Bioelectricity
Prof. Mainak Das
Department of Biological Sciences and Bioengineering
Indian Institute of Technology, Kanpur

Lecture - 9

Welcome back to the NPTEL lecture series on Bioelectricity. So, we have finished the eight lectures, now we are into the ninth lecture. In the last lecture, we talked about the microelectrode array and I requested you people please go online and check the real image of microelectrode array. So, one of the things what people are currently trying to do, so we will briefly talk about the applications of microelectrode array what people are trying to do. So and then we will move on to the intracellular recording. So, one of the things, what needs a bit of visualization is that think of this whole brain its thousands and thousands and thousands of neuron making multiple circuits out there something like that.

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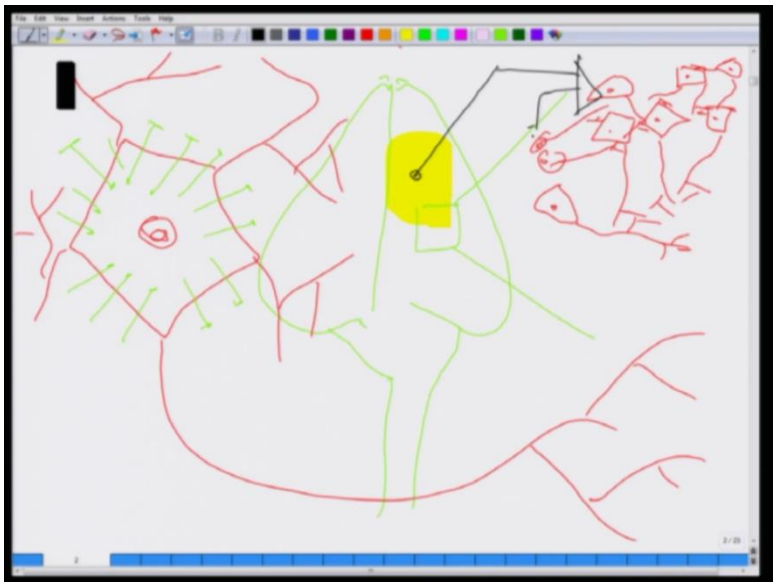

If you have to visualize the brain, it will be something like this. So for example, this is your brain, and this is the brain stem and here you have the brain. Now at any point, if you pick up any particular point and if you magnify what essentially you will see the series of neurons like this sitting out there, functionally dynamic state. It is a complex network something like this. And in between you have the glial cell and likewise series of supporting cells something like this. So, at one point of time, in this network, it has

been estimated that one neuron receives could receive the ability to receive signals from ten thousand other neurons. In other word, what that translates down into at one point of time a neuron on it is surface has ten thousand synopsis something like this.

Say for example, if this is single neuron and this is the axon, and here you have the dendritic tree something like this. This is the nucleus. So, this neuron at one point can receive signal from say ten thousand different sources like this. I could only draw certain feasible space I have; I mean I have limited space; I cannot draw all of them to just to show you. So, one of the critical challenge of the modern neuroscience as a whole is how really to understand, how a network functions, because in a complex brain, it is exceptionally challenging. Most of the time, when you are inserting certain electrode what you record is a field potential. What I meant by field potential is that say for example, I have a electrode cell, I am showing like like this, I have this electrode out there. This electrode will only measure something like this, and this region all the activities which are taking place out here in a broad region that is all it does.

So, a electrode recording like that cannot really pin point what is happening at a small will show like this or it cannot really pin point the cellular events which are taking place. What you are getting is something a aggregation of say ten thousand neurons at one spot or like you know five thousand neuron, of course, it depends on how smaller is the size of your electrode, how finer the electrode is you get the field study. The summation of the electrical activities of population of neuron that is essentially is helpful for several to understand the rhythms and several, I mean circadian activities all though there are several things leap and all those things, but that is not really the way you can figure out what exactly is happening at individual cells.

Say for example, I have a drug which is targeted to specific kind of neuron or say for example, I have a drug which is getting into the brain, I have no idea what it is doing to the individual cell, for that you need different approach. So, most of these approaches depends on individual culture model. What does that mean, there are two ways how you can approach use the electrical power or the bioelectrical techniques for understanding biological phenomenon. One is that you insert an electrode or you poke an electrode in a live animal this is one way. Where the live animal, the animal is moving around and you are recording, in a real time you are recording the events, which are taking place that is one way which is nevertheless one of the most powerful profound way to do it. But, that

would not give you any ideas I was mentioning you about individual cell what is happening.

In order to understand individual cell, you have to go down to at the cellular level and that you cannot do in the live system then you have to either take out the part of the tissue outside the system you can make a slice you can do the slice. On the slice, you can keep the slice alive for six to ten hours; and on that slice, you can do recordings. There is another way where you place the slice on the microelectrode array what I have shown last time. So, in that situation what you are essentially doing is, you are keeping the site to architecture intact.

