Sensors and Actuators
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Lecture - 50
Applications of microcantilever for Mechanical Phenotyping of breast

Hi welcome to this particular module, if you remember in last class we were discussing about the fabrication or process flow for fabricating the piezoresistive microcantilever right. And what we have seen? We have seen that for designing or fabricating such a sensor such a cantilever; we need to start with Silicon on Insulator that is called SOI wafer right.

You also seen that how we can open the window we can defuse the p plus first, then you can defuse p plus plus followed by the metal contact and followed by the front to back alignment and then DNI right.

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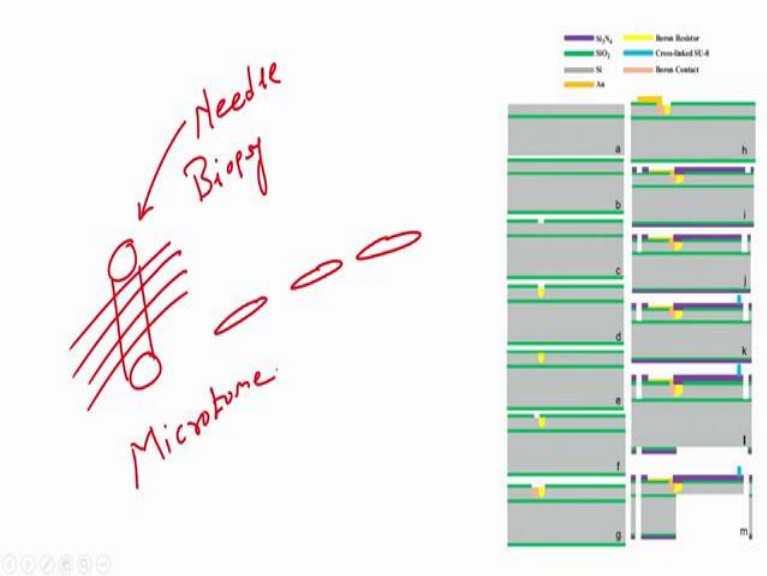

So, if you see the screen this is what we were talking about that in the last module we have seen this process flow in detailed.

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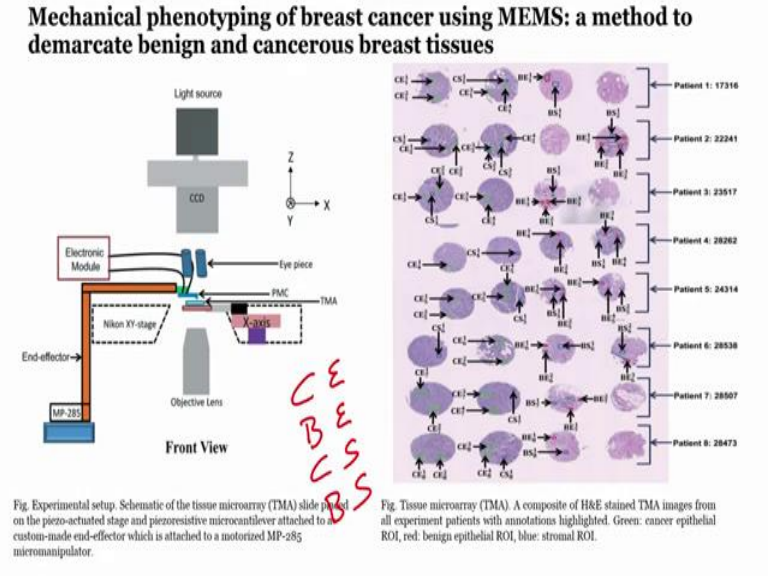

Fig. Experimental setup. Schematic of the tissue microarray (TMA) slide placed on the piezo-actuated stage and piezoresistive microcantilever attached to a custom-made end-effector which is attached to a motorized MP-285 micromanipulator.

Fig. Tissue microarray (TMA). A composite of H&E stained TMA images from all experiment patients with annotations highlighted. Green: cancer epithelial ROI, red: benign epithelial ROI, blue: stromal ROI.

Then we have also seen that if we want to understand the properties of the tissue. So, what happens is when the tissue is out right, it is tissue is out, this is taken out with a needle and this process is biopsy; so it is called biopsy needle. Then this tissue is sliced into and if into thin slices with a technique called microtome or is a equipment called microtome alright.

