Bio - Microelectromechanical Systems

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Module No. # 01

Lecture No. # 21

Hello and welcome back to this lecture 21 of BioMEMS. We will do a quick preview of what was done in the last class.

We talked about the self-assembly of nanoparticles and structures using DNA molecules, wherein, basically the complementarity of one strand with another was used as a property to assemble one or more nanoparticles. The strategy could be used for making inter connects at the Nano level wires etcetera.

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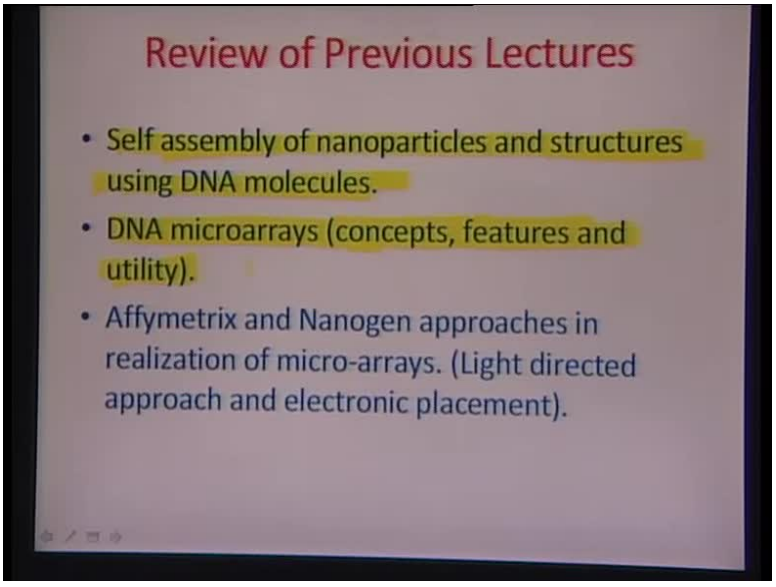

We also talked about DNA hybridization as a strategy to detection and discuss micro-arrays. They are libraries of different capture probes, which can be used to hybridize the DNA specimen, target DNA molecule. Then you can actually label the target with a Fluorophore so that as it goes on to the capture probe and gets immobilized onto the

surface. You could do a fluorescence read out and see whether there is a binding kinetics which is happening between the capture probe and the target DNA.

So, we talked about little bit of concepts about micro-array's features; what exactly hybridization is; how it can be done; how sensing can be done. Then we also discussed about the various utility aspects of such micro-arrays; RNA transcription profiling or HIV Re-sequencing - all these different things can be studied using DNA micro-arrays.

We also talked about two different companies and two different approaches that exist in the market as on date, for these different DNA micro-arrays. The two companies are Affymetrix and Nanogen. One of them, actually the Affymetrix is one which does a light directed synthesis of these captured probes. So, by using several photo masking strategies and chemical steps in between, there is buildup of molecule by molecule. So, you can have several combinations of these different ATCGs, in order to realize a variety of capture probes and different points of space over the whole array. Therefore, it could actually open and close pixels by doing multiple steps so that it could build exactly the molecule that you are looking at and have an information of sequence of the captured probe.

Nanogen on the other hand is company which actually gives you the chips and lets you assemble the capture probes that you want. So, you have to buy the capture probes separately. This is also known as the electronic way of immobilizing capture probes. There are a bunch of electrode arrays on a surface and you are using a positive potential to pull selectively. On some few goal posts, these molecules are negatively charged DNA molecules, which would thereby go and bind on the surface. There is a binding film like Poly-L-Lysine which is positively charged and there is electrostatic attraction between the negatively charged DNA and the surface.

So, you have built up a different over these different electrodes, all different arrays, and you could actually use a negative potential on the others so that you could direct only to the particular position, in which you want the capture probe. So, it has a very specific process for doing this immobilization of capture probe.

So, we talked about these different approaches; then also discussed about the DNA chip from Motorola which uses electrochemical sensing using a FeCN label and a bunch of different molecular linkers and wires as standing up on the surface upright as a brush

polymer; using of a signaling a capture probe in order to capture a certain target; position the FeCN sticks or the Ferro cyanide probe, very close to one of those molecular wires to take up the current, and then read out what is there - a sequence on the target DNA.

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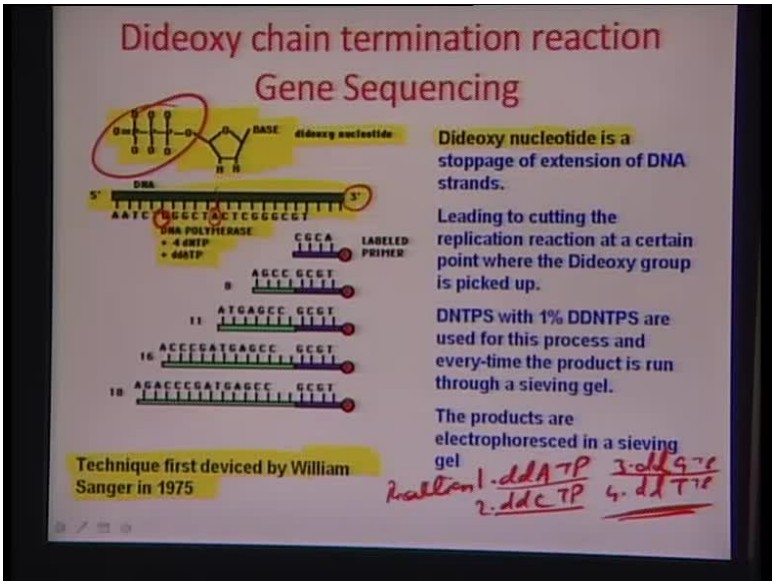

Today, we will actually start with new area which is also known as the fundamental process for gene sequencing. It was developed way back in 1975 by a scientist named William Sanger. This process has been popularly known in his name as - Sanger's reaction. So, this is again very interesting and fundamental level technique for identifying the sequence on the genes. By sequence, what I mean is, suppose there is a DNA molecule as shown here and reading out from the 5 prime to 3 prime, you can have this different groups A A T C T G; you know this G C and so there is a sequence of these molecules.

So, the sequence is a very important piece of information because it gives an address of the particular biological moiety or entity which contains this. It may be unique to a certain bacterial cell or a certain virus, and it may be able to identify if the virus is there in the ambient, just by looking at the sequence of the DNA that it has. So, it is very important information which is now, after the human genome project being recorded in a large database which is maintained at a global level and research gets on added, where these sequences are identified for any new stain, any new bacteria, any new virus, and it is put up there. It is also known as the N I S database.

Now, how do we identify the sequence? This very famous Sanger's reaction is all based around this molecule which is Dideoxy-Nucleotide. So, you can see this schematic of the molecule here. Therefore, there would be Dideoxy chain terminated, a Adenine, Dideoxy chain terminated Cytozine Guanine and Thymine respectively. So, there are four different Dideoxy chain terminated molecules.

Now, the way that this goes is that there are the several different constituents in the Sanger's reaction. There is an enzyme. This DNA polymer is which synthesizes the broken strands of the DNA. There are different four different DNTPs which is the normal A T C and G; it is not the Dideoxy chain terminated NTP. Then, we have four different reactions; so, this process is carried out in four different steps. In each of these steps, there is a different Dideoxy ATP, Dideoxy CTP, Dideoxy GTP and Dideoxy ATP.

So, let me just write all this down here for your convenience. So, you have reaction 1 with ddATP; number 2 with ddCTP; 3 with ddGTP, and 4 with ddThymine on Nucleotide.

The Dideoxy chain is an n group which stops the reaction. So, whenever you have the addition of a Dideoxy chain, it does not let the DNA chain replicate anymore. It stops the replication process. So, the chain is broken at that instance. So, suppose if i have a reaction wherein, in one go, the chain is broken along A, I can actually have a DNA molecule from the 3 prime and all the way to about A.

If the reaction goes all the way up to this particular G here, I can now have another reaction - the chain terminated from G; therefore, 3 prime to G will be there on the chain. So, whenever you put these different Dideoxy chain terminated agents, which is also about 1 percent by volume of the NTPS into the reaction, then, independently do the four reactions, wherever there is a thymine, this gets bonded to a ddATP, it automatically cleaves the reaction. So, there can be a fragment. Wherever there is a thymine, there is a fragment of that particular length.

So, if you try to put ddGTP on the reaction, wherever there is a cytosine on the principle DNA strand, the DNA will break. So, there will be multiple lengths of DNA available as end product of all these solutions or all these reactions, but mind you, these are terminated only at a single group. For example, if it is a ddGTP, it will terminate only at the cytosine groups in the DNA. If it is a ddTTP, then it will terminate only onto the

Adenine groups on the principle DNA. So, once these products are run through the gel, they have all varied lengths and you can easily fractionate them using Gillette Friesen. So, the smaller the DNA, the faster it moves; the longer it is, slower it moves, and we will have a read out like this.

