Bioelectricity

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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 the microelectrode array. So, one of the things that 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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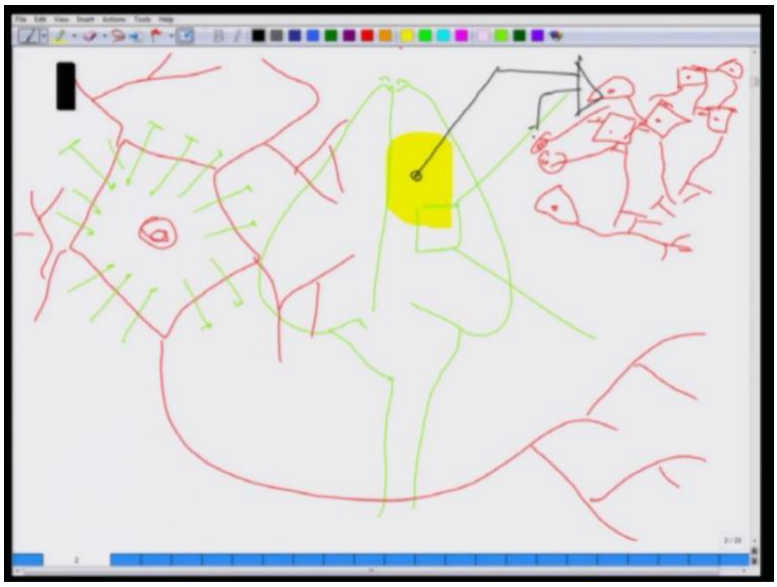

If you have to visualize the brain, it will be something like this. So for example, this is your brain, and this is the brain stem and here you have the brain. Now at any point, if you pick up any particular point and if you magnify what essentially you will see the series of neurons like this sitting 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 in 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 words, 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 a 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 a 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 challenges of 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 insert an electrode what you record is a field potential. What I meant by field potential is that say, for example, I have an electrode cell, I am showing like like this, I have this electrode out here. 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, an electrode recording like that cannot really pinpoint what is happening at a small will show like this or it cannot really pinpoint the cellular events which are taking place. What you are getting is something an aggregation of say ten thousand neurons at one spot or like you know five thousand neurons, of course, it depends on how smaller is the size of your electrode, how finer the electrode is you get a field study. The summation of the electrical activities of a population of a neuron that is essentially is helpful for several to understand the rhythms and several, circadian activities all though there are several things leap and all those things, 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 a different approach. So, most of these approaches depend on the 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 the 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 real-time you are recording the events, which are taking place that is one way which is nevertheless one of the most powerful profound ways to do it. But, that

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

In order to understand an individual cell, you have to go down to at the cellular level and that you cannot do in a 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 a 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 a microelectrode array that 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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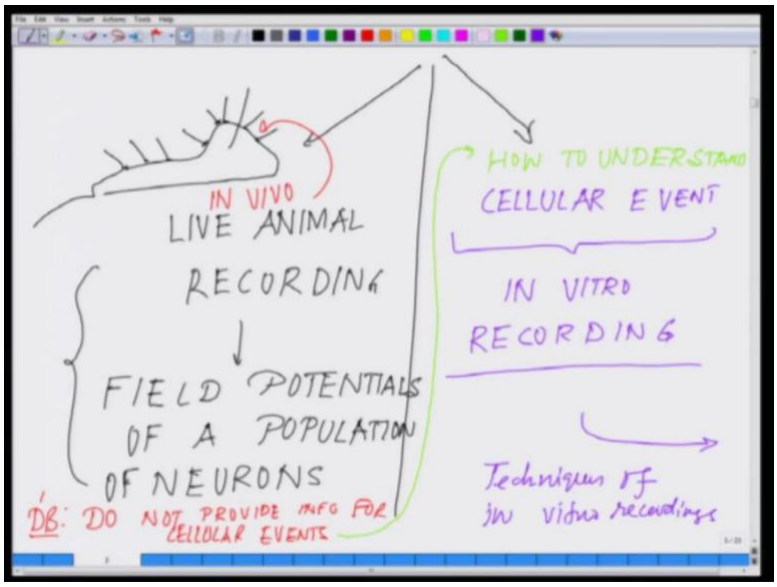

