Microscale Transport Processes

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Lecture No.#03

Lab on Chip

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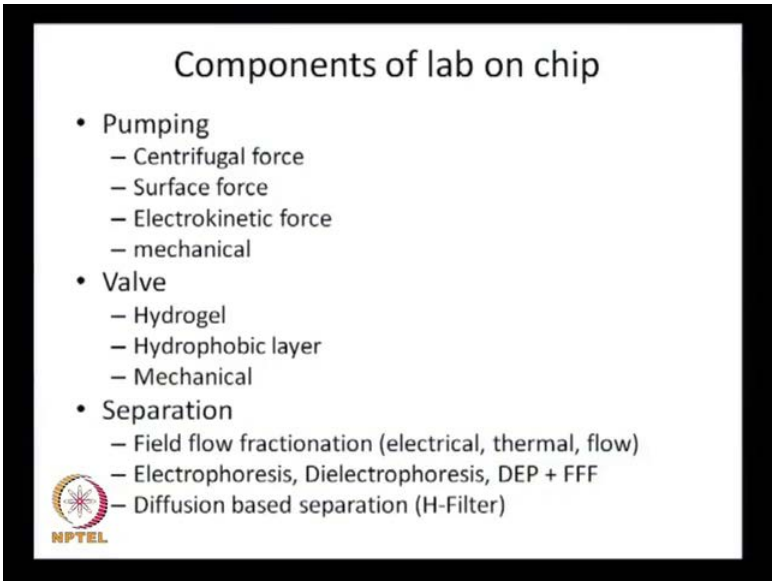

I welcome you all to this lecture on Microscale transport process.Here we are going to discuss, various components of lab on chip. As we mentioned in the last class that there are several components we must have in lab on chip device.

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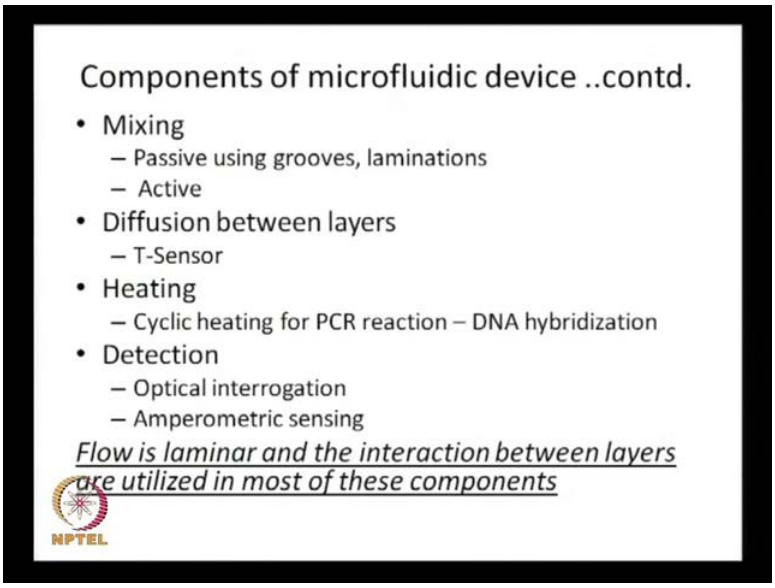

These components are suppose to be pumping, valve,separation,mixing, diffusion between layers, heating and detection;and there could be some other components also depending on the application that you are looking into.And in all thesecomponents, oneessential feature is that it is assumed flow is laminar. In fact, flow isvery much,in fact the Reynolds number is pretty small in flows in microchannels.Flow is considered laminar, and the interaction between layers are utilized in most of these components. So, this is unlike a typical flow in a channels where there could be turbulence,and there could be movement of ADsfrom one place to another; that is absolutely missing, because you are relying on two layers flowing like a pack of curge,one sliding against the other.And diffusion is taking place from one layer to the other.So, you are relying on this laminar flow that is for certain. So, these are some of things that you should remember.

Now, when it comes to pumping,of course you can have a pump that we have in a conventional sense;for example, you have seen centrifugal pumps,where you have an impeller rotating.Now, having such components in microscale is not something very easy.Of course, it is durable, it people have done it, but it is not very easy to implement. So, people have looked for other alternative routes that they canyou use to implement pumping action.And the action that they have isI havecategorized them, one is centrifugal force.

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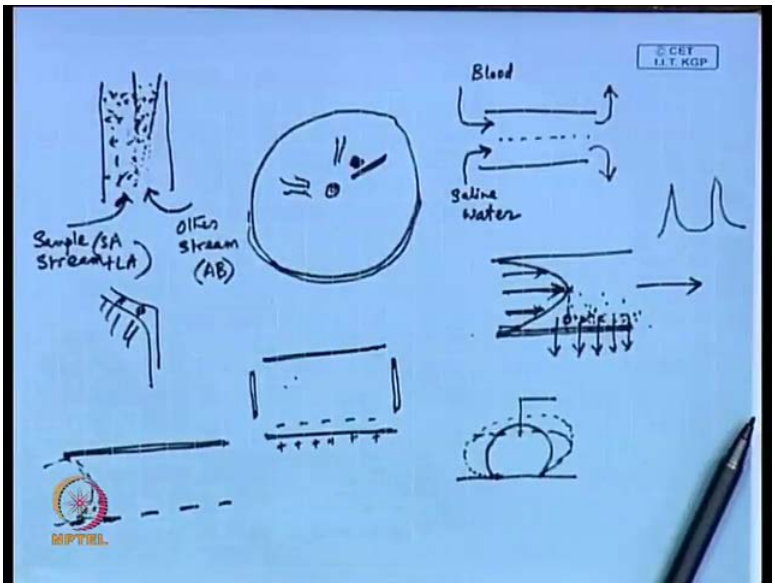

