

Dual-Modality Optical-Dielectric Cytometer for Microplastic and Single-Cell Analysis

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Abstract

Conventional analysis methods for particle characterization often rely on optical imaging or dielectric measurements independently, limiting the information that can be obtained. This thesis presents a non-invasive and label-free video cytometer capable of simultaneously measuring morphological and dielectric properties of particles in flow.

The dual-modality optical-dielectric cytometer utilizes a high-speed camera focused in a microfluidic channel with integrated coplanar electrodes. A polarizable particle placed in a non-uniform electric field experiences a translational motion, altering its velocity due to the parabolic flow profile in a microfluidic channel. Dielectrophoretic (DEP) analysis utilizes the polarizability of a particle compared to its background medium, described by the Clausius-Mossotti factor (KCM), to observe a positive DEP (pDEP) or negative DEP (nDEP) force acting on the particle to decrease or increase the velocity respectively for a particle on the lower half of the channel. An image processing algorithm analyzes images of cells passing over the electrodes to collect the trajectories and morphology of particles in flow. The optical images provide particle parameters such as the size, circularity, and eccentricity, as well as velocity data for DEP analysis.

Experiments were performed using polystyrene (PS) particles as a microplastic model and Chinese hamster ovary (CHO) cells as a biological cell model. Polystyrene microspheres verified the relationship between the applied voltage and the DEP force, as well as morphological differences between spherical and non-spherical particles. Two sizes of PS microspheres were differentiated and compared to morphological data from a cell counter and DEP simulations. CHO cells undergoing starvation were measured at two time points to capture mixtures of viable and non-viable cells at different viabilities. Experiments with CHO cells demonstrated the frequency dependent nature of the KCM using their dielectric response and displayed the associated morphological data. Viable and non-viable CHO cells were differentiated based on

their pDEP and nDEP response respectively, where the dielectric response was compared to simulations and the morphological data was compared with trypan blue staining results.

Combining optical and dielectric modalities provides complementary data with comprehensive particle information and establishes a foundation for future studies in drug development, diagnostics, and biological and environmental research.

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Chapter 1 – Introduction

1.1 Motivation

Optical analysis is an indispensable tool for particle analysis that has been used and optimized for centuries. These techniques enable the visualization of micro-scale structures that are otherwise indistinguishable. Morphological features can be detected using optical microscopy to understand the health and function of single cells [1]. Traditional microscopes are limited in their ability to visualize the complete structural and functional complexities of biological cells, but further insights can be gathered in combination with alternative analysis techniques, one common solution being labelling. Labelling through the use of dyes and fluorescent markers helps to highlight specific molecules and structures that are otherwise difficult or impossible to see. However, this process can be time consuming and many of these dyes and fluorescent markers may alter cellular physiology or cause irreparable damage to cells due to the toxicity [1], [2], [3]. Other more advanced systems like transmission electron microscopy can provide further insights into the internal structures of cells by accelerating electrons through a thin sample. The high-powered electron beam may cause damage to biological samples by breaking chemical bonds and causing radiation damage [4]. Dielectric analysis can be used as a complementary method with optical analysis to gain further insight into cellular properties while remaining non-invasive and label-free.

Dielectric analysis provides information about the electrical properties of cells, which can help to characterize cellular physiology. Dielectrophoresis (DEP) does this by analyzing the change in motion of a particle due to a non-uniform electric field. DEP analysis can reveal key electrical parameters including membrane capacitance, ion concentration, cytoplasm conductivity, and cell permittivity, providing insights into a cell's physiological state and enabling discrimination of cell types, which is crucial to single-cell analysis [5], [6].

Optical imaging analysis in conjunction with dielectric analysis provides complementary information on particles and cells. Correlating the morphological and dielectric properties of cells can improve accuracy of classification and characterization of single cells. This is useful to a variety of applications including drug development, disease monitoring, and diagnostics [5], [6]. Additionally, the application of this approach can be beneficial in areas such as environmental monitoring of microplastics and material characterization for microparticles [7], [8]. This thesis focuses on the development of a video cytometer that combines both imaging and DEP analysis of microparticles and cells, using samples of polystyrene (PS) microplastics and Chinese hamster ovary (CHO) cells.

1.1.1 Microplastics

Microplastics are an escalating global concern for both the environment and public health. These small particles predominantly originate from larger sources of commercial plastic debris that break down over time and are made from materials such as polystyrene (PS), polyethylene (PE), polypropylene (PP), and more. While plastics degrade and break down in the environment, they can adsorb toxic chemicals and pollutants, which can further exacerbate their impact. The morphological features including size, shape, and surface roughness, will all affect a particle's ability to penetrate biological systems and to adsorb chemicals due to the increased surface area. Contaminated microplastics can be exposed to humans through ingestion from food or water, inhalation from particles in the air, and absorption through the skin. The toxic effects of microplastics can present as a disruption to deoxyribonucleic acid (DNA), organ dysfunction, immune response, and more [7], [9], [10].

Microplastics are also commonly used as a beneficial tool for device calibration and verification. Microplastics of known size, material, and shape provide a predictable and reproducible sample prior to using biological cells due to their consistent size, refractive index, density, and dielectric properties [5], [11].

1.1.2 Biological Cells

Single-cell analysis of biological cells is critical in understanding the heterogeneity of a population. Traditional bulk measurements may obscure key complexities in cellular state, function, and interactions [12]. Knowledge of the cell-to-cell variation in a population helps to characterize important subpopulations that can appear as differences in metabolic activity, stress responses, growth rate, and more [13], [14]. A key focus in single-cell analysis is the characterization of viability. Viability reflects the health of a cell by indicating whether a cell remains metabolically active or is subjected to stress conditions [15]. Dynamic monitoring of cellular viability helps to indicate the longevity of a culture [13], [16].

Chinese hamster ovary cells are one of the most widely used cell lines in a variety of biomedical studies. CHO cell lines are well characterized and have become a valuable mammalian cell model. A primary benefit of these cells is their ability to be cultured in suspension while remaining tolerant to environmental variations. CHO cells undergo apoptosis when they experience external stress conditions such as nutrient deprivation or osmotic imbalances, and thus can be separated based on viability [5], [17], [18].

1.2 Cytometry Methods

Flow cytometers are widely used for the analysis of single cells or particles in a fluid suspension, commonly through fluorescent analysis. Recent advances have further developed both imaging and dielectric based flow cytometers. These techniques are individually well established and can function as complementary modalities. The following is a non-exhaustive overview of some relevant systems.

1.2.1 Imaging Flow Cytometry

Optical imaging microscopic flow cytometers utilize high-resolution cameras attached to microscope systems to image particles and cells in flow. These systems can gather high-quality images in multiple channels such as dark field, bright field, and fluorescence by using

objectives, filters, lasers, fluorescence, and other optical components. Lasers can excite fluorescent dyes to enhance cell identification and localization of subcellular structures. Advanced post-processing algorithms and machine learning techniques analyze morphological properties, intensity, and other key features to differentiate, characterize, or classify cells. Typical morphological features detected include area, perimeter, major axis, minor axis, aspect ratio, circularity, diameter, eccentricity, brightness, and intensity [19], [20], [21], [22], [23], [24], [25], [26].

The first commercial imaging flow cytometer was the ImageStream® and has since evolved to the Cytex® Amnis® ImageStream®X MkII imaging flow cytometer. This system uses a high-speed charge-coupled device (CCD) camera with time delay integration (TDI) to capture moving objects using bright field, dark field, and multiple fluorescent channels. The system diagram in Figure 1-1 demonstrates how up to 12 images per cell can be captured simultaneously, allowing for a rich set of morphological and subcellular information. The system configuration is versatile and can be used for both off-line and at-line applications. Off-line applications involve manual sample collection for experimentation, while at-line applications can automatically remove samples from the system. At-line formats connected to bioreactors can be automated to allow for continuous monitoring of cells. When capturing images, there is a measured velocity to ensure that the TDI of the camera successfully focuses and reconstructs images of cells. The field of view is small to focus on capturing localized particle images and ranges from 120×256 µm to 40×170 µm using the 20x and 60x magnification respectively. A major benefit of this system is its high throughput of up to 5000 objects/s with the 20x objective and a pixel size of 1×1 µm. The trade-off to enable a smaller resolution of 0.3×0.3 µm is a maximum throughput of 1200 objects/s. However, to enable a high throughput, there are risks of cells clumping together with higher sample concentrations. Samples must be carefully prepared in the ideal concentration range and with compatible fluorescent dyes. Fluorescent dyes that are excited at similar wavelengths may be excited and appear on images from lasers that

are close in wavelength. The system output provides large datasets containing morphological and fluorescent features that are used in conjunction with machine learning and artificial intelligence (AI) algorithms to distinguish cell types and assess physiological states. These algorithms are typically computationally heavy due to the large amount of data that is stored and may still require additional user input or interpretation [20], [22], [25], [27].

