Bio – Microelectromechanical Systems

Prof. Shantanu Bhattacharya

Department of Mechanical Engineering

Indian Institute of technology, Kanpur

Module No. #01

Lecture No. # 19

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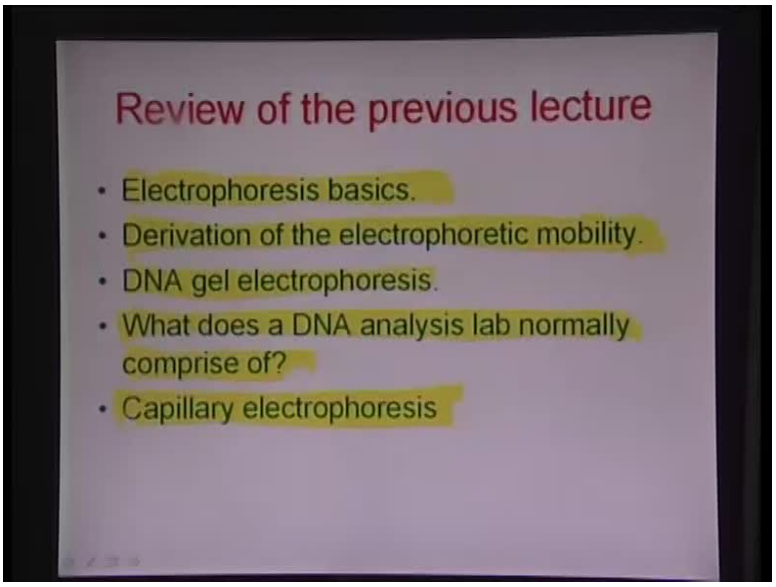

Hello and welcome back to lecture 19 of this Biomicroelectromechanical System. We will begin again by a quick review of the last lecture. We tried to cover some of the basics of the Electrophoresis process. It is the motion of a charge in an external electric field through a sieving media.

We also talked about certain basic derivations of electrophoretic mobility, velocity, so on so forth. We demonstrated some gel electrophoresis of DNA and laboratory scale where you can do size based fractionation and moving it through a gel and trying to make various mobilities on the basis of lengths as opposed to frictional forces that a molecule would experience as it moves through a series of sieves in the nanometer scale.

We also tried to estimate what does a DNA analysis lab normally comprise of? What kind of components it has? It has a DNA preparation unit, a mixture unit, commission

unit, the PCR, thermocycler, electrophoresis mechanism readout bench. We also talked about some concepts where these whole labs can be miniaturized onto a single chip level. We then described or started talking about Capillary electrophoresis which is an offshoot of the human genome project and the rapidity that was dictated by this project and we will try to continue in this area and explore this a little more.

Capillary electrophoresis is the electrophoresis of DNA, thin micro capillaries 70 to 100 microns in thickness. The advantages that capillary electrophoresis has to offer 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 transferred to the environment is increased tremendously which allows us to go for a higher amount of voltage or to sustain higher amount of fields without the gel getting melted.

Therefore, this can be utilized to an advantage wherein 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 one-sixtieth times in terms of times of fractionation from normal gel electrophoresis process.

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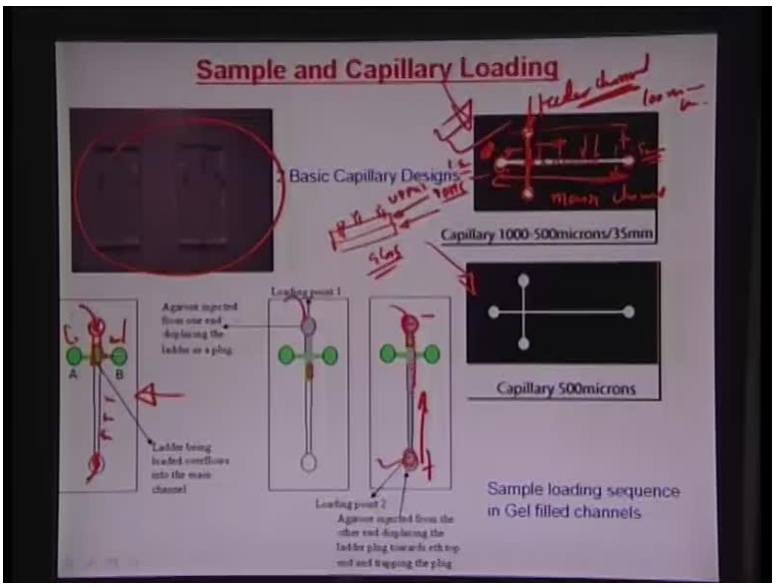

So, this is an illustration of how it would really look like. As you see in this figure here, we are talking about a case where there is a crisscross channel, this channel here, is for loading the DNA sample. The other white channel as you are seeing here is for

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 translate whatever has been trapped in here for the DNA into this particular capillary.

If you present something like a negative electrode here and a positive electrode here, there would be a tendency of these DNA molecules to move through this gel. As they move, they would fractionate and the sizes as is obvious here in this channel would be separated. You can do a readout by using a waveguide system which can be integrated on to the same planar level. This is a black and white mask. This is another mask of different dimension and these are some of the images of how the final device looks like.

It is a glass PDMS device. We have talked about replication processes using PDMS earlier. A mask is prepared; a master is prepared and the devices made in two layers to the lower glass transparent glass layer bonded to an upper PDMS layer, which has the channels carved in or molded in or replicated inside. There are ports essentially for loading, unloading the gel material as well as the samples. This is a scheme 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 flow in this empty channel, through this cross channel - the loading channel, and once this plug is formulated here there is liquid gel which is poured from both sides and this is adjusted accordingly.

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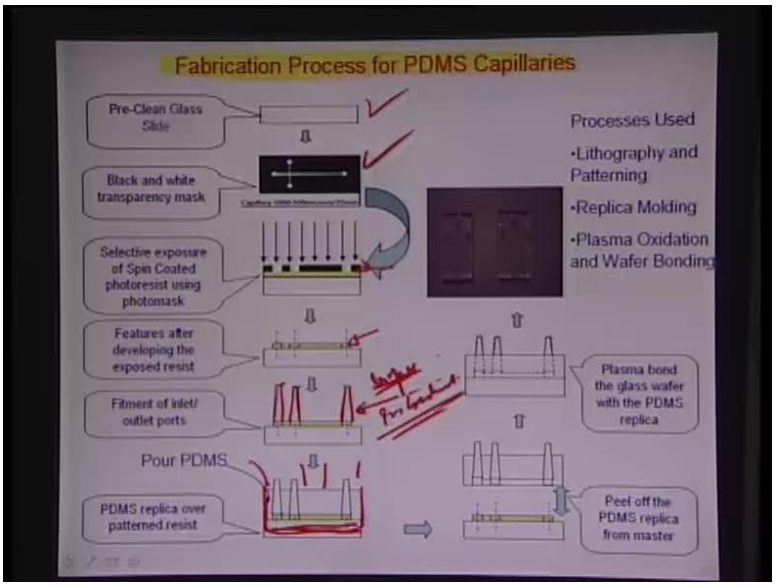

The idea is that this gel when it gets into the capillary, it gets almost immediately solidified. Therefore, you have to be very careful about the rapidity with which it needs to be done. There is the gel from this side and another gel solution from this side. When you allow it to cool, it develops a thin gel layer inside the capillary with a plug, the green plug here, containing the DNA. If you send positive and negative electrodes through these reservoirs, there is a tendency of this DNA to move towards the positive. As it moves, it fractionates and tries to light up.

