Continuous particle separation in a microchannel having asymmetrically arranged multiple branches

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A new method for continuous size separation and collection of particles in microfabricated devices, asymmetric pinched flow fractionation (AsPFF), has been proposed and demonstrated. This method improves the separation scheme of pinched flow fractionation (PFF), which utilizes a laminar flow profile inside a microchannel. In this study, multiple branch channels with different channel dimensions were arranged at the end of the pinched segment, so that the flow rate distributions to each branch channel were varied, and a large part of the liquid was forced to go through one branch channel (drain channel). In the proposed channel system, the flow profile inside the microchannel was asymmetrically amplified, enabling the separation of one-order smaller particles compared with PFF. After introducing the method, we examined the effect of the asymmetric amplification by controlling the outlet of the drain channel. Also, a mixture of 1.0~5.0 μm particles was separated, and erythrocytes were successfully separated from blood. The results indicate that the AsPFF method could be applied to the separation of much smaller-size particles, since more precise separation can be achieved simply by changing the geometries of branch channels.

Introduction

In recent years, there has been increasing interest in the separation of various kinds of particles, such as polymer beads, ceramics, drugs, cells, organelles, and microbes. Microfabricated devices have the potential to produce, separate, and analyze such small particles, since the microchannel dimensions are similar to their sizes, and the laminar flow can be precisely controlled. Accordingly, various methods for separating particles in microchannels have been proposed.

For selecting specific particles from a particular group, there are several methods, such as laser manipulation, laminar flow control, and dielectrophoresis (DEP). These methods are mainly used for the manipulation and analysis of individual cells. On the other hand, hydrodynamic chromatography (HDC) and field flow fractionation (FFF) are used to analyze the nature of a particle group, and both have been performed in microfluidic platforms. However, in the field of industrial production or chemical/biological research, preparative separation is one of the most important applications. To achieve large scale treatment, continuous particle separation is indispensable, and to this end, researchers have reported methods using acoustic separation, two-phase partitioning, split-flow thin (SPLITIT) fractionation, continuous DEP, or optical trapping with continuous flow. Recently, a new method for continuous particle separation was proposed using bifurcation of laminar flow around obstacles. This method is highly advantageous in the sense that particles follow the flow inside a specific microchannel geometry, so particle sizing can be continuously performed without the help of outer fields.

In our previous work, we proposed a concept of ‘pinched flow fractionation (PFF)’ for the continuous size separation of particles in a microchannel. This method is also advantageous in that it utilizes only the laminar flow profile inside a microchannel, and thus, complicated outer field control can be eliminated. To be more specific, liquids with and without particles are continuously introduced into a microchannel having a pinched segment, and particles are separated perpendicularly to the direction of flow according to their sizes by hydrodynamic force. In addition, separated particles can be collected independently by making multiple branch channels at the end of the pinched segment. However, in performing particle collection, not all the branch channels are effectively used, since even the largest particle goes through the center branch channel. Also, when the size of the target particles becomes much smaller than the width of the pinched segment, separation efficiency would be decreased, or sometimes separation would become impossible. Therefore, for the separation of small particles, it is necessary to make a small-sized pinched segment, even though this will be accompanied with fabrication limitation and channel clogging with contaminants.

In this work, we propose a new method, asymmetric pinched flow fractionation (AsPFF), with a microchannel equipped with asymmetrically arranged multiple branch channels at the end of the pinched segment. With this microchannel, liquid flow in the pinched segment is asymmetrically distributed to each branch channel, and the difference in particle positions near one sidewall in the pinched segment can be effectively amplified. This enables precise separation of small particles by a relatively large-sized pinched segment, so the highly precise...
microfabrication technique can be eliminated. Also, channel clogging will be prevented in AsPFF, which sometimes becomes a serious problem in PFF.

To control the flow rate distributions to each branch channel, we controlled flow resistance by adjusting the channel dimensions, i.e., width, depth, and length. This adjustment is advantageous because it realizes a purely geometrical separation, which is suitable for preparative separation of particles. In this study, we examine whether particles whose diameters are much smaller than the width of the pinched segment can actually be separated with the AsPFF method. In addition, we demonstrated the applicability of this method to biological substances such as blood cells.

