

# MICROFLUIDIC INTEGRATED OPTOELECTRONIC TWEEZERS FOR SINGLE-CELL SAMPLE PREPARATION AND ANALYSIS

Kuo-Wei Huang<sup>1</sup>, Yi-Chien Wu<sup>1</sup>, Sabbir Sattar<sup>1</sup>, Ji-Ann Lee<sup>2</sup> and Pei-Yu Chiou<sup>1</sup>

<sup>1</sup>Department of Mechanical and Aerospace Engineering, University of California, Los Angeles

<sup>2</sup>Department of Biological Chemistry, University of California, Los Angeles

## ABSTRACT

We report a novel microfluidic integrated optoelectronic tweezers platform for single- and multi-cell sample preparation and analysis. This platform allows users to selectively pick targeted individual cells from a population based on their optical signatures such as size, shape, and fluorescence colors, and transport them into isolated chambers using light induced dielectrophoretic forces. Single cell samples in these chambers can be cut into nanoliter digital liquid plugs by airflow, pushed to outlets, and extracted out of the chip for downstream analysis using commercial instrument such as polymerase chain reaction (PCR).

## KEYWORDS

Optoelectronic tweezers, single- cell analysis, dielectrophoretic forces, carbon nanotube thin film.

## INTRODUCTION

Single cell analysis is the trend for future biomedical diagnostics. Microfluidics has been shown to have great potentials in single cell analysis due to its capability in handling small liquid volume. However, microfluidics is weak in handling individual cells in fluid. Although numerous techniques utilizing physical mechanisms from electric, magnetic, acoustic, and optical forces have been applied for cell manipulation, most of them are limited to trapping, sorting, or concentrating cells. Optoelectronic tweezers (OET) promises a platform for high throughput single cell manipulation using light induced dielectrophoretic forces on a low cost silicon coated glass [1]. One of OET's current challenges, however, is in its difficulty of integration with other microfluidic components since it requires special electrodes to satisfy several interfacing issues [2]. This paper presents the first microfluidic integrated OET that utilizes SWNT embedded PDMS thin film as the electrode and multilayer PDMS channels to provide the valve functions for fluid control. Integration of OET with microfluidics promises a powerful platform for both fluid and cell manipulation. This platform is able to achieve multistep single cell sample preparation functions from on-chip cell selection, cell isolation, liquid plug formation, liquid plug transportation, and extraction for external single cell analysis using commercial high sensitivity instrument.

## EXPERIMENTAL

Figure 1 shows the schematics of a microfluidics integrated OET and the procedure of preparing single cell samples. This platform consists of an OET surface and multilayer PDMS channels. The multilayer PDMS have two layers of channels. The bottom fluidic channel contains aqueous solutions carrying biological cells or particles. The top actuation channel is used to control the membrane valve (Fig. 1A). OET performs cell manipulation in the region covered by SWNT thin film (Fig. 1A). The pneumatic valve is used to confine liquid while the OET is used to actively select target cells.

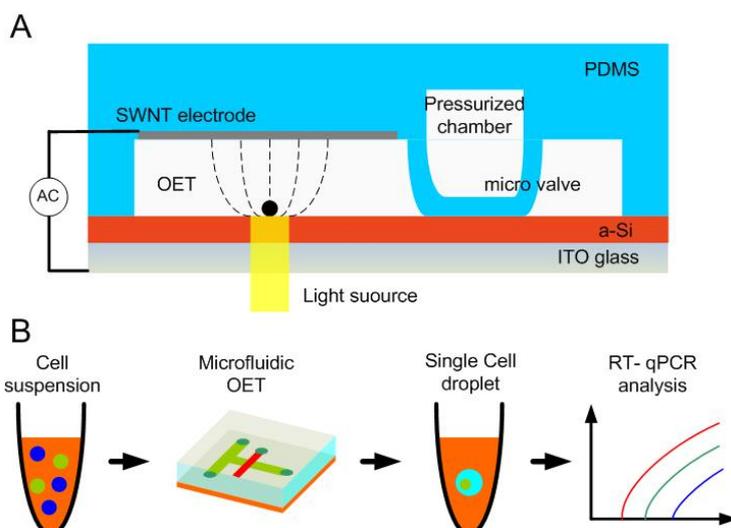


Figure 1. (A) Schematic of the microfluidic integrated OET platform. The SWNT electrode enables OET integration with multilayer PDMS based microfluidics. Cell manipulation in OET is achieved by light-induced DEP forces on a photoconductive surface. The elastomeric PDMS membrane valve controls fluid flows on the chip (B) The procedure of preparing single cell samples for analysis by this platform.

