Electronic Systems for Cancer Diagnosis

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Lecture – 57
Demonstration of Cleanroom Equipments
Impedance Analyser

Hello everyone, welcome to the course on Electronic Systems for Cancer Diagnosis as the course intend us to teach more about biosensors. Today, we are going to study more about an important type of biosensor called an impedance biosensor. What do you mean by impedance biosensor? A sensor which is fabricated with the intention to measure the impedance parameters, what is impedance parameter if and where does this come.

So, when we are talking about integrating a sensor and transducing the various physiological parameters of cells and tissues and understanding their mechanism with there, could be alterations in their physiological parameters and how do we leverage them and integrate that. And, understand and all of this requires biosensor, which provides as a platform to understand the various physical parameters, which we are intending to study based on alter alternating, alter based on the altering temperature or environment conditions.

Now, one such example is like I mentioned senor, which can measure the impedance parameters. So, it is already understand the literature talks about various parameters like the change in pH, the change in temperature, oxygen consumption, the ion concentration, the cell membrane, the potential varies, the membrane potential can be measured depending on different environment, where the cells are exposed to multiple challenges.

So, all of these parameters like I mentioned the different potential, pH, and the impedance is another important parameter, which also falls under these physiological parameters which can be used to study these the alteration that happens in cells. It could be due to disease or it could be due to some biology interaction. A kind of interaction is the protein-protein interaction like the antibody the antibiotic is say there is this, this antibiotic susceptibility.

So, you have an antigen, and then antibody. They are the two type of protein. So, when they interact the when they binding together, so not all combination that different that there multiple cells of antigen and antibody, so not everybody bind.

So, when there is right combination out of the n number of combination, when we write antigen and antibody combine, so this binding creates difference in potential. So, when the two bind, there is a change in impedance. This is how this parameter the potential which can be studied because of the binding the change in impedance which can which, which is the result of the right antigen; antibody bind is what is used in immunotherapy. And anti antigen antibody the antibiotic susceptibility test, all of these are multiple test, which are performed just to understand the right antigen and the antibody interaction.

So, what is the electrical parameter, which can be used to understand the right interaction, like I mentioned it is the change in impedance; now this impedance can be measured using a biosensor. Now, what cause into the biosensor, how do we fabricated, and how are these impedance parameters measured, the alteration study and how can they be characterized all of this requires and instrument called the impedance analyzer; more into the details about the impedance analyzer will be explained by me shortly.

So, when we talking about impedance analyzer, the instrument uses a four probe mechanism, what is a four probe mechanism? So, when we want to measure something, say resistance across the given resistor, you just hold it you have a multi meter, you hold the two leads and then measure the resistance.

So, so you change direct to where, where you can measure the resistance and then measure the resistance or you could change the voltage, you could change current, you could you could do multiple measuring with the help of two leads, but this is two point mechanism, but when we are talking about four point, it is mainly used when we need highly precise readings, high precise measurements. So, there is a parameter called the contact resistance. So, what happens is, when the let us say I take a multi meter now.

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So, here we have multi meter. Let us say I have a multi meter and then you do the resistance measurement and here you have two leads, the sensing and the gram. So, you so you have these two leads to do the measurement. It could be the voltage measurement, the current measurement, you could do the resistance measurement, all of this can be done using the two probes.

But when we see the contacts the electron the tip here offer some resistance, this is a parasitic resistance. The point of contact between the lead and the electrode the sensing the cell the point when the measurement has to be done. So, these two create a resistance called the contact resistance, this could be of high importance, when you are going precise measurements.

So, the amount of resistant what the biosensor could measure the impedance change, like one point which has to be considered even before you are doing the impedance is the cells, what we are talking about that their more insulating in nature. So, in order to measure, so you only to apply some voltage and then flow the current, so is not enough current that can flow through the flow, through these. They are not good conductors, like you can see a copper leader or like the aluminum or silver, they good conductors and you could flow current and measure, but when you comes to biological sensor tissues, they are not really good conductors.

