Nanobiophotonics: Touching Our Daily Life

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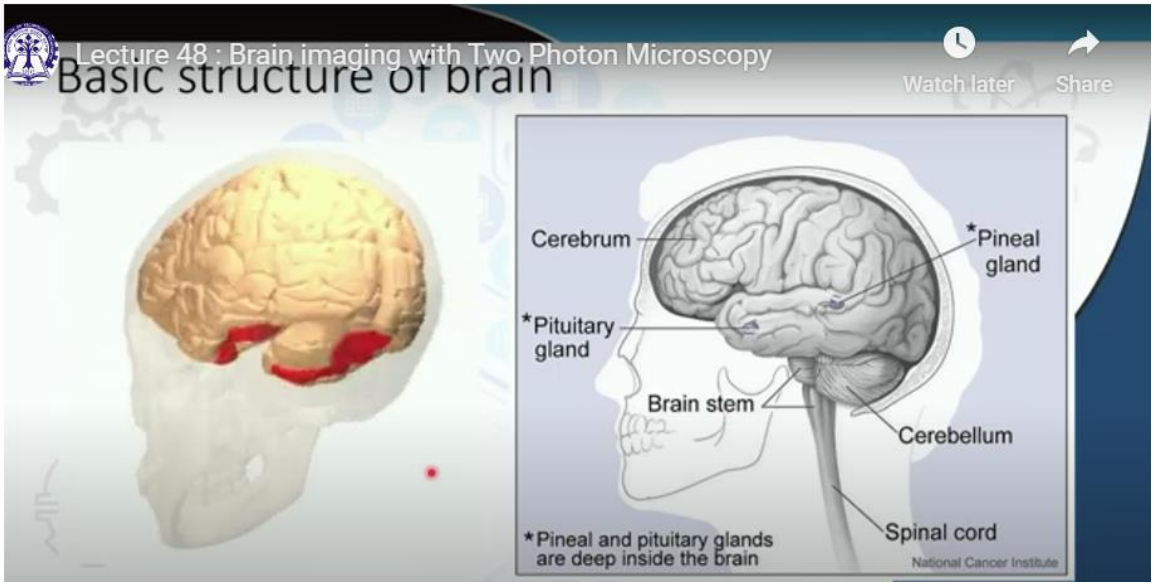
Lecture No. 48

Brain imaging with Two Photon Microscopy

Hello, and welcome. We will continue our discussion on the topic of Neurophotonics. Now, while I was designing the chapters for this particular course, there was no conscious effort from my side to actually differentiate specifically between optogenetics and neurophotonics. But now that I come to see the actual chapters part by part, I see that there is a distinct bias in me towards imaging of brain when it comes to study of neurophotonics. Whereas in optogenetics, I discuss about other thing like detection of disease or curing of disease during optogenetics. However, this needs to be clarified beforehand that both the topics of optogenetics and neurophotonics simply deals with interaction of light with brain matter, with brain matter, with neurons, with central nervous system, as well as peripheral nervous system.

So, please be aware there is, it does not mean that neurophotonics deals just with imaging of brain or optogenetics deals just with modification of brain or curing of brain diseases. Both have significant amount of overlap. However, the design is such that of this particular chapter of this particular module, that in module number 10, that is introduction to neurophotonics, I plan to discuss mostly the different types of imaging technologies available when it comes to brain and when it comes to using nanobiophotonics technology. So, today is lecture number 48 and today we are going to continue with the topic of brain imaging with two photon microscopes.



Now, I understand that I have taught you about neurons and cerebral, central nervous system as well as peripheral nervous system, but the organ of brain, the most important part of the central nervous system was not discussed in detail. Do not worry, I am not going to discuss brain as an organ in detail simply because I am not an expert, I am not a biologist, I am an electronics engineer, but you need to have certain amount of information. Thus far, I have given you the cellular structure, what happens when this organ, this brain is divided and you reduce it into its cellular components. The cells are neurons, mostly neurons and glia, neuroglia and we have discussed how neurons and glia work, myelinated sheath etc., nodes of run-visiver and how the information process through, but let us very briefly, very briefly before the electronics engineers leave this course discuss about the brain, about the human brain.

So, roughly very crudely, your brain can be divided into three main part, the top part is cerebrum, this part is cerebrum, the bottom part, the so called hind brain or small brain is cerebellum, which basically Latin for small brain, so this is here, this is the cerebellum part and then it is connected with the spinal cord using this thing called brain stream. They are very densely packed tissues, mostly, mostly made up of neurons as well as some other type of cells, but mostly neurons. So, this is the top part is cerebrum, there is a small, small, this red part as you can see is the cerebellum and it is connected, the entire brain is connected, the cerebrum and cerebellum is connected with the spinal cord using the brain stream. So, the cerebrum basically, which is generally, although wrongly called as brain, you do not consider these parts as brains or these parts are brains, you consider mostly this part as brain, but the entire three things cerebrum, cerebellum and the brain stream consist of the actual human brain. Now, the cerebrum part, cerebrum part that can be divided into gray matter and white matter.

So, this is the gray matter, the outer shell and inside it, there are white tissue structures,

which are called white matter. The outer side is grayish in color and it is therefore, named as gray matter and inside, inside these two lobes, inside this cerebrum, there is a whitish core, whitish core, which is called the white matter. Now, obviously, you know, it can be, the brain can be divided into two hemispheres, the left hemisphere of the brain, left side of brain and the right side of brain, inside it also, inside it also, there is left and right white matter. Why is white matter white and gray matter gray? Simply because the white matter, white matter contains mostly neurons with axons containing myelinated sheath. Remember myelins, myelinated sheath that is the lipid rich thing that protects the neurons.

So, the cell matter is less myelinated sheath matter is more inside the white matter and myelinated sheath is the lipid rich, fat rich and it has a whitish color. Whereas the outer portion, the outer portion of the brain is gray matter because it has a smaller number of myelinated sheaths, it mostly cell bodies and, and it has therefore, a grayish texture, grayish color. The whitish color comes from myelin sheath. Myelin sheath are this whitish lipid type sheaths that, you know, protects or that covers the neurons. Now, the cerebrum can also be divided into 4 parts.

