Sensors and Actuators
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Lecture – 58
Biosensors for ETM Phenotyping of breast cancer tissues for better prognosis

In the last module, the electrical and mechanical properties of tissue, how can we fabricate a flexible sensor with a PEDOT-PSS as a strain gauge and then insulating material and gold electrode over with there were SU-8 pillars were discussed. In this particular module, I want to emphasize and stress more on the chip for ETM properties E stands for electrical, T stands for thermal, M stands for mechanical properties.

Before we dive into this particular fabrication process you should have a very clear idea about the breast anatomy and also the breast cancer stages alright. So, I have got you two quick videos to look at and then we continue the class.

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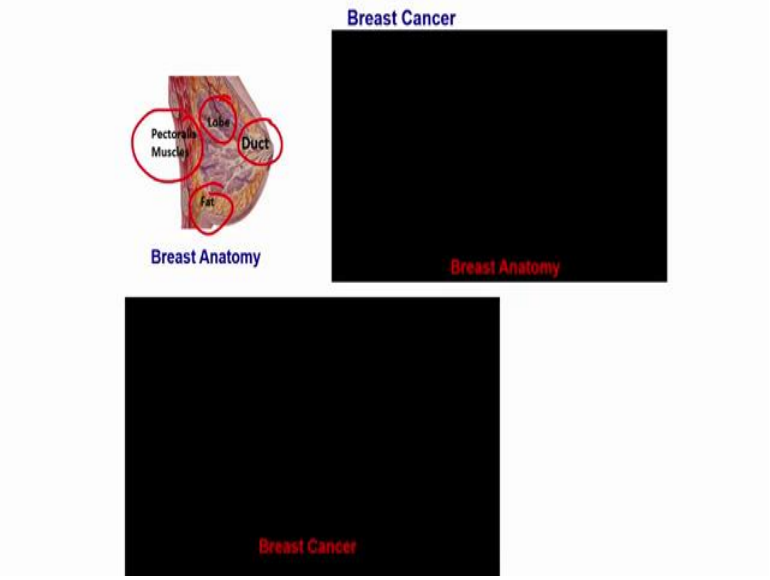

So, if you see the slide the breast generally not generally breast anatomy is such that it consists of ducts right, it consists of lobes you see lobes duct right and then there is a pictorial muscle and then there is fat alright. So, duct, lobe, fat and pectoral muscles. Now, if cancer occurs in the duct is called ductal cancer; it occurs in lobe – lobular cancer right it is both duct and lobe then mixed tumor cancer; if there is inflammation – inflammatory cancer; if there is a mucus – mucinous cancer.

So, several kinds of cancer are there and the very important is triple-negative breast cancer where all the markers are absent. So, this particular chip would probably help the oncopathologies to come up with a better diagnosis because we are adding three extra modalities or three additional modalities to the existing modality if it is a biomarker. If you see the video I am playing the first breast anatomy video.

Breast cancer is the most common form of cancer in woman today. Knowing more about your arm breast anatomy. It can help you early cancer detection and prevention. Your breasts are connected to small masses of tissue called lymph nodes by way of lymph vessels. The lymph nodes are responsible for collecting bacteria, cancer cells and other unhealthy material. You have groups of these lymph nodes under your arms above your collarbones and behind your breastbone as well as in other parts of your body.

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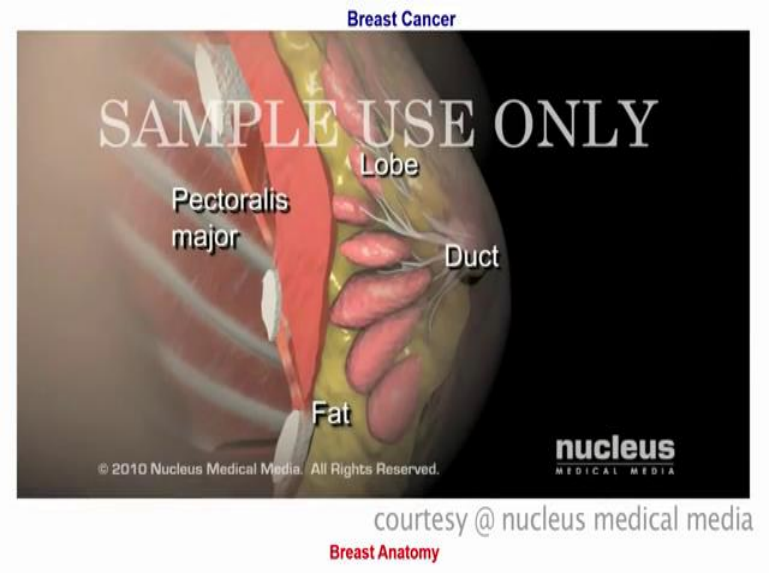

Each breast is made up of lobes, lobules and ducts. The lobes consist of smaller lobules that contain groups of tiny milk-producing glands. When a breast is producing milk it passes through the ducts into the nipple where it exits the body. Breast cancer most commonly develops in the lobules, glands and ducts of the breast.

So in this, you have seen in detail what I was talking on the slide about the anatomy of the breast. Now, let us see how the breast cancers different stages are there and let me play it.

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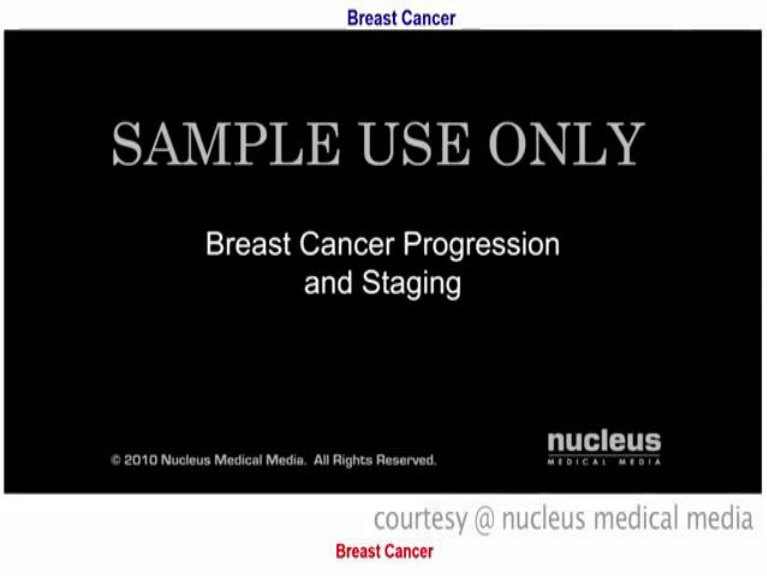

The staging of breast cancer refers to the extent of the disease. The cancer stage is based on several factors including the size of the tumor if any lymph nodes are involved, if the cancer is invasive or non-invasive and if cancer has spread to areas beyond the breast.

