High pressure inertial focusing for separation and concentration of bacteria at high throughput

F.J. Cruz and K. Hjort
Engineering Sciences, Uppsala University, Ångström Laboratoriet, Uppsala, Sweden
javier.cruz@angstrom.uu.se; klas.hjort@angstrom.uu.se

Abstract. Inertial focusing is a phenomenon where particles migrate across streamlines in microchannels and focus at well-defined, size dependent equilibrium points of the cross section. It can be taken into advantage for focusing, separation and concentration of particles at high through-put and high efficiency. As particles decrease in size, smaller channels and higher pressures are needed. Hence, new designs are needed to decrease the pressure drop. In this work a novel design was adapted to focus and separate 1 µm from 3 µm spherical polystyrene particles. Also 0.5 µm spherical polystyrene particles were separated, although in a band instead of a single line. The ability to separate, concentrate and focus bacteria, its simplicity of use and high throughput make this technology a candidate for daily routines in laboratories and hospitals.

Keywords. Particle separation, Bacteria separation, Inertial focusing, Microfluidic channel, High pressure.

1. Introduction
Inertial focusing is a phenomenon where particles migrate across streamlines in microchannels and focus at well-defined, size dependent equilibrium points of the cross section. In a straight system it is caused by the balance of two forces [1], Fig. 1.

Figure 1. Main forces on a particle in a straight microchannel.
a shear lift force directed towards the walls of a channel due to the shape of the velocity profile \( F_L \) (SHEAR GRADIENT) and a wall lift force directed towards the center due to interactions of the streamlines with the wall \( F_L \) (WALL EFFECT). The net lift \( F_L \) force was predicted by Asmolov [2].

\[
F_L = \frac{4\rho C_L U_f^2 a_p^4}{D_h^2}
\]

where \( \rho \) is the fluid density, \( C_L \) is the lift coefficient which is a function of the particle position across the channel cross-section and the channel Reynolds number, \( U_f \) is the average flow velocity, \( a_p \) is the particle diameter and \( D_h = \frac{4(hw)}{h+2w} \) the hydraulic diameter of the channel, with \( h \) its height and \( w \) its width. In curved channels the Dean flow enhances the lateral motion of particles and reduces the focus length [3].

We previously showed a set of scaling factors (Table 1) that maintain the magnitude of the lift forces and allow the transformation of a system that works for a certain size of particles into a system that successfully focuses smaller sizes. We also supported the idea with experimental results showing alignment of 1 µm particles and Escherichia coli (E. coli) in a spiral microchannel. However, the high pressure was on the limit for the E. coli. Its viability was similar before and after being focused at 50 µl/min (70 bar). However, at 100 µl/min (150 bar) 90% of the bacteria died. [4]

Table 1. Scaling law to transform a design that works for certain particle size to target another size. [4]

<table>
<thead>
<tr>
<th>Scaling Relations</th>
<th>Particle size</th>
<th>Height</th>
<th>Width</th>
<th>Flow rate</th>
<th>Focus length</th>
<th>Average speed</th>
<th>( \Delta P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale factor</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>( X^{-1} )</td>
<td>( X^2 )</td>
</tr>
</tbody>
</table>

In this paper, the aim is to show an alternative design that allows for inertial focusing faster and at lower pressure drops than with previous high throughput designs.

2. Experimental details

Microfluidic chips that could tolerate pressure up to 200 bar were dry etched (Tegal 110 S/DE) in silicon and anodically bonded to borosilicate glass, diced and finally silica capillaries were glued as connections.

Fluorescent polystyrene particles with diameters of 3, 1 and 0.5 µm and E. coli carrying Yellow Fluorescent Protein were suspended in deionized water at a concentration of \( 10^6 \), \( 10^7 \), \( 10^8 \) and \( 10^9 \) particles/ml, respectively.

A high pressure HPLC pump (Waters, model 515) was used to pump the samples.
3. Results
A design of straight channel with varying width showed excellent results already at 50 µl/min and approx. 30 bar, Fig. 4.

![Image](image_url)

Figure 4. (A) General view of a straight channel (4.6 mm in length) with varying width (B) Performance with 1 µm particles at 50 µl/min. (C) Performance with 0.5 µm particles at 50 µl/min (D) Analysis of the intensity signal. (E) Separation of 1 and 3 µm particles at 150 µl/min and approx. 100 bar.

4. Discussion
The design with straight channels with varying width is still under evaluation, Fig. 4. A preliminary study showed not only a faster alignment of 1 µm particles than in curved models but it also required less pressure for the same flow rate. It could also concentrate 0.5 µm particles to a narrow band enabling their separation.

The design can be shortened relieving some pressure drop. The system is expected to focus 1 µm particles at 50 µl/min demanding around 20 bar.
The separation between 1 and 3 µm is quite large, allowing for further discrimination of intermediate sizes.

5. Conclusion
In this work we used straight microchannels that vary their width. The new design aligned 1 µm particles faster than the spiral model and required less pressure. Furthermore, although not completely aligned, 0.5 µm particles were concentrated in a band and were separated from the main stream.

The ability to separate, concentrate and focus bacteria, its simplicity of use and high throughput make this technology a candidate for daily routines in laboratories and hospitals.

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References