Introduction to Cell Biology
Professor Girish Ratnaparkhi
Professor Nagaraj Balasubramanian
Department of Biology
Indian Institute of Science Education and Research, Pune
Introduction to Microscopy – Part 1

My name is Nagaraj, and I am going to continue this section that is the class after, set of classes after Girish has taught. And the intent of this set of classes is going to be to kind of introduce you to the cell. I think I have been at IISER, Pune for 12 years now and I run a research lab here, and my lab studies cells, and I have studied cells for a very long time too. And the thing that is really interesting and remarkable about cells is that we are still discovering new things about them.

So, there are things we know about cells, there are things we do not know about cells. And this set of lectures is kind of aiming to introduce you to some fundamentals about how to think about cells, how they are built. There is a lot of talk these days about making an artificial cell and bringing together many components to kind of define or make a cell. And to do anything like that, we will need to have a fair understanding of how cells are put together to begin with.

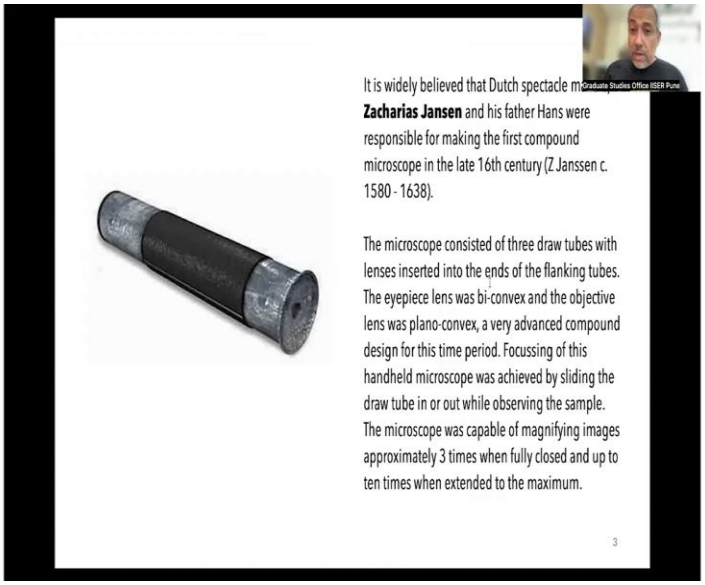
And so the intent of this class is to kind of give you that flavor of what cells are like. There are fairly interesting things and they do very complex processes and events very seemingly. I think something as simple as me talking to you today and you being able to wake up and understand everything that I am saying is driven by cells. There are cells in our brain that are firing, allowing me to communicate what I am thinking. And it is happening very, very rapidly. The thought about what word I am going to say next happens a fraction of a second before I am saying it.

And then this sound as it travels to you and you are able to tune in and listen into this, your brain also almost immediately processes every word that comes your way to kind of make sense of what I am trying to say. And all of this is happening because of cells. And so how cells work together, how are they put together, what is the architecture of cells like, contributes to everything that is happening here, by the way. So, that architecture is what we are going to try and introduce you to, I am going to try and introduce you to.

Clearly, you guys have been exposed to a lot of new things in the first set of lectures. And what we are hoping we will do is get you to think about cells in the remaining half. Think about how they are put together. My hope is at the end of this class if somebody set up lectures, if

somebody turns around you and say, tomorrow if you had to build a cell, how do we do this, you will have a sense of how to think about it. And I am going to quickly walk you through the thought process on why I think microscopy is something that I wanted you to be introduced to before we do cell biology.

