Lecture-03**
**Aberrations in Microscopy: General Concepts**

Welcome everyone to this NPTEL course on techniques of materials characterization and we are continuing with module 1, that is introduction to microscopy and basics of optical microscopy. And today we will be having the lecture 3 which will deal on the aberrations in microscopy and these are some general concepts, very general concepts of which can be spread over any kind of microscopes.

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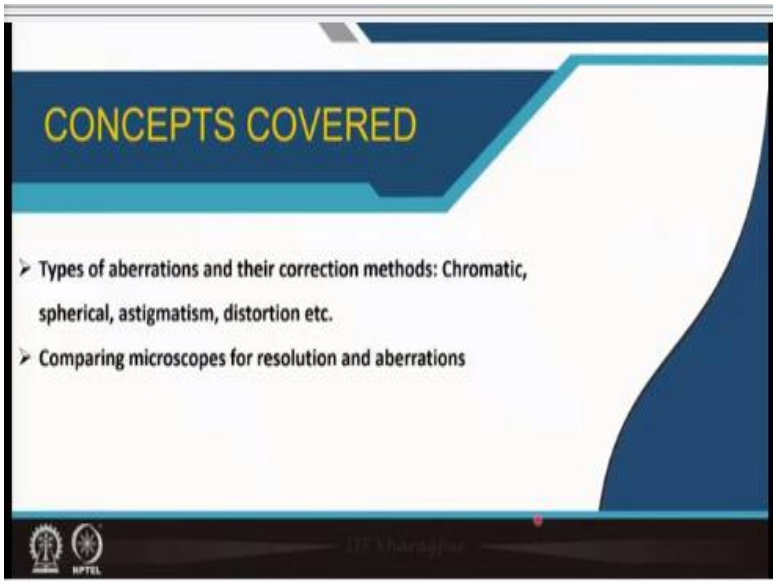


So, here we will be covering the types of aberrations and their correction methods and mostly what we will discuss in little later that aberrations that are most important for us. And we will be covering is chromatic aberration, spherical aberration, astigmatism, distortion etcetera. And then finally we will be comparing microscopes for resolution and aberration. Basically whatever we have covered in the second lecture and what we will be covering today based on that, where does different microscope stands? That is what will be our lecture today.

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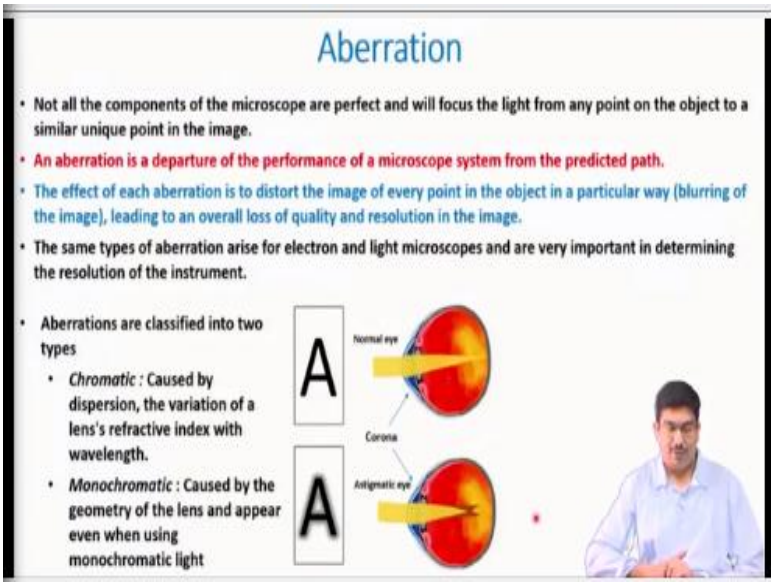


So, as we understand till now from lecture 1 and lecture 2 that for forming an image you just need an object and lens in between and finally in the image plane you are getting a nice little magnified image of this object. And the object can be self luminous; object can be illuminated from the back, from the front reflected whatever it is. But basically, this is all we have done and we have considered object, lens, image.

Now the problem is that in a real situation things are not so simple and not so straightforward also. Because not all the components of the microscopes are perfect and they will not focus lights from one object point to the similar unique point in an image. And that can happen because the object also is not a point object. Until unless now for a schematic purpose we were considering that points all the objects are like a point.

But in a true sense, the object has a finite dimension and you can imagine that this object has many, many points sources which are now going through the lens and equivalent image points they are creating. So, this entire process, object formation, lens, image, they are not perfect and of course they will be creating some different, some problems, some unique point for any point in the object there will not be any unique point in the image, there will be problems.

So, this is what is aberration, so aberration you can define it very generalistic term, the aberration can be defined as a departure of a performance of a microscope sister from the predicted path,

from it is predictive performance. And mostly the effect of any kind of each of these aberrations are felt in a distortion in a blurring. Usually the blurring of the image what we call in a common term the blurring of an image and this will lead to an overall loss of quality.

And more severely what we can able to see possibly, more severely the resolution in the image that we will be discussing in the end. So, this is the factor and that is why aberrations are so important to be considered in case of a microscopy. And this is again as I said general concepts we are still in the general concepts and these aberrations are equally valid for optical microscope and electron microscopes.

So, aberrations in the beginning or in the true sense aberration can be of two types. And before that let me tell you that in case you wonder that what is the importance an aberration, the same aberration whatever we are going to discuss for electron microscope and optical microscope same type of aberration happens for our human eye. Because our human eye is also an optical based system, it is also kind of a microscope and our microscope configurations are all based on human eye.

So, our human eye also suffers aberration and that is when we all go to an ophthalmologist or to a doctor for our eye checkup. And if you are having a glasses like me here that means we have aberration, some kind of an aberration in our human eye and this glass is basically a correction method for this that aberration. So, in a regular normal eye, what we are seeing able to see this A perfectly with no kind of defocusing and with perfect sharpness.

In this case if we have aberration we will not be able to see this and to rectify this we have this glass, so that is the importance of aberration. Anyway so aberration can be of 2 type one is a chromatic aberration which is caused by the variation of lenses refractive index with respect to the wavelength of the light or whatever the signal, so that is the chromatic aberration. And then of course, it has a counterpart that is called mono chromatic aberration.

