Abstract

Electrochemical impedance for lab-on-a-chip diagnostics

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Access to healthcare remains a pressing challenge globally. Portable healthcare solutions reduce infrastructure- and cost-related barriers to access in these limited settings. Lab-on-chip solutions aim to miniaturize clinical laboratory functions with integrated electronics to provide desired portable healthcare solutions. Planar metal electrodes can perform a multitude of laboratory functions depend on chemical and physical treatment and input electrical stimulus while being fabricated at incredibly low costs per chip. The electrochemical impedance between two such electrodes can be used as a biosensing element and intimately couples into signal transmission capabilities. In this work, we investigate how electrical impedance governs and constrains performance for high-throughput, planar electrode lab-on-chip assays using dielectrophoresis and the Coulter principle to separate and enumerate biological targets in physiological conductivity.

Physical geometry and solution conductivity determine the electrochemical impedance arising between two planar electrodes in solution. Displacement of a volume of conductive solution by an insulating particle produces volume-dependent changes in particle impedance. We demonstrate this principle for planar electrodes and investigate the physical origins of performance-limiting parasitics and their impact over a range of solution conductivities. Aggregating data from many particles passing through a single counter structure, we establish the ability to discriminate amongst target particles of different sizes in a simple and readily-miniaturized system.

We then investigate DEP electrode arrays and the role electrochemical impedance plays in performance degradation at high conductivity and high throughput conditions. Changes in electrode geometry alter loading of the voltage source driving DEP capture, negatively impacting device performance. DEP electrode designs must be optimized with these con-
straints in mind. This understanding extends to recommendations on permissible thickness for protective coatings and device architecture trade-offs for high-throughput performance.

Combining the impedance-based cell counter with the understanding of DEP performance in high-conductivity solutions produces devices capable of separating and counting target specimens from physiological samples. We demonstrate the ability to separate un-activated and activated murine T-cells from within a sample and the ability to distinguish the two populations electronically with our counter. Integrating these functions into a single microfluidic device yields an assay to monitor systemic immune response in patients from lymphocyte samples. The separated T-cells may also be cultured and interrogated for the specific antigen triggering their response. Future efforts with an additional on-chip separation step to isolate the lymphocytes from whole-blood samples to eliminate the need for prior centrifugation or extend this separate-and-enumerate schema to additional biological systems of interest.
Electrochemical impedance for lab-on-a-chip diagnostics

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Chapter 1

Introduction

Despite continued advances in the state of global healthcare, infectious disease remains prevalent in the world today. These diseases are responsible for significant losses in disability-adjusted life years, a measure of health outcomes that incorporates both mortality and reductions in quality of life from less-than-perfect health [1, 2]. Reducing disease burden for the most prevalent diseases is a simple and effective method for improving the global human condition. Reduction in total caseload depends on prevention of new infections, recognition of infection within patients, and subsequently administering the necessary treatment. We want to replace traditional methods of detecting infectious diseases within patients with measurement techniques making use of integrated silicon electronics, colloquially referred to as a “lab-on-a-chip” approach and thereby expand the global reach of diagnostic medicine.

Diagnostic detection is the specific identification of the markers of an infectious disease within a patient. The markers may be the pathogens (disease-causing agents) themselves, or chemical signals or proteins produced by the body in response to the infection. Specific detection is confirmation of the presence of a particular infectious agent, e.g. tuberculosis.

Pathogen detection has been accomplished traditionally via microscopy or culturing of bacterial cells [3]. Both approaches pose their own obstacles. Microscope image analysis by a trained professional remains the standard of care in much of the developing world [4, 5]. In these environments, reductions in the prerequisite individual expertise and hardware have
already been made: microscopes obviated by smartphone cameras, doctors in the field by remote transmission of acquired images, a.k.a. telemedicine, etc. [6, 7]. Visual identification can confirm a suspected diagnosis but proves challenging facing unknown pathogens. Culturing takes a sample and amplifies the population of infectious agent over many cycles of reproduction. The significant scale in sample size allows small amounts of sample to be tested against many different chemical recognition methods to identify an unknown microbe [8]. However, culturing has a cost: the growth time of the microbial culture [8–10]. Furthermore, not every pathogen of interest can be cultured [11].

A new generation of diagnostic techniques emerged to overcome these limitations, notably Polymerase Chain Reaction (PCR) and Enzyme-Linked ImmunoSorbent Assay (ELISA) [8]. PCR extracts and rapidly amplifies specific genetic material within the sample [9,12,13]. The amplified material is then tested against a range of genetic recognition elements for pathogen identification. For pathogens which cannot be cultured or require long cultivation times, PCR is a significant upgrade [11]. ELISA techniques dispense the sample over an array of differing recognition elements [14]. Each region binds a specific analyte, if present in the sample. The first binding event enables binding of a secondary recognition element, modified to include a fluorescent tag. After a final wash step, the user measures a fluorescence intensity signal proportional to the initial concentration of target analyte in the sample.

1.1 Motivation

Device engineers can improve pathogen detection capabilities in three methods: reducing the required infrastructure [15], reducing the procedural cost [16], and reducing the time-to-diagnosis [10]. Strides made in any of these target areas produce significant benefits in terms of global healthcare access and outcomes [15–17].

Procedural cost and prerequisite infrastructure are commensurate, but not completely interchangeable, aspects of healthcare provision. Healthcare services exist on a market across many schemes for provider reimbursement [18]. Provision is therefore sensitive to
the cost of services weighed against the impact on patient outcomes. Reduction in cost lowers the threshold for marginal utility required to render a given procedure the rational choice on a traditional supply and demand curve. Reduction of cost for services leading to improved outcomes directly benefits consumers able to pay either cost. The consumer who is only able to pay the reduced cost benefits tremendously – treatment is now an available option.

Access to infrastructure also partially dictates healthcare outcomes. While new techniques can eliminate the need for human visual expertise, the need for a fully-staffed wetlab remains a significant barrier to access in underserved communities globally [15]. In regions where providers are scarce due to low density of population or capital, patients face long travel times or the prospect of limited available services, if not both. Reductions in the facilities required for diagnosis and treatment increase the capacity for providing care in these resource-limited settings.

Detecting pathogens sooner affords healthcare providers more time to intervene [10]. Bacterial bloodstream infections arising from trauma and medical procedures have a 50% mortality rate worldwide [19]. Treatment efficacy decreases dramatically as the infection spreads. Detection speed increases either by detecting at lower concentrations in the same time interval or achieving detection at the same target concentration in a shorter time frame. Engineering procedures for resource efficiency reduces barriers in terms of cost and infrastructure; engineering new procedures for enhanced sensitivity should lead to reductions in the time to diagnosis.

Integrated circuits deliver chips with excellent reliability and scalability while reducing per-device cost on an absolute basis. The advent of portable electronics has furthered the ubiquity and availability of processing power in our daily lives. Developing biosensing modalities with electrical read-out capable of interfacing with chip-based electronics directly addresses cost and infrastructure as barriers to healthcare access for millions worldwide [15].

Invented by Leland Clark and Ann Lyons, the blood-glucose sensor for diabetes monitoring is the canonical example for electronic biosensing [20]. Researchers continue to develop
novel devices and techniques. Antibody-based detection [1, 21, 22] schemes have found multiple embodiments for electrical read-out. Researchers have developed chip-level analogues of ELISA [23, 24] and PCR [25, 26]. As long as the impetus to improve healthcare provision remain, efforts to transduce biological interactions into electrical signals will continue.

Detection approaches which do not require sample treatment prior to the sensing step greatly simplify device use. Both ELISA and PCR require chemical pretreatments, a significant hindrance to portable implementations. Physical manipulation of the sample on-chip also promises reduction in detection times. Mechanical or electrical separation and concentration can perform a pseudo-culture by artificially boosting the density of a small sample by aggregating the target in a local region. Researchers have used this approach to reduce the time-to-detection of PCR-based technologies [27]. It also presents an avenue to isolate the target of interest from a particular environment for ease of sensing [10].

1.2 Outline and scope

This dissertation presents work done to improve different electrochemical sensing modalities in anticipation of their combination for true lab-on-a-chip device functionality, aiming to combine cell sorting and counting with specific detection of target pathogens from whole blood environments. I extend the research of this lab and biosensing researchers worldwide. The thesis is structured as follows:

Chapter 2 introduces the basic concepts of electrochemical circuits.

Chapter 3 discusses the development of our impedance-based cell counter and its use as a T-cell assay.

Chapter 4 elucidates the working principles for cell counting circuitry.

Chapter 5 presents the ramifications of capture circuitry parameters on capture performance.

Chapter 6 summarizes the work presented in this thesis, reviewing the progress necessary to realize single-stream diagnostic potential.
Chapter 2

Principles and origins of electrochemical impedance

2.1 Introduction

We aim to develop diagnostic devices integrated with silicon electronics, reducing required cost and infrastructure. To do so, our measurement electronics must interface biological elements in their native environment. Biology exists and happens within ionic solutions. We must understand the electrochemical properties of these solutions to understand their behavior as we implement our desired sensing techniques.

2.2 Impedance spectroscopy

Thévenin’s theorem states that for any combination of an arbitrary number of passive elements, their impedances may be combined until the entirety of the circuit’s impedance has been captured in a single, frequency-dependent equivalent, containing all the information necessary to compute the circuit’s response to a given input current or voltage signal. The inverse of this problem is encountered experimentally. We measure the unknown circuit’s impedance at a given frequency by monitoring the output voltage in response to an input
current signal at that frequency. Repeating this process over a range of frequencies maps the equivalent impedance as a function of frequency. This is the process of impedance spectroscopy.

2.2.1 Electrochemical impedance spectroscopy

Performing impedance spectroscopy on metal electrodes immersed in ionic solutions is also known as electrochemical impedance spectroscopy (EIS). EIS is a widely-used technique [28, 29] for characterizing material systems such as protective organic coatings on metal electrodes [30], rechargeable batteries [31–34], and fuel cells [35–39]. The substrate electrodes, coating materials, and other chemical treatments impact the observed electrochemical behavior. The researcher then proposes a circuit model to explain the electrochemical behavior [30]. The model also must be as simple as possible within acceptably small error. Structural properties such as coating adhesion and defects, interface reactivity, and solution permeability are then inferred from changes in the EIS results [40]. To develop the intuition for these attributions, we must understand the physical processes which take place at the metal-electrolyte interface and elaborate the surface science contained within.

2.3 Physical phenomena and their discrete-element representations

2.3.1 Electrodes

Electrodes are indispensable in the performance of EIS measurements. Electrodes are conductors, material through which current readily flows, that contact non-metallic circuit elements such as electrolytic solutions. Up to three electrodes are necessary for EIS: the working electrode (WE), counter electrode (CE), and reference electrode (RE). The working electrode is the metal electrode whose electrode-solution surface is being probed in solution [41].

Counter electrodes are large-area pseudoreference electrodes capable of sinking large
amounts of current as necessary to establish a stable solution potential [42]. The large area of the counter electrode compared to the working electrode ensures that the DC potential applied to the working electrode is influenced almost entirely by working electrode surface kinetics.

Reference electrodes establish in the solution a potential with respect to a known thermodynamic equilibrium [43]. In addition to true reference electrodes, quasi- or pseudo-reference electrodes are commonly employed. Quasi- or pseudo-reference electrodes function similarly in establishing a steady potential but do not provide a true equilibrium, and instead must be referenced back to some known equilibrium indirectly [44]. The most common example of a quasi-electrode system is a silver-silver chloride wire which can be used to establish a stable potential in solution for experiments. Pseudo-reference electrodes are readily fabricated and immersed in the experimental solution.

### 2.3.2 The metal-electrolyte interface

When a metal electrode is immersed in an electrolytic solution, an ionic double layer forms at the metal-electrolytic solution interface. Mobile charge carriers within the electrode gather near the surface and an ionic distribution within solution counterbalances that charge [45–47]. The ionic distribution includes ions adsorbed on the metal surface, a diffuse region incorporating solvated ions of both polarities, and neutral molecules which influence the interface interactions [45].

For an ideal metal electrode, no ions cross the metal-solution interface while establishing equilibrium independent of the potential applied across the solution and electrode [45]. Instead, charge accumulates both within the metal electrode and in the adjacent solution. These layers of charge form a capacitance whose value depends on the magnitude of the electric field at the electrode interface. One consequence of the field-dependent capacitance, arising from the thermodynamics of the interface, is the notion of a differential capacitance:

\[
-\frac{dq}{dE} \bigg|_E = C \tag{2.1}
\]
where $q$ is the surface charge density of the metal, and $E$ the electric field between the electrode and solution. This differential capacitance is highly nonlinear in the applied potential and reflects changes in the physical structure of the ionic distribution.

The Helmholtz Planes

In 1853, Hermann von Helmholtz proposed a model for the solution side of the interface comprised of two distinct planes of ions, henceforth the inner and outer Helmholtz planes [45]. The inner Helmholtz plane is comprised of adsorbed ions due to covalent bonding or van der Waals forces. Solvated and hydrated ions in contact with, but not adsorbed to, the metal surface form the outer Helmholtz plane. The differential capacitance is dominated by the contribution of the inner plane, typically $32-34 \, \mu F/cm^2$ for a wide range of sodium chloride concentrations.
Figure 2.1: a) Abstract depiction of the electrode-solution interface with both positively-(purple) and negatively-charged (green) ions, depicting the working electrode (WE), Inner/Outer Helmholtz Planes (IHP/OHP), diffuse layer, bulk solution region, and counter electrode (CE). b) The equivalent circuit model, oriented so that the spatial arrangement of the circuit elements matches their physical origin c) Abstract representation of the electrostatic potential profile as a function of vertical displacement from the electrode-solution interface.

Guoy-Chapman-Stern Layer

The diffuse double layer consists of ions, mobile in solution, which gather with sufficient charge density to counterbalance the portion of the metal electrodes surface charge not neutralized by the Helmholtz planes [45]. Electrostatic and thermodynamic interactions govern the behavior of the diffuse double layer outside of the Helmholtz planes. Mathematical description of the diffuse double layer is constructed through the combination of electrostatics (Poisson’s equation):
\[ \frac{d^2 \Psi(x)}{dx^2} = \frac{-\rho}{4\pi \epsilon_r \epsilon_0} \]  \hspace{1cm} (2.2)

and thermodynamics (Boltzmann’s equation):

\[ n_i = n_0i e^{-qz_i \epsilon_r \epsilon_0 \Psi(x)/kT} \]  \hspace{1cm} (2.3)

where \( \psi(x) \) is the potential at a distance \( x \) from the metal-solution interface taken relative to the bulk of the solution, \( \rho \) the charge density of the ions in solution, \( z_i \) the charge state of ionic species \( i \), and \( n_i \) the density of ions per unit volume for all points with potential \( \psi \). This model neglects the work necessary to for an ion to displace the solvation shell of another ion as it closely approaches the metal electrode. The model therefore cannot be applied at distances closer than the outer Helmholtz plane. Substituting 2.3 into 2.2 and introducing a summation over ion species:

\[ \frac{d^2 \psi(x)}{dx^2} = \frac{-q}{\epsilon_r \epsilon_0} \sum_i n_0i z_i e^{-qz_i \psi/kT} \]  \hspace{1cm} (2.4)

from whence:

\[ \left( \frac{d\psi}{dx} \right)^2 = \left( \frac{n^d}{\epsilon_r \epsilon_0} \right)^2 = \frac{-2kT}{\epsilon_r \epsilon_0} \sum_i n_0i z_i e^{-qz_i \psi/kT} \]  \hspace{1cm} (2.5)

And thus we find \( n^d \), the surface charge density of the electrical double layer, the total charge per unit area in the column of liquid extending from the metal-electrode interface to the bulk solution:

\[ n^d = \sqrt{\frac{2kT \epsilon_r \epsilon_0}{\sum_i n_0i z_i e^{-qz_i \psi/kT}}} \]  \hspace{1cm} (2.6)

And in the case of a simple monovalent system:
\[ n^d = -4kT \epsilon_r \epsilon_0 n_{0i} \sinh (qz_i \psi/2kT) \] (2.7)

The integral capacitance of the diffuse layer is simply 2.7 divided by the potential at the outer Helmholtz plane. The differential capacitance is then:

\[ C^d = -2q\epsilon_r \epsilon_0 n_{0i} \cosh (qz_i \psi/2kT) \] (2.8)

These capacitances are quite large and in series with the capacitances between the metal surface and the outer Helmholtz plane. Therefore, the capacitance between the OHP and the metal surface dominates contributions.

**The Debye Layer**

We need to understand the length scale of the diffuse double layer. It remains to be seen how the potential behaves as one moves into solution from the metal-electrode interface. The previous derivation of the diffuse layer differential capacitance considers the potential in solution to be a known independent variable. We seek an expression for the position dependence of the potential within solution. Combining 2.5 and 2.7, we find:

\[ dx = -\sqrt{\frac{\epsilon_r \epsilon_0}{8kT n_{0i}}} \text{csch} \left( \frac{qz_i \psi}{2kT} \right) d\psi \] (2.9)

Approaching the Outer Helmholtz Plane, the potential takes the form:

\[ \psi (x) = \pm \frac{4kT}{zq} e^{-\kappa x} \] (2.10)

The constant Debye-Hückel length, \( \kappa \), has been introduced, dictating the decay length of the electrostatic field due the space charge of the ionic layer. The Debye-Hückel length depends upon both the valence and concentration of mobile ions:
\[ \kappa = \sqrt{\frac{2n_0 z^2 q^2}{e_r \epsilon_0 kT}} = 3.28 z \sqrt{c_i nm^{-1}} \]  \hfill (2.11)

at 25 °C, where \( c_i \) is the molar concentration of the solvent ion. At distances beyond the Debye length from the outer Helmholtz plane, charges are effectively entirely screened by the mobile ion distribution in the Gouy-Chapman-Stern layer.

### 2.3.3 The Warburg element

Up until this point, we have considered the case that no charge crosses the metal/solution interface. Charge transfer at the interface can occur via reduction or oxidation of ionic species. To understand the signature of such phenomenon, J.E.B. Randles originally investigated the consequence of applying a small alternating potential to a liquid mercury electrode in an aqueous solution [48]. Consider a small concentration of ions in solution, which can react with a low concentration, \( C \), of ions dispersed in the aqueous solution, and identically-low (for simplicity) concentration of metal atoms in the liquid mercury electrode.

Applying a small sinusoidal voltage perturbation between the mercury electrode and ionic solution with radial frequency causes a small current flow at some phase with respect to the voltage signal. The harmonic current oscillation establishes sinusoidal variations in the concentration of the metal in the mercury. Drift-diffusion dynamics cause the amplitude oscillation to decay exponentially with distance from the interface. The derived ratio of the current to the voltage is:

\[ \frac{I}{V} = \frac{n^2 F^2 AC \sqrt{\omega D/2}}{RT} \sin \phi \]  \hfill (2.12)

where

\[ \cot \phi = 1 + \frac{1}{k} \sqrt{\frac{\omega D}{2}} \]  \hfill (2.13)

To reproduce this relationship with conventional circuit elements, Randles proposed
modelling the circuit as a series resistance and capacitance with frequency-dependent amplitudes:

\[ R_{\text{Randles}} = \frac{RT}{n^2F^2AC} \left( \sqrt{\frac{2}{\omega D}} + \frac{1}{k} \right) \]  \hspace{1cm} (2.14)

and

\[ C_{\text{Randles}} = \frac{n^2F^2AC}{RT} \left( \sqrt{\frac{D}{2\omega}} \right) \]  \hspace{1cm} (2.15)

Both terms bear a magnitude dependence proportional to the square root of the perturbation frequency, quite unlike their macroscopic circuit element counterparts. Ionic diffusion dynamics give rise to this dependency. Noting structural similarities between the two terms, the sum of their impedances may be rewritten:

\[ R_{\text{Randles}} + \frac{1}{j\omega C_{\text{Randles}}} = \frac{RT}{n^2F^2AC} \frac{1}{k} + \frac{RT}{n^2F^2AC} \sqrt{\frac{2}{\omega D}} (1 - j) = R_{\text{ct}} + \frac{Z_W}{\sqrt{\omega}} \]  \hspace{1cm} (2.16)

where the combined impedance has now been explicitly separated into terms with and without frequency dependence, and \( j \) is the imaginary unit. The first term in 2.16 is the charge-transfer resistance, \( R_{\text{ct}} \), which is dictated by the kinetics of the reaction occurring at the metal-electrode surface. The second term is the frequency-dependent Warburg impedance, \( Z_W \), arising from the diffusion of ions over a semi-infinite length from the metal-solution interface.

### 2.3.4 The constant phase element

The model of the double layer and diffuse ion regions predicts capacitive behavior at the metal-electrolyte interface, with impedances inversely proportional to the excitation frequency. Empirically, sub-unity power law coefficients have been observed, necessitating the concept of the constant phase element (CPE) in EIS analysis [49–51]. The impedance of
the constant phase element may be expressed:

\[ Z_{CPE} = \frac{1}{Q_0 (i\omega)^n} \tag{2.17} \]

Where \( n \) is a constant ranging from 0 to 1 and \( Q_0 \) the pseudocapacitance. The impedance of the CPE recovers resistive (capacitive) behavior in the limit \( n \) goes to 0 (1) but typically ranges from 0.8-0.9. The constant phase element phenomenon is thought to arise from physical inhomogeneities at the electrode surface, giving rise to a local dimensionality interpolant between 2- and 3-D [52–55].

## 2.4 Circuit models

Physical understanding of the processes at the metal-electrode surface provide the intuition for proposing circuit models for a system of interest. The following section will provide a brief overview of circuit models commonly found in the literature and how the data is represented.

