Bio - Microelectromechanical Systems

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Module No. # 01

Lecture No. # 39

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Hello and welcome back to this last lecture 39 on biomicroelectromechanical systems. I have decided to take you through a brief glimpse of research from an industry perspective that is available in this particular area.

I have tried to cover two such areas where some practical demonstration of some examples are illustrated. One is in the area of lab on a chip technology developing silicon glass or PDMS silicon microchips. The second is actually MEMS for drug delivery applications, where we talk about micro needles etcetera.

The last section of this particular course, I would like to dedicate onto showing you guys the movie on photolithography process, which will also give you an idea of hands on about how such processes can be dealt with.

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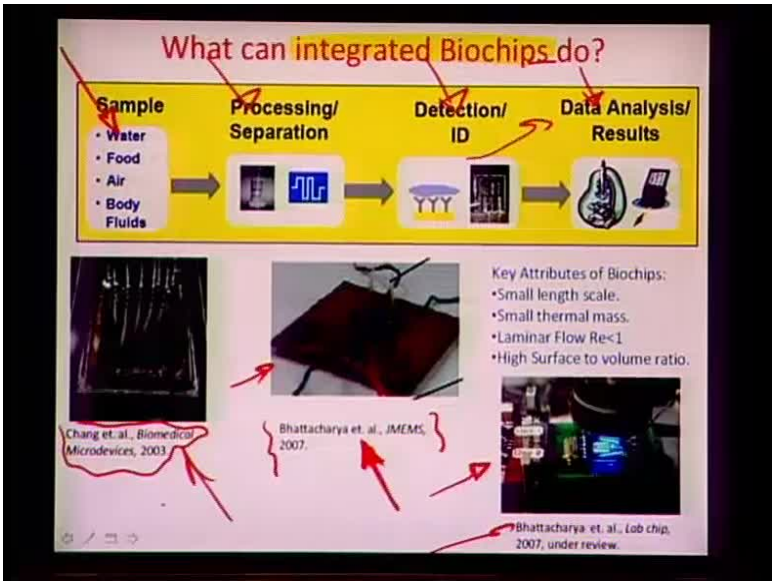

Let us right away look into lab on chip devices. We have been talking of and on about different lab on chip devices and systems. As you see here, I would like to just reiterate that integrated biochips are really capable of the whole integrated process varying from or starting from sample collection, sample processing or separation, some kind of a tagging or detection or identification in single transaction and then interpreting the data analysis and the results which come out of this transaction process.

The scope of biochips or the amount of work that biochips can do and deliver is huge. Now, there are several examples that are mentioned here, where we talk about different kinds of biochips. This right here is an example, which is borrowed from one of my collaborators work, which got published in biomedical micro devices in 2003. This is about finding bacillus anthraces or a chip scale using PDMS silicon hybrid micro device structure.

Here is an example from my own work based on finding out a PCR microchip for doing rapid amplification of miniscule samples of viral DNA. This right here is another example of my own work, where in we develop this of extended the technology that was

already available in chip form. To do an integration of two different processes dielectrophoresis DEP and PCR or Polymerase Chain Reaction we found out that we could go up to about 60 cells and do a successful real time PCR based identification using this particular protocol.

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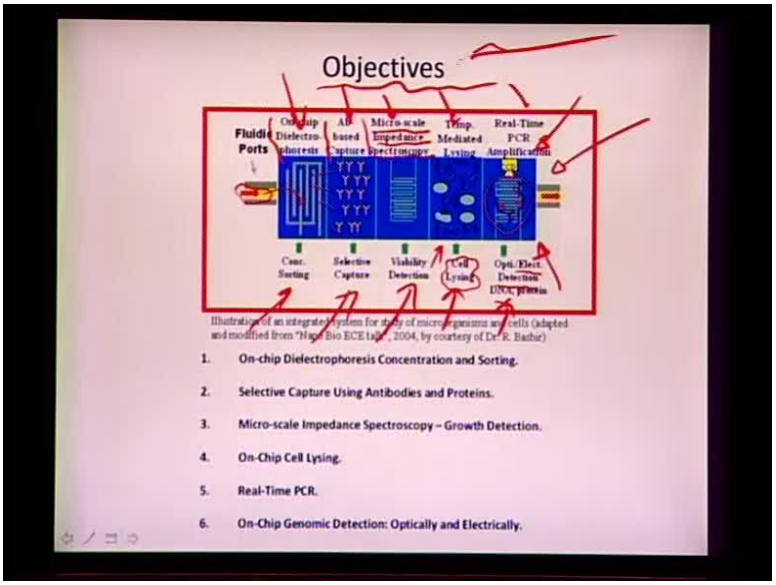

We will just illustrate time and again some of the work that has been done by various people including me around the world. If we look at the biochip, the first biochip in question it is really something which we have done before for investigating food and water bond pathogens. The biochip has a combination of several process steps as illustrated here.

As you are seeing here, the several different analysis mechanisms that are combined on such a chip and one of the reasons that is done so is owing to the fact that all the tests in molecular biology laboratory scale do not provide every information like for example the growth based test is really used to indicate the viability of a particular cell. The antibody base recognition is essentially upon the cell type. The PCR recognition based recognition is also the molecular identification and a stronger finding out of what type of species is there in a sample of interest or in analyze of interest.

Nobody has really looked at the combinatorial of all these processes and for the first time we did this initiative back in 2006 to 2007, where in this completely integrated platform

with scope for on chip dielectrophoresis, where cells would flow in and get captured our electrodes antibody based capture mechanisms, where antibodies are essentially nothing but y shaped structure with two like chains and a heavy chain and with an epitope side, which is useful for trapping bacterial pathogens or cells. As the bacteria or the sample containing the bacteria is flowed in here and the dielectrophoresis is used to locally concentrate the bacteria and the antibody is quoted onto the top of this particular electrode.

Therefore, whatever cells come very close to the surface get attached. Once it gets attached then basically tries to flow a low conductivity growth media and growth cells captured on antibodies. As they grow, there is a tendency of change of PH of the medium which can be highlighted by studying their impedance spectrometry or by changing the frequency. You can see what the impedance variation is.

So, the idea is as the growth increases and there are more number of cells, which are generated the ionic concentration of the media definitely changes because of the growth and I can trace the growth. If I can trace the growth based on the impedance change of the medium that is actually a great job because then viability indicator can be coupled to a microprocessor because of that. That is one step within this biochip.

The fourth step here is a temperature mediator lysing, where cells which are actually now grown on the chip are lysed open and the bacteria and the DNA kind of goes out in the atmosphere. Then, you use real time PCR amplification platform, which is on the same chip itself to identify based on optical means or as we have planned later on for purposes of electrical means of detection of DNA.

The objectives are many folds. First objective is on chip dielectrophoresis concentration and sorting, second is selective capture using antibodies and proteins, third is micro scale impedance spectroscopy for growth detection and viability indication of the particular bacteria, fourth is on chips lysing where the DNA the genetic material of the bacteria is pulled out and fifth is the real time based PCR product identification, which can be done using fluorescence and finally the sixth is the on-chip genomic detection, which can be either optically or electrically done.

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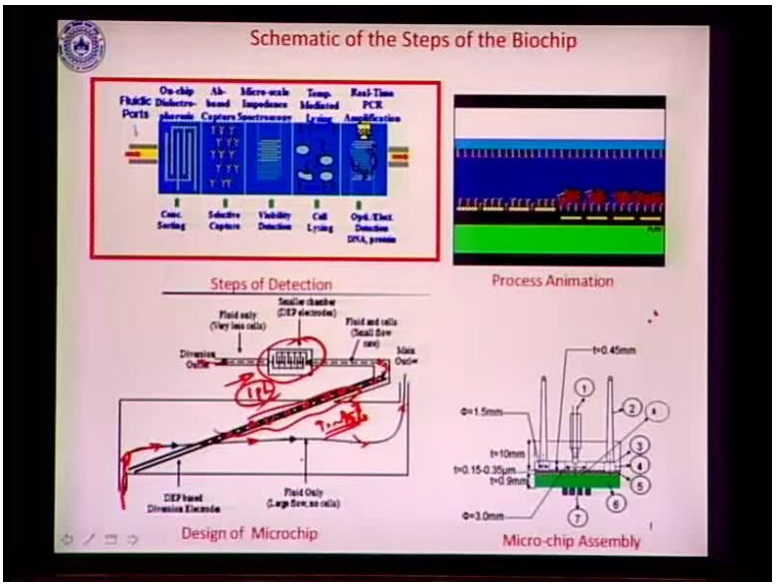

These are the kind of several fold objectives and goals that this particular biochip was supposed to have. We play a small animation to show what happens really the antibodies are flown in as you see on this particular silicon chip and they get settled and then you apply DEP or dielectrophoresis and capture rapidly the cells. They sit on the top of these antibodies lesser the red ones are specifically bound the other once are selectively flown away by using some kind of a wash solution. Then, you again apply a field and flow the low conductive growth medium, where you can see that the bacteria has grown many folds as you can see here.

As they grow the impedance is sensed and there is a trace or change or indication of the impedance, which would determine what cell types you are flowing in inside this particular chip. Then, this process is followed by the on-chip lysing and the PCR process all within the same microchip.

The way you actually do this the identification is by flowing the cell samples here and using this divergent electrode to pull of the cells and pack them selectively onto the smaller channel here. So that you can super concentrate the cells in this small chamber.

This is only about close to 1 Pico liters in size. Iit is very small chamber. All the bacteria is concentrated here and there is the set of electrodes, which would actually try to capture the bacteria and align it with these particular electrodes and this is the diversion outlet

from where the water carrying or the media carrying the bacteria is let out. It is a continuous fluidic circuit this way.

The sample which is lean in bacteria is flowed out of the solution here and the retention here is almost found to be close to 90 to 95 percent because of dielectrophoresis. We do not really need to worry about lossy components in this particular microchip situation.

