Electronic Systems for Cancer Diagnosis

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Lecture – 23
Understanding the methods and mechanism to study cell morphology

Hi, welcome to this series, here we will be understanding two different cases of electronic system that can be used for screening the oral cancer patients. So, there are two ways of screening; one is imaging, where you put the probe in the mouth, take the photo of the suspected region and send it to the clinician and get the you know the advice, whether the person should go for histology, where you have to remove the tissue right.

Second one is psychology and histology. Psychology is where you have to take the cells from the oral cavity and those cells are tested by the oncopathologist and if the cells are atypical, they the reserve comes as atypical or the person is ok. If there are the cells are atypical then the person has to go for histology, where the tissue is taken out and further the gold standards are using the gold standard the patient is given an diagnosis, whether a patient is suffering from a cancer or you can say malignant issue or a premalignant issue or it is a benign tissue right or it other patient is ok.

So, a patient is we do not have to worry, because that itself in the screening stage we can know. So, that the point is let us see how the change in the cells would help us to understand whether a person is susceptible or has a chance of having a particular disease, this these we are talking about is a cancer, so is chance of having a cancer alright. So, it is very sensitive and very crucial to understand the change in the cell morphology. Now, there are already worked around this area.

So, we will be talking about how these cells will look like and what is the cell morphology and how there are, what are the present way of understanding the morphology of the cells. And then what can we design as an engineer to a system that can help us to screen the patient in a effective, cost effective in a rapid manner alright.

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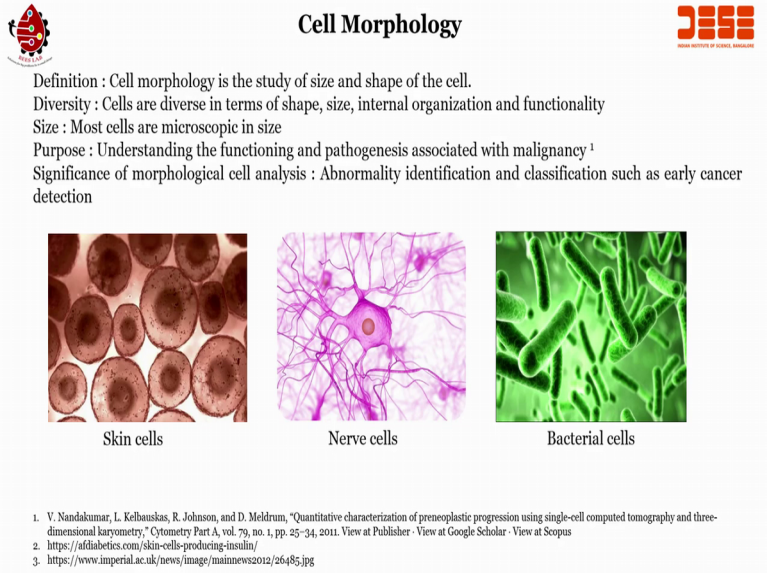

So, the topic today is to understand the cell and tissue morphology and in that let us first understand the cell morphology. Now, if I talk about cell morphology, the definition of the cell morphology would be to study, all size is the study of the size and shape of the cell right diversities. So, what are the cell diversities? Cells are diverse in terms of shape, size, internal organization and functionality. When I talk about size so most of the cells are microscopic in size right; and what is the purpose? Purpose is to understand the function and functioning and the pathogenesis associated with the malignancy.

Malignancy is the possibility of cancer alright; significant of morphological cell analysis here, the abnormality identification and classification such as early cancer detection. You see this is the extremely important part, where we do focus significance of morphological cell analysis. Here, why we have to understand cell morphology; because cell morphology if there is a abnormality in the identification that is; there is a change in the shape, change in the size, change in internal organization right. There are double nucleation lot of other stages are there, where we can understand that there is something wrong with the cells and that is why we can help for the early cancer detection. It can help in early cancer detection.

Now, you can see three different photos; one as skin cells, you can see, then there are nerve cells and finally, there are bacterial cells right. Now, bacteria is an enemy to human cells. In general but of course, there has small quantity of bacteria present in our body

itself which are good bacterias ok. We are talking about the bad bacterias. So, certain bacteria are good, certain bacteria are bad, if it is within the limited quantity within the body it is useful, above it is harmful one example is E. coli, one example is E. coli.

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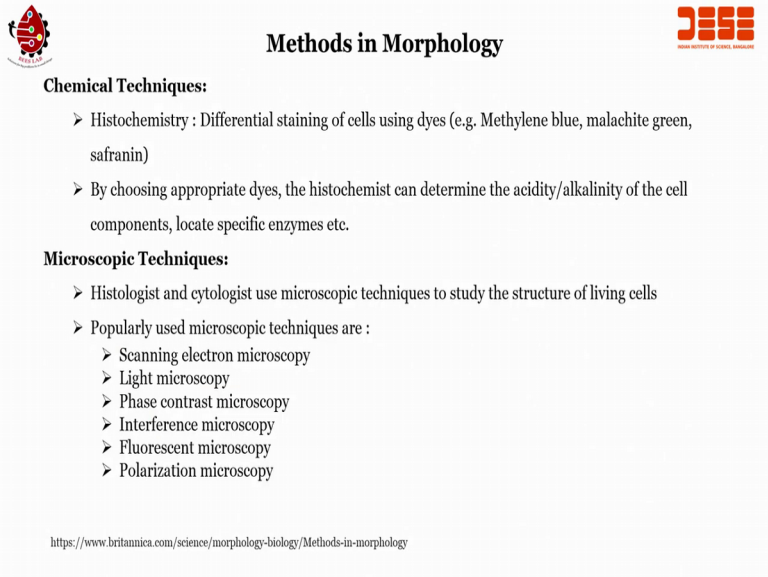

So, what are the methods in morphology? If you see we can divide into two different techniques; one is the chemical technique and second is microscopic techniques. So, if you talk about chemical techniques then there is histochemistry right and in histochemistry different cell staining of cells using dyes, methylene blue, right malachite green or safranin these are the dyes that are used in histochemistry to understand the change in the in the cell morphology.

Then by choosing appropriate dyes the histochemist can determine the acidity alkalinity of cells, also locate these specific enzymes alright. While, we talk about microscopic techniques then microscopy techniques, the histologist and cytologist use microscopic techniques to study the structure of living cells right. And what are the structure of living cells? We will talk in detail in the following slides, but what are the techniques or what are the microscopic techniques used. Then we can see that there are six different microscopy technique used right.

One is the SEM, which is Scanning Electron Microscopy, then second one is light microscopy, third one is phase contrast microscopy, fourth one is interference, then we have fluorescent and finally, we have polarization microscopy alright. So, there are six

different microscopy. Now, let us see each of those in detail. So, the good thing is I have videos for you.

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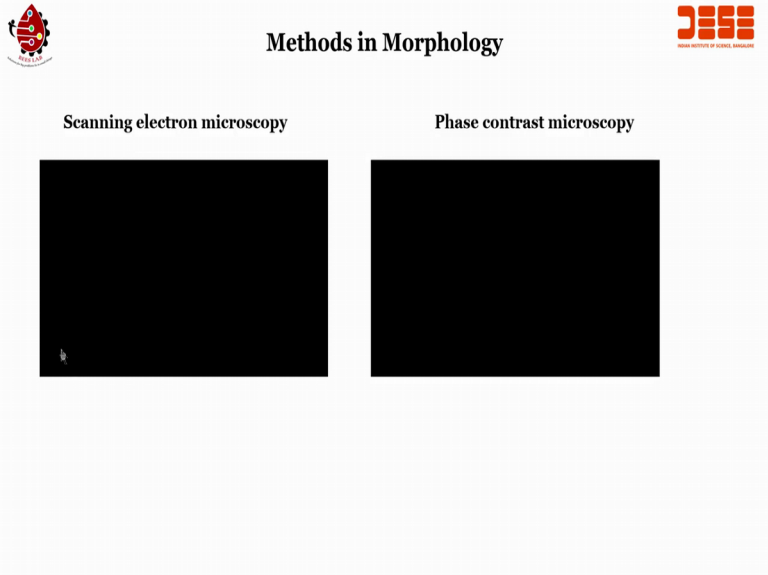

So, you can, you can go through the videos and it will be easier for you to understand right. There are short videos except for microscopy. So, it will not take much of your time when you look at the videos. So, let me play first the scanning electron microscopy ok.

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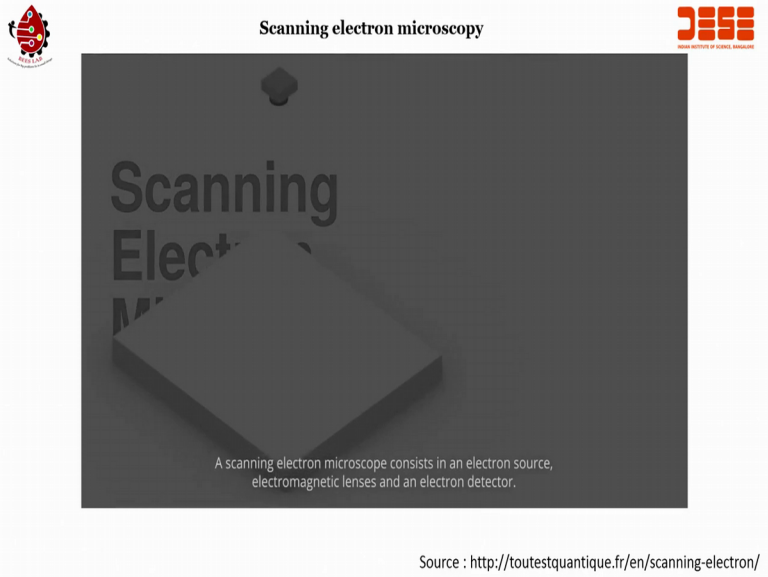

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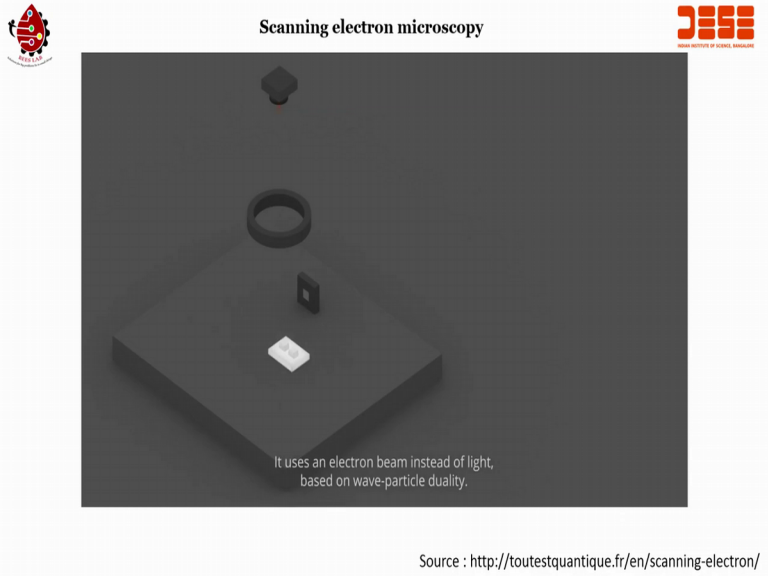

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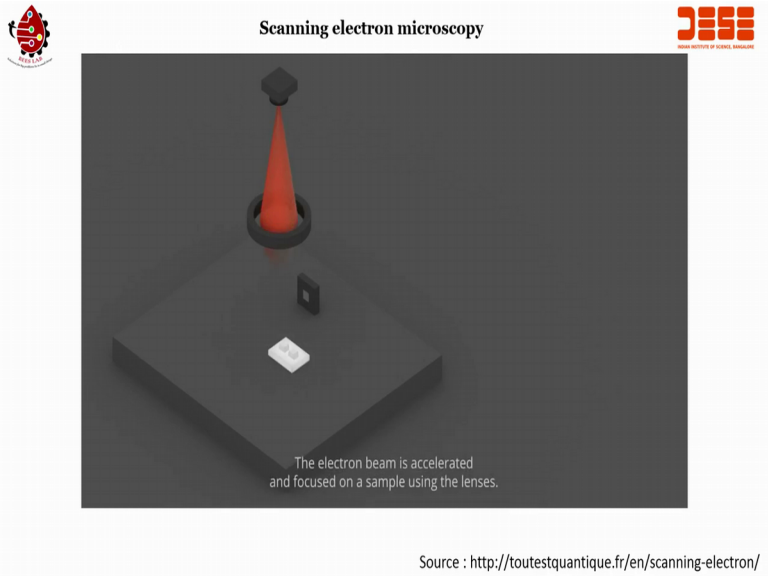

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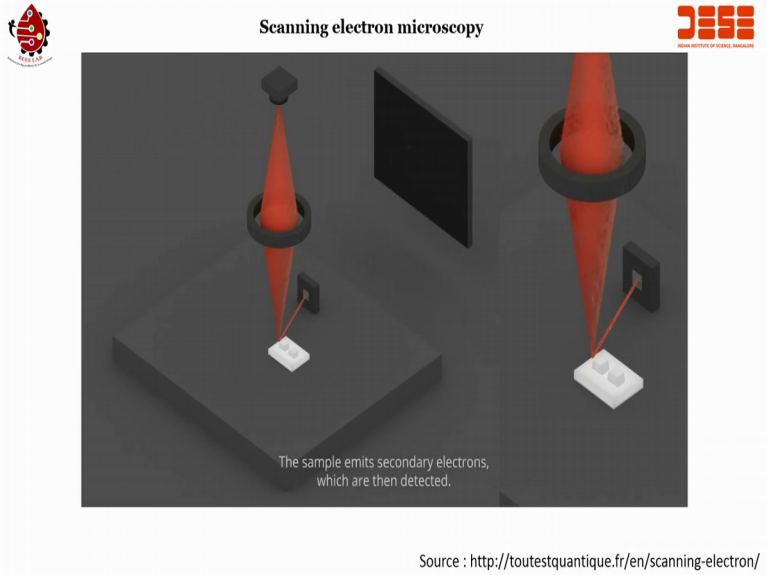

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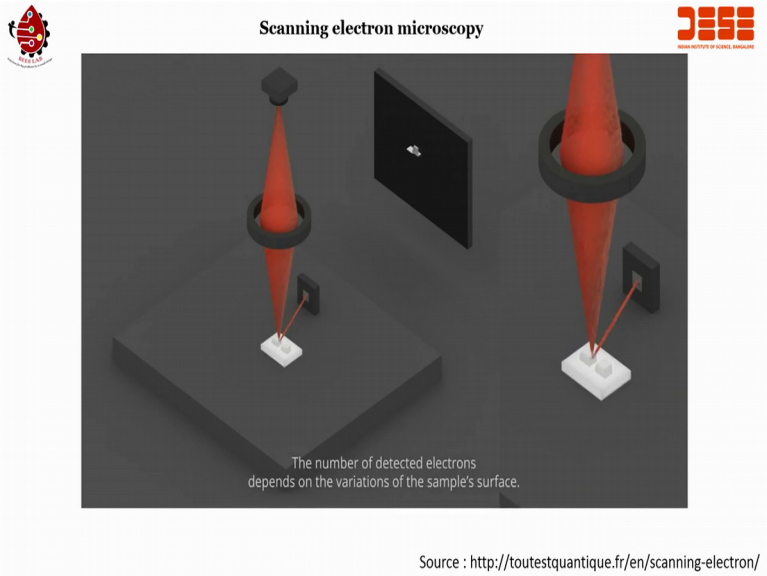

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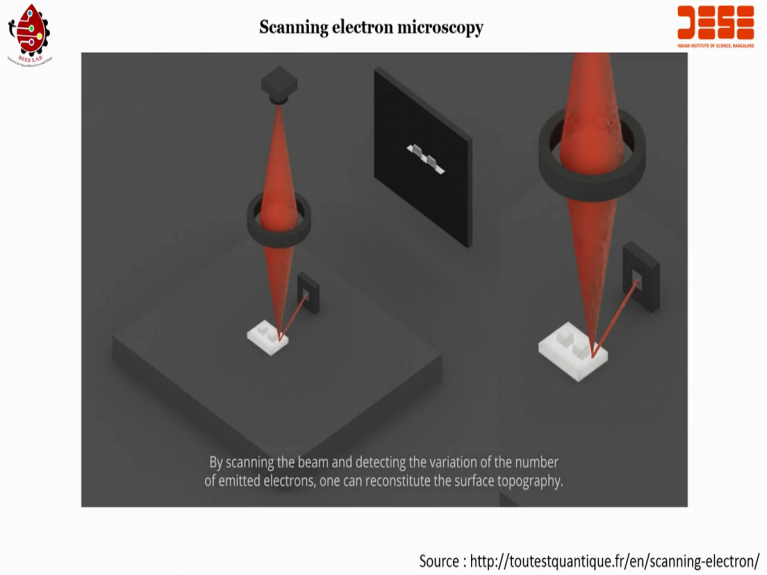

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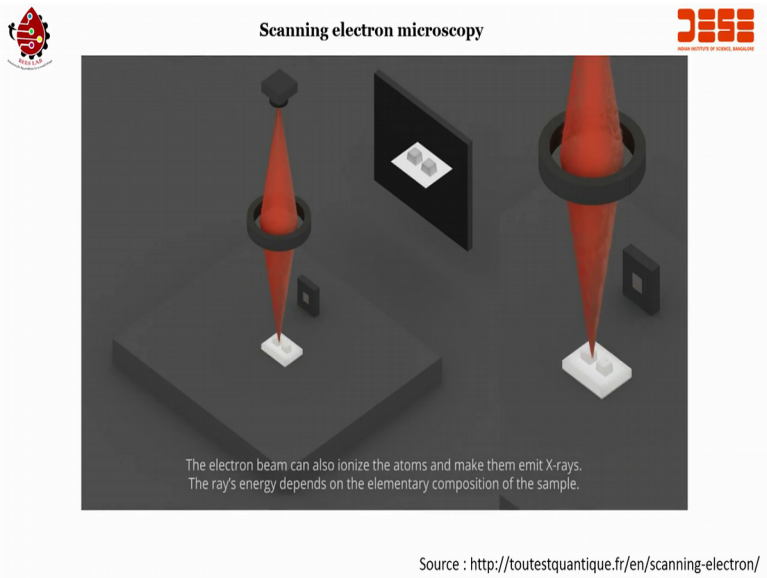

