Programmable micrometer-sized motor array based on live cells†

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Trapping and transporting microorganisms with intrinsic motility are important tasks for biological, physical, and biomedical applications. However, fast swimming speed makes the manipulation of these organisms an inherently challenging task. In this study, we demonstrated that an optoelectrical technique, namely, optically induced dielectrophoresis (ODEP), could effectively trap and manipulate Chlamydomonas reinhardtii (C. reinhardtii) cells swimming at velocities faster than 100 μm s⁻¹. Furthermore, live C. reinhardtii cells trapped by ODEP can form a micrometer-sized motor array. The rotating frequency of the cells ranges from 50 to 120 rpm, which can be reversibly adjusted with a fast response speed by varying the optical intensity. Functional flagella have been demonstrated to play a decisive role in the rotation. The programmable cell array with a rotating motion can be used as a bio-micropump to drive the liquid flow in microfluidic chips and may shed new light on bio-actuation.

Introduction

Precise manipulation of individual microorganisms with intrinsic motility is extremely invaluable to many applications in biology, chemistry, physics, and medicine. Cellular-level manipulation can be used to study cell behavior such as cell mechanical rigidity,1 cellular forces,2,3 motility,4 and interaction among cells.5,6 Single-cell manipulation can also facilitate the study of sample purification,7–9 drug discovery,10,11 medical diagnosis,12,13 and microsurgery.14–16

However, trapping swimming organisms has remained a significant challenge because of their high velocities (>30 μm s⁻¹). Existing techniques, including mechanical structure trapping,17,18 microfluidics,19–21 acoustic trapping,22,23 electrostatics,24 dielectrophoresis,25–27 and magnetic trapping,28 were previously used to capture particles and non-swimming cells, but their capability for selective trapping and manipulation of a swimming microorganism has not been demonstrated yet. Previous studies29–31 have demonstrated that optical tweezers could trap and manipulate motile microorganisms such as flagellated bacteria. However, the optical intensity of optical tweezers is approximately 10⁷ W cm⁻², which requires high-power lasers32 and can damage some cells,33 even resulting in cell death.34 The use of magnetic tweezers is another technology with the potential application in trapping and manipulating motile microorganisms; however, it requires additional magnetic labeling.35

Optically induced dielectrophoresis (ODEP) is an emerging particle manipulation method.36 In ODEP, optical illumination on a photoconductor substrate can create a nonuniform electric field. This spatially varying electric field results in DEP.37 Thus, trapping and manipulating microparticles or nanoparticles can be realized by combining the optical, electrokinetic, and hydrodynamic effects. ODEP was previously demonstrated with non-swimming cells,38–41 polystyrene beads,42 and semiconductor nanowires.43

In the present study, we demonstrated that ODEP could effectively trap and transport unicellular swimming algae, e.g., Chlamydomonas reinhardtii (C. reinhardtii), with an average swimming speed of 110 μm s⁻¹. Unique characteristics were observed in the trapping behavior of swimming algal cells compared with previous ODEP studies on non-swimming cells and polymer microspheres.36 We found that the trapped cells started to rotate and demonstrated that functional flagella played a decisive role in the rotation. We also realized homodromous rotation of a live C. reinhardtii cell array in an ODEP trap. The speed of rotation can be controlled by varying the optical intensity. The cells rotated with...
a frequency of 50–150 rpm. A light beam with an intensity of 4 mW cm\(^{-2}\) and mean wavelength of 463 nm was adopted in our experiment. The magnitude (50 kVpp m\(^{-1}\)) and frequency (50 kHz) of the electric field were chosen to ensure simultaneous trapping success and cell viability.