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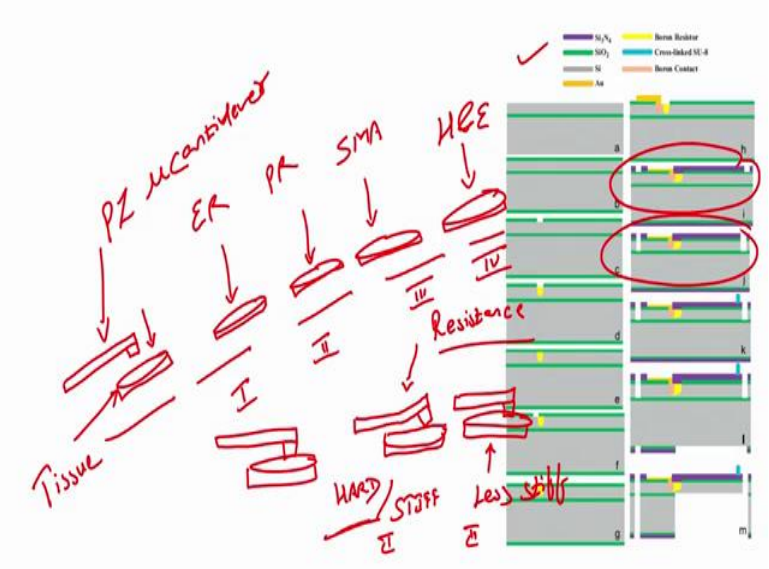

So, once a tissue is sliced then different biomarkers are tested right; it can be estrogen biomarker, it can be progesterone biomarkers, it can be SMA biomarkers right is SMA brown it is H and E staining; so lot of various possibilities are there ok.

Now, if I get this slices of tissue; since these are all uniform slices assuming do not do not worry about the figure. Assuming this all these slices I, II, III and IV are uniform in terms of thickness right and one more slice I get for my experiments which is I will take my piezoresistor and then there is a SU tip and I will indent this on this particular tissue.

I will indent my piezoresistive microcantilever cantilever on to this tissue and this cantilever will bend it will deform or bend depending on the tissue properties. If the tissue is hard the bending will be less if the tissue is soft is the tissue is hard bending will be more, if the tissue is soft bending will be less what does that mean? That if I have a tissue on which I am indenting my piezoresistive microcantilever; if the tissue is hard or stiff, then my piezoresistor will bend more right; when it touches the tissue is not it?.

So, if I just bring the tissue close by when it is touches the tissue it will bend more because this particular tissue is stiffer. But if the tissue is not stiff, then my cantilever will not bend much right this is less stiff compared to the first case; less stiff is more elastic right. So, then my piezoresistive cantilever will not bend. Now, what I am saying? It has a piezoresistor embedded on to the silicon wafer using this particular process flow; that means, that if I have the bending of the cantilever; then there is a change in the resistance.

Depending on how much a cantilever bends; my resistance would be more or less right. So, this study would help us to understand the elasticity of the tissue right and that is why these are all the tissue slices. You can see here using microtome and then we also discuss last time that the C stands for cancer, E stands for epithelial, B stands for benign, E stands for epithelial, C stands for cancer, S stands for stromal, B stands for benign, S stands for stromal.

So, benign stromal, benign epithelial, cancer stromal, cancer epithelial from patient 1 to patient 8; when we have this such a data and if I indent my this piezoresistive cantilever as shown in this particular figure right. Then depending on the elasticity my piezoresistive cantilever will bend and when it bends; I can and I can know the resistance

and convert it to a voltage depending and then from that we can understand what is the elasticity.

This is a inverted microscope, there is a eye piece and then there is a X Y stage, we have use Nikon MP 285 is a micromanipulators where we can indent the tissue with micron precision there is a end effector.

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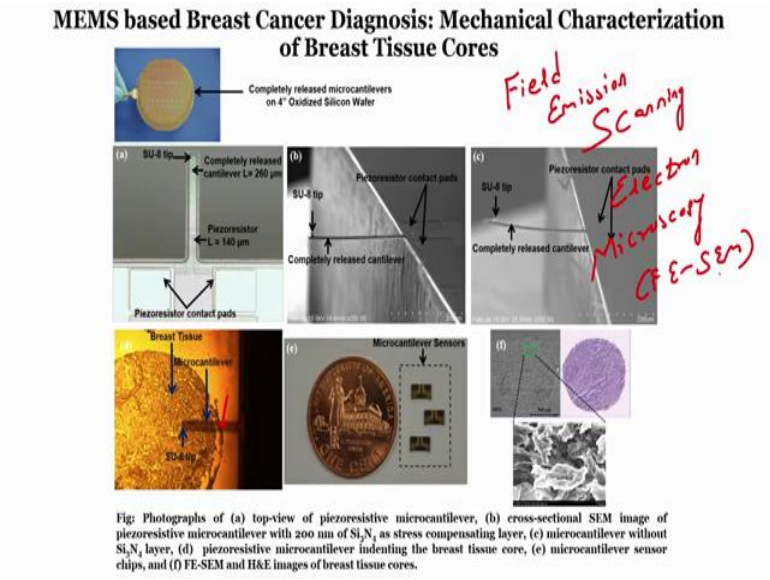

Fig: Photographs of (a) top-view of piezoresistive microcantilever, (b) cross-sectional SEM image of piezoresistive microcantilever with 200 nm of Si_3N_4 as stress compensating layer, (c) microcantilever without Si_3N_4 layer, (d) piezoresistive microcantilever indenting the breast tissue core, (e) microcantilever sensor chips, and (f) FE-SEM and H&E images of breast tissue cores.

