Dependence of the high frequency dielectrophoresis response on the lipid content of microalgal cells

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Mechanical Engineering

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May 2013
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entitled

Dependence Of The High Frequency Dielectrophoresis Response On The Lipid Content Of Microalgal Cells

be accepted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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May, 2013
Abstract

High frequency dielectrophoresis (>20 MHz) was used to demonstrate that the upper crossover frequency of microalgal cells is reduced as lipids accumulate within the cytoplasm. Currently, the majority of AC dielectrophoresis applications differentiate cells by exploiting differences in the lower crossover frequency, typically between 10-500 kHz. However, the single shell model also predicts another crossover in the 20-200 MHz range that is dependent upon the dielectric properties of the cytoplasm. This project demonstrates this effect with microalgal cells due to the relative ease in which the properties of the cytoplasm can be altered. *Chlamydomonas reinhardtii* cells were cultured in regular media and were observed to have an upper crossover frequency of approximately 75 MHz. When cultured under nitrogen-free conditions the same cells began to accumulate neutral lipids. The lipid content was verified via fluorescence microscopy and the upper crossover frequency was measured to be approximately 40 MHz. To measure the upper crossover frequency, two needle shaped electrodes were patterned onto a glass slide and the motion of the cells were observed as an AC signal was swept from 10-110 MHz at approximately $30V_{pp}$. It is found that an increase in lipid content reduces the effective conductivity of the cytoplasm thus reducing the upper crossover frequency.
Acknowledgements

Funding for this work was provided by the Oak Ridge Associated Universities Ralph E. Powe Junior Faculty Enhancement Award, the University of Nevada, Reno and support from the National Science Foundation GK-12 E-Fellowship Program, DGE 1045584.
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Symbols and Abbreviations

Variables

\( \varepsilon \) permittivity

\( \sigma \) conductivity

\( w \) angular frequency of the electric field

\( K \) Clausius-Mossotti factor

\( F_{\text{DEP}} \) Dielectrophoresis force

\( \phi \) Electrostatic potential

Abbreviations and Acronyms

DEP dielectrophoresis

pDEP positive dielectrophoresis

nDEP negative dielectrophoresis

\( F_{x2} \) upper crossover frequency

TEM Transmission Electron Microscope

FACS Fluorescence-activated cell sorter

\( V_{pp} \) Peak to peak voltage

\( C. \ Reinhardtii \) Chlamydomonas Reinhardtii
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

As technology evolves and expands it is becoming more compact and leading to discoveries of different properties and aspects cells. Properties of cells that were seemingly non-existent before are becoming essential to breakthroughs in medicine and biology. Dielectrophoresis (DEP) has been loosely described as far back as the early 20th century, however, it was not referenced until the 1950’s by Pohl [2]. Recent developments in microfluidics and microtechnology make this phenomenon relatively easy to study and continue to develop. Currently DEP has the ability to manipulate microparticles, nanoparticles, and cells. This manipulation is also useful for separation of cells. The properties of these cells are highly sensitive and causes different cells to be manipulated in different ways with the same experimental parameters. Cells can be manipulated on various properties although the particular properties in this research are part of the core region. The separation technique can also provide information about the properties of the cell without an intensive interrogation or destruction of the cell. This could be very beneficial to doctors and biologists alike.
The need to have a method for quick measurements of cells and other particles is growing. Advances in medicine demand quick tests with accurate results and the developments can only move as fast as the testing allows. The advances in microtechnology allow the development of different tests to fulfill the demand in biology and medicine. DEP has the potential to satisfy many of these needs with quick and accurate results. The testing would be quick and would not destroy cells so that they could be used in other tests. Researchers could perform many tests in a day as opposed to weeks or months. Although there are many different types of particles to choose from to test this theory, microalgae was chosen for various reasons.

1.1 Introduction to Dielectrophoresis

DEP refers to the motion of electrically neutral particles in a non-spatially uniform electric field [3–6]. It differs from electrophoresis in that it affects any non-charged particle that has different dielectric properties than the suspending medium. When placed in an electric field, a particle will polarize (i.e., develop a dipole moment) dependent on the polarizability of the particle. In a uniform field, the electrostatic forces on each side of the particle cancel resulting in no net force. In a non-uniform field, however, the force on one side will be greater and the particle will tend to move in that direction. The actual direction depends on the relative polarizability of the particle and the medium, as shown in Figure 1.1. If the particle has a greater polarizability than the medium, it will undergo positive DEP (pDEP) and tend toward regions with higher electric fields. Conversely, if the particle has a smaller polarizability than the medium, it will undergo negative DEP (nDEP) and tend away from
Figure 1.1: DEP refers to the motion of an uncharged particle in a non-uniform electric field. By changing the frequency of the field (or other parameters), the particle may experience positive or negative DEP. Taken from Hoettges [7].

regions with higher electric fields. Additionally, for AC fields, the polarizability of the particle is a function of the field frequency. Therefore, a particle might exhibit either negative or positive DEP depending on the frequency being used. Frequencies at which no net force is observed are called crossover frequencies. These crossover frequencies are dependent on properties of the particle, and are the focus of this work.

1.2 Microalgae

Microalgae technology continues to show promise for becoming a major source of renewable transportation fuel in the coming decades [8–11]. Due to their high growth rate and lipid content, microalgae have the ability to produce an order of magnitude more biodiesel per unit area than other land-based plants. This makes microalgae the only “oil crop” that could possibly meet the fuel demands of the United States. Additionally, their ability to grow in salt, brackish, or waste water means that there is little or no competition
with food production. Lastly, in contrast to bioethanol, microalgae biodiesel is significantly more energy dense, and therefore can be a drop-in replacement transportation fuel, including aviation. For the purposes of the research presented here, microalgae are a particularly interesting class of organisms in that the cells can be induced to accumulate neutral lipids in a variety of ways. Specifically, when particular strains of microalgal cells are stressed in a nitrogen free (N-free) environment they accumulate lipids [12–15]. The lipid accumulation in a microalgal cell can be quite dramatic [16], and thus microalgae is an excellent target for this study. This effect can be quite dramatic as seen in the transmission electron microscopy (TEM) images of *Chlorella protothecoides*, shown in Figure 1.2. The most common method to induce lipid accumulation is to culture the microalgae in nitrogen-free (N-free) media [8]. Additionally, there are limited reports that suggest the dielectric properties of microalgae might have some dependence on lipid content [17, 18]. This should come as no surprise since the dielectric properties, namely the permittivity and conductivity, of neutral lipids are significantly different than the aqueous cytoplasm.

