Sensors and Actuators
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Lecture – 44
Tissue Deparaffinization for Biosensors

So, we have seen in the theory class, right, that we are fabricating sensors to understand the change in the tissue properties right. So, which kind of tissue? I told you that it can be used for any tissue related cancer; it can be oral cancer, where you can take the tissue slide for histology or tissue slides for histology, you can use the breast cancer as an example of clinical model, you can take prostate cancer as an example of clinical model. So, in every case, you have to have a tissue.

When the surgeon operates the patient and takes out the tissue from particular organ right, this tissue is placed in a paraffinized medium, paraffinized to make it easier, it is a wax all right, to store and preserve the tissue for longer time and those things are kept in a tissue bank, alright.

So, then we have a tissue, or this tissue is sliced with the help of microtome and few of the slices are used for biomarkers, and remaining tissue is paraffinized and kept or stored in the tissue bank. Now, if we want to use those paraffinized tissue and study the mechanical properties, electrical properties or thermal properties, or let us say the optical properties like NIR, which is, near infrared rays how it diffuses through the tissue, what are the reflectance properties, then we have to deparaffinize it, that means we have to remove the wax is not it.

So, if we get the tissue from our clinical collaborator or if you are having a collaborator who is a clinician and in this case you require a oncopathologist, pathologist who works on oncology that means, pathologist expert in cancer. Because only oncopathologist are allowed to or they are taught of understanding the tissue properties and give a set of diagnosis back to the clinician, back to the doctor alright. So, they are specialized doctors on this particular domain.

Since they handle the issue, they are the right person to collaborate when we want to get the tissues for our studies ok; either you take your system to there in the oncopathology,

with the oncopathologist or you get the tissues from those people and you use in your laboratory. Now, both these things, in any case, whenever you use either animal samples like we are talking about rats brain or you use a human samples like blood, urine, the tissues right, then you need to get ethical clearance. Anything to do with animal or humans, you require an ethical clearance from the ethical committee that is either in the institute or with the collaborators institute, right.

Ethical clearance is that you are allowed to use this many subjects for your research, and it makes sense to use certain set of subjects. If I say, I want tissues from 1000 subjects, why? Is the ratio same right? What age group? Why I think that particular age group and this particular set of subjects would be good enough to come up with a novel solution and this is require for new research finding, right.

So, this all things are taken care before we get the ethical clearance. There is a template for each institute or each ethical committee that will be provided to you if you apply for the clearance. There is one set of things. It is very important to tell you this thing because in case if you work in the laboratory which works on this interdisciplinary field of using the engineering for clinical studies, then you require ethical clearance.

Second part of it is a bio-safety approval. Bio-safety approval states that your laboratory where you will be using those samples is approved to handle those samples. How you handle those samples, how you discard those samples right, whether you have a proper biohazard right, you have a autoclave in placed, where, where the students are using or the researchers are using facility, is it good enough for them to use it; so, a lot of things that comes when you get a bio safety approval. If you have both, you can start working on this kind of studies.

So, today, again, I will request Anil to show it to you how to deparaffinized a tissue that we get from the oncopathologist which is paraffinized one, what is the process alright. And he will talk about the de depaffinization steps in the lab component alright. If you have any questions, you are free to ask me through forum like I said; there will be a live session right as a part of this particular course, 1-hour session. And in that also, you are free to ask me all the technical questions related to this particular course including your experiment or including the experiments that we are showing it to you as a part of the lab component alright. So, I will request Anil to take over this particular lab. Thank you.

Welcome to this module. We have gone through a lot of sensors as part of this course. I have explained to you the rationale behind the design for each of these sensors. So, when you are you have made a lot of sensors, this is a course on sensors and actuators. I have also looked at several of the clinical applications at sensors and actuators can have, but did you actually see hands on about what goes in to actually use these sensors to measure from physical items or biological samples, ok.

You have all you have see in the sensors, you have seen that the sensor can measure that, the sensor can measure this, this sensor can measure this, but then how do you actually make the measurements what goes in to make such measurements. So, one such example I am throwing to explain to you today. Behind me, we are doing an experiment, it is part of that. So, I will explain to you what we are trying to do, we are not going to show you each and every step of it because it is a one-day protocol.

