Introduction to Biomicrofluidics
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Lecture – 02
Introduction to Biomicrofluidics (Contd.)

In the previous lecture we gave you a perspective of what is micro fluidics, what is biomicrofluidics and what could be the potential gamut of applications.

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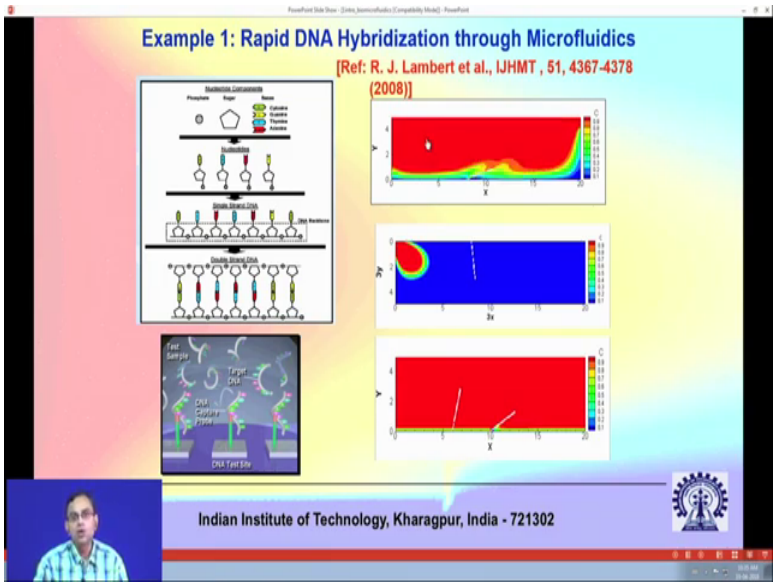

In this lecture we will talk about certain specific example applications based on some research work done by our research group. So, the first example is DNA hybridization, so what is DNA? You know of course all of you know the full form of DNA that is deoxyribonucleic acid. So, DNA has double strand and just to give you a basic feel of the DNA architecture, if you look at the top left part of the view graph. So, you have a single stranded DNA associated with a phosphate group which gives negative charge to the DNA or sugar and 4 nitrogen containing bases A T C and G.

So, if you look at each strand DNA strand it is linked with an alternate sugar and phosphate, sugar and phosphate and each base is connected with sugar. So, now how one strand of the DNA is linked with another strand look at the this part of the figure, so basically one base from one sugar it tries to combine with it is complementary base from

another sugar of another strand and which bases combine A wants to get combined with T and C wants to get combined with G and this is by virtue of hydrogen bonding.

So, this is a well known phenomenon and the question is how can you use these for diagnosing diseases. For example, so there are many diseases for which there is a particular sequence in the DNA for which you know there is a particular sequence of the bases which indicate a particular disease. For example, if you have a sequence like AT GG CC AA like whatever I mean some kind of sequence that particular sequence may be an indicator of the existence of certain type of disease, it may not be as simple as that. But, I am trying to simplify this to an extent that somebody with an engineering or a non biology background can understand and appreciate.

Now, how do you know that that sequence is present, so what you can do is if you want to interrogate whether one sequence is present you put a complement of that sequence on the wall of a micro channel or forget about micro channels say any channel. Now of course, why micro channels will be better is of course the reason is that I mean you can miniaturize the device, have lower sample consumption, although at all the advantages that we have talked about you can make a low cost device and so on.

Now, the as a first step what you do is you whatever DNA sample you want to interrogate you are you have to first prepare the DNA sample, how do you prepare that you break the cell and bring the DNA out of it this is called a Cell Lysis a very important procedure. So, after breaking the cell and bringing the DNA out of it you heat the DNA and if you heat the DNA the double stranded DNA will be broken into 2 single strands. This is called as melting of DNA or denaturation of DNA. Interestingly why this is called as melting of DNA is because, the double stranded DNA broken into single strands is almost like a melting transition a phase transition where you know solid gets molten into liquid phase, thermodynamically very much similar.

