Inducing self-rotation of cells with natural and artificial melanin in a linearly polarized alternating current electric field

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The phenomenon of *self-rotation* observed in naturally and artificially pigmented cells under an applied linearly polarized alternating current (non-rotating) electrical field has been investigated. The repeatable and controllable rotation speeds of the cells were quantified and their dependence on dielectrophoretic parameters such as frequency, voltage, and waveform was studied. Moreover, the rotation behavior of the pigmented cells with different melanin content was compared to quantify the correlation between self-rotation and the presence of melanin. Most importantly, macrophages, which did not originally rotate in the applied non-rotating electric field, began to exhibit self-rotation that was very similar to that of the pigmented cells, after ingesting foreign particles (e.g., synthetic melanin or latex beads). We envision the discovery presented in this paper will enable the development of a rapid, non-intrusive, and automated process to obtain the electrical conductivities and permittivities of cellular membrane and cytoplasm in the near future. © 2013 AIP Publishing LLC.

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I. INTRODUCTION

In general, AC electrokinetics (ACEK) focuses on the effect of external AC electric fields on the motions of fluids and micro/nano/bio entities in the fluidic media. ACEK can be used to achieve particle manipulation either by one or together with multiple electrokinetics forces.¹ This technique have been extensively utilized for the manipulation of micro/nano/bio entities, for example, microbeads,² quantum dots (QDs),³ and mouse embryos.⁴ It has also been used to facilitate other biomedical applications such as detection of DNA nanoparticles^{5,6} and drug delivery.⁷ Among ACEK manipulation approaches, Dielectrophoretic (DEP) force has been extensively explored for the separation,^{8–12} concentration,^{13–17} patterning,^{18–21} and trapping^{22–25} of particles and bio-entities in miniaturized microfluidic devices. Based on the distinctive electrical properties of various cellular phenotypes, it is possible to differentiate one type of cell from others or to separate multiple targets simultaneously, based on the specific application, by applying an appropriate DEP field.

Electrorotation (ROT), which is another non-invasive ACEK technique for cell manipulation, has been utilized to induce rotations in either a single cell^{26,27} or in coupled microspheres.^{28,29} This approach typically uses multiple electrodes^{26,27,30,31} and requires a phase difference between

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neighboring electrodes in order to create a *rotational* electrical field was first described in 1982.^{32,33} Applications for ROT include characterization of the dielectric property of the cells,³⁴ the development of electrofusion,³⁵ and the monitoring of the changes in electrical properties of a cell subjected to hypotonic stress.²⁶ In this paper, we present our discovery that cells with natural and artificial melanin can be induced to rotate in a non-rotating (i.e., linearly polarized) AC electric field. We term this phenomenon “self-rotation” of cells, and note that this phenomenon was theoretically predicted more than 30 years ago.³⁶ However, since this phenomenon is rarely observed, some researchers argued its existence. We will show here, using appropriately designed electrodes in a simple microfluidic device, self-rotation of selected cells can be induced repeatedly and controllably. We envision that this phenomenon can be applied to develop new technologies to rapidly and non-intrusively determine electrical properties of cells by establishing a rotation spectrum, i.e., the rotational speed as a function of the electric field frequency. Then, by analyzing the peaks of the frequency spectrum, the cell properties such as membrane capacitance and cytoplasmic conductivity can be estimated. Other potential applications include real-time identification and separation of specific cells by implementing custom-designed microfluidic devices and computer-vision algorithms to differentiate non-rotating cells and rotating cells, as well as cells with different rotational speed and axes.

