Advanced Neural Science for Engineers
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Lecture 51
Deep Brain Stimulation/Recording for Parkinson's-II

Hi everyone. This is the second half of the lecture on implantable micro-electrode arrays for Parkinson's. Until now what we have seen? We have seen how to what is Parkinson's, what happens when there is a freezing of gait, what are the regions in which the neurosurgeon will implant the electrodes, and what can be an alternative region which people are working on, and our efforts on using the surface plus deep brain to see whether it will improve the current in the treatment of Parkinson's. So for that, we need to work on an animal model, and for that, we have discussed about rats or rodents in detail.

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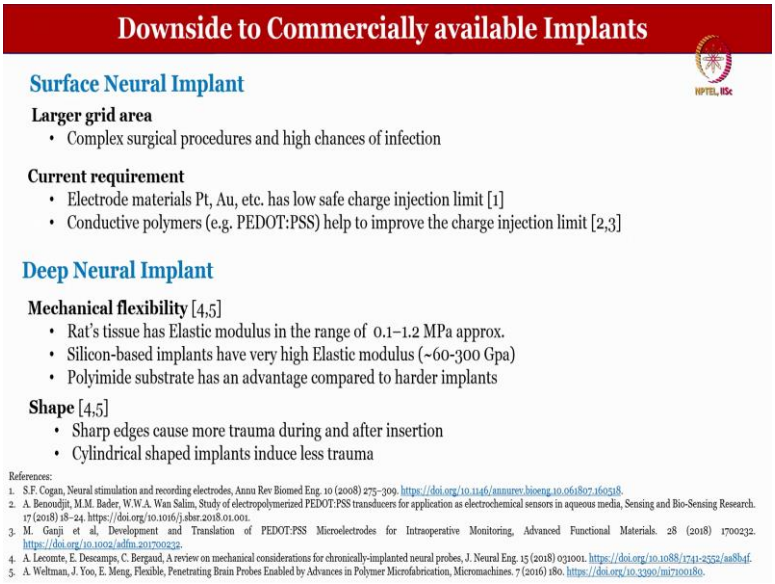

Now, let us understand the design and surface of the neural implant. So in the last slide, if you remember what we have shown is we have shown that, we have shown that what are the downside of the commercial available implants and we have also shown that what are the areas which are of our interest and in particular how we can fabricate the device.

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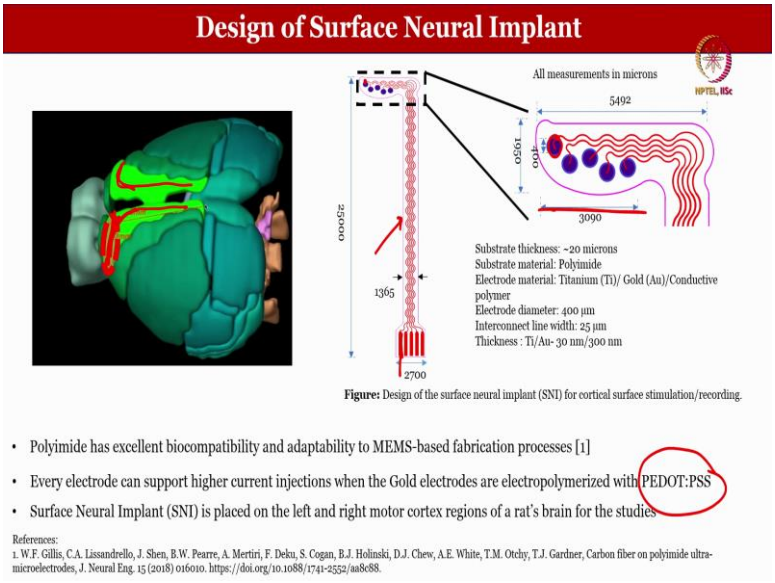

So let us understand how we can fabricate this particular device. So if you see this particular image in this image again our interest is of 3 mm by 8 mm that is the area that we are interested in and what else we are interested in? We are interested in the device that can be implanted in this particular region. So for that we need to fabricate this device and this device can be fabricated if you know how the micro-fabrication technology can be utilized or can be used.

So in this slide what you are able to see is the image where we want to implant the device and here on the side you are looking at the design of the surface neural implant. Now, we have talked about the deep neural implant and surface neural implant. This is for the cortical surface stimulation and recording.

If you see this design there are 5 electrodes- 1, 2, 3, 4 and 5 and each electrode is about 400 microns in diameter and the if you see the length is 3.9 which will fit somewhere in this area like this. It will fit like this, okay?

And then you have another device that will go in this area like this. Alright? So that is why we have designed this with this angle so that it can fit well in the brain. Now you can also see the wavy structures here like this.

The reason of that is to reduce the stress when the device is stretched. So it is a flexible device. I have told about the advantage of flexible device that it will damage the tissue less compared to the hard devices which are made up of silicon.

So the substrate thickness in this particular case which is the polyimide is about 20 microns. As I told you the substrate material is polyimide, the electrode which is the recording electrode and this one including the connecting lines are made up of titanium and gold and this and then on that we can also use conductive polymer like PEDOT:PSS. We have seen why PEDOT: PSS is important.

And then now, if you remember PEDOT: PSS, then I have shown it to you that the PEDOT: PSS had a certain advantage over gold and platinum that it will improve the charge injection cap limit. It will improve the charge injection limit. So it is better in terms of electrical stimulation.

