BioMEMS and Microfluidics
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Lecture - 16

Let us get started on this lecture 16 of Bio Micro electromechanical systems, quickly doing a brief review of the last lecture.

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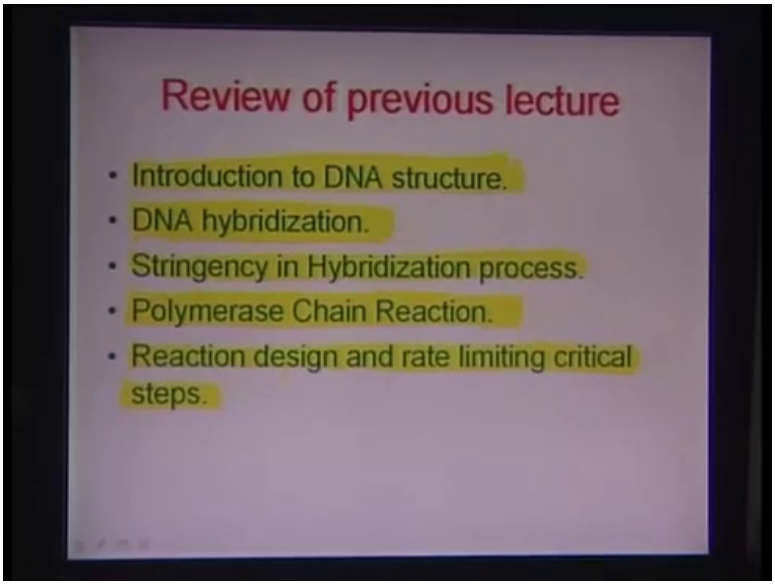

So, we talked about introduction to DNA structure described the double helical structure the Watson-Crick model of DNA details, how the base pairs are link to one other using different hydrogen bonds and that makes a the base phase quite complimentary. We also talked about aspect related to DNA hybridization and this used as a modality for detection of sequences rapid deduction sequences on the DNA stands.

So, you have to have a kind of capture probe on a library of this probes for able to detect a certain sequences, which could be potentially on the targets are the samples of interest. We talk about stringency in the hybridization process, how stringent hybridization are reduce stringency would cause reduction in the specificity of detection in the discuss the new totally new concept of how chemical signal can be amplified by copying a certain sequence.

In a molecular reaction n number of times using are we call the polymerase chain reaction it is one of the break technology of this d k, which has necessitated the huge are, which as 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 where the reaction can be rate

limiting are which that the reaction could be critical to limit in the rate of the reaction.

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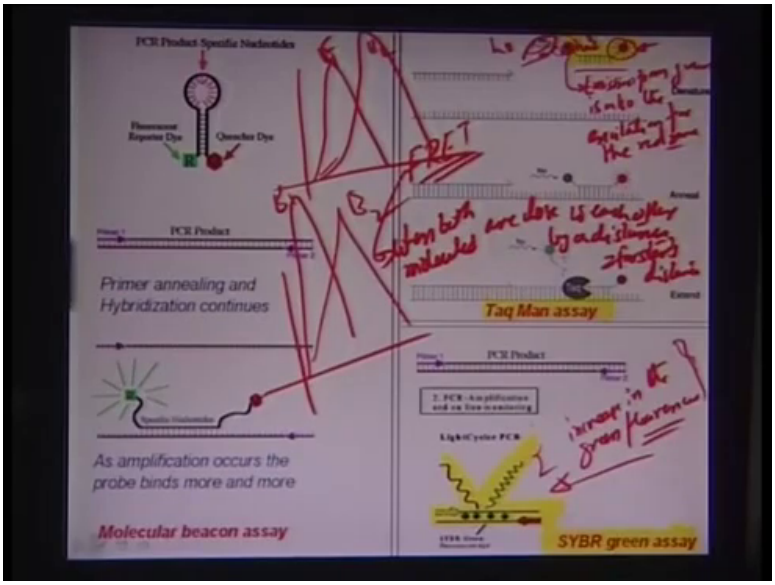

Now, today we will go ahead and do how once the DNA has been obtained how we really obtain this information about whether it is being copied are the sequences that we wanting to is being copied or not. Another words we need a mechanism to find out whether we can on a real time bases detects snap shot by snap shot, how different stages that this reaction is in as the DNA copies and becomes multiple force. So, because it is just molecules and they cannot, which as visualized we need to rely once a spectral information it could be an absorption spectra could be a florescence based spectra.

So, typically for detection we do need something which would be a high signal you know detection technique. And therefore, florescence in most of the cases is preferred for detecting the growing Indian stands along the PCR reaction of this process is also known as the q PCR quantitative PCR at the real time PCR. So, let us see what happens how what are the techniques, different techniques, where we can club the florescence died to the DNA molecule and also see how growth would be indicative of what stage the reaction is in.

So, the first technique that is very useful is called is SYBER green assay (a fluorophore) flora four it is essentially a very high quantum in fluorophore and this really different for RNA and DNA the way the bind to RNA maybe totally separate then, the way SYBER green ((Refer Time: 03:14)) binds to DNA this chemicals are available and have to be really stored in oil based emulsions for a better life time of a better quantum yield or self-life of this particular fluorophore die.

The basic operating principle is very simple it works in the principle of intercalation I explain the last time, what intercalation really means. So, if you have a DNA structure, which is more like cage like form. So, you have a this helical strange to helical strange of DNA with different base payers and then, this flourophore molecule goes and sits without really equivalent bonding in to this particular cage like structure and this result in a change in the orbital configuration and makes or attains an overall state we are florescence is possible.

So, if in that case there is an excitation frequency of let say certain lower wavelength it cells in emission frequency the green region for particularly Syber green. And the idea is that is copies of DNA molecules grow and as the grow along the certainly react along a successful PCR reaction we should be able to get a increase in the green florescence, which makes sense, because more the number of copies are more is the intercalation level of the lies actually available in plenty and the reaction I should be able to get a snap shot of a, what is the overall concentration at cell point of time in the reaction.

There is another say, which is very commonly used and, so there is a binding limitation in the SYBER green assay and let me just retrate this again that this is just by virtual of intercalation that this signal comes out. So, it is almost always obvious that irrespective of whatever be the sequence of the DNA or respective of whatever the nature of the DNA you should still get a signal. Because, the moment it is a double standard structure cage like structure this right get sense and start florescence.