How does centrifugal force works?Suppose, you have a CD type device;suppose you have a CD device,and in this device you have this sample that is going inthat sample goes in through the center. So, you have a CD type device, which can be put on a turn table. And it can rotate.Now and it,so it is a plate in which you have curved channels in radial directions in various,several channels in radial directions;and in some of the places, you havereagents stacked reagents put there and you introduce the sample at the center.Then what you do?You rotate the turn table. So, as you rotate the turn table, because of centrifugal action the fluid will start moving towards the periphery. So, this is an action that you can have this is a, this an action that you can have for pumping fluids. So, you have a CD type device on which you have channels curved and on top of that you have a lead and everything is happening in microscale.

Put the CD device on a turn table, if you introduce the sample at the center of the CD and rotate the CD,automatically the fluid will move towards the periphery. So, this is a centrifugal action that you can rely on for pumping fluids, because you can control the spinning rate and you can control the RPM; and by that you can control at what velocity the sample would be flowing towards the periphery. So, this can be a device.Similarly, you can have surface force.How that force look like?You have all studied capillary rise; if you dip a glass capillary inside water there would be rise of water through the capillary.

How does it happen?The that liquid has a weight, but that weight is balanced by surface tension force, because glass is hydrophilic;so you have,if the surface tension force is balanced,the weight of the liquid isbalanced by the surface tension force. So, surface tension can raise water through certain height.Now, if you have a tapping point, because anyway you are working with small scales, if you have a tapping points,little above the water level, you can have a continues flow of water. So, this is basically a surfaceusing surface force to pump a fluid.Next, you can have what you can have is Electrokinetic force.Electrokinetic force means, you haveyou canhave electrodes put there and you can mobilize the charges.And with the charge, some bulk liquid is also dragged;we willdiscuss this electrokinetic force in details later.And mechanical of course, you can haveactual movement,I mean something similar to centrifugal pump or reciprocating pump,you can have a moving element inside themicrochannel that is also a possibility.Butother elements are very much perused; other elements are very much looked into for pumping fluid.

The next item is valve.Of course, you can have valve in a conventional, the way you we have in a conventional sense, like you have a valve seat, you have acloser of the valve, you have another element that goes in and sticks to thevalves seat andso you can consider the valve to be closed. So, you can have such elements design such elements implemented in microscale, but that is not again very easy.However, other way is people have looked into and one ishaving a Hydrogel layer, because Hydrogel have some unique properties, it can respond to pHor thermal trigger.Either it can contract;I mean it can contract as well as it can expand depending uponthe external trigger.

So, if you put a Hydrogel pillar at the channel inlet, and if youput a trigger from outside and by the trigger, you can shrink the gel layer or you can expand gel layer. So by that way,you can block a channel or open part of the channel. So, Hydrogel can act as a valve in that case.Also there could be something called hydrophobic layer.If you have a hydrophobic element on the wall,you know that the contact angle;if there would be adverse contact angle.You might have seen already, when we dipglass capillary in mercury,how the interface would be;sothat gives you an example, how hydrophobic layer would have how hydrophobic surface would be actingtowards water.

So, if you have an aqua solution and if you put a hydrophobic layer,then because of this surface act, because of this surface tension and contact angle, you may have tocross a threshold pressure, and then only you can have a flow through the channel. So a hydrophobic layer, small hydrophobic layer put on the channel valve can act as a valve in a sense.The third element that we have here is separation in lab on chip device; you need to have separation accomplished.And for that there are various techniques that are available.First,I would you like to talk about is called what is called field flow fractionation.It can be of various types,for example, electrical, thermal or flow.The field flow fractionation, the way it worksis, suppose for example, I look at say flow field flowfractionation field flow fractionation of flow type.Suppose, you have a channel and on the wall, you have a membrane placed on the wall you have a membrane placed.I say that theflow would be laminar, sothe velocity profile would be parabolic like this.

And there is this membrane,soif you have solutes inside this,I mean the liquid that is flowing within the channel if that has solutes,then the soluteswould beas you collect fluid from this side I mean. So, you have a flow down this way and at the same time, you have some collection of fluid,at a very low rate you are collecting fluids from this side. So, when your collecting fluid collecting the solvent through this side and you are having solute been deposited, whatyou eventually have is something called concentration polarization.By that what I mean is you will see that the particles that are being held on the membrane surface, if the particle is smaller, it will have a larger diffusivity and it will be easy for that particle to go to the bulk, move to the bulk.

The particle that is being restrained by the membrane, if it is small, it can go to the bulk easily; if it is a bigger particle, it would be difficult for that particle to go to the bulk. So depending on the size of the particle, you will see a classification here, this is something called a polarization; you will have particles of certain sizes held next to the membrane and particles of other sizes are more close to the bulk,I mean more towards the center.Now, you are introducing a parabolic velocity profile from this side; so that means, this layer is moving faster than the other, this layer is moving little slowerand this layer next to the moving at the slowest atsmallest velocity. So, if you put something called a fractogram,if you analyze thefluent coming out of this, you will find peaks coming like this.And each peak represents particles of certain size. So, you can classifythe particles.