Now, once you have the melting of the DNA then this molten DNA it is or the single stranded DNA samples it is passed through a channel and the complimentary strands are already immobilized. These are called as capturing DNA or DNA probes which are located at the wall of the micro channel. So, if you look at this figure so you can see that at the wall of the channel you have that capturing DNA probes and in the sample floating are single stranded DNAs. If the strands are really complementary to the capturing DNA

these will be captured and a fluorescence will be emitted that will indicate that successful hybridization has taken place.

Now, how will this DNA hybridize? So, this DNA has to move through the sample and if there is no external force applied that DNA will move purely by diffusion, but diffusion is a very slow process. It may take years even, to complete one successful DNA hybridization. So, alternatively what technology people have been using for a long time it is DNA electrophoresis.

What is electrophoresis? Electrophoresis is essentially movement of a charged body relative to a fluid in the presence of an electric field. So, if you have a DNA we have clearly seen that it has a phosphate group by virtue of phosphate group it has negative charge. So, if you apply electric field the DNA will move and you can orient the electric field in such a way that the DNA will move very rapidly to the wall of the micro fluidic channel.

But the problem is that with electric field there is a joule heating in the sample and because of joule heating the hybridized double stranded DNA can be again broken into single strands. So, you cannot use a large electric field, so what could be a potential solution? So, we were working on this project on this research question with our collaborator Professor Marc Madous research group from the University of California at Irvine supported by one Indo US project and the solution that was arrived at and it was.

In fact, the research work of one of our joint students Ruth Lambert, I mean she was working on this problem as she visited our institute IIT Kharagpur for some time and this is the numerical solution to the problem design problem and which was eventually translated into fabrication. So, what was the solution? So, you look at this diagram, soon the wall of a micro fluidic channel you keep a mechanical actuator which is a flap made of a soft flexible material called as polypyrrole and there is an activation by which these flap oscillates.

So, when this flap oscillates what happens is that the DNA solution, this DNA solution this flap simultaneously acts like a mixer and a pump and once it is done let me run this movie again. So, once this is done the DNA solution it is virtually forced to move towards the wall where the capturing probes are located. So, the probes can be located anywhere in this case you have instead of one probe a couple of probes. So, I will tell

you that why more than one probe may be necessary because, if you have a sorry more than one flap is necessary the reason is that maybe with a single DNA sample you are trying to interrogate multiple sequences, whether sequence x is present or sequence y is present or sequence z is presence present like that.

Now, if you want to interrogate these kinds of multiple sequences, then what you can do is you can put multiple capturing probes on the wall and if all the DNA falls on one capturing probe other probes will not get the DNA sample. So, you have to optimize the distribution of the DNA on the wall of the channel, so what do you do here let me run this movie once more. So, you have in this example 2 flaps so these flaps are moving with certain amplitude and frequency and phase difference and what should be the best amplitude frequency and phase difference. So, that you know there is optimal distribution of DNA that is a very interesting design problem.

So, what I want to impress upon you is that see in this example we started with the consideration of DNA which is a pure biological entity and we ended up with a problem which is a computational fluid dynamics problem it is a fluid structure interaction problem, the dynamic interaction between a fluid and an oscillating flexible structure. So, the whole idea is to tell you that this is what about bioengineering that you know, starting with a fundamental problem which may be interesting for biologists you can translate that in the form of an engineering problem and try to solve the engineering problem to help the biologists that should be the motivation.