So, for graphical representation what I am trying to tell you, so these are the different techniques. So, this is the live animal. Say for example, if this is, these are recording techniques - live animal recording. So, you have electrode either implanted like this or you have a surface electrode like 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 in you cannot do it in the live animals, so this will give you field potential of a population of neurons. So, the drawback as DB. Do not provide information for a cellular event then this takes us to the next level, how to understand the cellular event. For the 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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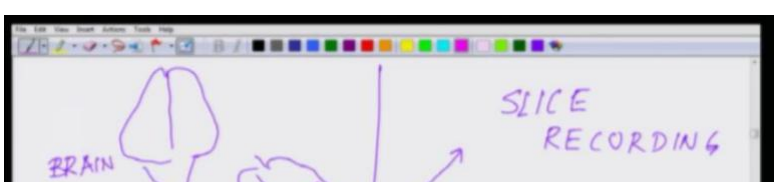

So, say for example, if I look at all the excitable tissues, so one of them is your brain and the spinal cord then you have the heart, here is the brain and SC. So, essentially you have two major techniques, one is slice recording, the other one is disassociated cell culture recording. What did you mean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

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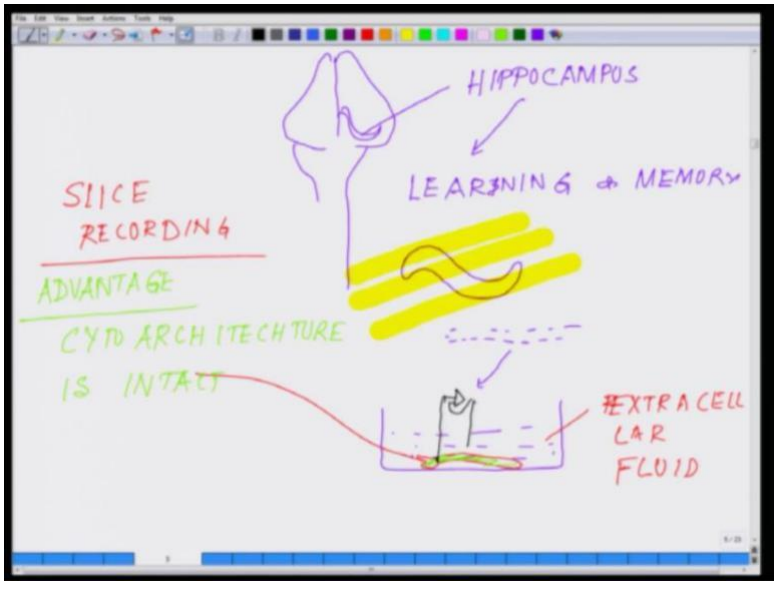

So, say for example, this part - this is the brain, you anesthetize the animal, you remove the brain, and you kill the animal. And we know that this is one of the areas which is called the hippocampus which is involved in learning and memory campus. We will talk more about it when we will talk about 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 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 extracellular fluid with a different energy source to make this tissue survive and when you approach already the cytoarchitecture is all maintained. Just to mention slice recording and advantage, cytoarchitecture is intact. Then on that what you are getting you are putting the electrode, portion black like this and you start doing the recording.

