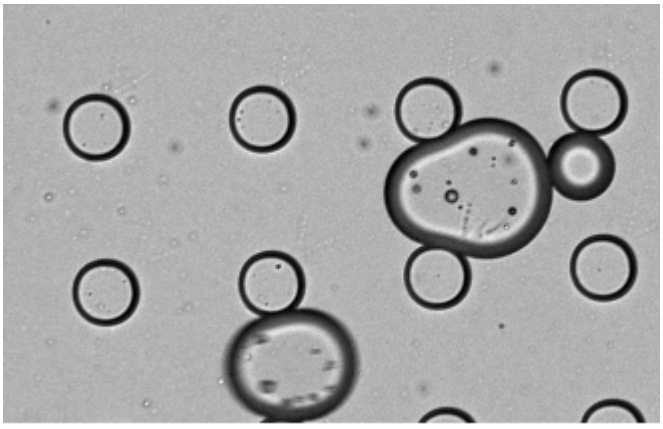
Oil drops in Bumper Array

By

Anette Lundqvist



Bachelor thesis
Spring 2007
Supervisors: Jonas Tegenfeldt and Jason Beech
Division of Solid State Physics
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Abstract

In this thesis, a model of cell separation in a bumper array is presented. The work was done using oil drops, separated in a bumper array. The aim of the model is to show whether the soft particle will be deformed due to forces exerted upon it as it moves through a bumper array. This will tell if the critical size will affect the critical size of the particle separation.
Measurements of the oil drops when they pass through a bumper array at different velocities were done. Tests show that the mean size of the oil drops decreases when the velocity increases. Because of the pour statistics the result are uncertain. To guarantee the results one may need more data.
A closer look at a small part of the bumper array shows that the drops deform a bit when they hit the posts. Especially the bigger drops deform to get through the posts array.
Another thing that was done was to see how solid particles behave under the same condition.
This was done with fluorescent beads at different velocities. This was done as a reference to compare the oil drop results with.

Abstrakt

I detta arbete presenteras en modell för cellseparation i en bumper array. Arbetet utfördes på oljedroppar som separerades i en bumper array. Modellen ska visa huruvida mjuka partiklar skulle deformeras när de träffade en pelare i bumpern. Detta kan avslöja om separation kan göras vid högre hastigheter eller inte.
En mätning på oljedroppar när de passerar genom en bumper array gjordes vid olika hastigheter. Detta visade att medelstorleken på partiklarna minskade när hastigheten ökade. På grund av de få mätvärdena är resultatet osäkert. För att säkerställa resultaten behövs fler datapunkter.
Vid en närmare titt på en liten del av bumpern visade det sig att dropparna deformeras när de träffar en pelare. Speciellt stora droppar deformeras för att ta sig igenom två pelare.
En annan sak som undersöktes var att se hur hårda partiklar uppför sig vid samma förutsättningar. Detta gjordes på fluorescerande kulor vid olika hastigheter. Anledningen till att detta gjorde var för att ha något att jämföra resultaten från oljedropparna med.

Acknowledgments

I want to thank my supervisors Jonas Tegenfeldt and Jason Beech for all help and supports that make this thesis possible to carry out. They came up with a lot of good ideas and things to try.

I also want to thank my brother Martin Lundqvist for all help with correcting the language. Last but not least I want to thank Hanna Nicklasson for all good talks and a nice company in the lab.

Contents

1. Introduction

This model of cell separation aims to show if soft particles have a special behavior when they are separated through a bumper array. The reason why oil drops are chosen is because of their homogeneity and that they do not clog the bumper array. They are very easy to create and when created they come out in a large range of different sizes.
The question to answer in this thesis is if the soft particles deform in a bumper array and if they deform more when the velocity increases. This is of interest when it comes to the separation condition, especially for cell separation, since most cells are fragile. If it turns out that the soft particles deform more at increasing velocity it may not be possible to separate the cells at high velocity. Another thing, which is a suggestion by J. A Davis at al. [1], is that the critical size of the particle could depend on the velocity.

2. Background

2.1 The Bumper Array

A bumper array is a microfluidic device that separates particles by their size. It contains posts at a well defined distance from each other, λ, measured from centre-to-centre of the posts. The posts in the following row is displaced by a distance, $\Delta\lambda$. $\Delta\lambda$ defines the angle θ so that each row are displaced perpendicularly to the direction of the flow. As can be seen in figure 2.1 this gives the relationship

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

where N is the period of post arrays. [2]

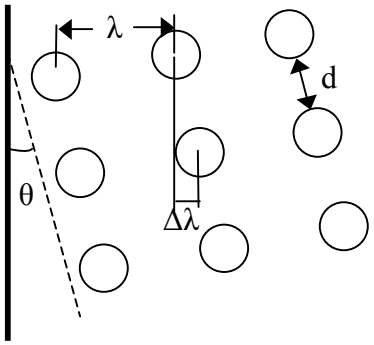


Figure 2.1. The Bumper array and how the posters are placed.[2]

With the period means that the post row one and the post row N have the same position measured from the side wall as each other.

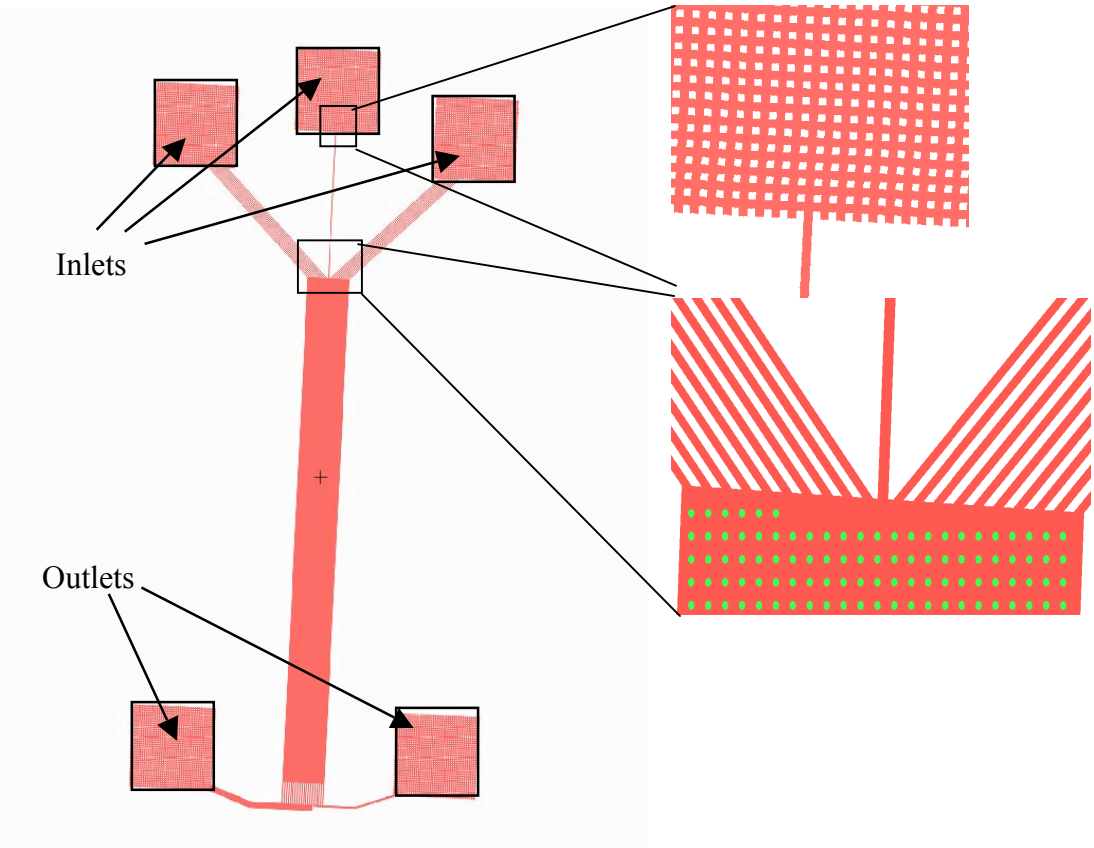


Figure 2.2. Design of the bumper array used in this project.

