Electronic Systems for Cancer Diagnosis

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Lecture – 60
Electronic System for Drug Screening

Hi, welcome to this module. This module will be quite an interesting one, this is an experimental module. In this module, we will be exploring a simulated drug screening scenario, where we use a micro fabricated electrode to understand how a drug testing, cancer cell drug testing could be done. Professor Hardik would have covered several aspects of cancer screening and cancer diagnosis devices. So, you might have heard about breast cancer, oral cancer and the several other types of cancer.

So, today what we are going to do is there is cancer can occur also in the brain. So, it is called glioblastoma. And what we are trying to do is we are trying to see how a drug screening or drug testing of cancer cells could be performed. We will not directly do the entire drug screening scenario, but try to show it to you in a very small module, so that you understand the whole process.

So, what is the whole before we get into the experiment, you need to know the basic things. So, what happens is that let us say a patient is diagnosed cancer, they go and if they are almost certain that it is a cancer, they take up biopsy which is a small section of the cancerous suspected cancerous tissue from your brain.

They so let we are talking about brain cancer, so they will take a small section of tissues from your brain and they do some tests on it ok. And some test will be are directly on the tissue and some test will be on cells derived from this tissue. So, this tissue is called primary tissue, because it is a first from the side of the person and from the primary tissue you can crush the tissue extract the individual cells from the tissue, because why see tissues are made up of cells right, cells are the individual living blocks ok.

So, you can crush the tissue, extract the cells and then do a culture, culturing of those cells. What is culturing? Culturing is where you extract the cells, you start with a let us say 10, 100 number of cells, you add growth environment that is favourable or susceptible for the cells to grow, which is called usually called a media. Onto this 100

cells and you allow them to grow and they multiply and you have a culture, these cultures are usually done on cell culture dishes. And at behind me you can see an incubator that is where, cell culturing is done.

So, you can see there, there is an incubator and that is where cell culturing is done. The parameters that are used for cell culturing are the same as the parameters that are existing inside our body, our inside body temperature is 37 degree Celsius, we have 5 percent CO 2 dissolved in blood and we have a 95 percent relative humidity. So, this environment is maintained inside the incubator, so that cells can grow in almost similar human inside the body conditions ok.

So, what happens? Patient comes, patient's tissue sample is taken, the tissue is crushed, cells are extracted, media favourable conditions for growth cells are added and the cells are cultured in a dish ok. And then conventional drug screening scenarios, what happens is that drugs some candidate drugs will be there, which the doctor wants to give to the patient ok. Instead of directly giving to the patient and looking for outcomes, what they do is they add the drugs on to these cultured cells and see over a period of time. Let us say 7 days or 14 days or 21 days and see how the cells are dying or are the cells able to survive and proliferate. What is proliferate? Proliferation is the growth and division of cells ok.

If these processes are inhibited and that when we measure the extent of inhibition of this processes then we call, that the drug is efficient or the efficacy of the drug is good ok, this is the conventional drug screening scenario. What we are trying to do is we are trying to employ this method on a micro fabricated platform, micro engineered platform and try to reduce the time of testing.

So, what we do is you might have seen the electrodes that we use. So, we have some device drugs screening device that we have made, which is an inter digitated electrode structure on which we have made a definite well that can contain a volume ok. And then what we do is we, so the cells when they grow, they grow on the surface of the dish cell cultured dish.

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So, I will show you cell cultured dish. So, this is a cell cultured dish, this is a 35 mm dish. So, cells can be cultured on this and there is also t 25 flask. So, I will show you the flask this is t 25.

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So, this is a t 25 flask, this is called a cell culture flask and what you see is actually cell that whatever you are going to use today. The cells of this are there in this, it is the colour of this shows the colour of the media ok. When the media is fresh that means, when the nutrients in the media are not consumed at all, the media will be pink in colour, when it

slowly gets consumed, the pink colour reduces and when it is totally consumed, when it is depilated of nutrients, it becomes yellow in colour.

