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Lecture-7
Materials Characterization
Fundamentals of Optical microscopy

Dr. S. Sankaran
Associate Professor
Department of Metallurgical and Materials Engineering
IIT Madras
Email: ssankaran@litm.ac.in

Welcome back the last class we looked at the phase contrast and optic stop microscopy and then we also have gone through the sum of the live demonstration a to appreciate the enhanced the I mean image contrast as compared to right field elimination. In this class we will move on to the next variant namely dark field microscopy, the dark field microscopy is a very simplest version similar to the bright field in a bright field as I mentioned the light rays falls on the object and get reflected and enter into the objective and those regions will appear bright and the light rays which are diffracted will escape the objective lens will appear dark from the object think in similar to grain boundaries and so on.

In dark field elimination it is the exactly the reverse will happen, briefly we look at it and then we will move on to the next variant.

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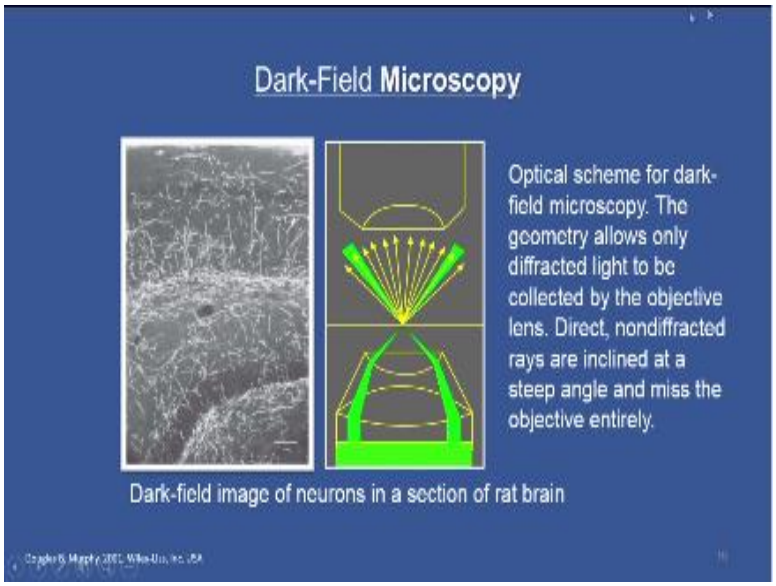

So let us look at the dark field microscopy the slide shows one typical example of the biological sample it is a dark field elimination you can see that all the features very minute features are clearly illuminated and if you look at the optical setup or the optical scheme for a dark field microscopy the geometry allows only the diffracted light to be collected by the objective and direct and non defected rays are inclined at a steep angle and miss the objective entirely.

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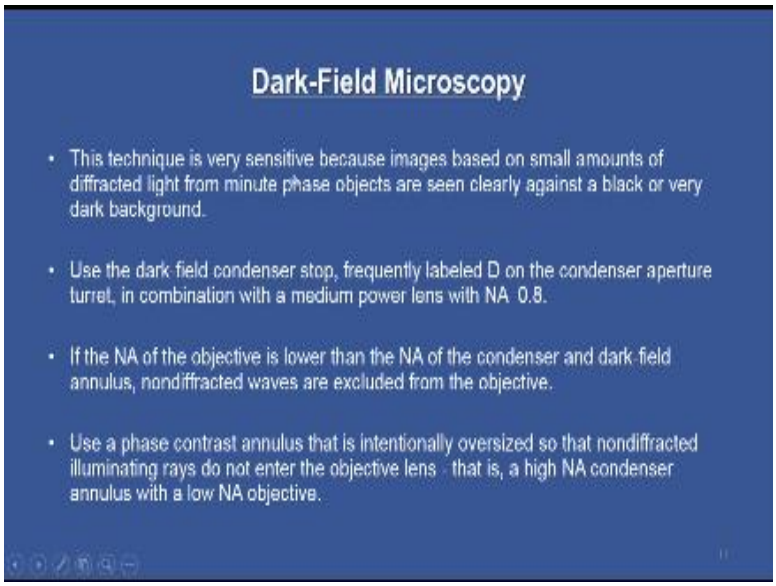

So it is a very simple design and this technique is very sensitive because image is based on small amounts of reflected light from minute phase objects are seen clearly against a black or very dark back ground. If the numerical aperture of the objective is lower than the numerical aperture of the condenser and the dark field analyst non diffracted waves are excluded from the objective.