Figure 2A is a microscopic image of a fabricated device. The fabrication method of SWNT electrode embedded in PDMS are shown in our prior work [2]. The device consists of a main channel and several branch channels with SWNT top electrodes. Only one pressure source and one pneumatic valve are used to control fluid flows in all branch channels. Fig. 2B-E presents the steps of preparing single cell samples. First, cell mixture is introduced into the main channel (Fig. 2B) when all the valves in the branch channels are closed. Target cells are selectively picked up by OET under a microscope based on their optical signatures (Fig. 2C) and transported into the T-junction regions in neighboring channels. The main channel is pressurized by airflow to leave an array of isolated liquid plugs with single cells (Fig. 2D). The airflow further pushes these liquid plugs across the valves to the outlets. Liquid plugs are extracted out from the chip and stored in standard PCR tubes containing mineral oil (Fig. 2E). The number of single cells contained in a liquid droplet can be arbitrarily tuned in the OET selection step. To perform quantitative reverse transcriptase polymerase chain reaction (RT-qPCR), RT-qPCR mixture is merged with the droplets and puts into a commercial real-time PCR system (Fig. 1B).

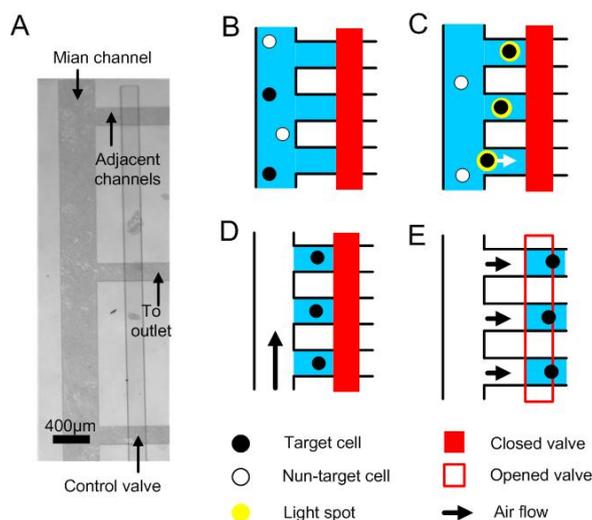


Figure 2. (A) A microscopic image of channels and valves on a microfluidic integrated OET platform. (B) Cell mixture is injected into the main channel. (C) Target cells are identified and selectively transported into branch channels by light beams. (D) The main channel is flushed by airflow to form liquid plugs in the branch channels. (E) The pneumatic valve is then open for pushing liquid plugs to outlets for extraction.

## RESULTS

To demonstrate the fluorescence-based cell identification and cell separation, HeLa cells stained with Calcein AM were imaged and separated from the population. Cell mixture was introduced into the microfluidic OET and inspected under a microscope in the bright field and fluorescence modes (Fig. 3A-B). The stained HeLa cell (circled by dotted line in Fig. 3) was clearly recognized in the population under the fluorescent mode. The target cell was pulled out and moved into an branch channel (Fig. 3C-F). Once the target cell was moved into the adjacent channel, airflow was then induced to remove the fluid in the main channel and create digital liquid plugs in the branch channels. The volume of the liquid plugs can go down to nano-liter. Fig. 3H shows the target cell stay at the same location in the branch channel after the liquid plugs form.

To demonstrate the downstream single or multiple cell analysis, we have measured the level of beta-actin mRNA of the cell samples prepared by microfluidic OET by RT-qPCR. Fig. 4 shows the amplification curves of cell samples and standard samples. The samples containing one or five target cells were prepared by the microfluidic OET platform. The standard curve was generated with known amount of human reference cDNA (Clontech) for the detection of the amount of the target cDNA. All qPCR was performed with a commercial machine (Lightcycler 480, Roche) and PCR kit (RNA Master Hydrolysis Probes, Roche Applied Science). Taqman primers and probe for human beta-actin were synthesized by Roche Applied Science. 1 μL cell droplet was directly merged with 9 μL reagents mixture and then subjected to RT-qPCR with 180 sec reverse transcription at 63°C, 30 sec pre-denaturation at 95°C, following by 45 cycles of denaturation at 95°C (15 sec), annealing 60°C (45sec) and elongation at 72°C (1sec). The threshold cycle ( $C_t$ ) value of the curves in Fig. 4 were analyzed by the 2nd derivative maximum method ( $y = -3.739x + 32.19$ ). The  $C_t$  value of single-cell samples was 34.20. The  $C_t$  value of five-cell sample was 30.61.

