Bio - Microelectomechanical Systems

Prof. Shantanu Bhattacharya

Department of Mechanical Engineering

Indian Institute of Technology, Kanpur

Module No. # 01

Lecture No. # 16

Let us get started on this lecture 16 of Bio-microelectromechanical systems, quickly doing a brief review of last lecture. We talked about introduction to DNA structure, described the double helical structure – Watson-Creek model of DNA details, how the base pairs link to one other using different hydrogen bonds and that makes the base pairs quite complimentary.

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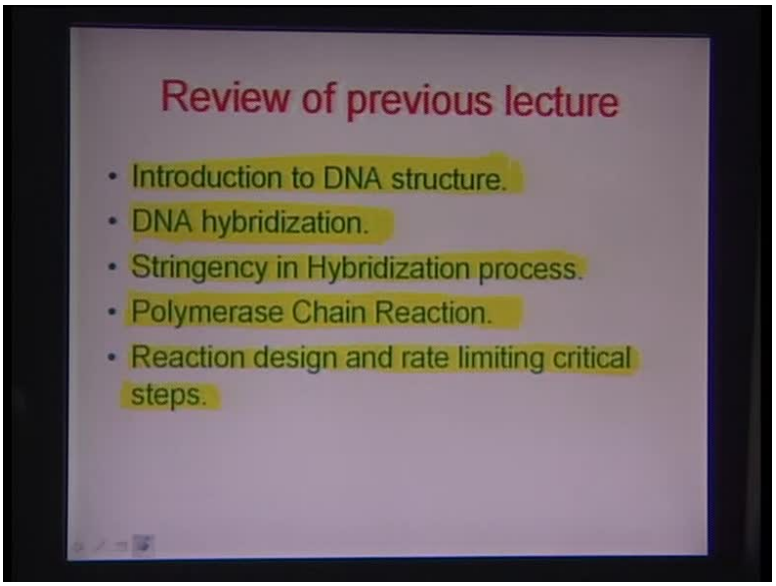

We also talked about aspects related to DNA hybridization and this used as a modality for detection of sequences, rep detection of sequences on the DNA strands. So you have a kind of capture probe and a library of this probe for being able to detect certain sequences which would be potentially on the targets of the samples of interest.

We talked about Stringency in the Hybridization process - how Stringent Hybridizations are reduced; stringency would cause reduction in the specificity of detection. We discuss

this totally new concept of how chemical signal can be amplified by copping a certain sequence in a molecule reaction n numbers of times or we call the Polymerase Chain Reaction. It is one of the breakthrough technologies of this decade, which necessitated the huge or which has essentially been the foundation of the huge molecular diagnostic industry.

We also talked about how to design certain steps of this reaction and way the reaction can be rate-limiting or which step the reaction could be critical to limit the rate of the reaction.

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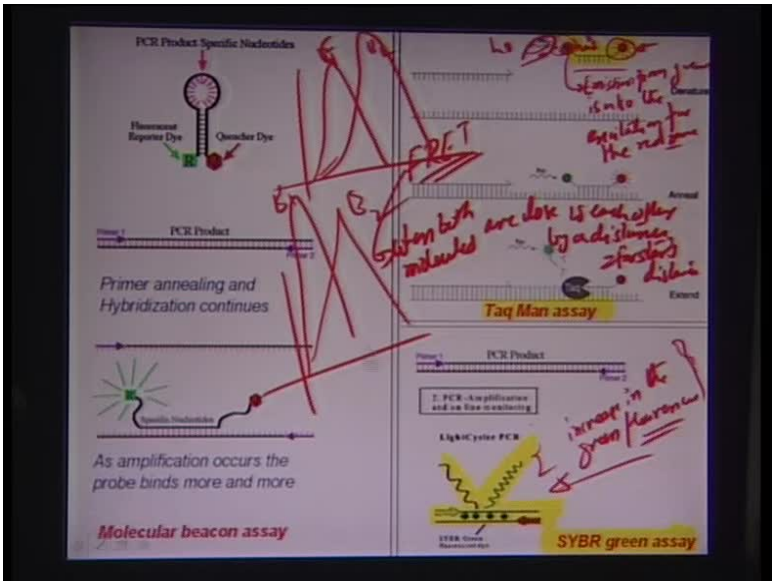

Now today, we will go ahead and do how once the DNA has been obtained. How we really obtain this information about whether it is being copied or the sequences that we are wanted to is, being copied or not. In other words we need a mechanism to find out whether on a real-time basis we can detect snapshot by snapshot of different stages that this reactions as the DNA copies and becomes multiple folds, because it is just molecules and they cannot be just visualized. We need to rely on spectral information and it could be an absorption spectra, it could be a fluorescence-based spectra.

So, typically for detection, we do need something which would be a high signal detection technique. Therefore, fluorescence in most of the cases is preferred for detecting the growing DNA strands along the PCR reaction. This process is also known as the queue PCR or quantitative PCR or the real-time PCR.

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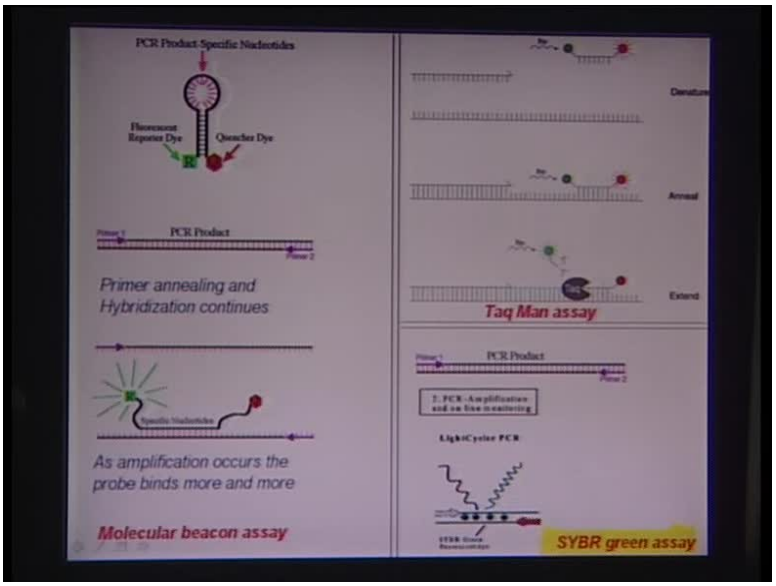

So let us see what happens; how one of the different techniques were we can club the frozen die to the DNA molecule and also see how growth would be indicative of what stage the reaction is in.

The first technique that is very useful is called SYBR green assay. SYBR green is a fluorophore - it is essentially a very high quantum meal fluorophore and these really different for RNA and DNA; the way the bind to RNA may be totally separate then the way SYBR green binds to DNA. These chemicals are available and have to be the really stored in oil based emulsions for a better lifetime or a better quantum yield shelf life of this particular fluorophore dye.

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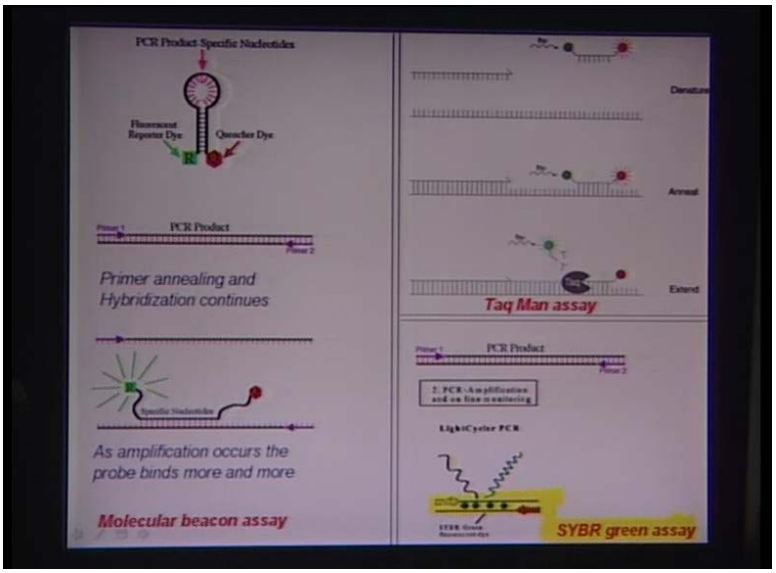

The basic operating principle is very simple; it works on the principle of intercalation. I explained last time, what intercalation really means. So if you have a DNA structure which is more like a cage like form, so you have this helical strands - two helical strands of DNA with the different base pairs and then this fluorophore molecule goes and sits without really covalently bonding into this particular cage like structure. This results in a change in the orbital configuration and makes or attains an overall state where fluorescence is possible.