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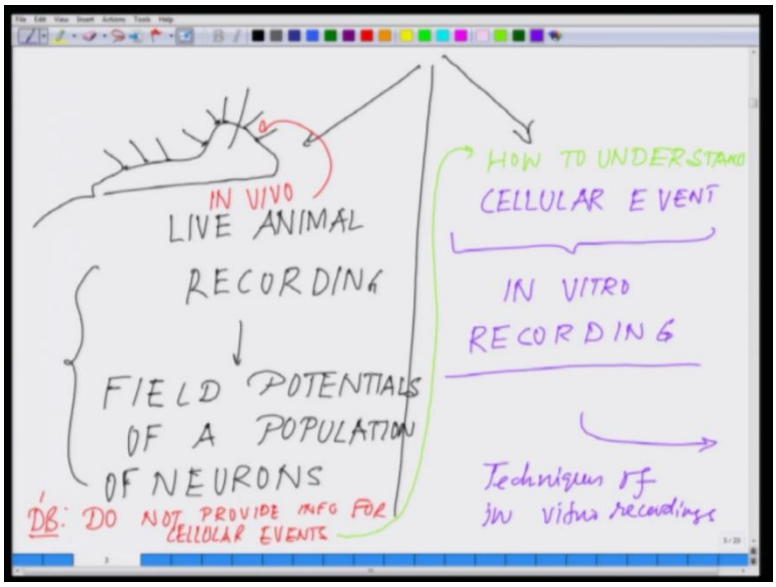

So, for graphical representation what I am trying to tell you, so these are the different technique. So, this is the live animal. Say for example, if this is, these are recording techniques - live animal recording. So, you have electrode either implanted like this or you have surface electrode like this, and the animal is alive. This is the spinal cord likewise you know and essentially what you are recording are the field potentials. The other set of recording out here, which is you cannot do it in the live animals, so this will give you field potential of a population of neuron. So, if I had to say the drawback drawback as DB. Do not provide information for cellular event then this takes us to the next level, how to understand cellular event. For cellular event, you need to have what we call in vitro recording and what we essentially call this technique as in vivo or in

animal recording. So, to in vitro recording, so in the next slide, we are moving different techniques of in vitro recording.

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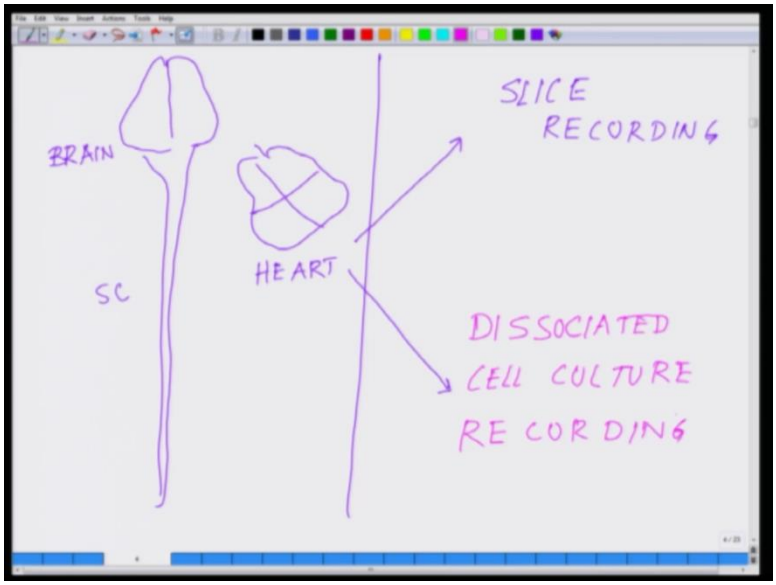

So, say for example, if I look at all the excitable tissues, so one of them is your brain and you spinal cord then you have the heart, here is the brain and SC. So, essentially you have two major techniques, one is slice recording, the other one is disassociated cell culture recording. What you meant by slice recording? So, in the next slide, we will talk about what meant by slice recording.

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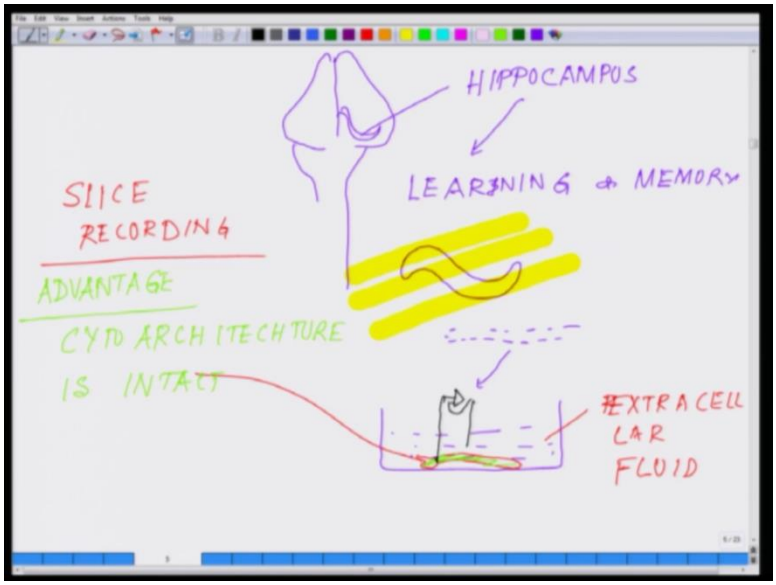

