BioMEMS and Microfluidics
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Lecture – 21

Hello and welcome back to this lecture 21 of a BioMEMS.

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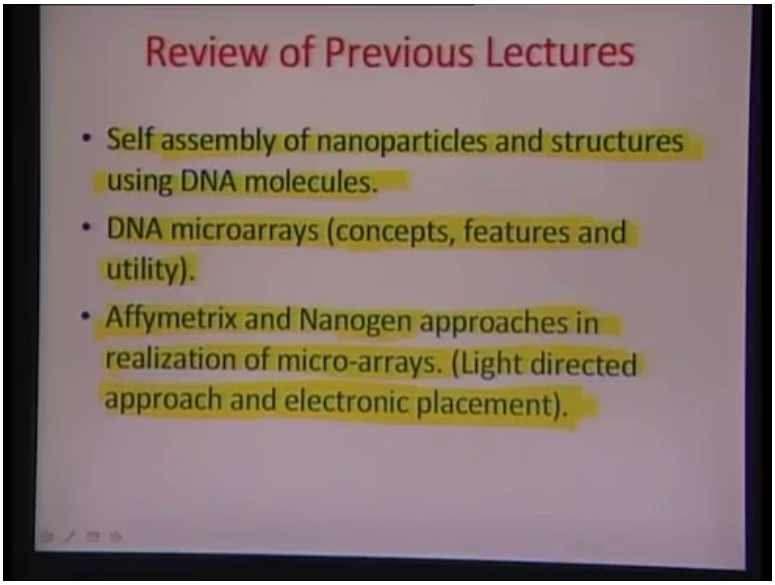

I will do a quick review of a what was done in the last class, so we talked about are the self-assembly of nanoparticles and structures using DNA molecules, where in basically the complimentary of one strand with in other was use this property to symbol one or more nanoparticles, in the strategy could be use for making inter connects at the nano level wires etcetera. So, we also talked about DNA hybridization as a strategy to detection and discuss micro arise. Essentially they are libraries of a different capture probes, which can be used to hybridize the DNA specimen target DNA molecule.

And then, you can actually label the target with the fluorophore, so that as it goes on to the capture probe and get is mobilized on to the surface you could do a florescence 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 the little bit of concepts about micro arrays features, what exactly hybridization is are how it can be done are how sensing can be done.

And then, we also discuss about the various utility aspects of such micro arrays essentially RNA perception profiling or HIV resequencing all this different things can be studied using

DNA micro arise. We also talk about two different companies or two different approaches that exists in the market as on date for these different micro DNA microarrays. The two companies are Alphamatrix and Nanogen one of them actually the alpha matrix is one, which does a light directed synthesis of this capture probes.

So, essentially by using several photo masking's strategies and chemical steps in between there is built up of molecule by molecule. So, you can have several combinations of these different A T C G is in order to realize variety of capture probes in different points of space over the whole array. And, so therefore, you could actually open and close pixels by doing multiple steps. So, that it could build exactly the molecules that you are looking at have an information of sequence on the capture probe.

Nanogen on the other hand is company, which actually gives you the chips and let us you sample the capture probes that you want. So, you have to by the capture probes separately and this is also known as the electronic way of a mobilizing capture probes. Essentially there are bunch of electrode arise on a surface and your using a positive potential to pull selectively on some few poss. goal post. these molecules is negatively charged DNA molecules, which would thereby go and bind on the surface there is binding film like polyamine seen just essentially positive charge and there is an electrostatic attraction between the negative charge DNA and the surface.

So, you have a built up different over this different electrodes all different kind of arrays. And it could actually use a negative potential on the others, so that you could direct only to the particular position, which you want the capture probe as it as a very specific process for doing this immobilization of capture probe. So, we talked about this different approaches and then, also discussed about of the DNA chip from Motorola, which uses electrochemical sensing using efficient label and a bunch of different molecular linkers and wires as standing up and surface up right is a best polymer.

And using of signaling and capture probe in order to capture a certain target and position the efficient six are the you know the Ferro seined probe very, very close to one of this molecule wires to take up the current and then, read out what is there a sequence on the target DNA.

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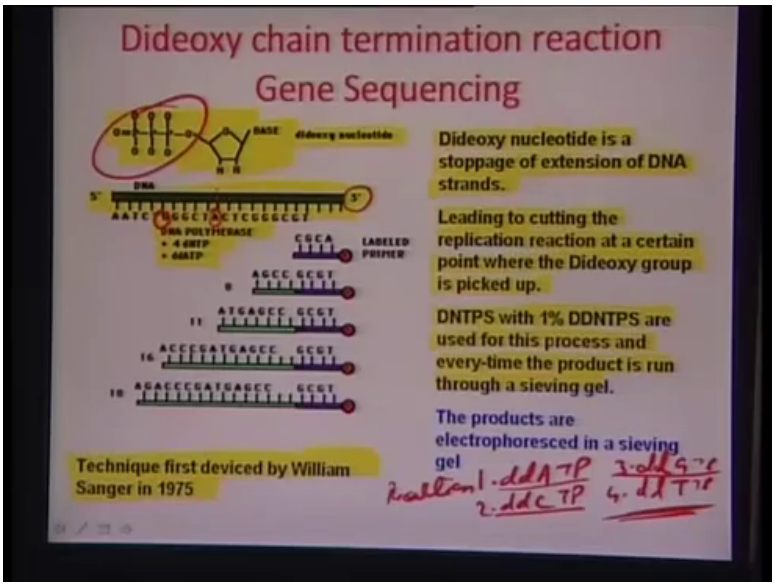

So, today we will actually start with new area, which is also known as a in the fundamental process for gene sequencing it was developed way back in 1975 by scientist name William Sanger on this process has been popularly known in this name a Sanger's reaction. So, this is again very interesting and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

So, essentially this sequences of very important piece of information, because it gives an address of a particular biological moiety or entity, which contains this. It may be unique to a certain bacterial cell or certain virus and it may able be to identify if the virus is there in the ambient just by looking at the sequence of the DNA there it has. So, it is a very important information which is now, after the human gene project been recorded in a large data base, which is maintain at a global level and re such gets on added, where this sequences are identify for any news stain any new bacteria any new virus and it essentially put up there it is also known as the nice data base.

Now, how to be identify the sequence this very famous Sanger's reaction is all base the round this molecule, which is Dideoxy nucleotide. So, you can see the schematic of the molecule here Dideoxy nucleotide. So, therefore, there would be the Dideoxy chain terminated A adenine the Dideoxy chain terminated cytosine guanine and thymine respectively, so there four different Dideoxy change terminated molecules.

Now, the way that this course is that there are the several a different consequence in the Sangers reaction there is an enzyme the DNA polymer is with essentially synthesizes you know the broken stands of the DNA. There are different four different DNTPS, which is the normal A T C and G it is not that Dideoxy chain terminated NTP and then, we have four difference reactions. So, this process is carried out in four different steps each of these steps there is a different Dideoxy ATP, Dideoxy CTP, Dideoxy GTP and Dideoxy ATP essentially.

So, let me just write all this down here for you convenience, so you have reaction one ddATP number two with ddCTP three with ddGTP and four with dd thiamine and neutrino tied the Dideoxy chain essentially is the end groups, which stops the reaction. So, whenever you have the addition of the Dideoxy chain it does not let the DNA chain replicated more it essentially stops the replication process, so the changes probe and that instants.

So, suppose if I have a reaction where in one way the chain is the broken along A essentially. So, I can actually have a DNA molecule, now from the three prime and all the way to about A if the reaction goes all the way up to let say this particular G here, so I can now, have the in another reaction the chain terminated from G. So, therefore, three prime to G will be there on the chain. So, whenever you put this different the Dideoxy chain terminated agents, which is also about one percent by volume of the NPTS into the reaction and then, independently do the four reactions, so wherever there is a thymine and this gets bounded to 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 put if you try to put this ddGTP on the reactions wherever that as a site to site on the principles DNA stand the DNA will break and, so there will be multiple lengths of DNA available is a n product of all these solutions or all the reaction would mind you these are terminated only at a single group like for example, if it is ddGTP it will terminate only act the site to site groups in the DNA, if it is ddDTP, it will terminate only on to the adenine groups on the principle DNA.

So, once this products are run through the gel they have all varied lengths and you can easily fractional them using gel electrophoresis. So, the smaller the DNA is the faster it moves, the longer it is a slow it moves and essentially will have a read out like this.