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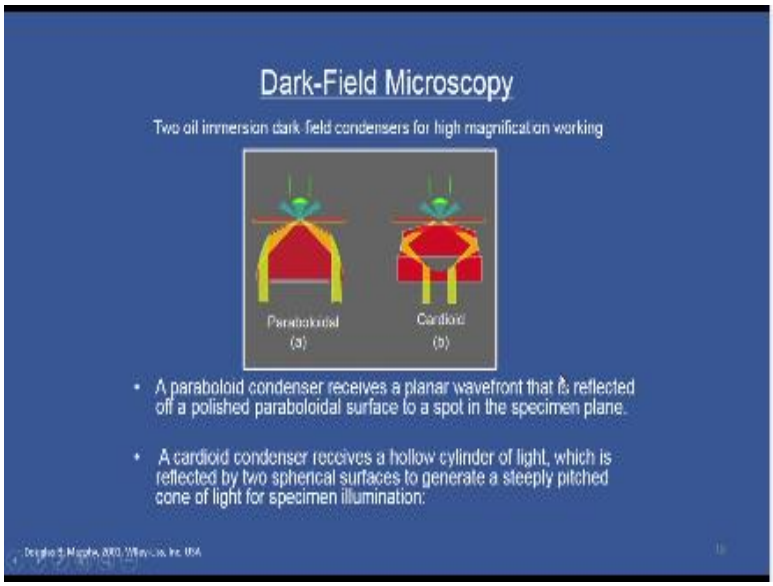

So you can have two kinds of oil immersion dark field condenser for this optical setup they are very simple in nature and you know that the principle of oil immersion objectives already we have seen that so I will skip and while quickly move on to the next variant polarization microscopy.

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Look at this slide very interesting microstructures taken from again microscopy you come and what you are now seeing is some of the microstructures of a polymeric material natural and synthetic polymers which is observed and the polarized light, what you are seeing here is a polygonal spherulites each one is a single entity turbulent and this is a polycarbonate specimen and this is a biological sample. So the reason I brought this slide again a similar to phase contrast from this website is because to appreciate the enhanced contrast which you get as compared to the bright field illumination.

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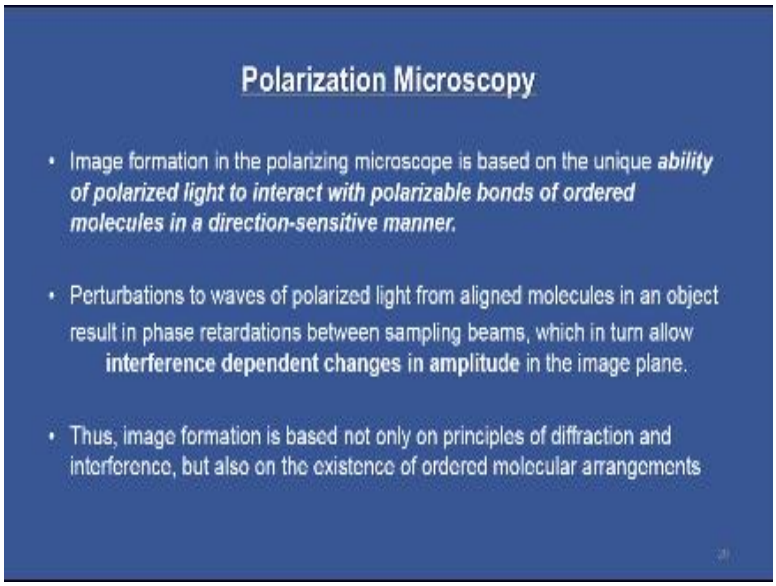

So if you look at the principles of polarization microscopy it goes like this the image formation in the polarizing microscope is based on the unique ability of polarized light to interact with polarizable bonds of the ordered molecules in a direction sensitive manner. Perturbations to the waves of polarized light from aligned molecules in an object result in phase retardation between sampling beams which in turn allow interference dependent changes in amplitude in the image plane.

So I will read it again the perturbations to the waves of polarized light from the aligned molecules in an object result in a phase retardation between the some sampling beams which in turn allow interference dependent changes in amplitude in the image plane, thus image formation is based not only on the principles of diffraction and interference but also on the existence of ordered molecular arrangements. So polarization microscopy is again another classical case of interference microscopy.

And if you look at the applications it is primarily for a transparent sample and it gives you a very accurate a quantitative results of optically sensitive constituents or optical properties of micro structural constituents in much more detailed manner compared to any other variants of

microscopy this is a unique feature in depolarization microscopy. We will now look at how this is other details in the polarization microscopy.

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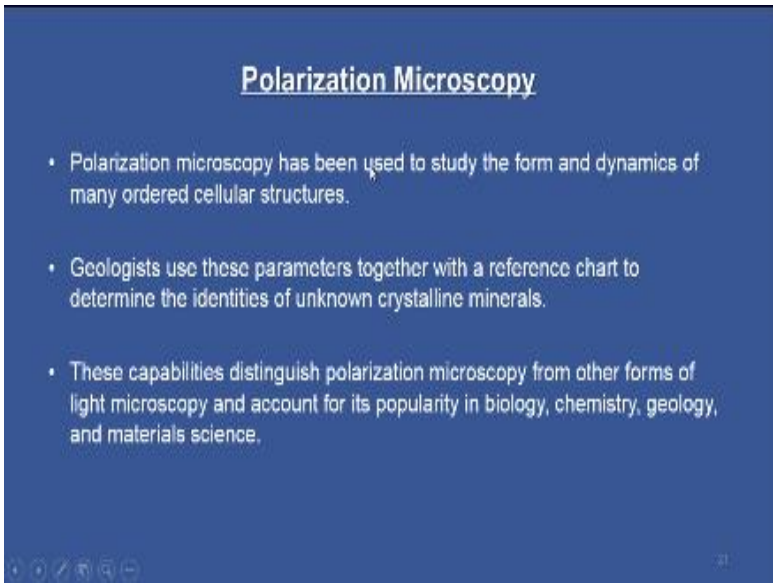

So polarization microscopy has been used to study the form and dynamics of many ordered cellular structures this is only with respect to biological sample and geologists also use this parameters together with the reference chart to determine the identities of unknown crystalline minerals. These capabilities distinguished polarization microscopy from other form of light microscopy and account for its popularity in biology, chemistry, geology and material science.