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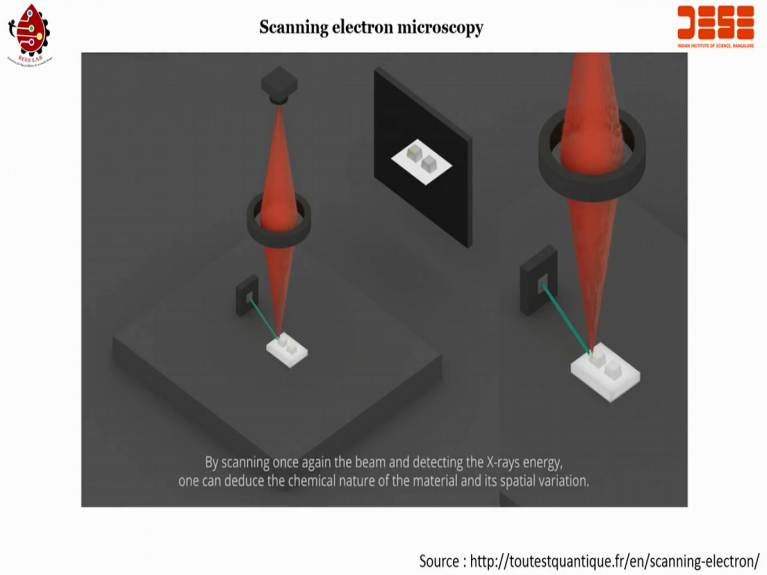

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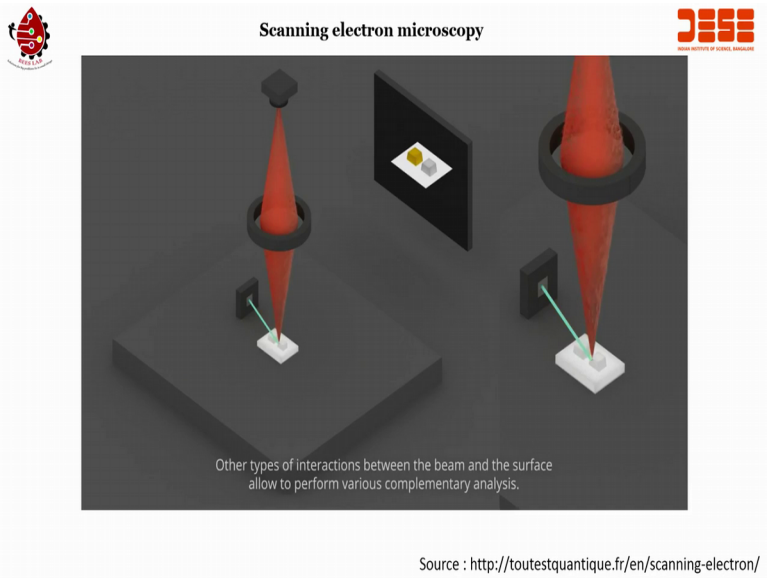

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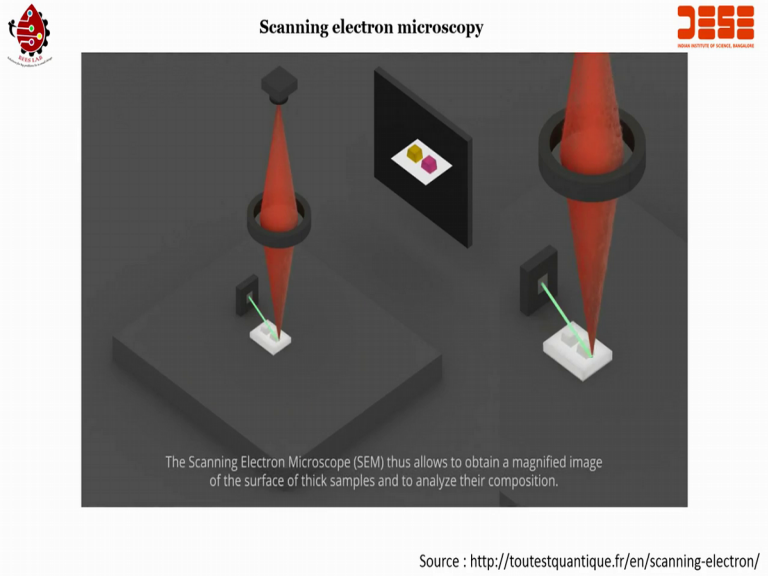

Now, as you have seen how the SEM can work right. SEM can not only be used for understanding their cells, but it can also be used to understand the extremely small structures in case of the MEMS based technologies. We have seen the SEM images in our early modules right.

So, this, but SEM can also be used to understand the cell morphology. Let us see the another microscopy, which is phase contrast microscopy. Let me play that video for you.

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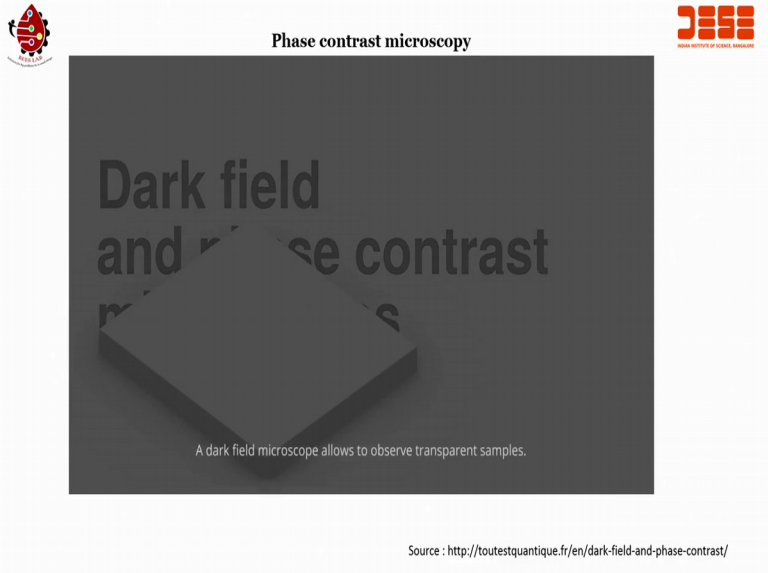

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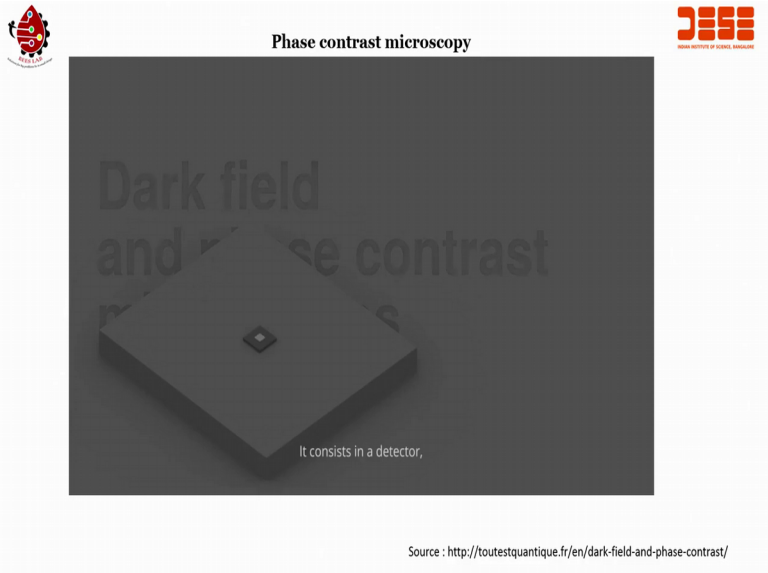

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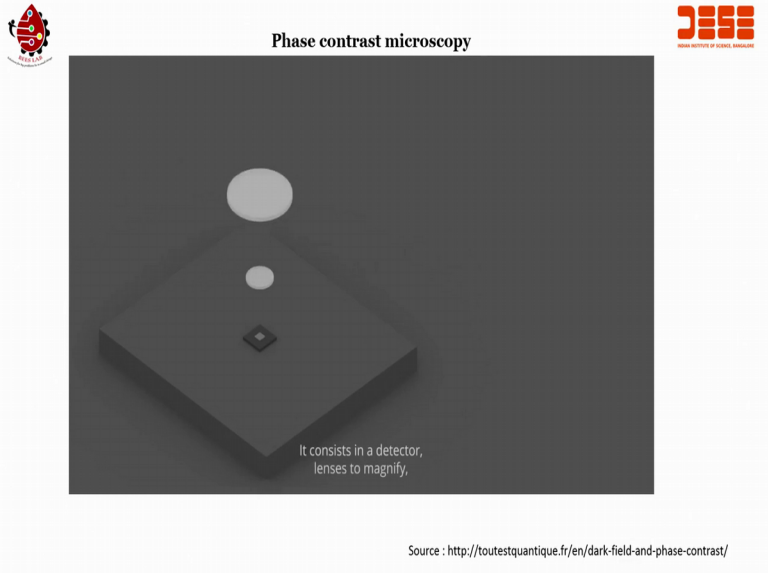

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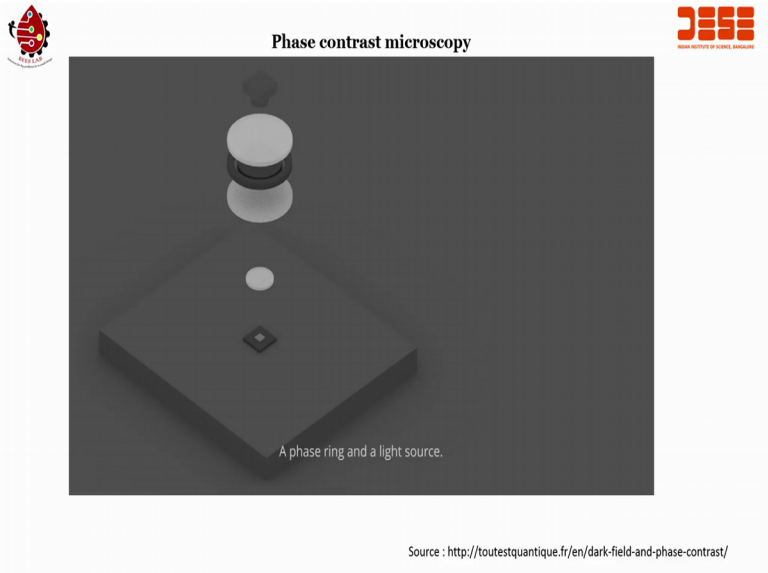

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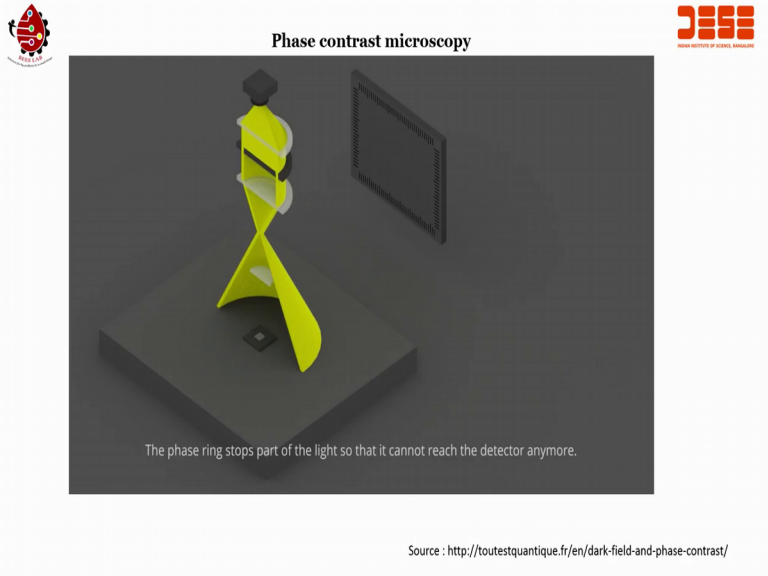

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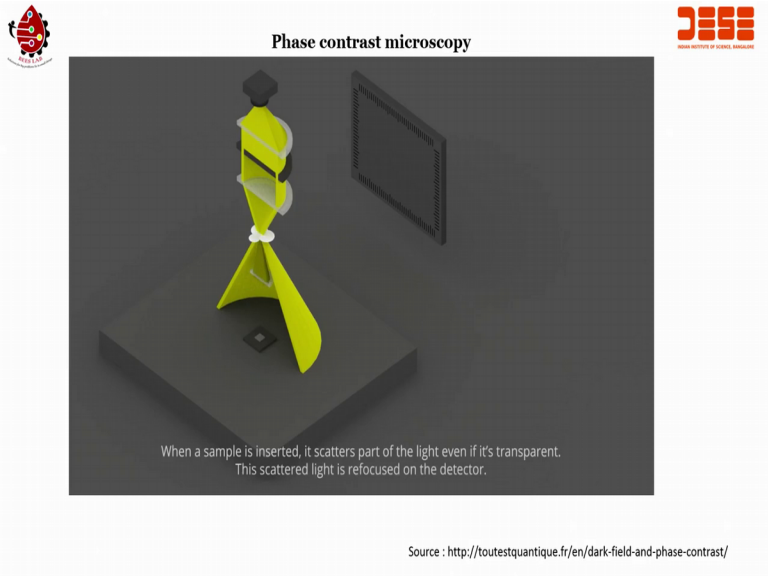

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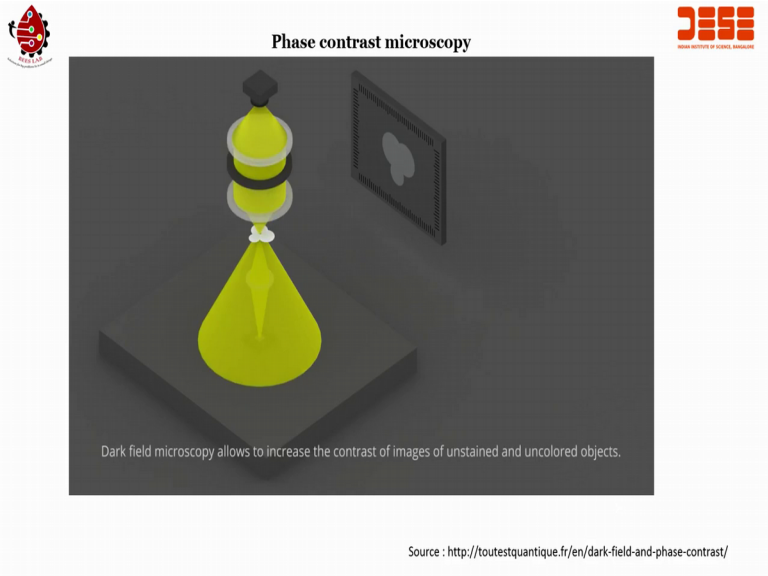

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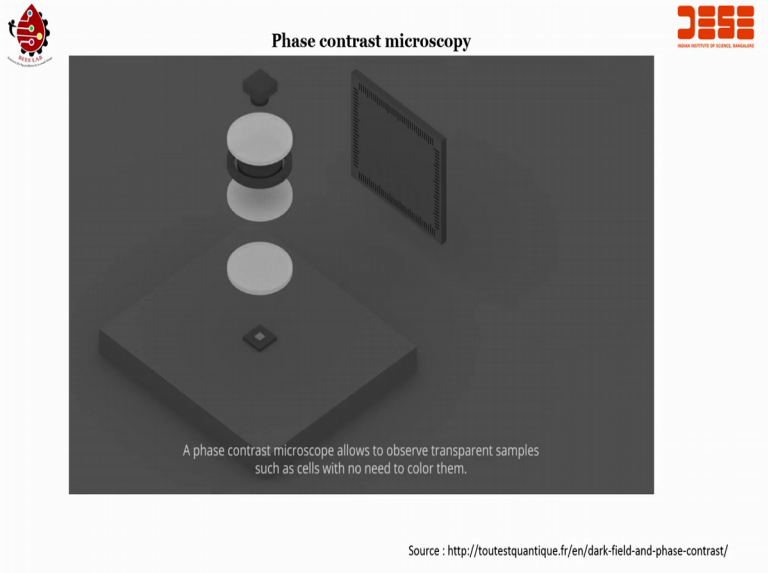

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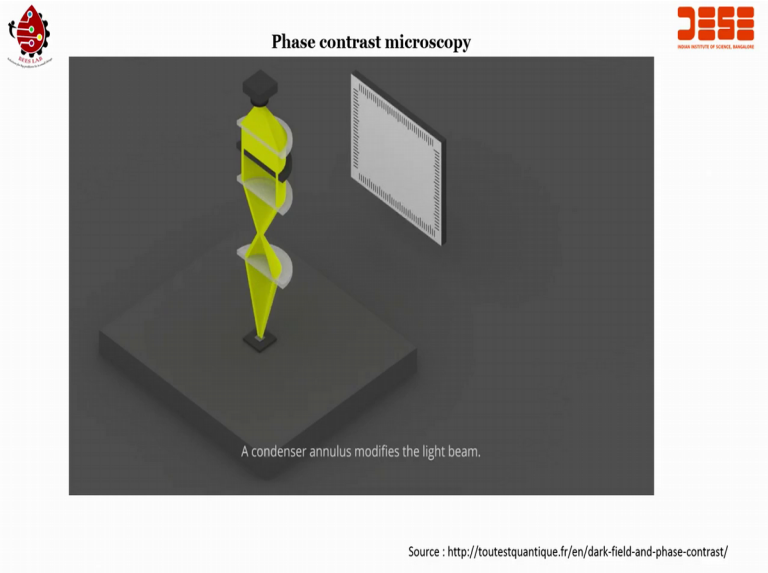

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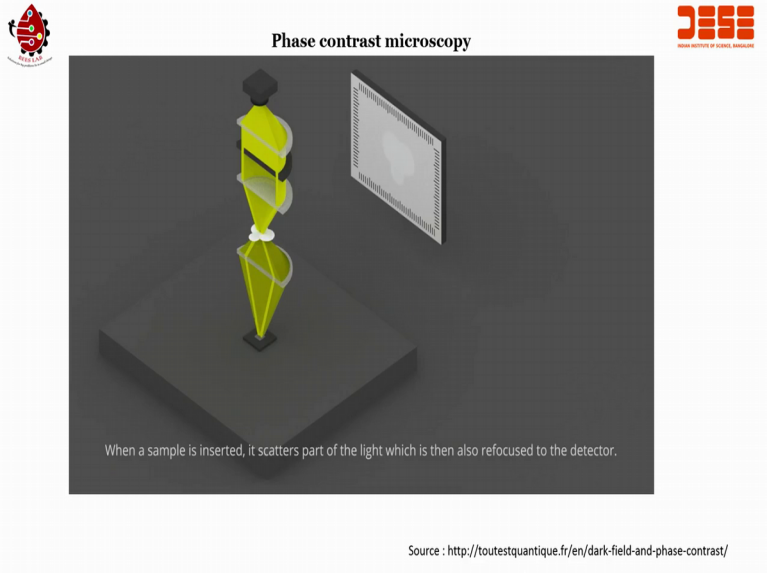