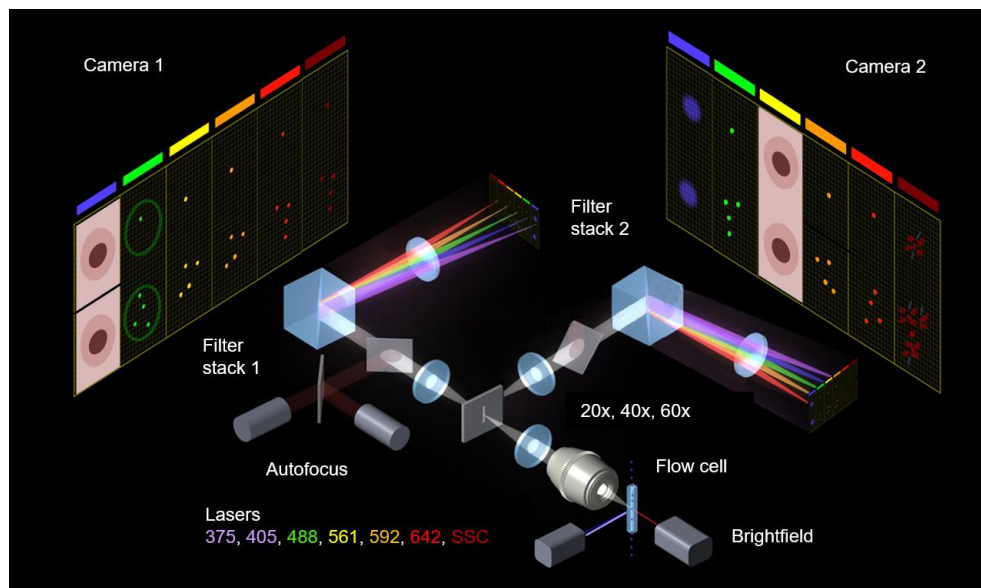


Figure 1-1: ImageStream® Mk II optical layout (Reprinted from [27], Copyright © 2024 with permission from Cytex Biosciences Inc.).

Other imaging flow cytometers focus on label-free methods to measure cellular properties. The Canty PharmaFlow™ captures images of particles in flow to analyze their size, shape, and concentration. This device favours versatility for multiple configurations including continuous monitoring of cells when connected to a bioreactor system. A study to evaluate the implementation of this device was performed by measuring the viability of CHO cells in [23]. Figure 1-2 shows the system configuration where particles are imaged using an 8.9 Megapixel CCD camera in bright field with an allowable frame rate of 30 to 60 fps. The microscope is equipped with a light-emitting diode (LED) illumination source and a 12x objective, which results in a field of view of 1315×867 μm . Raw images from the camera were analyzed using the CantyVision imaging analysis (CVIA) software to detect and identify morphological features

of cells. This software uses thresholding of the background to identify a particle's 2D shape as a binary image, then detects morphological features from the localized particle. The viability of cells is determined based on a machine learning model trained using images of stained cell populations. The morphological distinctions between viable and non-viable cells are primarily determined by the membrane integrity which arises in late-stage apoptosis. Due to this being the primary indicator for non-viable cells and a lack of information on the internal properties, this system demonstrated an underestimation in the classification of apoptotic cells compared to the annexin assay which is capable of detecting early-stage apoptosis. The variability in the measured viability of cells remains consistent with the current standard methods that use labelling to identify cells, such as trypan blue which has been known to overestimate viability. The system provides a real-time label-free method of acquiring cellular information which can be beneficial for many applications in biopharmaceutical studies [23], [28].

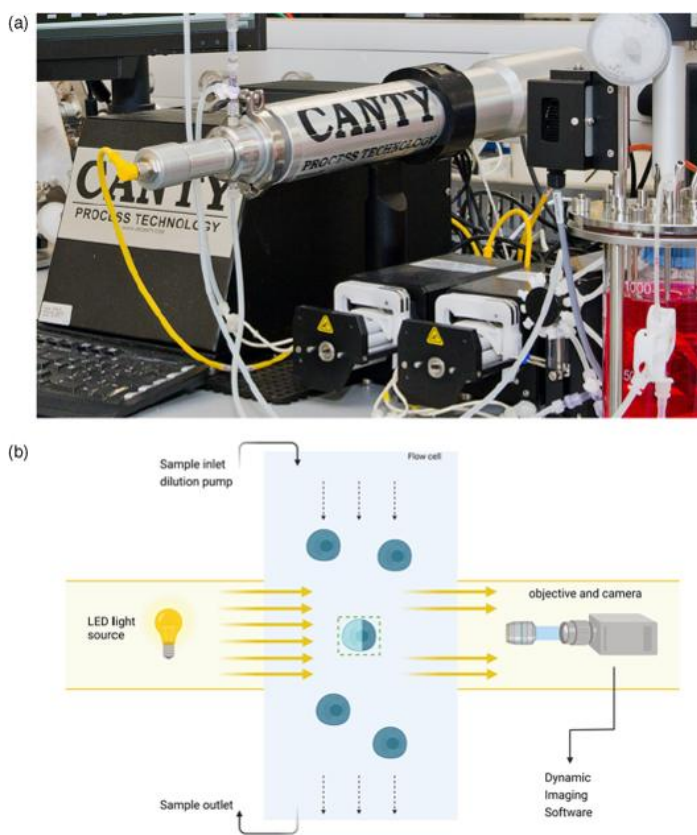


Figure 1-2: PharmaFlow imaging system a) physical set up and b) schematic diagram with cells in flow (Reprinted from [23]. Licensed under CC BY 4.0).

Another branch of imaging flow cytometers utilizes motion tracking of particles. An imaging flow cytometer that incorporates a real-time moving object detector (R-MOD) is presented in [24]. This system uses a high-speed complementary metal-oxide-semiconductor (CMOS) camera to detect particles in bright field with the process shown in Figure 1-3. The camera captures 500 fps over a 100×500 px viewing area in a polydimethylsiloxane (PDMS) microfluidic channel where each pixel is $1 \times 1 \mu\text{m}$. This system allows for a label-free method to measure and classify cells with the aim of integrating particle tracking in this system, primarily to avoid counting the same particle twice. The algorithm to track and identify particles begins by converting the image to a probability density function to locate particle positions on each frame. Particles are linked between corresponding occurrences in subsequent frames to capture trajectories while identifying occlusions for when particles enter and exit the field of

view and cases where particles overlap. A single image is chosen for each particle with a unique identification and is processed by a classifier. The classifier identifies features on the image of each cell and assigns a type using a convolutional neural network (CNN). This system was successful in classifying various sizes of polystyrene microspheres with high precision. Experimentation was also done with red blood cells and K562 leukemia cells to demonstrate the feasibility of this device with biological applications. The high-speed camera with the image processing algorithm allowed for particle tracking while minimizing the challenge other imaging cytometers face with counting the same particle more than once. The implemented classification is limited by the chosen model and would require additional training for alternative cell types [24].