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Stage 0 is considered non-invasive breast cancer. In this stage, there is no evidence that the cancer cells have spread into neighbouring breast tissue beyond the duct or lobule.

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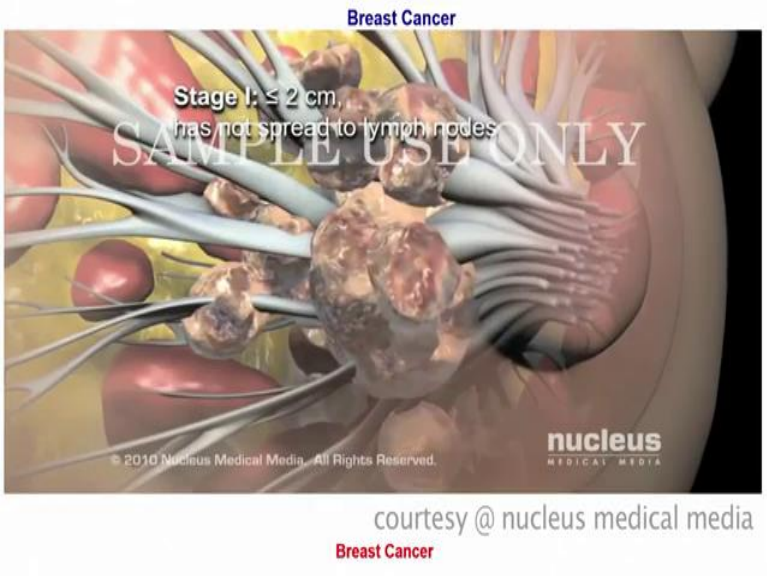

Stage I is considered an early stage of invasive breast cancer.

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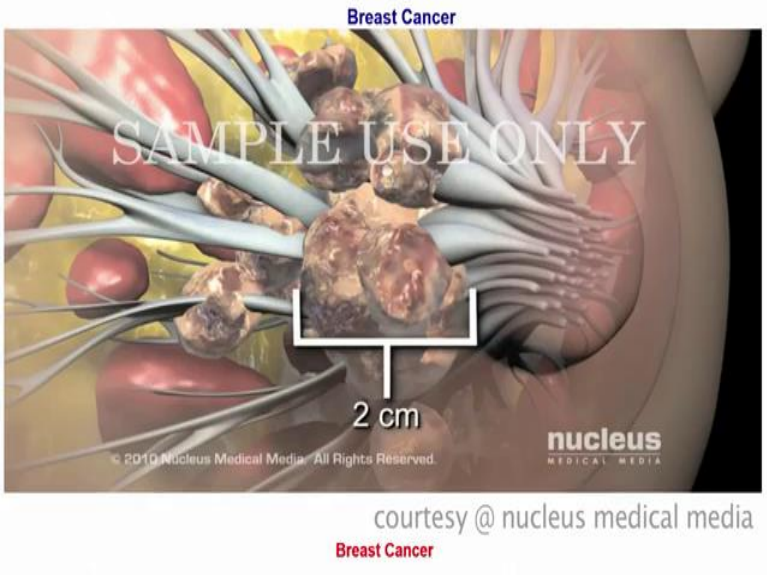

When measured the tumor is no more than 2 centimetres in diameter and there is no evidence that the cancer cells have spread beyond the breast.

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Stage II is divided into subcategories of IIA and IIB; stage IIA is invasive breast cancer where the tumor is either a maximum of 2 centimeters in diameter and has spread to the lymph nodes under the arm.

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Or the tumor is between 2 and 5 centimeters in diameter but has not spread to any lymph nodes. Stage IIB is a little different and that the tumor is either between 2 and 5 centimeters and has spread to underarm lymph nodes.

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Or the tumor is larger than 5 centimeters, but has not spread to the underarm lymph nodes.

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Stage III is considered a locally advanced cancer and it is also divided into subcategories of IIIA, IIIB and IIIC.

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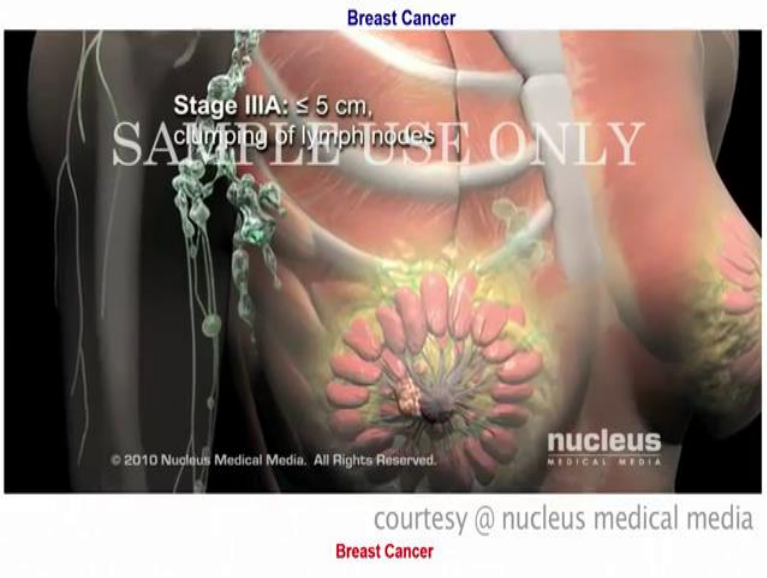

There are two main scenarios that can occur with stage IIIA breast cancer. One, where the tumor is no larger than 5 centimeters in diameter, but it has spread to underarm lymph nodes that are growing into each other forming clumps. Cancer may also have spread to the lymph nodes near the breastbone.

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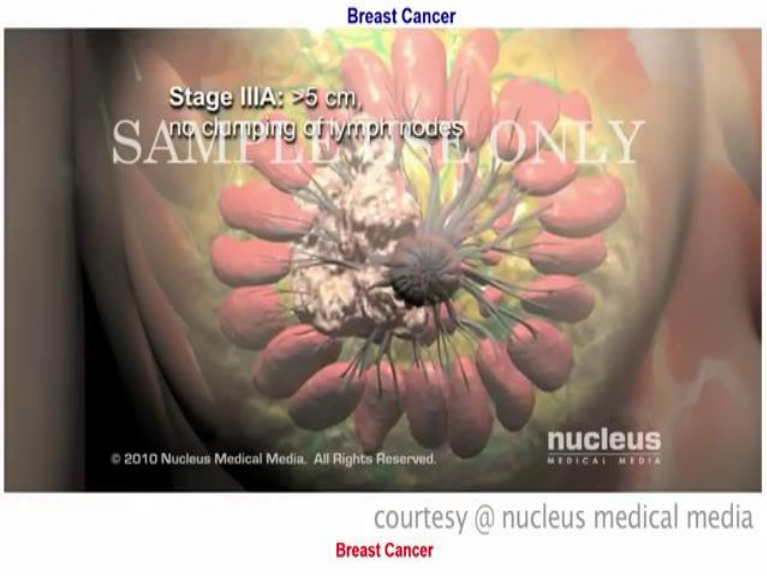

The second scenario for stage IIIA is very similar with the exception that the tumor is larger than 5 centimeters in diameter and the underarm lymph nodes do not adhere to one another or other tissues.