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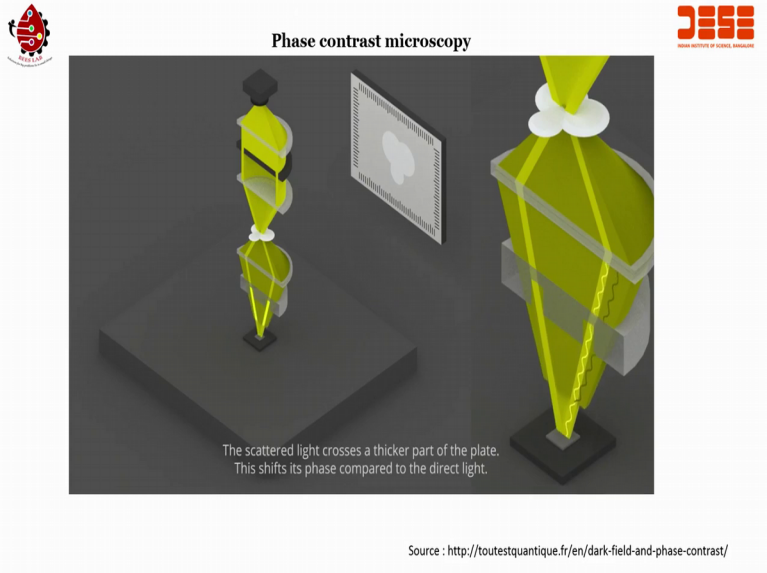

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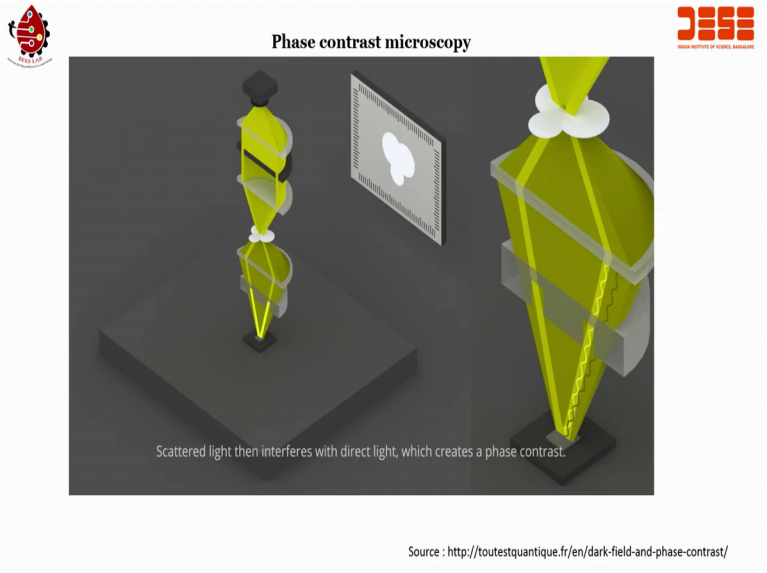

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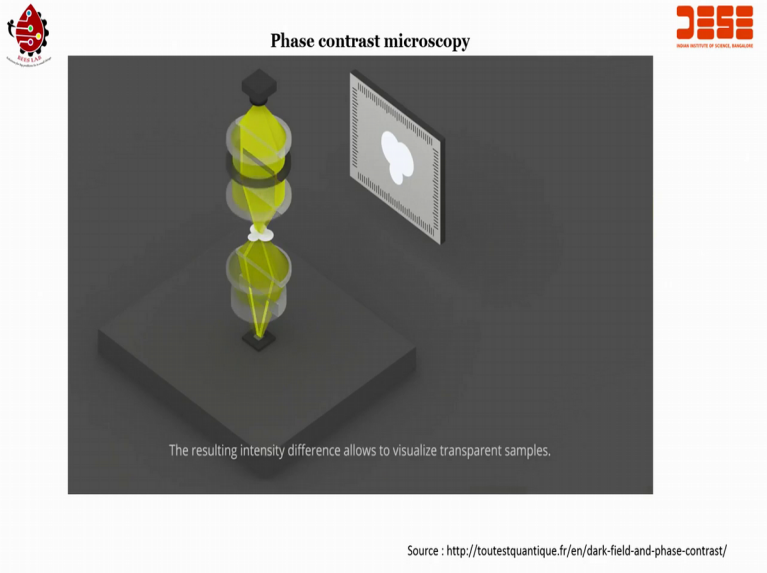

So, phase contrast microscopy ok; so, phase contrast microscopy you have seen now right. Now, there are several other microscopy, where you can understand the, whether it is bright field or it is fluorescence, because in some cases we are standing with h n e and we are with bright field. If there is a Dappy and there is a Catherine blue or there is a red dye or there is a green dye right, which is, so there is a fitzy and then there are lot of other nomenclature given for this particular dyes.

So, the point is our names given for the dyes and these dyes show a particular color when you look under the fluorescent microscope. So, we will also see how the bright field microscope looks like and then we will see how the fluorescent microscope looks like right. So, let us see the video of bright field and fluorescent microscope. I will first play bright field.

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Hey everyone with my chemistry basics here, let us talk about bright field microscopy.

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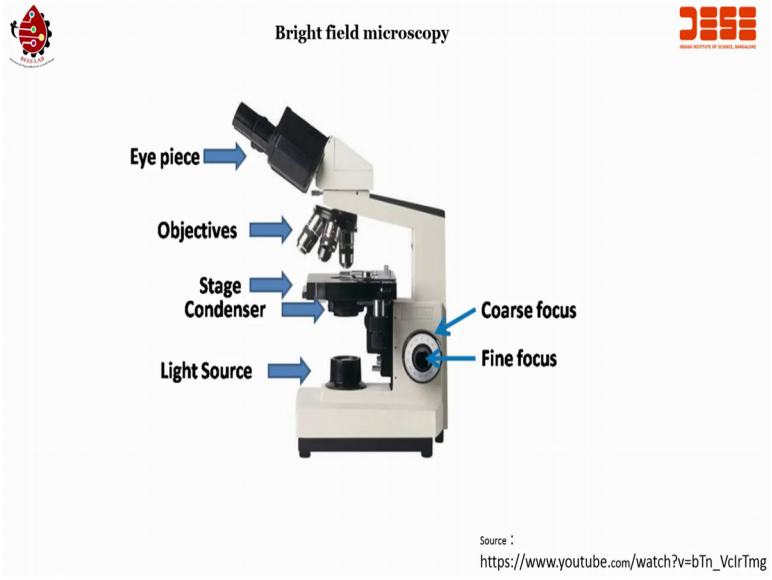

Bright field microscope is the most common and simplest of all optical microscope. A typical bright field microscope has many components that includes a light source, condenser, stage objective, eye piece, rough and fine adjustments.

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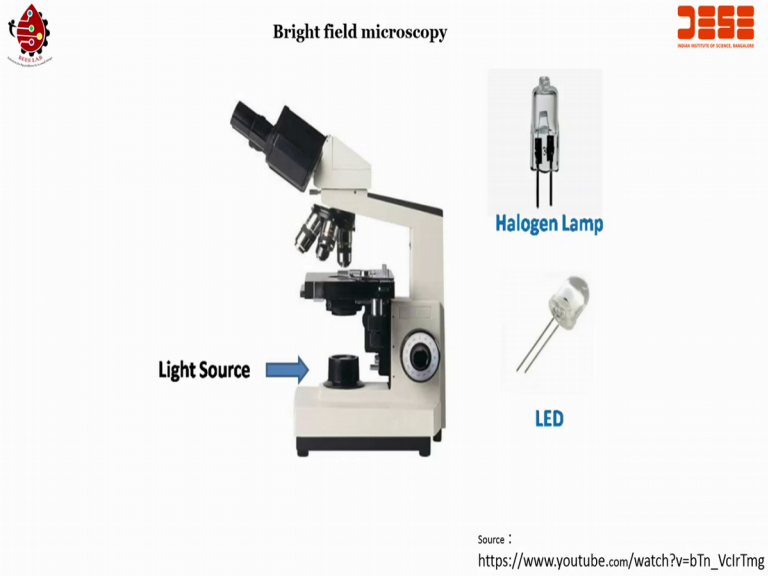

The light source can either be a halogen lamp or an LED.

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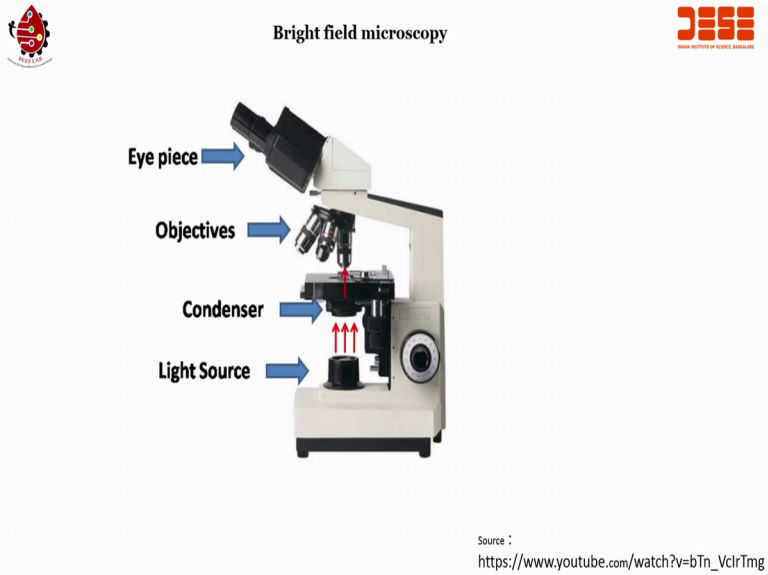

The condenser collects the light from the light source and directs the light to specimen, which is kept on the stage. The light passing to the specimen is collected by the objective lens and the final magnification is created by the eye piece.

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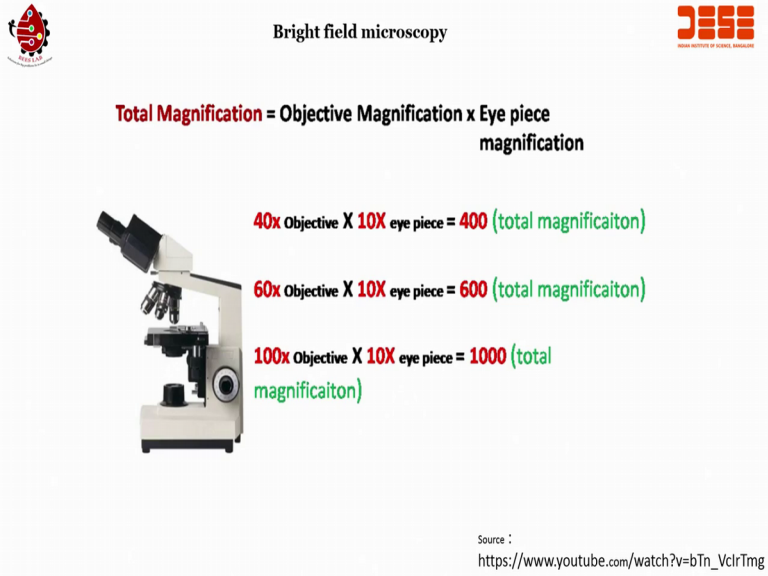

The total magnification of the specimen observed as the product of magnification of the objective and the magnification of the eye piece.

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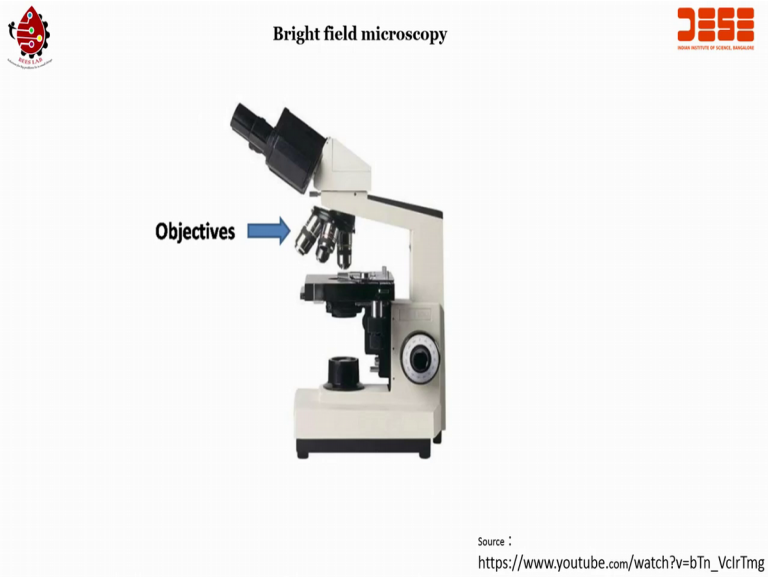

Now, let us talk about objectives. The objectives are the most important imaging components of the optical microscope and play a key role in magnification and resolution of the specimen. All the objectives have an information written on it.

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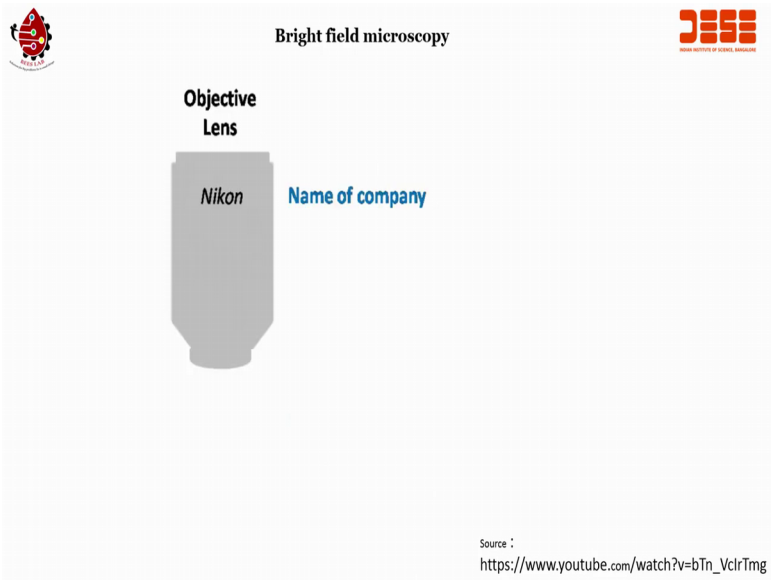

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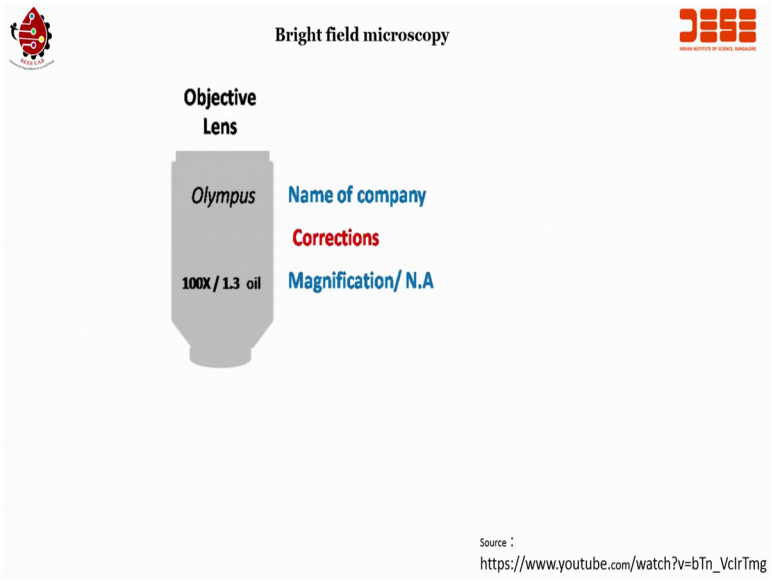

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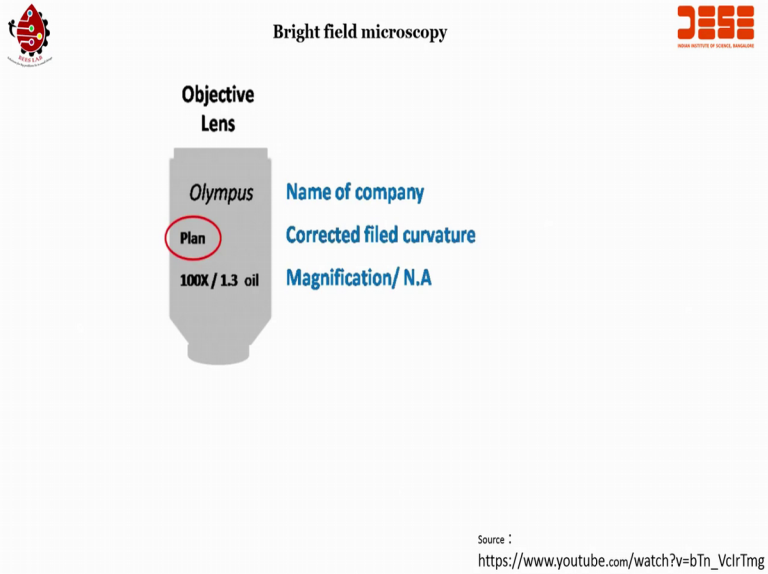

This includes the name of the manufacturing company, magnification and numerical aperture. The term plan is used to indicate corrected field curvature.