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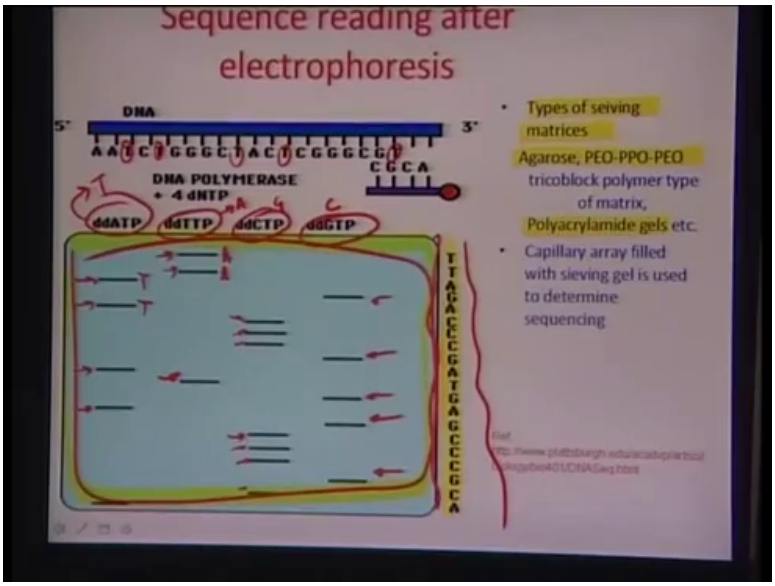

So, this is the ddATP, so essentially the chain termination here take place along the thymine groups. So, wherever there is a thymine on the DNA molecules you have fragments made of that level. So, essentially these thymine terminated processes are represented as gels let us say here, here, here, here and here, so gives an idea of the position are the length at which this particular group expression. So, wherever there is a ddTTP by Dideoxy TTP it terminates the molecule wherever there is adenine.

And, so therefore, if you run the products you have this different lengths of products, which are coming out, where there was actually and adenine on the parent DNA group. Similarly, the ddCTP will have products, which are kind of fractionated in a manor corresponding to wherever there is a G or a guanine and similarly, ddGTP the products terminated in a manor wherever there is a cytosine in this particular group. So, you have this different extension, where you can find out that how are where exactly what groups is located.

So, if you read this out is 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 A here and A here there should be a T here, similarly there is a T here. So, essentially this kind of gives you a feel of what exactly it is that you are looking at and of the DNA. And, if you just put the sequence all together something like this you get the total sequence on the DNA.

So, this is essentially the process in a nutshell slide if would like summaries, what happens. So, Dideoxy nucleotide is it kind of stopping mechanism for the extension any for the

extension 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 player as that on the Dideoxy n group it is picked up and the reactions stops there.

Similarly, the DNTP is with one percent ddNTPS are normally used for this process and every time the product is run through the sieving gel you have all different sizes, which comes up and the gel from, which you can read out really, what is sequence of the DNA and a essentially the read out this is also electrophoresis best in this case. Now, when we are talking about electrophoresis would like to just a and if read to way to again that there are different types of sieving matrices based on which you could have almost closed to a one base pair is a solution.

For example, the different products like, let say pluronics triblock copolymers polyethylene oxide polypropylene oxide polythene oxide this has essentially very small pores you know and that is absolutely case for and gel material with in capillaries is fell it results in reusable or washable capillaries essentially. Because, this material goes into liquid or gel depending on the ambient temperature there is an agar's material, which is most commonly use liberators for this wage of electrophoresis there are polyacrylamide gels there are product associated with hydroxide kill cellulose.

And a lot of different polymers of the kind, which would have essentially a porous contain and would also have some kind of a storage for the buffer to be there in the channels etcetera. So, these matrices are very often use to get this kind of read outs as illustrated here just about minute back and from that you would actually read, what is there on this particular DNA. And that there scene is to be a little problem that and the problem is the speed the rapidity, which you can read this out.

And, so therefore, there have been initiated are efforts in this BioMEMS and diagnostics area, where people of really try to evaluate that can all this mechanism of this reaction and gel and read out so and so forth. Be made you know May sensitive enough are rapid enough, so that it you could go to a very low time of detection level.

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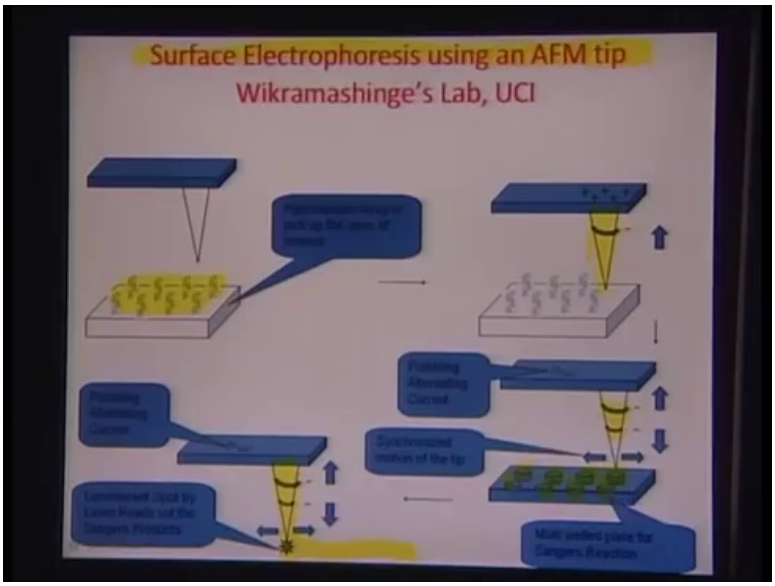

So, for that there is a group in a you know University of California, Irvine, who are working on this strategies based on the atomic force microscope essentially the idea is to be able to pick up molecules on a very small miniscule level and use a surface electrophoresis to shift the molecule back and forth in this particular manner by applying a potential. And the, once a these are all pickup using a hybridization an array are something and there is a primary level detection you could sequence by running a term the small vales here and running this angle reaction picking up the products.

And then, the idea is that the label is kind of in certain to the particular sequence to product you can see the florescence once it is move down with the pulsating electric field. So, as it crosses this self-luminous step here it kind of gives of florescence response from, which you can back calculate, what was there on the end on the group on the DNA essentially. So, you are at a stage picking up the correct DNA using hybridization and then, picking it up using an AFM probe using a small voltage at the depth of the probe.

And then, essentially you are trying to drop this back down into small, small numbers into different wells we should do this four reactions that, so associated with Sangers. And then, the products when they are all the different lengths they are kind of a labeled with certain fluorophore, which is also corresponding to the n group, which is their on the Dideoxy chain are which is terminated by that Dideoxy chain that particular molecule would come out at a certain rate, where it will come out a certain point of time air from the depth.

The way you read the florescence again you create a small self in spot here and then, using

AC signal you can actually send this down this DNA down you have picked up the DNA here in this case from this particular well if you can see and, so you have the send this different lengths down along the tip of AFM. So, that it kind of a goes one by one as it crosses the luminous point you get a certain florescence read out from, which you can find out, what was the base pair, which was chain terminated are which have that Dideoxy n group.

And from that you could also find out what is the sequential the DNA that you are picking up. So, this is essentially very interesting technique that people have been developing.

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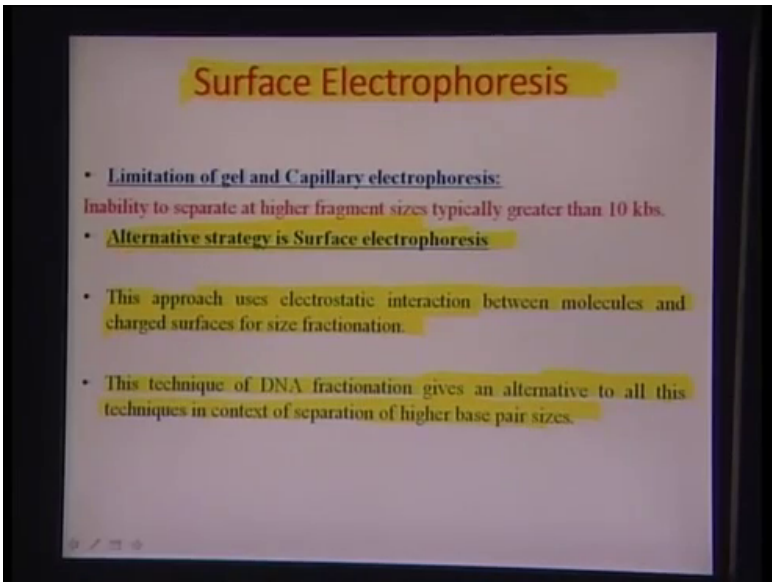

I would also like to illustrate a little bit about what surface electrophoresis would be actually able to do in this particular case as you have seen the first question, which comes to mind is that why is the people would do electrophoresis on a surface when there are already existing gels another materials we should do this electrophoresis beautifully. One of the reasons why electrophoresis taken from a gel or a volume in to a surface is essentially, because if you go above a certain base pair, let us say about 10 kilo base pairs also and the gel material respective of how big the pores are the has a tendency of a cutting down the resolution of DNA.

So, beyond the certain point let say if you are actually going above 10 kilo base pair and trying to translate and fractional DNA across this gel material there is a model called biased repetition model (BRM), which comes into picture which means that you know let us suppose the DNA is like a serpent, which is kind of a unfold that if you may remember we discuss this certain length when we are talking about electrophoresis.

