Techniques of Materials Characterization
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Lecture – 9
Differential Interference Contrast

Welcome everyone to this NPTEL online certification course on Techniques of Materials Characterization. So, we are now running on week 2 and we have so far studied various modes of optical microscopy. We saw bright-field mode, dark-field mode, reflected, transmitted microscopy and we have studied about some phase contrast microscopy and polarization microscopy. And today we will be studying about another mode of contrast enhancement that is called differential interference contrast, DIC.

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So, what we will be studying here is the first thing is the principle of interference contrast DIC, then how the image form and very important aspect of this interference contrast microscopy is depth perception, how that form and what are the requirements for DIC and finally we will compare this contrast enhancement technique with polarized light microscopy and phase contrast microscopy.

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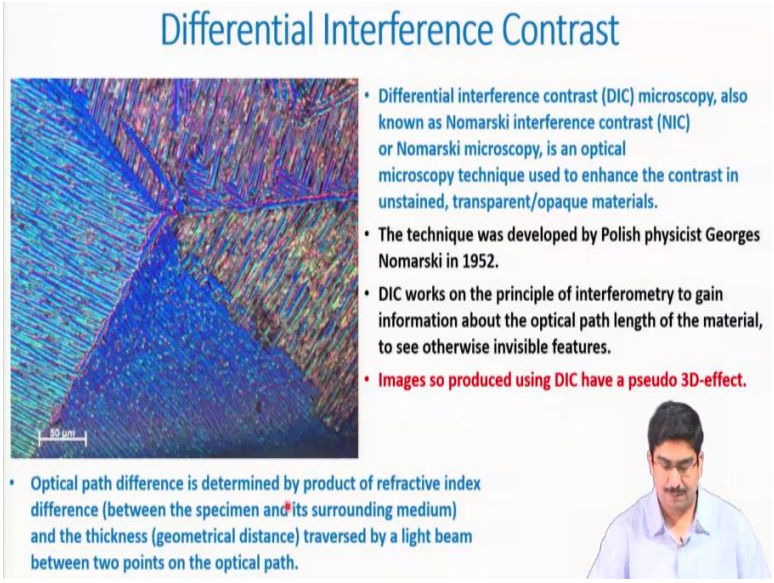

As I was telling in the last class, basically interference contrast is just like an advanced version of polarized light microscopy. Many of the components, hardware part is exactly the same. And many of the concepts which we discussed in the polarized light microscope like light polarization, like the birefringent materials, how the birefringent materials is basically causing the two rays, ordinary rays, extraordinary rays and all, everything remains the same here.

On top of that, you use something else and you use some more hardware and there are some more things in the image formation, which gives it the unique thing, depth perception which is not there in polarized light microscopy. We will come to that. So, this interference contrast microscopy is also known as Nomarski interference contrast or Nomarski microscopy. And here what we do is that we enhance the contrast generally for unstrained and transparent or opaque material.

So, this method can be used both for reflected microscopy as well as for transmission microscopy. It was discovered by a Polish physicist Georges Nomarski in 1952. And it works on the principle of interferometric. An interferometry basically it takes advantage of the optical path length. Just like in phase contrast, polarization light here also there is a phase shift, there is this optical path difference and all, but the way the contrast is generally slightly different, we will come to like that to this.

And here we produce this and unique 3D effect which is not there in any other microscopy. And this optical path difference we were talking about this optical path

difference is basically a product of refractive index difference between the specimen and the surrounding specimen. I mean if it is a single-phase material, then it is possibly the specimen and the surrounding medium.

If it is multi-phase material or if it has polycrystalline material of different grains that what we saw in case of a polarized light microscopy, so then the difference in optic refractive index and the thickness of the material, this we discussed in phase contrast microscopy as well. That these are the two effects which causes the phase shift and optical path difference basically between them.

The thickness, how long the light was traveling through that material or having certain refractive index and the difference so which we have two different regions with two different distant refractive index plus the thickness differs so that how light speed varies between them and when it light comes out, we will have a phase shift which is exactly having a retardation or optical path difference, which equals to the difference in refractive index multiplied by the thickness. So that is what is the optical path difference or phase shift whatever.

