Mathematical Aspects of Biomedical Electronic System Design

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Week – 12

Probability Distribution and Biomedical Systems Design

Lecture – 41

System Development for Tumor Delineation using ETM characterization

Professor 1: Hello everyone, welcome to the last module of Mathematical Aspects of Biomedical Electronic System Design. In this particular module, we are trying to summarize the work which of course require an understanding of tissue properties and little bit of mathematical analysis for modeling. So, throughout the course we have discussed about electrical, thermal, mechanical properties and how it will be useful in order to identify a particular disease condition.

For example, we have taken an example of breast cancer and how issue of cancerous tissue can be delineated from normal tissues using all these properties. So, in this particular work, one of my friends will summarize the system biomedical electronic system development and his analysis mathematical modeling briefly and it is part of an ongoing research.

So, we thought of conveying this recent progress or trend in terms of this tumor delineation using ATM characterization of tissue. So, he will be summarizing his work in the upcoming presentation. Thank you.

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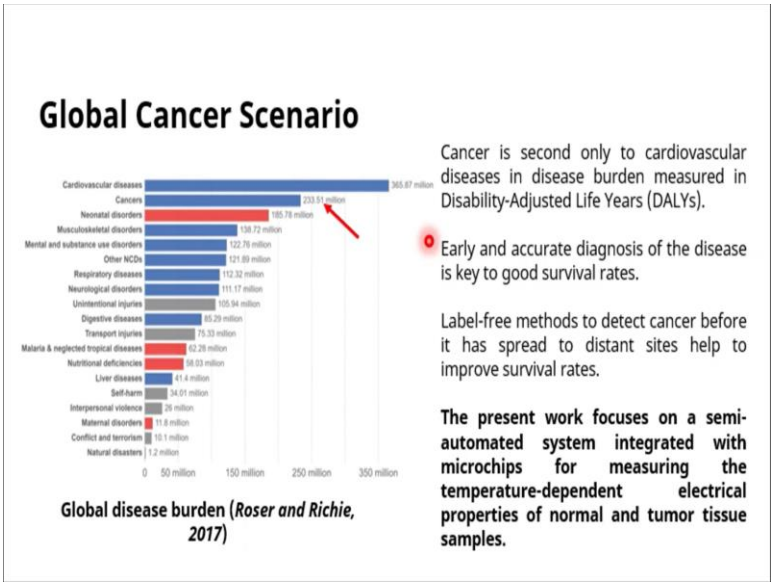

Professor 2: In an epidemiology study published in the Lancet in 2017, it was shown that cancers are only second to cardiovascular diseases in terms of disease burden across the globe, when measured in terms of Disability-Adjusted Life Years, what this metric measures is basically the number of years lost either due to death or disability due to different diseases. And it was shown that cancers are only second in terms of burden and second only to cardiovascular diseases.

When it comes to cancer diagnosis, early and accurate diagnosis of the disease is a very key component for good survival rates. If the cancer is diagnosed at an early stage, like stage 1 or stage 2, up to 95 percent survival rate can be achieved, as opposed to 10 to 20 percent when it is a late stage cancer like stage 4 or stage 3.

Label-free methods to detect cancer before it has spread to distant sites in a process called Metastasis can help to improve the survival rate because the disease can be diagnosed early. Our present work focuses on the development of a semi-automated system integrated with MEMS based microchips for measuring the temperature dependent electrical properties of normal and tumor tissue samples.

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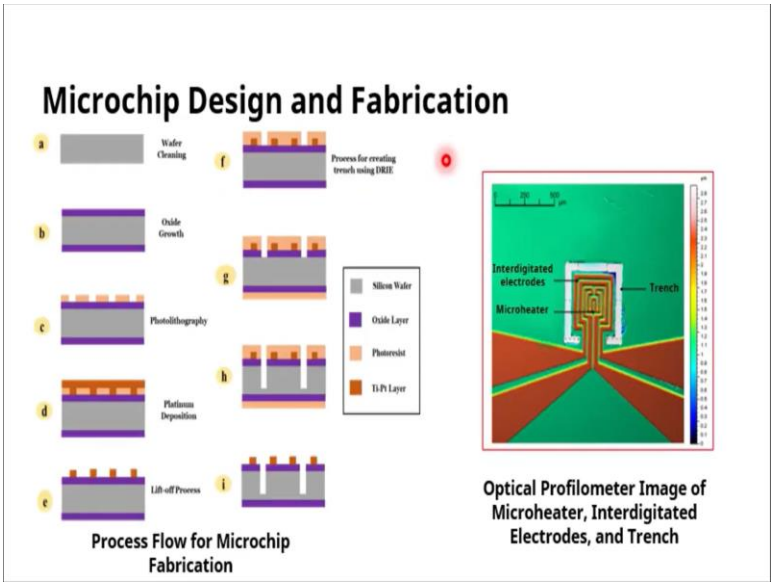

One of the key components of the semi-automated system is a mems based microchip, which is used to measure the temperature dependent electrical properties of the tissue samples. The microchips are fabricated on a four inch silicon wafer. The wafer is first oxidized using thermal oxidation, after this layer of titanium and platinum deposited using even evaporation or electron beam evaporation is patterned using the Lift off process.