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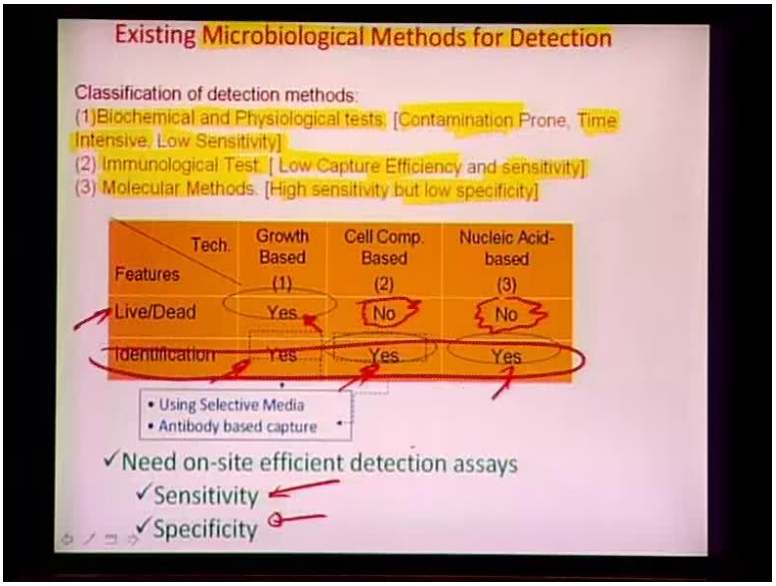

As I particularly described, if you look at the range of microvalve biological test laboratories methods, which are available for detection. There are methods related to biochemical and physiological tests, wherein they have disadvantages like contamination time intensitivity or timing intensiveness and low sensitivity.

There are immunological tests using antibodies, which lack from problems like low capture efficiency and sensitivity and then there are molecular methods, which would give an indication of detection of types by high sensitivity but it has a low specificity. You have lot of false positives which can get generated.

Now, the question is that some of this test like let us say the Para chemical or the physiological tests, which may include just plate culture and count or analytical profile index. These tests give very good idea about the cell viability or whether the cells are alive or dead as you can see here (Refer Time Slide: 10:30).

However, you cannot compartmentalize the cells or identify the cells. Neither can you identify the cells on the basis of its nucleic acids. The identification can be done by using the growth based method, the cell compartmentalization method and the nucleic acid based method.

So, the idea is to be able to plug in all these things together. So that on a single microchip you can actually sensitively and specifically detect on site the bacteria or pathogen load on a particular media.

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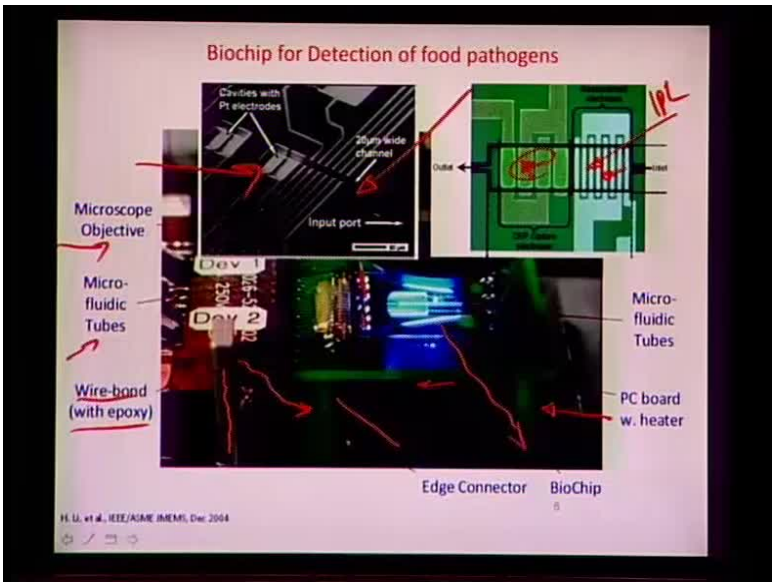

This right here illustrates some of the pictures, which are borrowed. This is actually a microchip borrowed from Doctor Busheer's group and this shows how this microchip has been constructed or realized. This is the small chamber, which is only about the close to one Pico liters and it is realized in silicon. There are open electrodes and then buried electrodes, the buried electrodes are used for dielectrophoresis and the open electrodes are used for impedance based sensing.

This is essentially how the TM image of this particular biochip would look like. The biochip is mounted on a PCB plate, which is an embedded heaters set wire is bonded to the PCB on one side and the chip on another side a microfluidic tubes, which go into the modules here on both ends.

You have a microscope objective and essentially you have a PCB or a printed circuit board with heater, which we do thermal cycling for PCR. You have edge connectors here wherein you can actually put this PCI kind of socket for inserting this PCB board. This is what the whole biochip really looks like. So, this in a nutshell can do all these other jobs that were shown just a minute back in this particular slide.

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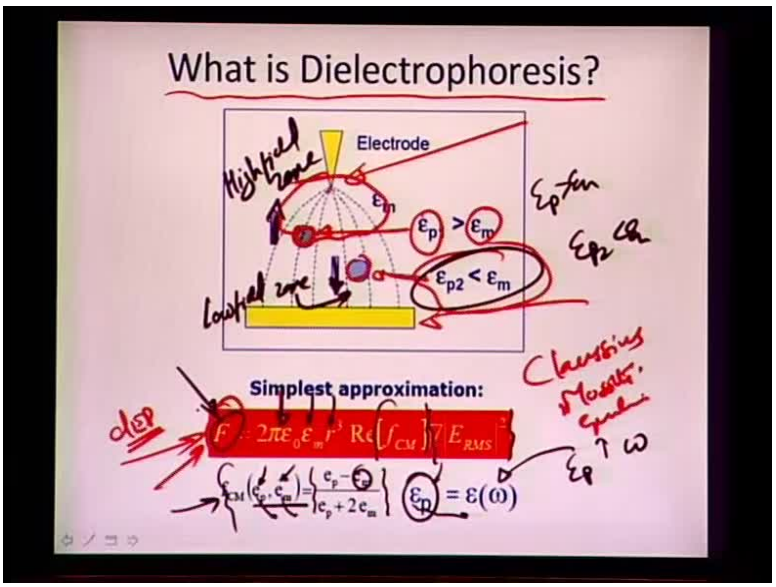

Now, let us understand one by one the different principles. The first principle is that the biochip uses dielectrophoresis. What really is dielectrophoresis? It is the polarization of neutral particles when you flow through a non-uniform electric field and this polarization would result in forces, where depending on the relative permittivity of the particle with respect to the media and the particle may either go towards the high field's zone or towards the low field zone.

Now, this here is an illustration. You have a non-uniform electric field been made up between a plate and a point and you have coiling of field lines in this particular zone or region. I have a particle here, let us say p 1 and p 2, where the dielectric constant of the particle is greater than that of the media in this particular case and the dielectric constant of the particle is lesser than of the media in this particular case.

So, without going into details; the dielectric forces in this case is also given by set of equations called Clausius Mossotti equations, wherein the component of force f is related

to this term here 2 pi epsilon 0 epsilon m 2 pi epsilon 0 epsilon m r cube times of the real part of this function f cm called Clausius Mossotti function, which is also given in terms of the particle dielectric constant and the medium dielectric constant. So, this function is given by e p minus e p m e m divided by e p plus 2 e m.

This is the dielectric constant of the particle. This is the dielectric constant of the medium. Further, both particles and mediums really depend on operating frequency. If you have an AC frequency, the value here the e p may really change with frequency may increase or decrease. If suppose it degrees then it goes beyond e m then the force here becomes negative in nature.

Therefore, one of these were the E p is more than E m and this value is positive has a positive d e p, which means the particle would go towards the high field zone. As you are seeing here in this illustration, the particle is trying to go towards the high field zone.

In the other case, when E p two is less than E m; as you are seeing here, this Clausius Mossotti factor would be negative, the force would be negative. In other words, the particle would tend to go towards the low field zone, as you can see in this particular illustration. So, it goes towards the low field zone and this is also a function of delta E RMS square magnitude and that is what the dielectric force really is in this particular case.

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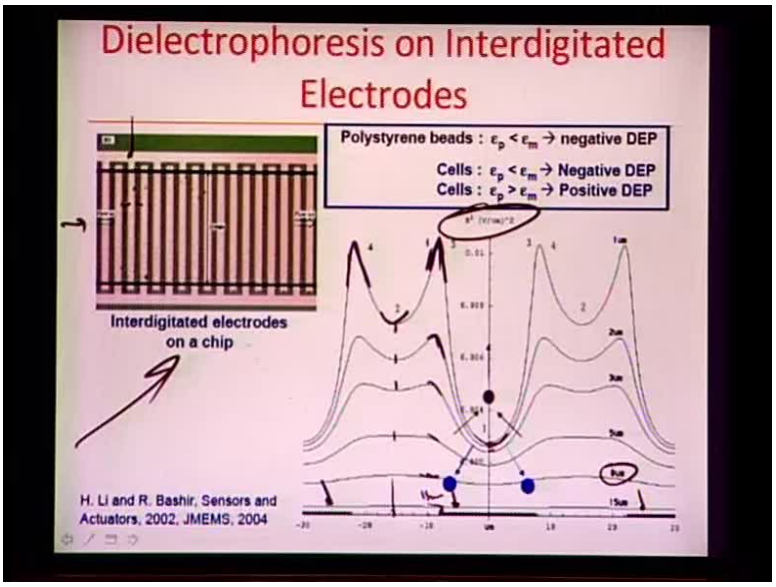

Now, the dielectric force can also be plotted with respect to the electrode position and you can see here these dark areas are the electrodes. If you see that above electrode for different kind of distances from the electrode, if you plot the square of the electric field you have plots wherein at the corners of the electrodes you can see a very near to the electrode, the square of the electric field is really very high. This goes on changing as you go away from the electrode almost to a certain static value, as you approach about 8 microns or even a little further 15 microns.

