LIST OF FIGURES

1. Acknowledgements

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Part 1

2. Introduction

The DLD is a new microfluidic device designed to separate small particles at very high speeds and with a very good precision. It has been around for just a few years but already it is showing plenty of promising applications.

Cell synchronization is important for many different biological assays but a good method for achieving this does not yet exist. We are hopeful that the advent of microfluidics and the DLD device will make it possible to create a device that can fill the needs of biologist.

This work I have tried to create a device that can sorts cells depending on their size and therefore their phase in the cell cycle. The thesis is divided into two parts. The first part is a theoretical part were all the theory necessary for understanding the DLD device is being presented. The second part is the experimental and practical part.

Part one starts with the biological background to understand what synchronization is and shows some different methods to achieve this synchronization. It continues to describe the field of microfluidics and what it can do towards cell synchronization. The theory and background of the DLD device is later described along with some examples of how it has been utilized so far.

The second part starts with the creation of our devices in chronological order starting with the design and manufacturing going to the experimental part were the devices have been tested.

3. Cells

The information in this chapter is largely taken from The WORLD of the CELL[1]. All living things are made up of cells. Cells are life. Just as so many other things in nature, living cells live out there lives in cycles, or suspended cycles for some specialized cell types.

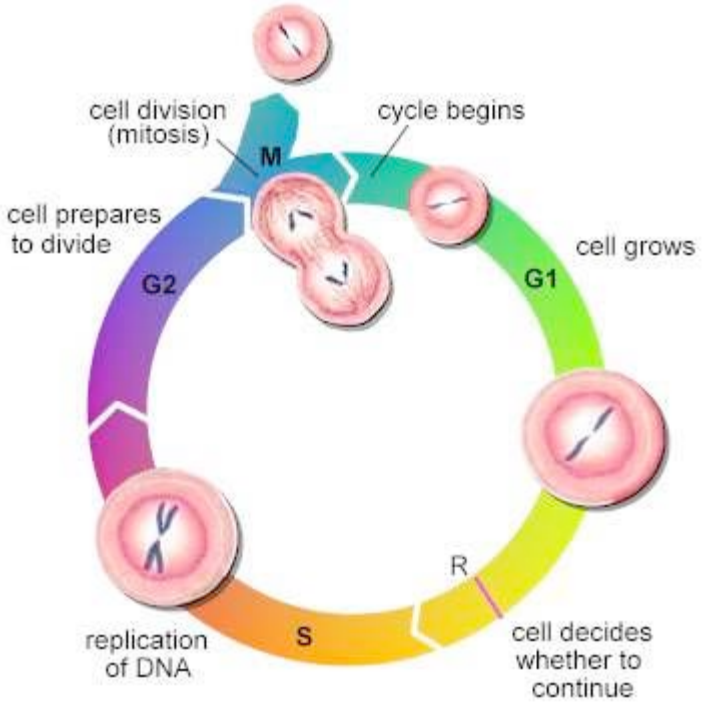

Figure 1. The cell cycle. Image from http://herb4cancer.files.wordpress.com/2007/11/cell-cycle2.jpg

3.1 Cell cycle

Cells grow. Throughout their life they synthesise protein nucleic, acids, carbohydrates and lipids. To accommodate all these new molecules the cell membrane grows and eventually the surface to volume ratio makes it necessary to divide. They grow out of their skin.

The cell divides into two daughter cells with, hopefully, identical DNA sequences as the mother cell. To ensure that the process goes well it goes through many discrete stages. The stages are called the cell cycle. The complete cycle usually takes around a day to complete, although this figure may vary between different cell types.

3.1.1 M phase

The M phase is the natural starting or end point of the cell cycle, depending on your point of view. In this phase the cell division occurs. It happens in two stages. First the nucleus divides, called mitosis followed by the division of the cytoplasm, called cytokinesis. The two new cells each with half of the volume of the mother cell have about 20% smaller radius. This phase, although being perhaps the most dramatic, typically lasts less than one hour for mammalian cells.

3.1.2 Interphase

Most of a cells life time is spent between the cell divisions. This growth phase is called the interphase. The cell components are mostly synthesized continuously over the whole interphase, so the cell size grows more or less linear during this phase. The interphase is divided into three other phases defined by when the DNA replication occurs. There is also one fourth phase in the interphase where it goes out of the whole cycle into a kind of hibernating phase where it don't divide anymore.

3.1.3 G_1 phase

This is the first phase in the interphase after the M-phase. The cell prepares for DNA replication by creating all the proteins etc that is needed. This stage varies most in time even among different cells in the same species.

3.1.4 S phase

In the S phase the cell replicates the DNA. It starts when the DNA molecule starts to copy itself and finishes when the cell has two complete sets of the DNA molecule. The time this takes is of course dependant on how much DNA the cell has to duplicate so it takes the same time, more or less, for all the cells in a species.

3.1.5 G_2 phase

This is the phase where the cell builds up the cytoskeleton and readies the cell for division. The newly copied DNA is scrutinized for any defects during this phase. It takes more or less the same time for all mammals.

3.1.6 G_0 phase

This is a dormant phase. Cells that are fully differentiated, like neurons, go to this phase indefinitely. Cells can also go to this state if the environment it lives in does not support further growth as an option to aptosis.

3.3 Cell culture

To make any kind of experiments concerning live cells it is important to be able to grow cells in an easy, clean and in a way so that you only have the interesting cell line.

To do this you need to have the conditions right, the right pH, right temperature, salinity, serum to nurture the cells and a clean environment free of contamination.

You will also need cells to seed out and let grow. This can be done by taking cells from a living or recently dead body, but it is far easier and more common to just by cell lines from one of the many companies available that provide this service.

4. Cell synchronization

Cell synchronization is to get a population of cells to go through the different phases of the cell cycle in synchrony. This is important for studies on the cell cycle, testing many cancer drugs and also reduces the noise in many biological experiments. Many substances you use in a lab only affects cells in a certain phase in the cycle so a study to see the effects of this substance while having a large population of unsynchronized cells will give a very unfavourable signal-to-noise ratio.

Many drugs treating, for example, cancer attacks the cell in a certain point in the cell cycle, so for lab tests of this type of drug it is important to have a synchronized cell population[2].

There are two main approaches to achieve synchronization of a cell culture divided into whole culture methods and selective methods. Whole culture methods involve adding chemicals to an existing culture of cells that will synchronize all of the cells. In the selective methods you try to sort the cells by different characteristics that relates to the cells phase in the cell cycle.

There is a debate however if it even is possible to synchronize cells or whether the cells that you get by synchronizing them with the methods at hand even lead to anything that can be used to represent life in vivo[3, 4].

4.1 Whole Culture methods

This is the most common of the methods used for cell synchronization. It gives large populations of well synchronized cells. The synchronization is reach by adding chemicals to a cell culture that arrest cells in a specific phase of the cell cycle and then releasing them in synchrony. The downsides are that it affects the cells metabolism by the chemicals used to arrest them.

4.3.2 Blockade

Blockade is a method where you halt the cells at a certain point in the cell cycle. Blockade is divided into two different methods; one is the use of inhibitors that block DNA replication or microtubular formation, that halts the cell in the S or in the G_2 phase, the other method is nutritional deprivation which halts the cell in the G_1 phase. When performing this type of synchronization you have to start the process by adding your agent or removing serum and than wait for all the cells in the culture to reach the phase were they are supposed to be arrested. Average cell cycle takes approximately 20 hours to complete and the whole process takes around five days to complete[5].

Among the agents used for DNA synthesis inhibition are; thymidine, hydroxyurea, cytosine, arabinoside, aminopterin. The major down side to theses agents are the fact that many of them are toxic. Also there is no guarantee that the cells will all halt in the S phase, you just get a majority of the cells in this phase. The cells that get arrested in other phases than S are usually non-viable after the cycle is resumed[5].

In[2] they showed that different blockade methods lead to morphological anomalies in the cells. It leads to abnormally large cells, this is because the cells still live and metabolize while not being able to leave the phase in the cell cycle.

For nutritional deprivation serum or isoleucine is removed from the medium for 24 hours and then added again. Upon restoration the cell cycle is resumed in synchrony. The degree of synchrony is high, over 80%, for the first cycle. For the second cycle it is around 60% and once you reach the third it's near random. This method only works for specific cell types[5].

4.3 Cell Selection

In cell selection the approach of the problem is a bit different and a bit gentler one. Instead of controlling the cells and making them stay in the same cycle, one simply selects the cells in a culture that are in the same phase and make a new culture of them. A bit like the old idiom if Mohammed will not go to the mountain, the mountain must come to Mohammed but in reverse.

4.3.1 Baby Machine

A method that lands somewhere between whole culture methods and cell selection is the Baby machine or Mitotic shake-off. This method comes from the 1960. The idea behind the machine is simply that you have a population of cells attached to a surface. When the cells on the surface divide either one or both of the daughter cells release the surface and float suspended in the cell medium. At regular time intervals, usually around an hour, you take out the medium and in it you will have cells that have very recently divided. These cells, which are of the same age, are assumed to be synchronized[5].

The merits of this method are that you have cells that have not been disturbed in their normal cycle by chemical agents, so they should be healthy and live on many cycles after synchronization. If you have the machine running continuously, and you don't run out of cells on your surface, you can have cells synchronized ready in short notice.

The disadvantages are that the yield is not great and you can only get small populations at a time. You only have cells synchronized within an hour or two. And it requires a rather big and cumbersome machine to work.

4.3.3 Cell Sorting

The other main foray into cell synchronization, apart from whole culture methods, is cell sorting. By sorting the cells by different criteria that are related to the phase of the cell in the cell cycle, the hope is that synchronization can be achieved. The characteristics sorted by can be cell size or DNA content. To sort by size one needs a method that can sort between the difference in cell size between the newly divided cells and the ones that are just entering M-phase so the resolution has to be around a micron to be able to recognize the difference. Since all of the whole culture methods disturb the cells a lot and many times lead to aptosis of the cell and might not even necessary lead to any kind of synchronization at all, the selective methods are preferred.

The simplest way to do this is to simply let the cells sediment at unit gravity[5] and then the size and density of the cells will decide the rate at which they sediment. This is however a slow process. In addition, it will be difficult to take out your different samples after the sedimentation has taken place.

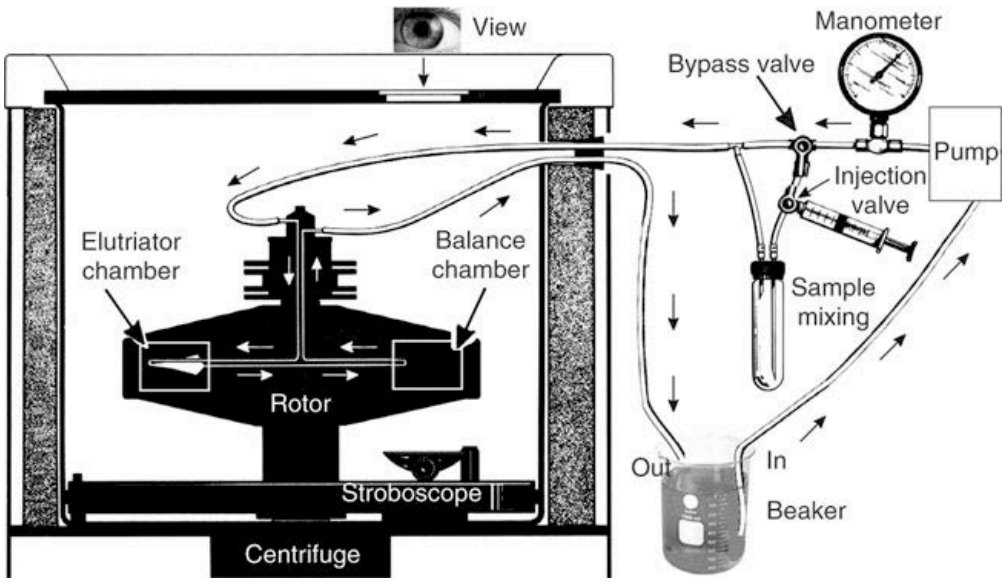

Figure 2. Centrifugal elutriation machine. Image from www.nature.com

Centrifugal elutriation[5] is a way to up the yield and improve the resolution of the aforementioned method. In this device, an elutriation chamber is spinning at a constant g-force balanced by the flow rate through the chamber. When this is in equilibrium cells will accumulate in different places of the chamber according to their sedimentation characteristics.

This method need a lot of complex and time-consuming preparations and the cells in the device will feel quite high mechanical stress[2].

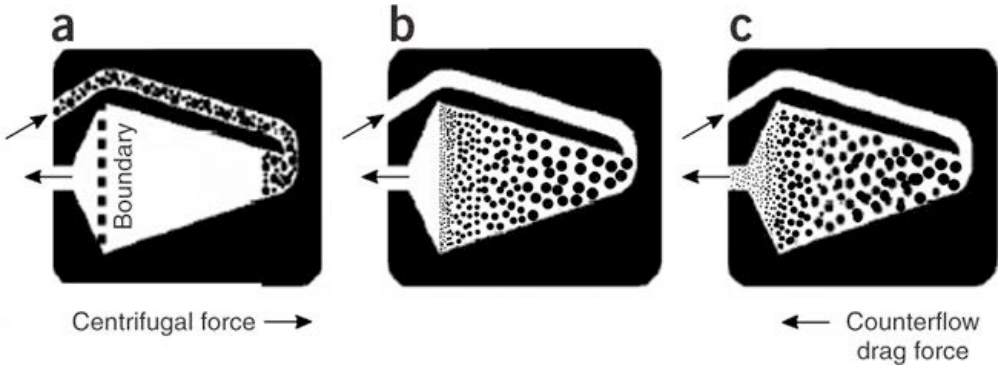

Figure 3. Centrifugal elutriation chamber in close. Image from www.nature.com

Another method to sort cells is to use **fluorescence-activated cell sorting[5]** (FACS). In this method one looks at the light scattering characteristics or the fluorescence emission. For fluorescence emission requires staining of the DNA with a non-toxic and reversible dye. In this method a single stream of cells are passed in front of a laser and several light scattering characteristics are observed.

Several new methods for cell synchronization are on the horizon in the field of microfluidics, the small size of these methods mean that they are ideal for performing tasks at the size level required.

4.3.4 Flow cytometry

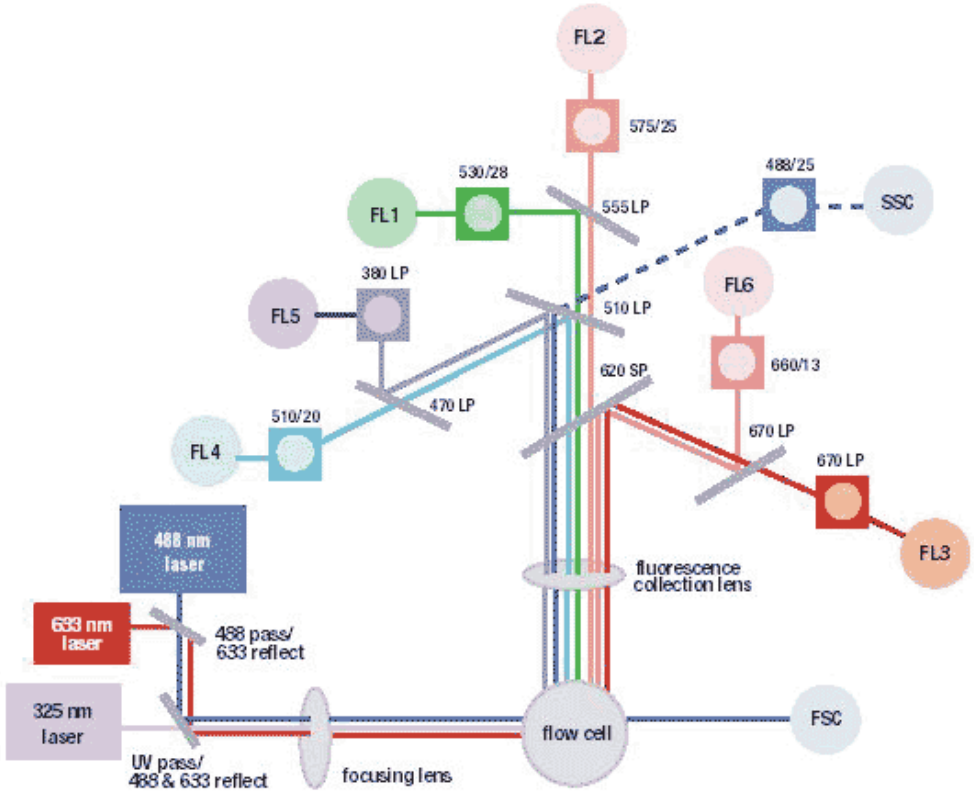

Figure 4. The beam path in a FACS. Image from Becton Dickinson.

Flow cytometry is a technique to count, analyze and sort particles or cells. It is a dynamic device that can sort cells according to user specified criteria in real time. The main use is as an analyze method but it also has many applications for sorting cells or other particles.

In the device a laser beam is directed at a stream of fluid in which the sample to be analyzed is suspended. The fluid flows so that each cell flows by one by one in front of the laser beam. A number or detectors placed around the stream measure fluctuations of the light intensity. The different detectors measure forward scatter (FSC), side scatter (SSC) and a number of detectors measuring different fluorescence spectra.

The particles in suspension flows through the laser beam and intensity fluctuations are registered. Forward scatter is related to the size, and somewhat to the surface characteristics of the particle, side scatter relates to the internal structure of the particle and to some extent the size of the particle. The fluorescence intensity is used mostly to see particles that have

previously been labelled with a fluorophor although most particles and cells have some intrinsic fluorescence properties.

The flow cytometer can analyze up to thousands of cells per second. The data can be plotted in several dimensions showing different populations of cells or particles in your sample.

4.3.5 FACS

Fluorescence activated cell sorting, or FACS for short, is a specified version of the flow cytometer that besides being able to analyze a fluid also has the abilty to sort the particles into different containers after the analysis has occured. The name FACS, an acronym that is owned by Becton Dickinson, is wrongly used for flow cytometer in many circles

In the FACS device instead of having the cells flowing by the laser beam in a sheet fluid the fluid is broken up into tiny drops in a way so that each drop has a high probability to only contain one cell/particle. Just before the fluid breakes up into drops it passes in front of the laser beam were a similar analysis to the flow cytometer occurs. The fluid gets charged either before forming drops or just as the drop formation occurs. These charged drops can then be deflected into different containers below the laser as they fall down by having metal plates charged in different ways.

The FACS machine, depending on the model, can sort cells real-time into many different populations. With a high accuracy, although the accuracy is connected with the speed at wich you run the machine. Unfortunatly the accuracy does not increase as the speed increases.

The benefits of a machine like this are that it is dynamic and has a very high precision. As method for analyzing samples it is very fast and relatively easy to use for the end user.

The downside is that it has a low throughput requires a full time operator and costs in the region of 2000000 skr. It taste does not outweigh the cost.

4.4 Conclusions

There is a whole host of methods out there to reach synchronization, the most common and widespread one is the blockade method which being relatively easy and gives a lot of well synchonized cells leads to many bad effects mainly being cell death or severe metabolic consequences.