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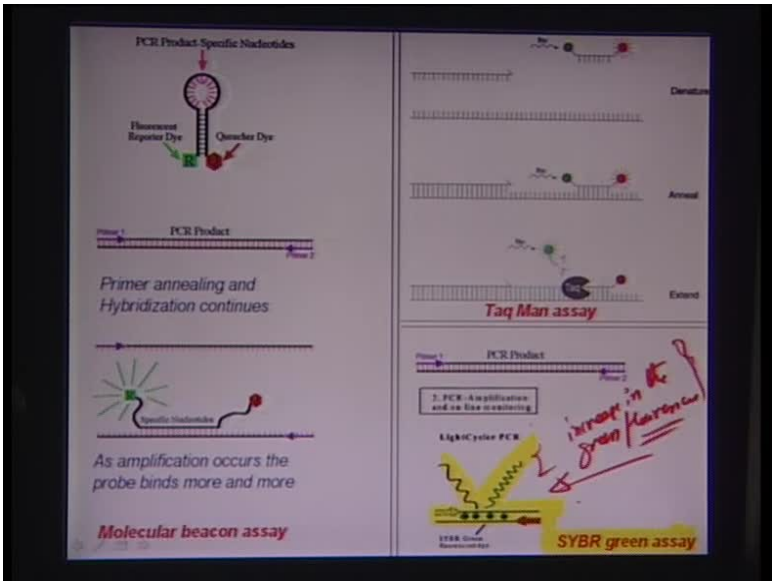

So in that case, there is an excitation frequency of let us say a certain lower wavelength; it results in an emission frequencies in green region for particularly SYBR green. The idea is that these copies of DNA molecules grow from as they grow along the certain reactor along a successful PCR reaction. We should be able together increase in the green fluorescence which makes sense because, more the number of copies are more is the inter collection level of actually available in plenty in the reaction. I should be able to get a snapshot of what is the overall concentration of certain point of time in the reaction.

There is another say which is very commonly used and so there is a binding limitation inside SYBR green assay. Let me just retry this again just by virtue of intercalation that the signal comes out. So, it is almost all are always obvious that irrespective of whatever be the sequence of DNA or irrespective of whatever be the nature of the DNA you should get a signal because the moment it is a double standard structure, a cage like a structure this I can send and start fluorescing.

In a PCR process as we know, there are about 30 40 cycles of different thermal steps. So you have to take this reaction through a step of 95, through a step of again about 50 degrees and then other step of 72 degrees. So due to this rapid thermal cycling that is always a tendency of the junk molecules to go away with time essentially if d natures or t natures all are jumbled up and beyond a certain time its starts really disintegrating because there are no specific end recognition sequences to pick up the junk. However, recognition sequences do pick up the target DNA and amplified many folds; let us say about a billion copies.

The success or failure of SYBR green based detection technique really bases itself from statistically significant number. So if you talking of comparison of billion molecules to molecules of a few 10s or few 100s of molecules, then it does not make any sense to compare because the signal in billion molecule is going to be many folds over the trace amount of junk molecules which are available.

The cleanliness of a PCR process in terms of what you are amplifying is what is remaining back after this rigorous thermal cycling step in combination with a protocol like intercalation might make a difference and may give you a good estimate of the fluorescence growth.

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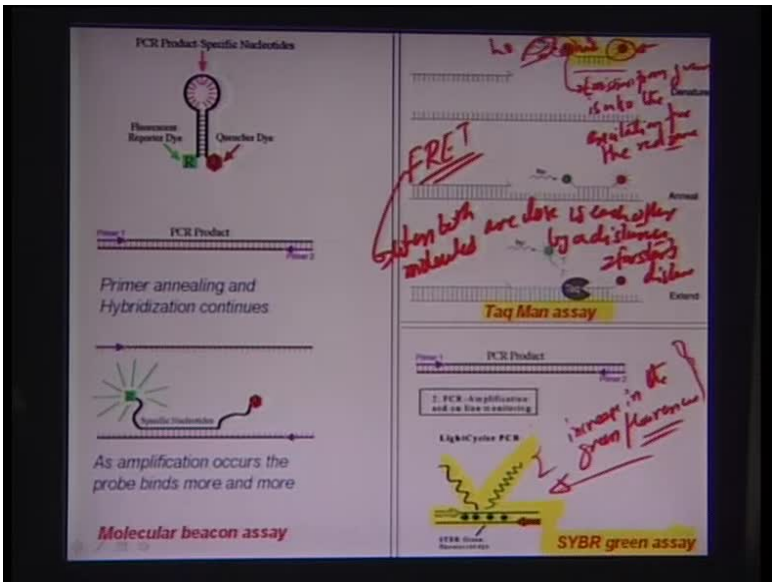

The second very useful technique which is used is and just suffices this condition; this necessary condition of non-specificity developed by techniques like intercalation is the Taq Man assay. Essentially, this is again based on a very simple principle. You design a primer in a manner that you have let us say a fluorophore f 1 and a fluorophore f 2 and this is based on again the principle of fret fluorescence resonance energy transfer.

The way it works is following. When you have this kind of a situation that you have a primer with one end which is having let us say red fluorophore, another which is have in green fluorophore and these 2 fluorophore are designed in a way that the emission from 1 let us say in this case the emission from green 1 is also the excitation for the other in this case, it is for the red 1. So the emission spectra of this particular dye molecule - green dye molecule is same as the excitation spectra of the red molecules. There is an overlap between the emission of the first molecule and the excitation of the second molecule, what you think will happen.

So when there is a wavelength which comes and hits the first molecule and provides h new energy, it starts to get excited and it starts to emit a certain frequency; let us say new one which is also good for exciting this red molecule. So, essentially there is almost always a reduced green fluorescence and an increase the red fluorescence just because of the fact that you have some coupling between the energies of both these molecules. But this happens only even the molecules are placed to certain distance which is equal to or

smaller than a very fundamental physical quantity called the forces distance. So there is a certain amount of distance between of spacing between these 2 molecules which can make it workably execute a fluorescence resonance energy transfer process.

This transfer of the emission signal from one into the exudation spectrum of the other is also known as fluorescence resonance energy transfer perfect. This can only happen when both molecules are close to each other by a distance equal to foster distance. So when they are beyond this, there is no coupling between the 2 molecules essentially and so the emission from one is lost into the ambient. Then there is a sudden decrease in the emission from the other because there is a decoupling action of excitation frequency.

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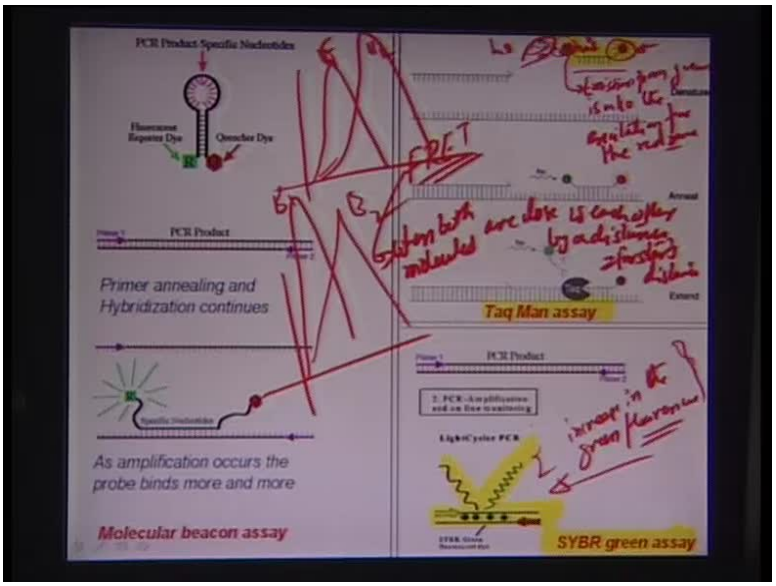

So essentially if you plot such a situation, let us say e 1 here is the emission peak for the first molecule and e 2 is the emission peak for the second molecule. So in case there is a separation of these 2 molecules beyond the foster distance, there is sudden growth in e 1 and sudden decrease in e 2, so that is what happens essentially. As you see here, level is clear all this out so that you can have a good idea; the different steps are indicated in this particular illustration here. You can see here that the primer comes and it kind of starts with a binding over it, separated by distance equal to forster's distance of a green fluorophore and red fluorophore.