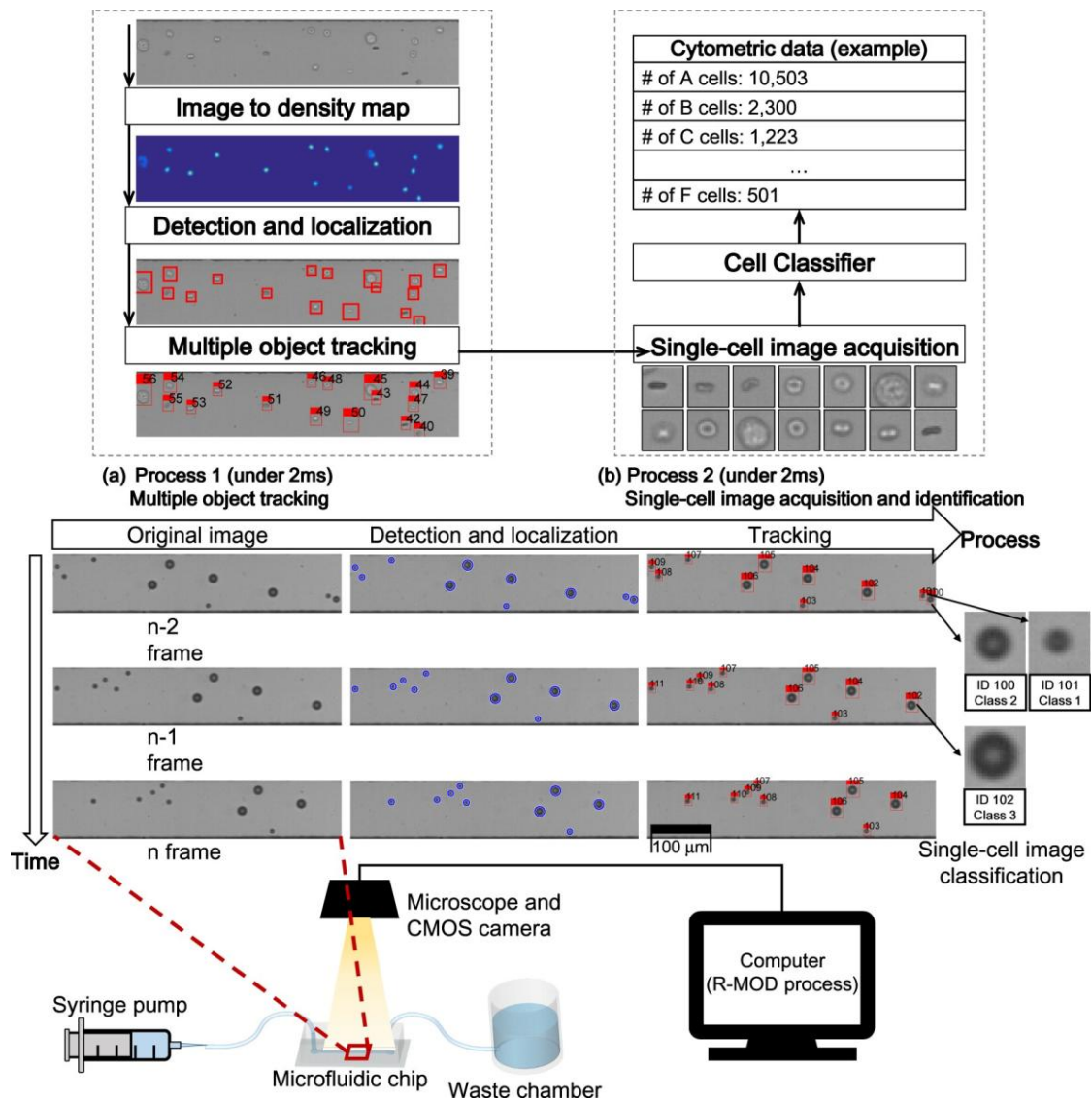


Figure 1-3: Schematic of the designed system and R-MOD (Reprinted from [24]. Licensed under CC BY 4.0).

1.2.2 Holographic Cytometry

An example of a newer evolution of imaging-based cytometers is holographic cytometers. Holographic cytometers use scattered field patterns to reconstruct 3D images of particles in flow. Lasers are employed as a reference beam to create interference patterns with particles passing through a microfluidic channel. The interference patterns in the acquired images are then reconstructed using computationally heavy numerical algorithms or machine learning.

This technology allows for information on both the phase and amplitude of cells, offering both internal and external information including size and refractive index [29], [30], [31].

1.2.3 Impedance Cytometry

A common method to measure dielectric properties of cells is impedance cytometry. This is a non-invasive label-free technique that works by measuring signal changes from the interaction of a particle passing through an electric field in a microfluidic channel, typically in the low frequency (LF) to high frequency (HF) bands. Integrated sensors such as coplanar electrodes or parallel plates measure signals as particles perturb the electric field and show a change in impedance, amplitude, frequency, capacitance, or other signal features that can be representative of the structure or dielectric properties of a cell. Analysis at low frequencies is generally used to infer size from the amplitude of the signal in relation to the volume of the particle while high frequencies can provide more information on the cell membrane, cytoplasm, and subcellular components. Data from these systems is complex and interpretation of the acquired signals requires careful system calibration, frequency selection, and advanced computational analysis [32], [33], [34]. An extension of impedance cytometry is microwave impedance cytometers which operate at higher frequencies in the microwave range. At higher frequencies, the permittivities of subcellular structures and other dielectric properties that are obscured at lower frequencies become observable. These systems are typically used in combination with other modalities such as a Coulter sensor to count the number of particles passing [8], [35], [36].

Impedance cytometry is employed as a method of non-invasive label-free detection and characterization of single cells. A system using coplanar electrodes is discussed in [37] to investigate differentiation of viable, necrotic, and apoptotic lymphoma cells. This system is displayed in Figure 1-4 and utilizes coplanar electrodes to simplify fabrication and to avoid potential alignment issues from implementing facing electrodes. Three electrodes run across the microfluidic channel with an applied signal on the centre electrode and two sensing electrodes

on either side that measure the difference in current through a transimpedance amplifier. This signal is demodulated using a spectroscopy to analyze the amplitude and phase of the signal peaks that arise from particles passing through the electric field. By using the ratio between the width and time of the signal, this system accounts for the difference in signal amplitude that occurs from the height of a particle in the channel. This eliminates the need for hydrodynamic focusing which is common in other impedance systems. Measurements were taken at frequencies of 0.5 and 10 MHz, where the electrical diameter was calculated using the amplitude of the signal at 0.5 MHz. This parameter, along with the phase at both 0.5 MHz and 10 MHz were used to classify cells as viable, necrotic, and apoptotic bodies. As cells become necrotic, there is damage to the plasma membrane which becomes apparent through the phase of the electrical signals. Apoptotic bodies are fragments of cells that form in late-stage apoptosis and can be differentiated based on their size which primarily relates to the amplitude of the signal at low frequencies. This system allows for differentiation using the size and internal properties of cells with a high throughput of ~ 200 cells/s while remaining non-invasive. A camera was implemented as a supplementary method to check whether cells were properly classified by correlating imaged cells with their detected signals. This confirmed the results of the impedance cytometer by providing additional visual information on a select number of cells. While this system was successful in the differentiation of cells, there are still possible sources of error that are common challenges in impedance cytometry. The system is sensitive to small environmental changes, and electronic noise makes signals difficult to detect. Signals may also be anomalous from more than one particle passing over the sensing region which can be a result of high sample concentration or other sample preparation and flow-related issues [37].

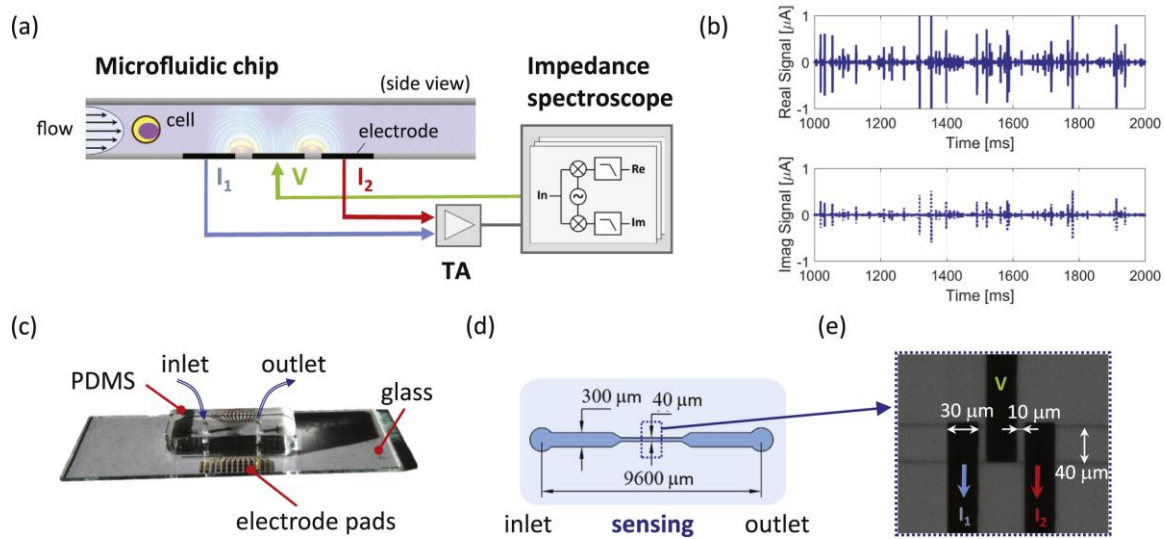


Figure 1-4: a) Operational schematic diagram b) example real and imaginary parts of the differential current demodulated at 0.5 MHz c) microfluidic impedance chip d) schematic diagram of the top view of the channel and e) the sensing region (Reprinted from [37], Copyright © 2019 with permission from Elsevier).

At higher frequencies, other internal properties such as intracellular molecules and organelles become apparent in their electrical signatures. The microwave flow cytometer presented in [38] uses a microwave frequency range to analyze two types of *Candida* yeast cells in a microfluidic channel. This system, shown in Figure 1-5, uses microstrip sensing electrodes that are attached to a vector network analyzer (VNA) to measure the changes in the signal when a particle passes through the sensing region and with an interferometer to improve the sensitivity of the signals. As particles pass through the sensing region, they perturb the electric field, producing a measurable change in the detected signal influenced by the electrical properties of the cell. Measurements are taken within a frequency range of 0.796 to 2.38 GHz for viable, heat-shocked, and drug induced cells to measure the change in permittivity when compared to the background medium. Since it is difficult to infer size from the acquired signals in this frequency range, a camera is used to capture and link images of cells to their acquired signals. Image processing is done using two methods, the bounding box and convex hull, to estimate

1.2 Cytometry Methods

the size. The size, shape, and orientation all affect the acquired signal and can appear as larger deviations to the average permittivity values which is problematic for non-spherical shapes. Despite this challenge, the average values were indicative of the populations, and the shape had a negligible effect in this study due to the predominantly spherical nature of these cells. Nevertheless, the effects from these features should be taken into consideration for alternative studies. This device demonstrated distinct differences between the three populations of yeast cells in a non-invasive label-free method and proved to be a beneficial foundation for future explorations on drug screening. There were apparent differences when comparing the yeast cells induced with an antifungal medication to the viable and heat-shocked cells that indicated varying responses to the drug within the population [38].

