BioMEMS and Microfluidics
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Lecture - 19

Hello and welcome back to lecture 19 of this Bio Micro Electromechanical System.

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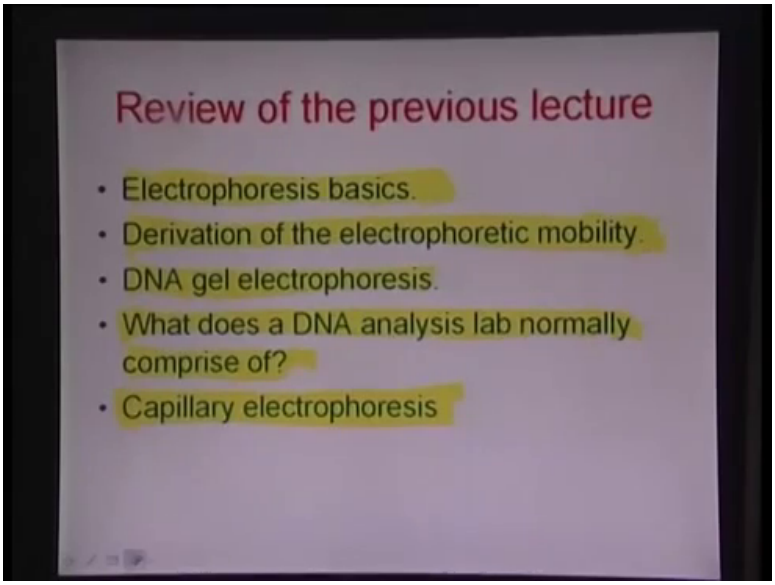

Now, we will begin again by a quick preview of the last lecture, we try to cover some of the basics of the electrophoresis process as you know normally do it, it is essentially the motion of the charge and an external electrical field through sieving media. We also talked about certain basic derivations of electrophoretic mobility, velocity so on so forth. We demonstrated some gel electrophoresis of DNA in laboratory scale, essentially something where you can do size based fractionation and moving it through a gel and trying to determine or make various mobilities on the basis of lengths as supposed to fictional forces that a molecule would you know kind of experiences as that move throws a series of sieves in the nanometer scale.

We also try to kind of estimate what does a DNA analysis lab normally comprise of, what kind of components it has the DNA perpetration unit, a mixture unit, the PCR thermo cycler, it has again electrophoresis mechanism as a read out bench and also talked about some concepts where these whole lab can be minimized on to a single chip level. We then described or it is started talking about capillary electrophoresis which is an off-shoot also the

human genome project on the rapidity that was the dictated by this project and we will try to kind of continue in this area and explore this little more.

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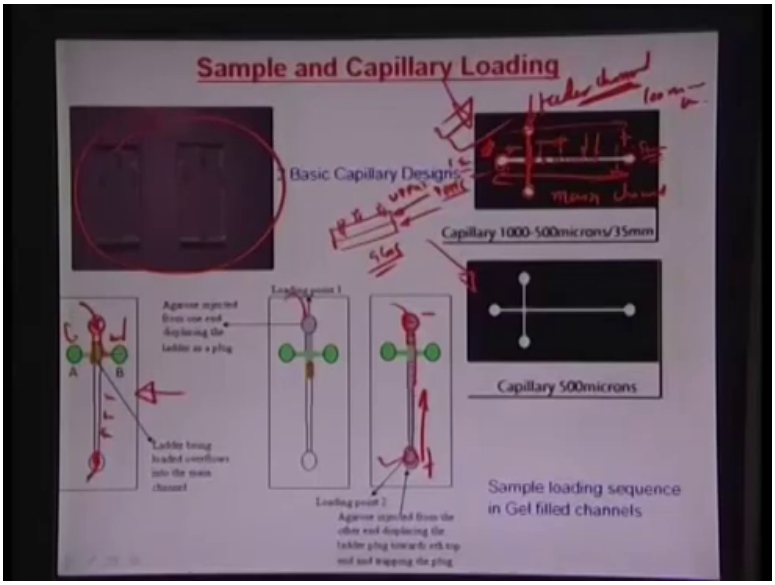

So, capillary electrophoresis essentially is the electrophoresis of DNA thin micro capillaries 70 to 100 microns in thickness and further advantages that capillary electrophoresis as to offer is in terms of higher surface area to volume ratio of the gel material which is filled in a channel and due to this surface area prominence there is a huge heat transfer coefficient. So, the way that heat is transfer to the environment is increase tremendously which allow us to go for higher amount of voltage or to sustain higher amount of fields without gel getting melted.

And therefore, this can be utilize to an advantage, where in these high voltages of fields can be used to rapidly translate and fractionate several base pairs of DNA, the rapidity can be to a scale which is almost 160'th times in terms of times of fractionation from normal gel electrophoresis process. So, this right here is an illustration of how it would really look like.

So as you see in this figure here, we have talking about a case where this crisscross channels, this channel here right now is essentially for loading the DNA sample, this channel here and the other white channel as you are seeing here is essentially for translating the DNA and causing the electrophoresis to happen, the loading can take place either electro kinetically, where the DNA is injected into this gel material here at this particular juncture by giving an electric field a separate electric field, a perpendicular electric field in this direction is

provided to kind of translate whatever has been trapped in here for the DNA and into this particular capillary.

So, if you essentially present negative electrode, so if you present something like a negative electrode here and a positive electrode here there would be a tendency of this DNA molecules to move through this gel and as they move they would kind of fractionate and the sizes as obvious here in this channel would be separated and you can do a read out by using either a capillary wave kind system something which can be integrate on to the same planet level, this is the black and white mask, this is another mask, where the different dimension and these are some of the images of how the final device looks like.

So, essentially it is a glass PDMS device we have talked about replication processes using PDMS earlier, a mask is prepare, a master is prepare and the devices made in two layers with the lower glass transparent glass layer bonded to an upper PDMS layer which has if the channels carved in or molded in or replicated inside and there are ports is essentially for loading and unloading the gel material as well as the samples and this is the scheme again, where it shows how the samples are loaded.

Whereas, you see there is a green plug like flow here of the DNA sample, there is a thin plug which has been inserted by continuous flour this empty channels through this cross channel, the loading channel. And once this plug is kind of formulated here there is liquid gel which is poured from both sides and this is adjusted accordingly, the idea is that this gel when it gets in to the capillary gets almost immediately solve define.

