Introduction to Biomicrofluidics
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Lecture - 20
Microfluidics for Healthcare (Contd.)

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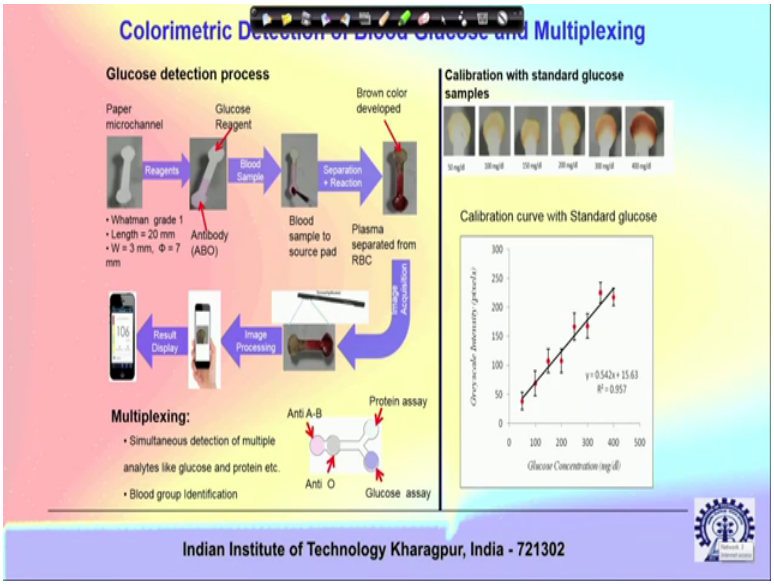

In the previous lecture, we were discussing about pathological intervention using blood samples by the help of paper based Microfluidics. So, now what I will do is, I will talk about some illustrative examples, what we have done in our lab as specific pathological tests. So, for example, this is the demonstrative device where in the slide you see there is calorimetric detection of blood glucose, and also multiplexing that there is glucose assay as well as protein assay in the same microfluidic device.

So, normally the not just blood glucose detection process, but any other detection process; where the blood plasma is involved, typically the blood plasma has to be separated from the whole blood. And then the analysis of the blood plasma is done. So, here you can see that in this example we have paper micro channels with Whatman grade 1, the length is 20 millimeter, width is 3 millimeter, and the diameter or the depth is about 7 millimeter. So, there is a glucose reagent that is loaded and there is a antibody which is loaded. So, then the blood sample is taken to the source pad all this steps are clearly given in the diagram.

Then there is a separation and reaction, and there is a brown color that is developed. Now, this color has to be analyzed. So, there is an image processing and there is a laser display. So, you can see in a right hand view graph, we have given that the colored image for various concentrations of blood glucose like starting from 50 milliliter deciliter to 400 milligram per deciliter. So, 50 milligram per deciliter is you know is quite a low value of the blood glucose. And 400 milligram per deciliter is substantially high right. The normal fasting will be typically less than 120 milligram per deciliter so, 400 is quite high.

So now, what we plot in the graph below which is very, very important; is the intensity the grey scale intensity of the images versus the glucose concentration. So, we make a mapping of this and a calibration curve. So now, if we get an intensity from another image that is taken, we make a plot in of that data point in this graph of course, we do not make a physical manual plot, but we use the function that is fitting the intensity versus glucose concentration in the mobile or in the computer we use that function has reference to, you know, co relate the image intensity with the corresponding blood glucose correlation, and then digital readout comes out. So, one can do the both protein assay and blood glucose assay in the in the device that is being demonstrated here.

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I will come to a second example; which is a very interesting example; this is paper based kit for antimicrobial susceptibility. So, this is the patent jointly filed in cooperation with

the Indian Council for Medical Research ICMR; for working on a topic which is very much relevant to the medical industry. So, as many of you have heard, that antibiotic resistance is a very serious problem. And antibiotic resistance comes from the fact comes from several facts. One is that over use of antibiotic that is whenever antibiotic is not needed antibiotics are given. And then the patient gets immuned to such antibiotics.

The other is that incomplete course of giving the antibiotics drugs; that means, let us say a course a proper course includes particular dosage of medicine taken for a particular number of days, but the patient if patient feels better stops taking the medication because of cost or whatever reasons. And this is one reason. The other possibility is that the bacteria during their evolution have adapted in such a way that they can resist the old antibiotics very effectively. So, all these things can occur simultaneously or I mean separately and that can lead to antibiotic resistance.