After this, another mask is used to create a thermal isolation trench around the micro-heater using the deep reactive ion etching process. Thus, the micro fabrication process flow for the chip microchip design is the two mask process. Once the design is completed, each individual microchip is diced out from the array on the 4-inch silicon wafer using an automated dicing machine.

Here, you can see the sensing elements on the microchip. The sensing elements consists of a micro heater at the center interdigitated electrodes around it, there is also a thermal isolation trench, which is around 280 micrometer beam to limit the heat generated from the micro heater from spreading across the bulk of the silicon wafer. While the micro heater is used to heat the tissue, the interdigitated electrodes measure the DC conductivity as well as the AC conductivity of the tissue samples.

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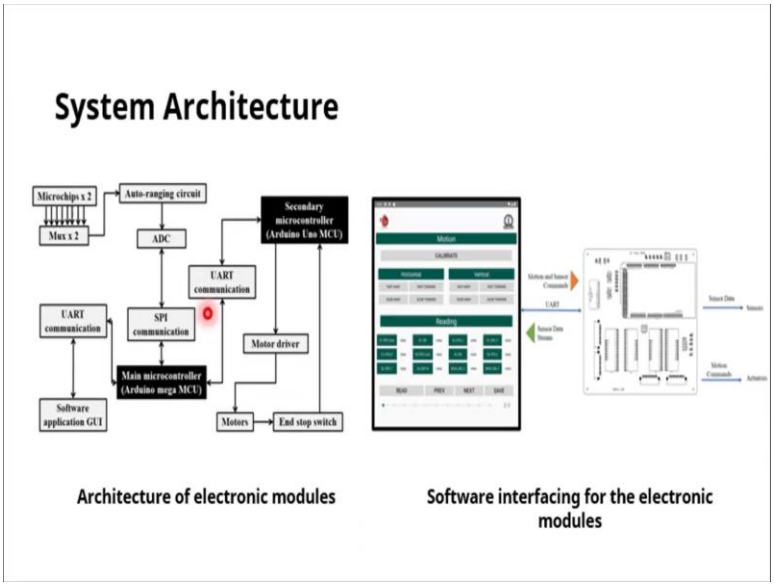

The system architecture of the electronic modules in the semi-automated system consists of a master-slave configuration of two Arduino microcontrollers. One is an Arduino Mega microcontroller and the other is an Arduino Uno. The Mega microcontroller controls both the activations of the system as well as the electronic measurements that are performed. The Arduino Uno, offer receiving commands from the main microcontroller controls the activations of the motor driver, and the in stop switches that allow for a calibrated probing of the tissue samples using the two intenders on the system.

The microchip sensing lines are connected to a digital muxing circuit, which are in turn connected to an auto-ranging circuit. The auto-ranging circuit measures the voltage drop across the sample under test, converts it into an ADC value, digital value and inputs in the microcontroller. The logic in the microcontroller then computes the unknown resistance value, which is required for us to perform the temperature dependent electrical characterization.

Now, this system overall is controlled by a software application running on a tablet PC. The software application communicates with the electronic module through the UART port or the universal asynchronous receive transmit port and commands for motion activations of the system as well as acquiring data from the sensor different sensing lines of the system are taken care of by the software application. Once the measurements are completed, the application also has an option to export the data captured into an Excel sheet for further analysis and arriving at conclusions.

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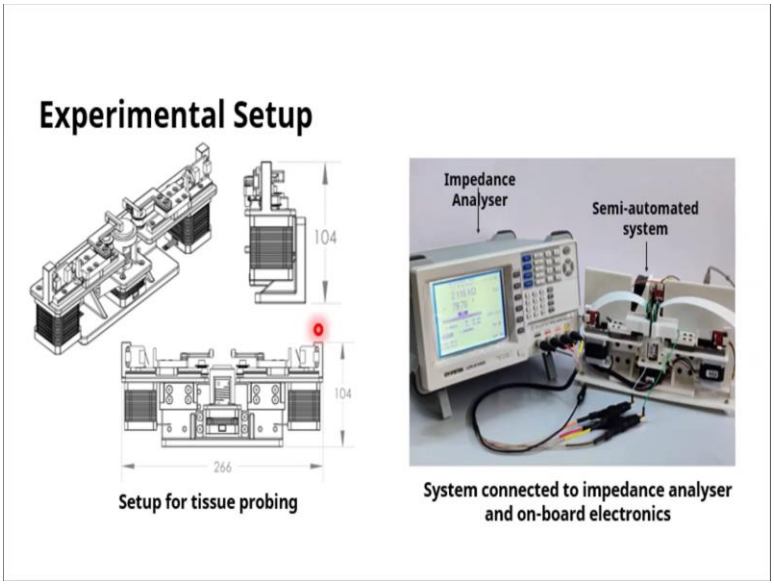

We have seen the design and fabrication of the microchips that are used for the temperature dependent electrical characterization and also the system architecture of the electronic modules in the semi-automated system. Now, we will see what is the product engineering of the system. So, the product engineering design consists of two intenders which are placed in, actually placed in the x direction. The microchips are mounted at the tip of this intenders you as you can see here, as an additional attachment on the intender tip, this additional attachment can be seen here, which is 3D printed using rapid prototype.