When you tell the protocol, a protocol is usually used, a word is used in biological experiments when you do a definite set of steps to arrive at a particular conclusion, you repeat the steps very diligently and then do it. So, it is a one-day protocol. I just explained what we are trying to do. So, idea is you might have seen several sensors, right and I our professor or other TAs would have explained to you that there are sensors which measure properties of tissues biological tissues. They may be mechanical properties, they may be electrical properties, they may be thermal properties, they may be chemical properties, optical properties and a lot of things can be measured.

Now, how do you get these tissues that is one question, how do you get these tissues. Now, tissues can be taken these are you might, remember that these are coming from actual patients. So, whatever sensor that you are doing, whatever measurements that you are doing, finally a patient somewhere who has suffering from a disease has given his or her flesh and blood for your experiment.

So, you should always have that respect towards the samples that you get, this is something this is some this is an ethical thing that you should always be aware of. Do not treat your samples just like that, like it just like does not have any value, it has immense value. It is the flesh and blood of somebody who is suffering from a very grave disease which you are trying to address. So, you should always have that respect towards what you are handling.

Now, the thing is, let us say we are trying to measure the physical properties of a tissues ok, cancer tissues for example. Let us say a patient, a lady, has breast cancer and she goes to the clinic. And already she is diagnosed, she knows that she is suffering from the disease, but the doctor said let us see, how is there your disease progressing. Let us see that and let us take a biopsy. So, a biopsy are of different types. There are fine needle aspiration cytology biopsies, where a fine needle like what you say your normal syringe needles like this.

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Syringed like this, this is not exactly a FNAC needle, but this must thin needles. You may not be seeing it, that is fine, very fine needles which you usually use to take blood are used to take cells from your breast, ok. Now, that gives only cells. Now, there are other biopsies called core biopsy.

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So, this is a core biopsy needle; it has about a diameter of 3 mm, ok. Now, this will be poked into the breast, and then you take out the sample. It is a very painful procedure. They will put local anaesthesia and take out the sample ok. Now, core biopsy needle will give you a definite size of chunk of tissue from the body. There are other biopsies called excisional or incisional biopsies which are surgical procedures these are not necessary.

So, these are not necessarily surgical processes such as biopsy procedures. Surgical procedures is when a patient will actually undergo surgery and they will remove a part of the breast or other body part that is called external biopsy or incisional biopsy, ok. This is core biopsy. So, for our projects, we use core biopsy. So, this core biopsy needle is there. So, we receive tissue samples in formalin fixed paraffin embedded tissue blocks, ok.

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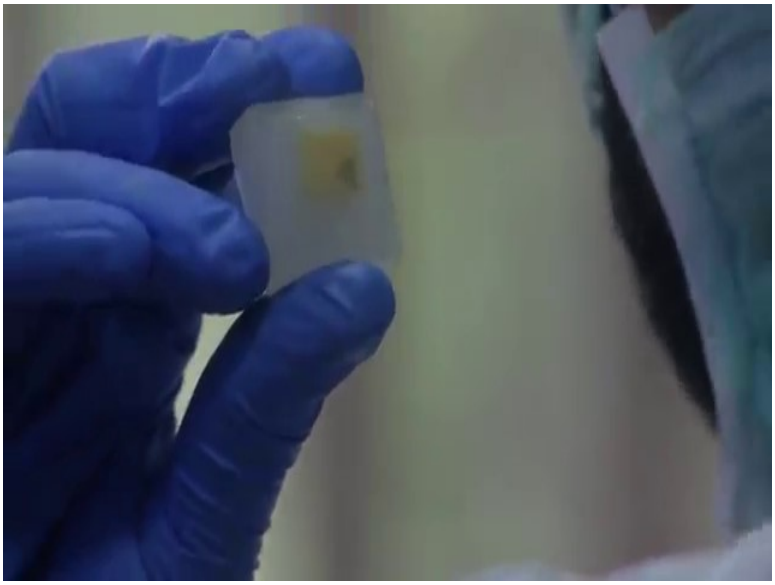

I will show you one such block. This is a tissue block ok, this is wax, the white color thing that you are seeing wax. If you look at the centre, you can see a yellow part, that is actually the tissue, tissue that is taken out from a patient. What they do is, they will take out the tissue from the patient, they will put it in formalin. The formalin will freeze the cells in their structure in a very layman's terms, and then that formalin fixed tissue will be embedded in paraffin or submerged in wax. Then solidified so that this can be preserved for a long time as also sections from this, very fine sections from this can be taken for chemical-biochemical analysis, ok.