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Unlike the other stages, in stage IIIB the tumor may be any size and has spread into the skin of the breast or chest wall. This stage may also include lumps on the skin of the breast or swelling of the breast.

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In stage IIIC, the tumor may also be of any size, but it is also have spread to lymph node areas above or below the clavicle, the chest wall and or the skin of the breast.

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Stage IV is considered distant metastatic cancer meaning cancer has spread to other organs and parts of the body.

So, what you saw you saw that there are several stages of breast cancers – stage 0, I, II, III, IV. Earlier you diagnose any disease you can have a cure for the disease right. So, early diagnosis, early screening is an important parameter in this field right. Earlier you screen, better; earlier diagnose, better. Unfortunately because of the lack of awareness generally when the women know that there is some problem and they go to the hospital it is already stage-III or stage II alright.

The reason is like I said lack of awareness. It is very important that we start awareness because we are now we are working on this research problem that a woman has to go for mammography which is a screening test for breast cancer. Age-wise there is a requirement if it is less than 40 once in a year about 40 twice in a year or vice versa you can just quickly see it, but the point is it is very important to go for mammography alright.

So, let us see the statistics and then you will understand why I am saying it. It is extremely important to screen and regularly test once in a year is not a big deal alright and a lot of government facilities gives it for free alright or very nominal price of like 80 rupees or 100 rupees. So, if it can save a life, it is worth spending 1000s, but at the same time, a lot of people cannot afford.

So, how about 100 right? So, if you see the government initiatives there are very good initiatives for healthcare in particular and if we are aware we can take help of those initiatives including I recently came to know that particularly in Karnataka state there is Government initiative where the Government will give about 1.2 lakh or 1.3 lakh something around that value for heart operation it is really important and good initiative alright.

So, but the point is we should be aware right if we are aware we can use initiatives ok. So, this I am talking a little bit slightly deviating the topic of sensor actuators because you had to learn sensor actuators when you have a disease, but why to have the disease, right why to not stop this thing. So, if you cannot stop at least why do not you screen it at an early stage? Right. So, that is our reason of I was talking about.

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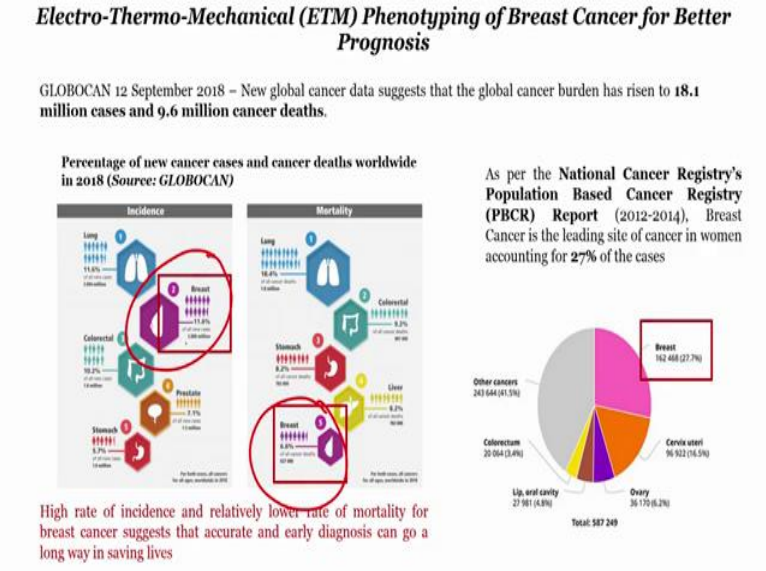

So, if you see the slide you will understand they we are talking about phenotyping of breast cancer not genotyping of breast cancer and according to GLOBOCAN the in September 2018, the cancer burden has raison to 18.1 million cases and close to 9.6 million cancer deaths. It is a huge value.

And you can see here that the incidence versus mortality the lung cancer leads to quite an extent you can see here incidence of lung cancer right 11.6 percents of all new cases about 2.094 million cases; where if you talk about breast cancer again close to 2.089 million cases; collateral colorectal I am sorry cancer which is about 10.2 percent of 1.8

million cases, followed by prostate cancer about 1.3 million cases and stomach cancer about 1 million cases.

When you look at the mortality ratio you will see that lung cancer 18.4 percent mortality; that means, 1.8 million deaths; when talk about colorectal you can see about 88 881000 patients die. Stomach cancer about 783000, liver 782000 while breast 627000 and this is for both sexes and all cancer for all ages worldwide. But, if you see the overall burden is huge alright. So, what we can see is that breast cancer is a leading site of cancer in women accounting for 27 percent of the cases and that is why a very important problem to address and to find a solution.

Now, the high rate of the incident and a relatively lower rate of mortality for a breast cancer suggested accurate and early diagnosis can go a long way in saving a life. You can see in terms of incident these are number 2, but in terms of mortality, it is a number 5. So, if we cannot get a better diagnosis better screening better sensors right then we can still reduce this mortality factor ok.

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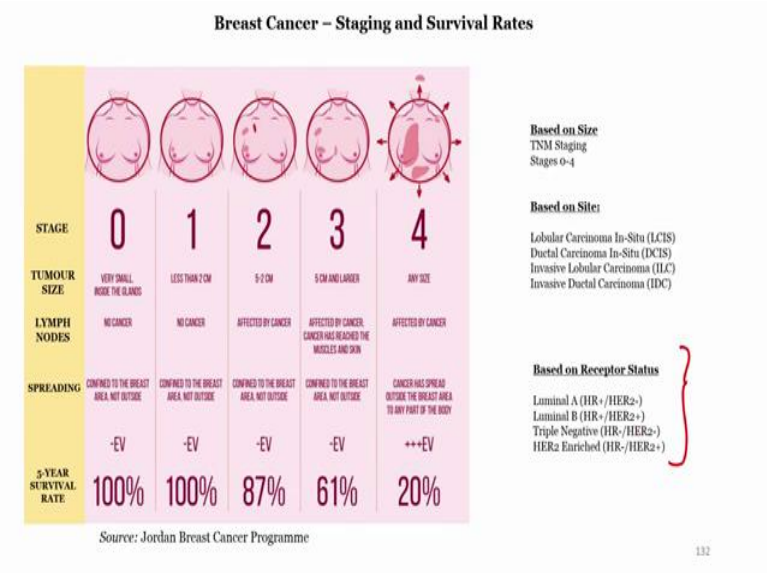

So, as you have seen in the video what we see is that the stages when you say stage 0 the tumor size is very small and inside the glands right; in lymph nodes there is nothing; spreading it is not spread and survival rate is about 500 is about 100 percent for 5 years survival rate. For 2 centimeter, stage I, again lymph node there is nothing and confined

to the breast area not outside which is a good point and 100 percent is a 5 years survival ratio. So, this two is where we should identify a patient alright.