And if you see the piezoresistive cantilever will look like this if you properly fabricate it if you miss a step. For example, in this particular step process flow; we have seen that there is a silicon nitride that we are using right. The silicon nitride will work as a counter stress when we use silicon dioxide.

If I do not use silicon nitride then you can see that the cantilever is bend right. So, there is a compression in the cantilever this compression can be that we can care of if I use silicon nitride. In this case, I deliberately have not use silicon nitride and that is why you can see this cantilever is having more stress because of the silicon dioxide. But in this case I have you silicon nitride as well as silicon dioxide and that is why the stress is being counted by the silicon nitride alright.

Now, there is a SU 8 tip and this; this cantilever is such that this is a back side. So, this side is here in the top and the SU 8 is touching this tissue; this is a breast tissue slice and this is how the E individual chips looks like right. And when we perform scanning

electron microscopy to understand the; the structure of the tissue, then what we found is when we go for FE SEM; FE SEM stands for field electron field emission; Field Emission Scanning Electron Microscopy, FE SEM alright. When you perform FE SEM of this particular slice; of the tissue which is taken from the breast, then we find that the normal tissues would have a much more smoother surface compared to the cancers tissue cancer tissue would be much more cores.

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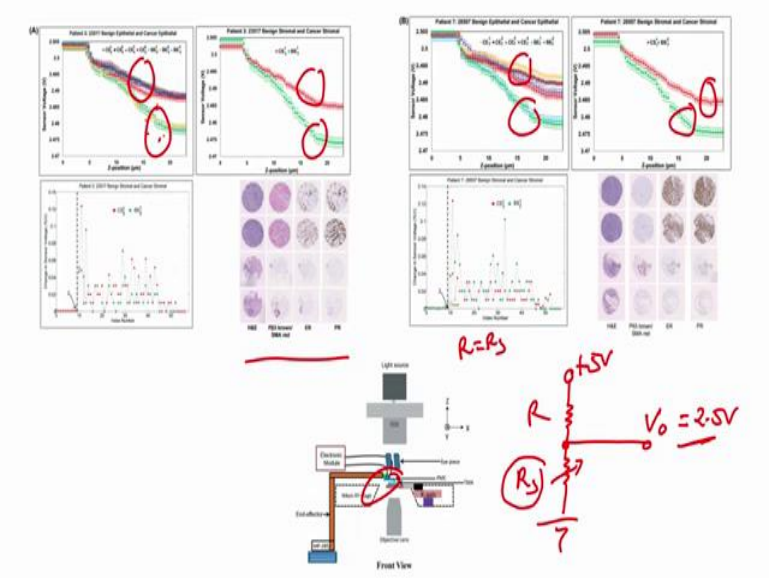

So, when we indent the tissue there is a change in resistance and if I just connect this resistor; this is a piezoresistor right. So, if I just connect the resistor in a potential divider circuit, what will I have?

If I apply 5 volts; if this is the R S right sensor and this is the resistor which is a fix resistor; here when R equals to R S, my output voltage V o would be 2.5 volts correct. Now, if my resistance of the piezoresistor this is R S is piezoresistor when the resistor piezoresistor changes depending on the elasticity of the tissue; I will have a change in voltage and that is why you can see here the sensor voltage changes for that a type of tissue here we can very clearly delineate the benign tissues which are this group of tissues compared to the cancerous tissues which are this group of tissues.

So, we can say that benign epithelial has a different elasticity compared to the cancer epithelial. Same way we can see that the benign stromal has a different value compared to cancer stromal. Here again if you compare another patient we just taken two example;

so we can understand you can see that the benign would have a different value compare to the cancer epithelial and here the benign stromal will have a different value than cancer stromal.

So, the point is that when I indent this tissue; I can clearly delineate the elasticity of the tissue based on the plot and based on the data that we obtained ah. Here is the such engine sensor voltage as you keep on indenting or inch when you go further and you can listen the change in the voltage. Also these are the ISC studies which is immunohistochemistry and you can see here HNEP 63 brown SMA red estrogen and progesterone markers ok.

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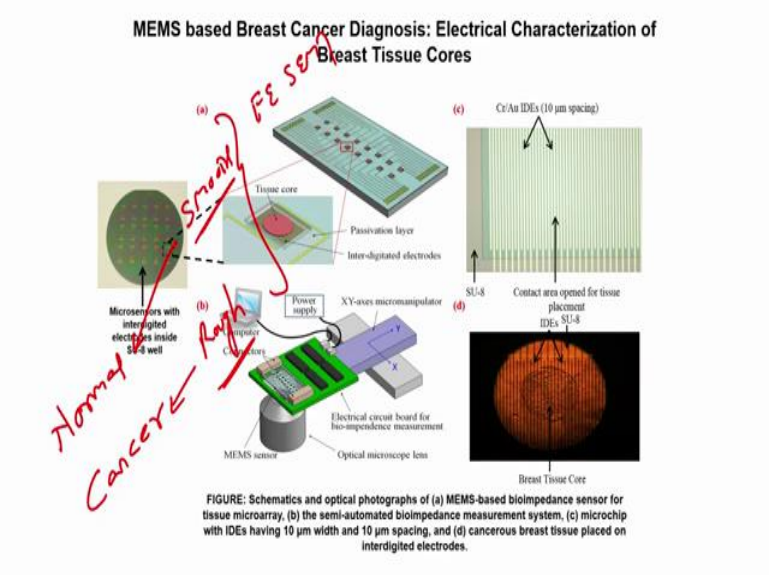

