Acoustic Focusing in Microdroplets

Ultrasonic manipulation of micro particles inside water emulsfied in oil in a microfluidic channel

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Abstract This thesis examines the possibility to combine droplet generation techniques together with acoustic particle manipulation in microfluidics to achieve focusing of particles inside micro droplets. In the thesis I introduce the reader to the general concepts of microfluidics, droplet dynamics, acoustics and microfabrication which are the basis for my experiments. The practical work focuses on the important steps in channel design, droplet generation and the possibilities to manipulate polystyrene particles with a diameter 10 μm inside channels with widths of a few hundreds of μm. The particles are manipulated inside water droplets dispersed in an continuous oil phase and the results include an examination of the droplet flow fields in interaction with the primary radiation force. I present evidence that the size and the flow speed of a droplet greatly affects the dynamics of the forces on particles inside the droplets.

Keywords: Microfluidics, Droplet generation, Segmented flows, Acoustofluidics, Particle focusing

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Of course with love to my mother whom without nothing would be possible.

Contents

1 Introduction

1.1 Background

The popular demand for the smaller hand-held technology that is fast, easy and accessible is growing. For a long time the computer industry has already paid attention to this demand, and as the techniques becomes cheaper and more accessible new ideas using the same fabrication methods appear. The micro fabrication of integrated circuits has developed methods to make small structures in different materials. These techniques are now being used to construct micro chips made for fluid experiments.

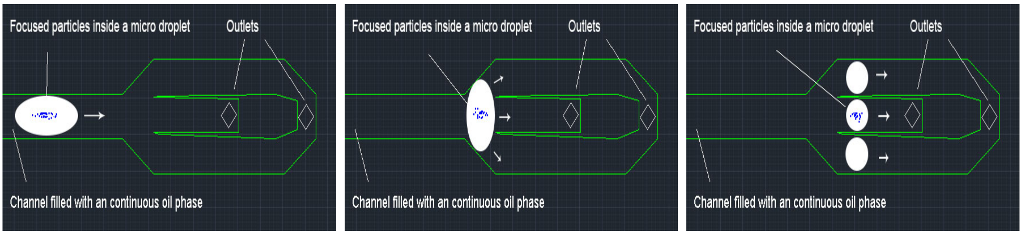
Studies in microfluidics have dealt with problems such as mixing, reactions, purification, cell trapping, labeling separation and more. An example is simply mixing two desired fluids in a micro channel. By decreasing the widths in the channels down to hundreds or tens of micrometers the mixing by diffusion becomes increasingly effective. By introducing small particles or cells in the fluids one can imagine the benefits for humans as medical diagnostics are often made using small cell samples.

If one is instead using two immiscible fluids such as oil and water it is possible just by a simple kitchen experiment to see the water form drops in the oil or the other way around rather than that they mix together. These droplets can on a smaller scale work as small micro environments for cells.

Introducing sound vibration has been discovered to affect small particles with a force [1]. One can imagine to combine these two ideas of a droplet and acoustic focusing and think of the possibility to manipulate particles inside the droplet environment.

Earlier work combining droplets and acoustics has included successful sorting of droplets with high frequency ultrasound in a microfluidic channel [2]. Studies has also examined the effect of acoustic waves on the ink-jet printer droplet dispensing. In this study they present that acoustic waves affect the droplet generation, and can be used to optimize the dispensing of droplets [3]. A recent study has used acoustic waves to provide control over droplet generation in the picoliter range for water in oil droplet generation [4]. Systems have also been developed for levitating droplets in air. The levitation effect does not only have practical possibilities but is also a beautiful demonstration of the acoustic force [5].

The experiments to focus particles inside droplets with the use of ultrasound is a new approach. Searching through article databases I did not find experiments that dealt with this phenomenon. The idea can be used to increase provide a purification and sorting of cells inside a droplet which could play an important role for cell in droplet experiments. The idea of an ideal case is presented in figure 1.1 where the green lines marks the micro channel structure and the white is a micro droplet with small blue particles trapped inside. Unfocused particles will be cleaned away from the centre droplet in this process while the bigger particles ideally will be kept in the middle droplet.



(a) A droplet with focused particles approaches the outlets of a a micro channel.

(b) The droplet approaches where the channel splits into three.

(c) The ideal case where the droplet is split into three smaller droplets keeping the focused particles in the centre droplet.

Figure 1.1

1.2 Motivation

In the fall 2012 I studied a course in microfluidics as a part of my degree in engineering physics. The course was stimulating and it was easy to see the direct applications of the field. I strongly believe that it is a field that has not yet reached it’s peak. The field has potential to be the basis for developing new practical instruments and reach new insight in fluid dynamics.

In one of the projects that were included in the microfluidics course me and a colleague studied the acoustics effects of ultrasound in droplets of water in air in a small micro chip. The project was a combination of practical work and good ideas, and I felt intrigued to follow up the project.

I will in this thesis introduce you, the reader to, general theory in microfluidics and droplet dynamics. I will also present my continued studies and experiments with the acoustic (sound) force effecting particles in micro droplets.

1.3 Overview of the thesis

The thesis is divided into two parts. The first part is conceptual and the second part is more practical. The first part includes ideas that are important from a theoretical point of view. The second part explains the practical steps and experiments in the project and ends with the most interesting results and a discussion with ideas and advice for future work.

Embracing simplicity I use $\tau = 2\pi$ [6] in the equations presented throughout the thesis.

Part I

Concepts and theory

2 Microfluidics

2.1 General

The field of microfluidics is a growing field and its potential application is far from fully discovered or developed. There has been extensive exploration of different applications of microfluidics. Among them are mixing [7], reactions, purification, trapping, labelling, separation and detection [8]. There are a few fundamental concepts to pay attention to when considering fluid systems on a smaller scale that will be discussed in this chapter. There are general concepts that cover both single phase flow systems and two phase flows. Then there are other that needs more attention when considering two phase flows that will be more crucial when considering droplet generation. The thesis will not present elaborate theory of the field but rather the most important concepts will be explained. For greater detail and further applications the reader is advised to look into [8].

Initially the definition of a fluid needs to be clarified. A fluid includes both gases and liquids in contrast to what the word might say to some people. A fluid is according to a common definition ”a substance that continually deforms (flows) under an applied shear stress.” This definition is also commonly used in graduate text books today. To visualise this property imagine a block with a solid and one with a fluid. If you put a force on to upper part both blocks will deform. However the fluid will continue to deform if there force is fix while the solid will find a new steady state.[9] See Figure 2.1

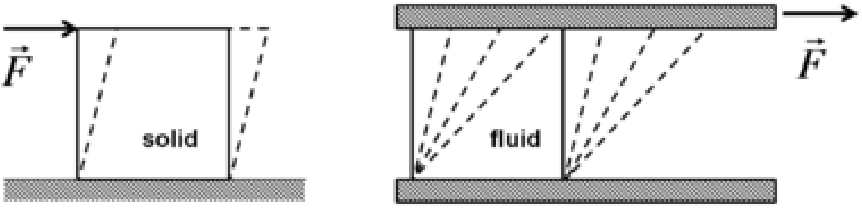


Figure 2.1: A general image of the difference between a solid and fluid. The fluid is continuously deformed while the solid is not [9].

The general understanding of a fluid is that it is modelled in one of two ways. Either a collection of individual interacting molecules or as a continuum in which a collection of certain properties are defined throughout space. A moving fluid can be characterized by the flow and the type of fluid organised in four different groups:

- Kinematic properties including linear and angular velocity, vorticity, acceleration and strain rate.
- Transport properties such as viscosity, thermal conductivity and diffusivity
- Thermodynamic properties such as pressure, temperature and density
- Miscellaneous properties such as, surface tension, vapour pressure and surface accommodation coefficient.

Knowledge of these properties are necessary to estimate and gain understanding about the fluids' behaviour. The behaviour may depend greatly on these flow properties but it is also determined by the interaction between the fluid and the vessel walls (see section 2.7). The three different types of fluids used in this project are air (gas), water (liquid) and oil (liquid). The interaction between these and the vessel walls will play a crucial role in experiments.

2.2 Laminar flow

In contrast to turbulent flow, laminar flow is a stable and controllable flow. The unorganised chaos of fluids that we usually see in our everyday life is in most cases of turbulent character. When we mix two liquids like coffee and milk they tend to randomly mix together, flowing without any traceable record of how they once were. In Laminar flow the mixing is mainly determined by diffusion instead of the fluids mixing turbulently. So what determines where the flow is turbulent or laminar? We know that in larger systems turbulence is more common, and we know that the velocity seems to affect a flowing stream. The governing equation in fluid dynamics is the Naiver-Stokes equation which states that given an incompressible fluid and constant viscosity:

$$\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla)\mathbf{u} = -\frac{1}{\rho}\nabla p + \mu \nabla^2 \mathbf{u} + \frac{1}{\rho}\mathbf{f} \tag{2.1}$$

where u is the flow velocity ρ is the fluid density, p is the pressure, μ is the dynamic viscosity, and f a collection of body forces.

From equation 2.1 one can derive when different forces are more important. If the large speed and characteristic lengths are governing the equation we will have a turbulent flow, but if dynamic viscosity is dominant, the flow will act more like the idea of a syrup going into a bowl of sour milk. Meaning that the tracks are traceable and that the process is reversible in time apart from the diffusion that has occurred.

2.3 Reynolds number

Reynolds number is a dimensionless number that is a standard measurement of which forces in the system it is that are dominant, and depending on the forces the flow will have different character. The number is derived from equation 2.1 and states that:

$$Re = \frac{\rho D_h u}{\mu} \tag{2.2}$$

were D_h is the Hydraulic diameter described in the next section, which can also be changed for a simple characteristic length in a micro channel typically as d shown in figure 2.2.

Normally Reynolds numbers above 2000 have more characteristic turbulent flow (see figure 2.2), while $Re < 1$ are considered to be laminar (see figure 2.2). In the higher numbers the turbulence is generally interspersed with laminar characteristics until it becomes clearly turbulent around 3000. The shape and structure of the channel will also come into play so the number is more a guideline in estimating the regime of the flow. Since the speed is an important factor, laminar flows can also occur in macro systems flowing at very low speed.

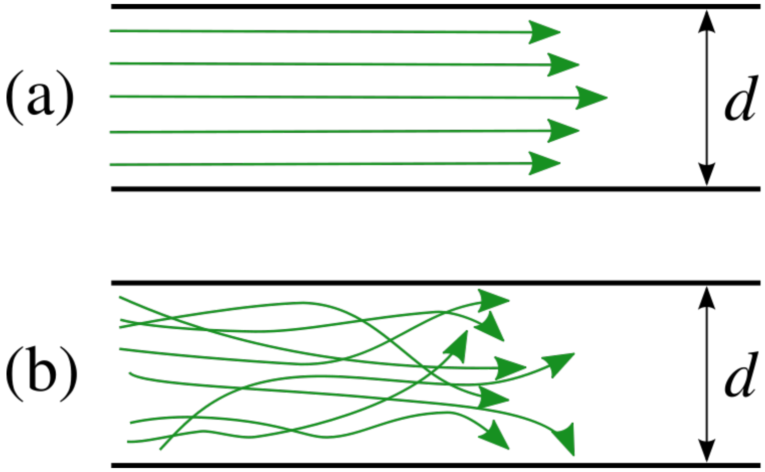


Figure 2.2: A image of a characteristic laminar flow on the top and a turbulent flow on the bottom

[10]

2.4 Hydraulic diameter

In microfluidics the channels are usually not constructed as perfect symmetrical circles where the flow in accordance is symmetrical. To estimate the flow in an arbitrary geometry a Hydraulic diameter is used which estimates the diameter of a circle corresponding to the geometry. It is calculated as:

$$D_h = \frac{4A}{Wettedperimeter} \tag{2.3}$$

where A is the cross-section area of the channel.
This diameter can then be used as a characteristic length of the system to give a good estimate of the Reynolds number and it should be used in calculation of the pressure drop according to the Hagen-Poisuelles (see section 2.5) law over the channel.

