Biophotonics
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Lecture 58
Optical Neuroimaging and Tomography

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Welcome back. We are at the home run or the home stretch, where only three more lectures remaining with this one included, where we are discussing about optogenetics and neurophotonics. So, today I have a very interesting topic, it is about neuroimaging and not just any other kind of neuroimaging, not MRI type neuroimaging, optical neuroimaging, and little bit of tomography.

So, let us understand once again that what exactly is our goal here? I know I have said this before, but for those of you who are still unclear little bit about the goals per se, I think I can give you an electronics engineering subtitle and electronics engineering analogy. So, suppose you have a motherboard, a PCB structure, Printed Circuit Board with different types of circuital elements. And this motherboard performs a specific function, and this motherboard has stopped functioning. It has gone bad. It is no longer working because some connection somewhere within that entire PCB, within this entire printed circuit board motherboard it is no longer working. Consider it as a motherboard of your computer in your CPU.

You have not made that circuit, but you want to know which part of the entire motherboard, which part is malfunctioning, which part is not working and you want to repair that. Now, use the same analogy to think about our brain. Consider our brain to be 1000 times have a more complicated motherboard circuit. And you want to first understand which part does what and if possible, repair some part which you think has gone loose, metaphorically, not literally. We say screw loose. So, that is the premise.

So, in your motherboard, electronics engineers will know, how do you do it? You take a multimeter, and you go on sticking those two prongs into different sites, those two probes into different areas and try to see if current flows through it and thereby you, this is a very common thing, you figure out which one is the loose connection, which one is the fused which has no circuitry etc., etc. That is what you do. Using a multimeter, you keep on probing different areas and thereby try to find out. You switch off different, all the other parts of the circuit board, all the other parts of the motherboard and you are probing one at a time the different circuital elements to see which one is working and what does that circuit, circuital element specifically do.

So, this is a transistor somewhere in this big motherboard that helps you amplify a specific signal which is related to the speaker. Say this is a communication, this is an LED. So, different areas, different circuits, each circuital element, each mini circuit, each sub-circuit performs a specific function, and you want to understand that. That is how you probe a motherboard something that you have not fabricated, something that you do not know, something whose circuital diagram or a blueprint something is missing.

Consider the same thing with our brain. You have obviously not fabricated; you have obviously not made it and it has been given to you. You want to understand what each neuron does by switching off the other areas of the brain but switching on only two parts. So, exactly the motherboard, you have a probe, you are touching different areas, rest of the areas are switched off. It is not connected with a voltage source or a battery.

Similarly, here, the circuital element or the neurons are switched off theoretically. Imagine it, theoretically. It has switched off. You are probing to different neurons connected who are sending neurochemical signals, current or potential from one area to another area and what does that has resulted in.

What kind of behavior, just the message or the connection between one neuron here and another neuron passing information between one another, what does this translate into behavior? Will that make the owner of the brain hungry, aggressive, hungry or aggressive or sad or sedated or starts running? What sort of behavior we expect when two specific neurons talk to one another. There is electronic potential change, electrical potential change and current flows. This is what optogenetic does.

It selectively locates some kind of an opsin protein, light activated, light sensitive protein into certain neurons, not all. Those neurons are switched on by sending light. By sending light these proteins present in the neurons activate and start sending electric, electrons start sending electric current, charge, a potential difference happens. And as a result, current flows.

And that neuron and the other neurons its counterpart start talking among each other, start communicating among each other by sending this neural signal, by sending this electric electrical signal, just like two elements of a circuit which you have connected with your multimeter start communicating, start sending electrical current and you know okay. So, this is not fused. They can communicate. And their communication results in this particular LED or this particular diode or these particular transistors getting on.

Same thing here, the connection between two different neurons, the communication between two different neurons result in the person or the animal, the owner of the brain, performing a specific behavior, because believe it or not, all our behaviors, all our patterns, all our moods, all our feelings, all our memories can be all contributed or attributed towards neuron communicating among each other. Neurons complicated-wise, but still they are communicating among each other. So, we want to probe one neuron at a time what is happening.

