Analysis of the dielectrophoretic properties of cells using the isomotive AC electric field

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Various microfluidic devices utilizing the principle of dielectrophoresis (DEP) have been developed to separate, concentrate, and characterize biological cells; however, their performance is still limited by a lack of quantitative characterization. We addressed this limitation by employing a method capable of accurately quantifying a cell's response to an imposed field gradient. In this study, a simple method using a newly designed Creek-gap electrode was proposed, and the electrokinetic behavior of cells was characterized by DEP velocimetry under the exposure of an approximately constant gradient of electric field square established along the gap of the electrodes. Together with the numerical prediction of the electric field based on three-dimensional electric field analysis, the magnitude of DEP forces and the real part of the Clausius-Mossotti factor of cells were deduced from their movement. Results demonstrated that the proposed method was applicable to the determination of the dielectrophoretic properties of cells. Published by AIP Publishing. <https://doi.org/10.1063/1.5031054>

I. INTRODUCTION

In microfluidics, dielectrophoretic (DEP) force has been widely used to separate, concentrate, and characterize cells due to electrostatic interactions^{1–3} and its localized nature.⁴ DEP relies on induced polarization effects on cells subjected to a nonuniform AC electric field. The polarization effect occurs due to differences in the dielectric properties of cells, and the resultant behavior of cells varies considerably depending on the field frequency. Thus, these unique characteristics of DEP have been utilized for, in particular, differentiation of subpopulations of cells, including live and dead cells,⁵ breast cancer sublines,⁶ circulating tumor cells,⁷ healthy and diseased RBCs,⁸ and differentiation of subpopulations of cells.^{9–11} Even though most DEP applications have focused primarily on the discrimination of cells, there is still undeveloped potential in the application of DEP technology to high-throughput analytical microdevices.

DEP techniques can measure the response of single cells to the electric field. This allows measurement of individual cells without interference from other cells or the cell aggregates surrounding them. One of the important characteristics of DEP is that by measuring the magnitude and direction of cell velocities over a range of field frequency, the dielectric properties of the cells can be extracted and used for cell analysis.

Currently, the most prevalent DEP cell-separation devices use a parallel-plate type flow channel. Because the magnitude of the DEP force is proportional to the gradient of the electric field square (∇E^2), the DEP force induced in the parallel-plate channel is largest at the edges of electrodes and decays exponentially with distance from the electrode surface. The fact that the induced DEP force is spatially nonuniform is, conversely, a primary disadvantage of current dielectrophoretic systems from the viewpoint of cell analysis. Certainly, nonuniform forces are suitable for separation applications; however, a constant DEP force field would enable

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straightforward analysis of cell movement by which DEP properties of cells could be determined. For analytical purposes, it would be preferable if the ∇E^2 could take a constant value throughout the regions of cell analysis. The concept of this type of electric field was first suggested by Pohl^{12,13} and was named the isomotive electric field.

In the previous studies, several designs have been proposed to distribute DEP forces more effectively, so that the cells undergo constant movement,^{14–17} although analysis was restricted to specific channel regions, and some cases, required extremely high-voltage applications that could affect cell viability. More recently, an isomotive electrode design was proposed by Allen *et al.*¹⁸ In their study, the analytically obtained geometry of electrode following the idea of Pohl¹² was utilized to produce a uniform DEP force in two different types of DEP microchannels: an electrode-based and an insulator-based DEP channels. To address the issue, we have developed an analytically approximated DEP device capable of measuring the dielectrophoretic properties of individual cells. The design of Creek-gap electrode was derived originally from the approximate solution of the definition of DEP force; $\nabla E^2 = \text{constant}$, which is quite different from the results of Allen *et al.*¹⁸ and Pohl¹² in both the functional form of the mathematical function of the electrode shape and the mathematical derivations. Advantageously, the Creek-gap electrode does not need a 120° bend angle of the channel such as the previous studies did to ensure the accurate isomotive motion of particles^{12,18} though the rigorous uniformity of the DEP force along the centerline of the electrode might be compromised in the Creek-gap electrode due to the approximation. The dielectrophoretic properties of cells were deduced from their uniform linear-motion going through a newly designed Creek-gap electrode, where the gradient of field square (∇E^2) was practically constant. In the examination area of the Creek-gap electrode, cells traveled straightly along the centerline of the electrode with a constant velocity, making it simpler and easier than ever to track the cells motion. The derivation of the basic principle of the method as well as the fabricated prototype that demonstrates its feasibility are discussed here, along with the sensitivity analyses of model parameters.

