Abstract

Electronic Particle Manipulation for Lab-on-chip Diagnostics

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Early disease detection is closely correlated with treatment success and is often a function of both testing sensitivity and testing frequency. Modern biomarker and cellular characterization diagnostics, such as those based on PCR, ELISA, or FACS operation, offer high sensitivity, but are often accompanied by high materials or infrastructure cost, rendering them insufficient solutions for resource-limited regions. Today, less than 50% of the world’s population has access to essential healthcare and, for nearly 10%, that access comes only at catastrophic levels of expenditure. Portable, lab-on-chip diagnostic solutions offer a promising combination of reduced cost and ease of operation, features necessary to increasing testing availability and frequency. Many on-chip analogues of conventional laboratory techniques have been successfully demonstrated. Planar electrodes in microfluidic environments can accomplish multiple sample processing and measurement functions by changing electrode geometry and electronic signal processing. Among those functions include the manipulation of cell positions with dielectrophoresis as well as the measurement of cell presence and volume by differential impedance monitoring.

We investigate both of these planar electrode systems individually and in combination for use in electronic lab-on-chip diagnostics. First we develop a system model for DEP devices to illuminate the degradation of device performance exhibited when these devices are attempted to be brought out of the lab and into the clinic. By this analysis, we enable informed design decisions to accommodate real-world limitations of sample conductivity and volumetric throughput. We then apply that understanding to the design and operation of DEP devices to electronically isolate and concentrate disease-associated bacteria from fluid samples. Concentration of target particles from a large volume sample can effectively lower the limit of detection for sensing measurements, serving to enable earlier detection. Additionally, instead of direct pathogen concentration, we take advantage of the immune
system’s extensive infection detection ability to monitor the pathogen-induced activation state of immune cells. We present a microfluidic device for continuous DEP separation of activated and naive T cells in physiological conductivity and on-chip quantification of both populations with an integrated coulter counter as a methodology of immune state assessment. To our knowledge, this is the first reported DEP-based device for label-free, fully electronic detection and simultaneous enrichment of activated T cells.
Electronic Particle Manipulation for Lab-on-chip Diagnostics

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Dedication

To Colleen.

For teaching me that everything is interesting if you’re curious enough to look.
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<tr>
<td>BSI</td>
<td>Bacterial bloodstream infection</td>
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<td>CTC</td>
<td>Circulating tumor cell</td>
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<td>DEP</td>
<td>Dielectrophoresis</td>
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<tr>
<td>DEP-FFF</td>
<td>Dielectrophoretic field-flow fractionation</td>
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<td>iDEP</td>
<td>Insulator dielectrophoresis</td>
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<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>pDEP</td>
<td>Positive dielectrophoresis</td>
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<tr>
<td>PDMS</td>
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<td>PECVD</td>
<td>Plasma enhanced chemical vapor deposition</td>
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<td>RBC</td>
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<td>SiO₂</td>
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<td>$f_{CM}$</td>
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<tr>
<td>$\omega$</td>
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Publications

S. Yosinski, Z. Kobos, P. Han, T. Fahmy, M. A. Reed, “Electronic label-free enrichment and assessment of T-cell activation,” To be submitted.

Z. Kobos, S. Yosinski, M. A. Reed, “Dielectrophoresis device design optimization for high conductivity and high throughput applications,” To be submitted.
Chapter 1

Introduction

1.1 Motivation for electronic diagnostics

Less than 50% of the world’s population have access to essential healthcare and, for nearly 10%, that access comes only at a catastrophic financial cost [1]. Early detection of many diseases is closely correlated with treatment success and is often a function of both testing sensitivity and testing frequency. Developed biomarker and cellular characterization diagnostics such as those based on genetic amplification, antibody-based immunostaining, or optical sorting of fluorescently tagged biomarkers, offer very high testing sensitivity. These methods also often come with high materials or infrastructure cost, rendering them insufficient solutions for the challenges faced in resource-limited healthcare settings. Alternative first-line diagnostic solutions are needed that overcome current cost barriers and infrastructure limitations.

Portable, lab-on-chip solutions offer a promising alternative diagnostic modality, as many viable low-cost chip-level analogues to conventional laboratory functions have been demonstrated. Among these on-chip laboratory analogues, planar electrode-based systems have been utilized for a host of relevant sample processing and measurement functions and offer the additional advantages of accessible single-chip integration. Additionally, many of these planar electrode processes can be performed using only electronic operation and readouts. By eliminating the need for optical microscopy, antibody stabilization, and expensive laboratory equipment, label-free, electronic biosensing modalities help to directly address
the cost and infrastructure limitations of conventional biomarker detection techniques [2]. Towards the goal of creating low cost first-line diagnostic alternatives, this work utilizes two label-free electronic techniques compatible with on-chip integration: electric field-induced biological sample processing and impedance-based cell detection.

1.2 Dielectrophoresis as a powerful particle manipulation technique

Electric fields applied to fluids or particles suspended in fluids can induce material movement through several mechanisms, collectively referred to as electrokinetic forces [3]. The three main mechanisms by which electric fields induce particle or fluid motion are dielectrophoresis, electro-osmosis, and electrothermal effects [4]. The electric properties of the particles and fluids as well as the frequency of the applied electric field dictate the relative contribution of each of these three forces to the net force on a particle in a fluidic system.

Dielectrophoresis (DEP) describes the force on neutral particles in response to an electric field [5]. Any particle than can polarize and form a dipole can experience DEP force, including all biological cells. The presence of an electric field causes rearrangement of mobile charges and reorientation of molecules having a dipole. The net effect of these reactions to the electric field is the formation of a net particle dipole. The degree of response is determined by both the electric field magnitude and a material’s polarizability. Polarizability describes material conductivity (the mobility of the charges within the particle) as well as the material permittivity (the ability of charge to accumulate in the material).

In a uniform electric field, equal number of charges within a neutral particle can accumulate on opposing sides of the particle, maintaining particle neutrality, but creating an induced dipole. The dipole will rotate to align with the electric field lines, but experience no net force to move in any direction as the force will be equal on both sides of the dipole as diagrammed in Figure 1.1a.

Non-uniform electric fields can be created by asymmetries in electrode arrangements or by positioning an insulating material between symmetric electrodes, a subfield of DEP called insulator DEP [6]. A particle dipole formed in response to a non-uniform field will
experience a net force in the direction of greater field strength (Figure 1.1b).

(a) Uniform electric field

(b) Non-uniform electric field

Figure 1.1: Diagram of dipole formation and forces on a particle in a uniform electric field (a) and a non-uniform field (b) showing no net force occurs on the particle in a uniform electric field, but a net force does result on a particle in a non-uniform field.

If a dielectric particle is surrounded by another dielectric material, like a suspending ionic fluid, charges within that surrounding material will also respond to the electric field, arranging charges near the particle surface to counterbalance any charge accumulation in the particle. Neutrality is maintained in both materials, but the degree to which charges in the surrounding medium counterbalance the charge accumulation in the particle depends on the ratio of the polarizability of each material.

In the case of equal polarizability, the charge on either side of the particle will be exactly counterbalanced by charges in solution, resulting in no net dipole formation and therefore no particle movement in either a uniform field (Figure 1.2c) or a non-uniform field (Figure 1.2d).

If there is a difference in polarizability between the two materials, however, charge accumulation will not counterbalance, resulting in a net dipole formation. If a particle is more polarizable than its surrounding medium, more charges will accumulate within the particle relative to the medium, resulting in a net force toward high electric field strength (Figure 1.2b). By convention the direction of net force toward high field gradient is positive. Thus this force is referred to as positive dielectrophoresis (pDEP). Likewise, if the medium is more polarizable than the particle, more charges will accumulate in the medium near the particle surface than within the particle itself, resulting in a net force away from the region.
of high field gradient as diagrammed in Figure 1.2f [7]. This force is referred to as negative dielectrophoresis (nDEP).

No matter the degree to which an induced particle dipole is counterbalanced by solution charges, the force on any net dipole is equally strong on opposing sides of the particle in a uniform electric field, resulting in no particle movement. In a non-uniform field, however, the relative polarizability of the two material components involved (the particle and it’s suspending fluid media) determine the magnitude and direction of the DEP force and resulting particle motion.
Figure 1.2: Taking material polarizability into account, the forces on a particle when fluid media polarizability ($\varepsilon_m^*$) is less than the particle polarizability ($\varepsilon_p^*$) (a) and (b), equal to the particle polarizability (c) and (d), and greater than the particle polarizability (e) and (f) result in no net force for particles in a uniform electric field (a,c,e), but influence the magnitude and direction of the net force on a particle in a non-uniform electric field (b,d,f).

While either a direct current (DC) or alternating current (AC) can produce an electric field, this work utilizes AC fields. Alternating current induced electrokinetics presents several advantages over it’s DC counterpart. Due to the nature of constantly reversing potentials in an AC electric field, electrochemical reactions and electrolysis are minimized
compared to direct current electric fields [4]. Additionally, the frequency-dependence of electrokinetic forces present another variable of tunable control for particle manipulation.

In an AC electric field in which the polarity of applied potential continually switches with some frequency, the rate of response of the dipole formation in the particle and the charge movement in the fluid matter. If the particle is more polarizable then it’s surrounding fluid, able to polarize more rapidly than the charges in solution can counterbalance, there will be a net force on the particle in the direction of the point at which the gradient of the electric field is strongest. This force toward areas of high field gradient is called positive dielectrophoresis, or pDEP. If, however, the fluid is more polarizable than the particle, solution charges will respond more quickly to the electric field both fully shielding any formed dipole on the particle and also creating bulk fluid movement toward the higher field gradient, effectively pushing the particle away from that area. This force away for areas of high field gradient is called negative dielectrophoresis, or nDEP.

The polarizability of the particle or fluid depends on a combination of the conductivity ($\sigma$) of the material as well as the permittivity ($\varepsilon$) of the material which is a complex value with frequency dependency. The DEP force that a particle experiences, therefore, is dependent on the dielectric properties of the particle as well as its surrounding fluid, the frequency of the applied AC field, and the spatial non-uniformity of the electric field (defined by the gradient of the electric field).

The net DEP force ($F_{DEP}$) on a particle subjected to a non-uniform electric field is given by

$$F_{DEP} = 2\pi a^3 \varepsilon_m Re[f_{CM}] \nabla E^2$$

(1.1)

where $a$ is the particle radius, $\varepsilon_m$ is the fluidic permittivity, $E$ is the electric field, and $f_{CM}$ is a term called the Clausius-Mossotti factor that describes the relationship between the frequency-dependent dielectric properties of the particle and the surrounding fluid that result in an induced dipole moment in an AC electric field [8]. For simple solid spheres, this factor is

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$

(1.2)

where $\varepsilon_p^*$ and $\varepsilon_m^*$ are the complex permittivities of the particle and surrounding fluid medium,
respectively. This complex permittivity describes the polarizability of a material and is given by

$$\epsilon^* = \epsilon - j\frac{\sigma}{\omega}$$

where \(\epsilon\) is the material’s dielectric constant, \(\sigma\) is its conductivity, \(\omega\) is the angular frequency of the applied AC electric field, and \(j = \sqrt{-1}\).

These equations show that the DEP force magnitude and direction are sensitive to the operational parameters of the voltage and frequency of the applied field, design parameters of the spatial arrangement of the applied field, the dielectric properties of the fluid and the particle, as well as the particle volume.

Whether it is filtering, concentrating, or purifying cells, particle manipulation is a crucial step in most biological assays. DEP presents an appealing tool for biological cell manipulation for diagnostics for several reasons.

**Increased testing access and frequency**

Able to generate forces on any cell type with only the application of an electronic field and requiring no chemical binding, antibodies, or cell contact, DEP presents a label-free and electronic particle manipulation method which is a very appealing tool in the context of more accessible first-line electronic diagnostic devices. Electronic based tests requiring no genetic or optical processing can come at significantly reduced material and equipment costs, while the relative ease of use can reduce personnel costs. Additionally, the simple fabrication requiring only one layer of conductive material for the formation of the DEP structure also lowers costs per test.

**Faster biosensing and earlier infection detection**

For small particles, diffusion to a sensing surface can take a long time. DEP offers a method of accelerating particles to a surface capture area on a shorter time scale than diffusion or gravitational forces alone could. Additionally, in the traditional and most common current method of pathogen detection, a patient sample is collected and cultured in order to expand the population of any bacteria present. This culturing step requires between
1 and 7 days of incubation time [9]. Other current detection methods decrease that time down to less than a few hours by utilizing molecular detection assays sensitive enough to detect a single colony forming unit per mL of blood [9]. The expensive cost per test as well as the technologically challenging implementation of these assays have prevented them from fully replacing the more lengthy traditional cell culture detection tests [9] and are not considered viable alternatives in resource-limited settings. DEP capture, however, presents an alternative method to cell culture for increasing the concentration of cells in a region.

**Increased diagnostic sensitivity**

Many applications require low limits of detection. Creating a localized region of particle concentration with DEP allows lower detection limits. Testing sensitivity can be increased by purifying and concentrating the particle of interest prior to detection. In diagnostic applications, lower detection limits mean earlier possible detection, resulting in more successful treatment outcomes.

**Adaptable assays**

Target particles all possess a unique set of dielectric properties, thereby conferring a degree of particle-specific selectivity in DEP operation. Furthermore, the response of particles is dictated by the frequency and magnitude of the applied electric field, both parameters that are dynamically tunable. Thus a DEP device can be either used for a range of applications simply by adjusting the operating conditions or utilized in a temporally dynamic fashion to alter DEP forces over time. Additionally, biological cells have complex structures, different material layers, and different sizes. Thus the sensitivity of the DEP force to the dielectric phenotype and size of a particle makes DEP a promising methodology for selective cell manipulation as well.

### 1.3 DEP in literature

A testament to the promising utility and wide ranging possible applications of DEP, an extensive array of DEP research has been conducted ever since Herbert Pohl’s first description
of the phenomenon of dielectrophoresis in 1951 [10].

DEP has been used to manipulate and study a host of biological particles, ranging in size from 10s of microns down to below 100 nanometers. Rare circulating tumor cells have been isolated [6,11,12]. White blood cells have been separated from red blood cells. Mammalian cells have been electroporated, and made to have synchronized cell cycles [13]. Bacterial cells have been concentrated from environmental and physiological samples [9,14,15].

Work in subcellular DEP has advanced greatly in more recent years, perhaps due to the increase in the accessibility of nanoscale fabrication. The magnitude of DEP force depends on particle volume, necessitating large electric field gradients for the manipulation of small particles on the order of $>10^6\text{ V/m}$ for sub-micrometer-sized particles [16]. Electrode designs with very small gap spacing have been used to achieve such high field gradients without the need for applying very high voltages.

AC electrokinetic techniques have also been employed to manipulate viruses [16–22], capture DNA [23–25], extract mRNA from cells [26], trap exosomes [27], and pattern polymer nanoparticles [21,25,28].

Beyond capture and concentration, separation of sub-micron particles has also been demonstrated for different viruses types and latex beads of varied surface chemistries [29]. Hughes et al. even demonstrate the ability to use viral DEP response to measure the dielectric properties of viruses subjected to different treatment, creating a methodology of assessment of viral infectivity [22].

**Discontinuous DEP retention**

Particle separation can be achieved by trapping a particle of interest in an area and allowing fluid or other particles to move past. DEP is regularly employed as the method of trapping particles from a fluid. DEP particle trapping most typically utilizes pDEP forces and so requires solutions of lower conductivities. Cells can have a tendency to adhere to the DEP electrodes when trapped [30], especially in low conductivity solutions that compromise cell health. Because upstream and downstream cellular culturing and quantification methods usually require high conductivity media on the order of 1 S/m, capturing cells in lower conductivity is less ideal than separating cells in physiologically relevant solutions [30].
Additionally the nature of this separation method necessitates discontinuous DEP trapping conditions (either voltage, solution conductivity, or flow rate) if the separated population is to be released from the DEP electrodes for further processing or study. Cellular adhesion can limit the efficiency of the release step.

**Continuous DEP deflection**

Another method of continuous DEP cell separation is DEP-deflection in which DEP electrodes preferentially push or pull one cell type towards a side of a microfluidic channel which then might flow into a separate outlet channel [6]. Electrode geometries for this type of separation are often angled with respect to the fluid flow direction in order to allow gradual lateral movement of the cells [6, 13, 30]. This deflection necessitates any velocity component perpendicular to the fluid flow direction. That movement could be to the side of a channel or even to different heights in a channel. As a consequence of these shifts in position perpendicular to fluid flow, particle velocity along the fluid flow also changes due to the parabolic velocity flow profile within microfluidic channels. This type of deflection in order to achieve altered velocity from flows streams is a general technique called field flow fractionation (FFF). When deflection is a result of DEP this is referred to as DEP-FFF. Utilizing disparity in cell densities, for example, cells can be drawn down by gravitational forces while being pushed up by nDEP forces from electrodes on the floor of a microfluidic channel. As these forces compete with each other, different cell types will have different elevations within the channel resulting in different flow speeds. They then can be separated according to their position in the channel.

Seo et al explored both divergent and convergent geometries for separating white blood cells from red blood cells in a high conductivity environment [30]. Red blood cells, being slightly more dense than white blood cells, experience a slightly lower equilibrium levitation height over the electrode surface in response to DEP force and gravitational force. The closer the cell to the electrodes, the stronger the electric field will be and thus the stronger the lateral DEP force on that denser cell, all other variables being equal [30]. By this disparity in cell density, and therefore lateral DEP force, Seo et al separated RBCs and WBCs with angled interdigitated DEP electrodes [30]. When the electrodes were angled out from the
center of the channel towards the channel edge (a divergent arrangement), the RBCs moved furthest to the channel edges. When the electrodes were angled from the channel walls in towards the center (a convergent arrangement), the RBCs moved furthest into the center. Interestingly, in both geometries, the cell population that was collected in the center of three outlet channels (WBCs in the divergent case and RBCs in the convergent case) was more purified than the population collected from the two side outlet channels [30].

1.4 Scope and novelty of this work

A wealth of DEP research supports the idea of DEP incorporation into diagnostic devices, and yet clinical devices utilizing DEP are rarely encountered. Several aspects of DEP operation present difficulties for successful incorporation into current clinical settings. Firstly, biological samples have a high fluid conductivity which can cause performance deterioration of DEP devices in several ways. Secondly, sample throughput high enough to allow for rapid processing times can also incur additional challenges. Cell population heterogeneity limits the resolution of selectivity that can be achieved. Additionally, the readout of the DEP-based test should also be electronic to lower costs and increase usability such that the devices could be adopted as less expensive alternatives for first-line diagnostics. Any DEP-based device that would be clinically useful must overcome these challenges.

This dissertation presents work toward the actualization of real-world electronic biosensors. We develop and validate a DEP system model for predicting the effect of device design parameters on DEP force generation, informing DEP device design for sample processing in clinically-relevant high conductivity fluids and high sample throughput. In addition to the design and operational system parameters, we also investigate the nuanced interactions of the material properties of the DEP device, fluid, and particle system components and the role they play in DEP performance limitations.

Informed by our experimental and theoretical work on DEP system performance optimization, we design and test devices for purifying bacterial cells from fluid samples. We validating their operational success with cellular relatives of two bacterial species involved in major world health issues: tuberculosis and \textit{E.coli} infections. With regard to infection
detection at low bacterial concentrations that does not require lengthy cell culture, we
demonstrate selective bacterial concentration from fluids containing red blood cells.

Integrating an electronic cell counter in line with a DEP device, we successfully enable
electronic on-chip quantification of particle populations and DEP performance assessment.
Toward low concentration sensing, we demonstrate the ability to detect single cells with
these on-chip counters. We then develop a novel fully electronic assay utilizing integrated
devices to selectively enrich and quantitatively assess immune cell activation as an indicator
of infection presence and progression.

1.5 Outline of chapters

- **Chapter 2** gives background information about DEP and other forces present on
  particles subjected to AC voltages within a fluidic environment and describes the
  basic multiphysics simulation developed for studying particle trajectory responses
  these environments.

- **Chapter 3** details device design, fabrication, and operation and presents main method-
  ologies used in this work for data collection and analysis of particle responses to DEP
  forces.

- **Chapter 4** presents and experimentally validates a DEP system model for the pre-
  diction of device design parameter effects on DEP performance, particularly in cases
  of high conductivity fluid operation.

- **Chapter 5** examines the interconnected effects of the material properties of the DEP
  system components on overall device performance and presents solutions for mitigating
  some common material-based performance losses.

- **Chapter 6** shows the ability to concentrate cells and selectively isolate cells from
  mixed cell populations with DEP by both capture-based structures and lateral move-
  ment structures which require lower DEP forces and are thus more amenable to high
  conductivity operation.
• **Chapter 7** demonstrates the integration of an electronic particle counter on-chip with DEP devices and characterizes counter performance through examination of experimental particle size detection and discrimination and device modeling.

• **Chapter 8** presents a novel DEP-based device with integrated counters for label-free electronic enrichment of activated T cells and quantitative immune activation assessment.

• **Chapter 9** presents preliminary work on device designs for enhancing throughput without increasing overall fluid volume, channel cross sectional area, or linear fluid flow speed.

• **Chapter 10** summarizes the major achievements and contributions of this work and discusses future directions of the research.
Chapter 2

Understanding electronic particle manipulation in fluidic environments

2.1 Modeling DEP force on complex particles

The broad landscape of DEP techniques and possible applications result from the simultaneously ubiquitous and discriminatory nature of DEP forces. All cell types and biological particles respond to DEP forces and, yet, the force on any particle type will be somewhat unique. DEP forces can be generated for simple homogenous spheres as well as more complex biological cells and sub-cellular particles. Recall that the overall DEP force on a particle is given by

\[ F_{DEP} = 2\pi a^3 \varepsilon_m \text{Re}[f_{CM}] |E|^2 \]  

(2.1)

where \( a \) is the particle radius, \( \varepsilon_m \) is the fluidic permittivity, \( E \) is the electric field, and \( f_{CM} \) is the Clausius-Mossotti factor describing the relationship between the dielectric properties of the particle and surrounding media.

Biologically relevant particles, such as cells and subcellular vesicles, can be non-spherical, non-homogenous, and non-solid, therefore necessitating more complex numerical models for accurate calculations of DEP forces. Cells are generally structured with a conductive fluid
core called the cytoplasm encased in a more insulating plasma membrane shell. Bacterial cells have an additional shell outside of the membrane called a cell wall. Both bacterial cells and eukaryotic cells can be spherical or non-spherical in shape. There are four main classes of particles utilized in the present work: a solid homogenous sphere (ie: polystyrene beads), a single shell inhomogeneous sphere (ie: immune cells), a single shell inhomogeneous oblate spheroid (ie: red blood cells), and a double shell inhomogeneous oblate spheroid (ie: rod-shaped bacterial cells). Schematic diagrams of these four cases are shown in Figure 2.1. For each particle case, the equation for complex permittivity calculation varies in order to take into account multiple shell layers and/or asymmetric particle shapes.

(a) Solid sphere

(b) 1-shell

(c) 1-Shell oblate spheroid

(d) 2-Shell prolate spheroid

Figure 2.1: Diagram of particle types for DEP modeling. Adapted from Ref [14] with permission from The Royal Society of Chemistry.

**Solid spheres**

Solid spheres, such as polymer microbeads, present the simplest case of particle permittivity calculations due to its homogeneity of material and symmetry of shape. The complex
permittivity of a zero-shelled sphere is given by

$$\varepsilon_p^* = \varepsilon - j \frac{\sigma}{\omega}$$

(2.2)

where $\varepsilon$ and $\sigma$ are the particle permittivity and conductivity, respectively, $\omega$ is the angular frequency of the applied electric field, and $j = \sqrt{-1}$. The CM factor is then calculated by

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2 \varepsilon_m^*}$$

(2.3)

where $\varepsilon_p^*$ and $\varepsilon_m^*$ are the complex permittivities of the particle and surrounding fluid medium, respectively. The ratio of the permittivity between the two materials dictates the magnitude and polarity of the CM factor and therefore the DEP force. Figure 2.2 shows how the CM factor changes as the ratio of the complex permittivity of a particle and the surrounding fluid media. When these values are equal (a ratio of 1), the CM factor is 0. As the media permittivity dominates, the CM factor approaches it’s minimum value of -0.5. As the particle permittivity dominates, however, the CM factor approaches it maximum value of 1. The magnitude of the CM factor determines the magnitude of DEP force, while the sign of the CM factor dictates the DEP force direction. For any set of conditions, the frequency at which the CM factor equals zero is called the crossover frequency.

Figure 2.2: The magnitude of the CM factor varies with the ratio of the complex permittivity of the particle and the surrounding medium. For particle permittivities greater than medium permittivities, the CM factor is positive. For particle permittivities less than medium permittivities, the CM factor is negative. When permittivities are equal, the CM factor, and therefore the DEP force on the particle, will be zero.
Figure 2.3 shows the calculated CM factor as a function of both media conductivity and frequency of the electric field. For all solution conductivities, the CM factor is negative at high frequencies. The CM factor is also predicted to be negative at low frequencies for high conductivity solutions. Only for low conductivity solutions at frequencies below about $500kHz$ would these particles experience positive DEP forces.

![Graph showing the calculated CM factor as a function of applied frequency and conductivity of suspending fluid.](image)

**Figure 2.3:** Calculated CM factor for polystyrene microspheres as a function of applied frequency and conductivity of suspending fluid

**Single-shell spheres**

Cells have a more complex DEP force response than solid particles. Some cells are comprised of two main material layers, an internal cytoplasm core and an encasing plasma membrane, as diagrammed in Figure 2.1b. In order to take the two layers into account, the overall particle permittivity now becomes a function of the radius of each layer and the permittivity of each layer’s material [31]. The complex permittivity of a single-shelled spherical particle is given by

$$
\epsilon_p = \epsilon_{mem} \left( \frac{a_0}{a_1} \right)^3 + 2 \left( \frac{\epsilon_{cyt} - \epsilon_{mem}}{\epsilon_{cyt} + 2\epsilon_{mem}} \right) \left( \frac{a_0}{a_1} \right)^3 - \left( \frac{\epsilon_{cyt} - \epsilon_{mem}}{\epsilon_{cyt} + 2\epsilon_{mem}} \right)
$$

(2.4)
where \( a_0 \) and \( a_1 \) are the radius of the particle’s membrane and cytoplasmic layer, respectively, and the subscripts \( \text{cyto} \) and \( \text{mem} \) refer to the cytoplasm and membrane layers, respectively [32]. As was the case for the solid sphere, the CM factor is calculated for this particle type by

\[
f_{CM} = \frac{\epsilon^*_{p} - \epsilon^*_m}{\epsilon^*_p + 2\epsilon^*_m}
\] (2.5)

**Single-shell oblate spheroids**

Single-shell oblate spheroids, such as red blood cells, also have the typical cellular membrane layer, but they also have a flattened shape requiring more complex numerical modeling. For non-spherical particles, the overall CM factor is an average of the CM factor along each major axis \((x, y, z)\) of the cell [14]. The complex permittivity of a single-shelled oblate spheroid is shown by Gagnon et al. to be

\[
\epsilon^*_p = \epsilon^*_m \frac{\epsilon^*_m + (\epsilon^*_\text{cyt} - \epsilon^*_m)(A_{ip} + v(1 - A_{op}))}{\epsilon^*_m + (\epsilon^*_\text{cyt} - \epsilon^*_m)(A_{ip} - vA_{op})}
\] (2.6)

where \( v \) is the volume ratio of the cell’s exterior to interior space defined by

\[
v = \frac{a^2c}{(a + d)^2(c + d)}
\] (2.7)

where \( a \) is the cell radius, \( c \) is the half length of the cell, and \( d \) is the thickness of the membrane. \( A \) is a depolarization factor that, for red blood cells, can be given by

\[
A_{op} = A_{ip} = \frac{1 + \frac{2}{3}(1 - \gamma^{-2}) + \frac{2}{3}(1 - \gamma^{-2})^2 + ...}{3\gamma^{-2}}
\] (2.8)

where \( \gamma = c/r \) [32].

The CM factor for these cells is calculated as

\[
f_{CM} = V_c \epsilon_m \frac{\epsilon^*_p - \epsilon^*_m}{\epsilon^*_m + (\epsilon^*_p - \epsilon^*_m)A_{op}}
\] (2.9)

where \( V_c \) is the cell volume [32].
Double-shell prolate spheroids

Bacterial cells also have a cell membrane and are a non-spherical shape, but they have additional layer external to the cell membrane called a cell wall. While some bacteria are roughly spherical in shape, some, such as *E.coli* and *M.smegmatis* have a rod-like morphology. Over the years the dielectric modeling of *E.coli* has evolved from Fricke et al.’s one-shell spherical model \[33\] to a two-shell spherical model incorporating an outer cell wall by Carstensen et al. \[34\]. Extending these two shells for a non-spherical morphology, Asamai et al. further advanced the accuracy of the DEP model of *E.coli* \[35\]. Here we have used the notation from Park et al. \[31\] of the equations presented by Asami et al. \[35\].

Allowing the radial dimensions perpendicular to the major axis to be equal, the complex permittivity of the particle across each \(x, y, z\) axis (denoted \(k\)) for a 2-shelled oblate spheroid is given by

\[
\epsilon_{1k}^* = \epsilon_{wall}^* + \frac{(\epsilon_{2k}^* - \epsilon_{wall}^*)A_{1k} + \lambda_1(\epsilon_{2k}^* - \epsilon_{wall}^*)(1 - A_{0k})}{\epsilon_{wall}^* + (\epsilon_{2k}^* - \epsilon_{wall}^*)A_{1k} - \lambda_1(\epsilon_{2k}^* - \epsilon_{wall}^*)A_{0k}}
\]

(2.10)

where \(\epsilon_{2k}^*\) is calculated similarly for the inner layer as

\[
\epsilon_{2k}^* = \epsilon_{mem}^* + \frac{(\epsilon_{cyt}^* - \epsilon_{mem}^*)A_{2k} + \lambda_2(\epsilon_{cyt}^* - \epsilon_{mem}^*)(1 - A_{1k})}{\epsilon_{mem}^* + (\epsilon_{cyt}^* - \epsilon_{mem}^*)A_{2k} - \lambda_2(\epsilon_{cyt}^* - \epsilon_{mem}^*)A_{1k}}
\]

(2.11)

where

\[
\lambda_1 = \frac{a_1 b_1 c_1}{a_0 b_0 c_0}, \quad \lambda_2 = \frac{a_2 b_2 c_2}{a_1 b_1 c_1}
\]

(2.12)

and \(A_{ik}\) is a depolarization factor along each axis \(k\) for the layers \((i = 1, 2, 3)\) given for the principle axis as

\[
A_{ix} = \frac{q_i}{(q_i^2 - 1)^{3/2}} \ln \left( q_i + (q_i^2 - 1)^{1/2} \right) - \frac{1}{q_i^2 - 1}
\]

(2.13)

and for the two minor axes, assumed equal, by

\[
A_{iy} = A_{iz} = \frac{1}{2}(1 - A_{ix})
\]

(2.14)

where

\[
q_i = a_i/b_i
\]

(2.15)
The CM factor is calculated by averaging the complex permittivity along each of the axes as

\[
I_{CM} = \frac{1}{3} \sum_{k=x,y,z} \left( \frac{\epsilon_{k} - \epsilon_m^*}{\epsilon_m^* + (\epsilon_{k} - \epsilon_m^*)A_{0k}} \right) 
\]  

(2.16)

where \(\epsilon_m^*\) is the complex permittivity of the fluid media.

The calculated CM factor for an example bacteria used in this work is shown in Figure 6.8a.

![Figure 2.4: Calculated CM factor for an E.coli bacterium modeled as a 2-shell oblate sphere as a function of applied frequency and conductivity of suspending fluid](image)

The cell wall of a bacterium can itself be very complex, having several layers and even large structures extending out from the cell surface [36]. Toward more accurate DEP models for complex bacteria, Hölzel expanded the two-shell spherical model to include a periplasmic space and an outer membrane in place of the cell wall layer for E.coli [37]. Bai et al. then modeled these three shells in a more accurate spheroid morphology to obtain a more accurate model of E.coli dielectric properties [38]. Sanchis et al. also used a three-shell model to characterize E.coli as rod-like particles and Staph aureus as a three-shell spherical particle [39]. In this work we treat the complex cell wall as a single layer, using values from literature for our calculations.
For all these particle types, the frequency of the applied electric field also dramatically affects particle response. Similarly, the properties of the suspending fluid media can dramatically alter the force magnitude and direction on the particles. Even in the case of simple solid spheres, overall particle conductivity is not independent of the suspending fluid. Particle conductivity is a function of both the internal bulk conductivity and its surface conductivity which is affected by particle size and even the surrounding fluid. Most materials in a fluid will have some surface charge. Mobile charges in the solution, such as ions, position themselves to counterbalance any surface charges on a material. The inner layer of these counter charges is called the Stern layer. Slightly farther from the surface the resultant alteration of ion arrangement is called the diffuse layer. Farther away still the ion arrangement becomes like that of the bulk fluid. Overall particle conductivity depends on these surface effects and can be calculated by

\[
\sigma_p = \sigma_b + \frac{2K_{s,i}}{a} + \frac{2K_{s,d}}{a}
\]  (2.17)

where \(\sigma_b\) is the bulk conductivity, \(a\) is the particle radius, \(K_{s,i}\) and \(K_{s,d}\) are the surface conductance values due to the Stern and diffuse double layers formed in solution, respectively [22]. The inner Stern layer depends mostly on the particle surface charge while the diffuse layer is more determined by the fluid conductivity and zeta potential [22]. While this first order effect of particle radius on particle conductivity contributes less dramatically to the overall DEP force experienced by the particle than the third order effect of particle radius as seen in Equation 2.1, it nevertheless does influence both the magnitude of the CM factor along the frequency space.

### 2.2 Non-DEP forces and reactions in the AC electronic and microfluidic environment

Besides the DEP force on particles in a fluid system, many other forces can affect particle movement.
2.2.1 Ubiquitous forces

Brownian motion

For very small particles, Brownian motion begins to compete with this sedimentation force. Brownian motion describes the random motion of small particles in a fluid. The movement of atoms in a fluid is more rapid than the movement of a larger suspended particle, resulting in collisions between atoms and the particle [40]. These random collisions cause random particle movement with a velocity related to the particle size and fluid viscosity. The diffusion coefficient \((D)\) is given by

\[
D = \frac{k_B T}{6\pi \eta a}
\]  

(2.18)

where \(k_B\) is the Boltzman constant \((1.3806 \times 10^{-23} \text{ m}^2 \cdot \text{kg} \cdot \text{s}^{-2} \cdot \text{K}^{-1})\), \(T\) is the temperature in Kelvin, \(\eta\) is the fluid viscosity in \(\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}\), and \(a\) is the particle radius in m [40]. The diffusion coefficient varies inversely with particle radius and linearly with temperature. It has a value of approximately \(2.19 \times 10^{-13} \text{ m}^2 \cdot \text{s}^{-1}\) for a \(1\mu\text{m}\) particle in water at room temperature and, if the temperature is raised to the boiling point of water (a 27% increase), the diffusion coefficient increases by the same amount to about \(2.78 \times 10^{-13}\) as shown in Figure 2.5. From the diffusion coefficient, particle velocity resulting from Brownian motion

![Figure 2.5: The diffusion coefficient of a particle in water varies inversely with particle radius and linearly with temperature](image)

can be calculated.

**Gravity and Buoyancy**

All particles in a microfluidic channel experience gravitational force directed downward, independent of the particle’s position in the channel. The gravitational force \( F_{\text{grav}} \) is defined by

\[
F_{\text{grav}} = \frac{4}{3} \pi a^3 (\rho_p) g
\]

where \( a \) is the particle radius, \( g \) is the gravitational constant directed downwards, and \( \rho_p \) is the mass density of the particle [41].

Simultaneously, the fluid surrounding a particle exerts a buoyancy force \( F_{\text{buoy}} \) directed upwards, opposing the particle’s weight and is defined as

\[
F_{\text{buoy}} = \frac{4}{3} \pi a^3 (\rho_m) g
\]

where \( \rho_m \) is the mass density of the surrounding fluid media [41].

Combining these two forces yields a net gravitational force \( F_g \) that scales linearly with particle volume and becomes negligible as the density of the particle approaches the fluid density [42], as shown by

\[
F_g = \frac{4}{3} \pi a^3 (\rho_p - \rho_m) g
\]

In a microfluidic channel, cells tend to settle at the bottom of the channel or tubing over time due to differences in the density of the cells and surrounding fluid [6]. The rate of sedimentation \( v_s \) can be calculated by

\[
v_s = \frac{4a^2 (\rho_p - \rho_m) g}{18\eta}
\]

where the sedimentation rate \( v_s \) [m · s\(^{-1}\)] and can be seen to scale with the square of the particle radius \( a \) [m] and linearly with the difference in densities between the particle \( \rho_p \) and surrounding fluid media \( \rho_m \) [kg · m\(^3\)], and inversely with the fluid viscosity \( \eta \) [kg · m\(^{-1}\) · s\(^{-1}\)], and \( g \) is the gravitational acceleration [m · s\(^{-2}\)] [43].
Sedimentation of a particle due to gravity has a terminal velocity \(v_g\) given by

\[
v_g = \frac{2}{9} \frac{a^2 g}{\eta} \left( \frac{\rho_p}{\rho_m} - 1 \right)
\]  

(2.23)

where \(a\) is the particle radius, \(g\) is the gravitational constant, \(\eta\) is the kinetic viscosity of the fluid, \(\rho_p\) and \(\rho_m\) are the densities of the particle and the surrounding fluid medium respectively \([44]\).

Gravity and DEP combined effect on particle elevation: Since both forces scale with the particle volume, the equilibrium elevation position of a particle is independent of size, but rather depends on frequency and field strength \([42]\). Particles subjected to negative DEP from planar interdigitated electrodes on the floor of a microfluidic channel experience several simultaneous forces. The DEP force acts on the particle in the direction along the channel as well as vertically and decays exponentially with particle height in the channel. Sedimentation force is directed downward and is independent of particle position in channel.

Wang et al. use a combination of DEP and conventional FFF to separate polystyrene beads of differing sizes (6, 10, and 15\(\mu\)m) as well as beads of differing surface chemistries \([45]\). In another work, DEP-FFF is used to separate human breast cancer cells from normal T-lymphocytes \([46]\).

Particles are forced by DEP into different fluid velocity streams as they are forced into differing vertical positions in the channel. Separation is accomplished by temporally coordinated fluid collection. While a powerful technique, because collection of each particle type occurs at a specified range in time, this approach as-is is not a continuous separation method.

Particles moving over interdigitated electrodes experience varied DEP force as their position changes in both the horizontal and vertical directions. Because the magnitude of the electric field gradient is greatest at the edges of electrodes, the horizontal component of the DEP force is also greatest when a particle is directly above the edge region. In contrast, the horizontal DEP force goes to zero for a particle above the center of the electrode or the center of the gap between the electrodes. Thus as a particle travels over interdigitated electrodes, the horizontal DEP force oscillates, being drawn either toward or away from
whatever electrode edge it is nearest, and being equally drawn toward or away from the edges when mid distance from two edges. Thus the horizontal DEP component actually switches directions as the particle passes over electrodes.

Similarly the vertical component of the DEP force is greatest when the particle is over the electrode edges. In this case, however, only the magnitude of the force oscillates with horizontal particle position and does not switch directions. Experimentally we can see the resultant oscillations in particle velocity as it passes over periodic electrodes. In order to model average particle velocity in these cases it is useful to utilize the periodicity of the electrode structures and use the average DEP force components. Although the horizontal DEP force oscillations contribute to the velocity fluctuations, the average horizontal DEP force is zero for a constant particle height and thus does not affect the average particle velocity \[45\]. Similarly the vertical DEP force oscillations move the particle into slower and faster fluid flow streams, but, by averaging the vertical DEP force component, we can use only the spatial average of the vertical DEP force when examining average particle velocity.

### 2.2.2 Electro-induced forces

The application of an electric potential to a fluidic environment can cause many different effects. Figure 2.6 diagrams how an electric potential can generate both an electric field gradient as well as a thermal gradient and how those cause different forces on particles in solution.
Electrophoresis

Electrophoresis describes charged particle motion induced by an electric field acting on particle charges. This applies to colloid particles in solution as well as to solution ions. The mechanism of force is the Coulomb force of the electric field ($E$) on a particle charge ($q$).

$$ F = qE $$  \hspace{1cm} (2.24)  

To calculate the total electrophoretic force, the Electric field is multiplied by the total particle charge ($Q$) is calculated by integrating surface charge density over the particle surface.

$$ F_{EP} = QE $$  \hspace{1cm} (2.25)  

Under AC electric fields, the time averaged force is typically treated as zero, but DC fields would result in a nonzero time averaged force [48].
Electrothermal flow

Electrothermal flow describes fluid motion induced by a thermal gradient. When electrical current passes through an electrically conductive fluid, Joule heating can occur [48]. The rise in temperature increases with the conductivity of solution and the square of the applied potential and goes inversely with the thermal conductivity of the fluid. An approximation of the change in temperature \( T \) can be calculated as

\[
\Delta T \approx \frac{\sigma V^2}{2k_m}
\]

where \( V \) is the applied voltage and \( \sigma \) and \( k \) are the electrical and thermal conductivity of the fluid, respectively. Flowing fluid over the electrodes reduces Joule heating at the electrode surface [12]. If more thermally conductive the materials surround the fluid, Joule heating can be further reduced [12]. A uniform electric field yields a spatially uniform delta T. In a nonuniform electric field environment, however, spatially varied changes in temperature are present and cause both fluid flow as well as spatially varied changes in solution permittivity and conductivity [48]. spatially nonuniform conductivity of solution can be caused by local ohmic heating [16].

Theoretical calculations of the electrothermal flow velocity and spatial profiles by Park et al suggest that fluid flow in space stays relatively constant as solution conductivity increases, but fluid velocity increases linearly with solution conductivity and to the fouth power with applied AC voltage [49].

Electroosmosis

Electroosmosis describes fluid motion induced by electric field acting on solution ions. First reported in 1999 by Ramos et al. [48]. During each half cycle of an AC electric signal applied to neighboring electrodes, ions in solution move to the electrode surface to counterbalance the charge on the electrode, forming an ion double layer on the electrodes. Ions moving toward neighboring electrodes of opposing polarity create bulk fluid flow towards the center of electrodes. These meeting flows are pushed upwards and a system or stable fluid vortices result. Particles can be moved to areas of stagnant flow and trapped over the electrode
Non-faradaic reaction

Non-faradaic reactions describe the changes that take place at the electrode electrolyte interface induced by electric current but wherein no electrons are transferred between the two materials. An ideally polarized electrode experiences no charge transfer to or from the electrolyte and can be modeled as a capacitor that experiences current only until it is fully “charged”. For the case of an ideally polarized electrode, an applied potential will attract solution ions to the electrode surface, but will not result in any electron transfer. Instead, an electric double layer forms at the interface, with a buildup of ion-bound charges balancing the buildup of charge at the electrode surface. In response to either positive or negative electrode potential, anions or cations can absorb to the electrode surface. This first section of the formed double layer, called the inner Helmholtz plane, contains the least mobile of the ion charges as adsorbed ions can bond strongly to the electrodes and remain present even if the potential of the electrode switches \[48\]. The extent of the adsorption heavily depends on the electrode material and solution ion species. In the next layer, the outer Helmholtz plane, electrostatic forces draw in solvated ions with opposing polarity of the electrode, independent of electrode material or ion species.

Faradaic reaction

Faradaic reactions describe the transfer of electrons between electrodes and a surrounding electrolyte solution \[48\].

2.2.3 Fluid-induced forces

Particles suspended in solution are introduced to DEP electrode structures through microfluidic channels. The following are basic principles of the fluid flow conditions in this work.
Laminar flow

Our microfluidic devices operate under laminar flow conditions as defined by a Reynold’s number much less than 1. The Reynolds number \((Re)\) is given by

\[
Re = \frac{\rho v D}{\eta}
\]

(2.27)

where \(\rho\), \(v\), and \(\eta\) are the fluid density, average velocity, and dynamic viscosity, respectively [50]. \(D\) is the characteristic channel dimension, such as the diameter in the case of a channel with a circular cross section.

Fluid velocity profile

In a microfluidic channel, fluid velocity varies nearly parabolically with position in the cross section of the channel such that the fluid media velocity \((v_m)\) is greatest at the channel center and lowest at the channel walls as shown by

\[
v_m(h) = 6\bar{v}_m \frac{h}{H} \left(1 - \frac{h}{H}\right)
\]

(2.28)

where \(\bar{v}_m\) is the average fluid media velocity, \(h\) is the height in the channel, and \(H\) is the total channel height [8].

Particle velocity profile

Particles in fluid flow do not travel at exactly the same velocity as the fluid surrounding them. The relation between the particle velocity \((v_p)\) and the media velocity \((v_m)\) is given by

\[
v_p = k_r v_m
\]

(2.29)
where $k_r$ is a retardation factor highly dependent on particle radius and position in the channel and defined as

$$k_r = \begin{cases} 
1 - \frac{5}{16} \left( \frac{a}{h} \right)^3 & \text{for } h > 1.1a \\
0.74 \cdot 0.5 - 0.2 \log(\frac{h}{a}) & \text{for } h < 1.1a
\end{cases}$$

where $a$ is the particle radius and $h$ is the height of the center of the particle in the channel [51].

**Drag**

The drag force describes the force on a particle moving through a viscous fluid. In low Reynold’s number flow conditions as in a microfluidic channel, the drag force ($F_{\text{drag}}$) can be written as

$$F_{\text{drag}} = -\gamma v$$  \hspace{1cm} (2.30)

where $v$ is the cell velocity and $\gamma$ is the friction coefficient defined by

$$\gamma = 6\pi \eta a$$  \hspace{1cm} (2.31)

where $\eta$ is the dynamic fluid viscosity, $a$ is the cell radius [6]. Particles that are larger or moving faster experience higher drag forces opposing their motion. For lateral DEP movement of particles within a channel with electrodes on the channel sides, the lateral equilibrium particle position across the channel (in the $y$ direction) would satisfy

$$F_{\text{DEP}_y} = F_{\text{drag}_y}$$

$$F_{\text{DEP}_y} - F_{\text{drag}_y} = 0$$

$$2\pi r^3 \varepsilon_m Re[K] \nabla E^2 - 6\pi \rho v_y v_y = 0$$

$$r^2 \varepsilon_m Re[K] \nabla E^2 = 3v_y \eta$$

$$v_y = \frac{r^2 \varepsilon_m Re[K] \nabla E^2}{3\eta}$$
In order for a cell to be captured by DEP force in the a fluidic microchannel, the DEP force must be at least equal to the drag force. Thus the maximum cell velocity would be

$$v_{\text{max}} = \frac{F_{\text{DEP}}}{6 \pi \eta r}$$

(2.32)

**Shear stress**

The shear stress on a particle ($\tau$) in a fluid can be calculated by the Law of Poiseuille as

$$\tau = \frac{4Q\eta}{\pi R^3}$$

(2.33)

where $\tau$ is the shear stress [N/m$^2$], $Q$ is the volumetric flowrate of the fluid [mL/sec] through a constriction of radius $R$ [m], and $\eta$ is the fluid viscosity [kg/(ms)] [43]. Physiological shear stress on cells in human typically ranges from 1-7N/m$^2$, while veinous shear stresses are approximately an order of magnitude lower at 0.1-0.6N/m$^2$. Murine shear stress values can be an order of magnitude higher than those of humans [43].

### 2.3 Modeling DEP-induced particle response in fluidic environments

Multiphysics simulations were made using COMSOL software to simulate particle response to simultaneous DEP and fluidic forces in a microfluidic channel. Electrodes can be defined with some geometry (Figure 2.7a, the fluid velocity can be simulated (Figure 2.7b), as well as the electric field formed by voltage applied to the defined electrodes (Figure 2.7c).
Figure 2.7: a) General channel geometry for COMSOL simulations for a channel $H$ high with electrodes on the channel floor, fluid inlet on the left, and fluid outlet on the right. b) COMSOL simulation of fluid velocity in a general channel. The red color near channel vertical center indicates highest flow rates and blue is lowest flow rates. c) COMSOL simulation of electric field resulting from an array of planar interdigitated electrodes of alternating polarities. Red color indicates highest positive voltage and blue is greatest negative voltage.

Introducing particles into this environment we can monitor their predicted motion as a function of fluid flow, electrode geometry design, particle or fluid dielectric properties, voltage, or frequency. Figure 2.8a shows an example of a particle trajectory change as the frequency of the applied field is altered from 100kHz to 80MHz. The crossover frequency for these conditions can be seen between 10MHz and 20MHz as the net DEP force on the particle switches from positive to negative DEP. Figure 2.8b illustrates the same scenario, but with a higher frequency resolution to more closely examine the crossover frequency.
Figure 2.8: Simulated *E.coli* response trajectories to a) wide and b) narrowed range of frequencies of applied AC signals in 0.001S/m fluid. The crossover can be seen to occur here between 1.6 and 1.7MHz [Simulation parameters: $V = 5V$, flowrate = 100um/s, $H = 50um$, $\sigma_m = 0.001S/m$, $a = 1.09um$, $\sigma_1 = 0.19S/m$, $\epsilon_1 = 61$, $\sigma_0 = 0.68S/m$, $\epsilon_0 = 10.8$, $\delta_0 = 25nm$]

Beyond a theoretical modeling and simulation work, we next design, fabricate, and experimentally utilize DEP for particle manipulation applications.
Chapter 3

Fabricating and operating DEP devices

The use of DEP for particle manipulation has a broad array of uses and applications. DEP-based devices adopt many electrode and fluidic geometries optimized for varied target applications. All DEP applications, however, require device designs that incorporate two major components: the ability to generate a nonuniform electric field and a system for fluid management.

Electric field generation requires the presence of charged particles. A pathway of conductive material is needed to introduce charged particles and can be made either from a permanently conductive material (such as a metal) or a material with induced-conductivity (such as photoconductive materials [52]). To achieve a nonuniform generated field there must be a spatial or temporal arrangement of the conductive material pathways.

