CHAPTER 6

Dielectrophoresis for Particle and Cell Manipulations

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Abstract

In this chapter, we will explore the use of dielectrophoresis (DEP) for particle and cell manipulation. This is a broad field, and so our aim will be to present the theory behind several dielectrophoresis techniques, as well as specific experimental recipes. Initially, we will present the concept and theoretical underpinnings of DEP and build a framework of approximations that connect our theoretical development to engineering design and experimental implementation. First, from a theoretical perspective, we will approach DEP techniques based on the properties of the driving electric field and introduce experimental techniques for manipulating these field properties. Next, from an experimental perspective, we will approach DEP techniques based on device geometries used to generate electric field nonuniformities and report specific methodologies for reproducing experimental results. Finally, from a design perspective, we will present DEP techniques based on the resulting motion of particles in the system and examine a variety of demonstrated applications.

Key terms  
dielectrophoresis  
insulating dielectrophoresis, iDEP  
negative dielectrophoresis, nDEP  
positive dielectrophoresis, pDEP
6.1 Introduction: physical origins of DEP

The term “dielectrophoresis” was used by Herbert A. Pohl as early as 1951 in describing the motion of particles in response to a nonuniform electric field [1]. In that work, “dielectrophoresis” describes the force—exclusive of electrophoresis—exerted on polarizable particles, as a function of their complex permittivity, in the presence of an externally applied, nonuniform electric field. In this work, we additionally include the torque experienced by a particle in a rotating electric field. We reason that these phenomena are related in that each is dependent, in some manner, on the relative complex permittivities of a particle and its surrounding media.

Dielectrophoresis originates from the response of matter to an electric field, and more specifically, from differences in this response across an interface. In an electric field, a perfectly conducting material will transport electrons instantaneously along the field, effectively making the electric potential uniform within the material. For a material that is perfectly insulating, electrons are immobile, and the electric current within is zero; thus the electric potential is defined by the charge distribution according to Gauss’ law. For most materials subjected to dielectrophoresis, the response to an externally applied electric field is neither that of a perfect conductor nor that of a perfect insulator, but rather behaves as a “leaky dielectric” or “lossy dielectric.” For harmonic fields, this property of matter is described using a complex, frequency-dependent permittivity, $\varepsilon$ (6.1). A material subjected to an electric field will respond, or polarize, in a manner that is dependent on its complex permittivity as well as the strength and frequency of the local electric field.

$$\varepsilon = \varepsilon - i \frac{\sigma}{\omega}$$  \hspace{1cm} (6.1)

Here, $i$ is $\sqrt{-1}$, $\sigma$ is the conductivity, and $\omega$ is the radial frequency of the electric field. The complex permittivity $\varepsilon$ is a function of electric field parameters (magnitude and frequency), thermodynamic parameters (temperature and pressure), and material parameters (composition) [2]. The real and imaginary components of $\varepsilon$ correspond to displacement and conduction current, respectively, and relate respectively to the localization of bound charge and the motion of free charge. The “leaky-dielectric” model implies that when matter within the system orients or charge redistributes in response to changes in the external electric field, there is a finite “lag” time, which is a function of frequency, between the electric field and the response of a material.

Fundamentally, dielectrophoretic forces (or torques) result from a nonuniform polarization along an interface between materials with different dielectric properties. This implies two things: (1) there is an interface between two materials that respond differently to an imposed electric field (described by different values of the complex permittivity, $\varepsilon$), and (2) the imposed electric field varies significantly along this interface. The Maxwell-Wagner interfacial polarization takes the form of electric molecular dipoles within the material. The creation of dipoles manifests at the interface between materials of different complex permittivity, due to different relative dipole strengths, as a “bound charge.” Bound charge is distinguished from “free charge” in that it manifests

Interestingly, it is also possible to observe a force with a uniform electric field, if the relative complex permittivities vary significantly along the interface.
itself only in response to an external field (i.e., it is induced) and spatial variation in permittivity and is not free to move through conducting media.

The electric field exerts a Coulomb force on these bound charges, whose sum along the interface is nonzero only when the field is nonuniform. Thus DEP allows a force to be applied to particles as long as the electric field is nonuniform and \( \varepsilon \) is different for the particle as compared to its surrounding medium. The frequency dependence of \( \varepsilon \) gives experimenters an extraordinary amount of flexibility with regards to what biological particles can be manipulated with DEP, while the favorable scaling of DEP forces as length scales diminish motivates DEP’s use in microfabricated systems.

### 6.2 Introduction: theory of dielectrophoresis

Now we wish to put the above description in mathematical terms that are relevant to the experimental researcher and recapitulate the canonical equations for the DEP force, \( F_{\text{DEP}} \).

In order to do this, we consider a particle submerged in an electrolyte, each described using complex permittivities (\( \varepsilon_{p} \) and \( \varepsilon_{m} \) for particle and media, respectively), under the influence of a harmonic electric field, \( \tilde{E} \). The electric field frequency is assumed to be low enough such that the permittivities, \( \varepsilon_{p} \) and \( \varepsilon_{m} \), can be considered constant. The electric field is created by applying a potential at some point in the device, and, as expected, we describe the electric field in general as the gradient of this potential:

\[
\tilde{E} = -\nabla \phi
\]  

(6.2)

We have made no assumptions yet as to the temporal or spatial properties of the field, except to say that it is harmonic. Owing to the mathematical simplicities stemming from the use of complex algebra in treating these sinusoidal functions, we represent the electric field as:

\[
\tilde{E}(\tilde{r}, \omega, \phi) = \Re \left[ \tilde{E}_{0}(\tilde{r}) e^{i(\omega t + \phi)} \right]
\]  

(6.3)

where \( \tilde{r} \) is a position vector, \( \tilde{E}_{0}(\tilde{r}) \) captures the spatial distribution of the electric field (still arbitrary), \( \Re \{ \ldots \} \) is the real component of the quantity in brackets, and \( \omega \) and \( \phi \) are the angular frequency and phase of the field, respectively. In this sort of complex analysis it is common practice to omit the harmonic portion in notation. As long as the system remains linear, and we consider only the steady state, we can continue to use principles of superposition, mesh analysis, and other analytical tools. Later, we can include the harmonic portion when we wish to calculate derivatives or time-averages of these harmonic quantities.

Moving forward, we present the basic theory of dielectrophoresis and discuss its limitations. We begin with a brief derivation of the dielectrophoretic force employing the commonly used dipole approximation for an isolated sphere in an infinite medium. Following this, we expand the discussion to illustrate the limitations of the electrodynamic and fluid-mechanical approximations.

In the most general case, the solution for the electromagnetic force (which includes DEP) on an arbitrary object is found by integrating Maxwell’s stress tensor. The Maxwell stress tensor formulation is the most general and powerful, but in problems lacking the
appropriate symmetry, solutions can only be obtained numerically or as close approximations. For those geometries with suitable symmetry, there are closed-form solutions and approximations available in literature. Relevant analytical [3–7] and numerical [5–11] references are summarized in Tables 6.1 and 6.2.

Consider a homogeneous, isotropic sphere in a semi-infinite, homogeneous, isotropic electrolyte, with an electric field applied along the z-axis. If the external field varies linearly over the characteristic particle dimensions, the polarization of a dielectric sphere in an electric field can be represented by replacing the particle with an equivalent, effective dipole located at the center of the particle. Such a dipole consists of two charges of equal magnitude and opposite sign at a position, \( \vec{r} \), separated by a vector distance, \( \vec{d} \). If the electric field is nonuniform over the dipole length, \( \delta d \), then the sum for electrostatic forces on the dipole is,

\[
\vec{F} = q \vec{E} \left( \vec{r} - \vec{d} \right) - q \vec{E} (\vec{r})
\]

(6.4)

where \( q \) is the dipole charge and \( \vec{d} \) is the vector from the negative dipole charge to the positive dipole charge, by convention. Expanding the first term in a Taylor series, we obtain:

\[
\vec{E} \left( \vec{r} + \vec{d} \right) = \vec{E} (\vec{r}) + \vec{d} \nabla \vec{E} (\vec{r}) + \ldots
\]

(6.5)

### Table 6.1 A Brief Summary of Literature Sources That Deal with Maxwell Stress Tensor Solutions and Dielectrophoresis

<table>
<thead>
<tr>
<th>No.</th>
<th>Author, Year</th>
<th>Title</th>
<th>Technique</th>
<th>Application</th>
</tr>
</thead>
</table>
6.2 Introduction: theory of dielectrophoresis

Table 6.2  Table 6.1 continued.

<table>
<thead>
<tr>
<th>No.</th>
<th>Comparison/ Validation</th>
<th>Relevant Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>[8]</td>
<td>effective dipole</td>
<td>MST formulation is necessary when particle size is on the order of electrode size, and particle-particle interactions play a significant role on this length scale.</td>
</tr>
<tr>
<td>[9]</td>
<td>n/a</td>
<td>MST solution matches experimental behavior for air/water interface. Electrowetting on dielectric is dominant in this case.</td>
</tr>
<tr>
<td>[5]</td>
<td>numeric solution</td>
<td>MST calculation is used to determine electrical force on a sphere and combined with the hydrodynamic stress tensor to yield net force due to concentration polarization.</td>
</tr>
<tr>
<td>[6]</td>
<td>effective dipole, numeric solution</td>
<td>Dipole approximation fails when particle size is the same order of magnitude as the characteristic length scale of the electric field. The dipole approximation is worse for a cylinder.</td>
</tr>
<tr>
<td>[10]</td>
<td>effective dipole</td>
<td>Dipole approximation becomes increasingly inaccurate as a particle approaches an electrode. MST is used to effectively calculate DEP force and deformation of spheres, CNTs, bacteria, and viruses.</td>
</tr>
<tr>
<td>[7]</td>
<td>multipole</td>
<td>The results show that a small number of multipolar terms need to be considered in order to obtain accurate results for spheres. The full MST calculation is only required in the study of nonspherical particles.</td>
</tr>
<tr>
<td>[11]</td>
<td>effective dipole</td>
<td>Point dipole model overestimates DEP force by 40% for particles with 10% variation in permittivity and size 25% of electrode size (when particles are &quot;close&quot; to the electrode). As particles approach electrodes or approach electrode size, even the quadrupole term is insufficient.</td>
</tr>
</tbody>
</table>

If the characteristic length scale of the electric field non-uniformity is large compared to the particle size, then we can neglect terms of higher order in (6.5) and plug (6.5) into (6.4) to find:

\[
\vec{F} = q \ddot{\vec{d}} \nabla \vec{E} \\
= \vec{p}_{\text{eff}} \cdot \nabla \vec{E}
\]  

(6.6)

which gives us the force on some unspecified effective dipole moment, but does not intimate how the properties of particle and media contribute to this effective dipole moment. There are several approaches to determine these details; the most common is to examine a homogeneous dielectric sphere in a dielectric media, and solve Laplace’s equation for the electric potential inside and outside the sphere, applying the appropriate boundary conditions at the interface. Solution by the separation of variables technique yields (6.7) and provides analytical results that are physically intuitive.

\[
\vec{p}_{\text{eff}} = -4\pi \varepsilon_m a^3 E_0 \log \left( \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \right)
\]

(6.7)

\[
\langle F_{\text{DEP}} \rangle = 4\pi \varepsilon_m a^3 M[f_{\text{CM}}] \nabla \left( \vec{E}_0 \cdot \vec{E}_0 \right)
\]

(6.8)

\[
f_{\text{CM}} = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}
\]

(6.9)

where the angle brackets denote time averaging, \(\vec{p}_{\text{eff}}\) is the effective dipole moment for a sphere, \(a\) is the particle radius, \(\varepsilon_m\) is the permittivity of the media, \(\varepsilon_p\) and \(\varepsilon_m\) are the complex permittivities of the particle and media, respectively, and \(M[f_{\text{CM}}]\) is the real part of the Clausius-Mossotti factor. This analytical expression for dielectrophoretic effects is...
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intuitive, tractable, and is applicable under numerous experimental conditions (limitations on the applicability of this form of $F_{DPE}$ will be discussed in Section 6.2.1). If we expand this analysis to a general electric field with spatially varying phase, we find an additional term that leads to electrorotation effects:

$$\langle \tilde{F}_{DPE}\rangle = \frac{\pi \alpha^2 \varepsilon_m}{2 \tan(\theta)} \Im \left[ f_{cm} \right] \Im \left[ \hat{E} \cdot \hat{E} \right] - 2 \pi \alpha^2 \varepsilon_m \Im \left[ f_{cm} \right] \Im \left[ \hat{E} \times \Im \left[ \hat{E} \right] \right]$$  \hspace{1cm} (6.10)

The first term in this equation is the well-known result for the time-averaged dielectrophoretic force, and appears in the presence of any nonuniform electric field (of the form prescribed). The second term arises only in the presence of a spatially nonuniform electric field phase, $\phi \neq 0$, and is the driving term for traveling-wave dielectrophoresis (twDEP). In both terms, forces scale with particle radius cubed, making particle size an important factor in most DEP experiments.

6.2.1 Limiting assumptions and typical experimental conditions

Though seldom explicitly defined in the literature, there are clear boundaries for the applicability of the dielectrophoretic force equations presented above. Particle shape, characteristic particle dimensions and device length scale, particle concentration, and characteristic length scale of changes in the electric field must be considered.

We will examine each of these parameters as variations from our “baseline case” (6.10): a sparse concentration of homogeneous, spherically symmetric particles in an infinite domain, under the influence of an electric field that is well approximated by a first order linearization. We will consider, as they affect dielectrophoretic forces and torques:

1. The limits of the spherical approximation, as particle shape deviates from sphericity and the dipole approximation fails.
2. The limits of the dipole approximation as the length scale of variations in the electric field decreases—due to shrinking channel dimensions, shrinking interparticle spacing, or increasing particle size—and our linearization of the electric field fails.
3. The limits of the effective permittivity model, and the Maxwellian equivalent body, as particle composition becomes increasingly complex.