II. EXPERIMENTAL SETUP AND METHOD

A. Principle of measurement

As shown in Fig. 1(a), we consider the situation in which a single spherical cell with a diameter d , and mass density ρ_c is immersed in a suspension medium with a mass density ρ_f and is moving along the x -axis at velocity v , over the surface of a horizontal planar plate. The cell experiences the DEP force, F_{DEP} , in its direction of movement (x). In addition, the Stokes drag force due to the presence of the surrounding medium F_d and the friction force between the surface of the cell and plate F_f act on the cell in the direction opposite ($-x$) to its movement.

FIG. 1. (a) Balance of forces acting on a spherical cell moving at velocity v over a horizontal planar plate. Forces are DEP force, F_{DEP} , Stokes drag force, F_d , and friction force between the surface of the cell and plate, F_f . (b) Conceptual illustration of the Creek-gap electrode. In order to produce an electric field, E , such that ∇E^2 is constant along the x -axis, we designed the Creek-gap electrode consisting of a pair of electrodes with a creek-shaped gap shape.

Suppose that the DEP force and the sum of these two resistant forces are balanced and that the cell moves with the constant velocity v . In this case, the force balance equation is described as

$$F_{DEP} - F_d - F_f = 0. \quad (1)$$

Meanwhile, the DEP force F_{DEP} is described by

$$F_{DEP} = 2\pi\epsilon_0\epsilon_f\left(\frac{d}{2}\right)^3 \text{Re}(\beta)\nabla E^2, \quad (2)$$

where ϵ_0 and ϵ_f are the vacuum permittivity and relative dielectric permittivity of the suspension medium, respectively. The symbol ∇ is the Nabla operator, and E is the nonuniform AC electric field imposed in the x direction. The value $\text{Re}(\beta)$ is the real part of the Clausius-Mossotti (CM) factor, β ,¹⁹ and

$$\beta = \frac{\epsilon_c^* - \epsilon_f^*}{\epsilon_c^* + 2\epsilon_f^*} \quad (3)$$

between the cell and the suspension medium, ϵ_c is the relative dielectric permittivity of the cell, and the superscript “*” indicates that the value is a complex number. The complex value of permittivity is defined as

$$\epsilon^* = \epsilon - j\frac{\sigma}{\omega}, \quad (4)$$

where σ is the electric conductivity of materials, ω is the angular frequency, $\omega = 2\pi f$, and $j = \sqrt{-1}$. If the nonuniform AC electric field E has a distribution such that the value ∇E^2 in Eq. (2) takes a constant value in the x direction, the cell travels continuously with a constant velocity, v , and thus the $\text{Re}(\beta)$ can be evaluated using Eqs. (1) and (2) as

$$\text{Re}(\beta) = \frac{F_d + F_f}{2\pi\epsilon_0\epsilon_f\left(\frac{d}{2}\right)^3\nabla E^2}. \quad (5)$$