So, when it is needed the most the antibiotic will not work for the patient. So, whether an antibiotic will work for a patient or not, we can make a simple low cost rapid test using the blood sample of the patient by confronting that sample with antibiotics; and see whether a particular antibiotic is working for that blood sample in killing the particular bacteria or not. And that for that simple low cost paper based devices can be used. Of course, this technology is at its infancy and lots of additional trials need to be met.

But I think this is one of the very critical applications that not just doing the routine diagnostics, but also studying antibiotics resistance or this kind of challenging propositions may need to be may actually be addressed by using the paper based technology.

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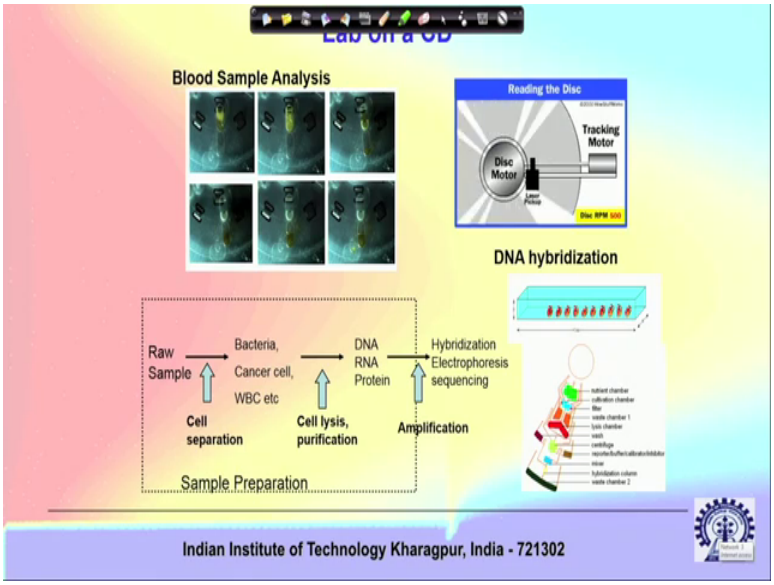

The next concept that I talk about is lab on a CD; which briefly we have touched upon earlier. So, in a lab on a CD the advantage is that in the paper based device to do a large number of tests you need to make a large number of channels on the paper. And there may be a limitation of fabrication and other things so that is itself an important design job.

Instead of lab on a paper, you could potentially have a device which you call as lab on a CD. So, the lab on a CD is a rotating microfluidic device; which looks like a CD used for external data storage, but it is actually a polycarbonate based material. There may be large number of micro channels on the CD, and if the CD is rotated by a single rotation fluid can move along the radial direction on all the micro channels simultaneously. And this is possible by centrifugal action.

So, then the advantage of this CD therefore, is that you can do a large number of tests simultaneously by actuating a single rotation. And the rotation can be actuated by a very simple motor. Not only that, many other possible tests could be done in conjunction with this; for example, you could have many other activities which are relevant to doing the test. Like for example, if you are doing a DNA RNA or protein analysis. The first stage before the doing the DNA analysis is to break the cell and bring the DNA out of it which is called as cell lysis.

So, for doing cell lysis CD can be used as a platform. So, staring from the raw sample to cell separation to cell lysis to DNA RNA amplification hybridization or electrophoretic sequencing, all this processes together can be done on a single CD based microfluidic platform.

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So, there could be several advantages associated with CD based device. Of course, the paper based device appears to be fugal and it is much more simple to design and fabricate. So, one could use either the paper based or the CD based device depending on the specific application.

Now, the next question; from point of care where do we move on? We move on to a concept which we call as extreme point of care diagnostics. So, as I mentioned earlier, that in the rural setting a big challenge is that the power supply is not very regular. There is dusty and humid and hot environment, and the storage of chemicals is not so easy. The trained man power is not available.

So, all these challenges lead to the consideration that from point of care diagnostics where we do the standard calorimetric detection based technology; instead of that in extreme point of care diagnostics, can we do the same thing with electro chemicals sensing, or electrochemical sensing based diagnostics? This is an ongoing area of research, and our research group is currently part of doing this exercise in a national initiative called as imprint; where we have collaborators from other institutes, and also

Professor Mark Madu from the University of California at Irvine is one of our consultance for this project.