And when you are using just a monochromatic light or monochromatic signal in order to avoid this chromatic aberration, still you can have some aberration because of the geometry of the lens

just because of that. So, chromatic aberration is something to do the source itself whereas the monochromatic aberration is mostly related to either the specimen or the object or the lens system which is there.

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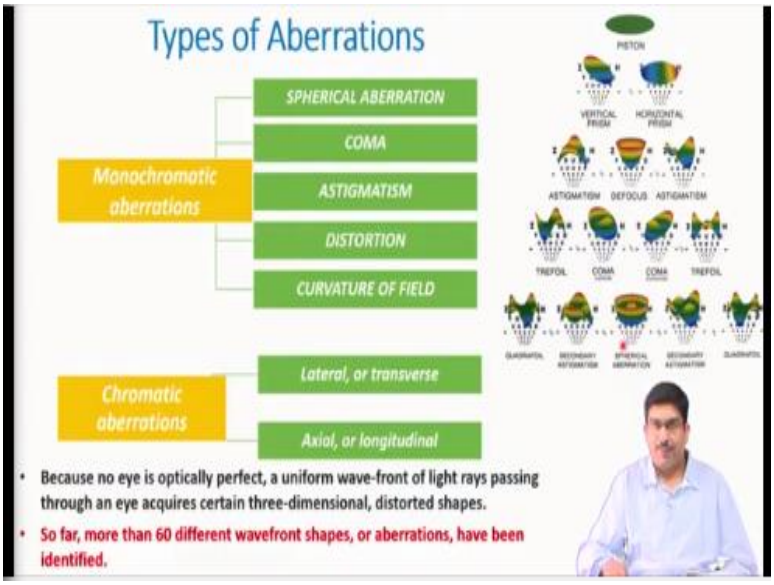


As I said there are within the monochromatic and chromatic aberrations there are many, many different kind of aberrations and we will just focusing on this spherical aberration and astigmatism and distortion. There are some other this if you have you ever encountered some image processing softwares photoshop or something. Then you will be able to see that all of this and how to correct them different filters and so on.

And chromatic aberrations again there are 2 different kinds but we will not be going into details we will be just restricting also within generalized description of this chromatic aberration. Just what I said that our human eye also suffers various kinds of different, different types of aberrations. And till now until up to now, almost 60 different types of aberrations are detected in our human eye.

And when this is what is showing that it is a general wavefront when it enters in our human eye, it can undergo these various types of aberrations. And these are what the wavefront will become the general simple wavefront will take the shape of these different, different wave fronts if we have this different types of aberrations with our human eye.

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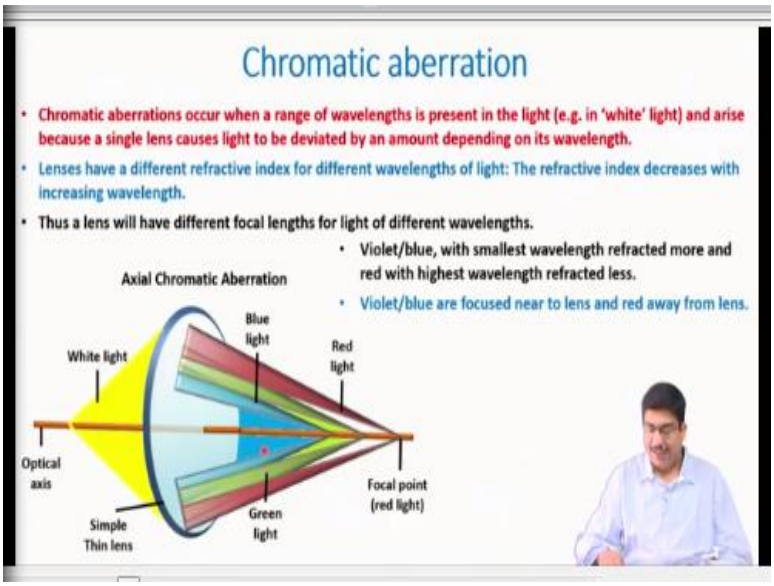


So, now first thing is chromatic aberration, of course chromatic aberration as I said, it appears because in the white light, you have different range of wavelengths in the light. So you have this complete VIBGYOR and depending on that blue and red light, their wavelength is completely different. And the sensitive the amount of the deviation that this lens causes to this different rays, it is sensitive to it is wavelength.

Basically what happens is that the lenses have different refractive index for different wavelengths. And finally the way it effects is that it causes them to focus at different, different points. So blue gets refracted and blue gets deflected far closer to this from the lens compared to the red. So, you have from the same point from every point the same thing is happening.

Basically I am just showing it from one point that is I am considering still that one single point source and the white light is going through here. And depending on the wave length the refractive index basically changes and refractive index decreases with increasing wave length. So, that is why you are getting this red focus at a much more distance from this lens compared to the blue.

Blue is getting focused much nearer to the lens that is what it happens when a white light goes through a lens any kind of lens.

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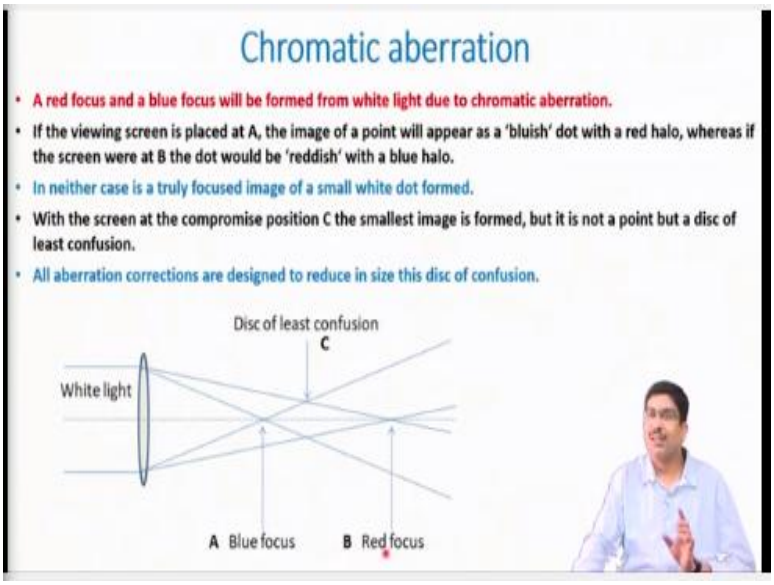


So, now in this process what is happening is that you have this white light and it passes through this lens and you have a blue focus and you have a red focus in this. So, now if you put your viewing screen somewhere over here along this blue focus, what you will get is a blue dot with a reddish hallow around because the red light is not focused in this plane. Exactly the different thing will happen or exactly the similar effect will happen if you put viewer imaging plane here on the red focus.