The design of the bumper array is quite simple as can be seen in figure 2.2. The large squares in the outlets and inlets are for making the alignments of the holes in the glass cover easier. For all dimensions on the bumper array see Appendix C.

2.2 Flow Properties

The flow in the bumper array is laminar, which means that streams of liquid flow parallel to each other. The only way mixing occurs in laminar flow is by diffusion. If the flow is not laminar it is called turbulent, that means that the parallel streams are mixed by turbulence. Laminar flow depends on the velocity of the flow, the cross-sectional dimension, the density of the fluid and on the viscosity of the fluid. These parameters indicate whether the flow is laminar or turbulent and is called the Reynolds number.

$$\mathrm{Re} = \frac{vl\rho}{\mu}$$

Here v is the velocity, l is the cross-sectional dimension, ρ is the density and μ is the viscosity.

Re is dimensionless and the value $\mathrm{Re} < 1$ corresponds to laminar flow. [3]

The flow in the channel is parabolic, so the flow speed is not equal everywhere in the channel. The speed at the wall is zero and increases in a parabolic way toward its maximum at the centre. Se figure 2.3.

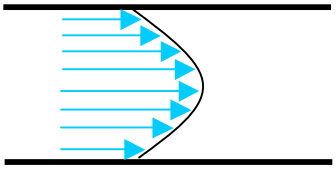


Figure 2.3. The Flow in the bumper array has a parabolic shape. [2]

It is because of the parabolic flow profile it is conceivable that soft particles deform in the bumper array. When the particles come near a post the flow is lower at the end near the post than the flow at the other end. This makes the particles roll and deform since the pressure at the particle is higher at the top of it.
When a particle comes into a bumper array it will follow a streamline. If the particle is smaller than the streamline it follows, it will continue in that one. When particles that are bigger than the streamline travel through the bumper array they cannot follow the streamline because they are forced by the post into the adjacent streamline. A particle that is smaller than the critical size and starts in streamline one at post row one, will in post row number two have changed its position to the *N*'th streamline. At the next post row the particle will be in the (*N*-1) streamline and when the particle reach the *N*'th post row it will be back in streamline number one again. See figure 2.4.

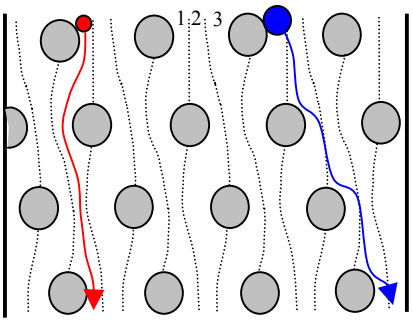


Figure 2.4. The streamlines and the way particles travel through a bumper array with $N = 3$

The size of the particle when it is at the edge of being bumped or not is called the critical size. All particles smaller than the critical size follow the streamlines and all particles above the critical size are being bumped. The critical radius can be calculated easily for a blunt flow profile with the formula

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

where d is the distance between two posts and N is the post period.

In the case of a parabolic flow profile one needs a correction factor α. The formula then becomes

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

For the bumper array in this project α is approximately calculated from [2]

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

That gives the bumper array a critical size of about 14 µm. This is only a theoretical value that is an approximation in two dimensions but an empirical calculated value in three dimensions is nearer 16 µm.
A well functioned bumper array that separates oil drops is shown in figure 2.5. The bumper is of the kind that were used in this experiment. In the figure one can see the small drops well separated from the bigger ones and how they go out in different channels.

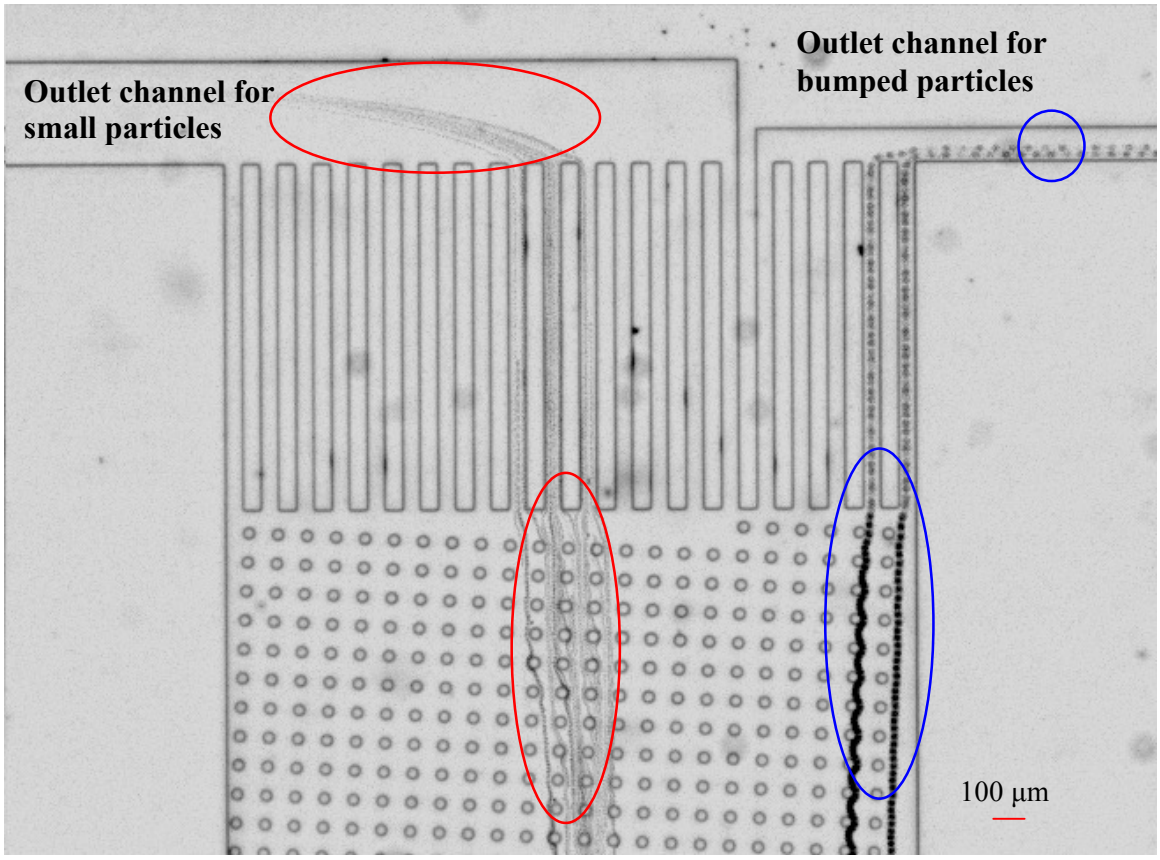


Figure 2.5. The blue circles show big oil drops that are bumped and the red circles show small oil drops that have followed the stream.

2.3 Diffusion

Since the flow in the bumper array is laminar, the only way that mixing in the streamlines can occur is by diffusion. Diffusion is caused by a phenomenon called the Brownian motion. This is a process that causes equilibrium between different concentrations. With the diffusion coefficient D one can calculate the root mean square of the distance a particle can travel in one dimension in time t.

$$< d^2 >= 2Dt$$

Where

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

is the Stokes-Einstein equation. Here k_B is the Boltzmann´s constant, T the temperature, η the viscosity and R_H the hydrodynamic radius of the particle.

