Nanobiophotonics: Touching Our Daily Life
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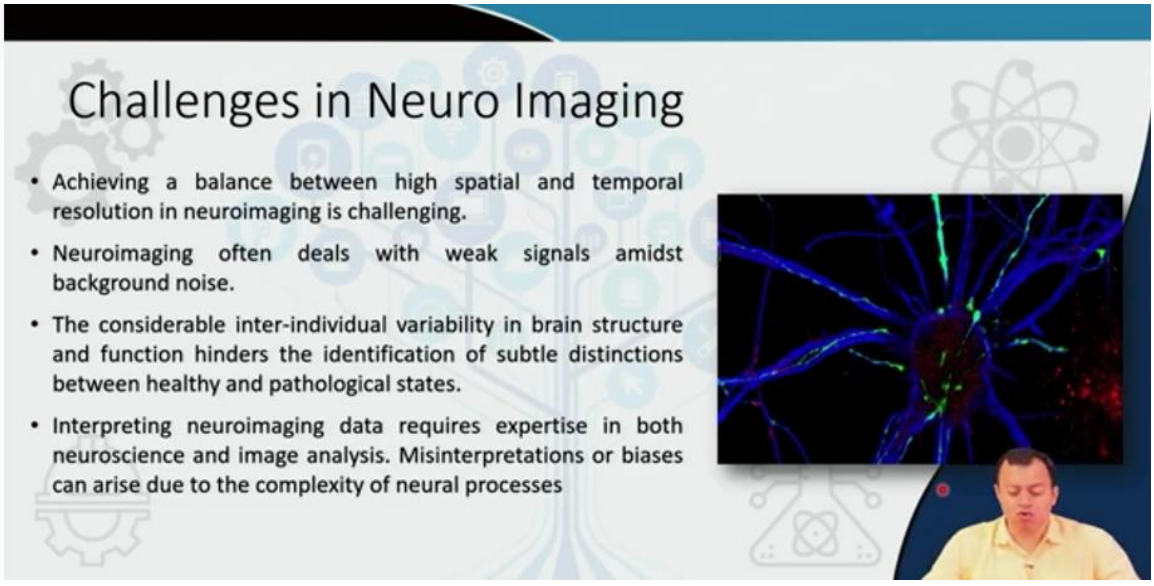
Lecture No. 47
Neuro imaging with Light-Sheet Microscopy

Welcome back. We are continuing our discussion on Neurophotonics and today I have another topic for you and that is Neuroimaging with Light Sheet Microscopy. It is also sometimes is used as LSM, but LSM has other connotation especially in life science laser scanning microscopy is also called LSM. So, this is usually LISM, but overall, we are going to discuss light sheet microscopy not laser scanning microscopy.

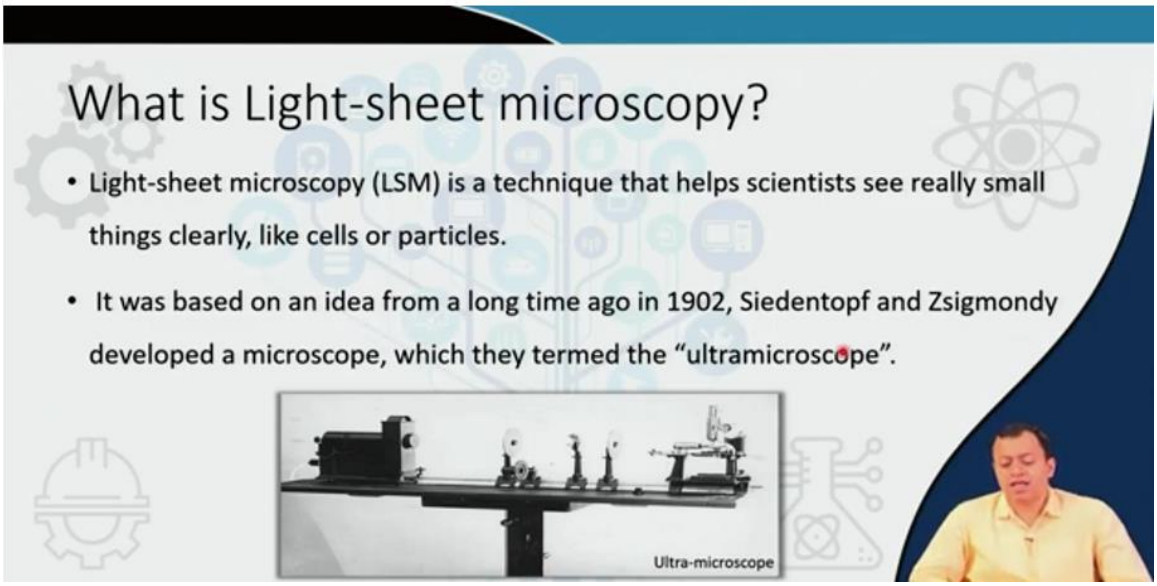


So, understand this that there are several challenges when you are trying to understand the working of a neurons or when you are trying to image neurons specifically in a live organism without you know taking the brain out and without any sort of dissection. So, achieving a balance between high spatial and temporal resolution in neuroimaging is very, very challenging.

Neuroimaging often deals with weak signal amidst background noise and there is huge amount of inter-individual variability in brain structure. Interpreting neuroimaging data requires expertise in both neuroscience as well as imaging analysis and therefore misinterpretation is very, very common. So in 18th century, early 19th century the gentleman Golgi what he did was he used to dissect animals, the brains of the animals try to dissect neurons, those nerves not neurons nerves of the animals cover them with some

sort of silver salts and then put the entire brain structure entire nervous system under the microscope shine light and try to see the staining that has happened by silver salts from which he came up with Golgi especially came up with all those beautiful axons, dendrites definition, then synaptic cleft. Golgi is the same person from with Golgi apparatus or Golgi bodies are named it is the same, same scientist.

