

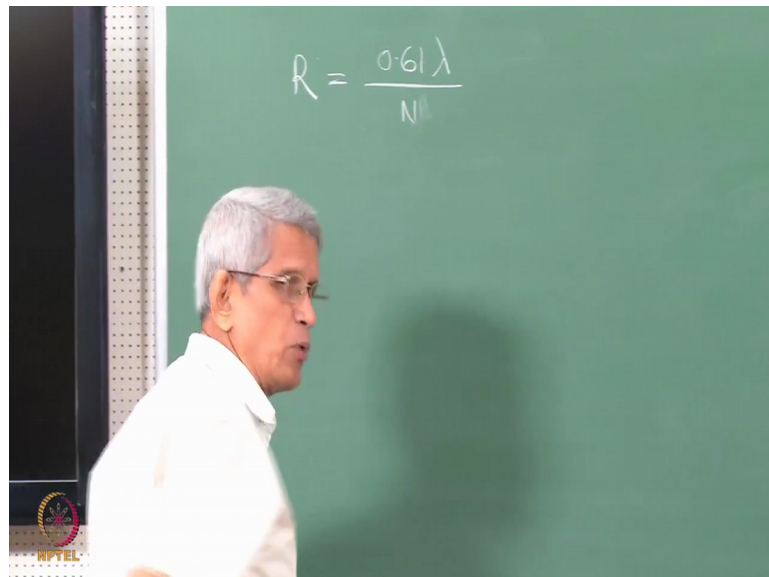
Electron Diffraction and Imaging
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Lecture – 14
Microscope – 02

Welcome to you all to this course on electron diffraction and imaging in the last class, we talked about the structure of microscope what all components microscope have little bit about the ray diagram how the images are formed at back focal plane as well as the image, but another aspect which is very important is resolution of a microscope then the other is about what all different modes in which microscope could be operated then the depth of field and depth of or depth of field or depth of focus which is also important for a microscope these are all the aspects which I will cover in today's class.

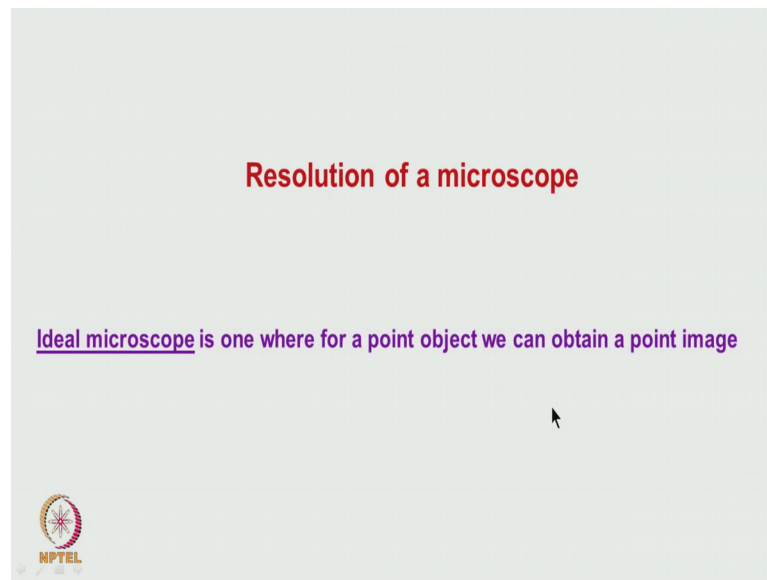
The first thing is before we talk about resolution of a microscope what do we mean by a resolution.

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Most of the instruments especially like in an optical microscope the resolution be generally write with that formula r equals 0.61λ by I take N into or N ; N is what is called as the numerical aperture, correct.

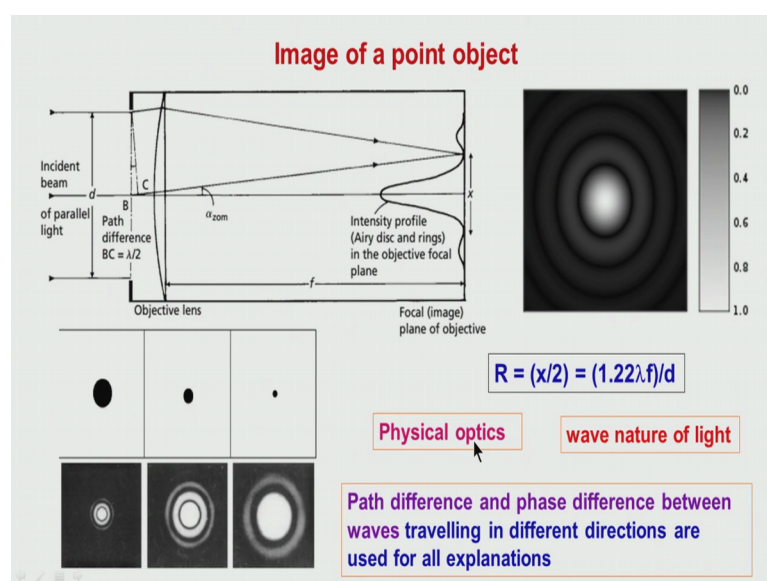
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This is for a camera or a lens where all the aberrations have been corrected that is this is something similar to for an ideal microscope or a perfect microscope we say that for a point object it introduced point image.

Let us look at how this comes normally an ideal microscope if we consider is the one where for a each point on that object we should have a point image that normally does not happen from this formula how does it occur.

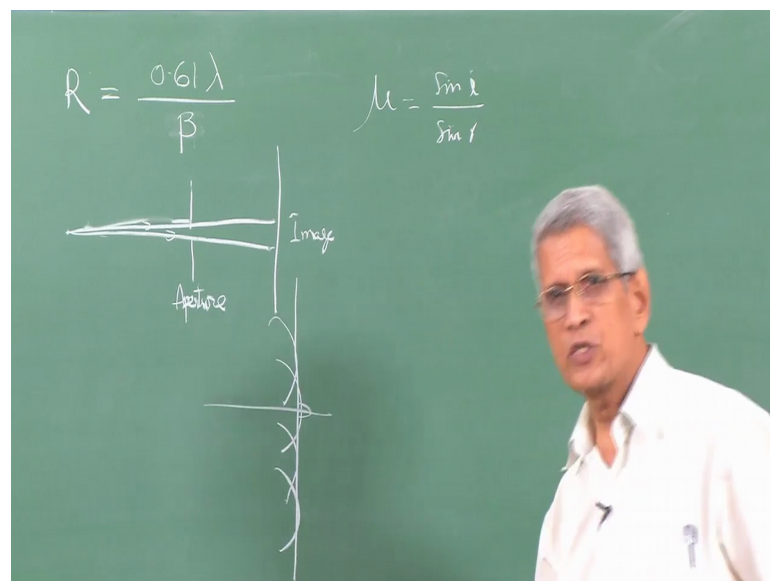
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Let us consider the case here the light is coming from an object which is very far away which we can consider it is a point and it is a parallel beam a lens is one which is focusing it onto the screen in this case how does that intensity comes normally we know that instead of the lens we could have an aperture also that does not matter, but when that aperture size is large when the light comes from a far away you get a shadow of object in that image nothing that you make the aperture smaller and smaller the shadow beam comes smaller and smaller that is what it happens is that is what is going to happen when there aperture becomes very small and of the order of the size of the wavelength of the radiation that is what the question which arises because when we operate microscopes where we are looking at resolutions or looking at objects which are at close to or the resolution which are very close to a wavelength of the radiation.

Let us look at what happens in that case in all these cases though we can draw a ray diagram like if it is a source is there if we have an aperture you can draw a ray diagram and this is the aperture.

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We can tell that at this point on this screen we are going to get an image with these sort of an ray diagram we could describe that image that is what essentially its being done, but quiet often we know that that is not the truth because here we consider light as a corpuscular one light essentially in most of the cases we have to consider it as a wave if we consider it as a wave then the intensity at every point depends upon contribution from

various points how it how it adds it that is essentially the phase difference between different rays which are reaching at a point that decides the net amplitude and that is what is going to decide the intensity that way if you look at it at a point which is going to be at the center of the light radiate this lens these 2 rays will have the same path length and they will add together.

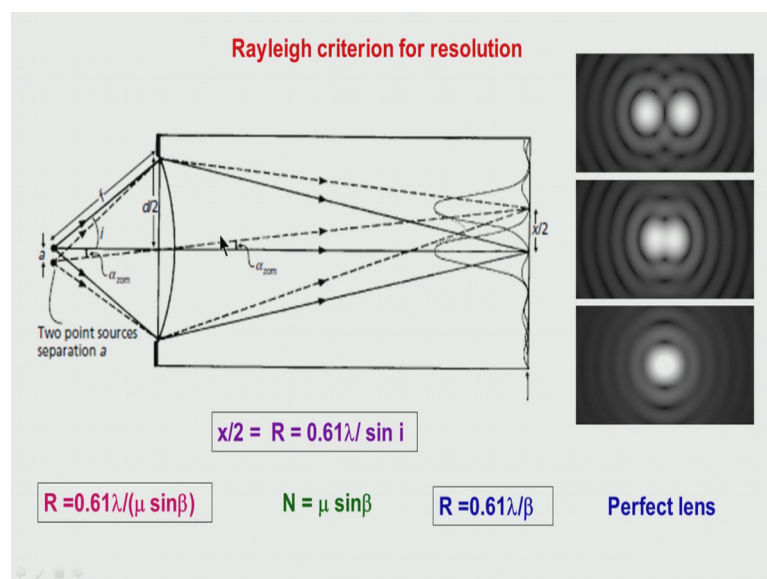
But let us move away from it then what is going to happen the ray which is going to come from here or the ray which is going to come from here they have different path lengths at some particular distance we find that the path the path difference becomes $\lambda/2$ then this is a destructive interference that is where the intensity falls off and reaches a valley small valley this is what essentially if we try to image this we can immediately see that we have a bright central spot then the dark region then again a subsidiary maxima and minima this is how we get this is called as a Fresnel diffraction or this called as an Rayleigh disc.

In this case qualitatively what happens is that if we look at it if we make the size of the aperture small or the lens size itself we can make it small because the lens size is something like an aperture because only that size which focuses the beam on to the screen the others are all going as a parallel ray as the aperture size becomes smaller and smaller what we can see in the image which is shown here is that the central disc size is becoming larger this is contrary to what we expect normally as the aperture becomes small the image size should become small and it becomes large.