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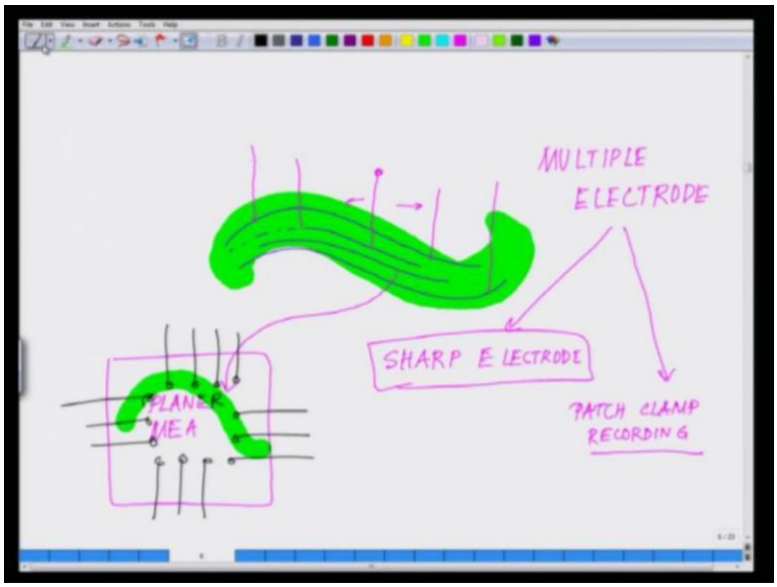

So, this is essentially is talking about the slice recording, and slice recording is very popular because your architecture remains intact, you can really poke there, so the circuits remain intact, you can really poke the electrode at a specific zone of the circuit and stimulate one circuit. And say 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 the hippocampus has something like this. So, the hippocampus has different circuits on its system something like this c a 1, c a 2,

c a 3 - these are the circuits within the hippocampus. We are coming in-depth on this one after the ward. 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 an electrode here, you can do a simultaneous recording from here or you can do it from here you can floe here likewise. Or you can stimulate here and you see how it is distributing on both sides likewise, and you can put multiple electrodes, so this is another advantage.

And you can do two kinds of the 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 it, but I definitely discussed it 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 meant. This whole circuit is placed on a planer meant. If you guys have seen it, it is something that looks like this. Please again I request you kindly go online and check the structure that will help you, and the circuits sit like this something like this.

So, you see these are different techniques that are being used to understand the functioning of the brain by extracellular. So, this is what I showed you in planer microelectrode array is an extracellular pattern of recording then you could have intracellular 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 a slice cannot last more than six to ten hours. It is really tough, 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, maybe 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 the drug will be acting over a period of months and maybe sometimes 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 animals, every drug discovery really takes 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, an 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 especially these kinds 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 lifeline 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 is drug kind of gets in the system. So, coming back where we aware of, what is the third technique, so we talked about the slice recording. Now we talk about the third set of recordings which is fairly old, yet fairly new also, there are two aspects of it.

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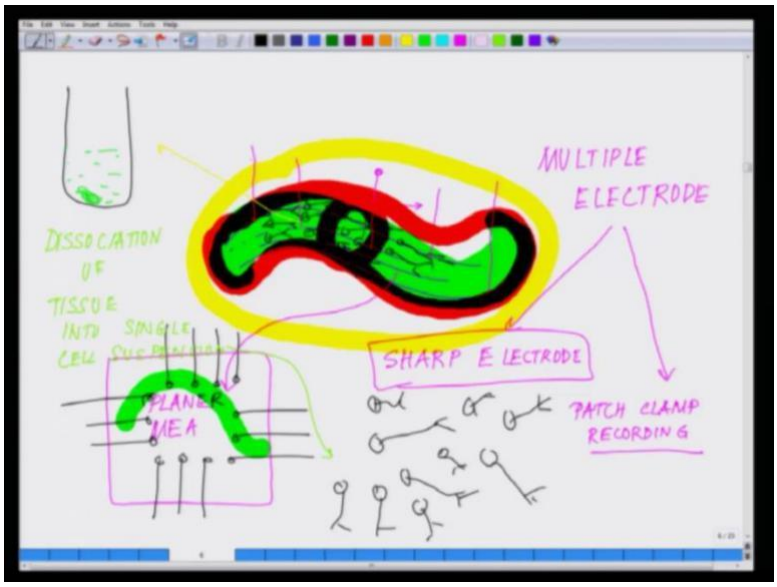