So Cael, Spanish scientist also work similarly where they used to put silver salt staining the brain with silver salt hopefully the brain of animal which is no longer alive and then try to image it. But all of those functions were difficult, caused errors, gave weak signals and it is very, very subjective regarding what you are looking at and the small change in ions depending on the type of neural circuitry going on what sort of electrical impulses action potential has been given.

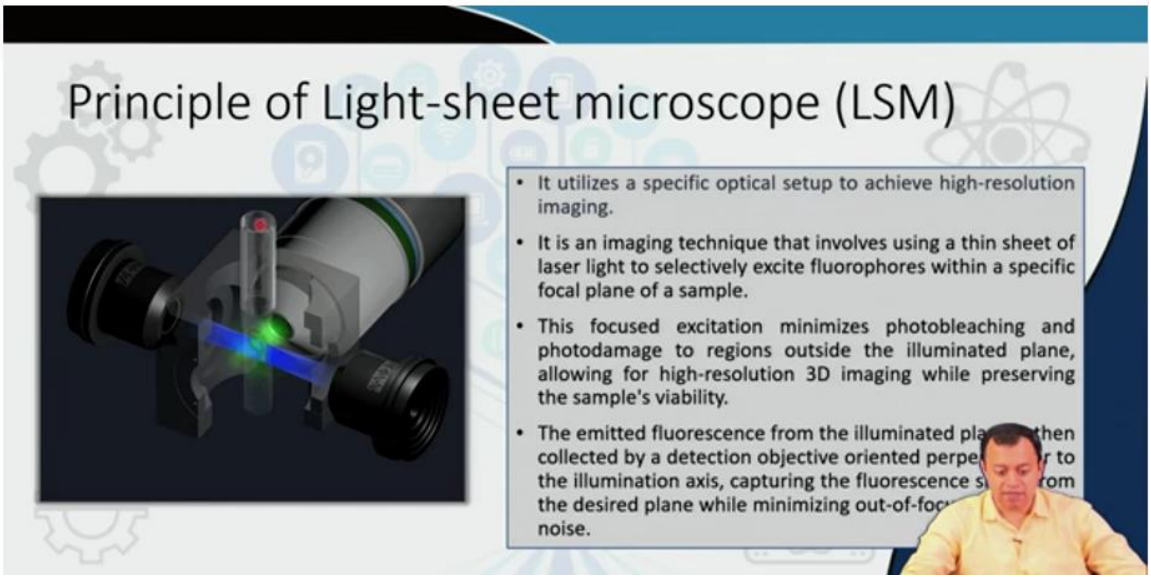


So, it causes huge amount of challenges that needs to be overcome. Light sheet microscopy it was said that could overcome several of this problem. Now light sheet microscopy is not at all recent.

In 1902 German scientist I frankly speaking I hope they are German and I cannot pronounce their name you try if you can pronounce their name this looks like Polish to me but this looks like German to me so I am just guessing. These two gentlemen came up with the term ultra microscope. So, what they did they saturated certain area, certain samples with metal nanoparticles, metallic particles and they illuminated the sample from say top, they shine light on the sample from top but the detection the measurement was from an orthogonal 90-degree angle. So, if you are shining light from the top you are measuring the sample scattering the light scattered from the side 90-degree difference this is input, this is output or if this is illumination you are measuring it from up or measuring

it from bottom. Usually what we have in a reflected microscope light comes falls and returns back so the entry and the output path is exactly the same it measures like you capture the same light and then you try to analyze it measure it at normal reflection microscope.

In transmission microscope light falls into a sample it penetrates through it, it transmits through it and you have your detector below and you are trying to measure it. Here it is 180-degree light is falling and you are measuring it from an orthogonal position and they said that this prevents now it seems very common sense to you why we do not do it but as you see that it cost becomes very high the light has to fall from here. If you measure it from an angle or measure it at a 90 degree or any other angle per say you are eliminating any other noise any other scattering that is caused by the input light simple. So, it was based on an idea long time ago and they called it ultra microscope this is that ultra microscope image and they claim that they help scientists to see really small things clearly like cells or particles.



So, basically this is what is happening the this is the sample box where you have your particular sample and it is illuminated from this side it is illuminated from this side it is illuminated from this side and at a 90 degree you are doing the detection.

It is illuminated from this side it does not have to be like this it can be like this but it can also be like this x and y are at 90 degree to one another x and y coordinates x and y coordinates and 90 degree to one another x and z coordinates are also 90 degree to one another yes. So, this is the principle of light sheet microscopy. It utilizes specific optical setup to achieve high resolution imaging it is imaging technique that involves using a thin sheet of light to selectively excite fluorophores. This focuses excitation minimized photo

bleaching and photo damage and the emitted fluorescence from the illuminated plane is then collected by detection. So, again let us try to understand what is happening.

You have understood the old light sheet microscope technique it illuminated from one side and capture the information the detection is from a 90-degree orthogonal angle. You can shine the sample the sample could be the sample could be the light could be shine here and you can you can detect it from there. What presently people are doing using some sort of cylindrical lenses which I will show you the illuminating light. The illuminating light is not just one single dot usually it is a point yeah when you are illuminating a sample it is usually a small spot usually circular that falls onto an area it gets reflect transmitted etcetera. Using cylindrical lens they are trying to make it a squarish or rectangular like a sheet like a sheet of paper a light falls a laser light falls like in a sheet not just one point not just a sphere not just a circle it is a sheet that is the sheet of light sheet microscopy.