**Methods and materials**

**ODEP system**

The three-dimensional view of the ODEP chip structure used in our experiments is shown in Fig. 1(a). The ODEP chip has a sandwich topology: the top layer is a glass substrate (20 × 20 mm) coated with conductive indium tin oxide (ITO) film. The objects (*C. reinhardtii* cells) to be manipulated are contained in the middle liquid layer, and another ITO glass substrate (20 × 20 mm) deposited with a thin photoconductive film of hydrogenated amorphous silicon (a-Si:H) is used as a bottom layer. The fabrication process of this bottom substrate was described in detail in our previous study.\(^{44–46}\) Briefly, the 100 nm ITO thin film is first sputtered on a 1 mm glass substrate. Then, a 1 μm a-Si:H film is deposited on the ITO film through plasma enhanced chemical vapor deposition. In the ODEP chip assembly process, the top and bottom glass substrates are first immersed in 99% v/v alcohol, sonicated for 20 min at a frequency of 59 kHz, rinsed with deionized water, and soaked for 4 h in 10% bovine serum albumin (BSA) solution, followed by second rinsing and drying using nitrogen gas. The BSA prevents nonspecific binding of cells onto the glass surfaces. Double-faced adhesive tape or a patterned polydimethylsiloxane (PDMS) is used as spacers between the top layer and a bottom layer of the ODEP chip to create a microfluidic chamber. The thickness of the double-faced adhesive tape or patterned PDMS is about 100 μm. The *C. reinhardtii* cell solution can be injected into the chip from the entrance of the microfluidic chamber.

The assembled ODEP chip is then placed on a translation platform with four degrees of freedom [Fig. 1(b)]. A signal generator (Agilent 33522A, USA) is used to generate an AC electrical signal that is applied between the top and bottom ITO electrodes. The commercial computer software (Adobe Flash) can generate different types of programmable light patterns, which can be projected from an LCD projector (VPL-F400X, Sony, Japan) through a condenser objective (Nikon 50×/0.5) onto the bottom a-Si:H substrate.

Compared with conventional DEP chips, non-uniform electric field is no longer generated by fixed metal electrodes. Here, the photoconductive a-Si:H film can be used to “generate” programmable localized virtual electrodes through its interaction with incident light. In the absence of an incident light, the conductivity of a-Si:H is approximately 10\(^{-11}\) S m\(^{-1}\), the a-Si:H film behaves as an insulator; therefore, the applied voltage mostly drops across the a-Si:H layer. The conductivity of the illuminated region in the a-Si:H film sharply increases by several orders of magnitude to 10\(^{-5}\) S m\(^{-1}\) due to the photon-generated electron holes. Most of the applied voltage drops across the liquid layer in this case, creating a localized virtual electrode, and a non-uniform electric field is generated. *C. reinhardtii* cells bear a DEP force in the liquid layer through their interaction with the optically induced non-uniform electric field, which is defined as the “ODEP force.”

**Two-shell model of cell morphology and ODEP force**

*C. reinhardtii* cells are the objects to be manipulated in this study. The ODEP force acting on a cell in an ODEP device is evaluated using eqn (1) under the assumption that the cell suspended in the medium is spherical.

\[
F = 2\pi R^3\varepsilon_m\text{Re}[K(\omega)]V E_{\text{rms}}^2
\]

where \(R\) is the radius of the cell, \(\text{Re}[K(\omega)]\) is the real part of the Clausius-Mossotti factor \(K(\omega)\), \(V\) is the gradient, and \(E_{\text{rms}}\) is the root mean square value of the electric field.
is the root mean square of the external electric field strength. Clausius–Mossotti factor $K(\omega)$ can be determined by

$$K(\omega) = (\varepsilon^*_{p} - \varepsilon^*_{m}) / (\varepsilon^*_{p} + 2\varepsilon^*_{m})$$  \hspace{1cm} (2)$$

$$\varepsilon^* = \varepsilon - j\sigma(\omega)$$  \hspace{1cm} (3)$$

where $\varepsilon^*$ is the complex permittivity that consists of physical permittivity $\varepsilon$, conductivity $\sigma$, and angular velocity $\omega$ of the external electric field. Subscripts $p$ and $m$ represent the particle and the medium, respectively. For the cell, the ODEP force is determined by the real part of Clausius–Mossotti factor $\text{Re}[K(\omega)]$ and $\sqrt{E_{\text{rms}}^2}$, $\text{Re}[K(\omega)]$ mainly contributes to the magnitude and positive or negative direction of the dielectrophoretic force, whereas $\sqrt{E_{\text{rms}}}$ determines both the magnitude and direction of the dielectrophoretic force.