Away from the electrode edge somewhere in between the electrodes, you have a potential well, which is created as you can see here just because of the fact that here probably the lines of forces are most separated and they are least dense so the electric field is smaller. Similarly, the same happens on the top of an electrode somewhere in the center, where the lines of force again are probably negligible. There is no question of lines of force in this particular region and the electric field is very it is an all-time blow in this particular region.

Therefore, if you have this kind of a interdigitated electrode set as you are seeing here with electrode space from each other as the capacitor plate, you can have this kind of a field distribution with a high field zone near the electrode edge, as you can see in both cases and a low field zone in between the electrode edge. Then, further lower field zone in between the electrode itself.

That is how this interdigitated electrode can be used to build up a non-uniform field. So, whatever happening here in terms of this pointed electrode and plane electrode can be replicated by building some kind of interdigitated electrodes like this to get a field distribution.

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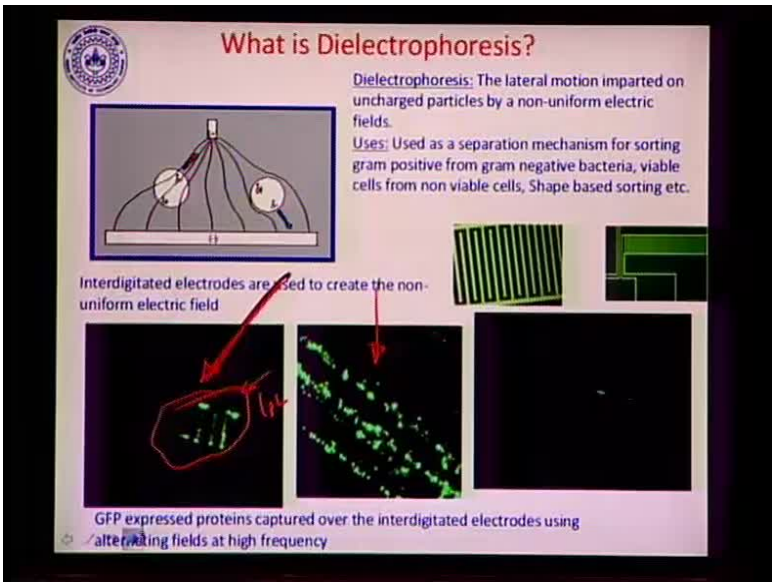

Now, this also illustrates how we can capture bacteria. This is flow green florescent protein expressing E-coli. This is h 1 57 o seven, it is a highly virulent bacterial stain and you can see that in its small one Pico liter size chamber, the cells are kind of aligning with the electrode. It is sitting more towards the top of the electrodes, which means it is now going into the low field zone and it is the inverse d b which is happening, which means that the particle dielectric constant is probably lower than the medium's dielectric constant.

This slide here is an illustration (Refer Time Slide: 18:32), where the same bacteria is captured on the deviating electrodes. If you may remember from our previous illustration here, the biochip that is in question here contains a set of deviating electrodes and a set of actual capture electrodes.

The first figure is on the capture electrodes here and the next figure is on the deviating electrodes. In terms of the actual SCM images, these are the capture electrodes. The deviating electrodes are somewhere you can see in this particular region, which can force the fluid to go into the small channel inletting this small one Pico liter size chamber.

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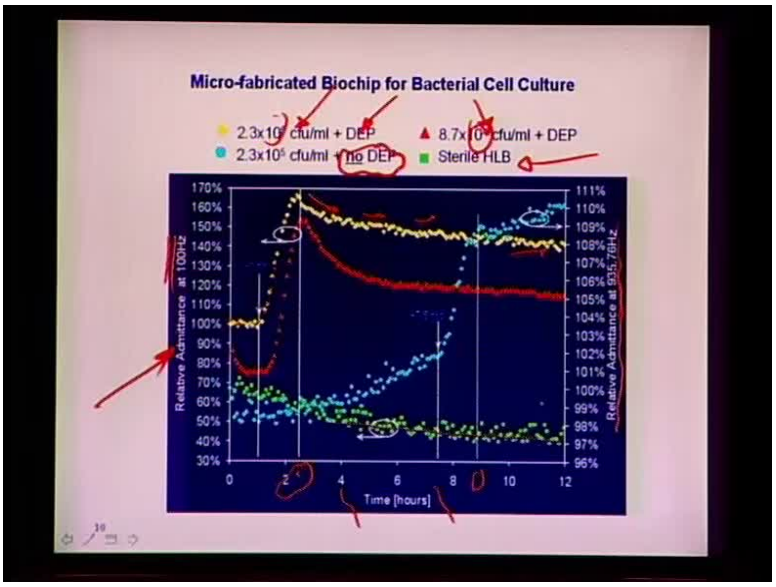

That is essentially what the power of DEP is. Now, you would actually able to see what kind of output responses the system would have in terms of relative admittance. Admittance is essentially the inverse of impedance.

As you can see here in this example, with time as the cells are growing into the chip captured over the electrodes the relative admittance is plotted at 100 Hertz in one end here and at 935.76 Hertz at the other end here. Now, you are seeing that as you have a solution of 2.3 ten to the power 5 colony forming units per mil of bacteria. You are using DEP, you are having response built by this yellow dots here of the relative admittance in terms of time.

It indicates that beyond 2 hours let us say between 2 and 3 hours two and half hours or so there is the maximum admittance, which is reached beyond which there is a downfall in the admittance. What it also leads us to believe is that the cell cycle of growth is really in this particular chamber for only about two and a half hours or so were the maximum growth rate is reached. Beyond which, as the cell cycle falls down or as the competitiveness grows up between the cells, competing for probably nourishment etcetera. There is a loss in viability and that is represented by fall down of the impedance spectrum as you can see here.

Similarly, for a different smaller concentration let us say 8.7 10 to the power 4 cfu per mil with DEP. You can have the similar kind of growth characteristics also the admittance in this scale is scaled down because of the initial change in the concentration from magnitude 10 to the power 5 to 10 to the power of 4. In case of no DEP, where no capture has been recorded hardly any cells are captured and you find out that the whole spectrum of the admittance gets changed and the admittance is high at a much higher growth cycle which is about close to 9 hours or so.

DEP does help in kind of demonstrating a more accurate signal in comparison to the no DEP based studies here. The background here is a sterile HLB, where there is almost 0 changes in admittance, almost no change in admittance as it goes with time. Of course, we will see that the admittances change slightly because the degradation of the ionicity of the sterile HLB takes place. HLB is basically the buffer of the growth medium in which the cells are kept.

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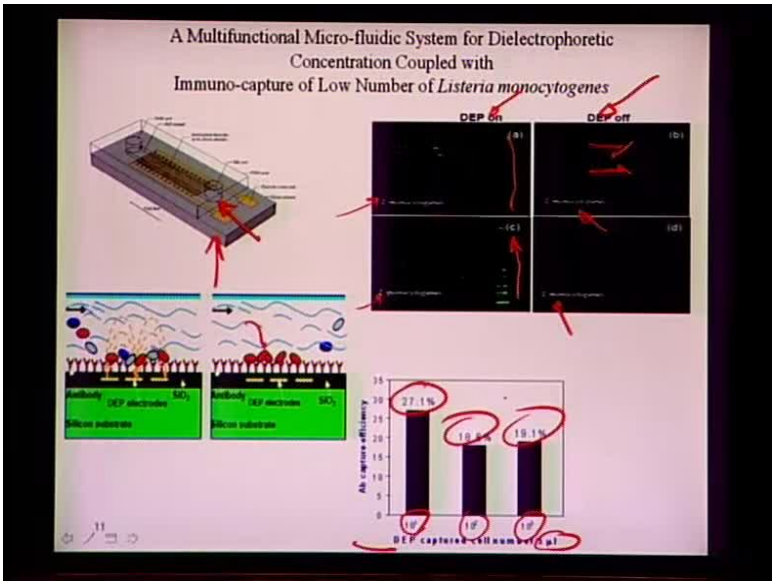

This is another illustration of what DEP can do. Here, you have a capturing chip with small PDMS channel it is hybrid micro device. This is the PDMS part. This is the silicon part. The silicon is coated with electrodes. PDMS seeded on the top of it and it has replicated channel chamber combination and here you are applying DEP and also capturing with antibodies to see whether there is a specific capture. You can see here with DEP on and off is a drastic change in the capture rate. The only capture events that

have happened is in this particular area here and here, whereas in DEP on the capture events has happened almost over all the electrodes as you can see here.

This is by the listeria monocytogene is V7 stain that is being used and is actually fluorescently labeled. So, per 5 micro liters the number of DEP cells, which are captured are about let us say for 10 cells, the capture efficiency is about 27.1, for 100 cells the capture efficiency is about 18.6, for thousand cells the capture efficiency is close to about 19.1 or so. Therefore, with DEP a lot of change happens to the otherwise one or two person capture efficiency.

