High Efficiency *E. coli* Concentration via Dynamic Light-induced Optoelectro-osmosis Flow on Organic Photoconductivity Biochip

S. M. Yang[*], C. Y. Lin[**], T. P. Wang[**], T. H. Punde[***], H. Y. Chang[**], L. Hsu[*], and C. H. Liu[***,****]

[*] Department of Electrophysics, National Chiao Tung University, Taiwan, R.O.C.
[**] Institute of Molecular Medicine, National Tsing Hua University, Taiwan, R.O.C.
[***] Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Taiwan, R.O.C.
[****] Department of Power Mechanical Engineering, National Tsing-Hua University, Taiwan, R.O.C.

ABSTRACT

We demonstrate a dynamic light-induced optoelectro-osmosis flow design for high efficiency *E. coli* concentration on an organic photoconductive material based biochip. Several traditional approaches of bacteria purification and enrichment utilize magnetic beads coated with antibodies to attract them. The efficiency of collecting magnetic beads is equal to sort bacteria. Our work provides a direct method to enrich the *E. coli* concentration with dynamic light images. Due to the applications of photoconductive material, TiOPc, two features are demonstrated, simple biochip fabrication processes and rapid *E. coli* collection. The biochip is fabricated only with spin-coating a thin TiOPc layer, 500nm, on the indium tin oxide (ITO) conductive glass. Besides, the *E. coli* are concentrated together within 30 sec toward the illuminating region by the surface charge slip. We also calibrate the dominating effects in different applied AC frequency region. In conclusion, our light-induced *E. coli* manipulation approach would expand the scope of the examination efficiency, for example, water quality.

Keywords: Photoconductivity, TiOPc,electroosmosis, dielectrophoresis, *E. coli.*

1 INTRODUCTION

For clinical case reports, rapid detection of *E. coli* is necessary for clinical diagnosis and treatment [1,2]. The measurement of *E. coli* number would provide an index to indicate the qulity of water. On-chip *E. coli* dection and analysis is one of the key issues in micrototal analysis systmes. Besides, microparticle manipulation by optoelectronic manipulation approach has been reported.[3-5] Light-driven methods provide the convenience for microparticle control.

In this article we demonstrate a dynamic light-induced optoelectro-osmosis flow design for *E. coli* concentration on an organic photoconductive material based biochip. With the different external AC frequency condition, light-induced electroosmosis flow and light-induced dielectrophoresis force are two methods to concentrate *E. coli*. Our work provides rapid and efficient concentration of bacteria with dynamic illuminating light image.

2 OPERATION PRINCIPLE

2.1 Light-induced Electroosmosis Flow

Utilizing projected light pattern to drive the liquid is the feature of light-induced electroosmosis flow. When the bulk fluid fulls the space of the chip, the electrical double layer (EDL), the diffuse layer and the compact layer of charge, is formed near the TiOPc surface. In the non-uniform electric field condition, the ions within the EDL would response and be driven via the tangential electric field component, E_t. The surface charge moving velocity,V_{EOF}, or named slip velocity, is defined by the Helmholtz-Smolchowski equation.[6-8]

$$V_{EOF} = -\frac{\varepsilon \zeta E_t}{\eta} \tag{1}$$

where ε is the permiitivity of the liquid, ζ is the zeta potential, E_t is the tangential electric field, and η is the fluid viscosity.

As the projecting light illuminating on the photoconductive material, the resistance of TiOPc within light pattern is diminished and the charges transport the photoconductive layer to form virtual electrode on its surface. This tangential electric field of light-induced virtual electrode would driven the ions near the TiOPc surface with a slip velocity and result in an overall bulk fluid flow to concentrate *E. coli.*