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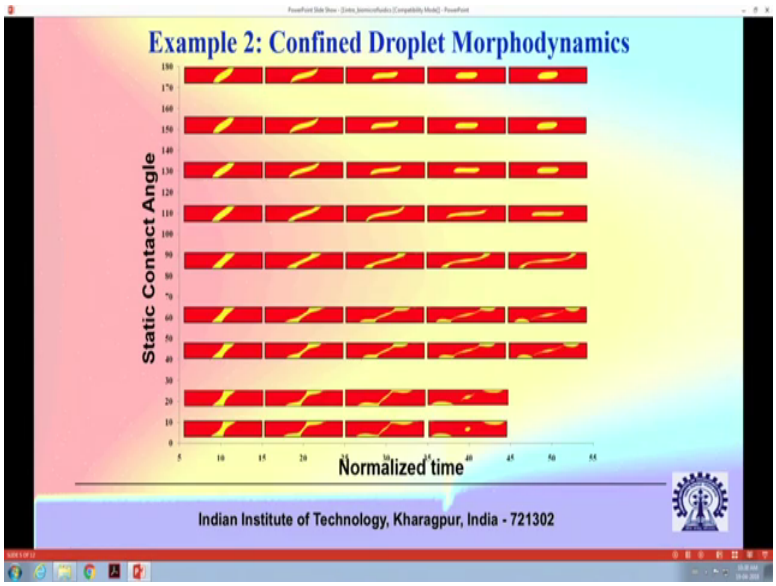

In the next example I will talk about confined droplet dynamics. This is a very interesting area and in micro fluidics droplets are studied for many reasons, I will not get into the details of this slide, but the whole idea is to let you know that how a droplet morphologically evolves based on the wettability of the surface on which it is there. So, that diagram is shown in this slide this is entirely a computational exercise.

Now, what motivates us to study droplets, see in the human body you have biological cells. So, cells are very complex we all understand that cells are structural and functional units of life, but it is very complex to analyze from a mechanistic viewpoint. So, to make simplifications we can start with something which is very crude which is much much away from a cell, but like a cell it has an interface which demarcates it from it is surroundings and that is a droplet which is dynamically evolving.

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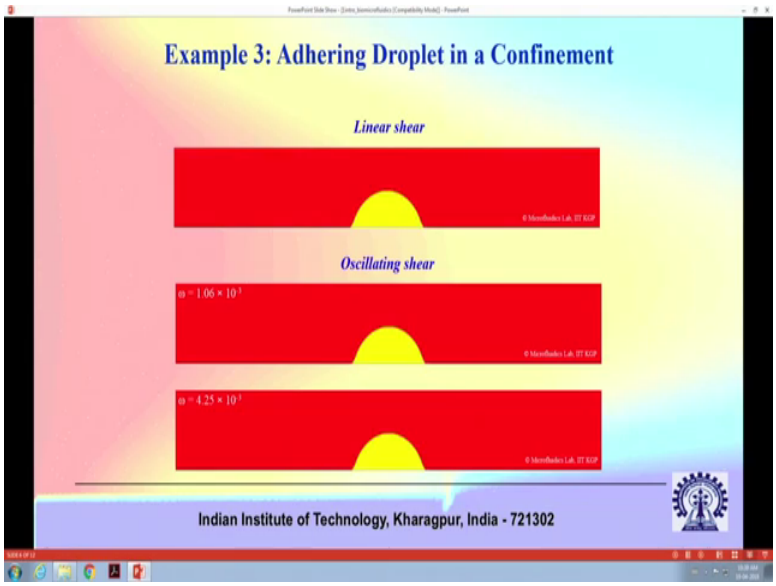

Now, in human body if you have cells these cells are subjected to oscillatory flows of blood instead of steady flows.

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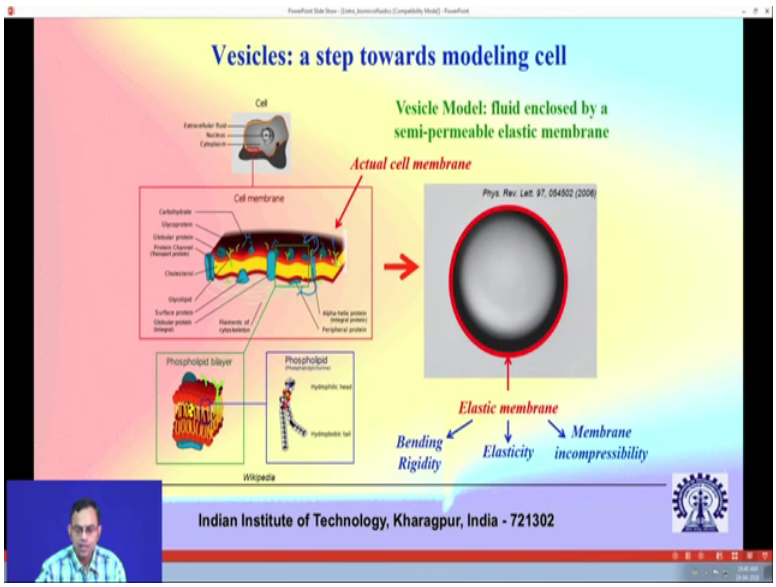