Now we do not know how do we relate it to the aperture size this is what essentially this Rayleigh formula what it say; the Rayleigh criterion what it says is that from here to here if you see this distance the central maximum to the first minimum that gives the radius of this disc and that is equal to some factor 1.22λ into f by d comes f is the focal length of the lens and d is essentially nothing, but the aperture size this essentially gives that angle β also essentially what we can make out from this is that as the aperture for the constant focal length of the lens as the d becomes smaller and smaller that aperture makes smaller and smaller or becomes larger and larger what we explain qualitatively the same thing is becoming clear from this expression which we derive it for the for a point object using a small aperture whose the size is of the order of wavelength how they with give an image the radius of the image this is all based on what you call it is a physical optics.

The ray diagram is also there, but other than that we have to incorporate the wave nature of the light and another aspect which have to be read this and the Hygen's principle Hygen's principles has to be used that is what it tells that from every point when the light propagates it comes as a spherical wave as they joined together they form a wave front again from every point at the wave front again spherical wavelets are emanated that is how the propagation takes place and the front which is normal to the propagation that is what we call it as the ray that is if waves are like this all the waves crosses are joining together it will not wave front and this we call wave front and the direction which is normal to this wave front is the ray diagram which we draw though we the draw the ray diagram to explain most of the things we should remember that when we have to explain how the intensities when are when we wondered to quantify that information we have to use the wave nature of life or the path difference; that means, that the light itself or the probe itself has to be considered as a wave which is coming and a wave which is propagating.

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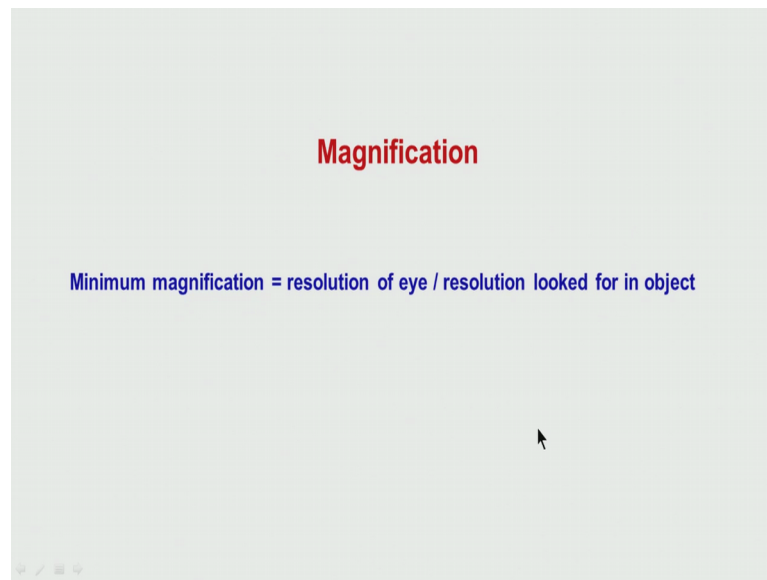
This we considered with respect to one point; that means, that even for one point without any aberration of the lens we do not get a point image we get essentially a size say which we call it as an Rayleigh's disc now let us consider a case where are 2 sources are there which are independent of each other from both of them we will be getting a Rayleigh discs like this then how are we able to differentiate them if these 2 are very far away at 2 different places they will be coming.

So, this Rayleigh disc we can see as 2 distinct ones here now if this separation between the object is brought closer together then this also comes together at some point they merge and still we can see them must distinct, but when the separation becomes smaller than that we are not able to see them as a separate disc we just observe them as a single disc; that means, that this feeling which we get is will vary from eye to eye of a person it is not going to be the same generally the variation in intensity may about less than fifteen percentage is going to be difficult for our eye to resolve. So, if the separation is such that between the middle and the other regions if the intensity is dips by fifteen percentage we can see that is what the Rayleigh criterion is all about.

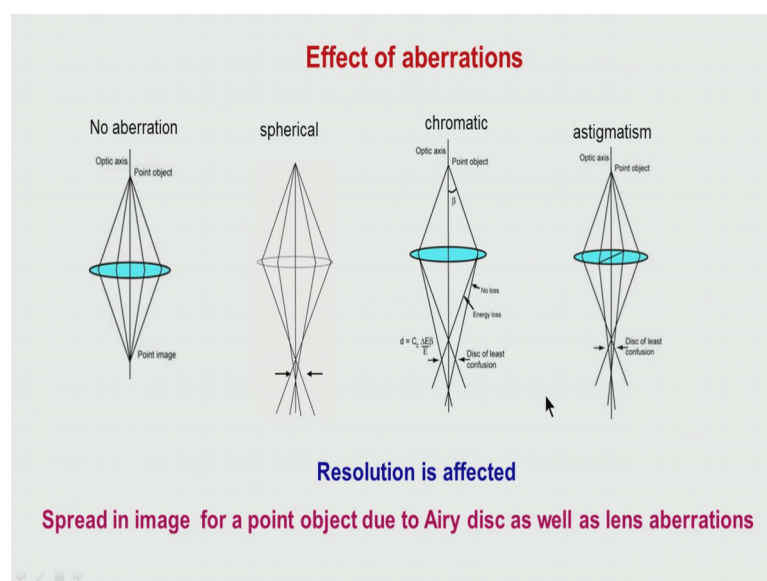
So, this is from this schematic diagram which has been shown if we see that this distance x by 2. So, how we define is that first the central maximum of one 2 where the next one that image of it when comes as the central maximum comes as the at the first 0 of the central maximum of the object this separation we call it as the resolution of the equipment or this is the separation between the object which can be resolved for this particular wavelength of the radiation.

So, we can see that this distance r equals this derivation one can do it and this turns out to be $0.61 \lambda \sin \theta$ with respect to the weight is being done otherwise normally we use for the angle which the object makes with the lens as maximum angle as θ then we can write it as $0.61 \lambda \sin \theta$ and what n is essentially is nothing, but the refractive index of that medium in an electron microscope the refractive index is one. So, and the angle θ is small. So, it can be written as just $0.61 \lambda \theta$ beta this is how it comes this is for a lens which is perfect. So, for a point object one should remember that we do not get a point image even or only an Rayleigh disc we get it and if we use a lens and separation between 2 objects this is the what the formula is going to this is what we call it as the Rayleigh criterion for resolution.

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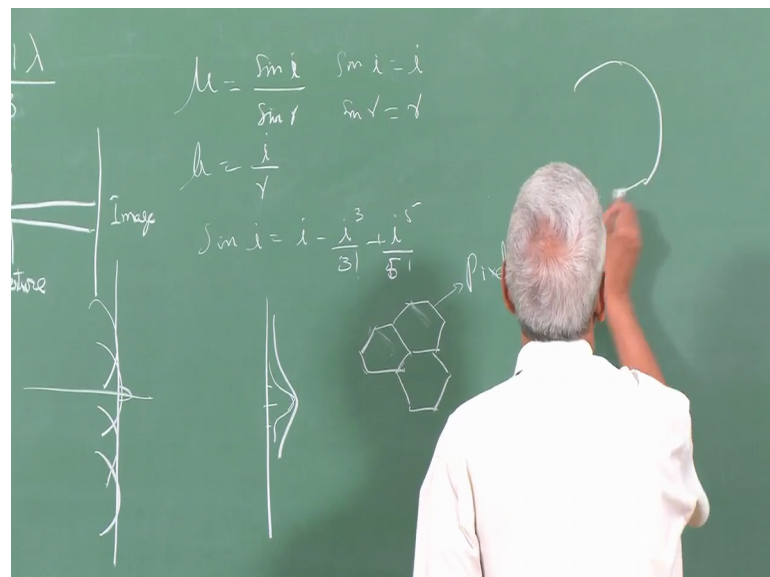


Then about magnification I had already mentioned now let us look at, but no lens is perfect. So, all lenses have some aberrations associated with it what will be the effect of that aberration on the first image which it creates. So, for a point object as we started with telling that for an ideal lens a point image will be formed that does not occur and the Rayleigh criterion tells what that value should be and that turns out to be this by beta this what that first criterion and then the lenses have different types of aberrations the one which is most important is what we call it as the spherical aberration of the lens spherical aberration what is spherical aberration is the rays which are travelling parallel

which are travelling closer to the optic axis they are focused at some point the rays which are travelling away from the optic axis they are focused at different points this gives rise to on the image plane a broad image that is what is shown in this slide.

This essentially comes because whenever we use geometrical optics to find out where an image will be formed for an object kept at a particular point in a lens the first criterion we assume that the rays are paraxial rays what the paraxial rays essentially means is that the incident angle and the refracted angle is extremely small because any ray which enters into a lens it has to be refracted and that is how it is being brought to our focus.

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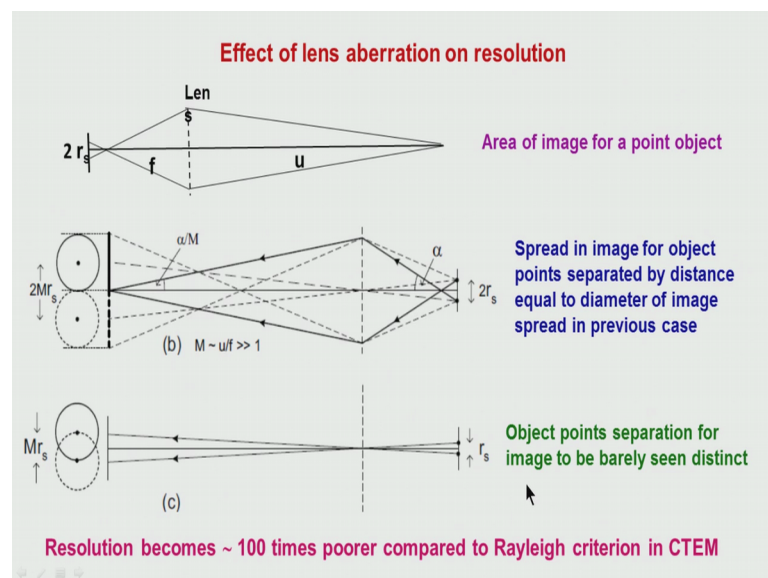
So, the refraction is given by the formula μ equals to we write $\sin i$ by $\sin r$ then this angle i and r are very small we can make $\sin i$ equals i $\sin r$ equals r . So, then this becomes μ equals i by r this is what only for small angles this condition is satisfied otherwise normally that $\sin i$ if you wonder to write we can write it as an expression i minus i cube by three factorial plus i to the power of five by five factorial this is the sort of a series in which it has to be done then only it will be an exact value.

So, we have for small values of i , it is very close to $\sin i$ that is how we this is the approximation which is responsible for this aberration because in a real lens we are assume paraxial rays, but in an actual lens it is not paraxial rays that is why we see this spherical aberration. So, if we have to avoid these effects due to spherical aberration if you put an aperture and cut off all the rays which are far away from the optic axis then

we can reduce the spherical aberration. So, this finally, what it leads to for a point object spread in image at the image plane chromatic aberration also does that same, but chromatic aberration has nothing to do with a lens we should always remember that chromatic aberration is to do with the wavelength of the radiation non mono chromaticity of the beam that is what is mainly responsible for there are other differ lens defects are there like astigmatism coma cu radius of curvature all this things we come to it shortly.