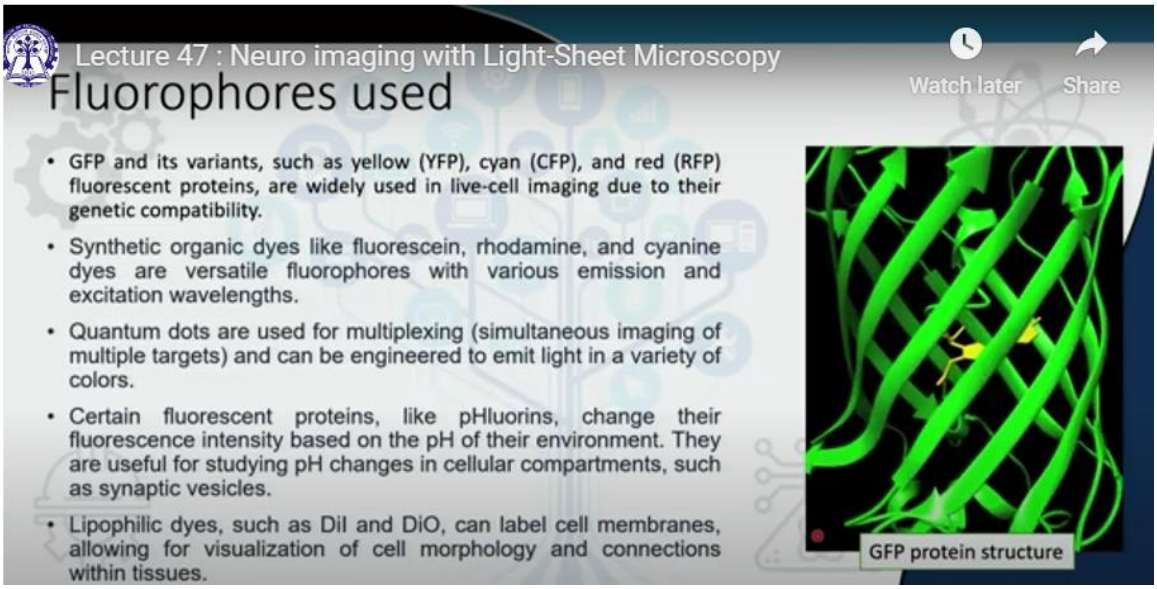
The laser which is coming like that this is a spot this is a spot instead of a spot you use lenses so that the laser light makes a rectangular a sheet like structure that that almost like slicing through the sample that is like slicing through the sample slicing through the sample as it slices through the samples it illuminates large number of area it illuminates large number of area not just pixel by pixel not just pixel by pixel you are not trying to scan individual areas individual pixels like you have done before. You are scanning an entire area using a particular wavelength or set of wavelengths as the light is slowly moved in this particular direction you are measuring the detector at an orthogonal angle at an orthogonal angle at a 90-degree angle in the same manner into the same manner. But if the light is falling in this direction your detection is in this direction if the light is falling in this direction your detection is in this direction and it helps prevent several problems this focused excitation minimized photo bleaching photo damage to the region outside the illuminated plane and it provides you a 3D imaging and a very high speed data rate you are not imaging pixel by pixel you are simply scanning plane by plane instead of using pixel you are sending a plane you know a sample is getting dissected by light not literal dissection you simply are illuminating it plane by plane and measuring it from side to side.

So this is something that is happening you have a sample you have an excitation laser system you have a cylindrical lens the cylindrical lens produces the point source of light into this kind of a light plane a beam of light that is squarish or rectangle ish and it illuminates the area it almost slices optically slicing the sample into like planes rather than points and you have this objective that tries to see the light that is being scattered when a plane by plane plane by plane plane by plane the light is moved or the sample is moved and the scattered light is collected by the cylindrical lens put into camera CMOS camera etcetera and you are simply measuring it analyzing it using a computer. So basically in light sheet microscope we are producing plane a sheet of light a sheet of light the sheet of light is made using cylindrical lens cylindrical lens can convert a point source into this kind of rectangular source the sample is slowly passed through the sheet of light different planes are sliced different planes of the samples are illuminated and whatever planes are illuminated they scattered some amount of light the scattered light are connected orthogonally at 90 degree at 90 degree this is the direction of the plane of the excitement light this is the direction of the plane.