The media that we are using is called DMEM which is Dulbecco's Minimum Essential Medium. It is a concoction prepared by one scientist long back, which has been known to be very good for cells to grow. So, another interesting thing that you need to be aware of its fine, this is a t 25 flask, this is a 30 mm dish, cells can be cultured in both. So, let me keep this aside ok.

So, another interesting thing that you need to know is that when the cells are cultured on such normal dishes, cells has a tendency to attach to the surface of the bottom surface of the dish, this is called attached growth cells by default grow only like that. So, even if you do shaking you try to remove the media, cells will still be attached very strongly to the surface, this is because of their cell addition, cell attraction; they have lot of forces acting between cells to keep them together, these are the building blocks by which how cells come together and form tissues ok.

But for us to do drug screening we cannot take such attached cells, because we cannot take them out. So, there is a process called trypsinization, where we add and enzyme called trypsin on to the cell mat of cell growth which breaks the cell to cell bonds and the cells get out of this attachment to the surface of the dish and they enter into a suspension, but they do not die. So, this is a non destructive way of making the cells a detached from the surface of the cultured dish, but this process is necessary for us to do our drug test drug testing.

So, before we do the drug testing we have we will have a cultured dish like this and then it will have cells attached to the surface. Then it will have cells attached to the surface, then we will add trypsin ok, then that will cause the cells to enter into a suspension, this suspension of cells we will transfer into an eppendorf tube.

I will show you what is an eppendorf tube is, so eppendorf this is eppendorf tube ok, this is where you can easily transfer cells, drugs and all. In this is a 1.5 millilitre eppendorf tube, we will transfer it into a eppendorf tube, then using micro pipettes and tips we will load the cells on to the micro fabricated wells, which are used for drugs testing and then we will see how drug testing can be performed. So, this is one part.

Now, how are we going to do this whole experiment? So, I am going to divide this into three parts; one is first we will see what cells are we trying to work on which is glioblastoma cells. So, first we will see attached to cells, how they are there directly looking at the t 25 flask. And we will see the bottom surface of the cells and see how the cells are looking. Then we will off the video, we will do trypsinization and show you after trypnizing what will be the liquid that looks like, we will transfer into an eppendorf tube and then use those to finally, test using impedance and the impedance based drug screen of the cells ok.

So, let us get into the first part we will see cells attached to the bottom surface of the t 25 flask. So, let us get to the first part. I have kept the t 25 flask which has the attached to cells on an inverted microscope and kept it focused on the cells, so that you can see it. First let us look into the t 25 flask.

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So, we can see that t 25 flask kept on the microscope this is an invented microscope that we have ok. If you see the flask is there, you can see the cap of the flask right. So, you have seen that its 5 percent CO 2 is maintained inside the incubator you know, but then if you are the flask is closed, how does the oxygen supply the CO 2 supply gets circulated.

So, if you see there are openings here at the tip of the cap, so there is a filter there. So, this filter is used for gas gaseous exchange, if gaseous exchange does not happen, the

cells will die. So, this is a very custom made flask for specially made for cell culture purposes ok, so that is a just a detour.

So, now let us come back. So, we have kept that t 25 flask cells are attached at the bottom surface of the flask, I have focused it; there are lenses below, now it is kept in 20 x objective lens 20 x magnification. So, I can see it through the eyepiece, but for you I have focused it on to the computer screen.

So, let us look at the screen. So, you can see the screen, you can see lot of cells here, they are elongated very nicely you can see the elongated morphology. They are elongated, because they are attached to the surface of the flask and they are trying to spread themselves out and be as flat as possible. Like physics, they are trying to be at the level of minimum entropy and they are spreading themselves out that is why you are seeing lot of cells are there, few cells are around, because they have died. So, all cells we cannot expect them to be alive some cells will die. So, whatever you are seeing around are actually dead cells, then most of them are elongated that they are attach the cells.

