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A Mathematical Model of Dielectrophoretic Data to Connect Measurements with Cell Properties

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Abstract

Dielectrophoresis (DEP) brings about the high-resolution separations of cells and other bioparticles arising from very subtle differences in their properties. However, an unanticipated limitation has arisen: difficulty in assignment of specific biological features which vary between two cell populations. This hampers the ability to interpret the significance of the variations. To realize the opportunities made possible by dielectrophoresis, the data and the diversity of structures found in cells and bioparticles must be linked. While the crossover frequency in DEP has been studied in-depth and exploited in applications using AC fields, less attention has been given when a DC field is present. Here, a new mathematical model of dielectrophoretic data is introduced which connects the physical properties of cells to specific elements of the data from potential- or time-varied DEP experiments. The slope of the data in either analysis is related to the electrokinetic mobility, while the potential at which capture initiates in potential-based analysis is related to both the electrokinetic and dielectrophoretic mobilities. These mobilities can be assigned to cellular properties for which values appear in the literature. Representative examples of high and low values of properties such as conductivity, zeta potential, and surface charge density for bacteria including *Streptococcus mutans*, *Rhodococcus erythropolis*, *Pasteurella multocida*, *Escherichia coli*, and *Staphylococcus aureus* are considered. While the many properties of a cell collapse into one or two features of data, for a well-vetted system the model can indicate the extent of dissimilarity. The influence of individual properties on the features of dielectrophoretic data is summarized, allowing for further interpretation of data.

Graphical Abstract

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CONFLICT OF INTEREST

Shannon Huey Hilton and Mark A. Hayes declare a conflict of interest with regards to Charlot Biosciences.

Keywords

Dielectrophoresis; electrophoresis; electrokinetic mobility; bacterial variations; biophysical properties; data modeling

INTRODUCTION

The ability to distinguish subtle biophysical differences in analytes is a major element in realizing the potential of dielectrophoresis (DEP). For cells, these differences are a result of mutations, gene expression levels, post-translational modifications, environmental effects, glycosylation variations, and lipid composition. They are difficult to assess, although tremendous efforts have been put forth to measure them. In bacterial cells, these physical variations can reflect anything from harmless, normal population variance to indications of transferred genes and possible acquisition of antibiotic resistance or an increase in virulence. Specific structures of the cells which are responsible for the physical changes have not yet been assigned to any quantitative changes in the dielectrophoretic data. Literature values for cellular properties are examined here for their effects on dielectrophoretic results, informing the qualitative features of dielectrophoretic data structures and defining reasonable values which can be expected.

Dielectrophoresis describes the motion of polarizable particles in the presence of electric field gradients. It was described and named by Herbert Pohl in 1951, although it was exploited before that time [1]. The phenomenon interfaces well with microfluidics, the advantages of which include small sample and reagent volume, portability, and often-fast analysis times, while bringing its own advantages, such as the ability to probe small differences in electrophysical properties of the cell through accurate and precise determination of electrokinetic and dielectrophoretic mobilities. In recent years, the study of

DEP has increased [2], encompassing many analytes ranging from DNA and proteins, to bacteria and yeast cells, to tumor and blood cells [3–16].

The theory of DEP has been well-developed by many researchers [17–19, 1, 20, 21]. Electrodebased DEP (eDEP) was originally used and continues to be implemented today. In eDEP, electrodes embedded into a device create the electric fields and gradients necessary for DEP to occur and particles to capture. The behavior of particles under applied AC fields and with varying frequencies has been extensively described [22, 23, 18, 1].

More recently, insulator-based DEP (iDEP) has emerged as an alternative to eDEP [24]. In iDEP, insulating features rather than patterned electrodes create the gradients in the electric field. Electrodes are placed farther away from these insulating features, often in an inlet and an outlet located at the two ends of a microfluidic channel.

The technique of iDEP allows for the use of DC fields, permitting electroosmosis and electrophoresis to be employed to move particles down a microchannel. While the forces in these systems are well known and many descriptive works for various analytes have appeared, less attention has been given to connecting the features of the dielectrophoretic data to the properties of the analytes.

Dielectrophoretic capture models have shown success in demonstrating experimental results closely matching predicted values [13]. Cells have been separated based on whether they exhibited positive or negative DEP at a given frequency, including mixtures of *E. coli* and *M. lysodeikticus*, and *S. cerevisiae* and *M. lysodeikticus* [9]. Cancer cells have also been separated from blood cells by trapping all cells under positive dielectrophoresis; the applied field was then adjusted to release blood cells, allowing the tumor cells to stay trapped [30]. With both eDEP and iDEP, AC fields have been used to assess the properties of cells and the frequency has often played a large role in this assessment. This technique has evaluated properties including the whole-cell membrane capacitance of neural stem cells [31], mitochondrial modifications [32], and the polysaccharides on the surface of cells for the characterization of blood types [33]. Using iDEP, live and dead *E. coli* cells have been separated [8]. Dielectrophoretic field-flow fractionation was used to separate circulating tumor cells from blood cells to an efficiency approaching 90% [5]. DEP has also been used to separate a mixture of two bacterial strains of the same species [34].

The fact that the physical properties of cells define their dielectrophoretic behavior is the underlying theme of these experiments. Differences in size, conductivity, surface charge, and other properties have been shown to result in different dielectrophoretic behaviors of particles [36–41]. Even slight differences in cells, such as the presence of a new enzyme in one bacterial strain versus another, can be discerned with a well-designed DEP system.