Optogenetics allow you to do this particular function, to image, to understand, to map cortex by cortex, neuron by neuron and get a whole, total complete understanding of which circuital element performs what function, which neuron or which group of neuron results in us mathematical calculation, which group of neuron results in us getting afraid, what neurons makes us laugh or sad or depressed. At least, that is the potential that biophotonics has shown.

None of this what I am saying is already achieved then we would have gone to the next civilizational quantum jump, but biophotonics has this capacity. So, today we are going to

discuss a bit about that and how exactly the mechanism of doing something of that sort. So, usually, you have an animal on whose brain you have transfected some amount of genes which can produce opsin and this opsin can upon excitement by light send signals, electrical signals, but what is the tool, what is the equipment, what is the mechanization behind it.

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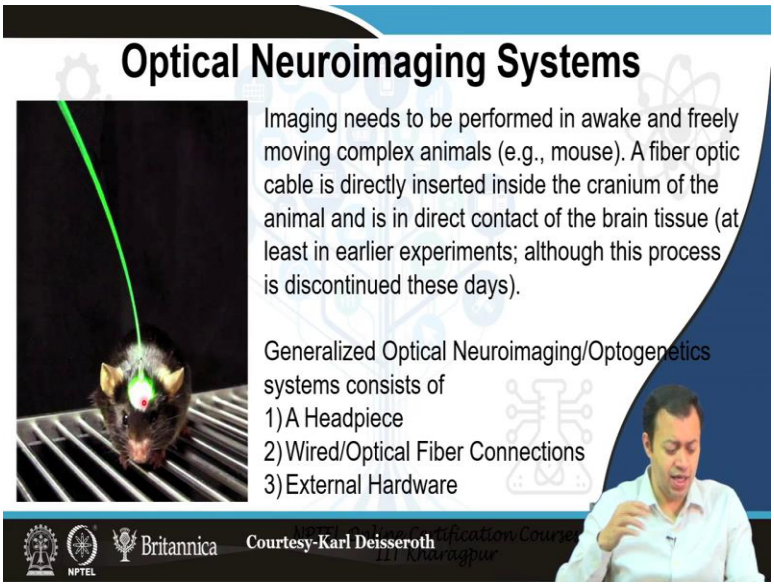

So, the mechanization is quite straightforward. So, you need to perform in awake and freely moving complex animal e.g. a mouse. We start with mouse and then only go to human being. You cannot do that to human being directly. A fiber optic cable is directly inserted inside the cranium of the animal. So, there is a fiber optic cable inserted into the cranium of the animal and is in direct contact of the brain tissue at least in the earlier experiments.

The very first experiment 2005, 2006, they did a direct connection with the brain tissue, but nowadays ethical committee and everything ethical consideration that process has long since been discouraged, because connecting something that close to brain tissue results in infection and the animal dying and it is still frowned upon.

So, nowadays it is close to the brain tissue not directly in contact, but previously it used to be directly in contact. And the total neuroimaging optogenetic system usually consisted of a headpiece, crown, a hat. Wire connected with it with optical fiber connection, wired or optical fiber connection. And external hardware, which is some sort of photodetector, some kind of an electric current detector, something to present in this headpiece and you shine light, you measure

the electric current that is happening. And you have already mapped which two neurons you have transfected with the opsin fluorophore. So, the image is something of that kind.

So, you see, this is the optical fiber. This is connected at the cranium of the mouse and this is sending light signals. And the mouse is more or less free and awake and it is still moving around. I have another more better picture for you. Some of you may dislike because of animal cruelty and whatnot. But remember, that is how somewhat science progress. I am not taking a controversial stance. I am saying that these things are being done. And hopefully, I hope, I do not know that they have gone through proper ethical clearance.