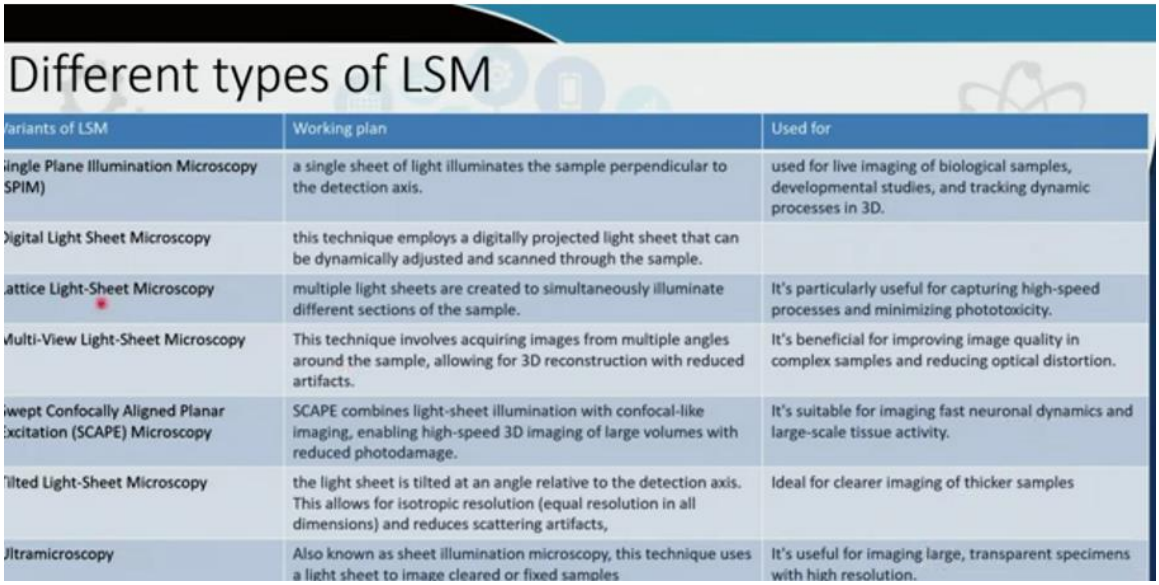
So if this is z direction this is I do not know y direction or x direction whatever and this is collecting it. So illumination system is often a laser to laser to generate the thin sheet of illumination the light is usually directed through optical elements like lenses mirrors and the beam shaping device to achieve desired thickness and shape of the light sheet sample holder is mounted using a precise positioning detection optics is some sort of an objective lens the lens collects an emitted fluorescence and for 3D imaging on multiple acquisition light sheet fluorescence microscope you have obviously used some sort of a fluorophore here to often include a sample strain a scanning or mounting stage. The advantage here understand is as this sample as this sphere is illuminated plane by plane and collected information the scattered information is collected you are able to get a three-dimensional

view of the particular sample. A three dimensional fluorescence image of the particular sample is possible you have previously in a three dimensional structure like a cell or a bacteria you have put fluorophores connecting a different part of your interest and then it is light wise sliced once one plane at a time and then the scattered light from that scattered light from this plane based dissection you are measuring through say particularly this angle or any other 90 degree angle the scattered light and from that you recreate the 3D image you recreate the 3D image of the slice of the sample and the different amount of scattering at different plane of illumination. And thereby recreate a three dimensional image you have obviously filter sets optical filters are used to selectively transmit specific wavelength of fluorescence emitted by the sample you have beam splitting and steering elements to direct the excitation of the light for the emitted fluorescence and of course data acquisition and analysis software specialized software is essential for controlling the imaging process and obviously analysis of the data.

This provides a very rapid analysis of samples because instead of doing a point by point scan you are doing plane by plane scan and immediately getting you slice by slice you have got a 3D image a 3D fluorescence image of the sample that you are trying to go through.



So, what are the fluorophores used the present fluorophore is obviously the GFP you know green fluorescence protein the there are other yellow fluorescence protein and cayenne and red cayenne or cayenne I do not know synthetic organic dyes like fluorescence, Rhodamine obviously these days quantum dots we are using silver nanoparticles, but several other of these type of proteins are used as fluorophores that could be utilized to label to attach with specific areas within the cell within the bacteria within the virus and then slice by slice we are getting information out of it.

