INTEGRATING OPTOELECTRONIC TWEEZERS FOR INDIVIDUAL PARTICLE MANIPULATION WITH DIGITAL MICROFLUIDICS USING ELECTROWETTING-ON-DIELECTRIC (EWOD)

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ABSTRACT
This paper presents the integration of two powerful technologies: manipulation of droplets (i.e., digital microfluidics) using electrowetting-on-dielectric (EWOD) and manipulation of individual particle inside the droplets using optoelectronic tweezers (OET). A novel platform for maintaining a viable cell culture environment is proposed as an application example, in which EWOD operations bring droplets containing cells, medium and waste into and out of the cell environment and OET operations address and manipulate the individual cells in coordination with the fluidic operations. Functions of EWOD and OET required to realize the concept are demonstrated.

1. INTRODUCTION
Electrowetting-on-Dielectric (EWOD) is a technique that enables droplet-based actuation of fluids ("digital microfluidics") using electrically controlled surface wetting properties. It requires very little power, and unlike channel-based microfluidics, it employs no failure-prone mechanical components like pumps or valves. Moreover, it can allow precise and simultaneous control over the volume and path of each fluid droplet [1-3]. Droplets can be created from reservoir, moved, cut and merged. Recent reports of digital microfluidics for biological applications [4, 5] have helped establish EWOD as a novel and promising lab-on-a-chip technology.

Optoelectronic tweezers (OET) employ light-induced dielectrophoresis to individually address and manipulate neutral or charged particles. This mechanism allows optical beams to pattern virtual electrodes with diffraction-limited resolutions. The virtual electrodes can be continuously addressed on a two dimensional surface as freely as optical tweezers, while requiring five orders of magnitude lower power [6]. Low power, massively parallel individual manipulation of micrometer size particles including cells has been demonstrated with OET, implying tremendous impact in the study of cell culture and related biological applications [7, 8]. For instance, individual cells can be picked and separated from a group of cells, using OET, based on difference in charge, dielectric properties or even color.

Particle separation and concentration using electrophoresis integrated with EWOD has been demonstrated before [9]. Binary particle separation was achieved using difference in polarity and mobility, and the droplet was subsequently cut with the particles separated. However, similar particles (e.g. same charge) cannot be manipulated differently with this technique. Using OET, each particle can be individually addressed based on difference in dielectric properties or simply visual differences (e.g. color, size) allowing greater flexibility and control. Integrating parallel manipulation of multiple droplets by EWOD with individual control over particles (cells) within each drop by OET promises to produce a uniquely powerful platform for cell studies.

2. DESIGN AND FABRICATION
The different requirements of EWOD and OET pose some challenges for their integration into a microdevice. Fig. 1 shows the cross-section of the integrated device. To maintain the transparency required by the optics, both the top and bottom chips are made on glass substrates sputtered with transparent but conductive indium tin oxide (ITO).

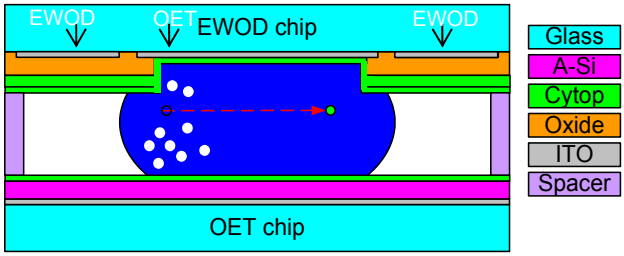


***Fig. 1**: Cross-section of device integrating EWOD and OET. Top glass substrate has EWOD electrodes except central electrode used for OET. Bottom glass substrate contains amorphous silicon for photoconductivity. Oxide and Cytop® are patterned on EWOD chip, and only a thin hydrophobic layer covers the OET region.*