In the bumper array the distribution of particles is dependent on diffusion since the stream of particle will widen if the diffusion is high. The diffusion depends on the velocity through the bumper array as can be seen by Peclets number [2]

$$P_E = \frac{vd}{D} = \frac{\frac{v}{d}}{\frac{D}{\overline{d^2}}} = \frac{Advective\ Rate}{Diffusion\ Rate}$$

$$P_E = \frac{(\Delta x)^2}{\sigma^2}$$

Peclets number should be as high as possible to get a high resolution for the streams. That will make it possible to see the difference between the distributions for the two separated streams of particles.

3. Fabrication

3.1 The Master

The master to the bumper array is done with the SU-8 method to a silicone wafer. [2] The process can be seen in figure 3.1.

A design is produced in a computer and then printed by laser on a mask made of glass with a chrome layer and a positive resist layer. The positive resist becomes weak where the laser has been. The weak positive resist is washed away with a developer and the mask is then held in chrome-etch. This leaves a hole down to the glass. The mask is then placed in a UV-light source and held over a master made of a silicone wafer with the negative resist SU-8. SU-8 hardens in UV-light and the SU-8 around washes away with a developer. The silicon wafer is then used as a master after treatment with anti-sticking fluorosilanes. [2] The anti-sticking is used as a anti-adhesion layer to avoid adhesion of the PDMS to the surface of the master.[4]

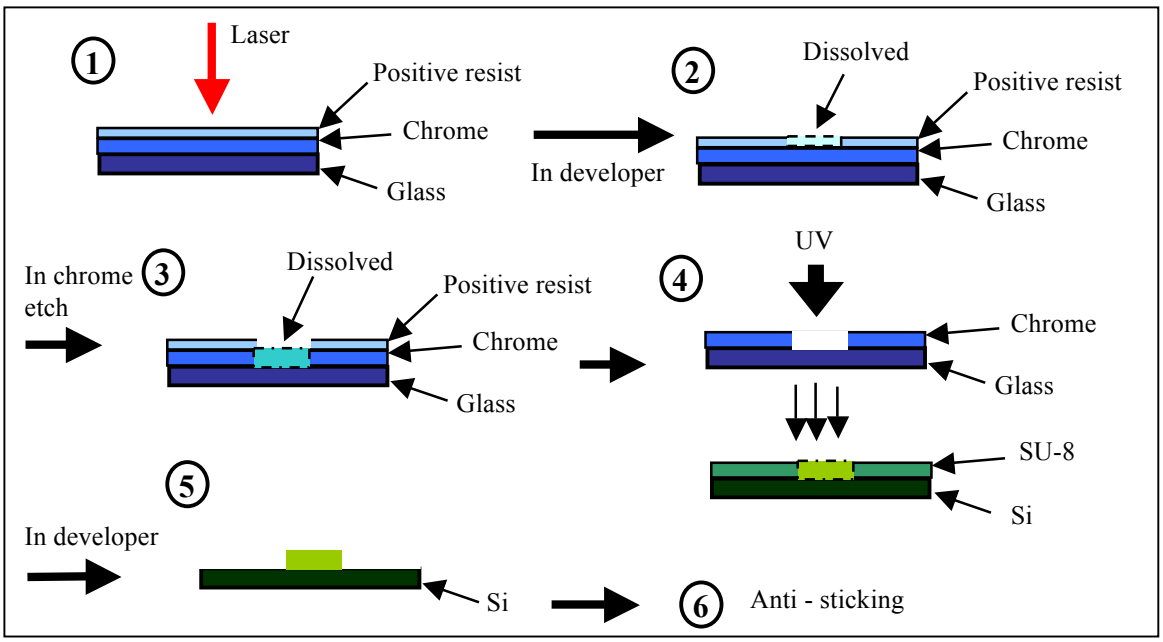


Figure 3.1. Show the process of master production.

3.2 The Bumper Array

The bumper array is then produced in PDMS (poly(dimethylsiloxane)). PDMS is a polymer that consists of repeating $–OSi(CH_3)_2-$ units. The presence of the CH_3 group gives the polymer hydrophobic characteristics at the surface. This makes the channel difficult to use with aqueous solutions. The surface is easy to make hydrophilic by oxidation in a plasma cleaner. The siloxane group then becomes a silanol. However if the surface isn't held in water afterwards it will go back to being hydrophobic. PDMS is a very good material, it is not expensive, it is very flexible and works well for detection in many optical methods because it is optically transparent down to 230 nm [3]. PDMS is poured on to the master and baked in an oven. When it comes out hard it releases from the master. The PDMS is then attached to a piece of glass and then ready to be used. The ready device can be seen in figure 3.2.

3.3 The Oil drops

There were two different oils used in the project, immersion oil for fluorescence and general microscopy and immersion oil for fluorescence microscopy. The buffer the oil drops were made in was either pluoronics or SDS (sodium dodecylsulphate). The reason why drops of oil where chosen is because of their ability not to clog the bumper when they are put in a buffer of a surfactant in low concentration. That is because the surfactant forces its hydrophobic end into the oil drop and lets the hydrophilic end stay outside in the buffer.

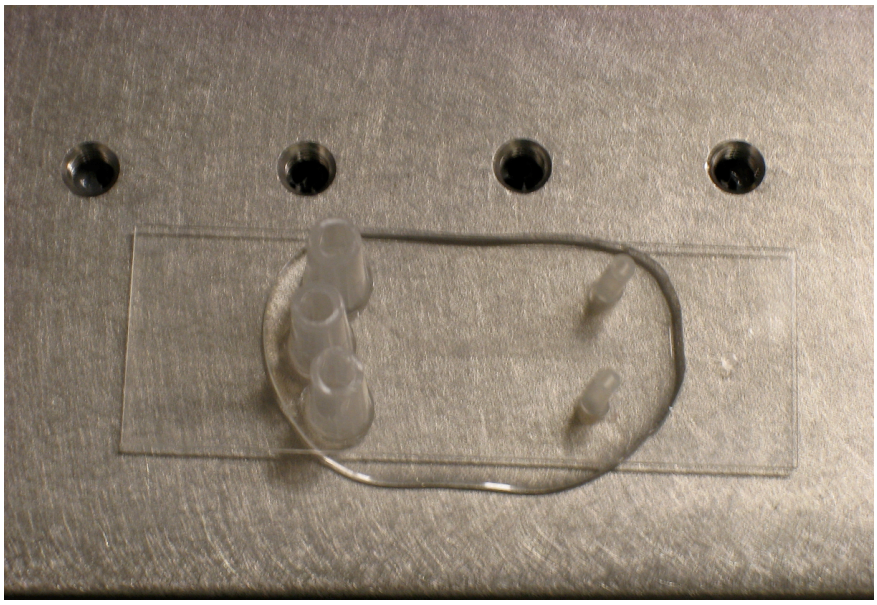


Figure 3.2. The whole device with the bumper array, glass cover and reservoirs.

4. Oil drops

4.1 Experiment

The first experiment was made on oil drops to see if they deform and if the oil drops forces to bump or not when the velocity increase.
The first measurement was done with setup 1 with syringe pump, but as it appeared it was better to use setup 2 with a vacuum pump and therefore the measurements was done using setup 2. See figure 4.1 for setups.
The vacuum-pump was connected to a pressure meter so the pressure could be monitored. The oil drops were put in the middle chamber and the buffer were put in the other two chambers. See setup 2 figure 4.1. As a buffer 0.1% pluoronics were used.
The bumper array was held under a microscope, and the picture of it could be seen on a computer screen.
The pressure was varied and at every level movies were taken. The pressure varies from around 2 mbar up to around 120 mbar in five levels.
A closer look at very high magnification was done to see if the drops really were deformed when they hit a post.