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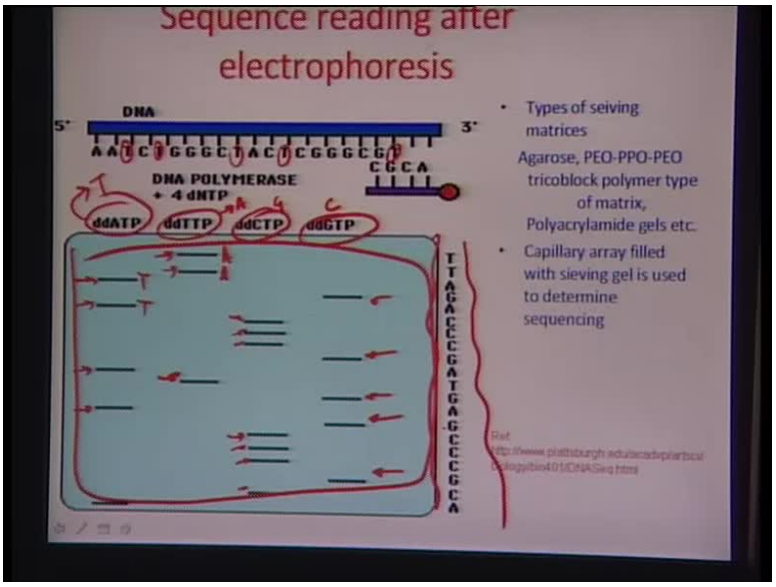

This is the ddATP. So the chain termination here takes place along the thymine group. So, wherever there is a thymine on the DNA molecules, you have fragments made up of that level. So, these thymine terminated processes are represented as gels. Let us say here here here and here (Refer Slide Time: 10:21). So, it gives an idea of the position or the length at which this particular group is present. So, wherever there is a ddTTP - Dideoxy TTP, it terminates the molecule wherever there is an Adenine. Therefore, if you run the products, you have these different lengths of products which are coming out, where there was actually an Adenine on the parent DNA group.

Similarly, in the ddCTP, you will have products which are fractionated in a manner corresponding to wherever there is a G or a guanine. Similarly, ddGTP - the products terminated in a manner wherever there is a Cytozine in this particular group. So, you have these different instances where you can find out, how or where exactly what group is located.

So, if you read this out as a whole, if you read this whole gel image and then try to see whatever comes, and corresponding to that what it is really. So, if there is a TTP in the

reaction, there really should be an A here and A here (Refer Slide Time: 11:25 to 11:29). There should be a T here; similarly there is a T here. So, this gives you a feel of what exactly it is that you are looking at of the DNA. If you just put this sequence altogether, something like this, you get the total sequence on the DNA.

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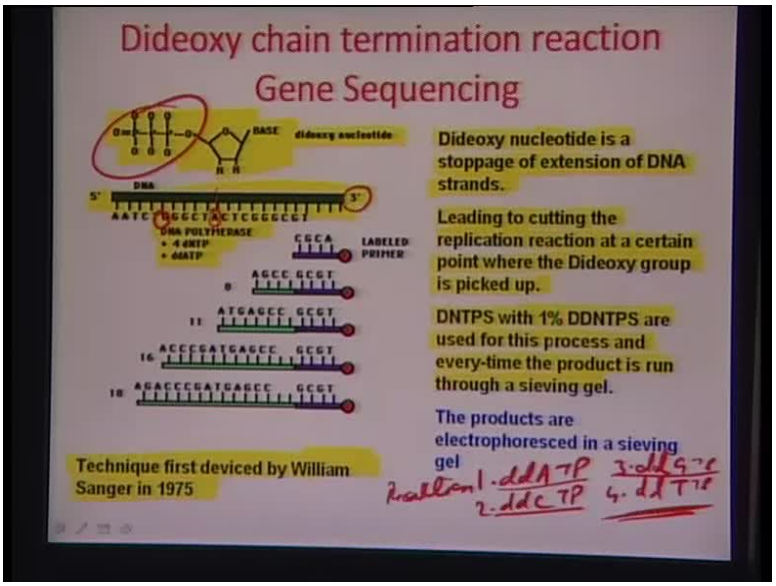

So, this is the process in a nutshell. If you would like to summarize what happens: Dideoxy Nucleotide is a stopping mechanism for the extension; any further extension of the DNA strand leading to the cutting. Cutting the replication reaction at a certain point where the Dideoxy group is picked up. So, if there are certain points which are corresponding to the complimentary pair as that on the Dideoxy and group, it is picked up and the reaction stops there. Similarly, the DNTPs; with one person, the DDNTPs are normally used for this process every time the product is run through a sieving gel. You have all different sizes which come up from the gel, from which you can read out really what is the sequence of the DNA. The read out process is also Electrophoresis, best in this case.

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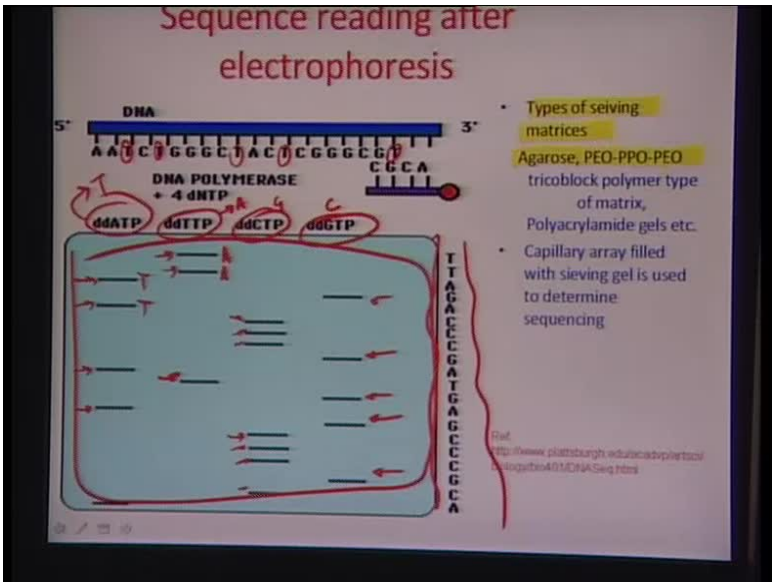

Now, when we are talking about electrophoresis, I would like to, just a retreat again, that there are different types of sieving matrices, based on which you could have almost close to a one base pair resolution. For example, the different products like let us say Pluronic exe, Tycho block polymer, Polyethylene oxide, Poly propylene oxide. Polyethylene oxide - this has very small pores and it is an absolutely transparent gel material within capillaries filled. It results in reusable or a washable capillary, because this material goes into liquid or gel depending on the ambient temperature.

There is a nagerous material which is most commonly used in laboratories for this work of electrophoresis. There are Polyacrylamide gels, there are products associated with hydroxyalkyl cellulose and lot of different polymers of the kind, which would have a porous content, and would also have some storage for the buffer to be there in the channels, etcetera. So, these matrices are very often used to get these kind of readouts as I illustrated here just about a minute back. From that, you could actually read what is there on this particular DNA.

Now, there seems to be a little problem though. The problem is the speed, the rapidity with which you can read this out. Therefore, there have been initiatives or efforts in this BioMEMS or diagnostics area where people really try to evaluate that - can all these mechanisms of these reactions and gel readout, so on and so forth; can it be made

sensitive enough or rapid enough so that you could go to a very low time of detection level?

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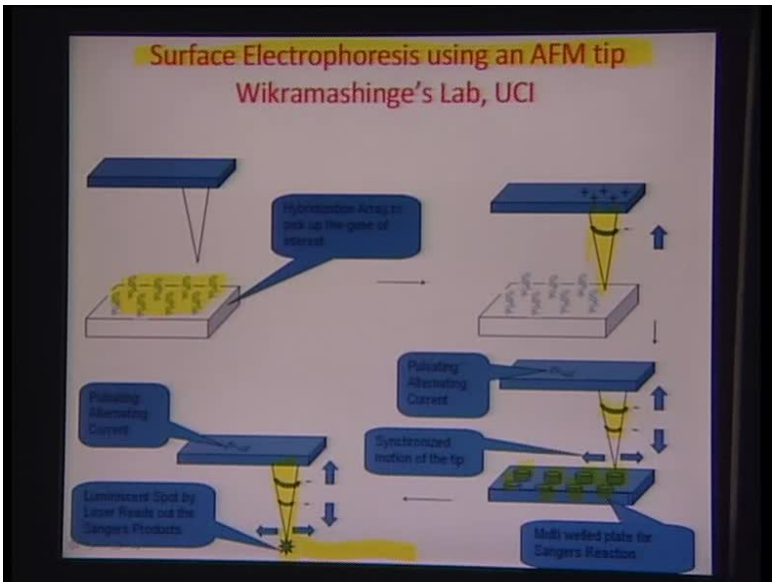

For that, there is a group in university of California at Irvine who are working on these strategies based on the atomic force microscope. The idea is to be able to pick up molecules on a very small miniscule level and use surface electrophoresis to shift the molecules back and forth, in this particular manner, by applying a potential. Then, once these are all picked up using hybridization array or something, there is a primary level detection. You could sequence it by running it on the small wells here (Refer Slide Time: 15:09) and running the Sanger's reaction, picking up the products. Then the idea is that, there is a label inserted into the particular sequence to product.

You can see the fluorescence once it is moved down with a pulsating electric field. So, as it crosses this self-luminous step here, it gives out of fluorescence response, from which you can back calculate what was there on the end, on the group, on the DNA. So, you are at a stage, picking the up the correct DNA by using hybridization and then picking it up using an AFM probe using small voltage at the tip of the probe; then, you are trying to drop this back down into small numbers, into different valves, which would do these four reaction steps associated with Sangers; then, the products when they are all different lengths and they are labeled with a certain Fluorophore, which is also corresponding to the n group which is there on the Dideoxy chain or which is terminated by the Dideoxy

chain, that particular molecule would come out at a certain rate where it will come out at a certain point of time from the AFM tip.

The way you read the fluorescence again, you create a small self-luminous part here. Then using an AC signal, you can actually send this DNA down. You have picked up the DNA here; in this case, from this particular well - if you can see. So, you have to send these different lengths down, along the tip of an AFM so that it goes one by one. As it crosses the luminous point, you get a certain fluorescence readout, from which you can find out what was the base pair which was chain terminated, or which had the Dideoxy ungrouped. From that, you could also find out what is the sequence of the DNA that you are picking up. So, this is a very interesting technique that people have been developing.