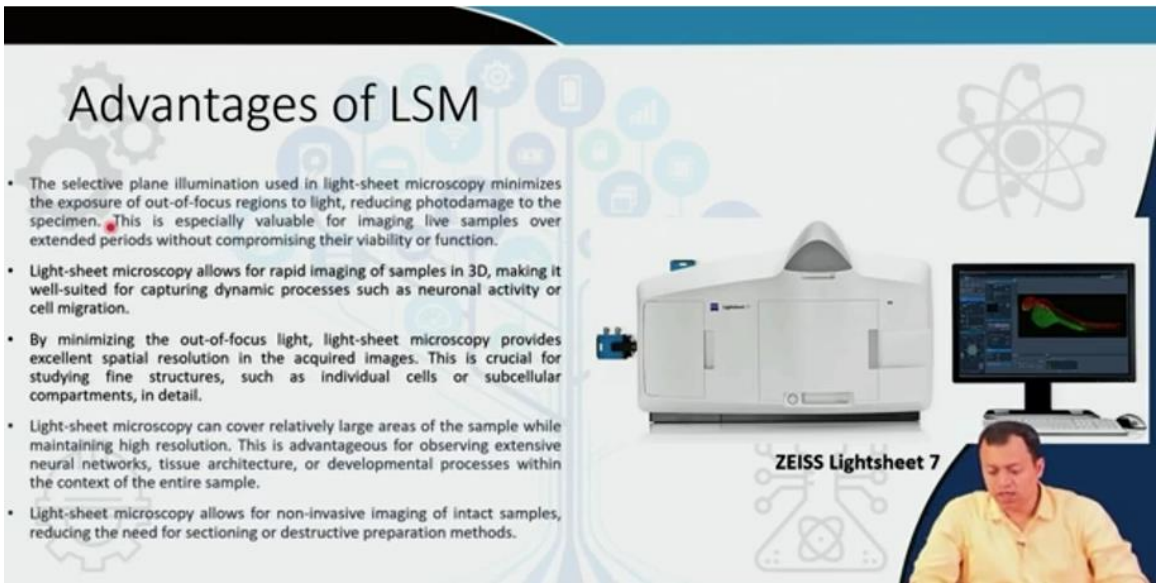


Different types of LSM

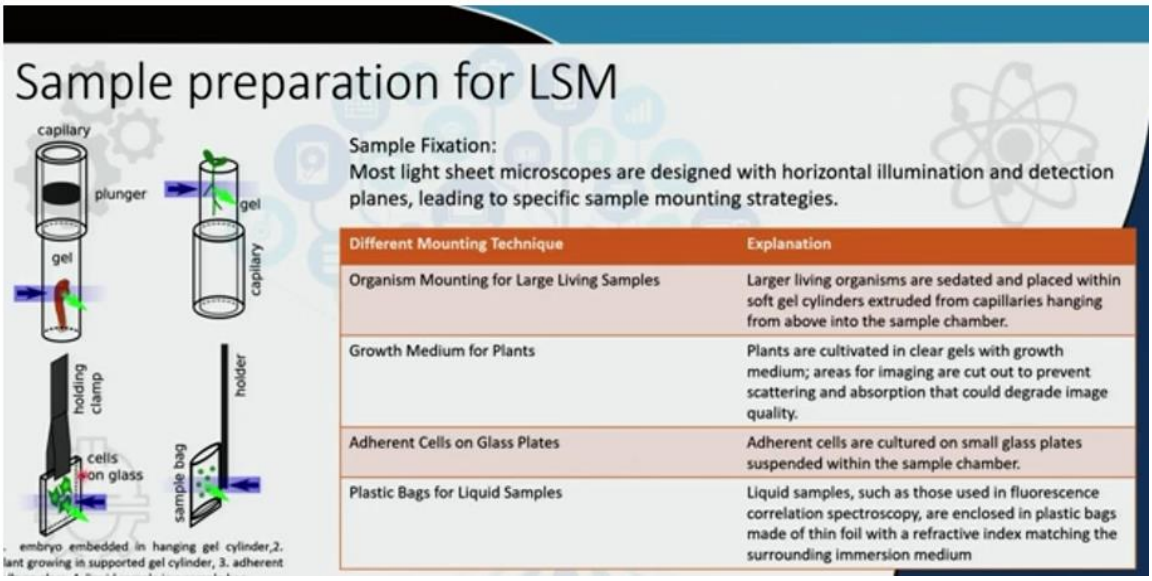
Variants of LSM	Working plan	Used for
Single Plane Illumination Microscopy (SPIM)	a single sheet of light illuminates the sample perpendicular to the detection axis.	used for live imaging of biological samples, developmental studies, and tracking dynamic processes in 3D.
Digital Light Sheet Microscopy	this technique employs a digitally projected light sheet that can be dynamically adjusted and scanned through the sample.	
Lattice Light-Sheet Microscopy	multiple light sheets are created to simultaneously illuminate different sections of the sample.	It's particularly useful for capturing high-speed processes and minimizing phototoxicity.
Multi-View Light-Sheet Microscopy	This technique involves acquiring images from multiple angles around the sample, allowing for 3D reconstruction with reduced artifacts.	It's beneficial for improving image quality in complex samples and reducing optical distortion.
Swept Confocally Aligned Planar Excitation (SCAPE) Microscopy	SCAPE combines light-sheet illumination with confocal-like imaging, enabling high-speed 3D imaging of large volumes with reduced photodamage.	It's suitable for imaging fast neuronal dynamics and large-scale tissue activity.
Tilted Light-Sheet Microscopy	the light sheet is tilted at an angle relative to the detection axis. This allows for isotropic resolution (equal resolution in all dimensions) and reduces scattering artifacts,	Ideal for clearer imaging of thicker samples
Ultramicroscopy	Also known as sheet illumination microscopy, this technique uses a light sheet to image cleared or fixed samples	It's useful for imaging large, transparent specimens with high resolution.

So, there are different variants of LSM we have single plane illumination microscopy lattice sheet microscopy lattice light sheet microscopy this is something that I have used where multiple light sheets are scattered. So, instead of having the illumination from here and detection from here you have multiple illumination multiple illumination and multiple detection you illuminate from here you illuminate from here you collect the data from here and you can you know try to get this information by rotating your detector and your illuminator at different areas making this you know as long as this 90 degree angle is almost matched you can make it in any xyz direction and thereby get a holography get a complete total image of the of the sample that you are trying to get. So, multi view light sheet microscopy ultra microscopy.

So, read it at your own leisure that they have been used for taking dynamic processes in 3D in real time what is happening in a particular bacterium as it is working the particularly useful for capturing high speed process with minimizing photo toxicity, escape microscopy this is imaging fast neural dynamics clearer imaging of thick sample. So, there are there are several views and all of them utilizes more or less the very same principle, but the amount or the angle of detection and illumination remains more or less 90 degree, but they can use multiple detector and multiple illuminator just not one detector one illuminator multiple detector at different angles and complementary orthogonal multiple illuminators at different angles.