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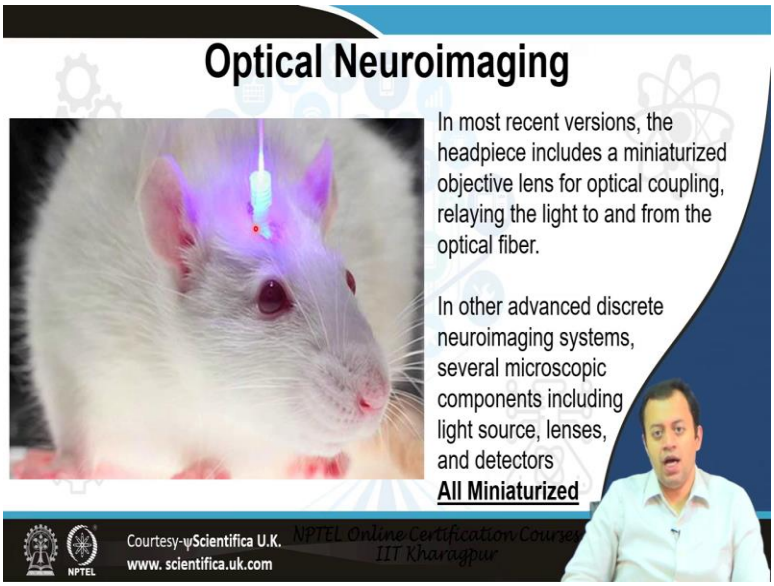

So, as I said, these days, we are not putting the fiber directly in contact with the brain tissue of the animal. These days we have a miniaturized objective lens that is focusing that the lens is at the end of the fiber that focuses light deeper still deeper, but in a free space into the brain tissue. And that the end of the fiber is connected with several advanced discrete neuroimaging system such as lenses, light sources, detectors, all miniaturized nanotechnology again, nanotechnology once again. And this is that picture. Do not finch. This is I hope that they have gone through.

So, you can see this thing is inserted into the cranium inside the skull of the mouse and you are sending light through it. This all contain miniaturized maybe light sources or lenses so that it could focus at different areas, different sorts of lights at, different sorts of laser wavelength laser pulses are done so that you can illuminate certain areas for a longer period of time, certain areas

for less period of time. You control the laser. And nowadays we are all so getting optical signal that has been scattered out. Remember optical biopsy, we were talking about.

The optical signal that is getting scattered or that is getting reflected by the light coming from here and then returning back we are analyzing it spectroscopically, thereby trying to analyze what kind of behavior changes in the head when the animal is fully awake, and it is freely moving. So, this thing is connected with a long cable, long wire. So, remember the image that I showed you in photodynamic therapy where optical fiber was brought in which was containing laser and which was inserted in someone's stomach and they were doing surgery. Remember that image.

So, imagine the same thing and no, well, they do take care of the animals' health as well, because obviously if the animal dies, what kind of brain activity are you going to get. So, that defeats the purpose. So, the idea is to keep the animal non-sedated, freely moving, and obviously alive and awake. That is the overall idea. So, you drill a hole. You have to do that. You have to drill a hole ensuring that the animal does not die. And then insert this sort of a tube, this sort of an optical fiber connecting all those miniaturized components on to its head. And then you send light, fluorophore, chromophore what not, different pulses, different intensity, different wavelength.

And then you measure either electrical current, the overall electrical conductivity changing of the brain because of the light that is affecting the opsin proteins, light sensitive proteins or you are sending some amount of light, the light is getting reflected back you are measuring or those of you who have already figured it out, we can also do some sort of tomography, optical coherence tomography that we were previously doing for our eyes or for our teeth can be done here.