(Refer Slide Time: 04:08)

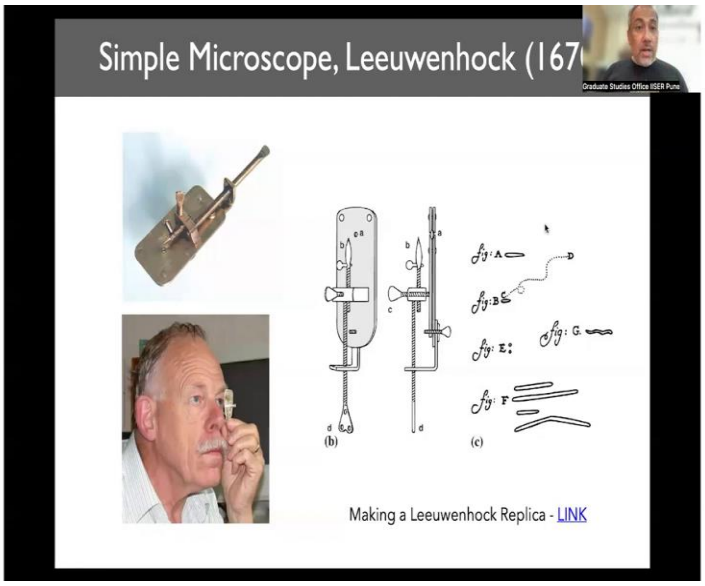


And it is a very interesting area, which has dramatically affected, how we think about cell biology. And the reason we think about microscopy in the context of biology of cells is rather simple actually, because a lot of our understanding of cells has emanated from the fact that there

is all this information that is available about cells and a lot of that has come from looking at cells and looking at what they do. So, that is vital to our understanding of cells.

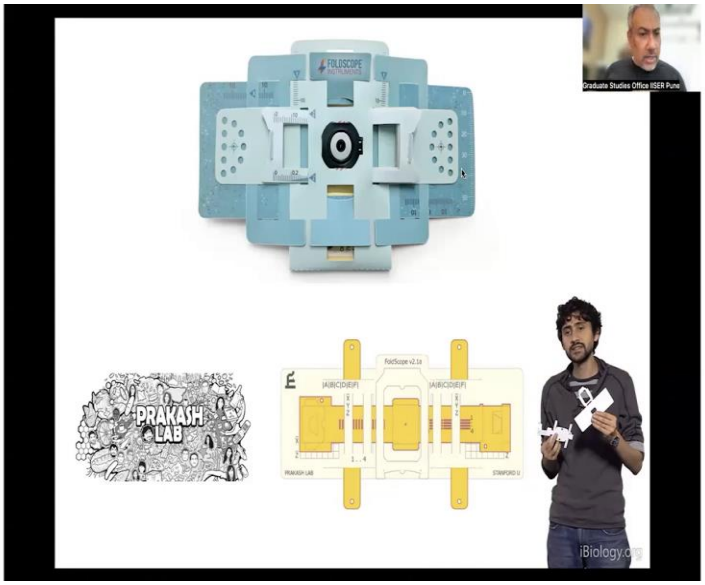
And it all began with the discovery of something that was the origin of a microscope by Zacharias Jansen, and this was somewhere in the 16th century, 1580 to 1638 is when he lived. And this looks more like a telescope than a microscope. But it allowed you to magnify things up to about three times. But that by itself changed what we were, what they were able to look at.

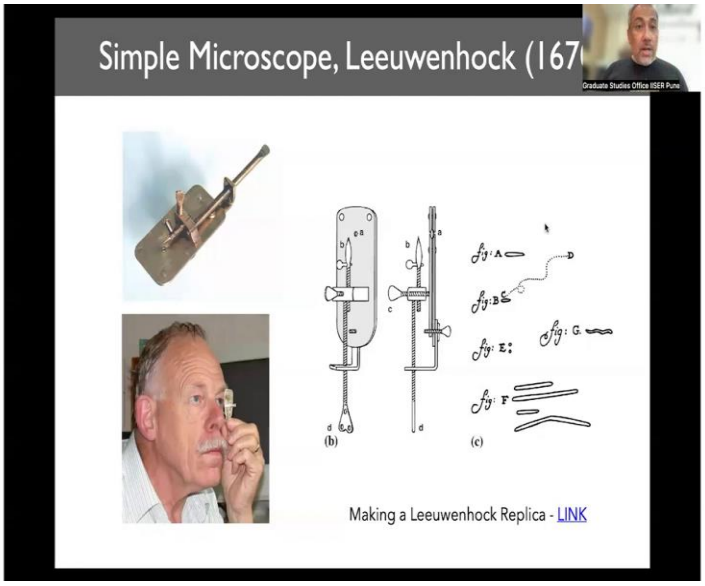
(Refer Slide Time: 05:28)



