Continuous Optical Sorting of HeLa Cells and Microparticles Using Optoelectronic Tweezers

Pei Yu Chiou, Aaron T. Ohta, and Ming C. Wu

Berkeley Sensors & Actuators Center (BSAC) and Department of Electrical Engineering and Computer Sciences, University of California, Berkeley,497 Cory Hall #1774, Berkeley, CA 94720-1774, USA
TEL: (510) 643-0808, FAX: (510) 643-6637, E-mail: pychiou@eecs.berkeley.edu

ABSTRACT

We demonstrate an automatic optical sorting technique that combines video image analysis, pattern recognition, and adaptive optoelectronic tweezers (OET) actuation on a continuously moving chip. This tool is capable of sorting cells and particles according to their visual characteristics at the single-cell level.

INTRODUCTION

Variations among individual cells of a population make it desirable to have the capability to identify single cells of interest, and sort these cells from the general population. Optoelectronic tweezers (OET), a tool that enables light-induced dielectrophoresis [1], is capable of single-cell manipulation. Unlike conventional dielectrophoresis (DEP), which uses static microfabricated electrodes to create non-uniform electric fields [2], the optically-defined manipulation patterns of OET are re-configurable in real time. In addition, OET requires 100,000 times lower optical power density than optical tweezers, enabling the use of an incoherent light source and direct imaging techniques for particle manipulation [3]. Light-induced dielectrophoresis has been demonstrated on a variety of cells, including *E. coli* bacteria [4] and *S. cerevisiae* yeast cells [5].

Previously, we have demonstrated automatic manipulation of individual particles in a random array by combining pattern recognition with OET actuation [6]. This operation, however, can only be performed within the field of view (1x1 mm^2) of a microscope. In this paper, we extend this technique to a *continuously moving* OET platform. Large manipulation area is attained by rastering the chip through the microscope field of view. Automatic concentration of HeLa cancer cells and sorting of 15- and 20-μm-diameter polystyrene beads have been successfully achieved.

SORTING PRINCIPLE AND RESULTS

Optoelectronic tweezers creates dielectrophoretic forces using optically-defined virtual electrodes. The structure of the OET device consists of a liquid layer containing the cells or microparticles under manipulation, sandwiched between an upper transparent 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.

The experimental setup is shown in Fig. 1(a). The system consists of a liquid-crystal spatial light modulator (Hamamatsu Photonics Corp.) to generate image patterns for optical manipulation. A 10-mW, 635-nm laser is expanded to cover the image-generating surface of the spatial light modulator (SLM). The patterned light is focused onto the OET device through a 10× objective lens. The OET device is mounted on a motorized stage.

The microscopic image in Fig.1 (b) is captured by a CCD camera and analyzed by an image processing software (Processing 1.0) to determine the particle characteristics such as size, color, texture, or shape. Based on this information, a corresponding virtual electrode pattern is generated in the SLM and projected onto the OET device. Particles are separated by applying appropriate OET patterns. As the OET chip moves continuously over the manipulation area, the randomly distributed cells are sorted into multiple lines.

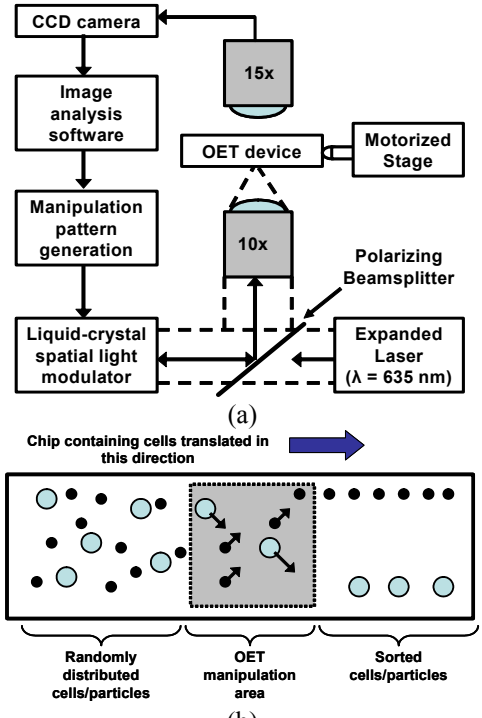

Fig. 1(a). Automated OET system for real-time image-analysis feedback control. (b) Schematic of method of chip-scale sorting using OET-based sorting.

To perform the sorting, the microscopic image is analyzed in real time as the stage carrying the OET device moves at a constant speed. Once a target cell is found, the corresponding pixels on the SLM are turned on, creating an optical pattern near the target cell. If a positive DEP force is induced, the cell will move towards the illumination area, while a negative DEP response will push the cell away from the optical pattern.