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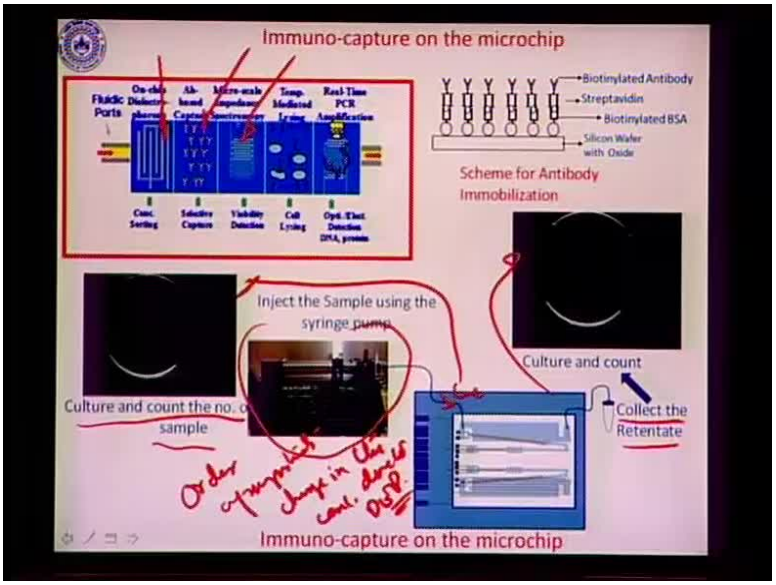

The way that the DEP capture can be illustrated is indicated in this particular example here, there is a syringe pump, which is used to fill this particular chamber and collect the retentate here. The DEP is switched on and you find out that because of the DEP what the inlet concentration here is? And try to plate culture in and what is the outlet concentration on this side? And try the plat culture it. So, you find out that there is almost an order of magnitude change in the concentration due to dielectrophoresis.

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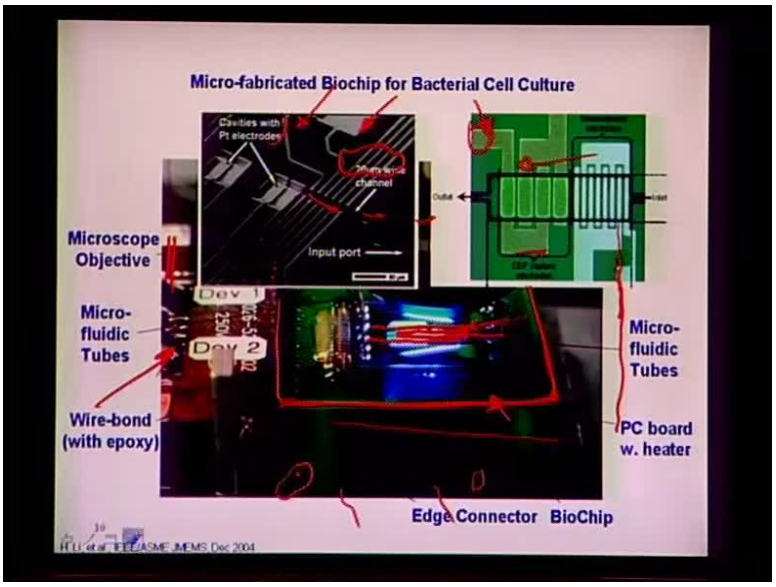

This is another very good illustration. We have already seen the DEP working. We have already seen the antibody based capture. We also had seen the growth cycle. Now, we turn to the next illustration, which is how we do on chip PCR on the same platform. If you may recall, when I talked about this microchip we also saw that there is this PCB green board with embedded heaters around it and there is a sort of serpentine RTD or Registered Temperature Detector, which is actually present on the top of this chip, which is not being illustrated here in the images but it is present on the top of the chip.

This is the platinum RTD. The concept is that as the temperature changes, the resistance would change and based on that, you can calibrate the temperature resistance relationship. Beyond which, you can actually see what the resistance is and predict what the temperature is and vice versa.

It is a control system that we want to design here do thermally cycle. As you already know PCR is a thermal cycling process of three temperature steps. I have illustrated it many times before. There is a step of 95 degrees, there is a step of 55 degrees and there is another step of 72 degrees and this follows in succession.

So here in this example, the control is provided by the RTD on the chip and the heater which is embedded onto this PCB is responsible for providing the power. These are actually built upon and a PID controller is built upon using lab view. There is a

programmable power supply, which would feed power onto this PCB breadboard and there is also a digital multimeter, which would sense the signal of this RTD but before that we need to calibrate the RTD to PCR reaction.

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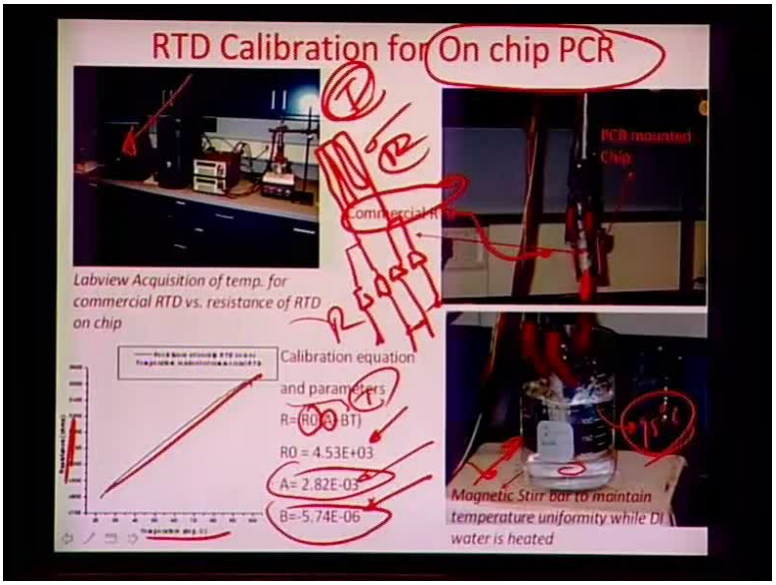

The way the calibration is done is given here in this illustration. It is a very simple way. As illustrated earlier, the RTD calibration here is very important to find out the coefficients in the equation for the temperature resistance variation. For that what we do is we can calibrate that with respect to a commercially available 4 wire thermocouple, which is available from omega. As you are seeing here on this figure, the thermocouple is placed somewhere here very close by and is made one integral part of this particular chip and this thing is lowered onto a beaker here, which has a magnetic stirrer. It is on a magnetic hot plate and therefore what happens is this heat up and then there is a stirring action. There is a continuous recirculation of water and it comes slowly all the way to about 95 degrees celsius or so.

In the meantime the lab view based acquisition system as can be illustrated here takes the data from the the 4 pointed probe thermocouple and the serpentine heater, which is also in the 4 wire configuration within the particular microchip.

This is the voltage divider circuitry, where you have readouts from these two and voltages given to this two. This is also used to avoid contact resistance. You take the data

of resistance from here and take the corresponding data of temperature from the commercially available RTD, the commercially available thermocouple and then compare these and draw a curve between the resistances. This is the acquired data and the temperature in degree Celsius.

You can actually do a linear fit on this curve and then try to find out what is the parameter R0, A and B. Here in this example, R0 is about 4.53 10 to the power 3 ohms 4.53 kilo ohms and the A parameter here for this equation is 2.82E to the power of minus 3 and the B parameter is minus 5.74 10 to the power of minus 6 respectively.

Once these are attained then every time you have resistance readout from here you could actually asked the lab view to back calculate what the temperature is? By plugging in these coefficients and that is exactly how the control mechanism happens.

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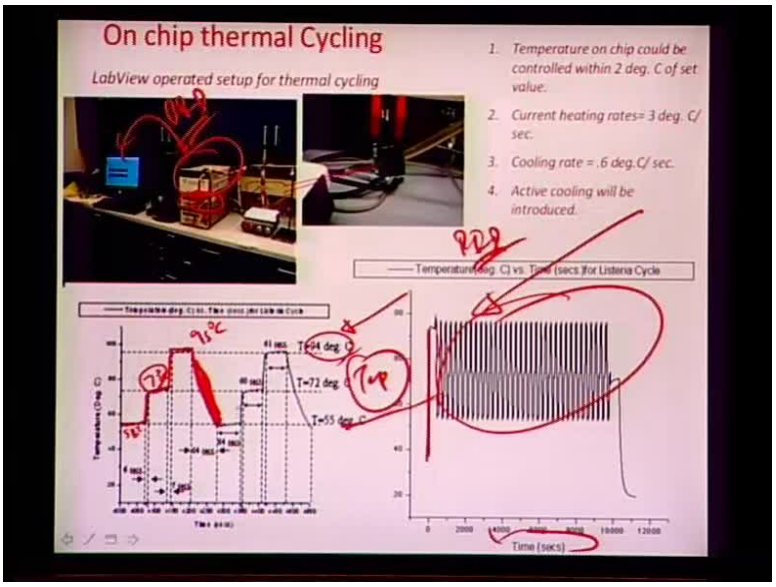

Therefore, you now next actually take this whole chip and then connect it to a programmable power supply as you are seeing here and a digital multimeter and you have a PID controller, which calculates of which actually initiates a set value of temperature. Let the heater go in power up to the level, where the temperature is achieved just as the temperature is achieved. There is a cutoff and there is certain switching off of the power due to which the power plot comes down.

Now, this really is a real time acquired signal using lab view of such a control system. You have to develop the PID parts experimentally using Ziegler Nichols or any other algorithmic method but the idea is that here as the heater power is switched on, there is a ramp up of the temperature and as it is switched down, there is a ramp down. The idea is that if I have a file which is executable it is may be a doc file, which contains the information about what time, what temperature is to be held on the top of the chip.

We can use that with the PID controller to control the sequence of temperature time steps as can be illustrated here. This is the temperature time curve; time is in seconds and temperatures in degrees Celsius. If I take just one plot or one section of this, it looks like this here as you see; this is the 95 degree point. This is about 55 degrees point or 52 degrees point and this is close to 72 or 73 celsius.

This is the three step temperature sequence of the PCR process and this goes rapidly. However, the only downside to this is that the cooling rate is not very high and for that there were several other modifications, which were introduced one of them being the introduction of a peltier cooler at the bottom of this particular chip, which would do forced cooling action. This curve would go down more although there were in that case problems related to the heeding up of the particular instrument.