To help determine what types of physical changes lead to a given outcome in dielectrophoretic measurements, this paper will introduce and develop a new mathematical model of dielectrophoretic data. This model will aid in the connection between the biophysical properties of analyte, specifically bacteria, and the data obtained from DEP experiments. Common properties of cells and their effect on DEP behavior based on the model will also be discussed. The typical high and low values for important cellular

properties which have appeared in the literature are used to plot and quantify expected behaviors of cells in dielectrophoresis. Some cell properties, such as whole cell conductivity, have major influence, where others, like surface softness, have minor effects. Some features strongly influence electrophoresis, while other affect dielectrophoresis, each with characteristic effects on the data structure and its quantification.

THEORY

Channel Effects

The motion of particles in a buffer-filled microchannel with an applied potential is described by a number of effects, each of which may influence the behavior of analytes and therefore the structure of data sets acquired from such systems. These effects include electroosmosis, electrophoresis, and dielectrophoresis. These each arise from various properties of the particle, the microchannel, the solution, the applied field, and the interactions between them. Higher order effects, such as relaxation, retardation, thermophoresis, and field-induced electroosmosis, among others, may also be present. However, these occur for a relatively narrow set of conditions that are unlikely to be present in currently-used operations of dielectrophoretic microdevice designs and will not be included in this discussion. In some specific applications, these may need to be introduced to fully account for experimentally observed effects.

Electroosmosis and Electrophoresis—Electroosmosis, the motion of the bulk fluid in a microchannel upon application of a longitudinal electric field, is described with the zeta potential (ζ) of the channel walls. Electroosmotic mobility (μ_{EOF}) is defined by the Helmholtz-Smoluchowski equation as

$$\mu_{EOF} = -\frac{\epsilon\zeta_m}{\eta} \quad \#(1)$$

where ϵ_m is the permittivity and η is the viscosity of the medium [42–44]. The fluid velocity (v_{EOF}) is

$$v_{EOF} = \mu_{EOF}E \quad \#(2)$$

where E is the electric field.

Electrophoresis refers to the motion of charged particles in a solution in the presence of an electric field. The size of the double layer surrounding these particles affects how these particles are considered. A particle is defined as small when the Debye-Huckel length is much larger than the particle and considered large in the opposite case. For cells under physiological conditions, the Debye-Huckel length is much smaller than the particle, which is generally the case for particles greater than 100 nm in diameter [43]. Here, only the large particle case will be considered. For a discussion of the case of small particles, see Tabeling 2005 and Nguyen and Wereley 2006 [43, 44]. In the large particle case, the velocity of the

particle again is the Helmholtz-Smoluchowski velocity [42–44]. The electrophoretic mobility can then be defined as:

$$\mu_{EP} = \frac{\epsilon \zeta_p}{\eta} \quad \#(3)$$

where ζ_p is the zeta potential of the particle (rather than the wall), with the electrophoretic velocity (v_{EP}) as

$$v_{EP} = \mu_{EP} E \quad \#(4)$$

Electroosmosis and electrophoresis are considered together as the electrokinetic force. These mobilities can be combined to become the electrokinetic mobility, μ_{EK} , given as the sum of the electroosmotic and electrophoretic mobilities. The electrokinetic velocity (v_{EK}) is

$$v_{EK} = \mu_{EK} E \quad \#(5)$$

Dielectrophoresis—Dielectrophoresis (DEP) refers to the motion of a polarizable particle in the presence of a nonuniform electric field [1]. With no electric field present, a dielectric particle will have dipoles distributed in random directions. When a particle is exposed to an electric field, the dipoles align with the field. A force is induced that is oriented either towards (positive dielectrophoresis) or away from (negative dielectrophoresis) regions of high field gradient. This force (F) is described by

$$F = 2\pi r^3 \epsilon_m f_{CM} \nabla |E|^2 \quad \#(6)$$

where r is the radius of the particle, ϵ_m is the permittivity of the medium, and f_{CM} is the Clausius-Mossotti factor. For a spherical particle, f_{CM} is

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad \#(7)$$

where ϵ_p^* and ϵ_m^* are the complex permittivity of the particle and medium, respectively. For DC fields, f_{CM} simplifies to

$$f_{CM} = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \quad \#(8)$$

where σ_p and σ_m are the conductivity of the particle and medium, respectively. The dielectrophoretic force can also be described for particles of other shapes [45, 18, 46].

The dielectrophoretic mobility, μ_{DEP} is defined as

$$\mu_{DEP} = \frac{\epsilon_m r^2 f_{CM}}{3\eta} \quad \#(9)$$

and the dielectrophoretic velocity, v_{DEP} is

$$v_{DEP} = \mu_{DEP} \nabla |E|^2 \quad \#(10)$$

From this, it is seen that the effect of the radius on the dielectrophoretic mobility is trivially understood, and thus will not be considered in more depth here.

Capture in DEP occurs when the flux of particles at a given point in the channel is zero. Written in terms of mobilities, this is

$$(\mu_{EK} E + \mu_{DEP} \nabla |E|^2) \cdot E > 0 \quad \#(11)$$

Rearranging this equation defines the condition that must be satisfied for capture to occur:

$$\frac{\mu_{EK}}{\mu_{DEP}} \leq \frac{\nabla |E|^2}{E} \quad \#(12)$$

The applied potential required for capture to begin to occur is called the capture onset potential.

Particle Properties

Properties of particles will affect their electrokinetic and dielectrophoretic mobilities, which in turn affect their behavior in a microchannel. These properties and their influence on these two mobilities are summarized here.

