Dynamic manipulation and patterning of microparticles and cells by using TiOPc-based optoelectronic dielectrophoresis

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Received February 11, 2010; revised April 28, 2010; accepted May 10, 2010;
posted May 20, 2010 (Doc. ID 123912); published June 3, 2010

We develop light-driven optoelectronic tweezers based on the organic photoconductive material titanium oxide phthalocyanine. These tweezers function based on negative dielectrophoresis (nDEP). The dynamic manipulation of a single microparticle and cell patterning are demonstrated by using this light-driven optoelectronic DEP chip. The adaptive light patterns that drive the optoelectronic DEP onchip are designed by using Flash software to approach appropriate dynamic manipulation. This is also the first reported demonstration, to the best of our knowledge, for successfully patterning such delicate cells from human hepatocellular liver carcinoma cell line HepG2 by using any optoelectronic tweezers. © 2010 Optical Society of America

OCIS codes: 230.2090, 230.4685, 230.0250, 350.4855.

Optical tweezers [1] and electrode-based dielectrophoresis (DEP) [2] are two well-known approaches to manipulate particles on the cellular scale. To combat the drawbacks of optical tweezers and DEP, which need either a high power laser or a nonflexible electrode, optoelectronic tweezers were invented [3]. Updated phototransistor-based optoelectronic tweezers have recently been reported with more than 500× higher photoconductivity than amorphous silicon [4]. The chip fabrication processes include boron and arsenic implantation, annealing, reactive ion etching, and dielectric filling. Utilizing photoconductive polymer is another method by which approach optoelectronic tweezers [5]. Three thin films (PEDOT, PSS/P3HT, PCBM/LiF) are formed in sequence onto indium-tin-oxide (ITO) glass. All the processes have to be executed in a nitrogen-filled glove box to prevent either water or oxygen from collapsing the polymer film. Specific facilities, such as plasma-enhanced chemical vapor deposition [6,7], ion implantation, reactive ion etching, and a nitrogen-filled glove box, are needed for fabrication of the above-mentioned optoelectronic DEP chips. A new optoelectronic DEP chip with easily acquired photoconductive material, an inexpensive optical system, and a friendly real-time control interface has been developed by our group and is reported in this Letter.

The organic photoconductive material comprising phthalocyanine pigments has been widely used as an electrophotographic sensitive member in laser printers because of its characteristics of strong illumination absorption [8,9] from the visible to the IR region [10]. In this research, we take advantage of TiOPc, which occurs within phthalocyanine pigments, to develop a new optoelectronic DEP chip for the dynamic manipulation of microparticles and cell patterning. Y-TiOPc is selected in our experiments because it is more sensitive than other crystal types [11]. There is only one process needed for fabrication of the chip substrate of TiOPc-based optoelectronic tweezers (Ti-OET). The fabrication process of our TiOPc-based chip substrate can be finished within 40 min. A thin TiOPc layer of about 200 nm is spin coated at 1500 rpm for 20 s on a 3 cm × 3 cm ITO glass. After baking at 130 °C for 30 min, the material property of TiOPc on ITO glass stays stable for months under normal operation. The structure of a Ti-OET chip consists of sandwiched layers of a top transparent ITO electrode, a liquid media, and a thin TiOPc layer on the bottom ITO glass. A 7 volts peak-to-peak (Vp.p.) ac bias with a frequency of below 20 kHz is applied between the top and bottom ITO electrodes, as illustrated in Fig. 1.

Figure 2 illustrates the experimental setup for the required optical system of our Ti-OET. The light source is provided by a Philips UHP mercury lamp and concentrated as a straight beam when it passes through a pair of focusing lenses (L1 and L2). To match the optimum absorbability efficiency of the TiOPc material, a bandpass filter is utilized to adjust the wavelength to the infrared region. Then, the image out of the digital micromirror display (DMD, with a spatial resolution of 1024 × 768 pixels) is programmed via a computer. The straight light is transformed into the designed image when it is illuminated and reflected from the DMD. The optical image contraction system, which consists of two lenses with 200 and 10 mm focal lengths (L3 and L4) and a dichroic mirror,