Now what will happen you will get the red dot that is the focused one with a bluish hallow, so that is exactly what will. So, you cannot place your image plane and either of these places you will be some portion of your object will be out of focus. So, then all we can do, we can put somewhere over here in a compromised position which is basically known as the disc of least confusion.

If you put your a image plane over here the object will more or less will be focused the best focusing will be attained in this position that is why it is called disc of least confusion. And all kind of aberration, corrections, chromatic aberration including is basically how to reduce the size of this disc of least confusion that is what it the correction methods all try.

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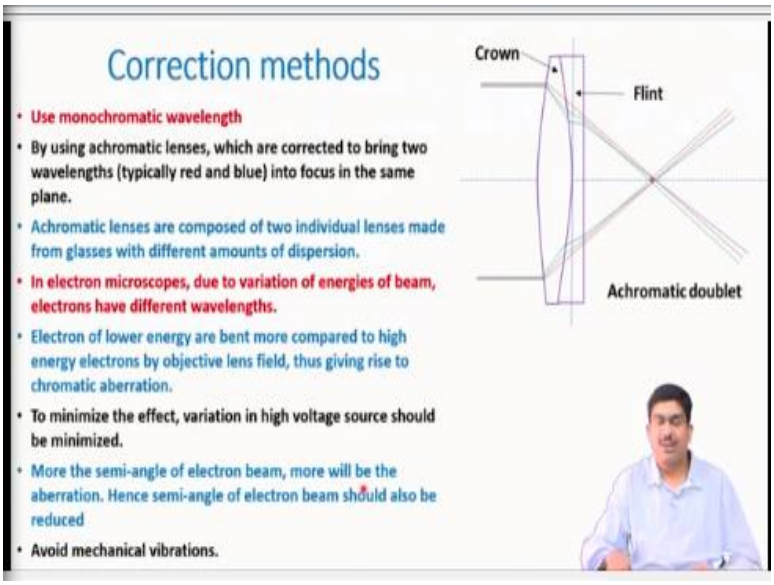


So, let us discuss about the correction methods for chromatic aberration simple, use monochromatic wavelength for your source. But the most difficult one and impossible nearly impossible whether you use a light source or whether you use electron source, getting a complete monochromatic light with single wavelength value is not possible. You can narrow down the range up to an extent of course but still you cannot have a single value.

So, chromatic aberration in this way you cannot really remove. The other ways you can do is basically you can use something called achromatic lenses and those achromatic lenses are usually a combination of lenses like this. You have a crown, they are called crown and flint and these are two different pieces of lenses the fuse together and this lenses basically are putting different effects on this glass.

So, they are basically when this white light passes through one of the lenses whatever the chromatic aberration happens by one of the lenses, the other one just basically corrects it because of the curvature of these lenses. So, that is how finally the image that forms here will have minimum amount of confusion. So, disc of least confusion will be of minimum size in this place.

And in electron microscope just like what I said like optical microscopes, like the light source you have different energy for the beam the electrons and electrons will have different wavelengths. So, chromatic aberration again is a reality in optical microscope and their

correction of this one is slightly different. So, you do something with the light source or the electron source itself, we will discuss about that.

Another way of doing it is basically to increase the size of the semi with aperture or the semi angle of the electron beam. So, if you increase the size if this semi angle is much higher for this electron beams for the aperture 10 more aberration will happen. Hence, you should try to restrict the semi angle within a limit within a value. But that will have another effect that we discussed earlier that it will be possibly in case of it will affect the resolution in the other sense.

Of course we can avoid mechanical vibration, because that also can affect the chromatic aberration. So, these are the correction methods for light microscope as well as electron microscopes.

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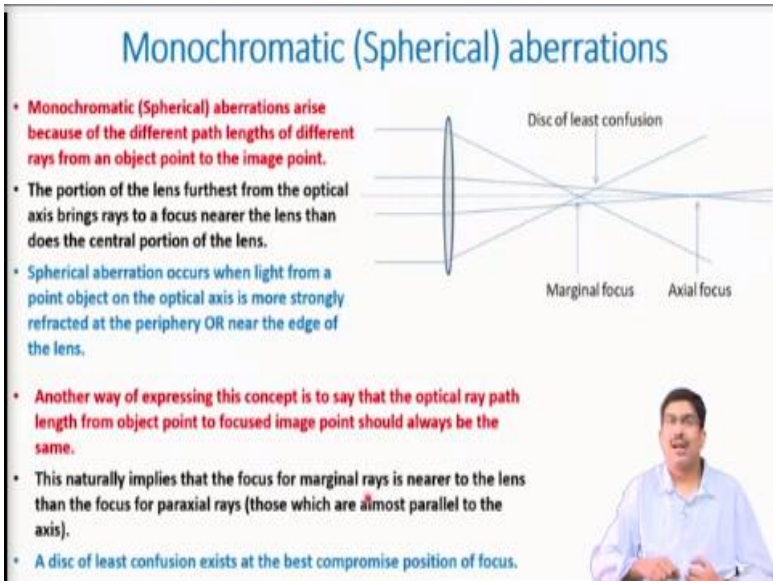


The other kind of aberration that will happen is for monochromatic beam, imagine you have a complete monochromatic beam and then still you will be getting some aberration because of the lens geometry itself. And this is calls for most important of this is spherical aberration, spherical aberration appears because of different path length of this rays which are travelling from the object plane to the image plane.