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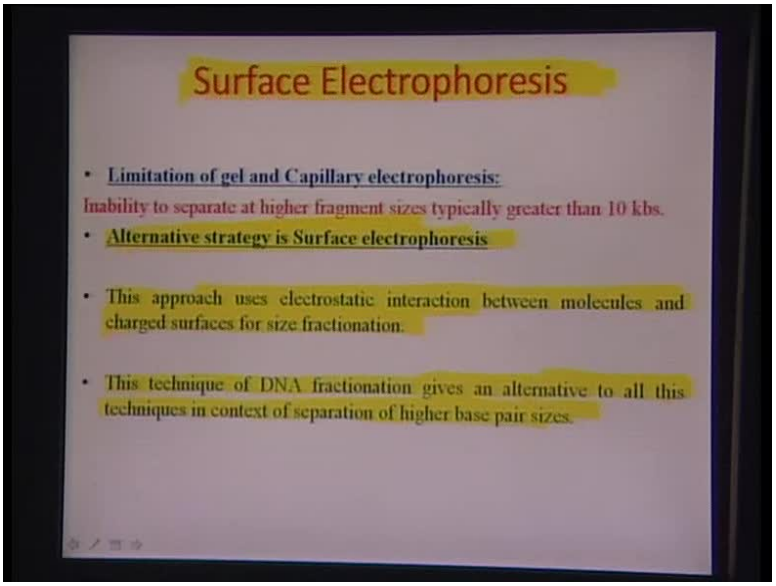

I would also like to illustrate a little bit about what surface electrophoresis would be actually able to do. In this particular case, as you are seeing, the first question which comes to mind is - why is it that people would do electrophoresis on a surface when there are already existing gels and other materials which would do this electrophoresis beautifully?

One of the reasons why electrophoresis is taken from a gel or a volume into a surface is because if you go above a certain base pair, let us say about 10 kilo base pairs or so, the gel materials irrespective of how big the pores are - that has a tendency of cutting down the resolution of DNA.

So, beyond a certain point, if you are actually going up of 10 kilo base pairs and trying to translate or fractionate DNA across this gel material, there is a model called bios repetition which comes into picture. It means that let us suppose the DNA is like a serpent which is unfolded; if you may remember, we discussed this at length when we were talking about electrophoresis. So, the head of the serpent goes into a series of pores, but then the DNA realizes it is too long to go through that bunch of pores into the next section, as a result of which it decides to stay back there and gets jumbled. So, it does not move forward anymore. So, this is an effect because of excessive friction between the walls of the capillary or walls of the of the pore through which the DNA is passing,

To avoid this problem, people or scientists have investigated this process using an alternative strategy called Surface electrophoresis.

So, this approach uses electrostatic interactions between the molecules and the charge surfaces for size fractionation. I will just explain this diagrammatically, a little bit. This technique of DNA fractionation gives an alternative to all these techniques in context of separation of higher base pair size. So, it is a very good alternative that the surfaces have to provide.

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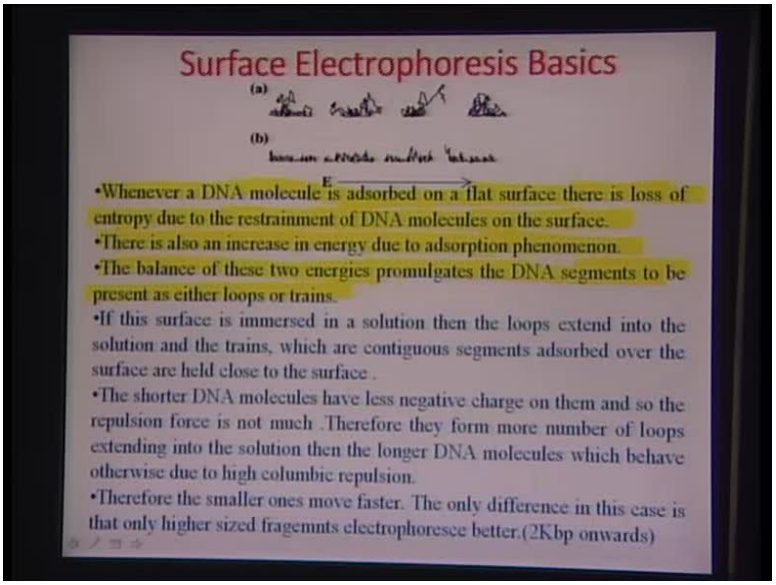

How the surface electrophoresis is done? This is out of a research work done at Benjamin choose group at Stony Brook.

So, if you look at that, when a DNA molecule is adsorbed on a flat surface, there is really a loss of entropy due to the restrainment of DNA molecules on the surface. So, there is also an increase in energy due to adsorption phenomenon. There is a balance of these two energies which promulgates the DNA segments to be present, either as loops or trains.

So, you are having a flat surface and you are putting this or if is adsorbing this DNA on the surface, and you have a layer of water over the surface and the DNA is still physically adsorbed on the surface over which the water is present. So, the DNA has a tendency to balance the adsorption energy over the local increase in entropy because of the extreme amount of negatively charged backbone. There is a configuration which comes out of it which is like loops and trains. So, portions of the DNA are looping into the solution as if they are hanging out and portions of DNA are adsorbed on to the surface of the solution.

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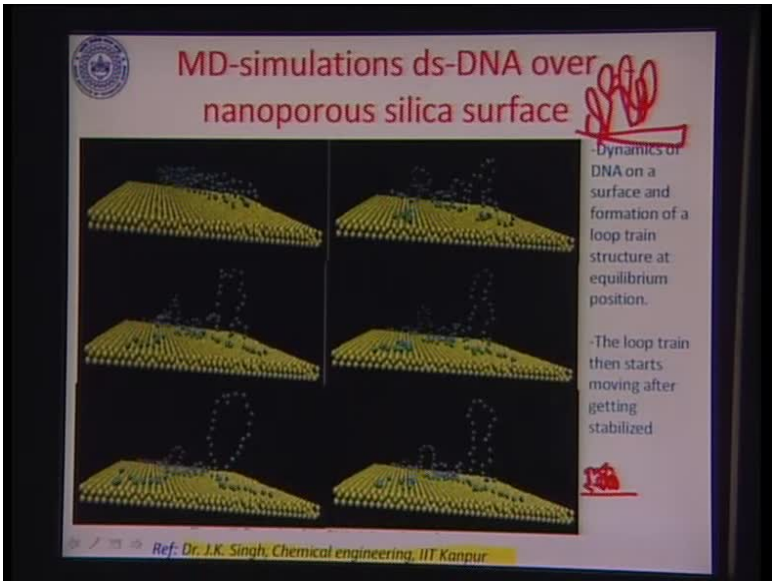

So, people have shown with molecular dynamics very interesting images and results which can call for it. Let us suppose, if you look at this particular figure here, you can see how using a ball and string model, this person has been able to show that there is really a tendency of this DNA to get folded into a loop as well as these trains. So, there are these trains here and there are these loops (Refer Slide Time: 21:07) which formulate.

Now, one interesting factor here is that longer the DNA molecule is, the more would be the train part or more would be the adsorb part. One of the reasons why that is so, is because the loops are a bunch of different negative charges. So, if the molecule is very long, you could consider that the charges are too much to be in the solution. So, there is a dense network of charge like this on a surface. So, if this DNA is long enough, there is a huge repulsion, if it were to be present as loops. So, it is definitely not a favorable energy configuration.

So, the other configuration that would be available would happen when they are really adsorbed very close to the surface and they are small. So, the loops are basically small and the train part of the adsorption part is more. So, we get one fact from all these that if the DNA molecule is a longer molecule, there is a tendency of the train part to be more as opposed to the loop part, which closes or closely adheres it or adsorbs it on the surface.

In this condition, if you try to apply an electric field and try to move it along the direction of the electric field, there is a tendency of the DNA to move slower by virtue of the adsorption energy, which comes into play every time you have to go to the next step or the DNA has to go to the next step, as opposed to the shorter DNA segment where probably because of less interaction or less entropy and longer loops and less adsorbed area or the DNA moves faster. So, this is actually a basis of size based fractionation of DNA, but just using surface without using bulk properties.

Therefore, all those other impacts which would have otherwise come from a three-dimensional gel metrics where there is a bunch of different pores through which this DNA is moving. There is a lot of friction between the DNA molecule in the pores and you almost always get a bundling, and you know a loss of resolution at higher base pairs. However in the surface case, the surface and the environment around it automatically adjust the DNA to have smaller loops or longer loops. It just makes it move slower or faster and there is a huge amount of resolution aspect in this particular case.

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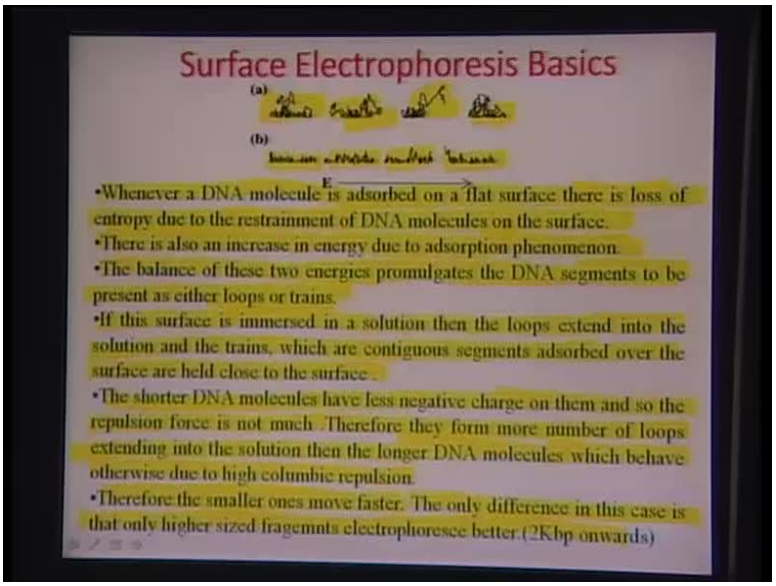

I would like to illustrate some of the work that has been already done. So, this is the case of shorter molecules whereas these are the longer ones. So, you have more trains than loops. So, in summary, whenever there is a DNA molecule adsorbed on flat surface, there is a loss of entropy due to restrainment of DNA molecule on the surface. There is also an increase in energy due to adsorption and the balance of these two energies promulgates the DNA segment to be present as loops or trains.