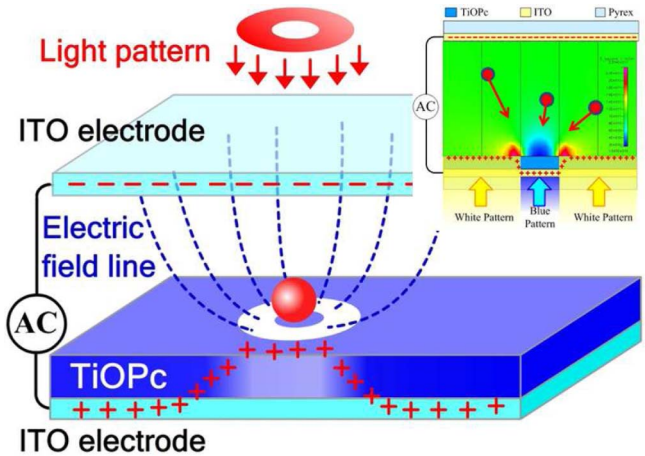

Fig. 1. (Color online) Operation principle of Ti-OET and simulation of the light-induced electric field.

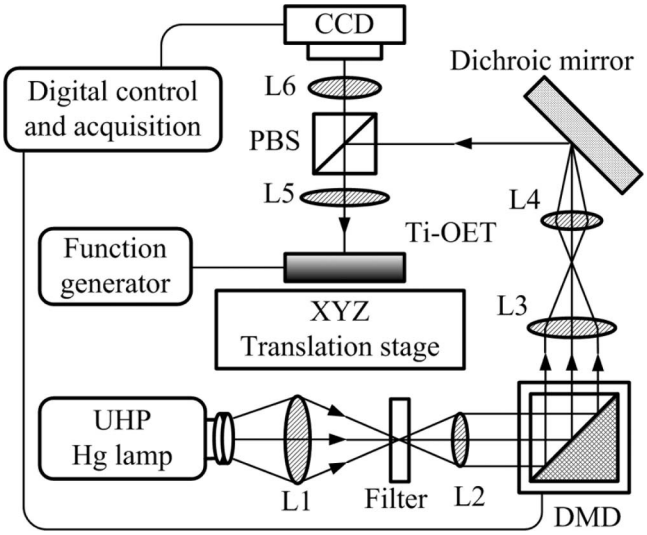

Fig. 2. Schematic diagram of the optical system setup for the light-driven optoelectronic tweezers.

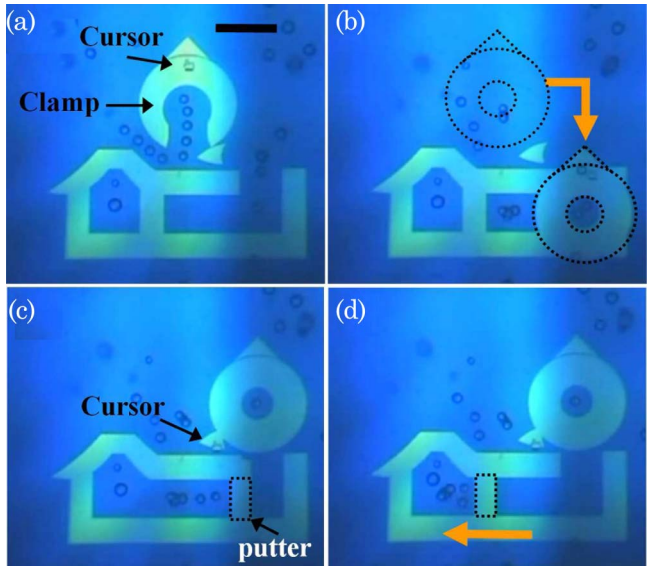

Fig. 3. (Color online) (a) Beads are captured by the light-induced ring-shaped nDEP clamp. (b) The trapped beads are transported by following the arrow path to the entrance of the house-shaped light pattern. (c) and (d) The light-induced nDEP putter pushes the beads leftward. All the demonstrations are operated via Flash codes and cursor action. The scale bar is 100 μm (Media 1).

is aligned to reduce the optical pattern size to 1/20. Finally, the image is projected to the Ti-OET chip through a polarization beam splitter and an objective lens. The image projected onto the Ti-OET chip and the real-time particle manipulation are formed and captured by a 10× object lens and a CCD, respectively.