So, the rays which are closer to the optic axis basically gets focused further away from this lens. And compared to the lens or rays which are travelling further away from the optical axis that means towards the edge of this lens system, they will get focused much closer to this lens system. Basically this is happening because of a principle that the total path length of this optical rays, if we imagine the object is somewhere over here.

And if we imagine the image plane is somewhere over here, then this total path length must be the same, path length means this one the length that this light ray travels. So, this lens should be the same and that is why this light will get focused the light which is further apart will be focused much closer to this lens system here. So, this is how this spherical aberration, this again the same thing like chromatic aberration.

You were getting two different focal points for the same lights or the light rays which are coming from the same object point. Just because of the lens geometry itself you will be getting two different focal points here. So, this is of course, here also you have a disc of least confusion that is referring here and the correction method will focus on how to reduce this disc of least confusion.

And the rays which are getting focused which are further away from the optical axis from the edge of this specimen they will create something called the marginal focus. Whereas the rays which are travelling closer to the optical, your optical path or optical axis, that means they are closer to this central point of the central they are travelling through the central part of this lens. That focus, they will be focused further away and that is called the axial focus.

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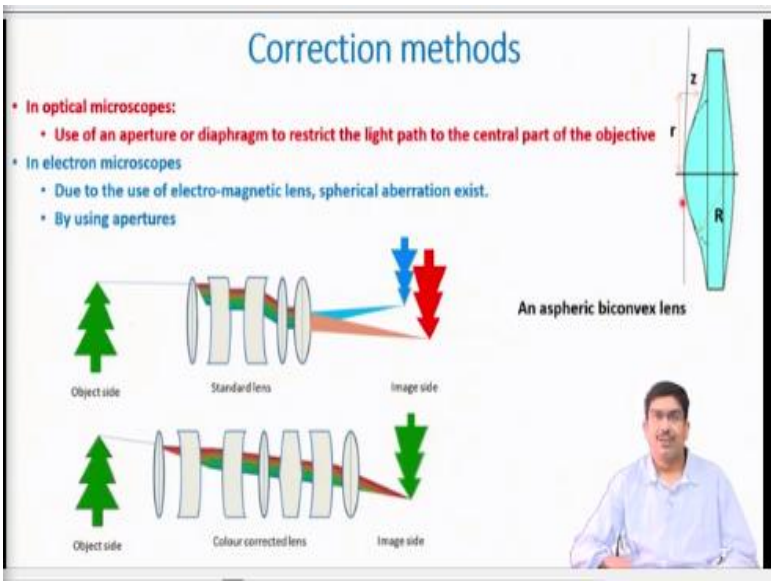


So, these two different focus points will be formed and this because of the lens geometry itself and that is will cause this spherical aberration, something called the spherical aberration. Correction method, for this just like what I showed for chromatic aberration here also you have to use a combination of lenses basically that will have different kind of a effect on the lights travelling.

So, if you can notice here this one is slightly thicker through this central part and thinner in the edge and this will change again the complete scenario, this will add a little bit adverse effect or little bit a completely sort of reverse effect on the rays travelling close to the optic axis compared to the rays travelling at the edges. So, they will cause this central with the rays travelling close to the optic axis they will try to converge or they will try to focus them much closer here.

So, they will try to bring this difference they will try to close this difference between marginal focus and axial focus by using this typical shape of the lenses here it is there. Electron microscopes, again you are using electromagnetic lenses spherical aberration exists and there it is not possible to do this kind of a correction, what these try to do there in order to reduce the spherical aberration to use aperture.

Basically what we can do we can try to do is to bring an aperture here and then restrict this rays which are travelling closer to the edge. And we just allowing those rays which are travelling

closer to the optic axis just by using bringing an aperture, virtual aperture you can imagine an aperture is brought here and that is how you can reduce spherical aberration in electron microscope.

And this has adverse effect basically what will happen is that this will reduce the brightness or the amount of intensity the amount of light that is passing through the lens. You are mostly you are chopping off most part of this incoming lights but that is how you can survive with it. And this is how you can see that finally by using this different type of, so this is a standard lens and by using a combination of lenses.

Finally, you can have this object and you can bring all of these red and blue lights to focus on a same point. And that is how you can get a better corrected finely corrected lights that chromatic aberration or spherical aberration corrected, you can get it here. So, this is how you do first level of aberration correction.

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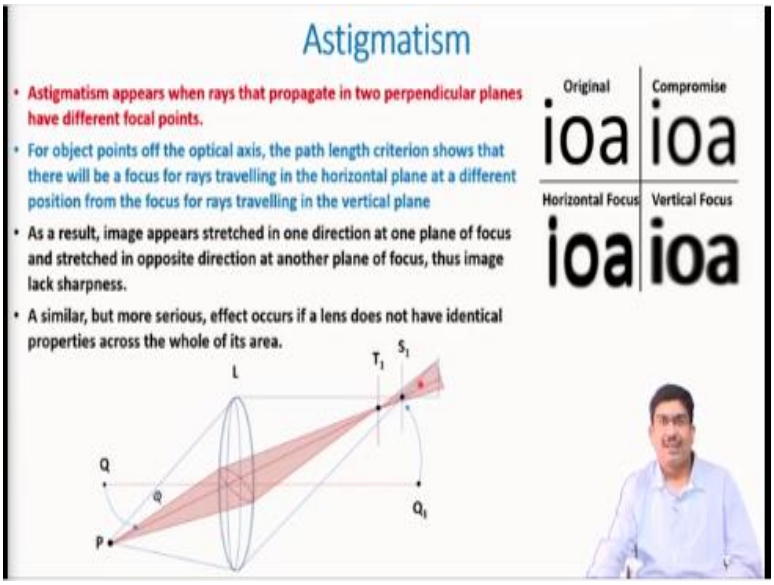


The next one very important distortion and aberration is called the astigmatism and it is somehow related to whatever we studied about the spherical aberration. So, here what happens is that the rays which are travelling on this plane that is on the horizontal plane and the rays which are travelling on the vertical planes, they will get focused at two different points? Because of the same optical path the ray path length should be the same.

And that is again will cause the same point from the same point source object here you are getting 2 different points of focus again a minimum least disc of least confusion kind of the situation will arise here. So, if you just consider if you put your image plane here that is a horizontal focus that is called this horizontal focus, you will be able to see the image gets dragged in the vertical direction.

