Spatial Cell Discrimination Using Optoelectronic Tweezers

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***Abstract*–The difference in dielectric properties of live HeLa and Jurkat cells are used to spatially discriminate the cells using optoelectronic tweezers. Spatial discrimination is verified by fluorescent tagging of the Jurkat cells.**

I. Introduction

When working with biological samples consisting of mixed cell populations, such as human blood, it is desirable to have the ability to discriminate among the different cell types. Optoelectronic tweezers (OET) is a tool that enables optically-induced dielectrophoresis for single-cell spatial manipulation and discrimination [1]. Unlike conventional dielectrophoresis (DEP), which uses static microfabricated electrodes to create non-uniform electric fields [2], the optically-defined manipulation patterns of OET are reconfigurable in real time. In addition, OET requires 100,000 times less optical power density than laser-induced optical tweezers [3], enabling the use of an incoherent light source and direct imaging techniques for particle manipulation [4].

Previously, we have demonstrated OET trapping of *E. coli* bacteria [5] and red and white blood cells [6], as well as the automated concentration of HeLa cells [7], and the selective concentration of live human B cells from dead cells [1]. In this paper, we use OET to achieve spatial discrimination between cell types in a mixed population of cells. Using a scanning line pattern, we exploit the difference in dielectric properties between Jurkat and HeLa cells to differentiate the mixed population. Successful differentiation is verified by fluorescence microscopy.

II. Experimental Details and Results

Optoelectronic tweezers creates dielectrophoretic force using optically-defined virtual electrodes, the details of which are explained elsewhere [1]. Briefly, the structure of the OET device consists of a liquid layer containing the cells under manipulation, sandwiched by an upper transparent planar electrode of indium-tin-oxide-coated glass, and a lower photoconductive surface of hydrogenated amorphous silicon (a-Si:H). Optical illumination increases the conductivity of the photoconductive layer and creates a virtual electrode to induce DEP forces for cell manipulation.

Dielectrophoretic force is a function of the frequency-dependent electrical properties of the cells under manipulation. As different cell types exhibit dissimilar electrical properties, DEP can be used to sort between cell types [2, 8, 9]. We use this capability to perform spatial discrimination between live Jurkat and HeLa cells using OET.

The OET setup consists of a digital micromirror device (DMD) spatial light modulator (Texas Instruments), which is programmed to create scanning-line optical patterns. The DMD is illuminated by a 625-nm LED (Lumileds, Luxeon Star/O). The image from the DMD is focused onto the surface of the OET device using a 10x objective lens. Observation of the cells is performed on an upright microscope (Nikon LV-100D) using DIC and epi-fluorescence.

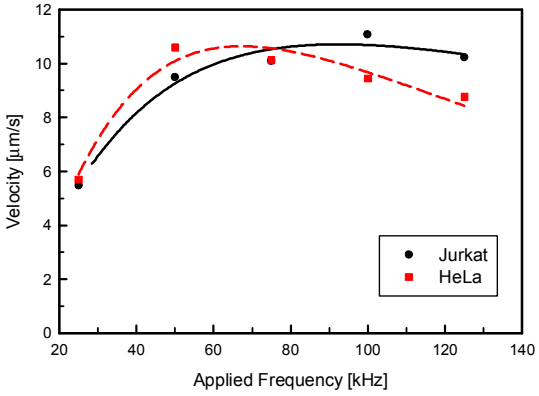

Fig. 1. OET-induced manipulation velocity of different cell types as a function of the frequency of the electric field. Cells are manipulated using a 15-µm-wide scanning line pattern.

Cultured Jurkat cells are labeled with a green fluorescent dye. The labeled Jurkat cells and cultured HeLa cells are washed with an isotonic buffer (8.5% sucrose, 0.3% dextrose), re-suspended in isotonic solution, and mixed together. The concentration of the mixed solution is approximately 5 x 10^5 cells/mL. Culture media is added to adjust the conductivity of the cell solution to approximately 2 mS/m. A 20-µL aliquot of cell solution is introduced into the OET device for manipulation. The cells experience a

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positive DEP force, and are attracted towards the optical manipulation patterns.

The maximum OET manipulation velocity of Jurkat and HeLa cells as a function of the frequency of the applied voltage is shown in Figure 1. The manipulation velocity is proportional to OET force. At an applied voltage of 10 Vpp at 100 kHz, sufficient variation in the OET force exists to differentiate between the two cell types.