So, the amount of current that good flow through them is very minute and so in order to measure the impedance, so you need to apply some voltage and then measure the amount of current that flows through them. So, all of this creates challenges now that the cells are not really good conductors, how do you flow them. Now, so it is already understood that you could apply some small voltage, and then externally force current to flow through them and then to the impedance measurement for that like this instrument cannot be used.

Like I said, the amount of impedance what we would be measuring is really minute and it requires high precision and sensitivity, which an instrument like this cannot often, and that is when you use the impedance analyzer and it has like I mentioned it has four probes. The sensing elements are divided into four, what happens when you divide them into four is so the two electrodes are used specifically only to force voltage and the remaining two the sensing electrodes are used the sensing buyers can be used only to measure the current.

So, now your voltage and current measurements are done by dividing the entire system into from two to four. Now, the now there is a drastic reduction, so the chances of eliminating the parasitic resistance, which can be cause because of the contact between the electrodes and the surface can be eliminated. So, this is where four point probe or the four terminal sensing mechanism comes into picture.

And let us see what are the different and how these four point probes can be used in order to measure the impedance of a biosensor. More about the details let us get a hands on, on the instrument which is here. So, this is the L C R meter also called as the impedance analyzer. In our case they are doing the impedance measurement otherwise, the different parameters like I mentioned based on the type of chemistry the changes in different parameters could be measured.

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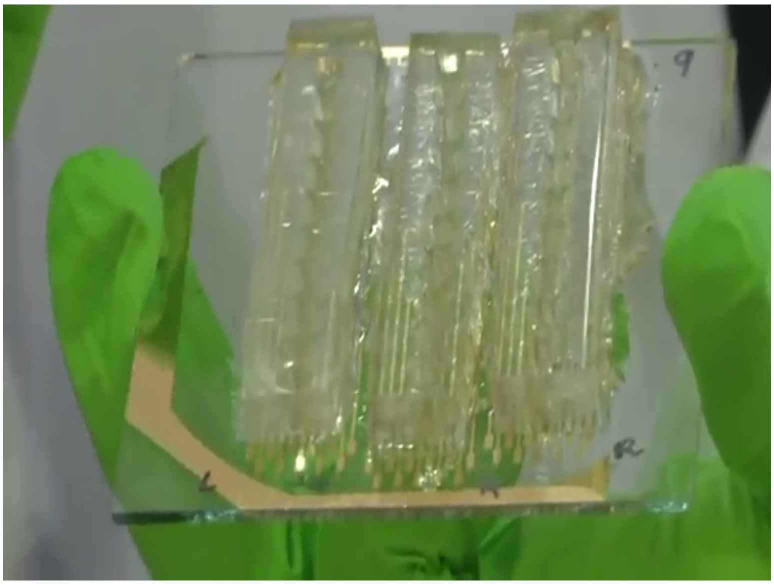

So, here this one example of a sensor; so, this is the biosensor so this is the biosensor in my hand, which measures the impedance. So, we call it an impedance biosensor and this is used to test the antibiotic susceptibility. So, what happens is how do you do the impedance test like, you could see here there are electrode pads.

Each of these are electrodes and when you force voltage on these electrodes, and then measure the corresponding current. So, this ratio of the applied sinusoidal voltage to the measured current gives you the impedance, so that is why we call this an impedance biosensor. This is a PDMS layer on top, which has micro wells.

So, there are three devices here and each of this micro well has inter digitated electrode, why is the electrode? So, when you load your sample like I mentioned the physiological parameters like the change in impedance, when there is a binding between the antigen and the antibody can be leveraged through these electrodes, which are inside each of these micro wells.

And these electrodes are externally given connection here, and these are the electrode pad such a bottom, where you could force voltage and measure the corresponding current; and hence we measure the impedance of the given sample. Now, let us see how we could integrate this would the LCR meter or the impedance analyzer and do the test.

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So, this is the LCR meter or the impedance analyzer as you can see like I mentioned this is a four probe device.

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Like a 4 terminal device here, L force, L sense, H sense and H force. So, each of these, so these two channels here are two force the voltage and the sense here are use to give the measure the current. So, both source and sense the entire mechanism is incorporated into this four channels coming out through the L C R enter here.