The accumulated lipids can be measured readily via fluorescence microscopy which involves the staining of microalgal cells with dyes that fluoresce in non-polar (or neutral) lipid environments. This technique was first demonstrated in 1987 by Cooksey et al. [19]. Briefly, the method used Nile Red to stain algal cells containing varying amounts of accumulated neutral lipids. Acetone was the carrier solvent. Upon excitation with 480-525 nm (green) light, the stained cells emitted 580 nm (yellow) light. The intensity of the emission could be linearly correlated to the amount of neutral lipids within the cell population. With the recent renewed interest in microalgae as a biofuel
source, several publications have improved fluorescent microscopy techniques. Chen et al. demonstrated that using high concentrations of DMSO as a carrier solvent and increasing the staining temperature give more quantitative results [20]; Cooper et al. recently demonstrated the use of BOPIDY 505/515 for fluorescently staining microalgae cells. These results using BODIPY appear to be far superior to Nile Red [21]. Fluorescence-activated cell sorters (FACS) are beginning to be used in microalgal research and take fluorescence microscopy one step further by using the fluorescent signal to sort microalgal cells. Briefly, a FACS interrogates the stained microalgal cells individually and electrostatically sorts them. A typical FACS has a throughput of 104 cells/s in sorting mode under ideal conditions. Purity and recovery are two common quality metrics for cell sorting and refer to the number of false positives and false negatives, respectively. Commercial FACS, depending on operating parameters, typically achieve a purity of >98% and recovery of >80% [22]. Based on this, microalgae are an ideal target to study DEP and are the main focus of this project.
Chapter 2

Literature Review

2.1 Motivation

Alternative methods to DEP for sorting particles are time consuming and require constant calibration that can have a large error margin. Although there have been many advances in cell sorting, [23–26] there are still many components of this method that make DEP a more desirable method of sorting. Kang et. al. describe the current state of cell separation technology. The largest is FACS although it is highly specific and selective it is complicated and must be calibrated for each type of particle [3].

2.2 Previous Work

Label-free cell characterization, separation, and sorting is a topic of growing interest to researchers in the biological sciences. There are many previous works that show DEP for membrane separation based on size[27–29], viability
type of cell and polystrene beads or liposome properties that are mostly used for research purposes. All of these methods are very valuable and are useful for many applications. However, there has been little research done on DEP that can separate based on the intrinsic properties of a cell. Several researchers have been able to demonstrate the ability to separate and/or sort cells at the lower crossover frequency which shifts due to changes in the dielectric properties of the cell membrane. This lower crossover frequency is often found in the 10-500 kHz range, but can be as high as 5 MHz.

Analytical models also predict that at high frequencies (20-200 MHz) cells should also be able to be differentiated based on differences in the dielectric properties of the cytoplasm. However, there is little experimental evidence to support the prediction of this upper crossover frequency. One reason for this is that standard bench top function generators are typically limited to maximum frequency of 20 MHz, and those with higher bandwidths are often unable to generate high frequency electric fields with sufficient amplitude to induce DEP motion. Despite this, the effect of changes in conductivity of the cytoplasm have been indirectly observed by measuring changes in the DEP spectra at frequencies up to 20 MHz by Valero et al. and Labeed et al. While these studies also predict a shift in the upper crossover frequency, this effect was not directly observed at the upper crossover frequency. Furthermore, the change in cytoplasmic conductivity was due to ion leakage past the cell membrane of non-viable cells. Gagnon et al. overcame this equipment limitation by dramatically altering the permittivity of the DEP media with the use of a zwitterionic buffer as opposed the more commonly used salt-based media. With this approach they were able to shift the upper crossover
frequency from an estimated 70 MHz into the 0.5-2 MHz range. Therefore by using a specialized DEP buffer, they were able to use standard bench top equipment. Another notable exception to this limitation is the seminal work by Holzel et al. who developed electrorotation spectra for yeast cells from 100 Hz to 1.6 GHz [45]. While these spectra do provide detailed knowledge about the cell, the equipment used was highly specialized and custom-built for these experiments.

Perhaps the most compelling example of cell being differentiated at the upper crossover frequency is the work of Castellarnau et al. who compared the DEP response of various mutants of *E. coli* with the wild type (5K strain) [46]. They observed that the upper crossover frequency of all cells was in the 55-110 MHz range and that upper crossover frequency of H-NS- mutants was shifted to the lower portion of this range. While their models suggest that this shift is due to changes in cytoplasmic conductivity, they only speculate that this may be due to differences in the structure of the DNA. Beyond this, it is unclear as to why the cytoplasm of the mutant strain might have a different conductivity.