In our previous work,³⁷ we have demonstrated the self-rotation phenomenon with pigmented cells (Melan-A) under a positive DEP force without using a rotating electrical field. The basic behavior of cells with respect to different DEP parameters was described. The conditions to induce the self-rotation phenomenon in the pigmented cells were discussed. The experimental results using both pigmented and non-pigmented cells (which did not rotate under a non-rotating electric field) were compared. Finally, the voltage and frequency dependence of the rotation speed of the Melan-A cells was demonstrated. As a continuation of that research, we herein report our recent results that correlate the rotation speed and the melanin content of the melanocytes, as well as our further experiments to induce self-rotation in cells that do not originally exhibit any self-rotation—we demonstrated experimentally that macrophages can also exhibit self-rotation after ingesting synthetic melanin or microbeads, i.e., after they become artificially “pigmented.”

II. THEORY

The first investigations using the DEP force as a tool for cell manipulations was by Pohl in 1951.³⁸ He observed the relative motion of a suspensoid in a medium due to polarization forces produced by an inhomogeneous electric field and defined the term “dielectrophoresis.” The DEP force is generated from a non-uniform electric field. When a polarized object is under a non-uniform electric field, a dipole moment is induced and the object will move towards the maxima or the minima of the electric field depending on its polarizability relative to the medium.³⁹ According to electromagnetic theory, the DEP force acting on a spherical particle, such as cells suspended in fluids is given by¹

$$F_{DEP} = 2\pi\epsilon_m R^3 \operatorname{Re}[K(\omega)] \nabla |E_{rms}|^2, \quad (1)$$

where R is the radius of the particle, E_{rms} is the root mean square (rms) value of the electric field, and $\operatorname{Re}[K(\omega)]$ is the real part of the Clausius-Mossotti (CM) factor, which is related to the dielectric constant of the particle and indicates the relative magnitude and direction of the force experienced by the particle. For single shell model, where cells are modeled as spherical particles, the shell and interior refer to the cell membrane and cytoplasm, respectively. The effective permittivity for this model is given by¹

$$\epsilon_p^* = \epsilon_m R \frac{j\omega\tau_c + 1}{j\omega(\tau_c + \tau_m) + 1}, \quad (2)$$

where R is the radius of the cells, $\tau_c = \epsilon_c / \sigma_c$, and $\tau_m = C_{mem} R / \sigma_c$. The subscripts c and mem represent the cell cytoplasm and membrane, respectively. C_{mem} is the capacitance of the cell membrane. The real part of the CM factor is expressed as¹

$$\text{Re}[K(\omega)] = \text{Re} \left[-\frac{\omega^2(\tau_1\tau_m - \tau_c\tau'_m) + j\omega(\tau'_m - \tau_1 - \tau_m) - 1}{\omega^2(\tau_c\tau'_m + 2\tau_1\tau_m) - j\omega(\tau'_m + 2\tau_1 + \tau_m) - 2} \right], \quad (3)$$

where $\tau_1 = \varepsilon_m/\sigma_m$ and $\tau'_m = C_{mem}R/\sigma_m$.

III. MATERIALS AND METHODS

A. Electrode design

As shown in Fig. 1(a), the microelectrodes used in the experiments described in this paper were four probe electrodes that were orthogonal to each other. The detailed fabrication procedure for the electrodes is presented in Sec. III B. The width of each electrode was $150\ \mu\text{m}$ with a semi-circular tip and the distance between the centerline of the neighboring tips was $200\ \mu\text{m}$. We noted that during experiments, no voltage with phase difference is applied among the quadrupole electrodes, hence the DEP force field created by this electrode pattern does not generate any *rotating* electrical/force field in the 2D electrode plane.

