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Contraction-extraction based particle separation in serpentine microchannels

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Abstract

There is an increasing need in today's world to connect different fields of expertise. Microfluidics is one of the many interdisciplinary fields that have emerged. This research area gained more and more importance in recent years. It combines aspects of physics, chemistry, engineering, and biology to fabricate devices that manipulate fluids at the micrometer scale. As a result, we can analyse and examine fluid flow and particle motion under different conditions. Microfluidic devices provide a quick and low-cost alternative in drug research, analytical device manufacture, and medical diagnostics, for example. As a result, the market for these devices has expanded substantially in recent years.

In microchannels the particles migrate to certain equilibrium positions. This movement is called inertial migration and it is caused by the forces acting on the particles. As a result, the particles are aligned and focused in the channel. Since particles with different properties have different equilibrium positions, separation can be achieved. Inertial microfluidics is one of the many branches of passive technologies. This field is based on the aforementioned inertial migration, and secondary flow. It is an innovative approach that exploits the effects inertial forces at intermediate Reynolds numbers ($1 < Re < 100$), to manipulate particles and fluids within microchannels. This field continues to evolve, offering promising solutions for challenges in diagnostics, drug development, and microscale engineering.

In this thesis I demonstrate the relevant microfluidic principles, dominant forces in inertial microfluidics, and particle motion in microchannels. Following the theoretical overview, I examine some of the current literature on inertial microfluidics in the field of particle separation and focusing. Working with the research group provided me with an understanding of the design, fabrication, and testing of microfluidic devices. I created my own microfluidic separators based on these articles and the knowledge I gained while working in the Biomicrofluidic laboratory. In this document, I provide a detailed

description of the design using CAD software and the fabrication process. I show some CFD (computational fluid dynamics) simulation results that help to understand how the devices operate. I describe the measurements performed with the finished devices and analyse the results to determine their characteristics and separation efficiency. I compare some of the results to previous measurements done in the laboratory, to further evaluate the effectiveness of the new devices. I will determine a set of parameters that will result in efficient separation based on the measurement results. Finally, I discuss the successfully completed tasks and compare them with my set goals, then talk about my future plans in mind.

Acknowledgements

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Chapter 1

Introduction

Microfluidics is an interdisciplinary field that combines aspects of physics, chemistry, engineering, and biology to design and fabricate devices, which contain chambers and channels through which fluids and particles can be manipulated, controlled, and analysed on a micro scale. There are active and passive microfluidic techniques for separating and focusing particles based on their physical properties (size, shape, density etc.). Active approaches use external sources to control the fluid flow through the channel. These devices, for instance, can be used in conjunction with external magnetic or electric fields. Electrophoresis, dielectrophoresis, magnetophoresis, acoustophoresis, and many other techniques are examples of these active processes. However, passive methods only alter fluid flow and focus particles by utilizing the channel geometry and internal hydrodynamic forces. This makes the implementation easier, also, these devices cost less to manufacture and operate. Pinched flow fractionation (PFF) [1], deterministic lateral displacement (DLD) [2], contraction-extraction arrays (CEA) [3], deterministic cell rolling (DCR) [4], and Dean flow-based [5] microfluidic separators are a few examples of passive separation techniques. This study is based on Dean flow-based microfluidic separation in asymmetric contraction-extraction serpentine channels, which is one of a wide variety of approaches.

1.1. Fundamental principles of fluid flow dynamics

There are some fundamental principles of fluid flow dynamics that need to be considered when designing and working with passive inertial focusing devices. These devices focus the particles into streamlines, according to certain equilibrium positions. Then the streamlines

of the particles are divided based on the size, shape or other physical properties of the particles.

In a circular pipe with constant cross section, laminar flow and Newtonian fluid, the flow is given by the Hagen-Poiseuille equation (1.1).

$$Q = \frac{\pi r^4}{8\mu L} \Delta P, \quad (1.1)$$

where Q is the volumetric flow rate, r is the radius of the pipe, μ is the fluid viscosity, L is the length of the pipe and P is pressure.

The velocity distribution is expressed in equation 1.2. As can be seen, the flow has a parabolic velocity distribution. This means that the fluid in the centre of the pipe has a higher velocity than the fluid near the walls.

$$v_z = \frac{1}{4\mu} \frac{\partial p}{\partial z} (r^2 - R^2), \quad (1.2)$$

where μ is the viscosity of the fluid, $\frac{\partial p}{\partial z}$ is the pressure gradient, r is the distance from the center line and R is the radius of the pipe.

The Navier-Stokes equations express momentum conservation and describe the evolution of a fluid's velocity field over time. Solving these equations makes it possible to predict the fluid velocity and pressure in a specific geometry. Comsol Multiphysics, a software designed for modelling and simulating a wide range of physical phenomena, including computational fluid flow (CFD) simulations, calculates the velocity and pressure profiles using this principle. For an incompressible Newtonian fluid the Navier-Stokes equation is as follows [6]:

$$\rho \left(\frac{\partial u}{\partial t} + u \cdot \nabla u \right) = -\nabla p + \mu \nabla^2 u + f, \quad (1.3)$$

where ρ is the density of the fluid, u is the velocity field, p is the pressure field, μ is the dynamic viscosity of the fluid and f is a vector field of external forces. At a low fluid velocity, the left-hand side of the equation is negligible and can be considered 0. In the regime, where $\left(\frac{\partial u}{\partial t} + u \cdot \nabla u \right) = 0$, we talk about Stokes flow. This is a common approximation in CFD simulations, but it can lead to incorrect results.

The Reynolds number (Re) creates a connection between inertial and viscous forces in fluid dynamics. It is a dimensionless number that can be used to assess whether a fluid flow pattern in a channel is laminar or turbulent [7]. Laminar flow occurs at low Reynolds numbers, where the ratio of inertial to viscous forces is low (indicating that viscous forces are dominant). It suggests that there is little to no mixing as the particles flow smoothly, in parallel layers. On the other hand, turbulent flow occurs at high Reynolds numbers, where the previously mentioned ratio of inertial and viscous forces is significant, and inertial forces are dominant. High fluid velocities exceeding a so-called threshold, which is determined by the Reynolds number, cause this to occur. In a turbulent flow the fluid is travelling in irregular pathways, and vortices can develop, causing lateral mixing. The Reynolds number can be calculated as follows.

$$Re = \frac{\rho v L}{\mu}, \quad (1.4)$$

where v is the velocity of the flow, L is the characteristic length of the channel, μ is viscosity of the fluid and ρ is fluid density.

This number is often referred to as channel Reynolds number, denoted as Re_c . If particles or an object is added to the fluid, another dimensionless number can be defined, that is called the particle Reynolds number (Re_p). This is used to describe the flow and its viscosity surrounding a particle or an object moving through a fluid. The particle Reynolds number can be expressed as:

$$Re_p = \frac{\rho v_f d}{\mu}, \quad (1.5)$$

where v_f is the velocity of the fluid relative to the particle, d is the diameter of the particle, μ is the viscosity of the fluid and ρ is the density of the fluid.

The particle Reynolds number (Re_p) makes it possible to analyse the flow past a circular cylinder. If $Re_p \ll 1$, then the viscous forces dominate on the particle. This is usually at slow flow rates, and the particles stay in the same distribution throughout the channel [8]. This regime, where $Re \rightarrow 0$, is called the Stokes regime. When the Re_p increases, inertial forces become more and more dominant on the particles. At the range of $1 < Re_p < 100$ both viscous and inertial forces acting on the particle are finite [9], and moving across streamlines is possible. This is where inertial microfluidics works, between the Stokes regime and the turbulent regime. The turbulent regime defines a threshold

above which the flow is turbulent, and the particles move in irregular paths.

1.2. Motion of particles in the channels

1.2.1 Dominant forces

Four dominating forces must be taken into account when utilising inertial focusing: viscous drag force, wall lift force, shear gradient lift force and in curved channels Dean force.

Viscous drag force

In fluid dynamics we talk about viscous drag force (F_d) if an object (e.g., particle) moves through the fluid, or when there is an object in the path of the fluid (e.g., bridges in rivers). This force acts against the relative motion of any object moving in the fluid. The magnitude of viscous drag force depends on the particle's size, shape, and the fluid's viscosity. Viscosity is a measure of a fluid's internal resistance to flow. High-viscosity fluids (e.g. honey), flow more slowly, while low-viscosity fluids (e.g. water), flow more readily. This force behaves differently if the velocity is low, in this case it is directly proportional to the velocity. This is usually in case of low Reynolds numbers.

If the velocity is high, then the drag force is proportional to the square of the fluid velocity. This is above the previously mentioned threshold, defined by the Reynolds number.

$$F_d = S \cdot c_d = \frac{\pi d^2 c_d}{4}, \quad (1.6)$$

where S is the cross-sectional area of the object or particle in the fluid, d is the diameter of the object or particle in the fluid and c_d is the drag coefficient, which is determined by the particle Reynolds number and relative velocity of the particles to the fluid.

According to the particle Reynolds number, the drag coefficient can be divided into four sections [9]. In this document two of these are discussed, the Stokes regime and the intermediate region. Other intervals are not relevant for inertial microfluidics, as above a certain threshold instabilities arise and the flow becomes turbulent.

In the Stokes regime ($Re_p \ll 1$) the drag coefficient is

$$c_d = 12 \frac{1}{Re_p} = 12 \frac{\mu v_f}{d} \quad (1.7)$$

Substituting this into the Equation 1.6 we get the magnitude of the drag force in the Stokes region.

$$F_d = 3\pi\mu d v_f \quad (1.8)$$

Increasing the particle Reynolds number, as we reach an intermediate regime, where $0.2 < Re_p < 500 \sim 1000$ the drag coefficient is as follows.

$$c_d = 12 \frac{\mu v_f}{d} (1 + 0.15 Re_p^{0.687}) \quad (1.9)$$

Again, substituting this into the Equation 1.6 we get the magnitude of the drag force in the intermediate region.

$$F_d = 3\pi\mu d v_f (1 + 0.15 Re_p^{0.687}) \quad (1.10)$$

Wall lift force

The existence of walls has a significant effect on the particles and it can not be neglected. The particles surrounded by walls behave differently than those that are in an unbounded flow. The disturbance in the flow field around the walls of the channel induces a force called the wall lift force (F_{LW}). Primarily this is caused by a single wall on one side of the particle. Because the particle size is substantially smaller than the dimensions of the channel, the other wall is too far away to have a significant effect on the particle's motion. The presence of walls causes a deceleration of the particle. This difference in velocity induces a lift force, which drives the particle towards the centre line of the channel.

Shear gradient lift force

Working against the wall lift force, the shear gradient lift force (F_{LS}) drives the particles towards the wall. This is caused by the lower pressure near the wall. This pressure difference is a result of the parabolic velocity profile in the channel. As mentioned above, the flow is slower down near the walls. As a consequence, the particles migrate towards the wall, until the shear gradient lift force is balanced by the wall lift force. A schematic

view of these forces can be seen on Figure 1.1. On this figure, the particles migrate to equilibrium positions, indicated by the yellow dashed line. In that position the two lift forces are balanced, and the particles stay in that position. The particles are indicated by green dots on the figure, the green dot-dashed line is the channel centre line. The parabolic velocity profile is also depicted on the left-hand side.

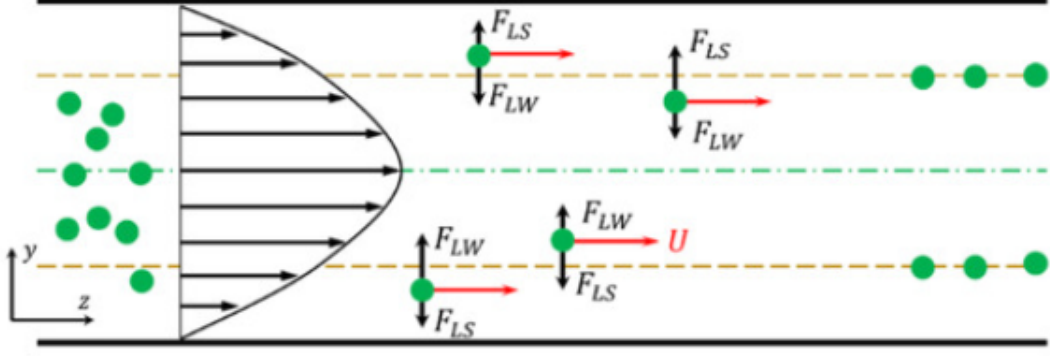


Figure 1.1: The flow field in a straight channel, the green dot-dash line is the centerline, the yellow dashed line indicates the equilibrium positions, the wall lift force (F_{LW}) and the shear gradient lift force (F_{LS}) are also apparent. This picture was adapted from the article of Zhang et al. [9].

Net inertial lift force

The wall lift force and the shear gradient lift force act against each other. The net of these forces, the net inertial lift force (F_L) can be calculated as:

$$F_L = \frac{f_L \rho U^2 d^4}{D_h^2}, \quad (1.11)$$

where ρ is the density of the fluid, U is the velocity of the fluid, d is the diameter of the particle, D_h is the hydraulic diameter of the channel, and f_L is a dimensionless lift coefficient, which is a function of the cross-sectional position of the particle [10]. When the net inertial lift force is zero, the two lift forces are equal and the particle is at an equilibrium position.

Dean force

The Dean force (F_{De}) arises if the fluid flows in a curved channel. It is due to the difference in velocity of the fluid between the inner and outer curve of the channel. The

fluid in the centre of the channel is swept towards the outer wall due to the centrifugal effect. The fluid near the outer wall then recirculates inward. This secondary flow in the channel causes the forming of two counter rotating vortices, called Dean vortices.

The emerging secondary flow is called Dean flow. The inertia of the fluid and the curvature of the channel determine the strength of this secondary flow. A dimensionless number can be defined as

$$De = Re \frac{D_h}{2r}, \quad (1.12)$$

where Re is the Reynolds number, D_h is the hydraulic diameter of the channel and r is the radius of the curvature. This number is called the Dean number.

Furthermore the Dean force (F_{De}) acting on the particles can be calculated as follows.

$$F_{De} = \frac{\rho U^2 d D_h^2}{r}, \quad (1.13)$$

where ρ is fluid density, U is the fluid velocity, d is the diameter of the particle, D_h is the hydraulic diameter, and r is the curvature radius of the channel.