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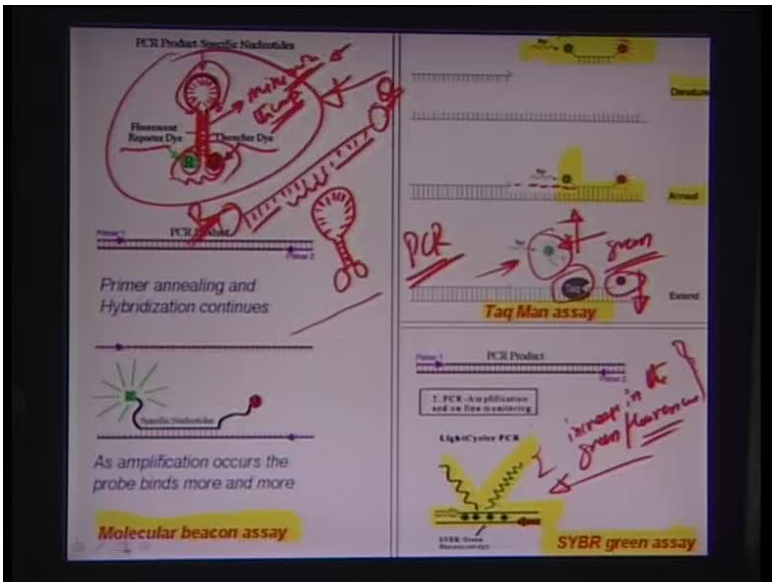

And bring denaturing step the molecules separate, then there is an annealing which happens in the sudden temperature were the primer goes and binds to one flanker, one end, of the DNA and beyond that there is an enzyme which starts cleaning of whatever is there on this particular end. This end grows and starts suturing or extending the DNA molecule in this manner on this fashion, so this is what the stack and designer does so. As it does that it liberates this fluorophore and makes it in a free molecules state in the solution immediately and there is an increase in the green signal because it is gone away from this red molecule; there is dip in the excitation emission of the red molecule where there is an increase in the emission of the green molecule.

So this can give you an illustration of whether the PCR process is going on well and that every step has tact man biting the DNA and creating this effect. This also gives real-time snapshot of PCR reaction. However, the problem in this case is the instrumentation is a lot more complicated because you essentially had find out or the scanned a solution to different emission spectra's and also provide two different excitation spectra's. Then you know the spectra as to be sensitive enough to detect what is the changeover between the two emission spectra as this process happens.

This has to be done at every cycle and has to be a data point in every cycle. There would be a gradual growth in the green fluorescence in the solution with a more number of copies and more number of this genetic termination of these labeled primers on the green

level of the primer as the reaction proceeds and the DNA grows. This assign steps are repeated again and again is a huge increase in the green fluorescence over the red fluoresces and that gives the success of the PCR process. So this is very interesting way of detecting whether reaction is going on successfully.

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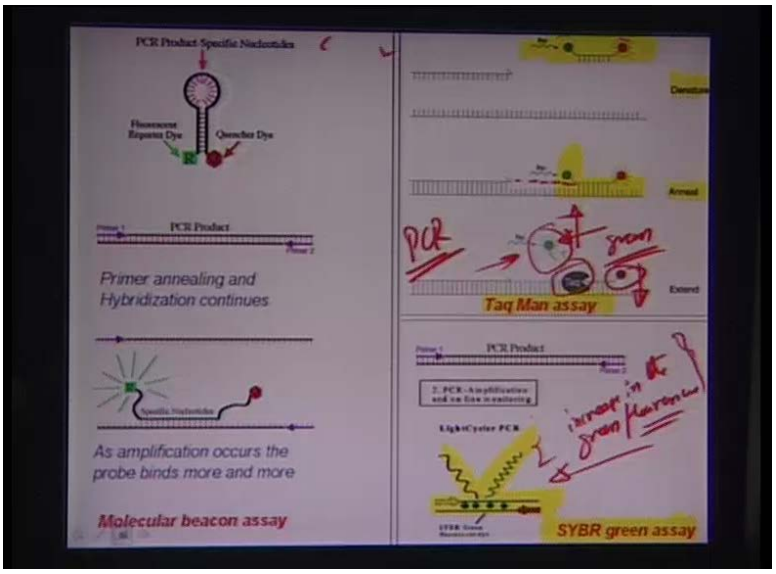

The third very interesting way, again call molecule beacons technology and what I would like to discuss here or share here is that not all PCR assays are available with this molecule beacons. We do have to be lucky in order to get something where we can use a molecule beacon for executing the PCR the sequences essentially what makes a difference.

Here in this particular reaction (Refer Slide Time: 13:46), there is a hairpin loop DNA molecule something like this. So there is a hairpin loop structure and by hairpin loop what I mean is that you have the certain amount of coupling. As you can see here, between the two strands and their certain base pairs with the exact complimentary is a feature that the couple to each other. So these lower ends or lower flanks of the DNA or the single strand DNA does bind to each other whereas, the molecules in a certain portion of the DNA over and above the once which have already bound or compliment are essentially not complimentary and they do not bind to each other.

So what happens is that, there is a development of this loop and this loop is bound essentially by this bounded portion of the single stranded DNA molecule. So you have a

molecule and your essentially folding it in a manner that a certain portion here is not compliment of each other whereas, the ends here are complimentary. So the fold up to form something like hairpin loop structure and we have these bond, the end flanks bonded to each other and these as the unbounded molecules.

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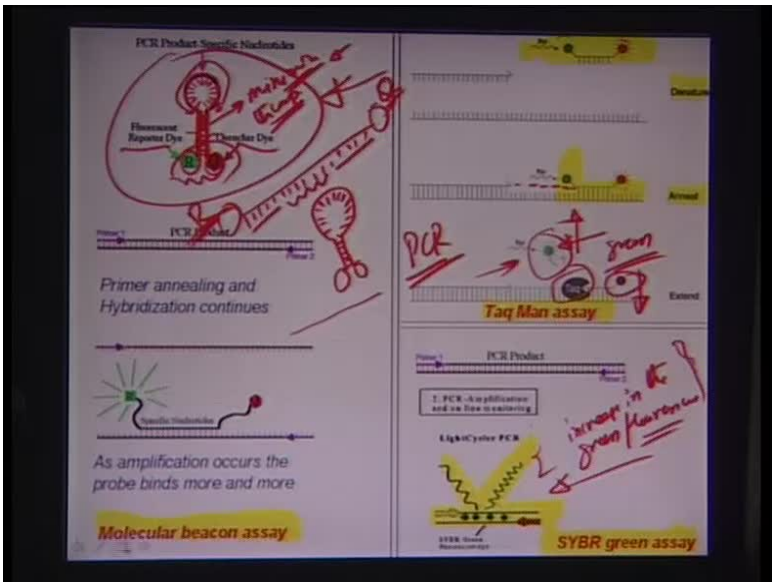

How can we make a difference here? So essentially if this DNA already had a reporter in a quencher molecule pretty much like the fluorescence of fluorophore and quencher. Quencher is something which quenches the fluorophore or it tries to draw the emission spectra of the fluorophore and essentially there is absorption, there is provision of absorption over fluorescence.

So a quencher with a fluorophore would mean that the quantum yield which is generated by the fluorophore is captured by the quencher and quenched essentially. So you have a lower signal, from lower energy signal or intensity signal of the particular emission frequency of the fluorophore or reported dye in this case.

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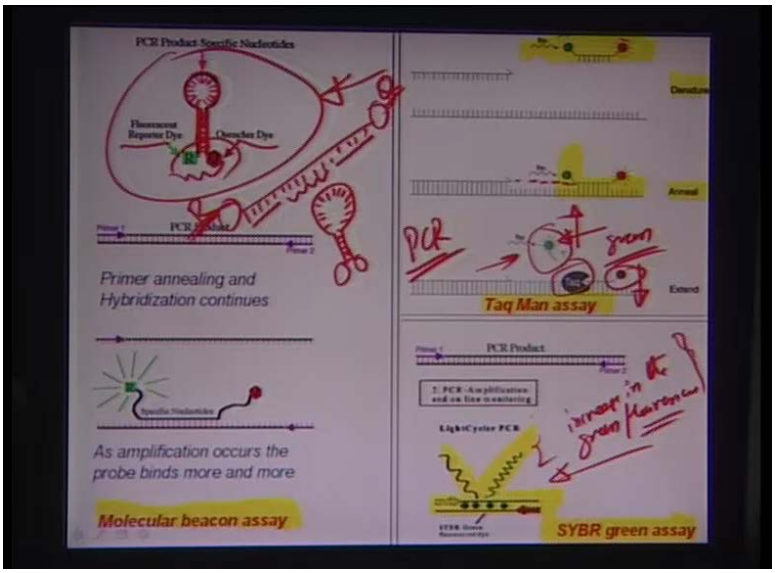

So you chemically immobilize the reporter at one end the quencher, another with an understanding that as the hairpin loop is formulated that is almost a close proximity of the report on the quencher molecule, as you can see here in this particular example. So the hairpin loop is folded and there is a development of these bonds, the reporter on the quencher molecules come close by and therefore, whatever is emitted by the reporter is essentially gathered by the quencher molecule, the overall fluorescence level is low.

Now what happens when it binds or its starts binding; so another important and interesting fact is that we can actually minimize the number of complementary make it just about enough to hold this hairpin loop and if we can do that by just leaving this making it shorter and this is our primer zone for a certain reaction to happen. So we can actually see whether this particular sequence of bases on this hairpin loop essentially of the loop area is complimentary to the end sequences of the target DNA as soon as it binds it kind of opens up. So you are binding this on let us suppose limits are all ball for the interest of the one more clarity.

