Microfluidics
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Lecture - 40
Few Applications of Microfluidics

Okay, so let us continue our discussion on particle separation. So in this lecture we will finish our discussion on particle separation, and then look at some of the applications of microfluidics okay. So we have been talking about particle separation, we talked about passive methods of separation, and now here we will talk about some of the active separation methods okay.

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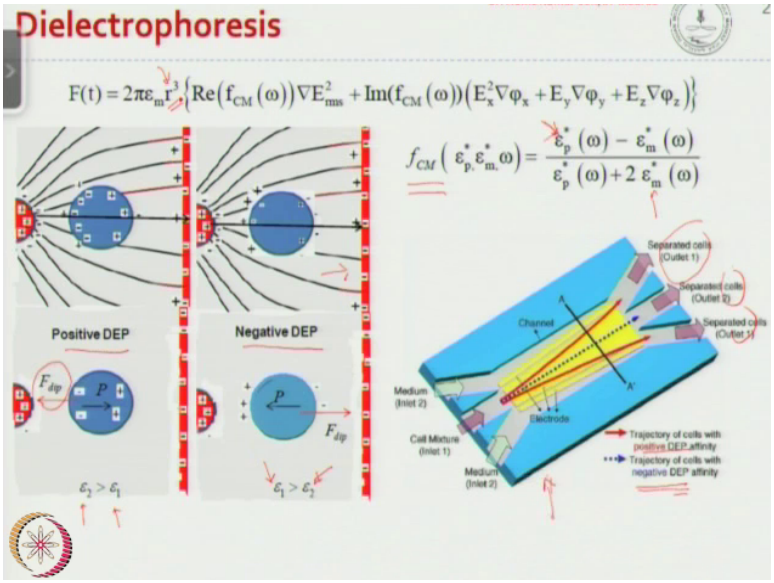

So the first active separation method that we will talk about is dielectrophoresis, the dielectrophoretic force is given by this expression okay 2 pi epsilon m which is the permittivity of the medium*the cube of the radius and the real part of the Clausius-Mossotti factor okay, and the gradient square of the gradient of the electric field. Now if you look at the Clausius-Mossotti factor it has 2 different components.

One is the complex permittivity of the particle and that of the medium okay, and which are functions of the applied frequency of the AC signal. Now here if you look at simple DC case, in one case the dielectric constant of the particle is more than that of the medium okay, so here the particle dielectric constant is more than that of the medium, and you can see that the net

dielectric force is towards the higher electric field gradient okay so that is known as positive DEP.

Here, in this case the medium has got higher permittivity than that of the particle, and the dielectric force is towards lower gradient of the electric field okay, so that is known as the negative dielectrophoresis. Now using this the Clausius-Mossotti factor will have different signs depending on whether the particle permittivity is higher or the medium permittivity is higher and as you can see here this has been utilized to separate particles okay.

So the 2 different particles in a sample exhibit different dielectric force, one type of particles or cells exhibit positive dielectrophoresis, so which have a tendency to go towards higher electric field or having positive electric field gradient in the direction of positive electric field gradient. Then one set of particles will exhibit negative DEP okay, they will tend to go towards lower electric field or negative electric field gradient.

So based on that principle a cell mixture has been separated okay, so the different outlets the different cells are being collected based on their nature of the dielectrophoretic force. Also the dielectrophoretic force can be used to separate particles based on the size of the particles okay, so if the size of the particle is big then for the same the relative permittivity the dielectrophoresis force is going to be higher for larger particles. So that also can be used to separate particles okay.

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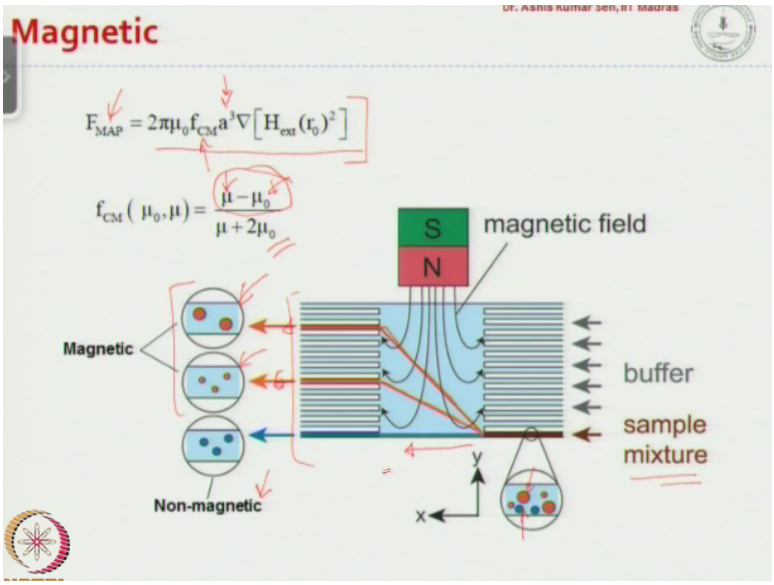

Next, we talk about magnetic based particle separation which also known as magnetophoresis, the magnetophoresis phoretic force is given by this expression. So it is a function of the size of the particle a cube and gradient of the magnetic field square of the magnetic field, and this is the magnetic Clausius-Mossotti factor equivalent to the electrical Clausius-Mossotti factor. So if this is depending on the permeability of the particle as well as the permeability of the medium okay.

So as you can see here we are introducing a sample mixture, which has a mixture of you know magnetic particles which are given by this red color, and these are magnetizable particles of different sizes, and this blue is a non magnetizable particle, the non-magnetizable particles are moving straight through the channel, because they are not perturbed by the magnetic field. So this is where we collect the non-magnetic particles.

The magnetizable particles get affected by the magnetic field, so there are different magnetic force act on the particle depending on their size and physical properties. So as you can see the larger size particles which subjected to larger magnetophoretic force, and smaller size particle will get subjected to smaller magnetophoretic force. So as a result the larger particles are collected here okay, it gets deflected more and gets collected here.

And smaller size particles deflected here and get selected here, so depending on the size, depending on the permeability of the particles with respect to the permeability of the medium the particles can be separated.

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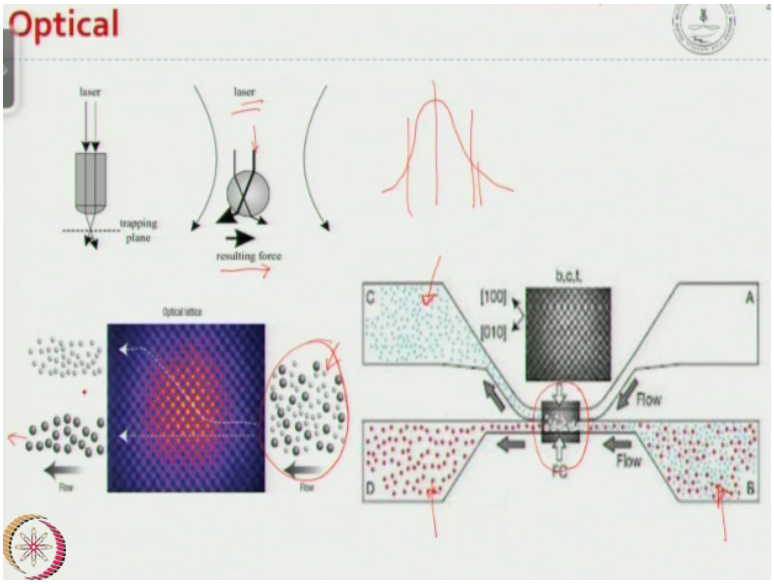

Next we talk about optical based particles separation, so in a typically when you talking about a light been, the beam will have a Gaussian profile okay, so the intensity will be higher at the middle and it will die down towards the edge of the beam. So when you have a laser beam coming here and it is interacting with particle, since the intensity will be maximum at the center and it will be reduced towards the edges the particle will be subjected to a resultant force towards the center of the beam okay.

Now in this case we are talking about creating an optical lattice structure here, and a sample that contains a particle mixture is allowed to flow through this optical lattice in the channel okay. Now what happens is that when you have this particle mixture coming in the larger particles which are affected more by the light beam tend to follow straight line and their collected here, and the unperturbed particles which are the smaller particles get deflected by the light beam and they are collected at this outlet okay.

So here the light beam is used as an actuation force to control the movement of the particles depending on size, and that is how they are separated.

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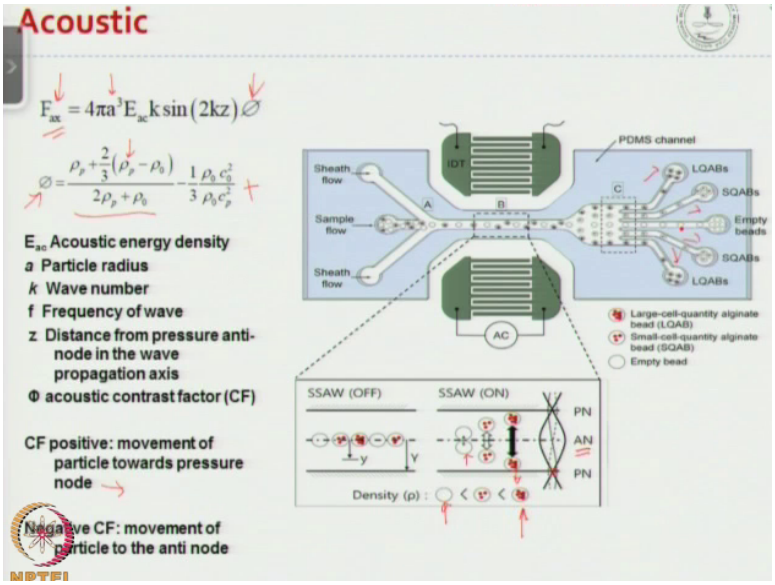

Here, we talk about acoustic based particle separation, the acoustic based particles separation this is the acoustophoretic force which is the function of size and it is also depends on the acoustic contrast factor phi, and phi is a function of the density of the particle and the density of the medium okay. Now the direction of the acoustophoretic force is dependent on the sign of the acoustic contrasts factor phi.

Now if a particle has got higher density if a particle have got higher density, then there is a larger chance that the acoustic contrast factor will be positive, and if the contrast factor is positive the particle have a tendency to go towards the pressure node okay. So as you can see here if this is positive the moment of the particle occurs towards the pressure node, so this density is higher okay so these particles go towards the pressure node.

So this pressure node can be established using interdigitated electrode pattern on the surface of the channel, and by adjusting the frequency of the wave and amplitude of the wave the pressure node and antinode can be created okay. So here in this particular case the pressure node is at the edge of the channel, whereas the antinode is at the center of the channel, now the particle that has got a higher density is migrating towards the pressure node so it goes to closer to the channel wall.