However, as you progress further where it is stage II and the tumor size is about 5 to 2 to 5 centimeter and now the lymph nodes are affected, you can see here the representative image the lymph nodes are affected and then further this is confined to the press not outside. So, still it can be cured and you can see that survival rate is 87 percent, but as you go to stage III and IV the difficulty or complexity increases.

Stage III is 5 centimeter and larger stage IV any size; stage III affected by cancer – cancer has reached to the muscles and skin you can see here, you can see the lymph nodes right where in this case can be anywhere including the tumor in brain; confined to the breast area not outside, but here it can be of any part of the body and this is about 20 percent only, this is also 61 percent only. What I prefer is how about we try to make a system that can have these things at least we can cover stage II alright.

So, based on tumor size stage 0 to IV based under sites then you can say that it is a lobular carcinoma in-situ, ductal carcinoma in-situ, invasive lobular carcinoma and invasive ductal carcinoma. Based on the receptors status you can divide into four states HER2 plus-minus luminal A, luminal B, triple-negative HR minus HER2 minus and HER2 enriched HR minus and HER2 plus. So, these are based on receptor status.

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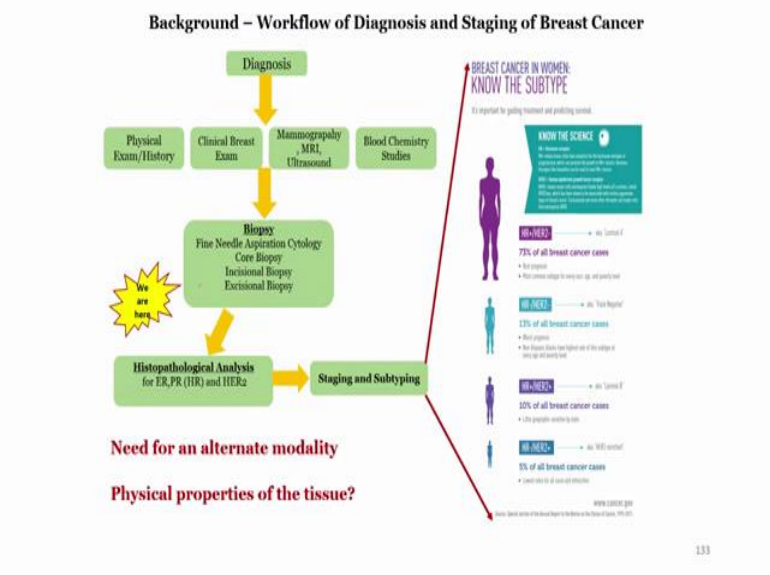

If I see the background or workflow of diagnosis and staging of breast cancer then you can see here that once it is diagnosed there can be many ways of diagnosing cancer. One is physical exam or history; second is a clinical breast exam which is called CBE; third is mammography, MRI, ultrasound; the fourth one is genotyping which is blood chemistry studies. We are talking about phenotyping.

So, we continue with a biopsy. If the biopsy is done then the cells are taken to study which is called cytology; so, cytology, then core biopsy, incisional biopsy and excisional biopsy. If further divided then there is a histopathological analysis where the all the biomarkers that I was talking about ER estrogen, PR progesterone, HER2 plus are studied and then based on that the staging and subtyping are there. So, this is the process or workflow for diagnosing on the staging of breast cancer.

So, 73 percent of all breast cancer is HR plus 2 HER minus HER2 minus where 13 percent which is also called triple-negative breast cancer very very dangerous HR minus and HER2 minus while 10 percent and 5 percents are for HR plus 2, HER2 plus and HR minus HER2 plus.

So, now, the point is we require an alternative modality and we need to study the physical properties of tissues. How about we study the physical properties of the tissue as an alternative modality? So, that is why we were somewhere in this particular domain biopsy domain alright.

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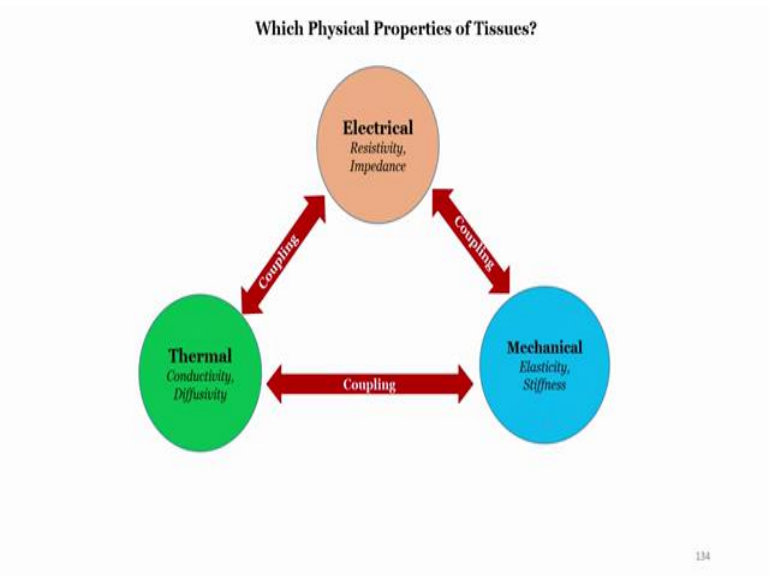

So, now why which physical properties of tissues we have to study? We can study the electrical properties which are resistive and impedance measurements; we can study the conductivity and diffusivity which is thermal conductivity; we can also study the mechanical properties like elasticity and stiffness.

If we couple all these parameters along with biomarkers can we bring the false positive and false negative signals down and can we also have a novel or alternative or additional modality is to reduce them or to not only reduce the false positive and false negative but also to diagnose triple-negative breast cancer. So, there is a bigger picture of this particular research.