Now, you know PCR process as we know that there are about 30, 40 cycle of different thermal steps. So, you have to take this reaction through a step of 95 through a step of again you know above 50 degrees, then other step of 72 degrees. So, due to this rapid thermal cycling there is always it tendency of the junk molecules to go away with time essentially if denatures are renatures at all is jumbled up and beyond a certain time it is starts really this in be create in, because there are no specific and the recognition sequences to pick up the junk.

However, then recognition sequences do pick up the target DNA and amplified many folds, let say about a billion copies. And the success are failure the syber green based detection technique really bases itself and the statically significant number. So, if you are talking of a comparison of billion molecules to molecules of a few tens if you 100 of molecules. Then it does not make any sense to compare, because the signal in case of be length molecule is going to be many, many foals over the trace some amount of junk molecules which are available.

So, the cleanliness of a PCR of processes in terms of what you are amplifying are what is remaining back after this rigorous thermo cycling step in combination with a protocol like intercalation might make a difference and might give you good estimate of you know the florescence growth. In the second very useful technique, which is used, where is adjust to supply this condition this necessary condition of non specificity develop by techniques like intercalation is the Taq Man assay.

Essentially, this again based on a very simple principle are you design a primer in a manner that you have, let say of fluorophore F 1 and fluorophore F 2 on this is base again on the principles of the fluorophore florescence energy transfer , the way it works is a following. Now, when you have this kind of a situation that you have a primer with one and which is having a let say red fluorophore another, which is green fluorophore. And these two fluorophores are designed in a way that the emission from one, let say in this case the emission from green one is also that excitation for the other and this case it is for the red one.

So, the emission spectra of this particular die molecule the green die molecule the same as the excitation spectra of the red molecules there is over lock between the emission of the first molecule and the excitation of the second molecule, what you think will happen. So, when there is a wave length which comes and hits you know the, the first molecule and provides h new energy it starts a to get exited 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 reduced green florescence and an increase that red florescence just, because of the fact that you have some coupling between the energies of both these molecules. But, this happens all in the molecules are placed to a certain distance which is equal to a smaller than you know very fundamental physical quantity call the furthest distance. So, there is a certain amount of distance between of spacing between this two molecules, which can make it to workably execute a florescence resonance energy transporters.

This transfer of the emission signal from one into the excitation spectrum of the other is also known as florescence resonance energy transfer or FRET. This can only happen when both molecules are closed to each other by a distance equal to the furthest distance. So, when they are beyond this there is no coupling between the two molecules essentially and so the emission from one is lost in to the ambient and there is a certain decrease in the emission from the other, because there is a decoupling action of the excitation frequency.

So, essentially if you plot such a situation, let say E 1 here is the emission peak for the first molecule and E 2 is the initial peak for the second molecule. So, in case a there is a separation of these two molecules beyond the distance of the furthest distance there is certain growth in E 1 and certain decrease in E 2. So, that is happens essentially.

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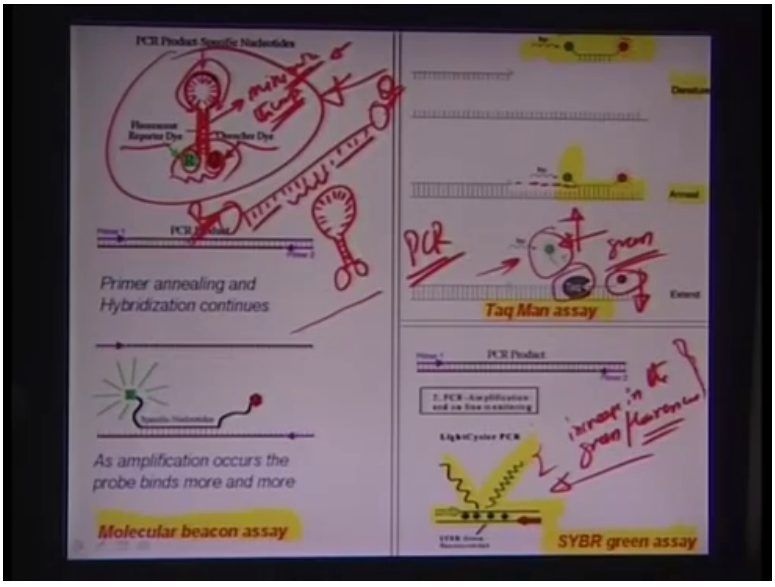

As you see here let me just clear all this out, so that you have a good idea would view. So, essentially this different steps are indicated by in this particular illustration here you can see here that the primer comes and it kind of starts with the binding over it separated by distance equal to furthest distance is of a green fluorophore and red fluorophore and the denaturing step the molecule separate, then there is a kneeling, which happens that is certain temperature with the primer goes and binds to one flank or one under the DNA and beyond that there is a enzyme, which starts cleaving of whatever is there on this particular end. And starts suturing or extending the DNA molecule in this manor on this fashion, so this what this stock design and does. So, as it does that it liberates this fluorophore and makes it a free let of free molecule state in the solution immediately there is an increase in the green signal, because it was gone away from this red molecule and there is a deep 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 Taqman biting the DNA and creating this effect. So, essentially this also gives a real times snap shot of a PCR are reaction; however, the problem in this case is the instrument is a lack more complicated, because you essentially have a find out scan that is a solution of two different emission spectra's and also provide to different excitation spectra's.

Then, you know the spectra has to be sensitive enough to detect, what is the change over between the two emission spectra as process happens and this has to be done at every cycle and there has to be a data point to the cycles. So, there could be a gradual growth in the green florescence in the solution with the more number of copies and more number of this enzymatic termination of this label the primers and the green level on the primer as the reaction proceeds in the DNA grows and this is enzyme steps are repeated again and again that is huge increase in the green florescence over the red florescence, that gives the success of the demonstrates success the PCR processes.

So, this essentially a very interesting way of detecting where the reaction is going on successfully. Other the third very interesting ways again call molecular beacon technology and, what I would like to discuss here shear here is that not all PCR assays are available with this molecule beacon we do have to be lucky in order to get something, where we can use a molecular beacon for executing and the PCR then sequences essentially what makes a difference and here in this particular reaction there is hairpin loop DNA molecule something like this.