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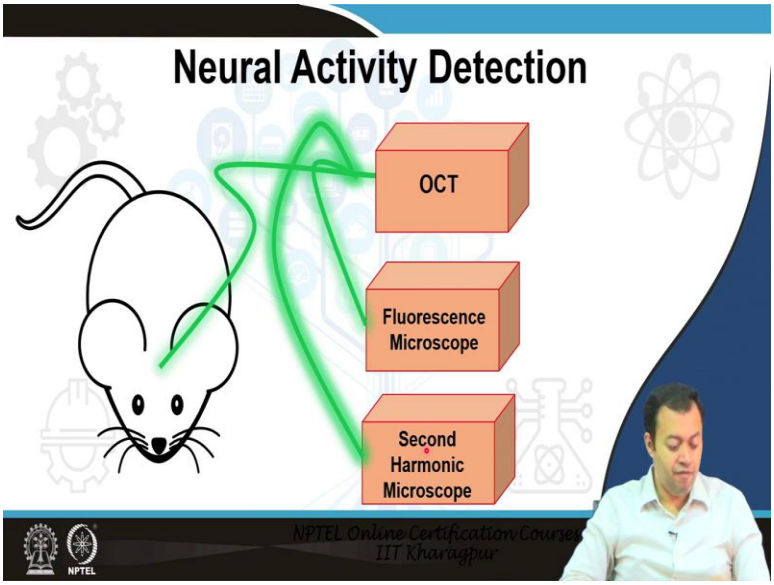

As a matter of fact, several things can be done here. If you have connected to the mouse's cranium of fiber, the other end of the fiber can be connected to several different areas, several different tools. You can connect with an optical coherence tomography machine. You can connect it to fluorescence microscope. You can connect it to second harmonic microscope. Remember, second harmonic microscope measures all those collagens, all those proteins that emits this second harmonic emission that those non-centrosymmetric molecules like collagen proteins, those which emit second harmonic radiations.

So, you can measure some sort of that kind of things, those type of proteins who are non-centrosymmetric as such. You can feed the animal with certain food that will contain some sort of a chromophore. And you can ensure, biotechnologists will tell you, how the different chromophore present in the food, get distributed into different areas of the body. Some may go into the brain, not just into the stomach and the digestive tract, may get lodged into the brain. You can ensure, this is how medicines are being discovered. So, thereby you can send light and different areas will fluoresce with a different frequency.

Obviously, absorption frequency and emission frequency in fluorescence are two different things. So, you measure that fluorescence using this fluorescence microscope. And obviously, obviously, you can do an optical coherence tomography. Optical coherence tomography by this time you already know.

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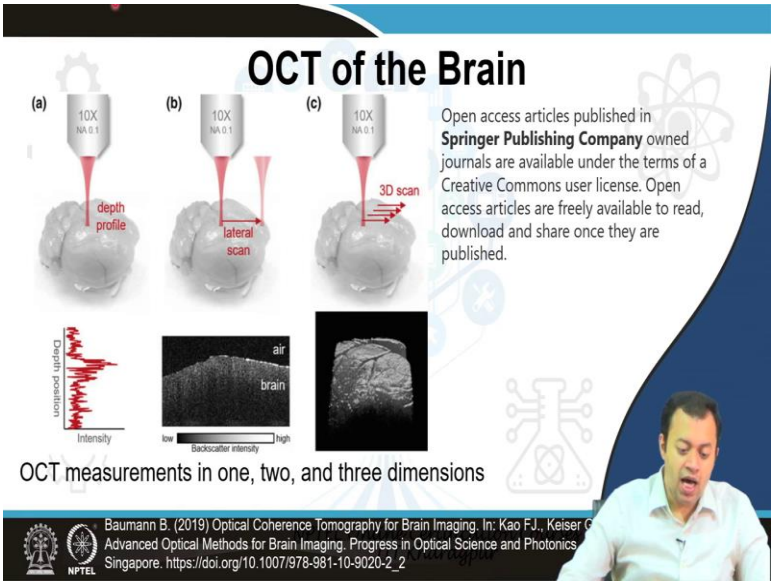