So, this is what is usually called in medical science as an FFPE block, formalin fixed paraffin embedded tissue block ok, so that you have seen. Now, ok, we started them from where we started from sensors, right. Why I am talking about formalin fixed tissue samples, biopsy everything. Why are we talking about all these things? If you are into product design, if you are into making some engineering solution that is useful to the society you should know all out what are the individual processes that are happening.

So, for you to define a sensor, for you to design a sensor in the best way possible, it is important that you understand the whole process, because your design will be more sound if you understand the whole process, ok. So, why are we telling all this to you because fundamentally this will define how the sensor that you use will measure the tissue sample that is used with the sensor, how it will measure the properties from the tissue sample correct.

So, this step that she is doing behind me is the preparation of the tissue sample, that finally, goes and talks to the sensor correct. Now, I told you that we have that formalin fixed tumor block right. We have seen the tumor block. Now, from the tumor block, from the paraffin block, we will use this biopsy punch and take out uniform 3 mm diameter, 10 mm, 10 mm height cylindrical tissue blocks, cylindrical tumor blocks or normal blocks, ok.

Now, what are the processes for you that you have to do to get the tissue? What you have to do is first there is a coating of paraffin, which is wax, you have to remove that. Then the paraffin has to removed and that is a fundamental goal. How do you do that? The blocks are first kept in an oven; you can see the oven behind me, it is kept in an oven for around 2, two and a half hours at around 60 degree Celsius. It will melt the wax, but it will not remove the wax from the tissue, you should remember that. It will melt the wax. Once the wax is melted, you take the tissue from, from inside the oven and put it in xylene, it is called xylene treatment.

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So, you have to put it in xylene in such an eppendorf tube, you can see the eppendorf tube in my hand, it is not focussed that is fine. It is, this is an eppendorf tube. It is a standard a vessel that is used. You put a xylene in the eppendorf tube, you put the tissue inside the eppendorf tube ok, so that is after you take out from the oven, so that a xylene what it does is, it will remove the paraffin from the tissue or the wax from the tissue ok.

Now, you know that the tissue is what the tissue is FFPE block, formalin fixed paraffin embedded. So, for you to get back the tissue, you have to remove the paraffin embedding and you have to remove the formalin fixation correct two things have to be done.

Now, what do you have done, you have put the tissue inside the oven, heated it. You heated it, and then you took it out the backs would be in a melted form, you put it in xylene ok. You put this in xylene, and a xylene will take out the paraffin from the tissue block. So, you have to, there are some defined, defined time, and the vortexing, centrifugation etcetera we have to do. And then what you will do is let me show you for a few things.

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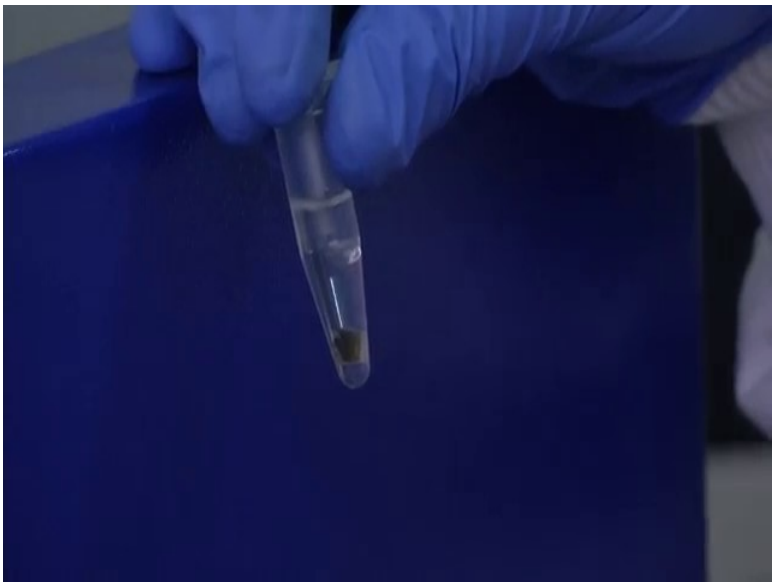