Now suppose,you if you have this device already calibrated with a known mixture of particles and then, if you introduce thisif you introduce an unknown sample into this device; and output that you get, if youlook it look at it(( ))the one that you havethe calibration that you have, you can figure out what would be the what is the what are the various components that are;what are the various sizes that are therein this fluid. So, this separation this can be this is referred as field flow fractionation of flow type.Now, here the flow has been put as a transverse gradient.Now, for other types of field flow fractionation,for example, the thermal one, there you have a thermal gradient in the transverse directions.Or electrical field flow fractionation, there you have this electrical gradient transverse to the flow direction. So by that way, you canby that way you canclassified by that way you can separateparticles depending on their sizes or depending upon their properties with a distance from the wall.

And since you have always a parabolic velocity profile,Icategoricallysaid that you are relying on laminar flow in the microchannel. So, you can get layersone layer at a time,middle layer first,because that is moving at a highest velocity, next layer and then the other layer. So, you can separate the particles you can get the fractogram with particles arrive eluting at the outlet at a different times. So, you can classify them.Similarly, there are other separation components, like you have this electrophoresis,Dielectrophoresisor Dielectrophoresisplus field flow fractionation.We will discuss this in details in electrophoresis, you have put electrodes and you apply electric field depending on the size and mobility, depending on the mobility of the ions; that means, size and charge of the ions, you can separate theyou can putit will be more closed to the electrode or away from the electro, depending on themobility of that ion mobility of the charge. So, you canfind outwhatthen if you study them.

For example, in electrical field flow fractionation,I will discuss this later that you impose this electric field in transverse direction. So, depending on the mobility of the ions, they will be located either next to the electrode or away from the electrode at various locations.Andsince you have the laminar flow taking one layer and the next layer,soyou will be collecting the particles at different times; andso, you can alwaysusing a calibration, you can always figure out particles of what sizes, where thereor what mobility where there in the original sample.

Then there is this diffusion best separation, which is referred as H-filter; it depends totally diffusion is does not have any electric field in water.I will discuss this; I will show draw a picture very soon. So, these are the some of the separation components. So, we if you think of conventional chemical applications, there are separation, possible, but when it comes to microscale, you are relying more on this laminar flow and relying more on this parabolic velocity profile and some diffusion orcertain responds to electric fields, sothese are the some of the things that you look into.Of course, you need mixing inmicrochannel and mixing isin a traditional sense in a microscale, it is done using an impeller, using a stirrer.Herethat is possible, you can haveelement within a microchannel, but implementing it in microscale is not very easy. So, there are alternative routes people have looked into,one issay passive mixing device using grooves or laminations.

You havealready heard of these passive mixers that mean, you have to strength flowing through a tube and then you have several baffles placed on the way,so that two streams they come together and then they divert. Soby that way, you increase the contact area between the two streams,andsoyou enhance the mixing. So, these are some of the elements that you would look into, when it comes to mixing in microscale.Then there is diffusion between layers, which is very important,I will discuss this very soonwhat is a T-Sensor?InT-Sensor diffusion people relying on this laminar flow parabolicor one layer sliding against the other, that means no cross flow no deformation; and then the diffusion from one layer to other this ispeople relying on this,to develop sensors. So this is a T-Sensor we are going to discuss very soon.Then there is this heating component also you should have in microscale,microscaleprocess.Andheatingcomponent one heating requirement,I have given an example here cycling cyclic heating for PCR reaction.

These reactionsthis requires heating insteps, within a very short time we have to accomplish several up and downin temperature.That means, you increase the temperature by certain degrees and then you decrease it, again you increase, again you decrease, again you increase, again you decrease. So, you have to do these are called thermal cycles and you have to expose the sample to such thermal cycles several such cycleswithin a very short time.

And typically, if you are if the mass is more, then accomplishing these cycles will not be very easy,because if the mass is more youthink of it.What would be the heat

requirement,what would be delta h?Heat requirement would be mass into specific heat into delta t. So, if delta t is known, specific it is known,so, if you have a large thermal mass. So, you have to injectso much of heat and that heat has to distribute through the entire material;and then the temperature has to uniformly rise everywhere within this material.And again you are cooling it down and you are doing it twenty times within a very short period. So that is very effectively done in a microscale and that is what I referred as cyclic heating for PCR reaction.

There are of course, all theseafter all these microfluidic elements microchannel devices a major application is in sensors. So, there has to be some detection element involved.This detection is done either by optical method,optical interrogation or amperometric sensing; that means, you find out the conductivity of thefluid that is passing by.Say for example, you are you have classified the sample classified, you have a sample and you have classified it into severalsay several components.And you had this parabolic velocity profile,so one component is eluting at some time, then the next component, then the next component it comes like this.Now, if you want to identify those components, one could be this optical interrogation, optical detection.Theother way to do it is by amperometric sensing; that means, you find out what is the conductivity of the sample that is coming out at a particular time, from the outlet and from that you can generate a fractogram. So, these are some of theelements that you have for lab on a chipdevice.