So, moving forward once we know, the, know that piezoresistive microcantilever can help us to understand the elasticity of the tissue we want to understand the electrical property of tissue. Now, why we want to understand electrical property? Because what we found like I said that in FE SEM; the normal tissues where having a different surface properties compared to the cancerous tissues or in another case the normal tissue another way normal tissues has much more cores are compared to cancerous tissue or sorry its opposite; the normal tissues as much more rougher compared to the cancerous tissues; this is also an correct statement.

One more statement for you normal tissues at much more smooth alright smooth compared to cancerous tissues. So, normal tissues are smooth and cancerous tissues are

rough. When you see this in the FE SEM, then what you understand? One is of course, smooth and rough and elasticity, but smooth and rough will also contribute to smooth and rough we are talking about the surface of normal tissue and surface of cancerous tissue.

So, the smoothness or the roughness of the tissue will also contribute to the resistance and that is why it is interesting to study; what is a change in the electrical properties of tissue as the cancer progresses. So, now for this we have fabricated micro you know interdigitated electrodes.

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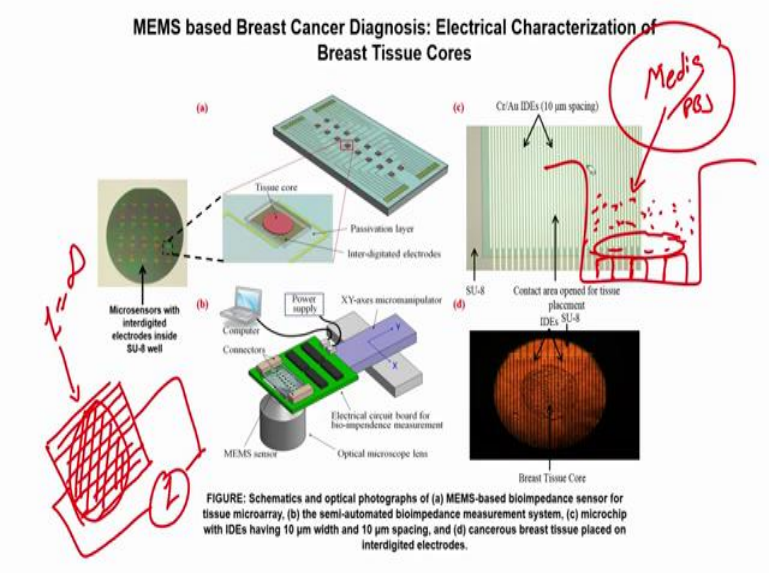

And interdigited electrodes is electrodes which are in this particular format which you can see on the screen right. These are all interdigited electrodes and as we can see they are not touching each other right. So, I can measure my electrical property from this particular set of electrodes; since they are not touching right now my Z should be infinite right; in ideally it should be infinite.

So, if I place a tissue on this particular interdigitated electrodes, then what will happen? There should be change in the impedance value or resistance value why we are talking about impedance, is that if I place the tissue I have to keep the tissue alive or at least keep the tissue properties intact. And that is why we will create a well in which this there will be interdigited electrodes and on that there is a tissue in which there is a saline

solution or a media that will keep the tissue properties intact right media or saline. So, that issue will not dry up and then we are measuring impedance.

Now, this media and PBS media or PBS will also contribute to the other properties; that means, it will contribute to the double layer capacitance and other properties; it is not as straight as resistance of the tissue. Now the new term that will come into picture would be impedance of the tissue. And that is why we will measure the impedance of the tissue that if you see the paper; there is a modelling in the paper in this particular paper which is in sensor as and actuators B.

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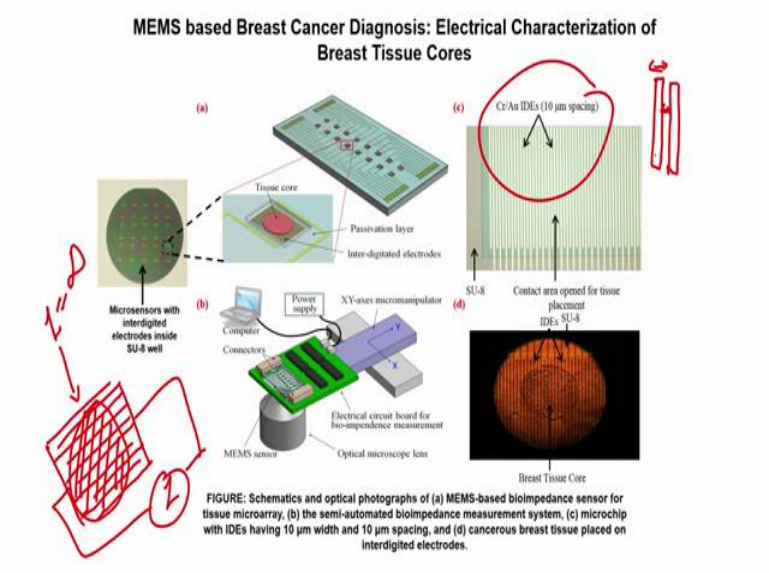