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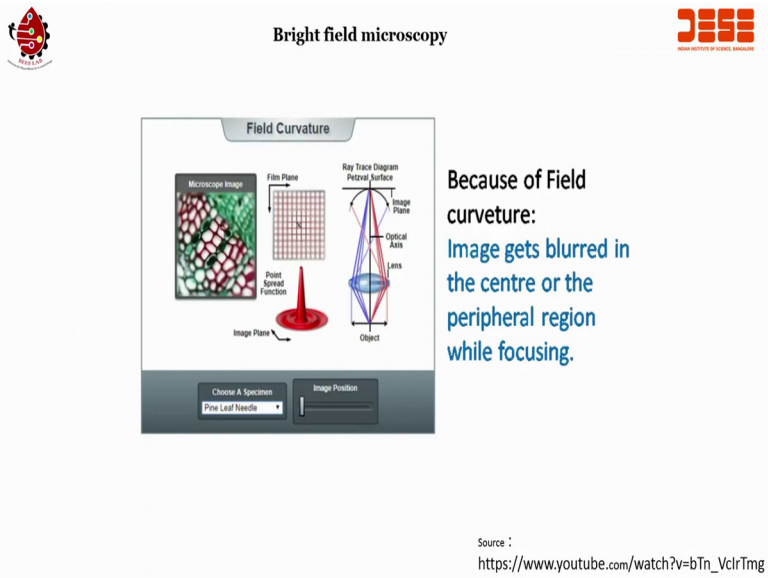

Field curvature is a common abrasion caused by the spherical surface of convex lens. In this phenomenon not all parts of an image are clearly focus. While, focusing in the center of an image the peripheral region gets blurred and while focusing in the peripheral region the central region gets blurred.

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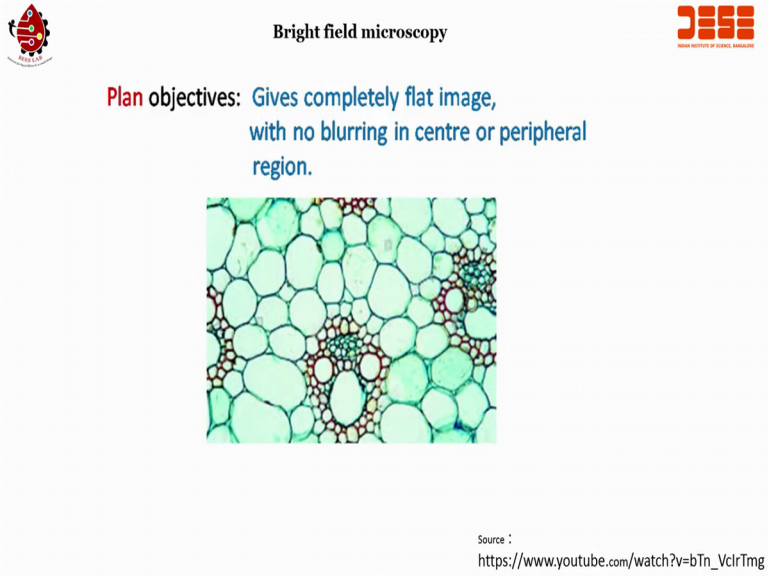

To avoid the problem of field curvature, modern objectives are designed to give a clear flat image, but no blurring in center or the edge.

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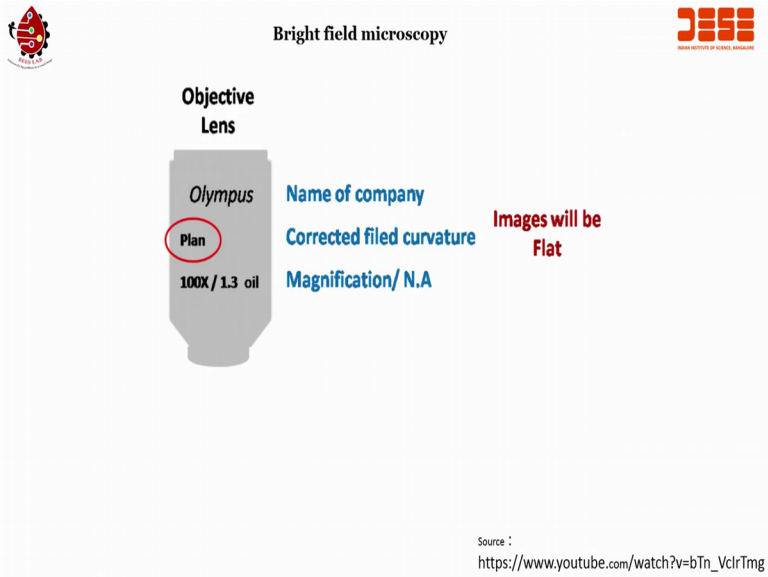

The objectives with corrections and field curvature are called plan objectives.

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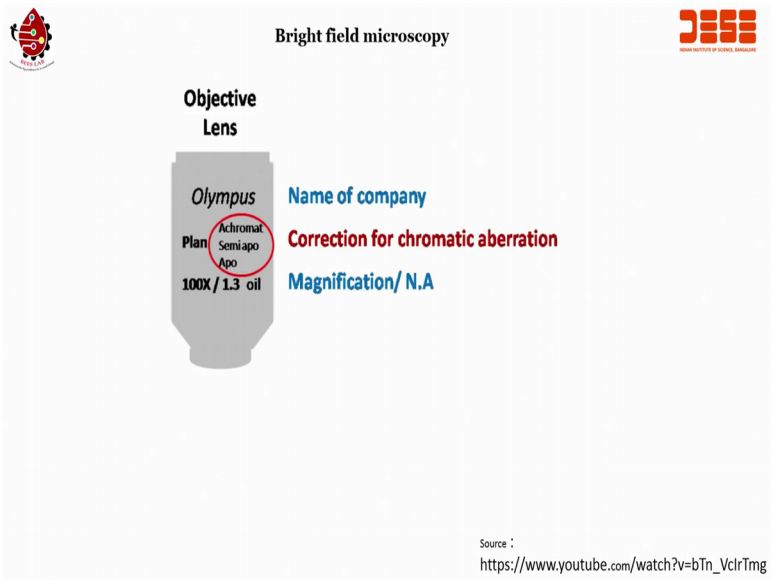

Corrections for chromatic aberration.

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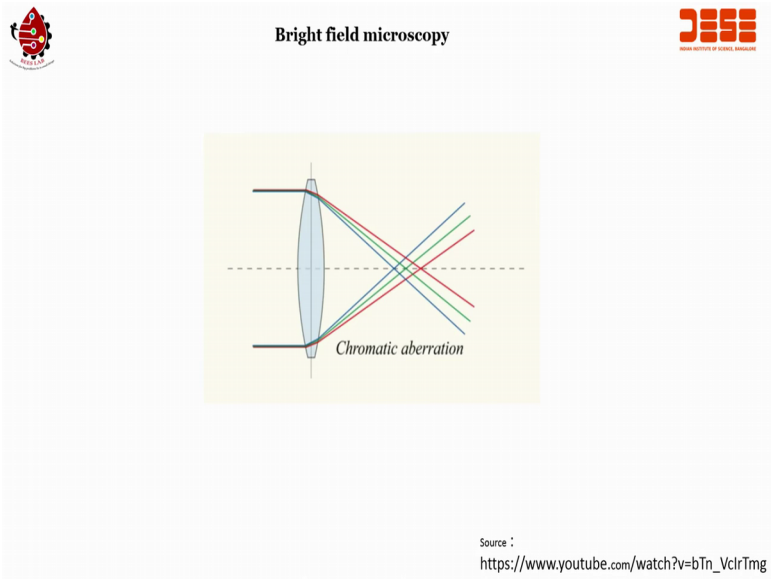

Chromatic aberration is a phenomenon in which the lens fails to focus all the colors of the light at the same point. It occurs, because the lens have different refractive index for different wavelengths of light.

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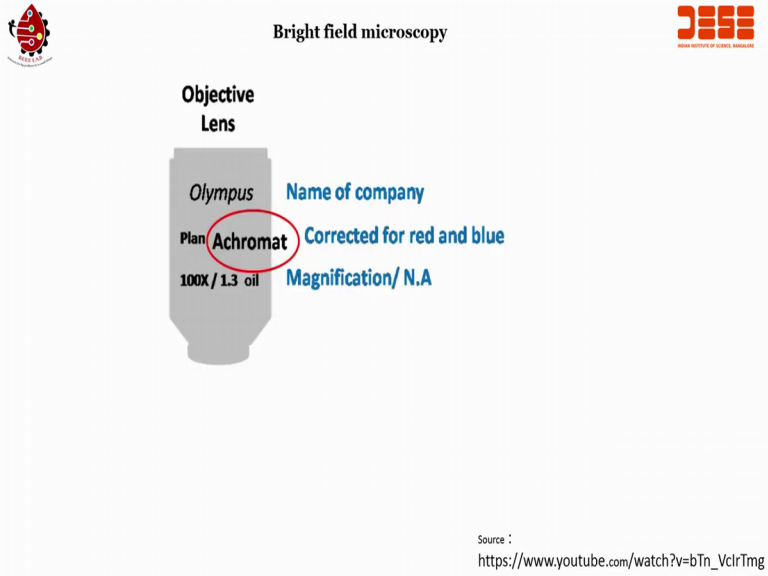

The term a chromatic lens means; it is corrected for two colors; red and blue.

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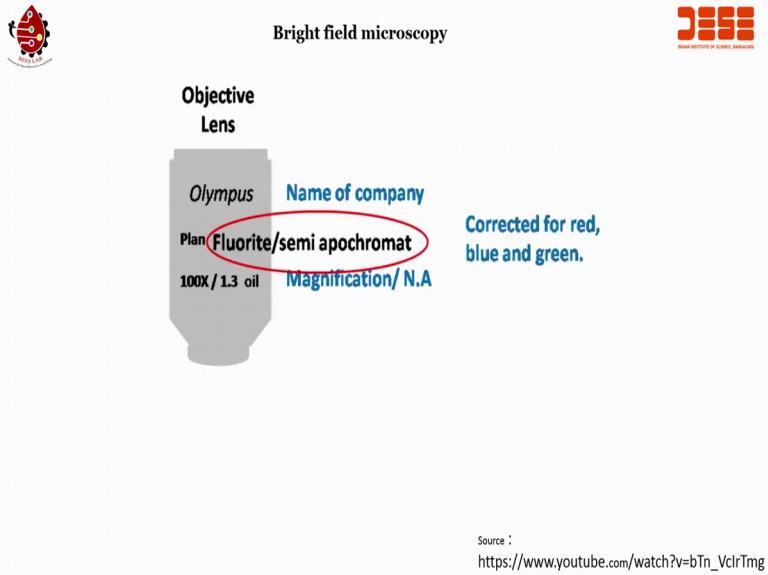

The term fluoride or semi apochromat means the lens is corrected for three colors; red, blue and green.

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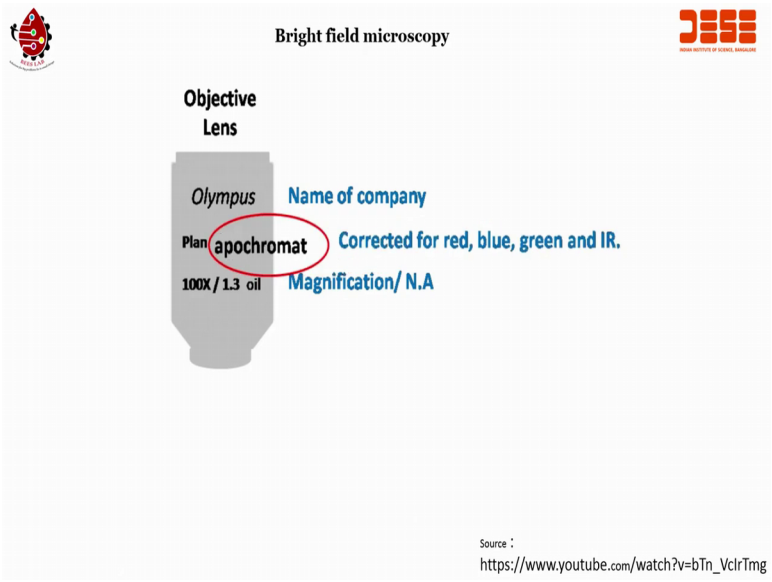

And the term apochromat means the lens is corrected for four colors; red, blue, green and infra-red.

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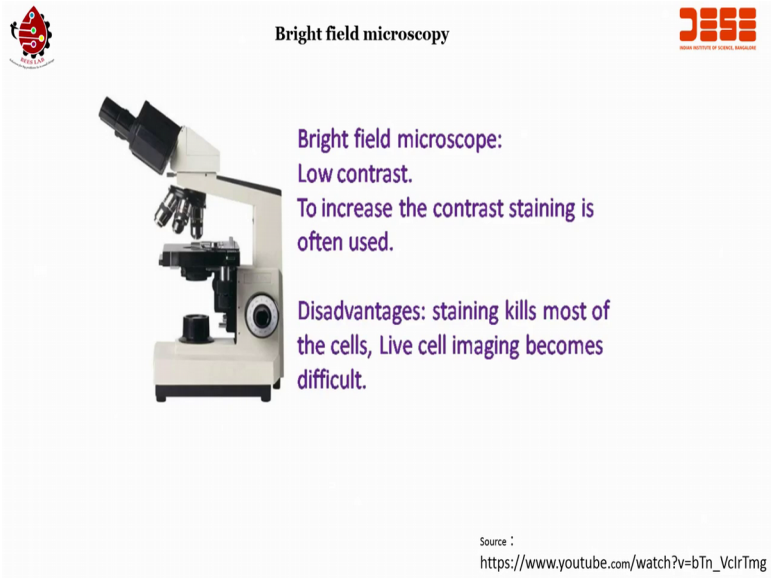

Bright field microscope typically has low contrast, as most of the biological samples transmit most of the light. To increase the contrast staining is often required. The disadvantage of staining is that most of the cells are killed and live cell imaging is difficult. Now, since here used the right field and seen the bright field microscopy, let us also see the fluorescent microscopy and let me play the fluorescent microscopy as well.

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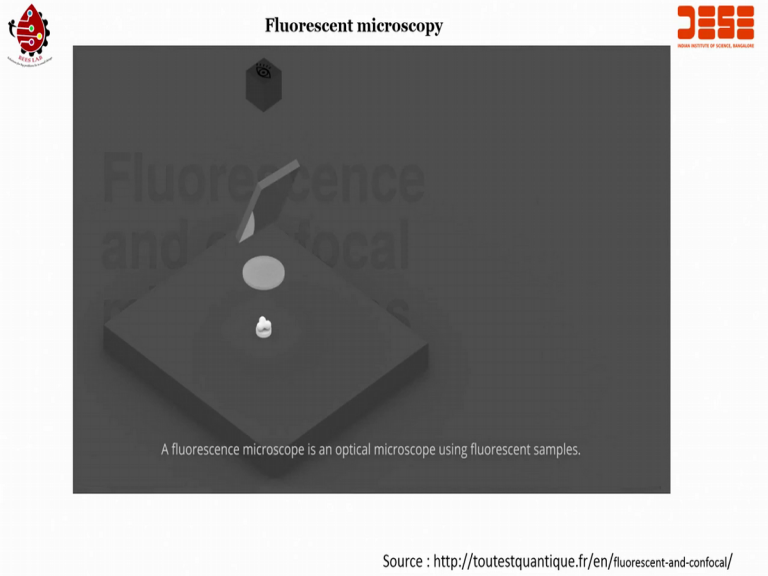

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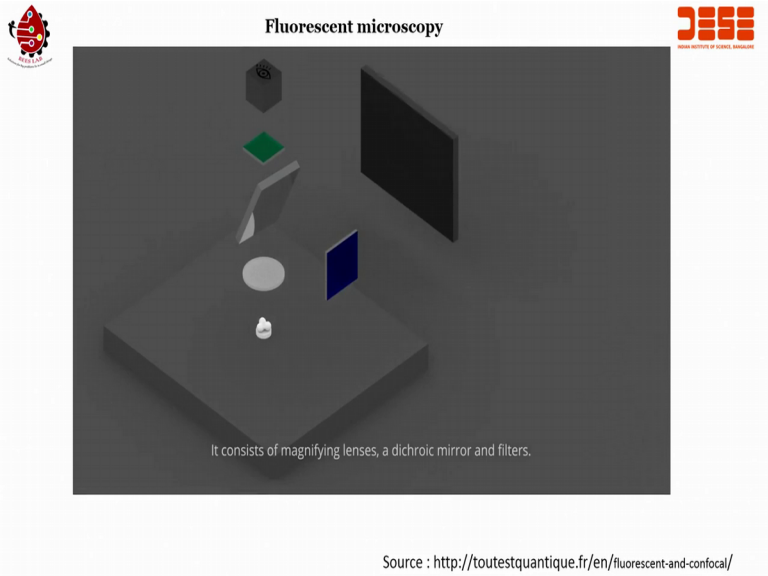

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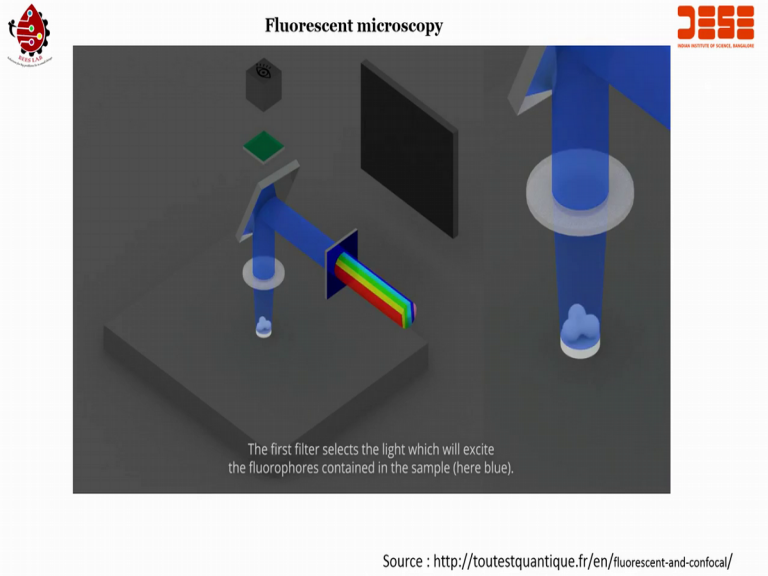