### 2.4.1 Nyquist and Bode plots

In control theory, Nyquist plots are an efficient means of visualizing the stability of the system response [56,57]. Nyquist plots present the real and imaginary portion of the impedance on the x- and y-axis, respectively, as shown in Fig. 2.2a. The surface phenomena probed via EIS are typically capacitive in nature. Capacitors have negative reactances and thus the imaginary component of the impedance is traditionally inverted when presenting the data. Each datapoint of the Nyquist plot is the response at a single frequency frequency varies along the curve. An ideal resistor has a strictly real, frequency-independent impedance. Its Nyquist plot is a single dot along the x-axis, whereas a lone capacitor produces a vertical line approaching the origin as frequency increases. In contrast, the Warburg element produces a line of unity slope. In the realm of electrochemical impedance spectroscopy, charge transfer processes manifest as semicircular arcs modeled as a parallel combination of a
resistor ($R_{ct}$) and a capacitor ($C_{DL}$). The radius and x-intercepts of these arcs contain valuable information about the reaction process.

Nyquist plots highlight the presence of time constants from charge transfer processes in the EIS spectra. In contrast, Bode plots make the frequency-dependence of the impedance explicit, as can be seen in 2.2b. This representation is convenient for predicting the system response to a given input signal in either the time or frequency domains. The choice of presentation depends upon conventions in the field as well as the aspect of the information that needs to be conveyed.

Figure 2.2: a) Nyquist plot of the impedance of the simplified Randles circuit shown inset. Datapoints taken at increasing frequency move counterclockwise. b) Bode plot showing the real and imaginary components of the impedance response as a function of frequency.

2.4.2 The Randles circuit

The Randles circuit is the fundamental circuit model employed for analysis of electrochemical circuits. Alternative models encountered in the literature are variations on the Randles model with increasing amounts of complexity as dictated by the physical realities of the system, as in Table 2.1. The Warburg impedance, $Z_W$, and the charge-transfer resistance, $R_{ct}$ are placed in parallel with the interfacial capacitance of the ionic double layer [48]. These impedance elements, representing the surface phenomena of the system, are in series
with a solution resistance, $R_s$, governed by the bulk conductivity of the electrolyte solution.

Figure 2.3: a) Randles circuit without a Warburg element (rapid diffusion condition). b) Diffusion-limited Randles circuit. c) Nyquist plot illustrating the influence of the Warburg element on the impedance signature.

The surface redox reactions are typically assumed to be completely reversible \cite{45,58,59} is that of the rapidly-reversible reaction. If the kinetics are rapid enough, the coefficient of the Warburg element is assumed to be negligible with respect to the charge transfer resistance, further simplifying the circuit behavior. Fig. 2.3a illustrates the Randles circuit model with the Warburg impedance incorporated, and Fig. 2.3b demonstrates the ramifications of this assumption for the Nyquist plot.

2.4.3 Further variations

Redox-less EIS

In the absence of redox reactions at the electrode-solution interface, the charge-transfer impedance ($R_{ct}$) of the Randles’ model becomes effectively infinite under normal operating conditions. When this condition is satisfied, such as in the absence of redox-active species or in the presence of a protective insulating layer, the circuit model for the interface simplifies greatly. The double-layer impedance in series with the solution resistance comprises the
Table 2.1: Common variants of the Randles circuit and the physical phenomena differentiating amongst them.

**Embedded Randles’ circuits**

Particularly in the study of multi-layered coatings, multiple redox reactions will appear between the solution and the working electrode [28]. Depending on the nature of the
system, these may appear as either sequential [22] or embedded [28] copies of the single Randles’ circuit when modeling the device performance data, as shown in Table 2.1.

**Alterations of the double layer**

The sample fabrication process also alters the circuit model necessary to effectively capture device behavior. The double-layer capacitance term in the Randles’ model may need to be replaced with a constant-phase element to effectively capture the surface kinetics, depending on the geometry of the working electrode.

### 2.5 Alternate geometries for EIS

Conventional implementation of EIS results in a macroscopic, layered hierarchy as current flows from one electrode to another. A different paradigm is required for studying microscopic phenomena with EIS [22, 60, 61]. Researchers turned to interdigitated electrodes (IDEs), fabricated with gaps as narrow as a few microns [22] to provide a new impedance sensing element. The small gap sizes greatly mitigates the influence of ion diffusion time for redox reactions at either surface [22]. The device geometry greatly enhances the surface-to-volume ratio of the sensor, greatly improving the sensitivity to small changes at the electrode-solution interface [42, 60].
The transition to IDE-based impedance sensing does not alter the fundamental physics behind the surface phenomena being studied. Due to the symmetry of the electrode structures, the circuit models themselves remain almost entirely unchanged: the additional copy of the metal/electrode interface model is indistinguishable from multiplying all fit parameters by a factor of two.

### 2.6 Conclusion

Impedance is a property of electrical circuits describing the relationship between the current passing through the circuit and the voltage forming across it. An electrical circuit comprised of many linear elements can be reduced to an equivalent impedance - a simplified circuit model which faithfully reproduces the response of the overall system. Understanding how to faithfully model these physical processes is the first step in characterizing the system of interest. Forming a cohesive circuit model containing and combining the circuit element representation of various physical phenomena permits meaningful inferences from the model circuit parameters. Monitoring changes in the model parameters over time further extends this technique to evaluate system dynamics on differing time-scales. Armed with sound
physical intuition, we can apply these principles in the design of biosensors that directly probe chemical samples throughout the rest of this work.
Chapter 3

Coulter Counter Fundamentals

3.1 The Coulter principle

Electrochemical impedance spectroscopy (EIS) probes the electrical properties of electrode-solution interfaces. Researchers monitored changes over time in the circuit elements representing physical properties at the interface. The solution resistance remained static throughout the analysis. In fact, experimentalists take care to ensure $R_{\text{soln}}$ is unchanging.

Inverting this paradigm leads to a new sensing modality, wherein the bulk solution between the electrodes forms the device sensing region. This formulation underpins the Coulter principle [62,63], in which the sensing element is the solution resistance of a narrow fluidic constriction between two electrodes. Particles passing through the constriction—such as red blood cells—alter the volume of conductive fluid within. The significant disparity in particle and solution conductivities produces a change in the channel impedance during each passage event proportional to the displaced volume of solution. Monitoring the impedance of the channel in real-time results in brief pulses containing constriction-dependent information about the number, size, and velocity of particles involved.

Wallace H. Coulter’s initial paper [63] described a benchtop instrument capable of obtaining cell size distributions on a half-milliliter sample in a matter of minutes. Orders of magnitude increases to the possible sample size and elimination of human error from visual
counts greatly improved test-retest validity for obtaining red blood cell counts. The principle of size-based discrimination to differentiate between cell species was also outlined: the mixture of sheep or goat’s blood with a human blood sample produced separate identifiable peaks in the cell size distribution, as did tumor cells floating in the bloodstream.

Figure 3.1: Commercially-available Beckman-Coulter Z Series Coulter counter weighs 30 lbs. and costs upwards of $11,000.

The first Coulter counter was not without limitations. The desire to improve performance has driven efforts to reduce the aperture size of the fluidic constriction and thus the minimum particle diameter that can be detected [64]. Approaches to reduce the frequency of clogging [65–67] and identify multi-particle passage scenarios [66] are necessary for performance in high-throughput conditions.

Impedance-based cytometry, the use of electrical impedance to count cells, remains a promising candidate for portable, lab-on-a-chip form factors. The past decade has seen an expansion of interest [65, 68–70, 70–77] in developing Coulter counter-based devices no longer confined to the laboratory benchtop.

The advantages that Wallace Coulter’s method held over visual or photoelectric approaches have been amplified by the revolution in integrated circuits that has taken place...
over the past six decades. Component reliability has increased, cost decreased, and computa
tional power for sizing has expanded exponentially. Researchers investigated different
electrode geometries to capitalize on this miniaturization and move past glass capillaries
and bulk electrodes. Variations include planar electrodes on the same [65, 69, 72, 77, 78] or
opposite [79, 80] faces of the microfluidic channel, various three-dimensional etch techniques
to deposite sensing electrodes on the fluidic channel sidewall [81, 82], or even the use of
highly-conductive solution regions to form fluidic contacts [83, 84].

3.2 Design considerations for portable flow cytometry

3.2.1 The measurement circuit

Desire to build a low-cost and portable flow cytometer has driven myriad design choices
throughout the development of our device. In the following sections, I will discuss the
operating principle of our device and elaborate on the logic underpinning the aforementioned
choices. The terms particle and cell will be used interchangeably throughout this discussion.
The small capacitance of cell membranes gives the appearance of an insulating particle in
the measurement signal for sufficiently low operating frequencies, typically below 1 MHz.
Figure 3.2: a.) The fluidic resistances $R_{\text{soln}}$ form part of the measurement bridge circuit. b.) The voltage difference between the two branches ($V_1, V_2$) of the bridge circuit is fed into a lock-in amplifier whose output c.) is recorded in time.

Our impedance-based flow cytometer adopts a three-electrode design, modeled after the cytometer presented by N.N. Watkins, et al., among others [65,72]. The circuit, as depicted in Fig. 4.1a, operates as an impedance bridge. A sinusoidal excitation signal ($V_{AC}$) at the middle electrode drives current flow through solution to the left and right sensing electrodes. Each of the sensing electrodes is connected to circuit ground by a resistor, henceforth referred to as the bridge resistor ($R_{br}$). The potential that forms at each sensing electrode ($V_1, V_2$) is governed by the ratio of the bridge resistor to the solution impedance ($R_{soln}$) between the excitation and sensing electrodes. Under ideal operating conditions, the solution impedances and bridge resistors are perfectly symmetric and thus both sensing electrodes are at identical potentials. When a non-conductive particle passes between the excitation and sensing electrodes, the solution impedance is temporarily increased, reducing the voltage measured at the sensing electrode. The process repeats as the particle subsequently passes between the excitation electrode and the other sensing electrode. In this manner, a passing particle generates a characteristic voltage signal encoding information about both its velocity and its size.
3.2.2 The AC approach

Employing a time varying voltage signal simplifies the measurement logistics compared to direct current (DC) approaches. Reference (or pseudo-reference) electrodes are necessary to establish stable DC potentials in solution \cite{71,78} but are difficult to microfabricate and cumbersome to integrate. Therefore, reference electrodes present a trade-off between simplicity
of design and measurement capabilities. Without the use of a reference electrode, a drifting DC potential complicates measurement attempts with a constantly-moving baseline.

3.2.3 Circuit model of the cell

At sufficiently low frequencies the cell membrane capacitance renders cells electrically indistinguishable from insulating particles [72, 80, 81, 85, 86]. However, researchers have also begun to use elevated frequencies in the MHz regime as part of their excitation signal [72, 80, 81, 85, 86]. At elevated frequencies, the impedance of the membrane capacitance is significantly reduced, allowing researchers to probe the inner conductivity of the cell cytoplasm. In this manner, cell populations of comparable size but differing in physiology may be discriminated from one another, enhancing the counter’s capabilities.

3.2.4 Microelectrode design

The implementation of planar microelectrodes for impedance-based sensing confers multiple advantages over other more-complicated geometries. The electrode definition requires only a few steps: photoresist coating, pattern definition, metal deposition, and a lift-off process. This simplicity compared to alternative electrode geometries significantly reduces per-device fabrication cost. The ease of fabrication simplifies combining the impedance sensor with additional sensing modalities (e.g., target capture, target recognition) into a single microfluidic sensing platform [77].

The extended emphasis on design simplicity suggests eliminating the third electrode in favor of a two-electrode approach. Such implementation is observed in much of the early Coulter counter work [64, 78, 87]. The additional resistive sensing element formed by the third electrode transforms the characteristic output signal from a single voltage peak to an antisymmetric peak structure. The elapsed time between the local maxima and minima of the antisymmetric structure reduces uncertainty in transit time measurements during flow conditions, compared to extracting particle velocity information from the full-width at half-maximum (FWHM) of a two-electrode configuration.
Differential measurement configurations are the critical competitive advantage of three-electrode approaches. The two solution impedance elements formed between the middle and the left and right electrodes, respectively, are nominally identical under all flow conditions and therefore no voltage forms across the bridge. Monitoring changes in the difference between these two impedances greatly enhances sensitivity by reducing the background signal upon which the transitory resistive pulse of a passing bead is imposed.

3.2.5 The fluidic constriction

Constriction diameter and signal magnitude

Design of the fluidic constriction is an integral aspect of the microfabricated Coulter counter performance. The Coulter principle depends upon the displaced volume of conductive solution by a passing particle. Therefore, the ratio of the volume of the target analyte to that of the sensing region, colloquially called the filling factor, strongly determines sensor performance. Consider an insulating spherical particle passing through a cylindrical volume of conductive solution. The effective change in solution resistance of the cylinder, measured between the circular faces, is given by [64,71]:

\[
\Delta R = \frac{4\rho L}{\pi D^2} \left( \frac{d^3}{D^2 L} \right)
\]

(3.1)

where \( \rho \) is the solution conductivity, \( L \) the length and \( D \) the diameter of the cylinder, and \( d \) the diameter of the particle. Here we have assumed \( L \gg D \). Given the cubic dependence on analyte diameter, the ideal constriction is roughly equal to the diameter of the largest analyte body in the envisioned end-user sample. Matching the diameter of the constriction to the largest target analyte maximizes sensitivity for a given heterogenous sample. It assumes that no debris larger than the largest analyte exists in the solution, or else the debris must be filtered out upstream of the constriction region to prevent it from blocking the channel.
Constriction diameter and clogging probability

A blocked channel effectively halts the device’s ability to count particles until the blockage is removed. During actively-driven fluid flow, large hydraulic pressures build up after clog formation. The resultant pressures can cause catastrophic mechanical failure of the fluidic channel, posing a significant biohazard to the end user when dealing with biological samples.

Figure 3.4: Illustration of the process of clog formation. a.) a single particle adheres to the PDMS constriction walls by chance and then b.) more incident particles adhere to the wall and original particle. c.) optical micrograph of catastrophically-clogged device.

Large debris within the sample is not the sole vector of clog formation. During normal operation, there is a finite probability that an incident particle will adhere to the side-wall of the channel. As the fluidic channel narrows down to the constriction diameter, wall-particle interactions become increasingly likely. A common failure mode observed in our fluidic channels is one such particle failing to become unstuck before a subsequent particle enters and adheres to the first. An aggregate quickly forms in the constriction region, driving jam formation and rapid onset of clogging. This process is illustrated in Fig. 3.4.

Researchers have investigated [67] the factors influencing the mean-time-to-failure (MTF) for clog formation in fluidic constrictions. Particle number density, flow rate, constriction
cross-section, and constriction length all influence this failure mode, as do particle rigidity and the geometry of the narrowing region approaching the constriction [67,69]. In the process of sensor development, we may manipulate all of these parameters to minimize clogging probability during measurement. Ultimately, the particle number density, rigidity, and flow rate are dictated by the end-user application. Engineering of the constriction geometry becomes the main approach to extend the MTF [88–90].

### 3.2.6 Ramifications of planar electrode geometry

![Figure 3.5](image)

**Figure 3.5:** a) top-down view of the metallization pattern for two chips, each of which contains several devices. b) PDMS (translucent grey) confines fluid flow over the electrodes. Different devices on different chips explored the ramifications of electrode transverse length, \( l \), inter-electrode gap distance, \( g \), and the constriction width, \( w \). c) Optical micrograph of a freshly-fabricated electrode structure with a microfluidic channel aligned and bonded.

The planar electrode geometry adapted in our sensing set-up greatly simplifies the device fabrication process. A single mask and a single metallization layer is all that is required for the Coulter counter sensing electrodes, reducing fabrication complexity and cost per sensing device.
The planar electrode geometry limits the size resolution performance of our Coulter counter structure. During device operation, an electric field forms when an electric potential is applied across the two electrodes. The electric field that forms is non-homogenous, as shown in Fig. 3.6. While the solution conductivity remains uniform over the entire sensing volume, different regions of the solution have non-identical contributions to the impedance between the two electrodes. The magnitude of the impedance-based signal acquires a marked vertical dependence [70, 79, 91, 92], as can be seen in Fig. 3.7a. This dependence produces a 20% dispersion in signal magnitude at fixed particle size, corresponding to a 7% uncertainty in diameters.

Figure 3.6: a) conceptual illustration of the field lines emanating from the planar electrode geometry born out by b) COMSOL simulation of the electric field profile for a pair of planar sensing electrodes generated by collaborators at the University of Alberta.
Solutions to the vertical dependence require either manipulation of the incoming particle stream or overhauling the electrode design. Researchers have implemented solutions using acoustic waves to focus the particles into the middle of the channel [91], sheath flows [76,93], and negative dielectrophoresis [94]. Alternatively, structuring the electrodes in three dimensions can greatly simplify [81] the electric field profile at the cost of complicating device fabrication.

### 3.2.7 Constrictionless Coulter counters

Coulter counter devices depend upon their constriction region to maximize the filling fraction of the incoming analyte and thus the signal magnitude. The detection threshold and size resolution of the counter is dictated by this fraction. Potential drawbacks to reducing the constriction volume have already been discussed.

The implementation of microfabricated planar electrodes offers an alternative solution: the fringing electric field of our planar electrode geometry. The density of electric field lines arcing from electrode to electrode falls off rapidly with distance from the electrode surface.
This has directly observable consequences for the sensor signal as a function of particle height within the channel.

For planar electrodes projecting into a wide solution channel, fringing occurs not only in the z-direction but also in the x-y plane where the electrodes terminate. Defining the x-axis along the direction of fluid flow, the density of field lines decays rapidly along the y-direction from the end of the electrodes. This spatial decay limits the volume of solution probed laterally past the end of the planar metal electrodes. In the manner, the effective volume sensed is confined electrically, rather than mechanically.

As a consequence, planar electrodes which just barely project into the side of a wide (~1 mm) microfluidic channel would only enumerate particles passing through the narrow width of solution flowing over the electrodes. Because of the self-limiting nature of the fringing electric fields, the sensing region does not feel the effects of the entire width of the channel. Thus good filling fractions may still be obtained despite the significant increase in overall channel width, eliminating the need for a constriction region.
Figure 3.8: a.) optical micrograph of a Coulter counter projecting slightly into the microfluidic constriction region. b.) Representative trace of a 4.45 μm bead in 0.01x PBS passing over the counter from a.). c.) A simple illustration of how this concept can be implemented with lateral-displacement structures to enumerate particles from the entirety of the sample.

To test this hypothesis, I bonded a 1 mm-wide microfluidic channel to a Coulter counter device such that the three counter electrodes projected slightly into the width of the channel, as can be seen in Fig. 3.8a. 4.45 μm diameter polystyrene beads, 10,000x-fold diluted in 0.01x PBS, were flown through the device at 12.5 μL/min. The absence of a constriction makes possible a fifty-fold increase in volumetric flow-rate compared to previous experiments, a key advantage of this technique.

Sheer probability dictates that some of the beads within the sample would be expected to flow over our Counter device to be detected. Indeed such an event can be observed in Fig. 3.8b. The significance of this demonstration cannot be understated from a commercialization perspective. This simple design changes greatly enhances commercialization potential, increasing volumetric throughput 50-fold. Eliminating the need for a mechanical
constriction also sidesteps a significant engineering challenge for product reliability.

The proof-of-principle demonstration only counted a small fraction of the sample within the population. Combined with lateral displacement via dielectrophoresis, as shown in Fig. 3.8c, the entire sample volume may be interrogated by focusing all the enumeration targets to the side of the channel occupied by the counter structure.

3.3 Experimental Design

3.3.1 Microscope and stage mount

Microscope

Our impedance-based cell counter aims to compete with fluorescence-based cytometers. Incorporating simultaneous optical imaging within our measurement system enables direct comparison to fluorescence-based approaches and simultaneous real-time verification of fluidic performance during the development process. To this end, all of our sensing experiments are conducted on the viewing stage of an Olympus BX51 microscope equipped with 5x, 10x, and 40x objectives as well as multiple filter lenses for fluorescence imaging. An Olympus DP70 camera system allows for image and video capture for later analysis. An Xcite Series 120Q laser source provides an intense source for fluorescence imaging.

Test fixture

Previous solutions for making electrical contact to devices on the optical stage involved lengthy cabling and a multitude of solder joints. This approach posed challenges for the mechanical positioning and connection stability when forming electrical contact. The extant cabling also had significant coupling to external noise sources as well as cross-talk between wires. A mechanically-robust and properly-shielded test fixture was needed.
As part of the test fixture, I designed a printed circuit board (PCB) which permitted electrical contact to individual pins on the device through coaxial connectors mounted on the board, while leaving sufficient clearance for the microscope objective lenses. The PCB made contact to the device via spring-loaded connectors projecting from the underside of the board.

I then designed a metal sample mount to mate with the PCB. A groove milled out of the sample mount, as shown in Fig. 3.9, allows devices to easily be loaded underneath the spring-loaded connector from the side. A slot (shaded blue) recessed in the center of the milled-out groove has been machined with lateral tolerances much tighter than the contact pads’ pitch to mechanically ensure in-plane alignment. Vertical alignment with the spring-loaded pins is likewise mechanically determine by the vertical displacement between the bottom of the slot and the height of the PCB.

The combination of the PCB and sample mount thus provides a secure and robust connection between the device and the coaxial connections on the PCB. Sample alignment in all three dimensions is achieved by the physical structure, removing a significant barrier
to reliability and ease-of-use. Furthermore, the metal sample mount and the ground plane of the PCB form a Faraday cage around the device to shield the device from external electromagnetic interference.

The sample mount was also machined with a second, larger contact area, shaded purple in the far left of Fig. 3.9a to permit interfacing device geometries which do not conform to the milled socket while still handling alignment in the vertical plane.