**Principle**

Fig. 1 compares the principles of AsPFF and PFF. In both methods, a liquid containing particles is introduced from Inlet 1, and a liquid without particles is introduced from Inlet 2. At this time, the liquid containing particles is focused on Sidewall 1 in the pinched segment, by controlling the flow rates from the two inlets. By this operation, particles are aligned to Sidewall 1 regardless of their sizes, causing a difference in the center positions of large and small particles. Next, at the boundary of the pinched segment and branch channels, particles are separated according to their sizes by the spreading flow profile. In this way, particles can be separated rapidly and continuously by means of only the hydrodynamic force inside the microchannel.

In the previous PFF method (Fig. 1(a)), the flow resistances of all branch channels are equal, so the introduced liquid flow is uniformly distributed to all branch channels. In this case, even the largest particles, i.e., particles whose diameter is equal to the width of the pinched segment, will go through the center branch channel, so half the branch channels (Branch 4 and 5 in Fig. 1(a)) are useless. When the number of the branch channels is increased, or when the pinched segment is narrowed, more precise separation will be achieved. However, these means are not adequate, since particles whose diameters are much smaller than the width of the pinched segment can not be separated.

In the AsPFF method (Fig. 1(b)), on the other hand, branch channels are arranged asymmetrically at the end of the pinched segment. In particular, one branch channel is made shorter and/or broader than the others (Branch 5 in Fig. 1(b)) in order to reduce its flow resistance. This channel is named the drain channel, since a large portion of the liquid will flow into it. So, the flow in the pinched segment is asymmetrically distributed to each branch channel. In this case, the flow near Sidewall 1 in the pinched segment, which contains aligned particles, is effectively distributed to branch channels. Therefore, the difference in particle positions near Sidewall 1 can be effectively amplified. Using this method, even when particle sizes are very small, or the difference in particle sizes is very small, separation can be performed. In short, by simply changing the width or length of a certain specific branch channel, a similar effect to that of a narrowed pinched segment can be achieved, without problems such as channel clogging.

In this study, the outlet of the drain channel was controlled to demonstrate the effect of the asymmetrically arranged branch channels. Also, the flow profile was visualized using small fluorescent particles to measure the flow rate distributions to each branch channel.

**Experimental**

**Materials**

Si (100) wafers were obtained from Furuuchi Chemical (Tokyo, Japan); negative photoresist, SU-8 2025, was obtained from MicroChem (Newton, MA, USA); PDMS (Sylgard 184) was obtained from Dow Corning (Midland, MI, USA); green, blue, and red fluorescent polymer microspheres (G700, G0100, G0500, B0200, and R0300) were obtained from Duke Scientific (Palo Alto, CA, USA); Tween 80 was obtained from Wako Pure Chemicals (Osaka, Japan). A blood sample from an ordinary person was used.
Microchannel fabrication and design

Microdevices were fabricated using the usual rapid prototyping and replica molding methods. First, channel patterns were transferred to a negative photoresist (SU-8 2025) layer coated on a silicon wafer, and the mold structures were fabricated. Then, PDMS prepolymer was poured onto the mold, and cured. After curing, the PDMS structure was bonded with a flat PDMS plate, and the microchannel was formed.

To regulate the flow rate distributions to each branch channel, the flow resistances were controlled by changing the dimensions of each branch channel. The laminar flow is dominant in the microscale, so when the flow is continuous, the pressure loss $\Delta P$ is given by the Hagen–Poiseuille equation:

$$\Delta P = \frac{32 \mu v L}{D^2}$$  \hspace{1cm} (1)

where $v$ is the average velocity of the liquid, $\mu$ is the viscosity of the liquid, $L$ is the length of the microchannel, and $D$ is the representative diameter of the microchannel. The representative diameter $D$ is given by the next equation when the cross-sectional shape is rectangular:

$$D = \frac{2wd}{w+d}$$  \hspace{1cm} (2)

where $w$ and $d$ are the width and depth of the microchannel, respectively. The flow rate $Q$ is given by the next equation:

$$Q = vdw$$  \hspace{1cm} (3)