So, essentially because of the aberration resolution is affected. So, even for a lens does not have any aberration there is a spread this adds to it. So, the total sum total; what is going to now decide that aberration? So, for a point; the object itself, so, whatever is the spread which we get it is due to spherical aberration plus the Rayleigh criterion.

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So, here what we are doing it is schematically trying to show or a qualitatively or pictorially I wanted to explain how the aberration makes the reduce makes the resolution words are; see for a point object for a lens what it does is this is what the image which it is going to create this is what the image here what is being done is that I have taken this is for a object for single object which is a point.

Suppose I have 2 points object are there which are separated by a distance which is quiet large then each one of them I will be getting an image in the image plane which is going to be like this, this is what essentially we get for both of them and since the 2 point objects are well separated now if you look at it, these images are separated and at some

critical distance they touch each other, but now the intensity maximum is here and it is of so we can see them as 2 distinct points.

Now, there is a particular critical distance between the object if we consider object which are still closer then there is at some critical distance now we can see that the discs overlap when the discs overlap at the center if we see it if the intensity variation is from the center to the middle of this between the spots if it becomes less than eighty five percentage we will be able to that from this we can make out that aberrations verses the resolution here we have considered only just the spherical aberration because that is a one of the which is most important and the. In fact, as we will go we see that this makes the resolution hundred times verses compared to what a Rayleigh criterion describes Rayleigh criterion dictates.

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Lens aberrations

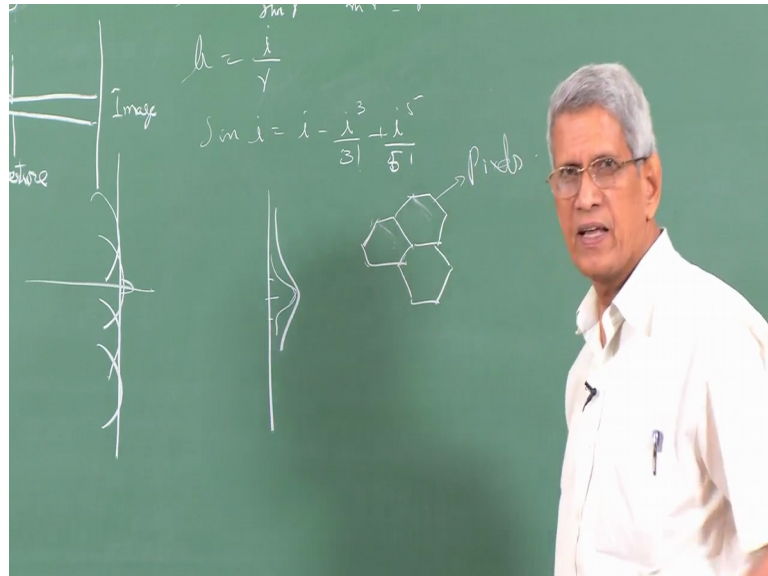
Spherical aberration Coma Astigmatism Curvature of field Distortion	$d_{Ry} = 0.61\lambda / \beta$ $d_s = C_s \beta^3$ $d_c = \beta \Delta f$ $d = (d_A^2 + d_s^2 + d_c^2)^{1/2}$ $d = (d_A^2 + d_s^2)^{1/2}$ $\beta = (0.61\lambda / C_s \sqrt{3})^{1/4}$ $d_{pp} = 0.9\lambda^{3/4} C_s^{1/4}$ For 200 keV electrons, $\lambda = 0.0025$ nm; $d_{pp} \sim 0.2$ nm
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Decreasing λ is effective way to improve point to point resolution HVEM

What aberration which we have in a lens which is inherent to a lens spherical coma that is for half axis ray that what will be the effect of spherical aberration that is coma astigmatism because the non planarity of that lens curvature of the field because there for an object which is perpendicular to the optic axis, we do not have to get an image in a one particular plane it may be curved that is one another is distraction of the image which occurs how do we quantify this for as far as the Rayleigh criterion we have looked at it we know the wavelength of the radiation what is angle which is the beam submits with the lens this the size of Rayleigh disc turns out to be 0.61λ by β spherical

aberration what it does is for a point object this gives C_s to the beta to the power of cube; that means, this is the spherical aberration coefficient.

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What does chromatic aberration do it is beta into Δf which is the defocus which it creates and all these effects if we consider it is that all of them the image distribution if we look at the image plane is essentially from the center there is a variation if this variation is essentially considered to be something like a Gaussian distribution if we take a Gaussian distribution many of these peaks which are adjusted one Gaussian peak and then we have another Gaussian peak which comes like this the total effect if you wanted to consider with respect to standard deviation is square of the standard deviation have to be added together and take a square root that will give the standard deviation of the overall effect that is what essentially is being done here.

So, this d equals d_a square; this is $d_d r$ square corresponding to Rayleigh criterion this corresponds to spherical plus this is due to chromatic since chromatic comes from the monochromaticity of the beam we assume that we are able to make a beam perfectly monochromatic then these 2 are the ones which are essentially going to decide the aberration the decide the resolution we can write it like this substitute like this, but if you look at the variation here it is inverse of beta here it is beta cube. So, we can optimize it and found for what value of beta we will get the minimum the best resolution that is that we can obtain by differentiating and then do it and the beta value turns out to be 0.61

λ by C s root three the whole to the power of one four and we can also find out what is going to be the from one point to another point in that object what separation which we can measure this is λ to the power of three by four than C s to the power of 1 by 4; that means, that small variations in λ can bring about lot of variation in the resolution and. In fact, here I am just showing the value which has been calculated for a 2 hundred k v electrons where λ is of the order of 0.0025 nanometer then the point to point resolution which we can obtain for the image of the object turns out to be point 2 nanometers. So, it is 100 times worser than what the Rayleigh criterion dictates.

So, this simple derivation shows how exactly one can calculate what will be the effect of different aberrations on the solution and. In fact, decreasing λ we can improve the resolution that is what all done initially and that is how people started making high voltages microscope there one million volt microscope three million volt microscope 2 million volt microscope where resolution becomes much better the other option which has been adopted now is that if we can make the spherical aberration small with a same normal operating voltage you can still get the best resolution those microscopes are called as aberration character microscopes. So, for now we have talked about the resolution how to calculate the resolution for a lens which has an aberration.

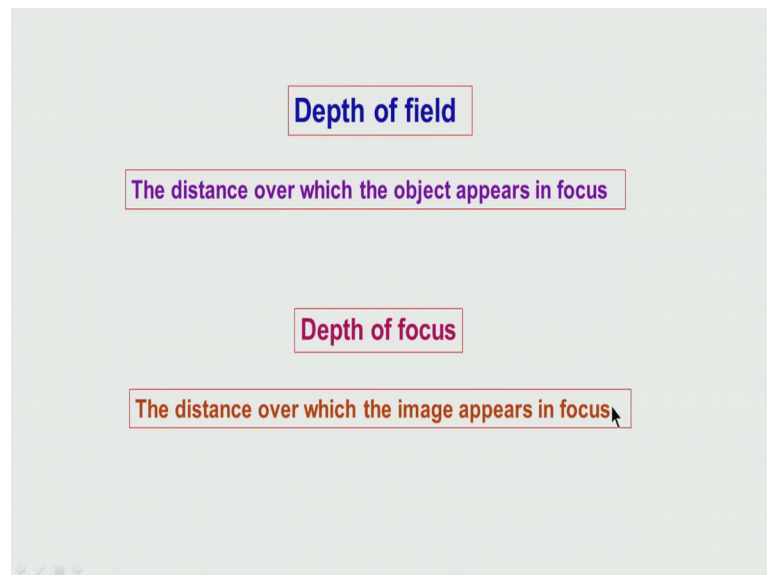
Now, I mentioned about depth of field and depth of focus these are also inherent property of a lens not only a lens it is also associated with the inherent property of the recording device as well because the best example which we can take is that when we take of picture using a camera we often see that picture that people or objects at the different positions in the object field they are all in focus only some distance beyond which you find that again it becomes AC, similarly on the focal plane also. In fact, there some distance over which even if we move the c c d camera or the film still the image will be image will appear in focus I using the word appear because it is essentially of feeling which we get it on that basis we decide I will talk about why I use that word appear.

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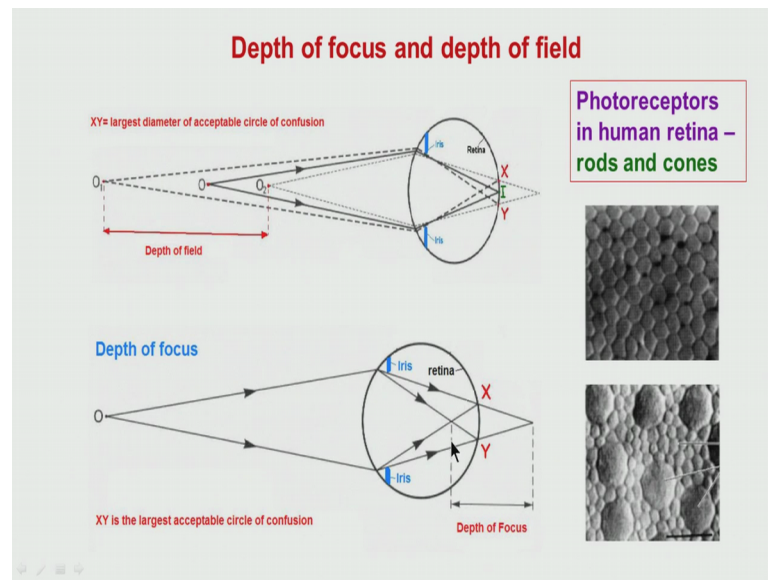
This is just a picture which I have taken of some cows which are grazing in a field. If you look at it, the cows which are at different positions are all in focus, but far away it is out of focus. The distance over which this is in focus we call it as the depth of the distance over which the object is in focus is defined as the depth of field.

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Similarly, on the image the distance over which the image is in focus is called as the depth of focus. To understand this we should just get an understanding about our eye itself how do we perceive an object and see that they are in focus.