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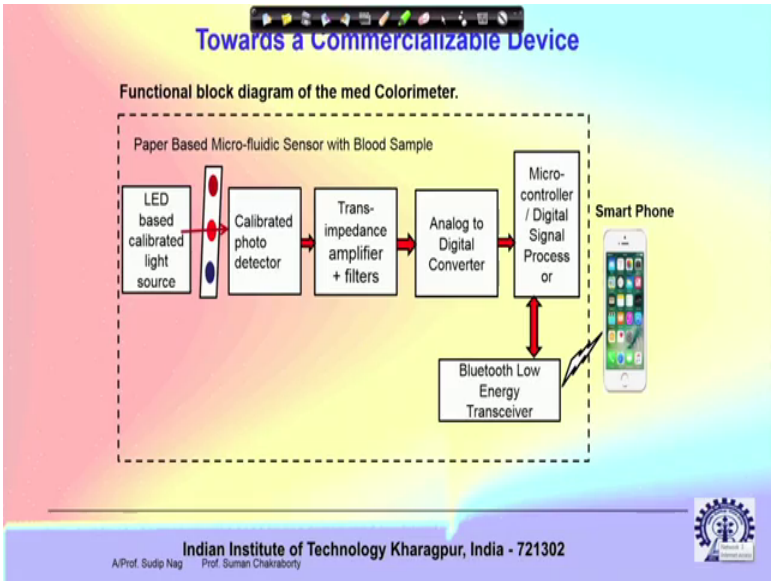

Now what next? So, we have started with a very simple idea of take a drop of blood. Load it on a device and do some test that you want. In laboratory even in field for one or two isolated cases, it may sound to be trivial. But how to make a commercial device out of it which can be used in the context of rural health care? So, the next agenda of my talk is towards a commercializable device.

So, there is needs to be an electronic box type of element; which essentially contains certain electronic components or electronic equipment, which will make sure that the colorimetric signal or electro chemical signal whatever signal, that is coming from the paper based or any other microfluidic device that is sensed, and then transformed into an electronic signal. So, our research collaborator Professor Sudeep Nag has developed a device; where you have a led based calibrated light source. There is a calibrated photo detector and there is a trans impedance amplifier and filter. And there is analogue to digital convertor and micro controller or digital signal processor.

So, these make sure that the colorimetric signal that is obtained from the microfluidic test that is essentially converted into a digital signal. And then by Bluetooth low energy transceiver this information is transferred to the mobile phone. And the mobile phone

now has the information, the mobile phone that the health worker or the patient is caring will now be having the information on the result of the diagnostic test.

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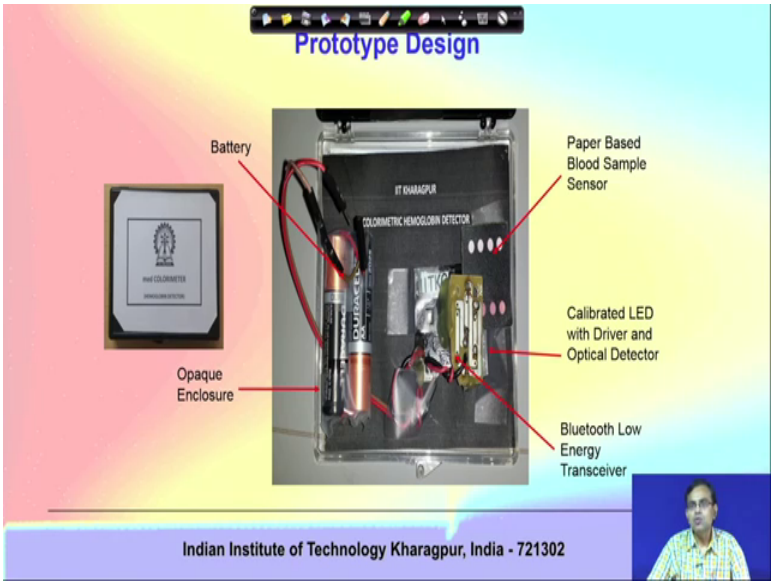

So, this is the prototype that we have designed at IIT, Karagphur and we call it mate colorimeter. And just to make you impressed about the kind of hardware that is needed for making this happen. This is the inside of the prototype. From the outside it will look like sleek box, but inside the box you will have the paper based blood sample sensor. Calibrated led with driver and optical detector, and Bluetooth low energy transceiver.