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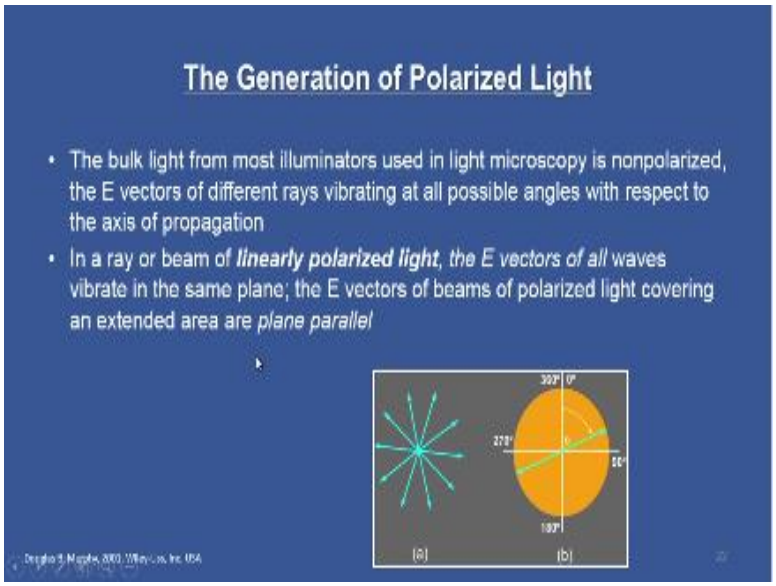

First we will introduce what is a polarized light and then we all know that the bulk light from a most eliminators used in a light microscopy is non polarized, that means the electric vectors of different race vibrating in all possible angles with respect to the axis of propagation. In array or a beam of linearly polarized light the electric vectors of all wave vibrate in a same plane so the e vectors of a beam of produce light covering an extended area are plane parallel, so this is the schematic which shows that how the vibrations of the electric vectors are represented.

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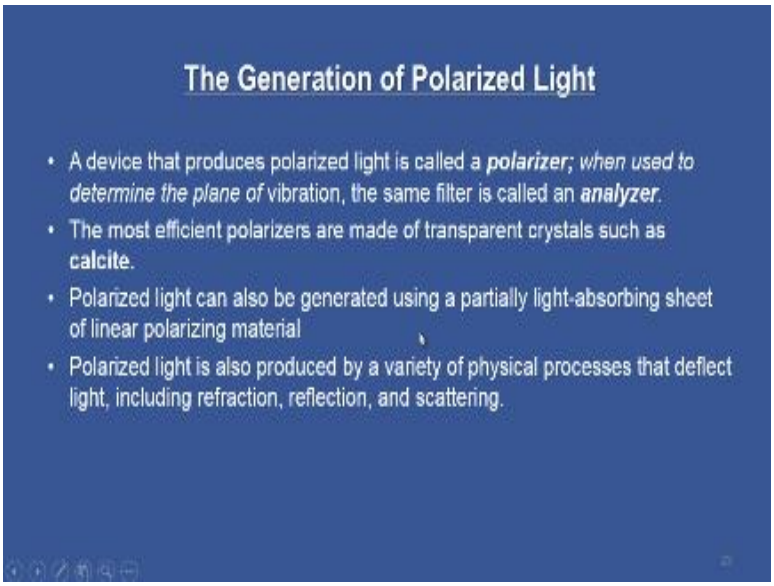

And we will see that much more details a device that produces a polarized light is called a polarizer, when used to determine the plane of vibration the same filter is called an analyzer please understand we have the same material which being called polarizer as well as analyzer when it is called polarizer when it is used to determine the plane of vibration the same filter is called an analyzer.

The most efficient polarizers are made up of transparent crystal such as calcite. Polarized light can also be generated using a partially light absorbing sheet of a linear polarizing material. Polarized light is also produced by a variety of physical processes that deflect light including refraction, reflection and scattering.