This spatial arrangement can take many forms and can be either permanent (such as a patterned metal arrangement) or induced, either in space (such as projected images on a photoconductive material) or in time (such as a multiplexed array or traveling wave). Permanent arrangements can be confined to a 2D surface (such as planar electrodes on a channel floor) or 3D configurations (such as electrodes on the side walls or the floor and ceiling of a channel).

Because electric field strength decays with the square of distance, fields generated by
planar electrodes on the channel floor decay through the vertical space of a fluidic region. More complex layers of parallel electrodes such as on the top and bottom or the sides of a fluidic channel have the advantage of providing a relatively stronger electric field over the entire fluid region, but the disadvantage of such designs come in the time and cost of fabrication. Additionally, complexity of electrode arrangement on channel walls can be limited and electrodes patterned on channel ceilings can limit visualization of particle motion within the channel.

The overarching goal of this work is to enable more accessible biosensing devices. To this end, we prioritize keeping fabrication and operational costs low. As such we use permanently conductive pathways to generate our electric field, arranged on a 2D plane to impart field non-uniformity. These planar metal electrodes are patterned onto the floor of a microfluidic channel. Throughout this work, variations on the planar arrangement of the electrodes are utilized to suit different target particles and applications.

This chapter introduces our base DEP device design and details the fabrication process for the devices. The electronic and fluidic components of device operation are explained as well as the general methods for data collection. While the exact data analysis process varies with each specific experimental design, some general heavily utilized analysis processes of particle detection and tracking are discussed.

3.1 Base device design

The base device design utilized in this work consists of an insulating base substrate patterned with planar metal electrodes and capped by side walls and a transparent ceiling to create a microfluidic channel. The channel allows particles suspended in fluid to be brought over the patterned DEP electrodes with controlled velocity, height, and without concentration changes due to evaporation.

Our basic chip layout is shown in Figure 3.1a. The footprint is typically 8mm wide x 40mm long x 0.5mm thick. Fluid introduced through the channel inlet flows over the DEP electrodes and out of the channel outlet. An array of twenty chip pads along one side of the chip allows external electronic interfacing for voltage application. From the chip pads
extend metal lead lines to connect each DEP structure within the fluidic channel. Each DEP structure consists of two electrode circuits and, therefore, two lead-ins and two chip pads as well. For twenty chip pads, then, up to ten independent DEP structures can be utilized on a single chip.

The basic DEP structures, shown in Figure 3.1b, consists of two circuits of electrodes, interdigitated to impart the applied voltage over an area of the channel floor while maximizing the electric field gradient by having opposing voltage polarity on each neighboring electrode. The number, length, and width of these interdigitated “fingers”, as well as the gap width between fingers can all be varied to optimize for a given particle. Additionally the edge profile of the electrodes, the angle of the electrodes with respect to the fluid flow, and the channel geometry and number of inlets and outlet channels can all be varied for different applications. Having multiple DEP structures in the same microfluidic channel allows for efficient experimental design in which the effects of a range of values for a given variable can be studied all in the same chip or several repeated structures can give repeat measurements without introducing any variation incurred by changing a fluid sample to new chips for each measurement.

Figure 3.1: General DEP device design. a) Layout of basic DEP chip with labeled features. b) Layout of an interdigitated DEP array with labeled features. c) Image of a fabricated DEP microfluidic chip.
Figure 3.1c shows an example of this base chip design after fabrication. Here the substrate is a silicon wafer covered by 2µm of insulating oxide. The metal electrodes are patterned in gold and the microfluidic channel is made of PDMS and has one inlet and one outlet. Next the details of chip fabrication are discussed.

### 3.2 Device fabrication

The choice to use planar metal electrode designs keeps device fabrication relatively simple and therefore the costs per chip low. In general, to fabricate a DEP device, we pattern conductive material on an insulating substrate, create a microfluidic channel to go over top of the electrodes, and then bond the two pieces together.

Our experimental data collection necessitates having at least one side of the device transparent to allow for imaging and tracking of particles in the fluid. Glass satisfies the need for electrode patterning compatible surfaces, bonding compatible surfaces, as well as transparency. In some cases we do utilize glass substrates for DEP devices. More commonly, however we pattern our electrodes on a silicon substrate, coated with an insulating top layer, with a transparent PDMS microfluidic channel bonded over top of the electrodes. In later chapters we will discuss the benefits and drawbacks we experienced with using glass vs silicon substrates in terms of heat insulation (Chapter 5) and electrical insulation (Chapter 4). The fabrication process details are diagrammed in Figure 3.2 and discussed in detail in the following sections.
Figure 3.2: Diagram of fabrication flow for DEP devices. a) Electrodes are patterned onto a substrate and passivated. b) A microfluidic channel mold is made and fluidic channels cast in PDMS. c) The microfluidic channel is bonded to the substrate and sample is introduced over the device.

### 3.2.1 Planar DEP electrode fabrication

The base of the DEP device consists of a substrate handle, patterned metal electrodes, and, optionally, a layer of passivating oxide over the electrodes.
**Electrode patterning**

To pattern the DEP electrodes in metal, a commercially available 100mm silicon wafer with 2µm of insulating thermally grown SiO₂ (Silicon Valley Microelectronics) or a borofloat 33 glass wafer (University Wafer) was cleaned with hot acetone followed by hot methanol and sonicated then rinsed in DI water and dried with nitrogen gas. The surface was further cleaned by oxygen plasma at 250 Watts for 1 minute (Glow Research, AutoGlow plasma system). Right before photoresist spinning, the substrate was dehydrated on a hotplate at 200°C for at least 5 minutes to assist with resist adhesion. Once cooled to 100°C, wafers were removed from the hotplate, aligned on a spinner (Laurell Technologies) and LOR5A photoresist was immediately spun onto the wafer for 1 minute at 3,000rpm and baked at 175°C for 10 minutes. A second layer of photoresist (Shipley S1805) was then spun on at 4,000rpm for 1 minute and baked at 120°C for 4 minutes. The resist was exposed to the desired pattern through either a transparency mask (CAD Art Services Inc) or a laser written chrome on glass mask at 115mJ in a mask aligner (EV Group, EVG620) then baked at 115°C for 1 minute. After developing the pattern in MF319 for 80-90 seconds with very gentle agitation, the wafer was rinsed very gently in DI and dried with nitrogen gas. An oxygen plasma clean was done at 150 Watts for 30 seconds to remove any resist residue left in the cleared areas of the pattern. Either 300nm of aluminum or 15nm of titanium followed by 285nm of gold were deposited on the wafer by electron beam deposition under vacuum less than $2 \times 10^{-6}$Torr (Kurt J. Lesker or Denton Vacuum). The metal is deposited directly onto the substrate in the areas cleared of resist and onto the top of the resist in areas where resist remains. The metal on the resist is lifted off by dissolving the remaining resist in hot NMP followed by a brief sonication in NMP followed by a progressive solvent clean with sonication in hot acetone, then methanol, then isopropanol, and DI then dried with nitrogen gas.

**Oxide deposition for electrode passivation**

Optionally, a layer of SiO₂ was deposited onto the DEP structures of each chip. If done, a 200nm layer of SiO₂ was deposited onto a chip by plasma enhanced chemical vapor deposi-
tion (PECVD) at 400°C (GSI). Deposition rates averaged 2.08nm/sec. Oxide thickness was measured by ellipsometry (Woolam) on a reference silicon sample present in the chamber during the deposition. To keep the resistance pathway to the active region of the DEP device low, all of the chip bonding pads were left without any additional oxide. This was done either by covering the region to be kept clear with a silicon block during oxide deposition or by etching the deposited oxide away after deposition. For etching, a layer of resist was spun over the deposited oxide layer and patterned in a similar manner used for electrode patterning. Once developed, the regions over the bonding pads to be etched were cleared of resist while the remaining chip had protective resist on it. SiO_2 was etched with 10:1 buffered oxide etch then rinsed well with DI and dried with nitrogen. Etch rates were monitored by ellipsometry measurements and continued until the PECVD oxide was fully removed.

**Dicing**

The wafer was then diced into individual chips to have microfluidic interfaces bonded. For dicing, the wafer was mounted onto a sacrificial carrier. To mount the DEP wafer, either a layer of wax (Crystalbond) or a layer of thick resist (Shipley) was applied to a carrier wafer.

For the wax mounting, the surfaced of the DEP wafer was first protected by spinning a layer of photoresist (Shipley S1818 or 1827) at 2,000rpm for 1 minute. This was repeated two more times and then the wafer was baked at 80-100°C for 1 minute. To build up a very thick layer, this was done a total of three times. The carrier wafer was then heated to 100°C and wax was applied to its surface. The DEP wafer was set on top of the melted wax and the heat removed to allow the wax to solidify, bonding the two wafers together.

A much better option for wafer mounting was ultimately chosen which utilized resist instead of wax as the bonding agent. While the bond strength was not noticeably better than with wax, the post-dicing cleaning of the wafer was found to proceed without issues of residue sometimes seen with the wax mounting. For the resist mounting, the carrier wafer received the thick three layers of spun resist but not baked. The DEP wafer was placed on top of this thick unbaked resist and the two wafers baked together on a hotplate at 115°C for 8 minutes. Then the thick three layer resist was spun onto the top of the mounted DEP
wafer and baked at 115°C for 3 minutes to protect the wafer surface.

The wafer was diced on a dicing saw. Afterwards, if mounted with dicing wax, the wax was removed by hot DI and sonication. The mounting resist and protective resist was removed by hot NMP and sonication, hot acetone and sonication, hot methanol and sonication followed by a DI rinse and nitrogen gas drying.

3.2.2 Microfluidic channel fabrication

The microfluidic channels were mostly fabricated in poly(dimethylsiloxane) (PDMS), a transparent and biocompatible material. Many material properties of PDMS make it well suited for microfluidic device fabrication: 1) It has high optical transparency allowing for little optical signal loss during imaging; 2) Its physical stability enables small micro-scale features to be reliably patterned into the PDMS; 3) It is biocompatible and therefore non-toxic to cells; 4) The fabrication process is rapid and low-cost; and 5) The surface chemistry enables secure bonding to both silicon and glass substrates [48]. PDMS microfluidic channels are formed by casting liquid PDMS onto a mold and then curing the material. The molds for PDMS channels were made out of SU-8, patterned on a silicon wafer substrate.

Microfluidic mold fabrication

Microfluidic molds were made on a 100nm silicon wafer substrate. The wafer was subjected to 4 minutes of 10:1 buffered oxide etch to remove the native SiO₂ layer from the silicon surface to increase adhesion between the wafer and the photoresist. After thorough DI rinse, nitrogen gas drying, and dehydrating the wafer at 200°C for at least 5 minutes, a negative photoresist (SU-8 2015, MicroChem) was spun onto the wafer with a spin coater (Laurell) at 500rpm for 10 seconds then at 2000rpm for 60 seconds. The resist was baked before exposure by putting the wafer on a hotplate at 65°C, ramping up the temperature to 95°C, holding at 95°C for 3.5 minutes, then reducing the temperature to 65°C.

A low pass filter was put between the light source and the wafer to be exposed in a mask aligner (EV Group, EVG620) to preferentially expose the SU-8 to longer wavelengths. The SU-8 was exposed through either a transparency film mask (CAD Art Services Inc) or a glass mask patterned with chrome. Total exposure energy was between 140 and 150mJ/cm².
done by repeated intervals of 5-10 seconds of exposure with 10 seconds of rest between each exposure interval. Post exposure bake was done on a hotplate at 65°C ramped up to 95°C, help at 95°C for 4.5 minutes, then reducing to 65°C. After this bake the exposed pattern was visible by eye. Unexposed resist was removed by submerging the wafer into a beaker of SU-8 developer (MicroChem) for 1 minute with very light agitation. After 1 minute the wafer was immediately transferred to a second beaker of clean SU-8 developer for an additional 1.5 minutes. IPA was sprayed onto the wafer to test development completion, as a white residue will form on contact of IPA with undeveloped SU-8. If development was incomplete, the wafer was resubmerged in the second beaker of SU-8 developer for an additional 20-30 seconds and re-checked for development completion. After completion was verified, the whole wafer was rinsed with IPA and dried with nitrogen gas and examined under a microscope. The SU-8 was fully cured by hard baking the wafer on a hotplate at 150°C for 10 minutes. The SU-8 height and roughness was measured with a profilometer (Alpha-Step).

Prior to casting PDMS onto an SU-8 mold for the first time, the wafer was silinized to prevent any PDMS adhesion. Silanization was done by placing the mold and an open vial of 100µL of either perfluorodecyltrichlorosilane or trimethylchlorosilane (Sigma Aldrich) into a vacuum chamber and pulling vacuum for 10 minutes then leaving the chamber closed for 30 minutes to allow vapor phase deposition to occur on the wafer surface.

**PDMS casting**

Microfluidic channels were cast with liquid PDMS then cured. A mixture of 10:1 base to curing agent PDMS components (Dow, Dowsil 184 Silicone Elastomer Kit) was measured out by mass in a weigh boat (Whatman) and mixed thoroughly for two minutes. The mixture was placed in a vacuum chamber and vacuum pulled for 30 minutes to debubble the PDMS. The liquid PDMS was then poured over the prepared SU-8 mold (with foil sidewalls to contain PDMS overflow) and debubbled in a vacuum chamber for an additional 30 minutes or until all bubbles had risen to the surface and been removed. The PDMS was then cured either in an oven at 80°C for at least 30 minutes or at room temperature for at least 24 hours.
After curing, the PDMS was removed from the wafer mold in a class-1000 cleanroom. Inlet and outlet holes were punched manually by a 0.75mm diameter hole punch (Electron Microscopy Sciences Rapid-Core). The wafer-sized PDMS was then diced into individual channels with a scalpel. Channels were cleaned by sonication in methanol or IPA and then sonication in DI and dried with nitrogen gas. Channels were then dehydrated by placing them on their sides on a clean silicon wafer on a hotplate at 50°C for at least 15 minutes. After dehydration, both the top and bottom of the PDMS channels were sealed with kapton tape (McMaster Carr) until ready to be bonded to device chips.

**Low temperature curing for accurate feature dimensions**

PDMS shrinks slightly at typical curing temperatures (60-85°C). In the case of noncritical feature sizes, this shrinkage may not be an issue. When feature sizes are critical or tolerances low, however, PDMS shrinkage during curing can dramatically affect final device performance. In order to reduce shrinkage in any channels with critical dimensions or low alignment tolerance between features, we cured PDMS at room temperature (20°C) instead of the typical elevated curing temperature (80°C). To assess the success of this protocol, features in the resulting channels were measured and the percent shrinkage of feature dimensions from the dimension of the features on the PDMS mold (1mm) was calculated. Figure 3.3 shows the clear reduction in PDMS shrinkage if cured at room temperature rather than at 80°C.
Figure 3.3: Feature size shrinkage in cast PDMS cured at room temperature (20°C) and a typical PDMS curing temperature (80°C) shows feature sizes are much more accurately maintained during room temperature curing compared to 80°C curing. Error bars are standard error of the mean.

Ultra thin PDMS for improved imaging

While the optical properties of PDMS are excellent for imaging, there are cases when the thickness of PDMS to be imaged through can degrade image clarity, even to the point of fully obscuring a feature of interest. When imaging small particles at high magnification in brightfield or very dimly fluorescent particles with fluorescence imaging, PDMS that is too thick can be problematic.

Typically our cast PDMS is 3-5mm thick. A 5x or 10x objective lens generally proceeds without issue at these magnifications. In cases where we need to image at higher magnification and when the clarity or fluorescence is critical, however, optical signals through 3-5mm thick PDMS can be insufficient.

In order to overcome this issue, we create microfluidic channel using an ultrathin PDMS layer. To cast ultrathin PDMS, we poured liquid PDMS onto an SU-8 wafer mold that had been mounted to a spinner. Because of the relatively low viscosity of uncured PDMS, the wafer being absolutely level was not critical. It was discovered any spinner capable of high rotational speeds could result in even spreading of a very thin PDMS layer. We utilized a handheld drill as a spinner with a custom attachment to hold the wafer in place during
spinning. After spinning, the wafer was debubbled and cured as described previously. With this method we successfully achieved ultrathin PDMS microfluidic channels having PDMS only 90µm thick as the channel ceiling on a channel 1000µm wide and 40µm high with less than 40µm of channel ceiling sagging. Figure 3.4 shows the dimensions of our conventionally cast PDMS microfluidic channels and our ultrathin spin cast PDMS microfluidic channels. In order to interface the microfluidic tubing with the thin PDMS, blocks of PDMS were cast and cured separately and then bonded onto the thin PDMS in the inlet and outlet areas. Bonding was done by applying a thin layer of uncured PDMS between the thin cured PDMS channel and the thick cured PDMS tubing blocks. Then the assembly was cured in an oven at 80°C for 30 minutes to cure the bonding PDMS layer.

![Figure 3.4: Examples of DEP devices with conventionally cast thick PDMS microfluidic channels (top view (a), side view (c), cross sectional diagram (e)) compared with spin cast ultrathin PDMS microchannels (top view (b), side view (d), cross section diagram (f)). In the diagrams, dimensions are in mm. The blue represents the PDMS material while the white space at the bottom represents the fluidic channel, gray notates the underlying silicon substrate. Not to scale.](image)

In order to compare the ability of the very thin PDMS to improve image quality, we introduce 1.75µm diameter fluorescent polystyrene beads into each microfluidic channel. Images of the beads in solution are then acquired in brightfield and fluorescence through
the ceiling PDMS layer. Figure 3.5 shows a comparison of images through conventionally thick PDMS and through our ultrathin PDMS for three objective magnification levels (10x, 50x, 150x). The objectives are Olympus LMPlan lenses. The numbers inset on each image give the exposure time of the image (in msec). The image comparison is made both for brightfield imaging (3.5a) and for fluorescence imaging (3.5b) to illuminate the different improvements that ultrathin PDMS can make for each imaging mode.

(a) Brightfield

(b) Fluorescence

Figure 3.5: A comparison of the image quality possible through conventionally thick and spin-cast ultrathin PDMS microfluidic channels with three different microscope objective lenses (10x, 50x, and 150x) in brightfield (a) and fluorescence (b) imaging modalities. Imaged particles in solution are 1.75µm diameter polystyrene beads. Values inset on each image give the image exposure time used (in msec). Diagrams of the PDMS cross section are not to scale.

At low magnification through the 10x objective lens, imaging through either conventionally thick or ultrathin PDMS yields good images of small particles. Particles can be seen through both thicknesses, though appear clearer in brightfield and slightly brighter in
fluorescence through the thin PDMS.

Through the 50x lens, the particles can no longer be visualized in brightfield through the thick PDMS, but appear rather clearly through the thin PDMS. In fluorescence, particles can be imaged through both thicknesses, but require a much longer exposure time through the thick PDMS. The particles imaged through the thin PDMS appear very bright and clear even at 10x the exposure time. This difference in necessary exposure time is crucial when particle motion is to be monitored, as is the case in many of our experiments.

At high magnification through the 150x objective lens, particles cannot be seen in brightfield through either PDMS layer. The ultrathin PDMS, however, allows for particle imaging in fluorescence that cannot be achieved at all through the thick PDMS.

Thus we see that ultrathin PDMS can enable particle imaging at higher magnification in both brightfield and fluorescence modes. We also have shown that ultrathin PDMS requires much lower exposure times in fluorescence imaging than thick PDMS, a critical improvement for studies of particle motion.

### 3.2.3 Bonding DEP devices and microfluidic channels

Once the DEP electrodes are patterned and optionally passivated and the microfluidic channels are made, the two are bonded together. In order to bond the PDMS microfluidic channels to the device chips, the bonding surfaces of the PDMS channel and the DEP device substrate were subjected to 40 Watts of oxygen plasma at 300mT for 20 seconds (Autoglow Glow plasma system). This treatment exposes silanol (OH) groups on the PDMS surface which can then form siloxane (Si-O-Si) bonds with the chip surface [53]. Once exposed to plasma, bonding should be done within a few minutes. Bonding does not work well if activated surfaces are disturbed prior to bonding. The PDMS therefore should not be bent and the surfaces to be bonded should not be brought into contact and then repositioned. The strategy of aligning the microfluidic channel to the device, therefore, becomes a critical step in ensuring a quality bond is made.

If alignment tolerance is large, the PDMS may be aligned by manually hovering the PDMS block over the DEP substrate, aligning by eye, and then placing the PDMS down. After the surfaces are brought into contact the sandwich is baked on a hotplate at 90°C or
at least 10 minutes. Heating provides the activation energy for the bond to form completely.

Once in contact, if the PDMS is removed or repositioned, the bond quality will suffer or fail entirely. When alignment tolerances are low enough that aligning the channel on the first try is improbable, a different approach that allows for repositioning of the channel without sacrificing bond quality should be utilized. To enable channel repositioning while simultaneously preventing any contact between the bonding surfaces, we use a spacer between the PDMS and the substrate. After oxygen plasma activation the spacer is applied to the substrate surface and then the PDMS set on top of the spacer. The PDMS can be moved around on top of the spacer to align channel features with electrode features.

A spacer ensures that no contact is made between the two surfaces and must not itself degrade the bonding surfaces. We utilize two kinds of spacers: solid spacers and liquid spacers. A solid spacer should have a very low friction so that it can be easily removed once the PDMS is correctly positioned and not contact any vital bonding area as shown in Figure 3.6a. A small cut out of weigh paper or a weigh boat (Whatman) has been found to work well. We have also successfully used liquid spacers such as methanol which will cover the entire bonding surface area but does not greatly impede the bonding surface groups (Figure 3.6b).

For solid spacers, once the PDMS is aligned, it can be gently pressed down to contact the chip surface in a region without spacers while the spacers were removed from the sides. After ensuring full contact between the PDMS and the chip surface the chip should be baked at 90°C for at least 10 minutes.

For liquid spacers, once alignment is reached, the liquid should be allowed to fully evaporate on a hotplate. The sandwich is then baked at 90°C for at least 10 minutes. During evaporation, the channel can shift so having either an object or tape to stabilize the channel position can help ensure alignment remains unchanged.
Another alignment strategy we have employed less frequently involves photolithographically defining the channel walls directly onto the DEP substrate in SU-8. This allows for very high alignment resolution. A channel ceiling must then be bonded on top of the SU-8 side walls. This bonding between either glass or PDMS channel ceiling and SU-8 side walls was never achieved with as strong of bonding force as that of PDMS directly with the substrate. Thus we most often utilized channels defined in PDMS bonded directly to the DEP substrate, using either solid or liquid spacers to overcome alignment limitations.

### 3.3 Device operation

The fabricated devices are mounted on a custom built aluminum stage which contains a recessed groove to align and secure the chip (Figure 3.7a). A printed circuit board (PCB) sits atop the stage mount, off to one side of the chip. The PCB is fastened to the stage-mount with metal screws which, along with stability, also helps establish a connection between a ground plane of the PCB and the larger aluminum holder. An array of spring-loaded pogo pins projects from the underside of the PCB (Figure 3.7c) and make electrical contact with the bonding pads on the loaded DEP chip (Figure 3.7d). Each of the 20 pins in the array is connected via PCB trace to a single female BNC connector mounted on the PCB. The combination of the stage mount and PCB provides a secure and robust connection for signal transmission to each DEP device.
Figure 3.7: Electrical interfacing with DEP chips. Devices were aligned on an aluminum holder (a) with the chip pads (b) near the aluminum wall. c) A PCB is mounted onto the holder with low profile spring loaded pogo pins extending down towards the aluminum surface and connected to BNC connections. d) DEP chips are placed under the pogo pins and the PCB screwed down to make stable electrical connection between the chip pads and the on-board BNCs.

Figure 3.8 shows a schematic of the components used for DEP operation. We use a Tektronix AFG3252 dual channel function generator to provide the AC voltage signal necessary to produce a DEP force. Two output channels were used, sourcing sine waves between 0.1 and 20 MHz, configured to be 180° out of phase with respect to each other. Each output channel was configured to expect a 50Ω load impedance and fed directly into a dual-channel, high frequency power amplifier (Tabor Electronics 9250). Typical voltage amplitudes used range between 0.1 and 5 V_{pp} from the Tektronix function generator with a subsequent ten-fold increase in amplitude provided by the Tabor amplifier. The difference
in the sourced voltage from channel 1 and channel 2 is the effective voltage at the device. Thus we are able to achieve a doubling in effective voltage by having two signals out of phase rather than applying voltage to one DEP electrode circuit and grounding the other. The voltage can be split off of each channel to an oscilloscope for signal monitoring and measurement (Tektronix DPO4104).

![Figure 3.8: Schematic of electrical, fluid, and optical interfacing with DEP devices.](image)

Particles to be manipulated are suspended in a fluid and loaded into a 1-10mL syringe (BD Biosciences). A volumetrically-controlled microfluidic pump (New Era Pump Systems, NE-1000) pushes the sample through a syringe needle (BD Biosciences, 26 gauge) and a length of PTFE tubing (Component Supply, 28 gauge) into the microfluidic channel inlet.

A custom LabView program is often used to simultaneously control the function generator, oscilloscope, and syringe pump. This is useful for coordinating condition changes when sweeping a range of frequencies or voltages or flow rates.

The aluminum mounting plate is also designed to fit into the microscope stage, as shown in Figure 3.9. The low profile spring loaded pogo pin electrical connectors were utilized to allow clearance of the microscope objective. The microfluidic inlets and outlets are punched
at an angle to allow clearance of the tubing as well. This allows particle responses to DEP to be optically monitored through a microscope (Olympus Bx651), recorded with an attached CCD camera (Olympus DP70) and analyzed with custom MATLAB programs.

Figure 3.9: Image of a DEP chip mounted with spring loaded electrical connections under a microscope objective. The low profile electrical connection design and the angled fluidic tubing allow clearance of the microscope objective for imaging.

3.4 Data collection and analysis

Once particles in fluid are introduced over a DEP electrode structure and the DEP electrodes electrically connected with an AC voltage source, particles can be subjected to DEP forces from the generated nonuniform electric field. By mounting everything under a microscope, particle responses can be optically monitored and measured. Two major data collection methods for studying DEP effects are employed: image capture and video capture.

3.4.1 Particle detection and tracking

Image capture allows examination of particle density and particle position at a given time point. Video capture allows examination of particle density and position over time, yielding further extraction of particle trajectory, velocity, and acceleration. Typically particles are imaged in fluorescence rather than in brightfield mode because small particles can be
much more clearly visible in fluorescence and the much higher contrast of particles against
background signal in fluorescent images makes particle detection much easier and robust
(See Figure 3.5 for example images comparing brightfield and fluorescence imaging of small
particles).

Analysis of particle DEP response from either images or videos begins by first detecting
every particle in each image or in each video frame. Particle detection is done by locating
islands of increased intensity while carefully eliminating background noise. Particle positions
are calculated by calculating the centroid of each detected intensity island. An example of
a frame from a video of flowing fluorescent particles is shown in Figure 3.10a in which red
circles are drawn around each detected particle which appears as a green dot.

For video analysis, after every particle is detected in every frame and particle position
calculated, particles are tracked from frame to frame by identifying a particle and calculating
the most probable particle in a subsequent frame, given the constraints of maximum dis-
placement extracted from the channel geometry and flow rate conditions of the experiment.
Figure 3.10b shows the tracks from 479 particles present in a 12 second video.

Registering to a reference image in which features of interest can be seen, such as the
edges of the microfluidic channel or the position of DEP electrodes allows for positional
information of the fluorescent particle image to be mapped into absolute positions on the
device. Device registration of regions of interest is either obtained automatically from
a brightfield reference image by detecting electrode positions or by obtaining user input
to select a feature of interest such an a channel edge or channel bifurcation point. By
positionally registering all images or videos In this way, many images or videos can be
analyzed and the extracted metrics compared between videos even if the device has been
moved between dataset collections, as commonly occurs.
Particle tracking requires balance of concentration, speed, and frame rate

Tracking particles necessitates a balance between particle density, particle speed, and particle intensity. In order to reliably track particles, the displacement of a particle from one frame to the next should be less than the average distance between two particles. If the particle-particle distance is less than the particle displacement per frame, identification of particles from frame to frame becomes difficult and much less reliable. This necessitates a balance be achieved between particle concentration, flow speeds, and frame rate of video capture.

The higher the particle concentration, for example, the shorter the particle-particle distance becomes. The faster the flow rate, the greater the particle displacement per frame. The lower the frame rate, the longer time occurs between frames and therefore the greater the displacement of particles from frame to frame. Low intensity particles require longer exposure times to be visible in an image and therefore lower possible frame rates. The combination of frame rate and particle velocity provides a lower limit on temporal resolution that can be extracted from video analysis. Our camera software allows pixel binning in which the incident light on groups of either 4 pixels or 16 pixels are combined into a single pixel. Binning pixels pools light and therefore decreases the necessary exposure time needed to visualize a particle. This sacrifices spatial resolution for the sake of increased temporal resolution.
resolution, but can be very useful to allow particle tracking.

**Particle intensity analysis in certain cases**

In some cases, individual particle detection is either not possible or not necessary. In these scenarios, image intensity can be used as an extracted metric rather than for particle locations. A study of particle sticking, for example, may have large areas of accumulated particles, with high degrees of particle-particle contact. In this case, intensity can be used as a substitute for number of particles, though always with the recognition that particle intensity does not correlate directly with particle size or with the number of particles present. Commercially sourced fluorescent microbeads show a relatively homogenous intensity for each particle. Cells, however, show great heterogeneity in their fluorescent intensity because of heterogeneity in cell sizes as well as in the success of fluorescent labelling. Additionally, the vertical position of a particle in a channel greatly influences its detected fluorescence intensity, as it can be out of focus and therefore appear larger and more dim at some heights. Analysis utilizing intensity rather than detection of individual particle must be careful to either control for heterogeneity through population level statistics or to conclude information only in regards to particle position, not particle concentration.

**3.4.2 Velocity extraction in regions of interest**

When particles can be individually detected and tracked through a video, particle position and velocity data can be extracted. Prior to velocity extraction, any particle present in the first frame of the video is discarded from analysis. This eliminates any particles stuck on the device from previous experimental conditions from affecting population velocity measurements. Additionally, any particle whose average position is within one particle length of a designated stuck particle is discarded from analysis. This is done to catch stuck particles that drop out of frame for a couple frames and get identified as a new particle upon reappearance.

Particle velocity is fit in two ways: with a linear fit over a small window moved along the whole particle trajectory trace (called a “walking fit”) or by doing a linear fit over a regions of interest. Generally these regions of interest are used to compare the particle movement
before and after a change in DEP signal has occurred. That change in signal can occur in time, such as a DEP signal being turned on several seconds after video acquisition has begun, or the signal change can occur in space, such as when a particle passes over a DEP electrode array as it flows down the microfluidic channel. In the case of the DEP signal changing in time, the region of interests are typically the particle trajectories or sections of particle trajectories that occur before and after the time in which the signal is changed. Analogously, if the DEP signal changes in space, the regions of interest are generally also the sections of the particle trajectory that occur before and after the time at which each individual particle experiences the change in signal. Different specific experiments may have different regions of interest, but here we will explain the example of velocity extraction in the case of a change in DEP signal occurring in space.

As a particle flows down a microfluidic channel, before it reaches the DEP electrodes, the DEP signal is effectively off. Once over the electrodes, the DEP signal is effectively on. A velocity fit is done for nine different regions of interest in this scenario (diagrammed in Figure 3.11a): 1) the entire particle trace (denoted “All time”), 2) all of the points before the particle reaches the start of the electrode array (denoted “Off all”), 3) all of the points before the particle reaches the start of a small buffer window before the beginning of the array (denoted “Off”), 4) the points when the particle is before the electrode array but within the buffer window (denoted “Off window”), 5) all of the points during which the particle is over top of the electrode array (denoted “On all”), 6) the points during which the particle is over the electrode array but outside of a small buffer window around the start of the array (denoted “On”), 7) the points during which the particle is over the electrode array and within the buffer window around the start of the array (denoted “On window”), 8) the points occurring near the end of the field of view (denoted “End”) to get as close to an ‘equilibrium velocity’ as possible with a limited field of view while also capturing particle velocity when no signal is applied, and 9) the points after the particles has passed over the array entirely (denoted “Off after electrodes”). The buffer window around the start of the electrode array is set to be twice the length of the electrode period, both before and after the electrode beginning.
Figure 3.11: a) Diagram of the different regions of interest used to fit the b) velocity for each particle tracked in a video in which DEP signal changes in space as particles flow over a DEP electrode array. c) Population level velocity averages for each region of interest.
The walking velocity fits show how each particle velocity changes in space as it nears and then passes over the electrode array. Figure 3.11b shows an example of the walking velocity fits for polystyrene beads in 0.1xPBS subjected to a 700 kHz AC signal. Each line is the walking velocity over space for a single tracked particle. There can be seen a dramatic shift in the velocities as they encounter the DEP electrode array (marked by the first dashed line at about 650 µm).

The velocity for each particle for each region of interest is fit with a linear regression. The average particle velocity in each region of interest is shown in Figure 3.11c. The error bars are the standard error of the mean. The velocity averages show a significant decrease once the particles are over the electrodes. Also, the spread of the velocities for the population decreases over the electrodes as well.

While a linear fit would not be very appropriately representative in the regions where velocity is changing, such as inside the buffer windows near the start of the electrode array, it is a useful fit for regions in which the particle velocity is in relatively stable equilibrium, such as in the “OFF” region and the “END” region. Thus the velocities in these two regions are most often utilized to extract a measure of relative velocity change. Examination of regions of changing velocity near the electrode start give information about response kinetics and force magnitude.

Any given particle velocity should equate to two vertical positions in the channel, both equidistant from the channel center. If the CM factor polarity is known and a particle tracked continually from an area of no signal until over top of the DEP device, the velocity profile could be used to extract particle height. For the analysis presented in this work, we chose to use measured velocity instead of an extracted height to avoid introducing additional sources of error.

3.4.3 Statistical analysis of particle motion

Once we have extracted particle concentration, position, and velocity of each particle, these metrics can be analyzed to extract a lot of information about the DEP force direction and magnitude experienced at the individual or population level. The exact analysis is very dependent on the experimental setup and the experimental variable of interest. The analysis
of particle movement under conditions of applied DEP signal requires careful statistical
error propagation as we accumulate many particle traces from each video taken and many
videos of each experimental condition. This section begins with a brief discussion of general
statistical definitions, followed by a discussion of the statistical methods used for particle
movement analysis.

General statistical definitions

Generally for a dataset of values, the average value ($\bar{x}$) is calculated as

$$\bar{x} = \frac{1}{N} \sum_{i} (x_i) \quad (3.1)$$

where $x_i$ is each individual data point, and $N$ is the number of data points used to calculate
the mean. The variance for a dataset (denoted as $s^2$ for sample variance or $\sigma^2$ for population
variance) is given by

$$s^2 = \frac{1}{N} \sum_{i} (x_i - \bar{x})^2 \quad (3.2)$$

In situations where both the mean and the variance are unknown and calculated from the
same data set, a bias-corrected sample variance must be used. This is given by

$$s^2 = \frac{1}{N-1} \sum_{i} (x_i - \bar{x})^2 \quad (3.3)$$

The difference between these two versions of the variance equation is that the bias-corrected
sample variance weights the sum of the squared differences by $1/(N-1)$ not $1/N$. In this work,
all variance calculations utilize this bias-corrected form of the variance equation unless
otherwise noted.

The standard deviation (denoted as $s$ for samples or $\sigma$ for populations) gives a measure
of the spread of the data and calculated as the square root of the variance.

$$s = \sqrt{s^2} \quad (3.4)$$
The standard error of the mean ($s_{\bar{x}}$ or $\sigma_{\bar{x}}$) gives a measure of the uncertainty of the extracted mean of the data and is calculated as the standard deviation divided by the square root of the number of elements used to generate the mean.

$$s_{\bar{x}} = \frac{s}{\sqrt{N}} \quad (3.5)$$

In general, when taking the ratio of two values ($x_1, x_2$) each with their own error ($s_1, s_2$), the error of the ratio ($s_{ratio}$) is calculated by

$$s_{ratio} = \left( \frac{x_1}{x_2} \right) \sqrt{\left( \frac{s_1}{x_1} \right)^2 + \left( \frac{s_2}{x_2} \right)^2} \quad (3.6)$$

Calculations of particle and population velocity

For the data analysis of particles tracked in a video, a least squares regression is sometimes used to fit a particle velocity ($v$) from experimental data. For every data point used to generate the fitted line, there is some error of prediction (the difference between the actual value and the predicted value). The standard error of the velocity from particle data of position over time is given as the standard error of the first fitted coefficient and is denoted as $s_v$.

The average velocity of all particles ($\bar{v}$) in each region can be calculated from the fitted velocities of each particle passing that region by

$$\bar{v} = \frac{\sum v_i}{N} \quad (3.7)$$

where $v_i$ is each fitted particle velocity and $N$ is the number of fitted particle tracks in that region of interest. Because the number of points used to generate a velocity fit for any particle is dependent on particle velocity, weighting the population average velocity by the track length imposes a bias towards the tracks of particles moving more slowly through a region. In order to prevent this bias, the population average velocity is calculated without weighting each particle velocity by its track length.

The standard error of the mean is used as the measure of uncertainty in the calculated
average particle velocity for each region of interest because the most important metric of
the uncertainty of the mean is how close it is to the mean that would be calculated from
each subsequent repetition of the experiment. The standard error of this population average
velocity ($\sigma_v$) is calculated by

$$\sigma_v = \frac{\sigma}{\sqrt{N}}$$

(3.8)

$$\sigma_v = \frac{\sqrt{\sum N_i (v_i - \bar{v})}}{\sqrt{N\sqrt{N - 1}}}$$

(3.9)

(3.10)

where the standard deviation used here in the calculation of the standard error of the mean
is bias-corrected. The errors in the velocity fits of each individual particle don’t need to be
explicitly included in this population mean error because that uncertainty of fit is already
rolled into the spread of the data, captured in the standard error calculation.

Calculations of particle and population velocity ratios

In the analysis of particle velocities, it is very useful to look at the ratio of velocities when
a particle experiences DEP signal and when the particle does not. In the case of the ratio
of the velocities of an individual particle in two different regions, the velocity ratio ($r$) is
given by

$$r = \frac{v_1}{v_2}$$

(3.11)

with the the error of the ratio of the particle velocities ($s_r$) calculated by

$$s_r = \left(\frac{v_1}{v_2}\right) \sqrt{\left(\frac{s_{v1}}{v_1}\right)^2 + \left(\frac{s_{v2}}{v_2}\right)^2}$$

(3.12)

where $s_1$ and $s_2$ are the errors of fit for the extracted velocity of the particle in regions 1
and 2, respectively.

Since we care most about the relative change each particle undergoes, it is beneficial
to look at the population average of the velocity ratios for each individual particle. This population average of individual particle velocity ratios ($\bar{r}$) is given by

$$\bar{r} = \frac{\sum_{i} r_i}{N}$$

(3.13)

where $N$ is the number of particle ratios used to generate the mean. The standard error of the population average of individual velocity ratios is calculated as

$$\sigma_{\bar{r}} = \sqrt{\frac{\sum_{i} (r_i - \bar{r})^2}{\sqrt{N(N-1)}}}$$

(3.14)

where $s_{r_i}$ is the standard error of the calculated velocity ratio for particle $i$.

In addition to the population average of particle velocity ratios, it can also be useful to look at the ratio of population average velocities ($q$) in different regions given by

$$q = \frac{\bar{v}_1}{\bar{v}_2}$$

(3.15)

where $\bar{v}_1$ and $\bar{v}_2$ are the population average velocities of particles in regions 1 and 2, respectively. This population ratio of the population average velocities has an error ($\sigma_q$) is calculated by

$$\sigma_q = \left( \frac{\bar{v}_1}{\bar{v}_2} \right) \sqrt{\left( \frac{\sigma_{\bar{v}_1}}{\bar{v}_1} \right)^2 + \left( \frac{\sigma_{\bar{v}_2}}{\bar{v}_2} \right)^2}$$

(3.16)

All of these values are calculated for each experimental data set comprised of the particle tracks from a single video. In order to combine the results from many repeats of the experimental data sets, the weighed average of any value $x$ from all data sets is calculated by

$$\bar{X} = \frac{\sum_{i} n_i \bar{x}_i}{\sum_{i} n_i}$$

(3.17)

where $n_i$ is the number of values used to generate each population average ($\bar{x}_i$) and $N$ is the number of data sets from which $\bar{X}$ is extracted. The averages across several data sets
of the particle velocity ($\bar{V}$), ratio of individual particle velocities ($\bar{R}$), and ratio of average particle velocities ($\bar{Q}$) are calculated in this manner. The associated errors of the mean from multiple data sets ($\sigma_x$) are calculated by propagating the standard errors of each population average ($\sigma_{x_i}$) as shown by

$$\sigma_x = \sqrt{\frac{\sum_i^N n_i(\sigma_{x_i}^2)}{\sum_i^N n_i}}$$

(3.18)

The standard errors of the multi-dataset average particle velocity, average ratio of particle velocities, and average ratio of population average velocities are denoted as $\sigma_{\bar{V}}$, $\sigma_{\bar{R}}$, and $\sigma_{\bar{Q}}$ respectively.

The ratio of velocities can contain a lot of information about the DEP force experienced by the particles. For our example video data, the ratio of the velocity in each region where the signal is on over the velocity in the mirrored region where the signal is off is shown in Figure 3.12. Here the ratio for each region is calculated in two different ways: as $\bar{R}$ and as $\bar{Q}$. The velocity ratio of the “END” region over the “OFF” region is most commonly utilized as a metric of relative DEP force as particle velocities in these regions typically are in a state of more equilibrium than the regions that include the point of signal change at the start of the electrode array.
Figure 3.12: Ratios of particle velocity during DEP signal on over velocities during DEP signal off for different regions of the device. The velocity ratio can be calculated in two ways: either by taking the population average of all individual particle velocity ratios (green bars) or by taking the ratio of the population average of all individual particle velocities (purple bars).

Table 3.1 summarizes the notations and equations used for the calculation of each relevant statistical value discussed above and its associated error extracted from particle tracking data.
Table 3.1: Notations and equations of statistical values extracted from particle trajectories from video data sets. Here \( n \) and \( N \) refer to the number of *** and *** values used in a calculation, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Value from a single particle trajectory</th>
<th>Population average from a single video</th>
<th>Population average from multiple videos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>Error</td>
<td>Value</td>
</tr>
<tr>
<td>Particle velocity</td>
<td>( v )</td>
<td>( s_v )</td>
<td>( \bar{v} )</td>
</tr>
<tr>
<td>Equation</td>
<td>(fit)</td>
<td>( \sqrt{\frac{\sum (x_i - v_{fit,i})^2}{n-1}} )</td>
<td>( \frac{\sum v_i}{N} )</td>
</tr>
<tr>
<td>Ratio of particle velocities</td>
<td>( r )</td>
<td>( s_r )</td>
<td>( \bar{r} )</td>
</tr>
<tr>
<td>Equation</td>
<td>( \frac{\bar{v}_1}{\bar{v}<em>2} ) ( \sqrt{\left(\frac{s</em>{r1}}{\bar{v}<em>1}\right)^2 + \left(\frac{s</em>{r2}}{\bar{v}_2}\right)^2} )</td>
<td>( \frac{\sum r_i}{N} )</td>
<td>( \frac{\sum (r_i - \bar{r})^2}{\sqrt{N \sqrt{N-1}}} )</td>
</tr>
<tr>
<td>Ratio of population average velocities</td>
<td>-</td>
<td>-</td>
<td>( q )</td>
</tr>
<tr>
<td>Equation</td>
<td>-</td>
<td>-</td>
<td>( \frac{\bar{v}_1}{\bar{v}<em>2} ) ( \sqrt{\left(\frac{\sigma</em>{\bar{v}_1}}{\bar{v}<em>1}\right)^2 + \left(\frac{\sigma</em>{\bar{v}_2}}{\bar{v}_2}\right)^2} )</td>
</tr>
</tbody>
</table>
3.5 Chapter summary

In accordance with our goal of creating low-cost devices for more accessible biosensing, we design and fabricate DEP-based microfluidic devices with planar metal electrodes and a transparent fluidic channel for optical performance characterization. The device is electrically interfaced with an AC voltage source through spring loaded electrical contacts and a custom designed printed circuit board. The low profile design allows the entire device with both fluidic and electrical connections to fit under a standard microscope objective lens. Through collection of optical images and videos, particle responses to applied DEP signals are examined and quantified. Over 29,000 lines of code were written to accomplish image and video cleaning, data processing, and analysis. From this processing, information about particle density, position, and velocity extracted from optical images and videos are utilized to quantitatively study DEP force responses and device performance. Using these quantification metrics, we next seek to inform device optimization and predict device performance.
Chapter 4

DEP system model for high conductivity performance optimization

Particle responses to DEP within microfluidic environments have been extensively modeled for the parameters found in the DEP force equation (Equation 2.1): solution and particle conductivity and permittivity, particle radius, and frequency. Electric field gradient profiles have been computed for many types of electrode and insulator geometries, some examples of which were shown in Chapter 2. Subsequently, CM factors have been modeled to approximately take into account the specific properties and architecture of many different particle types.

Amongst the majority of these studies lies an assumption of a direct relationship between the applied voltage and the voltage contributing to the formation of the electric field. DEP systems operating well under experimentally common low-throughput conditions (0.01 - 10µL/min) are often offered as a proof of principle of a device that could be adapted for real-world use by scaling up the design to increase throughput. High throughput designs are particularly necessary for applications in which processing times are limited or sample volumes are large. In medical DEP applications, long sample processing times delay diagnostics and treatments. High conductivity physiological solutions pose challenges for DEP
particle manipulation such as solution heating electrothermal flow and often constrain DEP forces to the negative DEP regime. The most straightforward method to overcome these conductivity limitations is to dilute high conductivity solutions until a suitable solution conductivity is reached. Sample dilution, however, compromises detection sensitivity \cite{16} and expands solution volume. Volume increase necessitates either increased processing time necessary for a given sample or increased processing rate.

The argument is often made that throughput could be enhanced by having multiple microfluidic channels with DEP arrays in parallel or by increasing the overall area of the DEP arrays. In reality, however, efforts to increase throughput have encountered difficulties not explained by conventional models of the DEP system, particularly in the realm of high conductivity fluids. In this chapter, we propose and experimentally validate a more complete model of the DEP system that takes real-world design and operation variables into account to more accurately predict DEP performance. Based on this model, the implications of device geometry and high conductivity operation are examined. Additionally, we discuss design considerations and tradeoffs for achieving real-world high throughput devices. This work was done in collaboration with Dr. Zachary Kobos.

4.1 DEP system circuit model

The basic conventional model for a DEP system is shown in Figure 4.1a, where AC signal sources are depicted connected to two electrode circuits. A fluid solution with some resistance \((R_{\text{soln}})\) bridges the space between the two electrodes. The voltage across the solution element dictates the DEP force magnitude achievable by the system. The main limitation of the conventional DEP system model is the assumption that the sourced voltage is equal to the voltage present at the electrode surface.

An important consideration is that circuit design elements as well as operational conditions can dramatically affect the voltage that is effectively available at the electrode surface for DEP action. Here we propose a more detailed DEP system circuit model to examine and predict the effects that these system variables have on the effective voltage at the electrode-solution interface. Figure 4.1b diagrams our proposed circuit model, which takes
into account several sources of resistance along the transmission pathway from voltage source to DEP electrode, the material properties of the device substrate, the geometry of the DEP electrode array, and the nuanced interaction of ionic fluidics with electrode surfaces.

Along the pathway from voltage source to electrode array in solution, several sources of resistance occur in series: an impedance of the voltage source output ($R_{out}$), macroscopic electronic connections and cabling used to interface with DEP devices ($R_{series}$), and on-chip resistance pathway of the connection between chip pads and DEP electrodes in solution ($R_{lead}$). The resistance of the DEP electrodes themselves ($R_{elec}$) is dependent on geometry and can have critical implications for DEP performance, especially in high conductivity solution environments.

Once at the electrode, two pathways between DEP electrode circuits occur in parallel: a pathway through the substrate ($C_{sub}$) and a pathway through solution. The solution pathway contains the conventionally understood solution resistance ($R_{soln}$), but also contains a capacitive element in the ionic double layer formed at the electrode-solution interface ($C_{DL}$). This element also allows us to consider the effects on DEP performance of adding a passivation layer on top of the electrodes.
Figure 4.1: Circuit diagrams of a) a conventional model of a DEP system typically assumed during simulations of DEP performance as a function of electrode structure and b) our circuit model of the full DEP system showing major elements that influence the magnitude of the voltage effectively available for DEP-driven phenomena.

With this circuit model, we can calculate the influence of each element on the voltage across solution and, therefore, calculate the effective DEP performance. Because DEP forces are heavily frequency-dependent, our circuit model needs to account for the frequency dependent behavior of each element. To this end, we convert each element into its complex impedance form which gives information about the resistance, capacitance, and frequency dependence of each element, enabling easier circuit analysis.
4.1.1 Complex impedance conversions of circuit elements

Treating all elements as circuit elements allows utilization of conventional circuit analysis for calculations of DEP performance implications. In order to study and experimentally verify the performance implications of this system circuit model, we first calculate the predicted device response to operational variables. The resistive and capacitive elements both contribute to the impedance of the device pathways and can be expressed simply as impedance elements rather than resistors and capacitors. Capacitors have both a real and an imaginary component of impedance while resistors have only a real component of impedance. Here we show the conversion of the circuit elements into their complex impedance forms, which are then used to define a DEP performance equation.

In order to calculate the effect of variation of each circuit element on the overall DEP performance, we can transform each circuit element into its complex impedance in which each element is described by a real and imaginary component with frequency-dependancy taken into account. Because DEP forces are highly frequency-dependent, it is important that any model of DEP performance incorporates the frequency-dependancy of all elements.

In the case of sinusoidal modulation of potential or current, the sinusoid function $\cos(\omega t + \phi)$ can be more conveniently represented by exponentials via Euler’s formula as

$$\cos(\omega t + \phi) = \frac{1}{2} \left( e^{+j(\omega t + \phi)} + e^{-j(\omega t + \phi)} \right) \quad (4.1)$$

where $\omega$ is the angular frequency of the AC signal, $t$ is the time, $\phi$ is the phase angle, and $j$ is $\sqrt{-1}$.