And on the top of that we have opaque enclosure of course, it is battery operated. So, currently research is going on, on how this battery operated technology could be improved in a sense that using low power or even the streaming potential; that is, generated from the transport of the blood sample within the device, this kind of device could be self-energized.

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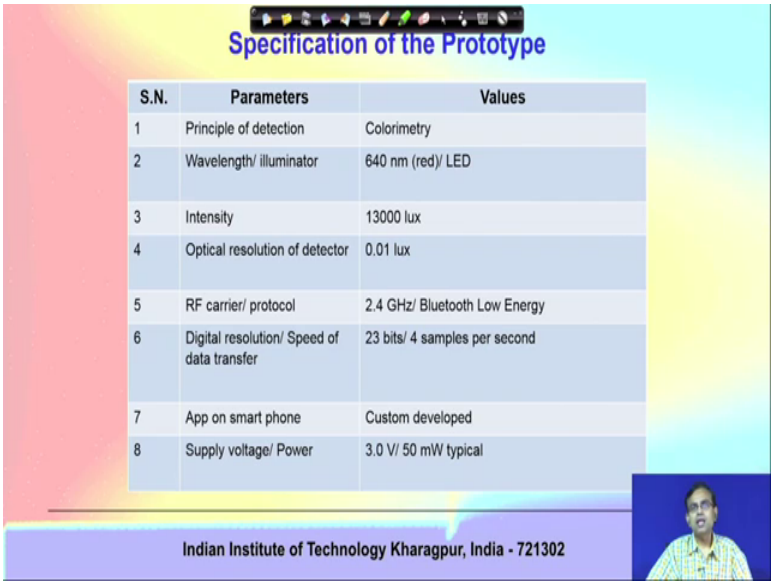

So, that could itself be a remarkable advancement in the technology, where such devices become more and more independent of the power supply. So, there are certain important parameters of this prototype. I will not read these parameters out, but I would just mention what are the important parameters wavelengths of the light source, intensity, optical resolution of the detector, RF carrier protocol, digital resolution or speed of data transfer and supply voltage or power. So, based on this information transfer to the mobile phone, smart mobile app can be developed. So, the patient himself or herself can click the mobile app and get the desired information on the diagnostic test.

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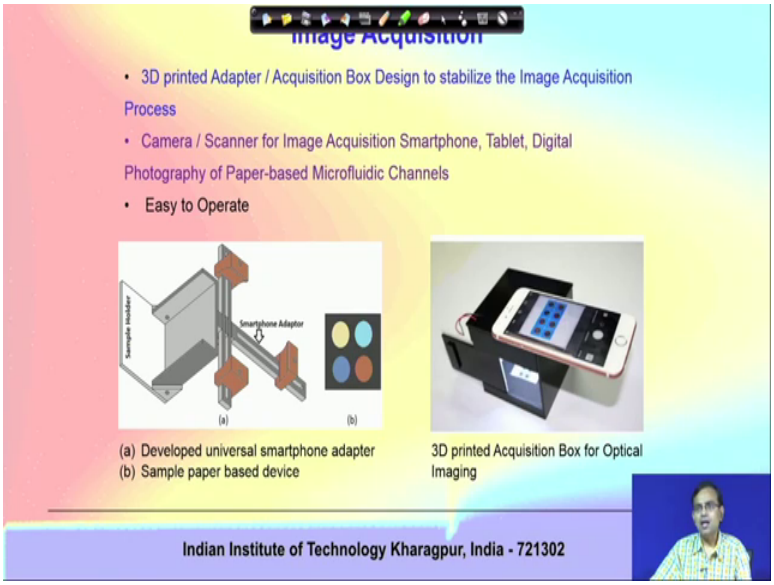

So, now in all this devices image acquisition is a challenge. And the challenge of image acquisition comes, because of various things. One is that the smart-phone camera or the camera itself that is not very rigidly placed. Or there is a disturbance from the ambient which creates the noise to the image that is grabbed. So, the ambiance collection factor has to be applied, but more importantly the camera has to be placed in an appropriate holder. So, currently we are trying to use the 3 D printing technology, to come up with the camera holder, that can hold this kind of camera for grabbing the colorimetric signal most effectively.