3.3.2 The electronics

![Circuit Diagram]

Figure 3.10: a) circuit diagram of the complete three-electrode structure, driven by the sine wave output of the b) function generator. The resulting voltage at the left and right sensing electrodes is measured by the c) PCB-mounted instrumentation amplifier before the signal is fed to the d) lock-in amplifier whose output signal is measured by e) the oscilloscope, controlled during acquisition by a f) MATLAB routine.

Function generators

A sinusoidal voltage source is required for our three-electrode bridge circuit. We used an Agilent 33120A function generator for the Coulter counter measurements as opposed to the built-in function generator of our lock-in amplifier. The Agilent 33120A demonstrated lower noise floors and higher spectral purity than the sine wave generator of our SR830 lock-in
amplifier, as measured on a network analyzer. The excitation signal, a 70 kHz sinusoid with 1 $V_{rms}$ amplitude, was rarely varied during the course of development.

**Lock-in amplifier**

We monitor the output signal from the bridge circuit during experiments with a Stanford Research Systems SR830 lock-in amplifier. Lock-in amplifiers exploit the orthogonality of sine and cosine functions to extract the amplitude of a specific frequency component of the input signal with high fidelity. This enables detection of the small changes in the bridge resistance during particle transit events expected at low filling factors.

An additional lock-in amplifier, the Stanford Research Systems 844, was used to characterize the impedance of our devices in the 25 kHz - 1 MHz regime. The additional order of magnitude in frequency range provided additional information about circuit electrical characteristics.

**Oscilloscope**

The transit time of the particles over our counter structure dictates the necessary sampling rate for measuring the bridge voltage. From the perspective of the Nyquist criterion, the minimum sampling frequency is $\frac{2}{\delta t}$, where $\delta t$ is the transit time of a particle passage. Researchers typically aim for a minimum of 20 datapoints per event, requiring sampling rates of 10-1000 kHz depending upon desired flowrate and constriction geometry. To satisfy this condition, we employ a Tektronix DPO4104 to record the analog voltage signals from the rear panel of the lock-in amplifier.

Furthermore, extracting particle size information from the shape of the voltage signal requires a sufficient number of datapoints per particle trace, with minimums in the literature between 10 and 20 points. Transit times of 0.1 ms correspond to sampling rates between 100 - 200 kHz which is hardly a stringent requirement in a laboratory setting, however there exists economic incentive to minimize the necessary sample rate when producing portable systems. The sampling rate also must be increased with increasing expected event frequency.
to resolve abnormal signatures arising from contemporaneous transits.

**The measurement circuit**

The printed circuit board comes equipped with the ability to interface with up to six counter structures. Each has a single Texas Instruments OPA-2227 operational amplifier configured as a dual-channel unity-gain voltage follower. A gain-bandwidth product of 8 MHz more than exceeds the necessary operating frequency of our Coulter counters. For a balanced bridge being driven by the typical $1\,V_{\text{rms}}$ amplitude, the equivalent peak-to-peak voltage occurring at either node is $2.83\,V_{\text{pp}}$. Given the specified slew-rate of $2.3\,V/\mu s$, operation up to $0.8\,\text{MHz}$ is possible. A dual-channel op-amp is chosen to avoid variance among individual integrated circuits which would contribute to a differential signal between the two terminals.

In addition to the dual-channel voltage follower, we also introduced a precision instrumentation amplifier for each Coulter counter measurement channel. An instrumentation amplifier produces a signal proportional to the difference between the two input terminals. Resultingly, signals common to both terminals are subtracted out. The efficacy to which signals common to both inputs are suppressed is referred to as the common-mode rejection ratio. The instrumentation amplifier can be configured to provide additional gain of the differential signal, elevating the signal of interest further over the suppressed background signal between the two amplifiers.

**The syringe pump**

To flow sample through the device, we use a New Era syringe pump (NE-1000). Typical sample flow rates range from 0.1 - 5.0 µL/min., corresponding to linear velocities of 5,000 - 250,000 µm/s. for particles within the constriction region and transit times across the entire length of the three electrode structure between 0.4 - 20 ms for our 20 µm x 20 µm constriction cross-sections.

Reducing processing time at a fixed sample volume requires increasing sample through-
put and therefore flowrate. A single droplet of blood, roughly 30 \(\mu\)L in volume, requires half an hour to process at flowrates of 1.0 \(\mu\)L/min. Mechanical and instrumentation limitations prevent arbitrarily increasing sample throughput. The microfluidic constriction for the counter region presents a significant hydraulic resistance, generating large back-pressures in the fluidic channel as flow velocity increases. As flowrates approach 10s of \(\mu\)L/min., the backpressure splits open the tubing inlet or breaks the adhesion between the channel and the substrate, causing leaks. Furthermore, increasing the flowrate reduces the mean time to clog formation within the channel, a problem exacerbated by the rigidity of polystyrene beads used for calibration experiments.

3.4 Counter performance evaluation

Prior to performing detection of heterogeneous biological populations, we must establish performance baselines for the counter performance. The limit of detection for particle diameter as well as the resolution of diameter-based classification is of particular importance as means of discriminating between biological specimens of varying size.

To avoid the complications of handling biological material as well as eliminate population heterogeneity as a variable for evaluating the sensor response, researchers developing fluidic Coulter counters have used polystyrene beads with tight size dispersions \([69, 76, 80]\). These experiments allow us to assess the sensitivity of the counter structure over a range of solution conductivities and particle densities.

3.4.1 Population analysis

We want to repeatedly record the response of the sensor to a known input to quantify its resolution capabilities. Passing the same particle through the same constriction is experimentally impractical. Instead, we rely upon the tight homogeneity of purchased polystyrene beads to approximate an identical input. We then pass many hundreds of beads through the counter structure while recording the output signal. Extracting bead size and transit time information from every recorded passage, we can map out the range of output re-
responses which correspond to the given input - a particle of known size in a known solution conductivity.

Figure 3.11: a.) A representative data trace containing two bead passage events, condensed into b.) a histogram to generate c.) a threshold parameter (red lines) based upon the standard deviation of the background noise. Threshold detection identifies the passage events which are then d.) fit with to extract particle size and velocity parameters, which are e.) mapped for thousands of such events acquired during the measurement.

Fig. 3.11 illustrates the process underlying the peak detection algorithm we use to generate the ensemble measurement statistics. Each recorded data trace lasts for a second and can contain some number of bead passage events. The program constructs a histogram of the time-domain voltage signal. We observe that the output noise of the lock-in amplifier has a Gaussian profile, and construct a histogram of the acquired signal. Curve fitting on the histogram bin counts extracts the standard deviation of the background noise which determines the threshold levels for peak detection of the individual trace. We then employ coincidence detection for particle recognition: within a finite time window (the expected
transit time based on flow rate and constriction geometry), the voltage signal must cross the positive threshold level with a rising and then falling edge before crossing the negative threshold with a falling and then rising edge. If all four of these crossings occur within the predefined time window, that section of the data-trace is flagged as containing an event. Curve-fitting of the antisymmetric peak structure within the event extracts the voltage amplitude (proportional to measured particle size) and elapsed time (measured velocity) between the maxima and minima of the signal.

We then construct a two-dimensional histogram of the velocity and size information to look at the dispersion in recorded events along both dimensions, reflecting both physical effects arising from the channel geometry (parabolic flow velocity profile, height dependence of the signal amplitude) as well as uncertainties arising from variances in the fitting algorithm. Of particular interest is the spread in measured values for the peak height, which determines our ability to use sizing alone to distinguish amongst incident particles or species.

### 3.4.2 Physiological conductivity

To assess the performance of the counter, I prepared a sample containing 4.45 µm (Spherotech PP-40-10), 6.42 µm (Spherotech FP-6056-02), and 8.87 µm (Spherotech PP-100-10) diameter beads. I filtered 1.0x PBS buffer twice to remove particulate matter from the stock solution, and subsequently used this mixture to dilute and rinse the beads. The nominal weight fraction of the three bead samples was used to calculate the nominal bead density per mL. The nominal densities determined the dilution ratios. The final sample contained all three bead populations with a nominal 670,000 beads/mL. I added 1.0% Tween-20 by volume as a surfactant to inhibit aggregate formation within the sample.

I then flowed the sample over the counter device at 1.0 µL/min. while recording 1600 one-second data samples. The algorithm of Fig. 3.11 analyzed the acquired data and extracted particle transit time and peak height for each detected event, as can be seen in Fig. 3.12b. Three separate populations are clearly visible. The cube root of the peak height
Figure 3.12: a.) Histogram of the peak heights of events acquired during the experiment as well as Gaussian fits of the histogram data to estimate the dispersion of the sensor events. The dashed vertical line represents the detection threshold of the algorithm for this dataset. b.) Heatmap of the detected particle sizes and transit times. c.) Linear regression of the is plotted, as it should be directly proportional to particle diameter \[69,95\]. Constructing a histogram of the peak heights reveals a trimodal distribution, as can be seen in Fig. 3.12a. Gaussian peak fitting extracts the mean signal amplitude and uncertainty for the three populations, plotted as a function of nominal bead diameter in Fig. 3.12c.

Linear regression (dashed blue line) of the peak locations gives the expected signal amplitude as a function of particle size for the given constriction. The intercept of the regression with the amplitude of the threshold for peak detection gives the limit of detection for the measured sensor, here \(\sim 2.8 \, \mu m\). Inspecting Fig. 3.12a, we can resolve particle diameter differences on the order of 0.5–1.0 \(\mu m\). The disparity in population counts despite nominally identical particle concentrations is unsurprising considering the age of the sample stock. Precision in counts was not a major consideration for this demonstration and thus some variance is acceptable.
3.4.3 Flowrate and transit time

Figure 3.13: Representative data traces of 4.5 µm beads in 0.1x PBS flown through a 50 µm x 20 µm cross-section constriction at a.) 8.0 µL/min., b.) 2.0 µL/min., and c.) 0.5 µL/min. Histograms of detected signal heights and widths for many such events, aggregated at flowspeeds of d.) 8.0 µL/min., e.) 2.0 µL/min., and f.) 0.5 µL/min.

While the signal arising due to a particle of a given volume passing through the sensing region should be independent of flow velocity, the lock-in amplifier itself places limits on the measurement bandwidth of the system. The output response time of the SR830 is dictated by the steepness of its bandpass filter as well as the integration constant chosen. For maximal signal-to-noise ratio during measurements at our targeted volumetric flow rate, a 30 µs time constant and 24 dB./decade roll-off were chosen. Per the SR830 datasheet, this generates a 99% response time of 300 µs [96]. No significant attenuation was observed for flow velocities up to 8.0 µL/min., or 0.5 mL/hr., as can be seen in Fig. 3.13. We observed consistent function over the range of flow-rates germane to the desired clinical applications.
of our sensor.

3.5 Impedance cytometry as an assay technique

The impedance-based flow cytometers provide information on the number and size distribution of incident particles [86]. The two subpopulations are readily resolved by the clear size differentiation (4-5 μm v. 8-12 μm) between activated and unactivated T-cells [97]. Operating a counter structure near the outlet of each stream, we can count the total population of activated and unactivated cells in the laminar flow of the original sample as well as in the exchange buffer. Thereby we can quantify the efficiency and purity of the dielectrophoresis separation as well as the ratio of unactivated to activated T-cells within the sample to assess immunological status.

3.5.1 The lymphocyte sample

Lymphocytes are a subtype of white blood cells involved in the body’s immune response [98, 99]. T-cells are a subset of lymphocytes with a surface coating of peptide-recognition molecules, the T-cell receptor. Foreign agents within the body are digested and displayed as cell fragments on the cell surface by antigen-presenting cells, the first line of defense in the body’s immune response. When a T-cell encounters these displayed fragments they become activated, amplifying the immune response. Activation greatly increases T cell metabolism as they rapidly grow in size and proliferate in number. The presence of activated T-cells within the blood stream is therefore a reliable indicator of the host immunological state, e.g. fighting off an infectious disease.

Our T-cells are primary cells, prepared directly from murine splenocytes, distinct from cell lines generated for modeling cell behavior under tissue culture conditions. The T-cells are in an unactivated state when initially prepared. To obtain activated T-cells, unactivated T cells are exposed to activation-inducing agonist antibodies, anti-CD3 and anti-CD28, for 72 hours. Both populations are suspended in 1.0x phosphate-buffered saline with 0.1% by volume of Pluronic F-127, a surfactant from Sigma Aldrich, to minimize cell adhesion to
the device.

## 3.5.2 Impedance-based discrimination

Figure 3.14: a.) Activated (orange) and unactivated (blue) T-cells passing through a constriction region with 20 µm electrode width and a 50 µm-wide constriction produce b.) markedly different Coulter counter signals. c.) Visualizing the dispersion in particle parameters reveals that unactivated and activated T-cells can be d.) readily differentiated by the signal magnitude.

After establishing the ability to separate the unactivated and activated cells, we set out to count and size them. Populations of activated and unactivated T-cells are readily differentiated from one another in mixed solutions, as can be seen in Fig. 3.14. Samples of
naïve and activated T-cells were prepared and flown through Coulter counter constriction regions at 0.4 µL/min. Sixteen hundred one-second data traces were acquired and analyzed to produce the results shown in Fig. 3.14c&d which shows a clear distinction between the two populations.

To convince ourselves of the results in Fig. 3.14, we next introduced samples containing a mixture of both naïve and unactivated T-cells, in 1:1 and 2:1 ratios. The combined signal contains a sum of both the naïve and activated signatures from Fig. 3.14d, and is shown below in Fig. 3.15.

Three sequential peaks are visible in the population histogram of Fig. 3.15. The broad, rightmost feature (shaded green) captures heterogenous size distribution of the T-cells after activation. The concentration of activated T-cells mixed into dilution was unchanged between Fig. 3.15a and Fig. 3.15b. Accordingly, we see no pronounced change in the peak magnitude between the two conditions. In contrast, the height of the naïve cell population peak (shaded yellow) halves when the mixing concentration of naïve cells in solution is reduced two-fold.

One last feature remains. Shaded red, there exists a pronounced peak unaffected by changes in the mixing concentration. Also visible as the bright and broad signature at
\(0.04 \Delta R/R\) in Fig. 3.14, this broad feature is attributed to debris within the sample. Such features are commonly seen in overly-sonicated preparations of polystyrene beads, fragmented by the extended sonication. The presence of a leftward lower bound on the debris peak feature is an artifact of the threshold parameter used for coincidence detection of features within the dataset.

Two separate physical origins of debris exist. Lysate within the sample is an inevitable byproduct of the T-cell harvesting process. Lysate consists of fragments of other cells, membranes and debris. Lysate can be filtered or rinsed away during sample preparation in future experiments now that it has been clearly identified as an issue. Investigations by my colleague, Shari Yosinski, into other potential origins of the debris revealed significant lysing of the T-cells themselves as they passed through the hypodermic needles used to couple our syringes into the microfluidics. Solutions to bypass the hypodermic needle and avoid this process remain to be determined.

### 3.5.3 Impedance-based measurements of activation kinetics

Size-based discrimination as a diagnostic criterion requires the ability to differentiate between naïve and activated T-cells after a prolonged period of growth. We have now demonstrated the ability to distinguish between the two. It remains to be seen how the growth process occurs over time for populations of T-cells after antigen exposure. The growth kinetics and size resolution of the sensor could both set a lower bound on time after antigen exposure for a detectable immune response.

Four distinct samples were prepared to investigate the T-cell growth kinetics. Naïve cells and cells that were exposed to antigen 72 hours prior were prepared as previously described. Additional samples which had been exposed to antigen 24 and 48 hours prior were also prepared using this same protocol.
Figure 3.16: Population distributions for cell sizes for a.) naïve cells and populations b.) 24, c.) 48, and d.) 72 hours after antigen exposure.

Changes in the size distributions over time are clearly visible in the four samples shown in Fig. 3.16. The naïve distribution (Fig. 3.16a) is the same as previously observed in Fig. 3.14. Twenty-four hours after antigen exposure (Fig. 3.16b), the naïve cells begin to undergo activation. The peak at 0.1 $\Delta R/R$ broadens, acquiring a rightward shoulder as cells within the population grow at varying rates. This process continues in the sample taken forty-eight hours after antigen exposure (Fig. 3.16c). A fraction of the population has reached the fully activated state while a fraction still has yet to undergo activation and appreciably change in size. A full seventy-two hours after activation, the naïve cell peak is fully suppressed only the broad activated population and debris signatures remain.
These results suggest a minimum of 36 to 48 hours necessary to observe significant fractions of activated T-cells within the sample to produce a clear and convincing response on size-based discrimination alone. It remains to be determined why some cells remain unactivated up to 48 hours after antigen exposure. Regardless, these results show the promise of our simple on-chip enumeration as a portable diagnostic tool.

3.6 Conclusion

In this chapter, we have demonstrated the implementation of an impedance-based sensor for particle sizing and enumeration using planar metal electrodes. Our sensor embodiment is suitable for lab-on-a-chip sensing applications. Monitoring impedance changes induced by insulating particles, we can detect, count, and discriminate based on size for a wide range of particle sizes and in a range of solution conductivities.
Chapter 4

Coulter Counter Design

Considerations

4.1 Circuit architecture

Researchers either employ a bridge circuit configuration or voltage amplifiers to measure the solution resistance for impedance-based flow cytometry. Voltage amplifiers are a straightforward solution for two-electrode systems where the fluidic resistance forms part of the amplifier feedback network. Robust bridge circuit designs are made possible by the presence of a third sensing electrode. Bridge circuit measurements are differential and therefore subtract out the background signal to provide high sensitivity to subtle changes in the solution impedance. Our Coulter counters were explicitly designed for this purpose. In this chapter, we will review the design considerations for the bridge circuit components necessary for our counter’s performance.
4.2 Bridge component values

Figure 4.1: a.) The fluidic resistances $R_{soln}$ form part of the measurement bridge circuit. b.) The voltage difference between the two branches ($V_1$, $V_2$) of the bridge circuit is fed into a lock-in amplifier whose output c.) is recorded in time.

4.2.1 Determination of the bridge resistance

The component values in the bridge circuit determine the performance limits of our Coulter counter measurement system. The equilibrium voltage, $V_{eq}$, for each branch of the bridge circuit is determined by the ratio of the solution resistance, $R_s$, to the value of the resistor forming the bottom half of the bridge, $R_b$, and the magnitude of the driving voltage, $V_{AC}$:

$$V_{eq} = \frac{R_b}{R_b + R_s} V_{AC}$$  \hspace{1cm} (4.1)$$

Eqn. 4.1 assumes the impedance of the double-layer capacitance is negligible with respect to $R_s$ at the operating frequency $\left( R_s \gg (j\omega C_{DL})^{-1} \right)$. The differential voltage forming across the two sides of the bridge circuit is thus:

$$V_{diff} = \frac{R_b}{R_b + R_s} V_{AC} - \frac{R_b}{R_b + R_s + \delta R_s} V_{AC}$$  \hspace{1cm} (4.2)$$

51
where we have introduced the term $\delta R_s$ to denote a small deviation in the observed solution resistance in the latter branch, as would occur during a cell passage event. We divide by the drive voltage, $V_{AC}$, to render both sides dimensionless, and solve:

$$\frac{V_{diff}}{V_{AC}} = \frac{R_b \delta R_s}{(R_s + R_b) (R_s + \delta R_s + R_b)}$$

(4.3)

To find the sensitivity maximum, we differentiate with respect to $R_b$ and set the resultant expression to zero:

$$0 = R_s^2 + R_s \delta R_s - R_b^2$$

$$R_b = \sqrt{R_s (R_s + \delta R_s)}$$

(4.4)

Inserting this solution for $R_b$ into Eqn. 4.3, we arrive at an expression for the maximum possible bridge circuit response for a given change in resistance:

$$\frac{V_{diff}}{V_{AC}} = \left[ 2 \left( \frac{R_s}{\sqrt{R_s (R_s + \delta R_s)}} + 1 \right) \left( \frac{R_s}{\delta R_s} + 1 \right) - 1 \right]^{-1}$$

(4.5)

In the limit of $R_s \gg 1, \delta R_s$, Eqn. 4.7 simplifies to:

$$\frac{V_{diff}}{V_{AC}} = \frac{1}{4} \frac{\delta R_s}{R_s}$$

(4.6)

corresponding to a signal amplitude of 2.5 mV per percent displaced volume per volt of excitation signal. This figure of merit is the upper performance limit for our device, contingent upon a perfectly-matched bridge circuit. The calculated bridge circuit response from Eqn. 4.6 is plotted in Fig. 4.2 as the ratio of the solution to bridge resistances varies, illustrating the sensitivity loss arising due to imperfect matching. Signal attenuation is less than a factor of two for bridge resistor mismatches up to a factor of 5.3x, indicating reasonable tolerance for slight variations in component values selected in terms of the ratio $R_s/R_b$. In subsequent sections we will discuss other physical considerations which attenuate
the sensor response to values below this theoretical maximum.

Figure 4.2: a) Output differential signal (solid blue line) as a function of the ratio between the bridge ($R_{br}$) and solution ($R_{soln}$) impedances, assuming a 1% change in impedance in one of the two sensing regions. Dashed red vertical lines indicate where bridge resistor mismatch has decreased the signal by a factor of 2. b) Volume displacement ratio as a function of particle diameter inside a constrictions of two different cross-sectional areas.

### 4.3 Frequency constraints

#### 4.3.1 Operating frequency

The choice of operating frequency is not entirely arbitrary. Physical considerations of the measurement circuitry itself form the first independent constraint on frequency of operation. Passing particles modulate the amplitude of the AC signal formed across the bridge. To resolve these modulations, the period of the excitation signal should be appreciably smaller than the expected transit time of particles over the sensor, that is:

$$f_{sig} \gg \frac{1}{\tau_{trans}}$$

(4.7)
4.3.2 Influence of the bridge capacitance

A bridge capacitor connects the two output terminals of the Wheatstone bridge configuration used to generate the sensing signal. This capacitor introduces a low-pass filter from the perspective of either sensing electrode. The filter attenuates high-frequency noise in the sensing environment, motivating its inclusion. Potential sources of high-frequency noise include monitor flicker, higher harmonics of the excitation frequency, or switching-mode power supplies. The value of the bridge capacitor must be chosen after establishing the operating solution impedance and bridge resistance of your device. The bridge capacitor must be chosen such that there is minimal, if any, attenuation at the signal frequency.