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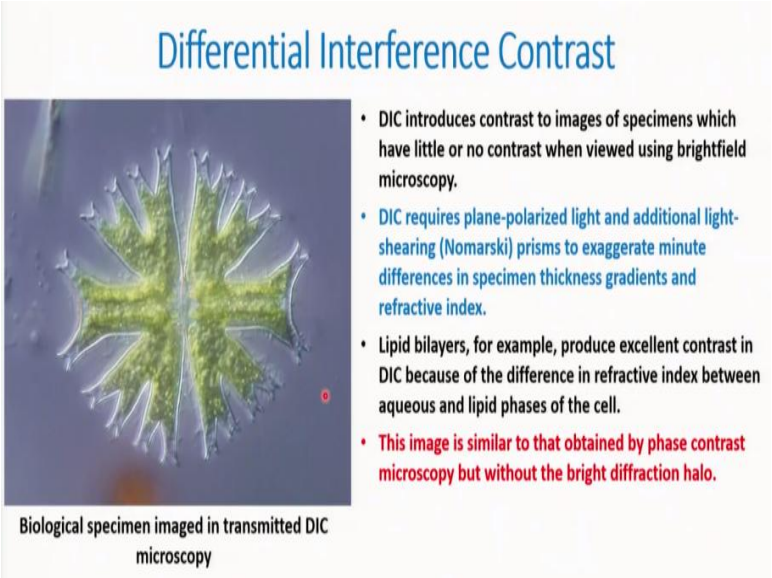

So, this interference contrast basically it says like works very good. For example, materials like birefringent material as I already said. So, birefringent materials it works very good and birefringent material does not really show very good contrast in case of bright-field microscopy, and using this interference contrast that can produce very nice contrast in this.

Of course, first thing it needs is a plain polarized light just like polarized light microscopy plus there are additional light sharing some additional components known as Nomarski prisms and this Nomarski prism what does it do is that it basically triggers or amplifies the minute difference in specimen thickness gradient and refractive index. So, whatever minute very little difference that you have in your optical path length that gets amplified by this Nomarski prism here.

For example, you can see this nice biological sample and this biological sample here the contrast generation between if you take this surrounding medium and this is typically because of the difference in refractive index and their thickness as well. Both of this creates this nice optical path length difference between rays traveling here and rays traveling here and finally this is what is used to generate the contrast.

So, in that way it is similar to phase contrast microscopy, but without that diffraction halo that is there, we will discuss about that also.

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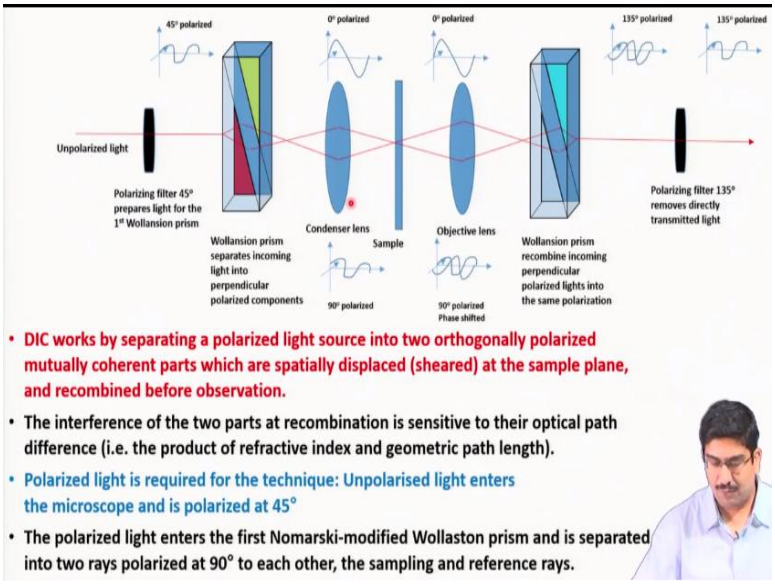

Now, let us talk about how this image or how is it forming this mechanism, the image formation mechanism here. So, first thing you have is unpolarized light to start with and then you have a polarizer. Just like polarized light microscopy, you have a polarizer here. Now, the point is this polarizer has a particular plane of polarization. It is generally kept in a way that you take or you finally from the polarizer you get a 45^{o} polarized light.

And that polarized light, this 45° polarized light from this polarizer then forms on another additional attachment that is called Nomarski prism or Wollaston prism. Now, what does this Wollaston prism do? Here, the Wollaston prism basically splits this ray, the ray 45° polarized light when it comes here, and this is a birefringent material, by now you are aware of birefringent material and extraordinary and ordinary rays.