So, there is a hairpin loop DNA structure and by hairpin loop what I mean is that you have a certain amount of coupling as you can see here between the two stands and there are certain base pairs, which are exact complimentary is a future the couple to each other and, so this lower ends or lower flanks of the DNA of the single strand DNA it does bind to each other. There are the molecules in a certain portion of the DNA over in a buff the once, which have already bound are comply mayor or essentially not complimentary in the 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 standard DNA molecule.

So, you have a molecule and you are essentially folding it in a manner that is certain portion here is a not complement here each other, where is the n here are complimentary. So, they kind of hold up to the form something like hairpin loop structure something like hairpin loop structure, now you have these at the bond the end flanks bounded to each other and these as the unbounded molecules, how can be make a difference here.

So, essentially if this DNA already had a reporter in a quencher molecule pretty much a like a florescence or 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 an absorption this problem was absorption over florescence. So, quencher with the

fluorophore would mean with the quantum mean, which is generated by the fluorophore is capture by the quencher quenched essentially.

So, we have a lower signal or lower energy signal or intensity signal of a in the particular emission frequency of the fluorophore are the reported die in the case. So, you chemically mobilize the reporter at one and the quencher another with an understanding that as the here loop is formulated there is almost close proximity of the reporter in the quencher molecule as you can see here in this particular example.

So, as 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 florescence level, over all florescence level is low, now what happens when it buying sorry such binding. So, another important interesting fact is that we can actually minimize the number of complementaries.

So, make it just about enough to hold this hairpin loop and if you can do that by just cleaving 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 the loop area is complimentary to the end sequences of the target DNA that as soon as a binds it kind of opens up.

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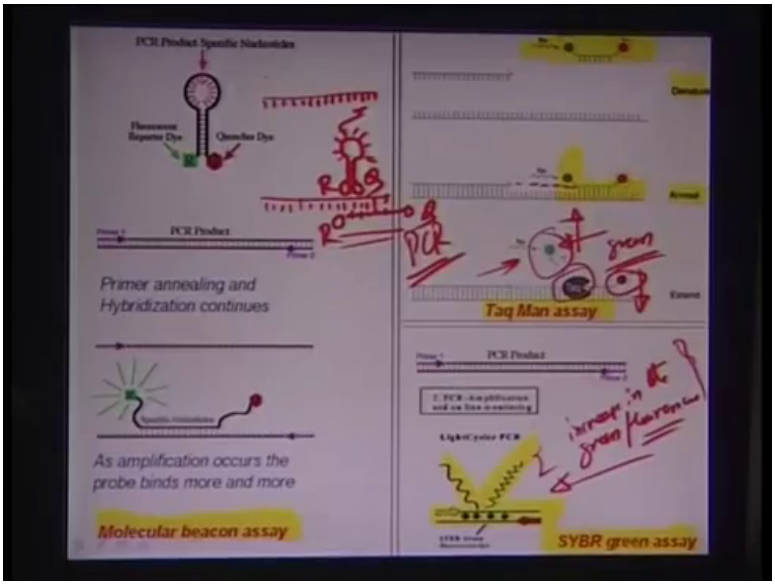

So, you are bounding this on let suppose let me just about all this R for the interest of the more clarity. So, we have suppose a looking into a denatured DNA and we want to find out how this stand would bind. So, let say we have, so we have denatured single strand DNA in

the approximately of a hairpin loop like structure with the reporter and a fluorophore. Let say we have this dangling size and bonds, so immediately as it is able to identify it is counterpart it is going to be bond and in the process of bonding it is also going to let say bonds in this particular area.

So, the reporter and the quencher is frightened out this hairpin loop is essentially straight and out to full size primer and in the way the reporter in the quencher molecules are separated by beyond a certain distance. And because of the separation there is a growth signal is a growth in a report a signal, because quencher is not for away from the reporter. And essentially that is what would cost are would define the success or failure of the PCR process.

So, essentially if the molecular beacon in a question the loop add up the hairpin loop actually goes and bonds it kind of opens the loop up. So, the small amount of base pairs, which are complimentary, which are holding the, the pin bath would actually split up, because the loop part is much more in size. And of course, it has much more molecular forces in the few bonds, which are making the pin part and in that case essentially as a flattened out the reporter and quencher would move away and therefore, there could be a certain growth in the in the reporter signal on the whole system.

So, it can give again an very good real times snap short are what step the particular real time PCR process is in. So, as more and more amplification would occur the probe binds more and more and of course, there would also be short of TaqMan where in whenever the enzyme come and cleans away the reporter molecule in close into the solution and then, that is move question out of being in your the quencher an automatically the florescence signal grows with time.

So, essentially these are the four different techniques or the four different assays, which are available for gradually monitory florescence some trying to see the real times snap short of any PCR process.

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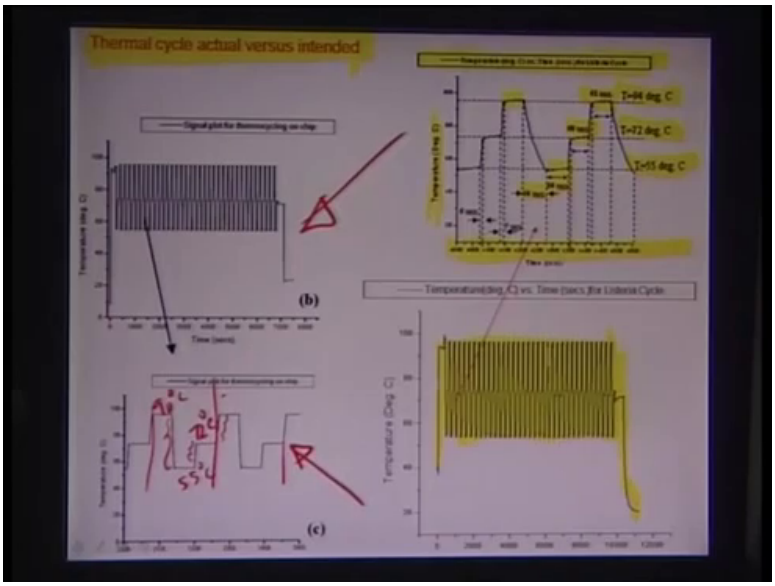

Let us talk a little bit about thermal cycle a very interestingly issue here is the time of the PCR reaction. And as we have to rapidly thermally isolate certain fluid in this volume almost always there is a issue related to thermal management he transfer across the fluid and these sum of this snap shots here taken from real times signal are micro snap PCR do show how this thermal cycling square be rapidly where it.