So, the head of the serpent goes into a series of a pores, but then the DNA realize too long to go through this bunch of pores into the next section as a result of which it decides to kind of stay back there gets jumble. So, it does not move forward any more. So, this is an effect because of excess fraction between the walls of the capillary are walls of the of the pore through which the DNA passing to avoid this problem people or scientist have investigated this process are using and alternative strategy called surface electrophoresis.

So, this approaches essentially use electrostatic interactions between the molecules and the charged surfaces for size fractionation I will just about explain this automatically in a little bit and this technique DNA fractionation gives 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, have to provide.

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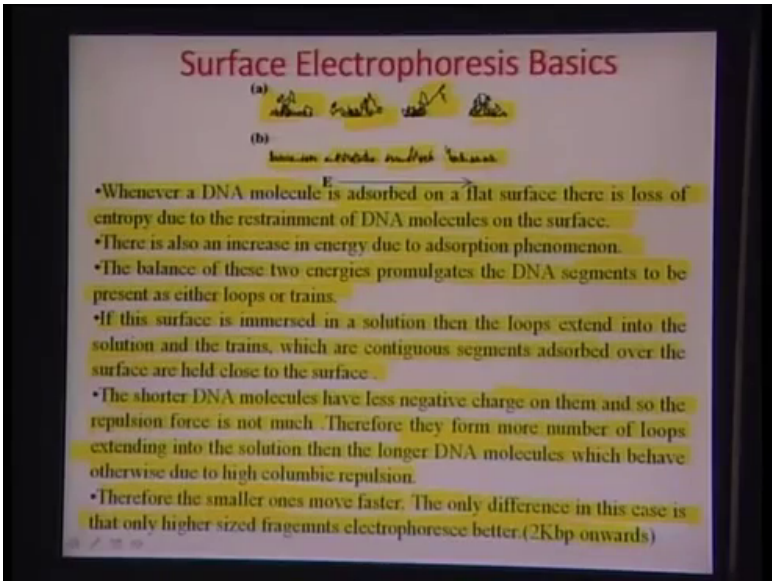

So, how the surface electrophoresis is done this is essentially out of a research work done and Benjamin choose group ((Refer Time: 19:22)). So, essentially if you look at that DNA molecule is adsorbed on a flat surface there is really a loss of entropy pretty much due to the restrainment DNA molecule on the surface. So, that is also an increase in energy due to absorption phenomenon and that is balance of this two energies, which promulgates that the DNA segments to be present as either loops or trains.

So, you are having a flat surface and you are putting this physisorbing are being is DNA on the surface and you essentially have layer of water over the surface and the DNA still adsorbed physically adsorbed in the surface over, which the water is present. So, the DNA

has a tendency to kind of balance the absorption energy over the local increasing the entropy, because of the extreme amount of negatively charged backbone and there is a configuration, which comes out of if it is essentially like loops and trains.

So, portions of the DNA or looping in to the solution as of they are kind of hanging out and portions of DNA are essentially adsorbed on to the surface of the solution. So, people of shown with molecular dynamics very interesting emerge as a results, which can call for.

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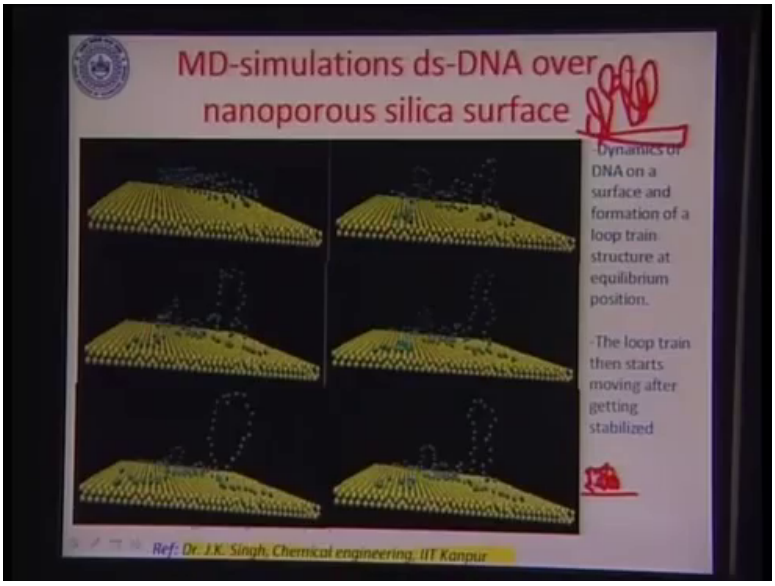

Let suppose, if you look at this particular figure here you can see how using a ball and string model this person here has been able to show that you know there is tendency of this DNA to kind of get folded into a loop as well as this strains. So, there are the strains here and there are this loops, which essentially formulate. Now, one interesting factor here is that longer the DNA molecules the more would be the train part or more would be adsorbed part, one of the reasons why that is show is, because you know the loops is essentially are a bunch of different negative charges.

So, if the molecule is very long you could consider that the charges at two much two be in the solutions. So, there is a dense network of charge like this on a surface like and, so if this DNA is long enough there is a huge repulsion if the if you towards to be presented loops. So, it is definitely not a favorable energy configuration and, so other configuration that would be available would happen when they are really adsorbed very close to the surface their small. So, the loops are basically small and the train part of the absorption part is more.

So, we get one fact from all these that the DNA molecule if it is a longer molecule it is a long

molecule there is a tendency of the train part to be more as suppose to the loop part how, which closes are close that at here is that adsorbed that on the surface. And this condition if you try fly to plain electric field and try to move it along the direction of the electric field there is a tendency of the DNA at move slower by virtual of the absorption energy, which come into play retain you have to go to the next step the DNA has to go to the next step.

As suppose to the shorter DNA segment where probably because of a less interaction and less entropy and longer loops and less absorbed area the DNA moves faster. So, this is a actually bases of size based fractionation of DNA, but just using surface without using bulk properties. And therefore, all those other impacts, which would have otherwise come from three dimensional gel kind of matrix, where there are bunch of difference force to which this DNA is moving and there is a lot of fiction between the DNA molecule in the pores you almost always get bundling and you know a loss of resolution at higher base phase.

However, in the surface case the surface and the environment rounded automatically adjust the DNA to have smaller loops or longer loops. And essentially it just makes it moves slow or faster and that is a huge amount of a resolution aspect in this particular case. So, I would like to illustrate some of the work that has been already done, so this is the case of a shorter molecules. So, this is essentially is a case of shorter molecules, whereas these are the longer once, so we have more trains the loops.

So, in summarily, so whenever there is a DNA molecule adsorbed on flat surface that is a loss of entropy did was strain want a DNA molecule and the surface that is also an increase energy it absorption and the balance of these two energies promulgates the DNA segment we present as loops or trains. If this surface is enough, then solution where the loops extend include the solution on the trains, which are contiguous segments adsorbed over the surface are held to close to the surface the short of the DNA molecules are they have less negative charge on them.

So, the repulsion force is not much and therefore, the for more number of loops extending into the solution, then longer DNA molecule, which behave otherwise due to high columbic repulsion the absorb more in the surface whether, then going into as loops. So, therefore, the smaller one should move faster, because there less absorb on the surface. The only difference on this case is when that only higher sized fragments electrophoresis better in this case typically this 2Kbp onwards, but essentially the effect can be more from 10Kbp, because just like agorase easily go up to 10Kbp.

Of course, there would be a solution loss, but the still it could make a detectable about up to by 10Kbp. So, the utility of this process is for beyond that although they can actually start starting from about 2 kilo base pair of size are the DNA molecule.

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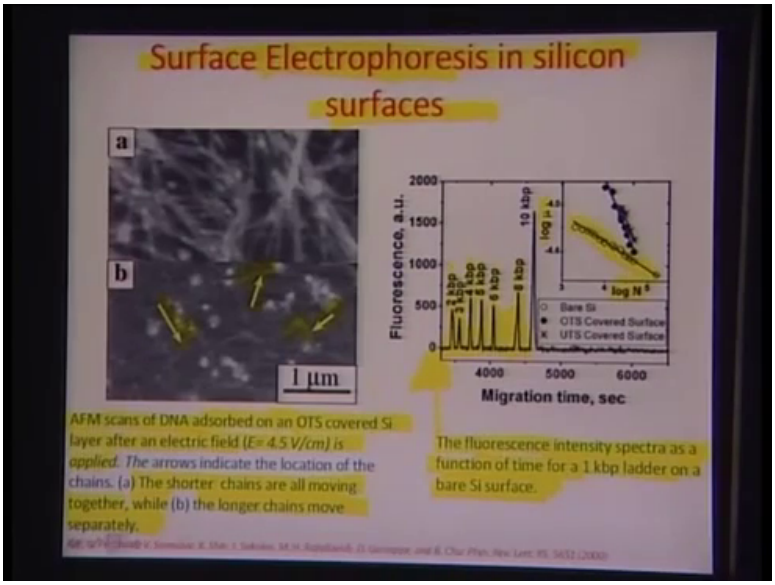

So, this is essentially taken from Benjamin choose crop where the talking about using this technique to detects the relative migration time of different sizes. So, here you can see you have a DNA of 2Kbp all the way to about 8Kbp 8 kilo base pairs. And you can see that if you see the log of mobility it kind of increases decreases as you increase the number of sizes this is the flat between logs of mobility of the DNA verses the log of number of base pairs.