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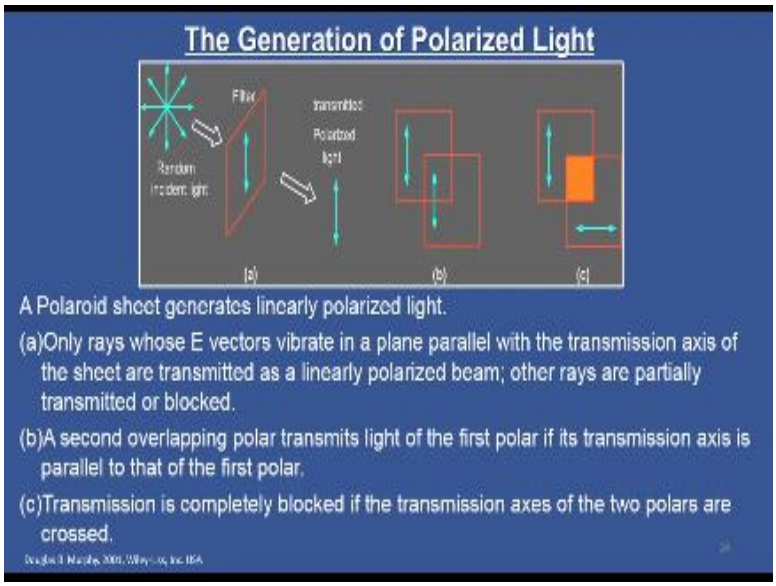

So now let us see how this filters are going to react with this polarized light or the filters how it allows this transmitted light in the optical path. A Polaroid sheet generates linearly polarized light that means this is a polarizer Polaroid only raised whose E vector vibrate in a plane parallel with the transmission axis of the sheet are transmitted as a linearly polarized beam other rays are partially transmitted or blocked, so you have the random light incident light which is passing through this filter and it allows only the waves which whose electrical vectors vibrate in the plane parallel to the transmission axis others is blocked.

The schematic b shows a overlapping polar transmits light of the first two polar if its transmission axis is parallel to that of the first polar. So since this directions are parallel the light will pass through this overlapping polar filter also and in the case of c the transmission is completely blocked if the transmission axis of the two polar are crossed since these two filters are kept in a a cross orientation the transmission is completely blocked so this is how the polarized polarizer filter will behave.

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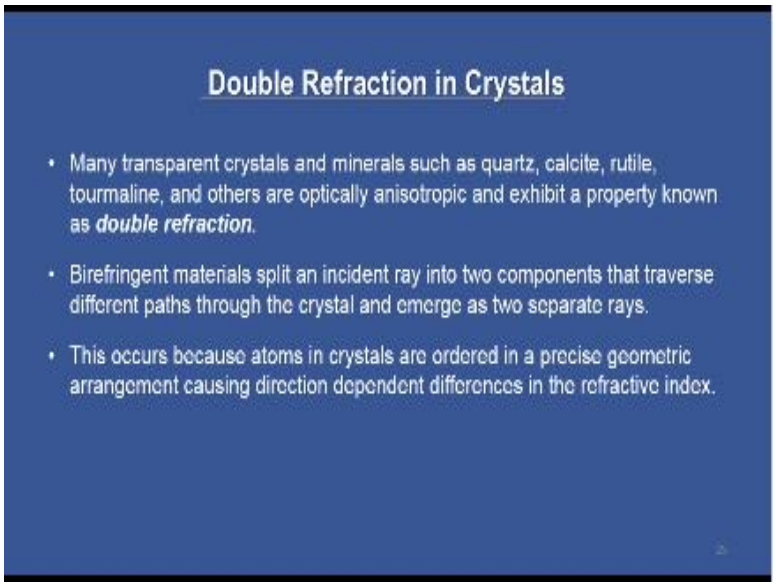

So now we will look at what is double refraction in crystals because we are going to look at the concept called birefringence and for that we need to know what is double refraction in crystals so many transparent crystals and minerals such as quartz calcite, retile and tourmaline and others are optically anisotropic and exhibit a property known as double refraction. Birefringent material split and incident ray into two components that traverse different paths through the crystal and image as two separate rays.

This occurs because atoms in the crystal are ordered in a precise geometric arrangement causing direction dependent differences in the refractive index see this is the fundamental reason why the polarized microscope is able to generate a special contrast. So if I look at this object which is made of birefrigerant materials that split an incident polarized light into two components one is called ordinary ray another is called extraordinary ray it is also been called as O array and E ray the ordinary ray means that will obey all the optical rules that is Snell's law and so on the extraordinary ray will not obey this optical rule that is the difference between an ordinary ray and an extraordinary ray and we will see that how these two rays and their amplitudes are manipulated to obtain a better contrast in this microscope.

So I will read this third Point again this occurs because atoms in crystals are ordered in a precise geometric arrangement causing a direction dependent differences in the refractive index, so we will see how this is being implemented in the optical scheme.