So, this is essentially is the 90 degree step this about 55 degrees and this about 72 degree Celsius and you repeating this every time in the process you have to actually ramp the temperature down in this region and ramp it up in these regions. So, you have to have proper thermal management for a getting you know better a proper thermal management for getting a getting a better in a cycling time essentially a lesser cycling time.

So, the ramp up and the ramp down rates very, very critical to determine the overall time step that the reaction would need. Of course, there is hold time it a certain step, because that is rate limited by other mechanism like diffusion and enzyme kinetics as we have been talking about before. But, then if you assume that the times of whole steps a different temperature are primarily fixed we do have the flexibility and luxury to vary the ramp out ramp down and ramp operates.

So, because squeeze the cycle more and make it less time consuming, so micro ship PCR are essentially, what is in that domain the ramp up and down the temperature while change in between two or more temperature states can be essentially squeezed and made almost very, very rapid. there are also techniques, which are available there are papers, where we talks

about maybe a droplet PCR, where there is a small micro- cantilever of made of conducting material, which is let all the way to droplet and that is a oil drop on the top and this cantilever, is able to heat the droplet.

Let us internally are a very high rate or a super fast feet as a result of, which you know PCR reaction can be accomplished as low as about closed to two minutes, where as in normal cases many put this vial size we know reactions inside the conventional thermo cycle with huge element in and blocks it would take as much is about 4 to 5 h 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 going to about surface based heating arrangement, then a volume based heating arrangements these are some positive impacts that micro system is make particularly in clinical diagnostics, which is in PCR a polymerase chain reaction.

So, this essentially show some plots here, which are signal plots acquired by national instrument lab view package on some real time micro chips, which we have developed over the past some years, which our lab has developed the past some years for different you know thermo cycling medalists. As you can see here the whole steps really are constant they are not varying much the but we do varying ramp up ramp down rate in tremendously from in this and. So, essentially this is almost reduce from five to about a hour of show and where a thermal can be possible with novel means intuitive designs one of them I really mentioned few minutes back.

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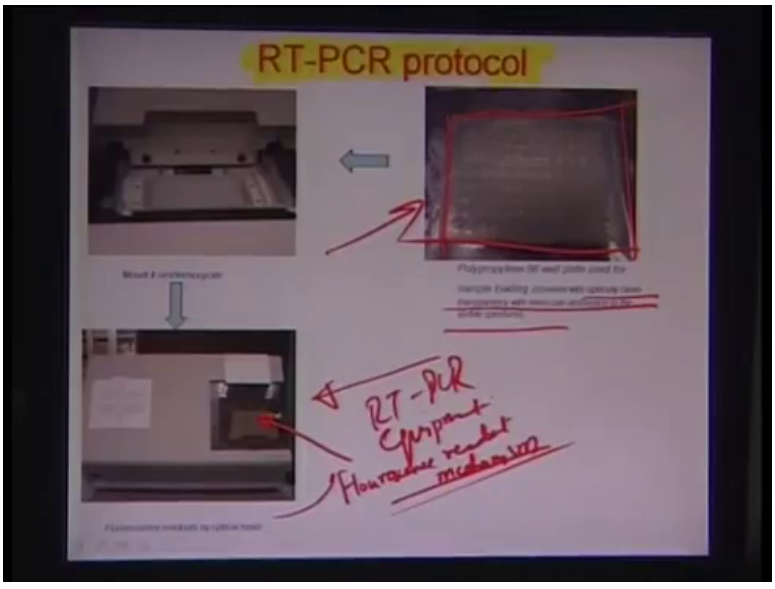

So, that is about the thermal cycle normally we do need to read out a florescence signal very quickly and for that especially in RT PCR or the QPCR reactions we do have separate reader unit. So, essentially there are two aspects here one is there has to be a reader head you can see this is again essentially we could from a strategy this is RT-PCR equipment and this here is also in the florescence read out mechanism.

So, what this mechanism is really it is a set of the detectors which would sit on the top of this wells here about 96 wells in which the 96 the reactions can be carried out in the single go. So, these are wells and the covered by using an optical clean transparency miniscule absorption. So, when you are talking about in optical detected scheme it is almost always necessary to certain that whatever emissions spectrum is generated by the particular die should not be absorb by a material which we come way in the light in the optical path.

So, what are the things which do come and which is important is a cover. The cover is more important. Because, you have to prevent thermal evaporation of the PCR fluid and when you are talking about small vial containing let say 25 microliters of a PCR solution you are heating it a closed come find in the environment was high is about the 93 to 95 degree Celsius there is almost a possibility there if leave it open it will evaporate.

So, you do have to cover it totally with some kind of polypropylene sheet let say cellophane paper, where it can give a leak proof joint. And then, you can actually press the head and give a grip a lot of pressure over these particular you know covered vials and at the same time the head can be use for optical read outs. So, there then over every well there is actually a detector and there is an array of detectors and this detectors sit on the top of this a small vials is containing each well over, which there is the cellophane paper.

Due to, which whatever is happening each well is a dependently report this detectors or having an electronic channels from one to 96, which record independently that some of the data you know partially this channels can be just the reference without the sample to manic at the background and partially the can actually be containing the sample. So, you have a growth and florescence and then you have a comparison between the reference and signal and that way you can actually evaluate a lot about close to at least you know about 60 to 70 samples maybe a little better about 80 samples making few of them as a references a just for a proper signal analysis module.

So, you can actually get the real times snap shot of about 80 reactions happening all are at a single go. So, it is essentially a very high through put process by decline, because of the

optical that is involved the instrumentation which is a provide is expensive and it is prone to the mechanic damage easily. So, therefore, in the trend now is slowly shifting from this a detector is into optical fibers, a bunch of optical fibers can do the same job even if left inside the solution. And, so therefore, and that is trend of making arrays or bundles, fiber bundles for picking up signals from this different multiple wells other more machines which are coming up now more with this a fiber technology.

