Introduction to Cell Biology
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Introduction to Microscopy – Part 2

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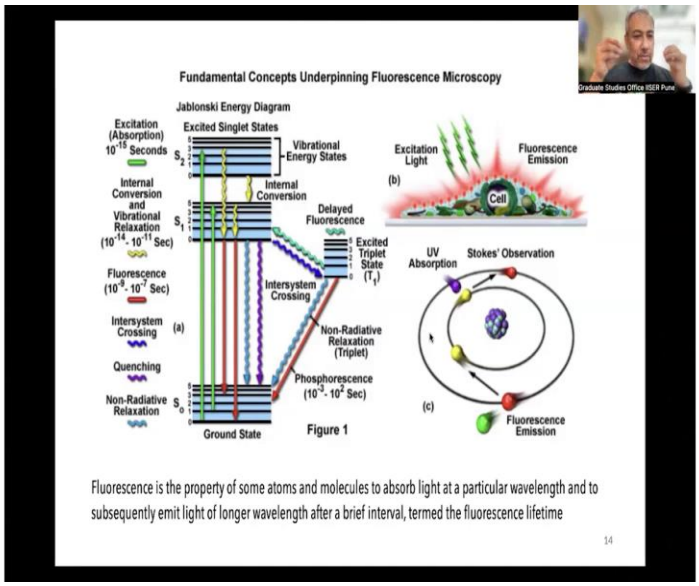

The other interesting thing about light microscopes, about microscopy also, that really came up from the fact that the, with, from the idea that you are able to hit something that is fluorescent in a cell or put something in cells that is fluorescent and hit it with light, and then measure the fluorescence energy that is emitted. So, instead of just looking at what is there, you are looking at the fluorescence that is coming out from a very specific region of the cell or a specifically labeled in some case protein inside the cell or an organelle inside a cell.

And that is determined by the fact that there is something that is fluorescent, which will absorb light at a certain wavelength and emit light at a different wavelength. And so you hit the cell or whatever it is that you want to observe with a light at a wavelength that will allow you activate the fluorescence of this object, and then you record the light that this is emitting to now be able to see this particular object and not everything else. And this allows you to be able to tag and detect very specific things inside the cell.

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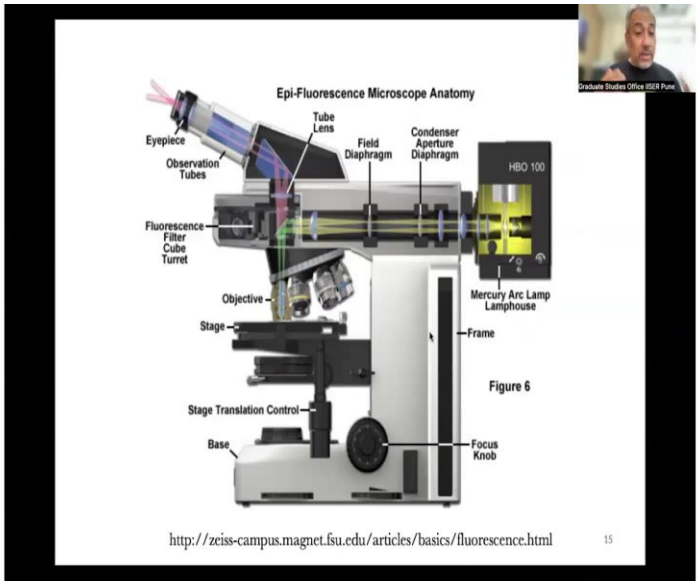

Fluorescence microscopy has really transformed how we view and think about cells.

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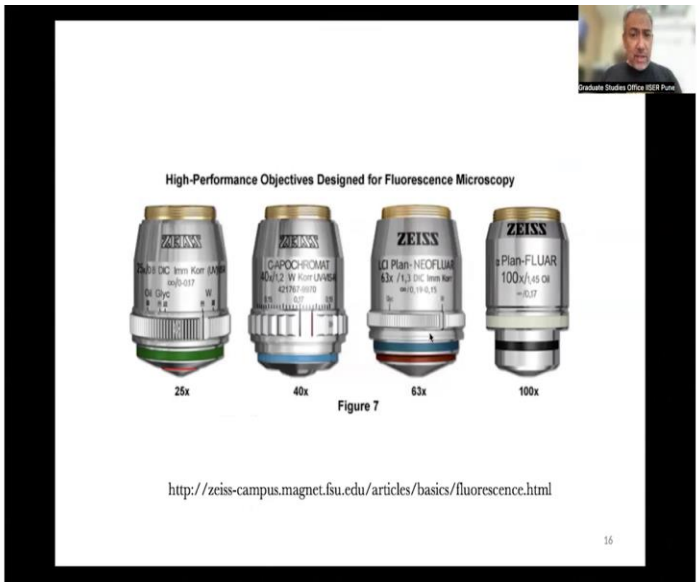

As I said, these are the different types of objectives. And as you can see, each microscope has a certain magnification. It will have a certain numerical aperture. And they together determine what exactly is the efficiency with which you can see stuff through this. Now, there are objectives which are just air objectives, where the numerical aperture that can be used to see anything using that objective is largely determined by the fact that there is air between the objective and the sample.