So, what does it do is that when this 45° polarized light falls here, this divides this Nomarski prism which is basically a birefringent material, this Nomarski prism divides it into two different rays and which are exactly orthogonal to each other, just like that ordinary and extraordinary rays, here the same two rays are produced and those two rays are now having polarization if one is 0° polarized and other one is 90° polarized.

So, two rays are produced from one single ray, you started with a unpolarized light and from there you then go to a 45° polarized light and after it passes through the Nomarski prism now you get two rays with exactly orthogonal to each other, one is 0° polarized, one is 90° polarized. And so these two rays usually are called one is a sampling ray and another one is a reference ray that is what it is called, but this you can call them 0° and 90° polarized ray does not matter.

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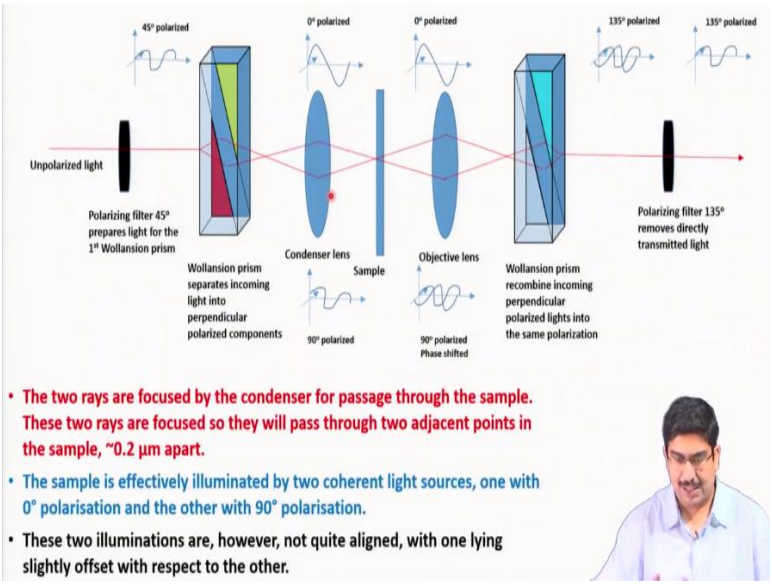

Now, these two rays again fall on the condenser lens and just like a normal light ray condenser lens basically focuses them on this specimen, but there is a slight difference between this. There is a slight trick here. So, from the condenser lens they get focused

slightly apart. So, these two rays are the focus where they fall on the specimen, these two points these are slightly apart from each other.

And that the distance between these two points is around 0.2µm. What is the significance of 0.2, I will come to that anyway. So, these two rays now from the condenser lens, these two rays which are produced one is 90 degrees and one is 0^{o} polarized, they are falling and they are focused on the specimen and they are focused slightly at a slight distance from each other and the distances is 0.2 micro that is what.

That means now what you can imagine is that the specimen is getting illuminated by two coherent light sources. They are obviously coherent light sources, they do not have any phase difference yet. So, they have they're perfectly coherent light. So, two coherent light sources are there and they have one with 0 polarization and one is 90^{o} polarization and with a slight offset between them.

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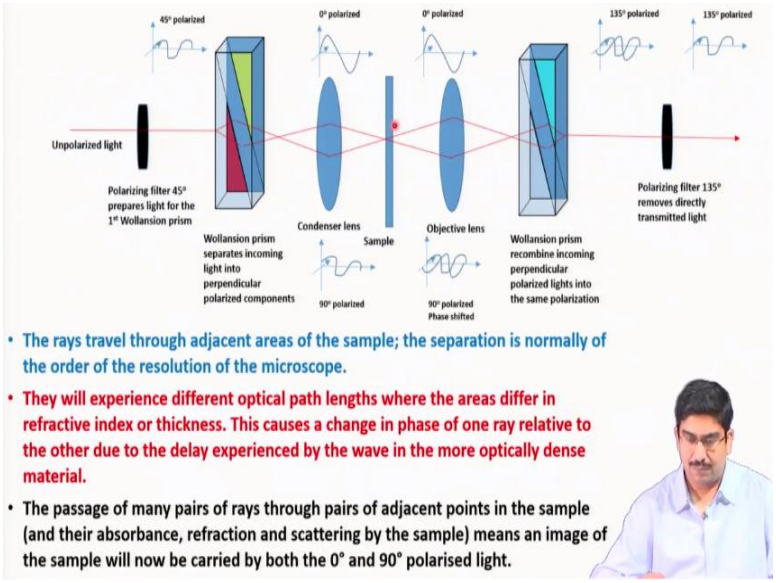

Now, what happened is why this 0.2 µm? If you remember as I said, this depends on the resolution of the microscope. Normally our human eye resolution as I said human eye resolution is 0.2 mm and normal optical microscopes has a resolution of around 200 nm. If you are using normal visible light it is around 200 nm is what the resolution of any optical microscope.