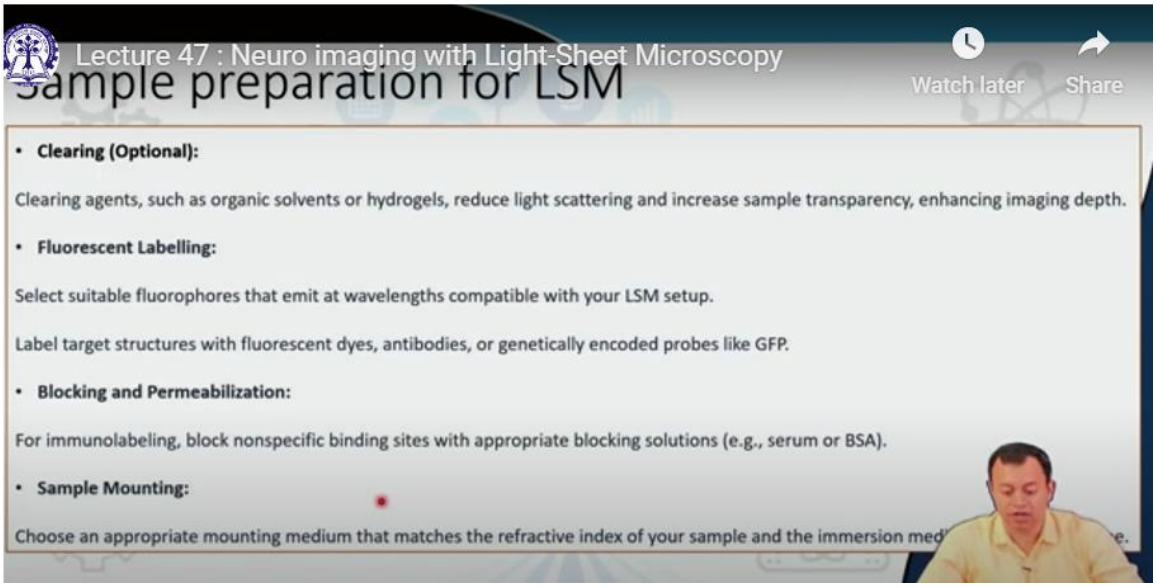


So, this is the quite popular one Zeiss light sheet 7 there are obviously several advantages light sheet microscopy allows for rapid imaging of samples in 3D making is well suitable for capturing dynamic processes by minimizing the out of focus light light sheet microscopy provides excellent spatial resolution can cover relatively large areas of the sample while maintaining high resolution light sheet microscopy allows for non-invasive imaging of intact sample reducing the need for sectioning or destructive preparation method.

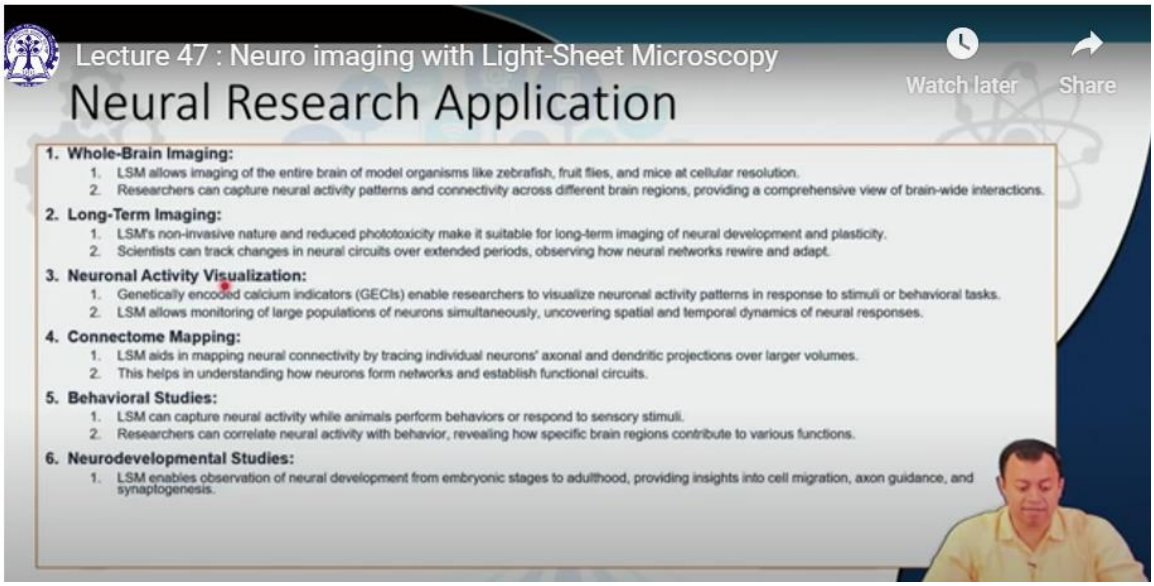


So, you do not need to destroy the sample or you know it is more or less non-invasive and it is also pretty much I mean how do you handle the samples there are like this holding clamps on which cells are put on a glass and then you are illuminating it like that extracting samples ah ah detecting it from like this you have a small sample bag or most importantly

you can have this cylinder capillary tubes like I saw before you put a zebra fish zebra fishes are usually transparent or plants or drosophila fly alive if you can you know stick it with something alive, but not moving ah and then illuminate it from here and get the detection from this particular angle you can illuminate it from here you can simultaneously illuminate it from here and get the detection from here and here and so on and so forth. So, you can have the growth medium of plants you can have cell glass plates you can have plastic bags for liquid samples you can have organism mounting like these kinds of clips clamps holding the sample. So, all of those things can be utilized using LSM.

