Programmable manipulation of motile cells in optoelectronic tweezers using a grayscale image

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This paper describes a grayscale optoelectronic tweezers (OET) which allows adjustment of the electric field strength at each position of OET. A grayscale light image was used to pattern vertical electric field strength on an OET. As an electric field depends on the brightness at each point, the brighter light patterns generate the stronger electric field in the OET. Its feasibility for application to cell manipulation was demonstrated by aligning highly motile protozoan cells in vertical direction. Depending on the brightness of each pixel, the behaviors of aligned cells varied due to the different electric field strength to each cell. © 2008 American Institute of Physics.

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Optical manipulation techniques such as optical tweezers have been used to study dynamic behaviors of motile cells. However, none of these technologies has been able to simultaneously apply different strength of force to individual cells. Recently, optoelectronic tweezers (OET) have emerged as a powerful tool for massively parallel manipulation of microparticles and living cells. This technology uses a photoconductive layer to allow an optical image to control the electric field and has been applied to the manipulation of living cells, microparticles, and nanoparticles. Until now, OET has been only operated by the binary optical image, which has two binary values (on/off transmission) for each pixel, and a control of the electric field strength at each pixel has not been exploited, while a change in the bias voltage causes a change in the scale of whole electric field.

Here we describe a grayscale OET, which allows adjustment of the electric field strength at each pixel using a grayscale image with a variable intensity value for each pixel. To demonstrate its functionality, we apply this technology to manipulate cells of the ciliated protozoan Tetrahymena pyriformis. The ciliates are known to swim by coordinating their ciliary beating.

The schematic configuration of the experimental setup of the grayscale OET is shown in Fig. 1. Overall setup consists of an OET device and a 0.5 in. liquid crystal display (LCD) module (L3P05S Series, Seiko Epson, Japan) installed in an upright microscope (Zeiss Axioskop 40, Carl Zeiss, Germany). In the OET part, a cell-containing solution was sandwiched between the photoconductive layer and the ground layer, and a double-sided adhesive tape was used as a spacer of 120 μm thick. Detailed procedures of the OET fabrication are elsewhere. Cell behaviors were observed and captured with a charge-coupled device camera (Pike F032C, Allied Vision Technologies, Germany). The brightness was measured using an illuminance meter (T-10M, Konica Minolta, Japan) and the bias voltage of 12 V pp at 10 kHz was produced from a function generator (AGF3022, Tektronix, OR).

A grayscale image was controlled by standard presentation software (Microsoft PowerPoint) on a computer.

Tetrahymena cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were cultured in a medium containing 0.5% peptone and 0.5% tryptone and 0.02% K2HPO4 (w/v). Cells were centrifuged at 500 × g for 2 min to collect a high density of cells and suspended in the experimental buffer (phosphate buffered saline solution with a conductivity of 22 μS/cm).

In order to manipulate swimming cells by the OET, the cells were introduced to the liquid layer of the OET and a vertical electric field was applied between the photoconduc-
tive layer and the ground layer in the OET. In a control experiment with a grayscale image and without an electric field, the image pattern gave no apparent effect on the free swimming of the cells. This result implies that there is little effect by the light. In addition, we conducted another control experiment with a vertical electric field and without the image. A transparent electrode was used instead of the photoconductive layer in the OET device, and an electric bias of 6 V_{pp} at 10 kHz was applied to make proper vertical electric field strength of 50 mV_{pp}/μm. Figure 2 shows a sequence of still images showing a typical example of the cell alignment where cells were observed using a top view. It is inferred that the cells freely swim when there is no electric field. When an electric field was applied, however, the cells become oriented into a vertical direction and appeared to be trapped. However, their ciliary movements were still sustained (data not shown), thereby allowing the aligned cells to move in the vertical direction. Accordingly, the lateral movement of the vertically oriented cells was decreased.