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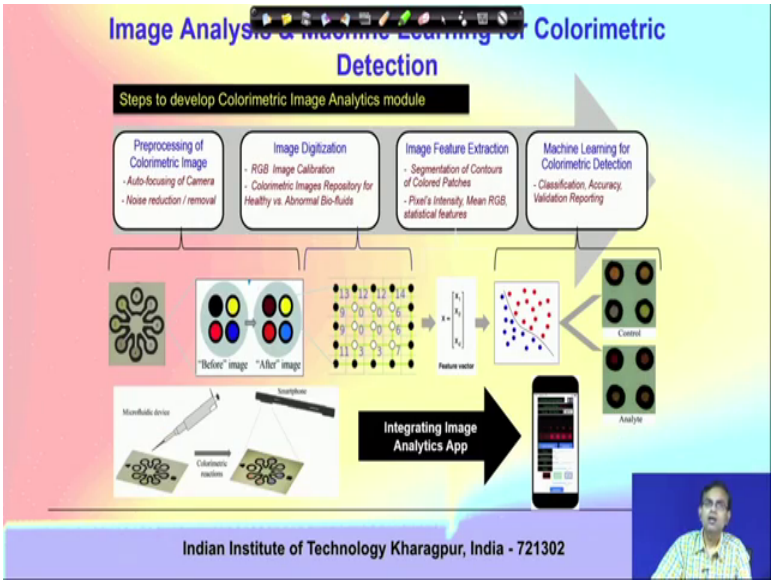

So, the other aspect of this research is image analysis for colorimetric detection. So, there is preprocessing of the colorimetric image digitization, image feature extraction. And also one can integrate machine learning for colorimetric detections so that the results from these microfluidic tests may eventually be used as a tool for decision making; in a by transferring these information to a large cloud, it is possible that one can use machine learning technologies to you know make further analysis of these results.

So, you can see the wide gamut of specializations required, starting from fluid mechanics, biotechnology, electrical and electronics engineering to image analysis and computer science and machine learning.

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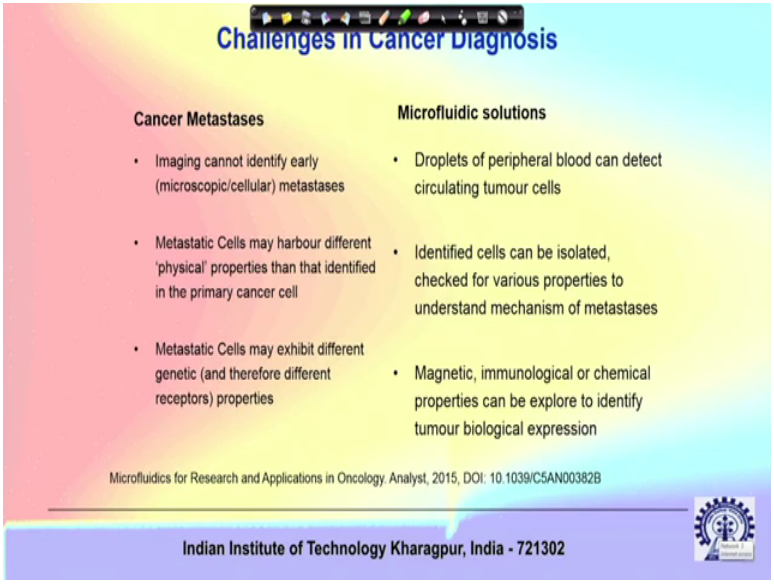

These are some of the activities that require to be executed to make such a device happen. Finally, in today's lecture I will come to some specific examples of challenges in cancer diagnostics. So, in cancer diagnostics, there are many many challenges. And I will come to one such challenge and try to provide a microfluidic solution for that. So, as you all know, that when cancer progresses, the most lethal stage of the cancer comes when the cancer cell from it is origin in the human body or in whatever animal body, the cancer cell moves from it is origin to a distant location by blood stream and creates a new cancer state at the new location where it has migrated. This is known as cancer metastases.