1.2.2 Inertial migration of particles

When $Re \rightarrow 0$, the inertia is negligible. Consequently, inertial microfluidics works in an intermediate range, $1 < Re < 100$ [9]. Here both inertial and viscous forces are finite, and the equilibrium positions of the particles are dependent on the shape of the cross section, Reynolds number, the physical properties of the particles. Segre and Silberberg observed the inertial migration for the first time in 1961 [11]. In their experiments, spherical particles migrated to an annulus between the centerline and the wall of a cylindrical pipe that was approximately 0.6 times the radius of the pipe. In bounded conditions, such as channels, it is caused by parallel and perpendicular forces to the main flow direction. This can be observed on Figure 1.2a. Because of the circles symmetry, there are many different equilibrium positions. In case of rectangular channel cross-section, the aspect-ratio of the channel has a huge impact on the number and location of the equilibrium positions. It is defined as the ratio between the height and width of the channel ($AR = height/width$). If the cross-section is a square ($AR = 1$) (Figure 1.2b), the particles migrate into four equilibrium positions, in the middle of each wall. In case of

a rectangular cross-section, two cases must be considered: high-aspect-ratio rectangular channel ($AR \gg 1$) and low-aspect-ratio rectangular channel ($AR \ll 1$). On figure 1.2c the high-aspect-ratio rectangular channel can be seen, and on figure 1.2d the low-aspect-ratio channel is demonstrated. In both cases, the number of equilibrium positions is reduced to two, and these are in the middle of the longer walls.

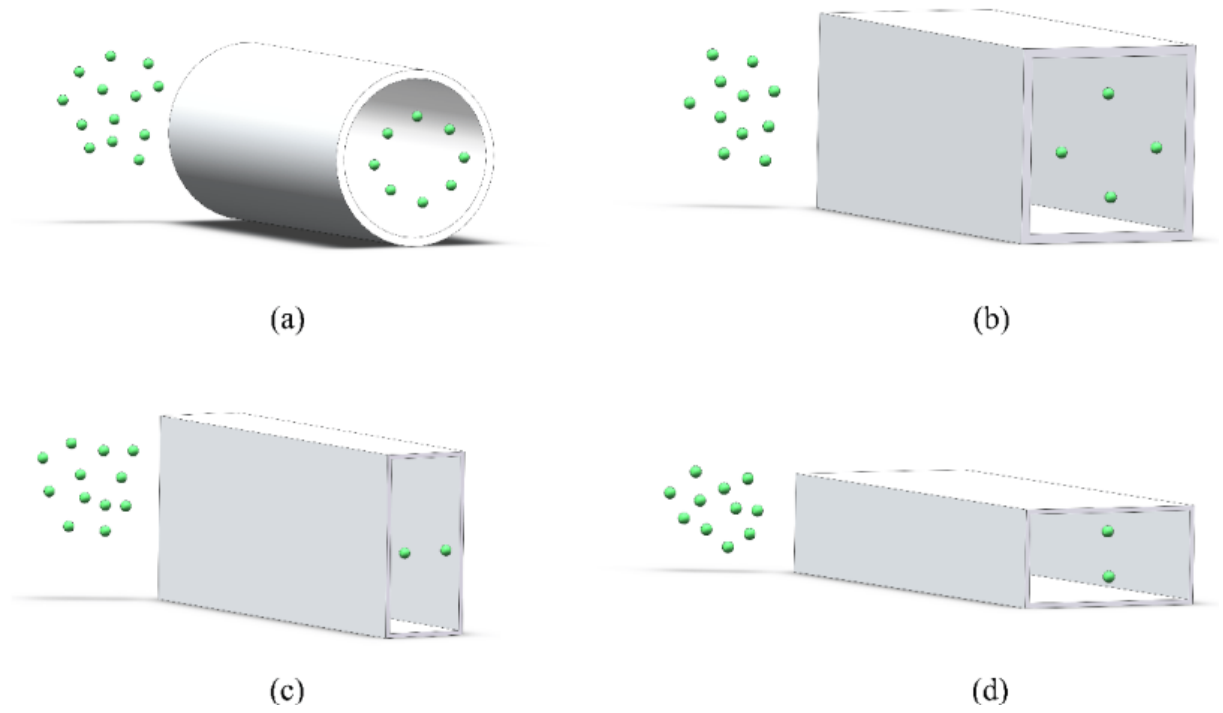


Figure 1.2: Equilibrium positions of particles in different cross-section channels, at intermediate Reynolds numbers. (a) circular pipe, (b) square channel ($AR = 1$), (c) high-aspect-ratio rectangular channel ($AR \gg 1$), (d) low-aspect-ratio channel ($AR \ll 1$). Figure was adapted from Sensors [12].

The effect of curvature

As mentioned before, even in straight channels particles migrate to equilibrium positions after a long enough distance. These positions can be altered and/or reduced by adding curvature to the geometry. In a curved channel the aforementioned Dean force arises, which results in a secondary flow in the channel. This secondary flow can reduce the number of equilibrium positions of the particles. Fewer equilibrium positions can lead to more precise separation of particles.

In a straight channel there is no secondary flow, therefore the Dean force (F_{De}) is zero. If the fluid flows in a curved channel, but $F_{De} \ll F_L$, then the Dean force is negligible and

the inertial lift forces drive the particles to equilibrium positions. Similarly, if $F_{De} \gg F_L$, then the lift forces have very little effect on the particles compared to the Dean force. This results in the particles following the direction of secondary flow. When the Dean force and the net inertial lift force is nearly equal, both of these forces act on the particle at the same time. This usually happens in the regime, where inertial microfluidics works.

1.3. Applications

Due to its ability to effectively manipulate particles and cells within microchannels, inertial microfluidics has found a wide range of applications in various fields. Inertial focusing is a well-known method in inertial microfluidics, where particles or cells are precisely aligned and focused into streamlines within a microchannel, facilitating their efficient sorting or analysis. It offers a label-free and high-throughput method for particle focusing, which is useful in applications that include e.g. cell sorting or microparticle separation. Since particles with different physical properties have different equilibrium positions, separation of for example circulating tumor cells from blood samples is possible [13]. This application is useful in cancer diagnostics and monitoring the conditions of cancer patients. Biomedical applications also include plasma extraction from blood samples [14]. Furthermore, these inertial microfluidics based devices are passive devices, hence they do not need any external sources to function. For this reason, they offer a cost effective alternative for the above mentioned applications.

Chapter 2

Overview of literature

In a straight channel the particles are migrating to equilibrium positions after a long enough distance. This was first observed by Segré and Silberberg, they experimented with a cylindrical pipe and the particles migrated to an annulus between the centre line and the wall of the pipe [11]. The channel's geometry has a significant impact on the particle's equilibrium positions. Even in a straight channel, the cross-section has a huge impact on the equilibrium position. To further alter these positions, a possible approach is to introduce curvature in the geometry. Curvature induces the appearance of Dean flow. This secondary flow, combined with the inertial lift forces, can alter the equilibrium positions of the particles, leading to more precise focusing.

This document is based around curved channels. Serpentine geometry is one of the possible design choices. The other design that uses curvature induced forces is the spiral geometry, but from now on the focus is on serpentine microchannels. Two types of serpentine geometries can be designed: symmetric and asymmetric. Both are sinusoidal geometries, but their curvatures are slightly different. Symmetrical serpentine channels have identical curves along the whole channel. In the other hand the asymmetrical design uses elliptical components to create a serpentine. This results uneven channel width at different parts of the serpentine. For example the positive half cycle of the sine wave is wider, than the negative half. Another possibility to introduce asymmetry into a serpentine is to have curves with different radii.

2.1. Comparison of straight, symmetric and asymmetric serpentine channels

In the experiments of Di Carlo et al [8], both symmetric and asymmetric serpentine channels were used. Additional forces emerge in these serpentine-shaped channels, which can change the equilibrium positions. As a control, they also used a simple, rectangular straight channel. This way, they could compare the effect of curved and straight channels and also symmetric and asymmetric designs. Their experiments were done with $9\ \mu m$ fluorescent particles. In the straight channel the increase of the flow rate, and with that the increase of the particle Reynolds number (Re_p) resulted in better focusing. Here four equilibrium positions were observed as previously demonstrated on figure 1.2 (b).

Both symmetric and asymmetric designs were able to reduce the number of equilibrium positions. Their research demonstrated that, compared to symmetric geometry, asymmetric geometry provides a more precise focusing. The device with the symmetric geometry focused the particles into two streamlines. On the other hand, they were able to focus the particles into one single streamline using the asymmetric geometry.

The length needed for focusing also differs in the different geometries. The longest distance is needed in the straight channel, here focusing was observed after $\approx 1\ cm$. Whereas in the asymmetric channel a much shorter distance, $\approx 3\ mm$ was sufficient. This is due to the arising secondary flow which results from the curvature in the geometry.

In the asymmetric channels, they also studied the size dependence of particle focusing. They experimented with various particle sizes, from $2\ \mu m$ to $17\ \mu m$. While $10\ \mu m$ particles were well focused, $2\ \mu m$ ones remained evenly distributed in the channel.

The experiments performed in the article demonstrate well the theory of particle focusing.

2.2. Enhanced asymmetrical serpentine channel

A sinusoidal asymmetric serpentine design was presented by Lei Wang and David S. Dandy [15]. They claimed that their device can concentrate particles as small as a micron and even sub-micron. They experimented with different flow rates and channel lengths. Their experiments with various flow rates showed, that above $6\ ml/h$ $2\ \mu m$ particles were

well focused and even 920 *nm* ones were following a predictable path. At this flow rate the Reynolds number exceeds 100, which means inertial forces acting on the particles are significant here. They also claimed, that the minimum length of the channel must be 4 *mm* to reach the desired focusing efficiency. This is much smaller, than those presented in other articles.

They also demonstrated that this device is able to focus bioparticles, more precisely cyanobacteria. This species can be genetically modified to utilize sunlight and CO_2 to produce biofuels [16], therefore successfully focusing these bioparticles can be useful for further investigation of their properties. To monitor the focusing of these bioparticles, the cyanobacteria was modified to express green fluorescent protein (GFP). GFP is frequently used as a molecular marker in biological and biomedical applications.

Their device has one inlet and right after that a filter region which serves to prevent clogging. This part was needed, because bioparticles were used in the measurements. The inertial separation region is an asymmetric serpentine, at the end of which is a widening where the separation into the outlets happens. On Figure 2.1 is the schematic view of their design. The height is uniform throughout the channel, it is 10 μm . The width of the small curves is 20 μm and at the large curves it is 80 μm . This is a 1:4 ratio between the narrow and wide parts of the serpentine. The length of the serpentine is 4 *mm*, which is much shorter, than presented in other articles.

The device was made out of thermoset polyester (TPE) instead of polydimethylsiloxane (PDMS). It has a similar fabrication process as PDMS, and it is also optically transparent. However TPE has a higher rigidity, which lowers its pressure induced deformation. This choice was made, because the experiments were done with a high flow rate. This causes high pressure in the narrow channel, thus the risk of deformation of the cross-section if PDMS is used.

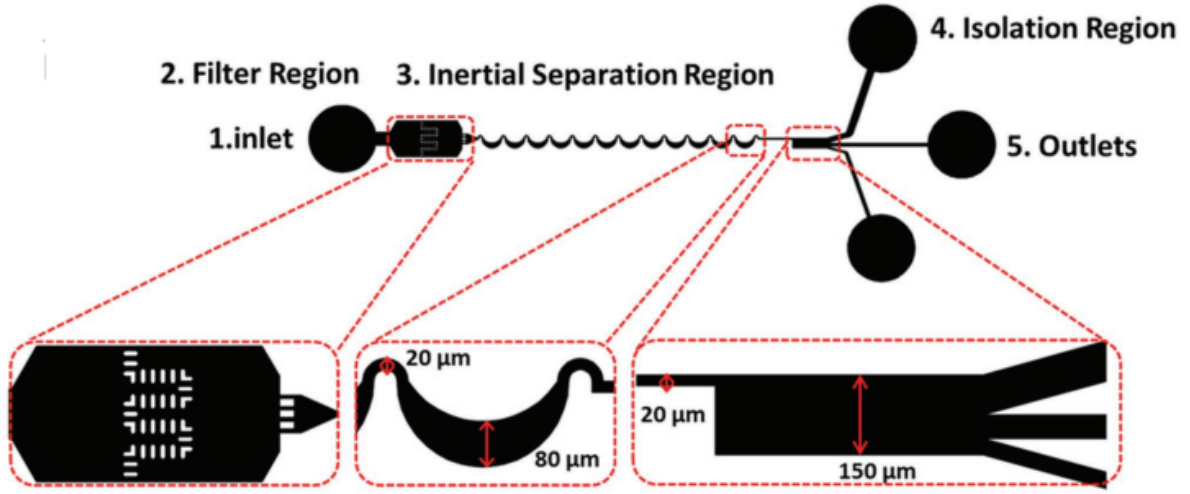


Figure 2.1: A schematic view of the device from the article of Lei Wang and David S. Dandy, the figure was adapted from Advanced Science [15]. The height of the device is $10\text{ }\mu\text{m}$, the length is 4 mm . The suspension is introduced into the device via the inlet (1.). Right after the inlet a filter region (2.) was inserted to prevent clogging. In the middle is the inertial separation region (3.), which is an asymmetric serpentine, the narrow part is $20\text{ }\mu\text{m}$ wide, the wider part is $80\text{ }\mu\text{m}$ wide. The end of the serpentine joins to a hydrodynamic opening. This is the isolation region (4.), where the separation of the particles into the three outlets (5.) happens.

Chapter 3

Materials and methods

Based on the literature presented above, three devices were created. One with the same dimensions of the serpentine, as in the article of Lei Wang and David S. Dandy [15] and one with the same 4:1 ratio, but with five times wider channels. The devices were designed in a way, that it can be compared with previous measurements done in the laboratory. They already experimented with asymmetric serpentes, but they used different ratios. It was an interesting path to take, to examine the effects of changing this parameter in the design. The third device is very similar to the second one, but the outlets join to the upper curve of the serpentine instead of the lower curve. This chapter discusses the design of these devices, the computational fluid dynamics simulations done with them, the fabrication technology and materials used in the laboratory, and methodology of the measurements and processing of the obtained data.

3.1. Design of the devices

The template of the device was designed using the Autodesk AutoCAD 2023 software. It is a computer-aided design application, that is used for designing and annotating 2D geometries and 3D models. For the first device the parameters given on Figure 2.1 were used for the exact sizes of the serpentine. The channel height was modified, because of the technology used in the laboratory. It is uniformly $20\ \mu m$ for each device, this is twice the height, than that of the device in the article. A few changes were made in the channel design compared to the articles. A longer serpentine and a different separation region was used. The filtration region was also left out, because there was no need for that in this

case, since no bioparticles were used. This device was named *CEA01*, because this was the first design. The geometry of *CEA01* can be seen on Figure 3.1.