So, we have also studied as a different example how oscillation in the flow is affecting the dynamics of blood sorry the dynamics of a droplet in a confinement. But I do not want to spend much time on that because that is: what is standard micro fluidics, I would essentially try to spend more time on the biological aspects.

So, now we have seen that to some extent we have tried to understand the dynamics of droplets, but cells are much different from droplets all of us understand from our perception that cells are not really like droplets. So, we need to upgrade the understanding from cells to the understanding of droplets, so there is an in between concept which is called as vesicle.

So, a vesicle is like a balloon filled with water, so a vesicle the has a membrane and therefore it is a membrane fluid interface that forms the interface of a vesicle unlike a fluid interface of a droplet. That mean, that vesicles are much more much much closer to cells as compared to droplets. Of course when you have a membrane fluid interface you add additional complexity to the fluid mechanics problem, you have bending rigidity elasticity membrane incompressibility all these aspects taken into account. But you know like in this viewgraph we are showing this vesicle as a crude version of the very complex cell which is shown in the left.

So, the question is that what demarcates the vesicle from a cell other than the architecture, the cell has a very complex architecture the vesicle does not have that complex architecture, the cell has a very complex functionality the vesicle does not have a very complex functionality but these are you know more subtle things. The primary point that I have to highlight, I like to highlight is that the a cell has life whereas a vesicle has no life.

So, question is how to model life it is a very interesting and open ended question in the world of fluid mechanics and in the world of thermal sciences, that a cell has certain energetic that is it is driven by certain free energy that is available with a living cell to perform certain operations. But how to couple that consideration of a living cell with the traditional equations in fluid dynamics which are also used to a large extent in micro fluidics, so this is a big research question of course, lot of research activities are going on towards understanding the dynamics of cells. And, in possibly the next slide I will try to bring in an example where I will try to show you that how biomicrofluidics can try to solve outstanding research problems in understanding the behavior of cells.

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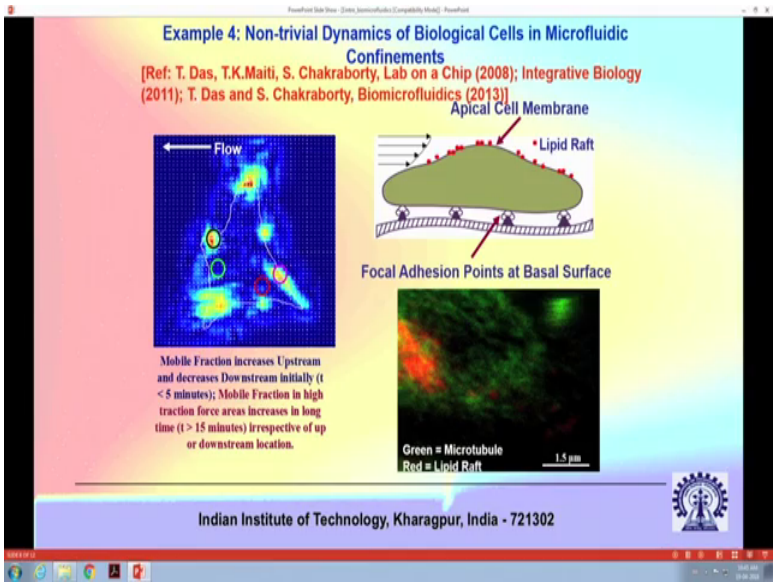

So, this is a problem which we tried to address h through one of my former PHD students Dr. Thamal Das, who is currently a faculty member at TIA for Hyderabad and this presentation is essentially part of the PhD work that he did jointly with myself and Professor Maiti. I will try to give you a broad picture motivation, the motivation is as follows let us think about cancer progression.