C. reinhardtii cells are heterogeneous particles that do not have single $\varepsilon^*_{p}$. However, effective $\varepsilon^*_{p}$ can be used to describe the cell behavior that accounts for the morphology and electrical properties of the different components of the cell. Here, a two-shell model is established. $C. \text{ reinhardtii}$ cell is modeled as a spherically symmetrical particle, which consists of a spherical cytoplasm with complex permittivity $\varepsilon^*_{cyt}$ surrounded by shells corresponding to the plasma membrane with complex permittivity $\varepsilon^*_{\text{mem}}$ and a cell wall with complex permittivity $\varepsilon^*_{\text{wall}}$, as shown in Fig. 2(a). First, the effective permittivity $\varepsilon^*_{\text{cyt,mem}}$, combined with the cytoplasm and plasma membrane, can be described as

$$\varepsilon^*_{\text{cyt,mem}} = \varepsilon^*_{\text{mem}} \left[ R_1^2 / R_3^2 + 2K(\omega) / R_1^2 / R_3^2 - K(\omega) \right]$$  \hspace{1cm} (4)$$

where $R_1 = R + \delta_{\text{mem}}$, $R$ is the radius of the spherical cytoplasm, $\delta_{\text{mem}}$ is the thinness of the plasma membrane, and $K(\omega) = (\varepsilon^*_{\text{mem}} - \varepsilon^*_{\text{cyt}}) / (\varepsilon^*_{\text{cyt}} + 2\varepsilon^*_{\text{mem}})$. Then, effective permittivity $\varepsilon^*_{\text{cyt,mem,wall}}$ of the cell can be further defined as

$$\varepsilon^*_{\text{cyt,mem,wall}} = \varepsilon^*_{\text{wall}} \left[ R_2^2 / R_3^2 + 2K(\omega) / R_2^2 / R_3^2 - K(\omega) \right]$$  \hspace{1cm} (5)$$

where $R_2 = R_1 + \delta_{\text{wall}}$, $\delta_{\text{wall}}$ is the thinness of the cell wall, and $K(\omega) = (\varepsilon^*_{\text{wall}} - \varepsilon^*_{\text{cyt,mem}}) / (\varepsilon^*_{\text{cyt,mem}} + 2\varepsilon^*_{\text{wall}})$.

To calculate effective permittivity $\varepsilon^*_{\text{cyt,mem,wall}}$ of the cell, the electrical properties of the biological cells are selected from previously measured values.$^{48,49}$ The $C. \text{ reinhardtii}$ cells are modeled as 10 $\mu$m-diameter spherical cytoplasmic spheres surrounded by a 4.5 nm plasma membrane and a cell wall with a thickness of 165 nm in its morphology. The cytoplasm is considered to have a relative permittivity of 60 and a conductivity of 400 mS m$^{-1}$. The membrane is modeled to have a relative permittivity and a conductivity of 2.3 and 10 $\mu$S m$^{-1}$, respectively. The cell wall has a relative permittivity of 60 and a conductivity of 50 mS m$^{-1}$. Here, the relative permittivity of deionized water (suspending medium) used in our experiments is considered to be 80, and the conductivity is measured as approximately 10 $\mu$S m$^{-1}$. The relationship between $\text{Re}[K(\omega)]$ and $\omega$ is shown in Fig. 2(b). When the frequency is less than 10 MHz, the value of $\text{Re}[K(\omega)]$ is positive and decreases with the frequency in a certain cell medium. The value of $\text{Re}[K(\omega)]$ approaches zero when the frequency reaches 100 MHz. However, when the frequency of the AC source with a certain voltage is below 10 kHz, we can easily generate bubbles on the surface of a-Si:H. The generated bubbles will influence the subsequent manipulation. Therefore, a frequency of 50 kHz is chosen.

