Fundamentals of optical and scanning electron microscopy

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Module – 02
Unit-4 Phase contrast, Polarized light, Differential interference contrast, Fluorescence microscopy
Lecture – 07
Dark field microscopy
Polarization microscopy
Double refraction
Generation of polarized light

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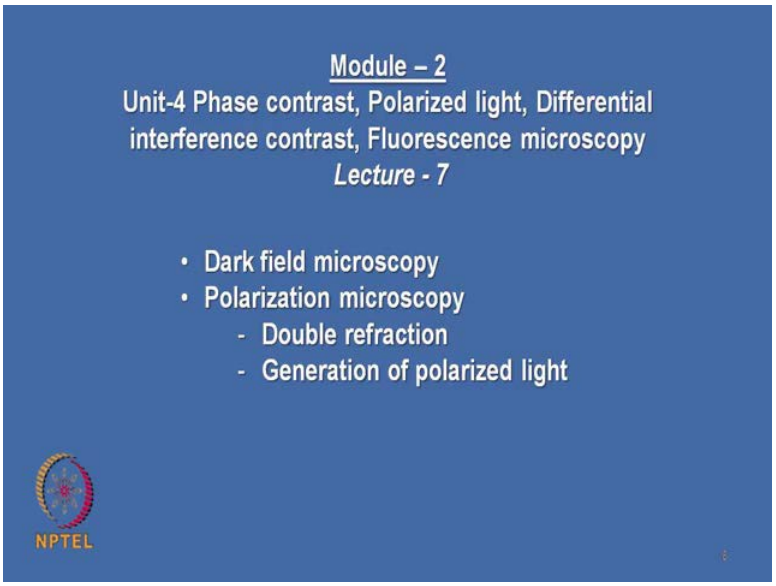

Welcome back. In the last class we looked at the phase contrast and opaque stop microscopy and then, we also have gone through the some of the life demonstration, to enhance the image contrast as compared to bright field illumination.

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 from the object and get reflected and enter into the objective and those regions will appear bright and that light rays which are diffracted will escape the objective lens will appear dark from the object region similar to green

boundaries and so on. In Dark-Field Microscopy, it is the exactly the reverse will happen. Briefly we will look at it and then we will move on to the next variant.

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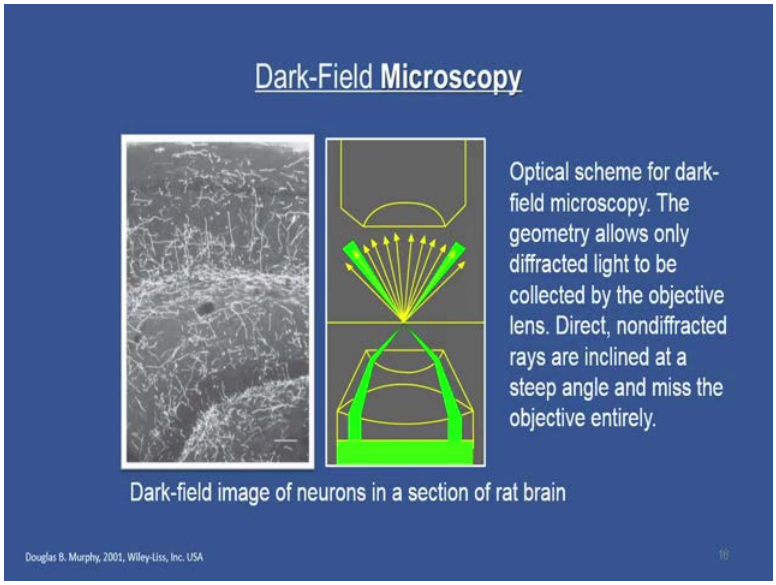

So, let us look at the Dark-Field Microscopy the slide shows. One typical example of the biological sample, it is the Dark-Field Microscopy you can see that all the features, very minute features are clearly illuminated and if you look at the optical set up or the optical scheme for Dark-Field Microscopy the geometry allows only the diffracted light to be collected by the objective lens. Direct and nondiffracted rays are inclined at a steep angle and miss the objective entirely.

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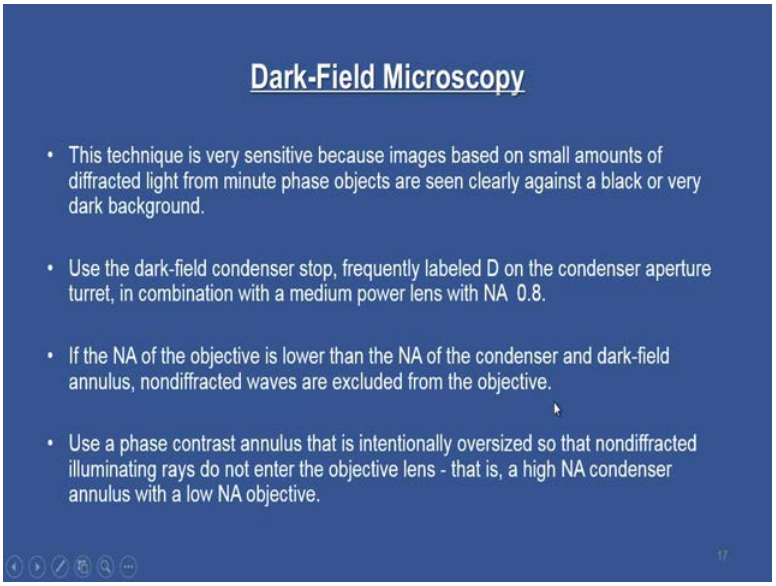

So, it is a very simple design. This technique is very sensitive because images based on small amounts of diffracted light from minute phase objects are seen clearly against a black or very dark background.