Now, the interconnect line width that is this wavy line that you see the line width, width of this is about 25 microns. The electrode diameter we already talked about which is 400 microns and the thickness of the titanium or gold or below gold (gold is over titanium). Titanium is used to improve the adhesion of gold on the polyimide. So the titanium thickness is about 30 nanometers and the gold thickness is about 300 nanometers.

So the reason for using polyimide is that it has excellent biocompatibility and applicability, adaptability to MEMS-based fabrication process. We can use the MEMS-based fabrication process but there is this deposition which is PVD or it is lithography, we can easily use.

Second is that every electrode can support higher current injections when the gold electrodes are electro-polymerized with PEDOT: PSS. This is another advantage of the, of using the polyimide and also the advantage of using PEDOT: PSS over gold because it will improve the charge injection currents or higher current injections.

The next one is, surface neural implant is placed on the left and motor cortex regions of rat's brain for these studies. So this is another advantage of a surface neural implant that we have designed in such a way that it can be placed on the left and right. So we can change the design or we can customize the design based on the requirement. So that is all about the neural implant, particularly the surface neural implant.

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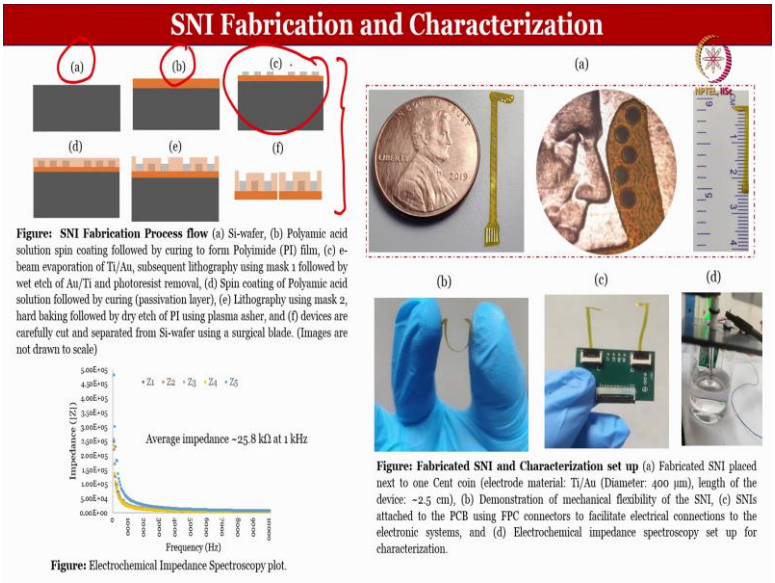

Let us understand how the fabrication is done, okay? So again you see that a device which is placed next to the cent to give you a perspective of what is the size of the device. You can see the device on the scale and there are 5 electrodes as you can see here- 1, 2, 3, 4, and 5. Each side and left area, there are 5 electrodes that can be used to stimulate that area or record from that area.

When we have this device, it is flexible in nature that you can see from (b) if we connected with the electronic module that you can see from (c). You can look at the electrochemical impedance spectroscopy which you can see from (d) and then you can also see that the average impedance value is about 28, 25.8 kilo ohms which matches with the literature.

Now, let us understand how to fabricate this entire polyimide. So it is a step-by-step. So I will show you each step. For here let us see whether you all understand this step and I will show it to you in detail okay in the next slide. So first is a silicon wafer, (a) is silicon wafer, easy.

Next (b) is you use the polyamic acid solution, spin code it on the silicon, and then cure it to form the polyimide which is PI film. The next one is that you e-beam the Ti/ Au, titanium at the bottom, gold at the top and then you perform the photolithography to have this design. So let us understand how to reach to this design and then we continue from (d), okay? To reach (c) what do you need to do? From (a) to (c) we will see in the next slide. Let us see.

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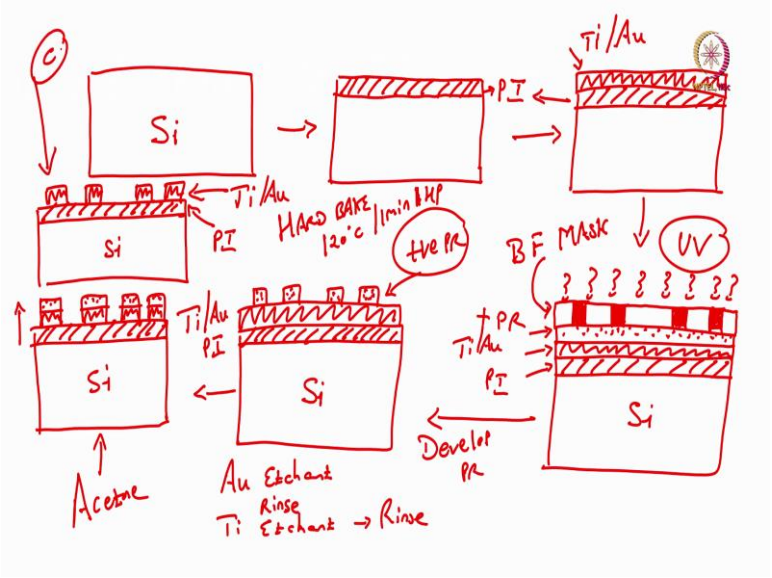

So you have a silicon wafer and then you can have a polyamide film. This is your PI film. Then you deposit titanium and gold. This is your titanium/gold. This is PI. This one is titanium/gold.

The next step is you have polyimide. On that you have titanium/gold. On there, you have a photoresist. Right? Now, let us say this is a positive photoresist. Next step is you load the wafer. Load the mask, I am sorry, load the mask and it is a bright field mask.