The selection methods are all time consuming and pricey and all give a quite small yield of cells per minute. While these machines may have some demerits from the cell arrest and release, the cells that you get from them are of a better quality and are more viable.

There is clearly a need for a new method that can do the things that a FACS can do but at a fraction of the cost and recent scientific breakthroughs point to a possible solution existing within the field of microfluidics.

5. Microfluidics

Microfluidics is like the name implies fluids in very small surroundings. With small length scales comes small Reynolds numbers, which lead to very pleasant and well behaved fluidic systems.

Microfluidics as a research field cropped up around 1980. It is interdisciplinary field that intersects engineering, physics, chemistry, microtechnology and biotechnology. The fabrication of devices borrows it's techniques from the electronics industry, with CAD programs and lithography being used while the way you use the devices are more related to biology or chemistry.

The reason for borrowing so many techniques from the field of electronics is that they had the means to create very small and detailed devices at the relevant length scale.

The two major milestones in this field have been technology based. The first one came when micromachining in silicon made it possible to make complex structures at a very small length scale. The other milestone has been when the field went from utilizing silicon as the major material used to utilizing plastics. This shift made it possible to create many cheap "chips" at just a fraction of the cost of a silicone wafer.

5.1 Theory

Small things don't always behave as big things and we are lucky that they don't. Thanks to this scaling effect we have the possibility to use microfluidics. As we scale down our devices water start to flow less like the turbulent fluid we see in our faucet and behaves more like a slow flowing glacier. This happens because the ratio between inertial forces and viscous forces are remarkably different from fluids flowing in our "big" world.

5.2.1 Navier-Stokes equation

In most microfluidic devices one has a flow of a fluid of one sort or another. To make a mathematical model of a flow it's easiest to break up the fluid into many small elements, as is common in many mathematical models. Each of theses small elements are subject to forces and move accordingly[6]. These forces can come from a variety of sources such as pressure forces, viscous forces, gravitational forces and centripetal forces.

Pressure forces come from having a pressure difference over a body of fluid. It can come from having lower pressure on one side, sucking the fluid

forward, or having higher pressure on one side pushing the fluid forward. All this considering that you are at a somewhat normal atmospheric pressure.

Viscous forces are forces between elements in the fluid. Essentially a fluid with high viscosity, high viscous forces, is very thick and flows slowly. Viscosity also comes into play with the friction between the fluid and any surface that contains it. This makes it harder to push fluids through channels with a high surface-to-volume ratio.

Gravitational forces can for the most part be neglected in microfluidics since the effects are small in comparison with the induced flow from pumps and such.

Centripetal forces is important in the aforementioned centrifugal elutriation devices, but will not be used in this project.

The equation that governs the flow in a microfluidic device is the Navier-Stokes equation.

$$\rho \frac{\partial v}{\partial t} = \nabla P + \rho f$$

This general form of the equation has ρ is the fluid density, v is the velocity and f is the body force vector. The tensor P represents the surface forces applied on a fluid particle. This equation is basically Newton's second law of motion for a fluid.

It is a very complex and powerful equation, the solution of it gives the trajectories and velocities of all the elements of the fluid. The solution to this equation in its general form has never been found and in fact, you can win a prize of a million dollars by simply showing the existence of a solution. The practical use of this equation is always in a simplified form.

5.2.2 Reynolds Number

Reynolds number is central to microfluidics. It is the ration between inertial forces and viscous forces in a fluid[6]. It is defined as

$$\mathrm{Re} = \frac{D_H \rho \langle v \rangle}{\eta}$$

D_H is the hydraulic diameter of the channel, ρ is the fluid density, $\langle v \rangle$ is the flow velocity and η is the viscosity of the fluid. The hydraulic diameter is dependant on the size and the geometry of the channel.

What the Reynolds number can tell us is whether the flow in a device is turbulent or laminar. A value over 2000 leads to turbulent flow, a value under

30 gives laminar flow. The range in between the two the fluid can be in either mode.

A laminar flow is a flow where two parallel streamlines flowing next to each other does not mix like a flowing glacier. This is very different from how fluid flows from a faucet for example where it is a very turbulent fluid. Laminar flow is paramount to most microfluidic devices since it is the predictability that comes from laminar flow that is being used in most

In the device used in this project the Reynolds number will be well inside the laminar region. As shown in a master thesis made by Jason Beech.

5.2.3 Flow

Flow can be induced in many different ways, with pumps, syringes, capillary flow and electrophoresis to name a few. In this project only pressure driven flows induced by pumps have been used so only this option will be used.

Depending on which kind of method one uses to induce flow, the flow will follow different flow profiles. Flow profile shows if the flow has the same speed all over the channel through which the fluid flows, or if it differs close to the edges. Electrophoresis for example gives a very flat flow profile, meaning that the flow speed is more or less homogenous. Using pumps and inducing pressure driven flow causes a parabolic flow profile, the flow is faster in the middle of the channel and zero at the channel wall. This leads to considerations when designing a device such as the deterministic lateral displacement since streamlines will not all have the same size all over.

For low reynolds numbers as in most microfluidic devices there is a very easy way to calculate the flow that like the way Navier-Stokes equation relates to Newton's second law of motion relates to Ohm's law. To calculate the flow the equation looks like

$$Q = \frac{\Delta P}{R}$$

Where Q is the flow, R is the resistance and ΔP is the pressure difference applied over the channel.

The resistance depends on the geometry of the channel in question the width and depth but also if it is a capillary tube or a square channel.

5.2.4 Diffusion

Diffusion is the random movement of particles in a fluid induced by the random motions that occur among the molecules that comprise the fluid. By the random nature of diffusion it can leads to effects that may have to be

taken into consideration in separation science, some applications utilize diffusion while for others it is a problem.

Since microfluidic, devices in general, have a low reynolds number and has laminar flow, no mixing between different fluids occur due to turbulence. All the mixing comes from diffusion. The molecules in the fluid vibrate due to their thermal energy and bump into each other and also into larger particles inside the fluid. These bumps create the brownian motion explained by Einstein in his miracle year of 1905. Due to its random nature this process will strive to create an even concentration of particles or other substances throughout the fluid.

The distance a particle can traverse in one dimension in time t depends on the diffusion coefficient according to

$$d^2 = 2Dt$$

The diffusion coefficient, D, is

$$D = \frac{k_B T}{6\pi\eta R_H}$$

kB is Bolzman's constant, T temperature, η the viscosity of the fluid and RH the hydrodynamic radius of the particle. Because the distance is quadratic the time it takes for a particle to diffuse will change dramatically depending on how far it needs to travel. Inside cells a lot of transport of molecules rely on diffusion to just lead them right, and this works for most small and spherical cells, but for neurons that can be up to meters long the time scale it would take for things to reach from one side to the other would be years.

Since diffusion by its nature is a random process it fits very poorly when trying to create a device that works in a deterministic manner. To have a quick test to see how much effect diffusion has in your device there is the Péclet number[7]. This is defined as:

$$P_e = \frac{Advectiverate}{Diffusiverate} = \frac{v/d}{D/d^2} = \frac{vd}{D} = \frac{\langle d^2 \rangle}{\Delta x^2}$$

Where v is the flow speed, d the characteristic dimension of the array and D the diffusion coefficient of the particles being separated. This number should be as high as possible to minimize effects of diffusion in the device. For the device used in this project diffusion will not be a factor.

5.2.5 Radius

To have a paragraph written about radii can seem unnecessary, it is common knowledge what this is. However, what happens in the case of a particle that is not spherical? There are several definitions in use like:

Radius of rotation, this is the radius of the sphere you get by rotating the object around its centre of mass.

Mass Radius, the radius of a hard sphere of the same mass and density of the object.

Hydrodynamic Radius is the radius of a sphere that diffuses at the same rate as the object.

The Hydrodynamic radius is the most interesting for our application since it is believed that it is the hydrodynamic radii that decide which streamline the particle will choose to follow. This size also governs diffusion that will be described in coming paragraphs.

Which ones are important for us?

Cells are however, at least the ones used in this study, close to spherical when they are suspended in solution. However, they will feel a shear force negotiating the device and bumping against the obstacle in the array, this force might deform the cells since they are soft particles. There is a possibility that this will affect the performance of the separation, but the quantity of this is yet not known.

This is true for “normal” Newtonian mechanics and such but for fluids and microfluidics it is the hydrodynamic radius that is the interesting quantity.

5.3 Cells in microfluidics

When using living cells in microfluidic devices there are some problems that you are bound to come across that has to be solved to get a good working device. Depending on the purpose of the device, there will be a slight difference in problems faced.

The biggest problem will be to make a device that keeps the cells alive after going through the device. The biggest threat against the cells is mechanical forces that can come from high pressures of small channels. Since the channels are almost exclusively very small in microfluidics the pressure inside them due to even a very modest pressure difference over the entrances or exits can be very high. Thus there is a speed limit on a device working with cells.

The other big problem that has to be addressed will be clogging. Clogging occurs very easily from biological material since many cells are designed to stick to things as one of their major functions. Clogging can be faced by treating the surface of the device with anti-sticking agents or mixing chemicals into the buffer used that prevents them from sticking. In choosing the method by how to avoid clogging one also has to consider what the non-clogging method does to the cells.

Other problems that will be faced can depend a bit on what the device function is. Some devices are used to be a method for analyzing samples. If this is the case throughput is not an important factor. But if the device is supposed to sort a sample for further use it is important to be able to get a useful amount of sample in a reasonable time scale.

5.4 Surface Passivation

In pretty much every application in microfluidics where one deals with particles or cells or molecules it is important to get your sample through the device without clogging or sticking to the surfaces of the device, unless, of course, that is your goal with the device. To this extent, there is a wide variety of methods in use to passivate the surface. Some methods involve coating the walls of the device with a substance that will prohibit unspecific binding to the surface while some other involve adding chemicals to the buffers used that reverse these effects or compete with the interesting molecules for binding sites.

Pll-PEG [8, 9] is one method used to passify a surface. One end of the PEG molecule has a poly-L lysine anchor and the other side just has PEG chains. The anchor binds to negatively charged surfaces and the PEG end stand out like whiskers from the surface, not letting molecules in the solution to reach the surface.

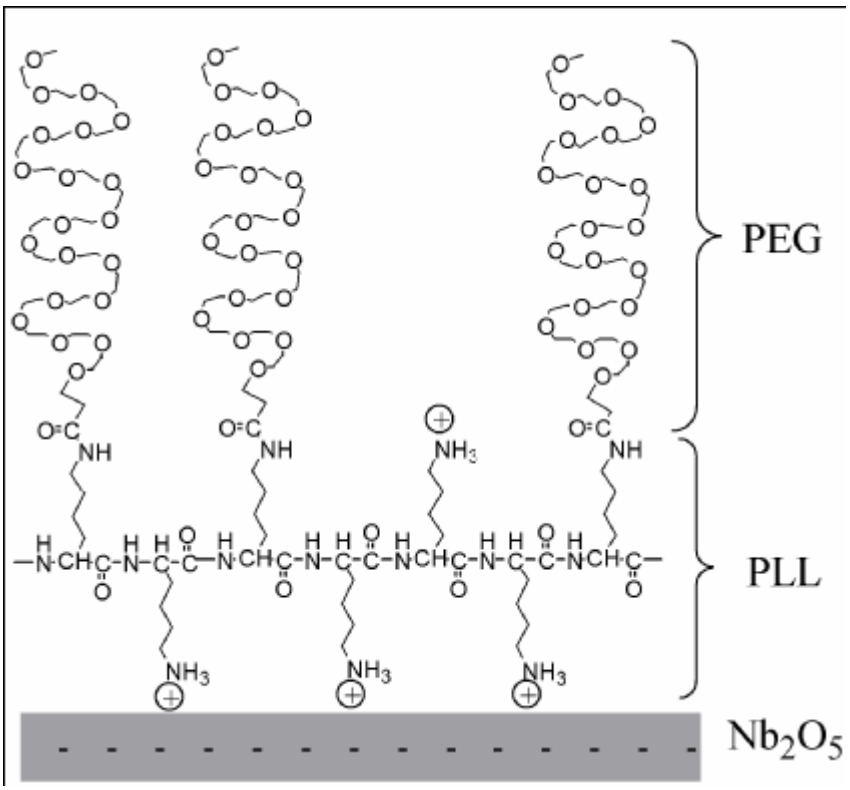

Figure 5. The Pll-PEG chain. Image from [9].

5.5 Fabrication and materials used

The technology used to create devices that utilize effects on the micrometer scale has spawned largely out of the semiconductor industry. Almost all of the fabrication methods used today are the very same methods used to create microchips.

Many materials are being used in microfluidics and the dominating material has changed since the birth of the field. In the beginning most devices were made from silica wafers[10], this is the most natural material to use since it is the material used in the lithography process. This material however is expensive and it is very time consuming to make devices so nowadays most work in Poly(dimethylsiloxane), PDMS, a cheap and easy to use plastic. With PDMS the silica wafer only have to work as a master that can be utilized to cast many PDMS devices in a fast easy and cheap way[11-14].

The steps included to create a device from PDMS will be briefly described. This is not the only way to fabricate devices but it is the one utilized in this project and the most common method used today.

The first step in the process is to transport the pattern to a silicone surface. This is done by creating a design for the master in a CAD program, printing this design on a lithography mask and subsequently expose a silica wafer coated with resist.

Once the pattern is transferred, etch away the excess material and subject the master to an anti-sticking treatment. Fluorinated alkyl derivates or fluorinated polymers are the most commonly used. These are very similar to the coating used on frying pans. This creates a monolayer on the surface that acts in a fashion similar to Pll-PEG.

Once the master is done it's easy to cast PDMS devices, a process that takes merely a couple of hours. To get these devices to work the channel created has to be sealed. For PDMS this is done by bonding together the PDMS to glass by exposing them to oxygen plasma. This will induce the formation of silanol groups on the surface. When these surfaces are pressed together they will create covalent Si-O-Si bonds that are stronger than the PDMS material itself.

Once the device is sealed it just needs to be hooked up to the pumps or syringes used and it is ready to use.

5.6 Applications for cell synchronization

The only paper published so far that has utilized microfluidics for cell synchronization purposes have been, Selection of mammalian cells based on their cell-cycle phase using dielectrophoresis by Kim[2].

They have created a device that sorts cells depending on size using a long channel with electrodes along it that will push them to one side depending on the size of the cell. They have conducted a test that shows that cells go from 10 μm in G_1/S phase to around 20 μm in the G_2 phase.

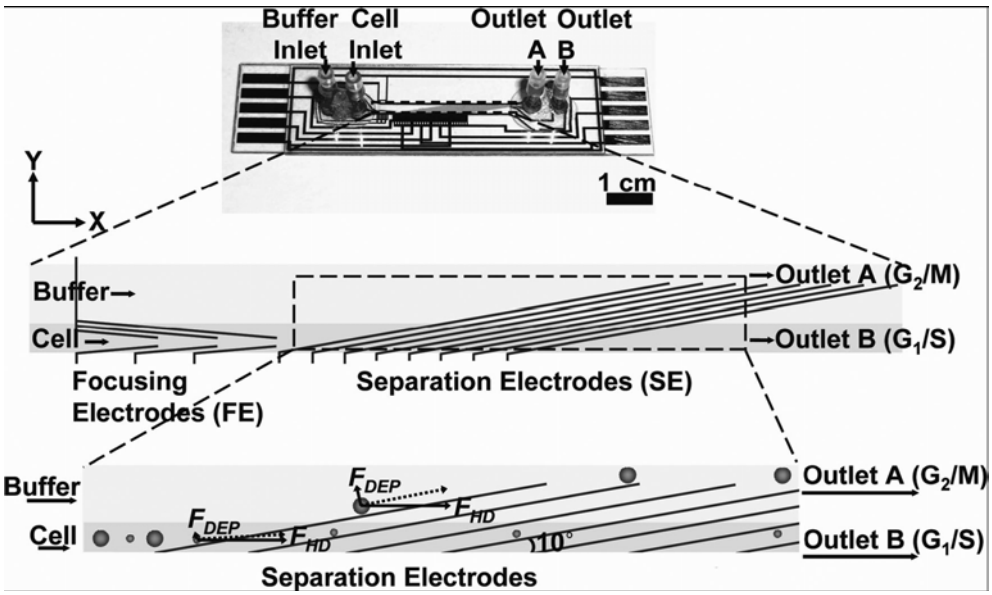

Figure 6. The prototype used by [2].

The cells are introduced in the lower entrance and then pushed against the lower wall. Once the cells reach the separation part of the channel, the cells will feel a push away from the wall because of the electric field. The reason they chose having negative DEP is to have the cells move away from the electrodes to decrease the chance of hurting the cells. The cells experience a force because of the electric field that will depend on the size of the cell. Hence, the bigger G_2 cells will be pushed further away from the wall and will be extracted in the upper exit. The smaller ones will stay closer to the wall were they entered and will be extracted in the lower exit.

This device has been showed to be able to synchronize cells within 96% of the population in one run. The yield though is only $2 \cdot 10^5$ cells per hour so far.

Other microfluidic devices have been used to separate cells although the main goal is not to achieve cell synchronization. A version of field flow fractionation, FFF, was used in[15, 16]. The basic principle used in these two papers are the same as the one used in this thesis although it all the separation occurs in a very small area. The small area lead to a much simpler device but can also lead to lower resolution and the device has to be very finely tuned to have the right ratio in the different streamlines.

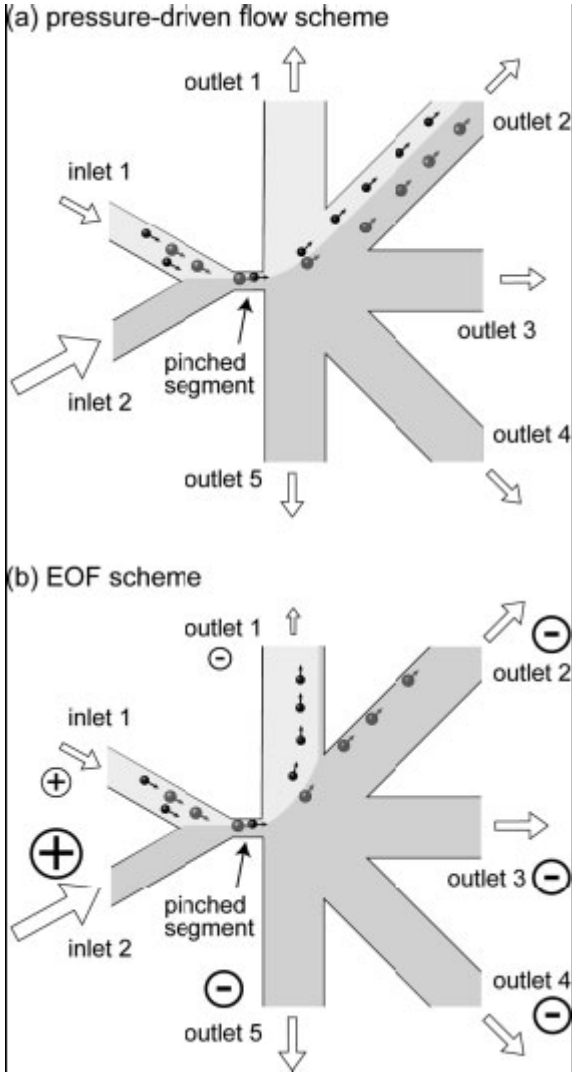

Figure 7. A FFF device in action. Image from [16].