If the numerical aperture of the objective is lower than the numerical aperture of the condenser and the dark-field annulus, nondiffracted waves are excluded from the objective.

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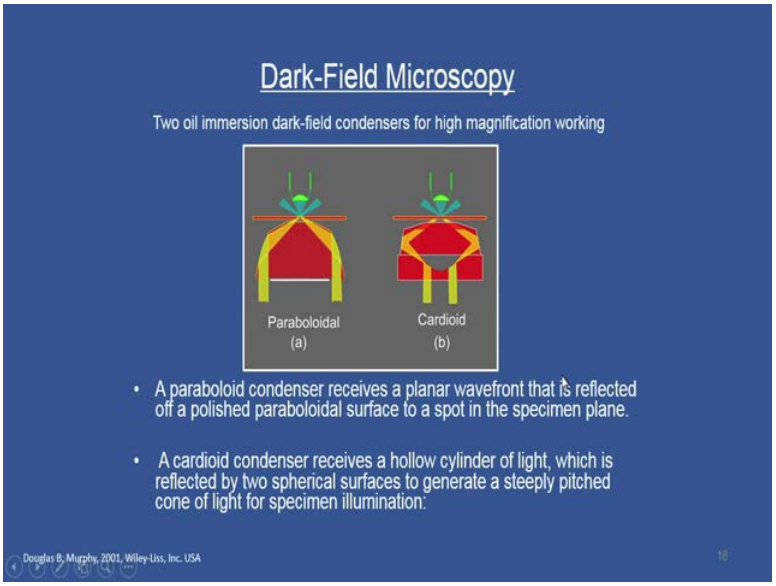

So, you can have two kinds of oil immersion dark-field condenser for this optical set up they are very simple in nature and you know that the principle of oil immersion objectives already we have seen that, so I will skip. And we will quickly move on to the next variant Polarization Microscopy.

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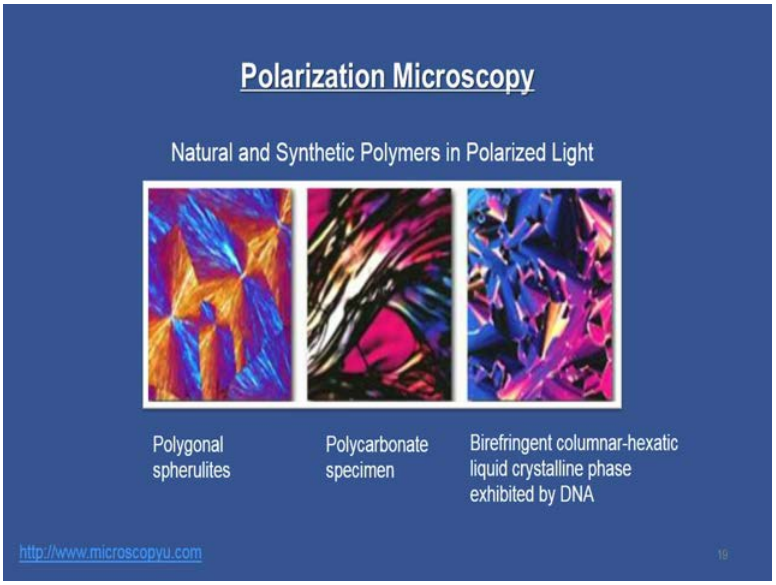

Look at this slide very interesting micro structures taken from again microscopy u dot com. What you are now seeing is some of the micro structures of a polymeric material, natural and synthetic polymers which is observed on the polarized light. What you are seeing here is Polygonal spherulites, each one is a single entity spherulite 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 compare to the bright field illumination.

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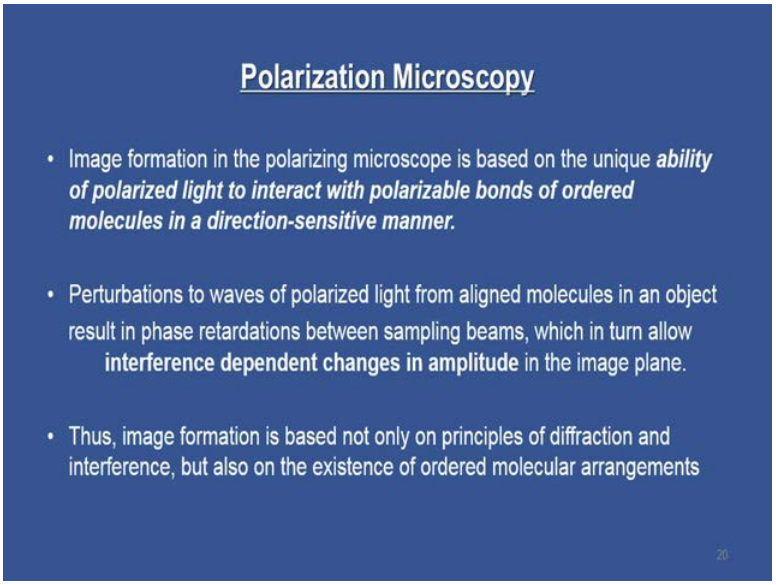

So, if you look at the principles of Polarization Microscopy it goes like this, the image formation in the polarizing microscope is based on the unique ability of polarized light to interact with polarizable bonds of the ordered molecules in a direction-sensitive manner. Perturbations to the waves of polarized light from aligned molecules in an object result in phase retardations 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 retardations between a 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 a 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 the Polarization Microscopy.