The way you make these devices using fabrication strategies for microfabricated PDMS glass capillaries in the following manner. Take a mask here and you pre clean 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 photoresist, may be SU-8 obtained 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 close or place this master inside Petri dish and pour PDMS on the top of this master. Before doing that, you have to do some surface pretreatment here, where you can actually make the surface highly hydrophobic, so that it can be able to not go do away with sticking - without sticking to the PDMS when it hardens from the liquid state. 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 plain glass slide and that would give you a device something looking like this. You have these small ports here in all four sides and this crisscross channel that you can see are real-time optical micrographs of such devices. These are some detailed results and this is now been published as well.

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 a voltage of 300 volts after 25 seconds, you see that there is a plug like flow of the DNA. This is the ladder that you are translating. This is a 100 to 1000 base pair DNA ladder.

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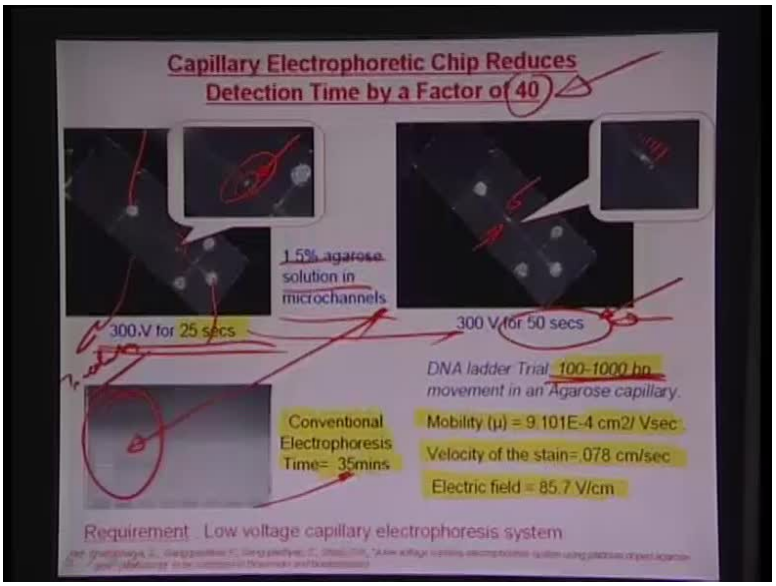

When you are flowing it for about 25 seconds, you see that there is a small stain that is developed and lit up. This whole assembly is placed over a transilluminator. This is placed over a transilluminator and that is why you can see or visualize this particular stain of interest here. Use a 1.5 percent agarose solution in these micro channels. If you keep applying this for another 25 seconds, it splits up into these various stains or bands in the same capillary as you can see. This is one of the finest examples of capillary electrophoresis.

The mobility can be calculated in such stains as this 9.1 10 to the power minus 4 centimeter square plus per volt second. Velocity of the stain would be roughly about 0.078 centimeter per second and the electric field that is used is almost close to 85.7

volts per second. If you compare time wise to the conventional electrophoresis, how much time reduction happens in this case? In the conventional electrophoresis normally occurs in 35 minutes or so. This is the convention electrophoresis where the ladder is split up about 35 minutes as opposed to be only 50 seconds here. So, there is almost close to one-thirtieth or one-fortieth time reduction, if you shift from the conventional to the capillary. This is basically a reduction factor of 40. It is a huge advantage to the industry, because we essentially look for rapid ways and means of doing this DNA fractionation and this is one of the very best methods which are available in the capillary where you can do the same job.

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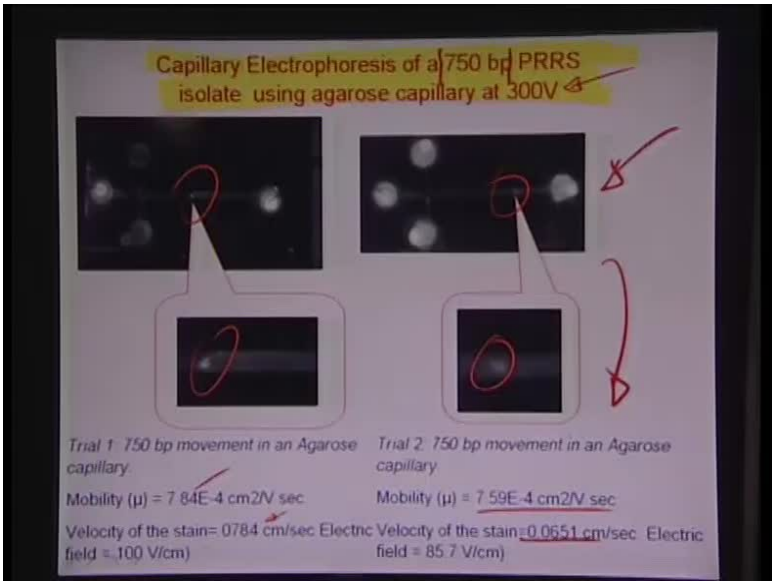

This is another illustration where you can show electrophoresis happening inside a capillary using a 750 base pair porcine reproductive and respiratory virus syndrome. It is a dreaded disease in the swine herds in nine states and across the other part of the world. It is a viral disease; the virus is recognized by the 750 base pairs of a certain section of the genome of the virus isolate. Using agarose capillary in about 300 volts, we have been able to successfully demonstrate electrophoresis in terms of the stains as you can see through in the capillary.

The mobility is calculated at about 7.84 10 to the power minus 4. The velocity of the stain is about 0.0784 centimeter per second in a field of 100 volts per centimeter. In this second trial here, the mobility slightly changes to 7.59 10 to the power minus 4

centimeter square per volt second and the velocity of the stain is 0.065 centimeter per second, the electric field about 85.7 volts per centimeter.