What you do essentially here, so from this diagram itself, let us start to draw. What you do here, say for example, I wanted to have understood about the hippocampus. What I will do is that I will pull out the hippocampus I will break the hippocampus in the sense I will disassociate the cells of the 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 neurons sitting like this, like this, thousands and thousands of neurons sitting like this. And they are making circuits at a

different level and this is just the 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 likewise in extracellular fluid, then you break the tissue, and this breaking of the tissue is called disassociation, disassociation of tissue into a single-cell suspension. What we mean by single-cell suspension, means now you have all these individual components that I was drawing are separated out something like this. In that process of course, the tissue undergoes a lot of damage or something, but those, which survive are important for you.

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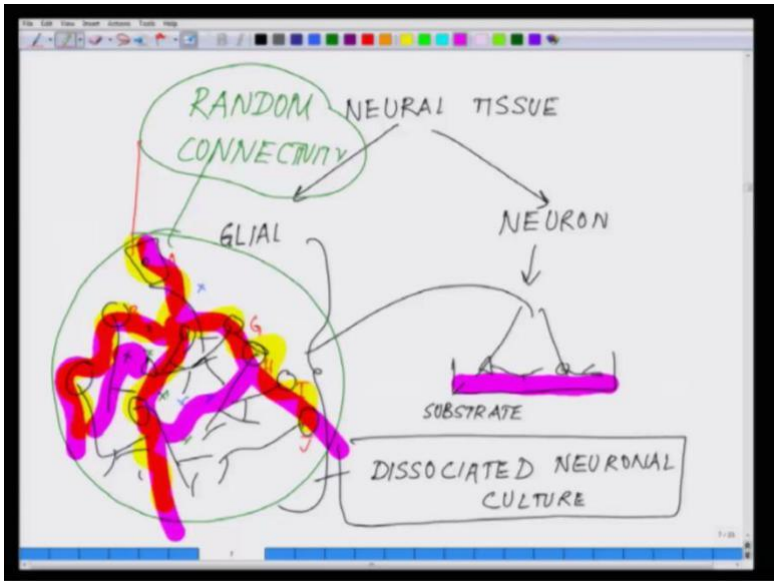

Now you take these neurons and the accessory cells depending on you have the different mode of that you can purify at this stage you can, so if you have the neural tissue in case

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 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 likewise. It will be a random connection between different neurons, this is a dissociated neuronal culture. So, these dissociated neuronal cultures, 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 figure out what they do.

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

So, this network is forming in a very very random manner, you really cannot dictate that how many synapses 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 has moved, I really cannot trace it, because the signal may move like this, the signal may move like this, the signal may move like this, the 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 over the number of cells which will be involved in generating that signal. So, you really do not know, and moreover, you really cannot in a random circuit, it is really difficult to keep a tab at the changes at individual synopsis because, at an 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, now 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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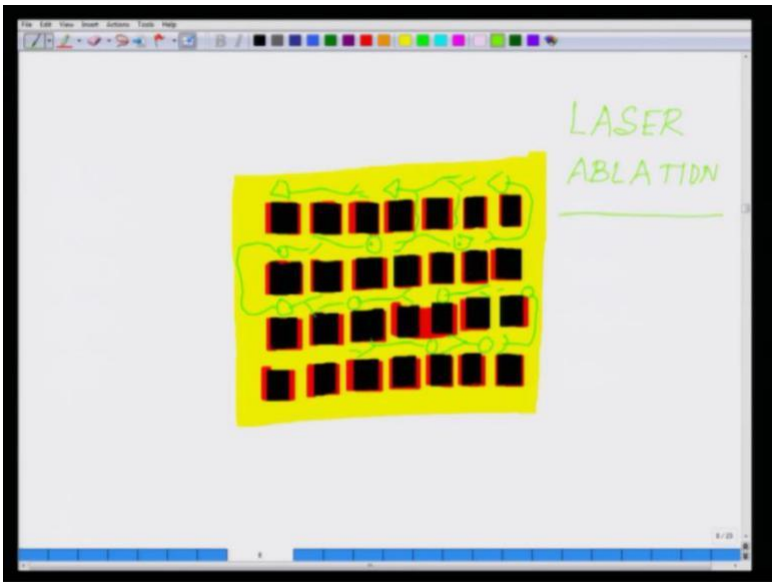