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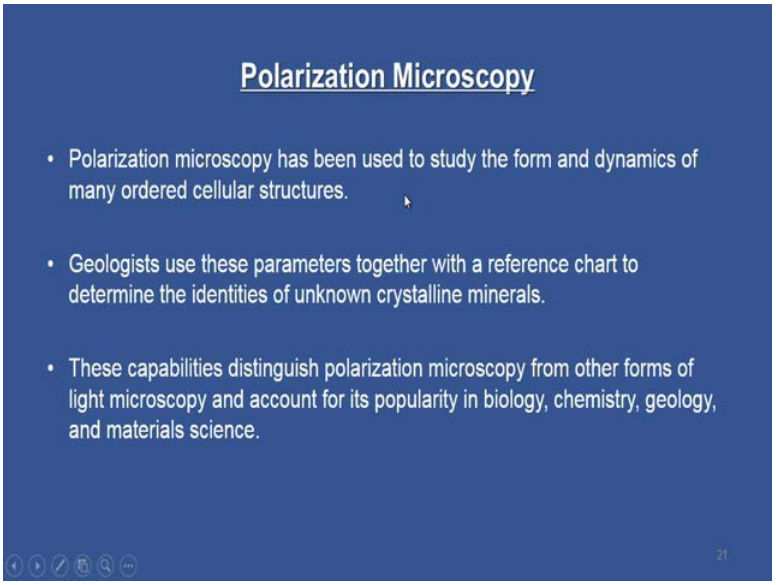

We will now look at, how this is other details in the Polarization Microscopy. So, Polarization Microscopy has been used to study the form and dynamics of many ordered cellular structures. This is only with respect to biological sample and geologists also use these parameters together with a reference chart to determine the identities of unknown crystalline minerals. These capabilities distinguish Polarization Microscopy from other form of light microscopy and account for its popularity in biology, chemistry, geology and materials science.

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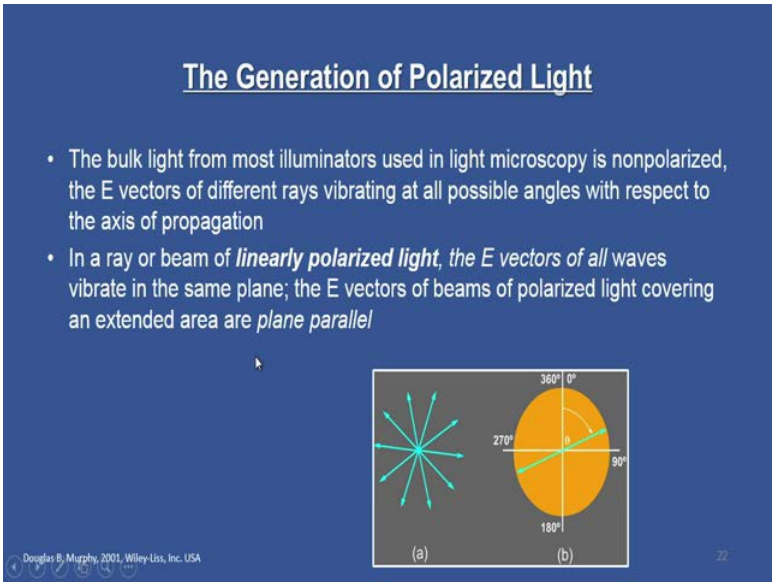

First we will introduce what is a polarized light and then, we all know that the bulk light from a most illuminators used in a light microscopy is nonpolarized, that means, the electric vectors of a different rays vibrating in all possible angels with respect to the axis of propagation.

In a ray or a beam of linearly polarized light the electric vectors of all waves vibrate in a same plane; so the E vectors of a beam of polarized 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 and we will see that in much more details.

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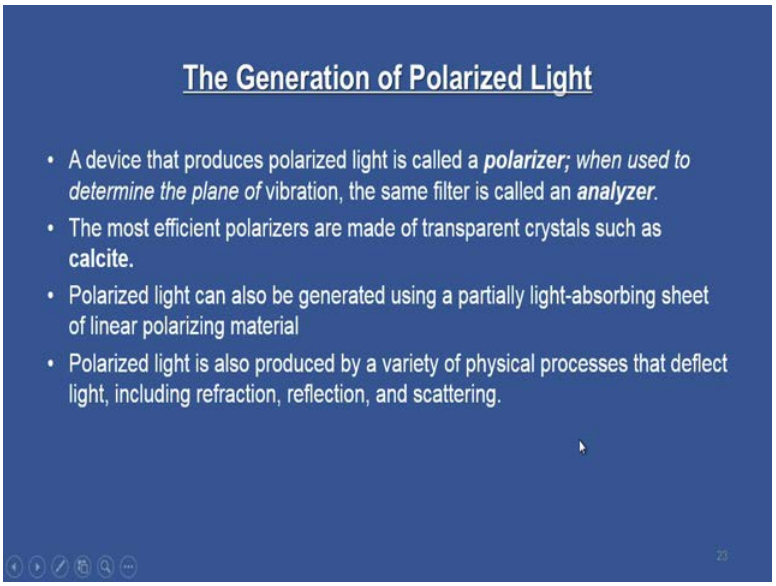