So, therefore, you have to very careful about the rapidity with which it is needs to be done, so there is the gel from this side and then non other gel solution from this side when you allow it to cool it develops a thin del gel layer inside the capillary with a plug the green plug here containing with DNA. Now, if you sent a positive and negative electrodes through these essentially reservoirs there is a tendency of this DNA to move towards the positive and as if move it kind of fractionate and tries to light up.

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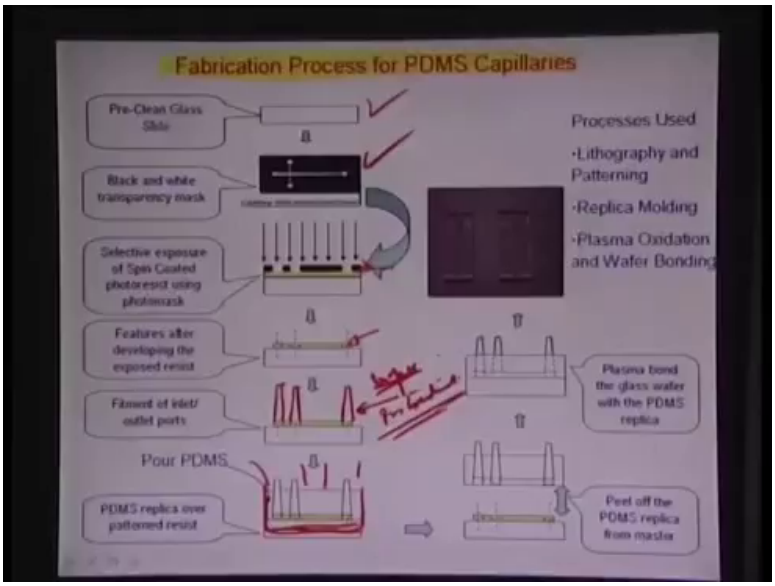

So, the way you make this devices using fabrication strategies for micro fabricated PDMS glass capillaries and the following manner you take a mask here and essentially you pre-clean in a glass slide and then spin coat some photoresist material and expose selectively using this black and white transparency mask. So, that you can actually create these features on the photoresist material, this is the negative tone water resist may be as you wait obtain from a company called Microchem.

You then follow it by actually pasting these inlet outlet ports over this particular master which is formulated and then essentially closer place this master inside a you know kind of Petrie dish and pour PDMS on the top of this master. Before doing that you have to do some surface pre treatment here that you can actually make the surface highly hydro feel, hydrophobic, so that can be able to not do away with sticking without sticking to the PDMS when it hardens from the liquid state.

And then once this is done you can remove the ports along with the PDMS with the channel like structures and then plasma paste or plasma bonded over this lower glass slide plain glass side and that would give you a device something looking like this. So, you have these small, small ports here null four sides and this essentially is the crisscross channel that you can see these are real time optical micro graphs of such devices. So, these are some detail results and this is now been published as well.

So, as you see here the following observations are made if you apply 300 volts across these two electrodes or these two reservoirs with DNA somewhere here and with the voltage of 300 volts after 25 seconds you see that there is a plug like flow of the DNA. So, this really is the and this is the ladder that you are translating.

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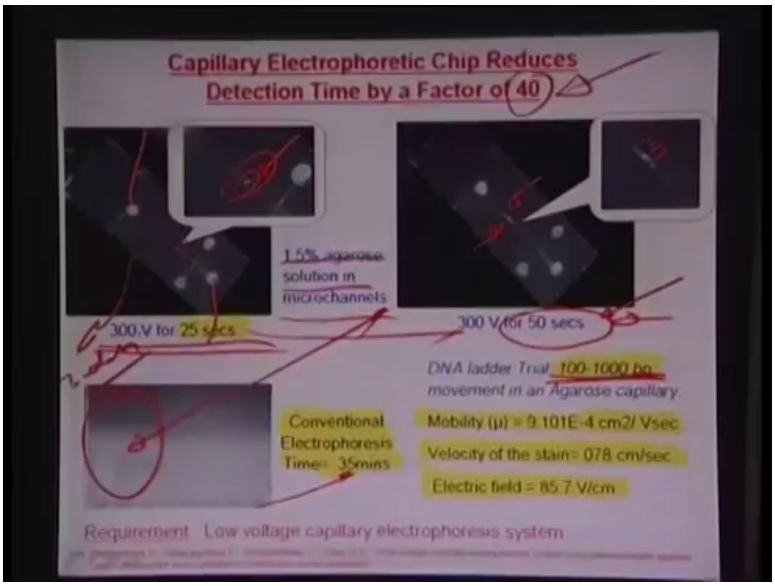

So, this is essentially a 100 to 1000 base pair DNA ladder. So, when you are flowing it for about 25 seconds you see that there is a small stain that is developed and lit up and this whole assembly is essentially placed over trans illuminator, this is placed over a trans illuminator and that is why you can see are visualize is particular stain of interest here. Now, use a 1.5 percent agarose solution in this micro channels. Now, if you keep applying this for another 50 seconds and other 25 seconds it kind of splits up into this various stains or bands and the same capillary as you can see, this is one of the finest examples of capillary electrophoresis.

And essentially the mobility can be calculated in such stains as this 9.1 into 10 to the power minus 4 centimeter square plus per volt second, velocity of the stain would be about roughly 0.078 centimeter per second on the electric field, there is use almost closed verify 0.7 volts per second and if you compare time wise to the conventional electrophoresis how much time, reduction happens in this case and the conventional electrophoresis normally occurs in about 35 minutes also, this is a conventional electrophoresis, where the ladder is split up about 35 minutes as suppose to only 50 seconds here.

So, there is almost close to you know 130'th or 140'th time reduction, if you shift from the conventional to the capillary. So, this is basically a reduction of 40 factor of 40, so it is a huge advantage to the industry, because we essentially look for rapid ways and means of doing this you know DNA fractionation and this is one of the best very best methods which are available in a capillary, where you can do the same job.