In light of these reports, it is clear that more work is needed to understand the DEP response of cells at the upper crossover frequency. To explore this high frequency regime, microalgal cells were chosen due to the ease in which the cytoplasmic properties can be changed.
Table 2.1: Summary of current DEP literature for cell sorting and/or separation. Note that the excitation frequency is typically less than 1 MHz and that at these frequencies, the separation criteria is limited to particle size or membrane properties. Castellarnau et al. is a notable exception and reports separating cells at low and high frequencies.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Particle Type</th>
<th>Excitation Frequency</th>
<th>Throughput (particles/sec)</th>
<th>Separation Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castellarnau, et al. [46]</td>
<td><em>E. coli</em></td>
<td>0.5-5 MHz</td>
<td>N/A</td>
<td>Membrane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80-100 MHz</td>
<td>N/A</td>
<td>DNA Structure</td>
</tr>
<tr>
<td>Choi, Kim, and Park [47]</td>
<td>Latex microspheres</td>
<td>200 kHz</td>
<td>$2.67 \times 10^4$</td>
<td>Size</td>
</tr>
<tr>
<td>Doh and Cho [31]</td>
<td><em>S. Cerevisiae</em></td>
<td>5 MHz</td>
<td>$1.3 \times 10^3$</td>
<td>Membrane</td>
</tr>
<tr>
<td>Gallo-Villanueva, et al. [48]</td>
<td><em>S. capricornutum</em></td>
<td>DC</td>
<td>$1.22 \times 10^4$</td>
<td>Membrane</td>
</tr>
<tr>
<td>Hu, et al. [37]</td>
<td><em>E. coli</em></td>
<td>500 kHz</td>
<td>$1.00 \times 10^4$</td>
<td>Membrane</td>
</tr>
<tr>
<td>Kim, et al. [27]</td>
<td>Mammalian cells</td>
<td>800 Hz</td>
<td>$5.00 \times 10^1$</td>
<td>Size</td>
</tr>
<tr>
<td>Kim and Soh [49]</td>
<td><em>E. coli</em></td>
<td>500 kHz</td>
<td>$1.33 \times 10^4$</td>
<td>Size</td>
</tr>
<tr>
<td>Li, et al. [38]</td>
<td>Yeast cells</td>
<td>100 kHz</td>
<td>$8.00 \times 10^3$</td>
<td>Membrane</td>
</tr>
<tr>
<td>Oblak, et al. [39]</td>
<td>Mouse melanoma</td>
<td>0.1-50 MHz</td>
<td>N/A</td>
<td>Membrane</td>
</tr>
<tr>
<td>Vahey and Voldman [50]</td>
<td><em>S. Cerevisiae</em></td>
<td>500 kHz</td>
<td>$2.50 \times 10^2$</td>
<td>Size</td>
</tr>
<tr>
<td>Wang, et al. [40]</td>
<td>Breast cancer cells</td>
<td>20-40 kHz</td>
<td>$3.80 \times 10^4$</td>
<td>Membrane</td>
</tr>
</tbody>
</table>
Chapter 3

Theory

3.1 Dipole Theory

The theory and equations derived here has been adapted from Jones et. al. [51] and Pehtig et. al [25]. Particles placed in an electric field will tend to develop a dipole moment in which the positive and negative charges within the particle will separate. For particles that have a net positive or negative charge the application of an electric field will result in movement of the particle and is also known as electrophoresis as shown in Figure 1.1. For neutral particles in a uniform electric field the particle will develop a dipole, but the forces on each side of the particle will neutralize resulting in no net movement. However, if the neutral particle was placed in an electric field that is non-uniform, then the dipole would cause different magnitude forces on either side of the particle that will cause a net movement. The force on a single change in an electric
Figure 3.1: A single dipole that has been split into two different charges. $r$ is the radius between a point and the charge, $d$ is the distance between the two charges. $d \ll r$.

The field is given by the following equation:

$$\vec{F} = q\vec{E}(\vec{r})$$  \hspace{1cm} (3.1)

where $q$ is the charge of the particle, $\vec{E}$ is the electric field that the particle is suspended in, and $\vec{r}$ is the radius between a point and the dipole. This can be applied to a dipole that has been characterized into separate and opposite charges as shown in Figure 3.1. In this case the force on the dipole will be

$$\vec{F} = q\vec{E}(\vec{r} + \vec{d}) - q\vec{E}(\vec{r})$$  \hspace{1cm} (3.2)
where \( d << r \). This equation can be simplified using a Taylor expansion. The electric field can then be expressed as

\[
\vec{E}(\vec{r} + \vec{d}) = \vec{E}(\vec{r}) + \vec{d} \cdot \nabla \vec{E}(\vec{r}) + \frac{\vec{d}(\vec{d} - 1)}{2} \cdot \nabla \vec{E}(\vec{r})^2 + ... \tag{3.3}
\]

The third and following terms can be neglected because \( d^2 \) is very small compared to the first two terms. The expanded version of the electric field can then substituted into the force equation with the following is the result

\[
\vec{F} = q\vec{d} \cdot \nabla \vec{E} + ... \tag{3.4}
\]

If the limit is taken as \( |d| \to 0 \) and keep the dipole moment finite, the result of the force on an infinitesimal dipole is

\[
\vec{F}_{\text{dipole}} = \vec{p} \cdot \nabla \vec{E} \tag{3.5}
\]

Where \( \vec{p} \) is the dipole moment for a pair of charges and is equivalent to the following expression

\[
\vec{p} = q\vec{d} \tag{3.6}
\]

Equation 3.5 implies that there is a dipole force only if the particle is in a non-uniform field. A neutral particle will not move unless the electric field is non-uniform. However the particle can rotate because of the torque that is induced from the electric field. Torque is defined as the cross of the dipole moment \( \vec{p} \) and the electric field \( \vec{E} \). For a single particle that has been separated into two separate charges as in Figure 3.1, the torque can be expressed as the
following
\[ \vec{T}^e = \vec{p} \times \vec{E} = q\vec{d} \times \vec{E} = \frac{\vec{d}}{2} \times q\vec{E} + \frac{-\vec{d}}{2} \times (-q\vec{E}) \] (3.7)

Equation 3.7 implies that there can be a torque within a uniform field, unlike the force, with a neutral particle. We can then use this equation for a single cell and apply it to many cells that would be present in a DEP sample.