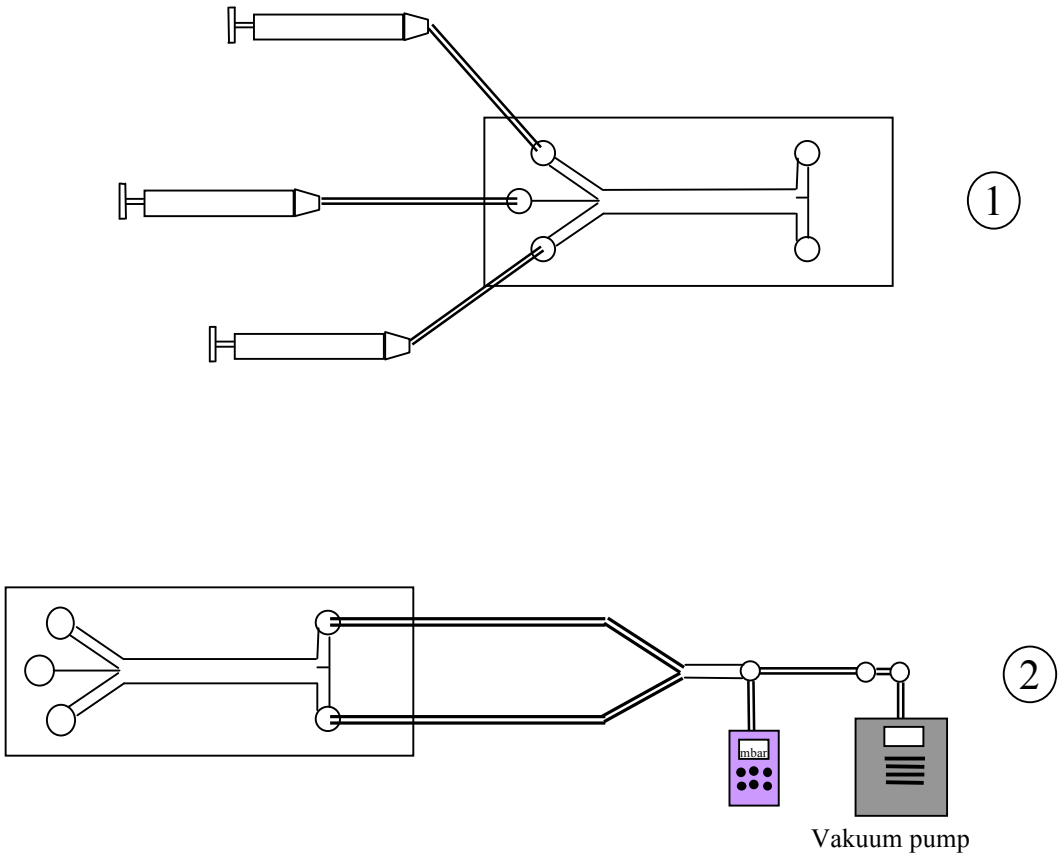


Figure 4.1. Shows the two different setups used in the experiments.

4.2 Analysis

The measurement was done on every oil drop that became bumped the whole distance between the 20 posters. The only exception was at the lowest pressure where the drops didn't manage to reach the 20th poster before the movie was over. Then were the distance the drops traveled over the whole movie taken instead. Their size and velocity were noted. The velocity was calculated from the full distance between the 20 posters and the total travel time, the number of frames times the average frame rate. The size was then plotted against the velocity for every pressure-level used. Then the mean size for every bumped drop for every level was plotted against the mean velocity for each level. The mean size was also plotted against the pressure but since the relation between pressure and the mean velocity is linear, the velocity was used.
An uncertainty in the velocity was calculated by the standard deviation

$$\sigma^2 = \frac{1}{N-1}\sum_{j=1}^{N}\left(x_j - \langle x\rangle\right)^2$$

where

$$\langle x\rangle = \frac{1}{N}\sum_{j=1}^{N} x_j$$

gives the mean velocity.
The uncertainty in the size was estimated by how many pixels wide an oil drop was and how big a pixel was.

4.3 Results

The size and the velocity for every bumped oil drop were measured. Then the size was plotted against the velocity for every pressure level. The uncertainty in the velocity was calculated to

$\sigma^2 = 6.7*10^{-6}$	For the first plot
$\sigma^2 = 2.4*10^{-5}$	For the second plot
$\sigma^2 = 7.7*10^{-5}$	For the third plot
$\sigma^2 = 1.4*10^{-4}$	For the fourth plot
$\sigma^2 = 5.3*10^{-4}$	For the fifth plot

The uncertainty in the particle size was estimated to about 5 µm.

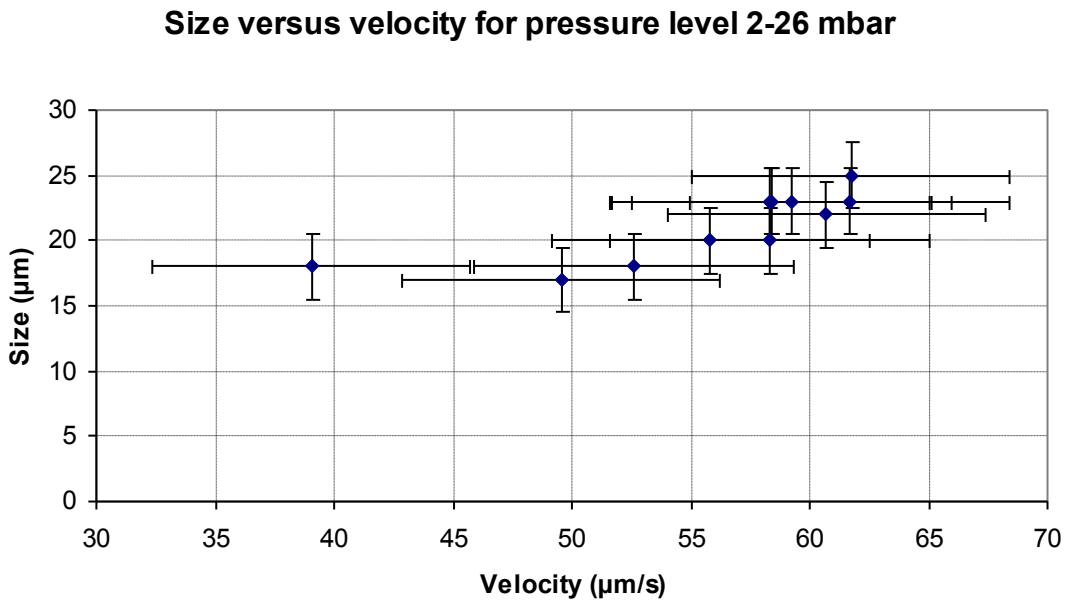


Figure 4.2. A plot with error bars for pressure level 2-26 mbar.