We will also consider, as they affect drag forces and terminal particle velocity:

1. The limits of the spherical approximation, as particle shape deviates from sphericity and the drag coefficient takes on a different form.
2. The limits of the isolated particle approximation, as particle concentration increases.
3. The limits of the infinite domain assumption, as channel dimensions decrease.

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2 Throughout this chapter, however, we will continue to make the assumption that the double-layer thickness (Debye length, $\lambda_d$) is small in comparison to channel and particle dimensions.
6.2.1.1 Assumptions and approximations for $F_{\text{dip}}$

**Spherical approximation** While many of the particles in DEP experiments are not spherical, the spherical approximation, and the simple multishell models associated with it, is often used. Spherical symmetry leads to analytical solutions that are intuitive and easy to apply. There are, however, situations where these approximations are inappropriate (e.g., cellular samples of rod-shaped bacteria). We will present analytical solutions for the dipole moment of an elliptical particle and compare this result to the spherical moment presented earlier (6.7). In all of these cases we will assume that the major axis of our ellipsoidal particle will be aligned with the applied electric field and that the field varies only along this direction; a reasonable assumption for isotropic particles in non-rotating fields.\(^3\)

The shape of a polarized particle will influence the electric field it creates. As particle shape deviates from spherical, the applicability of the equivalent dipole representation based on a spherical particle rapidly decreases. Consider a prolate ellipsoidal particle with a long axis, $a$, and equivalent minor axes, $a_2 = a_3$. In general, the effective dipole moment will have three components, one along each axis due to the variation of the electric field along that axis. We have assumed that the particle aligns instantaneously with the field, and that the field varies only along the major axis. In this case, the effective dipole moment will be:

\[
\tilde{p}_{\text{effective},1} = 4\pi a_1 a_2 a_3 e_m f_{CM,1} \tilde{E}_{a,1}
\]

\[
f_{CM,1} = \frac{\tilde{\varepsilon}_p - \tilde{\varepsilon}_m}{3 \left( \tilde{\varepsilon}_m + (\tilde{\varepsilon}_p - \tilde{\varepsilon}_m) L_1 \right)}
\]

\[
L_1 = \frac{a_2^2}{2 a_1^2} e_m \left[ \ln \left( \frac{1 + e}{1 - e} \right) - 2e \right]
\]

\[
e = \sqrt{1 - \frac{a_2^2}{a_1^2}}
\]

where the subscript 1 denotes a component along the 1-axis. $\tilde{p}_{\text{effective},1}$ is the effective dipole moment in the 1-direction. $f_{CM,1}$ is the Clausius-Mossotti factor from our previous representations, but now depends on the axis under consideration. $L_1$ is the “depolarization factor” along the 1-axis [12].

Under typical experimental conditions ($E_0 = 1 V/\mu m$, $2\pi \omega = 1$ MHz, $f_{CM} = 0$ when $2\pi \omega = 0.1$ MHz, $a_2 = a_3 = 1 \mu m$), for a prolate ellipsoid with complex permittivity shown in Table 6.3, a 30% variation in major axis length leads to 10% error in the effective dipole moment. This error quickly rises to 50% as the aspect ratio of the ellipse approaches 2:1. In the limiting case of a long, thin ellipsoid ($e \rightarrow 1$), the relative error between the real $f_{CM}$ and the spherical approximation thereof can be shown to be equal to the spherical approximation of $f_{CM}$, so the relative errors of the spherical approximation approach zero near crossover points, and can range from 1 to -0.5.

\(^3\) A particle consisting of an isotropic material (or an effectively isotropic material as will be discussed later in Section 6.2.1.1) will obtain three effective moments along its major and semimajor axes and experience a torque on each moment. For isotropic materials, the major axis has the largest moment, leading to a torque that will align the major axis with the external field.

\(^4\) For Cartesian coordinates, \([1, 2, 3]\) corresponds to \([x, y, z]\).
### Table 6.3  Typical Experimental Conditions Used To Calculate Errors Associated with the Spherical Approximation

<table>
<thead>
<tr>
<th>Material Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon_m$</td>
<td>$80\varepsilon_0$</td>
</tr>
<tr>
<td>$\varepsilon_s$</td>
<td>$2.6\varepsilon_0$</td>
</tr>
<tr>
<td>$\sigma_m$</td>
<td>$5.5 \mu S/m$</td>
</tr>
<tr>
<td>$\sigma_s$</td>
<td>$100 \mu S/m$</td>
</tr>
</tbody>
</table>

While the errors associated with the spherical approximation can be large, it remains a valuable tool for a first-pass at device design, especially in applications of sorting, screening, or trapping where subtle variations in the magnitude of polarization response are unimportant. In particular, the addition of multipoles and ellipsoidal adjustments does not alter the behavior of the sign of $f_{c_{DEP}}$.

When performing ROT or DEP spectra experiments in an attempt to draw conclusions about particle structure or composition, a practice common in biological assays, spherical models are generally insufficient—ellipsoidal, cylindrical, or spheroidal models are available and allow for more accurate structural inferences.

**Dipole approximation**  When considering the electric field produced by a polarized particle of spherical shape, the characteristic length scale of the system is important. Since higher-order multipole effects drop off more rapidly than dipole fields, the field produced by a nonspherical particle becomes equal to that produced by a spherical particle as the distance from the particle center increases. Close to the particle, however, the effects of higher-order multipoles are apparent. As the characteristic length scale of the device shrinks, owing to reductions in channel dimensions or increases in particle concentration, the contributions of higher-order multipoles becomes significant. Additional multipoles are also important when considering rapidly varying electric fields and nonspherical particles. Several different geometries have been considered in detail in the literature [6, 7, 13–19]. A selection of these references is summarized in Tables 6.4 and 6.5.

The characteristic length scale of the particle and electric field under consideration must be examined to evaluate the applicability of classical DEP representations such as that shown in (6.7). When the electric field varies significantly over the length scale of the particle, then, in order to model the induced moment, we must replace the particle with an equivalent multipole. Green and Jones [17] compare the results from up to 9 moments to analytical solutions for spheres and ellipsoids, and show that higher-order moments are necessary to accurately obtain equivalent potential solutions for nonspherical particles.

While multipolar solutions are necessary when attempting to determine the effective moment or potential field surrounding a particle, the spherical approximation retains significant utility in estimating particle behavior in many experimental settings. The application of these approximations should be undertaken with care in light of the ultimate goal of the experiment. When using DEP for transport, trapping, or screening, the spherical approximation often leads to uniform errors (which are obfuscated by experimental uncertainties) and the qualitative correctness of the spherical approximation often suffices. In screening or electrorotation studies that depend critically on the
### Table 6.4 A Brief Summary of Literature Sources That Deal with Multipole Moment Solutions and Particles of Various Geometries in Dielectrophoresis

<table>
<thead>
<tr>
<th>No.</th>
<th>Author, Year</th>
<th>Title</th>
<th>Technique</th>
</tr>
</thead>
</table>

### Table 6.5 Table 6.4 continued

<table>
<thead>
<tr>
<th>No.</th>
<th>Application</th>
<th>Relevant Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]</td>
<td>The effective $f_{CM}$ of isolated and randomly clustered spherical and cylindrical particles.</td>
<td>For closely spaced particles (clusters) an effective dipole factor can be defined based on the MIM and MGA. Experimentally distinguished DEP characteristics of isogenic mutants. Presented a confocal, multishell, ellipsoidal model for $f_{CM}$.</td>
</tr>
<tr>
<td>[13]</td>
<td>An ellipsoidal, multishell model of <em>E. coli</em> was used to determine the differences between isogenic mutants.</td>
<td>Dielectrophoresis of erythrocytes is predicted using the short-cylinder model for field frequencies between $10^{5}$ and $10^{6}$Hz.</td>
</tr>
<tr>
<td>[14]</td>
<td>Erythrocyte dipole moments are modeled using a short-cylinder model as an approximation.</td>
<td>Predicts a discontinuous electrorotation spectra due to the influence of dipole moments along the three coordinate axes.</td>
</tr>
<tr>
<td>[15]</td>
<td>Traditional formulations for DEP and ER with numeric techniques allow for consideration of the interaction of these phenomena.</td>
<td>The use of ellipsoidal dielectric models of DEP and ER resulted in more accurate interpretation of results. Changes in cell dielectric properties was observed after Smrn of experimentation, a fact that should be considered by other researchers.</td>
</tr>
<tr>
<td>[16]</td>
<td>Ellipsoidal dielectrophoretic and electrorotation spectra more accurately explain experimental data.</td>
<td>Higher order moments for various particle geometries are calculated. Particle shapes include sphere, oblate-and prolate-ellipsoids, cylinder, and erythrocyte. Higher order moments can be used to more accurately determine the DEP force, and are particularly important in the case of non-spherical particles. In addition, the approximation of short-cylinders using prolate spheroids in particular poorly in light of the multipolar analysis.</td>
</tr>
</tbody>
</table>

magnitude of induced polarization, however, the spherical approximation should be examined critically for accuracy.

**Maxwellian equivalent body** An axisymmetric, anisotropic particle can be well-approximated by replacement with a particle with an effective, isotropic, complex permittivity: the Maxwellian equivalent body [20]. Many models assume a homogeneous, isotropic (equivalent) material, and in so doing, also assume that particle structure or isotropy is unimportant. For certain applications, this is true (e.g., separations and trapping applications where these components are not of interest), but when attempting
to predict behavior (rather than measure) or gain insight into changes in internal structure, particle anisotropy can affect particle response through changes in $\tilde{\varepsilon}_{\text{eff}}$ [16, 21–29, 30, 31]. The complex permittivity, as we have defined previously, applies for isotropic materials only. In general, the permittivity takes on a tensorial character, $\tilde{\varepsilon}$. We can define our coordinate axes to coincide with the principal axes of $\tilde{\varepsilon}$, and in so doing, obtain a diagonal matrix. Furthermore, the work of Simeonova et al. [20] showed that the Maxwellian equivalent body can be used in cases where the anisotropy is axisymmetric, yielding:

$$\tilde{\varepsilon}_{\text{eff}} = \tilde{\varepsilon}_{1,\text{effective}} \frac{E_1}{|E|} + \tilde{\varepsilon}_{2,\text{effective}} \frac{E_2}{|E|} + \tilde{\varepsilon}_{3,\text{effective}} \frac{E_3}{|E|}$$  \hspace{1cm} (6.12)

In the specific case where the electric field is linear and along the direction of the major axis, the permittivity tensor will reduce to a scalar component along the direction of the field.

6.2.1.2 Assumptions and approximations for $F_{\text{drag}}$

Spherical approximation

In order to determine the velocity of a particle undergoing DEP, we consider equilibrium between the DEP force and the viscous drag. For low Reynolds number, the viscous drag is given by

$$F_{\text{drag}} = \frac{1}{2} \rho u^2 A_p C_{\text{d, sphere}}$$  \hspace{1cm} (6.13)

$$C_{\text{d, sphere}} = \frac{24}{Re}$$  \hspace{1cm} (6.14)

$$F_{\text{drag}} = 6\pi \mu u a$$  \hspace{1cm} (6.15)

where $A_p = \pi d^2 / 4$ is the particle cross-sectional area perpendicular to the direction of flow, $d = 2a$ is the particle diameter, $Re = \rho ud / \mu$ is the particle Reynolds number, and $C_{\text{d}}$ is the drag coefficient. We also know the analytical form of the DEP force and can balance this against the drag force to give the velocity at equilibrium:

$$F_{\text{DEP}} = \pi \varepsilon_m a^3 [\text{Im}[f_{CM}]] V \left( \tilde{E} \cdot \tilde{E} \right) = F_{\text{drag}}$$  \hspace{1cm} (6.16)

$$\pi \varepsilon_m a^3 [\text{Im}[f_{CM}]] V \left( \tilde{E} \cdot \tilde{E} \right) = \frac{1}{2} \rho u^2 A_p \frac{24 \mu}{\rho u d}$$  \hspace{1cm} (6.17)

\[5\] These references primarily consider electrorotation phenomena rather than transport by dielectrophoresis. However, electrorotation spectra and dielectrophoretic spectra are related by the Kramers-Kröning relationships:

$$\text{Re}[f_{CM}(\omega)] = \frac{2}{\pi} \int_0^\infty \frac{x^3 [f_{CM}(x)]}{x^2 + \omega^2} dx + \text{Re}[f_{CM}]$$

$$\text{Im}[f_{CM}(\omega)] = \frac{2}{\pi} \int_0^\infty \frac{x^3 [f_{CM}(x)] - \text{Im}[f_{CM}]}{x^2 + \omega^2} dx + \text{Im}[f_{CM}]$$

where $f_{CM}$ is the limit of the complex Clausius-Mossotti factor as $\omega \to \infty$ [12].
\[ u = \frac{\varepsilon_a a^2}{6\mu} R \left[ f_{CM} \right] \nabla \left( \bar{E} \cdot \bar{E} \right) \]  

(6.18)

We use an equilibrium relation because the characteristic equilibration time (the time for particles to reach their terminal velocity) is very short compared to experimental time scales. The constant term that relates the dielectrophoretic force to the resulting particle velocity, then, is termed the “dielectrophoretic mobility”:

\[ \mu_{DEP} = \frac{\varepsilon_a a^2}{6\mu} R \left[ f_{CM} \right] \]  

(6.19)

When a particle is not spherical, however, the expression for drag coefficient changes as a function of particle shape and orientation. This variation is expressed through changes in both \( A_p \), the cross-sectional area perpendicular to the flow, and \( C_{gr} \), the drag coefficient based on an effective particle diameter. The effective particle diameter is the diameter of a sphere of equivalent volume. For isotropic materials, particles will typically orient with their long axis parallel to the external electric field.

Consider an ellipsoid with axes \( a_x, a_y, \) and \( a_z (a_x > a_y > a_z) \). The dipole moment of such a particle can be broken up into components along each axis. Moments will be proportional to the effective permittivity (along a particular direction) and the electric field along a particular direction.

\[ p_i \sim \varepsilon \cdot a_i \bar{E} \cdot \hat{a}_i \]  

(6.20)

\( p_i \) is the dipole moment vector along the axis \( i = [1, 2, 3] \), \( \varepsilon \) is the permittivity, \( \bar{E} \) is the electric field, and \( \hat{a}_i \) is a unit vector along one of the axes \( [1, 2, 3] \).