If this surface is immersed in solution, then the loops extend into the solution and the trains which are contiguous segments adsorbed over the surface are held close to the surface. The shorter the DNA molecules are, the lesser is their negative charge. So, the repulsion force is not much. Therefore, they form more number of loops extending into the solution. Then, the longer DNA molecules behave otherwise due to high columbic repulsion; they adsorb more on the surface rather than going into loops.

Therefore, the smaller ones should move faster because they are less adsorbed on the surface; the only difference in this case is that only higher size fragments electrophorese better in this case. Typically this 2Kbp onwards, but the effect can be felt more from 10Kbp because just like agarose could easily go up 10Kbp; of course they would be a resolution loss, but still you could make a detectable up to about 10Kbp. So, the utility of this process is felt beyond that, although they can actually start, starting from about 2 kilo base pair size of the DNA molecule.

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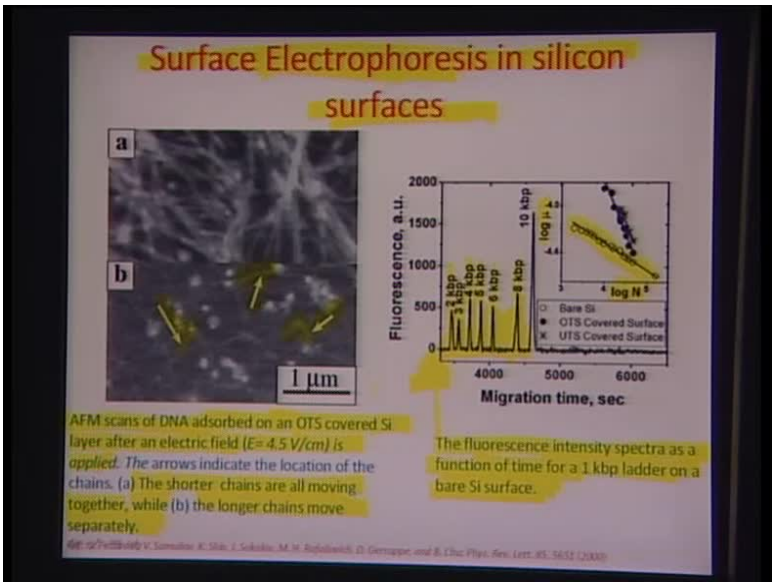

So, this is an excerpt taken from Benjamin choose group where they are talking about using this technique to detect the relative migration time of different sizes. So, here you can see you have DNA of 2Kbp all the way to about 8Kbp - 8 kilo base pairs. You can see that if you see the log of mobility, it decreases as you increase the number of sizes. This is the plot between logs of mobility of the DNA versus the log of number of base pair. So, if the number goes up here, the mobility goes down, this is imperfect agreement of what our theory really is. You have illustrated this from this particular plot - migration time versus fluorescence.

So, one more interesting factor which choose group as shown is that you can really detect with an AFM, the DNA adsorbed on an OTS covered Silicon layer after an electric field of 4.5 volt per centimeter is applied. So here, the arrows that you are seeing indicate the shorter chains and the way they are moving. So, the shorter chains are moving together, if you see; these arrows signify them. These are the shorter chains here (Refer Slide Time: 26:25). The longer chains move separately which is probably not shown in this particular figure, but Benjamin choose group has observed this.

So, a very repeatable data on a surface of DNA has been recorded and repeated. The fluorescence intensity spectra as a function of time for a 1 kilo base pair ladder on a bare Si surface is given in this particular graph as we discussed before.

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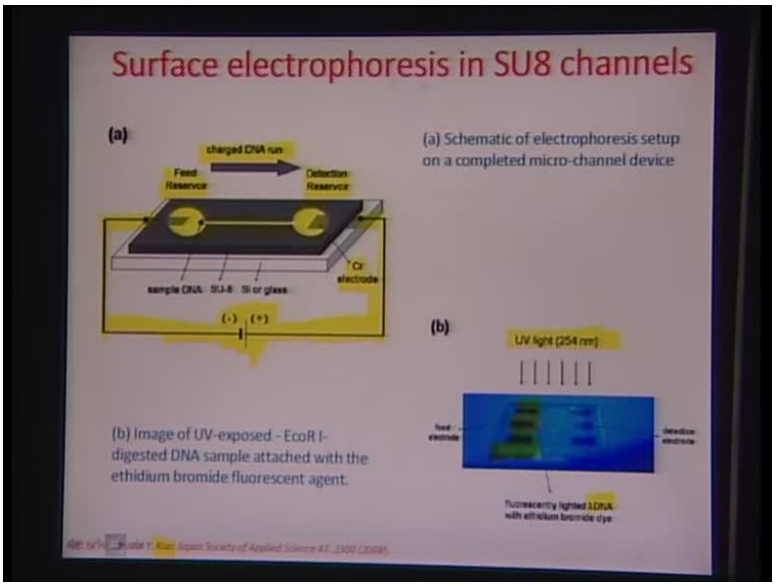

So, we will like to show you, how some of the detection can be done in this. In fact, paper by Coattol at the University of Tokyo, what he talks about is that you have micro fabricated architecture here; you are talking about an SU-8 based micro-channel with some reservoirs; this is mounted in the top of two electrodes which are made up of Chromium and on a glass plate; you connect these electrodes through an external battery, external source as indicated here (Refer Slide Time: 27:46 to 28:01) and basically run the charged DNA from the field reservoir side up to the detection reservoir side.

So, here, of course they have shown using UV light at 254 Nanometers the labeled DNA - the fluorescently lighted DNA with the EtBr - Ethidium Bromide; this is of course lambda DNA that moves all through. So, you can see these figures of the reservoirs (Refer Slide Time: 28:17) and they can see that with time this fluorescence spreads from this reservoir all the way up to the detection reservoir.

What they also report is an impendence based signal connected from the electrode at the detection reservoir and they see peaks coming as these DNA molecules would go through and pass or hit the particular electrode - a positive electrode made up of Chromium. So, this endorses the concept of surface electrophoresis. So, our group has done some work on electrophoresis as well and this the most recent excerpt from the one of the works reported in the American Institute of Chemical Engineers 2009 meeting.

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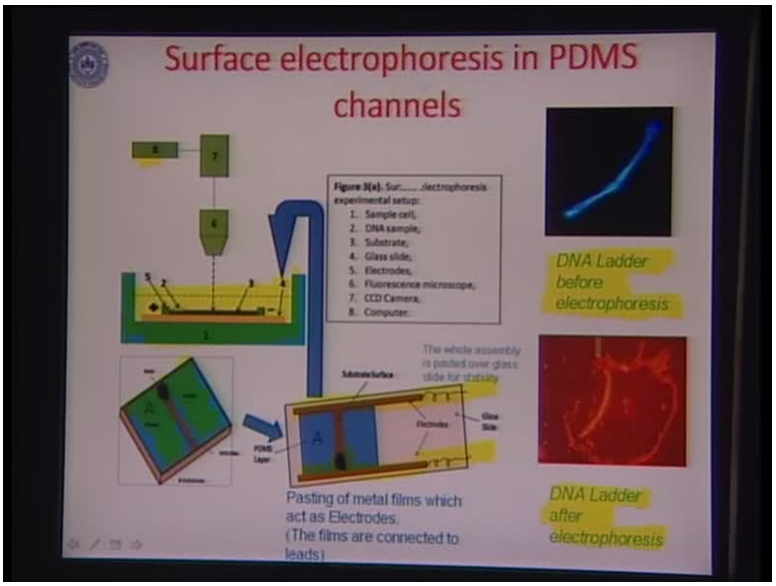

This talks about a surface - Silicon surface, with the potential across it wherein you are actually physisorbing some DNA molecules on one side of the surface. You have PDMS upper layer which is acting as a cover and also gives a micro-channel in between, through which the DNA should be translated. You have actually pasted electrodes - film electrodes on both sides so that you can apply a strong field from where the DNA is adsorbed this side, all the way to the other side here (Refer Slide Time: 29:38). Then, you have connected these through external wires and this whole assay is placed inside a very small flow cell as you are seeing here; in this, number 1 is the flow cell and you are filling this - covering this whole thing up with buffer solution.

So, the DNA loops into the solution and forms the same exact same configuration as was reported by choose group. So, it is brought under an inverted fluorescence microscope which gives it a read out and this shows some of the illustrations of how DNA ladder would behave on doing surface electrophoresis. So, this is the dye doped DNA. Similarly, this is after a few minutes of electrophoresis - surface electrophoresis has occurred. So, you can see there is a marked difference between the ways that the ladder looks before electrophoresis is opposed to after electrophoresis; this is simply physisorbed on to the Silicon surface.