So, this is your original image and if you keep it in this position, you will be getting all the points will be dragged in the y direction in the vertical direction. If you keep your image plane somewhere over here again exactly reverse will happen all the points will be dragged on the horizontal direction. So, this is the vertical focus, this is the horizontal focus and you can bring it to a compromise position somewhere in the middle disc of least confusion and you can get this kind of a best focusing out of this, so this is the astigmatism.

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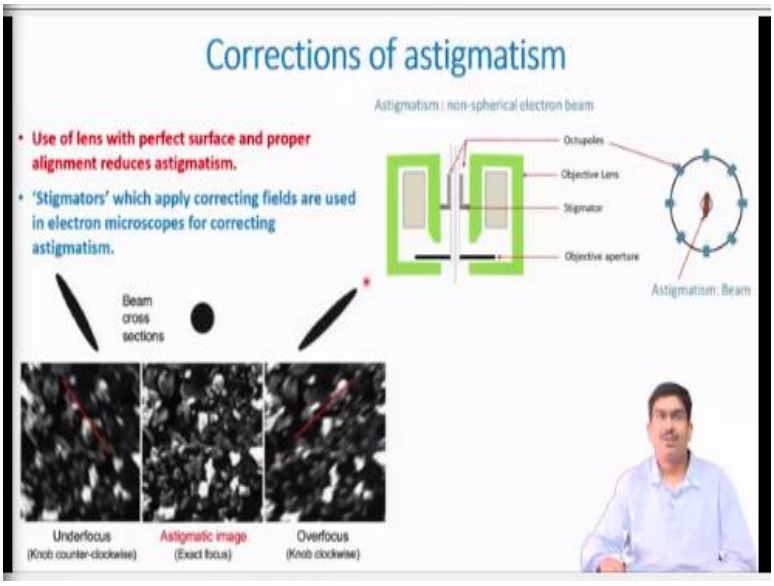


Correction of astigmatism is again this is how the effect can be say first of all the detection of astigmatism itself is very, very difficult. And this we will discuss when we discuss about the next one that is called the distortion. So, detection of this kind of astigmatism, this effect of astigmatism is very, very difficult. And for that you need some kind of a most commonly you need a spherical feature under the microscope.

If you have a spherical feature and if you just change the focus if you bring it under focus that means you are changing between this horizontal focus and the vertical focus. If you do that and if you change the shape in one certain direction, then you can know that you have astigmatism. So, detection of astigmatism this is a way you can do it under any microscope. Detection is extremely difficult that is the first problem with astigmatism.

In order to correct that what you can have is something called the stigmators and stigmators are typically used for electron microscope, these are like you have some scan calls and this will put exactly what this compound lenses they do for a monochromatic spherical aberration, the similar kind of effect they will give and they will produce, they will correct this astigmatism for a electron microscope.

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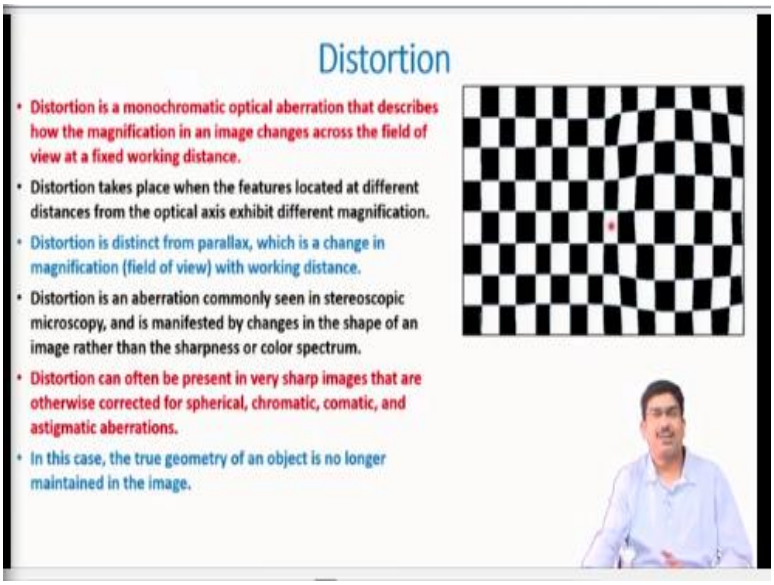


The next kind of aberration that we can consider is called distortion and distortion is again it happens even for a monochromatic beam. And in some sense even when the image is completely corrected for spherical aberration, chromatic aberration and all other astigmatic aberration you can still have distortion into it. So, if you notice in this image so what is happening is that this part looks like a nice checkerboard and everything is symmetric?

In this part we will see that all this checkerboard some of them are distorted and this distortion is more prominent when you go to the edge of this image. Central part, it is still better but if you go

to the edges the distortion effect is much more. So, distortion basically happens when if you imagine that this is your central optic axis then you have a difference in magnification from the central axis, the magnification changes as you move away from the central axis.

Then this kind of problem will start happening and this distortion is slightly different from another effect called parallax. Basically, in distortion this change in magnification from optic axis, this happens over the same working distance. In case of a parallax this change of magnification happens if you change this working distance that is the difference.