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The two probes, so these are the 4 leads and we have used buyer binding. So, in order to make measurements using the impedance sensor which I mentioned, otherwise you could remove these excel connection and then you can see the prototype dense. So, these are the two probes. Even before we start using, let us calibrate the device even before any measurement is taken.

So, initially if you could see the display they are multiple measurements, see the capacitance and inductance, reactance, susceptance, impedance, advertence all of these parameters depending on your application can be measured. So, when you are talking about AC circuits and when you are having sensors and you directly want to measure the admittance. So, you could use this option and then do the measurements. Now, we have in the impedance mode.

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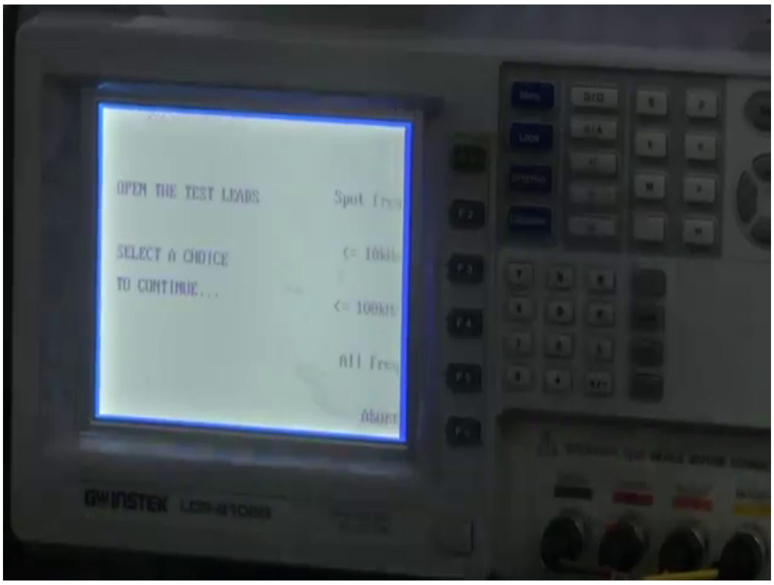

Let us say we want to calibrate, open circuit trend here means open circuit calibration. So, when I saying OC trim over what frequency range the calibration has to happen.

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We can select they are different frequency range, you could chose all frequency. And now the two leaves the two electrodes in my hand, I will open circuit configuration and then we do the OC trim. Now, that this complete let us do the short circuit trim.

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So, this is a short circuit trim and then I will give it for all the frequencies. So, there is a throughout the so now there is a failed, because initially I cannot hold it in the short circuit connection. So, there was so initially there was an open circuit configuration. Now, let me keep it in contact, now that both of them are in contact. Let us do the now then it says the short circuit trim failed. Now, the connected them correctly, let us do the short circuit trim and choose all frequency operation.

Now, both of them are in contact and throughout the frequency range yeah this short circuit, you know short circuit configuration the a calibration has been performed. So, now that we have finished this, you could go to the measurement very good measure for 10 milli volt say you could do the setting. So, here you could use the option and then set the voltage, which you want to force on the sensor and frequency at what, what is the frequency sweep. This here is the penti locals, so you could choose the entire or you could choose the options for the applied voltage and the frequency setting.

All of this once performed, let us see after calibrating the open circuit and short circuit, let us connect this to the impedance analyzer. The sensor which we have here and do the measurement also in order to give a proper for the ease of performance, here there is a software which is linked to the impedance analyzer.

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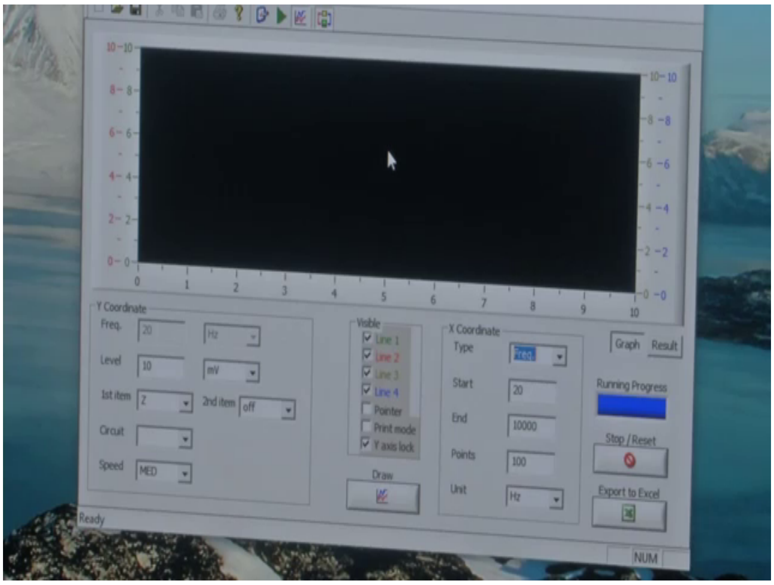