So, let us just get to a higher magnification just to see how these cells are. So, when you go to higher magnification usually you need to change the brightness. So, this is 40 x magnification; actually not 40 x, it will be 400 x, because eye piece will have a 10 x magnification. So, you can see the cells more clearly, how they are very much elongated. So, I can even go to a different place in the flask, everywhere it will be same.

So, different places will have different number of dead cells. So, it is a very dense cell culture, lot of cells are here you can see it, they are all elongated ok. So, these are the cells. Now, next step is we will add a trypsin to detach them from this attachment at the bottom of the flask, then we will transfer it to an eppendorf tube. So, we will do that and I will show you the eppendorf tube.

We you will not actually saw show the trypsinization tube, because we have already like parallelly doing it, because we have multiple cultures. So, we will just I will just show you the eppendorf tube which has the suspended cells.

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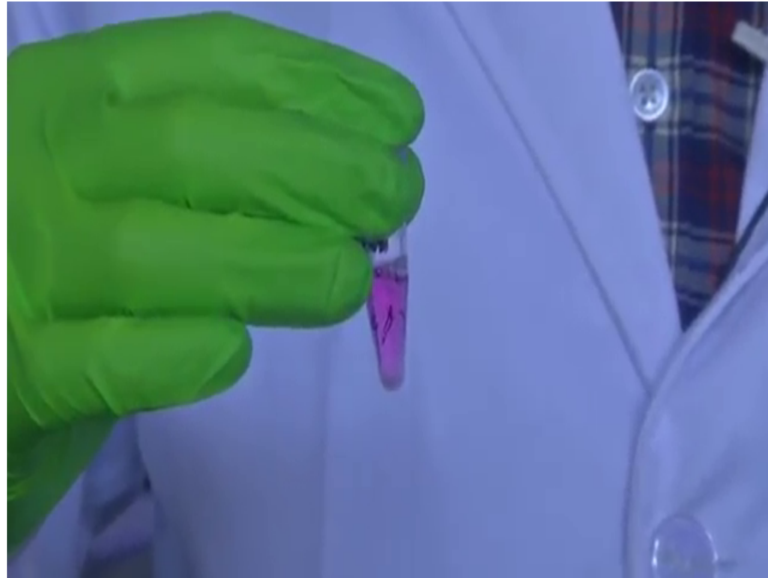

So, we have trypsinizaed the cells in the media we have added fresh media ok. You can see the cells at the bottom was this centrifuged ok, these are not centrifuged there is a process, you can see this is the cells after trypsinization, we have added fresh media. So, for the cells to take nutrients, this is why it is pink in colour fresh media is added, this is the drug that we will be testing with it.

Right now we have used a very high concentration of drug ok, for because of confidentially purposes I cannot reveal what drug is this, but this is very high concentrated drug. This is not a practical concentration, because for practical concentration we have to test it for at least a day, because where do you see somebody eating a drug and suddenly getting cured of cancer, so that does not happen.

So, there is that because other if you use a high concentration, it will have side effects in our body. But for showing to you in this module, just to show that the drug can kill the cells and see how that causes a change in impedance which is the modality with which we are doing drug screening, I have used a high concentration of drug.

And this is the cells, so the cells are in suspension in the media, you can do a process called micro centrifugation ok; where you keep this in the centrifuge, it will rotate and it will collect particles depending on its density. So, still you can see the cells getting collected at the bottom here, lot of cells are there. There is a white pellet at the bottom.

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There is a white pellet at the bottom, which is most of the cells getting collected there, the media is there ok. And this sample will be using for our drug testing ok, cool. So, how are we testing it we are using micro fabricated electrodes correct, what modality are we using we are using impedance sensing modality, but what does it mean. So, we add the cells, we measure the impedance; what is the impedance of the cell suspension, then we add the drugs ok.