So, this is what that resolution, because of that resolution limit we are creating a gap not more than or not less than rather, not less than 0.2. If you create a gap between them less

than 0.2, less than 200 nm, then we will not be able to use them in future through this objective lens because the condenser lens and objective lens because of the numerical aperture, the resolution, the diffraction related resolution itself is around that limit.

So, we will not be able to distinguish them later when we fuse those two rays to make the final image that is why the limit 0.2 is so important. So, now these two rays again they fall on the specimen and they basically get scattered, diffracted whatever that happens. So, that is exactly the same rays like what happens for a bright field-image, so same thing here also.

These two rays create their own bright field images, again they are getting absorbed, they are getting scattered whatever but these two are the two direct rays just works like two direct rays and they will be just carrying forward towards this objective lenses. But now what happens is that since they are slightly different and they have a different polarization as well.

So their optical path length will also be different because there is number one they can encounter two different phases altogether that is a separate, but even if it is a single phase material because of the birefringent, so this material itself also has to be birefringent that is another point. So DIC cannot be used for isotropic material, it has to be an anisotropic material okay.

So, this birefringent material now it is here and what happens is that these two rays are falling over here and again they will have when they are going out, so they will be like they will have absorption, they will work like a direct beam, they will have absorption, some amount of light will be scattered, some amount of diffracted and so on. But within themselves they will have a phase shift because now they are sort of traveling in orthogonal to each other.

So, they are facing different refractive index during their path. So, they will have a optical path length difference within this. Now, what happens is that after this objective lens they passes through a second Nomarski prism. So, here there is another just like analyzer polarizer I have two different prisms, in DIC mode analyzer polarizer is still

there and I will have two different prisms, two different Wollaston prisms and Nomarski prism in the here.

One will be in condenser lens side, one will be in the objective lens side, I need to have them that is what makes the difference, how I will come to that. So what you can imagine before these two falls here, these light rays which started all the way from this Nomarski prims, Wollaston prism here, that light ray is now carrying two bright-field images with them okay and they are still 0^{o} and 90^{o} polarized with respect to each other.

Only their intensity, their optical path length everything that they have a phase shift, there is like you can imagine that some part of the light gets scattered because they are falling at two different places, so some difference is there, optical pathway difference is there. But basically, these two are carrying the image, you can imagine two-bright field images that is what it is carrying by these two images. okay.

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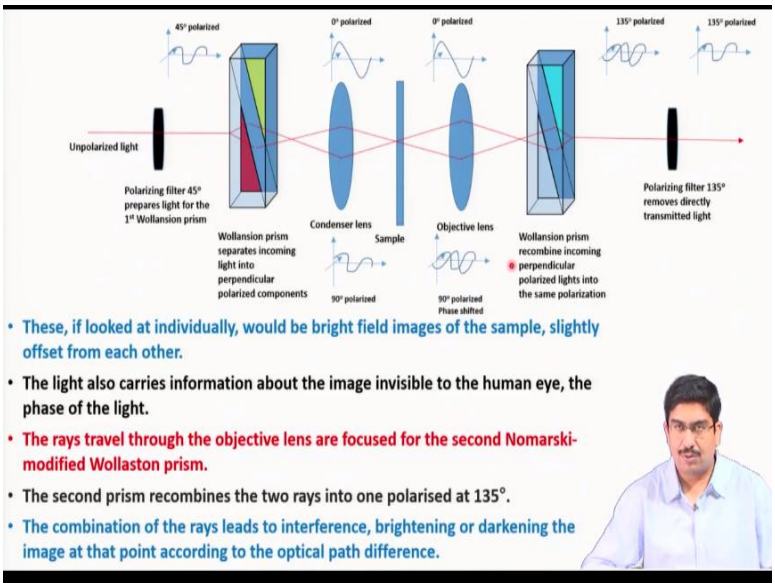

And what happens is that so these two are having a phase difference, of course which you cannot detect by the eye, plus they are also carrying some amount of amplitude difference that is because of that bright field what I said the diffraction effect and all they are losing some intensity and so on. So, they are basically carrying amplitude difference as well as phase difference between them.