Whereas the particle having lower density is going towards the antinode meaning the center of the channel. So based on that principle as you can see here particles of different density are separated at different outlets okay, so that is the principle based on which acoustophoresis works. So we have looked at different techniques of active separation, earlier we looked at the passive particle separation methods.

Now we will move on and talk about some of the applications of microfluidics okay. The first application that we can talk about is the use of microfluidics for separation of microparticles, so we will talk about how a microfluidic device can be designed, fabricated and experiments could be performed to demonstrate separation of particles okay.

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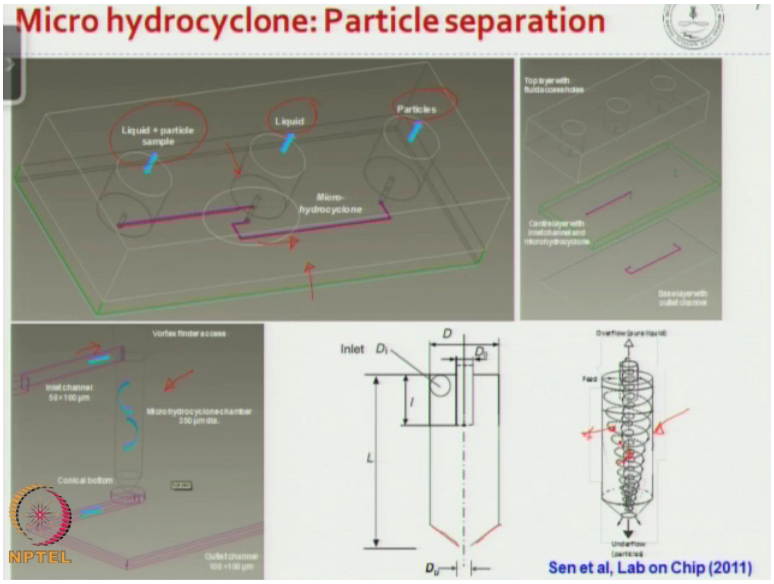

So here we talk about a micro hydrocyclone that has been used to separate particles, so this is a micro hydrocyclone based microfluidic chip, so it has 1 inlet where the liquid and particle sample come in, and the liquid will it comes into this the hydrocyclone structure it comes into the hydrocyclone structure, and the liquid exit from the top of the hydrocyclone where at the bottom the concentrated particles come out and exit here okay.

So this device is used for separating liquids and particles from a liquid particle sample. So this is as you can see here, this is a closer view of the hydrocyclone, so it is basically consists of a cylindrical chamber with frustoconical bottom, now the sample is injected in a direction

tangential to the cylindrical chamber okay. And when we do so, the sample takes a helical path going down okay.

So when it follows a helical path as you can see here because of the centrifugal force the particles go towards the wall and to satisfy a mass conservation the liquid pure liquid comes towards the center okay. So the particles will have a net outward force acting on them, and that outward force will also act down so it is outward and down, so the particle will follow the spiral and going down.

And the pure liquid will come towards the center and it also will have a vertical component of the velocity so they will move up okay. So the particles are spiraling down and the liquid is spiraling up okay, so that is how the particles and liquids are going to be separated.

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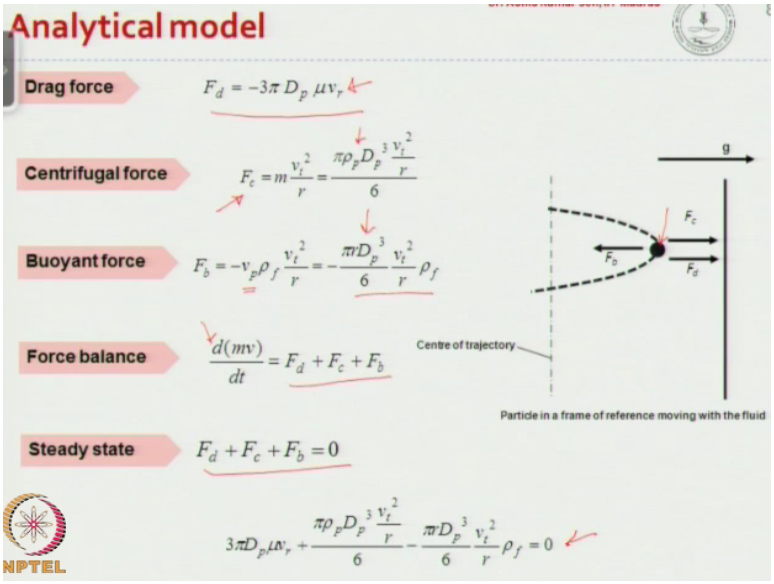

So before we designed the device you know the in analytical model was developed to look at the physics based on which this particular separation is achieved, so there are different forces that act on a particle that is going through the trajectory of the spiral path. One is the drag force which is given by the -3 pi*the diameter of the particle mu*vr, mu is the viscosity, and vr is the radial component of velocity.

And this is the centripetal force which is m vt square/r, so we can express m in terms of density and diameter of the particle. The third force is the buoyant force which is -of vp volume of the particle*the density of the fluid and vt square/r which we can express in terms of diameter of the particle and density, so this is the expression for buoyancy force. So we can do a force balance okay, so d m v /dt is the summation of the three different forces that we talked about. And in the steady state the summation of the force is going to be 0, so all these forces add up to 0.

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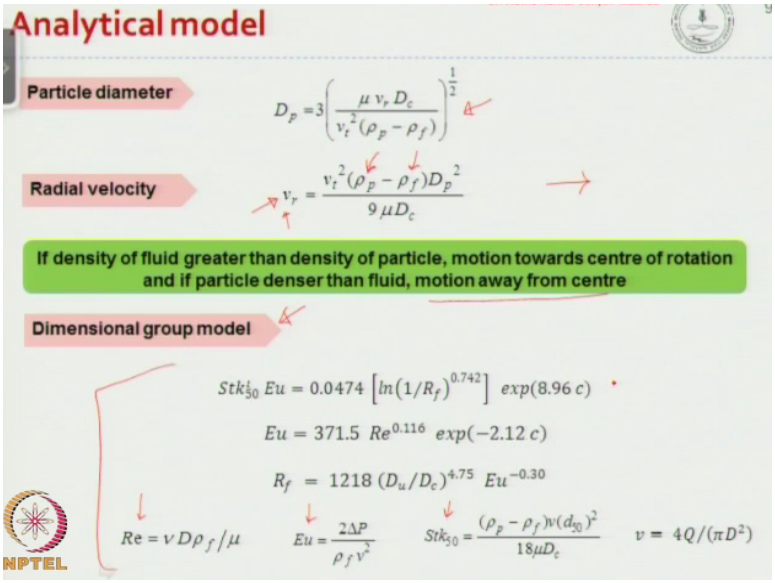

So if you do so we can get an expression for the diameter of the particle, and we can also get an expression for the radial velocity okay. Now if you see this expression, if the density of the particle is larger than the density of the fluid the particle will get subjected to a positive radial force in the outer direction okay, so you know and in for many of the bio-particles that we encounter in microfluidics the density of the particular is greater than the density of the fluid.

So you would have a net positive radial velocity acting on them, so that would separate the particles towards the channel wall towards the hydrocyclone wall okay, so you know if the density of the fluid is greater than the density of the particle the motion is towards the you know the motion is outward, and so that is why the particles go outside you know towards the periphery of the micro hydrocyclone.

The design of the hydrocyclone was based on the dimensional group model, which has been established for micro scale and meso scale hydrocyclone, and we had extended that for the micro hydrocyclone. And here so these are some important parameters the Reynolds number, the Euler number and Stokes number which was used to design the micro hydro cyclone.

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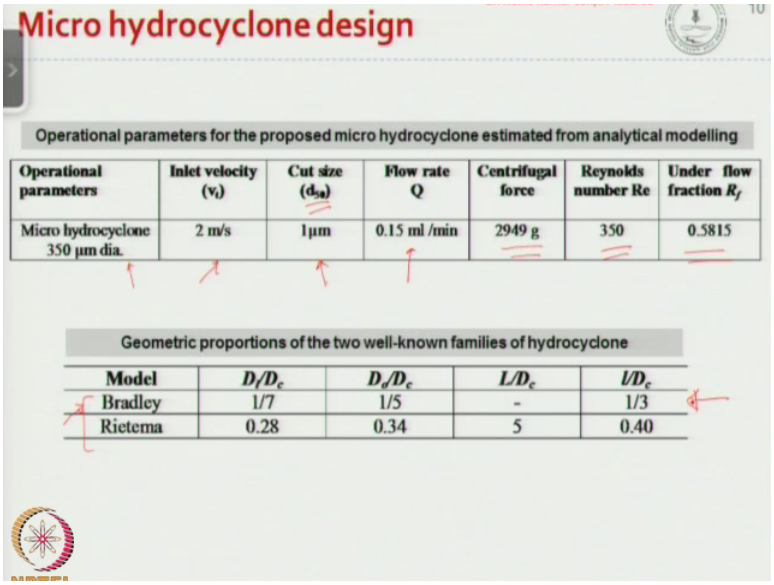

And based on the design these geometrical parameters were identified, so the hydrocyclone had 350 micron diameter, this is the inlet velocity this is the cut size, cut size is the minimum size of the particle that can be separated with 50% efficiency that means only 50% of the particle will come down okay, so that is the minimum size of the particle that is possible okay that is known as the cut size. So flow rate typical flow rate is 0.15 milliliter per minute.

And from there we could estimate the centrifugal force, Reynolds number and the underflow fraction how much liquid and particle are going down at the bottom. So these are 2 different models Bradley model and Rietema model, and the Bradley model offers lower cut size of the particle, so we have used the Bradley model in this you know design of the micro hydrocyclone, so these are different parameters that are specific to the Bradley model.