In some cases there are water objectives, which means you can have a small layer of water between the objective and the sample and that now changes the numerical aperture in such a way that there is just enough light to be able to see stuff that you would not with say just an air objective.

And then you have oil immersions objectives. And one of the common mistakes here is that you need to know which objective is oil and which objective is air and which objective is water. They are not interchangeable. So, you cannot take an air objective and put oil in it and think that you are now going to be able to see things better. You will not. So, the objective also is designed in such a way that this adding or presence of oil or water could affect the way light enters it. So, the objective along with what you use to regulate the numerical aperture can determine how visualization happens.

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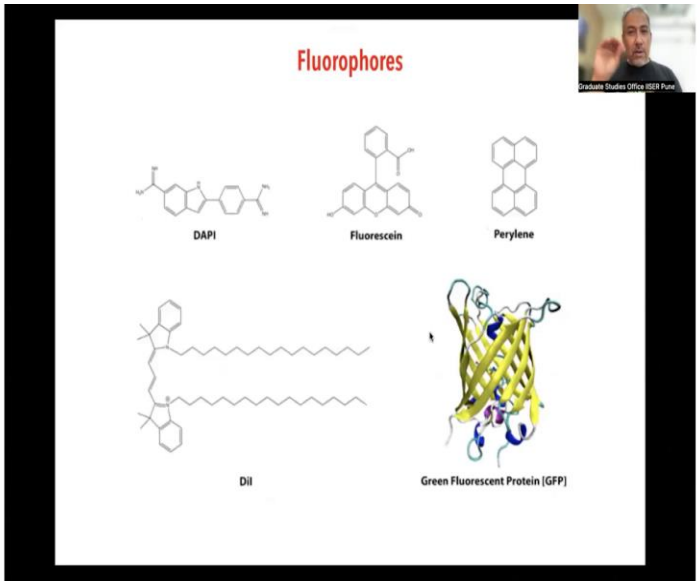

As I said earlier, there are fluorophores, which are fluorescent and can now look at, allow you to see things inside the cell. There are many different dyes, so they could be just chemical compounds. There are proteins like the green fluorescent protein, that you may hear more about in the future as well, which is, can be used to tag a protein.

So, you can take a protein and you can add this tag to it, and this can be done by cloning, taking the DNA of the protein that is of interest, taking the DNA of the green fluorescent protein,

adding it side by side, and you guys now learnt about translation, and you translate the entire protein.

And so you have the protein now with a fluorescent tag. It is like adding a bulb to the protein. And this bulb will glow the moment light of a particular wavelength hits it. And when it, when I say it glows, that means it is emitting light in a particular wavelength. And so, so that allows you to now see things these molecules in such a way that you would not be able to otherwise. And so, this can be used to look at different aspects of how cells or different things inside the cells.

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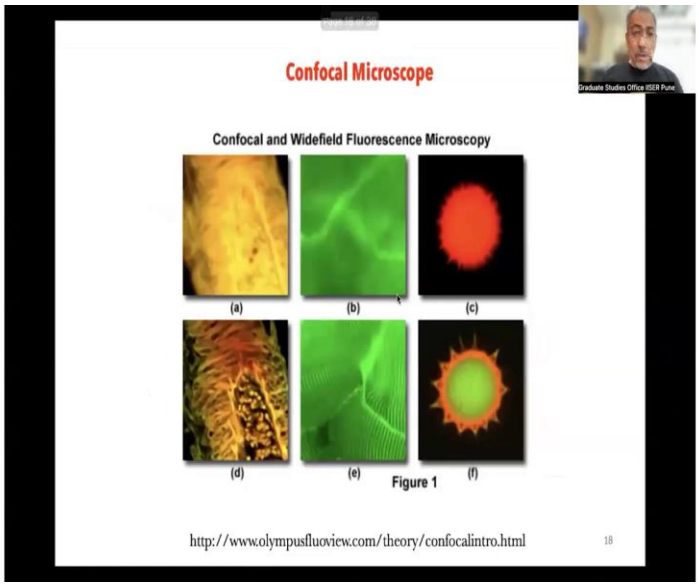

The other really major advance, so you had the conventional microscopy, you had the ability to now use these objectives to see cells in such a way that you could not before, you had fluorescence microscopy where you could tag certain things with a fluorophore and now look at that fluorophore inside the cell or that fluorescent molecule inside the cell, rather than just the cell, the other real major advance that happened with microscopy is something called confocal microscopy. And confocal microscopy is conceptually, I wanted you to quickly understand what confocal microscopy does that is different.