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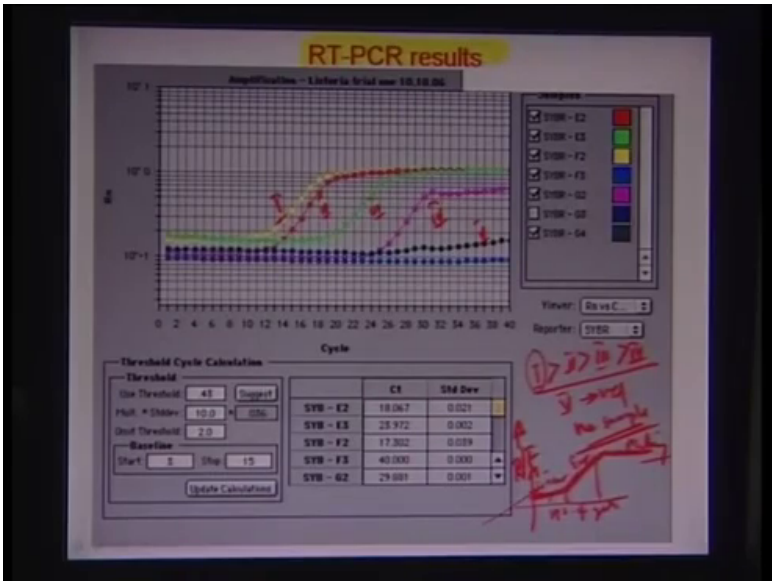

So, these are some real time PCR results as obtained from. So, this here illustrates. So, are the Q-PCR or RT-PCR data as you can see here these really correspond to different concentrations initial concentrations of the template. So, these are let say sample one, two, three, four five are containing you know an increase order of a concentration of a initial template. So, this is how the initial template is the different in different from the first one is the highest template concentration in forth from as the least fifth essentially in the reference, so this is no sample.

So, there is no DNA to start with there is just other components in this reaction nothing increases a florescence and other interesting fact that is in the trend as you are seeing essentially linear, exponential and plateau. So, there are three friends here the linear, the exponential and the plateau why this happens is again something that I think of is spoken back in my previous lecture. So, this is number of cycles and this is let say the relative florescence RF and the growth in that.

So, what happens here is that in this region there is very less primer or enzyme and other

molecules available because. So, this is diffusion limited region and diffusion is not fully taken place. So, get in micro on this would mean that this 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 almost all way round the reaction I have in started now to copy a very fast or rate at you reach a certain cycle here there is a plateau phase which means now one or more of the reactants or constituents are depleting. Because of the concentration depletion there is a contractual reduction and the growth rate of the florescence. So, that is why this particular trend is visible it comes.

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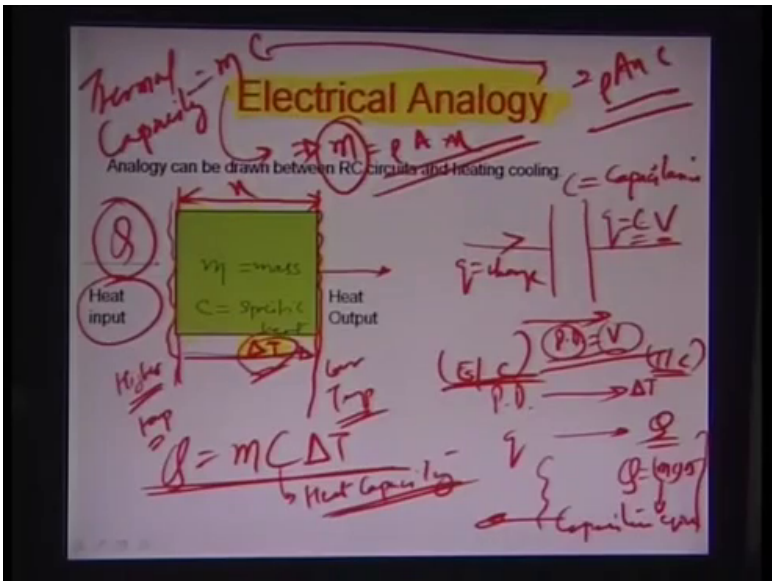

So, one interesting factor which comes in to picture while designing PCR chips is essentially how you will try to characterize the chips thermal and what you need to do is certain how much time it would take for the chip to cycle to a certain temperature. So, let us actually draw some kind of an electrical analog for these kind of problems we consider any thermal system to be a bunch of resistances, capacitances and circuits. So, let us draw the anagogic one by one.

So, if we look at this slide here really you can know the there is a let say mass which you are heating up this right here is the mass and if the mass is produced by the represented by the m and specific heat of the material C, assume the temperature; that means, ΔT between both ends of this mass. So, ΔT is temperature difference this may be on a higher side of them temperature and; obviously, because in this side high is the higher side of the temperature by there should be a heat flow from one end to other, so there is a heat input cross the sense.

So, this is the surface with a higher temperature and there is a heat output here from this particular surface this is the surface where lower temperature all the difference ΔT . So, if we assume that q is the heat flow not the heat flow rate this is amount of heat that is flowing inside system. So, Q also can by represented as m C ΔT right. So, this kind of looks familiar or with something like a capacity this is called heat capacity.

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

So, let us draw the capacitor, so this is the capacitor let say you have capacitance value equal to C, you have charge molecule which is flowing. So, this capacitors and essentially there is a potential difference of V here. So, what cases the charge really to flow as a potential difference here what causes the heat here to flow is the temperature difference here. So, it kind of analogous, so potential difference in an electrical circuit is the same as temperature difference in a thermal circuit and the quantity and electrical circuit which is flow is the charge Q as supposed to in the quantity in the thermal circuit which is in the heat Q also.

Therefore, as we know here Q is equal to C into V, capacitance the voltage. So, therefore, in the thermal circuit Q is mC ΔT where m c is the capacitance equivalent of thermal circuit. So, the thermal capacity such a circuit is also mass times of this specific heat capacity of the material. So, explore further mass can also be return as the density times of area times of the depth of and then in this case let us actually forget about the depth of this time the area times of the length here assuming that this length here is about x.

So, area times of x that is what the masses, so the thermal capacity here should be represented as row A x times of c that is what how you can percent the thermal capacitance let us also look at if there is something analogues to resistance in electrical circuit as in a thermal circuit.