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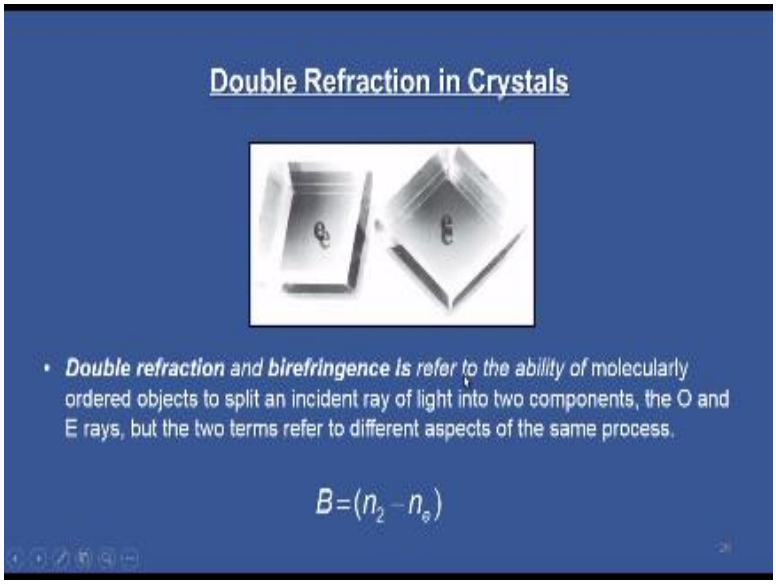

And before that let us clarify much more details about double refraction in crystals, so look at this very interesting crystals you see that letter appears as if you have one on the front plane one is lying on the back plane let us see what is the reason for this double refraction and the birefringence is referred to ability of a molecular ordered objects to split an incident ray of light into two components the O and E rays what is ordinary and extraordinary rays. But the two terms refers to the different aspects of the same process.

B that is birefringence $C=n_2-n_e$ that means n_o it should be should be an e the difference between these two refractive index.

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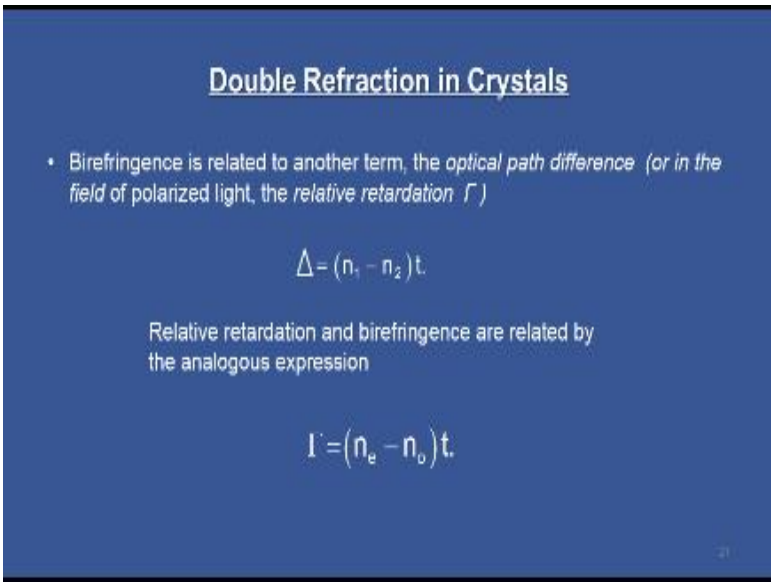

Birefringence is related to another term the optical path difference or in the field of polarized light the relative retardation so optical path difference and related retardation or the similar term but relative retardation is used in polarized light scheme otherwise it is in generally in optical literature it is reference to optical path difference which we have already seen $\Delta=(n_1-n_2)t$ where t is the thickness of the glass or the medium.

Relative retardation and birefringence are related by an analogous expression that is γ capital γ=ne that is refractive index of extraordinary ray minus refractive index of ordinary ray times the thickness, so these parameters will be useful when we talk about the enhanced amplitude of the resultant wave which come out of the object which produces a better contrast in the poultry slide scheme.

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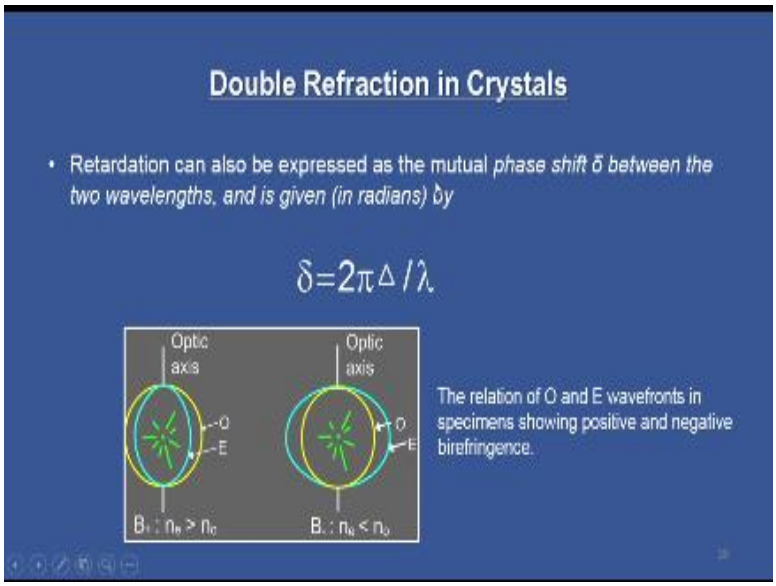

Retardation can also be expressed as the mutual phase shift ′ between the two waves and it is given in radians by $\delta=2\pi\Delta/\lambda$ look at this schematic you will appreciate this much more as we move along in this lecture. The schematic the first schematic shows this is an optic axis and then you have ordinary ray and this is an extraordinary ray suppose if these two are there and you have the positive B value when refractive index of extraordinary ray is greater than the ordinary ray and you have the reverse scheme when you have the refractive index that is a negative B value when you have refractive index of extraordinary ray is smaller than the ordinary ray.