So, as I told you that first we will heat it in the oven, the oven is here. We have heated in the oven, we took it out we put the tissue sample in xylene in an eppendorf tube. The tube that I am holding in my hand is called an eppendorf tube, it is 1.5 ml eppendorf tube ok. You have you can see the black colour substance inside the tube, that is a tumor sample, ok. It is dipped in xylene. This is what it does is it will remove the wax, that is melted which is we just came it was kept in the oven. You cannot keep it for a long time in xylene, we need to immediately do it; otherwise, it will become it will make this tissue stiffer.

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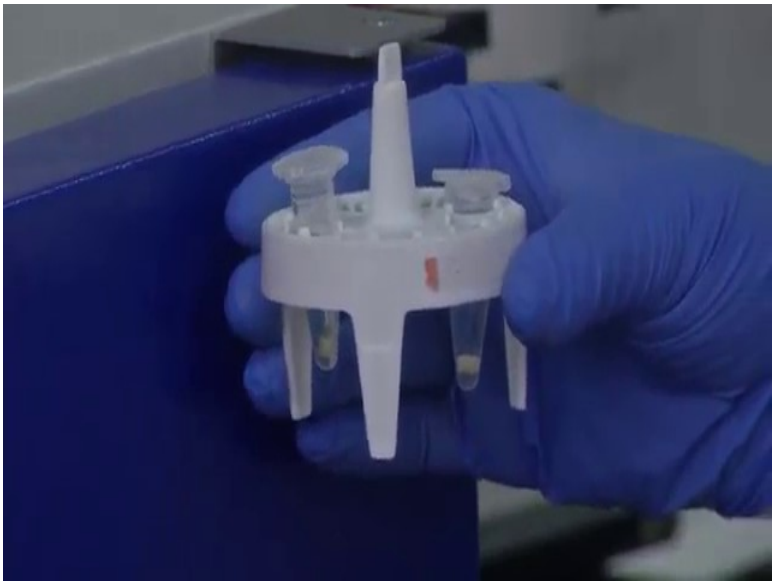

So, we have one sample and such eppendorf tubes can be held using this carrier, you can see and we have another sample here, ok. So, both samples you have seen. And the next thing what we have to do is let us zoom out. What we have to do is then is to vortex it. So, vortexing is very violent shaking, we have an equipment for micro vortexing, small level vortexting here; let us see, let us focus there.

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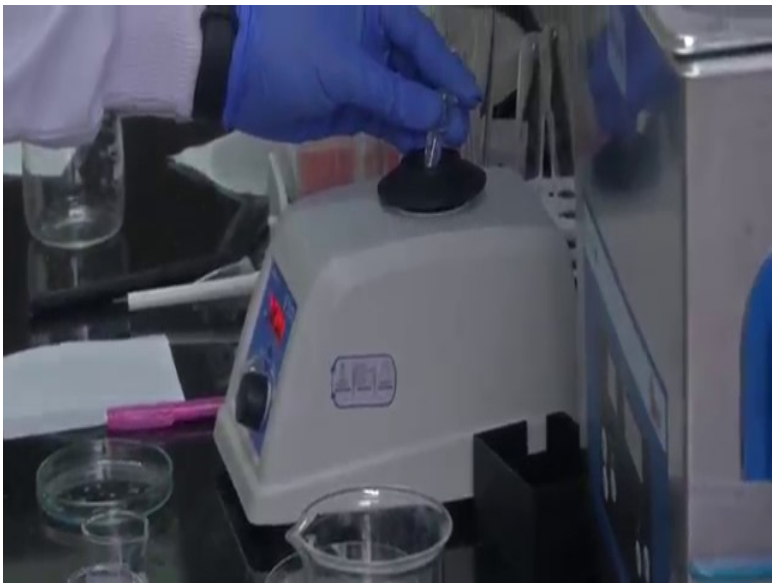

So, here we have an equipment for vortexing. So, I will do it. So, if I press it, it will start shaking the sample, so that is done.