When they fall on the Nomarski prism afterwards they recombine. Those two gets recombined because now these two are getting through another birefringent material. So

here, they split up, this one ray they get split up, those two rays are getting interference happens between them and those two here they get recombined. When they recombine, of course these two will undergoes and when they recombine, they will now become another ray which is polarized at 135^{o}.

So 135^{o} polarization is there after this analyzer. So, there will be an analyzer here and that analyzer will create 135^{o} polarized light. So just the same principle as polarized light here. If you take out these two Nomarski prisms basically and if you consider not two rays but one single ray, it is exactly the same. You have this cross polarization between them, here it is 45, here it is 135.

So, you have the cross polarized, polarizer and analyzer is still kept in a cross polarized condition or configuration and same thing will happen. But these Nomarski prisms what they do is that this one split that polarized light into two components, this one recombines those two into one single polarized light that is what it happens. And that is the extra thing that this DIC technique will have.

So, this interference between these two bright-field images now will have, obviously there will be an interference between those two and this will lead to some brightening or darkening of the image according to their path length difference. So these two, you can imagine that these two bright-field images have slightly offset with them with a phase shift as well and after interference this will create some amount of contrast between them.

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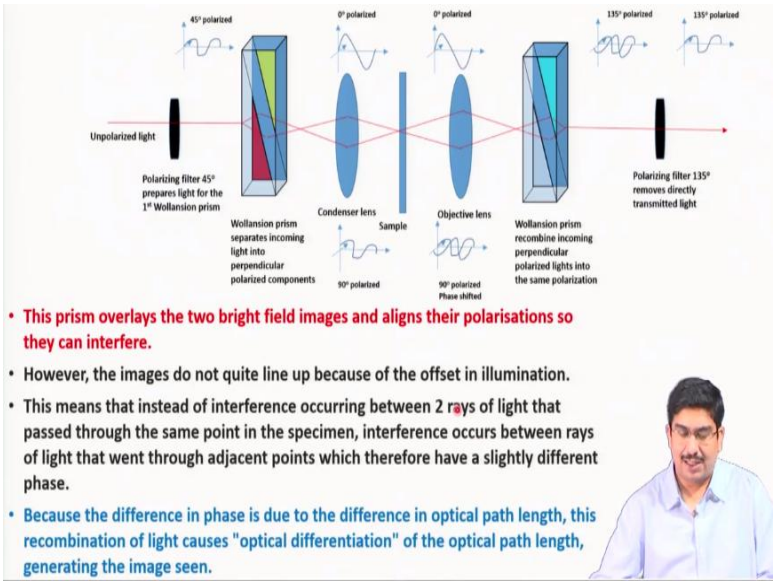

So, that is what it happens as I already discussed this Wollaston prism basically recombines these two bright-field images. And finally, it will pass through this analyzer and on this site I will get one single image because of this interference and this interference will create some amount of optical differentiation in their optical path length and that will generate the depth perception. So, that is what we will discuss now. How this depth perception comes and how these images are formed ultimately in DIC mode.

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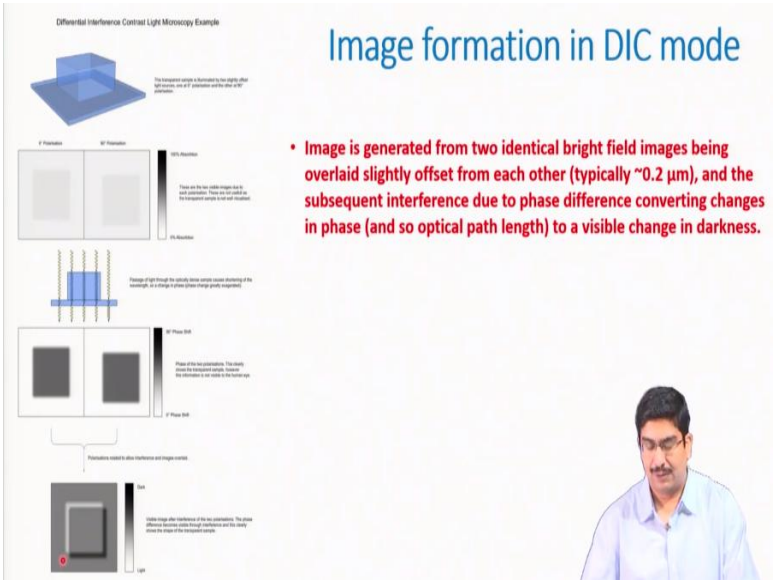

So, I start with this specimen which has this kind of a depth, just imagine that we have this kind of a feature that we are seeing. So, this definitely has a completely different depth from the other side okay. So, basically the same mechanism and light is passing through the same thing. So, let us imagine that this specimen over this there is another region here, which has a completely different depth.