There are 4 different areas or regions of the cerebrum that could simply be opened up. As you can see, this side, this side, this can open up, this side is slightly distinctive feature, this side is slightly distinctive feature and the, and the below part of the cerebrum has also distinctive feature. It can simply be opened up and you can easily distinguish, it can be differentiated, 4 regions of each hemisphere, of each hemisphere. So, these 4 regions or areas or zones are called lobes and they can be differentiated at this zone or this lobe, it is at the front, therefore, it is called the frontal lobe. This is at the bottom, this part, this part, you see this part, this part, this part that is the temporal lobe.

This is parietal lobe and the bottom part and the back of behind is the occipital lobe. Your vision is mostly dealt in this particular area. Remember, I discussed about your retina connected with optical nerve that basically goes here. So, your occipital lobe mostly deals with vision, the frontal lobe, the frontal lobe deals with short term memory, attention, you know, concentration, etcetera. The parietal lobe, the parietal lobe mostly deals with integration of sensors, integration of sensory cortex, different sense organs, the information that are being sent by different sense organs, your eyes, your ears, etcetera, they are integrated, they are processed here.

Though vision is in the, most part of the vision is in the occipital region. This is the temporal region, the temporal region is also dealt with higher cognitive functions such as sometimes of memory as well as, well, this could also be allowed with attention, but this is mostly with language comprehension. Language comprehension is mostly, remember language is a very, very higher functions that only we, the human beings in this entire

planet has developed. Other primates have gestures or sounds, but under no circumstances they could be called as language. So, language development is poetry, literature, all of those things that are the product of language is a very high sophisticated cognitive function.

So, they are mostly available here. The cerebellum part, the cerebellum part, the hindbrain, the red part, the cerebellum part is mostly associated with motor functions, your movement. And the brainstem is the connection between spinal cord and the different area, cerebrum and cerebellum. Bellum means small, small brain and this is big brain. Big brain and small brain, both of them are connected with the spinal cord using brainstem.

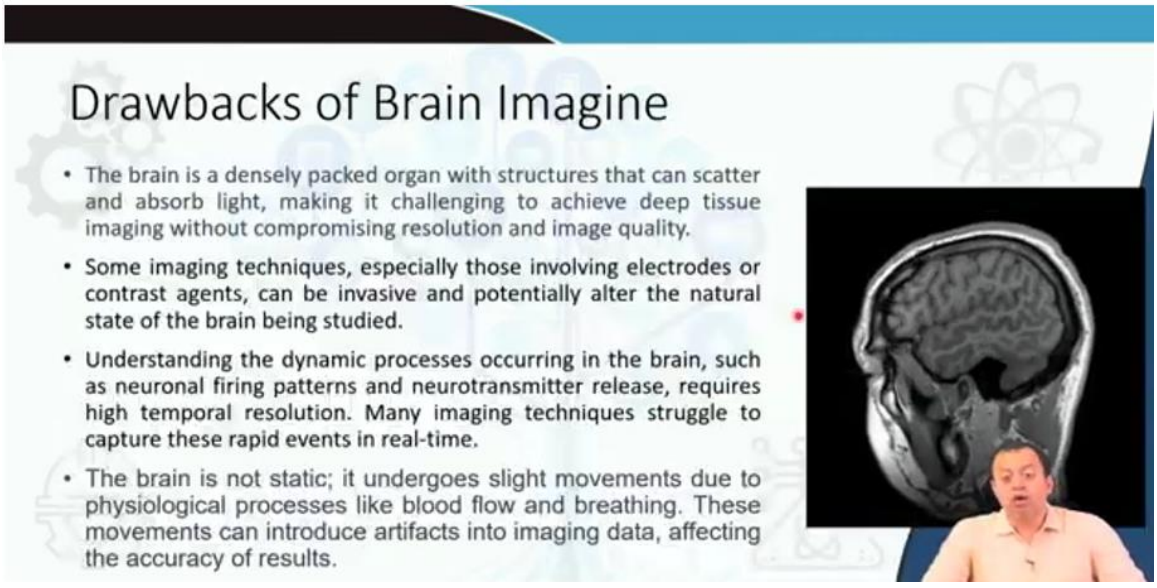
Brainstem is simply that connector, it brings in all the electrical connection from the spinal cord, which has bundle of nerves as you have seen, they carry all the information, the brainstem allows that information to be connected to pass through different areas of the brain. So, there are of course, glands associated with it, pineal and pituitary glands are deep inside the brain. So, this is the easiest way or the simplest or the crudest way in which I could describe the organ brain to you, needless to say, needless to say, this is the most complicated object, most complicated thing in the entire universe that human beings have encountered. So, under no circumstances, I can reveal all the mysteries of the brain or make you a brain expert, an expert in neurology or neurobiology or neuroscience in just one slide. But this is the crudest or the simplest way in which the organ brain and its different functions could be described, grey matter, white matter, etc.

I ask you to read a bit more about it, because obviously, I am not an expert in neurobiology or as such. So, we now want to image it, we now want to make a three-dimensional image, we want to see how each neuron is firing, we need to know how each individual neuron is working, both individually as well as in tandem with group of neurons and thereby connecting with, connecting itself with several bodily functions. Also, also here I must mention that when I just said that this part deals with language, this part deals with movement, this part deals with vision, there is a strong argument in the sense that the entire brain works simultaneously together, though different areas might have different functions, these functions are not always very much clear cut as I just said. There is an argument, there is an argument that, so if this part is damaged or this part is damaged, will the person not be able to walk or will the person not have a capacity to speak any language or understand any language. So, the answer is not clear cut yes or no, right, the answer is clear cut yes or no.