So, during these metastases, circulatory tumor cells are shred in the blood stream. And currently there is a growing tendency that instead of doing a traditional biopsy, this circulatory tumor cells can be isolated and characterized, and then based on simple blood test cancer diagnostics for advanced stage cancers can be done. And this technology in the medical world is commonly known as liquid biopsy. And in the general scientific world, it is called as analysis of circulatory tumor cells or CTC

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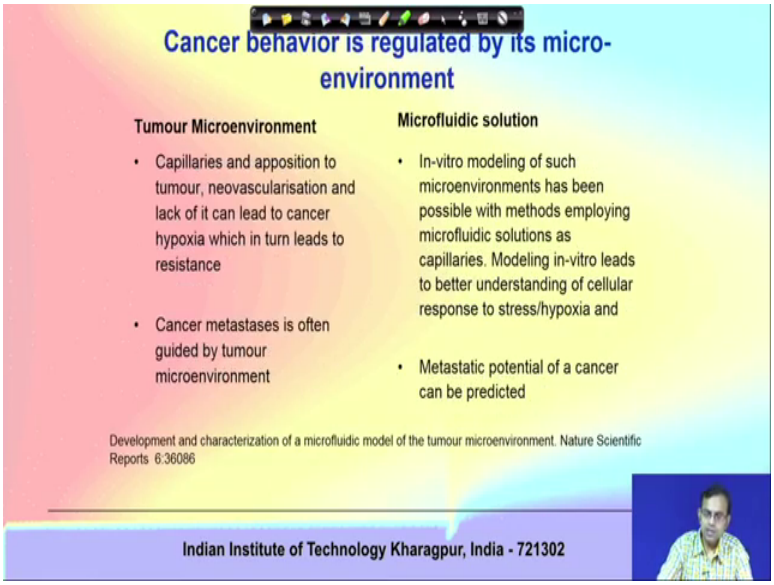

So, when the tumor cells circulate, and it is possible that in vitro modeling of the microenvironment through which the cancer cell is circulating is created; that means, an engineering device is created; which mimics the blood vessels across which the tumor cell is circulating and proliferating, and using the understanding on the basis of what happens in the in vitro device, it is possible to have a decision or make a decision on the stage of cancer by using one drop of blood from the patient. And using this actually the metastatic potential of cancer can be predicted.

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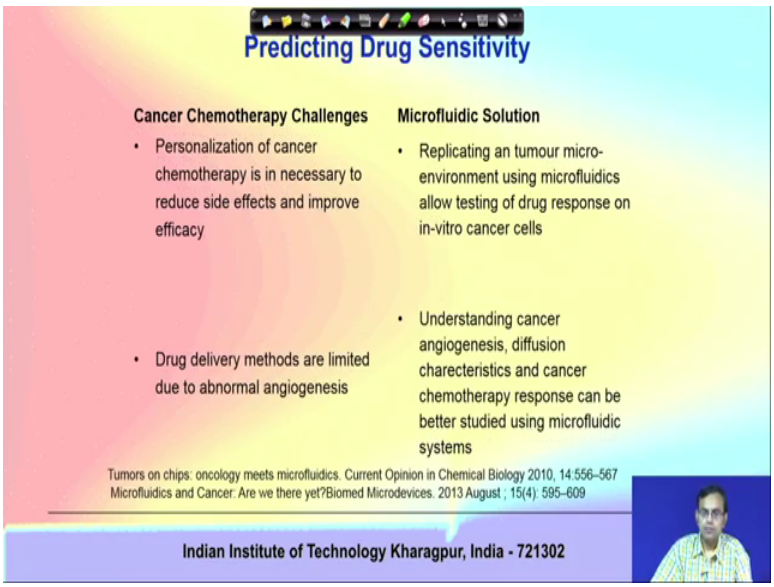

Not only that, when we isolate the tumor cells, how do they respond to drugs? So, in the in vitro diagnostic platform, one can pass different drugs, and make a test of which drug will work for that particular type of that particular type of cancer in one particular patient. So, this is a very personalized medical approach; which is growing prevalent in the modern era. So, we are now going for personalized treatment, and we see very often that treatment of not just cancer for any disease. A particular medicine will work for one particular patient, but it may not work for another patient having similar symptoms and similar conditions.

So, very rapidly one can make the assessment of whether the drug which you are interested to apply on a patient will work or not, or particular chemotherapy drug for example, will it work or not. So, instead of waiting for by making trials on the patient and see that eventually whether it works or not, or instead of going through experience based understanding; one can make engineered microfluidic devices test that drug on the blood sample or actually on the isolated tumor cell from the blood sample, and then figure out whether it is able to successfully come back the cancer under such conditions.