And we wait for like 1 or 2 minutes, because a drug concentration very high, this drug will kill the cells ok; some cells will die, some cells will not die. Once the cells die, they are cell walls rupture, they are intracellular contents enter the medium, they are more ionic. So, they are the overall impedance of the cell suspension reduces. We again measure the impedance, if we see a change in impedance between the point where no drug was added and the point when the drugs was added, then we can tell that the drug is working ok, this is the basic simple hypothesis of our testing methodology.

Next step, we will show you the micro fabricated sensor as well as the testing set up, how are we measuring the impedance of the drug testing ok. And that and then we will directly start measuring it and we will see how things are playing out, it will be a very interesting exercise, so hold on. So, we are coming to the last and most exciting part of this module. We have made the set up to do the drug testing ok. So, first thing the core of this is the micro fabricated sensor, so let us look at it.

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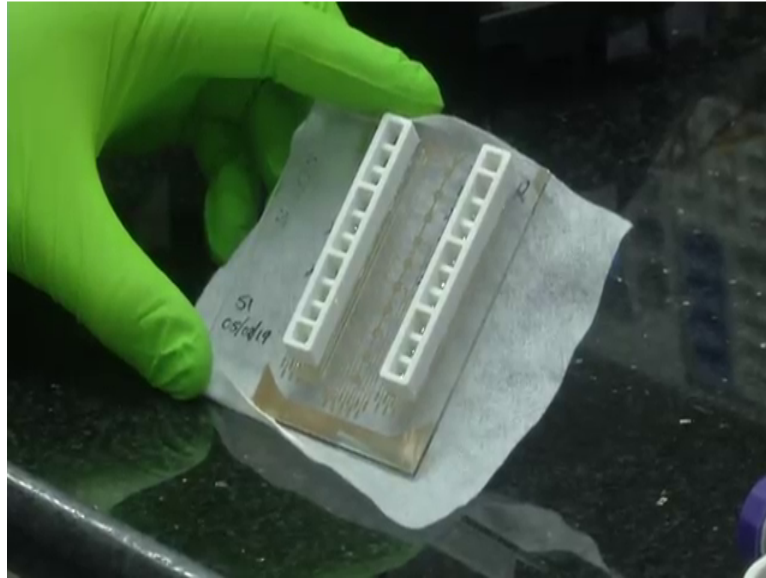

So, we have the sensor here which have electrodes, I think you have already seen this sensor before, so I will not get into the full details of it. So, we have the electrodes and we have made 3D printed wells and attach term to the electrode structure. So, we have each individual wells, where we will put our samples.

So, what we will do now is to one of these wells, I will select this well ok, I will add our cells first, I will add our cells into it, then we will measure the impedance ok. And then after that we will add the drug to the same well, what will happen then, the drug will kill the cells; some cells will die, some cells will not. This will cause a change in impedance of the solution that is in the well, we will again do the impedance measurement and we will show that: what is the difference between both these impedance measurements.

So, the difference if there is a difference, then that means that this can this method can be used for a rapid drug screening process ok. Ideally a drug screen test will be carried out for a period of like 12 hours to show this for you, I have made as I told before I have made a higher concentration of the drug.

So, for efficient, for correct proper experimentation, you need to have a statistically significant experimentation process. So, what we do usually is we would repeat whatever we are doing in at least 3 wells, it is called n equal to 3 technical repeats. We repeat in 3 wells and we do measurements from all 3 wells and all 3 have to be consistent that is how you do experiments.

Now, for you to show it to you we will do it one well and show you the basic hypothesis working. What is the hypothesis? Impedance value measured of cell suspension changes, when the drug is added there is the hypothesis ok, to prove the hypothesis, we will use 1 well. To show that the experiment succeeds, we need to have statistically significant vessels for that we need to do technical and experimental repeats.

What is the difference between technical repeat and experimental repeats or biological repeats, technical repeat is you will use one sample, but you load this due to the same experiment on 3 wells that is technical repeat. What is biological repeat? You do you repeat the same experiment on different time points using different samples, even when using different samples you get relatively same results, then you that it means that your experiment is statistically significant and experimental repeats and technical repeats are consistent that is how an experiment is designed and executed ok, this is generic information.