4.3.3 The double layer

The capacitive double-layer at the counter electrode-solution interface presents an additional impedance in the bridge circuitry. Since the operating principle of the counter relies on detecting changes in the net impedance between two counter electrodes, and the double-layer impedance would not be modified appreciably by passage of a particle well overhead, the counter should be operated at frequencies where the impedance of the ionic double layer is negligible in order to maximize the signal magnitude for the counter system.

4.3.4 The cell model

We have previously assumed a frequency-independent particle conductivity. The picture becomes more nuanced for biological mediums. Cell samples of interest typically possess one of two outer layers: either a cell membrane (semi-permeable) or cell wall (impermeable). These outer layers surround a conductive inner medium, the cytoplasm. By configuring the Coulter to record both magnitude and phase information, or simultaneously monitor at multiple frequencies, researchers can also measure the electrical properties of these outer layers, allowing further discrimination amongst similarly-sized species of bacteria [72,80,81,85,86].
Figure 4.3: Discrete-element circuit model of a cell with a single membrane.

The counter response to a passing cell has two frequency regimes: at low frequencies the membrane impedance is very high, and at high frequencies the membrane impedance is small compared to the internal impedance of the cell [72, 80, 81, 85, 86]. The low-frequency signature encapsulates the relevant size information, whereas the high-frequency signature conveys information about the outer layer of the cell.

4.3.5 Realities of high frequency operation

The upper cutoff for the operating frequency is determined by the physical embodiment of the counter itself. Parasitic capacitances are unwanted capacitances arising between conductive elements within a circuit due to their physical proximity. As the operating frequency increases, the impedance of this parallel pathway falls off. At sufficiently high frequencies, parasitic capacitances dominate the behavior of the bridge circuitry.

4.4 Influence of parasitic capacitances

Parasitics are fundamentally unavoidable but the impact of these parasitic capacitances can be thoroughly minimized with careful design. Stray capacitances arise in the Coulter counter measurement circuitry in parallel with the bridge capacitor, solution impedance, and bridge resistor, as illustrated in Fig. 4.4.
Figure 4.4: Circuit schematic of the measurement bridge circuit, incorporating the capacitance of the double-layer at the electrode-solution interface as well as parasitic capacitances through the substrate ($C_1$) and across the bridge resistors ($C_2$).

### 4.4.1 Bridge capacitor

Parasitic capacitances in parallel with the intentionally-placed bridge capacitor will increase the effective value of the bridge capacitor, decreasing the cut-off frequency of the low-pass filter formed. Operating at frequencies above the cut-off frequency will result in significant attenuation of the measured voltage. Extending the maximal possible operating frequency requires minimizing stray capacitances in parallel with the bridge capacitance.

### 4.4.2 Solution resistance

A parasitic capacitance in parallel with the solution resistance replaces the solution resistance with an equivalent impedance in the bridge circuit. Parallel impedances combine reciprocally and therefore the smaller term dominates the equivalent combination. Even if the two terms are comparable in magnitude, the parallel combination suppresses sensitivity to changes in the solution resistance. At sufficiently high frequencies, the parasitic capacitance $C_1$ becomes the sole determinant of the impedance of the parallel combination, suppressing all observable changes in the solution resistance due to passing particles or cells.
The frequency at which this occurs is determined by the value of the solution resistance and the magnitude of the parasitic capacitance:

\[ R_{\text{soln}} \gg \frac{1}{j\omega C_1} \]  \hspace{1cm} (4.8)

The solution resistance depends upon the conductivity of the sensing solution as well as the geometry of the sensor electrodes. These constraints are predefined by the counter’s target application. The operating frequency may be reduced to an extent governed first by the expected transit time of particles and also by the presence of the ionic double-layer at the electrode-solution interface. Eliminating sources of the parasitic capacitance is the most straightforward means of satisfying Eqn. 4.8 but becomes increasingly difficult as the magnitude of \( C_1 \) diminishes.

### 4.4.3 Bridge resistance

As discussed previously, the maximum bridge circuit response to a particle passage event occurs for the case that the solution impedance is equal to the bridge resistance. If a parasitic capacitance forms in parallel with the bridge resistance, this can have significantly deleterious effects. The solution resistance is typically on the orders of hundreds of k\( \Omega \) and comparable values are chosen for the bridge resistor as well. A small parasitic capacitance in parallel with this bridge resistance will cause the effective impedance to fall off dramatically with increasing frequency, and thus the sensitivity of the counter system.

The parasitic capacitances are unintentional and therefore by no means equal. Resultingly, the equivalent impedance of the two bridge resistors will have slightly different frequency dependencies. In addition to component tolerances on the bridge resistors themselves, this contributes an additional background signal: with the solution resistances perfectly matched, there is a non-zero voltage difference across the bridge. This increases the dynamic range required by introducing a background signal comparable to or larger than changes induced by passing particles.
4.5 Origins of parasitic capacitances

4.5.1 Coaxial cabling

Coaxial cabling used to interface with benchtop laboratory equipment introduces an unwanted capacitance into the system. Coaxial cable acts as a distributed circuit element with a capacitance per unit length \[ C_{l} = \frac{2\pi \epsilon_{r} \epsilon_{0}}{\ln(D/d)} \] (4.9)

where \( D \) is the inside diameter of the coaxial shield and \( d \) the outside diameter of the inner conductor. Commercially-available coaxial cabling has capacitances of 50-100 pF/m. When interfacing directly with the bridge circuit for measurements, this places a sizeable capacitance in parallel with the bridge resistor even for reasonable cabling lengths. The frequency at which the cabling capacitance impacts the magnitude of the bridge resistance is given by the corner frequency \( (f_{3dB}) \) of the parallel combination of the bridge resistor and cabling:

\[
f_{3dB} = \frac{1}{2\pi R_{br} C_{cable}}
\] (4.10)

yielding a corner frequency of 31.8 kHz for a 100 kΩ bridge resistor and a 50 pF cabling capacitance to estimate the magnitude of the effect.

4.5.2 Substrate

The contact pads for interfacing the device were fabricated with areas of 1.5 mm² atop 2 μm of silicon dioxide insulation isolating the electrodes from the silicon wafer handle. The thick insulator provides excellent isolation of the electrode pads for DC signals. We would expect to observe the same behavior at signal frequencies owing to the macroscopic separation between pads but this is not the case.
The wafer handle, from Silicon Valley Microelectronics, has a conductivity between 13-30 Ω-cm and a thickness of 525 µm, corresponding to sheet resistances of 247-571 Ω/□. The pad width is 1.5 mm and the spacing between pads is 0.27 mm, corresponding to roughly 1/6th of a square. Ignoring the effects of skin depth and carrier mobilities (reasonable assumptions at the signal frequencies), the resistance between pads underneath the silicon dioxide is 41-95 Ω. This resistance is small with respect to the solution or bridge resistances. Regarding it as a short when estimating the parasitic capacitance between contact pads, we can consider two neighboring pads to be capacitively-coupled plates with only 4 µm of dielectric between them. We may then estimate this capacitance:

\[ C = \frac{\kappa \epsilon_0 A}{d} \]  

(4.11)

where \( \kappa \) is the relative permittivity of our insulator (3.9 for SiO₂), \( \epsilon_0 \) is the relative permittivity of free space, \( A \) the area of the plates, and \( d \) the separation between them. Conductance measurements, such as those shown in Fig. 4.5, found a net parasitic capacitance of 15 pF between pads for dry chips on silicon, in excellent agreement with this estimate when accounting for additional sources of parasitic capacitance in parallel with the pad-to-pad mechanism.

4.5.3 Printed circuit board

Small parasitics arise between contact pads due to the metal traces on the printed circuit board design. The macroscopic separation between traces, 0.06”, limits the magnitude of this effect but from Eqn. 4.11 it contributes roughly 1.1 pF of capacitance per inch of parallel wiring at this separation. This additional contribution likely accounts for most of the discrepancy between the calculated 12.9 pF and 15 pF for the pad-to-pad capacitance.
Figure 4.5: Device impedance measurements taken without a chip connected, a dry chip, and three concentrations of phosphate-buffered saline (PBS) to demonstrate the effect of a) 2 µm of silicon dioxide versus b) an entirely-insulating glass substrate for both low-frequency (LF) and high-frequency (HF) regimes.

**Extending the frequency range**

The lower bound of operating frequency is dictated by the target throughput and target size. The upper constraint is dictated largely by the aforementioned parasitics which decrease the bandwidth of the measurement system. As shown in Eqn. 4.11, reducing the cross-sectional area of the contact pads will reduce the magnitude of the parasitic coupling between counter electrodes. Macroscopic alignment becomes increasingly challenging as pad size shrinks, restricting adoption of this solution during the benchtop development phase.

Fig. 4.5 shows the significant influence of the choice of substrate on the device impedance. A voltage signal of varying frequencies was applied to the middle electrode of the counter structure, and one of the adjacent sensing electrodes was connected to the inverting input of a voltage amplifier with a 100 Ω feedback resistor. Measurements were taken with different concentrations of phosphate-buffered saline flowing through the channel at 2.0 µL/min.

Impedances measurements in lower conductivities on silicon show the significant influence of the parasitic capacitance between the contact pads in dictating the device behavior. This can be inferred by comparison to the measurements taken without solution in the
Figure 4.6: a) computed impedance change for the b) sensing region circuit model in response to a 1% change in solution resistance, demonstrating the signal attenuation caused by the parasitic capacitance of the c) the silicon substrate in contrast to d) devices fabricated on glass. Measurements for a 4.5 µm bead in 0.01x PBS at 0.5 µL/min. for a 20 µm channel width and gap.

channel in Fig. 4.5a. This stands in stark contrast to the large differences in measured conductivities on the glass substrate seen in Fig. 4.5b. Improvements to the printed circuit board design increase the measured impedance two-fold in the absence of a chip (blue lines).

To ensure complete solution exchange between experimental conditions, the flow rate was increased one order of magnitude in between datasets. The low frequency measurements were recorded first for each condition, beginning at 100 kHz and descending in frequency. Measurements did not begin until approximately five minutes after returning the flow rate to 2.0 µL/min. The devices exhibited some a weak dependence of conductivity with flowspeed. There are slight discrepancies observed in Fig. 4.5b, thought to stem from insufficient settling time for the flow speeds in the system.

The resultant improvement in SNR can be observed in Fig. 4.6. The impact of the substrate is markedly more dramatic at lower solution conductivities (wherein the solution resistance is higher). While less consequential in the high-salinity of whole-blood environments, we desire lower conductivity for other applications for which fabrication on glass becomes essential.
4.6 Conclusions

Developing a robust Coulter counter requires conquering various sources of parasitic capacitances to elevate the signal above sources of measurement noise.

Cabling capacitance

Miniaturization of the counter electronics has the added benefit of isolating the counter from capacitive loading of coaxial cables. The current PCB contains both dual-channel buffer amplifiers as well as an instrumentation amplifier. The signal amplitude at either $V_1$ or $V_2$ increases over an order of magnitude when buffered by one of the active amplifiers, highlighting the significance of proper isolation.

Bridge capacitance

Moving the buffer amplifier circuitry to be spatially adjacent to the spring-loaded header reduces geometric capacitances arising from trace lengths in the PCB. In future iterations the bridge capacitance can be likely be eliminated, as even negligible parasitics combine with our sensor impedances to form corner frequencies in the hundreds of kHz.

Present designs connect each counter structure to both a dual-channel buffer amplifier as well as an instrumentation amplifier for prototyping. Now that the instrumentation amplifier has been validated, the buffer amplifier structures can be eliminated. The reduction in trace length will decrease the parasitics further and has the added benefit of eliminating the off-state capacitance to ground of the buffer amplifier inputs.

Substrate capacitance

Replacing the silicon substrate with glass greatly improved counter performance by eliminating capacitive coupling between the contact pads. Transitioning back to silicon for mass production is possible provided the area of the contact pads is reduced. Even a ten-fold reduction in pad size would suffice. Another avenue for commercialization is replacing glass
with another insulator for disposable test chips. The electrical properties of the chosen insulator need to be considered as well when making this substitution.
Chapter 5

Dielectrophoresis for lab-on-chip applications

Integrating additional functionality with on-chip enumeration expands potential applications of our system for portable point-of-care diagnostics. The ability to manipulate the position of cells within our device allows for the capture and concentration of rare targets from within the sample or to physically separate out the target from the sample background. We investigated the use of dielectrophoresis to achieve these functions within our microfluidic lab-on-chip system.

5.1 Principles of dielectrophoresis

Dielectrophoresis is the forced exerted by an electric field acting on the dipole moment of a charge-neutral particle. The particle’s polarizability governs its response to the external field and depends on both the mobility of charge within the particle (conductivity) as well as the particle’s ability to accumulate charge (permittivity). Under the influence of an external electric field, positive and negative charge carriers within the particle re-arrange. This spatial arrangement of opposing charge distributions constitutes a dipole.
Figure 5.1: a) An ideal dielectric sphere polarizes in response to an external electric field. b) The dielectric fluid medium partially responds to the polarization of the sphere.

Suspending the neutral particle within a fluid medium complicates the response. An external electric field applied across the fluid will drive the re-arrangement of charge in both the neutral particle as well as the fluid. Charge within the fluid will move to respond to the external electric field and counter-balance the dipole of the neutral particle. Depending on the polariability of the particle and the fluid medium, the particle dipole will be either partially-, completely-, or over-balanced. The counter-balancing dictates the effective dipole moment observed by the particle in the presence of an external electric field.

In the case of a uniform electric field, no net force is exerted on the dipole. The interaction between the field and the spatial charge distribution of the dipole exerts a torque which rotates the particle into alignment with the external field. In the presence of an electric field gradient, however, the particle experiences a force acting along the gradient lines. This force, dielectrophoresis, induces motion towards either the maxima or minima of the gradient depending upon the orientation of the induced dipole.

5.1.1 Motivation for dielectrophoresis

Dielectrophoresis boasts incredible appeal for point-of-care diagnostics. Cells, viruses, and other biomarkers are permealizable and therefore experience the dielectrophoretic force. The
Actuating mechanism is the interaction of an applied electric field with a particle in solution. Microelectrode structures are readily fabricated to manipulate the target within the sample. Different cell species have differing frequency responses, allowing some selectivity of the target analyte through the choice of operating frequency. Dielectrophoretic manipulation does not rely upon the presence of chemical binding elements to selectively interact with the desired analyte, and in this manner is said to be label-free. The ease of fabrication and lack of a need for additional chemical treatments greatly simplifies some aspects of implementation for point-of-care diagnostics, hence the appeal.

Detection of biological agents at very low concentrations is limited by diffusion of the target to the sensing element. The electric field gradient generated for dielectrophoresis reaches microns into solution, actively driving analyte motion to overcome diffusion limitations on the measurement time-scale. These limitations are exacerbated by sample dilution which is often required to manipulate the sample conductivity into a suitable regime for other detection mechanisms. Dilution reduces the concentration of the target analyte, demanding a compensatory increase in sensitivity. Dielectrophoresis may be used to capture and concentrate the target from solution either before or after dilution to bolster the local concentration of analyte, reducing demands on sample volume throughput and thereby decreasing the time-to-diagnosis.

5.2 Derivation of the dielectrophoretic force

Let us derive an expression for the dielectrophoretic force in order to better understand the balancing act between the solution and particle polarizability. Consider the dipole, \( \vec{p}_m \), of the solution in the presence of an external electric field, \( E_0(\vec{r}) \). The electric potential (\( \Phi_m \)) at a distance \( r = |\vec{r}| \) from the center of dipole is:

\[
\Phi_m = \frac{\vec{p}_m \cdot \vec{r}}{4\pi \varepsilon_m r^3}
\]  

(5.1)

where \( \varepsilon_m \) is the permittivity of the fluid medium. If we now displace the solution dipole
with a spherical dielectric particle of permittivity $\varepsilon_p$ and radius $a$, we find the new potential a distance from this particle to be [101]:

$$
\Phi_{\text{eff}} \approx \frac{(\varepsilon_p - \varepsilon_m) a^3 \vec{E}_0 \cdot \vec{r}}{(\varepsilon_p + 2\varepsilon_m) r^3}
$$

(5.2)

and by visual comparison to 5.1, we see that the effective dipole moment of the particle is thus:

$$
\vec{p}_{\text{eff}} = 4\pi \varepsilon_s \frac{(\varepsilon_p - \varepsilon_m) a^3 \vec{E}_0}{(\varepsilon_p + 2\varepsilon_m)}
$$

(5.3)

Provided the size of the particle is small compared to the length-scale over which the the electric field varies, the force exerted on this effective dipole by the external field becomes:

$$
\vec{F}_{\text{DEP}} \approx (\vec{p}_{\text{eff}} \cdot \nabla) \vec{E}_0 = 2\pi \varepsilon_m a^3 \frac{(\varepsilon_p - \varepsilon_m)}{(\varepsilon_p + 2\varepsilon_m)} \nabla \vec{E}_0^2
$$

(5.4)

wherein the fractional term, comprised of the permittivities of the solution and the medium is known to as the Clausius-Mossotti (CM) factor. The value of the CM factor can range from $-1/2$ to 1 depending on which of the permittivities dominates the expression, and the sign of the CM factor dictates the direction of the force the particle feels in the external electric field.
Figure 5.2: Plot of the Clausius-Mossatti factor as a function of frequency for red blood cells in saline solutions of differing conductivities. Reproduced with permission from Shari Yosinski.

Plotting the CM factor for red blood cells in saline solution, as in Fig. 5.2, illustrates how changes in both frequency and solution conductivity alter the competition between cell and solution polarizability. At very low conductivities, there is a wide range of frequencies for which the CM factor is positive. The red blood cells experience positive dielectrophoresis (pDEP) and are pulled towards the metal electrodes. At high conductivities (1 S/m), the CM factor remains negative for the entire range of frequencies shown. The red blood cells experience negative dielectrophoresis (nDEP) and are pushed away from the electrodes. At intermediary conductivities (such as 0.1 and 0.19 S/m) there exist narrow regions where the red blood cells experience pDEP. The dielectrophoresis behavior is readily modulated by tuning the signal frequency over just a narrow range.

5.2.1 Dielectrophoresis of cells

Expanding this result to cells, we must abandon the assumption that both the medium and particle are ideal dielectrics. Instead, each possesses conduction mechanisms that allow for
the internal motion of ionic charges. These conduction mechanisms may be modeled as characteristic resistances [102]. When charge re-arranges itself to form a dipole, it flows through these conduction channels and thus the characteristic resistance, dissipating some electrical power. The effect is most pronounced for time-varying electric fields, requiring constant motion of the dipole charges and therefore continuous Ohmic losses. Incorporating the effect of these Ohmic losses into the model for the dielectrophoresis requires substituting the complex permittivity:

$$\varepsilon_{p,m} \rightarrow \tilde{\varepsilon}_{p,m} = \varepsilon_{p,m} + \frac{\sigma_{p,m}}{j\omega} \quad (5.5)$$

where $\sigma_{p,m}$ is the conductivity of the particle (cell) or medium, respectively and $\omega$ is the angular frequency of the external electric field. Substituting the complex permittivities of Eqn. 5.5 into Eqn. 5.4, we obtain a complex-valued expression for the DEP force, the time-average of which is found by evaluating the real component.

Thus, we observe that the conductivities of the cell and the surrounding medium, as well as the frequency of oscillation for the electric field also impact the CM factor and therefore the DEP force observed. This is the mechanism by which DEP becomes frequency-dependent and species-selective.

5.2.2 Competing forces

Stokes’ force

Microfluidic channels, well-suited for handling small volumes of biological sample, experience viscous flow. The cells within the sample experience a force proportional to their velocity relative to that of the fluid medium. The Stokes’ force on a small sphere of diameter $r$ flowing through this channel is:

$$F_{Stokes} = 6\pi\eta rv \quad (5.6)$$
where $\eta$ is the ratio of the fluid viscosity to fluid density, and $v$ the relative velocity between the particle and the fluid. The force of dielectrophoresis must be strong enough to overcome this viscous drag in order to capture particles in the flowing stream. Alternatively, for a given DEP force strength, there is a maximum flow velocity for which successful capture can occur.

**Sedimentation**

The force of gravity is countered by the buoyancy force as the cells flow in the suspended medium. If the density of the cells, $\rho_p$, exceeds the density of the fluid environment, $\rho_m$, they will eventually settle to the bottom of the tubing or the channel, out of the flow [103]:

$$F_{\text{grav}} = \frac{4\pi}{3} r^3 (\rho_p - \rho_m) \quad (5.7)$$

where $r$ is the radius of the cell. Sedimentation presents an annoyance upstream, wherein cells may settle out of flow before reaching the counter structure. The DEP capture force must also oppose it in the vertical direction above the plane of the electrodes to maintain the position of the captured cell.