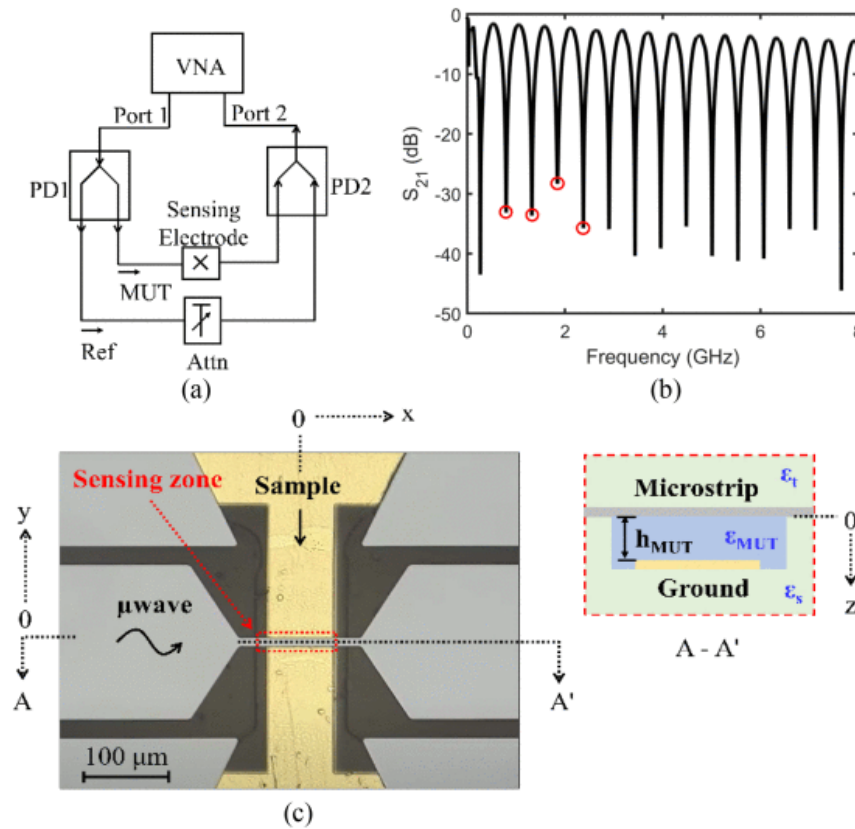


Figure 1-5: a) Schematic diagram c) example broadband S_{21} magnitude with frequencies used circled in red c) the sensing region and d) the sensing zone diagram (Reprinted from [38], Copyright © 2023, IEEE).

A recent study explores the effect of non-spherical objects on microwave signals. This system in [35] analyzes PS particles flowing in a microfluidic channel with an integrated split-ring resonator and a Coulter counter, shown in Figure 1-6. The split-ring resonator appears as coplanar electrodes in the sensing region with two different gap widths to account for the vertical location of the particle in the channel. This is a high-frequency capacitive sensor that is measured using a VNA. The Coulter counter is a low-frequency resistive sensor that is measured using a trans-impedance amplifier followed by a lock-in amplifier. The Coulter sensor is primarily used to measure the electrical diameter of the particle, while the split-ring resonator is used to measure analytes. The amplitudes from both signals are collected along with the phase from the high frequency signal and are used as inputs for the machine learning model to detect size, shape, and orientation. The change in capacitance in the signal is related to both the volume of the particle and the Clausius-Mossotti factor which describes the polarizability of the particle using the permittivity, eccentricity, and orientation. The machine learning model uses the random forest regression algorithm which relies heavily on the training data for classification accuracy and may cause errors when extrapolating outside the given data. A camera is used to compare the captured results with the results from the machine learning model to enable confident use of electrical signals to measure particles in future studies. The image processing algorithm used to measure particles may have some error due to pixelation, motion blur, resolution, noise, and distortion. Overall, this system provides an important step in gathering morphological properties from microwave systems and demonstrates the feasibility of using machine learning for feature detection. However, some deviations remain in comparison to the simulated models due to a series of physical parameters including the height of the particle in the channel, changes in orientation as it changes throughout the sensing region, and asymmetry in the particle shape [35].

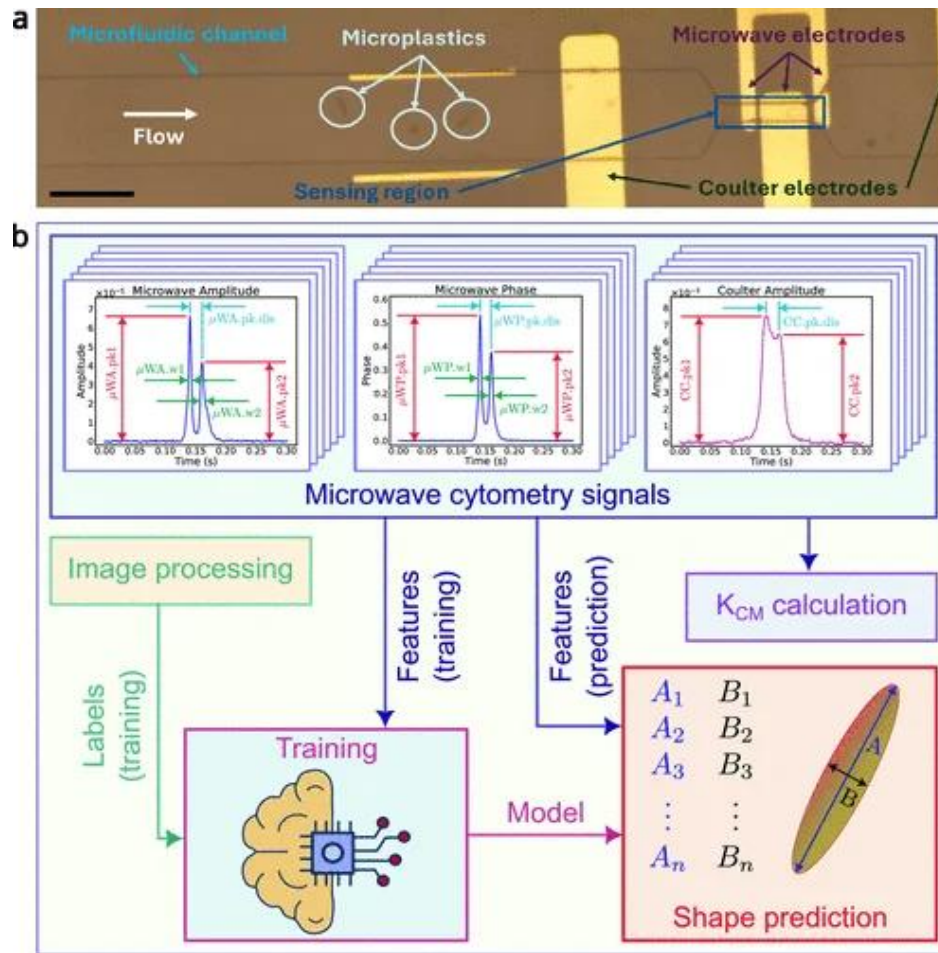


Figure 1-6: a) Sensing region image with a 200 μm scale bar and b) methodology flow diagram (Reprinted from [35]. Licensed under CC-BY 4.0).

1.2.4 Dielectrophoresis Cytometry

Dielectrophoresis cytometers measure dielectric properties by using non-uniform electric fields to manipulate a particle's motion in a microfluidic channel. Particles in flow will experience a DEP force resulting in a translational motion along the gradient of the electric field intensity. The translational motion is related to the frequency dependent polarizability of particles and thus relates to their dielectric properties which can be correlated to the physiological properties of cells. This allows for identification, sorting, discrimination, and differentiation of particles and cells [5], [6], [36].

A common use for DEP analysis is physical separation of cells. The study in [39] demonstrates a device capable of sorting various sizes of PS microspheres as well as viable and non-viable yeast cells. This system, shown in Figure 1-7, uses a diffusive mixer to create a monotonic gradient in electrical conductivity of the suspension medium laterally across the channel. Coplanar electrodes run diagonally across the channel to guide cells from the area of high medium conductivity to low medium conductivity. As the conductivity of the medium decreases, the DEP force becomes weaker and the drag force begins to dominate. The particles shift from moving along the diagonal electrodes to the flow direction in the channel. Particles with different electrical properties will realign with the flow at different locations in the channel. The lateral location in the channel is captured using an image processing algorithm with fluorescent imaging. Fluorescence is used to check whether the sorting was successful between three sizes of PS microspheres and for viable and non-viable cells. This imaging method allows for the trajectories to be seen along with the distribution of fluorescent intensities across the width of the channel. Experiments were performed with a range of frequencies for the applied signal to find the ideal frequency for separation. Particles with varying dielectric properties were sorted under optimal conditions, but there are many factors that can influence the effectiveness of this device such as the applied frequency, electrode design, particle sizes, medium conductivity, concentration, and flow rate. This device focuses on cell sorting rather than characterization of particles [39].