2.5 Pressure driven flow

When considering the flow through the channel, the different type of flow determines a characteristic flow profile. In a pressure driven flow, the flow though the channel can in equivalence with circuit theory be seen as a pressure difference (Δ P) corresponding to a potential, a flow (Q) corresponding to a current and a fluidic resistance (R) corresponding to an electric resistance. These relate in microfluidics just as ohms law and we have:

$$\Delta P = Q * R \tag{2.4}$$

where the resistance R is derived from the Hagen-Poiseuilles equation as:

$$R = \frac{16\mu L}{\tau r^4} \tag{2.5}$$

Q is the flow and Δp is the pressure drop over the channel. In the project setup (as will be described further in the practical part) syringe pumps are used where a flow rate is selected. From this the pressure drop over the channel and also the velocity field inside the channel can be calculated.

Generally when a pressure is applied to drive a fluid through a channel a characteristic flow profile and certain properties arise due to interaction with the channel wall and with the fluid itself. The fluid close to the wall will have lower velocity, and when the velocity can approximated to zero it is called a no-slip condition. If the channel where the fluid flow is considered to be circular the relation between the speed in the profile and the pressure applied is:

$$u(r) = \frac{(r_0 - r_2)\Delta p}{4\mu L} \tag{2.6}$$

where $u(r)$ is the flow speed, r_0 the radius of the flow profile, r the distance from the centre of the channel, Δp pressure difference over the length of the channel, μ the viscosity and L the length of the channel.

The flow profile will have an importance in the droplet generation explained further in the next chapter. The interaction between the liquid and the wall are crucial for both stable droplet generation as well as keeping them stable inside the channel.

2.6 Stokes drag force

When solving the Naiver-Stokes equation 2.1 in the laminar regime the force on a particle inside a fluid can be calculated. This force will act as a counterforce to a particle moving in some direction and is expressed as:

$$F_{drag} = 3\mu\tau r_p u \tag{2.7}$$

where μ is the dynamic viscosity, r_p the particle radius and u the velocity.

In acoustic microfluidics this force is balancing the acoustic radiation force (see section 5.2). It can also be used to calculate the terminal velocity of the particles. It is central to know this force and how both speed viscosity and size affects this force, to predict the movement of the particles in a micro channel.[1]

2.7 Hydrophobicity

The hydrophobicity of a channel is important to consider, since it determines the character of interaction between the liquid and the channel walls. If the channel is hydrophobic the water will have a contact angle that is above $\tau/4$ and if it is hydrophilic which is the contrary it will have a contact angle that is less than $\tau/4$. This is due to interfacial properties that will be discussed more related to this project in section 3.3. The flow profile will be characterised by the contact angle in response to the surface which is seen in figure 2.3.

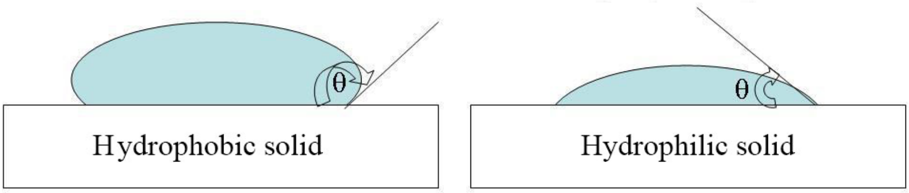


Figure 2.3: The pictures shows characteristic hydrophobic and hydrophilic contact angles for droplets. The different angles come from either the liquid changing its properties or that the solid surface changes its properties.

3 Droplet dynamics

3.1 General

The droplet generation was the first main fluidic goal of this project. In this chapter some of the basic ideas needed when considering droplet generation and droplet stability will be discussed. I will also go through different channel dimensions that are commonly used in experiments.

The benefit of using droplets in microfluidics is that droplets can be viewed as micro-reactors in which samples are confined. Small volumes of e.g. cells can be confined inside a droplet, and inside these small reactors they can be manipulated. [11] Experiments in chemistry or biochemistry are usually conducted in small parallel volumes and experiments within droplets. In e.g. single cell analysis many challenges can be overcome by using droplets [12] and with new techniques and tools from microfluidics the possibilities and benefits of using droplets expand. The ability to separate cells or particles from an outside environment like channel walls gives the ability and enhancement of applications such as mixing, diagnostic methods, particle sorting and many more. [13][14]

3.2 Two-phase segmented flow

The most important ingredient in droplet formation is the presence of two immiscible fluids. That they are immiscible means that there will be an interface between the two fluids. The two phases, a continuous phase (in this study oil or air) and a disperse phase (water) will have their different characteristic properties as e.g. a gas might have a high compressibility while a segmented fluid will not. The dominating characteristics of the fluids in their interaction will be an interplay between the interfacial tension, the velocities of the fluids and their viscosity. [15]

3.3 Surface tension

As surface tension is the main additional problem with respect to single-phase flow I will try to discuss it in a bit more detail. There are two different definitions of this property and both can be of use with benefit depending on the problem.

The first definition states that interfacial tension is a force per unit length that pulls the interface. The magnitude is given in γ (Nm^{-1}). If there is a spatial imbalance of significance (an interface) there will be a flow along this interface from the low to the higher values of tension. It can be imagined as a high tension that pulls towards itself with higher intermolecular forces with a goal to minimise the surface while a low surface tension will rather make the fluid to spread out to increase contact with surrounding fluids or surfaces.

Interfacial tension can also be defined as energy per unit area (Jm^{-2}) which acts as in order to reduce the total free energy. Since the minimum surface of a volume is a sphere, an isolated droplet have a tendency towards this shape. With walls present the shape adapt to the circumstances present and will be deformed to more elliptic shapes depending on the volume.

As mentioned in the hydrophobic section 2.7, if the interfacial tension is low the liquid will flow easier along the surface as the molecules are more attracted to the surface than the attraction between themselves, and give a hydrophilic contact profile. In the other case given a high interfacial tension the contact angle will be higher giving a hydrophobic contact angle.

3.4 Weber, bond and capillary number

The Reynolds number is the most commonly used dimensionless number to give an idea about the characteristics of the flow in microfluidics. However if we consider low Reynolds number regimes we can examine other dimensionless number that can give an idea about which forces dominate depending on the circumstances.

The Weber number compares inertial effects with the interfacial tension and is defined as $We = \rho U^2/\gamma$, where U represents a characteristic velocity scale, ρ the density and γ is the surface or interfacial tension between the two fluid phases. Considering low Reynolds number this number is low so that the interfacial properties will dominate over inertial. In certain instances e.g. in droplet break-off or at significantly higher speeds, the inertial effects can come into play and dominate the interfacial tension. In the experiments conducted this number is considered to be low due to the relatively low velocities.

The Bond number is in microfluidics defined to evaluate the gravity effect in relation to the interfacial tension as $Bo = \Delta\rho g l^2/\gamma$. In the cases of flow with oil and water and of water and gas in microfluidics the $Bo << 1$ which means that we can ignore the effect of gravity compared to the interfacial tension.

One of the more important numbers to consider is the Capillary number $Ca = \mu U/\gamma$ where μ is the viscosity of the liquid, U is a characteristic velocity. This number compares the viscous stresses with the interfacial stresses. A low Capillary number will indicate that the interfacial stresses dominate so that the dispersed fluid will produce spherical ends. At higher velocities and higher viscous forces the droplets will be more deformable and shear stress will dominate so that symmetrical properties will be harder to observe. [15]

3.5 Rayleigh forces

Important to notice in the droplet generation point is that the break-off is due to a so called Rayleigh-Platau instability. For a non-viscous fluid the theory states that droplet break off occurs if you got a small elongation of e.g. water in air the elongation will break off at $L = \pi D$. In the circumstances of the water-oil combination and small channels used in microfluidics as well as the wetting of the walls will play a role in the stability of the elongation. The interplay in water-oil generation will be more complicated due to that the interfacial tension will be important.

3.6 Flow fields

Inside a droplet there will be flow fields induced by the presence of the immiscible interface. These fields of movement inside the droplet will affect any particles trapped inside. The field depends on how the droplet moves forward and how the immiscible surfaces interact. The movement will result in recirculation zones shown in Figure 3.1. The moving droplet does not slide gently but can rather be thought of a droplet rolling forward against outer walls. The flow field then correspond to a flow similar to Poiseuille-flow (see section 2.5) onto which counter-rotating recirculation rolls are induced.

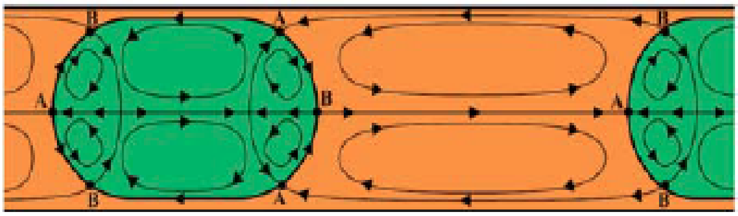


Figure 3.1: Flow fields inside a droplet with counter rotation caused by the droplet "crawling" rather than sliding forward

The full 3-D flow fields in rectangular micro channels have been visualised by-PIV [16][17] by confocal microscopy, and with numerical simulation for low viscosity drops. This is an interesting effect that will come to interact with the

PRF that will be discussed in Chapter 5.

4 Introduction to acoustics

4.1 Sound waves

Sound is pressure waves in a media of particles [18]. When discussing waves one way to think is to imagine particles connected by springs that sit together in an large network. Each particle is affected by those around it and will affect them back. Say for example that we strike the particles in one spot in this large network with a hammer. This will create a high pressure oscillating to a low pressure in this point that will spread out by the connected particles as waves, as the particle continues to oscillate. In practice different wave properties occur depending on the medium and the molecules and how they are coupled together. Transversal waves are when the oscillation is perpendicular to the propagation direction and longitudinal waves are when the oscillation is in the same direction as the propagation. In solids both of these waves occur but in fluids such as those used in this project, only the longitudinal waves are able to propagate.

The theory describing the waves are based on the wave equation which given one spacial dimension states that:

$$\frac{\partial^2 s}{\partial x^2} = \frac{1}{c^2}\frac{\partial^2 s}{\partial t^2} \tag{4.1}$$

where s is the displacement, x the position in the wave and c the sound speed.[18]

A common way to express the solution for a displacement as a function in time is to express it as:

$$s(x,t) = A \cdot sin(\tau(\frac{t}{T} - \frac{x}{\lambda}) + \alpha) \tag{4.2}$$

As the sound wave propagates as compressions variations, the wave in equation 4.1 can also be expressed as variations in gauge pressure p:

$$p(x,t) = p_0 \cdot cos(\tau(\frac{t}{T} - \frac{x}{\lambda}) + \alpha) \tag{4.3}$$

$$p_0 = B \cdot k \cdot A \tag{4.4}$$

where p_0 is the gauge pressure amplitude, B the adiabatic bulk modulus and A the displacement amplitude. The pressure is phase shifted by $\tau/4$ in reference to the displacement, meaning that the pressure will peak where the displacement is at its lowest.

The medium where the sound travels becomes important to calculate the speed of the sound. The propagation speed is given by.

$$c = \sqrt{\frac{K}{\rho}} \tag{4.5}$$

where K is the bulk modulus and ρ the density of the material. The sound speed then gives a general relation between a certain wavelength and a frequency as

$$f = \frac{c}{2w} \tag{4.6}$$

where f is the frequency and λ is the wavelength.

4.2 Acoustic impedance, reflectance and transmission

In the respect to to different media used in this study it is important to consider the acoustic impedance of the different media. Acoustic impedance indicates how much sound pressure is generated by the vibration of molecules of a particular acoustic medium at a given frequency. The acoustic impedance will determine what the reflection and transmission for a sound wave at a boundary of two different media. The characteristic (acoustic) impedance of a medium is an inherent property of a medium measured in Rayls and is given by:

$$Z_0 = \rho_0 c_0 \tag{4.7}$$

where ρ_0 and c_0 are the density and speed of sound in an unperturbed medium.