Equation 3.5 is an approximation and works very well for a single cell. The addition of more cells complicates the system as the forces from the other cells must be taken into account. The effective moment method is a method that can help to describe the forces in a system with many particles. This method focuses on the most accurate version of the dipole moment \( \vec{p} \) that should be used in equations 3.5 and 3.7. The electrostatic potential \( \phi_{\text{dipole}} \) due to a point dipole with the effective moment \( p_{\text{eff}} \) in a dielectric medium is

\[ \phi_{\text{dipole}} = \frac{p_{\text{eff}} \cos \theta}{4\pi \varepsilon_1 r^2} \] (3.8)

where \( \theta \) and \( r \) are the polar angle and radial position and \( \varepsilon_1 \) is the effective permittivity of the medium that the dipole is suspended in. The effective permittivity is an average of the media permittivity and how the other particles in the system change the permittivity. We can also describe the electrostatic potential inside and outside a particle

\[ \phi(r, \theta) = \begin{cases} -E_0 r \cos \theta + \frac{A \cos \theta}{r^2} & \text{if } r > R \\ -B r \cos \theta & \text{if } r < R \end{cases} \] (3.9)

The boundary conditions can be applied for this system to find the coefficients \( A \) and \( B \). The two parts of Eqn 3.8 must be continuous at the boundary where
\( r = R \). The separated charges that result from the induced dipole moment can be described using the electric displacement field. This displacement field, \( D \), can be described from the following equation

\[
D = \varepsilon E
\]  

(3.10)

where \( \varepsilon \) is the permittivity and \( E \) is the electric field. Equation 3.10 is dependent on position and can be applied as a boundary condition to Equation 3.9 as given by the following

\[
\varepsilon_1 E_r(r = R, \theta) = \varepsilon_2 E_r(r = R, \theta) \quad (3.11)
\]

where \( \varepsilon \) and \( E \) is the permittivity and electric field at the respective charge. Substituting Equation 3.11 into 3.9 along with the first boundary condition the following can be calculated

\[
A = \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} R^3 E_0 \quad \text{and} \quad B = \frac{3\varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} E_0 \quad (3.12)
\]

Which leads to an equation for \( p_{eff} \) by equating Eqn 3.8 and 3.7.

\[
p_{eff} = 4\pi \varepsilon_1 A \quad (3.13)
\]

If this is applied to a dielectric sphere, then the expression becomes

\[
p_{eff} = 4\pi \varepsilon_1 K R^3 E_0 \quad (3.14)
\]
Figure 3.2: Spherical shell with radius $R_1$ and permittivity $\varepsilon_2$ enclosing a homogeneous sphere of radius $R_2$ and permittivity $\varepsilon_3$ all in a medium of permittivity $\varepsilon_1$

where,

$$K(\varepsilon_2, \varepsilon_1) = \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1}$$

(3.15)

$K$ is known as the Clausius-Mossotti function and provides the strength of the effective polarization with $\varepsilon_1$ as the permittivity outside the sphere, and $\varepsilon_2$ as the permittivity inside the sphere.

A similar approach can be taken for a sphere that has a dielectric shell. In this research particles are modeled with a dielectric shell as shown in Figure 3.2. To get the Clausius-Mossotti factor for a sphere with a dielectric shell an equivalent permittivity of the inner core and the shell must be found. This can be done using the same method as before with an added electrostatic potential for the shell. There are 4 constants that must be solved for which then lead to an expression for the effective permittivity as follows

$$\varepsilon_{2\text{eff}} = \varepsilon_2 \left\{ \frac{a^3 + 2\left( \frac{\varepsilon_3 - \varepsilon_2}{\varepsilon_3 - 2\varepsilon_2} \right)}{a^3 - \left( \frac{\varepsilon_3 - \varepsilon_2}{\varepsilon_3 - 2\varepsilon_2} \right)} \right\}$$

(3.16)
Eqn 3.14 can replace \( \varepsilon_2 \) in Eqn 3.13 and the Clausius-Mossotti factor becomes

\[
K(\varepsilon_{2\text{eff}}, \varepsilon_1) = \frac{\varepsilon_{2\text{eff}} - \varepsilon_1}{\varepsilon_{2\text{eff}} + 2\varepsilon_1} \tag{3.17}
\]

This method can be applied for many concentric spheres by working from the innermost sphere and working outward. We must also account for the dielectric loss. This means the complex permittivity of the cell and medium must replace the permittivity terms in the CM factor. The complex permittivity is described in Eqn 3.16

\[
\varepsilon_1 \rightarrow \varepsilon_1' + \frac{\sigma_1}{jw} \quad \text{and} \quad \varepsilon_2 \rightarrow \varepsilon_2' + \frac{\sigma_2}{jw} \quad \text{and} \quad \varepsilon_3 \rightarrow \varepsilon_3' + \frac{\sigma_3}{jw} \tag{3.18}
\]

Where \( \sigma_1 \) and \( \sigma_2 \) are the conductivities of the particle and the medium respectively and \( w \) is the frequency of the AC electric field. The effective CM factor then becomes

\[
K'_{\text{eff}} = \frac{\varepsilon_2'_{\text{eff}} - \varepsilon_1'}{\varepsilon_2'_{\text{eff}} + 2\varepsilon_1'} \tag{3.19}
\]

This also implies that there is an imaginary component to the CM factor. Substituting the complex CM factor into the \( \vec{F}_{\text{DEP}} \) shows that there is an imaginary part of the force. If the time average of the force is taken, \( \langle \vec{F}_{\text{DEP}} \rangle \), the result is the following the following equation for the DEP force

\[
\langle \vec{F}_{\text{DEP}} \rangle = 2\pi\varepsilon_1 R^3 Re[K'_{\text{eff}}] \nabla E_{\text{rms}}^2 \tag{3.20}
\]

The imaginary term is accounted for in the torque of the particle. The time average also changes the \( E_0 \) term into a root mean square of the AC electric field. By further examining Equation 3.18, certain aspects of the equation can
be noted. First, $F_{DEP}$ is proportional to the volume of the particle. Secondly, the DEP force depends on the magnitude and sign of $K$. Thirdly, $F_{DEP}$ is also proportional to the dielectric permittivity of the medium that the particle is suspended in. The real part of the CM factor determines whether the particle experiences positive DEP force (pDEP), when $K_{eff}^\prime > 0$, or negative DEP force (nDEP), $K_{eff}^\prime < 0$.