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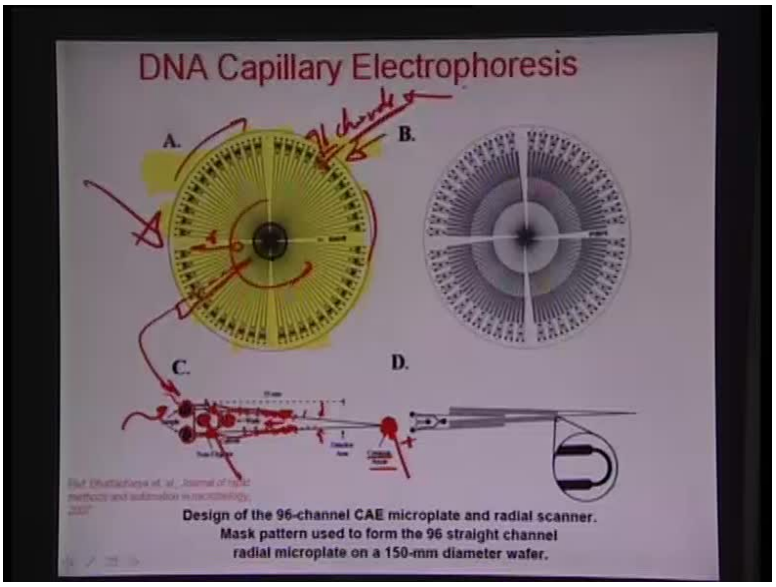

Some of the facts and figures which are important for knowing about this electrophoresis process a little bit more. Another fantastic example is from this paper published way back in the early 2000s by the Mattis group at UC Berkeley. Here what is Mattis and his group is trying to show is a set of capillary 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.

If you look at one of the channels here, let us say we want to have a look at this one channel, you have two channels in this one unit of the channel that is mentioned here - two independent channels converging into 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 are two of the detection arms on both sides and these arms are adjusted in a manner that you have the sample ports outside and you have a crisscross channel on both sample ports from both sides of these main detection arms and these are all connected integrally to something like a waste collection center. Then you have a cathode, which is placed at southern end of the micro channel and anode which is placed somewhere in the waste reservoir.

Then the idea is that when you load the sample here and make it negative, the sample negatively charged by putting another electrode. The sample electro kinetically gets injected into this direction arm and this direction arm and then the cathode and anode combination of these two across these two ends are able to drive the small samples which are caught in this detection arm all the way through the arm.

In the process, there are stains which are formulated in these two regions here and 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 stains are there on this capillary.

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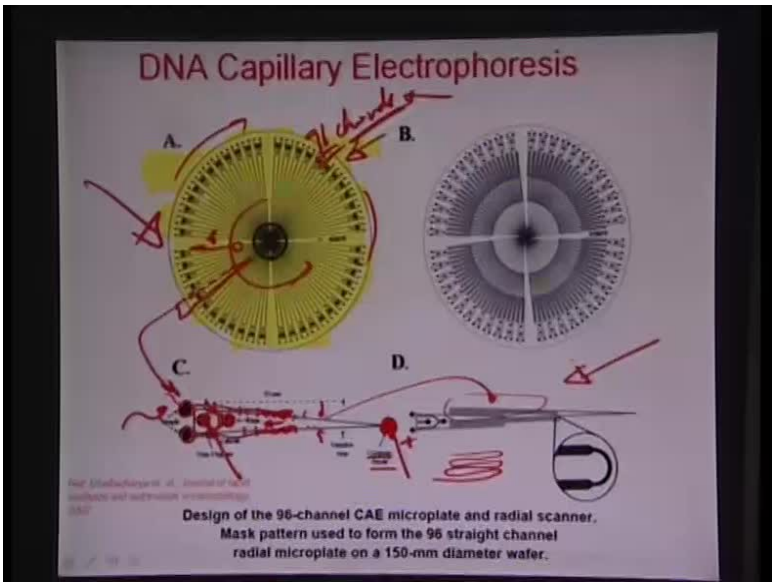

So, there is a unique combination of the rotary motion of this particular micro capillary containing CD and in relation to the radial motion of the detector sensor assembly. That can help you to identify atleast 96 – these are all 96 in number. 96 separate channels are there in this CD like platform. You can actually at a time read about 96 PCR reactions using this particular capillary. So, this is also a very high throughput process that mattis and his group have developed before. You can also take this particular channel and introduce a lot more turns and accommodate a lot more length of this channel.

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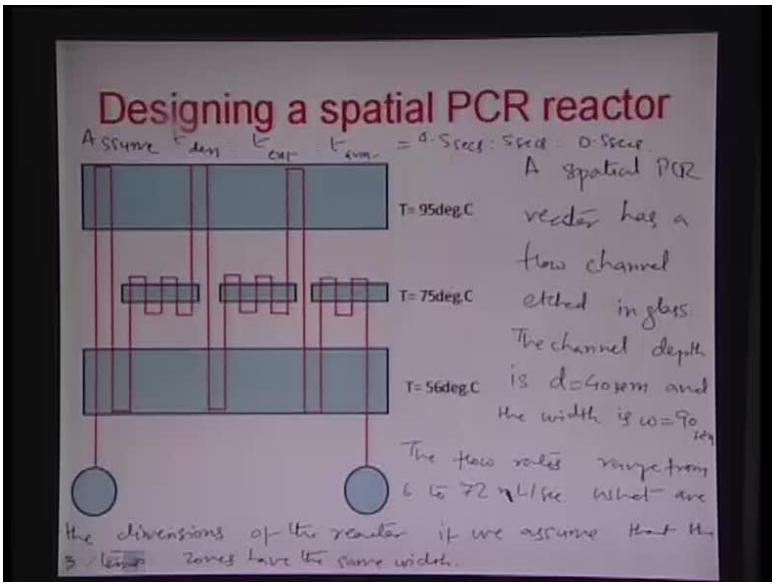

The only thing here is to be a little careful in designing the way that these channels will twist and turn and that should not be done at the cost of the resolution loss of the DNA. There is some interesting work which has been done in this capillary array electrophoresis area by various researchers around the world.

After doing these electrophoresis basics, I would like to turn your attention towards another very important aspect known as the design of a spatial or a space domain PCR reactor. When we were talking about the PCR reactor in general, we assume that there is a small chamber contained in time and space and the temperature contained at the fixed spatial location and contains a small volume of this PCR fluid. We assume that the thermal cycling is done on this fixed spatial chamber through on a temporal basis, with the time varying basis. These devices are also known as the time domain devices for PCR micro reactors.

However, one issue with these devices is the very fact that you have to heat a large amount of thermal mass associated with the wafer or the substrate of the base which would contain this chamber in it. Therefore, every time you have to ramp up the whole mass to a certain temperature and ramp it down in order to be able to quickly do thermal cycling. However, this is a very inconvenient module because the thermal mass and particularly the materials that we use in microfabrication, silicon glass and then the

polymers, they are all poor thermal insulators. Therefore, it does not give a very easy solution in terms of a rapid ramp up or ramp down.