So, the number goes up here as you see the mobility goes down, which is in perfect agreement of whatever theory is really you have illustrated this from this particular plot here of the migration time verses florescence. So, one more interesting factor which choose as groups shown is that you can really detect with in AFM the DNA adsorbed on an OTS covered silicon layer after an electric field of 4.5 volts by centimeter is applied.

So, here essentially these arrows as you are seeing kind of indicate the shorter chains and the way they are moving. So, the short a change are all moving to gather if you see you know these arrows kind of signify them these are the shorter chains, here essentially these are the shorter chains and the longer chains kind of move separately, which is probably not shown in this particular figure, but Benjamin choose group as observed this essentially. So, essentially very repeatable kind of data on a surface of DNA has been recorded repeated and a florescence intensity spectra as a function of time for 1kilo phase per ladder on a bare a size

surfaces is given in this particular a graph as discuss before.

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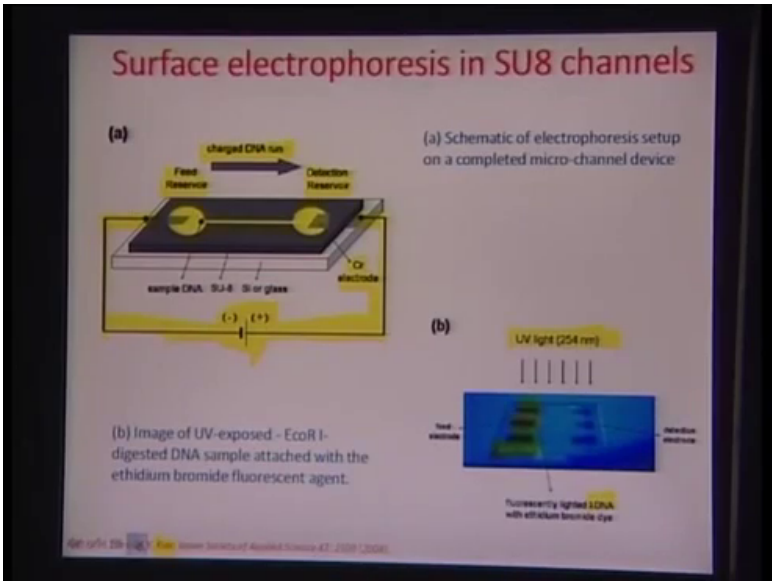

So, I like to show you the how some of the detection can be done in this in fact, paper by *Co et al*., but university of Tokyo and, so what it takes about is that you have a micro fabricated architecture here you are talking about next we way based micro channel with some reservoirs and this is a mounted in the top of two electrodes, which are made up of chromium. And essentially on a gun class plate and you connect this electrodes when external battery external source has indicate here.

And the basically run the charge DNA from the feed reservation side, which is this after the detection is was side, which is this. So, here of course, they have shown using UV light at 254 nanometers the label DNA the florescence lighted DNA with TPR ((Refer Time: 28:06)) might this is also force lambda the DNA that kind of moves all through you can see these a figures of the reserves and they can see that the time the florescence splits from the reserve all the way up to the detection reservoir, what they also report as is implement base signal from connected from the electrode at the detection is where.

And why the see picks coming as this DNA molecules would kind of go through and pass of heat the particular electrode a positive electrode made up of this chromium. So, this kind of gives or endorses for those of this concept of surface like electrophoresis.

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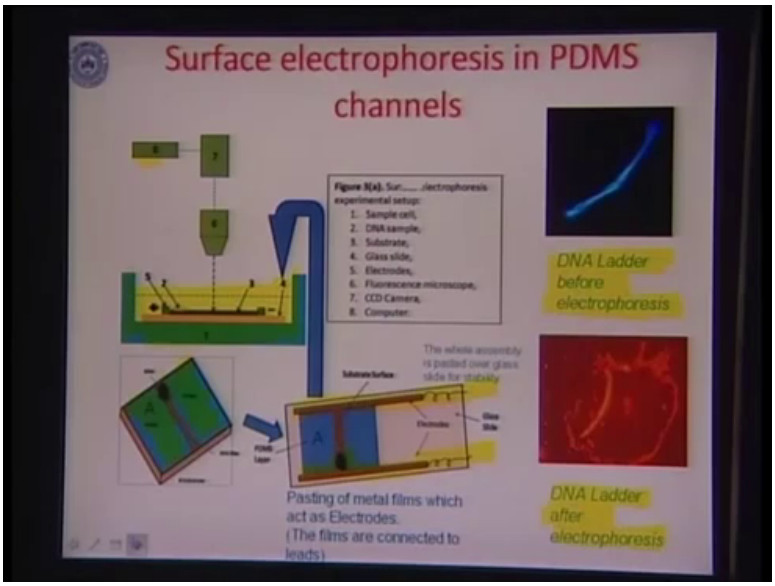

Our group of some works an electrophoresis well of this is and most is a syntax from, then one of the one of the works imported and the American institute of chemical engineers 2009 meeting. So, essentially this talks about a surfaces silicon surface with the a potential a crosshead, where in you are actually physiosorbing are being some DNA molecules and one side of the surface we have a PDMS upper layer, which is acting was a cover and also give a micro channel in between through, which the DNA should translated and we have actually pasted electrodes film electrodes and both sides.

So, that you can apply a strong field from where the DNA is adsorb this side all the way to the other side here. And then, you are connected these the external wires and the sole assays place inside a very small flow side as you see here in this number one is the flow cell and you are feeling this paper covering this whole thing up that the buffer solution. So, the DNA kind of loops into the solution and forms the same exacts and configuration as was reported by Choose group.

So, it is essentially brought and the inverted florescence is microscope as you can see here, which kind of a gives these a read up and these a show some of the illustrations of a how a DNA ladder would behave by doing surface like electrophoresis. So, this is the die depot DNA, similarly this is after in a few minutes of a surface like electrophoresis occur. So, you can see there is a mark difference between the ways that the ladder looks before electrophoresis supposed to after electrophoresis, which simply physics are done to the, the silicon surface.

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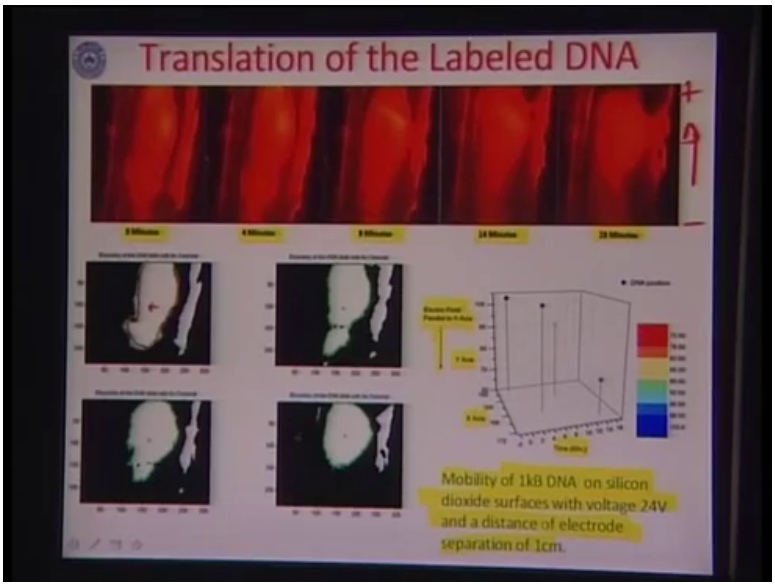

I have a some other data to share with you here is a again the translation of DNA molecules of across a surface of different instances of time you could see all from 0 minutes all the way about 23 minutes. And you could see that, because you have applied a positive and negative potential in the following manner you have seen that there is a translation of DNA towards the positive potential you can actually do through image analysis scan on the overall boundary and try to detect the centroid.

And then, calculate mobility this right here is illustration where we are talking about the mobility of a such DNA molecules. And we find out there are although there are electric field is a applied only parallel y access there is almost always the Brownian motion along the x access, which causes the DNA two move ether as post to illustrate away in the direction of the electric field.

So, the mobility of a one kilo base DNA on silicon dioxide surfaces with the voltage of the 24 volts has been plotted here in this graph and this is a corresponding to a separation distance of the electrode of around 1 centimeter also.