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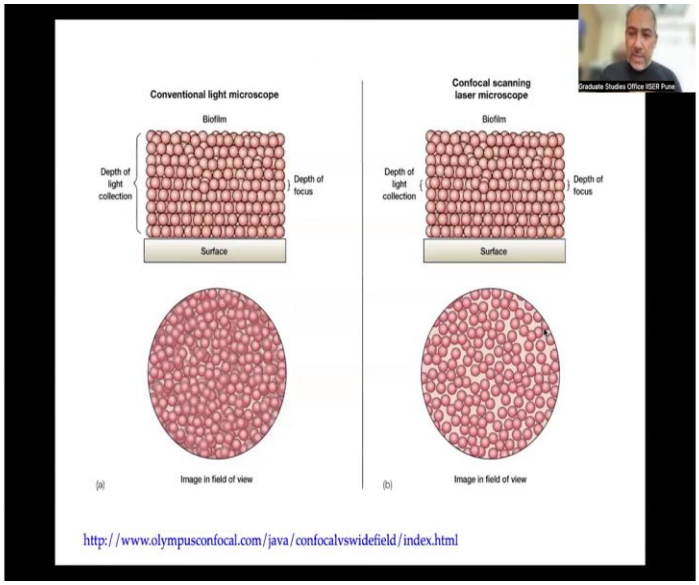

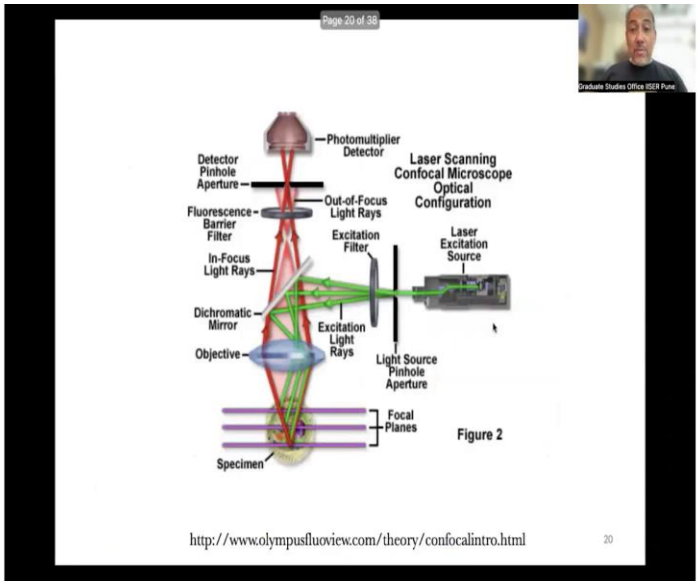

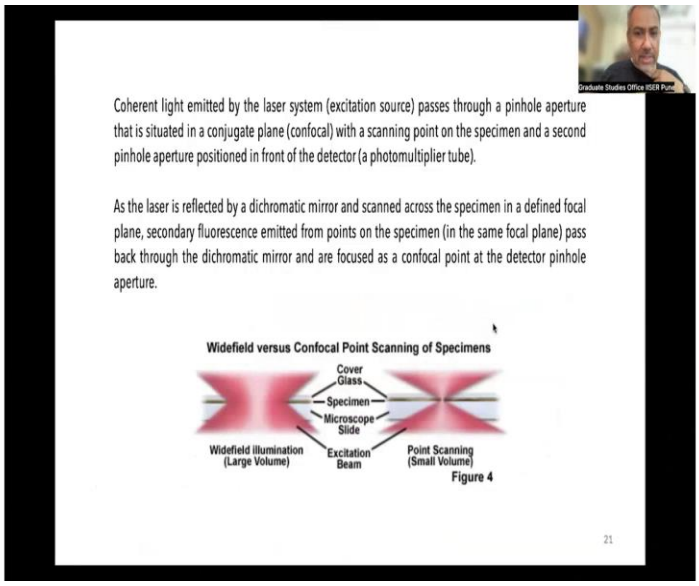

And what it does different is very beautifully illustrated here. Now, if you have an object and it has many different layers in it, because it is an object that is a three dimensional object, even if it is a cell, even if it is very small, it has a certain depth to it. And when you visualize something, and imagine you are visualizing something that is a stack of things like this, bunch of balls that are sitting on the surface. And when you use the objective, the focus, you are focusing actually, on a very small area. So, the depth of focus that you see here is where you are focusing on.

