HIGH DYNAMIC RANGE ELECTRICAL PROFILING OF SURFACE EXPRESSION VIA FLOW-RATE-MODULATED-MAGNETOPHORESIS

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ABSTRACT

Flow cytometry is commonly used for profiling surface expression of cells fluorescently labeled against antigens of interest. The instrument, however, is highly complex and expensive, making it practical to operate only in specialized laboratories. As a cost-effective and portable alternative, we recently developed a microchip-based cytometer that combines magnetophoretic cell sorting with the Microfluidic CODES sensor technology. Here, we report a technique to enhance the dynamic range of measurements using this device.

KEYWORDS: Flow cytometry, Lab-on-a-chip, Microfluidics, Surface antigens, Magnetophoresis, Microfluidic CODES, High dynamic range measurement

INTRODUCTION

Membrane antigens are important proteins that regulate cell function. Flow cytometry is the current gold standard for quantitative analysis of cell surface expression and is widely used in applications ranging from basic research to clinical studies. However, a typical flow cytometer is often bulky, highly complex, expensive and can only be operated by trained personnel, all of which are limiting its use to well-equipped facilities. As a low-cost and portable alternative, we have previously introduced a cytometry approach by coupling magnetophoretic cell sorting with the Microfluidic CODES sensor technology and developed a microfluidic device that can electrically profile cell surface expression [1]. In this paper, we improve our technique by modulating the sample flow rate to increase the dynamic range of measurements while preserving the frugality and simplicity of the original design.

THEORY

In our microfluidic cytometer, magnetically sorted cells are directed into discrete outlets, which are concurrently monitored by a code-multiplexed electrical sensor array to quantify fractions. While discretization of cell sorting process via multiple outlets provides robust differentiation between subpopulations, the number of outlets dictates the dynamic range of measurements. Besides increasing the number of outlets, the dynamic range can be enhanced by varying the sample flow rate during processing. This is because cell exposure to magnetic force varies with the flow rate and two cells sorted into the same outlet under different flow rates are interpreted to represent different cell surface expression levels. This is analogous to high dynamic range (HDR) digital photography, where an image is taken under varying "light" exposures. In our case, we analyze the sample under different "magnetic force" exposures and collectively analyze the aggregate data from various flow rates to achieve an HDR.

EXPERIMENTAL

Our device (Figure 1a) consists of three components; a polydimethylsiloxane (PDMS) microfluidic layer that was fabricated via soft-lithography, a glass substrate with the electrical sensor network that was fabricated via lift-off process, and an on-chip permanent magnet to create a magnetic field gradient. In operation, immunomagnetically labeled cells deflect in the transverse direction under the externally-applied magnetic field according to their surface expression [2] and their deflection is electrically measured by code-multiplexed Microfluidic CODES sensors [3] monitoring the 8 microfluidic channels at the end of the sorting chamber.

Magnetic and hydrodynamic characteristics of the device were examined by finite element analysis (FEA) using COMSOL Multiphysics (Figure 1b). Specifically, the trajectories of 8 μm-radius cells with different number of magnetic particles were simulated under different flow rates. Based on these results, a look-up table was created. We then applied our device to profile epithelial cell adhesion molecule (EpCAM) expression of SK-BR-3 breast cancer cells. The cells were pre-labeled with anti-EpCAM conjugated 1 μm-diameter magnetic beads. The distribution of the number of magnetic beads across the cell population was independently measured using a custom image processing software in MATLAB.

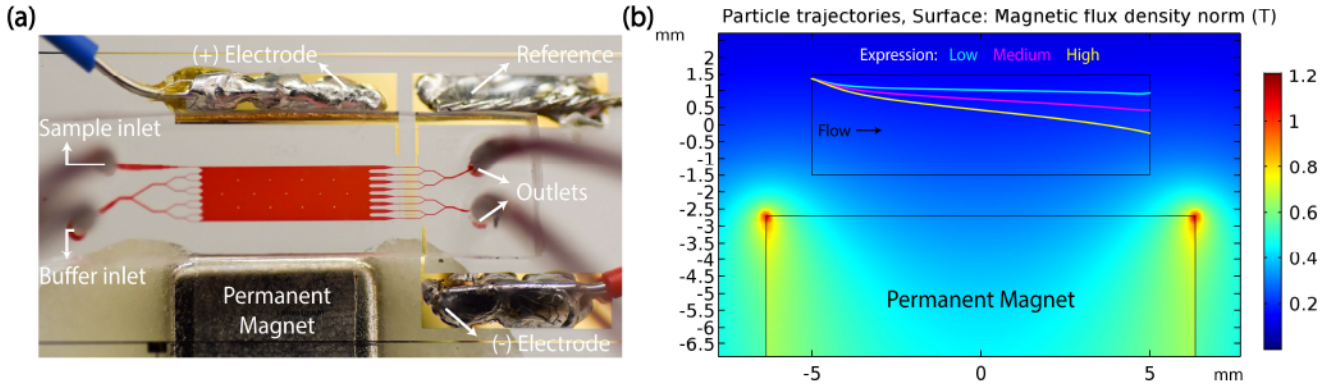

Figure 1: Design of the microfluidic device for surface expression analysis. (a) A photo of the microfluidic device with individual components labeled. The sensors are excited from the reference electrode and a bipolar electrical signal is acquired from the positive and negative electrodes. (b) Finite element analysis of the magnetic and hydrodynamic characteristics of the device for the calibration of experimental results. Cyan, magenta and yellow lines represent the trajectories of a 8μm radius-cell labeled with 10, 40 and 80 magnetic beads, respectively, under a 15 mbar drive pressure.