For a nonrotating electric field, the particle will experience torques along each moment, tending to align the particle to the sum of moments. In a rotating electric field, there is still a torque on the particle, however, and orientation is a function of the frequency of rotation in addition to the factors determining the magnitude of torque on each moment of the particle [15].

For the viscous drag on a particle, we present approximate analytic results from [32] for the special case of a prolate spheroid \( (a_z > a_y = a_x) \)—useful due to its similarity to rod-shaped particles:

\[ C_{D, \text{ellip}} = \frac{128}{Re} \frac{(1-e^2)e^3}{2e + (3e^2 - 1)\ln \left( \frac{1+e}{1-e} \right)} \]  

(6.21)

where we have again used the eccentricity, \( e = \sqrt{1 - a_z^2 / a_x^2} \). The resulting dielectrophoretic mobility for prolate spheroid with its long axis parallel to the direction of the applied electric field is found by combining (6.21), (6.11), and (6.6):
\[ \mu_{\text{DIP}} = \frac{2e + (3e^2 - 1)\ln\left(\frac{1+e}{1-e}\right)}{e^2} \frac{a_1 a_2 \varepsilon_m}{24 \mu} \left( \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + (\varepsilon_p - \varepsilon_m) L_3} \right) \]  

\[ L_3 = \frac{a_1^2}{2a_1 e^2} \left[ \ln\left(\frac{1+e}{1-e}\right) - 2e \right] \]  

(6.22)

The first term in parentheses contains geometric corrections to the drag coefficient and in the second term, these corrections are made by including \( L_3 \).

For a rod-shaped bacterium with a 2:1 major/minor axis ratio, the electrodynamic correction is on the order of 20%–40% far from the crossover point, while the drag correction is roughly 60%.

**Infinite domain assumption** In many fluid mechanics and electromagnetics problems, it is common to invoke “boundary conditions at infinity.” For instance, it is useful to argue that as distance from the origin goes to infinity, a parameter of interest (such as velocity potential or electric potential) must converge to an externally imposed value. However, in applications where channel dimensions approach the particle diameter, this assumption is typically poor. The electrodynamics of the problem is influenced by the proximity of the boundaries on the Laplace equation, requiring more terms in the multipole expansion, while the drag relation is influenced by the proximity of the boundaries on the Stokes flow equations, requiring drag adjustments.

As a particle moves through the surrounding fluid, it will exert a pressure on the surrounding fluid. We will consider only incompressible fluids, and so, this pressure will be transduced to fluid velocity. Solutions to the fluid velocity field produced by a moving particle in an infinite fluid are readily available. However, as the moving particle approaches a wall, the fluid velocity field resulting from the moving particle will be retarded due to the no-slip boundary condition at the wall. When the force, \( \vec{F} \), on a particle is directed parallel to the wall, the terminal particle velocity is modified by the scalar coefficient, \( B \):

\[ \vec{u} = \frac{B}{6\pi \eta a} \vec{F} \]  

(6.23)

The scalar coefficient, \( B \), decays to 0 as \( x \to a \), and approaches 1 for \( x > 10a \). A plot of the mobility coefficient shows the retarding effects of the wall as \( x \) increases [33]. At distances greater than one particle radius, the scalar coefficient, \( B \), can be approximated as:

\[ B = \left[ 1 - \frac{9a}{8x} + \frac{1}{2} \left( \frac{a}{x} \right)^3 + \ldots \right] \]  

(6.24)

**Isolated particle assumption** As with the infinite domain assumption discussed above, the assumption that particles are isolated in a media may break down at low interparticle spacing distances, caused by high particle concentrations or by particle localization due to hydrodynamic and/or electrokinetic forces. We examine briefly the
6.3 Materials: equipment for generating electric field nonuniformities and DEP forces

There are myriad different DEP devices and techniques, so much so that dividing them cleanly into categories proves difficult. The dielectrophoretic effect is fundamentally tied to the electric fields, which can be defined by spatial and temporal characteristics, and so we choose to divide DEP approaches by spatial and temporal characteristics accordingly. We will consider DC or sinusoidal AC fields, with variations in temporal (frequency, phase) and spatial (geometry) character, as they relate to the generation of DEP forces.

6.3.1 Electric field frequency

The frequency dependence of the DEP force is perhaps its most alluring feature, and as a result, the majority of DEP techniques employ sinusoidally varying electric fields.

6.3.1.1 AC fields

AC electric fields are the primary field type employed in dielectrophoresis applications, owing to the availability of straightforward analysis and experimental implementation
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techniques. AC fields of high frequency (>10^3 Hz) can be safely applied to microfabricated electrodes in microfluidic channels without significant buildup of electrolytic products (hydrogen and oxygen bubble production at the anode and cathode, respectively), significant changes in temperature, or significant changes in pH.

**Equipment and experimental setup**

The most common and straightforward method for generating a sinusoidal AC electric field is to employ a function generator connected to electrodes. Function generator performance can range from low to high according to the experimental requirements. Higher quality instruments offer finer control over the characteristics of the output waveform (phase, frequency, amplitude) and offer a wider range of operating parameters, achieving higher frequency and power output. The power output of these devices is typically low, on the order of milliwatts for frequencies in the kilohertz range, and tends to decrease as frequency increases beyond one megahertz. These limitations are primarily the result of component slew rate limitations, which will be higher as performance (and cost) increase. In the kilohertz and megahertz range, it is common for generators to offer a DC-offset option, which will allow for the simultaneous use of AC and DC electric field components. This has been utilized by some experimenters to actuate DEP and electrophoresis/electrosome independently [36].

In general, the DEP frequency response characteristic extends beyond the megahertz range, and for experimental applications requiring field frequencies in the gigahertz range, an RF signal generator must be used. These signal generators work quite well for low-power applications, where microfabricated electrodes are used and can generate high electric field gradients due to their close proximity. There are situations where higher-voltage signals must be applied, such as in insulative dielectrophoretic applications, where electrode spacing is necessarily orders of magnitude larger, requiring concomitant increases in applied voltage. General amplification of arbitrary signals is possible for low frequency (~1 kHz) signals at a reasonable cost [36]. In order to amplify higher frequency, arbitrary signals (including a DC-bias) to the kilovolt range, more specialized equipment is currently required. Amplification of high frequency sinusoidal waveforms can be accomplished using RF-amplification equipment.

In this discussion, we have concerned ourselves with only sinusoidal or DC signals, but this does not represent the limits of possible waveforms. Indeed, some researchers have shown that the addition of multiple frequency components (or the higher frequency spectral distributions present in sawtooth, square, or triangular waveforms) can be beneficial to a particular DEP application [37]. Most function generators can generate these waveforms; more complex waveforms, however, require an arbitrary waveform generator.

6.3.1.2 DC fields

While the majority of DEP approaches use sinusoidally varying electric fields with relatively high frequencies, recent advances in microfabrication techniques have made spatial variation of the electric field magnitude possible on relevant length scales (typically in insulative-DEP techniques; see Section 6.3.3.2) and opened the door to the applications of low-and zero-frequency electric fields. DC fields are interesting because they allow combination of linear and nonlinear EK effects and offer simplified analysis and straightforward implementation.
6.3 Materials: equipment for generating electric field nonuniformities and DEP forces

**Equipment and experimental setup**  DC electric fields offer simplicity of implementation, and combine transport of fluid and particles via electroosmosis and electrophoresis. DC electric fields drive electrophoresis and electroosmosis by the familiar mechanisms, and can also drive dielectrophoresis (see Section 6.3.3.2 for an explanation of the mechanism). This combination is convenient, as it allows analyte transport and manipulation with only one field.

DC fields will tend to drive larger currents and lead to the production of electrolytic products at electrode interfaces. For this reason, DC dielectrophoresis applications are typically carried out by placing insulating constrictions in the electric current path and electrodes in external reservoirs, allowing the electrolytic products to escape to the atmosphere [38, 36]. Brask et al., in a non-DEP application, employ palladium electrodes, taking advantage of the high hydrogen permeability of palladium to minimize hydrogen bubble formation at the electrode surface.

**Driving DEP via DC fields from external reservoirs usually requires high voltages. These can be generated and controlled relatively easily with a DC high-voltage power supply, though the electrical resistance of the channel should be considered when planning for power and thermal management of the system. A short, wide channel filled with high conductivity buffer (such as 1M phosphate buffered saline) will have a low electrical resistance. This will lead to a larger current draw from the power supply, increased rates of electrolysis, and increased Joule heating. Lower conductivity solutions and long, narrow, shallow channels will ameliorate these difficulties. As electrophoresis and electroosmosis are both dependent on $\vec{E}$ and dielectrophoresis is dependent on $\nabla \cdot (\vec{E} \cdot \vec{E})$, these effects can be tuned independently using the field magnitude and the geometry of the device (specific geometries and fabrication techniques will be discussed in detail in Section 6.3.3).**

### 6.3.2 Electric field phase

In addition to the field frequency, the phase (and the spatial variation of phase) can be used in system design as well. If the electric field phase ($\phi$) is spatially uniform, then it can be neglected when calculating steady-state dielectrophoretic effects. However, when the phase is spatially nonuniform, additional force and torque components exist, which open up a wider range of possible applications and measurements.

One particularly useful method for taking advantage of spatial variations in phase is what has become known as “traveling-wave” dielectrophoresis (twDEP), which uses a spatially-varying phase to propel particles down a channel. Recall from Section 6.2, (6.10), that the dielectrophoretic force contains two components: the irrotational, “traditional” dielectrophoresis component and the curl-dependent, “traveling-wave” dielectrophoresis component.

The canonical example of twDEP is to use an array of electrodes patterned in a microfluidic channel. The electrode array is composed of alternating, independently driven electrodes with different phase. The electrode array is aligned at an angle to the direction of flow. These signals—irrespective of phase—are used to levitate particles against gravity within the flow field due to irrotational, negative dielectrophoresis, and the varying phase is used to drive particles transverse to the direction of flow according to the imaginary component of the Clausius-Mossotti factor.
In much the same way that spatially varying phase leads to traveling wave dielectrophoresis, a rotating electric field will induce a particle “electrorotation” response. In this case, the dielectrophoretic effect is instead expressed as a torque and is dependent on a different aspect of the particle’s electrical properties, namely the imaginary component of the Clausius-Mossotti factor ($\Im \{ \tilde{F}_{\text{CM}} \}$).

$$\langle \tau \rangle = -4\pi \varepsilon_0 a^2 \Im \{ \tilde{F}_{\text{CM}} \} \left[ \Im \{ \tilde{E} \} \times \Im \{ \tilde{E} \} \right]$$ (6.27)

The results of an electrorotation experiment typically consist of measured rotation rates as a function of electric field rotation frequency, or “ROT spectra.” ROT spectra are related to DEP spectra by the Kramers-Krönig relation (see footnote 5), and thus ROT spectra have large magnitudes at the frequencies where DEP forces are changing.

**Equipment and experimental setups** Creating spatially varying phase signals in microfluidic systems is most often accomplished using arrays of interdigitated electrodes. By applying a different phase to various electrodes in an array, a spatially varying field is produced. The electrode array itself is often fabricated at an angle to the microfluidic channel, because the twDEP forces that are created drive particles perpendicular to electrode orientation. This configuration leads to sorting as a function of the imaginary component of the Clausius-Mossotti factor transverse to the direction of flow. The electric signals applied to elements of the electrode array generally consist of four sinusoidal signals with phase magnitudes $\phi = 0$, $\pi/2$, $\pi$, and $3\pi/2$, in order. This ABCD-configuration effectively generates an electric field maximum that propagates perpendicular to the array at a frequency equal to the driving frequency. Chang et al. showed that separating particles with similar (but distinct) $f_{\text{CM}}(\omega)$ functions via twDEP techniques can be improved by using two (or more) signals simultaneously, with different frequency. The first frequency is chosen to optimize the levitation of particles above the electrodes (i.e., $\Re \{ f_{\text{CM}}(\omega) \} < 0$ for both particles), and the second is chosen to optimize the transport of particles along the array as a function of $\Im \{ f_{\text{CM}}(\omega) \}$[37].

The design and fabrication of twDEP devices is slightly more challenging than simpler electrode configurations because of the need for four different signal paths. This requires multilayer fabrication techniques, but these are well understood and can be readily accomplished in appropriate clean-room facilities.

Rotating electric fields can be created by forming a quadrupolar configuration and driving each electrode at a different phase (again, $\phi = 0$, $\pi/2$, $\pi$, and $3\pi/2$ form the components of the ABCD configuration). Fabrication of these devices is single-layer process, and can be accomplished using standard photolithographic techniques. However, the planar quadrupolar configuration can induce nDEP and force particles away from the electrodes (in the z-axis direction, if the electrodes are in the x-y plane). Particles will then be out of the range of influence of the electrodes or trapped against the microchannel wall, inducing a force that can confound a ROT spectra analysis. This difficulty has been overcome by using an octode cage: two planar, quadrupolar electrode arrays are assembled facing one another, displaced in the z-axis direction [39]. This configuration creates an electric field null in the center of the three-dimensional configuration. This is effective for trapping, but a field null yields no net torque. In order to conduct the electrorotation study, one quadrupolar array is offset by a few degrees.
(rotated about the z-axis in space or by adding a phase increment between the signals driving the quadrupolar arrays), creating a minimum, not a null, in the center of the octode cage [39, 40].

In short, electrorotation and twDEP are two useful techniques that depend on the phase of the electric field and the imaginary component of the Clausius-Mossotti factor, $\Im[f_{ca}]$. Electrorotation studies can be used to gain a large amount of information about an individual particle. ROT spectra can be used to determine $\Im[f_{ca}]$, and $\Re[f_{ca}]$ can be found via the Kramers-Kröning relationships (see footnote 5). Quadrupolar arrays and other ROT measurement techniques work well for single particle studies, but have low throughput, making them inappropriate for sorting applications. twDEP has been used to successfully separate particles transverse to the direction of fluid flow, making it an effective continuous-flow sorting technique. Combined with multiple frequencies, twDEP can be applied to a wide range of experimental conditions, with judicious choice of the driving waveform [37].