Electro-orientation is the ability to align nonspherical particles immersed in a solution of different permittivity with an electric field. Compared to dielectrophoresis (DEP) or electrorotation, it is similar but different in many aspects. DEP occurs in a nonuniform field and electrorotation occurs in a rotating electric field, while electro-orientation occurs for nonspherical particle even in a uniform electric field. Electro-orientation was applied to study the several types of cells such as red blood cells and bacteria. Recently, it was reported that electro-orientation occurred during the OET manipulations of nanowires and red blood cells.

Figures 3(a)–3(d) show a typical alignment behavior of swimming cells in the grayscale OET. The brightness of each pixel in the grayscale image was linearly increased from the left to the right (see Ref. 16). When there was a uniform dark image at 0 s, the cells at any position swam freely and looked as an elongated shape. However, as the image appeared gradually from 0 to 6 s, the cells at the bright region were aligned. These cells did not swim freely, vibrated at almost the same place and looked as a circular shape. According to the brightness of each pixel, cell alignment behaviors were different due to the different electric filed. At the bright region, more cells were aligned than the cells at the dark region. When the image disappeared gradually from 8 to 14 s, the aligned cells got their original shape again and swam freely one after another. After the image disappeared all over, every cell swam freely and returned to its original elongated shape. In addition, there was no apparent damage of cells. The various strength of the electric field was also applied for each swimming cells at the same time and on the same stage. To measure the alignment ratios, the pattern of light image is designed to have five areas of different brightness (1677, 3140, 10 036, 24 722, and 43 872 lux) [Fig. 3(e)]. The aligned cells were marked with arrows, while free swimming cells were unmarked. The ratio of cell alignment according to the light contrast was calculated in each area using following definition: (alignment ratio) = (number of aligned cells)/(number of total cells). The alignment ratio increased gradually from 4.3% (at 0% contrast) to greater than 96.0% (at 100% contrast) according to the light contrast [Fig. 3(f)].

To confirm the electric field relationship according to the brightness, we calculated the vertical electric field in the grayscale OET [Fig. 4(a)]. We assumed that the vertical electric field was affected by only the brightness of that pixel and was not affected by the brightness of surrounding area. The brightness was measured to be from 1677 to 43 872 lux at the contrast of brightness was 0%–100%. We get the photoconductivity value of 1.237–32.377 μS/cm from the measured photoconductivity-brightness relation. The voltage across the liquid layer was calculated from an equivalent circuit model [see inset of Fig. 4(a)], and the vertical electric field was calculated by dividing the voltage across the liquid layer with the thickness of the liquid layer. The vertical electric field increased according to the brightness and its range was from 35 to 70 mV_{pp}/μm.

To verify that the direction of the electric field is almost vertical, we compared lateral and vertical electric fields. Figure 4(b) shows a section view according to the dotted line AB in Fig. 3(c). Both lateral and vertical electric fields were calculated using a commercial computational fluid dynamic simulation package.
and did not get its normal shape or swim freely again. However, the cells were damaged when we applied strong electric field, for example over 100 mVpp/μm. Some cells were divided into two or more segments, lysed, and did not get its normal shape or swim freely again even though the electric field disappeared. These phenomena have been described as deformation and electrical breakdown of the cellular membrane. Therefore we examined the shapes and behavior of cells at the final step in each experiment to confirm that the cells have not been seriously damaged.

It is clear that the grayscale OET is able to control the electric field intensity and has all advantages of the OET. Namely, the OET manipulation can be used not only at a single cell level but also at a multiple-cell level. In addition, the complex, multistep manipulation is possible and the required brightness is much smaller than that of conventional optical tweezers. One of the anticipated applications of this platform is a motility assay of cell. It would be useful to study a signaling pathway of the motile cells by an attractant or a blocker of a specific receptor. These experiments need a single-cell study as well as massively parallel analysis.

In summary, we have developed a grayscale OET which allows adjustment of the electric field strength at each position of OET. The electric field strength was controllable independently in space and time, so that the method was applied to the local alignment of swimming cells. The calculated electric field also showed that the vertical electric field increased according to the brightness. This platform will be a powerful tool in studying cell motility.

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