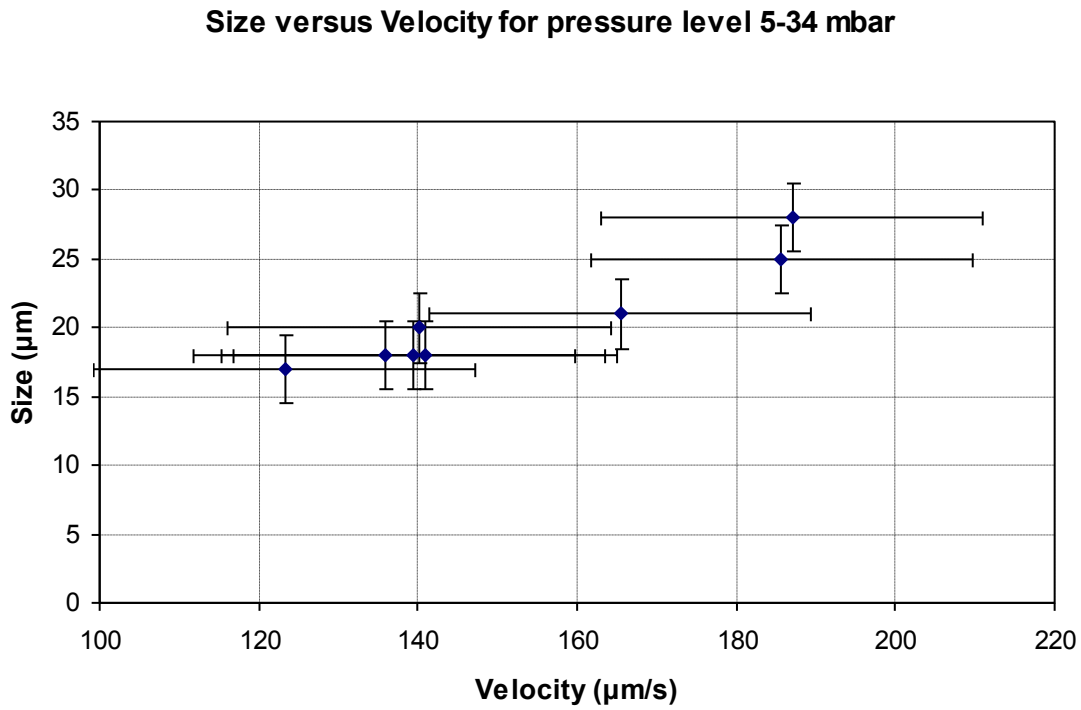


Figure 4.3. A plot with error bars for pressure level 3-34 mbar.

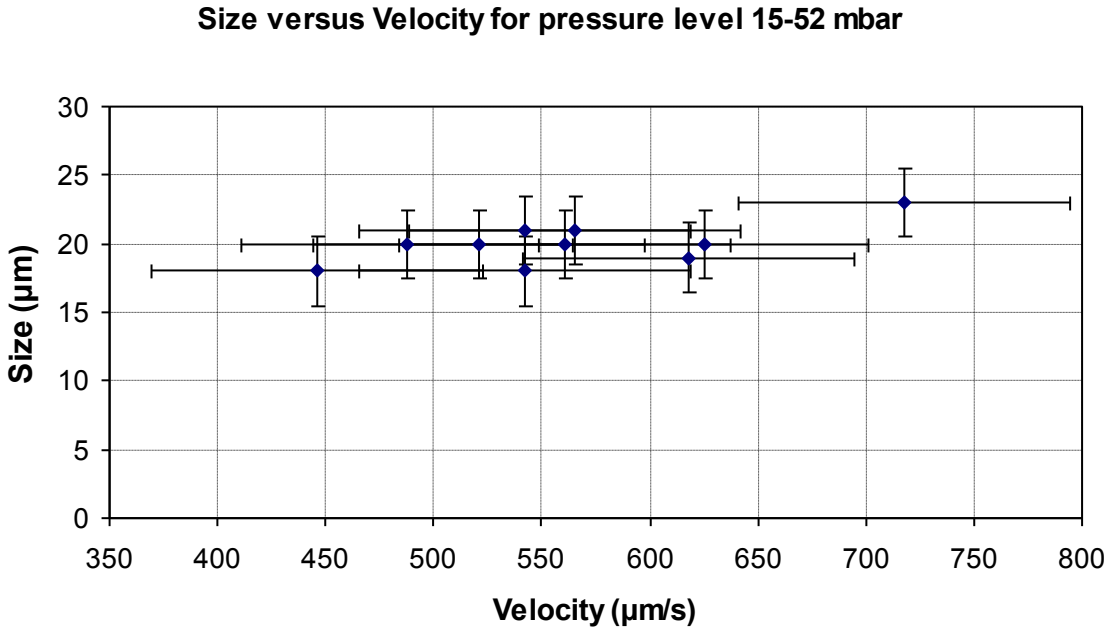


Figure 4.4. A plot with error bars for pressure level 15-52 mbar.

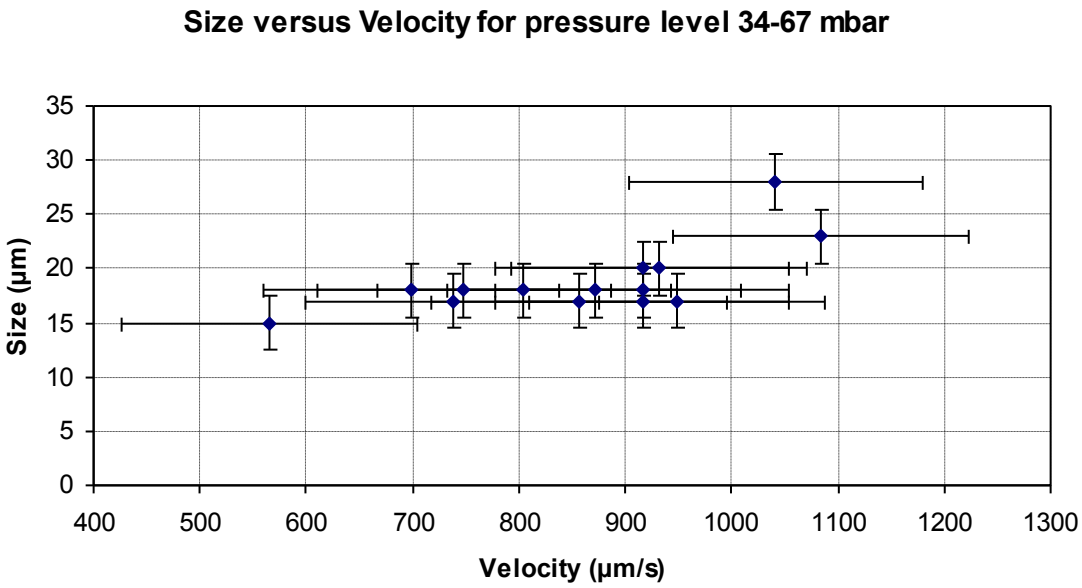


Figure 4.5. A plot with error bars for pressure level 34-67 mbar.

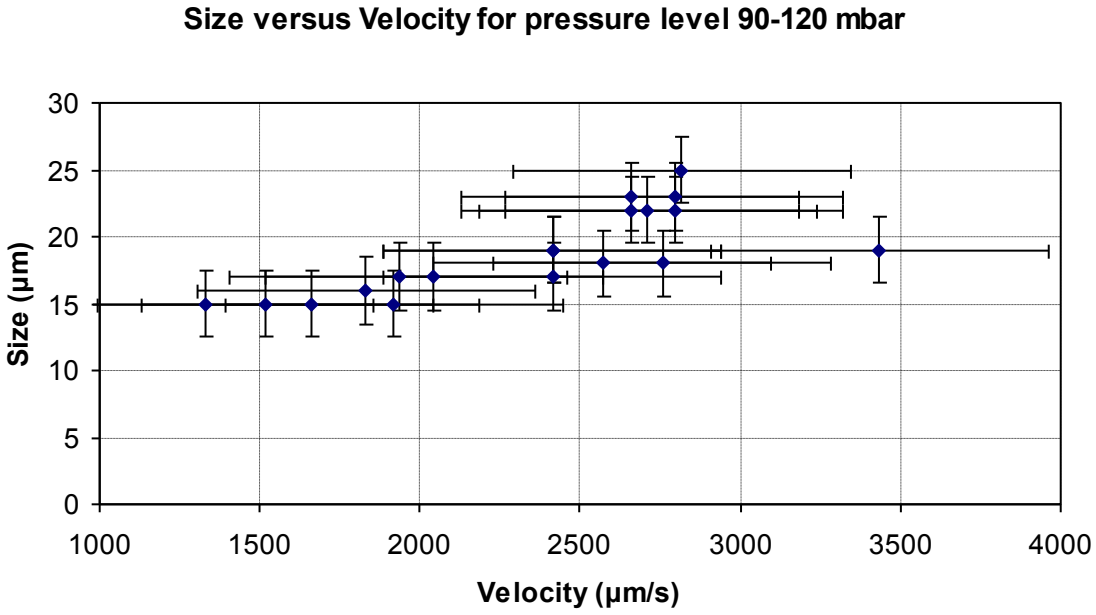


Figure 4.6. A plot with error bars for pressure level 90-120 mbar.