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So, now, what vortexing does is the wax that is there in the tissue right, it will remove the wax violently or in a very abrasive manner from the tissue correct. Now, next step what we have to do is centrifugation. What is and what does centrifugation do, it will separate out individual constituents. So, you know that in this liquid, in this liquid, you have seen it in up close, now you are not seeing it up close that is fine. In this liquid, you have wax that is separated out; in this liquid you have wax that is separated out from the tissue and the tissue itself. But you need to separate it out properly correct.

For that you use the centrifugation apparatus. What it does is, it will rotate this tube at certain rpm ok, and then it will separate it out based on mass. Wax, because it is lighter, it will go up and the tissue will remain at the bottom. So, that is why a centrifugation step is required. So, if we do vortexing like this, next sample. Next, on the other side we have a centrifuge. The oven has finished that is what sound was.

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Now, on the other side of the table, we have a micro centrifuge which is here. Put these tubes in the micro centrifuge and centrifuge it for 2 minutes at a 4500 rpm. So, you are keeping it inside the centrifuge, and then she has kept it inside the centrifuge, it is a micro centrifuge. There are different types of centrifuges that are available. It is a very small portables centrifuge, she has closed it. Now, she will fix the rpm, and it will start rotating. You may not be able to see the rotation inside because it is a very smooth machine. So, it has started rotating, no, it has not yet started rotating. She has to increase the rpm, so that it to start it will start rotating and power should be there.

Yeah, now it started rotating, yeah, now if you let us zoom in further yeah it is rotating inside. For 2 minutes, we will do this centrifugation. So, let that happen, let us come back. So, now, what we have done, we have taken tissue sample using the biopsy punch, correct, we kept it inside the oven, heated it at 60-degree celsius to melt the wax. Took out the samples, put it in xylene, vortexed it, centrifuged it, here we have a currently centrifuging it.

After that what we will do is, now what I told you, what are we trying to do, we are trying to get the tissue back from the FFPE block state. What is FFPE again formalin fixed paraffin embedded box, correct. Now, paraffin embedding we are removing now through this oven heating, xylene treatment and all. Now, we have to remove the formalin fixing correct. So, the xylene treatment is will get over by the by the time we

finish the centrifugation. It will take out the eppendorfs. Then, we will take out the tissue samples from inside and put in different concentrations of ethanol. ok.

What does ethanol do, we will put in four different concentrations of ethanol 100 percent ethanol, 95 percent ethanol, 70 percent ethanol and 50 percent ethanol, this is the protocol. Again, I repeat we will put it four different concentrations of ethanol 100 percent ethanol, 95 percent ethanol, 70 percent ethanol and 50 percent ethanol. What it does, 100 percent ethanol does two things; one is already there is a coating of xylene on your tissue sample correct that has to be removed first.

This ethanol will remove the xylene from the tissue sample. Then, what it does is, what does formalin fixation do; formalin fixation actually kind of like takes a snapshot in time of the tumour or the tissue. It freezes the issues, all the cytoskeletal structures, everything, it forms formaldehyde bonds linkages between all the cells and their cytoskeletal structures.

So, for the tissue to regain its normal stiffness, it has to break these formalin bonds, we just, which has been found by the fixing. For us to make or preserve it cells or tissues for a long time, formalin fixing is important, that is why we do it. But for us to use the tissue when we are actually doing experiment that is not good, we need to break those linkages. So, ethanol does that now. But why do you use four different concentrations? Now, everything has a shock, you cannot suddenly introduce lot of water into it. For formalin fixed one, it is a very very dehydrated state of the tissue. There is very less water in the tissue ok. And then you are putting in paraffin blocks. 100 percent ethanol has absolutely no water in it because it is a 100 percent ethanol.

So, we are not directly putting in 50 percent ethanol, because there will be lot of the water, the tissue will be hydrated very fast. So, it becomes like a shock to the tissue, it can affect the tissue structure. So, it has to be done in a mild, sober slow away that is why we use different steps.