For each main type of circuit element - a resistor, inductor, or capacitor - we can use the exponential form of the sinusoid wave to calculate the ratio of voltage over current for each element. This ratio of voltage across to current through an element, provides a measure of the effective resistance of the element, also known as impedance, denoted as $Z$, with units of Ohms.
Impedance of a resistor

For a resistor, the relationship between voltage and current is given by Ohms Law as

\[ V = IR \]  \hspace{1cm} (4.2)

where \( V \) is the voltage across a resistor, \( I \) is the current, and \( R \) is the resistance (in \( \Omega \)).

If we let the voltage be modulated sinusoidally in frequency, as is the case in our DEP operation, the voltage is given as

\[ V = e^{j\omega t} \]  \hspace{1cm} (4.3)

then Ohm’s law becomes

\[ e^{j\omega t} = IR \]  \hspace{1cm} (4.4)

and it follows that the impedance of a resistor (\( Z_R \)) is simply given by

\[ Z_R = \frac{V}{I} = \frac{e^{j\omega t}}{I} = \frac{e^{j\omega t}}{V_R} = R \]  \hspace{1cm} (4.5)

Impedance of a capacitor

Unlike resistors, capacitors store charge as voltage is applied. The ability to store charges in response to an applied potential is referred to as capacitance (\( C \)), and is measured in farads. For the case of DC potentials, capacitance is defined as

\[ C = \frac{Q}{V} \]  \hspace{1cm} (4.6)

where \( Q \) is the accumulated charge on the capacitor (in coulombs) and \( V \) is the applied potential across the capacitor (in volts). One farad, then, is defined as one coulomb of charge accumulated per volt of potential across the capacitor. In a parallel plate capacitor, the electric field between plates is uniform and therefore the work (force times distance) required to move a charge from one plate to the other is constant over the distance between the plates. Voltage is a measure of this work per unit charge and can therefore be expressed
as

\[ V = \frac{Fd}{q} \]  \hspace{1cm} (4.7)

where \( F \) is the force on the particle of charge \( q \) as it is moved a distance \( d \) between the two parallel plates. The electric field \((E)\) is the force per charge and so the voltage between plates can also be described as

\[ V = Ed \]  \hspace{1cm} (4.8)

where \( E \) is the electric field and \( d \) is the distance between capacitor plates. The electric field can be expressed as the ratio of charge density \((Q/A)\) on the two plates over the permittivity of the dielectric material between the two plates \( \varepsilon \) shown by

\[ E = \frac{Q}{A\varepsilon} \]  \hspace{1cm} (4.9)

where \( A \) is the area of one of the capacitor plates. Substituting this definition of the electric field back into the equation for the potential of the field across the capacitor, we see that the capacitance can be defined as

\[ C = \frac{Q}{V} = \frac{Q}{Ed} = \frac{Q}{A\varepsilon d} = \frac{\varepsilon A}{d} \]  \hspace{1cm} (4.10)

From this we can see that larger capacitor conducting areas or smaller distances between conducting elements of a capacitor lead to increased ability to store charge for a given applied voltage.

For a capacitor, the relationship between voltage and current is given by

\[ I = C \frac{dV}{dt} \]  \hspace{1cm} (4.11)

then, with the voltage again expressed as a sine wave, the current becomes

\[ I = C e^{j\omega t} \]

\[ I = C j\omega e^{j\omega t} \]  \hspace{1cm} (4.12)
and it follows that the impedance of a capacitor \((Z_C)\) is

\[
Z_C = \frac{V}{I} = \frac{e^{j\omega t}}{Cj\omega e^{j\omega t}} = \frac{1}{j\omega C}
\]  
\[(4.13)\]

**Impedance of an inductor**

For an inductor of inductance \(L\), the relationship between voltage and current is given by

\[
V = L \frac{dI}{dt}
\]  
\[(4.14)\]

if we let the current be given as an oscillating sine wave given by

\[
I = e^{j\omega t}
\]  
\[(4.15)\]

then this relationship becomes

\[
V = L \frac{d}{dt} e^{j\omega t}
\]

\[
V = Lj\omega e^{j\omega t}
\]  
\[(4.16)\]

and it follows, then, that the impedance of an inductor \((Z_L)\) is

\[
Z_L = \frac{V}{I} = \frac{j\omega Le^{j\omega t}}{e^{j\omega t}} = j\omega L
\]  
\[(4.17)\]

Thus the impedance for a resistor, capacitor, and inductor \((Z_R, Z_C, \text{ and } Z_L \text{ respectively})\) are given by

\[
Z_R = R
\]  
\[(4.18)\]

\[
Z_C = \frac{1}{j\omega C}
\]  
\[(4.19)\]

\[
Z_L = j\omega L
\]  
\[(4.20)\]

From these equations, we can see that the impedance of a resistor is constant in frequency space, while the impedance of capacitors and inductors are frequency-dependent. Addition-
ally the impedance of resistors are real numbers while those of capacitors and inductors are complex numbers. At very low frequencies the impedance of a capacitor is very high, acting akin to an open circuit as the frequency approaches zero. Conversely, at very high frequencies the impedance of a capacitor is very low or approaching zero and acting as a short in the circuit as frequency approaches infinity. For this reason, a capacitor can be used as a high pass filter in a circuit. This frequency dependent behavior of capacitors will become important in the optimization of DEP performance.

The general form of the impedance of an element is

\[
Z = R + jX
\]  

(4.21)

where \( R \) is the resistance of the element and \( X \) is the reactance of an element, both in \( \Omega \). The reactance of a capacitor and inductor (\( X_C \) and \( X_L \) respectively) can be calculated from the imaginary component of their impedance equations above. For capacitors, the reactance is

\[
jX_C = \frac{1}{j\omega C} \\
X_C = \frac{1}{j^2 \omega C} \\
X_C = -\frac{1}{\omega C}
\]  

(4.22)

and for inductors, the reactance is

\[
jX_L = j\omega L \\
X_L = \omega L
\]  

(4.23)

**Impedance of the electrode-solution interface**

When a potential is applied to an electrode in an ionic solution, mobile charge carriers in the electrode, as well as in the solution, can respond to the applied voltage. Mobile charge carriers within the electrode move toward or away from the electrode-solution interface in response to the applied AC voltage. Mobile charge carriers in the ionic solution (ions)
arrange near the surface to counterbalance this charge accumulation at the electrode surface. Because mobile charge carriers from the electrode and the solution are typically not exchanged between the two materials, the layer of charges on the electrode surface and the layer of opposing charges in solution create a capacitor.

The capacitance of the layer depends on the ionic mobility and the frequency of the applied field and is an important consideration in the circuit as the electric potential drops across this capacitive double layer. In an ideal capacitor, the impedance varies inversely with frequency raised to a power $n = 1$, as shown in Equation 4.19. Because of the complexity of this double layer element, it does not behave as an ideal capacitor, but rather exhibits a frequency dependence less than unity. Therefore we must modify the impedance equivalent of this circuit element. We model the double layer as a non-ideal capacitor with a constant phase and a frequency dependence $n$ between 0 and 1.

The capacitance value ($C_{dl}$) is also scaled from that of an ideal capacitor. The impedance of this double layer ($Z_{dl}$) then is given by

$$Z_{dl} = \frac{1}{C_{dl}(j\omega)^n}$$  \hspace{1cm} (4.24)

where $C_{dl}$ is the capacitance of the double layer, scaled from an ideal capacitor due to the complexity of this layer, and $n$ is between 0 and 1. The impedance of the double layer decreases as the frequency of the applied AC signal increases. Thus operating at high frequency can help overcome the potential drop in voltage across this double layer element.

**Equivalent circuit calculations for complex impedance elements**

In the analysis of circuits, impedances in series add together in the same style as resistors in that the total impedance of $n$ individual elements in series ($Z_{series}$) is the sum of each
element \(Z_i\). For complex impedances this sum of series impedances is calculated by

\[
Z_{\text{series}} = \sum_{i=1}^{n} Z_i
\]

\[
Z_{\text{series}} = Z_1 + Z_2 + \ldots + Z_n
\]

\[
Z_{\text{series}} = (R_1 + jX_1) + (R_2 + jX_2) + \ldots + (R_n + jX_n)
\]

\[
Z_{\text{series}} = (R_1 + R_2 + \ldots + R_n) + j(X_1 + X_2 + \ldots + X_n)
\]

\[
Z_{\text{series}} = R_{\text{tot}} + jX_{\text{tot}} \quad (4.25)
\]

\[
Z_{\text{series}} = |Z_{\text{series}}|e^{j\phi} \quad (4.26)
\]

The total impedance of elements in parallel also sum in the same manner as resistors in parallel. The total impedance of \(n\) individual elements in parallel \((Z_{\text{parallel}})\) is the reciprocal of the sum of the reciprocal impedances of each element \(Z_i\)

\[
\frac{1}{Z_{\text{parallel}}} = \sum_{i=1}^{n} \frac{1}{Z_i} \quad (4.27)
\]

The calculation of the total impedance and phase values in parallel are more involved than in the case of elements in series, so rather than express the sum as a general for of \(n\) elements in parallel, here we will show the calculation for the total impedance of just two elements in parallel. This is calculated by

\[
\frac{1}{Z_{\text{parallel}}} = \frac{1}{Z_1} + \frac{1}{Z_2}
\]

\[
Z_{\text{parallel}} = \frac{Z_1 Z_2}{Z_1 + Z_2}
\]

\[
Z_{\text{parallel}} = \frac{(R_1 + jX_1)(R_2 + jX_2)}{(R_1 + jX_1) + (R_2 + jX_2)} \quad (4.28)
\]

After rationalization of the complex numbers, we can obtain \(R_{\text{tot}}\) and \(X_{\text{tot}}\), respectively, as

\[
R_{\text{tot}} = \frac{(R_1 R_2 - X_1 X_2)(R_1 + R_2) + (X_1 R_2 + X_2 R_1)(X_1 + X_2)}{(R_1 + R_2)^2 + (X_1 + X_2)^2} \quad (4.29)
\]

\[
X_{\text{tot}} = \frac{(X_1 R_2 + X_2 R_1)(R_1 + R_2) - (R_1 R_2 - X_1 X_2)(X_1 + X_2)}{(R_1 + R_2)^2 + (X_1 + X_2)^2} \quad (4.30)
\]
The final impedance can be expressed by the same general form as for elements added in series

\[ Z_{\text{parallel}} = R_{\text{tot}} + jX_{\text{tot}} \]  \hspace{1cm} (4.31)

\[ Z_{\text{parallel}} = |Z_{\text{parallel}}|e^{j\phi} \]  \hspace{1cm} (4.32)

Once we have the cumulative real and imaginary components \((R_{\text{tot}}\) and \(X_{\text{tot}}\)) of the impedance for either type of equivalent circuit, the magnitude and phase of this cumulative impedance for elements in series or in parallel \(|Z|\) and \(\phi\) respectively) can be calculated by

\[ |Z| = \sqrt{R_{\text{tot}}^2 + X_{\text{tot}}^2} \]  \hspace{1cm} (4.33)

\[ \phi = \tan^{-1} \frac{X_{\text{tot}}}{R_{\text{tot}}} \]  \hspace{1cm} (4.34)

**Cutoff frequency of a resistor and capacitor in parallel**

When a resistor and capacitor are in parallel, the fraction of current flowing through each element is frequency-dependent because the impedance of the capacitor is a function of frequency. The frequency at which the impedance of each element is equal is called the cutoff frequency \((f_{eq})\) and occurs when

\[ Z_R = Z_C \]

\[ R = X_C \]

\[ R = -\frac{1}{C\omega} \]

\[ R = -\frac{1}{C2\pi f_{eq}} \]

\[ f_{eq} = \frac{1}{2\pi RC} \]  \hspace{1cm} (4.35)

Below this frequency, the impedance of the capacitor will be higher than that of the parallel resistor causing the majority of current to pass through the resistor. Above the frequency, however, the capacitor’s impedance is less than that of the resistor and therefore the majority of current flux will occur through the capacitor. In our DEP circuit, the presence
of capacitors can have dramatic implications for the frequency-dependent effects of each circuit element on the overall DEP performance of the system.

With this conversion of circuit elements in terms of their complex impedance, we can now examine the effects of each element on the actual DEP force generation.

### 4.1.2 Equation for effective voltage available for DEP

Regardless of what circuit elements are considered present in the DEP system, the voltage potential across the solution is the critical determinant of DEP force of the whole system, rather than the output voltage of the AC source. From our proposed system circuit model, we can define a measure of the effective voltage as the fraction of the voltage across solution \((V_{\text{soln}})\) over the applied AC source voltage \((V_{\text{AC}})\) as

\[
\frac{V_{\text{soln}}}{V_{\text{AC}}} = \frac{Z_{\text{soln}}}{Z_{\text{tot}}}
\]

where \(Z_{\text{soln}}\) is the impedance of the solution resistance and \(Z_{\text{tot}}\) is the total impedance of the circuit. Now that we have converted all the circuit elements into their complex impedance form, the total impedance of the circuit can be expressed using equivalent circuit analysis for different sections of the circuit. Figure 4.2 shows the same circuit model of the DEP system as shown previously, only now the resistances in series external to the microfluidic region have been combined into a single element \(Z_{\text{ext}}\).

![Figure 4.2: Diagram of DEP circuit with sections defined for equivalent circuit analysis](image)

Using the labels for each circuit section shown in the diagram, we can express each
circuit section with the following equations

\[ Z_{tot} = Z_{ext} + Z_{int} \]
\[ Z_{ext} = 2(Z_{out} + Z_{series} + Z_{lead}) \]
\[ Z_{int} = 2Z_{elec} + Z_{C} \]
\[ Z_{C} = \frac{Z_{D}Z_{sub}}{Z_{D} + Z_{sub}} \]
\[ Z_{D} = 2Z_{dl} + Z_{soln} \]

Combining these all together we can express the total device impedance as

\[ Z_{tot} = Z_{ext} + 2Z_{elec} + \left( \frac{1}{2Z_{dl} + Z_{soln}} + \frac{1}{Z_{sub}} \right)^{-1} \] (4.37)

The fraction of effective voltage for one pair of electrodes then is

\[ \frac{V_{soln}}{V_{AC}} = \frac{Z_{soln}}{Z_{ext} + 2Z_{elec} + \left( \frac{1}{2Z_{dl} + Z_{soln}} + \frac{1}{Z_{sub}} \right)^{-1}} \] (4.38)

Table 4.1 shows the complex equation for each impedance element in Equation 4.38. It also shows how the DEP design specific features influence the value of each element. Both the materials used as well as the geometry of the electrode structure are taken into account.
Table 4.1: Description of circuit impedance elements. Terms $L$, $W$, $H$, and $t$ refer to length, width, height, and thickness, respectively. Subscripts lead, elec, gap, ch, and soln refer to the lead lines, IDE electrodes, gap between electrodes, fluidic channel, and the solution, respectively.

<table>
<thead>
<tr>
<th>Element</th>
<th>Complex equation</th>
<th>Sub equation</th>
<th>Terms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Z_{out}$</td>
<td>$R_{out}$</td>
<td>-</td>
<td>-</td>
<td>Output impedance of voltage source, typically 50Ω</td>
</tr>
<tr>
<td>$Z_{series}$</td>
<td>$R_{series}$</td>
<td>-</td>
<td>-</td>
<td>Resistance pathway of macroscopic connections and cabling</td>
</tr>
<tr>
<td>$Z_{lead}$</td>
<td>$R_{lead}$</td>
<td>$R = \rho \frac{L_A}{A}$</td>
<td>$L = L_{lead}$</td>
<td>Resistance pathway from chip connection to DEP electrodes $\rho = \rho_{elec}$</td>
</tr>
<tr>
<td>$Z_{elec}$</td>
<td>$R_{elec}$</td>
<td>$R = \rho \frac{L_A}{A}$</td>
<td>$L = L_{elec}$</td>
<td>Resistance pathway down DEP electrodes $\rho = \rho_{elec}$</td>
</tr>
<tr>
<td>$Z_{soln}$</td>
<td>$R_{soln}$</td>
<td>$R = \rho \frac{L_A}{A}$</td>
<td>$L = W_{gap}$</td>
<td>Resistance pathway between electrodes through solution $\rho = \rho_{soln}$</td>
</tr>
<tr>
<td>$Z_{sub}$</td>
<td>$\frac{1}{j\omega C_{sub}}$</td>
<td>$C_{sub} = \frac{\varepsilon A}{d} = Q_{sub}A$</td>
<td>$d$ handle dependent, $\varepsilon$ is material dependent</td>
<td>Capacitance between electrodes through substrate $A = L_{elec}W_{elec}$</td>
</tr>
<tr>
<td>$Z_{dl}$</td>
<td>$\frac{1}{(j\omega)^n C_{dl}}$</td>
<td>$C_{dl} = \alpha \frac{\varepsilon A}{d} = Q_{dl}A$</td>
<td>$0 &lt; n &lt; 1$, $d$ handle dependent, $\varepsilon$ solution dependent</td>
<td>Capacitance at electrode-solution interface $A = W_{ch}W_{elec}$</td>
</tr>
</tbody>
</table>
Using the complex equations for each element, we can rewrite Equation 4.38 as

\[
\frac{V_{\text{soln}}}{V_{\text{AC}}} = \frac{R_{\text{soln}}}{R_{\text{ext}} + 2R_{\text{elec}} + \left( \frac{2}{(j\omega) n C_{\text{dl}}} + R_{\text{soln}} \right)^{-1} + j\omega C_{\text{sub}}}^{-1}
\]  

(4.39)

If we define a value \( A_E \) for the surface area of a DEP electrode as

\[ A_E = L_{\text{elec}} W_{\text{elec}} \]  

(4.40)

and a value \( A_X \) for the cross sectional area of a DEP electrode as

\[ A_X = W_{\text{elec}} L_{\text{elec}} \]  

(4.41)

and a value \( A_C \) for the cross sectional area of the fluidic channel as

\[ A_C = W_{\text{ch}} H_{\text{ch}} \]  

(4.42)

we can express the effective voltage in terms of the material and geometric design of a DEP device by

\[
\frac{V_{\text{soln}}}{V_{\text{AC}}} = \frac{\rho_{\text{soln}} \frac{W_{\text{gap}}}{A_C}}{R_{\text{ext}} + 2\rho_{\text{elec}} \frac{L_{\text{elec}}}{A_X} + \left( \frac{2}{(j\omega) n Q_{\text{dl}} A_E} + \rho_{\text{soln}} \frac{W_{\text{gap}}}{A_C} \right)^{-1} + j\omega Q_{\text{sub}} A_E}^{-1}
\]  

(4.43)

This gives the fraction of the applied voltage that is dropped across the solution electrode for the case of one pair of DEP electrodes. We now appreciate the importance of the device design dimension and material choice on the overall DEP performance.

In order to calculate the change in the voltage across solution as we increase the number of electrode pairs in parallel in an interdigitated DEP device, we can return to our circuit diagram and now treat the circuit as containing two elements - an impedance element of everything external to the interdigitated DEP electrodes (denoted as \( Z_{\text{ext}} \)) and an impedance element of everything between and including the interdigitated electrode pair (denoted as \( Z_{\text{int}} \)), as diagrammed in Figure 4.3a.
Figure 4.3: Diagram of the DEP system circuit model divided into an external impedance element $Z_{ext}$ composed of all resistive elements in series with the DEP IDE electrode array and an interior impedance element $Z_{int}$ comprised of a single IDE electrode pair element $Z_1$, which encompasses electrode resistance and the pathways through solution and through the substrate between the two electrodes. As an approximation for $N$ IDE electrodes in parallel, we represent $Z_{int}$ as $N/2$ electrode pair elements in parallel.

For the case of 1 pair of electrodes that we have been considering so far, the voltage drop across the solution element ($V_{soln}$) is a fraction of the voltage across $Z_{int}$ given by

$$V_{soln} = V_{int} \frac{Z_{soln}}{Z_1} \quad (4.44)$$

where $V_{int}$ is the voltage drop across $Z_{int}$ which is equal to the voltage drop across $Z_1$, the impedance of 1 pair of electrodes and the solution pathway and substrate pathway between them. We can define the fraction of impedance across solution to that $Z_1$ block as a constant because that relationship would remain constant even if other elements outside of the $Z_1$ block were added.

$$\alpha = \frac{Z_{soln}}{Z_1} \quad (4.45)$$

$$V_{int} = V_{AC} \frac{Z_{int}}{Z_{ext} + Z_{int}} \quad (4.46)$$

where $Z_{int} = Z_1$ if there is one pair of electrodes or $Z_{int} = Z_1/N$ if there are $N$ pairs of electrodes in parallel.

We now treat the internal block of the circuit as a single element that is repeated in
parallel for each pair of DEP electrodes, as diagrammed in Figure 4.3b. As we add more electrode pairs in parallel, the voltage across the interior block decreases because the net impedance of that block decreases. The voltage across the interior block for \( N \) electrode pairs in parallel then goes as

\[
V_{int} = V_{AC} \frac{Z_1}{N} \left( \frac{1}{Z_{ext}} + \frac{Z_1}{N} \right)
\]  

(4.47)

Since they are in parallel the voltage across the entire interior block equals the voltage across each \( Z_1 \) block and thus the voltage across solution can be expressed in terms of \( N \) electrode pairs in parallel as

\[
V_{soln} = \alpha V_{AC} \frac{Z_1}{N} \left( \frac{1}{Z_{ext}} + \frac{Z_1}{N} \right)
\]

(4.48)

From this we can extract our effective drop in voltage across solution for \( N \) pairs of electrodes in parallel as

\[
\frac{V_{soln}}{V_{AC}} = \frac{\alpha}{N \frac{Z_{ext}}{Z_1} + 1}
\]  

(4.49)

where \( \alpha \) is the fraction of the impedance across solution over the net impedance of a single repeating unit of an electrode pair, \( Z_{soln}/Z_1 \). Substituting the definition of \( \alpha \) back in, we can simplify this equation to

\[
\frac{V_{soln}}{V_{AC}} = \frac{Z_{soln}}{NZ_{ext} + Z_1}
\]

(4.50)

where \( V_{soln} \) is the voltage drop across solution, \( V_{AC} \) is the sourced voltage, \( Z_{ext} \) is the net impedance of everything external to the parallel block of repeated electrode pairs, \( Z_1 \) is the net impedance of the internal block containing a single electrode pair unit, \( N \) is the number of repeated electrode pairs in parallel. The impedance of the single block \( Z_1 \) was shown previously to be

\[
Z_1 = 2R_{elec} + \left( \frac{2}{(j\omega)^nC_{dl}} + R_{soln} \right)^{-1} + j\omega C_{sub} \right)^{-1}
\]  

(4.51)

This treatment of a pair electrodes as a repeating unit is a convenient way to calculate the relationship between the effective voltage and the number of repeating units. This approach reflects all the pathways from one electrode to the next within a pair of electrodes, but neglects the interaction from one pair of electrodes to the next. In reality the circuit di-
agram for many electrodes in parallel would look like an infinite grid of infinitesimally small pathways of resistance along each electrode and parallel pathways between each electrode resistance pathway from one electrode to each of its neighboring electrodes. The factor $\alpha$ describing the relationship between the impedance across solution and the impedance of a single repeating unit might shift as additional pathways between electrode pairs are taken into account.

To test the validity of this treatment, we simulated a limited grid of resistance pathways and extracted the voltage drop across solution for larger and larger simulated grids representing more electrodes in parallel. We found the same relationship of the decay in voltage drop across solution as the number of electrodes increases using the simulation of limited grids as with the calculation approach shown here. Thus, while there may be a missing scaling factor, the similarity of the simulated trend indicates that this calculation approach is a reasonable approximation of the impedance of $N$ repeated units of electrode pairs.

Now that we have a model proposed for predicting the performance of a DEP device as materials and electrode geometries are altered, we seek to compare predicted device responses to experimentally measured device performance.

### 4.2 Methods of model validation

In order to examine the efficacy of our model in predicting device performance, we compare the predicted DEP force to an empirically extracted metric of relative DEP force as we sweep a circuit parameter. If the model is reflective of the effect of circuit parameters on the DEP performance, the trends of predicted force and empirically extracted force should be the same. In this section we discuss the methods by which we compare our device model to experimental measurements. We first measure or calculate impedance values specific to our device components and geometry. We then conduct experiments monitoring particle response to DEP forces as we vary certain circuit elements.

Since our circuit model predicts the effective voltage available for DEP force generation and our empirical measurements extract a DEP force metric from particle movements in response to DEP forces, we also employ multiphysics simulations to gain insight into
the expected bridge between predicted DEP force magnitude on particles and empirically measured particle movements through fluid in response to that force magnitude.

4.2.1 Device impedance measurements

Characterizing our DEP devices allows us to make predictions about DEP performance using our circuit model. Total device impedance measurements were taken by measuring the current through a device for a given sourced voltage. The current through the device was measured by sourcing an AC voltage connected to one side of a DEP electrode circuit. The other side of the DEP circuit was connected to an inverting amplifier with a 100Ω resistor. Figure 4.4a diagrams the measurement circuit. The amplitude of the output voltage was recorded on two lock-in amplifiers - a Stanford Research System 830 and a Stanford Research System 844 - to allow measurement of device impedance over a broader range of frequencies than capable by either lock-in individually. The current through the added resistor \( R_1 \) is equal to the current across the device. With a measured output voltage, the current through \( R_1 \) is calculated. With that current and the known input voltage, the total impedance of the device is calculated as

\[
Z_{\text{device}} = -R_1 \frac{V_{\text{in}}}{V_{\text{out}}} \tag{4.52}
\]

By measuring the device impedance over a range of frequencies, the device impedance can be fit with our circuit model Equation 4.39 to extract the total device resistance as well as the electrode-solution double layer parameters. This is made possible by measuring devices fabricated on a fully insulating substrate (glass), whereby any substrate capacitance contribution can be ignored. With no substrate capacitance term, the total device impedance is given by

\[
Z_{\text{tot}} = R_{\text{ext}} + 2R_{\text{elec}} + R_{\text{soln}} + 2 \frac{1}{(j\omega)^nC_{\text{dl}}} \tag{4.53}
\]
If the total resistance is collapsed into a single term, $R_{tot}$, the measured device impedance can be fit to the function

$$Z_{tot} = R_{tot} + \frac{2}{(j\omega)^nC_{dl}}$$

(4.54)

to extract the total device resistance $R_{tot}$, the double layer capacitance $C_{dl}$, and the double layer frequency dependence $n$.

(a)

(b)

Figure 4.4: Frequency dependent device impedance is a) measured with an inverting amplifier circuit and b) the data used to fit parameters in from our device model

With the ability to fit our actual device values from device impedance measurements then allows the prediction of DEP performance for varied device circuit elements.

4.2.2 Multiphysics simulation of DEP particle response

Simulations of particle trajectories in a fluid channel in response to both fluid flow and DEP force were made using COMSOL multiphysics software.

In the case of positive DEP, we can model the capture efficiency of the device as a function of the number of electrodes over which particles pass. In the simulation, 100 particles are released at a microfluidic inlet and brought by bulk fluid flow over a planar IDE DEP array. The conditions are set such that the particles experience a positive DEP force and consequently travel toward the electrodes in response to applied voltage signals as shown in Figure 4.6a.

Particle trajectories and the position at which particles are captured on the electrodes are extracted for varying parameters such as number of electrodes, applied voltage, flow rates, electrode widths, and electrode gap widths. This model assumes the conventional
DEP circuit model in which the sourced voltage equals the voltage present at the electrode-solution interface. Henceforth in this chapter, this model of pDEP particle response will be referred to as the “pDEP capture efficiency model”.

When particles experience positive DEP force, particle capture on the electrodes is a very convenient metric for DEP performance. This is essentially monitoring the particle’s change in position perpendicular to the DEP electrodes and extracting information about the position along the channel at which particles reach a certain threshold height in the channel. In the case of capture, that lower threshold height is the electrode surface.

In cases of negative DEP, when particles are pushed upward in the channel instead of pulled downwards, we also want to utilize an extracted measure of the change in the particle’s position perpendicular to the DEP electrodes as a metric for DEP performance. Because the DEP force decreases rapidly with distance, DEP force on particles declines as the particle responds by moving up in the channel, away from the electrodes. If the channel ceiling is high enough, there will exist an equilibrium height at which DEP, drag, and sedimentation forces balance for that particle. Monitoring particle trajectory along the channel length and width allows straightforward extraction of particle velocity in the $x$ and $y$ directions. If particle velocity along the length of the channel was a product solely of the balance of fluid flow at that height and particle drag forces, then the particle height could reasonably be extracted from particle velocity along the channel. Because of the parabolic nature of the fluid flow profile, this extraction requires first mapping the particle velocity to one of two possible heights in the channel and then, determining which of those two positions is correct. If the direction of DEP response (negative or positive) is known and the trajectory of the particle spans a region without and with DEP force applied, then the height can be determined by the shape of the particle’s velocity in space when encountering the DEP array.

While height extraction from velocity along the channel is possible, and in some cases preferable, route of analysis, a more immediate metric for DEP performance can be extracted directly from the measured particle velocity itself. If the nDEP force is stronger than some threshold, the average velocity of a population of particles will decrease as force increases because they are pushed toward the lower flow speed regions near the channel ceil-
ing. For any applied electric field gradient magnitude, particles nearer the channel bottom will experience more force than particles in the upper sections of the channel. If the electric field gradient magnitude is high enough to allow particles in the lower half of the channel to cross the vertical center of the channel, then the slowing of the overall population velocity reflects the applied voltage well. In the case, however, of forces insufficient to drive particles in the lower half of the channel across the midline, any particle increase in height comes with a corresponding *increase* in velocity. Figure 4.5 illustrates the velocity of a particle as it rises linearly in a channel.
Figure 4.5: Simulated particle velocity profile resulting from vertical movement in a channel. In the a) upper half of the channel, any vertical movement upwards is accompanied by a decrease in velocity. b) velocity will peak as a particle crosses the channel center. c) Within the lower half of the channel, particle ascension results in velocity decrease.
In order to test the use of particle velocity as a metric of DEP performance for nDEP situations, we created another multiphysics model which will be referred to as the “nDEP velocity model”. This model is set up very similarly to the pDEP capture efficiency model and monitors DEP-induced particle trajectory in a microfluidic channel environment. Again, this model is constructed based on the assumption of the conventional DEP circuit model. The difference for the nDEP velocity model is the extracted metric of DEP performance is the particle velocity at a defined region, rather than the position of particle capture. Our experimental data collection setup has a finite field of view for recording video and images of particle motion. In order to make our model compatible with our experimentally obtained datasets, we extract particle velocities from defined regions relative to the start of the electrode array that fit within our experimentally-constrained field of view for data collection. Thus the particle velocity measured is not an equilibrium velocity which might require long distances to reach under conditions of low force, but rather a velocity in a region after the start of the electrode array. This “end” region is defined to be 100µm long, beginning 500µm after the start of the DEP electrode array.
Instead of defining the capture efficiency as the metric for DEP efficiency, we calculate the change in velocity ratio ($\Delta v/v_0$) from simulated particle trajectories in the same way that we extract that metric from experimentally obtained particle trajectories. In these COMSOL simulations, all geometric and operational conditions are set to match those used experimentally, including channel dimensions, electrode dimensions, fluid flow rates, fluid conductivity, and particle size. The only parameter not matched to our experimental conditions is the voltage applied. As this chapter aims to show, the sourced voltage does not equal the effective voltage available for DEP action. Thus a voltage was chosen to maximize the dynamic range of the simulated DEP response as a function of the number of IDEs to best illuminate the effect of IDE number on DEP efficiency. The simulated voltage was $1V_{pp}$ while the experimental voltage sourced was $12V_{pp}$. All parameters used can be
found in Table 4.2.
Table 4.2: Parameters used in COMSOL simulation of nDEP particle response to varied number of interdigitated electrodes at varied voltages

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>1000000</td>
<td>Hz</td>
</tr>
<tr>
<td>Voltage</td>
<td>0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.8, 5.2</td>
<td>V</td>
</tr>
<tr>
<td>Channel height</td>
<td>12.7</td>
<td>um</td>
</tr>
<tr>
<td>Flow rate</td>
<td>525</td>
<td>um/s</td>
</tr>
<tr>
<td>Fluid concentration</td>
<td>1000</td>
<td>mol*mm³</td>
</tr>
<tr>
<td>Fluid conductivity</td>
<td>0.019</td>
<td>S/m</td>
</tr>
<tr>
<td>Fluid relative permittivity</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Fluid density</td>
<td>1000</td>
<td>kg/m³</td>
</tr>
<tr>
<td>Fluid dynamic viscosity</td>
<td>1.00E-03</td>
<td>Pa*s</td>
</tr>
<tr>
<td>Particle density</td>
<td>1050</td>
<td>kg/m³</td>
</tr>
<tr>
<td>Particle diameter</td>
<td>1.77</td>
<td>um</td>
</tr>
<tr>
<td>Particle conductivity</td>
<td>0.004519774</td>
<td>S/m</td>
</tr>
<tr>
<td>Particle relative permittivity</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Electrode width</td>
<td>25</td>
<td>um</td>
</tr>
<tr>
<td>Electrode gap width</td>
<td>25</td>
<td>um</td>
</tr>
<tr>
<td>Number of electrodes</td>
<td>2, 4, 8, 16, 32</td>
<td>-</td>
</tr>
<tr>
<td>Channel width</td>
<td>1000</td>
<td>um</td>
</tr>
<tr>
<td>Channel length before electrodes</td>
<td>300</td>
<td>um</td>
</tr>
<tr>
<td>Channel length at electrodes</td>
<td>eln*(elw+elgw)</td>
<td>um</td>
</tr>
<tr>
<td>Channel length after electrodes</td>
<td>700</td>
<td>um</td>
</tr>
<tr>
<td>Channel length total</td>
<td>ch_in+ch_mid+ch_out</td>
<td>um</td>
</tr>
<tr>
<td>Initialize probe value for stop condition</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Number of particles to release</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Time step size study 2</td>
<td>0.01</td>
<td>s</td>
</tr>
<tr>
<td>Time total study 2</td>
<td>5</td>
<td>s</td>
</tr>
<tr>
<td>Slip velocity</td>
<td>0</td>
<td>m/s</td>
</tr>
</tbody>
</table>
4.2.3 Relative DEP force measurements

In order to experimentally test and validate our circuit model’s reflection of the reality of DEP system variables’ effect on performance, we monitor the DEP response of suspended particles in solution while varying a circuit parameter and then extract a metric of DEP force from particle motion.

Device design for testing circuit variables

In order to experimentally test and evaluate our circuit model, DEP devices were designed to systematically vary circuit elements in isolation. Generally, each chip was fabricated on an 8mm x 40mm substrate footprint and had 20 contact pads on chip as diagrammed in Figure 4.7a. Each contact pad was connected to one half of an interdigitated DEP electrode array so that 10 DEP devices in total were patterned onto each chip. Particles are physically brought over the DEP electrodes by a microfluidic channel that is bonded over the electrodes.
The electrode pairs terminate in an interdigitated (IDE) finger structure (Figure 4.7b). The width of the lead-in lines varied between 10 and 500um to test the effect of the series resistance on effective DEP force. The electrode width was kept constant at 25µm. The width of the gap between each electrode finger varied between 10 and 50um to vary the solution resistance element. The electrode resistance was varied by changing the number of electrode fingers from 2 to 160 as well as by changing the electrode length from 0.75mm to 3mm.

From Table 4.1 we see that the changes to one feature dimension can have implications for the values of several other elements. The length of the electrodes, for instance, not only changes electrode resistance, but also the solution resistance, the double layer capacitance, and the substrate capacitance.

In one set of devices, in order to isolate the electrode resistance changes from the solution
resistance changes with longer electrode lengths, the microfluidic channel width over the IDE electrodes was kept constant. In another experiment, the channel width was varied from 0.5mm to 2mm as the IDE finger length increased in order to keep the ratio of exposed metal to solution constant. The substrate capacitance was varied by fabricating devices on two different substrates: conductive silicon covered by 2um of insulating silicon dioxide (SiO$_2$) or on glass (Figure 4.7c left and middle respectively).

The double layer capacitance at the electrode-solution interface was varied by fabricating devices with either no oxide layer over the metal electrodes or with a 200nm layer of insulating SiO$_2$ (Figure 4.7c middle and right respectively). In addition to IDE structures, there were some test structures on chip such as a wide metal bar connecting lead lines to enable measurements of lead-in and series resistances. Table 4.3 summarizes the chip features varied, a description of the element that each feature affected, and the specific values tested.

Table 4.3: DEP device features varied for examining effects of circuit elements on DEP performance

<table>
<thead>
<tr>
<th>Feature varied</th>
<th>Description</th>
<th>Values tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{series}$</td>
<td>External series resistor</td>
<td>0, 20, 50, 100, 200, 350, 500 $\Omega$</td>
</tr>
<tr>
<td>$W_{lead}$</td>
<td>Lead-in width</td>
<td>10, 25, 50, 100, 250, 500 um</td>
</tr>
<tr>
<td>$L_{elec}$</td>
<td>Electrode length</td>
<td>0.75, 1.5, 3 mm</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of electrodes</td>
<td>0, 2, 4, 8, 16, 32, 160</td>
</tr>
<tr>
<td>$W_{ch}$</td>
<td>Channel width</td>
<td>0.5, 1, 2 mm</td>
</tr>
<tr>
<td>$W_{gap}$</td>
<td>Gap width</td>
<td>10, 25, 50 um</td>
</tr>
<tr>
<td>$C_{sub}$</td>
<td>Substrate material</td>
<td>glass, silicon w 2um SiO$_2$</td>
</tr>
<tr>
<td>$C_d$</td>
<td>Passivating oxide thickness</td>
<td>0, 200 nm</td>
</tr>
<tr>
<td>$\frac{W_{ch}}{L_{elec}}$</td>
<td>Fraction metal exposed to solution</td>
<td>$\frac{1}{3}, \frac{2}{3}, \frac{4}{3}$</td>
</tr>
</tbody>
</table>

**Fabrication**

The device fabrication process was the same as described in Chapter 3 with the electrodes being patterned in 300nm of aluminum and optionally having 200nm of deposited PECVD
SiO$_2$ added on top of the electrodes. The microfluidic channels bonded to the devices were 12.7µm high.

**Electronic chip interface**

The fabricated devices are mounted on a custom aluminum stage and electrically interfaced through spring-loaded pins mounted to the underside of a PCB as detailed in Chapter 3. PCB traces connect each pin to a female BNC connection used to connect to our AC voltage source.

AC voltages are sourced as two sine waves between 0.1 and 20 MHz, 180° out of phase with each other. Both signals are amplified through a high frequency power amplifier (Tabor Electronics 9250), yielding a ten-fold increase in signal magnitude for each channel. Sourced voltages were typically 1.2 V$_{pp}$ prior to amplification. These amplitudes were chosen such that the incoming particles experienced significant DEP-induced slowing over the DEP electrodes without becoming captured to render our measurements sensitive to shifts in the DEP force without saturation of particle velocity range.

The sourced voltage begins to roll off at high frequencies. Figure 4.8 shows the measured amplitude after two sourced voltage signals (180° out of phase) are sent through the amplifier, the PCB with a device connected, and to an oscilloscope for measurement. The signal amplitude begins to roll off above $1 \times 10^7$ Hz. This is important to consider when operating at high frequencies, as the DEP response will also decrease as frequency increases above 10MHz.
Particule and Fluid Sample

We flowed fluorescent polystyrene beads over our interdigitated electrodes for particle tracking video analysis. The beads (Polysciences, Inc. 178675) were 1.75µm in diameter and fluoresced green under excitation. The beads were diluted 4,000-fold into 0.1x PBS and introduced at a rate of 0.4µL/min. The low flow rate was chosen to ensure a sufficient number of video frames were recorded per particle transit over the DEP electrode array to enable high resolution particle velocity extraction. The dilution was chosen to ensure a high bead density during recordings while not being too high as to overwhelm the tracking algorithm.

The 0.1x PBS buffer was chosen to emphasize the significance of design variations on device performance in contrast to lower-conductivity solutions in which the relative effects of most system variables on the effective voltage becomes less significant. Additionally, this solution conductivity eliminates the need to factor in the frequency-dependent CM factor. The solution conductivity was measured with a conductivity meter (Hanna Instruments, EC215) to be 0.19S/m. The CM factor was calculated for polystyrene beads in this solution.
conductivity and is plotted in Figure 4.9. For this conductivity, polystyrene beads are expected to experience negative DEP for all frequencies utilized in this study. Moreover, the CM model for polystyrene beads under these conditions is nearly constant over the frequency range utilized, varying by only 0.53% over the entire frequency range used. Thus we can reasonably ignore the CM factor influence for experiments of varied frequency, which simplifies the DEP force extraction from particle motion. Furthermore, it was preferable to operate within the negative DEP regime because cell capture on the electrode surface would add an additional complicated circuit element required to be taken into account in data analysis.

![Figure 4.9: Calculated CM factor for polystyrene beads in 0.1xPBS (with measured conductivity = 0.19S/m) for the range of frequencies utilized in circuit model validation experiments. CM factor magnitude varies by only 0.53% over the entire range](image)

**Data collection**

Measurements of particle motion in response to DEP signals for varied circuit parameters were obtained by recording videos of fluorescent beads subjected to DEP forces. Our experiments are conducted on the viewing stage of an Olympus BX51 microscope under 5x magnification. Following optical excitation (Xcite Series 120Q), fluorescent images and videos were recorded with an Olympus DP70 camera system for later analysis.
Data processing and analysis

DEP-induced changes in particle velocity were analyzed by tracking individual particles from fluorescent movies acquired at 30 frames per second. MATLAB programs were written to detect each particle in every frame then connect particle trajectories between frames. From these tracks we extract the velocity information over both time and space for every particle present. We calculate the average population velocity when particles are not subjected to any DEP forces ($v_0$) as well as the average population velocity when particles experience DEP force over the electrode array ($v_{on}$). A small spatial buffer window directly before and right after the start of the DEP electrode array was ignored in the calculation of particle velocity in order to more accurately calculate the equilibrium velocity in each region without skewing the velocities with transitional values as particles equilibrate. This buffer window was set to equal to 3x the pitch of the IDE electrodes (150µm for IDE arrays of 25µm electrodes and 25µm gaps) both before and after the start of the DEP array. The velocity during DEP signal was extracted from a region at the end of the field of view so that the particle positions had a chance to equilibrate. This “end” region is located between 500 and 600µm after the start of the electrode array. The fractional change in velocity that particles experience when subjected to DEP forces over the device are extracted as

$$\frac{\Delta v}{v_0} = \frac{v_0 - v_{on}}{v_0} \tag{4.55}$$

By setting up the fractional slowing this way, higher DEP forces (which result in slower particle velocities) have a higher fractional slowing metric, allowing more direct comparison with the voltage ratio. This fractional slowing, used as a metric for DEP force, is compared with the trends in fractional effective voltage from Equation 4.39 at the electrode-solution interface as predicted by our circuit model to examine the predictive power of our system circuit model. The DEP force increases with voltage squared so the comparison metric from our circuit model is the square of the effective voltage.

$$F_{DEP} \propto \left(\frac{V_{soln}}{V_{AC}}\right)^2 \tag{4.56}$$
In our experimental conditions, particles experience negative DEP and are therefore pushed upwards by the DEP force over the DEP array as shown in Figure 4.10a. Because of the parabolic nature of the fluid velocity profile, particle velocity near the channel floor or ceiling is very low, but passes through a maximum velocity at the vertical center of the channel. Thus, as a particle’s vertical trajectory through the channel passes through the channel center, the particle velocity will increase, peak as it crosses the channel center, and then begin to decrease the higher up the particle is pushed.
Figure 4.10: a) Particles flowing down a channel experience faster flow speeds near the center of the channel. As particles experience nDEP as they pass over a DEP electrode array, DEP force will push particles higher in the channel and consequently, into different fluid flow speeds. b) Videos of particles monitor particle (green dots) motion over DEP arrays (vertical grey bars). c) Extracted velocity profiles of every particle show particle response to encountered DEP forces.

When the DEP force is greater than the threshold force needed to push particles up to
the top half of the channel, there is a close to linear relationship between DEP force and equilibrium particle velocity. If DEP force is too low to push particles into the upper half of the channel, a small force will actually result in particle velocity increase as particles ascend closer to the center of the channel where they experience higher fluid flow speeds. For this experimental data, analysis was focused on the velocity rather than on an extracted particle height because, for cases in which the DEP force is strong enough to push particles past the vertical center of the channel, velocity becomes a good approximation of particle height without introducing potential additional error into the system from an extra data processing step. This near-linear relationship between height and velocity breaks down at very low DEP forces, when particles’ final positions may be either in the bottom half or near the vertical center of the channel. Generally our measurements were taken with voltage ranges high enough to push particles into the upper half of the channel and so enable us to reasonably utilize particle equilibrium velocity in our relative DEP force calculations, with the exception of a study of oxide coatings. In that experiment, discussed in more detail later, the dynamic range of the force over the conditions studies was very high, necessitating measurements taken at very low DEP forces at low frequency.

Figure 4.11 shows the extracted velocity ratio for particle trajectories subject to DEP with varied applied source voltages. The velocity ratio, reflective of the amount of particle slowing and therefore related to the DEP force, is plotted against the square of the sourced voltage. This is proportional to the DEP force on the particles. There exists an approximately linear relationship between the velocity ratio and the square of the sourced voltage, and therefore with the DEP force, when the force is greater than a lower threshold.
Figure 4.11: Velocity ratio from a population of particles subjected to DEP forces from varied sourced voltages show a near linear relationship between velocity ratio and \( V^2 \) for forces that are above a lower threshold

In order to further assess the validity of this method of empirical extraction of a relative DEP force, we simulate particle responses to DEP in fluidic channels in a multiphysics software and compare the predicted particle response to those we obtain experimentally.
Figure 4.12: Particle velocity ratio extracted from simulated particle trajectories in multiphysics software plotted against the square of sourced voltage show a very similar trend to extracted velocity ratios from empirical data in which a negative velocity ratio occurs at low voltages followed by a region of near linear relationship between velocity ratio and $V^2$

4.3 Effects of system variables on DEP performance

With our circuit model developed, our DEP devices made to experimentally vary circuit parameters, and our system of relative DEP force extraction validated by both experimental measurements as well as multiphysics simulations, we can now examine the effect of circuit variables on DEP performance. Comparing the performance effect predicted by our circuit model to the effect measured experimentally will allow us to assess the validity of our circuit model. We look at the effect of device materials, geometry, and operational conditions on the expected DEP force. Through a combination of experimental data, circuit analysis, and multiphysics simulations we examine the nuances of DEP performance optimization.
4.3.1 Solution resistance ratio

Since the DEP force on particles in solution depends on the electric field generated in solution, the voltage differential across solution is the critical voltage in the system that determines the DEP force magnitude. In any circuit the voltage drop across a resistor in series is proportional to the fraction of the total circuit resistance that resistor contributes.

The solutions used in DEP devices vary widely in conductivity, with typical values ranging from $1 \times 10^{-6}$ to over 2 S/m. The resistivity ($\rho$) of solution is the inverse of its conductivity.

When the solution resistance element ($R_{\text{soln}}$) contributes a significant portion of the total resistance in the circuit, a substantial voltage drop will occur across solution, yielding a large electric field and large DEP forces. Such is the case for low conductivity fluids where solution resistance is high. In high conductivity fluids, however, the solution resistance contributes less to the overall circuit resistance and therefore the voltage drop across solution can dramatically decrease. Figure 4.13 shows the dramatic effect that the solution resistance has on the effective DEP voltage. If the solution resistance contributed 100% of the total circuit resistance, the DEP force achieved would be 100% of the maximum possible DEP force for any given applied voltage. In other words, the effective voltage across solution would equal the sourced voltage. This is the background assumption of conventional DEP models.

If, however, the solution resistance contributed less than 100% of the total resistance, the effective voltage decreases at frequencies above about $10kHz$. If the solution resistance contributes only 50% of the total circuit resistance, the DEP force will only be about 25% of the maximum DEP force. Thus, if not properly understood and designed for, high conductivity solutions can significantly decrease DEP device performance.
Figure 4.13: The fraction of solution resistance ($R_{\text{soln}}$) to the total circuit resistance ($R_{\text{tot}}$) significantly affects the fraction of the maximum DEP forces a device is able to achieve.

This predicted decline in performance with decreasing solution resistance has serious implications for DEP manipulation of particles at high conductivity. From a throughput standpoint, high conductivity DEP is favorable because most physiological samples are high conductivity samples. Any necessary sample dilution to decrease conductivity also decreases net sample throughput by the same degree.

To examine this predicted performance dependence on the solution resistance ratio, we first experimentally increase the total circuit resistance while leaving the solution resistance constant by adding additional transmission resistance.

### 4.3.2 Transmission resistance

The presence of any external resistance in series with the solution resistance element will impact the transmission of the voltage signal driving DEP. The pathway from voltage source to active region of the DEP electrodes has several resistive elements in series that contribute to voltage loss even before the signal ever reaches the DEP electrode-solution interface.

To illustrate the impact of the solution resistance ratio on device performance, we introduced a series resistance in line with our device, as indicated in Figure 4.14a. By adding
in another resistance element in series with the transmission pathway, we alter the total circuit resistance without changing any other circuit element and therefore have an isolated measure of the effect of the solution resistance ratio on DEP performance.

Measurements of velocity ratios were taken for series resistance values ranging from 0 to 500 Ω and plotted against the voltage ratio predicted from our circuit model. As can be seen in Figure 4.14b, less voltage drop across solution occurs as the series resistance increases. This means that there is less DEP force for higher series resistances.

The uncertainty in the impedance model voltage predictions is dominated by the standard error in the series resistance parameter extracted from fitting. From this prediction of the effective force across solution as well as the experimental measurement of the amount of DEP-induced particle slowing, we can appreciate how higher resistance in series with the DEP device causes a significant rolloff in performance.
Figure 4.14: a) Circuit diagram showing the series resistance $R_{\text{series}}$ which was altered to determine b) the effect of series resistance on the predicted relative DEP force (dashed line ---) and the empirically measured particle velocity ratio (squares □). This data are in good agreement between our system circuit model prediction and experimentally measured metric of the effective DEP force for decreasing DEP performance with increasing series resistance. In practical terms, this demonstrates the importance of minimizing any resistance in series with a DEP electrodes, especially when operating in high conductivity solutions.
Next we examine the effects of altering the solution resistance through a commonly proposed mechanism of increasing throughput - increasing fluid channel widths.

