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Experimental study of the inertial focusing phenomenon for micron-scale particle separation purposes

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Abstract

Since 2007, inertial microfluidics is continuously gaining popularity in several fields of particle manipulation. In biomedical research the most significant field is the focusing and separation of micron-size particles. Compared to conventional microfluidics, which relies on Stokes flow, inertial devices are working in an intermediate regime between Stokes and turbulent flow, where the fluid inertia is not neglectable, leading to the lateral migration of particles to stable equilibrium positions.

Since in biomedical research analysing body fluids is common task, inertial microfluidics became an important field of research. It has the advantage of being passive, which means in this case that no external force field is needed. The devices have simple geometry and high throughput, they can potentially outperform traditional flow cytometry. The procedure can be label-free, meaning that no biochemical labelling is required, the movement of particles depend only on physical properties like size or deformability. Due to their compact size, these chips can be much more available and easy-to-use in the point of care.

The progression in the field of micro-machining techniques like direct laser writing, hot embossing and soft lithography, makes the prototyping and mass manufacturing of these devices more and more available. During these years research groups developed several channel designs for particle separation purposes, beginning with straight channels, followed by curved and serpentine channels, as well as contraction extraction arrays, but the working principle of all these devices is originating from the inertial focusing effect, which was popularised back in 2007.

For this report my aim is to investigate the foundation of inertial microfluidics by designing, fabricating and testing several Inertial Focuser devices with different flow control methods, a wide range of particle sizes, different channel geometries and initial particle positioning achieved by hydrodynamic focusing. I am also going to thoroughly present

the history and physical background of the inertial focusing phenomenon as well as the designing steps and testing methods I followed during my work. Overall, the results are suggesting a promising and not yet amply described method for particle separation with sheath flow aided inertial focusing.

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Chapter 1

Introduction

1.1. Biomedical relevance

The analysis of cells and other biomolecules is an important task in biomedical research. Since in most cases these samples are in the form of a fluid like blood or urine, the manipulation of these particles inside the fluid is essential. In the last 15 years inertial microfluidics, which is capable of processing these complex biological samples, became a relevant researching field. The biggest advantage of inertial microfluidics compared to other techniques is that it has the ability to perform label-free, size-based separation of particles with high throughput, while having minimal effect on the targets viability and integrity. For biomedical purposes the most common targets in inertial microfluidics are circulating tumor cells (CTCs), white blood cells (WBCs), reproduction related cells, blood plasma, extracellular vesicles and pathogens.

In clinical practice complete blood cell counts is mostly carried out by traditional flow cytometry. Although it is a popular technique, there are several efforts aiming to further increase throughput and bringing the testing at the point of care by miniaturizing the devices, increasing the cost efficiency and decreasing the need of trained personnel by simplifying the usage. In 2010 Di Carlo et al. presented a microfluidic cell positioning device for extreme throughput flow cytometry purposes [1]. They achieved throughput of 1 million cells per second, which is two order of magnitude higher compared to traditional flow cytometers. Since traditional flow cytometry heavily relies on a serial focusing method the advantage of inertial focusing is based on the ease of parallelization.

Target cell enrichment is also a common task of traditional flow cytometry. During such procedure the detection of the target cells is based on immunolabeling, but this often

requires special biomarkers and the labeling process can be time consuming, expensive or potentially cell-damaging. Overall, there is no guarantee that for a given target acceptable biomarkers are available. To overcome these challenges Hur et al. designed a device to enrich adrenal progenitor cells [2]. Traditionally, progenitor cells are distinguished from fully differentiated cells based on intracellular cholesterol levels, which is measure by fluorescent immunolabeling. However with the help of inertial focusing the cells were separable simply based on size .

In case of an infection it is crucial to be diagnosed at an early stage. However, this is a challenging task, due to the low concentration of parasites in the blood. The polymerase chain reaction (PCR) can help to overcome this difficulty, but this requires a pure sample of the target DNA, which is rarely the case. The popular method is to manually analyse the blood sample by microscope, but usually this is only accurate, if the concentration reaches 50-100 parasite/ μl . In 2015 Warkiani et al. introduced an inertial microfluidic channel which was able produce pure enough sample to use PCR [3]. Overall, they were able to detect malaria infection from blood at 2 parasite/ μl concentration.

1.2. The inertial focusing phenomenon

Inertial microfluidics works in the middle regime of laminar flow, between stokes flow and turbulent flow. Under these circumstances there are two main forces, which are responsible for developing and maintaining the equilibrium positions of particles [4]. The wall-induced lift force, which is driving the particles away from the walls in the direction of the channel centre and the shear gradient induced lift force, which is caused by the curvature of the velocity profile, driving the particles from the centre in the direction of the walls. The particles will migrate, until they reach an equilibrium position, where these forces are balancing each other out.

1.3. The structure of this report

The aim of my study is to gain a comprehensive understanding of the inertial focusing effect. Although currently the focus in inertial microfluidic research is shifted towards more complex geometries, the effect of inertial focusing is relevant in these cases as well, and yet no accurate models exist to describe the particles motion even in the simplest channel geometries, suggesting that our understanding is not complete.

2. Chapter Fundamentals of inertial focusing. This chapter is a summary about the most important discoveries related to the inertial focusing phenomenon from 1961 until recent years. The physical background of particle motion in inertial fluid flow is introduced here in detail as well.

3. Chapter Experimental approach. This chapter is about the introduction of the measurement setup, the experimental data collected during measurements and the evaluation techniques used to analyse this data.

4. Chapter Results. In this chapter the results of each measurement are presented. Through an iterative process of designing, manufacturing, and testing several devices I was able to achieve promising results with a sheath flow aided Inertial Focuser.

5. Chapter Conclusion. These are my overall thoughts about this project based on my experience including my opinion about in which directions the research could be continued in the future.

Chapter 2

Fundamentals of inertial focusing

2.1. Dynamics of inertial migration

2.1.1 Segre-Silberberg annulus

The inertial focusing effect was introduced before the science of inertial microfluidics even existed. In 1961 Segré and Silberberg presented a report about the motion of millimeter sized particles flowing through a circular tube which had a diameter of 1 cm [5]. They observed that at low channel Reynolds number 2.2, if the length of the tube was sufficient enough, then the particles were arranged in a circular manner near the channel walls by the outlet (figure 2.1). They also determined that the radius of this circle was 0.6 cm. This is called the Segre-Silberberg annulus or the tubular pinched effect.

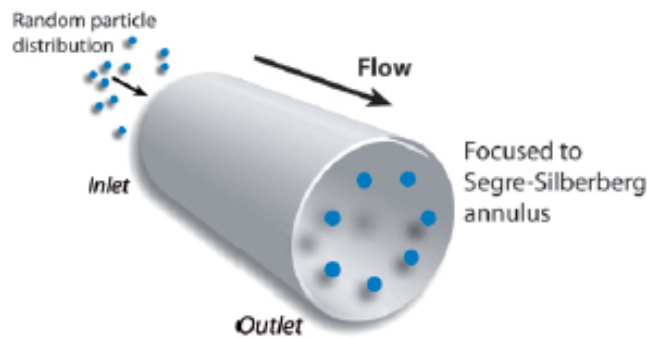


Figure 2.1: Schematic representation of the Segre-Silberberg annulus discovered in 1961. The initially randomly distributed particles after sufficient time are arranged in a circle with radius of 0.6 times the radius of the tube. Source of the image: [6]

At that time the theory of fluid dynamics could not explain the migration of particles and until the early 2000s it was not experimentally proved that this would work in the micron scale as well. However, it has an importance because it led to further scientific analysis of the inertial flow, and generally, it is still a good practice in the field of microfluidics to be inspired by higher scale technologies.

This phenomenon led to the acknowledgement of the need of the Navier-Stokes equation 2.1 to describe fluid dynamics,

$$\rho\left(\frac{d\mathbf{u}}{dt} + \mathbf{u} \cdot \nabla \mathbf{u}\right) = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{f} \quad (2.1)$$

where ρ is the density of the fluid, $\mathbf{u}$ is the fluid velocity field, p is the pressure field, μ is the viscosity of the fluid and $\mathbf{f}$ is a vector field of external forces [7]. It is necessary because simply the Stokes equation implies a symmetric force acting on particles, which could never cause them to migrate across streamlines, it has to be the doing of inertial effects. To describe the ratio of the inertial components to viscous ones the channel Reynolds number (Re_c) is defined as:

$$Re_c = \rho \cdot \frac{U_m \cdot 2 \cdot H \cdot W}{\mu \cdot (H + W)} \quad (2.2)$$

where H and W are the channel height and width respectively U_m is the maximum flow velocity, ρ is the density, μ is the viscosity of the fluid [7]. Low channel Reynolds number ($Re_c \ll 1$) is characteristic for Stokes flow meaning that the flow is dominated by viscous effects and lateral migration does not occur. Inertial microfluidics works in the region where the channel Reynolds number is between 0.5 and 2000.

2.1.2 Magnus lift force

Before the 1960s the theory of inertial dynamics was lacking about the individual forces. The Magnus lift force (equation 2.3, sometimes refereed as rotation induced lift force) was described and explained by Rubinow and Keller also in 1961 [8].

$$F_{ML} = \pi \cdot a^3 \cdot \rho \cdot \Omega \times U_{rel} \quad (2.3)$$

$$U_{rel} = U_{fluid} - U_{particle} \quad (2.4)$$

where ρ is the density of the fluid, U_{rel} is the particle velocity relative to the fluid velocity 2.4, Ω is the rotational angular momentum and a is the diameter of the particle. This force is present because the direction of rotational angular momentum vector continuously changes, thus it will not be the same than the direction of flow velocity vector. Due to

the constant rotation, the flow velocity will be different each side of a rigid spherical particle, resulting a pressure difference as well, causing it to migrate laterally (figure 2.2) [9]. Although this is a lateral force, under the circumstances of the tubular pinched effect it is directed towards the centre of the channel, so it could not explain the phenomenon.

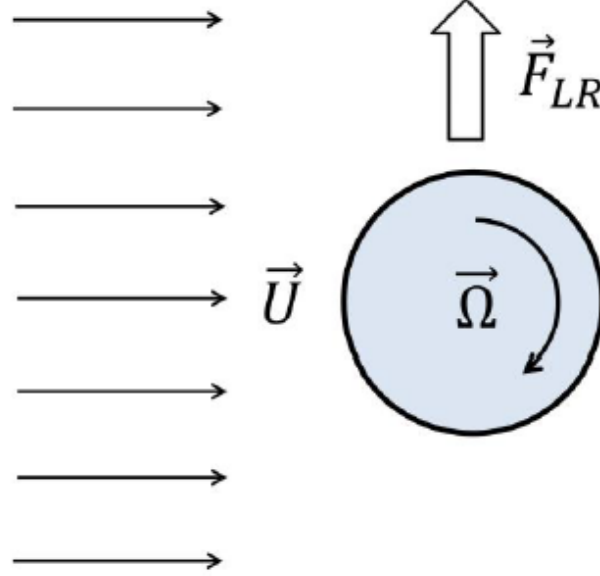


Figure 2.2: The Magnus lift force acting on a spherical particle perpendicular to the streamlines in uniform flow. Source of the image: [9]

2.1.3 Saffman lift force

In 1965 Saffman took a different approach and introduced the theory of a force (equation 2.5), which is independent from angular momentum, considers inertial effects and the presence of the walls [10]. This force was based on the fact that in shear flow the fluid velocity is different each side of the particle. The shear effect causes the particles to spin, and this is further increased by the drag effect of the wall, making the particle lagging the flow [9]. The lateral force resulted by this is called the Saffman lift force (or slip-shear induced lift force). It is always acting in the direction of the maximal relative velocity between the particle and the fluid (figure 2.3). The formula (equation 2.5) was derived by Saffman using the matched asymptotic expansion method, but he could not explained the Segre-Silberberg annulus.

$$F_{SL} = 81.2 \cdot U_{rel} \cdot r^2 \cdot \sqrt{\frac{\gamma}{\mu}} \quad (2.5)$$

where U_{rel} is the particle velocity relative to the fluid, r is the particle radius, γ is the velocity gradient, μ is the viscosity of the fluid. Although this equation neglects the

rotation of the particle, it does not mean it is truly independent from it. Usually the Magnus and Saffman lift forces are acting simultaneously, because the slip-shear effect is likely to induce rotation, so using simple superposition on them would not be accurate [9].