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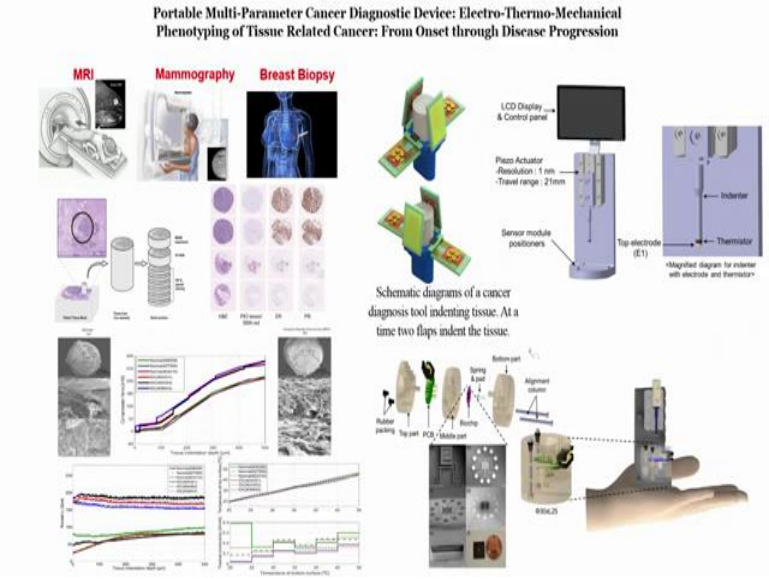

If you again see the screen you will find that this is how the MRI is done right. Most of you have seen there is a separate lecture is required for making understand how MRI operates. For now, you just understand that MRI and mammography both are used for screening the patient. If there is a suspicion region then the patient is asked for a biopsy. A breast biopsy is done with the help of a needle and the tissue is taken out like you can see here and tissue is sliced further. When you slice tissue further with microtome then you can get different biomarkers.

So, here you can see H and E staining, P63 brown SMA, red estrogen and progesterone which is PR, ER, SP 63 SMA and H and E ok. Now, what we are planning is can we measure the electrical mechanical and thermal properties of this tissue and for that what

we can do? So, now, you take this particular example this very very important and then we will see that how can we fabricate this particular tissue right this particular chip and place it for fabricating for understanding the issue property.

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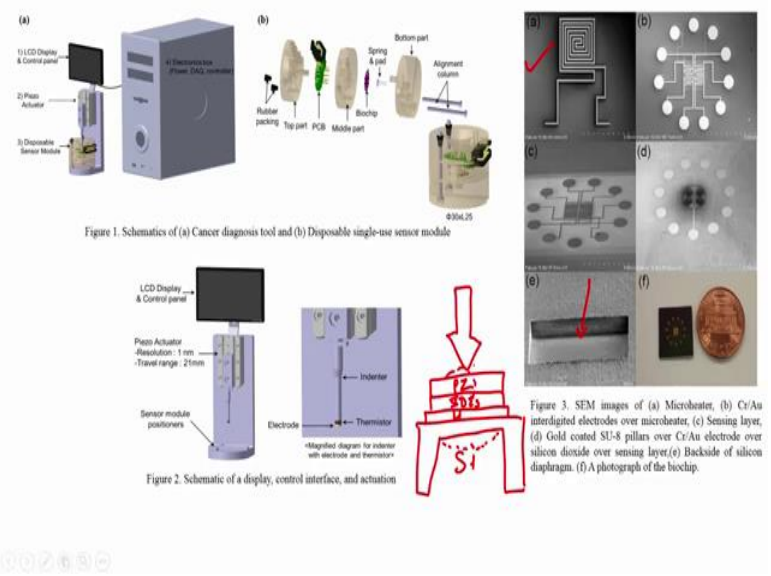

So, if you see this chip let me see the next slide, yes. Now, this is a chip that we have to fabricate alright. What is this chip consists of? This chip consist of a heater which you can see here right and then on that heater there is an insulating material. Now, you all know how can you fabricate heater it is very easy right you deposit a metal, then spin code photoresist, soft bake, expose it with a mask, developing the photoresist, then hard bake, then etch the metal, then strip off the photoresist with acetone and you have a heater.

After the heater you have an insulating material; on insulating material, you can have interdigitated electrodes. Once you have interdigitated electrodes again you know it how can you fabricate, you deposit metal and use lithography technique to form interdigitated electrodes. On these interdigitated electrodes you can deposit the sensing material and this sensing material is your piezoresistive material. Now, if you have a piezoresistive material; that means, say I will just use a block diagram to make it easier.

So, there is a heater at the bottom which is I will call H; on that there is let us say interdigitated the digital electrodes which I call IDEs on which you have the sensing material so, I will say piezoresistor; so, PZ alright. Now, if I apply force, then the resistor

piezoresistor which are this particular four blocks. This should show me the change in the resistance, but it will only show the change in resistance when my silicon will bend, but silicon is hard.

So, what can I do that is why I can now create a diaphragm and this is what a diaphragm you can see here. The diaphragm is created, so that when I apply a force then this diaphragm will bend and when it bends there is a strain in the piezoresistor and thus there is a change in the resistance. So, this is the point. Now, if I on the piezoresistor again so, let me again show it to you what exactly this block is so that you understand very clearly.

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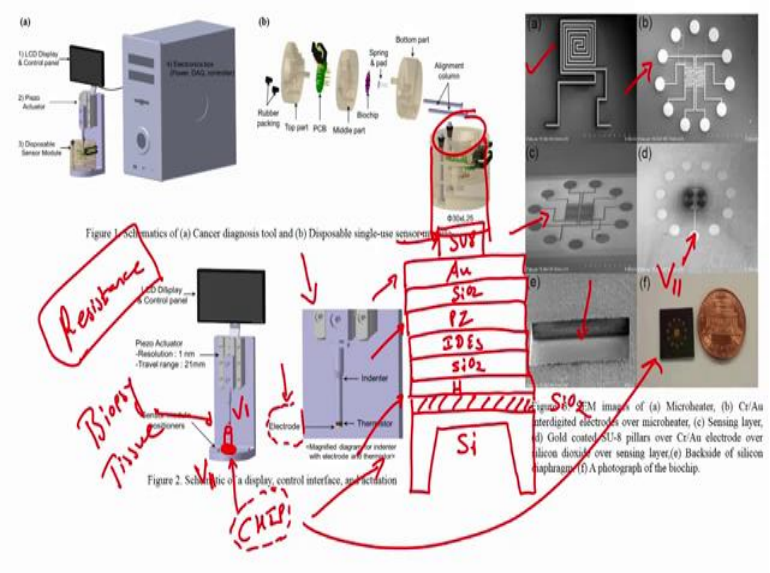

I am just showing a block diagram it becomes easier that way. So, silicon and of course, you have oxide oxidize silicon wafer. So, we will have SiO_2 right SiO_2, on that you have your heater. So, I will write H or heater on that you have again silicon insulate dioxide than on that you have interdigitated electrodes is IDEs, on IDEs you have piezo resistor so, I have PZ here on that you have on piezo resistor you again have an insulating material which is SiO_2; on that you have gold pad which is Au; on gold pad you have a SU8 pillars. So, SU8 pillars I will just draw like this representative SU8 right.