They have done, you know, studies on both human beings as well as an animals, studying human beings whose cerebellum has been damaged or trying to replicate that thing in mice models, animal models and yes, it does affect the motor function, it does affect the human beings ability to, you know, judge distance or make a precise movement, the so called

hand-eye coordination. You probably have heard several cricket players have fantastic hand-eye coordination. As soon as the ball comes, they know when to strike the bat, the ball is coming in cricket very fast, you need to strike the bat at a specific specific microsecond time to hit it because if you hit any other second the ball will simply come and hit you either you or your wicket. So, this brain hand-eye coordination all of those things though mostly dealt by cerebellum, but it does not simply automatically mean that the person will have zero ability as soon as this part is damaged or a person will, you know, have no more attention or cannot concentrate if just one part of the frontal lobe is damaged. Of course, the person will have difficulties, of course, the person will have difficulty in movement if the cerebellum is damaged and all of those things, but there is something called the plasticity of the brain, the flexibility of the brain in which other part starts taking over if one part of the brain is damaged other parts have the capacity to, you know, divide that work and help that person to regenerate to lead his or her daily life.

So, those things that I said that this part is for language, this part is for vision, this part is for sensing that is average or in general. So, be absolutely aware of them and of course, we have still to know the mysteries of the brain just like the universe is a mystery to us there is one universe outside our body and there is one universe that is inside our body. So, this is the brain which is a universe in itself and every single day we want to, you know, we are trying harder and harder to understand to unravel the mysteries of it.

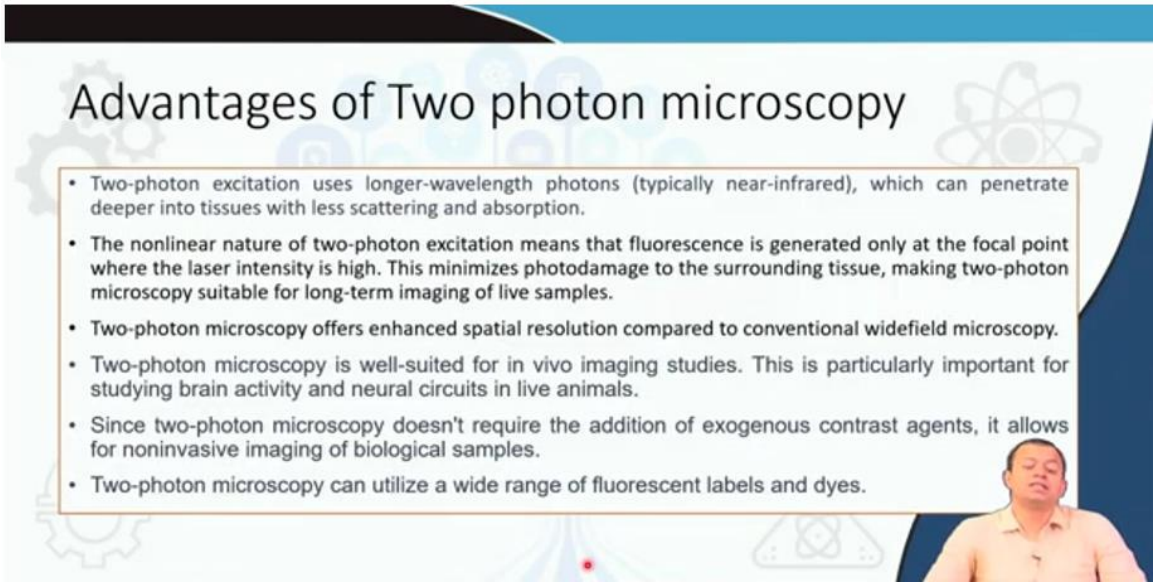


So, what are the problems with brain image? Well, mostly it is inside this hard skull which is difficult to penetrate, which is difficult and it is quite fragile. This is one of the most important organs of your body.

So, any you know tweaking with it will can could be dangerous, but nevertheless the brain

is densely packed organ with structures that can scatter and absorb light making it challenging to achieve deep tissue imaging. Some imaging tissue techniques such as electrodes or contrast agent can have several side effects and can damage the brain. Understanding the dynamic process occurring in the brain such as neuronal firing pattern, neurotransmitter release requires high temporal resolution which are difficult for you know run of the mill technique like MRI, magnetic resonance imaging or you know CAT scan. You will not be getting with magnetic resonance imaging or CT scan, coherence tomography scan, cellular level information and each neuron firing. And of course, the brain is not static, it undergoes slight movement due to physiological processes like blood flow and breathing.

These movements can introduce artifacts into the imaging data affecting the accuracy of results. So, what to do? There are several different techniques that we will see in the next two lectures as well, but in today's lecture let us deal with two photon microscopies.



So, two photon microscopy uses the non-linear process. You know the non-linear process in which two or more than two photons can be sent each having half of the energy or each having intermediate energy. Remember your non-linearity lecture from laser for bio photonics module.

You instead of sending one higher energy photon that excites electron of a fluorophore from lower level to upper level and then the electron moves from upper to lower emitting a low energy photon. Here you excite, you excite using non-linear process you send two photons in this particular case non-linearity second order non-linearity you excite two photons. One photon is intermediate of the band gap. So, if the band gap is saying one electron volt say one electron or two electron volts if the band gap between the lower level

and the upper level is say two electron volt, three electron volts let us be realistic three electron volt semiconductors have three electron volt compound semiconductors have three electron volt three electron volt. So, you send an input photon of 1. 5 electron volt and another input photon of 1.5 electron volt, intermediate stages or you can send one input photon of one electron volt another of two electron volts. So, the total photon is equal to three and this individual photon are generating a virtual state, a virtual state that should not have been existing in first place, but in non-linearity some weird things happen as we have seen a virtual state opens up inside the band gap where the photon can simply go and jump and come up. So, two photon excitation uses longer wavelength photons typically near infrared which can penetrate deeper into the tissue. Remember the previous lecture that brain tissue has this optical window the transmission between 650 to around 900 nanometers.