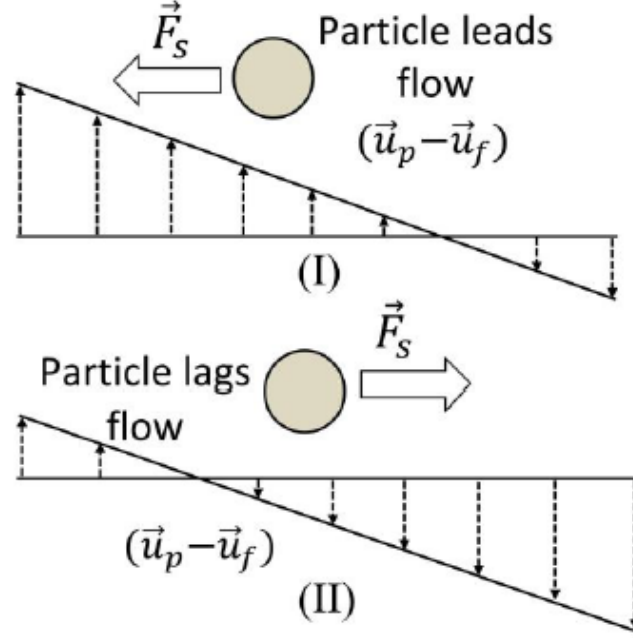


Figure 2.3: The Saffman lift force acting on a spherical particle in shear flow. The direction is based on the relative velocity between the particle and the fluid. Source of the image: [9]

2.1.4 Inertial lift force

In 1974 Ho and Leal and two years later Vasseur and Cox were able to identify the force, which was necessary to analytically confirm the experimental results of Segré and Silberberg [11] [12]. They described this force being proportional to the shear rate, directed to the channel walls. This is later called the inertial lift force, it is three order of magnitude greater than the Magnus lift force (equation 2.3) and one order of magnitude greater than the Saffman lift force (equation 2.5). Overall, this force is responsible for the lateral migration of the particles. That is the reason why in application usually the Saffman and Magnus forces are neglected, as well as gravity and buoyancy, since these forces have no practical effect on the particles lateral movement, compared to the inertial lift force. In 1999 Asmolov described the magnitude of the inertial lift force, and introduced the formula of the particle Reynolds number (equations 2.6-2.8) [7].

$$F_{IL} = \frac{\rho \cdot U_m^2 \cdot a^4}{D_h^2} \cdot f_c(Re_c, x_c) = \frac{\mu^2}{\rho} \cdot R_p^2 \cdot f_c(Re_c, x_c) \quad (2.6)$$

$$D_h = \frac{2 \cdot W \cdot H}{W + H} \quad (2.7)$$

$$R_p = \frac{U_m \cdot a^2}{\frac{\mu}{\rho} \cdot D_h} = \frac{U_{rel} \cdot \rho \cdot a}{\mu} \quad (2.8)$$

where H and W are the channel height and width respectively, U_m is the maximum flow velocity, U_{rel} is the relative velocity (equation 2.4), ρ is the density, μ is the viscosity of the fluid, f_c is a lift coefficient proportional to the channel Reynolds number (Re_c , equation 2.2) and the particle position inside the channel (x_c) and it is zero if the particle is in equilibrium. D_h is the hydraulic diameter and R_p is the particle Reynolds number. It is important to notice that since F_{IL} is directly proportional to a^4 , the bigger the particle is, the faster it migrates to equilibrium and this can be exploited for size based separation.

Based on this theoretical background, the first experiments with micron-sized particles were conducted by Di Carlo et al. in 2007 [13]. In 2009 after further analysis of the inertial lift force it was shown that it is actually composed of two forces, the wall induced lift force and the shear gradient induced lift force [6].

2.1.5 Wall induced lift force

The channel wall effects the nearby particles by slowing them down compared to the fluid and causing pressure to build up between the wall and the particles [14]. This happens because if the wall is on the right side of the particle, the streamlines will evade it from the left. By doing so the flow will accelerate on the left side of the particle causing higher pressure on the wall side. This results a force driving it away from the wall in the direction of the centre as shown in figure 2.4.a. This is called the wall induced lift force (F_{WL}):

$$F_{WL} \propto \frac{\rho \cdot U^2 \cdot a^6}{D_h^4} \quad (2.9)$$

where ρ is the fluid density, U is the fluid velocity, a is particle diameter and D_h is the hydraulic diameter (equation 2.7).

2.1.6 Shear gradient induced lift force

The parabolic velocity profile of the Poiseuille flow also induces an inertial lift on the particles, meaning that the relative velocity magnitude is lower on the centre side of the particle and higher on the wall side [14]. To resolve this difference the shear gradient induced lift force drives the particles in the direction of the wall figure 2.4.b. This is the shear gradient induced lift force (F_{SGL}):

$$F_{SGL} \propto \frac{\rho \cdot U^2 \cdot a^3}{D_h} \quad (2.10)$$

where the parameters are the same as in the equation 2.9. The F_{WL} is dominant close to the channel walls, the F_{SGL} is dominant near the centre. The regions inside the channel, where the lift forces are equal, from the equilibrium positions (figure 2.5).

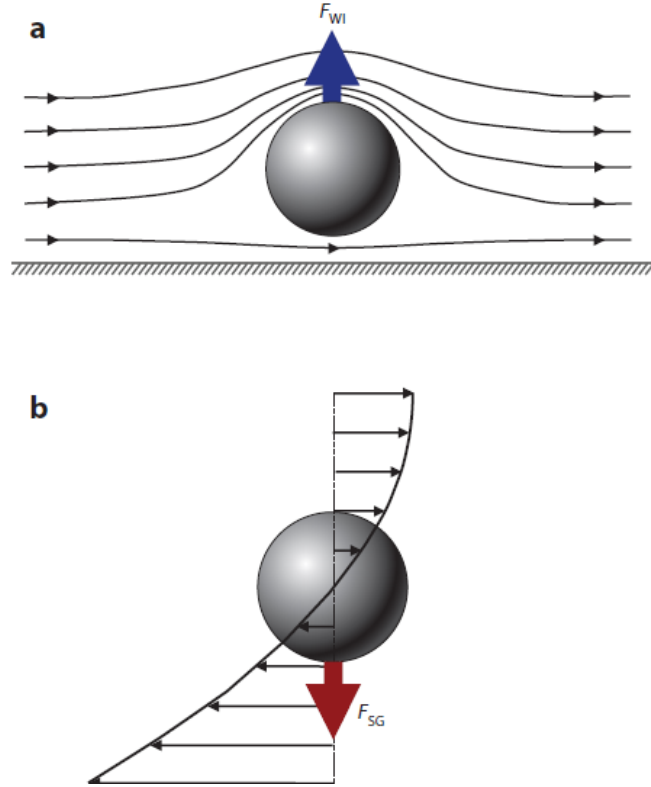


Figure 2.4: Schematic representation of a) streamlines regarding the wall induced lift force, b) relative velocity profile regarding the shear gradient induced lift force. Source of the image: [15]

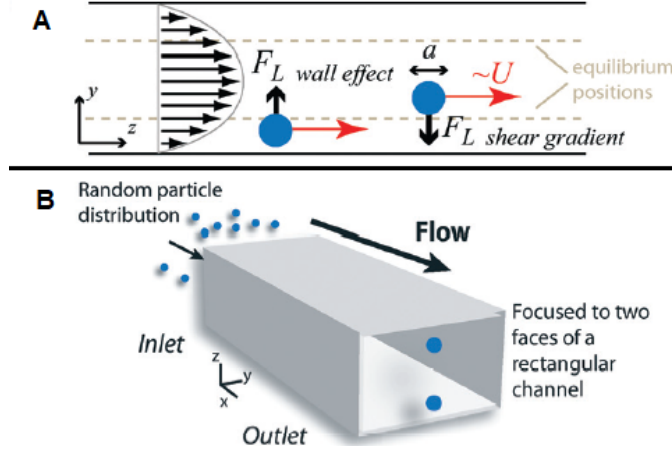


Figure 2.5: (A) Development of particle equilibrium positions in a straight channel, due to the balance between wall-induced and shear gradient induced lift forces. (B) Particle equilibrium positions in a low aspect ratio rectangular channel. Source of the image: [16]

2.1.7 Viscous drag force

The force in the direction of flow exerted by the fluid on a solid particle is called drag. If the particle is a sphere, we can express the drag force as:

$$F_{drag} = \pi \cdot r^2 \cdot f_{drag} \quad (2.11)$$

where r is the radius of the spherical particle and f_{drag} is the drag coefficient. f_{drag} has different expressions based on the particle Reynolds number (Re_p , equation 2.8) of the system. [17] The first region is the Stokes region with Stokes drag called Creeping flow, if

$$10^{-4} < Re_p < 0.2$$

then

$$f_{drag} = 6 \cdot \frac{\mu \cdot U_{rel}}{r} \quad (2.12)$$

and

$$F_{drag} = 6\pi \cdot r \cdot \mu \cdot U_{rel} \quad (2.13)$$

The next case is when

$$0.2 < Re_p < 500 \sim 1000$$

which is the Inertial region and the flow type is Laminar, then

$$f_{drag} = 6 \cdot \frac{\mu \cdot U_{rel}}{r} (1 + 0.15 \cdot Re_p^{0.687}) \quad (2.14)$$

and

$$F_{drag} = 6\pi \cdot r \cdot \mu \cdot U_{rel}(1 + 0.15 \cdot Re_p^{0.687}) \quad (2.15)$$

Finally in the case when

$$0.2 < Re_p < 500 \sim 1000$$

we are in an Intermediate region, close to the lower boundary of Turbulent flow, then

$$f_{drag} = 0.22 \cdot \rho \cdot U_{rel}^2 \quad (2.16)$$

and

$$F_{drag} = 0.22 \cdot \pi \cdot \rho \cdot r^2 \cdot U_{rel}^2 \quad (2.17)$$

Technically there is a fourth term for fully developed Turbulent flow when

$$Re_p > 2 \cdot 10^5$$

but it is too chaotic to be relevant in inertial microfluidics. In summary the drag force is responsible for the motion of particles in the direction of the flow. The second case describes most applications in inertial microfluidics.

2.1.8 Diffusion

Particles inside a liquid frequently collide with the fluid molecules. The random motion of particles resulted by these collisions called Brownian motion [18]. Based on the Einstein-Smoluchowski theory the root mean squared distance diffused by the particle is expressed as:

$$d = \sqrt{6D \cdot t} \quad (2.18)$$

where t is the time interval and D is the diffusion coefficient expressed as:

$$D = \frac{k \cdot T}{6\pi \cdot \mu \cdot r} \quad (2.19)$$

where k is the Boltzmann's constant, approximately $1.3806488 \cdot 10^{-23} \frac{J}{K}$, T is the absolute temperature, r is the particle radius. Inertial microfluidics typically used in room temperature and the viscosity of biological samples can be high, which usually means low diffusion coefficient and diffusion effect. But for mixing type of task it can be important to estimate the relevance of diffusion. For this the Péclet number is defined as:

$$Pe = \frac{L \cdot U}{D} \quad (2.20)$$

where L is the channel length, U is the fluid velocity and D is the diffusion coefficient 2.19 [19]. The Péclet number can be interpreted as an importance ratio between convection and diffusion. In microfluidics diffusion is only neglectable, if the Péclet number is small.