### 4.3.3 Channel width

If we test the effect of solution resistance on DEP performance by varying the solution conductivity, we will introduce an immense variation in the particle response due the CM factor also changing with the solution conductivity. But the solution resistance depends on more than just the conductivity of solution.

As we posited in Table 4.1, the solution resistance between two electrodes is proportional to the gap width between two electrodes and inversely proportional to the width of the microfluidic channel. Increasing channel width decreases the solution resistance as it allows essentially more parallel pathways of conduction between neighboring electrodes. The only other circuit variables that are predicted to show a dependence on the channel width is the double layer capacitance, increasing as the electrode-solution interface area increases.

We therefore test the effect of solution resistance on our system by empirically measuring the DEP performance as a function of channel width while keeping the electrode configuration constant. With a constant electrode geometry and experimental setup, we expect that that altering the channel width only alters $R_{\text{soln}}$ and $C_{\text{dl}}$.

To experimentally measure the relative DEP force with increasing channel width, we fabricated DEP devices with identical electrode structures, but with three different channel widths (0.5, 1, and 2mm). Impedance measurements of the device were taken for each channel width and shown in Figure 4.15b. As predicted, the wider channels yield an overall lower device impedance, in part due to the decreased solution resistance.
Figure 4.15: a) Fluidic channel width alters the solution resistance and the double layer capacitance elements in the system circuit. b) Device impedance measurements show decreased impedance with increased channel width.

Over these devices, we introduced particles at adjusted volumetric flow rates of 0.2, 0.4, and 0.8 µL/min to achieve a constant average linear flow rate of 525 µm/sec over all devices (channel height = 12.7 µm). DEP signals were applied and particle responses monitored. Particle velocity ratio is plotted along with the circuit model prediction of the effective force available from the devices for the three channel widths in Figure 4.16.

Figure 4.16: a) Diagram of increasing fluidic channel width over unchanged DEP electrodes and b) the predicted and experimentally measured decrease in effective DEP force with wider channels.
A method often proposed in literature for increasing device throughput is the widening of fluidic channel areas over a DEP electrode array to allow for increased volumetric throughput while maintaining low linear velocities. Here we have shown that the DEP force generation decreases with increasing channel width. For a constant linear flow speed, this decreased DEP force results in overall decreased DEP performance of the device.

4.3.4 Number of electrodes

Increasing the number of DEP electrode structures within the fluidic region is a strategy often offered in literature for improving device throughput performance. Longer DEP arrays would provide a more sustained force on particles (spatially and temporally) allowing for more complete particle manipulation in conditions of low particle acceleration.

Here we seek to determine whether the addition of more interdigitated electrode (IDE) fingers indeed serves to increase the efficiency of the DEP system, as is commonly assumed in literature. To do so, we first simulate the trajectory of particles subjected to DEP in a microfluidic environment for varying number of electrode fingers. The simulation model was made in which particles are captured by positive DEP forces as they flow down a microfluidic channel as described in the pDEP capture efficiency model in Section 4.2.2. Recall that these COMSOL simulations assume the sourced voltage is equal to the voltage present at the electrode-solution interface. They show relationships between voltage across solution and resulting particle responses in a fluidic environment, but do not take into account any alteration to the voltage across solution that might result from altered device geometry.

Simulations of particle capture efficiency were performed for varied number of interdigitated electrodes and applied voltages. Figure 4.17 shows how capture efficiency increases with the number of available capture electrodes present in the DEP array for any given applied voltage.
Figure 4.17: Simulated pDEP capture efficiency model showing the increase in capture efficiency with the number of available DEP capture electrodes as well as the voltage applied. (Simulated in COMSOL assuming conventional DEP circuit model)

By examining the number of electrodes required to achieve a desired capture efficiency, we find that the number of electrodes required to achieve that capture increases inversely with the square of the applied voltage as shown in Figure 4.18. Because the DEP force is also proportional to the squared voltage, we can conclude that the number of electrodes ($N$) necessary to achieve a desired DEP capture efficiency increases inversely with the relative DEP force ($F_{DEP}$) on the particles or

$$N \propto \frac{1}{V^2} \propto \frac{1}{F_{DEP}}$$  \hspace{1cm} (4.57)
Figure 4.18: Simulated pDEP capture efficiency model showing how the number of electrodes required to reach a certain (x%) capture efficiency scales inversely with the square of the voltage applied to the electrodes. The same data is in each plot, but a) shows the trend vs voltage while b) replots that trend in terms of $1/V^2$ to illuminate the linear relationship. (Simulated in COMSOL assuming conventional DEP circuit model)
In terms of desired capture efficiency, we can fit a proportionality ($\alpha$) constant between $N$ and $V^2$ from these modeled values with a simple polynomial function. For this simulated dataset, that proportionality constant can be expressed as

$$\alpha = 3.7C^2 - 220.4C + 2341.5$$

(4.58)

where $N$ is the number of electrode fingers, $C$ is the desired capture efficiency, and $V$ is the voltage at the electrode-solution interface. The coefficients would change with different experimental conditions, but the general relationship of the number of fingers needed to get a capture efficiency being inversely related to the square of the applied voltage should remain.

The decrease in effective force at each electrode does not necessarily mean that the performance of the system as a whole will deteriorate. For most DEP devices, success is defined not by the magnitude of DEP force, but rather by the extent of particle response in the space and time that the particle passes through the device. In the case of particle capture, for instance, successful operation need only achieve capture before the end of the electrode array. Similarly, with DEP deflection devices, the metric of device success hinges on the extent of particle movement in a direction perpendicular to fluid flow. The speed of the fluid does not hinder that component of movement, but only degrades performance by necessitating certain rates of movement. Thus for the judgement of device success, the effective force should not be fully decoupled from the influence of fluid flow in cases where design choices alter the length of time or space over which a particle experiences DEP forces.

To look into this relationship more quantitatively, we first model particle trajectories in a channel in response to both pDEP and nDEP. In a simulation, a group of 100 particles, uniformly spaced along the height of the channel, are released at $t = 0$, 300µm before the start of the DEP IDE array and drawn by fluid flow over the DEP array. For small numbers of IDE fingers, particles experience a brief repulsion toward the channel ceiling. Particles nearest the DEP electrodes at the channel floor experience the strongest repulsive force and therefore there is a slight increase in particle velocity as lower particles get pushed toward the higher velocity central flow streams. With the addition of more electrodes, the duration
of the repulsive force on each particle increases, allowing particles to be pushed further toward the channel ceiling. This is not at all unexpected since the duration of the DEP force has increased and, in these simulations, the effective voltage is assumed (inaccurately) to be equal to the applied voltage, regardless of the number of electrodes. Figure 4.19 shows the simulated particle trajectories in the channel for the case of 2 and 16 electrodes in terms of both particle height in the channel and particle velocity.

(a) Height (N = 2)  
(b) Height (N = 16)  
(c) Velocity (N = 2)  
(d) Velocity (N = 16)

Figure 4.19: Dashed lines (−−) indicate the extent of the electrode array. Dotted lines (...) indicate the region from which velocity for signal on is pulled. This region is chosen as the end region of the field of view that can be obtained experimentally to allow more accurate comparison between model predictions and empirical measurements.

Extracting the velocity ratio ($\Delta v / v_0$) from these simulations for varied number of electrode fingers, we can plot the predicted velocity ratio vs N as shown in Figure 4.20.
Figure 4.20: Velocity ratio extracted from COMSOL simulation for increasing number of DEP electrodes

Seeking to achieve higher throughput rates, it is often proposed in literature to extrapolate successful DEP systems to larger areas by increasing the device area and/or increasing fluidic cross sectional area. As we have seen from both the pDEP capture efficiency model and the nDEP velocity model, increasing the number of fingers is indeed projected to increase the DEP efficiency of the system. The primary assumption in these models is that the conventional DEP circuit model holds true in which the voltage at the electrode-solution interface is independent of the number of electrode fingers.

From our proposed DEP system circuit model, however, increasing the number of electrodes would affect the voltage present at the electrode-solution interface. Increasing the number of electrode subunits increases the total metal area exposed to solution and thereby decreases the solution resistance. Additionally, it affects the capacitive double layer and the electrode resistance as well. From our circuit simulation of a limited grid of electrode and solution resistors, we find agreement with our analytical circuit model that the effective voltage would decrease exponentially with increasing number of electrodes as shown in Figure 4.21.
Thus we have two models, a system circuit model taking into account electronic nuances but no particle-fluid dynamics and a multiphysics simulation taking into account particle-fluid dynamics but no electronic nuances.

The two models predict opposite trend in DEP performance with increasing number of electrodes. The interplay of this predicted decay in force with more electrode fingers and the simulated increasing capture probability predicted by the COMSOL model. Figure 4.22b shows the predicted voltage ratio from the circuit model (left axis) and the predicted velocity ratio that would be achieved with increasing numbers of electrode fingers if the voltage at the electrode-solution interface remained constant. These two trends are in complete opposition to one another. This suggests a tradeoff exists between these two competing effects and that the actual maxima for DEP performance is achieved at a finite number of electrode sub-units.

Figure 4.21: The simulated effective voltage dependency on the number of electrode fingers
Figure 4.22: Increasing the number of electrodes (a) leads to (b) a prediction of decreased effective DEP force due to altered circuit element values, but also a prediction of performance enhancement due to the increased DEP force duration on particles.

We empirically examine the combination of these competing effects on DEP performance by measuring the equilibrium velocity while doubling the number of interdigitated electrode fingers among different devices (Figure 4.23). At first, as the number of fingers - and thus repeating units - increases from 2 to 8, the equilibrium velocity of the particles over the DEP region increases. With more electrodes comes more sustained DEP force and particles can be pushed further up into the channel. Further increases in the number of electrode fingers, however, has the opposite effect. As the number of electrodes increases from 8 to 32, the velocity ratio decreases, indicating a decline in the effective voltage at the electrode-solution interface. With greater number of electrodes, the decreasing solution resistance reduces the magnitude of the voltage driving the DEP force.
Empirically, the velocity ratio of particles subject to forces from DEP arrays of varied number of electrodes shows a maximum peak performance that is the result of two competing effects: the decrease in effective voltage with increasing $N$ and the increasing spatial and temporal application of DEP force as the particle travels over longer arrays.

As mentioned, the COMSOL simulation of was run with all the variables in the system mirroring the experimental setup except for the voltage, which was set to 1V rather than the experimentally sourced voltage of 12V. We chose 1V to have a high dynamic range in our simulation. Were the voltage set lower, we expect that the magnitude of the experimental velocity ratio would be less than the COMSOL predicted velocity ratio at $N = 8$.

The most important takeaway from these models and experimental validation is that increasing the number of DEP electrodes helps increase DEP efficiency, but only to a certain extent. As the addition of more electrodes beyond that extent hinders overall DEP performance due to decreasing effective voltage. Thus this shows the importance of optimizing the number of DEP electrodes in a device, based on the solution conductivity and fluid velocity used for that application.
4.3.5 Passivation layer over electrodes

In some cases it is desirable to coat DEP electrodes with a passivating layer to prevent particle adhesion, protect against electrode corrosion, and promote long-term device stability. Often the passivating layer is an insulating oxide. This oxide layer acts to increase the distance between the two conducting materials - the electrode and the electrolytic solution - and will therefore decrease the overall capacitance of the double layer. This increases the impedance of this element in series with the solution resistance.

The impedance of the capacitive double layer \( Z_{dl} \), as discussed previously is given by

\[
Z_{dl} = \frac{1}{(j\omega)^nC_{dl}}
\]  

(4.59)

with \( n \) between 0 and 1 for a double layer due to it’s structural complexity. The capacitance \( C_{dl} \) can be calculated for simple structure geometries by

\[
C_{dl} = \frac{\varepsilon_r \varepsilon_0 A}{d}
\]

(4.60)

where \( \varepsilon_r \) is the relative permittivity of the dielectric material between two conducting plates of area \( A \) separated by a distance \( d \) and \( \varepsilon_0 \) is the permittivity of free space \( (8.85 \times 10^{-12} \text{F/m}) \).

For a double layer, the capacitance may be scaled by a factor \( \alpha \) to account for complexity. For interdigitated electrodes, the total capacitance is equal to the capacitance between two electrodes multiplied by the number of repetitions of the pathway there are (ie: \( N - 1 \)). This is shown by

\[
C_{dl} = \alpha \frac{\varepsilon_r \varepsilon_0 A(N - 1)}{d}
\]

(4.61)

where \( N \) is the number of interdigitated electrodes. Thus the impedance of the double layer is proportional to the separation distance of the capacitor and inversely proportional to the total area of the electrodes and the \( n \) power of the frequency.

\[
Z_{dl} \propto \frac{d}{\omega^n A(N - 1)}
\]

(4.62)

From this we see that the thicker the oxide layer, the greater the voltage drop across it.
will be, resulting in less voltage drop across the solution resistance element. In the case of an insulating oxide coating, then, operating at high frequencies becomes even more critical for mitigating the decrease in DEP performance that an increased double layer impedance would incur.

Devices were fabricated with and without a 200nm coating of insulating SiO\textsubscript{2} over the electrode surfaces. As a capacitive element, the impedance of the passivating layer is frequency dependent. Impedance measurements were taken of each device and model parameters fit to calculate a predicted voltage across solution for each device. Figure 4.24 shows the measured device impedance and fits for each device. Readily apparent can be seen the increased overall device impedance that the oxide layer causes. The increase in impedance is itself frequency-dependent and is much less pronounced at higher frequencies.

![Figure 4.24: a) Introducing a passivation layer over electrodes influences the capacitance of the double layer formed at the electrode-solution interface. b) Measured impedance of devices with and without a 200nm SiO\textsubscript{2} insulating oxide layer over the electrodes.](image)

Particles in 0.1xPBS (0.16S/m) were introduced over the device and their motion monitored as they encounter the DEP electrode array. Particle DEP responses were measured for a range of frequencies from 100kHz to 20MHz to illuminate the effects of a passivation layer on DEP performance. Figure 4.25 shows the relative DEP force predicted by our circuit model as well as the empirically measured velocity ratio of particles plotted against the frequency of the applied AC signal. Without oxide, the presence of the capacitive double layer in series with the solution resistance causes significant attenuation of the voltage available for DEP. The passivation coating serves to further increase the impedance of the
double layer element and results in a deterioration of DEP performance. At frequencies above about 5MHz, however, the empirical data shows no significant difference in DEP performance between the two devices.

![Cross sectional diagram of devices without (top) and with (bottom) a passivating oxide coating over the DEP electrodes.](image)

**Figure 4.25:** a) Cross sectional diagram of devices without (top) and with (bottom) a passivating oxide coating over the DEP electrodes. b) The predicted relative DEP force (dashed lines - - -) and the empirically measured velocity ratio (solid lines —) of each device type decay at lower frequencies. Devices with oxide (squares □) show a much lower DEP force than devices without oxide (circles ○) at frequencies below about 5MHz.

The CM factor calculated for polystyrene beads in this solution conductivity is nearly constant over this frequency range, exhibiting only a 0.53% variation in magnitude from 100kHz to 20MHz as was shown in Figure 4.9. Therefore, the performance changes with frequency can be reasonably attributed to voltage transfer across solution rather than a change in CM factor with frequency. Additionally, for any given frequency, the two device configurations can be compared directly because the CM factor will be identical at any given frequency.

Passivating layers over the electrodes can be useful for surface compatibility and device stability. Operation of these devices, however, should remain at high frequencies in order to avoid significant performance attenuation. Since the double layer capacitance is in series with the solution resistance, the smaller the capacitive impedance of the double layer, the
higher the voltage drop across solution. Thus, larger electrode areas or thinner passivation
layers will ease performance attenuation.

Interestingly, for the case of widening electrodes to increase throughput, the dependence
of performance on electrode area in terms of double layer capacitance stands in contrast to
the performance effects of both the solution resistance and the electrode resistance. As dis-
cussed previously, as electrode length and channel width increases, the electrode impedance
increases and the solution impedance decreases, both of which decrease the effective voltage
available for DEP. The double layer impedance, however, will decrease with larger area,
improving voltage drop across solution. This tradeoff existed in our experimental study of
widening channels and, from that data, we can clearly see that the benefit gained from the
decreased double layer impedance when widening the channels was not sufficient to mitigate
the performance loss due to decreased solution resistance.

4.3.6 Substrate material

Next we examine the other capacitive element in our circuit model - the capacitance between
electrodes through the substrate material. In addition to the conduction pathway between
electrodes through solution, there exists a parallel pathway through the substrate itself. This
substrate capacitance ($C_{\text{sub}}$) is influenced by the substrate material as well as the electrode
area and the distance between electrodes. The more insulating the substrate material and
the lower the operating frequency, the less voltage will be lost to this inefficient substrate
pathway. Our DEP devices are fabricated on either a borofloat 33 glass substrate or a 2µm
thick insulating thermally grown SiO$_2$ layer atop a silicon wafer handle, as diagrammed in
Figure 4.7.

Here we use a relative permittivity of 3.9 for SiO$_2$ and 4.6 for glass.

For the devices fabricated on 2µm SiO$_2$ on top of a silicon wafer handle, the distance
$d$ used is the distance of the pathway travelled through the insulating oxide, which is a
total of 4µm from pad to pad. The resistance of the silicon handle can be neglected from
the distance because of it’s relatively low resistivity (13-30 Ωcm. And for the solid glass
substrate, the distance is the distance between neighboring electrodes.

Between two neighboring chip pads, each with an area of 1.35mm$^2$ with a distance of
0.47mm between them, the expected capacitance on a SiO$_2$ on Si substrate is 11.65pF. This was measured experimentally to be 15pF. The proximity of these values indicate that this is a reasonable approach for calculating capacitance across the SiO$_2$-Si-SiO$_2$ sandwich.

For the devices used in these experiments, the default IDE structure contains 16 electrodes, each 25µm in width, 1.5mm long, separated by 25µm gap. The calculated total expected substrate capacitance for devices on a SiO$_2$ on Si substrate is 4.85pF and only 0.916pF for devices fabricated on glass. With over 5x the capacitance of the glass substrate, the insulating SiO$_2$ may affect the overall device performance.

Thus, we see that increasing device area by either lengthening electrodes or by increasing the number of electrodes causes a proportional increase in the substrate capacitance. Electrode resistance, on the other hand, increases with longer electrodes but decreases as more electrodes are put in parallel. Ideally, the substrate capacitance and the electrode resistance are as small as possible to allow more voltage drop across solution. Thus we conclude that if device area should be increased to increase throughput, it is preferable to increase the number of electrodes, rather than the length of electrodes from the consideration of substrate capacitance and electrode resistance.

The implications of this substrate capacitance become most practically important as the area covered by electrodes grows large and also as the gap size between electrode circuits becomes small. Proposals in literature for expanded device area or multiple parallel devices in order to increase sample throughput may suffer performance losses from this inefficient substrate capacitance.

Increasing the number of electrodes or increasing electrode length will both increases the capacitance proportionally. Transitioning from a set of 10 electrodes 1mm long to a set of 1000 electrodes 1cm long, for example, will increase device capacitance by 1000x. Even when fabricated on glass, this changes the substrate capacitance from 0.37pF to 407pF (for electrodes with a width and gap with of 25µm).

Additionally, as DEP work increasingly focuses on more nanoscale particle manipulation, the necessity of very small gap sizes between electrodes may also result in a loss of performance due to now non-negligible substrate capacitance unless the electrode area is also scaled down in dimension along with the gap wide to maintain that ratio.
4.3.7 Channel height and flow rate

Like increasing channel width, increasing channel height allows for higher throughput without sacrificing the average linear flow speed. The DEP force decreases with the square of the distance away from the electrode. So, intuitively, particles more distant in a taller channel will feel less DEP force and therefore require longer time/space to respond to the DEP field. Longer response times would either be achieved by lowering the flow rate (thereby negating the improvement in throughput) or by having electrode arrays with larger area along the axis of fluid flow in the channel. As we have just shown, increasing the electrode array by number of fingers or by finger length results in lower DEP efficiency. A reasonable question, then, would be whether the reductions in efficiency outweigh the improvements to volumetric throughput.

Albrecht et al. model DEP-induced particle patterning kinetics on planar interdigitated parallel electrodes in a fluidic chamber under conditions of no fluid flow [54]. As would be intuitive, they found that the patterning time generally decreases with decreasing fluid chamber height. Using \( h' \) as a metric relating chamber height \( (h) \) and electrode pitch \( (d) \) as

\[
h' = \frac{h}{d}
\]

they found that the time required to pattern particles \( t_{\text{pat}} \) increased exponentially with chamber height for chamber heights greater than the electrode pitch \( (h' > 1) \) as

\[
t_{\text{pat}} \propto \exp(2\pi h)
\]

This is somewhat intuitive considering the electric field strength \( (E) \) also decays exponentially with distance above the electrodes \( (z) \) as

\[
E \propto \exp(-2\pi z)
\]

For thinner chamber heights, however, Albrecht et al. found that this trend dramatically reverses due to the electric field nonuniformity being confined nearer to the electrodes and therefore more uniform within the space between electrodes [54]. Compared to particles
in higher chamber heights, particles near electrodes in a very thin fluidic chamber would move faster, while those farther from the electrodes would move slower. When fluid flow is present, however, particles are continually brought near electrodes and thus would have the opportunity to encounter the region of high nonuniformity rather than rely on the relatively uniform electric field lines between electrodes resulting from thin chamber heights. Thus, under fluid flow conditions, we would not expect the decrease in patterning time to reverse for very thin chamber heights.

While the absolute lower limit of chamber height is the size of the particle going through the chamber at maximum compression, there are limitations to consider as the height approaches the particle diameter. Keh and Chen model the increase in drag force on a particle as the diameter of that particle approaches the chamber height \([55]\). Their published results of the increase in the drag force on a rigid spherical particle with diameter \(a\) in a fluid chamber of height \(h\) as the particle diameter approaches the chamber height are plotted in Figure 4.26. Their model shows that a particle that is 10\% of the channel height experiences approximately a 10\% increase in drag force. A particle whose diameter is 50\% of the channel height, however, will experience about an 85\% increase in drag force \([55]\).
Figure 4.26: Relative increase in drag force \((F/F_0)\) with varied particle radius \((a)\) to channel height \((h)\) ratios (Data gathered from Ref [55] Huan J. Keh and Po Y. Chen, “Slow motion of a droplet between two parallel plane walls”, Chemical Engineering Science, 2001)

With the ultimate goal of increased throughput for a device, the interplay between number of electrodes, channel height and linear flow speed were modeled. Figure 4.27a shows the number of electrodes needed to compensate for increased channel height for constant flow speeds. Similarly, Figure 4.27b shows the number of electrodes needed to compensate for increases in fluid speed for varied channel heights to maintain throughput.
Figure 4.27: The simulated number of electrodes necessary for particle capture for coupled channel height and fluid speed tradeoffs
4.4 Implications for real-world throughput enhancement

Our DEP system circuit model can be used to predict the voltage drop across solution and, therefore, the effective voltage available for the generation of DEP forces. When optimizing throughput, however, both the DEP force generated as well as the overall volumetric flow rate of the sample affected performance. As we have shown, there are tradeoffs between DEP force magnitude and sample throughput when device geometry is altered. When seeking to optimize device throughput, then, the connection between both metrics must be understood and optimized. As we saw in the case of widening the fluidic channel, there is less DEP force generated but also allows a range of lower flow speeds for which the overall particle response was improved.

To examine this interplay between DEP force and throughput we will define the axis of our microfluidic channel such that the $x$ direction occurs along the fluid flow direction down the channel, and $z$ is the height within the channel, perpendicular to the fluid flow direction. For many DEP applications, overall successful device performance necessitates the movement of particles a certain distance perpendicular to the fluid flow within a certain amount of distance down the fluid channel. In pDEP capture, particles must reach the electrode surface by the time they reach the end of the DEP array to have a chance at being captured. In nDEP field flow fractionation, particles must be pushed into a certain flow stream either vertically or laterally before the end of the DEP electrode structure. So we can define our device performance $P$ that reflects the fraction of the required particle displacement achieved by the time a particle has reached the end of the DEP electrode structure as

$$P = \frac{\Delta z_{\text{max}}}{\Delta z_{\text{required}}}$$

where $\Delta z_{\text{max}}$ is the displacement in $z$ reached by the end of the electrode structure and $\Delta z_{\text{required}}$ is the necessary displacement in $z$ for successful device operation. For simplicity sake, we will consider only the DEP force perpendicular to the fluid flow and treat the fluid velocity as an average velocity so that the particle velocity in the $x$ direction ($v_x$) is a constant. Since we only want to extract some relationships that are generalizable to multiple DEP applications, we will also treat the DEP force as a constant value in space.
Before encountering the DEP electrodes, the particle’s velocity perpendicular to the fluid flow \((v_{z,0})\) is 0. After some time subjected to DEP force the particle position in \(z\) is given by

\[
z_t = z_0 + v_z t + \frac{1}{2}a_z t^2
\]  

(4.67)

where \(z_t\) is the particle’s position at a time \(t\), \(z_0\) is the initial particle position in \(z\), \(v_z\) is the initial velocity in \(z\) which is 0 since the defined \(z\) axis is perpendicular to fluid flow, and \(a_z\) is the acceleration in \(z\). Since

\[
\Delta z = z_t - z_0
\]  

(4.68)

and

\[
F_{DEP,z} = ma_z
\]  

(4.69)

where \(F_{DEP,z}\) is the DEP force in the \(z\) direction and \(m\) is the mass of the particle, Equation 4.67 simplifies to

\[
\Delta z = \frac{1}{2m} F_{DEP,z} t^2
\]  

(4.70)

again, with the simplification that the DEP force is an averaged force over the space travelled by the particle. Expressing \(t\) in terms of time distance travelled down the channel \((\Delta x)\) and average linear flow speed \((v_x)\) we see that

\[
\Delta t = \frac{\Delta x}{v_x}
\]  

(4.71)

If particle response occurs before the end of the electrode array, the maximum time allowed for particles response is

\[
t_{max} = \frac{L_x}{v_x}
\]  

(4.72)

where \(L_x\) is the length of the electrode array along the direction of fluid flow. Combining these equations gives an expression of the maximum displacement in \(z\) \((\Delta z_{max})\) as

\[
\Delta z_{max} = \frac{F_{DEP,z} L_x^2}{2m v_x^2}
\]  

(4.73)
where $t$ has been converted to $t_{\text{max}}$. Since

$$F_{\text{DEP,z}} \propto V_{\text{soln}}^2$$  \hspace{1cm} (4.74)$$

and

$$v_x \propto \frac{Q}{A_{ch}}$$  \hspace{1cm} (4.75)$$

where $Q$ is the volumetric flow rate and $A_{ch}$ is the cross sectional area of the fluidic channel ($A_{ch} = W_{ch}H_{ch}$), the displacement in $z$ over the length of the DEP electrode array can be expressed as

$$\Delta z_{\text{max}} \propto \left( \frac{V_{\text{soln}}A_{ch}L_x}{Q} \right)^2$$  \hspace{1cm} (4.76)$$

Note here that $A_{ch}L_x$ is the volume of solution over top of the electrode array, which we can rewrite as

$$S = W_{ch}H_{ch}L_x$$  \hspace{1cm} (4.77)$$

giving us a final relationship of the maximum displacement in $z$ of

$$\Delta z_{\text{max}} \propto \left( \frac{V_{\text{soln}}S}{Q} \right)^2$$  \hspace{1cm} (4.78)$$

Thus the maximum particle displacement perpendicular to the fluid flow depends directly on the square of the voltage across solution and the square of the volume of solution over the DEP electrode array, and inversely on the square of the volumetric throughput.

The maximum throughput of a device, then, for any necessary $\Delta z_{\text{required}}$ has the relationship

$$Q \propto \frac{V_{\text{soln}}S}{\sqrt{\Delta z_{\text{required}}}}$$  \hspace{1cm} (4.79)$$

where $Q$ is the volumetric throughput of the device, $V_{\text{soln}}$ is the voltage drop across solution, $S$ is the solution volume over top of the DEP electrode array, and $\Delta z_{\text{required}}$ is the particle displacement perpendicular to the fluid flow direction needed for successful performance of the device.

As we have shown with our circuit model, $V_{\text{soln}}$ depends heavily on device geometry.
as does, of course, the solution volume $S$ over the DEP electrodes. Even $\Delta z_{required}$ often depends on device geometry. In some applications, for example, $\Delta z_{required}$ might need to be at least equal to the channel height or half of the channel width.

Using our default device design ($N = 16, W_{ch} = 1 mm, W_{elec} = W_{gap} = 25 \mu m$, and fabricated on a glass substrate) as a starting point, we investigate the influence of several device design and operational conditions on the calculated relative DEP force. In Figure 4.28a we show the effect of varied substrate capacitance values as a function of frequency. All else held constant, increasing the inter-electrode substrate capacitance will degrade performance at elevated frequencies as the competition between the solution resistance and substrate capacitance parallel branches plays out. Increasing the substrate capacitance by an order of magnitude lowers the effective DEP force at frequencies above 1MHz. Reducing the substrate capacitance from 10pF to 1pF, however, does not result in improved performance.

Figure 4.28b illustrates the effect of the double layer capacitance on effective voltage versus frequency. Unlike the substrate capacitance, the double layer capacitance can dramatically influence the effective DEP force by shifting the frequency at which the force begins to roll off. Beyond some high frequency threshold, any double layer capacitance will yield similar effective DEP forces. As the capacitance decreases, however, the performance at any frequency below that threshold decreases. This would be the case for increasing the thickness of a passivation layer, for example. The thicker the layer, the smaller the double layer capacitance, the higher the frequency of operation should be to avoid performance attenuation.

Figure 4.28c demonstrates the important effect of solution conductivity on DEP force for several relative device areas. Higher conductivity solutions degrade the effective DEP force and this degradation becomes more pronounced the larger the device area becomes. Large area devices with a fixed external impedance have greater constraints in terms of solution conductivity. If a solution were diluted in order to lower the conductivity, the available voltage would improve. If, however, the device area were scaled accordingly to overcome the throughput loss of sample dilution, the effective voltage also lowers accordingly. Thus there is no net gain in the effective force generated by diluting samples if the device area
is also scaled up. We also can see that the reduction in effective force becomes much less significant when operating in lower conductivity solutions.

Figure 4.28d similarly shows the relationship between effective force and device area for several solution concentrations. The decline in force as device area increases and as solution conductivity increases can dramatically impact performance.

![Figure 4.28](image)

Figure 4.28: Modeled relationships of illustrating the dependencies of the effective DEP force generated on system design and operational parameters. a) The substrate capacitance for a fixed device area, the combined double layer capacitance due to passivating oxide layers of varied thickness both have a strong frequency dependency. c) Higher solution conductivity degrades performance especially with increased device area. d) Similarly, device area reduces performance, and more dramatically so for high conductivity fluids.

### 4.5 Chapter summary

In this chapter we have examined how device design and operational conditions influence the effective voltage available for DEP force generation as well as the tradeoffs between device area, flow rate, and voltage for optimizing throughput.

We proposed a DEP system circuit model incorporating device geometry and material properties. Arguing that the differential potential across solution is a critical determinant of the generation of DEP force in the system, we use circuit analysis to predict the effects of...
design and operational parameters on DEP force. We developed an experimental methodology of extracting a metric of relative DEP force for particles experiencing negative DEP in flow conditions. By analyzing DEP-induced velocity changes as particles are pushed into different vertical flow streams, we extract a relative change in velocity that mirrors relative DEP force for forces sufficient to push particles above the vertical midline of the channel. We validate this methodology by measuring velocity ratio as we experimentally sweep voltage amplitude and show a region of voltages for which a linear relationship occurs between relative DEP force and extracted velocity ratio (Figure 4.11). Using multiphysics simulation, we also model this same system and extract the same velocity ratio as a function of relative force and again verify a region of linear dependence of velocity ratio on relative DEP force (Figure 4.12).

With this experimental method extracting a metric for relative DEP force, we design and fabricate DEP devices with systematically varied parameters to test the success of our circuit model’s prediction of relative DEP force for many varied device parameters (Figure 4.7).

As our work focuses on the use of DEP devices for cell manipulation for clinical applications in which cell suspension solutions have high conductivity, we perform experimental tests in a 10x dilution of physiological conductivity yielding solution conductivity of about 0.16S/m. The conductivity of solution dramatically influences the degree to which circuit elements influence predicted DEP performance. Higher conductivity environments are much more susceptible to DEP performance deterioration for any given device design change (Figure 4.13).

Both our predicted relative DEP force and our experimentally measured relative DEP force show a decrease in performance with increased transmission resistance (Figure 4.14), increased fluidic channel width (Figure 4.16), or increased number of interdigitated electrodes (Figure 4.23). An insulating passivation layer over the electrodes, which can be useful for preventing device damage, was also studied and found to degrade performance at low frequencies, but to cause very little performance decrease at high frequencies (Figure 4.25).

While the strength of DEP force on particles is certain of critical importance for the
overall performance of the DEP device, design decisions must also take into account device throughput. Design changes to increase throughput include increasing the fluid flow speed or increasing the channel cross section by widening the fluidic channel or increasing the channel height. Each of these fluid throughput changes necessitate a corresponding change in electrode design. Wider channels necessitate widening the DEP electrode array, increasing channel height or fluid flow speeds necessitate adding more DEP electrodes to spatially compensate for the decreased DEP force on particles higher up in the channel or the increased distance over which particles travel before being sufficient affected by DEP forces. We’ve shown that widening the channel and electrode array or adding more electrodes incurs a loss of DEP force magnitude. A tradeoff exists, then, between force magnitude and throughput.

In order to study this tradeoff, we predict relative DEP force with out circuit model and experimentally measure relative DEP force, but we also incorporate a multiphysics simulation to inform the effect of fluid forces on DEP response times.

Beyond the device geometry and operational conditions, there is yet another facet of design to consider that impacts overall device performance - the stability and compatibility of system materials. In the next chapter we will more deeply explore the nuanced performance tradeoffs that accompany the material properties of the particles, the device components, and the suspending solution.
Chapter 5

Material considerations for DEP optimization

Many of the design choices discussed in Chapter 4 have nuanced effects on DEP performance beyond both the effective DEP force generation and the achievable sample throughput. In this chapter we discuss major material-based causes of device performance degradation from the perspective of material stability and material compatibility. The solution conductivity, applied voltage, frequency, electrode metal, electrode passivation layer, and the surface chemistry of the device and particles all contribute to issues of solution heating, electrode degradation, particle adhesion, and particle loss due to sedimentation. We first discuss the interconnected nature of all of these material-based performance limitations and then explore strategies to prevent or mitigate these issues. We also look at the implications of the tradeoffs of damage prevention strategies on DEP performance.

5.1 Major material-based issues in DEP performance

During DEP operation, material properties of the device, solution, and particles all affect overall performance by contributing to issues such as particle adhesion, bubble formation, electrode damage, and particle loss due to sedimentation. Figure 5.1 diagrams the interactions of the system material properties that contribute to the major material-based device issues discussed in this chapter.
Heating of the electrodes and solution is a major cause of device damage, bubble formation, and deteriorated cell health which leads to particle adhesion. Solution conductivity, thermal capacity of the device substrate, sourced voltage, and frequency all contribute to heating. Device damage degrades the lifetime of a device and bubbles present in solution disrupt fluid flow lines, impacting particle DEP response patterns.

Particle adhesion prevents DEP-induced particle motion and limits device usability. The materials and chemical treatments of surfaces, solution conductivity, magnitude of DEP forces, and fluid flow rates can all affect particle adhesion to the device or other particles. Additionally, heating and bubbles can contribute to increased particle adhesion, especially for biological cells.

In this section we discuss briefly some of the major causes of material-based device performance degradation issues including solution heating, electrode degradation, bubble formation, particle adhesion, and particle sedimentation.

Figure 5.1: Diagram of the interactions of system material properties on common issues of DEP optimization
Solution heating

Heat generation during DEP operation can negatively impact DEP performance in several ways. Heating of the solution can create bubbles as well as electrothermal fluid flow, both of which alter the fluid streamlines in the device. Disruption of fluid flow characteristics will impact DEP-induced particle trajectories, thereby changing overall DEP performance. Heating can also degrade the device materials themselves, decreasing device stability and lifetime. Biological particles are also very sensitive to the temperature of their environments. Cell adhesion and cell death can increase at elevated solution temperatures, negatively impacting DEP operational success.

Heating in response to DEP operation depends on many factors but is ultimately caused by the generation of a large power density in the fluid region [56]. Current flowing through a resistive element can generate heat through a process called Joule heating or Ohmic heating. For this to occur, electrical energy is converted to thermal energy through collisions by accelerating charge carriers as they respond to the voltage across a resistive element. The power generated during this heating process is given by the Joule-Lenz law as

\[ P \propto I^2 R \]  

(5.1)

where \( P \) is the power converted from electrical energy to thermal energy, \( I \) is the current through a resistor with a resistance \( R \). Utilizing Ohms’s law we can rewrite this as

\[ P = \frac{V^2}{R} \]  

(5.2)

where \( V \) is the voltage drop across a resistance \( R \). In the case of AC fields, \( R \) is the real part of the impedance. Power has the units of energy per time or Joules per second, called Watts. As shown by the Joule heating equation, higher currents produce greater thermal energy production for any given resistive element.

As we have shown in Chapter 4, current passes through the resistive elements of the DEP electrodes and the solution. The voltage drop across the solution resistance (\( V_{soln} \))
determines the magnitude of the DEP force generated \( (F_{dep}) \) as

\[
V_{soln}^2 \propto F_{dep} \tag{5.3}
\]

and here we see that that same effective voltage across solution is related to the heat generation by

\[
V_{soln}^2 = P_{soln}R_{soln} \tag{5.4}
\]

where \( P_{soln} \) is the power generated in solution and \( R_{soln} \) is the resistance of the solution. Therefore we see that the heat generation is proportional to the DEP force over the solution resistance

\[
P \propto \frac{F_{dep}}{R_{soln}} \tag{5.5}
\]

From an operational standpoint, this means that for any given device design and solution conductivity used, any increase in DEP force comes with an increase in heat generation [56]. Also we can see that the heat generation is proportional to the solution conductivity for a given effective voltage. Thus if a minimum force is required to achieve desired particle motion, the heat generated in that application of force will scale with the solution conductivity.

The change in temperature \( (\Delta T) \) can be approximated as

\[
\Delta T \approx \frac{\sigma V^2}{2k} \tag{5.6}
\]

where \( V \) is the voltage drop across solution, \( \sigma \) is the solution electrical conductivity, and \( k \) is the solution thermal conductivity [57]. Recalling that electrical conductivity is inversely proportional to the resistance of solution, we can see that this equation agrees with Equation 5.5.

The formation of this temperature gradient can actually alter the electrical properties of the fluid. Both the solution electrical conductivity and the solution permittivity are altered by temperature as

\[
\frac{1}{\sigma} \frac{\partial \sigma}{\partial T} \approx 0.02 \tag{5.7}
\]

\[
\frac{1}{\varepsilon} \frac{\partial \varepsilon}{\partial T} \approx -0.004 \tag{5.8}
\]
where $\sigma$ and $\epsilon$ are the solution electrical conductivity and permittivity at room temperature, respectively [57]. Thus a temperature gradient can generate a gradient in the electrical properties of the solution [57–60]. In some cases this increase in solution electrical conductivity with increased temperatures can create a positive feedback loop for further temperature increases. These gradients of solution electrical properties drive electrothermal fluid flow [61].

Beyond the alteration of fluid flow streams which would affect particle trajectories and, therefore, DEP performance, increased temperatures by themselves also can negatively impact cell health which can increase particle-particle adhesion as well as particle-device adhesion. Even temperature elevations of a few degrees can begin to affect cell membrane properties and cell functions.

**Electrode oxidation**

In some cases heat generation can damage DEP electrodes. Aluminum, for example, is a commonly used material because of its low cost. At elevated temperatures exposed aluminum can convert into aluminum oxide [62]. In heated solutions this reaction can occur until completion, leaving no aluminum metal remaining in the fluidic channel and thereby completely destroying the DEP device. Figure 5.2 shows an example of a device fabricated with 300nm of aluminum metal on a glass substrate before and after soaking in hot deionized water. After 5 hours in water at about 95°C, the electrode surfaces have been completely oxidized.
Figure 5.2: Aluminum electrodes before (a) and after (b) soaking in 95°C DI water for 5 hours show aluminum oxidation can proceed quickly at elevated temperatures.

**Electrode degradation**

Apart from aluminum oxidation at elevated temperature, electrodes of other materials can also be damaged in response to DEP signals. Many researchers have reported DEP operational constraints resulting from frequency and conductivity related electrode damage [63–65]. This damage is typically seen at either low frequencies or high solution conductivities. Sanchis et al., for example, report electrode degradation and bubble formation even in very low conductivity solutions of 0.0025S/m when a 12V signal was applied below 1kHz to IDE arrays of 50µm wide electrodes with a 10µm gap fabricated of 80nm of gold with a 15nm chrome adhesion layer [39]. The dependency of damage on conductivity and especially on frequency may indicate that the damage is caused by heating, electrolysis [64,65], or Faradaic reactions [66].

Figure 5.3 shows examples of how device damage differs with the electrode material as well as with the frequency of the applied voltage. Covered with a high conductivity solution (1xPBS), at high frequencies (10MHz), neither gold (5.3a) nor aluminum (5.3b) electrodes
show damage even up to 50V. Small bubbles begin to form just outside the edge of the PDMS microfluidic channel on both electrode structures, but are much more pronounced in aluminum devices. At lower frequencies (100kHz), however, the same electrode structures in gold are damaged at 12.5V and aluminum electrodes damaged at only 5V.

Interestingly, the manner in which damage occurs differs between the two metals. Gold electrodes are damaged by a gradual thinning from the electrode edges until a connection is severed (5.3c). Aluminum electrodes, however, degrade in a very different manner, with connections severed not along the electrode edges in the channel, but rather across the electrodes at the edge of the channel (5.3d). Aluminum electrode damage also occurs more consistently at lower voltages than gold electrodes of identical geometry.

Thus we see that electrode damage is influenced by the electrode material as well as by the voltage and frequency of the applied field.
Figure 5.3: Interdigitated arrays of electrodes fabricated in gold - (a) and (c) - and aluminum - (b) and (d) - under 1xPBS fluid show material-dependent differences in damage [(a) vs (b) and (c) vs (d)] as well as frequency- and voltage-dependent differences [(a) vs (c) and (b) vs (d)].

Bubble formation

Bubbles can form in solution in response to DEP signals in several different ways. If solution heating reaches the solution boiling point, bubbles can form in solution. Additionally, at very low frequencies, electrolysis reactions might occur. These reactions transform water molecules or ions in solution into other products which can be gas-phase products.

The bubbles formed by these processes should form in solution, as we see on the aluminum device in Figure 5.3d, or they can form initially outside of the microfluidic channel, just on the other side of the bonded channel edge in the gap between electrodes. The bubbles formed just outside of the channel boundary differ in appearance from those formed in the fluidic channel as they are smaller and grow in number with time but do not generally
grow in size. Figure 5.4 shows the formation of these small bubbles over a short period of time. Into a channel, 1xPBS was introduced at 1µL/min and a signal applied to a 25µm linear DEP array at 10MHz and 30V. The device was imaged over time and the formation of small bubbles is shown to begin just outside of the channel border even at 1 minute (5.4b). Over the next 11 minutes, more bubbles form between the electrodes outside the channel edge and, after 3 minutes, small bubbles can be seen over the first electrode within the channel bounds (5.4). After the signal is left off for 3 hours, the device shows no visible signs of damage or remaining bubbles (5.4d).
Figure 5.4: Formation of small bubbles in high conductivity. In high conductivity solutions and high voltages (here was used 1xPBS solution and a $30V_{pp}$ signal applied at 10MHz), small bubbles appear first between electrodes just outside of the channel border (b). At slightly longer time points, bubbles can be seen also forming over the electrodes within the channel, beginning at the electrode with the highest potential (c). Device recovers its initial appearance over time after the signal is removed (d). A closer look at the darkened area shows a morphology composed of small neighboring circles (e).
The location of the bubble formation is interesting and gives clues as to the cause and possible prevention methods. Topographically the electrodes are 300nm thick. The PDMS, though flexible, should not conform completely to the electrode edges, leaving some space at the electrode edges that fluid might be able to penetrate. It is possible that the confined fluid volume accounts the size restriction of bubbles formed in this region. If this is the case, it suggests that the fluid volume or fluid flow matters in the generation of bubbles. This indicates that the fluid in the main channel provides a thermal sink and, in the absence of that, heat buildup increases and bubbles result.

The presence of bubbles is problematic for several reasons. First, bubbles act as new fluid barriers in the channel, causing fluid flow alterations. Second, the bubbles have a lower thermal conductivity than the solution they replaced, decreasing the overall thermal diffusivity of the device and contributing to further temperature increases. Last, bubbles can increase particle adhesion.

**Particle adhesion**

Particle adhesion is a major issue DEP device performance issue. The encounter of biological cells with bubbles can lead to cell health deterioration, possibly due to increased shear forces as cells traverse along the liquid-air interface. The bubble itself can also act as an anchoring point onto which cells can adhere, creating massive cellular chains extending down the channel. Figure 5.5 shows an example of *E. coli* bacterial cells that have adhered around a bubble in solution. As more cells encounter the barrier, they too can adhere, growing the cell aggregation, greatly deteriorating DEP performance.
In addition to particles adhering onto a nucleation site in a channel, particles can adhere to each other or to device surfaces for many reasons. Chemical interactions of particle surfaces can increase adhesions. Any cell damage can increase the likelihood of adhesion. Cells can be damaged by high temperatures, high shear stresses, improper solution conductivities, or deformations from high DEP capture forces. Even non-biological particles can undergo irreversible damage when subjected to high DEP capture forces. Chen et. al, for example, showed latex microsphere breakdown after 5 minutes of DEP capture force (100kHz, 5Vpp) [67].

**Particle sedimentation**

Besides particle loss due to adhesion, particles can also be lost due to sedimentation along the fluidic pathway. The size and density of the particle influences the rate at which it settles. Because of low flow near microfluidic surfaces and particle-surface adhesion, particles that have settled to a fluidic surface can remain at that surface. Any particle-particle adhesion can exacerbate this effect as larger aggregates will have faster sedimentation rates. The resultant reduction in particle concentration over time attenuates DEP performance and
also impedes accurate sample quantification.

5.2 Operational and material considerations to limit degradation and bubble formation

Having experimentally seen the negative outcomes of solution heating, electrode damage, and bubble formation, we now seek to mitigate these material-based device issues through operational parameters and material choices.

Passivating aluminum to prevent oxide growth in elevated temperatures

Aluminum presents an attractive alternative to gold for the fabrication of patterned electrodes because of the potential material cost savings. One inhibiting factor in making the transition from gold to aluminum is that aluminum oxidizes in water, and does so quickly at elevated temperatures [68]. At room temperature this process can proceed relatively slowly, while at higher temperatures, the reaction can quickly produce thick oxides. During the fabrication process, oxide growth can be minimized by keeping all DI water rinse steps at room temperature. In DEP experimental conditions, however, elevated temperatures from Joule heating can cause problematic electrode degradation. Exposure to 80°C water even for less than 10 minutes can result in the growth of about 300nm of aluminum oxide [62]. In efforts to overcome this operationally-induced oxidation, we tested the use of a passivating silicon dioxide layer deposited on the aluminum surface prior to exposure to hot water. Devices of 300nm aluminum were fabricated and a 200nm layer of SiO₂ deposited over half of the metal area by PECVD. The device was then submerged in 95°C DI water for 16 hours. After drying the chip with nitrogen, the formation of aluminum oxide was examined optically as well as with a profilometer to measure the height and roughness of the electrode area. Figure 5.6 shows the growth of aluminum oxide on aluminum exposed to hot water, both with and without a deposited passivation oxide layer. It also demonstrates that a passivating silicon dioxide layer deposited on aluminum can prevent aluminum oxide growth in the same conditions. The same tests were also done with devices of 15nm of titanium covered by 285nm of gold. No change in height or roughness on the electrodes occurred.
after hot water treatment, on the bare gold or the gold under the passivating oxide layer (data not shown).

Figure 5.6: Aluminum oxide growth can be prevented with a deposited silicon dioxide layer. Silicon dioxide (187nm thick) was deposited on only the top half of the aluminum (300nm thick) structure shown. a) shows the structure before any exposure to DI water and b) shows the same structure after 16hrs of exposure to DI water at 100°C with the change in the height of each section denoted by $\Delta h$.

The success of aluminum protection with a passivating oxide layer allows low-cost aluminum to be used under higher temperature operational conditions than bare aluminum.

**Substrate material for better heat dissipation**

Our observations of bubble generation suggest that heat sinking into fluid or device substrates may play a crucial role in limiting heating and subsequent damage and bubble formation. To test the ability of a substrate with higher thermal capacity to limit solution heating and damage, we fabricated DEP devices in gold on two substrates: thermally insulating borofloat 33 glass and more thermally conductive silicon that has a 2µm thick SiO$_2$ layer between the electrodes and the silicon. We also fabricate the same set of device with a 200nm SiO$_2$ layer on top of the electrodes to act as a barrier to electrode damage.

Because of our work analyzing the importance of material properties on the effective voltage available for DEP, we know that the substrate capacitance and double layer capacitance are effected by these different materials. In order to control for the effective voltage at the electrode-solution interface, rather than using an applied voltage as a metric of force, we use particle velocity ratios as previously described.

For these four sets of devices, we introduce particles in flow over the device and slowly increase the voltage until there is either damage of the electrode or bubble formation in
solution. Figure 5.7 shows the velocity ratio as a function of the applied voltage for each device type. Additionally, the end of the scans shows the voltage at which damage or bubbles occur, which are a sign of solution heating.