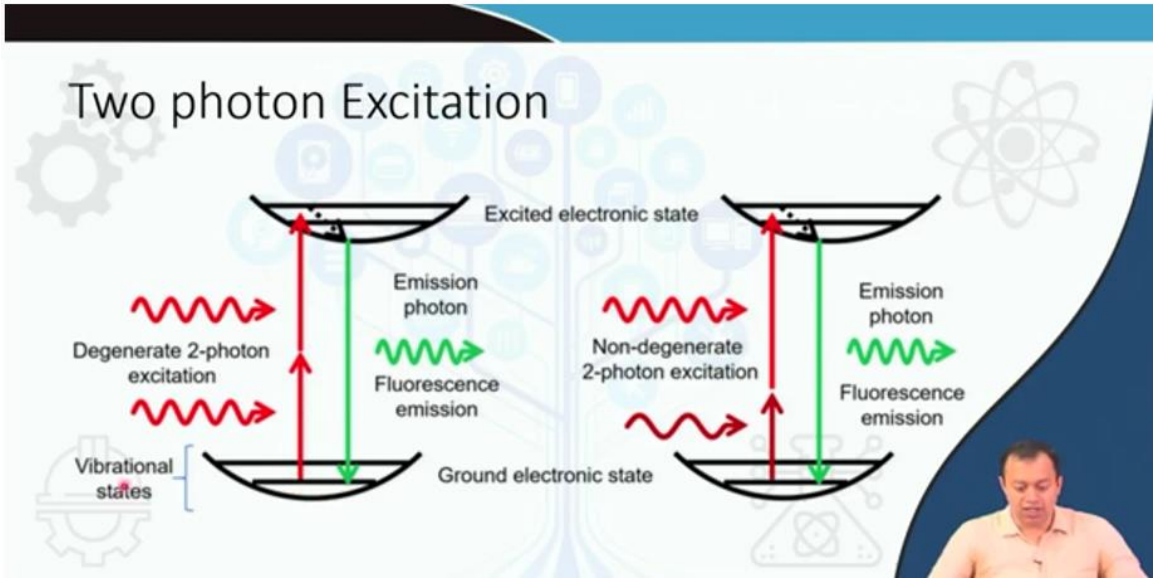
So, you what you can do you can make fluoro force. So, the basic idea here is what I have mostly dealt with is the neuronal activities the electrochemical potential changes the resting potential the action potential mostly deals with calcium ions, calcium ions, sodium ion, potassium ions, calcium ions. So, let us take calcium ions. So, there could be certain fluoro force which will be made to tag with calcium ions only. So, there are fluoro force which are made to tag with calcium ions only and these fluoro force works on a non-linear principle.

These fluoro force works on a non-linear principle i.e. they will absorb two photon and will emit one photon out. Now, if you have made your fluoro force in such a way that this falls within that optical window which brain tissue does not absorb. Brain tissue does not absorb 600 nanometer to 950 nanometer 650 nanometer to 900 nanometer and you are sending these two photons one after another or simultaneously whatever they can penetrate deeply through the brain they can penetrate deeply through the brain the brain is not absorbing it the brain is not absorbing it, but the fluoro force which is tagged with a specific area specific molecule of the brain is absorbing it and is then emitting the light.

So, you can thereby see certain portion of the brain and you by this time know that this portion this light is coming from only the calcium ions and how the movement of the calcium ions can be mapped in real time because this particular photon between 650 to 900 nanometer is not absorbed by brain tissue. It is not absorbed by brain tissue it is only absorbed by the fluoro force which is being made to tag with the calcium ions. Calcium ions being just an example it can be made to tag with it can be made to label with it can be made to connect with other areas as well. But overall this is the idea of two photon microscopy. The non-linear nature of two photon excitation means that fluorescence is generated only at the focal point where the laser intensity is high.

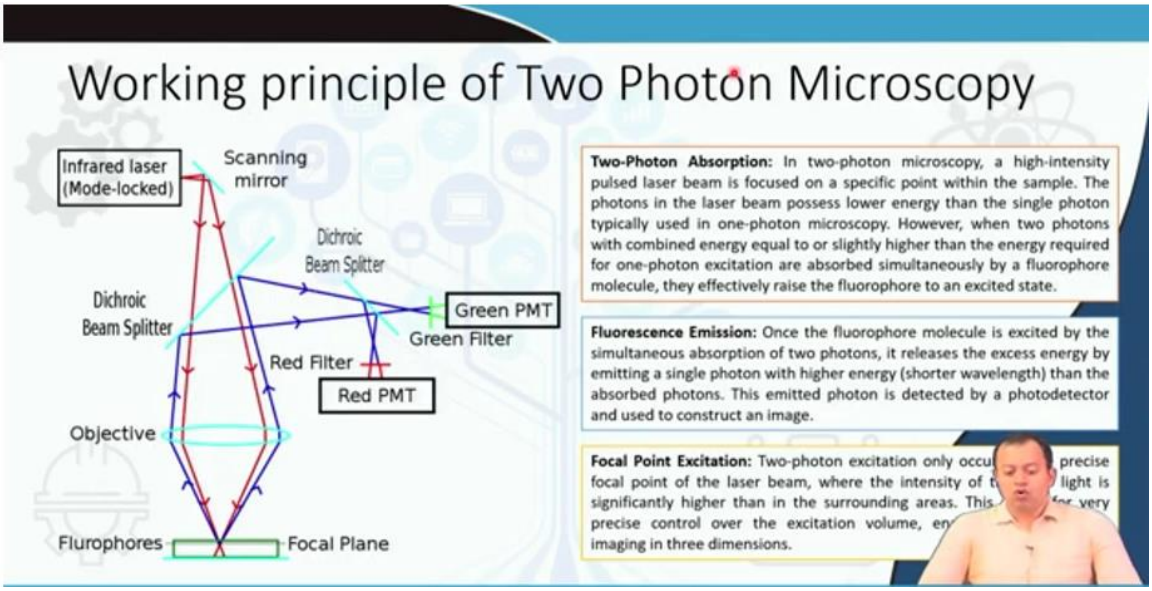
The minimized photo damage to the surrounding tissue because only that area where the fluoro force is there will absorb the laser intensity other areas will not absorb because that that particular near infrared frequency is not absorbed by brain tissue. Two photon microscopy offers enhanced spatial resolution. Two photon microscopies are well suited for in vivo imaging studies vivo vivo vitality this is life. So, in live imaging studies since two photon microscopies does not require the addition of exogenous contrast agent it is allowed for non-invasive imaging. So, you can also sometimes go through without fluoro force, but anyway I think using a fluoro force is better.