But the depth of light collection which is from where light is collected into the objective is not just in the depth of focus, it is from above and below as well. So, when you look at an object that looks with that kind of depth of light collection, what you see is that not only can you see that particular plane, but you can see be diffused light that is coming from above and below. That essentially means your image that is in that particular plane of focus is kind of altered or is made fuzzy by the fact that you are collecting light from above and below as well.

What a confocal microscope does, which is quite remarkable is interesting, is that it will allow you to visualize at a particular plane. But more interestingly, it will activate or shine light only in that particular plane, and it will also collect light from only that particular plane. So, that means an object that looks like this now the depth of light collection is also restricted, you can see that your object has, what you are seeing has been dramatically clarified, because light coming from above or below is no longer collected.

So, this makes visualization really interesting and really clear in ways that would not be possible otherwise. I am not going to get into these aspects.

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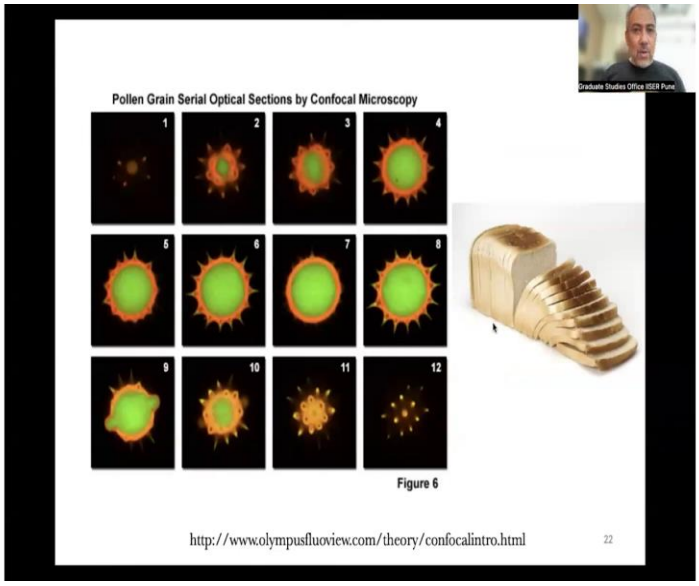