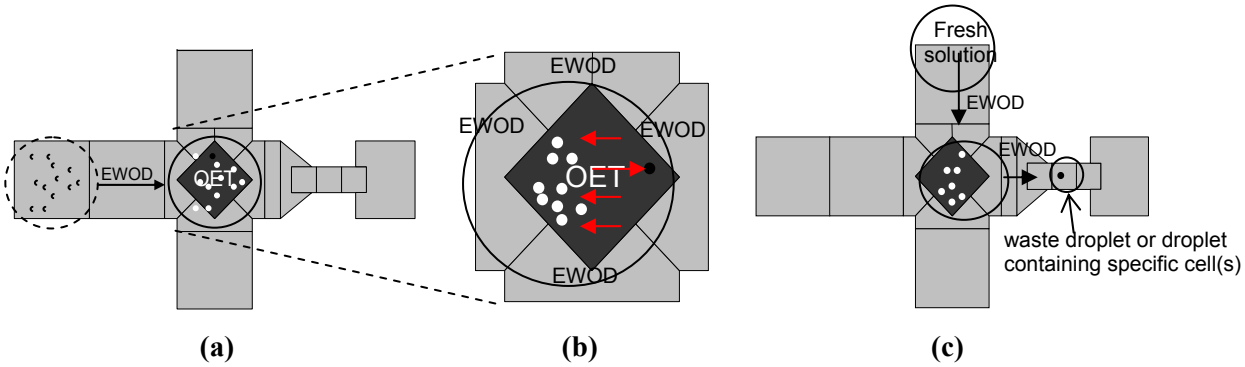


Fig. 2: *Schematic showing the motivation for integrating EWOD and OET. Light gray electrodes are for EWOD, dark gray electrode is for OET: (a) Droplet containing cells is brought in using EWOD; (b) Using OET, cells are swept to left and individual cell(s) is(are) picked based on dielectric properties or visual differences (e.g. color). Droplet position relative to the OET pad can be adjusted by EWOD side-electrodes; (c) Using EWOD, desired cell(s) is(are) taken by generating a droplet or waste medium is removed as droplets. Fresh droplet can be added from other directions to replenish cell medium.*

To prevent electric breakdown and current leakage across the droplet, EWOD operation requires a relatively thick dielectric layer (typically 0.5 to 1.5 micron of silicon dioxide or parylene) in addition to the hydrophobic top layer of Teflon or Cytop [1-5].

However, OET relies on controlling the electric field gradient across the droplet to produce the dielectrophoretic (DEP) force by optically manipulating the resistivity of the photoconductor. In the dark state, most of the voltage drop is taken up by the photoconductor. Optical illumination over a specific area increases the photoconductivity in this region, causing most of the voltage to drop across the droplet and producing the required field gradient for DEP. The presence of a thick dielectric in the cross-section would absorb most of this voltage and weaken the DEP force available in the droplet.

The thick dielectric layer covering the EWOD electrodes is therefore patterned away from over the OET electrode. This implies that OET is ineffective over the EWOD electrodes and the OET electrode cannot be used for EWOD actuation. Bringing the droplet into and out of the OET region (where particles can be manipulated) must be performed using EWOD electrodes surrounding it. This entails a trade-off between relative sizes of the OET electrode and EWOD side-electrodes. Larger side-electrodes make droplet manipulation easier, but reduce the area available for particle manipulation by OET. The present electrode layout (Fig. 2) was chosen as a result of this trade-off.

Fig. 2 illustrates the concept that can be implemented using an integrated EWOD/OET device. A droplet containing the cells is brought in and positioned over the OET region using the adjoining EWOD electrodes (Fig. 2(a)). Using OET, all cells are swept to one side and individual cell(s) are picked and separated to the other side, based on difference in visual or dielectric properties (Fig. 2(b)). EWOD is used to create a smaller droplet containing just the selected cell(s) (Fig. 2(c)). Maintaining a viable cell environment also requires replenishing of medium. Using EWOD operations, wastes and toxins can be removed as droplets, and fresh droplets containing nutrients, drugs, etc. replace them while cells are held by OET.

In fabricating the device in Fig. 1, the top glass substrate contains the electrodes for EWOD operation and one electrode for the OET region. The electrodes were patterned into a 1400 Å ITO layer. The contact pads for each electrode were patterned with Au/Cr for easier addressability. A 7000 Å layer of PECVD SiO_2 was deposited over the electrodes, followed by a 2000 Å layer of Cytop by spin-coating. Both these layers were patterned to remove the relatively thick dielectric from above the OET area. A thin layer of Cytop (~200 Å) was spin-coated over the region to maintain hydrophobicity. The bottom substrate has 1000 Å of ITO on glass, over which is deposited 50 nm n+ hydrogenated amorphous silicon (a-Si:H), and 1 µm of intrinsic a-Si:H. A thin Teflon®layer (~200 Å) is spin-coated.

3. EXPERIMENTAL SETUP

The experiemental setup of this integrated OET and EWOD system is shown in Fig. 3. 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 5× objective lens. The microscopic image is captured by a CCD camera and analyzed by an image processing software 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. This system allows automatic, real-time interactive control of the optical patterns for parallel single particle manipulation.