So coming back to the basics again, so I drew that I told you that there is a substrate. Say for example, let us try to understand it say for example, you have a culture substrate like this. Imagine this is the culture substrate; all the cells will grow on it everywhere. On these culture substrate, say for example, if you have a way that you could introduce some pattern, say for example 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 backfill it with something which one promotes. So, essentially what you are getting now look at it. So, essentially now cells so say for example, I backfill 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 backfilling agent. So, that is ensuring that those ablated surface. So, what we those who are not understanding ablation, let me just explain. What you do essentially do is say, for example, you have the substrate like this, you take a mask, and if you 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 in which you are exposed to the laser beam is the one which is blank. So, it turns 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 backfill it? You did it in such a solution, which will only sit on top of those ablated regions 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 that 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 to 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 I showed you. So, this is done by a technique which is called laser ablation. You ablated using a 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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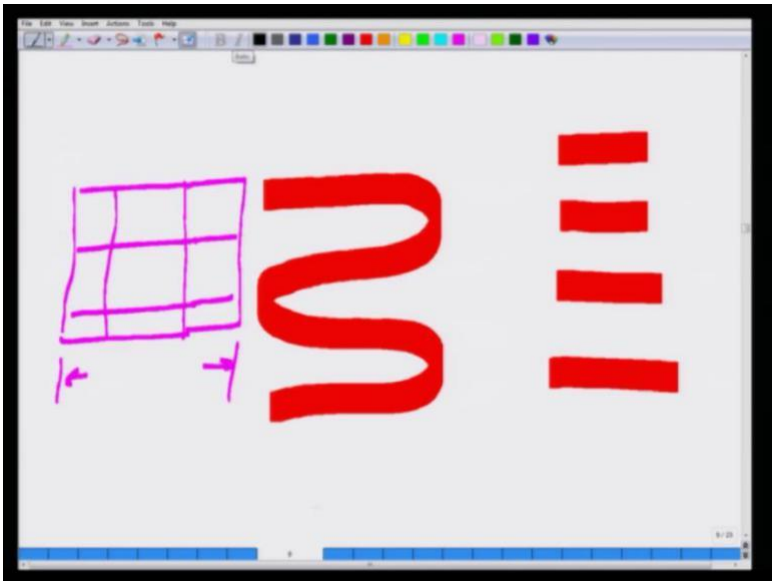

Say for example, you on your word document, you draw the circuit, narrow it down and on the cartridge, where you fill the old inject printer, you fill the ink. You throw away the ink sterilize it, and on top of that you put this either the substrate you want to do. So, what will you do the jet printer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