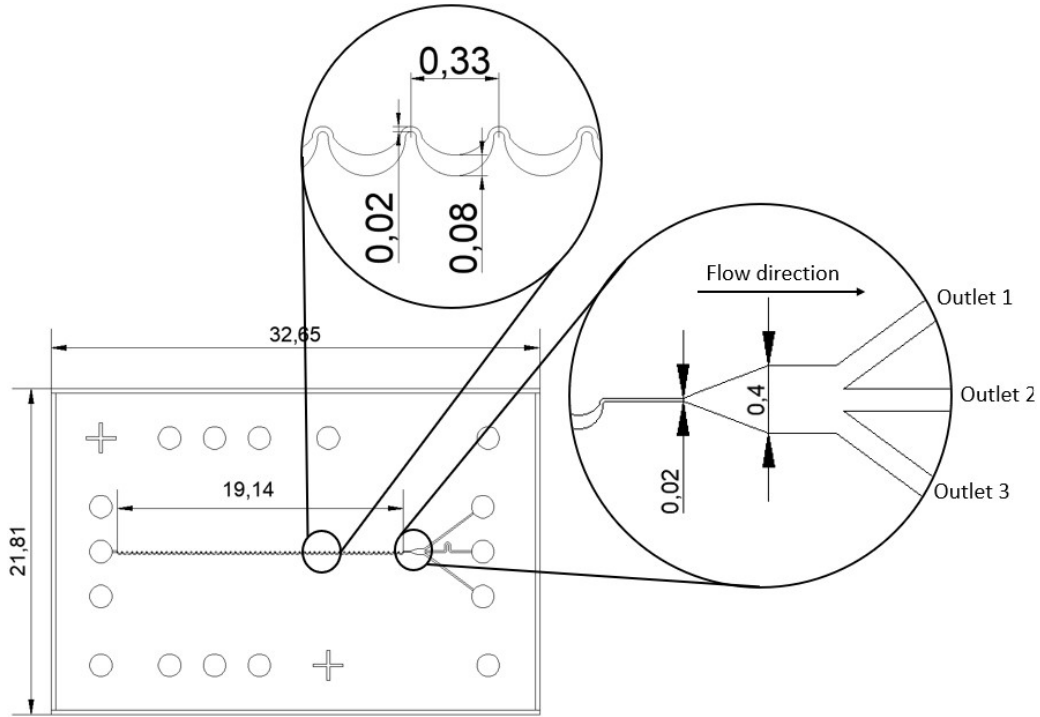


Figure 3.1: Images showing the CAD design of the device based on the article (*CEA01*). A top view of the whole channel geometry can be seen and two enlarged regions, one of the serpentine and one of the outlets. The height of the device is 0.02 mm , and the unit of measurement is in mm.

For the second device, as mentioned before the same 4:1 ratio was used, as in the article, but with a five times wider channel. This was important, so that it can be compared with previous measurements. It also allows a higher flow rate, which is useful, given that the time required for measurements is an important consideration when developing microfluidic devices. This device was named *CEA4x-v1*, because of the wide part of the serpentine is four times wider than the narrow part. Also this is the first version of this type of device. The finished design can be seen on Figure 3.2. The length of the focusing region is 19.2 mm .

In the previous experiments in the lab, the outlets in all devices were aligned with the lower curve of the last turn of the serpentine. The question arose, as to whether this affects the efficiency of the separation. The idea of the third device came from this question. This is version 2 of the device *CEA4x-v1*, hence the name *CEA4x-v2*. It is very similar to the first version, but the outlets join to the upper curve of the serpentine

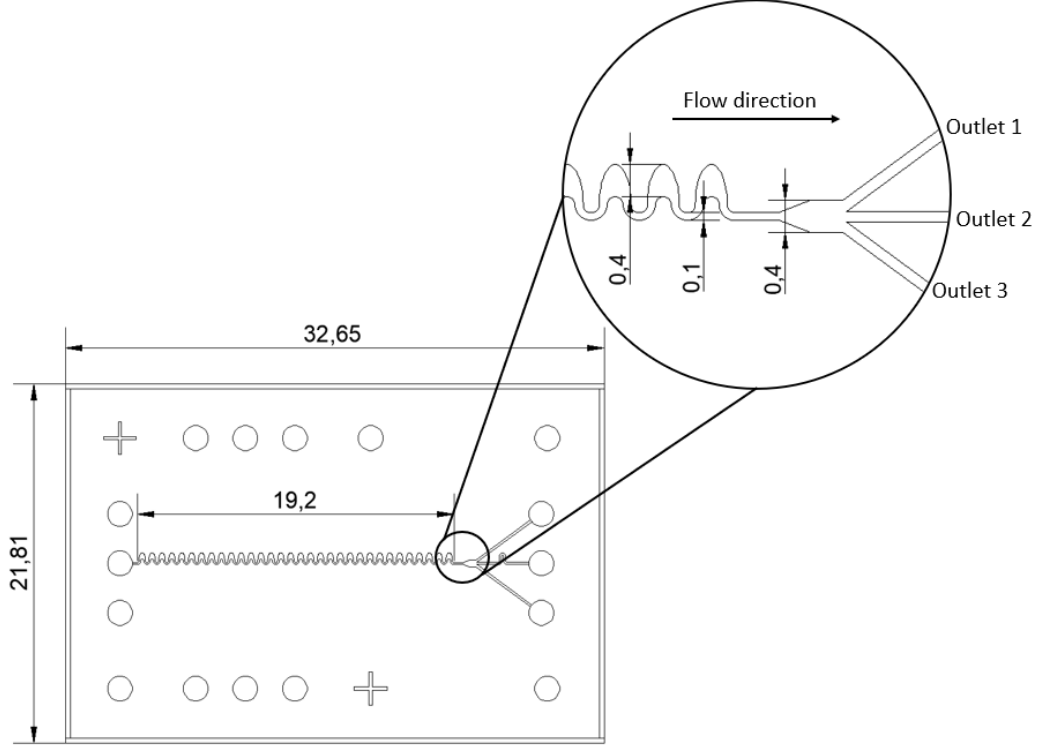


Figure 3.2: The design of the second device, *CEA4x-v1*. A top view of the geometry of the whole channel can be seen and two enlarged regions, one of the serpentine and one of the outlets. The height of the device is 0.02 mm , and the unit of measurement is in mm.

instead of the lower curve. This device can be seen on Figure 3.3.

There were multiple things to pay attention to while creating the design. One of them was, that everything had to be a closed polyline. Another important one was to not have overlapping shapes. On Figure 3.1, 3.2 and 3.3 are the finished designs of the three devices. The pictures are annotated and some parts were enlarged for better understanding. The dimensions are meant to be interpreted in mm. These are 2D designs, therefore the height can not be represented, but it is uniformly 0.02 mm . The length of the focusing region is about 19 mm , at the end of the serpentine a hydrodynamic opening is designed, where separation into the two outlets can happen. This part is uniform for all three devices. The outlets are numbered from top to bottom, later on they will be referred to as outlet 1, 2 and 3.

For comparison a fourth device was used in the measurements. This device was included in previous experiments done in the laboratory. It has the name *CEA3x*, because in the serpentine region it has a 3:1 ratio between the wide and narrow parts. The geometry of this device is presented on Figure 3.4.

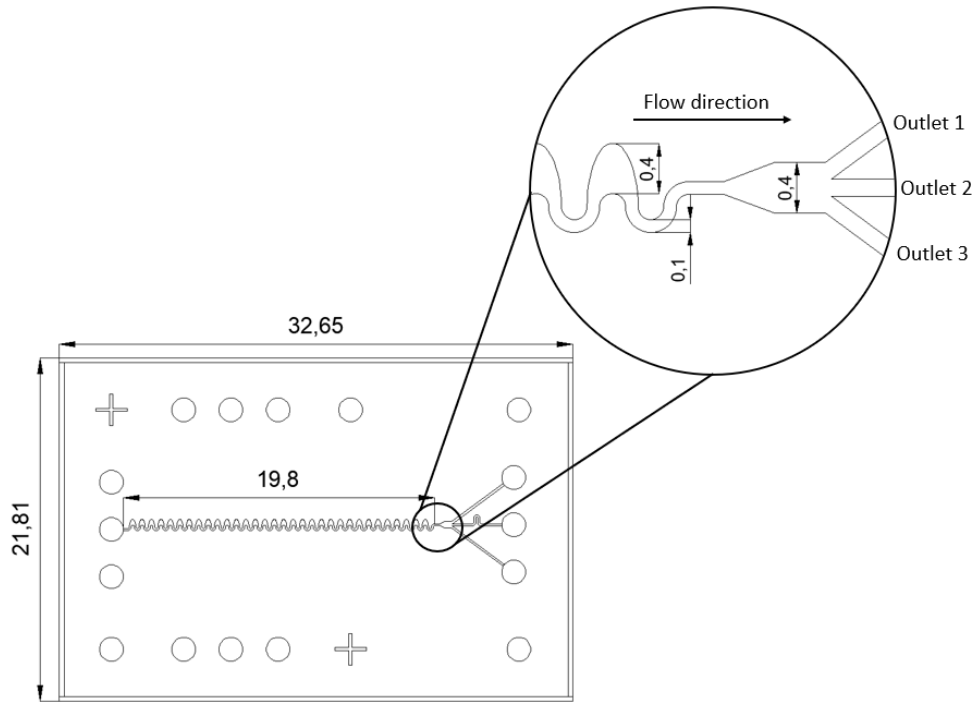


Figure 3.3: The design of the second device, *CEA4x-v2*. A top view of the geometry of the whole channel can be seen and two enlarged regions, one of the serpentine and one of the outlets. The height of the device is 0.02 mm , and the unit of measurement is in mm.

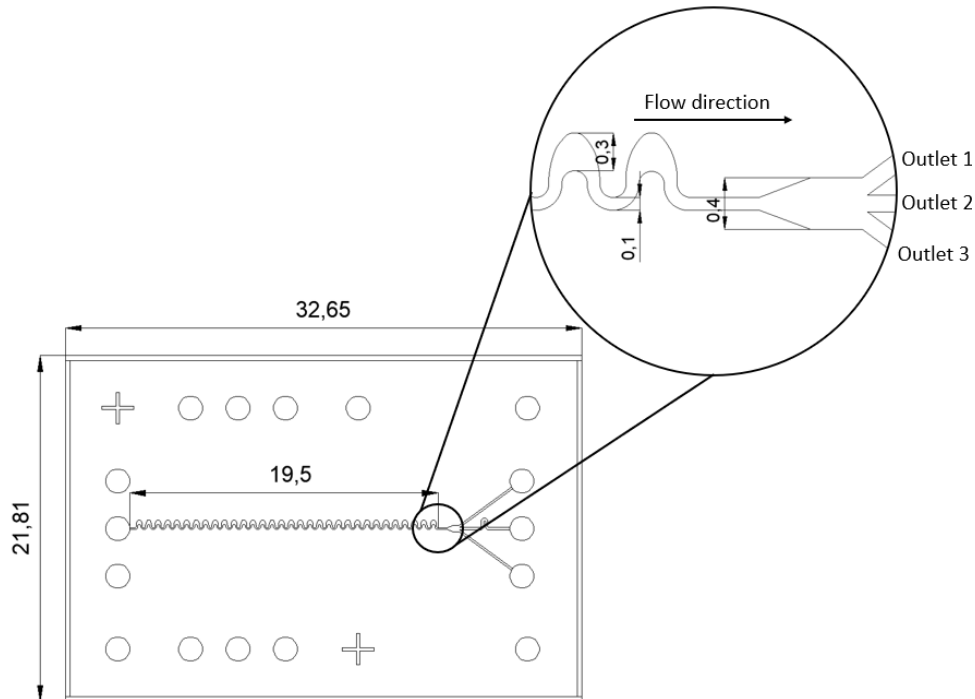


Figure 3.4: The design of the device *CEA3x*. A top view of the geometry of the whole channel can be seen and two enlarged regions, one of the serpentine and one of the outlets. The height of the device is 0.02 mm , and the unit of measurement is in mm.

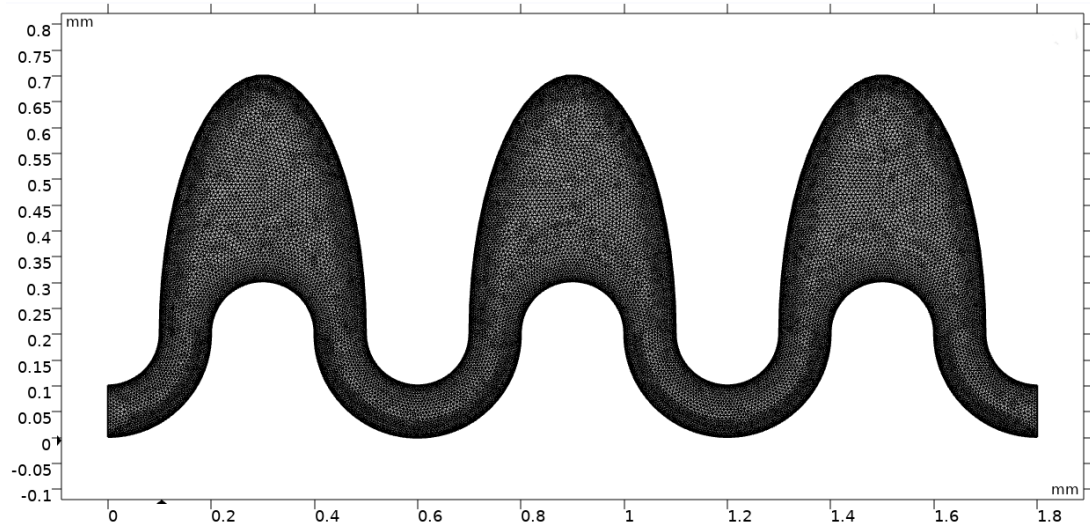
3.2. Computational fluid dynamics simulation

Computational fluid dynamics (CFD) uses numerical methods and algorithms to solve problems that involve fluid flow. It connects digital computers and fluid dynamics to simulate the behaviour of fluids. CFD simulations help to gain a better understanding of the operation of the devices and allows a cost-effective and efficient analysis. However CFD simulations require making various assumptions and simplifications to represent complex physical systems. These assumptions can introduce inaccuracies in simulations. With that said these simulations are very useful, but they have to be treated with caution. The CFD simulations presented in this document were made by using Comsol Multiphysics 5.5.