### 3.2 Re[K] single-shell model

Plotting $F_{cm}$ vs the frequency provides significant understanding of the behaviour of the cells. A typical plot is shown in Figure 3.3. We see that at low frequencies the cells undergo nDEP. As the frequency is increased, the response passes through a lower crossover frequency, $F_{x1}$, and the cell experiences pDEP. At even higher frequencies, an upper crossover frequency is observed, $F_{x2}$, as the response returns to nDEP. Additional shells can be added to the single shell model to examine the effect of internal cell structures, such as a highly conductive nucleus or, as in this case, a large non-conductive region of lipids. Using these models, the factors that change the DEP response of cells can be examined. For cells exhibiting two crossover frequencies, the lower crossover frequency is dependent primarily upon membrane properties and the higher crossover frequency is dependent primarily on the properties of the cytoplasm as shown in Figure 3.3 [1]. Referring back to Table 2.1, the majority of DEP publications separate cells with differing lower crossover frequencies. For the common example of separating viable from non-viable cells, the physical difference is that the damaged membranes of the non-viable cells have different dielectric properties than the intact membranes of the viable cells.
Figure 3.3: The lower crossover frequency is primarily determined by the dielectric properties of the membrane. This project is concerned with the upper crossover frequency which is largely dictated by the dielectric properties of the cytoplasm. Adapted from Hughes [1]
Therefore in these cases, a frequency can be found such that the non-viable cells undergo nDEP while the viable cells experience pDEP [31]. This effect is distinct from any changes in DEP response due to changes in the conductivity of the cytoplasm due to leakage.

As mentioned, the exact value of the upper crossover frequency is primarily dependent on the dielectric properties of the cytoplasm, but there are very few reports that discuss differentiating cells at the upper crossover frequency. Castellarnau et al. compared the DEP response of various mutants of *E. coli* with the wild type (5K strain) [46]. They observed that the upper crossover frequency of all cells was in the 55–110 MHz range and that upper crossover frequency of H-NS mutants was shifted to lower frequencies. While their models suggest that this shift is due to changes in cytoplasmic conductivity, they only speculate that this may be due to differences in the structure of the DNA. Beyond this, it is unclear as to why the cytoplasm of the mutant strain might have a different conductivity. Nonetheless, this report is the most compelling example of cells being differentiated via high frequency DEP. Labeed et al. measured the DEP induced cell collection rate of human chronic myelogenous leukaemia (K562) cells at various stages of apoptosis (programmed cell death)[44]. They observed that as the apoptosis process progressed, the cell collection rate at the higher frequencies was reduced. They argue that as the cells died, their membranes began to leak leading to a lower cytoplasmic conductivity. It should be noted however, that they were not able to generate electric fields with frequencies greater than 20 MHz. Broche et al. analyzed these results [41] and by using a symbolic software package, they derived that
the upper crossover frequency can be directly estimated with

\[ F_{x2} = \frac{1}{2\pi} \sqrt{\frac{\sigma_c^2 - \sigma_c \sigma_m - 2\sigma_m^2}{2\varepsilon_m^2 - \varepsilon_c \varepsilon_m - \varepsilon_c^2}} \]  

(3.21)

For sufficiently low media conductivities, this equation predicts that the upper crossover frequency is dictated by the conductivity of the cell’s cytoplasm. While these studies support the overall hypothesis of this proposal, they only offer a glimpse into the behaviour of cells undergoing high frequency DEP. In order to conclusively determine whether the upper crossover frequency is dictated primarily by the conductivity of the cytoplasm, more experimental data are needed. It would be ideal to observe cells with variable, yet quantifiable, cytoplasmic conductivity in high frequency electric fields and observe their response while holding other quantities as constant as possible.

For complex particles, such as a cell, the polarizability of the particle, and thus the crossover frequency(ies), can be affected by intrinsic internal and external structures [36, 50]. This has formed the basis for several devices that are capable of label-free sorting of cells. For example, several groups have been able to isolate viable from non-viable yeast [31, 32, 38, 50, 52], and Gascoyne et al. demonstrated the ability to separate normal murine erythrocytes from erythroleukemia cells [33]. Of particular interest to the work proposed here, Gallo-Villanueva et al. recently demonstrated the ability to separate viable from non-viable microalgae [48]. A summary of these reports is provided in Table 2.1.
3.3 Matlab modeling

The primarily concern is with shifts in $F_{x2}$ and therefore it is worth examining what parameters affect this frequency. Broche et al. [41] simplified the full Claussius-Mossoti factor using a computer algebra system to find that in cases where the core radius is more than 20 times the shell thickness and Equation 3.21 can be used to determine $F_{x2}$. Thus for biological cells, $F_{x2}$ depends only upon the permittivity and conductivity of the media and cytoplasm. It should be noted that $F_{x2}$ only exists for cases where the conductivity of the media is lower than the cytoplasm and the permittivity of the media is higher than the cytoplasm as shown in Fig 3.4. It was worth making a model of the DEP response to understand the concepts of DEP. However, the values for the algae in terms of conductivity and permittivity are not well known. Lipids however can be considered the same across many algae cells. The values used in Figure 3.5 were taken from a few different papers but mostly from snow algae values taken from [17]. These values are also shown in Table 3.1. Furthermore, DEP media is typically much less conductive than the cytoplasm and has a roughly linear dependence on the cytoplasmic conductivity as illustrated in 3.6.

<table>
<thead>
<tr>
<th>Properties</th>
<th>$\sigma_{\text{shell}} (\frac{m}{m^2})$</th>
<th>$\varepsilon_{\text{shell}}$</th>
<th>$\sigma_{\text{cytoplasm}} (\frac{m}{m^2})$</th>
<th>$\varepsilon_{\text{cytoplasm}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle 1 with low/no lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>Particle 2 with High lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.2</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure 3.4: The typical DEP response predicted by the single shell model has two crossover \( F_{x2} \) frequencies. The lower crossover frequency, \( F_{x1} \), is dictated primarily by membrane properties, while the upper crossover frequency, \( F_{x2} \), depends on the properties of the cytoplasm. Modeling parameters are based on Muller et al. and Wu et al. [17, 18]: 
\[
\begin{align*}
\sigma_{\text{media}} &= 0.06 \text{S/m}, \quad \varepsilon_{\text{media}} = 80, \\
\sigma_{\text{membrane}} &= 2 \times 10^{-5} \text{S/m}, \quad \varepsilon_{\text{membrane}} = 8, \\
\sigma_{\text{cytoplasm}} &= 0.5 \text{S/m}, \quad \varepsilon_{\text{cytoplasm}} = 50,
\end{align*}
\]
\( R = 6 \text{m}, \quad \delta = 7 \text{nm} \). In cases where the media is more conductive than the cytoplasm (\( \sigma_{\text{membrane}} \leq 0.6 \text{S/m} \)), no crossover exists. In cases where the permittivity of the cytoplasm is higher than the media (\( \varepsilon_{\text{cytoplasm}} \geq 90 \)), no upper crossover frequency exists.
Figure 3.5: Using 3.21, the effects of cytoplasmic conductivity and permittivity on the upper crossover frequency can be easily seen ($\sigma_{\text{media}} = 0.06 \text{S/m}$, $\varepsilon_{\text{media}} = 80$). At lower cytoplasmic conductivities, the cytoplasmic permittivity has a smaller effect.
Figure 3.6: Using 3.21, the effects of cytoplasmic conductivity and permittivity on the upper crossover frequency can be easily seen \((\sigma_{\text{media}} = 0.06 \text{S/m}, \varepsilon_{\text{media}} = 80)\). At lower cytoplasmic conductivities, the cytoplasmic permittivity has a smaller effect.
Chapter 4