So we will see how this two things are visualized in an optical scheme as we move along the relation of ordinary and extraordinary wave fronts in the specimen showing the positive and negative birefringence is summarized in this schematic.

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So let us now see how the generation of elliptically polarized light by a birefringent specimens, so what we are now assuming here is the object which we are looking at our viewing are analyzing under the polarized light microscope will exhibit the Birefringence then and then how that material or object react with the plane polarized light and then what happens this is what we are going to look at so let us see the generation of elliptically polarized light by a Birefringent specimens the wave forms of elliptically and the circularly polarized light you have two forms ordinary ray and extraordinary rays.

Follow in the same propagation axis but vibrating in a mutually perpendicular planes cannot interfere but can be combined by a vector addition the sheet of cellophane held against a single polarizer, on a light box is an example of this behavior a cellophane is a birefringence sheet made up of parallel bundle of cellulose so which will help to produce elliptically polarized light let me now run this schematic.

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Animation you can see that suppose if you assume this is one wave plane polarized in one plane the other one is a plane wave which is perpendicular polarized. If the phase shift is dynamically happens between these two then you can see that elliptically polarized light is possible I will play this again to appreciate how this elliptically polarized light is generated and you have this ordinary ray and extraordinary ray they are vibrating in a mutually perpendicular plane and if the phase shift the mutual phase shift is dynamic then you will be able to see this the wave propagation in an elliptical manner.

So this is elliptically polarized light so I hope this animation helps you to appreciate this concept of elliptically polarized light.

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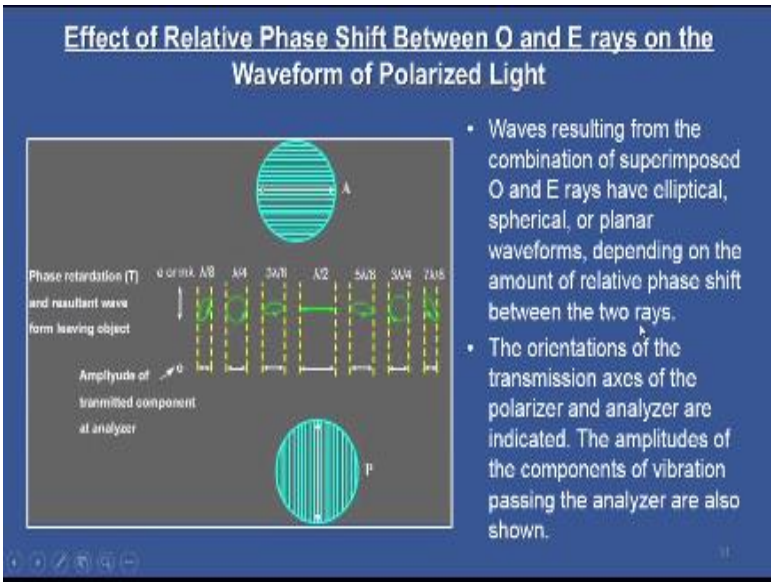

And now we see that the phase shift which I talk about in between the e and o raise how it is understood the effect of relative phase shift between ordinary and extraordinary rays on a wave form of a polarized light look at this we have a two Polars one is analyzer and this is a polarizer they are kept in two perpendicular directions and let us go through the points and then we will get into the details of this schematic waves resulting from the combination of superimposed ordinary and extraordinary rays have elliptical spherical or polar wave forms depending upon the amount of relative phase shift between the two rays.

So what I have just shown in the previous schematic clearly shows that depending upon the first relative phase shifts either, you can have elliptically polarized light like we have seen in the animation but you can also have a spherical and circular wave forms. So that is what being summarized in this slide, so you look at this is the phase retardation and resultant wave form leaving the object and this is the amplitude of the transmitted component at analyzer. So you see that the amount of phase shift that is γ by 8 produces this kind of an amplitude this amount of amplitude.

Let me say γ by 4 produces a different level of amplitude and three by γ 3 γ by 8 produces again different and you see that γ by 2 produces the maximum amplitude of the analyst at analyzer so it is the orientation of the transmission axis of the polarizer and analyzer are indicated the amplitudes of the components of the vibration passing through the analyzer also shown. So suppose if I rotate these two with respect to their orientation all this amplitudes are possible so that means you have this polarizer and analyzer the two Polars depending upon the rotation and their orientation axis.

You are able to manipulate the amplitude of that trans transmitted light which is coming to the coming from the specimen to the analyzer so this is the key to appreciate the manipulation of the amplitude of the transmitted beam from the birefringence specimen.