Figure 2 illustrates the sorting of HeLa cells. The stage carrying the OET device moves from the left to the right at a constant speed of 5 μm/s. The cells enter the active manipulation area from the left side of the screen and trigger the SLM to switch on the pixels below the cells. The cells experience a positive DEP response at an ac frequency of 100 kHz, and are pulled to the bottom of the screen. The bright pixels on the SLM are turned off once the corresponding cell reaches the bottom of the screen. The trajectories of the cells are recorded in Fig. 2(c). The linear trajectories show that the cells experience a constant DEP force across the active area.

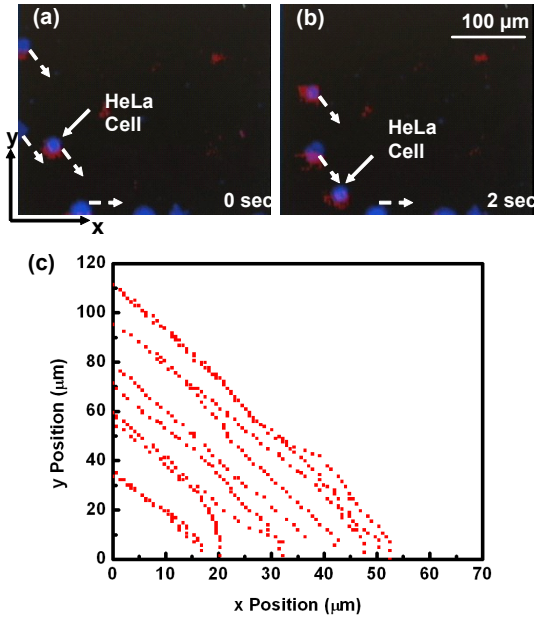

Fig.2 (a)(b) HeLa cells concentrated at the bottom of the images. (c) Trajectories of the HeLa cells entering the active area from the left side of the image.

In addition to cells, sorting of microparticles has also been demonstrated. Figure 3 shows the sorting of 15- and 20-μm-diameter polystyrene beads. The beads enter the active area from the left of the screen, due to the microscope stage moving at a velocity of 10 μm/s. Fig. 3(c) shows the particle trajectories of these two sizes of particles. The 20 μm beads are swept to the bottom in the active area, while the 15 μm particles are pushed to the top.

In our current system, the throughput is limited by the refreshing rate of the SLM, which is 5 frames/s. Moving the stage at a speed larger than 15 μm/s introduces a time delay of the optical patterns projected onto the OET surface. The highest particle concentration that has been successfully sorted in this system is 1600 beads/mm^2, corresponding to a sorting throughput of 120 beads/min in the active area.

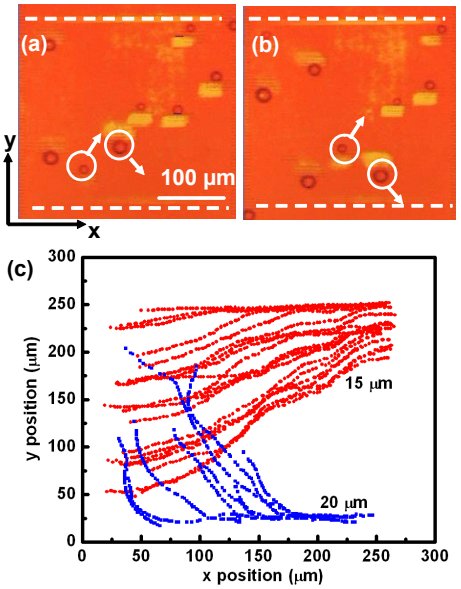

Fig. 3 (a)(b) Continuous particle sorting on 15- and 20-μm-diameter polystyrene beads. The dashed lines show the position of the optical guiding bars. (c) Trajectories of the beads entering the active area from the left of the image.

CONCLUSION

We demonstrated a fully automatic, real-time cell and microparticle optical sorting system that is capable of sorting cells based on the visual appearance. The sorting of HeLa cells and two sizes of latex beads has been shown. The throughput of the current system is approximately 120 cells/min, limited by the refreshing rate of the SLM.

ACKNOWLEDGMENT

The authors would like to thank the members of Prof. Luke Lee's group for assistance with HeLa cells. This project is supported by the Institute for Cell Mimetic Space Exploration (CMISE), a NASA University Research, Engineering and Technology Institute.

REFERENCES

[1] Chiou, P. Y., Chang, Z., Wu, M. C., *Optical MEMS*, 2003.
[2] Pohl, H. A., *Dielectrophoresis*, Cambridge: Cambridge University Press, 1978.
[3] Ohta, A. T., Chiou, P. Y., Wu, M. C., *CLEO*, 2004.
[4] Chiou, P. Y., Wong, W., Liao, J. C., Wu, M. C., *MEMS*, 2004.
[5] Lu, Y.-S., Huang, Y.-P., Yeh, J. A., Lee, C., *Asian and Pacific Rim Symp. on Biophotonics*, 2004.
[6] Chiou, P. Y., Ohta, A. T., Wu, M. C., *MEMS*, 2005.