6. Deterministic Lateral Displacement

The deterministic lateral displacement is a new microfluidic device that can sort particles in a fluid depending on size in a fast non clogging manner. It was first conceived by Huang et al in 2004[17].

DLD is a specialized version of field flow fractionation (FFF) a technique to separate particles by applying a force to drive particles toward one side of a device. This can be read in [15, 16, 18].

The DLD device is made up of a periodic array of obstacles that divide up the laminar flowing liquid into many streamlines, while also applying forces to particles in the fluid. Theses forces act perpendicular to the direction of the flow and decide which streamline particles will follow.

6.1 Theory

The DLD is basically a sieve but the holes in it are much larger than the holes in a traditional sieve. This leads to one major advantage over traditional sieves; it does not clog as a traditional sieve does. This theory can be read about in detail in [6, 19, 20]. In this chapter section all the theory needed to understand the workings of the DLD device will be presented in a clear and easy to grasp way.

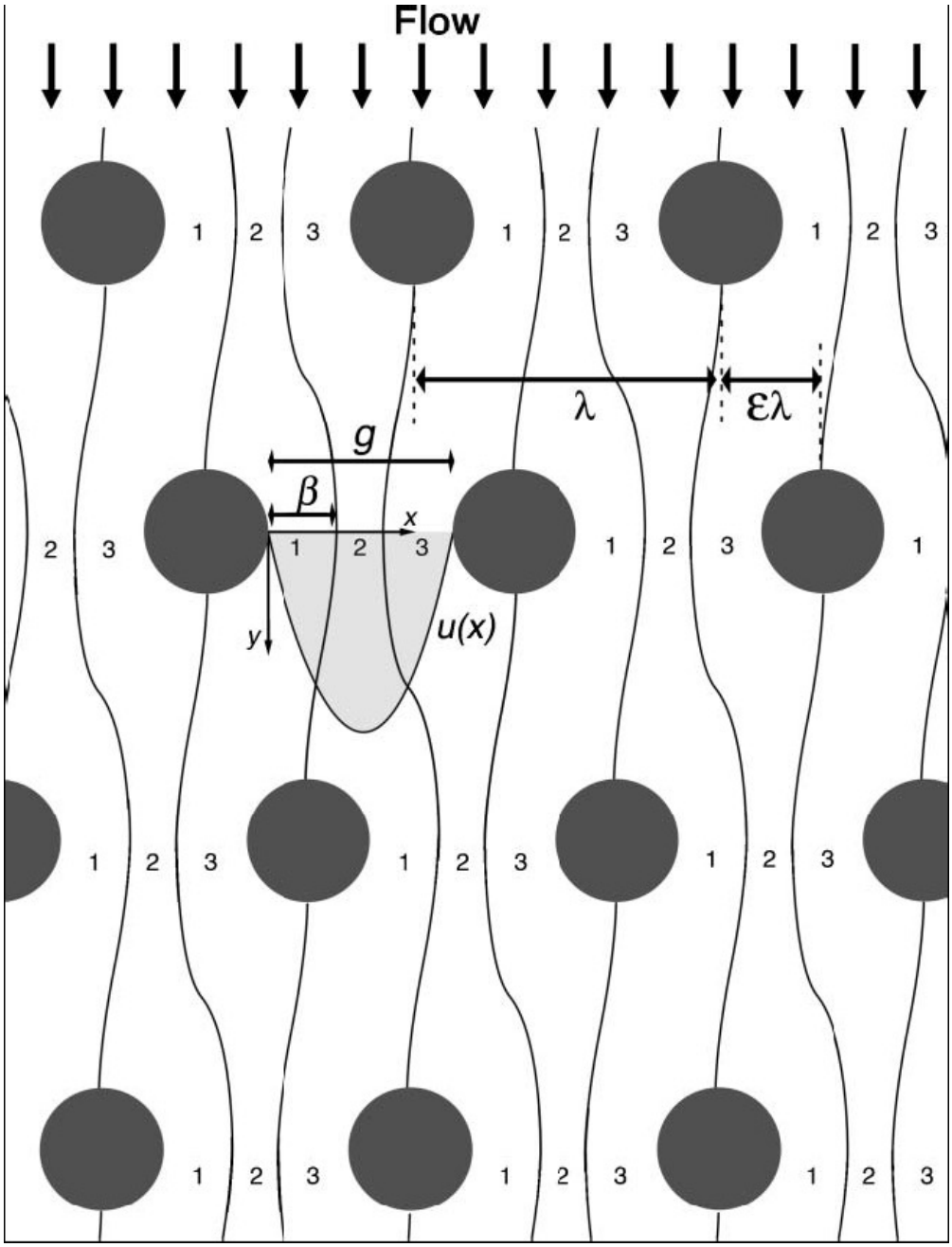

Figure 8. The flow through a DLD device. Image from [20].

As previously stated, the DLD device consists of a channel with a matrix of periodic posts. These posts have a lateral shift between each row so that the next row is not exactly in line with the previous one. The lateral shift between rows is called ελ. The period of this of the matrix is defined by how

many rows it takes before the pillars align again counted along the wall of the device in the direction of the flow. The period N of the device is defined as

$$N = \frac{\lambda}{\varepsilon\lambda}$$

The angle of separation in the device is given by

$$\tan\theta = \frac{\varepsilon\lambda}{\lambda} = \frac{1}{N}$$

The fluid flowing through the device is being divided into streamlines as seen in figure 8. A particle entering the device wants to stay in the same streamline as it entered into. When it reaches a pillar, the particle will be pushed laterally by the obstacle. If the particle is small it will go around the pillar following the streamline, however if the particle is large enough that its hydrodynamical center reaches the next streamline, it will try to follow this new path. But at the next obstacle the procedure will repeat and thus the particle will be bumped. There are two modes of movement that particles can take in the DLD; either it will be in the "zigzag" mode following one streamline through the device or it will be "bumped" as the other mode is called. The "bumped" particle always be pushed one row to the side on its way through the device and end up towards the accumulation wall

6.1.2 Critical Radius

The critical radius, or sometimes diameter, is the minimum radius particles need to have to start flowing in the "bumped" mode in the device. Due to symmetry, the amount of streamlines between posts is the same as the period of the device. The bumping starts when a particle is large enough to reach the next streamline when it gets pushed; this happens if the particle has a radius larger than $\varepsilon\lambda$.

$$R_c = \varepsilon\lambda$$

Or more commonly

$$R_c = \frac{g}{N}$$

General fluidics tells us that the flux in each streamline has to be equal and because the device is being run by pressure driven flow the flow profile is parabolic. All of this means that the streamlines are not identical in cross sectional area, the streamlines closer to the wall need to be larger since the flow speed is slower there. This means we need to have a correction term in the equation

$$R_c = \alpha \frac{g}{N}$$

The correction factor is calculated using the following, at a point between the two posts they can be approximated with two infinite walls. Assume that they are at a distance g from each other in a coordinate system where x is perpendicular to the flow and y parallel. The parabolic velocity profile is given by

$$v_y(x) = \frac{4v_{\max}}{g^2}\left(gx - x^2\right)$$

Assume that the depth of the channel is w>>g. The partial flow between a post and a plane a distance Rc from the post in the x direction is then given by

$$\phi(R_c) = w\int_0^{R_c} \frac{4v_{\max}}{g^2}\left(gu - u^2\right)\partial u$$

The relative flow of the partial flow is

$$\phi(R_c) = \frac{\phi(R_c)}{\phi_{tot}} = \frac{w\int_0^{R_c} \frac{4v_{\max}}{g^2}\left(gu - u^2\right)\partial u}{w\int_0^{g_c} \frac{4v_{\max}}{g^2}\left(gu - u^2\right)\partial u} = 3\left(\frac{R_c}{g}\right)^2 - 2\left(\frac{R_c}{g}\right)^3$$

Assuming $\phi(R_c) = \frac{1}{N}$ and substituting Rc with $\alpha \frac{g}{N}$

$$\alpha \approx \sqrt{\frac{N}{3}}$$

6.2 Devices

The usage for the DLD device over the four years the technology has been around has been mostly to understand how it works and create a functioning theoretical model to describe the device. It is very much still running around with the practice wheels still on.

Most of the separation that has been done has been made with polystyrene beads that are easy to use and come in well known sizes.

There has been work done with biological material most of them using the device to analyze blood. Here are some of the groups currently working with the DLD device and what they have accomplished so far.

6.2.1 HUANG

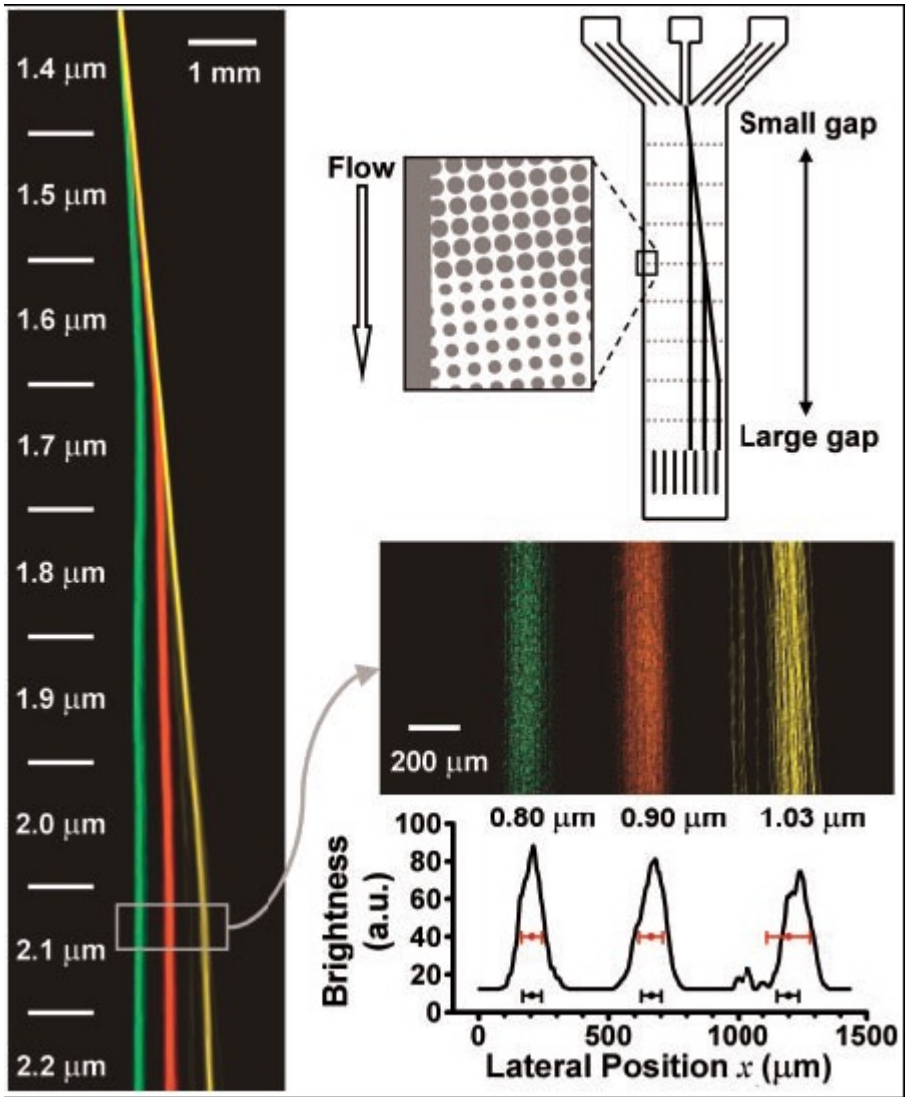

Figure 9. The first DLD device ever published. Image from [19].

In his paper in science [17] the DLD was first presented to the world of science. It lays the ground for all the theory concerning the device.

The group has made some initial test of separating populations of plastic beads and came to the conclusion that the resolution could come down to ~10nm with their current technology. This is a very high precision for a continuous separation, better and faster conventional flow techniques.

Since this is the first paper no real applications have been tested concerning biological uses of the device.

6.2.2 INGLIS

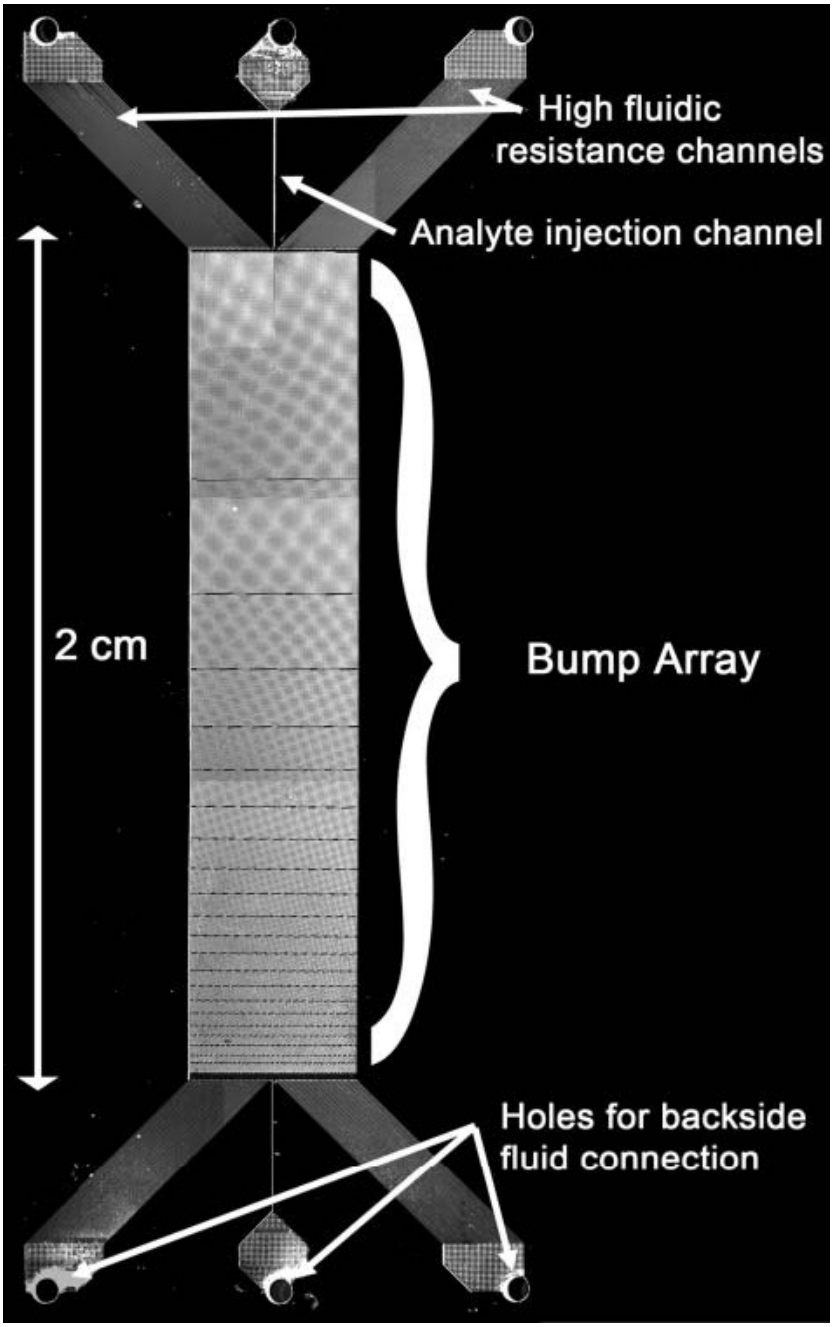

Figure 10. The first DLD device from Inglis et. al. Image from [20].

One of the most prolific groups working with the DLD is Inglis group. There early work has been to establish a working theory for the DLD and a way to calculate the critical size in the device, the most important characteristic of

all. They take up the torch were Huang et al left of in the paper [20], deepening the theory of the DLD by adding a correction factor for the parabolic flowprofile between two posts. They compare the results of their experiments and Huang's and come to a conclusion about the critical size, they show that the parabolic flowprofile will lead to an increase in the critical size of the device.

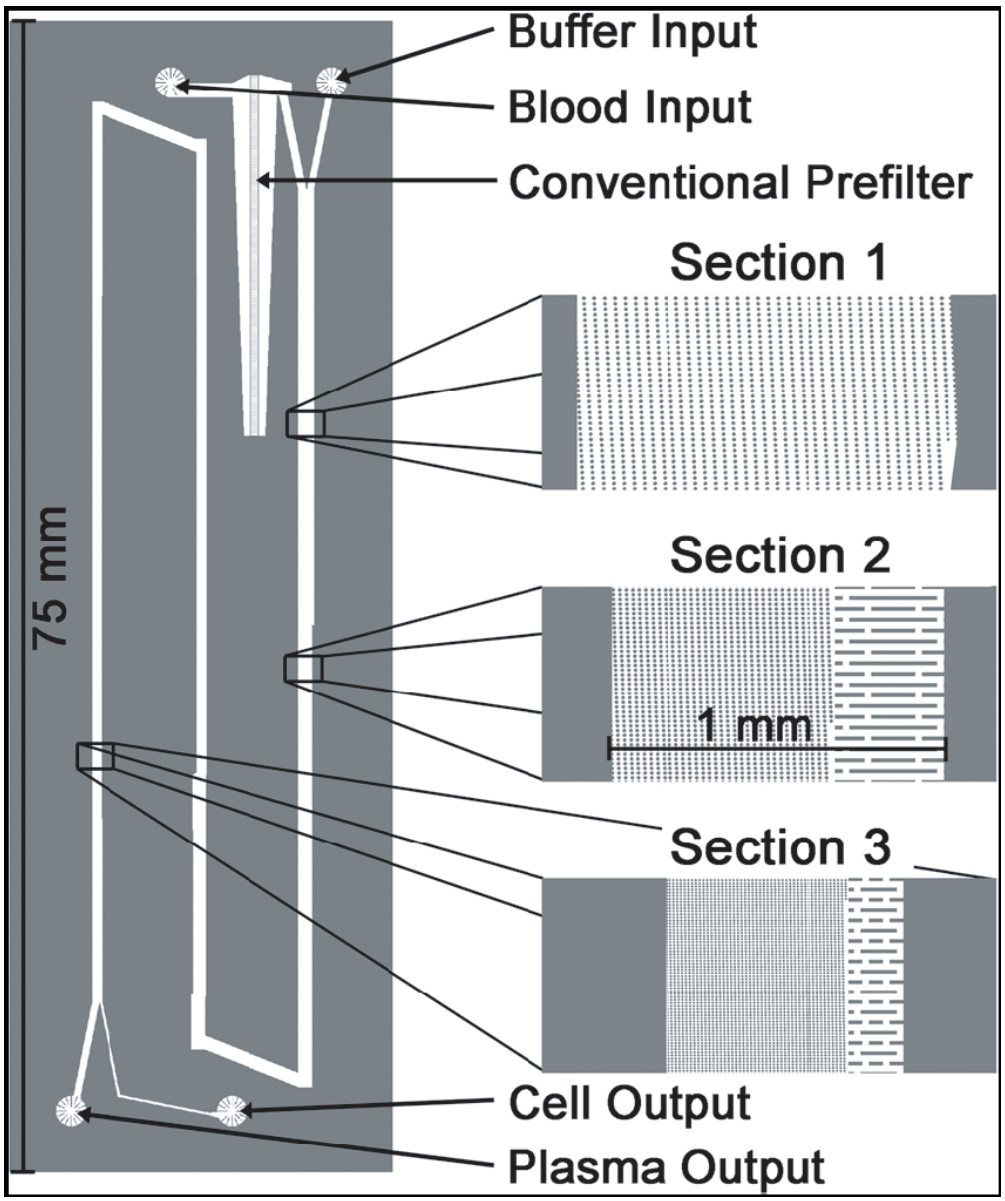

Figure 11. A more advanced device from Inglis et. al. Image from [21].