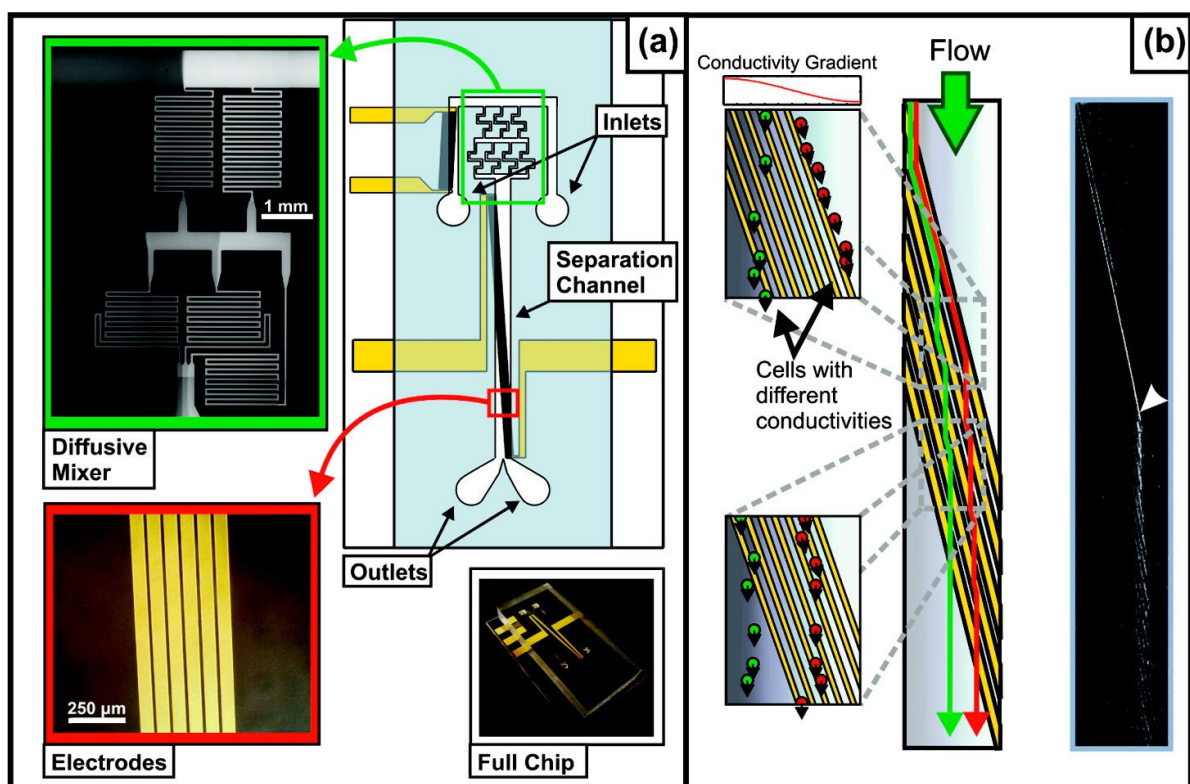


Figure 1-7: a) Device layout diagrams and images and b) device operation diagram and image (Reproduced with permission from [39], Copyright © 2008, American Chemical Society).

A more common method to sort cells is by deflection rather than by equilibrium. The device studied in [40] uses a label-free method to sort two types of cells based on their dielectric properties. The microfluidic channel in Figure 1-8 has one inlet and two outlets for each of the sorted cell types. Cell separation occurs using a single array of castellated microelectrodes with an AC signal applied to generate a non-uniform electric field. Each of the seven electrodes in the array are 80 μm wide with an 80 μm gap between. As cells flow through the non-uniform electric field, they experience a force that causes a translational motion towards or away from the electrodes. Viable T-cells experience a positive DEP force, due to their dielectric properties, that pulls them towards the electrodes and into the top inlet after passing through the field region. Non-viable T-cells are pushed away from the electrodes from a negative DEP force and into the bottom inlet. Fluid dynamics simulations were performed and found the ideal

1.2 Cytometry Methods

medium conductivity and frequency to be 0.42 S/m and 1.5 MHz. A population of viable and non-viable T-cells was experimentally tested with a 1:1 ratio at a range of frequencies. The region where the microfluidic channel branches into two outlets was imaged with a camera to measure the separation efficiency. The viable and non-viable T-cells were sorted with a 93% separation efficiency at the ideal frequency and medium conductivity for separation, with an applied voltage of 10 Vpp. This device is beneficial for continuous sorting of cells from a single inlet while remaining label free, enabling applications in point-of-care therapeutics. The flow rate in this system must be carefully set to ensure particles have sufficient time to interact with the electric field [40].

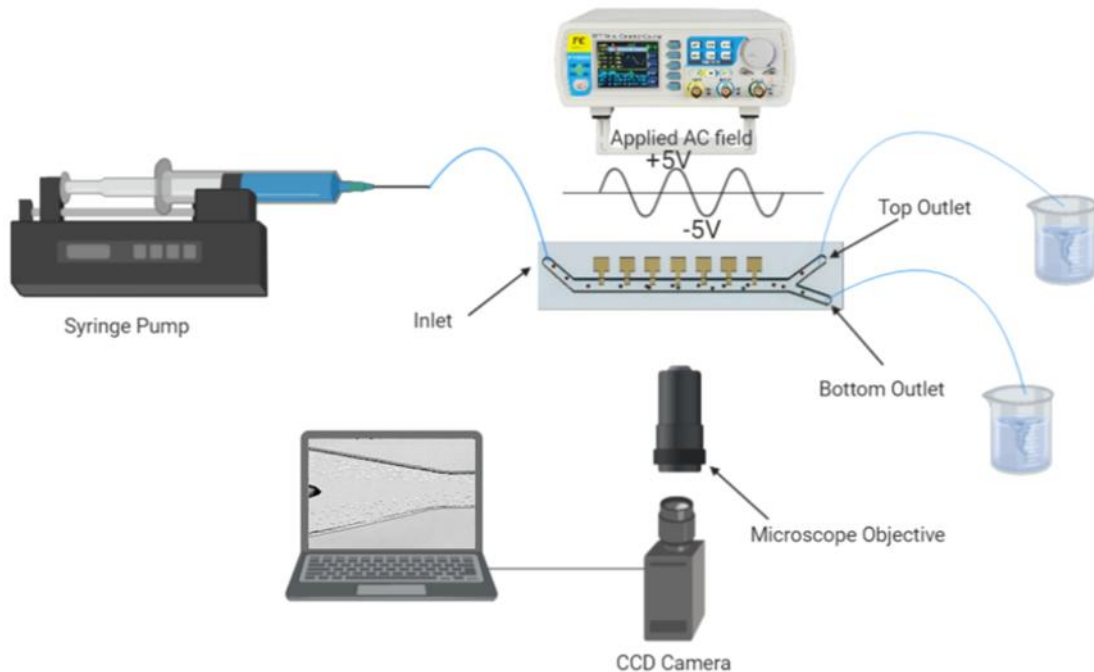
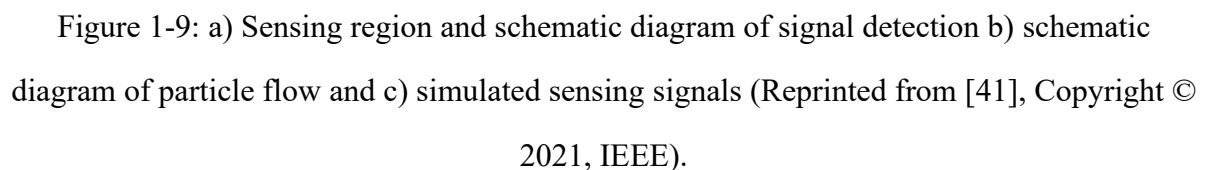


Figure 1-8: Schematic diagram of the experimental system for particle separation in a microfluidic channel (Reprinted from [40]. Licensed under CC-BY 4.0).

Many DEP cytometers focus on separation or sorting of cells rather than characterization beyond crossover frequencies. A past study done at the University of Manitoba focuses on characterization of CHO cells using a combination of DEP and microwave cytometry in [41]. As seen in Figure 1-9, this system uses coplanar electrodes along the bottom of the channel to

measure particle signals and to apply a DEP signal. Sensing electrodes before and after the DEP actuation consist of three coplanar electrodes with asymmetric gap widths to determine the particle's height in the channel by measuring the microwave impedance. A signal at LF-HF is applied to a centre electrode between the sensing regions to apply a DEP force to particles passing by. The change in height is measured before and after the DEP actuation to observe the effect from the applied force. The translational motion experienced by the particle relates to its internal properties. The device is tested using a mixture of viable and non-viable CHO cells across multiple experiments. This system uses a large range of frequencies from 300 kHz to 400 MHz to capture a large spectrum that indicates a variety of cellular properties including both morphological and subcellular information. This system provides a beneficial platform for a non-invasive label-free method to characterize a large frequency spectrum for cells. This system demonstrated differentiation and characterization of viable and non-viable CHO cells [41].