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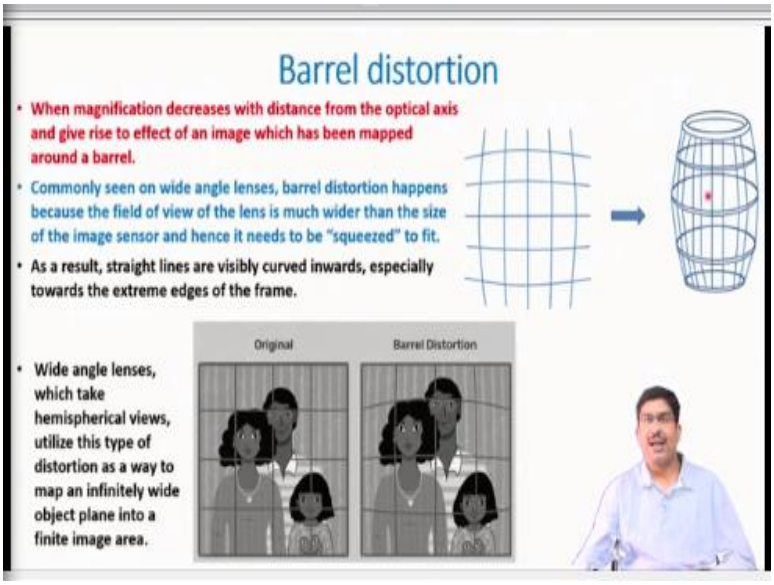


So, distortion basically does this and there are two different types of distortions again, first one is called a barrel distortion. So, what happens is barrel distortion is that? From the optic axis if you go further away, the magnification decreases as you are moving away from the optic axis magnification basically decreases. So, that this portions which are at the near the edges of this images, they are kind of squeezed compared to the images which are in the close to the central section or close to the optic axis.

And this ultimately produce an effect which is similar like a barrel. So, the central part is a little fatter compared to the top part which is kind of squeezed into it. The same kind of distortion and basically the distortion is more prominent when you take a digital image using a camera, you

should be able to see the distortion effect if it is there. For example, if you have an wide angle lenses, sometimes you tend to use a wide angle lens.

And there what happens is that you trying to map or try to capture an image of a very wide object plane into a finite image area within your detector. And then what it will do is that it will produce a barrel distortion, it will try to squeeze those areas which are at the edges close to the edges of the image within this small finite area and it will produce a barrel distortion.

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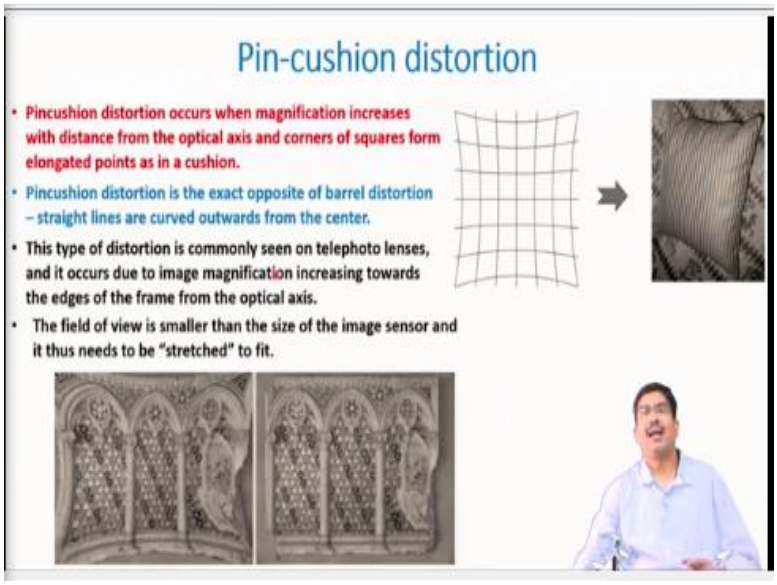


Exactly opposite to barrel distortion is something called pin-cushion distortion. And pin-cushion distortion in here what happens is that magnification increases as you are moving away from the optic axis. So, that means here this part, the parts which are in the edges of the image these are something like a squeezed images. Whereas the part which are close to the optic axis is something looks like they are sort of pushed.

Actually they are the right kind of a magnification but this area the magnification is much higher. That is why finally the kind of shape that it will produce is looks like this kind of a pin-cushions where the central part is looks like it is squeeze and the edges looks like they are stretched away. So this also, again happens for a digital photography and that is what I tried to show it here.

And this happens when you have something called a telephoto lens, when you are using telephoto lens, then this kind of distortion will happen. What happens is that here the field of view, it is much smaller than the image sensor, the sensor that you have in your digital image is much bigger than the field of view itself. If you are zooming it too much with your telephoto lens, then this pin-cushion distortion will happen.

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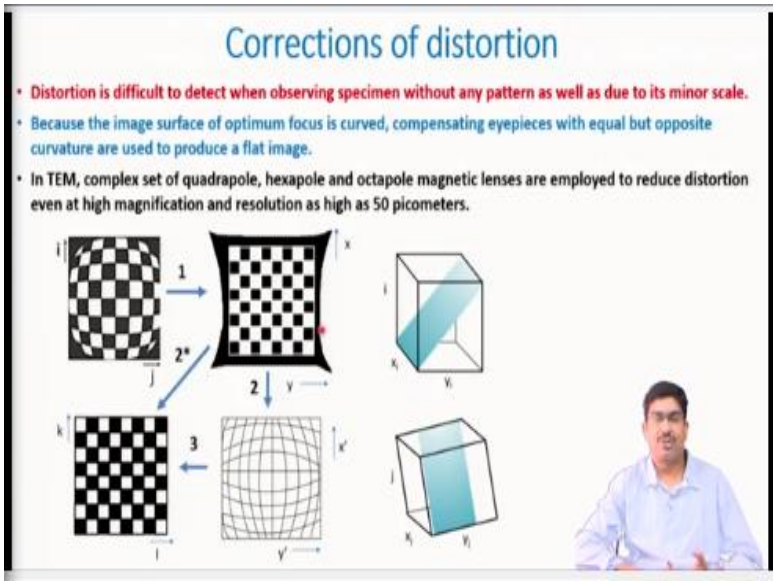


Correction, again same comment like the aberration, detection is the first correction here both for aberration and for distortion. If you are able to detect this distortion or aberration astigmatism, then you should be able to correct them, so detection is very, very important. And here the problem is that unlike astigmatism, where you can still try to find out a spherical object and try to see over focus, under focus and try to find out the right whether you have astigmatism or not.

Here you are it is very, very difficult to find out distortion in the first place for that you have to move and you have to understand. You have to first you have to have a regular pattern like this in your material, least kind of a regular pattern should be there. And if you see and that you must check it with something else, it must be a known regular pattern. And then if you put it in your system in your microscope and if it shows this kind of distortion, this kind of a change in magnification you know you have a distortion that is what.