The reflectance and transmission of a sound wave through a media is given by a relation of acoustic impedances between the two materials. The reflectance is given as:

$$R = \frac{Z_2 - Z_1}{Z_1 + Z_2} \tag{4.8}$$

and the transmission is

$$T = 1 - R = \frac{2Z_2}{Z_1 + Z_2} \tag{4.9}$$

where Z_1 and Z_2 are the characteristic acoustic impedance for the two materials. [18]

4.3 Standing waves and resonance

When two waves interact the interference pattern can be calculated by superposition. Standing waves arise when two identical waves traveling in opposite directions meet. By adding two waves with opposite signs of the phase and rearranging the displacement is calculated as:

$$s(x,t) = s_1 + s_2 = 2A \cdot sin(\frac{x}{\lambda} + \alpha)cos(\tau(\frac{t}{T} + \alpha) \tag{4.10}$$

The result is that two factors, one depending on position and the other on time determine the displacement. If we ignore the phase shift α we can se that the position dependent term will alternate from minima to maxima every quarter of a wavelength. These are the displacement nodes alternating with antinodes, as seen in figure 4.1

The pressure can in the same way as above be calculated for the two waves as:

$$p(x,t) = p_1 + p_2 = 2A \cdot cos(\frac{x}{\lambda} + \alpha)cos(\tau(\frac{t}{T} + \alpha) \tag{4.11}$$

Due to the change from sinus to cosinus in the pressure term the displacement and the pressure nodes are at displacement maxims and vice versa.

The standing wave is a commonly occurring and very well used acoustic phenomenon. A music tone is a standing wave with a certain frequency and we can see it in for example a guitar string. In figure 4.1 we can see how the displacement and pressure of two different harmonics are visualised with reflectance between two walls.

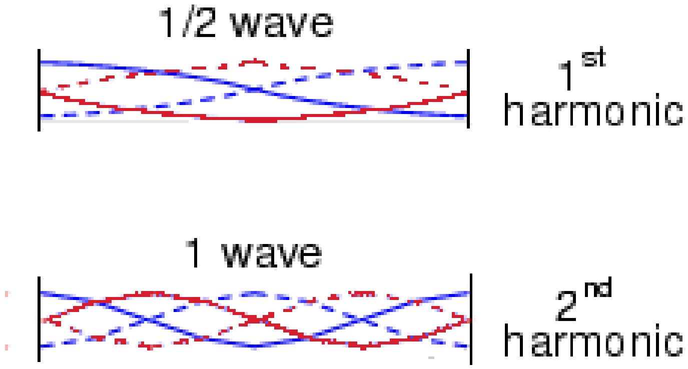


Figure 4.1: Figure of two harmonics of a standing wave. The red is the oscillation of displacement and the blue of pressure. The first shows half a wavelength that matches the length between the walls and the second the double frequency for which one wavelength matches the length.

The boundary conditions for the sound wave will determine if the wave in the end point will have a pressure node or maxima. In a guitar string the displacement at the ends is fixed to zero, and for a sound wave inside a less dense material with lower acoustic impedance than a denser surrounding media, the displacement node will be at the wall.

4.4 Piezoelectric actuators for ultrasound

To generate the sound vibration needed for microfluidics, usually piezoelectric actuators are used. Since the standing waves that we want to use is of wavelength as hundreds of micrometers, the frequency is of the range of megahertz.

The Piezoelectric actuators couple electric and mechanical behaviour in a controllable way. More clearly put is that the material will vibrate depending on the applied voltage, or in the opposite fashion polarise under a mechanical stress. This effect arises from non-centrosymmetric charge distribution in the crystal unit cell. A deformation changes bonding angles in the crystal and change the charge distribution giving a polarisation.

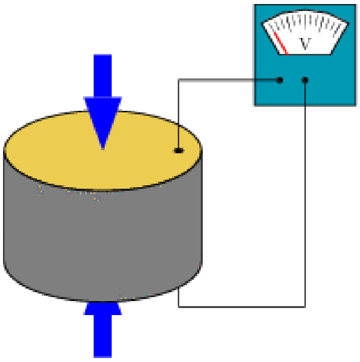


Figure 4.2: A piezo material deforms depending on the applied voltage. An alternating current is able to give high frequency sound vibrations

These piezoelectric materials are able give small deformation at a high efficiency and precision and is therefore commonly used in a variety of fields. By applying an alternating current (AC) a oscillating behaviour occurs. The equation determining the vibrations from the voltage are described in more detail in [19]. At negative voltage the material contracts and at positive it expands and the vibration of the actuator will therefore produce a mechanical oscillation at the same frequency as the electrical signal. The actuator will peak in performance at a resonance frequency where half wavelength correspond to the thickness of the crystal. The thickness must therefore be chosen carefully depending on which frequency is desired. [20][21]

5 Acoustic forces on particles

5.1 General

The variation in the pressure depending on the phase of the wave is the cause of a so called radiation force. A particle acquires an acoustic energy potential dependent on the position in the sound wave. There are two main forces in consideration, one is called the primary radiation force (PRF) and affects all particles to be drawn to the pressure nodes and another force called the secondary radiation force, which only affects closely located particles to be drawn to each other in an acoustic field. Particles under the influence of a passing sound wave will have a negligible influence of the acoustic forces. But particles under the influence of a standing wave field will have a greater influence since the waves are superpositioned and the pressure amplitude will build up significantly. In this section I will discuss the primary radiation force, which is central in this project to get a focusing effect on particles in the micro droplets.

5.2 Primary radiation force

The PRF was first described by King in 1934 [22]. The force has been investigated and the system of factors that affects this force are density and compressibility (ρ and β) differences, between media and particles, the pressure amplitude of the sound wave P, wavelength λ particle radius a and the position in the wave field $2ky$. The dependence of these factors as denoted by Gor'kov [1][23] is shown in equation 5.1.

$$F_r = 2\tau a^2(ka) \cdot E_{ac}\phi(\beta, \rho) \cdot sin(2ky) \tag{5.1}$$

where the equation has different parts that will be described further. A pre factor $2\tau a^2(ka)$ collects the radius dependence of the force and the effect of the frequency dependance. The benefit of micro scale system lies in that the force is stronger with the higher frequencies that are present in micro scale systems. Seen in figure 5.1 the force related to the size of particles is shown.

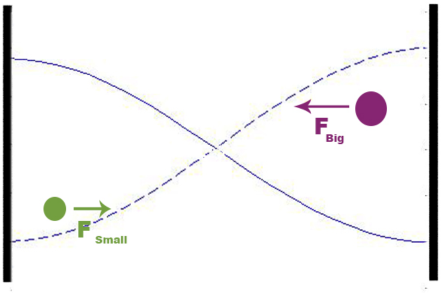


Figure 5.1: The figure shows how the primary radiation force is size dependent. A larger force acts on the bigger particle.

The second term E_ac in the equation is called the energy factor which is the energy of the standing wave. This factor is calculated as:

$$E_a c = \frac{p^2}{4\rho_0 c_0^2} = \frac{p^2 \cdot \beta_0}{4} \tag{5.2}$$

where p is the pressure amplitude and β is the compressibility. These two couple to account for the acoustic energy since compressibility is the volume change per applied unit of pressure at a specific temperature.

The next factor in equation is the so called acoustic contrast factor, ϕ, shown in equation 5.1. The factor highlights the force dependence of the relation in compressibility and density between particles and surrounding media and is calculated as:

$$\phi = \frac{\rho_a + \frac{2}{3}(\rho_a - \rho_0)}{2\rho_a + \rho_0} - \frac{1\beta_0}{3\beta_a} \tag{5.3}$$

where ρ_a and ρ_0 are the densities and β_a and β_0 the compressibility for the particle and the surrounding media.

A consequence of this factor that can be seen is that a switch of the dominating density will change the sign of this term. The effect of this can be seen in figure 5.2 where the denser particles are moved to the pressure node and the less denser particles the the antinodes. This has been used for example in experiments with separation of blood cells and blood fats. [24] [25]

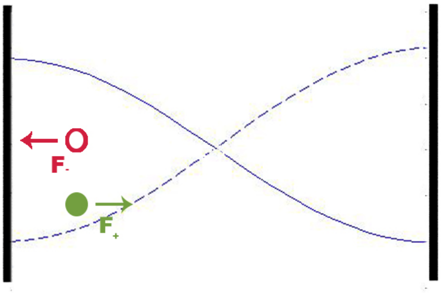


Figure 5.2: Two particles with different contrast factor experience different directions of the force.

The last factor $sin(2ky)$ in equation 5.1 is the dependence on the wave field position. $k = \frac{\tau}{\lambda}$ is the wavenumber and y is the distance from the pressure node. Smaller forces will be exerted on particles in the centre (at the pressure node) and the edges (pressure antinode) of the channels due to this term.

Part II

Method, experiments and results

6 Overview of the method

The progress of the work is divided into different practical steps that were carried out in this thesis. An overview of the steps and goals of the process can be simplified into the following.

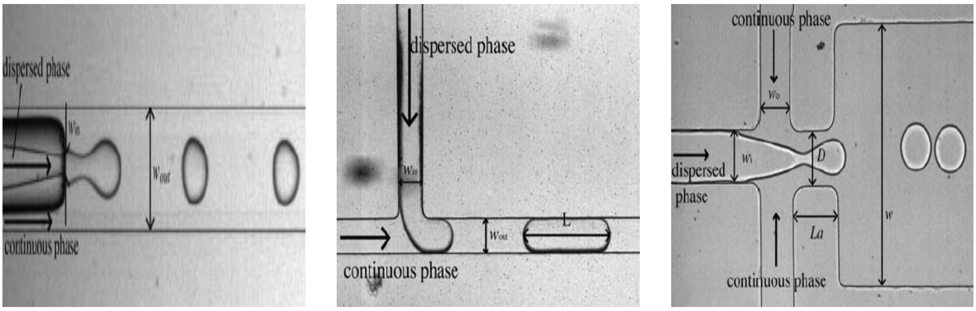
- Channel design
- Fabrication of micro chips
- Preparation of the micro channel (including: application of tubings, attachment of piezo and surface treatment of channel walls)
- Preparation of fluids (including: adding surfactants and polystyrene beads)
- Setup with computer controlled syringe pumps to control the flow speeds
- Adjustment of flows and ultrasound to generate droplets with acoustic focusing effect
- Splitting droplets with beads focused in the middle

In the following chapters these steps will be dealt with in more detail.

7 Channel Design

7.1 General

The idea when constructing a channel is to make the droplet break off as efficient as possible. Generally three main geometry approaches are used 1) breakup in co-flowing streams, 2) breakup in cross-flowing streams, 3) breakup in elongation strained flows or flow focusing channels [15]. In this project the two latter approaches are used. Typical channel designs are shown in figure 7.1.



(a) A co-flow chip design made for droplet generation

(b) A cross-flow T-chip design for droplet generation

(c) A flow focus design for droplet generation

Figure 7.1: Different channel chip designs used for droplet generation [15].

7.2 T-junction

T-junctions are one of the more simple channel constructions for droplet generation. It was first used by Thorsen et. al. to generate droplets in different oils [26]. The dispersed and the continuous phase meet each other in 90 degrees. When the dispersed phase flow is low compared to the continuous phase flow the viscous stress will break of the droplet once it overcomes the interfacial tension. As the dispersed flow increases in respect to the continuos phase it is able to block the continuos phase flow as it grows, thus forcing the interface to neck and pinch of the droplet. Increasing the flow even more will eventually form into two parallel flows. This speed required to enter this regimes have been seen to decrease with an increase in viscosity of the dispersed phase [27].

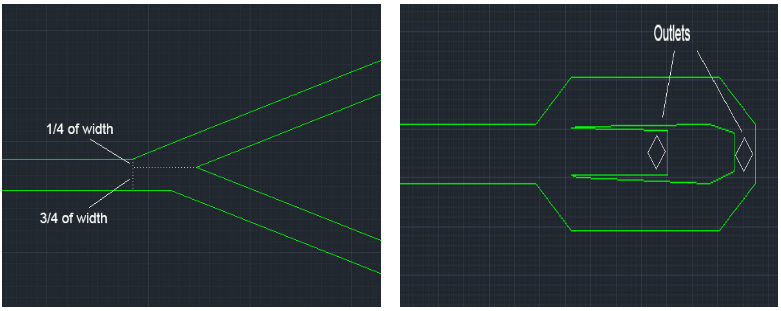
7.3 Flow-focusing devices

In a flow focusing geometric setup the dispersed phase is squeezed by two counter flowing streams. In this setup four different regimes can be observed: squeezing, dripping, jetting and thread formation. In the squeezing regime which is the most stable from droplet generation point of view, the break of is due to the capillary instability (Rayleigh-Plateau see section 3.5). When the speed of the dispersed phase is increased the new regimes are entered until a stable thread formation in the middle of the channel is formed so that the fluids will flow parallel in the channel.