Another approach that was very intelligently thought by group in Epitoxword, Andria's man's group way back in about the early part of this particular decade was about space domain PCR reactors. Instead of one location and heating at various temperatures of that location, what this group for the first time thought was -why not have three different heating zones on the same chip - one heated to 95, one heated to 72, another heated to 50. Then you move the PCR fluid around in these three different zones by a serpentine path and in a serial manner so that the cycle which is a 95 followed by a 50 followed by a 72 is followed and at the same time PCR gets executed only by virtue of the motion of the droplet in these differentially heated areas of a single surface. This is a very normal approach and also called space domain PCR devices.

I would like to draw your attention to how such a device can be designed. It is a challenging engineering problem. You have three different heating zones maintained at different temperatures and you have a serpentine path over the zones in which you are flowing or circulating the fluid. So, when it goes to the first zone, you have to give it time enough for the volume to go up to the temperature of the surface, that is let us say 90 degrees and 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 hold the drop in 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- any link step actually. Similarly, it goes true for the extension step. 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 or give sufficient time for the denaturation, the annealing, and extension process of PCR to occur in an easier manner.

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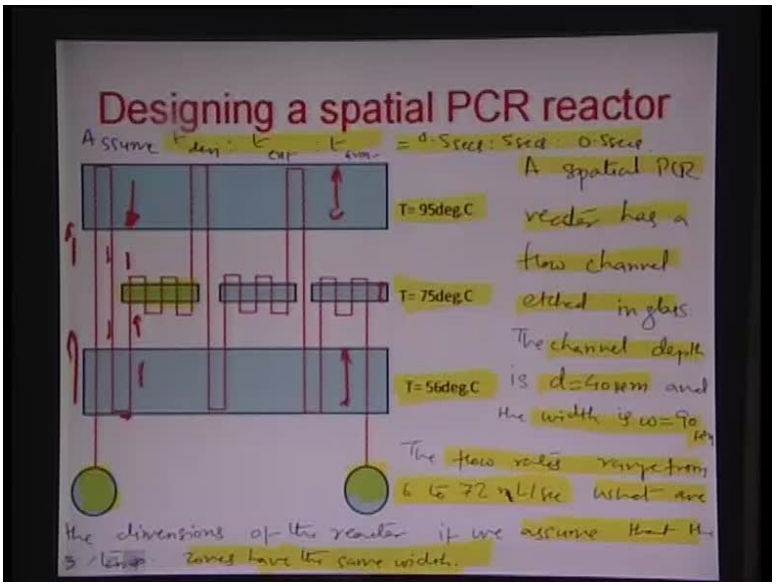

Let us do an example here. We now start designing such a spatial PCR reactor. As you see here, there are different zones - 95, 75, and 56 designed in a manner so that you can use probably micro fabrication techniques to design this kind of a thing. You have the 75 degrees only enroute between the 56 degrees and the 95 degrees. There is an input output reservoir here and the understanding is that the droplet would be moved in a direction so that it is able to rest on this particular area whatever the temperature is for the time duration where the whole effect can be felt in terms of a successful annealing, a successful extension, and a successful denaturation time.

The assumptions that we have to make for solving this particular question is that the denaturation, extension, and annealing time are in the ratio of 0.5 is to 5 is to 0.5 seconds respectively. We also know that the spatial reactor has flow channel etched in glass with a channel depth of about 40 microns; the width is around 90 micro meters. We also have flow rates ranging from 6 to 72 nanometers per second; that is the kind of flexibility that we have. We have to design the dimensions of this different differential temperature zones; the length dimensions or the width dimensions of the differential temperature zones. If we assume that all the zones have the same width - we are assuming that the three temperature zones here have the same width.

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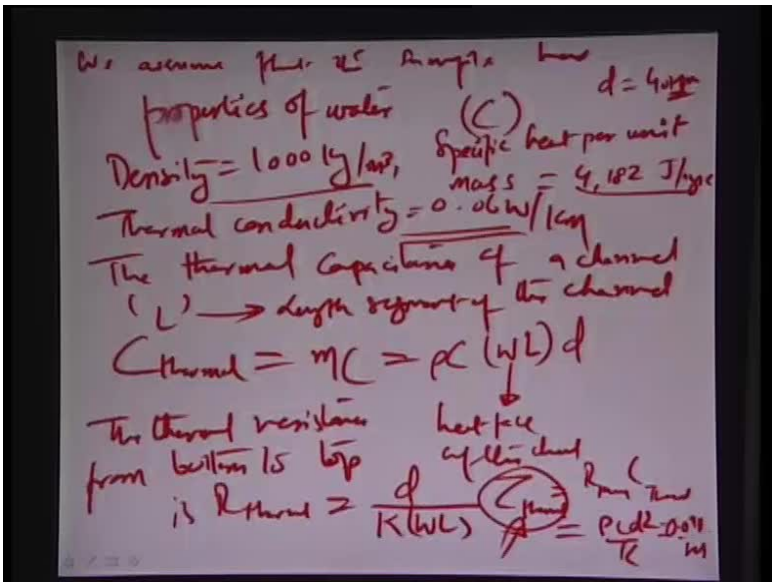

We have to first take into consideration some properties. We assume that the sample has properties of water. All PCR fluids are basically aqueous based. Density would be about 1000 kg per meter cube, specific heat capacity per mass of water is about 4182 joules per kg Kelvin, thermal conductivity about 0.06 watts per Kelvin meter.

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 m times of C. Mass is density of the medium times area times volume. You have to see what the area in this case is. The width of the channel being W and let us assume L to be the total length of the segment, WL is the facing area, so, this is the heat face of the channel.

Of course, the heat has to travel through the thickness of the channel d; so, WL d is what the volume of the channel would also be. Now, if we 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, which is the depth d by K times of square of L, in this case, square of the area. 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 is- in this case rho c d square by K. This comes out to be - assuming these various values of density specific heat per unit mass C the d value of about 40 microns and also the

thermal conductivity of 0.06 watts per Kelvin meter comes out to be - 0.011 seconds. That is how the whole time constant of this thermal circuit would be denoted.