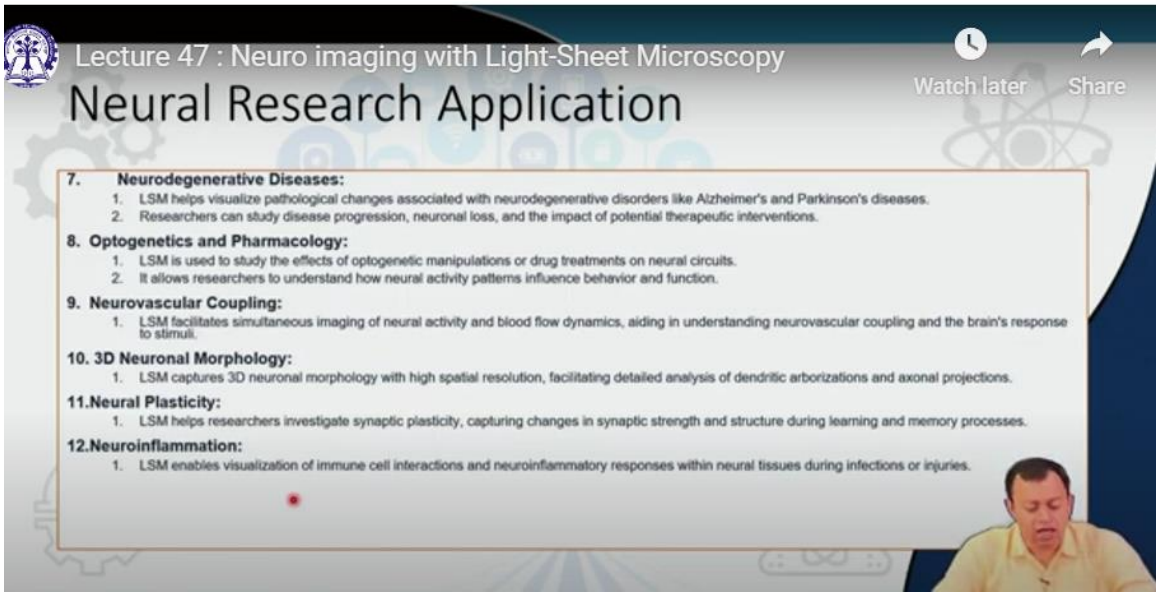


Ah fluorescence labeling has to be done in LSM. So, that um light sheet fluorescence microscope is also used ah to denote this LSFM label targeted structures with fluorescence dyes antibodies or genetically encoded probes like GFP and ah you go for the measurement.

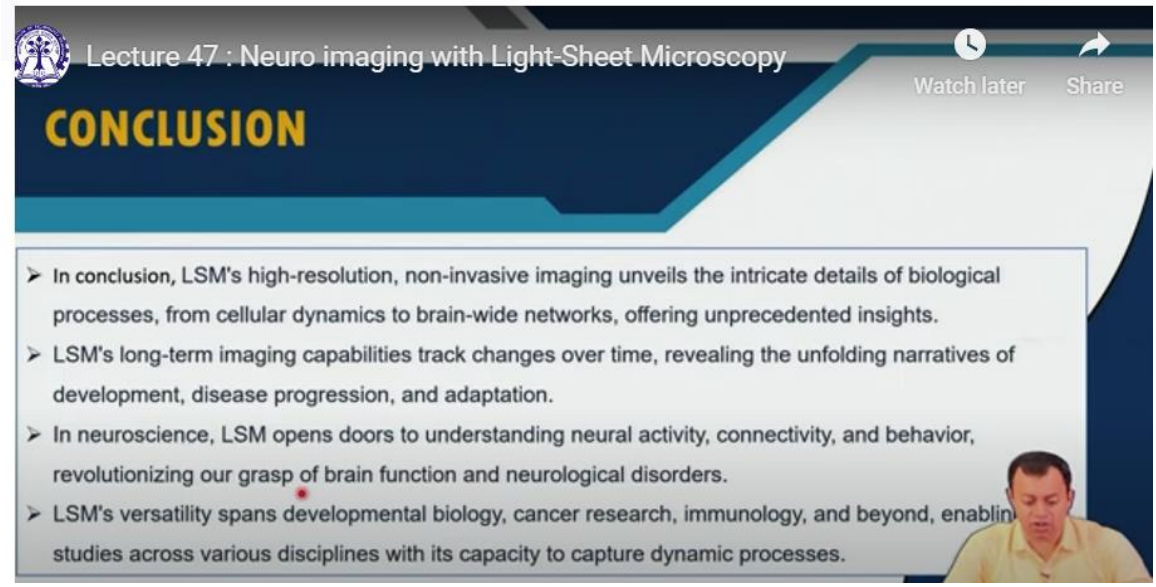


So, several different areas can be applications are there which can be utilized by LSM LSFM you have neuronal activity visualization especially in zebra fish zebra fish are optically transparent you can see the internal organs of a zebra fish ah from from naked eye you can now fluoresce it with say GFP or something like that. So, various areas of a particular organ especially brain can be illuminated by this green protein and then you slice it using the LSM and then measure and then get a ah 3D image of the brain while the zebra fish is still alive yeah.

Ah you have connectosome mapping you have behavioral studies LSM can capture neural activities where animal perform behaviors or response this is still challenging I mean as the ah animal is alive, but if it is moving obviously your focus and everything goes goes bad and then you have to redo it. So, like everything else it has its own ah challenges associated. Neuro development studies your ah take a larva of say zebra fish I know because these are the type of work that I am being doing ah you have done something. So, that the larva has ah the brain development of the larva at different stages could be used in light sheet measurement and you can see the 3D image of the brain of the larva ah that is the young stage of the zebra fish at when the age of the larva is 1 day, 2 day, 3 day, 10 days, 1 month, 3 month, 4 month, 6 months and you can have a real time development of the brain as it passes through different stages of its maturity right.