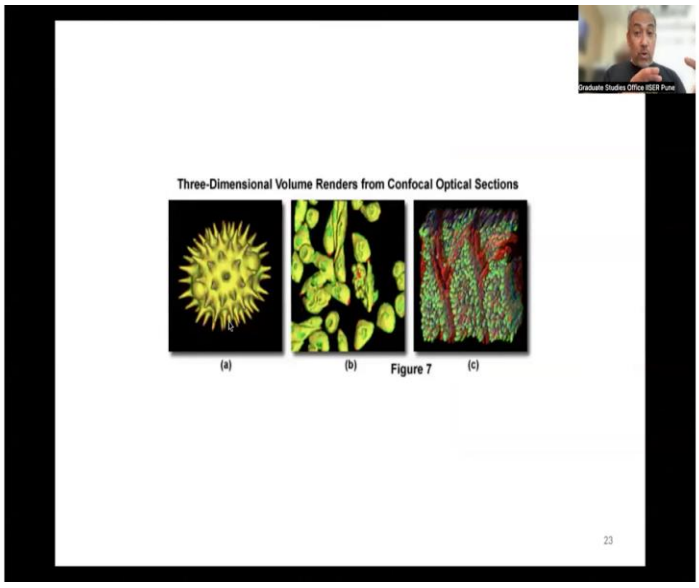

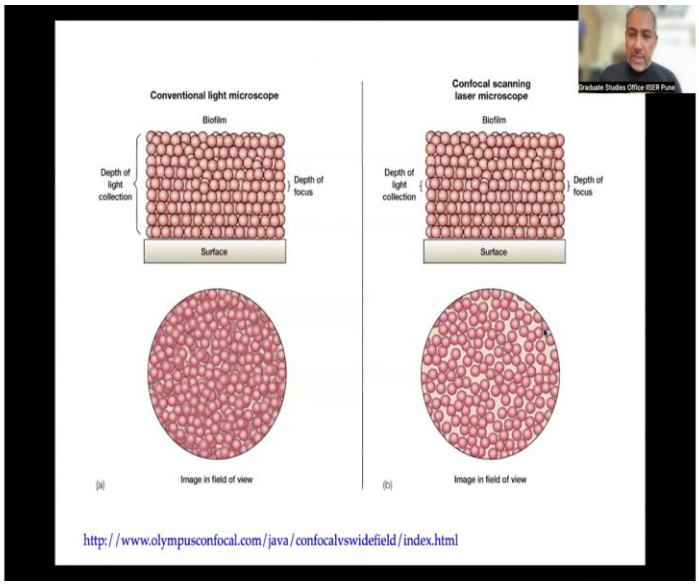

What I wanted you to look at is also this other interesting aspect of a confocal microscope, which is because of the fact that you are able to collect light from one particular plane, you can actually move the plane and collect light in sections. This essentially is like chopping a piece of bread, and taking each slice and visualizing each slice, and then putting all the slices together to see the entire slice of bread, entire loaf of bread. And that is what now you can do by taking optical sections.

So, you can take, this is a image of pollen, and you can see that you can take sections from one end to the other, and then put all of it together to create this three dimensional structure. This a that you are seeing is essentially this pollen that has been imaged in a confocal. And you can create a three dimensional image of the cell or whatever object that you are visualizing using a confocal microscope in a way that would not have been possible otherwise. This, in my opinion, was a real game changer in microscopy. And along with the kind of change in visualization that those early microscopes produced, confocal changed how we view things.

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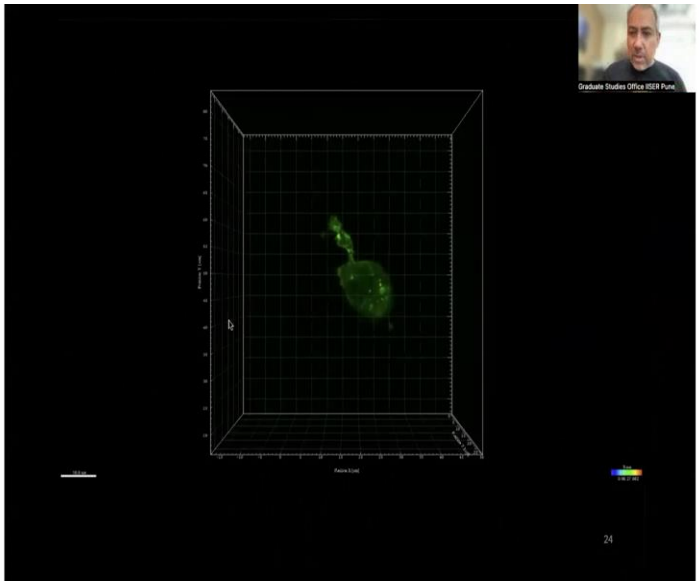

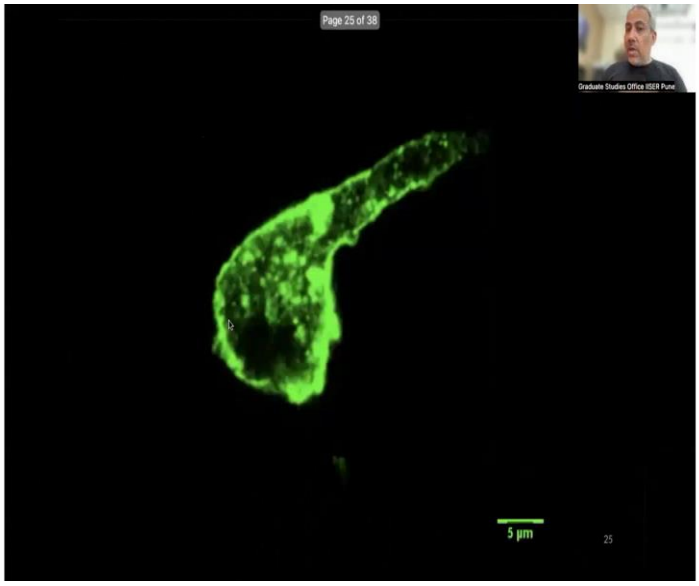

It allowed us to see things and this is a movie that is not playing, because I have a PDF file here, but you are essentially looking at a cell that is in a gel and it is made a small protrusion and you can visualize the cell, not only as a cross section and then put it together, but you are also able to see this in time, which means it will do section, section, section, go to the bottom, go back section, section, section.

And every, suppose it takes two seconds for it to take sections from top to bottom and go back up, you have a three dimensional image, that is one or two seconds apart. And now you can put a movie together right of these and play them back to back and see things that are changing inside

the cell accordingly. So, you are also able to visualize things that are moving inside the cell as well.

