A novel optoelectrofulidic system for cells/particles manipulation and sorting

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ABSTRACT

A novel optoelectrofulidic system integrated optical image concentration and alignment system, dielectrophoresis phenomenon, microfluidic and friendly real-time control interface is first reported in this article. A new application of photoconductive material oxotitanium phthalocyanine (TiOPc) for microparticle applying has been first described and demonstrated by our research group. Basis on the special character of the photoconductive material, a TiOPc-based optoelectronic tweezers (Ti-OET) is utilized for single and massive cells/particles manipulation. The objects wanted to be manipulated are defined with different behaviors (e.g., press, release, drag and move) using Flash® software when the cursor acts on them. It also reveals the application for biological application to form the cells trapping with three sorts of cells, HMEC-1, HepG2 and HEK293t.

Another application of our optoelectrofulidic system is to fabricate a TiOPc-based flow cytometry chip which can be used for sorting the 15μm diameter particles with 105 μm/s velocity. When the 10Vp.p. voltage and 45 kHz AC frequency apply on the top and button ITO electrode, the illuminated light pattern will become a spatially virtual switch inside the microchannel. The dielectrophoresis force between top ITO glass and button photoconductive layer controlled by the friendly interface will concentrate the cells/particles as a straight line and individually direct each one in different paths.

In summary, we have established an optoelectronfulidic-based chip and spatially virtual switch system which are applied for cell pattern and particles sorting. In the future, this easy manipulation approach can place the full power of optoelectronfulidic chip into the biological operators' hands.

Keywords: TiOPc, Optoelectronic Tweezers (OET), TiOPc-based OET (Ti-OET), Dielectrophoresis (DEP), Cell manipulation

1. INTRODUCTION

In recent years, there has been a dramatic proliferation of investigation concerned with manipulation of individual polystyrene beads and human cells. These fundamental researches provide more knowledge about cellular behavior when they encounter the other cells or exist under the changing environment. Optical tweezers [1-2] and electrode-based dielectrophoresis (DEP) [3-6] are two sorts of well-known non-invasive methods to manipulate single and massive cells. The former require a high numerical aperture objective lens, however its short working distance limits the application region and reduces the cell numbers for manipulation. Optical tweezers also need a high power laser which intensity is about 1×10^6 W/cm^2 to increase the force for trapping, nevertheless, the biological specimens would be harmed by optical or thermal damage from laser source. The latter reveals that the particle polarized by external non-uniform electric field could be attracted or pushed in order to control particle's position and moving direction. Although DEP manipulation system is easy to be fabricated in produce process of Micro Electro-Mechanical Systems (MEMS), its adaptability of dynamic control is limited by the fixed electrode pattern.

Another method to manipulate particle combined the features of both optical tweezers and dielectrophoresis is invented and named as optoelectronic tweezers (OET). By changing the optical pattern projected on a photosensitive surface, the particle suspended in low conductivity media could be manipulated with the alteration of induced electric field. Numerous different specimens could be controlled under OET chip such as polystyrene particles[7], nanowires,[8] DNA,[9-10] and biological cells[7]. The OET platform supply a simple approach to manipulate small particle, however

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the fabrication process of OET chip need special instrument in clean room and cost high-priced expense, for example, depositing a heavily doped hydrogenated amorphous silicon (a-Si:H), or intrinsic a-Si:H on Indium Tin Oxide (ITO) conductive glass[11]. After many times experiments, our research group has been found that the material of TiOPc is a suitable, more convenient and simple material to produce a chip with OET phenomenon.

In recent years there has been a rapid increase of research concerned about the technology of semiconductor laser and it is applied widely in laser printer, copier application, and digital xerography[12]. Because the wavelength range of semiconductor laser is around 780nm ~ 840nm which is infrared region, the investigation to develop high sensitivity and absorbability material has increasingly been the major object of study for commercial purpose .[13] Phthalocyanine pigments have been utilized as electrophotographic sensitive members because of their strong absorption from visible to infrared region[[14-15]. Therefore TiOPc, within phthalocyanine pigments, has been the first choice to fabricate the OET chip. There are several different crystal structures of TiOPc, for instance, a, b, g, m, Y, A and B-type and Y-TiOPc is selected in this report because it is more sensitive than other types.[16] The experimental results about using cheap TiOPc as new material show its effect is close-fought to the tradition expensive OET chip fabricated with a-Si. A maximum beads movement velocity of 70 um/s is performed within a Ti-OET force of 10 pN.