So the area which has this chrome, it is a chrome mask. We have seen that you can have bright field mask, we have dark field mask and the material that is used to pattern this mask is chrome. That is why it is also called as a chrome mask. So this is our bright field mask.

After you load the bright field mask you know when you sprinkle photoresist what is the next step? Next step is always a soft bake. Soft bake is done as I have told you. Easy to remember- 90 degrees, 1 minute on a hotplate. But if you have an oven, it is different. We have seen that. For the oven it will become 40-45 minutes. Again you need to optimize this soft bake timing okay depending on what kind of whether you are using a hot plate or using an oven depends on that.

So once you spin code photoresist and you soft bake it, then you can load the mask and then you have to expose the photoresist through the mask using your oh-huh, the beauty of technology and the limitation of the technology. Okay, we are back. Good. So now this is your UV.

So if you expose it through the mask, it is a positive photoresist, so whatever the design is there in the mask will be there on the photoresist or the unexposed region will become stronger. This is your PI. This is Ti/Au, silicon, and your photoresist. You see the area which is not exposed is stronger.

After exposing the photoresist with UV, you have to unload the mask, develop the wafer, develop the photoresist in the photoresist developer. When you do that, you have this particular pattern. The same pattern that is on the mask is replicated on the photoresist because you are using a positive photoresist. After that you have to perform the hard bake.

Hard bake is done at 120 degrees centigrade, 1 minute on hot plate. All right? After hard bake, the next step is you have to dip it in gold etchant and rinse it. So first dip it in Au enchant, then rinse, then Ti etchant, then rinse. If you do that, you will have, I am sorry, titanium/old protected by the photoresist. Correct?

This is what you have. And then if I did this wafer in acetone then what will I have? So if I dip this wafer in acetone I will have a PI with my titanium/gold; PI with a titanium/gold. Correct? This is the PI; PI and on that titanium/gold. So where are we? What is this thing? This thing is our (c). Let us see, okay?

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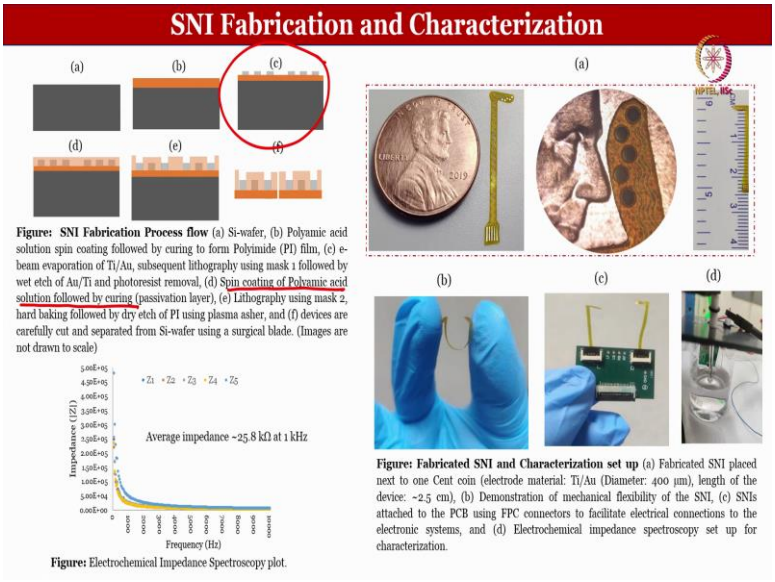

You see here, this is where we are. Now the next step is if you see (d), spin coating of polyamic acid solution followed by curing passivation there. So again we had to coat the PI on the (c).

So if you want to do that, let us remove all these things and we will start with the gold, titanium/gold on the PI and then we coat the PI as an insulator. PI is a substrate. PI is an insulator. It is a very interesting concept.

Because the polyimide can also work as an insulating material, can also work as a substrate because we can have a 20-micron thickness. We can vary the thickness. It is flexible in nature. It is biocompatible. So a lot of advantages of using the polyimide in the MEA fabrication. MEA stands for micro-electrode arrays. All right? So our slate is clean and let us start drawing again.

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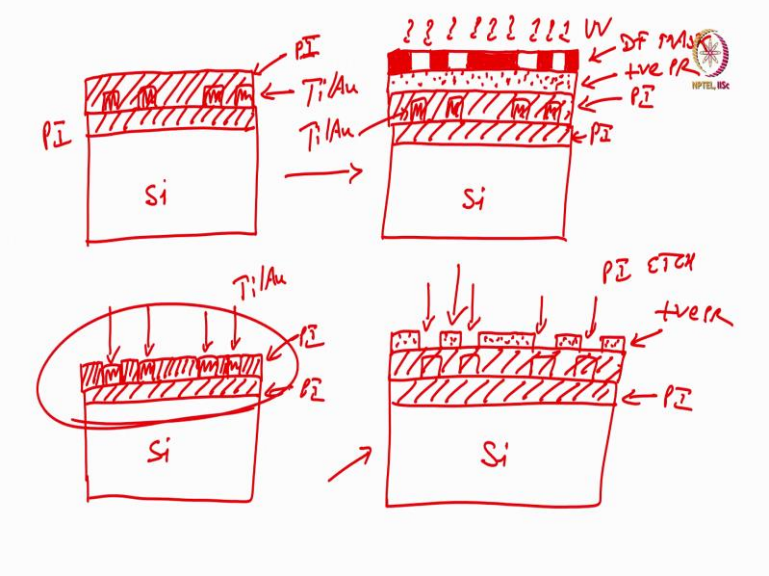