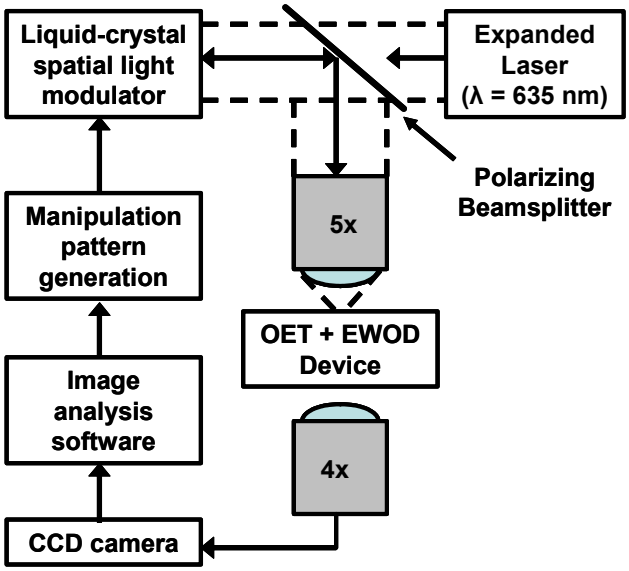


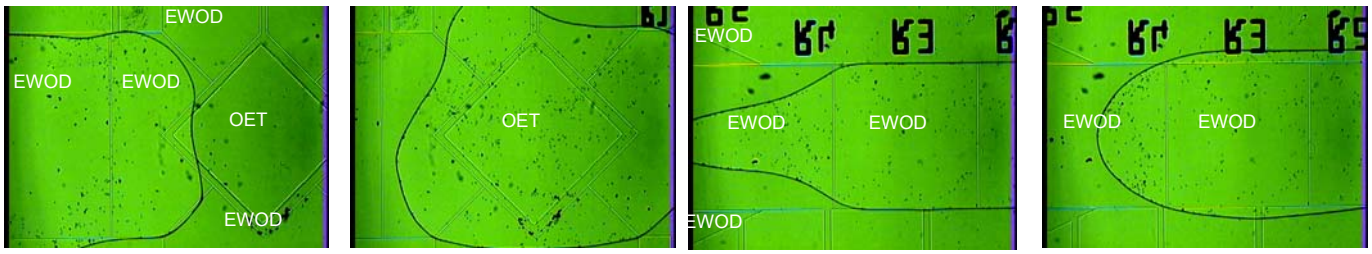
Fig. 3: *Experimental setup of the real-time interactive and automatic OET manipulation system.*

4. RESULTS AND DISCUSSION

Fig. 4 demonstrates the operation of EWOD on the integrated device. A droplet containing 20 micron polystyrene beads was moved into the OET region using the EWOD side-electrodes (Fig. 4(a),(b)). The side-electrodes can also be used to pull the droplet in different directions, so that particles initially outside the OET region can be "captured" with OET. Cutting of the droplet was also demonstrated (Fig. 4 (c),(d)) using the adjoining EWOD electrodes. A dynamically generated optical pattern was used to manipulate individual particles in the droplet using OET. Particles were identified using an image-processing software and swept to the left (Fig. 5(a)). A mouse-controlled optical pattern can also be used to sweep (Fig. 5(b)). Nearly all particles moved (Fig. 5(c)) except one or two, which appeared to be stuck. Next, a particle was user-picked (by mouse-click), triggering the optical pattern to move only this particle rightwards (Fig. 6).

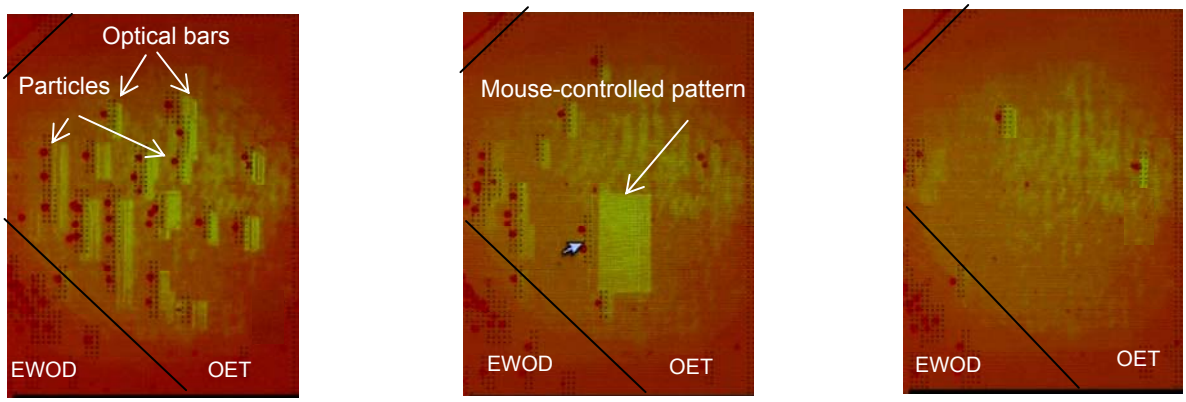
5. CONCLUSION

Functions of both EWOD (viz. cutting, moving and merging) and OET (viz. parallel manipulation of individual particles) have been demonstrated. Currently we are developing to generate droplets containing specific type and number of particles by combining these two operations, while replenishing with fresh droplets. The success promises to develop into a novel platform integrating individual cell control with digital microfluidics. Simultaneous controllability of multiple droplets with EWOD and parallel manipulation of individual cells with OET within each droplet are anticipated to provide a powerful technique for cell studies.