There are no clear scaling laws between the geometry and speed for these different behaviours. In this project some of these regimes where clearly observed. The squeezing regime is clearly the most beneficial for droplet generation and was in the practical work determined experimentally by variation of flow rates.

7.4 Droplet splitting

To be able to split droplets a technique with unsymmetrical ends was used. The splitting of the different channels can be seen in the complete channels in section 7.7 and in a highlighted view in figure 7.2. In the glass channels to the outlets are displaced 1/4 of the width from the centre. The idea was to make an unsymmetrical splitting. The silicon chip was symmetrical in both ends. It also had a splitting where the channel splits up into three, each with the same size as the channel before the split. The purpose is to separate the focused particles from clean water and thereby increasing the concentration of particles in one of the resulting droplets and decreasing in the others.

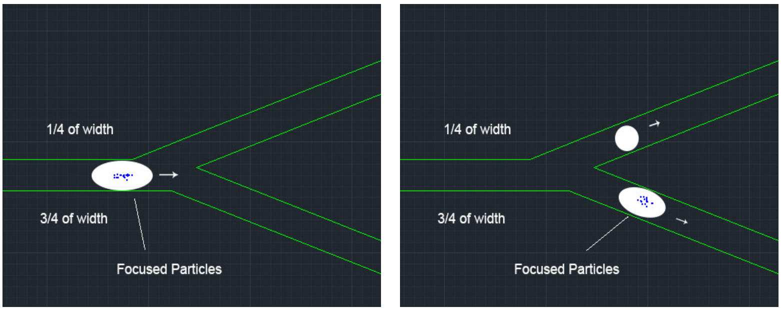


(a) The splitting design for the glass chip. An unsymmetric split, with the purpose of divinding the droplet unsymmetrically.

(b) One end of the silicon chip. The channel split into three branches. Two of which share one outlet seen in the right of the picture.

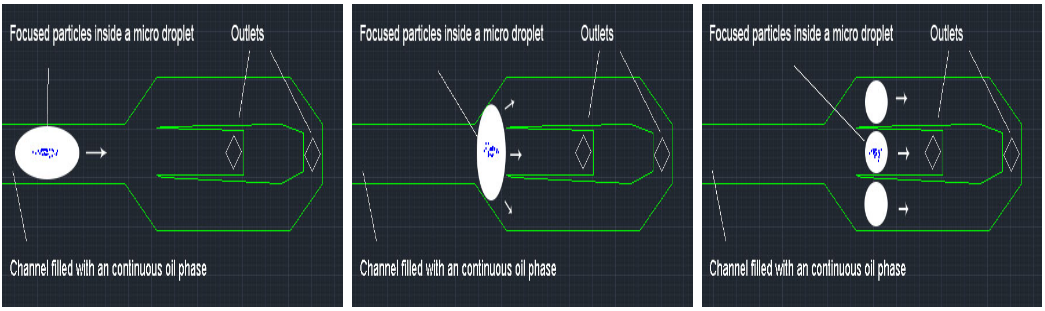
Figure 7.2

The idea of the splitting process for the two designs can be seen in figure 7.3 and 7.4.



(a) (b)

Figure 7.3: Ideal splitting of a droplet in the glass channel design

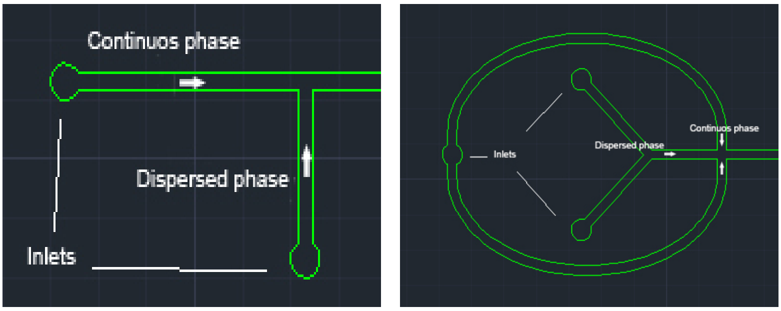


(a) (b) (c)

Figure 7.4: Ideal splitting of a droplet in the silicon channel design

7.5 AutoCad drawing

A section about different materials for channels will be discussed in the next chapter. The design for the glass channels used in this project is drawn in AutoCad. The silicon channels were reused from previous projects by the acoustic group at the department and a the polymer chip used was a collaboration with Prem Kumar from KTH. The design of the glass chip followed. How the chips are obtained from the Cad drawings to reality channel is explained in the next chapter. In figure 7.5a and figure 7.5b the important parts for droplet generation points are shown.



(a) A t-flow design drawn in AutoCad for the masking in the micro fabrication (see chapter 8)

(b) A flow-focus design drawn in AutoCad for the masking in the micro fabrication (see chapter 8)

Figure 7.5

7.6 Design to match acoustic frequencies

The width of the channel were designed to match the peak frequencies of common piezo actuators. The piezo elements that are usually used have their peak frequencies at 2, 4 and 6 MHz. Therefore the width of the channels were designed as 375 μm 187.5 μm and 125μm which can be calculated from equation 4.6.

In the graph below the focusing frequency as a function of the width of the channel is shown. Multiples of nodes can also be obtained by increasing the lengths or by increasing the frequencies, so that an integer of half wavelengths matches the width of the channel. The multiple nodes correspond to different harmonics described in chapter 4.

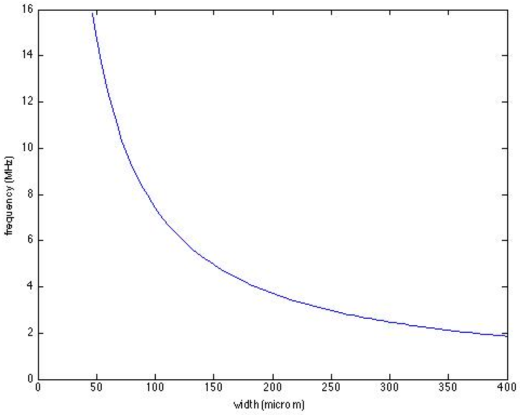


Figure 7.6: A graph showing the frequencies needed for half a wavelength to match a standing wave over different widths of a channel. The graph is calculated using the sound speed in water of 1482 m/s.

7.7 Summary of channel designs

The depth of the channels were limited by effects of isotropic etching that will be explained in the next chapter (see section 8.4). The channel designs are visualised in figure 7.7, 7.8 and 7.9 . The width depth and length are summarised in table 7.1. The isotropic (glass) and the anisotropic (silicon) etching affects the cross-section of the channel and can be seen in section 8.4 in8.3. Standing waves will be slightly affected by this change in geometry but the acoustic focusing has been shown to work [28].

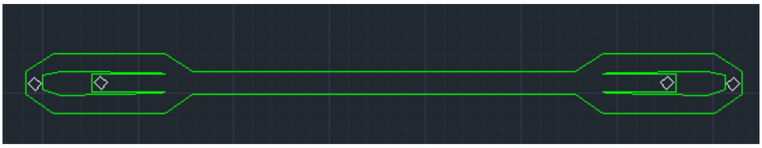


Figure 7.7: The lines in the figure shows the design of the silicon channel used in the project. The white squares shows the inlets and outlets of the channel.

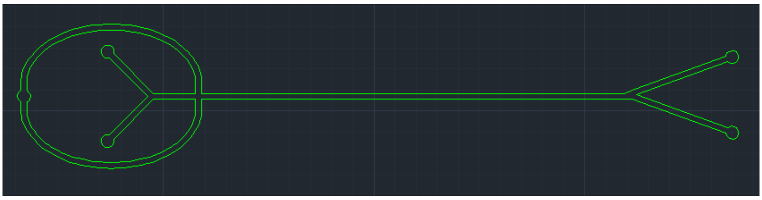


Figure 7.8: Flow focusing design for a glass channel, with a midpoint of the exit split located $1/4$ of the width from the top for droplet splitting

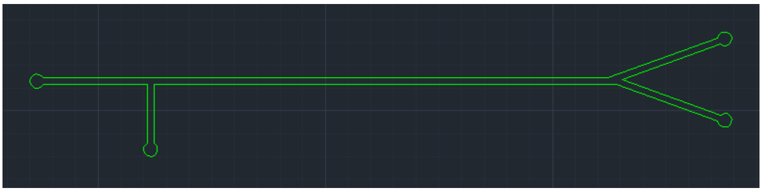


Figure 7.9: A simple cross flow t-design for a glass channel, with a midpoint of the exit split located $1/4$ of the width from the top for droplet splitting

Chip Design	Width	Depth	Length
Glass Flow-Focus	125 μm	48 μm	2 cm
Glass Flow-Focus	375 μm	48 μm	2 cm
Glass T-Focus	125 μm	48 μm	2 cm
Silicon Design	375 μm	150 μm	4 cm

Table 7.1: Width, depth and lengths for the different channels used

8 Microfabrication

8.1 General

In microfluidics one is adviced to choose a material fitting the purpose of the study. There are basically three main groups of materials, silicon (semiconductor), glass and plastics (polymer). The suitability of the different materials are dependent on the application due to their different properties.

8.2 Materials

8.2.1 Silicon

Silicon was used early since the methods of fabrication were easy to obtain from the integrated circuit industry. It has a crystal structure that makes some limitations in wet etching. A silicon crystal has structured bonds between atoms that create a difference in molecular density with respect to different directions in the crystal. Due to these differences the etching process may be anisotropic. This enables a few different shapes and cross sections that can be made, but also limits the possibilities since the different directions (or so called crystal planes) needs to be considered. Some of the possible structures are shown in figure 8.1

(a) (b) (c)

Figure 8.1: The figure shows typical structures that can be constructed in silicon. The anisotropic etching in the silicon crystal creates boundaries on the types of structures [25].

The etched silicon channel is usually covered with glass on top to cover the channel that enables visibility to the experiments and do not interfere too much with the acoustics properties of the channel.

The silicon has a high acoustic impedance in relation to water and oil (see chapter 10) which makes it very well suited for acoustic applications. A lot of the early experiments in Lund were made in silicon chips and it is still a commonly used material in microfluidics.

8.2.2 Polymers

With plastics and polymers such as a commonly used material as Polydimethylsiloxane (PDMS) a lot of possibilities with structure arises. PDMS is reasonably cheap and there are methods to create small structures. The easy and low cost fabrication methods, make this material quickly growing in usage. When considering large scale productions the polymers would hold an advantage over both glass and silicon. However, the acoustic properties of polymers are when working with acoustofluidics not as desirable.

8.2.3 Glass

Glass has many favourable properties and is commonly used in combination with the other materials. Channels are also possible to make solely in glass. The inert properties that glass posses gives a lot of the practical advantages. From our common day life we see through glass and the same applies in micro fluidic studies. Visibility is of course of great advantage, and even the silicon chips are used in combination with glass for this purpose. When fabricating glass structures it does not possess the same crystal structures as silicon but will etch in a isotopic manner, which enables possibilities for new structures of channels. The downside is that the depth will at maximum always be half of the width when using wet etching. In the next section I will explain the etching process and the different steps in this process to get a better idea about the fabrication.

8.3 Overview of fabrication process

Glass chip fabrication follows the normal lithographic procedure as silicon with small differences. The overall procedure for silicon can be seen in figure 8.2 and will be explained briefly.

The process start with the substrate being coated with thin (about 20nm) silicon dioxide protection layer. Then a photoresists is deposited on top by spin coating. A mask is put in front of the photoresist that exposes parts which are illuminated with UV-lithographic methods or a laser. Depending on if the photoresist used is positive or negative it is either the solvability of the photoresist will be changed. If it is positive as in the figure 8.2 the exposed photoresist is removed with a solvent. After this, the exposed silicon dioxide is etched with hydrofluoric acid (HF), leaving the desired structure exposed in the silicon. The photoresist layer is removed and after this the desired structure is etched into the silicon with e.g. KOH (potassium hydroxide). The last steps is to remove the remaining silicon dioxide and seal the channel with typically with a glass lid.