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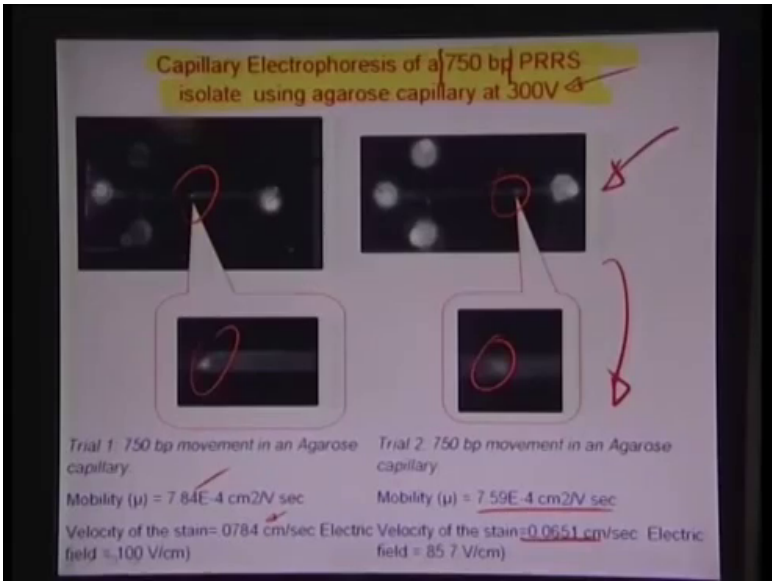

So, this is another illustration where you can show electrophoresis happening inside a capillary using the 750 base pair posing reproductive and respiratory virus syndrome. It is essentially a dreaded disease in the swine herds in United States and across the other part of the world. So, essentially this virus is recognized, so it is a viral decease and the virus is recognized by the 750 base pairs of certain section of the genome of the virus isolate. So, using agarose capillary and about 300 volts we have been be able to successful demonstrate electrophoresis in terms of these stains as you can see through in the capillary.

So, the mobility is calculated here above 7.84 into 10 to the power minus 4 velocity of the stain being about 0.0784 centimeter per second in a field of 100 volts pre centimeter and in this second trail here the mobility slightly changes to 7.59 into 10 to the power minus 4 centimeter squared per volt second and the velocity of the stain is 0.065 centimeter per second electric field about 85.7 volts per centimeter in some of the fraction figures which are important for knowing about this electrophoresis process a little bit more.

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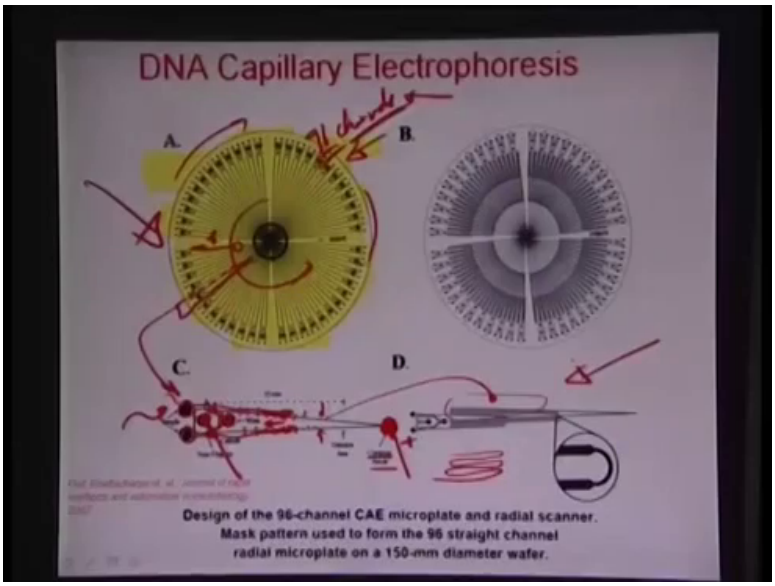

Another fantastic example is this from this paper published way back in the you know the early 2000's by essentially the mattes group apart you see bugle. So, essentially here what mattes group is trying to show is a set of capillary array this is instead of one capillary, many capillaries and a radial plate like a CD and there is a principle which is involved in the detection of DNA stains in this particular illustration.

So, if you really look at one of the channels here, let us say we want to have a look at this one channel, essentially something like this. So, you have two channels in this one structure or one unit of the channel that is mentioned here independent channels, two independent channels converging in to this port here, which is also at the center and has a common anode. So, it is a positively charged plate which is also a dispensing reservoir. These two are the detection arms on both sides and these arms are just in a manner that you have the sample ports outside and you have a crisscross channel on both sample ports from both sides of this main detection arms and these are all connected integrally to something like a waste, you know collection center and then you have a cathode which is placed at this other end here of the micro channel and anode, which is placed somewhere here in the waste reservoir and then when you the idea is that when you load the sample here and make it negative the sample negatively charged by putting another electrode. Other sample kind of electro kinetically gets injected into this detection arm and this detection arm and then the cathode and anode combination of these two across these two ends are able to you know these two ends this and this end they are able to drive the small samples which are called in this detection arm all the

way through the arm and in the process there are stains which are formulated in these two regions here.

And essentially this CD has an additional advantage that you can actually calibrate and place a reader detector system which can actually go back and forth radially and can what all stage of there in this capillary. So, there is a unique combination of the rotary notion of this particularly, you know micro capillary containing CD and in relation to the radial motion of the detector sensor assembly and that can help you two kind of identify at least 96 these are all 96 number, the separate channels are 96 separate channels are there and the CD like platform.

So, you can actually at a time read about 96 particular reactions PCR reactions using this particular capillary. And so this is also very high through put process that Mattes group has develop before. You can actually also take this particular channel and introduce a lot more turns in a accommodate a lot more length of this channel, the only think here is to be a little careful and designing the way that this channels will twist and term and that should not be done at the cost of the resolution laws of the DNA. So, there are some interesting work which has been done in this capillary array electrophoresis area the various research around the world.