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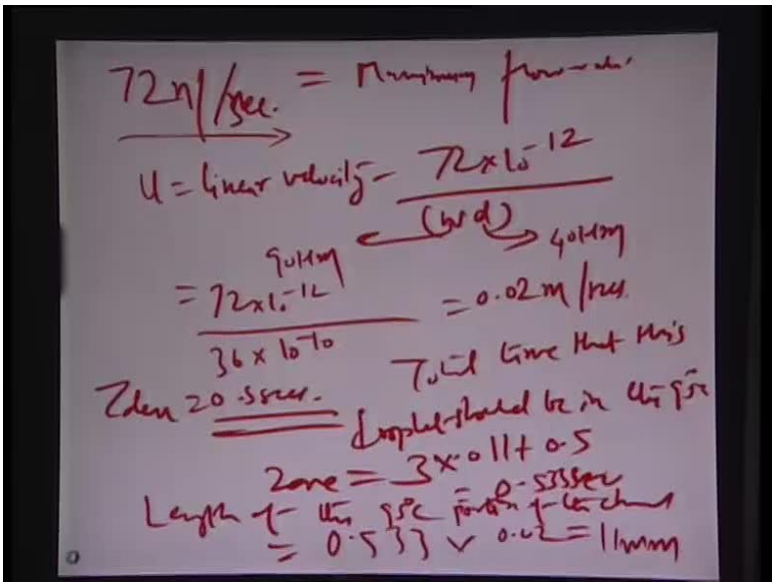

For designing any MEMS based system, what is important is also its reliability. The question is that there is a small droplet which we are moving through different zones of heating on a chip and you have to be 100 percent sure that the droplet gets the time that it needs for executing the whole denaturation time along with the time that it would need for going to that temperature which is 3 times of its time constant.

Assuming that you have a maximum velocity of flow, you can give the maximum tolerance to the system. Because anything lower than that would be definitely able to get the time of heating and the time that the whole denaturation would take normally otherwise to get completed. Therefore, we will do all the designing based on the maximum flow rate which is about 72 nano liters per second as given in the problem statement.

Let us first find out what the linear velocity u would be, in case the velocity of flow or the volume rate of flow is maximum. Linear velocity is actually the volume divided by the area of cross-section which is w into d in this particular case. d is about 40 microns and w is about 90 microns. So, it is 72 10 to the power minus 12 divided by 36 into 10 to the power of minus 10 and this comes out to be equal to about 0.02 meters per second. The time required for passing through the denaturation zone, assuming the denaturation

time to be about 0.5 seconds as has been given in the problem statement again is the total time that this droplet should be in the 95 degrees zone is 3 times of time constant, that is 0.011 seconds times 3 plus 0.5, this comes out to be 0.533 seconds.

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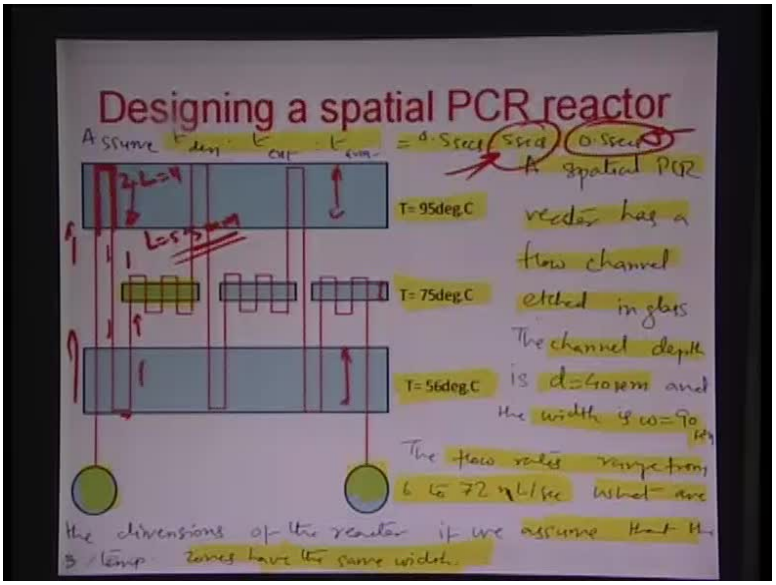

Therefore, the length of the 95 degree Celsius portion of the channel should be equal to this time here - 0.533 times of 0.02, which is actually about 11 mm. Since we were actually talking about a flow in this denaturation zone which is twice the length, the length becomes equal to 5.5 mm. We do assume that this portion here is not very major length in comparison to the length of zone. This turn is neglected because of it is smaller magnitude. The length of the zone that we have been looking at is about 5.5 millimeters. Now the same length is true for the extension case, because we do not have different time in the annealing area. But for the extension case as you can see here, the time is about almost 10 times. So, let us look at how much length will be able to cover in the extension zone.

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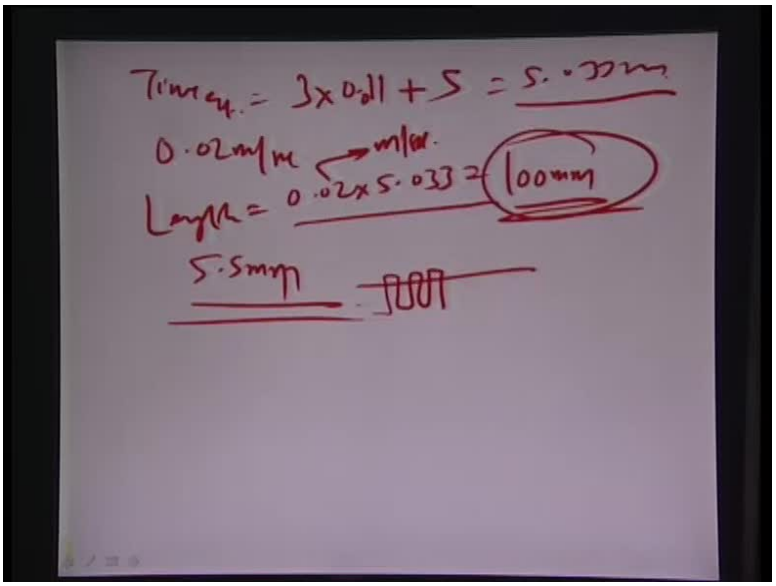

The time needed for the extension to happen is 3 times of 0.11 01 seconds plus 5 seconds, so, 5.033 seconds and the velocity of 0.02 meters per second. The total length that it would need to stay or cover the total channel length that would need to be in this extension zone is 0.02 times 5.033, which is what the total extension length would be. So, this is about 100 mm. Assuming this was in meters per second, it is about 100 mm. The very fact that you have one turn which is corresponding to about 5.5 mm, it is a design constraint that you cannot go over this in all the three temperature zones.

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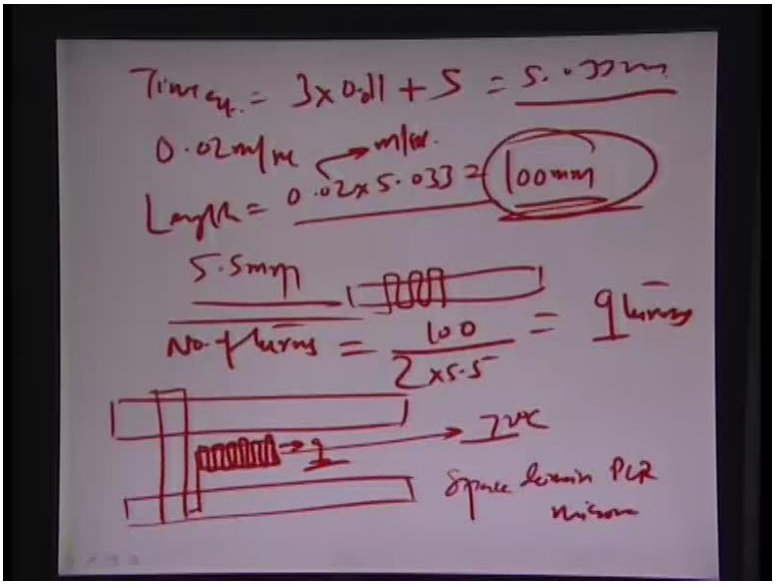

So, you have to actually ask the channel to serpentinely return in this extension area as can be seen here in the figure. So, you have to ask the channel to turn multiple times in this particular area, so that it can get heated as it goes into the serpentine path. The number of turns that this channel would need to execute in the 72 degrees area is 100 divided by 2 into 5.5, which is about 9 turns.