So, first we put in 100 percent ethanol; that 100 percent ethanol will remove the xylene from the tissue. Then it will actually start breaking the formaldehyde bonds; and then 100, from 100 percent ethanol, we shift it to 95 percent ethanol little bit water is there. And that 95 percent ethanol is prepared in distilled water, obviously, so water will be there.

So, water will go into the tissue slowly and parallelly the formaldehyde formalin bond breaking will happen, then 70 percent little bit more water, 50 percent a bit more water. Finally, when you take it out of 50 percent ethanol, you will put it in distilled water pure distilled water. So, then the tissue is expected to come back to its normal consistency as much as possible and it is ready for measurements. For our studies, for engineering studies, we you want to measure full tissue or full tumor cell blocks and measure its properties.

Biological protocols, they want sections, 6-micron, 10-micron sections from these tumors and they do a biological methods called immunohistochemistry, immunocytochemistry and histopathology and all. I am not going to details of that because that is beyond the scope of this course. Our idea is to only teach you what goes behind, what happens behind the scenes in preparing your sample to measure with your sensor ok.

So, now, the centrifugation is done. She has completed centrifugation. Now, you can see the eppendorf tubes on the micro centrifuge. So, now, she will take it out. So, we are taking out the samples from the micro centrifuge, yes. Now next step is to do the ethanol washes. So, now let us see the preparation set up for the ethanol washes.

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So, this is the set up for the ethanol washes ok. So, we have 4 different concentrations of ethanol 100 percent ethanol is here 95 percent ethanol is here, 70 percent ethanol is here,

50 percent ethanol is here. Finally, once these washes are completed, we will put in distilled water which is here, ok. Now, let us take the centrifuge. So, she is taking out the, yeah, she is taking out and putting that sample in that tissue in the 100 percent ethanol vessel.

So, one sample is put already. Now, she will put the other sample. First taken and put using a tweezer ok. Now, for 3 minutes, we will keep in 100 percent ethanol. So, we have kept in a 100 percent ethanol for 3 minutes ok. So, now one and a half minutes are done. Once 3 minutes is over, we will transfer further tissue from there to here as you know that it is a mild the rehydration treatment ok. While this happens, I will tell you there is another equipment here.

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So, this is a sonicator. I think you might have seen it before in this course, but this is an ultrasonic bath. What it can do is, if I let me switch it on. So, what you can do is, it can like your centrifuge like your vortex. Vortex is a very violent shaker correct, this is vortex machine; you can see my hand, but this guy is an ultrasonic vibrator. So, it will give mild ultrasonic vibrations to clean or to vibrate your substrate, it you in case you want to clean it.

So, you can set, you can heat it with this control here, you can heat it to a different degree, and then you can vibrate it also. So, if I start, you can hear this unique sound it is because of the ultrasonic vibrations in the water inside. So, there will be water inside and

ultrasonic vibrations, this can also be used instead of vortexing. If you are very careful about the, if we do not want to add that much mechanically ah force your sample, you go off you want to mild you do a mild vibration of your sample, you can use this ultrasonic bath, you can hear the sound you; can have an even have a timer. So, now, 3 minutes is done. Now, let us take out and keep it in the next concentration.

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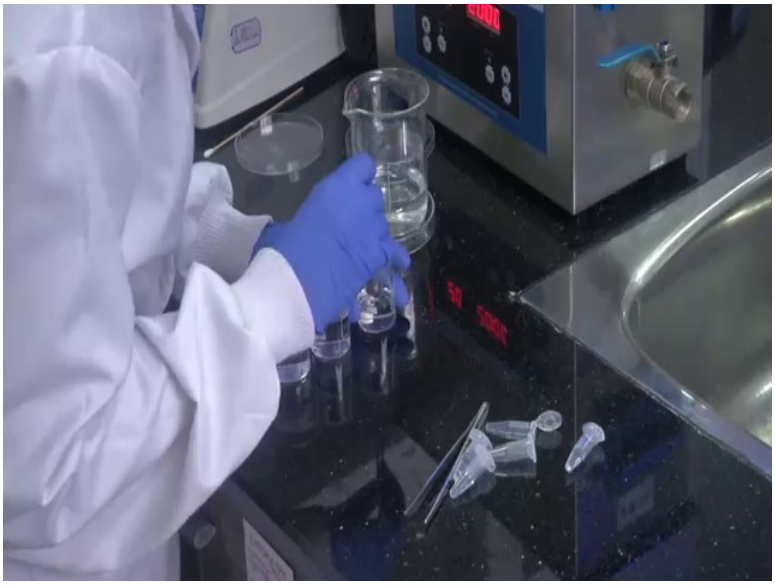

So, she is taking it out and she has kept it in the 95 percent ethanol concentration. So, you can see the samples falling into the ethanol, yeah, you can see the samples inside the ethanol bottle here.