B. Fabrication of microfluidic chips

A sealed microfluidic chip (Fig. 1(b)), which was composed of an irreversibly bonded glass substrate (Sail Brand, China) to a polydimethylsiloxane (PDMS) microchannel layer, was fabricated to perform the cell rotation experiments. A $500\ \text{\AA}$ thin film of Cr, as an adhesion layer, followed by a $3000\ \text{\AA}$ thick layer of Au was deposited on the glass substrate by sputtering and the metal microelectrode was patterned using standard micro-photolithographic and wet chemical etching processes. A PDMS pre-polymer (SYLGARD 184 Silicone Elastomer Kit, Dow Corning, MI, USA) was mixed with its curing agent in the volume ratio of 10:1. This PDMS was patterned with a microchannel that was casted using a negative mold and peeled off after curing. The negative mold was fabricated by patterning a $500\ \mu\text{m}$ thick photoresist (SU-8; MicroChem Corp., MA, USA) onto a poly(methyl methacrylate) (PMMA) substrate. Then, a permanent bond was formed between the PDMS layer and the glass surface by exposing them to an oxygen plasma discharge. Finally, interconnections were inserted into the PDMS layer and served as an inlet and outlet. During experiments, a function generator (HP 8111A, CA, USA) was used to apply a sinusoidal AC signal between appropriate electrodes. A syringe was used to introduce the appropriate cell solution (i.e., Melan-A or macrophages in a concentration of 10^5 cells/ml in a $0.2\ \text{M}$ sucrose solution) into the microchannel. The cell movement was observed via an optical microscope (JVC KY-F55B, NJ, USA).

C. Cell preparation

Murine Melan-A cells (provided by the courtesy of Prof. Dorothy C Bennett, St George Hospital Medical School, London, UK) were cultured in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS), $100\ \mu\text{g/ml}$ streptomycin, $100\ \text{IU/ml}$ penicillin, and $200\ \text{nM}$

FIG. 1. Illustration of the microfluidic chip. (a) Schematic of the microelectrode pattern used for DEP manipulation of cells. Electrodes with the same label (i.e., *a* or *b*) were short-circuited to the same pole of the AC electric field. (b) A photo of the microfluidic chip. The red dashed line indicates the position of the $500\ \mu\text{m}$ wide microchannel.

12-O-tetradecanoylphorbol-13-acetate (TPA). A549 cells (CCL-185, ATCC, VA, USA), which is a human epithelial carcinoma cell line, were cultured in Ham F12K medium supplemented with 10% FBS, 100 $\mu\text{g/ml}$ streptomycin, and 100 IU/ml penicillin at 37 °C in a humidified atmosphere of 5% CO_2 . The Murine B16 melanoma cell line (NBL6323, ATCC, VA, USA), HaCaT cell line (provided courtesy of Prof. Petra Boukamp, German Cancer Research Center, Germany), and murine RAW 264.7 macrophage line (TIB-71, ATCC, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% FBS, 100 $\mu\text{g/ml}$ streptomycin, and 100 IU/ml penicillin at 37 °C in a humidified atmosphere of 5% CO_2 . Unless specified, the cells used in the analysis were restricted to 30 passages. Polystyrene latex beads (LB-11, Sigma, MI, USA) with mean diameter of 1.1 μm and synthetic melanin (M8631, Sigma, MI, USA) were resuspended in culture medium and co-cultured with RAW264.7 cells, respectively. After several hours (i.e., 2–6 h), the beads or melanin granules were phagocytosed by RAW 264.7 cells.

IV. RESULTS

A. Numerical simulation

The finite element method (FEM) was used to numerically simulate the DEP field using a commercial software package (COMSOL Multiphysics, Sweden). Using the COMSOL time-harmonic analysis module and the assumption of a three-dimensional quasi-static current field, Maxwell's equations for a model of the liquid chamber of the DEP chip were solved to simulate the electrical field and DEP force field distribution therein (Figs. 2(a)–2(c)). Single shell model aforementioned was adopted, where cells were approximated as spherical particles. This

FIG. 2. Simulation of the DEP force and the electrical field exerted on a cell with a 5 μm diameter using the orthogonal electrodes. (a) DEP force field distribution. Red arrows indicate the DEP force vectors, and the surface color indicates the norm of electrical field magnitude at the surface of the electrodes. (b) 2D distribution of the cross-sectional view between two neighboring electrodes. Red arrows indicate the DEP force vectors, and the surface color indicates the cross-sectional distribution of the x-component of the vector ∇E_{rms}^2 . (c) AC electrical field distribution. Red arrows indicate the electrical field vectors. (d) Comparison of the magnitude of three main AC electrokinetics forces and force due to Brownian motion exerted on the cell as a function of the applied frequency.