So, let us start of so, just to just for brief thing. So, this is a micromanipulator you have already cover this, this is impedance analyser.

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Micromanipulator, impedance analyser, the impedance analysers probes are connected to the micromanipulator.

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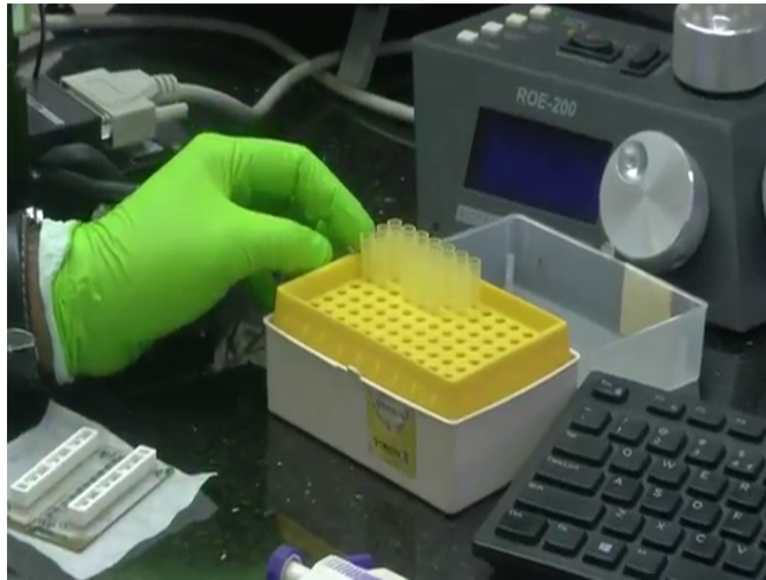

This is the tip box, where we have micro tips to load the sample. This is the micropipette, it will load the sample 20 to 200 micro litre micropipette. This is the control unit to control the micromanipulator it is switched on now. Then finally, we have the software.

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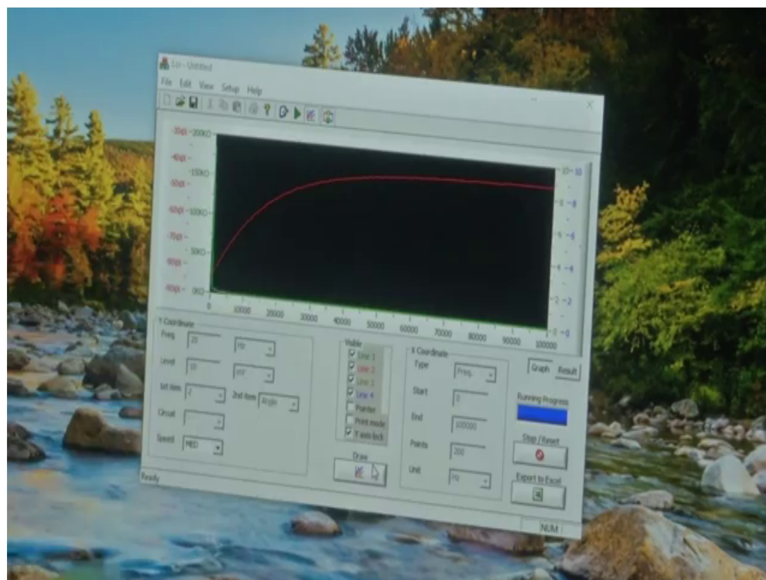

This is the software, where we will be seeing the impedance results ok. So, this is our test set up ok, you need to be very aware that everything is in place including a glass cylinder to put your tips, waste everything should be [FL] when you start doing your experiment.