TiOPc is utilized for new material of OET chip. Besides, the commercial and universal software of FLASH (Adobe) is applied to establish the real-time manipulation interface. This software supplies a friendly environment and control panel for user to manipulate each object real-time just moving the cursor. The integration of new TiOPc material, a real-time manipulation interface and experimental results of our Ti-OET chip with polystyrene particles will be reported as follows.

2. DESIGN AND FABRICATION

2.1 Principle of Ti-OET

The material which could generate electron-hole pair upon illumination is named photoconductive substance. This sort of material has been popularly utilized in copier application, laser printer, and digital xerography[12]. Titanium oxide phthalocyanine (TiOPc) within an organic molecular crystal has been developed as a photoreceptor with a high infrared sensitivity.[15,17] This material has several sorts of crystal structure and synthetizing approaches for each type of TiOPc are reported.[18,19] Utilizing crystal transformation is a general method to produce Y-TiOPc, Other elementary type of TiOPc is fabricated and then they are synthesized together to form Y-TiOPc. In this experiment Y-TiOPc is chosen for this OET chip fabrication based on its special photoconductivity.

2.2 Operating Principle

Because of the different conductivity of one particle and its environment medium, the phenomenon of particles polarized as a dipole in the solution under non-uniform electric fields is wildly known as dielectrophoresis(DEP). The time-averaged DEP force,[20-21] F_{DEP}, acting on a spherical particle of radius r suspended in a medium with relative permittivity ε_m is given by

$$F_{DEP} = 2\pi r^3 \varepsilon_m \operatorname{Re}[f_{CM}(\omega)] \nabla E_{rms}^2 \qquad (1)$$

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

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

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

$$\varepsilon^* = \frac{\varepsilon - j\sigma}{\omega} \qquad (3)$$

where σ is the conductivity and ω is the angular frequency of the applied electric field. According to eq. (2), $f_{CM}(\omega)$ indicates that the force vector acting on the particle varies with the frequency of external electric field and depends on the permittivity and the conductivity of the particle and the circumstance medium. If $f_{CM}(\omega)>0$, the particle is more polarizable then the medium and then attracted toward the regions with large electric-field gradient. If the $f_{CM}(\omega)<0$ and the medium is more polarizable then the particle, the DEP force repels the particle toward the region with the local electric-field minimum. The former is named as positive DEP and the latter is known as negative DEP.

2.3 Ti-OET Chip Fabrication and Structure

For the purpose of investigating new material for OET, a heavily doped hydrogenated amorphous silicon (a-Si:H) or intrinsic a-Si:H deposited on ITO glass have been substituted for TiOPc layer. A 3cm $\times$ 3cm square ITO glass having a 500 $\mu\ell$ TiOPc drop on it have been placed on a spin coater. After two continuous spin processes, one is 1000 rpm 10 sec and the other is 2500 rpm 30 sec, a thin TiOPc layer about 230 nm have been formed on the ITO glass. The structure of TiOPc could be hardened to prevent dissolved through 120 $°C$ and 30 minutes baking.

Figure 1 illustrates the structure of Ti-OET chip which consists of sandwiched layer of a transparent indium-tin-oxide (ITO) electrode, liquid media, a thin TiOPc layer and a bottom ITO electrode. In the experiment two sorts of beads sizes, 8 μm and 15 μm, are used suspended in the liquid media. An AC bias is applied between the top and bottom ITO transparent electrodes.

A real-time manipulation interface is programmable with the software of FLASH. The objects wanted to be manipulated are defined with different behaviors (e.g., press, release, drag and move) when the cursor acts on them. The pattern could be project on the Ti-OET chip with a general commercial projector. Two lenses with 200mm and 10mm focal length and a reflective mirror are aligned in the light path to reduce the optical pattern size to 1/20 times. The projected image and real-time particle manipulation situation are formed by a 10X object lens and captured by a charge-coupled device (CCD).

In the experiment operation, the particle wanted to be controlled could be observed from a real-time video and manipulated by the screen of the other person computer. The frequency is tuned by the function generator below 100 kHz and 10V peak-to-peak.