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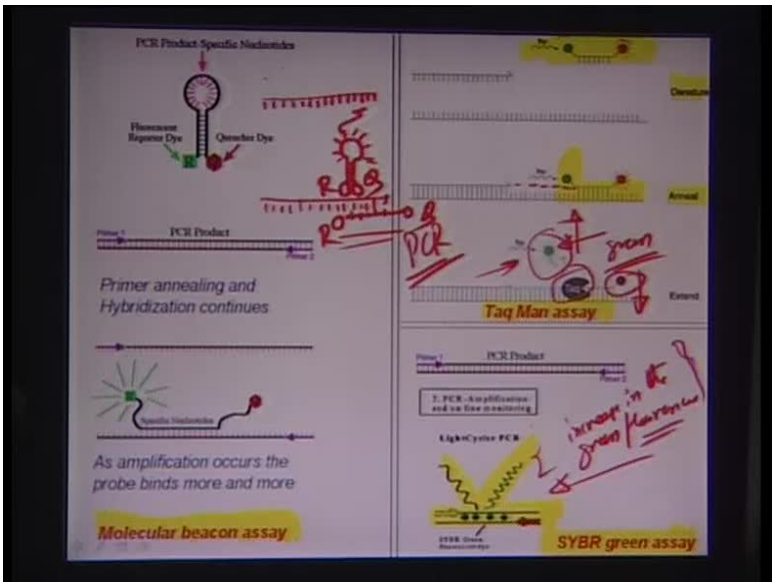

So, suppose we are looking into a denatured DNA and we want to find out how this strand would bind. So let us say we have denatured single standard DNA in the proximity of a hairpin loop like structure with the reporter and a fluorophore.

Let us say we have these dangling sites or bonds. So immediately as it is able to identify its counterpart is going to bond; in the process of bonding it is also going to let us say bonds in this particular area. So the reporter on the quencher is straightened out this hairpin loop is essentially a straightened out into a full-size primer of and in the way the reporter on the quencher molecules are separated by beyond a certain distance and because of the separation there is a growth in the reporter signal because the quencher is now far away from the reporter and essentially that is what would cause would define the success or failure of the PCR process.

So essentially if the molecule Beacon in question the loop part of the hairpin loop actually goes on bonds it kind of opens the loop up. So the small amount of base pairs which are complementary which are holding the pin part would actually split up because the loop part is much more in size. Of course it is much more molecular forces then the few bonds which are making up in part and in that case essentially as it flattens out the reporter and quencher would move way therefore, there would be a sudden growth in the reporter signal on the whole system.

So it can give again a very good real time snapshot of what a step in the particular real time PCR process is in. As more and more ratification would occur the probe binds more and more and of course, there would also be a sort of Taq Man where in whenever the enzyme comes and cleaves away the reporter molecule it goes into the solution and then there is no question of being near the quencher and automatically the fluorescence signal grows with time.

So essentially these are the 4 different techniques of the 4 different assays which are available for gradually monitoring fluorescence and trying to see the real time snapshot of any PCR process.

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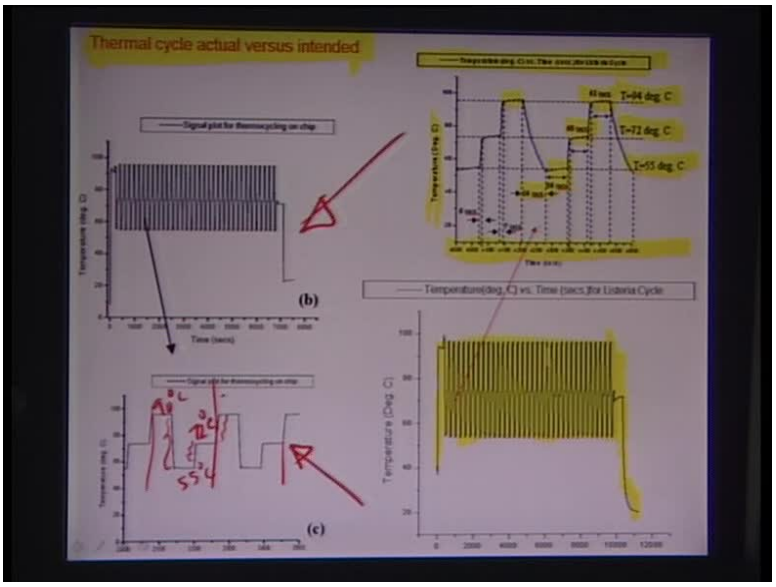

Let us talk a little bit about thermal cycle, a very interesting issue here is the time of the PCR reaction. As we have to rapidly thermally oscillate a certain fluid in its volume almost always there is an issue related to a thermal management or heat transfer across the fluid; these are some of the snapshots here a taken from real time signals on microchip. PCR do show how these thermal cycles can be rapidly carried. So this is essentially the 90 degree step, this is about 55 degrees and is about 72 degree Celsius step. You repeat this every time in the process you have to actually ramp the temperature down in this region and ramped up in these regions.

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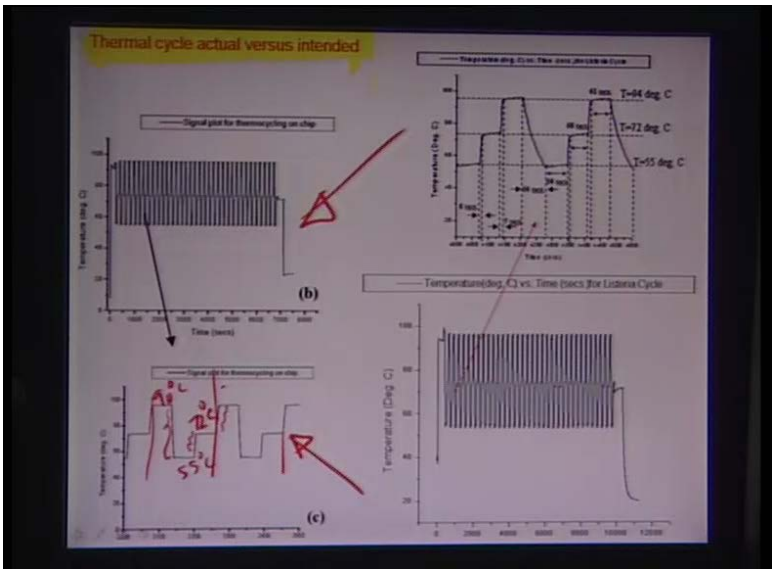

So you have to have a proper thermal management for getting a better proper thermal management for getting a better cycling time, essentially a lesser cycling time. So the ramp up and the ramp down rates are very critical to determine the overall time step that the reaction would need. Of course there is a whole time in the certain step because that is rate limited by other mechanisms like diffusion and enzyme kinetics as we have been talking of before but, then, if we assume that those times of whole steps at different temperatures are primarily fixed; we do have the flexibility and luxury to carry the ramp out, ramp down and ramp up rates so that we can squeeze the cycle more and make it the less time consuming.

So microchip PCR essentially works in that domain where the ramp up and down the temperature while it is changing between two or more temperature states can be essentially squeezed and made almost very rapid there also of techniques which are available. There are papers where it talks about maybe a droplet PCR where there is a smaller micro cantilever of made up of conducting material which is the lead all the way to droplet and there is oil drop on the top. This cantilever is able to high the heat the droplet internally at a very high rate super-fast speed.

As a result of which PCR reaction can be accomplished in as low as about close to 2 minutes whereas, in normal cases when you put these while size reactions inside conventional thermo cycles with huge aluminum blocks, it would take as much as about 4 to 5 hours sometimes. So diagnostics can be made really rapid by taking care of the

volume of interest, volume of the sample to a smaller scale and also by trying to go into a more surface based heating arrangement than a volume based heating arrangements; these are some of the positive impacts that microsystems make particularly in clinical diagnostics using PCR polymerase chain reaction.

So this essentially shows some plots here, which are single plot is acquired by a national instrument lab view package on some real time microchips which we have developed over the past are some years which our lab has developed over the past years for different thermal cycling modalities. As you can see here, the whole steps really are constant and they not very much but, would be to vary ramp up, ramp down rate immensely, tremendously. So essentially this is almost reduced from 5 to about an hour or so and their thermal management can be possible with normal means of intuitive designing, one of them I really mentioned few minutes back; so that is about the thermal cycle.