Two photon microscopy can utilize a wide range of fluorescent level and dyes they are trying with this certain inherent protein that can absorb can show non-linear non-linearity, but that is that is not something that we are going to discuss today. We will discuss with the normal the general two photon microscopy which uses fluorescence level and dyes and since that particular frequency is optically transparent it needs to be it needs to have less scattering and it can penetrate deeper inside the brain.



So, this is what I was talking about. So, this is the lower level and this is the upper level you can have two degenerate photons photon 1 and photon 2 this is the intermediate the virtual state within the band gap which should not exist which should not exist, but does exist and it allows the electron to go from first absorbing here into the virtual energy state then to the real energy states where it undergoes through non-radiative transition and coming back with an emitted photon with an emitted photon. The same thing can happen in degenerate two photon excitation photon both photons are exactly the same frequency in non-generate two photon excitation it is two different type of input photons, but adding up adding up making the making the band gap.
So, this could be anything remember it does not matter what the input value is if this is the

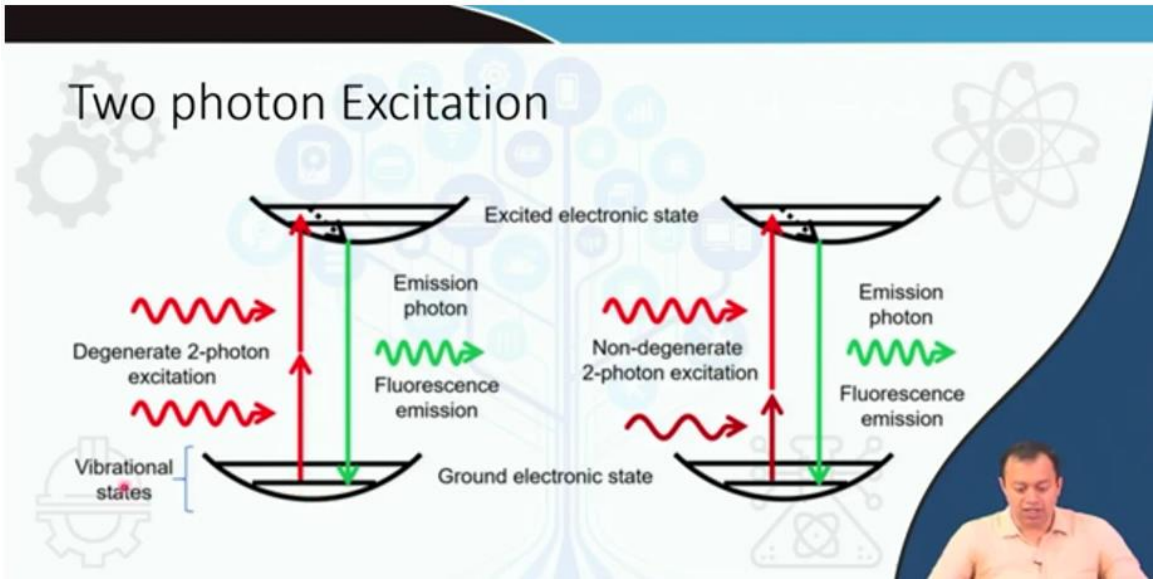
S1 level and there are S2, S3, S4 above as long as this is above the threshold it can take you here any frequency per say the emission will be the fluorescence emission will be of the exact same frequency because any higher level any higher level S2, S3, S100 are highly unstable and no electron can stay there for less than few femtosecond, attosecond, picosecond.



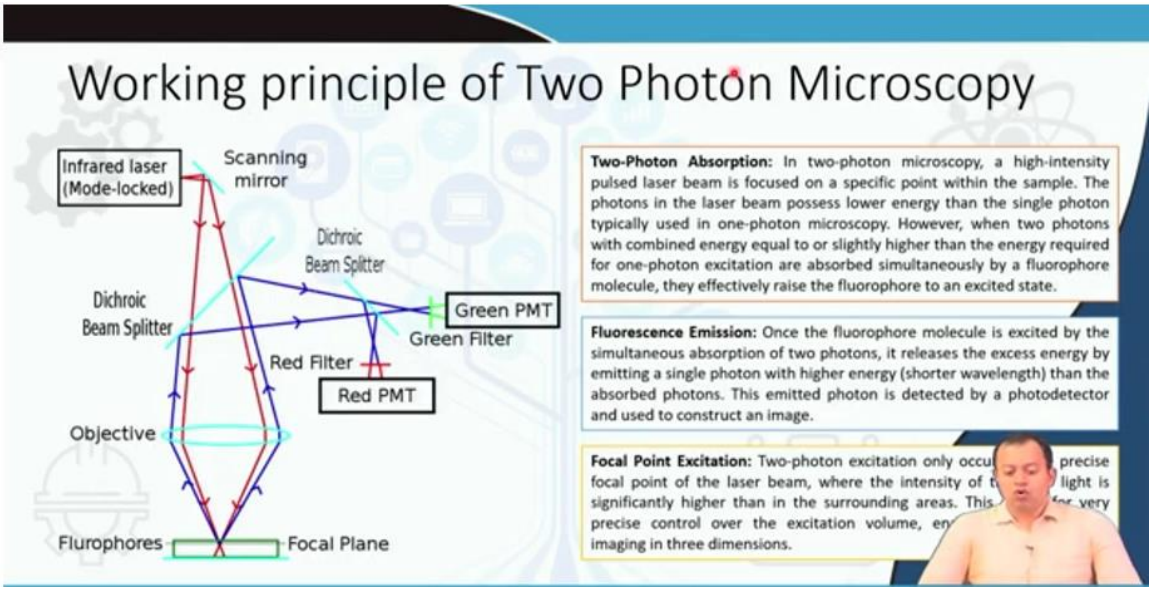
So, the working so you if you have understood this frankly speaking you have understood the entire chapter, but now let us discuss a little bit about the instrument. So, this is the working principle you can I will not be reading the entire thing you can read it at your own leisure time, but just to go through two-photon absorption a high intensity pulse laser beam is focused on a specific point within the sample the sample being your brain tissue the photons in the laser beam possesses lower energy than the single photon typically used in one photon microscopy. However, when two photons with combined energy equal to or slightly higher than the energy required for one photon excitation simultaneously by fluorophore molecules they effectively raise the fluorophore to an excited state, but this also reduces damage if you have a higher energy single photon. Fluorescence emission once the fluorophore molecule is excited by this simultaneous emission of two photon it releases the excess energy by emitting a single photon with higher energy than the absorbed photons the emitted photon is detected by a photoreactor.