For each oxide condition, the silicon substrate can reach both a higher applied voltage and achieve a higher velocity ratio before damage occurs. This suggests that the heat sinking benefits of the silicon substrate outweigh the slight effective voltage deterioration that would occur from the increased capacitance of this substrate. Additionally, for each substrate type, the devices with a passivating SiO$_2$ layer over the electrodes achieves higher velocity ratios before either heating or damage occurs as well. Again, even though the effective voltage across solution should be lower in the case of this oxide layer, the higher velocity ratio achieved indicates an overall net advantage to passivation in these circumstances.

![Particle velocity ratio for increased voltages applied to DEP electrodes fabricated on either glass (dotted lines ....) or silicon (solid lines —) substrates and with either no oxide over the electrodes (yellow), or 200nm of oxide over the electrodes (green). The voltage sweep ends when there is any device damage or bubble formation.](image)

Figure 5.7: Particle velocity ratio for increased voltages applied to DEP electrodes fabricated on either glass (dotted lines ....) or silicon (solid lines —) substrates and with either no oxide over the electrodes (yellow), or 200nm of oxide over the electrodes (green). The voltage sweep ends when there is any device damage or bubble formation.
5.3 Limiting particle sedimentation

In most microfluidic DEP experiments, a sample of particles suspended in fluid is introduced to the DEP device through some system of fluidic tubing. Over time the concentration of particles passing over the DEP device can tend to decline and more dramatically so for larger particles.

Particle loss in the system is problematic as it continually alters the conditions for device performance analysis or sample collection and analysis. Several factors may be at work to limit particle concentration incident at the DEP device. Gravity-induced sedimentation may cause particles to settle to the bottom of the syringe or inlet tubing and therefore never make it into the channel. Passive particle-surface adhesion may further reduce particle concentration along the route to the DEP device. Additionally mechanical restrictions may decrease particle passage if particles tend to aggregate at any point along the pathway. As size-dependent effects, particle loss due to either settling or mechanical restriction would be further enhanced in the event of any particle-particle adhesion resulting in particle aggregates.

In order to explore and prevent particle loss in our DEP system, we studied each possible contributing factor: gravitational sedimentation, mechanical restriction, particle-device adhesion, and cell health deterioration which can decrease cell concentration through cell-cell adhesion, cell-device adhesion, or cell death. We then tested possible solutions to prevent loss from each factor in order to optimize particle incidence over the DEP device over time. In this section we explore methods for mitigating particle loss due to sedimentation. In later sections, we address particle loss due to adhesion.

Gravity-induced sedimentation force is always present in a system, but the rate of sedimentation determines whether that force will affect particle concentration on time scales relevant to DEP device operation. Generally, large or dense particles, such as mammalian cells or large microbeads, can settle in solution on a time scale shorter than a typical run time for a DEP experiment or measurement. Concentration of these particles at the device, therefore, declines over time.

In our experimental setup, solution is dispensed from a syringe by a volumetrically
controlled pump, passed through a syringe needle, a length of PTFE tubing, flowed through the microfluidic channel to the DEP device, and then on to an outlet tube for optional collection. This passage is diagrammed in Figure 5.8a.

Figure 5.8a demonstrates the decline in particle concentration along the fluid pathway from syringe to outlet tubing for large (8.87 µm) and medium (4.45 µm) diameter polystyrene beads. At each point along this fluidic pathway, the number of particles present declines, and does so more aggressively for larger particles. The more rapid loss of larger beads indicates the contribution of one or more size-dependent particle loss factors such as either gravitational settling or mechanical constriction. For this experiment, a sample of mixed beads in 1xPBS was flowed at 5 µL/min through four pathways in parallel to enable simultaneous sample collection at each pathway point of interest at specified time points. For each time point, samples were collected from the original vial (A) by mixing the vial contents and pipetting a small aliquot and from collection points (B) through (E) by collecting sample output into a vial during continued flow for 3 minutes. The concentration of each type of particle from each collected sample was measured on a hemocytometer. The size differences between large and medium beads were large enough to reliably distinguish by eye as seen in Figure 5.9.

The data in Figure 5.8 shows the fraction of the original particle concentration that remains at each point along the fluidic pathway. Particle concentration measurements at each point were taken simultaneously from 4 parallel fluid pathways as soon as fluid exited the outlet tubing at point (E).
Figure 5.8: a) The pathway for particles during our DEP operation typically includes A) particle preparation in a vial, B) syringe ejection into C) a 28 gauge syringe needle connected to D) a short length of PTFE tubing that enters E) the microfluidic channel inlet. After passing over the DEP device in the microfluidic channel, particles finally exit the device through F) another short length of PTFE outlet tubing. b) The fraction of original particle concentration remaining decreases with distance along the fluidic pathway, with more rapid decline for larger particles.
Even at a collection point early in the pathway, there can be seen a rapid decline in particle concentration over time. The output of the syringe needle at 0, 20, and 40 minutes is shown in Figure 5.10. The enhanced reduction in concentration of larger particles over time indicates concentration reduction results from either gravitational settling or mechanical constriction.
The rate of sedimentation is given by

\[
v_s = \frac{4a^2(\rho_p - \rho_m)g}{18\eta}
\]  

(5.9)

where the sedimentation rate \(v_s\) [m/s]) can be seen to scale with the square of the particle radius \(a\) [m]) and linearly with the difference in densities between the particle \(\rho_p\) [kg/m\(^3\)] and surrounding fluid media \(\rho_m\) [kg/m\(^3\)], and inversely with the fluid viscosity \(\eta\) [kg/(ms)] [43]. The sedimentation rates vs. particle size for polystyrene beads with a density of 1.05g/cm\(^3\) and cells modeled with a common cell density of 1.08g/cm\(^3\) suspended in an aqueous fluid of density 1g/cm\(^3\) are plotted in Figure 5.11. The calculated sedimentation rates for particles commonly used in this work are given in Table 5.1. The inner diameter of a 1mL syringe commonly used to dispense samples to microfluidic devices is 4.64mm. The time required to settle this distance for small (1.77µm), medium (4.45µm), and large (8.87µm) polystyrene beads is 15.5h, 2.4h, and 0.6h respectively. A small bacteria cell such as E.coli would take 17h to traverse this distance (assuming no autonomous cell movement), while a much larger mammalian cell, such as a 10µm T cell would settle the entire distance in only 18 minutes! The high settling rate for large cells presents a challenge for DEP device operation, as it dramatically restricts the time window available for testing as well as continually alters the cell concentration incident to the device.
Figure 5.11: Calculated sedimentation rates of polystyrene beads (density = 1.05g/cm³) and cells (density ≈ 1.08g/cm³) in water (density = 1g/cm³). The sedimentation rate increases as the square of the particle radius. Cells, having a higher density than polystyrene beads, sediment faster for any given particle size.

Table 5.1: Sedimentation rates for particles commonly used in this work in an aqueous environment with fluid viscosity similar to water (1cP). Cell diameters and densities listed are approximate.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Diameter (um)</th>
<th>Density (g/cm³)</th>
<th>Sedimentation rate (um/min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene beads</td>
<td>1.77</td>
<td>1.05</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Polystyrene beads</td>
<td>4.45</td>
<td>1.05</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Polystyrene beads</td>
<td>8.87</td>
<td>1.05</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>1.24</td>
<td>1.08-1.1</td>
<td>4-5</td>
<td>[69]</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>8</td>
<td>1.13</td>
<td>272</td>
<td>[70]</td>
</tr>
<tr>
<td>Unactivated T-cells</td>
<td>8</td>
<td>1.08</td>
<td>167</td>
<td>[71]</td>
</tr>
<tr>
<td>Activated T-cells</td>
<td>10-12</td>
<td>1.08</td>
<td>262-377</td>
<td></td>
</tr>
</tbody>
</table>

The length of horizontal tubing that can be used before particles settle through the
The entire tubing inner diameter can be calculated by

\[ L = \frac{4 \times 10^6 Q}{v_s \pi d} \]  

(5.10)

where \( L \) is the length of horizontal tubing (in mm) that particles at the top of the tube will travel before reaching the tubing bottom, \( Q \) is the volumetric flow rate in \( \mu \text{L/min} \), \( v_s \) is the settling rate in \( \mu \text{m/min} \), and \( d \) is the inner diameter of the tube in micron. For our typical 28-gauge tubing diameter of 381\( \mu \text{m} \), if fluid is introduced at 1\( \mu \text{L/min} \), we would expect that all small beads will settle to the bottom of the tube after 668mm while the larger 8.87\( \mu \text{m} \) beads would settle after only 26mm. This is assuming an average linear flow speed as particles settle in the tube and no hydrodynamic lift forces. Thus this is a conservative estimation.

Overcoming the limitation of particle settling could be approached from several angles. Either a setup must be equipped to allow settling to occur at its regular rate without negative impact or the settling rate should be reduced such that the time scale of settling is much larger than the time scale necessary for DEP operation. In the first case, vertical fluid reservoirs or the continual mixing of the fluid sample could allow more stable particle concentrations over time despite particle settling. In the second case, increasing the fluid density to approach the density of the suspended particles would cause the particle settling rate to approach zero, thereby reducing particle settling rates in the system. We tested withdrawing samples from a continuously mixed source vial and also density matching the fluid to the suspended particles.

5.3.1 Continuous mixing to combat sedimentation losses

In order to infuse a DEP device with a consistent particle concentration, we tested the ability to pull solution from fluid reservoirs that were constantly mixed to mitigate settling. Three parallel setups were made consisting of a microfluidic channel connected by teflon tubing to a syringe in a syringe pump on one side and to a vial on the other side. Polystyrene beads (6.42\( \mu \text{m} \) diameter) were either loaded into a syringe and pushed into the microfluidic channel or loaded into a vial and pulled through a microfluidic channel by the syringe pump.
set to withdraw mode as diagrammed in Figure 5.12a. In one of the setups with fluid pulled through the channel, the solution reservoir was continuously mixed by being mounted on a shakerplate. In order to minimize solution evaporation during the experiment, all vials were closed with only a small hole drilled in the top to allow a snug fit between the tubing and the vial. The tubing in the vials extended to the base of each vial to collect fluid from the bottom. Flow rates for all parallel setups were set to 0.4µL/min throughout the experiment. Samples collected at the end of the inlet tube after 2 hours of flow were imaged on a hemocytometer and bead concentrations calculated for each. Samples were collected from the end of the inlet tubing rather than the outlet tubing in order to negate any particle loss that might occur within the microfluidic channel.

Figure 5.12b shows the bead concentration at the end of each outlet tube after 2 hours of flow as a percentage of the original bead concentration loaded into the supply reservoir (syringe or vial). There was significant improvement of bead concentration with the pulling methods compared to the pushing method as well as significant improvement in the continually mixed solution reservoir compared to either static reservoirs. Thus pulling from a continually mixed reservoir presents a possible approach for overcoming particle settling over time during DEP operation. Unfortunately, each of the two solution pulling setups resulted in significant bubble introduction into the channel. No bubble sources were noticeable in either fluid reservoir and no bubbles seen in the inlet tubing. Bubbles first appeared in the channel itself and continued into the outlet tubing and bubble generation was unaffected by ceasing vial mixing, all suggesting that air was being introduced at the junction of inlet tubing and microfluidic tubing inlet. No solution leakage was observed at any channel inlet or outlet during the experiment. Possibly pulling solution through a channel requires a tighter seal between tube and channel than necessary for pushing solution through. While this seal might be achieved with a device redesign or setup reconfiguration, initial efforts to seal the inlet junction more tightly on existing devices were not sufficient to eliminate bubble introduction. The concentration of beads in even the best case setup tested was less than 40% of the original concentration after 2 hours, indicating that all methods would require further modification in order to maintain a stable particle concentration over the DEP devices.
Figure 5.12: **a)** Diagram of three parallel setups for particle introduction into a microfluidic channel: Pushing solution from a syringe [A], pulling solution from a reservoir [B], and pulling solution from a continuously mixed reservoir [C]. Samples were collected for analysis from the end of the inlet tubes marked by an asterisk (*). **b)** Bead concentration at the end of the inlet tube after 120 minutes of fluid flow, relative to initial bead concentration. Solution pulling improved concentration compared to pushing and continuously mixing pulled solutions further improved concentration, though still resulting in an over 65% loss of sample concentration after two hours.
We also tested intermittent syringe rotation to limit particle settling (data not shown). Syringe was rotated 180° every three minutes to counteract the sedimentation rate. While syringe rotation seemed a viable option for limiting settling within the syringe itself, each rotation event creates transient fluid velocity changes and would do little to limit settling within the inlet tubing. We therefore utilized syringe rotation for many experiments when flow rate fluctuations would not be problematic.

Pulling solution from the bottom of a fluid reservoir improved particle concentration maintenance compared to pushing solution from a syringe. Continuously mixing solution in a reservoir further improved particle concentration maintenance. Even with mixing, particle concentration decreased 65% over two hours (Figure 5.12b). Furthermore, pulling solution introduced bubbles into the microfluidic channel and would therefore necessitate some device or setup redesign to be a viable solution for limiting particle settling. Intermittent syringe rotation is a more readily adoptable fix for counteracting settling, but has the disadvantage of creating flow rate fluctuations during rotation. In cases when these fluctuations would not be problematic, syringe rotation was implemented. In order to further limit particle settling beyond that achieved by either syringe rotation or pulling solution from a continuously mixed reservoir, while avoiding fluid flow fluctuations or possible bubble introduction, we proceeded to explore another approach to limiting particle settling through density matched particle suspension solutions.

5.3.2 Density matching fluids to combat sedimentation losses

Another approach to reducing sedimentation in a system is to reduce the sedimentation rate by minimizing the disparity between particle density and solution density. Cells have a higher density than water, so we explored the efficacy of mitigating settling by increasing solution density. Solution density can be increased with the addition of heavy metal salts, but that also confers an increase in conductivity which is not always a welcome characteristic in DEP operation, as we have seen. Fortunately, several nonionic solution additives exist for increasing solution density such as sucrose or commercially available additives for generating density gradients.

Cell populations can be separated by density using a centrifugation density gradient.
Two commercial density gradient additives, Percoll (Sigma Aldrich) and Ficoll 400 (DOT Scientific), were tested for their ability to limit particle settling as well as maintain cell viability. Several concentrations of Ficoll were tested and the resulting solution density and conductivity measured. Figure 5.13 shows the density and conductivity measurements of solutions of 1x T-cell growth media supplemented with 8.5% sucrose (J.T.Baker), 1% pluronic F-127 (Sigma Aldrich), and concentrations of Ficoll varied from 0% to 30%. Increasing amounts of Ficoll increases fluid density as expected and simultaneously decreases fluid conductivity as Ficoll is less conductive than the base 1x T-cell media. Of the solutions measured, the 30% Ficoll solution matched most closely with the range of lymphocyte densities found in literature ($\approx 1.08 \text{g/mL}$) [71].

![Figure 5.13: Densities and conductivities of solutions containing 1x T-cell growth media with 8.5% sucrose, 1% pluronic, and varied concentrations of Ficoll 400. Increasing Ficoll concentration increases fluid density and decreases fluid conductivity. Error bars on density measurements are standard error of the mean from multiple measurements. Conductivity measurements were not repeated.](image)

To test the viability of cells in high density solutions, naive T cells were centrifuged at $200 \times g$ for 7 minutes and resuspended in solutions containing Ficoll concentrations of 0, 10, 20, and 30% in a base solution of 1x T-cell growth media, 8.5% sucrose and 1% pluronic. After an incubation of 2 hours, cells were diluted 1:1 with 0.4% Trypan blue (Corning) and
their viability measured on a hemocytometer. Results, shown in Figure 5.14, demonstrate that cell viability remains uncompromised by Ficoll, even up to Ficoll concentrations of 30%.

![Graph showing cell viability vs Ficoll concentration](image)

Figure 5.14: Naive T-cell viability after 120 minutes in solutions of 1x T-cell growth media, 8.5% sucrose, 1% pluronic, and varied Ficoll concentrations. Viability is maintained even at Ficoll concentrations up to 30%. Error bars are standard deviation of multiple measurements of a single fluid sample.

With the viability of cells shown to be uncompromised in these high density solutions, we tested the efficacy of Ficoll solutions for limiting cell settling by loading syringes with cells suspended in each prepared solution, pumping the solution, and measuring the cell concentration and viability over time for each solution.

One potential drawback of utilizing high density fluids in DEP operations is the increase in fluid viscosity that often accompanies increases in fluid density. Shear stress on suspended particles increases linearly with fluid viscosity as shown by

\[
\tau = \frac{4Q\eta}{\pi R^3}
\]  

(5.11)

where \(\tau\) is the shear stress \([\text{N/m}^2]\) on a particle in a fluid with viscosity \(\eta\) \([\text{kg/(ms)}]\), and \(Q\) is the volumetric flow rate of the fluid \([\text{mL/sec}]\) through a constriction of radius \(R\) \([\text{m}]\) [43].
In anticipation of the increased shear stress cells will experience in fluids of higher Ficoll concentrations, samples were collected from the output of a 26-gauge syringe needle rather than out of the wider syringe tip in order to assess any decline in viability with increased fluid viscosity. Solution was pumped at 0.1µL/min between sample collections and at 5µL/min for 3 minutes during sample collection at 0, 40, 80, and 120 minute time points.

Cell concentration and viability were measured for each sample with Trypan viability staining and hemocytometer. Figure 5.15a shows the cell concentration of samples collected from the syringe needle over time for various Ficoll concentrations, and Figure 5.15b shows the corresponding cell viability for those same samples. Cell concentration declined quickly over time for all samples except the 30% Ficoll solution, whose density most closely matched that expected for the suspended cells. The viability of cells exiting the syringe remained very near 100% for the 30% Ficoll solution, showing that the expected increased shear force on the cells was not high enough to cause cell death. Interestingly, while the viability measurements of the lower Ficoll solutions have very large errors due to small cell counts, the trend indicates a decrease in viability for lower Ficoll concentration solutions upon exit from the syringe needle. For both the cells not subjected to any fluid flow (Figure 5.14) and for the cells exiting the syringe needle, cells suspended in the 10% Ficoll solution show the worst viability performance. It should be noted that the change in viability is not a statistically significant finding, but only an indication of a trend that may warrant further exploration.

Along with increased shear stress, higher viscosity fluids exert more drag force on suspended particles. As shown previously, the drag force \( F_{\text{drag}} \) on a particle moving through a fluid is given by

\[
F_{\text{drag}} = -6\pi \eta a v
\]  

(5.12)

where \( v \) is the cell velocity relative to the fluid velocity, \( a \) is the cell radius, and \( \eta \) is the dynamic fluid viscosity [6]. Since drag force opposed any particle movement in response to DEP force, increasing fluid viscosity has implications for DEP particle response. The DEP force will need to be increased linearly with any fluid viscosity increase in order to achieve the same particle movement.
Figure 5.15: Cell concentration (a) and cell viability (b) for naive T cells suspended in solutions of varied Ficoll concentrations exiting a 26G syringe needle. The base solution is 1xT-cell media + 8.5% sucrose + 1% pluronic. Even with the increased shear stress expected for higher Ficoll concentrations, cell viability remains high for the 30% Ficoll solution even after 2 hours.
These experiments show that we are able to limit nearly 100% of cell settling by matching fluid density to that of the suspended cells (Figure 5.15a). We also show that the higher density fluids do not themselves cause a decline in cell viability (Figure 5.14). Furthermore, we demonstrate the maintenance of cell viability in high density fluids even when increased shear stress is expected as cells exit a syringe needle (Figure 5.15b).

5.4 Preventing particle adhesion

Overcoming particle loss in the pathway before the DEP device by limiting settling in the solution reservoir and inlet tubing greatly increases the particle concentration incident at the DEP device and helps stabilize that concentration over long time periods. Once inside the microfluidic channel, however, particle loss due to particle-device adhesion can dramatically alter particle concentration incident at the DEP device. After DEP particle capture, particles often remain stuck to the capture area even after the DEP signal has been turned off. Particles can adhere to channel or electrode surfaces even in the absence of DEP forces. Additionally, any particle-particle adhesion can limit particle concentration by accelerating particle settling or by creating mechanical restrictions from larger particle aggregates. Many factors influence particle adhesion, including electric charge [72] and hydrophobicity of the particle and device materials [73], ionic strength [73] and pH of the suspending fluid, mechanical surface roughness, cell surface proteins, extracellular organelles [74], cell secretions [75], cell responses to elevated temperatures and shear forces [43], and the general state of cell health.

In this section we explore the various causes of particle adhesion and, informed by the theory, study the efficacy of some adhesion prevention strategies. We quantitatively measure adhesion prevention for a range of materials including inert microspheres, mammalian immune cells, and bacterial cells with extracellular organelles. We study the use of chemical channel coatings and solution additives to alter channel and particle surface properties to mitigate passive adhesion. Informed by particle adhesion theory, we also look at the effect of electrode passivation and applied voltage on DEP-induced particle adhesion.
5.4.1 General particle adhesion theory

A major theoretical model for particle-surface interactions, called the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory, describes adhesion of colloid particles to a substrate as a result of two main forces present between the particle and surface: van der Waals interactions and electrostatic interactions \([76,77]\). An extended version of the DLVO theory additionally incorporates understanding of hydration interactions to further elucidate adhesion mechanisms \([78]\). In the cases of biologically active particles, such as immune cells, bacterial cells, or platelets in whole blood, additional cell-specific adhesion mechanisms are considered in addition to the classic electrochemical forces in order to understand and prevent undesired particle adhesion.

**Van der Waals forces**

Van der Waals forces are a class of weak forces between atoms or molecules at very close proximity. When separated by about 0.5nm, electron cloud density shifts can create an instantaneous atomic dipole which can interact with that of a nearby atom. The resulting force is called London dispersion force. In molecules with shared electrons, one atom may be more electronegative than its bonded atom, creating a molecular dipole that is more probabilistically constant than the instantaneously atomic dipoles formed by electronic density shifts. Forces between molecular dipoles are called dipole-dipole interactions. Both of these force types contribute to the overall Van der Waals forces between very near atoms or molecules. Van der Waals force decays rapidly with separation distances for distances greater than the van der Waals contact distance at which positively charged atomic nuclei begin to repel each other. As an additive force, van der Waals forces for surfaces in intimate contact can be calculated as the cumulation of individual nearest neighbor interactions between the two materials. The energy of the van der Waals interaction \((U_{vdw})\) for a macroscopic sphere and a plate is given by

\[
U_{vdw} = -\frac{A}{6} \frac{a}{d^2}
\]  

\((5.13)\)
where \( a \) is the particle radius, \( d \) is the distance of separation, and \( A \) is the Hamaker constant which gives account of the molecular scale interactions between the two surfaces as a function of atomic density and the polarizability of each component atom in the materials [78]. By convention the negative sign indicates lower energy from attractive forces.

At extremely small separations, repulsion between atomic orbitals and atomic nuclei dominates over any attractive van der Waals forces. The energy of this repulsive short-range force, called the Born force, between a sphere and a plate surfaces is given by

\[
U_B = \frac{A}{168} \frac{a d_0^6}{d^7}
\]  

(5.14)

where \( d_0 \) is the equilibrium distance between the two surfaces [78]. From the combination of these equations, we can see that the energy of van der Waals attraction increases inversely with the square of the separation distance, but then at distances closer than the equilibrium distance, repulsive Born forces rapidly increase and dominate.

**Electric double layer repulsion**

In addition to these forces, material surface charge also influences surface interactions. Particles in solution can readily acquire surface charges due to preferential ion absorption and dissociation of surface groups, very often resulting in a negatively charged surface in neutral pH conditions [78]. Solution ions redistribute in response to surface charges, with ions being attracted nearer to surfaces with opposing charge, creating an electrical double layer of ions. Surfaces of like charge will have mirroring electric double layers that repel each other when in proximity.

The interaction energy between the electric double layers on a sphere and plate both with constant surface potential is given by

\[
U_{dl} = \varepsilon_m \pi a \left( (\psi_p + \psi_s)^2 \ln \left( 1 + e^{-\kappa d} \right) + (\psi_p - \psi_s)^2 \ln \left( 1 - e^{-\kappa d} \right) \right)
\]  

(5.15)

where \( \psi_p \) and \( \psi_s \) are the surface potentials of the plate and sphere, respectively, \( \varepsilon_m \) is the permittivity of the fluid media, \( a \) is the sphere radius, \( d \) is the separation distance, and \( \kappa \) is
the reciprocal double layer thickness (also called the Debye length) \cite{78}. The ionic strength of the solution greatly influences the electric double layer as seen in the calculation of $\kappa$

$$\kappa^2 = \frac{1000eN_A}{K_BT} \sum \frac{1}{\epsilon_m} z_i^2 M_i$$ \hspace{1cm} (5.16)

where $z$ is the valence number of the ions and $M$ is the molarity of the ions \cite{78}. $K_B$, $T$, $N_A$, and $e$ are the Boltzmann constant, absolute temperature, Avogadro’s number, and the electron charge, respectively.

Plotting the net energy as the summation of all these forces with the convention of attractive forces being negative (attractive van der Waals forces) and repulsive forces being positive (Born force and electric double layer forces) can yield an interesting energy barrier profile, as sketched in Figure 5.16.

![Figure 5.16: Sketch of possible interaction energy between a macroscopic sphere in very near contact with a plate surface shows two energy minimums can exist as attractive van der Waals forces dominate over repulsive Born and double-layer forces. Surface adhesion states are closely tied to the presence of these energy minimums.](image)

As the surfaces are brought closer to contact, attractive van der Waals forces become stronger. As the separation distance narrows further, the repulsive electric double layer forces begin to dominate. Because van der Waals forces increase faster with decreased distance than the double layer forces, van der Waals forces again can dominate over the electrical double layer repulsion. As the separation distance decreases beyond the equi-
librium distance between neighboring nuclei, however, the repulsive Born forces quickly outpace the van der Waals forces and the energy becomes very large.

Thus, as a function of distance, there can exist two energy minimums due to van der Waals forces dominating over the double layer at small distances and then again at very small distances before the Born repulsive force takes over. When surfaces have come to exist within the smaller secondary minimum at greater distances, adhesion is considered reversible. For high ionic strength solutions, the electric double layer is very thin and thus, attractive van der Waals force can dominate at much smaller separation distances, increasing the adhesion energy of the secondary minimum. Consequently, in solutions of high ionic strength, surfaces even with the same surface charge, can experience a net attractive force towards each other leading to adhesion [78]. If surfaces come to exist in the deeper primary energy minimum that can occur at much closer separation distances, however, adhesion requires more energy input to reverse. This adhesion is sometimes nearly irreversible, requiring large amounts of energy to overcome the energy barrier to separate the surfaces.

These very close range forces can have dramatic impacts on DEP devices. The very high DEP capture forces can bring particles into extremely close contact with a surface. Depending on the materials involved, this can result in adhesion in either the reversible energy minimum or the nearly irreversible energy minimum. The higher ionic strength particle suspension fluids should generally lead to slightly stronger adhesion within the secondary minimum regime.

**Hydration interactions**

Hydrophobicity also plays an important role in particle adhesion. Hydrophobic particles adhere more to hydrophobic materials such as PDMS and less to hydrophilic surfaces such as SiO$_2$, glass, and gold. Significantly for DEP operation, hydrophobic interactions can become stronger at elevated temperatures, up to a point before molecular denaturing occurs.

**Surface roughness**

Surfaces with macroscopic mechanical roughness can allow larger areas of intimate contact with a particle, thus increasing adhesive forces. Microscopic roughness, on the other hand,
may actually decrease adhesion of rigid particles by limiting possible contact points. For particles with more flexible surfaces, however, microscopic roughness can exacerbate adhesion as particles deform to establish increased intimate contact area and therefore increased cumulative van der Waals forces.

5.4.2 Surface treatments to reduce adhesion

Many areas of research have contributed to understanding and manipulating the interactions between cells and surfaces. Major strategies for minimizing cell-surface adhesion include altering the hydrophobicity of the surface, modifying the surface charge, and mechanically increasing minimum separation distances.

Hydrophobicity modification

Bacteria adhere more readily to hydrophobic surfaces. Even hydrophilic surfaces can have great bacterial adhesion if hydrophobic molecules such as hydrocarbons or fatty acids are adsorbed onto the surface [78].

As a hydrophobic surface, PDMS can therefore promote bacterial adhesion. Several methods can be used to alter PDMS hydrophobicity [79]. Exposure to oxygen plasma renders PDMS hydrophilic, but only temporarily [53]. More commonly, PDMS is chemically treated with adsorbed molecules. In a study of molecular interactions with a PDMS membrane, Boscaini et al. found that both polar and non-polar compounds interacted strongly with the PDMS surface [80].

Wang et al. quantified the absorption of several commonly used molecules in microfluidic PDMS channels and related absorption rates to molecular hydrophobicity. Hydrophobicity of a molecule was quantified by its partition coefficient (logP), measured by the ratio of the molecule’s concentration in a hydrophobic solvent compared to that in a hydrophilic solvent. They found that hydrophobic molecules (logP > 2.62) exhibited over 90% absorption into the PDMS, while hydrophilic molecules (logP < 2.47) absorbed less than 10% [81]. This general rule does not apply to proteins containing methyl or alkyl groups, however, which, even though these molecules are hydrophilic, show ready absorption onto a PDMS surface due to van der Waals forces [82].
Proteins can readily absorb onto many surfaces and render them more hydrophilic [82, 83]. D’Amico et al, for example, added bovine serum albumin (BSA) to a microchannel prior to DEP experiments (at 5mg/mL for 1 hour) or added BSA into DEP sample solution (at 0.1mg/mL) [9]. Jeyachandra et al. measured the adsorption of BSA onto hydrophobic and hydrophilic surfaces. They found that BSA coverage of the hydrophobic surface reached a maximum of about 50%, but covered about 95% of the hydrophilic surface [84].

**Surfactants and mild detergents**

Detergents composed of a hydrophilic head and a hydrophobic tail region can be used to prevent adhesion. Non-ionic detergents, a class of detergents with an uncharged head group, act as mild surfactants and prove able to disrupt lipid-lipid and lipid-protein associations while generally not disrupting any protein-protein associations or denaturing proteins. Tween-20 and Triton-X 100 are both examples of these non-ionic detergents. Gadish and Voldman used a combination of hydrophobic coating and an anionic detergent (Alconox) to facilitate the release of pDEP captured beads and spores [85]. Park et al. added 1% of the detergent Triton-X 100 into DEP samples and buffers in order to reduce cell adhesion in a microfluidic channel. [14].

**Electrostatic modification**

It is common that surfaces in ionic solutions become negatively charged. It can therefore be beneficial to make sure that both surfaces are negatively charged to increase electrostatic repulsion. Additionally, the presence in solution of positive cations can create electrostatic bridges between negatively charged surfaces. Introducing a cation binding additive such as EDTA which binds Ca$^{2+}$ can limit the cation concentration and therefore better take advantage of surface charge to limit adhesion.

**Increasing separation distance**

Physically distancing a particle from a surface will reduce the strength of any attractive forces. Polyethylene glycol (PEG) or other polymer brush layers accomplish adhesion prevention in this manner [86]. It can be important that both the particle and the channel
surface be modified with the same polymer to avoid creating a polymer bridge that binds both the surface and then the particle in solution.

Pluronic is an interesting surface modifier that can bind well to both hydrophobic and hydrophilic surfaces and can adopt a brush-like conformation when bound to a hydrophobic surface. D’Amico added Pluronic into DEP sample solution (at 0.1mg/mL) to reduce cell adhesion to DEP electrode substrates [9]). Boardman et al. also found that pre-treating polypropylene and acrylic devices with Pluronic F127 significantly decreased passive adhesion of bacteria to devices [72]. For treatment devices were submerged in 0.5g/L Pluronic F-127 and sonicated for 10 minutes.

These general strategies of surface modification must be employed thoughtfully for systems of different combinations of particle and channel surface properties to achieve successful adhesion mitigation. In the following sections we experimentally measure the efficacy of several surface treatments on reducing adhesion of T cells and E.coli cells.

5.4.3 Surface treatments and solution additives to reduce T-cell adhesion

In order to reduce the adhesion of healthy mammalian immune cells in DEP devices, we measured the efficacy of several channel coatings and solutions additives at preventing particle-device adhesion for murine T cells in channels composed of both hydrophobic and hydrophilic materials.

Before the experiments, T-cell viability was measured and confirmed to be over 95%. Starting with healthy cells was important to be able to test sticking due to passive causes rather than sticking due to cell lysis or cell death and also to limit the occurrence of any cell clumping that might affect cell passage through the microfluidic pathway. Twelve parallel microfluidic pathways were set up consisting of a 1mL syringe, a 26-gauge syringe needle, a short length of 28-gauge PTFE tubing, a PDMS microfluidic channel bonded to a borofloat 33 glass wafer, and another short length of teflon tubing from the outlet of the channel to a small 1.5mL vial (Eppendorf) for sample collection (see Figure 5.17).
Figure 5.17: Setup of twelve parallel PDMS microfluidic channels bonded to a glass wafer for examination of channel coatings and solution additives to prevent particle adhesion. Each inlet is connected to a syringe in a syringe pump. Each outlet tube empties into a vial enabling simultaneous sample collection from all twelve channels. All tubing lengths and channel lengths are identical between the 12 samples.

Channels were initially coated with a control coating of air or 1xPBS, or with 1xPBS supplemented with one of the following additives: 1% BSA, 0.00005% EDTA, 10% sucrose, 1% Tween-20, or 1% Pluronic F-127. After channel coating, cells were suspended into either a control solution of 1xPBS or 1xPBS supplemented with one of the following additives: 10% sucrose, 20% sucrose, or 1% Pluronic F-127.

Cell solutions were introduced to the microfluidic channel at 20µL/min using a volumetrically controlled syringe pump (New Era Pump Systems). Solution samples were collected from the outlet tube at 5µL/min for 7 minutes to verify that cells had passed all the way through the channel. Cells in solutions were introduced to the channel through the same syringe needle and tubing pair used to coat the respective channel to maintain any channel coating that might be present along the needle and length of tubing as well. For the duration of the experiment that followed, cells were pumped at 2µL/min and samples collected from the outlet tubes from all twelve channels simultaneously for 6 minutes starting at each time point: 0, 20, and 40 minutes. Hemocytometer measurements were taken on each output solution sample and cell concentrations calculated.

Figure 5.18 shows the cell concentration present in the fluid exiting the outlet tubing after 40 minutes of cell flow. Lower cell concentrations indicate increased cell loss due
to adhesion or sedimentation. Likewise higher concentrations of cells indicate lower cell adhesion or lower sedimentation losses. The sedimentation rates are expected to be equal for all solutions except for the solutions containing sucrose, which increases the solution density and should therefore, if anything, lower sedimentation losses.

In a study of lymphocyte adhesion, Groth et al. found that blood peripheral lymphocytes adhered almost twice as much to hydrophilic substrates compared to hydrophobic substrates under static fluid conditions. Additionally, they show that nearly 100% of adhered lymphocytes remained attached to hydrophilic surfaces even under fluid flow creating 5 N/m² of shear stress, while less than 50% of adhered cells remained on the hydrophobic surface under the same flow conditions [87].

In our system, the channel floor is glass which is hydrophilic and the channel walls and ceiling are PDMS, which is hydrophobic. We would expect, therefore, that T cells would adhere to the hydrophilic channel floor if there were no surface modifications.

For samples in which the cells are suspended in pure 1xPBS (blue bars), it can be seen that a channel coating of 1% BSA increased overall T-cell adhesion compared to the control samples. Since BSA is known to adsorb onto PDMS and render surfaces more hydrophilic, we posit that T-cell adhesion worsens due to the increase in hydrophilic interactions present with BSA-coated surfaces. A channel coated with 1% Tween-20, on the other hand, shows improvement in limiting cell adhesion compared to our controls.

Interestingly, a channel coating of 1% Pluronic (red bars) worsened cell adhesion for cells suspended in 1xPBS. When cells were suspended in 1% Pluronic, however, there was dramatic improvement in cell passage through the device. Pluronic is a surfactant with a polyethylene oxide triblock polymer. The three polymer blocks are composed of a middle block of hydrophobic poly(propylene oxide) (PPO) flanked on either side by a hydrophilic surface binding poly(ethylene oxide (PEO) block. On a hydrophilic surface, the flanking polymers bind, but on a hydrophobic surface, the central block binds, leaving two flanking polymer blocks free, creating a brush-like conformation [72]. This brush polymer layer increases the separation distance between the surface and particles. It also effectively decreases the hydrophobicity of the surface. If Pluronic is used solely as a channel coating, we would expect the Pluronic to render the PDMS surface hydrophilic. However, because
of a brush-like conformation, we would anticipate it to not increase T-cell adhesion dra-
matically. We would also expect the creation of a polymer bridge, however, between the
hydrophilic glass surface and the hydrophilic cell surface, leading to an increase in adhesion.
The hydrophilic blocks of Pluronic are highly hydrated, however, so the interaction strength
between hydrophilic blocks should be low.

When Pluronic is also present in the cell suspension solution, however, the flanking
hydrophilic blocks of Pluronic should associate more with the cell surface, leaving the central
hydrophobic block and the highly hydrated hydrophilic block more exposed for interactions.
We propose that Pluronic coating on both surfaces might lower overall T-cell adhesion by
slightly lowering the hydrophilicity of the cell surface and the glass surface simultaneously,
as well as preventing intimate contact with the now hydrophilic PDMS because of the
brush-like conformation.
Figure 5.18: Naive T-cell concentration measurements of samples collected from channel outlets after 40 minutes of flow through microfluidic channels with varied coatings with cells suspended in a control fluid of 1xPBS and through microfluidic channels with a coating of 1% Pluronic with cells suspended in 1xPBS containing varied additives. Error bars are the SEM for concentration measurements from a single collected fluid sample at the time point.

In a similar effect, but for probably quite different reasons, a 10% sucrose channel coating did not improve cell passage compared to a control channel with no coating, but when 10% sucrose was present in the cell suspension solution, the overall cell passage increased by an order of magnitude. We hypothesized that sucrose in solution would increase cell concentrations by decreasing settling rates and perhaps aid slightly in preventing adhesion by lowering the solution conductivity. A sucrose concentration of 10% in solution performed very well at increasing cell concentrations. A higher percentage of sucrose, however, resulted in worse cell loss than our controls. The sucrose percentage that would yield a solution with matched osmolality to typical cell cytoplasms would be 8.5%. A 20% sucrose solution, therefore, may damage cell health due to the osmolality imbalance. Health-compromised cells are much more prone to adhesion.
With these experiments, we have shown that a channel coating of 1% Tween-20 or a
channel coating of 1% Pluronic with cell suspensions in either 10% sucrose or 1% Pluronic
are most effective at preventing loss of murine T cells in channels that contain both hy-
drophobic PDMS and hydrophilic glass. Next we test solution additives for preventing
adhesion of the other major cell type in our DEP work: bacterial cells.

5.4.4 Solution additives to reduce bacterial adhesion

Having more complex surface features and interactions than immune cells, bacterial cells
require their own testing for solution additives that would limit surface adhesion.

Bacteria (including \textit{E.coli}) in solution generally carry a negative surface charge and
many common substrate surfaces likewise have a net negative surface charge. Thus the
surfaces of substrate and bacteria might repel each other. This would normally bode well
for issues of particle adhesion. Unlike most non-biological particles, however, the surfaces
of bacterial cells are highly complex and highly dynamic, able to respond to environmental
pH, ion concentrations, and nearby surfaces or other particles [88].

Some bacteria have evolved very sophisticated mechanisms to adhere to surfaces. Struc-
tures such as flagella, fimbriae, and fibrils extend from the surfaces of bacteria to aid in
movement, environment sensing, and surface adhesion. Some bacterial species also actively
secrete polymeric substances that can surround the bacteria [88] and adsorb onto surfaces,
pre-conditioning the surface for later bacterial adhesion [89]. The strength of bacterial ad-
hesion to surfaces can increase over time. Reversible adhesion can occur within minutes,
but irreversible attachment can occur on the order of hours [90,91]. Once at the surface,
\textit{E.coli} can even alter their surface charge to increase their adhesion [92].

Much research has been done in the field of bacterial adhesion that has implications for
bacterial DEP manipulation. Studying biofilm formation over long time periods, Janjaroen
et al. concluded that \textit{E.coli} adhesion increased in solutions of higher ionic strength, and
also on surfaces of greater roughness [93]. Using a bacteria-coated AFM tip, Ong et al.
measured the adhesion forces between \textit{E.coli} and varied surfaces [94]. They found that
surface hydrophobicity enhanced bacterial adhesion and that the thickness of the bacterial
outer layer of lipopolysaccharides on the cell surface reduced adhesive forces. In a similar
vein, Otto et al. examine the surface interactions of *E. coli* in varied solution conductivities on hydrophilic and hydrophobic surfaces [73]. For bacterial cells that lacked extracellular fimbriae, adhesion was stronger at higher ionic strengths and on hydrophobic surfaces. *E. coli* that had external fimbriae, on the other hand, exhibited lower levels of adhesion across all conditions. The authors propose that the presence of fimbriae decrease the intimate contact between the two surfaces, thus lowering adhesion.

In order to test solution additives that are effective in limiting *E. coli* adhesion, *E. coli* cells were cultured on Lauria broth agar plates supplemented with 10mg/mL ampicillin (Sigma Aldrich) for selection and fluorescently labeled with BacLight™ red bacterial stain (Life Technologies) at 14µL working solution per 1mL of concentrated cells suspended in 1xPBS. After staining, *E. coli* cells were diluted 100x into one of eight prepared solutions as listed in Table 5.2 to be tested for adhesion prevention.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Additive concentration</th>
<th>Additive</th>
<th>Base solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>- -</td>
<td>1x PBS</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>99.9%</td>
<td>DI water</td>
<td>1x PBS</td>
</tr>
<tr>
<td>3</td>
<td>10%</td>
<td>Glycerol</td>
<td>1x PBS</td>
</tr>
<tr>
<td>4</td>
<td>8.5%</td>
<td>Sucrose</td>
<td>1x PBS</td>
</tr>
<tr>
<td>5</td>
<td>1%</td>
<td>Pluronic F-127</td>
<td>1x PBS</td>
</tr>
<tr>
<td>6</td>
<td>1%</td>
<td>Tween-20</td>
<td>1x PBS</td>
</tr>
<tr>
<td>7</td>
<td>1%</td>
<td>BSA</td>
<td>1x PBS</td>
</tr>
<tr>
<td>8</td>
<td>100%</td>
<td>Lympholyte</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5.2: Solutions tested for the limiting *E. coli* adhesion

Each sample of *E. coli* in solution was loaded into a syringe and flowed through a PDMS microfluidic channel (11.6µm high x 1mm wide x 25mm long) bonded to a borofloat 33 glass wafer. Initially cells were introduced at 10µL/min until all samples exited the channel
outlet. Flow rates were kept at 0.4 µL/min thereafter for the remainder of the experiment. Each channel was imaged in fluorescence at 5 locations along the channel every 30 minutes for 2.5 hours. Adhered cells appear as red areas while areas without any stuck cells show no fluorescent signal. Figure 5.19 shows example images of channel areas without and with adhered bacteria.

(a)  
(b)

Figure 5.19: Example fluorescence images showing the absence and the presence of stuck E.coli cells along a horizontally oriented microfluidic channel. a) E.coli in a 1% Tween in 1xPBS solution show no adhesion in the channel after 30 minutes of flow. b) E.coli in 1x PBS without any additives adhere strongly to the channel after 30 minutes of flow.

A MATLAB program was written to quantify the intensity of the fluorescence above a background threshold for the length of each channel for each time point. The cumulative intensity of adhered bacteria over time for each sample are plotted in Figure 5.20. From this data we see that the high conductivity 1x PBS solution with no additives performed the worst out of all solutions tested for adhesion prevention. Adhesion was less, but still very noticeable for 10% glycerol and 8.5% sucrose solutions. The low conductivity 0.001x PBS solution, 1% Pluronic, 1% Tween, 1% BSA, and pure Lympholyte solutions all showed very good reduction of E.coli adhesion even after 2.5 hours. The rate of adhesion for each solution condition was calculated by a least squares regression fit of the intensity over time data and the results are plotted in Figure 5.21.
Figure 5.20
Figure 5.21: The rate of *E. coli* adhesion in a microfluidic channel over the course of 2.5 hours for a) all *E. coli* suspension solutions tested and for b) only the five most successful tested solutions (shown alone to more clearly elucidate their differences). Error bars are RMSE of the fits for each rate of change of intensity.

From the fitted rates of cell intensity increase over time, it can be seen that the greatest adhesion occurred when bacteria were suspended in 1xPBS. Altering nothing but the ionic strength of solution by diluting down to 0.0001xPBS drastically reduces cell adhesion. Lower ionic strength solutions have larger electric double layers which, according to the DLVO theory, would increase the separation distance at which double layer repulsion forces begin,
thereby decreasing the domination of attractive van der Waals forces.

Notably, all tested solution additives improved cell adhesion prevention even though the base solution was 1xPBS for all solutions with additives (with the exception of Lympholyte, which was used as commercially purchased solution). The conductivity of the Lympholyte solution is 0.95S/m while that of the 1xPBS based solutions were about 1.7S/m.

Interestingly, while 1% BSA coating on a channel was the worst tested treatment for reducing T-cell adhesion (Figure 5.18), 1% BSA in solution performed the best at preventing *E. coli* adhesion. Since *E. coli* has been shown to adhere more to hydrophobic surfaces [73,94], we hypothesize that bacteria would have less adhesion to glass than to PDMS, and BSA in solution can adsorb onto hydrophobic surfaces like PDMS, rendering them more hydrophilic and thus reducing bacterial adhesion.

As with BSA, the addition of 1% Tween-20 to 1xPBS also reduced adhesion to levels even lower than those of bacteria in 0.0001xPBS. Pluronic also performs very well, achieving in 1xPBS adhesion reduction similar to that of 0.0001xPBS.

From these experiments we have shown that *E. coli* adhesion can be significantly mitigated by lowering the ionic strength of solution or by the addition of 1% of BSA, Tween-20, or Pluronic into high ionic strength solutions. These tests and those done for T cells demonstrate the ability to prevent passive cell adhesion in the absence of any applied DEP force. Next we look at what effects on cellular adhesion occur when DEP force is utilized.

### 5.4.5 Voltage and passivation effects on adhesion

Beyond chemical coatings and solution additives for preventing passive cellular adhesion, we also explored the effect of DEP forces on adhesion. The theory of adhesion suggests that irreversible adhesion can increase if particles comes very near the surface or if particle deformation occurs upon contact with the surface. Higher operating voltages conferring higher attractive pDEP forces might result in such scenarios, leading to greater adhesion.

To test this hypothesis, we tested the sticking of cultured bacteria with voltage on a PEG coated device. Bacteria were stained with BacLight™ (at 4µL/mL of cells) in 1xPBS, incubated for 1 hour, then washed and resuspended in 0.001xPBS. A DEP device (gold interdigitated electrodes coated by a 200nm PECVD SiO₂ passivation layer) was coated
with PEG to help reduce sticking. Introduced to the DEP device through a 17µm high x 1mm wide microfluidic channel, bacteria were captured with a 10MHz signal at a flow rate of 1µL/min, corresponding to a 0.98mm/sec average flow speed. The device was imaged right before and after 20 seconds of capture, and then rinsed with the DEP signal turned off for 20 seconds at 100µL/min (98mm/sec average flow speed). Once flow returned to the original capture flow the device was imaged again. Repeated cycles of capture and release were done for low and high capture voltages. Using our particle detection MATLAB program, bacteria in each image were counted and the percent of captured cells that remained stuck to the device after rinsing at high flow velocity was measured. This percent cell adhesion is shown in Figure 5.22 for both high and low capture voltages.

![Figure 5.22: Bacterial cell adhesion increases with pDEP capture voltage](image)

We can see that the extent of sticking has been found to increase with increased capture voltage. Higher voltages might lead to greater sticking by the attractive DEP force overcoming surface repulsion and bringing cells in very close contact with the surface, leading to near irreversible adhesion. The health of the cell under these DEP conditions might also be a contributing factor to the voltage-dependance of the irreversible bacterial adhesion. The nonlinear dependence of adhesion on voltage, however, suggests that there may be a threshold force at which sticking becomes irreversible.

Since passivating the DEP electrode surface with an oxide can be beneficial in some
situations, we then tested the effects of such passivation on voltage-dependent adhesion. In our passivated devices, electrodes were coated with 200nm of SiO\textsubscript{2} via plasma-enhanced chemical vapor deposition (PECVD). Effect of the oxide layer on effective voltage available for DEP manipulation has already been addressed in Chapter 4, but here we will look into how the oxide surface compares to bare metal for bacterial adhesion. The floor of the device is a 2\textmu m thick thermally grown SiO\textsubscript{2} layer on which metal electrodes are deposited. If electrodes are passivated with a 200nm layer of SiO\textsubscript{2}, that layer covers the electrodes as well as the channel floor. Figure 5.23 shows a cross section diagram of the two devices used for testing the effect of oxide passivation on bacterial adhesion under various DEP trapping conditions.

M.\textit{smegmatis} bacteria were fluorescently labeled with BacLight\textsuperscript{TM} red bacterial stain (4\textmu L per 750\textmu L cells), and suspended in 0.01x PBS. Cells were introduced via volumetric syringe pump to DEP devices with either no SiO\textsubscript{2} coating or a 200nm SiO\textsubscript{2} coating over an interdigitated array of gold electrodes. While cells were flowing at a desired flow rate, a DEP signal of 16MHz was applied to the electrodes to capture bacteria for 20 seconds. After capture the signal was turned off and the flow rate raised to 100\textmu L/min for 20 seconds to help remove any captured cells. After stabilizing the flow rate at the next desired flow rate for 20 seconds, the DEP signal would be turned on for capture. The process was repeated for a range of flow rates from 0.1\textmu L/min to 10\textmu L/min and three sourced voltages: 0.05, 0.25, and 0.5 $V_{pp}$ which, after amplification, correspond to approximately 0.5, 2.5, and 5 $V_{pp}$ respectively on each of two DEP circuits, 180° out of phase with each other. Images of the DEP array were captured after each step (capture, release, and flow stabilization) for each condition examined. A MATLAB program was written to detect and count the number of bacteria present on the DEP array from each image. From the images at each step of the condition, the number of particles captured for each condition as well as the number of captured bacteria that remained adhered to the array after the 100\textmu L/min rinse step were calculated.