Materials and Methods

4.1 Algae Cultivation and Preparation

*Chlamydomonas reinhardtii* (strain 90, UTEX, Austin, TX) was cultured from slant using the growth medium reported in Sager and Granick [53]. Separately, a nitrogen-free medium was prepared from the same recipe by omitting the ammonium nitrate. The cells were grown at 27°C while being continuously bubbled with air with light and dark periods of 18 hours and 6 hours, respectively. After the cells had grown into a dense culture, a portion of the culture was spun down and rinsed with the N-free medium 3 times before being resuspended in the N-free medium. As previously mentioned, the absence of nitrogen stresses the cells which retards cell growth and promotes the accumulation of lipids within the cells. The N-free cells were checked every few days for lipid accumulation by dying a portion of the culture with Bodipy 505/515 (Invitrogen, Grand Island, NY) [21]. The dye solution consisted of a 1:10 mole ratio of Bodipy 505/515 dissolved in dimethyl sulfoxide. Fluores-
Figure 4.1: The cultivation set-up for *C. Reinhardtii*. The algae is kept in a hood with a thermostat set at 80°F. House air is plumbed to the water trap and then plumbed to the different containers of algae with a needle valve for each flask to adjust the flow of air into the containers.

Fluorescence was checked using a fluorescent filter (Chroma, Model 49002 ET-GFP, Bellows Falls, VT) on a Nikon TE-2000S inverted epifluorescent microscope (Melville, NY). The picture of the set-up is shown in Figure 4.1.

After enough lipids had accumulated in the N-free cells, portions of the regular culture and N-free culture were separately spun down and rinsed with DEP medium 3 times. The DEP medium consisted of deionized water adjusted to 0.064 S/cm conductivity with potassium chloride as measured with an Extech Exstik II conductivity meter (Nashua, NH). Glucose was then added (85 g/L) in order to raise the osmotic pressure of DEP medium without affecting its conductivity [11][6].

Culturing algae in Reno, NV had unexpected hurdles and it took several months to get a clean sample of algae that could be used in experiments. The lack of humidity was a significant problem because the growth solution could be completely dehydrated overnight. This problem was overcome with
the addition of a water trap in line with the supplied air. Controlling the temperature was also an obstacle because of the lack of insulation in the lab. A heater with a thermostat was placed in the hood to keep a near constant temperature of 80°F inside the hood. The largest obstacle was contamination from a fungus or from a bacteria, or some other unknown source and could easily have come from the building. Algae that has a fungus growing with it will clump together as shown in Figure 4.2 and DEP experiments become inaccurate and difficult to perform.

Figure 4.2: An example of *C. Reinhardtii* that has been infected with a fungus. The algae tends to clump together and is no longer a good sample for DEP testing.
Figure 4.3: A simple two-needle dielectrophoretic set-up on a microscope slide. Cells with low lipid content (green) are attracted toward the field while high lipid cells (red) are repulsed.

4.2 Microfabrication of Electrodes

The first needles that were used for this experiment were Signatone tungsten catwhisker 1 $\mu$m needles (Model number SE-SM, Gilroy, CA). These needles were effective when they could be aligned properly. The needles were arranged as shown in Figure 4.3. The experiments done with this set-up were promising but overall there was not any real substantial evidence to support the hypothesis. There were inherent problems with the needles moving, and they would break easily. To overcome this the needles were placed on a glass slide and the Polydimethylsiloxane (PDMS) was poured over the top to create a seal with the PDMS and the glass that would create a well for the algae sample to be placed and then to also secure the needles. This method, although better, was still unreliable and it was difficult to observe algae in DEP conditions.

The next round of experiments consisted of micropatterning a glass slide that was sputtered with chrome and gold (UVH Sputtering, Morgan Hill, CA). These slides were covered with positive dry film photoresist from Think and
Tinker, Ltd. (Dupont Riston M115i, Palmer Lake, CO), that is sensitive to UV light. The photoresist was then covered with a mask that would prevent UV light from hitting it in specified places. Patterns with made using Solidworks to create a mask which was then sent to CAD/Art Services (Brandon, OR) to create a dark field mask using a photoplot. These masks were then placed on the photoresist and exposed to UV light using a Bachur and Associates LS 100-2 NUV exposure system (Santa Clara, CA). After an exposure time of 0.4 seconds the chip sat for 5 minutes. The photoresist was then developed using sodium carbonate that was also purchased from Think and Tinker, Ltd. (Palmer Lake, CO) which left the desired pattern of hardened photoresist on top of the gold. After developing the photoresist the exposed gold and chrome were then removed. Finally the remaining photoresist was removed using Sodium Hydroxide. This procedure is shown in Figure 4.4. To use the electrodes on the chip copper tape was put on the pads as shown in Figure ... The copper tape was heated in order to increase the adhesion to the gold.