So, this is the software here connected to the pc there are multiple options, which are given here. Now, let us say I stop and then see this; you could choose the frequency, you could chose the voltage. The like I said the force voltage is said to 10 milli volt and then you could choose what is the measuring parameter, you could it could be the capacitance, inductance, reactance, admittance or the DC resistance in our case let begin impedance.

And then we have the speed. So, at what rate the measurement has to be performed; if you required the measurement has to be perform into faster rate, you could chose pass however, it could compromise on the accurate results, which are obtained. So, in order to get accurate results keep it to medium and then you could choose frequency on voltage. And then they start the number of points were sampling has to be done; the start can be end frequency these are the different options, which are provided here.

So, let us see how we can plot the entire operating throughout the entire operating frequency range. Let us see the different impedance values that can be captured using the tool and the impedance analyzer their system, which will be integrated with our biosensor.

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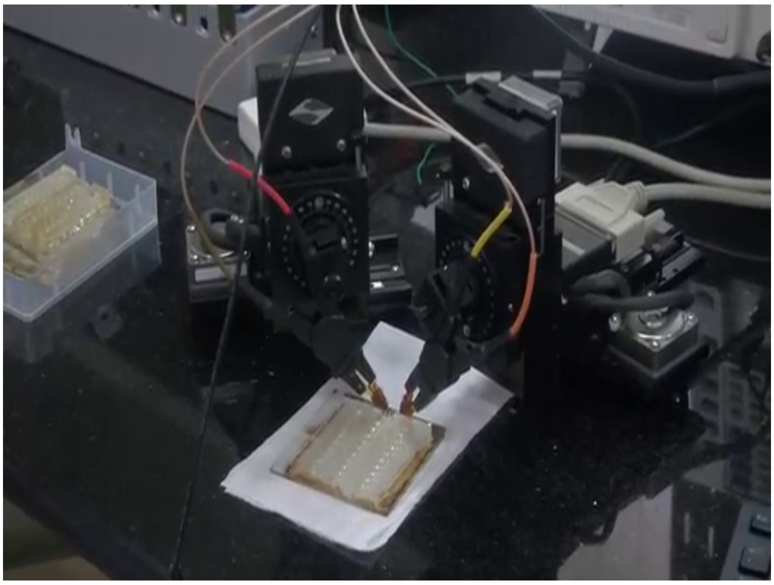

Like it one of the previous modules, I am already mentioned the use of a micromanipulator. So, you could use this does the tools lot here to connect then indenter, like I can mentioned earlier, if you want to study the tissue biomechanics or in our case now we have a biosensor, which is placed here. So, how do we like I already showed you, the different electrode placement.

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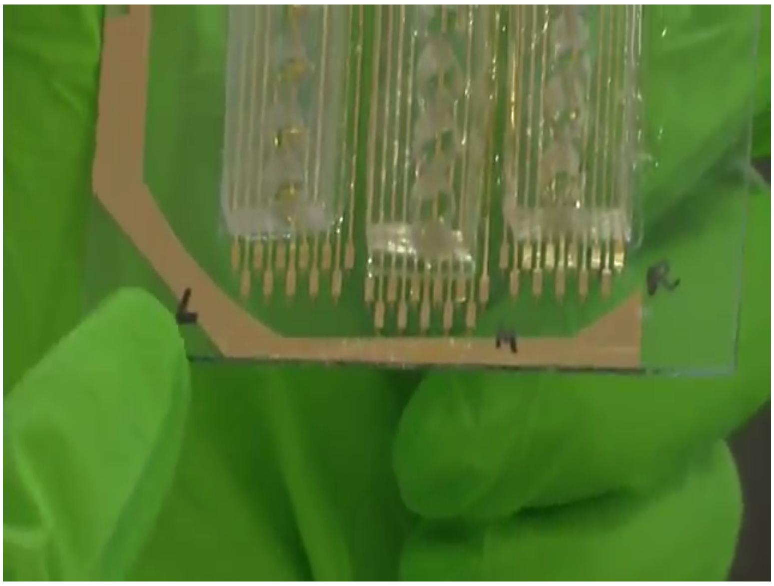