2.2. Equilibrium positions

As I mentioned previously the equilibrium positions are regions inside a channel, where the wall induced lift force (equation 2.9) and the shear gradient induced lift force (equation 2.10) are balancing each other out. At the beginning of this report I also introduced the Segre-Silberberg annulus (figure 2.1), if particles traveling in a circular channel, the equilibrium positions forming a circle in a cross sectional view, and its radius is 0.6 times the channel radius.

Although in macroscopic scale circular tubes dominating other cross section geometries, due to microfabricational challenges square and rectangular channels are much more common in the micron scale. These rectangular channel are described with the aspect ratio $\frac{H}{W}$, where H is the channel height and W is its width. If the aspect ratio is greater than one, it is considered high, otherwise it is considered low. In 2007 Di Carlo et al. experimentally found four equilibrium positions in a square cross-sectional channel [13]. These positions were located symmetrically, near the middle of each channel walls (figure 2.6). These results confirmed previous theoretical estimations of Asmolov [7].

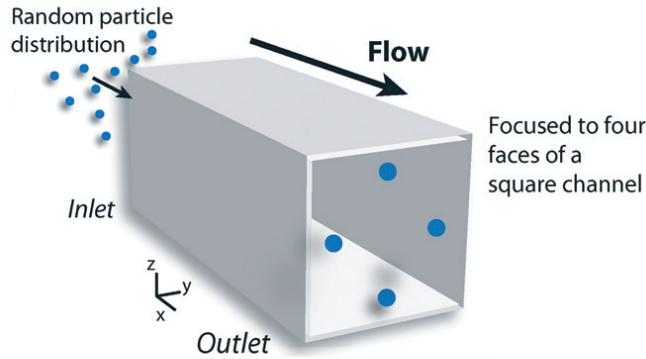


Figure 2.6: Schematic representation of the four equilibrium positions in a square cross-sectional channel Source of the image: [6].

Then in 2009 Di Carlo et al. both numerically and experimentally found that the distance between these positions and the centre is reciprocally proportional with the $\frac{a}{H}$ ratio, where a is the particle diameter and H is the channel cross-sectional dimension in the direction of particle migration (figure 2.7) [20]. If the $\frac{a}{H}$ was around 0.2, they get the same results as Segre-Silberberg in 1961. They also investigated the case of a rectangular

channel [20]. Based on numerical simulations with low and high aspect ratio, they found that the effect of the shear gradient in the direction of the shorter walls and the counter effect of these walls are close to zero near the centre of the channel, which is probably the reason why the number of equilibrium positions in such channel is reduced to two, near the middle of the wider sidewalls (figure 2.5.B). This phenomenon was experimentally observed before, in 2008 [21].

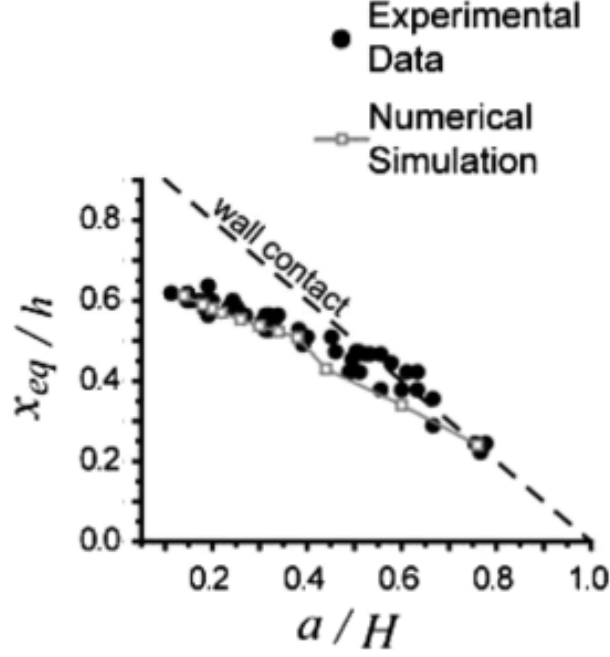


Figure 2.7: Strong reciprocal relation between the position of the equilibrium and the a/H ratio of the system found by Di Carlo et al, where a is the particle diameter and H is the cross-sectional dimension of the channel in the direction of the particle migration. Source of the image: [20]

2.3. Hydrodynamic focusing

Hydrodynamic focusing in microfluidics means using sheath flow for particle manipulation. Although it is not an inertial technique, it can be adapted easily to inertial devices by using additional side inlets and some particle free buffer solution, for example water. When the inlets are adjoining, the sample flow in the middle will be surrounded by the sheath flow and depending on the individual flow rates the width of the focused sample stream can be adjusted (figure 2.8) [22]. For this report only equal side flow rates will be

considered. Using equation 2.21 the width of the focused stream can be calculated.

$$W_f = \frac{Q_i \cdot W_o}{\gamma \cdot (Q_i + 2 \cdot Q_s)} \quad (2.21)$$

where W_f is the width of the focused stream, W_o width of the outlet channel, Q_i is the sample flow rate, Q_s is the side flow rate and γ is the velocity ratio, which approaches 1.5 as the aspect ratio approaches infinity and approaches 1.0 as the aspect ratio approaches zero.

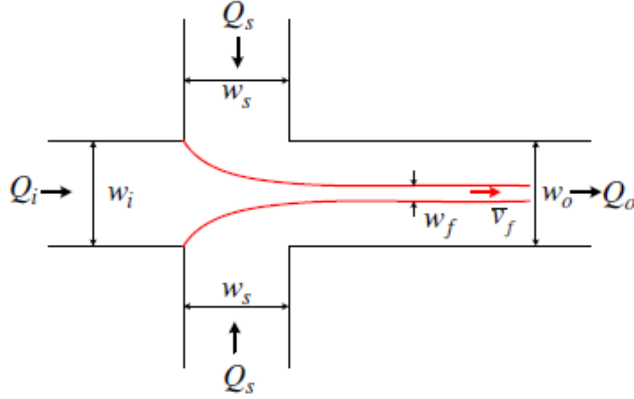


Figure 2.8: Schematic representation of the hydrodynamic focusing effect from a top view, in the case when the flow rate of the side inlets are equal. Source of the image: [22]

Chapter 3

Experimental approach

3.1. Channel design and fabrication

Designing microfluidic devices is not an easy task, because most geometrical parameters have an impact on the functionality and there are non-geometrical ones as well, like the physical properties of the particle and the flow control method. Even in the case of a simple inertial focuser, which is a straight rectangular channel with high (>1) aspect ratio, the width, height and length of the channel are quasi independent parameters, given the aspect ratio is greater than 1. Although theorists suggested some ideas to aid the choosing of these parameters, these formulas are based on some own numerical simulation or experimental results, containing hypothetical coefficients, which makes them hard to use as a third party research group [4] [23].

That is the reason why the parameters of the first device in this report will be arbitrary, and my aim is to make such parameter changes for the next device, which will improve the functionality. Because of this iterative process, the individual designs will be detailed with the inherent results in the next chapter. The blueprints were created using AutoCAD, a computer aided design software application developed by Autodesk. For conventional reasons the input is on the left, the output is on the right hand side of all devices.

For each device the mold was fabricated using a photoresist. It was spin coated on the surface of a silicon wafer. After heat treatment soft lithography can induce the polymerization of the photoresist along the channel geometry. After polymerization the unpolymerized resist can be solved in the appropriate solvent. The finished mold is filled with Polydimethylsiloxane (PDMS), which is a viscoelastic, silicon-based organic polymer. After solidification the PDMS is easily removable from the silicon wafer. To

seal the bottom of the channel the solid PDMS is covalently bound to a microscope slide using plasma treatment. The fabrication process took place in a laminar flow cabinet to decrease the chance of dust contamination.

3.2. Measurement setup

3.2.1 Flow control

An other important factor is the method of flow control, the most common ones are pressure driven flow and displacement pumping. Since

$$\Delta P = QR_f \quad (3.1)$$

where ΔP is the pressure drop, Q is the flow rate and R_f is the flow resistance, the biggest difference between the two is that in the first case the flow control device guarantees a desired constant pressure and the flow rate will change based on the resistance. In the second case a syringe pump pushes the sample with a predefined constant flow rate, meaning that the pressure will depend on the flow resistance of the channel.

3.2.2 Sample preparation

The devices were tested with a suspension of Polystyrene micro-beads manufactured by Micromod, TWEEN 20 (polyoxyethylene(20) sorbitan monolaurate) surfactant and HyClone Cell Culture Grade Water. The concentration of micro-beads in the stock-solution according to the manufacturer is $50 \frac{mg}{ml}$ regardless of the diameter of the particles, meaning that the $\frac{particle}{ml}$ concentration strongly depend on the volume of the bead. Since evaluating huge number of particles is challenging, due to the manual particle detection involved in the evaluation steps, the concentrations were adjusted according to the bead diameter. For the 1- and 2-micron particles $1 \frac{\mu l}{ml}$, for the 3- and 4-micron particles $2 \frac{\mu l}{ml}$, for the particles between 5 and 8-micron $5 \frac{\mu l}{ml}$ and for the 10-and 12-micron particles $10 \frac{\mu l}{ml}$ testing sample was used. 9- and 11-micron particles are not manufactured by the company. These numbers still did not mean equal $\frac{particle}{ml}$ concentrations, but the evaluation became manageable. The TWEEN 20 stock-solution was 20 times diluted to avoid crystallization and than used in $10 \frac{\mu l}{ml}$ concentration in each sample. The purpose of mixing the surfactant to the sample is to prevent the Polystyrene micro-beads to bind to each other and to the channel walls.

3.2.3 Measurement process

To achieve pressure driven flow the ElveFlow OB1 MK3+ pneumatic flow control device and for displacement pumping syringe pumps were used. To get real time feedback the microfluidic chip was placed at an Olympus CKX53 inverted microscope. The connections between the flow control device(s) and the inlet(s), as well as between the outlet(s) and the Eppendorf tube(s) were established via Teflon tubes. During the measurement a gray scale video footage was recorded by the outlet from a fixed position with UI-3180CP-C-HQ Rev.2.1 USB 3 industrial camera and inherent software application. The video was captured with low FPS (~ 2 frame per second) to ensure no particle appears on more than one frame. The processed sample is collected to Eppendorf tubes for further analysis in Bürker chamber.

3.3. Evaluation methods

3.3.1 Real time observation

Real time feedback from the microscope is necessary for evaluating the credibility of the measurement. Firstly, every design is tested for pressure tolerance. Pure water is flowed through the device on higher pressure or on higher flow rate than during the real measurement, to make sure the Teflon tubes will not be releasing from the device and there will be no leakage in the system, so under the circumstances it has a point to test the devices with particles. Secondly, if the device has multiple outlets, the flow resistance of each outlet should be the same. Theoretically, if the outlets have the same cross section and overall length on the blueprint, than they should have the same flow resistance even if some of them are not straight. However, photo lithography not necessarily produces truly perpendicular walls, thus if a design changes at the outlet region, it is good practice to check the equality of outlet resistance by using pure water and measuring the weight of the produced samples. If the differences between Eppendorf tubes are taken in to account, than equal weights imply equal volume, which means equal flow resistance. On the other hand, if the volumes are not equal the particles will tend to follow the flow through the channel with the lowest flow resistance. Continuous supervision is also important during measurements with real sample, because bubbles or some dust can stuck inside the channel or even inside the outlet Teflon tubes and have a disturbing effect on the particles. Some fabrication errors can cause the PDMS to detach from the microscope slide, leading to leakage, thus influencing the results.