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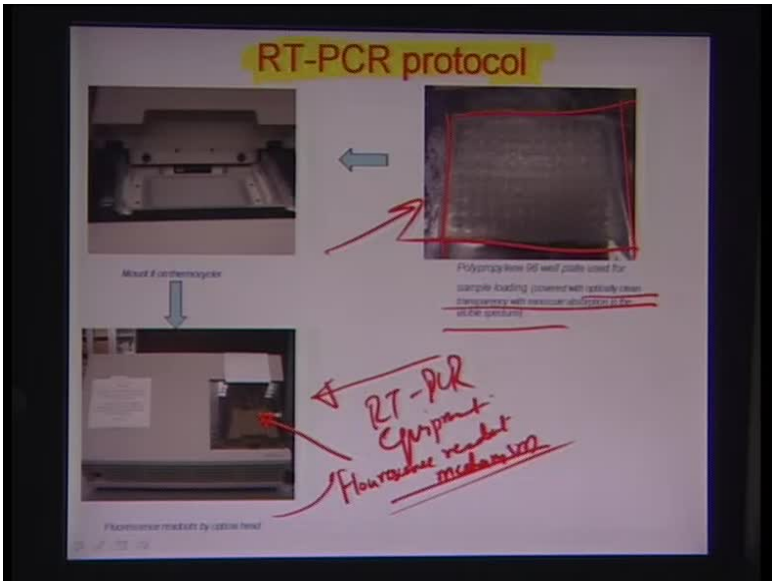

Normally, we do need to read out the fluorescence signal very quickly and for that in especially in the PCR or QPCR reactions we do have a separate reader unit. So essentially there are 2 aspects here; one is there has to be a reader head you can see, this is essentially equipment from strata gene, this is RT-PCR equipment and this right here is also the fluorescence readout mechanism. So what this mechanism is really? It is a set

of detectors which would sit on the top of these wells are about 96 wells in which 96 different reactions can be carried out in a single go.

So these are wells and they are covered by using an optically clean transparency miniscule absorption. When you are talking about an optical detection scheme is almost always necessary to certain that whatever emission spectrum is generated by the particular dye should not be absorbed by materials which come in the way of the light or an optical path.

So one of the things which do come and which is important is a cover. The cover is more important because you have to prevent a thermal evaporation of the PCR fluid and when you are talking about a small vial containing let us say 20 to 25 micro liters of the PCR solution, you are heating it in a close confined environment to aside about 93 to 95 degree Celsius; there is almost a possibility there if leave it open it will evaporate.

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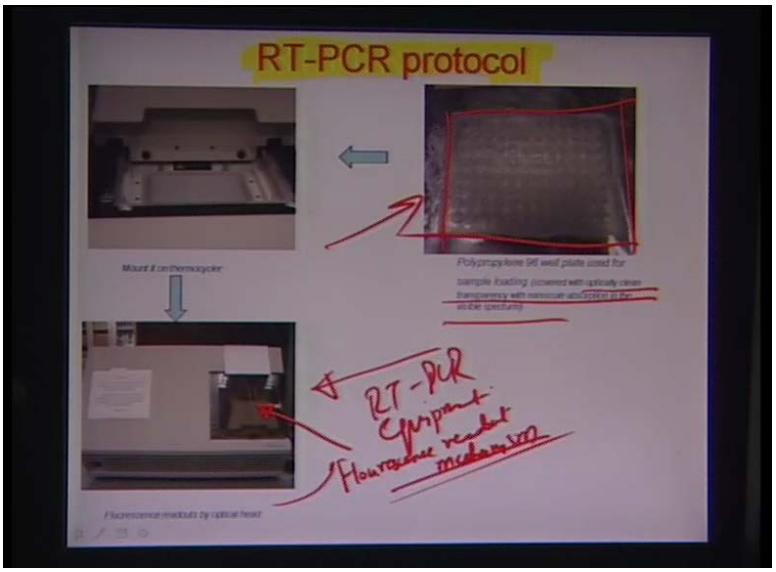

So you do have to cover it totally with some kind of polypropylene sheet or let us say cellophane paper where it can give a leak proof, seal proof joint and then you can actually press the head and they do give a lot of pressure over these particular covered vials; at the same time a head can be used for optical readouts. So there then over every well there is actually a detector and there is an array of detectors and this director sits on the top of these is a small vials containing each well over which there is the cellophane paper and due to which whatever is happening in each well is independently reported.

This detectors are having electronic channels from 1 to 96 which record independently some of the data partially, these channels can be just the reference without the sample to manic of the background impartially they can actually be containing the sample.

So you have a growth in fluorescence and then you have a comparison between the reference and signal and the way you can actually evaluate a lot about close to do at least about 60 to 70 samples, may be even a little better about 80 samples making few of them as a references just for proper signal analysis module. You can actually get to the real time snapshots of about 80 reactions happening all at a single go, so it is essentially a very high throughput process but, again because of the optics that is involved the instrumentation which is the provided is pretty expensive and it is prone to getting damaged easily.

Therefore the trend now is slowly shifting from these detectors into optical fibers. A bunch of optical fibers can do the same job even if left inside the solution and therefore, there is a trend of making arrays of bundles, this fibers bundles for picking up signals from these different multiple wells and there are advance more machines which are coming up now more with this the fiber technology.

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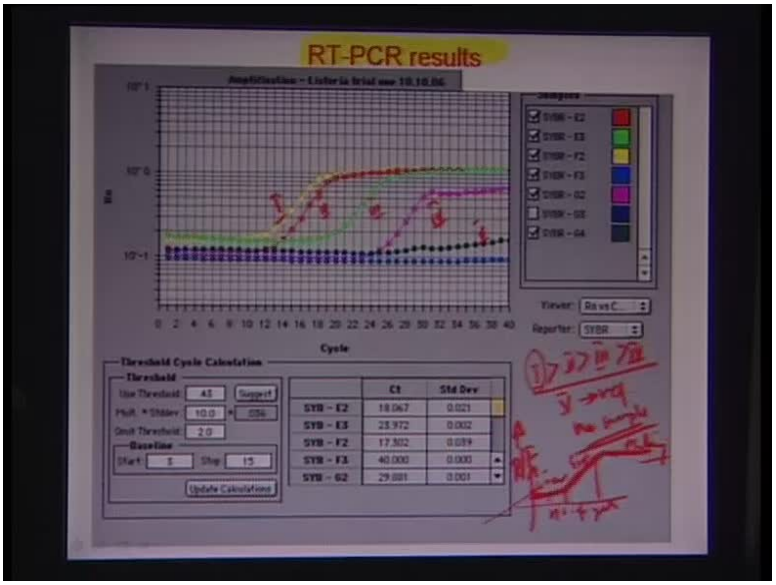

So these are some real time PCR results as obtained from so this here illustrates so the QPCR or RT PCR data as you can see here these really correspond to different concentrations, initial concentrations of the template. These are let us say sample 1, 2, 3,

4 and 5 containing increased order of concentration of the initial template. So this is how initial template is different in different for the first one has the highest template concentration in the fourth one has least the fifth is essentially the reference, so this is no sample; so there is no DNA to start with a there is just the other compliments in this reaction nothing get amplified and nothing increases the fluorescences.

Another interesting factor is the trend, as you are seeing essentially linear the exponential and plateauing. There are 3 trends here: the linear, exponential and the plateauing. Why this happens is again something that I think I have spoken back in my previous lecture. This is number of cycles and this is let us say the relative fluorescence RF, the growth in that so happens here is that in this region there is very less primer or enzyme other molecules available because this is diffusion limited regime diffusion is not fully taken place. So getting micro on this would mean that the number of cycles would really be compressed more towards left and the exponential is really the area where you have almost all diffusion complete molecules are always of almost all around the reaction and we have started now to copy a replicated a very fast rate after you reach a certain cycle. Here is a plateauing phase which means now one of more of the reactant or constituents are depleting and the because of the concentration, depletion there is a gradual reduction in the growth rate of the fluorescence.

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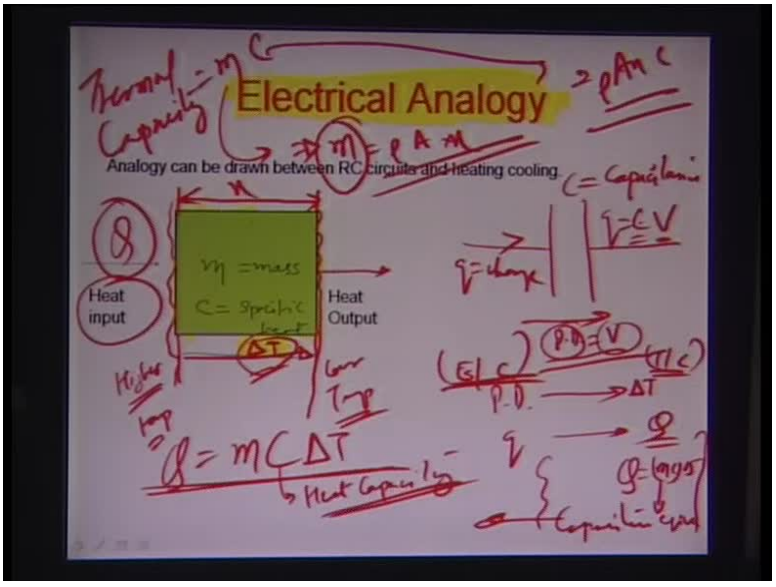

So that is why this particular trend is visible or it comes so. One interesting factor which comes into picture while designing a PCR chips is essentially how you will try to characterize the chips thermally and what you need to do to certain, how much time it would take for the chip to cycle to a certain temperature.