The external pads are very close to each other. Now, if I am sure you could even not see the numbering, which is provided on this. So, how do we manipulate this manually, there could cause a lot of errors and in could even need to damaging the sensor. So, how do we precisely manipulate this micrometer variation and distance from one electrode to other. All of this can be easily handle with the help of the micro manipulator.

In this case we are using the micromanipulator and the sensing electrodes of the LCR meter let me connect each of this to the tool handle here. So, now we have use the two manipulators and connected to the electrode pads of the third electrode, the third the there are three devices like I mentioned. So, the third micro well, which will be used now for experimenting so, I have use the 3 and 0 on the electrode pad terminals in order to connect to the manipulator.

Now, let us use the software to see the over the frequency see sweep, how do we measure the impedance of this, now the well is empty. So, just the pad resistance let us see what is the impedance, there can be seen in the software.

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So, initially we have taken care of do in the calibration; open circuit, short circuit calibration of the two sensing electrodes for what would be using for measurement. And once calibration is done in the measurement phase after connection, let us see initial they so we have to connect to the compound and once the connection is established, we can see the different options we have set the frequency level. So, 10 millivolt is the applied voltage to the sensor.

Now, if we use the draw option here, now you could see how the readings are in progress. So, over the given frequency starting from 20 years so, then there are 100 so the here you mentioned 10 points. So, starting from 20 to 100 Hertz will the 10 points where the frequency is being captured, this frequency is when the electrode they, they, they just the plane electrode the sensor and the electrode pads. So, the well has nothing in it, it is just empty.

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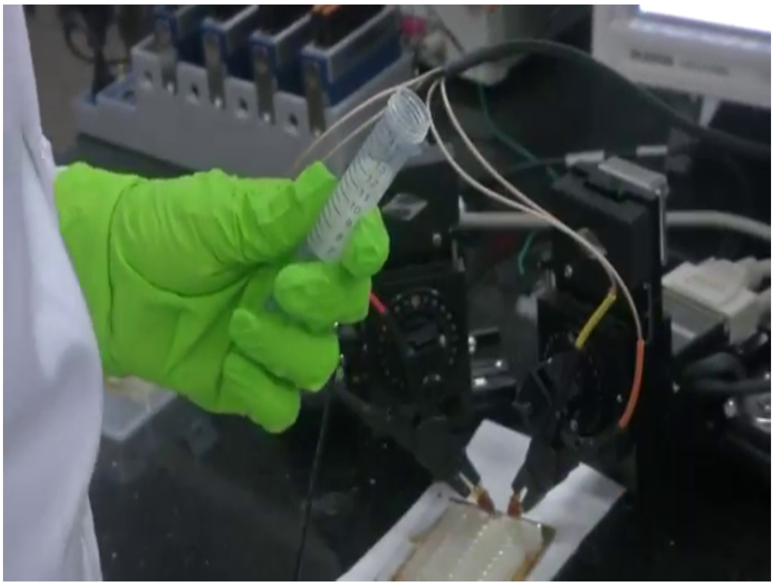

Now, let us say I fill that with water here, what I have is DI water I would use a micropipette.

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So, this is the pipette, here in my hand.