COMSOL multiphysics is used to compute non-uniform electric field $\sqrt{E_{\text{rms}}^2}$. Here, an axially symmetric model is adopted. The simulation module and its corresponding boundary settings are the same as those used in our previous work described in ref. 50. The following experimental parameters are chosen: the relative dielectric constant of the liquid is 80; the relative dielectric constant of a-Si:H is 11.7, which can reach up to 21.7 with incident illumination; the conductivity of the cell solution is 10 $\mu$S m$^{-1}$ (measured by a Cond 3110 conductivity meter); the conductivity of a-Si:H with incident illumination is $4 \times 10^{-5}$ S m$^{-1}$; and the dark conductivity of a-Si:H is $1 \times 10^{-11}$ S m$^{-1}$ (measured by a Keithley 2410 source meter). The applied voltage and frequency are 5 Vpp and 50 kHz, respectively. Geometrically, we modeled an optically-projected spot with a diameter of 20 $\mu$m to function as virtual electrodes. The finite element method numerical analysis result of the potential and the direction of the electrical field distribution caused by the optically projected “spot” pattern is shown in Fig. 3(a). The white arrows indicate the direction of $\sqrt{E_{\text{rms}}^2}$, which mainly point from a dark area toward an illuminated area. These results indicate that the cells bear an ODEP force from a dark area toward an illuminated area according to the value of $\text{Re}[K(\omega)]$, as shown in Fig. 2(b). Fig. 3(b) shows the DEP force distribution on a 10 $\mu$m $C. \text{ reinhardtii}$ cell 5 $\mu$m high above the a-Si:H film surface. We can see from the result that the $C. \text{ reinhardtii}$ cell will bear a large piconewton ODEP force around the light spot, and a maximum force of approximately 62 pN occurs at the edge of the light spot. Here, the experimental parameters and corresponding ODEP force were chosen to satisfy two conditions, namely, trapping success and cell viability, which will be confirmed from the experiment and analysis presented in the next sections.
Sample preparation

Experimental strains were obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wuhan, China). The *C. reinhardtii* used in the present study were grown in tris-acetate-phosphate medium at 25 °C. The culture was illuminated with a series of fluorescent grow lights with a 12/12 h light/dark cycle. The cells were grown to a density of $5 \times 10^6$ cells per ml. The cells were centrifugated at 2000 rpm for 5 min and suspended in deionized water before the subsequent experiments.

Images and data analysis

The trapping process of the cells was monitored in real time using a charged coupled device (DH-SV1411FC, DaHeng Image, China) and a microscope (Zoom 160, OPTEM, USA). The images were transmitted to a computer through USB and processed by in-house developed customized software. Videos of cell motion were imported into MATLAB (Code, ESI†), and converted to individual frames. With the developed MATLAB program, the position of the *C. reinhardtii* cell at different instants can be extracted, the trajectory of the cell can be got, and the velocity can be further calculated combined with traced time.

Results and discussion

Trapping and transportation of swimming algal cells

The trapping and transportation of swimming algal cells were experimentally verified according to the proposed approach. Here, the algal cells were diluted to a density of $5 \times 10^5$ cells per ml, then injected into the liquid layer of the ODEP chip. The experimental demonstration of trapping and transportation of the swimming algal cells are shown in Fig. 4 (Movie S1, ESI†). Without an ODEP force (AC source was turned off), the algal cells exhibited a significantly random and forward motion [Fig. 4(a) and (b)]. When an algal cell was close to a spot (AC source was turned on), it experienced an ODEP force, and the direction of the cell changed. According to the above analysis, the direction of the ODEP force exerted on the algal cell points from the dark area toward the illuminated area, which means that the cell stayed on the spot [Fig. 4(c) and (d)]. The trapped swimming cells could be carried by a mobile light spot (bright spot) then transferred to any location in two dimensions [Fig. 4(e) and (f)]. The trapped cell started to rotate, which will be explained in detail in the next section. Here, the big blue circle can be used not only as a “barrier” to prevent a redundant cell to go into an effective region (four small blue spots within the big blue circle) but also as a “source” of the cell. If the rotation ability of some cells in the effective region decreases, we can choose appropriate cells from the source then transfer them to a designated location in the effective region and replace the inefficient cells. When the ODEP force is removed, the cell restarts its translational motion, and the trajectories of the cells after being released are provided in ESI† (Cells Releasing), demonstrating that no
discernable damage was incurred by the cellular motor system caused by the ODEP force used in these experiments [Fig. 4(g) and (h)].