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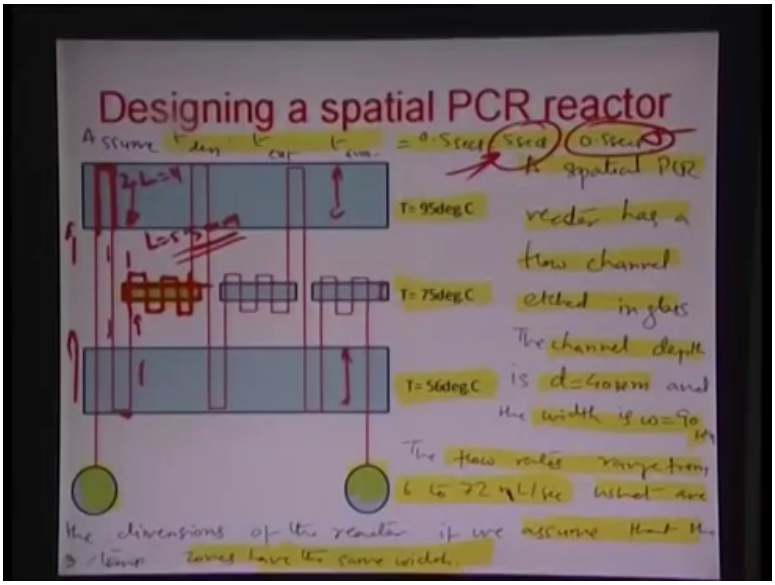

So, after doing this electrophoresis base x I would like to turn your attention towards another very important aspect known as the designer spatial or a space domain PCR reactor. So, when

we are talking about you know the PCR reactor in general be assume that there is a small chamber contain in time you know in space and the temperate contain in the fixed special location and contains the small volume of the PCR fluid and we assume that the thermal cycling is done on this fixed special chamber through you know on a temporal bases, so with the time varying bases.

So, these devices are also known as the time domain devices for PCR micro reactors; however, one issue with these devices as the very fact that you have to kind of heat large amount of thermal masses associated with the vapor or you know the sub stated at the base which would contain this chamber in it. And therefore, every time you have to ramp up the whole mass to a certain temperature in ramp it down in order to be able to quickly do thermal cycling.

However, this is very inconvenient module, because thermal mass and particular material that is we use in micro fabrications, silicon glass then your polymers they are all poor thermal insulators. And therefore, it does not give very easy solution in terms of a rapid the ramp up or ramp down. So, another approach that was very intelligently thought by a group in up at Oxford Andriasmann's group way back in about in early part of this particular decade was about space domain PCR reactors.

So, instead of one location and heating at various temperature of that location, what this group for the first time though is that why not have three different heating zones on the same chip one heated to 95, one heated to 72 and other heat is 50 and then essentially move with the PCR fluid around in this three different zones by a certainty in a path and in a serial manner. So, that the cycle which is essentially we are 95 followed by a 50 followed by a 72 is followed and at the same time PCR get executed only by virtue of the motion of the droplet in this differentially heated area of a single surface.

So, this is called, this is a very novel approach and also called a space domain PCR devices. So, I would like to draw your attention to how such a device can be designed. It is essentially a challenging in engineering problem. So, you have three different heating zones at the different maintain at the different temperature and you have a serpentine path over the zones in which you are flowing yourself circulating the fluid.

So, if goes to the first zone, you have to give it time in enough for the volume to go up to the temperature of the surface that is let say 90 degrees and then also you have to wait long

enough for the whole denaturation step to happen. So, the total amount of time that this small droplet should be present in the 90 degrees heated area is about the ramp up time of the mass of the droplet to go to 90 degrees plus the denaturation time.

Similarly, the amount of time that is needed to whole the droplet the 50 degrees area is essentially the ramp down time to the 50 degrees area times of the amount of time it would take for the sample to be present in the extension step the any link step actually, similarly goes to for the extensions that. So, you have to design effectively the velocity of this droplet in a manner. So, that length which is also equal to velocity into time would be able to accommodate are given sufficient time for the denaturation the kneeling and the extension process of PCR to occur in easier manner.

So, let us do an example here we now start designing such a special PCR reactor as you see here there are different zones 95, 75 and 56 design the in a manner. So, that you know you can use probably micro fabrication techniques to design this kind of a think. So, you have the 75 degrees only root between 56 degrees and the 95 degrees, there is an input output reservoir here and the understanding is the droplet would be moved in direction. So, that it is able to rest on you know this particular area whatever the temperature is for a time duration, where the whole effect can be felt in terms of successful and healing and successful extension in a successful denaturation time.

So, the assumption that we have to make for solving this particular question is that the denaturation extension in the kneeling time and ratio of 0.5 the 5 is 0.5 seconds respectively. And we also know that this special reactor has flow channel etched in glass with the channel that 40 microns, the width is around 19 micro meters, we also have flow rates ranging from 6 to 72 Nano liter per second that is the kind of flexibility that we have and we have to design the dimensions of this differential temperature zones. The lengths dimensions or the width dimensions of this differential temperature zones, if we assume that all the zones are same width, so, we are assuming that the zones, the three temperature zones here has have the same width.

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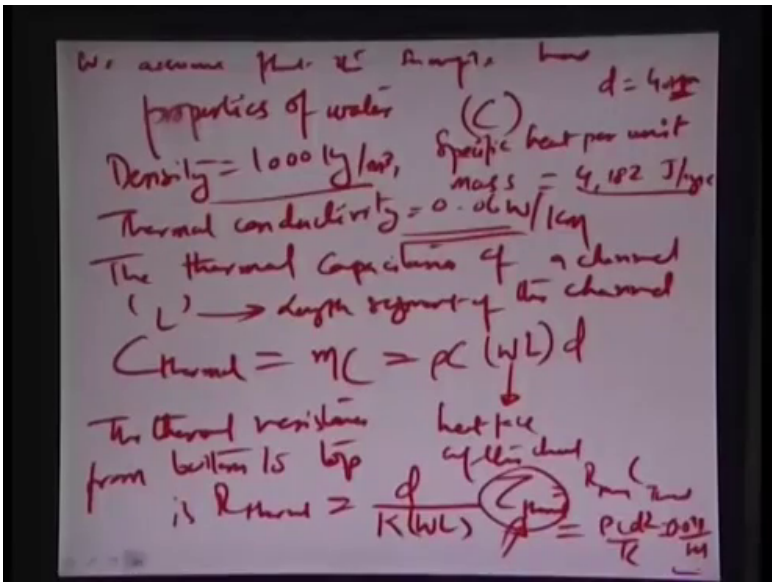

So, we have to first take in to consideration some properties. So, we assume that the sample has properties of water all PCR fluids are basically that was based density would be about 1000 kg per meter cube specific heat capacity per unit mass, what is about 4182 joules per kg Kelvin, thermal conductivity about 0.06 watts per Kelvin meter. So, the thermal capacitance if you look at of this particular channel assuming that the length segment is about L. So, you have L as the length segment of the particular channel.