In his second paper [21] they are working with blood to first separeate up blood in its components and then secondly sort out all the cells from blood to just have serum left.

The first experiments goal is to use the DLD as a cheap and easy FACS machine to analyze blood, by using a narrow stream of sample and using small quantities of sample. The device is built up by having many DLDs in a row each with a progressively smaller critical size, thus sorting out new components of the blood in each step. The analysis takes place by analyzing videos captured showing the exit of the device and counting where the different particles end up. The larger components will exit further towards the accumulation wall than the smaller ones. These results are the compared to analysis made with a flow cytometer showing that the DLD could possibly be used as a cheap alternative to the costly flow cytometer.

In the second part they use a device with a broader stream of sample and filter out all of the particles in blood to just leave clean plasma. The broader stream is chosen so that the throughput can be improved.

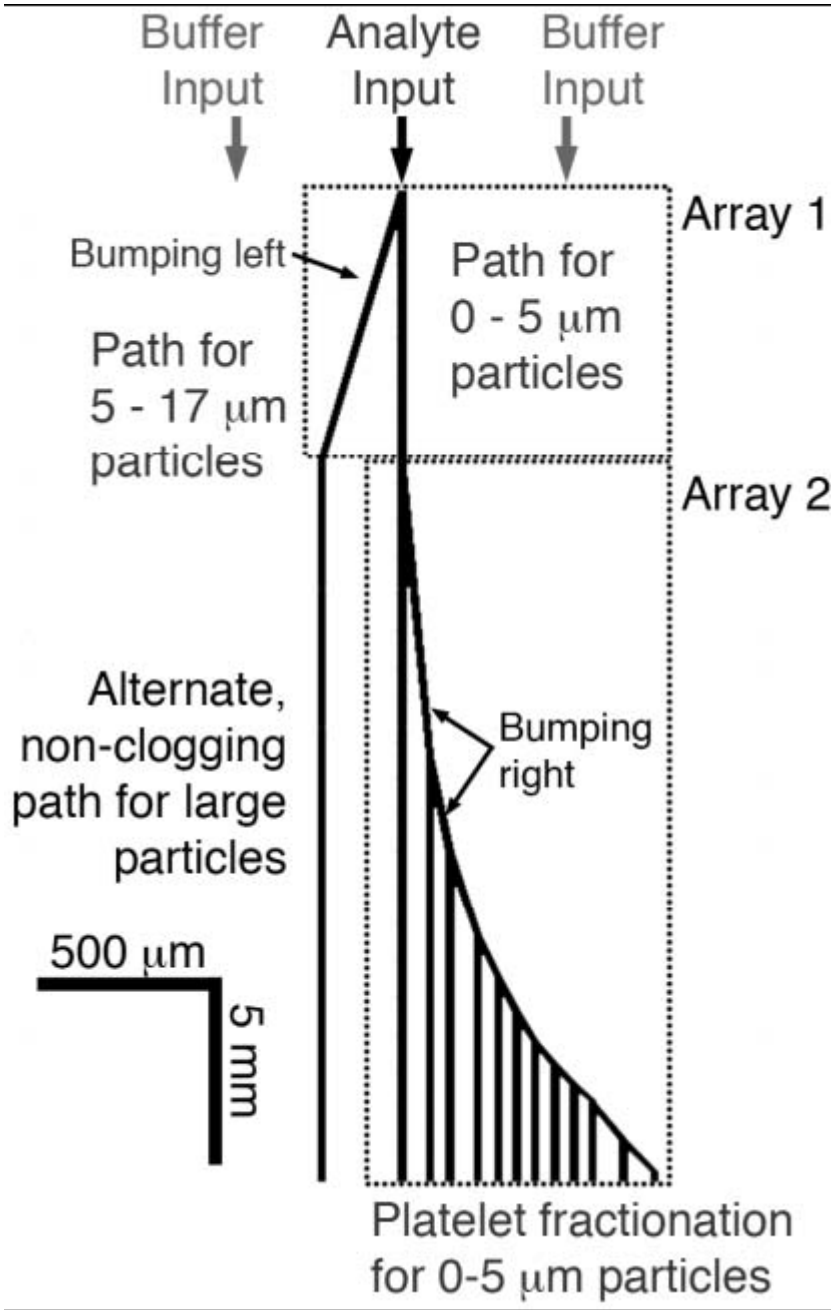

Figure 12. A DLD device used as a cheap FACS. Image from [22].

The third paper [22] also takes use of the DLD as a FACS to analyse blood platelets that have or have not been activated with thrombin at 4 degrees. They show that activation of the platelets changes the morphology of them

6.2.3 BEECH

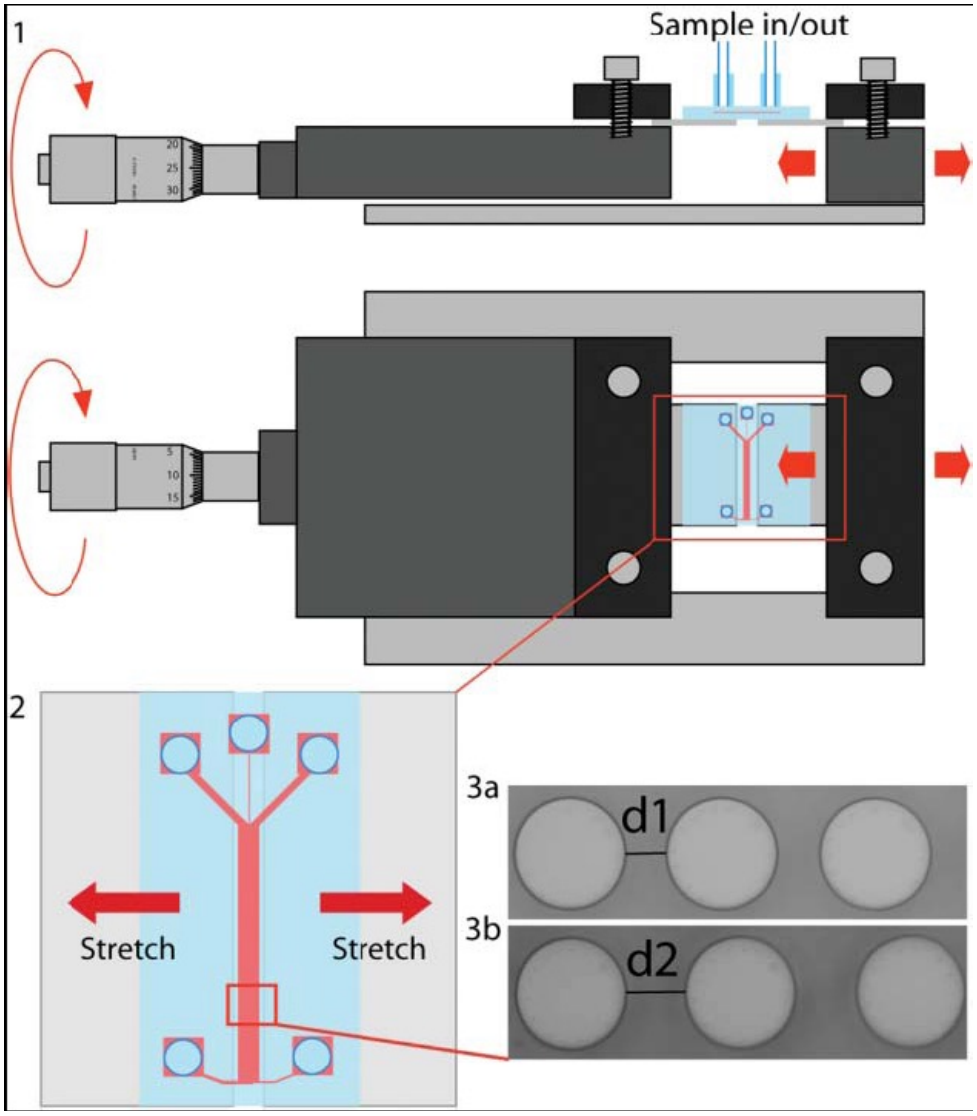

Figure 13. An ingenious way to create a dynamic DLD device. Image from [23].

Beech and Tegenfeldt [23] have focused on making more usable DLD devices, trying to make them more adaptable and thus more commercially viable.

In his paper Beech et al has created a DLD device all in PDMS that can be stretched with micrometer precision thus altering the distance between the posts of the array. Larger distance leads to a larger critical size. He has showed a working device that can dynamically alter its critical size during operation.

Part 2

This is the experimental and practical part of this thesis. The goal of the experiments where, to firstly get a working prototype up and running by over coming obstacles such as clogging and secondly separate populations of cells so that synchronization can occur.

This part starts out with the practical part of creating a device from drawing board to ready prototype and follows with the experimental setup and then the results from the experiments.

7. Design

7.1 Version 1

The design of this prototype was made by Jason Beech, further design considerations can be found in [7].

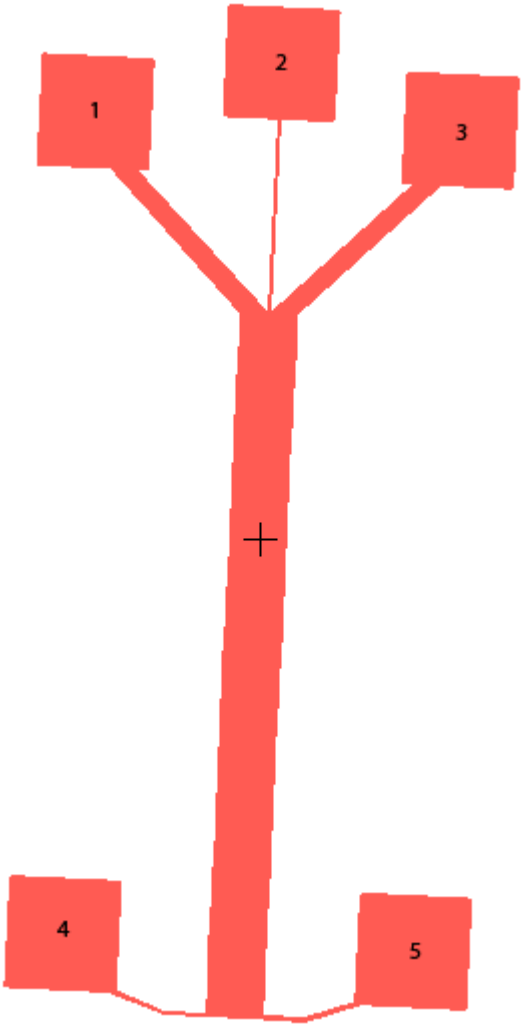

Figure 14. The design of the first chip.

The main thought behind this design is to have maximum resolution. The choice of cut-off size has been chosen at a size that we believed to be the size of the cells we would use. The size was measured in the thesis [7] and was found to be between 10µm and 22µm. Thus a critical size of 14µm was chosen.

The entrances have been numbered 1 to 5, the first three ones are the entrances to the device and the other two are the exits. Of the exits number 4 is the exit where the bumped sample is supposed to exit and exit number 5 is the one where the smaller particles are supposed to exit.

The reason for choosing channel number 2, the sample channel, so small in comparison is to get a narrow and well focussed stream that will give a good resolution. Of course, since the buffers channels, 1 and 3, are both comprised of 10 channels they have 10 times more throughput meaning that most of the liquid in the bumper will be just buffer. The long and very narrow channel 2 may give rise to a strong shear force that the cells are subjected to in this channel which can lead to the breaking of the outer membrane.

7.2 Version 2

Figure 15. Design of the second chip.

The main problem encountered with the first device was that the cut-off size was set to low so that almost all cells were larger than the critical size. This had to be solved. By not knowing the exact size that we needed and now having the ability to do lithography on larger silicon wafers we opted on doing several devices covering a wider range of cut-off sizes on a single master.

A second problem recognized in the first trials where the small amount of sample that we could put through the device, the amount of sample being approximately 20µL/h. To attack this problem we wanted a larger entrance

for sample. This gave us the idea to make a device with only two entrances for simplicity and speed, the lone sample channel from the first version had been cut away by Occam's razor. We wanted more throughput to be able to do flow cytometer analysis on the sample that had been run through the device.

This design is much simpler than version 1 with only two entrances and two exits. Number 1 and 2 are the entrances where the smaller one, 2, is the one where the sample is introduced. The length of the device was chosen so that a particle entering close to the right wall would be, if the size is right, be bumped all the way over to the left wall before it reaches the exit. The walls were of a different design than version one with the wall following the first row of posts. This was to prevent the particles in the bumper to be able to stick between the wall and a post close to it and also to be able to get particles flowing close to the wall to go out towards the centre of the device.

The reason for choosing the sample entrance and exit a third of the width was to improve on resolution of the device.

This device is simpler and should have a much higher throughput than version 1 at a cost of resolution.

The gaps between posts in the devices were chosen to be 48, 50, 52, 54, 56 and 58 μm. This was to span the range of version 1 and also go beyond it and thereby hopefully find the size we were looking for. These gap sizes led to critical diameters being; 12.50, 13.0, 13.50, 14.0, 14.6 and 15.1 μm.

8. Fabrication

8.1 Master

The master for PDMS casting was made by UV-lithography on a silicon wafer coated with a ~30µm thick layer of SU-8, a positive resist from Microchem.

The pattern was developed and checked under a microscope for imperfections caused by an over/under exposure of UV-light or by over over/under developing the SU-8. Once a good master had been created it had to be treated with an anti-sticking agent.

8.2 PDMS

PDMS, Sylgard-184, was mixed at a mass ratio of 10:1 and placed in a vacuum chamber for 40 minutes to remove all air bubbles. The PDMS was then poured over the master and baked for one hour at 80ºC to harden.

8.3 Device Preparation

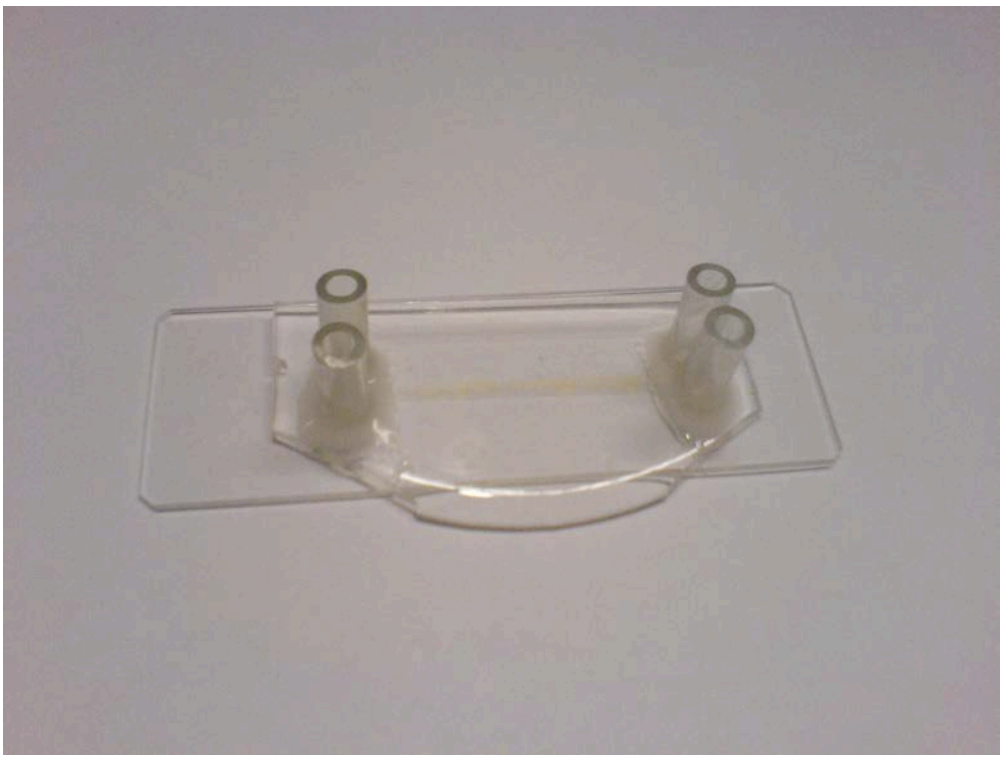

Figure 16. A Ready device. The picture shows a second version device**.**

The finished PDMS device was cleaned and holes were made for the reservoirs. The device and a cleaned glass-slide was then exposed to oxygen plasma for 30 seconds both to bond the two parts together for a tight seal and to create the negative surface charges needed for the Pll-PEG to attach to.

As soon as the device has been bonded Pll-PEG was introduced to make use of the negative surface charges in the channel before they dissipate.

The next step was to attach the reservoirs by gluing them with silicone glue.

9. Experimental Setup

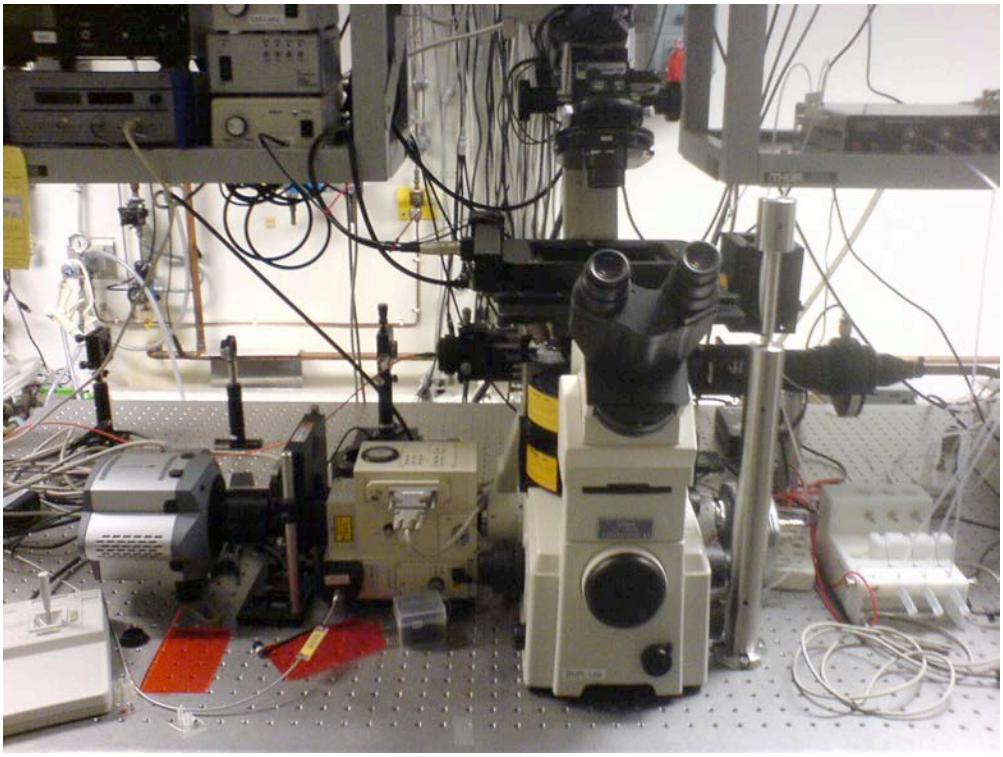

Figure 17. The experimental setup**.**

The experiments were recorded with a Nikon Eclipse TE200-U microscope fitted with an Andor Luca camera and later in the project with an Andor Ixon and Andor confocal setup.