In Eq. (5), the Stokes drag force, F_d , acting on the cell moving over the plate with the velocity v can be obtained as²⁰

$$F_d = Kv \quad \left(k = 1.7 \times 6\pi\frac{d}{2}\eta\right), \quad (6)$$

where η is the viscosity of the suspension medium. Moreover, the friction force, F_f , acting between surface of the cell and the plate is given as

$$F_f = \frac{4}{3}\pi(\rho_c - \rho_f)\left(\frac{d}{2}\right)^3\mu g, \quad (7)$$

where μ and g are the coefficient of kinetic friction and the acceleration of gravity, respectively. Accordingly, when the mass densities of the cell, ρ_c , and suspension medium, ρ_f , are known, and the ∇E^2 takes a constant in the x direction, the value of $\text{Re}(\beta)$ can be readily evaluated by measuring the cell diameter, d , the cell velocity, v , and the coefficient of kinetic friction, η .

B. Design and characterization of Creek-gap electrode

The design concept of the Creek-gap electrode is illustrated in Fig. 1(b). In order to produce a nonuniform electric field, E , such that the value of ∇E^2 is constant along the centerline (x -axis) of the gap, a Creek-gap electrode was contrived consisting of a pair of planar thin

electrodes with a creek (mouth of a river)-like shape between them. The exact geometrical shape of the gap was determined as follows. Consider the electric field E along the x -axis passing through the gap center. Suppose that the value of ∇E^2 has a constant value, a , namely,

$$\nabla E^2 = a. \quad (8)$$

Because the Creek-gap electrode is symmetric about the centerline

$$\frac{\partial}{\partial y} E^2 = 0 \quad (9)$$

along the x -axis. Therefore, Eq. (8) can be reduced as follows:

$$\frac{\partial}{\partial x} E^2 = a. \quad (10)$$

Integration of both sides of Eq. (10) with respect to x yields the functional form of the electric field along the x -axis

$$|E| = \sqrt{ax + b}. \quad (11)$$

Here, the constant of integration, b , is determined by the boundary conditions. On the other hand, the electric field in the vertical direction across the gap can be approximated as

$$|E| \cong \frac{V}{2y}, \quad (12)$$

where $2y$ is the lateral gap separation at an arbitral position on the x -axis, and V is the difference of the applied AC voltage between the electrode pair. As discussed later, this assumption gives relatively good approximation to the condition $\nabla E^2 = \text{constant}$ along the centerline of Creek-gap except both the inlet and the outlet regions of the electrode gap. Accordingly, following the field approximation of Eq. (12), the shape of the Creek-gap electrode, y , can be expressed as a function of x for the AC voltage V between the electrodes

$$y = \frac{V}{2\sqrt{ax + b}}. \quad (13)$$

The Creek-gap electrode was produced using a standard photolithographic procedure. Briefly, a glass slide [75 mm (L) $\times$ 25 mm (W) $\times$ 0.7 mm (t)] was used as a substrate for the electrode. A thin aluminum film (300-nm thick) was used as the electrode material and vacuum-deposited on the glass substrate. The positive photoresist (S1805G, Rohm and Haas Electronic Materials) was spin-coated onto it, and then baked on a hot plate at 90 °C for 3 min. The resist layer was exposed to UV light through a positive mask image of the electrode patterns. The exposed photoresist was developed and baked on a hot plate at 130 °C for 3 min. The uncovered aluminum region was etched with a commercially available acid mixture (a mixture of phosphoric, acetic, and nitric acids) at 40 °C for 1.5 min. Finally, the photoresist was removed with AZ 100 remover (AZ Electronic Materials). The design and specification of the electrode substrate are shown in Fig. 2. The shape of the Creek-gap was specified using Eq. (13) with the following boundary conditions: $y = 1.0$ mm at $x = 0$ mm and $y = 50$ μ m at $x = 1.0$ mm. With these geometric specifications of the Creek-gap electrode, the constants in Eq. (13), a and b , were calculated as

$$\begin{cases} a = 1.0 \times 10^{11} V^2 & (\text{V}^2/\text{m}^3) \\ b = 2.5 \times 10^5 V^2 & (\text{V}^2/\text{m}^2) \end{cases}, \quad (14)$$

where V is the AC voltage to be applied across the pair electrodes. Thus, the shape of the Creek-gap electrode used in the experiment was expressed as