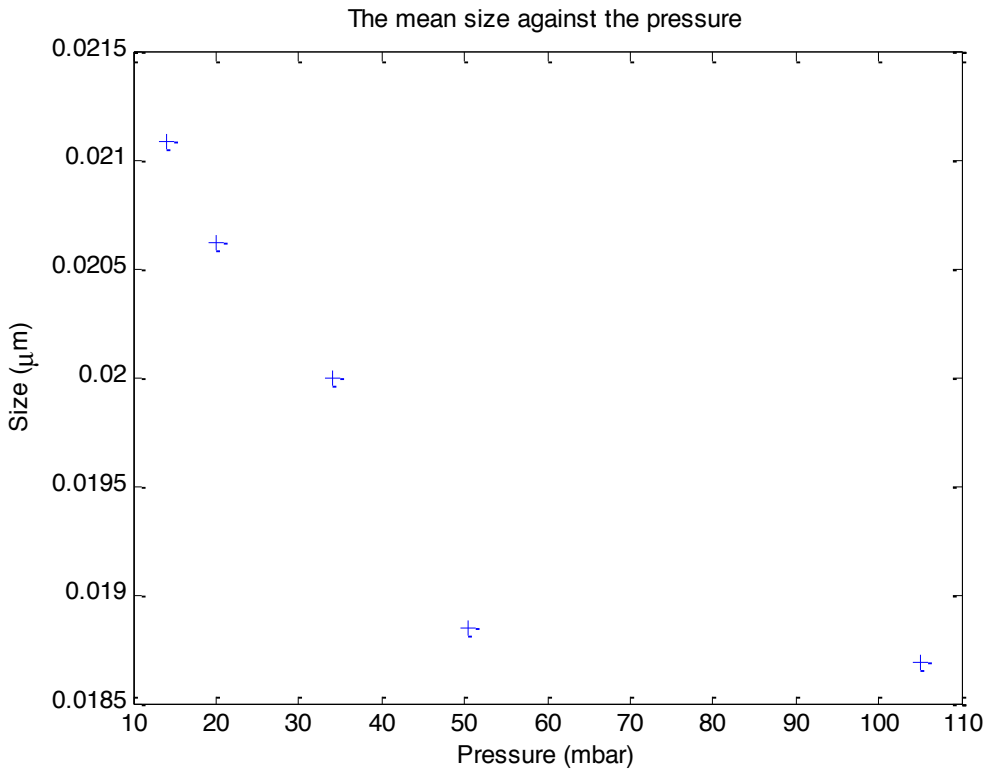


Figure 4.7. A plot which shows how the particle size decreases when the velocity increases.

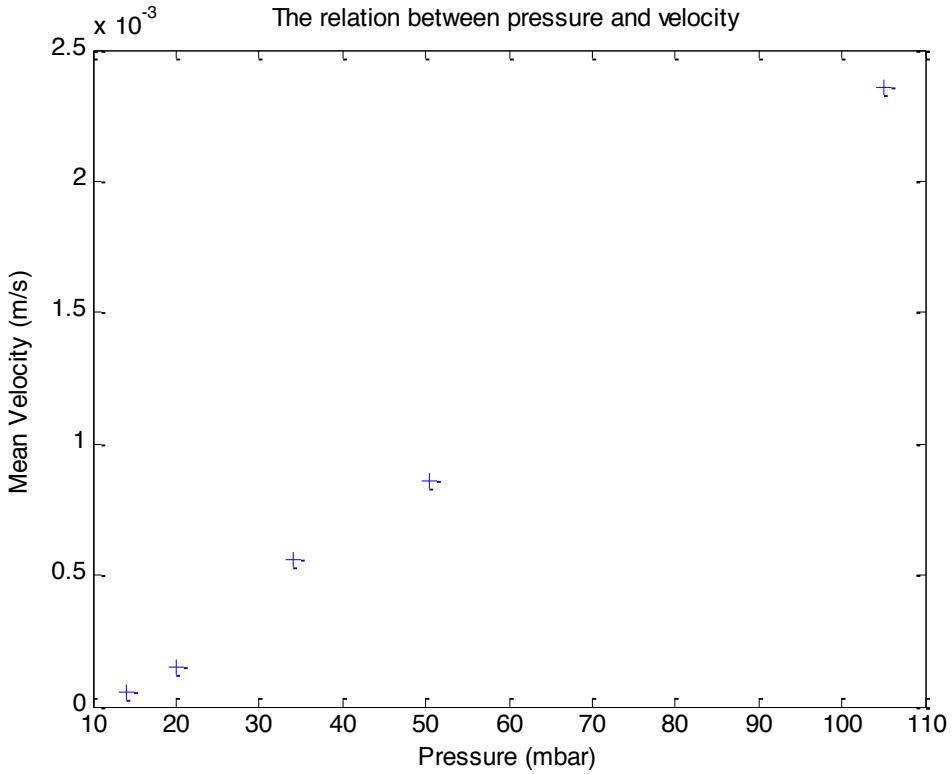


Figure 4.8. The relation between the velocity and the pressure.

Figure 4.8 shows the relation between the velocity and the pressure. Since the relation between pressure and mean velocity is linear either of these quantities can be used when plotting the size dependence.

A big oil drop were seen when search for deformation was done. The big drop showed how it easily deformed to get through the post array even if it was too big.

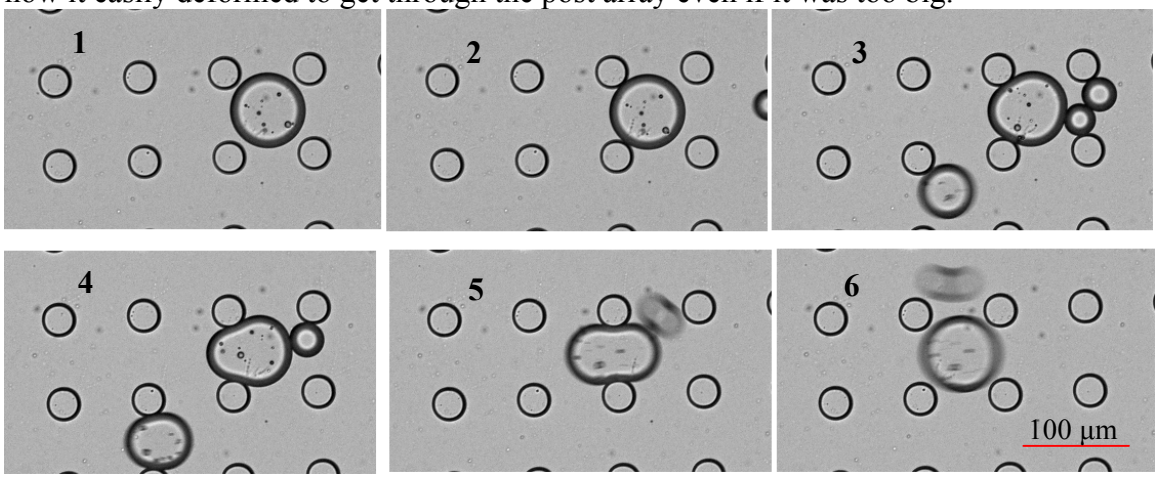


Figure 4.9. A big oil drop deforms itself to get past the post array.

5. Beads

5.1 Experiment

The experiment with the beads was done to see whether the velocity changes as a function of which trajectory the beads take through the bumper array. A comparison for the distribution at the different pressures would show any sign of change. If a change for the beads is shown one would have to take that into account for the oil drops.
For this experiment setup number two was used. Two sizes of beads were used; a red one with size 10 µm and a green one with size 16 µm. The beads were put in the middle chamber and the buffer in the other two. The buffer used was 0.1% pluronics. The pressure varies from around 3 mbar up to around 180 mbar in five levels.

