

Negative Dielectrophoresis Force based High Efficiency and High Throughput Cell Sorting System Harnessing Paired Electrode Arrays and a Meso Size Channel

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ABSTRACT

In this paper, we present a high efficiency and throughput cell sorting system employing repeated electrode arrays and a meso size channel. The paired twin electrode arrays generate a negative dielectrophoretic (n-DEP) force and deflect cells according to dielectric properties. The proposed dielectrophoresis activated cell sorter can achieve high efficiency even under strong hydrodynamic force caused by a large flow rate for high throughput because target cells are continually deflected by the n-DEP force while passing through each electrode pair. To find the optimal flow rate of the sorting system, we implemented numerical simulation under a signal-off condition. Consequently, the target cells, K562 cells, are effectively separated along an electrode pair under a voltage of 6 Vp-p with 100 kHz and a flow rate of 20 $\mu\text{l}/\text{min}$ and 60 $\mu\text{l}/\text{min}$ in each outlet. The proposed sorting system showed a high deflection ratio of $98.0 \pm 0.3\%$ and high throughput over 38,000 cells/min.

Keywords: Dielectrophoresis, Negative dielectrophoretic force, Cell sorting system, Paired electrode array

1 INTRODUCTION

In the clinical pathology and diagnosis field, many cell sorting systems such as fluorescent activated cell sorters (FACS), and magnetic activated cell sorters (MACS) have been developed for the detection of cancer cells and manipulation of biological particles [1]. FACS and MACS have been major techniques for the past few decades. However, they have a limitation due to the labeling process which causes cell damage and requires high cost. Therefore, various cell sorting systems were proposed utilizing external forces, such as ultrasonic, hydrodynamic and dielectrophoretic (DEP) forces [2]. Above all, dielectrophoresis activated cell sorters (DACS), which exploit the intrinsic dielectric properties of target cells, are highlighted as an alternative cell sorting method [3-6]. Nonetheless, the DACS performing in micro channels have a limitation of throughput, even while they achieved high efficiency. In this paper, we present a high efficiency and high throughput cell sorting system which is comprised of paired electrode arrays and a meso size channel. In order to improve separation efficiency by continuous separation at

each pair of electrodes, micro electrodes were deposited sequentially on the silicon and glass plate, respectively. To enhance throughput which is representative limitation of micro channel based DACS, the channel of the system was designed with a scale of meso size. Finally, a separation experiment is carried out with a live leukemia cell line (K562 cells).

2 WORKING PRINCIPLE AND EXPERIMENTAL SYSTEM SETUP

2.1 Basic principle of cell sorting system

The direction of input cells is determined by vector summation of gravity, hydrodynamic force, and n-DEP force (Figure 1). In case of the target cells, a live leukemia cell line (K562 cells), they move toward outlet-A due to a dominant n-DEP force as shown in Figure 1-(a). In case of the non-target cells, dead K562 cells, they move toward outlet-B as shown in Figure 1-(b) since the hydrodynamic force is dominant over the n-DEP force. The hydrodynamic force is controlled by a flow regulator at each outlet and the DEP force is expressed as shown below [7].

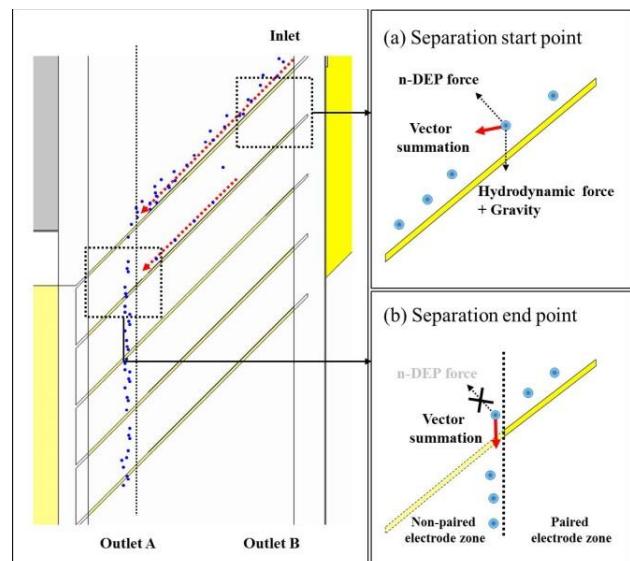


Figure 1: Working principle of the proposed sorting system: (a) the n-DEP force is dominant at the paired

electrode zone, (b) the hydrodynamic force is dominant at the non-paired electrode zone.

$$F_{DEP} = 2\pi r^3 \varepsilon_m \varepsilon_0 Re [f_{CM}] \nabla E^2 \quad (1)$$

Where r is the radius of the target particle, ε_m is the permittivity of the experimental buffer, ε_0 is the dielectric constant at the vacuum state, f_{CM} is the Clausius-Mossotti factor which determines the direction of the DEP force, and E is the electric field according to the input voltage condition.

