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

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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 ($F_L^{\text{SHEAR GRADIENT}}$) and a wall lift force directed towards the center due to interactions of the streamlines with the wall ($F_L^{\text{WALL EFFECT}}$). The net lift ($F_L$) force was predicted by Asmolov [2].

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

Eq. 1

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 $D_h = \frac{4(hw)}{h+w}$ 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.

The microchannel was 6.6 µm deep and consisted of a series of expansions-contractions along 3-4.6 mm. The narrow parts were 20 µm that expanded until 60 µm.

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. 2.

![Figure 2](image)

**Figure 2.** (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 (green) and 3 (red) 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. 2. 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