So let us actually draw some kind of an electrical analog for these kind of problems. We consider any thermal system to be a bunch of resistances and capacitances and circuits. So let us draw analogy one by one; so if you look at the slide here really another there is let say mass which are heating up, this right here is a mass and the masses detected represented by the term m and specific heat of the material is c, we assume a temperature difference delta t between both ends of this mass. So delta t is temperature difference, this may be on a high side of temperature and obviously because of the side of the higher side of temperature there should be a heat flow from one end to another. So there is a heat input across this ends, so this is the surface with a higher temperature and there is a heat output here from this particular surface. So this is the surface whether lower temperature over the differences about delta t.

So if we assume that Q is the heat flow, not the heat flow rate just the amount of heat that is slow inside the system, so Q also can be represented as mc dealt t. So this kind of looks familiar or its rhymes with something like capacity right this is called heat capacity.

So essentially this is the ability to store the ability of a material, to store heat the capacity of a material or the ability of the material to store heat and equal in the electric circuit is capacitances but, there it is the capacity or the ability of a system to store charge instead of the heat. So Q in the thermal system essentially an equivalent of charge in the electrical system and it is very well founded that there should be analog terms for V - the voltage in an electrical system, to the temperature in the thermal system and the also the capacitance in an electrical as opposed to the thermal system.

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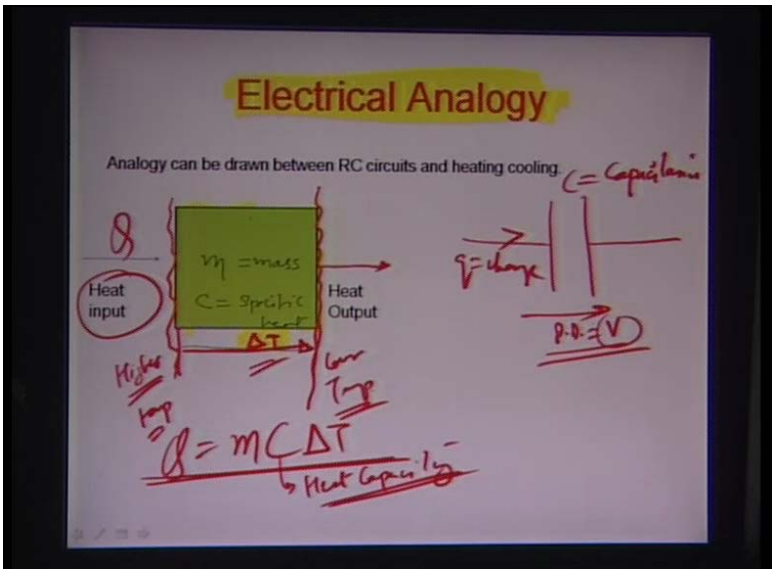

So let us draw the capacitor. This is the capacitor; let us say you have capacitance value equal to c, you have a charge small q which is flowing through this capacitor and essentially there is a potential difference of v here. So what causes the charge really to flows the potential difference here; what causes the heat to here flow is temperature difference here, so it is kind of analogues.

So potential difference in an electrical circuit is same as temperature difference in a thermal circuit. The quantity an electrical circuit which flows the charge q as opposed to the quantity in thermal circuit which is the heat q and therefore, as we know here q is equal to c into v, the capacitance into voltage and therefore, the thermal circuit q is m c delta t where m c is the capacitance equivalent of thermal circuit.

So, thermal capacity of such a circuit is also mass times of the specific heat capacity of the material. To explore further, mass can also be written as the density times of area, times of the depth. In this case, let us actually forget about depth at this time the area times of the length here and assuming that this length here is about x. So its area time of x, that is what the masses and so thermal capacity here should be represented as the rho ax times of c there is what or how you can represent the thermal capacitance.

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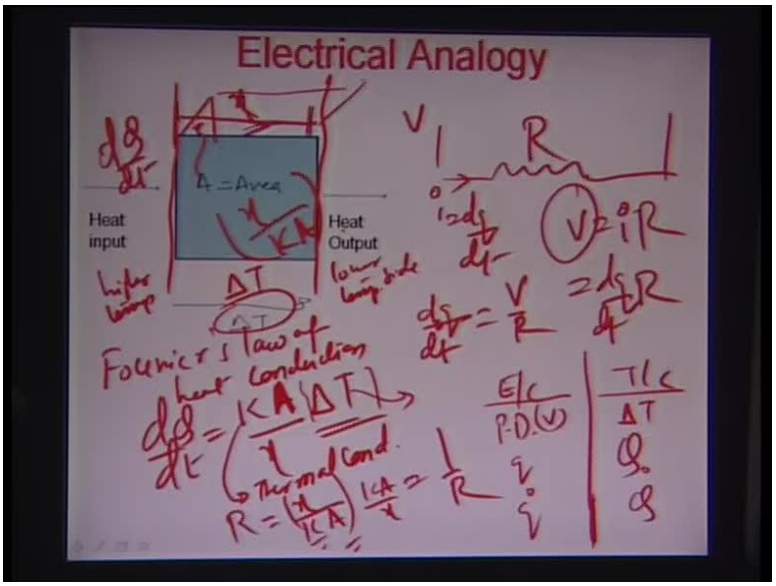

Let us also look at is there is something analogous to resistance in an electrical circuit as in a thermal circuit. So for doing that we need to assume the rate of flowed of charge. So basically in case of the equivalent of a resistance what we can do is we have to consider the rate of flow of charge. So if we consider the equivalent model that we drew earlier between the electrical circuit and the thermal circuit the potential difference an electrical circuit was corresponding to the delta T or the temperature in the thermal circuit and the charge Q in an electrical circuit was equivalent to the heat actually in the thermal circuit.

So here by the Fourier's law of heat conduction, if we really look at the heat transfer between these two face here and here are the dQ by dt in the rate of flow of heat is essentially represented by the KA by x times of delta t; the k here is the thermal conductivity the material. Of course, the area is the cross section perpendicular to the direction of the heat flow; so if this were assume to be like a cube, the area a is really the area of this particular face. The x is essentially the distance between the two flanks of this particular mass and delta t v in the temperature difference across it. Therefore, this is certainly the higher heat or higher temperature side, this is the lower temperature side and delta t essentially is nothing but, the difference between on two temperatures.

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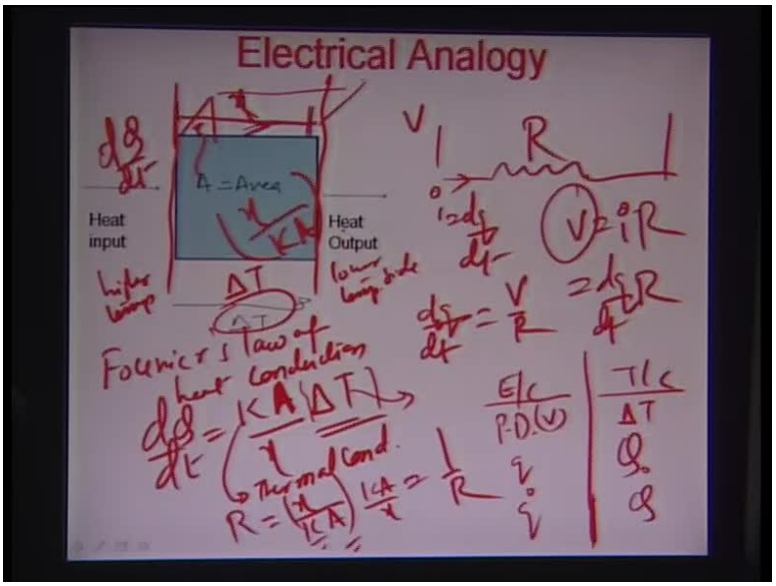

So if we put circuit equivalent, this can be more analogous to a resistance that you have a current across it which is also the rate of flow of charge. So essentially q dot in the electrical circuit it is essentially equivalent to on the heat flow rate q dot and dQ by dt on the thermal circuit as we know here, the potential difference being V can be equated to i times of R there is dq by dt times of R. So since the potential difference also equivalent to our dt here, therefore, dq by dt or i is V by R.

So delta t is the equivalent of V and essentially k by x is essentially same as 1 by R or R is the thermal resistance in this cases, x by K times K is a thermal conductivity, A is the area of cross section perpendicular to the direction of the heat flow and x is the length across which the heat flows causing temperature difference of delta t across both its surfaces.