So, here I think I need to tell you usually in fluorescence the input photon is higher energy the output photon is of lower energy here remember you are doing non-linearity. So, here the first photon is only raising the electron at the mid level it is not going all the way up. So, this photon is of this energy this relatively low energy only and then come similar photon another one or a slightly dissimilar one that takes to the next level. So, it is like this and this a step in between this step is half this step is half usually it is discrete 1 2 3 4 now

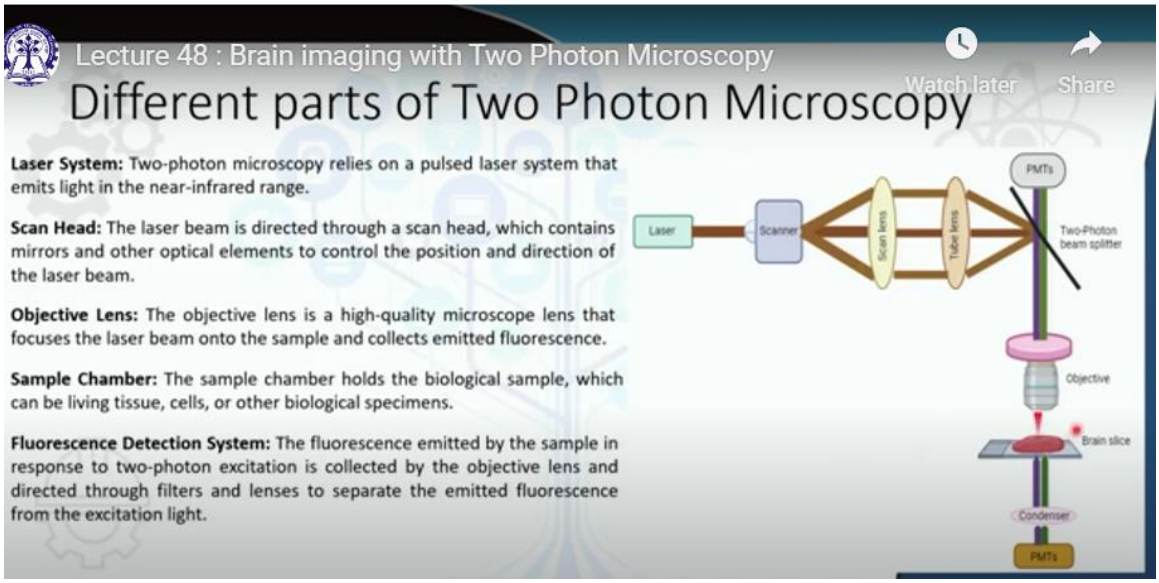
it is 0.5 0.5. Now, 0.5 previously you used to send 1 electron volt and used to get 0.75 electron volt out 1 electron volt say say 1 electron volt input 0.7 electron volt output some amount is lost.



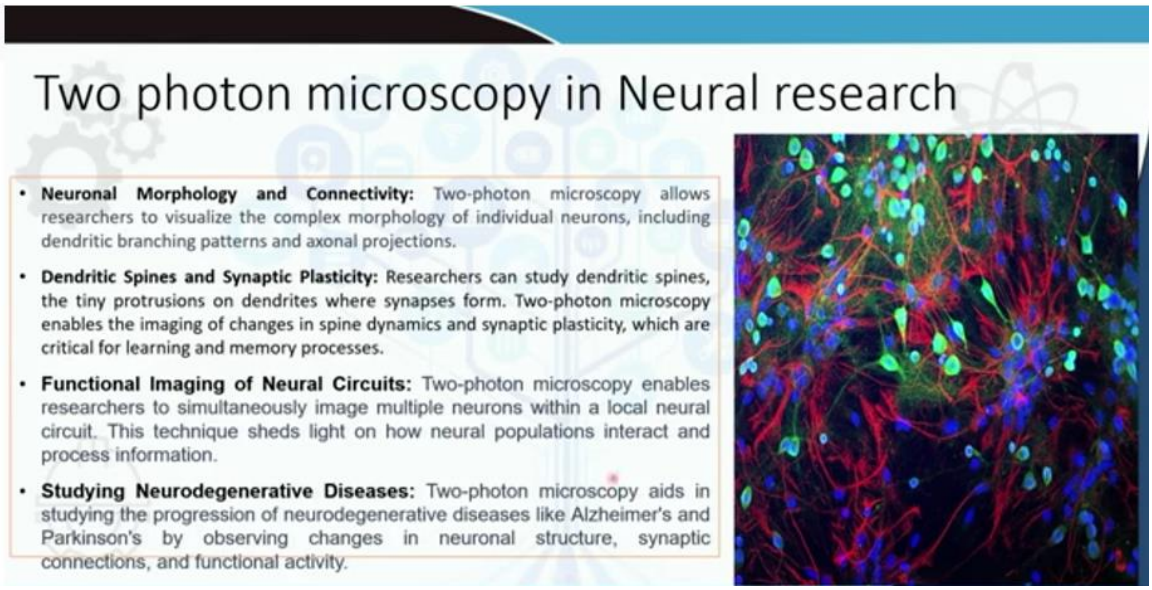
Now, you are sending 2 0.5 electron volt 0.5 electron volt result is same 1 electron volt, but the individual photons are of lower energy the output is same 0.75 electron volt. So, individually you can say that you are sending in a lower energy photon in the input not 1, but 2 and they are adding up to produce the same effect.