Now, the semi-automatic system has a height of about 10 centimeters and a length of about 26 centimeters. While being flexible flat cables from the microchips connect to the electronic modules packaged inside the semi-automated system, these flat cables measure the resistance properties of the tissue, the impedance or the frequency dependent electrical properties of the tissue are measured using an externally connected impedance analyzer, which can measure from 10 hertz up to 5 megahertz.

Together, the impedance analyzer and the semi-automated system constitutes our experimental setup. While the electronic components inside the semi-automated system are able to heat the tissue and also perform the electrical resistance or conductivity measurements from the sample. The externally connected impedance analyzer measures the frequency dependent electrical properties of the tissue.

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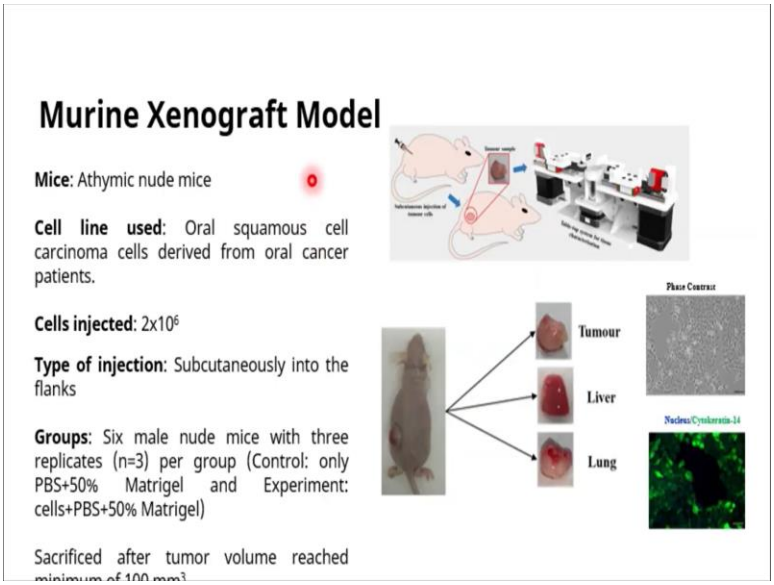

The tissue samples for the study are obtained using a murine xenograft tumor model. For this Athymic, nude mice or immune compromised male nude mice is used as the animal. Oral squamous cell carcinoma cells derived from oral cancer patients is used at the cell lines that are used to inject into mice to create the tumors.

These cells before injection were confirmed for their epithelial nature and their epithelial morphology using phase contrast imaging and also by staining of the nucleus and the cytokeratin, which is an epithelial marker. Once this was confirmed, 2 $\times 10^6$ cells, or 2 million cells, suspended in PBS and metric gel was injected subcutaneously into the flanks of the animal, which is basically under the skin and then near the abdomen of the animal.

6 male animals were taken for the study and divided into two groups of three animals each. While one group was a control were only PBS and 50 percent matrigel was injected into the Advil, the other group formed the experimental group. In the experimental group, the 2 million cells, and PBS and matrigel were injected into the animal subcutaneously.

The animals were then monitored for a couple of weeks, and they were sacrificed once the tumor volume reached a minimum volume of 100 millimeter cube. While sacrificing, while the tumor was extracted for the study from the control group of the healthy animals, the healthy organ tissues from the liver and the lungs were also extracted as control experiments for the state. Thus,

we will compare the tumor and the liver and lung in the study for their temperature dependent electrical properties.

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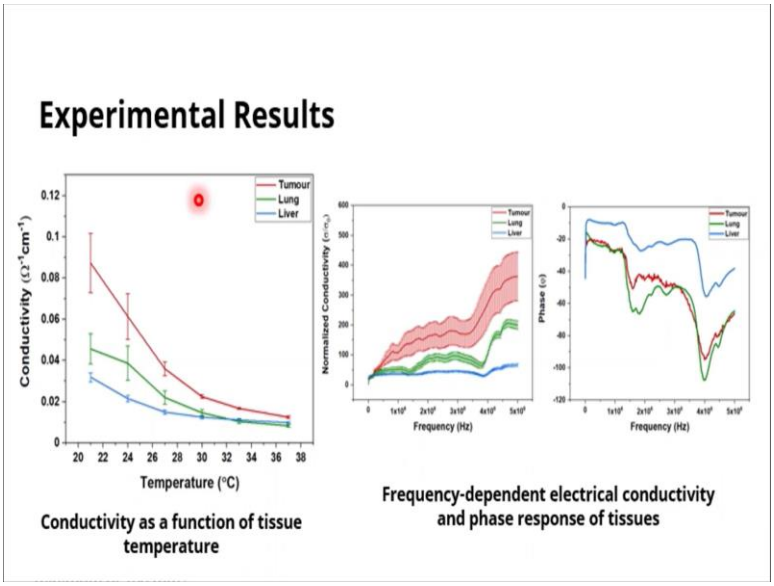