FIG. 2. The design and specification of the Creek-gap electrode. A glass slide vacuum deposited with a thin aluminum film was used as the electrode pattern. The shape of the Creek-gap was specified by Eq. (13) with the following boundary conditions: $y = 1.0 \text{ mm}$ at $x = 0 \text{ mm}$ and $y = 50 \text{ } \mu\text{m}$ at $x = 1.0 \text{ mm}$.

$$y = \frac{1}{\sqrt{1.0 \times 10^{11}x + 2.5 \times 10^5}}, \quad (15)$$

and the strength of the gradient of the field square was from Eqs. (9) and (10)

$$\frac{\partial}{\partial x} E^2 = 1.0 \times 10^{11} V^2, \quad \frac{\partial}{\partial y} E^2 = 0 \quad (V^2/m^3). \quad (16)$$

The electrode substrate was fabricated at the Semiconductor Center of Kitakyushu Foundation for the Advancement of Industry, Science and Technology.

C. Experimental setup and procedure

Figure 3 is an illustration of the experimental setup. The test section of the setup consisted of the electrode substrate on the bottom and a cover glass on the top, separated by a 0.1-mm gap created with a silicon-rubber spacer. The spacer had a 5-mm square hole at the center to hold the cell suspension. In order to suppress cell adsorption on the electrode substrate, the top surface of the substrate was coated with 0.5%(w/v) ethanol solution of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer (NOF Corporation, Lipidure[®]-CM5206) before the experiment. For the application of the AC voltage on the test section, a function generator (Tektronix, AFG3101) was used. To monitor the applied voltage, we used a digital oscilloscope (Tektronix, TDS2012B). The test section was placed on the stage of a phase-contrast inverted microscope (Olympus, CKX41) and connected to the function generator after the square hole in the spacer was filled with the cell suspension. A sinusoidal AC voltage of $V_{pp} = 20.0 \text{ V}$ peak-to-peak and

FIG. 3. Illustration of the experimental setup. The experimental test section consisted of the electrode substrate on the bottom and a cover glass on the top, separated by a 0.1-mm gap created using a silicon-rubber spacer. The spacer had a 5-mm square hole at the center to hold the cell suspension.

frequencies of $f=20$ and 40 kHz were applied. We confirmed that an input voltage of $V_{pp}=20.0\text{ V}$ could induce sufficient DEF forces on the cells to drive them without any problems of temperature increase or electrolysis. The behavior of cells was observed using the microscope and a CCD camera system (GR-D8T2), and video signals were sent to a personal computer for monitoring and recording.

D. Cell preparation

Human breast epithelial cells (MCF10A) were used in this study. MCF10A cells were cultured in DMEM/F12 Ham's Mixture (Thermo Fisher 1330032) supplemented with 5% horse serum (Thermo Fisher 16050122), 20 ng/ml of EGF (Thermo Fisher PHG0311L), $10\text{ }\mu\text{g/ml}$ of human insulin (Sigma I9278), 0.5 mg/ml of hydrocortisone (Sigma H0135), and 100 ng/ml of cholera toxin (Sigma C8052). Cells were fed twice a week and grown to confluence before sub-culturing. Cells grown to confluence were washed once with phosphate-buffered saline w/o calcium and magnesium [PBS(-)] (Nissui, 08190) and exposed to 0.05% trypsin (Sigma, T4799) and 0.02% EDTA (Sigma, E6758) for $15\text{--}20\text{ min}$ before the action of trypsin was stopped with the cell culture medium. Cells were then centrifuged at 600 rpm for 5 min , and the cell pellets were resuspended in the culture medium and transferred to other $\phi\text{ }35$ culture dishes. All cells were grown in a humidified incubator and maintained in a $37\text{ }^\circ\text{C}$, 5% CO_2 atmosphere.