3.3.2 Time-lapse images

Time-lapse images are created from the outlet video using ImageJ software application. Z-projection is used to merge the frames together. The median projection type returns only the static part (background) of the footage. Depending on the intensity of the illumination, the particles can appear as dark or bright spots. The minimum intensity option returns the minimal pixel value for each pixel position from the stacked frames, creating an image containing all the dark particles. The maximum intensity option does the same for bright particles. By subtracting the minimum intensity image from the median or the median from the maximum intensity image the particles will be highlighted as bright spots on dark background. On the minuend image the channel walls can be replaced with a white line, so it remains visible on the final image. This is a qualitative result, if the focusing is successful, the particles form two joint lines near the channel walls and exit by the side outlets. One problem is that with small particles only a fraction of them will be in the plane of focus and some of them will not be visible at all. An other one is that to avoid detecting the same particle twice, the video is recorded with low FPS, thus some particles may not be detected at all.

3.3.3 Particle distribution histogram

In ImageJ the particles can also be marked using the multi-point tool even across frames. The origin is on the upper right corner and the scale can be changed knowing the pixel size of the footage given the magnification. The region of interest can also be cropped to the part between the channel walls. With these settings the position of the particles in μm can be saved to a .csv file, where the y coordinates are between 0 and the channel width. By opening the file in Microsoft Excel this interval can be discretized into bins, and the occurrences of particles in each bin can be counted using the Data Analysis tool. The particle position histogram is created by normalizing and plotting the data. This is a more detailed representation, than the time-lapse image, but the problems are the same as well, and since the manual particle detection is time consuming, the number of particles was limited to ~ 300 .

3.3.4 Particle counting with Bürker chamber

The ratio of particles exiting through each outlet can be approximated using the processed samples and the Bürker chamber method. The Bürker chamber is a laboratory tool to estimate cell concentrations in a sample. Usually it is made out of glass with the

contour of a 3 mm times 3 mm square etched in the middle, which is further divided into smaller and smaller squares with known side lengths. The chamber can be filled up with a few μl of sample. The particles can be counted on some regions with the same area and since the chamber has a fix 0.1 mm height, by averaging the number of particles in a given volume the $\frac{\text{particle}}{\text{ml}}$ concentration of a sample can be approximated. Figure 3.1 show the design of a Bürker chamber and the counting decisions at the boundaries.

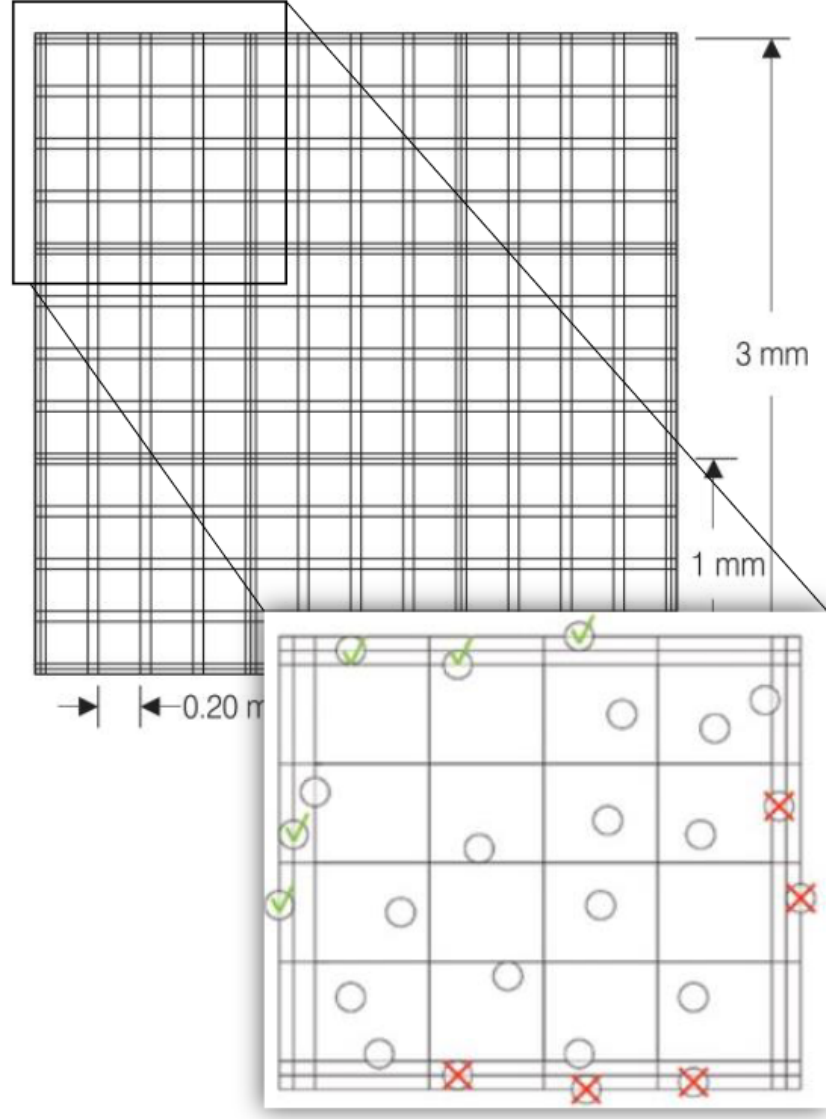


Figure 3.1: Design of a Bürker chamber and particle detection rules at the boundaries, to avoid counting the same particle twice.

Early on the particles on the images from the chamber were counted manually using ImageJ and plotted via Excel. From the IF04 device to make the counting faster I wrote a Python script to aid the particle detection. The script is available through the link in

the Appendix (page number 45). It is based on the scikit-image library and performs contour detection in a specified size range. This size is not the true particle size, but the number of coordinates (n) needed to describe the given contour, which is an ndarray with shape $(n, 2)$ generated by the `skimage.measure.find_contours` function, and n scales well with the true size. It returns the number of particles detected, the original image with the contours printed on it and the ten most frequent detected contour sizes to help adjust the range. When the Bürker images are captured, the region of interest is set to 1040x2080 pixel, which covers different areas depending on the magnification as figure 3.2 shows. This is necessary, because the script would count all the particles on the image, regardless of the counting rules by the boundaries. The script can make false positive and false negative errors as well, thus manual corrections almost always needed, but it still speeds up the process. However, particle counting is the most time consuming technique from the three, so it was only used after the footage based methods showed promising results.

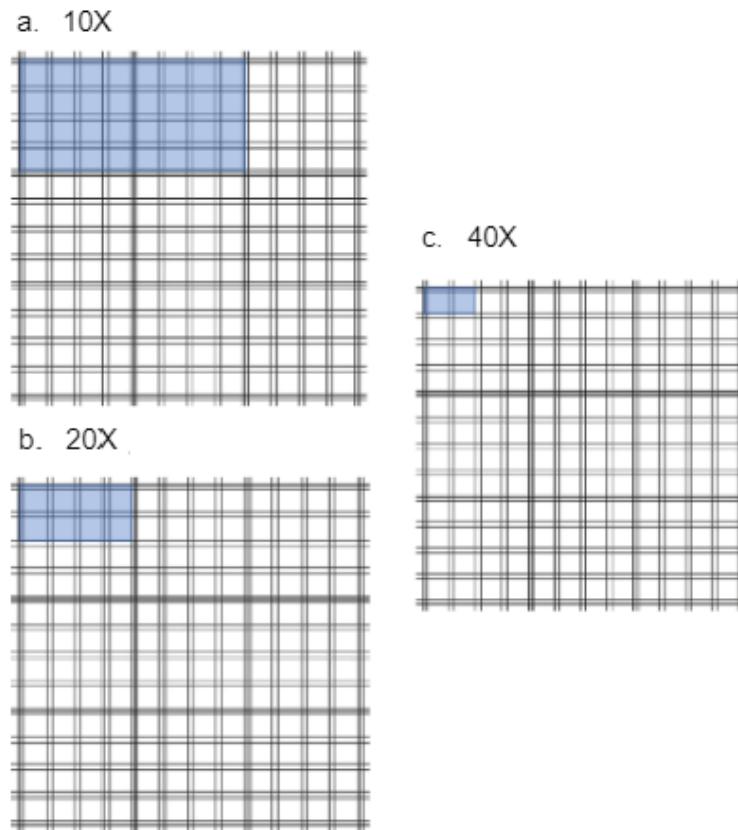


Figure 3.2: The area of a 1040x2080 pixel image depending on the magnification.

Chapter 4

Results

4.1. Measurement 01

The figure 4.1 shows the design of the Inertial Focuser 01 device (IF01). The parameters were chosen arbitrary, except the fact that it should be a high aspect ratio channel, to ensure lateral particle migration instead of vertical. The length was set to 10 *mm*, the width to 30 μm and the height to 50 μm . There is an expansure at the outlet part similarly to the inlet to make the particles visible by decreasing their speed. It is important that the particles keep their relative distance from the centre after the expansion as well. If the particle was $0.6W_1$ away from the centre in the narrow part, it will be $0.6W_2$ away from it in the wider one, where W_1 , W_2 are the width of the narrow and wide parts respectively. This template (figure 4.1) is a square with 21.81 *mm* side length. In this report this will be considered the unit template size.

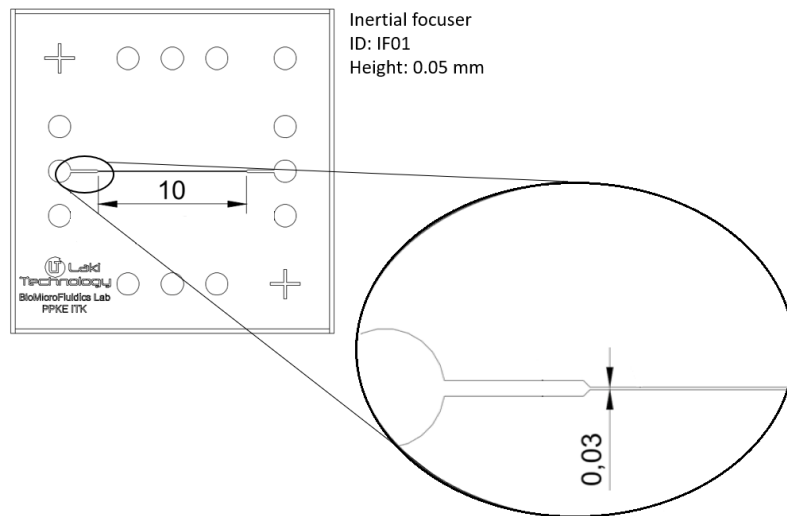


Figure 4.1: Design of IF01 Inertial Focuser. Distance is measured in mm.

The device was tested with the largest available particles (12-micron), since bigger particles reach equilibrium faster, as I mentioned in subsection 2.1.4. For flow control pressure driven flow was used, because it has the advantage, that the sample can be continuously mixed during the measurement using a magnetic mixer and magnetic stirring bars, thus particles can not sediment. The pressure was set to 800 mbar, because it was also important, that the individual particles should be visible on the outlet footage. Higher pressure involves higher speed, thus motion blur as well. Figure 4.2 shows the time-lapse image and figure 4.3 shows the particle distribution histogram. Although the focusing is incomplete, the particles are concentrating around 0.6 time the width each side, agreeing with the Segre-Silberberg annulus (2.1.1).