In low Reynolds number environments, such as the interior of plastic syringes or teflon tubing, competition between the force of gravity (Eqn. 5.7) and viscous drag (Eqn. 5.6) sets a terminal velocity on sedimentation rate for particles in solution, $V_t$:

$$V_{\text{term}} = \frac{2r^2 g (\rho_p - \rho_m)}{9 \eta} \quad (5.8)$$

Considering a constant particle density within syringe or teflon tubing. From this initial distribution within the circular inner diameter, particles sediment out from solution at a variable rate. Assuming particles only sediment out when they reach the bottom of the cylindrical interior (ignoring adhesion at the walls), the fraction of particles remaining in suspension as a function of elapsed time can be readily computed, as shown in Fig. 5.3.
As can be seen from Fig. 5.3, particles settle much more rapidly in teflon tubing than within the syringes. The calculations here are shown for polystyrene beads in water which should settle less rapidly than cells in solution [104]. The analysis is carried out in the absence of fluid flow to provide a rule-of-thumb heuristic for settling times that is in reasonable agreement with empirical observations. Laminar flow introduces a parabolic flow velocity profile within the channel and therefore a buoyancy force acting on particles in the slower flow streamlines. A recent dissertation investigates the impact of constriction diameter, flow velocity, and particle size on the magnitude of this force [105].

**Electrocapillary forces in microfluidic environments**

Liquid droplets on a metal electrode experience a surface tension which depends upon the polarization of the metal electrode and the capacitance of the electrode-solution interface [106]. The surface tension exerts a force on the droplet. This force, electrocapillarity or electrowetting, can be controlled with an external applied potential to manipulate the contact area of the metal-droplet interface. Electrocapillary changes in the surface ten-
sion require the applied electric potential to be DC, or sufficiently low in frequency such that significant polarization of the double-layer can occur [107–109]. We do not expect electrocapillary effects to influence device behavior in the frequency regimes used for dielectrophoresis. The double-layer capacitance contribution to device impedance is typically negligible at these frequencies, as will be shown below.

5.3 Device

5.3.1 Chip fabrication

The devices used for dielectrophoresis are fabricated in the cleanroom by another member of our group. The electrode structures are lithographically patterned in photoresist atop a Borofloat-33 glass wafer. Metallization with 200 nm of aluminum follows. Devices are either allowed to form a native oxide upon exposure to atmosphere or are subsequently coated in a layer of 200 nm of plasma-enhanced chemical vapor deposition (PECVD) silicon dioxide as an insulating coating. The wafer is then diced and cleaned. At this point, the chips are available for use.

![Image of interdigitated electrode](image.png)

Figure 5.4: Optical micrograph at 5x magnification of a typical pair of interdigitated electrode. This particular device has an electrode-electrode gap of 25 µm, sixteen electrode fingers, and a 1 mm channel width.
5.3.2 Microfluidics fabrication

To fabricate the microfluidic channels, my colleague created an imprint mold. SU-8 photoresist was photolithographically defined to create a nominally 20 µm feature height for the microfluidic channels. Polydimethylsiloxane (PDMS, Dow Corning Sylgard 184) was mixed in a 10:1 ratio and poured over the mold. The mixture and mold were de-bubbled for thirty minutes in a vacuum chamber before being cured for one hour at 70° C. The PDMS “wafer” was subsequently peeled from the mold. Individual microfluidic channels were cut from the mold, cleaned, and hole-punched to form inlet and outlet ports. The microfluidic channels were then bonded to the individual chips after UV-ozone treatment by heating the aligned PDMS-chip combination in an oven for fifteen minutes at 70° C, after which devices were ready for use. This process is described in more detail in Appendix A. A microscope image of an interdigitated electrode structure is shown in Fig. 5.4. The microfluidic channel sidewalls are visible as parallel vertical lines on the left and right boundaries of the image.

5.4 Realistic modeling of dielectrophoretic devices

The simplest derivation of the dielectrophoretic force consider the polarizable particle experiencing an AC potential gradient between two parallel plate electrodes [110, 111]. Variations in the electrode design geometry alter the spatial profile of the potential gradient which alters device performance, an effect which physics-based simulations effectively capture [112–114].

Trouble arises when these computations cast the DEP force term as a function of the potential at the electrode-solution interface [101, 112–116]. Theorists and experimentalists alike have equated this potential with the externally-applied potential when optimizing device design. They experience significant deviations from expected performance in the operating regimes where this assumption breaks down. We must incorporate a fuller understanding of electrochemical impedance and real-world limitations to understand the conditions where this occurs.
5.4.1 Developing the full circuit model

Consider the infinitesimal of solution volume used in computing the DEP force experienced by a particle. The potential appearing at the boundaries of this solution volume generate the potential gradient which establishes the magnitude of the DEP force. As we expand the boundaries of the solution volume into consideration, the infinitesimal solution resistance element becomes approximated by the familiar solution resistance element invoked during discussions of electrochemical impedance spectroscopy. Fig. 5.5 depicts the process of dipole formation in an external electric field for a particle well above the Helmholtz planes of the metal electrodes.

Figure 5.5: A dielectric particle interacts with the electric field gradient in the fluid medium and has its dipole moment partially shielded by solvent ions. The ionic double layer around the planar electrodes influences the magnitude of the electric field in the inter-electrode region. Ions not shown to scale.

Electrode-solution interface

As the volume expands to its logical limit, the boundaries of the volume approach the electrode-solution interface. The impedance of the diffused double-layer and the potential drop which forms across it is the first term not taken into consideration when modeling the
behavior of DEP structures. For solution saline concentrations exceeding 1 mM, the length scale of the diffused layer is less than 10 nm. Comparing this to the typical size scale of cells being manipulated via DEP, on the order of microns, we can conclude that the potential gradient dropping across the double-layer itself will only exert act upon an incredibly small volume fraction of the cell, if at all. Therefore, the true potential determining the magnitude of the DEP force for device capture is the proportion of the applied voltage signal that forms across the solution resistance, between the double-layers of the two electrodes.

**Electrodes**

As previously discussed (Section 4.5.2), the impedance between two electrodes in solution contains two parallel conduction pathways: the capacitance between the two electrodes through the substrate in parallel with the electrode-solution-electrode circuit. Parameters governing the inter-electrode capacitance include the length and width of the electrodes as well as the gap between them [117–119].

Deposited electrode leads enable connection to macroscopic circuit elements (e.g., coaxial cabling) with fabricated contact pads. The lead-ins themselves also possess a finite resistance per unit length which induce Ohmic losses between the contact pad and the IDE region. The transmission line formed by the cabling connection to the voltage source instrumentation introduces an additional impedance, as does the output impedance of the voltage source itself (typically 50 Ω).

**Substrate capacitance**

Even in the absence of solution conduction, the large footprint of the interdigitated electrode structures and close physical proximity produces a capacitance between the two electrodes which may be measured directly in the dry state. This capacitance is a strong function of the electrode geometry and choice of substrate. For large-area designs, the capacitive loading can overwhelm the output capabilities of most voltage sources, preventing observation of DEP-driven phenomena.
5.4.2 Ignored inductances

A complete analysis of the dielectrophoresis circuit model cannot be achieved without consideration of the inductances formed by sharp bends in the electrode structure, occurring in the IDE structure and potentially in the electrode leads themselves. The operating frequencies for this work ranged between 100 kHz – 20 MHz and would require inductances on the order of 10-1000s of µH to pose a significant contribution to the overall device impedance, contrast with the “nH inductance expected from back-of-the-envelope calculations.

The full circuit model

![Diagram of a) Typical circuit schematic assumed when simulating DEP circuit performance as a function of electrode structure contrasted with b) a more realistic model of the full circuit which influences the force magnitude.]

Integrating these different circuit elements into a single model, we arrive at the circuit of Fig. 5.6b. We have assumed no charge-transfer at the electrode-solution interface which motivates our selection of gold for the electrode material. The resistance of the interdigitated electrodes and structure of the leads, $R_{elec}$, is here depicted to be symmetric but this need not be the case. The output impedance of the function generator ($R_{out}$) and any additional series resistances ($R_{series}$) occur in series with the device. Contributions from the substrate capacitance ($C_{sub}$) and double-layer capacitance ($C_{DL}$) can be separated by contrasting the device impedance in the presence and absence of solution in the channel.
5.4.3 Ramifications for the capture force

From visual inspection of Fig. 5.6b, multiple impedance elements exist in series between the solution resistance and the voltage generator. The potential formed across the solution region is the potential driving DEP capture and is therefore in principle sensitive to DEP circuit parameters, such as the interfacial polarisation as discussed by Glascoyne, et al. [120]. Demierre et al. [83] addressed the influence of a series resistance in-line with a DEP capture region when using fluidic side-channels as electrical contacts. The entire transmission pathway impacts the magnitude of the signal observed across the solution resistance, and we may write:

\[
\frac{V_{\text{soln}}}{V_{\text{appl}}} = \frac{Z_{\text{soln}}}{Z_{\text{total}}} = \frac{R_{\text{soln}}}{R_{\text{out}}} + \left( \frac{1}{2(R_{\text{elec}}+Z_{\text{CPE}}) + R_{\text{soln}} + j\omega C_{\text{sub}}} \right)^{-1}
\]  

(5.9)

wherein \( R_{\text{out}} \) is the output impedance of the function generator, typically 50 \( \Omega \), \( C_{\text{sub}} \) is the capacitance of the electrode structures coupled through the substrate, and \( Z_{\text{CPE}} \) the constant-phase element representing the double-layer capacitance of the planar electrode structures. Optimization of design parameters that neglects their impact in voltage transmission as described in Eqn. 5.9 will produce sub-optimal performance.

There exist three separate frequency regimes embodied within Eqn. 5.9. In the highest range of applied frequencies, both the double layer and the substrate capacitances have negligible impedance, at which point the voltage across the solution resistance drops precipitously, eliminating the ability to manipulate particles via dielectrophoresis.

In the intermediary regime, the impedance of the substrate capacitance is comparable to or much greater than the solution resistance, whereas the double-layer capacitance remains virtually shorted. In this regime, the maximal applied voltage drops across the solution resistance for a given electrode geometry and is therefore the desired operation regime.

At frequencies below this intermediary regime, the impedance of the double-layer capacitance is no longer negligible. With decreasing frequency, larger and larger proportions of the voltage appearing at the metal-solution interface drop across the double-layer capacitance,
effectively screening out the bulk of the DEP signal from particles in solution.

5.5 Experimental verification of the circuit model

Transitioning from a theoretical hypothesis to electrode design guidelines requires experimental verification of the predicted behavior. We present a series of investigations to demonstrate how device performance is impacted by design variations from the perspective of this voltage transmission framework. Operating at higher linear flow velocities, we use the competition between the Stokes force and the DEP force to shift the equilibrium velocity of incident particles flowing over our DEP electrodes. The magnitude of this shift is determined by the competition between the DEP and Stokes force acting on the particle in that region [121].

5.5.1 Methodology

The measurement

![Diagram](image)

Figure 5.7: a) particles flowing through a microfluidic channel move at an equilibrium velocity, $v_{off}$, determined by the Stokes force. Over the IDE region, the Stokes’ force competes with the DEP force, reducing the equilibrium velocity $v_{on}$. b) Tracking equilibrium particle velocity along the direction of fluid flow thereby probes the DEP force magnitude.

Particles flowing in a microfluidic system quickly reach an equilibrium velocity due to the Stokes’ force exerted by the fluid medium. When passing over the interdigitated electrode arrays, the particles experiencing pDEP experience an additional force opposing their direc-
tion of motion, reducing their equilibrium velocity. For full pDEP capture, the equilibrium velocity is reduced to zero. Multiple examples in literature have attempted to map the real component of the CM factor by analyzing cell velocities under laminar flow from microscope video recordings [121–123].

This process is illustrated in Fig. 5.7, depicting the position as a function of time as a particle passes over the interdigitated electrode array, located at $x_1$. In generating the position-time traces for the hundreds of particles passing over the IDE region, we perform sequential image analysis to track and trace the position of particles frame-by-frame from recorded videos. The beads are fluorescently-tagged, and therefore we employ fluorescence imaging with a laser excitation source and optical filter to maximize the particle-background contrast. The change in equilibrium velocities occurring between $x_1$ and $x_2$ as the particle as it passes over the array is proportional to the magnitude of the DEP force. The fractional change in velocity that particles experience when subjected to DEP forces over the device are extracted as

$$\frac{\Delta v}{v_0} = \frac{v_0 - v_{DEP}}{v_0} \quad (5.10)$$

We monitor this fractional slowing as a measure of the time-averaged strength of the DEP force and compare it with expected trends predicted by Eqn. 5.9. Multiple difficulties arise in extracting the precise force dielectrophoresis exerts on the passing particles. Force, proportional to acceleration, is related to the second derivative of position. Optical approaches measure the position as a function of time, and therefore extracting the acceleration requires differentiating twice with respect to time. Evaluating multiple orders of numerical derivatives inherently amplifies measurement noise, here generated both by uncertainty in the position as well as uncertainties in frame-to-frame timing interval. The dielectrophoretic force also acts on the particles in three dimensions and thus our top-down microscopy averages over the ensemble distribution of vertical positions within the channel. We use our findings to make best-practices recommendations for the design of DEP electrode structures optimized for function in high-throughput and high-conductivity scenarios.
The sample

We flowed fluorescent beads over our interdigitated electrodes for particle tracking video analysis. The polystyrene beads (Polysciences, Inc. 17867-5) were 1.77 \( \mu \text{m} \) in diameter and fluoresced green under excitation. The beads were diluted 4,000-fold in 0.1x PBS and flown at a rate of 0.4 \( \mu \text{L/min} \). The low flow rate was chosen to ensure a sufficient number of frames were recorded per particle transit. The dilution was chosen to ensure a high number of beads passing during recordings while not being so high as to overwhelm the tracking algorithm computationally. The 0.1x PBS buffer was chosen to reduce the solution resistance and thereby emphasize the significance of design variations on device performance in contrast to lower-conductivity solutions. As can be seen from inspection of Eqn. 5.9, the largest influence of electrode design is expected to be seen when the solution resistance is comparable to the electrode resistances.

Operating conditions

Solution was flown through the microfluidic channels at rates between 0.2–1.0 \( \mu \text{L/min} \), depending on the width of the microfluidic channel under investigation. The linear flow speed, and thus the viscous drag force, varies inversely with channel width at a given flow rate. The effect of the dielectrophoretic force is in opposition to this drag force. The flow rates were chosen such that the magnitude of the two forces would be comparable to improve detection.

The electronics

We use a Tektronix AFG3252 function generator to provide the AC voltage signal necessary to produce a DEP force. Both output channels were used, sourcing sine waves between 0.1–20 MHz configured to be 180° of phase with respect to each other, a mode of operation known as bipolar DEP. Each output channel was configured to expect a 50 \( \Omega \) load impedance and fed directly into a dual-channel, high-frequency power amplifier (Tabor Electronics 9250). Typical voltage amplitudes were 1.2 \( V_{PP} \) for the Tektronix function generator.
with a subsequent ten-fold increase in amplitude provided by the Tabor amplifier. These amplitudes were chosen such that the incoming beads experienced significant slowing over the DEP electrodes without becoming captured to render our measurements sensitive to shifts in the DEP force.

Furthermore, the instantaneous forces experienced by the particles are rapidly changing. The dielectrophoretic force varies not only as the particles pass over the electrodes but also depends on the particles’ height within the channel. The laminar flow profile of a microfluidic channel is fastest in the center, thereby introducing variance in the drag force arising from vertical height as well as the lateral position within the channel. These factors combine to render evaluation of the dielectrophoretic force magnitude challenging to put in their appropriate context. The desired end functionality of dielectrophoretic capture is a change from the initial equilibrium velocity to nil in the electrode region. Equilibrium velocity shifts therefore are a suitable proxy measure of the DEP force and an experimentally-relevant metric for performance evaluation.

Naively, one would expect to monitoring the fraction of captured particles to evaluate performance. However, capture is an unbounded threshold condition; a bead cannot be more captured by DEP forces exceeding those necessary to reduce the equilibrium velocity. For a given input voltage, there will be a range of electrode geometries for which the voltage across the solution resistance is sufficient for high capture and a range of geometries for which the voltage is insufficient for any capture. The only nuance in the measurement lies in the interpolant regime in which some, but not all, incident particles are captured. This regime is not a priori guaranteed to span a wide range of geometries, nor include any of the extant devices for a given set of operating conditions.

Measuring changes in the equilibrium velocity, however, avoids the pitfalls of capture-efficiency based performance evaluation. Sensitivity lost due to excessive capture force is avoided entirely by eschewing capture altogether, operating the experiment below that threshold. Evaluating differing equilibrium velocities allows us to then make comparisons between a range of electrode geometries, all of which achieve no capture for the initial conditions chosen.
5.5.2 Additional series resistance

The presence of an external resistance in series with the solution resistance element will impact the transmission of the voltage signal driving DEP. Typical origins include the output impedance of the voltage sources driving capture and the electrode leads transmitting the signal to the microfluidic region. To illustrate this phenomenon, we introduced a series resistance as indicated in Fig. 5.6b in line with our device. For each value of the series resistance used, the particle-tracking software identified the location of the fluorescent beads from frame to frame (Fig. 5.8a), computing the velocity in the region of the video with and without the DEP force. The measured impedance of the electrodes (Fig. 5.8b) was used to compute the expected voltage across the solution resistance and thereby the relative strength of the DEP force the particles experienced. As expected, the equilibrium velocity over the interdigitated electrodes increases as the series resistance is increased, indicating a decrease in the strength of the DEP force on the particles.

5.5.3 Number of fingers

Increasing the number of electrode structures within the fluidic region is another strategy for improving device performance, particularly for capture. Particles not captured by the first pair of electrode structures have additional chances to be captured during subsequent interactions with the DEP force as they pass over the repeating electrode sub-units. Accordingly, COMSOL simulations predict asymptotically-increasing capture probability as the number of repeating sub-units is increased.

As a consequence, then, it was posited that the only upper bound on capture electrode area was the maximal permissible footprint of the device. Akin to expanding channel width, increasing the number of electrode sub-units increases the total area exposed to solution and thereby decreases the solution resistance and thus the DEP force exerted. Competition between this phenomenon and the increasing capture probability predicts that the global maxima for capture probability is achieved at a finite number of electrode sub-units.

We empirically demonstrate this by measuring the change in equilibrium velocity while
Figure 5.8: a.) Particle-tracking software extracts particle velocities as they pass over the interdigitated electrodes. b.) Electrochemical impedance measurements extract circuit parameters characterizing the electrodes. c.) The DEP force experienced by passing particles is proportional to the squared magnitude (blue dashed line) of the voltage across the solution resistance element. With increasing series resistance, the ratio of the particles velocities off and on the DEP region (brown squares) approaches unity, indicating decreasing DEP force magnitude.
Figure 5.9: Changing the number of electrode fingers alters device performance. 

a.-c.) Top-down view of IDE structures with differing numbers \( N_F \) of electrode fingers. 

d.) Initially, the equilibrium velocity (brown sq.) over the DEP electrodes decreases with an increasing number of electrode fingers until influence of the decreasing voltage outweighs the increasing number of interactions with DEP force.

doubling the number of interdigitated electrode fingers from device to device. At first, as the number of fingers – and thus repeating units – increases, the equilibrium velocity of the particles over the DEP region decreases, as can be seen in Fig. 5.9d. Further increases in the number of electrode fingers, however, has the opposite effect, as the decreasing solution resistance reduces the magnitude of the voltage driving the DEP force. A fit in the expected form of \( a^2 / (a + b \times N_F)^2 \) interpolates the predicted voltage from Eqn. 5.9 plotted in Fig. 5.9d. Losses in magnitude outweigh the increasing capture probability of additional subunits, constraining the number of fingers to a geometry- and conductivity-specific optimum.

5.5.4 Channel width

Increasing fluidic channel width is a common tactic to increase volumetric throughput for DEP-actuated devices [124]. Increasing the width produces a commensurate decrease in the
solution resistance of the fluidic region. We placed a microfluidic channel of varying widths (0.5, 1.0, and 2.0 mm) over identically-fabricated electrode structures, as shown in Fig. 5.10a.-c. We correspondingly adjusted the volumetric flowrate (0.2, 0.4, and 0.8 µL/min.) to maintain a constant linear velocity – keeping the Stokes’ force constant across all three channel widths. Each doubling of the channel width correspondingly halves the solution resistance of the channel, consequentially decreasing the effective voltage seen across the solution (Fig. 5.10d) which is again interpolated with the fitting function $a^2 / (a + b \cdot w)^2$.

The voltage predictions of the device impedance model are contrasted with conventional approaches which do not modify the Dirichlet boundary conditions as the number of fingers are varied, here populated with data from the $N_F = 16$ case from the previous experiment which should be nominally identical to the 1 mm channel width condition.

Increasing channel height is another means of increasing volumetric throughput at constant linear flowrate. The fringing electric fields between planar metal electrodes driving the DEP capture decay in strength with increasing vertical distance above the electrode surface. The fraction of cells passing far above the electrode surface scarcely experience the DEP force. Increasing channel heights thereby increases fractional waste of the inlet samplet.

### 5.5.5 Protective coatings

Insulating layers are preferable to inhibit electrolysis at the electrode-solution interface, reduce the likelihood of cell adhesion, and reduce the probability of electrode corrosion by the sample [125–128]. These protective coatings introduce an additional series impedance in-line with the solution resistance and therefore impact the magnitude of the DEP force between the electrodes. The voltage transmission model also directly informs physical design limits on the effective capacitance permissible when coating the electrodes with a protective, insulating layer.
Figure 5.10: Increasing throughput by increasing width sacrifices DEP efficiency. The solution resistance of the channel decreases with increasing channel width and with thus the magnitude of the DEP voltage (dashed blue line).

Figure 5.11: Profile illustration of our devices a.) with and b.) without oxide and the corresponding impact on c.) impedance measurements for the devices in 0.1x PBS solution.