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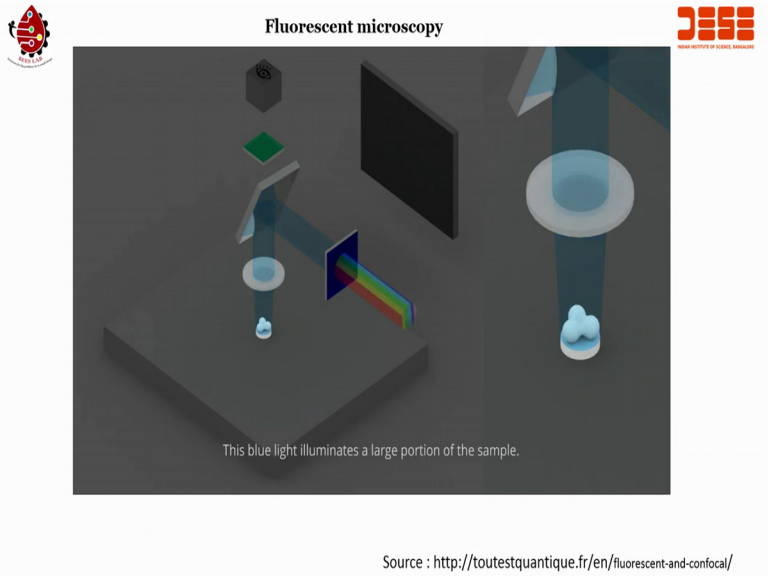

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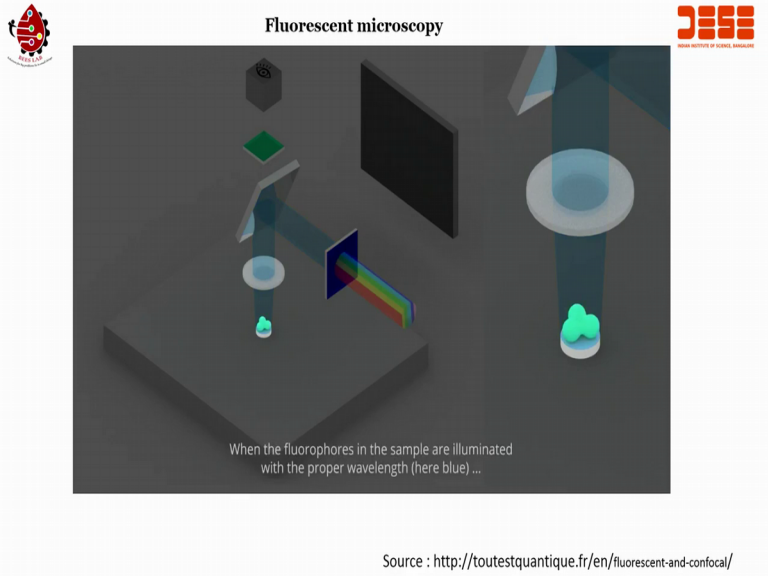

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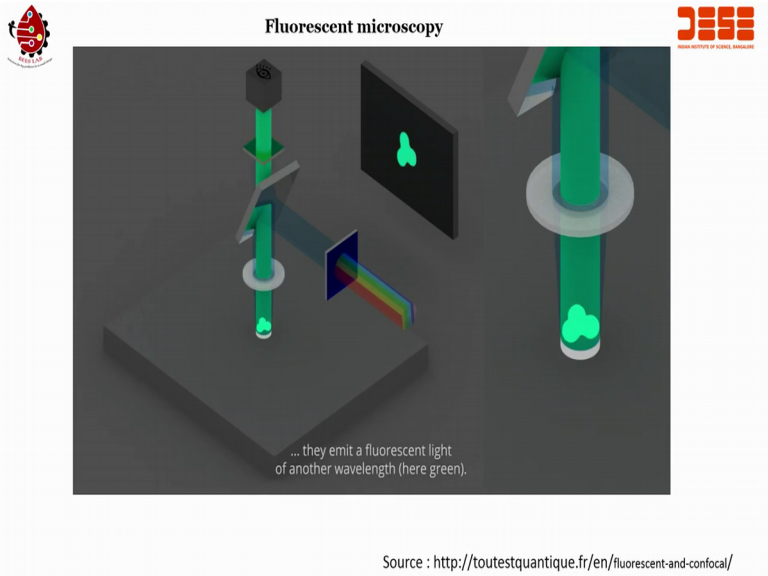

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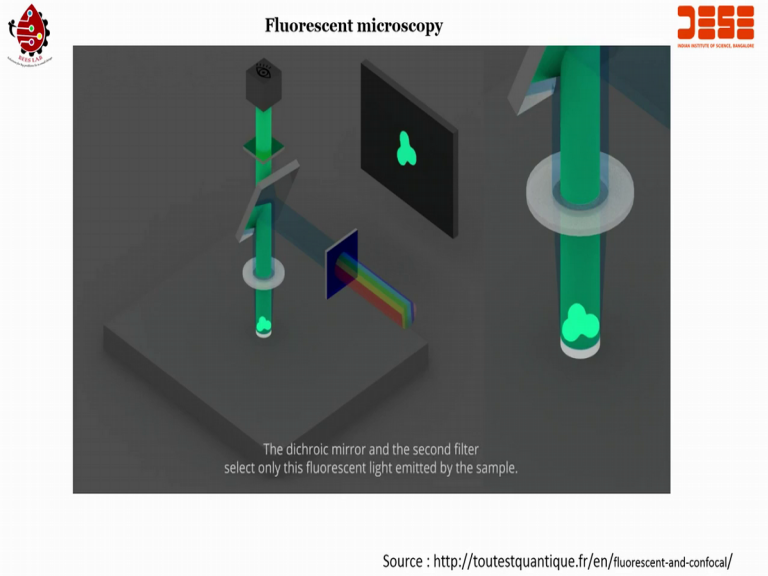

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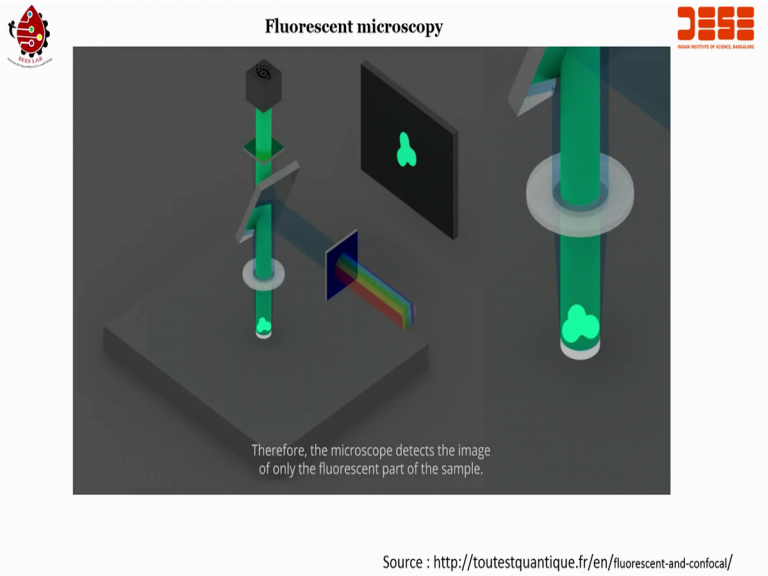

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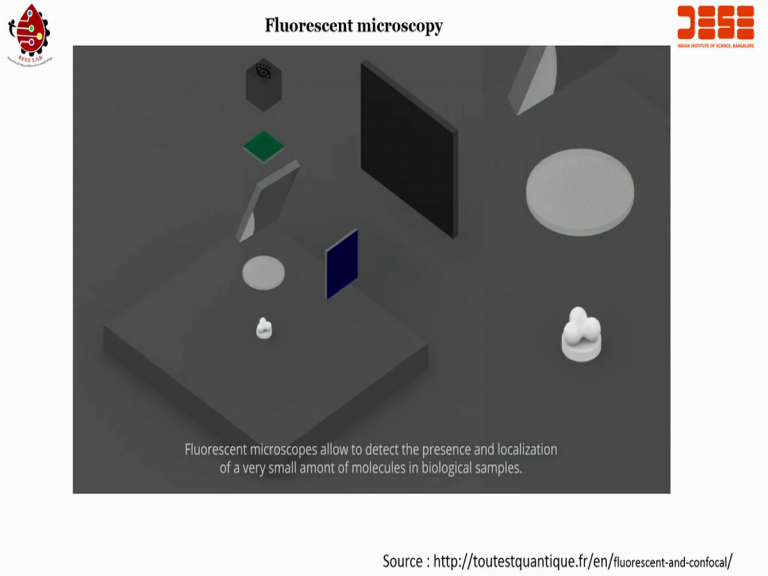

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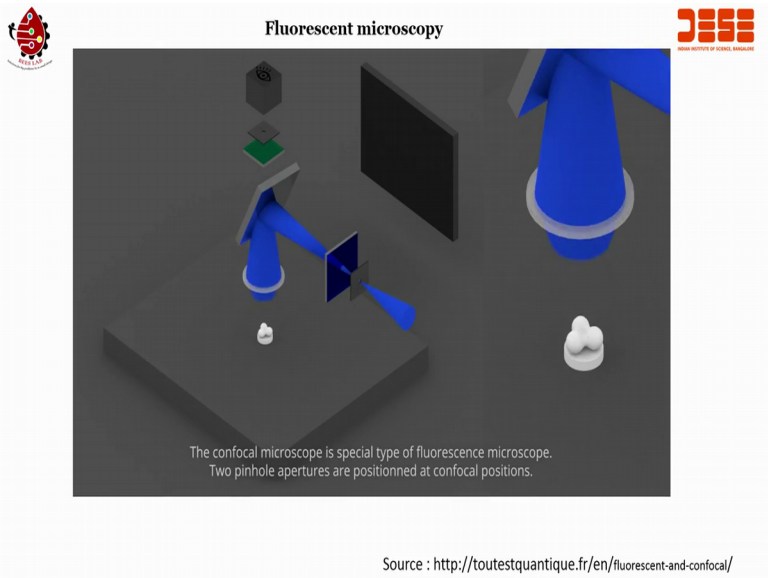

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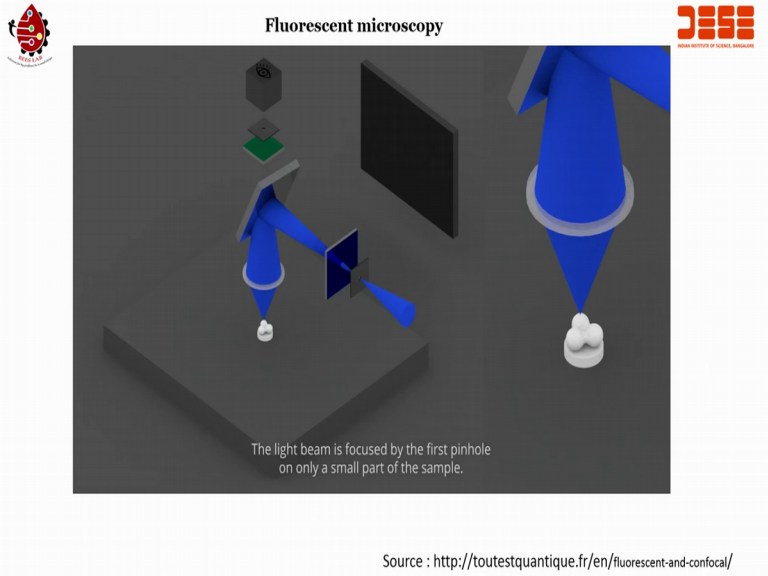

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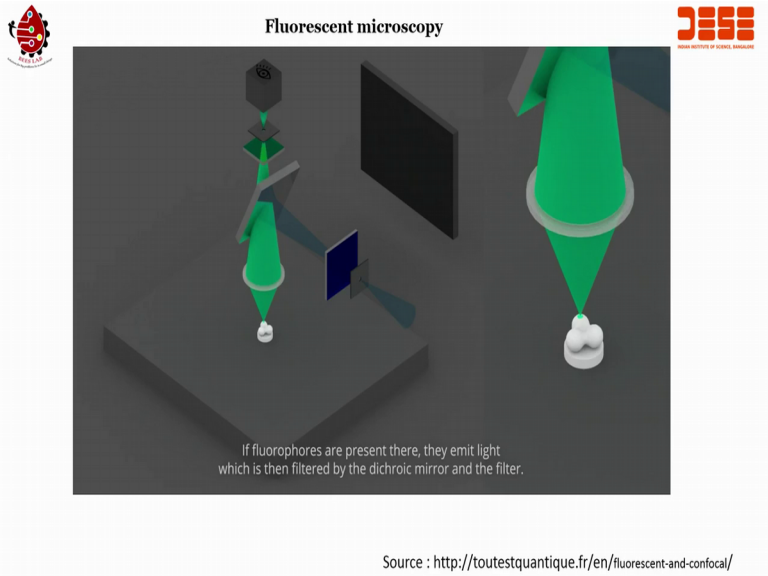

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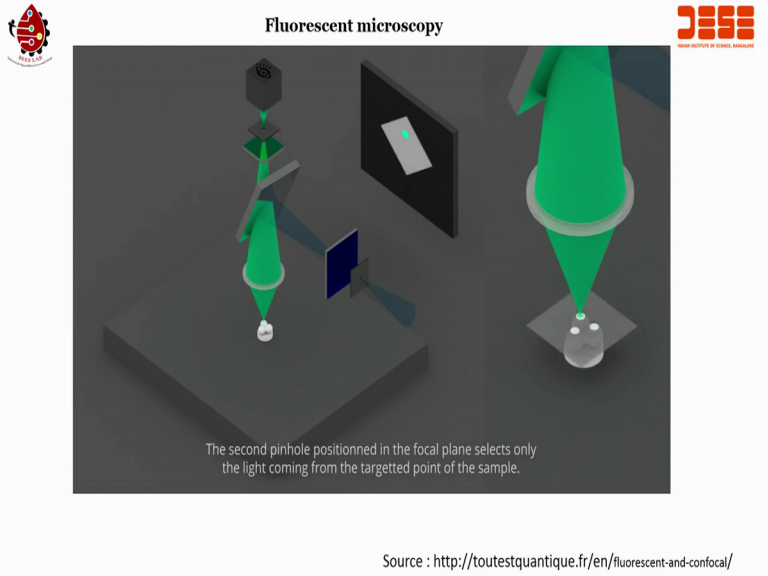

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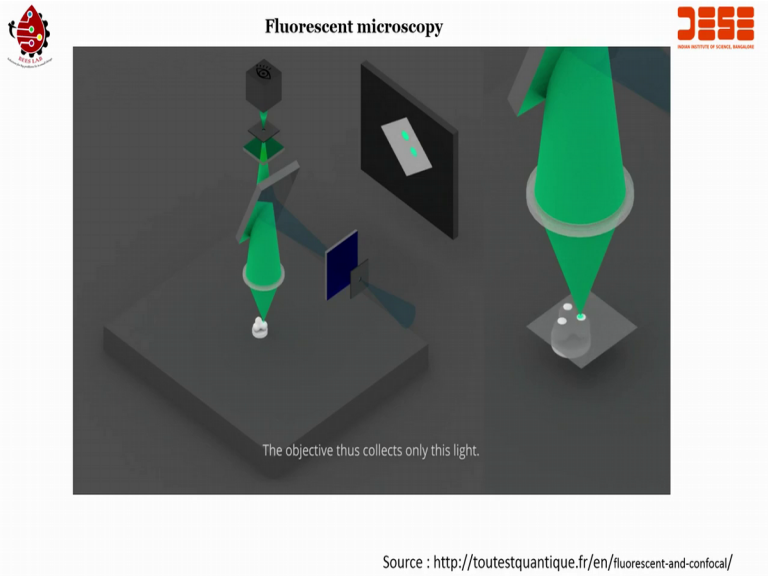

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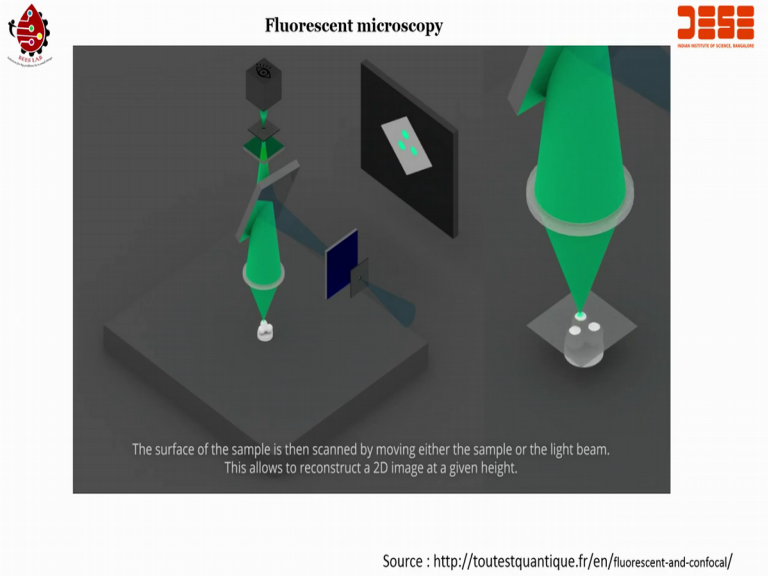

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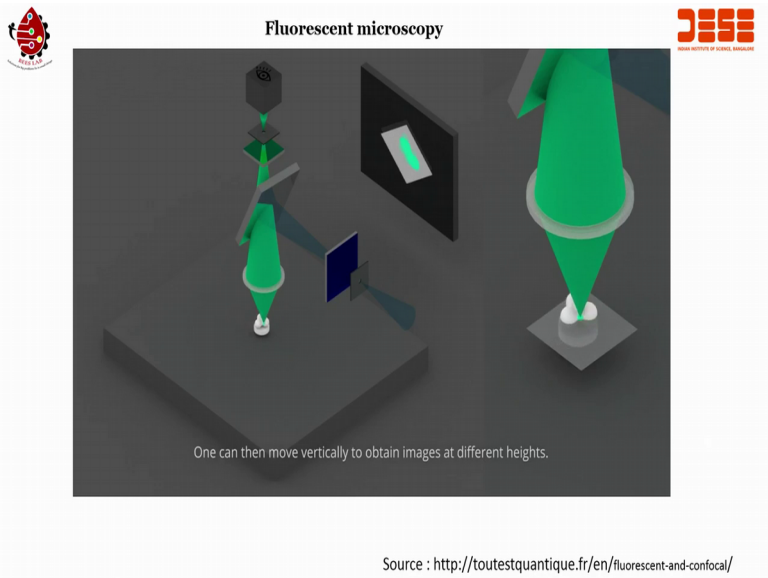