The experiments were performed using cells with passage numbers of $8\text{--}10$. For the experiment, cultured cells were collected and centrifuged once, and the volume was replaced with an isotonic and non-electrolyte 300-mM mannitol solution (Sigma M4125). A suitable volume was then pipetted into the square hole of the test section. The cell diameter d , obtained experimentally, was $d = 17.5 \pm 0.4\text{ }\mu\text{m}$ (mean $\pm$ SE, $n=45$). The mass density of the cell was represented by that of the cytoplasm of MCF10A,²¹ as $\rho_c = 1.05 \times 10^3\text{ kg/m}^3$, since an exact measurement of single cellular mass density is very difficult.²² On the other hand, an equal value of mass density has been obtained for human lung cancer cell,²³ meaning that this value may give a reasonable value for the mass density of major cell types of soft tissues. The coefficient of kinetic friction between surfaces of the cell and the slide glass, μ , was determined by sliding down cells on the inclined slide glass without acceleration and then noting the tilted angle θ , using $\mu = \tan \theta$.

III. RESULTS AND DISCUSSION

A. Prediction of the electric field distribution

Prior to the experiment, numerical simulation of the electric field, E , induced in the gap of the pair electrodes was performed to predict the distribution of ∇E^2 along the centerline of the gap. The commercially available FEM software ANSYS Maxwell was used for the three-dimensional (3D) field analysis. Figure 4 shows (a) the numerical simulation model of the electrode substrate and (b) the computational grid system. The geometries and dimensions of the glass substrate, metal electrodes, and silicon rubber spacer of the model used for the numerical simulation were exactly the same as those used in the experimental test section.

For the suspension medium (300-mM mannitol solution), values of the electric conductivity, σ_f , and relative permittivity, ϵ_f , used in the numerical simulation were $\sigma_f = 40\text{ mS/m}$, and $\epsilon_f = 80.0$, respectively. An applied voltage of $V_{pp} = 20.0\text{ V}$ was assumed. For the computational grid system, the surface and tetrahedral elements were generated automatically using ANSYS meshing. Finer grids were further generated over the immediate periphery of the metal electrodes to ensure sufficient spatial resolution of the electric field. The number of the computational grids was increased until the simulation results converged satisfactory, and $n \sim 60\,000\,000$ FEM elements was the final number adopted for the numerical simulation.

Figure 5 shows (a) the contour plots of the electric field distribution and (b) profiles of the electric field strength $|E_{\text{rms}}|$, and the value of ∇E_{rms}^2 along the centerline (x -axis) of the gap at a height of $z = 8\text{ }\mu\text{m}$, which corresponds to the height of the center of the mass of the spherical cell from the top surface of the glass substrate. Here, the subscript "rms" means that values are

FIG. 4. (a) Numerical simulation model of the electrode substrate. (b) Computational grid system. The geometries, dimensions, and dielectric properties of materials of the simulation model were the same as those of the experimental test section.

root-mean-squares. As expected, the predicted value of ∇E_{rms}^2 was almost uniform along the centerline of the gap, except in regions on both the left- and right-side ends of the Creek-gap. In detail, the accuracy of the uniformity of the value of ∇E_{rms}^2 in the region between $x = 0.2 \text{ mm}$ and $x = 0.8 \text{ mm}$ in Fig. 5(b) is found to be $\nabla E_{\text{rms}}^2 = (2.20 \pm 0.17) \times 10^{12} \cdot \text{V}^2/\text{m}^3$ (MEAN $\pm$ SD). The predicted value of ∇E_{rms}^2 at the plateau in the central part of the profile ($x = 0.2\text{--}0.8 \text{ mm}$) was $\sim 44\%$ of the theoretically obtained value [equivalent to the value of the coefficient $a = 5.0 \times 10^{12} \text{ V}^2/\text{m}^3$ (rms value) in Eq. (10)]. This was because a 2-D approximation was adopted in the theoretical model. The approximated 2-D model overestimated the strength of ∇E_{rms}^2 by more than 2.3 times.