Fluorescence excitation light came from a Prior Lumen200 light.

Pressure was applied using KNF Lab labport pumps or by a fluigent setup.

All polystyrene beads were purchased from Duke scientific.

Buffers used have been Hepes, Pluronics or in the case of living cells. serum (RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. Cell culture medium components were purchased from Biochrom KG, Berlin, Germany).

10. Surface Passivation experiments

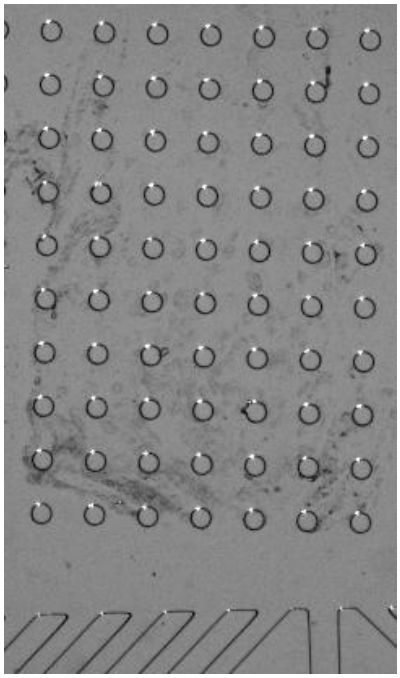

Figure 18. Mysterious gunk at the entrances. Possibly caused by DNA from lysed cells.

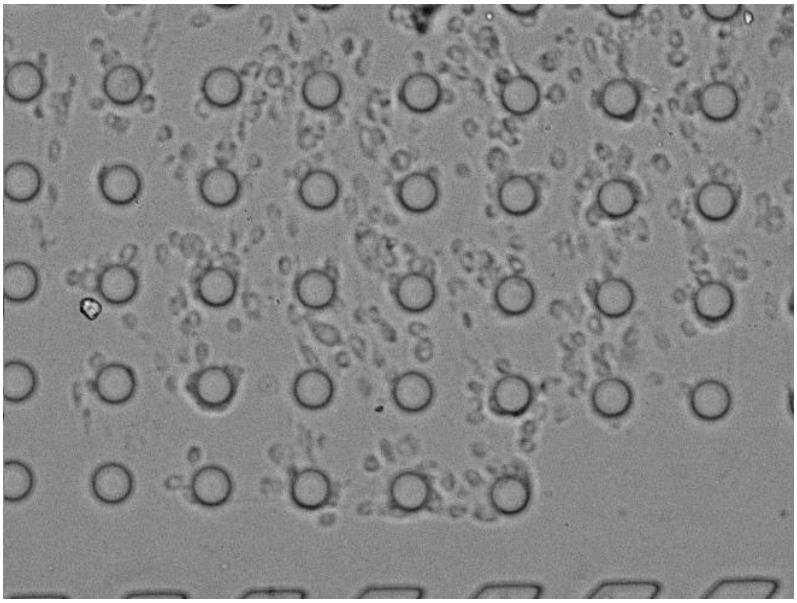

Figure 19. A close-up of the gunk

The first experiment to be done was to test different ways to pacify the surface to that the actual interesting measurements could be made. To this

extent we ran two devices treated in different ways to see which ones had the longest life span. All the runs were performed under the same conditions with the same pressure to drive the device.

The different methods used to treat the surface were oxygen plasma and Pll-PEG. Another method was also planned on being used, Poly-HEMA, but the way this material had to be dispersed in the device made it impractical for microfluidic use. Adding BSA to the buffer had already been tried by [7] so a second try was not necessary.

An untreated device could run for approximately 10 minutes before it started to severly clogg up, see figure 16 and 17, close to the entrance of the device. Strings of what is assumed to be DNA got stuck around the posts and very soon the device was useless.

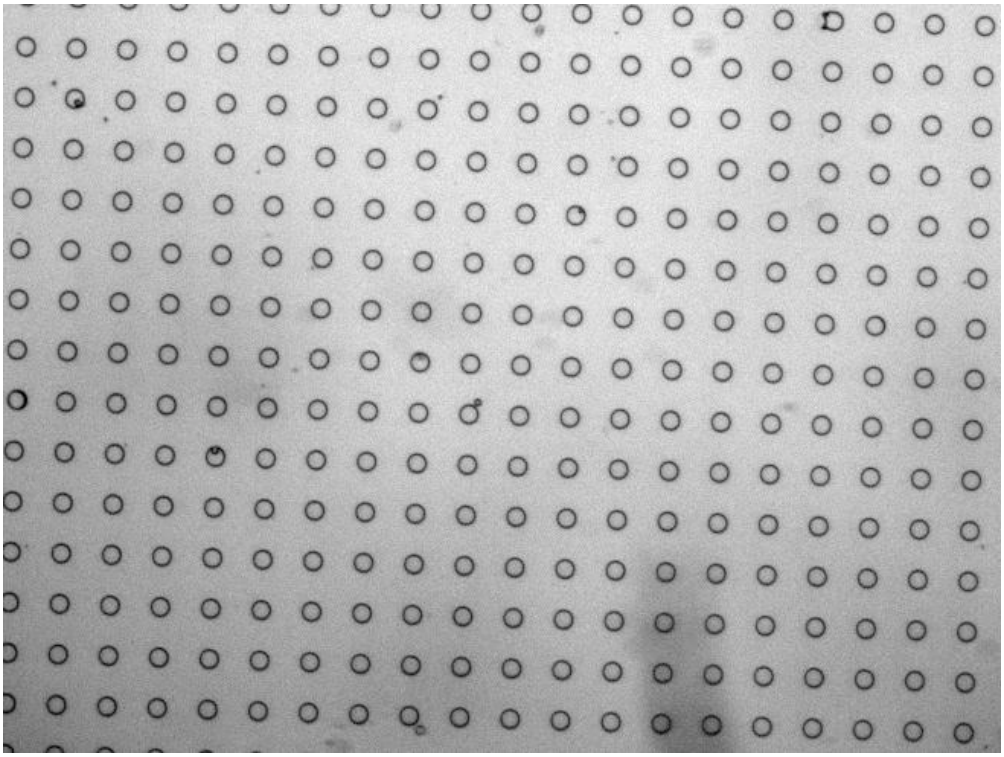

Figure 20. A picture of a treated device. The gunk has not yet appeared and the device is working well.

A device treated with Pll-PEG however, could run for periods of time up to a couple of hours without severe clogging. A remarkable improvement over a untreated device.

We were planning on testing a substance callen Poly-HEMA as well but due to the handling of the substance and its application to the device we decided that it was not going to meet our demands.

In conclusion we found that treating our devices with Pll-PEG gave a remarkable result for surface passivation. Pll-PEG made it possible for us to decide for our self how long a test would be, not the device itself. All future devices were cells are being separated will be coated with Pll-PEG.

11. Beads in version 1

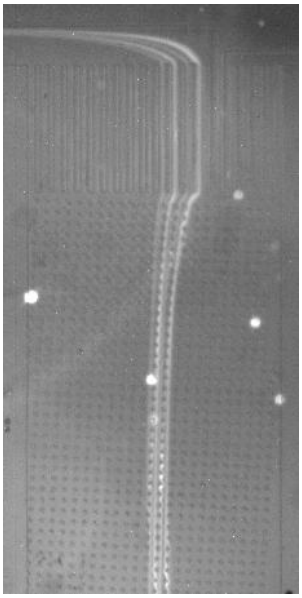

Figure 21. 10μm beads run through the DLD. The picture is a composite of 500 frames.

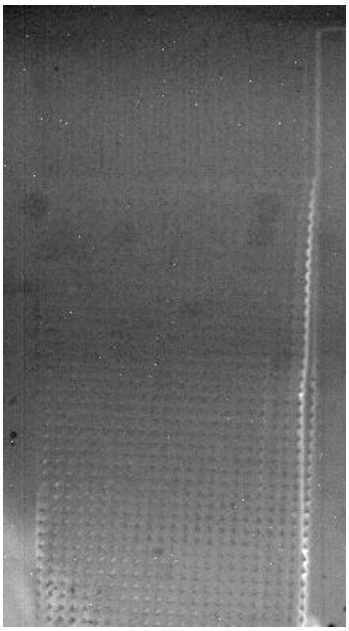

Figure 22. 16μm beads run through the DLD. The picture is a composite of 500 frames.

To see that we had a separation in the device we separated populations of beads of sizes 10μm and 16μm. This was to understand how the device works and get some hands-on experience on separating things and using all the tools in the experimental setup.

The results show a clear and fast separation between the two populations showing that the device works well and at high speeds. All of the 16μm beads were led of to the side, figure 20, and most of the 10μm ones flowed straight through, figure 19. The 10μm ones that did get bumped were all clusters of two or more beads stuck together.

Tests were made to if it was preferable to bond the device to a glass surface spin coated with a thin layer of PDMS to have all the sides of the channel comprised of the same material, or to bond it to the traditional glass substrate. Any difference could not be seen so for convenience we opted to keep using just clean glass slides.

The clear separation between the 10 and 16 μm beads shows that the device works well and that the theory for understanding the critical size in the device is working since that the critical size of the device was 14 μm, according to known theories.

12. Cells in version 1

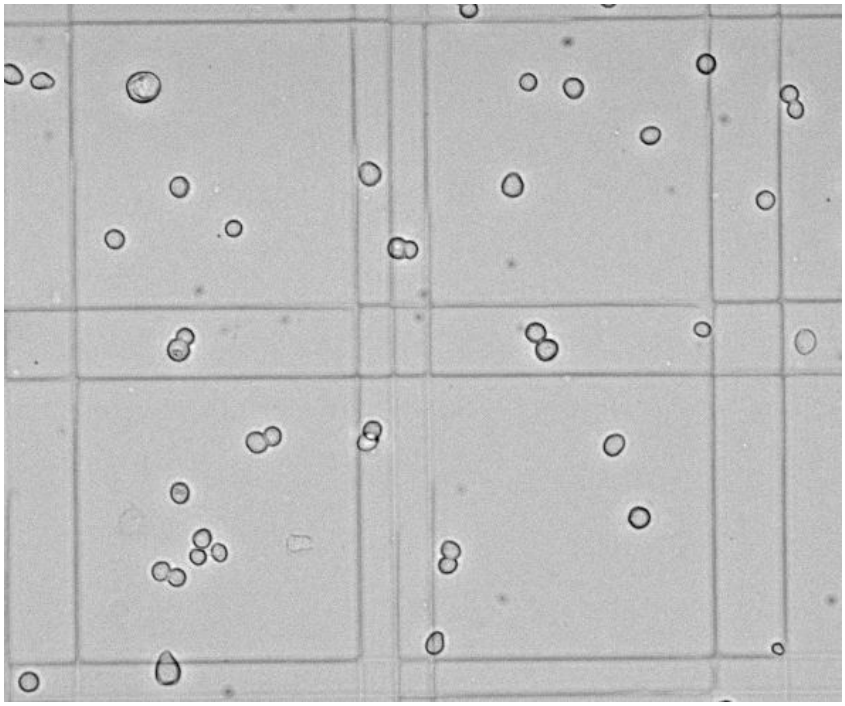

Figure 23. L929 cells in a burkerchamber.

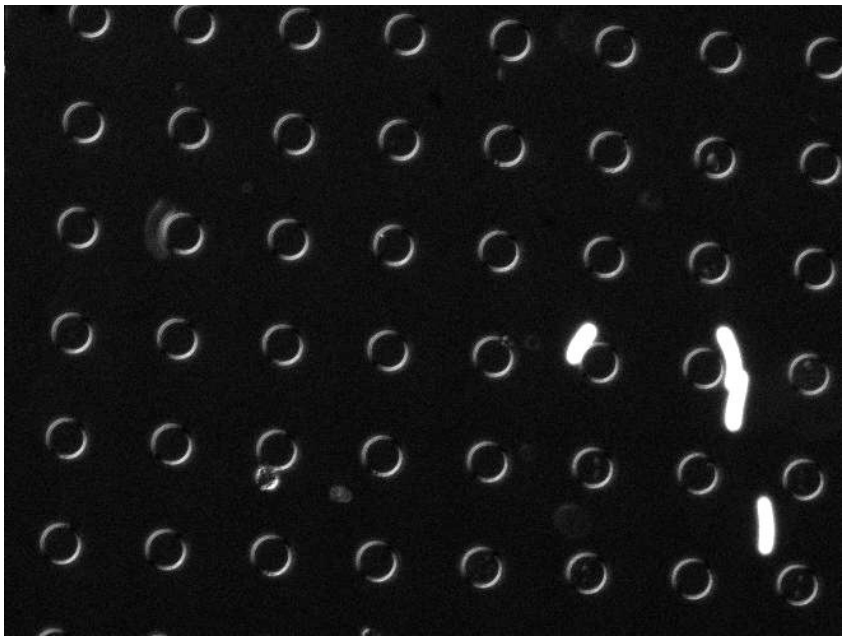

Figure 24. Cells and 10µm beads in the device at the same time. The cells are being bumped in the left side of the screen.

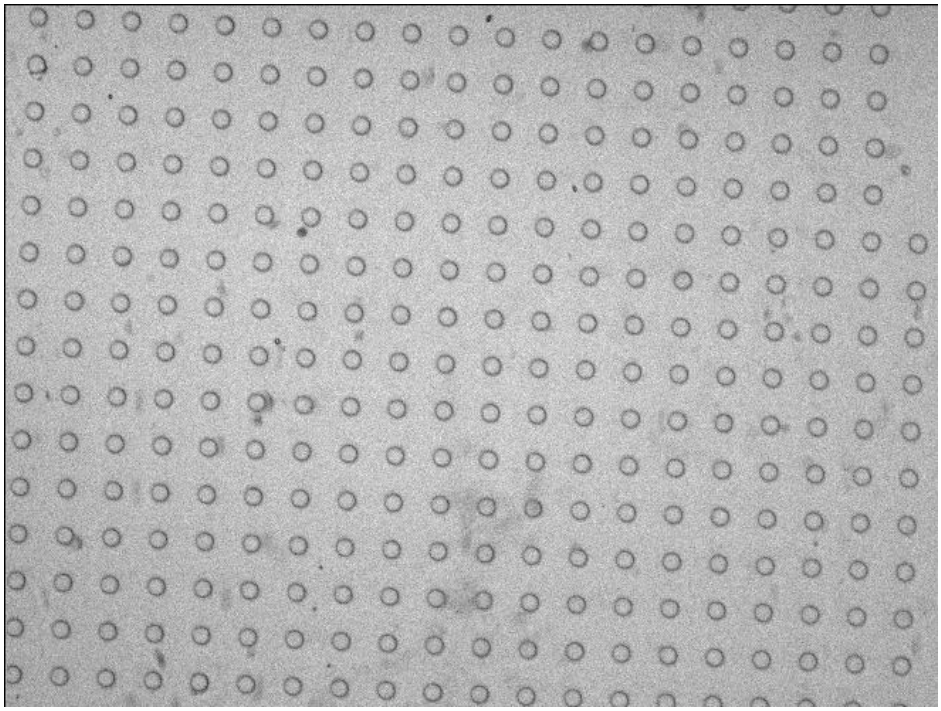

Figure 25. L929 cells in the device.

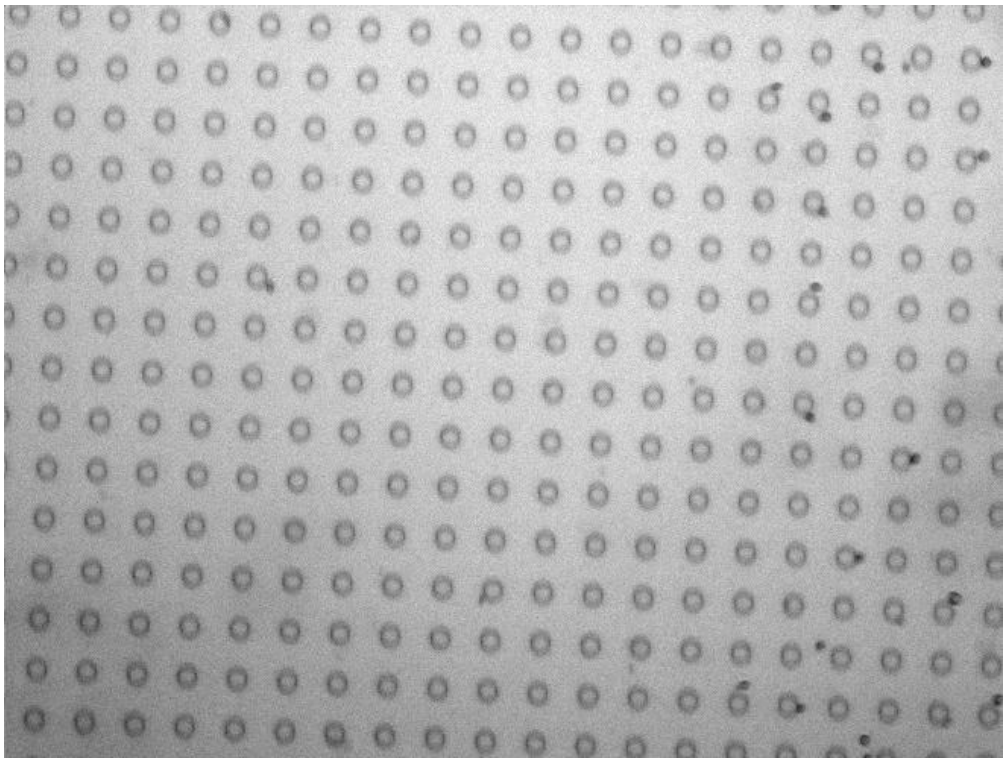

Figure 26. Close-up of L929 cells in the device.

Once the problem of clogging had been sufficiently overcome it was clear that the device was sorting out all of the cells. Figure 21 shows a picture of the device where 10 μm and cells are being separated at the same time. The cells are one the left side of the picture and beads to the right. Cells are being bumped while the beads all go straight through the device. This shows that the device is actually working and is not only just separating out everything. Nearly all of the cells were being bumped in this device, this led us to the conclusion that a new device was needed.

Another problem that needed to be addressed was the poor throughput in the device. One hour of continuously running the device only 20μL of sample had gone through the device. If the device was going to be used for any practical use and to be able to do FACS measurements on our samples we needed to improve the throughput.