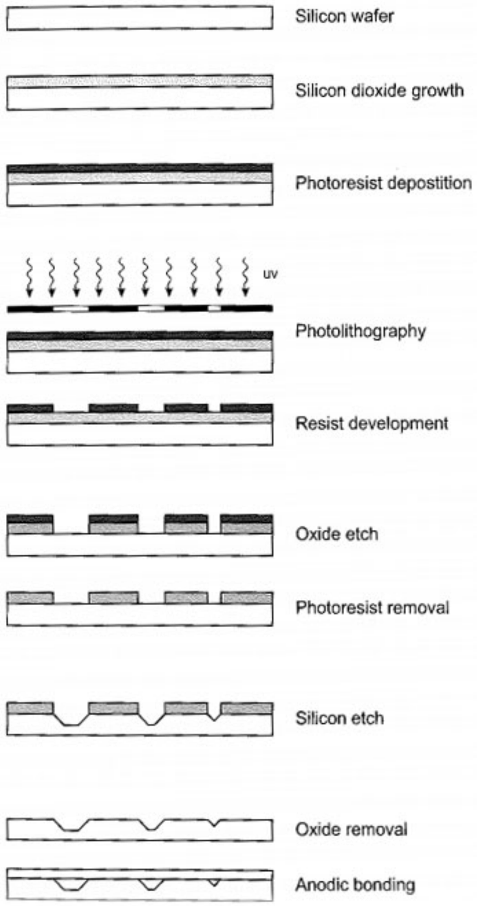


Figure 8.2: The different steps in a micro fabrication process

The Lithography method is particularly sensitive to dust and particles. These will directly affect the pattern in the process. Another common problem is that the bonding does not keep tight but small air holes are stuck next to the channel. This will affect the flow and be crucial to its function. For complete details of the process used in the glass etching used in this project to etch glass channel see Appendix A. The practical work was done by my supervisor M. Tenje who helped me to etch and prepare the channels.

8.4 Etching

There are two different profiles in wet etching; Isotropic and anisotropic. Isotropic means that the etching is equal in all direction and will give a channel appearance seen to the left in figure 8.3. The appearance will in the isotropic case be a more circular shaped channel structure and the depth and width will be related as $x = w - 2d$ as seen to the right in figure 8.3.

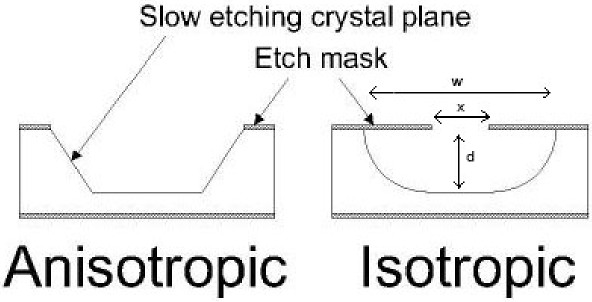


Figure 8.3: The different etching profiles. The anisotropic characteristic for silicon and the isotropic that is characteristic for glass.

Due to these etching profiles the desired depth and width should be considered in the mask design. Isotropic etching is the case in glass since all the direction in the glass have a similar structure, which is in difference to the crystal structure of silicon.

In contrast to isotropic etching anisotropic etching is when some planes are etched faster than others. This is the case in the crystal structure of silicon. By choosing a proper mask some beneficial structures can be created. For example straight walls and quadratic channels can be created, that can be of advantage as the particles are able to be focused in two dimensions. The planes etches at different speeds so that in relation to the slowest most dense plane the other etches 20 to 100 times faster.

8.5 Bonding

When bonding glass-to-glass, the general method is to clean the surfaces with sulphuric acid, piranha wash [29][30] or similar cleaning methods and then bonding the surfaces by allowing van der Waal bonds to occur between them. This method is very sensitive to dust and it is important to conduct in a clean-room environment. The method used when sealing the glass chips in this projects uses the van der Waal bonding principle.

9 Droplet generation

9.1 General

In this chapter the practical details of how the droplet generation was accomplished is presented. There are several dynamic problems that appear when working with droplets both in generation and keeping the stability of droplets. Depending on the application, the wish might be to decrease the chance of two droplets combining and keep the stable environment of single droplets (eg. see [12]).

Adjustments were made to increase the control of droplet generation. The practical details will be discussed in this chapter that relates to the concepts mentioned in chapter 3. Different flow regimes can occur when the droplet mix, split or during the droplet formation and this can cause instabilities in the droplet generation.

Small variations of the system can change the whole behaviour because of non linear dynamics in the generation processes, which can make a small initial condition to cause a greater change [15]. In the project the surface treatment of the channel and surfactants (see section 9.2) greatly changed the dynamics of the generation point. At times there was dust stuck in the channel that greatly affected the generation. If water got stuck in some place near the generation the whole system was affected.

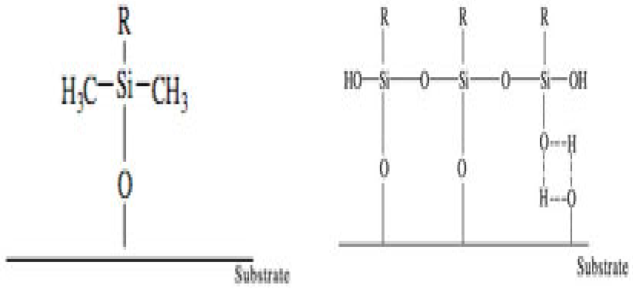
Two different combinations of fluids are tested in a two-phase-flow in this project in order to generate droplets. The first is water in oil and the second is water in air. The dynamics in the two different combination differs greatly, not only in fluid dynamic properties but also in acoustic properties. However, the best attempts to generate water droplets in air were very unstable even though multiple different channel designs were tested. I believe this is due to that air is compressible, which makes it very hard to work with in lower volumes, together with water. The focus will therefore be on the water-in-oil droplet generation.

9.2 Hydrophobicity

The hydrophobicity of the channel walls will be important for the droplet generation, and there are treatments that can be done with the wall to increase the hydrophobicity of the wall. At first a commercial product called Aquapel was tested to increase the hydrophobicity of the channel walls. No chemical details about this product are available from the supplier. After unsuccessful attempts to achieve the hydrophobicity needed with this product a repel-silane was tested instead. With greater success the hydrophobicity achieved with this chemical was visible instantly.

In a repel-silane aliphatic hydrocarbon substituents or fluorinated hydrocarbon substituents (the R group in figures 9.1), are the hydrophobic entities which enable them to induce surface hydrophobicity. It binds to a surface and with an oxide part to the substrate while the hydrocarbon substituents or fluorinated faces into the channel. The binding of the silane to the surface was in some cases under hydrolytic conditions since some of the channel had been run through with water on several occasions before the silane. Others had closer to anhydrous conditions. Problems were experienced with these channels that had already been run though they still had wetting tendencies in some places. This could have been due to dirt or water that stuck to the channel, which inhibited the hydrophobic treatment of the repel-silane, or that the reaction is more complex under these conditions. For details in the binding process of the silane see [31].

The different binding and how the silane is attached to the surface are shown in to anhydrous conditions in figure 9.1a and for hydrolytic conditions in 9.1b.



(a) The chemical biding of a Silane to a substrate surface with a hydrophobic R-group under anhydrous conditions

(b) The chemical biding of a Silane to a substrate surface with a hydrophobic R-group under hydrolytic conditions

Figure 9.1

The Repel Silane used with best success in this project was Dimethyldichlorosiliane, 2% in Octamethylcyclotetrasiloxane.

9.3 Surfactants

As discussed above and in chapter 3 the interfacial forces seems to play an important role in droplet generation and stability. The surfactant molecules are molecules that are drawn to the surface between two fluids, and these a role in the interfacial tension. Depending on the type of fluids some molecules will naturally migrate and self assemble as a layer at the interface and stabilise the surface. The introduced molecules has an important consequence as it will introduce a tangential stress jump called Marangoni stress. The idea of how surfactants can locate along a droplet surface incapsulating and decreasing migration of cells, DNA and RNA can be seen in 9.2.

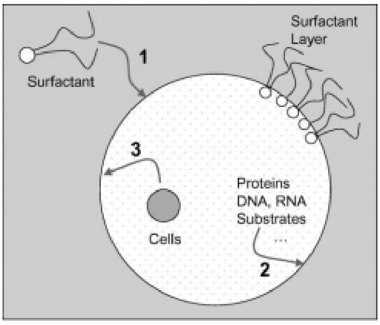


Figure 9.2: In the picture a drop of water in oil is shown that is typical for encapsulation of biological molecules (DNA, RNA or proteins) and/or cells. Surfactants adsorb to the interface (arrow 1), forming an interfacial surfactant layer, that increases the stability of the surface. The layer also prevents the adsorption of biomolecules and cells to the interface (arrows 2 and 3) [32]

.

The surfactant used in this project is Krytox, which is a surfactant with a performance studied for droplet stability in Flourcarbon oils [32]. The oil used are is a type of flour carbon oil called 3M Novec 7500 Engineered Fluid. For material safety data sheets (msds) for the oil see [33]

In addition to their critical role in stability for the droplets, surfactants can also prevent the adsorption of biomolecules at the drop interface. This effect is of use when conducting e.g. cell experiments inside droplets [12]. A small test was made together with Prem Kumar from the group who conducted these experiments. The objective was to test the droplet generation with a surfactant that is called krytox in PDMS chips that they use in their experiments. A picture showing successful droplet generation is seen in figure 9.3. These chips were significantly smaller with a width of 50 μm and yielded extremely high droplet generation rate in comparison to the silicon and glass chips. No continued experiments with acoustics in these chips were however conducted due to acoustic properties of PDMS.

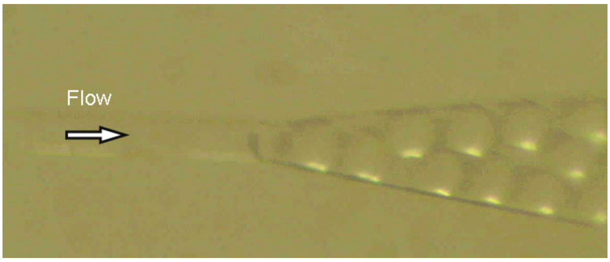


Figure 9.3: Stable droplets in a PDMS chip. The droplets are stable and a decreased probability of merging is obtained by the surfactant.

9.4 Practical setup and usage of syringe pumps

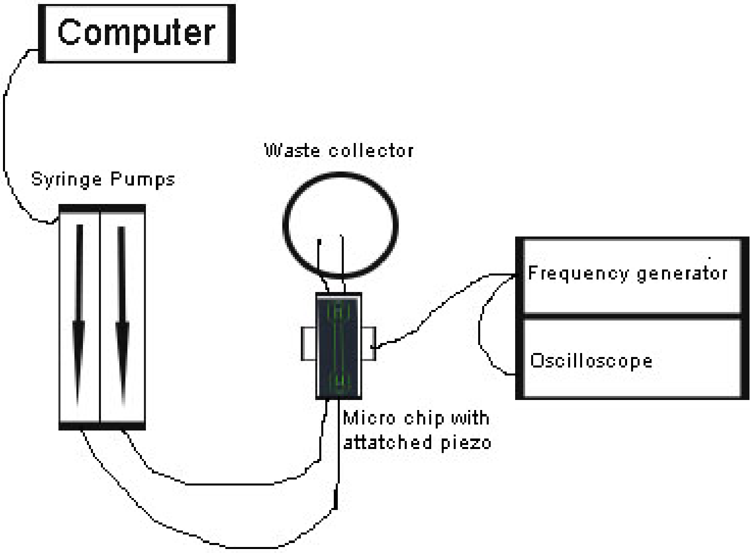


Figure 9.4: An overview of the setup. The microchip is illuminated with lamps and looked at through a microscope from above

(For details on the instrumentation used see Appendix B)

When the preparation of the fluids and the channel was complete a computer controlled syringe pump system is used to manipulate the fluids into the channel. Two methods were tested: The first was to force the fluids through the inlets by applying a pressure to the syringe pumps shown in figure 9.4 without controlling the flow of the outlets. The other was to attach empty syringe pumps to the exits of the channel and having tubings connected with the inlets put into reservoirs of the oil and water.