Conductivity and Permittivity—The conductivity or permittivity of a sphere made up of multiple shells is described by the multishell model [47, 18, 40]. In this model, a cell is assumed to be a collection of concentric spheres representing the cytoplasm (conductivity σ_1 , permittivity ϵ_1 , and radius R_1), the membrane (conductivity σ_2 , permittivity ϵ_2 , and radius R_2), and the cell wall (conductivity σ_3 , permittivity ϵ_3 , and radius R_3). The conductivity of the two inner shells is given by

$$\sigma_{2shell} = \sigma_2 \frac{\left[\left(\frac{R_2}{R_1} \right)^3 + 2 \left(\frac{\sigma_1 - \sigma_2}{\sigma_1 + 2\sigma_2} \right) \right]}{\left[\left(\frac{R_2}{R_1} \right)^3 - \left(\frac{\sigma_1 - \sigma_2}{\sigma_1 + 2\sigma_2} \right) \right]} \quad \#(13)$$

and the overall conductivity of the three shells is

$$\sigma_{3shell} = \sigma_3 \frac{\left[\left(\frac{R_3}{R_2} \right)^3 + 2 \left(\frac{\sigma_{2shell} - \sigma_3}{\sigma_{2shell} + 2\sigma_3} \right) \right]}{\left[\left(\frac{R_3}{R_2} \right)^3 - \left(\frac{\sigma_{2shell} - \sigma_3}{\sigma_{2shell} + 2\sigma_3} \right) \right]} \quad \#(14)$$

Thus, factors which affect the conductivity of the cell wall, the membrane, or the cytoplasm will change the overall conductivity of the cell.

The same model can be applied to the permittivity of each layer of a cell. The permittivity of the inner two shells is

$$\epsilon_{2shell} = \epsilon_2 \frac{\left[\left(\frac{R_2}{R_1} \right)^3 + 2 \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + 2\epsilon_2} \right) \right]}{\left[\left(\frac{R_2}{R_1} \right)^3 - \left(\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + 2\epsilon_2} \right) \right]} \quad \#(15)$$

and the overall permittivity of a three-shell system is

$$\epsilon_{3shell} = \epsilon_3 \frac{\left[\left(\frac{R_3}{R_2} \right)^3 + 2 \left(\frac{\epsilon_{2shell} - \epsilon_3}{\epsilon_{2shell} + 2\epsilon_3} \right) \right]}{\left[\left(\frac{R_3}{R_2} \right)^3 - \left(\frac{\epsilon_{2shell} - \epsilon_3}{\epsilon_{2shell} + 2\epsilon_3} \right) \right]} \quad \#(16)$$

This pattern can be followed to determine the conductivity or permittivity for a system of four or more shells. The shell model is more complex for an ellipsoid [48, 49].

Surface Charge Density—Ohshima and Kondo, along with others, have described the electrophoretic mobility of a particle as it relates to the charges on the surface of the particle [50–52]. These equations well-describe soft particles such as bacteria and have been used to determine the surface softness (λ^{-1}) and surface charge density (N_c) of bacterial cells (N in the original work). With a specific set of assumptions described in the original work, they found the expression for the electrophoretic mobility to be

$$\mu_{EP} = \frac{\varepsilon_m \varepsilon_o}{\eta} \frac{\frac{\psi(0)}{\kappa_m} + \frac{\psi_{DON}}{\lambda}}{\frac{1}{\kappa_m} + \frac{1}{\lambda}} + \frac{zeN_c}{\eta\lambda^2} \quad \#(17)$$

where z is the valence of the charged groups on the particle, e is the electric unit charge, and κ_m (the Debye-Huckel parameter of the surface charge layer), $\psi(0)$ (the potential at the boundary between the surface-charge layer and the solution), ψ_{DON} (the Donnan potential), and λ are as described in the original work [51]. Each of the parameters κ_m , $\psi(0)$, and ψ_{DON} are influenced by the surface charge density and parameters z and/or N_c . The softness parameter is larger for cells with more polymer on the surface, and small as cells become “hard”. For the limit where the softness parameter becomes zero (hard particles), this equation reduces to the familiar Smoluchowski equation, which is commonly used to describe electrophoretic mobility. This analysis has been used to study analytes including bacteria, microgel particles, and latex particles bound to immunoglobulin G [53–56].

Charge Distribution and Multipoles—Pysher and Hayes, Anderson, and Fair and Anderson discuss how multipoles affect the electrophoretic mobility [57–59]. Multipoles affect the zeta potential of a particle. Generally, particles are considered to be a point charge with spherically symmetric charge distribution, but this assumption does not hold true for biological particles.

Multipole expansions of the zeta potential have been described. The model developed by Anderson gives the electrophoretic mobility, using the first three multipole moments of the expansion of the zeta potential, as

$$\mu_{EP} = \frac{\varepsilon}{4\pi\eta} \left[\beta_0 I - \frac{1}{2} \beta_2 \right] \quad \#(18)$$

where β_0 is the monopole moment, β_2 is the quadrupole moment, and I is the identity tensor. This shows that the quadrupole moment is affected by the distribution as well as by the magnitude of the surface charge in a particle.

Pethig summarizes the dielectrophoretic force as it is affected by multipoles [19]. The DEP force acts on the n th-order multipole in a spherical particle according to the equation

$$\langle \mathbf{F}_{DEP_n} \rangle = Re \left[\frac{p_n [\cdot]^n (\nabla)^n \mathbf{E}}{n!} \right] \quad \#(19)$$

where p_n is the effective n th-order multipolar moment given by the equation

$$p_n = \frac{4\pi\epsilon_m r^{2n+1}}{(2n-1)!!} CM_{(n)} (\nabla)^{n-1} E \quad \#(20)$$

and $CM_{(n)}$ is the general multipole form of the Clausius-Mossotti factor, given by the equation

$$CM_{(n)} = \frac{\epsilon_p^* - \epsilon_m^*}{n\epsilon_p^* + (n+1)\epsilon_m^*} \quad \#(21)$$

with $[\cdot]^n$ representing n dot products performed on the dyadic tensors, $(\nabla)^n$ representing n vector ∇ operations, r as the radius of a spherical dielectric particle, and E as the sinusoidal steady-state field vector to which the particle is subjected.