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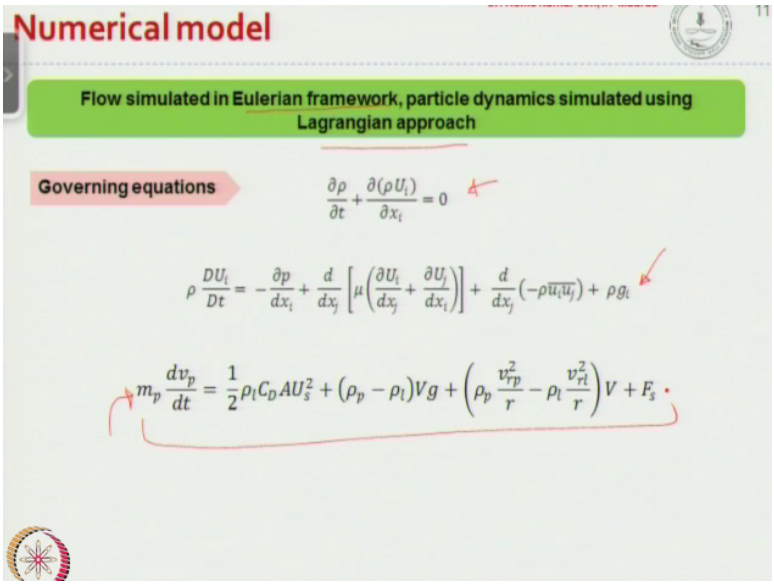

So first the numerical simulations we have performed, so it is basically simulating the flow in Eulerian framework okay, so Eulerian Framework are used to solve the flow, and the dynamics of the particle is solved using the Lagrangian approach. So these are the governing equations, this is the continuity or conservation of mass equation, and these are the momentum equations, and this is the particle tracking equation.

So this takes a force balance on the particle to track it inside the fluid it okay. So what we do is we first solve the flow field okay, and then inject the particles of the inlet, and the location of the particle is tracked using the particle tracking equation, and once the particle position and the velocities are estimated its effect on the flow field is calculated, and this process is continued in the subsequent iterations okay until the solution is converged.

So at every time step we identify we solve the flow field then from that the parameters are used to solve for the particle, the effect of the particle is again taken into the flow field, and this continues until and converse solution is obtained at each time step okay, and then we proceed to the next time step okay.

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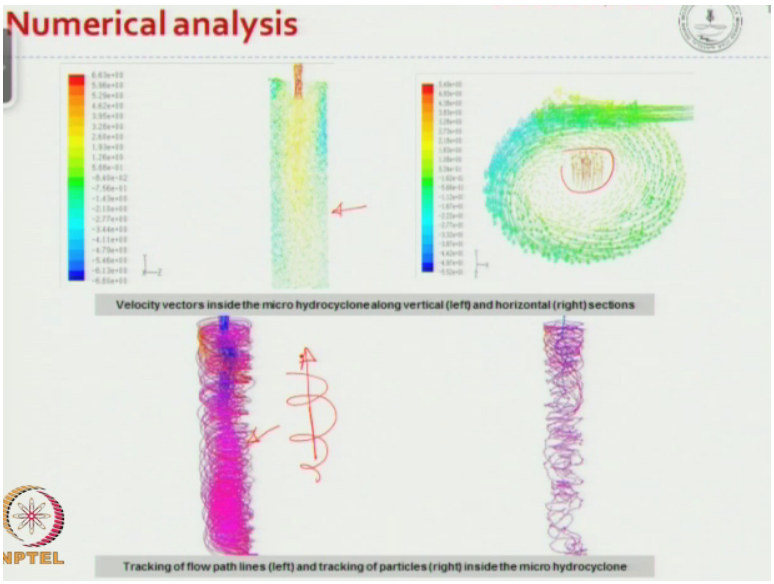

So here this shows the solution here this is the numerical simulation, here you can see that the particles have a tendency to go and follow the helical path, and it has the net downward velocity as you can see, and the liquids have a net upward velocity at the center. So this is confirmed by the tracking lines as you can see the particles are spiraling down as it goes okay, so it spirals down and the liquids are going up at the center.

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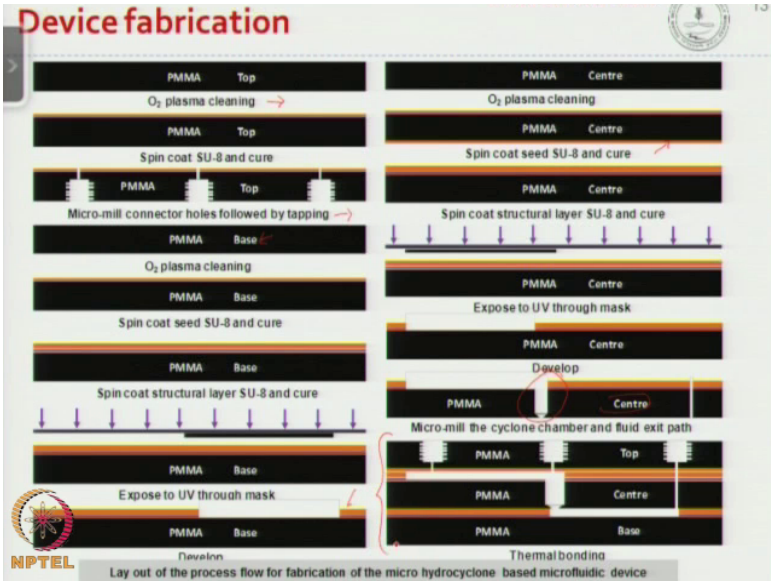

So after the simulation the device was fabricated, so it is fabricated based on a combination of you know photolithography and bonding. So it starts with a PMMA wafer, cleaning with oxygen plasma, and then the interconnectors where micro-milled using micro-milling, and then followed

by tapping, and then the surfaces this is for the top surface so the top surface was cleaned by oxygen plasma, then spin coated with SU-8 and cure.

And then the inter connector connection holes are machined, and the taps where done to so that the threaded connector can be accommodated, this is base on which the spin coating was done, and there was exposure and development to create the channel structure, and the center layer this is the center layer which is cleaned, and you know photolithography was done by UV exposure through the mask, then it was developed.

And the hydrocyclone was micro-milled using special milling to with an inverse taper at the bottom, and this was done in the center layer. And once the top base and the central layers where fabricated they were bonded thermally okay using thermal bonding.

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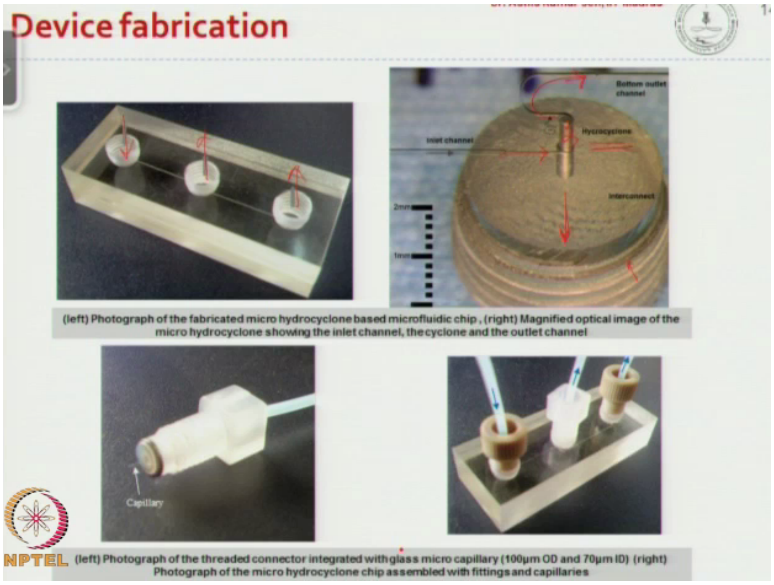

So this is the device that was fabricated, so it has the sample inlet and here the liquid comes out, here the particle come out. As you can see here this is the channel that comes into the hydrocyclone, and the particle spiral down and exit here, and the liquid move up, and this is the connector in the background that you see, so the liquid exit here and the particles exit there okay. So you know this was the device that was fabricated.

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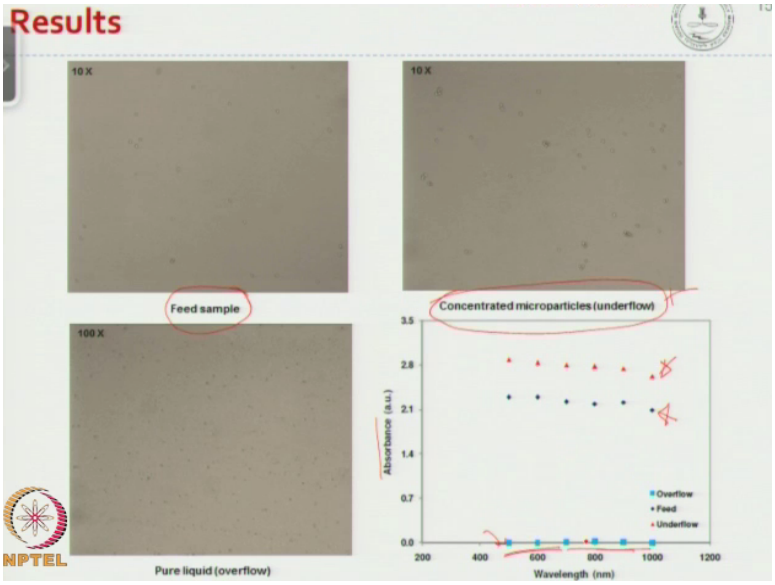

And experiments were performed with particles, so as you can see here this is the feed sample that is put into the device, and this is pure liquid that was collected on the top of the hydrocyclone, and as a result this is the concentrated political that was collected down okay. And also the absorbance measurements were performed, you can see that this is the you know absorbance that is offered by the feed what comes in as the sample.

And this is the absorbance of the concentrated microparticles the separated microparticles which absorbance is higher as compared to the feed, and the overflow which is pure liquid has got negligible absorbance okay meaning there is no particles present in the pure liquid in the overflow okay.

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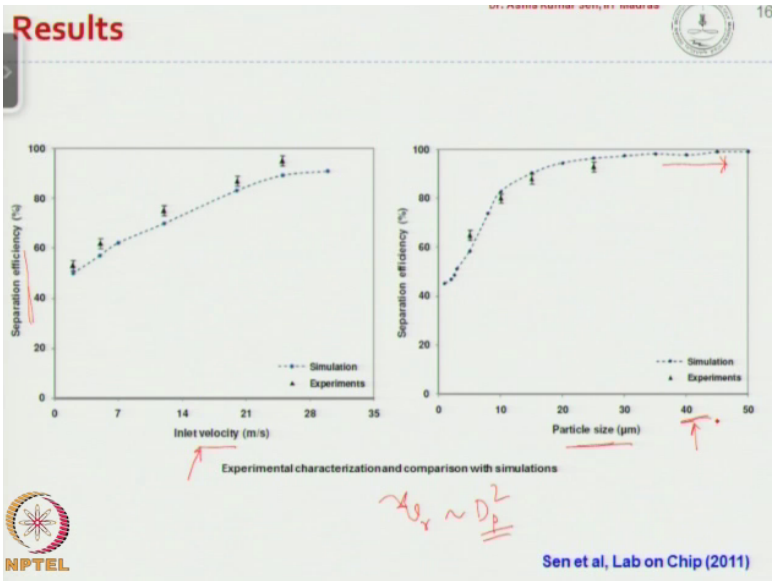

So here we did you know the effect of inlet velocity on the separation efficiency was studied using both simulation and experiment, as you can see that the separation efficiency increases with the inlet velocity. So as the inlet velocity increases the centrifugal force also increases which leads to higher separation efficiency. Here, you can see the increase in the particle size versus separation efficiency.