Two electrode structures differing only in the presence of a 200 nm of PECVD silicon
dioxide coating were compared to illustrate the coatings influence on device performance. The equilibrium velocity for passing particles was measured while the signal frequency ranged from 100 kHz to 20 MHz. The impedance of the oxide coating varies accordingly. Fig. 5.12a. illustrates the change in equilibrium velocity as the particles pass over the DEP region for the device without the PECVD coating. The slowing effect of the DEP force is in line with expectations from the voltage transmission perspective, neglecting variations in the CM factor of the polystyrene beads in the range of frequencies investigated when comparing to the voltage predictions but the comparison between devices at a fixed frequency remains valid. Contrast this with the performance of the device with a 200nm PECVD coating, as seen in Fig. 5.12b. DEP slowing rapidly vanishes at lower frequencies in a sharp transition between 7 MHz and 1 MHz where the impedance of the oxide attenuates the signal.

Figure 5.12: the expected voltage (blue stars) differs greatly when comparing devices with (a.) and without (b.) the 200nm deposited oxide as a function of the signal frequency. This effect is observed in the equilibrium velocity ratios (brown squares) at lower signal frequencies.
5.6 Conclusions

At elevated physiologically-relevant conductivities, simulations to enhance performance must incorporate loading of the voltage source into the Dirichlet boundary conditions. In high conductivity we want to maximize the performance to reduce operational demands (such as power and heat dissipation) while still achieving the desired functionality. This lowers the barrier to implementation for portable lab-on-a-chip applications.

As the electrode area exposed to solution or solution conductivity increases, the consequences of the low-impedance load manifest in weakened capture and Joule heating challenges [129, 130]. Joule heating constraints are a particular concern for operation in physiological salinities. We have also demonstrated existence of optimal/maximal array size for DEP capture. Competition exists between the number of momentum impulses,($\alpha N_F$), and their magnitude from the applied external voltage.

We may rewrite Eqn. 5.9:

$$\frac{V_{\text{soln}}}{V_{\text{AC}}} = \frac{\rho/A}{R_{\text{tot}} \left(1 + \frac{\left(j\omega Q_0 A\right)^{-n} + \rho/A}{\left(j\omega C_{\text{sub}}\right)^{-1}}\right) + \left(j\omega Q_0 A\right)^{-n} + \rho/A}$$

where $R_{\text{soln}}$ has been redefined as $\rho/A$ to make explicit the dependence of the solution resistance on the area of the channel exposed to solution. Likewise, $C_{\text{sub}}$ and $Q_0$ have had their area dependencies ($A$) separated out. We can combine Eqn. 5.4 and Eqn. 5.11 by inserting the definition $\vec{E} = -\nabla V (\vec{r})$. The spatial profile of the potential is dictated by the electrode geometry. If we assume a fixed geometry, therefore, we may separate the potential $V (\vec{r})$ into a spatial profile $P (\vec{r})$ which governs the gradient between the DEP electrodes and a circuit parameter-dependent function (Eqn. 5.11) which dictates the amplitude of potential multiplying the spatial function. Thus, our expression for the DEP force becomes:

$$\vec{F}_{\text{DEP}} = 2\pi \varepsilon_m a^3 \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) \left(\frac{\rho/A}{R_{\text{tot}} \left(1 + \frac{\left(j\omega Q_0 A\right)^{-n} + \rho/A}{\left(j\omega C_{\text{sub}}\right)^{-1}}\right) + 1}\right)^2 \nabla |\nabla P (\vec{r})|^2$$ (5.12)
Figure 5.13: Illustrations in changes in $V_{\text{soln}}^2/V_{\text{DC}}^2$ due to variations in a) the self-capacitance of the interdigitated electrode structures at fixed device area (0.8 mm$^2$, b) the interfacial capacitance due to the presence of an oxide coating, c) the concentration of the saline buffer solution for different device areas exposed to solution, and d) the area of the device exposed within the fluidic channel.
We explore the effect of these parameters in Fig. 5.13. We start with rounded values approximating the $N_F = 16$ device from Fig. 5.9, a design commonly employed in our lab. We then adjust the parameters one-by-one to illustrate how variations in each would impact device performance per Eqn. 5.11. The value of parameters held constant are denoted in the top left (a., b.) or bottom left (c., d.) inset corners of the figures. Some secondary parameters were also varied within each plot to give a richer understanding of the interplay of the several variables, these values are reported directly adjacent the line to which they correspond.

Capacitive coupling ($C_{\text{sub}}$) through the substrate arises between the DEP electrodes. $C_{\text{sub}}$ is an extensive quantity, depending upon the electrode density (the inter-electrode gap length) and the total area of the electrode structure. The dielectric properties of the substrate also impact this term, which forms in parallel with the solution impedance and interfacial capacitance. $C_{\text{sub}}$ sets an upper bound on the operational frequency for DEP capture, as shown in Fig. 5.13a. For our typical structures fabricated on glass, the capacitance is negligible. Some attenuation in the DEP force magnitude is predicted at higher frequencies for larger values of the substrate capacitance term, constraining fabrication options.

A large pseudo-capacitance forms at the electrode-solution interface in conductive solutions. Ion concentration (solution conductivity) and device area govern the magnitude of the pseudo-capacitance. In Fig. 5.13b, we consider the effects of variations in $Q_0$, the series combination of this pseudo-capacitance with the capacitance of a protective coating deposited over the device region. The impedance of the smaller capacitor dominates series capacitor combinations. Due to the atomically-thin nature of the ionic double-layer, the deposited coating is the determining factor. As the thickness of the coating increases, the effective capacitance decreases, shifting the curves rightward in Fig. 5.13b. This is in line with our results from Fig. 5.12. Within our framework, the maximal permissible coating capacitance is determined by the solution resistance of the device and the desired operating frequency, $R_{\text{soln}} \gg \frac{1}{2\pi f C_{\text{coating}}}$. This simple guideline allows for protective coatings with no marked detriment to device performance. Fabrication of dielectrophoresis electrodes nor-
mally involves noble metals such as gold or platinum to minimize reactions at the electrode surface. Protective electrodes enable use of cheaper metals in device design for significant cost savings.

The Clausius-Mossatti factor and physiological needs of the biological target constrain the choice of solution conductivity for DEP devices. The solution resistance and interfacial capacitance scale inversely and linearly with conductivity, respectively. Changes in the solution conductivity for a fixed device design will alter the voltage driving DEP capture per Eqn. 5.11. This effect is plotted for two order-of-magnitude variations in device area exposed to solution in Fig. 5.13c. As the solution conductivity decreases, device performance becomes less and less sensitive to design variations. Conversely, performance varies as we alter the area of device (A) exposed to solution for a fixed solution conductivity, as shown in Fig. 5.13d. In low conductivity regimes, the device area may be scaled aggressively before performance limitations take hold. At higher conductivies, performance is highly sensitive to device footprint for a fixed $R_{tot}$.

Fig. 5.13 shows the influence of experimental factors chosen after defining the electrode geometry. This assumption is necessary for Eqn. 5.12 to hold. The spatial pattern and circuit parameters are in fact coupled through the geometry design process, a fact which must be taken into consideration when optimizing device design.

The scaling parameters varied in Fig. 5.13 depend heavily on the value of the total external series resistance, $R_{tot}$. Minimizing losses from series resistances requires reducing the output impedance of the voltage source and metal leads. Increasing the width and thickness of the electrode leads while reducing length. Integrated circuit solutions for voltage sources can reduce the output impedance below the 50 Ω convention for benchtop electronics, mitigating some attenuation. Researchers should be aware that there are diminishing returns to these increases for the electrode leads as the series resistance contribution approaches a few Ω at most. Finger resistance should be primarily address through minimizing the excess finger length. Further study into the interplay of the electrode width/gap on the DEP force, but such design changes also alter the gradient profile driving the DEP capture and therefore require a more nuance and target-specific view but remains an active area of
investigating for performance engineering.

Maximizing the solution resistance will improve performance, all else held constant. Possibilities include decreasing the area exposed to solution, widening the inter-electrode gap, reducing solution conductivity. This is most readily done by adjusting the conductivity of the sample solution used and helps to explain the prevalence of DEP in the literature conducted at lower conductivity: with low conductivity/large resistance, other design considerations are unlikely to have a significant deleterious impact on performance.
Chapter 6

Future Outlook

Improving global healthcare outcomes requires reducing the cost and infrastructure necessary to provide treatment to patients around the globe. Innovations in the biomedical device space are increasingly important to achieve these goals, particularly in the realm of portable diagnostics. Present efforts at miniaturizing common diagnostic procedures still require complex sample pretreatment or additional subsequent steps. This thesis research investigates the role electrochemical impedance plays in the design and function of lab-on-a-chip diagnostic techniques.

Our work began developing an impedance-based sensor for the enumeration and sizing of biological particles in solution. We implemented planar electrodes as low-cost sensing elements and developed the measurement circuitry and code necessary to detect and discriminate amongst particles of varying sizes in a range of solution conductivities at low filling factors in our constriction region. We identified the role device geometry and parasitic capacitances play in limiting the performance of the impedance-based sensor and identified solutions applicable in the laboratory and in production – of particular importance for sensing in lower conductivities and when implementing high-frequency impedance sensing for cell species discrimination.

Translating this technology to a portable form factor requires miniaturization of the relevant hardware to board-level circuit analogues. Refinement to the front end of the dif-
ferential bridge measurement circuitry is of the utmost importance for the next generation. The spring-loaded connectors make prototyping convenient but ultimately a smaller form factor will be required. Multiple integrated circuit solutions exist to generate the counter’s sinusoidal excitation signal, including direct digital synthesis and phase-locked-loop techniques. Modern lock-in amplifiers perform digital demodulation of the input signal to achieve the remarkably high dynamic ranges commercially available. Appropriate selection of an analog-digital converter and microprocessor could likewise perform the same function in a portable platform.

Understanding the role that electrochemical impedance played in biosensing contexts, we examined the influence different facets of electrode design held over the performance of DEP structures. The dielectrophoretic force depends on the fraction of the externally-applied voltage formed across the fluidic medium. Losses in transmission due to the output impedance of the voltage source, resistance of the source electrodes, and polarization of the electrical double layer must be considered when designing electrodes for dielectrophoretic manipulation of cells in solution.

Increasing the number of repeating sub-units of the electrode structure is a common solution to improving capture performance. Our experimental results demonstrate that this only provides benefit until voltage transmission losses overwhelm the marginal return of an additional sub-unit. Our results also indicate that anti-fouling coatings may be deposited to protect the metal electrodes from cell adhesion without degradation in device performance up to coating thicknesses dictated by device geometry, solution conductivity, and desired operating frequency.

For DEP arrays of considerable size, significant performance improvements can be achieved by splitting the large array into several sub-arrays driven by independent voltage sources to mitigate attenuation due to the output impedance of the source itself. Particularly in high conductivity solutions, such as physiological samples, Joule heating remains a significant challenge. A stronger DEP force requires increased power dissipated across the solution resistance. The resultant heat can damage the sample or electrodes and thus sets the operational upper bound. Development of thermal management techniques to reduce
Figure 6.1: Stills taken from fluorescent microscopy video recordings of lateral separation of activated from unactivated T-cells. a.-c.) Activated T-cells (fluorescing red) experience lateral displacement as they pass over the angled electrode structures, whereas d.-f.) unactivated T-cells (fluorescing green) pass mostly unaffected. Vertical blue lines indicate the edges of the PDMS channel.

sample heating.

Understanding the inherent challenges in performing dielectrophoresis in physiologically-relevant conductivities, we designed electrode arrays to manipulate the lateral displacement of cells within our sample to enhance the functionality of our impedance-based assay. Dielectrophoresis and the Stokes’ force have different dependencies on cell diameter. Under carefully chosen conditions, we can separate activated and unactivated T-cells as shown in Fig. 6.1a-c. This approach has been previously demonstrated in the literature [131].

Physiological samples are inherently messy. Separating the enumeration and analysis target into a parallel fluid stream isolates it from the environment containing debris and up to billions of cells per mL which comprise the fluidic background signal. This confers two distinct benefits. Only the purified side stream needs pass through a constriction region for enumeration, greatly reducing the clogging probability during operation.

Furthermore, physically filtering the incoming fluid stream in this manner greatly simplifies the computational complexity of enumeration. Isolating the target from a high number of background count relaxes the rejection thresholds for false positives and false negatives at the same error rate in terms of events per volume. This feature is particular desirable for
background signals which are comparable in size to the intended target which necessitates additional discrimination mechanisms to distinguish between the two populations.

The current generation of devices integrate both the DEP separation and Coulter counter enumeration onto a single microfluidic chip, having established both operational capabilities separately. We expect to then quantify the separation efficiency of our assay and purity of the sample within the exchange buffer stream as illustrated in Fig. 6.2. Two separate inlets, one connected to the sample and the other to the buffer solution, flow in side-by-side in the wider microfluidic channel before passing over the separator structure. In the absence of an applied DEP signal to the separator, Fig. 6.2a, the parallel laminar flows continue through the device, separating at the junction before passing over a counter structure en route to two outlets in the bottom of the figure. An applied DEP signal, Fig. 6.2b, drives lateral separation of the activated T-cells (purple spheres) as well as a few unactivated T-cells (red spheres) into the buffer stream. These pass through the right outlet channel where they are enumerated by the right Coulter counter.
Figure 6.2: Incoming sample and an adjacent exchange buffer stream flowing through our device a.) without and b.) with a dielectrophoresis signal applied to the separator electrodes. c.) Without lateral separation (DEP off), both species of particles pass through the left Coulter counter constriction region while not passing through the right counter. When a DEP force is applied, lateral separation drives particles into the exchange buffer stream, producing counts from the right counter structure.

In the absence of an applied DEP signal, the counter in the left channel detects both populations in the outlet stream whereas the right channel sees few, if any, events. Histograms of the detected events for both counters are shown in the top row of Fig. 6.2c. When the DEP signal drives lateral separation of the activated T-cells, the bulk of the activated population in the left channel (the sample stream) is depleted and instead detected in the outlet of the buffer channel by the right channel counter. A fraction of the unactivated T-cells are also separated by the DEP signal. The DEP signal therefore drives changes in the detected cell distributions as measured by both counters, shown in the bottom row of Fig. 6.2c.

Presently, we are working on establishing good separation on the present devices. Enumeration was performed using Coulter counter structures fabricated on a glass substrate to mitigate the influence of parasitic capacitances from the bonding pads, whereas the initial
separation structures were fabricated on silicon wafers. The combined devices have been fabricated on glass and are experiencing issues with electrode integrity while applying the DEP drive signal. We are currently investigating the origin and potential solutions to this phenomenon at this point in time.

We then developed a diagnostic assay combining lateral displacement with enumeration and sizing which could deliver valuable information about patient health status. Our efforts centered on enumerating the ratio of activated to unactivated T-cells in physiological saline as an indicator of patient immunological function. We also explored other means of integrating dielectrophoretic manipulation and impedance-based cell counting for biosensing applications.

The logical progression for the immunological assay is extending the result from sample in physiological saline to separation in whole blood. A two-step buffer exchange process would eliminate the need for sample centrifugation prior to analysis. Additional efforts are needed to ensure reliable operation at low target concentrations, such as in the case of circulating tumor cells which can be found in concentrations as low as 1-10 cells/mL. We can then extend this assay schema to a range of applications where speed of detection and portable form factors are of the utmost importance.

Although we have presented proof-of-concept demonstration for the combined dielectrophoresis and Coulter counter diagnostic subsystem, much engineering remains to be done translating this technology from the laboratory to a handheld form-factor capable of bringing rapid diagnostic screening to low-infrastructure settings. Beyond the challenges we have already addressed, mechanical engineering for sample processing and handling, biosafety considerations, and more remain to be sorted out as part of a broader and concerted effort to bring this concept to fruition.
Appendix A

Integrating DEP & Coulter counter: capture & count

Researchers are continuously investigating additional applications of dielectrophoresis for lab-on-a-chip biosensing applications, combining it with other on-chip technologies to tackle specific design challenges and demonstrate utility in additional contexts [94, 114, 116, 129, 132–134]. The present research is no exception, and we have explored several ways to make use of dielectrophoresis to simplify design constraints for our impedance-based flow cytometer and produce a more compelling diagnostic system.

Dielectrophoretic electrode structures have been widely used for cell capture [135, 136] and sample concentration [114, 115, 137, 138]. We investigated how sample concentration could be used to enhance the performance of our counter subsystem. In low particle density regimes, the volume of solution per particle greatly exceeds the volume of the constriction region. DEP capture enhances the local concentration of particles within the volume of fluid above the electrodes. This presents no computational for the counter software provided that the particle density does not produce a high incidence rate for simultaneous passage of multiple particles.

At high capture efficiencies, very few particles will pass over the counter structure while the DEP signal is on. When the DEP signal is turned off, a packet of concentrated particles
Figure A.1: a.) incoming particles trapped on the DEP electrode structure are then b.) subsequently released for enumeration. c.) Only a few particles escape the electrodes while the capture signal is applied, in contrast with d.) the output response when the packet passes over the counter. e.) The frequency of bead passage events peaks sharply in time shortly after the release.

will leave the electrode structure and travel downstream. The process restarts when the capture signal is applied once more, in a process illustrated by Fig. A.1.

To show this detection scheme in action, 4.45 µm diameter polystyrene beads were 2,000x-fold diluted in 0.01x PBS and flown through the device at 0.5 µL/min. A capture signal of 4.0 MHz and 5.0 V_{PP} concentrated the incoming beads at the electrode structure. The counter region was simultaneously monitored optically and electrically. While the capture signal was applied, a few single bead passage events were observed as the DEP electrodes failed to capture some beads. Shortly after the capture signal was turned off, a large number of beads was observed passing through.
Appendix B

Experimental protocols

B.1 Sample preparation

B.1.1 Particle concentrations

Particle concentration within the sample is an important choice for the experimentalist. We have found that particle densities between 0.1-1 million/mL function best for acquiring a significant number of events within a reasonable measurement time-frame without presenting excessive clogging risk (for 20 \( \mu \text{m} \times 20 \, \mu \text{m} \) channel cross-sectional areas).

While cell line densities are typically provided in terms of counts per mL, polystyrene beads come shipped reporting the weight percentage by volume, and must be converted to particle densities:

\[
[C] = \left( \frac{\% \text{wt.}}{\text{vol.}} \right) \frac{1}{\frac{\pi \rho d^3}{6}} \quad (B.1)
\]

where \([C]\) is the concentration per unit volume for particles of density \(\rho\) and diameter \(d\). Reducing the bead density to the require range requires differing dilution factors for different bead diameters. These dilution factors range from 100x (for large diameter beads) up to 10,000x at smaller diameters. Typical dilutions are most easily performed in a two-step process: a preliminary 100x dilution and, if necessary, a subsequent dilution stage to
achieve the desired concentration.

### B.1.2 Phosphate-buffered saline preparation

Debris in beakers of the stock PBS solution used for bead dilution is the most prevalent cause of clog formation. Detritus which has fallen into the stock over of time makes its way into the diluted sample. Filter pillars are fabricated in the microfluidic pattern upstream of the constriction region with a nominal pitch equal to the width of the constriction cross-section and should therefore block all particles too large to pass through the constriction.

For large debris slightly smaller than the constriction, there exists a finite probability it will interact with the PDMS channel and become stuck. Flowing particles may rapidly stick to the adhered debris until the channel region is completely clogged. The filter pillars do not always bond to the surface of the microfluidic chip on account of their small area. Consequentially, long and slender debris pieces have been observed to pass through the filter pillars. Passed debris rotating as the channel narrows down will immediately clog the constriction entrance.

To avoid this problem, the stock buffer solution should be thoroughly washed prior to use. The protocol is as follows:

1.) Gather the beaker of stock solution, two 50 mL Falcon tubes, one 10 mL falcon tube, one 10 mL syringe, and one syringe filter.

2.) Open the syringe, and withdraw 10 mL of stock solution from the beaker.

3.) Place the syringe filter on the tip of the syringe.

4.) Slowly empty the contents of the syringe into the first 50 mL falcon tube. The solution should come out droplet by droplet. This will take a few minutes - the slow rate of fluid transfer is necessary to avoid forcing debris through the filter paper.

5.) Remove the syringe filter from the syringe, and withdraw the contents of the 50 mL falcon tube into the 10 mL syringe.

6.) Discard the first Falcon tube, and place the filter tip back on the syringe.

7.) Repeat steps 4-6 using the second 50 mL Falcon tube.
8.) Repeat steps 4-6 using the 10 mL Falcon tube. You now have thrice-washed buffer solution which should be free and clear of debris.

9.) Discard the filter tip and the 10 mL syringe.

10.) Optional: add 10-100 µL of Tween-20 solution into the buffer to act as a surfactant and inhibit particle aggregation.

Note that we use 50 mL Falcon tubes for ease of withdrawing solution via a 10 mL syringe. Typically, 1 mL syringes are used for actual experiments and therefore the 10 mL Falcon tube suffices when withdrawing thrice-washed solution for experiments.

B.1.3 Washing the beads

As an additional precaution, you may centrifuge and wash your diluted bead solution to remove any suspected contaminants in the bead stock. Take your diluted bead solution and load it into a 0.5 mL Falcon tube for centrifuging. Place an identical Falcon tube full of DI water directly opposite your sample tube in the centrifuge, taking care to mark which is which. Place the plastic cover securely on, and close the lid. Centrifuge the sample at 2500 g for 10 minutes. Remove the actual sample and carefully pipette out the supernatant liquid from the top so as not to disturb the densely-packed beads. Pipette in 400-500 µL of thrice-filtered buffer, briefly (~10 seconds) vortex the sample to re-suspend the beads, and place the sample tube back in the centrifuge. Repeat this process to centrifuge the sample twice more.

When this process is concluded, vortex the beads to re-suspend them. You may briefly sonicate the beads to break up any aggregates which might have formed during the process. Do not vortex polystyrene beads for longer than 30-60 seconds. Especially for larger beads, prolonged sonication leads to fragmentation, producing a wildly heterogeneous population in terms of diameter.
B.2 Device handling

B.2.1 Wetting the device

Flowing a sample containing cells or beads through a dry microfluidic device will invariably result in some fraction of particles adhering or stuck to the microfluidic channel and chip surface. The best method to avoid this occurrence is to wet the channel with solution prior to measurement. Typically, we will flow the same PBS buffer solution as will be used in the subsequent experiment. The addition of 0.1-1.0% Tween-20 to the wetting solution coats the channel with surfactant, reducing sticking probability.