So, C thermal will be given by... So, we given by m times of C which is again mass is essentially density of the medium times area times of volume and I have to see what the area and this cases the width of the channel being W and let us assume L to be the total length of the segment, W L is the facing area. So, this is the heat phase of the channel and of course, the heat has to travel through the thickness of the channel d. So, W L d essentially is what the volume of the channel would also be.

Now, if you look at the thermal resistance in this case, the thermal resistance from bottom of the channel to top of the channel is estimated as R thermal is the depth d by k times square of L and in this case square of the A or you know square of essentially the area. So, area in this particular case is also W times of L and therefore, the time constant of this thermal circuit which is also equal to R thermal into C thermal it is essentially in this case rho c d square by k and this comes out to be assuming this various values of density, specific heat per unit mass c and the d value of about 40 microns and also the thermal conductivity 0.06 watts per Kelvin

meter comes out to be 0.011 seconds there is how the whole time constant of this thermal circuit would be denoted by...

$$R_{thermal} = \frac{d}{k(WL)}$$

So, for the designing and memes base system what is important is also it is reliability and here the question is that there is a small droplet which we are moving through different zones of heating and a check and you have to be 100 percent sure that the droplet gets the time that it needs for executing the whole denaturation time along with the time that it would need for going to that temperature which is three times of it is time constant and assuming that you have a maximum velocity of flow you can give the maximum tolerance to this system, because anything lower than that would be definitely able to get that time of heating and the time that the whole denaturation would take normally otherwise to get completed.

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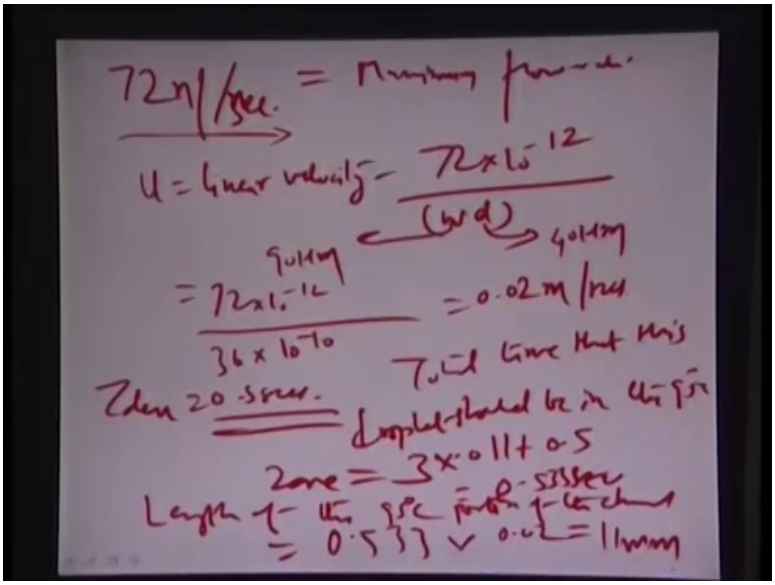

So, therefore, we will do all the designing based on the maximum flow rate, which is about 72 nanoliters per second is during the problems statement, this is the maximum flow rate. So, let us find out what the linear velocity u would be in case the velocity of flow of the volume rate of flow is maximum. So, linear velocity is actually the volume divided by the area of cross section, which is w into d in this particular case these about 90 microns and w is about one side these about 40 microns and w is about 90 microns. So, it is 72 * 10 to the power

minus 12 divided by 36 10 to the power of minus 10 and this comes out to be equal to about 0.02 meters per second.

So, the right time required for passing through the denaturation zone assuming the denaturation time to be about 0.5 seconds has been given in the problem statement again is essentially the total time that this droplet should be in the 95 degrees zone is 3 time of time constant 0.011 seconds time 3 plus 0.5 comes out to be 0.533 seconds and therefore, since the length of the channel needed in the denaturation zone is only.

So, therefore, the length of the 95 degree Celsius portion of the channel should really be equal to this time here is 0.533 * 0.02 which is actually about 11 mm. And since, we were actually talking about a flow in this denaturation zone, which is twice the length and the length becomes equal to 5.5 mm we to assume that this portion here is essentially not very major, you know length in comparison to the length of the zone, this term is essentially neglected, because of it is a smaller magnitude.

So, the length of the zone that we been looking is really about 5.5 millimeters. Now, the same length is this true for the extension case, because would not have... So, the annealing case, because would not have a different time in the annealing area for the extension case as we see here, the time is about almost 10 times. So, let us look at how much velocity will be able to for how much length will be able to cover in the extensions over.

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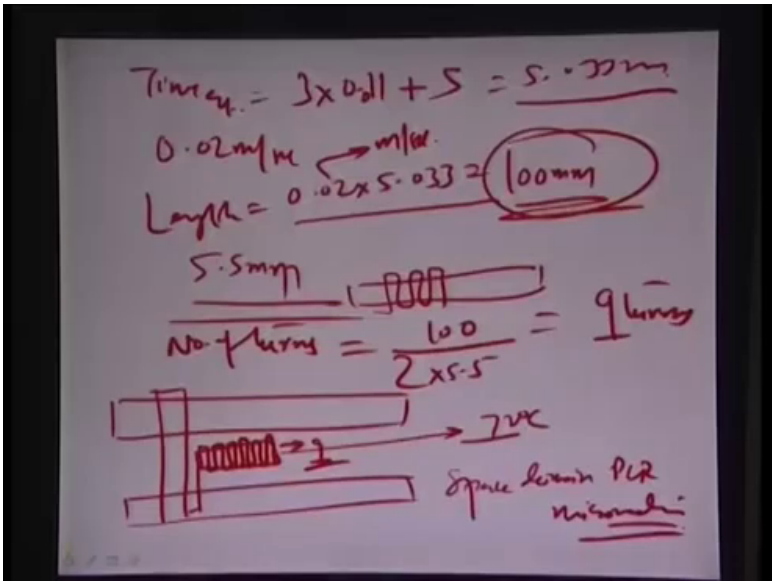

So, in the time needed or the extension to happen is essentially 3 time is 0.1101 seconds plus 5 seconds or 5.033 seconds at the velocity of 0.02 meters per second, the total length that it would need to stay or covered the total channel length or could need to being the extension zone 0.02 times 5.033 which is essentially what the total extension length would be, so this about 100 mm assuming this was a meters per second, this about 100 mm.