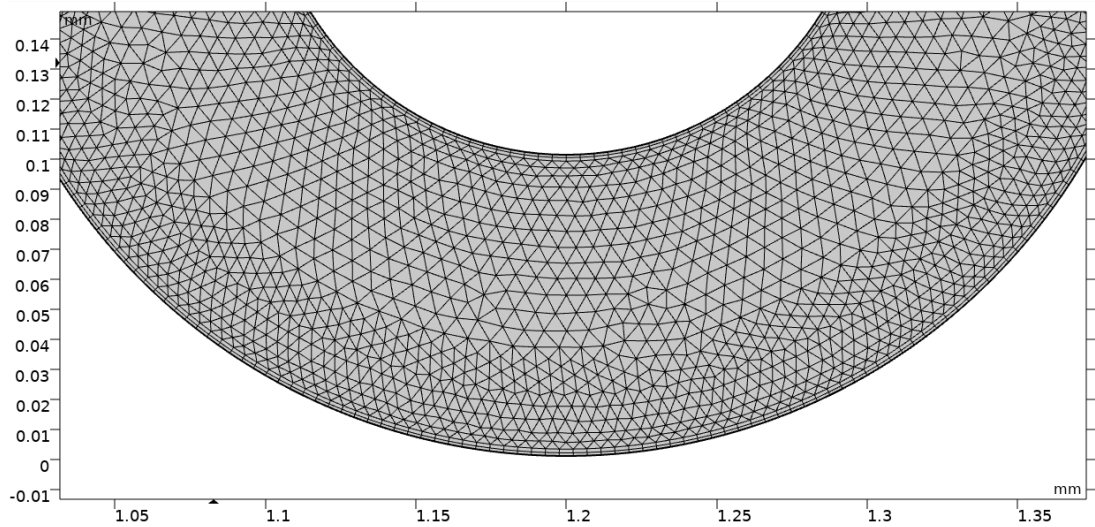
For these simulations only part of the design was used, the parts that are not relevant for the calculations were cut off. This was necessary to reduce the computation time. These modifications were done in Autodesk AutoCAD 2023, just like the original design. The drawing had to be exported from AutoCAD as a .dxf file, and imported into Comsol Multiphysics. After defining the inlet and the outlets, as material, water was added to the simulation. The dynamic viscosity and the density of the material is defined as a functions, dependent on the temperature. This parameter is globally defined as 293.15 K . Having done the calculations, the dynamic viscosity of the water is $0.001\text{ Pa} \cdot \text{s}$, and the density is $998\frac{\text{kg}}{\text{m}^3}$. The simulations use these values in the calculations.

The next step was calculating the mesh. It was important to choose the right mesh for the calculations, because it has a huge effect on the computing time and also the accuracy of the results is strongly linked to the mesh size. There are multiple mesh types to choose from regarding the size and shape of the elements. In this design a physics-controlled mesh, that is automatically adapted to the models physics settings, was used. The element size can also be chosen, the default setting is *normal*. In this model this setting was modified to an *extremely fine* element size. This means that the size of the elements is in the range of $0.0903\text{ }\mu\text{m}$ to $7.83\text{ }\mu\text{m}$. This was possible, because the simulated part of the geometry is relatively small. This element size gives a more accurate result, than one with bigger elements. On Figure 3.5a the built mesh can be seen and on 3.5b an enlarged part of the mesh is depicted. As can be seen on these figures, the elements making up the mesh are very small. This will predictably lead to good results

during the calculations.



(a)



(b)

Figure 3.5: The mesh plotted on the device *CEA4x-v1*. The element size is *extremely fine*, which means the size of the elements in the mesh are between $0.0903 \mu m$ and $7.83 \mu m$. (a) the whole calculated mesh, (b) an enlarged part of the mesh.

The the pressure profiles and the velocity profiles were calculated for the $3x$ and $4x$ devices and streamlines were plotted on these. For these simulations a stationary study was used, and laminar flow as physics. Comsol Multiphysics uses the Navier-Stokes equation for calculating the velocity and pressure profiles. It assumes, that the left side of the equation is zero. This simplifies the calculations, but it might not be the best approximation. Stokes flow implies laminar flow, but this statement is not always correct

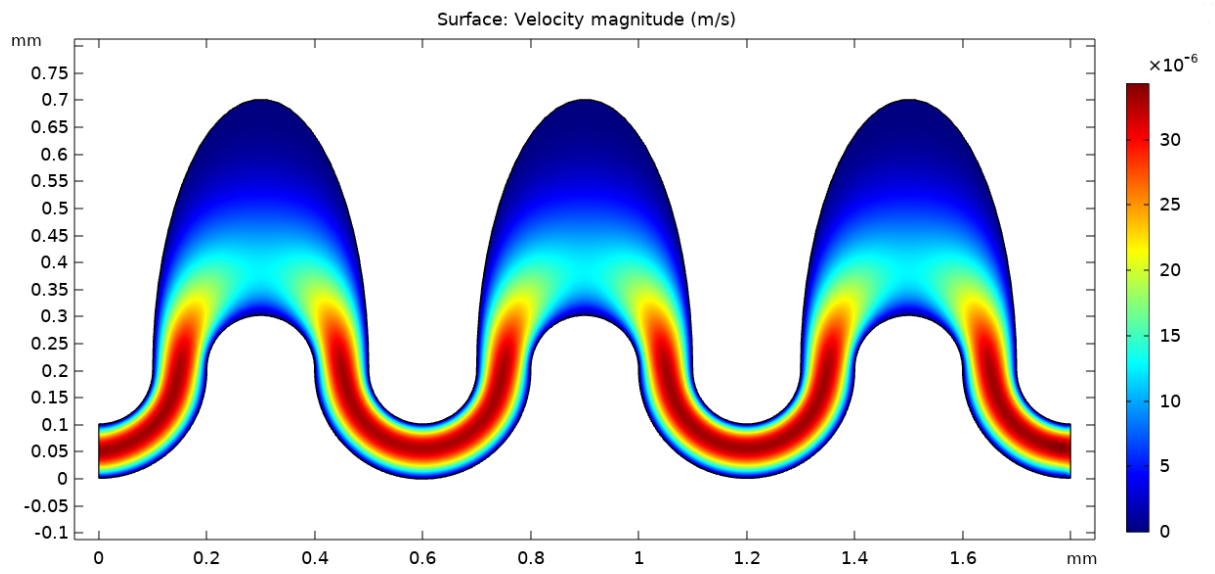
backwards. Laminar flow does not necessarily mean the flow meets the characteristics of the Stokes flow. Therefore, when simulating laminar flow, assuming Stokes flow can in some cases lead to inaccurate result. With that said, these simulations are still a good starting point for understanding the operation of the device, but they have to be interpreted with caution.

The inlet and outlet were defined after importing the geometry. This was done by selecting the walls of the geometry, where the inlet and outlet would be. For the inlet a boundary condition had to be set. For this fully developed flow was chosen, which means that the velocity profile across the pipe's cross-section remains constant and does not change along the length of the pipe. This setting assumes, that the flow has had sufficient length to become fully developed. The flow rate of the fluid also needed to be defined here, this parameter was set to a constant 8 ml/h for every simulation.

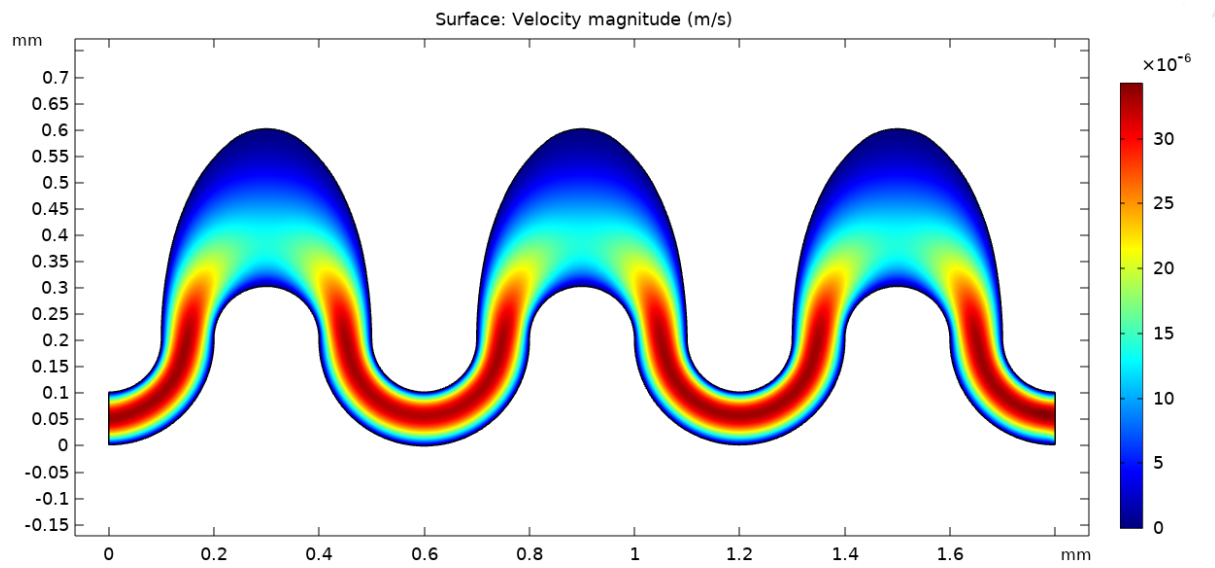
CFD simulations were done on the devices *CEA3x* and *CEA4x-v1*. The simulation for *CEA4x-v2* was not needed, because the serpentine part of this device is identical to the serpentine of *CEA4x-v1*. As mentioned before a stationary study was calculated, the computation time of the study was about $5 - 10 \text{ s}$. The result of this simulation was the velocity profile and pressure profile of the fluid flowing in the channel. The streamlines in the channels were also plotted on the velocity profile. These can be seen on Figure 3.6 and Figure 3.7.

The velocity profile (Figure 3.6) shows, that the fluid near the walls is slower than in the middle of the channel. This is as expected and corresponds to what was explained in the theoretical introduction. Also in both devices the velocity of the fluid is significantly higher in the narrow parts of the serpentine. In the widening, the velocity decreases. Since in case of *CEA4x-v1* this part of the channel is by $100 \text{ }\mu\text{m}$ wider, the velocity is slower here, than in *CEA3x*. This difference between the two devices could lead to difference in their focusing efficiency too.

In case of the streamlines plotted (Figure 3.7) it can be observed, that the narrow part is roughly the same in both devices. At this part the streamlines are close to each other, because of the high velocity and the narrowing of the geometry. As the channel widens and the velocity decreases, the streamlines move away from each other. The distance of the streamlines from each other is larger in case of *CEA4x-v1*. This is also due to the fact, that this geometry is wider here, than in the design of *CEA3x*.



(a)



(b)

Figure 3.6: The calculated velocity profiles of $CEA4x-v1$ (a) and $CEA3x$ (b). The dark red indicates the highest velocity and the dark blue indicates the lowest velocity. The fluid is the fastest at the narrow segments, while as the channel widens the flow slows down.

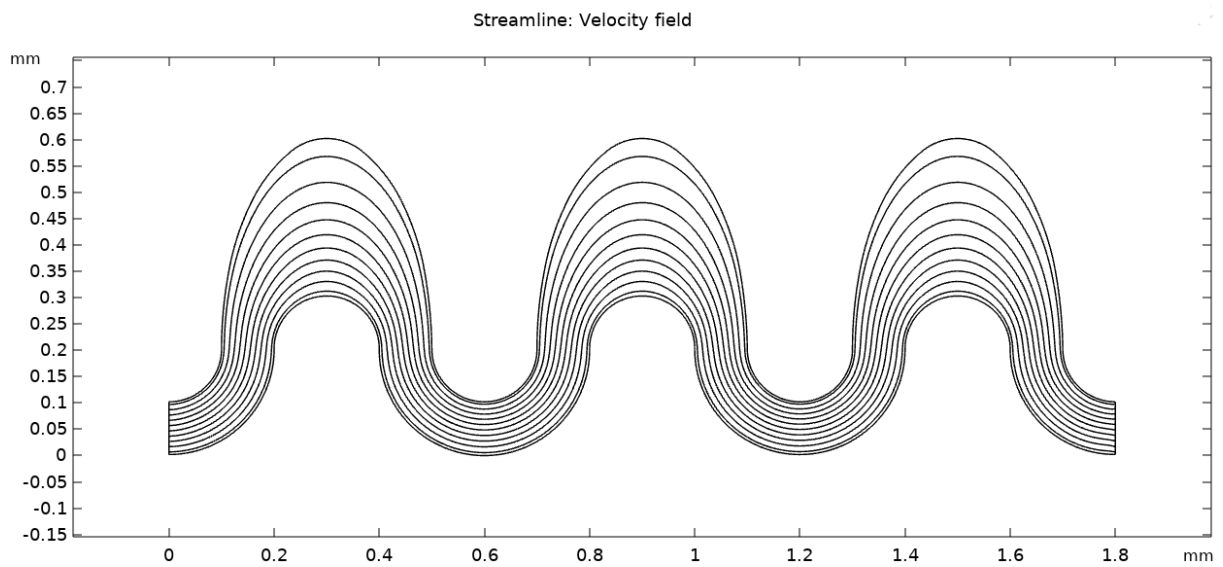
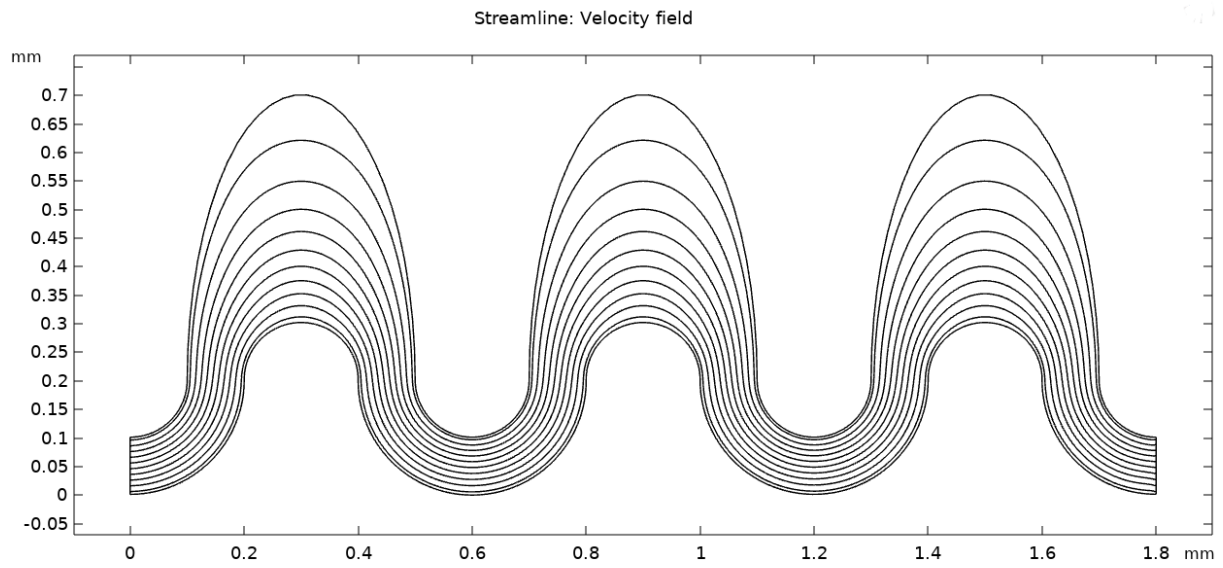


Figure 3.7: The plotted streamlines on $CEA4x-v1$ (a) and $CEA3x$ (b). The streamlines are closest to each other at the narrow parts of the serpentine and they move away from each other as the channel widens. In case of $CEA4x-v1$ (a) the distance of the streamlines is larger in the widening, than in $CEA3x$ picture (b).