For nonspherical particles, p_n becomes

$$p_n = 4\pi\epsilon_m r_{int}^{n+1} \frac{2n+1}{2} \int_0^\pi \phi_{r_{int}} P_n(\cos\theta) \sin\theta d\theta \quad \#(22)$$

where $P_n(\cos\theta)$ are the Legendre polynomials, using spherical coordinates, $\phi_{r_{int}}$ is the electric potential due to the particle over a spherical surface of radius r_{int} .

Recent efforts focused on developing more accurate models of electrophoresis and dielectrophoresis include the polarized solute-solvent interface [60–65]. This approach indicates a greater degree of descriptive differentiation for complex particles such as proteins and cells. Given the potential high-resolution separations theoretically predicted and the experimental results beginning to support this high-resolution capability, more nuanced theoretical descriptions will likely be of greater applicability.

MATERIALS AND METHODS

Modeling of the effects of various cell properties on dielectrophoretic data was performed with Matlab (version 9.5 R2018b, MathWorks, Natick, MA). The equations governing each property presented above were used in combination with the piecewise function of the mathematical model developed (see below) to determine the expected features of dielectrophoretic data of varying cellular properties.

Finite element commercial software (COMSOL version 5.1 with AC/DC module, COMSOL, Inc., Burlington, MA) was used to interrogate the distribution of the electric field and find values of E_{ave} (V), E_{max} , and $\nabla|E|^2_{max}$, as they relate to the specific channels used in these studies. The microchannel has a decreasing size of constrictions, formed by triangles, from the outlet to the inlet and has been previously described in-depth [6, 34, 16, 41, 12]. The second microchannel used to demonstrate the applicability of the model to other

systems uses multi-length scale insulators, and has also been previously described [66]. Here, this channel was 3.2 cm long with a constriction of 37 The values used were determined from centerline measurements.

RESULTS AND DISCUSSION

Development of a Mathematical Model of Dielectrophoretic Data

A mathematical model is developed for dielectrophoretic data, when either the electric field or time is varied. When one of these is altered, the capture behavior of two or more particle populations can be compared [67, 36, 68–70, 6, 34, 38, 7, 71–74, 12]. This model of the data relies on electrophoresis and electroosmosis for particle transport and assumes a DC field is present, but could be modified to account for an AC field with a DC offset. Differences in the either the electrokinetic or dielectrophoretic mobility of particles will produce unique capture conditions (equation 12). While the model, as developed, uses fluorescence intensity as the metric of detection, other concentration-sensitive methods could be used with only a slight adaptation of the model. Possible other detection methods include impedance and light or dark field illumination [75], but could also include other methods to measure the number of bacterial cells present. Particle properties will also be considered and expected variations in the data due to changes in these properties noted. The model developed here will advance the ability to compare particles based on their behavior in the microchannel.

Assuming a homogeneous population of particles with average velocity v in a microchannel, the particles will travel distance $d = vt$ in time t . The particles that will reach the gate within time t are then, before any potential is applied, found within a volume with length d ending at the capture zone, height h the height of the channel, and width w , the average width of the channel (Figure 1a). The number of particles (N) that reach the capture point in time t is

$$N = nvtwh \quad \#(23)$$

where n is the average particle density.

The average electric field (E_{ave}) is the average magnitude of the field across the entire channel and reservoir length, whereas the local magnitude of the electric field (E) varies dramatically within a microchannel due to the insulating features. The local maxima of the electric field magnitude (E_{max}) occurs near each gate and is associated with the location of capture. The applied potential (V) is the voltage applied across the entire microchannel and provides a convenient way to relate E_{ave} and E_{max} to this same externally controlled value. The values $E_{ave}(V)$ and $E_{max}(V)$ are the average electric field and the local maximum magnitude of the electric field, respectively, as a function of the applied potential.

The number of particles can be determined relative to the electric field using equations 5 and 23:

$$N = n\mu_{ek}twhE_{ave} \quad \#(24)$$

Fluorescence intensity (FI) is established by multiplying N by nominal integrated fluorescence signal for an average particle, γ :

$$FI = \gamma N = \gamma n \mu_{ek} t w h E_{ave} \quad \#(25)$$

The value of γ for the fluorescence signal from an average particle may be substituted for the signal detected in a different method, if the overall signal is concentration-dependent. A correction factor, s , accounts for potential staking of the analyte, scattering of the light, saturation of the detection method, or other issues in detecting all analytes. When using fluorescence as the detection method, this also accounts for the particles not visible due to the depth of the channel, either due to stacking or to the depth-of-focus. The expression for the observed fluorescence intensity then becomes

$$FI_{obs} = \gamma n \mu_{ek} t w h s E_{ave} \quad \#(26)$$

Data is captured and assessed via two methods, holding t constant and varying E_{ave} , or by holding E_{ave} constant and varying t (Figure 1b).

Varying Potential—The magnitude of the local electric field varies as a function of applied potential (V). An expression for how this varies within a specific system can be determined using COMSOL. For the specific channel under study, this relationship was found to be

$$E_{ave}(V) = 0.24 \cdot V \quad \#(27)$$

This expression, $E_{ave}(V)$, can then be substituted into equation 26 to determine the fluorescence intensity as a function of applied potential, resulting in

$$FI(V) = \gamma n \mu_{ek} t w h s E_{ave}(V) \quad \#(28)$$

For the specific channel under study, equation 27 can be combined with equation 28 to find

$$FI(V) = \gamma n \mu_{ek} t w h s \cdot (0.24V) \quad \#(29)$$

To extend this work to other microchannel geometries, other expressions for $E_{ave}(V)$ could be found and used in place of the values shown here.