Once the ODEP force exerted on the cell body dominates the inherent propulsive force of the algae, successful trapping can be realized. Here, a series of experiments and theoretical analysis was carried out to evaluate the propulsive force of the algal cell. Initially, we tracked the swimming paths of the *C. reinhardtii* cells, and the typical results are shown in Fig. 5. Real-time movie clips of the translationally moving cells were captured and converted to individual frames. The motion of each cell was considered as a “trajectory,” and its movement was traced using MATLAB software. Self-rotating cells were avoided in all the quantifications. Fig. 5(a) shows the trajectories of the freely swimming *C. reinhardtii* cells, which demonstrated a significant random forward motion. The velocities of the swimming cells were measured from the extracted motion trajectories. A histogram of these velocity data is shown in Fig. 5(b). The velocities of 180 swimming *C. reinhardtii* cells ranged from 60 to 160 μm s⁻¹. We fitted a Gaussian to the peak moving velocity, avoiding non-motile cells, and found a modal velocity of 111.35 μm s⁻¹. We also performed a theoretical analysis on the motion of the cells. The swimming of *C. reinhardtii* requires techniques that are very different from those used by macroscale swimmers. To understand this phenomenon, we referred to the Navier–Stokes equations,⁵¹ which completely define a fluid flow. For a fluid with constant density $\rho$ and constant viscosity $\eta$, the Navier–Stokes equations are given by the following vector equations:

$$
\rho \left( \frac{\partial}{\partial t} + u \cdot \nabla \right) u = -\nabla p + \eta \nabla^2 u
$$

(6)

$$
\nabla \cdot u = 0
$$

(7)

where $u$ is the velocity vector field and $p$ is the hydrodynamic pressure scalar field. We considered a steady flow with typical velocity $U$ around a body with characteristic length $L$. The Reynolds number $Re$ is classically defined as the ratio of the typical inertial force to the viscous force obtained by combining eqn (6) and (7)

$$
Re = \frac{\rho U L}{\eta}
$$

(8)

In our experiment, a medium with $\rho \approx 10^3$ kg m⁻³ and $\eta \approx 10^{-3}$ Pa s was used. *C. reinhardtii* with $U \approx 111.35$ μm s⁻¹ and $L \approx 10$ μm has Reynolds number $Re \approx 1.11 \times 10^{-3}$. At low Reynolds number, the flagella of *C. reinhardtii* deform to create paddling motions. The kinematic equation of the *C. reinhardtii* cell can be described as

$$
m \frac{d^2 x}{dt^2} = -3\pi \eta d \frac{dx}{dt} + F_{\text{thrust}}
$$

(9)

where $x$ is the position of the cell, $m$ is the cell mass, $d$ is the diameter of the cell, and $F_{\text{thrust}}$ is the propulsive force generated by its flagellum. The first term on the right side represents the viscous drag force from the surrounding fluid. When the *C. reinhardtii* cells swim in an equilibrium state, their thrust force is equal to the drag force exerted on them. Liquid drag force $f$ can be expressed as

$$
F_{\text{thrust}} = f = 3\pi \eta v d
$$

(10)

where $v$ is the velocity of the *C. reinhardtii*. We assumed that the cell is spherical with diameter $d$ of 10 μm. Then, we estimated a propulsive force of 10.49 pN according to our velocity measurements. The propulsive force was much higher than that of the bacteria (0.39–0.64 pN) reported by another group.⁵² Therefore, the speed of a *C. reinhardtii* cell is essentially a balance between the propulsive force generated by its flagellum, which is a reflection of the flexural rigidity, and the drag from the surrounding fluid. In the above ODEP force analysis, when the applied voltage and frequency of 5 Vpp and 50 kHz is adopted, the maximum force can reach up to 62 pN. This is approximately six times larger than the propulsive force of the *C. reinhardtii* cell, which should be large enough to trap and transport the *C. reinhardtii* cell. Therefore, the corresponding parameters were used in our trapping conditions.

**Analysis of the rotation of *C. reinhardtii* cell**

In the freely swimming state, the *C. reinhardtii* cell exhibits a randomly forward motion. In the trapping state, the optical spot functions as a “pivot”, and the translational motion of the cell can be converted into a rotary motion around the optical spot.

The self-rotation behavior of white and red blood cells in an irrotational AC electric field has recently been reported.⁵³,⁵⁴ In the aforementioned reports, all cells did not have any internal means of motion, whereas the trapped *C. reinhardtii* cells in our study have an intrinsic linear motion capability. To explore the interplay between the

![Fig. 5](image-url)
intrinsic force generated by the flagellar motor and the ODEP force, 2 mM NiCl was used to treat the *C. reinhardtii* cell, which has been used to fairly inhibit a flagellar function. Then, the cells were trapped by the ODEP force, and their motion was observed. The trapped cells ceased to rotate after being treated with NiCl. We can verify that the rotation behavior of the *C. reinhardtii* cell mainly depends on the intrinsic motor action of the cell.