The pressure profile (Figure 3.8) shows, that the pressure is highest at the inlet and gradually decreases towards the outlet. The high pressure at the inlet suggests that a significant driving force is required to push the fluid into the device. As the fluid traverses the serpentine, it encounters flow resistance due to the geometry of the channel. The channel has a low aspect-ratio and there are very narrow parts, this explains the high pressure at the inlet. This simulation gave fairly similar results for both devices. There is a slightly higher pressure in case of *CEA3x*, this is a result of the narrower serpentine design.

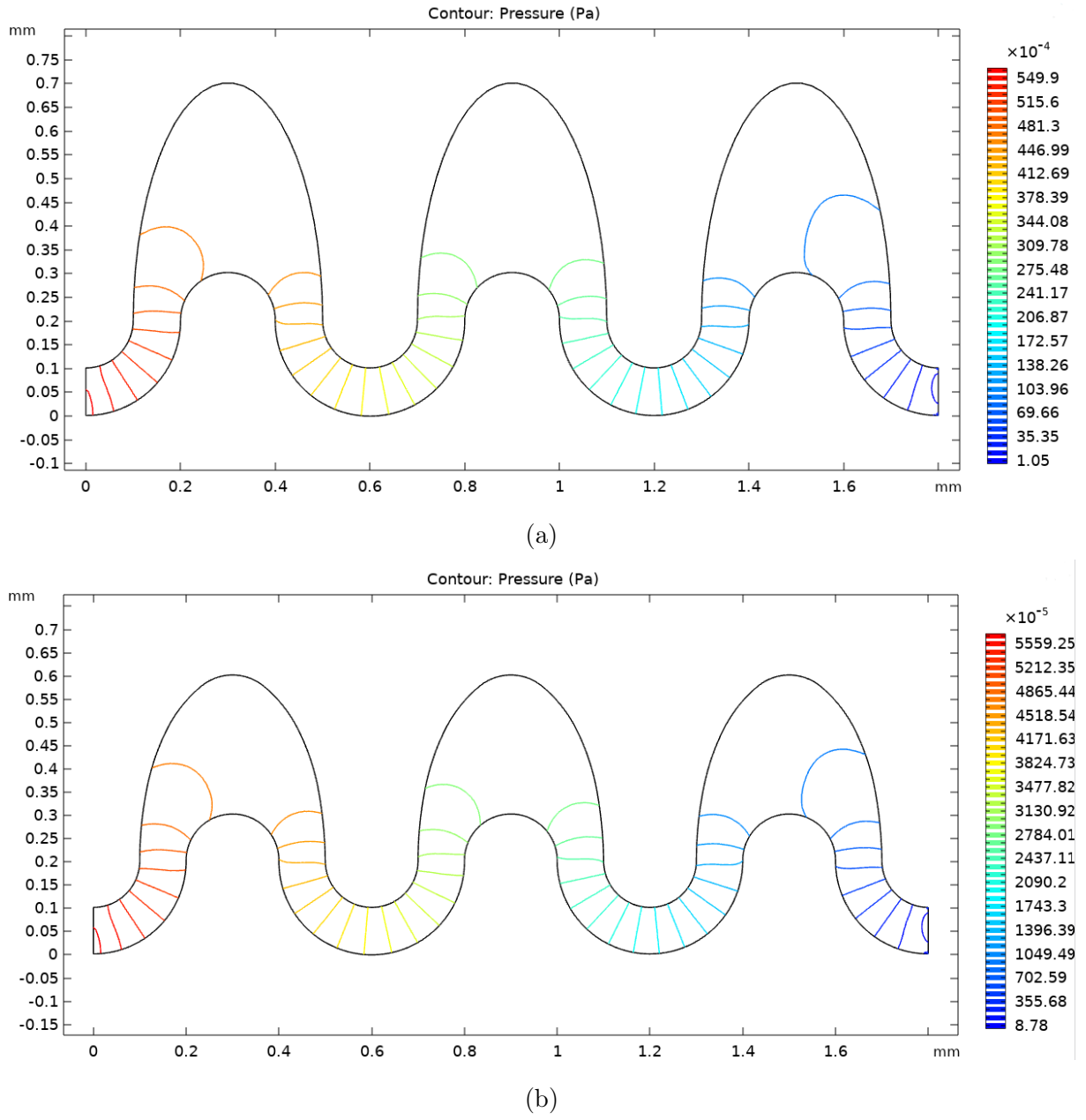


Figure 3.8: The pressure profiles of $CEA4x-v1$ (a) and $CEA3x$ (b). The pressure is highest at the inlet and gradually decreases towards the outlet. A coloured legend can be seen on the right side, the unit of measurement is Pa .

3.3. Fabrication technology

The devices were produced using a fabrication method known as the PDMS-glass technique. Polydimethylsiloxane (PDMS) is frequently used in the creation of microfluidic devices [17]. It is an elastomer, and due to its deformability, it is simple to mold with micrometer accuracy. Since it is optically transparent down to 300 nm, optical detection is possible. This aspect of PDMS is crucial, because it makes constant monitoring possible during the measurements. Another important feature of PDMS is biocompatibility, which makes it suitable for utilisations involving cells and tissues. Furthermore, it allows for the interchange of gases like oxygen and carbon dioxide with the environment, which is necessary for applications including cells or tissues. Its widespread application in the creation of microfluidic devices was made possible by both its wide range of beneficial qualities and its low cost.

The process of creating the device started with making a master mould. For this step a photoresist material was poured on a silicon wafer. A photoresist is material, that if illuminated with a specific light, undergoes a chemical change, making it either more soluble (positive photoresist) or less soluble (negative photoresist) in a developer solution. This means, that in case of a positive photoresist, the parts that are exposed to the light become more soluble in a developer solution, allowing them to be easily dissolved and removed. Negative photoresist materials behave the opposite way, the parts that are exposed to the light become less soluble in a developer solution, so the parts that are not exposed will be removed later. Photoresist materials are engineered to provide high-resolution patterning capabilities. They enable the creation of intricate and fine features on the substrate, essential for advanced microfabrication processes.

To ensure that the photoresist was spread evenly, spin coating was used. After that, a prebake step followed, which allows the photoresist to set slightly. A direct laser writer was used to transfer the channel pattern. Since a negative photoresist was used, the material polymerized in the areas that were exposed to the laser beam. After the exposure was done, a post bake took place to complete polymerisation process. The parts that were not exposed were removed, leaving only the desired pattern on the wafer, this step is called development.

After the master mould was done, the soft lithography step followed. There are two parts to PDMS, and they should be combined in a 1:10 ratio (curing agent to base). Once combined, the material was uniformly poured into the mold and heated to 65 °C in the oven for about an hour to complete polymerization. By doing so, the PDMS can solidify and the pattern on the polymer will be transferred from the mould. The PDMS had been taken out of the mould after baking. The sides of the PDMS block were cut off with blade, to clean up its edges. Next the inlets and outlets were pierced with a biopsy needle. These little holes will be where the teflon tubes connect to the device.

The surface properties of the PDMS and glass were modified with plasma treatment, then pressed together to create a Si-OH bond between the two materials. This is a strong bond, that seals together the two materials. After the bonding is done, the fabrication of the device is considered finished, and it is ready to use. One of the finished devices can be seen on Figure 3.9. The channel was filled with blue food coloring to make it more visible.

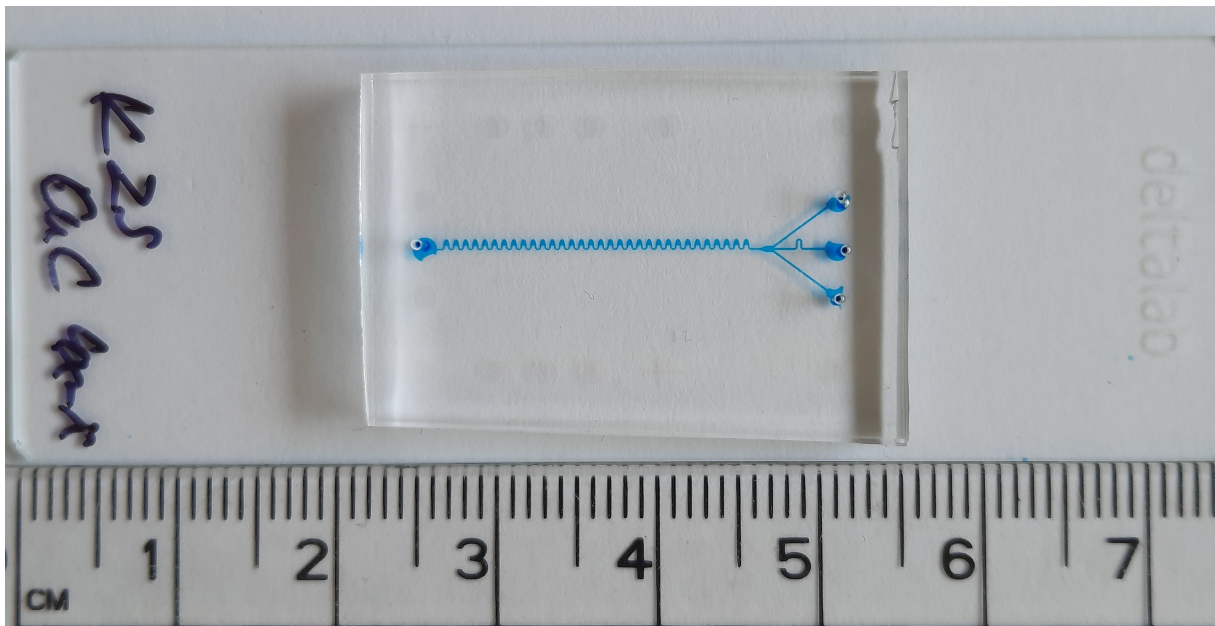


Figure 3.9: One of the finished devices. The channel was filled with blue food coloring to enhance its visibility.

3.4. Materials

At the beginning of each measurement the sample solution was prepared. This was done by measuring out distilled water, Tween 20 stock solution diluted with distilled water in 1:20 ratio, then adding some microbeads of the desired sizes. These microbeads are plain micromer[®] particles, produced by micromod Partikeltechnologie GmbH. They are white polystyrene particles, that can serve as a size scale, or in this case as the particles to be focused and separated. The particles are available in the size range of 25 *nm* to 10 μm . In the measurements presented in this document particles in the size range of 1 μm to 10 μm were used. The specifications of these particles are in Table 3.1. Each particle size has a unique product ID. For every particle size, the concentration of the solution is 50 *mg/ml*. However, the 1 μm particles have a different mass, than the 10 μm ones for example. As a result of this, the number of particles per ml differs for each particle size. The bigger the size, the less particles are in 1 *ml* of solution.

Product ID	Diameter (μm)	Concentration (<i>mg/ml</i>)	Particles per ml
01-00-103	1	50	$9.5 \cdot 10^{10}$
01-00-203	2	50	$1.2 \cdot 10^{10}$
01-00-303	3	50	$3.4 \cdot 10^9$
01-00-403	4	50	$1.4 \cdot 10^9$
01-00-503	5	50	$7.4 \cdot 10^8$
01-00-603	6	50	$4.3 \cdot 10^8$
01-00-703	7	50	$2.7 \cdot 10^8$
01-00-803	8	50	$1.8 \cdot 10^8$
01-00-104	10	50	$9.3 \cdot 10^7$

Table 3.1: Plain micromer[®] particle specifications. Each particle size has its Product ID on the website of micromod Partikeltechnologie GmbH. They sell 10 *ml* of the product with a concentration of 50 *mg/ml*. The amount of particles per milliliter is depicted, it is based on the size of the particles. The smaller the particle, the higher amount of it is in 1 *ml* of product. The parameters were adapted from the website of the manufacturer, micromod Partikeltechnologie GmbH [18].

From now on the Plain micromer[®] particles will be referred to as microbeads or just particles. These particles were mixed into the sample used in the measurements. Because of the differences in the number of particles per milliliter, a different amount of microbeads were added, based on their size. The exact volumes of each ingredient can be seen in Table 3.2.

Microbeads		Distilled water (μl)	Tween 1x (μl)
Size (μm)	Volume (μl)		
1	1	989	10
2	1	989	10
3	2	988	10
4	2	988	10
5	4	986	10
6	4	986	10
7	4	986	10
8	4	986	10
10	4	986	10

Table 3.2: Materials used for sample preparation. Quantity of distilled water, Tween 1x solution, and microbeads based on the size of the microbeads used to create 1 ml sample.

3.5. Methology of the measurements

Throughout the measurements, there were a lot of things to pay attention to. The most important one was that, while preparing the sample and during the measurement, care had to be taken to keep dust and other contaminations out. This was important, because these contaminations in the sample can clog the devices, making them unusable. To avoid this, the working platform and its surroundings were carefully cleaned prior to each measurement. Gloves were also worn while preparing and performing the measurements. Owing to these precautions and precise work, there were no significant problems with clogged devices.



Figure 3.10: The assembled working platform. On the left side is the syringe pump on an elevated platform, the device is on the microscope and the outlets are placed in centrifuge tubes.

3.5.1 Measurements for particle counting

When the sample was done, in the next step, the working platform was put together. Since the devices have one inlet, one syringe pump was needed in the measurements. A stepper motor ensures that the flow rate is constant throughout the experiment. The diameter of the syringe (9.7 mm) and the flow rate needed to be set on the syringe pump. Teflon tubes were connecting the syringe and the device, and also the outlets and the centrifuge tubes. About 2 ml of the sample solution was in the syringe in each measurement and a little more than 1 ml was pumped through the channels and collected at the outlets in separate centrifuge tubes. The device was set up on an inverted light microscope to allow the inspection of the channel. The microscope was also equipped with a camera, so taking pictures and constant monitoring was possible during the measurements. The setup can be seen on Figure 3.10.

The flow rate was set to 8 ml/h for the measurements that included the use of the

Bürker chamber, this parameter was predetermined by the research group, so that my measurements could be compared with previous experiments. After starting the syringe pumps, I collected the outlets in one centrifuge tube until the flow stabilised in the device and then the samples from the three outlets were collected separately during the measurements. The measurement continued until enough samples were collected for the processing with Bürker chambers. Each particle size was measured three times, independent of each other.