5.2 Analysis

To see whether the streamlines changed for the different pressures a comparison of the distribution near the end of the bumper was done. For each film taken, with the bumper at different pressures, a Z-projection was done. This is an overlay of all slides of the film producing a single composite picture of the whole event. From this composite picture the distribution of beads was measured.
A problem with the Z-projection came up as only the lowest pressure and the highest pressure could be taken for a comparison. Due to that all of the big particles for all pressure levels were followed to see if they were bumped.

5.3 Results

From the count of big particles there could not be seen anyone that didn't get bumped at any pressure level.

From the (figure 5.1) no conclusion could be drawn.

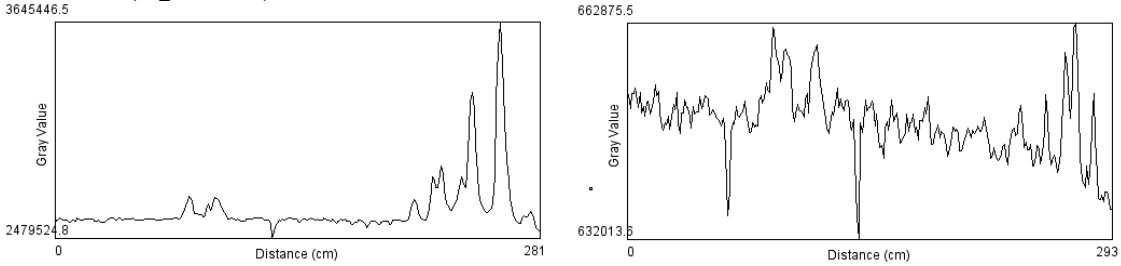


Figure 5.1. The distribution for the lowest pressure level to the left and the highest pressure level at the right.

Figure 5.2 shows a picture taken from the lowest pressure and is taken just at the small beads that should go straight through. But a few of them seem to have be bound together and therefore gets bumped.

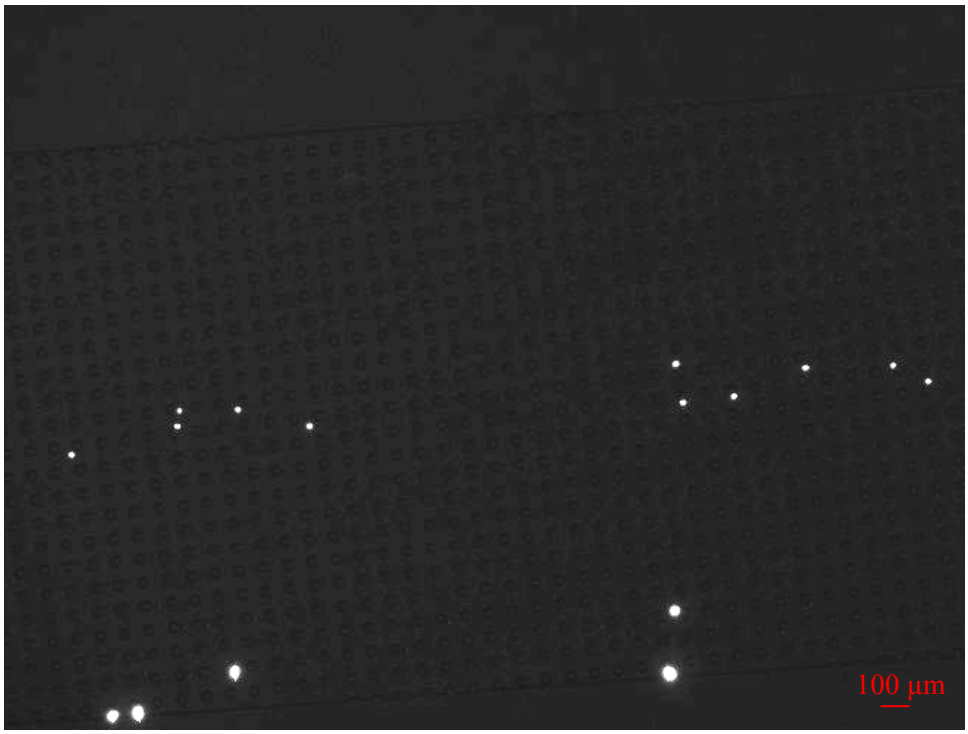


Figure 5.2. The small particles at the lowest pressure.

6. Discussion and Conclusions

By measure the size and velocity for oil drops it could be seen (figures 4.2 - 4.6) that for every pressure level the velocity increases for increasing particle size. This depends on the parabolic flow profile in the bumper, a bigger particle feels more of the velocity near the middle where the flow is higher and therefore gets a higher velocity.
When the mean value for the sizes at the different pressure levels where plotted against the pressure the result become a bit unexpected. For increasing pressure the particle size seems to decrease. The big question is then why? A few theories have come up.
This could be exactly what the project wanted to show, the oil drops deform more at higher velocity and therefore they look smaller when measured. But this is a bit odd too, because the particles may follow the stream when they are smaller in diameter since they become smaller than the critical size. So maybe this is not the fact in this case.
It could be just a coincidence. The size does not vary much so in this case the reason could be that, when measure at a high pressure there were smaller oil drops than at a lower pressure.
Another thing that may be the case is camera effects. At lower velocity the camera has time to take a good picture at the oil drop and one could see the entire drop. But at higher velocity the camera has difficulties having time to take the picture so the oil drops looks smoothed out, motion blur. Maybe one cannot see the whole particle when the measurement is conducted.
A more thorough analysis is needed to find out if this is true.

It was very hard to see if there was any deformation to the oil drops in the bumper array. The big oil drops were definitely deformed when they got through two posts if they were bigger than the distance between them. The smaller oil drops seem in some cases to be deformed, but the result is uncertain.
To see if the oil drops really deform one need to look at a place in the bumper where all particles are at the same circumstances. One thing is to look at the outlet for the bumped oil drops where there are no posts interrupting the flow and all particles have been bumped. An example on how it can look when measured at the outlet channel for the bumped oil drops can be seen in figure 6.1.

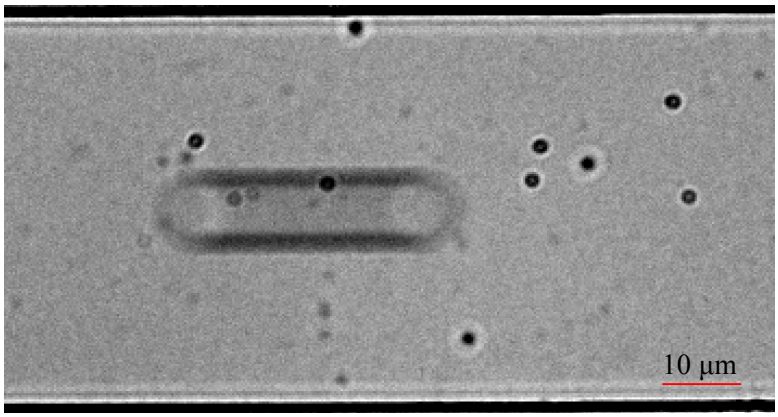


Figure 6.1. The outlet channel for bumped oil drops.

A problem that surfaced in the beginning of the measurements was when using the first setup. With the syringe no big drops were produced, only very small ones. But when changing to the second setup all sizes were seen. The discrepancy could be due to a big oil drop clogging the syringe pump or the pipes making only the small drops go through.

The few results from the beads experiment only showed that the critical size probably don't change for the hard particles. This mean that the bumper array does not deform at higher velocity. The reason why the analysis could not be done was because there was to much background light in the pictures. That caused very much disturbance in the distribution plots, so the peeks were not seen.
When measurement is done next time all the light has to been turned off so only the beads are seen.