### 6.3.3 Geometry

The temporal aspects (frequency and phase) of electric fields in DEP systems are, of course, critical. However, the magnitude of the DEP force in micro- and nanoscale systems is central to DEP’s burgeoning importance in the last 10 years. Micro- and nanofabrication techniques have opened the door to applications of DEP at the cellular length scale, where subtle variations in particle composition and changes in the dielectrophoretic response can be observed on a particle-by-particle basis. By altering the geometry of energized electrodes or the channel boundaries that define the electric current path, dielectrophoretic effects can be tuned for numerous applications.

#### 6.3.3.1 Electrode configurations

As previously discussed, changing the shape and orientation of electrodes, in addition to modulating the frequency and phase applied, can give rise to dielectrophoretic particle trapping, dielectrophoretic sorting, electrorotation, and traveling wave dielectrophoresis effects. Specific geometries will be discussed in Section 6.4.

Castellanos et al. examined the case of two adjacent electrodes in detail in [41]. Their results detail the relative effects of DEP and hydrodynamics on the motion of particles adjacent to the electrode array (Figure 6.1). The electrodes were energized with a 5V potential and separated by a distance of 25 μm. Their findings concluded that, in general, motion due to gravity and dielectrophoresis vary with particle volume, and dielectrophoresis will dominate provided an appropriate electric potential is chosen (frequency and magnitude). Brownian motion decreases with increasing particle size, depending on 1/a. For particle sizes in the micron regime, this can be overcome by dielectrophoresis with a relatively low electric field. A similar argument can be made for the effects of buoyancy.

Depending on the depth of the channel, both positive- and negative-dielectrophoresis can be observed in electrode-based systems. Positive dielectrophoresis (pDEP) is observed as some fraction of passing particles become trapped on the electrode structures. Negative dielectrophoresis (nDEP) will only be observable if the distance between the electrode array and the opposite channel wall is short, and particles become trapped against the channel wall owing to nDEP and hydrodynamic forces. If the chan-
nel is too deep, particles will be forced away from the array (by dielectrophoresis) and across (by hydrodynamic forces, either electrokinetic or pressure driven). In the absence of trapping (i.e., in a deep channel), it can be difficult to characterize the magnitude of nDEP forces, as deflection toward or away from the viewer is difficult to quantify in traditional microscopy.

**Equipment and experimental setup** The fabrication of arbitrary electrode geometries has been an integral part of the development of dielectrophoresis as a viable technique for particle manipulation. The patterning techniques used are those of the microfabrication or MEMS industry, with electrode structures being fabricated via sputtering or electron-beam evaporation of metals, usually gold.

Devices with three-dimensional elements must be fabricated in planar configurations and then subsequently aligned using a mask aligner and bonded. The primary difficulty associated with octode cages (and other such three-dimensional structures) is that associated with visualization of trapped or sorted particles. Also, fabricating arrays of these structures can only be accomplished through complicated multilayer fabrication processes. Other geometries—point-lid, circle-dot, and grid-electrode—also require the use of multiple layers of electrodes leading to a concomitant increase in the complexity of device fabrication. In situ fabrication of three-dimensional dielectrophoretic traps in an array-able format has been achieved by electroplating gold onto an SU-8 mold [42]. The resulting post-trap configuration, allows for easier array fabrication and visualization of trapped particles.

Typically, electrodes are fabricated on glass substrates owing to the well-defined deposition process and visualization capabilities. Depositing metal onto the surface,

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7 Gold is chosen because it offers good conductivity, low production of electrolytic products at a reasonable cost (compared to platinum or palladium), and has well-established protocols for deposition.
6.3 Materials: equipment for generating electric field nonuniformities and DEP forces

however, limits the efficacy of traditional diffusion-based wafer-to-wafer bonding techniques, and so PDMS covers are generally used to define microfluidic channels [43–52]. Polymers other than PDMS have also been used, but adhesion remains a difficulty.

6.3.3.2 Insulative configurations

Insulative dielectrophoresis refers to a subset of DEP techniques that use insulating constrictions in the electric current path to create regions of high or low electric field magnitude. This is done by altering microchannel geometry in a glass, silicone, or plastic substrate. A simple analysis demonstrates the concept: consider a microchannel made of insulating material with two different regions filled with a conducting fluid. Treating each region separately as a one-dimensional problem, we can apply Ohm’s law and find, for the electric fields in region 1 and 2:

$$\vec{E} = \nabla V \rightarrow \vec{E}_n = E_n \hat{x} = \frac{V_n}{L_n} \hat{x}$$  \hspace{1cm} (6.28)

$$E_1 = \frac{V - V_2}{\frac{V}{w_1 d}} \frac{\alpha L_1}{w_1 d}$$  \hspace{1cm} (6.29)

$$E_2 = \frac{V_2}{\frac{V}{w_2 d}} \frac{\alpha L_2}{w_2 d}$$  \hspace{1cm} (6.30)

Solving for $V_2$ and substituting, we can determine a relationship between $E_1$ and $E_2$ as a function of the channel geometry:

$$E_2 = E_1 \frac{w_1}{w_2}$$  \hspace{1cm} (6.31)

This is the fundamental principle that leads to the utility of insulative DEP (iDEP) configurations. In addition, there are practical advantages to iDEP; the lack of internal electrodes simplifies fabrication considerably, as electrodes are typically placed in reservoirs external to the microfluidic channel. External electrodes avoid complications due to electrolysis and electrolytic products at low frequencies (which can occur when electrodes are placed inside microfluidic channels), allowing for low-frequency operation.

Insulative or electrodeless dielectrophoresis has been primarily used to concentrate samples for later analysis, but we will also explore some designs that accomplish separation based on particle characteristics.

**Equipment and experimental setup** The great appeal of iDEP devices is due to the simplicity of their operation and design. Their fundamental mode of operation does not involve integrated electrodes, inducing variation in electric field gradients via variations in channel geometry. Without integrated electrodes, devices can be fashioned from a single material, greatly simplifying the fabrication process. This process will vary depending on the substrate, but can often be accomplished on the benchtop, outside of a cleanroom.
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Substrate choice will depend on the needs of the experiment. Glass devices are well characterized, with well-known physical and electrokinetic properties. Fabrication techniques for glass are well established, but are usually limited to quasi-two-dimensional configurations due to available wet etching techniques. Multilevel glass devices require careful alignment of photomasks to achieve multiple etch steps [53, 54]. Polymeric devices—polystyrene, Zeonor, TOPAS—have seen increasing use recently, owing to their low cost and greater flexibility. They achieve greater flexibility by leveraging existing silicon fabrication techniques and using the silicon device as a master for hot-embossing [36]. PDMS—a seemingly ubiquitous substrate for microfluidic devices—has not been used to a large degree in iDEP devices. This is due to its geometric variability due to high compliance and relatively high ion permeability (and resulting conductivity) making it a poor insulator. Glass devices, while more difficult to fabricate, can be reused consistently and will perform consistently, given their detailed characterization. Polymeric devices, on the other hand, offer simpler fabrication and lower cost, lending themselves to disposable applications.

6.4 Methods: data acquisition, anticipated results, and interpretation

In this section, we provide general recommendations for DEP techniques, followed by specific protocols for select applications. We have divided the protocols into two main classes based on device characteristics: (1) electrode-based, and (2) electrodeless or insulating devices. First, we will outline some general considerations when working with each type of device. Following that, we examine some of the more common, representative experiments and summarize the relevant experimental parameters for each.

6.4.1 General considerations for dielectrophoretic devices

Electrolysis

Metal electrodes in aqueous media can lead to the generation of gas bubbles at the electrode-media interface. In order to conduct current through the media between electrodes, electrons are donated at the anode, creating anions and/or eliminating cations; the reverse then occurs at the cathode to return electrons to the circuit. These Faradaic reactions (often electrolysis of water) generally produce gaseous products near this interface. In many cases, the amount of gas generated is small enough to remain in solution, but the characteristically small volumes used in most eDEP devices lead to decreased storage capacity for these products, and bubbles can form as a result. The specific chemical reaction that is responsible for gas generation depends on the media and the metal used in electrode fabrication. The relevant parameter for estimating the magnitude of these effects is typically current or current density per half cycle, so high-frequency techniques have little or no problems, while low frequency techniques can be challenging. iDEP devices have fewer problems with bubble generation due to electrolysis because electrodes are placed in relatively large, open, external reservoirs.
6.4 Methods: data acquisition, anticipated results, and interpretation

Media conductivity

Electrolyte concentration influences media conductivity (and therefore electric current and electrolysis), as well as the viability of biological samples. When working with cells or other biological samples, suspending solutions generally contain high concentrations of salts (~1M). High conductivity buffers have low electrical resistance, thus requiring more current to maintain an applied electric potential. Two major confounding effects are associated with large electric currents in dielectrophoresis devices: Faradaic reactions (discussed above) and Joule heating.

Joule heating is the result of ions flowing through the media, leading to a volumetric heat transfer rate that is proportional to the media conductivity and the square of the applied potential:\footnote{It is important to note that “high conductivity” buffers are still poor conductors when compared to the conductivity of metal electrodes. Because of this, electric potential changes occur almost entirely across the media, driving the electric field source term (6.32) in the metal electrode to zero.}

\[
q^v \sim (\nabla v)^2 \sigma \tag{6.32}
\]

where \(q^v\) is the volumetric heat transfer rate to the fluid as the result of the applied electric potential \(v\). The total heat transfer increases with increasing conductivity, and the total integrated heat transfer will increase over time as the system heats up\footnote{This equation only models the heat transferred to the fluid media; a complete analysis will include the heat transferred from the fluid to its surroundings. This relationship is intended to illustrate the dependence of heating on potential and media conductivity. A full solution will also consider the dependence of conductivity on temperature.}.

Electrode configurations in iDEP and eDEP devices vary; eDEP devices have closely spaced electrodes in a microfluidic channel, while iDEP devices have electrodes placed in external reservoirs. The large interelectrode distance in iDEP devices means that the applied electric potential may be orders of magnitude larger than in eDEP devices; however, the electric field magnitude will tend to be comparable in both types of devices, and Joule heating can occur in both cases.

Thermal effects

Variations in temperature, in both iDEP and eDEP devices, can influence a number of experimental parameters (e.g., conductivity, viscosity, and cell viability). We will briefly outline potential sources for temperature variation, possible outcomes, and strategies for maintaining isothermal conditions. Most researchers consider isothermal conditions within their devices—as we have above. Depending on experimental parameters, however, heating may become important, and the isothermal assumption may fail to explain certain results.

Given the electric field source term of Joule heating (6.32), any device region with high field magnitude is a potential source of thermal fluctuations. iDEP techniques can experience local changes in temperature in constricted regions, far from electrodes. Electrode-based DEP techniques can also generate temperature changes, especially near electrodes where current densities are typically highest.

Changes in temperature lead to myriad changes in experimental parameters, including fluid viscosity and conductivity. Changes in viscosity change the particle drag coeffi-
cient, making measurements of dielectrophoretic or electrophoretic particle mobilities inaccurate. Local changes in viscosity, $\eta$, due to temperature variation, lead to changes in the local fluid Reynolds number and complicates the normally tractable low-Reynolds number Navier-Stokes equations. Even a $5^\circ$C temperature change will lead to a 10% difference in the viscosity of water [55]. Fluid conductivity is also a function of temperature; the conductivity of electrolyte solutions at 25°C increases approximately 2%/°C. Impurities tend to buffer these variations in conductivity, so variations should be verified experimentally in temperature-sensitive experiments. Local changes in conductivity create locally varying electric fields, which can complicate dielectrophoretic force predictions and lead to nonlinear fluid currents and vortices [56, 57].

There are a few strategies for avoiding thermal variations in dielectrophoresis experiments. Lower conductivity buffer solutions can ameliorate the effects of high electric fields; many DEP experiments use deionized water as the running buffer for this reason. The dependence of dielectrophoretic effects on the gradient of the electric field also suggests that decreasing the characteristic length scale of a particular device may improve performance at lower electric field magnitudes [41]. Finally, choice of substrate can also improve thermal performance. A material that readily absorbs and dissipates heat (such as glass) will offer improved thermal performance over plastics (polycarbonate, cyclic-olefin polymers, etc.).

**Particle adhesion**

Depending on the substrate and particle, particle adhesion left unmanaged may be significant and, in some cases, prohibitive. Many techniques are available to ameliorate the effects of adhesion, such as surface coatings or surfactants in solution. An in-depth discussion of this topic is beyond the scope of this text; indeed there is a large body of literature on the subject [58], but we discuss a few common adhesion mechanisms, such as hydrophobic, electrostatic, and chemical interactions.

The hydrophobic properties of a particle-substrate system can affect particle adhesion. In an aqueous solution, hydrophobic substrates will promote adhesion of hydrophobic particles. Polystyrene particles are hydrophobic and, as a result, can adhere to hydrophobic substrates such as polycarbonate, cyclic-olefin polymers (TOPAS, Zeonor), and PDMS. Finally, biological samples present a complicated set of interactions that will depend on the specific analyte, which can be either hydrophobic or hydrophilic, positively or negatively charged, and have complicated chemical interactions with the substrate. Bioparticle adhesion is a broad topic that is covered extensively in the literature and remains an active area of research [59, 60].

Charge-carrying particles and surfaces will interact with one another electrostatically. Carboxylate-modified polystyrene particles carry a negative surface charge, which will repel them from negatively charged surfaces. The opposite is true for some amine-functionalized particles. Biological particles are invariably zwitterionic but usually net negative. Charge characteristics will also vary as a function of solvent pH, which will change the charge state and surface potential of both particle and substrate.

