ISO-DIELECTRIC SEPARATION: A NEW METHOD FOR THE CONTINUOUS-FLOW SCREENING OF CELLS

M.D. Vahey[1] and J. Voldman[1]
[1]Massachusetts Institute of Technology, USA

Abstract

We introduce the first implementation of a method for the continuous-flow sorting of cells based specifically upon differences in their electrical properties. The method, which we call iso-dielectric separation (IDS), uses the dielectrophoretic (DEP) force in a liquid of spatially varying conductivity to map any electrically distinguishable phenotype to a unique position along the width of a microfluidic channel. The method is analogous to iso-electric focusing, with dielectric properties replacing surface charge as the basis for separation. IDS leverages the correspondence between the physiological state of a cell and its electrical properties to separate cells based upon such characteristics as viability or production of biomolecules [1]. Because IDS selects cells according to their generic electrical differences, it is possible to screen for such production without regard to the specific molecule being produced.

Keywords: Dielectrophoresis, continuous-flow screening, cell separations, conductivity gradient

1. Introduction

Conventional DEP separation methods typically sort cells based upon either the magnitude or the sign of the DEP force [2, 3], and are thus either strongly size-dependent, since the DEP force is proportional to cell volume, or intrinsically binary. Since screens generally involve heterogeneous populations with variations in cell size, these techniques lack the requisite sensitivity. An alternate method, DEP field-flow fractionation [4], balances the gravitational force with DEP levitation to sort cells approximately independent of their size. This approach, however, is not amenable to continuous-flow operation, since cells must be injected and collected in spatially confined plugs, and the separation occurs along the axis of flow. Furthermore, small cells such as bacteria do not settle to the defined heights needed for DEP-FFF. Because IDS offers continuous-flow sorting of particles into a continuum of electrical conductivities, it is possible to resolve highly diverse cell populations into arbitrarily many subpopulations with high throughput, independent of the sizes of the constituent cells.

2. Theory and Design

The IDS device consists of a 10 – 20 μm high PDMS microfluidic channel bonded to a Pyrex chip with gold electrodes patterned on it. Two fluids doped to different electrical conductivities enter the device and are sampled by a diffusive mixer to create a linear conductivity gradient in the separation channel. The cells enter this channel confined to the more highly conducting fluid; this way, the electrodes arranged across

the channel's diagonal serve as an n-DEP barrier, deflecting the cells across the channel in the direction of decreasing conductivity. This continues until the cells are sufficiently close to their iso-dielectric point that the drag force overwhelms DEP, and the cells are carried to one of the device's outlets for collection.

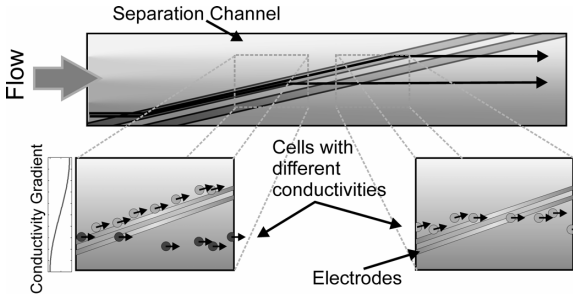

Figure 1: Device concept and operation. Electrodes along the channel's diagonal serve as DEP barriers, forcing cells to traverse the conductivity gradient until the DEP force is overwhelmed by drag and the cells flow downstream for collection.

Coupling between the physical domains relevant to the device's performance complicates the design of the device. We overcome these challenges by leveraging the advantages of microfabrication to create precisely defined geometries with disparate length scales. To maximize both the time over which the gradient attenuates by diffusion as well as the DEP force, we use a wide, shallow channel. Also of importance is the polarization of the electrically heterogeneous fluid by the electric field and the electrohydrodynamic flows that are induced by this interaction. Confining the electric field to a small fraction of the channel's width results in a locally uniform conductivity and mitigates this coupling.

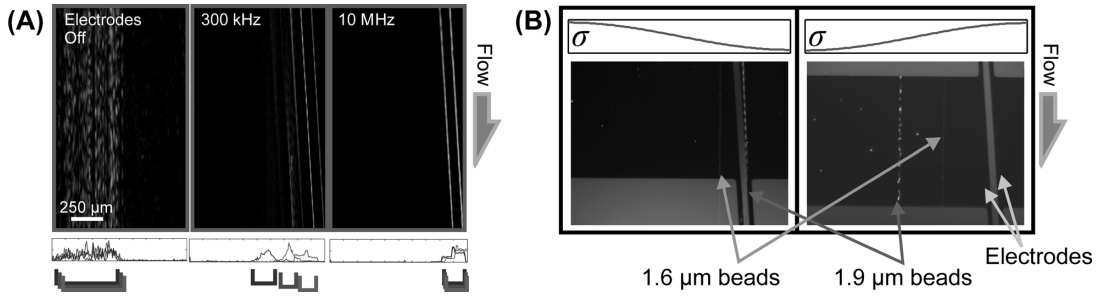

Figure 2: (A) Simultaneous separation of three types of polystyrene beads. (B) Selecting for conductivity in the presence of size variations; reversing the direction of the conductivity reverses the order in which the beads separate.