The profile of ∇E_{rms}^2 along the centerline was also examined at heights $z = 0 \mu\text{m}$ (bottom end of the spherical cell) and $z = 16 \mu\text{m}$ (top end of the spherical cell), and there was found to be almost no difference between them (not shown). Therefore, it could be assumed that the electric field had a uniform distribution, at least in the vertical direction, within the length scale of the cell diameter from the glass surface. Thus, the induction of a uniform DEP force was realized on cells placed on the centerline of the gap.

B. Cell velocity measurement

Figure 6 shows snapshots of the transient behavior of cells moving through the Creek-gap at times $t = 0, 10, 20$, and 30 s after the application of an AC voltage of $V_{\text{pp}} = 20.0 \text{ V}$ with a frequency $f = 20 \text{ kHz}$. Because the cells exhibited a negative DEP property (pushed away from the electrodes) in this range of the field frequency,⁵ all the cells began to move in the direction of the gap expansion (from right to left in Fig. 5) immediately after the AC electric field was applied and soon after continued to move at a constant velocity. Simultaneously, cells also began to form chain-like clusters growing in the lateral direction, and the clusters continued to move with their orientation perpendicular to the direction of movement without slowing down. The chain-like clusters were formed by the presence of dipole-dipole interactions working

FIG. 5. (a) Contour plots of the electric field distribution. (b) Profiles of the electric field strength, $|E_{\text{rms}}|$, and the value of ∇E_{rms}^2 along the centerline (x -axis) of the gap at a height of $z = 8 \mu\text{m}$.

FIG. 6. Snapshots of the transient behavior of cells moving through the Creek-gap at times $t = 0, 10, 20$, and 30 s after the application of an AC voltage of $V_{pp} = 20.0$ V with the frequency $f = 20$ kHz. The direction of cell movement is from right to left.

between neighboring cells in the direction parallel to the field lines [Fig. 1(b)]. It was also observed that the velocity of cells or clusters remained constant in the central part of the gap. However, cells decelerated gradually as they approached the left-side end of the gap, where the value of ∇E_{rms}^2 dropped rapidly (Fig. 5).

Measurement of the cell velocity was performed on cells moving within the central part of the Creek-gap, where the plateau of the profile of ∇E_{rms}^2 was created (Fig. 5) after cell velocity became constant. Cell velocity was calculated from the distance of cell movement and the time required to travel it. Figure 7 shows average cell velocities measured under the exposure of an AC voltage of $V_{pp} = 20.0$ V for two different field frequencies, $f = 20$ and 40 kHz, with error bars representing the standard error of the mean (mean $\pm$ SE). Number of cells measured was $n = 30$ for each frequency. Comparison of the two groups was performed using student's t test. A two-tailed P value of less than 0.005 was considered statistically significant and is indicated with the symbol “*.” Because the value $\text{Re}(\beta)$ at the frequency 20 kHz was more negative than that at 40 kHz,^{5,24} the DEP force induced on cells at 20 kHz was greater than that at 40 kHz, resulting in faster cell velocity at 20 kHz.

C. DEP force and real part of CM factor evaluation

Estimations of the DEP force induced on cells were $F_{DEP} (=F_d + F_f) = 0.76 \pm 0.05$ and 0.53 ± 0.04 pN for $f = 20$ and 40 kHz, respectively. The representative variables of the physical

FIG. 7. Cell velocities measured with the applied voltage of $V_{pp} = 20.0$ V for two different field frequencies, $f = 20$ and 40 kHz (mean $\pm$ SE). Error bars represent the standard error of the mean. The symbol “*” indicates $P < 0.005$.

properties of MCF10A used in the force calculation are listed in Table I. The order of the magnitude of DEP forces calculated in this study was similar to the previously reported values,⁸ and thus the method of DEP force measurement proposed here gave reasonable estimations of DEP force.