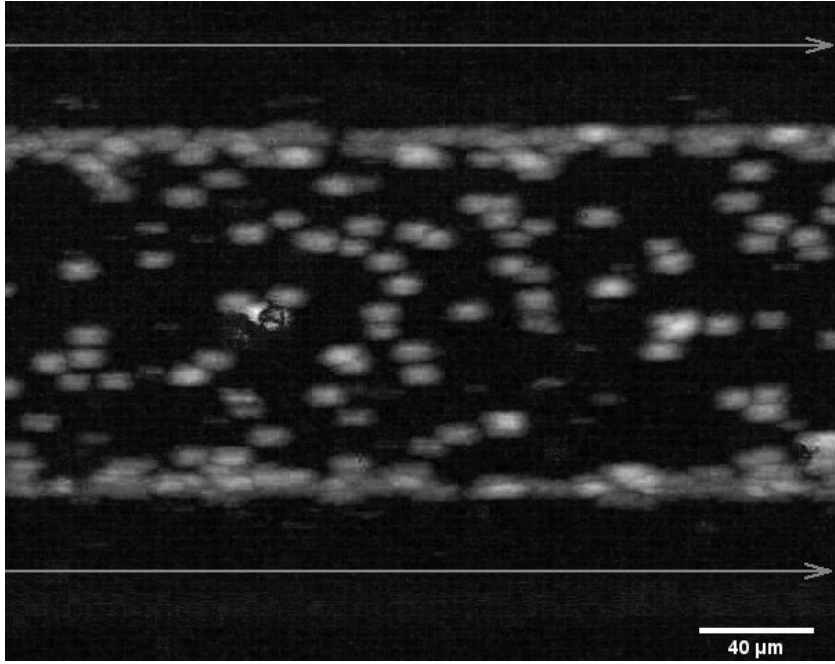


Figure 4.2: Time-lapse image captured by the outlet of the IF01 device.

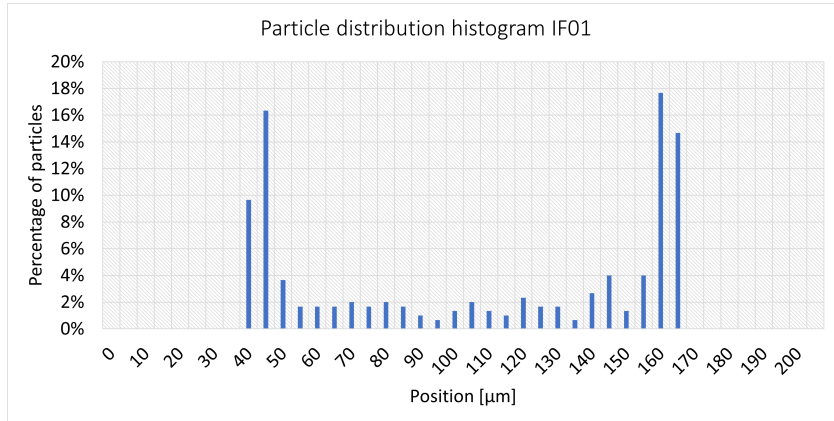


Figure 4.3: Particle lateral position histogram after focusing with the IF01 device.

4.2. Measurement 02

Based on the results of IF01 it was concluded, that the particles did not spend enough time in the focuser channel to reach equilibrium, thus the Inertial Focuser 02 device (IF02) was designed to be longer, 13 mm. Figure 4.4 shows the design. The template size was also increased to the double of the unit template size. It was expected, that due to this length increment, the focusing will be more complete.

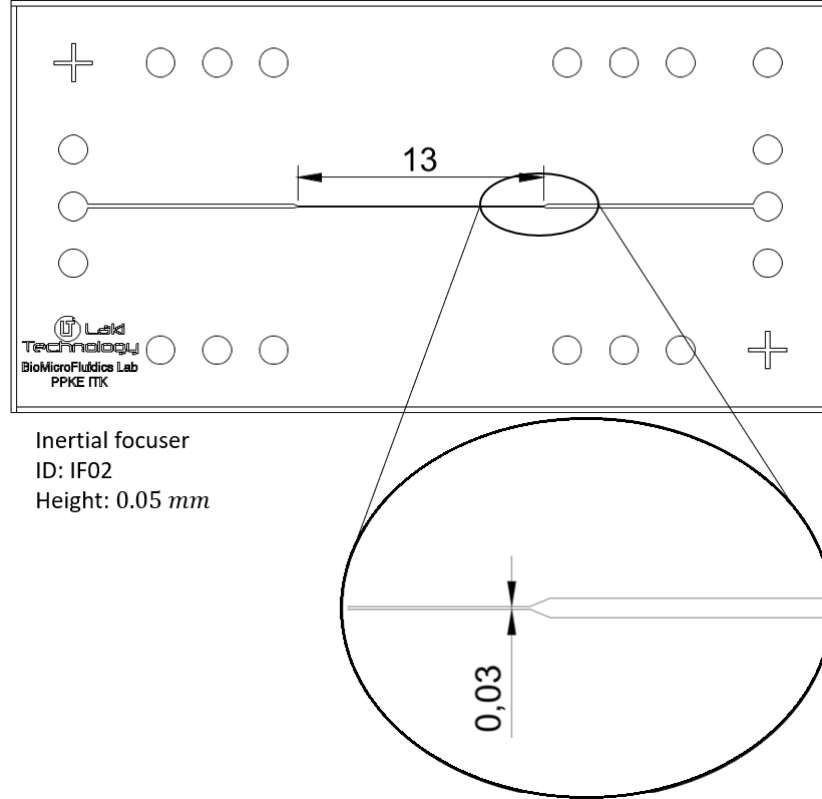


Figure 4.4: Design of IF02 Inertial Focuser. Distance is measured in mm.

Longer channels have higher resistance, meaning the pressure should be higher as well to reach the same flow rate (equation 3.1) without the risk of high motion blur, so the pressure was set to 1600 mbar. Figure 4.5 shows the time lapse image. It is visible that only significantly fewer particles could not reach one of the equilibrium positions compared to the results of the IF01 device. Figure 4.6 shows the particle distribution histogram and here we can see the same as on the time-lapse image, but with more details especially regarding the ratio of focused and not focused particles. Only a very low percentage of particles is not in equilibrium.

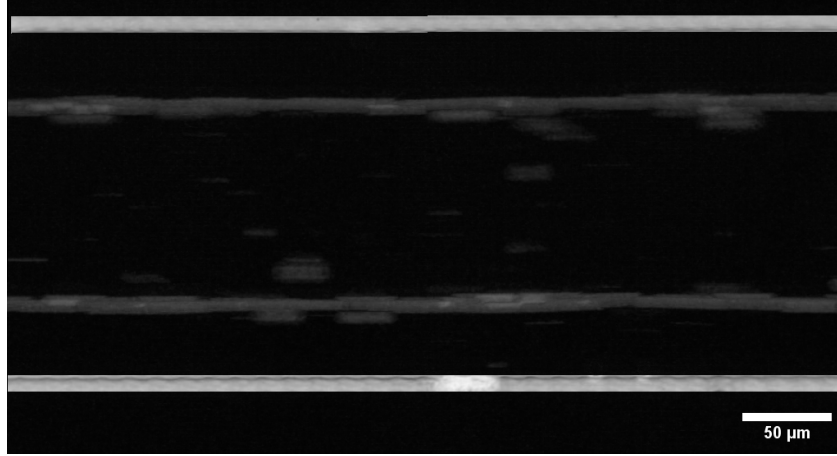


Figure 4.5: Time-lapse image captured after 13 mm focusing with the IF02 device.

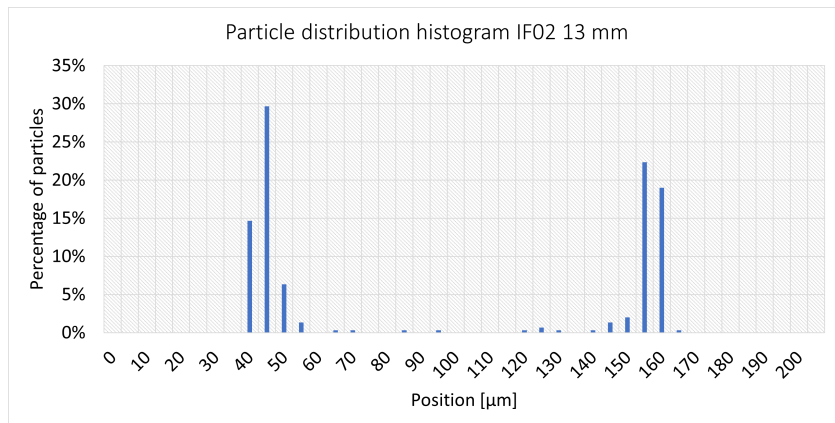


Figure 4.6: Particle lateral position histogram after 13 mm focusing with the IF02 device.

4.3. Measurement 03

For the Inertial Focuser 03 (IF03) device, the length was increased again to see whether the focusing efficiency can be further improved or not. Figure 4.7 shows the design. This time a 20 mm long focusing channel was used.

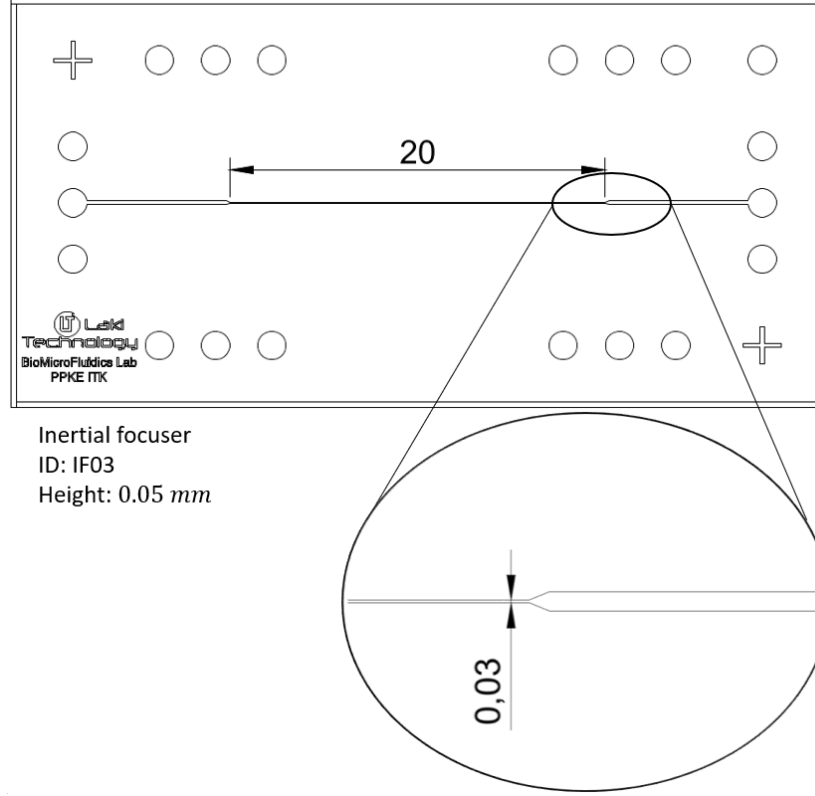


Figure 4.7: Design of IF03 Inertial Focuser. Distance is measured in mm.