2.2 Light-induced Dielectrophoresis

When the operation frequency condition is near MHz range, the dielectrophoresis effect would be generate to trap the microparticle on this TiOPc-based chip. In this article we utilize this effect to generate *E. coli* columns structure. The time-averaged DEP force, F_{DEP}, acting on a spherical particle of radius r suspended in a medium with relative permittivity ε_m is given by [9]

$$F_{DEP} = 2\pi r^3 \varepsilon_m \,\mathrm{Re}\left[f_{CM}(\omega)\right] \nabla E_{rms}^2 \tag{2}$$

where E_{rms} is the root mean square of the AC electric field and $f_{CM}(\omega)$is the Clausius-Mossotti factor, given by

$$f_{CM}(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{3}$$

where the ε_p^* and ε_m^* are the complex permittivity of the particle and medium, respectively, and

$$\varepsilon^* = \frac{\varepsilon - j\sigma}{\omega} \quad (4)$$

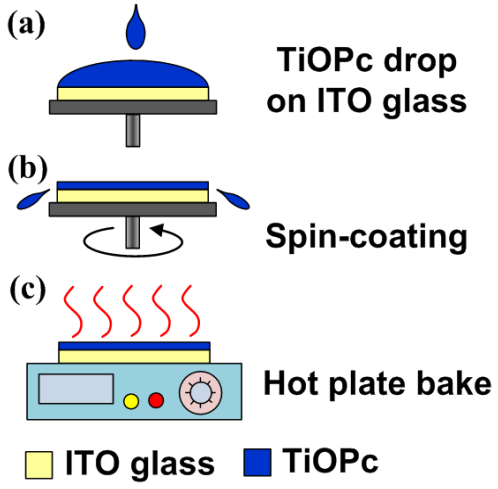
where σ is the conductivity and ω is the angular frequency of the applied electric field.

The light-induced electroosmosis flow and light-induced dielectrophoresis are performed in different external AC frequency. The former is near kHz range and the latter is about MHz range on our TiOPc-based optoelectronic chip.

3 EXPERIMENT RESULTS

3.1 Chip Fabrication

The simple fabrication processes and chip structure are shown in Fig. 1. The optical system of optoelectronic dielectrophoresis with novel organic photoconductive material, TiOPc, has been reported [9]. Simple step is able to fabricate the TiOPc-coated substrate. Firstly, a drop of TiOPc solution about 500 µl is placed on the surface of conductivity ITO glass, Fig. 1(a). Next, a unifom TiOPc layer is formed by the spin-coating process with 1000 rpm, Fig. 1(b). Finally, the TiOPc-coated ITO substrate is placed on a hot plate and baked with 130 ^{o}C to hard the TiOPc layer structure, Fig. 1(c). After these steps, 500 nm thickness TiOPc layer is fabricated on the ITO substrate. These steps are simple and no needed to operate them in clean room. The dried TiOPc is not dissolved in water, but it would be dissolved in alcohol solution. Therefore, the operation area of TiOPc is defined by using alcohol to eliminate the useless region of outer TiOPc coating area shown in Fig. 1(d). For experiment operation of *E. coli* concentrating, the *E. coli* is suspended in DD water and sandwiched between top ITO glass and bottom TiOPc-coated substrate with height 100µm spacer, Fig. 1(e).



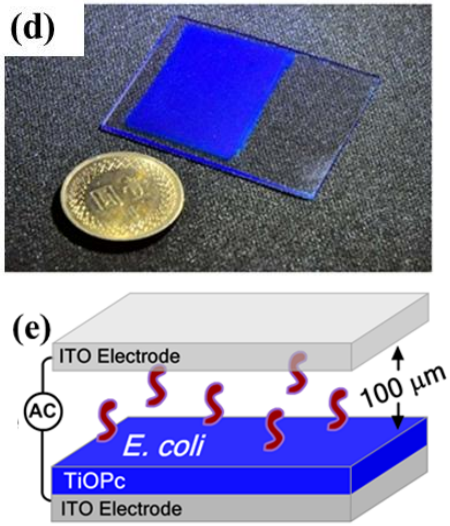


Figure 1. (a)~(c) Single step chip fabrication process. (d) TiOPc-coated ITO substrate. (e) Schematic diagram of TiOPc-based chip.

3.2 Electric Field Simulation

When the light pattern is projected on an organic photoconductive layer, the charges transport it to form a non-uniform electric field as shown in Fig. 2. The electric double layer, a diffused and compact layer, are generated a surface slip to drive the *E. coli* together toward the illuminating region. To identify the electroosmosis flow effect induced by the electric-field gradient of virtual electrode on the TiOPc surface, a software CFD-ACE+ is utilized to simulate the non-uniform electric field and induced flow direction shown in Fig. 2. While the light is projected on the TiOPc layer, the conductivity of TiOPc within the illuminated region increase and charges transport through the photoconductive materal and assemble as a virtual electrode pattern. Comparing with the top whole ITO electrode, the small virtual electrode induce non-uniform electric field in the bulk fluid. The yellow color of Fig. 2 illustrates the non-uniform electric field distribution and the blue arrows show the induced flow direction. The liquid outside the illuminating region is driven toward the light pattern by the moving ions of electric double layer. Following this process, the *E. coli* are concentrated within the illuminating region by the light-induced electroosmosis flow.