So, when cancer progresses the most lethal stage of cancer comes when the cancer cell metastasizes; that means, the cancer cell from it is origin moves to a distant location within the human body through the bloodstream and creates a new cancerous trait at the new location, in the process the cancer cell has to proliferate through different blood vessels. And, if you imagine the hierarchy of blood vessels in the human body you have large arteries, large veins, small arteries, small veins arterioles venules micro capillaries.

So, in these the micro capillaries are like micro channels and they are extremely narrow and the when the cancer cell invades the cancer cell has to proliferate through micro capillaries. It is such a stressful condition for the cell that under normal circumstances any normal cell would not be able to invade that path, but a cancer cell successfully does it. So, the question is what is the physics that governs the cancer cell to undergo such remarkable behavior, if we can understand that it is possible that we can implement certain measures to arrest this rapid movement of cancer cells and the end stage cancer can be arrested which is a big problem in the cancer research community. We all

understand that significant advancements have been made in the early stage cancer, but in the end stage cancer I mean still there are lots of challenges for treatment.

So, now this problem is studied with the help of this experimental setup, where we have a micro channel which is made of a material polymeric material called as PDMS and the PDMS is made ultra soft and it is coated with a biocompatible material called as AP TMS APTS. So, that you know it is possible that the cell tries to adhere to the micro fluidic channel with a typical biocompatibility. After the cell adheres to the micro fluidic channel we administer of fluid flow.

Now this channel is a very special channel this channel is made very soft, so that with a fluid flow when the cell deforms the micro channel walls also deform and there are fluorescent beads embedded within this micro fluidic channel, so that these beads get displaced. And, if we take an image of the beads and then we can figure out that how the displacement of these beads are occurring and by an inverse Fourier transform we can try to figure out a force field which is responsible for this displacement.

So, the in this figure you can see that the force field around the cell is shown the red colored region is high stress concentration and the blue colored region is low stress concentration now what we do with this. You can see when the cell is standing on the surface it is standing with the help of it is legs which are called as focal addition points. Now, when this stress is there this first the focal addition points understand that there is stress on the cell, it is very much similar to the fact that when we are standing and somebody kicks us at our back we first feel destabilized on our feet, the cells are also failing feeling destabilized on their feet.

So, once this destabilization is there the message that there is a mechanical stress on the cell passes through this entire cell by a mechanism called as mechanotransduction this is a very hot topic in research and then the message ultimately reaches the most distant part of the cell membrane which is called as apical cell membrane. In the cell membrane there are physically two types of entities: one is lipid which is a liquid type, another is lipid raft which is relatively harder or solid type.

So, when the cell membrane understands that there is a stress it has to respond to it how does it respond, we have figured out that the obvious response is that the lipid raft tries to escape from the cell membrane to inside the cell. So, that it does not have to survive in

the stressful condition which is imposed on the cell membrane, that means that the cell membrane now has less lipid rafts and the cell membrane has more lipid. So, this escaping of lipid rafts from cell membrane to inside the cell I can tell you a very loose analogy that let us say a dacoit has attacked somebody's house then somebody who is not.

So, brave like me what I will try to do is that instead of fighting with the dacoit directly I will try to hide myself and my family members inside the bathroom, so that the dacoit cannot attack us. So, in that way what we are what the lipid raft is trying to do is it is trying to go to inside the cell membrane. So, when is it is trying what is getting into the cell membrane the cell membrane is becoming more fluidic, because it is more fluidic it is more malleable and then in a highly stressful condition, it can have a unique shape adaptation to survive the stress and this mechanism is of course there also for normal cells. But cancer cells have a very rapid response to the stress by lipid raft internalization and therefore cancer cells are much better stress responsive and they can survive the stressful condition in the micro capillaries.

So, now how can this be used for administering better cancer treatment. So, now a days there is a great advancement in targeted drug delivery, so what can be done is there are certain chemicals which are known as lipid raft inhibitors. So, you can directly if you directly inject the lipid raft inhibitors to the cell membranes, if this technology could at all be implemented then the cell membranes will retain the lipid rafts the lipid rafts will not go from cell membrane to inside the cell. The cell membrane will remain stiff and in the process they will not be able to undertake the metastasis, so the metastasis could be prevented if this could be used as a technological intervention.