A device that produces a polarized light is called a polarizer; when used to determine the plane of vibration, the same filter is called an analyzer. Please understand, we have the same material which is been called polarizer as well as analyzer. When it is called polarizer, when it is used to determine 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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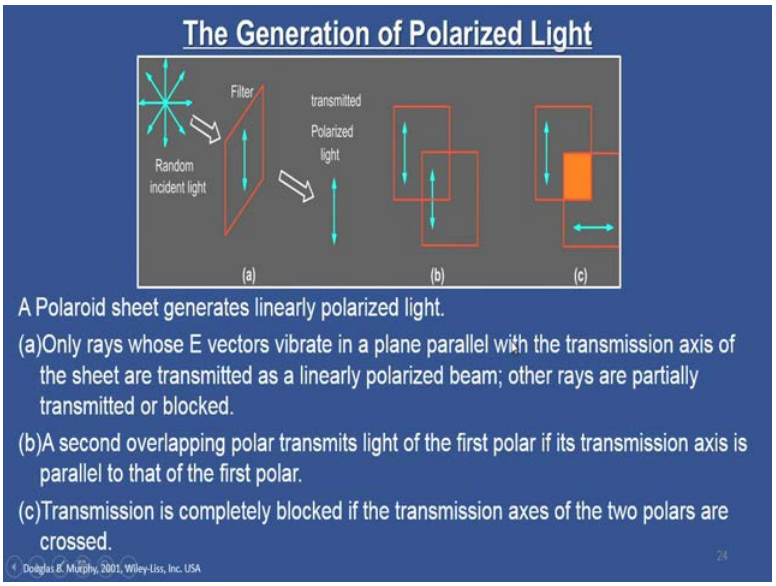

So, now let us see how this the filters are going to react with this polarized light or the filter how it allows this transmitted light, in the optical path. A Polaroid sheet generates linearly polarized light; that means this is the polarizer, Polaroid only rays whose E vectors vibrate in a plane parallel 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 rays are blocked. So, you can see that only this direction of vibration is allowed others is blocked.

The schematic-b shows a overlapping polar transmits light of the first polar if its transmission axis is parallel to that of the first polar. Since these directions are parallel, the light will pass through this over lapping polar filter also. In the case of c, the transmission is completely blocked if the transmission axes of the two polars are crossed. Since these two filters are kept in a crossed orientation, the transmission is completely blocked. So, this is this how the polarizer filter will behave.

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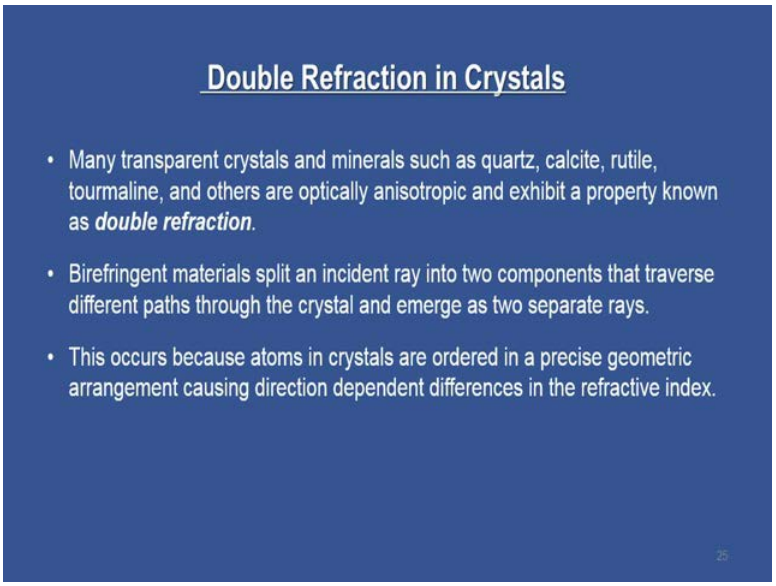

So, now we will look at what is Double Refraction in Crystals because we are going to look at the concept called birefringents and for that we need to know what double diffraction in crystals is. So, many transparent crystal and minerals such as quartz, calcite, rutile, tourmaline, and others are optically anisotropic and exhibit a property known as double refraction. Birefringent materials split an incident ray into two components that traverse different paths through the crystal and emerge as two separate rays.

This occurs because atoms in the crystals are ordered in a precise geometric arrangement causing direction dependent differences in the refractive index. See this is a fundamental reason, why the polarize microscopy is able to generate a special contrasts. So, if I look at this object which is made of birefringent materials that split an incident polarized light into two components; one is called ordinary ray, another is called extra ordinary ray. This is also been called as O ray or E ray. The ordinary ray means that will obey all the optical rules that is (Refer Time: 12:57) so on. The extra ordinary ray will not obey this optical rule. That is a difference between an ordinary ray and an extra ordinary 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 direction dependent differences in the refractive index. So, we will see how this is being implemented in the optical scheme, and before that let us clarify much more details about double refraction in crystals.

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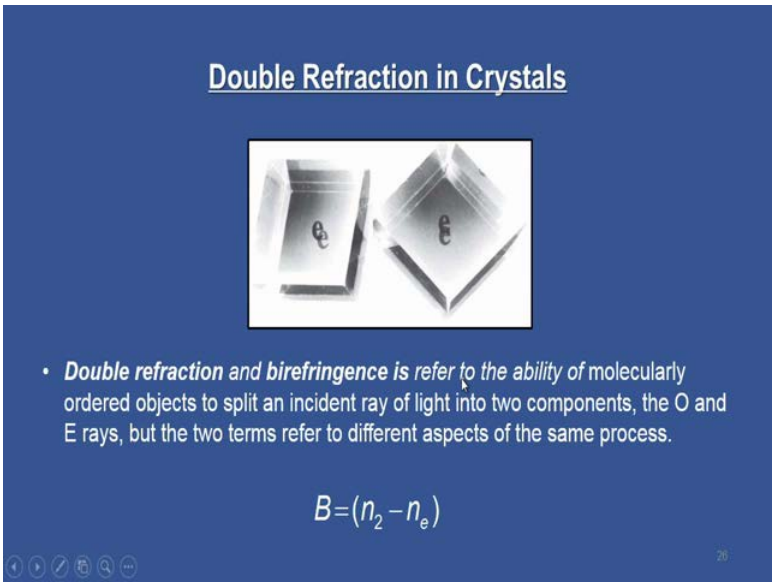