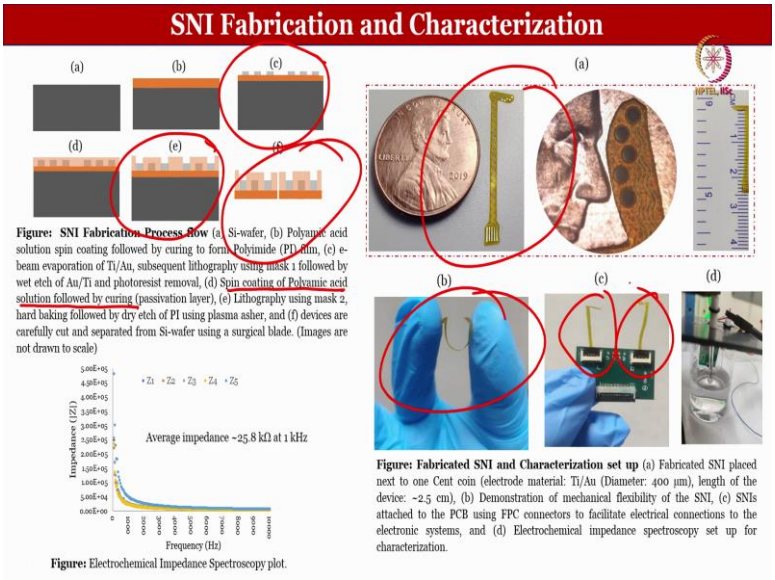

So we have your silicon wafer and PI and titanium/gold. On that we can have one more layer of PI, one more layer of PI, okay? So and you know how to coat it- polyamic acid followed by curing forms the polyimide.

The next step is, after this the next step is to load the wafer; to load this particular titanium/gold, PI, PI, Ti/Au, spin coat positive photoresist, soft bake it and then use a dark field mask, use a dark field mask so that we can remove PI on the contact electrodes and recording electrodes.

These are dark field masks dark field mask and then uploading dark field mask ultra-violet light exposure. Now dark field mask is there, and then positive photoresist is there, positive photoresist unexposed area becomes stronger. So what we will have?

We will have your PI, bottom PI, then we have these electrodes and then we have the top PI and photoresist. Right? Area which is not exposed becomes stronger. After this next step? The next step is I will etch the PI from these regions, PI etch, okay?

If I do that what will I have? I will have a silicon wafer with polyimide, let me just, with polyimide and titanium/gold. Very difficult to differentiate but this is a contact, this is a contact, this is a contact and this is a contact, okay?

These 4 contact regions does not have your, does not have the PI on it. So this is all your titanium/goal. The remaining area has a PI because you can etch PI to the windows that you can see in this particular diagram. Where are we now?

We are here in (e) and then once it is there, we can remove this, we can strip it off the PI from the silicon wafer to form the flexible sensor as you can see in this one or this one or this one or even infact this one which is connected, isn't it? So this is how the flexible sensor is fabricated with n number of electrodes. In our case it is 5 electrodes.

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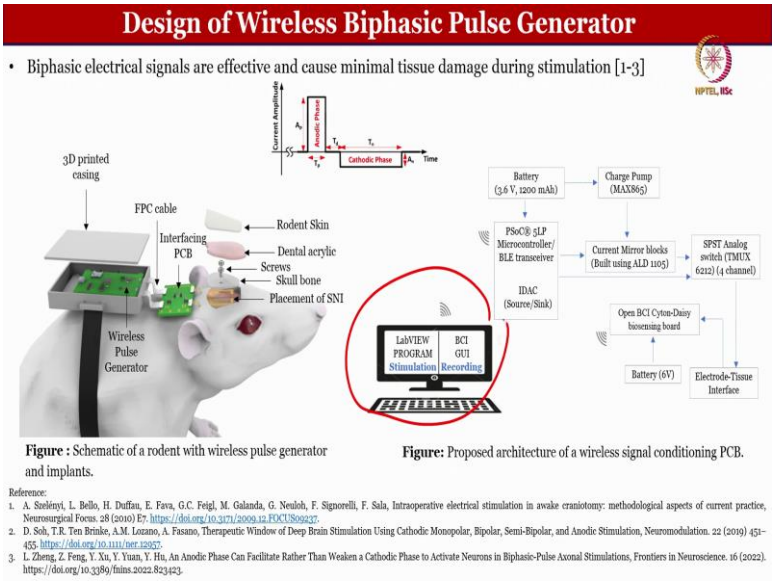

So once you do that the next step is to design the wireless biphasic pulse generator. Now you need to understand that we have we are using deliberately biphasic electrical signals because they are effective and cause minimal damage during the stimulation. So you can see the anodic phase followed by a cathodic phase. The deliberation, deliberately the anodic phase, the time is smaller compared to the cathodic phase and in this case, the schematic of a rodent with a wireless pulse generator is clearly seen.

There is a 3D printer casing where there is FPC cable, interfacing PCB which is interfaced with these particular electrodes. There is a placement of surface neural implant, skull bone is there, screws are there for reference and ground, dental acrylic is there and rodent skin is there. So this is how the electronics is connected to the brain of the rat.

For details, we can also understand that if you understand a bit of the electronic side, then you will know that how the PSoC microcontroller will work. You need to have a 3.6-volt battery. There is a charge pump and there is the current mirror block, there is an analog switch and then this whole thing is connected to your electrode-tissue interface and also the signal that comes out is given to the OpenBCI, and for OpenBCI biosensing board there is a power supply of 6 volts.