13. Beads in version 2

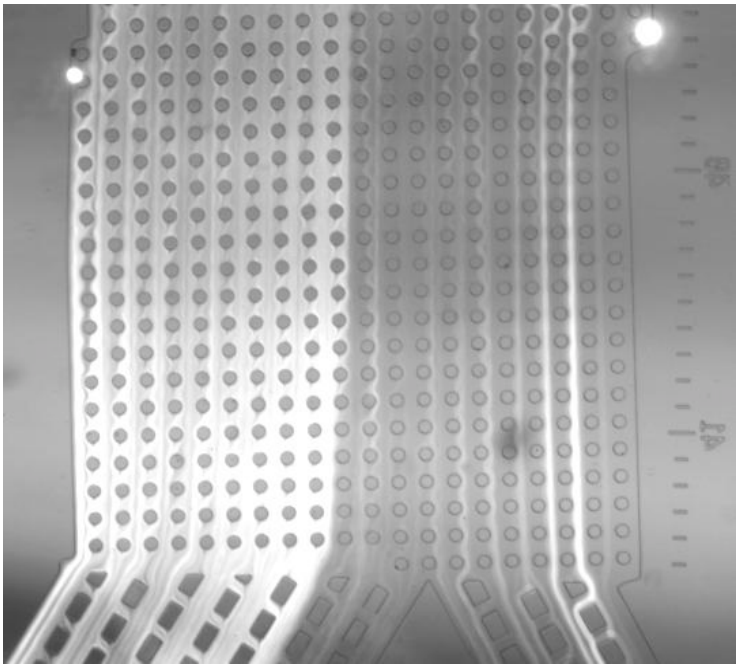

Figure 27. 10 µm beads in the new device. The beads should not be bumped in this device. The particles that have been bumped are clumps of two or more beads stuck together.

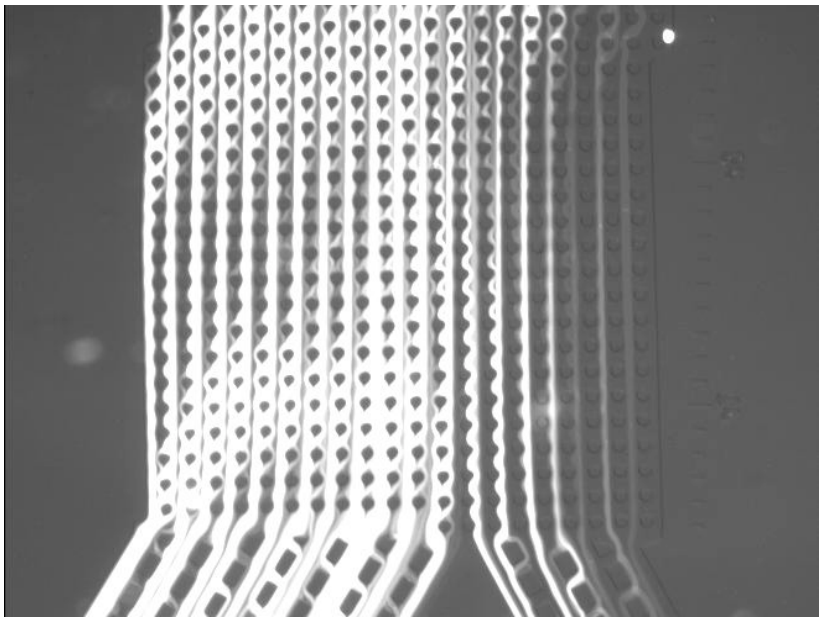

Figure 28. 16 µm beads in the new device. The beads should have been bumped to the right side of the device.

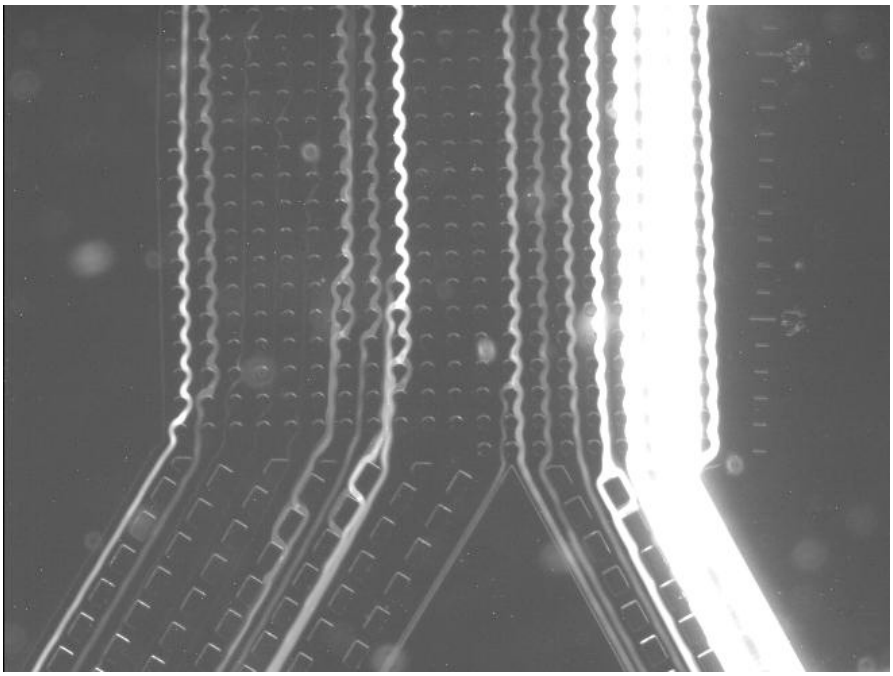

Figure 29. 27 μm beads in the new device. Nearly all of the beads are being bumped, as the should.

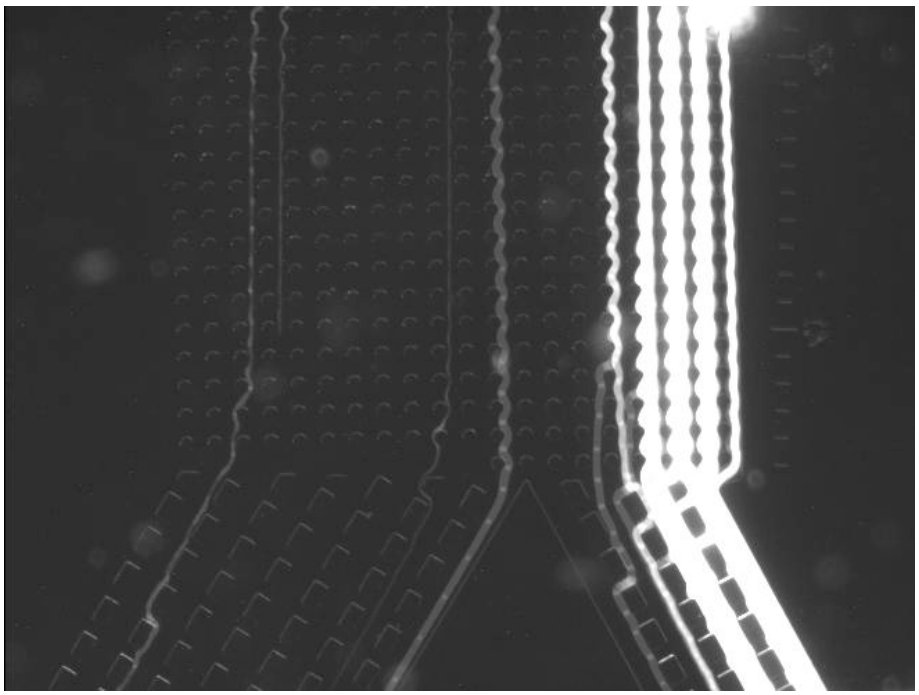

Figure 30. Another picture of 27 μm beads in the new device.

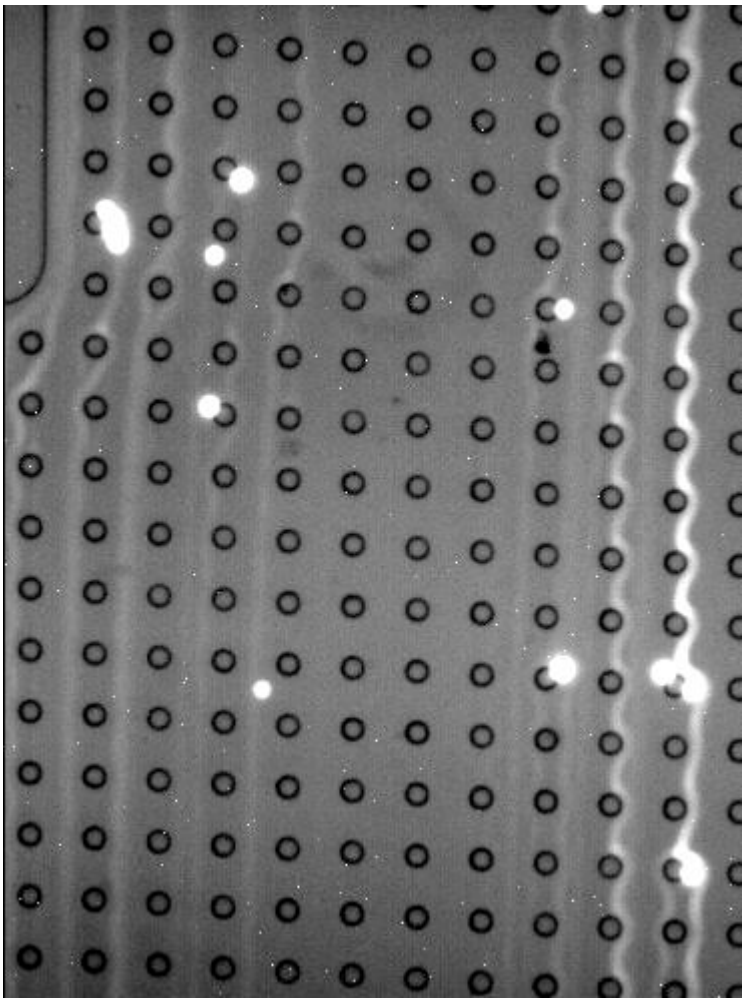

Figure 31. 16 µm beads in the new device. The beads are divided up into two populations.

The device does separate beads. The 10µm beads went straight through the device, figure 25, as expected and the 16µm beads, figure 26, got separated out, or some of them did. This was not expected. Because of this unfortunate characteristic 27µm beads, figure 27 where also introduced these were all separated out, luckily, as expected.

In the runs with 16µm the beads were clearly divided into two different streams as seen in figure 28, it was not a continuous distribution over all of the exit channels. The 16µm beads that didn't get sorted out were all next to the channel wall of the device, even in the devices were the cut-off was supposed to be well below 14µm, far under even the smallest of the beads, the beads have a 10% coefficient of variance which still means that almost all

of the beads have a diameter over the critical size. This is suggesting a wall effect not considered during the design of the device.

14. Cells in version 2

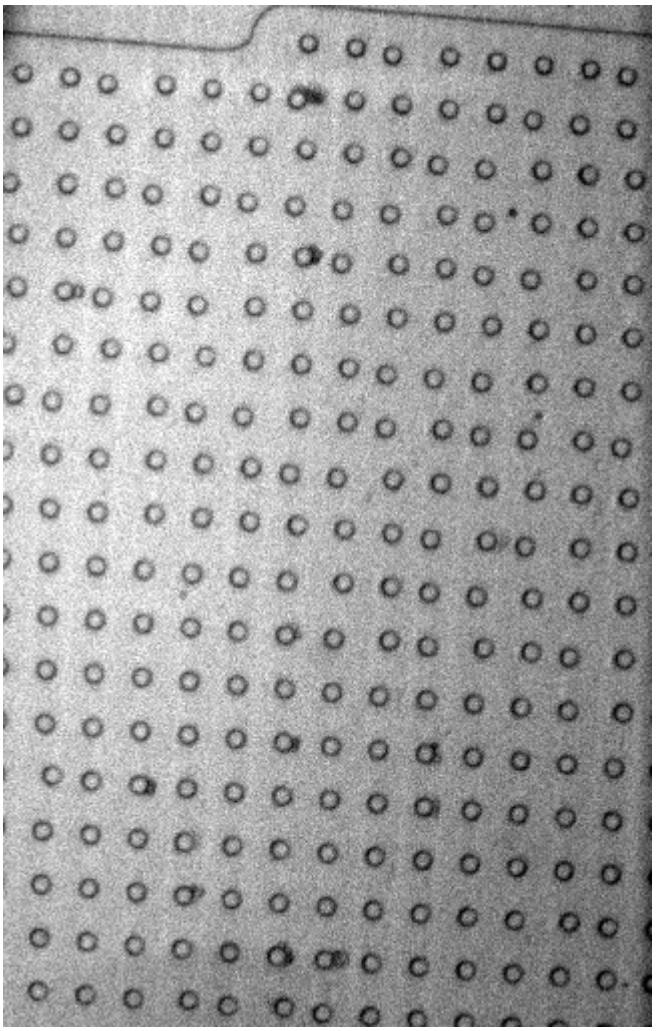

Figure 32. L929 cells in the new device.

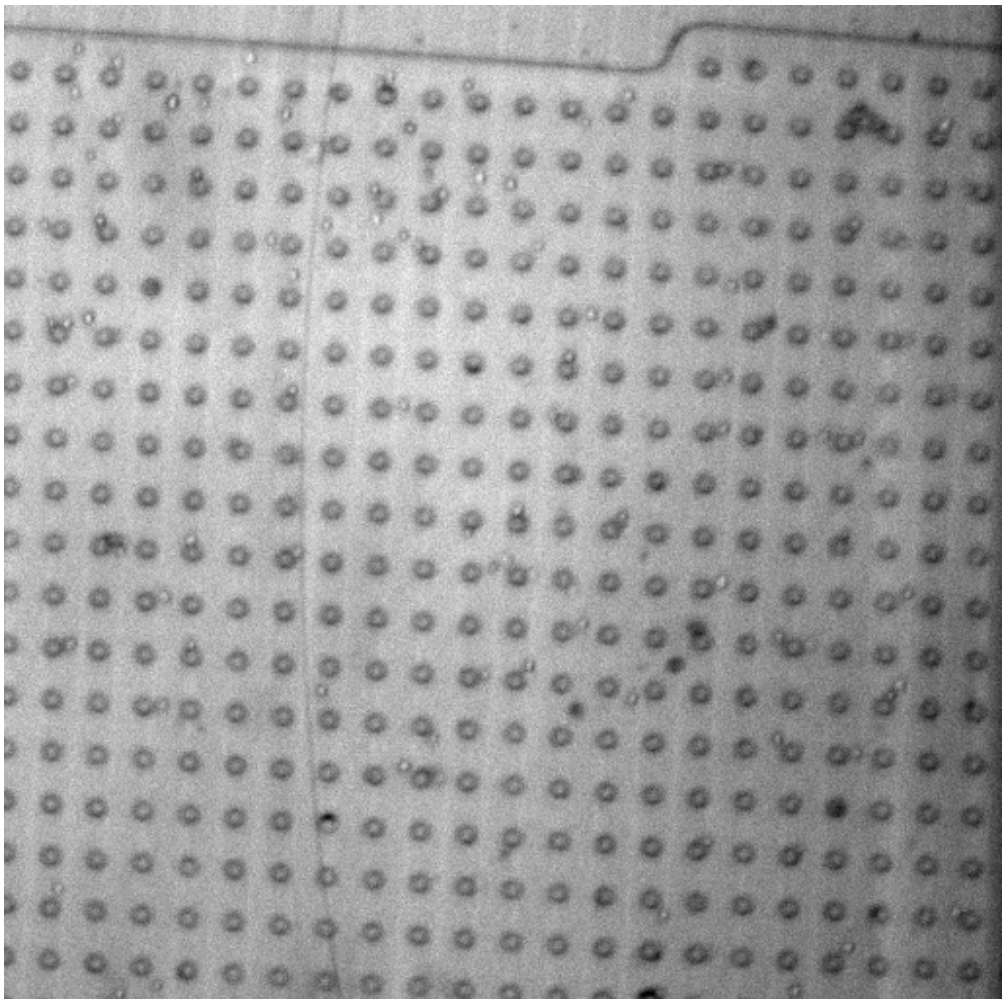

Figure 33. A close-up of L929 cells in the device. Some of the cells are being bumped.

Since the unfortunate edge effects in the device that lead to particles not moving away from the wall or the first couple of rows in the device a perfect result was not expected in the trials with cells. However, the cells that would be lead of had to be over the critical size as was shown in the runs with 10μm beads.

One big difference when running the device with cells instead of beads was that the cells were spread out more over all the exits in device showing a larger spread in size among cells, something that was also to be expected.

The main reason to creating this device after version 1 apart from trying to find the perfect critical radius for separation was to improve the throughput. The throughput in this device was greatly improved over version 1 up to

320μL of sample per hour. Making it much easier to get samples to perform FACS or running beads two times through the device.

15. FACS

The reason for upping the throughput of the device was to be able to have a more practical device for commercial use, be able to run enough sample through the device that it would be possible to run the sample twice through the device and to be able to do FACS measurements.

The FACS measurements were performed in a FACSCalibur system from BD Biosciences. The first test to be performed was to calibrate the system. The relevant measurement for determining size in the cells is the FSC (forward scattering). The problem is that the FACSCalibur is measuring a relative value. So using the same settings in all of the measurements particles with a known size was run to create a calibration diagram. The particles used were 3, 5 and 16 µm beads from dukes scientific. Another reason for doing this test was also to check the resolution of the FACSCalibur system since the beads had already been measured in a separate TEM measurement.

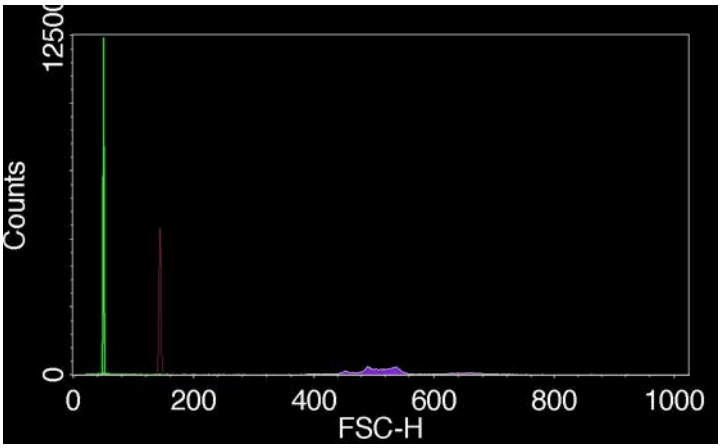

Figure 34. Test of FACS precision 3, 5 and 16 µm beads

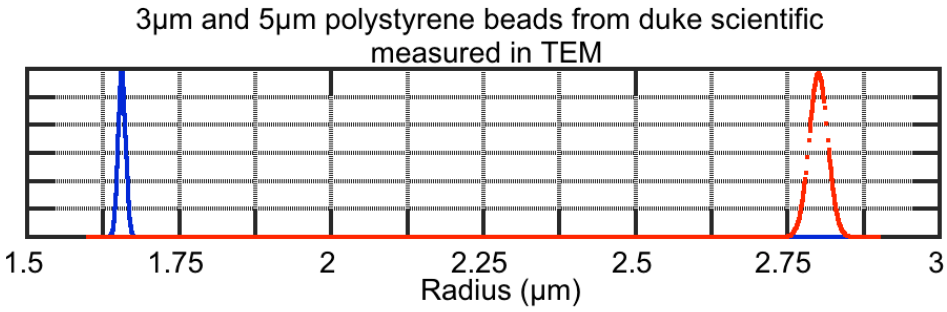

Figure 35. Results from TEM measurements of beads.