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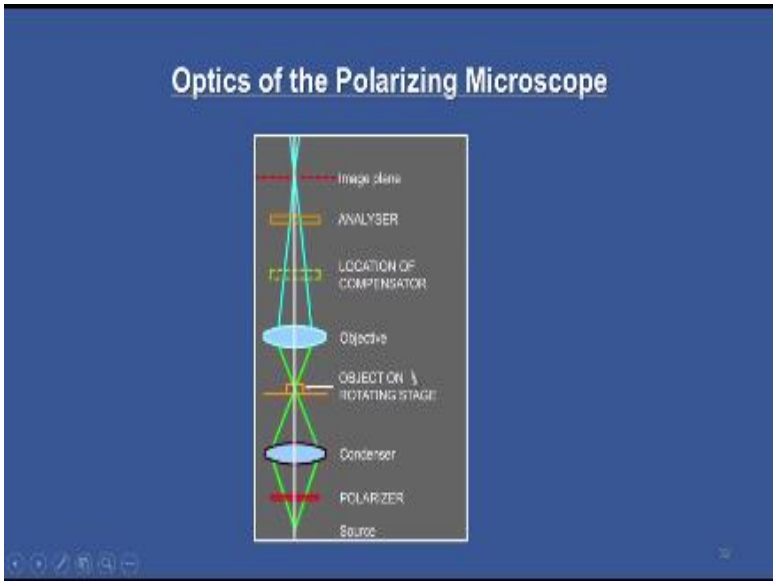

So this is the optical scheme for the polarizing microscope you have the polarizer the light source and you have a polarizer it could be a whatever the BF just mentioned in the slide could be any material which is which can do this job polarizing activity and then you have a condenser and then you have the specimen on a rotating stage again objective and this is the another important aperture or device called a compensator or retarder here we will see the what is the function of

the retarded the retarded will give the specific space phase shift between ordinary and extraordinary ray.

And then you will get the relative retardation because the ray which is coming out of the specimen and this will be compared and then you will get the relative retardation and you the material characteristics so you have the analyzer and then finally the image plane so image plane you have the both rays are interfering and then producing the contrast.

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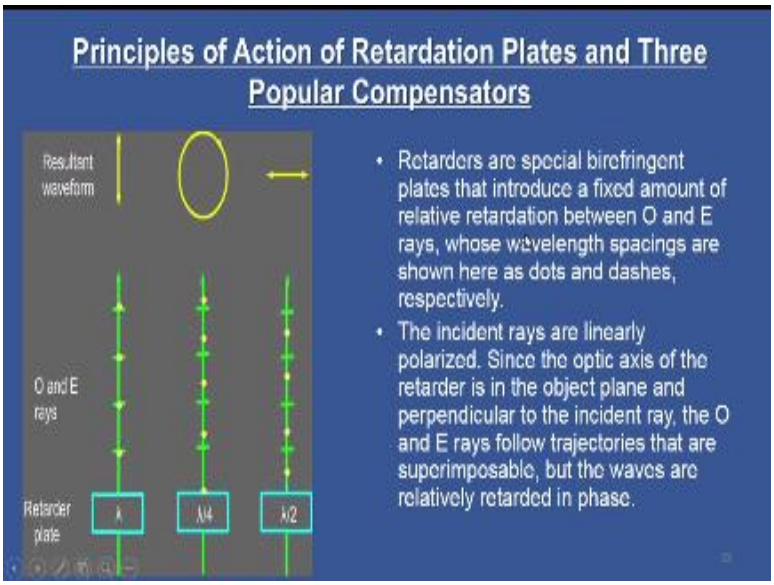

So just I mentioned about the retardation plates so let us see what are the principles of the actions of retardation plates are and the three popular compensators the retardation plate or compensator they are owned on the scene, so look at this schematic let us look at the remarks first and then we get into the details of the schematic retarders are special by refrigerant plates that introduce a fixed amount of relative retardation between ordinary and extraordinary raised whose wavelength spacing are shown here as a dot and dash dashes respectively.

So here dots and dash this is the wavelength of extraordinary and ordinary ray so the incident rays are linearly polarized since the optic axis of the retarder is in the object plane and

perpendicular to the incident ray the ordinary and extraordinary day follow a trajectories that are super impossible, but the waves are relatively retarded in phase. So you can see that the both raised ordinary and extraordinary race they have a similar wavelength but if they are retarded by γ by 4 then how you will see the waveform here and if it is γ by 2 it is completely a different orientation compared to the incident wave form so this is how the retardation plate will also operate.