So, say for example, this part - this is the brain, you anesthetize the animal, you remove the brain, and you kill the animal. And we know that this is one of the area which is called hippocampus which is involved in learning and memory campus. We will talk more about it when we will talk about learning and memory involved in learning and memory. So, what you do essentially you take out that organ like this and then you make slices something like, you make slices like this. So, you essentially get you can make slices in different in different ways. So, you essentially get a very thin tissue like this and then you put them in a chamber immolating the condition of the brain either tissue is sitting like this. So, here is the extra cellular fluid with different energy source to make this tissue survive and when you approach already the cyto architecture is all maintained. Just to mention slice recording and advantage, cyto architecture is intact. Then on that what you are getting you are putting the electrode, which I showing black like this and you start doing the recording.

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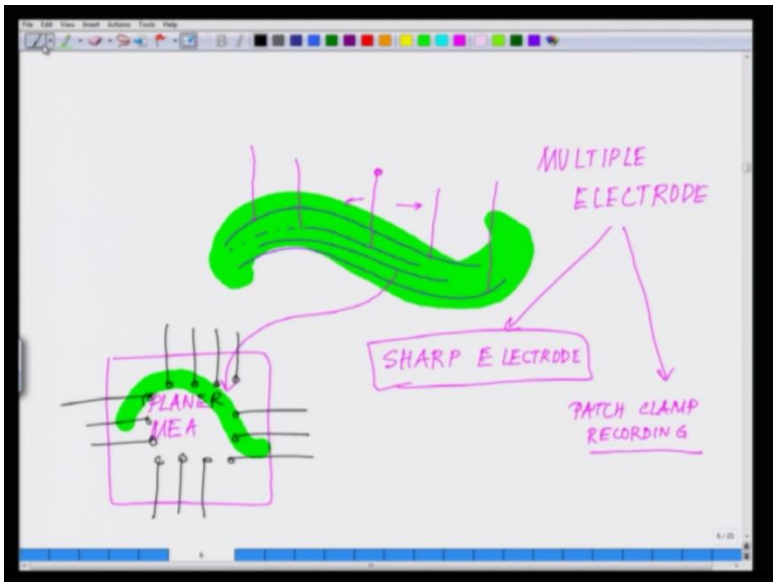

So, this is essentially is talking about the slice recording, and slice recording is a very popular because you architecture remains intact, you can really poke there, so the circuits remain intact, you can really poke the electrode at a specific zone of the circuit and stimulate one circuit. And say for example, in this diagram if I just highlight this further. So, it will be something like this, if this is the part of the tissue, so for example, if this is the hippocampus is almost like this and it is known that hippocampus has something like this. So, hippocampus has different circuits on its system something like this c a 1, c a 2,

c a 3 - these are the circuits within hippocampus. We are coming in-depth on this one after word. So, now, what you can do now to study this different circuits and connectivity you can poke electrode here, you can stimulate here ,you can do a recording from here or you can poke a electrode here, you can to a simultaneous recording from here or you can do it form here you can floe here like wise. Or you can stimulate here and you see how it is distributing on both sides likewise, and you can put multiple electrode, so this is another advantage.

And you can do two kind of recording here, you can do sharp electrode recording which I have already talked and you can also do something called which I have not discussed yet patch clamp recording. We have not discussed about it, but this definitely discussed with people, and some people even tried to take this whole circuit and take this whole slice and put it on top of a microelectrode array on a planer mea. This whole circuit is placed on a planer mea. If you guys have seen it, it is something looks like this. Please again I request you kindly go online and check the structure that will help you, and the circuits sits like this something like this.

So, you see these are different techniques which are being used to understand the functioning of the brain by extra cellular. So, this is what I showed you in planer micro electrode array is a extra cellular pattern of recording then you could have intra cellular electrode and you could have patch clamp. Patch clamp, I have not discussed with you people what I am going to discuss after this. So, this is one way, but there is another way which is the third way and which is so you have to realize the drawback of slice recording is this. Slice recording cannot last more than six to ten hours. It is really trucked off, because it is three-dimensional tissue out there, which is already taken out from the system, it is not really adapted.

So, for maybe you can make it last of eighteen hours maybe, may be a day if you are exceptionally good, but the problem is that whenever we talk about the drug trials, chronic situations, chronic experiments the story changes. Why the story changes, because it will realize that say for example, I put a drug, and drug will be acting over a period of months and may be sometime years. And most of the animal trials are really costly, whenever an on long term effects at the cellular level at times get missed.

