# TWO-DIMENSIONAL CELL SORTING DEVICE EMPLOYING PINCHED-FLOW FRACTIONATION AND MAGNETOPHORESIS

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**ABSTRACT**

A simple but versatile microfluidic system is presented to continuously sort cells according to two factors at the same time. The principle employs the combination of pinched-flow fractionation (PFF), which enables the continuous size-dependent particle sorting, and magnetophoresis for affinity-based cell selection. Cells conjugated with magnetic nanoparticles are initially focused onto a corner of the narrow junction called pinched segment, and then they are separated in the horizontal direction according to size with the help of the spreading streamline, while separated in the vertical direction by applying the magnetic field, achieving the 2D separation. The separated cells are individually recovered through the multistep outlet array structures. In the experiment, we demonstrated the continuous separation of JM (human lymphocyte cell line) cells by using three-layer microfluidic devices having 4 × 4 outlets, showing the system's potential as a new tool for cell separation.

**KEYWORDS:** Cell sorting, Pinched-flow fractionation, Magnetophoresis, Microfluidics

## INTRODUCTION

Sorting of specific cells from a complex mixture is a critical process for clinical diagnosis/treatment and biological research applications. Conventional methods for cell separation include physical filtration, centrifugation, fluorescence activated cell sorting (FACS), and immunomagnetic techniques. These methods utilize the differences in cell characteristics including size, density, or surface marker molecules, however, there are limitations either in the separation efficiency, throughput, or availability. In the past decade, microfluidic devices have emerged as a new platform to precisely manipulate small samples including cells, bacteria, and biomacromolecules. Researchers have developed new microfluidic technologies for cell sorting utilizing the stably-formed laminar flow profile [1,2], magnetophoresis [3], dielectrophoresis [4], acoustic force [5], and so on. Although these studies demonstrated the potential of microfluidic system for separating cells, cell sorting based on multiple factors at the same time would be more useful for actual clinical and biological applications. In this study, a 2D-cell sorting system has been proposed, by combining the previously-developed PFF scheme [1] and magnetophoresis [3], in which cells are separated in vertical and horizontal directions in a microchannel based on two individual factors.

## PRINCIPLE

The principle is shown in Figure 1. Cells, conjugated with magnetic immunoparticles, are continuously introduced in to the microchannel and initially focused onto a corner of the pinched segment with the help of the sheath flow. Then, the spreading laminar-flow profile separates cells in the horizontal direction in the broadened channel based on size; this separation scheme is called "PFF". In the downstream, the magnetic field applied in the vertical direction moves cells conjugated with a larger number of magnetic particles upward greater than those labeled with less particles. There are two critical points to realize 2D cell sorting; (1) cells should be perfectly focused on the corner in the pinched segment regardless of size, and (2) separated cells in the horizontal and vertical directions should be individually recovered. For these purposes, we designed and fabricated three-layer glass-PDMS-PDMS microdevices as shown in Figure 2, equipped with a pinched segment with the different-depth structures (Fig. 2 (b)) and the multistep outlets (Fig. 2 (c)).

## EXPERIMENTAL

Microfluidic devices were fabricated using soft lithography and replica molding techniques (Fig. 2 (d)). The microchannel network consists of (1) three inlet channels, (2) pinched segment, (3) drain channel, (4) broadened channel, (5) application area of magnetic field, and (6) 4 × 4 outlet channels. The width and depth of the pinched segment were 40 and 30 μm, respectively, while those of the broadened channel was 1000 and 30 μm, respectively. The rectangular through-holes were made on the middle PDMS plate with a thickness of ~1 mm. The channel depth is ~30 μm, except for the deep region near the pinched segment (d = ~380 μm). Magnetic field was generated by placing a neodymium magnet (~1 × 1 × 4 cm, with a magnetic induction of 380 mT) at a distance of ~1 mm above the separation channel.