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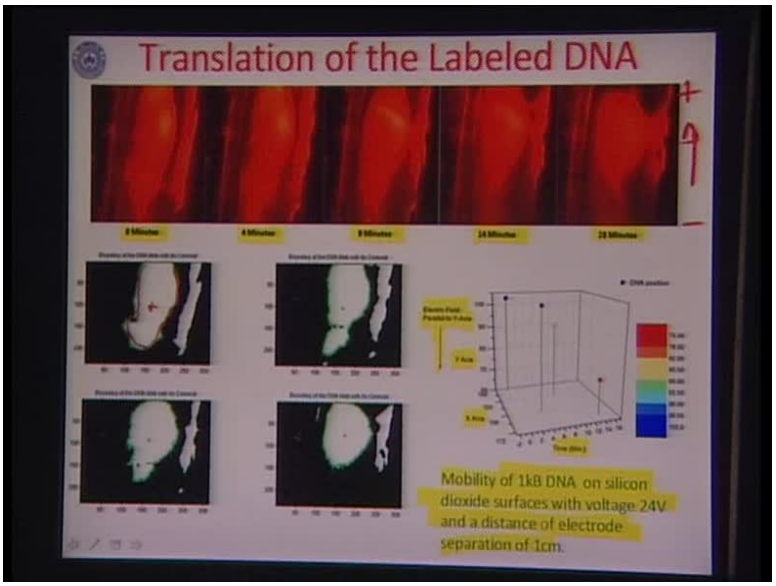

I have some other data to share with you. Here is again the translation of DNA molecules across the surface of different instances of time; you could see all from 0 minutes all the way to about 23 minutes. You could see that because you applied a positive and negative potential in the following manner, you are seeing that there is a translation of DNA towards the positive, potentially.

You can actually do, through image analysis scan on the overall boundary and try to detect the centroid and then calculate mobility. Here is an illustration where we are talking about the mobility of such DNA molecules. We find out that although the electric field is applied only parallel to the y axis, there is almost always the Brownian motion along the x axis which causes the DNA to move hither to hither as opposed to going straight away in the direction of the electric field. So, the mobility of 1 kilo base DNA on Silicon dioxide surface of the voltage of 24 volts has been plotted here in this graph and this is corresponding to separation distance of the electrode of around one centimeter or so.

So, this is a perfect bidirectional process. As you can see in this particular illustration what we did here is really we just went here and changed the direction of the electric field. Here this is negative and this is positive (Refer Slide Time: 31:56). You can see slowly that the DNA emerges back into the field of view for this different time intervals,

as can be illustrated from 0 minutes all the way to about 10.03 minutes. Then we plot the mobility in the same mechanism as we did before and still see the Brownian motion.

So, this is a real problem because you can get a one-dimensional mobility model because of which we are trying other alternatives like a super focusing the DNA, onto very small track, so that we can eliminate totally the Brownian motion. The super focusing can be obtained by using a different surface energy pattern on a surface where we can really make a narrow hydrophilic pattern, where all the DNA would rapidly converge. We are not sharing the data at this time, but I just wanted to make an illustration of what research is possible in this particular area of surface electrophoresis.

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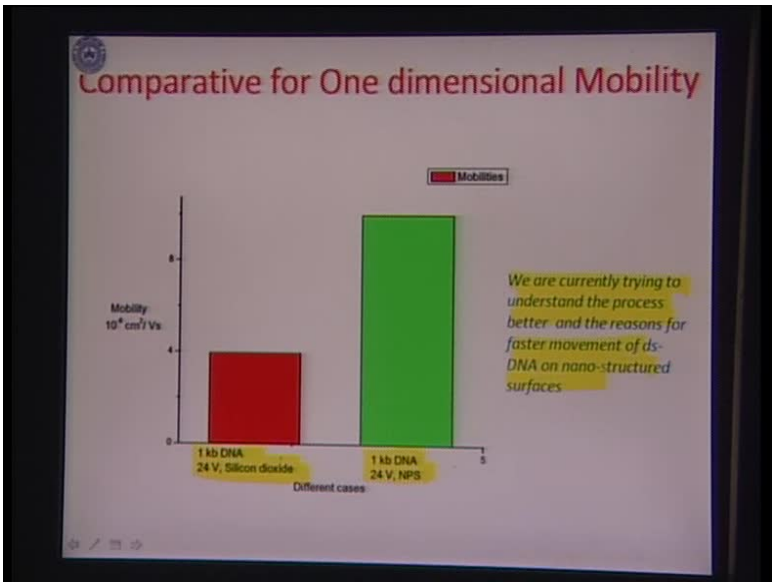

So, this is a study that we did, where we can show that, on a higher energy surface like Silicon dioxide is opposed to a lower energy surface like porous structure, porous matrix made up of Silicon, we have differential mobilities which we report. We are trying to understand the process better, but this is how surface electrophoresis can be done.

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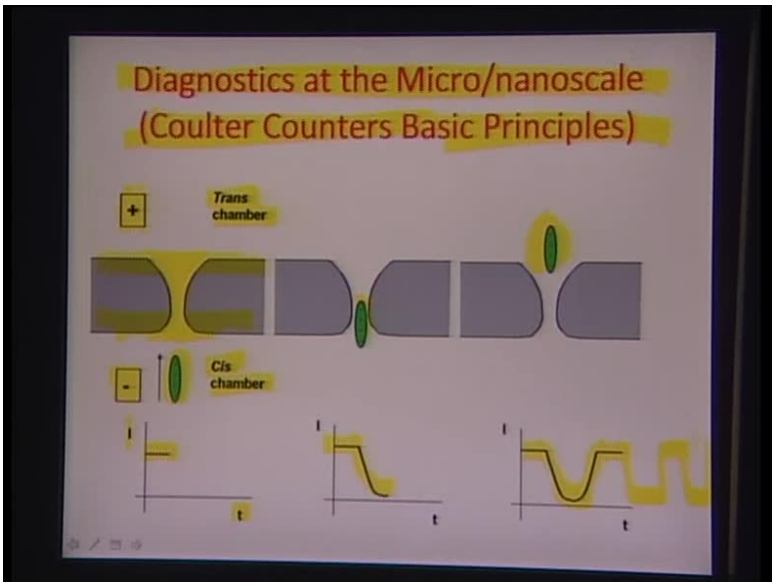

Now, I would like to illustrate another very novel technique of BioMEMS, which BioMEMS has to offer based on various fabrication protocols or various kind of structures and features that can be fabricated using micro systems fabrication technology. So, this is really the diagnostics at Micro Nano scale and is also the basic principle behind Coulter Counters. So, we are talking about a cell counter by the name of Coulter Counter.

The whole goal here is the following: You have a small pore which is close to the size of a single cell. This pore or this porous membrane is kept as the only communicating medium between two fluid cells, where one side is rich in the cells and another side is a lean mixture. If assuming that these cells are negatively charged and you apply a positive bias on the other side of the fence or the other side of the pore, there would be a tendency of these cells to go and go into the pore, and go straight out into the other side.

While doing that, it momentarily blocks the pore and blocks the ion current which flows in the reverse direction as opposed to the direction of the movement of the cells. So, this is simply based on this principle. So, you have a negatively charged cell here and you have a micro pore in Silicon which is made like this. This is the Trans side or Trans chamber; this is the Cis side. You are applying a potential across this particular porous membrane in a manner that this negative cell is dragged into the trans chamber side (Refer Slide Time: 35: 03 to 35:16).

So, the idea is that you keep on monitoring the ion current; that means the current I with respect to the time t across this membrane. The moment the cell goes and blocks the membrane, there is the dip in the ion current and when it goes to the other side as you are seeing here the current takes up back. So, if I can count these dips with time, I can be able to ascertain how many cells pass per in time from this particular micro pore and this could be a basis of counting the number of cells that are there in the ridge side of the solution. So, this is the basic principle of Micro Nano scale coulter counters.

Based on that, based on this Micro scale Coulter Counter, you can extend the same concept on to the Nano scale.

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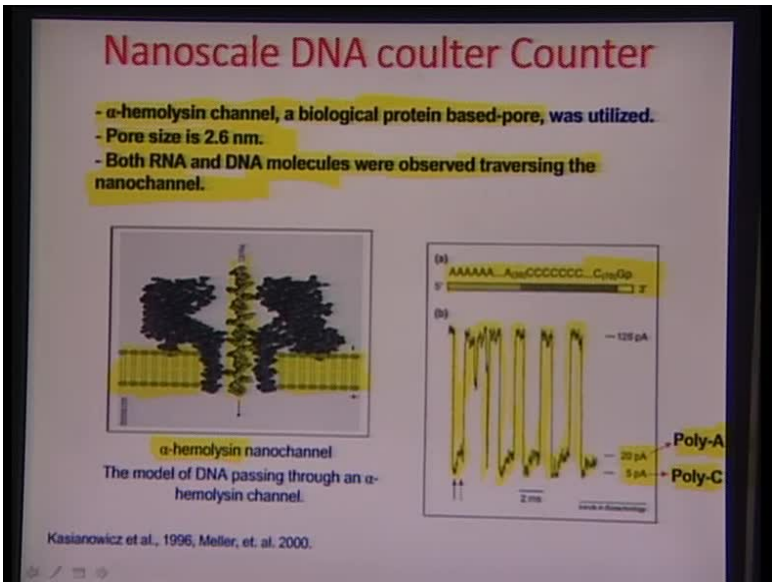

In fact, one of the first few articles which came out in this area was really from Kasianowicz group and would like to illustrate one of those examples here, where they talk about self-assembly of a protein by the name of alpha-hemolysin on the top of a lipid bilayer of cell. So, if you see here, this is the lipid bilayer that they are talking about and you are assembling. So, you are actually putting this protein alpha-hemolysin on such a lipid bilayer and you assume that it self-assembles. So, there is a tendency of this protein to go and embed to the lipid bilayer and self-assemble itself.

What is also very interesting and important here is that if you look at the alpha-hemolysin as a protein molecule, it really has a pore within it. It has a small narrow channel and this also access a biological protein based pore. There are lot of charge

transfer process, across particularly our central nervous system, where we use this charge intake and charge outside delivery in order to transmit between the cells. These are called exocytosis or endocytosis based processes. They are proteins on the cell wall which would open and close, and give way to the charges.