Flash software (Adobe Systems Co., Ltd.) is used to develop our manipulation interface. When the light pattern is projected onto the Ti-OET chip, the dark region without illumination has a high impedance. Most of the applied voltage drops across the TiOPc layer. Thus, the electric field in the liquid is too weak to influence the microparticles. The TiOPc conductivity increases when it is illuminated by the light. The induced charge is distributed as the light pattern, forms a "virtual electrode" and generates a nonuniform electric field between the top ITO glass and the bottom TiOPc surface. The particles are, then, either trapped toward or repelled from the illuminated image. The operation principle and the light-induced electric field are illustrated and simulated in Fig. 1. Based on the DEP theory [12], the microparticles and the cells suspended in the medium would be manipulated by either moving the position or changing the shape of the light pattern.

Figure 3 demonstrates the light-induced negative DEP (nDEP) manipulation of 15 μm polymer beads by using a real-time moving cursor. By taking advantage of the Flash software, we design two action modes, which generate different light patterns, for the functions of clamping and transporting beads. The first mode generates a light pattern of a closed/open ring. When we click on the ring itself, it becomes a clamp with an opening at the bottom. The ring becomes closed when the cursor is not pressed on the ring. The second mode is a drag mode. When we click and hold on the triangle at the top of the ring, we can drag the ring-shaped light pattern to the desired position.

To demonstrate the dynamic manipulation of beads, the light-induced nDEP clamp, first, is dragged to capture the target beads. In Fig. 3(a), the opening will keep appearing when the cursor presses on the ring. The beads are captured at the center of the clamp when we release the cursor. Figure 3(b) shows that we press the cursor on the top triangle and drag it along the arrow path to transport the trapped beads to the entrance of the house-shaped light pattern. Then, the trapped beads are released out of the nDEP clamp. In Figs. 3(c) and 3(d), when we press the cursor on the other triangle, near the house-shaped light pattern, a rectangular putter appears and moves leftward to push and collect the beads. The formation and movement of the light-induced putter is also conducted via Flash codes. In short, the beads can be captured, transported, pushed, and collected by the cursor action. All the demonstrations are based on light-driven optoelectronic nDEP by using our Ti-OET chip.

Cellular patterning, which arranges the specific cells into the basic unit tissue morphology to rebuild tissue-mimetic blocks, is becoming important for biomedical applications [13]. Here we report the first successful demonstration of liver-cell patterning by using our light-driven optoelectronic nDEP chip. In this demonstration, the delicate liver cells, HepG2 cells, are used. HepG2 cells are harvested from subconfluent cultures by trypsin/EDTA (Sigma), suspended in the DEP manipulation buffer (8.5% sucrose and 0.3% dextrose in ddH_2O; conductivity, 10 mS/m), and sandwiched between the top ITO glass and the button TiOPc substrate surface.

When the AC potential of 6 Vp.p. at 30 kHz is applied to the Ti-OET chip, HepG2 cells are randomly distributed first and then trapped within the contours of the unilluminated region by the light-induced optoelectronic nDEP, as shown in Figs. 4(a) and 4(b), respectively. When we increase the applied frequency to megahertz, the nDEP turns into light-induced positive DEP that is an attractive force for cells. Figures 4(a)–4(c) show the real-time image recording for our liver-cell patterning demonstration by using the light-driven optoelectronic nDEP manipulation. The continuous time-varying change of the light pattern is implemented via Flash codes. Figure 4(d) shows the more obvious cell pattern by prelabeling HepG2 cells with

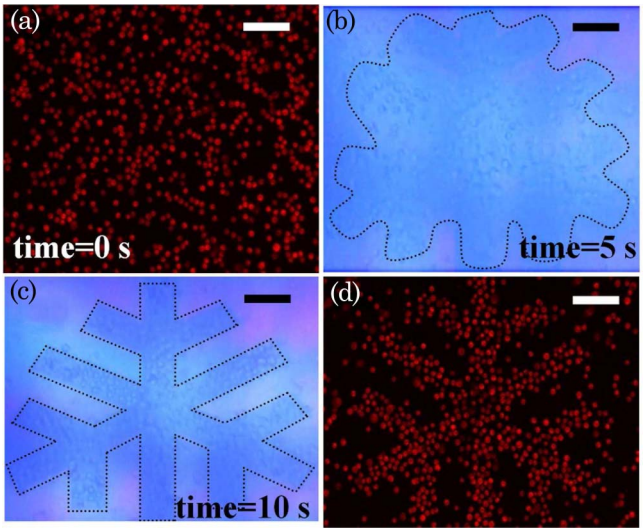

Fig. 4. (Color online) (a)–(c) Real-time image recording for our liver-cell patterning demonstration by using light-driven optoelectronic manipulation. (a) and (d) Fluorescent microscope images for on-chip light-induced cell patterning in the beginning and after 10 min, respectively. The scale bar is 100 μm. (Media 2 is demonstrated by using latex beads instead of cells for a more visible demonstration).