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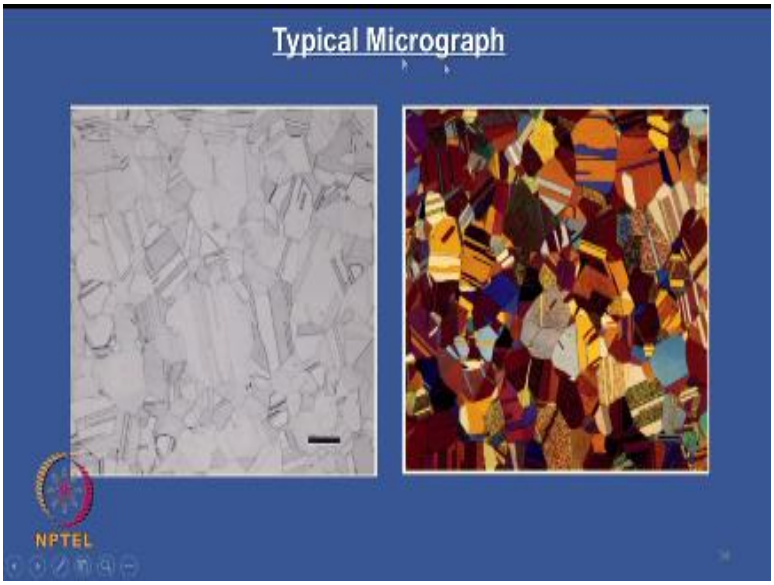

And you see that this is a typical micrograph of a polarized microscopy the material is under the examination is brass and you see that bright field elimination gives a kind of contrast a polarized light gives her extraordinary a contrast now I will take you to the lab.

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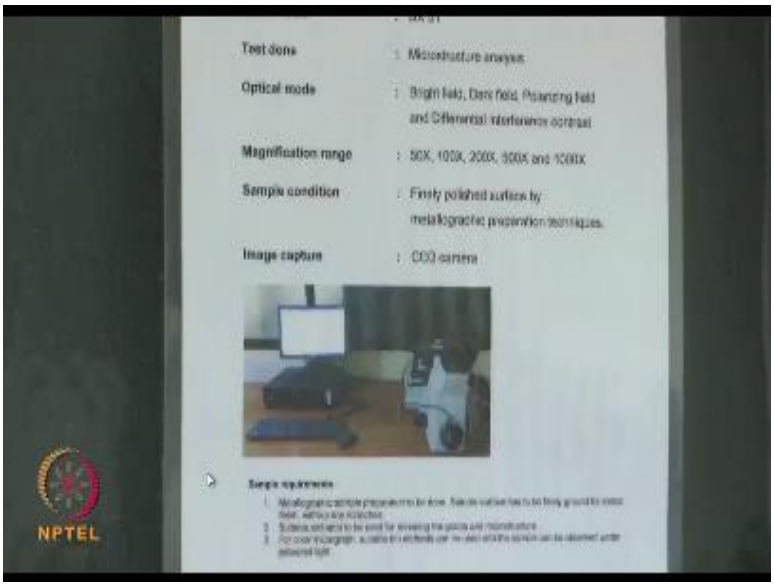

Where I am going to show a live demonstration about this polarizing microscope and you will appreciate whatever we just discussed so this is in our lab now olympus microscope similar to what I have introduced in the previous classes so what now you are seeing is an inverted microscope.

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So I do not have to explain the details which you know already the parts of this microscope and so on what I will now concentrate is about the a polarizer analyzer setup so if you have listened to the lecture clearly we will now witness this polarization analyzer orientation here, so one is polarizer is kept here and this is an analyzer which are just and there is a rotation knob to change the orientation of these two. So what you are now seeing is a polarizer is kept in this orientation and you can see that there is a mark here which indicates the plane of vibration of the waves in that direction and you have the analyzer and you can also see the mark here which is in the perpendicular direction of the polarizing polarizer.

So you can now clearly see in much more closer view of the polarizer and then this is an analyzer and you can also see that there is a knob here a rotating knob which is identified by this white spot you can move this in the circle that is you can rotate these two to change the orientation of this analyzer so whatever just we have seen in the slides it is exactly the geometry is exactly kept here the polarizer and analyzer are kept in two perpendicular direction and now we will see how this is going to change the contrast of the microstructure.

So now we are seeing that that knob is being moved to appreciate the orientation of the analyzer so depending upon the orientation you will the retardation also will be there and the various the ship to the phase shift will be according to this orientation shift so now we will see one particular microstructure of is a titanium alloy this is in a bright-field mode now we will insert this a polarizer and analyzer in the optical path like this and then, now you see what kind of microstructure you are seeing.

So now you are seeing in a complete extinction condition that means your phase shift is bored γ by 2 it is a extinction condition so now you will slowly rotate this analyzer so your phase shift changes and then you see that the microstructure is getting much more clear with all the details you are able to see as I mentioned this is a titanium alloy you can see that a lot of twinings in the grain and the grain interior now you will see that he is again rotating this analyzer and your image will become almost like a bright field image so that means the phase shift between these two which contributes to the contrast is not there so as long as you are able to do this manipulation of retardation or I would say that phase shift you will be able to get the better contrast in the specimen you will see that it is going back to these right field mode and then is coming back you can see this operation again this is an extinction condition again that means it goes to a complete orthogonal orientation.

So now at a specific spaceship you will be able to arrive at the best possible results so I hope you appreciate this demonstrations how the polarizer and analyzer and give a better contrast as compared to the bright field illumination and we will look at the other variant of the microscope like a differential interference contrast in the next class thank you.

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