assumption is reasonable for our cell rotation experiments, since all the cells were harvested from the culture flask by trypsinization first and then resuspended in the solution, where their irregular morphological appearance became very close to spherical. The cell diameter was set as $5\text{ }\mu\text{m}$, which is based on the approximation of Melan-A cells we observed. The liquid conductivity was set as 0.37 mS/m , which is the same as experimental measured data. Simulation results of the electric field distribution and the DEP force field exerted on a single cell using the orthogonal electrodes are shown in Figs. 2(a) and 2(b). By applying a frequency of 22 MHz and a voltage of 16 V to the orthogonal electrodes, the cells were attracted to the substrate surface due to the positive DEP force. As shown in the simulation result of electrical field distribution (Fig. 2(c)), the maximum electric field occurs at the hemispherical edge of the electrode tips, making it easier to trap cells in this region. If there are a sufficient number of cells available in the solution, two parallel chains of cells will be formed, connecting the neighboring electrodes. For the given electrode geometry and applied DEP parameters, a linearly polarized AC electrical field was generated. Hence, this confirms that there was no existence of a rotating electrical field.

In addition, we compared the magnitude of the four types of forces exerted on the cells in the microchannel, i.e., the DEP, AC-induced electrothermal (ACET) flow, AC-induced electro-osmosis (ACEO), and Brownian motion. As shown in Fig. 2(d), the DEP force is approximately three, five, and sixteen to nineteen orders of magnitude larger than the ACET, Brownian motion, and ACEO, respectively, from 1 MHz to 100 MHz . This indicates that DEP force is the dominant force during cell manipulation in the $500\text{ }\mu\text{m}$ wide microchannel within the frequency range applied for cell rotation (i.e., 10 MHz to 22 MHz). The equations used to perform the simulation are summarized in Table I. For Brownian motion, since the temperature increase due to the ACET effect was negligible ($\sim 3\text{ K}$), the average room temperature (i.e., $T = 300\text{ K}$) was used for the calculation.

B. Self-rotation behavior of pigment cells from different passages

The Melan-A cells used in the experiments are immortalized murine melanocytes originally with pigment granules in their cytoplasm. However, the melanin content decreases at high passages and the cells become lighter in color. The repeatable and controllable self-rotation was only observed in the motion of the pigmented cells, as opposed to the non-pigmented cells (such as A549 and HaCaT cells). This implied that the existence of the melanin in the cell might contribute to this rotation phenomenon. Based on this hypothesis, it is reasonable to assume that differences in the melanin content will also be reflected on the rotation speed. Therefore, experiments were designed to compare the rotation speed of pigmented cells that are of the same species but from different total number of passages, i.e., passage 28 (P28) and passage 44 (P44).

TABLE I. Equations describing the AC electrokinetics forces exerted on the cells.^a

Forces	Governing equations ⁴⁰
DEP	$2\pi\epsilon_m R^3 \text{Re}[K(\omega)] \nabla E_{rms} ^2$
ACEO	$1/8\epsilon_m V^2 \Omega^2 / \eta R / (1 + \Omega^2)^2 f_r$
ACET	$0.5\epsilon_m [(\alpha - \beta)(\nabla T \cdot E)E^* / (1 + (\omega\tau)^2) - 0.5\alpha E ^2 \nabla T]$
Brownian	$(K_B \cdot T \cdot \pi^{-1} \cdot R^{-1} \cdot \eta^{-1} / 3t)^{1/2}$