So, this is the structure of this chip. What is at the bottom? There is a heater which you can see here then there is an insulator and then there are interdigitated electrodes which you can see in this particular diagram. On interdigitated electrodes, there is a piezo

resistor which you can see in this diagram right. On that, there is silicon dioxide over which there are gold pads you can which you can see here and then over the gold pad, there are SU8 pillars which also you can see over here alright. So, this is the chip.

And, on the backside of this silicon what you have? You have etching which is your diaphragm right. So, this is the chip. Now, if I place a tissue on this chip; that means, I have tissue and this tissue is placed on this chip like this alright and if I place this chip here alright, in between what is there? There is tissue like this ok. This is my chip, which chip? This one aright or you can say this one.

Now, if I apply a force through my indenter this is my indenter alright this is my indenter. If I apply a force through indenter what will happen if I apply a force on this tissue this is my biopsy tissue. So, if I apply a force then my piezoresistor on the chip will show a change in resistance right my piezoresistor on this chip will show a change in resistance depending on how much force it can observes right or it is translated through the tissue.

There is F_1 force that I apply through indenter and my chip will get F_2 force this F_2 will depend on the elasticity of the tissue or this stiffness of the tissue alright. So, if I know the difference of the force then I can measure the elasticity or the stiffness of tissue, got it? This force change I can measure with the help of a piezo resistor.

Now, let us see the second property. This remains as it is alright. Now, you see if you see the indenter which in this figure in this figure if you see the indenter there is a electrode and the mod tip of the indenter you can see electrode that tip of the indenter; that means, if I apply voltage here which is let us say voltage one potential of the voltage is here and on the chip there are there is a gold pad with SU8 pillars right gold pad with SU8 pillars.

So, if I apply a voltage here as another pad of this potential then what will happen if I apply voltage between V_1 and V_{11}, then depending on the resistance of the tissue my current will change right if I apply potential difference that is voltage between two pairs V_1 and V_{11} it is like two electrodes ok. So, electrode 1 and electrode 11; electrode 11 is on the chip, electrode 1 is on the indenter this one right.

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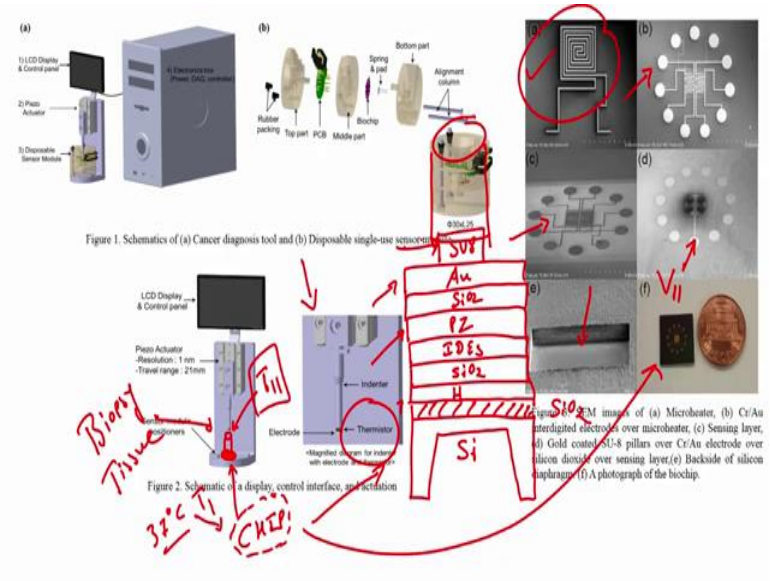

So, if I apply a voltage between those two electrodes and if there is a tissue in between then I can see the change in current and that change in current will be because of the resistance of the tissue. So, if I know the voltage, if I know the current I can measure the resistance of the tissue. So, first, we are measuring the elasticity now we are measuring the resistance.

Let us see the third one. What our chip has? Our chip has a heater is not it a chip has a heater here right. So, if I apply a voltage to the heater it will heat; that means, I can heat my chip let us say at 37-degree centigrade and tissue are placed on this chip. Now, if you see indenter, indenter has thermistor the and this thermistor would be on the top of the tissue here.

So, let us say T_{11} and the chip is at T_1. So, because the chip has a heater intender has a thermistor I can measure what is a change in the temperature if I apply 37-degree centigrade what is a temperature that tip of the tissue which is T_{11} and from that difference I can measure the thermal conductivity of the tissue.

So, now, I can measure elasticity or mechanical properties, I can measure resistance or electrical properties, I can measure the change in temperature or thermal conductivity of the tissue. So, all three properties I can measure with the help of a microfabricated biochip or ETM for measuring the ETM properties of tissue or phenotyping of the tissue. Now, this is for particularly and then where is this tissue kept this tissue is kept in the

casing that you can see here and this casing is with the help of 3D printing material 3D printer alright. So, I will show it to you how 3D printer works in one of the lab class 3D printers alright.

So, if you see here there is a top panel, there is a PCB, there is a middle part, here is our biochip this biochip is this one alright. Then there is a spring and there is a bottom part when you put everything together you have a cone in which you can place the tissue alright. So, this is how it works.

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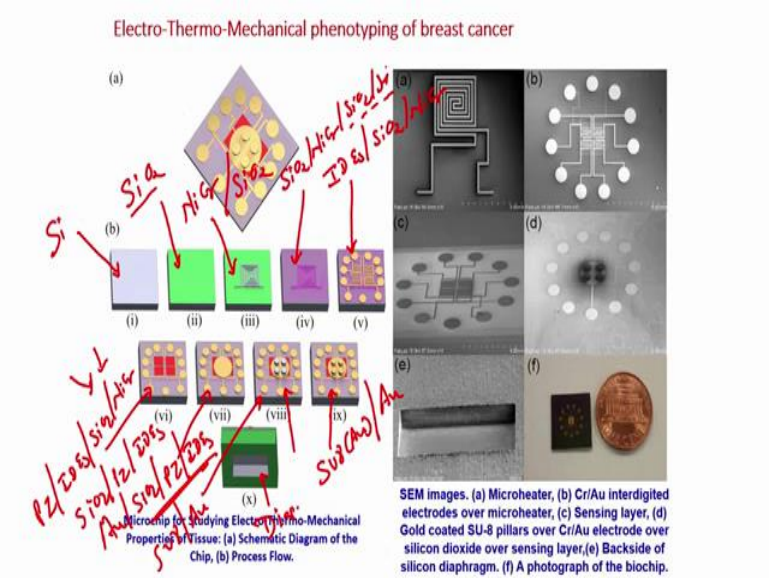

Microchip for Studying Electro-Thermo-Mechanical Properties of Tissue: (a) Schematic Diagram of the Chip, (b) Process Flow.