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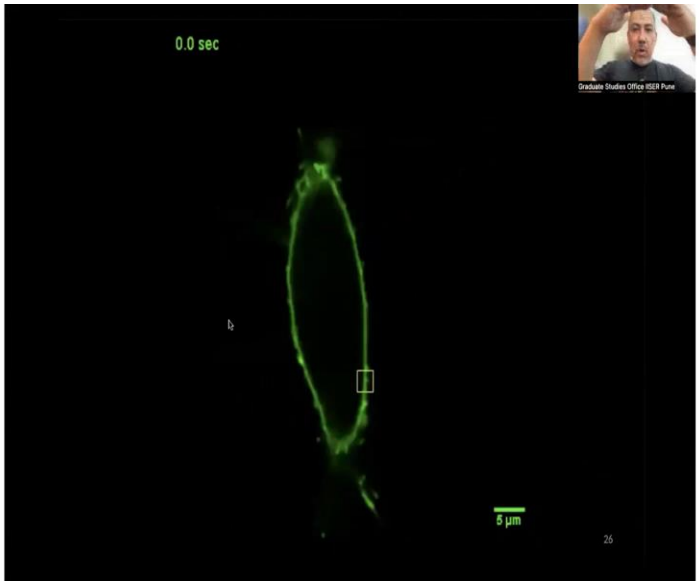

You are also able to do things like this is a protein that is on the membrane of the cell, and that box that you are seeing is a box that we go and bleach. There is something called photo bleaching, which is essentially, you shine light and you completely remove the fluorescence in there, inside that box. And then you watch the rest of the proteins come and fill that area. And this allows you to now watch the rate at which things are moving inside the cell. So all this is now possible because of confocal microscopy.

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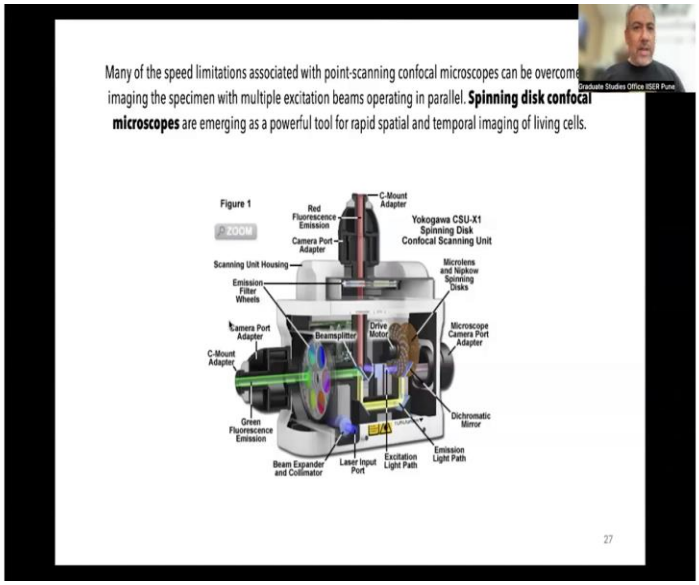

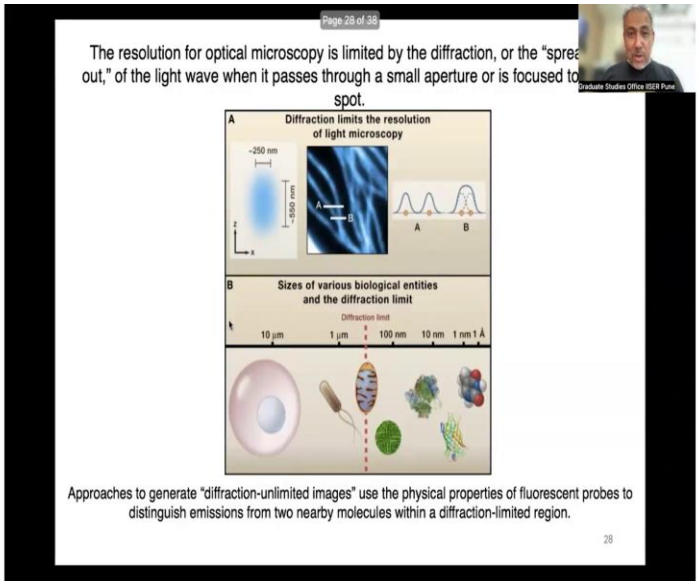

There are more advanced versions and I am not going to get into that in the interest of time and also this is something that you can go read on your own. There is something called a spinning disk confocal microscope, which does one better than the existing confocal microscope.