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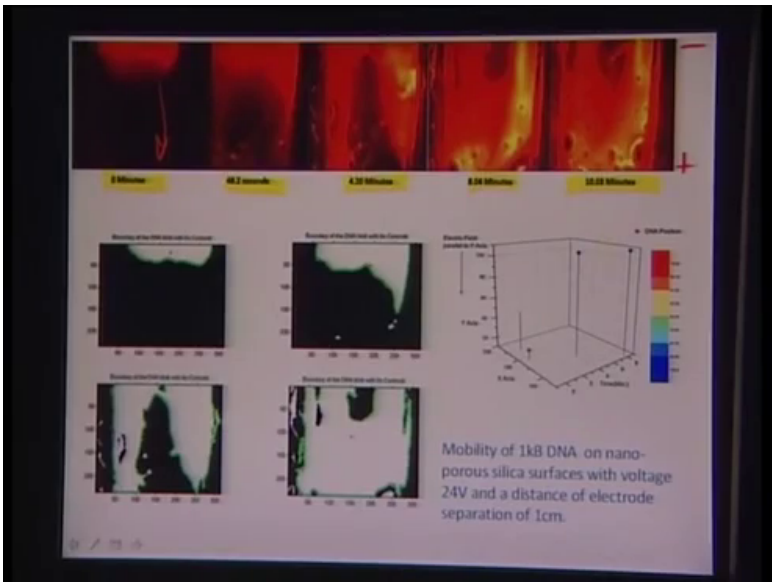

So, this is the a perfect by directional process as you can see in this particular illustration, what we did here is really we just went here and change direction of the electric field here this is negative and this positive. And you can see slowly that the DNA kind of a emerge back into the field of view for this different time intervals as can be illustrated form 0 minutes all the way to above 10.03 minutes.

And then, we kind of plot the mobility and the same mechanism as we did before and still see the Brownian motion. So, this is a real problem because as you can get one dimensional mobility model, because of which we trying other alternative like kind of super focusing the DNA on to very small track. So, that we can eliminate totally the Brownian motion in the super focusing can be obtain by using different surface energy pattern and a surface where we can really make an arrow hydrophilic pattern where all the DNA would rapidly converse and we not a sharing the data at this time. But, I just wanted to make illustration of a what kind of research is possible in this particular area of surface electrophoresis.

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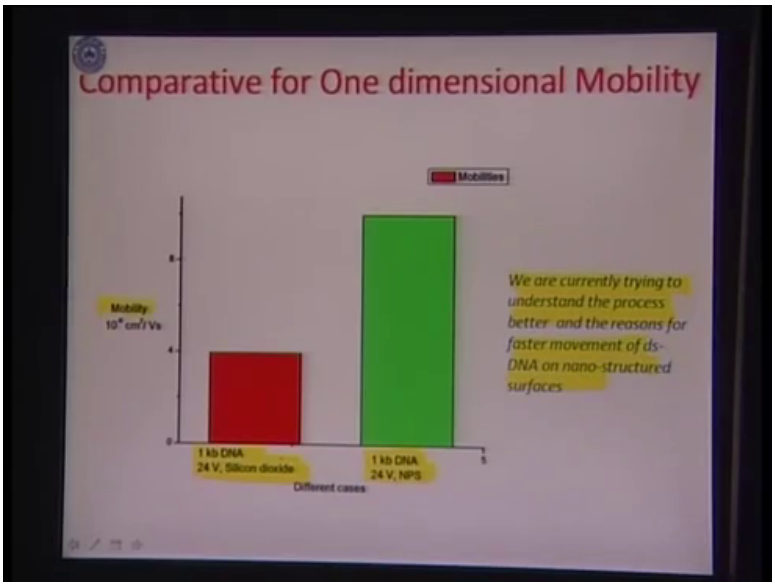

So, this is a mobility studies that we did where we can show that you know on an energy on a higher way on a surface like silicon dioxide is supposed to a lower energy surface like a pores structure pores matrix made up on silicon. We have

made up a silicon we have differential motilities, which we report and we are trying to understand the process better.

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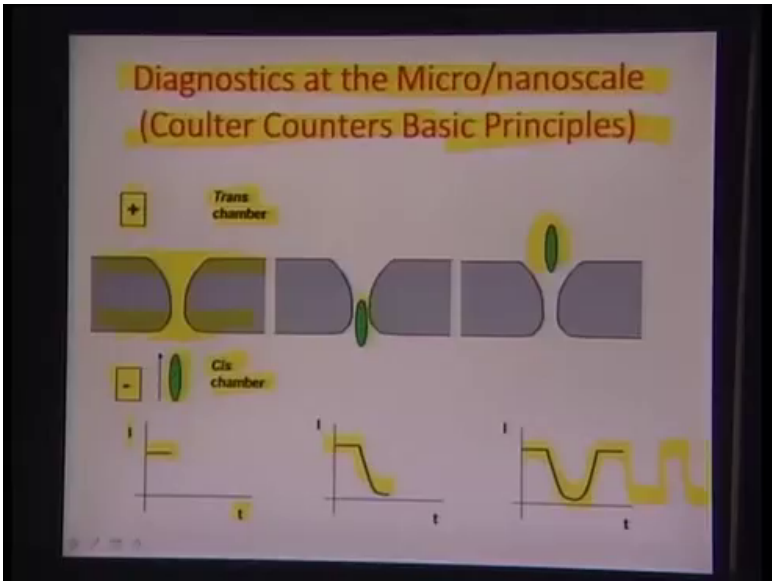

And essentially this is how surface electrophoresis can be done, so I now would like to illustrate another very novel technique of a BioMEMS, which BioMEMS has to offer a based on various fabrication protocols are various kind of structure and features that can be fabricated using micro system fabrication technology. So, this is really the diagnostic at micro nanoscale and is also the basic principle behind Coulter Counters.

So, we are talking about some kind of cell counters by the name of Coulter Counter. So, the whole goal here is the following, so you have a pore small pore, which is closed in the size of a single cell and this pore this pore is membrane is kept as the only communicating medium between two fluid cells, where one side is rich in this cells and other side is essentially lean mixture and if a assuming that this cells are negatively charged and you apply a positive bias.

On the other side of the famous on the other side of the pore there would be a tendency of the cells to go and going to the pore and go straight out into the other side and while doing there it kind of momentarily blocks the pore and blocks the ion current, which flows the reverse direction has suppose to the direction on the moment of the cells this is simply based on this principle.

So, you have negatively charge cell here and you have a pore micro pore silicon, which is made like this, this is the trans side trans chamber this is reside, where in a applying a potential across this particular pore is membrane in a manner that this negative cell is dragged

into the cross chamber side. 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 and the moment the cell boss and blocks the membrane there is the dip in the ion current and when you goes to the other side as you are going here the current kind of takes up back.

So, if I can count this dips with time I can be able to a certain, how many cells pass per in time from this a particular micro pore and this could be a bases of counting the number of cells that there in the rich side of the solution. So, this is the basic principle of micron nano scale Coulter Counter based on that based on this micro Coulter Counter you can extend the same concept on to the nano scale in fact, where the first one of the first few articles, which came out in this area was really from a Kassianowicz group group.

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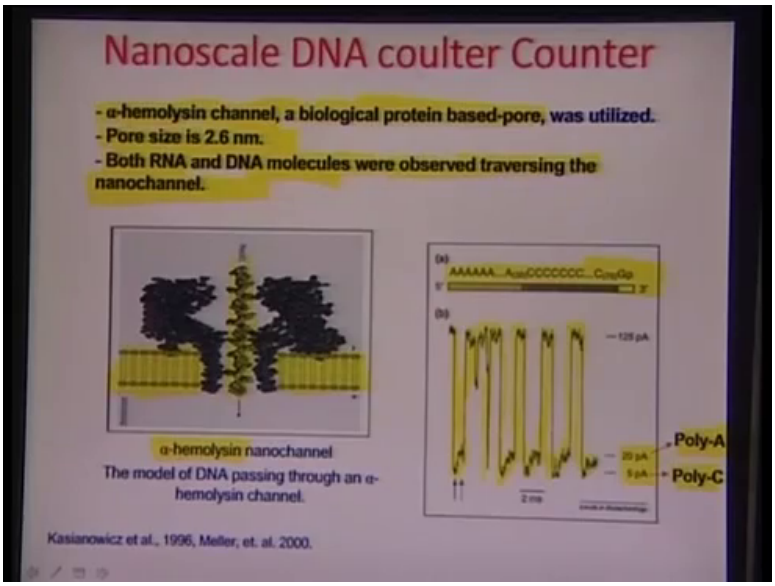

And would like to illustrate one of that example here, where the talk about self-assembly of a protein by the name of the alpha hemolysin on the top of the lipid bylayer of a cell. So, if you see here this is essentially the lipid by layer that the talking about in you are assembling self is, so you are actually put in this protein alpha hemolysin on such a lip it by layer and it assume that itself assemble. So, there is a tendency of this protein to kind of a embedded to the by layer and kind of self-assemble itself, what is very also very interesting.

And important here is that if you look at the alpha hemolysin has a protein molecule it really has a pore within you know right as a small narrow channel and this also access the biological protein based pore there are lot of a charge transfer process across particularly our central neural system nervous system, where we use a this charge in take and charge outside

delivery in order to transmit between the cells and these are calling endocytosis based process, where there are essentially you know proteins on the cells involve which you know open and close and give way to the charges.