So, the pore size illustrated on this alpha-hemolysin is about 2.6 nanometers. I have indicated before that 1 helical turn of a DNA is of about 2 to 3 nanometer in size. So, it very well matches with the 2.6 nanometer pore size on the alpha - hemolysin protein. So, as the result of this, if you are assembling a structure across Cis Trans chamber just in the manner that you did for a cell, but in this case, you make one side rich in DNA concentration and other side lean. In applied electric field, you should see the similar effect as a drop or a dip in the current versus the plot to detect a single molecule or single DNA molecule if it passes by.

So, that is exactly what this principle is. Here, the recording that was made using this technology showed that you have these small peaks and dips which are coming out just because of the blockage of ion current; a DNA goes and blocks one of the pores. What is also very important for me to tell you here is that you know this really is a size effect. That means if you have a molecule poly A as opposed to poly C, and if you look into the molecular structure of C – cytosine, it is little bigger; it has two 6 carbon 5 carbon ring. Adenine on the other hand is a little bit smaller molecule, but here, there is a really a marked difference in the current values.

In case of poly A, the current is slightly higher than in case of poly C, which means that if the size is more, this DNA here which is moving through this porous membrane as a more size, it takes a while for the DNA to come out. So, the average current which would be able to get generated in the case of a loosely fitted molecule is slightly higher in comparison to the current which you can generate by a tightly fitted molecule in the pore. Also, what is more interesting is that, it takes a little more time for the DNA to pass through this pore once it is tightly fitted as opposed to the loosely fitted.

Now, in a sense, this is a break through. It gives us a feeling that may be with this strategy, someday we could be able to sequence the DNA in terms of what basis it has; just based on the response of the current with time. This is really the holy grails in the industry how to electronically be able to sequence. Now, in fact, there are companies

which are doing this electronic based sequencing and at least there are few products which are tried to be made commercial very soon about electronic sequencing. There are lot of novel ideas and lot of projects which are in this direction of how to quickly detect the sequence electronically, and people have dream in the range of something like a nanosecond base pair kind of read out of these processes.

So, in this particular work, though both RNA and DNA were tried and they were observed traversing through the nanochannel structure right and that gave a new direction to this whole area of manufacturing Nano scale pores.

So, one problem about this particular work is that lipid bilayer being very fragile system, which can be effected by temperature, pH - lot of these parameters. People started thinking of can we really produce synthetic pores made up of materials which are may be thin wafers or polymeric materials? So, there are few groups in the world which have developed some of the synthetic nanopores and one of the strategies that people have normally followed is using SOI - Silicon on Insulator wafer, as I would be just illustrating in this particular example.

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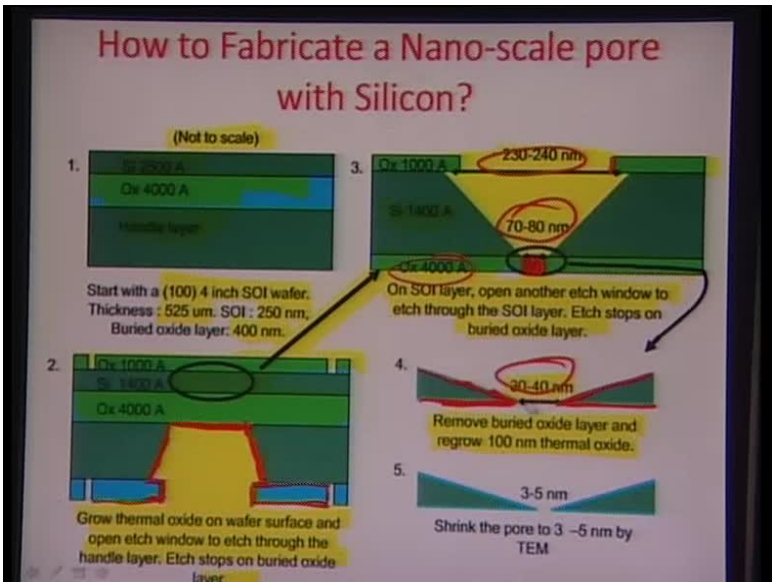

Here, as you see there is an oxide layer of about 400 angstroms. There is a Silicon layer on the top of it of about 2500 angstroms and this whole is assembled onto a handle layer which is a much thicker wafer - a couple of microns; probably couple of 100 microns. Although it is not to scale, it is a 4 inch SOI wafer with this handle layer. Thickness of

the handle layer is about 525 micrometers. The SOI is about 250 nanometers; embedded oxide is about 400 nanometers.

So, what you do is that you first grow thermal oxide on the wafer surface. Here, you can see on these two sides (Refer Slide Time: 42:42) you are growing the thermal oxide. It is about 1000 angstroms about 100 nanometers thick and this you do all sides of the wafer; therefore, both surfaces have this thermal oxide.

So, once you grow thermal oxide on the wafer surface, you open an etch window somewhere in one of the sides. As you can see in this particular illustration here, this etch window (Refer Slide Time: 43:14) has been opened in the oxide where you make Silicon as the selective layer and Silicon dioxide as the etching layer. You design the h m t in that manner and use photolithography process to carve small window of sacrificial resist, and create a via through which you could actually etch away the oxide and create a hard mask of the oxide on the surface of the Silicon.

Once that is done, you can etch through the handle layer, just as illustrated in this particular example, using an anisotropic etch process. So, it is at an angle of about 54.34 degrees.

An isotropic etch, as will be talking about in details later on, is an etch system where we use either some hydroxide to perform an electron exchange process as the layer etches away. So, you are converting Silicon into the corresponding Silicon hydroxide and dissolving it away into the solution. However, what is important for me to mention is that at certain crystal planes on the Silicon surface, the electron release is limiting the rate of formulation of hydroxide. Because of the higher density of atoms, there is more binding of electrons. So, at a plane which is also called 1 1 1, along that direction the etch step is the slowest. Eventually whenever you do this, etching is an averaging effect; it shapes in the shape of a 1 1 1 plane which is at an angle of about 54.34 degrees with respect to the vertical. So, that is how you get this window here (Refer Slide Time: 45:06).

So, you have to use an etchant combination one which is selective to SIO 2 and one which is selective. So, it cannot etch this oxide. Once it hits the oxide, however it etches the Silicon. So, it is etching Silicon and etch i selective to oxide, and in the other system is etching the Silicon dioxide and selective Silicon.

So, you have to follow process 2 first and then process 1. So, you can hit the right shape over this handle layer. So, etch top is the buried oxide layer in that particular case; as you are seeing here (Refer Slide Time: 45:42). So, then what you do is you actually take this upside down.

Now, you have an illustration here, where you are talking about again doing a etch window from the top side and I am not showing the bottom side here; the bottom side is actually shown here in this case (Refer Slide Time: 46:04). So, you take this particular top portion as you are illustrated by this circle and then you are doing an etch from the top side.

So this is that oxide 1000 angstroms layer which you had grown earlier on the top of this Silicon layer which is 1400 angstroms. Here this is thermal oxide so you can say that earlier the Silicon was 2500 angstroms, but because oxidation is diffusion driven process, the oxide layer formulated is about 1000 angstroms making the Silicon the pure Silicon about 1400 angstroms or so.

So now what you do is open another etch window here as you are seeing in this portion and here (Refer Slide Time: 46:49). You are using again photolithographic masking step and resist which is selective to Silicon, but can etch away oxide very easily. So, you can open at 230 to 240 nanometers etch window and then again perform another isotropic etch. There would be some undercut in this case as well so that this whole area here (Refer Slide Time: 47:09) can be removed from the 1400 angstroms Silicon layer. You can design the thickness here in a manner, because this is 1400, through calculations you can show that the lower part here is only reduced to about 70 to 80 nanometers; it is the very small part that remains at the very end of this pit.

So, you have a case where you have opened an etch window from the backside and then use the same process step to open an etch window from the front side as well. Effectively, you are just left with this 4000 angstroms oxide layer in between - the one which you saw here in between. So, this is only about 400 nanometers thick and then you have the advantage of the luxury of having a 70 to 80 nanometer hole on one side. So, you can actually use E beam lithography and now etch your hole which is about close to 30 to 40 nanometers in this particular region.

You can also use the focus time beam for creating such a pore. So, you could actually design a system wherein you can actually open a 30 to 40 nanometer film or small hole in this particular region here (Refer Slide Time: 48:29). In this particular 70 to 80 nanometer region, you can create a small hole which is about 30 to 40 nanometers.

So, once this is created, you put an oxide layer around it. So, you again grow; you first remove the buried oxide; so, this is the buried oxide layer. As you are seeing, this 400 angstroms layer; here is the buried oxide; so you are removing the buried oxide almost in totality, except a portion which is about 30 to 40 nanometers. Then, you regrow the 100 nanometer thermal oxide; in this particular region, you are re growing the oxide. So, from this step to this step, after you have opened the small hole here through FIB about 70 to 80 nanometers, you are trying to remove this layer altogether and also try to grow so that you can shrink this pore from the general about 70 to 80 nanometers all the way to about 30 to 40 nanometers. Once this 30 to 40 nanometer is created by etching away this oxide from the bottom, you can actually regrow thermal oxide on the sides here.

So if you see here, this blue layer in this particular region which are now marking red is the regrown oxide. So, you have step one where you are removing a small etch window on the back side; you are removing an etch window and etching on the front side so that you reduced to 70 to 80 nanometers pore; you cut using an E beam lithography, this 230 to 240 nanometers width here of this particular oxide and then with an FIB, try to drill away this area. Alternately, you can also remove the oxide altogether and grow or regrow about 100 nanometers of thermal oxide around this area so that you are left with about 30 to 40 nanometers.