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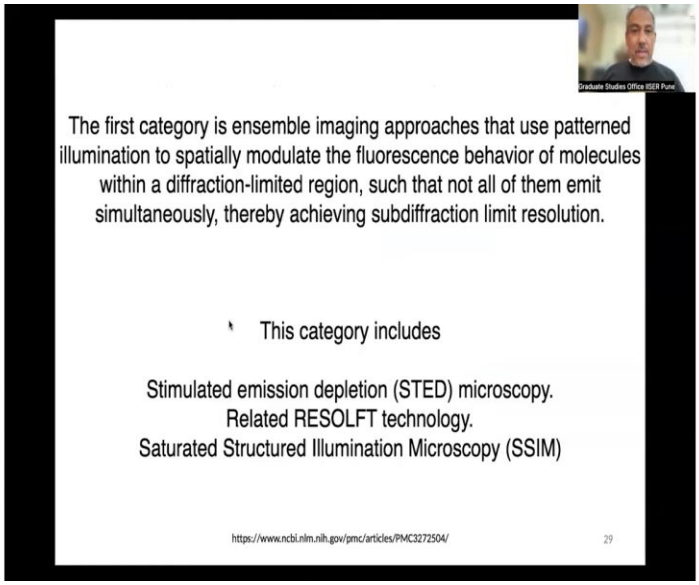

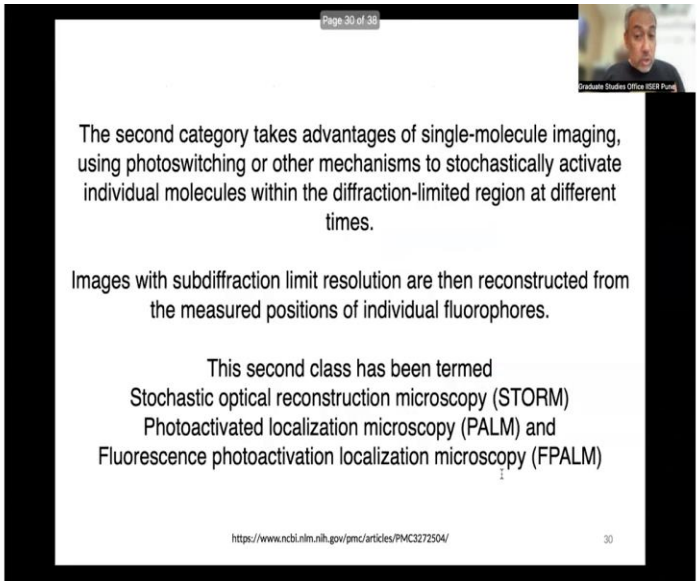

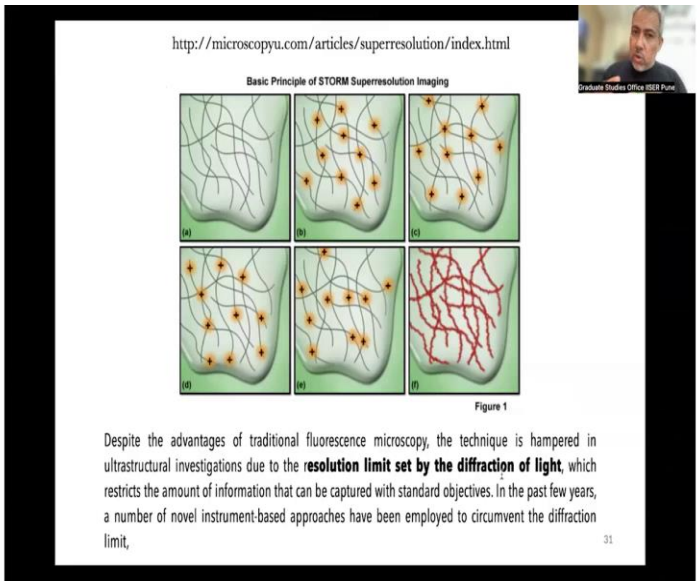

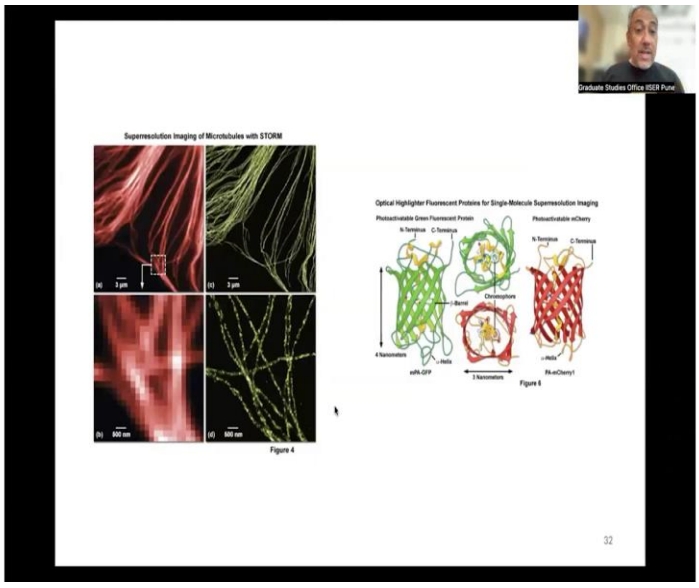

And there is, there are now new microscopy systems called STED and there is a system called STORM, which also affects the resolution of the image and allows you to now resolve things better. So, if you have a fluorescent strand that looks like this, the quality of that image can be improved dramatically by STORM microscopy. So, these are similar images which are taken with a regular confocal microscope and with the super resolution imaging microscope.