And the fact that they were able to look at new things allowed for us to now start noticing things that were happening that we could not otherwise. The very simple microscope that Leeuwenhock developed, this is a replica of the same. And as you can see the optics were very simple, the light source was actually white light and he was able to look at things, look at movement of stuff. And just the idea of being able to look at these things, things that are happening in a drop of water, for example, from a pond, can be quite remarkable if you have never seen them before. And it is this curiosity that even today is being exploited. So, Leeuwenhock made this microscope in 1670.

(Refer Slide Time: 06:28)

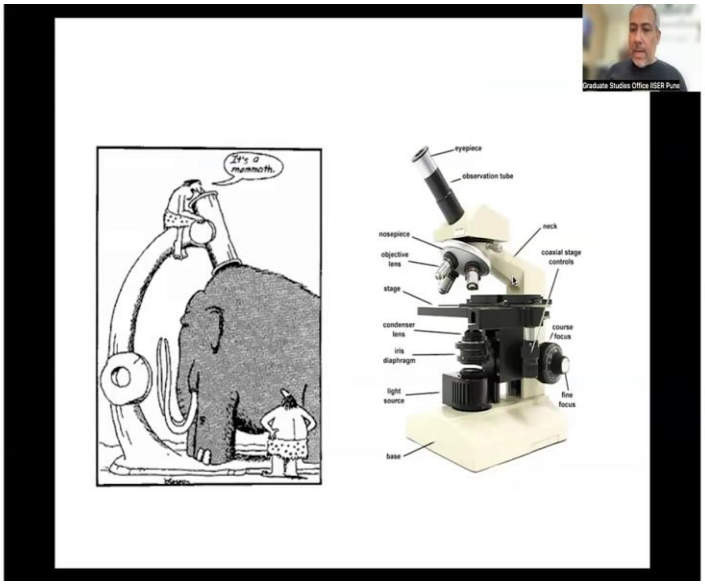




And I do not know how many of you know about Manu Prakash, and this foldscope that they developed a few years ago. And it is a remarkable thing. Manu's lab is in Stanford, and they develop, among other things, tools, they, and this is a paper microscope, which kind of takes its inspiration from what Leeuwenhock built so many years ago. And this paper microscope, the amazing thing about this is, it gives you significantly higher resolutions, and you are able to see things like, for example, drop of water from your local pond or even from the tap in a way that really sparks your curiosity.

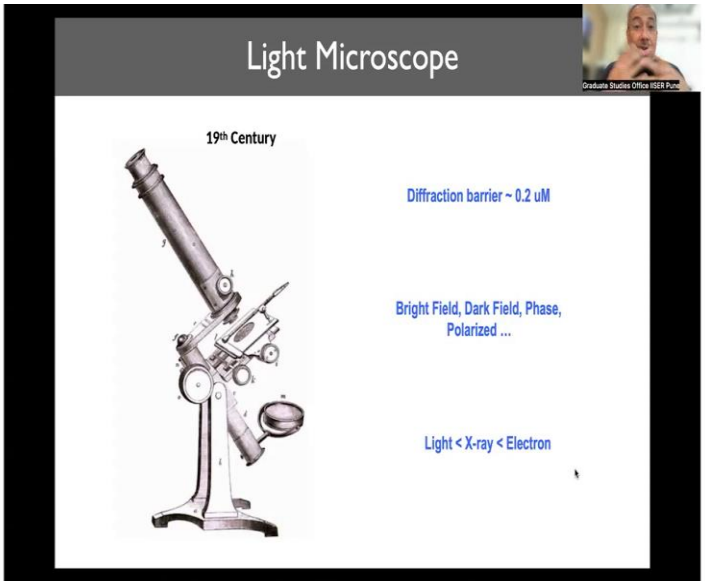
So, if you never handled or looked at a foldscope, go look it up. Foldscope is available to buy in India as well. There is a company called Guddy Lab which sells foldscope in India, and it comes like a small pencil box. And it is a really remarkable thing to see. And what you realize is there is an immense power to be able to look at things at a magnification that allows you to see that they exist, that there are things that move around that cells, which you normally would not see, once you start being able to see these things, you are now curious about how they are being put together, how are they functioning, why are they doing, what they are doing and how are they doing what they are doing. So, that is the real power of being able to look at something.