^aHere, ϵ_m is the permittivity of the medium, R is the radii of the cells, $K(\omega)$ is the CM factor, ω is the angular frequency of the AC power, E is the electric field, V is the amplitude of the AC power, and η is the dynamic viscosity of the medium. Ω is the non-dimensional frequency, expressed as $\pi\omega\epsilon_m R \lambda_d / 2\sigma$. λ_d is the thickness of the electrical double layer and is 25 nm when the medium conductivity, i.e., σ , is $1 \times 10^{-3}\text{ S/m}$. f_r is the friction factor of the cell in the medium, defined as $6\pi\eta R$. T is the temperature of the medium, $\tau = \epsilon_m / \sigma_m$, K_B is the Boltzmann constant, and V is the volume of the cells. For DI water at room temperature, approximately, $k = 0.6\text{ J}\cdot\text{m}^{-1}\text{ s}^{-1}\cdot\text{K}^{-1}$, $\rho_m = 1\text{ g}\cdot\text{cm}^{-3}$, $\alpha = -0.4\%\text{ K}^{-1}$, $\beta = 2\%\text{ K}^{-1}$, and $\partial\rho_m/\partial T/\rho_m = 10^{-4}\text{ K}^{-1}$.

Under an optical microscope, P28 cells appeared to be black since they contained a larger amount of melanin, while P44 cells were almost pale as they contained little melanin. During experiments, it was found that both P28 and P44 cells exhibited a strong attractive motion towards the microelectrodes when an electric field at 16 V and 22 MHz was applied. Also, both passages of cells exhibited self-rotation. However, the rotation of P28 cells was consistent even after repeatedly switching between a sine wave to a square wave with 10 s for each waveform cycle, for the total duration of 5 min. However, the rotation of P44 cells stopped after approximately 1 min and could not be repeated under the same experimental conditions. Although the ability of Melan-A cells to self-rotate gradually diminished at higher passages, the basic response of these higher passage cells in the presence of an AC electrical field were still quite different from that of other non-pigmented cells, e.g., HaCaT cells. The reason is that the behavior of these cells, either observed as an attraction towards or a repulsion away from the microelectrodes, were primarily determined by the physical and dielectric properties of the cells instead of the melanin content. Specifically, the existence and amount of melanin in the cytoplasm did not noticeably affect these basic cell responses towards applied DEP force field. The differences in the observed behavior of low passage pigmented cells, high passage pigmented cells, and non-pigmented cells are summarized in Table II for P28 Melan-A cells, P44 Melan-A cells, and HaCaT cells, respectively.

C. Artificially—induced cell rotation

The results discussed above suggest that the existence of melanin is the key factor to cause self-rotation phenomenon in the cell. However, it remains unclear whether the interaction of a unique electronic property of the melanin resulted in the rotation, or if the distribution of melanin inside the cell disturbed its mass balance. In order to further explore the role that melanin played in the self-rotation phenomenon of pigmented cells, experiments were conducted to determine whether it was possible to induce self-rotation in cells that do not normally exhibit self-rotation in the presence of an AC electric field.

Macrophages are cells that can ingest foreign bodies (e.g., microparticles) via phagocytosis. The same aforementioned experimental setup and DEP parameters were used to test the behavior of macrophages and no rotation of these cells was observed. Next, the macrophages were fed with either synthetic melanin or latex particles and were re-collected after a few hours. These foreign substances entered the macrophages via phagocytosis and were clearly visible under the microscope, as shown in Fig. 3(a). In this group of photographs, the macrophages were transparent in appearance prior to ingesting any foreign substances (i.e., 0 h). As time elapsed, the grey area inside the cells slowly became darker, which was caused by the accumulation of melanin inside the macrophages. The grayscale intensity of this area corresponded to the amount of melanin and latex beads ingested via phagocytosis, as established in Fig. 3(b). Since melanin is darker in color than the latex beads, the grayscale intensity level for melanin was higher after ingestion. In addition, the macrophages after phagocytosis exhibited self-rotation in the presence of a DEP field. This behavior was very similar to that of the Melan-A cells. Therefore, we have demonstrated that this self-rotation phenomenon can be artificially induced.