When the light pattern projected on the Ti-OET chip surface, the TiOpc at the dark region without illumination has the higher impedance. Most of the applied voltage drops across the TiOPc layer and the electric field in the liquid is too weak to influence the particle. The conductivity of TiOPc would increase when the chip surface is illuminated under defined optical pattern and the impedance drops in the region with light. The shape of light pattern becomes a “virtual electrode” and produces a non-uniform electric field between up ITO glass and bottom TiOPc surface. Therefore, the particle suspended in the medium would be manipulated by the change of the light pattern based on the DEP force.

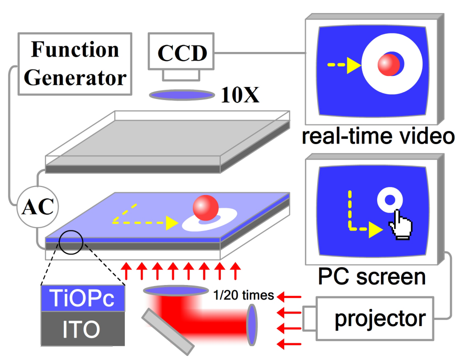

Fig.1 The Ti-OET chip and real-time particle manipulation system. From the first pc screen the pattern could be controlled just utilizing cursor. The programmable pattern is projected on the TiOPc layer in 1/20 times through an optical contraction system. The electric field is applied by a function generator and the beads control situation is observed by the CCD camera.

3. RESULTS

3.1 Pushed Bead walking in Labyrinth

In this real-time beads manipulation interface designed by FLASH software the beads could be moved not only using the cursor but utilizing the movie object which is just appeared when the defined button are pressed or released. The process which one bead is moved follow a moving object like a wedge which is controlled by two defined buttons is illustrated as Fig.2 (c)~(i).

First, two part of object, one is a moving wedge object and the other is a pair of triangle with opposite direction, are created. Second, the actions of these two triangle buttons are defined and connected to the moved object. When one button is pressed the wedge object will move and when the other button is press the wedge object will move in opposite direction. Finally, the controlling button, moving wedge object and a labyrinth are combined together like Fig.2 (b).

When the triangle buttons are pressed, the wedge object will appear and move in one direction which is defined following the button. With a series of cursor pressing actions, the beads could be pushed forward, backward, turn left or turn right in the labyrinth. The orange arrow in Fig.2(c)~(i) describes the moving direction of wedge object and bead. The result of these experiments reveals that utilizing a defined controlling panel could make beads manipulation more convenient.

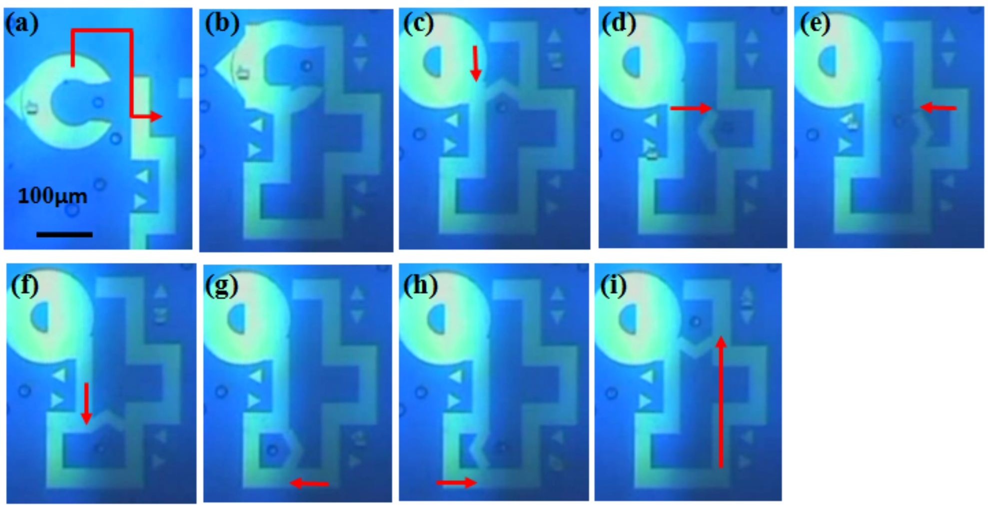

Fig.2 (a)(b) One beads is grabbed and delivered into the Labyrinth. (c)~(i) The moving wedge shape pattern which direction is controlled by the bottom outside the maze pushes the beads along the dark path.