Certain applications, such as antibody assays and ELISA tests, purposely promote the adhesion of specific particles via chemical interactions. Such adhesion reactions depend on a number of conditions: pH, concentration of product and reactants, temperature, and interaction time.
Transport
In both iDEP and eDEP devices, analyte transport can impact device operation. A fundamental challenge when dealing with small planar electrodes in eDEP devices is how to bring particle suspensions into close enough proximity that the electric field variations caused by the electrode can manipulate the particles. Potential solutions are to use small channels or particle focusing techniques. Transport of fluid and analyte in iDEP systems is also of critical importance. Where pressure-driven flow is used, variations in channel geometry that create electric field gradients can break the similitude of electrical and fluidic potential field solutions, leading to difficulties in predicting particle paths. Alternatives are to use very low flow rates in pressure driven flow, or to use electro-osmotic/electrophoretic effects for media and analyte transport.

Small channel dimensions are the most common solution to ensure that particles interact with DEP forces. However, as channel dimensions shrink, the point approximation for particles (meaning particle motion does not impact fluid behavior) becomes less applicable. A good rule of thumb is that if the bulk DEP relations presented in this chapter are to be used, channel dimensions should be at least ten times larger than particle diameter\(^{30}\).

Particle-focusing techniques can be used, in both eDEP and iDEP devices, to ensure interaction between particles and DEP forces. Fluid flow focusing techniques use variable volumetric flow rates in multiple input channels to control the location of particles in the flow downstream of the intersection. Three (or more) channels converge near the dielectrophoretic section of the device. Two channels contain “sheath fluid” which does not contain any particles while the third channel contains the analyte particles. By setting the volumetric flow rates of the sheath fluid channels higher than the flow rate of the analyte channel, the fluid injected from the analyte channel will be focused by a factor of the ratio of volumetric flow rates [61].

Fabrication uncertainty
Insulative or electrodeless DEP techniques rely on variations in channel geometry defined by an electrically insulating substrate. Changes in channel geometry leading to changes in electric field magnitude can be estimated by defining a characteristic “constriction ratio” of bulk channel dimensions over constricted channel dimensions. The increase in electric field magnitude due to constrictions in channel geometry will be proportional to the constriction ratio [36]. Variations in channel geometry from manufacturing defects or mechanical strain before or during testing can lead to significant operational changes within the device. Due to the potential significance of these defects, substrate choices should include consideration of material properties such as hardness and modulus. A low modulus material, such as PDMS, might “sag” in wide, long channels (over 1 cm\(^3\)). The quality of channels fabricated in high modulus materials such as polycarbonate or Zeonor avoid this issue. Esch et al. examined the quality of devices fabricated with hot-embossing techniques—often used to fabricate devices in these high-modulus materials—as a function of master material choice (e.g., silicon, SU-8, copper) and device design [62]. They found that wet etching of the master material will yield nanometer-scale surface roughness that will transfer to the plastic, and is not recommended for dielectrophoresis applications. Also, high aspect ratio features and

\(^{30}\) If trapping of particles becomes significant and agglomeration occurs, the effective diameter of agglomerates must be considered.
designs with high feature density will be prone to defects at corners and near features on the order of 5 \mu m. Similar confounding effects are observed in eDEP devices. Variations in channel geometry, due to channel “sag,” or imperfections in electrode geometry can also lead to erratic electrokinetic effects.

### 6.4.2 Electrode-based dielectrophoresis

Given the general considerations above, we will now examine more specific applications. We will cover canonical electrode-based dielectrophoresis techniques and describe example experimental recipes. For each technique, we will summarize the general configuration of the experiment and then describe what the reader might expect to observe while conducting the experiment. Throughout this section, we will refer to Tables 6.6 and 6.8, which summarize experimental device parameters and solution parameters, respectively. Each experimental technique is identified with a number that corresponds to entries in Tables 6.6 and 6.8 at the end of this section.

#### 6.4.2.1 Filtering/binary sorting

Binary sorting or filtering is one of the most common applications of dielectrophoresis. In these experiments, dielectrophoresis is used to separate one type of particle from a flowing solution based on size or material properties. In this way, a subset of particles (or all particles) are removed from a flowing solution. The goal of these experiments is either to remove a contaminant from solution or to capture particles for analysis.

**Experiment ID #1: interdigitated electrode array**

**Discussion:** The interdigitated electrode array is one of the most common electrode configurations used in DEP studies. The electrode array consists of two sets of electrodes, grounded and energized, that alternate spatially. This creates a nonuniform field in the region of the electrode array that can be used to trap particles against a flow.

Interdigitated electrode arrays have been used extensively to retain particles of interest from a microchannel flow, or to filter out unwanted particles from an analyte stream. Electrodes are typically gold and patterned on a glass substrate via a lift-off procedure. There are only a few relevant design parameters to optimize: electrode width, interelectrode distance, electrode length, and fluidic channel depth. With these parameters set, variations in applied electric field magnitude and frequency are left to vary in the experiment.

Electric fields and particle motion are easily modeled (and have been calculated analytically in [63]) in electrode systems and can lead to consistent results when accurate particle models are used [41, 64]. The advantages associated with planar, interdigitated electrode arrays are simplicity of fabrication and analysis. Disadvantages of the interdigitated electrode array are the potential for permanent particle adhesion during positive dielectrophoresis and the inherent “binary” separation achieved under a particular set of experimental conditions.

Trapping in these devices is usually due to pDEP, which attracts particles to electrodes. Occasionally, these devices will operate via nDEP, repelling particles from the array and trapping them against the opposing channel wall, but this regime is less common and more difficult to quantify. The interdigitated electrode array is enclosed in a
channel of some sort to sustain pressure driven flow. A common and well-characterized channel can be fabricated using a silicon master and PDMS. Fabrication of the PDMS channel is covered briefly in Chapters 1, 2, and 3. Bonding the PDMS channel to the glass substrate is generally carried out by plasma cleaning, taking care to align the channel perpendicular to the electrode array. Once the channel is bonded to the glass device, particles can be introduced in a dilute suspension via a syringe pump.

**Methods:** For the device characteristics described in Table 6.6 and solution characteristics described in Table 6.8, we would typically observe the following. As particles flow past the interdigitated electrode array, application of an electric field at a frequency of 10 kHz will induce positive dielectrophoresis, attracting particles from the fluid to the electrodes. Particle accumulation may be observed via bright field or fluorescence microscopy. Changing the electric field frequency to 1 MHz will induce negative dielectrophoresis, reversing the direction of dielectrophoresis and forcing particles into the stream; accumulated particles will be effectively released.

### 6.4.2.2 Trapping

Several electrode configurations have been used by investigators to trap single particles or small populations for close observation or manipulation \[39, 42, 65–68\]. In general, these devices are used to examine the response of samples to changes in buffer solution or other external stimulus on a particle-by-particle basis.

**Experimental ID #2: quadrupole traps**

**Discussion:** Quadrupole traps consist of four electrodes placed in an “x” or “cross” configuration. Opposite electrodes are energized, with the remaining two grounded or energized 180° out of phase. This configuration creates an electric field minimum in the center of the array and a maximum at the region of closest interaction between adjacent electrodes. A device in this configuration will trap particles via positive dielectrophoresis in the high field regions. Particles repelled from the high field regions by negative dielectrophoresis may be observable near the central minimum, but typical channel dimensions are large such that particles will be forced away from the plane of the electrodes. A channel depth of approximately 10 μm would be necessary to observe particle aggregation via nDEP for micron-scale particles and the experimental parameters listed in Table 6.6. The quadrupole trap is typically used to trap particles via positive dielectrophoresis from a static suspension. Therefore, the channel geometry is largely irrelevant for pDEP, quadrupole trap configurations.

**Methods:** Quadrupolar traps tend to aggregate particles in the high field regions between adjacent electrodes due to positive dielectrophoresis. Particles are introduced using a syringe pump or by applying pressure by hand, but observation takes place at zero flow rate. For device parameters found in Table 6.6 and particle and solution details found in Table 6.8, particle aggregation will be observed between the electrodes for electric field frequencies between 10 kHz and 1 MHz. Below 8 kHz, no aggregation will be observed, due to dissipation from negative dielectrophoresis, unless channel depth is small, as discussed above.
Dielectrophoresis for Particle and Cell Manipulations

Experiment ID #3: circle/dot traps

**Discussion:** Electrode-based DEP traps generally consist of geometries that tend to trap single particles. The goal is usually to create a system of addressable particle traps to observe individual particle responses to a stimulus or to study biological particle interactions as a function of distance. Methods used to achieve addressable trapping include quadrupole cages, circle-dot, and “DEP microwell” geometries (Figure 6.2) [69].

Common to all of these geometries is the goal of trapping an individual particle or a small number of particles in one location. To achieve this, electrode geometries are designed to create pointlike regions of high (or low) electric field magnitude. Individual addressing is possible in many cases, but usually limited by fabrication of control lines or addressing traces [70].

Circle/dot traps are a single-particle trapping technique that benefits from easy multiplexing and parallelization. Traps are formed by a two-layer lithographic technique and consist of a conducting “dot” exposed on one layer, surrounded by a circle fabricated on a second layer. This configuration traps particles via positive dielectrophoresis, drawing particles into the high field region in the center (dot). A key advantage of this technique is that particles of interest can be released into the flowing

![Image of microchannel and DEP traps](image-url)

**Figure 6.2** (a–c) Various electrode-based DEP trap geometries.
buffer by grounding a particular row and column, and collected in an output reservoir or via another collection scheme. This allows easier addressing of individual traps in large arrays. Typical addressing scales with the number of traps, but in this case, it scales with the square root of the number of traps [70].

**Methods:** Device operation involves sample loading, trapping, extra sample clearing, and release phases. Sample can be loaded by filling the device with particles and energizing circle electrodes (columns) to $+2V_{peak}$ and dot electrodes (rows) to $-2V_{peak}$ at a frequency of 1 MHz. The dielectrophoretic trapping force can be modulated by changing the voltage on the circle and/or the dot. The strongest trapping forces will occur when both the dot and circle are energized. If either the circle or the dot is grounded, the dielectrophoretic force will be reduced, but not zero. The analyte suspension can be introduced until all traps are filled. After the loading phase, the excess analyte can be removed by flowing buffer solution over the array at a relatively low flow rate (0.06 mL/hr). This flow should continue through the release phase. If both circle and dot are grounded, then the dielectrophoretic force will be zero, and any particle in the trap will be released. This configuration will release only particles at the intersection of a grounded row and a grounded column. Observations can be made on a particle-by-particle basis in each of these traps while varying the buffer solution.

**Experiment ID #4: castellated electrode traps**

**Discussion:** Castellated electrode arrays have been used to align or trap particles. They consist of interdigitated electrodes with “castellations” along their length. This creates alternating regions of high and low electric field magnitude, corresponding to regions of close proximity between castellations and regions of separation in between castellations, respectively. Arrays can be formed by aligned or offset castellated electrodes placed adjacent to one another.

Castellated electrode arrays have been used to focus particles from a well-mixed solution into a line of particles for subsequent analysis. This technique has been used in DEP sorting devices and micro-scale dielectric spectroscopy experiments, where the electrical response of individual particles is examined [71, 72]. Both straight and castellated interdigitated electrode arrays can sustain a fluid flow and potentially trap particles against fluid drag forces using DEP. This characteristic has been used to measure not only the sign, but also the magnitude, of DEP forces as a function of frequency. This is accomplished by measuring the number of particles collected by the array at varying electric field frequencies. Castellated electrode arrays, however, are most typically used to concentrate samples (in the high electric field regions, under positive DEP) or to pattern particles at a specific location. The advantage of castellated interdigitated electrodes is the localization of high electric field regions.

In our experimental example, Sebastian et al. utilized aligned, castellated electrode arrays to create localized aggregates of mammalian cells. Such aggregates “could be used as artificial microniches for the study of interactions between cells” [73]. Experimenters trapped layers of stromal cells and Jurkat T-lymphocytes in the high field regions.

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11 This is equivalent to a 180° phase differential between the circle and dot.
between castellations (Figure 6.3). Trapping fields were maintained until three-dimensional aggregates were formed containing both cell types.

**Methods:** Aggregation of mammalian cells (described in Table 6.6) using this array (described in Table 6.8) can be demonstrated in two phases: sample loading and sample aggregation. During the sample loading phase, cell suspensions can be introduced to a flow chamber via a syringe pump. In the aggregation phase, the electrodes can be energized at 1 MHz, to voltages ranging from 0 V\textsubscript{peak} to 20 V\textsubscript{peak}, and the cells can aggregate between castellations for 5 to 12 minutes. During this time, low conductivity, 480-mM D-sorbitol solution should be slowly circulated throughout the flow chamber to wash away ions released from the cells, preventing changes in the local conductivity that would confound dielectrophoretic trapping effects [73].

### 6.4.2.3 Sorting

Electrode-based designs used to sort particles based on size or frequency-response are less common than trapping techniques, but have still been well characterized in the literature [74–76]. These designs typically involve a spatially varying parameter such as electrode geometry or electric field phase that leads to a corresponding distribution of particles. We present two of the most conceptually illustrative techniques.

**Experiment ID #5: angled or curved electrodes**

**Discussion:** Angled electrodes can be used to separate particles based on DEP response or as a preconcentration system to create a localized stream of particles. Castellated electrodes have been used as “concentrators” as well, but the mechanism is slightly different. Castellated electrodes act on particles using negative dielectrophoresis (referring to the sign of $f_{\text{extr}}$, meaning particles are directed away from regions of high electric field) and focus by the cumulative action of a series of high field regions generated between two parallel castellated electrodes. Angled electrodes, in contrast, rely on negative dielectrophoresis to trap particles against fluidic drag forces. Dielectrophoretic forces and fluidic drag forces parallel to the direction of flow balance, and a net force parallel to the electrode results.

The parameters that determine particle behavior in this case are particle size (nearly always a significant contributor to DEP response; see (6.10) and Section 6.2.1.1), flow

![Figure 6.3](image-url)  
(a) Bright field image of cell aggregates formed between castellated electrodes. (b) Red fluorescent labeled Jurkat cells trapped close to the castellated electrode array. (c) Green fluorescent labeled stromal cells trapped on top of Jurkat cells. (From [73].) Copyright Institute of Physics. Used with permission.
velocity, particle position within flow (fluid velocity will vary with distance from channel walls in Poiseuille flow), electric potential, electric field frequency (through $f_{cell}$), and particle properties (again, through $f_{cell}$).