Conditions of Capture—At low values of applied voltage, no capture takes place. The above expression only holds true when capture is occurring. A function is necessary that describes the full data, including the region of no capture, the capture onset potential, and capture with a linear rise in FI . The behavior is best described by a piecewise function. At

values less than the capture point, $y = 0$, while at values higher than the capture point, FI will increase linearly as described above. The intercept of this function is related to the applied potential at which capture begins to occur ($V = c$). Setting $FI(c) = 0$ and using algebra to determine the intercept of the linear rise part of the piecewise function results in the expression

$$FI(V) = \begin{cases} 0 & \text{if } V < c \\ 0.24\gamma n\mu_{ek}twhs(V - c) & \text{if } V \geq c \end{cases} \quad \#(30)$$

Capture Onset Potential—COMSOL was used to find finite element software-derived

values for the function $\frac{\nabla |E|^2_{max}}{E_{max}}(V)$ in the specific device under study

$$\left[\frac{\nabla |E|^2_{max}}{E_{max}}(V) \right] = [1.4 \cdot 10^7] \quad \#(31)$$

When the value of V is set to the onset capture potential (c), the entire equation can be set equal to $\frac{\mu_{ek}}{\mu_{dep}}$. Doing this and then solving for c yields

$$\left[\frac{\nabla |E|^2_{max}}{E_{max}}(c) \right] = [1.4 \cdot 10^7] \cdot c = \frac{\mu_{ek}}{\mu_{dep}} \quad \#(32)$$

$$c = \frac{1}{1.4 \cdot 10^7} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \quad \#(33)$$

Again, an expression can be found for other microchannel systems, and the appropriate values substituted in this equation.

This expression can be substituted into the piecewise function of equation 30:

$$FI(V) = \begin{cases} 0 & \text{if } V < \frac{1}{1.4 \cdot 10^7} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \\ 0.24\gamma n\mu_{ek}twhs \left[V - \frac{1}{1.4 \cdot 10^7} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \right] & \text{if } V \geq \frac{1}{1.4 \cdot 10^7} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \end{cases} \quad \#(34)$$

This reflects that the fluorescence intensity of captured particles, as well as the capture onset potential, is related to known parameters. This final function can be fitted to data collected

from a specific particle population to find properties of that population. Differences in both μ_{EK} and μ_{DEP} will affect the shape of the data when the potential is varied (Figure 1b). As before, an expression for other microchannel systems can be found by using the appropriate expressions for $\frac{\nabla |E|^2_{max}}{E_{max}}(V)$ and $E_{ave}(V)$.

One such system, previously described in-depth [66], uses a multi-length scale insulator rather than the triangles of the system used in the case above. For this system, it was calculated that

$$E_{ave}(V) = 0.29 \cdot V \quad \#(35)$$

and

$$\frac{\nabla |E|^2_{max}}{E_{max}}(V) = [1.9 \cdot 10^6] \cdot V \quad \#(36)$$

Thus, the final equation can be written as

$$FI(V) = \begin{cases} 0 & \text{if } V < \frac{1}{1.9 \cdot 10^6} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \\ 0.29 \gamma n \mu_{ek} t w h s \left[V - \frac{1}{1.9 \cdot 10^6} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \right] & \text{if } V \geq \frac{1}{1.9 \cdot 10^6} \left(\frac{\mu_{ek}}{\mu_{dep}} \right) \end{cases} \quad \#(37)$$

which demonstrates how this model could be applied to other dielectrophoretic systems.

Varying Time—Rearranging equation 29, holding the applied potential V and therefore E_{ave} constant, and allowing t to vary gives

$$FI = 0.24 V \gamma n \mu_{ek} w h s t \quad \#(38)$$

where expressions for $E_{ave}(V)$ from other devices can be substituted.

Features of Data and Interpretation with the Model

The equations derived for FI reflect that differences in the electrokinetic mobility appear as a change in both the slope in voltage- and time-based data, as well as in the voltage at which capture begins to occur. Differences in the dielectrophoretic mobility, however, will only appear as a difference in the onset voltage and the slope of the data remains unchanged (Figure 1). Other devices and systems can also be interpreted with this approach by using the data from other microchannel geometries [67, 36, 76, 38, 77–79, 74, 40, 80] and these general features of the data would remain unchanged, although quantitative details will vary

depending on the configuration. Specific dimensions and operating parameters for a common device design were used in the calculations presented here. Some other systems may require additional factors, such as the addition of pressure driven flow [81].

Typical data demonstrates a linear increase in FI with increasing V , consistent with equations 29 and 34, starting at the value of c where capture begins [6, 34]. There is a limited range of linearity, beyond which the signal falls below the values predicted by the model. The non-linearity has two possible origins: saturation of the capture zones with particles (causing release or visual occlusion) or preceding capture zones collecting particles, reducing the available pool.

Applications with Known Particle Properties

Various particle properties are known to affect μ_{EK} , μ_{DEP} , or both, but with unique mechanisms for each. Variations in these properties can cause differences in capture onset potential and/or slope, and the magnitude of this difference will depend on the property. Here, differences in bacterial cell conductivity and permittivity, surface charge density, and zeta potential, and their effect on the data according to the model developed will be considered. The results discussed below and shown in Figures 2–5 are a direct application of the model to real data, from values for these properties found in the literature. The influence of multipoles will also be discussed. Shape, deformability, and the mobility of surface charges will be briefly considered within the influence in multipoles.