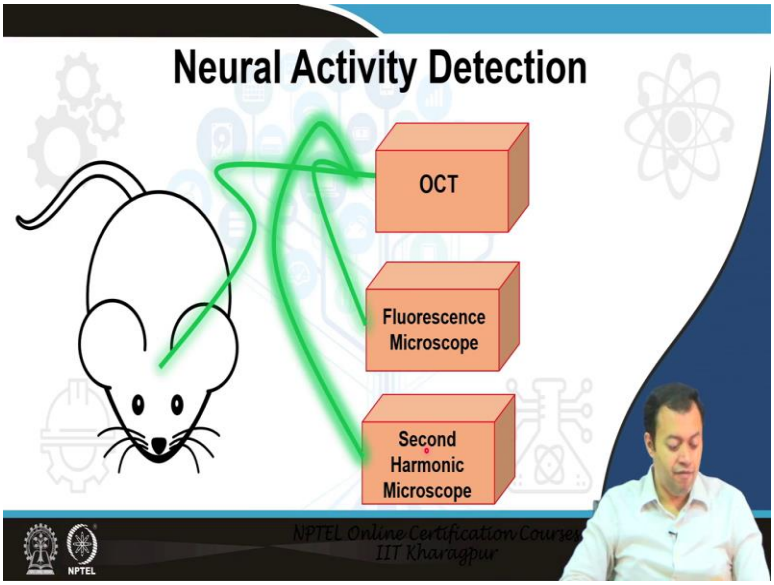

And I have a very cool picture here. This is what they are going to do. They are using this optical fiber with a 10X numerical objective. They are putting the light here. And you remember by changing the distance, the coherence of the two waves matching changes, the interference pattern changes and by that you understand the overall depth of the tissue. Remember, if you do not, then go back to the lecture in which I discussed optical coherence tomography that was for your eye to understand how the retina has performed.

Here we are using it into the brain by inserting the optical fiber directly into the cranium, into the skull of the animal, inside the skull of the animal. And by putting this up and down you can get a

depth profile. You can move it side by side to get a lateral scan. And when you do both of them together depth profile as well as lateral scan, you can get a three-dimensional image of the brain. This is air and this is the brain tissue.

And depth profile and intensity and all of those things can be measured. This total information when combined with your fluorescence microscope, second harmonic microscope as well as some of the octogenetical tool in which the current is measured by firing specific pulses of light all of these three or four, remember this tube, this fiber can contain many small fiber and different frequencies of light can be shown simultaneously.

So, 1 micron or 2 micron or 8 microns could be the diameter of each fiber. And remember, the width of our human hair is around 800 micrometer or 500 micrometer or even less. So, 1 micron, 2-micron, 10 micron or 20-micron fibers, a bunch of them could be tied together and sent inside the cranium. It is still risky, but we have to perform. How else do you think we will be able to understand how the brain works? And if you think that that is not important enough, well then maybe this is not the right area for you to invest in.

So, anyways, we are thereby able to measure the optical, the depth profile, a three-dimensional scan of the brain. And these are highly developing subjects. These are highly classified and highly developing subjects. And I have to put a disclaimer here that Springer Publishers have allowed for freely available to download and share once they are published. So, I hope they do not sue me for showing you this, because this picture is taken from this particular reference. I am quoting the name and I am quoting everything so that this, they do not take it seriously. This is simply for teaching you.

But this, these are highly advanced and sometimes classified information. So, not all the time I can get copyright to show this to you. I can get the permission to break the copyright and show this to you. So, again, I am asking you just do an Internet search on the optical coherence tomography of the brain for those of you who are interested to know the depth profile of the brain. So, these, this is my reference, and I am grateful that they allowed me, or I hope they allowed me based on this disclaimer, what sort of information you can image.

And combine this with the other sort of information, other sort of microscope, other analysis, there is a pretty good chance, at this present moment it is only chance, there is a pretty good

chance that we will be able to understand image or diagnose or map overall the entire circuitry of the brain. Remember, this is the, not just any human brain, any mammalian brain, even if it is a as tiny an animal like a mouse, it is tremendously complex. And no other organ in its complexity comes closer.

I think, I will in this time get a concurrence from life science students and medical students. No other organ, neither your heart nor your lung nor your digestive tract nor your kidney nor your liver comes into the entirety of the complexity of the brain even in an animal such as a mouse. So, it is incredibly difficult for us to understand the complexity or understand which part of the brain perform what particular function. We may be able to image it. But to understand deeper insight into which part does what is complicated, especially cell by cell neuron by neuron.