So, so the pore size illustrated here on this alpha hemolysin is about 2.6 nanometers and as an indicated before one helical turn of a DNA is of about 2 to 3 nano meters in size are also very well matches with the 2.6 nano meter pore size on the alpha hemolysin protein. So, as a result of which if you are assembling this kind of a structure across a session a chamber just in the manner that have been done for a cell.

But, in this case you make one side rich in DNA concentration in other side lean and then apply eclectic field you should see in the similar kind of effect as a drop or you know a dip in the current verses T plot to detect the single molecule single DNA molecule if it passes by. So, that is exactly what this principle is here the recording that was made using this kind of technology shown that you have these small, small dips, which are coming out just because of block with the find current as 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 the size effect.

So, what is also very important for me to tell you here is that it is really is size effect; that means, if you have let say a molecule poly A as opposed to poly C, C is if you look in to the molecular structure of C cytosine it is little bigger as to six carbon five carbon brings essentially adenine on the other hand is a smaller little bit smaller molecule. But, you can see here there, there is a really mark difference in the current values in case of poly A the current is lightly higher than in case of poly C, which means that you know if the sizes is more, let say this is 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 we should be able to get generated in the case of loosely fitted the molecule is slightly higher in comparison to the current, which you can generate by a kind of tightly fit in the molecule in the pore and also what is more interesting is that in takes a little more time for the DNA to pass through this pore once it is tightly fitted as a suppose to the loosely fitted. But, this a in a sense is kind of break through it gives as feeling that some day may be with this kind of strategy the we could be able to sequence of the DNA.

In terms of what bases it as just based on the response of a of the current with time this is really how the in the industry how to electronically be able to sequence there are in fact companies now which are doing these electronic based sequencing and at least there if you

product, which are tried to be made commercial very soon about electronic sequence there lot of novel ideas and lot of projects which are in the direction of how to quickly electronically detect this sequence and people have dream in the range of something like a Nano second the base pair the kind of read out of these kind of a processes.

So, in this particular work though that the both RNA, DNA what tried and they where adsorbed traversing though the nano channel structure right and that kind of case a new direction to this whole area of manufacturing nano scale pores. So, one problem though about this particular work is that lipid bylayer being the very fragile kind of system which this which can be effected by temperature pH lot of this parameters people standard thinking of a k can be really produce synthetic pores made up of material which are may be thin wafers polymeric metrical.

So, there are few groups in the world which have developed some of the synthetic nano pores in that why this static is that people are normally followed is a using SOI silicon on insulator wafer as we just illustrated in this particular example.

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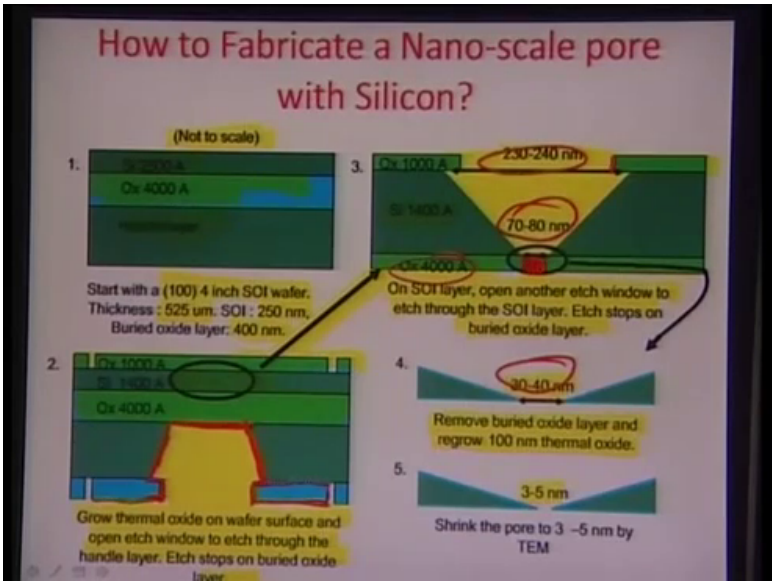

So, here as you see there is a there is an oxide layer of about 400 Armstrong's there is a silicon layer on the top of it about 20 by 100 Armstrong in this whole is assembled on to the handle layer, which is thicker wafer couple of microns problem couple of 100 microns and this although it is not scale. So, it is a four inch SOI wafer with this handle layer thikness of the handle layer is about 525 micro meters this SOI is about 250 nano meters, buried oxide layer is about 400 nano meters.

So, what you do essentially is that you first grow thermal oxide on the wafer surface here you can see on these two sides essentially you growing the thermal oxide is about 1000 Armstrong about 100 nano meters thick and this you do all sides of the wafer. So, therefore, both surfaces having essentially this thermal oxide. So, once you grow the thermal oxide in the way for surface you open a etch window somewhere in the one of the sides as you can see.

In this particular illustration here this etch window as been opened in the oxide, where you essentially make silicon as the selective layer and silicon dioxide as the etch layer it is design the in that kind of nano and manner and use photolithography process to carve a small window of a sacrificial resist to create a via though, which we could actually etch away the oxide and create mask the hard mask of the oxide in the surface of the silicon once that is done you can etch though the handle layer just as illustrated in this particular example using anisotropic etch process.

So, essentially it is angle of about fifty four point three four degrees and isotropic etch as will be talking about details later on is next system, where use either some kind of hydroxide to perform and electronic change process as the layer etch is away we are essentially converting silicon in to corresponding silicon hydroxide and dissolve in way to this solution either, what is important for me to mention is that a certain planes and certain interested planes one the silicon surface the electron release is essentially limiting the rate for formulation of hydroxide, because of the higher density of atoms there is more binding of electrons in.

So, at a plane with which is also call 111 along that direction etch step is slowest. Eventually whenever you do this h in averaging effect the it kind of shapes in the shape of a one frame which is a number of 54.34 degrees with respect to the vertical. So, that is how you get this kind of a window here. So, you have to use etchant combination one which is selective to SIO2 and one which is selective. So, it cannot h this oxide once it hits the oxide either it etches the silicon, so it is etching silicon , etch I selective to oxide and in the other system is etching the silicon dioxide and selective silicon.

So, you have to follow processes two first and then process one, so you can hit the right shape over this handle layer. So, the etch stop is the buried oxide layer in that particular case right as you see here, so then what you do actually take this upside down. So, on the now you have and illustration here, where you are talking about again doing the etch window from the top side. So, you are actually and I am not showing the bottom side here the bottom side is

actually shown here in this case.

So, you take this particular top portion as you are illustrated by this circle and then, you are doing in etch from the top side. So, essentially this is that oxide 1000 Armstrong layer which would grow earlier and top of this silicon layer which is 1400 Armstrong's essentially and here this is a thermal oxide. So, you can say that earlier silicon was 2500 Armstrong, but because oxidation is diffusion even process the oxide layer formulated is about 1000 Armstrong making the silicon the pure silicon about 1400 Armstrong are, so now, what it.

So, is essentially you open other etch window here as you are seeing in this portion and here essentially are using again photolithographic masking step and resist with is selective to silicon, but can etch away oxide very easily. So, you can open a 230 to two 240 nano meters etch window and then again perform and other isotropic etch they would be some under cut in this case as well. So, that this whole area here can be remove from the 1400 hundred Armstrong silicon layer and you can design the thickness here in the manner.

So, because this is 1400 you have through calculation as you can show that the lower part here only reduced about 70 to 80 nano meters it is a very small part that kind of remains at the very end of this pit. So, you have a case where you have opened an etch window from the back side and then, use the same process step to open in a etch window from the front side as well and effectively you are just left with 4000 Armstrong oxide layer in between the one which you saw here in between this is only a about 400 nano meter.

And then, you are the advantage the luxury of having a 70 to 80 nano meter hole on one side. So, you can actually now use E beam lithography and now etch or you know hole, which is about close to 30 to 40 nano meters in this particular region you can also use the focus time being for creating such a port. So, you could actually design a system where you can actually open a 32 to 40 nano meter from a small hole in this particular region here in this particular 70 to 80 nano meter region you can create a small hole, which is about 30 to 40 nano meters.

So, once this is created you put an oxide layer around it, so you again kind of grow you first remove the buried oxide. So, this essentially the buried oxide layer as we are seeing this you know this 400 Armstrong layer here is the buried oxide, so you removing the. So, the buried oxide almost in totality except portion which is about 30 to 40 nano meters and then, you essentially regrow the 100 nm thermal oxide in this particular region you regrow in the oxide.

So, for this step this step after you have open the small hole though FIB above 70 to 80 nano meters you are essentially try to remove this layer all together and also try to grow that you

can shrink to this pore from the general you know about 70 to 80 nano meter all the way to above 30 to 40 nano meter. And once this 30 to 40 nano meter is created by etching away this oxide from the bottom you can actually kind of a regrow the thermal oxide on the sides here.