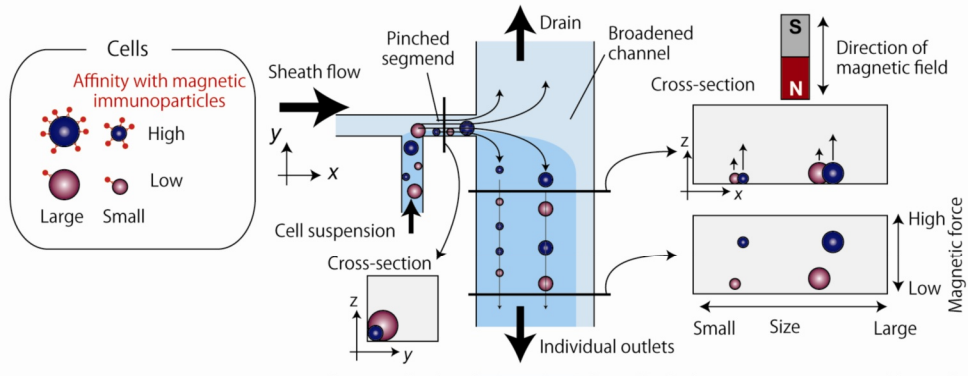


***Figure 1.*** *Principle of 2D cell sorting system by using PFF and magnetophoresis. Cells are initially labeled with magnetic immunoparticles. By introducing the cell suspension and sheath-flow, cells are focused onto one corner in the pinched segment. Then, cells are separated in the horizontal (x) direction according to size, with a help of the spreading streamline (PFF). In the downstream, magnetic field is applied in the vertical (z) direction, to move high-affinity cells to the upper, achieving 2D cell sorting.*

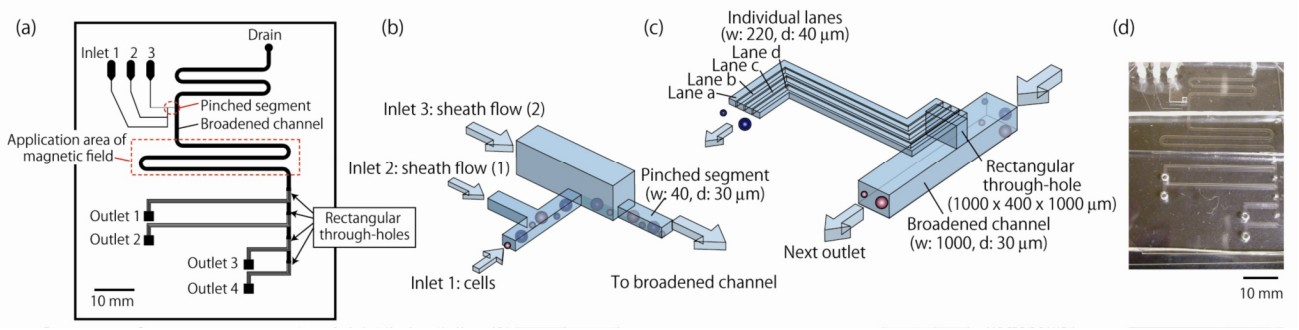


***Figure 2.*** *Design of the microdevice and its detail structures, composed of three-layer glass-PDMS-PDMS plates. (a) Whole microdevice design, having three inlets, one drain, and 4 × 4 outlets. (b) The pinched segment composed of different-height structures, to effectively focus the cells onto the corner. (c) Structure of the outlet junction for individually recovering 2D-separated cells. Each outlet channel is divided into 4 sub-lanes. (d) Photograph of the microdevice. (b) and (c), not to scale.*

## RESULTS AND DISCUSSION

First, we evaluated the fluid-focusing efficiency in the pinched segment, by introducing 1-µm fluorescent polymer particles from Inlet 1 and observing their positions in the cross section of the pinched segment by using confocal laser microscopy. As shown in Fig. 3 (a, b), when the sheath flow-rates were sufficiently high (3000 µL/h), fluorescent particles were located near the corner with both the width and height of less than 8 µm. This result indicated that cells with a diameter larger than ~10 µm would be perfectly focused on the corner with the help of the different-height structures of the pinched segment. Also, we measured the outlet flow-rates as shown in Fig. 3 (c), which well corresponded with the theoretical estimations.