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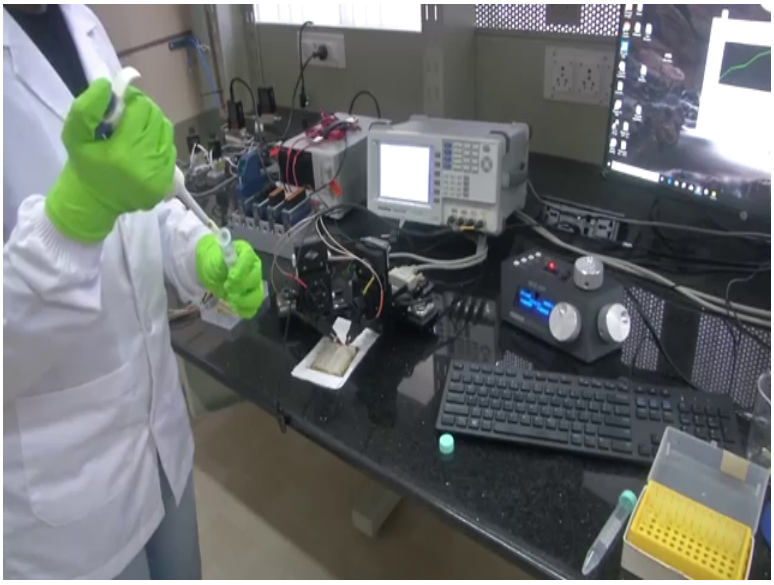

And then let us use the. So, 100 micro liter sample, 100 micro liter of DI water is taken in this pipette.

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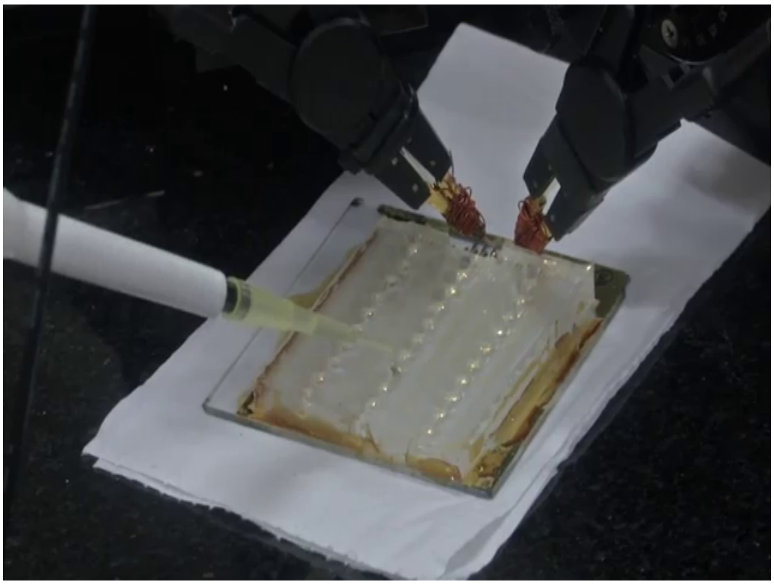

Now, I would fill that in the third well. So, the in the third well goes the 100 micro liter of DI water what I had taken. Now, now that the well is filled with water.

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Let us do the frequency sweep and check the impedance measurement. As you see there is the drastic change in the measurement here. So, this was the empty well and here. So, as you can see this is a capacitance here. So, we have change the item which is measuring here is the capacitance. So, we need the impedance measurement.

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So, I am going to stop the entire process, again change this 10 milli volt is fine. So, we need to do the impedance measurement and do the frequency sweep over this. So, this is find 10 points and then let me draw, this is showing 180 kilo ohm, the impedance is in kilo ohm with the DI water. So, we have lost the readings, because it was in the capacitance the measurement was in capacitance mode the empty well, the amount of impedance the empty well measure was not captured, but now we can see the impedance will kilo ohm. So, it, it is dropping over the as this frequency increases, so this is with the DI water.

Now, I will take back the pull back the DI water, which was put in the third microwave. And then discarded here, and then let me take another pipette and then now let us know that we have use DI water.

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Let us try using salt water. So, 100 micro liter of salt water is what I have in the pipette going to the third well.

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So, here I fill the third well with salt water. Let us see what is the impedance.