So, if I please and you can see here in this schematic; the interdigited electrodes each are having 10 micron width this is a width. And distance between two electrodes which is this one is also 10 microns; 10 micron spacing, 10 microns width and then on this when you place the tissue this you can see here this is a SU 8 well is made up of SU 8 material so that you can hold the saline solution or media inside the well.

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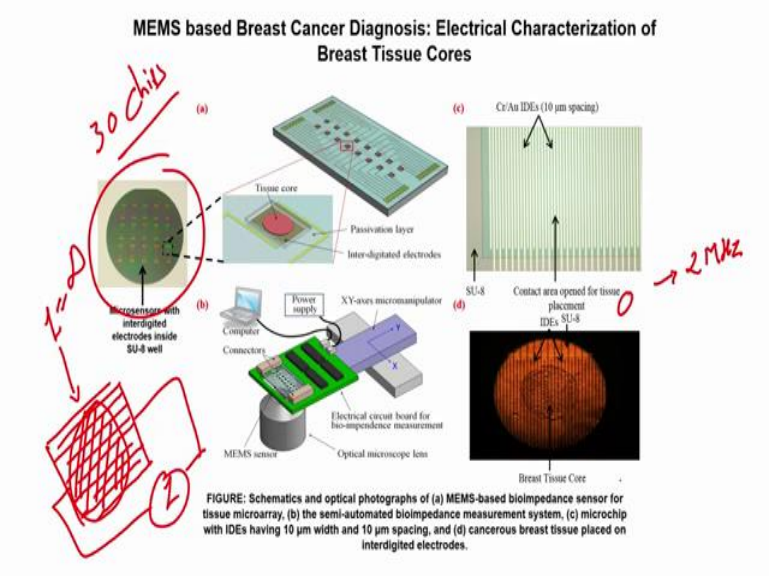

Now, this is a complete 4 inch wafer; where we can see many chips, in fact, you can see 30 chips where you can perform the experiments. So, you have to perform the frequency sweep from 0 which is DC voltage to let say 2 megahertz and you will find out the change in the impedance as well as the phase of; if I place normal tissue and then cancerous tissue.

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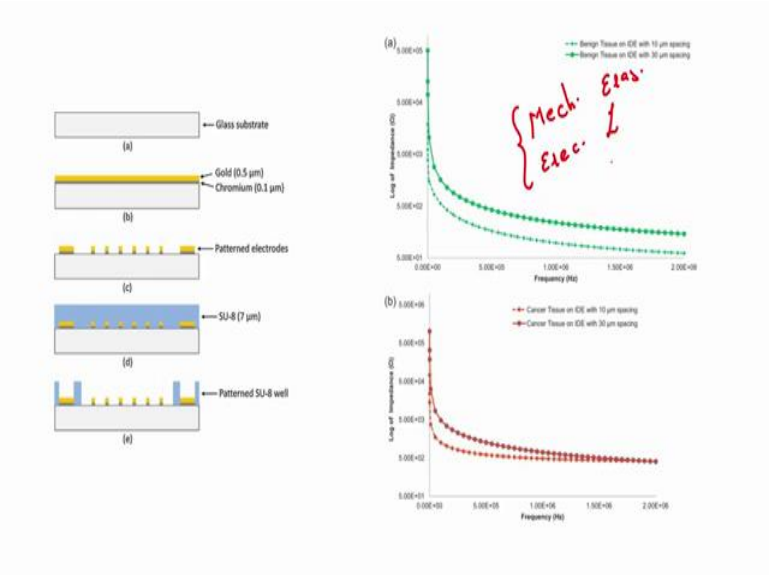

So, we can see here there is a change in the impedance and for normal tissues compared to when you look at the cancerous tissues, we also compared that if you have 10 micron spacing or 30 micron spacing; what is the change.

But what you need to focus here is that the y axis. So, if you see the y axis what you will find out in both the cases there is benign and cancer that the impedance for benign is different than impedance of the cancerous tissues alright; we can here very clearly see that.

So, the point is can I use electrical properties to delineate normal and cancer like I was using in earlier cases mechanical properties which are elasticity. So, now we can in a way we can understand that the mechanical properties which is the elasticity of the tissue and the electrical properties which are the impedance of the tissue can be used for delineating whereas, distinguishing the normal or benign and cancerous tissues.