So, this is where I leave you. This is where I am leaving you. I am not going into further detail because frankly speaking, I am facing great difficulty in understanding the brain. So, irony, my brain is not capable to understand brain. So, it would not do any justice to you by me teaching you the entirety of the brain and which part we understand does what.

So, I will leave it, leave you here. Regarding the brain imaging, I strongly recommend you to go and look for neural imaging, not just with optical neural imaging, but several other type of neural imaging. And try to ascertain the enormous challenges and complexities that we face in some kind of a neural imaging and then corroborate it co-relate it with the behavior.

So, what is changing inside which part of the brain which is resulting in a specific behavior of the animal. This is a fascinating subject. And I cannot do any justice to you by giving you half information or quarter information. So, I will leave this part here. There is another topic coming up. But that would be more or less the extent that I am comfortable going because otherwise I might not be able to teach you as I expect or as you expect of me.

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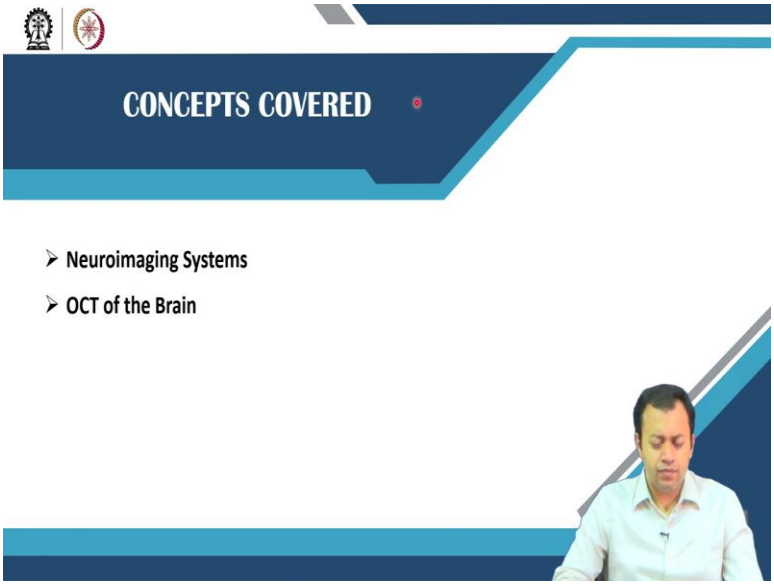

So, these are some of the topics that we discussed today.

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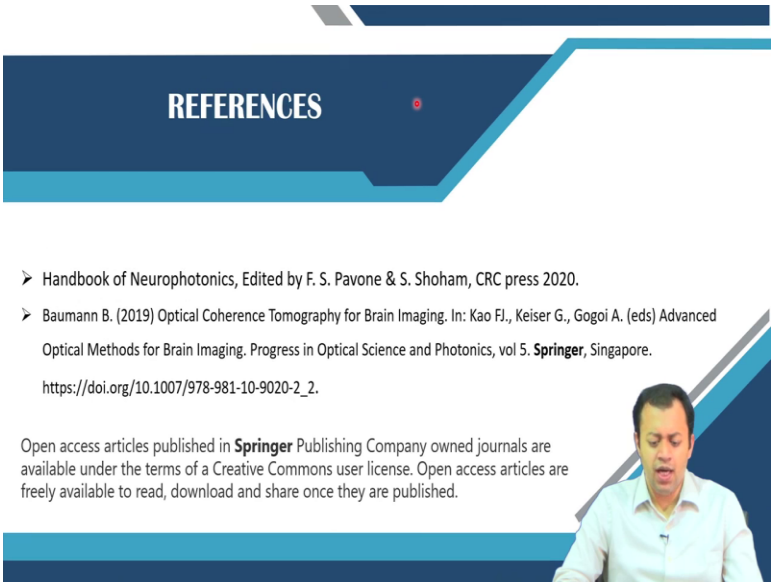

And please, please go through the references that I have given you. This is the book that I am following. This is the book that is helping me out and, along with some of the papers. And again, this is the disclaimer that I need to tell to Springer Company so that they allow me there, for me to show though that particular image to you. And I will see you in next class. Thank you. Thank you very much.