So it is a and then you can always use the LabVIEW programming for stimulation and your graphical user interface can be used with your brain computer interfacing modules. So you want to know more about this simulation, you can look at any of these particular references. If you do not find the references, please let me know I will send it across.

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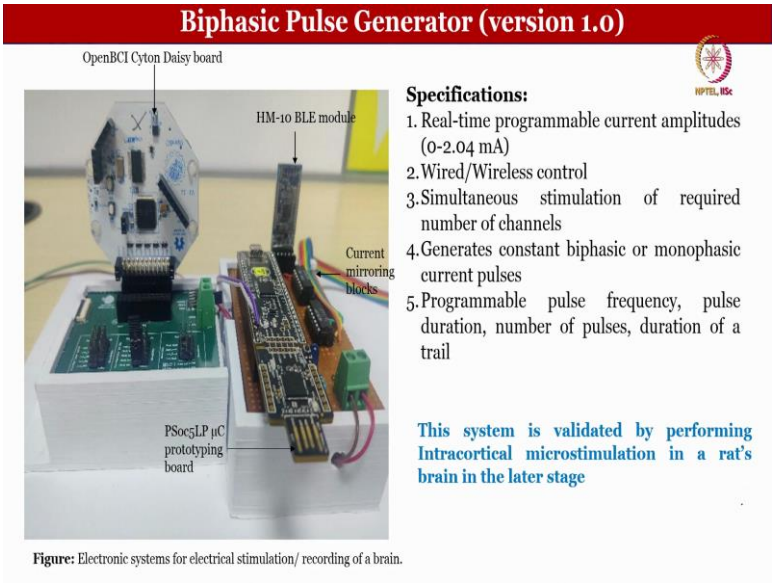

Figure: Electronic systems for electrical stimulation/ recording of a brain.

So while doing this we bought a PSoC LP microcontroller prototyping board that is easier for us to start working with and already there are current mirror blocks and there is a HM-10 BLE module for Bluetooth transmission. There is an OpenBCI Cyton Daisy board which you can see here in the schematic and the specifications that we used for this particular version where it can be real-time programmable with an amplitude of 0.2 to 0.4 milliampere current.

It can be wired as well as wireless control. The simultaneous stimulation of required number of channels is possible. Generates constant biphasic or monophasic current pulses. It can is programmable pulse frequency, pulse duration, number of pulses duration of trail, so all things are very easily programmable. We have validated this by performing intracortical micro-stimulation in a rat's brain at a later stage.

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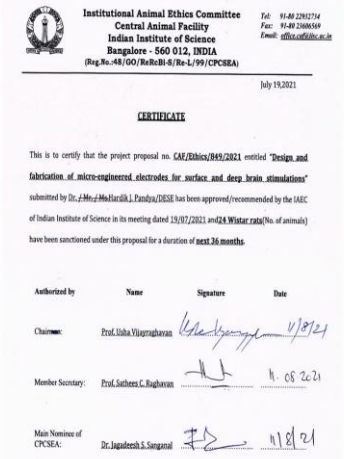
Experiments

Approvals for Animal Experiments



Institutional Animal Ethics Committee
Central Animal Facility
Indian Institute of Science
Bangalore - 560 012, INDIA
(Reg.No.:48/GO/ReRcBi-S/Re-L/99/CPCSEA)

Tel: 91-80 22932734
Fax: 91-80 23606569
Email: office.caf@iisc.ac.in

July 19,2021

CERTIFICATE

This is to certify that the project proposal no. CAF/Ethics/849/2021 entitled "Design and fabrication of micro-engineered electrodes for surface and deep brain stimulations" submitted by Dr. / Mr. / Ms.Hardik J. Pandya/DESE has been approved/recommended by the IAEC of Indian Institute of Science in its meeting dated 19/07/2021 and24 Wistar rats(No. of animals) have been sanctioned under this proposal for a duration of next 36 months.

Authorized by	Name	Signature	Date
Chairman:	Prof. Usha Vijayraghavan		4/8/21
Member Secretary:	Prof. Sathees C. Raghavan		4.08.2021
Main Nominee of CPCSEA:	Dr. Jagadeesh S. Sangamal		4/8/21

Figure: Ethical clearance certificate obtained from IAEC, IISc.

INDIAN INSTITUTE OF SCIENCE
BANGALORE -560 012
Institutional Biosafety Committee (IBSC)

IBSC Clearance Certificate

The following proposal was cleared by the IBSC members after the online meeting held on 22.09.2021

Title of the Project: "Design and fabrication of microengineered electrodes for surface and deep brain stimulation of Parkinson's diseases"

Principal Investigator: Dr. Hardik J Pandya
Department of Electronic Systems Engineering
Indian Institute of Science
Bangalore-560012

Clinical Collaborator: Dr. Shaheri Girishan K V
Ramaiah Memorial Hospital

Prof. Usha Vijayraghavan
Chair
Institutional Biosafety Committee
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Prof. Ganesh Nagaraju
Member Secretary
Institutional Biosafety Committee
Secretary
Institutional Biosafety Committee (IBSC)
Indian Institute of Science
Bangalore - 560 012

Figure: Certificate obtained from IBSC, IISc.

In vivo experiment: Recording brain signals

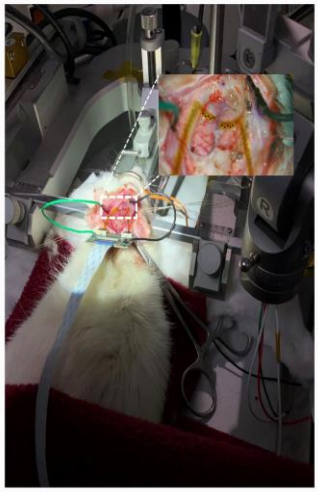

Figure: Anesthetized rat's head fixation on a stereotaxic apparatus followed by surgery and recording ECoG signals from sensorimotor cortex regions.