One interesting point here to be noted is that if you heat this region using a focus beam, let us say, you are using an E beam - an electron beam, to heat this particular area, you see that this oxide reflows and tries to have self-closing gap. So, this 30 to 40 nanometers can be reduced to about 3 to 5 nanometers, as you can see here (Refer Slide Time: 51:18). So, you are re growing the oxide and you are trying to flow it so that it becomes self-closing.

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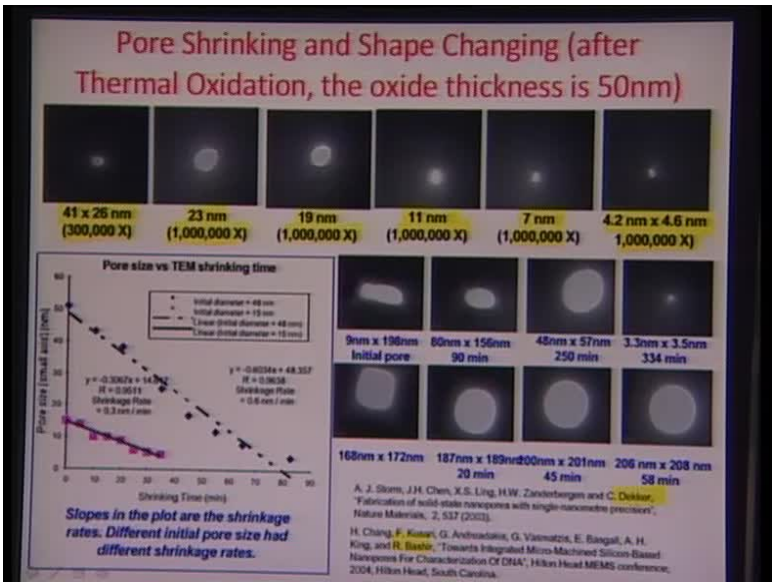

It is illustrated here in the example, if you can see from this particular work by reported by Dekker (( )) and later on Bashir (( )).

What they are showing here is that this is an excellent visualization process. So, you take a TEM and try to start visualizing the pore when it is about 300000 X; you can see this 41 into 26 nanometer pore, and then mind you, the magnification here is about the 1000000 X which is about 3 times this particular value, and here you are trying to investigate a 23 nanometer pore. Then, if you look at the pore with time, as you are focusing the electron beam, the oxide reflows and the pore starts becoming smaller and smaller. So, it starts self-closing till it attains about 4.2 nanometers times 4.6 nanometers.

You stopped the visualization process in this particular instance. So, you are taking the bigger hole here which is regrown with an oxide on the surface and trying to focus the E beam and causing self-heating so that there is a flow of this oxide. Self-closure of the pore is the smallest pore size here which is about 4.2 nanometers times 4.6 nanometers. This is how size of a DNA would be or it rhymes very well with the size of a DNA.

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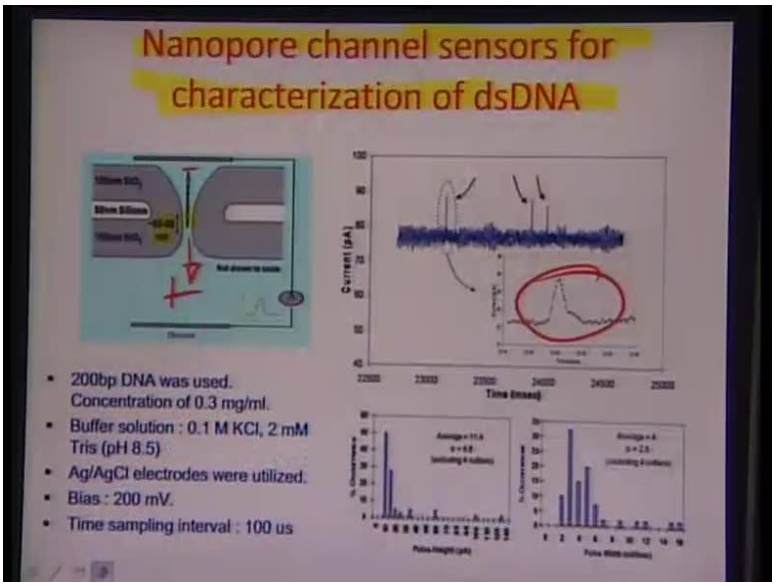

So, you can make a synthetic nanopore in this manner which you could later on use for DNA translation and also try to understand of gauge whether it can do some sequence. So, this is how this nanopore channel sensors are characterized particularly for double-stranded DNA structure.

So, you can see that there is a micron sized pore or there is a nanometer size pore here which is in length about 50 to 60 nanometers and in diameter is about close to 4 to 5 nanometers. DNA is translated from the Cis side to the Trans side. So, you can think of this side to be the positive bias here, this side to be the negative bias, and the DNA translating across this pore. What is very interesting to note here is that you get upward peaks like this (Refer Slide Time: 53:30 to 53:40)

So, the peaks here are in the reverse direction. They are as if there is a large ion currents suddenly being released after the DNA blocks and is about to cross over.

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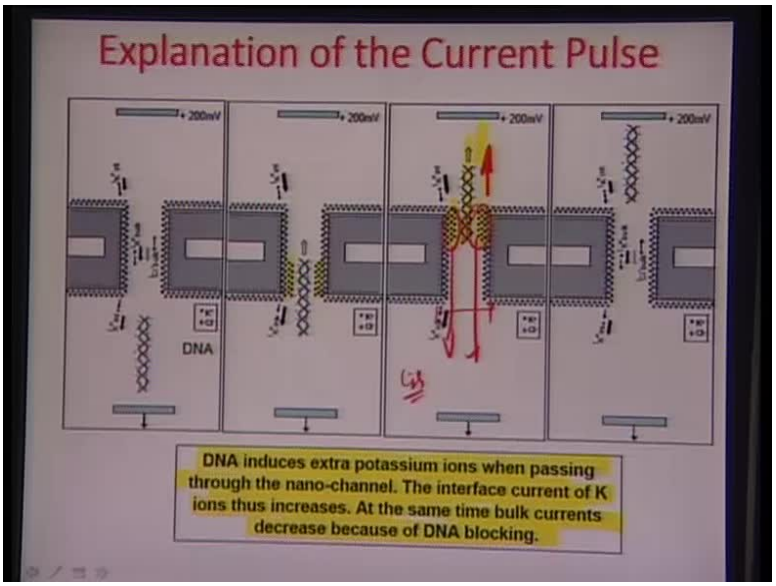

So, this paper reported by Bashir atal which I just illustrated earlier here in this particular example, tries to explore this principle - why is it that we are getting a peaking action rather than a dip in the current level? So, one explanation which is possible is given by this tendency of the DNA to carry a counter ion cloud around it; you know we have been discussing off and on in the earlier lectures that DNA is having a very strong charge on the backbone, negative charge on the backbone. So, if it is flows in a solution and its electrophoresing in a solution, there is a tendency of this DNA to grab together a counter ion cloud around it and this shields the overall charge. As we have modeled before electrophoresis leads to a reduction in the charge by delta z and also increase in the radius - the overall radius, because it is moving along with the whole ion cloud in the center.

Now, the idea here is that as the DNA travels into this region and it grabs together this ion current along with it, and as it goes out into the other region, or it not only introduces a negative charge in this region, but also the positive counter ion group which it is carrying along. That is the reason for sudden spurt of increase in the charge in the ion pore.

So, you can think of it that as this is the positive ion, it drags along the positive ion. It leaves into this side of the solution and the positive ion starts coming back once it crosses through the pore. It comes back in this direction, the DNA is moving in this

direction, and this whole positive ions here (Refer Slide Time: 55:55) which come back or pumped back into the Cis side from where the DNA has translated into the Trans side. Because of this, there is a huge increase in current rather than reduction in current.

So, this is an opposite effect than the Microscale Coulter Counter in which you have no such effect because you talking about a micron scale object. Whatever flow is across that micron scale gap is a bulk flow, but here because we talk about more in terms of almost to a single ion level what is going on, this counter ion effect becomes very important.

So, you can think of this counter ion cloud which is going with DNA into the pore. When the DNA escapes, it is released back into the solution in the reverse side, as a result of which there is an increase or a peaking effect rather than a dip down as is normally observed in other cases this is more. Also because the Silicon surface here develops the charge dual layer because you are talking about the same range is that of the dual layer between the Silicon and the translating molecule. So, this process is lot more complicated than the normal Microscale Coulter Counter.

So, you can conclude by saying that in this case the DNA induces extra potassium ions when passing through the Nano-channels and the interface current of the potassium ions increase suddenly. At the same time, bulk currents decrease because of the DNA blocking. So, the overall effect is an increase in the current because of a sudden spurt of release of these ions back into the solution.

So, particularly in this example, 200 base pair DNA has been used with the concentration of about 0.3 milligram per mil. If the electrodes used for this measurement are AgCl based electrodes, as this AgCl itself is non-dissolvable in Nachos solution, you have a coating of AgCl on the top of Ag. That is the way you use these electrodes; we have talked in details in the electrochemistry section of this course.

