AUTOMATED HIGH-THROUGHPUT CHARACTERIZATION OF CELLS USING MULTIMODAL ELECTRICAL AND OPTICAL CYTOMETRY (MULTIMEOC)

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ABSTRACT

We demonstrate an automated high-throughput method—multimodal electrical and optical cytometry (MultiMEOC)—for simultaneously measuring the electrical and optical properties of cells. MultiMEOC utilizes microscopic imaging of particles moving against electrodes to extract the electrical and optical properties on a per-cell basis. We used the method to discriminate mammalian HL-60 cells based upon viability, and were able to decompose complex mixtures containing both cells and beads. Finally, we used MultiMEOC to discriminate primary neutrophils based upon their activation state. MultiMEOC can perform multimodal characterization and discrimination of cells efficiently and therefore evaluate the separability of cells based upon intrinsic properties.

KEYWORDS

High-throughput characterization, Multimodal, Dielectrophoresis, Neutrophil activation, Cytometry

INTRODUCTION

Cell separation based on intrinsic properties such as size[1], shape[2], and electrical properties[3] has diverse applicability, from cancer[4] to stem cells[5]. Intrinsic properties are typically widely distributed in any given population (e.g., factor of two variation in cell size), and devices that separate based on a single intrinsic property may not perform optimally (e.g., leukocyte subpopulations have overlapping size distributions[6]). Unfortunately, no devices exist that can acquire multimodal intrinsic information (size, shape, electrical properties) simultaneously on large numbers of cells. Here we describe multimodal electrical and optical cytometry (MultiMEOC), an automated high-throughput method that combines microscopy with DEP to measure multimodal intrinsic properties in thousands of cells.

RESULTS

To acquire multimodal intrinsic information, we combine microscopy with dielectric characterization in a flow-through device (Fig. 1). The method takes images of cells, thus acquiring size, shape, and other morphological data, while simultaneously acquiring electrical information. Since we have multiple pieces of information from each cell, we
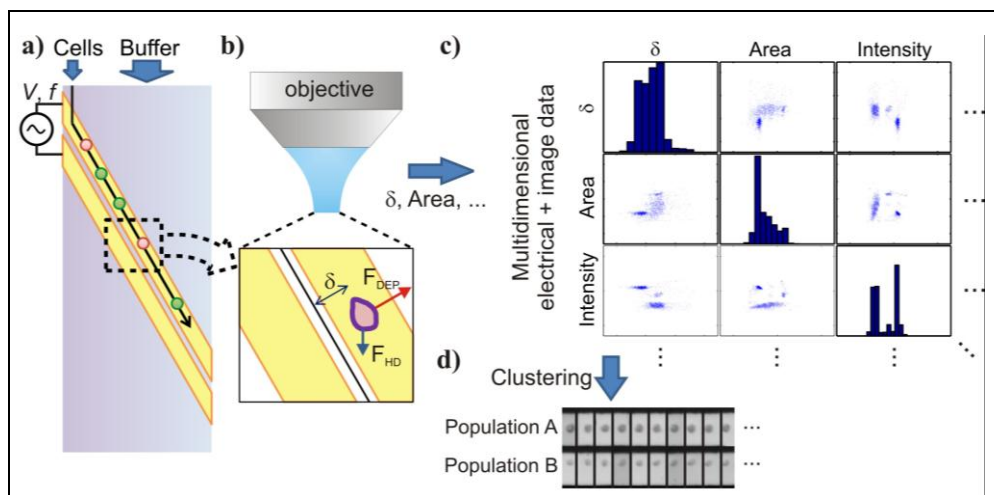

Figure 1: Overview.   a) Mixed cell populations introduced into a microfluidic channel flow along the electrodes due to the combined action of hydrodynamic (F_{HD}) and DEP (F_{DEP}) forces. b) As the cells pass by the microscope objective, we take images, from which we extract the distance from the cells to the electrode centerline (the balance position, δ) and optical information (area, morphology, intensity, etc.). c) The multi-dimensional information enables thorough single-cell analysis of various intrinsic properties within the population, here shown as two-dimensional scatter plots of pairs of intrinsic parameters. d) We can cluster the multi-dimensional data and then gate the clusters to examine the underlying cell images, thus virtually de-mixing the population.

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136

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are able to analyze and cluster the intrinsic properties of complex mixtures of cells in a multi-dimensional space.