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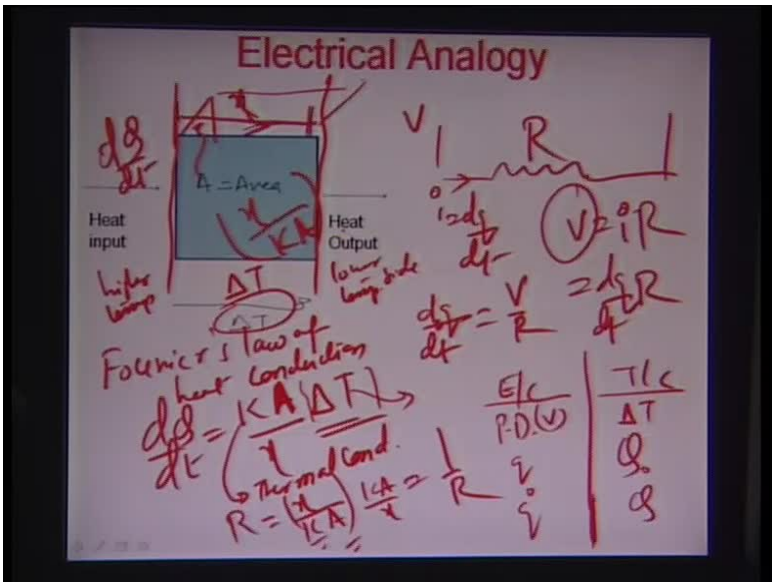

So the resistance equivalent, thermal resistance equivalent, in this particular thermal circuit is x by K times of A. So that in a sense the complete electrical analogy of what thermal resistance or thermal capacitance would be, in fact there is an approach called bond graph modeling; where these kind of concepts are very often used for kind of developing an analogy between electrical side and the various other physical phenomena it may be heat transfer, may be you related to optical phenomena and there is an analogy developed.

So since we have a resistance of the capacitance here we can always draw an RC circuit see that with a potential difference across RC circuit or assuming that you know you have a temperature difference in a thermal circuit and kind of series or parallel combination of the thermal resistance of the thermal capacitance, can we really develop something called the time constant?

Once you develop that, the other question would be how relevant it is? What is the physical meaning of the time constant? And can we get some influence about the systems thermal behavior - the lab or lead or the thermal response based on what this time constant is.

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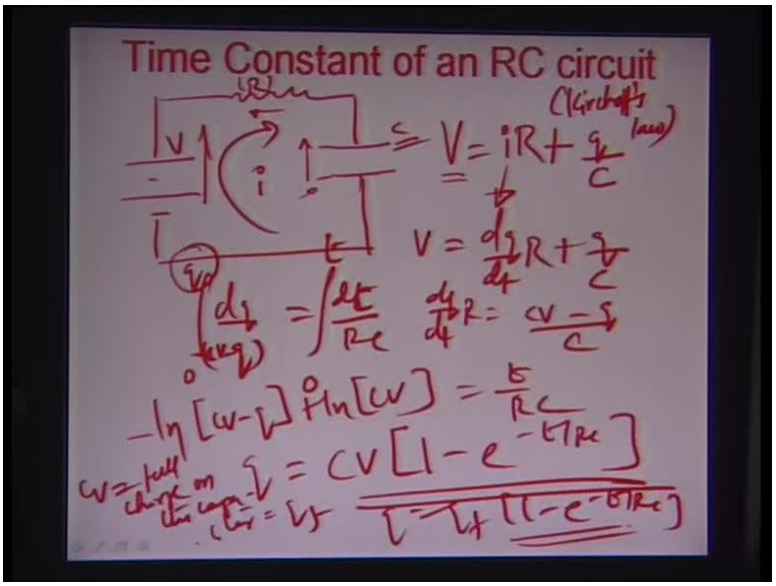

So let us look at preparing univalent RC circuit and trying to see what the time constant of a RC circuit would be. Let us suppose we have this resistance capacitance circuit connected to the potential difference and this value is a resistance value, this is the capacitance value and this completely closed the circuit. The capacitance c, the resistance here R and we assume further that subject to availability of the potential V we have a current of the circuit i which is generated because of the potentially. So V essentially is also equal to by Kirchhoff's law, iR plus or the other voltage across the capacitance which is actually Q the charged and let actually right small q here in the interest of clarity divided by the capacitance C.

So that is what the voltage drop is across the capacitor and there is a drop across the resistor and together the drops are also the same as the voltage which is provided in the interest of conservation of energy, this is Kirchhoff's law. If we remember, we apply this law in one of the electric kinetic pumping design modules for doing circuit calculations and try to find out the iron current as well as the fluids.

So let us for this equation, we essentially again is written as the dq by dt, R plus q by c current as we know the rate of flow charge dq by dt. So if we rearrange this equation we are left with dq by q and we assume varies between 0 charged in the capacitance of the very outset to borrow full grown charge of let us say a small q and this essentially is sorry this is the cv minus q, so dq by dt are is cv minus q by c.

This essentially is equal to the integral of the dt by RC, dt being where between 0 and t; t is a full time over which the charge of the capacitor grows from 0 to q. So if we solve this equation, we are left with minus ln CV minus q plus ln CV; so essentially t by RC in other words q here and the charge CV times of 1 minus e to the power of minus t by RC.

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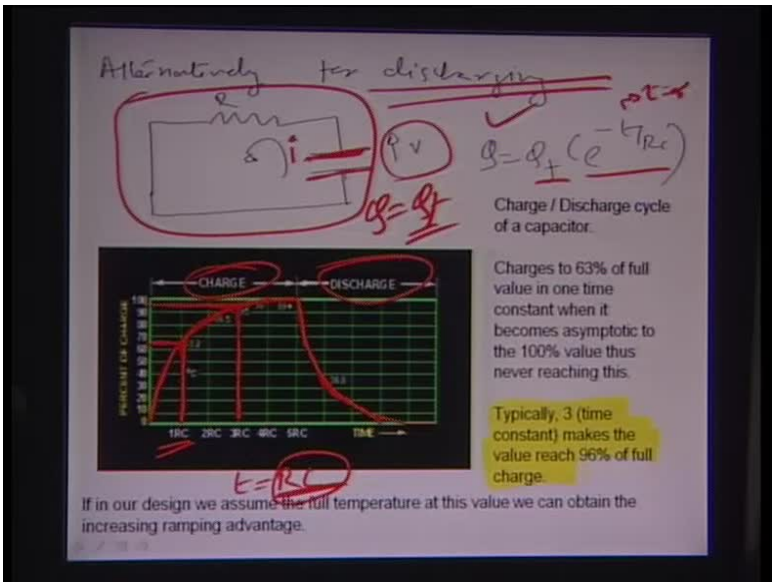

So CV also is a representative of the full charge on the capacitor. It is called qf, so q is qf times of 1 minus e to the power of minus q by RC. Automatically for the discharging circuit when we assume that there is no voltage source as you can see here and this particular illustration of the voltage of the capacitor which has been developed over its state is going to feed and the circuit of the current i and drop across the resistor. So in that case, one of the discharging time for the discharging process and Q become Qf e to the power of minus e by RC, so a time equal to infinity the Q essentially becomes actually 0, because this is minus.

So 1 by e to the power of infinity is nothing but, 0, alternately time t equal to 0 the Q is actually grown to its full value Q as just before the start of the discharging process. So very interesting the aspect about these two charging and discharging cycle times are there if you plot the percentage of charge with respect to the time as you are seeing this illustration here on this is really the growth cycle. This is essentially is the way that the charging happens in the circuit following expression Q equal to Qf 1 minus e to the

power minus t by RC and this is the exponential decay in the charge Q is equal to Qf e to the power of minus t by RC.

So this is the charging cycle; on this part of the discharging cycle also what is interesting here to know is that if you have a time constant the t of this RC this corresponds to about 63 percent of the total charging. If you have one time constant RC resistance into capacitance you have about 63 percentage of the charging and it takes about three time constants for the charging to be above 95 percent. So essentially we are looking at 3 time constant for the capacitor to charge to almost 96 percent of its full charge.

This is an interesting proposition because if we want to apply this to other pc or microchips we may as well develop a strategy of trying to figure out, how much time it would take for the ramp up, ramp down mechanism of the particular chip.

If I actually make an electrical equipment and an electrical analogue in terms of the thermal capacitance, thermal resistance and thermal potential difference which is also the temperature I would be able to make the electrical analogy and trying to see that what kind of time would be needed for our thermal circuit to go to a certain level. Let us say about the 96 percent of the full thermal load the cycle can take at a particular temperature. So in that if we designed assuming the full temperature at this value, we can obtain the full advantage of increasing ramping rate.