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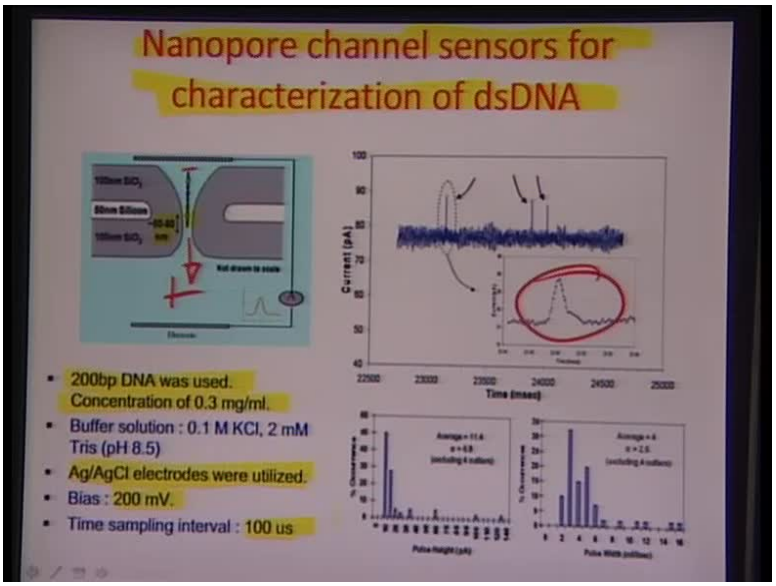

The bias voltage used in this case is about 200 millivolts and the time of sampling is about 100 microseconds which we would also get using a high resolution DAQ card - Data Acquisition Card.

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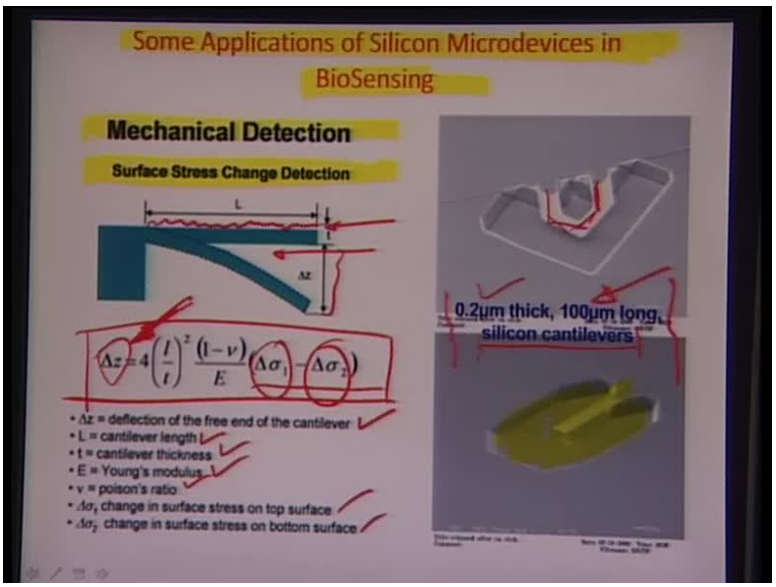

So, this has actually become a very major research area nowadays in BioMEMS, as to how you could actually measure the single molecular translation across these Nano pores; is it of some utility or effect in the diagnostics area?

So, there are other forms of sensing or detection which can be used with these MEMS kind of architectures. We have been talking earlier about Silicon micro devices. So, this is the direct application of Silicon micro device in Bio Sensing. This talks about mechanical detection or a mass based detection which was one of the frontier areas of sensing, these days.

So, here, what this process is all about is defined by a change in surface stress across a small thin film micro cantilever. So, you can see here, this is the micro-cantilever which is coming all the way into this small pool and it is separated from the base. So, it is like a cantilever which is pointed into a gap here like a swimming pool. So, it is a diving board in a swimming pool kind of arrangement.

Here, if you do something in the surface of this cantilever in a manner that the surface energy has a differential on the top and the bottom surface, you put some molecules; you change the surface energy somehow by binding it to some hydroxyl groups; or some other SAMs layer - Self-Assembled Monolayer in a manner that there is a differential between the bottom surface of the cantilever and the top surface of the cantilever. So, you have something immobilized onto this region here on the top so that you have a difference between the bottom and the top surfaces of the cantilever.

So, there is a bending because of this. It is a mechanical bending and this bending is also defined by something called the Stoney's equations. It is given in this particular illustration here. In this case delta z is this particular distance that the cantilever moves. It is also directly proportional to a difference in the surface energies on the top and the bottom surface of the cantilever. So, delta sigma 1 and delta sigma 2 here are the differences of these energies on the top and bottom surfaces, which occur because of this chemical change or chemical activation of the surface.

Therefore, dealt z, the change in the z motion of the particular cantilever is also directly proportional to the difference of the cantilever delta sigma 1 minus delta sigma 2. So, delta z again is the deflection of the free end of the cantilever, L is the overall length of the cantilever, t is the thickness, E is the young's modulus, and v is the Poison's ratio. Delta sigma 1 and 2 are change in surface stresses on the top and bottom surface.

So, this is the mechanism of detecting if really there is a change. You can produce a yes - no type of answer of whether there is a surface change. I will just in a minute show

another slide which talks about how you could detect DNA or recognize DNA of different base pairs based on this technique, by just immobilizing some capture probes on the top of such micro cantilevers. Really, these cantilevers could be of all different shapes; this for instance is more like a v shaped cantilever.

You know this is actually a straight i shaped cantilever. So, some typical dimensions of these cantilevers illustrated here is about 200 nanometers thick film, over which you make these cantilevers and they can project as long as about 100 microns. So, really it has to be a steady film, steady structure which can go up to 100 microns and still be integral, although the thickness is only as low as about 200 nanometers.

So, the aspect ratio is about 5 times and they are normally made in Silicon because the process is very well acknowledged and found out. However, there are initiatives to also make cantilevers in other alternate polymeric material with certain changes in their strength properties or modification in these strength properties.

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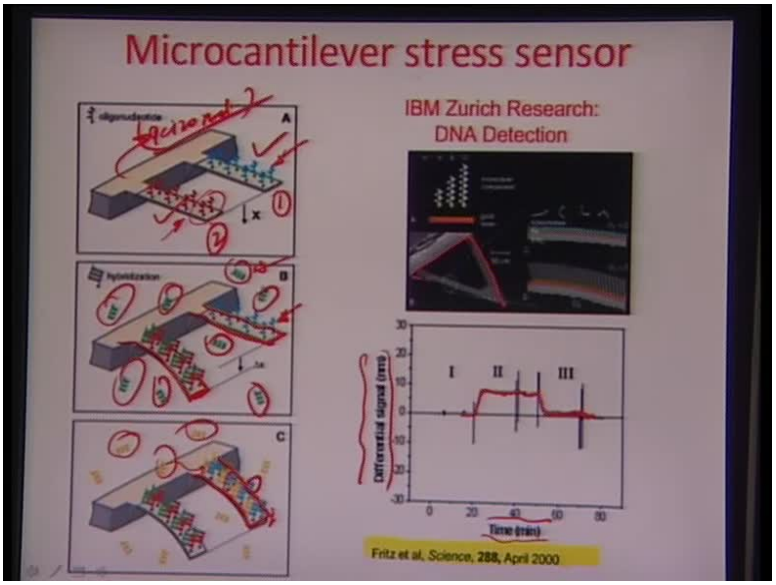

So, let us see what we can do with this. This is really a very fine example bought from IBMs research plan on Bio Sensing. So, this is also corresponding to a paper reported by (()) in science which talks about how such instruments or techniques can be used for bio detection.

So, here, in this illustration as you see, there are two different capture probes which are put on both sides on a series of two cantilevers. So, there is cantilever 1 and cantilever 2 on which… there are these blue probes which are fitted on one side and red probes which are fitted on another side.

So, there are two different capture sequences all together and in the first instance there is a green target. Here, you can see this green target which is corresponding or which is complementary to the red target, which is already mobilized onto this particular layer of the cantilever. So and this cantilever is also coated with the Piezo material. So, you have transduction of mechanical into electrical signal across this surface of the cantilever.

So, now when you flow this green probe which is only specifically able to bind to the red probe leaving the blue probe, there is always a surface reaction at this cantilever end here. As you are seeing, the DNA is binding here, the red probe which was there on the cantilever is binding to the green target which is flowing by, whereas in the other cantilever here, there is no binding - there is no significant binding because this blue probe here does not really bind; it is not complimentary with the green probe (Refer Slide Time: 64:03 to 64:27).

So, what would happen? There is a change in the signal generated and if this were to be the reference signal and there was the reference 0 in this area, there would certainly be differential which is being introduced here. So, this is a differential signal that is reported with time in minutes as the binding takes place. So, as you see here, the binding is ensuring that there is a pickup in the differential signal. Then, there is continuity here. Until now, what we do is we flow another probe of similar nature, but now this time this is complementary to the blue probe here.

So, there is another target which we are flowing here which is complementary and it has the same number of base pairs on both sides cases to the blue probe here (Refer Slide Time: 65:11). Now, the blue binds to this yellow target and yellow does not bind to all of these bound DNA only. Therefore, this cantilever now also bends. So, this corresponds to a change back of the signal, all up to 0. So, the differential signal which arose here because of a binding of the green target with the red probe changes back to 0 because of the binding of the yellow target with the blue probe.

So, this is how you could really make fast DNA detection possible, by looking at just bending mechanisms associated with a pair of different cantilevers. This is an illustration of how these cantilevers would typically look like. You have all different shapes and sizes for these cantilevers. This was actually reported as a module by IBM and it is Zurich research center for the first time, where they talked about how DNA hybridization can be carried out using mechanical signals. So, this was one of the first initiatives in the area of how sensing can be in terms of transaction into mechanical signals from chemical signals.

So, in essence, this is some of the range of work that has been covered in the area of DNA related detection and sensing using BioMEMS kind of architectures.

This brings us to the end of this particular lecture. So, in the next illustration, next lecture, we would cover different topics or aspects related to proteins. Then, we will look at how the living cells normally correlate between the sequences of the RNA and the proteins, and we will find out more. It is in fact a very interesting topic of process called transcription and translation within a cell.

Thank you.