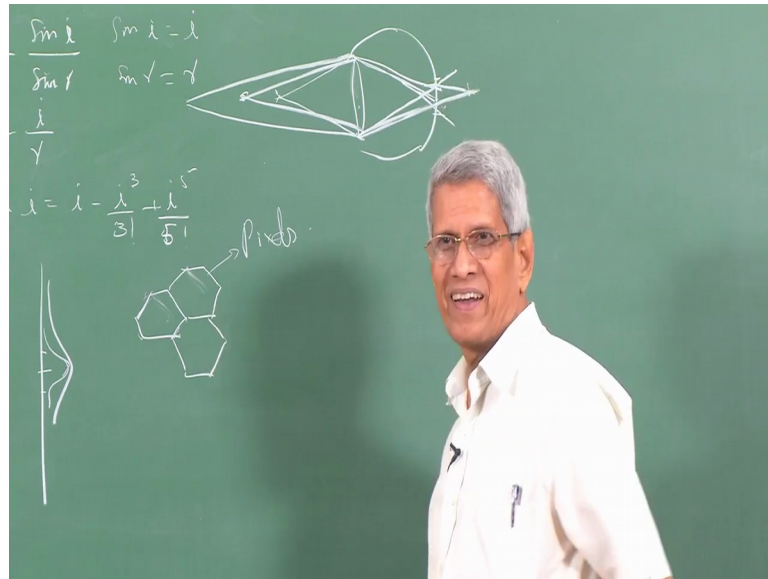
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This is because finally, in our eye we have a lens and the retina which is there the retina is the one which is able to sense the light radiation which comes and sends that information to our brain and an image is being formed how does the retina looks like that is the most critical part of it these photoreceptors are essentially what is called as rods and cones if you look at this picture this is essentially nothing, but like this are small segments which are connected together each one of them is a separate photo sensor whatever is the intensity of the radiation which falls onto it the whole thing is collected together it considers the some intensity which has come; that means, this has a finite size this is exactly what happens in the case of a c c d camera also where we call each of them we will be calling them as pixels; pixels have some finite size in the case of our eye this cones have got about something like four to five microns square is the size which they have.

So, suppose we assume that that is the finite size of one such pixel or the color cone which is there. So, if this is that size if we have an object which is here the ray which comes and enters here it gets focused here and this whole thing is falling on one pixel it is counted suppose the object is the focal its focused being that object is at this particular point when that ray comes this ray also will be forming an image at the back.

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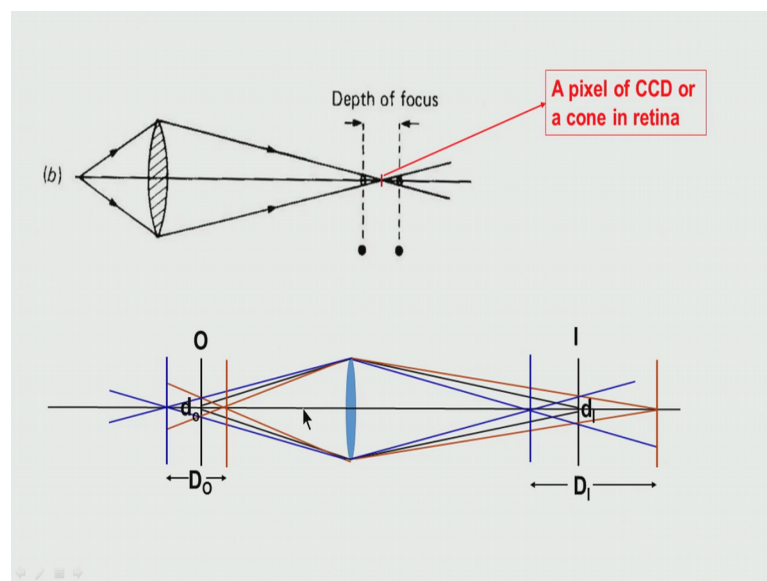
Because essentially what happens is that the eye this is a lens the ray which comes like this it forms at this particular point if this is the pixel size the ray which comes from here this what it will do it will focus it at this particular point in front of it and still if we see it the ray which is coming from here though this is focused here all the rays which are coming from this which are captured by the eye by the lens is essentially reaching this pixel.

So, as far as our eye is concerned the entire information has come whether object is here or here or is that object I keep it here then what it can happen is that it may come like this it may be forming. So, this also all the; that means, that because of the finite size of the detector which we use it the rays are being captured. So, there is a distance over which if we move beyond this; what is going to happen is that the rays will be going beyond this onto other pixels. So, this pixel there is a reduction in intensity then we say that we are not able to see that as a sharp object. So, this is how images are formed because of this what is essentially is going to happen is that there is a distance over which the object appears in focus and in this distance also if we can move it from here to here it does not matter where we keep the this one over this distance image is always going to appear as the in focus. So, this is called as depth of focus this is called as the depth of field.

Here this is just showing that eye when we go far away from the center of the retina then the cones are going to decrease and that what is called as the rods are there the rods are

the ones which has highly sensitive for vision that is are under dim radiation and the cones are the ones which collects further intense radiation and color sensitivity is there in the cone this is going into a different area. So, I will not talk about it now. So, the same way if we have an object here if and if it is focused in front or if it is that same ray if it is focused below behind or if it is focused here because of may be the lens problem still it is going to be collected for this particular distance as if it is in focus with this is called as the depth of focus.

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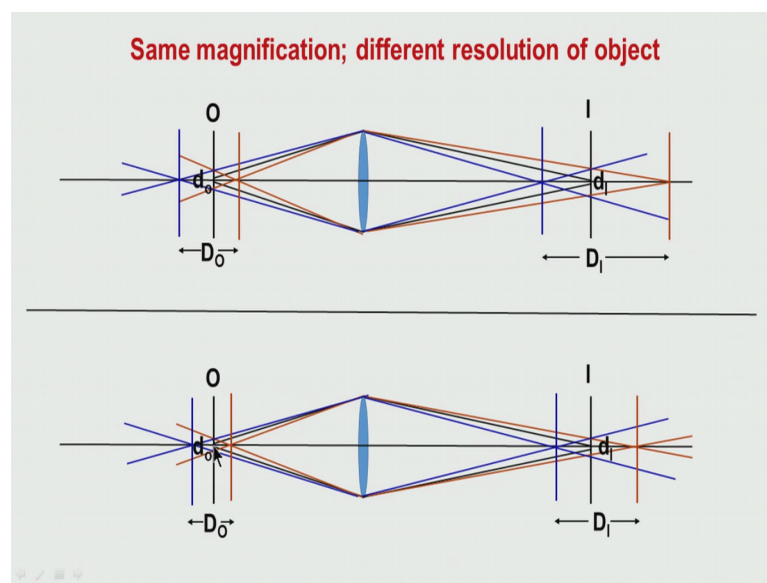
In the case of c c d that each pixel size that will decide at what distance the depth of focus is going to be there and here what I have done is that. So, far I talked about with respect to depth of focus and a depth of field and how do e relate it to the resolution that is something which we have to look at it because does it effects the resolution.

Let us take this case here for a; this is an object plane and this is the lens it forms an image in these plane if by because of some aberration we assume that the; this point is we are trying to focus it somewhere here. So, if we are trying to focus it here if you look at the ray diagram it looks as if the ray from here is coming like this ray from here is coming like this and it is forming an image here; that means, that this distance which is called as the d_0 this distance what is within this we cannot see this is what it limits the resolution and similarly from here also one is an over is over focus if it is an under focus if we do it that same thing which happens. So, between so, we can now decide what is

the area on the object which we want to resolve then depending upon that we can find out over focus and under focus regions which are going to be in focus that essentially tells us that there is for particular resolution or the particular separation between the object which we wanted to resolve very clearly there is a particular distance over which they are in focus; that means, if we use a sample of a thickness which is only of this particular size for a specific resolution we will be able to see all the region in focus this is what the consequence of it.

Similarly, in the image we can also find for that same separation between the object what is the distance over which the image will be in focus because we are using a c c d camera with some specific resolution or even if we look with our eye it is finally, behaves exactly like a c c d camera pixels of a c c d camera.

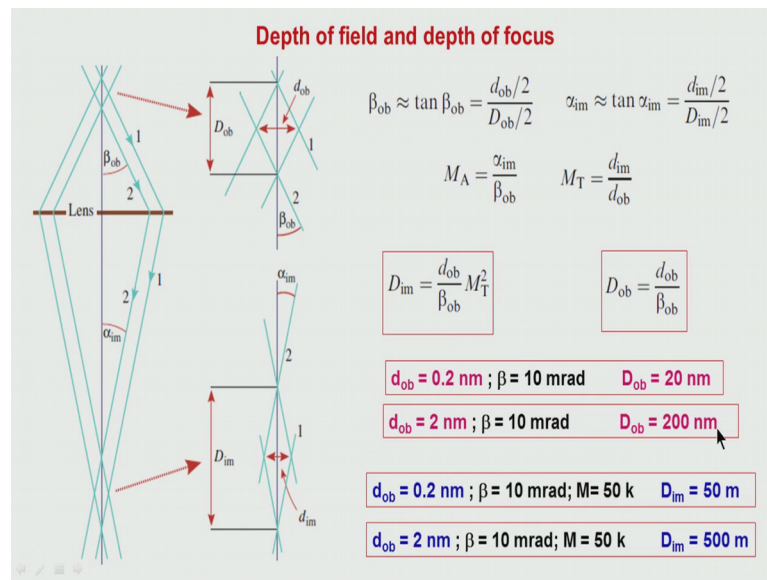
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Here what I have done is that for one particular object separation I have shown it what is going to be the separation for there is for the resolution of that object on the sample if you wanted to have what should be the depth of focus depth of field and for which what will be the depth of focus.

Now, what I have done it is here I have made the resolution much more stringent or means that the separation between the objects which I wanted to be resolved is made smaller then you can see that the depth of field has reduced similarly the depth of focuses also reduced this is essentially all are qualitative ray diagram.

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But how do we quantify it for quantification I will not go into a derivation, but I am just giving some formulas. So, this angle beta is essentially given by if you look at this particular diagram what is the object size which we wondered to see to from the ray diagram we can make out that this is the separation for the over focus and the under focus condition where within which this much region will be in focus this we can see it for the image plane also finally, then we know what the magnification of that lens substituting this we get some formula for that d image that is what the depth of focus or the depth of image is what the object size is divided by the angle which it makes with respect to the lens and the magnification square at that time similarly the depth of object equals this distance where that object is in focus is given by what is the resolution which we are looking divided by the angle which that objects are meet with the lens.