So, if you see here this blue layer in this particular region which are now mark in red is the regrown oxide. So, you have step one there you are removing a small etch window in the back side, where removing a etch window and etching on the front side. So, that you are reduce with reduce to 70 to 80 nano meter pore you cut using E-beam lithography kind of a this 230 to 240 nano meter width here you know of this particular oxide and then you with in FIB kind of try to drill a way with area are alternately you can also remove the oxide all together and grow or regrow about 100 nano meter of thermal oxide around this area.

So, that you are left with about 30 to 40 nano meters one interesting point here to be noted is that if you heat this region using a focus beam. Let say you are using and Ebeam the electron been you kind of heat this particular area you see that this oxide kind of reflows and tries to have self-closing gap. So, this 32 nanometers 30 to 40 nano meters can be reduced about 3 to 5 nano meters as you can see here you are regrowing the oxide and you are trying to flow it that it kind of become self-closing.

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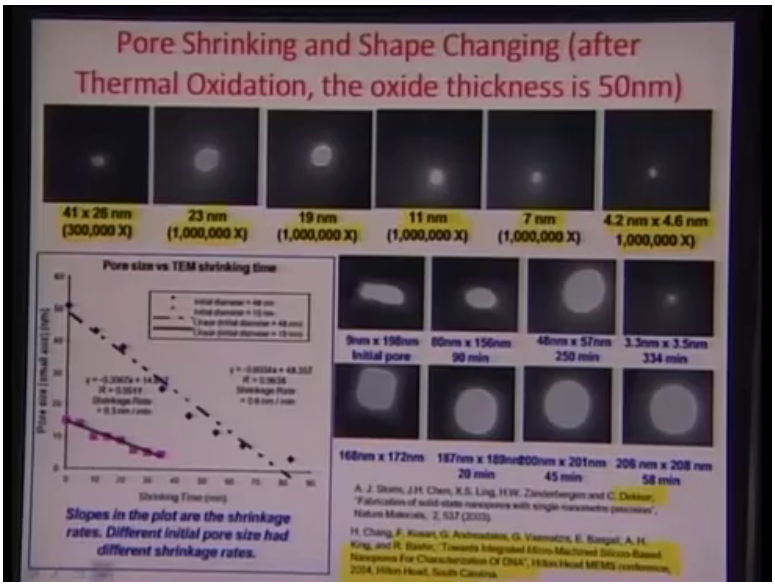

This is illustrated here in the example if you can see from this particular works by reported by Bashir et al. later on the at all what they are showing here is that this is the excellent visualization process you take a TEM and try to start visualizing the pore when it is about

300, 1000 x you can see this 41 into 26 nano meter pore and then the mind you the magnification here is about a 1000 etch which is about three times this particular value and here you are trying to investigate the 23 nano meter pore.

And then, if you look at the pore with time as you are focusing the electron beam the oxide reflow in the pore kind of starts becoming smaller and smaller. So, it starts self-closing tell it about 4.2 nano meter times 4.6 nano meters and you essentially stop the visualization process in this particular instance. So, you are taking the bigger hole here which is regrown with an oxide in the surface and trying to focus the E-beam in and causing cell heating.

So, that there is a flow of this oxide in self-closer of the pore you could see the smaller pore sides here, which is about 4.6 nano meter times 4.6 nano meters. So, this is how size of a DNA would be the very well size of a DNA. So, you can make a synthetic nano pore in this manner, which would later on use for DNA translation and also try to understand gage whether can do some sequencing.

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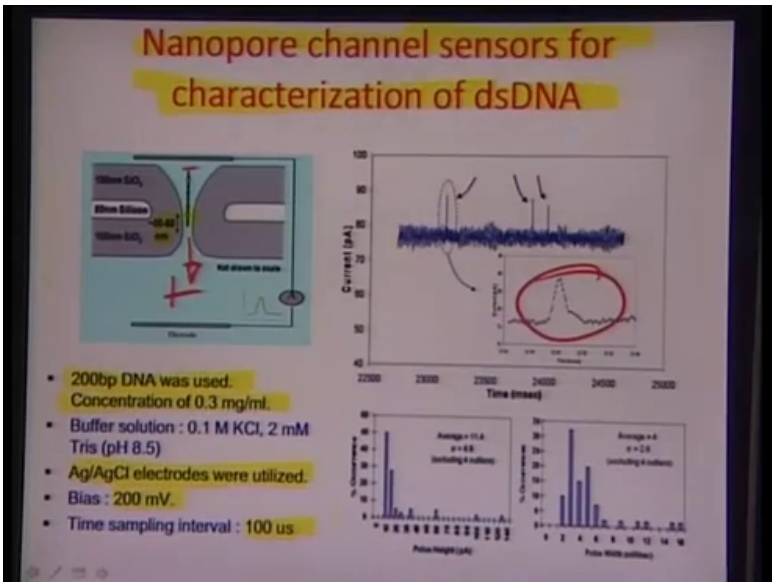

So, this is how this nano pore channel sensors are characterized special practically for double standard DNA structure. So, you can see that there is a micron size pore there is a nano meter size pore here, which is a you know length about 50 to 60 nanometers and in diameter is about close 4 to 5 nano meter and the DNA kind of is translated from cis side to trans side. And, so essentially you can think of this sight to be the positive buyers here in the DNA translating across the pore, what is very interesting to note here is that you get upward peaks like this.

So, the peaks here essentially at in the reverse direction they are as a there is a large ion current can be realize after the DNA blocks and is about to crossover. So, this paper reported by Bashir et al., which I just illustrated earlier here this in this particular example it tries to kind of explore in this principle that y z that we are getting the peaking action rather than you know depth in the current level and one explanation which is possible is.

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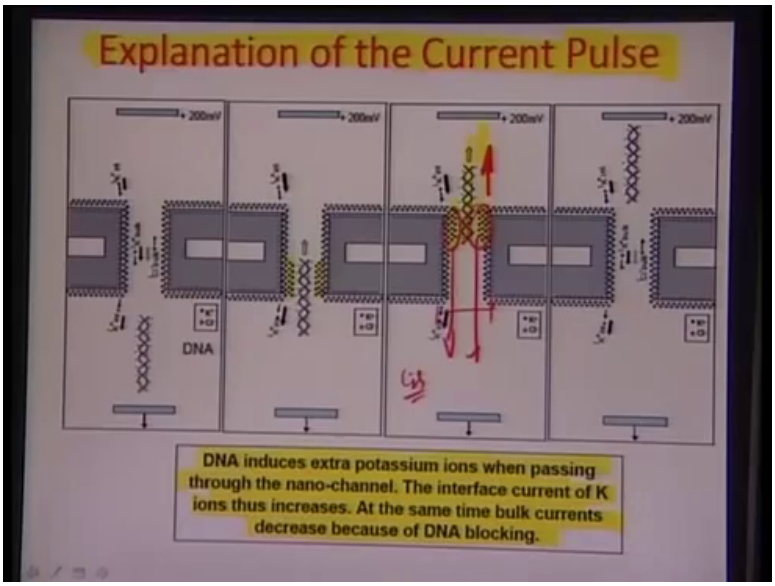

Given by this a tendency of the DNA to kind of carry a counter ion cloud you know we have been discussing of an on the earlier lecture that the DNA is essentially have in a very strong charge on the back bone negative charge in back bone. So, if it flows in a solution and essentially electrophoresing in 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 a model before electrophoresis at kind of leads to a reduction in the charge by reduction delta g and also increase in radius overall radius.

Because, it is moving along with the whole iron cloud in the center now the idea here is that as the DNA travels into to this region and it kind of grabs together. So, this ion current along with it and as it goes out into the other region it now, only kind of introduces are it not only kind of a introduce about negative charge region. But, also the positive counter ion group which is carrying along with it that is the reason for sudden spot of increase in the charge in the ion pore.

So, you can think of a that as this is a positive ionic kind of drags along the positive ion and it leaves into this side of the solution of the positive irons starts coming back one it cross

through the pore and it comes back in this direction as a DNA is moving in this direction and these whole positive ions here, which come back around or pumped back into the cis side from where the DNA has translated into the trans side essentially because of this there is a huge increase in current rather than reduction in current.

So, this essentially is an opposite effect then the micro scale Coulter counter in which you have no such effect because you know we talk about a microns scale object whatever flow is a cross that microns scale cap is a bulk flow. But, here because we talk about more in terms of almost a to single ion level what is going on this counter ion effect very becomes very important. So, you can think of that this counter ion cloud is which is going with the DNA into the pore on the DNA escape it is kind of released back into the solution in the reverse side as a result of which there is an increase or a peeking effect rather than as dip sown as a normally observed in other cases this is more.

So, also because the silicon surface here essentially developed charge dual layer you know because a you are talking about same range that of the dual layer between the silicon and you know the translating molecule. So, this process is lot more complicated than the normal micro scale Coulter counter. So, essentially you can conclude by saying that in this case in the DNA induces the extra potassium ions potassium through the nano channel interface currents of a the potassium ion increase suddenly and the same time bulk current decrease because the because of the DNA blocking.