Now let us look at some of the experimental results from the study. We performed two types of temperature dependent or electrical characterization of the samples. One was the conductivity measurements as a function of tissue temperature. Using the micro heater, we heated the tissue samples and measured the resistances. The measured resistances were then computed to arrive at the conductivity values of the samples.

What was observed was that the tumor samples had an overall higher conductivity value as compared with the lung and liver. This could be due to the reported leaky blood vessels that are there in the tumor, which leads to an increased water content and blood content in the tumor as compared to lung and liver. This in turn increases the conductivity of the tumor samples which are freshly excised.

Along with the temperature dependent conductivity measurements, we also perform a frequency dependent conductivity measurement of the samples. The tumor samples were observed, to have a higher anomalous conductivity as compared to the lung and the liver as you can see here. Additionally, as you can see, after a particular frequency the content normalized conductivity suddenly increases. And this is due to the percolation threshold being reached for the percolated transport which is usually seen in composite materials, even tissues or organ tissues healthy

organ tissues or tumor tissues are also known to have a composite nature because they consist of extracellular matrix cells, etcetera.

Now, since tumors are known to be a site of intense proliferation of cells, intense vasculature matrix remodeling, they are known to be more disordered as compared to healthy well ordered structure tissues. As a result, the tumor tissues are observed to have an early onset of this highly conducting regime as compared to the liver or the lung. As you can see from here, tumor tissues are having an earlier frequency at which their conduct normalized conductivity suddenly increases as compared to the lung and liver. We also measured the phase response of the tissues and we did not see any significant differences in the phase response.

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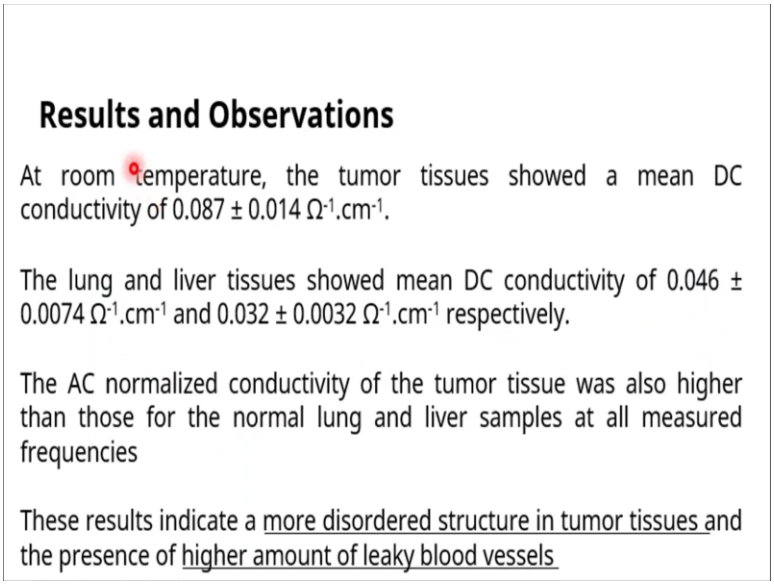

To summarize the results obtained, what we see is that at room temperatures, the tumor tissue showed a mean DC conductivity of 0.087 ± 0.014 per ohm per centimeter. Compare to this the lung and liver tissues showed a mean DC conductivity which was lower than those for the tumor, averaging around 0.046 and 0.032 per centimeter for the lung and liver respectively.

The AC normalized conductivity of the human tissues was also found to be higher, as compared to the normal lung and liver samples at all measured frequencies. We also observed that tumor tissues have an early onset of AC conduction as compared to normal lung and liver samples. These results indicate a more disordered structure in the tumor tissues and the presence of higher amount of leaky blood vessels at the tumor site.

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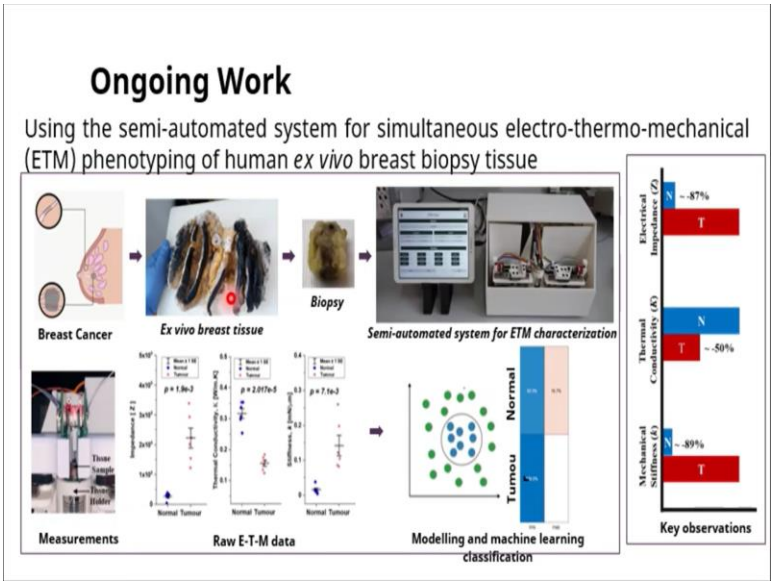