And from the analytical model we have seen that the radial velocity varies as the particle square okay the diameter of the particles square. So as a result, as you increase the particle size the radial velocity is going to increase, then that gives a higher separation efficiency, but it saturates down for a larger particle size where some kind of choking effect comes in there okay.

So the particle size becomes too large as compared to the overall diameter of the hydrocyclone itself, some kind of choking effect comes in and the separation efficiency does not increase further with increase in particle size. So now we look at a technique that can be used for detection of microparticles okay, which is based on what is called microflow cytometer.

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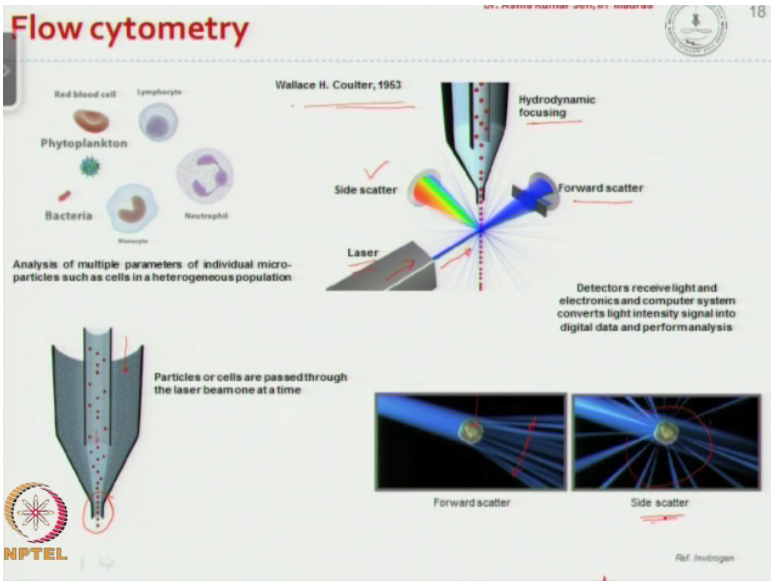

So this is the method of flow cytometry that is used for detection of microparticles, so different microparticles like blood cells, phytoplankton's, bacteria they can be all detected using micro flow cytometer. So this is the principle of the Coulter flow cytometer as you can see here, it basically has got 2 components, one is the fluidic component, the other one is the optoelectronics.

The fluidic component tries to focus the particles that we want to detect by a pure fluid into a single stream of particles, so the particle move single file one by one through a stream, and these particles are you now are interfered by a light beam that is produced by a laser, and as the particle crossed the light beam the particles will scattered light, so the scattered light in the forward direction above which is about you know 5 to 10 degrees to the direction of the incident light which is called forward scatter.

And the light in all other direction is known as side scatter okay, so here you see how the sheath fluid is going to focus the sample to enable the particles to move single file, and this is a particle which is trying to cross the light beam, and this light here is known as the forward scatter, and the light in all other direction is known as side scatter.

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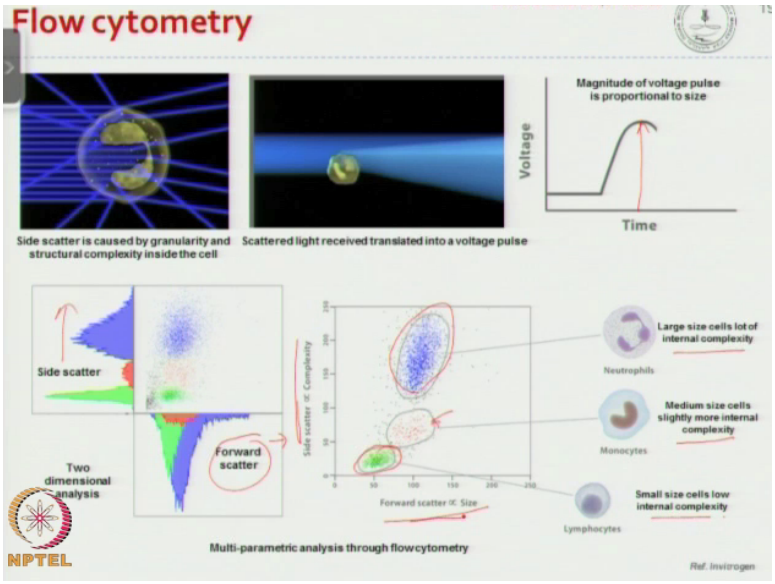

So the forward scatter is caused by the size of the particle, but the side scatter is caused by the internal structure of the particle okay. So when the particle crosses you know the light as you can see here the light signal is collected by what is called a photomultiplier tube and what is known as PMT, and the PMT basically converts the light energy into electrical energy okay. So this electrical energy can be collected using a data acquisition system.

And on the voltage time spectrum as the particle crosses the beam you would see a peak in the spectrum, so depending on the number of particles crossing the beam you will have as many peaks in the voltage time spectrum, and using this voltage line spectrum we can also you know come up with something which is called 2 dimensional analysis, 2 dimensional plot, where we would show the forward scatter in the x-axis and side scatter in the y axis.

So this is known as 2 dimensional plot in flow cytometry. So here as you can see on x axis we have forward scatter proportional to the size, and side scatter which is proportional to the complexity. And based on the 2-dimensional plot different blood cells have been categorized, so here in this colony we have lymphocytes which is small size cells with low internal complexity, and followed by that we have medium sized cells with slightly more internal complexity.

And then we have neutrophils which are larger size cells with lot of internal complexity. So that is how flow cytometry can be used to you know the counting of the particle by counting the peak, they can also be used for characterization of the particle using 2 dimensional scatter plot.

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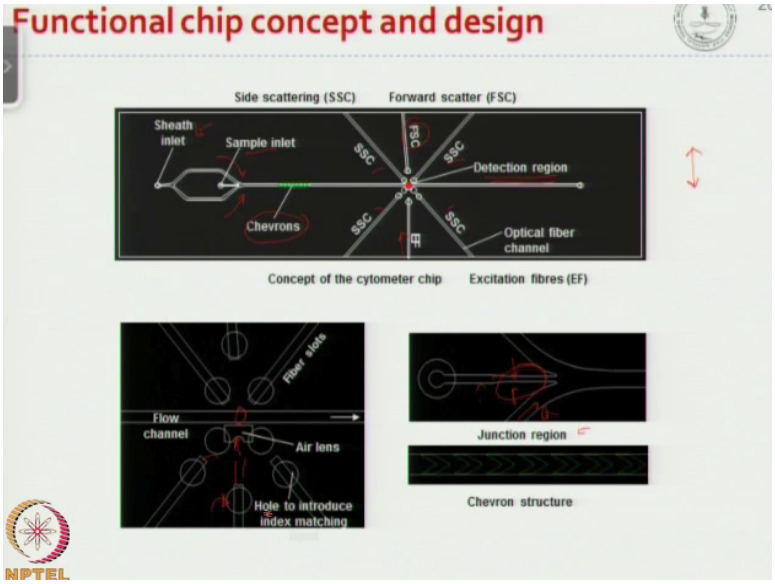

Now to fabricate a microfluidic chip as in a microchannels are 2 dimensional in nature, so this is one example of the chip that was fabricated, so here one important requirement is to focus the sample particles okay. So to do focusing in the horizontal direction we would have sheath through it is coming from both sides to focus the particles, to focus in the vertical direction we would have this chevron structures at the bottom and top of the channel to focus the particles in the vertical direction okay.

So you know particles will move single file through this detection region, and we have this excitation fibre which will excite light at certain wavelength, and we will be collecting this forward scatter as well as side scatter lights okay. And these optical fibers can be used to collect this forward scatter and side scatter lights which can be connected to photomultiplier tube to convert it into electrical energy, and using a data acquisition system we can acquire the data.

Some of the design considerations here, typically the sheath fluid flow rate will be you know 10 to the 100 times higher than the sample flow rate, and so there could be a possibility of backflow okay, so since this pressure will be very high as compared to this pressure because this flow rate

is going to be several orders of magnitude higher than the sample flow rate, then there could be a possibility of the backflow.

Now to prevent backflow the resistance of the channel is increased by reducing the channel size okay, so that is one design consideration. The other optical designs that have been used so you know there is this is an air lens which is machine pocket, which is used for collimating the beam that is coming from the fibre and going into this detection region, and you know when you insert these optical fibers there is always going to be a gap between the ends of the optical fibers and the ends of this slots. So to fill the gap you know the index matching liquid is introduced through this holes okay.

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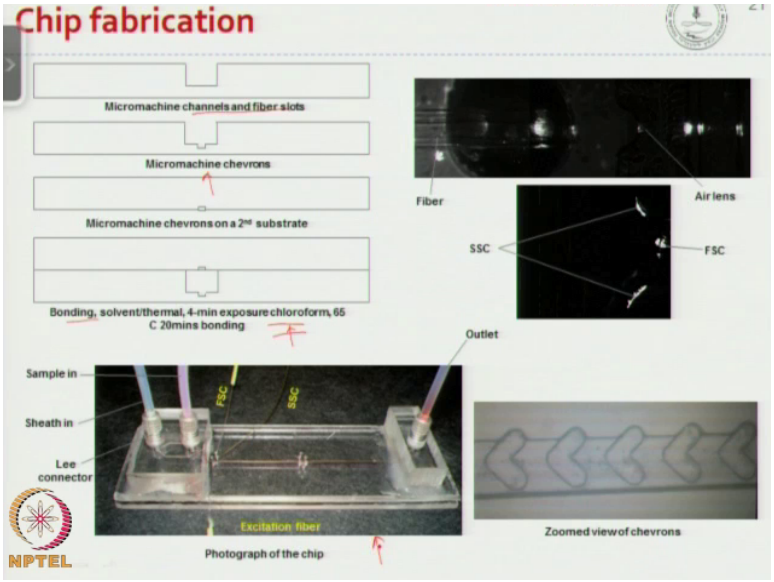

So this is the chip fabrication process, it uses micro-milling and bonding, so it is basically micromachining the channels and the fibers slots, and then micromachining the chevrons at the bottom of the channel, and then a bonding the 2 different wafers using the solvent bonding okay, here chloroform used for the solvent bonding process. So this is an image of the chip that has been fabricated using this method okay, and this is the microflow cytometer chip that we are talking about.