This if you do a simple calculation suppose we say that this d object I will put 0.2 nanometer because I want a resolution of 0.2 nanometer in the sample then if the beta equals 10 milli rad then the d object turns out to be 29 nanometers; that means, that sample of only twenty nanometer size only we have to use it if we want very clear the full sample to be the image suppose the sample size is larger others will give a diffuse background and the contrast will become much worse; that means, that when we want better and better resolution the sample has to be thinner and thinner that is the information if I want about 2 nanometer then what happens is that this is about something like 2 hundred nanometer; that means, that now the sample can be if I

wanders to see precipitate size of 2 nanometer is to be resolved not 0.2 nanometer then I can use a sample which is 2 hundred nanometer 2000 Armstrong thickness or thousand Armstrong thickness which we can totally prepare. So, now, we can understand what essentially is that the difference similarly for the same one I can calculate what is the depth of field in the image. So, here you can see that for point 2 nanometer for a normal microscope its gives about something like 50 meters; that means, that from the image plane twenty five meters up twenty five meters down the image is going to be in focus if we place camera anywhere we will be able form the image only thing is that image we magnified or demagnified depending upon whether we keep it in front of the image plane or at the back of that image plane that is all which is going to happen for 2 nanometers this turns out to be five hundred meters still it is very large.

So, now you can understand that what is the effect of this depth of field and depth of focus and how it is related to the resolution this is very important factor in getting good images in the microscope and not only that this dictates that we should know what is the resolution with which we wanted to see depending upon that the sample has to be prepared and then also it tells that at what distances the recording device can be kept and image. So, the essential advantage is that because of this large depth of focus is that we can focus it to one region and that region we may not have sufficient space to keep a camera we can keep it somewhere lower down or higher up and record it that is a greatest advantage in the microscope that is what is shown in these. So, within this region which we consider since the electron beam passes through the sample all the information in this region is in focus in the recording device. So, it is essentially a 2 dimensional there is the three dimensional information each projected and recorded in a 2 dimensional one this is like a perspective projection that is what we get it here, but if the sample becomes very thick normally what happens is that this we talk about with respect to only just lens considering it as the characteristic of the lens with particular focal length and the wavelength of the radiation.

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Depth of field and depth of focus

$d_{ob} = 0.2 \text{ nm}$	$\beta = 10 \text{ mrad}$	$D_{ob} = 20 \text{ nm}$	$M = 50 \text{ k}$	$D_{im} = 50 \text{ m}$
$d_{ob} = 0.2 \text{ nm}$; $\beta = 10 \text{ mrad}$; Maximum sample thickness = 20 nm				
$d_{ob} = 2 \text{ nm}$	$\beta = 10 \text{ mrad}$	$D_{ob} = 200 \text{ nm}$	$M = 50 \text{ k}$	$D_{im} = 500 \text{ m}$
$d_{ob} = 2 \text{ nm}$; $\beta = 10 \text{ mrad}$ Maximum sample thickness = 200 nm				

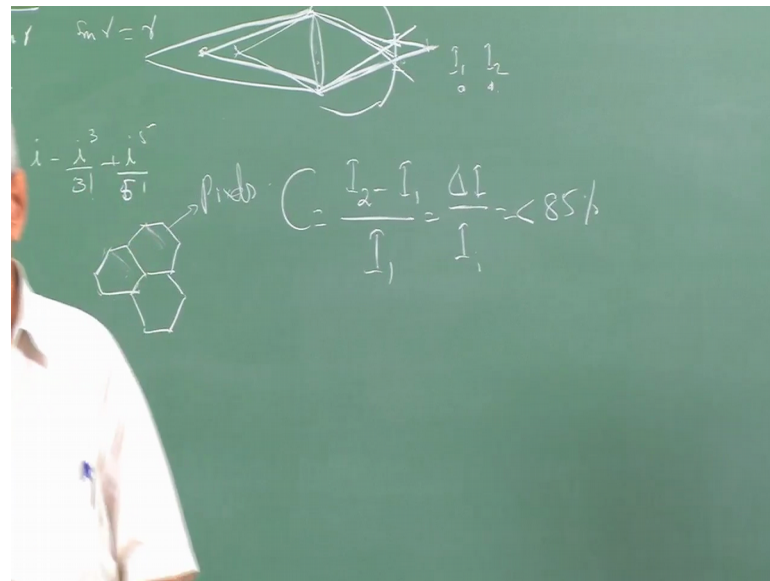
3-D information is recorded in 2-D projection
in focus for this thickness of the sample

Higher thickness – chromatic aberration increases

But if the sample thickness becomes large 2 things one spherical aberration increases chromatic aberration increases because as the beam passes through that sample lot of inelastic scattering will takes place they will spoil that. So, this we have to take care of it for this purpose its always better to have as thin as a sample as possible.

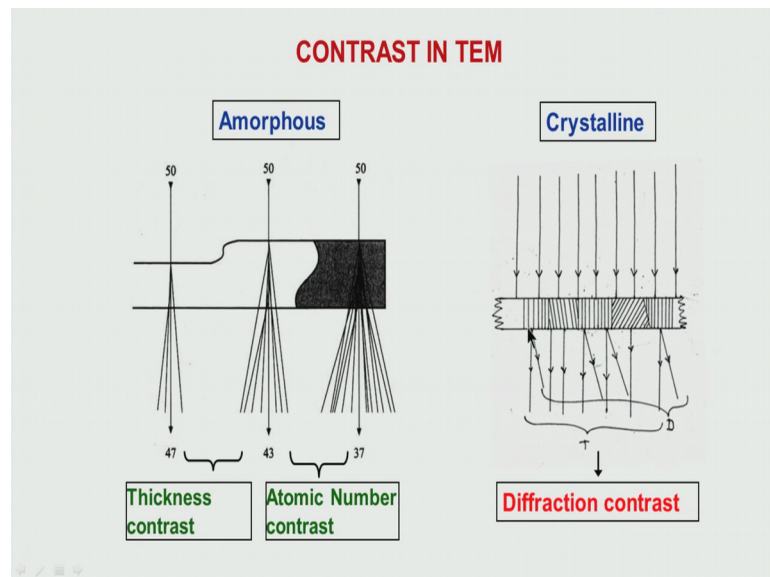
So, far we have taught about resolution this let us talk about contrast finally, when we have see some images there should be contrast what is a contrast; contrast is nothing, but intensity variation from region to region if it can be perceived by our eye then we see that we are able to distinguish features.

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So, this we can dif as from 2 regions if one I 2 minus I 1 by I o1ne I will write it if it is I 1 at one region another I 2 is this region in between these 2 regions if this variation in contrast ΔI , if this turns out to be less than eighty five percentage our eye can very clearly see it.

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So, now we have look at how contrast arises in a microscope suppose we keep a sample which is amorphous in an amorphous sample what is essentially it is going to happen is that is the sample of uniform thickness as the beam passes through because of different

type of specimen beam interaction in the incident beam direction the intensity can get reduced and if the thickness remains that same and the sample contains element of a particular type which is uniformly distributed it will be a uniform intensity we will be getting it as a background we will not see any contrast in that sample we can say, but if the thickness of the sample increases then the absorption will increase. So, this will give rise to reduction in intensity; that means, that if from one region to another region if there is a variation in intensity is going to be there in the sample that variation in intensity that is if there is a variation in thickness of that sample and that will be reflected as variation in intensity, now we can see as a contrast in that sample.

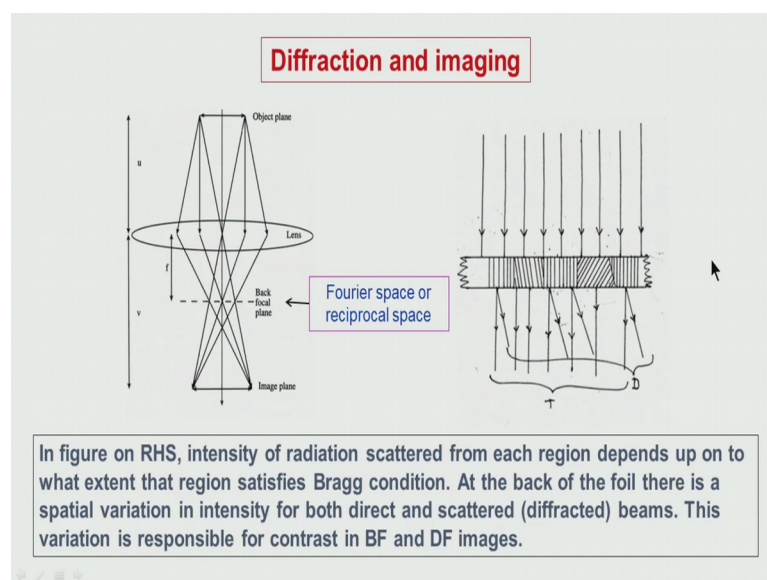
Next if we look into a region different regions contain different composition this region contains one element aluminum you assume that this region contains uranium then from in this one which contains high is a element for the electron beam which enters this will be absorbed more for the same thickness. So, the intensity will be different. So, intensity will be varying from region to region this way also we can get contrast. So, this contrast we call it as a thickness contrast this contrast variation from here to here we call it as atomic number contrast in most of the materials when we look at a microstructure different phases have different composition. So, both the thickness as well as the mass contrast both of them will be simultaneously occurring in the sample all the sample this is what it happens in the case where we are not considered that that the sample is diffracting because it is not a crystalline sample even in crystalline sample this should happen what happens in the case of a crystalline sample.

In the case of a crystalline sample the as the beam falls on that sample we consider the beam to be coherent beam and since atoms are arranged in a periodic fashion at some scattering directions the intensity of the beam for some angle if the planes are oriented in such a way that has the incident beam enters for some direction the scattered beam intensity will be high for some directions you find that scattered intensity is less for other direction; that means, that converse is in the primary beam direction as the beam passes through some orientation of the sample the intensity of the primary beam is quite high as the sample is being tilted the intensity changes that can happen because in a polycrystalline material different regions are oriented in different way with respect to a beam. So, we find that intensity variation will come.

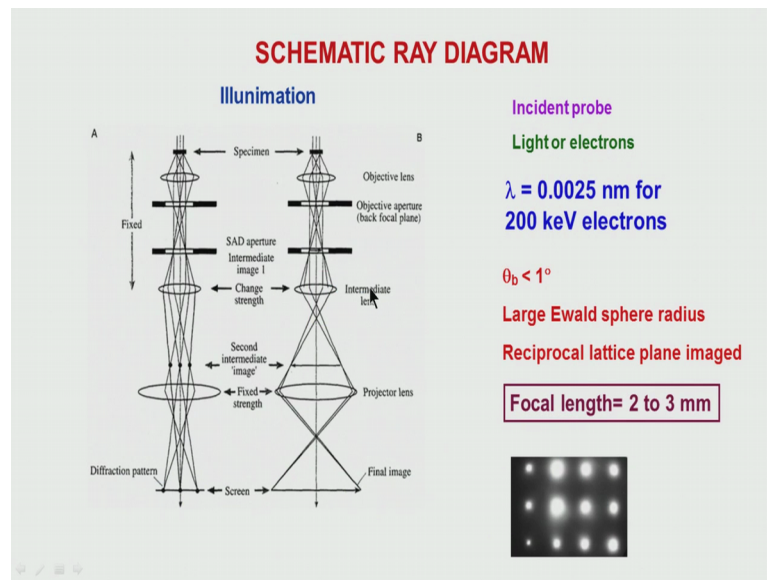
Why this is occurring this is because here the planes because especially in an electrons microscope the Bragg angle is less than one degree. So, it is almost the planes which are parallel to a beam only give rise to diffraction. So, depending upon how the planes are oriented from region to region in a grain there will be a variation in intensity depending upon that in a transmitted beam also if we look along these direction you find that there is a variation which is going to happen suppose some defects are present. So, the defects also or regions where nothing, but where the atoms are displaced from the correct lattice side that will also gives rise to variation in the scattered intensity. So, that will also come as variation in than this we call it as a contrast. So, the contrast arises in a crystalline material due to diffraction and this contrast effect could be very strong much more stronger than this mass thickness contrast.