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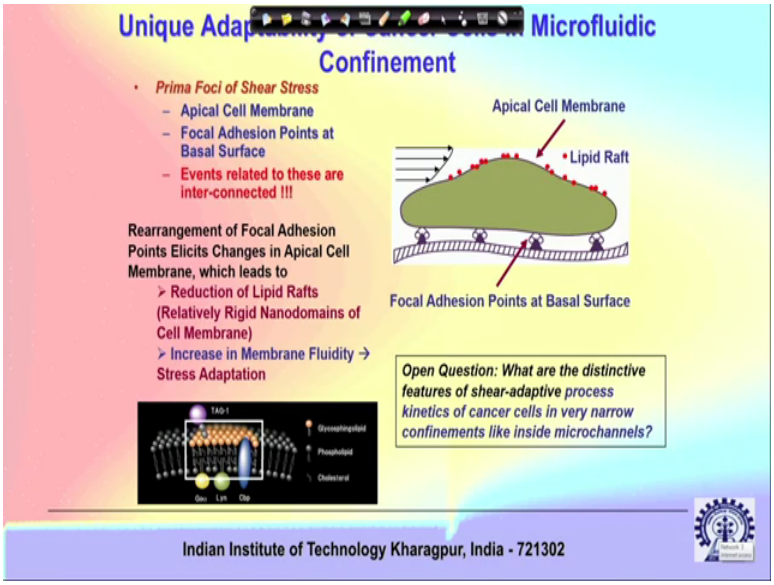

So now when we come to the issue of the interaction of drug with a cancer cell, there is a interesting issue also of how a cancer cell adopts in a microfluidic confinement. Because when a cancer cell adopts in the human body in presence of a drug, one has to isolate the chemical effect of the drug with the physical effect of the stress that is coming from it is

surroundings or microenvironment; which is called as tumor microenvironment in the biological world. So, to make a model of that, one of my former PhD student Dr. Kamal Das who is now faculty member at the TIFR, Hyderabad, had made a very interesting device which is called as ultrasoft PDMS based traction force microscopy on a chip.

So, in simple terms what he has made is a very flexible microfluidic platform, on which a tumor cell or a cancer cell is sitting. So, then what happens is, that if there is a stress on the cell there are imbedded particles or fluorescent beads in the base of the microfluidic channel, and this beads will be displaced. If the cell is adhering to the channel, and if the if there is a stress on the cell and the stress and the cell gets deformed the beads which are there in contact with the boundary of the microfluidic channel, this beads will be displaced. Now, by taking an image of the displaced beads by sing an appropriate microscopy and camera, it is possible that, it is possible that what can be done is a that by inverse Fourier type of analysis the stress which is there on a cell could be predicted.

So now if we come to this diagram, there is a flow on the cell. So, when there is a flow on the cell, first the because of the stress in the surroundings, the legs of the cell are first disrupted or disturbed. These legs are called as focal adhesion points. So, because of the disturbance on the legs of the cells there is a mechanical stress. And this mechanical stress transmits from the bottom of the cell to the most distant part of the cell membrane which is called as epical cell membrane.

So, in the epical cell membrane when it reaches when the disturbance reaches the epical cell membrane, and it does by a remarkable process called as mechanotransduction; the cell membrane now responds in a certain way. So, the cell membrane could have lipid and also it could have lipid raft, which is a solid type of entity in the lipid. So, what happens is that because of the stress on the cell membrane, the lipid raft tries to hide from the stressful location. So, what the lipid raft tries to do is, it tries to escape from the cell membrane and it goes to inside the cell. And then the cell membrane therefore, becomes more fluidic. Because it becomes more fluidic, it becomes more malleable, and it can have a unique shape adaptation to overcome the stressful barriers in the microfluidic confinement.

Where is the microfluidic confinement in the human body? So, in the human body in the hierarchy of blood vessels, you start with large artery, large veins, small arteries, small

veins, arterioles, veinoles and then micro capillaries. These micro capillaries are like micro channels. Perhaps, like this kind of micro channels are very uniquely made by nature. It is very difficult to mimic this, but when the cancer cell tries to move through these micro capillaries by this unique lipid raft dynamics, the cancer cell can survive the stressful condition. And we have shown by research that, the lipid raft dynamics although it is there also in normal cells, but in cancer cells the lipid raft response is much faster.