Conductivity and Permittivity—The conductivity and permittivity of the cell directly impact the dielectrophoretic mobility (equations 7–9) and helps to define the capture onset potential. Changes in μ_{DEP} do not influence the slope of the FI (equation 34 and Figure 1b).

Differences in conductivity were examined using high and low literature values for various microorganisms. The overall conductivity of a cell can range from about 0.002 to 0.1 S/m for gram-negative bacteria, a range of about two orders of magnitude. In gram-positive species, this range is about 0.015 S/m to 0.15 S/m, about one order of magnitude [82]. These values are used to model expected dielectrophoretic behavior, and provide graphical evidence of reasonable changes in dielectrophoretic behavior arising from this variable (Figures 2a, 2d and 2g). Values for other properties not mentioned specifically are averages of values found in the literature. The same holds true for the considerations of zeta potential and surface charge density below.

The magnitude of the difference in capture onset potential, however, is dependent on the conductivity of the medium. When the cell and the medium conductivity are similar, there is a larger difference in capture onset potential (Figure 2). This is more clearly shown for gram-positive species; the difference in capture onset potential is much greater when the medium conductivity is closer to the values of the particle conductivity (Figure 2a–c). Varying the medium permittivity or the viscosity of the medium will shift the potential at which capture occurs but does not affect the difference in capture onset potentials between two species. Varying medium conductivity could be exploited to determine if the differences seen in capture onset potential are likely related to the conductivity of the particles, or if other possibilities should be considered.

As reflected in the shell model, the conductivity or permittivity of any layer of a cell can affect the overall conductivity or permittivity, and thus each layer can affect the dielectrophoretic mobility (equations 13–14) [18, 78]. These relationships, for both a sphere and an ellipsoid, show that a change in thickness in any layer will affect the overall conductivity of the particle. An increase in conductivity of the cell will lead to an increase in the capture onset potential. The same is true for an increase in the permittivity. The impact of a single layer will be affected by the radius of that layer. For three layers that are the same thickness, a change in the outermost layer would cause the largest change in the overall conductivity or permittivity; however, this is not the case in bacterial cells where the three layers are the cell wall, the membrane, and the cytoplasm, each with its characteristic thickness (Figure 3).

The cell wall and the cytoplasm conductivities can vary by about an order of magnitude, with reasonable values of the conductivity for each being between 0.5 and 0.05 S/m (Figure 3a and 3c). The membrane conductivity also varies by approximately an order of magnitude, with a reasonable value being 10^{-7} S/m [17, 83, 82]. As this variation generates a difference in the capture onset potential in the 6th decimal place, two orders of magnitude were considered, changing conductivity from 10^{-7} to 10^{-9} S/m (Figure 3b and 3d).

This modeling shows that a change in conductivity solely in one layer of the cell will cause a much smaller effect on the capture onset potential compared with whole cell conductivity (Figures 2 and 3). The same logic can be applied to permittivity to examine the effect of a variation in each layer. The effect on total conductivity of a cell differs to varying degrees depending upon which layer is changed. A difference in conductivity in either the cell wall and the cytoplasm could solely be responsible for a small difference in overall conductivity and thus the capture onset potential. A variation in the membrane conductivity, however, could only be responsible for a very small difference in the capture onset potential. Thus, it is unlikely that a difference in capture onset potential between two cell populations could be assigned exclusively to a difference in the membrane conductivity.

The ability to separate live and dead cells with dielectrophoresis has been attributed to the difference in cell conductivity. When a cell dies, especially through heat treatment, the membrane becomes more permeable, resulting in an increase in conductivity of about four orders of magnitude, from about 10^{-7} S/m to about 10^{-3} S/m [84]. This also allows an efflux of cytoplasmic ions, resulting in a decrease of cytoplasmic conductivity, from 0.44 S/m for live cells to 0.9 S/m for dead cells [85]. Modeling this interesting case shows a similar result to Figure 3a and c, with the dead cells requiring a higher potential for capture. This is consistent with what was shown by Lapizco-Encinas et al [8], where dead *Escherichia coli* cells are captured at locations of higher field gradient than live *E. coli*.

Zeta Potential—The zeta potential for a cell is a defining property reflecting the interplay between surface charge and the ionic solution surrounding the cell. This variable appears in a linear relationship to electrokinetic mobility in equations (equations 1 and 3). As such, a difference in zeta potential will cause a difference in both the slope and the capture onset potential (equation 34). Even a small difference in zeta potential will give a noticeable difference in both the capture onset potential and the slope of the dielectrophoretic data

(Figure 4). The literature shows that the zeta potential can vary between strains of a single species, as demonstrated by *Streptococcus mutans*. At pH 7, the zeta potential for this species can range from about -20 to -35 mV [86]. It is unsurprising, then, that different species have even larger differences in their zeta potentials. For example, the zeta potential of *Rhodococcus erythropolis* is -102 mV [87], while *Pasteurella multocida* has a zeta potential of -25 mV [88] with an intact capsule. The non-capsulated derivative of *P. multocida* has a zeta potential of -15 mV at the same pH of 7 (Figure 4) [88].

These real values for zeta potential found in the literature can be used in the mathematical model to show how such differences will affect dielectrophoretic behavior. Small differences in the zeta potential of the cell, such as that arising from the presence vs. absence of the capsule, will give a notable difference in the capture onset potential. Larger zeta potential variations logically result in even larger variations in the capture onset potential. From this, disparities in capture onset potential up to about 450 V, and perhaps larger, which accompany a small change in slope could be assigned to a difference in zeta potential. However, as noted, differences in conductivity could also contribute to difference in capture onset potential.