Figure 4.8 and 4.9 are showing similar, but rather better results with 20 mm focusing. This is suggesting that with constant pressure and channel cross section the focusing efficiency increases with the channel length but not linearly only with a decreasing rate. The $\frac{a}{W}$ ratio is 0.4, which means as I mentioned before (subsection 2.7) that the equilibrium positions should be approximately half way between the walls and the centre. Based on the distribution histogram (figure 4.9) the particles are 45-55 μm away from the walls. If we take into account that the material PDMS may expand a bit, thus increasing the width of the channel and decreasing the $\frac{a}{W}$ ratio, than the equilibrium positions are where they were expected.

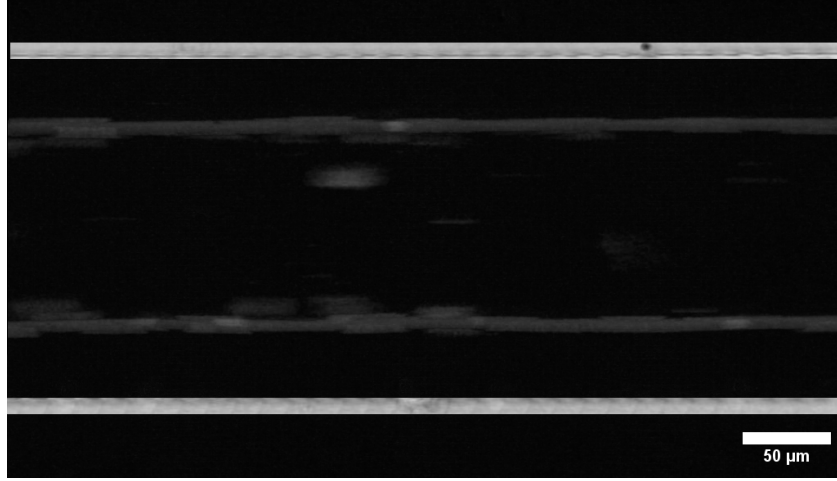


Figure 4.8: Time-lapse image captured after 20 mm focusing with the IF03 device.

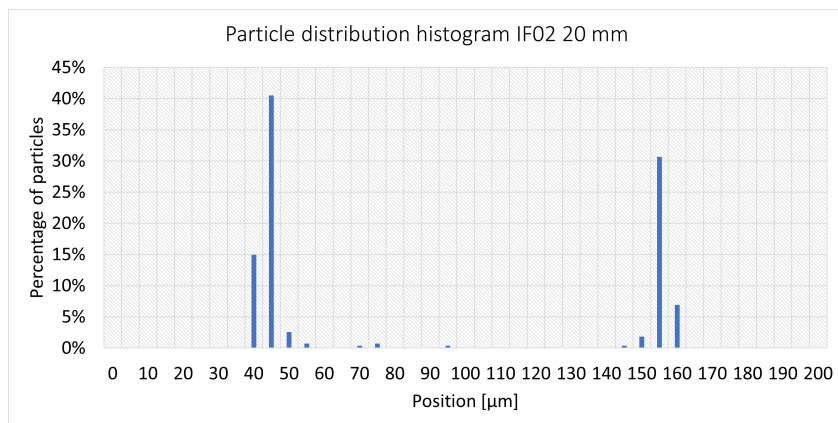


Figure 4.9: Particle lateral position histogram after 20 mm focusing with the IF03 device.

4.4. Measurement 04

After the promising results of the IF03, the next reasonable step was to do particle counting as well. For this, the Inertial Focuser 03 was redesigned with three outlets (IF03OUT, figure 4.10) named OUT1, OUT2 and OUT3 from top to bottom. The result 4.11 confirms the previous ones. It is important to notice, that the fraction of particles exiting through the two side outlets is not necessary equal. The reason behind this probably the random distribution of particles by the inlet.

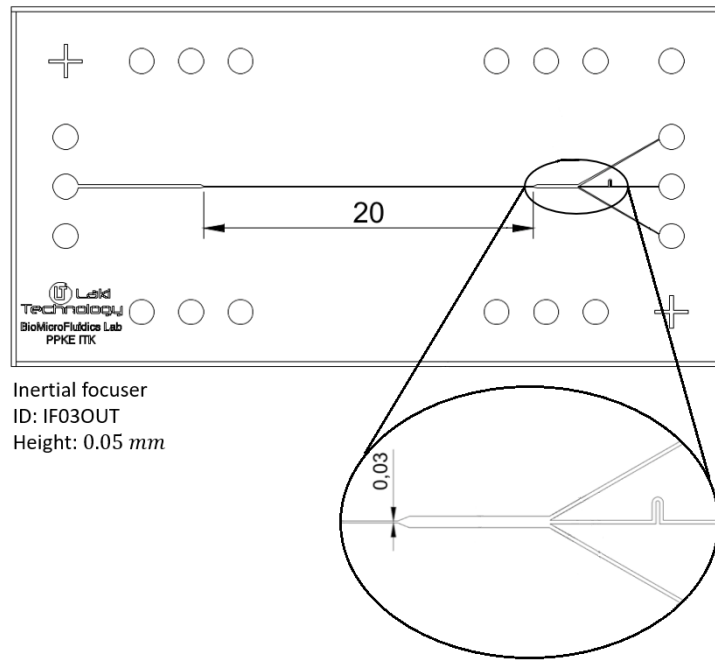


Figure 4.10: Design of IF03OUT Inertial Focuser. Distance is measured in mm.

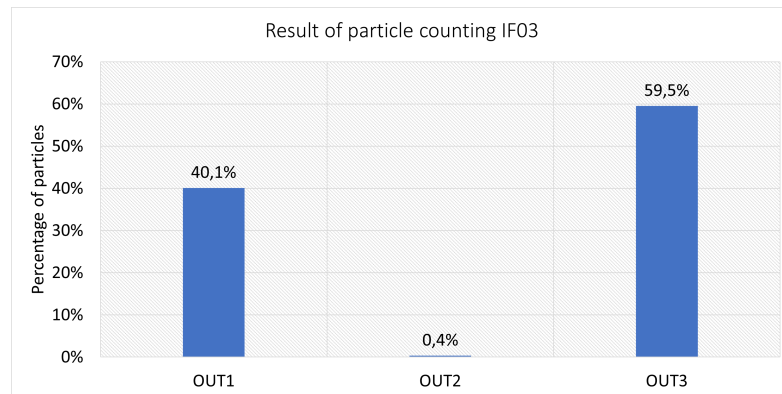


Figure 4.11: Result of particle counting after focusing with the IF03OUT device based on one measurement.

4.5. Measurement 05

Although the IF03 design was able to focus 12-micron particles almost perfectly, the usage of the devices was somewhat difficult. Due to the small cross section, the channel was often clogged by the particles or by some pollution of the sample. To overcome this problem the channel cross section was increased in the IF04 version. This device was designed parallel with the HIF01 device in the next section and for comparability the length of the IF04 channel was also reduced to 19 mm and the flow control changed to displacement pumping with $8 \frac{ml}{h}$ flow rate. For a more detailed analysis the device was tested with 1-8 and 10-micron particles. The time-lapse images, 4.13 for 5-micron and 4.14 for 10-micron particles, already show that as expected, the focusing of 10-micron particles was more successful. The detailed outlet distribution can be seen on figure 4.15, which confirms the previous observation, the focusing becomes less effective for smaller particles. Based on the position of the outlets, there are small enough particles so their distribution between the outlets is almost even and the tendency is that as the particle size increases, the focusing becomes more and more complete.

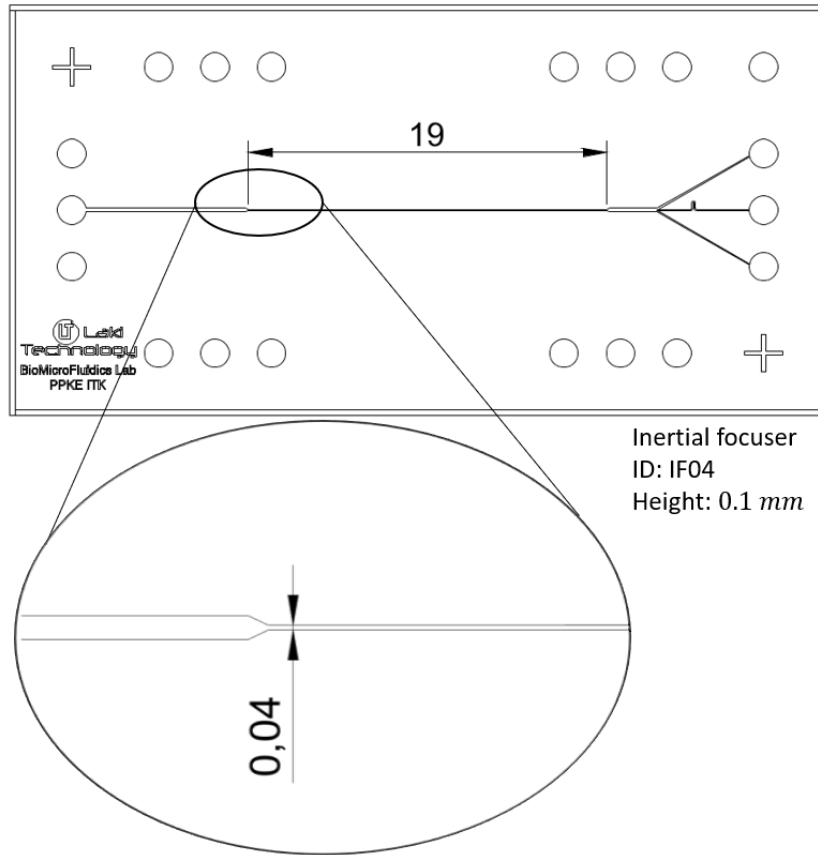


Figure 4.12: Design of IF04 Inertial Focuser. Distance is measured in mm.

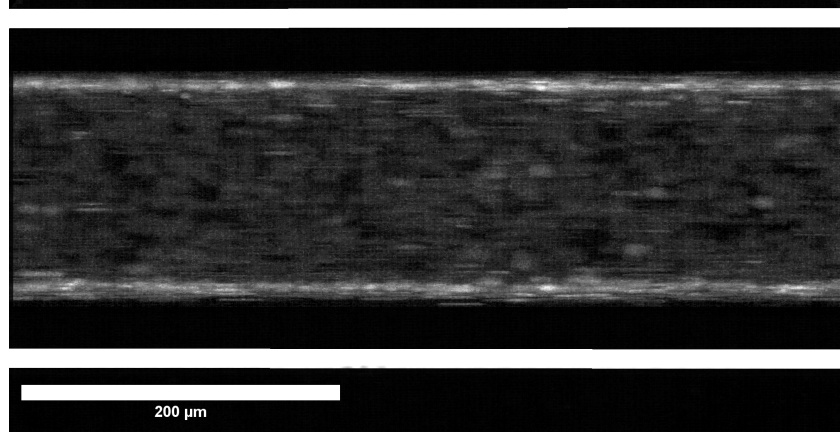


Figure 4.13: Time-lapse image of 5 μm particles captured by the outlet of IF04 device.