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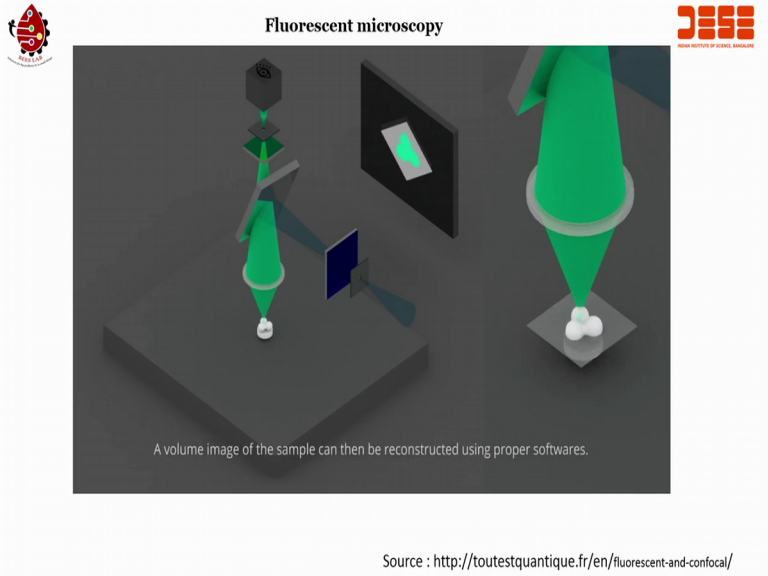

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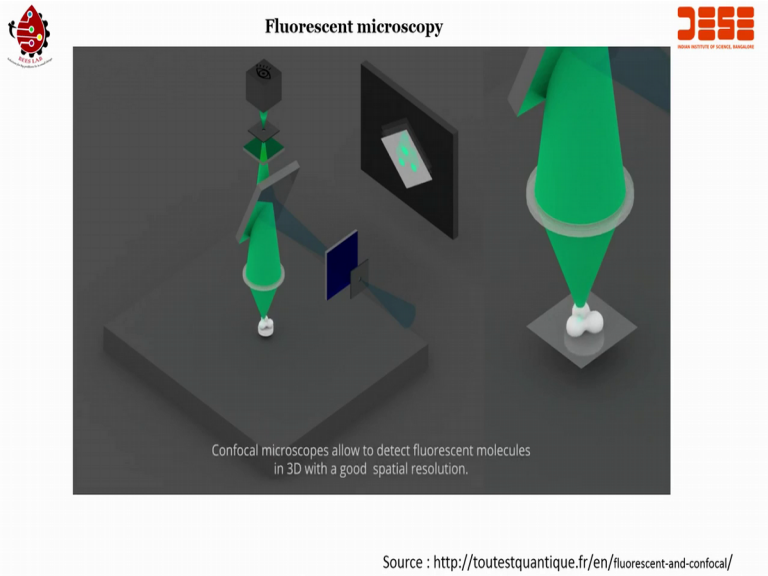

So, I will as a part of this course, we will show it to you how the bright field microscope works like right and we also see inverted microscope, we will see a stereo microscope, we will also see a metallurgy microscope. So, we will see three different microscope. So, understand the, how the cells looks like, how the devices looks like ok. Now, then the even complex microscope comes as a polarization microscope and interference microscope.

So, let us see the videos of polarization microscope as well as interference microscope and then we will go to the how can we do the biomedical research, when you understand the cell morphology ok. So, let us, let me play it, both the videos. first I will play polarization microscopy followed by the interference microscopy. So, I am playing fluorescence microscopy.

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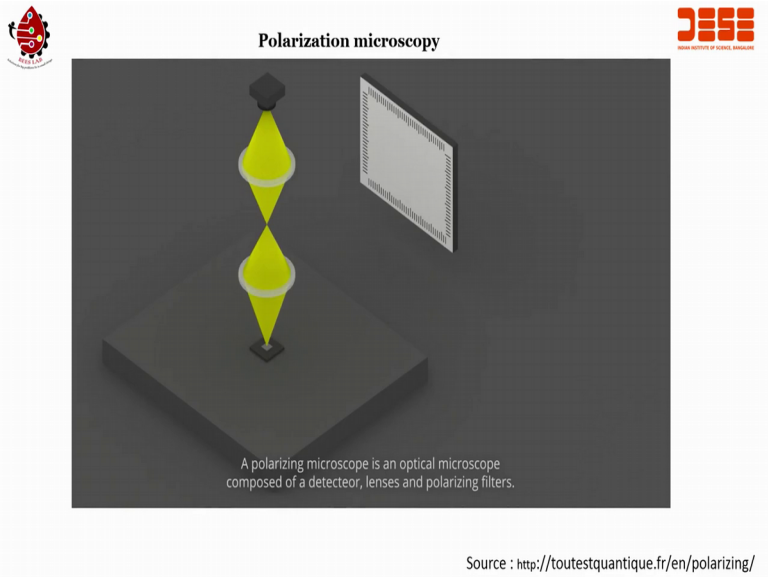

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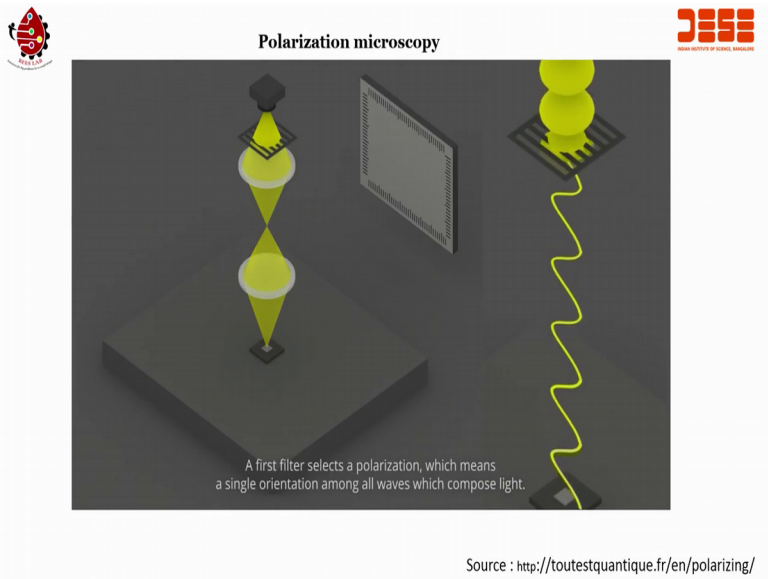

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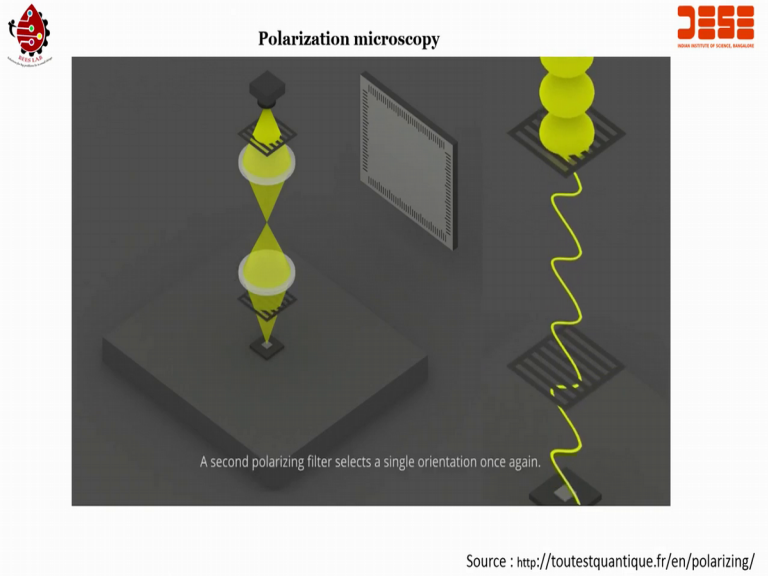

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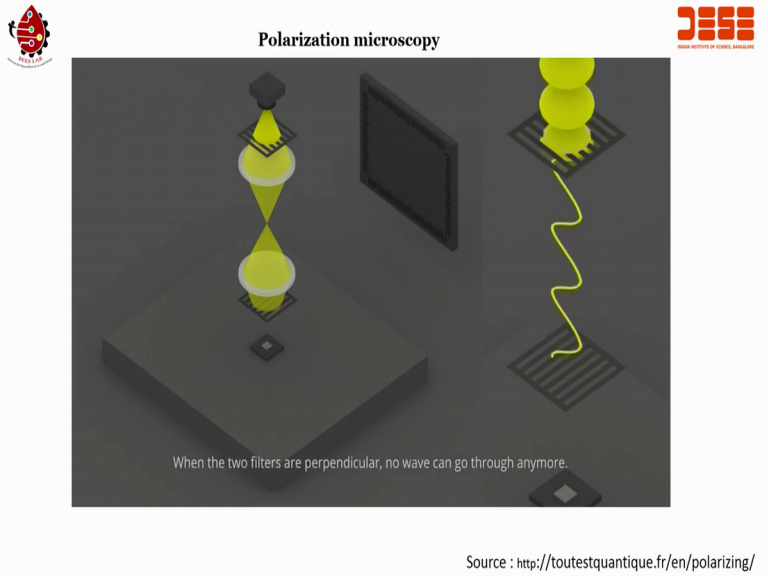

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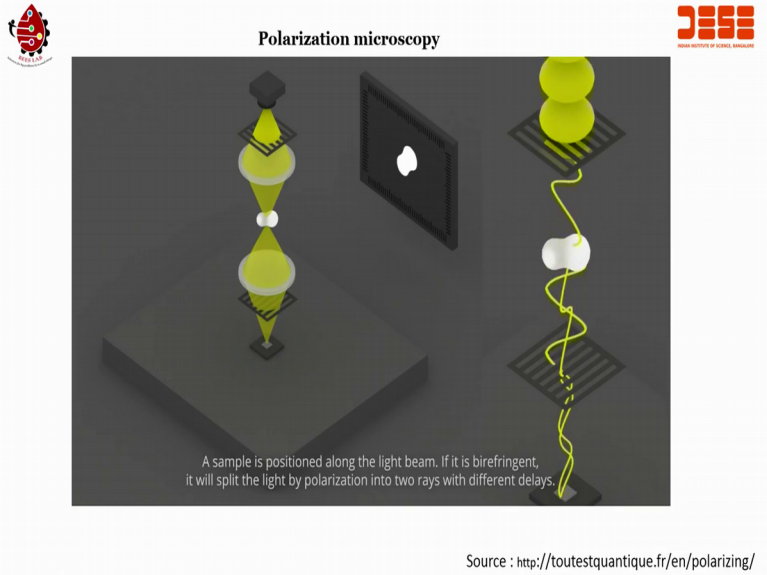

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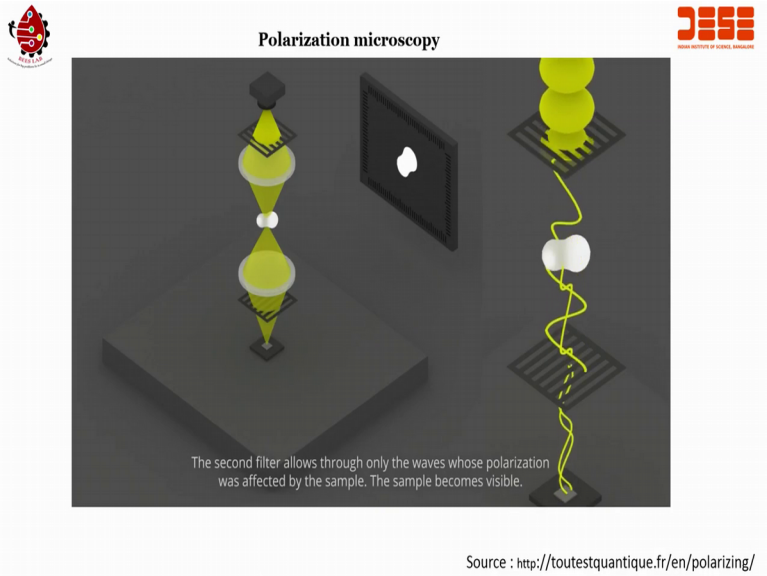

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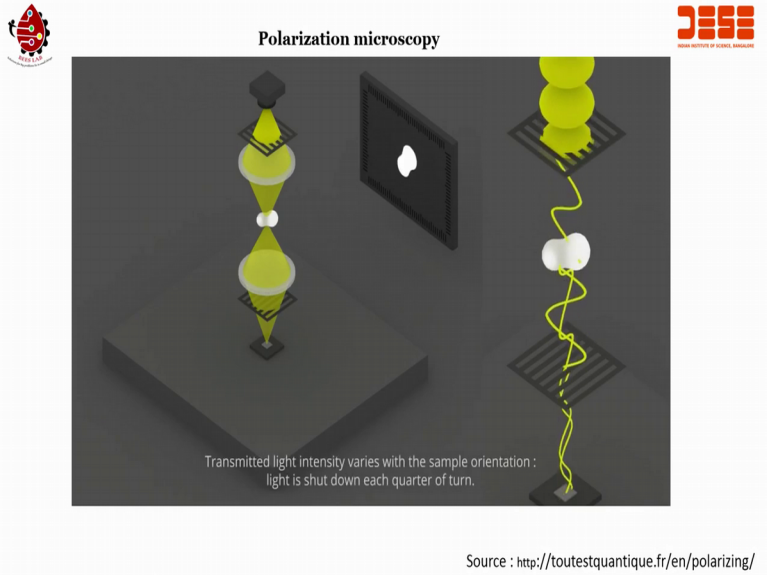

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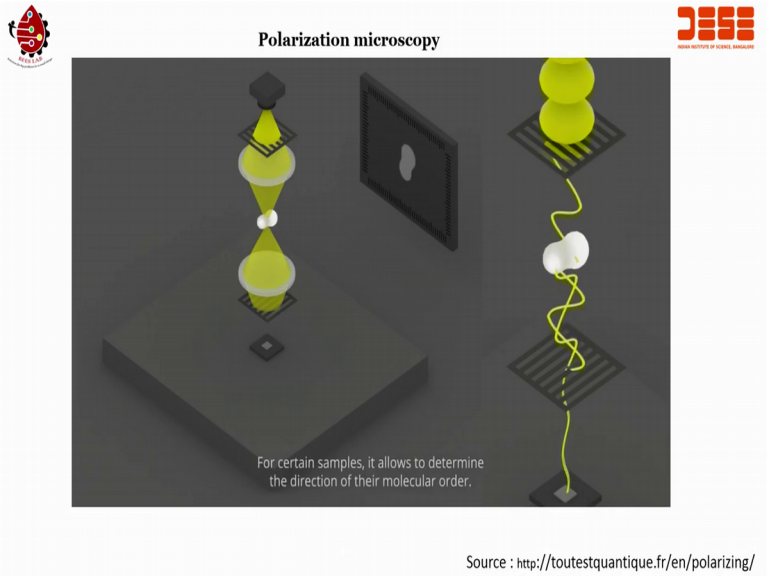

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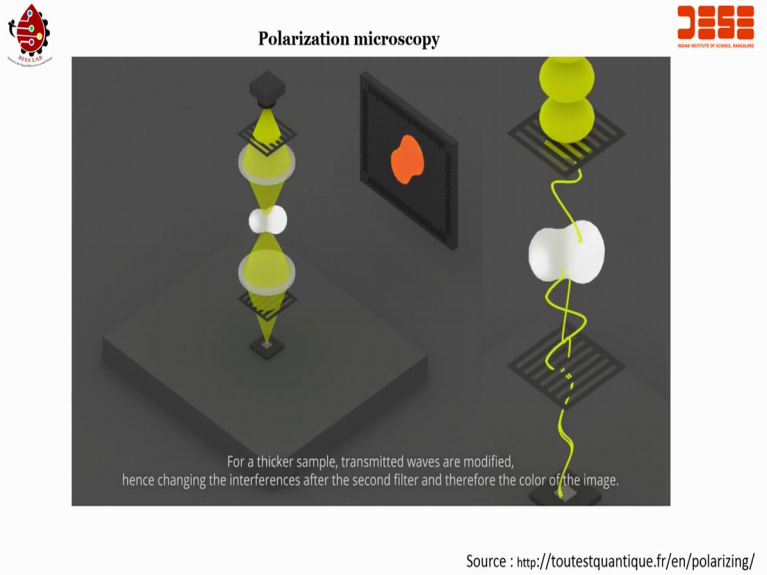

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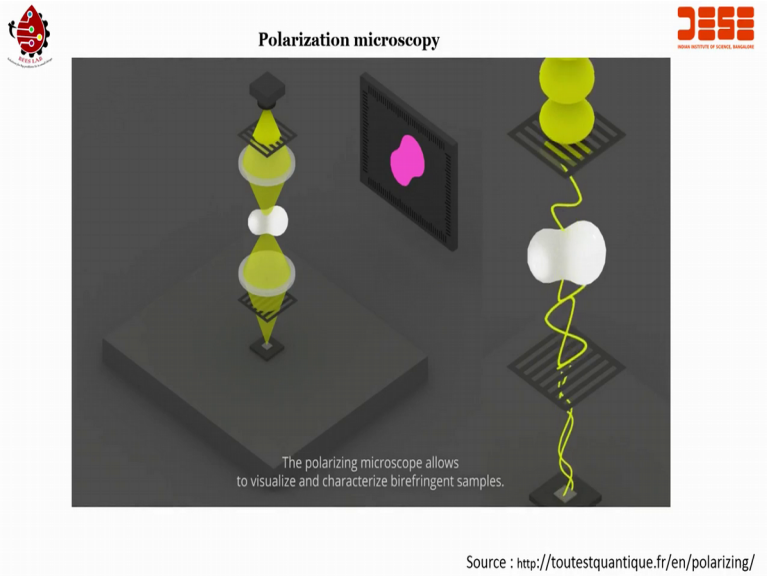

Now, since you have seen polarization microscopy let me also play the interference microscopy.