Figure 5.24a shows the number of particles captured on DEP arrays without and with a SiO\textsubscript{2} passivation layer for all three voltages tested. These capture values are averaged over repeated data acquisitions for a range of flow rates from 0.1 to 2.15\textmu L/min. At low
voltages more cells are captured on the bare electrodes. This is not unexpected, given the voltage drop over the oxide layer as discussed in detail in Chapter 4. Though the effect of the oxide layer on the voltage should be limited in the operating frequency of 16MHz, any decrease in effective voltage should lead to decreased capture. Surprisingly, however, at higher voltages (5V), the trend reverses and more cells are captured on the oxide-coated electrodes than on the bare electrodes. Suspecting that a difference in adhesion behavior between the two materials might cause the reversal, we examined the fraction of captured cells that remained adhered to the DEP array even after the 100µL/min rinse step (Figure 5.24b). For all voltages, the fractional sticking of the bacterial cells is greater on the oxide-coated devices than the bare metal devices. Additionally, the fractional sticking generally increases with voltage for the bare metal devices, but remains relatively more constant for the oxide coated devices. This suggests that the adhesion mechanism on bare gold relies on voltage-dependent forces, while the adhesion of bacteria on SiO₂ relies at least somewhat on voltage-independent mechanisms.

![Cross section diagram of devices made without (left) and with (right) a SiO₂ passivation layer over the metal electrodes. Not to scale.](image)

Figure 5.23: Cross section diagram of devices made without (left) and with (right) a SiO₂ passivation layer over the metal electrodes. Not to scale.
Figure 5.24: Examination of the a) capture and b) adhesion rates of *M. smegmatis* captured from 0.01x PBS solution flowing over interdigitated capture arrays with and without a 200nm SiO$_2$ coating over the electrodes. Each bar is averaged data taken with an identical set of flow rates ranging from 0.1 to 2.15µL/min. Error bars are the standard error of the mean for each condition.
The increase in adhesion with voltage for bare metal electrodes has important ramifications for DEP operation optimization. While firm conclusions should not be drawn without further investigation with higher sample numbers and greater range of voltage studied, these initial findings indicate that the force at which a bacterial cell is brought into contact with a surface can dictate the strength of adhesion developed on a short time scale (in this case less than 20 seconds). Thus, in cases where release after DEP capture is desirable, it may be preferable for DEP forces to be kept as low as possible in order to minimize unwanted voltage-dependent adhesion.

5.4.6 Limit adhesion by limiting cell death

During DEP operation, many stressors to cell health can occur. The solution conductivity and osmolarity should be compatible with cell health. Solution heating due to voltage application can damage cells, as can shear stresses from high flow rates.

In order to test whether adhesion varied with cell health, adhesion of \textit{E.coli} of varied health was monitored. Viable \textit{E.coli} were fluorescently labeled green and non-viable \textit{E.coli} were fluorescently labeled red. Cells were washed and suspended in 0.001xPBS buffer and flowed over an IDE DEP array at 5\(\mu\)L/min and then captured with 25\(V_{pp}\) signal at \(1 \times 10^7\) Hz. Cells were then released with the removal of the DEP capture signal and an increase in flow rate to 100\(\mu\)L/min. Figure 5.25 shows an image before DEP capture, during DEP capture, and after the device was rinsed with high flow rate and no DEP signal applied. The non-viable cells remain adhered to the device at much higher rates than the healthy cells. This increased adhesion in unhealthy \textit{E.coli} compared to healthy cells demonstrates the importance of maintaining cell health during DEP operation.
Figure 5.25: Viable *E. coli* (stained green) and dead *E. coli* (stained red) are a) flowed over an IDE DEP array, b) captured with a DEP signal and then c) released with the removal of the DEP capture signal and a 20x increase in flow rate. Dead cells exhibit increased adhesion following DEP capture.
To maintain cell health the solution and cell should be compatible. Matching the osmolarity of the suspending media with that of the cell cytosol limits the influx of water and subsequent swelling of a cell with damaged membrane [95]. Sucrose addition allows for osmolarity matching without alteration of solution conductivity. The resultant isoosmotic media has been shown by Pakhomova et al to limit short-term cell swelling and cell death following short electroporation pulses. In this work, the authors report that either a 4:1 or 7:3 mixture of RPMI cell media with isosmotic sucrose in water solution (290 mOsm/kg) limits cell swelling after exposure to nanosecond pulsed electric fields (600 pulses with a 300ns duration, 7kV/cm amplitude, and 200Hz frequency) [95].

Additionally, heating of the solution should be minimized and bubbles in the channel which can increase shear forces avoided. Cells with severely compromised health may also physically break apart, further decreasing particle concentration at the DEP device and limiting device performance. Thus we see that the maintenance of cell health becomes a critical component in the optimization of DEP performance.

5.5 Regenerating fouled or damaged devices

During DEP operation, particle adhesion or fluidic leakage can affect performance and render a device unusable. Here we show methods by which a chip can be regenerated and reused after either cellular adhesion or fluidic leakage.

5.5.1 Cleaning biofouled surfaces

Cells in microfluidic channels can adhere to surfaces or deposit chemical substances onto chip surfaces. This biofouling not only can interfere with fluid movement, but can also provide positive feedback for additional fouling. The removal of biofilms and cellular debris, then, is crucial for preserving the lifetime and functionality of DEP devices. We sought to regenerate the device surfaces while maintaining the microfluidic bond strength. PDMS is known to swell in the presence of solvents which can compromise PDMS bonding to the chip surface. We therefore needed a cleaning solution made without solvents that was compatible with PDMS and our chip materials.
Wanting to remove lipid-based materials, we utilized the chemical reaction called saponification, in which a base in water can convert esters into carboxylates and alcohols. Seeing that Hoek et al. report no damage to PDMS after 24 hours of incubation with aqueous 1M NaOH solution [96], we chose to use this as our base solution.

A device used for T-cell manipulation incurred T-cell adhesion during DEP operation. Figures 5.26a and 5.26b show the chip surface through brightfield and fluorescence filters, respectively. The T-cell residue fluoresces red, showing significant T-cell residue even after the chip was flushed with DI water.

We then flushed the device with a 1M solution of sodium hydroxide in water for 1 hour and imaged the device. Figures 5.26c and 5.26d show the same area of the chip in brightfield and fluorescence, respectively. The PDMS channel and chip surface show no additional damage and the T-cell residue has been completely removed. Chips were then rinsed with DI water to remove any NaOH from the channels.
These tests show that a 1M solution of sodium hydroxide in water could be flushed into the channel for an hour to effectively remove cellular debris without damage to the device materials or fluidic channel bond. We utilized this method after many cellular DEP experiments to thoroughly clean chip surfaces.

5.5.2 Removal and rebonding of PDMS microfluidic channels

In the event of catastrophic microfluidic leakage or clogging, it can be useful to be able to replace the microfluidic channel to reuse the more costly device base. With our collaborators in Dr. Jie Chen’s group at the University of Alberta, we were able to successfully remove permanently bonded PDMS microfluidic channels and also re-bond new PDMS channels onto cleaned device bases.
To remove bonded PDMS, chips were first sonicated in DI water to test whether a chemically gentle mechanical method could be used to remove PDMS. After sonication for about 1 hour and an overnight soak, no effect on PDMS adhesion was noticed (Figure 5.27a).

We then sonicated chips in a 1:1 mixture of acetone (Macron) and acetonitrile (Sigma Aldrich) for two hours. After soaking in the mixture overnight, chips were sonicated again for another four hours. After this, the bulk of the PDMS could be peeled off of the chip surface quite easily (5.27b). In some places a residue appeared left behind and so the chips were soaked again overnight, rinsed in DI water and examined again, but no change in the PDMS residue was noticed.

To try to remove this residue, a 0.1M solution of potassium hydroxide in isopropanol was prepared by dissolving 0.56g of KOH pellets (Mallinckrodt) into 100mL of IPA (J.T.Baker). Chips were soaked in the KOH solution for 1 day, rinsed with DI water, dried, and examined. Chip surfaces had markedly increased hydrophilicity after this KOH treatment. The PDMS residue on the chip surface had been dramatically reduced, though a residue or damage still appeared on the gold electrodes (5.27c).

After 2 hours of sonication in 0.1KOH in IPA, the residue on the metal electrodes had diminished, but was still clearly visible (5.27d). To try to remove this, chips were then sonicated in a 1M solution of sodium hydroxide (Macron) in DI water for 2 hours, rinsed, dried, and examined. This treatment removed all remaining residue except for some ghost marks on the electrode where the edge of the channel had originally been (5.27e).

To see whether that remaining marking would be removed by NaOH, chips were soaked for 10 days in 1M NaOH solution and examined. After 10 days, the ghost markings remained and also damage to the metal occurred (5.27f). There was even damage to the chip substrate through the 2µm thick SiO2 layer by this point, and significant dissolution of the exposed silicon on the chip sides and back.
Figure 5.27: PDMS removal from a silicon dioxide coated silicon chip with patterned gold electrodes. Chips were sequentially a) soaked and sonicated in DI water which had no effect on PDMS bonding, b) soaked and sonicated in 1:1 acetone:acetonitrile which allowed the bulk of the PDMS to be removed, c) soaked in 0.1M KOH to remove the PDMS residue from the chip surface which was further aided by d) sonication in 0.1M KOH. e) After sonication in 1M NaOH the remaining residue on the metal was removed except for ghost markings of the original PDMS channel edge. f) Further soaking in 1M NaOH for 10 days did not change the markings and began to damage the metal electrodes.
After successful PDMS removal, chips that had not been left to soak in NaOH for the additional 10 days were cleaned and new PDMS channels were bonded in the usual method. Leak tests performed on the new devices at experimental flow rates showed no fluidic leakage indicating that the bond strength achieved was at least sufficient to withstand the pressures used in our experimental setup.

5.6 Chapter summary

In this chapter we have explored the nuanced interactions of material-based system properties in overall DEP device performance limitations (Figure 5.1). Solution conductivity, voltage, and frequency impact the extent of solution heating in the device. The presence of a passivating layer over aluminum can prevent heat-induced aluminum oxidation (Figure 5.6). The electrode metal impacts the stability and the manner of electrode degradation in response to DEP signals (Figure 5.3). The thermal capacity of the substrate and fluid impact the increase in temperature at the DEP electrodes (Figure 5.7). The formation of bubbles in solution should be avoided as they alter particle trajectories, fluid speeds, and can damage cells through increased shear forces.

We experimentally tested the tradeoff in performance between different substrate materials and found that, despite smaller capacitance, heat sinking substrates improved device performance. Similarly, the passivation of electrodes with an oxide layer resulted in a net gain in achievable DEP performance before damage or heating occurred.

Beyond electrode damage and bubble formation, particle loss anywhere in the system limits device performance and decreases performance evaluation accuracy. Fluid pathway design, particle density, and fluid density all impact the extent of particle loss due to settling in the system. We have shown decreased particle loss through continuous sample agitation (Figure 5.12b). This occurred at the expense of the introduction of bubbles into the fluidic channel and so would require further design alterations to be implemented as a successful solution to particle settling. We were able to achieve nearly 100% prevention of particle loss due to settling by density matching the fluid to the particle density, however, with no issue of bubble introduction (Figure 5.15a).
Particle loss can also be caused by particle adhesion, either to device surfaces or to other particles. We discussed the various mechanisms by which adhesion occurs and then experimentally tested several surface modification strategies for limiting adhesion of mammalian immune cells (Figure 5.18) and *E.coli* bacterial cells (Figure 5.21). We show that some surface modifiers, such as BSA, can have directly opposing effects on particle adhesion depending on the particular makeup of particle surface.

Informed by the DLVO adhesion model, we also tested the extent of cell adhesion with increased pDEP force. It was shown that the rate of irreversible adhesion increased with increased DEP force (Figure 5.22). Additionally we tested the effect of a passivating oxide layer on voltage-dependent adhesion (Figure 5.24). We also demonstrate the importance of cell health in preventing cell adhesion (Figure 5.25).

Finally, in the event of irreversible adhesion or biofouling, we demonstrate a method for removing cells and cellular debris with sodium hydroxide that is compatible with all device materials (Figure 5.26). In cases of irreparable fluid leakage or particle adhesion, it is desirable to be able to removing the fluidic channels and reuse the device base. We demonstrate a protocol for successfully removing bonded PDMS fluidic channels and rebonding new channels (Figure 5.27).

With this increased understanding of the material-based device performance limitations and strategies tested for avoiding or mitigating these issues, especially in the case of high conductivity solutions and biological particles, we next seek to utilize DEP devices to isolate and concentration cells in high conductivity environments.
Chapter 6

Label-free cell isolation and concentration for rapid infection diagnostics

6.1 Motivation for rapid bacterial isolation and concentration

Pathogen detection is important for a wide range of applications including water-quality monitoring, antibiotic efficacy testing, and infection detection. Research into bacteria operation and behavior remains vital to discoveries regarding human, animal, plant, and environmental health. As an bacteria are organisms on the order of 1µm in size, microfluidic manipulation strategies are useful for bacterial study and detection [85]. Bacteria sensing modalities range from staining identification of cultured colonies to identification of genetic markers by PCR. All current sensing and characterization modalities have limits of either the concentration of bacteria necessary for measurement or time required for sample processing or both.
Earlier diagnosis

The ability to actively concentrate any bacteria present in a sample before detection or characterization measurements are performed lowers both the limit of detection and/or the time required for sample processing including lengthy culturing times. Particle concentration has been achieved by centrifugation, physical membrane filters, and fluid vortices made by acoustic microresonators. Because of its ability to act on micro-scale particles, be incorporated into other microfluidic devices, act without any required labelling or tethers, manipulate or capture particles in a reversible way, and be dynamically tuned to select the desired particle of interest, DEP presents a promising system for actively concentrating bacteria prior to detection or characterization measurements.

Limiting antibiotic resistance

Infection symptoms can be caused by a wide range of foreign invasions and can be bacterial, but also viral or fungal in origin. Antibiotics, which target only bacteria, are often prescribed in response to infection symptoms, before the cause of the infection is known. The overuse of antibiotics in cases of viral or fungal infections leads to increased antibiotic resistance. Additionally, broad spectrum antibiotics are typically employed, further exacerbating the antibiotic resistance crisis.

The ability to confirm the presence of bacteria or to characterize the bacterial species responsible for an infection before the administration of antibiotics begins may help reduce the rapid spread of antibiotic resistance.

In addition to genetically inherited resistance, bacteria can also show persistence in the presence of an antibiotic. Unlike resistance which is the result of genetic changes and is thus inheritable, persistence is a transient property of certain bacteria gained while in the presence of antibiotics. Bacterial persistence in populations exposed to antibiotics may be responsible for treatment failures, prolonged treatment regimes, and relapses following treatment [97]. In 2014 Elitas et al. utilized DEP to separate out live M.smeqmatensis bacteria from antibiotic-treated M.smeq populations as a measure of bacterial persistence against antibiotics.
Sanchis et al. proposed the use of DEP bacterial characterization to classify bacteria as either Gram-positive or Gram-negative. Distinguishing even between these two major types of bacteria would inform antibiotic selection, as most Gram-negative bacteria, with their outer wall structure, are resistant to penicillin [39].

The need for rapid, accessible tuberculosis diagnostics

According to the World Health Organization’s published 2018 Global Tuberculosis Report, tuberculosis (TB) is one of the top ten causes of death worldwide and is the leading cause of death from a single infectious agent [1]. Claiming 1.6 million lives in 2017, TB remains a serious threat to world health. Both the infection incidence rate and the case fatality ratio are highest amongst lower income nations. Globally, 16% of all cases of TB are fatal [1]. The existence of drug-resistant TB and extensively drug-resistant TB remains a health crisis. The World Health Organization also calls for the detection and treatment of latent TB infections as a preventative measure.

A 2017 WHO/World Bank report on universal health coverage found that less than 50% of people worldwide have access to essential health care. Thus any hope of overcoming the tuberculosis global health crisis must include increased access to TB diagnosis and treatment.

TB detection begins with collection of a sample of patient sputum that has been coughed up from the lungs. Sputum samples contain a mixture of pulmonary mucus and saliva. These samples are then either processed for molecular or genetic sensing or cultured between several days and up to one month [98,99]. The resultant bacterial growth can then be tested for speciation and antibiotic susceptibility.

Efforts to increase the detection and treatment success of TB (currently only 55%) require more available TB diagnostics testing in areas of low health care coverage, drug susceptibility testing, access to treatment, and new treatment regimes. Successful new TB diagnostics should be created appropriate to the target regions of use, namely areas of low income and low health care service coverage. Thus an ideal new TB diagnostic would require no expensive accessory equipment, be able to be operated without need of a cold-storage chain, be able to give rapid point of care results, and also provide information about
antibiotic resistance.

Access being a function of financial and personnel resources, we seek to lower the cost of TB detection and lower the specialization of personnel and equipment necessary for TB detection through multifunctional lab-on-chip devices. Dielectrophoresis-based devices may present a promising combination of those abilities as they are inexpensive to manufacture, can be operated in a portable system, can rapidly isolate and concentrate bacteria from fluid samples, and can be combined with other on-chip functions such as identification and drug susceptibility testing.

Work in this field has already begun offering promising indications of the viability of DEP-based TB point-of-care diagnostics. In the same genus and exhibiting similar cellular architecture and properties, Mycobacterium smegmatis is often used as a nonpathogenic substitute for M. tuberculosis in research testing [100]. In 2010 Zhu et al. demonstrated the ability to selectively differentiate between live, dead, and latent M. smegmatis populations [100]. Dormant M. tuberculosis cells have a thicker cell wall [101] and are smaller than their active counterparts [102], resulting in a differential DEP response between the cell subpopulations. Using an array of DEP carbon electrodes in 2014, Martinez-Duarte reported the ability to capture M. smegmatis cells that remained viable after treatment with isoniazid, a front-line TB treatment drug [103]. Thus with the ability to isolate latent cells and also provide information about drug susceptibility, DEP may be a promising component of new rapid POC TB diagnostics.

In this chapter we examine the use of DEP as a tunable bacterial concentrator for two major bacteria involved in global health conditions: Escherichia coli, a major cause of food and water-borne infections worldwide [104], and Mycobacterium smegmatis, a nonpathogenic relative of the tuberculosis-causing bacteria Mycobacterium tuberculosis. Each pathogenic bacteria of interest comes with a set of challenges for rapid DEP isolation. M. smegmatis must ultimately be isolated from sputum samples and E.coli should be separated from blood samples. Both of these sample types are highly conductive as well as highly viscous, creating challenges for the development of rapid bacterial isolation and concentration. To this end, we explore the use of DEP for simultaneous purification and concentration of bacteria from highly conductive and viscous fluid samples. Sample throughput is a big
concern for the clinical viability of DEP-based sample processing devices. In light of our findings on the decrease in efficiency with increasing DEP electrode array area (Chapter 4), we develop two novel DEP device designs to help overcome sample throughput limitations without increasing electrode array area.

6.2 Capture and concentration of \textit{M.smeqmati} from fluid samples

Isolation and concentration of \textit{M.smeqmati} bacteria from solution are critical functions in rapid detection and diagnostic processes. In order to inform the real-world detection of \textit{M.tuberculosi}, we first model the DEP frequency response of \textit{M.smeqmati} and then experimentally map the pDEP capture response in low conductivity fluids. In later sections we explore the ability to isolate these cells at high conductivity and in high viscosity fluids, as well as examine a method by which pDEP concentration may be accomplished from high-conductivity, viscous samples without sacrificing throughput.

6.2.1 Modeling \textit{M.smeqmati} DEP response

The bacteria responsible for TB infections, \textit{Mycobacterium tuberculosis}, are rod shaped, Gram-positive bacteria [105]. Though Gram-positive, they exhibit a more complex cell wall architecture than typical Gram-positive bacteria [106]. The central cell cytoplasm is surrounded by an inner plasma membrane. Exterior to the inner membrane is a cell wall consisting of a thick peptidoglycan layer encapsulating a limited periplasmic space. From the exterior of the peptidoglycan layer extends a lipid layer of mycolic fatty acid chains, giving the bacterium a waxy outer coating. This cell envelope may be partially responsible for the virulence of \textit{M.tuberculosi}, allowing the bacterium to avoid host cell antimicrobial efforts, prevent antigen presentation, and even confer increased antibiotic resistance [106]. Figure 6.1 shows a diagram of a typical Gram-positive bacteria.
In Zhu et al’s work, the effective conductivity of active, dead, and dormant cells was found to be 0.056, 0.095, and 0.0812S/m respectively and ascribed to differences in the state of the cell wall for each subpopulation. Suspended in a low conductivity solution (0.0002S/m), live cells were allowed to be captured by pDEP onto an interdigitated castellated array (30µm characteristic dimension) with a 20V_{pp} potential applied at 120kHz, under conditions of no fluid flow. Subsequent rinsing out of the dormant cells which were not as strongly captured enabled separation of the two populations. Dormant cells experienced pDEP at higher frequency (1MHz) in these conditions. Because of the dramatic difference in DEP response of the populations, they captured dead bacteria with pDEP at 80kHz at much higher conductivity (0.09S/m) from mixed populations of dead and dormant bacteria [100].

In order to provide insight about the expected DEP response of M.smegmatis, we employed a two-shell oblate spheroid model to map the CM factor for varied frequencies and media conductivities. The interior layer is the cell cytoplasm. The first shell is the inner plasma membrane and the second shell is a combined 'cell wall' consisting of the periplasmic space and the encompassing peptidoglycan layer. This model ignores the presence of free lipids projecting outward from the peptidoglycan layer. The CM factor is plotted for a range of solution conductivities and frequencies in Figure 6.2.
Table 6.1: Values used to model CM factor for *M. smegmatis* as a 2-shell spheroid [105]

<table>
<thead>
<tr>
<th>Shell layer</th>
<th>Long axis radius (um)</th>
<th>Short axis radius (um)</th>
<th>Conductivity (S/m)</th>
<th>Permittivity</th>
<th>Layer thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytoplasm</td>
<td>0.99</td>
<td>0.3</td>
<td>0.3</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>membrane</td>
<td>0.997</td>
<td>0.307</td>
<td>$5.5 \times 10^{-6}$</td>
<td>5.65</td>
<td>7</td>
</tr>
<tr>
<td>wall</td>
<td>1.037</td>
<td>0.347</td>
<td>$1.33 \times 10^{-4}$</td>
<td>13</td>
<td>40</td>
</tr>
</tbody>
</table>
Figure 6.2: Numerical DEP response model of *M. smegmatis* as a two-shelled oblate spheroid for a) a wide range of conductivities between 0.0001 and 1S/m and b) a narrowed range of conductivities between 0.01 and 0.1S/m to show the predicted conductivity at which pDEP becomes possible.
The broadness of the pDEP peaks, especially at lower conductivity, suggest that particle separation by frequency specificity would be challenging. In such cases, a system which could isolate particles at very low force magnitudes would benefit from operating at a conductivity between 0.01 and 0.07 S/m where there is a narrow window in frequency space at which these cells will experience positive capture forces. The absolute values may be shifted due to the approximation of the complex cell wall architecture as a single layer. It is desirable, therefore to experimentally examine the CM factor of M.sme 

### 6.2.2 Experimentally mapping M.sme frequency response

Here we empirically examine the pDEP capture response of M.sme to find the maximum conductivity at which pDEP concentration can be achieved for several solution conductivities. Additionally, the broadness of the capture peak in frequency space will inform us about the specificity of particle separation available for use later on.

* M.sme samples were obtained from our collaborators at QuantuMDx Group Ltd (Newcastle Upon Tyne, UK), and cultured on agar plates. Plates were made with Middlebrook 7H11 agar (BD Biosciences), reconstituted with DI containing 0.5% glycerol, sterilized by autoclave at 120°C for 15 minutes, supplemented with 10% Oleic Albumin Dextrose Complex and 20 µg/mL Kanamycin once cooled, poured into culture dishes, and allowed to solidify at room temperature. Plates were then sealed and stored upside down at 4°C until use. Bacteria was introduced to the plate by streaking a sample with a sterilized culture loop over a warmed agar plate. Plates were covered and inverted and bacteria allowed to grow at 30°C.

Before experiments, bacteria were collected from the culture plate and suspended in 1xPBS. The kanamycin used in the plates conferred selection for a plasmid containing green fluorescence protein (GFP), but the produced native fluorescence intensity was sometimes insufficient for optical imaging and so additional fluorescence staining was used for optical visualization of bacterial cells. Bacteria were fluorescently labeled with BacLight™ red bacterial stain (B-35001, Life Technologies) at 6 µL of 100 µM working solution per mL of cell suspension. After a 1 hour incubation, cells were washed by centrifugation and...
resuspended into the desired final buffer solution.

Bacterial suspension samples were introduced over a DEP capture device at 1µL/min through a microfluidic channel 42µm high by 1mm wide, corresponding to an average linear flow speed of 397µm/sec. DEP capture structures were interdigitated electrodes aligned perpendicular to the direction of fluid flow, with a characteristic dimension of 25µm. The electrodes were patterned in 285nm of gold with a 15nm titanium adhesion layer, and coated with 200nm of PECVD SiO₂.

Capture measurements were performed under continual flow. For each frequency to be measured, the DEP signal was turned on and pDEP bacteria capture allowed to proceed for 20 seconds. At the end of the capture period, a fluorescence image was acquired showing the captured bacteria on the DEP array. After capture the flow rate was increased to 70µL/min to release any captured cells from the array before the next capture and then stabilized again at 1µL/min before the next capture signal was applied. Measurements were taken for solutions of 0.001x, 0.01x, and 0.1x PBS for a frequency range of 0.1 to 10MHz. Fluorescence images of the DEP capture array were taken before and after each capture period and before and after each elevated flow release step to allow for analysis of particle capture as well as particle sticking.

An image processing program was written to locate and count individual captured bacteria on the DEP array. Figure 6.3 shows example images taken just before and just after a capture voltage is applied for 20 seconds. In this example, *M.smegmatis* bacteria (seen as red dots) are in 0.001xPBS and subjected to an AC signal of 5V at 126kHz. On the right of each full image is a zoomed in region showing more clearly the bacteria (red dots) and the indication of the automated particle detection of each bacteria (red circles).
Figure 6.3: Fluorescent images of *M. smegmatis* over an interdigitated DEP electrode array a) just before and b) after application of a DEP capture signal (5V, 126kHz, 20sec, 397μm/sec average linear flow speed). Bacteria show up as small red dots and a red circle indicates the automated detection of a particle.

The amount of captured particles was used as a metric for quantifying the relative DEP force. Capture counts were defined as the number of bacteria present on the electrode array after DEP capture minus the number present right before the DEP signal was turned on. Additionally a measurement of sticking was defined as the percent of bacteria captured in each step that remain on the device after the elevated flow release step. Results of the number of captured bacteria for a range of frequencies and 3 different conductivities are shown in Figure 6.4. Generally, the magnitude of the positive DEP force decreases with
higher solution conductivities. Even with twice the applied voltage, the pDEP capture rate for 0.1xPBS remains zero, suggesting that \textit{M. smegmatis} experiences only negative DEP at 0.1xPBS for this frequency range.

The two-shell oblate spheroid model used to calculate the predicted relative CM factor (shown previously in Figure 6.2) was evaluated at conductivities matching those of the solutions used experimentally. The predicted CM factors for these solutions are shown in Figure 6.5. The predictions for the positive CM factor range align very well with the pDEP capture response. The model also illuminates the nDEP regime, which cannot be gleaned by our pDEP capture study. From the model we can see that the absolute magnitude of the CM factor is greater for the nDEP regime at high conductivity than even the maximum pDEP response at low conductivity. This suggests a device design utilizing nDEP rather than pDEP for \textit{M. smegmatis} manipulation would be more efficient.

The frequency at which maximum pDEP capture occurs can be seen more clearly when the experimental capture curves and predicted CM curves are normalized to the maximum capture for each dataset (experimental or predicted), shown in Figure 6.6. From this plot we can see good agreement between the model prediction and the experimental results. Additionally, we can see that the frequency of maximum capture shifts towards higher frequency with higher solution conductivity, as predicted by our model. The maximum capture occurs at about 1.26MHz for 0.001x PBS (0.0019S/m) and at about 15.8MHz 0.01x PBS (0.019S/m). The experimental capture data shows a slightly wider spread than the predicted CM model. This may be due to cell population heterogeneity.
Figure 6.4: Experimental map of positive DEP capture response for \textit{M.smegmatis} (Error bars are the standard error of the mean of repeat capture measurements)

Figure 6.5: Calculated positive DEP CM map for \textit{M.smegmatis} for conductivities corresponding to experimental data shown in Figure 6.4
From this study of pDEP frequency response in varied solution conductivities, we see very good experimental agreement with the numerical prediction of *M. smegmatis* pDEP capture conditions. At at least 0.19S/m (and above) there is no expected pDEP forces on the bacterial cells. Clinical sputum samples have a higher conductivity than this and so it is expected that pDEP capture of *M. smegmatis* could only be accomplished by first lowering the sample conductivity. The typical method to lower the conductivity is to dilute a sample with a low conductivity fluid, such as deionized water. This comes with a negative tradeoff in throughput as every dilution proportionally increases the volume of fluid to be processed. However, given the high viscosity of sputum (about 12Pa·s), sample dilution may be necessary for processing, regardless. Next we endeavor to capture target bacteria from more realistically complex samples containing multiple cell types. In later sections we explore ways to separate particles in both high conductivity samples and high viscosity samples without sacrificing throughput.
6.3 Simultaneous separation and concentration of \textit{E.coli} from blood cells

Beyond capture of bacterial cells from a solution, clinically relevant detection requires isolation of bacterial cells from other cell types present in the clinical sample, such as epithelial cells in a sputum sample or blood cells in a blood sample. Here we employ DEP to selectively isolate bacterial cells from samples containing a mixture of bacterial and non-bacterial cells. Because of its importance to global health, we utilize \textit{E.coli} in these isolation experiments.

6.3.1 Motivation for \textit{E.coli} manipulation

Because clinical samples for \textit{E.coli} are often taken as blood samples, we examine the possibility of using DEP to selectively isolate and concentrate \textit{E.coli} from RBCs. Previous work in this arena has shown very promising results. Notably, D’Amico et al. report bacterial isolation from blood samples spiked with 1000 CFUs per 200ul of blood \cite{9}. Utilizing an upstream membraneless dialysis system, they were able to decrease the sample solution conductivity 100x in order to operate within the pDEP regime, while not as significantly expanding the volume of solution. Kuczenski et al. also separate \textit{E.coli} from blood, with volumetric throughput of 0.58\mu L/min.

6.3.2 Modeling \textit{E.coli} and RBC DEP response

To gain an understanding of the relationship between the DEP response of \textit{E.coli} and RBCs, we first simulate the CM factor for both particles. \textit{E.coli} bacteria are rod-shaped cells with a conductive cytoplasmic interior surrounded by a plasma membrane and a cell wall. External to the cell wall is a cell capsule composed of long-chain polysaccharides \cite{36}. The cell wall consists of an outer membrane enclosing a periplasmic space that itself contains a peptidoglycan layer, as diagrammed in Figure 6.7 \cite{107,108}. Additionally extending from the capsule are long structures called pili, fimbriae, and a flagella which function to exchange genetic material, adhere bacteria to surfaces, or facilitate movement. Such a complex cell architecture presents a considerable challenge to accurately predicting the cell DEP response. We therefore adopt a 3-layer oblate spheroid model for \textit{E.coli}, consisting
of the interior cytoplasm, the plasma membrane, and the cell wall. Though the wall consists of multiple components, we use approximated values from literature to condense the periplasmic space, peptidoglycan layer, and the outer membrane elements into one layer (shown in Table 6.2).

![Diagram of a gram-negative bacterial structure showing a peptidoglycan layer (PG) in the periplasmic space and lipopolysaccharides extending out from the cell surface](image)

**Figure 6.7:** Diagram of a gram-negative bacterial structure showing a peptidoglycan layer (PG) in the periplasmic space and lipopolysaccharides extending out from the cell surface (Adapted with permission from Ref [107] T.J.Silhavy, D.Kahne, and S.Walker, “The Bacterial Cell Envelope”, Copyright 2010, Cold Spring Harbor Perspectives in Biology)

<table>
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<th>Shell layer</th>
<th>Long axis radius (um)</th>
<th>Short axis radius (um)</th>
<th>Conductivity (S/m)</th>
<th>Permittivity</th>
<th>Layer thickness (nm)</th>
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Table 6.2: Values used to model CM factor for *E.coli* as a 2-shell oblate spheroid [35]

The other cells in our sample of interest are red blood cells (RBCs). RBCs have a much simpler set of layers, consisting of an interior cytosol surrounded by a plasma membrane. They do, however, have a complex biconcave shape. To model this, we employed both a 1-shell spherical model as set up in [31] as well as a 1-shell oblate spheroid as set up in Gagnon et al for comparison [32].
Table 6.3: Values used to model CM factor for RBCs as a 1-shell sphere [109]

<table>
<thead>
<tr>
<th>Shell layer</th>
<th>Long axis radius (um)</th>
<th>Short axis radius (um)</th>
<th>Conductivity (S/m)</th>
<th>Permittivity</th>
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<td>-</td>
<td>$1 \times 10^{-6}$</td>
<td>4.44</td>
<td>9</td>
</tr>
</tbody>
</table>

The values for cell radius here for the 1-shell spherical model are smaller than the large radius of an RBC. Since this model assumes a spherical particle, the radius is chosen to approximate the volume of an RBC, were it spherical. An RBC has a volume between 80 and 96 fL which corresponds to a sphere with a radius between 2.67 and 2.84 µm. The radius used is slightly smaller in keeping with the model presented by Valero et al [109].

Figure 6.8a and Figure 6.8b show the simulated CM maps for *E.coli* and RBCs, respectively, for the same set of environmental conditions.
Comparing the expected DEP response behavior of these two cell types, we see the
major difference occurs at low frequencies (below about 300kHz) for mid-range solution conductivities (between 0.001 and 0.1 S/m). In this regime, \textit{E.coli} tends to have a higher CM factor than RBCs. One ideal approach to separate two particle types would be to operate under conditions in which one particle experiences a capture force while the other experiences either very low force or force in the opposing direction. \textit{E.coli} and RBCs are predicted to fall into this situation for low conductivities, but only at low frequencies below about 100kHz. At these low frequencies, electroosmosis forces can begin to dominate over DEP forces. To prevent both cell and electrode damage and to avoid confounding electroosmotic effects, it is preferable to operate at higher frequencies.

6.3.3 Isolation of \textit{E.coli} from RBCs

From the simulations we see that the expected CM factor for both cells align very closely at high frequencies (above 1MHz), making separation by differential DEP force direction a challenge. We therefore take advantage, not only of slight differences in CM factors between two cell types, but of the difference in their sizes. When a particle experiences pDEP force, it is pulled towards the area of highest field gradient, which in our case are the electrode edges. For cells with a diameter much smaller than the characteristic dimension of the electrodes, when drawn to one electrode edge, the force on the particle from a neighboring electrode is very small compared with the force at the capture electrode. As a cell size approaches the characteristic dimension of the electrode array, however, the force from a neighboring electrode on a captured cell is a much greater percent of the capture force. Thus we propose that the net capture force on a particle can depend on the relationship between the particle size and the electrode array dimensions. We propose this effect to be in addition to contributions of the electric field distribution expected by smaller distances between electrodes and those of larger DEP forces expected from larger particle volumes and also larger drag forces being experienced by larger particles. Thus we chose a characteristic electrode dimension of 25µm for the capture of \textit{E.coli} (\sim 2μm in length) in the presence of RBCs (\sim 7μm in diameter).

Informed by these simulations and seeking to use pDEP to capture \textit{E.coli} and also to use high conductivity solution which would be closer to physiological sample conductivity,
we chose to operate in 0.1xPBS (with a measured conductivity of 0.18S/m). The simulated CM factor for both particles at this conductivity (plotted in Figure 6.9) show an expected maximum positive CM factor for *E.coli* at about 10MHz in this solution. While the RBC CM factor is also positive at these conditions and indeed even greater in magnitude, we rely on the theoretical reduction of DEP force for large ratios of cell diameter to electrode dimensions to selectively capture the *E.coli* cells without capturing the much larger red blood cells.

![Figure 6.9: Simulated CM factor for *E.coli* and RBCs in a solution of 0.1xPBS with a conductivity of 0.18µm](image)

In order to test this selective capture, we fluorescently label both cell types for imaging to determine performance evaluation. After staining, cells were resuspended into 0.1xPBS (with a measured conductivity of 0.18S/m) and loaded into a syringe pump. Sample was introduced to a DEP electrode array and flowed at 0.02µL/min. Figure 6.10a shows the interdigitated circular electrode array used in this experiment. In the image the light areas are the metal electrodes and the dark areas are the gaps between electrodes. The radius distance between electrodes as well as the radial width of each electrode is 25µm. An AC
voltage was applied to each set of interdigitated electrodes, 180° out of phase to each other to maximize the voltage delivered to the electrode surface. Under continuous flow, the frequency space was scanned from $1 \times 10^5 \text{Hz}$ to $1.1 \times 10^8 \text{Hz}$. Fluorescence images were acquired for each frequency. The RBC fluorescence stain is visible as an orange color under the GFP filter used to visualize E.coli. Because of our slow flow speeds, we captured long exposure images in order to be able to clearly show the difference between cells that are flowing slowly by the electrodes and cells that are captured.

Figure 6.10b shows both cell types flowing by when there is no voltage applied to the electrodes. In the absence of any DEP force, the RBCs (appearing as elongated orange particles) and E.coli (appearing as fine green lines) passed the electrode array unhindered during the long image exposure. When a signal of $100kHz$ is applied (c) both cell types can be seen captured in a pattern over the center of the electrode indicative of electroosmosis dominant capture. At $1MHz$ E.coli are captured by pDEP while RBCs still experience electroosmosis capture (d). At the predicted peak pDEP frequency for these conditions, $10MHz$, E.coli are firmly captured with pDEP while RBCs do not experience enough net force to overcome their drag force in the fluid flow (e). By $100MHz$ neither cell type experiences a capture force sufficient to overcome fluid drag (f). The simulation predicts that both particles experience negative DEP at this frequency. From this capture data, it cannot be determined whether the cells experience nDEP or just insufficient pDEP to be captured. The predicted maximum pDEP frequency aligns very well with the E.coli capture seen experimentally with both occurring at $10MHz$. 
Figure 6.10: Selective capture of E.coli in the presence of RBCs. a) A circular interdigitated electrode array is used to manipulate the two cell types in mixed cell sample (electrode metal appears light while gaps between electrodes appears dark) b) When no AC signal is applied to the electrodes E.coli (small green particles) and RBCs (larger orange particles) flow past the electrode array unhindered. DEP signals of c) 0.1MHz, d) 1MHz, e) 10MHz, and f) 100MHz can be seen to differentially affect each particle population. At the predicted peak pDEP capture frequency of 10MHz RBCs are not captured while E.coli are selectively captured and concentrated (5V, 0.1xPBS).
In this experiment selection of one particle over another is a binary function: either a target particle is captured or it is not captured. For any given flow conditions, a particle will require some threshold force to overcome the drag force to be stopped by a DEP electrode array.

If two particles both experience the same polarity of DEP force (pDEP vs nDEP), however, separation must rely on the difference in force magnitudes rather than force direction. According to the DEP force equation (2.1), the force magnitude on a particle scales with the CM factor and the particle volume. For similar CM factors, larger particles should always experience more DEP force than smaller particles. The threshold force for capturing a particle from flow would be higher for large particles as well as the drag force is larger, but only scaling with particle diameter (5.12). Thus it would be expected that a larger particle would experience a greater net force scaling with the square of its diameter.

As illustrated in this experiment, however, the small particles experienced a net DEP force greater than the threshold force necessary for their capture, while the much larger RBCs did not. One possible explanation for this is our proposed theory that the larger the ratio of particle diameter to electrode dimension, the smaller the net DEP force on that particle as it will constantly feel non-negligible DEP forces in opposing directions at every position along the electrode array.

6.4 Continuous particle isolation by lateral movement

6.4.1 Theory of DEP lateral movement

Because of the binary nature of the selection methodology present for E.coli from RBCs, forces greater than a threshold capture force are necessary to isolate one cell type from another. Clinically relevant sample fluids generally have high viscosities and high conductivities, creating situations in which the threshold capture force is increased because of increased drag force with viscosity and the ability to reach that threshold becomes limited because of solution heating. Additionally the threshold force becomes more difficult to reach as the CM factor magnitude generally becomes smaller with higher conductivity solutions.

In highly conductive physiological solutions, cells often are only able to experience nDEP.
Because nDEP forces push cells away from the electrode edges and up towards the channel ceiling with DEP force decreasing rapidly with distance. Trapping cells with nDEP requires either higher operating voltages capable of arresting cell movement near the channel ceiling, lower channel heights, or lower flow rates. Operating at high voltages in highly conductive solutions is problematic, as discussed previously, due to heating and bubble formation, and lowering channel heights or flow rates come with negative impacts on device throughput.

In order to enable particle isolation with nDEP in high conductivities, we utilize electrodes that are angled with respect to the fluid flow direction. For IDE arrays with electrodes perpendicular to the fluid flow, as shown previously, nDEP forces pushing particles away from the electrode, oriented perpendicularly to the electrode edges. Thus the net force in this case is oriented along the axis of fluid flow. If, however, the electrodes are angled with respect to the fluid flow, there enters an x-component of the net force which propels particles toward the side of the channel. Using angled electrodes, Sea et al. have shown DEP-induced lateral separation of blood cells [30] and Hoettges et al. have demonstrated EO-induced lateral movement of nanoparticles [110]. While capture requires a DEP force equal to or greater than the fluid drag force, lateral movement requires a DEP force only to have some force component perpendicular to the fluid flow direction. The magnitude of force required to achieve operational success depends, then, on the fluid flow speed, not because of the need to counteract that flow, but because the flow speed will limit the response time available for particle movement.

Here we utilize a method for selectively isolating particle populations from samples of mixed particles that requires a lower threshold for DEP force than retention-based separation methods. Instead of isolating particles by capture, here we design and test devices to isolate particles by lateral movement.

For device fabrication, interdigitated electrode arrays are patterned at an angle to the direction of fluid flow rather than perpendicular to flow. The shift to lateral movement devices only requires modifications to the electrode design, not the fabrication process, thus devices were fabricated in the same manner as those of previously discussed DEP devices.
6.4.2 pDEP Lateral movement

Lateral movement devices were tested to see if they could successfully move particles from one side of the channel to the other using pDEP force. For this test, *E. coli* were fluorescently stained with BacLight™ red bacterial stain and suspended in 0.01xPBS was introduced to the DEP array. For this test, no fluidic focusing was done. An AC signal at 10MHz was applied to produce a pDEP response from the *E. coli*. (*E. coli* was shown previously to experience pDEP at 10MHz in 0.1xPBS Figure 6.10e) and, from the simulations, is expected to experience only greater magnitude of pDEP force as the conductivity is decreased from 0.1xPBS to 0.01xPBS (Figure 6.8a). Videos of cell movement at various flow rates and voltages were captured. A particle tracking and analysis program was written to detect every cell in each video frame and track each cell from frame to frame. From these tracks, measurements of position and velocity can be obtained for each particle and population statistics computed.

An example of such particle movement tracks is shown in Figure 6.11. Here *E. coli* were subjected to 7µL/min volumetric flow rate and 0.8V before amplification, yielding about 8V V on each of the two electrode circuits, 180° out of phase with each other. The tracks of 43 bacterial cells are shown overlaid onto an image of the approximate position of the electrodes, showing that the particles are located on the electrode edges and are moving along the edges over time. The average velocity of non-stuck cells is 4.5321µm/sec in the x direction and 4.9433µm/sec in the y direction. Here, stuck particles are defined as particles whose average x velocity and average y velocity are less than 0.25µm/sec. The slight difference in the x and y velocities appears due to a slight rotation in the device.
Figure 6.11: Lateral movement tracks of 32 nonstuck \textit{E.coli} experiencing pDEP on angled IDE electrodes in 0.01xPBS. Each color is an individual particle with each data point being the particle position in each video frame over time. Particles move along the electrode edges.

After obtaining the successful lateral movement of cells within a microfluidic channel in low conductivity, we then seek to accomplish lateral movement in higher conductivities, in which cells usually experience negative DEP forces rather than positive DEP forces.

6.4.3 nDEP lateral movement in high conductivities

Positive DEP is effective for particle capture in low conductivity solutions, but the capture force decays rapidly with increased conductivity. Low conductivity solutions osmotically stress living cells and require volumetric dilution of physiologic samples, thus limiting the use of pDEP for manipulation of living cells [49]. Negative DEP force, however, is greater in high conductivity solutions and presents a more attractive method for cellular manipulation in physiologic samples. In high conductivities, electrothermal flow and electroosmosis effects become strong, and often overcome the DEP force on particles [49], especially at low
frequencies. Thus it is beneficial to operate at higher frequencies and to utilize as low a voltage as possible for DEP manipulation in high conductivities to avoid solution heating and device and sample damage.

By requiring lower voltages, particle isolation by lateral movement provides a promising alternative to binary DEP capture, which requires higher voltages, especially when operating in the nDEP regime (as is typically the case at high conductivities). Here we attempt to use negative DEP to laterally move particles from one side of the channel to the other.

For this experiment, we utilize fluorescent polystyrene beads (1.75µm diameter, Polysciences, Inc. 178675) suspended in 1xPBS with 1% Pluronic F-127 added to prevent particle adhesion. Sample was introduced by syringe pump over the lateral movement DEP array at a flow rate of 0.2µL/min. The microfluidic channel was 1mm wide by 17.1µm high, yielding an average linear flow speed of 195µm/sec. The DEP electrodes had a characteristic dimension of 60µm, angled at 45° to the direction of fluid flow (shown in Figure 6.12a). Two AC signals 180° out of phase were applied to the two electrode circuits. The frequency was kept constant at 20MHz and the voltage was swept from 0.3 to 8V prior to signal amplification which further increased the voltage by about a factor of 5, rolling off at higher voltages. For each applied voltage, videos were taken of the particle response over the electrodes.

A MATLAB program was written to analyze the videos, first by tracking particles through each video. Figures 6.12b-h show the particle traces for all beads in videos taken with 3-8V applied prior to amplification. From these traces at the lower voltage (3V, Figure 6.12b), particles can be seen proceeding down the channel without any lateral displacement in the x direction. As the voltage is increased from 3V to 7.5V, particles traverse laterally more and more. Above 7.5V there is actually a decay in the horizontal motion. At this voltage, in such high conductivity, electrolysis began to occur, generating bubbles most likely altering the fluid flow rate.

Then the ratio of the velocity in the x direction (horizontally across the channel) and the particle’s velocity in the y direction (vertically with the fluid flow) was calculated for
each particle trace. The average velocity ratio \( r \) for each condition was defined as

\[
r = \frac{1}{N} \sum_{i}^{N} \frac{v_{x,i}}{v_{y,i}}
\]

where \( N \) is the number of particles tracked in each video, \( v_x \) and \( v_y \) are the fitted particle velocities in the \( x \) and \( y \) directions, respectively. Particles that are stuck on the device are excluded from this calculation. This velocity ratio is plotted for each voltage in Figure 6.13. The error bars are the standard error of the calculated mean. This data shows quantitatively what we see in the particle traces, that the lateral movement of particles increases with increasing voltage up until 7.5V.
Figure 6.12: Particle trajectories showing nDEP-induced lateral particle movement increases with voltage. Polystyrene beads traveling over an angled DEP electrode array (a) show almost no lateral movement with an applied DEP signal of 3V (b) but as the voltage is increased to 4.5, 5.0, 6.5, 7.0, and 7.5 V (c, d, e, f, and g) respectively, the lateral movement becomes more pronounced. At 8.0V solution heating generates bubbles, decreasing lateral motion. Voltages listed here are the values prior to amplification with an approximate 10x gain.
Figure 6.13: The velocity ratio of lateral velocity over velocity in the direction of fluid flow for each particle increases with voltage until 7.5 V. At 8 V bubble generation negatively affects lateral motion. Data points are the population average of many individual particle velocity ratios. Error bars are the SEM.

We have previously shown the ability to selectively separate a cell type from a population of mixed cells (E. coli from RBCs). Here we have demonstrated the ability to laterally move particles using both pDEP and nDEP forces on the same device architecture and shown the effect of voltage on lateral movement. Furthermore, we have demonstrated the ability to manipulate particle position in fully conductive solutions by taking advantage of the lower operating voltages necessary for lateral movement with an angled DEP electrode structure. We successfully achieved lateral movement at full conductivity at voltages lower than the voltage at which solution heating becomes an issue. As an alternative to the binary DEP capture based separation, in the next section we will explore DEP-induced lateral particle motion for particle separation.
6.5 Continuous lateral particle sorting

A slight change in the microfluidic channel design allows for an angled DEP electrode array to be used as a continuous lateral particle separator. To create a separation function, a second microfluidic inlet and outlet are added. The function of the second inlet is to initially focus particles over one side of the channel so that they will be moved to the other side of the channel only if subjected to sufficient DEP force. The second outlet serves to physically collect any particles that have been successfully moved laterally in the channel by DEP. The basic design of these lateral movement devices is shown in 6.14.

![Diagram of particle sorting by differential lateral DEP force on two particle types. Not to scale.]

In order to test the ability to separate different particle types with DEP-induced lateral movement from angled electrode arrays, we introduce two populations of polystyrene beads: small green fluorescent beads that are 1.75µm in diameter (Polysciences) and larger red fluorescent beads 6µm in diameter (Spherotech). By using two particles of the same material we can illuminate the separation ability of the device simply in terms of DEP force magnitude without any uncertainty about additional factors of each particle’s CM factor. The device channel was passivated to prevent particle-device adhesion by incubating overnight with a solution of 2% Tween-20 in 1xPBS. Beads were suspended in a solution of 0.0001xPBS
supplemented with 1% BSA and 1% Tween-20 to help prevent bead aggregation and bead-device adhesion. Beads in solution were pumped by syringe into the left-hand microfluidic inlet at 0.2µL/min while the same solution without beads was pumped into the right-hand inlet at 0.8µL/min. With the channel dimensions of 1mm wide by 18.8µm high, this yields an average flow speed of 887µm/sec.