So, in all crystalline material the contrast is essentially coming from diffraction phenomenon and in the diffraction phenomenon we consider the beam to be a wave which is coming or even if you take as a ray what is the path difference which is introduced for the different raise coming to a particular point that is how we try to calculate the intensity at every point this one should remember because that is the way or the quantification is carried out in a microscope this is what essentially explained here.

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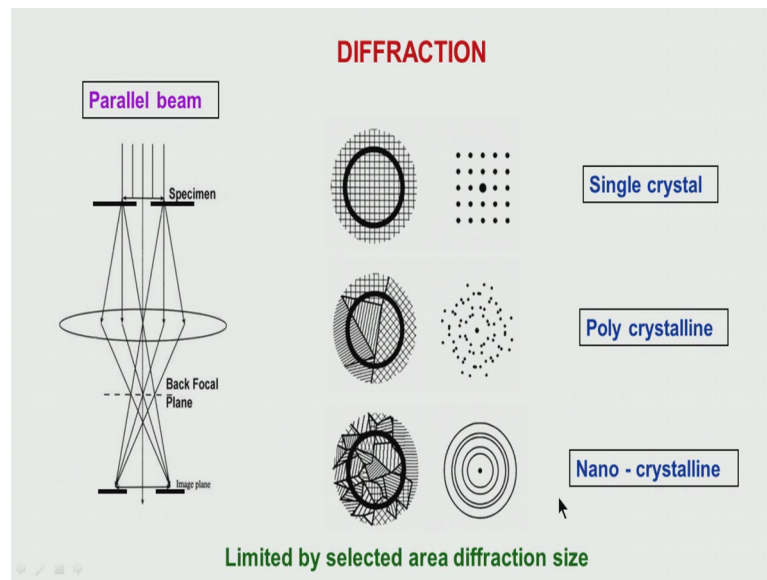
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Now, let us look at how do we form the image the image which we can form we will consider as a ray diagram which is shown here if you look here the beam which is scattered it is a crystalline specimen we are assuming it as the beam passes through the sample at the back focal plane we get a diffraction pattern there is an image plane where we get that image and for other lenses which we use it which one because now you have 2 planes are there the beams are converging the back focal plane converge they are the beams which are scattered in from different points in that object, but in a particular direction they converge and image is one where the beams which are scattered from a particular point in different directions they converge.

So, if we take a back focal plane as that object for further lenses we get that diffraction pattern on that screen if we use the image plane then we will be getting an image one should learn how to draw this ray diagrams this may be I will take it as a tutorial where I will just tell you how ray diagrams have to be drawn for lens system because these very important to understand this because one should not try to remember this ray diagram from memory because I always expected to drawn because it will be a problem which will come from specific values. So, one cannot from memory draw ray diagrams.

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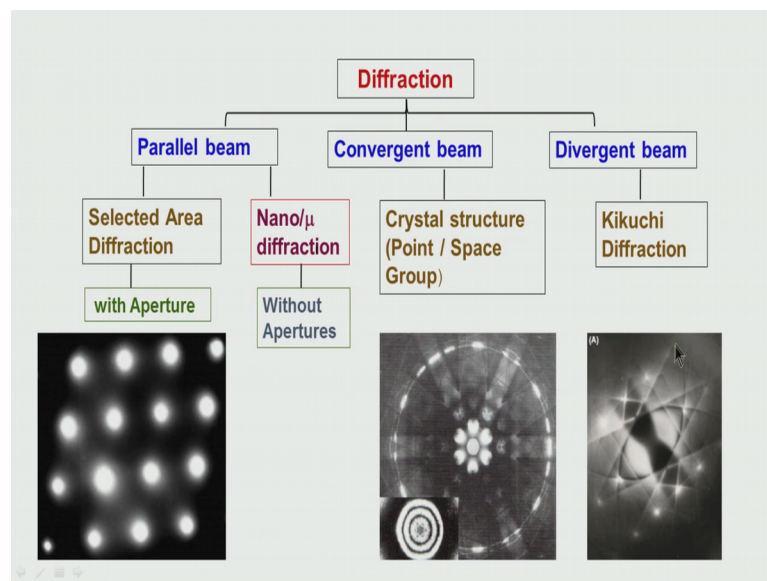


Another important aspect which I have to consider is that let us look at the diffraction in a diffraction we assume that it is a parallel beam which is falling on that sample as the beam falls on that sample at the back focal plane we have the diffracted beam in the image plane is here since it is an electron beam I can focus the beam to a very small size parallel beam and fall it on a specific area and get a diffraction, but when I try to do it the intensity will get reduced considerably suppose I want a diffraction pattern from both the regions then either you play with the beam or I other way we in we can do it in front of the specimen keep a parallel beam put an aperture suppose that area of interest is about ten nanometer; that means, putting an aperture of ten nanometer is very difficult as we have seen if you put an aperture of ten nanometer there is a Fresnel diffraction effects are going to be there image one clear what is the other way we can do it if we put an in the image plane if I put an aperture that is equivalent to putting a virtual aperture on the object plane which is demagnified that way using a larger aperture we can effectively choose any area on the sample this is what is this sort of diffraction when we do it we call it as a selected area diffraction in a selected area diffraction we put an aperture in the image plane that decides from which area or the sample though the beam is falling on the large area which area of the sample is being chosen for diffraction.

Suppose we choose an area which is essentially stands out to be one single grain. So, from that grain we can get a single crystal diffraction pattern suppose the area which we choose contains more grains then the diffraction pattern from each of the grain is will

come and this is how a pattern will appear you can see that com complexity which is coming if that aperture that which we put it contains lot of grains with various orientations we assume that is a random orie all random orientations are possible then we will be getting essentially a ring pattern which is observed for nono crystalline particles. So, far I taught about diffraction with respect to a parallel beam we can make the beam convergent.

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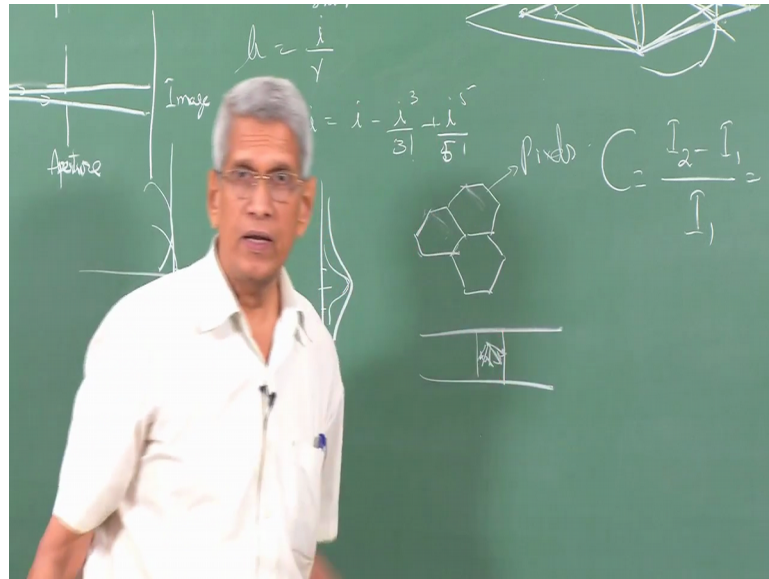


We can make the divergent also will also give raise to some the same type of a diffraction pattern or whether they give raise to some special information. So, far I talk about parallel beam when we do it the selected area diffraction this is with an aperture we can get a single crystal pattern or when the aperture size cannot be made very small if you wandered from extremely small area we can make a beam as a parallel beam and do a nano or micro diffraction. In fact, specifically how it is being done is be be made slightly convergent. So, that we can get a very fine beam, but apertures are not used that we can do it the ka if the beam which is being converged onto a sample it is as if simultaneously we are getting diffraction from beams which are falling on the sample at different orientations.

So, this sort of diffraction if we take it this gives rise to a pattern which is like this where to only the 0th order zone higher order Laue zone also we can get simultaneously image this way almost the full reciprocal lattice the various reciprocal lattice sections we can

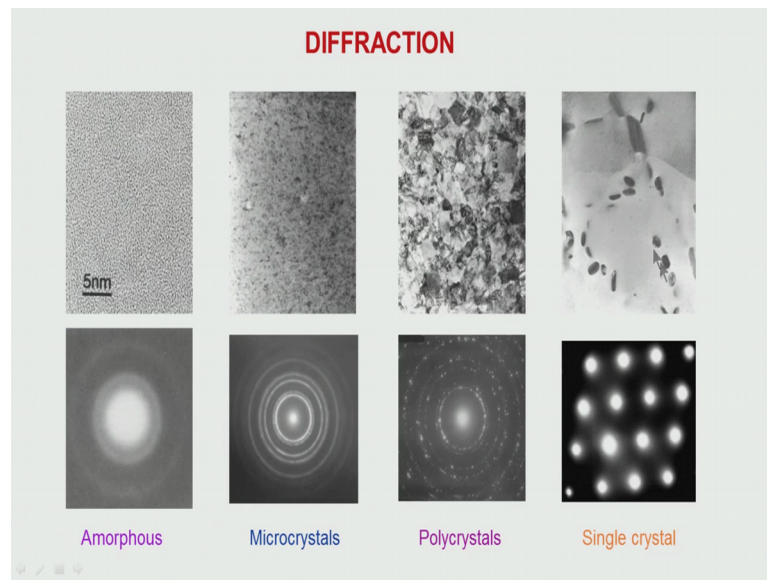
get information about how the diffraction is occurring from this we can get about the crystal structure point groups space group determination all these things could be done this will be talked about in a separate lecture later the divergent beam is when the beam is falls onto the sample if a scattering is taking place inelastic scattering takes place from a specific point that is the beam has lost some energy due to Plasmon oscillations.