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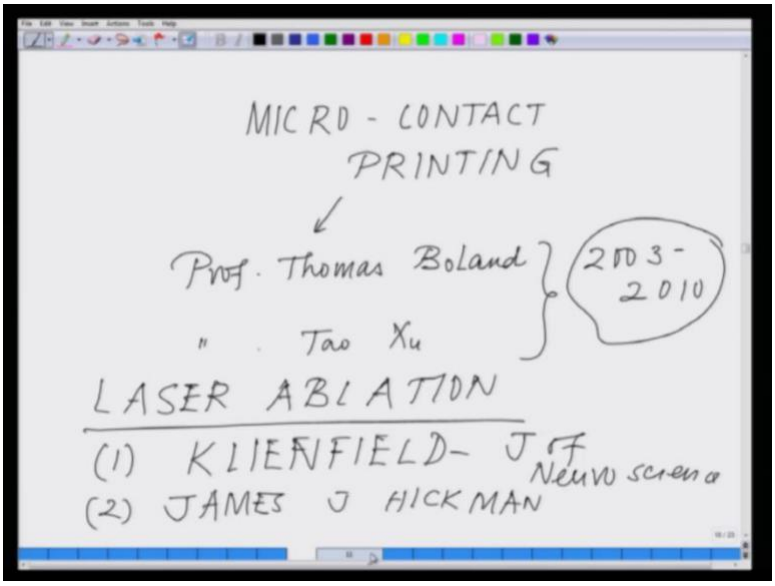

So, for this, I will recommend you, kindly go through some of the extra materials which I expect you to see the papers for microcontact printing one of the pioneering people you should refer to the work of Prof. Thomas Boland; currently, he is in the University of Texas at Alpaso; Prof. Tao Xu, these people have done very significant amount of work on microcontact printing. It is worth reading some of their work; how they have done it using very very simple most of this work was published from 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 microcontact 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 this 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 the 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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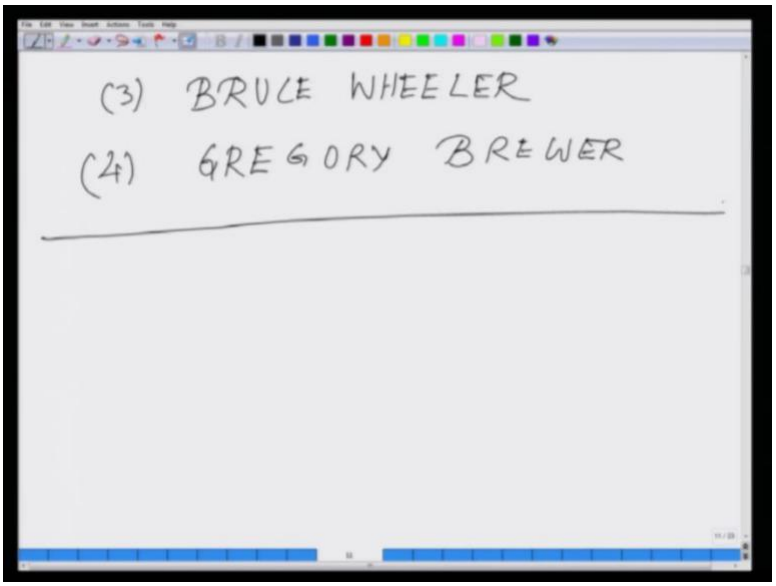

You should look through the work of Bruce Wheeler who also has done a very significant amount of work in this area and you have Gregory Brewer. These are the people who have done a significant amount of research in this area, and it is defiantly I will recommend you people please go through some of their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

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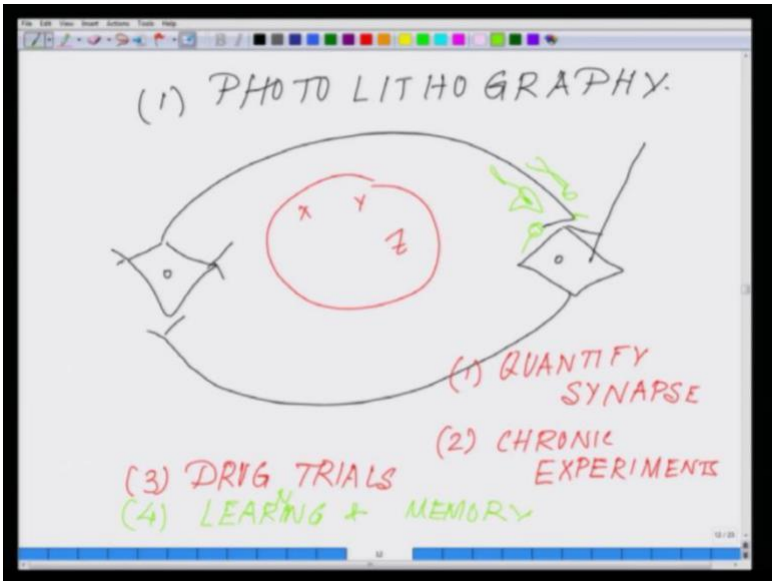