Appendix A - Fabrication facts

The Master

The master was produced with the SU-8 method and wasn't a part of this thesis, the production was made by Jason Beech and the recipe can also be found in [2].
This recipe gives a 20 μm layer when used with SU-8 2010 and is recommended by Microchem.

Substrate preparing
Ensure that the silicon substrate (2" 100 wafer) is clean and planar.
Bake in convection oven at 200° C 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 wafer directly upon the removal from the oven in order to minimize the amount of water that can adsorb 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 one third of 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.
20 seconds at 500 rpm to spread the coating evenly over the wafer.
120 seconds at 1000 rpm to obtain 20 μm layer.
These times are longer than those recommended by Microchem but gave more even coatings of SU-8 with smaller edge beads.
Allowing the sample to relax on a level surface for 1-2 hours can minimize edge beads.

Pre-Baking
Pre- and post-exposure baking was performed on a hotplate with vacuum.
1 minute at 65°C.
Ramp to 95°C- takes 4 – 5 minutes.
2 minutes at 95°C.
Relax for 10 minutes on a separate hotplate at 35°C.

Exposure
25 seconds at 12.5 $mJcm^{-2}$.

Post exposure bake
1 minute at 65°C.
Ramp to 95°C (takes 4 -5 minutes).
Ramp to 65°C (by setting the hotplate to 5°C – 8 minutes).
Relax 10 minutes on second hotplate at 35°C.

Developing
Sonicate at low amplitude (50 V) for 2 minutes in SU-8 developer.
The developer is rinsed away with isopropanol and the wafer dried with nitrogen.

A white film indicates the presence of undeveloped resist, in which case further developing is required. This should be done in 1-minutes step, with intermediate rinsing, until the white film is no longer seen.

Hard baking
200°C in a convection oven. These causes reflow reducing the size and number of cracks.

Anti-sticking treatment of master
The silanisation process was performed using the same equipment and method as described by M. Beck in reference [5], with the only exception that 150°C were found to give the best results, namely fewer excess deposits on the surface of the master.

PDMS

PDMS in monomer form is mixed well with a catalyst that cause the monomers to crosslink. The PDMS is in ratio 9:1 to the catalyst.
Then PDMS is placed in vacuum for about 30 – 40 minutes to get rid of all gas bubbles.
The PDMS is poured on to the master carefully so that no gas bubbles are formed.
Bake at 80°C for 1 hour.
The hardened PDMS is removed carefully from the master.

Channel holes

The holes were made in either the glass cover or in the PDMS. The holes in the glass cover were made by 50 μm aluminum oxide particles in a micro-sand blaster. The holes in PDMS were made by a sharp cannula.
Over the holes rubber tubes were attached to form reservoirs.

Bonding PDMS to glass

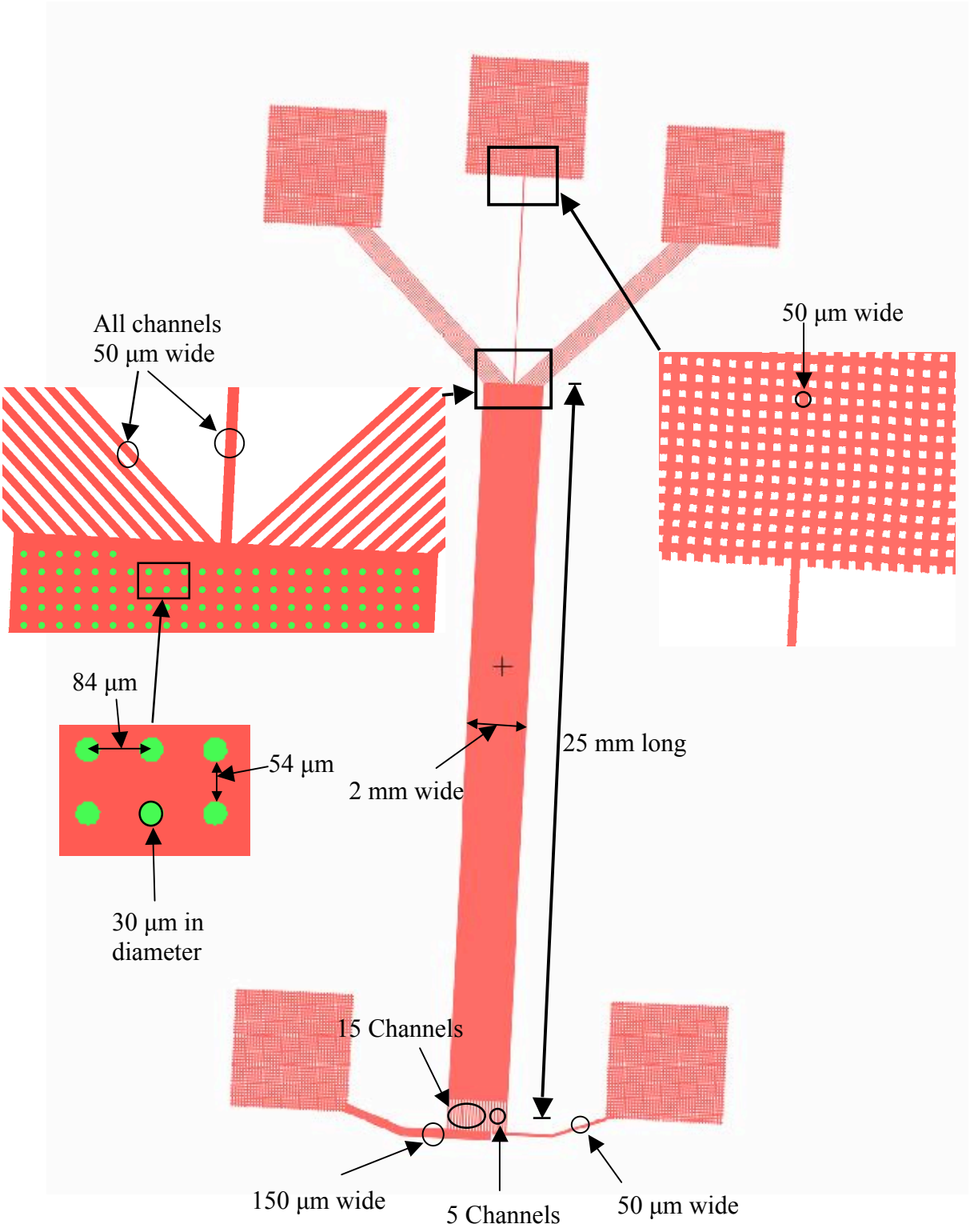
The PDMS and glass slide is placed in a plasma preen and exposed to oxygen plasma at a pressure of 8 mbar for 1 minute.
The PDMS and glass are put together with high accuracy and a light pressure with the fingertips.
Then immediately placed in Milli-Q water so the PDMS remains hydrophilic.

Appendix B – Equipment facts

The equipment that was used in the experiments is listed below.

- The microscope is a Nikon Eclipse TE2000-U
- The camera is an Andor Luca.
- There were two different syringe pumps used, Aladdin–1000 and SP210IWZ SYRINGEPUMP.
- The syringes are BD Plastipak, 1 ml.
- The glue that was used to attach the tubes to the device is Wacker Elastosil A07.
- There were two sorts of oil used, Immersion oil for fluorescence and general microscopy Formula: CODE 1261 TYPE DF and Immersion oil for fluorescence microscopy Formula: CODE 159 TYPE FF, both from Cargille.
- For ultra sonication were used a Branson 1510.
- The vacuum pump was made of the brand KNF.
- The micro-sand blaster is a Microetcher by Danville Materials.

Appendix C – The Dimensions of the Bumper Array



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