Say for example, if you go back to the slide where I showing you the real animal situation. Say for example, I injected a drug into this animal. Now this is circulating all over the body, all over the place. So, it is really tough to know exactly what is happening at the individual cellular level; and over a period of time, how it works. Chronic experiments are really tricky and really tough to do. And on top of that with animal ethics and the cost of animal, every drug discovery really take a huge amount of funding, huge and that is how when the drug comes to market it becomes so costly, it is not costly because this drug is out of the world. It is costly because it has to go through all the different channels of screening and those screening takes enormous money, enormous amount of funding is required, so that capital investment essentially jack up the price of a drug when it comes to the market.

So, now coming back, what is the other technique, and specially these kind of drugs are exceptionally costly when you talk about the nervous system or the cardiac system which are kind of you know pretty much your life line a cardiac drug. It is not easy, I mean it is really tricky, you have to go through all possible channels of hoops before there are drug kind of gets in the system. So, coming back where we aware about, what is the third technique, so we talked about the slice recording. Now we talk about the third set of recording which is fairly old, yet fairly new also, there are two aspects of it.

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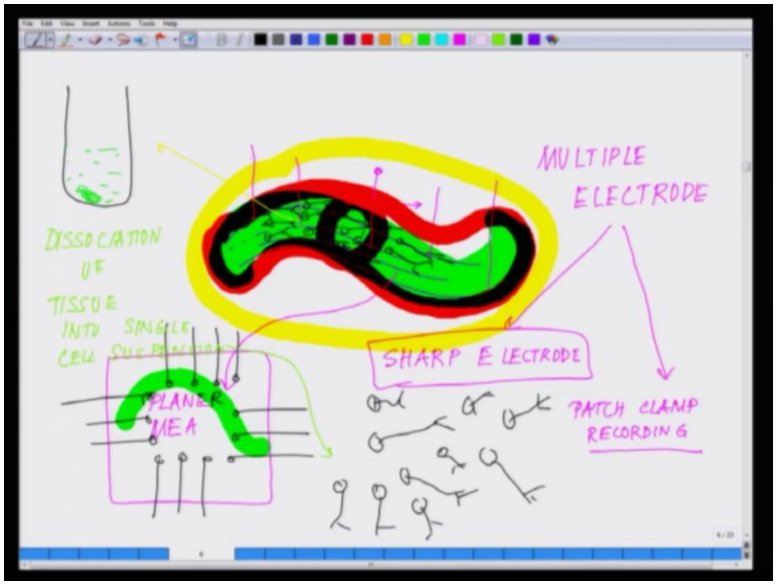

What you do essentially here, so from this diagram itself, let us start to draw. What you do here, say for example, I wanted to have understand about the hippocampus. What I will do is that I will pull out the hippocampus I will break the hippocampus in the sense I will disassociate the cells of the hippocampus by doing by using different enzymes or different mechanical ways. So, if you look at this circuit now, so at the cellular level if you try to look at this circuit, the circuit is essentially nothing but series of neuron sitting like this, like this, thousands and thousands of neurons sitting like this. And they are making circuit at different level and this is just the of top layer I am showing in multiple layers and likewise. They are arranged in a specific array, specific circuit and everything.

Now what we do is that you take this hippocampus take this out, take this whole thing out or you can take any part of it if you are very good at dissection or something. Then take this out and say for example, you have collected that part of the tissue out here like wise in an extra cellular fluid, then you break the tissue, and this breaking of the tissue is called disassociation, disassociation of tissue into single cell suspension. What we mean by single cell suspension, it means now you have all these individual components what I was drawing are separated out something like this. In that process of course, the tissue undergo a lot of damage or something, but those, which survive are important for you.

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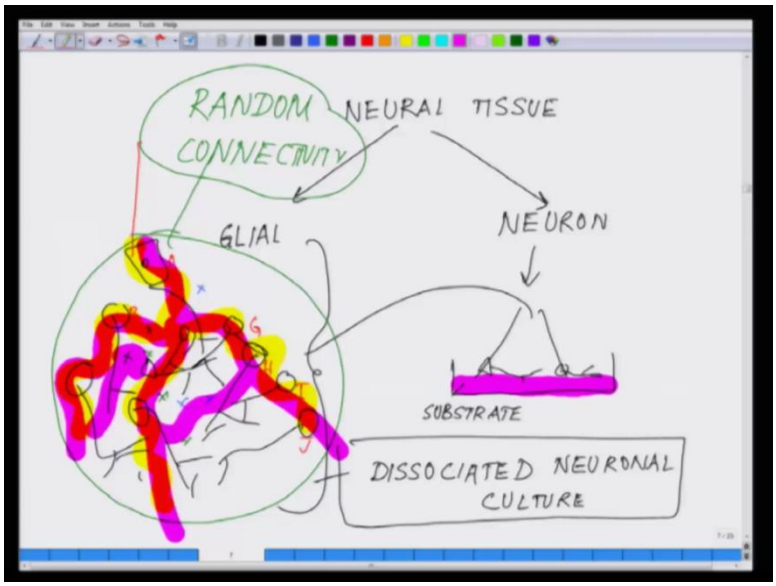

Now you take these neurons and the accessory cells depending on you have the different mode of that you can purify at this stage you can, so if you have the neural tissue in case

of say neural tissue, you can purify the neural tissue, you can have the glial cells separated which are the supporting cells. You have the neurons separated out. And then you put them in a dish to grow of course, they would not grow just in the thin layer, you need do you a bit of a homework, you have to coat this dish with something on with they prefer to grow some kind of substrate on which these neurons will grow.