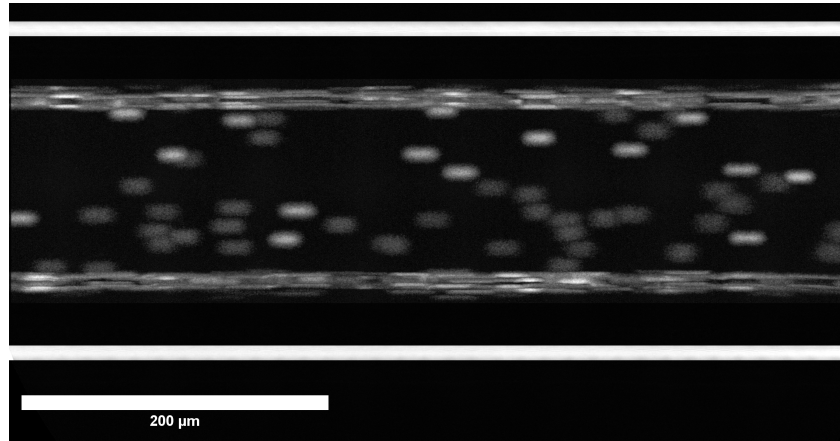


Figure 4.14: Time-lapse image of 10 μm particles captured by the outlet of IF04 device.

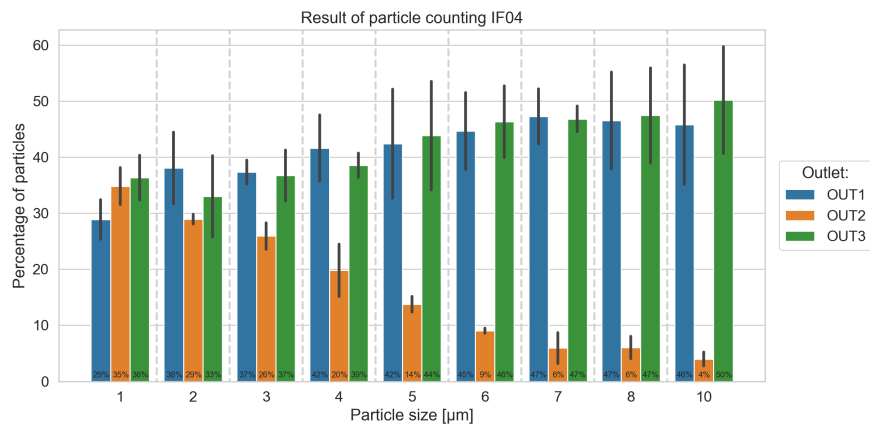


Figure 4.15: Result of particle counting after focusing with the IF04 device in 3x9 independent measurements. The average and standard deviation are presented.

4.6. Measurement 06

The results of IF04 can be interpreted as the majority of small particles entering the focuser around the centre of the channel are exiting through the middle outlet as well. Based on this with the next design, the phenomenon is inspected in the way that all particles enter the inertial part close to the centre. This is achieved via hydrodynamic focusing, thus these type of devices are called Hydro-Inertial Focusers (HIF). For flow control displacement pumping was used, because it is common practice to start the sheath flow before the sample to avoid the contamination of the side inlets with particles. With pressure driven flow this is more complicated, because the puffer can flow back in the sample inlet. The flow rate was set to $8 \frac{ml}{h}$.

Figure 4.16 shows the geometry. The additional side inlets are for the sheath flow, which performs the hydrodynamic focusing of the particles before the inertial part begins. According the equation 2.21 considering equal flow rates for the inlets the width of focused stream can be described as $\frac{W}{3\gamma}$ where γ is between 1.0 and 1.5 and it is closer to the upper boundary as the aspect ratio increases. To achieve a tighter stream of particles, the inlets were joined in the higher aspect ratio region, but doing so the length of the inertial part was decreased by 1 mm. The exact width of the focused stream is not known, but it has to be less than $\frac{W}{3}$ because of γ , meaning that the particles not being effected by the inertial focusing, should exit though the middle outlet, since it is $\frac{W}{3}$ wide. On the other hand, bigger particles should migrate to equilibrium as before.

On the time lapse images 4.17 and 4.18 it is visible that in either case there are no particles on the walls side of the equilibrium positions and the 5-micron particles localized much more densely in the middle. The distribution histogram shows 4.19 the opposite of the IF04 case. As expected, a very high percentage of the particles under 5-micron is now exited through the middle outlet. Unfortunately the sheath flow negatively influenced the inertial focusing of the rest, but it still shows a tendency to improve as the particle size increases. Although this only led to an even distribution in the case of 10-micron particles, there is reason to believe that the improvement continues for larger ones as well.

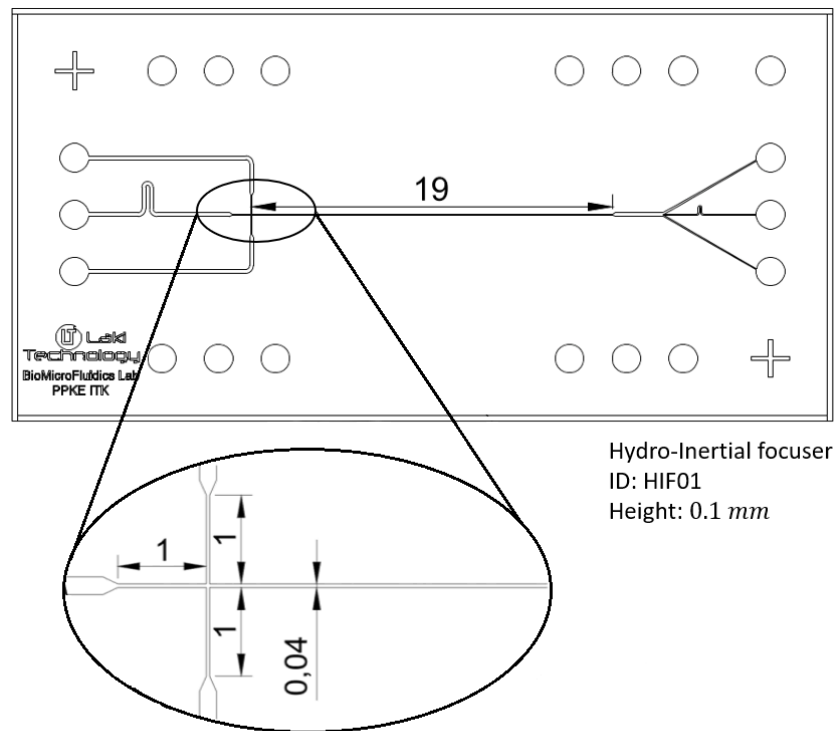


Figure 4.16: Design of HIF01 Hydro-Inertial Focuser. Distance is measured in mm.

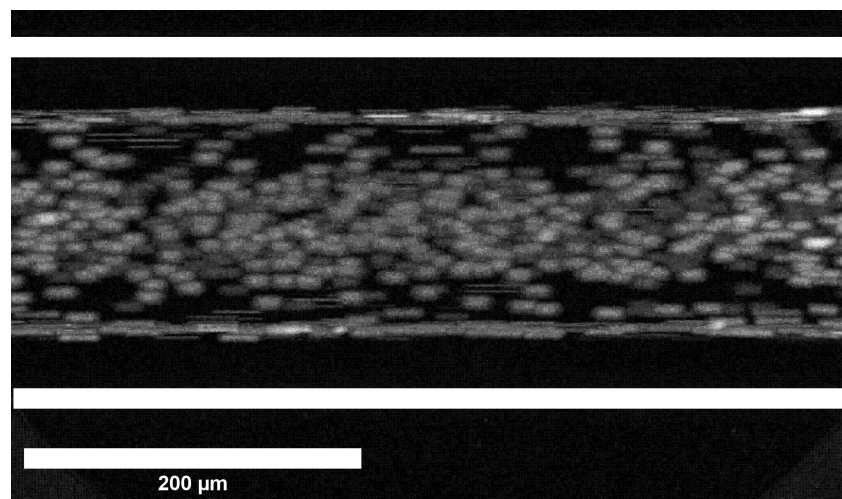


Figure 4.17: Time-lapse image of 5 μm particles captured by the outlet of the HIF01 device.

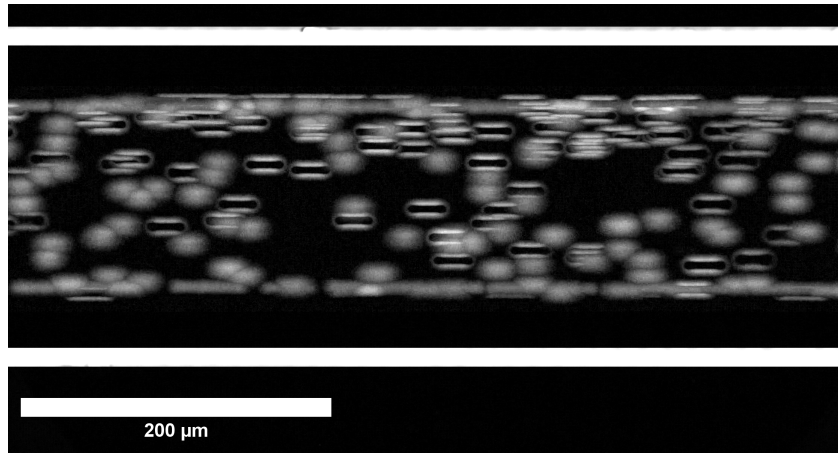


Figure 4.18: Time-lapse image of 10 μm particles captured by the outlet of the HIF01 device.

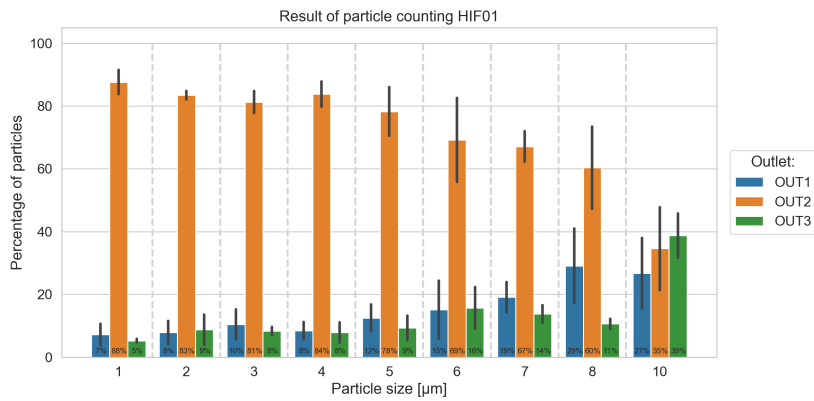


Figure 4.19: Result of particle counting after focusing with the HIF01 device in 3x9 independent measurements. The average and standard deviation are presented.

4.7. Measurement 07

For a future comparability with other project in the lab a 31 mm long version of the IF04 devices named Inertial Focuser 05 (IF05, figure 4.20) was also designed and tested. The expectation was that in the increment of length will result better focusing even with smaller particles, since it takes more time flowing through the channel, and this should compensate their lower migration speed.

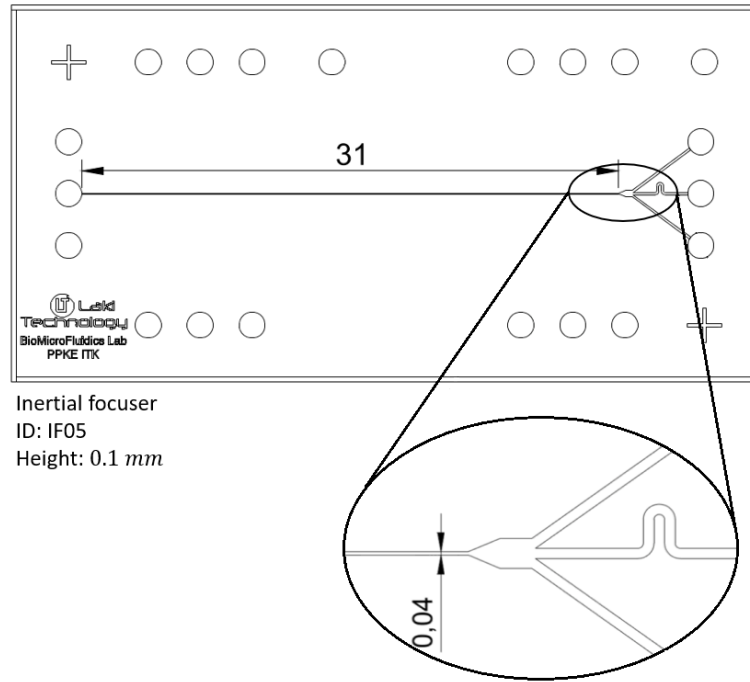


Figure 4.20: Design of IF05 Inertial Focuser. Distance is measured in mm.