I mean you can go to the other end of the world. So, there are techniques that are being used in laser ablation and then there is something called photolithography and this will get a lot of references in Prof. Hickman's work photolithography and professor Gregory's work and Prof. Kline Fields to work and of course, Prof. Bruce Wheeler work. So, we talked about the microcontact 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 the 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 nowadays, you can develop like two neuron circuits like this. These are you will see these kinds of circuits are being developed single two neurons. So, now in these two neurons, 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 experiment 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 reduced 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 dynamics of the glial cell and on top of that here is

the control model where you can study learning and memory. So, these kinds 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 circuits 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 are that you start from the whole animal, you come at the single-cell level and then it all has to merge.

So, there is no one technique that 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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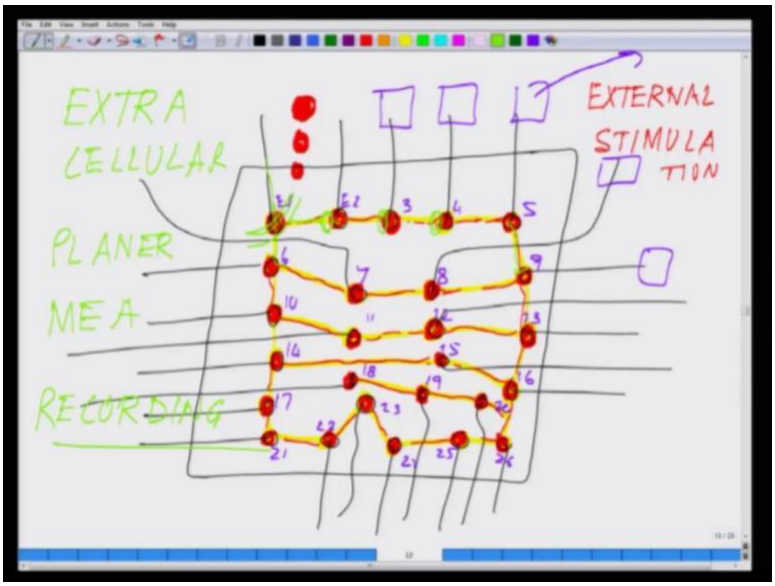

So, another approach in the same line which is you know hybrid approach which is been followed, I am going to draw is I introduce you to the microelectrode arrays. Let me draw a microelectrode array and tell you what is that approach, it is a very interesting approach. Say for example, you have these 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 the rest of the places where you see yellow are the only places where the cells will grow; the 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 said twenty to thirty microns, and those lines say for example, they were an aspect ratio of 10 to 20 microns or maybe 10 microns. And specifically, except the places where the electrodes are their aspect is slightly more maybe this is 20 micron and the lines are said 5 microns 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 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 into it. So, cells will preferentially 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 disassociated 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, in several ways you can do. You can even simulate this circuit, in order for this whole process to take place, and they will form a very controlled network. A network that you can monitor in 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 of what is happening in which electrode. Now once a network is formed, say for example, I give a signal out here, I give a stimulation out here now how this stimulation is moving along this circuit, I can monitor in real-time. How the synopsis is 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 strengthened I can study all these things now. What and then based on this 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 modern microelectronics, we are able to access a single neuron on top of an electrode. And this is all could be done using an extracellular recording, these are all extracellular planer MEA or

microelectrode array recording. So, this is the advantage that the 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 the 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 intracellular 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.