So, again the very fact that you have one term which is corresponding to about 5.5 mm it is design constraint that you cannot go over this in all the three temperature zones. So, you have to actually ask the channel to serpentinely turn in this extension area as can we seen here in the figure. So, this area essentially is to ask the channel to term multiple times in this particular area. So, that it can get heated as it goes into these second team path.

So, the number of turns that this channel would be to execute in the 72 degrees area 100 divided by 2 into 5.5 which is essentially about 9 terms. So, you have to design the PCR in a manner. So, that we have a space here which comprises of about 9 terms of this particular channel is the spaces with heated to about 72 degrees Celsius. So, the healing zone characteristics the same as the denatures of zone although in the extension zone you need to secondly call the channel of 9 times in order to get the total time of the extensions with that the whole DNA pair can be copied. So, this in a nutshell is how you design of space domain PCR device or a PCR micro reactor.

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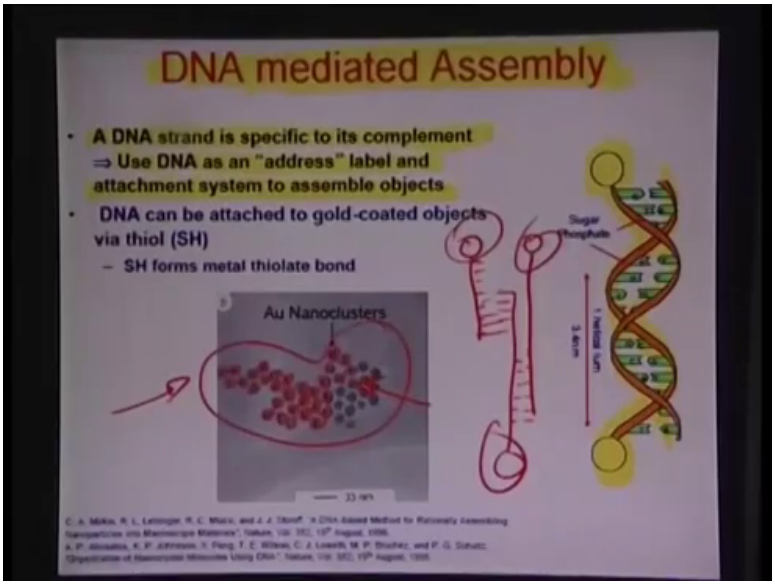

So, I would now like to actually delve into another very interesting area of bio sensing and that is essentially what you can do using the self assembly skills of a DNA molecule deoxyribonucleic acid molecule. So, what we are going to do now is to kind of try and discuss some of the various detection diagnostic protocols that has been promulgated by the complementary team itself in the DNA chain as ((Refer time: 36.22)) one other.

So, one of the first papers in this area is that generated by Alivisatos and this group essentially is about gold nano clusters. So, what he found out is DNA mediator assembly process away back in 1996. So, here as you see there are two complementary strands of DNA which are exactly complementary when the time to each other with high tendency and what he essentially done is that he puts these gold on a particles on both these strands separately and let themselves assemble. So, that there can be you know it an array or an arrangement of these gold nano particles.

So, the moment that these two strands of totally complementary to each other and in fact, that this is not only binding kinetics that one may have here one may have something like say you have this DNA molecule with the gold nano particle here and you have maybe another molecule with kind of complementary structure, which is corresponding to few base pairs here and then it has it is own base pairs which again combine to the third molecule. So, it combines to another third molecule here which has another of this particle.

And so therefore, there are several such strand which be just by means of confidentiality between the strands would keep binding to each other. So, there is how this self assembly would DNA mediator itself assembly forces would happen, how you get know whether the DNA or self assembling is by looking at what happens after giving sufficient time for this particles to assemble. So, this is essentially a scanning electron micrograph of these different goal particles as you can see the form these clusters.

So, these are gold nano clusters which is indicative of the fact that they are bound together or help together. Now, by the DNA functionization which is gold nano particles have individually on their surface. So, essentially this is one of the first experiment which demonstrated that DNA mediator self assembly how that this suppose can be used or putting together nano particles. So, essentially at DNA strand specific two it is complement. So, use DNA as an address level an attachment system to assemble objects. And other DNA can also be attached to this goal particles by using Thiol chemistry. So, you modify one end of the

DNA through a thiol SH group and essentially absorb the SH onto the gold nano particle which you know just by pure absorption, it can kind of conjugate or it kind of job and then become integral with one of these site change of the DNA. So, SH forms a metal thiolate bond which is also the bases of the attachment here.

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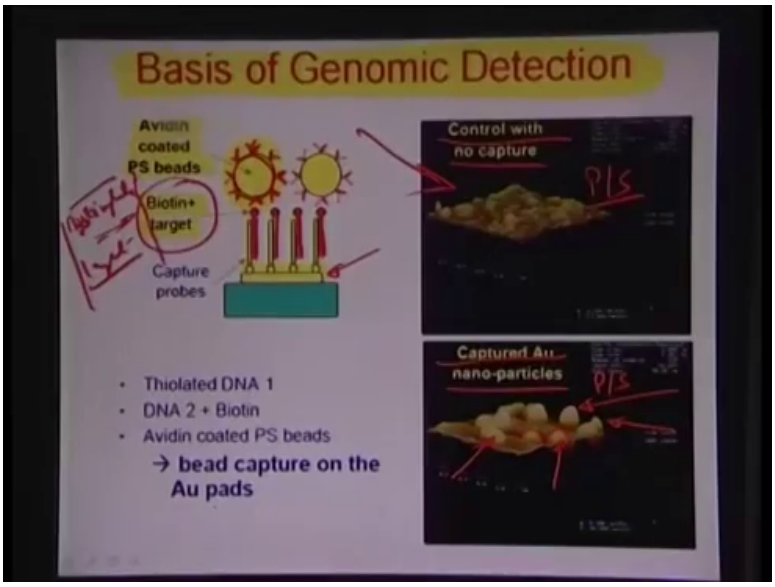

And another very interesting work and another very interesting work which has been probably reported by the same group is one of the fundamentals of genomic detection and this is one of the first few works which indicate the concept of DNA hybridization. Here of course, what happens is that do you have these Avidin coated polystyrene beads as you can see these are the Avidin mighties on the top of the polystyrene beads. And Avidin essentially again a vitamin which bonds very well to you know biotin which is again you know some kind of a protein.