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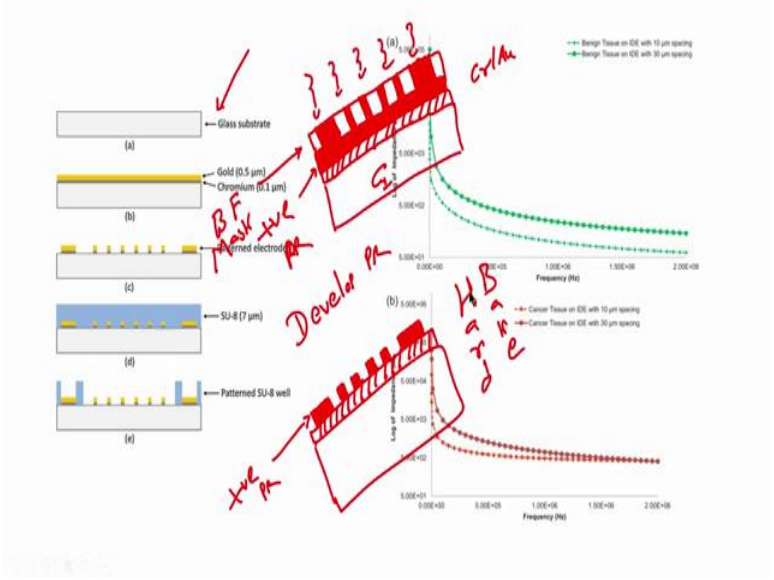

See, if I want to understand how can I fabricate such kind of electrodes? It is very easy; you take a glass substrate, then you deposit a gold film. Now, I told you earlier also whenever you want to deposit gold film you have to have the chromium as a base material because chromium will help in a better addition of gold.

So, I have chrome gold; after chrome gold I can patterned this electrodes with a very simple process called photolithography. So, I have a glass, I have here chrome gold let us

say this is chrome gold, this is my glass on this I will spin coat photoresist right. I will spin coat photoresist assuming that let say it is a positive photoresist; positive photoresist PR; then next type would be soft bake right.

Soft bake 90 degree centigrade; 1 minute hotplate followed by loading a mask; followed by loading a mask. So, my mask would have this particular pattern and this will be my bright field mask. So, this is my bright field mask; after this mask, I will expose this wafer with UV lithography or UV rays followed by developing photoresist. Once I develop the photoresist; what will I have? I will have my glass wafer with my chrome gold and photoresist left in the area which was not exposed by UV; since my photoresist is a positive photoresist.

So, the characteristics of the positive photoresist is that the area which is not exposed will be stronger; area which is not which not exposed will be stronger. So, this is my positive photoresist alright. After this I will go for hard bake; hard bake is (Refer Time: 21:56) 120 degree centigrade for 1 minute on hot plate.

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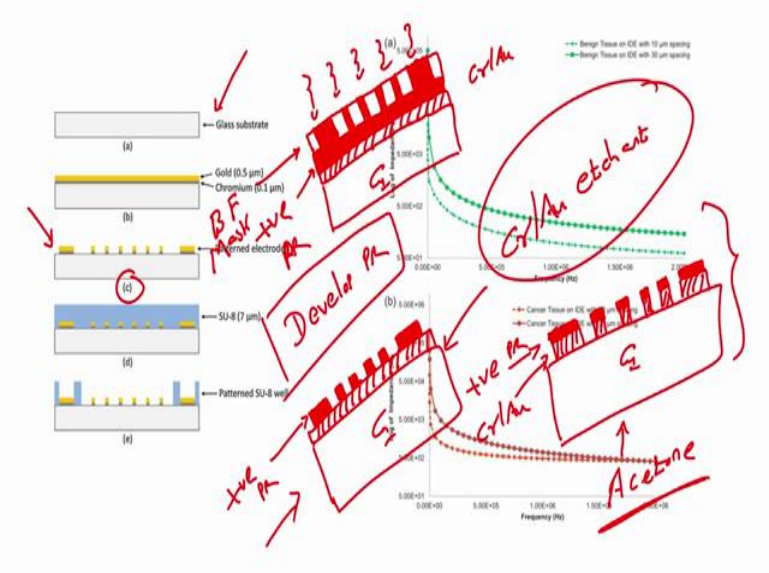

Followed by followed by dipping this wafer in chrome gold etchant. See if I dip this wafer in chrome gold etchant, then what will happen? I will have my glass wafer and my chrome gold protected by the photoresist like this is not it? It is very easy right.

Steps process flow; once you understand process flow it becomes very very easy to fabricate any sensor ok. So, what we have? We have positive photoresist, we have chrome gold and we have glass correct. After this I will dip this wafer in acetone; if I dip this wafer in acetone. What will happened? Acetone will strip of the photoresist and I would have this particular pattern sorry this is pattern num in C.

Again let us quickly see once again what we are discussed; we have glass wafer then we have chrome gold after I after we have chrome gold we have a positive photoresist spin coated on chrome gold followed by a soft bake followed by loading a mask exposing the wafer.