TABLE II. A comparison of the self-rotation of pigmented cells from different passages and non-pigmented cells.

	Basic response to the DEP field	Self-rotation	Remarks
Pigmented cells (Melan-A P28, B16)	Strong	Yes	Repeatable, controllable
Pigmented cells with reduced melanin (Melan-A P44)	Strong, Similar to Melan-A P28 cells	Yes	Unrepeatable
Non-pigmented cells (HaCaT, A549, macrophage)	Weaker than pigmented cells	Rarely observed	If any, unrepeatable, slower, and susceptible to flow

FIG. 3. Macrophages with melanin or latex beads ingested. (a) Photograph of cells experiencing a positive DEP force and attracted to the edge of the microelectrodes. For each group, i.e., melanin or latex beads, the phagocytosis duration was 0 h, 3 h, and 6 h, respectively. (b) The grayscale intensity of the ingested area after different durations. For 0 h, the transparent area in the center of the macrophages was used as a reference to measure the intensity; for photos after 3 h and 6 h, the area where melanin/latex accumulated was used to measure the intensity. Each data point is presented as a mean value $\pm$ SD ($n=3$).

D. Quantification of the cell rotation velocity

1. Dependence of rotation speed and pigmentation

As reported in our previous work,³⁷ pigmented cells would rotate under a sufficient positive DEP force. It was further demonstrated that changing certain DEP parameters could lead to variations in the rotation speed of the cells, i.e., the self-rotation speed was controllable in a repeatable manner. Cell rotation videos were examined frame by frame to estimate the rotation speed of the cells using the following equation:

$$S = \frac{5f}{\sum_{n=1}^5 R_n} \times 60, \quad (4)$$

where S represents the rotation speed with the unit of rpm; f represents the frame rate, which is 25 fps in this case; and R represents the frames needed for a single cell to complete one revolution. For each cell under the same condition, 5 consecutive revolutions were examined to get the average value of the rotation speed. Furthermore, experiments were conducted to verify the voltage and frequency dependency of the cell rotation speed using different waveforms (Figs. 4(a) and 4(b)). The rotation speed increased with increases in the frequency and/or voltage. Also, for both of these aforementioned DEP parameters, a square wave delivered the highest rotation speed followed by a sine wave and a triangular wave. This difference between waveforms became more significant at high frequencies and voltages. This phenomenon could be explained by the fact that a square wave is composed of the infinite number of harmonics. This can be verified by a fast Fourier transformation (FFT), which will show that a square wave is expressed as an infinite series of sinusoidal waves. Therefore, comparing sine and square waveforms at the same frequency, the latter one contains many higher frequency components other than the designated frequency of the wave.⁴¹

2. Characterization of the rotation speed of macrophages

The self-rotation speed of macrophages that had ingested foreign objects was also analyzed using the same image-based estimation method as with Melan-A cells. A square wave was applied during these experiments. For both groups, macrophages with a longer phagocytosis duration exhibited slower self-rotation speeds (Fig. 5(a)), i.e., we have tested phagocytosis duration of 2, 3, 5, and 6 h. Since we had previously demonstrated that the melanin/latex beads content would become higher over time (Fig. 3(b)), it is reasonable to assume that this decrease of

FIG. 4. Quantification of the self-rotation speed with respect to DEP parameters. (a) The voltage dependence as the frequency was kept constant at 22 MHz. (b) The frequency dependence as the voltage was kept constant at 16 V. In both figures, each data point is presented as a mean value $\pm$ SD ($n=3$).

rotation speed is directly related to the number of foreign particles. One possible explanation might be that the particles have a higher probability of becoming evenly distributed in the macrophages due to the larger amount ingested by the macrophages given the longer time. Hence, the longer phagocytosis duration may decrease the initial imbalance in the mass distribution in the macrophages. Or, the other explanation could be that the overall mass of the macrophages have increased, so given the same input energy for rotation, their rotational torque have