So, from each and every point the light will be going through. So, this plane polarized two lights will be falling on this specimen for each-each pair. So, there will be a pair of images or pair of lights which will be carried through and finally there will be two different images produced which will have two images with different contrast altogether because of their optical path length.

Plus these two will have a slight offset because they are like physically also these two images have this 0.2µm offset between them plus they will have some amount of phase shift that means some amount of amplitude difference as well. Ultimately when I recombines them through another Wollaston prism and analyze the system and I make them superimpose, you can just imagine that these two bright-field images are now superimposed.

So, certain regions, of course there will be going interference, certain regions there will be constructive interference, certain regions there will be destructive interference and all other regions there will be a modification in the final amplitude that is it.

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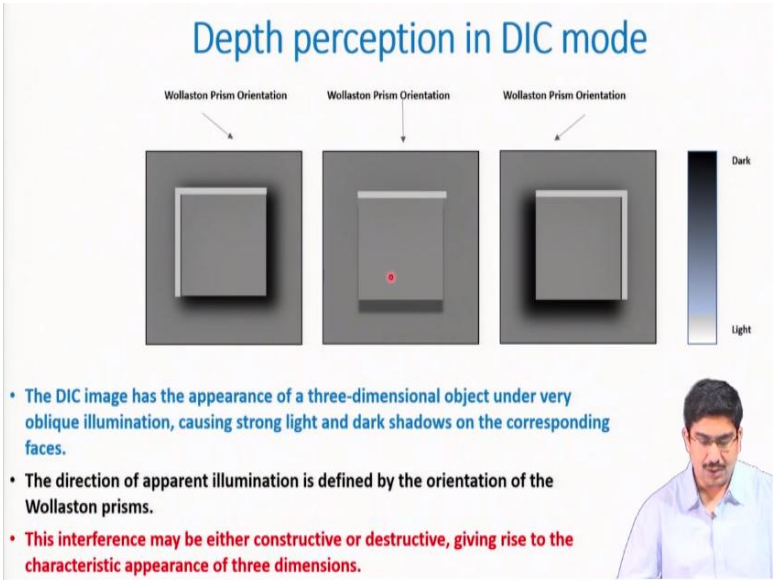

And this what it will create is something called shadow effect. So, if you look at here that shadow effect is basically responsible or if you can look at here, the shadow effect is responsible for this depth perception. Because now what you can imagine that you are seeing it from top, this you are seeing it from the top and on a planet. So, this is a 3D section, 3D component and you are just projecting that on a 2D section.

And on the projection on the process of light is falling over here and it is creating a shadow and that shadow will create some regions. Because of this height difference some regions will be shadow, some regions will be dark, some regions will be bright and that will create this shadow effect and that will be responsible for basically this ultimate depth perception.

And that depth perception or three-dimensional object perception that you can change basically by changing this Nomarski prism, the second one. The second Nomarski prism you can change that the way this interference happens, the way the superimposition happens. So if you have superimposition of this sort, you will see the shadow in one way and this if you have this Wollaston prism or the superimposition of this two brightfield images.

If you have in other way around, then you will have another type of shadow formation, but whatever it is finally you will be getting something like depth perception in the DIC mode.

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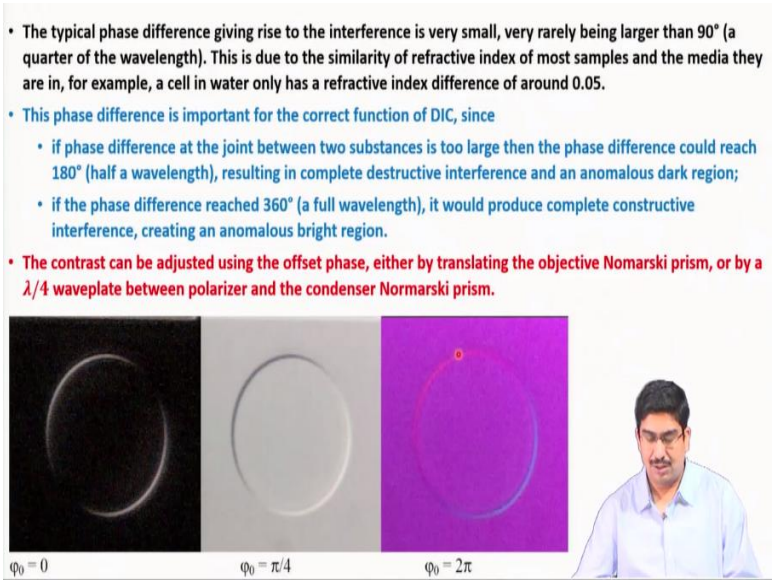