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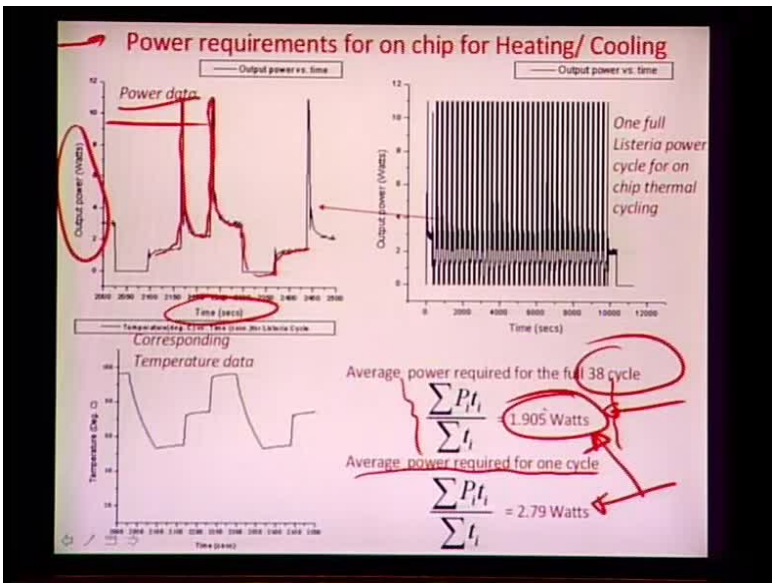

The power requirements of the chip this is done in a state of the art way. What you do is you acquire the voltage and current values over a substantial amount of time and then you actually calculate the output power multiplying that.

This is how a power profile would look like. Powers as you may see here is getting higher as there is a temperature ramp up requirement and it falls down as there is a sudden switching off of the heater because of ramping down.

This is the applied power by the voltage source. So, I can really compute what is the average power by taking into the consideration the full 38 cycles and the average power as you see here is about 1.905 watts about 2 watts, which is very small because the time the size of the particular PCR chamber or PCR chip is also very small.

The effective thermal mass that we have to take through these thermal cycles is very less. As the result of which it is a low power processor, it makes it more field friendly for field analysis. The average power required for one cycle is slightly higher because there is a cycle of idle time particularly, where the power is a kind of hovering over a constant level.

Having said this is the power requirement for heating and cooling of the microchip, let us look at some of the results that we got from these experiments. We started working with a listeria monocytogenes as V7 stain wherein a performance regulatory portion of the genome was investigated for doing PCR. The primers were forward and reverse primers were prepared designed and the PCR process was otherwise tested using conventional system.

Here, it is a real time process which has a cyber-green molecule. You may be aware from the previous lectures that cyber-green based PCR is something wherein wherever there is a increase in the DNA concentration there is a growth in fluorescence because full scale DNA bonds to the cyber-green molecule and fluorosis green and there is a change green intensity.

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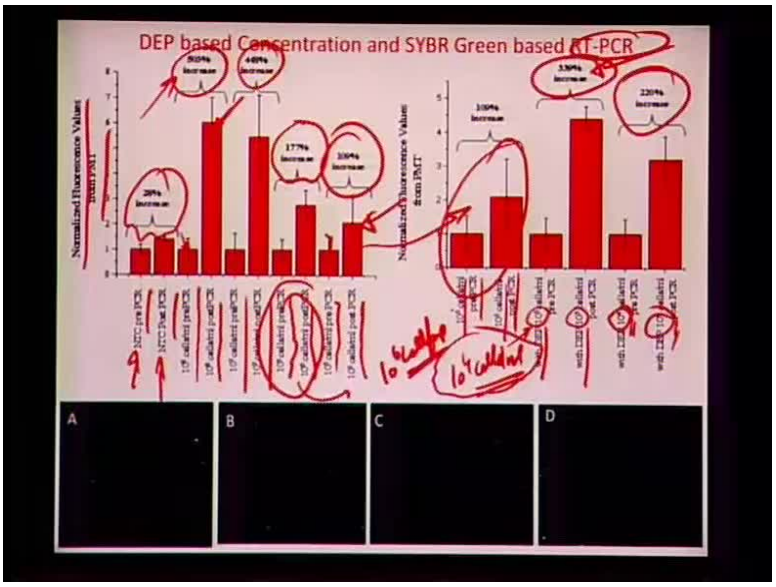

If you look at this particular plot, this is the normalized fluorescence value readout from a PMT. This is the no template control pre-PCR before and after pre and post PCR samples do not show much increase. If we see the error bars are more or less within each other and so there are at the same level. Whereas, if you take 10 to the power 8 cells per mil, pre-PCR and post PCR show substantial amount of difference there is almost a 503 percent increase.

Now, this growth here it is an end point detection wherein after the whole 38 cycles of PCR are completed 38 thermal cycles. We take the fluorescence readout and before they even begin, we take the fluorescence readout so that is what the comparison is.

There is an increase in 503 percent. In case of 10 to the power 7 cells per mil, pre and post PCR shows an increase of about 448 percent growth. This comes down further, when it goes 10 to the power 5 cells, it comes to about 177 percent increase and for 10 to the power 5 cells per mil again, it comes to as low as 109 percent increase.

But you see here, the error bars of both the processes are more or less within each other. This is not really a good data point and we can say that for this microchip 10 to the power 6 cells per mil really is the ultimate concentration which this chip can take up and amplify.

Now, let us add a DEP component to this and capture all these cells and make the cells more concentrated. You talk about 10 to the power of 5 cells per mil pre-PCR and post here and this is as you see the error bars are into each other this is data point repeated here.

If you applied with DEP then there is a substantial increase. You see this goes all the way to about 339 percent, which means the pre-concentrating effect of the DEP is letting more number of cells to be actively captured within the microchip even though the concentration is as low as 10 to the power 5 cells per mil. This actually if you can translate to 10 the power of 4 cells per mil a pre and post shows about 220 percent increase with the error bar still substantially away from each other, which means we can really go at least two orders here, I would say from 10 to the power 6 to 10 to the power 4 cells per mil and still be able to successfully demonstrate the RT-PCR process even though the cells concentration is miniscule.

So, DEP or dielectrophoresis has this additional effect of pre-concentrating the mixture so that you can have a better signal in this particular case. That is in a nutshell what the effect of DEP on PCR processes are when you are doing colony post PCR on a particular chip.

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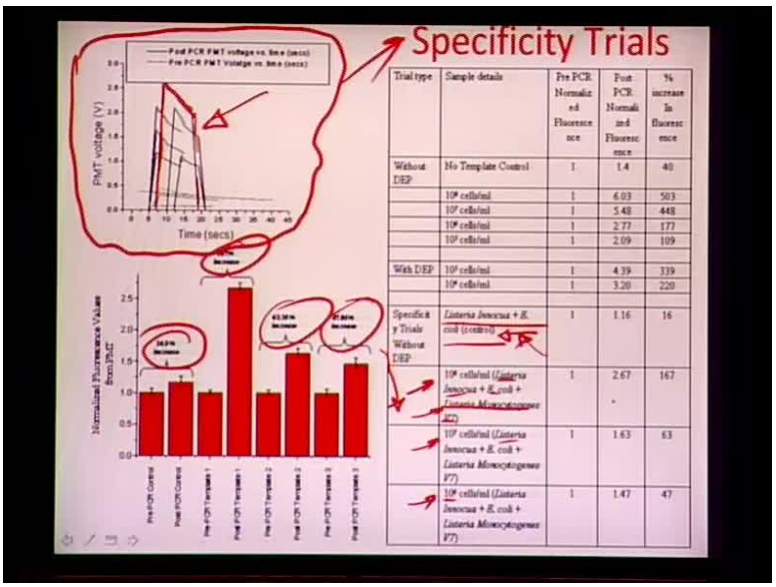

The way you take the data is that every time you have completed a fluorescence cycle. You scan the surface over 10 different areas and this areas actually do not vary, they remain same, Then, you are taking this PMT data or in terms of PMT voltage by just switching on and off the shutter. So that for a very brief amount of time peak intensity after which there is decay in the signal which happens because of overexposure.

We really do not consider the decay part; we consider the highest points here to make the average or calculate the average and report the average fluorescence value. We have also tried to investigate the specificity aspect of the PCR process.

As you know, there may be a tendency because of doing multiplex sometimes in assays particularly if you have multiple cells and the FEKC of a PCR processes based on how to design a set of unique primers for picking up or identifying one species out of several competing species in a solution.

The essence of a multiplex assay is basically to design of specific primer sequence to pick up one particular cell. Therefore, if the primary supposed to amplify let us say 385 base pair long region of a particular cell, the signal should only come because of this amplification and not something which is which goes unnoticed, which is non-specific in nature.

Here is to test this particular illustration. We actually mix a bunch of different cells and try to understand the sequence of amplification. So here in this particular example, for the specificity trials what we do is we take four different templates, one which is a control and having listeria innocua and E-coli h 157 o seven stain.

Another in which 10 to the power 8 cells per mil of listeria innocua E-coli and LMV-7 listeria monocytogene is V7 stain with a specific primer design on twelve MV7 and in the second template, we have 10 to the power 7 cells per well of listeria innocua equal i and listeria monocytogene is V7 and the third we have 10 to the power 6 cells per mil of listeria innocua E-coli and LMV7. These are the different stains that you have now you perform or undertake a PCR process and study that PCR rate on that. As you see here between the pre-PCR and post PCR in the control, the increase is 16.5 percent with the error bars within each other. This is not sufficient, the values that are indicative of the

fact that you are because of the absence of LMV7 here in this particular control sample, there is no fluorosis growth and so the primer is very specific.

Now, when you do it for the 10 to the power 8 cells per mil and then go all the way up to 10 to the power 6 cells per mil with template 1 2 and 3. The growth rates are 167 percent, 63.35 percent and 45.05 percent increase and the error bars are substantially away from each other. So, which means that we can really pick up the listeria monocytogenes as V7 stain with in a mixture of this equal i and listeria innocua cells very accurately using the set of primers that have been designed.