Varying properties of the system such as the concentration of particles, the zeta potential of the channel wall, the conductivity of the medium, or the permittivity of the medium will have little to no effect on the difference of capture onset potentials between two species. It does, in the case of zeta potential of the wall, shift the forces required for capture. However, both the concentration and the permittivity of the medium have a large impact on the difference in slopes between two species. Without knowing these parameters accurately for a specific system, it would be difficult to attribute a difference in slope to any one property, including zeta potential differences.

Surface Charge Density—Surface charge density and surface viscosity have analytical solutions for their electrophysical effects. Surface viscosity is approximated with so-called surface softness (λ^{-1}). This softness parameter arises as the inverse of a measure of the friction of a liquid flowing through the surface layer of the cell [53]. Cells characteristically have some number of polymers on the surface, and as such the inclusion of the softness parameter gives a more accurate description of cells. Both the surface charge density and the softness can vary in bacteria due to differences in the lipids forming the membrane, the amount and type of glycosylation in the capsule and/or peptidoglycan layer, and the presence of proteins at the surface of the cell. An increase in the surface charge density (N_c) or a decrease in surface viscosity (η), represented by the softness parameter (λ), will increase electrokinetic mobility (equation 17). This increase in μ_{EK} will cause an increase in the slope of the line (Figure 5, equation 34), and an increase in the capture onset potential.

Both the density of charged groups and the λ factor have been found for various bacterial species. Example gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) species, for which N_c and λ are found in the literature, are used to demonstrate typical behaviors. For *E. coli*, the surface charge density at a pH of 7 is 0.145 M (or $1.74 \cdot 10^{-5}$ mmol/m²), while for *S. aureus*, it is 0.025 M (or $3.00 \cdot 10^{-6}$ mmol/m²). The λ factor for *E. coli* is 1.5 nm^{-1} , while for *S. aureus*, it is 0.53 nm^{-1} [52]. Using these values, the

electrophoretic mobility is affected (equation 17), and thus the capture onset potential and the slope of the data (equation 34). The capture onset potential and slope of the data for the modeled particles are summarized in Figure 5d. These two populations are representative of a large range of surface charge densities that will be found on bacterial cells; however, there may be other species or strains with much larger or smaller surface charge densities. Considered separately, the surface charge density has a noticeable impact on the electrokinetic mobility, and thus both the capture onset potential and the slope (equation 34), while a difference in the λ factor gives little to no discernable change (Figure 5).

The magnitude of the change in both capture onset potential and slope, when two differing species are considered, is smaller for the surface charge density than for zeta potential. Larger variations in capture onset potential, as well as in slope, could more likely be ascribed to zeta potential alone than to surface charge density alone. However, these two parameters are very closely related, and likely will change together. As discussed above, variation solely in overall conductivity will only result in a difference in the capture onset potential and will leave the slope unchanged.

As with population differences in zeta potential, varying system properties, including particle concentration, medium permittivity, or medium viscosity, will not cause much of an effect on the difference in capture onset potential of two populations with differing surface charge densities. The concentration, medium permittivity, and medium viscosity, however, do appear to affect the difference in slopes. As discussed above, this makes it difficult to attribute a change in slope to any one specific property.

Experimental dielectrophoretic data for a strain of *S. epidermidis* (ATCC 35983) [34] can be compared to the results from the model using the surface charge density from that strain that was determined by Kiers et al. [89]. The capture onset potential for this strain, using the same channel that is used for the modeling presented here, was determined experimentally to be 443 ± 51 V. Kiers et al. found the surface charge density of this strain to be 0.028 M (or $3.36 \cdot 10^{-6}$ mmol/m²), with a λ of 1.9 nm⁻¹. Setting the surface softness and surface charge density as that found by Kiers et al., and other parameters as in that experiment, results in a calculated capture onset potential of 416 V. This is within the standard deviation of the experimentally-determined capture onset potential, showing that this model accurately reflects experiment.

Charge Distribution/Multipoles and Dipoles—The presence of permanent dipoles can affect both the electrophoretic and dielectrophoretic mobilities. Altering the dipole will cause a difference in both the slope and the capture onset potential. A distribution of charges in a sphere is well represented as the potential distribution of a system of multipoles [19]. The shape and deformability of a cell, as well as the mobility of surface charges, all will affect that system of multipoles.

Typical DEP relationships, such as equation 6, consider only the dipole approximation of all multipoles in a particle. While using the equations that include the higher-order multipoles can provide a refined value, it does not add any new information for most systems [90]. Nevertheless, exceptions to the assumption of the dipole as a good approximation do exist

[46, 19]. In general, the dipole force dominates for small particles relative to the size of the electric field inhomogeneity, but the multipole force terms become important as particles become larger relative to the field variations [91, 46, 19, 92]. One such case is described by Washizu [93, 92], where a particle is located near the center of quadrupole electrodes, and another is when the particle is larger than one-tenth of the electrode spacing [90].

CONCLUSION

Very subtle cellular differences have allowed differentiation and separation in dielectrophoretic systems, but it is difficult to connect this with known biological structures. The mathematical model of the data presented here provides a starting point to discover this needed connectivity. The bracketing of literature-based high and low real-world values of conductivity, zeta potential, and surface charge density allow for an understanding of likely and unlikely possibilities within these systems.

The biophysical properties of a cell are a summation of many values, and DEP gives a single value. Thus, many properties are collapsed and will appear similar in such a system. Furthermore, a single change in the makeup of a cell may change multiple parameters. When the type or extent of the cellular difference is unknown, this work can help in assigning the origin of the differences. For a well-vetted system where likely differences are known, this model can indicate the extent of a dissimilarity between two populations, or perhaps the expression of a specific protein.