As a follow up to the study that we have just shown, our ongoing work focuses on using the semi-automated system that we have just shown, for simultaneous electro-thermo-mechanical phenotyping of human ex vivo breast biopsy tissues. Apart from the electro thermal phenotyping, we have also added a mechanical sensing aspect to the system by incorporating a piezo resistive 4 sensor into the setup but on the Z indenter here.

Now, patients having breast cancer specifically ductal carcinoma in situ or invasive ductal carcinoma, the samples extracted from such patients using breast biopsy. These samples were used for the electro thermal mechanical phenotyping of the breast biopsy tissues. The results obtained from the phenotyping studies namely the impedance, the thermal conductivity of the sample, and the mechanical stiffness of the sample were also entered as inputs to a training and classification model specifically, a support vector machine classifier to classify tumors and normal samples as and when the samples are tested.

To summarize we have measured electrical impedance, thermal conductivity and mechanical stiffness of breast biopsy samples and this work is ongoing. So far, what we have observed is that the tumor tissues which are ex vivo formalin fixed tissues are also to have a higher electrical impedance as compared to normal and also higher mechanical stiffness as compared to normal. Whereas the thermal conductivity for the human tissues is found to be almost half as compared

to normal tissues. We are still in the process of acquiring more data and improving the classification algorithm and in getting more raw data as a part of the study.

Professor 1: So, we have seen the tissue properties can provide useful information about the state of thesis and using different types of properties we can identify or classify or locate a particular region or whereas lesion is there or where tumor is there. Moving ahead we have seen so far three electrical, mechanical and thermal properties.

Moving ahead, we have also tried to analyze optical properties and acoustic properties of tissue and we have developed a system to base the tumor delineation using this optical and acoustic properties. Additionally, it is very important to note is that how these different modalities are resulting into a better delineation like only optical property yields to one particular accuracy or better performance, but if you use even a two acoustic property will give you some other accuracy.

But if you use both of them or if you merge even all the properties together, then if you are converging towards the better and better results. So, how this multimodal system and its analysis would be helpful in order to moving towards a better characterization and better classification or better identification of tumor region. One of my colleagues will summarize his work briefly in the coming presentation.

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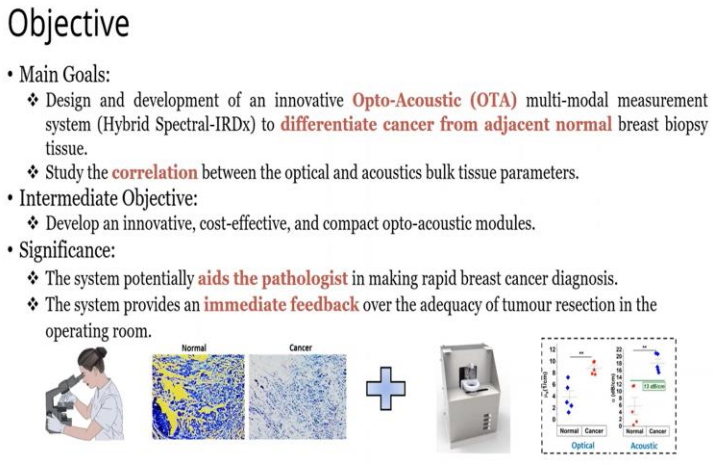

Professor 2: The main objective of the work was to design and develop multimodal system which can quantify the optical as well as acoustic properties of the tissues. In these properties, we can differentiate the cancer from the adjacent normal tissues. Second objective was to correlate the optical versus the acoustic properties.

The intermediate objective was to develop innovative cost effective and a compact Opto-Acaustic modules. The significance of the work was to develop a system that can aid the pathologist to make rapid breast cancer diagnosis and also to give an immediate feedback over the adequacy of tumor resection in operating room.