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Now, we are in 95 percent ethanol. Yes, you can clearly see it. So, here we will keep for another 3 minutes and then we will transfer to 70 percent and 50 percent ethanol. Finally, we will put it in distilled water for washing that is the process I do not want you to wait till that much time. So, this is the process of the deparaffinising. So, why finally, we will have a sample that is tissue, and which can be used in the tool or sensor to measure ok.

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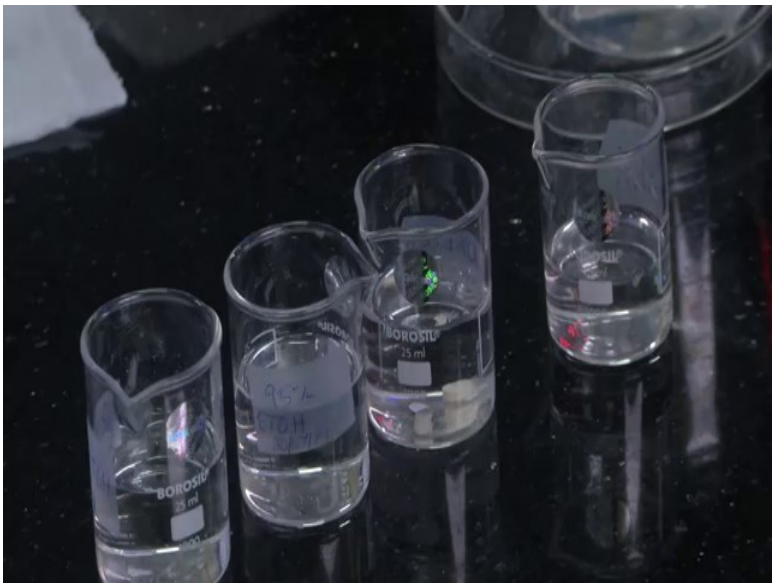

Let us come back. It has become whiter know; it has become whiter know.

Yeah, it is because we kept it in drying for long.

Is it?

Yeah.

Last time, it was not this much no? Now, what we can do?

We can just shut it down like for once when you do that I mean we repeated the steps twice nah, we will just make it once.

That is what I was telling that time well fine ok. You can complete this and keep it in the eppendorf.

Ok.

As you might have observed towards the end of our conversation now, we are also trying to optimize a process here ok. So, the protocols that we are trying to execute, the protocols are all available for tissue sections. None of the protocols are available for whole tissue blocks, but for our applications, for our engineering application, we need the biological sample to be in blocks.

So, what we are trying to do here is we are trying to optimize the time of different procedures so that the tissue becomes like the original tissue that is taken out from the patient as much as possible, not exactly it is never possible. So, it will become as soft as possible, so that is what I was talking to her like should we do that xylene washed two times should we do it once only.

So, we are having a technical discussion, like should we do it or not and how much time should we put in ethanol, so that is how you arrive at a protocol. You do three-four experiments, and then you arrive at a protocol and then that you keep as a standard, standard process and then you repeat for these samples. So, I think you got a good overview of what happens behind the scenes before you actually test with a sensor, you need to prepare the biological sample, you need to give enough respect to the biological sample that is at your disposal.

So, what we have done; we have done deparaffinising of formalin fixed paraffin embedded tissue blocks, tumor blocks. And we have tried to deparaffinising it and we are still in the process of doing that. At the end of it, you will have a tissue. I think in the video, you have seen me trying to look at the tissue sample, how soft it is whether the consistency is proper and all that is how you do it. And then you load it onto the sensor

or your product and measure the properties that you are looking for. Hope you understood and enjoyed this session. We will meet you in another session.

Thank you.