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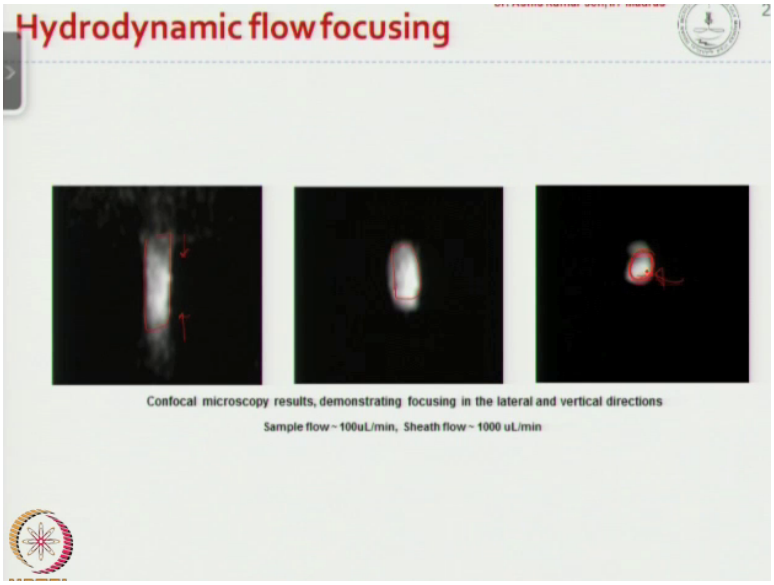

So first hydrodynamic flow focusing was demonstrated, so this is here this is as somewhere at the beginning of the channel where the fluid is touching the top and bottom walls, and then after few pairs of chevrons, this fluid has been focused in the vertical direction, so this is the size of the liquid stream. And here a perfect three-dimensional focusing has been achieved, where the stream width, the diameter of the stream is the size of the stream equal to of the order of the size of the particles, so the particles can move only single file.

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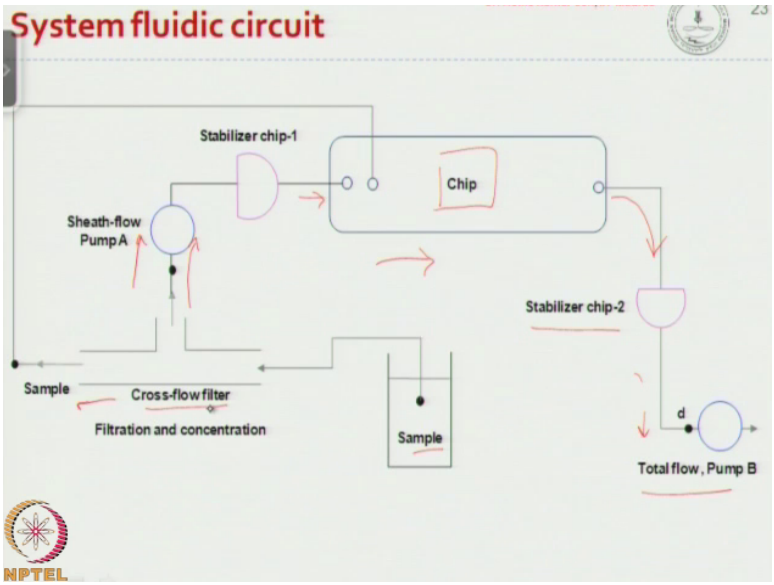

So this is the system fluidic we have sample coming in here, and it gets into a cross flow filter which basically filters the pure fluid which is used for focusing the sample. And here concentrated sample is coming in which is being focused by the pure fluid, so that all the

detection is happening here, and then the particles exit okay. And this the fluidic transferred is controlled by the 2 different pumps.

One pump is using for pulling and this pump is used for pushing, and this push-pull arrangement has been used to transport the fluid through the chip.

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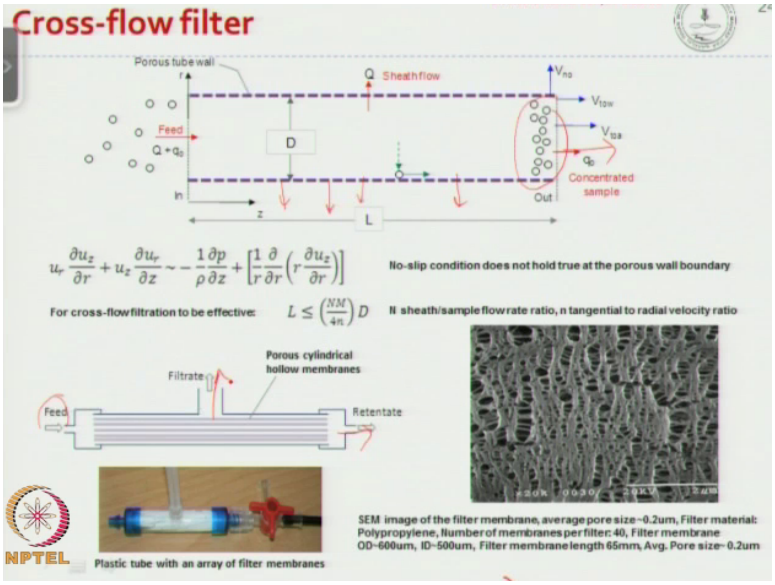

So here as we discussed there is a cross flow filter being used for filtration of the sample and the sheath fluids, and this cross flow filter basically uses a you know uses an array of hollow cylindrical porous membranes, where the particles can come in and the sheath fluid can exit around the perimeter, and the concentrated particle exit at the other end. So that is how we can bring in sample and concentrate the particle and simultaneously collect the pure fluid.

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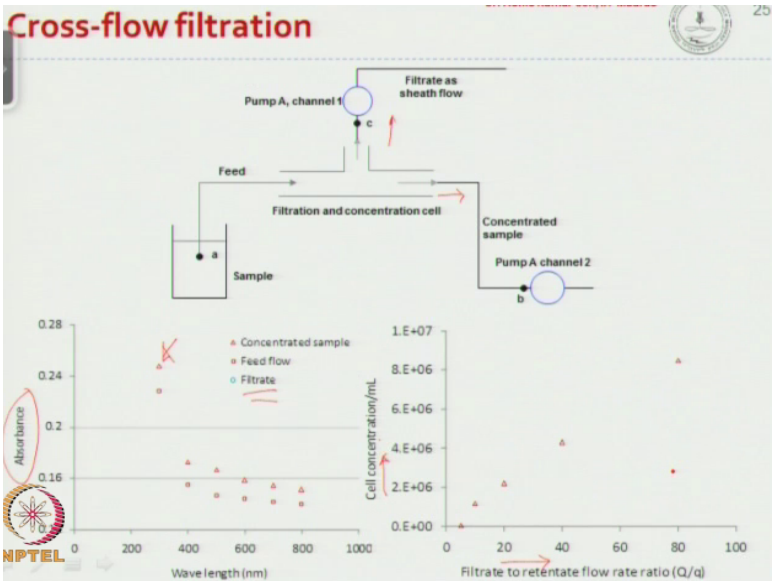

So this mechanism of the cross flow filtration was first demonstrated using absorbance's measurements, and it was found that the concentration of the concentrated sample the absorbance of the concentrated sample is much higher as compared to the feed flow rate, and the filtrate or the pure fluid does not have any particle present. Also it is studied here the effect of the filtrate retentate flow ratio, this is the retentate which is comes out as the concentrated liquid, filtrate it is pure fluid.

So the filtrate to retentate flow rate ratio and it effects on the cell concentration okay, so what it tells that as the filter retentate flow rate ratio increases the cell concentration is increased okay, so what it means that there is no clogging that is being observed in this cross flow filter.

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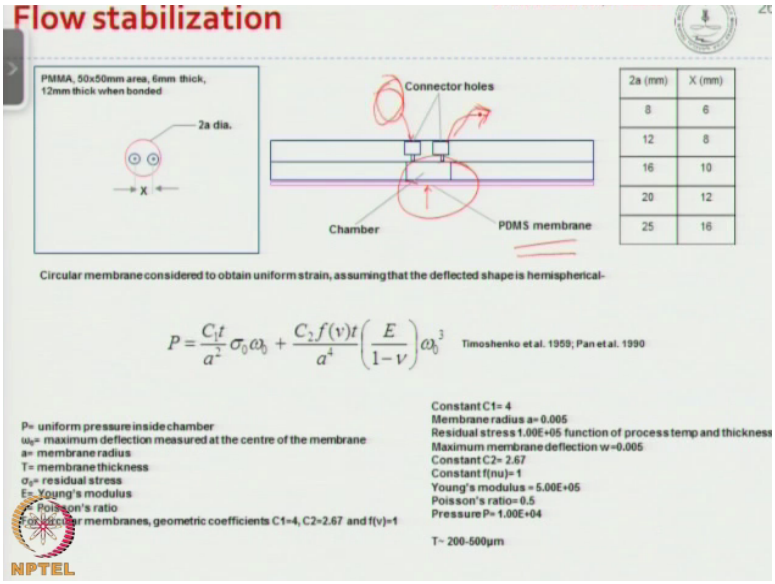

Another mechanism which has been used in this particular micro flow cytometer is a flow stabilizer, as we know that you know to count the particles we need the particle single file, but at the same time this location of the particles need to do not change with respect to the light beam okay, and to achieve that the flows should be very steady, and most of the microfluidic pumps including change pumps are pulsatile in nature.

So to make them stable you know one such mechanisms is included as you can see here, so this is the flow stabilizer chip it has an inlet connected to the supply, and this is connected to the flow cytometer chip, and this chamber has got a flexible membrane so this membrane this is a PDMS membrane which is flexible, so this arrangement basically takes out any pulsation that is coming in the fluid from the pumps, and it provides absolutely steady flow into the flow cytometer chip.

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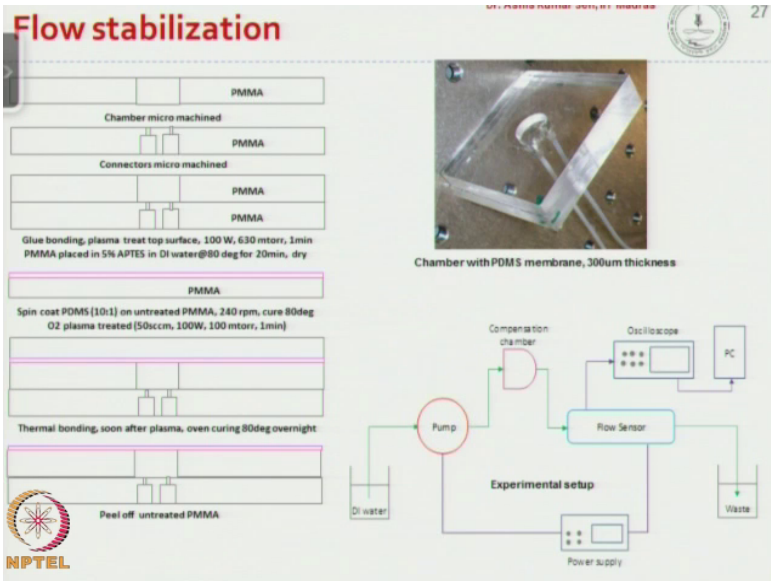

So this is the fabrication approach that has been used to fabricate this flow stabilizer chip.