So, look at this, very interesting crystals. You see that letter appears as if you have one on front plane, one is lying on the back plane. Let us see what is the reason for this? Double refraction and the birefringence is refers to ability of a molecularly ordered objects to split and incident ray of light into two components, the O and E rays, that is ordinary and the extraordinary rays. But, the two terms refers to the different aspects of the same process. B that is birefringence is equal to n 2 minus n e; that means n o should be n e the difference between this 2 refractive index.

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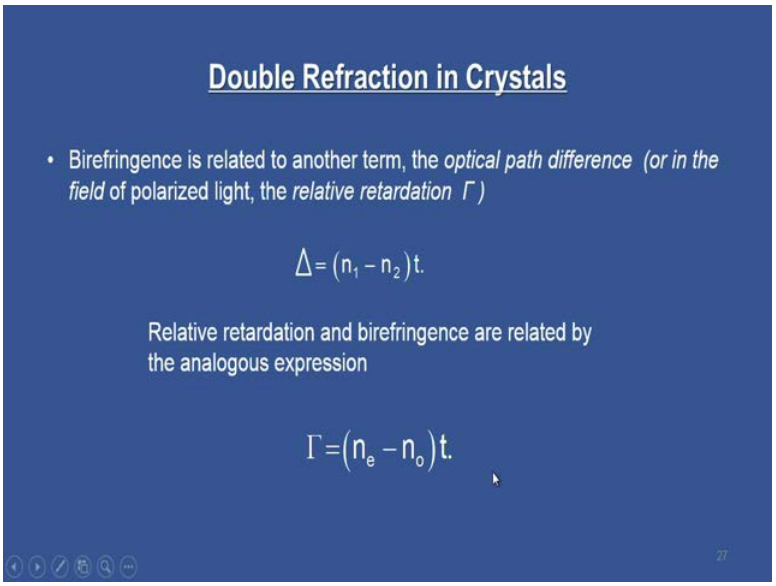

Birefringence is related to another term, the optical path difference or in the field of polarized light the relative retardation. So, optical path difference and the relative retardation on the similar term, but relative retardation is used in polarized light scheme otherwise, it is in generally in optical literature it is reference to optical path difference. Which we have already seen delta equals to n 1 minus n 2 times t; where t is the thickness of the glass or the median.

Relative retardation and birefringence are related by an analogous expression that is gamma, capital gamma is equal to n e 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 enhance aptitude of the resulted wave which come out of the object which produces a better contrast in the polarized light scheme.

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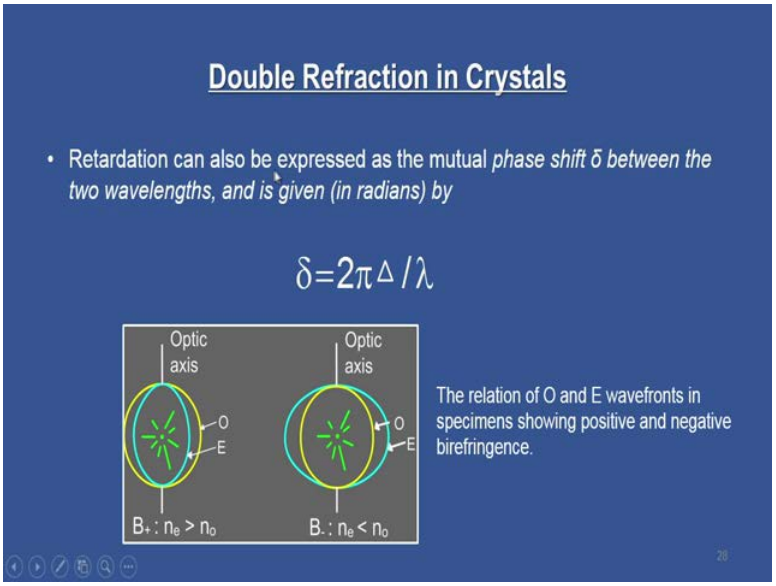

Retardation can also be expressed as the mutual phase shift delta between the two waves. And it is given in radians by small delta equal to 2 pi delta divided by lambda. Look at this schematic; you will appreciate this much more as we move along in this lecture. The schematic, the first schematic shows this is an optic axis and then you have ordinary ray and this is an extraordinary ray and 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 these two things are visualized in an optical scheme as 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 Birefringent S Specimens. So, what we are now assuming here is, the object which we are looking at are viewing or analyzing under the polarized light microscope, will exhibit the birefringence. Then, and then how that material or object react with the plain 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 Birefringent Specimens. The wave forms of elliptically and circularly polarized light, you have two forms; ordinary ray and extraordinary rays following the same propagation access, but vibrating in a mutually perpendicular planes cannot interfere, but can be combined by a vector addition. A sheet of cellophane held against a single polarizer on a light box is an example of this behavior. Cellophane is a birefringent sheet made up of parallel bundle of cellulose which will help to produce elliptically polarized light.