3.2 microparticle sorting

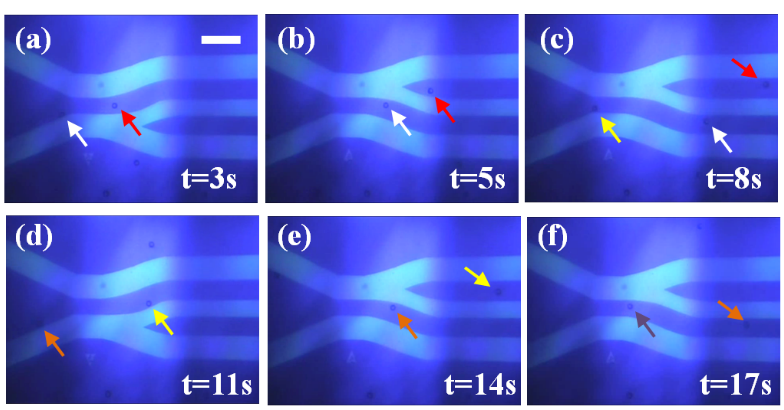

Fig.3 Experimental results of optoelectrofluidic-based cytometry system. The size of the micro-particle is 15μm, and the flow rate is about 105 um/s. The beads are continuously separated by the light pattern switch. The scales bar is 50 μm.

Figure 3 illustrates the microparticle sorting ability of the Ti-OET chip. The 15μm polymer beads are moving from leftward to rightward with the liquid flow. The continuous beads are restricted within the non-illuminating area by negative DEP force. The bead moving direction is controlled by the switch light channel. We manipulate the light pattern to guide the bead direction. For example, in Fig. 3(a)(b) the bead indicated with red arrow is guided into the upward channel and in Fig. 3(b)(c) the bead indicated with white arrow is controlled into the downward channel. This sort of micorparticle sorting technology provides a convenient approach to separate different microparticle on the TiOPc-based optoelectrofulidic chip.

3.3 Cell manipulation

Figure 4 shows the cell manipulation capability on this Ti-OET chip. The cell is suspended in the DEP buffer which is component with fewer ions. The cell are attracted and trapped with the light pattern. This phenomenon illustrates that the cell manipulation force is positive DEP force. When we move the light square pattern, the cell restricted within the square is manipulated follow the moving light square. The cell manipulation results reveal that cell manipulation behaviors of the different kind cells are not unlike very much. Relative with the surrounding liquid, all of the materials with cell membrane are wealthy with ions. We use three sorts of cells, HMEC-1, HepG2 and HEK293t to be manipulated and the results of cell manipulation are the same. Therefore, these cell manipulation methods provide the biological application on this TiOPc-based chip.

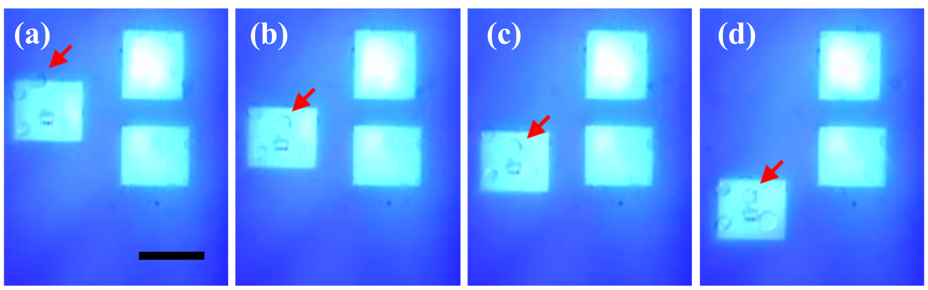

Figure 4. Cell manipulation within illuminating region by positive DEP force. The scale bar is 50 μm.

4. CONCLUSION

In summary, we have given a novel application for photoconductive material, TiOPc, to establish a TiOPc-based optoelectronic tweezers (Ti-OET) system. The beads on TiOPc chip could be real-time manipulated with ordinary FLASH software. These general material, simple fabrication process and easy manipulation system could expand the application of OET technology. The single bead trapped with negative DEP force is caught, delivered and pushed in particular direction and path by virtual electrode formed with designed light pattern. It have the ablility to manipulate single particle such as polymer beads or cells. This investigation of novel TiOPc material would expand a new research region in cellular and biological applications.

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