Figure 8 shows the relationship between the field frequency, f , and the real part of the CM factor, $\text{Re}(\beta)$. In the figure, closed circles with bars represent mean $\pm$ SE of the values of $\text{Re}(\beta)$ calculated using Eq. (5), and the solid curve represents the analytical prediction obtained from the modified form of β [Eq. (3)] for a single-shell model of the biological cell.¹⁹ The measured electric conductivity of the suspension medium, σ_f , was 40 mS/m, and the other physical properties used for the calculation are listed in Table I. The value of $\text{Re}(\beta)$ at $f=40$ kHz showed good agreement with the analytical prediction; however, $\text{Re}(\beta)$ at $f=20$ kHz underestimated the analytical prediction by 34.0%. This may come from the underestimation of the Stokes drag force acting on the cells. The dipole-dipole attractive interactions exerted between cells at frequency $f=20$ kHz was greater than at $f=40$ kHz, resulting in the formation of longer chain-like clusters. From the perspective of fluid mechanics, spherical particles forming a line cluster experience greater Stokes drag force than that they experience individually when in an isolated state.²⁵ This effect becomes more apparent when the length of cluster increases.

D. Sensitivity analyses

In order to evaluate how the value of $\text{Re}(\beta)$ is affected by small changes in individual model parameters (i.e., the field frequency f , cell mass density ρ_c , coefficient of kinetic friction μ , cell diameter d , and gradient of the field strength square ∇E_{rms}^2) separately, sensitivity analyses were performed. The sensitivity factor, p , is defined as the sensitivity of a variable, y , to small changes in an independent variable, x .²⁶

$$p(x) = \frac{dy/y}{dx/x}, \quad (17)$$

where dx is the small changes in the independent variable x , and dy is the associated changes in the variable y . In this study, the function y represents $\text{Re}(\beta)$ and is assumed to be a function of the variables mentioned above as follows:

$$y(=\text{Re}(\beta)) = F(f, x_1, \dots, x_i, \dots, x_N), \quad (18)$$

where x_i ($i=1-N$) is the i th member of N parameters. When considering values of $\text{Re}(\beta)$, an appropriate expression for dy/y is the relative rms deviation of the $\text{Re}(\beta)$ resulting from a small relative change dx/x in the dominant parameter of interest, x . Thus, the factor of the sensitivity of the $\text{Re}(\beta)$ with a small change dx in x_i at $x_i = x_{i0}$ is given as

TABLE I. Variables used in the analysis.

Variables	Values	Reference
Cell diameter	$d = 17.5 \times 10^{-6}$ m	21
Cell mass density	$\rho_c = 1.05 \times 10^3$ kg/m ³	
Medium mass-density	$\rho_f = 1.00 \times 10^3$ kg/m ³	
Viscosity of the medium	$\eta = 0.89 \times 10^{-3}$ Pa·s	
Coefficient of kinetic friction	$\mu = 0.18$ (=tan 10°)	
Gradient of E_{rms} square	$\nabla E_{\text{rms}}^2 = 2.20 \times 10^{12}$ V ² /m ³	
Vacuum permittivity	$\epsilon_0 = 8.85 \times 10^{-12}$ F/m	
Relative permittivity of medium	$\epsilon_f = 80.0$

FIG. 8. Relationship between f and $\text{Re}(\beta)$. Closed circles with bars represent mean $\pm$ SE calculated using Eq. (5), and the solid curve represents the analytical prediction. The electric conductivity of the suspension medium was $\sigma_f = 40$ mS/m, and the other physical properties used for the calculation are listed in Table I.

$$p(x_{i0}) = \frac{\sqrt{\frac{[F(f, x_1, \dots, x_{i0} + dx, \dots, x_N) - F(f, x_1, \dots, x_{i0} - dx, \dots, x_N)]^2}{F(f, x_1, \dots, x_{i0}, \dots, x_N)^2}}}{dx/x_{i0}}. \quad (19)$$