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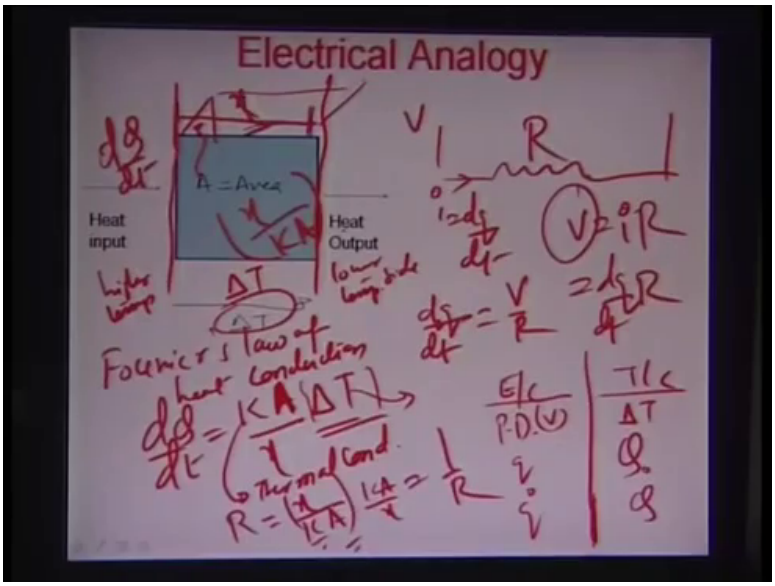

So, for doing that we need to assume the rate of flow of charge, so basically in case of the equivalent of a resistance what we can do is we have to consider the rate of flow of charge. So, if we consider the equivalent model that we do earlier between the electrical circuit and the thermal circuit the potential difference on an electrical circuit corresponding to the ΔT to the temperate 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 a the Fourier's law of a heat conduction, if we really look at the heat transfer between these two phases here and here the dQ/dT , the rate of flow of heat essentially represented by now, $\frac{dQ}{dT}=\frac{kA}{x}\Delta T$, the K here is in the thermal conductivity in the material and of course, A is the area of cross section perpendicular direction of the heat flow. So, if this for assume to be like a Q, so the area A is really the area of the this particular phase the x essentially the distance in the distance between the two plans of this particular mass and ΔT being the temperature difference of cross.

Therefore, this is certainly the higher heat or higher temperature site, this is the lower temperature site and ΔT essentially is nothing, but the difference between the two temperatures. So, if you put a circuit equivalent this can be more analogous to a resistance that you have a current across which is also the rate of flow of charge. So, essentially Q dot in the electrical circuit is essentially equivalent to in the heat flow rate Q dot d Q by d T on the thermal sector as we know here the potential difference being V can be equated to I times of R that is d Q by d T times of R.

Since the potential difference is also equivalent to our ΔT here therefore, $\frac{dQ}{dT}=\frac{V}{R}$. So, ΔT is the equivalent to T and essentially K by x is essentially same as one by R, R then thermal resistance in this case $R=\left(\frac{x}{kA}\right)$, K is the thermal conductivity, A is the area of cross section perpendicular to the direction of the heat flow and x is the length cross which the heat flows causing a temperature flow of ΔT cross both its surfaces.

So, the resistance equivalent thermal resistance equivalent this particular thermal circuit is $\frac{x}{kA}$. So, that in a sense the complete electrical analogy of what thermal resistance or thermal capacitance would be. So, be. In fact, there is an approach called bond graph Modeling where this kind of concept very often used for you know kind of developing an analogy between the electrical side and the various other physical phenomena it may be heat transfer or may be a related to optical phenomena and there is analogy developed.

So, since we have a resistance and a capacitance here we can always draw a R-C circuit see that with the potential difference across the RC circuit are assuming that you know have temperature difference in a thermal circuit and a kind of serious of parallel combination of the thermal resistance and the thermal capacitance can we really develop something called a time constant. And once we develop that other question would be how relevant it is what is the physical meaning of that time constant and can we get some influence about this systems thermal behavior the lag or the lead of the thermal response you know based on what this time constant. So, let us look at preparing the equivalent R C circuit trying to see what the time constant of R C circuit would be.

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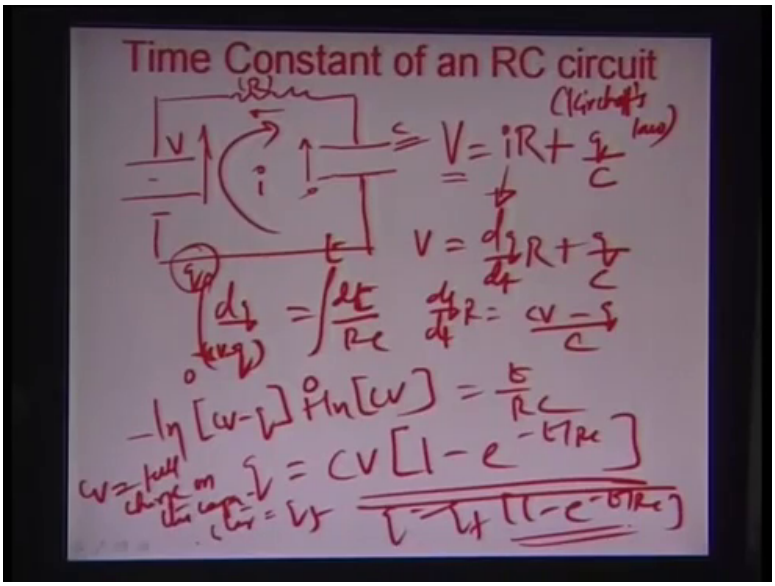

So, let suppose we have this resistance capacitance circuit connected to potential difference and this value is resistance value, this is the capacitance value. And then, completely closed circuit the capacitance C the resistance here is the R and we assume further that subjected to availability of the potential V we have a current in a circuit I which is generated, because of the potential V.

So, V essentially is also equal to by Kirchhoff's law i R plus the voltage across the capacitance, which is the free q in the charge lets actually right small q here may interest of clarity divided by the capacitance C. $V = iR + \frac{q}{C}$ So, that is what the voltage drop is across the capacitor and there is a drop across the register together it is drops around also the same as in the voltage which is provided by the interest if the conservation this is Kirchhoff's law if we remember we applied this law in one of the electric kinetic pumping design modules for doing circuit calculations and try to find out in the ion current as well as the flow rates.

So, let us all this equation, so V essentially written as d q by d T R plus q by C current as we know as a rate of flow charge d q by d T i.e. $V = \frac{dq}{dT}R + \frac{q}{c}$. So, if we rearrange this equation we are left with dq by q we can assume the q variation betweens zero charge in the capacitance of the very outset to out of dull ground charge of let say the small q, and this a essentially sorry this is the C V minus q. $\int_0^q \frac{dq}{q} = \int_0^t \frac{dt}{RC}$. So, $\frac{dq}{dT}R = \frac{CV - q}{C}$ and this

essentially is equal to in the integral of a d T by R C d T being where in between 0 and T, T is the full time over which in the charger capacitor varies from 0 to q.