From eqn. (1) and (3), the flow rate $Q$ is expressed as follows:

$$Q = \Delta P \times \frac{D^2 w d}{32 \mu L} = \Delta P \times \frac{1}{R}$$  \hspace{1cm} (4)

where $R$ is the flow resistance in the microchannel, which is analogous to the resistance in an electrical device. In this study, the pressure losses between inlets and outlets are the same for all branch channels, and the viscosity of liquid and the channel depth are uniform. Therefore, the flow rate is dominated by the representative diameter $D$, channel width $w$, and channel length $L$, expressed as follows:

$$Q \propto \frac{1}{R} \propto \frac{D^2 w}{L}$$  \hspace{1cm} (5)

Based on eqn. (5), we calculated the channel dimensions and designed a microchannel as shown in Fig. 2. This microchannel is used for separation and collection of particles. The pinched segment width was 20 $\mu$m, and the microchannel depth was approximately 20 $\mu$m. Thirteen branch channels were connected to the pinched segment. Twelve of the thirteen branch channels, Branch A channels, were designed so that the flow resistances were larger than that of Branch B (drain channel). Branch A was composed of two channel segments with widths of 200 and 50 $\mu$m, and lengths of 3 and 10.6 mm, respectively. The width of Branch B was 200 $\mu$m, and the length was 1.5 mm. From eqn. (5), the ratio of the flow rate of one Branch A to that of Branch B was calculated to be approximately 1 : 48. Consequently, 80% of the total liquid was expected to flow into the drain channel. In this work, this microdevice was used for all experiments.

Operation

In this work, green fluorescent polymer spheres, whose diameters were 1.0 or 5.0 $\mu$m, blue fluorescent polymer spheres, whose diameter was 2.1 $\mu$m, and red fluorescent polymer spheres, whose diameter was 3.0 $\mu$m, were used as model particles. These particles were suspended in 0.5% (w/w) Tween 80 aqueous solution. By using the surfactant, cohesion of particles could be prevented. The concentrations of 1.0, 2.1, 3.0 and 5.0 $\mu$m particles were approximately 1800, 2000, 1300, and 7000 per 1 mL solution, independently. To visualize the flow profile inside the microchannel and to measure flow rates, small green fluorescent polymer spheres with diameters of 0.71 $\mu$m were used at a concentration of 500 per 1 mL. Their movements were observed using an ICCD camera (C2400-89V; Hamamatsu Photonics, Hamamatsu, Japan).

For the biological application, the blood cells of an ordinary person were separated as model particles. 3 $\mu$L of whole blood was suspended in phosphate buffered saline, and the concentration of blood was approximately 0.3%. Separation phenomena were observed under an optical microscope, and
moving images were captured using a CCD camera (DXC-151; Sony, Tokyo, Japan).

The solutions with and without particles were continuously introduced into the microchannel from Inlet 1 and Inlet 2 in Fig. 2, respectively, using syringe pumps (KDS200; KD Scientific, New Hope, PA, USA). Flow rates were controlled independently. Numbers of particles were counted for one minute, and about 260 particles were counted for each condition.

**Results and discussion**

**Effect of asymmetric flow distribution**

Initially, we tested the effect of asymmetric flow distribution on particle movement. At first, the outlet of the drain channel (Branch B) was closed with a PDMS membrane, so that liquid would not flow into the drain channel and liquid would be divided equally into Branches A. Next, the PDMS membrane at the outlet of the drain channel was removed, so that a large portion of the liquid would flow into the drain channel. In this state, the flow in the pinched segment was asymmetrically distributed. Fluorescent microspheres with a diameter of 5.0 \( \mu \text{m} \) were used. The flow rate of the particle-containing solution for Inlet 1 was 20 \( \mu \text{L h}^{-1} \), and that of the solution without particles for Inlet 2 was 1000 \( \mu \text{L h}^{-1} \). A difference in the branch channel containing the particle effluence was observed between the two conditions.