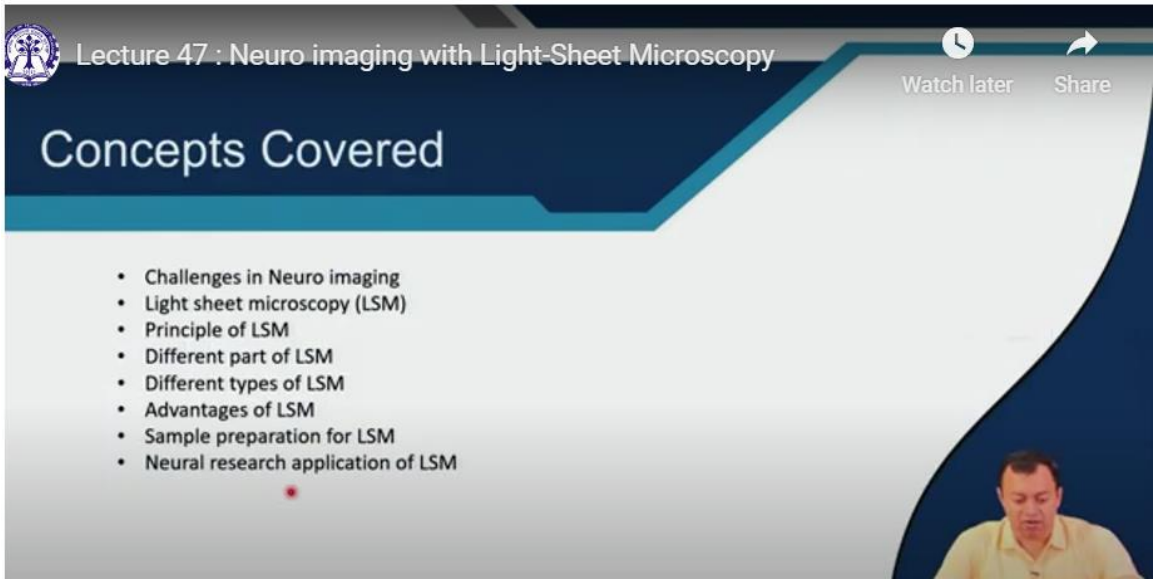


So, similar other if you can look into the brain you can map the brain in a in a 3D manner you can look into various ah diseases that are associated with the brain neuroinflammation, epilepsy, if you have given some sort of a ah drug a pharmacological ah chemical and then try to see how it is affecting the brain if something has attacked the brain a pathogen has managed to attack the brain and then ah particular set of drugs are been given how those two are interacting inside the brain at a at a at a at a at a real time manner ah light sheet microscopy can help you in a very nice way where a complete 3 dimensional view of the entire thing can be can be achieved right neurodegenerative diseases etcetera all of those things could be detected.

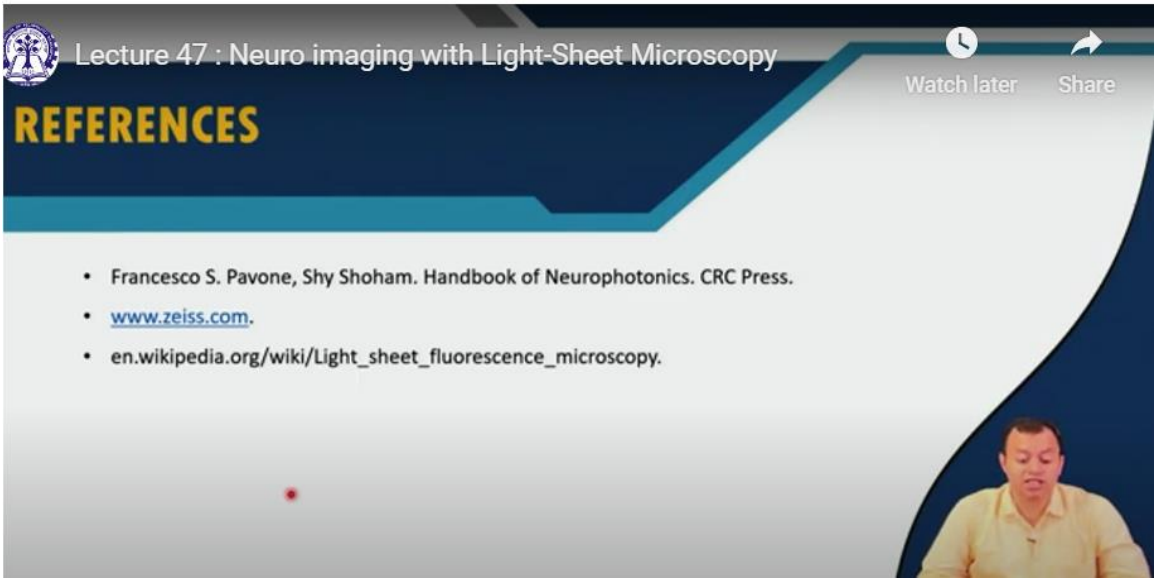


So, in conclusion LSM's high resolution non non invasive imaging unveils the intricate details of biological processes from cellular dynamics to brain wide networks offering unprecedented insights LSM's long term imaging capabilities track changes over time

revealing the unfolding narrative of development in neuroscience LSM opens door to understand neural activity connectivity behaviour revolutionizing our grasp of brain and LSM's fertility spans developmental biology cancer research immunology and beyond and various disciplines are there to capture the ah dynamic process.



So, these are the concepts that I covered today and these are the references please go through this ah Zeiss dot coms ah light sheet microscopy they have several beautiful



videos in social media, youtube etcetera where you can see they are copyrighted and did not decide to give it here, but I will ask you to go and look into light sheet microscopy of mouse brain or zebrafish brain and you will see the ultra high ah resolution images of brain and in a 3D in a 3D format and how what happens to the brain as it go as it performs a

particular function as it goes on developing as some sort of pathogens have attacked. So, Zeiss have several beautiful images beautiful videos on this topic I ask you to ah search for it. Thank you very much I will see you in the next class. Thank you.