In the computer program measurements of the syringe diameter and volume are used to calculate the dislocation that is needed to control a constant flow speed in $\mu l/min$ that is setup in the program. The pumps apply a mechanical force by dislocation, pushing the fluids through the tubing into the channel. For the $1ml$ syringes a controlled flow could be achieved down to about 1 $\mu l/min$.

Different flow speeds and flow ratios were used (from (5/5 $\mu l/min$ water/oil up to about 100/100 $\mu l/min$). Lower flow speeds caused the flow and generation to be more unstable, while higher flow speeds emptied the syringe pumps too fast and gave no direct benefit to the experiments. Different size of droplets were generated by changing flow speeds and ratio. Temporary generation dynamics caused the sizes to change. No relationship between flow ratios or speeds with droplet sizes was obtained although a range of different sizes were generated.

To greater illuminate the beads inside the droplets and how they were affected by the acoustic force (see section 5.2) the flow rates were lowered and were also stopped under the microscope. To stop droplets inside the channel the flow rates were lowered and stopped and the outlets of the channel were plugged.

10 Acoustic effect on droplet generation and particle focusing

10.1 General

After the droplets were generated the goal was to apply a sound field to focus polystyrene beads of sizes $10\mu m$ and $3\mu m$ inside the droplets. To manage this an acoustic field with frequency matching the width of the channel was applied. According to the theory in section 5.2 the particles would experience a force towards the centre of the channel.

The transmission of the sound and in the transition between different materials is given by equation 4.8. In table 10.1 the reflection and transmission between different materials are listed with values from table 10.2 used.

Material	Density (kg/m^3)	Sound Speed (m/s)	Z (Mrayl kg/sm^2)
Silicon	$2.33 \cdot 10^3$	8433	19.7
Glass	$2.2 \cdot 10^3$	5500	12.1
Water	$1 \cdot 10^3$	1461	1.48
HFE 7500	$1.6 \cdot 10^3$	664*	1.06
Air	1.2	343	$405 \cdot 10 - 6$

Table 10.1: [18]

*The sound speed in the HFE oil was measured in an experimental setup. A signal from a transducer was sent through a setup with a small sample and reflected after the sample back to the transducer which worked as a receiver. The time difference between the sent signal and the received signal was obtained. A sample of MilliQ water was used as a reference sample and the ratio between the time for the signal to travel in the water sample and the time for the signal to travel in the HFE oil sample gave a relation of the velocities, since the length L through the setup with the sample was constant and $L = v \cdot t$.

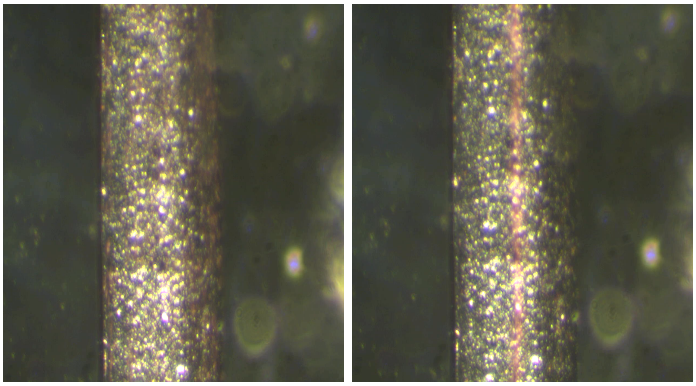
Materials	Reflectance	Transmission
Silicon/Water	0.86	0.14
Glass/Water	0.78	0.22
Silicon or glass/Air	1	0
Water/Oil	0.17	0.83
Water/Air	1	0

Table 10.2

In an air-water system it is fair to account that most part of the sound travels directly to the water since the reflection between silicon and air is too high to transmit any energy in comparison. In the water-oil system the reflection inside is a bit lower since the difference in acoustic impedance is lower. A considerable amount of water will also be transmitted from the channel to the oil. As mentioned in chapter 9 the water-oil system was used because the droplet generation proved to be more functional in these experiments, although an water/air system posses favourable acoustic properties.

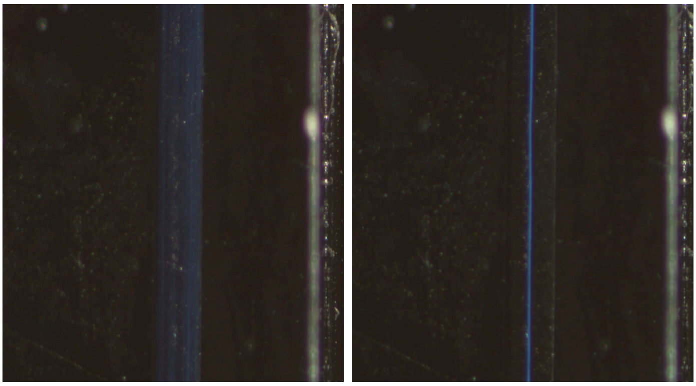
10.2 Single phase flow focus

The first step in a single phase flow is to make sure the frequency is set right. This is done with polystyrene beads in a single phase water flow. The frequency is adjusted until the beads clearly focus in the centre of the channel. This has been done in previous studies and has been applied for cell sorting and different types of cell and particle manipulation projects. In my setup I added polystyrene beads that were 3 or 10 micrometers in diameter. The difference in effect of the acoustic focusing can be seen in figure 10.1.



(a) Single phase flow with unfocused 3 μm particles

(b) 3 μm particles focused in a single phase flow by an applied voltage of 10V



(c) Single phase flow with unfocused 10 μm particles

(d) 10 μm particles focused in a single phase flow by an applied voltage of 10V

Figure 10.1: A more narrow focus can be seen for the larger particles. The PRF on these particles are stronger which makes them better suited for my experiments.

The frequency that was used in the silicone chip of 375 μm width was 2.08 MHz. The same frequency was used when focusing inside droplets. Different speeds of the plugs were tested. From down to 5 $\mu l/min$ up to 200 $\mu l/min$. Different ratios of water were also tested from 20% water generating smaller droplets to 50% generating larger droplet of water.

In the glass chips the experiments to focus the particles were hard to visualise due to a problem with a very shallow channel. Not enough particles were able to enter the channel to make the focus effect clear. The experiments were therefore continued in the silicon channel. With a deeper channel with wider openings the effect would probably be similar to the silicon channel because the reflectance of the sound in the glass is strong.

In the polymer chip the droplet generation has been well studied by groups such as [12]. However due to the lack of reflectance of sound in the polymer walls the acoustic focusing effect is small and was not studied further in this project.

10.3 Acoustic effect on droplet generation and flow

The sound waves was seen to affect the interfacial tension between two fluids by vibration of the surface. The droplet generation is definitely affected by the sound. The droplet size decreases and a small Releigh-Platau length seems to be needed for droplet break-of. The effect of droplet generation under acoustic influence was only visible in the oil-water flow, due to the stability of droplet generation in this scenario.

The voltage applied to the piezo during the experiment was altered from low voltage up to 22V Peak to Peak. At this voltage the piezo becomes hot and the wave form from the frequency generator less sinusodal. The energy that is actually transmitted to the channel depends not only on the applied voltage but also the effectivity of the piezo (the performance of the piezo varies with shape matching layers and cutting and have been studied in [34] and how well attached the piezo is to the surface of the chip.

Above 16 Volts the droplets experienced intense vibrations that caused them to split up into smaller and droplets in a very unstable generation point.

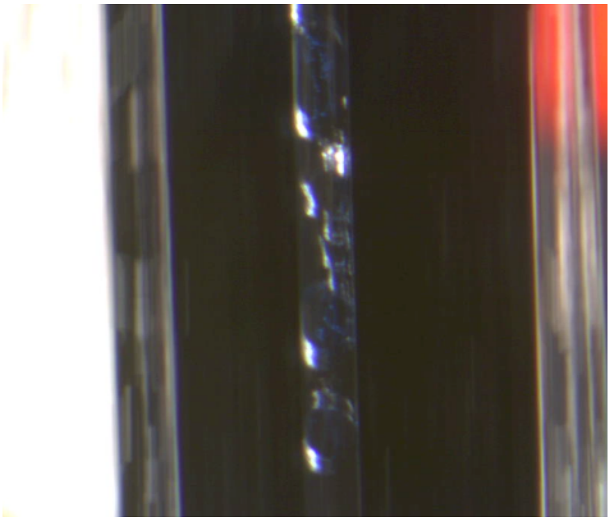


Figure 10.2: Splitting into smaller droplets caused by a high voltage applied to the piezo actuator

In the particle focus experience a stable well suited generation and flow of the droplet were found around 10-16V, under which condition the droplets generated could be consistent in size throughout the channel.

10.4 Acoustic focusing in droplets

Stable droplet generation was successful for all the different types glass, polymer and silicon chip with the two-phase system of oil-water. The best acoustic focusing results was achieved with the silicon chips, due to reasons explained in this chapter. This will be the focus of chapter 11.

The $10\mu m$ particles were used due to their ability to focus better than smaller $3\mu m$ particles as seen in figure 10.1. A high speed flow of 100/100 (oil/water) $\mu l/min$ was used to make the particles enter the channel fast enough to avoid sedimentation to the bottom of tubings.

The experiments were conducted with a continuation of high throughput of droplets of different sizes. Different sizes was achieved by different ratios of oil and water and was also obtained at high water/oil ratios.

A voltage of 10-16V was used due to circumstances described in the previous section.

11 Summary of results

11.1 Results for droplet generation

11.1.1 Surface treatment and surfactants for droplet generation

The surface treatment and surfactants were seen to have a crucial impact on the dynamics of the droplet generation. The usage of Aquapel did not result in any successful increase of hydrophobicity of the glass channels. A air bubble was trapped in the glass syringe that was used to store the Aquapel, which could have reacted with the fluid.

Sufficient surface treatment was achieved with the repel-silane presented in section 9.2 which also showed less trouble to be stored in a syringe as well. The increase of hydrophobicity in both the flow focusing glass chip and the silicon chip was sufficient to enable droplet generation of water in oil. Wetting issues when trying to generate water droplets in air were more severe and were never solved in any of the chips used.

11.1.2 Droplet generation with and without wetting problems

Stable droplet generation was successful in the glass chips shown in figure 7.8 and in the silicon chip form figure 7.7. Certain steps were shown to be critical for the droplet generation to work in a proper way.

- Surface treatment with repel-silane (see section 9.2)
- Add surfactant (krytox) to the water phase
- Flush the chip thoroughly with oil
- Adjust the flow of the two phases to generate droplets

Wetting issues critically changed the dynamics of the generation point. If the channels had been wetted with water before the repel-silane treatment it never recovered from these problems. One example of wetting problems experienced in a glass chip is shown in figure 11.1.

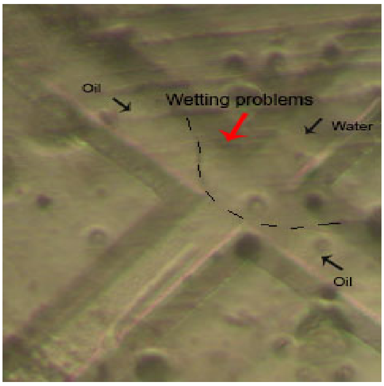


Figure 11.1: In the figure the interface between the oil and the water is the black dotted line. On the channel walls the water shows hydrophilic behaviour by wetting in critical flow focusing point

The t-channel chip were never recovered from wetting issues and no successful droplet generation can be presented for those structures.

A video of the best (successful) droplet generation without repel-silane and krytox where the channel clearly has wetting problems can be seen in the link below.

https://vimeo.com/72201150

The result of droplet generation after proper surface treatment and with surfactants was too hard to visualise in images due to the frame rate of the camera. A successful experiment with the repel-silane and krytox in a glass chip can be seen in the link below.

https://vimeo.com/72189025

In the silicon chip stable uniform droplets were generated with ratios with flow speeds from 10/10 to 100/100 $\mu l/min$ water/oil, while droplets with a variation in size were generated with lower flow speeds around 5/5 $\mu l/min$ water/oil. The dynamics in the flow focusing caused the droplet generation to be unstable, but the varied size distribution was desired for some of the acoustic experiments. The droplet generation also became unstable when the ratio of water was higher than the oil. Also for lower flow rates under 0.5 $\mu l/min$ the syringe pumps became unstable.