Ethanol proves superbly effective for wetting PDMS channels. However, it must be avoided at all costs. We have observed cracking and peeling of PDMS within the microfluidic channel after prolonged exposure (~30 minutes) to ethanol and other solvents. These PDMS fragments clog channels irreparably. We suspect this is related to their ability to dehydrate the PDMS polymer matrix but have not investigated this effect further.

B.2.2 Avoiding tears

For our microfluidic systems, we typically employ thin-walled, 28-gauge Teflon tubing (Component Supply Company STT-28-C). The choice of gauge is not a significant constraint for the linear flow velocities at which we operate our devices. Thin-walled tubing performs markedly better than its regular-walled counterparts. The inlet and outlet tubing exits our device vertically and subsequently curves away under the influence of gravity. Thin-walled tubing has significantly less mass per unit length and exerts far less torque on the PDMS microfluidics.

This torque puts stress on the PDMS leading to tears at the PDMS-tubing interface. Tears must be avoided to maintain reliable flow rates during experiments. Depending on the severity of the tear, it will either introduce oscillations in the volumetric flow rate as small droplets of solution escape through it or slow down the intended flow rate as some solution continuously leaks out into the ambient.
Accidental collisions during operation of the lateral stage mount causes tears as well. The microscope objective stands directly between the researcher and one of the microfluidic ports during use. Excise caution during translation of the sample stage.

### B.2.3 Patching tears

Removing and re-bonding the entire microfluidic channel can be done as a matter of last resort in the case of catastrophic clogs or stubborn tears. It is more practical to patch over smaller tears. Taking a razor blade, cut a square piece of PDMS to be your patch. Fresh PDMS is softer and more pliable - the more recently the patch and microfluidic channel have been made, the more likely this procedure is to be successful.

Punch a hole in the PDMS patch with the same diameter as the inlet/outlet holes. Under the microscope objective, observe both surfaces of the patch for tearing adjacent to the punched hole. Adhere and then rip off Scotch tape from the surfaces of the PDMS patch as well as the surface of the microfluidic channel to remove contaminants.

Load both the patch and the device into our UV-ozone machine and run it for 10-15 minutes to activate the surface bonds on both PDMS faces. Taking whichever side of the patch was face-up for this step, press it face-down onto the microfluid channel. Ensure that the patch hole is aligned with the outlet hole in the microfluidic channel, inspecting it from the top and the sides.

Mix up a small amount of fresh PDMS. Taking a thin film on the tip of a toothpick, gently apply PDMS to all four sides of the patch-channel interface. Be careful so as to not nudge the patch out of alignment while doing so. The freshly-mixed and uncured PDMS will caulk and seal the two pieces of PDMS together. Put the caulked and patched microfluidic channel into the oven at 70 °C for twenty minutes to cure the PDMS. The device should now be ready for operation.
B.2.4 Solving clogs

The strategy for removing clogs depends upon the severity of the clog when it is first caught. Always halt sample flow immediately if a clog has formed. The first line of defense is to manipulate the channel directly. Taking the broad side of a pair of tweezers, press down on the PDMS channel in the immediate vicinity of the area downstream of the clog. If the clog is not yet too severe, this will generate a hydraulic pressure to send the clog constituents back upstream. As this pressure relaxes, the constituents will flow towards the constriction region again. This process, after a few repetitions, can remove minor clogs altogether as the offending particles are given a few chances to make it through the constriction without reforming a clog. Apply tweezer pressure gently to avoid abrading the PDMS, which distorts optical path lengths and renders crisp imaging futile.

If the clog is more severe, do not allow the device to dry out. Remove the device and submerge it in de-ionized water within a clean container. Sonicate the device for thirty minutes, remove it from DI, and check to see if the clog remains. If possible, ensure that the fluidic channel is vertical to allow individual polystyrene beads and bead fragments to sediment downward through the device under the influence of gravity.

B.3 PDMS Recipe

To mix up PDMS, I have adopted the following recipe from Wei-wei Cui, who was a visiting Ph.D. student in our lab. The procedure is as follows:

1.) Gather a Down Corning Sylgard-184 PDMS kit, a plastic weighing boat, a 10 mL syringe, a 3 mL syringe, and a toothpick.
2.) Using the 10 mL syringe, measure out and dispense 30 mL of the PDMS base into the weighing boat. With the 3 mL syringe, measure 3 mL of the activator chemical and dispense it into the weighing boat. Dispose of both syringes. *Note*: in general, this 10:1 ratio performs best for microfluidics. The volumes specified here are used when making microfluidic channels on a 4” patterned wafer.
3.) Mix the contents of the weighing boat thoroughly with the toothpick. Anywhere for 2-5
minutes should suffice.

The PDMS is now ready to be used for any application. The steps that follow outline how this is used to imprint microfluidic patterns into PDMS microfluidic channels using a wafer mold.

4.) Take the wafer mold and lay it flat on a 10” x 10” sheet of aluminum foil.
5.) Using Scotch tape, tape the entire perimeter of the wafer mold to the aluminum foil. This prevents liquid PDMS from flowing underneath the wafer.
6.) Fold up the sides of the aluminum foil until it resembles a petri dish. Use tape and remove excess foil where necessary to ensure the aluminum foil forms a good wall at the edges of the wafer.
7.) Pour in the contents of the weighing boat from step 3.
8.) Place in the designated vacuum chamber and pull vacuum for thirty minutes, or until bubble evolution from the PDMS has stopped.
9.) Turn off the vacuum and gently vent to atmosphere, removing the wafer from the chamber.
10.) Cure the PDMS wafer, either for 20 minutes at 70 °C or 24-36 hours at room temperature. Curing at elevated temperatures will shrink the PDMS between 1-3% for all dimensions. For patterns where alignment is required over large scales, the longer room-temperature cure must be performed. This solution was tested and implemented by Shari Yosinski, another former Ph.D. student in our lab.

Some tears occur prior to device handling altogether, such as when cutting individual microchannels from the PDMS. Each channel has an outline defined in the mold, leaving a visible imprint in the PDMS after separating it from the wafer. Individual channels are cut from the mold with a razor blade. Align the blade carefully to the channel outline and press straight downward, firmly through the resistance of the PDMS. Allow time for the blade to sink through the PDMS. The resistance of the PDMS can cause the blade to wrench, tearing through and destroying a channel.

Inlet and outlet holes must be punched once the channels have been cut. We use a 0.75 mm hole punch for use with 28-gauge tubing. The hole-punching process can lead to
inlet/outlet tearing. Always double-check your channel afterwards. To avoid tears, place the PDMS channel atop a sacrificial block of PDMS. Insert the punch straight down and do not adjust its angle once it has entered the PDMS. After punching completely through the channel, grasp the channel firmly on both sides of the punch and slowly remove it. The sacrificial block lets the punch go cleanly through while providing a supportive substrate to hold onto without warping the microfluidic channel. Treating the channel gently during the punching procedure significantly reduces the likelihood of tears.
Appendix C

Stage mount and PCB

Coulter counter measurements in high-conductivity solutions required adequate shielding, as previously mentioned in Section 3.3. A metal stage mount was constructed which automated sample alignment in all three dimensions, greatly reducing the experimental time spent per device over previous implementations.

The metal stage mount also acted as a Faraday cage for our measurements. Surrounded by a multitude of instruments in close proximity, our Coulter counter devices were enveloped in ambient 60 Hz noise. The metal stage mount was electrically connected to the ground plane of the PCB through metal screws which tightened into metal pads around the through-holes contained within the PCB. I took a three-pronged electrical cable, cut off the female adapter, and safely terminated the two live wires. The terminated live leads were heat-shrunk to the cable itself to ensure they were well-passivated and would not make accidentally make physical contact. I soldered an alligator clip connector to the neutral earth, the third terminal of the power cord. This alligator clip was kept contacted to the sample mount, and the cord plugged into the same single surge protector powering all the counter equipment.

The neutral earth connection was necessary to handle the radiated signal amplitude at 60 Hz that would otherwise couple into the measurement system when the microfluidic pumps were activated. As a precautionary measure, the microfluidic teflon tubing was
encased in a mesh metal sheath and the syringe itself wrapped in aluminum foil to form a pseudo-coaxial shielding around the microfluidic conductor (which at high conductivities formed a nice antenna to couple into the 60 Hz aggressor signal). This shielding ensemble was kept in physical contact with the metal stage mount to ground it as well and isolate the electrolytic sample from the ambient noise.

C.1 Stage Mount

For reference, in addition to the image included in Fig. 3.9, I have included a full PDF of the design for the metal stage mount. The particular dimensions are chosen specifically for our wafer thickness and the height and compression length of our spring-loaded pogo header used to make electrical contact.

One design feature that has not yet been mentioned is the presence of four grooves, most clearly visible in quadrant B1 on sheet 3 of 4. The PCB design contained several rows of BNC coaxial adapters mounted onto the board with through-hole connectors for mechanical stability. These grooves were necessary to allow the board to sit flush with the stage mount despite the through-hole connections.

The stage mount has two sets of screw holes, spaced one inch apart along the fixture. In this schematic, the grooves do not come far enough forward when trying to use the outer chip-interfacing area. As shown in this schematic, the grooves do not extend far enough into the stage mount to permit the PCB to sit flush when using the outer area. An additional inch had to be milled out in the student machine shop after the error was discovered.
Figures 1 and 2 are provided as supplementary images for the Microscope PCB Stage document. The diagrams illustrate the dimensions and tolerances specified in the drawing. Details such as material, finish, and notes are also included to provide comprehensive information for the assembly process.
Microscope PCB Stage
Screw-hole locations

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C.2 Printed circuit board

The printed circuit board design for the devices was quite simple. The top and bottom layers were flooded with copper ground planes. An array of through-hole, coaxial BNC jacks were mounted at the distal end of the PCB, far away from the microscope objectives.

The twenty pin pogo header was divided as follows: two initial pins (pins 1 and 2) for a single DEP array included on almost every chip, followed by six three-pin pairings (pins 3-20). On some chips, each three-pin group corresponded to a single Coulter counter structure. Other chips had more DEP electrode structures after the initial two pins, and only used the last two or four groupings for Coulter counter devices.

The boards were designed to accommodate this modularity. Each counter structure grouping had the necessary passive bridge components, a single dual-channel buffer amplifier, and a single instrumentation amplifier connected to it. The buffer amplifier had the inverting and non-inverting pins for each channel directly adjacent to one another. The surface-mount pads intended for these pins could be soldered together to create straight-through connections for DEP electrodes on boards intended for chips with fewer than six counter structures.

To prevent any electrical hazard from unintentional shorting and preserve battery life, each individual IC was given its own power switch controlling the flow of current from a pair of 9V batteries which powered the whole board. Battery power being used to reduce noise. In future iterations, both ICs could share a common power switch with no significant loss in functionality.

Both the buffer circuitry and the instrumentation amplifiers were included to validate the performance of the instrumentation amplifier against the known working buffer circuit approach. Furthermore, inclusion of the buffer amplifiers permitted the modular design during rapid prototyping stages of the instrumentation apparatus. Future iterations could eschew the buffer amplifiers altogether to save precious trace length and cut down on parasitic input capacitances which might still be limiting bandwidth of the devices at present. The sole argument in favor of keeping the buffer amplifiers is that they provide a good sanity
check in higher conductivities. Amplitudes between 20-60% of the input signal should be observed in 1.0x PBS, for instance.

The following page contains the schematic capture process for a single iteration of the counter structure measurement circuitry interfacing the PCB. Removal of additional counter bridge circuits greatly cleans the presentation, but this design as shown would be repeated for pins 3-17 on the header before moving to board layout.
Passives

Buffer amp

Instr. amp

BNC connex.

Power switch
C.3 PCB Layout

The PCB layout is conceptually quite straightforward and can be divided into three main sections. The first section is the array of BNC adapters placed over the grooved slots in the stage mount which allow interfacing with all 20 pins of the POGO header contacting the device. The second section contains placement of two battery holders for the 9V batteries as well as the array of switches that control power to the integrated circuits. The final section, visible in Fig. 3.9 is nearest the microscope objectives and contains the bridge circuits and buffer/instrumentation amplifiers used to generate the counter signal.

![Component Placement Diagram](image)

Figure C.1: Representative component placement for one Coulter counter structure interfacing both a dual-channel buffer amplifier (U6) and instrumentation amplifier. Additional routing removed for clarity.

To minimize parasitics, placement of the counter components should be as close to the pogo header as possible and all elements of a single measurement circuit should be in close physical proximity. Fig. C.1 illustrates a layout pattern for the components which minimizes overall path length between traces and the number of vias required to route the circuit.

Current devices make use of 1210 and 1208 package sizes for the passives which are
quite easily manipulated for individual soldering placement. The use of stencil masks and an IR reflow oven permits the use of even smaller components but requires a certain degree of expertise in hand-eye coordination to manually place and a quality soldering iron tip to repair. Reducing the physical dimension of the passive components would greatly reduce footprint constraints on spacing, allowing for placement closer to the pogo header especially with multiple counter structures on a single PCB. Presently, decoupling capacitors are on the board between the supplies and ground are directly adjacent the battery connectors as a space-saving measure. With smaller footprints, the decoupling capacitors can be placed directly adjacent the IC rail supplies without forcing the counter structure measurement circuits to occupy an excessive board area.

On the following page you will find an overview of the entire PCB layout on the final iteration of boards sent to fabrication and used throughout this dissertation.
Appendix D

Appendix C: MATLAB Scripts

D.1 Overview

This appendix presents the MATLAB scripts used to acquire time-domain voltage readings from the Coulter counter measurement system as well as subsequently analyze the data to identify passage events and extract parameters about the size and velocity of these passages.

The code is presented here such that it may be directly copied into the MATLAB interactive development environment with no editing by the user. No special packages or drivers should be required to execute these scripts, just the base MATLAB software (last ran on 2018a and 2018b) with an active license.

The explanation of the code is contained within the comments, provided throughout in green. On occasion, where a deeper discussion of a design choice is merited beyond the contents of the comments, I will break out of the code text to explain the logic behind said choice.

D.2 Coulter counter data acquisition

```matlab
%% DPO4104 Coulter Counter Signal Acquisition
% Zachary Kobos, Department of Electrical Engineering, Yale
% University. New Haven, CT 06511. zachary.kobos(at)yale.edu.
% Last updated October 16th, 2018.
clear all;

%% INSTRUMENT COMMUNICATION
% First we scan for an available VISA resource channel
% at the address we expect to find the oscilloscope. The
% DPO4104 oscilloscope we presently use has a USB-serial
% communication interface.
```
instrObj = instrfind('Type', 'visa-usb', 'RsrcName', 'USB0::0x0699::0x0401::C021409::0::INSTR', 'Tag', '');

% Create the VISA-USB object if it does not exist, otherwise
% use the existing interface object.
if isempty(instrObj)
    instrObj = visa('tek', 'USB0::0x0699::0x0401::C021409::0::INSTR');
else
    fclose(instrObj);
    instrObj = instrObj(1);
end

%% ACQUISITION PARAMETERS
% The parameters which the user may wish to adjust are
% aggregated here to minimize errors introduced by losing
% track of what settings have been changed and where. Use
% the MaxRecordLength variable, which will later configure
% the number of datapoints per acquisition, to set the input
% buffer size to prevent time-outs and "dropping" parts of
% the oscilloscope reading moving forward.
MaxRecordLength = 1e5;
instrObj.InputBufferSize = 2*MaxRecordLength;

The maximum record length can be varied in orders of magnitude from $10^3$–$10^6$ samples per trace. As previously discussed in Section 3.3.2, there are certain experimental considerations which constrain the desired sampling rate and thereby the minimum number of samples sufficient for adequate performance. For our typical flow velocities (dictated by flow rate and channel constriction geometry), $10^5$ is more than adequate.

One key limitation in the function of this program for real-time data recording is overhead time. The oscilloscope has a single memory buffer and cannot acquire a new trace without overwriting the previous one. The time required to transfer the buffer contents via serial communication to the MATLAB PC sets an upper bound on the efficiency of the routine (the acquisition window of the oscilloscope divided by the entire duration of the program required capture and store the data). The transition from 100 kSamples/s. to 1 MSamp/s. incurs a significant (50%) penalty in acquisition efficiency and therefore should be avoided wherever possible.

% Scale is the voltage per vertical division. HorScale is the
% seconds per horizontal division. There are ten vertical
% and horizontal divisions in the oscilloscope acquisition.
% Ten times the horizontal scale divided by the max
% record length gives the sampling rate of your acquisition.
% Note that the lock-in amplifier output can range from
% -10 V to 10 V at full-scale for a given sensitivity setting.
% Depending on the volume fraction of your target analyte
% to counter, you'll want to adjust the vertical scale
% accordingly. Start conservative.
Scale = 0.5;
HorScale = 0.1;

% Do we want single-channel or dual-channel measurements?
% We can either measure the in-phase and out-of-phase
% component of the differential signal, or take input off of
% two lock-in amplifiers' in-phase components to monitor two
% counters at once.
twochannel = 1;

% Connect to instrument object in order to begin serial
% communication.
fopen(instrObj);

%% INITIALIZATION
% First, let's autogenerate the save folder for the program to
% run with. This will spit out a warning if the directory
% already exists. I'm sure there is a way to check for
% the existence of the directory and avoid the warning but it
% doesn't impair the functional performance of the script.
clock_init = clock;
folder = char( strcat('C:\Users\Reedlab ThinkPad\Documents\MATLAB',num2str(clock_init(1)),'.',num2str(clock_init(2)),'.',num2str(clock_init(3)),'','Coulter Counter Traces\'));
mkdir(folder);
cd(folder);

%% RESET
% Set if = 1 to reset the instrument settings. This prevents
% any manual settings from previous users interfering with the
% programmatic acquisition.
RESET = 1;
if(RESET==1)
    fprintf(instrObj, ':HEADER OFF;DESE 255;*ESE 255;*SRE 255;*CLS ;
    );

% This command specifies the data encoding format. The
% binblockread command, used later, expects the LSB first.
% See the instrument manual for more information.
fprintf(instrObj, 'DAT:ENC RIB');

% Specify that the reference levels for any measurement
% functions are to be calculated relative to HIGH and LOW
% on the TTL lines. DDT 211 executes some pre-stored and
% presently-unknown commands, TRIG FORC forces a trigger
% event for the first acquisition of the reset, and VERB OFF
fprintf(instrObj, 'MEASU:REFL:METH PERC');
fprintf(instrObj, '* DDT #211; TRIG FORC; ');
fprintf(instrObj, 'VERB OFF; ');
else
end

% Now we specify the start and end data points for data
% transfers. We want to make sure we transfer the whole
% waveform. We use the MaxRecordLength variable to tell
% the oscilloscope just how many points we're looking for
% during a serial transfer. We also use this variable to
% explicitly define the number of data points per sample
% later in the program. The present implementation ensures
% that the two values are kept synchronized.
fprintf(instrObj, char(strcat(' :DAT:STAR 1;& DAT:STOP', '{', '}', num2str(MaxRecordLength), ';')));

%%% CONFIGURE THE OSCILLOSCOPE
% Choose the input impedance of Channel 1. Your options are
% [MEG, SEVENTYF, FIF] corresponding to 1 Meg, 75, and 50
% Ohms respectively. You MUST set the input impedance to
% 1 mega-Ohm before selecting AC coupling - AC coupling is
% not available at the lower input impedances. We then select
% the input coupling - AC, DC, GND.
fprintf(instrObj, ':CH1:IMP MEG;&CH1:COUP AC; ');

We’ve chosen to use AC coupling on the oscilloscope input to maximize the dynamic
range of our measurement system, as configured. Theoretically, the voltage signal should
have zero DC mean after demodulation. However, physical imperfections throw the bridge
circuit out of balance, resulting in a relatively-constant DC background for each measure-
ment circuit during operation. AC-coupling the input discards this background. As a
consequence, our vertical scale is dictated not by the size of the background signal but by
the magnitude of the transients generated by particle passages. Decreasing the vertical
scale magnitude correspondingly decreases the magnitude of the least significant bit of the
oscilloscope and thereby increases the resolution of the measurement system.

Furthermore, the oscilloscope has an input noise floor whose magnitude is also governed
by the vertical scale setting. Reducing the vertical scale directly improves the signal-to-noise
ratio of the overall system during this final digitization step. For these reasons, I would
highly recommend AC coupling where possible in future implementations, include efforts
at miniaturization.