So, your system I mean like it should be calibrated for this distortion time and again, you cannot do it online when you are checking a specimen that is what. Now the collection method is simple basically if you have a defect, if you have someone kind of distortion you bring a compensating another kind of distortion. This is to say, if you have a barrel distortion if you have something similar to this.

Parallel distortion, what you can do is that you can bring a pin-cushion distortion over that. In that barrel distortion, you put a pin-cushion distortion and then you can hope that finally you will get both of these will cancel each out and you will be able to get something like a corrected image here. Most often this is done offline that is through an image processing software you can check this distortion, you can correct this distortion.

There is no online way of doing it unlike chromatic aberration, spherical aberration and astigmatism distortion is very difficult to detect and distortion is not you will not be able to correct them online with help of some kind of a hardware, it is difficult.

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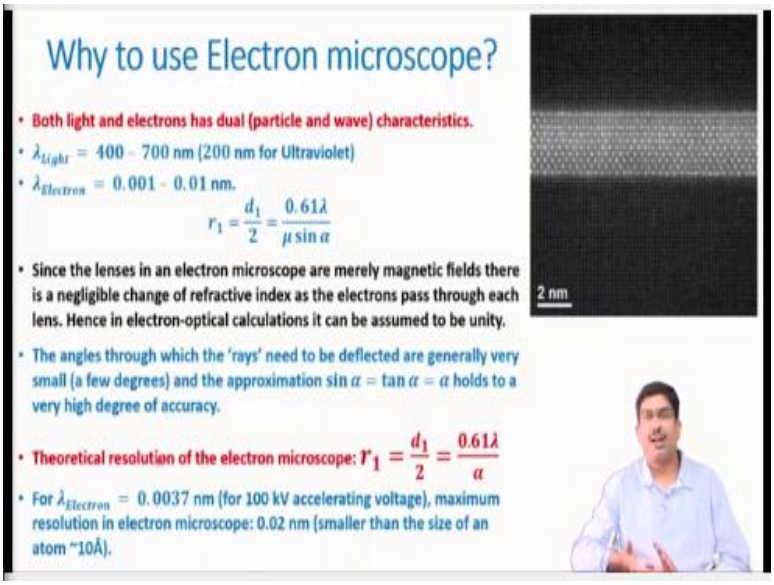


And mostly it is done using software and image processing. Now based on all the things we discussed about resolution, aberration. Let us see where does our optical microscopes and electron microscopes stand, what is the difference between them? So, we can imagine that both

lights and electrons has dual particle and wave characteristics and they are characterized by this kind of a lambda wavelength of light.

We can imagine this is within 400 if you use visible light it is within 400 to 700 nanometer, if it is ultraviolet we can give go or down to almost 200 nanometer. Even with that kind of resolution we can get just by diffraction effect is something like 200 nanometer at the max 150 nanometer. $\lambda_{electrons}$ again it is much, much smaller than the $\lambda_{light}$ and that is why we can get very low resolution possible, very high level of resolution we can go down to very small distance then also we should be able to see using electrons.

We are basically modifying, instead of modifying this numerical aperture now we are modifying this lambda value that is one**.** So, what happens is for an electron microscope, if we take this formula for resolution, what happens for an electron microscope? Basically in the electron microscope there is nothing, it is an electromagnetic lens, so there is no medium within that lens.

So, we can very safely say that this refractive index (μ) view of the medium is 1, it is unity. The second thing that happens for an electron microscope is that the α value the semi angle for these apertures are usually kept very, very small. Just I have discussed about the aberration and that is mostly to correct the aberrations. That is why this approximation is very much valid there, you can imagine that sin α = tan α = α.

So, this for a very small value of α, this approximation can be made. So, theoretical resolution of optical microscope or electron microscope we can imagine that this formula comes down to this 0.61λ/ α not sin α just simple α. And now if we put different types of values, that is λ value usually for 100 kv accelerating voltage, we have an wavelength of 0.0037 nanometer.

The maximum resolution just because of diffraction effect that we can expect is around 0.02 nanometer that means around 2 angstrom or even sorry around this comes out to be around 0.2 angstrom which is even smaller than the size of an atom which is around 10 nanometers. This is one image I think I have shown you previously and explained that this is taken with a transmission electron microscope and this almost represents the atomic columns. Look at the

scale bar and look at the size of this, these are almost atomic columns. So, these are the maximum level of resolution possibly you can achieve with a TM.

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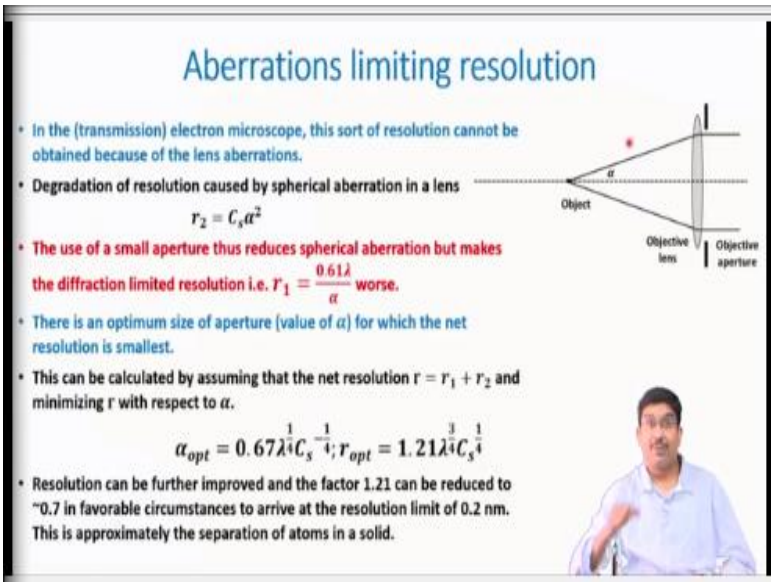


So, that means this sort of resolution 0.2 angstrom, 0.2 angstrom means you are supposed to see within the atoms itself which is still yet not possible. This kind of resolution we cannot get because the resolution also is limited by aberrations and this is how they do. So, if we imagine only spherical resolution, I am imagining that there is no chromatic aberration, astigmatism or anything.