So, if you solve this equation we are a left with $-\ln[CV-q]+\ln[CV]=\frac{t}{RC}$ minus l n C V minus q plus l n C V essentially equal to T by R C and in other words q here by the $q=CV\left[1-e^{\frac{-t}{RC}}\right]$ alright. So, CV also is representative of the full charge in the capacitor let us call it Q_f .

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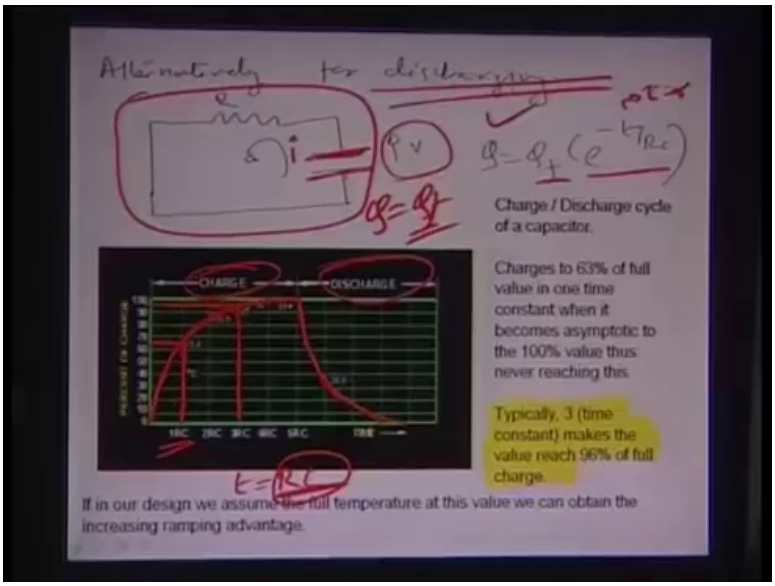

So, q is $Q=Q_f\left(e^{\frac{-t}{RC}}\right)$ alternately for the discharging circuit when we assume that there is no voltage source as you can see here. And this particular illustration the voltage of the capacitor, which has been develop over which plates is going to feeds the circuit with the current I and there is drop cross the resistor. So, in that case now the discharging time of the discharging process in the Q comes to $e^{\frac{-t}{RC}}$.

So, time T 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 zero alternatively time T equal to 0 the Q is actually grown to full value Q_f just before the start of the discharge processes. So, very interesting aspect about these two charging into charging cycle times of that if you plot the percentage of charge with respect to the time as you see in this illustration here and this really is the growth cycle right.

So, this is essentially let us give me one minute. So, this essentially is the way that the charge happens in the circuit following the expression Q equal to Q of $1 - e^{\frac{-t}{RC}}$ and this is the exponential decay in the charge Q is equal to Q to the power of minus t by R C. So, this is the charging cycle this part is the discharging cycle also what is interesting here to know is that few have a time constant T of this R C this corresponds to about 63 percent of the total charging.

So, if you have one time constant RC resistance in the capacitance we have a 63 percent of the charging and takes about three time constants for the charging to be above 95 percent. So, essentially and we are looking at three 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 fly this to our PCR microchips we may as well develop a strategy of trying to figure out how much time it would take of the ramp up ramp down mechanism of the particular chip if I actually make a electrical equivalent and electrical analog.

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

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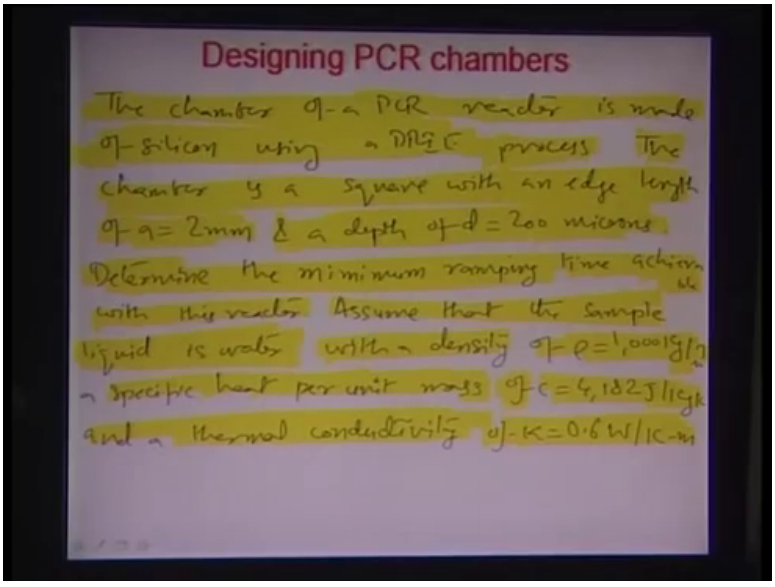

So, in this particular case as you seeing here there is a chambers of PCR that which is made a silicon using deep reactive ion etching process. So, this chamber is essentially a higher expect ratio chamber used or etched away using gas plasma the deep reactive ion etching, the chamber is a square size more than h length of about 2 milli meters and there is depth of d equal to 200 microns and this particular chamber. So, therefore, you have a square of 2 mm and 2 mm and depth about 200 microns.

So, the chamber is essentially pretty much in high value on the length aspect and a really micro in the depth aspect and this is how normally micro architecture are there designed in other advantage why this almost thousand times increase in the dimensions in the in the linear x y dimensions chamber is because the idea as to actually spread volume of fluid over a thin surface area which essentially you are taking a bulk volume of the fluid. So, the idea is really is to kind of spread over thin film and bulk.

So, you can increase the transfer, so in the example you have to determine then minimum rumpling time, which is achievable and with this particular reacted design at assume if the example if it is water most to the PCR fluid is actually an aqueous based fluid. So, the little bit of ionic content here and there, given their density is around 1000 k g per meter cube as the normal case with water this specific heat point area per unit mass of a 4182 joule kg degree Kelvin and there is a thermal conductivity associated of k equal to 0.6 watt per Kelvin meter.