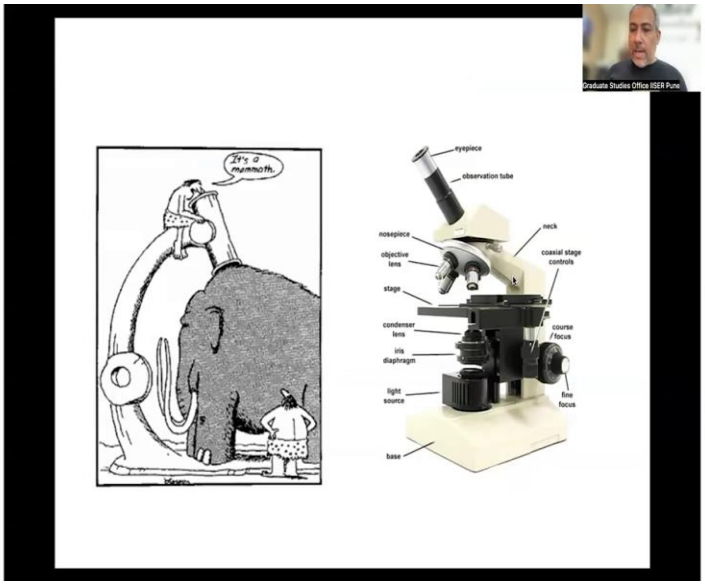
(Refer Slide Time: 08:16)

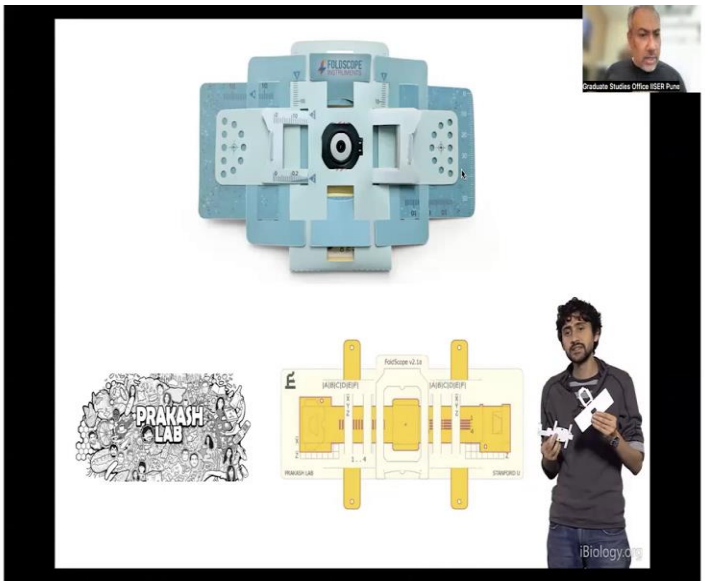


And clearly, the microscope, as many of you may have used, has gone a long way in kind of raising that curiosity. It is never been perfect. So, it is also possible that what you see you do not fully understand, but that is fine. As long as you see and you make observations based on that and you are curious enough to find out or think about what is actually happening, the hope is with time you will have an understanding of the same as well.