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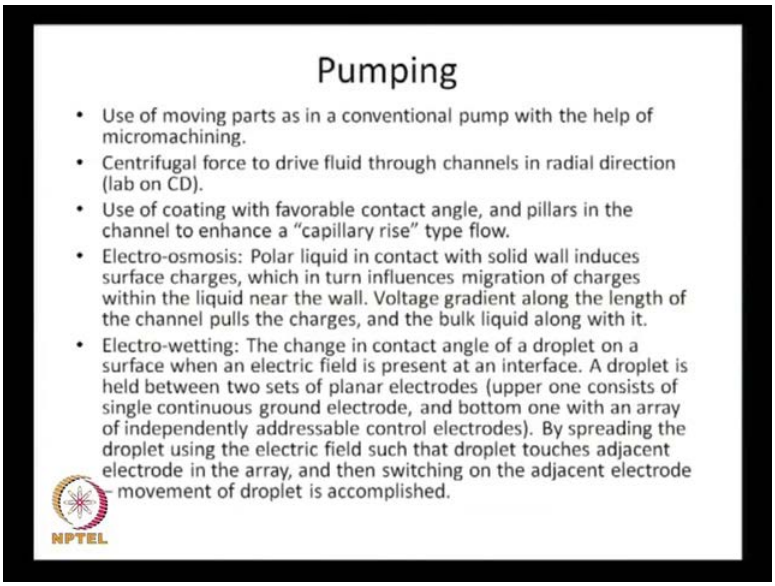

Now, let us look to this quickly this,what are the pumping elements that we have here.First of all,use of moving parts as in a conventional pump with the help of micromachining thiscan be done.There can be a moving part, but this can this is not the only way to do and this may not be very easy to accomplish;so, there are other methods which are in demand here.Centrifugalforce drive fluids through channels in radial direction, which is referred as lab on CD,this is used in certain industry these lab on CD is used. So, these areI already I have discussed here.The third one is use of coating with favorable contact angle and pillars in the channel to enhance a capillary rise type flow. So, you can in a channel, you can have favorable contact angle as I said capillary rise of water in a glass capillary.

Now, one thing is there the capillary diameter has to be,I think what we have studied is it should be less than say 5 millimeter or there was threshold,I mean,if we cross the diameter, you see these capillary rise is notsubstantial. So you can,sowhat you can have is to induce these capillary rise, to induce this surfacetension driven flow, you can introduce small pillars on in the channel.So that means if this is the channel, within the channel you can introduce small pillars. Sothis pillars will provide extra surface area,sothat you can have enhanced flow.The third, the fourth element what you have here is electro-osmosis,I said electrokineticpumping. So electro-osmosis, it is basicallylike this; a polar liquid in contact with solid wall induces surface charges, which in turn influences migration of charges within the liquid near the wall.

Voltage gradient along the length of the channel of pulls the charges and the bulk liquidalong with it.What you have is, you havea wall and you have electrodes placed here.Now,polar liquid in contact with solid wall induces surface charges. Sosurface is where?This is the surface; this is the wall,sothis is the surface.On the surface, there are some surface charges generated.SayI sayI write plus,we will discuss this later, we will solve the, we willget in to the theories of it.Ifyou have certain charges induced,soautomatically the... So, this polar liquid in contact is solid wall induces surface charges. So, the negativecharges within the liquid, they will start getting accumulated on the wall. And then this electrode,they will be pulling these negative charges say.While pulling it, it will pull the bulk liquid along with it. So that is that is how we generatesome flow.Of course,the channel this wall should be very close to each other,I mean if you

have a large pipe and if you expect this kind of flow to take place that may not be verymuch possible or feasible.

The other element that we have is electro-wetting.Here, the change in contact angle of a droplet on a surface, when an electric field is present at an interface.I have supposethe wall and on which I have a droplet sitting there; this is a droplet.Now, if I introduce some voltage difference,if I introduce an electric field,I will find that this contact anglethis changes, this contact angle changes, if I introduce an electric field there. So, if this contact angle changes,sothen this would be more spreading.For example, this was the liquid and later on, when youbring in the electric field,the liquid cannot grow, but the volume remain same, but the liquidthe contact angle will be different. So, if you have an arrangement, where you have suppose,one ground electrode on top and several control electrodes in the bottom.

And suppose, you have a droplet sitting here,a droplet is there and you apply electric field using the electrodes. So, this would more spreading and by while spray while this dropletsspreadsit will go to the next, it will touch the next electrode.Then what you do is you switch off the,you have theseelectros are they can be control separately. So you switch off this electrode and introduce electric field through this electrode. So automatically this droplet will be picked up by this electrode and the same process will continue. So, you will see this droplet rolling down, this droplet will be rolling down through this electrode assembly by this(( )).

So that is what exactly what I mean by this electro-wetting here.The change in contact angle of droplet on a surface, when you an electric field is present at an interface.Adroplet is held between two sets of planer electrodes,I described here; two sets of planer electrodes, upper one consist of single continues ground electrode and the bottom one,with an array of independently addressable control electrodes.By spreading the droplet using the electric field, such that droplet touches adjacent electrode in the array and then switching on the adjacent electrode, movement of droplet is accomplished. So, this is one method by which you can move droplet within a microchannel.