Angled electrodes have a few advantages that have led researchers to utilize them to preferentially guide particles or trap them. By angling the electrodes, with reference to the channel, it is possible to take particles trapped against a channel wall by nDEP (see Section 6.4.1.1) and displace them transverse to the direction of flow. In this manner, they have been used to preferentially direct particles to different outlets or focus particles into concentrated streams [77–80].

Schnelle et al. extended the concepts of angled electrodes and achieved a continuous flow separation by creating curved electrodes patterned on the top and bottom of a microfluidic channel. The curved electrodes were aligned with each other on top and bottom and shaped as shown in Figure 6.4 [39]. This curved electrode configuration balances negative dielectrophoresis against fluidic drag forces to achieve spatial separation of analytes in a continuous flow regime. The nDEP force generated at the electrodes is directed parallel to the radius of curvature of the electrode while the fluid drag force is directed parallel to the channel walls. The component of the nDEP force parallel to the direction of fluid flow will tend to trap or stop the forward motion of the particle. The component of the nDEP force perpendicular to the direction of flow will tend to move particles along the electrode. As the local angle of the electrode changes, the nDEP force opposing fluid drag decreases, eventually allowing the particle to pass the electrodes.

**Methods:** This configuration can be used to achieve continuous-flow separation of polystyrene particles as a function of size [74]. Sample experimental device and solution parameters are listed in Tables 6.6 and 6.8. A phosphate buffered saline solution with polystyrene particles of varying diameter can be introduced to the channel by constant pressure-driven flow. Electrodes with angled and curved geometries can be energized to

<table>
<thead>
<tr>
<th>ID</th>
<th>Application</th>
<th>Configuration</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Binary Sorting</td>
<td>Interdigitated Electrode Array</td>
<td>Gold electrodes on glass, channel in PDMS</td>
</tr>
<tr>
<td>2</td>
<td>Trapping</td>
<td>Quadrupolar Electrode Array</td>
<td>Gold electrodes on glass, channel in PDMS</td>
</tr>
<tr>
<td>3</td>
<td>Trapping</td>
<td>Circle-Dot Electrode Array</td>
<td>Gold electrodes on Si/SiO₂ (double layer fabrication); Channel in 3M tape/glass coverslip</td>
</tr>
<tr>
<td>4</td>
<td>Trapping</td>
<td>Aligned Castellated Electrode Array</td>
<td>ITO Electrodes on glass; Channel in tape/glass coverslip</td>
</tr>
<tr>
<td>5</td>
<td>Sorting</td>
<td>Paired Curved Electrodes</td>
<td>Gold electrodes on glass; channel in glass</td>
</tr>
<tr>
<td>6</td>
<td>Sorting</td>
<td>Traveling-wave dielectrophoresis</td>
<td>Gold electrodes on glass/Si₃N₄ insulator (double layer fabrication); channel in SU-8 and PDMS</td>
</tr>
<tr>
<td>7</td>
<td>Electrorotation</td>
<td>Polynomial quadrupolar electrodes</td>
<td>Gold electrodes on glass</td>
</tr>
<tr>
<td>8</td>
<td>Concentration</td>
<td>Insulating Post Array</td>
<td>Isotropically etched glass, channel in bonded glass coverslip</td>
</tr>
<tr>
<td>9</td>
<td>Sorting</td>
<td>Curved Insulating Constriction</td>
<td>Zeonor 1020R</td>
</tr>
</tbody>
</table>
5.3 V$_{ac}$ to manipulate the particles. As the particles enter the device, they can be concentrated into a stream at the edge of the channel via a straight-angled electrode “aligner.” The mechanism here is similar to that previously described for interdigitated electrodes. The “aligning” electrode pair can be designed to be wider than the sorting (curved) electrode pair, leading to higher nDEP forces at the same voltage compared to the narrower curved electrodes [74]. Once aligned at the edge of the channel, particles can then be deflected by the sorting (curved) electrode pair, resulting in a spatial separation that depends on the local fluid drag and dielectrophoretic forces. Depending on device design, output flow can be divided into a number of separate outlets. Schnelle et al. divided the output of their device into four separate channels.

Experiment ID #6: traveling-wave dielectrophoresis

Discussion: Travelling-wave dielectrophoresis (twDEP) is the result of an electric field with a spatially varying phase, and is typically created by using an interdigitated electrode array with four separate electric potentials of varying phase applied to individual electrodes (Section 6.3.2). This leads to a levitation force as well as a transverse force that can be used to sort particles according to (6.10). Cui and Morgan designed such a device and demonstrated motion of polystyrene particles via twDEP [81].

Methods: Sample experimental device and solution parameters are listed in Tables 6.6 and 6.8, respectively. In this example, the experiment proceeds in two phases: loading and sorting. Initially, a suspension of particles can be introduced to the channel via pressure-driven flow. Once the channel is filled, the flow can be stopped and the particles allowed to come to rest. At this point, the electrode array can be energized to 1V$_{peak}$ and 1 MHz. When the array is energized, particles will experience a negative dielectrophoresis force levitating them above the array. In addition, the spatial variation in phase will tend to drive the particles perpendicular to the array (in either direction, depending on the sign of $\Im(f_{col})$). Terminal particle velocity can also be measured, and will be directly related to the magnitude of $\Im(f_{col})$.

6.4.2.4 ROT spectra

Electrorotation techniques provide information about electrical phenotypes on a particle-by-particle basis. Because it traps particles in one position and can measure fre-
quency-dependent properties in situ, electrorotation is an ideal technique for observing the electrical response of a single particle to various stimuli.

Experiment ID #7: electrorotation

Discussion: In order to measure the rotation spectra of a sample, individual particles must be trapped and examined under a wide range of frequencies. This is typically accomplished using a quadrupolar set of electrodes.

Chan et al. utilized a quadrupolar electrode array (Section 6.4.1.2) to conduct electrorotation studies on uni- and multilamellar liposomes [24]. Liposome membranes were constructed from the phospholipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol. Liposomes encapsulated a solution of Percoll, which increased their density and made handling easier. The use of constructed liposomes was designed to validate multilayer, effective permittivity models [12].

Based on their data, Chan et al. determined that a single-shell effective permittivity model can be used for uni- and multilamellar liposomes. However, if the liposome contains distinct compartments, the single-shell model fails, and multishell modeling must be employed. In addition, it was confirmed that the electrorotation response is a function of internal as well as membrane properties.

Methods: Electrorotation experiments involve trapping a particle in a single location, subjecting it to a rotating external electric field, and observing the rate of particle rotation as a function of the electric field frequency. Consider the experimental conditions summarized in Tables 6.6 and 6.8. A polynomial, quadrupolar electrode array can be fabricated, after which particles can be introduced to the chamber surrounding the electrode array at a low concentration (10^4 liposomes/mL). Once introduced, particles can be allowed to settle. After settling, the trap can be energized, liposomes can be trapped within the array, and the trapped liposomes can be observed to measure their rate of rotation. As the frequency changes, particles will go through regimes where the rotation rate matches the electric field as well as regimes where particle rotation lags electric field rotation or does not rotate at all. When conducting these measurements, only the liposomes that are many diameters from one another and near the center of the array should be recorded. These precautions ensure that particle-particle interactions are minimized and that the electric field near the rotating particles is spatially uniform.

6.4.3 Insulative dielectrophoresis

Insulative techniques, which rely on compressions or expansions in channel geometry to generate electric field nonuniformities, have been used for a number of applications. Constrictions create locally high electric field regions that can induce deflection [82, 83], trapping [54, 84–86], or sorting via negative dielectrophoresis [36, 87].

In order to understand iDEP systems, it can be beneficial to examine a simple constriction in the electric current path (similar to the theoretical development presented in

\[ \text{12} \] The effective permittivity technique replaces \( \varepsilon_r \) for a homogeneous particle with \( \varepsilon_{r, \text{effective}} \). The derivation involves several iterations of the Laplace equation solution technique. Ultimately, the dipole moment becomes proportional to \( \varepsilon_{r, \text{effective}} \). A more detailed discussion can be found in [12].
Section 6.3.3.2), and from there, build up our understanding of more complicated systems such as an angled constriction, and finally our experimental example: a curved constriction.

**Perpendicular constriction:** In a system with a simple constriction (Figure 6.5), there are two modes that can be explored: trapping particles against fluid drag via negative DEP [36] or deflection of particles from a known, controlled position to another output position based on positive- or negative-dielectrophoretic response [82].

Constriction configurations such as these are useful for trapping or concentrating particles against fluid drag forces using negative dielectrophoresis, but trapped particles must be released before forming a bolus that may clog or alter fluid flow patterns. Constrictions such as these have also been used to alter the flow patterns of particles passing through the constriction [82]. A variable constriction was constructed by introducing an immiscible fluid in a side channel. As the immiscible (insulating) fluid was introduced in the side of the main flow channel, surface tension formed a circular constriction. The amount of constriction could be varied by changing the amount of immiscible fluid in the side channel, thereby changing the size of the “bubble” constricting the main channel [88].

**Angled constriction:** Angled constrictions are an extension of the rectangular constrictions considered earlier. A constriction in channel width can become a constriction in channel depth with a simple change of variables. However, practical fabrication considerations lead to an additional degree of freedom that was not possible with constrictions in width: the angle of incidence, \( \theta \). The DEP forces generated by the insulating constriction act primarily perpendicular to the constriction, while fluid flow (in the low Reynolds number limit) retains its direction. A summation of forces at the constriction yields an expression that depends on the magnitude of the dielectrophoretic force and the angle of the constriction. If the DEP force is large enough, for example:

![Figure 6.5](image_url) nDEP trapping of carboxylate-modified fluorescent polystyrene beads. Channel width is 1 mm, 200 \( \mu \)m in the constricted region, and channel depth is 100 \( \mu \)m. (From the authors' lab; unpublished work.)
particles will be stopped at the constriction and “deflected” parallel to the constriction. Angled constrictions have been used to preferentially separate particles and concentrate particles of interest in a single step [87].

**Experimental ID #8: postarray**

**Discussion:** Trapping experiments using post-array type devices offer the most common example of insulative dielectrophoretic techniques. Post-array configurations have been used for microbe isolation and detection [54, 84, 85].

The mechanism of action in a post array is largely similar to that of a perpendicular constricted: a constriction in the current path causes a concomitant increase in the local electric field magnitude, leading to an electric field gradient that drives DEP (Section 6.3.3.2). Post-array trapping techniques involve flow of a suspension of analyte past a large array of insulating posts. Fluid flow can be either pressure-driven from a syringe pump or electrokinetically driven via an external electric field. The advantage of electrokinetically driven flow is that a single DC voltage can be applied to induce fluid flow as well as dielectrophoretic trapping. This approach was used successfully by Lapizco-Encinas et al. to separate live and dead *Escherichia coli*.

Like electrode-based devices, iDEP devices have a wide range of variability in the geometry used to shape electric fields. In the case of post-array type devices, the geometric variables that affect the electric field are the post shape (circular, elliptical, square, etc.), gap between posts, and the array angle to the applied electric field. The post shape affects the rate of change of the local electric field between the posts; the gap itself determines the magnitude of the electric field between the posts; and the angle will have a relatively minor impact on the variation of the electric field magnitude. Lapizco-Encinas et al. report that a square array of circular posts yielded the best results (See Tables 6.6 and 6.8).

**Methods:** Trapping and separation of live and dead *E. coli* can be observed in an insulating post-array, given device and solution characteristics summarized in Tables 6.6 and 6.8, respectively. Once the device is fabricated and filled with particle solution, care must be taken—as must always be done in such experiments—to eliminate pressure-driven flow due to reservoir height differences and/or surface tension phenomena. Once the reservoirs are loaded and pressure differences eliminated, flow can be initiated by applying an electric field via electrodes in the external reservoirs. As the applied potential increases, fluid flow rates ($\propto \vec{E}$) and dielectrophoretic forces ($\propto \nabla (\vec{E} \cdot \vec{E})$) will

---

13 Since dielectrophoresis depends on the gradient of the squared electric field magnitude, changes in either the peak value of the electric field—between the posts—or the bulk value—“away” from the posts—will alter the dielectrophoretic force. In this case, the angling of the array will tend to shift insulating posts into what would normally be the “bulk” region for subsequent posts in the array. This will lead to an increase in the “bulk” value of the electric field, and a decrease in the gradient, leading to a minor decrease in the dielectrophoretic force. Lapizco-Encinas et al. do not report significant variation in performance as a function of the angle of the electrode array. In nontrapping regimes, however, Cummings and coworkers have found that the angle of the array will play a significant role in the “streaming” dielectrophoresis effects observed under nontrapping conditions [38–89].
increase. As the applied potential reaches 160V \( |E| = 16 \text{ V/mm} \), nDEP forces will begin to dominate fluid drag forces, and live bacteria will trap between posts in the array. Increasing the potential to 400V \( |E| = 40 \text{ V/mm} \) will lead to trapping of both live and dead cells. Finally, at a potential of 600V \( |E| = 60 \text{ V/mm} \), discrete banding of samples based on differences in dielectrophoretic mobility should appear.