(Refer Slide Time: 08:52)





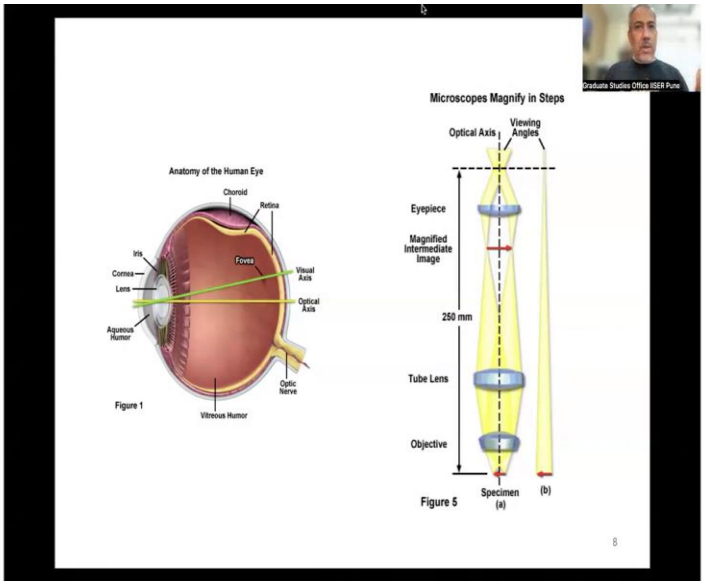


Now, the thing to remember here is that a lot of the visualization that happens through microscopes, particularly the light microscopes, so everything including this particular microscope or Manu's foldscope all light microscopes. So, essentially, the source of light that is being used to look at stuff has a significant impact on how you can resolve things.

And this is something to go think a bit about that what exactly is this diffraction barrier. It is something that allows you to be able to separate two objects as being two distinct objects provided the beam of light or in some case x-rays or electrons, if you are using an electron microscope, is able to distinguish between these two objects as being two distinct objects.

So, if you have two objects that are really close to each other, the magnification at which you are looking at it, and the way the light source is able to separate these two objects significantly affects your ability to see them as two distinct objects. And that is limited by in light by the fact that light has a certain diffraction barrier.

(Refer Slide Time: 10:30)

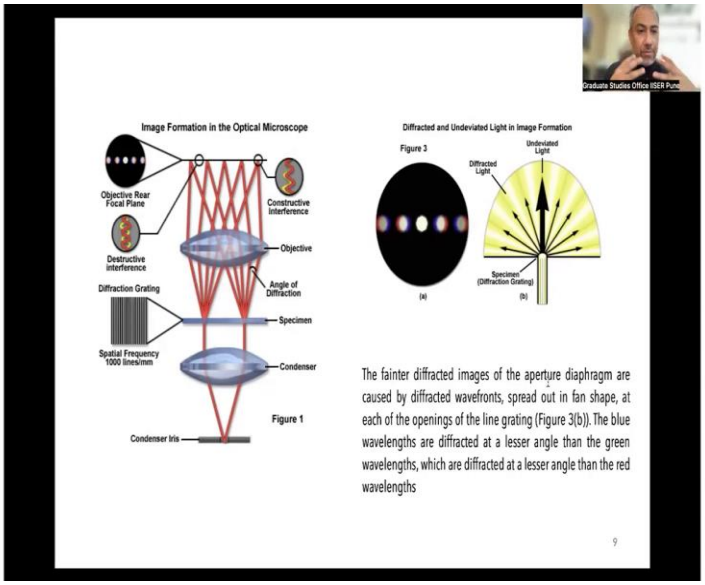


And there are, of course, classic examples of how light is used to perceive objects, the fact that you are looking at a screen and are able to see what is happening is driven by the eye and how it is able to observe light and focus it and allow you to see things. And those kinds of principles are built into the microscope as well, where along with the light source, you have a set of objectives or lenses that allow you to magnify the object.

And then there is a receiver at the end. And the receiver at the end in a light microscope could be your eye, because it is actually looking at stuff, or in case of more advanced microscopes, it could be a recorder of some kind, something that perceives the image. And now this image is transmitted in such a way that you can see it on a computer screen.