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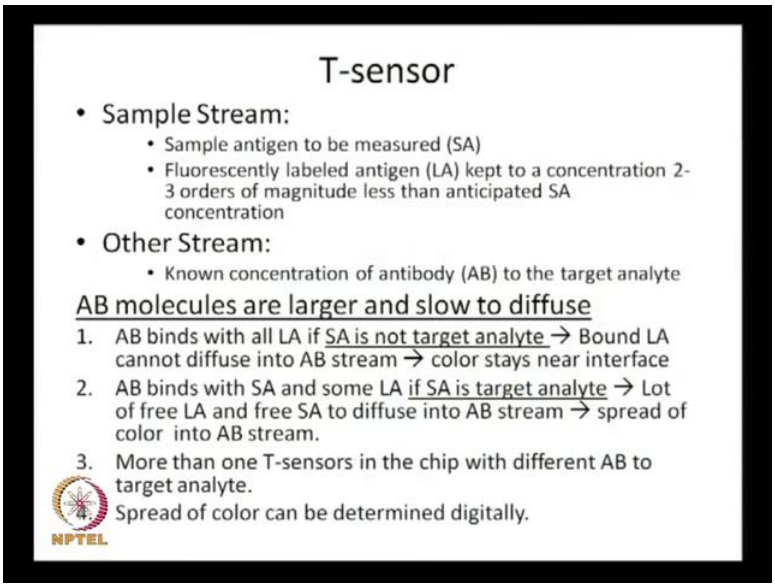

Next, what we want to discuss is at T-Sensor,because this is some element which you have probably not seen in a in microscale.This is unique device, but this is this become once upon a time this was very popular in microfluidic literature.ThisT-Sensor,it is basically nothing but a channel, it is nothing but a channel through whichtwo, there is provision for two liquids to enter into the channel,two liquids can enter into thechannel. So ideally you expect the liquid to bemoving like this,parallely, like two layerthe two layersthey are moving parallely. So, this is ideally that is what you expect, this is a channel with two liquids introduced. So, they will be moving parallelly, one layer sliding against the other.Now if you have, if you allow diffusion to take place in between, then you expect, suppose one is say having red color and other is colorless and one can mix with the other.

So in that case, you can have the color moving from one layer to the other. So, if the red color is, ifthis is say this is red color and this is say blackthis is a black color, you will findthere would be some amount of diffusion. So, the interface would bemore interface would be little blurred,because one layer will diffuse into the other.Now, what you have in a T-Sensor here is that you have a sample stream and other stream. So one is your, one is sample stream and otherthe other stream I am calling it say other stream.Now, sample stream contains sample antigen that you want to measure, and also a fluorescently labeled antigen that is kept to a concentration two to three orders of magnitude less than anticipated sample antigen concentration.

So, you have a sample streamthat contains samples antigen and a fluorescently labeled antigen,which we refer as LA. So, there is SA and there is this LA. So, one stream that is the sample stream,this sample stream contains SAand LA. So, SA plus LA that is what sample stream contains.And this other stream, that contains known concentration of antibody AB to the target analyte. So what that means is I want to find out what pathogen is there in a sample. So, I have several antibodies present and I would like to find out which antibody goes with the sample. So, I say I have say five antibodies and I know four will not go with the sample, the only one will go with the sample that is that target;that but that I do not know,I do not know what is this sample and antigen. So, sample antigen is something which we do which I do not know and I want to find out, what is the sample antigen.

So, I have introduced some amount of fluorescently labeled antigenthat is LA, which I introduced with the sample stream, but theLA concentration is very small.And then,I am having this two streams flowing side by side through this channel. So, the implication of this is that AB molecules, these antibody molecules they are larger and slow to defuse.Now,AB binds with all LA, if SA is not target analyte.SeeAB binds with LA;AB binds with SA,only if that is the corresponding AB.Because,I have five such AB's present, five such antibodies present, out of them only one can bind with the bind with SA, other will not.So, AB binds with all LA, if SA is not target analyte. So, bound LA cannot defuse into AB stream;once this LA is bound with the AB, because AB is a bulk here. So, color stage near interface, because LA gives you the color.LA gives you the color,so LAso, color stays near interface, because LA which gives the color that is bound with thebound with AB and AB is a bulkier molecules,soit cannot now defuse.

So, it will stay next to the interface.AB binds with SA and some LA, if SA is target analyte. So, if SA is target analyte, because SA is there in very largequantity; at the very outside I said that LA concentration is intentionally kept two to three orders of magnitude less than anticipated SA concentration. So, if in the event SA is the target analyte, ifin the even AB binds with this analyte;soin that case, there will be lot of free LA available.And this and free SA as well, because AB is all in fix quality and if this is binding both with LA and with SA. So there will be free SA,free LA available and this can defuse into AB stream.

So, AB stream means which stream, the other stream. So, I had originally sample which is SA plus LA and I have another stream, which is AB. now two are moving side by sideand then I will find that in most of the cases,there would be say this is the color. So, these color is penetrating maybe this is more deep and this is light. So, this part is probably light lighter, but there would be a penetration of color into the, into this other stream, which is predominantly AB. this penetration of color is possible only if this SA,if the AB that I introduced is actually the AB corresponds to the sample antigen.Butthis penetration of color is not possible, if this AB that I introduced is notit does not correspond to the sample antigen. So, I haveseveral such channels in place,I have several such and suppose,I can assumethis is my sample and this sample can contain say ten such pathogens,it is possible.I want to find out which one ispresent there.