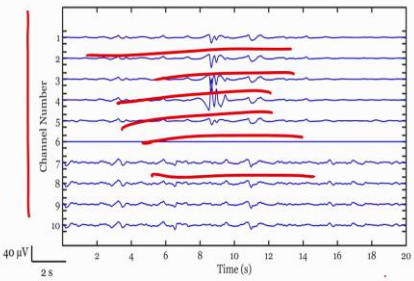

Figure: Recorded baseline ECoG signals from the sensorimotor cortex regions on the left and right hemispheres of a anesthetized rat's brain.

Now let us see the experiment. So we have Ethical Clearance and the institute biosafety clearance to perform the experiments. In this study, you can see that how the electrodes are placed in the area that we were looking at earlier, in the earlier slides. And when you measure the signals and record baseline ECoG, then you can see that from number of channels, how these ECoG signals are being recorded.

This is this shows for 20 seconds recording and it is on the left and hemispheres of an anesthetized rat's brain. So this is an actual study. We are not just talking about theory, we are not talking about just fabrication, we are not talking about simulation but actually experimenting this particular electrode by implanting it onto the rat's brain and this is a schematic of the same.

So when you really say about advanced neural science for engineers, this is what advanced neural science means that when you fabricate a device, you should not be only able to design it or simulated but you should also be able to design the electronics for that, you should design these sensors for that and use it in an implant.

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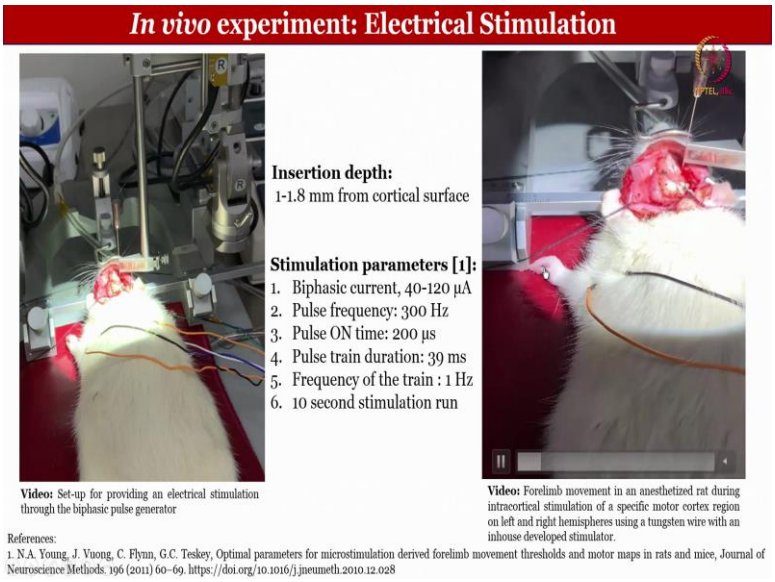

Now, the insertion depth of 1 to 1.8 millimeter from the cortical surface. This is for the when you implant a wire for electrical stimulation. So this is the setup for providing electrical stimulation through the biphasic generator. I will just play a video so you can see and then let me just show you the stimulation parameters.

It is a biphasic current, 40 to 120 microamperes. The pulse frequency is 300 hertz, pulse ON time is 200 microseconds, duration is 39 milliseconds, frequency is 1 hertz and for 10 seconds duration run.

So when you actually see what happens is that you can see that here the how the rat's paw is moving? That is because you are in the motor area. So forelimb moment in an anesthetized rat during intracortical simulation of specific motor cortex regions on left and hemispheres using a tungsten wire with an inhouse developed stimulator. Again see this movement here. That is because of the electrical signal that is provided to the rat's brain, alright? That is how it is and let us go to the next slide.

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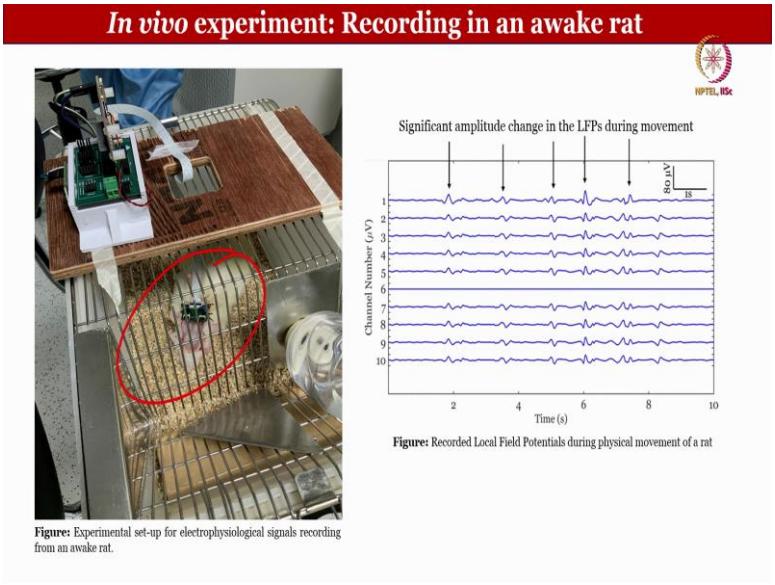