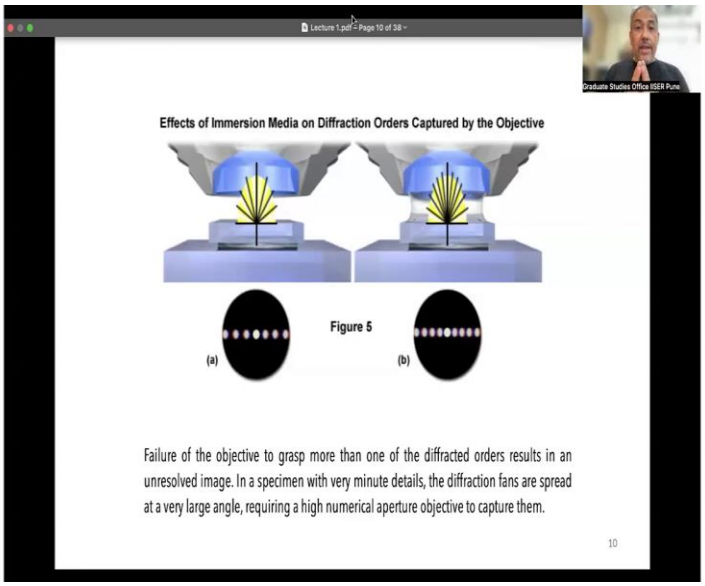
So, the idea of the eye as an analogy is that, along with objectives that are present, there is a sensor that actually responds to the light that is being focused. And now this allows you to visualize things. There are many of us who might be color blind and are not able to distinctly separate out colors and there are many other changes that could happen, which means for the eye to be able to perceive something, all these machinery have to work properly to be able to allow you to visualize something, and that is the same with microscopes as well.

(Refer Slide Time: 12:19)



So, I am not going to get into the details of how diffraction works. And if you are curious, go read a little bit about it. The critical thing for you to remember here is that the extent of diffraction that happens is going to determine the nature of the image that you are going to be able to visualize.

(Refer Slide Time: 12:45)



So, this makes a difference in also how much light is able to get into the objective that allows you to now see things very well. And air has a certain diffraction that is the moment light comes through, the way the light distributes is different when the medium that is there between the

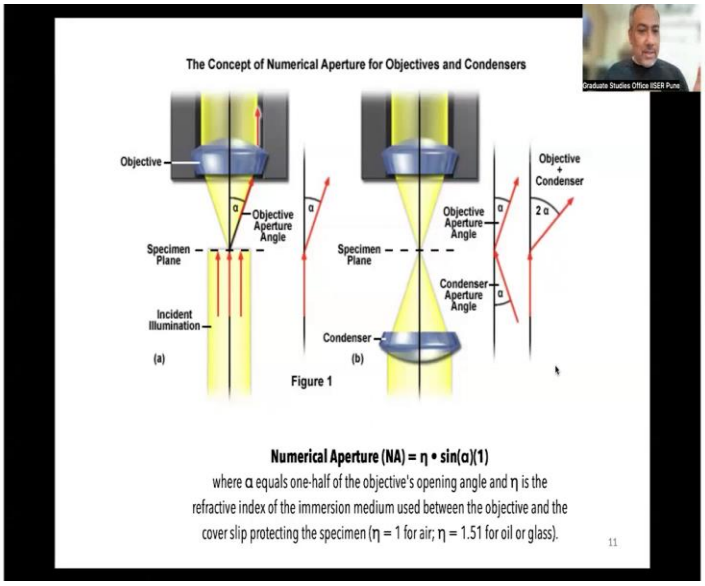
objective and your sample is air. And that can be altered by using the way a medium that allows light to now make it, more light to make it into the objective.

One of the good examples of this, and I do not know how many of you have note is that, if you have a very dark room and a small source of light to be able to, for your eye to perceive that room, to begin with, if you come from a bright source, so if you have a tube light on in that room, and you turn it off, you will not be able to see much at all, and this probably happens to all of us. But you give yourself some time for your eye to adjust. The amount of the sensitivity changes pretty dramatically. And this allows you to now see things in the dark in a way that you could not about a couple of minutes ago.

Now, that is an example of how the sensitivity of light, of your eye has changed over a period of time. And in part that is contributed by the fact that the eye is able to allow more light to get in. So when it is very bright, the amount of light could be regulated. When the light goes down the amount of light getting into the eye could be increased. And that allows for you to see things now in such a way that you could not clearly some time back.