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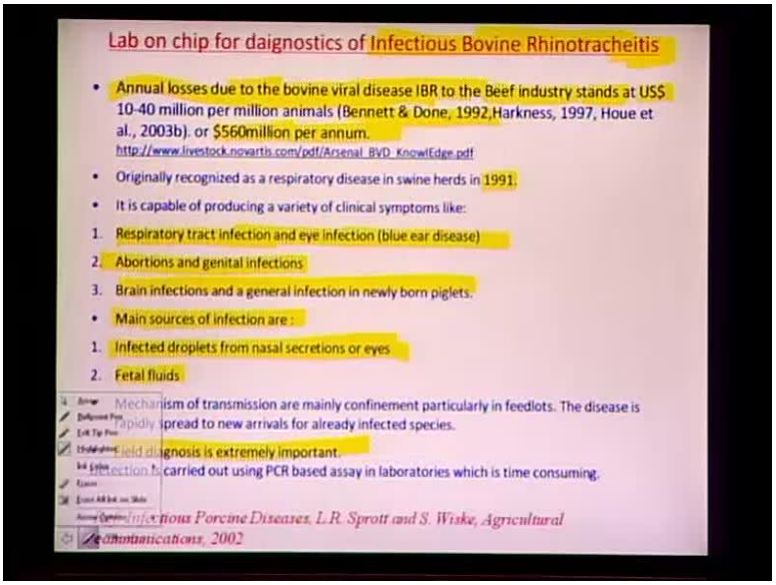

In these kind of microchips can really be used to determine the efficacy of picking up of a particular strain of bacteria over the others particularly in multiplex PCRs. Let me now go a little bit ahead and show you another example of previous work or an accept, which had been done by our laboratory. This is actually for the lab on chip phase diagnostics of this is infectious bovine rhinotracheitis.

I would like to give a little bit of background information, in order to do this particular study. When we studied this or when we started this research it was dictating or it was being dictated by the need of the beef industry and it was really a very pertinent problem that is infectious bovine viral diseases would kind of eradicate lots of lots.

So, the annual losses due to these bovine viral diseases alone particularly the disease IBR, Infectious Bovine Rhinotracheitis it is also called the red nose diseases happens because of a virus. This stood at roughly US 10 to 40 billion million dollars per million animals. As the data reported from this Bennett and Done in 92 and Harkness in 1997 Houe et al in 2003 and the annual overall losses that the beef industry would face because of this disease alone was about 560 million US dollars.

What were the symptoms that were recognize this was initially recognized in 1991 for the first time in bovine herds? It was capable of producing variety of clinical symptoms light like respiratory tract infection, eye infection, then they would be abortion and genital infections on the animal, then there would be brain infections and general infection newborn calves, actually because of this disease.

The main sources of infection really in this particular case are from nasal secretions or eye or fetal fluids and that makes the disease more dangerous to the feedlot. There the transmission mechanics of this virus being through the arousal route, wherever there is a even one unhealthy species in an otherwise healthy feedlot, the infection was certain in less than 4 hours' time and so a quick diagnostics or a quick check was really the need of the day, where from the blood samples or even the sperm samples of the infected animal. Just on symptomatic basis, we could also have a solid diagnostics which would talk about whether the animal was really infected and in case it was infected the animal was decided to be thrown out of the feedlot otherwise it would turn the whole feedlot diseased.

Therefore, in this kind of a situation it was pertinent that some tool be developed which was do field diagnosis and the very first idea, which came for detection diagnosis is the PCR based identification system.

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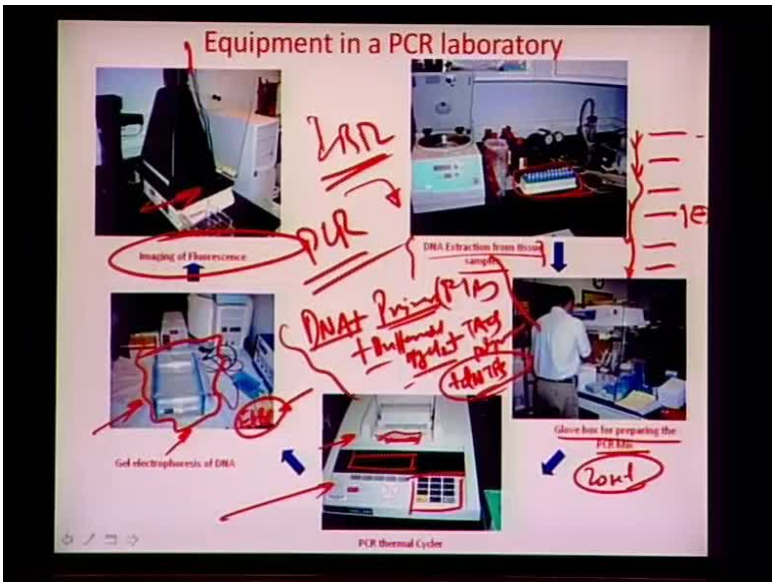

Let me just go through quickly about how a normal PCR laboratory would typically work and then the idea is to put everything together in a small chip level. The PCR laboratory here as you see begins with the DNA extraction from the tissue sample they set of these 96 this 40 wells, which have vacuum sucking the fluid through membranes which would really lies and at a certain PH the DNA would bind to such membranes which would be later on eluted as pure DNA.

This was a method, which would be developed in normal PCR laboratories for extracting pure DNA samples from the lipid layers and other infection or the other nonparticipating junk which would be there in the cells apart from the DNA. Once the DNA is extracted for analysis, you have to prepare the PCR mix which would be including DNA, the primers both forward and reverse, the buffer solution which would typically be in the C l 2 kind of a solution which would keep a certain PH level.

So, you have DNA, you have primers, you have a buffer solution and then you will also have to give the stack polymerase which is essentially the enzyme which would do all the amplification. After providing all these species in and then you will also have to provide the DNPT's which would be essentially giving the independent ACCT and G tags as the DNA is expanded through this chemical process of amplification.

I have already talked in details about PCR before. So, I need not give any illustrations here but the idea is that this sample is mixed in about 20 microliters size voile made up of polypropylene material highly hydrophobic. Then, these are centrifuged and vortex repeatedly in sequence so that all the mixture diffuses the mixes before we start the thermal cycling process.

This is how a thermal cycling really works and looks. This is system from applied bio systems, the company and here this is almost of the size of a desktop computer as you can see. You have LCD based screen, where you can do the programming. These are essentially the interaction modes with the machine and there is a PID controller inside. This is a 96 well aluminum huge block which is thermally cycle rapidly.

The idea is its high throughput process, there are 96 tubes you can load into this particular block and do the thermal cycling all at once.

Once this thermal cycling is accomplished and the DNA gets amplified, the testing is done through this gel material here as you can see. This right here is an illustration for that you have a box here, which contains agarose gel with pockets carved in it where this DNA solution can go in. You have also several pockets one for a DNA ladder, which would essentially do electrophoresis and provide different base pair size mobility's and would indicate what size a base pair we are amplifying and then if the amplified sample is run parallel to it and it formulates a stain between any of these two marks you could really identify the size segment.

The amplification whether it has happened or not based on the primer which is used for selection and the segment that is amplified would really give a full data about what is the nature of DNA and it would be used for identification of the IBR dreaded IBR virus.

Once this is done, it is imaged using this fluorescence setup here. It is an inverted fluorescence set up with a Trans-eliminator, which is at the bottom and it can give to different UV based wavelengths. There is a camera on the top and then there is a filter and basically the DNA is actually mixed with the dye material, which is in ETBR Ethidium bromide. We can actually get stains or signals which are at a certain frequency which would be indicative of the presence of absence of the DNA.

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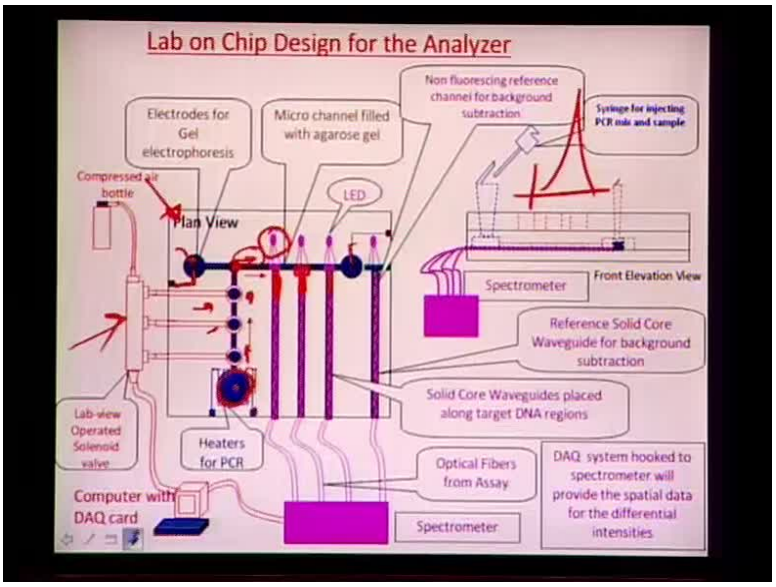

So, that is what the equipment in the PCR laboratory would typically look like. Our purpose here as an engineer was to really try to design in an savor all these different protocols, which can be done within the molecular biology laboratory can be integrated onto a similar chip and that is exactly what we did here.

As you look here, this is about a 2 inch by 2 inch silicon wafer. As the plan view, there are certain channels in PDMS which is made and pasted on the top of it using clean modeling protocols.

This is the small PCR chamber, which is actually sitting over the top of these embedded heaters, which are actually in the silicon chip, which are drawn by means of lift off and deposition process. There is a inverted thermocouple, which is placed on the top of this chamber and also there is an RTD as I will be illustrating later on which can sense very accurately what is the surface temperature of this particular chip.