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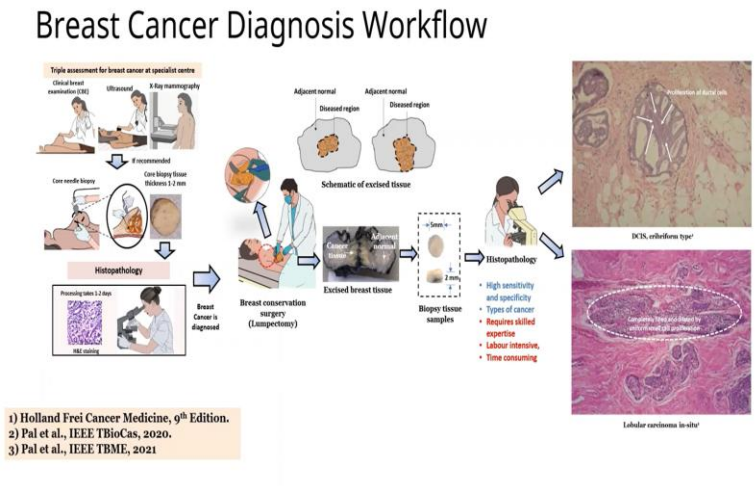

As you can see over here, the breast cancer diagnosis workflow, it starts with the triple assessment at the health center, it would include at the ultrasound, X-ray mammography and if found suspicious, a collateral biopsy is performed were a small chunk of tissues is excised and given to the Histopathologist, the Histopathologist slices this tissue into hundreds of micrometers thin slices.

It is it then stained and then observed under bright field microscope. If the breast cancer is diagnosed, one of the approaches is to perform breast conservation surgery that is the lumpectomy where the surgeon remove the breast tissues. So, here you can see that this is a cancerous tissue and this is adjacent normal tissues.

So ideally, the case should be that the diseased tissue should be in the center and the adjacent normal, adjust tissue should be the normal tissues. However, there could be undesirable cases where the diseased tissue would be present at the peripheries of the exact tissue. This could also mean that the diseased tissue might be present in the body of the patient that may lead to the reoccurrence of the cancer.

To assess this the surgeon removes the small chunks of tissues again and then gives to the pathologist to know whether it is normal or cancerous tissue. In histopathology test has the positive such as it is having high sensitivity and specificity. It can differentiate different types of cancers as you can see over here, but it requires skilled expertise. It is labor intensive and time consuming. In this project, we actually proposed two techniques, optical as well as acoustic technique.

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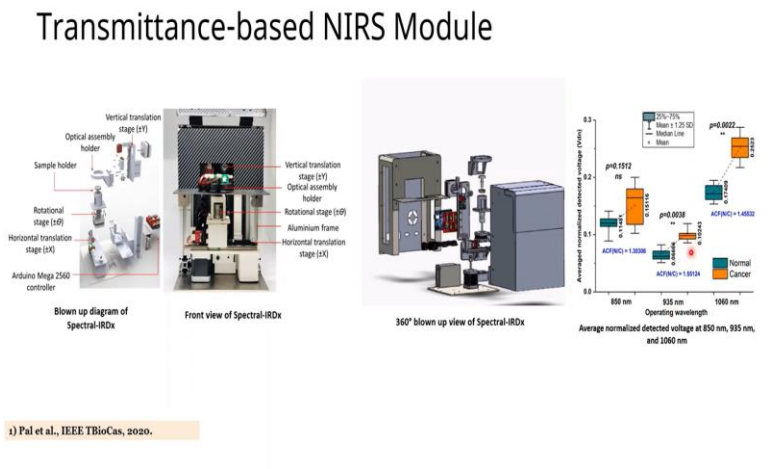

First one is the transmittance based near infrared spectroscopy module. And over here you can see that this is the blown up diagram of the spectral IRDx we call the system as spectral IRDx tool and is the actual design that we have developed. So here you can see there are three translation stages. The one is the horizontal translation stage that moves the sample, the vertical translation stage that moves the optical assembly holder and the rotational translation stage which rotates the sample holder to operate under different wavelengths.

In this you can see that we have worked with 850 nanometers, 935 nanometers and 1016 nanometers, and we were able to differentiate normal from cancer with good statistical significance.

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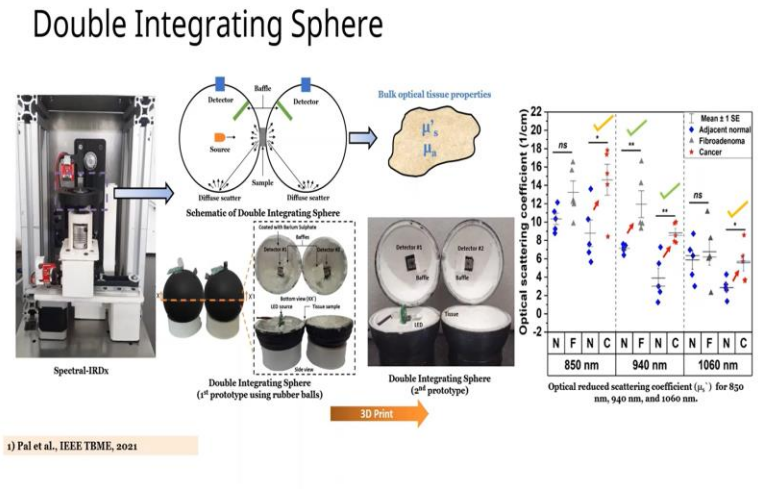

The next was to quantify the optical properties of the tissues. So, we use the double integrating sphere, where the source of the light could be continuous wave. This is how the schematic looks like; the source led incidents over the sample tissue over here, which actually backscattered as forward scatter the light. And that backscattered light is detected by the detector number 1 and forward scattered light is detected by the detector number 2. With this detected voltages, we can actually back calculate the scattering coefficient and the absorption coefficient by solving the inverse problem.