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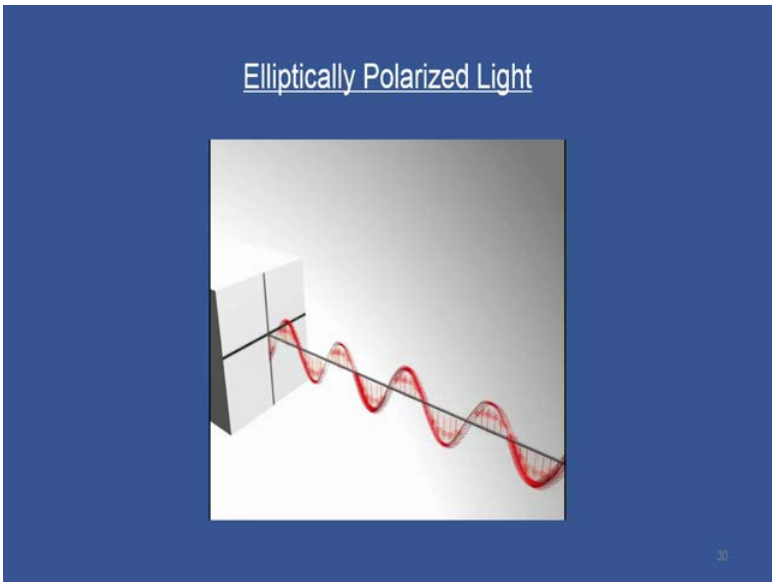

Let me now, run this schematic animation. You can see that; suppose, if you assume this is one wave plane polarized in one plane.

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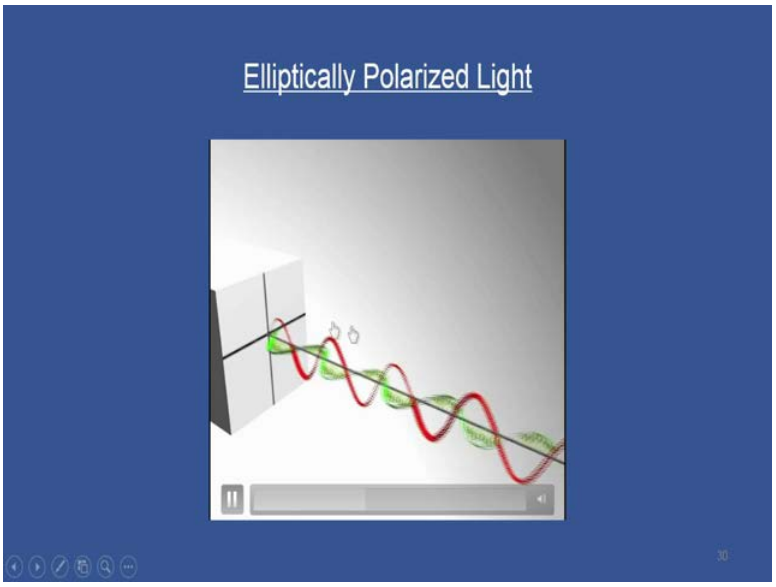

The other one is a plane wave which is perpendicularly polarized.

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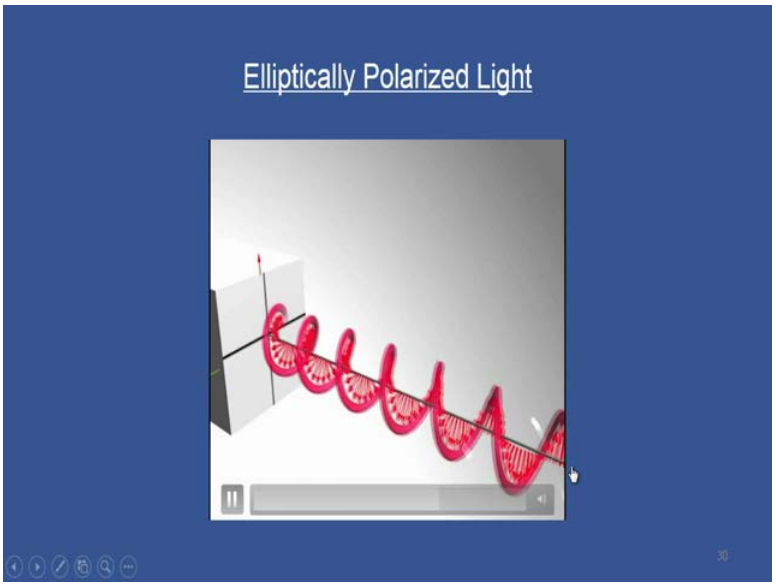

If the phase shift is dynamically happens between these two, then you can see that elliptically polarized light is possible.

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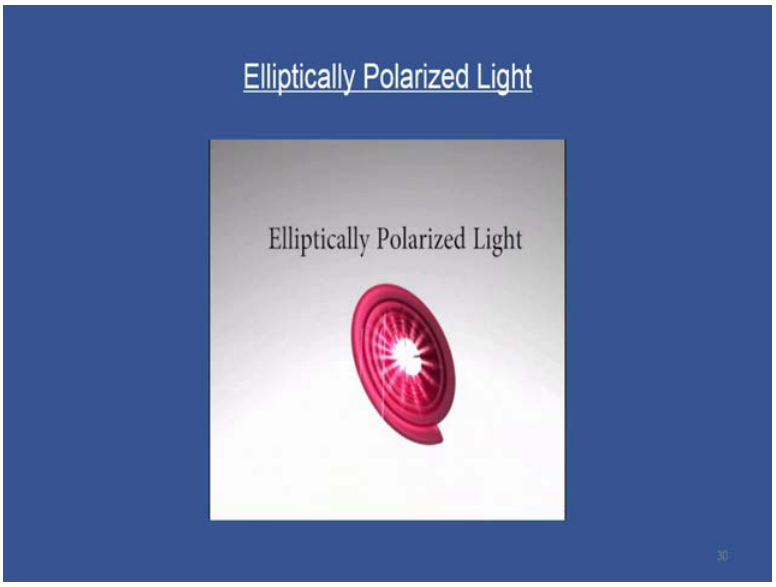