3.5.2 Measurements with varying flow rates

For this type of measurement the working platform was assembled as shown on Figure 3.10, but the outlets were collected in one centrifuge tube. It was not necessary to collect the three outlets separately, because the essence of the measurement was where the particles were located within the channel. The sample collected from the outlets could be reused during the measurement. Therefore less materials were needed for each experiment. To make this possible, extensive caution was required to prevent contamination of the sample.

In the measurements $3\ \mu m$, $6\ \mu m$ and $10\ \mu m$ sized particles were used. Each measurement started with an initial speed of $3\ ml/h$, then after the flow stabilised a picture was taken of the last turn of the serpentine and the outlets. After the picture was taken, the flow rate was increased by $1\ ml/h$. This process was repeated until the flow rate reached $37\ ml/h$. This was the maximum velocity, that all three devices could handle. This process was done with all three bead sizes and with three different devices (*CEA3x*, *CEA4x-v1* and *CEA4x-v2*). Each measurement took about 30 minutes to an hour.

3.6. Processing of the obtained data

3.6.1 Particle counting with Bürker chambers

Different sized microparticles were measured in the experiments. To evaluate the efficiency of the device, Bürker chamber was used. It is a thick glass slide, with a grid of perpendicular lines etched on it with laser. The lines of this grid are of specific distances from each other, allowing cell counting in a specific area, in which the volume of the fluid

is known. Therefore concentration of cells can be determined.

Before use, the cleanliness of the chambers had to be checked. If they were not properly clean, a soapy wash was needed to achieve the desired outcome. When the chambers were clean enough, a thin glass cover was placed on the top of them. About 10 μl of the sample was loaded under the glass cover using a pipette and the chamber was placed under the microscope. Pictures were taken of each of the nine sections of the chamber for manual counting later. After making sure everything was documented, the chambers needed to be washed off before the sample dried on them.

The number of particles were counted manually. Depending on the size of the particles, usually six different sections were counted and the mean of those was calculated. The number of particles are calculated with Equation 3.1.

$$N_i = \text{average}(n) \cdot 9, \quad (3.1)$$

where N_i is the number of particles in the Bürker chamber of Outlet i., n is the number of particles in one 4x4 section of the Bürker chamber. Great emphasis was placed on consistency while counting. On Figure 3.11 you can see which particles were included or excluded from the calculations of a section. The green checked particles were included, and the red x marked particles were excluded from the calculations.

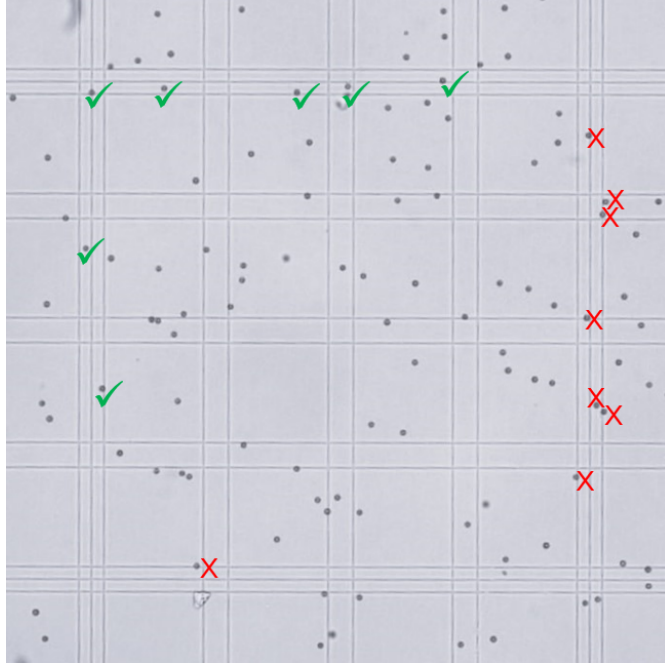


Figure 3.11: The method of counting the particles in the Bürker chamber. The green checked particles were included, the red x marked particles were excluded from the calculations.

The percentage distribution of the particles was calculated with the equation:

$$N_i\% = \frac{N_i}{N_1 + N_2 + N_3}, \quad (3.2)$$

where $i = 1, 2, 3$, and $N_i\%$ shows what percentage of particles went to the i . outlet. After the percentage distribution was obtained, the values for each particle size was plotted on a diagram. Being that three individual measurements were performed for each particle size, therefore the mean of these results was plotted along with the standard deviation of the data.

3.6.2 Comparing the effect of varying flow rates on different sized particles

The pictures from the measurements for each particle size in all three devices were edited together for comparison. The processing of these pictures was done with the help of ImageJ, which is an image processing software developed at the National Institutes of Health. With ImageJ, the distance between the upper wall and the streamline of the particles was measured on each picture. An example of the measured distance can be seen

on Figure 3.12. The comparison and evaluation of the images and distances were done by visual analysis. From the measurements with ImageJ diagrams were made for better understanding and visualisation.

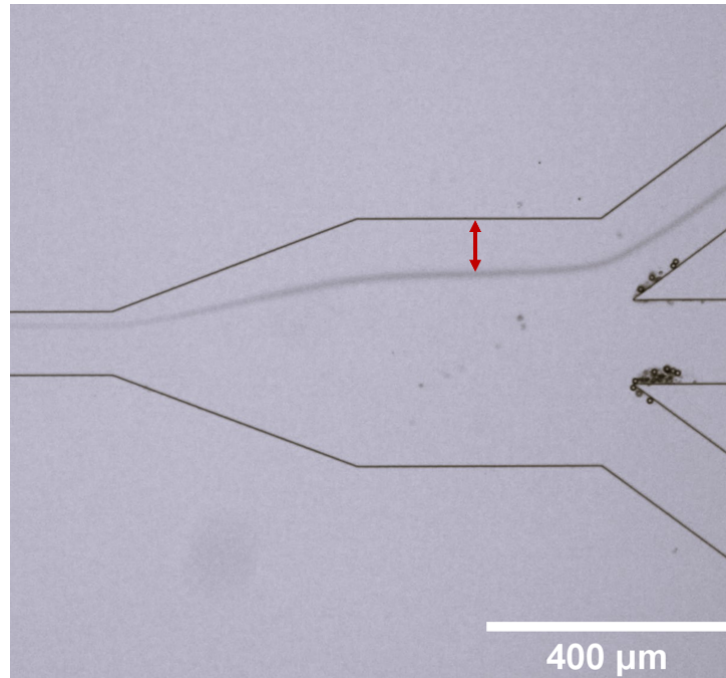


Figure 3.12: An example of the distance measurement. ImageJ was used to determine the distance along the red arrow. The scale bar on the bottom right is set to $400\ \mu m$, which is the width of the channel at its widest part.

Chapter 4

Results

This chapter discusses the results of the performed measurements. The results are composed of Bürker chamber calculations and image processing with ImageJ. The Results of the Bürker chamber calculations are based on three measurements with each microbead size, independent from each other. The data represented on the diagrams is the average of these three measurements.

4.1. Measurement 1

The first measurement was done using the device *CEA01*. In case of this device, the experiment was performed with $5\ \mu m$ and $3\ \mu m$ particles. First, $5\ \mu m$ particles were attempted to be measured at an $8\ ml/h$ flow rate, but this turned out to be too much for this device. At $8\ ml/h$ the pressure was so big, that the teflon tubes could not stay in place, thus the experiment had to be shut down. The reason for this was, that the height and width of the channel are small, and the serpentine is relatively long. After this incident it was decided to use $5\ ml/h$ flow rate with this device. As expected the particles were focused in outlet 2 for both $5\ \mu m$ and $3\ \mu m$ sized particles. However it was visible, that the $3\ \mu m$ particles were less focused, they had a wider streamline than the $5\ \mu m$ ones. Lei Wang and David S. Dandy [15] experimented with smaller particles, but the dimensions of their device were also much smaller. They came to the same conclusion, that smaller particles have a wider streamline, thus bigger particles are more focused. This device and the measurement with it served as the basis for the planning and execution of further experiments with the other devices.

4.2. Measurement 2

To determine the focusing efficiency of the device $CEA4x-v1$ and $CEA4x-v2$ a series of measurements were done with different sized particles. In every experiment the flow rate was set to 8 ml/h . The outlets were collected in three different centrifuge tubes. Each particle size was measured three times, and all three measurements were independent of each other. After the measurements the obtained data was evaluated using the Bürker chamber. Equation 3.2 was used to calculate the percentage distribution of particles between the three outlets. Since three independent measurements were performed for each particle size for both devices, the standard deviation of these measurements was calculated as well.

The results of these calculations are depicted on figure 4.1 and 4.2. The percentage distribution of the different sized particles between the three outlets, and the standard deviation of the measurements are indicated. It can be seen that both devices successfully focused the particles into outlet 1 down to $2\text{ }\mu\text{m}$. The $1\text{ }\mu\text{m}$ particles were almost evenly distributed between outlet 1 and outlet 2, but about $4 - 7\%$ more went to first outlet. These results show, that the position of the outlets (whether they join to the upper or lower curve of the last turn of the serpentine) did not make much of a difference, it has no to minimal effect on the focusing and separation of the particles.

These results show, that both devices are efficiently focusing particles down to $2\text{ }\mu\text{m}$. In the article of Lei Wang and David S. Dandy [15], they focused particles as small as 920 nm into predictable paths, but they also had a much smaller channel. The channel cross section and the aspect ratio has a huge impact on the equilibrium positions. Note that their device was five times narrower and the height of their channel was half of the ones presented in this document. However $CEA4x-v1$ and $CEA4x-v2$ have much bigger dimensions, this allows to test them for bigger particles as well. Despite these differences in the designs, the results show, that both $CEA4x-v1$ and $CEA4x-v2$ were able to focus small, $1\text{ }\mu\text{m}$ particles into predictable paths. These particles are not much bigger, than the ones used in the measurements by Lei Wang and David S. Dandy, which was 920 nm .

For comparison on figure 4.3, the results of the previous measurements performed in the laboratory on the device $CEA3x$ can be seen. The flow rate was set to 8 ml/h in these measurements and various particle sizes were used. Based on the diagrams, it can be

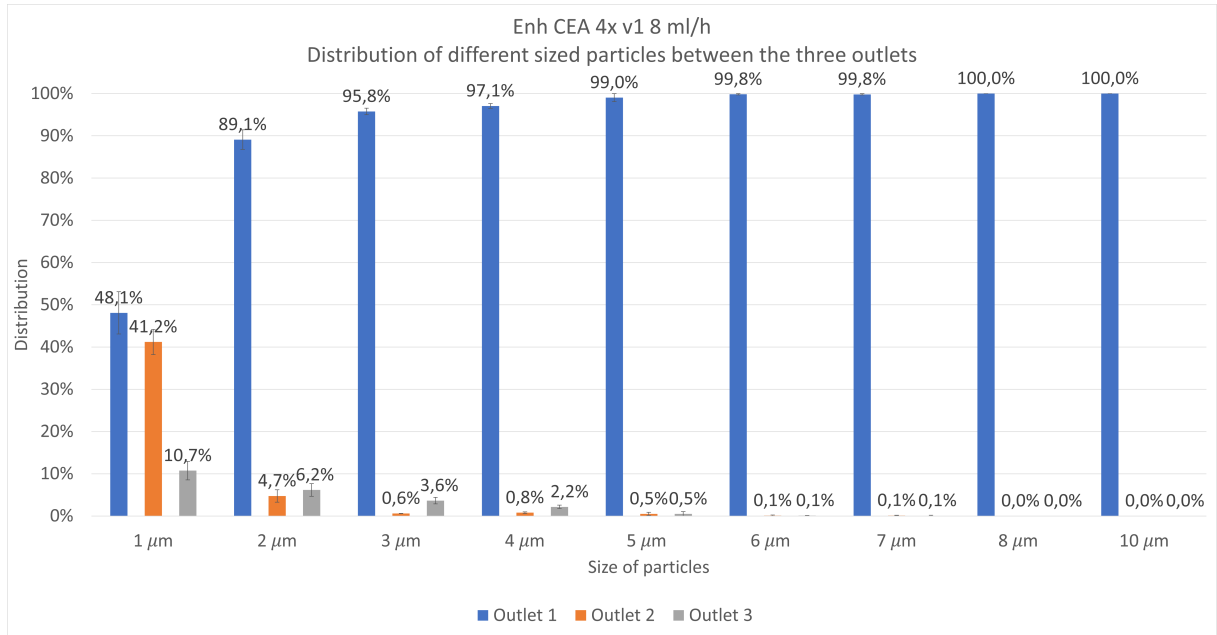


Figure 4.1: The results of the measurements made with the device *CEA4x-v1*, calculations were made by using Bürker chamber. Distribution of different sized particles between the three outlets and standard deviation of the three individual measurements.

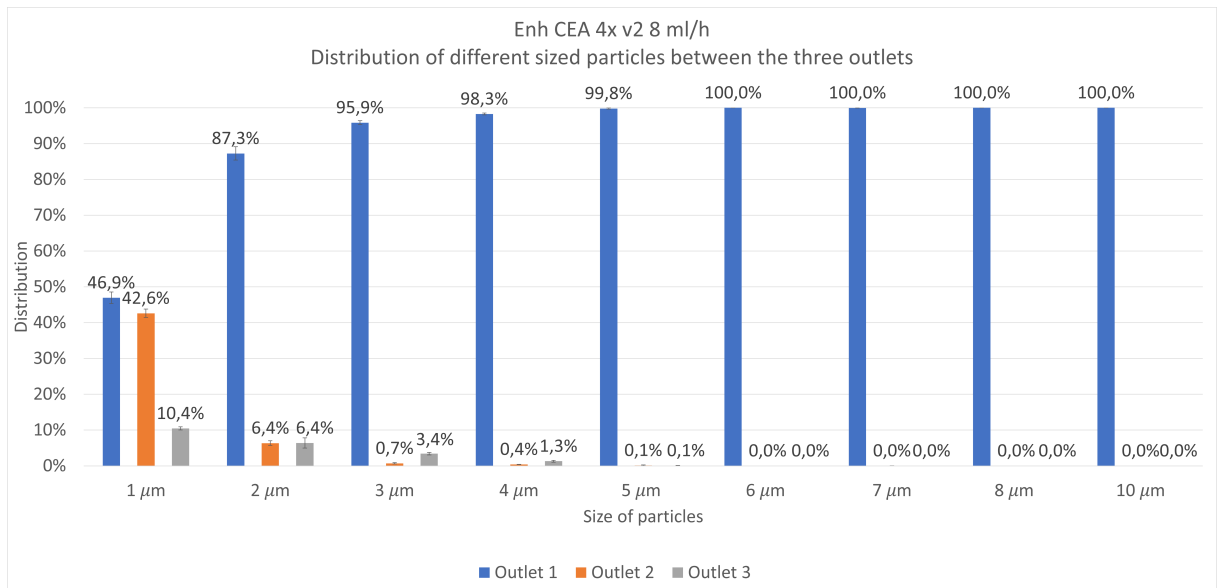


Figure 4.2: The results of the measurements made with the device *CEA4x-v2*, calculations were made by using Bürker chamber. Distribution of different sized particles between the three outlets and standard deviation of the three individual measurements.

concluded, that the devices with the 4:1 ratio (*CEA4x-v1* and *CEA4x-v2*) have a more precise focusing ability than the one with 3:1 *CEA3x* ratio. The percentage distribution improved by 3 – 5% due to the bigger widening.