So, I have the target. So, I identify the targetantigens and I get the antibodies and I put them in place and I flow them side by side; and I find, in one case there is diffusion of color deep into that other stream, deep into the antibody stream.And in other cases, the color remains mostly next the interface. So from that I can figure outwhich one is the target antigen. So, which pathogen I have in this sample. So, what we have here is that I said AB binds with SA, lot of free LA etcetera and spread of color into AB stream.More than one T-Sensor in a chip with different AB to target analyte, spread of color can be determined digitally.You can have an light source, you can have a light source and you can have a detection element placeplaced on the next to the wall of the microchannel.

And from that, theit can sense a digitally, it can go through some algorithm and it can tell you,this is the pathogen directly with thoughin thatfrom that scanner.I mean, we do not have to look at this and find outhow much the rate color as gone in and make decisions on your own,it is not that way.If it is it you can acquire the data digitally and have a software to analyze this and software to tell youthis is the pathogen.So, this is device which can be used to find out which pathogen is there in a sample.

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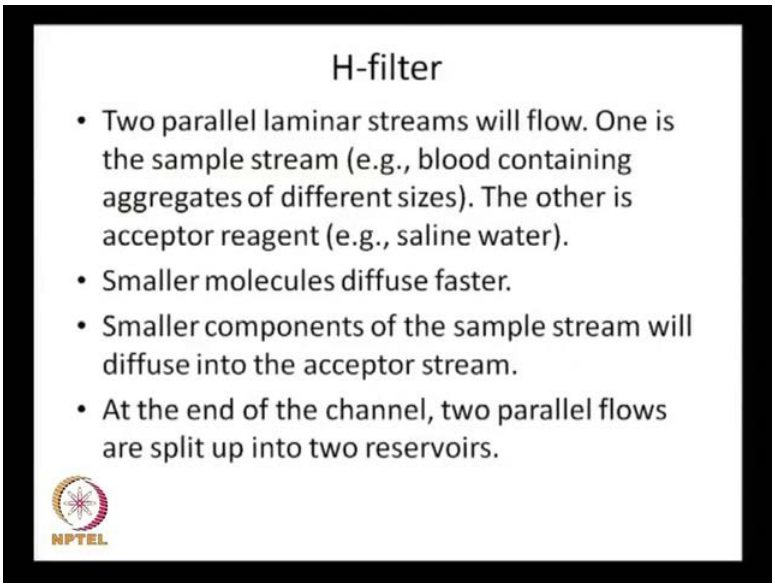

There is another element of interest here,I mean which is uniquethese elements are unique.I mean, you we thought that we know about flow andmacromacroscale processes, but in microchannelmicroscale processes there are some simpleelements that cando lot ofimportant work.Here you have two parallel lamina streams, two parallellamina streams will flow; one is the sample stream, such as blood containing aggregates of different sizes and the other is acceptor reagents, such as saline water. So, what you have in this case is you have a channel through which you havetwo streams flowing and they are flowing parallel to each other, one is blood containing aggregates, blood containingaggregates of different sizes and the other one is saline water.So, you have these two streams flowing side by side.The smaller molecules, the smaller components, smaller particles in blood thatyou will find that will be very easyfor these particles to defuse into the water stream;whereas, larger ones the diffusion will be easy.

So, if you collect these two streams out, what do you have?You split the two flows into reservoirs. So, what you find there is the upper stream that will contain blood without the smaller particles or smaller aggregates, because smaller ones have gone to the saline water. So, if you want to separate the smaller ones from blood or if you want to concentrate the blood or focus on thebigger particles within the blood;soyou can have such device.So, I said at the very outside that these laminar flow.Anddiffusion between two parallelparallely flowing layers, these would beusedin several elements of this microscale device and they do a very unique andvery useful job.

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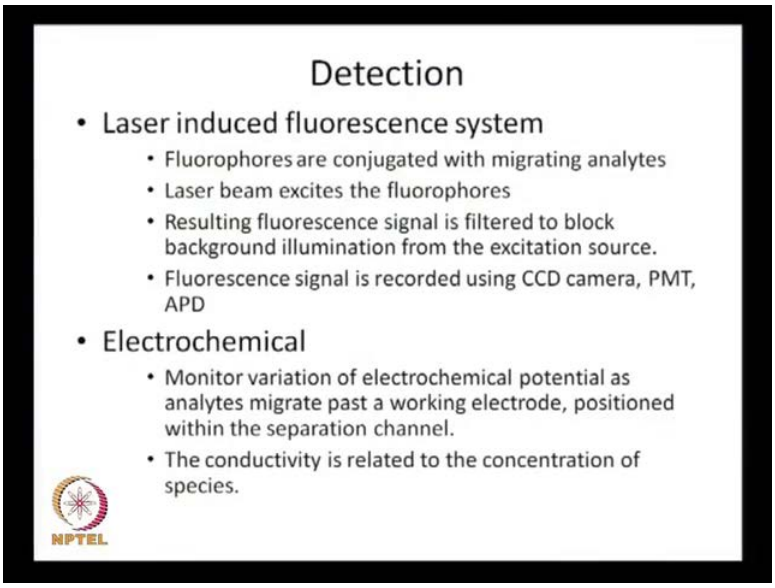

We discussed aboutthis detection, we said there are two types of detections possible; one is a optical interrogation that is what we said, laser induced flouresence system.And the other is the electrochemical method.In case of laser induced flouresence system,fluorophores are conjugated with migrating analytes; laser beam excites the fluorophores, resulting flouresence signal is filtered to block background illumination from the excitation source and flouresence signal is recorded using CCD camera.What these means is that you intentionally put a fluorescing,you intentionally put some fluorophores,they are conjugated with migrating analytes.