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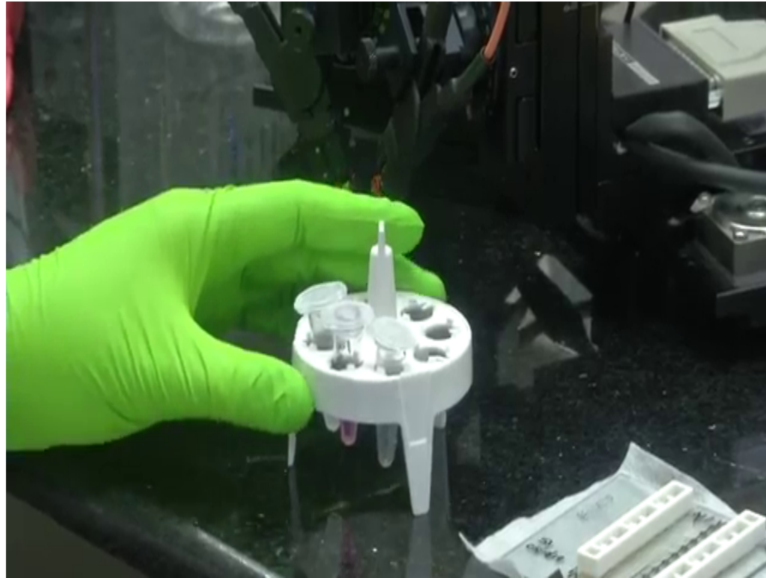

We have our samples here on this sample stand, we have our cell suspension, we have our drug here, here we have our drug cool. So, let us start the experiment. What I will do is, I will choose one well here.

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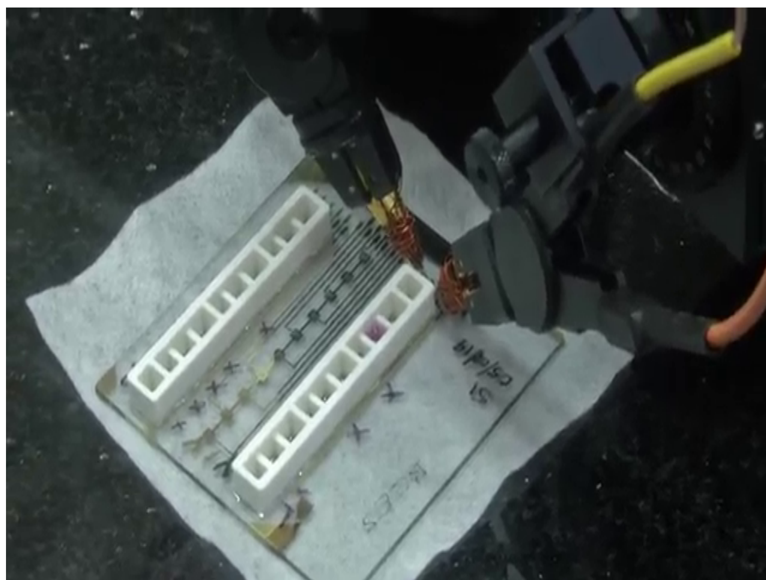

Now, we will start the experiment. So, what we will do is, we will load our sample in one of these wells ok and then see how the measurements are coming out. So, let me take the third well here or let me take the 8th well here. So, I will take the 8th well here, 1 well and we will load the sample ok. I will set the volume to 30 micro litre. So, we are

changing the micropipette to 30 micro litre or let us keep it at 40 micro litre. So, we add take up tip pipette tip, you take the sample open the sample.

So, before we do that as we all know the cells are at the bottom, you can see the pellet. So, let me just shake it up a little bit, so that cells are in the suspension. So, now cells are properly in suspension, before they settled down let us take the sample. So, the sample is there in the tip, sample is there in the tip, now I am going to load it. I will add another 10 micro litre to it, because there is still volume in the well. Let me add another 20 micro litre to it.