Since the positive photoresist and bright field mask are there whatever the pattern is there on the mask will be patterned on the wafer that we already know. Another way is that the area which is not exposed will get stronger and that is why after the performing the (Refer Time: 24:06) lithography; if I develop this photoresist or after performing the UV exposure, if I develop this wafer develop the photoresist develop the photoresist in there in a photoresist developer what will I have?.

I will have this particular wafer by the photoresist in the area which was not exposed by UV will get stronger and a photoresist whether where it was exposed by UV will get weaker followed by I will put this dot dip this wafer in a chrome gold etchant. When I dip this wafer in chrome gold etchant the chrome and gold will get etch and the photoresist will be still there.

Which now what I will do is I will dip this wafer in acetone is a photoresist stripper and when it is strips a photoresist; then what will happen? We will have this particular pattern which is shown in shown in C. Now, after that what we want? We want this interdigitated electrodes to be inside a SU 8 well; is not it?

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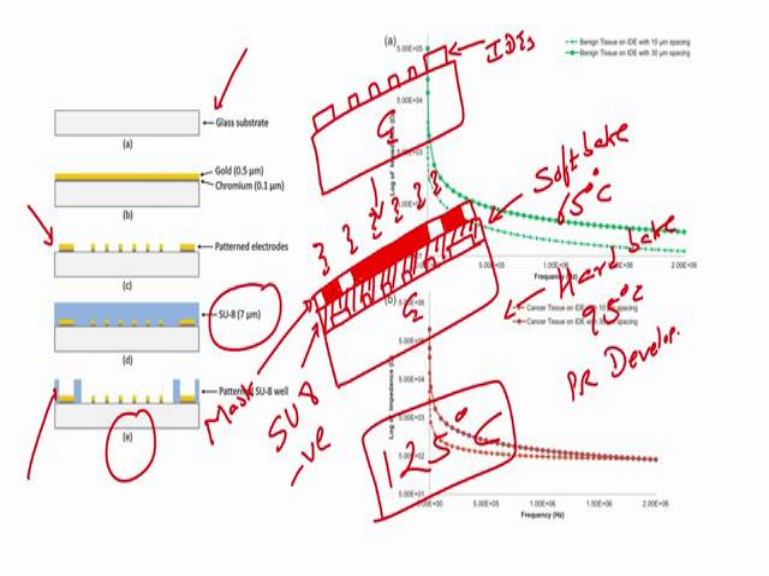

So, what we will do is now after this; I will take this wafer which is my which is shown in schematic C; I will take this wafer like this alright. And on this we will we will spin coat SU 8; we will spin coat SU 8. So, if I spin coat SU 8 right; this is SU 8 on the interdigitated electrodes, then I will perform soft bake. Now, in case of SU 8, the soft bake is done at 65 degree centigrade and the time depends on the thickness of the SU 8 material alright. If the SU 8 is thicker the time is more SU 8 is thinner time less.

So, after soft bake I will perform exposure and exposure; that means, we have a mask; we have a mask and SU 8 will act as a negative photoresist; that means, the area which is not exposed will get weaker; area which is not exposed will get weaker. So, I have this area; I will have this area correct. So, what I will do is; I will have a mask this is my mask in the area which is not exposed; this one, this one and this one you getting it? Now, if I use such a mask what will happen? SU 8 is a negative photoresist ok.

So, if I use mask like this and then expose the wafer; then expose the wafer followed by hard bake. In this case after exposing; you have to go for hard bake not like other kind of photoresist and hard bake is done at 95 degree centigrade.

Again the time depends on the thickness of photoresist followed by photoresist developer. There is a difference between SU 8 photoresist and other photoresist. Then in other photoresist we go for soft bake at 90 degree followed by loading the mask exposure developer hard bake. But in this case we are going for soft bake then exposure then hard

bake than photoresist developer and when you develop the photoresist because the area which is not expose SU 8 is negative photoresist.

So, area which is not exposed with UV light will get weaker and that is why after developing with photo photoresist developer the pattern would look like schematic number e right; the you can see here the area which was not exposure photoresist can got developed. And then if you want to make the material harder which is photoresist you want to make it harder you can further bake it at 125 degree centigrade for sometime right.

Again depend on depending on the type of the photoresist depending on the type of the thickness of the SU 8 that you are using. Once you do that what you have is that you have your interdigitated electrodes inside the SU 8 well. And now you are chip is ready for testing the electrical properties of the cancerous and the normal tissues. Now, we are talking about breast cancer, but you can talk about any tissue related cancer in this particular case.