So, this is the substrate; and on top of the substrate, you have the neurons growing like this. So, if you get a top view of this something like a top view little look like this, neurons are all over the place like wise. It will be a random connection between different neurons, this is a dissociated neuronal culture. So, these dissociated neuronal culture, now you can approach the individual cell with individual electrodes. You can have a sharp electrode like this. You can approach the individual cell, and you can monitor several events you could put x, y, z compounds out here say for example, compound a, compound b likewise and there is another compound out here or a third compound out here likewise, and we can figure out their what they do.

So, this technique gives you an access to the individual cells in disassociated culture this is what it does, but it comes with a drawback. Drawback is that I told you in the previous slice preparation the cyto architecture is maintained. In other word, if you go back, so the circuit is all maintained out circuit is not getting destroyed, but out here what you did, once you disassociate everything, then there is a random connectivity; you do not have really a control on their connectivity. So, there is a random connectivity.

So, this network is forming in a very very random manner, you really cannot dictate that how many synopsis are forming, you really cannot have any control, but eventually it becomes really cumbersome to detect. Say for example, think of a practical situation if this is I level at this as A, and I level this as B, this as C, this is D, this is E, this is F, this is G, this is H, this is I, and J likewise. Now say for example, a signal is getting originated from here, and I am seeing the signal is all over the place. Now I really do not know how the signal have moved, I really cannot trace it, because signal may move like this, signal may move like this, signal may move like this, signal may take back turn and likewise signal may have connectivity like this. So, there is no way I can figure out how the network exactly functions.

So, network behavior is really tricky, it is almost the same situation as when you do a field potential measurement. You really have no control about the number of cells which will be involved in generating that signal. So, you really do not know, and more over you really cannot in a random circuit, it is really difficult to keep a tab at the changes at individual synopsis, because at individual synopsis, it is again getting connectivity from multiple sources, because it is random. There is no way that you can control that connectivity, because anything gets from connection with anybody, so that makes the story very complex and that is something you do not wish to happen.

But if any technology, if you need to a directed or you know completely patterned growth, you need some different kind of technology then starts within this disassociate culture the current technology which most of the people in the area of bioelectricity or bioelectrical accordance are following. So, they are trying to develop build circuits out of this disassociated cell how they are doing so…

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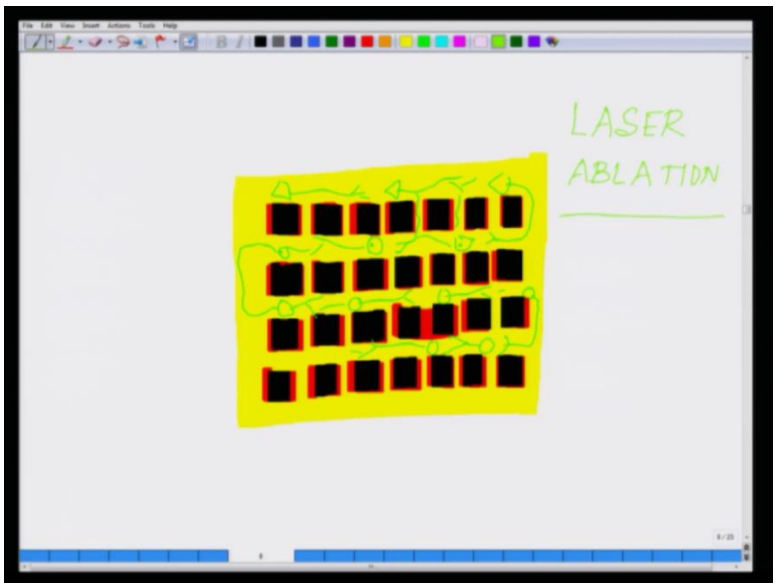

So coming back to the basics again, so I drew that I told you that there is substrate. Say for example, let us try to understand it say for example, you have a culture substrate like this. Imagine this is the culture substrate; all the cells will grow on it everywhere. On these culture substrate, say for example, if you have a way that you could introduce some pattern, you do something like this, you ablate this part of the circuit. What I meant by ablation means I am removing that particular yellow color compound from here. So, if

you remove the yellow nothing will grow there provided. You back fill it with something which one promote. So, essentially what you are getting now look at it. So, essentially now cells so say for example, I back fill it what I meant by that on these zones you fill it with another something.

So, on black, I really cannot draw anything, because everything will, let us see if we could do it with red, this is the back filling agent. So, that is ensuring that those ablated surface. So, what we those who are not understanding ablation, let me just explain. What you essentially do is say for example, you have the substrate like this, you take a mask, and if imagine this is the substrate and I have the mask and I keep the mask here. If I keep the mask here and I put a laser beam or something. So, at this part, where the mask is covering it nothing will get ablated; rest of the places will all get ablated; does that make sense? So, that is exactly what I am trying to tell you.