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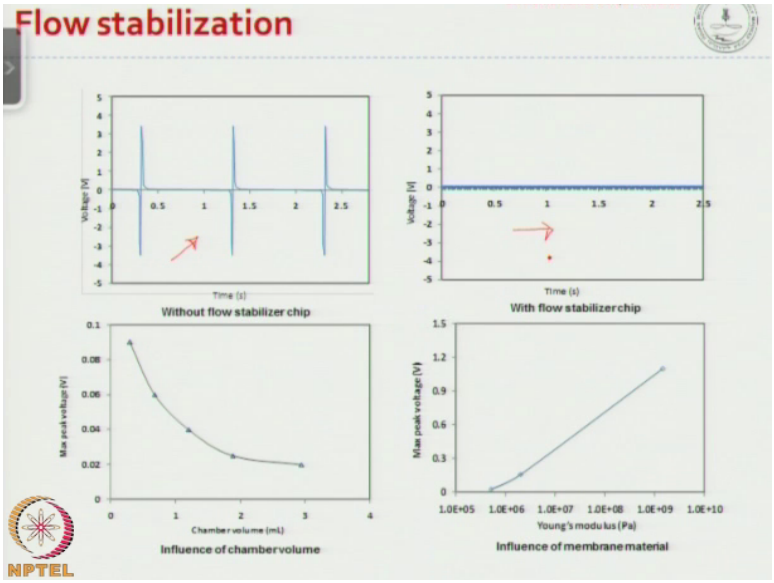

And here we show that by using the flow stabilizer chip that typically you know oscillating flow becomes steady with the time, which is essential for flow cytometer applications.

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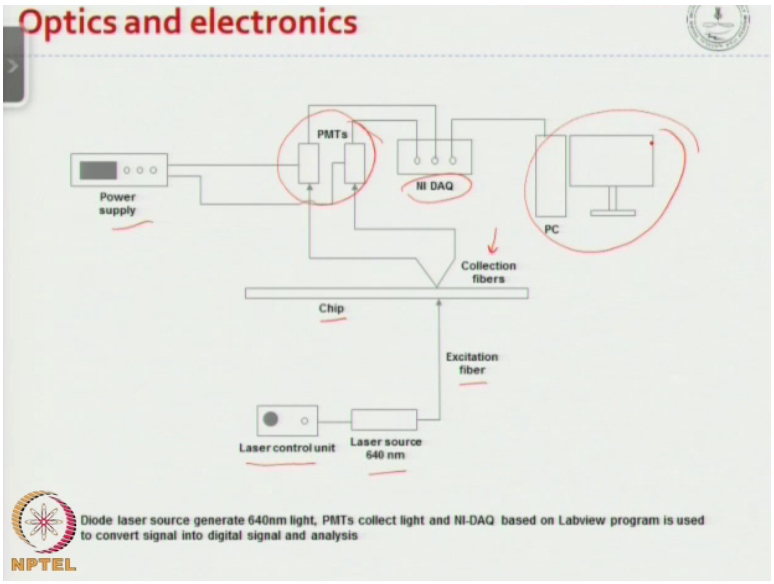

So this is the general arrangement of the experimental setup, we would have a chip, and we have already shown the fluidics circuit, here we show the optoelectronics, so here the laser control unit is arriving a laser source at 640 nanometer, this is the excitation fibre, and these 2 are the collection fibers we are collecting light and they are taking it to photomultiplier tube, which you know driven by this power supply.

And the PMTs convert this light energy into electrical energy which is red by a data acquisition system through a software interface like Labview and red on a PC okay. So that is the general arrangement of the optoelectronics.

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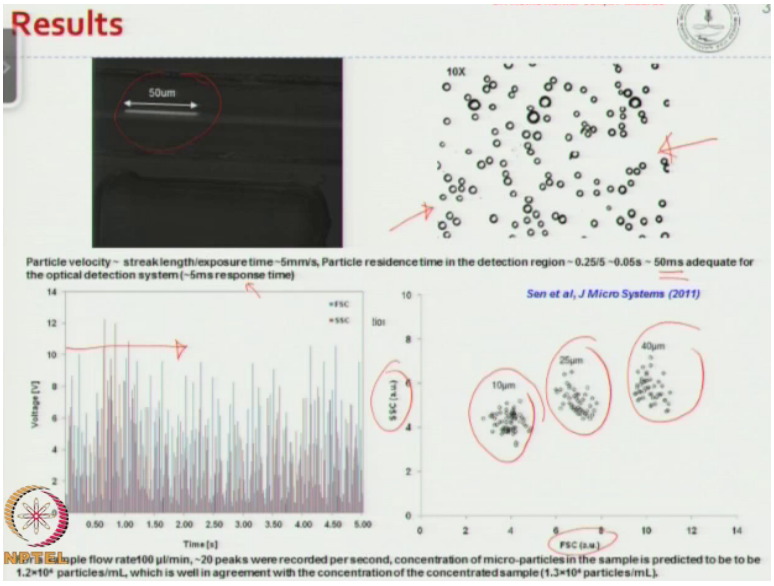

So another important parameter to check here was the residence time of the particle in the detection window, so we could find from the vertical velocity using optical measurements, we could find the residence time is about 50 millisecond which is adequate for the optical detection system that was used which has a response time of about 5 millisecond. Now next, a mixture of particles where put into the microflow cytometer.

And this is the voltage time spectrum shows the number of the particle that are crossing through the deduction point, and by counting each of these peaks in the spectrum we can and knowing the flow rate we can calculate the concentration of the particle in the original sample okay. And here based on the 2-dimensional plot based on the forward scatter and the side scatter we are able to separate the particles of different sizes are present in the mixture okay.

So that talks about a microflow cytometer that has been used for counting of particles, and detection of different particles present in a sample okay. So next we move on and talk about a platform that has been used for detection of bacteria.

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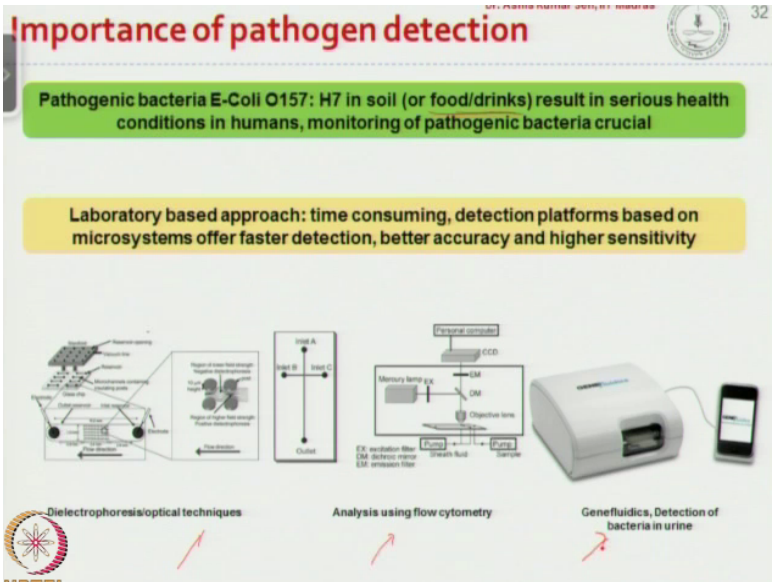

So detection of pathogenic bacteria has great importance in a variety of industries in food and chemical industry, in drinks, and so you know there are a number of platforms have been developed for detection of bacteria.

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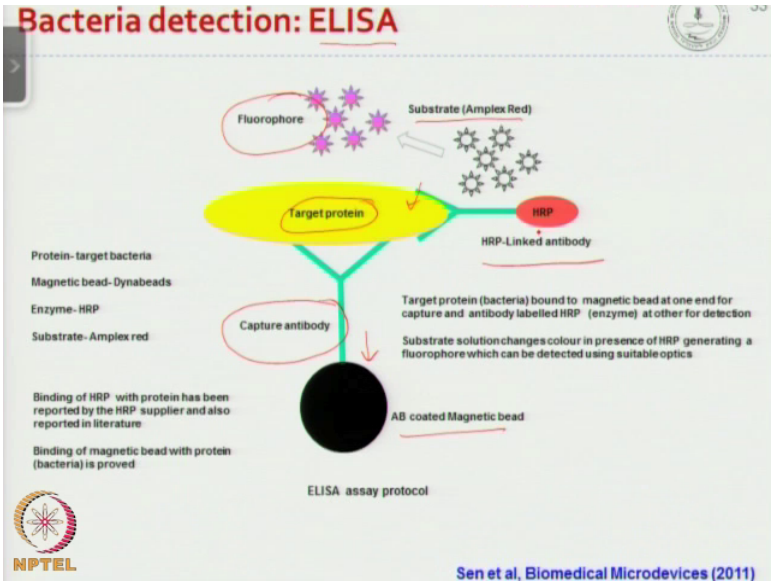

And here we present a microfluidics system that has been developed for detection of bacteria which is based on the ELISA technique which is Enzyme-linked immunosorbent assay okay. So essentially this is the target protein which is the bacteria, and the first we try to capture the bacteria inside the microfluidic device and that is done by capture antibody which is coated onto a magnetic bead, so that capturing is done by this magnetic bead.

And once we capture the bacteria inside the microfluidic chip, we bring in an antibody enzyme which is coated with an antibody to bind this bacteria, and once this HRP enzyme is bound to the target bacteria we introduce a substrate solution which generate a fluorophore in present of the enzyme, so that is the basic mechanism.