So, you have to essentially designed this manner that you know you can determine the minimum ramping time, which is achievable by this system. So, let us use theory that you have this formulate in terms of electrical analogy. So, essentially we need to find out the thermal capacitance in the resistance in this case.

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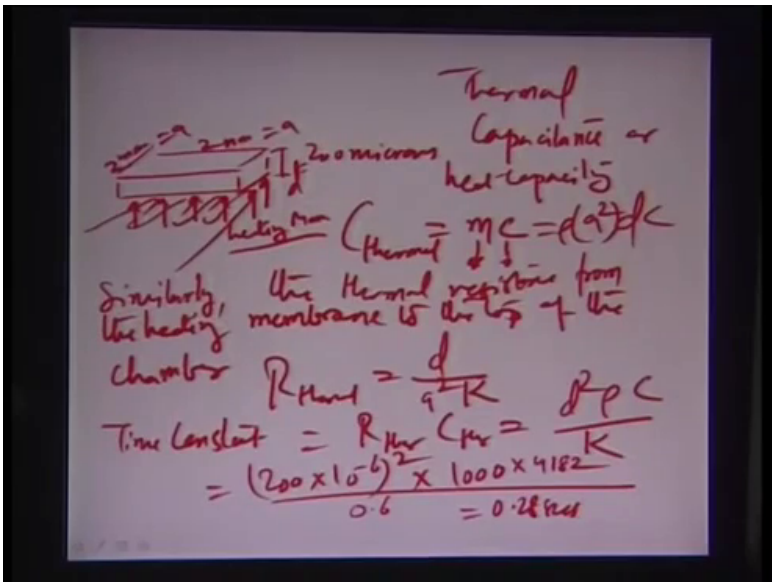

So, you have first let us draw the chamber you have the case where you have 2 mm by 2 mm by chamber and essentially the depth of the chamber is about suppose to 200 microns and assume a heat transfer from bottom to top. So, there is set of heaters let say set of micro heaters in the bottom of this chamber and this actually feeding this particular chamber. So, from this case the thermal capacitance, heat capacity C thermal the essentially and m times of C time is mass of this volume C is the heat capacity, now the material.

So, essentially it is again rho times of area cross section which is square of a the times of in the depth in this case the depth being from the heating sight to open of the possible side is d. So, assume this to be a and this to be b. So, you have rho squared rho a squared d the total mass and then of course, you have the specific heat C, similarly if you determine thermal resistance that is from the heating member to top of the chamber. So, this is the heating membrane in heaters and this is top of the chamber, so from in the heating membrane into the top of the chamber.

So, let us call it R thermal. So, in this case would be actually x by k a. So, x essentially is d here if you just remember the electrical analogy that, which is the top are which is three about slide back the area of cross section a square. So, we have d divided by a squared K and essentially the time constant in this case which is $R_{thermal}$ $C_{thermals}$ as nothing but, d squared rho C by K. So, if assume d of a 200 micron that is 200 into 10 to the power minus 6 squared times of density of water, which is about 1000 kg per meter cube times of specific heat capacity, which is about again 4182 joule per kg per Kelvin divided by the K value thermal conductivity, which is 0.6 Watts/K meter we obtain times constant of a 0.28 seconds. Time

constant $R_{thermal} C_{thermals} = \frac{d^2 \rho C}{K} = \frac{\left(200 \times 10^{-6}\right)^2 \times 1000 \times 4182}{0.6} = 0.28\, sec$

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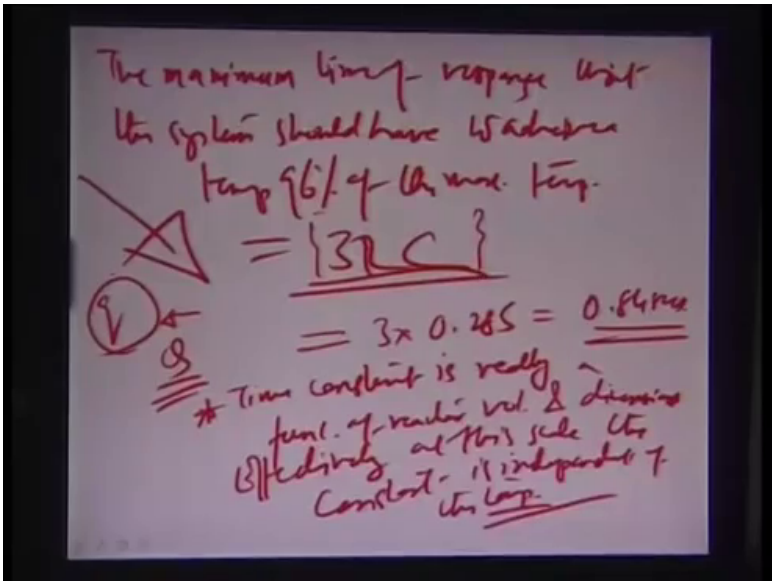

Essentially the maximum time of response that the system should have to achieve the temperature 96 percent of the maximum temperature, which in this case is probably 90 degrees and 94 degrees should be 3 RC as we saw in the RC example another q, which was the essentially the electrical equivalent of a the heat. So, q which was essentially and the electrical equivalent of a the heat Q in the case of capital Q and a case was about 63 percent of the full charge once a the time reached one time constant and was about 96 percent of full charge as it reached about three times RC.

So, the maximum time that you can allow for the system to ramp up to this particular 96 percent of maximum temperature, now our cases about three times of 0.28 seconds, which is about 0.84 seconds. So, take on message here really is the time constant it is really function of reactor volume. So, if you can make the volume smaller and smaller you could make the time constant smaller and smaller and the volume and the time depend in the dimensions.

So, effectively at this scale of the constant is dependent of the temperature. So, that is exactly what we have to consider for designing microsystems. So, would like to finish this lecture and just by you know trying to just retrate that times we need to probably draw these kind of analogy is together solution of what would be the maximum time that become allow for a system to go to certain temperature state by going to ramp up cycle as soon.

The next lecture will take on to little more details of how we really design the volume based

on time constant. And then, look into some other aspects of how the DNA polymerase is works on you know DNA molecules of files to extend zipped up or zipped down molecule make copies inside the human body we also like to kind of a review basic electro-kinetic techniques, which is also used for finding out the length of a DNA base pair and is critical to diagnostic industry. So, I would like to close as lecture with this out.

Thank you.