The result is shown in Fig. 3. When the outlet of the drain channel was closed, 95.5% of particles went through Branch A2, as shown in Fig. 3(a). On the other hand, when the outlet of the drain channel was opened, 96.0% of particles went through Branch A4, as shown in Fig. 3(b). From this result, it was confirmed that the drain channel contributes to the shift in the branch channel containing the particle effluence, since most of the liquid flows into the drain channel.

We assumed that the particle position is represented by its center, and neglected the flow rate distribution in the pinched segment. In short, the flow rates into each branch channel are proportional to their virtual widths in the pinched segment. Also, it was assumed that the radius of the particle is equal to the distance from the sidewall to the center position of the particle in the pinched segment. So, the effluent channel (Branch AN) can be predicted by the next equation:

\[
\frac{w_p \times (1-a)}{n_B} \times (N-1) < \frac{D_p}{2} < \frac{w_p \times (1-a)}{n_B} \times N
\]

\((N = 1, 2, \ldots, n_B)\)

where \(w_p\) is the width of the pinched segment, \(a\) is the ratio of the flow rate into the drain channel to the total flow rate, \(n_B\) is the number of Branch A, and \(D_p\) is the particle diameter. This equation is valid under the following condition:

\[
\frac{D_p}{2} < w_p \times (1-a)
\]

When the particle diameter does not follow this equation, the particle will go through the drain channel.

In this study, the microdevice was designed so that 80% of the liquid flow would be distributed into the drain channel, and the rest of the flow would be divided equally into Branch A channels. Based on eqn. (6), when the outlet of the drain channel was closed \((a = 0)\), the effluent branch channel of 5.0 \( \mu \text{m} \) particles was predicted to be Branch A2, and when the outlet of the drain channel was opened \((a = 0.8)\), the effluent branch channel would be Branch A7. The difference in theoretical and experimental values was significant when the outlet of the drain channel was opened. To reveal the factors responsible for this difference, the flow rate into each branch channel and drain channel was measured.

**Visualization of flow profile**

Flow rates into each branch channel were measured by visualizing the flow profile, using fluorescent microspheres with a diameter of 0.71 \( \mu \text{m} \). The liquid with particles was introduced into the microchannel from both inlets. These particles are so small that it can be said that their movement represented the flow profile in this microchannel. The numbers of particles which entered into each branch channel were counted. The flow rate of the particle-containing solution for Inlet 1 was 20 \( \mu \text{L h}^{-1} \), and that for Inlet 2 was 1000 \( \mu \text{L h}^{-1} \).

The flow profile in this microdevice is shown in Fig. 4(a). As can be seen, it was confirmed that the flow near Sidewall 1 in the pinched segment was effectively distributed into twelve Branch A channels. On the other hand, the flow near Sidewall 2 was distributed into Branch B. In addition, a delineated track never intersects with other tracks, showing the possibility for particle separation in a size-dependent manner.

The numbers of counted particles are shown in Fig. 4(b). As can be seen, the numbers of particles entering into each Branch...
A channel were almost uniform, which shows the uniformity of flow rates. The number of particles into the drain channel was calculated to be $5.2 \times 10^3$ per minute. Considering that the particle count was proportional to the flow rate, the ratio of flow rates to one Branch A channel and drain channel was 1 : 18.9, and $a$, the ratio of the flow rate into the drain channel to the total flow rate, was 0.61. The theoretical value of $a$ was 0.8, and the difference in these values may have been due to the inaccuracy of microdevice fabrication, or to a pressure

difference caused by the liquid in the outlet holes. Based on the experimental value $a$ and eqn. (6), the effluent branch channel of 5.0 μm particles was calculated to be Branch A4, which corresponded well with the experimental result.

Separation of small particles

Next, we examined whether a mixture of small particles could be actually separated, even when the particle size is one-order smaller than the width of the pinched segment. First, 1.0 and 2.1 μm particles were separated. Next, as a separation of particles with various sizes, particles with 1.0, 2.1, 3.0 and 5.0 μm diameter were separated. In each experiment, the flow rate of the particle-containing solution for Inlet 1 was 20 μL h$^{-1}$, and that of the solution without particles for Inlet 2 was 1000 μL h$^{-1}$.