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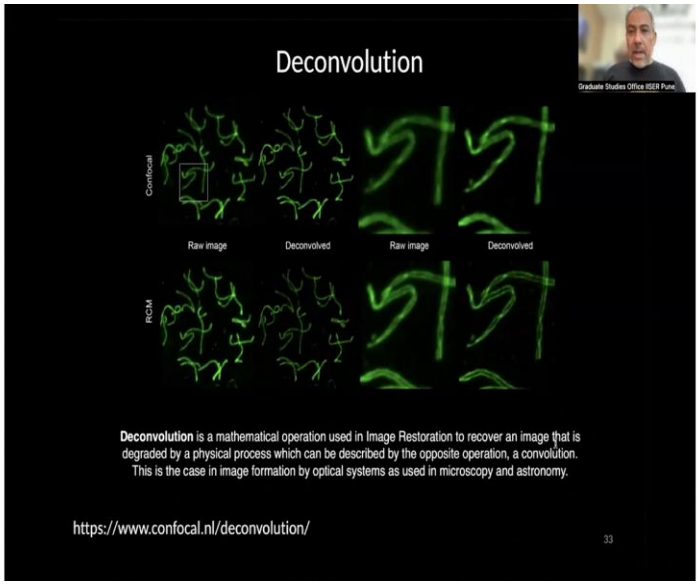

What I will also say a little bit about is along with the development in tools to visualize, there are algorithms that are written to process the image. That means these are mathematical algorithms that are written to essentially clean up the image, but do it keeping in mind what the fluorescence is like, where exactly the fluorescence is, what is the region of say bleed through in the fluorescence. If this is where the light source is, but when you record the image, you are seeing fluorescence in this neighbouring region, how do I determine that this is the specific light source and remove all of this and let us see only this much. This can be done mathematically now.

And there is a very, very powerful beautiful tool called deconvolution, which is nothing but a mathematical algorithm that was written to clean up images. So, you can take a confocal image and then you can deconvolute it, which means you are not actually changing the, how you record the image, you are only cleaning up the image as a post processing. After you have recorded the image you are processing it in such a way that you can now see things that would have been difficult to see otherwise. We in the lab, for example, use a deconvolution software that is made by this company from Holland, called the Huygens and it is an incredibly powerful tool.

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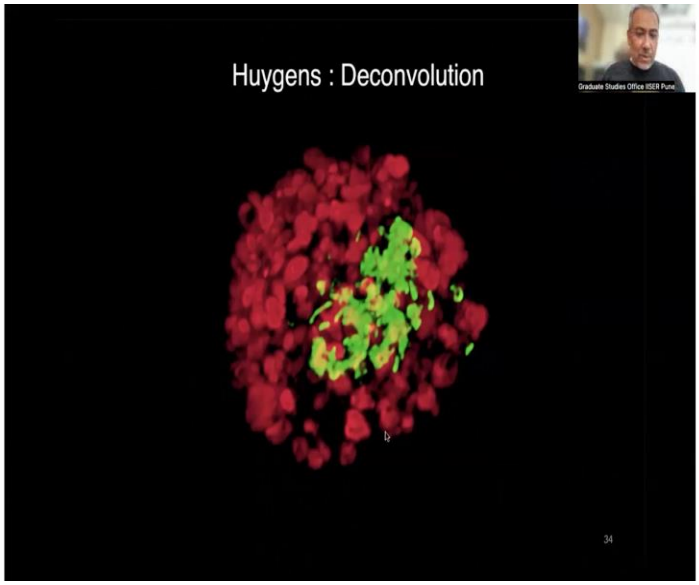

It allows us to shoot deconvoluted images such as this. This is actually a cell in which the Golgi has undergone restructuring and its filled up the entire cell. And this is an image which is a confocal image. So, we have taken sections and then we have deconvoluted it using such an algorithm to get this clarity in the architecture in a way that we would not have had otherwise. So, we can we can do stuff like this in this system in a way that would not be possible otherwise. So, that is where I will stop.