Recall that the dielectrophoretic force is proportional to the permittivity and conductivity of both particle and media, as well as the frequency of the applied electric field. Closer examination of (6.1) yields that as \( \omega \rightarrow 0 \), the complex permittivity becomes \( i/\alpha \), and \( f_{cm} \) becomes:

### Table 6.7 Table 6.6 continued

<table>
<thead>
<tr>
<th>ID</th>
<th>V0-peak</th>
<th>Channel (length x width x depth)</th>
<th>Characteristic Length Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1V</td>
<td>2 cm x 100 ( \mu \text{m} ) x 50 ( \mu \text{m} )</td>
<td>Inter electrode spacing, 10 ( \mu \text{m} )</td>
</tr>
<tr>
<td>2</td>
<td>1V</td>
<td>Not Applicable</td>
<td>Inter electrode spacing, 25 ( \mu \text{m} )</td>
</tr>
<tr>
<td>3</td>
<td>2V</td>
<td>Not Applicable</td>
<td>Electrode width, 10 ( \mu \text{m} ); Ring diameter, 50 ( \mu \text{m} )</td>
</tr>
<tr>
<td>4</td>
<td>0–20 ( V_{peak} )</td>
<td>Not Applicable</td>
<td>Electrode width, 50–250 ( \mu \text{m} ); Electrode spacing, 50–250 ( \mu \text{m} )</td>
</tr>
<tr>
<td>5</td>
<td>5.3V</td>
<td>(( \sim 1 )) cm x 300–800 ( \mu \text{m} ) x 40–140 ( \mu \text{m} )</td>
<td>Alignment electrode width, 15 ( \mu \text{m} ); Curved electrode width, 7 ( \mu \text{m} )</td>
</tr>
<tr>
<td>6</td>
<td>1 ( V_{peak} )</td>
<td>(( \sim 1 )) cm x 300 ( \mu \text{m} ) x 70 ( \mu \text{m} )</td>
<td>Electrode width, 10 ( \mu \text{m} ); Electrode spacing, 10 ( \mu \text{m} )</td>
</tr>
<tr>
<td>7</td>
<td>Not Available*</td>
<td>Not Applicable</td>
<td>Diagonal electrode spacing, 400 ( \mu \text{m} )</td>
</tr>
<tr>
<td>8</td>
<td>160–600 ( V_{DC} )</td>
<td>10.2 mm x Not Applicable x 10 ( \mu \text{m} )</td>
<td>Post diameter, 200 ( \mu \text{m} ); Center-to-center distance, 250 ( \mu \text{m} )</td>
</tr>
<tr>
<td>9</td>
<td>50 ( V_{DC} ), 750 ( V_{AC} )</td>
<td>1 cm x 2500 ( \mu \text{m} ) x 100 ( \mu \text{m} )</td>
<td>Constriction, 10 ( \mu \text{m} ); Constriction ratio, ( r = 10 : 1 )</td>
</tr>
</tbody>
</table>

* It is the author’s opinion that voltages on the order of 1V should be sufficient.

### Table 6.8 Experimental Solution Characteristics for DEP Applications Described in Section 6.4.*

<table>
<thead>
<tr>
<th>ID</th>
<th>Particle</th>
<th>Size</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carboxylate-modified polystyrene</td>
<td>1 ( \mu \text{m} ) diameter</td>
<td>1.9 ( \times ) 10^6 particles/mL</td>
</tr>
<tr>
<td>2</td>
<td>Carboxylate-modified polystyrene</td>
<td>2 ( \mu \text{m} ) diameter</td>
<td>2.4 ( \times ) 10^7 particles/mL</td>
</tr>
<tr>
<td>3</td>
<td>Silver-coated polystyrene</td>
<td>20 ( \mu \text{m} ) diameter</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>4</td>
<td>Jurkat and AC3 stromal cells</td>
<td>(~ 10) ( \mu \text{m} )</td>
<td>5 ( \times ) 10^5 cells/mL</td>
</tr>
<tr>
<td>5</td>
<td>Unmodified latex</td>
<td>6.4, 10, 15, and 20 ( \mu \text{m} ) diameter</td>
<td>Not Available</td>
</tr>
<tr>
<td>6</td>
<td>Unmodified polystyrene</td>
<td>10 mm diameter</td>
<td>Not Available</td>
</tr>
<tr>
<td>7</td>
<td>Uni-, multilamellar liposomes</td>
<td>Variable, 2.5–12 ( \mu \text{m} )</td>
<td>Not Applicable, 10^8 liposomes somes/mL</td>
</tr>
<tr>
<td>8</td>
<td>Viable and heat-treated <em>Escherichia coli</em> cells</td>
<td>1 ( \mu \text{m} )</td>
<td>6 ( \times ) 10^6 cells/mL</td>
</tr>
<tr>
<td>9</td>
<td>Carboxylate-modified polystyrene</td>
<td>1.75 ( \mu \text{m} ) and 2 ( \mu \text{m} ) diameter</td>
<td>2 ( \rightarrow ) 2.4 ( \times ) 10^7 particles/mL</td>
</tr>
</tbody>
</table>

* All solution percentages are w/v. IDs correspond to data listed in Table 6.6.

* The expression for electric torque contains \( a^2 \) dependence, but this balances against the viscous torque (drag), leading to a rotation rate that is independent of size.
\[ f_{CM} = \frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \] (6.34)

It has been reported that, in deionized water, the membrane conductivity of live cells is significantly lower than that of dead cells, leading to a significant difference in \( f_{CM} \) for the two populations and an observable separation (Figure 6.6). The key advantages of post-array techniques stem from their simplicity of operation. After fabrication, the only necessary component is a power supply that will drive flow and preferential trapping.

**Safety considerations:** In order to obtain the electric fields required for separation (on the order of 200 V/mm) a high voltage (on the order of 2 kV) power supply must be used. High voltages are hazardous; experimenters should be trained in the proper usage of all equipment and safety measures.

**Experimental ID #9: curved constriction**

**Discussion:** Further refinement of the angled constriction in channel depth leads to a continuously varying angle of constriction throughout the channel. The resulting curved constriction in channel depth (Figure 6.7) operates on the same basic principle. Dielectrophoretic forces are still perpendicular to the constriction, but now this force is directed along the radius of constriction curvature. Particles are either trapped and “deflected” at the constriction or allowed to pass. Again, summing forces on a deflected particle at the constriction yields a component parallel to the constriction. As a deflected particle traverses the constriction, the angle between the direction of bulk flow and the DEP force (normal to constriction curvature) changes, until the inequality (6.33) is violated or until the particle reaches the channel wall [36].

Hawkins et al. [36] demonstrated particle trapping in a system with a simple constriction using a relatively low-voltage DC signal to drive electrokinetic flow and a high-voltage AC signal to achieve particle trapping via negative dielectrophoresis. They also demonstrated a separation utilizing a curved constriction in channel depth to separate polystyrene particles on the basis of size.

**Methods:** Sample experimental parameters used to separate particles using a curved constriction in channel depth are similar to those described earlier for trapping in an

**Figure 6.6** Trapping and subsequent separation of live (green) and dead (red) *Escherichia coli* in an insulating post array. (a) 16 V/mm, live cells only are trapped, (b) 40 V/mm, trapping both live and dead cells with observable banding, and (c) 60 V/mm, trapping of both live and dead cells with separation of populations. (From [84].) Copyright American Chemical Society. Used with permission.
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![Figure 6.7](image)

**Figure 6.7** A schematic of a constriction in channel depth. Input and output reservoirs, channel top not shown. Inset: a top view of device fabricated in Zeonor 1020R polymer substrate. (From [36].)

insulating post array and are listed in Tables 6.6 and 6.8. External reservoirs can be utilized for sample introduction and application of electric potential via platinum electrodes. As with the post-array experiment (Section 6.4.2), pressure-driven flow due to reservoir height mismatching or surface tension effects should be eliminated by careful observation and the use of large external reservoirs.

Once the sample is loaded, a low, DC potential of 25V can be applied to induce dielectrophoretic and electroosmotic flow. After flow is established, an AC signal at 1 kHz can be applied in addition to the DC potential. The magnitude of the AC signal is typically 5 to 10 times larger than the DC signal ($125 - 250 V_{peak}$). At low AC potentials ($< 100 V_{peak}$), little change will be observed. As the AC potential is increased, deflection of particles at the perpendicular section of the curved constriction will be observed. Once particle deflection is observed, the field amplitude can be increased further to observe additional deflection of particles. In an experiment where two particle populations (e.g., 1.75- and 2-µm diameter) are initially uniformly mixed and distributed throughout the channel, deflection will lead to three distinct regions in the output section of the device. Low $\theta$ values will lead to downstream regions containing no particles, as they have all been deflected by the constriction. The next region, with higher values of $\theta$ will contain smaller particles only. The lower dielectrophoretic force associated with smaller diameters will lead to less deflection. The last region, downstream of higher $\theta$ values, will contain both particle types, with a higher population of larger particles due to deflection.

### 6.4.4 Summary of experimental parameters

Tables 6.6, 6.7, 6.8, and 6.9 summarize sample experimental parameters for devices discussed in this section.
6.5 Troubleshooting

Tables 6.10, 6.11, and 6.12 summarize a few of the common problems associated with microfluidic dielectrophoresis experiments and some potential solutions.

6.6 Application notes

Dielectrophoresis applications range from fractionating particles based upon their "electrical phenotype" [69] to precise manipulation of single particles for property interrogation to new strategies for the creation of engineered tissues and organs. With the emergence of multistep lab-on-a-chip devices and the use of miniaturized bioanalytical techniques in a microdevice, DEP has become an attractive option for particle and cell manipulation. Owing to the favorable scaling of dielectrophoretic effects at shrinking length scales, the utility of electrokinetically driven flows at these length scales, and the flexibility of microfabrication techniques, integration of these effects is common.

We categorize applications in this section based upon the goals of the user. The first category includes applications that control the relationship between the particle location and the fluid (e.g., holding or trapping particles relative to a fluid volume). The second category includes applications which control the relationship between particle types, usually dynamically, as the particles progress through a device or fluid stream (e.g., particle sorting and fractionation). The third category includes applications in which control the position of particles relative to each other. While many examples overlap two or more of these categories, these categories nonetheless serve as a framework for classifying relevant applications based upon user goals. This section will serve only as an overview for readers, highlighting specific examples rather than providing a comprehensive review of the applications of DEP. These examples provide context for the detailed discussions throughout the chapter on the methods used to implement DEP.

6.6.1 Particle trapping

The most common use of DEP is to fractionate a particle suspension by statically trapping particles within a fluidic channel. Variations in electric field are induced in a fluid

<table>
<thead>
<tr>
<th>Table 6.9</th>
<th>Comparison of Conclusions from Table 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>Fluid</td>
</tr>
<tr>
<td>1</td>
<td>Deionized water</td>
</tr>
<tr>
<td>2</td>
<td>Deionized water</td>
</tr>
<tr>
<td>3</td>
<td>50% sucrose/DI water</td>
</tr>
<tr>
<td>4</td>
<td>1% BSA, 1% Triton X-100</td>
</tr>
<tr>
<td>5</td>
<td>450 mM D-sorbitol</td>
</tr>
<tr>
<td>6</td>
<td>Deionized water</td>
</tr>
<tr>
<td>7</td>
<td>Deionized water</td>
</tr>
<tr>
<td>8</td>
<td>5% mannitol</td>
</tr>
<tr>
<td>9</td>
<td>Deionized water</td>
</tr>
<tr>
<td>10</td>
<td>Deionized water</td>
</tr>
</tbody>
</table>

* Phosphate buffered saline solution was added to stabilize the conductivity of the solution at 1.7 mS/m.
† Volumetric flow rates were not available for this experiment.
‡ An electrolyte solution was added to bring the solution conductivity to 1 mS/m.
Table 6.10  Tips and Suggestions for Common Problems Encountered in Dielectrophoresis Experiments

<table>
<thead>
<tr>
<th>Problem</th>
<th>Potential Causes</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inconsistent flow rate</td>
<td>In pressure-driven flow:</td>
<td>Fix pump</td>
</tr>
<tr>
<td></td>
<td>Check pump for failure</td>
<td>Use pump rated for low flow rates</td>
</tr>
<tr>
<td></td>
<td>Check pump flow rate</td>
<td>Fill device with compatible, low surface tension fluid (e.g. methanol), then thoroughly flush with working fluid</td>
</tr>
<tr>
<td></td>
<td>Check device for bubbles at inlet, outlet, and electrodes</td>
<td>For bubbles forming at electrodes:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce fluid conductivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Choose different electrode material (see Section 6.4.0.1)</td>
</tr>
<tr>
<td>In electrokinetic flow:</td>
<td>check power supply</td>
<td>Make sure load (channel) resistance is high for high-voltage applications</td>
</tr>
<tr>
<td></td>
<td>check electrodes for contact</td>
<td>use a lower conductivity working fluid</td>
</tr>
<tr>
<td></td>
<td>check for bubbles in device</td>
<td>reduce channel cross section</td>
</tr>
<tr>
<td></td>
<td>check for bubbles at electrode</td>
<td>increase channel length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>remove bubbles from current path (see above)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If electrode contact area is small, bubbles here can also break the current path, take measures to reduce electrolysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reduce fluid conductivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>choose different electrode material (see Section 6.4.0.1)</td>
</tr>
</tbody>
</table>

Table 6.11  More Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Potential Causes</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle adhesion</td>
<td>Electric field too high</td>
<td>Lower applied potential</td>
</tr>
<tr>
<td></td>
<td>Hydrophobic interactions</td>
<td>See Section 6.4.0.1</td>
</tr>
<tr>
<td>No or low particle DEP</td>
<td>$f_{CM} = 0$</td>
<td>Change $\sigma_{s}$ (usually decrease)</td>
</tr>
<tr>
<td>response</td>
<td>Check fluid conductivity</td>
<td>Change $\omega$</td>
</tr>
<tr>
<td></td>
<td>Check particle conductivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Check electric field frequency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In trapping experiments:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>if $f_{CM} &gt; 0$, channel depth too large</td>
<td>Decrease channel depth</td>
</tr>
<tr>
<td></td>
<td>if $f_{CM} &lt; 0$, flow rate too high</td>
<td>Decrease flow rate</td>
</tr>
<tr>
<td></td>
<td>Particles not close enough to electrodes (or constrictions in iDEP)</td>
<td>Flow focusing (Section 6.4.0.1)</td>
</tr>
<tr>
<td>Particle aggregation/</td>
<td>Particle concentration too high</td>
<td>Dilute particles</td>
</tr>
<tr>
<td>clogging</td>
<td>DEP chaining</td>
<td>Lower $</td>
</tr>
<tr>
<td>Bubble generation during</td>
<td>Electrolysis or boiling</td>
<td>See Section 6.4.0.1</td>
</tr>
<tr>
<td>experiment</td>
<td>Leaking channel</td>
<td></td>
</tr>
</tbody>
</table>

channel and exert a positive or negative DEP force, causing the particle of interest to be statically confined at or near a peak or valley in the electric field distribution. Particle confinement often occurs as a colloidal suspension propagates through the channel. While positive DEP (pDEP) is most frequently used, owing to the simplicity of system design, negative DEP (nDEP) has been used as well, owing to the lower fields it applies to cells. In order to hold or trap specific populations of particles from a fluid suspension, (1) the DEP forces on the particles of interest must be greater than the hydrodynamic and
gravity forces on the particles, and (2) differences in dielectrophoretic mobility must exist to discriminate populations of particles.