3. Results and Discussion

We have performed experiments using a broad array of particles and cells, including polystyrene beads, vesicles, and yeast. To demonstrate the analog nature of IDS, we flow three types of beads with different effective conductivities into the device simultaneously; at frequencies between 100 kHz – 1 MHz, we are able to resolve the three subpopulations, as shown in figure 2A. In the absence of a conductivity gradient, the same three beads are not resolved under the conditions of voltage, frequency and flow rate used in our device, suggesting that it is conductivity rather than size that governs the order of separation. This is supported further by the reversal of the IDPs of

two types of polystyrene beads upon reversing the direction of the conductivity gradient and using p-DEP as the basis for separation (figure 2B).

For a biological demonstration of IDS, we separate yeast based upon viability. Heat treatment of the cells compromises their membrane, causing the conductivity of the cytoplasm to decrease, as small ions are no longer confined within the cell [5]. As a result, viable cells exhibit successively higher electrical conductivities as the frequency is increased from ~100 kHz to ~1 MHz. Figure 3 shows the frequency- and conductivity-dependent separation of viable yeast cells from non-viable ones, with optimal separation occurring at ~600 kHz.

4. Conclusions

Technologies for continuous-flow screening of cells are currently in great demand. We have demonstrated through experiments involving both biological and synthetic particles a proof-of-concept for IDS as an emerging method for high throughput screens. The ability to sort cells based upon a phenotypic marker (viability) and perform non-binary separations in a method that is specific to electrical conductivity prepares us to apply IDS to screens of mutant libraries.

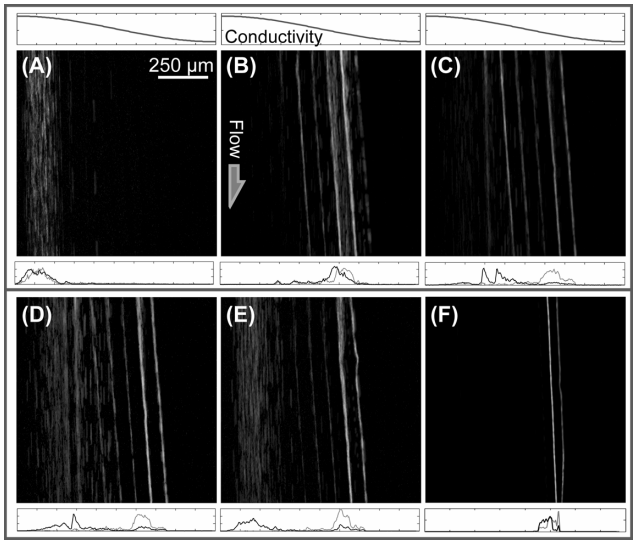

Figure 3: Live/dead separation of yeast using IDS with (A) electrodes off, (B) 200 kHz, (C) 400 kHz, (D) 500 kHz, (E) 600 kHz, and (F) 50 MHz.

Acknowledgements: This work was supported by NIH grant (EB005753), MIT Buschbaum Fund, and the Merck/CSBi Graduate Fellowship.

References

1. Stephanopoulos, G., *Metabolic fluxes and metabolic engineering.* Metab Eng, 1999. **1**(1): p. 1-11.
2. Wang, X.B., et al., *Selective dielectrophoretic confinement of bioparticles in potential energy wells*. Journal of Physics D (Applied Physics), 1993. **26**, p. 1278-1285.
3. Markx, G.H., M.S. Talary, and R. Pethig, *Separation of viable and non-viable yeast using dielectrophoresis.* Journal of Biotechnology, 1994. **32**(1): p. 29-37.
4. Huang, Y., et al., *Introducing dielectrophoresis as a new force field for field-flow fractionation.* Biophysical Journal, 1997. **73**(2): p. 1118-29.
5. Huang, Y., et al., *Differences in the AC electrodynamics of viable and non-viable yeast cells deteremined through combined dielectrophoresis and electrorotation studies.* Phys. Med. Biol., 1992. **37**(7): p. 1499-1517.