Once the PCR fluid is inserted inside here on pure DNA sample and rapidly, thermally cycled the amplified DNA is transported using the set of peristaltic micro pumps onto this particular channel here, which is a small pocket carved in this gel filled channel as you can see.

This as the capillary channel really. As the gel has the DNA here and you have these two set of electrodes, which are embedded on both sides and you apply a voltage redundancy

of the DNA to move and translate depending on what size you have been amplifying it can move at a certain rate and go through these wave guides which are placed on the same chip.

The idea is that if we know the size segment that we are amplifying we will put an LED here and opposite to it a waveguide at a place, where within a fixed electric field the DNA had it been of the same size at the target would have easily gone. There would be fluorescence response as a peek as the DNA passes through this waveguide at that particular fixed time and that would be indicative of whether the particular sample we amplified here was of that particular segment size or not and which would have mobility as per plan.

This would be a kind of quick check on whether there is a presence or absence of the IBR virus within the sperm sample or the blood sample of the infected species. The primary means of diagnostics would be symptomatic. If there is some symptom, where you can see some kind of strange behavior by the animal species it would quickly take a sample from the species and run through check. It would give response are positive or negative responses, which would be declaring whether the species is infected or not.

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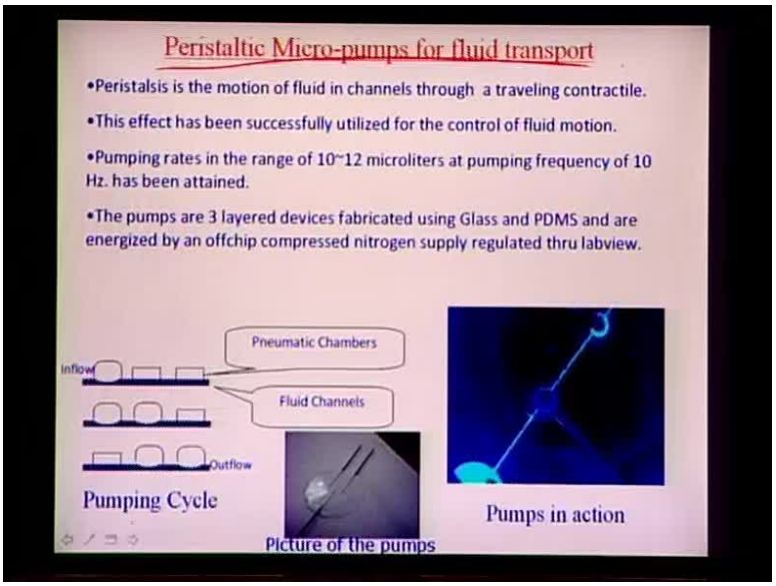

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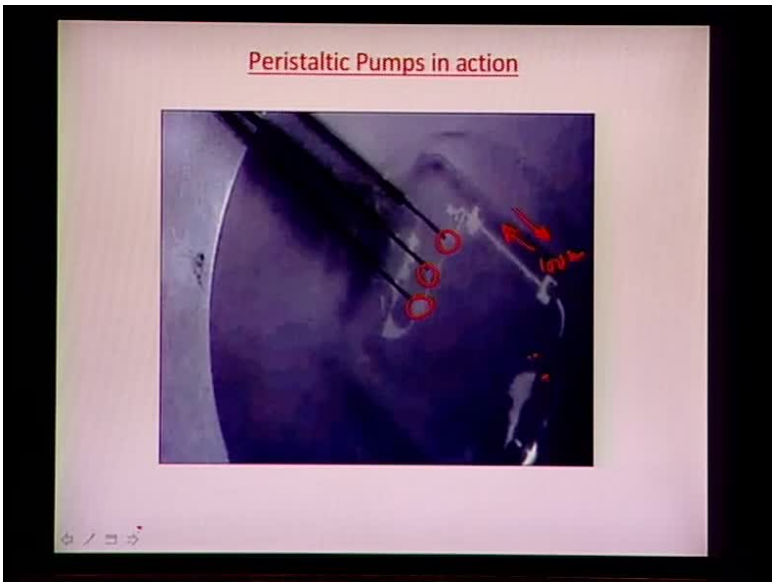

All set and done different aspects of these devices were built up, I have already discussed the peristaltic micro pumps in great details. So, I need not illustrate. This really is an example of working micro pump devices, you can see this as the channel through which the fluid flows back and forth and it is a bidirectional system this is the small hundred micron channel and the fluid here is a fluorescence dye. These are the three pockets or three blisters, which operate in a certain sequence and you can see this fluids flowing back and forth.

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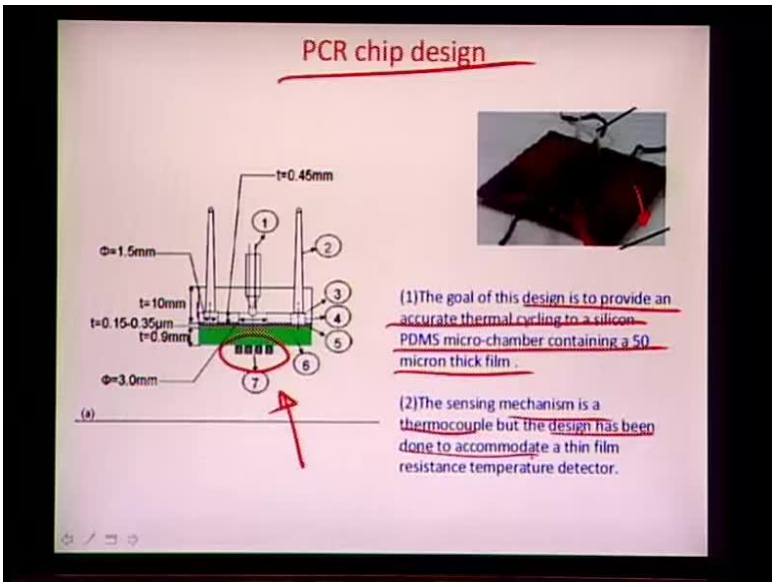

This is the PCR chip design, which we made using this heaters set and a thermocouple built on the top of this. The whole PCR was put in a breadboard on a PCB board, where we could easily connect it through wire bonding so that the small heaters could be supplied with the voltage. The goal of this design was to provide an accurate thermal cycling to silicon a PDMS micro chamber containing 50 micron thick film of SOG. The sensing mechanism was a thermo couple but the design that was primarily done was to accommodate a thin film resistance temperature detector.

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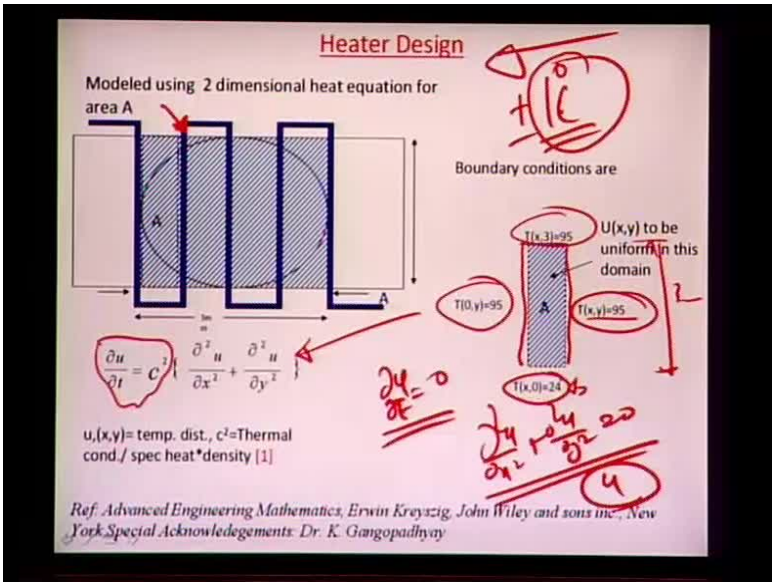

This is how we design the heaters and this is very interesting how we did that. We use the two dimensional heat transfer model and assumed the transience to be 0 so du by dt was assumed to be 0 in this particular case for solutions. Why we wanted to do so is because PCR is really all about holding at a certain temperature step rather than trying to get to that particular temperature step. The ramp ups and downs in a microchip PCR are considerably less in comparison to the whole times that the chip would have.

Effectively, almost all the time we are doing PCR thermal cycling, we are concerned with uniformity of the hold process of the hold time of the particular solution. We design something serpentine heater as you are looking at here, by shape it is apparent what kind of heating system it is. The PCR process is very sensitive to temperature variations and it kind of dictates for or calls for almost plus minus 1 degree celsius accuracy in the temperature.

Our goal here to design this heating system was to somehow incorporate this accuracy in this heater. As we solved this equation d t u by d x 2 plus d t u by d y 2 equal to 0, we try to develop solutions of u in terms of x and y by the boundary conditions assuming that we had three sites where we had this serpentine heater placed on one side, which is room temperature. We assume the boundary conditions to be t 0 y equal to 95 t x 3 equal to 95. We assume that this total chip length about three mm and this is x y equal to 95 and this t x 0 equal to 20.