With only spherical aberration this is how the resolution is calculated $r_2 = C_s\alpha^2$, which is again the semi angle that semi angle for this aperture that is used here with the objective lens. And if we take this one and we try to reduce so reducing, improving resolution means we are basically trying to reduce the value of this one.

So, in order to reduce this what we need to do is to use as small an aperture semi angle as possible. But if you do that what will happen is that this resolution the diffraction related resolution will again going back. So, if you reduce α, $r_1$ will increase that is not a good news for resolution. So, there is like a compromise between aberrations and the diffraction related resolution.

And this final resolution we can imagine that this final resolution is a linear combination of these two. And if we get the optimum value of α this is coming out to be this and this optimum resolution that we can get out of any kind of microscope can be is considering spherical resolution aberration is coming out to be around this value. And putting all other values what we can get out of here is the resolution limit of around 0.2 nanometer.

That is around some 2 angstroms around 2 angstrom is the limit that we can get with all kinds of corrections with spherical aberrations. And this is what possibly the separation distance of atoms and the image that I showed you here is basically having this kind of resolution.

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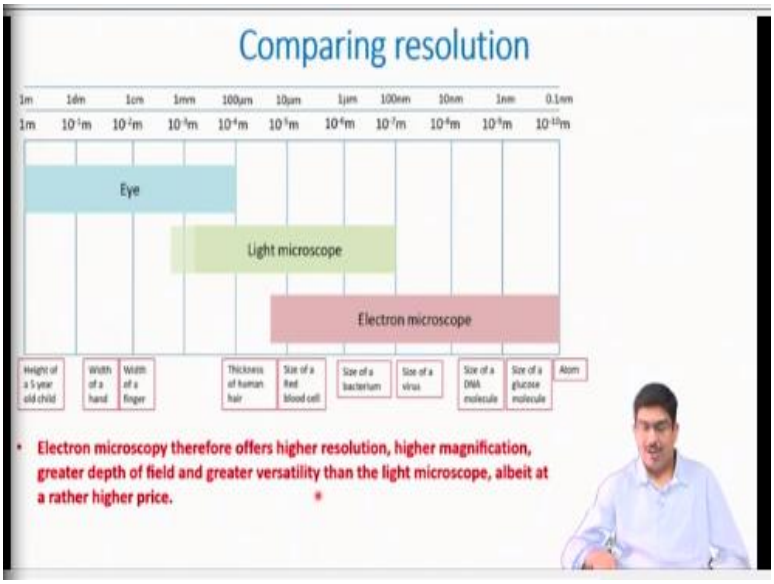


So, if we now compare different types of microscopes, in terms of resolution our human eye can give you all the way around 100 μm or so, light microscopes can give you around this level 100 nm with the best possibility. And electron microscopes can go all the way down to around 0.1 nm around 10 angstroms or so, around 1 angstrom or so.

So, electron microscopes of course offer you higher resolution, higher microscopes, higher magnification, greater depth of field which we will see in a minute and greater versatility but at a much, much higher cost and that cost comes because of some other factors.

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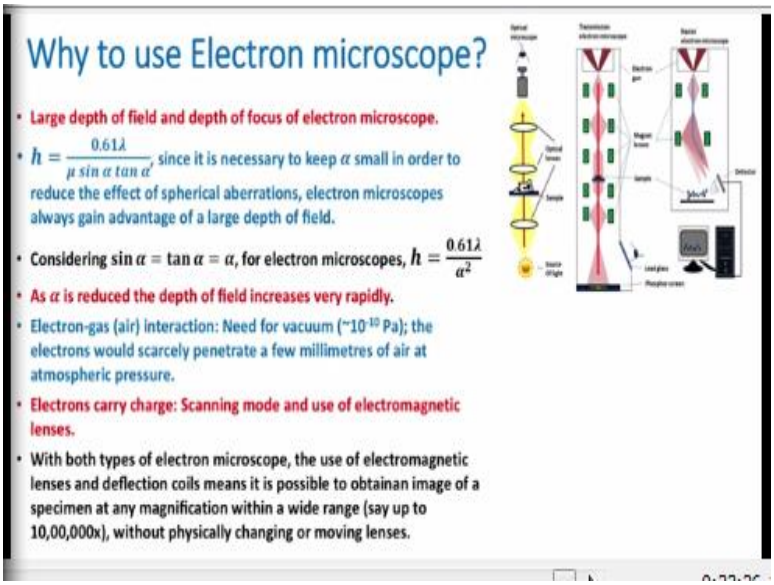


So, this revolution one of the positive side of the resolution to improved resolution by using very small value of α for electron microscope is that. There is a advantage of getting higher depth of focus for electron microscopes, because depth of focus is calculated you know from this formula here $0.61\lambda/(\mu \sin\alpha \tan\alpha)$. If you now use the same approximation that $\mu = 1$, in this case and sin α tan α boils down to α only.

So, this depth of field can be calculated as this $H = 0.61\lambda/\alpha^2$. And if we reduce α in order to get the best resolution from spherical aberration plus optimized α for very small for spherical reducing spherical aberration. And getting the diffraction related resolution reasonable amount that will come down to a very high depth of field much higher than optical microscope.

Because depth of field if you reduce this one depth of field becomes much higher and that is the advantage of electron microscope. The problem with electron microscopes is that since electrons are charged particles, they will interact with the gas. If you have if you cannot control the atmosphere within the electron beam, within the column through which the electron passes there the atmosphere needs to be controlled.

That means you have to create a very high level of vacuum in here and often that leads to around $10^{-10}$ Pa, so that kind of a vacuum you have to maintain here. And the advantage of course with this charged particle is that you can use electromagnetic lenses that it is own advantages we will

discuss it later. And we can get go for scanning mode, so the scanning mode is not so easy, there are certain optical microscopes for scanning mode, we will briefly discuss them.

And but scanning mode is a very regularly used for electron microscope because electrons can be deflected by this electromagnetic lenses. And with using all of this you can very easily go to a very, very high magnification almost up to millions or a slightly less than this. So with this we are closing here with this lecture and in the next lecture we will be discussing particularly about optical microscopes.

And then we will be discussing their modes, basic modes, components and so on, and different modes of optical microscopes in the next few lectures, thank you.