RESULTS AND DISCUSSION

During measurement, immunomagnetically labeled cell suspension and the buffer (1X phosphate buffered saline) were pneumatically driven into the device under 5, 10, 30 and 50 mbar pressures. The electrical signal from the sensor network was sampled into a computer and decoded with a custom LabVIEW program. We compared the experimentally obtained distribution of sorted cells to outlets with FEA-predicted cell distributions under different drive pressures (Figure 2). The two data fit well except under low flow rates, where, some of the high expresser cells were trapped by the permanent magnet at the bottom of the deflection chamber, leading to the underestimation of the cell count deflected towards the 8th outlet. Consequently, cell counts from the 8th outlet under 5 and 10 mbar drive pressures were not used in the data analysis.

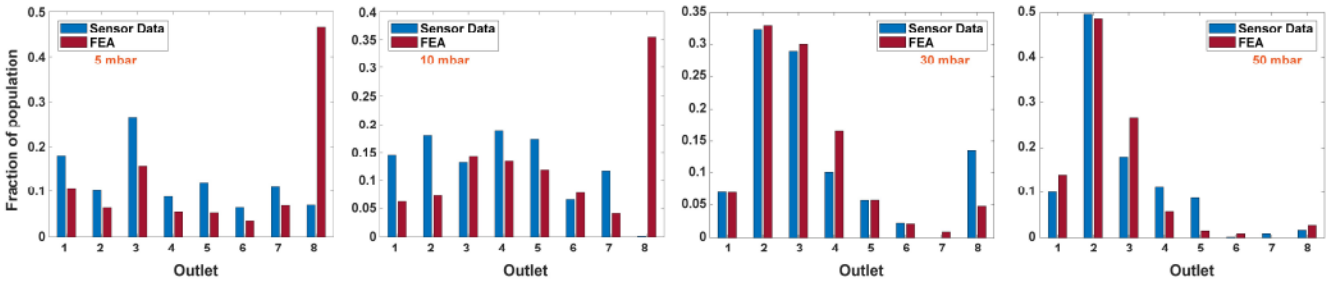

Figure 2: Comparison of experimental results obtained under different flow rates and finite element analysis results. Under low flow rates, some high expresser cells were trapped by the magnetic field. This led to an underestimation of the cell counts in the 8th outlet of 5 and 10 mbar experiments.

The cell counts from different outlets were converted into magnetic load distribution in MATLAB using a look-up table. The aggregate data from different flow rates were collectively analyzed and a magnetic load distribution histogram was created and compared to the data obtained via image processing (Figure 3). First histogram in the Figure 3 shows the magnetic load distribution obtained by solely using the data recorded under 5 mbar drive pressure. As expected, the data collected at 5 mbar drive saturated above a certain magnetic load and could only discriminate cells with low magnetic load. By adding data from higher flow rates, we were able to increase the dynamic range, which resulted in a magnetic load distribution that gradually converged to the data obtained from the image processing-based analysis of the cell population.

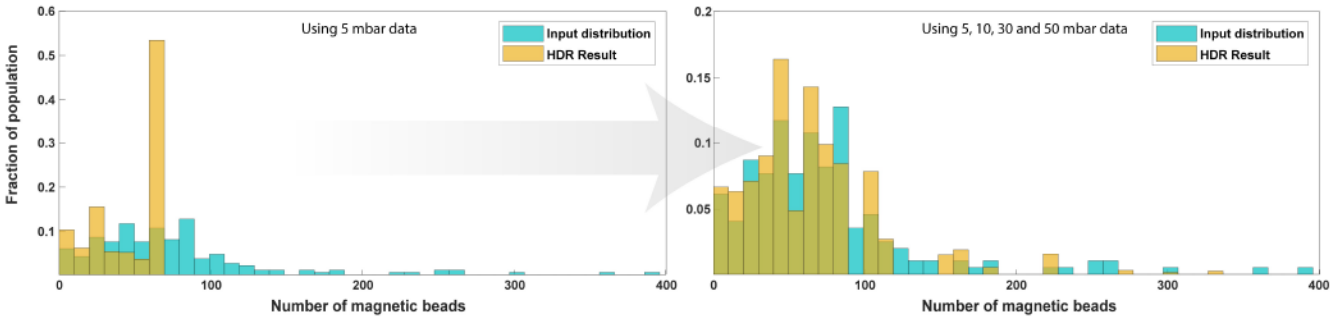

Figure 3: Improvement of the HDR results with additional information from different flow rates. The effective dynamic range of the system increased by collectively analyzing the aggregate data from different flow rates.

CONCLUSION

We have demonstrated a high dynamic range implementation via flow modulation to increase our magnetophoretic cytometry approach for profiling of cell surface expression. This new approach successfully increases the dynamic range of our system without affecting the physical design aspects of the device.

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