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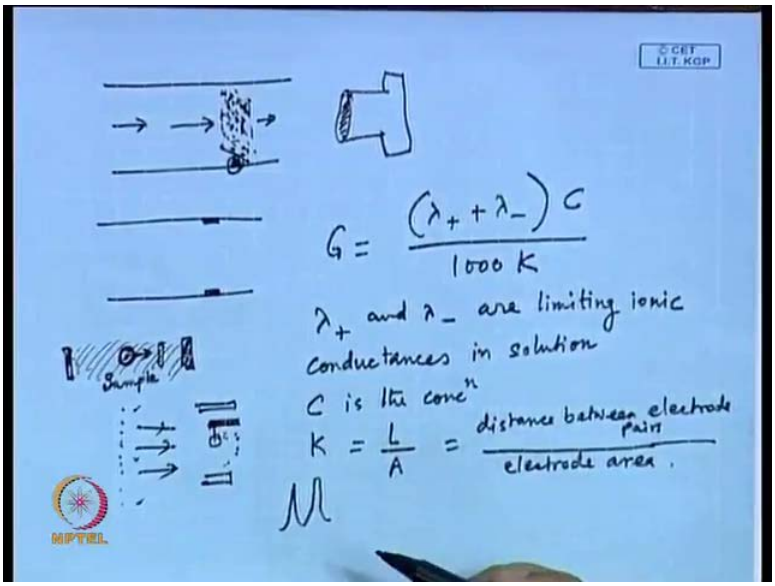


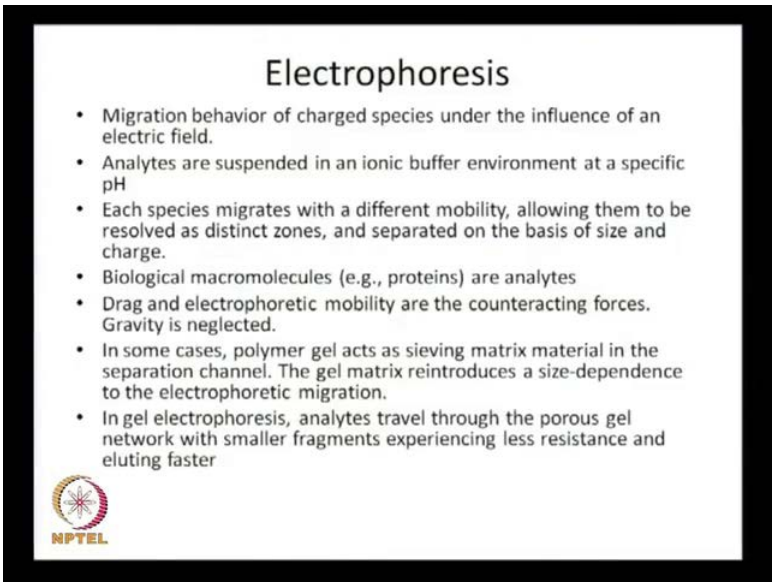
That means, I have a channeland I havesomesomeanalytes flowing through this;andI am introducingfluorophores that arefluorophores are conjugated with migrating analytes.What that meansis, suppose I have say calcium ionand that calcium ion I want to identify.So, I introduce a fluorophore, which would be,I introduce a fluorescing agent that ties up with calcium. So, they are ties up with calcium and then what we do is we put a lasered beam.We put a laseredbeam, sowe put the beam here; and withinthis beam say I put a laser beam,I illuminate this layer by using a laser source using this light source;I illuminate this layer.Andthis is continuously this stream is flowing through this. So, I illuminate this layer,I illuminate this layer means,I produce a light of certain wavelength; that is what the purpose of laser is.

It will produce light of certain wavelength that will go and heat the fluorophores.And you get the light; you get a fluorescence signal, which is probably at a different wavelength.And that signal is captured by a photo detector, say foror simply a camera; suppose this is a camera,by aby a CCD camera, by PMT photo multiply tube or APD,which is avalanche photodiode.By somephoto detector, you detect the light that is emanated that light that is coming out form this fluorophores. So, you intentionally add some chelating agent that ties up with the ion that you want to measure, say calcium ion you are measuringso it ties up with calcium.

And then, you find out what is the concept,then you introduce a light of certain wavelength and you know that the light, this light willwhen the they when it hits this fluorophores, you will get a fluorescence signal of certain wavelength.Nowon this camera or on this photo detector, if you have some kind of screen, some kind of filter to block the other the illuminations of other wavelengths; and only focus on that particular wavelength which this fluorophores can generate,then you can get a fair idea, what to what is exactly the concentration of the sample that you have. So, you havethis resulting fluorescence signal is filtered to block background illumination from the excitation source, fluorescence signal is recorded using CCD camera PMT or APD. So, this is a laser induced fluorescence system,this particularsource, this laser source and the detector, etcetera; these would be a part of the scanning device. So, these may not haveto be a part of themicrochannel, but this would be next to the microchannel and if this microchannel this microscale this wafer, if this is opticallytransparent, then it can be held the laser that that light source on the detector can be held next to it.