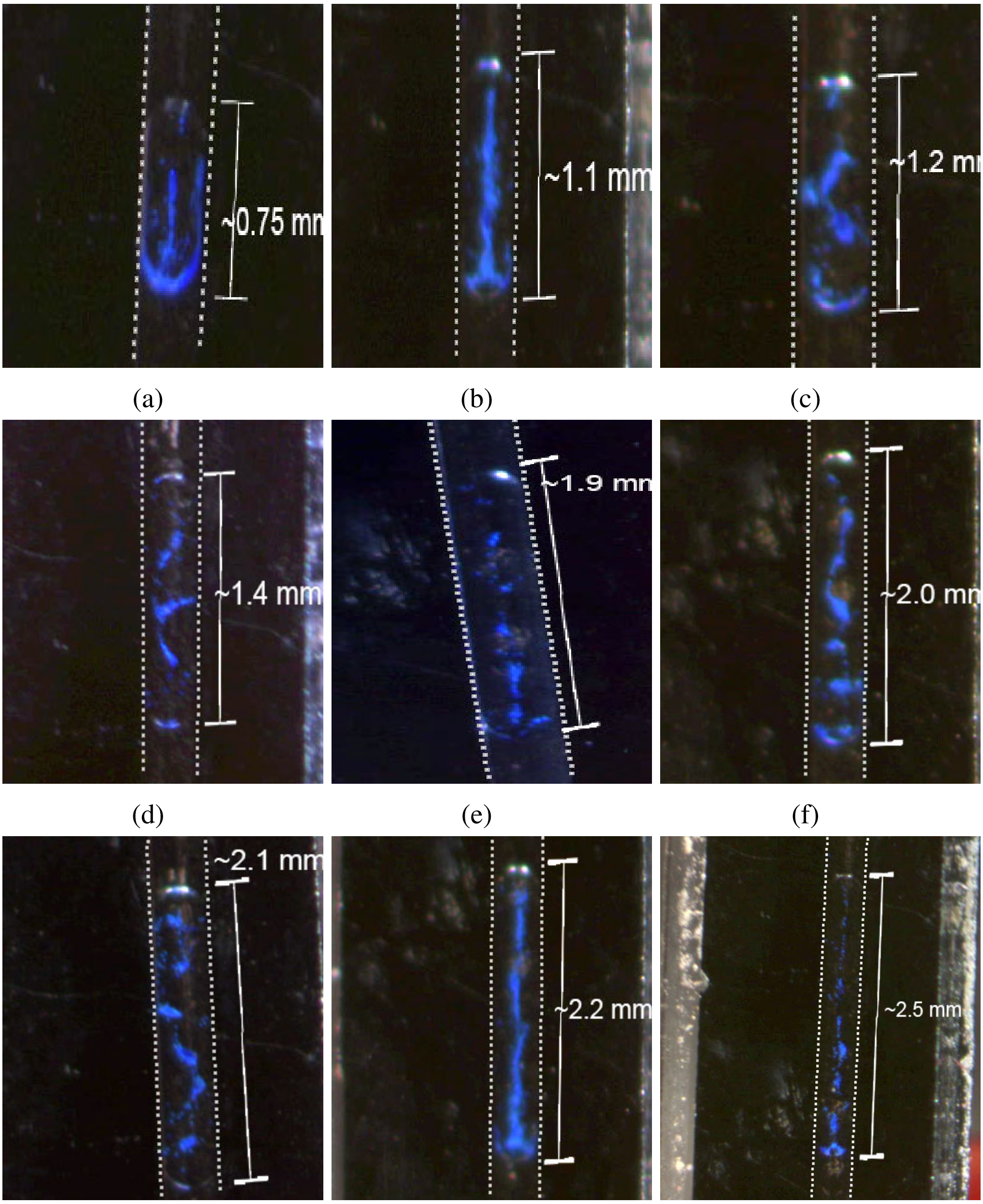
The size found not to correlate with the flow speed or ratios used. Sometime instead of an increase of size the frequency of generation increased. No reproducible relationship between flow speeds and droplet size or frequency of the droplet generation was found in the chips used.

Seeing the frames of droplets using flow speeds above 10/10 $\mu l/min$ in the silicon chip was hard with the equipment used. To clearly see the acoustics effects in droplets very low flow speeds (5/5 water/oil) was used.

11.2 Results for acoustic focusing in droplets

11.2.1 In stopped flow

When the flow was stopped and enough beads were caught inside a droplet the focusing effect was clearly visible for 10 μm particles. In figure 11.2 pressure nodes across the length of the droplets can also be seen.



(a) (b) (c)

(d) (e) (f)

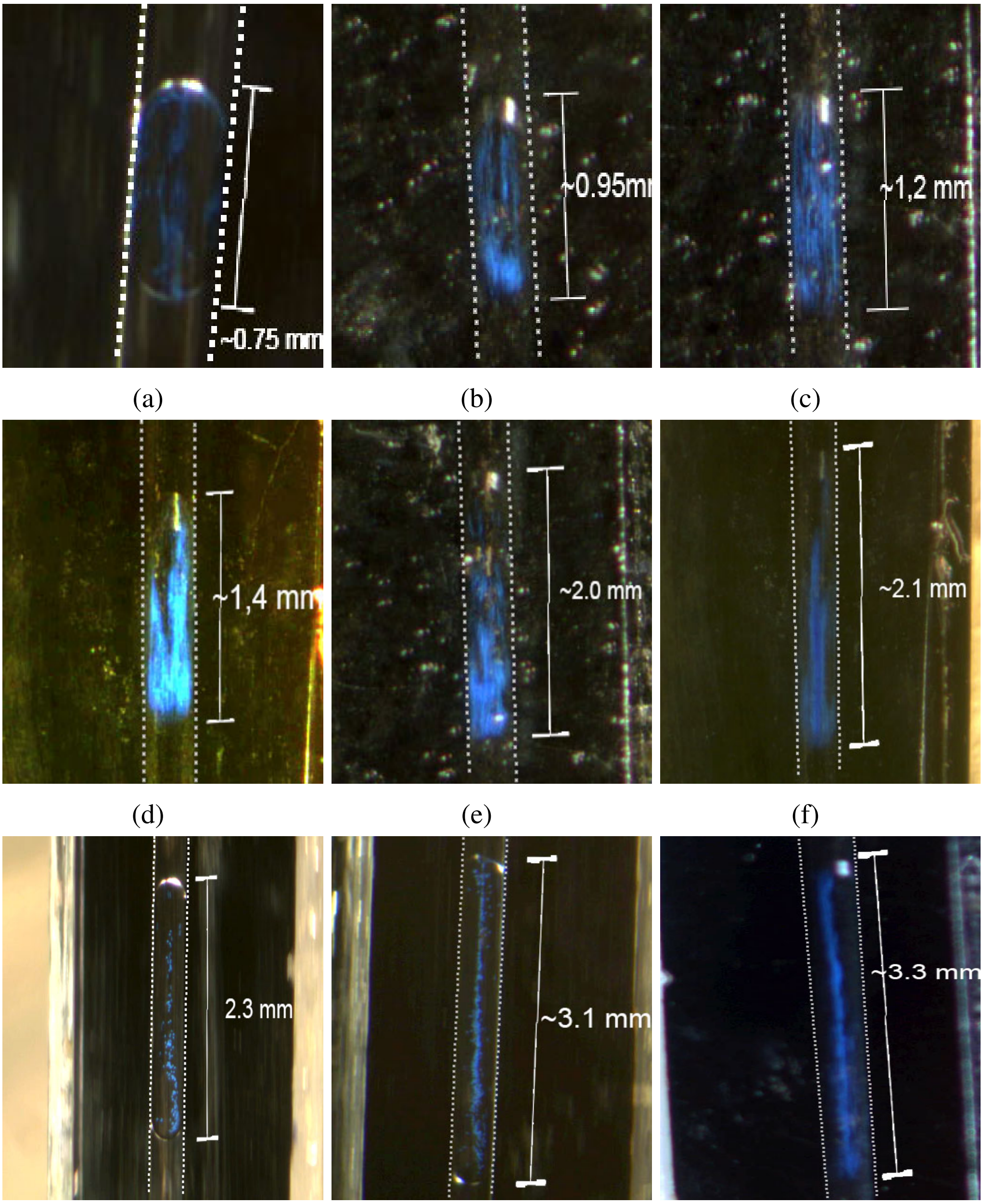
(g) (h) (i)

Figure 11.2

The amplitude of the frequency applied was 10V and the frequency 2.08 MHZ. Each pictures shows a characteristic behaviour of the focusing effect in the size of droplet it is presenting.

11.2.2 In slow flow speed

In a low speed flow bigger droplets showed more tendency to be able to keep particles focused. Smaller droplets did not show the focusing effect as seen in figure 11.2



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 11.3

The amplitude of the frequency applied was 10V and the frequency 2.08 MHZ with a 5/5 water/oil flow. Each picture shows a characteristic behaviour for the size of droplet it is presenting. The behaviour was seen multiple times but enough beads were needed for the effect to seen.

11.2.3 In high flow speed

Since no frames could be taken to illuminate these experiments in a good way I will put links to the experiments below.

For flow speeds of 30/30 $\mu l/min$ water/oil with acoustic field given from 16V applied to the piezo. In the video stable droplet generation but no clear focusing effect can be seen.

http://vimeo.com/72344317

For flow speeds of 100/200 $\mu l/min$ water/oil without acoustic field. In the video a hint what seems to be stable droplet generation without focusing effect can be seen.

https://vimeo.com/72336317

For flow speeds of 100/200 $\mu l/min$ water/oil with acoustic field given from 12V applied to the piezo. In the video a hint what seems to be stable droplet generation but no clear difference in focusing effect from without acoustic field.

https://vimeo.com/72336318

11.3 Results for splitting droplets

11.4 Droplet splitting

A channel splitting was located at the exit of the channel (see chapter 7). The idea is that after the focusing, the channel splitting will result in a split droplet with particles manipulated to the centre outlet of the channel. No control of the outlet flows was used in these experiments, and manipulation of the droplet splitting needs further work to be fully examined. In the glass chips the flow had the tendency to choose one outlet and stick to it. It could have been that some of the outlets had been blocked or obstructed for different reasons.

An example of how the droplets looked in the splitting on the silicon chip is seen in figure 11.4. The figure shown is with low speed flow (10 $\mu l/min$ total). How the effect works in higher speed flows was unable to be seen with the camera used, and will be needing a high speed camera to be seen in greater detail.

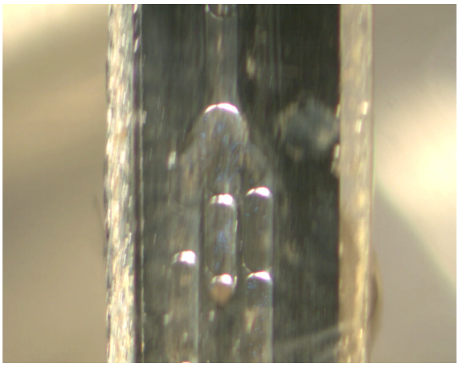


Figure 11.4: Splitting droplets in the exit of the silicon channel

The splitting mechanism was never developed, however, the droplet were seen to have a strong surface tension that did not let them split easily. If this can be manipulated through the concentration of krytox or another surfactant remains to be investigated. A video of droplets splitting can be seen in the video link below:
https://vimeo.com/73596986

12 Discussion and conclusions

12.1 Wetting and droplet stability

The first and main issue has been wetting on the channel walls. The Aquapel which was a commercial water repellant (used for e.g. car windows) that was first tried have been tested and used previously in PDMS chips [12]. Since the desired acoustic effect required glass or silicon channels, the benefit of using this cheap and easy accessible windshield treatment was lost. No information about the chemical function was given, and it remains unclear why it didn't work as well as the latter repel-silane that was used.

The surfactant added did create stability for the droplets but did not have any crucial effect on the droplets generation. The generation was tested without the krytox added and worked just as well. This tells me that the addition of krytox is mainly needed to keep droplets from merging with each other, but have no crucial effect on the droplet generation.