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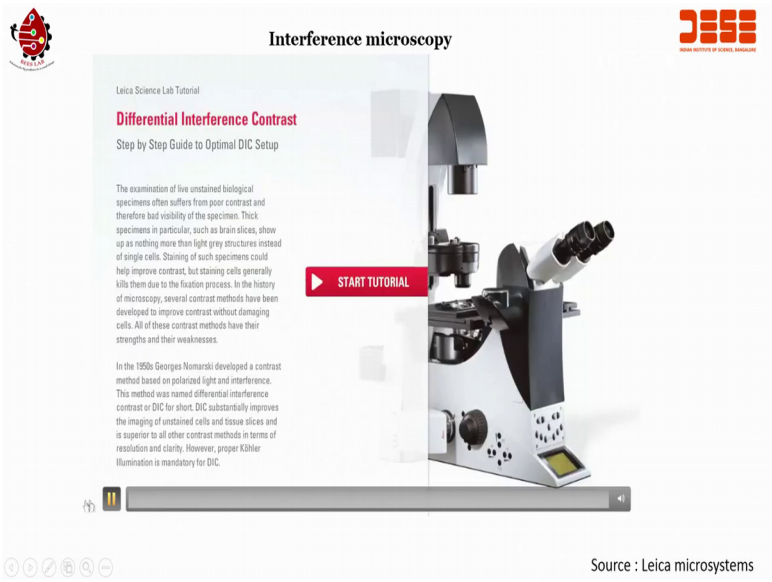

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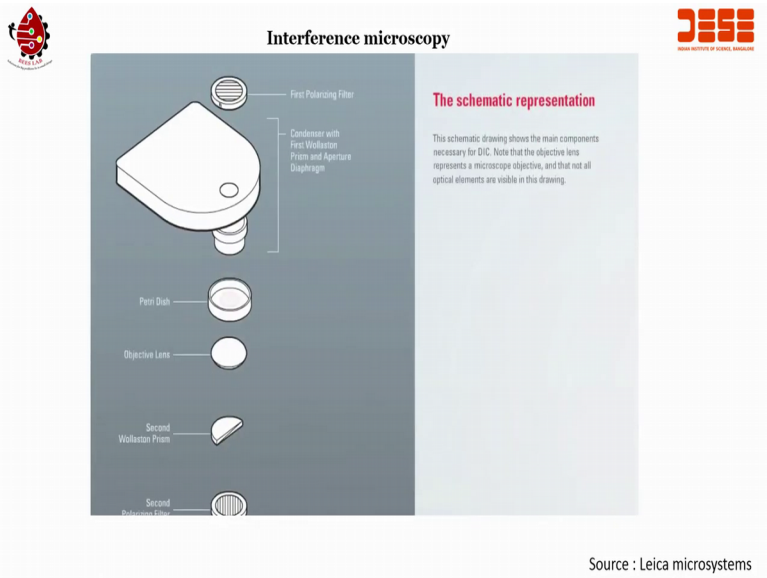

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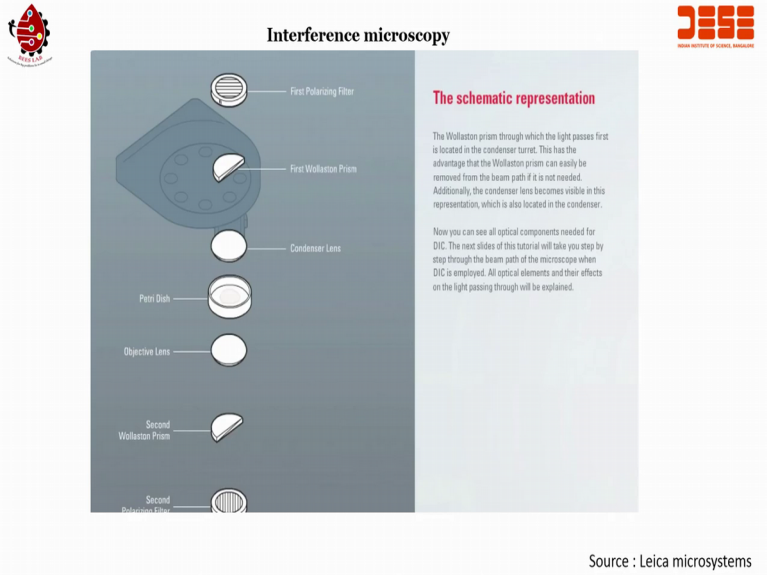

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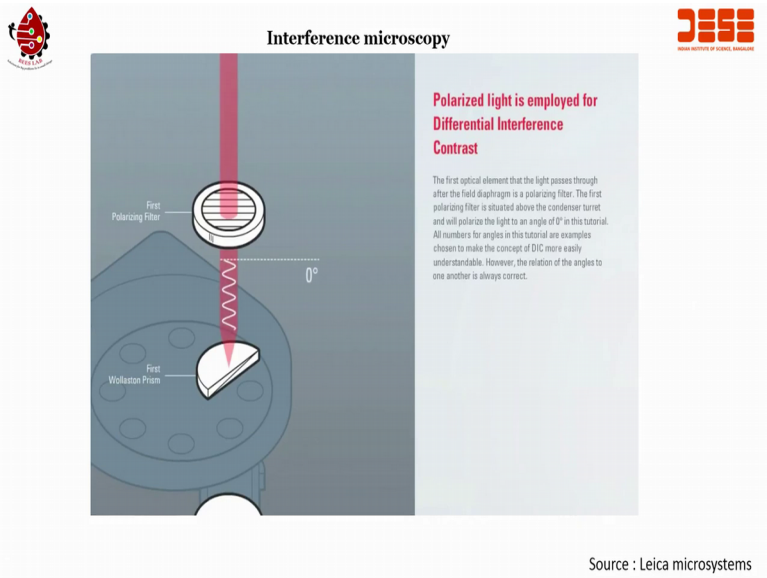

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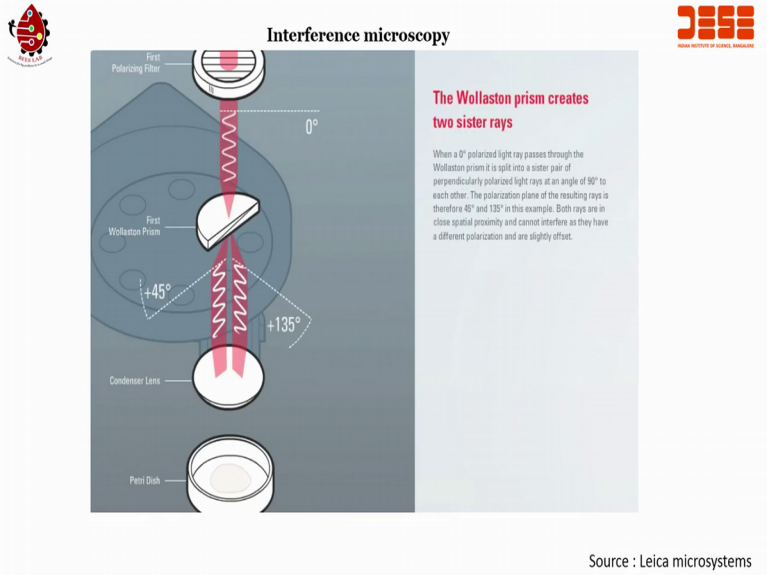

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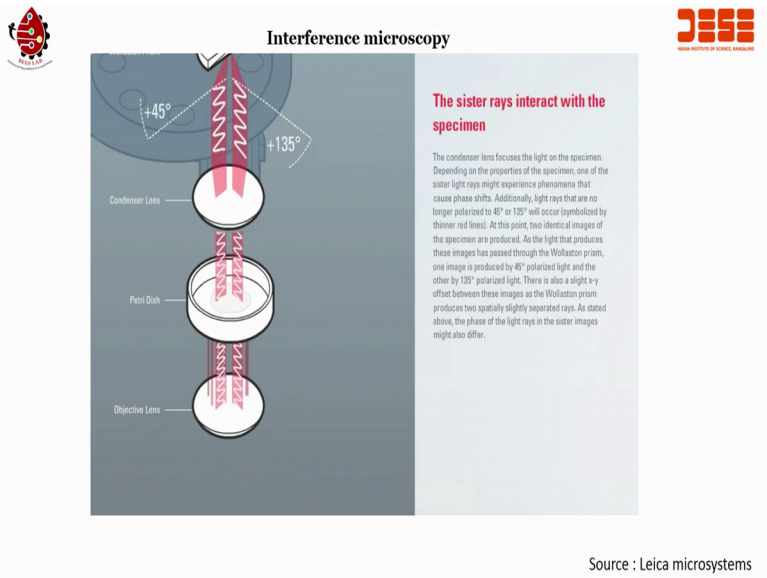

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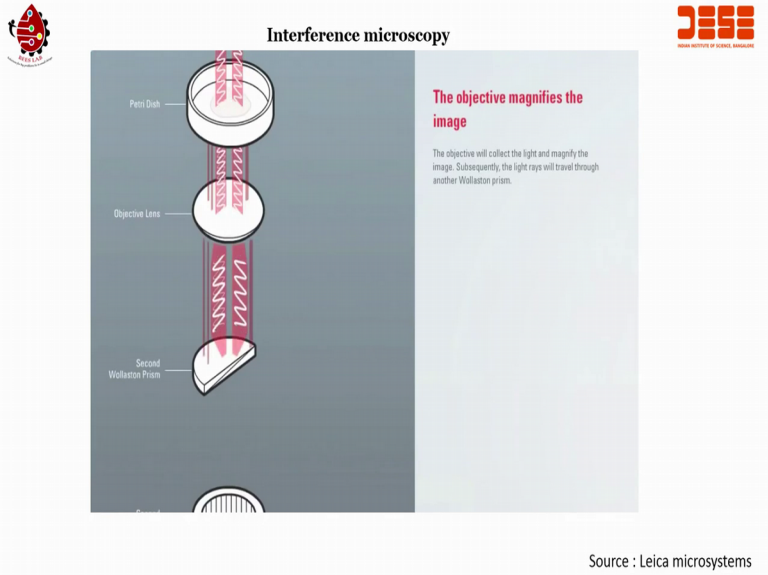

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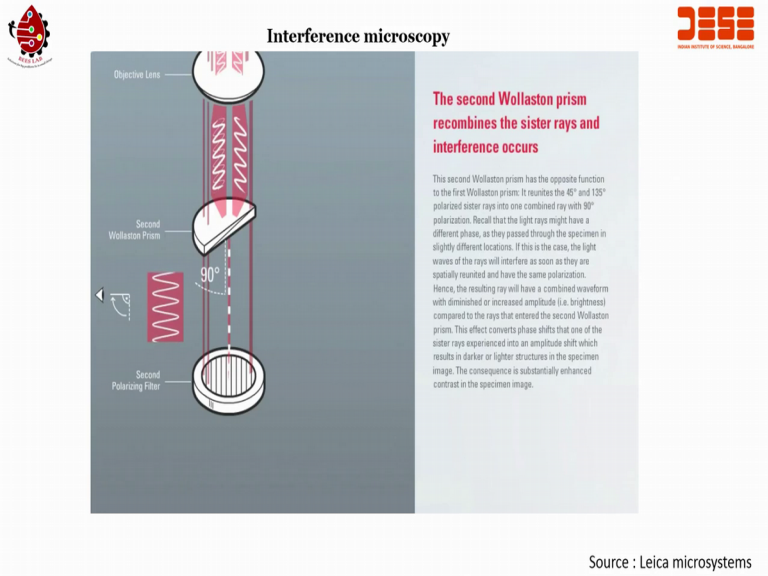

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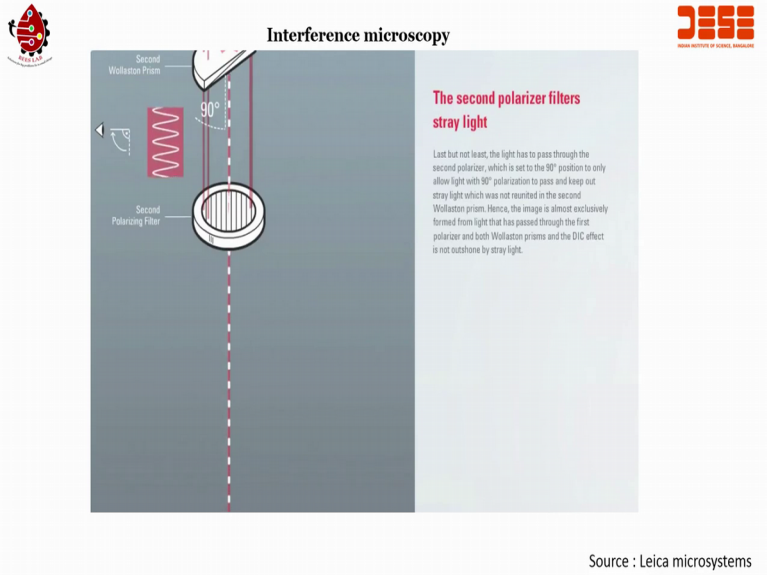

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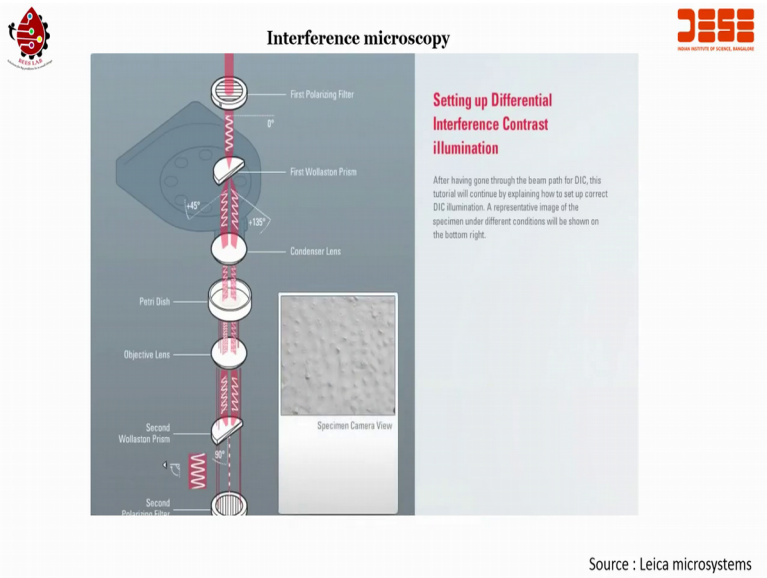

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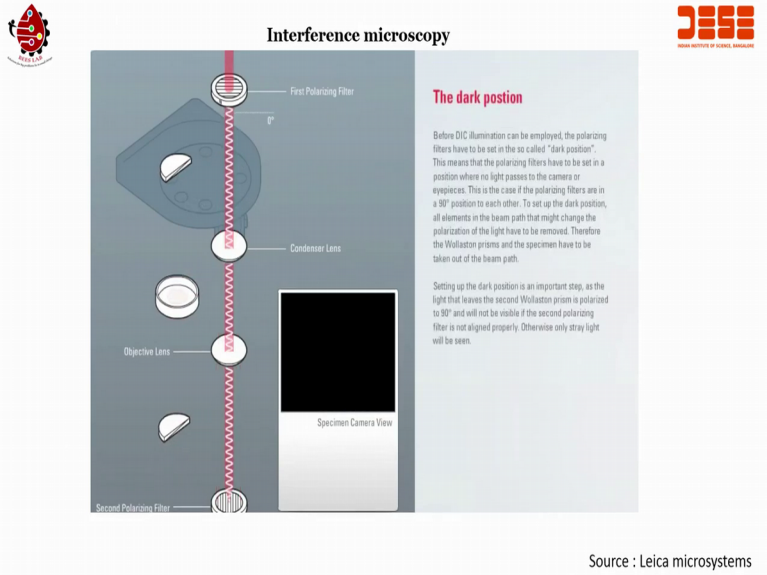

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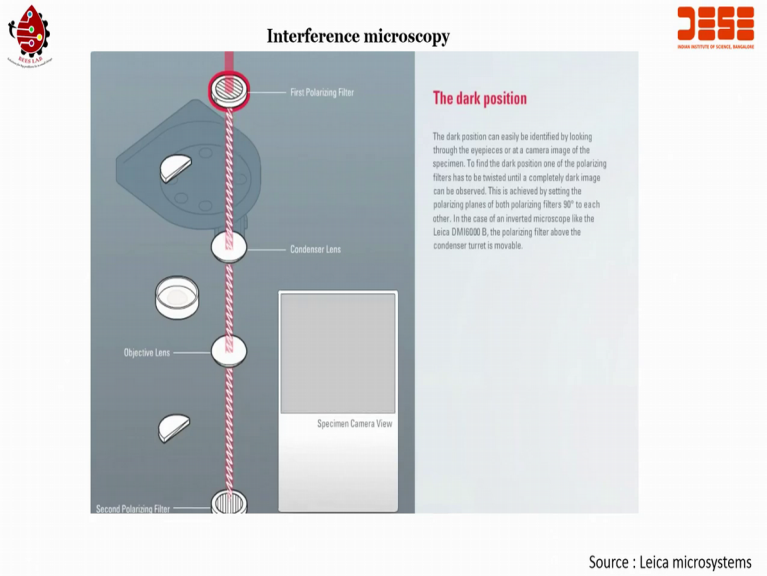

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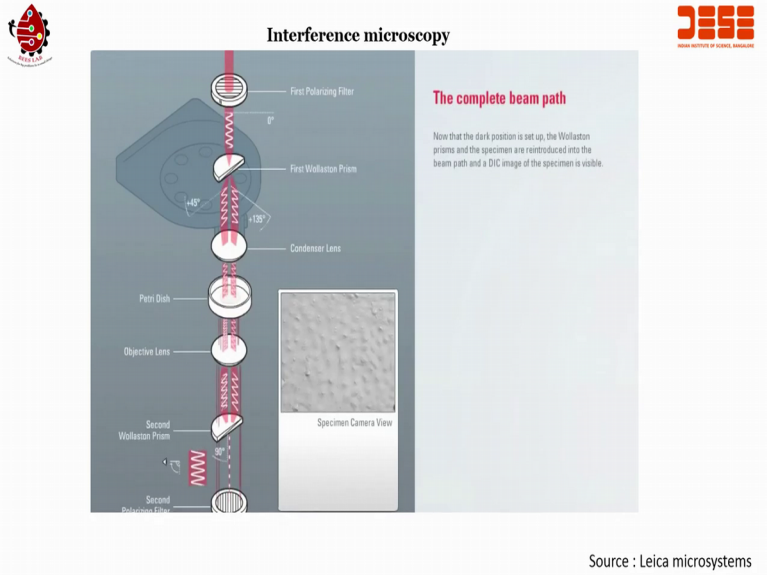

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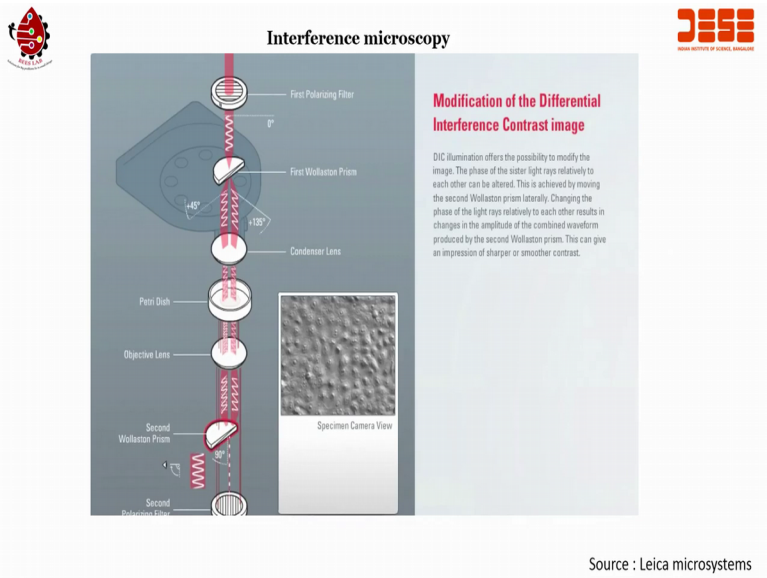

Alright guys; so, what we understand? We understand there are a chemical techniques and there are optical techniques or microscopic techniques. We have seen six different microscopes and here in each of those videos you were able to understand, how each microscope would work for would work and how we can use it for so; this all three, all six microscopes can be used for understanding the cell morphology.