The proposed DACS is capable of achieving high efficiency even under a strong hydrodynamic force due to a caused by large flow rate for high throughput because the target cells are continually deflected by the n-DEP force while passing through 5 electrodes pairs.

2.2 Electrode array fabrication procedure

The micro electrode arrays are fabricated on two plates, which are silicon and glass as shown in Figure 2. Additionally, to minimize the change of resistance in one electrode, Au is deposited with a thickness of 6000 Å. The detailed procedure is as follows.

(a) Silicon and glass plate with chemical and mechanical planarization process (300 μm), (b) Oxide for insulation (silicon plate only) and positive photoresist (PR, AZ 7220) deposition, (c) Patterning through photolithography, (d) Developing PR, (e) Cr/Au layer (500 Å / 6000 Å) deposition. (f) Eliminate PR through lift-off process, and completion fabrication.

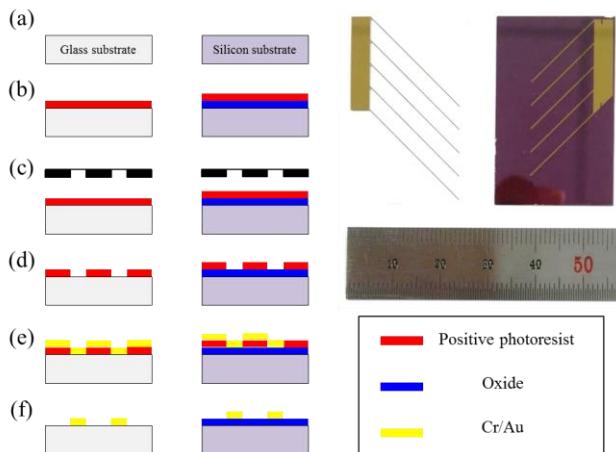


Figure 2: Micro electrode array fabrication procedure.

2.3 System configuration of cell sorting system

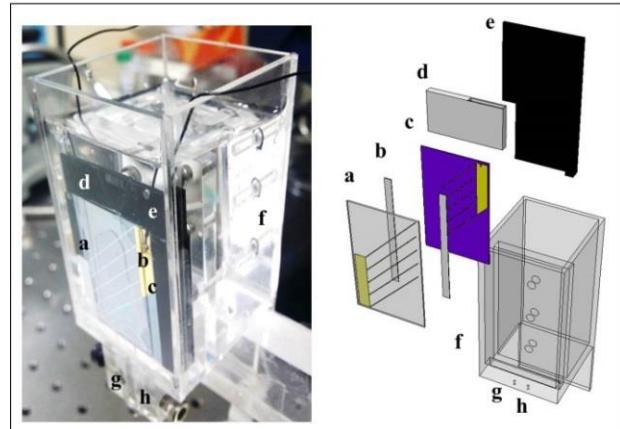


Figure 3: Configuration of the cell sorting system.

As shown in Figure 3, the proposed sorting system is comprised of a glass plate (a) and a silicon plate (c) which has a electrode array on each plate, 200 μm width PDMS side wall (b) to make a meso sized channel, Inlet (d), Supporter (e) and container (f). When assembling the two plates, electrodes on each plate are paired to generate a n-DEP barrier. The PDMS side wall enable to maintain the height of the channel of 200 μm. The inlet part for cell loading is allocated on the top of the channel and the supporter holds the assembled channel. Lastly, a reservoir keeps experimental buffer.

2.4 Experimental setup

The experimental system is set up as shown in Figure 4. It is comprised of (a) a CCD camera for monitoring cell separation, (b) a 3-axis micro stage for fine control of focal length from the CCD camera to the position of cell separation, (c) a function generator for applying input voltage with a specific frequency, (d) an optical microscope for counting the number of cells before/after the experiment, and (e) a cell sorting system.