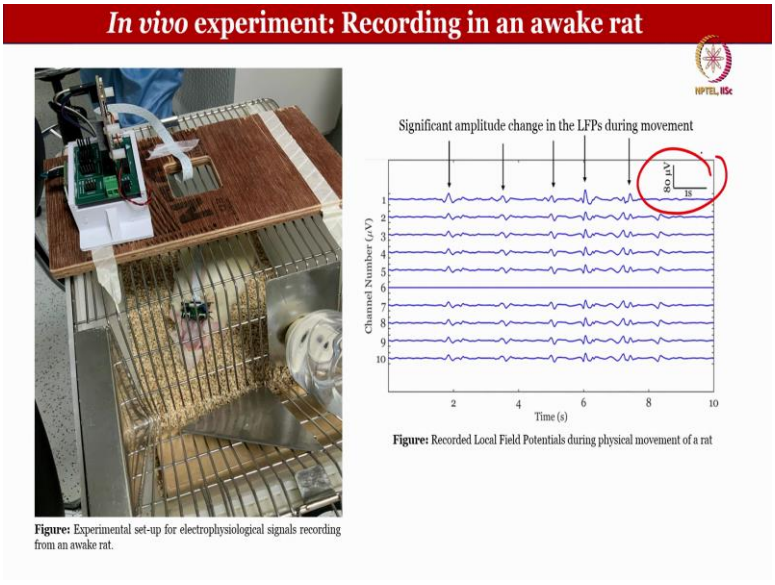

So this is how the experiments are done. This is the in vivo experiment or recording in an awake rat. You can see the rat which is awake here and electrical signals stimulation can be given here. This is a wired version as you can see that wires are coming from the red spring. The wireless version is better than the wired version because it will allow the rat to freely move on the treadmill or a catwalk system.

Right now it is all about how long the rat can be alive without causing any kind of infections, and whether we are faithfully able to record the signals from all the channels. So we were able to do that.

You can see that local field potentials during the movement is very clearly observed when we go from 0 to 10 seconds. The y-axis is for 80 microvolts and the x-axis is for 1 second. For here it is for this is the one that I am talking about. Here the recording is done for 10 seconds and the number of electrodes are 10; 5 on each side.

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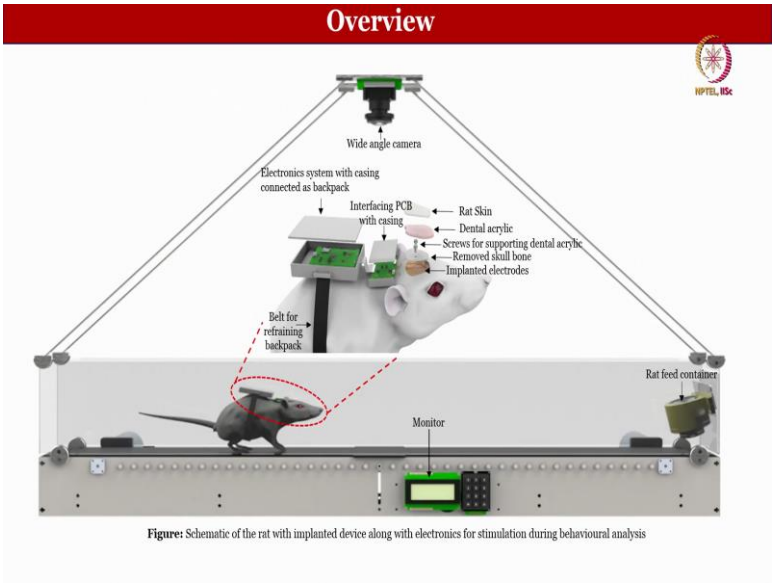

Figure: Schematic of the rat with implanted device along with electronics for stimulation during behavioural analysis

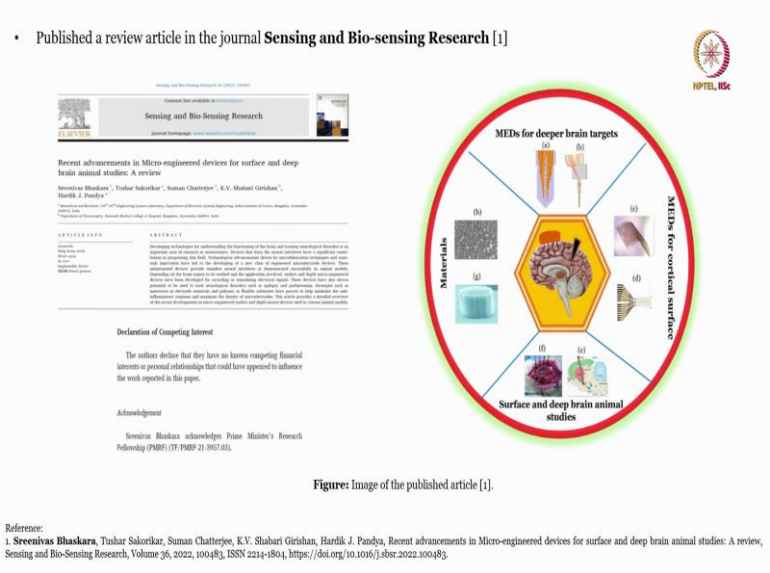

Figure: Image of the published article [1].

Reference:
1. **Sreenivas Bhaskara**, Tushar Sakorikar, Suman Chatterjee, K.V. Shabari Girishan, Hardik J. Pandya, Recent advancements in Micro-engineered devices for surface and deep brain animal studies: A review, Sensing and Bio-Sensing Research, Volume 36, 2022, 100483, ISSN 2214-1804, https://doi.org/10.1016/j.sbsr.2022.100483.