Now, the sample is loaded and we are ready to make take our first measurement. So, let me just connect it. So, I am focusing it on the contact pads, so he will so we have my lab mate with me here Arun. So, he was just adjust the this micro manipulator, so that it moves down please adjust it. So, you have to make sure that you have a very strong contact with your contact pads, otherwise your measurements will not come out properly.

Now let us this, this contact is made with the reference electrode. Now, we have to go to the electrode of the well which is well number 8, it has what happened is that it has reached the end of the set as a direction and still could not make contact. So, these things will happen, what we have to do is we have used this as micro manipulator right. So, we have to push it down further little bit, so that we can reach the bottom. So, we have adjusted the height of the contact, so that it will reach the bottom. So, now he is adjusting it again, come it come down further please come down, again keep coming down yeah, so we have made contact.

So, let us just confirm the contact of the reference electrode also, yes both are nicely in contact now for this well. You can see the pink colour, where the sample is loaded, the electrodes are there underneath and the contact pads are measuring from this electrode are in place. You have connected the impedance analyser's probes on those contact pads.

So, now its time to measure it, so let us look at the software; so, now this is the interface as you might have seen in previous lectures. So, for metre show you both the differences, so what we will do is we will measure both impedance and angle. So, first item is impedance which is selected, what we will do is, we will use an excitation voltage of 10 milli volt and we will also measure angle. What is the angle? The imaginary angle for the complex impedance with which you can resolve the impedance into resistive component

and or capacitive and inductive component; which is the r plus j omega c or r plus j omega 1 by j omega l, 1 by j omega c or r plus j omega l.

So, then what we will do, we will start with 0 frequency and we will end it 100 kilo Hertz and we will take 200 points ok. So, when you click; when we click draw, it will start acquiring the impedance data; click draw yes, so it is taking the data. So, we are having the measurements being carried out. So, it will take time, because it is scanning through frequencies, because it is an impedance analyser it takes the impedance values from a range of frequencies which we specify.

Here we have specified from 0 Hertz to 100 kilo Hertz. So, the green colour that your seeing on the screen is the impedance value and the red colour is a phase information. You can see the real time impedance value plotted with green and the phase information plotted with red here ok. Things will be more interesting, when we add the drug and see how the changes that is what I was thinking, why you are holding it.

The problem is that if I measure this know, then we will not able to see any change, because that guy also we will come on this range only. What I will tell that we have got a spectrum, now we will measure it at 10 kilo Hertz [FL] its recording. So, I should be speaking.

We will take off (Refer Time: 26:33) I know coming in this way, you can come from (Refer Time: 26:42).

So, it has finished acquiring the data from 0 Hertz to 100 kilo Hertz. So, the thing is that if you see it has almost flattened out after 10 kilo Hertz correct, its almost same value. So, there is no utility in taking data from all these frequencies. So, let us do one thing, we will change the frequency that we are acquiring stop and reset.

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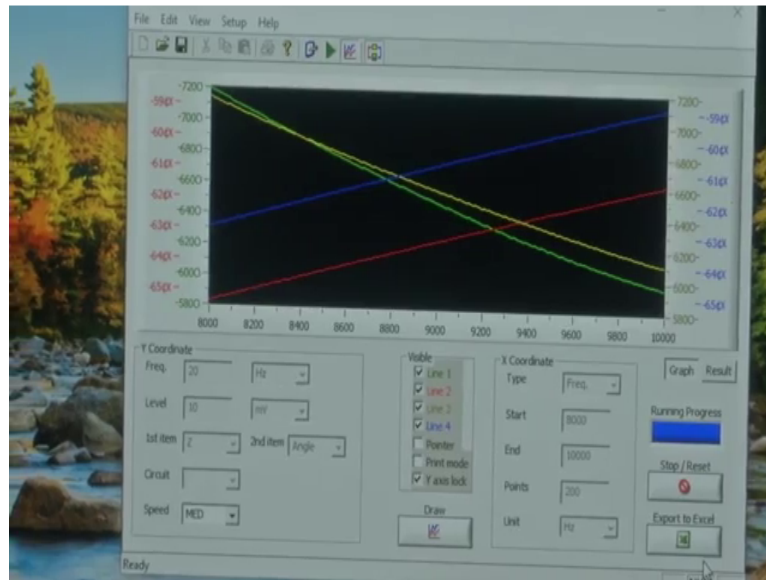