The interdigitated angled electrode array had a characteristic dimension of 25µm with a shallow angle of 60° relative to the fluid flow direction. Electrodes were fabricated in 285nm of gold with a 15nm titanium adhesion layer on top of 2µm of insulating thermal SiO₂ on a silicon substrate. No SiO₂ was deposited on top of the DEP electrodes for this device. Two 0.5V 10MHz AC signals 180° out of phase with each other were amplified by approximately 10x and applied to the two electrode circuits.

Videos were captured of the particles over the device with the DEP signal off (Figure 6.15), DEP signal on (Figure 6.16), and immediately after the signal was turned off again (Figure 6.17). In part (a) of each of these figures, a cumulative image of all of the frames of the video shows the trajectories of the two particle types (green and orange) over the DEP electrode device. Dashed lines on the images indicate the position of the microfluidic channel walls. For each frame of the video, the intensity of each particle type was extracted and cumulated for every position across the width of the channel. This intensity profile was normalized to the maximum intensity for each particle and plotted against the position in the channel (part (b) of each figure).

When there is no DEP signal applied (Figure 6.15) both particles flow down the far left side of the channel, focused there by the faster flow rate of the right-hand buffer inlet stream. Particles show no lateral movement. When the DEP signal is turned on, the larger particles (appearing orange), immediately begin to move laterally along the electrodes all the way over to the right-hand side of the channel. The smaller beads (appearing green) do not show a shift in x position. Turning the DEP signal off again immediately releases all large beads from the right side of the electrodes as well as any beads that were midway through transit along the electrodes. This total release of particles is vital for the ability to physically collect the separated particle population from the device outlet.
Figure 6.15: Particle positions during DEP signal off. a) A cumulated image of the trajectories of two bead populations (1.75\(\mu\)m green beads and 6\(\mu\)m orange beads) over an angled DEP electrode array shows particles experiencing no lateral movement. b) The positional intensity distribution of each particle population across the width of the channel, normalized to the max intensity for each population. Dashed lines on each figure indicate the position of the fluidic channel edges.
Figure 6.16: Particle positions during DEP signal applied to the electrodes. a) A cumulated image of the trajectories of two bead populations (1.75µm green beads and 6µm orange beads) over an angled DEP electrode array shows the smaller particles experiencing no lateral movement, while the larger particles all move laterally into the right side of the channel. b) The positional intensity distribution of each particle population across the width of the channel, normalized to the max intensity for each population. Dashed lines on each figure indicate the position of the fluidic channel edges.
Figure 6.17: Particle positions immediately after the DEP signal is turned off. a) A cumulated image of the trajectories of two bead populations (1.75µm green beads and 6µm orange beads) over an angled DEP electrode array shows the small particles experiencing no lateral movement while the large particles are all released from their positions on the electrode array, allowing collection from the device outlet. b) The positional intensity distribution of each particle population across the width of the channel, normalized to the max intensity for each population. Dashed lines on each figure indicate the position of the fluidic channel edges.

This demonstration shows successful separation of particle populations from a mixed sample by DEP-induced lateral motion with the ability to gather both purified populations from device fluidic outlets. A video of this demonstration of lateral separation of beads is
in the Supplemental materials (S6-5_video_demonstrating_lateral_separation_of_beads.mp4). Here the DEP signal is off at the start of the video, then is switched on and separation is allowed to continue for several seconds. Then the DEP signal is turned back off, showing successful particle release from the electrode array. Toward the goal of continuous label-free electronic isolation of cells from samples of high conductivity and high viscosity, we next examine the effect of viscosity on particle separation in high conductivities.

6.6 Examining DEP separation efficiency in viscous samples

Because the lateral motion is a balance between DEP forces and drag forces and because the drag force is directly proportional to the solution viscosity, we expect that lateral motion in high viscosity fluids would require more DEP force or slower flow rates than in low viscosity environments. In an attempt to study viscosities affect on lateral movement as well as to examine its effect on particle separation, we perform particle separation experiments similar to the one described above, but alter the solution conductivity and viscosity of the suspending mediums to approach more physiologically relevant solutions.

Voltage scans of particle separation were performed in four different suspending fluids. The conductivity of the media was carefully controlled to be either low (0.0017S/m) or high (1.2S/m). The viscosities were either low, medium, or high. The value of the fluid viscosity was not measured, but only qualitatively defined by monitoring bubble rise times in each. Each fluid contained 1% BSA and 1% Tween-20 to prevent particle adhesion. In Chapter 5 high density media containing Ficoll and sucrose was used to successfully reduce issues of large particle settling. In order to evaluate the viability of this high density media in lateral cell separation devices, we included this media in the separation tests. Table 6.4 shows the components of all four solutions used. PBS forms the base of two low viscosity solutions. Lympholyte® (Cedarlane), a dense cell separation media, confers a mid-level viscosity to solution 3. Ficoll-400 (Dot Scientific Inc.) and sucrose in a base of T-cell media (TCM) provide a high viscosity fluid for solution 4. DI or PBS was used to adjust the conductivity of each solution as needed to make all three high conductivity fluids have equal conductivities of 1.2S/m. T-cell media is composed of RPMI cell media
supplemented with 10% heat-inactivated FBS, 10mM Hepes, 1% non-essential amino-acids, 2mM L-glutamine, 1mM sodium pyruvate, 0.05mM β-mercaptoethanol, and antibiotics.

Table 6.4: Components of four solutions used for particle separation testing

<table>
<thead>
<tr>
<th>Solution base</th>
<th>Measured conductivity (S/m)</th>
<th>Qualitative viscosity</th>
<th>Additives to alter viscosity</th>
<th>Additives to prevent adhesion</th>
<th>Additives to adjust conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001xPBS</td>
<td>0.0017</td>
<td>low</td>
<td>-</td>
<td>1% BSA</td>
<td>-</td>
</tr>
<tr>
<td>1xPBS</td>
<td>1.206</td>
<td>low</td>
<td>-</td>
<td>1% BSA</td>
<td>1% Tween-20</td>
</tr>
<tr>
<td>Lympholyte</td>
<td>1.202</td>
<td>medium</td>
<td>-</td>
<td>1% BSA</td>
<td>1% Tween-20</td>
</tr>
<tr>
<td>TCM</td>
<td>1.207</td>
<td>high</td>
<td>8.5% sucrose</td>
<td>1% BSA</td>
<td>1% Tween-20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30% Ficoll</td>
<td></td>
<td>PBS</td>
</tr>
</tbody>
</table>

The experimental setup was the same as used for the bead separation testing outlined in the previous section. Briefly, two polystyrene bead populations (1.75µm and 6µm) were added to each of the four suspending medias and introduced over an interdigitated DEP electrode array, angled at 45°, and with a characteristic dimension of 25µm. The electrodes did not have any SiO\textsubscript{2} deposited on their surfaces. The flow rates for the left-hand particle inlet and the right-hand blank solution inlet were 0.2µL/min and 0.4µL/min respectively, yielding an average flow speed in the 1mm x 18.8µm channel of 532µm/sec. Two AC signals at 10MHz, 180° out of phase with each other were amplified by about 10x (Tabor Electronics 9250). Datasets were acquired for a range of voltages for each tested media sample. The voltage range for each sample was chosen to encompass a regime in which the voltage was too low to cause lateral movement as well as the regime in which the voltage was too high to allow lateral particle movement because particles were just being captured on the electrodes or a voltage at which heating caused solution bubbling to occur.

Data acquisition was done with fluorescent imaging. Before the DEP signal was turned on, a reference image was acquired. Once the DEP signal was turned on, 100 images were captured with a frequency of 1 image per second. Immediately after 100 images were acquired the DEP signal was removed and an additional 50 images taken at the same frequency. Imaging was done just downstream of the end of the DEP electrode array so that particle positions would reflect their ultimate position without their process of movement bearing any contribution on the measurement of separation efficiency. For instance particles
that move slowly should not be weighted more highly than particles moving quickly as would occur if image intensity extraction was used on images of the angled device area. Additionally particles often get captured at the end of the electrodes near the channel wall and would disproportionately contribute to a measurement of particle position if remaining at the wall during multiple images. The 50 images taken just after the DEP signal is turned off serve to monitor the amount of particles that were reversibly captured. For this separator design it is ideal to physically isolate the purified sample from the microfluidic outlet for further examination. Any irreversibly captured particles would not arrive at the outlet and so we did not allow their numbers to contribute to the separation efficiency metric obtained through optical imaging either.

Using the reference image to get the exact channel position for the images, the intensity of each particle population for every position across the channel width was extracted. The entire width of the first 100 images was used in this calculation. The right half of the 50 images taken after signal removal were also included to measure the particles that had reversibly captured on the array. The left half of these images were ignored because, in the absence of DEP signal, that would just contain a mix of the two particle populations from the inlet proceeding down the channel in the absence of any DEP forces. Once extracted, the positional intensity of each population was used to calculate two metric of separation efficiency: harvest efficiency and purity.

Harvest efficiency \((E)\) is a metric for the fraction of the target particles that are harvested in the separation outlet compared to all target particles in the channel as given by

\[
E = \frac{T_{50}}{T_{100}}
\]

where \(T\) is the fluorescence intensity of the target particles. Subscript values refer to the percentage of the channel width from the separation side (ie: 50 refers to the right-most 50% of the channel only and 100 refers to the right-most 100% of the the channel, the entire channel width).

Purity \((P)\) describes the fraction of target particles compared to all particle types har-
vested in the separation outlet as

\[ P = \frac{T_{50}}{T_{50} + N_{50}} \]  

(6.3)

where \( T \) and \( N \) are the fluorescence intensity of the target and non-target particles, respectively. Subscript values refer to the percentage of the channel width from the separation side (ie: 50 refers to the right half of the channel only and 100 refers to the entire channel width). Ideal separation occurs when both harvest efficiency and purity are at unity. In real-world systems there is often a tradeoff required between these two metrics. Depending on the application, it may be preferable to prioritize the maximization of either harvest efficiency or purity over the other. In diagnostics for example, the occurrence of a false negatives may be more costly to patient health than false positives and thus the harvest efficiency of the target would be prioritized more highly than the purity. In this experiment the smaller green 1.75\( \mu \)m beads are the non-target particles and the larger orange 6\( \mu \)m beads are the target particle.

An example of one of the images taken separation is shown in Figure 6.18a. Here small beads appear green and the larger target particles appear orange. The image is downstream of the separation electrodes so that particles are no longer traveling laterally, but only flowing down the channel at the horizontal position at which they exited the DEP electrode array. Dashed lined indicate the In this image, taken with the DEP signal applied at 2.1V, green particles have shifted slightly to the right of where they appear when there is no signal applied, but they do not cross beyond halfway across the channel. The orange particles, however, show a large shift in horizontal position. Many have reached all the way to the right-hand channel wall, while others exit the DEP array at a position midway across the channel.

The positional intensity of each particle population is extracted and cumulated from all 150 images taken and is plotted in Figure 6.18b. Again we see the spread of green particles on the left half of the channel while the majority of the orange particles have been fully separated to the channel wall, with some orange particles spread amongst the right half of the channel.
In our devices, a microfluidic bifurcation separates the channel into the two outlets for sample recovery. We can calculate the harvest efficiency and the purity of the target (orange) particles that will reach the right fluid outlet from this positional intensity. For example, if the bifurcation occurs the channel midpoint (relative position = 0.5), then the right-most 50% of the fluid will be collected. The harvest efficiency of the target particles in that fluid is about just under 100%. Likewise the purity is nearly 100%. If, however, our channel bifurcated very near the right channel wall (such as at a relative channel position of 0.9) the fluid collected would still have a high purity, but the harvest efficiency would be lower, as many of the target particles in the middle region of the channel would not be collected. We can likewise calculate the harvest efficiency and purity that would be achieved for devices with bifurcations placed at every point across the channel width. Figure 6.18c shows this plot for this dataset. This calculation is useful for informing device design. Once a particular sample and operating conditions are known, the channel design can be optimized to either maximize the harvest efficiency or the purity or to achieve a balanced tradeoff between the two, depending on the specific application.

In order to compare the separation performance across many voltage and solution conditions, we extract the harvest efficiency and purity calculated for the case in which 50% of fluid is collected in each outlet. In this example, the harvest efficiency and the purity are both just below 90% half way across the channel.
Figure 6.18: a) A stacked fluorescence image shows the position of small green particles and larger orange particle downstream of a DEP lateral movement array. b) The positional intensity of each particle type shows a dramatic shift to the right for larger orange particles. c) The smaller the portion of the collected fluid from the channel’s right side, the higher the purity of the target particle in that fluid and the lower the harvest efficiency. If fluid is collected from the right half of the channel, both separation efficiency metrics are near 90%.
These two metrics of separation efficiency, for our case in which 50% of the fluid is collected into each side channel outlet, were calculated for each applied DEP voltage used for each of the four medias tested. Figure 6.19 shows the harvest efficiency and purity for the voltage ranges tested for each of the four solutions of varied conductivity and viscosity.

Figure 6.19a shows the separation efficiency for the low conductivity and low viscosity 0.0001xPBS media. For these parameters there is an increase in both separation metrics with increasing voltage. With higher voltages the target particles are moved more to the right, raising the harvest efficiency. Likewise the purity increases at similar conditions. An ideal separator would have both the harvest and purity reach unity at the same condition. At voltages above 7V, the purity begins to decline indicating that some non-target particles have begun to travel to the right side of the channel at those voltages.

For particles in the same viscosity solution, but at higher conductivity (1xPBS, Figure 6.19b), the harvest efficiency and purity again climb together as voltage is increased. The performance peaks at around 12V after which there is a decline in both metrics. A decrease in harvest efficiency at increased voltages indicates the occurrence of particle trapping on the electrodes.

Maintaining this conductivity, but in a slightly more viscous fluid (Lympholyte, Figure 6.19c), the behavior of the harvest efficiency is restored. No suffering as voltage is increased, the harvest efficiency staying near unity shows that there is not target-particle capture on the electrodes as the move across the channel. Thus there seems to be an improvement in particle movement or in preventing particle adhesion with the Lympholyte media. The purity of this sample generally declines with voltage, showing that non-target particles are also moving more easily to the right side. For all three of the fluids discussed thus far, all have a window of voltages at which both the separation efficiency and the purity are near 90%.

The high viscosity solution, however, never reaches that success. Figure 6.19d shows the separation efficiency achieved in the high conductivity, high viscosity TCM + 30%Ficoll solution. While the purity of the collected sample is high, the harvest efficiency never exceeds 40%. Particle retention occurs along the electrodes preventing lateral movement. Additionally, as the conductivity or viscosity are increased, higher voltages are required to
achieve the maximum performance.

Interestingly, the separation metrics are more coupled in low viscosity solutions, indicating a higher particle selectivity in those conditions. Since more viscous fluids impart more drag force on a particle, scaling with particle radius, the net force on particles moving laterally will decrease with higher viscosity, but the ratio of net forces of the two particles will remain the same and equal the ratio of the particle radii.

(a) Low $\eta$, low $\sigma$ (0.0001xPBS)  
(b) Low $\eta$, high $\sigma$ (1xPBS)  
(c) Medium $\eta$, high $\sigma$ (Lympholyte)  
(d) High $\eta$, high $\sigma$ (TCM + Ficoll)

Figure 6.19: Separation efficiency for beads in fluids of varied viscosity ($\eta$) and conductivity($\sigma$). a) Low viscosity buffer with low conductivity (0.0001xPBS), b) Low viscosity buffer with high conductivity (1xPBS), c) Medium viscosity buffer (Lympholyte) with high conductivity, and d) High viscosity buffer (TCM + Ficoll) with high conductivity. Harvest efficiency gives the ratio of amount of harvested 6µm target particles to the total amount of target particles in the whole channel. Purity is the ratio of target particles to total particles (target and non-target) in the harvested section of the channel. Bifurcation into the outlets occurs at the midpoint of the channel.
6.7 Chapter summary

In this chapter we have utilized DEP for cell concentration, isolation, and separation. Using a non-pathogenic analogue to the tuberculosis causing bacteria, we demonstrate a method for mapping the pDEP CM factor for cells as a function of frequency by measuring the extent of DEP-induced cell capture (Figure 6.4). Comparing this to a mathematical model of the CM response, we see good agreement with our experimental data (Figure 6.6).

We then show how pDEP cell capture can be used to isolate cells from a population of mixed cells. We show selective capture of *E. coli* in the presence of red blood cells. We also show the frequency dependence of the separation (Figure 6.10).

Capture-based cell separation requires DEP force magnitude to compete with the full fluidic drag force magnitude in order to achieve successful cell isolation. As we have shown in previous chapters, DEP force magnitude can be hindered by device design and the application of high voltages can damage devices and result in cell adhesion issues. We therefore present an alternative continuous separation method in which cell isolation can be achieved with lower DEP force requirements. This lateral movement is demonstrated to work effectively for both pDEP forces (Figure 6.11) and nDEP forces (Figure 6.12).

We then show the ability to achieve size-based lateral separation of a population of microspheres from a mixed population of two bead sizes (Figure 6.16). We show the tradeoff between harvest efficiency and sample purity that occurs during separation (Figure 6.18). In our work we are motivated to utilize DEP in high conductivity fluids and also to use fluids of increased density to mitigate particle settling. High density fluids often come with increased viscosity as well which increases the drag force opposing particle movements. To assess the effect of these fluid types on separation efficiency, we measure the separation efficiency metrics for particle separation as a function of voltage for solutions of varied conductivity and viscosity (Figure 6.19). In accordance with our DEP system circuit model and particle CM factor models, particle separation in higher conductivity solutions requires higher voltages. As solution viscosity is increased for high conductivity solutions, the tradeoff between harvest efficiency and purity becomes more dramatic.

Having successfully achieved label-free electronic bacterial concentration, bacterial sep-
aration from red blood cells and lateral particle separation, we next seek to incorporate a label-free electronic system for cell quantification on chip with our DEP devices.
Chapter 7

Integrating on-chip coulter counters for continuous electronic particle detection

7.1 Motivation for integrated on-chip electrical detection

The current state of the art technology utilized for particle separation and classification is flow cytometry or fluorescence-activated cell sorting (FACS). In this technique, fluorescent labels are first bound to biomarkers of interest. Then labeled cells passing through a narrow fluidic region are optically probed and classified by their combined fluorescent markers and physically sorted accordingly. With the ability to output a purified cell population while also providing quantitative readout of the composition of cells in the original mixed sample, FACS is a powerful characterization tool. With its high cost and complexity of operation, however, it is not a viable diagnostic option for settings with resource or healthcare access limitations [2].

Fortunately, the primary functions of FACS - particle sorting and mixed population characterization - have promising chip-level alternatives. Previously we and others in the literature have demonstrated the use of DEP as an alternative method for particle sorting [6, 9, 12–15, 30, 32, 45]. In this case, quantification of sorted populations was accomplished
optically, either on-chip or off-chip. Off-chip manual cell counting is routinely accomplished at low cost using a hemocytometer, but requires a microscope and several minutes per sample. Off-chip automated cell counting with commercial coulter counters can be faster, but incurs equipment costs anywhere from $2,000 to over $100,000.

In order to further progress toward fully electronic, affordable, and portable diagnostics, quantification of the cell population of interest should also be electronic and should not require secondary devices or expensive equipment. Here we design and integrate an on-chip electronic cell counter into our DEP separation chips for real-time quantification of mixed cell populations and real-time assessment of DEP performance.

This work was done in collaboration with Dr. Zachary Kobos.

7.2 The Coulter principle

Wallace Coulter first reported the ability to electrically determine the size distribution of small particles suspended in fluid [111]. Demonstrating the ability to electrically distinguish between several cell types, Coulter began a field of study around the technique that became known as coulter counting.

The most basic electronic coulter counter consists of two electrodes, separated by a material, typically a conductive fluid, as diagrammed in Figure 7.1a. The resistance between the two electrodes reflects the impedance of the material and the geometry of the space between the electrodes. When no particles are present between the two electrodes, the resistance is simply the resistance of the solution $R_0$. A particle passing into the sensing volume near the electrodes replaces a volume of solution (Figure 7.1b). If the conductivity of the particle and that of the replaced solution differ, a net change in the solution resistance between the two electrodes results. Measurement of this solutions’ resistance change can be used to detect the passage of individual particles, as well as extract information about particle size and velocity.
Figure 7.1: Two parallel electrode plates separated by a solution. a) In the absence of any particles the net resistance between the electrodes is due solely to the solution resistance ($R_0$). b) The presence of a particle with different resistance than the surrounding fluid, however, will alter the net resistance between the two electrodes ($R_1$).

For a rectangular prism solution sensing volume, the sensing volume ($V_{soln}$) is simply

$$V_{soln} = whL \quad (7.1)$$

where $w$, $h$, and $L$ are the width, height, and length of the solution area between the electrodes, respectively. The resistance between the electrodes ($R$) through a solution of resistivity $\rho$ is given by

$$R = \frac{\rho L}{wh} \quad (7.2)$$

assuming that $L >> w, h$. When no particles are present in the sensing volume, the solution resistivity is simply that of the bulk solution. When an insulating particle is present, however, the effective resistivity of the solution ($\rho_{eff}$) increases approximately as

$$\rho_{eff} \approx \rho_0 \left(1 + \frac{3}{2}f_{fill}\right) \quad (7.3)$$

where $f_{fill}$ is the filling fraction of the particle in the solution and $\rho_0$ is the resistivity of solution in the absence of any particles. If a single particle is present in the sensing volume the filling fraction is $V_{particle}/V_{soln}$ or

$$f_{fill} = \frac{\pi d^3}{6whL} \quad (7.4)$$
where \( d \) is the diameter of a spherical particle. Thus the presence of a single particle gives an effective solution resistivity \( (\rho_1) \) of

\[
\rho_1 \approx \rho_0 \left( 1 + \frac{\pi d^3}{4 \, w h L} \right) \tag{7.5}
\]

From these equations we can calculate the change in resistance due to the presence of a particle in the sensing volume as

\[
\Delta R = R_1 - R_0 \\
\Delta R = \frac{\rho_1 L}{w h} - \frac{\rho_0 L}{w h} \\
\Delta R = \frac{L}{w h} (\rho_1 - \rho_0) \\
\Delta R = \frac{L}{w h} \left( \rho_0 \left( 1 + \frac{\pi d^3}{4 \, w h L} \right) - \rho_0 \right) \\
\Delta R = \frac{\pi \rho_0 d^3}{4 w^2 h^2} \tag{7.6}
\]

where \( R_0 \) is the resistance when no particle is present and \( R_1 \) is the resistance when 1 particle is present. The relative change in resistance then would be

\[
\frac{\Delta R}{R_0} = \frac{\pi d^3}{4 wh L} \tag{7.7}
\]

From this equation we can see that the relative change in resistance is proportional to the volumetric filling fraction.

\[
\frac{\Delta R}{R_0} = \alpha f_{fill} \tag{7.8}
\]

With a proportionality constant \( \alpha = 3/2 \) for fully insulating particles, but this proportionality constant will vary if the particles are less resistive than a conventional insulator.

These calculations are for the simple case in which coulter counter electrodes are parallel plates separated by the sensing volume (and in which \( L >> w, h \)). Particles here are also assumed to be insulating.

Unlike simple insulating particles discussed thus far, biological cells have a conductive interior, surrounded by a capacitive membrane. Care must be taken in order to maximize
the change in the net impedance of solution when a cell is present in the sensing volume. Utilizing a low enough frequency voltage signal (typically below 1MHz) for measurements allows the small capacitance of the cell membrane to shield the conductive cell interior, resulting in a particle that appears as an insulating particle.

Beyond detection of the presence and relative volume of a cell, coulter counters have also been demonstrated to be able to probe the internal electrical properties of cells. By operating at higher frequencies (above 1MHz), the impedance of the cell membrane capacitance of a passing particle is reduced. Thus the internal properties of the cell such as its cytoplasmic conductivity can be studied on a single-cell level \[112,113\].

We seek to create an accessible and portable device that can accomplish the major functions of FACS (particle sorting and subpopulation quantification) all electronically and all on-chip. Towards that goal, we have previously demonstrated the ability to electronically sort particles on-chip with DEP. Here we present the design and integration of a coulter counter onto DEP-active microfluidic chips to enable on-chip electronic particle quantification. We then demonstrate simultaneous DEP particle manipulation and coulter counter enumeration of particles. Finally we evaluate the performance characteristics of the coulter counter and examine its ability to measure size distributions of particle subpopulations from a mixed sample containing particles of three different sizes.

### 7.3 Coulter counter design and on-chip integration

#### 7.3.1 Design considerations

The on-chip coulter counter structure we utilize most closely resembles that created by Watkins et al. in which three electrodes cross under a narrow fluidic region \[114\]. Their counter is incorporated onto a multistep chip to first preferentially lyse red blood cells and enumerates white blood cells. They then capture CD4 or CD8 T cells onto an antibody functionalized surface and then does a final count to quantify the concentration of CD4 or CD8 T cells.

Only two electrodes are necessary for a coulter impedance measurement: a working electrode on which a sourced voltage is applied, and a sensing electrode from which the
voltage relative to the source electrode is measured as shown in Figure 7.2a. The voltage difference between the two electrodes is monitored in time \( (V_1 - V_0) \), utilizing time points during which no particles are passing over the electrodes to measure the background voltage. With this measurement strategy, the background voltage is subject to any global impedance fluctuations and therefore the differential signal of a particle passing can vary in time and be affected by any condition changes.

In order to eliminate the effect of any system impedance fluctuations over time, we adopt a three electrode measurement setup in which the sourced signal is applied to a center working electrode that is flanked by two independent sensing electrodes as diagrammed in Figure 7.2b. The difference in the relative voltage between each side sensing electrode \((V_1, V_2)\) and the working electrode \((V_0)\) is extracted and the difference between these two voltages \( (V_{diff}) \) is measured as

\[
V_{diff} = (V_0 - V_1) - (V_0 - V_2) \tag{7.9}
\]

This output differential voltage shows an asymmetric spike as a particle passes over the three electrodes. Figure 7.2d show a data trace of a single particle passage event with labels corresponding to the labeled particle positions in Figure 7.2c. In the absence of any particles in the sensing volume, the differential voltage is zero (point a). As a particle comes over the first pair of electrodes, a spike in the differential voltage indicates a local increase in solution resistance between the first two electrodes (point b). When directly over the center electrode, symmetry is restored and the signal briefly passes through zero (point c). Then when the particle is over the second pair of electrodes, a negative spike is seen (point d). After the particle exits the sensing volume, the differential signal again returns to zero (point e).
Figure 7.2: Coulter counter theory. a) Particle presence over an electrode pair can alter the net impedance measured between the two electrodes. b) Measuring a differential impedance between the two pairs of electrodes allows for local background subtraction in real time. c) As a particle passes over the two electrode pairs, d) the differential voltage yields an asymmetric peak in time for each event. Labeled points on the time trace in (d) correspond to the particle position in (c).

Incorporating this additional electrode pair that shares a common sourced voltage electrode overcomes any global impedance changes because the differential impedance of one pair acts as the background level for the differential impedance of the pair over which a particle is passing. This also allows coincident detection of particle passage which can help distinguish real particle passage events from signal noise. Additionally, the distance between the two asymmetric peaks enables easy extraction of particle velocity data since the peaks in time are coupled with particle location in this geometry.
The magnitude of impedance change during each particle passage event depends on the disparity in conductivity between the particle and any surrounding fluid, the distance between the particle and the sensing electrodes, and the relative volume of displaced solution in the overall sensing volume.

Despite a highly conductive interior cytoplasm, a cell appears very insulating at lower frequencies due to the small capacitance of the surrounding cell membrane (below 2-3MHz) [115]. The impedance contribution of the capacitive membrane \( Z_C \), however, decreases with increasing frequency \( \omega \) as shown by

\[
Z_C = \frac{1}{j\omega C}
\]

(7.10)

where \( C \) is the membrane capacitance and \( j = \sqrt{-1} \). If operating in high conductivity fluids, which is more optimal for cell health, the coulter counter differential signal magnitude will be greater the more resistive the cell appears in the sensing volume. Thus, the lower the frequency of the applied voltage, the greater the impedance of the cell, and the greater the disparity between the cell and the background fluid impedance. In our experiments with cells in conductive fluids, we operate at about 70kHz to yield a high differential signal while avoiding several issues that can occur with DC operation in conductive fluids such as electrode charging and solution heating (discussed in detail in Chapter 5).

For large distances between particle and electrode or for small fractions of the sensing volume displaced by a particle, the resultant change in solution resistance will be relatively small. If, however, the distance to the particle can be minimized and the volume fraction replaced can be maximized, change in solution resistance increases, allowing for better detection. In order to enable impedance detection of single particle in solution flowing over planar electrodes, we increase the signal magnitude through three approaches. First, the difference in conductivity between the fluid and particle is considered in order to maximize signal while still operating in conditions necessary for cell health. Second, we constrain the distance between the particle and the sensing electrodes. Finally, we constrain the volume of sensed solution over the electrodes to maximize the occupied volume fraction.

Due to the electric field strength decaying with distance from the electrodes, the ability
to measure a replaced solution volume also decays with particle distance above the sensing electrodes. Therefore, we mechanically constrain the particle height above the electrode surface by a thin microfluidic channel. Similarly, because the fractional volume of the particle compared to the total sensed fluid volume is proportional to the measured impedance change, constraining the total sensed solution volume is critical for sensitive detection. We accomplish this volume fraction maximization through either a mechanical constriction of the entire fluid flow volume over narrow sensing electrodes or by an electrical constriction of the particles to be sensed over narrow electrodes. The latter strategy is discussed in more detail in Section 9.1.

**Theoretical maximum throughput for limited sampling rate**

Depending on throughput needs, the sampling rate of the system may pose a limitation. The minimum sampling rate, \( f_s \), needed to detect a particle passing over the counter is given by

\[
 f_s = \frac{n}{t} \tag{7.11} 
\]

where \( f_s \) is in data points per second and \( n \) is the number of data points desired per cell crossing event and \( t \) is the time required for a particle to pass over the counter. For a given velocity, the time \( t \) necessary for a particle to pass over the counter is

\[
 t = \frac{l}{v} \tag{7.12} 
\]

where \( l \) is the path length of a particle to pass over the counter device (in \( \mu \text{m} \)) and \( v \) is the linear velocity (in \( \mu \text{m/sec} \)). Counter length can be expressed in terms of the electrode width (\( w_{elec} \)) and the gap width (\( w_{gap} \)) by

\[
 l = 3w_{elec} + 2w_{gap} \tag{7.13} 
\]

The average linear fluid velocity (\( v_{avg} \)) can be calculated from the volumetric flow rate by

\[
 v_{avg} = \alpha \frac{Q}{A} \tag{7.14} 
\]
where $v_{avg}$ is the average linear velocity of fluid in the channel (in $\mu$m/s), $Q$ is the volumetric flow rate (in $\mu$L/min), $A$ is the channel cross sectional area (in $\mu$m$^2$) expressed simply as

$$A = w_{ch}h$$  \hspace{1cm} (7.15)

where $w_{ch}$ and $h$ are the channel width and height respectively (in $\mu$m), and $\alpha$ is a unit conversion factor given by

$$\alpha = \frac{1 \times 10^9}{60}$$  \hspace{1cm} (7.16)

Due to surface friction between channel walls and flowing fluid, the maximum linear velocity occurs in the center of the channel and is 1.5x the average linear fluid velocity:

$$v_{max} = 1.5 \alpha \frac{Q}{A}$$  \hspace{1cm} (7.17)

Substituting these equations into Equation 7.11, the minimum sampling frequency necessary for particle detection can be expressed in terms of linear velocity or volumetric throughput and device geometry as

$$f_s = \frac{n}{t}$$

$$f_s = \frac{n v_{max}}{l}$$

$$f_s = 1.5 \alpha \frac{nQ}{lA}$$  \hspace{1cm} (7.18)

$$f_s = 1.5 \alpha \frac{nQ}{hw_{ch}(3w_{elec} + 2w_{gap})}$$  \hspace{1cm} (7.19)

where $f_s$ is the minimum necessary sampling rate (in Hz), $n$ is the number of data points desired per cell crossing event, $t$ is the transit time of a particle over the counter (in sec), $v$ is the maximum fluid velocity over the counter (in $\mu$m/sec), $Q$ is the volumetric flow rate (in $\mu$L/min), $A$ is the channel cross sectional area (in $\mu$m$^2$), $l$, $w_{ch}$ and $h$ are the channel length, width and height, respectively (in $\mu$m), $w_{elec}$ and $w_{gap}$ are the counter electrode width and inter-electrode gap width, respectively (in $\mu$m), and $\alpha$ is a unit conversion factor ($\alpha = 1 \times 10^9/60$).

Lower limits on the number of sampled data points per passage event ($n$) in literature
are approximately 10, whereas 20 being is a more preferable limit. The channel width and height \((w_{ch} \text{ and } h)\) should not be less than the maximum expected particle diameter. Increasing these values allows greater throughput for a given linear velocity. As any of these geometry dimensions are increased, however, the sensing volume also increases, leading to a proportional reduction in signal strength. Thus it is advantageous to keep the sensing volume as small as possible for a given particle size. This necessitates balancing the trade-off between sensing signal strength and electrical sampling frequency limits that restrict operation at high flow rates.

We chose to make the inter-electrode gap width \((w_{gap})\) also greater than the maximum expected particle diameter to maximize the differential signal between the upstream and downstream electrode pairs. For convenience, the counter electrode width \((w_{elec})\) used was usually equal to the gap width. For initial comparisons, we can define a characteristic device geometry dimension \((D)\) as

\[
D = w_{ch} = h = w_{gap} = w_{elec}
\]  

(7.20)

In such cases, the minimum sampling frequency can be expressed as

\[
f_s = 1.5 \alpha \frac{nQ}{D^2(3D + 2D)}
\]

\[
f_s = \frac{1.5 \alpha}{5} \frac{nQ}{D^3}
\]

\[
f_s = 0.3 \alpha \frac{nQ}{D^3}
\]  

(7.21)

If we express the volumetric flow rate also in terms of the characteristic geometry dimension, we can re-write the minimum sampling frequency as a function of data points desired and maximum linear fluid velocity as

\[
f_s = 0.3 \alpha \frac{n}{D^3} \left( \frac{v_{max}A}{1.5 \alpha} \right)
\]

\[
f_s = 0.2 \frac{nv_{max}D^2}{D^3}
\]

\[
f_s = \frac{n}{5} \frac{v_{max}}{D}
\]  

(7.22)
Figure 7.3a shows the minimum sampling frequency necessary to detect particles passing over the counter at varied volumetric flow rates. Several device geometries are shown in which the characteristic dimension is varied from 1 to 50µm. The minimum number of data point per trace is set to 10, the lower limit of what is found in literature. A dashed horizontal line marks the 100kHz sampling frequency limit of the lock-in amplifier used in the measurement setup.

For any given throughput rate, the narrower channel geometries necessitate higher sampling frequencies due to faster particle speeds. While increasing channel cross sectional area would allow decreased minimum sampling rates needed for particle detection, that would simultaneously decrease signal magnitude as the passing particle would occupy a smaller volume fraction of the sensing region. One possible method for decreasing the necessary sampling frequency while keeping the cross sectional area constant, is to increase the electrode width. This would allow a longer particle transit time while keeping the path length of solution resistance constant from the constant electrode gap width.

The maximum linear flow speeds and maximum volumetric flow rates able to be used for different characteristic device geometries are shown in Figure 7.3b, assuming a maximum sampling frequency of 100kHz and a minimum number of 10 data points per trace. Under these conditions, for example, a device with a characteristic dimension of 20µm would have a maximum throughput of 16µL/min. In order to obtain 10x higher resolution (100 data points per trace), the maximum throughput drops to 1.6µL/min.
Figure 7.3: Examination of throughput limitations. a) Sampling frequencies necessary for detection of particles passing over counter electrodes with varied characteristic dimensions at different flow rates ($n = 10$ points per event). A dotted line indicates the throughput limitations for a $100$kHz maximum sampling frequency. b) For a sampling rate of $100$kHz and a minimum $n = 10$ points per event, the maximum throughput and linear flow speed increase with increasing device dimension.
Theoretical maximum particle concentration to avoid coincident detection

When particles pass through the sensing volume of a coulter counter one at a time, the signal shows isolated peaks for each event. The signal is additive for each concurrent event. If two particles pass by at the exact same time, the signal will show the addition of two peaks at the same time, effectively doubling a regular signal peak amplitude. Similarly, if a particle enters the sensing volume before the previous particle has exited, the signal will show the addition of two peaks separated slightly in time, resulting in a peak shoulder, bimodal peaks, or even overall lower peak magnitudes if the particles are over each electrode pair at the same time.

In order to easily distinguish individual passage events and to extract particle size from the data, concurrent events should be avoided. This necessitates a limit on the particle concentration in the suspending fluid such that only one particle exists within the sensing region at any time. The theoretical maximum particle concentration can be expressed as

\[ C_p \leq \frac{1}{V_s} \]  

where \( C_p \) is the particle concentration and \( V_s \) is the sensing volume. The exact sensing volume depends on the applied field and the geometry because electric field decays rapidly with distance from the electrodes. For cases where the electrodes are all on the floor of the fluid constriction, if the applied voltage is assumed to be high enough to enable sensing at the channel ceiling, we can use that channel height as an approximation for the minimum extent of the sensing region extending out laterally from the electrodes. In cases in which the electrode pitch is greater than the channel height, the operating voltage would need to be higher than this minimum value. To estimate a generalized sensing volume, we can calculate the fluid volume over the three electrode region as

\[ V_s = h w_{ch} (3 w_{elec} + 2 w_{gap}) \]  

where \( h \) is the channel height and \( w_{ch}, w_{elec} \) and \( w_{gap} \) are the channel, electrode, and gap widths, respectively. Thus, for an example device with a characteristic dimension (\( D \), the
maximum particle concentration could be expressed by

\[
C_p \leq \frac{1}{V_s}
\]

\[
C_p \leq \frac{1}{hw_{ch}(3w_{elec} + 2w_{gap})}
\]

\[
C_p \leq \frac{1}{D^2(5D)}
\]

\[
C_p \leq \frac{1}{5D^3}
\]

\[
C_p \leq \frac{1}{5D^3}
\]

(7.25)

This relationship is shown plotted in Figure 7.4. For example, for a characteristic dimension of 20µm, this approximation gives a maximum particle concentration of \(2.5 \times 10^7\) particles/mL. In a blood sample, red blood cells exist in a concentration between about 4.2 and \(6.1 \times 10^9\) cells/mL, about 100x the max particle concentration for a device with characteristic dimension of 20µm. In order to process 6.1E9 particles/mL, a characteristic dimension of 3.2µm would be needed, which is smaller than the dimension of a red blood cell. Thus we can see the importance that device geometry plays in increasing both signal magnitude and also sample throughput.

![Figure 7.4: Maximum particle concentration for varied characteristic counter geometry lengths such that only one particle is within the sensing volume at a time](image-url)
7.3.2 Device design

Three parallel conductive electrodes are used for impedance particle detection as diagrammed in Figure 7.2. The planar geometry is utilized to allow compatible integration into the fabrication flow of DEP structures without necessitating any additional layers or costs. The center electrode is the working electrode (WE) onto which an AC current is applied. The two neighboring electrodes serve as a counter electrode (CE) and a reference electrode (RE) from which voltage is extracted. Both of those voltages are either amplified and the differential of the two is measured in a LockIn amplifier or the differential voltage between the two outer electrodes with respect to the center working electrode is directly extracted on our PCB and the differential output then amplified and measured with a LockIn amplifier.

Devices were made to test and optimize on-chip coulter counter performance. As mentioned, the basic design of the coulter counters consist of three electrode fingers spanning the width of a narrowed fluidic region. An AC signal is sourced onto the middle electrode while the difference in impedance measured between either side electrode and the center source electrode is output. As a particle passes over the electrodes between the first and center electrode, the impedance between these two electrodes increases, yielding a spike in the differential signal. Similarly, as the particle passes between the center and third electrode, the impedance at the third electrode increases, giving a negative spike in the measured differential impedance signal between the two electrode pairs.

The impedance due to a particle passage could be measured from only one electrode pair, but the addition of a second electrode pair allows measurement of the differential impedance which is both less impervious to noise fluctuations and also yields easily extractable information about the length of time taken for the particle to pass over the electrodes. The magnitude of the differential impedance measurement reflects the magnitude of change in the effective conductivity of the solution volume between the source and sense electrodes. The larger the disparity between the conductivity of the solution and the conductivity of a passing particle, the greater the measured signal. Likewise the larger the solution volume fraction occupied by the particle, the larger the measured signal. The electrode lengths are kept as short as possible to minimize the solution volume between the two electrodes such
that a passing particle occupies as large a solution volume fraction as possible, maximizing signal magnitude.

In order to have both very short sensing electrodes and for every particle in solution passing over the electrodes for counting, all of the particles must be physically brought over a narrow region over the electrodes. This is typically accomplished by restricting the microfluidic channel width to mechanically funnel all particles over the counter electrodes. Funneling particles over the counter by a narrowing microfluidic channel ensures 100% of particles pass over the electrodes and serves to increase the magnitude of the measured signal due to increasing the fill fraction of the sensing volume. It is desirable to get the highest fill fraction possible for the detection of the particle of interest while balancing throughput tradeoffs and limiting particle clogging at the constriction.

**Geometries for variable testing**

A wide range of device geometries were designed and fabricated in order to test the fill fraction limits of detection in our system.

Generally, each basic coulter counter test chip footprint was 8mm x 40mm with one linear DEP IDE array followed by six coulter counters, each with its own microfluidic constriction and microfluidic filter region as shown in Figure 7.5a. Particles are physically brought over a narrow region over the electrodes by a fluidic constriction over the coulter counter electrodes located at the center of the channel as shown in Figure 7.5b. In order to mitigate clogging of the constriction region by particle aggregates, large debris, or air bubbles, an upstream microfluidic filter is implemented to block any particle aggregates, air bubbles, or sample debris larger than the constriction from entering the narrowed channel as diagrammed in Figure 7.5c. Once through the filter region, particles pass over the coulter counter electrodes in a narrowed fluidic constriction as shown in Figure 7.5d.

In order to test and optimize coulter counter performance, several chip design variables were systematically varied on different devices. On the counter devices, both the width of each electrode and the width of the gap between each electrode varied. The width of the gap between each electrode varied between 10 and 100um, with electrode width to gap width ratios varying between 0.2 and 5. The channel width of the constriction region varied...
Figure 7.5: Basic coulter counter chip design. A) Full chip layout with one DEP linear array and six counter devices on an 8mmx40mm footprint. B) Coulter counter unit cell consisting of a filter, a narrowed fluidic constriction, three electrodes. C) Microfluidic filter to prevent particle aggregates or large debris from clogging the coulter constriction. The gaps in the filter are the same width as the constriction region. D) Coulter counter electrodes span a narrow fluidic constriction region.

between 10 and 200um. The filter region upstream of each counter device had gaps equal to the width of the neighboring constriction region. The electrode width of the linear DEP IDE array varied between 10 and 100um, with a gap width equal to the electrode width. Table 7.1 details the geometry of the six types of coulter counter test chips.
Table 7.1: Coulter counter chip designs with varied parameters. (All width values are given in um, “w” indicates longer electrode fingers in order to span a larger fluid channel width.)

<table>
<thead>
<tr>
<th>Chip type and purpose</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Type 5</th>
<th>Type 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count small particles</td>
<td>Count medium particles</td>
<td>Count large particles</td>
<td>Vary electrode width</td>
<td>Vary gap width</td>
<td>Vary channel width</td>
</tr>
<tr>
<td>Pad numbers</td>
<td>Structure type</td>
<td>Metal width</td>
<td>Gap width</td>
<td>Fluid width</td>
<td>Metal width</td>
<td>Gap width</td>
</tr>
<tr>
<td>1,2</td>
<td>DEP</td>
<td>10</td>
<td>10</td>
<td>1000</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3,4,5</td>
<td>Counter</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>6,7,8</td>
<td>Counter</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>9,10,11</td>
<td>Counter</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>12,13,14</td>
<td>Counter</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>15,16,17</td>
<td>Counter</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>18,19,20</td>
<td>Counter</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Upstream filtering to limit clogging

The narrowed fluidic region incurs some operational drawbacks: increased particle speed over the electrodes, elevated fluidic pressure, and, most notably, potential clogging of the constriction region if particles aggregate or are present in too high of concentrations. In order to mitigate clogging of the constriction region, an upstream microfluidic filter is implemented to block any particle aggregates, air bubbles, or sample debris larger than the constriction from entering the narrowed channel.

Shrinking device geometry increases detection signal strength but comes at the cost of limiting achievable throughput. Additionally, as the constriction volume decreases, mechanical clogging of the constriction can become more of an issue. Constriction blockage can be caused by large debris in a sample, by particle adhesion to the channel walls, or by particle-particle adhesion leading to large aggregates.

If a blockage occurs in the constriction, fluid flow to the counter is restricted, altering particle analysis accuracy. A severe blockage can totally restrict all fluid flow, rendering the counter unusable. During blockage formation, large hydraulic pressure buildup can also lead to channel leakage or rupture, permanently disabling the use of that counter device. Figure 7.6 shows an example of a counter constriction that has been fully blocked by incoming particles, leading to a pressure buildup and subsequent channel leakage demonstrated by the presence of particles outside of the original channel boundary walls.
Several design and operational variables influence the length of time a constriction device will run before clogging occurs, referred to as the mean time-to-failure. Design variables such as a larger constriction cross sectional area, a shorter constriction length, and a more gradual narrowing of the channel walls approaching the constriction region all help to increase the mean time-to-failure of a coulter device.

On the operational side, lower particle concentrations and slower flow speeds lower the likelihood of clogging. Surface treatments to reduce particle-device and particle-particle adhesion can also increase the mean time-to-failure. For a given geometry, smaller particles and particles with lower rigidity are less prone to clogging, such as highly deformable RBCs.

In iterative cycles of design, fabrication, and experimental testing, our counter constriction design evolved from a 45 degree approach angle (Figure 7.7a) to an 18 degree approach angle (Figure 7.7c-d). We also implemented an upstream mechanical filter to block the passage of any large debris or particle aggregates from encountering the constriction region. Originally, the filter region approached angle was 90 degrees (Figure 7.7c), but, similarly to the constriction itself, we found decreased clogging at the filter and easier passage of bubbles if the approach angle was more gradual (Figure 7.7d).
Figure 7.7: Coulter counter constriction design evolution. Initially a fluidic approach angle of about 40° was used (a) but a more gradual approach angle of about 18° was adopted to decrease clogging (b). An upstream filter was incorporated to prevent any debris or aggregates larger than the constriction width to encounter the constriction (c). Finally, the approach angle for the filter passthroughs was also decreased from 90° to 20° to allow for easier pass through of appropriately sized particles and bubbles (d).

Figure 7.8 shows an image of the filter region after fabrication in PDMS. The presence of the filter is particularly effective in preventing channel clogging from large debris or particle aggregates. The angle of the lead in to the pass through regions help allow bubble passage.
Figure 7.8: Optical image of a filter region used to prevent the passage of any debris or particle aggregates larger than the downstream coulter counter constriction region

**Clearing a clogged constriction**

In the event that a constriction does become clogged, several approaches can be utilized to recover device function, used either individually or in combination to re-establish full fluid flow. Back flushing fluid from the opposite direction can help dislodge a clog. If the particle aggregation is made of cells, chemical treatments that degrade adhesion proteins and convert lipids of cell membranes into water soluble components can be utilized. Additionally, mechanical sonication in the presence of DI water can help loosen and remove blockages, as demonstrated in Figure 7.9 in which a clogged counter is submerged in DI water and sonicated for 18 minutes to clear the constriction blockage.
Figure 7.9: A μm coulter constriction blocked by particles a) before and b) after 18 minutes of sonication in DI water

### 7.3.3 On-chip integration with DEP devices

By designing coulter counters with planar electrodes, device fabrication can follow the same fabrication steps used for DEP device fabrication. Thus, coulter integration onto DEP chips can incur no extra fabrication costs, consistent with our goal of a low cost device with several integrated functions. The counter electrodes are kept unpassivated by any insulating oxide to maximize the electric field in the sensing volume. Due to the small feature sizes and smaller alignment tolerance of the counter structures, alignment of the microfluidic channels to the silicon base chip for bonding was accomplished with the use of either mechanical or liquid spacers (as described in Section 3.2.3).

Electrical contact between source function generator and on-chip devices was accomplished in the same manner as for DEP operation interfacing as described in Chapter 3. Briefly, chips were placed into a socket of a custom-designed aluminum mounting plate. A custom-designed PCB was screwed into the mounting plate, tethering the PCB ground plane to the larger metal mounting plate. The signal from a function generator was sent via BNC cables to PCB-mounted BNC jacks and then passed to patterned breakout contact
pads on the chip surface via right-angle spring-loaded contact pins mounted on the PCB. Horizontal alignment of the chip to the PCB is accomplished by the placement of the chip socket in the holder and the threaded screw holes for the PCB mounting. Vertical alignment of the chip surface to properly engage the spring-loaded pins is accomplished by precision milling the chip socket depth such that secure contact is made once the PCB is screwed down in place to the mounting plate. The PCB design was updated from that used for DEP interfacing. Recall that the end signal to be ultimately detected is the differential voltage between \( V_1 \) and \( V_2 \). Sending both \( V_1 \) and \( V_2 \) to a lock-in amplifier for differentiation and amplification is an option, but in order to reduce noise and prevent amplification of background noise between the PCB and the lock-in, the voltages from each of the two sensing electrodes was differentiated and then amplified all on board with an instrumentation amplifier before being sent to a lock-in amplifier for detection.

### 7.4 Coulter counter performance evaluation

Once fabricated and successfully integrated onto DEP chips, the performance characteristics of the coulter counter devices were tested. Initially, the ability to operate two different electrical systems on the same chip was tested to determine the viability of simultaneous DEP manipulation and on-chip coulter counter quantification.

#### 7.4.1 Particle detection

Particles to be detected are suspended in fluid and passed over the counter electrodes through bulk fluid movement by syringe pumps (New Era Syringe Pumps NE-1000). A sinusoidal AC voltage of about \( 100mV_{rms} \) and \( 70-100kHz \) is sourced (Agilent 33120A) and applied to the center working electrode of the coulter counter.