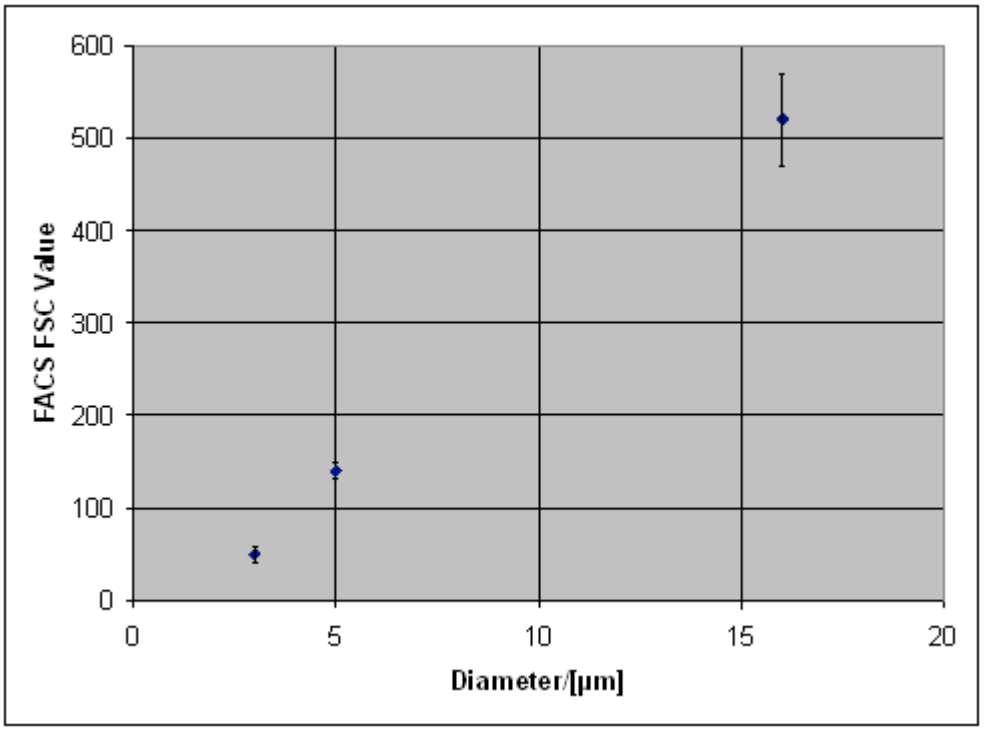

Figure 36. FACS readout as a function of particle diameter

The calibration showed a linear or at least a very close to linear connection between radius and FACS FSC value. It is possible that it is actually a polynomial of a higher degree but for our purposes it can be approximated to a linear function. The error in the measurement is quite large for the 16 µm beads, this probably stems from the handling of the beads. The 3 and 5 µm beads came already in solution but the 16 µm ones were delivered dry in a powder form and the mixed in solution by hand. We have been lead to believe that these beads tend to have some distortions in their size configuration.

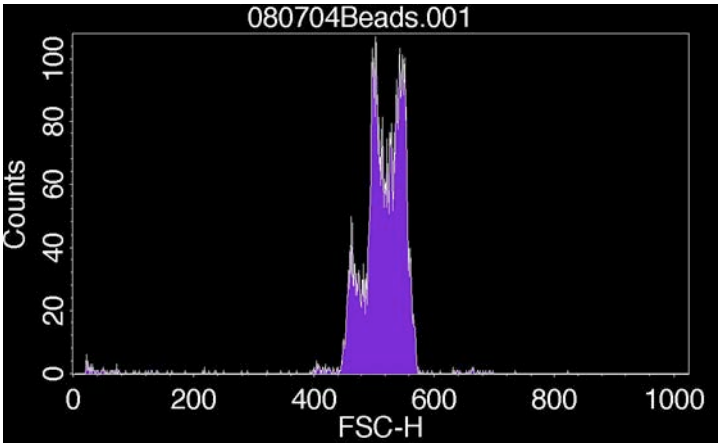

Figure 37. 16µm beads control

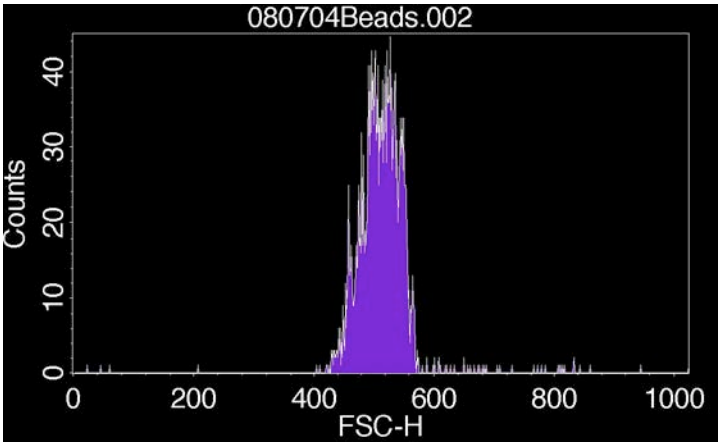

Figure 38. 16µm beads bumped in a device with critical size 12.5µm

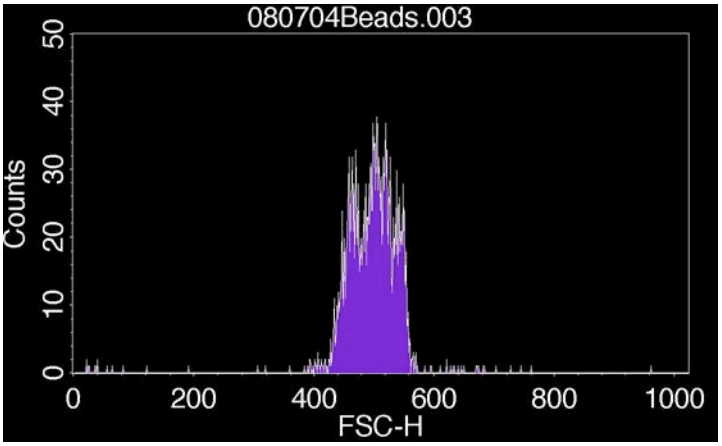

Figure 39. 16µm beads not bumped in a device with critical size 12.5µm

Beads in these tests were the beads had been run through a device with a cut-off size of 12.5 µm. The size distribution of the beads was from 13.5 µm to 17 µm. In this device all of the beads should have been diverted since they all are over the cut-off size. Not all of the beads have been diverted as can be clearly seen but the distributions seem to have a different characteristic in the two exits. Smallest beads seem to be less common in the bumped exit unlike the ones that went straight through the device.

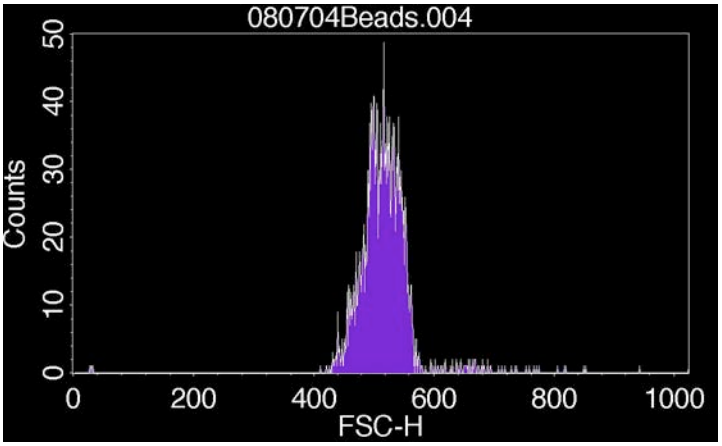

Figure 40. 16µm beads bumped in a device with critical size 15µm

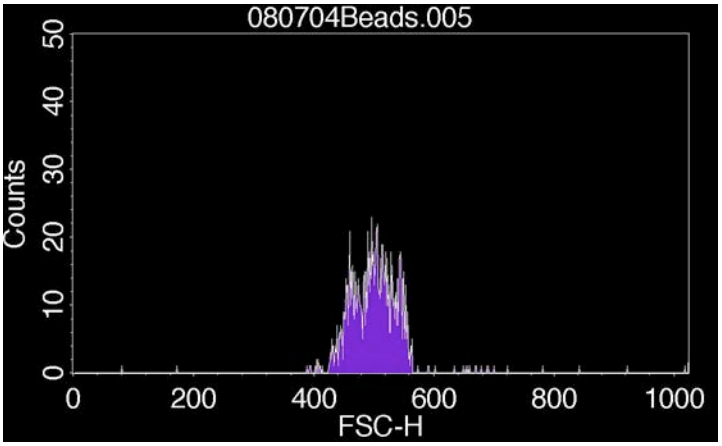

Figure 41. 16µm beads not bumped in a device with critical size 15µm

Beads in this device should have beahaved differently that from the smaller device. Here the cut-off is 15 µm so some of the smaller beads in the distribution shouldn't have ended up in the bumped exit at all. Once again the smaller beads seem to be less common in the exit were the bumped beads ended up. Some still seem to have got there, this could be from beads bunching up into aggregates that get bumped and then split up after they were pipetted out of the device.

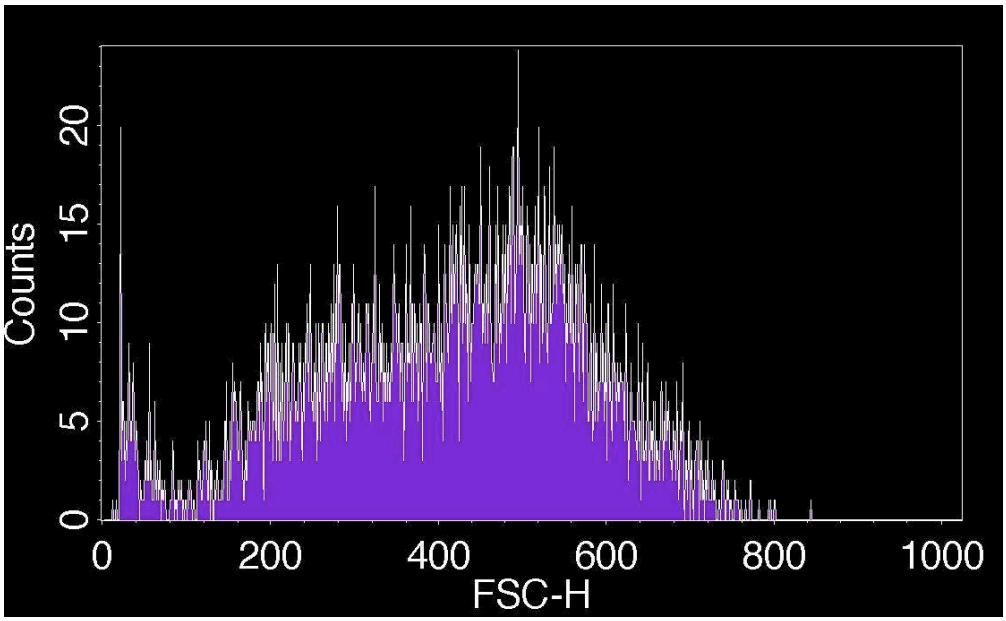

Figure 42. L56 cells control

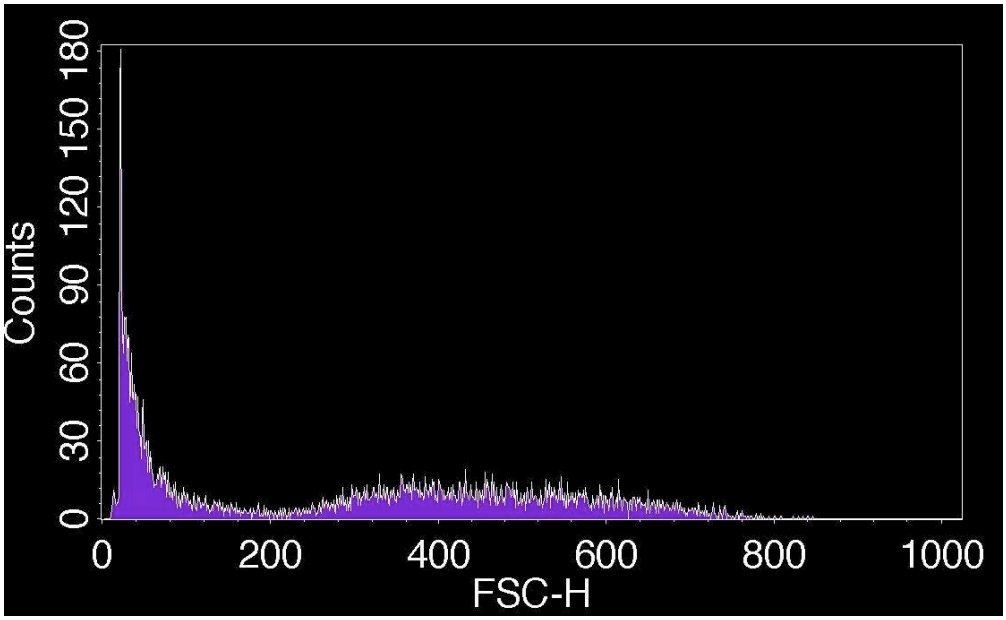

Figure 43. L56 cells bumped in a device with 12.5µm critical size

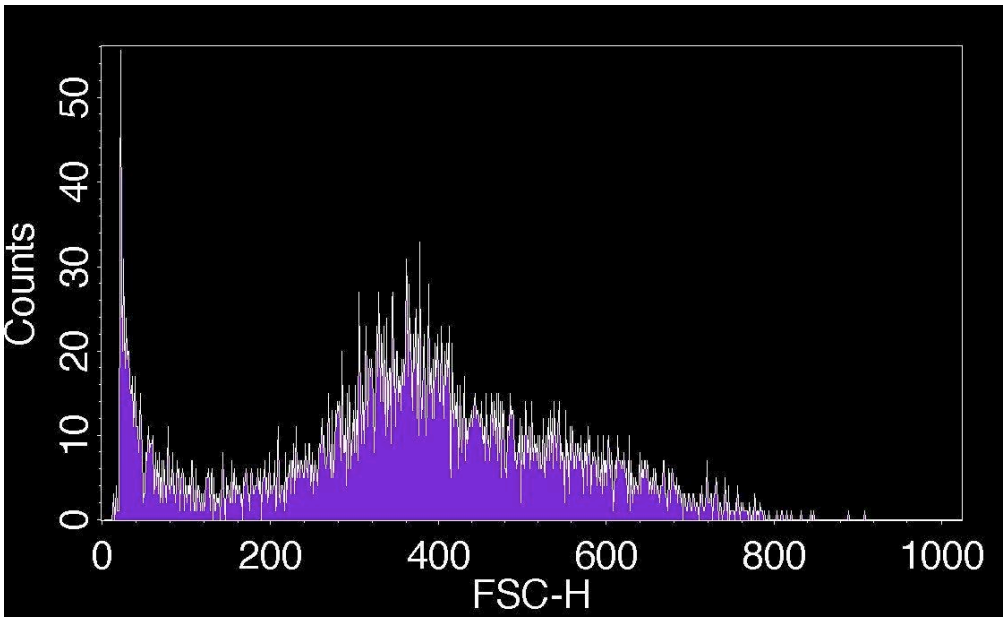

Figure 44. L56 cells not bumped in a device with 12.5µm critical size

The L56 Cell distribution seem to have undergone a separation in these tests. In the sample from the unbumped exit the distribution seem to favour a population of cells with smaller sizes. In the exit were the bumped cells should have ended up the cells seem to have an even distribution over the whole spectrum, this might be because the cells have been damaged or broken while they were being bumped towards the exit.

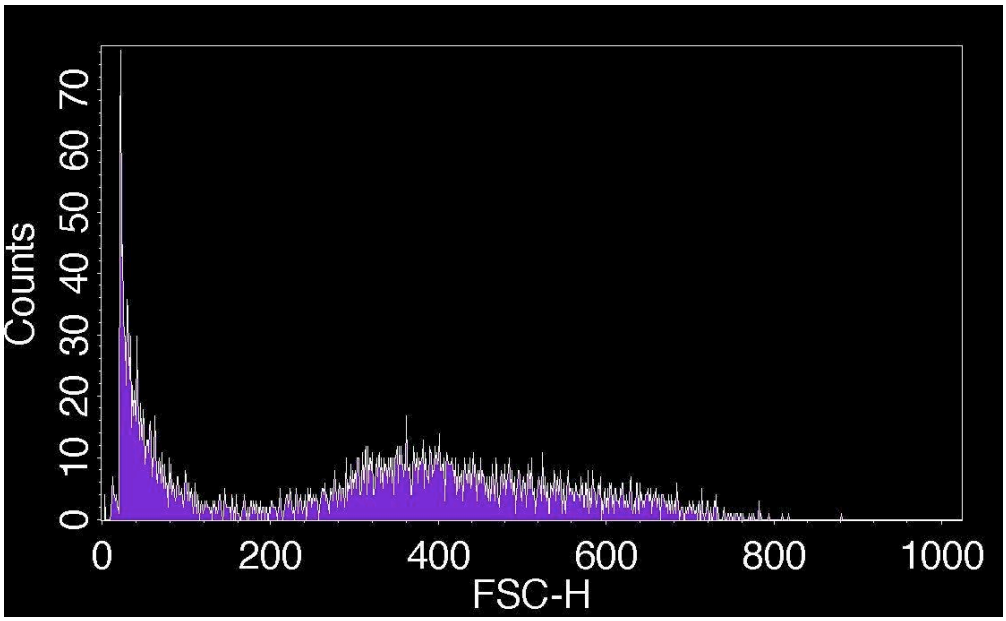

Figure 45. L56 cells bumped in a device with 15µm critical

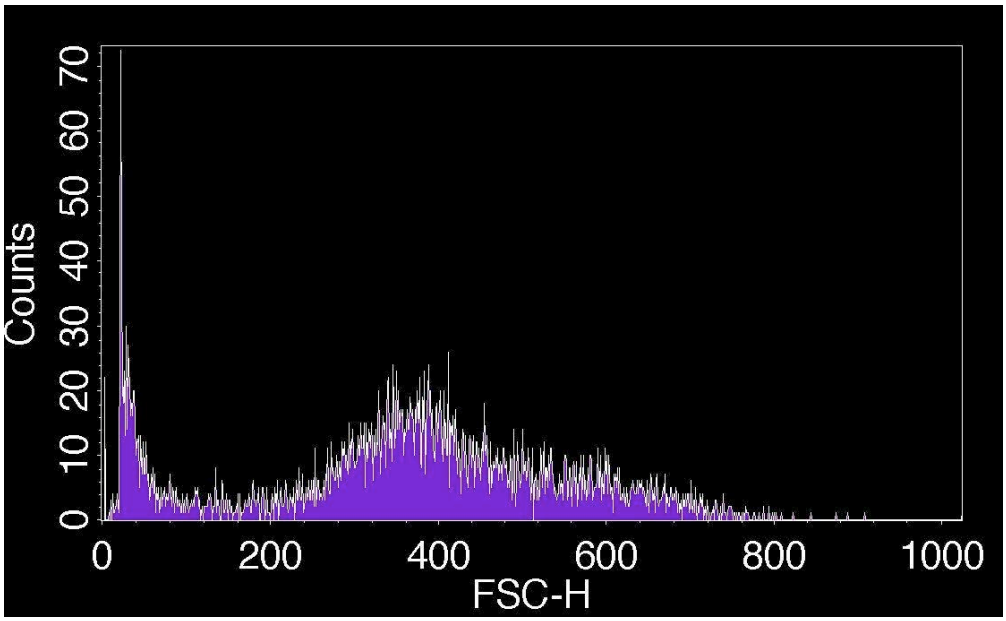

Figure 46. L56 cells not bumped in a device with 15µm critical size

The result for the is very similar to the results from the smaller device the difference being that the cells coming out of the bumped exit is bigger and more cells seem to have come out unscathed on that side than the other device.