To obtain the electrical properties of the cells, we developed an optical method of measuring the DEP force, which we term the "DEP spring". The method uses a microfluidic channel with two coplanar electrodes on the channel bottom, placed at an angle
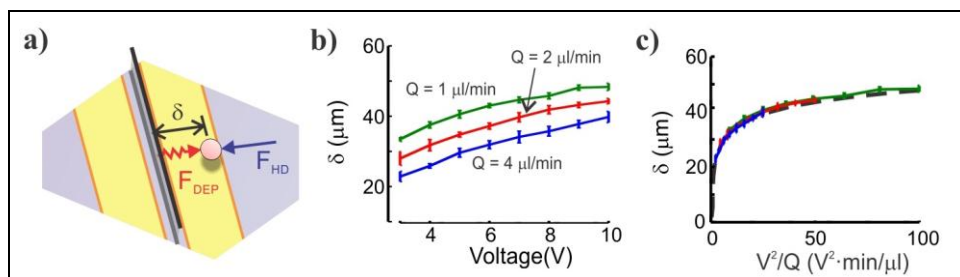

Figure 2: The DEP spring. a) The balance position, δ, is determined by competition between DEP (F_{DEP} which is ~V^2 and electrical properties) and hydrodynamic (F_{HD}, which is ~ Q) forces. Therefore, we can extract the electrical properties from δ. b) Measured balance positions of 6-μm beads. c) Scaling the balance positions from b) according to the electrical and hydrodynamic forces causes them to collapse onto each other and into agreement with numerical modeling (---).

to the flow (Fig. 2a). The electrodes are energized with an electric field to create a repellant negative-DEP force barrier. When a cell is pushed against this DEP barrier by the hydrodynamic force, the position of the cell relative to the barrier — the *balance position* — provides information into the electrical properties of the cell. The balance position is essentially the equilibrium deflection of a nonlinear spring with stiffness proportional to the voltage square and flow rate proportional to the applied force.

To validate the DEP spring, we measured the balance positions of 6-μm polystyrene beads at different flow rates and voltages (Fig. 2b). As expected, the balance position increases with increasing voltage (stiffer spring) or decreasing flowrate (smaller applied force). The balance positions all
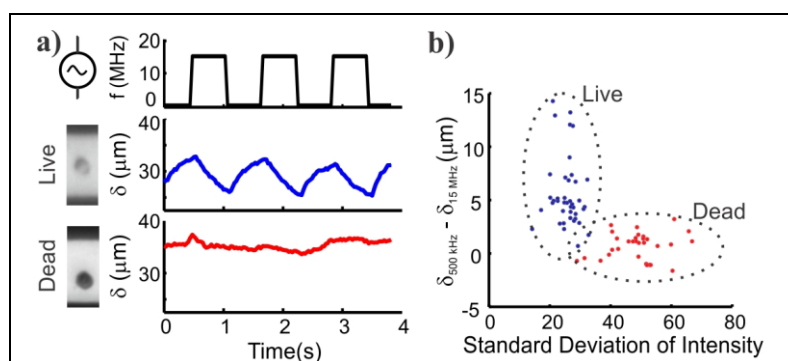

Figure 3: Discrimination of live and dead cells using MultiMEOC. a) Introducing a mixture of live and dead HL-60 cells and switching between two frequencies (15 MHz and 500 kHz) (top), some cells are observed to have varying δ (middle) while others have constant δ (bottom). b) Correlating the balance position with image information (trypan blue staining of viability), we find that the cells with varying δ are live cells, whereas dead cells have constant δ.
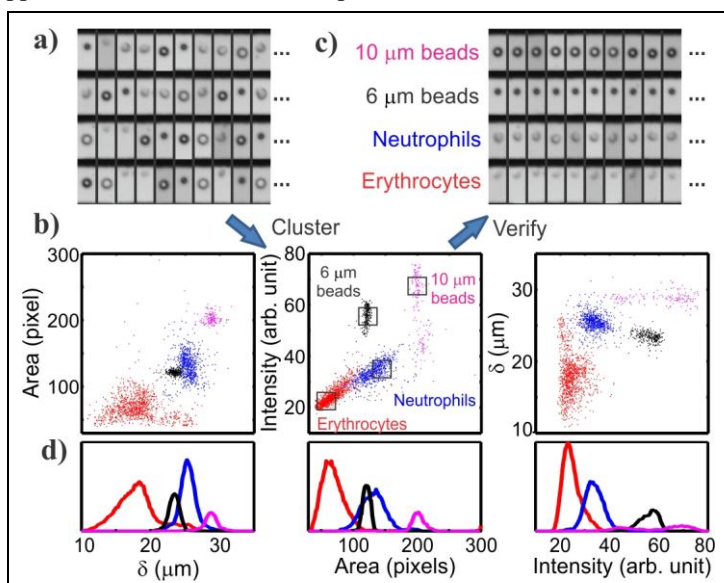
collapsed onto the analytical model when they were scaled according to the applied forces (Fig. 2c), highlighting the quantitative nature of the approach.