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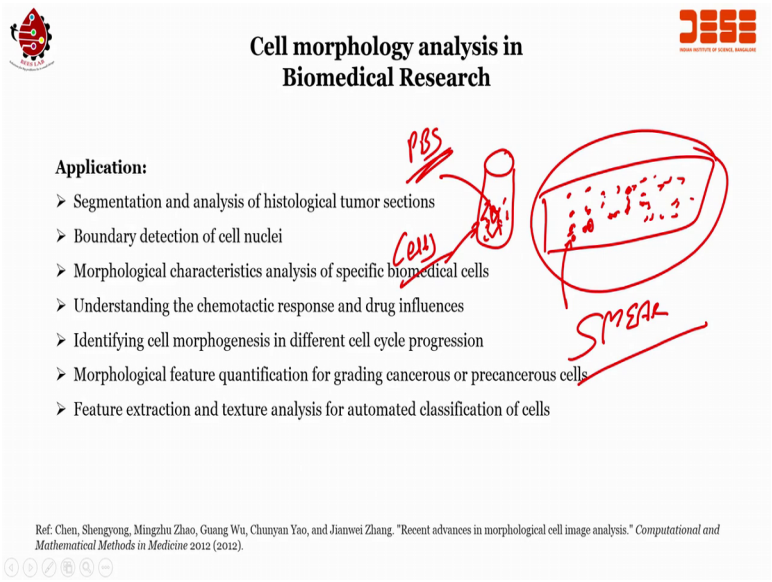

Now, if I want to further understand what are the research components or biomedical research components, if you want to understand the analysis of the cell morphology then we can see that if the applications are in to understand the segmentation and the analysis of histological tumor sections, boundary detection of cell nuclei, morphological characteristics analysis of specific biomechanical cells, understanding the chemo tactic responds and drug influences.

Identifying cell morphogenesis in different cell progression morphological features quantification for grid cancerous or precancerous cells and finally, feature extraction and texture analysis for automated cell classifications right or classifications of cell. So, there are several application if we understand the morphology and we want to do the analysis right. So now, to understand this, right let us see what can we do?

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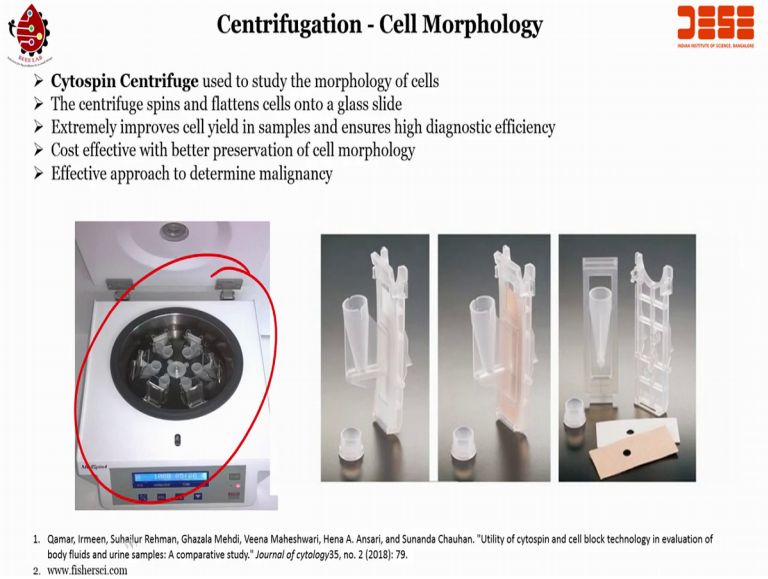

So, before we go to that the, the way it is carried out the current technique that we use to screen the patient, let us first understand a technique called Cytospin alright. Before, cytospin let me draw a slide. So, you understand why he had to go for cytospin. Now, generally when the cells are taken from the mouth ok; the cells are taken from the mouth with the help of a cytology brush, when it is taken from the mouth or oral cavity, it is placed in a solution, in a solution call phosphate buffered saline or P B S. So, there are cells in PBS.

These cells in the pathology lab, they are smeared on the glass slides, they are smeared on the glass slides. So, how the smearing works? You take the cells, put a drop here and then take another slide and then you push the slide or smear the slide across the bottom slide. What will happen? When you do that, when you do that the cells will be this, it gets spreads like this. This is for smearing alright.

The disadvantage of this particular technique, disadvantage of this particular technique is there is a double nucleation sorry, there is a the cell will get it will be clumped, another way we can say it will be clumped right. So, it is very difficult to identify the boundary of itself and it will not be uniformly distributed, not be uniformly distributed. This is with the help of smearing the cells SMEAR, S M E A R right. You understand when you take the cells from the oral cavity. You place in the you place along with PBS and when you take it out and you smear it, then you will find the clumping of cells and clumping of

cells will not help the oncopathologist to clearly identify whether there is a change in the morphology of the cells or not.

So, to avoid that we can use a system called cytospin. I will discuss in detail about cytospin, in the next module, but let us see right now, how the cyto cytospin works? Cyto centrifuge, cytospin centrifuge used to study the morphology of cells the centrifuge spins and flatten cells onto a glass slide extremely. it is, it improves the cell yield in samples and ensures high diagnostic efficiency. Also it is cost effective with better preservation of cell morphology and effective approach to determine the malignancy alright.

So, we can see this particular cytospin centrifuge system and we have the video for that as well and then you can see here the loading system for the cells and you load the system in the cytospin so that you can load the glass slide here and the cells here. So, at a very high speed the cells will be smeared on this glass slide and the advantage will be by using this technique, you can have you can preserve the cell morphology and you can have a uniform distribution of the cells. Alright, that these two advantages you can preserve the cell morphology and you can have uniform distribution.

Now, why it is important to have uniform distribution, because if I have uniform distribution of the cells right, then I can identify, whether the morphology of this cell is different than this cell or all the cells looks alike right. Also there are a lot of things that we need understand double nucleation cell to nuclear set up a same ratio and whether the cell shape is change right. So, lot of things are there that we had to take care when we are understanding the morphology of cells. So, the spinning on the glass slide with the help of cytospin will help us to preserve the cell morphology compared to the smearing.

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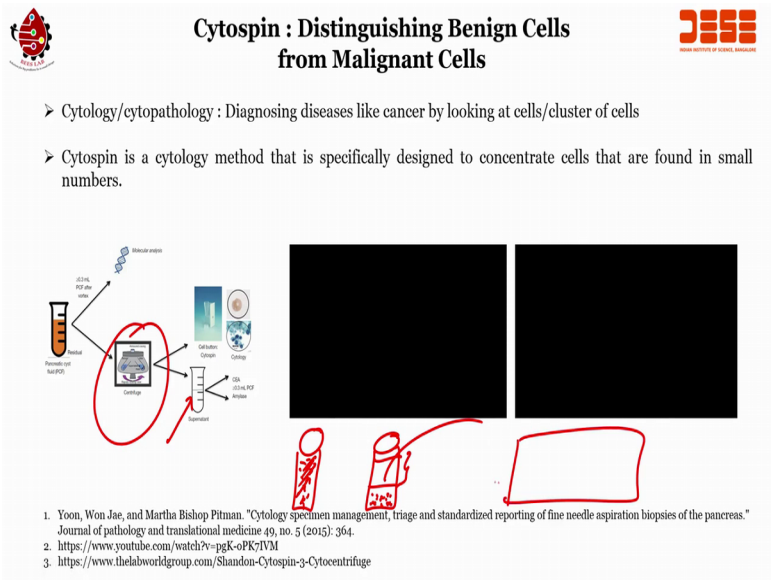


Now, how to use cytospin and why? Cytospin we have to use it for understanding or for delineating benign and malignant cells right. Why it is very important? Now, I already told the importance of that that the importance is; so, that we can understand the cell morphology right, cell morphology is so that we can understand whether the cell shape is change or not. Now, the diagnosing disease like cancer, at looking at the cluster of cells is a help of cytology. Cytospin is a cytology method that is specifically designed to concentrate cells are found in a small numbers.

So, there are two things one is a cytology which is cytospin based. Cytology is the study of cells by the way, cytopathology is study of the cells whether there is a change in the shape or (Refer Time: 32:42) the from the pathological angle. cytospin is a system that is used to smear the cells on the glass slide right. So, cytospin is a cytology method to that is specific to design and concentrate the cells that are found in small numbers. Now, if you see here you see, there is two system one is the centrifugation and then another one is the supernatant. So, see in this particular case let us say if I have a blood and I want to separate the blood cells, then I will use the centrifuge.

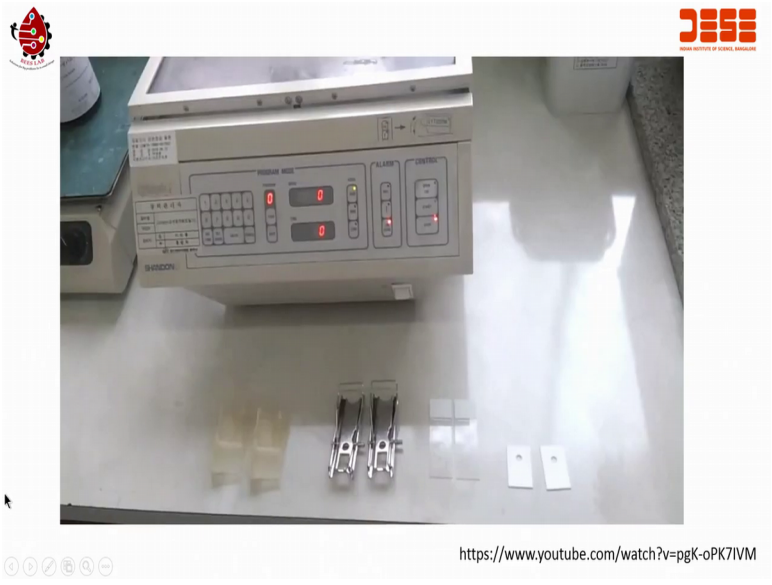
So, centrifuges that it will put the cells in the in the bottom and that blood cells would be at the bottom, because they are the heaviest one and you will see the layer of the cells and finally, there is a supernatant. So, in general, if you have just cells in a PBS and you want to wash the cells multiple times, you can use centrifuge. What will happen that

whenever you wash the cells right and you put in this, in this particular device then what will happen?.

When you centrifuge it, this cells; so, let me just draw it correctly that help you to understand properly right. So, here the cells are everywhere along with the PBS solution. When you do centrifugation or when you centrifuge the cells then the cells would be at the bottom and the top will be supernatant and that supernatant we can discard it, we can throw it out alright. So, because this we do not require it. So, when you have to wash the cell multiple times you can use centrifuge when to when you want to smear the cell on the glass slide, you have to use cytospin alright.

Cytospin also works in the centrifugation technique, but the system is such that the design of the, cytospin is such that it will smear the cells on the glass slide, instead of just separating out like supernatant and the cells at the bottom alright. So, let me play these two videos and you will understand the things in detail. Anyway like I said, I will tell you the importance of cytospin in my next module, you will understand it better alright. Let me play this first video.

(Refer Slide Time: 34:53)



(Refer Time: 34:55) cytospin I just (Refer Time: 35:00) previously in the ok, we (Refer Time: 35:38) from the first you know (Refer Time: 35:40).

(Refer Slide Time: 35:40)

In this you know (Refer Time: 35:42) make them to do (Refer Time: 35:59).

(Refer Slide Time: 35:59)

(Refer Slide Time: 36:03)

Go.

(Refer Slide Time: 36:23)

(Refer Time: 36:25) some (Refer Time: 36:26); obviously, the right. Now, let me play the second video.

(Refer Slide Time: 36:31)

(Refer Slide Time: 36:42)

This is the Laurell W S 650 H Z spin code. It has a maximum rotational speed of 12000 RPM to controller allows operator in a action in real time during the process execution including buzzing time, stopping and continuing on from that point. The closed ball design allows most coating materials to dry in a quiescent state increasing uniformity and minimizing particle contamination. This convenient tabletop unit is powered by 100 and 20 volts AC single phase. It also requires vacuum, to be at least 15 inches of mercury for more.

(Refer Slide Time: 37:26)

(Refer Slide Time: 37:40)

First, I like to give a demonstration of the speeds, so that I am going to use a other timing strip. First, this is the contact control panel completely self contained, I am going to call up a program here. Program for and what we will first do is we will change the spin speed to let us say 4000 RPM. Here we go again, leave a raspberry pi vacuum backing. It tells you that you ready and ok, when it is done in order to go on and run another program or any other process, we must open the way this signals and then it is ready for another one.

What I will do next one, here is a double back to let us say 8000 RPM and we would be open it. We done, we always say done and I have to (Refer Time: 40:40) vacuum is satisfied and your nitrogen purge, it will tell you that it is ready, would you want more spin and would you this at the maximum RPM of 12000 so on, on this it is done.

(Refer Slide Time: 41:40)

Next, we are going to spin a small wafer. It has this very-very nice little adapter here, that you can fit right on top with the standard chuck raspberry pi vacuum.

(Refer Slide Time: 42:10)

And we will spin this wafer with the natural spin next.

(Refer Slide Time: 42:52)

I have this chart here and this is wafer size versus spin speed um; obviously, the smaller the workpiece the (Refer Time: 42:59) need to spin and I given a little guideline here, first we ran (Refer Time: 43:04) up to 12000. Now, we are going to run a 100 millimeter wafer as suggested up to 6000.

(Refer Slide Time: 43:24)

This epsilon 50 (Refer Time: 43:21) is coming here fly vacuum and we will also edit that speed down to 6000 RPM and open the (Refer Time: 43:58) off the vacuum those are wafer.

Now, the spin coater has the capability from actually being able to spin of 4 to 100 millimeter or 8 inch wafer.

(Refer Slide Time: 44:57)

However, it is the now requirement what are we do. So, you have to remove the top and take off this plastic splash board, exact just this enough from (Refer Time: 45:05) It actually has the capability spin a 8 inch wafer.

(Refer Slide Time: 45:17)

However, what is important also in particular unit, is that has the capability of spinning a 4 5 inch by 5 inch square substrate. I have a piece of glass here.

(Refer Slide Time: 45:25)

And I am use the demonstrate in the (Refer Time: 45:29) and even though it is approximately seven half inches across here, if you look through the top, it still comfortably fits in between the splash you. I am going to spin this one. Since, this is closer to the very larger size which they recommend between two and half and 3.5 k r p m. I am going to spin this 3500 and here we go. Vacuum first, there we go ready, light, sound, start sizzlers. Here, we have it very versatile, very compact, lightweight, fix in anywhere and very easy ok.

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So, this will be the end of this particular module and in this module like I said we are just understanding the morphology of the cells and how different techniques are used to understand the cell morphology right. And, in the next module we will look in detail, how this cytospin system works.

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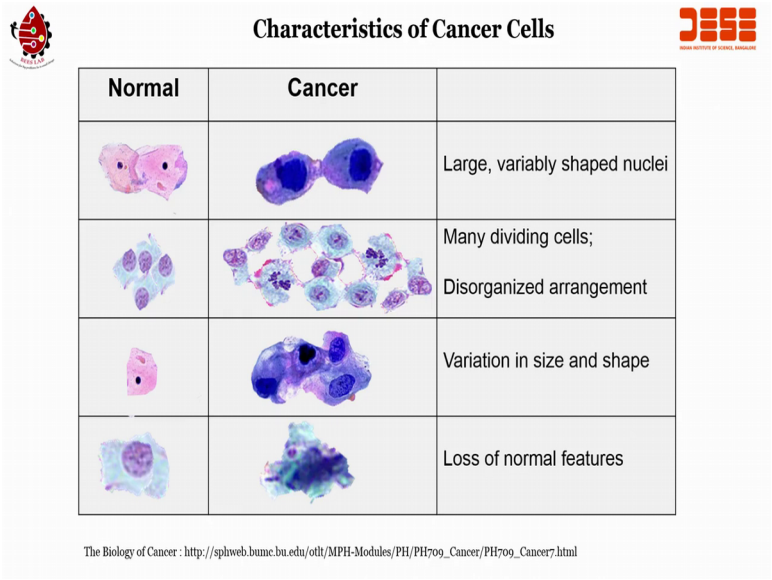

And along with the cytospin system, we will also talk about how the characteristics of the cancer cell helps us to understand, whether the characteristics of the cells helps us to understand, whether the cells are from normal region or from the cancerous region alright. So, this is a last slide for this particular module and in the next module, we will talk about the remaining stuff along with the details about cytospin system, because I want to teach you how easily cytospin system looks like and why we want to go for cytospin. So, I will make a PPT for you.

So, that you understand the importance of cytospin alright, but for now, just go through these videos these are more like informative videos. So, that you understand how the system works like right, but again to understand the cell morphology and to delineate the cells based on the cell morphology is our goal right. But, and from that we will understand whether the cells are atypical that is it has change the morphology or it they are normal alright. And if there are atypical then the patient is asked to go for the histology.

So, we will talk that thing in the next module, till then you take care, I will see you in the next class bye.