So, this is the first prototype, we slice the rubber tissues into halves and then we place the LED's, tissues and the baffles and the detectors over here. And then we code the internal surface of the spheres with barium sulfate, which reflects almost 98 percent of near infrared light source. The second prototype was 3D printed as you can see, this is a more sophisticated design and we then place the tissue and take more measurements.

What we observe is that the red points that you see over here are for the cancer and the blue ones are for the normal. We observed that the cancerous tissue were having higher scattering coefficient as compared to normal, I had all the three wavelengths 850, 940 as well as 1060

nanometers and the difference of scattering coefficient between normal and cancer were found to be statistically significant, especially at 940 nanometers.

We also differentiate or we also calculate the scattering coefficient for fibroadenoma tissues and compare it with the normal tissues. Over here you can see that in the fibroadenoma tissues the scattering coefficient was more as compared to the normal tissues.

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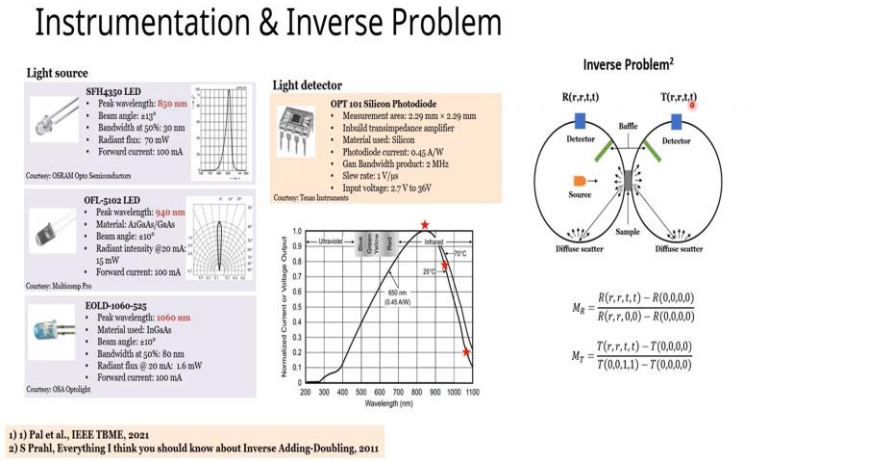

To talk about the instrumentation, we work with three different LED's so for 850 nanometers we use the LED from Osram Opto semiconductors, you can see that it has a very low beam angle. And then we also put a Fresnel lens in front of the LED to focus the light source. And then we use for the 940 nanometers we use the multicomp pro LED light source again a very low beam angle and low power LED source.

For the 1060 nanometers we use the OSA Opto light led over here as you can see. The detector we used OPT 101 from Texas Instruments and the good part of this detector is that the silicon photodiode is connected with the trans impedance amplifier within this chip. So, we do not need to have an external circuit attached to this photodiode.

This is how the spectral response of the photodiode looks like. The star are the points where we are actually measuring our data. Inverse problem was solved using the inverse adding doubling method proposed by Scott Prahl. This is a Monte Carlo code and we use the data from the detectors and then we back calculate the scattering coefficient and absorption coefficient.

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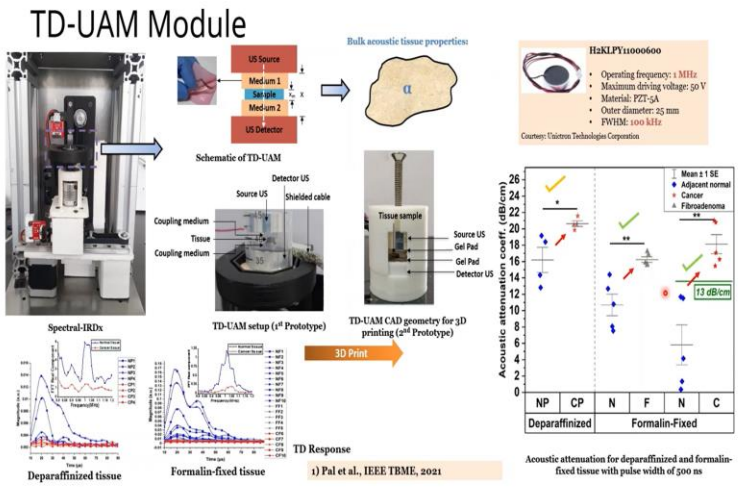

The second system that we propose was time domain ultrasound acoustic measurement module. Over here we again observe over here is the ultrasound source and this is ultrasound detector and then it has a coupling medium. So, the, so to reduce the impedance mismatch between the transducer and the tissue sample.