Say for example, I put a mask, the zone which you are exposed to laser beam are the one which are blank. So, it burns out those spots. What is left with all the yellow that things so grow, and now where you have the black spots, there what you do is that you back fill it. You did it in such a solutions, which will only sit on top of those ablated region that compound would not sit on the top of the yellow, so that is what I trying to do. So, this is that next compound by which you ensure this black compound is the one which will not allow any cellular growth at particular black surface likewise.

Now you have a pattern situation; this pattern situation will allow the neurons to grow like this. The neurons could grow if their thickness is like this, something like this. If this thickness is good enough for a neuron to grow then there may be connectivity coming out like this, possibilities are there, but there are ways to you know control that. Now what you are seeing essentially is, you are trying to control the position of the neuron, and you really can do it in a very interesting way. There are several geometries, which you can follow. So, this is one geometry which I showed you. So, this is done by a technique which is called laser ablation. You ablated using laser. There is another way you can do it. You can make these circuits using your old style inject printers what you do is that you print the circuit.

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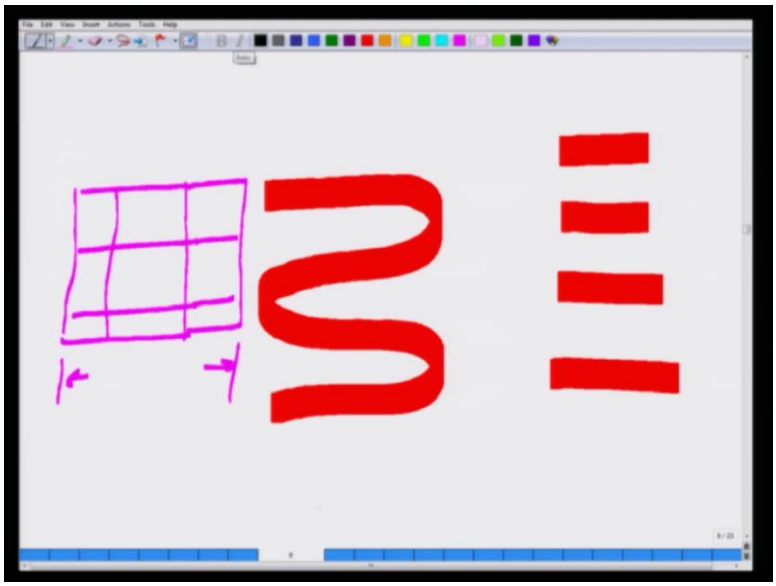

Say for example, you on your word document, you draw the circuit, narrow it down and on the cartridge, where you fill the old inject printer, you fill the ink. You throw away the ink sterilize it, and on top of it you put this either the substrate you want to do. So, what will you do the jet printer will make, say for example, a circuit like this say for example, I want a circuit like this, I want the cells to grow like this. So, make a circuit like this or it can even make a circuit like only lines or it can make a circuit like or it make a circuit like this. So, there is several ways you can make circuits, and these are some of the different ways what I am trying to highlight, and you can control the dimension.

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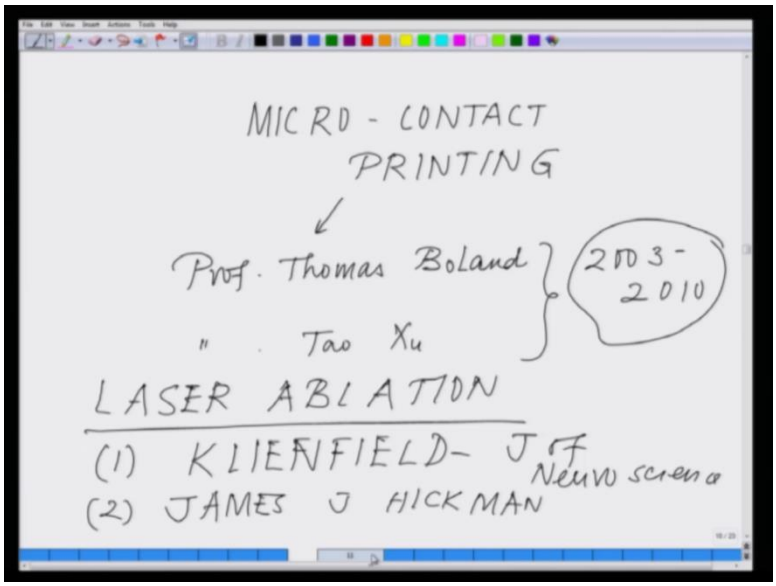

So, for this, I will recommend you, kindly go through some of the extra materials which I expect you to see the papers for micro contact printing one of the pioneering person you should refer to the work of Prof. Thomas Boland; currently he is in university of Texas Alpaso; Prof. Tao Xu, these people have done very significant amount of work on micro contact printing. It is worth reading some of their work; how they have done it using very very simple most of this work was published during 2003 to 2010. Now, also some of the work they are publishing and they are absolutely phenomenal I mean the way they have done all these things just with very crude techniques around them. They could really do very nice micro contact printing and some very well documented papers are there from their side. So, this is one group paper I like you people to look at it.