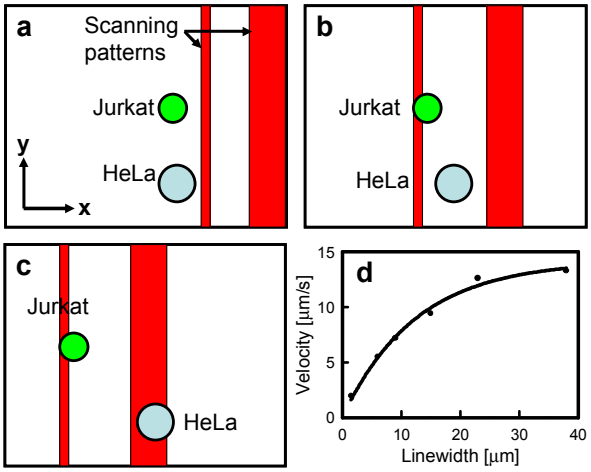

Fig. 2. Optical pattern for OET-enabled cell discrimination. (a) The cells before the lines are scanned in the negative *x* direction. The thin leading line produces a weaker OET force than the thicker trailing line. (b) As the pattern is scanned, the Jurkat cells have a sufficiently OET strong force to be retained by the leading line, while the HeLa cells do not experience sufficient force. (c) The trailing line provides enough force to transport the HeLa cells, while the Jurkat cells continue to be retained by the leading line, achieving spatial discrimination. (d) OET manipulation velocity of HeLa cells as a function of the width of the optical pattern.

In order to spatially separate the HeLa and Jurkat cells, a scanning line optical pattern is used to exploit the differences in OET force on the cells. This is shown schematically in Figure 2. A 15-µm-wide leading line and a 23-µm-wide trailing line are separated by ~40 µm, and are simultaneously scanned at a rate of 13 µm/s. The leading line produces a weaker OET force than the thicker trailing line, as the manipulation velocity of cells exhibits a dependence on the width of the optical pattern (Fig. 2d). Thus, as the two lines are scanned across the OET device, the Jurkat cells, which experience a stronger OET force, are held by the leading line. The leading line does not produce sufficient force to transport the HeLa cells against the viscous drag, which are subsequently attracted to and transported by the trailing line. After the scan is completed, the cells retain a spatial separation equal to the spacing of the two scanning lines.

The results of the optical line scanning on a mixed population of Jurkat and HeLa cells are shown in Figure 3. An initial group of both cell types is present (Fig. 3a). As the scanning pattern moves from right to left across the field of view, the Jurkat cells are transported by the 15-µm leading line (Fig. 3b). The HeLa cells are transported only slightly by the leading line pattern, and cannot maintain the velocity of the translated leading line. The 23-µm trailing line then attracts and transports the HeLa cells. Scanning of the line patterns was repeated twice to achieve the desired separation between the Jurkat and HeLa cells. After the third scan, the two cell types are spatially separated (Fig. 3c). A second HeLa cell is also visible, which moved into the field-of-view during the scanning of the optical pattern. Using fluorescent imaging, it is verified that the cells on the leading line pattern are the fluorescent-labeled Jurkat cells (Fig. 3d). In this image, the unlabeled HeLa cells do not appear.

III. Conclusion

We demonstrate the OET-enabled spatial discrimination of live Jurkat and HeLa cells by exploiting the difference in electrical properties of the two cell types. A two-line scanning pattern results in a separation of the cell types with a distance equal to the separation of the line patterns.

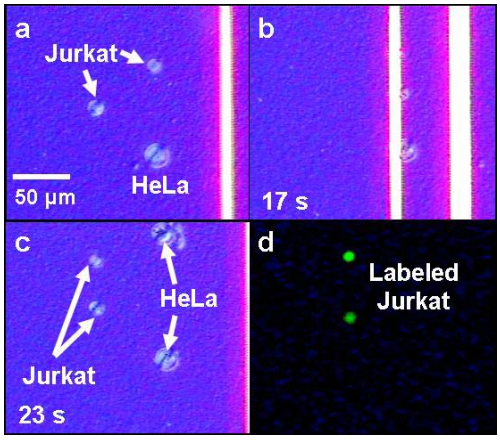

Fig. 3. OET-enabled spatial discrimination of live Jurkat and HeLa cells. (a) The initial cell positions before the optical pattern is scanned from right to left across the field-of-view. (b) The cells are attracted to the leading line. The HeLa cell is starting to lag the scanning line. (c) The cells after the scan is completed, showing spatial separation. An additional HeLa cell has moved into the field-of-view during the scan. (d) Fluorescent image of the cells in (c), verifying that the leading cells are the fluorescent-labeled Jurkat cells.

Acknowlegment

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