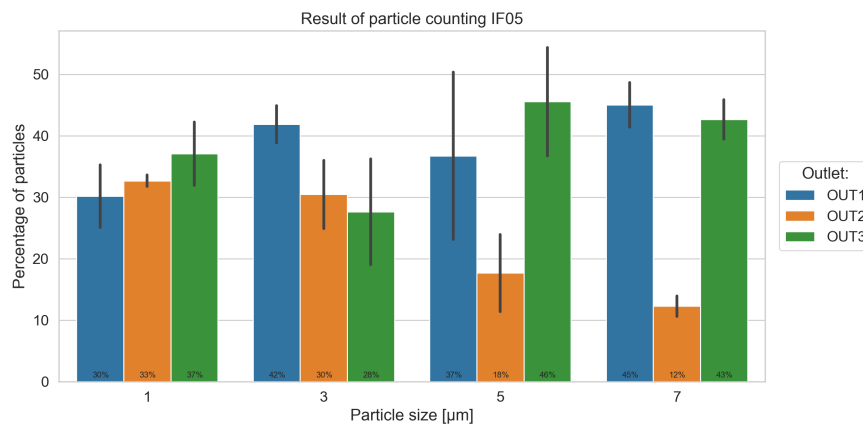


Figure 4.21: Result of particle counting after focusing with the IF05 device in 3x4 independent measurements. The average and standard deviation are presented.

According to the results of the IF04 device (figure 4.15) for particles between 1 and 7-micron the percentage decreases in the middle outlet is almost linear and above 7-micron the difference is not significant, thus the number of particle sizes was reduced, the IF05 device was tested with 1-, 3-, 5- and 7-micron particles. Looking at the figure 4.21 the distributions are very similar to the IF04 case (figure 4.15). This suggests that further increasing the length will not improve the separation efficiency.

4.8. Measurement 08

Finally the the 31 *mm* inertial channel was also combined with hydrodynamic focusing in the Hydro-Inertial Focuser 02 device (HIF02). Figure 4.22 shows the geometry. Based on the very similar results in the case of IF04 and IF05, for the HIF02 device somewhat similar results were expected as in the case of HIF01.

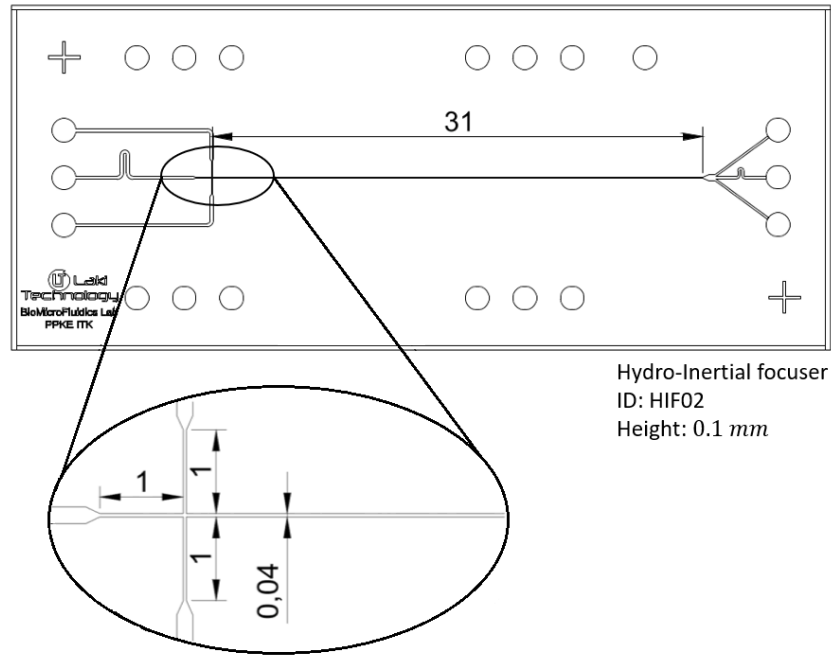


Figure 4.22: Design of HIF02 Hydro-Inertial Focuser. Distance is measured in mm.

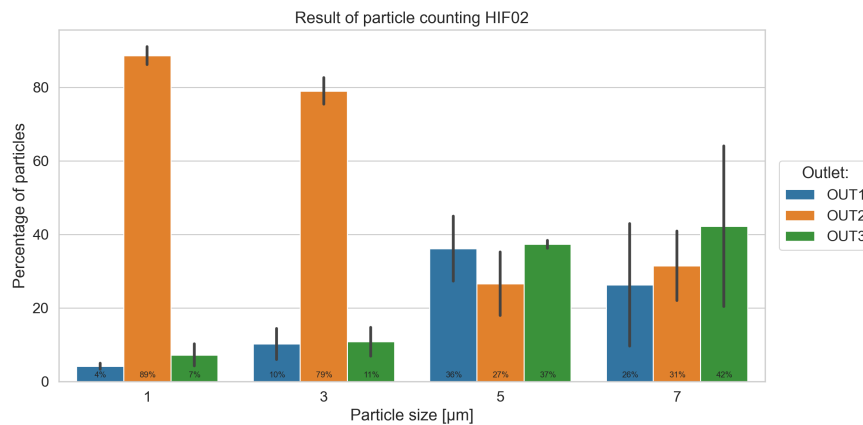


Figure 4.23: Result of particle counting after focusing with the HIF02 device in 3x4 independent measurements. The average and standard deviation are presented.

However, as figure 4.23 shows the length increment helped the 5- and 7-micron particles to overcome the effect of hydrodynamic focusing compared to the HIF1 case without influencing the smaller particles. An other observation is that these longer devices are much more sensitive to the outlet resistance, even the curvature of the outlet Teflon tubes can make a difference making the measurement process harder to do and the number of resultless measurements increased significantly.

4.9. Separation task

Finally the best performing design, HIF01 was tested with 3- and 12-micron particles mixed together to separate the two. It was expected that according to the tendency in focusing efficiency of the device (4.19) the 12-micron particles should be large enough to be focused, and the behaviour of 3-micron ones should be the same. Figure 4.24 show the result of three independent measurements. It is confirmed, that the presence of the 12-micron particles have neglectable effect on the 3-micron ones, as well as that the tendency of improvement observed before continues with larger particles, hence only 15% of 12-micron particles exited through the middle outlet, compared the 37% of 10-micron ones previously.

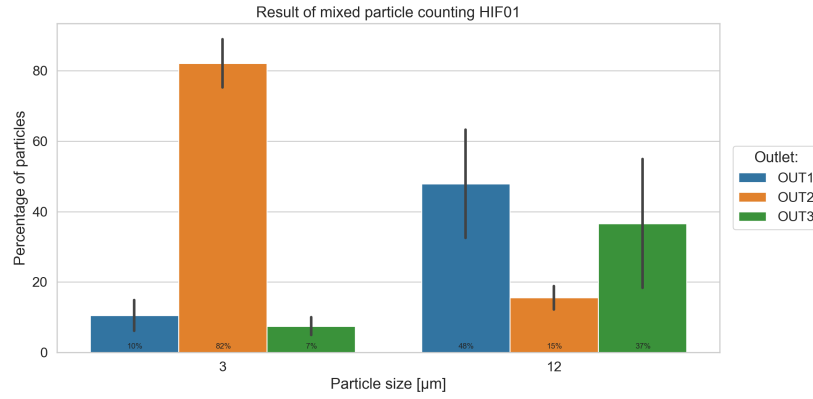


Figure 4.24: Result of particle counting of a mixed sample after focusing with the HIF01 device in three independent measurements. The average and standard deviation are presented.

Chapter 5

Conclusion

5.1. Summary

The aim of this project was to develop a great understanding about inertial focusing phenomenon, which is foundation of inertial microfluidics, thus it must be considered with all the most complex geometries as well, like Construction-Extension Array, Serpentine or Spiral channels. Throughout this report inertial focusing was studied theoretically by reviewing the physical background and key scientific discoveries from 1961 until recent years and through experiments as well. It was described in detail what kind of experimental data was collected and how evaluation methods, like time-lapse image, particle distribution histogram and particle counting work for microfluidic data.

5.2. The results

It was shown how the focusing efficiency changes through a wide range of particle sizes. The inertial focusing of the particles tend to be more and more complete as the diameter of the beads increase. Compared to an almost even distribution in the case of 1-micron particles only 4% of the 10-micron ones exited through the middle outlet (figure 4.15). The combination of inertial focusing and hydrodynamic focusing was also introduced as a possibility to improve the particle separation, by changing the initial particle position. With the aid of sheath flow particles was positioned at the centre of the channel, thus the smaller ones, less effected by the inertial forces, could not reach the side outlets. Using a Hydro-Inertial Focuser 82% of 3-micron particles were successfully separated from 12-micron ones compared to 26% in the simple Inertial Focuser case (figure 4.24). For a particle separation point of view using these devices, if the target is below 5-micron and the rest is larger, inertial focusing would achieve higher purity, but lower efficiency,

than hydro-inertial focusing. For a target above 5-micron in a suspension with smaller particles, this will be the opposite, for higher purity one should use hydro-inertial focusing and for higher efficiency simple inertial focusing.

The influence of the length of the channel was also investigated both with IF and HIF devices. The results were suggesting that in the IF case the increase in focusing efficiency is neglectable above 19 *mm* focusing length. On the other hand, for HIF devices a longer inertial part helped to reduce the negative effect of hydrodynamic focusing on larger particles without disturbing the positive effect on smaller ones. However, with constant flow rate there is a price to pay for longer channels, because the number of errors during a measurement tend to increase as well, thus it makes the usage of the devices more difficult.

5.3. Future plans

In experimental research as a direct proceeding of this work one could try different initial conditions. In the case of both devices the flow rate could be increased, specifically for HIF devices the effect of unequal flow rates could be tested. Since hydrodynamic focusing is problematic with pressure driven flow and it results diluted samples it would be interesting to find some other methods to achieve size independent positioning of the particles. The research could also take a direction where one is trying to close the size gap between the particles that are separated from each other. This would probably require the usage of some more complex geometry.

For practical purposes the next step could also be to test the devices with biological samples. Polystyrene beads are common models in microfluidics, but unlike cells PS beads are rigid, and deformability can not be taken into account. The viability of the cells is also crucial question, if we only want to detect the presence of some organism, for example bacteria among alga cells, viability is less of an issue, but if the task is to collect rare cells for further analysis, it is very important to know against how much mechanical stress can the cell resist.

Another aspect is the fabrication method. Photolithography is a great prototyping tool, but solid PDMS is elastic, thus the channel may expand, due to the high pressure. Other methods like hot embossing or injection molding would make rigid materials available for fabrication.

Finally the area of numeric computer simulations could also help to measure our understanding about the behaviour of particles in fluid flow. In inertial microfluidics in silico models are lacking, which could be used in the designing phase, in order to reduce the number of experimental measurements needed to reach the desired functionality.

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Appendix

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