So, lipid raft very quickly understands the stress, and it make sure that very rapidly the cancer cell makes lipid raft internalization from the cell membrane to inside the cell and makes the cell to adopt to the stressful environment. So, as a resistive measure; for example, for advanced stage cancer, one could have a targeted drug delivery of lipid raft inhibiters. So, in the cell membrane, if you could target lipid raft inhibiters, then the lipid raft will not be allowed to move from cell membrane to inside the cell. They will still remain on the cell membrane and therefore, the cancer cell cannot have this kind of shape adaptation to survive the stressful condition, and that is how the metastasis can possibly be prevented.

So, this discussion gives us a clue that from understanding the biophysics of cancer progression, how can a medical protocol actually be designed.

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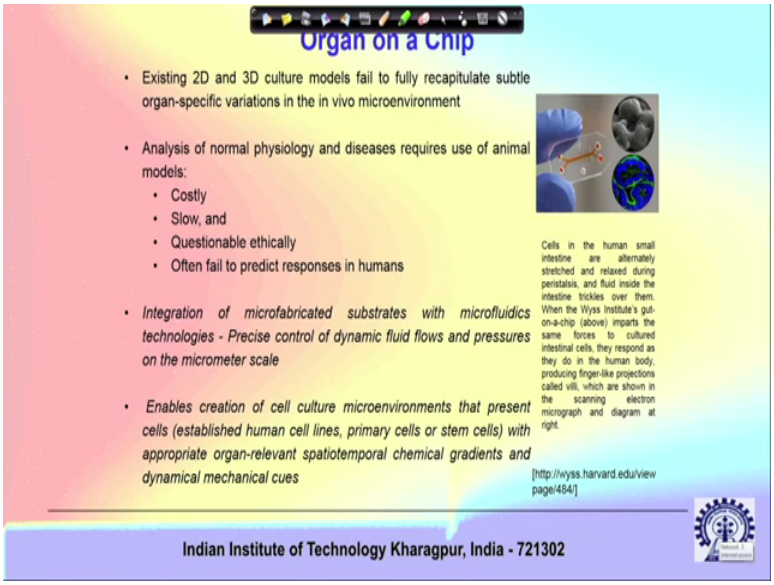

Again what next? So, we have talked about the influence or impact of the drug or stress on a cancer cell. But in reality in human body you do not have one cancer cell, you could

have a large number of cancer cells, and therefore, it is very important to understand how a organ behaves in response to a fluidic or chemical stimulus, not just a single cell.

So, therefore, from the concept of cancer cell on a chip or cell on a chip we go for organ on a chip, or may be at least a tumor on a chip; where we construct or a mimic or we construct or mimic an organ like or a tumor like environment within the microfluidic device.

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And then what else? Eventually we plan for some research which has already been initiated globally is called as human body on a chip. It might appear to be like a science friction, but it is not literally a human body on a chip, but all of us understand that when there is a treatment of a particular disease going on, then it could have side effect on other organs, for which the treatment is not targeted.

So, for example, a toxic chemical as a drug could have an adverse effect on liver or kidney. So, instead of making mimicking a single organ on a chip, one could mimic a collection of multiple collection of disparate cells, and then put them under the embeds of different organs like heart mimicking cells, lung mimicking cells, kidney mimicking cells, liver mimicking cells, and make connected microfluidic networks across that. And then this mimics in some way at artificially engineered miniaturized system that replicates the human body in an artificial environment. This we call as human body on a chip.

So, if this human body on a chip is effectively used for making drug trials, then expensive are involved animal trails where there are large number of ethical and other issues could be minimized. Those could only be made in the last stage, but in between engineered devices could give answer to outstanding biological problems. And that is what bio microfluidic research is all about.

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Finally, I would like to acknowledge the scientists and students, who have contributed for this part of the last 2 lectures; that I have presented and their names and photographs are there in the slide that I have just shown.

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And also so, these are the scientists and various departmental colleagues' former students and researches, other researchers and students who have contributed significantly to this work that I am presenting today.

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And, also I would like to thank the collaborative partners and funding agencies, for allowing us to do a important research in this domain. And I believe that with further sustained efforts with this research we will be in a position to make a difference in the healthcare scenario, not just in the Indian context, but also in the global context

Thank you very much.