So the overview again. If you see that there is a wide-angle camera, there is a rat with a backpack that will move on the catwalk system. We have interfacing PCB with casing, electronic system with casing connected as a backpack, and the remaining things we have already discussed.

This is a complete system developed in-house in the Department of Electronic Systems Engineering which is my department and in the lab which is in the ground floor that is exclusive for developing this system, okay? And then all the experiments we need to perform in center animal facility of the institute.

So when you do that, you need to also understand and review what are the literature. Very important to first review the literatures and then only start your work. So when you do the review of literature, then you will actually understand what are the existing micro-engineered devices for surface and deep brain animal studies and based on that you can optimize your design or use the current design so that your time can be saved. So this is all about the microelectrode array stimulation in the rat's brain for Parkinson's disease.

Now, you do understand that in spite of our best effort the syllables that you see and the syllables that you that I was able to cover it is a little bit different. The reason is that we have covered 70 or 80 percent of the syllabus what was shown to you as Week-1 to 12, whether you talk about the micro-engineered arrays, fabrication of thin film deposition techniques, techniques for acquiring neurological signals, characterization, MEA for needle potentials, flexible MEA fabrication, deep brain stimulation, what are the needs system using 3D printing, non-invasive BCI which is what according the data from the neonates, recent trends

in brain-computer interfaces, epilepsy basics, basics of EEG, approaches for seizure detection and whatnot.

That some of, introduction also we have discussed about how the brain veins system how is the anatomy brain, whether it is an axon, how the electrodes are placed, this comes in the introduction basics. Some things we were not able to cover was computational neurobiology, modeling cells functions and local networks, complex networks in specific cases, and large-scale network and optimization. These things were, we were planning and in fact, Dr. Vikas was going to talk more on those subjects if the time permitted but because of his ill health, he is not able to do that.

So what I will do is even we have crossed more than 30 hours as a part of this particular course, I will try to cover some of the topics that we have proposed in week 1, 2, 3, 4 as 1, 1 and half hour of the class so that we can at least know what are the different computational techniques that can be used for this particular application, whether it is your modeling of cell functions, or complex networks, or large scale networks, and optimization.

So and I am really sorry, the reason is not to keep on talking on one technology but the reason is to try to explain using this online mode in depth so that tomorrow when you take this course it should be fruitful. You should be able to understand how the fabrication is done.

When you understand how fabrication is done, then you can understand because you see the computational neurobiology techniques you can always read. Of course, it is very difficult. It is not so easy topic to pick up and read.

But before understanding neurobiology and all these things, if you also understand the fabrication and implantation and the equation and the stimulation, that becomes very interesting actually from both Vikas and my point of view and that is how a little bit of not dot by dot comparing what we propose and what we are teaching.

But like I said, almost 75 to 80 percent is taught with the best of our efforts. And then you do understand that we wanted you to look at the experiments. Experimental laboratories is very difficult for most of the students that I have been teaching in the last several years through this particular platform.

Most of the students does not have or do not have the access to the laboratory facilities. So that is why I spent a lot of time on recording those experimental labs so that you have some flavour of understanding how the things are done.

It is very difficult if you really see that to record the experiments and to try to replicate those experiments through the online mode is not so easy. Right? So with our best efforts, we want to cover as many topics as possible within the 30 hours of timeframe.

However, since some of you may have a question about what should be there in that computational neurobiology I will take about an hour and a half, depending on the time just to go through these topics so that we can cover the course.

Again, both sincere apologies from Dr. Vikas and I am just conveying to you because of the issues. He was not able to cover some of the topics and not able to participate but he is there.

He is advising us on all the topics. He is helping us with the assignment. He is helping us with the neurosurgery part and also helping us with the implantation part- how the design should be there, how the electrodes are there, and that is how I can teach you that part of the course.

Because without a neurosurgeon telling where exactly to implant or what to implant where the needle should go, engineers cannot teach. So that is a reason. So but if you have any questions about understanding in detail, please feel free to contact me.

Also, see the assignments sometimes that we give it to you, all does not have to be just exactly topic-wise. I also want to do a little bit of homework and see what are the different applications. If you understand lithography, what kinds of different photoresists are there?

Suppose I teach you UV lithography and I asked you on e-beam lithography, that does not mean that exactly topics should match. I want you to explore and learn something beyond what we are teaching in this particular course but as far as the exam is concerned exam would cover the thought process by yourself, the efforts that you do, and the course and syllabus and topics that we have covered in this particular class.

So but still if you are confused, feel free to contact me and I will have and I will spend time to clear your doubts, if it is not clear through the 30 hours of our coursework. In fact 31 hours because 1 hour, I will be taking in the next as part of the next remaining topics of this course.

So till then, I hope that you are gaining something, you are learning something, it is useful, it is advanced enough for you to talk as advanced neural science for engineers. If you have any questions feel free. Guys, this is not so easy topic to digest and to do it.

The fabrication part that I am showing it to you today I am working in this area from last almost 15 years or close to 15 years as a part of my master's program or Ph.D. program or post-doctorate program or last 6 years of my life here as a faculty in the institute.

So after that, it is it becomes slowly and gradually easier for you to understand how to fabricate it, how the device should look like, what should be the thickness, what kind of stimulation you can do. But try to understand it. Try to understand it, grasp it.

And there will be doubts, and there will be questions that we are supposed to answer as much as we can and that is why your NPTEL platform is there. In the forum please keep on asking and be curious. I wish you all the best for the remaining class, remaining time. Any questions? Shoot me an email. And I will see you in the next class. Take care. Bye!