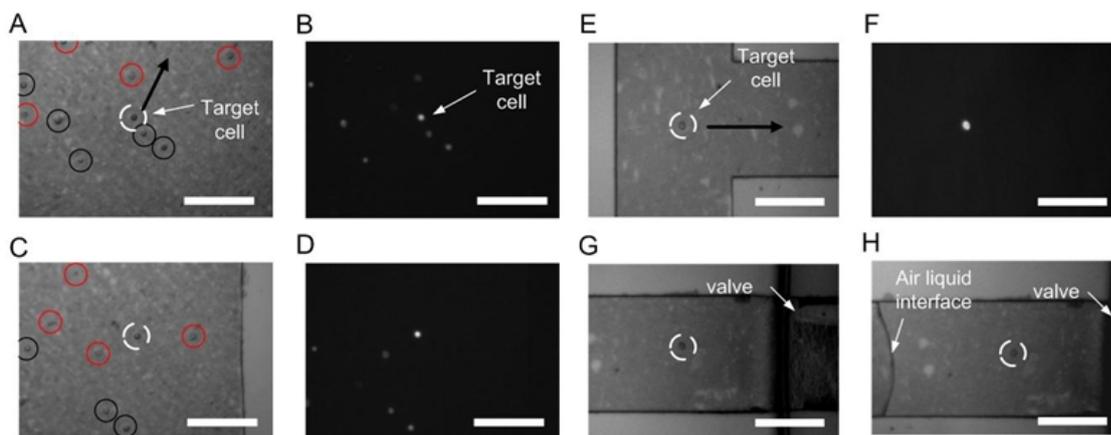


Figure 3. Images of single cell preparation on the microfluidic OET. (A) The initial position of a target HeLa cell is circled with white lines. (B) The fluorescent image of (A). (C)(D) The target cell is selected and moved away from other cells. (E)(F) The target cell is moved to the entrance of a perpendicular channel. (G) The target cell is inside the branch channel. (H) A liquid plug is formed in front of the valve after airflow in the main channel. Scale bars are 200 $\mu$ m.

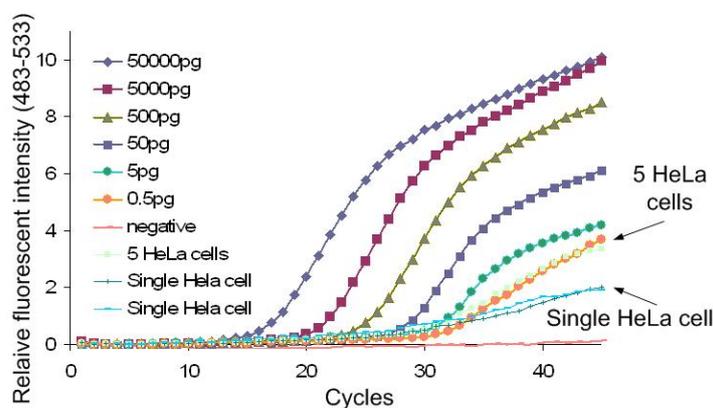


Figure 4. qPCR of cDNA converted from Beta actin mRNA extracted from single and 5 HeLa cells. Data is compared with curves showing amplification of serial dilutions of reference human cDNA.

## CONCLUSION

In conclusion, we have demonstrated a microfluidic integrated OET platform for single-cell or multi-cell sample preparation. The platform employs light-induced DEP and PDMS microfluidic channels to sort target cells based on their optical signatures into isolated droplets for downstream analysis. The optical cell manipulation function simplifies the design of microfluidic device for single cell analysis. Single-cell/multi-cell samples have been successfully prepared for analyzing the level of beta-actin mRNA by real-time RT-PCR.

## ACKNOWLEDGEMENTS

The authors thank Chimei-Innolux Inc. for fabrication of OET chips, Prof. Jiang Zhong for discussion about single cell analysis. This project is supported by NSF CAREER Award (ECCS 0747950) and NSF DBI-0852701.

## REFERENCES

- [1] P. Y. Chiou, A. T. Ohta, and M. C. Wu, "Massively parallel manipulation of single cells and microparticles using optical images," *Nature*, vol. 436, no. 7049, pp. 370-372, 2005.
- [2] K.-W. Huang, S. Sattar, J. F. Zhong, C.-H. Chou, H.-K. Tsai, and P.-Y. Chiou, "Electrodes for Microfluidic Integrated Optoelectronic Tweezers," *Advances in Optoelectronics*, vol. 2011, 2011.

## CONTACT

Kuo-Wei Huang tel: 1-310-825-9091 or kuowei@ucla.edu