% Set the measurement bandwidth to 20 MHz, the smallest
% bandwidth available on the scope. Other options are FUL,
% TWO, 150E+6, corresponding to full bandwidth, 250 MHz,
% and 150 MHz respectively. We also set the vertical offset to
% zero.
fprintf ( instrObj , ':CH1:OFFS 0.0;:CH1:BAN TWE;');

% Here we set the channel one vertical scale.
Ch1VertScale = Scale;
fprintf ( instrObj , char ( strcat (':CH1:SCA',{'}num2str(
    Ch1VertScale),{'})));

% Lastly, we enable the channel for measurement.
fprintf ( instrObj , ':SEL:CH1 ON;');

if ( twochannel == 1 )
    % Repeat configuration process for Channel 2.
    fprintf ( instrObj , ':CH2:IMP MEG;:CH2:COUP AC;:CH2:OFFS 0.0;');
    fprintf ( instrObj , char ( strcat (':CH2:SCA',{'}num2str(
        Scale),{'})));
    fprintf ( instrObj , ':CH2:BAN TWE;:SEL:CH2 ON;');
else
end

% Disable any window zooming and any horizontal delay from the
% trigger condition. HIR specifies high-resolution acquisition
% for lowered noise.
fprintf ( instrObj , ':ZOOM:MOD OFF;:HOR:DEL:MODE 0;:ACQUIRE:MODE HIR;');

% We then set the horizontal scale (time per division) as well
% as the horizontal position of the start point of the time
% trace (in percent) of the trigger event.
fprintf ( instrObj , char ( strcat (':HOR:SCA',{'}num2str(
    HorScale),{'})));
fprintf ( instrObj , ':HOR:POS 0;');

% We use the MaxRecordLength variable to set the horizontal
% record length, a.k.a. the number of measurement data
% points saved per oscilloscope trace. The curly brackets are
% necessary to get the concatenated command to contain a
% space and work as intended.
a = char ( strcat (':HOR:RECORDL',{'}num2str(MaxRecordLength))
    );
fprintf ( instrObj , a);

% As well setting the expected number of bytes (2) per data
% point. We set the byte order to transmit the LSB first.
fprintf ( instrObj , ':DAT:WID 2;:WFMO:BYT_NR 2;:WFMO:BYT_OR LSB;');

% Set the oscilloscope to record a single sequence with
Automatic triggering so that you can use Force Trigger
% to continuously bring in fresh acquisitions.
fprintf(instrObj,'TRIG:A:MOD AUTO;:ACQ:STOPA SEQ;');

for N = 1:1:1;
%% ACQUIRING THE WAVEFORM
% We then program a for loop to repeatedly read the voltage
% traces and collect measurement data. The maximum value
% of the for loop should be adjusted based on the expected
% or desired duration of the experiment.
clock_init = clock;

%% ENABLE CONTINUOUS ACQUISITION
% Enable acquisition, tell the instrument to not respond to
% other commands until after the instrument is ready. We
% force a trigger event and acquire the waveform. This
% minimizes downtime in the program waiting for acquisition
% to occur. The pause is the time required for the acquisition
% with a 2% overhead programmed in to avoid any close calls.
fprintf(instrObj,':ACQ:STATE ON;*OPC;')
pause(0.05)
fprintf(instrObj,:TRIGGER FORCE;')
pause(10.2*HorScale)

% Based on Status Byte polling routines I've implemented in
% LabVIEW previously. We construct a logical array, the
% seventh bit of which is the Master Status Summary (MSS).
% MSS goes high when the status byte has been enabled AND
% there is a message (the resulting waveform) available in the
% output queue. Currently this is disabled because it's failed
% to function as desired -- it will function perfectly during
% repeated acquisitions and then cut out after some time
% and cease functioning, hence the 10.2*HorScale pause.
i = 0;
h = false(1,7);
while(i ~= 1)
    h = or(h,logical(de2bi(str2num(query(instrObj,'*STB?')))));
i = h(7);
end

%% READ THE DATA
% We send the command asking the oscilloscope to send the
% waveform data, then immediately we read it. The last read
% is to clear the carriage return from the buffer that is
% typically sent at the end of the data.
fprintf(instrObj,':DAT:SOU CH1;:CURVE?;')
raw = binblockread(instrObj,'int16');
fread(instrObj, 1);

% Here we use the sample rate and the zero-point of the % horizontal settings to construct our x-domain data (time) % for the plot. The time-domain signal is simply an array % the length of MaxRecordLength which increments % from t=0 to t=10*HorScale.
xzero = 0;
ptcnt = MaxRecordLength;
xincr = 10*HorScale./MaxRecordLength;
x = (((0:(ptcnt-1)) .* xincr) + xzero);

% We hardcode zero offset for the y-domain data in the initial % stages of the acquisition as well, therefore eliminating the % need for queries as to that setting.
yoffs = 0;
yzero = 0;

% Since we're using hi-res mode with 2-byte data values, the % y-axis is split into 2^16 values. There's ten divisions % along the y-axis, so 10*Vertical Scale/2^16 gives the % theoretical vertical multiplier. The scope has some % additional tolerance beyond this nominal range, giving % rise to the 102.4 multiplication factor. The net formula is:
ymult = 10.24*Ch1VertScale/(2^16);
y1 = ((raw - yoffs) .* ymult) + yzero;

% If two-channel measurement is enabled, we will read the % second channel here. For explanations of all commands, % consult the preceding section.
if(twochannel==1)
    fprintf(instrObj, ':DAT:SOU CH2::CURVE?;
    raw = binblockread(instrObj, 'int16');
fread(instrObj, 1);
    ymult = 10.24*Ch2VertScale/(2^16);
    y2 = ((raw - yoffs) .* ymult) + yzero;
else
end

fprintf(instrObj,:ACQ:STATE OFF);

%% DATA HANDLING
% Set the if statement equal to 1 to have the acquired data % plotted after each acquisition, otherwise just let the % program run and watch the oscilloscope.
if(0)
    if(twochannel==1)
        figure;
        % Show the data here...
    end
end
plot(x,y1,x,y2)
    legend('Channel 1','Channel 2')
else
    plot(x,y1);
end
xlabel('Time (s)'); ylabel('Voltage (V)');
title(strcat('Vert. Scale = ',num2str(Scale)));
else
end

% Save the raw data. We pull another clock reading to track
% the elapsed time of the loop iteration as well generate a
% closer-to-accurate timestamp for the file save. Note that
% if our loop runs more than once per second, we'll end up
% overwriting the raw data so we ought to be careful.
% Presently the program is continued to acquire data in one-
% second intervals which avoids this problem.
clock_save = clock;
filename = strcat('DPO4104_voltage_traces_',num2str(clock_save(4)),'h',num2str(clock_save(5)),'m',num2str( round(clock_save(6))),'ss.mat');
if(twochannel==1)
    save(filename,'x','y1','y2');
else
    save(filename,'x','y1');
end

% We keep track of our measurement efficiency (time per
% acquisition versus duration of acquisition) to evaluate
% performance as I tweak the code.
iteration_time = clock_save - clock_init;
efficiency(N) = (10*HorScale)./(60*iteration_time(5)+
    iteration_time(6));
end

%% DATA PROCESSING
% Here would be the ideal place to start analyzing the data if
% real-time analysis was desired during experimentation. If
% you're going to implement that, you should seriously
% consider breaking the various functions we have outlined
% above into sub-functions called within a larger program.

%% HOUSEKEEPING
% Close the serial communication channel, but I'm not sure
% what other sorts of best-practices (clearing out buffers
% and such?) we should implement in this routine.
fclose(instrObj);

It is satisfactory to leave the data analysis for post-processing for our present experimental purposes. Beyond demonstrating proof-of-principle, the device should be capable of providing real-time feedback, or fast feedback after all the data has been acquired. Depending on the clinical objectives, such routines should be implemented in the above data analysis section.

The following code performs the data analysis. An overview of this process is given in Section 3.4.1, with an accompanying graphic illustration (Fig. 3.11). The following code is not capable of true real-time analysis, as it constructs an estimator of the background noise from the entire one-second data trace. This is a moot point in contexts where only the aggregate count is clinically-relevant. Potential solutions require implementation of a dynamically-updating noise estimators. Suggested solutions from the literature include Weiner and Kalman filters and are a natural extension of this thesis research by subsequent students.

It is important to note that the data analysis routine is configured to analyze and process the entire contents of a directory. The directory creation commands should be updated in the previous code to add subdirectories if multiple experimental conditions are being evaluated on a single data, or the files moved into an appropriate subdirectory when the acquisition has stopped.

### D.3 Coulter counter data analysis

clear all;
mat = dir('*.mat');

% Ask a few preliminary input questions before we start the % for loop over all the files so we only have to answer once. % 0.001 is cubic meters per liter, 1e-6 is liters per ?L, and % 1/60 is seconds per minute.
xdim = 1e-6*input('What is the channel length (um)?');
ydim = 1e-6*input('What is the channel width (um)?');
zdim = 1e-6*input('What is the channel height (um)?');
flowrate = (0.001)*(1e-6)*(1/60)*input('What is the flowrate ( uL/min)?');
transit_time = (xdim*ydim*zdim)/flowrate;
EXTRACTED_DATA = [];

for q = 1:length(mat);
load(mat(q).name);
tic;
% The program is configured to only handle the y1 or y2 % data, whereas conceivably both could be used to construct R
and perhaps clean things up. Zeroing the phase prior to running the program will avoid this ambiguity. We also subtract out any residual mean that `survived' the AC coupling.

data=y2;
data=data-mean(data);

Many of the counter structures used to evaluate the performance of the system do not have a preferential flow direction. The program assumes event signatures go positive before going negative and therefore a global multiplicative inversion is sometimes required.

if(0)
data=-1*data;
else
end

MaxRecordLength=length(x);
dt = (max(x)-min(x))/MaxRecordLength;
width = round(1.0*transit_time/dt);

The signal can ostensibly contain spikes of varying magnitudes. For instance, our 1.7um/8.7um bead pairing has spikes which are over two orders of magnitude different in intensity, but both of which are distinguishable from the noise floor. In order to extract information about the noise floor, we construct a histogram of the y-domain signal, fitting it with a Gaussian function:

N_bins = 400;
set(0,'DefaultFigureVisible','off');
h = histogram(data,N_bins);
set(0,'DefaultFigureVisible','on');
counts = h. Values;
edges = h. BinEdges;
MLEst = @( param ) param(1).*exp(-0.5.*((edges(1:N_bins)+edges((1:N_bins)+1))./(2.*param(2))).^2);
objfcn = @(param) MLEst(param) - counts;
x0 = [counts(N_bins./2 + 1), std(data)];
opts=optimset('display','off');
fit=lsqnonlin(objfcn,x0,[0,0],[inf,inf],opts);
thresh=3.*fit(2);

We construct four flags, A, B, C, and D. The four flags search for the rising and falling edge crossing of the positive (A,B) and negative (C,D) threshold value within the data trace. Each flag contains three elements. The
first is its Boolean truth value (1 = flag activated, 0 = flag unactivated). The second is the loop integer at which the flag was activated, which lets us position the signal in time. The final element is a countdown timer. We're searching for an A-B-C-D pattern here. If we don't detect B in a certain amount of time after A, we reset and start looking again. Signal number keeps track of how many events are detected within the trace, and we initialize signal array to save information about the event locations.

FLAG_A = [0,0,0];
FLAG_B = [0,0,0];
FLAG_C = [0,0,0];
FLAG_D = [0,0,0];
MASK = zeros(length(data),1);
signal_number = 0;
signal_info = [];

for i=3:1:length(data);
    if(data(i)>=thresh & data(i-1)<=thresh & data(i-2)<=thresh & FLAG_A(1)==0);
        FLAG_A = [1, i, 1.25*width];
    else
        end

    if(data(i)<=thresh & data(i-1)>=thresh & data(i-2)>=thresh & FLAG_A(1)==1 & FLAG_B(1)==0) ;
        FLAG_B = [1, i, 0];
    else
        end

    if(data(i)<=-1*thresh & data(i-1)>=-1*thresh & data(i-2)>=-1*thresh & FLAG_A(1)==1 & FLAG_B(1)==1 & FLAG_C(1)==0)
        FLAG_C = [1, i, 0];
    else
        end

end
% Finally we have Flag D when the signal goes high
if(data(i) >= -1*thresh & data(i-1) <= -1*thresh & data(i-2) <= -1*thresh & FLAG_A(1) == 1 & FLAG_B(1) == 1 & FLAG_C(1) == 1 & FLAG_D(1) == 0)
    % implement this condition to give a "pause" between
    % detecting a FLAG C condition and a FLAG D condition
    % in case the signal happens to have appreciable noise
    % right around your chosen threshold.
    if(i-FLAG_C(2) >= 0.01*width);
        FLAG_D = [1,i,0];
    else
        end
else
    end

% Variables to be deleted, used for debugging issues with
% flag triggering while developing the code.
FA(i) = FLAG_A(1);
FB(i) = FLAG_B(1);
FC(i) = FLAG_C(1);
FD(i) = FLAG_D(1);

% Upon successful recognition, we define a MASK that is
% unity for the time domain within which we detected an
% event and zero elsewhere. MASK is a debugging tool
% used to visualize the algorithm's identification of
% events.
if(FLAG_A(1) == 1 & FLAG_B(1) == 1 & FLAG_C(1) == 1 &
    FLAG_D(1) == 1)
    MASK(FLAG_A(2):FLAG_D(2)) = ones(FLAG_D(2)-FLAG_A(2)+1,1);
    signal_number = signal_number + 1;

    % We now use the location of the A+B flags to guess at
    % the signal center before using a find min and find
    % max function to provide a better estimate for the
    % true center of the signal. Note that in the case of
    % a noisy signal where one of the peaks is not the
    % local maximum or minimum, you'll get something
    % kinda randomly within the signal window which will
    % lead to a poor fit. N adjusts the width of the
    % fitting window.
    N = 1;
    signal_center = round((FLAG_B(2)+FLAG_C(2))/2);
    start = max(signal_center - 2*(signal_center - FLAG_A(2)),1);
stop = min(signal_center + 2*(FLAG_D(2) - signal_center), length(x));
height_est = (1/2)*((max(data(FLAG_A(2):FLAG_B(2))))-min(data(FLAG_C(2):FLAG_D(2))));

% now we use the width (peak location plus or minus one
% times the expected transit time from channel dimensions
% and nominal flow rate to compute the spans over which
% we expect to see bead events. We also pass along the
% extracted min and max to avoid running find functions
% again later.

signal_info(signal_number,:)=[start,stop,signal_center,
height_est];

% And we reset the flag vectors to search for the next
% peak.
   FLAG_A = [0,0,0];
   FLAG_B = [0,0,0];
   FLAG_C = [0,0,0];
   FLAG_D = [0,0,0];
else
end

% Countdown timer within each iteration - we only have a
% certain window after the first event to detect a proper
% coincidence. When the counter hits zero, we reset the
% flags. We decrement the timer within Flag A, floor zero.
% Likewise for Flag A's timer. However, here, we only
% decrement A's timer if Flag B is low.

if(FLAG_D(1)==0)
   FLAG_A(3) = max(FLAG_A(3)-1,0);
else
end

% Here we check if the flag's internal countdown timer has
% returned to zero. If so, we set the Boolean portion of
% the flag vector to zero in order to renew the pattern
% search sequencing. Note we do not have to handle a
% timer for C, as C going high triggers a detection event
% and a reset of all three flag vectors.
if(FLAG_A(3)==0);
   FLAG_A(1)=0;
   FLAG_B(1)=0;
   FLAG_C(1)=0;
else

% We round the entries in signal_info to avoid any issues%
% or warnings when we go to use the signal span data to%
% feed into our fitting functions.

[n_loops,~]= size(signal_info);

% Configure some settings for our least-squares solver that%
% we're going to throw at every set of peak data that we end%
% up dealing with.

opts = optimoptions(@lsqnonlin,'Algorithm','trust-region-
                   reflective','Display',...%
                   'off','TolPCG',1e-9,'FunctionTolerance',1e-12,'%
                   StepTolerance',1e-12,...%
                   'ScaleProblem','Jacobian','MaxFunctionEvaluations',4e12);

% We define our fitting function as a first derivative%
% Gaussian in the time domain.
Gauss = @(A,t) -1*(t-A(1)*1e2).*((A(3).sqrt(2*pi*A(2).^6)).*%
                   exp(-1*((t-A(1)*1e2).^2)/(2*A(2).^2));

% These three variables track the height, width, and goodness%
% of fit for each event detected within a given data trace.
GAMMA = [];%GAMMA = [
HEIGHT = [];%HEIGHT = [
CHI = [];%CHI = [

for i = 1:1:n_loops;

CHI(i)=0;

% Clean up our notation for the start and stop indices.
start=signal_info(i,1);
stop=signal_info(i,2);
signal_center=signal_info(i,3);
height_est=signal_info(i,4);

% A few lazy guesses for the initial parameters from which to%
% start the solver. We use the midpoint of the max and%
% minimum peaks to estimate the center-crossing of the signal,
% then we use their half their distance as an estimator for%
% the width term. Absolutely no clue why we divide down the
% peak-to-peak height by a factor of fifty, this appears to be 
% another just-so parameter in our code.

A0(1) = 1e-2*(x(signal_center) - x(start));
A0(2) = abs(x(stop)-x(start))/2;
A0(3) = abs(height_est)/50;

lb = [0,0,0];
ub = [(2*width./MaxRecordLength)/1e2,(2*width./MaxRecordLength ),10];

% We need to ensure that the data is in row format, otherwise 
% the solver function is evaluating the sum of the squares of 
% a massive square matrix and that's why the solver kept on 
% crashing. We also introduce the notation xtest. The 
% peak-search function that occurs in a prior loop iteration 
% executes on the raw x values, and therefore the a,b,c,d 
% values that we get passed include that.

% Crucial for good performance is keeping the SCALE of the 
% three parameters within your fitting function roughly equal. 
% For us, this is the case when the peak center parameter is 
% scaled down by two orders of magnitude. We've been testing 
% the algorithm on the first detected peak. In order to avoid 
% issues on peaks detected very late in the dataset, we 
% construct the variable xtest which has the left-most 
% (minimum) x-value of the dataset subtracted. In this manner, 
% we preserve the relative scale of our three parameters 
% across several orders of magnitude in the time domain 
% (milliseconds to a full second in the oscilloscope trace).

if(isrow(data(start:stop))==0)
    test = data(start:stop)'; 
    xtest = x(start:stop) - x(start); 
else
    test = data(start:stop); 
    xtest = x(start:stop) - x(start); 
end

objfcn=@(A) Gauss(A,xtest) - test; 
[A,resnorm,residual,exitflag,output,lambda,jacobian] = 
    lsqnonlin(objfcn,A0,lb,ub,opts); 
CHI(i) = 1 - resnorm / norm(data(start:stop)-mean(data(start: 
    stop)))^2;

% We can activate the PLOT_EACH_FIT variable to have the 
% program ask us if the fits look good before including them 
% in the compiled dataset.
PLOT_EACH_FIT = 1;
if(PLOT_EACH_FIT==1)
    if(CHI(i)>=-1.0)
        figure;
        plot(x(start:stop),data(start:stop),'blacksq',x(start:stop),Gauss(A,xtest),'r--', 'LineWidth',1.5)
        xlabel('Time (s)'); ylabel('Oscilloscope voltage reading (V)');
        legend('Detected peak','Gaussian derivative fit');
        title(['Peak event number' + string(' ') + num2str(i) + string(' ') + 'CHI: ' + string(' ') + num2str(CHI(i))]);
        prompt = 'Is this a good fit? Y/N [Y]: '
        str = input(prompt,'s');
        if isempty(str)
            str = 'Y';
        end
        close(gcf)
        if(str=='Y')
            HEIGHT(i) = A(3);
            GAMMA(i) = A(2);
            GOODFIT(i) = 1e2*A(1)*length(data)+start;
        else
        end
    else
    end
else
    if(CHI(i)>=0.7) % 85
        HEIGHT(i) = A(3);
        GAMMA(i) = A(2);
        GOODFIT(i) = 1e2*A(1)*length(data)+start;
    else
    end
end
end

% Convert the extracted height and width parameters into
% column vectors to append to the overarching tracker
% that we're using.
if(iscolumn(GAMMA)==0)
    GAMMA = GAMMA';
else
end
if(iscolumn(HEIGHT)==0)
    HEIGHT = HEIGHT';
else
end
if(iscolumn(CHI)==0)
CHI = CHI';
else
end

% Now we append the column-vectored height and width
% information to our extracted data array, which we'll
% want to plot at the end of it all. Let's also reset GAMMA
% and HEIGHT to null to avoid accidentally creating any weird
% Frankennmatrices that are non-zero along the first row and
% first column only. We want them to be vectors, after all.

EXTRACTED_DATA = [EXTRACTED_DATA; GAMMA, HEIGHT];
GAMMA = [];
HEIGHT = [];

time_elapsed(q) = toc;
end

%% DATA VISUALIZATION
% Now what we really care about are the height of the signal
% and the time that elapses from the maxima and minima. We
% are simply using the fitting procedure to extract noise-
% robust parameters from the data. We therefore convert the
% extracted parameters into the height and peak-to-peak
% information we desire.
for i = 1:length(EXTRACTED_DATA);
    peak_height(i) = sqrt(1/(2*pi)) * (1./EXTRACTED_DATA(i,1)
    .^2) * EXTRACTED_DATA(i,2) * exp(-0.5);
    peak_height(i) = sqrt(1/(2*pi)) * (1./EXTRACTED_DATA(i,1)
    .^2) * EXTRACTED_DATA(i,2) * exp(-0.5);
    peak_to_peak(i) = 2e3*EXTRACTED_DATA(i,1);
end
plot(peak_to_peak, peak_height.^(1/3), 'sq')
xlim([0,1e-3*width])
grid on
xlabel('Peak-to-peak time (ms)')
ylabel('Peak voltage height (V)')

% Use this code to generate heatmaps that are constrained in x
% and y. SENS divides the maximum front-panel input level
% (user-specified during the experiment) by 10 V to get the
% conversion factor between front-panel input and rear-panel
% output. We can then optionally toss data-points which are
% physically non-sensical but somehow evaluated to represent
% a good-quality fit. We then bin the data along two
% dimensions (transit time and cube root of peak height) to
% construct a heatmap of particle size and velocity extracted
% from the dataset.
if(0)
    SENS = 1000/10;
    d=find(peak_height./SENS>=0.01);
    peak_height(d)=[];
    peak_to_peak(d)=[];
    b=find(peak_to_peak>=width./5e2);
    peak_to_peak(b)=[];
    peak_height(b)=[];
    hist3([peak_to_peak', (peak_height'./SENS).^(1/3)],'
CDataMode','auto','FaceColor','interp','Nbins',[100,100],'EdgeColor','none')
    view([0 90])
    colorbar
else
end
Bibliography