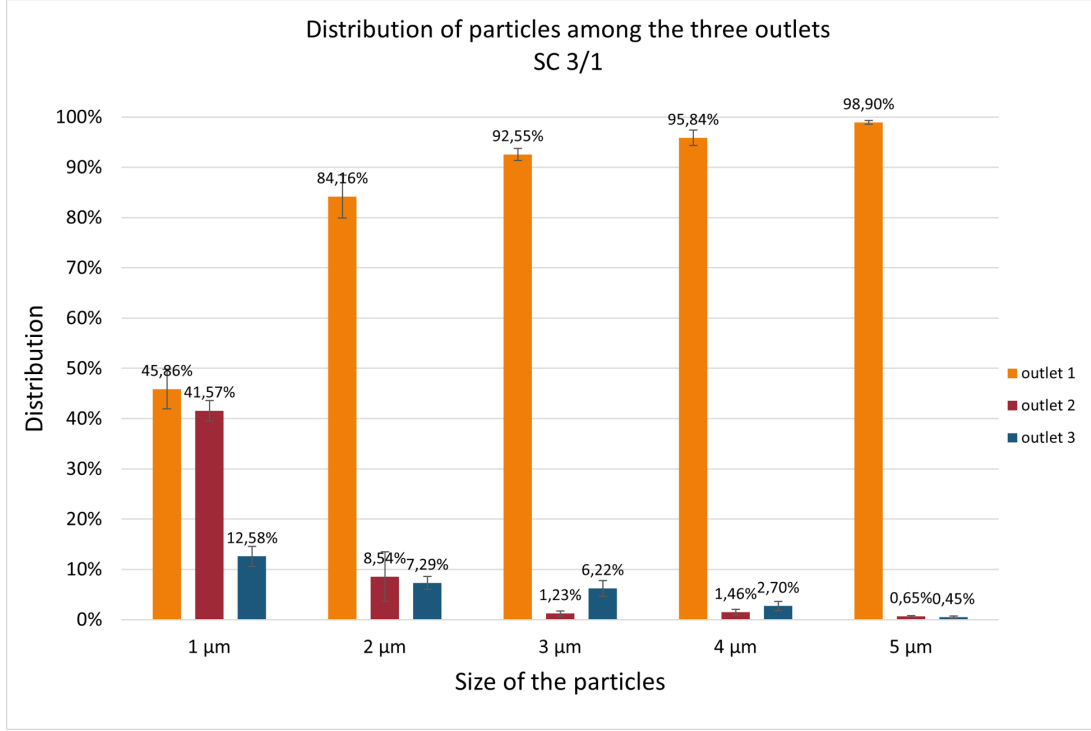


Figure 4.3: The results of the measurements made with the device *CEA4x-v2*, calculations were made by using Bürker chamber. Distribution of different sized particles between the three outlets and standard deviation of the three individual measurements.

4.3. Measurement 3

The third type of measurement was performed to compare the effect of varying flow rates on different sized particles. This aspect was tested on three devices, *CEA4x-v1*, *CEA4x-v2* and *CEA3x*. In general, from this experiment it can be concluded, that the 4:1 ratio results in a more stable focusing, than the 3:1 ratio.

Three diagrams were made from the measurements, one for each device. These can be seen on figure 4.4. The distance from the wall was measured between 3 *ml/h* and 25 *ml/h*, because above 24 – 25 *ml/h* the particles started to be less and less focused and the distance could no more be measured accurately. This scattering can be observed first for the 3 μm particles, and on the pictures this can be seen as their streamline gets wider. It manifests on the diagrams (Figure 4.4) in that, the blue lines start to get closer to each other again. For the bigger particles a bifurcation is formed, for *CEA4x-v1* and *CEA3x*, where the outlets join to the bottom curve of the serpentine, the streamlines are shifted towards the top wall of outlet 2. In case of *CEA4x-v2*, where the outlets join to

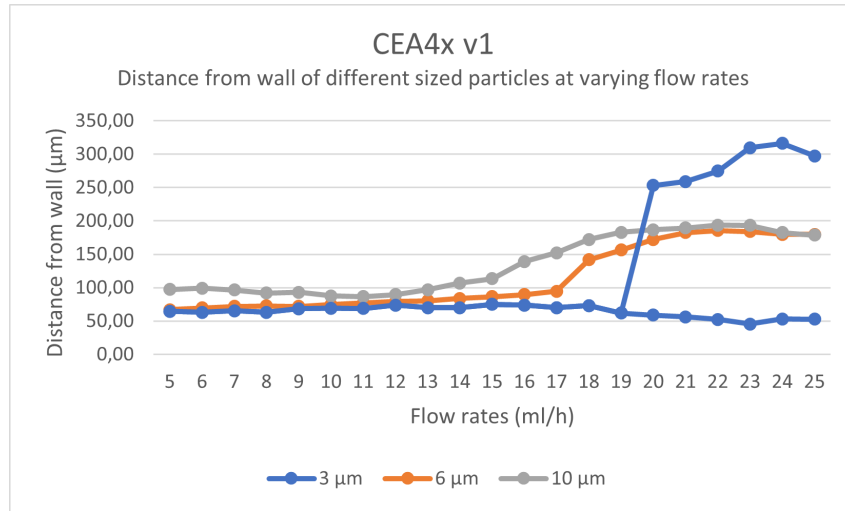
the upper curve of the serpentine, this shifting is in the opposite direction, the particles are pushed towards the bottom wall of outlet 2. On the diagrams this is apparent in the downward or upward shifting of the orange and grey lines between 23 – 25 ml/h .

At the beginning of each measurement, the flow rate was set to 3 ml/h . At this rate the particles were not completely focused. As the flow rate increased all three sizes of microbeads focused into one streamline. This was first apparent in all three devices at 8 ml/h . The bigger, 10 μm and 6 μm particles were focused in thinner streamlines and at a lower flow rates, than the 3 μm particles.

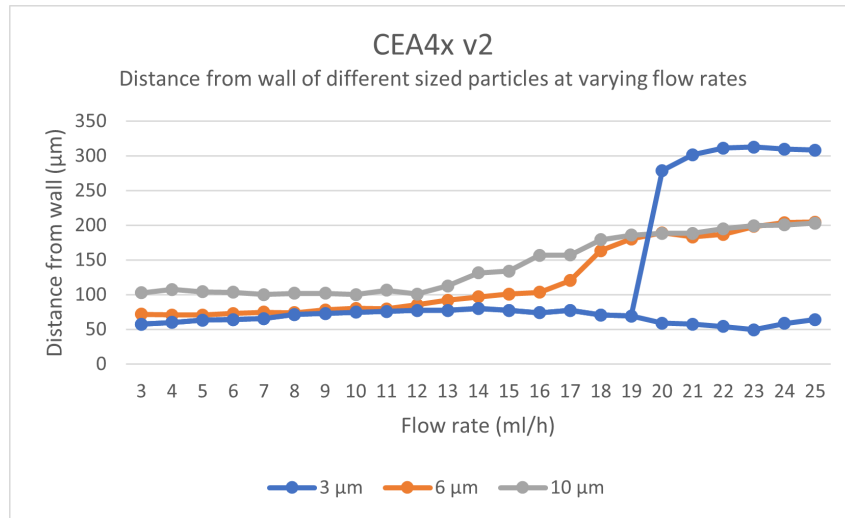
As the flow rate increased, the streamlines started to move to the middle of the channel. The first separation appears at 16 ml/h . Here the 10 μm particles are shifting from outlet 1 to outlet 2, while the 6 μm and 3 μm ones are still exiting through outlet 1. The best separation at this flow rate is achieved with *CEA4x-v2*. This can be seen on figure 4.5. In *CEA4x-v1* and *CEA4x-v2* at 18 ml/h the 6 μm microbeads also shift to the second outlet. In *CEA3x*, at the same flow rate, 6 μm particles go to both the first and second outlets.

Further increase in the flow rate results in the separation of the streamline in case of 3 μm particles. This phenomenon starts at 20 ml/h and becomes most stable at 24 ml/h . The 3 μm particles migrate into two individual streamlines, that separates the particles into outlet 1 and outlet 3. At the same flow rate 6 μm and 10 μm particles are still focused into one streamline and exit through outlet 2. This phenomenon can be observed in *CEA4x v1* on Figure 4.6. Additional images can be seen in Appendix A.

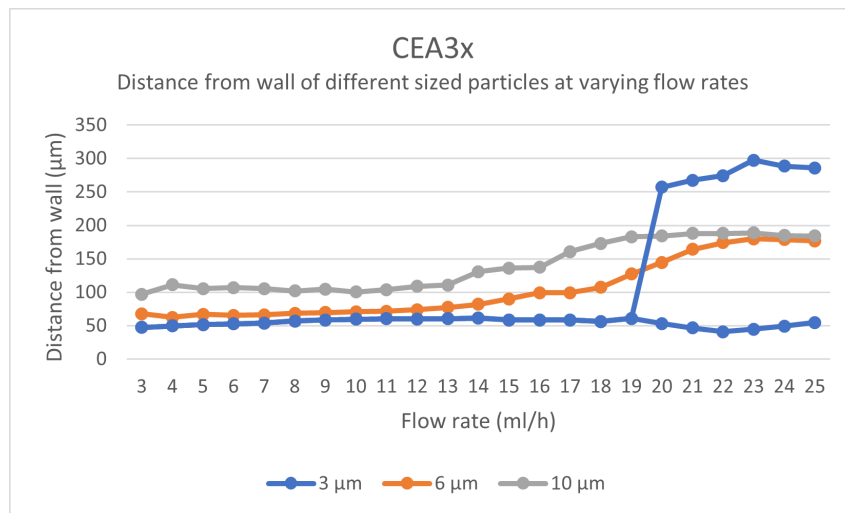
Above 24 ml/h , the high flow rate resulted in instabilities and first the 3 μm , then the 6 μm particles were less and less focused. The reason for these instabilities is the appearance of vortices in the serpentine. The smaller the particles, the easier it is for them to get caught in these vortices. These first appear at the beginning of the serpentine, then as the flow rate increases, they reach the end of the serpentine. An example of these vortices can be seen on figure 4.7.



(a)



(b)

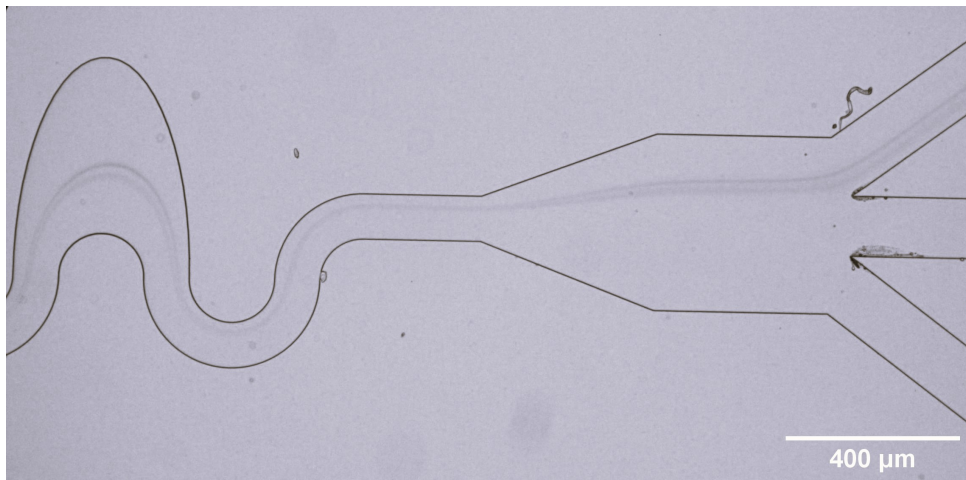


(c)

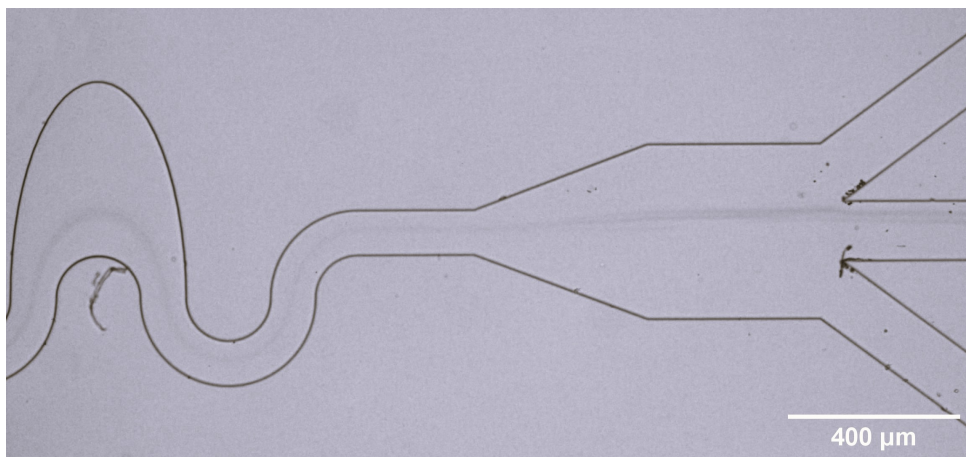
Figure 4.4: Distance from wall of different sized particles at varying flow rates in each device (*CEA4x-v1* (a), *CEA4x-v2* (b) and *CCEA3x* (c)). The 10 μm and 6 μm particles are moving away from the wall, as the flow rate increases. At 20 ml/h separation of 3 μm particles into two streamlines can be observed.