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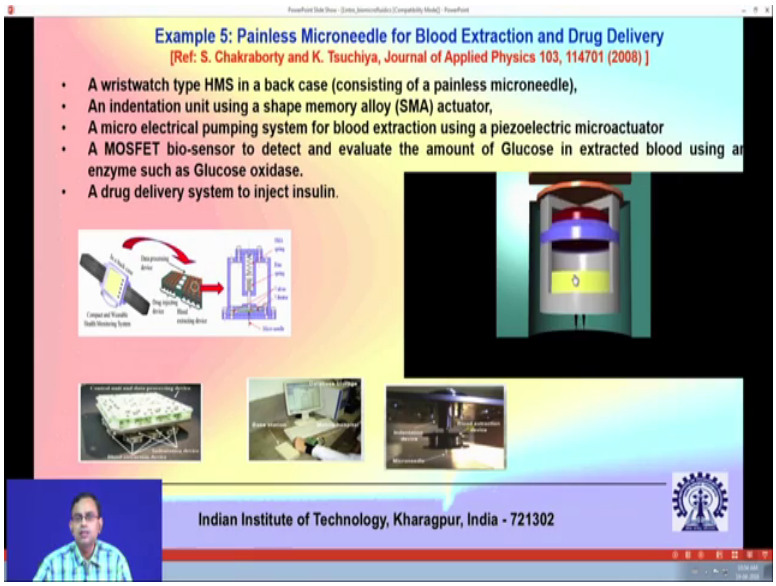

I will talk about a few more examples and this example is a very unique example where we worked on a research problem of painless micro needle development for blood extraction and drug delivery. This work was done in collaboration with Professor Tsuchiya from the university the from the Tokai University located in Kanagawa in Japan. So, the whole objective of this was to design a micro needle for diabetes management, diabetes management was considered to be a case study but it could be applied to any situation.

So, look at the device this looks like a wristwatch a very deceptive device, but on the back of the wristwatch you have micro needles. These micro needles are designed in the by taking clues from the mechanism by which a mosquito sucks blood. So, essentially when a mosquito sucks blood a suction pressure is developed, so this device tries to develop a suction pressure and suck a drop of blood in a painless manner.

By the way the mosquito's blood sampling is mechanically a painless process, the kind of irritation that we feel after the mosquito bites is because of an irritant chemical that it spreads during the bite. So, when the mosquito bites a suction pressure is created the same suction pressure is created in this kind of a device and mosquito's labium is like a god made micro channel. So, using the labium of the mosquito just in the way in which the blood is sucked similarly the blood is sucked in this device and my surface tension driven flow the we will study surface tension driven flow later on in one of the lectures.

The blood goes to a blood glucose sensor located in the same wristwatch where it is a mosfet based sensor and there is an enzyme called as glucose Oxidase with which it reacts and the there is a detection calorimetric detection based on that the glucose level is detected and there is a smart insulin delivery system also associated with the same device. So, this is a very unique device and this entire device is designed based on bio micro fluidic principles.

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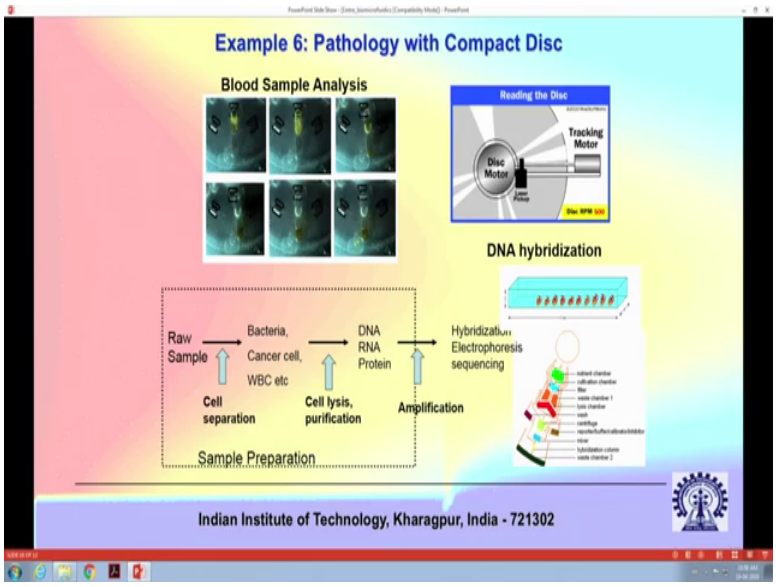