Now, there are other methods as well.For example, this electrochemical methods isalso there.In this method,what is written here is monitor variation of electrochemical properties, as analytes migrate past a working electrode, positioned within the separation channel, the conductivity is related to the concentration of species; that means, in the channel,you have electrodes positioned within the separation channel. So, you have electrodes position within the separation channel and you find out the conductivity asthis quantity.Here this lambda plus, these and lambda minus these are limiting ionic conductance limitingionic conductances in solution;C is the concentration;K is the cell constant.K is the cell constant that is basically that L is distance between,sothis is basically distance between electrode pairsdivided by the electrode area. Soby lookig at this conductivityand by having a precalibration done, you can figure out whichcomponent is eluting from the channel at what time. So you can identify the components.

(Refer Slide Time: 45:22)



I mentioned about this electrophoresis as one of the separation element.What we have in this electrophoresis here is that migration behavior of charge species under the influence of an electric field.Hereanalytes are suspended in an ionic buffer element at a specific pH.Each species migrates with a different mobility, allowing them to be resolved as distinct zones and separated on the basis of size and charge.Biological macromolecules are, biological macromolecules such as proteins are analytes.What we are talking about here is basically I have two electrodes.I havetwo electrodes and in between I have this

sample; so I have say one ion here, it has a size, it has a charge.And depending on what size and what charge it would be attracted towards certain electrodes.That is what we are talking about.Migration behavior of charge species under the influence of an electric field,analytes and suspended in an ionic buffer element at a specific pH;sothe pH isat a, at this is done at a specific pH.

Each species migrates with a different mobility.Now these, see these particles would be experiencing what.These particle under this electric field, it would be pooled and at the same time, there would be a drag experienced by this particle.So depending on,so there would be a force balance, drag and electric phonative mobility they are counteracting forces and typically gravity is neglected. So each species migrates with a different mobility, allowing them to be resolved as distinct zones.

So, if you start this process and may be freeze after sometime, take away the electric field; what you will find is, these particles they have rearrange, they haverepositioned themselves depending on their mobility. So, this is one way ofsay forming the or resolving the resolving this sample as into indistinct zones, separated on the basis of size and charge. So, you have distinct zones, suppose this sample containsa, b, c, d, e, f, g, h, out of them a, h, and k these belong to the same category, as far as the size and charge is concerned. So, they will band together in one location.Some other components, they will band together in another location. So, that is that would be eventually that is what is going to happen, if you have such system in place.And in some cases, on top of this, you have a polymer gel placed between these electrodes. So, basically sample is diffusing through this sample is moving through this polymer gel.

So in some cases, polymer gel acts as sieving matrix material in the separation channel.Gel matrix reintroduces a size dependence to the electrophoretic migration.That is in gel electrophoresis,analytes travel through the porous gel network with smaller fragments experiencingless resistance and eluting faster.Do you understand what a gel is, hydrogel it is basically made of water and small amount of polymer and cross linker is present there. So, this polymer chains they are cross link; that means, itsit could be possible that this cross linker that forms some kind of coordination complex betweenwith the polymer. So, there are several dangling chain of the polymers and they are tied together by the cross linker. So, you form anetwork here and water remains trapped within this network;so that is typically a hydrogel. So that is a gel we see.

Nowthis so, it has a structure. So gel has a structure, because these chains are all ready cross linked; and water remains strapped within this cross linkednetwork. So, this cross link network this acts as a sieving matrix; that means, if the moleculewith a sample I said contains a, b, c, d, e, f, etcetera, out of them, the components that are small that can move through this network easily, where as the components which are big that cannot move through this network.So byso, you are inyou are reintroducing, you are introducing another size dependence; you are introducing another size, another classification technique within the channel. So, this isbasically the,sothis is referred as gel electrophoresis. So, in gel electrophoresis analytes travel through the porous gel network with smaller fragments experiencing less resistance and eluting faster.

Now I want to,I want you to think of thisthat the picture that I had here, the picture that I have here, now if you invert this invert it this way, you have the electrodeshere and the sample is placed in between. So, if you move it by 90 degree.And then you have a laminar flow through this in laminar flow in this direction.Let us do not confuse ourselves with the presence of gel,you havethe two electrodes and thenyou orient them you rotate this by 90 degree. Sodepending on the size and the charge, the particles as I said a, d, and h, would besitting in at one layer. So, if you have the two electrodes present there and if you have the sample,sothey would be a attracted to,I said it is moved by 90 degree; it isoriented,it is rotated by 90 degree. So, if after sometime if you switch off, if you freeze then thisyou will find one layer, one band with a, g, andh, depending on the size and charge; another band with some other components that has similarmobility.

So, you will be producing bands within, sowithin this channel.And now, if you have are slowing, if you are having thislaminarflow, if you have a parabolic velocity distribution, one layer sliding against the other at different velocity. So, what you will find this you would be eluting layers based on their size, and charge. So, you will be producing as I said fractogram; one size, and then another size, and then another size. So, this is exactly what you call electrical field flow fractionation.I just now mentioned field flow fractionation of electrical type, where you have electric field to put the components in bands, and then eluting it using a parabolic velocity profile that is exactly what is electrical field flow fractionation.And that is what you can accomplish using electrophoresisin arotated frame.I would like to stop here.In the next class,I willprobably

complete thislab on a chipcomplete this discussion on lab on a chip, and then we willproceed further onbasics ofmicrofabrication.I stop here today.