The value of sensitivity $p(x_i)$ to the one parameter x_i was calculated by keeping the other parameters constant. A large value of the sensitivity factor $p(x_i)$ means that the value of F [$=\text{Re}(\beta)$] was greatly altered in response to changes in parameter x_i at the given frequency f . Thus, a more accurate estimate of x_i can be derived from the optimization procedure of fitting the model to the experimental data. Figure 9 shows the sensitivity curves for $\text{Re}(\beta)$ parameters, calculated using Eq. (19). The range of variation for the mass density and cell diameter was set so as to encompass the previously reported values.²⁷ The sensitivity of each parameter was calculated with respect to the nominal values: $\rho_c = 1.05 \times 10^3$ kg/m³, $\mu = 0.18$ (tan 10°), $d = 17.5 \times 10^{-6}$ m, and $\nabla E_{\text{rms}}^2 = 2.20 \times 10^{12}$ V²/m³. The value of $\text{Re}(\beta)$ had a different sensitivity depending on different parameters. Results demonstrated that $\text{Re}(\beta)$ was the most sensitive to changes in the mass density of the cell. Moreover, the sensitivity of mass density varied dependently on frequency and was more sensitive at $f = 40$ kHz than at 20 kHz. Contrarily, the cell diameter was more sensitive at $f = 20$ kHz than at 40 kHz.

In cell separation process, the relative difference in the values of $\text{Re}(\beta)$ between different field frequencies is more important than the difference in absolute values of $\text{Re}(\beta)$. From this

FIG. 9. Sensitivity curves for various $\text{Re}(\beta)$ parameters calculated using Eq. (19).

point of view, the accuracy of the measurement of cell velocity and the appropriate modeling of the drag force is important rather than the correctness of the estimations of cell density and ∇E_{rms}^2 .

IV. CONCLUDING REMARKS

Accurate estimation of the real part of the Clausius-Mossotti (CM) factor, $\text{Re}(\beta)$, is crucial for the development and optimization of cell manipulation and separation devices utilizing dielectrophoretic (DEP) force. In this study, in order to estimate the $\text{Re}(\beta)$ value of cells exhibiting negative DEP in a simple and straightforward way, we proposed a Creek-gap electrode that enabled the creation of a practically constant DEP force in the direction of the cell movement. The velocity of moving human breast epithelial cells (MCF10A) exposed to a nonuniform AC electric field was measured, and the DEP force induced on cells was calculated using the equation of balance of forces acting on cells. The $\text{Re}(\beta)$ values of cells were estimated using calculated cell velocity and the numerically predicted value of the gradient of the field square.

The results demonstrated that the value of $\text{Re}(\beta)$ at the field frequency $f = 40$ kHz showed relatively good agreement with the analytically obtained value, while the value of $\text{Re}(\beta)$ at $f = 20$ kHz underestimated the analytical value by 38%. This may have been due to the model approximation of the Stokes drag force acting on clustered cells. We then performed sensitivity analyses of the $\text{Re}(\beta)$ value in response to the model parameters. Results demonstrated that $\text{Re}(\beta)$ showed different sensitivities to different parameters, and if the mass density of the cell, ρ_c , was precisely known, ρ_c would become a dominant model parameter constraining the accuracy of $\text{Re}(\beta)$.

So far we have described above, the Creek-gap electrode is easy to fabricate for its relatively simple geometry and structure and is favorable for analysis of negative DEP cells. However, it is hard to apply the proposed method to the analysis of positive DEP cells because positive DEP cells are captured at the electrode edges.²⁸ This problem will be resolved by, for instance, adopting an insulator-based Creek-gap whose concept of the device operation is similar to the isomotive DEP system¹⁸ using insulative microchannel.

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