SEM images. (a) Microheater, (b) Cr/Au interdigited electrodes over microheater, (c) Sensing layer, (d) Gold coated SU-8 pillars over Cr/Au electrode over silicon dioxide over sensing layer,(e) Backside of silicon diaphragm. (f) A photograph of the biochip.

So, let me go to the next slide. Here you can see a process flow quickly. There is a silicon, then you have an oxidized silicon, then you have a microheater, on that you have a silicon dioxide again, on which you have an interdigitated electrodes, after interdigitated electrodes you have a piezoresistor; after piezoresistor you have insulator and over which you have a gold pads, on gold pads you have a SU8 pillar and then you perform the liftoff technique to coat SU8 pillars with gold. Once you do that on the backside you can create a diaphragm alright.

So, I will write down here silicon, silicon dioxide, nichrome heater on silicon dioxide, then silicon dioxide on nichrome right, on silicon dioxide on silicon this you understand this thing you understand I am not writing every time. Then you have interdigitated electrodes on silicon dioxide on your heater; here you have piezoresistive material on

your interdigitated electrodes which are sitting on silicon dioxide which is on nichrome and which is on silicon dioxide further then you have a gold pad.

So, then you have an insulator. So, you have insulator; so, SiO_2 on piezoresistor on IDEs I will stop writing down these particular things. Now, you have gold on SiO_2 on piezoresistor on IDEs. Here you have this one you have SU8 on gold pads and then I will I am not writing this thing then you have SU8 which is conducting on the gold pad and finally, you have diaphragm right by etching the backside of the silicon wafer. So, this is the process flow for creating a chip that can measure the electrical, thermal and mechanical properties of tissue.

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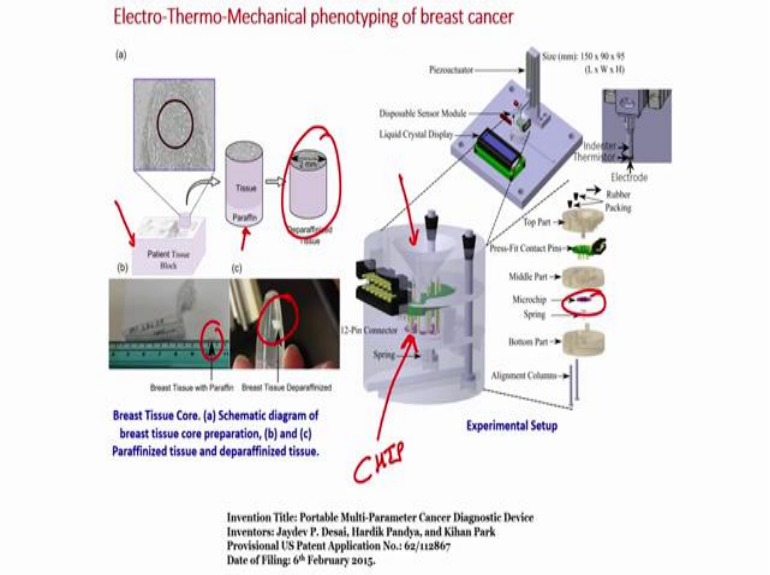

We will show it to you in one of the lab class paraffinized and deparaffinized how to deparaffinized the tissue. When you get the tissue generally it is covered with wax and this is called patient tissue box or block and then this tissue is taken out, you can see the paraffin. It will remove the paraffin and then this is your deparaffinized tissue you can see here the paraffinized tissue versus the deparaffinized tissue alright.

Now, this tissue you have to place in this particular conical or cone-shaped holder and if you see here the chip or a microchip or biochip here right is placed inside this particular 3D printed case holder alright. So, I will move to the next section.

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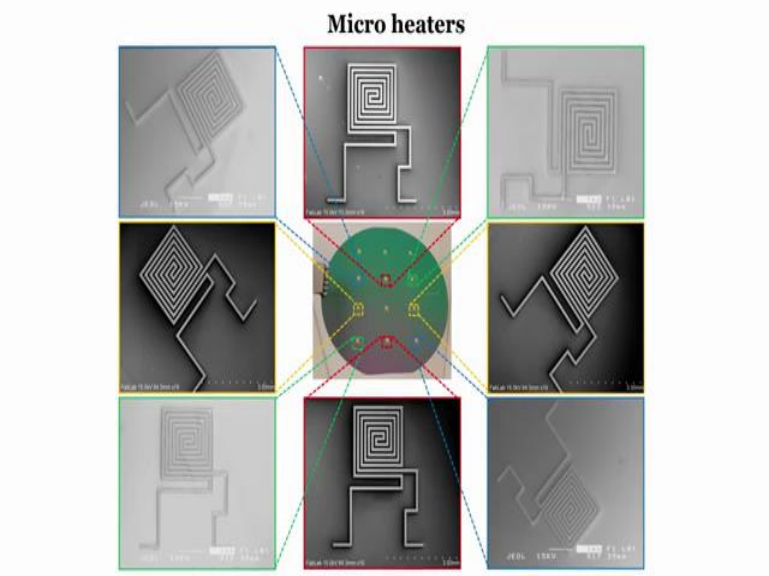

And, you already know this how to fabricate micro heaters right. I will go to the next section.

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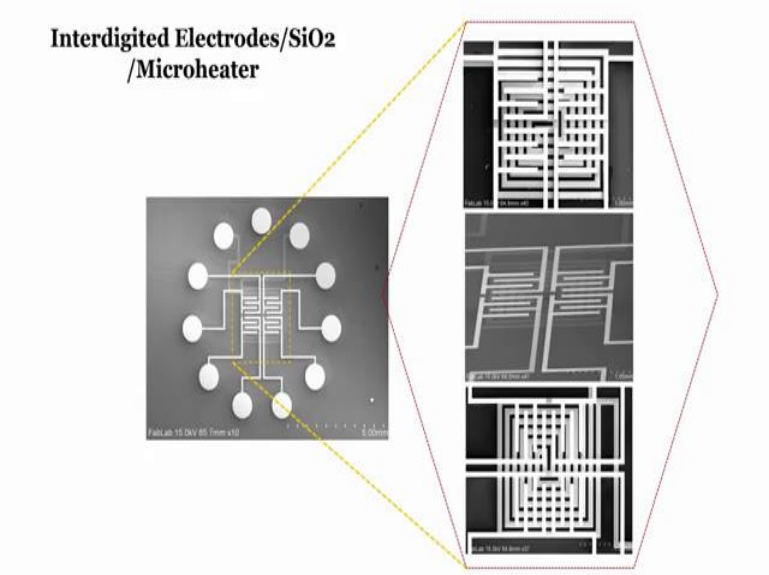

These are the interdigitated electrodes, SEM images of it and these are on the there is a microheater over which. So, there is a microheater right on which there is an insulator on which there are interdigitated electrodes.