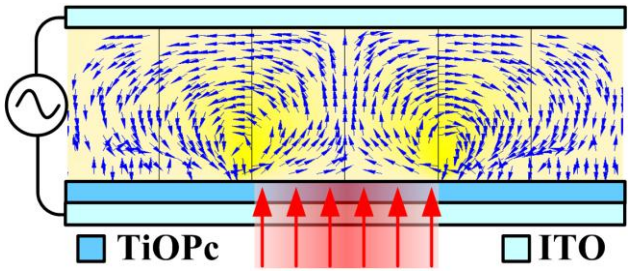


Figure 2. The Schematic diagram of optoelectronic concentrator and the computer simulation results of light-induced electroosmosis flow. Charge transports through the organic photoconductivity material within illuminating region to form a virtual electrode. This assembled charge pattern generates light-induced electroosmosis flow (indicated by small arrows) to drive and concentrate the *E. coli* suspended in the buffer toward the center of illuminating region.

3.3 *E. Coli* Concentration

While a round light pattern is projected on the TiOPc layer, it would form a virtual electrode and generate non-uniform electric field in whole bulk fluid. The tangential electric field would induced electroosmosis flow on TiOPc surface to concentrate *E. coli* from non-illuminating region toward illuminating region. The *E. coli* concentration within local light pattern is increased. Fig. 3 illustrates that *E. coli* gets concentrated to form a 3D vertical cluster with height H. After the AC voltage is witched OFF, the electroosmosis flow stops to collect *E. coli*. The *E. coli* momentum caused by gravity is large enough so that they rebound from the TiOPc surface and spread outward to form a ripple ring as shown in Fig. 3(A). In general the Brownian motion of *E. coli* without the momentum of gravity is not able to form this ring. The *E. coli* quantity of acroos the cross-section line are shown in Fig. 3(B)(D)(F). The analysis of *E. coli* density in Fig. 3 (C)(E)(G) illustrate the volume and shape variation of the 3D cluster of *E. coli*. The collected *E. coli* are spreaded out within 1 second.

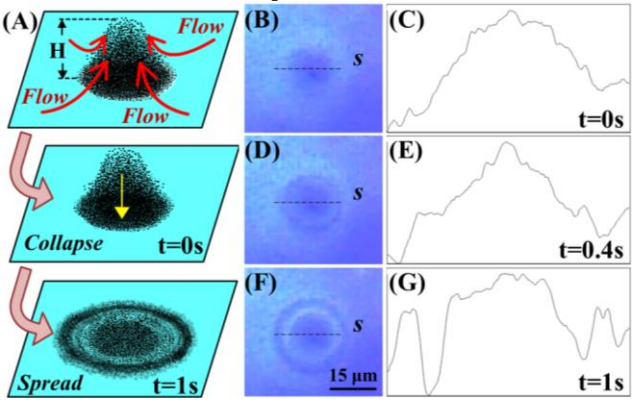


Figure 3. (A) When the applied voltage is turned ON, the *E. coli* are concentrated by the flow to form a structure with height, H. Once the voltage is turned OFF, the 3D structure of *E. coli* collapses down due to the gravitational force. The *E. coli* collide with the surface and bounce back in the outward region to form a *E. coli* ring. (B)(D)(F) show the collapsing and spreading process of the *E. coli* structure. (C)(E)(G) *E. coli* density at the line of cross-section, s, shown in the corresponding images on the left.

The concentrating process of *E. coli* via surface slip is shown in Fig. 4. This process leads to the increase in *E. coli* concentration with illuminating region. After 30 sec *E. coli* collection, a dark region at the position of the light pattern indicates the high density of *E. coli*.