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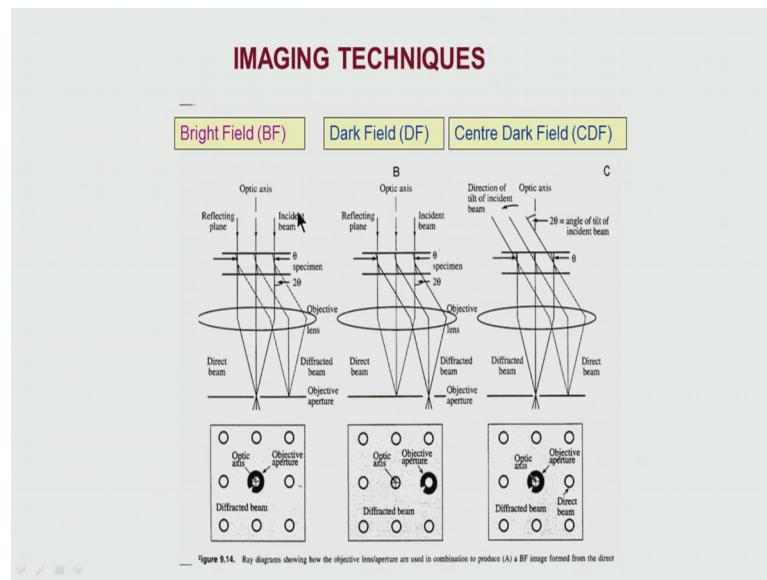
So, from this region the beam is scattered in various directions this beam could be diffracted by the plane. So, it is a divergent plane which gets diffracted this pattern we call it as a kikuchi diffraction pattern this is how it is looks like and this kikuchi diffraction patterns are very sensitive to sample orientation. So, this could be used to find out whether the beam is exactly at the correct Bragg condition or how much is the deviation to determine all this quantitative information we could use kikuchi diffraction this also will be talked about in a separate class.

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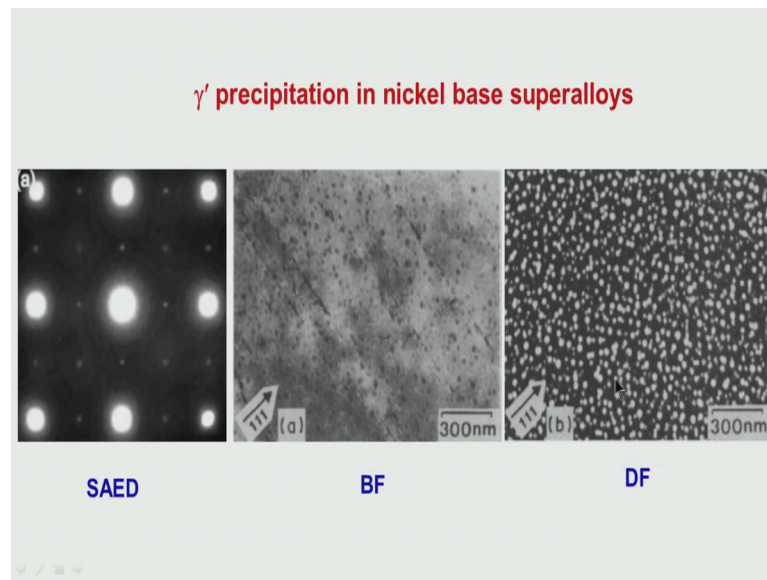
In a diffraction pattern if we look at it from an amorphous region we get a ring pattern and now we can see that if the thickness is that same we get a uniform contrast there is no variation and in a micro-crystals we can get a ring pattern diffraction pattern if we look at that image from region to region we can see that some regions are bright some regions are dark this is because how the crystals are oriented with respect to a incident beam in a poly crystalline materials semblance of ring is here, but we see spots in some things, but not continuous here also we can see the contrast when its becomes grain becomes very large single crystal diffraction pattern and we can see different grains and a second phase particles could be here all the contrast which is coming is because of diffraction effects.

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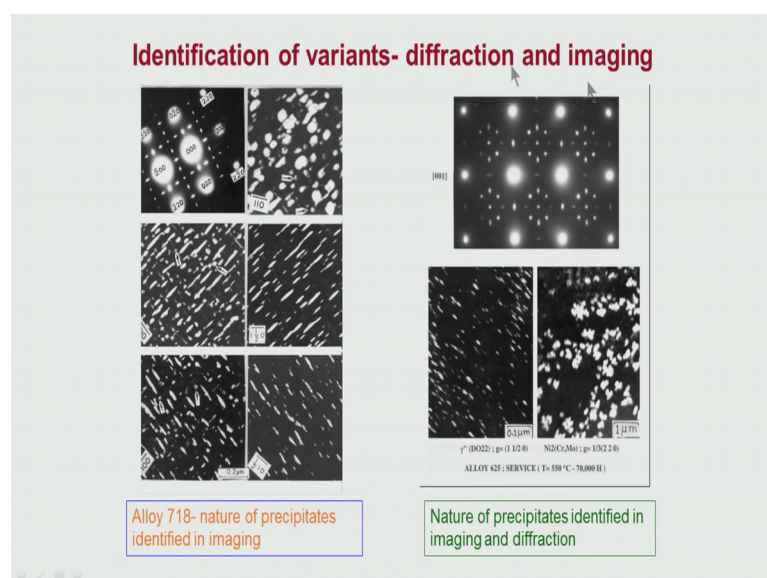
So, far we talked about diffraction and we just showed some images this all this images are taken what we call it as a bright field image what is a bright field image and there is a dark field image there are these are all the 2 things which you might have heard off when a diffraction is taking from a sample if we cut off the rays which are scattered in a other directions by putting an aperture at the back focal plane where the diffraction pattern is formed then only this beam is which is which we magnify we get that image which is called as a bright field image and if you put an aperture around this diffracted beam and get the image this is equivalent to viewing the sample in this direction of the beam or the various direction as if we view each of this directions give different information and that is how we can get the information about that is complete information about that sample and generally what happens is that if we use this sort of a dark field the beam is a way from the optic axis. So, the lens aberration increased to reduce the lens aberration we use a technique called as a center dark field what is done in a center dark field essentially is that we tilt the beam. So, that the diffracted beam is at the center and the direct beam is away.

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Using these we can get images. So, this is what I am showing it here is diffraction pattern which is taken from a gamma prime precipitates in nickel base super alloys you can see the central spot and there are weak super lattice reflections could be seen I put an aperture around it and get an image and this is the bright field image where we can see this dark regions are precipitates which are there now I put an aperture around this spot this super lattice reflection now I can see the particles which are there and how they are distributed their particle size all this information which I can get it in these particular case which I considered there is only one type of a precipitate.

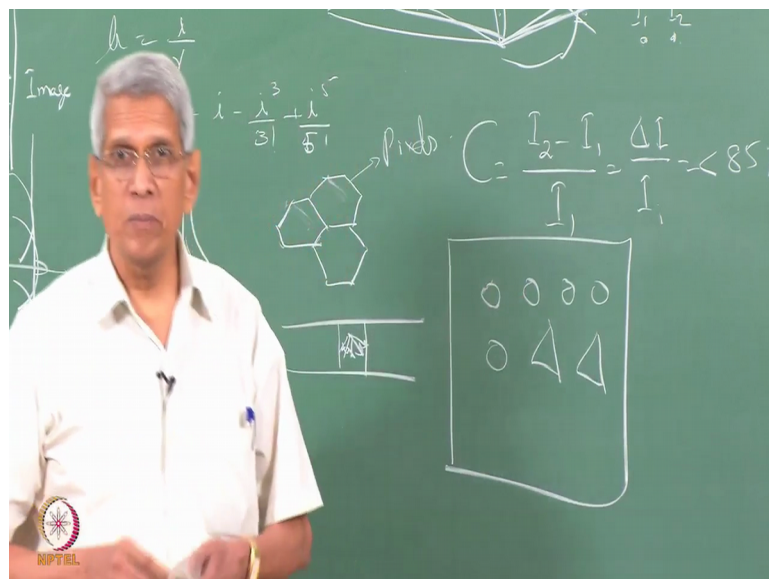
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There are cases where we can have more than one type of precipitate here there are three variants of the same phase id there and choosing different diffraction spots taking dark field image I can identify how different variants are oriented in the matrix that information we could see it this will be explained later in detail in other classes.

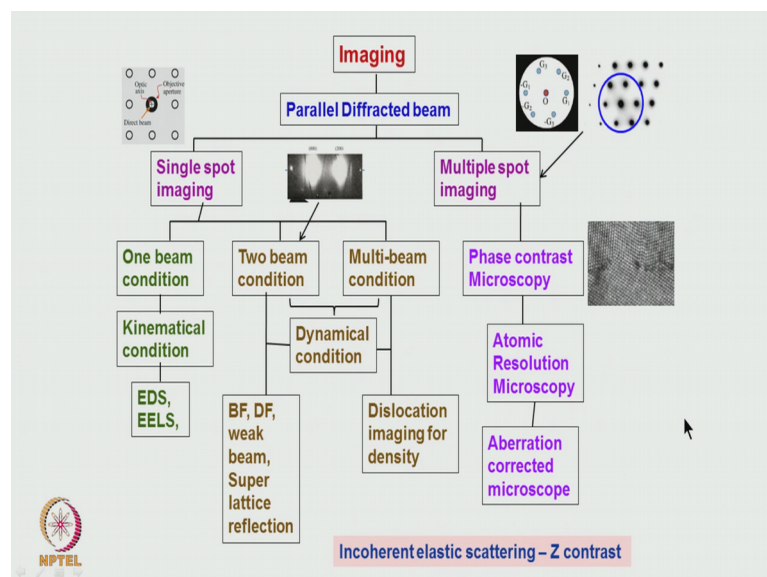
In these particular case if we consider the diffraction pattern because just what I am doing is gradually decreasing the complexity of the diffraction pattern here after analysis we find that there are 2 types of precipitates are there putting difra spots that is putting a aperture around different super lattice aperture I could identify the various types of precipitates and how they are distributed its easier said than done because this takes a lot of effort to analyze this pattern and the normally what we do it is that when we get a pattern like this you put apertures around it and get the images and then do the analysis later that is what is being done, but the whole thing takes quite a bit of if we have understand that transformation taking place in the material and crystallography and an experience electron microscopy one will be able to looking at the diffraction pattern one will be able to make out which are the spots which we should use for getting dark field image essentially what you can make out is that using a bright field image we find that variation in contrast is there that tells that there could be different types of cases present and if there are morphological differences which we see in the pattern because what may happen is that the region which we are seeing it some regions the precipitates appear like this.