biocompatible DiD fluorescent dyes for the identification at the excitation/emission wavelengths of 530/565 nm under a fluorescent microscope. Such rapid/flexible cell patterning reduces cell damage in the process of cell manipulation and promotes the efficiency of cell patterning for tissue engineering.

According to the time-averaged DEP formula [2], the light-induced DEP force, $F_{\text{Ti-OET}}$, acting on a spherical particle of radius r suspended in a medium with relative permittivity ε_m is

$$F_{\text{Ti-OET}} = 2\pi r \varepsilon_m \text{Re}[f_{\text{CM}}(\omega)] \nabla E_{\text{rms}}^2, \quad (1)$$

where E_{rms} is the rms of the effective ac electric field. $f_{\text{CM}}(\omega)$ is the Clausius–Mossotti factor [12], which depends on the complex permittivity of both the particle and the medium, the conductivity, and the angular frequency ω of the applied electric field. When the particle is driven with a constant velocity on the Ti-OET chip, the driving force resulting from the light-induced DEP equals the viscous drag. Because it is complicated to get the exact values of individual parameters, an indirect approach to estimating the light-induced DEP force is based on the Stokes law [14], i.e., $F_{\text{Ti-OET}} = 6\pi r \eta \nu_{\text{Ti-OET}}$. Here, η is the medium viscosity and $\nu_{\text{Ti-OET}}$ is the steady-state velocity of the particle.

The steady-state velocity of the light-driven bead in the operational medium with applied voltage of 7 Vp.p. at 20 kHz is measured as 74 μm/s. The corresponding light-induced force $F_{\text{Ti-OET}}$ acting on the microparticle is 10.5 pN. The light-induced optoelectronic DEP force on the microparticles versus the applied ac voltage is experimentally characterized as shown in Fig. 5. The Ti-OET chip yields the maximum light-induced $F_{\text{Ti-OET}}$ force of 10.5 pN under 7 Vp.p. at 20 kHz and the minimum of

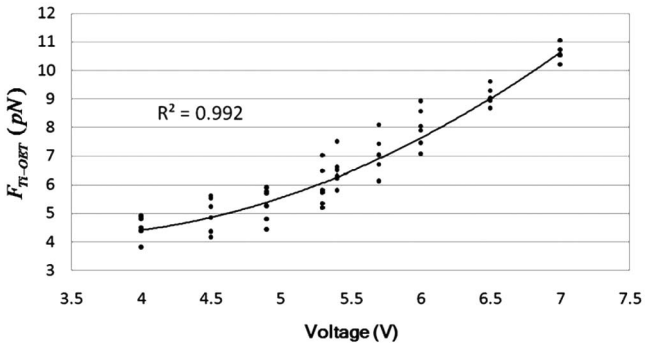

Fig. 5. Characterized relationship between the light-induced DEP force and the applied voltage at 20 kHz for 15 μm polymer beads on our Ti-OET chip.

4.5 pN under 4 Vp.p. with observable and stable microparticle movement.

Light-driven optoelectronic tweezers, which take advantage of the organic photoconductive material TiOPc, are developed as reported in this Letter. With Flash software, we successfully demonstrated the dynamic manipulation of microparticles and cell patterning by using this light-driven optoelectronic DEP chip. Human liver cells (HepG2) are utilized for the cell patterning demonstration. To the best of our knowledge, this is the first reported demonstration for successfully patterning such delicate cells (HepG2) by using any optoelectronic tweezers. The rapid light-pattern formation with changing the contour, as demonstrated in this report for light-driven optoelectronic manipulation, has potential for many biological applications, such as tissue engineering technology. This investigation of TiOPc-based optoelectronic manipulation is expected to expand a new research area in cellular and biological applications.

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