I will play this again, to appreciate how this elliptically polarized light is generated and you have this ordinary ray and extraordinary ray, they are vibrating in a mutually

perpendicular plane and if the mutual phase shift is dynamic then, you will be able to see this wave propagation in 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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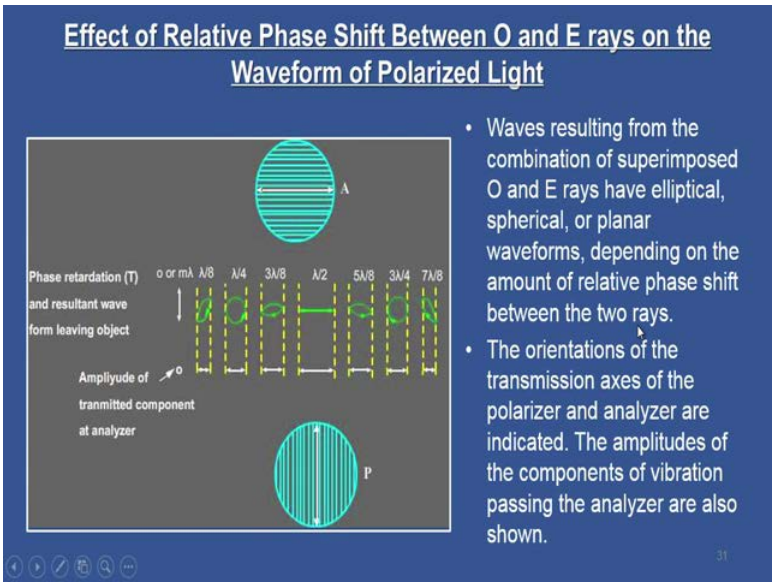

And now we will see that, the phase shift which I talk about in between the E and O rays. How it is understood? The effect of relative phase shift between ordinary and extra ordinary rays on the way form of a polarized light; look at this, we have a two polars - one is analyzer and this is a polarizer. They are kept into 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 super imposed ordinary and extraordinary rays have elliptical, spherical or polar waveforms, 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 phase relative phase shifts either you can have elliptically portrays light like we have seen in the animation, but you can also have a spherical and circular waveforms. So, that is what we been summarized in the slide.

So, you look at this. This is the phase retardation and resultant waveform leaving object and this is the amplitude of the transmitted component at analyzer. So, you see that the

amount of phase shift that is lambda by 8 produces this kind of an amplitude this amount of amplitude let me say. Lambda by 4 produces a different level of amplitude and 3 lambda by 8 produces again different, any you see that lambda by 2 produces the maximum amplitude at the analyzer. So, it is the orientation of the transmission access of the polarizer and analyzer are indicated. The amplitudes of the components of the vibration passing through analyzer are 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 the polarizer and an analyzer the two polars, depending upon the rotation and their orientation access, you are able to manipulate the amplitude of the transmitted light which is coming from the specimen to the analyzer. So, this is the key to appreciate the manipulation of the amplitude of the transmitted being from the birefringent specimen.

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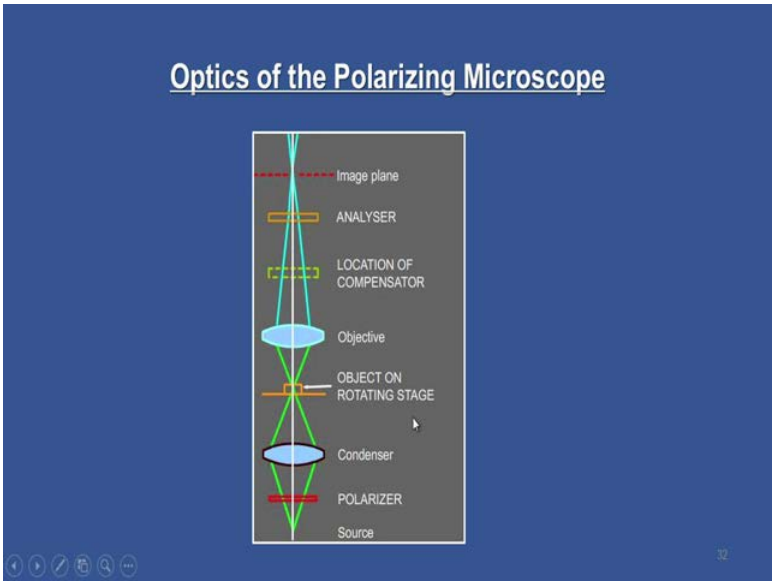

So, this is the optical scheme, for the polarizing microscope. You have the polarizer, the light source and you have polarizer. It could be whatever we have just mentioned in the slide could be any material, which can do this job polarizing activity and then you have a condenser and then you have the specimen on your rotating stage. Again objective and this is the another important aperture or device called a compensator or retarder.

Here we will see what is the function of a retarder. Retarder will give the specific 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 will get the material characteristics. So, you have the analyzer and then finally, the image plane. So, image plane you have the both race are interfering and then producing the contrast.