1.3 Objectives and Scope

Optical and dielectric analysis of single cells in flow are traditionally performed as independent modalities, providing distinct but complementary information. Optical analysis methods typically focus on measuring cell morphology and fluorescent intensities, whereas dielectric analysis measures the dielectric properties of cells in response to an electric field. The primary objective of this work is to combine optical and dielectric analysis to obtain the benefits of both individual analysis methods by capturing the morphology and dielectric properties. This thesis presents a dual-modality optical-dielectric cytometer that utilizes a high-speed high-resolution camera to capture real-time images and measures the DEP response of particles passing through a microfluidic channel. This system enables a non-invasive label-free method to analyze microparticles. Experiments were initially performed using 10 and 15.7 μm polystyrene microspheres, then non-spherical polystyrene microplastics were added, followed by experiments with CHO cells to explore differentiation based on viability.

The theoretical background relevant to the proposed device is in Chapter 2. The concept for dark field microscopy is explained along with the morphological changes that appear as cells undergo apoptosis. This chapter also reviews the fundamentals of dielectrophoresis in a microfluidic setting and the electrical models of particles and cells.

Chapter 3 details the hardware implementation for the chosen camera and the custom dark field light source. The fluid delivery fixture for this device was modified from the developed system by Emerich Kovacs III in [42]. This chapter details the system configuration and fabrication process for the microfluidic system. Additionally, this chapter includes the image processing algorithm that was created to process images of cells in motion to determine their morphology and trajectory information.

Chapter 4 demonstrates the simulated results from COMSOL Multiphysics using parameters from previous members of the Biosensors Research Lab with experience analyzing CHO cells. These simulations demonstrate the trajectories for PS microspheres and CHO cells to

1.3 Objectives and Scope

demonstrate the translational motion that occurs from the non-uniform electric field that forms from a DEP signal applied to the coplanar electrodes.

Experiments in Chapter 5 show the results for 10 and 15.7 μm PS microspheres and a comparison with non-spherical PS particles. These results utilize the consistent dielectric properties of microplastics to demonstrate the relationship between the DEP force and the applied voltage using a voltage range from 0 to 6 Vpp. Additionally, the morphological properties are shown for the two sizes of microspheres and the non-spherical particles, allowing for morphological differentiation based on size and shape with each sample. Further analysis is done to compare the detected properties from the sample using two sizes of PS microspheres with morphological data measured using a cell counter and to simulated data showing the dielectric response.

Chapter 6 details the results of experiments performed with mixtures of viable and non-viable CHO cells at a range of frequencies from 100 kHz to 10 MHz to explore the changes in dielectric response in relation to the Clausius-Mossotti factor while tracking the morphological features. A chosen frequency of 6 MHz is used to differentiate viable and non-viable cells based on their dielectric response and is compared to simulated data. The morphological data from the differentiated populations are also compared to data collected with a cell counter.

Finally, Chapter 7 discusses the evaluation of the device and summarizes the findings from the results of the device. Potential future improvements are also outlined to modify the system hardware and software as well as other potential applications.

Chapter 2 – Background

The optical-dielectric cytometer combines imaging and dielectrophoretic analysis. This chapter details the relevant background and theory for this device. Dark field imaging captures particles in flow with high-contrast images to aid in morphological detection. The dielectric properties of particles are detected using DEP, where microplastics are modelled as homogeneous spheres and biological cells are modelled using the multi-shell model.

2.1 Dark Field Illumination

Dark field microscopy (DFM) is commonly used to visualize unlabelled biological cells. It is particularly beneficial for transparent objects due to the high contrast compared to conventional bright field illumination. Dark field illumination is created when an opaque disk, called a light stop, that is wide enough to cover the field of view is placed over the illumination source to block any direct light from entering the objective, resulting in a black background. The edges surrounding the opaque disk are open and create a cone of light focused at the focal plane and then the light bends past the objective in the shape of an inverse cone. This light will illuminate any particles in focus, resulting in some of the light reflecting or refracting into the objective, allowing for high-contrast images [43], [44].

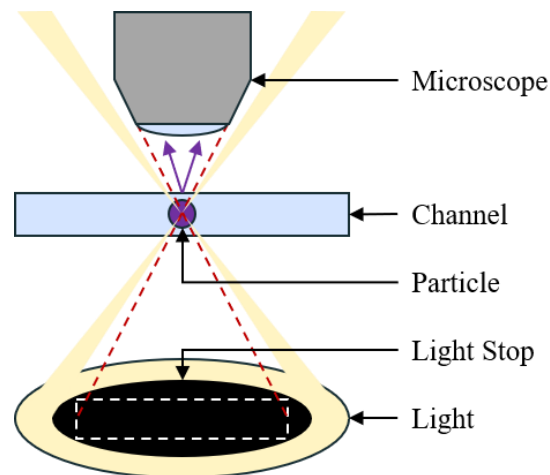


Figure 2-1: Basic diagram for dark field illumination.

When using dark field illumination for biological cells, the edges of the cell and sometimes the internal properties can be seen due to the refraction and reflection of light as it reaches the boundaries [44]. Morphological features can be identified to determine characteristics about a cell that can provide insights into the health and viability. As CHO cells undergo apoptosis, they experience visible morphological changes. The first notable morphological change seen when the cell membrane is illuminated is cell shrinkage. The cells used in this thesis are estimated to shrink from an average diameter of 12.5 to 11 μm [7], [23], [41]. If internal structures are visible, the condensation of chromatin in the nucleus and DNA fragmentation may be seen. Next, blebbing becomes apparent on the plasma membrane, decreasing the circularity of the cell. In the late phase of apoptosis, the cell membrane breaks apart and apoptotic bodies form [23], [45]. The size, eccentricity, and circularity can be used as morphological indicators for biological cells.

2.2 Dielectrophoretic Analysis

Dielectrophoresis is used as the analysis method to measure the dielectric properties of cells. DEP allows for the characterization of polarizable particles when subjected to a non-uniform electric field. The non-uniform electric field induces an electric dipole in the particle from the redistribution of charges. The charges at the ends of the effective dipole are exposed to different field strengths, producing a net DEP force and causing a translational motion [6].

The DEP force comes from the differences in the polarization of the particle and medium. The Clausius-Mossotti factor (K_{CM}) is used to describe this relationship as,

$$K_{CM} = \frac{\tilde{\epsilon}_p - \tilde{\epsilon}_m}{\tilde{\epsilon}_p + 2\tilde{\epsilon}_m} \quad (2.1)$$

Where $\tilde{\epsilon}_p$ and $\tilde{\epsilon}_m$ are the particle and medium complex permittivity. The complex permittivity is described as,

$$\tilde{\epsilon}_p = \epsilon_p - \frac{j\sigma_p}{\omega} \text{ and } \tilde{\epsilon}_m = \epsilon_m - \frac{j\sigma_m}{\omega} \quad (2.2)$$

2.2 Dielectrophoretic Analysis

Where $\varepsilon_p = \varepsilon_{rp}\varepsilon_0$ and $\varepsilon_m = \varepsilon_{rm}\varepsilon_0$ are the particle and medium permittivity, σ_p and σ_m are the conductivities of the particle and medium, ω is the angular frequency, and $j = \sqrt{-1}$ [5], [46]. The Clausius-Mossotti factor is crucial in determining the direction and magnitude of the force with a dependency on the applied AC frequency. The DEP force acting on a particle induced from the non-uniform electric field is described as,

$$\vec{F}_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}\{K_{CM}(f_{DEP})\} \nabla |\vec{E}_{RMS}^{DEP}|^2 \quad (2.3)$$

Where r is the radius of the particle, f_{dep} is the applied DEP frequency, and E is the electric field [5], [6], [36]. Along with the DEP force, when a particle is suspended in a microfluidic channel with laminar flow, it will experience several other forces such as gravity, buoyancy, drag, and lift. The combination of forces acting on a particle can be defined as,

$$\vec{F}_{tot} = \vec{F}_{DEP} + \vec{F}_{g-b} + \vec{F}_{lift} + \vec{F}_{drag} \quad (2.4)$$

The gravity and buoyancy forces acting on the particle are,

$$\vec{F}_{g-b} = -\frac{4}{3}\pi g r^3 (\rho_p - \rho_m) \hat{y} \quad (2.5)$$

Where g is the acceleration due to gravity, r is the particle radius, and ρ_p and ρ_m are the particle and medium densities. The particle will also experience a hydrodynamic lift force which can be described as,

$$\vec{F}_{lift} = -\frac{6C\eta r^3 \langle v \rangle \text{sgn}(y)}{H \left(\frac{H}{2} - |y| - r \right)} \hat{y} \quad (2.6)$$