So, you have to design the PCR in a manner so that you have a space here which comprises of about 9 turns of this particular channel with spaces heated to about 72 degree Celsius. The annealing zone characteristics of the is same as the denaturation zone, although in the extension zone, you need to serpentinely call the channels about 9 times in order to get the total time of extension so that the whole DNA pair can be copied. This in a nut shell is how you design a space domain PCR device or a PCR micro reactor.

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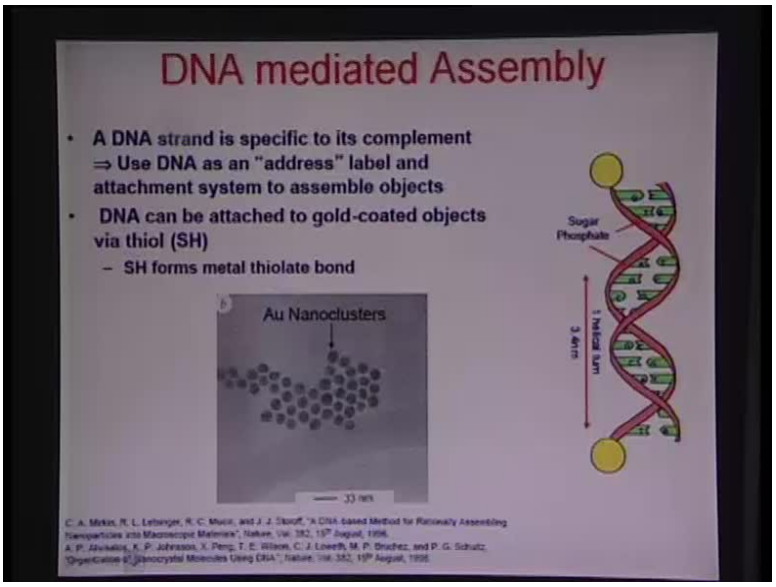

I would now like to actually delve into another very interesting area of biosensing and that is essentially what you can do using self-assembly skills of a DNA molecule, deoxyribonucleic acid molecule. What we are going to do now is to try and discuss some of the various detection diagnostic protocols that have been promulgated by the complementarity itself in the DNA chain. It itself gets assembled over one another. So while the first papers in this area is generated by Alivisatos and his group is about gold nanoclusters. What he found out is a DNA mediated assembly process way back in 1996.

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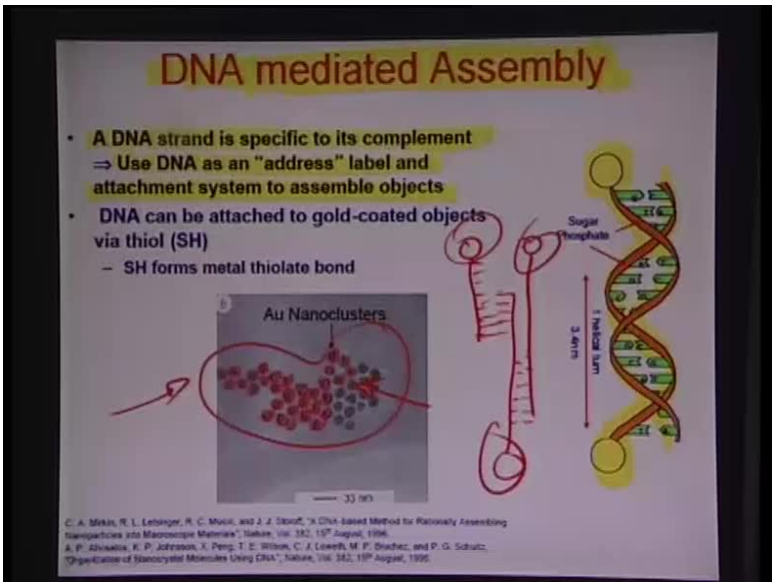

There are two complementary strands of DNA which are exactly complementary when they bind to each other with high stringency and what he had done is that he puts these gold nano particles on both these strands separately and let them self-assemble so that there can be an arrangement of these gold nano particles.

The moment these two strands are totally complementary to each other and in fact that this is not the only binding kinetics that one may have here. One may have something like- let us say you have this DNA molecule with a gold nano particle here and you have another molecule with a complementary structure which is corresponding to few base pairs here, then it has its own base pair switching and bind to a third molecule.

So, it binds to another third molecule here which has another of these particles. Therefore, there are several such strands just by means of complementarity between the strands which keep binding to each other. So, that is how DNA mediated self-assembly process would happen. How you get to know whether the DNA is self-assembling is by looking at what happens after giving sufficient time for this particle to assemble.

This is a scanning electron micrograph of these different gold particles as you can see. They form these clusters. These are gold nano clusters which is indicative of the fact that they are bound together or held together now by the DNA functionisation which is gold nano particles have individually on their surface.

This is one of the first experiments which demonstrated that DNA mediated self-assembly -how this approach can be used while putting together nano particles. A DNA strand is specific to its complement. So, use DNAs as an address label, adjust level attachment system to assemble objects and the DNA can also be attached to the gold particles by using thiol chemistry. You modify one end of the DNA through a thiol SH group and adsorb this SH onto the gold nano particle, which just by pure adsorption can conjugate or it can absorb and then become integral with one of these side chains of the DNA. So, SH forms a metal thiolate bond which is also the basis of the attachment here.