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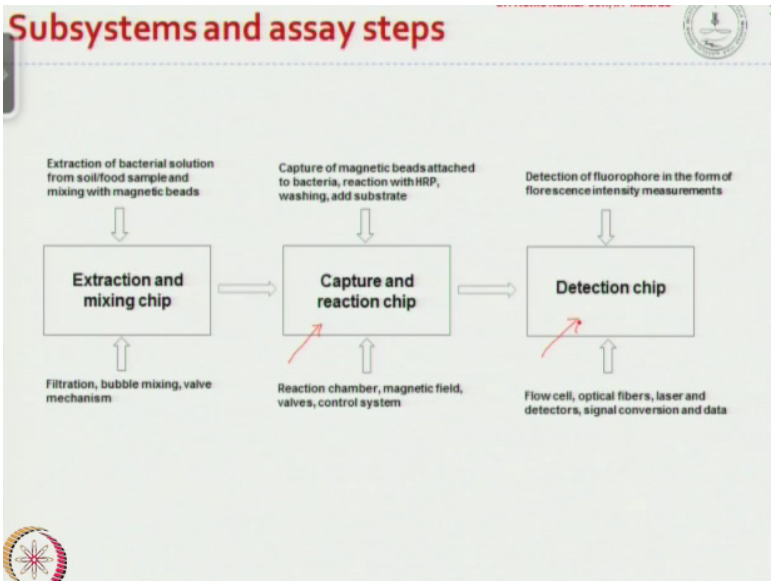

So the system that we talk about has 3 different chips, one is the extraction chip which extract bacteria from soil, and the second chip is the capture chip which captures bacteria inside a microfluidic device, and the third chip is the detection chip which actually detects based on the fluorescence measurement,

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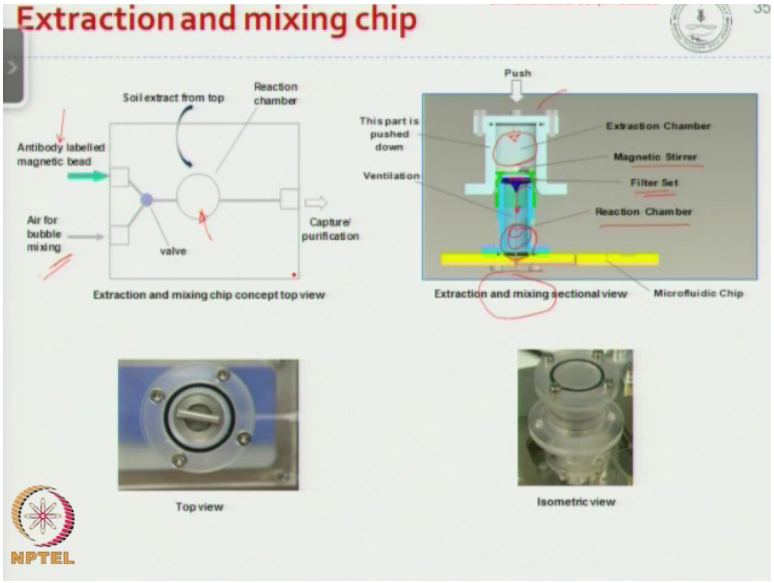

So this is the extraction chip, it has the plunger arrangement so you know here the top cover is taken out and the soil and buffer is introduced into the chamber here, and inside the chamber we have a magnetic stirrer which is driven by a motor underneath the chip, and this magnetic stirrer stirs the buffer and the soil solution. So after the soil buffer solution is mixed well then this plunger is pushed down, so here we have a filter set.

And this filter has a membrane size pore size of the order of 0.2 micron okay, so you know this from the soil sample only bacterial solution is extracted into this reaction chamber, so this is the top view of so we extract the bacterial solution mixed with buffer through this filter on to this reaction chamber. And in this reaction chamber the bacteria is mixed with the antibody coated magnetic bead, and this is done by the bubble mixing.

So basically is pumped in through one of the inlets which moves through the sample that is present here and escape through a ventilation that is given here, so that mixing you know that bubble displacement mixes the antibody and the bacteria inside this reaction chamber. And once this mixing is done you know the antibody magnetic bead and the bacteria is pumped into the next module.

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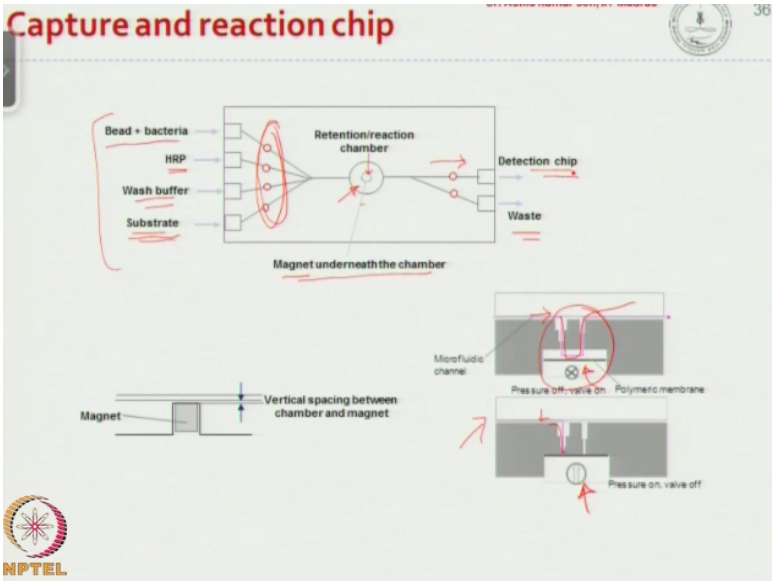

So this is the capture and reaction module, here the capture is done by a magnet underneath this chamber, so as this bead bacteria solution flow over the magnet, the bacteria attached to the magnetic bead are captured. So then we wash these captured bacteria several times to remove any dirt or any you know very fine soil particles that they may still be present, so that goes to the waste, and after washing we introduce the enzyme which binds to the bacteria.

So the antibody coated on the enzyme binds to the bacteria, and after binding we again wash the bound solution several times, because any unbound enzyme will get removed which may otherwise give false positive. And so then after washing only the enzyme which will be bound to the bacteria which is bound to the magnetic bead held by the magnet will be present. Then we introduce the substrate solution which will react in presence of the enzyme to generate a fluorophore.

So these different fluids are you know infused into the chamber through different valves the pneumatic valves which are based on this principle, so basically you know this is the pneumatic valve we have discussed when you are talking about valves, so this is the fluidic channel we create a point of this connectivity in the channel in the form of a valve, and here this is the inlet this is the outlet.

And this is connected to a pressure supply when the pressure is off, this pressure is higher than the opening pressure so there is continuity so fluid can go, and when this pressure is more than this fluidic pressure then the continuity is lost and the valve is closed. So based on that fluids are being infused selectively through different channels okay different fluids are infused into the chamber. Now what we generate here is fluorophore after we incubate the enzyme with the substrate and we take it to the detection chip.

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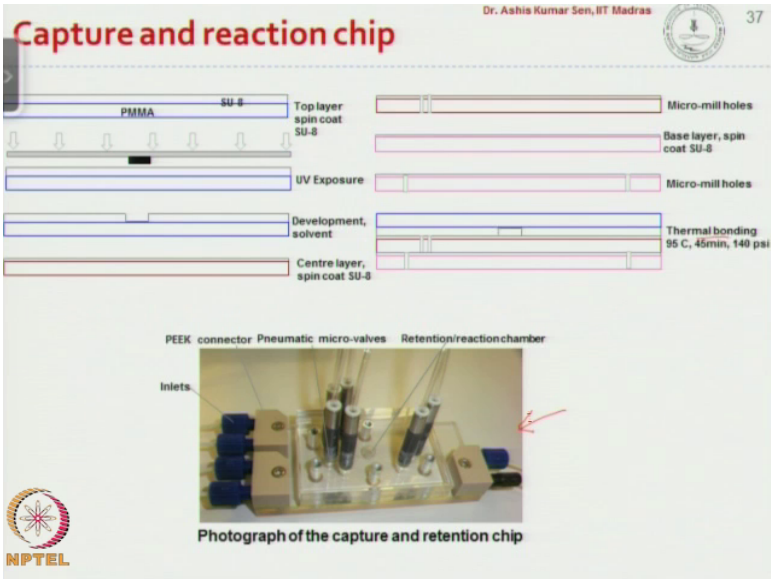

So this is the fabrication process how this capture reaction chip was fabricated, and it was fabricated based on a combination of photolithography and micro-milling followed by thermal bonding. So this is the chip that you can see here.

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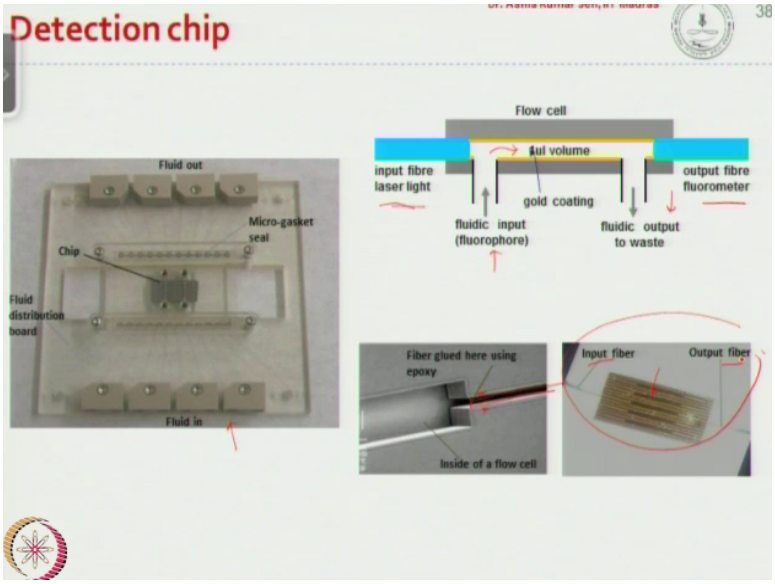

So this is the detection chip that we used to detect the fluorophore based on fluorescence measurement, so essentially we have a flow cell and an inlet and outlet, and the flow cell is interfaced with a input fibre and output fibre. So this is the photograph of the chip that was fabricated and this shows the inside of this flow cell, so this is the flow cell, and here the optical fibers are introduced and the glued around here, this is what you see this is the chip input fibre and output fibre.

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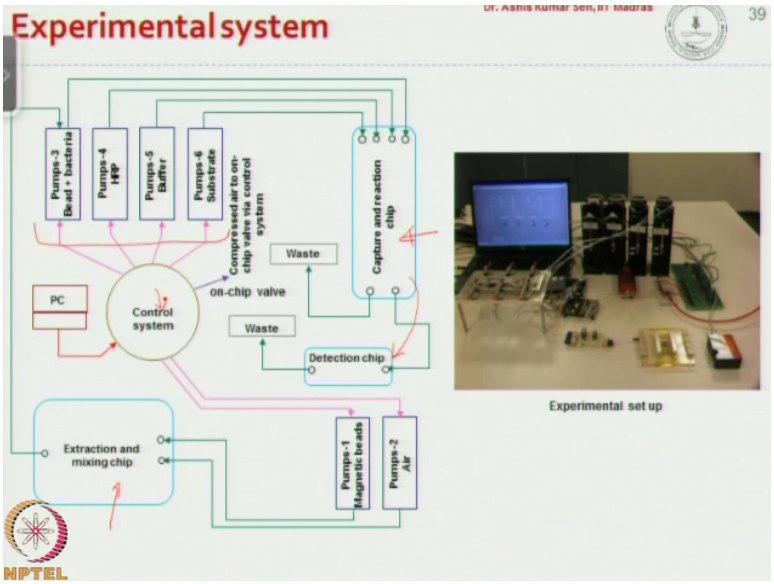

So this is the experimental setup with you know different modules, this is the extraction module so after the extraction and mixing with antibody the bacteria solution is infused to the capture and reaction chip, from there the fluorophore is generated which taken to the detection chip. And all these you know different types of different pumps and valves are actuated using a control system.