And we will take from let us say 8 kilo Hertz to 10 kilo Hertz only. Start frequency will be 8000 and end frequency will be 10000 ok and then everything else remains same. So, we will draw again, this decision we can make only after doing a first sweep of the frequencies and see that where it is changing. So, we saw that there is a sharp fault in the value and then so we are going to look at a more interesting frequency range.

So, now we are plotting the impedance and the phase information from 8 kilo Hertz to 10 kilo Hertz. It will acquire 200 samples and then we will see. So, this is all a still a signal acquisition. So, it takes time, what is happening is we are acquiring signal data from real world and then trying to show it on a software. So, you should understand the engineering challenges involved in making such a system. So, even this much time is a big achievement from instrumentation point of view [FL].

You put everything in that [FL].

I put everything in that.

So, we have finished the acquisition of a signals. So, keep looking at the software, now I will add 20 micro litre of drug to the same well. You have seen the drug, now we will add 20 micro litre of drug a 20 micro litre of the drug, come down, further down, wait. So, I have added 20 micro litre of drug to the well, we have to wait for at least 2 minutes for the drug testing to have an effect. We will wait for 2 minutes.

Student: Boss.

So, we have added the drug we have waited for around 2 minutes to for the drugs to take effect, now it is eureka moment, let us do the measurement and see if there is a difference. So, these are very micro level measurements. So, even if we get around 10 ohms or 5 to 10 ohm difference which is repeatable, which is repeatable that itself is a good achievement and the marker of drug efficacy.

So, we are doing the measurement now let us see how things happen. So, it is getting plotted. So, this is the second after adding drug ok, measurement after adding drug. So, another thing to notice is that I have added only 20 micro litre of drug to this.

So, let us see if there will be any a difference in the values. If we see a clear demarcation between the green and the yellow lines which is the impedance, then we know that there has been an effect. And also the red and the blue lines, only when it completes if we will know; so, there as a small update that came.

So, it is taking the measurements from 8 to 10 kilo ohms. So, as you can see; as you can see there is a clear difference between the green and the yellow lines. And there is even more star difference between the blue and the red lines, but you can see is that as I have told you that there is maximum change happening from 0 to 10 kilo Hertz and after that it is constant right. When we look at the spectrum up to 100 kilo Hertz, now then we change the frequency with which we are seeing it.

So, then if you see when it starts of the yellow line is below the green line and there is a crossover point, after which the yellow line is above green line and at that point the impedance also, the angles are also there is clear difference fixed, constant difference in the angle measured between the blue and the red lines. So, this you can see as a significant difference of the signals between when we have added the drugs and before adding drugs. So, this is how we can this signal we can use as a measure of drug efficacy ok.

So, I think you have got a very interesting experiment you have seen that. So, we have seen a very interesting experiment you have see we have seen how signals are changing and how which frequency is to choose. So, many learning’s from this experiment. So, but

this was a very short experiment ideally this should not be carried out in the short time, but for educational purposes we have tailored the experiment in that manner.

But ideally this drug testing should be carried out at least with micro litres also for a period of at least 12 hours, for a realistic concentration of drug to be used against the cells. We have used a very high concentration, which is not a realistic scenario.

But even then conventional method take if 7 days and up to 2 weeks to show give drugs cleaning results, which can which we can use this method, we can get it in 1 to 2 days. So, these are the advantages of incorporating engineering methods into to solve biological problems. I hope this example of the screening will stoke further interests in you to explore this field, which is a very budding and exciting field.

Thank you; see you in the next module.