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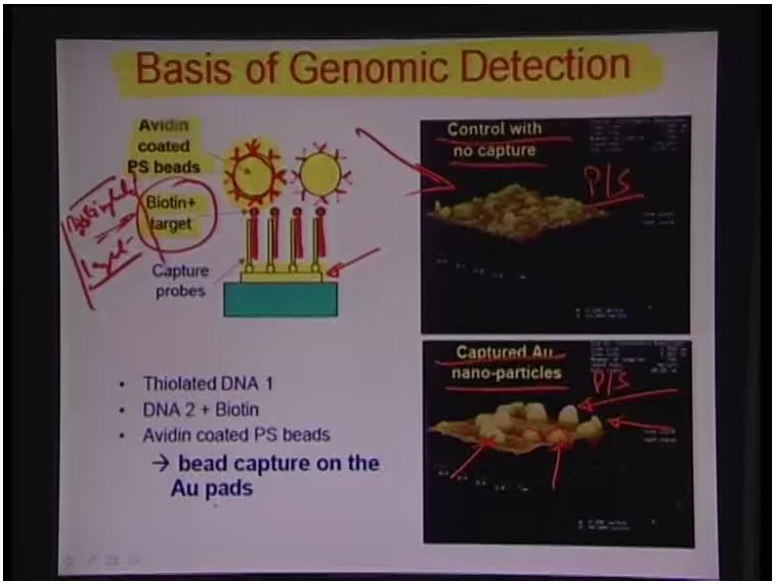

Another very interesting work which has been probably reported by the same group is one of the fundamentals of genomic detection. This is one of the first few works which indicate the concept of DNA hybridization. Here of course what happens is that you have these Avidin coated polystyrene beads as you can see. These are the Avidin moieties on the top of the polystyrene beads. Avidin is a vitamin which bonds very well to biotin which is again some kind of a protein. What you do here is you coat Avidin on these polystyrene beads and you make a series of capture probes with some particulates at the end which could actually be immobilized on the top of may be a gold substrate.

So, you are immobilizing this half stranded DNA molecule which is also a captured probe on to this gold surface using some thiolated chemistries. Now what you do is, you bind the target with a Biotin. So, you biotinylate the target. There are ways and means of

doing it and I am not going to get into the chemistry details of that. There are kits now available through which you can buy about in lead proteins, DNA, or all sort of biological entities can be biotinylated which means that Biotin group can be added on to the entities externally. Once this Biotin target has been realized, then you flow the target and it is going to find its capture probe and getting bound to the capture probe at the location that it is intended for and the Biotin moiety on the capture probe are able to trap the Avidin which you have already quoted on the surface of this polystyrene bead.

Therefore, whenever there is a target binding to a certain capture probe, it is indicated by a polystyrene bead the with the Avidin surface functionlisation in turn binding to this half or this semi DNA or this small part of the DNA tucking out of the hybridization. This bonding here between the yellow and red is also known as DNA hybridization. So, how do you detect such a mechanism? We use an AFM for doing that. Here if you look at the two scans, in the top scan there is a control sample with no capture.

So, there are no polystyrene beads which are immobilized as opposed to the captured gold nano particles. You will see here that these polystyrene beads have been immobilized on to the surface by virtue of the Avidin coated to the biotin conjugated onto the target DNA bound to the immobilized capture probe on the surface. So, by virtue of this, it is like a hook which holds the polystyrene bead in place and therefore, you can easily see that the presence or absence of a right capture probe corresponding to the target would just make a difference in terms of laying out a signal as a bound polystyrene bead over the surface.

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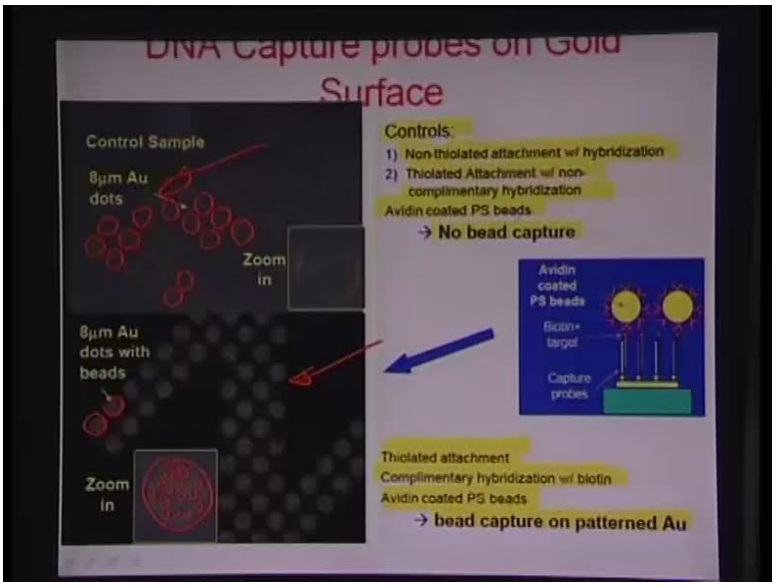

This is another very interesting example of how you can make hybridization arrays between a target and a captured probe in DNA molecules. These are some other illustrations where the exact same thing has been done but here as you see, there about 8 microns gold dots over which the capture probes have been immobilized by using Thiloated chemistry. These dots here that you can see are 8 micron gold dots, made of gold and what we do here is that in some of the dots we do not bind any capture probe and in others we do bind capture probes.

So, you are making some control and some reference you know presides of binding. Wherever these dots have the presence of the capture probe, there is always a capture of the polystyrene beads so that the machine seen here in the close up view, the small moieties here are the little polystyrene beads. The tiny beads have got captured because of the presence of the capture probe. So, optically you could actually - using an optical microscope - realize whether there is some binding going on between the target DNA and the capture probe just by looking at how these gold dots are whether they are with polystyrene bead or not.

So, the controls for some of these experiments are non thiolated attachment to hybridization, thiolated attachment with non-complementary hybridization, and essentially all PS beads all polystyrene beads are Avidin coated so then there is no bead capture indicative of there is a control. So, if there is a Thiolated attachment however and

a complementary hybridization with Biotin, every 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 only 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.

Now we come to another very interesting area which is a DNA hybridization micro array. This area is essentially a very big business nowadays; billions of dollars are involved in this diagnostics. One area is about building of DNA micro arrays. These arrays can be used for a variety of applications including hybridization arrays and for different applications like this RNA special monitoring applications, sequencing, HIV resequencing, in general trying to detect target DNA or sense some target DNA so on so forth.