Becker et al. created an “electroaffinity column” based upon differences in dielectrophoretic mobility for separating leukemia cells from blood [90]. They created a wide channel with offset, castellated electrodes (see Section 6.4.1.2) along the bottom and injected diluted human blood that was spiked with HL-60 leukemia cells. Erythrocytes (red blood cells) and leukemia cells were captured from the blood with the application of a $5 \text{ V}_{\text{peak}}$ 200-kHz AC electrical signal to the electrodes. An elution volume was flowed through the device and the frequency of the electrical signal was decreased to 80 kHz, causing the release of blood cells but retention of leukemia cells. At 80 kHz, Becker et al. calculated the polarizabilities of the leukemia cells to yield a strongly positive DEP force and the erythrocytes to have a repulsive force. As a result, blood cells were eluted and the retained leukemia cell population was of high purity. The prototype electroaffinity column created by Becker et al. was able to sort approximately 1000 cells/sec. This technology is scalable, enabling higher throughput of cell sorting by increasing the size of the channel and electrode area and thus allowing a greater volumetric flow rate of sample through the device.

Another application of DEP was demonstrated by Yasukawa et al. [80], who used an nDEP “cage” to confine particles within a desired volume and subsequently perform an enzyme-linked immunosorbent assay (ELISA). An ELISA is a quantitative assay that involves the conjugation of fluorophores or chromogens to target surface molecules by antibody coupling for the measurement of selected molecules. The authors patterned electrodes on the top and bottom surface of a channel to create a “caged” area. The downstream electrodes were activated, and polystyrene latex microspheres were concentrated into a stream using preliminary focusing electrodes (see Section 6.4.1.3). Once the desired quantity of microspheres was collected, the upstream DEP electrode was activated to close the “cage” and prevent additional particles from entering. The authors then performed an immunoreaction to attach antibodies and subsequently fluorophores to the microspheres by adding reagents to the fluid stream. Fluorescence intensity was measured with conventional microscopic techniques. At the conclusion of the assay, both electrodes were deactivated and the labeled particles were released down the channel in the fluid stream.
In a variation on the typical pDEP capture of particles, Urdaneta and Smela [91] used
electrodes at multiple frequencies to capture two separate populations of yeast cells (live
and dead) from a heterogeneous cell suspension. Urdaneta and Smela took advantage of
being able to tune the effective Clausius-Mossotti factor \( f_{\text{eff}} \) or frequency response and
thus create regions of p- and nDEP that were opposite for each cell type. They created
three planar electrodes, one grounded and two electrodes at varying frequencies and
amplitudes, in two different geometries within a fluidic compartment. An equal part
mixture of live and dead yeast cells was injected into the device and the two cell popula-
tions were separated with applied signals at 5 kHz and 5 MHz, respectively. Multiple-
frequency DEP (MFDEP) has been used for electro-rotational spectra [92], particle levita-
tion [93], and traveling wave DEP (twDEP) [94] applications.

Separation and trapping of cells by their electrical phenotype using DEP enables the
efficient capture of a high purity population of cells. As mentioned earlier, Becker et al.,
as well as others, used the “electroaffinity” column to demonstrate the ability to separate
different cell types from each other, including the clinically applicable separation of
tumor cells from nonpathologic cells [90]. In addition to separating a specific cell type
from a heterogenous population, DEP has also been utilized to separate same cell popula-
tions to discern physiologic differences such as activation of mitosis [95, 96], exposure
to drugs [97–100], induced cell differentiation [3, 101, 102], and cell death [91,
103–105]. Using DEP-based devices to potentially separate the same type of cells based
upon physiologic parameters is a powerful tool to identify responses of cells to various
soluble agents. Besides the direct application of DEP trapping to biological problems,
capturing cells also enables a practical method of manipulating cells and particles within
a multistep microfluidic lab-on-a-chip device. Investigators have used DEP “gate”
electrodes to enable the passage of selected particles based upon dielectrophoretic mobility
[106]. This idea can be extended to incorporate multiple gate electrodes to control spatial
and temporal particle motion through a microfluidic chip. Likewise, the ability to
localize cells within a specified area, either on a 2-D surface or within a 3-D cage, poten-
tially creates a platform to investigate responses of a population of phenotypically
similar cells to different chemical environments.

6.6.2 Particle sorting and fractionation

Many continuous flow separation techniques use dielectrophoresis to sort, fractionate,
or enrich particle populations dynamically within a fluid stream. These methods use
either positive or negative DEP (or both) in microfluidic devices to deflect particles trans-
versely such that an enriched population of the desired particles passes into one of
several downstream exits. The heterogenous electric fields are created either through
variations in fluidic channel geometry (insulative DEP, iDEP) or electrodes (elec-
trode-based DEP, eDEP) along the channel in various configurations. Application of a
voltage across a channel with varying geometry, or across electrodes along the length of
the fluidic channel, creates locally nonuniform fields and a resulting DEP response from
the particles or cells in the fluid stream (see Sections 6.2 and 6.3).

Many continuous-flow dielectrophoresis systems utilize insulative configurations.
Cummings and Singh [89] explored iDEP using insulating postarrays and dc electric
fields to trap particles or align them in streams. The authors used posts of various shapes
and adjusted the orientation of the postarrays within the electric field to induce a p- or
nDEP response and thus concentrate or deplete streams of microspheres or reversibly immobilize the microspheres on the insulating posts. In another application, Hawkins et al. [36] used a curved ridge to create an insulative constriction region within a microchannel to separate 1.75-μm from 2-μm microspheres in a heterogenous population (Figure 6.8). Electrokinetically driven flow within the device was established via a dc-offset, ac electric field and particles were deflected along the curved constriction to change the particle’s position within the channel (see Section 6.4.2). The particle deflection depends on the orientation of the constriction and thus Hawkins et al. created a tunable system to separate particles based upon dielectrophoretic mobility.

Continuous flow separations have also been demonstrated in electrode-based systems. Hu et al. [77] devised a device that uses DEP to sort cells in a manner analogous to a fluorescence-activated cell sorting (FACS) device (Figure 6.8). In a manner analogous to fluorescent staining for FACS, the authors conjugated beads to some bacterial cells via antibody coupling and mixed the bead conjugated bacteria with nonconjugated bacterial cells. The newly made bead-bacterial cell conjugates are sensitive to a DEP force due to the increased size of the complex. Hu et al. injected the bacterial cell suspension into their device and utilized angled electrodes (see Section 6.4.1.3) to induce a nDEP force on the labeled bacterial cells to deflect them into a separate collection channel. The device collected 95% of the labeled cells with a throughput of 2–3 × 10⁷ cells/hr, a rate comparable to conventional cell sorters. Braschler et al. [107] introduced fine control of a particle’s deflection along the length of a channel using multiple frequency eDEP (Figure 6.9). Two electrode arrays are patterned on either side of a fluidic channel with each array excited at different frequencies. The difference in frequencies and the ability to tune the system allows the user to balance the DEP forces on the particle and control a particles’ position within the channel as it travels down the length of the electrode array.

Continuous flow techniques have also been used to create temporal separation of particles within a flow. Taking advantage of differing dielectrophoretic mobilities, several groups have used DEP for field flow fractionation (FFF-DEP) [108, 109]. As a particle

![Figure 6.8](image_url) **Figure 6.8** Time-lapse (60 seconds) fluorescence microscopy image. 1.75-μm diameter polystyrene spheres (green) are sorted to the center of the channel, and 2-μm diameter polystyrene spheres (red) are sorted to the bottom of the channel. Copyright American Chemical Society. Used with permission.
Figure 6.9 DEP was used to continuously separate bacteria conjugated with beads from a heterogeneous sample (a) by Hu et al. [77]. Angled electrodes deflect bacteria conjugated beads into a buffer stream that exits the device through a collection channel (b). Bruschler et al. [107] used MFDEP to focus a stream of particles within a channel (c). The differential application of two frequencies to electrodes along each side of a channel enables tunable control of a particle's position. (a) copyright National Academy of Sciences. (b, c) copyright Royal Society of Chemistry. Used with permission.

stream progresses through a microfluidic channel, DEP forces oppose sedimentation (gravity) forces and particles with a weaker DEP response will settle to a lower-velocity streamline within the Poiseuille flow profile. Differences in fluid velocity across the cross-section of the channel cause elution of the DEP-responsive particles at different times. Thus, in a manner similar to a separation column, particles with high DEP response will be eluted from the device subsequent to those with weaker DEP response.

An advantage of DEP is the ability to separate particles or cells without the need for secondary affinity coupling steps to achieve separation. Traditional techniques such as FACS, flow cytometry, ELISA and affinity chromatography require a pretreatment with fluorescent molecules (FACS, flow cytometry), or specific antigens conjugated to the particles (ELISA, flow chromatography) prior to separation. This bypass of prelabeling eliminates use of additional reagents and saves processing time. Like many of the commonly cited benefits of microfluidic systems—low Reynolds number, laminar flow, low reagent volumes—dielectrophoresis benefits from the same favorable scaling at small scales. The DEP force is proportional to the gradient of the electric field squared, or the inverse of the characteristic length cubed, and thus the DEP force increases dramatically in these microfluidic environments.
The use of DEP as a continuous-flow separation technique has been demonstrated for several types of particles including polymeric spheres [36, 82], bacteria [110], yeast [111], and mammalian cells [112]. Similar to trapping applications, investigators have used DEP in continuous flow applications to separate populations of the same cell with physiologic differences. Examples include separation of stem cells from their differentiated progeny [113] and mammalian cells based upon their cell-cycle phase [112]. These continuous-flow, DEP fractionation devices enable rapid sorting of cells and particles which can be scaled up to fractionate larger populations. Separation of large populations of phenotypically similar cells is of great interest to biologists and tissue engineers who investigate drug interactions with phenotypically varied cells and create in vitro tissue models from pure cell populations.

### 6.6.3 Single-particle trapping

In addition to bulk processing of large numbers of particles, the appropriate electrode design enables manipulation of single particles or small groups thereof. These methodologies have enabled significant advances in microscale bioanalytical techniques. From a biological perspective, the utility of DEP to manipulate individual particles for the discrimination of properties is promising and opens up new tools to investigate single cells or cell-cell interactions. In general, strategies to address the fine manipulation of particles involve either (1) the use of electrode arrays that are individually addressable, or (2) the use of light on photosensitive conducting materials to dynamically alter the electrode configuration.

An electrode post-array was used by Voidman et al. [68] to create a microfabricated cytometer to trap and release individual cells with temporal control (Figures 6.10 and 6.2(b), Section 6.4.1.2). They used an array of posts to create several quadrupole DEP traps. The nDEP traps captured calcein labeled HL-60 cells and maintained the position of the cell for more than 40 minutes. While the cells were trapped, the investigators were able to perform fluorescence measurements in the device and selectively release individual cells at different time points. Similarly, Taft and Voidman [70] improved on the original cell sorting array by creating addressable traps using a circle-dot pDEP electrode design that is highly scalable (see Section 6.4.1.2). Shih et al. [114] developed a device for sorting particles with trapping, detection, and sorting regions. The cells were captured in quadrupolar traps, analyzed in real time in a second region of the chip, and then passed into a wide channel with several electrodes patterned on the lower surface (Figure 6.10). The electrodes are addressed appropriately to create nDEP in all areas except a path to the correct outlet channel. The authors were able to successfully sort microspheres with a protein coating from uncoated microspheres into one of five output channels.

While electrode arrays are usually implemented in a 2-D configuration, Albrecht et al. [115] used pDEP and photopolymerizable hydrogels to precisely position chondrocytes within a 3-D environment (Figure 6.10). They injected cells within a prepolymer solution between two plates patterned with electrodes. The electrodes were energized and DEP caused clusters of cells to form based upon the electrode geometry. Once the cells were patterned, the prepolymer was polymerized using UV light, locking cells into position within a hydrogel. This methodology of using DEP to spatially control cell-cell spacing is a significant step forward in the field of tissue engineering, because conventional techniques are not able to control intercellular spacing. These 3-D, in vitro
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Figure 6.10 A dynamic cytometer was created by Voldman and colleagues [68] with an array of quadrupole traps (a). Individual cells were captured within the respective traps, fluorescence measurements made, and then selectively released. Shih et al. [114] used nDEP to dynamically control which outlet particles are sorted into (b). DEP has also been used to pattern cells with controlled spacing in a 3-D environment (c) for the investigation of the role of cell signaling on cell growth and tissue formation. Albrecht and colleagues [115] created aggregates of cells with a patterned electrode with evenly spaced trapping regions. Prepolymer was flowed into the channel and photopolymerized to create a hydrogel encasing the cell aggregates. Serial patterning and polymerization can then be used to create engineered tissue analogs with controlled cell localization within a 3-D environment. (a) copyright American Chemical Society, (b) copyright IEEE, and (c) copyright Nature Publishing Group. Used with permission.

models allow the investigation of local micro-scale cell signaling and the resulting impact on cell growth and tissue formation.

As evidenced by these examples, DEP’s strength is in manipulation at the single-particle/single-organism level. While techniques such as optical tweezers and ultrasonic particle manipulation already exist to trap and isolate single particles, DEP systems, in addition to being simpler, cheaper, and potentially more parallelizable, have the advantage of inducing either positive or negative forces. Under the appropriate experimental conditions, this ability leads to selective trapping or controlled aggregation of particles within novel trap designs or 3-D cages. In addition to these direct applications, DEP has also proven useful to control cells and particles in multiple-process lab-on-a-chip devices using gates, traps, and particle aligners to regulate spatial and temporal trafficking through microdevices [116, 117].
References

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