DIGITAL PHOTOGRAPHY TECHNIQUES IN MICROFLUIDICS: EXPOSURE BRACKETING FOR HIGH DYNAMIC RANGE MAGNETOPHORETIC CYTOMETRY

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ABSTRACT

Due to the vast difference in surface expression levels among cell populations, flow cytometers must possess a dynamic range sufficiently high to accommodate such variations. We recently introduced a microchip-based flow cytometer that combines magneophoresis and distributed Coulter sensing. Inspired from digital photography techniques, we implemented exposure bracketing in magnetophoretic cell sorting to enhance the dynamic range of cell surface expression measurements with our electronic cytometry chip.

KEYWORDS: Flow cytometry, Dynamic range, Microfluidics, Surface antigens, Magnetophoresis, Coulter sensor

INTRODUCTION

Cell-based diagnostics, immunophenotyping of hematological cells and histocompatibility testing of transplants rely on qualitative and quantitative characterization of cell membrane antigens. However, the expressions of membrane antigens may vastly vary even within the same tissue; hence, the measurement technology must accommodate a dynamic range wide enough to cover those variations [1]. Here, we report a high dynamic range operation inspired by exposure bracketing in digital photography for the all-electronic magnetophoretic cytometry platform we introduced as an alternative to flow cytometers based on immunofluorescence [2].

EXPERIMENTAL

Our magnetophoretic cytometer (Figure 1a,b) utilizes a free-flow magnetophoresis chamber where immunomagnetically labeled cells are differentiated according to their surface expression under an externally-generated magnetic field gradient. Magnetophoretic trajectories of the cells together with the cell size are then transduced into signature electrical waveforms by distributed Coulter sensors. These trajectories and cell size data are then processed to calculate cell surface expression via flow-rate specific look-up tables (Figure 1c) generated by finite element analysis.

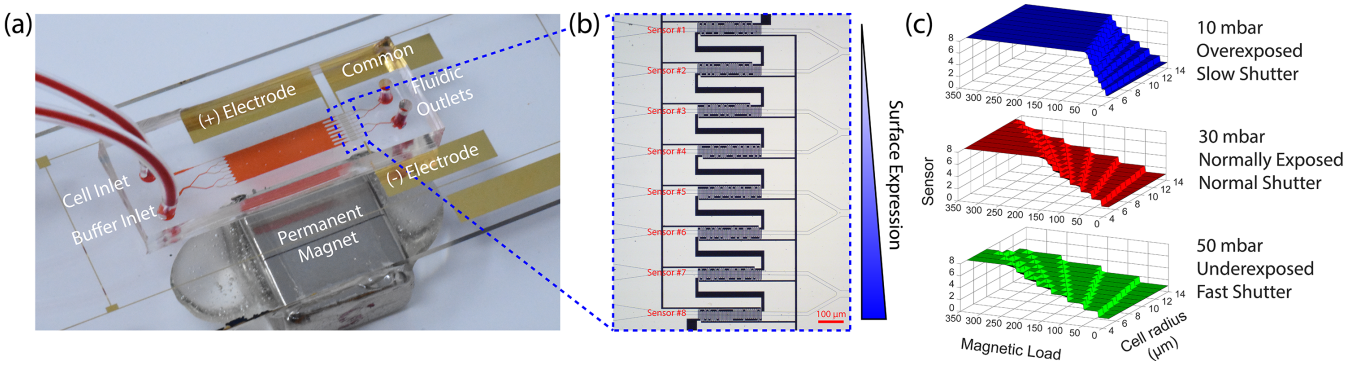

Figure 1:Design and operation of our magnetophoretic cytometer. (a) Photo of a fabricated device. (b) Microscope image of the electrical sensor network that monitors cell trajectories. Each sensor generates a 31-bit digital code for a multiplexed read-out. (c) Simulated look-up tables mapping the sensor identity, cell size and magnetic load under overexposure, normal exposure and underexposure settings. For each exposure setting, flat region indicates the magnetic load range saturating the sensor.