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In some precipitates have a different shape it is possible that this sort of shape may be due to a another particle this by using dark field image we can try to find out what this precipitates are suppose I put a dark field image from some specific reflection if both of them belong to the same precipitate and they have the same orientation only the way they have been cut it appears then all of them will appear bright. So, there are ways in which it has to be done that part of it is what is being covered in the practical electron microscopy and that is not what I am doing it in this class because here I am talking about essentially about the principles of electron microscopy and how the image formation, but in a brief nutshell what I wanted to that this is the way.

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We form the bright field and dark field images in imaging also I talked about a simple bright field and dark field from a parallel diffracted beam in a parallel diffracted beam when we take it there are many ways in which it could be use it if we choose the beam in such way that only the central spot is bright all other spots are weak I call it as a single spot imaging in this case only one beam is strong and this condition we call it as a kinematical condition; that means, if all the diffracted parts are weak essentially only one scattering event is taking place that is the condition under which we should do EDS or eels in a microscope this is the way it has to be done and then other case is that we get a diffraction pattern.

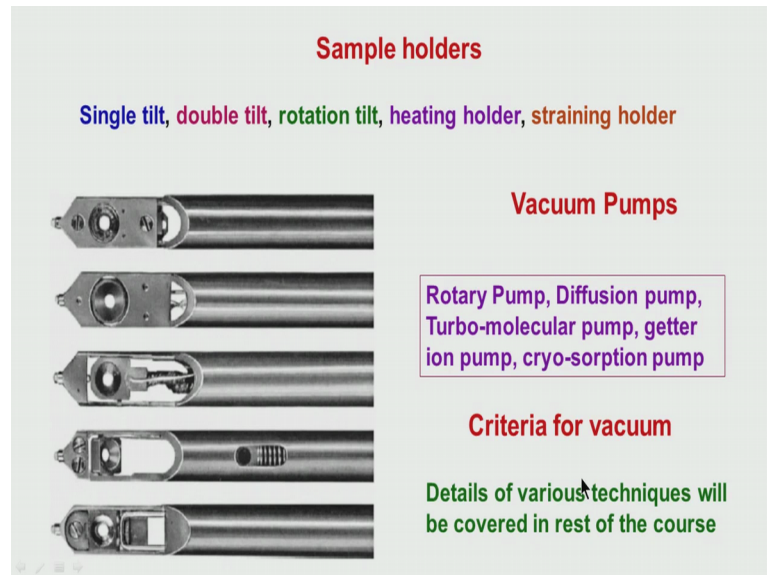
Symmetry choose only 2 beams central beam and an another beam by tilting that sample and this condition is called as a 2 beam condition this is the condition which is used most of the time to get bright field or dark field or weak beam image various types of image which are being formed and specifically if you wander to I did, find out the dislocation density it is better to use a better multi beam a symmetry this one and put an aperture around the central beam then because we know that when a defect analysis beam done depending upon the which type of G vector which is being used that if it can be present or it can absent if not the defect is present, but the in the image we do not see it that does not mean that the defect is not there that is especially important for dislocation imaging that is where we have to be careful about it if we use a multi beam condition one may satisfy the $G \cdot b = 0$ another will not because of that we will able to image all the dislocations simultaneously.

So, that we can get the true dislocation density even the parallel beam suppose we put an aperture around all the spots and then try form an image this is what is being done is that then from different regions when the beam come and join together there is a phase difference which is created and this phase difference will come as interference contrast these called as phase contrast microscopy and when multiple beam we use it this is what we call it as a some dot image pattern we get it this is called as a high resolution microscopy this also will be all this aspects will be covered separately in different classes in detail and if we can make the lens aberrations almost 0 or less and there are in phase contrast microscopy there is a way to correct for that aberration make the aberration minimum. So, that we see this sort of pattern and when the chromatic not chromatic when the spherical aberration is being made very small in such cases those microscopes on aberration corrected microscope they give an atomic resolution microscopy.

There is an another type which I mentioned right at when we started in the last class that there is called as an STEM in a STEM; the beam can be made as small as possible about point one nanometer size in such a case as the beam scans through the sample surface depending upon whether its falling on an atom or in between atom positions there will be a variation in intensity we can essentially we can map the atomic positions as we do in the case of scanning tunneling microscope or atomic force microscopy that is also give is atomic resolution ma images of the sample we can get it these as all these different types of imaging techniques will be covered in later classes what is the type of sample holder

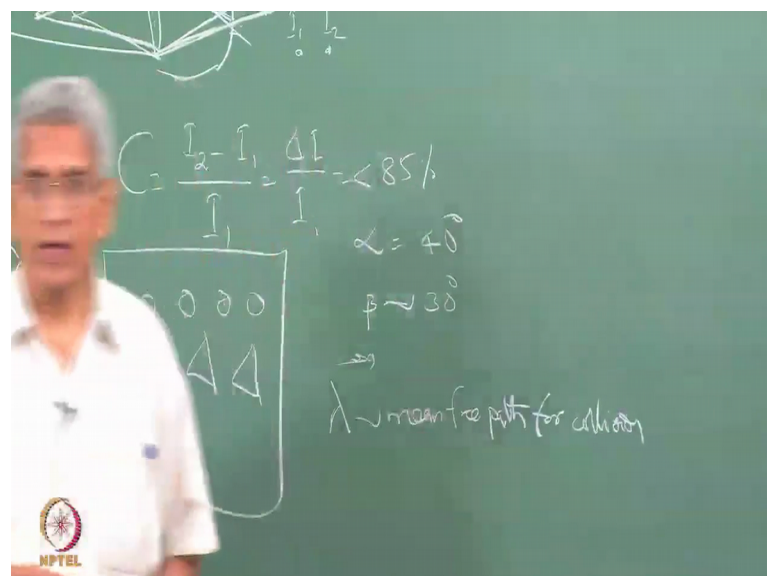
which we use it there are various types of sample holders are available single tilt holders double tilt rotation and tilt heating holder straining holder so that there are various ways in which in situ microscopy could also be formed in the microscope itself.

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And the keeping a sample in the double tilt or a rotational holder depending upon the tilt availability which is there, orient that cross as sample in whichever beam direction we want within the limits normally the tilt which is given in a microscope one tilt is about forty degree another tilt they call it as a beta this is about the order of 30 degree.

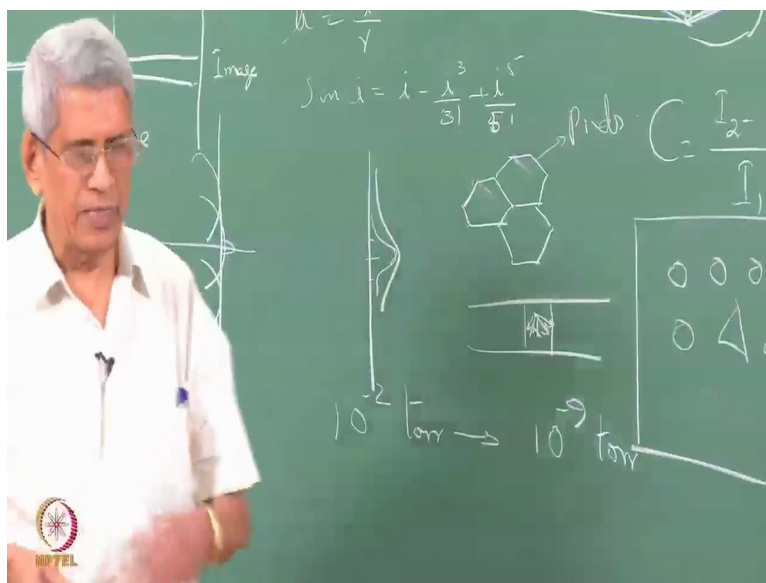
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This tilt covers more stuffs the zones which we wanted to see in conventional in typical cubic materials another thing which you should remember is that we are talking about electron microscope electron beam which we have to use it; that means, that electrons strongly interacts with matter it can get scattered its energy can be lost.

So, we require a very high vacuum. So, vacuum could be obtained using rotary pump diffusion pump turbo molecular pump getter ion pump cryo sorption pump.

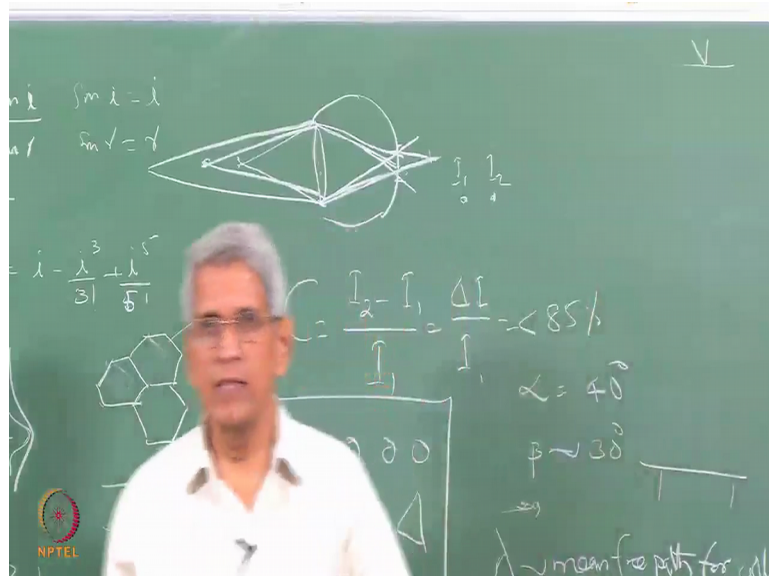
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There are various types of pumps which gives vacuum from around ten to the power of minus 2 tore to about ten to the power of minus nine tore of order vacuum could be generated in that sample this details you can see it in a book which I will not be going into, but what is essentially important is that what is the order of vacuum which we require because all pumps are available which pump you should use for a particular system that is dictated by that is by the kinetic theory of the gases if we understand that can we can that is if molecules are moving even in this room because of the number of molecules which are there as they are travelling they as they travel in this direction they encounter an another molecule it collides with it we can find out what if the separation average distance it travel it has to travel before it collides with it this is called as the mean free path for collision.

Generally the term lambda is used to define this one should not confuse it with wavelength of the radiation as we adequate it the number of molecules get reduced the

volumes remains that same the mean free path increases the minimum vacuum should be that when an electron is emitted from a electron gun from the top on the filament till it reaches the photographic device.



So, that is how we can. So, the 2 thing the one requirement from the detection side another is from the sample side and from the gun side the put a condition on what sort of vacuum which has to be used. So, that is why some differential vacuum is used in this cases this is the criteria for vacuum various techniques which are it we covered all will be covered that is what all the various techniques which are available in the microscope and their brief principle and a aberration and the theory will be covered in the rest of the course I will stop here now.