Obviously, the results are you know less damage and you can utilize the window the transmission window etcetera. Two photon excitation only occurs at the precise focal point of the laser beam where the intensity of the laser light is significantly higher than the surrounding area this allows for a very precise control over the excitation volume.

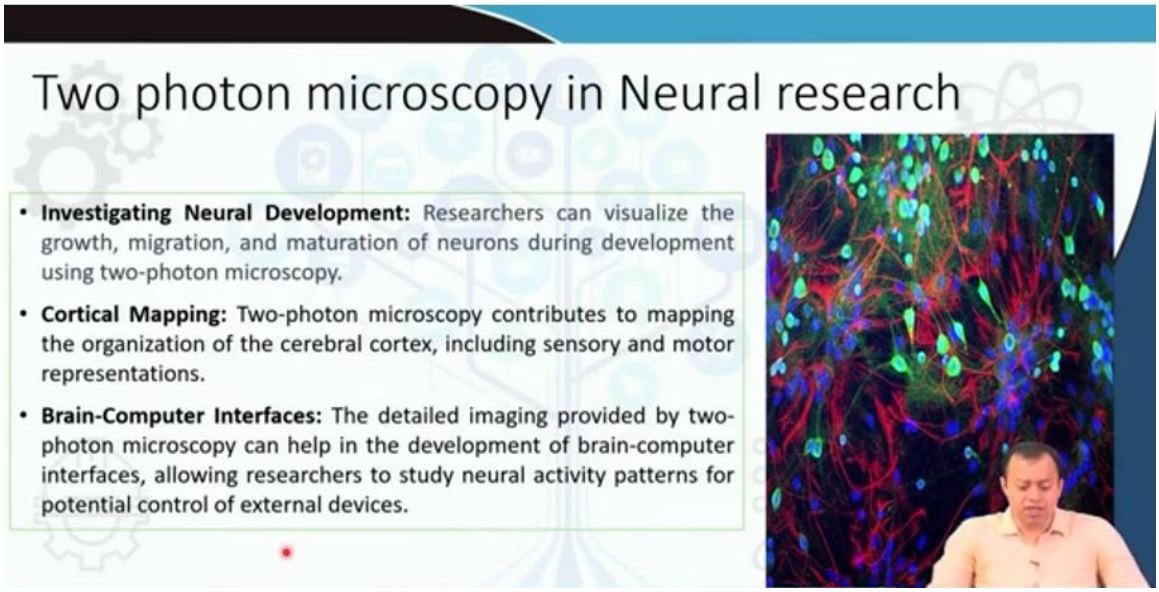


So, different part is simply here is your brain slice though you can do two photon microscopy in live live mouses as well you have a laser system a two photon microscope relies on pulse laser system you either send pulses photon 1 photon 2 simultaneously two photons together or slightly differ gap between two photons scan head the laser beam is directed through a scan head. So, through this objective lens sample chamber and fluorescence detection system you have obviously, photo multiplier tubes the detection system emitted by the sample in response to two photon excitations is collected by the objective lens and directed through filters for processing of data. Obviously, you have photo detector image acquisition system and optical filters. So, that any other stray light noise etcetera could simply be reduced.

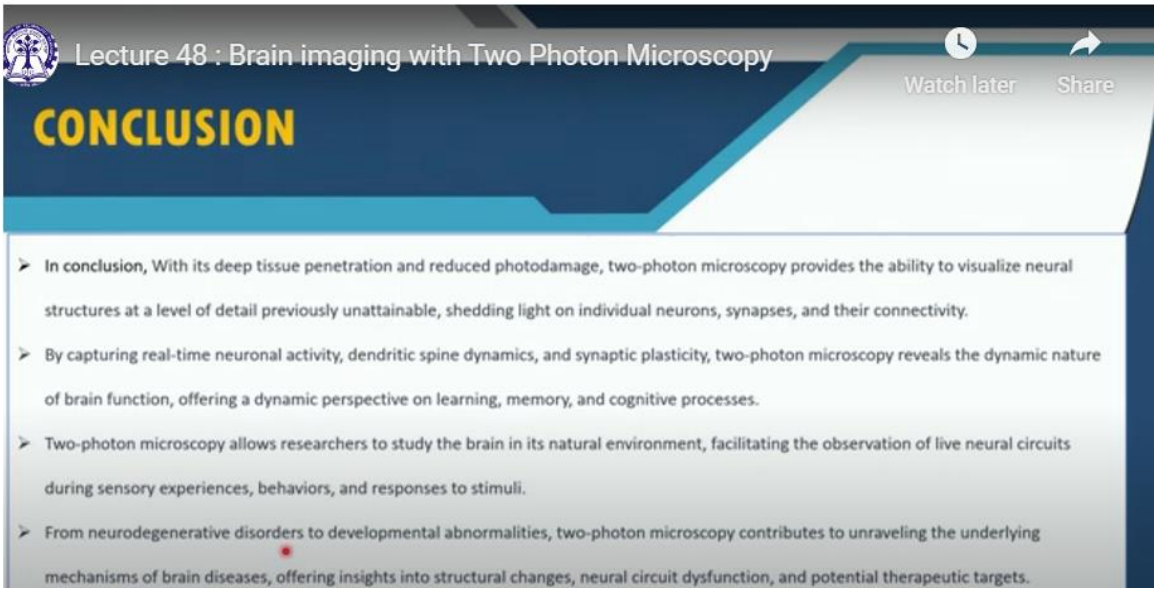


So, these are some of the images of two photon you know neuronal imaging system two photon fluorescence microscopy image of neurons in a brain cells.