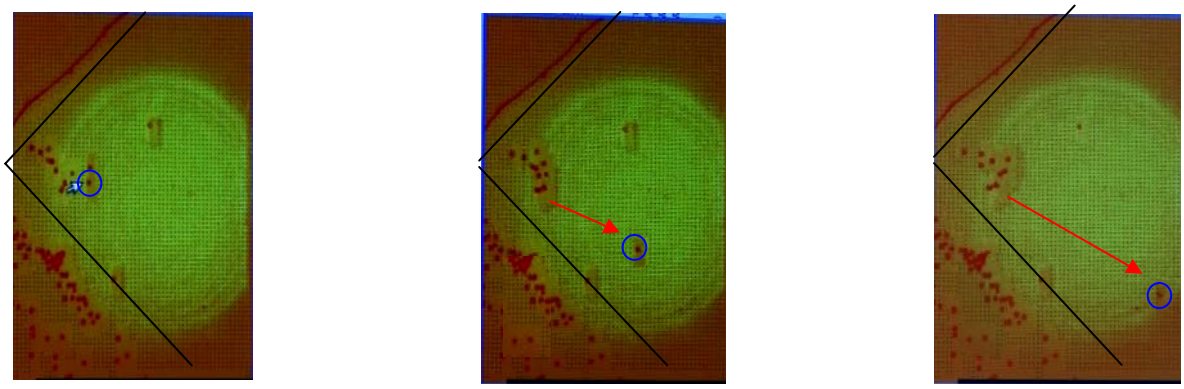
(a) **(b)** **(c)** **(d)**

Fig. 4*: Droplet manipulation by EWOD. (a, b) EWOD operations of bringing droplet with beads into OET region; (c, d) EWOD cuts droplet out of OET region as in Fig. 2(c). The dielectric pattern inside the OET electrode (rotated square) marks active OET region. Surrounding EWOD electrodes are used to move droplets into, out of and around OET region.*



(a) (b) (c)

Fig. 5: *Particles swept by OET. (a) Each particle (dark dot) is pushed to left by an optical bar pattern next to it. (b) Optical patterns, e.g. bar controlled by mouse (or keyboard) can also be used to sweep. (c) Nearly all particles in the OET region have been swept to the left (most are out of view). Edges of OET electrode were added in figures for clarity.*



(a) (b) (c)

Fig. 6*: Individual particle control by OET. (a) Specific particle (circled) is chosen by clicking the mouse, triggering the optical pattern to produce rightward movement of that particle. (b, c) chosen particle is moved away from the rest, as indicated by the arrow. Edges of OET electrode were added in figures for clarity.*

6. ACKNOWLEDGEMENTS

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REFERENCES

1. Cho, S.K., H. Moon, and C.-J. Kim, *Creating, Transporting, Cutting, and Merging Liquid Droplets by Electrowetting-Based Actuation for Digital Microfluidic Circuits*. J. MEMS, 2003. **12**: p. 70-80.
2. Fan, S.-K., *Digital Microfluidics by Cross-Reference EWOD Actuation: Principle, Device, and System*, in *Mechanical and Aerospace Engineering*, Ph.D. Dissertation, 2003, UCLA, Los Angeles.
3. Moon, H., et al., *Low voltage electrowetting-on-dielectric*. Journal of Applied Physics, 2002. **92**(7): p. 4080-4087.
4. Wheeler, A., et al., *Electrowetting-Based Microfluidics for Analysis of Peptide and Proteins by Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)*. Analytical Chemistry, 2004. **76**(16): p. 4833-4838.
5. Srinvasan, V., V.K. Pamula, and R.B. Fair, *An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids*. Royal Society of Chemistry, 2004. **4(4):**p. 310-315.
6. Chiou, P.Y., A.T. Ohta, and M.C. Wu. *Microvision-Activated Automatic Optical Manipulator For Microscopic Particles, Proc. IEEE Int'l. Conf. MEMS, Miami, FL, Jan 2005* pp. 682-5.
7. Chiou, P.Y., A.T. Ohta, and M.C. Wu, *Massively parallel manipulation of single cells and microparticles using optical images*. Nature, 2005. **436**: p. 370-372.
8. Chiou, P.Y., et al. *Cell addressing and trapping using novel optoelectronic tweezers*, *Proc. IEEE Int'l. Conf. MEMS*. Maastricht, Netherlands, Jan 2004, pp. 21-24.
9. Cho, S.K. and C.-J. Kim. *Particle Separation and Concentration Control for Digital Microfluidic Systems*. *IEEE Int'l Conf. MEMS,* Kyoto, Japan, Jan 2003, p. 686-9