With this, we can actually quantify the acoustic tissue properties of course attenuation for example. So, this is the first prototype that you can see over here, we place the ultrasound transducer, then we have the coupling medium, then the tissue again the coupling medium and then the ultrasound. We then 3D print the design as you can see over here which is now actually more sophisticated design.

For this we used the 1 megahertz ultrasound transducer which is built using PZT-5A material piezoelectric material and it had a FWHM of 100 kilohertz. To this transducer will give an input pulses of varying pulse widths starting from 200 nanoseconds to 1000 nanoseconds and we found that at 500 nanoseconds, the system was having highest sensitivity.

So, this is how the time domain response looks like for the deparaffinized and formalin fixed tissues. You can observe that for the blue graph that are for normal tissues the transient response is time domain response like higher as compared to the cancerous tissues, in the case of both deparaffinized as well as in formalin.

From this we gave this to this mathematical model the time domain response the peak value of the time domain response and from there we calculate the acoustic attenuation coefficient and we found that for the cancerous tissue, the acoustic attenuation coefficient was more as compared to the normal tissues. We also observed the fibroadenoma issues were having also higher an acoustic attenuation coefficient as compared to the normal tissues. And for both the fibroadenoma cancers, when we compare it with the normal tissues, both were found to be statistically significant, especially for formalin fixed tissues.

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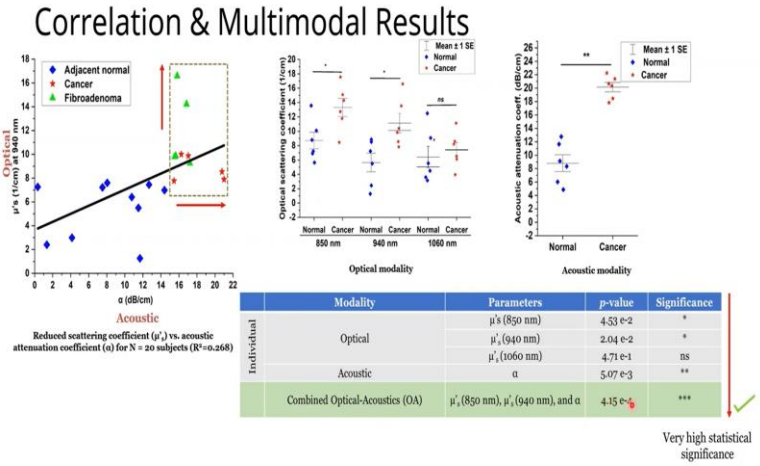

We then try to understand the correlation and the multimodal results. So, we then perform the multimodal measurements for n = 6 tissues. And we found that again we showed the same trend that the scattering coefficient for cancer was more as compared to the normal tissues. And the acoustic attenuation coefficient for cancerous tissue was more as compared to the normal tissues.

We tried to understand the correlation between optical and acoustic property, so we tried to plot the normal as well as cancerous as well as the fibroadenoma tissues. And in the x axis, we have the acoustic coefficient and y axis we have the reduced scattering coefficient. And we found that the first thing was that the optical properties correlated positively with the acoustic properties and all these cancerous and the fibroadenoma tissues lie on the first quadrant of this graph.

We then try to understand for individual test, we see the statistical significance. And then we saw that as we go on increasing the number of tests, for example, combined optical and acoustic modality, the statistical significance actually increased a lot, see from the p value over here.

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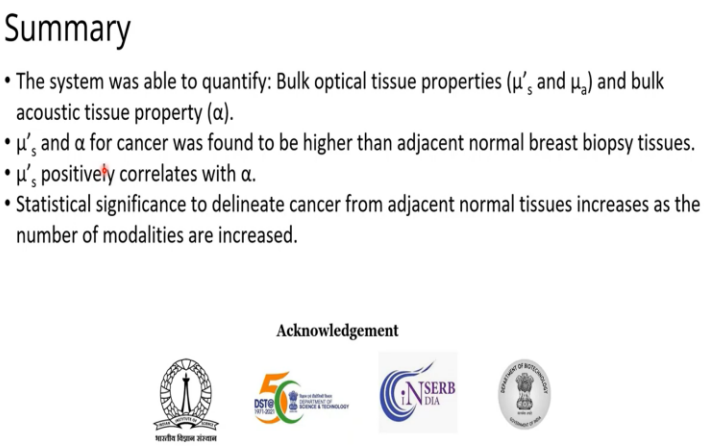

I would like to summarize the work that we performed over here, we were able to design and develop a system that would quantify the optical as well as the acoustic properties of the tissues. We found that the scattering coefficient and the acoustic attenuation coefficient for cancer was higher as compared to the normal tissues. The scattering coefficient correlates positively with the acoustic attenuation coefficient. And we found that the statistical significance improves as you go on increasing the number of modality of test.