In an immunofluorescence analysis, the brightest cell may be orders of magnitude brighter than another due to the wide variation in surface expression, hence current flow cytometers utilize logarithmic amplifiers to achieve a high dynamic range [1]. Unlike immunofluorescence, our technique measures surface expression by acquiring the magnetophoretic trajectories of immunomagnetically labeled cells via distributed Coulter sensors. As the dynamic

range is determined by the number of sensors implemented in our device, having a sensor with wide-enough dynamic range would require design changes and increase the hardware complexity. This limitation is analogous to sensor saturation in digital imaging; hence, digital photography techniques can be utilized to achieve a high dynamic range without altering the device design. A high-dynamic-range image in digital photography is achieved by taking the same image with different exposure times, a method called exposure bracketing [3]. The camera takes an underexposed (i.e., fast shutter), a normally exposed and an overexposed image (i.e., slow shutter) which are later computationally stitched into a single high-dynamic-range image. We adapted this technique in our technology and applied exposure bracketing via modulating the flow rate of the cells, which is equivalent to the shutter speed.

RESULTS AND DISCUSSION

During the experiment, immunomagnetically labeled SK-BR-3 breast cancer cells were driven into the cytometry chip at 10 mbar, 30 mbar and 50 mbar pressure rates to simulate overexposure, normal exposure and underexposure, respectively (Figure 2a). The cell distribution acquired at each flow condition was converted to magnetic load distribution via their look-up tables, and a high-dynamic-range profile of the surface expression was achieved by utilizing the properly exposed data points ("pixels") of each flow rate and, the results were compared to independent microscopic measurement of magnetic load (Figure 2b). The results showed a correlation coefficient of 0.86 and p-value of 6.9×10^{-15}, confirming flow modulation inspired by digital photography as a compelling method for high dynamic range in magnetophoretic cytometry.

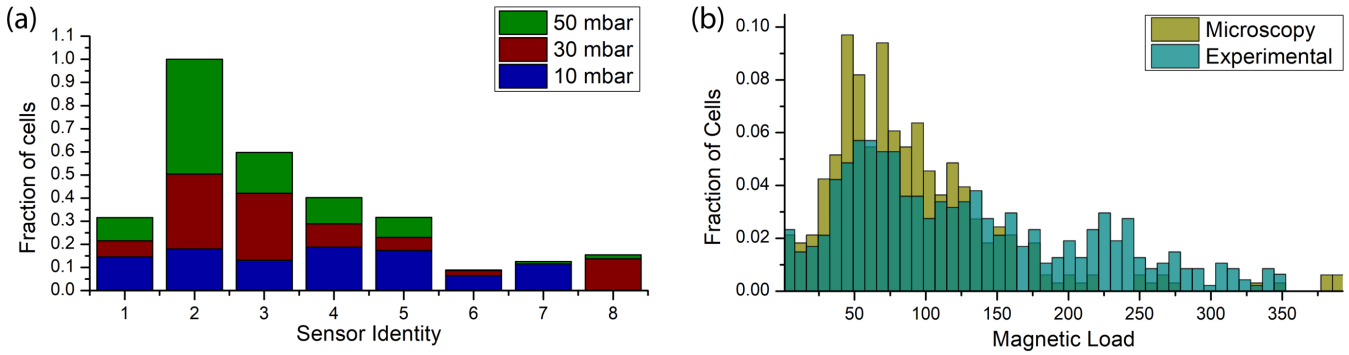

Figure 2: Experimental results. (a) Distribution of magnetically sorted cells per sensor during the experiments under 10 mbar, 30 mbar and 50 mbar pressure rates.(b) Comparison of results from the microchip with enhanced dynamic range to results from analysis of microscope images of cells using a custom-built image processing algorithm.

CONCLUSION

We enhanced the dynamic range of cell surface expression measurements by implementing a magnetic field exposure bracketing technique in our electronic cytometry chip. Monitoring magnetophoretic trajectories of labeled cells under different magnetic exposures enabled an effective dynamic range that was considerably higher than what could be achieved with a single exposure acquisition.

ACKNOWLEDGEMENTS

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