FIG. 5. Measurement of the self-rotation speed for macrophages that had ingested either synthetic melanin or latex beads. (a) Phagocytosis duration versus rotation speed. The macrophages were allowed to engulf the particles for 2 h, 3 h, 5 h, and 6 h, respectively. (b) Frequency dependence of the rotation rate of macrophages after 2 h of phagocytosis as compared against P28 Melan-A cells. The voltage was kept constant at 16 V. (c) Voltage dependence of the rotation rate of macrophages after 2 h of phagocytosis as compared against P28 Melan-A cells. The frequency was kept constant at 22 MHz. In all three figures, each data point is presented as mean value $\pm$ SD ($n=3$).

decreased. Moreover, the rotation speeds of the macrophages with melanin and with latex beads were similar as no distinctive difference between the two curves was observed.

Other than the duration of phagocytosis, the rotation speed of these macrophages could be varied by controlling the DEP parameters in a similar manner to the pigmented cells. In Figs. 5(b) and 5(c), we compared the frequency and voltage dependency of macrophage rotation after 2 h of phagocytosis and in Melan-A cells with both under an applied sine wave. The macrophages exhibited similar trends to the Melan-A cells since their rotation speed decreased under lower voltages and frequencies. However, the rotation speed of these macrophages was typically lower than the Melan-A cells for the same DEP parameters. Considering the fact that macrophages after 2 h of phagocytosis showed the fastest rotation among all the macrophage groups, we can conclude that artificially induced self-rotation is feasible, but the rotation speed varies depending on the dielectric property of different cells. In addition, the rotation speeds of the macrophages with melanin and with latex beads were similar under the same input voltage magnitude (Fig. 5(c)), as no distinctive difference between the two curves was observed. However, the rotation speeds of the macrophages with melanin and with latex beads do have noticeably different dependence on applied electric field frequency (Fig. 5(b)), which is a further indication that the dielectric property of cytoplasm of cells is important in determining the self-rotation properties of cells.

3. Axis of rotation

In addition to the rotation speed, a study of the axis of rotation of pigmented cells and rotating macrophages may prove insightful. Collecting the video sequences of cells with distinct rotation information, the axis of rotation was determined manually via visual inspection. In a right-hand-rule coordinate frame as defined in Fig. 6, for Melan-A cells, we observed that approximately 86% (37/43) of the cells rotated counter-clockwise on the surface and along the edges of the electrode (i.e., on the x - y plane), which makes the rotation axis perpendicular to the electrode surface (i.e., along the z -axis). For the rest of the approximately 13% (6/43) of the cells, the rotation axis was parallel to the electrode surface (i.e., parallel to the x - y plane). However, for macrophages ingested with melanin/latex beads, approximately 92% (48/52) of the rotating cells were observed on the edge of the electrodes and perpendicular to the chip surface, with the rotation axis parallel to the electrode surface (i.e., parallel to the x - y plane). For

FIG. 6. Illustration of the axis of rotation for different types of cells in the DEP force field. The black arrows through the cells indicate the rotation axes, which were determined by the right-hand rule based on the direction of the cell rotation. In this illustration, the rotation axis of the pigmented cell is along the z -axis; the rotation axes of the macrophages with ingested melanin/latex beads are parallel to the x - y plane. Also, the non-pigmented cell and macrophage prior to phagocytosis are not rotating.

the rest of the observed melanin-/latex beads-ingested macrophages, cell rotations were irregular and lacked consistent rotational axis. The mechanism behind this difference in rotational axis is unclear and remains under investigation. However, we suspect that it is related to the location of the melanin/latex beads inside of the cell and their distribution around the center of mass.