Laser ablation and all these work I expect you please go through the work of there are few people whose work will be really looking one will be, this is one of the very old paper Kline Field in journal of neuroscience. It is a very seminal paper Kline Field; and you should go through the work of James J Hickman, he has done extensively extensive work in that area.

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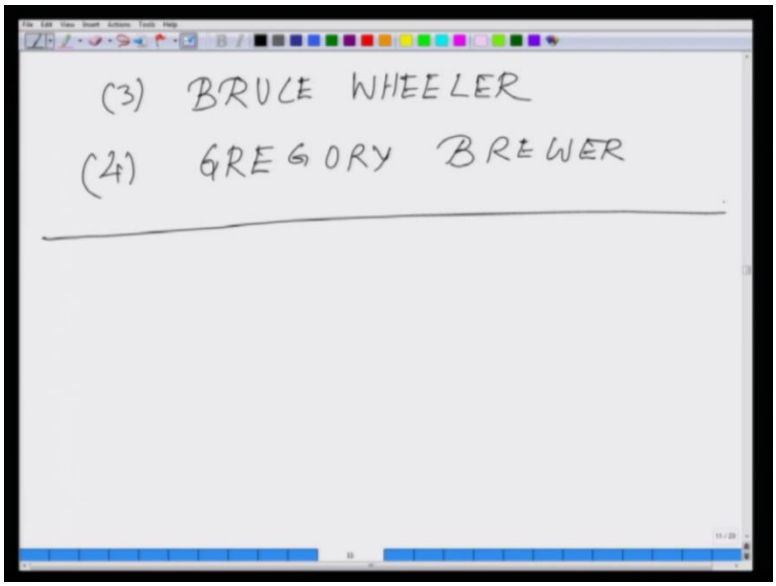

You should look through the work of Bruce Wheeler who also have done very significant amount of work in this area and you have Gregory Brewer. These are the people who have done significant amount of research in this area; and it is defiantly I will recommend you people please go through some of their work, they have worked in wide areas, but definitely, they have made some seminal contribution in these kinds of printing circuits. So, current status is like with this.

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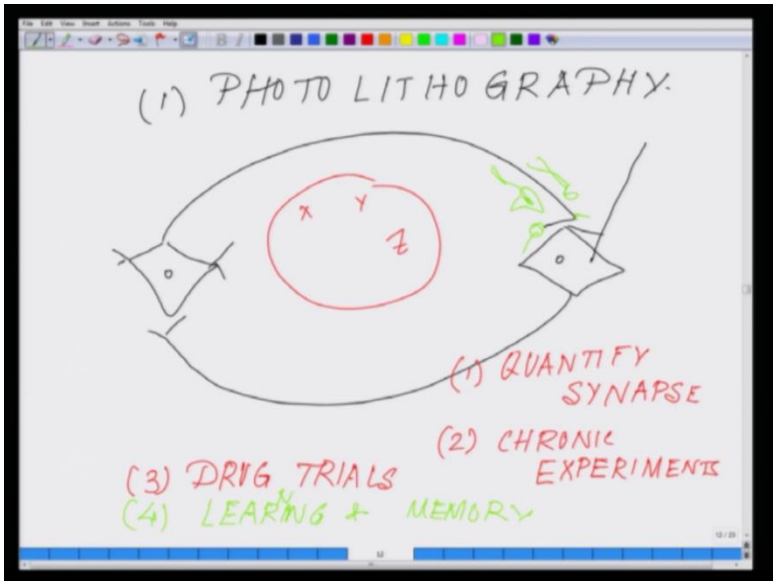

I mean you can go to other end of the world. So, there are techniques which are being used in laser ablation and then there is something called photolithography and this will get a lot of references in Prof. Hickman's work photolithography and professor Gregory work and Prof. Kline Fields work and of course, Prof. Bruce Wheeler work. So, we talked about the micro contact printing where you should look through the work of Prof. Thomas Boland, and there are few other people who have done very significant work. I will come back to that in a next lecture. So, these people have shown that they actually can guide the neuron in a specific trajectory a single neuron.

So, you will see some of these circuits like you know you can now a days, you can develop like two neuron circuit like this. These are you will see these kind of circuits are being developed single two neuron. So, now in this two neuron, you can really approach with a single electrode, you can have x y or z compounds all over the place, you can really quantify the synapse. So, what all you can do, you can quantify synapse – one, you can do chronic experiments for a long period of time and this chronic experiments could be these circuits could survive for more than a month or so if you are really good at it.

So, they may chronic experiments you can quantify the synapse, you can do cheap drug trials they deduced on the cost of drug trials. On top of that you can introduce the supporting cells like you know the glial cells, you can study the glial cells dynamics and on top of that here is the control model where you can study learning and memory. So, these kind of control circuits you can make series of them. I mean as you at the authors whose papers I have mentioned you or the those who have made some kind of contribution if you will read these papers, you will realize you can make series of such circuit to approach a single cell in a very elegant way. And you can really understand the network behavior in a very simplistic reductionist approach; of course, it comes with its drawback, because you rebuilding the circuit. So, you know there will be some error here and there, but the way biology works is that you start from whole animal, you come at the single cell level and then it all has to merge.