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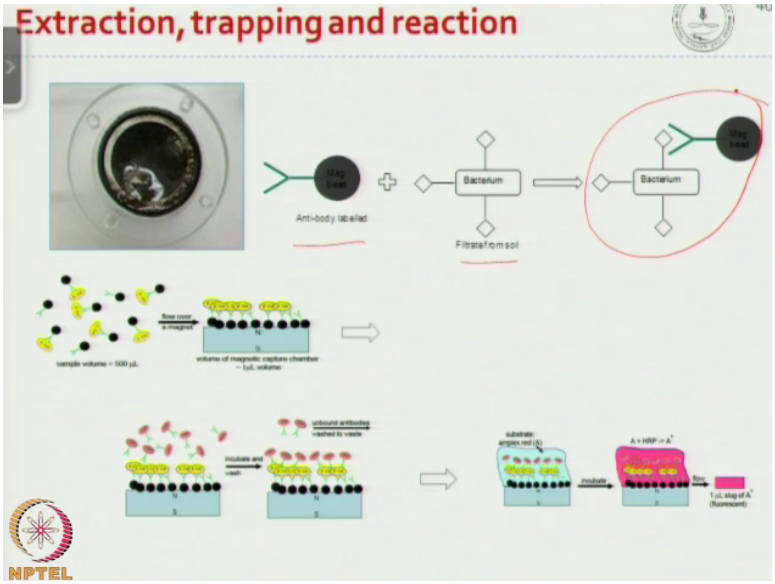

So this is the extraction trapping that is shown here, so antibody magnetic bead reacts with the bacteria, so they form a bond as you can see here.

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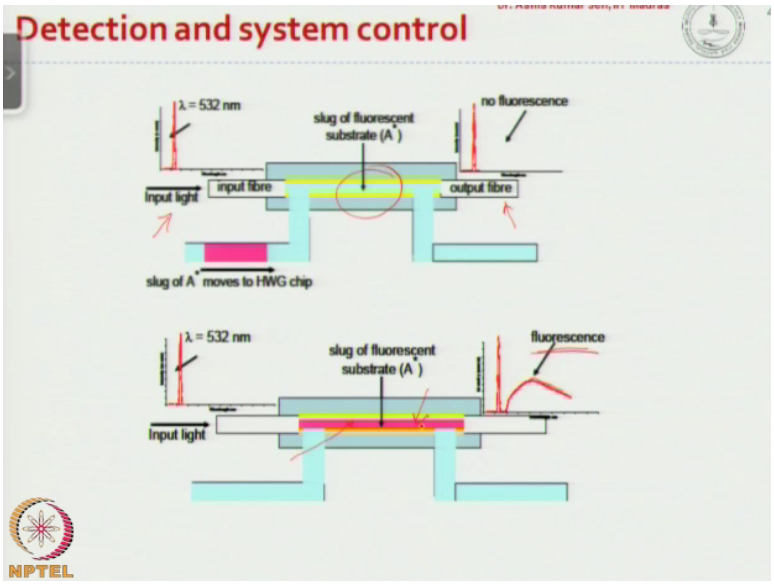

And this is the detection system that is shown here we have the input light that is coming at 532 nanometer, and this is the output light, and here we do not have any fluorogenic fluorophore present, and as the slug of the fluorophore is introduced into the flow cell, we would see a peak in the fluorescence okay. And this fluorescence characterizes the fluorophore here okay.

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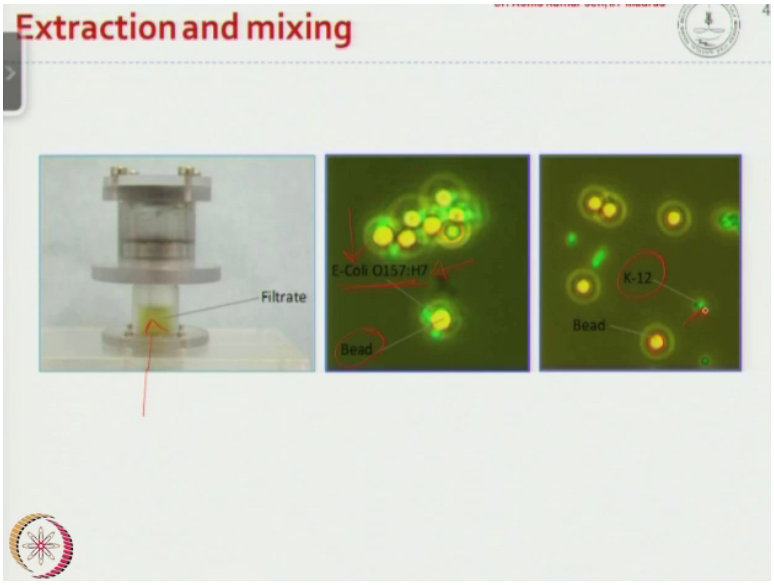

So this is what we see these are the extracted bacteria solution, and this shows that the there is good binding between the magnetic bead and E.coli 0157 H7 which we are interested to detect, and here K-12 was used as the control you can see that the magnetic bead is specific only specific to E.coli 0157 H7 and it does not bind with K 12.

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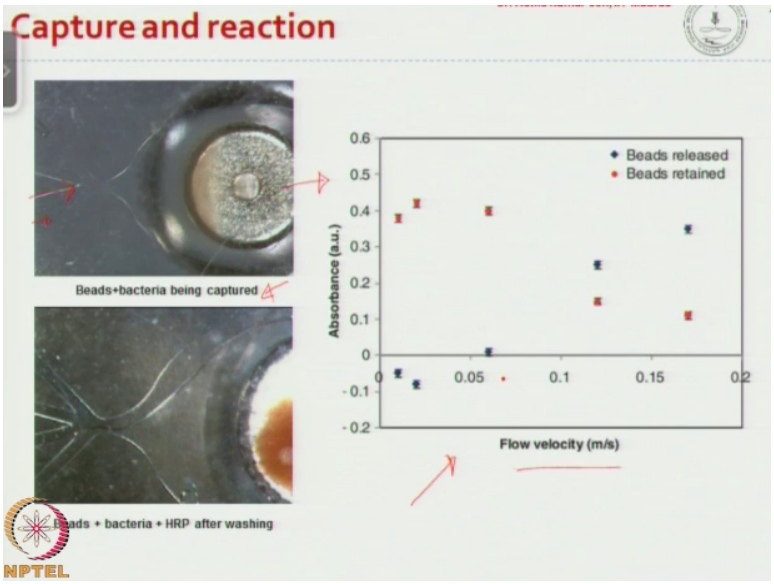

And here we are showing photographs of how the beads and bacteria are being captured using the magnet underneath the chamber, and this is very important study that was made what is the flow rate at which we need to flow the bead bacteria solution for the trapping to occur. If we the flow rate is very high the kinetic energy is more compared to the magnetic energy, then you would have the bead bacteria are getting lost some of them are not getting trapped.

But if you know drive at very low velocity it will take a longer time for the trapping to achieve. So there is an optimum velocity which we found about you know 0.05 meter per second which can be used to capture the bead and bacteria without losing any bead.

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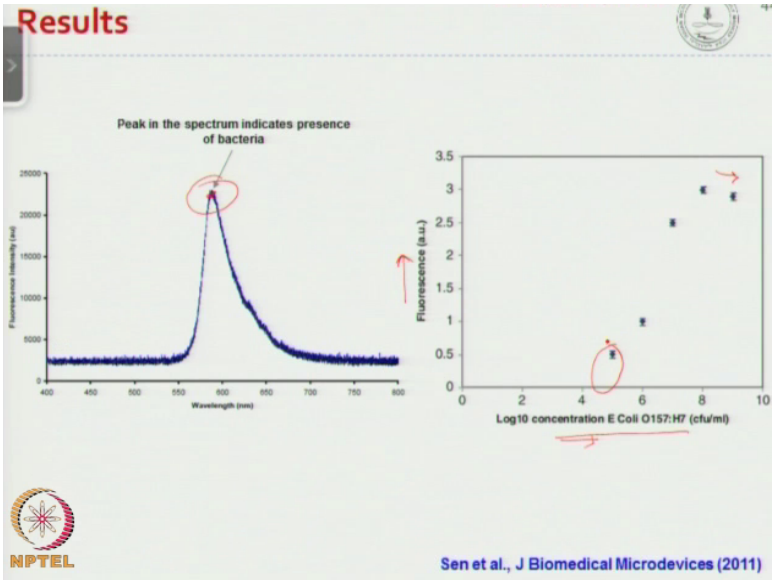

And you know this shows after we put in after we capture and we put in you know the HRP incubate the sample, and then we bring in the substrate solution we have a fluorogenic compound, then that fluorophore is taken into the detector chip, and in the detector chip based on the fluorescence intensity we get a spectrum. So as you can see depending on the intensity we see the fluorescence intensity spectrum we see a peak okay.

And this peak is representative of the fluorescence intensity that is come because of the fluorogenic compound, and so the mechanism works like this if there is bacteria present that will be attached to the magnetic bead which is held by the magnet, and if there is bacteria present the HRP is going to be bound to the bacteria, and if there is no bacteria present HRP will be washed if there is bacteria present HRP will be present, which will react with the substrates to give a fluorogenic compound.

And this fluorogenic compound or fluorophore is taken into detector chip for detection. So here if you have a fluorophore that we see a peak in the spectrum. Now here we study the effect of the concentration of E.coli with fluorescence, so as we can see that as the concentration of the E-Coli increases the fluorescence also increases, but it reaches a limit where it stabilizes okay, and the minimum concentration of the E.coli that can be detected is about 10 to the power 5 cfu/ml okay.

And which compares very well with some of the existing methodologies that have been used for detection of bacteria okay. So we have looked at a few applications of microfluidics, first we looked at applications of microfluidic for separation of microparticles, then we looked at applications of microfluidics for counting of particles, and then we looked at how microparticles can be used to detect bacteria okay.

So with 3 different examples we complete our discussion on the applications of microfluidics and with that let us stop here.