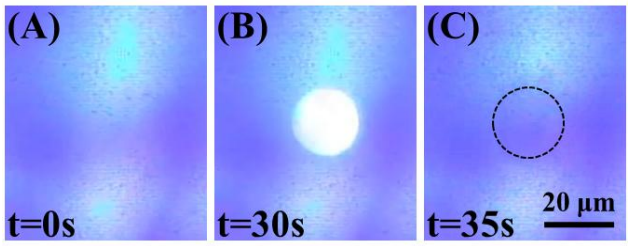


Figure 4. The process of *E. coli* concentration at 5Vpp, 10 Hz. (A) Before illuminating the *E. coli* are spread everywhere. (B) A circular light pattern is projected on the surface to generate light-induced electroosmosis flow to concentrate the *E. coli*. (C) Comparing with the Fig. 4(A), the shadow at the center of the image indicates the *E. coli* concentration.

The circle dark dotted line in Fig. 5 indicate the locations of projceting light pattern. These two images show the high efficiency *E. coli* patterning wherein the *E.coli* is not only attracted towards the illuminating region but they also get confined in the region between each light spot in 30 sec indicated in dark region.

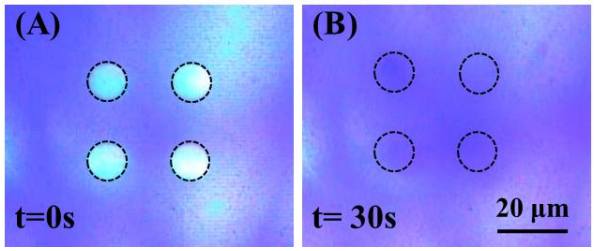


Figure 5. Four spots for patterning *E. coli* at 5Vpp, 10Hz. (A) Four circular light pattern not only drive the *E. coli* to the illuminating region, but also collect the *E. coli* toward the area between each circle. (B) Large numbers of *E. coli* are concentrated within 30 sec.

3.4 3D *E. Coli* columnar structure

We categorize the electromechanics of *E. coli* shown in Fig. 6(E). At 10k ~10MHz frequency range, the DEP phenomenon mainly dominates the acting force of the *E. coli* structure formation. As the chip structure in Fig. 6(A), the external AC voltage applied on top ITO glass and bottom substrate would generate vertical electric field to form *E. coli* peral chain. Thick columnar structure of *E. coli* takes place at 5Vpp and 1MHz. Initial formation of thin filaments and subsequent transformation of these filaments into column takes place as shown in Fig. 6(A). In the absence of applied voltage, *E. coli* are scattered everywhere as shown in Fig. 6(B). At 5Vpp and 10MHz, the columnar structures are formed. Fig. 6(C) shows the observing record from Z axis direction and each dot is a single 3D *E. coli* columnar structure. When the voltage is turned OFF, the columnar structure collapses, Fig. 6(D).

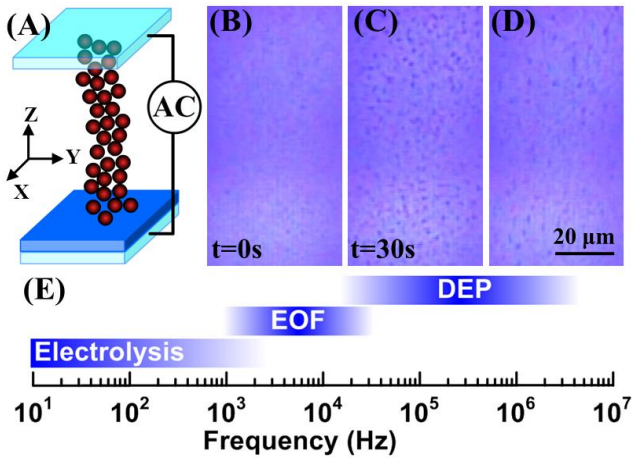


Figure 6. Frequency-dependent electromechanics of *E. coli* (A)The columnar structure of *E. coli* between the top ITO glass and the bottom substrate. (B) The *E. coli* spread uniformly on the surface which are too small to be observed at 5Vpp, 10 kHz. (C) When the applied frequency increases to 1MHz, the DEP principle dominates the force to form *E. coli* columns observed from the Z direction of Fig. 5(A). (D) The columnar structure collapses and the *E.coli* spreads in horizontal direction. (E) The operational frequency calibration of *E. coli* at 5Vpp, 1 ~ 10 MHz. EOF is utilized for *E. coli* concentration and DEP is applied to establish *E. coli* columnar structure.