The result of 1.0 and 2.1 μm particles separation is shown in Fig. 5. As can be seen, particles with 1.0 μm diameter moved mainly toward Branch A1, and those with 2.0 μm diameter moved mainly toward Branch A2. As measured, 99.0% of particles with 1.0 μm diameter went through Branch A1, while the rest went through Branch A2. 76.4% of particles with 2.1 μm diameter went to Branch A2, while the rest went to Branch A1. Under the condition that $a$ is 0.61 in eqn. (6), the effluent branch channel of 1.0 μm particles was calculated to be Branch A1, and that of 2.1 μm particles was calculated to be Branch A2, which shows good correspondence with the experimental results. If there is no drain channel, and flow in the pinched segment is uniformly distributed, separation of 1.0 and 2.1 μm particles would be impossible, even though there are 12 branch channels. It was thus confirmed that the role of the drain channel is indispensable when the difference in particle sizes is small, since the difference in particle positions in the pinched segment can be effectively amplified. Also, even when the particle sizes are one-order smaller than the microchannel dimensions, particle separation could be performed.

Also, separation of particles with various sizes was successfully performed. As a result, 1.0 and 2.1 μm particles were moved toward the same branch channels as in the experiment above. Particles with 3.0 μm diameter moved toward Branch A2 and A3, and 5.0 μm particles moved mainly toward Branch A4. As measured, 49.5% of particles with 3.0 μm diameter

![Fig. 4](image-url) Visualization of flow profile in microchannels. Particles with 0.71 μm diameter were introduced from both inlets. (a) Delineated tracks of microsphere movement. (b) Numbers of particles counted in each branch channel. The particle count in Branch B (drain channel) was calculated from the particle concentration.

![Fig. 5](image-url) Separation of particles whose diameters are 1.0 or 2.1 μm.
went through Branch A2, while 50.5% of particles went through Branch A3. 85.7% of particles with 5.0 μm diameter went through Branch A4, while the rest went through Branch A5. So, it was confirmed that the AsPFF method could be applied to the separation of various-sized particles.

Separation of erythrocytes

In the case of biological particles including cells and organelles, the shapes are not always spherical. We therefore examined whether particles whose shapes are not spherical could also be separated using this microdevice. As model biological particles, we used erythrocytes with a disciform shape. The flow rates were the same as in the previous experiments, i.e., that of the blood-containing solution for Inlet 1 was 20 μL h⁻¹, and that of the solution without blood for Inlet 2 was 1000 μL h⁻¹.

Fig. 6(a) shows a photograph of the erythrocyte separation. As measured, approximately 80% of erythrocytes went through Branch A3, while the rest went through Branch A2. From eqn. (6), it is expected that particles whose diameters lie between 2.6 and 3.9 μm can be collected from Branch A3, when the particle shape is spherical. Since the diameter of erythrocytes is 7–8 μm, and the thickness is approximately 2 μm, it was supposed that erythrocytes were aligned in the pinched segment as shown in Fig. 6(b). Therefore, in the case of the separation of particles whose shape is not spherical, the particle behavior is dominated by its minimum length, such as the thickness or minor axis. Also, it was considered that the liquid introduced from Inlet 1 was distributed into Branches A1 and A2. Therefore, with this microdevice, blood constituents not containing blood cells could be collected from Branch A1, showing this method’s possibility for isolation of specific cells from a complex mixture.

Conclusions

The current study presented a novel method for accurate and continuous particle separation using a microfluidic device. By forming a drain channel to receive a large portion of the liquid flow, high separation performance could be achieved, since the liquid flow near one side wall could be effectively distributed. The mixture of 1.0–5.0 μm particles was successfully separated in a microchannel whose pinched segment width was 20 μm. Also, it was demonstrated that this method is suitable for separation of biological substances whose shape is not spherical, such as blood cells. It is expected that this method will be applied to the separation of much smaller-size particles, since particles whose diameters are much smaller than the pinched segment dimension can be separated. Also, this system can be incorporated into lab-on-a-chip technologies as one of micro-unit operations.

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Fig. 6 Separation of erythrocytes. (a) A photograph of separated erythrocytes, which are indicated by white arrows. (b) Schematic diagram of the erythrocyte alignment and separation.

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