We then developed an automated system composed of computer-controlled syringe pumps, function generators, and automated microscope that can automatically control flowrate, electrical conductivity, DEP force, and imaging parameters to measure the balance position of cells in different media conductivities and frequencies, able to acquire ~1000 cell images/sec. The automatic control and multimodal information allowed measurement of the balance position at different frequencies (Fig. 3a) to distinguish between live and dead cells, which was verified simultaneously with the trypan blue stain (Fig.

Figure 4: Resolving complex populations. a) Images of a mixture of 10-μm beads, 6-μm beads, neutrophils, and erythrocytes taken with MultiMEOC. b) Scatter plots of δ, particle area, and intensity extracted from the images. It is possible to distinguish the four populations using a combination of intrinsic parameters across the three scatter plots. c) The classified populations can be verified by gating and examining the images of particles in each gated region. d) Single-parameter histograms show overlap of the four sub-populations, showing that one intrinsic parameter is not sufficient for classification.
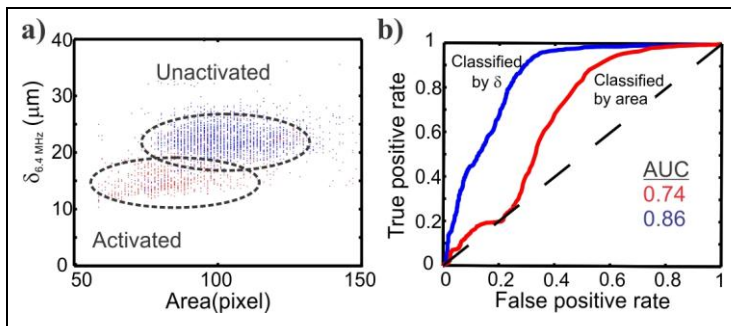
Figure 5: Discrimination of neutrophil activation. a) Unactivated and activated (via PMA) human neutrophils were analyzed with MultiMEOC. Shown are scatter plots of two intrinsic properties of the two populations. b) The receiver operating characteristic is a standard approach to compare separation methods. The higher area-under-curve (AUC) obtained with electrical properties (via δ) indicates that they are better than size for distinguishing neutrophil activation.

3b). To further show the utility of obtaining multimodal information, we introduced a mixture of four particle types into the device (Fig. 4a). Using a combination of intensity, size, and electrical information, we could distinguish the four populations (Fig. 4b), which could be examined by going back to the images of the clustered populations (Fig. 4c). In contrast, using any single parameter cannot effectively discriminate the populations, demonstrating the power of using multimodal information (Fig. 4d).

Finally, we used MultiMEOC for a medically relevant application, namely to compare different intrinsic approaches to separating activated versus unactivated immune cells. Immune cell activation is important in a number of diseases, including sepsis[7], and there is interest in identifying and potentially separating activated immune cells for diagnosis or treatment. Intrinsic identification and separation of such cells is advantageous because it is faster than label-based approaches and avoids artifactual activation caused by the labeling procedure. We acquired multimodal information from primary human neutrophils (purified from fresh blood) in activated versus unactivated states (activated with phorbol-myristate-acetate). By rapidly testing 21 different combinations of media conductivities and frequencies within 1 hour, we found the two cell states differ in both δ and size in media with conductivity 0.8 S/m and at a frequency of 6.4 MHz. Examining balance position and size (Fig. 5a) shows that electrical properties are superior to size for resolving the populations, as evidenced by the highest area under the curve (AUC) of the receiver operator characteristic (Fig. 5b).

CONCLUSION

We developed MultiMEOC, an automated high-throughput cytometry system for acquiring electrical and optical information of single cells in flow. MultiMEOC uses a novel optical method for measuring DEP forces. Using the method, we characterized and discriminated live and dead HL-60 cells, complex mixtures of beads and cells, and different activation states of neutrophils, all by extracting multiple parameters for each particle. The multimodal information acquired by MultiMEOC allows study into the distributions of intrinsic properties of cells, which will in turn guide the design of intrinsic separation technologies.

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