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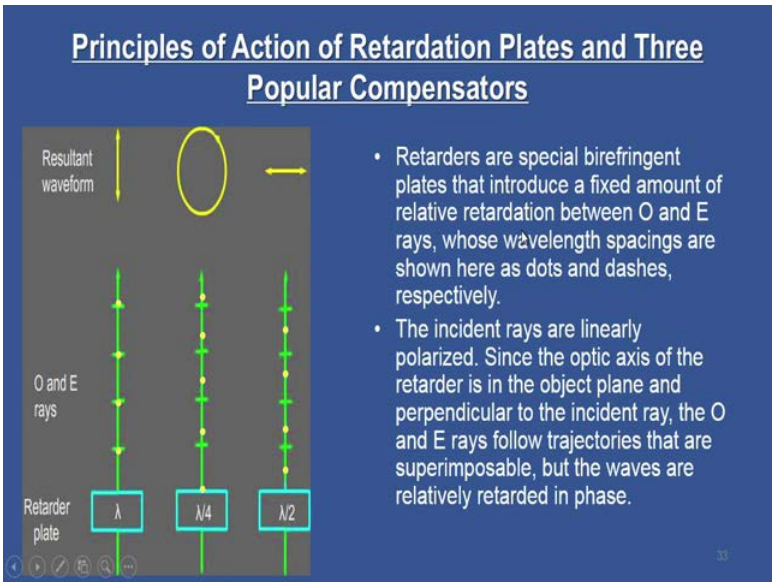

So, just I mentioned about the retardation plates. So, let us see what is the principles of the actions of retardation plates, or and the three popular compensators. So, the retardation plate or compensator, they are one and the same. So, look at this schematic. Let us look at the remarks first and then we will get into the details of schematic.

Retarders are special birefringent plates that introduce a fixed amount of relative retardation between ordinary and extraordinary rays, whose wavelength spacing are shown here as a dots and dash dashes respectively. So, here dots and dash this is a wavelength of extraordinary and ordinary ray.

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 ray

follow trajectories that are superimposable, but the waves are relatively retarded in phase. So, you can see that, both rays; ordinary and extraordinary rays they have a similar wave length, but if they are retarded by lambda by 4, then how you will see the wave form here. If it is lambda by 2, it is completely a different orientation compare to the incident wave forms. So, this is how the retardation plate will also operate.

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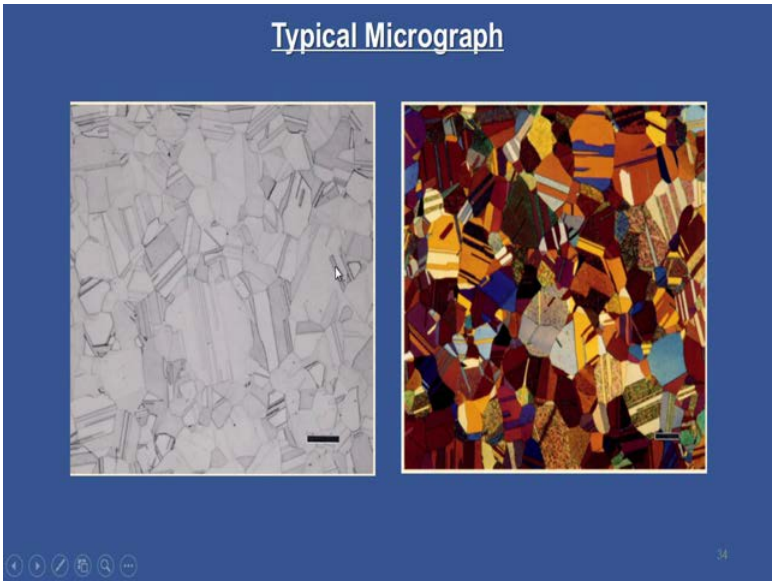

And you see that this is a typical micrograph of a polarized microscopy. The material under the examination is a brass and you see that bright field illumination gives a kind of contrast, a polarize slide gives the extraordinary a contrast.

Now, I will take you to the lab, 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 in Olympus microscope. Similar to what I have introduced in the previous classes. So, what now you are seeing is an inverted microscope. So, I do not have to explain the details which you know already the pots of this microscope and so on. What I will now concentrate is about the polarizer analyzers set up. So, if you have listened to the lecture clearly, we will now witness this polarizer and analyzer orientation here. So, one is a 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. You can see that there is a mark here which indicates the plane of vibration on 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 polarizer.

So, you can now clearly see in much more closer view of the polarizer and then this is an analyzer. 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 is kept in two perpendicular directions. Now we will see how this is going to change the contrast of the micro structure. 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 shift with the phase shift will be according to this orientation shift. Now, we will see one particular micro structure of is a titanium alloy. This is 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 micro structure you are seen.

Now, you are seeing it in a complete extinction condition; that means, you are phase shift is about lambda by 2 it is an extension condition. Now, you will slowly rotate this analyzer. So, your phase shift changes and then you see that the micro structure is getting much clearer with all the details you are able to see.

As I mentioned, this is a titanium alloy you can see that lot of twinnings 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 a 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 bright field modes and then, it is coming back. You can see this operation again this is an extension condition again, that means it goes to a complete orthogonal orientation. So

now, at a specific phase shift, you will be able to arrive at the best possible results.

So, I hope you appreciate this demonstration, how the polarizer and analyzer give a better contrast as compare to the bright field illumination. We will look at the other variant of the microscope like a differential interference contrast in the next class.

Thank you.