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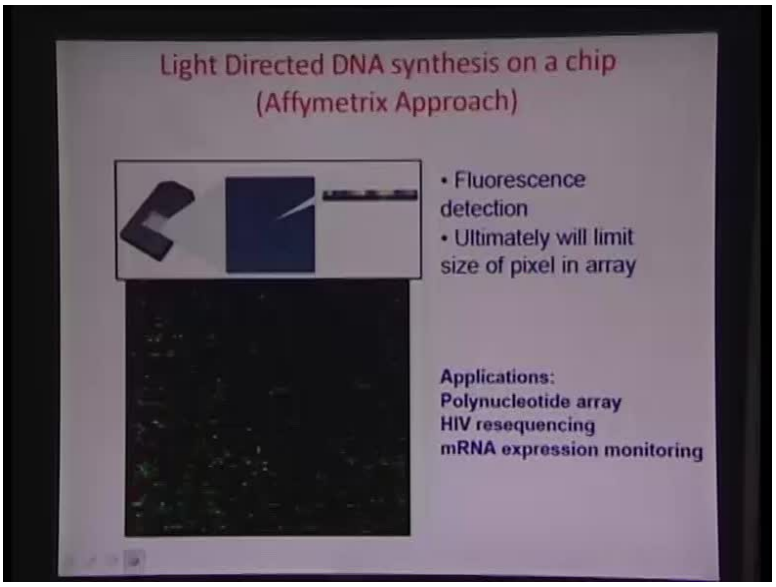

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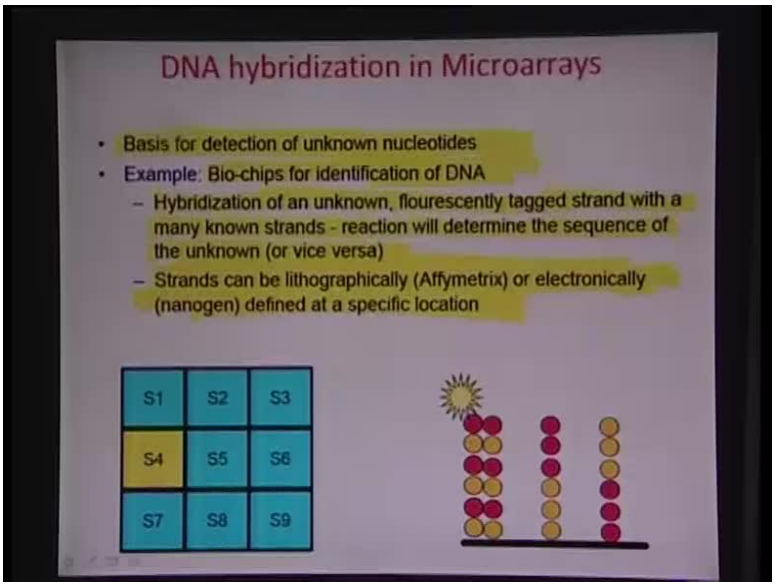

A very interesting aspect of all this is how do you build such microarrays or what is the basic principle or a basic mechanism of such micro-arrays. By definition, hybridization is the basis of detection of the unknown nucleotides. Examples that come into picture are bio-chips for identification of DNA. Hybridization of an unknown fluorescently tagged strand with many known strands reaction will determine the sequence of the unknown or vice versa. So, if you have an unknown target and you are wanting to hybridize it with something which is a known – some capture probe with known sequence and you are able to somehow fluorescently tag this strand with many known strands. The reaction will determine the sequence of the unknown or vice versa. So, strands can be lithographically or electronically defined at a specific location.

There are two companies in the world which principally makes these micro arrays. One of them is Affymetrix which does molecular buildup for the capture probes using light directed synthesis and the other company which builds these is Nanogen. They 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 the microscale architecture that they would provide. Let us look at what these micro-arrays are.

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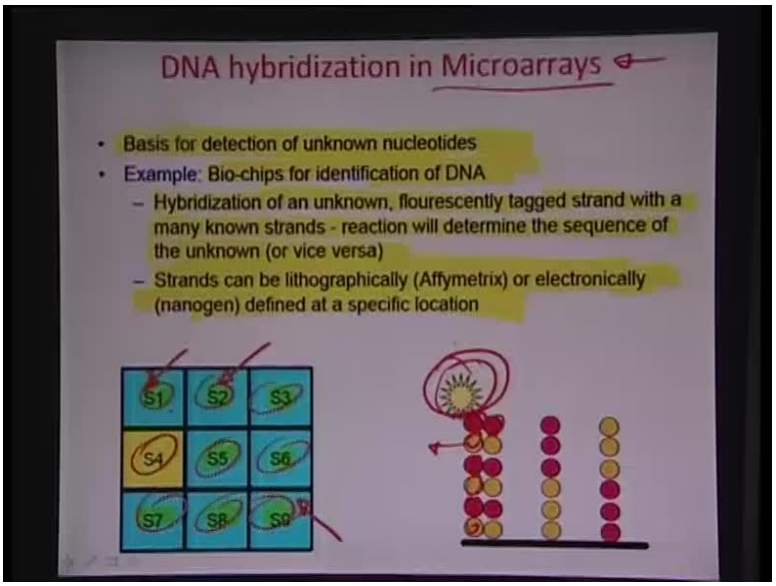

It is all about building different sequences or a library of different sequences of DNA onto a single chip level. In the left figure, if you look at these numbers here S1, S2, S3, S4, S5 all the way up to S9, these are different locations on the same chip. If we can somehow direct specific sequence of a capture probe of single-stranded DNA onto this area S1 of the first column and first row or the second column and first row, we have an accurate information of what sequence is there in S1, what sequence is there in S2, so on so forth up to what sequence is there in S9.

Then, if you bind DNA with a 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 immobilized the capture probe on this plate and the DNA which is fluorescently labeled is getting immobilized to the already immobilized capture probe. So, it is a hook between the plate and the new target molecule in that way gets bound.

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

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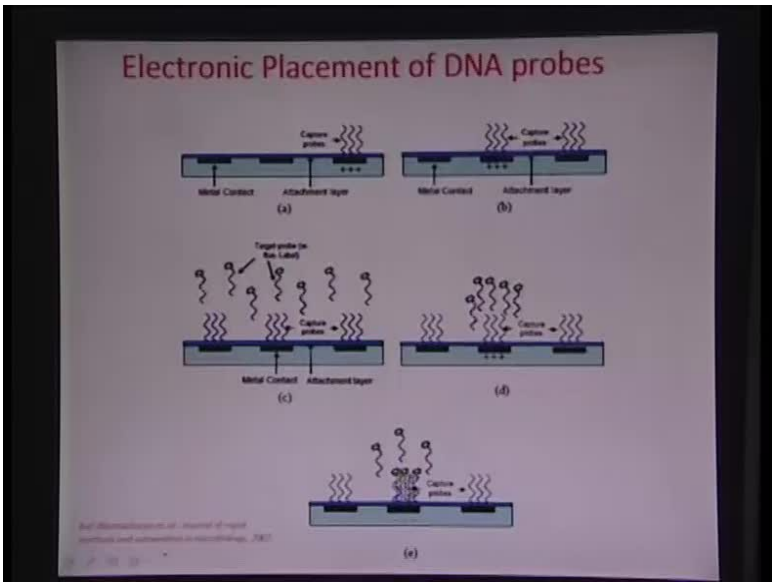

That brings us to the end of this lecture. What is interesting here is that we would now be in probably the next lecture working on some of the ways and means of placing these capture probes on the arrays or building these capture probes on the arrays. We will be looking at some of the companies like Nanogen and Affymetrix and the way they immobilize capture probes and build micro arrays and that will be covered in the next lecture. Thank you.