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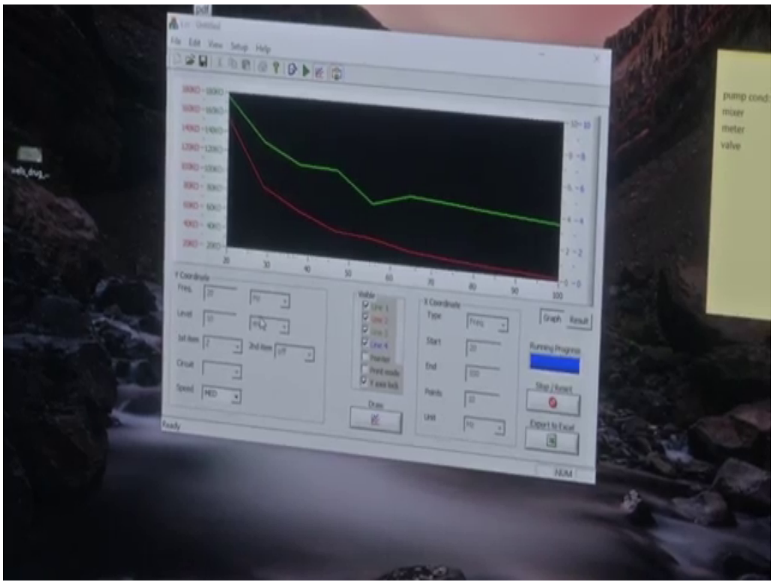

You could see there is a drastic drop in the impedance like, we all know salt water is a good electrolyte, they you can see the difference in the resistance. So, there over the given frequency there is a drastic drop in the impedance. So, this was using the DI water, this green line here indicates the impedance measurement over this frequency from 20 Hertz to 100 Hertz. The impedance measurement of DI water and this red what we see here is the impedance over the given frequency range, when you apply 10 microvolt across the 2 electrons.

So, when you force 10 micro volts and across this frequency, you can see the amount of impedance, the difference in impedance what the DI water gives and the salt water gives. So, this was a simple example you could use the same experiment to perform the different antigen, antibody analysis that the different test can be performed using multiple samples. And then study the cells, that is the physiology of the cells and understand the electrical parameters like the impedance.

And let me just discard the entire. So, this is the borosil tumbler which is used to discard all the pipette which were used. So, and in when you are talking about the doing experiment with biology samples, always ensure you do not reuse them that could cause that could be a major reasons for contamination. So, once that is discarded, so this is how the impedance biosensor is connected to each of the arm of the, two arms of the micromanipulator. And the different electrode terminals can be connected can be pointed using these manipulators and this goes to the L C R meter, where you can sense the voltage and measure the current with the other two leads.

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And, like I already explained previously the entire set up.

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You can manipulate the movement using these are on the, the control system of the micromanipulator the z, x and y arms and you could also choose the 1 and 2 option, and then set the distance. So, this we have just tested for the third well, that are multiple devices, which are in the single chip here. All of these can be tested for the proof if you could even calibrate each of these devices, and then test the performance of each of the device, which is on the single glass vapour.

Now, let me just remove the salt-water put in the third micro well and then discarded on. So, this was brief explanation about how to use micro how to use the micromanipulator and the L C R meter. Like we mention in order to measure the impedance, the every time there is bond that is the antigen, antibody and then the two link results there is a change in impedance. And this phenomenon can be leverage to understand the right combination of antigen and antibody and do the antibiotic susceptibility test protein-protein interaction, and you could even do the individual therapy.

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The multiple options, this was one such an example where we used this device if you can see inter digitated electrodes each of this on top of each of these electrode is the micro well, where we loaded samples. And the electrode pads externally when you force the 10 milli volt, on these electrode pads using the L C R meter. So, here you force the 10 milli volt on these electrode pads and then do the corresponding current measurement and do the impedance, calculation for the entire set of electrodes.

So, now that we have seen how and impedance analyzer can be used, you could use then to do the various studies like the immunotherapy and other this. I am sure, you understand how to operate how the software can be used and how different parameters can be changed, like the frequency sweep the amount of voltage that you force in the various parameters, electrical parameters that can be measure the impedance, admittance, depending on the type of application. So, this is how you would integrate a biosensor, which the entire electronic system and then do the analysis.

Thank you.