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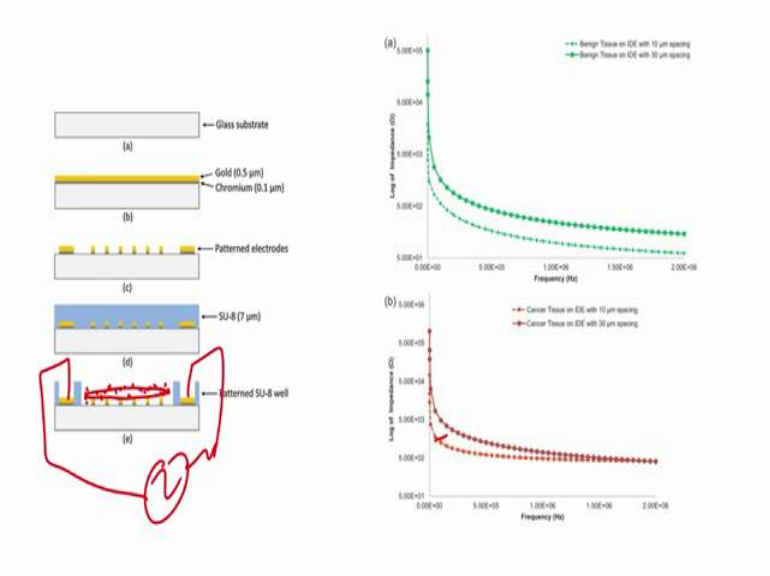

If I load the tissue on this right; if I load with some saline solution or media I can measure the impedance of this particular tissue right across the terminals right. So, this is how you are measuring the or this is how we can measuring measure the electrical properties of the tissues.

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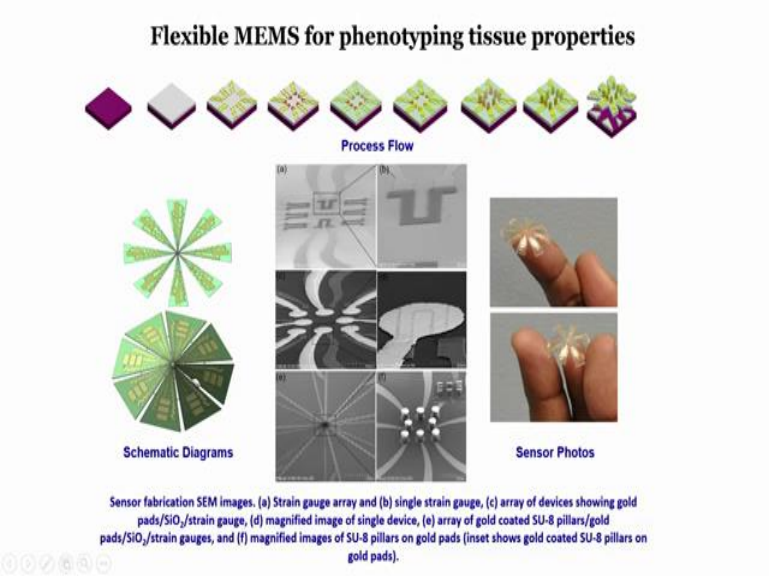

So, since we have learn electrical properties and we have learn the mechanical properties of tissue; the next step would be to understand the electro mechanical properties of the tissue.

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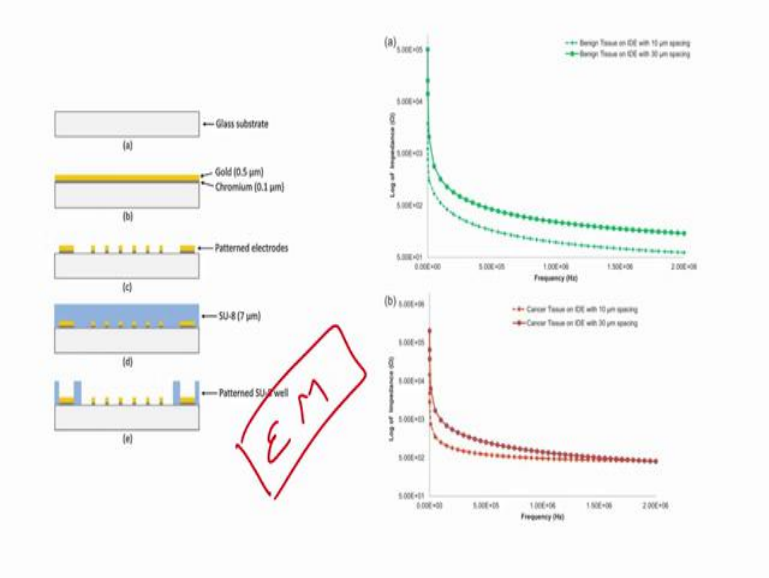

Now, we have done individual mechanical properties which is the stiffness and we have also try to understand the electrical properties which is the impedance; how about we see both the parameters together there is electrical and mechanical parameters together.

So, that we will see in the next module; for this module just understand this much things. In the next module, what we will discuss is how can you design flexible sensor that can perform both electrical and mechanical studies together or phenotyping of tissues electrical and mechanical simultaneously right.

So, till then you have any questions please ask me, ask my teaching assistants. There is a forum right you have to solve questions by yourself; you cannot not ask the solutions of the question of the assignment. The forum is to ask doubts if you have any; do not ask for the new ideas this is doubts from the topics that I am teaching you alright.

You can free you are free to ask anything from the from the course content. And next module let us see how can we fabricate a sensor which is flexible in nature and we can also measure both the properties electrical and mechanical simultaneously; till then you take care have a nice day bye.