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Abbreviations:

DEP	dielectrophoresis
eDEP	electrode-based dielectrophoresis
iDEP	insulator-based dielectrophoresis
ζ	zeta potential
μ_{EOF}	electroosmotic mobility
ϵ	permittivity
η	viscosity
v_{EOF}	electroosmotic velocity
E	electric field strength
μ_{EP}	electrophoretic mobility
v_{EP}	electrophoretic velocity

μ_{EP}	electrokinetic mobility
v_{EK}	electrokinetic velocity
F	dielectrophoretic force
r	radius
f_{CM}	Clausius-Mossotti factor
ϵ^*	complex permittivity
σ	conductivity
μ_{DEP}	dielectrophoretic mobility
v_{DEP}	dielectrophoretic velocity
λ^{-1}	surface softness
N_c	surface charge density
z	valence of charged groups
e	electric unit charge
κ_m	Debye-Huckel parameter
$\psi(0)$	potential at the boundary
ψ_{DON}	Donnan potential
β_0	monopole moment
β_2	quadrupole moment
I	identity tensor
p_n	nth order multipolar moment
$CM_{(n)}$	general Clausius-Mossotti factor
$\phi_{r_{int}}$	electric potential due to the particle
v	velocity
d	distance
t	time
h	height
w	average width
N	number of particles

n	average particle density
E_{ave}	average electric field magnitude
E_{max}	electric field local maximum magnitude
V	applied potential
$E_{ave}(V)$	average electric field magnitude as a function of the applied potential
$E_{max}(V)$	electric field local maximum magnitude as a function of the applied potential
FI	fluorescence intensity
γ	nominal integrated fluorescence signal for an average particle
s	stack factor
FI_{obs}	observed fluorescence intensity
c	capture onset potential

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Fig. 1. Schematic diagram of the channel (a, top), typical particle collection pattern (a, bottom), and data structures resulting from changes in electrokinetic and dielectrophoretic properties (b). Notable variables used in the mathematical model include channel width, w , and the distance, d , from which particles can reach the gate and capture (a, top). This distance increases with increasing voltage (V) or increasing time (t). The channel height, h , is perpendicular to the paper. This channel is on the microscale, with dimensions of about 1 mm for w and about 20 μm for h . The number of particles captured and a brighter fluorescence intensity result from larger d (a, bottom). An increase in μ_{EK} (dashed line) will result in a larger slope in time-dependent data, along with a larger slope and higher onset capture potential in voltage-dependent data (b, left). An increase in μ_{DEP} (dashed line) will result in a smaller capture onset potential in voltage-dependent data, but will not affect the slope (b, right); time-dependent data will not be affected by changes in μ_{DEP} . The opposite cases (solid lines) induce the opposite effect.

Fig. 2.

Calculated dielectrophoretic data for model cells with high and low values of overall cell conductivity (Gram-positive: a, b, c; Gram-negative: d, e, f) with respect to a range of medium conductivities (left to right: low, 0.0005 S/m; mid-range, 0.0009 S/m; high, 0.003 S/m). The high (dashed red line) and low (solid blue line) overall cell conductivity literature values are used to calculate dielectrophoretic properties, while all other properties were assigned an average from across the literature. In f, the low cell conductivity value is smaller than the medium conductivity, leading to positive dielectrophoresis. The capture onset potential for this situation is outside the plotted x-axis (at $6.0 \cdot 10^3$ V), and thus the line is not plotted. A table summarizes the changed variables and resulting capture onset potentials for each situation (g).

Fig. 3.

Calculated biophysical data from modeled cells in a dielectrophoretic system influenced by changes in the conductivity of the cell wall (a, blue solid 0.05 S/m, red dashed 0.5 S/m), membrane (b, blue solid $1 \cdot 10^{-9}$ S/m, red dashed $1 \cdot 10^{-7}$ S/m), and cytoplasm (c, blue solid 0.05 S/m, red dashed 0.5 S/m). A representative high (red dashed) and low (blue solid) value for conductivity of each layer was chosen. The shell model (equation 14) is used to determine the effect of the conductivity. Larger x - and y -axis values consistent with other data presented is shown (insets), allowing for visual comparison of the effect of a single layer to that of the overall conductivity. The table summarizes the changed variable in each plot and the capture onset potential obtained from the modeling (d).

Fig. 4.

Calculated biophysical data of modeled cells of varying zeta potential in a dielectrophoretic system. Zeta potentials corresponding to those of *Rhodococcus erythropolis* (−102 mV, red dashed line) and *Pasteurella multocida* (−25 mV, blue solid line) were plotted (a). Within a single species of bacteria, capsulation can make a difference in zeta potential: zeta potentials corresponding to capsulated (−25 mV, blue solid line) and non-capsulated (−15 mV, red dashed line) strains of *P. multocida* (b) were plotted. The table shows the different zeta potentials used in each plot and the calculated capture onset potentials and slopes (c).

Fig. 5.

Calculated biophysical data of modeled cells in a dielectrophoretic system influenced by changes in surface charge density, N_c , and the softness parameter, λ . Representative values found in the literature for common bacteria are plotted: *Staphylococcus aureus* (solid blue line, $N_c = 0.025$ M, $\lambda = 0.53$ nm⁻¹) and *Escherichia coli* (dashed red line, $N_c = 0.145$ M, $\lambda = 1.5$ nm⁻¹) (a). With λ held constant at the *S. aureus* value (0.53 nm⁻¹), only N_c is varied (b). With N_c held constant at the *S. aureus* value (0.025 M), only λ is varied (c). The table summarizes the values for surface charge density and surface softness used in each plot, and the calculated capture onset potentials and slopes (d).