Now that depth perception also you can tweak it, you can modify it how it will be done and that modification that phase difference basically is very important. The phase difference that gives rise to the interference that is very important because what happens is that typically the phase difference is very small, very small, typically it is less than 90^{o} and this is basically because the sample and the media.

If you are using some biological sample, already we discussed in a phase contrast microscopy that typically the refractive index difference or optical path difference is very small in them. The they do not have much of a refractive index difference that is why the DIC mode works very good in reflection mode. The reflection mode because if you have a polycrystalline material, we will see some examples.

If you have polycrystalline material and depending on the orientation the refractive index can vary a lot and that can cause you and the thickness also if you can create some thickness difference you can etch the material, create thickness difference, then the optical path length you can really increase this difference and finally you can get very good contrast. But you have to be really careful about this contrast or phase difference.

Because if your phase difference is almost like 180^{o}, if it reaches 180^{o} the phase difference when you sort of superimposing this to bright-field images if the phase difference in certain regions is around 180^{o} then that will be completely destructive interference will happen. You will get a complete dark region like this. So, if you have this kind of a 180^{o} difference between the phases, these two regions, which your two bright-field images that you can imagine.

Then you will have the entire region will look like completely dark. Completely different scenario will happen if you have something like 360^{o} of phase difference, then you will have complete constructive interference and you will have everything all regions will have very high intensity. So, that you can change it or you can suitably change this using the Nomarski prism.

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So, now the requirement for DIC. As I said the DIC it is just an advanced technique to the polarized light microscopy. So, the first thing you need to have is DIC prism, polarizer, everything you have to have and additional component you have this DIC, this DIC prism or Nomarski prism or Wollaston prism. So, here you have this birefringent material and generally you have a knob here, which is creating a tension in this prism and changes this birefringent, this angle of polarization and so on that is what it does.

And that has a big impact on the final image that you get. So, in your material in the microscope you need to have those slots and those slots like you have to have polarizer. You have to introduce the polarizer and then you have to introduce you have to have the provision for introducing this DIC prism both in this condenser lens side as well as the objective lens side.

And typically, this Wollaston prisms they are made of crystalline substrate like quartz which is again quartz silica, which is again a birefringent material and you typically used to have two layers of this crystalline material and due to their variation in refractive index depending on the polarization of light, they split the light according to this polarization. And you can change the tension and you can create which angle they will have or how the polarization will happen there.

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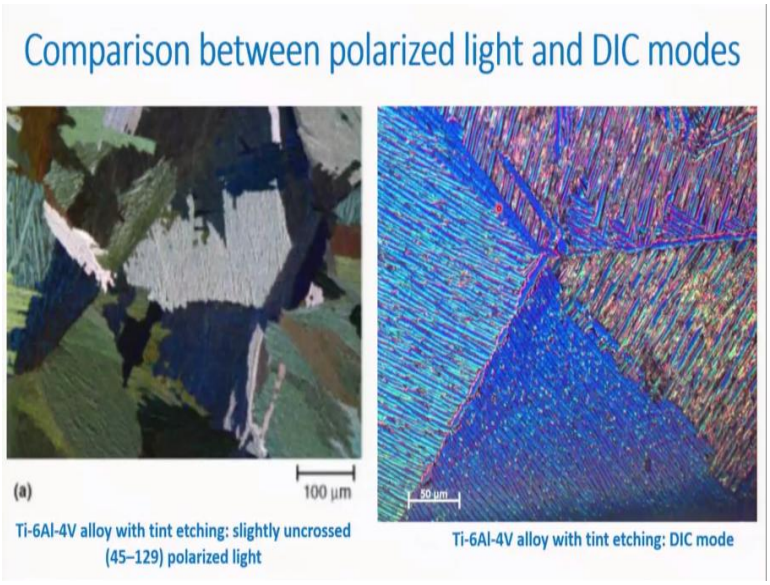

Now, let us compare these images between the reflected light and the DIC mode. So, this is what is the polarized light microscope and this is what the DIC interference contrast microscope. The same material Ti64 (Ti6Al4V) alloy and this is something that I have shown you before and something that I captured during my research. So if you use polarized light microscopy, this is what you will be getting.