So, overall effect is an increasing the current because certain spot release these ions back into the solution. So, particularly in this example a two hundred base per DNA has been used to the concentration about 0.3 mille gram per ml and if the electrodes used for this measurement are the Ag/AgCl based electrodes as you know this Ag/AgCl itself is non dissolvable in solution you have a coating of AgCl the top of Ag that is the way you use these kind of electrodes.

We have talked details the electro section of this course the bias voltage use in this case about 200 milli volt in the time of example in about of 100 micro seconds which would also get using a high resolution the DAQ card the data acquisition card. So, this is essence is actually it is become a very major research area about now a day's in BIOMEMS. How you could actually measure single molecular translation across these nano pores is it of some utility or effect in the diagnostic area.

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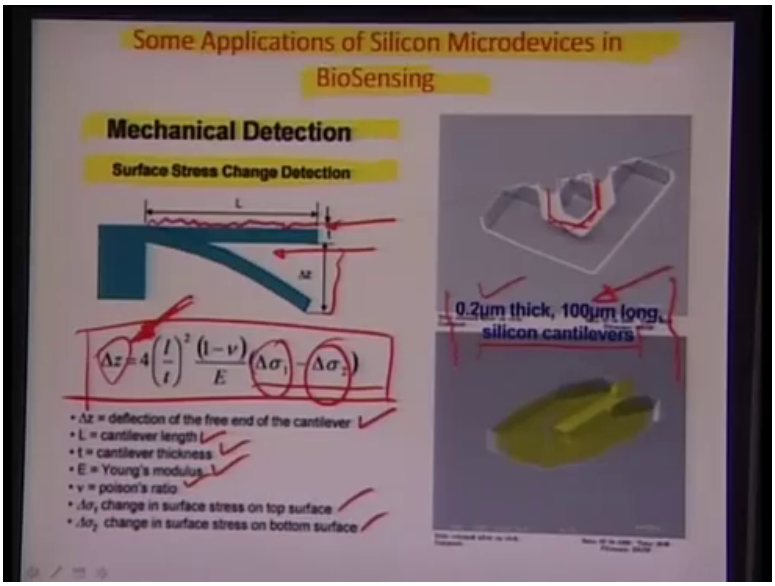

So, there are others forms of sensing or detection which can be used with the MEMS kind of architecture and we are talking about earlier about silicon micro devices. So, this is the direct application of a silicon micro device in bio sensing and this talks about mechanical detection about mass base detection, which was kind of another frontier areas of a sensing these days. So, here what this process is all about is define by change in surface stress across a small thin film micro cantilevers.

So, you can see here this is the micro cantilever which is coming all the way into this small spoon and it is separated from the base it is like a it is like a cantilever which is a pointed into a gap here like a swimming pool. So, it is a diving port in a swimming pool kind of arrangement and here if you do something in the surface of this cantilever in a manner that the surface energy as a differential on the top and the bottom surface you put some molecules you change the surface energy somehow by binding it to.

Let say some hydroxide groups or some other Self-assembled monolayers (SAM) in the manner there is a differential between the bottoms surface of the cantilever and the top surface of the cantilever in this manner. So, you have a something immobilized on to this region here on the tops, so that you have a difference between the bottom and the top surfaces of the cantilever here. So, there is a bending because of this is a mechanical bending that this bending is also define by something called STONEY's equation is given in this particular illustration here.

So, the bending delta z in this case delta z is this particular distance in the cantilever moves is

also directly propositional to a different in the surface energy on the top and the bottom surface of the cantilever. So, delta sigma 1 and delta sigma 2 here are the difference in surface energies on the top and bottom surface which occur, because of this chemical change chemical activation of the surface. And, so therefore, delta z here the change in the G motion of the particular cantilever is also directly propositional to the different delta sigma 1 minus delta sigma 2.

So, delta d delta z again is a reflection of the free end of the cantilever L is the overall length of the cantilever t is the thickness, E is the Young's modules mu is the Poisson's ratio and delta sigma 1 and 2 are change in surface stress on top and bottom surface. So, this is essentially a mechanism of a detecting if really there is a change you can produce a yes no type of answer of whether there is a surface chain just in the minute shown.

Another slide, which talked about how you could detect DNA or recognize DNA of different base pairs based on the technique by just mobilizing some capture probes in the top of such cantilevers such micro cantilevers and really this cantilevers could be of all different kind of shapes this for instance is more like a v shape a cantilever. As you can see you know this is actually a straight I shaped cantilever such some typical damnations of these cantilever are illustrate here is about 200 Nano meters thick film over which you make this cantilever and they can project as long as about 100 microns.

So, it is really a very it has to be a sturdy structure which can go up to 100 microns still be integral all though the thickness is only as well as about 200 Nano meters. So, this is what the aspect ratio is about 5 time and there are normally made in silicon, because a the process is very well acknowledged and found out however there are initialed to also make cantilever in other alternate polymeric material with certain changes in this Strength property modification in the strength properties.

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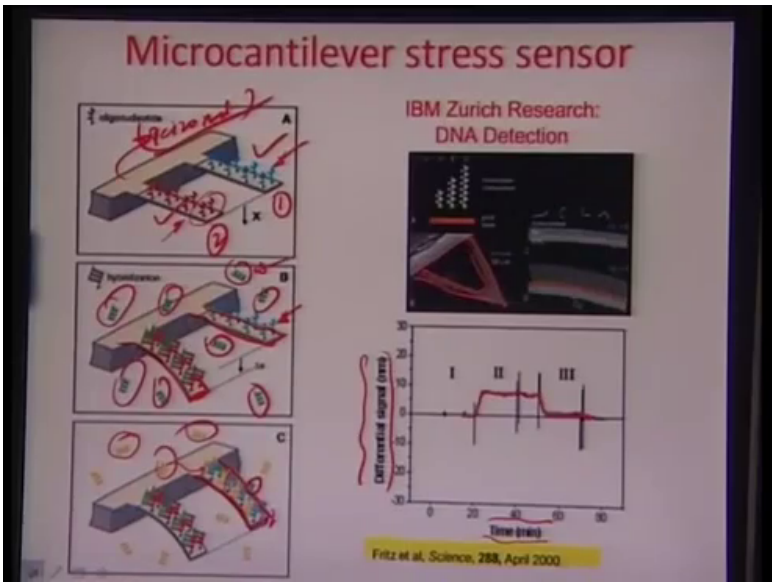

So, let see what we can do with this is really an example very find example borrowed from you know IBM research plan in sensing. So, this is also corresponds to paper reported by Fritz et al., which talks about how such instrument or techniques can be used from by a detection. So, here in this illustration as you see there are two different kind of capture probes, which are put on both sides of on series of two cantilever.

So, there is cantilever one and cantilever two here one which there are blue probes which are fitted are one side and red probes which are fitted in other side. So, there are two different capture sequence altogether right and what we do is that in the first instance there is a there is a green target here you can see this green target, which is corresponding or it is complimentary to the red target, which was already immobilized on to this particular area of the cantilever. So, and the cantilever also coated with the piezo material.

So, you have a kind of transaction 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 and here as you seen the DNA is binding here the red probe, which was there in the cantilever you bind to the green target, which is flowing by where other in 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 in the green probe.

So, what would happen is that there is a change in the signal generated and if this where to be the reference signal and there was a reference 0 in this area there would certainly it

differential, which is being introduce here. So, this is the here differential signal that is reported with the time in minute as the binding takes place as you see here the binding is kind of ensuring that there is a pickup in the differential signal. And then, there is continuity here until, now what we do is we flow in other probe of similar nature, but now this time this is complimentary to the blue probe here.

So, there is another target which you are flowing here which is compliment here it as same number of base pairs and both the size cases to the blue probe here. So, now, the blue by into this yellow target and yellow does not bind to all of these bound DNA only and therefore, this think also bends this cantilever also bends. So, this corresponds to change back of the signal all up to 0 also differential signal, which arose here, because of binding of the green target with the red the probe kind of changes back to zero because of the binding with the yellow target with the blue probe.

So, this is how you could really make DNA, fast DNA detection possible by looking at just bending mechanism associated with a pair of the cantilevers. this is an illustration of how this cantilever will typically look like you all different shapes and sizes for this cantilever as you can see here and this was actually reported as a module by IBM minutes Zurich research center for the first time that the talked about how DNA hybridization can be carried out using mechanical signals.

So, this was kind of one of the first initialed in the area of how sensing can be in terms of transduction into mechanical signal from chemical signals. So, this in essence some of the kind of range of work that has been covered in the area of DNA related detection and sensing using BioMEMS kind of architectures. So, this kind of brings us to the end of this particular lecture.

So, in the next illustration next lecture we would cover topics different topics aspects related to proteins then we will look at how the living cell normally correlates between the sequences of the RNA and a the proteins and you will find out more as. In fact, a very interesting topic of the processes called transcription and translation within a cell.

Thank you.