### 4.3 Experimental Procedure

A needle pattern was used due to the ease of observing positive or negative DEP [1] as shown in Figure 4.5. Conductive copper tape and alligator clips were used to connect the glass slide to the electronic equipment. Sine waves were generated with a Rigol D64162 signal generator (Oakwood Village, OH) capable of generating sine waves with frequencies up to 160 MHz which was amplified with an Ophir 5322 Rev.x1 RF amplifier (Los Angeles, CA). A Fairview Microwave -3 dBm attenuator (Model SA3N511-03, Allen, TX) was placed between the amplifier and the electrodes to help match the load to the
Figure 4.4: Cross-section of Electrode fabrication process. A) A glass slide with Gold and Chrome sputtered on top. B) Photoresist is laminated onto the slide and C) patterned. D) The desired electrode geometry is protected with patterned photoresist and unwanted Gold is removed with a wet etching process. E) Unwanted Chrome is removed with a wet etching process. F) The photoresist is removed to expose the patterned gold electrodes.
amplifier and reduce the amount of reflected power. This set-up was capable of 30 Volts peak-to-peak ($V_{pp}$) amplitudes in the given frequency range as monitored by an oscilloscope (Model 1202CA, Rigol Technologies) probing the electrodes. The experimental set-up is shown in Figure 4.6. The glass slide was placed on the inverted microscope and a camera (Nikon DS-L2) was used to take pictures of the algae.

Cells were placed on the glass slide where they were observed for a period of 3 minutes for frequencies ranging from 20 to 110 MHz in 5 MHz increments. It should be noted that as the frequency changed, the $V_{pp}$ varied as much as 40 $V_{pp}$ across the range of frequencies. This frequency change is shown in Figure 4.7. We suspect that the impedance of the experimental set-up was highly frequency dependent at the high frequencies used in this study. Therefore, the amplitude was adjusted at each individual frequency in order to apply a 30 ±3 $V_{pp}$ signal across all frequencies. After the 3 minute observation, cells were removed from the slide before more cells were placed onto the slide, in order
Figure 4.6: Photo of the experimental set-up. A) Function generator B) RF Amplifier C) Oscilloscope D) Inverted fluorescent microscope E) Camera controller.

to minimize the effects of evaporation on the small drop of DEP media. This process was repeated until the microalgal movement had been observed over the entire range of frequencies. Pictures were taken at 1 minute intervals with a 40x objective (Nikon MRH08420).
Figure 4.7: Plot of the amplitude change as a function of frequency. The load across the electrodes changed as a function of frequency which also changed the voltage.
Chapter 5

Results and Discussion

C. reinhardtii cells cultured in regular media were observed to have an $F_{x2}$ of approximately 75 MHz. These cells did not fluoresce when stained with BODIPY 505/515, indicating no significant levels of accumulated lipids. On the other hand, the microalgal cells grown in N-free media were observed to have an $F_{x2}$ of approximately 40 MHz, a shift of approximately 35 MHz. These N-free cells did fluoresce when stained, indicating lipid accumulation, as expected. The results of these experiments, illustrated in Figure 5.1, strongly support the hypothesis that microalgal cells can be differentiated on the basis of lipid content via high frequency dielectrophoresis.

There are two possible mechanisms that could be responsible for the shift in $F_{x2}$ with the increase in lipid levels within the cell. As mentioned, the accumulated lipids are non-polar; a fact supported by the use of BODIPY 505/515 which fluoresces in non-polar environments. The lipids, due to their oily nature, have a lower conductivity and permittivity than the rest of the cytoplasm and therefore reduce the both the effective conductivity and the effective permittivity of the cytoplasm. Based on Equation 3.21, either of these effects
Figure 5.1: Micrographs of pDEP and nDep. A) Microalgae cells grown in N-free media fluoresce when stained and undergo pDEP at 30 MHz. B) At 55 MHz, these cells have been pushed away from the electrodes indicating nDEP. The N-free cells are approximately the same size as the regular cells but appear smaller due to the bright fluorescence of the lipid rich regions of the cell. C) Microalgae cells grown in regular culture experience pDEP at 70 MHz. D) At 85 MHz these cells have been pushed away from the electrodes due to nDEP except for cells that have adhered to the electrode. The regular cells do not fluoresce when stained due to a lack of lipids. The micrographs have been converted to greyscale and uniformly adjusted for proper contrast.
The shift in the upper crossover frequency is primarily due to changes in the cytoplasmic conductivity due to the increase in lipid content ($\sigma_{\text{media}} = 0.064 \text{S/m}$, $\varepsilon_{\text{media}} = 80$). In order to help separate these two effects, the effective conductivity of the cytoplasm was calculated for a range of assumed cytoplasmic permittivities. These results, plotted in Figure 6.1, indicate that the reduction in $F_{x2}$ is primarily due to changes in the effective cytoplasmic conductivity. While the cytoplasmic permittivity does affect the calculated effective cytoplasmic conductivity at lower lipid levels (i.e. higher conductivities), this effect is much smaller at higher lipid levels. Furthermore, changes in cytoplasmic permittivity cannot account for the entire shift in $F_{x2}$. Therefore, these experimental results, coupled with the analytical model, support our hypothesis that lipid accumulation within an algal cell shifts $F_{x2}$ to lower frequencies by reducing the effective cytoplasmic conductivity of the cell.

The Matlab model in Figure 3.5 was then adapted using these new values as shown in Table 5.1. These values use the same initial values for the permittivity but instead the conductivities are changed using values from Figure 6.1 for the
Figure 5.3: The shift in the upper crossover frequency is primarily due to changes in the cytoplasmic conductivity due to the increase in lipid content ($\sigma_{\text{media}} = 0.064 S/m, \varepsilon_{\text{media}} = 80$).

conductivity when the permittivity is 50. These results were then put into the Matlab model and the following results are shown in Figure 5.3.

Table 5.1: Summary of initial values used and the results using the crossover frequencies that were calculated from DEP experiments.