So, essentially what you do here is you coat Avidin on this polystyrene in deeds and you make a series of capture probes with some particulates at the end which could have actually be immobilized on the top of maybe a gold substrate. So, essentially you are immobilizing this half and standard DNA molecule which is also captured probe onto this space surface using time thiol chemistries.

Now, what you do is are you bind the target with the biotin. So, essentially biotin late the target, there are ways and means of doing it and I am not going to get into the chemistry details of that, but essentially there are kids are available through which you can divert

simulate proteins DNA or all sort of a biological entities can be bio stimulated which means that about biotin group can be added on to the entities externally.

And essentially wants this biotin target has been realized you know this when you flow the target and it is going to find it is capture proven getting bound to the capture probe at the location that it is intended for and the biotin mighty on the capture probe are able to trap the Avidin which you have already coated on the surface of this polystyrene b. So, therefore, whenever there is a target abiding to a certain capture probe it is indicated by a polystyrene big bead with the biotin surface with the Avidin surface functionization in term binding to this half or this semi DNA or this small part of the DNA taking out of the hybridization as say this bonding here between the yellow and red is also known as DNA hybridization.

So, how we detect such a mechanism, so user name for doing that here if we look at the two scans in the tops scan essentially there is a controls sample with no capture. So, there are no beads which are immobilized polystyrene beads which are immobilized as suppose to the captured gold nano particles you can see here that these polystyrene beads have been immobilized on to the surface by virtue of the Avidin coated to this target DNA or that the biotin you know conjugated on to the target DNA bound to the immobilized capture probe on the surface.

So, I virtue of this it is like a hook which holds the polystyrene indeed in place and therefore, that you can easily see that the presence or absence of a right captured probe corresponding to the target would just make a different in terms of laying out a signal is a bound polystyrene bead over the surface. So, this on the very interesting example of how you can make hybridization arrays between target in a capture probe DNA molecules, these are some other illustrations where the exact same think has been done, but here as you see other about 8 microns gold dots over which the capture probes have been immobilized by using thiolated chemistry.

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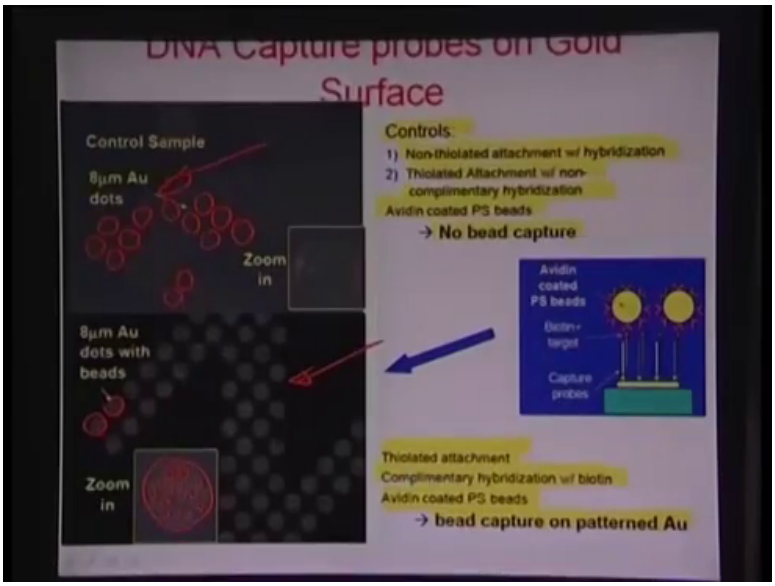

So, essentially these dots here that you can see or 8 micron gold dots made of gold and what we do here is that in some of the dots you do not bind any captured probe and in others we do bind capture probes. So, essentially you are making some control and some reference you know sides of binding. So, wherever there are these dots which kind of have the presence of the capture probe there is already is a capture of the polystyrene bead as can be seen here in the closer view these small mighties here at the little polystyrene beads, the tiny beads which have in captured. Because, the prevents of the capture probe.

So, optically you could actually using an optical micro graph in optical micro scope realize whether there is a binding going on between the target DNA and the capture probe just by looking at how this gold dots or whether they are with polystyrene bead or not. So, the controls for some of these experiments on non thiolated attachments with hybridization, thiolated attachment with non complementary hybridization and essentially all PS beads all polystyrene in beads are have been encoded.

So, then there is no bead capture indicated of there is a control. So, if there is a thiolated attachment however and the complementary hybridization with biotin Avidin coated polystyrene beads capture on the pattern gold surface which gives us an idea of what is the particular target. Because, if it is complementary on the then it is going to bind and we do have information on the capture probe which is going to bind it.

So, we can just figure out what is the complementary of the capture probe which is essentially the target in this particular case. So, now, we come to another very interesting area which is a DNA hybridization micro arrays, now this area is essentially a very big business now a day's billions of dollars are involved in this diagnostics one area, which is about building of DNA micro arrays, this arrays can be used for a variety of replication including hybridization arrays and for different applications like you know this RNA special emanating applications sequencing HIV re-sequencing in general kind to detect target DNA or sense some target DNA so and so forth.