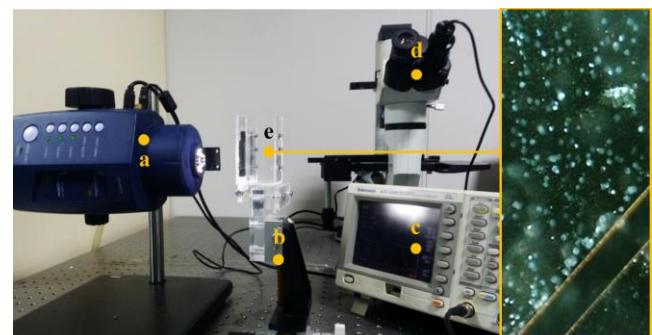


Figure 4: Experimental system setup for investigating cell separation.

3 RESULTS AND DISCUSSION

3.1 Numerical analysis

To find the optimal flow rate for the proposed sorting system, we carried out numerical simulation using COMSOL Multiphysics®. As shown in figure 5, simulation is implemented under the voltage signal-off condition. In case of the signal-off condition, all of the cells under a high flow rate must move toward outlet-B for high throughput. For the flow rate condition for each outlet, four cases are simulated as follows:

Case (a): Outlet-A: 16 $\mu\text{l}/\text{min}$, Outlet-B : 20 $\mu\text{l}/\text{min}$

Case (b): Outlet-A: 15 $\mu\text{l}/\text{min}$, Outlet-B : 20 $\mu\text{l}/\text{min}$

Case (c): Outlet-A: 12 $\mu\text{l}/\text{min}$, Outlet-B : 30 $\mu\text{l}/\text{min}$

Case (d): Outlet-A: 20 $\mu\text{l}/\text{min}$, Outlet-B : 60 $\mu\text{l}/\text{min}$

Case (a) is not a proper condition since all of the cells move toward outlet-A. Case (b), (c) and (d) meet the separation working principle which means the cells move toward outlet-B. Considering throughput, therefore, we select case (d) which has the highest flow rate of 20 $\mu\text{l}/\text{min}$ and 60 $\mu\text{l}/\text{min}$ in outlet-A and B, respectively, for the cell separation experiment.

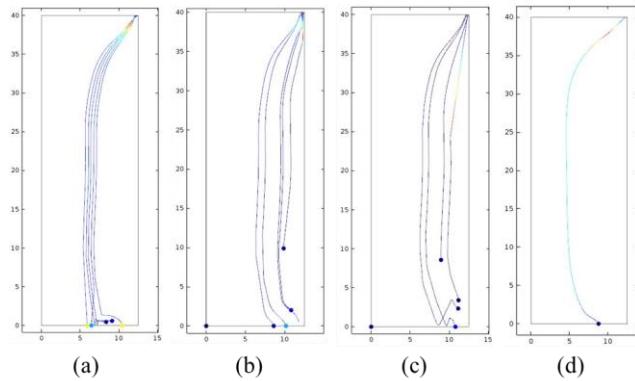


Figure 5: Particle trace under each flow rate: (a) is improper for the proposed sorting system, (b)~(d) are proper for the sorting system.

3.2 Experimental result

In order to generate an adequate n-DEP force and hydrodynamic force, we select optimal experimental input conditions which are a voltage of 6 Vp-p with 100 kHz, a flow rate of 20 $\mu\text{l}/\text{min}$ and 60 $\mu\text{l}/\text{min}$ in outlet-A and B respectively, and buffer conductivity of 0.2 S/m. As a target cell, K562 cell (leukemia cell line) is employed [8].

At first, when K562 cells arrived at the first electrodes pair, which consists of two electrode arrays, they are repelled by the n-DEP force, vertically against the DEP barrier. As a result, over 90% of the target cells move toward the end of the paired electrode zone (Figure 6. (a)) due to vector summation among n-DEP, hydrodynamic

force and gravity. Few cells passing through the first pair are deflected at the second electrode pair or the next pair (Figure 6. (b)). Since there are 5 electrodes pairs, separation efficiency can be maximized even under a large flow rate. In the separation process, deflected target cells adhere to each other and form some groups. When groups of cells arrive at the pairing end point, they fall down toward outlet-A (Figure 6. (c)). Consequently, the proposed sorting system showed a high deflection rate of $98.0 \pm 0.3\%$ and high throughput over 38,000 cells/min.