<table>
<thead>
<tr>
<th>Properties</th>
<th>$\sigma_{\text{shell}} \left( \frac{\Omega}{\mu} \right)$</th>
<th>$\varepsilon_{\text{shell}}$</th>
<th>$\sigma_{\text{cytoplasm}} \left( \frac{\Omega}{\mu} \right)$</th>
<th>$\varepsilon_{\text{cytoplasm}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial values without lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>Results without lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.43</td>
<td>8</td>
</tr>
<tr>
<td>Initial values with High lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.2</td>
<td>8</td>
</tr>
<tr>
<td>Results with High lipids</td>
<td>$1 \times 10^{-6}$</td>
<td>50</td>
<td>0.25</td>
<td>8</td>
</tr>
</tbody>
</table>
Chapter 6

Conclusion

The major consequence of this work is that particles/cells can be separated based on intrinsic values vs. the traditional membrane properties. It is also an effective method compared to the more common separation techniques, i.e. FACS. Microalgae was used because of its ease in growing cells that have noticeable differences in their cytoplasm properties. C. Reinhardtii in particular could grow large amounts of lipids and is a fast growing algae. The algae was grown in an ideal environment and then a stressed N-Free environment that caused the accumulation of lipids within the cell. These differences in cytoplasm properties for C. Reinhardtii were experimentally verified using DEP theory by measuring the crossover frequencies for each cell. In particular the Clausius-Mossotti factor was investigated and used for modeling. The Clausius-Mossotti factor simplified the $F_{DEP}$ equation and simplified the models. These models were used to show that in theory that cells could be manipulated based on intrinsic properties.

Although this is simply a verification of the theory, the opportunities for fast and simple separation are there. Medicine and biology could use these
techniques to quickly run tests and speed up research. This is a realistic idea, but more work is required to provide a suitable method for these disciplines.

6.1 Future Work

The next step is to quantify how the lipid content of a microalgal cell changes the cell’s high frequency DEP response. The basic premise behind this idea is that we can measure the lipid content of a microalgal cell via fluorescence while simultaneously observing the cell’s DEP response. Analytical and numerical models would need to be developed to support these experiments and provide additional insight and verification of the observations. Microalgae could be observed over a period of days as the lipids gradually accumulate, and a sensitivity study of the DEP response as the cells change could be done.

In order to improve testing time a high throughput screening (HTS) device that can sort microalgae on the basis of lipid content via high frequency DEP could be built. To our knowledge, this would be the first demonstration of label-free HTS for lipid accumulation within microalgal cells. This device could be designed as a continuous flow instrument with the goal of matching or exceeding the performance of a commercial FACS with regard to throughput. The most common approach used to sort cells via DEP is to use the DEP force to move the cells laterally within the microchannel as shown in Figure 9. In this case, the frequency of the electric field and the conductivity of the supporting medium are the two "knobs" available to separate the algal cells on the basis of lipid content. Because the effects of these two parameters are largely orthogonal, this gives us two different methods to separate the cells, either on the basis of the upper crossover frequency or the conductivity of the
Figure 6.1: In order to achieve separation in a continuous flow device, the DEP force moves the cell laterally within the channel. In this case, the direction of the DEP force should depend primarily upon the lipid content of the cell.

medium. Both methods depend on changes in the effective conductivity of the cytoplasm.

In order to expand this research, a comprehensive study to see what other cells can be separated using high frequency DEP would be effective. The goal would be to develop criteria that can be used to predict whether a cell type would be a good candidate for sorting via high frequency DEP. In particular, high frequency DEP could have much broader applications when compared to low frequency DEP since it probes the interior of the cell as opposed to the cell membrane. This would also provide preliminary data with which can provide more applications.
Bibliography


[45] R. Hlzel, “Electrorotation of single yeast cells at frequencies between 100 hz and 1.6 GHz,” *Biophysical Journal*, vol. 73,


Chapter 7

Appendix

7.1 Growing Media

Stock Solutions

K-Pi stock (pH 7.0):

- Dipotassium Phosphate ($K_2HPO_4$) 14.3 g/l
- Monopotassium Phosphate ($KH_2PO_4$) 7.3 g/L

Ca-N-Mg stock:

- Ammonium Nitrate ($NH_4NO_3$) 16.0 g/l
- Magnesium Sulfate $7H_2O$ ($MgSO_47H_2O$) 4.0 g/L
- Calcium Chloride ($CaCl22H2O$) 2.0 g/L

Ca-Mg(N-free) stock:

- Magnesium Sulfate $7H_2O$ ($MgSO_47H_2O$) 4.0 g/L
• Calcium Chloride (CaCl₂2H₂O) 2.0 g/L

**MOPS-Tris buffer stock (2.0 M MOPS):**

• MOPS(free acid) 418.6 g/L
• Titrate MOPS with solid Tris Base to pH 7.2

**Hutner’s trace elements Stock:**

• EDTA(free acid) (Free acid) 16.0 g/l
• Zinc Sulfate (MgSO₄7H₂O) 4.0 g/L
• Boric acid (H₃BO₃) 2.0 g/L
• Manganese Chloride (MnCl₂4H₂O) 16.0 g/l
• Iron Sulfate (FeSO₄7H₂O) 4.0 g/L
• Phosgene (CoCl₂5H₂O) 2.0 g/L
• Copper Sulfate (CuSO₄4H₂O) 16.0 g/l
• Ammonium Heptamolybdate ((NH₄)₆Mo₇O₂₄4H₂O) 4.0 g/L
• Deionized water 750 ml

For the Hutner’s trace elements, the mixture must be boiled and then cooled slightly. The pH is then adjusted using Potassium Hydroxide (KOH). The clear solution is then diluted to 1000 ml with deionized water. It should have a green color that changes to purple on standing. Using the stock solutions, the following is the mixture for the growth media and for the nitrogen free media. The mixtures are per liter of water.

**Growth Media:**
- K-Pi Stock 10 ml/L
- Ca-N-Mg Stock 25 ml/L
- MOPS-Tris Stock 10 ml/L
- Hutner’s trace elements Stock 1 mL/L

**N-Free Media:**

- K-Pi Stock 10 ml/L
- Ca-Mg Stock 25 ml/L
- MOPS-Tris Stock 10 ml/L
- Hutner’s trace elements Stock 1 ml/L

Media was made just before inoculation and autoclaved before algae was introduced.