So, there is no one technique which is perfect and there can never be on technique which is perfect. So, the whole idea is you know having multiple techniques trying to tell you or trying to unrevealed truth of nature this is what we are always trying all throughout like. We are trying different techniques, so this is one approach.

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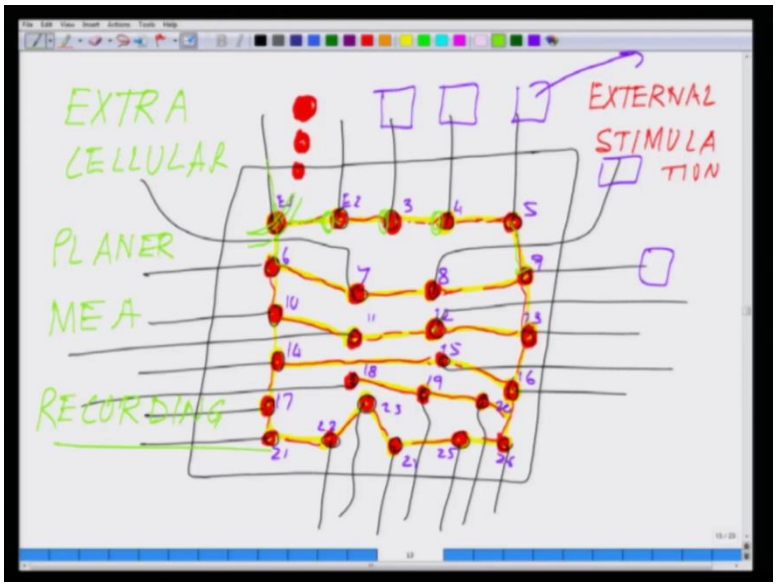

So, another approach in the same line which is you know hybrid approach which is been followed, I am going to draw is I introduce you to the microelectrode arrays. Let me draw a microelectrode array and tell you what is that approach, it is very interesting approach. Say for example, you have these microelectrode arrays sitting out here like this, now you may have electrodes like this. So, if you can pattern this, say for example, I have a pattern like this. The cells will follow this trajectory something like this. Now I connect this like this. So, now rest of the places where you see yellow are the only places where the cells will grow; rest of the place cells will not grow. So, I modify the surface of this planer microelectrode array in such a way that cells will grow all along those electrodes they are connecting the electrodes.

The dimension of the electrode is say twenty to thirty microns, and those lines say for example, they were aspect ratio of 10 to 20 microns or may be 10 microns. And specifically, except the places where the electrodes are there aspect is slightly more may be this is 20 micron and the lines are say 5 micron thick. So, on a five micron surface, it is really tough for a cell to sit, but the cells will sit preferentially will sit on top of the electrode, because this electrode regions have more surface area, it is around 30 microns or 20 to 25 five microns. So, when you put the disassociate cells in this chamber what will happen?

So, for example, so if I represent the cells with red, so now, I am putting the cells in to it. So, cells will preferentially will try to sit here, because these are the zones where they will try to migrate to on top of the electrodes because that is where they will get the maximum surface area to grow. These are all disassociate cells. So, once they will sit like this, what they will try to do, they will try to send out processes like this. They will try to send out processes like this to connect with each other. They can do it in like this, like this, several ways you can do. You can even stimulate this circuit, in order for this whole process to take place, and they will form a very controlled network. A network which you can monitor in a real life something like this. So, they will start forming network inside you keep this whole system inside in incubator and you monitor it, as they are forming the network

As they are forming the network, so what you can do, you can give an external stimulation for network formation and stimulation. And you can register the electrodes say for example, I registered them as E 1, E 2, 3, 4, 5,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26. So, I can register the electrodes and you really can monitor that activity at the individual channels. At the individual channels of the amplifier, you can monitor the activity what is happening in which electrode. Now once a network is formed, say for example, I give a signal out here, I give an stimulation out here now how this stimulation is moving along this circuit, I can monitor in a real time. How the synopsis are forming out here, how the synopsis forming here, how it is forming here, how which circuit is getting more strengthen, how it is getting more strengthen I can study all this things now. What and then based on this now I can back calculate what is probably happening in the brain.

So, if you look at it there are profound scopes of, which is open up with the advancement of a modern microelectronics, we are able to access a single neuron on top of a electrode. And this are all could be done using extra cellular recording, these are all extra cellular planer MEA or microelectrode array recording. So, this is the advantage with which microelectrode array offers in order to study the circuit from a very reductionist approach. It is not a holistic approach, it is a very very reductionist approach; you are building the system from the base again from grass root, brick by brick you are building the system.

So, I will close in here for this class; and in the next class, we will talk about the other end of the intra cellular recording, where we will be approaching a single ion channel because once I will introduce the ion channel then I will talk to you about the structures and the details of the ion channels.

Thanks a lot.