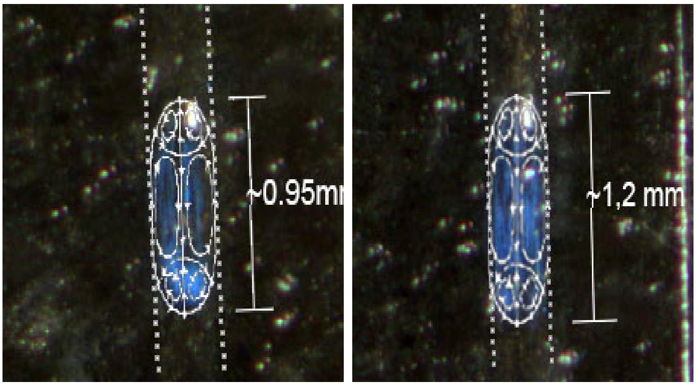
12.2 Particle entering

The next problem was to get enough beads into the droplets to be able to visualise the acoustic effect. Some different particles were tested. The smaller red particles from figure 10.1 took longer to sediment than 10 μm beads, but did not work as well with the acoustic effect. A type of fluorescent beads were tested as well but they sedimented even faster which made me abandon these attempts. To get enough particles into the chip I shook the syringe frequently. There are also other, potentially better suited, techniques, for example by a rotating magnet inside the syringe as a co-worker at the department just made a device for.

In the glass channel the problem with beads entering was never solved, and the reason for this I believe is due to the depth of the channel. As this channel was only approximately $50\mu m$ in depth and the particles of size 10 μm would already take up 1/5 of the channel. Taking this into account together with the isotropic etch profile (seen in figure 8.3) it is understandable that the particles had difficulties to enter the channel. In a more shallow channel the particles would also sediment a lot faster. The conclusions is that for the glass channel to work a deeper channel would be needed.

12.3 Droplet size and acoustic focusing

The images in figure 11.3 and 11.2, shows the main results of this study. For low flow speeds a relationship between the size of the droplet and the focusing effect can be seen. The surface relationship to the droplet volume of the droplet seems to play an important role for how strong the rotating flow fields inside the droplets are. The PRF is approximately the same for any droplet size, but the surface to volume ratio seems to change the effect of the flow fields. The smaller the droplet is, the stronger the flow fields will be. In figure 12.1 The flow fields as they are dominating the PRF are shown.



(a) (b)

Figure 12.1: Flow fields inside droplets are shown to give the idea of how particles moved inside.

In some of the pictures particles seems to stick to the ends of the droplet, and be caught in rotations in these places. This was seen in more experiments and it seems to agree with the flow field picture in section 3.1 where smaller flow fields are located at the ends of the droplets. When the droplets are still in the stop flow pictures the acoustic effect dominates these flow fields but it can still be seen that particles stick to the surface of the droplet. It was seen that even a very small movement starts the rotation of particles inside the droplet. For droplets longer 2.5 mm the PRF seems to be dominating the rotations under these conditions.

In higher flow speed shown in videos presented no visible focus could be seen as even though the droplets could not be seen separately in these experiments. If a strong enough focusing effect would be present a more clear blue line would be visible. It remains unclear if this was due to that the size of the droplets were too small in these experiments or if the flow field effect increases with higher speeds. Better camera equipment would be needed to highlight this issue better.

In droplets that are stopped inside the channel a focusing effect is clearly visible. The pictures show a tendency to focus the particles in smaller dots across to droplet length as well. These foci could either be reflections against the droplets edges, but can also be effects from other reflection inside the channel. If the focusing is from the reflection against the droplet wall the number of nodes that is expected to be seen is listed in table 12.1

Droplet length (mm)	Droplet length / $(\lambda/2)$	Pressure nodes inside droplet (excluding surface nodes)
0.75	2	1
1.1	2.9	~ 2
1.2	3.2	~ 2
1.4	3.7	~ 3
1.9	5.1	~ 4
2.0	5.3	~ 4
2.1	5.6	~ 5
2.2	5.9	~ 5
2.5	6.7	~ 6

Table 12.1: In the experiments a frequency of 2.08 MHz was used. The ratio of the length between the droplet and the wavelength of the sound gives an idea of what harmonics that could focus the particles across the length of the droplet

If the oil has lower acoustic impedance than the water the sound will have a pressure node at the ends of the droplets and depending on the harmonics a number of pressure nodes in the middle. The reflection is low between the water and oil (0.17 from table 10.2) which gives an idea that this effect will be quite weak. It was in the experiments seen and can also been seen in the pictures that the effect was low. A very small movement would get the particles out of their focus. As seen in figure 12.2 the focusing can be seen but is not perfectly clear. In the other pictures in figure 11.2 it is even more unclear how many and where the points of foci should be.

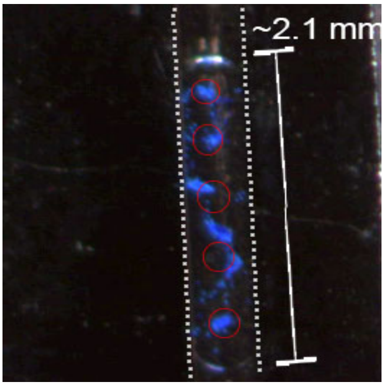


Figure 12.2: Focusing points seen in red with a number that match the theory of how many nodes that there should be (see table 12.1)

When I reflect further it still remains unclear if this effect was due to the internal reflections in the channel or inside the droplet. The length between pressure nodes across the length of the complete channel land across the length of the droplet would be the same, and it is to hard to say an exact distance from the edge of the droplet to the first focus point. The strongest conclusion that can be drawn is that flow fields in stopped droplets disappear in stopped flow and that the PRF clearly dominates the particles.

If another continues phase with an acoustic impedance more different from water is chosen then this effect would probably be seen stronger and could dominate over the reflections inside the channel. An idea to test how strong this effect is could also be by using a polymer channel where the reflections from the channel are significantly lower.

12.4 Future goals and ideas

After reviewing the results of this project there are still several several areas that could benefit from future work. I think the flow fields will continue to be an issue in smaller droplets. Experiments in higher flow rates with larger droplets with a high speed camera or a stroboscope would definitely be interesting to look at.

For experiments in lower and higher flow rates it would also be interesting to experiment with the splitting process. If by controlling the outlets the splitting can be controlled and then one might be able to separate the particles in the middle section of the droplet. As krytox affects the stability of the droplets it would also be interesting to experiment with different surfactants and different concentrations. Experiments without krytox might be beneficial in the splitting process.

A suggestion that simplifies the entering of particles is of course to make a channel deep enough to let the particles enter freely. The sedimentation of particles should definitely be taken into account when designing the channel.

Different oils might give different acoustic properties which could enhance the performance of the focusing. Since the particles experienced most trouble at the surface of the droplet an oil with higher acoustic impedance than the water would be interesting to try as a continuous phase. A pressure anti-node at the surface would be beneficial when trying to focus them in the centre of the droplet.

13 Popular scientific summary

Microfluidics is a field that, as the name states, deals with fluids on the scale of micrometers. Lund university is performing cutting-edge research in the field and companies using the research are starting to emerge. A past example that the research has led to is the inkjet printer that deals with small volumes of ink that are disposed on a paper.

In this thesis I have experimented with two everyday types of fluids, water and oil, for the purpose of medical technology. The two fluids do not mix, which we can see in a salad dressing at the dinner table. The oil ends up on top of the water, in a layer or in smaller drops. Exciting videos showing and experimenting with this can easily be found on youtube for the one who likes the visual effects. The fact that they do not mix is in this project used to make controllable drops of water inside an oil, the size about half a millimetre (these small drops are also called droplets).

A setup to produce the droplets is a so-called micro channel that is constructed using similar techniques as integrated circuits. An example of a structure producing droplets can be seen below. The oil enters from the sides and pinches of small droplets off water.

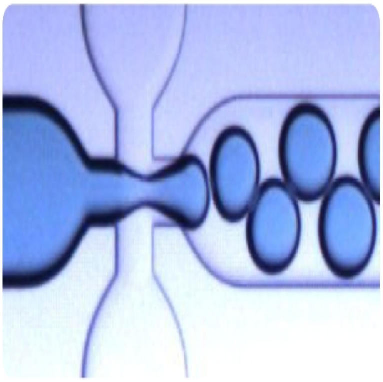


Figure 13.1: A construction used to generate small droplets of water. The oil coming from the sides pinches off the droplets. (Image taken from: http://www.dolomite-microfluidics.com/)

Just like we humans can feel when we feel like we are living inside a bubble, a drop of water can be its own protected environment. Making controlled experiments with cells inside droplets can be beneficial in medical research and diagnostics since these are not disturbed from outside influences. In this project I used test particles that substitutes the cells but were of the same size. These particles were trapped inside the water droplets.

Finally ultrasound is used to manipulate these particles, since small particles have been proven to be affected by sound. A frequency generator that supplies a voltage variation is attached to a piezomaterial. This piezomaterial vibrates mechanically when a voltage variation is applied. With a quickly switching voltage it gives high frequency vibrations. The pressure variations from these is the ultrasound. The frequency of this sound is set so that half a wavelength matches the channel width. This gives a standing wave phenomenon over the width of the channel that will continuously affect the particles.

The idea is to focus the particles tightly into the centre of a droplet and after doing so, to cut the droplet into smaller drops keeping the particles in only one of them. By cutting it into pieces the middle one of the smaller drops will increase its concentration of particles while the concentration in the others will decrease.

The final goal was never reached since there were other strong forces working on the particles inside a drop and a splitting of a droplet the size of a millimetre is not that easy. The results of the thesis show that in a droplet the size of a few millimetres it is possible to focus the particles but that the cutting of the droplet needs to be edged!

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Appendices

A Glass fabrication process

Droplet glass/glass chip fabrication - Maria Tenje March 2013

1. **Mask fabrication**
   4" Borofloat glass wafer with Cr and resist on is used. Wafer thickness: 1.1 mm. Mask writer DWL66 is used to define the drawn CAD mask on the glass wafer with laser writing.

2. **Mask development**
   Develop the mask pattern in the resist using AZ326 for 70 s. Rinse in water 3 min. Dry with nitrogen. Develop the mask pattern in the Cr layer using Cr etch. Rinse in water 3 min. Dry with nitrogen.

3. **Wafer stock out**
   Take a 4" Borofloat glass wafer with Cr and resist on. Wafer thickness: 0.7 mm.

4. **Exposure**
   Place the mask wafer with the Cr side downwards in close contact with the resist-coated side of the glass wafer. Expose using the MA4 mask aligner. Expose for 8 s.

5. **Pattern development**
   Develop the mask pattern in the resist using AZ326 for 70 s. Rinse in water 3 min. Dry with nitrogen. Develop the mask pattern in the Cr layer using Cr etch. Rinse in water 3 min. Dry with nitrogen.

6. **Glass etch**
   Coat the backside of the glass wafer with blue tape for protection. Etch the chip structure in 100:28:72 of $HF:HNO_3:H_2O$ in a plastic beaker placed on a rocking table. Etch rate ~1.3 µm/min. Rinse the wafer thoroughly in water afterwards.

7. **I/O holes drilling**
   Drill holes for inlets and outlets using a 300 µm diameter drill.

8. **Resist and Cr strip**
   Place wafer in Acetone 1 min to remove all resist. Rinse in water 3 min. Dry with nitrogen. Place wafer in Cr etch 1 min to remove all Cr. Rinse in water 3 min. Dry with nitrogen.

9. **Wafer for bonding**

B Instrumentation

Instrumentation:
Pumps: neMESYS Pumps
Syringes: Bd plastic syringes 1 ml (sometimes with larger volumes)
Frequency generator Hewlett Packard 33120A Arbitrary waveform generator
Oscilloscope: Tektronix TDS 1002
Microscope: Nikon SMZ-2T
Camera: eo Edmund USB camera
Piezo: Ferroperm Piezoceramics 2, 4, 6 MHz Actuators
Amplifier: Costume made 10x amplifiers by the institution
Lamps: Directable tabletop lamps

Computer software:
neMESYS UserInterface Software

Fluids:
HFE 7500 (see msds [33])
MiliQ Water

Miscellaneous:
Beads: Sigma Aldrich: Micro particles based on polystyrene, (blue 10 μm and red 3 μm)
Tubings: TFE teflon Tubing 1.58 OD x 0.3mm ID