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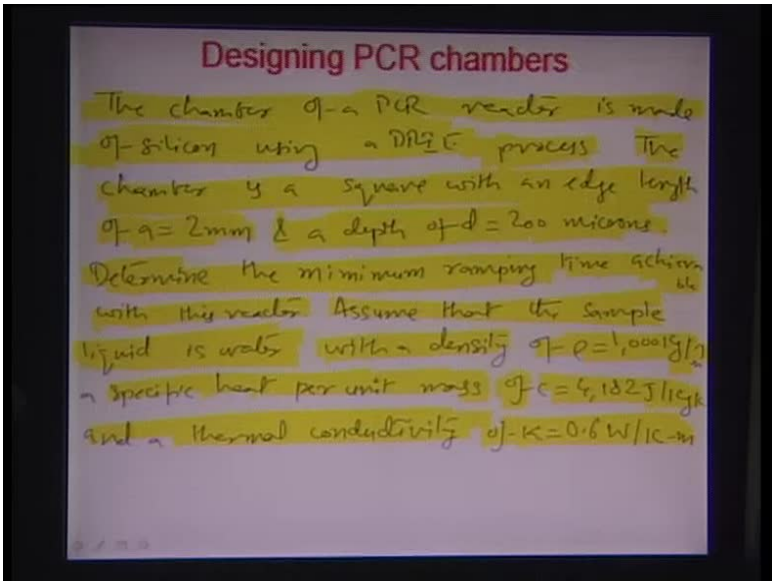

Now let us kind of tried to illustrate that little more with an example. In this particular case as you seeing here, there is a chamber of PCR reactor which is made of silicon using a deep reactive ion etching process. So the chamber is essentially a high aspect ratio chamber used or etched away to using gas plasma the deep reactive ion etching.

The chamber is a square size with an edge length of about 2 millimeters and there is a depth of d equal to 200 microns, this particular chambers. Therefore you have a square of 2 mm into 2 mm, a depth of about 200 microns; so the chamber is essentially pretty much mean a high value on the permanent aspect and a really micro in the death aspect of this is how normally micro architectures are designed. Another advantage why this almost 1000 times increase in the dimensions are in the linear of the x y dimensions of the chamber because the idea is to actually spread volume of fluid to over a thin surface area. So essentially you are taking a bulk volume of the fluid to the idea really is to kind of spread over thin film from bulk, so it can increase the heat transfer.

In this example you determine the minimum ramping time which is achievable and with this particular reactor design and you have assume to the sample liquid is water. Most of the PCR fluid is actually an aqueous based fluid with a little bit of ionic content here and there the densities around 1000 kg is permitted, q as is the normal case of water is a specific heat per area operant mass off 4182 joules per kg degree kelvin and there is a thermal conductivity associated of K equal to 0.6 watch per kelvin meter and so you have essentially design the chamber or design this in a manner that you can determine minimum ramping time which is achievable by this system.

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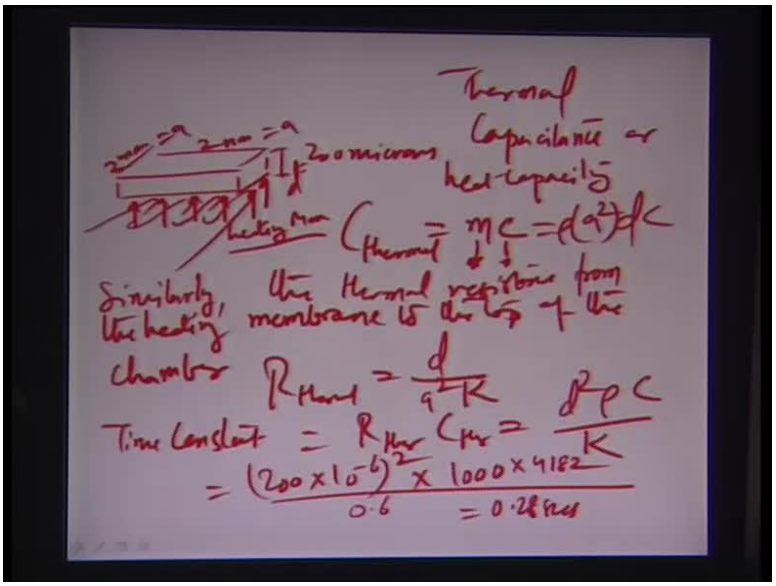

Let us use the theory that we have just formulated in terms of electrical analogy. So essentially we need to find out the thermal capacitance of the resistance in this case. You have first, let us draw the chamber you have a case where you have a 2 mm by 2 mm chamber and essentially the depth of this chamber is about close to 200 microns and assume a heat transfer from bottom to top. So there is set of heaters, let us say a set of micro heaters at the bottom of this chamber and it is actually feeding this particular chamber.

So in this case the thermal capacitance, heat capacity it is a C thermal essentially m times of C, m is the mass of this volume and C is the heat capacity of the material. So essentially it is again rho times of area of cross section which a square of a times of the depth; in this case the depth being from the heating side to at the open of the atmospheric side is d. So we assume this to be a and this to be d, so you have rho square, rho a square d is total mass and then of course, you have the specific heat C. Similarly, if you determine thermal resistance and that is from the heating membrane to the top of the chamber; so this is the heating membrane and heaters and this is the top of the chamber so from the heating membrane to the top of the chamber (Refer Slide Time: 52:25).

So let us call it R thermal, so this case it would be actually x by k a. So x essentially is d here, if you just remember the electrical analogy that analogy we just dropped or which is true about the slide back. The area cross section a square, so we have d divided by a

square k and essentially the time constant in this case which is R thermal, C thermal – it is nothing but, d square rho C by k if you assume d of 200 microns 200 10 to the power minus 6 square times of density of water which is about 1000 kgs per meter cube times of specific heat capacity which is about again 4182 joule per kg per kelvin divided by the k value and thermal conductivity which is 0.6 watts per kelvin meter.

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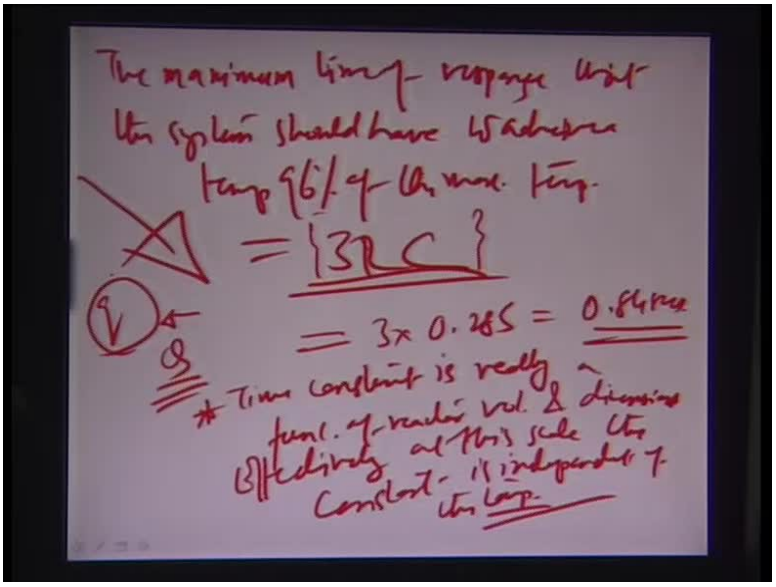

We obtain time constant of about 0.28 seconds, so essentially the maximum time of response that the system should have to achieve a temperature 96 percent of the maximum temperature which in this cases probably 90 degrees and or 94 degrees should be 3 RC as we saw in the RC example that q which was so essentially the electrical equivalent of the temperature, sorry, the heat, the q which was essentially an electrical equivalent of on the heat dq in a case or capital Q in our case was about 63 percent of the full charge. Once the time is reached one time constant and was about 96 percent of the full charge as it reached about 3 times RC.

The maximum time that you can allow for the system to ramp up to this particular 96 percent of maximum temperature in our cases about 3 times of 0.28 seconds is about 0.84 seconds. So take on message really a is that the time constant, is really function of reactor volume and so if you can make the volume smaller and smaller you can make the time constant smaller and smaller and the volume in turn depends on the dimensions.

So, effectively at this scale, the constant is independent of the temperature. So that is exactly what we have to consider for designing microsystems. So would like to finish this lecture just by you know trying to just reiterate that at times we need to probably draw these kind of analogy to get a quick solution of what would be the maximum time that we can allow for system to go to certain temperature state by going to ramp up cycle. So, in the next lecture will take onto a little more details of how do we really design the volume based on such time constants and then look into some other aspects of how the DNA polymerases works DNA molecules and tries to extend zipped up or the zipped down molecules make copies inside the human body.

We also like to kind of review of basic little kinetic technique electro freezes which is also used for finding out length of a DNA base pair and is critical to diagnostics industry so would like to close with this note. Thank you.