You will get a color depending on the orientation of different grains, you can imagine that these are different grains. So, you will just get a color based on the orientation of the grains. In the DIC mode what additionally you will be getting is this kind of a depth perception and this is this. I can go on and talk about this for long, but basically this depth is coming because of the thickness difference.

So you have basically etch this material I have etched this material in a particular way so that this depth is created. Really in the specimen in real sense there is a depth, there is an uneven surface that is created and that adds to this refractive index difference and the optical path length difference. So, number 1 you have this different orientation between grains that is giving you this refractive index difference.

Plus you are creating this thickness difference, finally what you are creating between them is really and this is a multiphase material. So, these lines which you are seeing here this is another phase and that is why finally what you are getting is this depth perception here which is missing in this case. You are getting it in some sense, some case, but you

are not getting it this nicely or this good. So, this depth perception is very important and this is what you get for your DIC technique.

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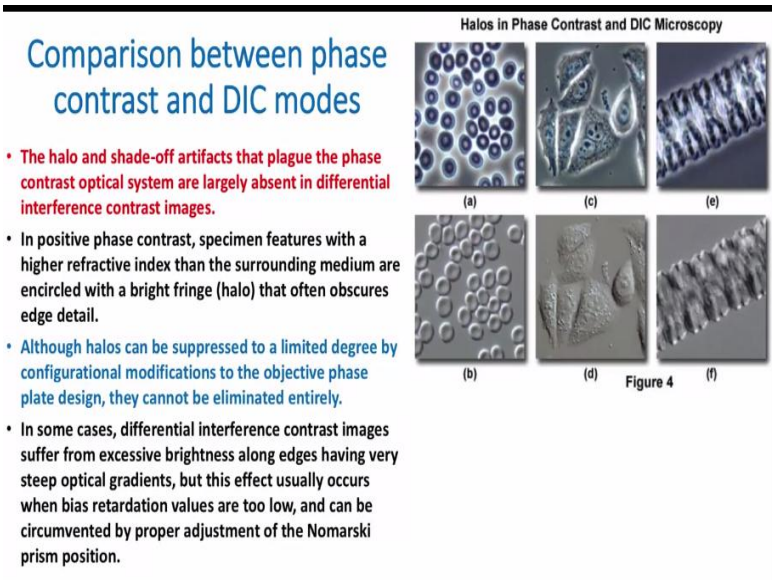

And in some sense as I said DIC technique also uses the phase contrast microscopy the same principle it uses. The difference in their phases, the optical path length difference between rays and then finally they recombine, they interfere and then reduce the contrast. So, in some sense DIC technique also uses same methodology or same kind of principle as phase contrast microscopy.

But the difference is that in DIC we do not get this halo and shadow this shade-off artifacts which are there for phase contrast microscopy, DIC does not have that. It will not produce because as I explained in phase contrast microscopy that this is produced due to the presence of that you have the phase top in the objective lens side and that phase top not only allows the direct beam.

But it also allows the diffracted beam and the diffracted beam finally goes and creates this kind of halo, shade-off and halo effects and positive contrast mode, and that also we discussed that in positive contrast mode in phase contrast microscopy. So, specimen which features higher refractive index and the surrounding media that are encircled with this bright halo here.

But that if you look at the same images of same material and same regions under DIC mode, you will not be seeing, you are seeing the depth perception here, you are seeing

the depth perception in all of these images but you will never be seeing this kind of a halo here. Of course, what you can see is something that we discussed this kind of an effect, this brightening effect maybe there.

If their phase difference is something where you have constructive interference, then definitely you tend to have the similar kind of effect, but if you look at here that same these brighter regions are not continuous, it is not surrounding the entire material. So it is bright in certain regions, but complementarily it is dark in certain other regions. So that is because you are having this shadow effect.

You are having these two bright-field images superimposed on each other which has taken a slightly different locations, two locations which are slightly apart from each other that is why after interference you are getting some regions which is very bright, but there will be some other regions which will be dark. And you can modify this, you can make them of equal intensity by modifying this Nomarski prisms by modifying their strain and so on.

So with this, we are over with this differential interference contrast and in the next class we will be discussing about the fluorescence microscope which is another mode of, exactly not contrast enhancement, it is a completely different mode altogether. It also gives you some amount of contrast enhancement but through some other mechanisms. And we will be discussing this in the next class. Thank you.