I will conclude my talk or my introductory lecture with some example of pathological intervention and pathological intervention is a very typical application of biomicrofluidics, we will talk about the details of these later on. But as I told you in the very beginning of this particular lecture that instead of going for an elaborate medical test with you know a very sophisticated medical laboratory, we can essentially take one drop of blood urine or saliva and load it in a small miniaturized hand held low cost device.

And, we can invent or innovate certain principles based on which there is a quick readout and a quick results readout methodology that is adopted with the device. And, there is a electronic display maybe integrated with a Smartphone also which gives the result of the test with minimal cost rapidly and with lot of accuracy this is our hope this is our imagination and certain devices have already been developed to cater these needs.

We will discuss these devices as we discuss the chapter micro fluidics for healthcare in one of the later lectures, I would not like to elaborate on that. But I would just like to emphasize that this is: what is the hallmark of biomicrofluidics that essentially make low cost that is affordable. But very accurate and rapidly performing medical device for outstanding socio economical leads.

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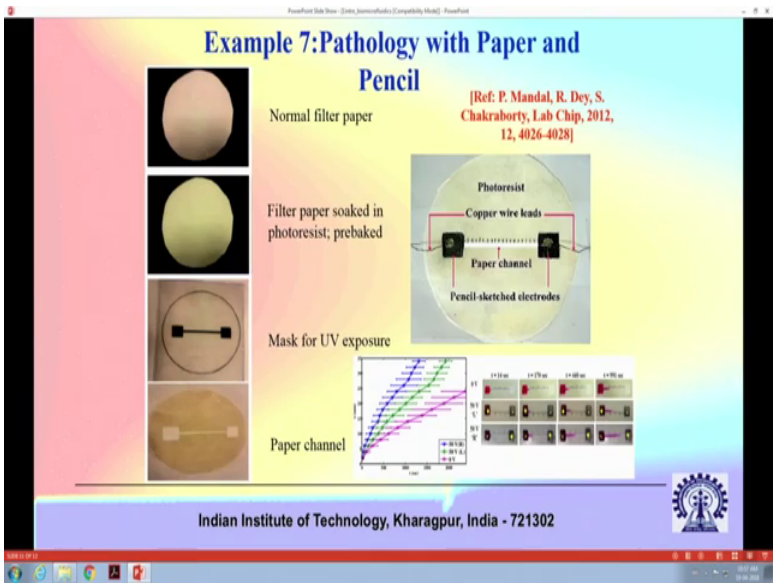

So, the previous device that was there is called as lab on a compact disc or lab on a CD, where you essentially have a rotating disc on which you drop one drop of blood and it can do large number of tests. You can do it also on a paper strip with on which we sketch pencil sketch electrodes, it is a very unique device invented by our group called as paper and pencil device I will talk more about this device later on.

So, the whole objective is to develop a device where you can do rapid medical diagnostics implement it in rural places in a low cost paradigm and create socio economical transformations which bring a rapid change in paradigm in which medicine or medical intervention is done in the broad societal scale.

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So, with this little bit of note I thank you for your kind attention I hope that we have given you enough food for thought and at the end I would suggest you to like prepare a mindset where you do not essentially have to be a biologists to work on biomicrofluidics. But, essentially you need to know certain basics of cells and DNA and maybe in general genomics and proteomics and Professor Maiti will go through some introductory biology for engineers in some of the subsequent lectures.

Thank you very much.