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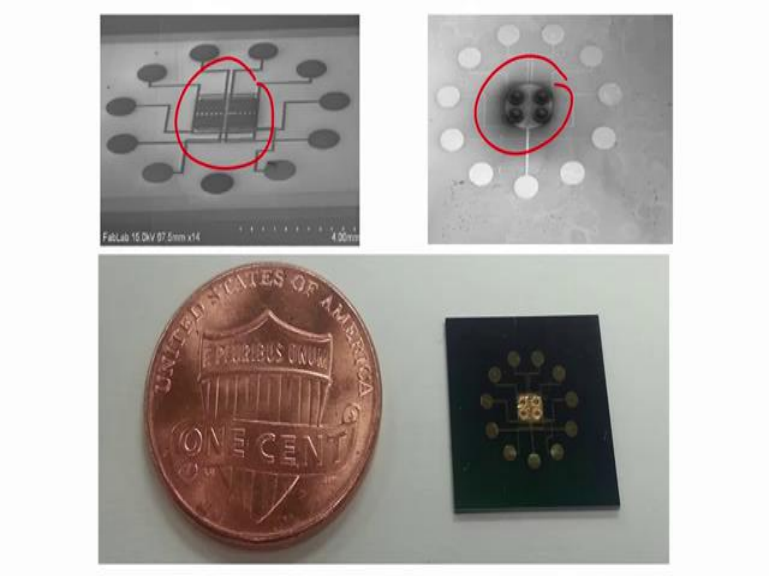

And, then if I go to next slide you can see here the piezoresistive materials or interdigitated electrodes you can see a gold pads on the on so on the piezoresistor there is silicon dioxide on which there is a gold pad which SU8 pillars are there and you can here see the chip against one cent the reason of using cent is that the size of the coin does not change that is why the photograph is with one cent.

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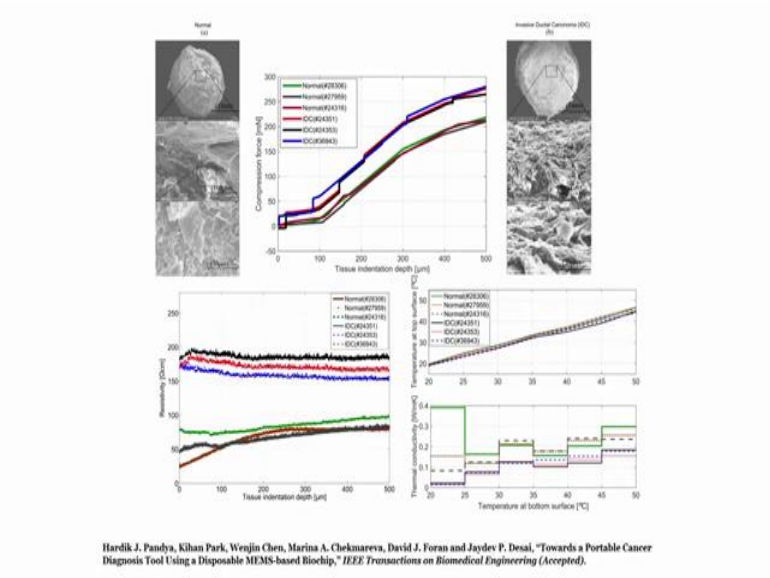

Hardik J. Pandya, Kihan Park, Wenjin Chen, Marina A. Chekmareva, David J. Foran and Jaydev P. Desai, "Towards a Portable Cancer Diagnosis Tool Using a Disposable MEMS-based Biochip," *IEEE Transactions on Biomedical Engineering (Accepted)*.

And, here you can see the results, you can very clearly see that the tissue that lies in the cancerous region would be having different properties mechanical properties compared

to normal tissues. And, the same way if you see this particular graph you can see very clearly the resistivity of the cancerous tissues is different than the resistivity of the normal tissues. And, from this graph even it is very close when you understand thermal conductivity you will understand that the cancerous tissues show a different kind of thermal conductivity compared to the normal tissues.

So, now this is only for 6 subjects as you can see here 3 normal and 3 cancer. If I increase it to 6 subject to 200 subjects then I will have a better or we can have a better understanding of how to correlate these three properties with the existing morality that is your biomarkers right. So, if any question please feel free to ask me in the forum so that we can discuss further.

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And, to go ahead with this now I will show it to you this particular tool.

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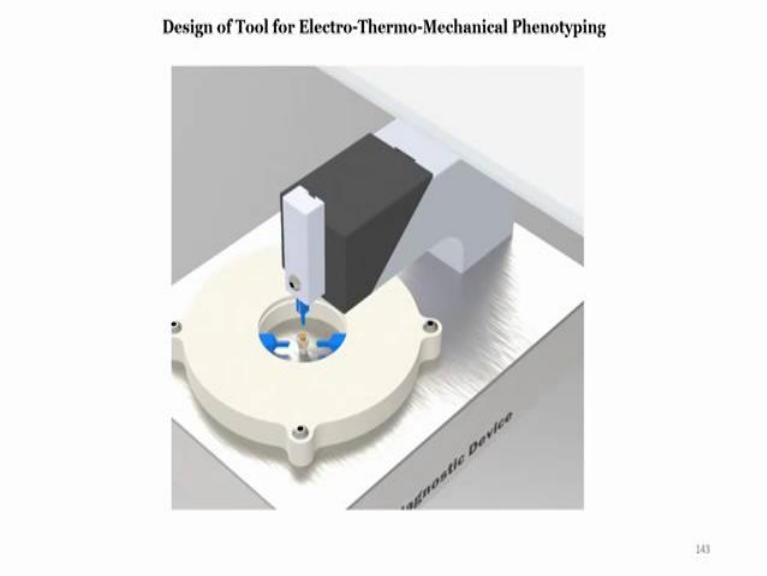

So, what we mean is you can see the video and in this video what you can see is that there are indenters right and these indenters the press against tissue and when the indenters press against a tissue there is a third indenter also. So, once the tissue is there you press it and then you rotate the tissue, again you press it and you press it from the top. So, you are covering most of the surface of the tissue which is a better you know properties or it is important to understand or cover more area of tissue because the tissue is an isotropic um.

And, now let us see another chip that we can fabricate which has a different kind of design, but it has an interdigitated electrodes and heater for understanding the same tissue properties which are your electrical and thermal properties ok. So, what we will do is we will cover that section in the next module is a small section so, you understand that not only the way I have taught you in this section how the chip can be fabricated, but there is another way of fabricating the chip as well.

And, let us discuss this thing in the next module so that not to increase this module to a more than it is required in terms of length right too many things continuously will bore a person. So, I will see you in the next class with a new chip that can also perform the electrical and thermal properties or you can measure the electrical and thermal properties of the tissue right. Till then you take care, I will see you in the next class. Bye.