V. DISCUSSION

A. Self-rotation vs. ROT

To the best of our knowledge, all previous cell rotation studies in the literature were mostly focused on ROT. The self-rotation phenomenon reported in this paper is different from traditional ROT in three important aspects. First, there was no rotational electrical field in our experimental design. Second, ROT typically requires the cells to be suspended in the solution and is usually accompanied with translational movement, unless the electrode design restricts the translational motion of the cells, such as when using a quadrupole electrode pattern with a DEP phase difference at each electrode. In our study, the rotation of the cells typically occurred at a fixed location after they were trapped on the electrodes or had formed a chain of cells between electrodes. No translational movement was observed while the cells were rotating. Finally, some ROT examples were reported based on coupled cells in a DEP force field; however, the self-rotation observed in this study does not rely on contact with neighbouring cells. Single cells were also able to rotate on the edge of the electrode where the DEP force was a maximum.

B. The mechanism behind the self-rotation phenomenon

Despite the efforts made to connect the existence of melanin with the self-rotation phenomenon and to elucidate the fundamental mechanism for cell self-rotation by comparing the rotation behaviour of cell species with and without natural pigment and cells with artificial pigment (i.e., macrophages with ingested melanin/latex beads), the mechanism responsible for the self-rotation still remains unclear. One possible explanation is that given the quadrupole electrode design with its polarity symmetry, the relatively high frequency, and the quantity of cells,⁴² the Maxwell force was most likely to be tangential and only exists at the bottom or side surface of the cells. As a result of the DEP force field, the cells were settled gravitationally and the high frequency field penetrated only the low conductivity/permittivity lipid bilayer of the part close to the electrodes and produced a large tangential component there. Such tangential force on one localized surface position imparted torque and generated rotation. The variation of rotational axes may due to the asymmetry of cell shape and cell orientation relative to the field with the latter factor being sensitive to the distribution of ingested particles. A summary of the experimental observations for various types of cells under a non-rotating electric field is provided in Table III. However, we have shown that the existence of melanin played a significant role in inducing self-rotation of cells. One of the unique properties of the melanin is that it is semi-conductive,^{43–45} which leads to our speculation of whether the electrical property of melanin is the reason for the rotation. However, consider the macrophages that ingested latex beads, which do not have the same semiconducting property as melanin—these cells exhibited a very similar rotation behaviour as the macrophages, which ingested melanin, both in terms of rotation speed and rotation axis. Hence, it is reasonable to conclude that the observed self-rotation phenomenon is more likely to be caused by the mass distribution rather than the unique electrical property of melanin granules.

C. Data analysis limitations and solutions

The rotation speed and rotation axis were estimated by visually examining the recorded video data frame by frame, which was labor intensive, time consuming, and researcher-skill dependent. Issues such as the optical transparency of the cell itself, the inconsistency of the melanin distribution within each cell, the variation in the background contrast (e.g., in some cases, half of a cell was contrasted against the brighter Au electrode surface while the other half was against the darker area between electrodes), and the layering of cells when the total

TABLE III. Summary of the self-rotation phenomenon observed in various types of cells.

Cell type	Cell name	Cell rotation
Non-pigmented cells	A549 Alveolar epithelial cell line	Rarely
	HaCaT Keratinocyte cell line	
Pigmented cells	Melan-A melanocytes	Repeatable, controllable
	B16	
Macrophages	Macrophages	None
	Macrophages + melanin	Repeatable, controllable
	Macrophages + latex beads	

number of cells in the microchannel was large, all contributed to the challenge of efficiently and effectively obtaining useful rotation information. Therefore, it is desirable to pursue a faster and more systematic computer-vision-based approach for data analysis. We have made some initial progress in implementing a hybrid optical flow and template block-matching algorithm to automatically calculate the rotation speed of the pigmented cells.⁴⁶ These calculated rotation results matched data from the visual inspection methods very well. Current efforts also focus on improving the accuracy of automatically measuring the rotation speed using different types of cell models, as well as the development of an algorithm for accurate identification and tracking of the axis of rotation.

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