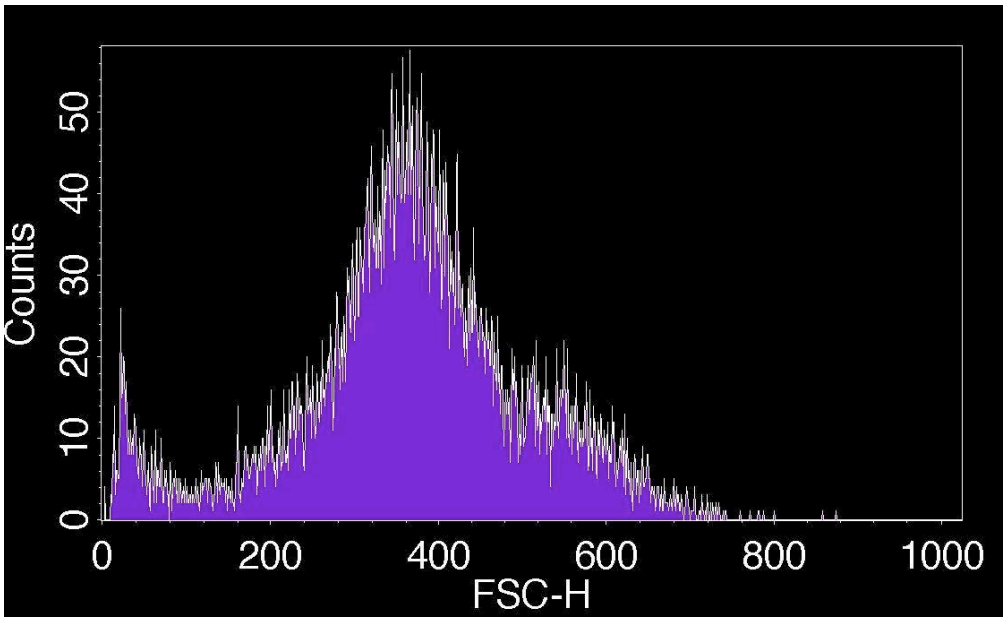

Figure 47. L929 cells control L929 cells bumped in a device with 15µm critical size

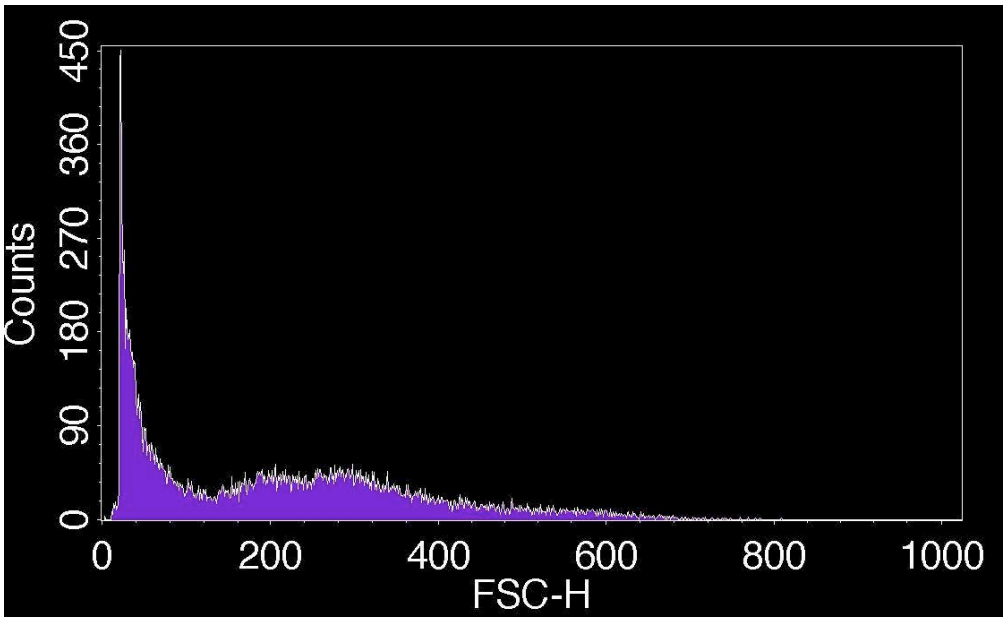

Figure 48. L929 cells bumped in a device with 12.5µm critical size

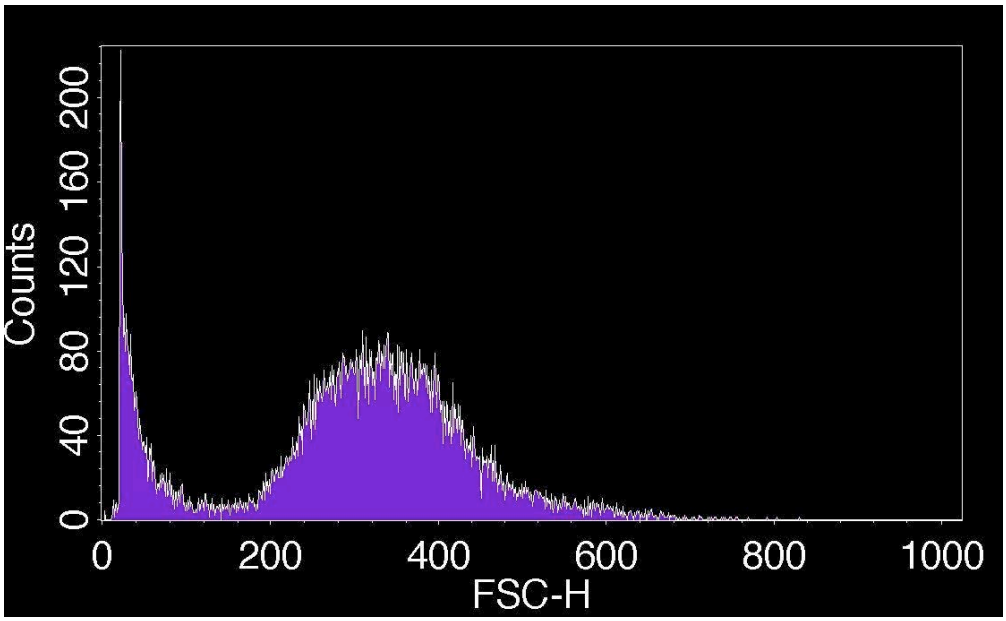

Figure 49. L929 cells not bumped in a device with 12.5µm critical size

L929 Once again in these tests the cells in the smaller exit seem to have shifted there distribution towards the smaller end of the scale. The bumped side show almost no selection.

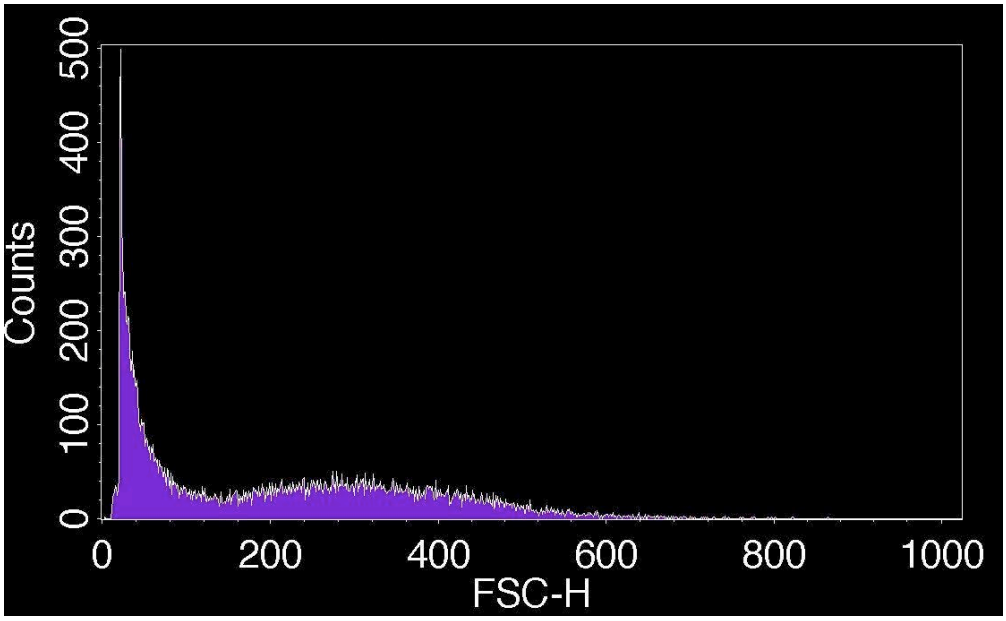

Figure 50. L929 cells bumped in a device with 15µm critical size

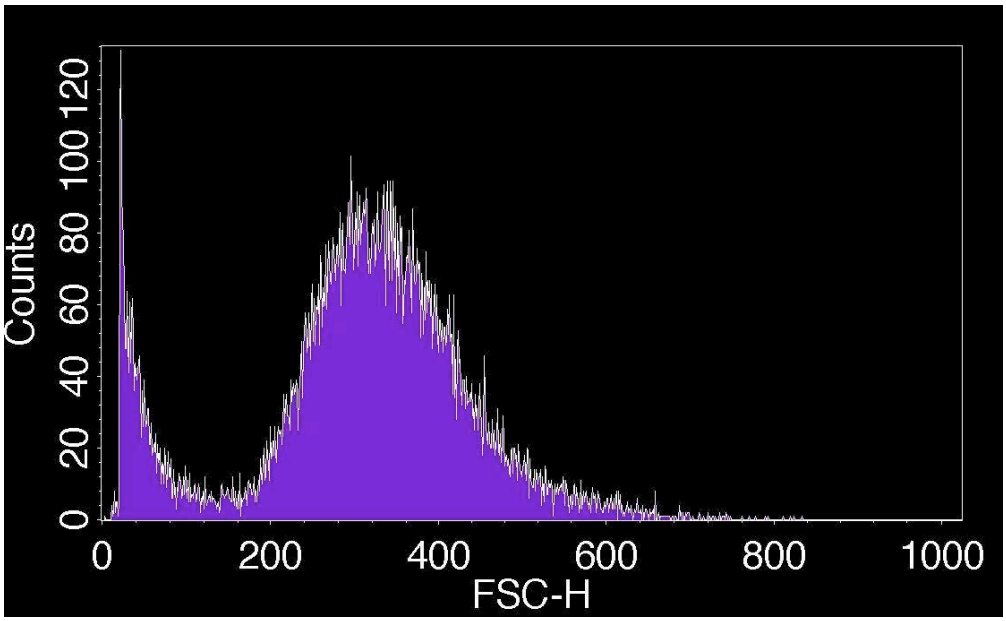

Figure 51. L929 cells not bumped in a device with 15µm critical size

Here once again as in the earlier tests the bumped exit doesn't show much of a selection but the cells exiting in the unbumped exit show a distribution around a smaller size.

16. Discussion

This project was set out to investigate if the DLD device could be used for cell synchronization applications. This involved many steps and problems to overcome to be able to reach the goal: a cheap and effective method to be able to sort out cell populations fast and easy. If this can be reached there is a great need in the biological community.

The first problem to overcome was to be able to reduce the clogging in the device, a very important problem to overcome to be able to use the device. The use of Pll-PEG greatly improved the performance so much so that other options for reducing clogging was not even needed to look at. In adding Pll-PEG to the device also makes the preparation of the device easier since filling the channel with fluid just after bonding while it is still hydrophilic. Adding fluid in the device early also reduces chances of air bubbles in the channel which ruins the separation.

The tests with beads in version 1 were simply made to test that the separation in the device was actually working. Sure enough they separated and showed that the critical size actually lies between 10μm and 16μm as the theory was predicting, indicating that it is correct and also showing that the correction term in the theory is really needed.

The preliminary tests with cells showed that whole cells suspended in the medium can be run through the device and that the device is sorting them out. Major problems with clogging had been overcome, although the problem will never be completely overcome. Adding the beads was done to show that the device was running correctly and that the separation was actually occurring. All the cells were sorted out, this lead to the creation of version 2.

The initial results of the new design were a bit disheartening, but it was perhaps a bit naïve to not consider wall-effects in the bumper. An explanation of the wall-effect could be that the bifurcation of streamlines around the pillars that occur in the middle of the device cannot occur since the wall blocks this part of the fluid. My suggestion is that the streamlines get broader closer to the wall because of this by the streamlines who were supposed to go towards the wall are merged with the ones who go between the posts and the wall. The broader streamlines lead to a larger critical size as was showed with the 27μm beads. This effect could reach out a few rows out into the device gradually lowering the critical size until the size from the theories is reached. It may be that future devices should be designed so that you avoid having cells close to the wall when entering the device. It could also be possible to utilize this effect in a device that doesn't rely on a homogenous critical size.

The flow cytometer measurements were made to verify the separation. Although it is hard to really tell if a separation has really occurred it did tell us a lot about the size distribution among the 16 μm beads. The precision of the apparatus was very good as was seen in the tests with the smaller beads of 3 and 5 μm sizes. Unfortunately one of the parameters we were trying to retrieve could not be extracted. We wanted to find out how much sample had gone through during the collection of the data. This was important to find out the relation between the concentrations in the different exits. When performing flow cytometry with the cell samples it seemed as though the samples had a very low concentration, this might have been because the cells have stuck to the walls of the container and are no longer suspended in the medium.

18. Outlook

It has been showed that it is possible to make a microfluidic cell sorter that sorts cells in real-time. There are however hurdles to overcome still. To make a device that is commercially viable the issue of throughput has to be addressed. Separation of quite large quantities of sample in a short time is needed. One possible solution for upping the throughput is to run many devices in parallel, something that the small size of the device could be fairly easily done. If a chip runs for a long time things like sedimentation of the sample has to be considered also.

Using the DLD for living cell applications requires some deeper understanding of the device. Questions like: What is the maximum speed of the device? What is the maximum speed cells can survive? Is there a limit in concentration of sample that can be separated?

A more dynamic DLD device would be favourable since the size of cells of different kind varies and there is also a variation in size between different populations of cells. Using a device as have been suggested by Beech for example might be a good idea to get the tuneable separation needed.

APPENDIX

Mask Fabrication

The pattern was transferred from blue-print to the mask by a Heidelberg mask generator for version one. Version 2 was transferred by a Dutch company called Deltamask.

Master fabrication in SU-8

This recipe was gave a channel depth of ~30μm in SU-8 2050 based on the recommendations from Microchem.

Substrate preparation

- Ensure that the silicon wafer (4" <100> wafer) is clean and planar
- Bake in convection oven at 200C for 30 minutes to remove surface water and promote the adhesion of SU-8 to the surface.
- Spin coating of SU-8
- SU-8 should be applied to the wafer directly upon removal from the oven in order to minimize the amount of water that can absorb to the surface
- Holding the wafer in one hand, and pouring from the SU-8 bottle with the other in as even a manner as possible, about half the wafer is covered.
- The wafer is then tilted so that the SU-8 flows over the entire surface
- The wafer is centralized on the vacuum pad in the spinner and the following spin cycle is performed
- 12 seconds at 500 rpm – to spread the coating evenly over the wafer
- 60 seconds at 2000 rpm – to obtain the 30 μm layer

To ensure a good surface a surplus of SU-8 had to be poured over the wafer. When this was spun the surplus would be cast of the wafer and many times find its way back to it as droplets. To avoid this a piece of filter paper was hold close to the edge to catch the surplus to good effect.

Pre-baking

- 8 minutes at 65ºC
- 8 minutes at 95ºC

Exposure

- Exposure time was 12 seconds with an intensity of 14mW

- Post exposure bake
- 2 minutes at 65ºC
- 7 minutes at 95ºC

Developing

- The wafer was submerged in developer for 7 minutes and then for an extra 30 seconds in fresh developer.
- After the developer the wafer was washed with isopropanol and dried with nitrogen.

Hard baking

200ºC in a convection oven. This causes reflow reducing the size and number of cracks.

Anti-sticking treatment of master

The steps to perform the anti-sticking treatment was taken from the references [6, 24].

- Hydroxylate the master in nitric acid, wash it thoroughly and dry it
- Transfer it to an anhydrous glove box environment (nitrogen atmosphere, water content <1 ppm)
- The master, situated in a Petri dish with a little hole near the edge of the cover, is placed on a hotplate
- The hotplate is heated to 150ºC. Wait for about 10 minutes so that the temperature equilibrium is reached
- Inject a few microliters (how many depends on the size of the Petri dish) F13-TCS (tridecafluoro-1,1,2,2-tetrahydrooctyl-trichlorosilane) with a microsyringe through the hole in the Petri dish. Cover the hole immediately with a glass slide. Wait for 2 hours.
- Open the Petri dish chamber and wash off excess F13-TCS with anhydrous hexane inside the glove box. The master can then be taken out of the glove box.

Baking the PDMS

The devices are made from Sylgard-184 purchased from Dow Corning and sealed with glass slides.

- The PDMS is mixed thoroughly with a hardener at 10:1 mass ratio
- The PDMS is placed in a vacuum chamber for 40 minutes to remove any air bubbles

- The PDMS is poured onto the master and baked at 80ºC for 40 minutes

Making the holes

- Holes in the PDMS are made with a sharpened small pipe
- Holes in the glass slide are drilled with a Dremel tool fitted with a dental drill bit.

Bonding the device

- The glass slide and the PDMS was carefully cleaned with isopropanol
- The PDMS and the glass slide are exposed to oxygen plasma at a pressure of 8 mBar for 30 seconds.
- The PDMS and the glass is then carefully put together right after the oxygen plasma with the finger tips
- The device is directly filled with a solution of Pll-PEG and Hepes to coat the surface against non-specific binding.

Gluing the tubes

The fluidic connections were made using silicone rubber tubing glued on with Wacker Elastosil A07

Cell preparation

Cells were cultured by Kersti Alm at the department of cellular biology. Trypsin was added at room temperature for 10 minutes to release the cells from the surface and suspend them in the medium. Cell medium was added after 10 minutes and the cells were kept at 0ºC to keep cells suspended in the medium.

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