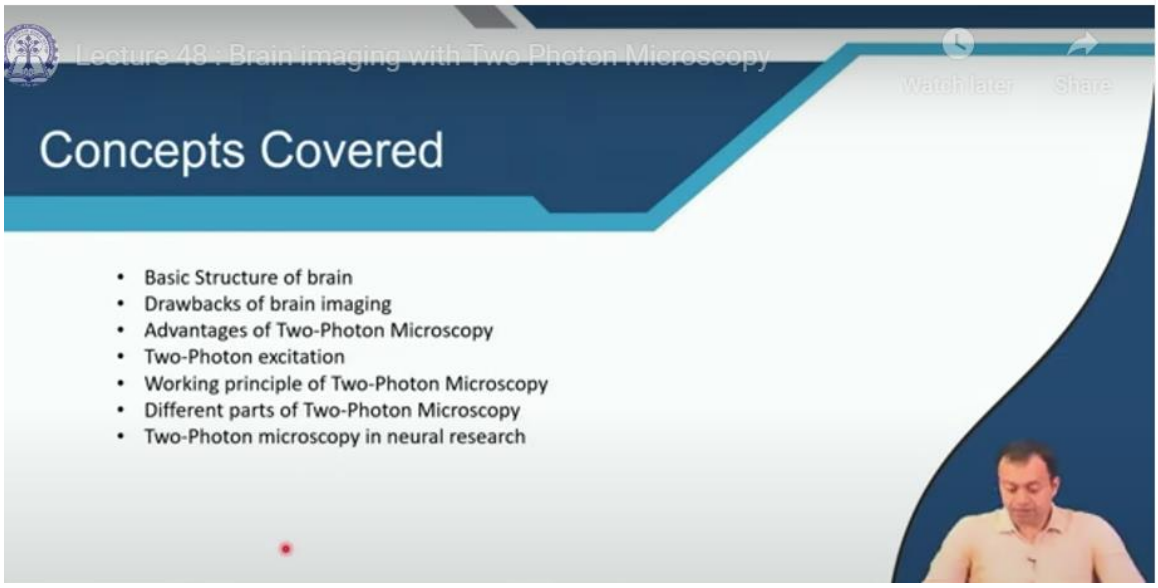
So, in neural research you can do understand obviously, the morphology and the connectivity allows researcher to visualize the complex morphology of individual neurons including didentric branching patterns and axonal projection remember axons are the long chain and the didentric are the branches dendritic sorry not didentric dendritic dendritic spines and synaptic plasticity researchers can study spines and tiny protrusion on dendrites where synapses form synapses are this gap between physical junction the gap between neurons versus axons versus dendrites functional imaging of neural circuits two photon microscopy enables researcher to simultaneously image multiple neurons and two photon microscopy aids in studying neuro generative disease like Alzheimer Parkinson's. Alzheimer Parkinson's are these days very very hot topic when it comes to neuroscience and detection neurodegenerative disease we still know very very little about these diseases diseases of the brain which is associated with misfolding of proteins, but we still do not know because of the misfolding the disease has happened or because of the disease the proteins have misfolded people have you know difficulty dementia people are forgetting things old people have had these problems motor neuron diseases all of those things.



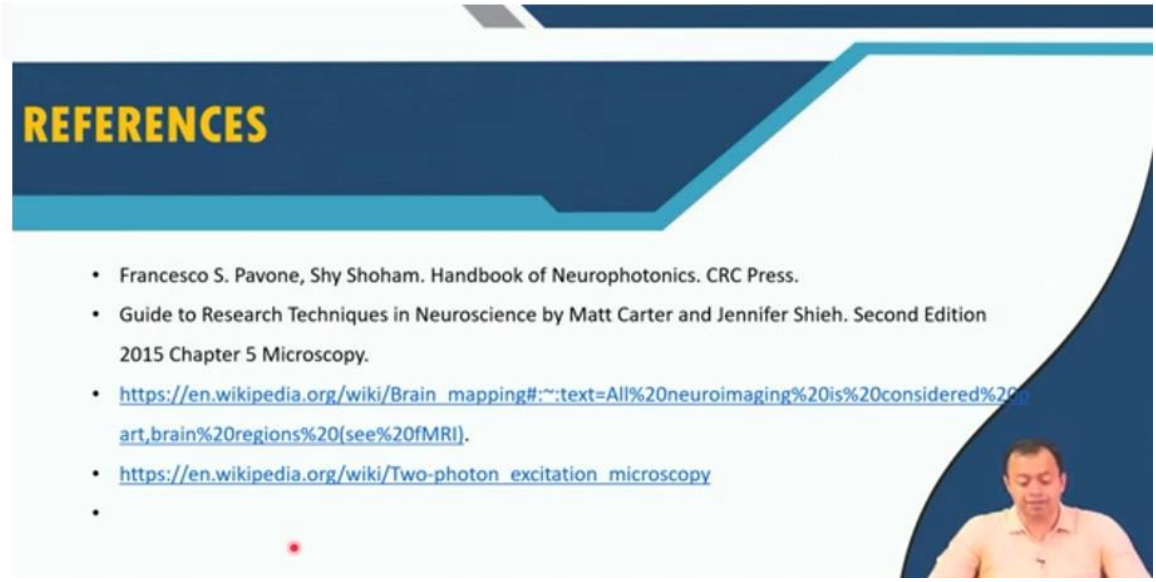
So, two photons can also be used for cortical mapping brain computer interface investigating neural development you can try from a larval stage and see an animal move animal grow animal become old and you know constantly map the brain with regular interval especially in zebra fish and other such animal models and you can get a fuller understanding of relatively fuller understanding because we do not have fuller understanding of course of the of the development of the brain.



So, in conclusion with its deep tissue penetration and reduced photo damage two photon microscopy provides the ability to visualize neural structure at a level of detail which was previously unattainable very helpful for detection of neurodegenerative disorders we can it can allow researchers to study the brain in its natural environment and individual parts of the nervous system could also be detected.



So, these are the topics that I covered today and these are my references Wikipedia surprisingly have fantastic section on the human brain and cognitive neuroscience as well as normal neuroscience not that cognitive neuroscience is abnormal, but you get my point just go through some.



So, especially for electronics engineer electrical engineers who think this is too much biology just go through some lectures or some cartoons two minute three minute each available in YouTube and that describes the brain the human organ brain very very simply to anyone from a non-medical background right I was greatly benefited by that. So, go through it right. Thank you very much I will see you in next class.