The impedance between the source electrode and the upstream and downstream sensing electrodes can either be amplified and differentiated by a lock-in amplifier or they can be differentiated and amplified directly on the PCB followed by detection of the output differential signal with a lock-in. By differentiating the voltage between the two sensing electrodes before lock-in amplification, amplification of the background noise can be minimized. An
example of the raw differential signal output from the counter is shown in Figure 7.10. After either lock-in detection of the differentiated signal or lock-in differentiation of the two sensing electrode signals (Stanford Research Systems SR830 DSP), the overall differential voltage is read and recorded from an oscilloscope (Tektronix DPO4104), and exported to a computer for analysis.

![Figure 7.10: Raw data output from the on-chip coulter counter shows two asymmetric peaks for each particle passage event](image)

In order to verify counter detection of real particle passage events and determine any background signals in the system, fluid samples with and without particles added were measured. Additionally, particle concentration was altered in real time using upstream DEP particle capture and release. Simultaneous imaging to monitor particle passage showed clear coulter counter signal differences between particle counts during DEP capture and after DEP particle release, demonstrating the coulter counter detection signal corresponding to particle passage events. Figure 7.11a shows an optical image of the constriction region and the corresponding data trace from the coulter counter when particles were being captured upstream with DEP signals and therefore no particles were passing over the counter. Figure 7.11b shows the optical image and corresponding data trace after the particles upstream were released by turning off the DEP capture signal. Particles can be seen as small black dots in the fluid approaching the constriction region. Because device geometry and sample flow rate are known, particle concentration can be obtained from the frequency of particle passage events extracted from the data traces.
In order to distinguish a real passage event from any background noise, a few selection criteria are employed. Due to the three-electrode design, real events have two coincident asymmetric peaks as a particle passes the upstream then downstream pair of electrodes. Event detection begins by flagging any event in which two neighboring peaks have opposing polarities. Each detected event is fit with two Gaussian peaks and the peak height and
peak-to-peak transit time are extracted data traces of differential voltage amplitudes over time as shown in Figure 7.12a.

In order for the event to be considered a ‘real’ event, the peak amplitudes must be greater than a chosen noise threshold. Additionally, the time delay between two peaks of a possible event, extracted as the distance between subsequent peak positions in time must be within a range of possible values given the device geometry and operational flow rate conditions. Particle concentration is calculated by

\[ C_p = \frac{f_p}{Q} \]  

(7.26)

where \( C_p \) is the concentration of particles (in particles/mL), \( f_p \) is the particle frequency (in particles/sec, and \( Q \) is the volumetric flow rate (in mL/sec).

Beyond particle concentration, peak heights and peak-to-peak transit times contain information about particle size and velocity, respectively. The extracted peak-to-peak transit times can be used to calculate particle velocity since the electrode geometry is known. Plotting peak height and peak-to-peak transit time for each particle on a heat map illuminates the distributions present in a population of particles and indicates any relationship between the two factors. Figure 7.12b shows a heat map of peak heights and transit times for a population of polystyrene microparticles.

\[ \text{(a)} \hspace{1cm} \text{(b)} \]

Figure 7.12: a) Raw data trace of a single particle passage event with arrows indicating extracted peak height and transit time from Gaussian fits of both peaks. b) Heat map of the peak heights and transit times of a population where each data point represents a single particle passage event.
As long as the geometry of the counter electrodes is known, particle velocity can be calculated from transit time. The spread in transit time is due in part to the positional dependence of fluid velocity (and therefore particle velocity) within the constriction region.

While particle size does dramatically influence peak height, the calculation of particle size from peak height requires a more nuanced examination. The following section looks into the problems and possibilities of using coulter counters to extract particle size information.

7.4.2 Particle size discrimination

Beyond electronic enumeration of particles present in a fluid sample, we sought to extract information about individual particle sizes, thereby enabling the simultaneous enumeration of multiple particle subpopulations from a sample of mixed particles. In order to test the on-chip coulter counter’s ability to discriminate between subpopulations in a sample of mixed particle sizes, polystyrene beads of three known diameters were mixed and introduced over the counter. Average sizes for each bead population were nominally 4.45, 6.08, and 8.87µm (Spherotech).

Suspended in 1xPBS buffer solution, beads were introduced over a counter by a syringe pump (New Era Pump Systems NE-1000) at 1µL/min. Counter constriction width and height were 20µm and 17.3µm respectively, yielding an average linear flow rate of 48.2mm/sec. Data was collected in 1600 1-second scans and analyzed to detect real passage events and extract peak height as well as peak-to-peak transit time. Since the signal amplitude is proportional to particle volume, the cube root of signal amplitude is proportional to particle diameter. Figure 7.13a shows the population distribution of the cube root of extracted peak heights. The vertical dashed line indicates the lower threshold for noise elimination. Fitting each peak in the distribution with a Lorentzian function gives an average particle size and indication of spread. Figure 7.13b shows the same data but plotted as a heat map, incorporating the corresponding peak-to-peak transit time for each particle passage event. Plotting the nominal given diameter against the cube root of the differential voltage magnitude demonstrates a linear dependance of the peak height on particle volume - or a linear relationship with the cube root of the peak height and particle diameter - as expected (Figure 7.13c). From this linear dependence we can extract the proportionality
constant for these experimental conditions and use that as a calibration factor for relating the coulter counter signal with polystyrene bead diameter for this device dimensions.

Figure 7.13: a) Measured amplitude of the coulter counter detection of three polystyrene bead sizes shows distinct peaks for each bead size present in the sample. b) A heat map of the peak height and corresponding transit time for each particle. c) Particle diameter is proportional to the cube root of the peak height.

The limit for particle detection occurs when the cube root of the peak height is less than the noise threshold used in data analysis. For this channel geometry, the limit is about 2.8µm, shown graphically by the x-value of the intersection of the calibration fit line the peak height noise threshold line. Upon initial examination the large spread in peak heights for each nominal bead size indicates a resolution for size distinction around 0.5-1µm. This resolution is sufficient for many applications, but in order to determine how to further improve the device, we examined this spread of sizes in more detail.

First, we examined the size distribution of the beads themselves, starting with the sample of 8.87µm beads that show the maximal peak height spread. An optical image of the bead sample was acquired on a microscope (Olympus Bx51) with a 10x objective lens (Figure 7.14a). A program was written to detect all beads within the image, ignoring any beads
near image edges or clumps of more than one bead, and calculate the average diameter of each individual 2D particle circle (Figure 7.14b). Using a standard calibration for the image resolution and objective lens magnification, the particle diameters were converted from pixels to real distances and are plotted in Figure 7.14c. The same Lorentz distribution fitting function was applied to fit the diameters of all particles from the optical image. From this data we see that the variation in particles sizes from the nominal diameter is quite dramatic, with a coefficient of variation (standard deviation divided by the mean) of 8.53%.

The fits of the coulter counter data of peak heights is replotted with the values of the cube root of peak height converted to particle diameters using the calibration curve acquired in Figure 7.14d. Comparing the fits of the subpopulation of 8.87µm beads obtained from the coulter counter with those obtained from the analysis of the bead sample image (Figure 7.14e), we can see that the absolute average diameter obtained from each measurement varies slightly between measurement techniques (by about 0.5µm). This is likely due to the original nominal value of average bead diameter (used in the calibration curve generation) being no longer accurate. A shift in average bead size in an older sample of beads is not surprising, given that beads on one end of the size distribution may have been more prone to settling during extraction from the sample bottle. Over time, this bias may skew average particle sizes slightly larger. The important parameter from this comparison is not the absolute average size of the bead population. Rather, it is the spread of sizes present that matters in our determination of the size discrimination resolution of the coulter counter. Therefore we overlaid the normalized fitted curves from both the coulter counter data and the image analysis data, aligning their peaks (Figure 7.14f).

This comparison shows that the majority of the spread of sizes from the coulter counter data can be explained as actual differences in the bead sizes. Using full width at half maximum peak height as a measure of distribution spread, we calculate that 62.8% of the spread of the coulter counter size data is due to real variation in particle sizes within the 8.87µm bead sample. This indicates a much better size-discrimination resolution for the counter than original calculations based on the assumption of negligible spread in actual bead sizes within each subpopulation.
Figure 7.14: An optical image of nominally 8.87μm beads (a) is processed to detect and measure the actual diameter of each particle (b). A histogram of these beads shows a large spread in actual particle sizes (c). The histogram of particle diameters measured by the coulter counter for 3 different nominal bead sizes shows a spread in measured diameters as well (d). Comparing the coulter counter fitted peak and the fitted peak of bead sizes from the optical image show very similar spreads (e). When plotted as a relative particle diameter to exclude the uncertainty in most common particle size from the commercial sample, the majority of the spread of fitted sizes comes from the actual distribution of particle sizes (f).
The remaining spread in peak heights, then, must be due to factors other than variations in particle size. Possible sources include variations in distance of the particle from the sensing electrodes, variations in particle conductivity, or a source of inaccuracy or noise in the measurement system.

The manufacture of polystyrene microspheres utilizes a common material stock and so non-negligible variations in particle conductivity are unlikely. In order to assess whether the remaining spread in the data is caused by a fault in the counter measurements, we modelled what the signal variations would look like for expected particle positional variations within the channel.

Because of the decay in electric field strength, the signal strength of the counter counter signal decays with the square of the distance of the particle from the electrodes. In a channel higher than the particle size, particle height variation should result in variation in signal amplitude. For a given particle height and volume, we can calculate the expected relative signal amplitude. Figure 7.15a shows the expected relative signal strength for particles of three different sizes for all allowed heights in a 20µm tall channel. Here we used the same average particle sizes as used experimentally to generate the microbead calibration curve. We have allowed particles to be located anywhere in the channel, but with a minimum distance between particle edge and channel wall of 10% of the particle radius. The larger volume particles yield the largest signals for any given height. Heights here are defined as the particle center and, therefore, we see that smaller particles have a wider range of possible positions within the channel.

Our coulter counter data shows variability not only in peak heights, but also in transit times. Similar to signal amplitude, transit times should vary with particle position in the channel. The cause of the variation in this case, however, is the variation in fluid velocity with location in the channel. Having a parabolic flow profile with respect to both the vertical position and horizontal channel position, particles exhibit a related distribution of velocities with position. Figure 7.15b shows the possible velocities of particles for three sizes. These velocities are calculated from particle positions only at the horizontal center of
the channel, but at every allowed height within the channel. Fluid velocity is calculated by

\[ v_f = 6 \bar{v}_f \frac{h}{H} \left( 1 - \frac{h}{H} \right) \]  

(7.27)

where \( v_f \) is the fluid velocity at a given \( h \) height in a channel of total height \( H \) and average linear flow velocity \( \bar{v}_f \). From the fluid velocity, particle velocity \( v_p \) is calculated as

\[ v_p = v_f \beta \]  

(7.28)

where \( \beta \) is

\[ \beta = 1 - \frac{5}{16} \left( \frac{r}{d} \right)^3 \]  

(7.29)

where \( r \) is the particle radius and \( d \) is the particle distance from the channel wall. These equations are valid for distances greater than 1.1 \( r \) from the channel wall. Accordingly, we restrict particle positions to have a minimum separation distance of 10% of the particle radius between the bead edge and channel wall.

By generating a random normal distribution of particle heights along the horizontal channel center, we can plot the relative signal strength and corresponding transit time expected for particle passage events. Figure 7.15c shows this plot for three particle sizes. By restraining particles to the horizontal center, this plot shows the lower bound on transit times. For any horizontal shift in position away from the center, the transit time would increase while the signal strength would remain unchanged, filling in some of the area to the right of the plotted curves. Comparing this expected distributions to our experimental data in Figure 7.15d we see general trend agreement.
Figure 7.15: Numerical modeling of the effect of particle locations within a channel on a) relative signal strength, b) particle transit times, c) overall distribution of the signal and transit times expected for a particle in a given allowed channel position all compared for three bead sizes equivalent to those used in d) the experimental particle detection of a mixed population of three bead sizes.

The experimental distributions of peak height and transit times show a slightly larger spread for smaller particles. This agrees with the model of signal distributions resulting from changes in particle location within the channel. Larger particles have slightly more limited possible positions in the channel and, therefore, more limited spread of transit times and less variation in signal magnitudes.

This analysis also shows the same trend towards slightly longer minimum transit times for larger particles, as would be expected from fluid dynamics of larger particles. Interestingly, the spread of signal strength shows more overlap in the modeled data then the
experimental data, indicating that the particle positions in the channel are more focussed by fluid flow. If we constrain the spread of the randomly generated particle heights, we observe better agreement with the experimental data. Figure 7.16a shows the histogram of heights with a standard deviation of 0.1. The resultant particle signal and transit times for this distribution of particle heights is shown in Figure 7.16b. More tightly constraining the particle heights distribution standard deviation of 0.05 (Figure 7.16c) yields particle transit times much more in line with the experimentally measured coulter counter data (Figure 7.16d). The overlap in the signal strength for each particle distribution is significantly decreased with the increased constraint of particle positions. Additionally, the spreads of transit times are reduced and match the experimental spreads much more closely.

Figure 7.16: a) Random heights generated from a normal distribution with a standard deviation of 0.1 yields b) bead transit times with a large spread. c) Drawing particle locations from a more constrained distribution of heights with a standard deviation of 0.05 d) greatly restrains the resultant spread in particle transit times.
By constraining the particle heights in the channel discrimination ability of the counter would increase dramatically. Consider the medium sized beads in Figure 7.16b. A small percent of the beads modeled are able to be absolutely distinguished from the population of larger or smaller beads. In Figure 7.16d, however, a much larger percent of all beads can be identified. Limiting the spread of particle velocities as they travel over the counter, therefore, decreases the overlap in the populations coulter signals and improves device performance.

7.5 Chapter summary

Toward the goal of creating fully electronic physical particle sorting and on-chip electronic particle quantification, we have integrated a digital coulter counter structure into our DEP devices. Details of the theory and measurement setup are discussed (Figure 7.2). Chip designs are detailed for testing different device geometries (Table 7.1) and mitigating a common problem of constriction clogging through implementation of an upstream fluidic filter (Figure 7.8).

Using beads, we demonstrate the ability to electronically detect particle passage (Figure 7.10). The number of particles and particle concentration can be calculated from the number and frequency of detected events, particle velocity can be calculated from the peak-to-peak transit time, and particle size extracted from the magnitude of the signal. We then demonstrate the ability to count particles in real time while DEP signals are manipulating particle movement (Figure 7.11).

Attempting to utilize coulter counters to discriminate between subpopulations of particles of mixed sizes, we measure the peak height and transit times of particles of three different sizes in a mixed population (Figure 7.13). If the particle sizes were equal to their reported nominal value, the resolution of size discrimination for our counter would be about 0.5-1µm. An investigation into the actual sizes of one of the bead population, however, revealed that over half of the spread of the fitted peak height is due to actual size variations in the particles themselves (Figure 7.14).

We then investigated the source of the remaining resolution limitations. Beyond particle
size, particle position also influences signal amplitude. As long as the channel height is
greater than that of the particle diameter, some systematic peak height spread will exist
due to z-position spread (Figure 7.15), but physically constraining particle height limits the
spread of peak heights due to vertical positioning (Figure 7.16). This increases the counter
resolution for particle size distinction.

By measuring a mixed sample of definable particle sizes (such as polystyrene beads)
to characterize the extent of peak height spread in a population due to vertical height
differences, we can better attribute peak height spreads to real size differences in more
heterogenous biological samples.

Besides size and position in the channel, material conductivity is the main particle
property that can influence measured peak height. We anticipate that biological cells will
exhibit greater variation in conductivity within a cell subpopulation and thus have an
intrinsically lower resolution for size-based discrimination than polystyrene beads.

Having verified the ability to simultaneously operate DEP and count and distinguish
particle subpopulations electronically on-chip in real time, we next utilize our integrated
coulter counter to quantify the DEP response of different cell types from a population of
mixed cells.
8.1 Motivation for activated T-cell detection and purification

Clinical diagnostics often operate by detecting the presence of a specific biomarker or pathogenic agent. Instead of directly detecting the presence of a certain pathogen, an alternative diagnostic strategy takes advantage of the immune system’s extensive ability to detect pathogen presence.

Certain immune cells in the body present surface molecules or release cytokines in response to pathogen presence. One type of immune cell that responds to the expression of these surface-bound stimulatory molecules or released cytokines is called T cells. T cells can be divided into three major categories according to function: cytotoxic T cells, helper T cells, and regulatory T cells. Prior to encountering an activating antigen signal, an immune cell subpopulation called helper T cells exist in a quiescent or naive state. Naive CD4+ T cells differentiate in response to these stimulating antigen signals into several types of T cells, each performing different functions and showing distinct surface marker expressions and cytokine secretion profiles. During this process of T-cell activation, T cells grow in size and increase their rate of proliferation as they are prepared for antigen-specific effector response.
The state of T-cell activation in a body is a critical immunological indicator of the presence and progression of disease states [116]. In the case of an infection, detection and quantification of activated T cells serves as an indicator of the presence and severity of the infection. Activation of the immune cells in response to pathogen presence in the body can therefore be monitored as a method to detect the presence of an infection or as a quantification method to evaluate treatment efficacy. Current methods for assessing disease through specific immune activation requires knowledge of the cognate antigen, the use of antibody-based immunostaining, or measurement of transient ion fluxes [116]. These techniques are limited by either costly processes, lengthy processing times, or signal transience and are therefore of more limited use for rapid, accessible, point-of-care approaches [2].

Several groups have reported promising advancements toward a fully electronic T-cell assay. Mishra et al. demonstrate on-chip impedance detection of CD4+ T cells bound to antibody-functionalized surfaces [117,118]. Watkins et al. take a similar approach but do total cell count before and after CD4+ T cell binding to antibody-functionalized surfaces occurs [119]. Also utilizing an antibody-functionalized surface, Cheng et al. isolated CD4+ T cells from 10µL of whole blood with cell affinity chromatography [120,121]. Captured T cells were quantified by conventional optical microscopy. After lysing red blood cells from a blood sample, Holmes et al. perform impedance detection on white blood cells. By using two frequencies for measurement, they are able to distinguish between T cells and monocytes and neutrophils [122].

In this chapter, we present a fully label-free device for continuous electronic DEP separation of activated and naive T cells in physiological conductivity, with integrated on-chip electronic quantification of both populations as a methodology of immune state assessment. To our knowledge this is the first reported DEP device designed for electronic label-free detection and enrichment of activated T cells.

This work was done in collaboration with Dr. Zachary Kobos and Dr. Patrick Han.
8.2 Device design and experimental setup

When activated, T cells undergo several changes that result in an increase in size. As demonstrated in Chapter 6, differences in particle sizes can be utilized to separate particle subpopulations. Though the drag force on particles increases linearly with particle radius, the DEP force increases with the radius to the third power. Beyond electronic label-free purification of T-cell populations, however, we also seek to provide an on-chip detection and quantitative measure of the concentration of each cell population. As demonstrated in Chapter 7, an on-chip coulter counter can be used to detect particles and even give indications of differences in particle sizes.

Here we combined these two electronic label-free methods of sample purification and quantification onto a single microfluidic device to first enrich activated T cells from unactivated T cells and then measure the concentration of each subpopulation. Both of these functions are done on a single microfluidic chip, all electronically, and without need for cell labeling or cell contact with any surface or binding molecules.

Figure 8.1 shows the basic chip design used for these experiments. A mixed sample of cell types is introduced into a left-hand microfluidic inlet, while a buffer solution is put into a right-hand inlet. The buffer solution is used to focus all of the cells onto the left side of the microfluidic channel prior to their arrival at a DEP separator (Figure 8.1b). An applied AC voltage on the separator electrodes provides lateral force on the cells, preferentially moving some cells more than others (Figure 8.1c). Downstream of the separator the microfluidic channel bifurcates into two channels, bringing cells in either half of the channel to one of two coulter counters (Figure 8.1d). Each coulter counter has a voltage applied to the center of three electrodes and a differential voltage is extracted from the two side sensing electrodes, relative to the sensing electrode. The difference in this extracted voltage between the two pairs of electrodes is amplified and fed into a lock-in and then output to an oscilloscope (Figure 8.1e). Data collection from the oscilloscope and analysis is done with a MATLAB program in the manner described in detail in Chapter 7. The only difference in the coulter counter setup from that work to this is the utilization of two coulter counters simultaneously instead of a single one. A modified chip-interfacing PCB and duplicate counter measuring
setup are therefore used to enable simultaneous data collection from both counters.

Figure 8.1: Chip design for enrichment of activated T cells from mixed population of activated and unactivated T cells with downstream quantification of activation.
8.3 Examination of T-cell activation

Before testing T-cell separation, the two cell populations of interest are first generated and then molecular indicators of activation are studied for each cell population to determine whether size-based differentiation is a viable method for T-cell activation state differentiation.

To obtain T cells, murine splenocytes were harvested. Red blood cells were lysed with ACK lysis buffer and the remaining cells stained with CSFE. CD4+ T cells were then isolated using negative selection kits (StemCell EasySep Mouse CD4). To produce an activated T-cell population, T cells were then exposed to anti-CD63 and anti-CD28 in culture for up to three days. After three days, another sample of murine T cell was harvested and purified in the same manner, but without any activating stimulus. We refer to this population of cells that are not given activation stimulus as naive or unactivated T cells.

Both cell populations were then studied to assess the viability of utilizing size disparity as a metric for T-cell activation. Images showing the size of T cells not subjected to an activating stimulus and those that have been activated for three days are shown in Figure 8.2a and 8.2b, respectively. While a large size variation exists, there is a clear increase in the average cell size in the T-cell population stimulated with anti-CD63 and anti-CD28.

An indication of cell size distribution was also obtained with FACS. In a FACS measurement an output voltage is proportional to the intensity of light incident on a detector. Detectors positioned in line with the light source collect forward scattered light (FSC) while a detector placed at 90° from the light path collects side scattered light (SSC). The intensity of FSC is proportional to cell diameter while the intensity of SSC gives an indication of the granularity of a cell. Figure 8.2c shows the SSC and FSC intensity for both prepared cell populations. From the FSC values we see that there exists a lot of overlap in the sizes of the two cell populations. This agrees with the images of the two cell populations in which no very large cells are seen in the naive cell population image, but a non-negligible percentage of cells in the activated T-cell population image are similar in size to the naive cells. From the images as well as the FSC data, we see that the activated cell population does show a much larger average size, as well as a much larger spread of sizes, than the naive cell
Several molecular indicators of T-cell activation were measured by FACS for each population as well. CD4 is a marker expressed by T-cell populations and CSFE was used as an inverse indicator of cell proliferation. As a parent cell population with CSFE divides, each subsequent generation will have less CSFE present and, therefore, lower CSFE signal intensity. The measurements of CD4 and CSFE for naive and activated cell populations are shown in Figures 8.2d and 8.2e respectively. The merged plot in Figure 8.2f shows that cells in both populations show similar levels of CD4 expression, but the activated T-cell population has lower CSFE intensity, indicating higher rates of proliferation for these cells.

CD69 expression was also measured as an early molecular indicator of activation. CD69 expression begins quickly after activation ($\leq 4hr$), making this a useful marker for distinguishing cells that have been stimulated to activation states but have not yet undergone all of the cellular changes that result in cell enlargement [123]. The overall DNA content of the cells were also measured using 7-amino-actinomycin D (7-AAD), which fluoresces when associated with DNA. FACS measurements of CD69 and 7-AAD are shown in Figures 8.2g and 8.2h for the naive T cell and activated T cell populations, respectively. The merged plot of the two populations in Figure 8.2i shows a clear disparity in both of these values between the two populations. T cells subjected to activating stimulus after harvest show higher expression of CD69 as well as greater intensity of 7-AAD DNA marker than cells not subjected to an activating stimulus.
Figure 8.2: a) Naive CD4+ T cells and b) CD4+ T cells that have been exposed to activating antigen for 3 days show disparity in average cell size. c) A FSC plot of both populations shows a larger spread of cell sizes for cells in the activated population. T-cell marker CD4 and CSFE proliferation stain for d) naive and e) activated cells and a f) merged plot show increased proliferation of activated cells. Early activation marker CD69 and DNA stain 7-AAD for g) naive, h) activated and a i) merged plot of both populations show a clear distinction in activation levels between populations and a greatly increased spread of 7-AAD for activated cells.

The disparity in the molecular signatures of the two cell populations and the corresponding disparity in the cell sizes between the two populations demonstrate the viability
of using T-cell size as a first level differentiation metric for activation. Interestingly, the overlap in these indicators with the measured values of the cells not stimulated is less than the overlap seen in the cell sizes.

The clear elevation in the early activation marker CD69 indicates that the vast majority of cells in the activated T-cell population have indeed experienced activation signaling. The significantly wider spread in the DNA content and clearly decreased CSFE intensity of the activated T-cell population indicate that these cells are quickly proliferating but are at a wide range of stages of cell division at any moment in time.

This possible explanation for the wide spread of cell sizes present in the activated cell population would suggest that a size-based cell selection method would gather only the larger of the T cells from the activated T cell population. The overlap in sizes between cells of the activated and unactivated populations shows that an upper limit of either harvest efficiency or purity of separation exists below unity. As a metric for overall activation state, the concentration of this larger subportion would reflect the extent of activation and proliferation and would therefore still reflect the overall extent of T-cell activation. Thus the use of size-based differentiation as a method of immune T-cell activation state assessment remains valid.

8.4 DEP response of T cells in high conductivity

Before attempting to do any separation of two populations of T cells we first need to determine if we can successfully manipulate T cells with DEP and, if successful, explore the range of operational conditions for doing so. Here we begin by mapping the relative DEP response magnitude for naive T cells.

Murine T cells were harvested and isolated as described previously with no activating stimulus given. Cells were stained with 1.5µL of 5mM CSFE stock solution per 200µL of cells in a 50:50 mixture of T-cell media and 1xPBS. After incubating and being protected from light for 20 minutes at room temperature, an additional 1000µL of T-cell media was added and the sample was centrifuged at 200g for 7 minutes. Cells were resuspended in a solution of T-cell media supplemented with 8.5% sucrose, 30% Ficoll-400 (Dot Scientific Inc.) to
increase density to reduce cell settling during long experiment times, and 1% Pluronic F-127 (Sigma) to reduce cell adhesion. Solution conductivity and density were measured to be 1.206 S/m and 1.0824 g/mL, respectively. Viability was examined by staining with Trypan Blue and counting in a hemocytometer and measured 96.1%.

Loaded into a syringe, suspended cells were introduced by syringe pump into a 1 mm x 18.2 µm microfluidic channel over an interdigitated DEP electrode array with a characteristic dimension of 50 µm. Electrodes were oriented at 90° with the respect to the fluid flow direction. Pumped continually at 0.4 µL/min (yielding an average linear flow speed of 366 µm/sec), T cells were subjected to applied AC signals of varied frequency.

In the method of CM mapping shown for bacterial cells in Chapter 6, cells were either captured by DEP or were not captured and the relative magnitude of DEP force was extracted from the relative number of captured cells for varied applied frequency. As it requires final forces strong enough to capture particles from fluid flow, this system is best suited for examination of positive DEP rather than negative DEP forces. Because we sought to operate in physiologically relevant high conductivity solutions which generally yield negative DEP forces for biological cells, we used our developed method of measuring a relative DEP force that works for both positive and negative DEP regimes.

This method of extracting a relative DEP force magnitude from the change in particle velocity is described in detail in Chapter 4. Briefly, for each applied signal several videos were captured of cells flowing down the channel and over the DEP electrodes. The DEP electrode array was positioned to begin about halfway down the field of view for the videos as shown in Figure 8.3a. Cells were then tracked throughout the videos and their DEP response trajectories analyzed using video processing and analysis MATLAB programs written in-house. Because of the positioning of the field of view incorporating a section of channel without and with the DEP electrode array, every particle tracked throughout both halves of the field of view contains its own control data. When in the top portion of the field of view before the electrode, there is effectively no DEP signal applied to the particle. As a cell continues on over the array, the DEP signal is effectively introduced to the particle. The particle will experience a change in its velocity that is dependent on the magnitude of the DEP force. Particle velocity is fit for the region of no DEP signal between the top
of the field of view until 200µm before the start of the electrode array (shaded red region in the image). This distance is 2 x the electrode period to make sure that the fit does not incorporate the beginnings of particle responses to the applied electric field. The particle velocity during DEP signal is fit from 500 to 600µm after the start of the electrode array to allow particles time to respond (shaded blue region in the image).

Figure 8.3b shows the velocity of each tracked particle for a single video taken with a 6MHz signal applied to the DEP electrodes. Within the region of no DEP signal (red area on the plot), each particle’s velocity is relatively constant. As the particle nears the start of the electrode array (marked with a dashed line), particle velocity changes in response to the applied electric field. By the time these particles reach the zone shaded blue, they have experienced an overall decrease in velocity.

Fitting the velocities in each of these zones of signal off and signal on, we can plot a histogram of the particles velocities in each region of interest. For the case when no DEP signal is applied to the electrodes, the velocities of particles in each region are very similar because no DEP force exists to alter particle movement (Figure 8.3c). When a signal is applied, however, there is a shift in velocity between the two regions (Figure 8.3d). For all the particles in all the videos taken for a given condition of applied voltage and frequency, we calculate the ratio of the change in velocity divided by the original velocity when no signal is applied for the whole population of particles as

\[
\frac{\Delta v}{v_0} = \frac{\bar{v}_0 - \bar{v}_{on}}{\bar{v}_0}
\]  

(8.1)

where \(\bar{v}_0\) and \(\bar{v}_{on}\) are the population averages of the particle velocities during signal off and on, respectively. The error of each individual particle velocity fit function is propagated through the population averaging.

This velocity ratio is plotted for a range of frequencies of applied AC signals in Figure 8.3e. In this figure the two peaks at 0.46MHz and 1.7MHz show that the DEP effect is greatest at these frequencies for naive T cells in this high conductivity solution. The notable decrease in velocity ratio around 1MHz indicates a crossover from one DEP polarity to the other between the two peaks. This is somewhat unexpected given the conductivity of this
solution (1.206S/m) and the CM maps we have modeled for other cell types. The control data point for videos taken when no DEP signal was applied yields a velocity ratio of 0.0005 with an error bar of 0.0098.
Figure 8.3: Frequency map for naive T cells in high conductivity. a) Image of the beginning portion of a DEP electrode array showing the regions of velocity extraction when particles feel no DEP force (shaded red) and after experiencing DEP force (shaded blue). b) Velocity traces for particles show a decrease in velocity as they encounter the DEP electrodes. Histograms of velocity comparing population velocity distributions in the signal off region and signal on region when c) no voltage is applied and d) when a 20V signal is applied. e) The velocity ratio as a metric for relative DEP force shows clear frequency dependence even at high conductivity.
Because this method of mapping relative DEP force relies on velocity changes which would occur whether there was pDEP or nDEP forces, we need to determine which of those two regimes the cells are experiencing at the high frequency regime. In other work, DEP polarity has been determined by the equilibrium position of particles relative to an electrode structure such as a IDE electrodes [67], quadrupole electrodes [18], or castellated designs [29, 100, 124].

To do these experiments, particles in solution are passed over an electrode structure with no signal applied. After signal is applied, particles are allowed to positionally equilibrate for some length of time. When DEP forces are small, this equilibration can take a long time but also might never provide enough force to actually result in the movement of the particles in solution. To help overcome the limitation of DEP polarity mapping for small force magnitudes, we designed a DEP device that brings a particle over each area at which it might experience maximum force and then gradually directs the particle in that equipotential channel to a parallel array to be measured (Figure 8.4a). This allows illumination of polarity when forces are smaller.

To accomplish this, we introduce cells over an array of interdigitated electrodes that initially have slight angles relative to the fluid flow (Figure 8.4b), but decrease until they run parallel with fluid flow (Figure 8.4c). The more parallel the electrode is, the less drag force component occurs along the direction of particle movement necessary to align particles with electrode features. By having the electrodes initially angled, particles flowing down the channel will encounter the electrode feature at which they experience maximum DEP force without necessitating any competition with drag force to get particles to those locations. Particles experiencing positive DEP forces would feel maximum DEP force positioning them at the edges of the electrodes, while particles experiencing negative DEP forces will be positioned either between electrodes or over the middle of electrodes.

When no DEP signal is applied, the positional intensity of the T cells in the device show no clear alignment (Figure 8.4d). When a DEP signal at 20MHz is applied, however, T cells show alignment along the centers of the electrodes (Figure 8.4e). This positional intensity along the electrode centers indicates the occurrence of negative DEP force, at these conditions of high frequency and high conductivity.
Figure 8.4: DEP polarity mapping for unactivated T cells. a) a diagram of a microfluidic DEP device in which b) tapered electrodes have a gradually decreasing gap spacing until they become c) an interdigitated electrode array running parallel to the direction of fluid flow. Monitoring the position of particles over this array when there is d) no DEP signal applied and when a e) DEP signal is applied (at 20MHz) maps out the final positions of cells, indicating whether they experience negative or positive DEP force or insufficient DEP force to come into alignment.

Having demonstrated the successful manipulation of T cells with negative DEP forces in high conductivity solutions, we now test the ability to electronically detect and discriminate T-cell sizes with our on-chip coulter counter also operating in high conductivity solutions.

8.5 Coulter counter detection and discrimination of T-cell activation states

In order to test the coulter counter’s ability to detect T cells and quantify the extent of T-cell activation in a sample of mixed T cells, we generated T-cell populations without and with activation stimulus as described previously. Cells were left unmixed and resuspended in 1xPBS supplemented with 0.1% Pluronic F-127 (Sigma) to limit cell adhesion. Each population was separately introduced over a coulter counter device (diagrammed in Figure

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8.5a) with a fluid constriction cross section of 50µm wide and 20µm high. Cells were pumped at 0.4µL/min yielding an average linear flow rate of 6.7mm/sec through the counter constriction region. Counter data was acquired as previously described in Chapter 7. Briefly, a 100mV70kHz signal was sourced onto the center of three electrodes and the differential voltage between each side electrode and the center was extracted. The difference between these two values is amplified on board, detected by Lock-in, and exported for analysis.

After 3 days of activation, T cells exhibit a change in size. With a larger displaced volume in the coulter counter sensing region, activated T cells produce a much larger differential voltage signal (to the third power of the increase in cell diameter). Figure 8.5b shows a representative differential voltage trace for a cell from the naive T cell and the activated T cell samples.

The heat map generated from the cube root of the peak height voltage and the transit times for both populations combined shows two population clusters (Figure 8.5c). While there is certainly some overlap in values between subpopulations, with reasonable thresholding, statistics on the two populations can be extracted, in the same way a FACS readout is binned for two related factors. A histogram of the cube root of the peak heights as a metric for particle diameter shows the presence of two populations with some overlap (Figure 8.5d). Thresholding between these two peaks allows for extraction of an estimation of the concentration of each subpopulation as well as the average and spread of cell sizes for each population. In both populations there also occurs an additional peak at the lower values of relative particle size.
Figure 8.5: Coulter counter discrimination of activated T cells from unactivated T cells. a) A mixed population of T cells pass over the counter eliciting b) larger differential voltage responses for the larger activated T cells. Extraction of the population distribution of c) peak heights and transit times and d) the number of cells counted with different peak heights allow quantification of the concentration of the two cell populations.

To verify whether the two populations that show up in the heat map and the histogram of peak heights and transit times actually reflect naive and activated T-cell subpopulations, and to determine the origin of the small particle peak, the ratio of each population was altered and the measurement repeated. The histograms for a 1:1 and a 2:1 ratio mix of naive to activated T cells are shown in Figure 8.6a and 8.6b respectively. The extracted cube root of the relative change in voltage shows the presence of three major subpopulations. By increasing just the amount of naive cells in the second measurement, it can be determined that the second peak, marked in blue, corresponds to naive T cells. The larger activated T cells compose the third peak.
The notable stability in the first peak when naive cell concentration is doubled indicates that the contents of this peak may originate from either the activated T-cell sample or the mixing buffer. The relative size of this lower peak in Figure 8.5d in the activated T-cell population compared to its more limited prominence in the unactivated T-cell population also suggests its origins relate to the activated T-cell population. We therefore attribute the first peak to cellular debris. In general the viability and stability of the activated T-cell population is much lower than that of the unactivated cell population. Thus the appearance of cellular debris occurring primarily in the activated sample is not very surprising.

\[ (a) \quad (b) \]

![Figure 8.6: Coulter counter histogram of the cube root of relative peak heights for mixed population of cells with a) a 1:1 and b) a 2:1 ratio of cells from naive vs activated populations](image)

In our initial examination of the two populations of T cells, there was a question of the cause of the great disparity in sizes seen in the activated T-cell population. Recall that the overlap in cell sizes between the two populations seemed to be greater than the overlap in the measured activation metrics between the two populations. In order to study the progression of T-cell size change after activation, T cells were stimulated for 0, 1, 2, and 3 days and profiled with the coulter counter. Figure 8.7 shows the distributions of the cube root of the relative change in the coulter signal for each of these samples.
In the naive T-cell distribution, there is notable absence of the activated T-cell peak. By one day of activation, there can be already be seen the development of a shoulder towards the activated T-cell peak location. At day two of activation, there exists two distinct and populations present - unactivated and activated cells - both in substantial proportions. Here we also see the relative magnitude of the debris peak begin to increase. By the third day of activation stimulus, the presence of unactivated cells has diminished dramatically and the debris peak has increased further. There does seem to exist a slight remaining population of small cells within the three day sample, however, as the peak at the unactivated cell size never disappears completely.

In the image of 3-day activated T cells presented earlier (Figure 8.2b), there was also a portion of cells of a smaller size than the more predominant large T cells. If those smaller cells are activated cells in an earlier state of division, this data showing the increase in the proportion of large T cells over time indicates a time dependent shift in the average cell size in a population of T cells exposed to activating signals. For the remainder of experiments utilizing activated T cells, therefore, we activate cells for 3 days to ensure a high proportion of the large activated T cells.

The data obtained from the on-chip coulter counter measurements not only serve to quantify cell concentration, but also to illuminate the activation state of the individual cells.
measured. At the population level, these measurements can be used to quantify the extent of activation that a population of T cells has undergone. Electronic and label-free, this characterization method presents a much less costly approach by which T-cell activation can be roughly assessed compared with conventional FACS measurements. We now seek to achieve a similarly accessible analogue to the other major FACS function - physical population sorting.

### 8.6 Electronic separation and on-chip quantification of naive and activated T cells

Having demonstrated the ability to manipulate T cells with DEP in high conductivities (8.4), shown the ability to separate particle populations by size (6.5), and explored the validity of using T-cell size discrimination as a basis for first-level assessment of T-cell activation states (8.3 and 8.5), we now seek to take advantage of the increase in T-cell size that occurs after activation to utilize DEP to separate activated T cells from unactivated cells and electronically quantify each population.

Unactivated and activated cell populations were prepared as described previously, with three days of activation for the activated cell population. After counting to determine concentration, cells were mixed, centrifuged at 200g for 7 minutes, and resuspended into media to obtain the desired final cell concentration of both cell types. The suspending media was a T-cell media composed of RPMI 1640 (Invitrogen) with 10% heat-inactivated FBS (HyClone), 10mM Hepes, 1% non-essential amino-acids, 2mM L-glutamine, 1mM sodium pyruvate, 0.05mM β-mercaptoethanol, and antibiotics. Final cell concentrations used were typically between $1 \times 10^6$ and $3 \times 10^6$ cells/mL of each cell type. Cells from the unactivated cell population were fluorescently labeled with Cell Trace Yellow and cells from the activated population were fluorescently labeled green with CSFE.

The mixed cell population was introduced into the left-hand inlet of the DEP separator at 0.2µL/min while the same suspending buffer without any cells was introduced into the right-hand inlet at 0.5µL/min. With a channel cross section of 1mm × 18.8µm this yields a net average linear flow speed of 621µm/sec over the DEP device and an average linear
flow speed of 6.2mm/sec over coulter counter constriction region which each have a cross section of 50µm × 18.8µm.

T-cell separation using negative DEP to preferentially induce lateral motion for activated T cells takes advantage of the increase in cell size that activation incurs. The DEP separation structure was an array of interdigitated electrodes with a characteristic dimension of 40µm, oriented at 45° to the direction of fluid flow, fabricated in 285nm of gold with a 15nm titanium adhesion layer, on a silicon substrate with 2µm of thermally grown SiO₂ between the silicon and electrodes. A DEP signal was sourced at 1.3Vpp before about a 10x amplification gain from two channels at 6.8MHz, 180° out of phase with each other, and applied to each electrode circuit. Each center coulter counter electrode received a 100mV, 70kHz signal.

With the DEP signal off, both cell types proceed down the left side of the microfluidic channel over the DEP electrode array and to the left of the channel bifurcation and through the left hand coulter counter. Images in fluorescence for each fluorescent label were captured at the channel bifurcation to show the positional spread of each cell population. About 200 images were cleaned to remove background noise and then stacked to create a cumulative image shown in Figure 8.8a. Here both cell types (unactivated cells appearing red, activated cells appearing green) progress down the left side of the channel. In Figure 8.8b a histogram of the positional intensity of each cell type shows that none of the cells go down the right half of the channel. Likewise the coulter counter data acquired simultaneously from each counter shows the presence of the smaller unactivated cell peak and the larger activated cell peak on the left counter, while the right counter shows only the small background peak (Figure 8.8c).

Turning the DEP signal on, larger T cells from the activated population begin to move laterally across the channel towards the right. At the channel bifurcation, the cumulation of images show all green activated cells have moved to the right half of the channel (Figure 8.9a). The majority of the red cells from the unactivated population proceed down the left half of the channel. Some, however, have moved far enough to the right to proceed down the right channel after the bifurcation. A plot of the positional intensity for each population is shown in Figure 8.9b. This clearly illustrates the successful lateral movement of activated
T cells to the right channel along with a small portion of the red unactivated cells. The
coulter counter data collected during DEP signal application for each channel is shown in
Figure 8.9c. In the left channel we see a large peak of unactivated cells and a small shoulder
of larger activated cell sizes. The right-hand coulter counter data shows a large activated
cell population, as well as a small unactivated cell population.
Figure 8.8: With no DEP signal applied to the separator, a) cumulative optical images show the trajectories of cell from the unactivated (red) and activated (green) T-cell populations all proceeding down the left side of the channel. b) Positional intensity histogram of each population shows all cells present on the left. c) Coulter counter cell counts from the left-hand channel show both cell types prominently, while the counts on the right-hand channel show only the small background peak.
Figure 8.9: With a DEP signal applied to the separator, a) cumulative optical images show the trajectories of cell from the unactivated (red) T-cell population proceeding mostly down the left side, and some down the right side while all of the activated (green) T cells proceed down the right side channel. b) Positional intensity histogram of each population shows the enrichment of activated T cells in the right channel. c) Coulter counter cell counts from the left-hand channel show predominantly naive T cells, while the counts on the right-hand channel show predominantly larger activated T cells.

From the optical data, we can calculate the harvest efficiency and purity of harvested ac-
tivated T cells as a function of the channel bifurcation position. The plot of these separation efficiency metrics for when there is no DEP signal applied (Figure 8.10a), illustrates that for any harvest efficiency above 10%, the sample purity will only be 50%. This means that the collected cells have no activated T-cell enrichment compared to the original sample. When the DEP signal is turned on, however, activated T cell are enriched in the collected fluid. For our channels which have a bifurcation 50% of the way across the channel, our harvest efficiency is 88.4% with a purity of 83.7%. Since both metric are never simultaneously at 100%, a tradeoff between the two exists. Depending on the application, sacrificing harvest efficiency for purity or vice versa may be more optimal.
Figure 8.10: Harvest efficiency and purity measurements from optical data for activated T-cell separation for DEP signal off (a) and DEP signal on (b)

8.7 Chapter summary

Towards the goal of developing low-cost diagnostic alternatives, we have developed a device to assess the state of immune activation by measuring concentrations of activated CD4+ T cells from mixed population of activated and unactivated CD4+ T cells (Figure 8.1).

We discussed the validity of utilizing size-based discrimination to separate activated
T cells. We show the ability to continuously separate label-free naive and activated T cells in solutions of physiological conductivity with a lateral nDEP separator (Figure 8.9a). Separated activated T cells are directed to a different outlet channel than unactivated cells. A coulter counter integrated into each channel enables real-time on-chip quantification of T-cell sizes and DEP separation performance (Figure 8.9c). Coulter counter performance is validated with varied mixed ratios of naive murine T cells and activated T cells (Figure 8.6) and for T cells at varied states of activation (Figure 8.7). Activated T cells were enriched with a harvest efficiency of 88% and a purity of 84%.

To our knowledge, this is the first reported device designed for label-free DEP enrichment and electronic detection of activated T cells. We envision this system to have broad applications both for bench-side quantification of T-cell activation responses as well as bedside assessment of infection presence and monitoring of disease state progression.

For any bedside or bench-side measurement, sample throughput is a critical concern. We have previously demonstrated the performance tradeoffs incurred when increasing throughput through solution conductivity dilution, channel cross sectional area increase, or fluid speed increases. In the next chapter we present preliminary work for device designs and methods for increasing throughput without altering effective DEP forces, solution volume, or fluid flow speeds.
Chapter 9

Throughput enhancement techniques for real-world DEP applications

In this chapter we present several designs and show preliminary results for increasing device performance and throughput.

9.1 Constrictionless counter counter to enable higher throughput

In Chapter 7 it was shown how the sensing ability of a coulter counter depends on the fraction of solution volume displaced by a passing particle. Narrowing the fluid in a channel to shuttle all particles over a very narrow fluid constriction region over the coulter sensing electrodes allows particles to replace larger volume fractions of the sensing fluid volume. The sensing volume is constrained by the channel dimensions and particles are brought to the counter by spatial confinement. This confinement brings several challenges such as higher probabilities of constriction clogging, throughput limitations due to sampling rates limitations, increased fluid pressure, and increased shear stress on passing particles.

As an alternative we present a device that takes advantage of the distance-dependent
decay of electric fields to constrain the counter’s sensing volume and brings particles over the counter by lateral DEP movement. By locating the counter at the very edge of the microfluidic channel, the sensing width is defined by the electrode length into solution rather than the channel width. The rapid decay of the electric field with the square of the distance limits the fluid volume that the counter can sense. Angled DEP electrodes can shuttle particles over the channel wall and the counter, either selectively or ubiquitously. Figure 9.1a shows a diagram of the device design. An image of a fabricated chip is shown in Figure 9.1b. A trace of a passing 4.5µm bead suspended in 0.01xPBS is shown in 9.1c, demonstrating the counter’s ability to acquire quality signals even without the mechanically constrained sensing volume.

Figure 9.1: Diagram of a constrictionless side counter device design utilizes DEP to shuttle particles over an electrically confined sensing volume. b) A fabricated device used to c) detect 4.5µm beads in 0.01xPBS

By allowing constrictionless counting, this design will help overcome the challenges incurred by traditional coulter counting under fluidic confinement.
9.2 Positive feedback electrode designs to amplify particle separation

We designed special lateral movement electrodes to enhance particle separation. For any lateral movement, the force on the particle to move laterally increases. In this way, the electrode structure provides a positive feedback loop on any lateral movement, amplifying separation. Compared to a standard lateral movement structure (Figure 9.2a), we increase the x-component of the DEP force as a function of x position in the channel by gradually decreasing the gap width (9.2b), gradually increasing the electrode angle (9.2c), or by inputting a buffer gradient to increase the CM factor (9.2d).
Figure 9.2: A typical lateral movement DEP structure (a) and three designs to amplify particle separation by providing a positive feedback loop for lateral movement by gradually decreasing the gap width (b), gradually increasing the angle (c), or gradually decreasing the solution conductivity (d).

Devices were fabricated in gold with a titanium adhesion layer with an 18.8µm channel.
Polystyrene beads in 0.0001xPBS + 1% BSA + 1% Tween-20 were introduced over different lateral movement devices and their trajectories optically recorded and tracked. For parallel angled electrodes, which are most commonly used for lateral DEP, angles tested included 30, 45, and 60° with respect to the x axis. Figures 9.3a, 9.3c, and 9.3e show the particle trajectories for parallel electrodes angled at 30, 45, and 60°, respectively. While there is a difference in particle lateral movement between the devices, the velocity in the x direction does not change with the x position. Figures 9.3b and 9.3d show particle trajectories for our separation amplification devices with increasing angle and decreasing gap width, respectively. For these devices, there is an increase in lateral movement as a function of x position in the channel.
Figure 9.3: Conventional parallel electrode arrays oriented at (a) 30°, (c) 45°, and (e) 60° don’t show any difference in lateral movement as a function of x position. The separation amplification designs, however, with (b) gradually increasing angle or (d) gradually decreasing gap width show increased lateral movement as the particle moves laterally, providing a positive feedback system.
Chapter 10

Summary of achievements and contributions

Toward the goal of advancing the accessibility of DEP-based diagnostic devices in resource-limited settings, we have developed and experimentally validated a numerical system model for evaluating and optimizing DEP device design for clinically-relevant high conductivity and high throughput applications.

By integrating on-chip coulter counters in line with DEP structures, we realized fully electronic and label-free particle manipulation and quantification devices.

Using these devices, we developed the first fully electronic label-free assay for the purification and quantification of activated T-cell populations for the purposes of immune state assessment.

Advancing toward actualizing DEP-based clinically-relevant sample throughput capabilities, we designed and preliminarily tested several novel performance enhancement techniques. Among these are a constrictionless coulter counter that utilizes lateral DEP to focus particles over the counter electrodes, allowing for higher fluid throughput without issues of fluid pressure, constriction clogging, or sampling rate limitations.

By redesigning several lateral movement DEP electrodes to yield increasing lateral DEP force as a particle moves more horizontally in a channel, particle movement can be subjected to positive feedback, enhancing lateral separation.
In a field that is vibrant with continual advances, I hope to have contributed to a holistic approach to device design and optimization as well as by adding some device designs for novel capabilities into the DEP arsenal, so that we continue to collectively advance toward accessible healthcare solutions.
A master summation Haiku

*Boo bacteria*

*I haven’t slept in eight years*

*Tada! Microchip.*
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