(a)

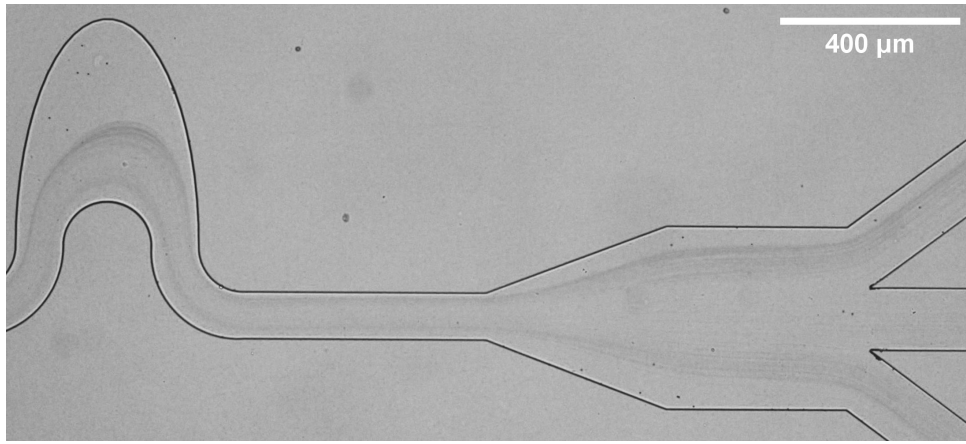


(b)

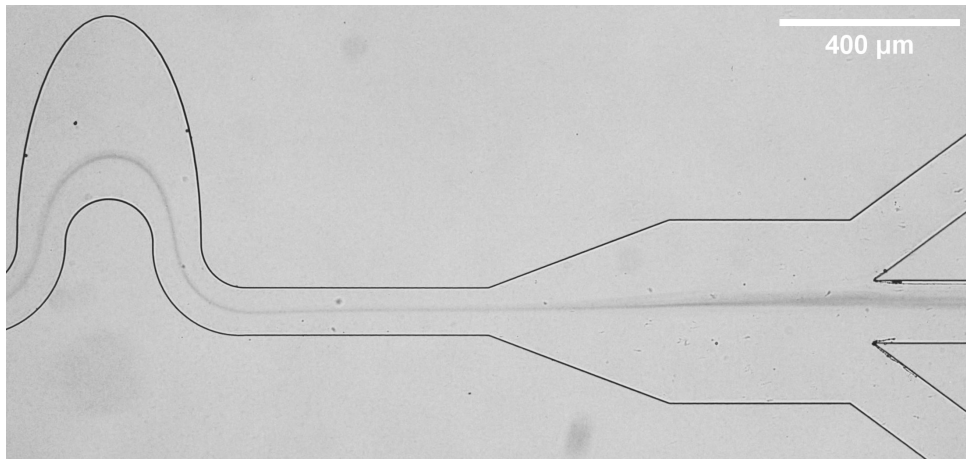


(c)

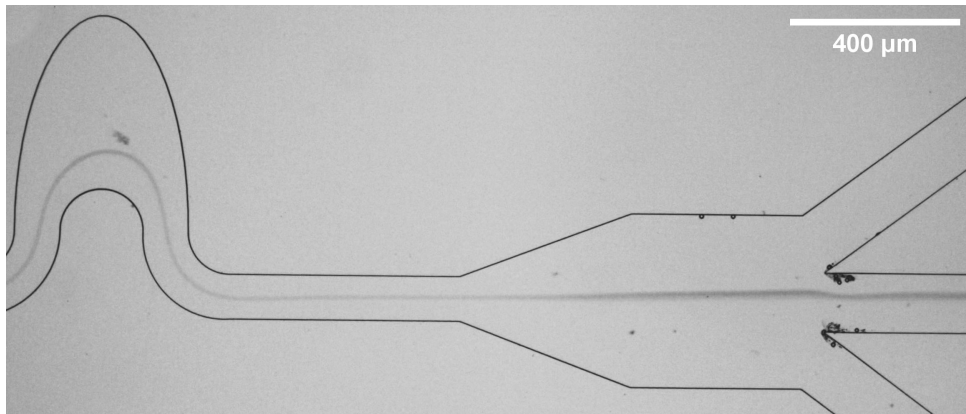
Figure 4.5: Separation of $10\ \mu m$ particles (c) from $3\ \mu m$ (a) and $6\ \mu m$ (b) sized particles in the device *CEA4x-v2* at a flow rate of $16\ ml/h$. The darker lines in the channel are the particle streamlines. The $3\ \mu m$ and $6\ \mu m$ particles are exiting through outlet 1, while $10\ \mu m$ particles are going to outlet 2.



(a)

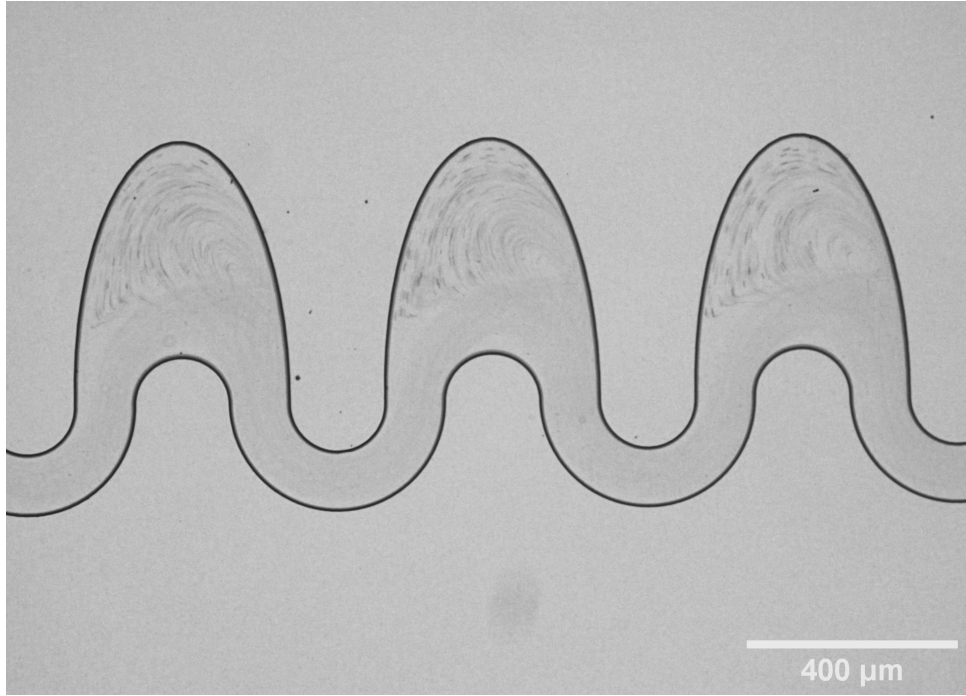


(b)

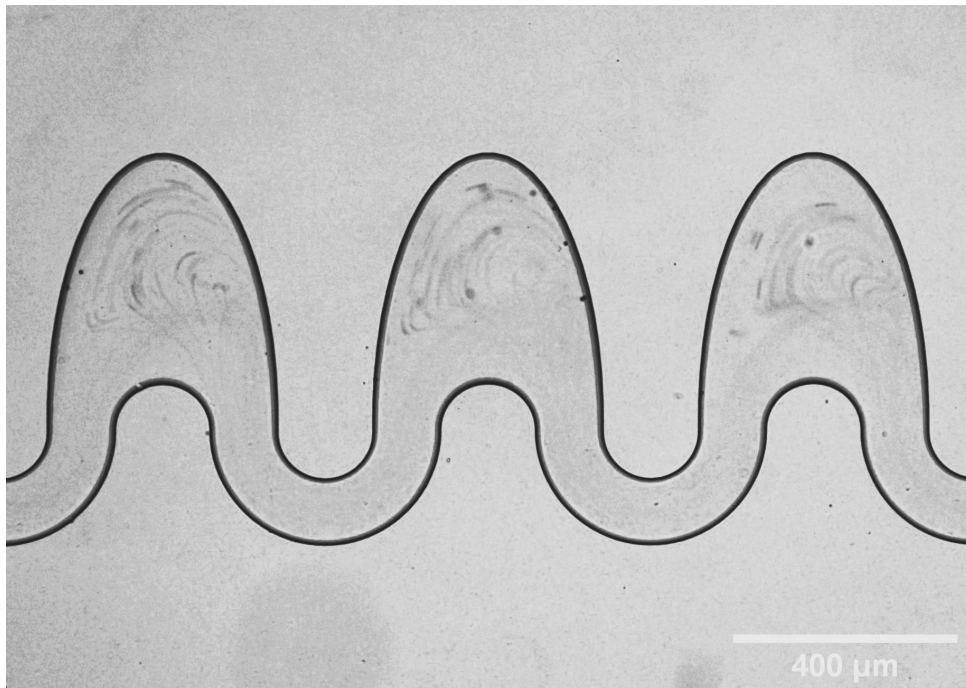


(c)

Figure 4.6: Separation of $3\ \mu\text{m}$ particles (a) from $6\ \mu\text{m}$ (b) and $10\ \mu\text{m}$ (c) sized particles in the device *CEA4x-v1* at a flow rate of $24\ \text{ml/h}$. The darker lines in the channel are the particle streamlines. The $6\ \mu\text{m}$ and $10\ \mu\text{m}$ particles are exiting through outlet 2, while $3\ \mu\text{m}$ particles are going to outlet 1 and outlet 3.



(a)



(b)

Figure 4.7: Vortices forming in the device *CEA4x-v1* at 30 ml/h flow rate, with $3\text{ }\mu\text{m}$ (a) and $6\text{ }\mu\text{m}$ (b) particles. The particles follow the paths of the fluid and get caught in the vortices formed in the widening of the serpentine. In the narrow part of the serpentine the particles are evenly distributed, in the widening the vortices are visible as little circular grey lines.

Chapter 5

Conclusion

5.1. Discussion

CEA01 proved to be a well focusing device, however it had troubles with higher flow rates. The pressure was too big in the channel, which lead to the teflon tubes shooting out of the device. The reason for this is, the channels height and with are small, while the serpentine part is long. The device was only tested for $5\ \mu m$ and $3\ \mu m$ particles and Bürker chamber was not used to evaluate the efficiency of the separation. More tests are needed to determine if it can focus smaller particles too, as the article said. Although this channel differs from the one presented by Lei Wang and David S. Dandy [15]. They had a shorter channel with lower aspect-ratio. This device was a good experiment for the measurements with the other devices. It also helped to acquire the necessary knowledge, and practice to perform the measurements.

The measurements for particle counting with Bürker chamber showed, that both *CEA4x-v1* and *CEA4x-v2* are efficiently focusing particles down to $2\ \mu m$ particles. At $8\ ml/h$, almost 90% of $2\ \mu m$ particles went to outlet 1. In case of bigger particles more than 95% focusing efficiency was achieved. For $5 - 10\ \mu m$ sized particles even 99 – 100% was achieved. $1\ \mu m$ particles had the biggest distribution between the three outlets. This was to be expected, given that the magnitude of net lift force acting on small particles is much smaller than that on larger particles. However, almost 50 % of the particles went to the first outlet. Since three individual measurements were performed with each particle size, the standard deviation could be calculated. Both *CEA4x-v1* and *CEA4x-v2* has a relatively small standard deviation for each particle size. There were no major outliers

among the measurement results. $1\ \mu m$ particles also had the biggest standard deviation, this is due to the less effective focusing of this sized particles at $8\ ml/h$ flow rate. Despite the fact, that $1\ \mu m$ particles were less focused, they still followed a predictable path. These particles are not much bigger, than the ones used in the measurements by Lei Wang and David S. Dandy, which was $920\ nm$. They also said, that they could not focus these particles into one single particle streamline, but they were able to drive them into predictable lines. Given that almost 90 % of $1\ \mu m$ particles exited through outlet 1 and outlet 2, the conclusion can be drawn, that some focusing was reached, but it was not as precise as with bigger particles. Also, the collected data are consistent with the results presented by Lei Wang and David S. Dandy.

The comparison to the previous measurements done in the laboratory, with the device *CEA3x*, showed, that the 4:1 ratio allows a more precise focusing than the 3:1 ratio. An improvement by 3 – 5 % in focusing efficiency is apparent based on these results. This is a result of a more stable flow in the $4x$ geometries.

The measurements with varying flow rates showed, that the velocity of the flow and the size of the particles has a significant effect on the equilibrium positions of the particles. Based on observations during measurements and image analysis, it can be concluded again that the 4:1 ratio results in a more stable focusing, than the 3:1 geometry, since *CEA4x-v1* and *CEA4x-v2* provided a more stable streamline, than *CEA3x*. This was manifested in the width and stability of the streamlines. This observation agrees with the results from the Bürker chamber particle counting.

At $16\ ml/h$ separation of $10\ \mu m$ particles happens. At this flow rate the streamline of $10\ \mu m$ particles shifts from outlet 1 to outlet 2. This separation is most prominent in *CEA4x-v2*, it has the most precise focusing at this flow rate. The other two devices have a slightly wider streamline of the $10\ \mu m$ particles, therefore some of these particles are still exiting through outlet 1. However most of the $10\ \mu m$ microbeads go to outlet 2 and separation from $6\ \mu m$ and $3\ \mu m$ particles can be observed in all three devices. Not much later, at $18\ ml/h$ $6\ \mu m$ particles also shift to the second outlet in case of *CEA4x-v1* and *CEA4x-v2*. For *CEA3x* $6\ \mu m$ particles are distributed between outlet 1 and outlet 2 at $18\ ml/h$. This device needs a higher flow rate, about $19 - 20\ ml/h$ to fully shift $6\ \mu m$ particles into outlet 2.

As the flow rate is increased, at $20\ ml/h$ the streamline of $3\ \mu m$ particles starts to

separate into two individual lines. This becomes stable in all three devices at 24 ml/h . Here the 3 μm particles are going to outlet 1 and outlet 3, while 6 μm and 10 μm particles go to outlet 2. Again the particles are separated at this flow rate.

Overall the difference in geometry between $CEA4x-v1$ and $CEA4x-v2$ did not result in a significant difference in focusing efficiency. However in the measurements with varying flow rates $CEA4x-v2$ seemed to have the most stable flow, this is also indicated by having the smallest standard deviation in the Bürker chamber counting.

These results show, that a simple asymmetric serpentine channel geometry allows high efficiency focusing of micro-particles. These devices are highly dependent on various parameters, including fluid velocity and particle size. It is important to consider these factors, when designing the devices.

5.2. Future plans

Since these devices showed promising results, I would like to continue experimenting with them and further investigate their focusing and separating ability. There are some parameters that were not changed during the measurements, for example the length or aspect-ratio of the channel. The question also arises as to whether a broader channel, say with a 5:1 ratio, could improve focusing efficiency further.

Another interesting path I would like to take is to experiment with different samples. More precisely, samples that resemble better the characteristics of blood samples. Future studies and measurements like this would be useful to determine whether these devices are suitable for e.g. blood plasma extraction, or separating white blood cells from red blood cells. This could potentially lead to an application for separating circulating tumor cells from blood samples, which would be useful for diagnosing and monitoring cancer patients.

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Appendix A

Appendix

Additional information for the measurements with varying flow rates can be found in the following online storage: <https://drive.google.com/drive/folders/1DSTijq66MkPcV4MIVY5e6CtkCfv3QT7Ifbclid=IwAR2GFpu2AHLiHla4f4crNF85HICwWprstaPWvvITw5Pk-X2e5UH9ojKuTA8>