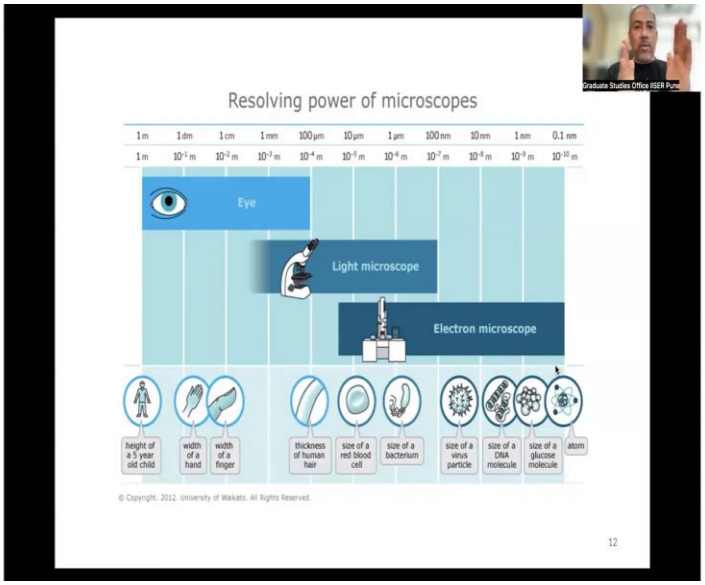
In a loose sense that is what is happening with microscopes as well. And the idea here is to be able to collect more light and send it into the objective of the microscope so that we are able to see things better.

(Refer Slide Time: 15:07)



So, light gets collected in these different ways and gets directed into the objective and this is governed by the numerical aperture. What you need to remember here is that the numerical aperture is the definition of how which light actually can get into the objective and the condenser.

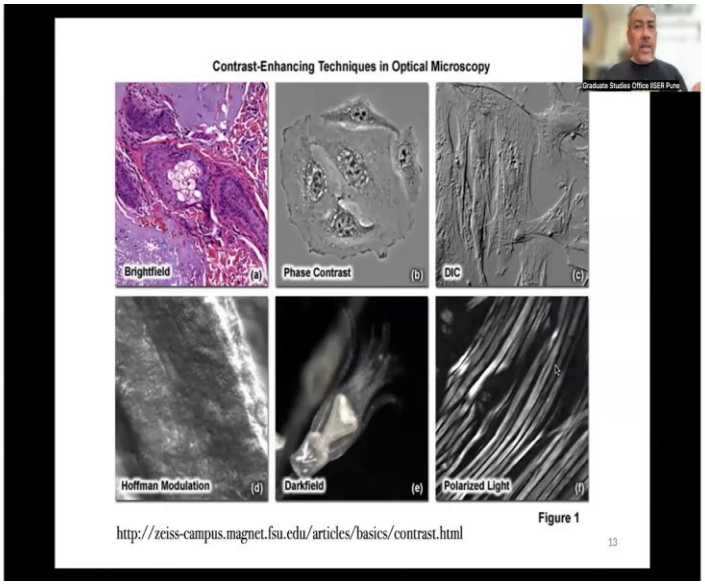
(Refer Slide Time: 15:32)



So, every objective if you see, its resolving power can be variable depending upon; one, as I said, the magnification that it has; and things like the numerical aperture has a significant effect on the resolution that you are having.

Also, as I said that the beam of light or the beam that you are not just light of say electrons that you are using to illuminate the sample has a significant effect on the quality of resolution, because light has a certain wavelength and that ensures that it will be able to only separate two objects that are, that have a separation that is more than the wavelength of light. So, if they are really, really close chances are, the wavelength of light becomes limiting in determining or being able to resolve these two objects.

(Refer Slide Time: 16:33)



There are many different kinds of microscopes, and this is just a kind of way to give you a sense of what kind of light sources can be used to enhance the contrast and be able to see things that you otherwise may not be able to see. So, even with light microscopes, there are variations here, bright field, phase contrast, DIC, dark field, polarized light microscope, all essentially using light to illuminate the object slightly differently for you to, for, to allow you to be able to see things.