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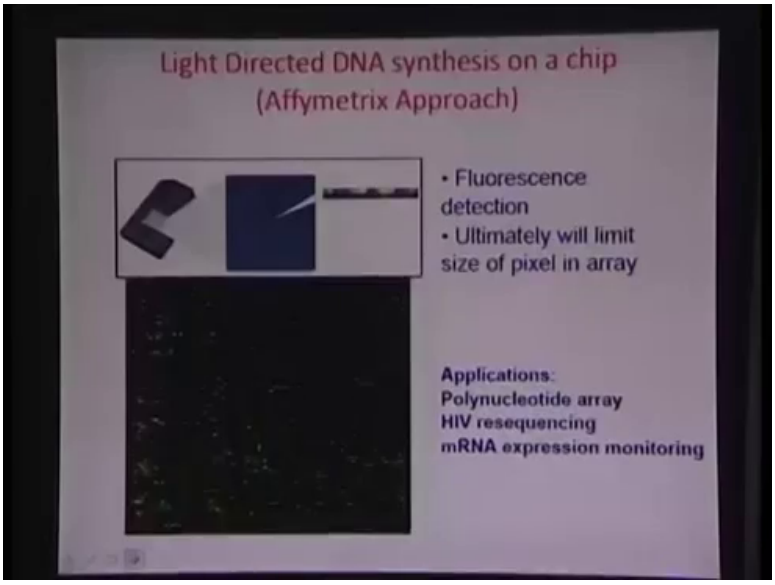

And the very interesting aspect of all the how you do build such micro arrays or what is the basic principle or basic mechanism of such micro arrays.

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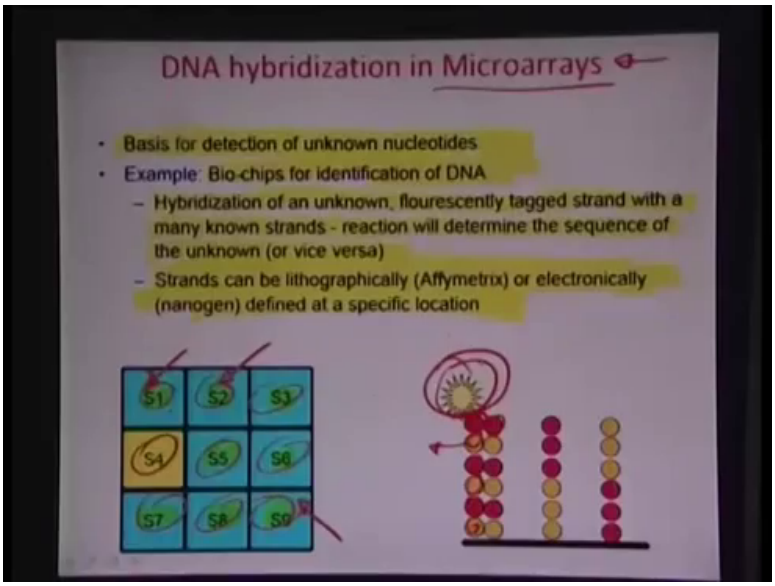

So, definitional hybridization essentially is the basis of detection of the unknown nucleotides, examples that come into picture or biochips for identification for DNA hybridization of an unknown fluorescently tagged strand with the many known strands of reaction will determine the sequence of the unknown are vice verse. So, if you have an unknown target and you wanting to hybridization it with something which is known some captured probes which is unknown sequence.

And you are able to somehow recently tagged this strand with the many known strands the reaction will determined the sequence of the unknown are vice versa. So, strands can be lithographically or electronically define that specific location. So, there are two companies in the world which principally makes these micro arrays one of them is asymmetric which does molecular build up for the capture probes, you know using light directed synthesis and the other company which build these or Nanogen they kind of a give this more to the user and they just build the electronics, where in electronically you can direct a particular capture probe to a certain site of interest within you know the micro scale architecture that they would provide.

So, let us look at what these micro arrays are. So, essentially it is all about building difference sequences or library of different sequences of DNA on to single chip level. Here, in the left figure essentially if you are looking at these numbers here S 1, S 2, S 3, S 4, S 5 all the way up to S 9 these are different location on the same chip and if we can somehow direct specific

sequence of capture probe single strand DNA on to this area S 1 of the first column and first row or the second column and first row and we have an accurate information of what is what; that means, what sequences there in S 1 what sequences there in S 2 so and so forth up to what sequences is there in S 9.

Then, if you know we bind the DNA with the fluorescent label which is the target DNA essentially on to this and wash this plate only the bound will stay back, because you have already immobilize the capture probe on this plate and the DNA which is fluorescently labeled is getting mobilized to the already immobilized captured probe, so it is essentially a hood between the plate and the new target molecule and that way gets bound.

So, in case if you wash this plate the binding goes away of the binding stays back or the bound DNA stays back and you still have the fluorescent signal which is indicated that there is binding or there is complementarity in the inlets input strand, the target strand based on which you can find out what is this sequence, because you have already know what is the sequence on the capture probe. So, this is essentially is what the DNA hybridization principle is and the micro array the name as the name is indicated this essentially it is an array of these different sites for doing or immobilizing different capture probes on to this particular site.

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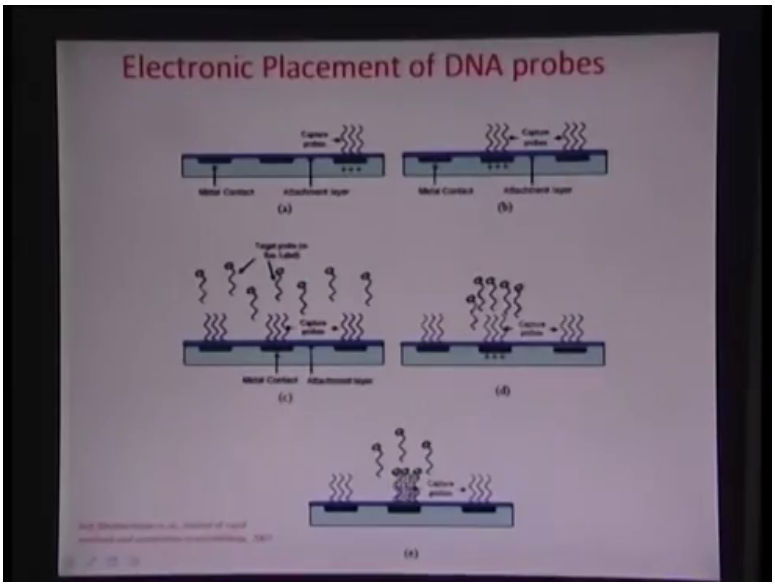

So, basically that kind of brings as to end of this lecture, what is interesting here is that we would now be in probably the next lecture working on some of the ways and means of placing this capture probes on the arrays or building these capture probes on the arrays. And

we will be looking at some of the companies like Nanogen, Affymetrix on the way they immobilize this capture probes and build micro arrays and should essentially that will be covered in the next lecture.

Thank you.