4 CONCLUSTION

In the former works, we developed the TiOPc-based optoelectronic dielectrophoresis chip and focused on the microparticle manipulation in cell scale. The simple fabrication processes with TiOPc material is easy to apply for optoelectronic applications. The microparticles, polystyrene beads, liver HepG2 and fibroblast 3T3 cells, diameter are about 10 μm. In this work we utilize this approach to concentrate *E. coli* which size is about 1 μm. Accorrding to DEP formula, when the microparticle size is diminished, the DEP force is reduced in three times. At this moment, electroosmosis flow would dominate main operation effect in low frequency range, kHz. In this article, we develop a bacteria manipulation platform of rapid *E. coli* concentration via dynamic optoelectro-osmosis flow. The *E.coli* are driven from non-illuminating region toward light pattern by the slip velocity of surface ions. When enough quantity *E. coli* are concentrated, they would form 3D structure which is observed by the *E. coli* ring as the strucure collapses. In high frequency of MHz range, DEP force is able to generate 3D *E. coli* columnar structure. Finally, we calibrate the operation frequency range of optoelectronic mechanism on TiOPc-based chip for *E. coli* manipulation. The optoelectronic approach to collect and recognize *E. coli* concentration would expand the scope of the examination efficiency for water quality.

ACKNOWLEDGEMENTS

This project was financially sponsored by the National Science Council (Grant No.98-2120-M-007- 003). We extend special thanks to the staff of the Biophysical Laboratory at National Chiao Tung University and the Micro-Systems and Control Laboratory (MSCL) at National Tsing Hua University for their assistance. We are also thankful to Prof. Hwan-You Chang and the staff of his Microbiology and Biotechnology Laboratory at National Tsing Hua University for their support. We also thank Prof. Ming C. Wu at University of California, Berkeley, Prof. Pei-Yu Chiou at University of California, Los Angeles, and Prof. Gwo-Bin Lee at National Tsing Hua University for sharing optoelectronic approaches experience and helpful discussion.

REFERENCES

[1] Rachmilewitz D, Karmeli F, Shteingart S, Lee J, Takabayashi K, Raz E. "Immunostimulatory oligonucleotides inhibit colonic proinflammatory cytokine production in ulcerative colitis." Inflamm Bowel Dis. 12, 339–45, 2006

[3] Arash Jamshidi, Steven L. Neale, Kyoungsik Yu, Peter J. Pauzauskie, Peter James Schuck, Justin K. Valley, Hsan-Yin Hsu, Aaron T. Ohta, and Ming C. Wu, "NanoPen: Dynamic, Low-Power, and Light-Actuated Patterning of Nanoparticles", Nano Lett.,9 (8), pp 2921–2925, 2009

[4] Pei Yu Chiou, Aaron T. Ohta, and Ming C. Wu, "Massively parallel manipulation of single cells and microparticles using optical images", Nature 436, 370-372, 2005

[5] Wei Wang, Yen-Heng Lin, Ruei-Syuan Guan, Ten-Chin Wen, Tzung-Fang Guo, and Gwo-Bin Lee,"Bulk-heterojunction polymers in optically-induced dielectrophoretic devices for the manipulation of microparticles", Optics Express, 17, 20, 17603-17613, 2009

[6] A. Ramos, H. Morgan, N. G. Green and A. Castellanos, AC Electric-Field- Induced fluid flow in microelectrodes, J Colloid Interface Sci., 217, 420, 1999

[7] J. Lyklema, 1991, Fundamentals of interface and colloid science, London, UK: Academic Press, 1991.

[8] J. K. Valley, A. Jamshidi, A. T. Ohta, H. Y. Hsu, M. C. Wu, "Operational Regimes and Physics Present in Optoelectronic Tweezers", J. Microelectromech Syst., 17, 2, 342-350, 2008

[9] Shih-Mo Yang, Tung-Ming Yu, Hang-Ping Huang, Meng-Yen Ku, Long Hsu, and Cheng-Hsien Liu, "Dynamic manipulation and patterning of microparticles and cells by using TiOPc-based optoelectronic dielectrophoresis", Optics Letters, 35, 12, 1959-1961, 2010

*Cheng-Hsien Liu, Tel: +886-3-5715131#33706; liuch@pme.nthu.edu.tw
Shih-Mo Yang, Tel: +886-3-5715131#33793; g913328@gmail.com