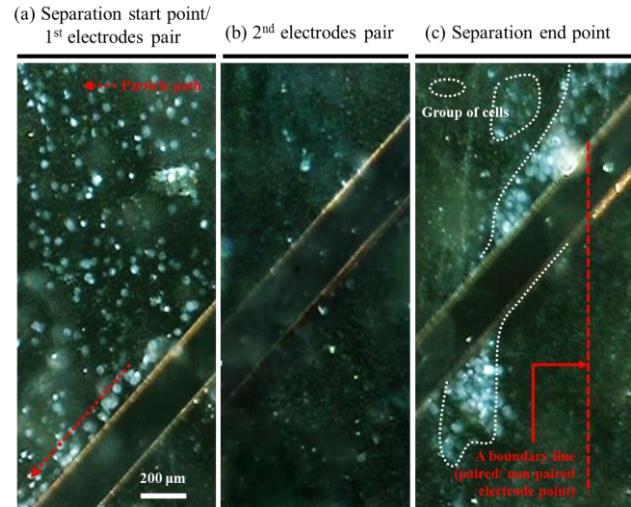


Figure 6: Movement of target cells under input condition (Voltage of 6 Vp-p with 100 kHz, flow rate of 20 $\mu\text{l}/\text{min}$ and 60 $\mu\text{l}/\text{min}$ in outlet-A and B, respectively): (a) 1st electrodes pair below inlet and (b) 2nd electrodes pair below inlet, (c) End of paired electrode zone.

4 CONCLUSION

In this article, we present a novel cell sorting system using the n-DEP force. Compared with a conventional cell sorter, we developed a separation technique under low voltage without a labeling process and with a simplified structure of the cell sorter. Furthermore, without a micro syringe pump, we controlled flow rate precisely using the micro flow regulator. To improve throughput and efficiency, a meso size channel as well as paired electrode arrays were employed. Conclusively, we achieve a high deflection rate of $98.0 \pm 0.3\%$ and high throughput over 38,000 cells/min under the condition of a voltage of 6 Vp-p with 100 kHz and a flow rate of 20 $\mu\text{l}/\text{min}$ and 60 $\mu\text{l}/\text{min}$ in outlet-A and B, respectively. In the near future, to evaluate the performance of the proposed sorting system for various target cells, we will consistently implement the separation test utilizing different cancer cells.

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REFERENCES

- [1] K. Bernath, M. Hai, E. Mastrobattista, A. D. Griffiths, S. Magdassi and D. S. Tawfik, "In vitro compartmentalization by double emulsions: sorting and gene enrichment by fluorescence activated cell sorting", *Analytical Biochemistry*, 325, 151-157, 2004.
- [2] D. R. Gossett, et al, "Label-free cell separation and sorting in microfluidic systems", *Analytical and bioanalytical chemistry*, 397, 3249-3267, 2010.
- [3] M. Alshareef, N. Metrakos, E. J. Perez, F. Azer, F. Yang, X. Yang and G. Wang, "Separation of tumor cells with dielectrophoresis-based microfluidic chip", *Biomicrofluidics*, 7, 011803, 2013.
- [4] V. Gupta, I. Jafferji, M. Garza, V. O. Melnikova, D. K. Hasegawa, R. Pethig and D. W. Davis, "ApoStrem, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood", *Biomicrofluidics*, 6, 024133, 2012.
- [5] D. Lee, D. Kim, Y. Kim, K. Park, E.J. Oh, Y. Kim and B. Kim, "A Negative Dielectrophoresis and Gravity Driven Flow based High Throughput and High Efficiency Cell Sorting System", *Journal of Laboratory Automation*, 19, 60-74, 2014.
- [6] J. Lee, Y. Kim, D. J. Beebe and B. Kim, "Harnessing gravitational, hydrodynamic and negative dielectrophoretic forces for higher throughput cell sorting", *Biochip J*, 6, 229-239, 2012.
- [7] H. Morgan and N. G. Green, "AC Electrophoresis: Colloids and Nanoparticles", Research Studies Press, 15-54, 2003.
- [8] D. Lee and B. Kim, "A cell sorting system for the evaluation of drug sensitivity using negative dielectrophoresis", *International Journal of Precision Engineering and Manufacturing*, 16, 609-613, 2015.