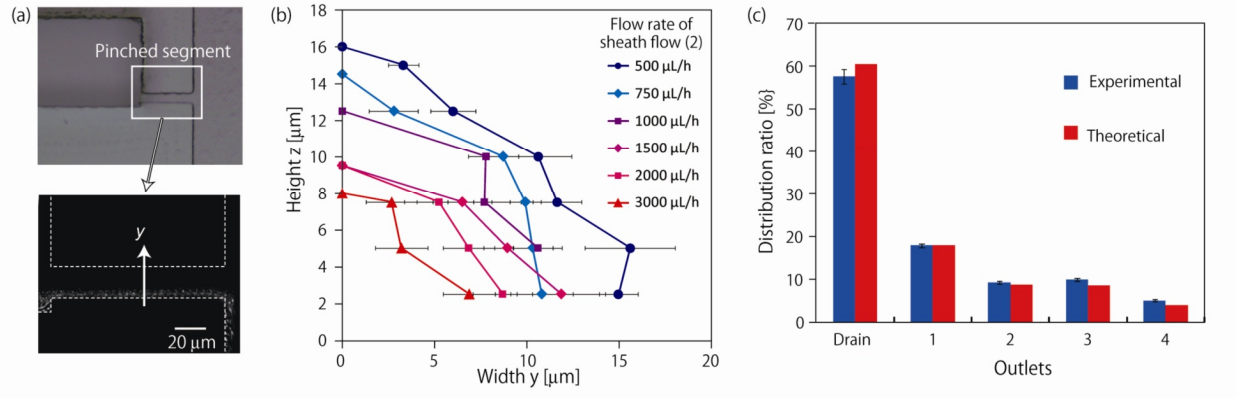


***Figure 3.*** *Evaluation of the microdevice. (a, b) Effects of the structure and the sheath-flow rate 2 on the focusing efficiency in the pinched segment, examined by introducing fluorescent particles (Φ = 1 µm) from Inlet 1, and keeping* $Q_1$ *(cell/particle suspension) and* $Q_2$ *(Sheath-flow rate 1) constant (50 µL/h each). In (b), the lines show the boundary of the particle-containing region (close to the corner with small y and z values) and the sheath flows. (c) Theoretical and measured volumetric flow rates distributed to each outlet/drain.*

Then, we sorted JM cells (human leukemia cell line; average diameter of 13.2 μm) labeled with anti-CD3 antibody-conjugated magnetic nanoparticles (Φ = 50 nm). Cells and fluorescent particles (Φ = 9.9 μm) were suspended in PBS containing 1% BSA, and introduced into the microchannel from Inlet 1 at a flow rate of 50 μL/min. The sheath flow-rates 1 (from Inlet 2) and 2 (Inlet 3) were 50 and 3000 μL/min, respectively. The number of cells/particles flowing through each lane of each outlet was counted. As a result, cells were mainly recovered from Outlet 3-c when the magnetic field is not applied. On the other hand, the outlet position shifted to Outlet 2-c, when cells were exposed to the magnetic field for ~40 sec, which was attributed to the magnetic force applied to the cells. Note that the recovering positions of 9.9-μm fluorescent particles were not changed by the magnetic-field application, showing the effect of magnetic field for cell separation and the possibility for 2-D cell sorting. The separation efficiency would be improved by using a microchannel having a larger number of outlets, and optimizing the strength and the time of magnetic-force application.

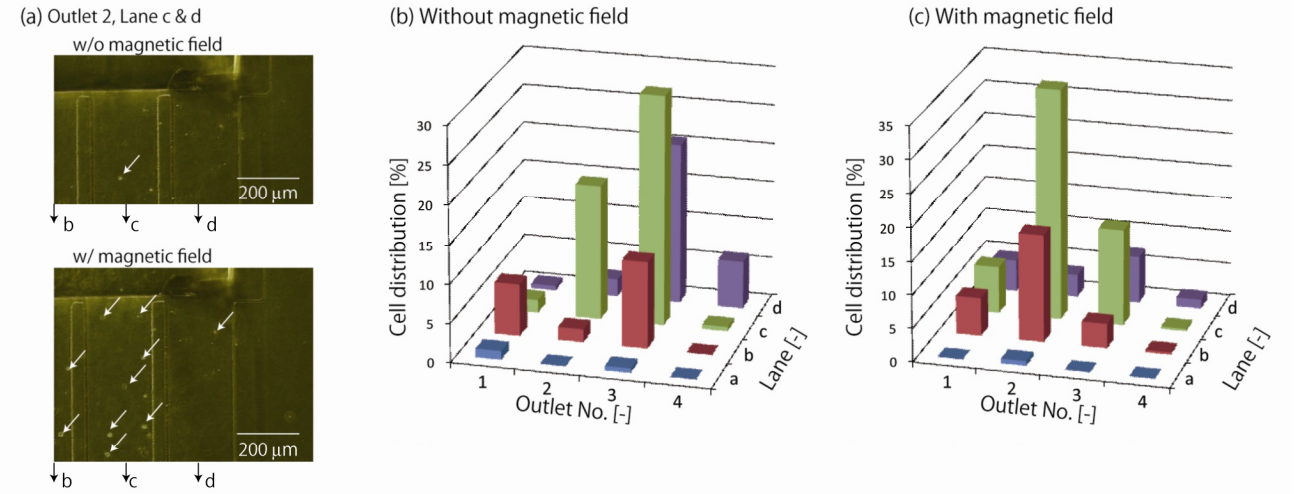


***Figure 4.*** *2D sorting of JM (human leukemia cell line) cells by using anti-CD3 conjugated magnetic nanoparticles (Φ = 50 nm). (a) Cells flowing through the Outlet junction 2, with and without applying magnetic field. Cells are pointed with white arrows. (b, c) Counts of cell numbers flowing through each outlet, with and without applying the magnetic field.*

## CONCLUSIONS

A new microfluidic system has been presented to easily achieve two-dimensional cell sorting based on size and surface markers. The presented system will be a useful means for selecting specific cells from a complex mixture, due to its high functionality and the easiness and simplicity in operation.

## ACKNOWLEDGMENTS

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