Fig. 6 show the free-body diagrams of the cell in the ODEP chip. Specific force *F* and torque *M* are generated by the flagellum motors. $F_{\text{dep}}$ is the ODEP force, and $F_r$ and $F_f$ are the stamina and friction forces exerted by the substrate, respectively. The density of the *C. reinhardtii* cell (1.05–1.07 g cm$^{-3}$) is approximately equal to that of the deionized water used in our experiment. Thus, the gravity of the cell can be balanced by the buoyancy exerted on the cell. Under the action of all the forces, the cell rotates its body at certain speeds in the optical spot. A large number of cells were analyzed, and their rotational speed was obtained using a number of frames converted from a real-time movie. The measured rotational speed ranged from 50 to 120 rpm. Torque *M* can be derived from this speed under low-turbulence conditions.

$$M = -8\pi\eta r^3\omega$$

where $r$ is the radius of the cell and $\omega$ is the angular velocity. The calculated torque values are in the range from $1.6 \times 10^{-17}$ to $3.9 \times 10^{-17}$ N m for the rotating *C. reinhardtii* cells.

### Rotating speed regulation

The rotation speed of the *C. reinhardtii* cell is controlled by varying the optical intensity. Fig. 7(a) shows the changes in the rotation speed with time. The rotation speed was reduced by 9.5% 10 min after being trapped. The relationship between the rotation speed and optical intensity is shown in Fig. 7(b). The cell speed reversibly changed along with the light intensity in a fast response speed. When the optical intensity was approximately 300 mW cm$^{-2}$, the rotation speed could reach up to 150 rpm. The torque generated by a bull-sperm flagellum during an isometric stall was approximately $3.9 \times 10^{-15}$ N m. To obtain a similar torque value, a rotation frequency of 200 Hz can be expected. Meanwhile, the rotary frequency in our experiment was approximately 1–2 Hz, which has just reached the peak output of approximately 1% only of the dynein molecules. The dynein functions as a gear in response to an external load, as previously demonstrated. In the state of random motion, only a subset of the motor molecules was available. Meanwhile, more motor molecules will be activated by the optical, thermal, or chemical stimuli during flagellar beating, which can well explain the increase in the rotation speed with the light intensity.

### Pattern of swimming algal cells

Different patterns of algal cells can be realized by an ODEP trap. The location of the swimming algal cells can be determined. Fig. 8 (Movie S2, ESI†) shows the different types of cell patterns, which could be patterned in a horizontal line [Fig. 8(a)], a vertical line [Fig. 8(b)], a triangle [Fig. 8(c)], and a 3 × 3 array [Fig. 8(d)]. The directions of the rotation of all the trapped cells in the pattern are the same. The inefficient cells can be easily replaced. The site-selected live *C. reinhardtii* cells array with homodromous rotation can be seen as a dynamic micrometer-sized motor array, which can
be used as bio-actuators in microfluidic applications. The emergent dynamic behavior of the hydrodynamically coupled microrotors is expected to be investigated using the proposed programmable cell pattern method.

Conclusions

In this paper, we have proposed a dynamic trapping and transportation of individual swimming microorganism approach with high precision, which does not need any special surfaces or additional labeling on the biological samples and eliminates any risk of heating or photodamaging the biological samples. Unique characteristics were observed in the trapping behavior of the swimming algal cells compared with the previous ODEP studies on non-swimming cells and polymer microspheres. The trapped cell started to rotate. We also demonstrated that functional flagella played a decisive role in the rotation. Homodromous rotation of live *C. reinhardtii* cells array in the ODEP trap was realized. The rotating speed of the cell can be reversibly adjusted by the optical intensities with a fast response speed. Different types of patterns of live algal cells can be flexibly realized by this optoelectrical method. The programmable cell patterns with controllable rotating motion offer great potential for investigating the emergent dynamical behavior of hydrodynamically coupled microrotors and may find future applications in bio-actuation.

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Notes and references