Where C is the lift coefficient, η is the medium viscosity, $\langle v \rangle$ is the average flow velocity in the channel, H is the channel height, y is the deviation of the particle from the centre of the channel described as $y = \frac{H}{2} - h$, where $(-\frac{H}{2} + r \leq y \leq \frac{H}{2} - r)$, and h is the height of the particle from the bottom of the channel [5], [36], [47]. The drag force is described as,

$$\vec{F}_{drag} = \begin{cases} 6\pi r \eta (\vec{v}_m - \vec{v}_p) \hat{x} \\ 6\pi r \eta (\vec{v}_m - \vec{v}_p) \lambda \hat{y} \end{cases} \quad (2.7)$$

2.2 Dielectrophoretic Analysis

Where $\vec{v}_m$ and $\vec{v}_p$ are the velocity of the medium and particle and λ is the drag correction factor. The drag correction factor is the ratio of the force acting on a sphere between confining walls and the same sphere at the same velocity in an unbounded fluid [5], [48]. The cross-sectional flow profile in a laminar channel is parabolic, meaning the velocity will be fastest in the centre and slowest towards the edges. The flow velocity of the medium is described as,

$$\vec{v}_m = 6\langle v \rangle \frac{h}{H} \left(1 - \frac{h}{H}\right) \hat{x} \quad (2.8)$$

The parameters for the above equations are shown in Table 4-1 for the fluid dynamic simulations in Chapter 4. Using coplanar electrodes at the bottom of a microfluidic channel creates an arch-shaped electric field, where the electric field is stronger near the electrodes and weaker further away. A negative DEP (nDEP) force occurs when a particle is less polarizable than the medium, whereas a particle will experience a positive DEP (pDEP) force when it is more polarizable than the medium. A particle on the lower half experiencing a nDEP force will be pushed away from the electrode array and thus will speed up. For a pDEP force, a particle on the lower half of the channel will be pulled towards the bottom of the channel and thus will slow down.

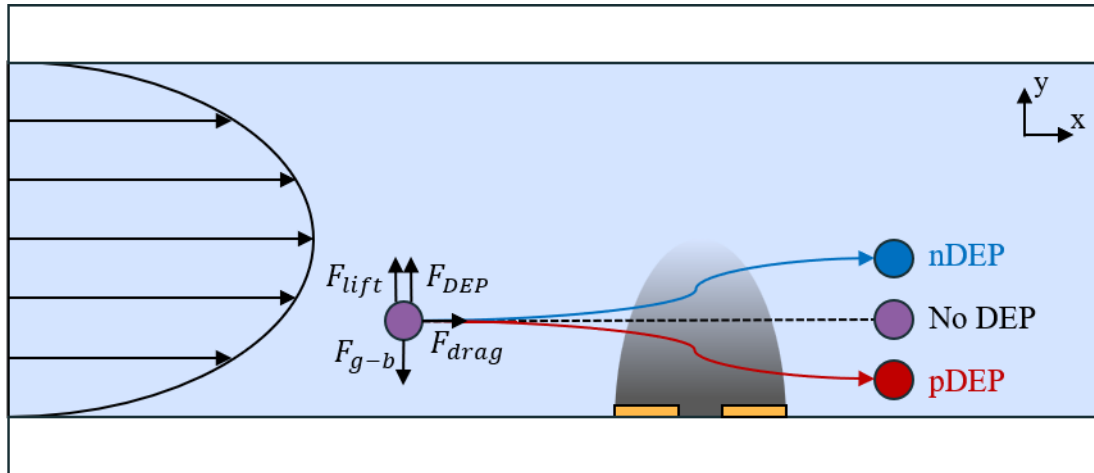


Figure 2-2: Fluid profile in a microfluidic channel showing the forces acting on a polarizable particle passing over two coplanar electrodes creating a non-uniform electric field.

2.3 Dielectric Model of Non-Biological and Biological Cells

2.3.1 Homogeneous Particles

When a particle is placed in a conductive fluid, an electric double layer forms at the interface of the particle and the fluid. For a small particle, the ratio of surface area to volume is larger, which results in a larger effect from the surface conductivity since ions can move more readily along the particle surface. Taking this into consideration, the effective conductivity of the particle (σ_p) is written as,

$$\sigma_p = \sigma_{bulk} + \frac{2K_s}{r} \quad (2.9)$$

Where σ_{bulk} is the bulk conductance, K_s is the surface conductance, and r is the particle's radius [36], [46]. Thus, the complex permittivity becomes,

$$\tilde{\epsilon}_p = \epsilon_p - \left(\frac{j\sigma_{bulk}}{\omega} + \frac{j2K_s}{\omega r} \right) \quad (2.10)$$

The real part of the Clausius-Mossotti factor is plotted for polystyrene microspheres given the parameters in Table 2-1 and shown in Figure 2-3. There is a high contrast in dielectric characteristics of polystyrene particles compared to the background medium, as seen from the real part of the KCM. Particles with a high dielectric contrast to the background medium will have a stronger, more consistent response to the electric field making this useful for characterization. At all operable frequencies, particles will experience a nDEP force, where above 1 MHz the value plateaus.

Table 2-1: Properties of Polystyrene Microspheres [5], [36], [49]

Parameter	Symbol	Medium	Polystyrene
Relative Permittivity	ϵ_r	76.4	2.5
Surface Conductance [S]	K_s	-	2.5e-9
Bulk Conductance [S/m]	σ_b	-	1e-12
Medium Conductivity [S/m]	σ_m	5e-4	-

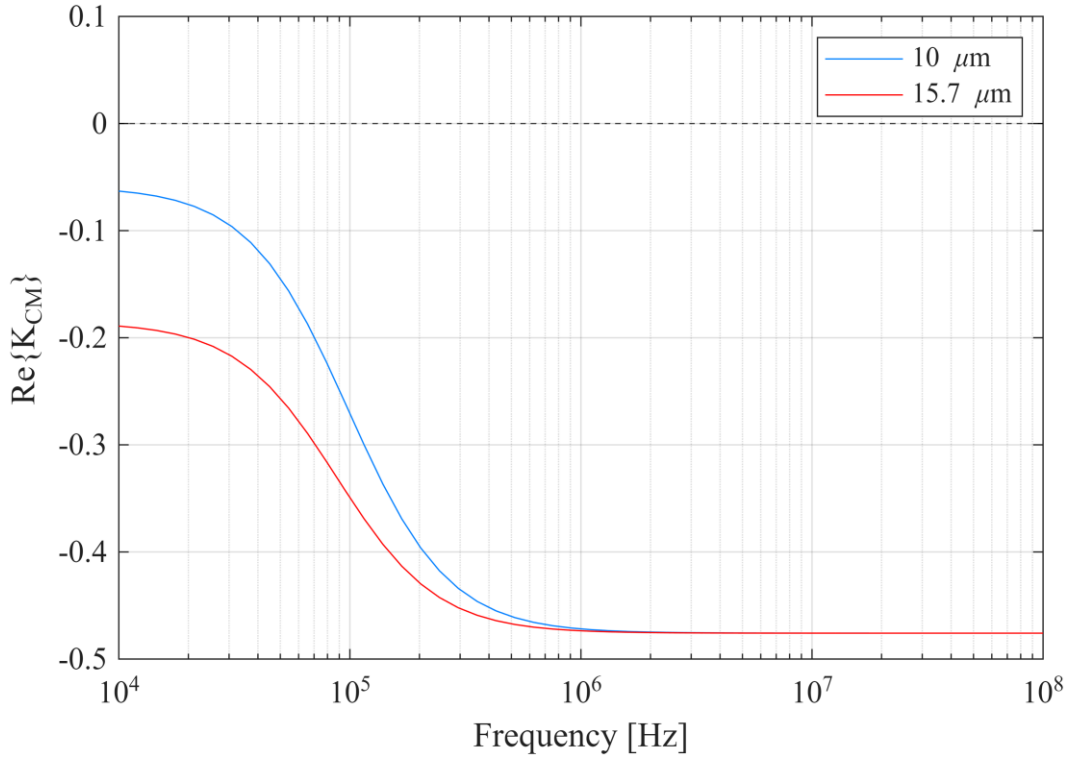


Figure 2-3: Real part of the Clausius-Mossotti factor at a frequency range from 10 kHz to 10 MHz for 10 and 15.7 μm polystyrene microspheres in 0.1% Tween 20.