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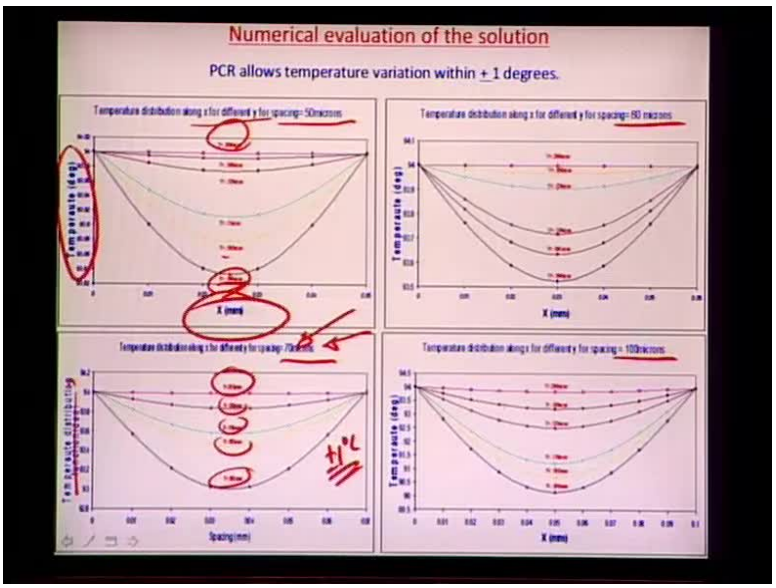

We assume this boundary conditions and try to solve and u as a function of x and y was plotted. As you see here for different values of y spacing's that means for different values of this length of the heater, you could actually get the plot between temperature and the x distance. You can see that along somewhere along the center the temperature as the y is more the temperature is actually the not very uniform. As the y is the temperature is getting more and more towards the uniform site.

At a particular x value, which is also probably the middle of this architecture here, you find out that depending on what the y values are you could have a range of different values of temperatures. Essentially, what you do is you find out for spacing's distribution for temperature distribution on x for different y for spacing's of the heater 50 microns,

60 microns, 70 microns and 100 microns. Then, try to see what is the optimum spacing which would be able to still at accommodate an RTD in the system by giving some space for the RTD component to come in as you can see here and still be able to attain this plus minus 1 degrees means so right here as you see is varying from 94 to 93 this kind of plus minus 1 degree is controlled.

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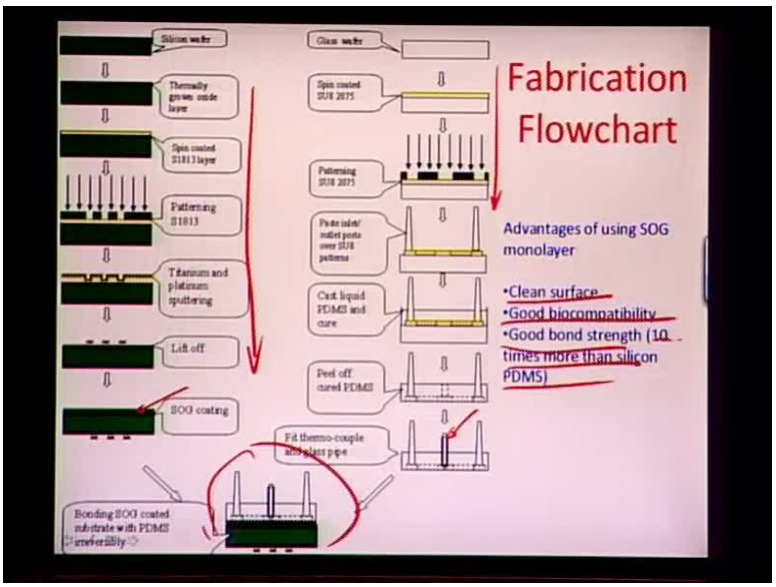

Therefore, in this kind of a situation you can easily say that for a 70 microns heater spacing, we can have a uniform temperature distribution of plus minus 1 degree Celsius. This is all the fabrication flowchart is constructed. You have a process for making the heaters and a process for making the PDMS upper part with channels and thermocouple. Then, you bond the two assemblies together in order to figure the make the complete hybrid device and here as you are seeing you use the layer of SOG or spin on glass as you can see here, just because of cleaner surface good comparability and a good bond strength which is about 10 times more in the PDMS surface.

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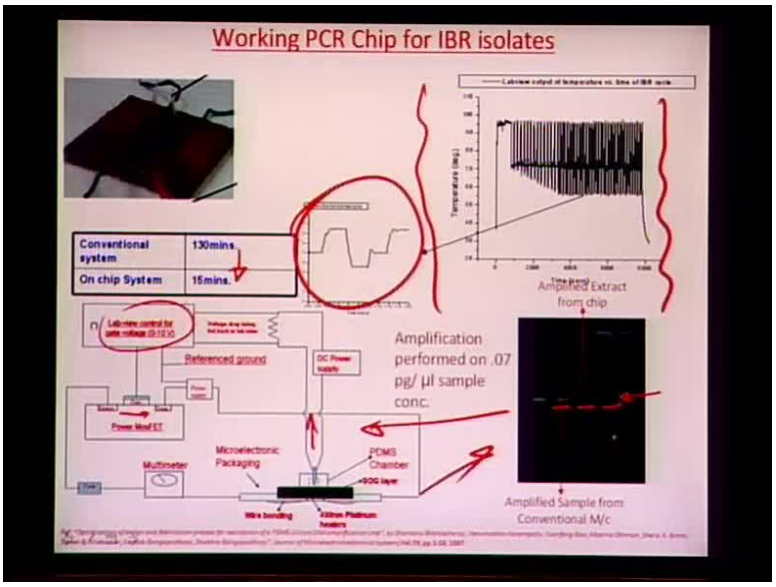

This is how the thermal cycling would look like of the particular embedded system. This is one snapshot of it and what we found out is that from a conventional system, we have almost reduced the total on chip time of thermal cycling by about the one tenth. This is by reducing the ramp up ramp down times and compressing the whole thermal cycle.

This is how the control system would typically look like, you had the temperature output from the thermocouple, which would control a PID in the lab view and this is based on the pulse rate modulation technology, where it would determine how many times you can switch off and on this gate region.

So that, the current between source and drain kind of increases and decreases over a certain period of time and that causes the quick heating or the slow cooling or slow heating or the passive cooling of the particular chip.

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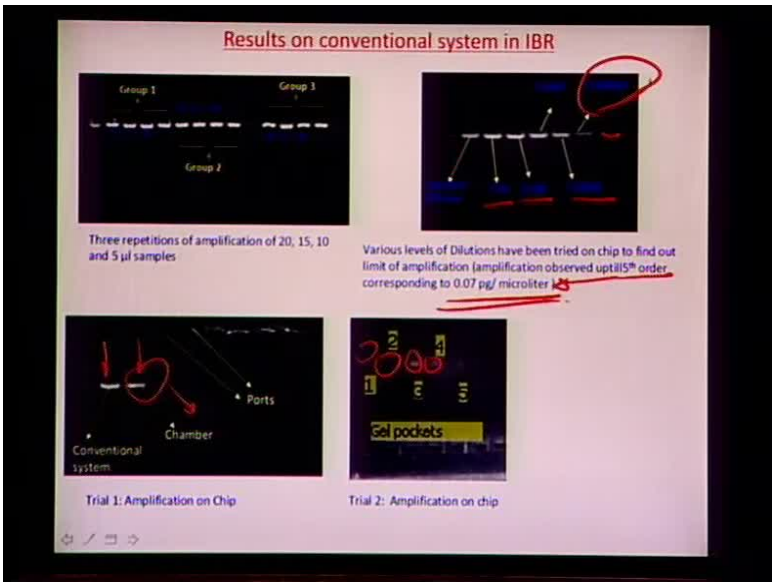

This right here is illustrates what some of the results as you can see these are amplified products, which come as stains in the gel from the conventional machine but in the chip samples, as you can see in the chamber you have a repetitive amplification. As you can see here, this is again another illustration of how the amplification came on a gel plot. So, whatever size we are amplifying on the conventional system is also getting equally amplified with equal intensity in the gel system.

What is also important here is to mention that if you have a PCR product, PCR initial template and the dilute the template one tenth one hundredth and one thousandth you still get a good signals up to one thousandth here and this right is here one is to probably 100000 dilution beyond which the signal would fall down.

If you can actually go to the fifth order would be corresponding to 0.07 Pico grams per microliters, which is actually a pretty low level of amplification that this chip could take.

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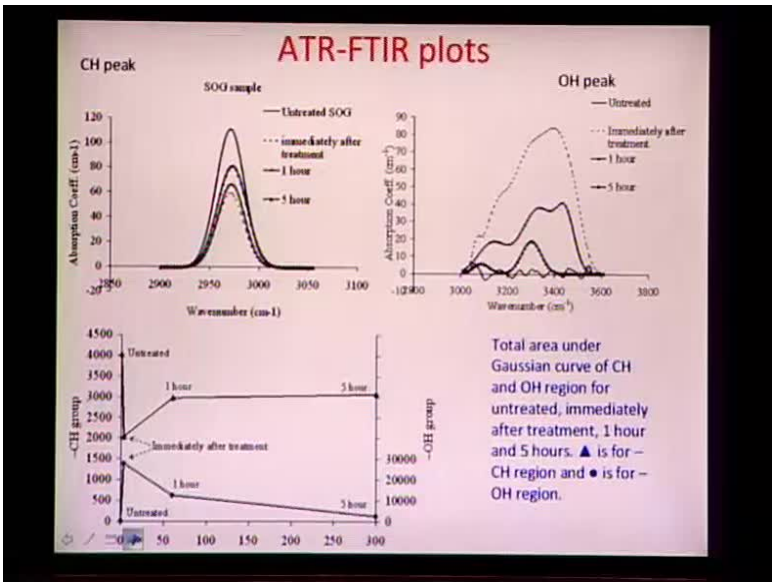

So, the reasons for the chip why it could take this application have already been discussed in the previous lecture. It is because of this gradual mutilation and slow hydroxylation process, which causes a very sturdy non adhering surface to the DNA stains, which causes free DNA available within the solution and that is why the amplification efficiency automatically increases and the lowest template concentration automatically increases.

So, with this we come to the end of this lecture. So, I would like to illustrate a few more research topics probably along with the movie in the next lecture.

Thank you.