2.3.2 Biological Cells

The homogeneous model oversimplifies key complexities when modelling biological cells. Shell models are used to approximate the permittivities and conductivities of the key structures in a cell. The single shell model represents the cytoplasm and the cellular membrane. The effective complex permittivity of the shell and the core in a single shell model can be calculated using Equation 2.11.

$$\tilde{\epsilon}_{eff} = \tilde{\epsilon}_{mem} \frac{2(1 - v)\tilde{\epsilon}_{mem} + (1 + 2v)\tilde{\epsilon}_{cyt}}{(2 + v)\tilde{\epsilon}_{mem} + (1 - v)\tilde{\epsilon}_{cyt}}, \text{ where } v = \left(1 - \frac{d_{mem}}{r_c}\right)^3 \quad (2.11)$$

This equation can be used sequentially with multiple shell layers. Using a three-shell model approximates the nucleus, nuclear membrane, cytoplasm, and cell membrane. Working from the core to the surface, an equivalent homogeneous particle can be calculated with the process in Figure 2-4.

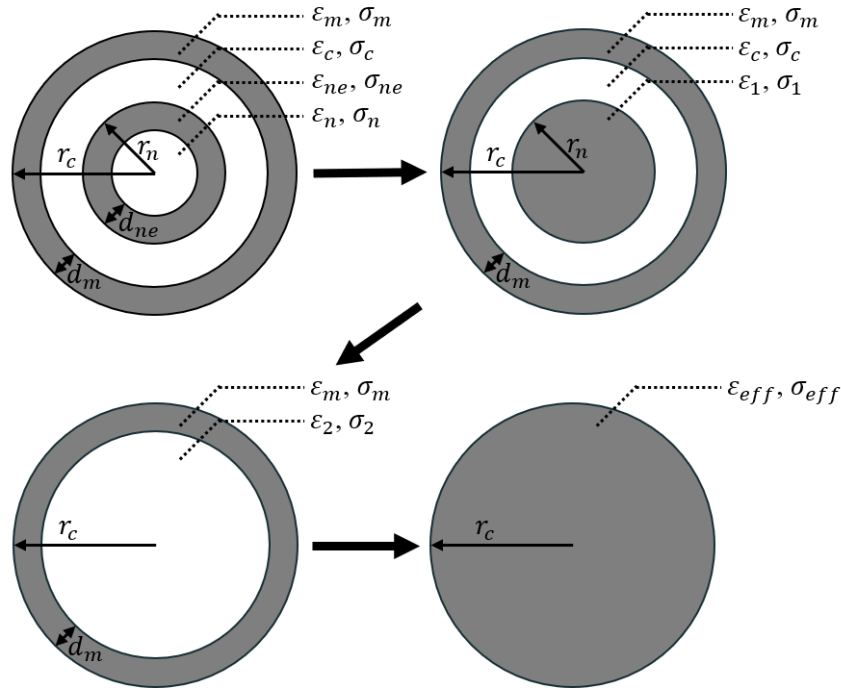


Figure 2-4: Recursive calculation process of the effective complex permittivity of a multi-shell model accounting for the dielectric properties of the nucleus, nuclear membrane, cytoplasm, and cell membrane of a biological cell.

The process to calculate an effective complex permittivity is done using the following equations.

$$\tilde{\epsilon}_1 = \tilde{\epsilon}_{ne} \frac{2(1-v)\tilde{\epsilon}_{ne} + (1+2v)\tilde{\epsilon}_n}{(2+v)\tilde{\epsilon}_{ne} + (1-v)\tilde{\epsilon}_n}, \text{ where } v = \left(1 - \frac{d_{ne}}{r_n}\right)^3 \quad (2.12)$$

$$\tilde{\epsilon}_2 = \tilde{\epsilon}_{mem} \frac{2(1-v)\tilde{\epsilon}_c + (1+2v)\tilde{\epsilon}_1}{(2+v)\tilde{\epsilon}_c + (1-v)\tilde{\epsilon}_1}, \text{ where } v = \left(1 - \frac{r_c - d_m - r_n}{r_c - d_m}\right)^3 \quad (2.13)$$

$$\tilde{\epsilon}_{eff} = \tilde{\epsilon}_m \frac{2(1-v)\tilde{\epsilon}_m + (1+2v)\tilde{\epsilon}_2}{(2+v)\tilde{\epsilon}_m + (1-v)\tilde{\epsilon}_2}, \text{ where } v = \left(1 - \frac{d_{mem}}{r_c}\right)^3 \quad (2.14)$$

From the effective permittivity, the real part of the Clausius-Mossotti factor can be calculated based on the parameters shown in Table 2-2 for viable and non-viable CHO cells, with the resulting data in Figure 2-5. Above the zero crossing at ~720 kHz, the force acting on viable CHO cells switches from nDEP to pDEP. Measurements above the crossover frequency enable

2.3 Dielectric Model of Non-Biological and Biological Cells

particle differentiation using their differential velocity, separating particles based on their positive or negative DEP response.

Table 2-2: Dielectric parameters of viable and non-viable CHO cells [5], [41]

Parameter	Symbol	Viable CHO	Non-Viable CHO
Cellular membrane thickness [nm]	d_{mem}	5	5
Average particle radius [μm]	r_c	6.25	5.5
Nuclear envelope thickness [nm]	d_{ne}	40	40
Nucleus radius [μm]	r_n	3.44	3.03
Membrane permittivity [F/m]	ϵ_{mem}	$8.5\epsilon_0$	$5\epsilon_0$
Membrane conductivity [S/m]	σ_{mem}	1×10^{-6}	1×10^{-6}
Cytoplasm permittivity [F/m]	ϵ_c	$54.5\epsilon_0$	$54.5\epsilon_0$
Cytoplasm conductivity [S/m]	σ_c	0.53	0.07
Nuclear envelope permittivity [F/m]	ϵ_{ne}	$11.5\epsilon_0$	$11.5\epsilon_0$
Nuclear envelope conductivity [S/m]	σ_{ne}	1×10^{-3}	1×10^{-3}
Nucleus permittivity [F/m]	ϵ_n	$120\epsilon_0$	$120\epsilon_0$
Nucleus conductivity [S/m]	σ_n	1.5	0.56

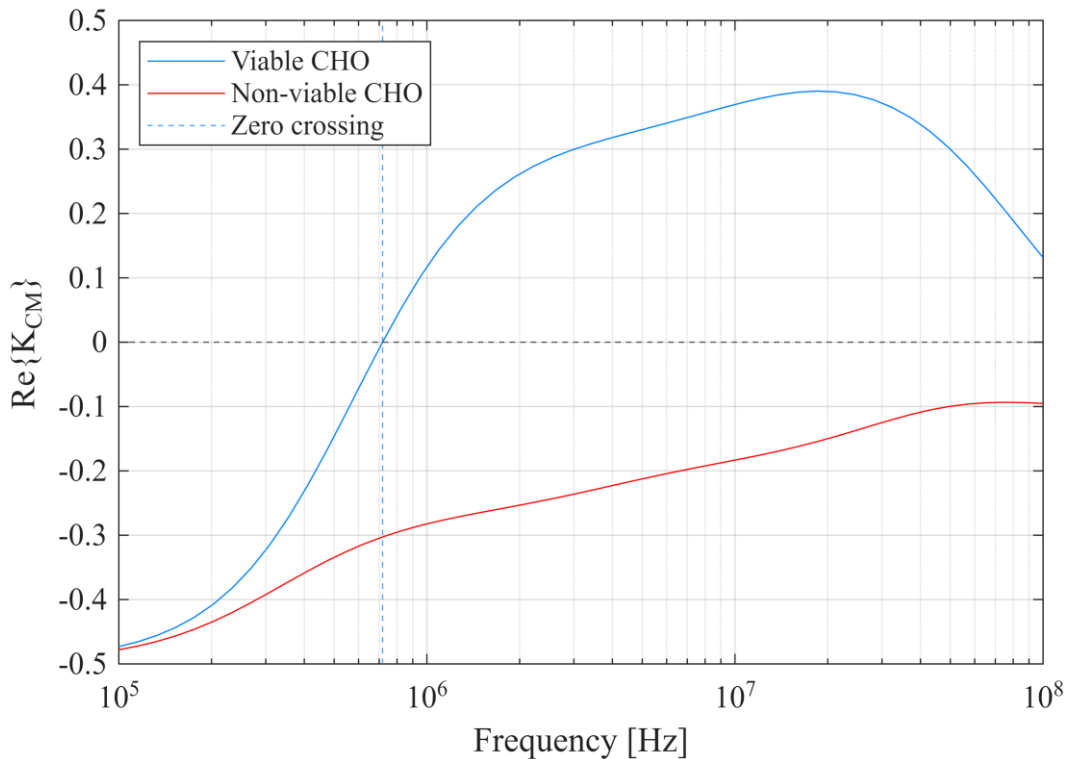


Figure 2-5: Real part of the Clausius-Mossotti factor for viable and non-viable CHO cells in a 0.17 S/m media at a frequency range from 10 kHz to 10 MHz.

Chapter 3 – Materials and Methods

3.1 Hardware Design

The hardware for this system can be split into two sub-systems, the imaging system and the fluid delivery system. The imaging system has a camera attached to a microscope with a light source below enabling dark field illumination. The fluid delivery system consists of sample and flushing syringes that guide the fluid through the fabricated microfluidic chip and into a disposal reservoir.

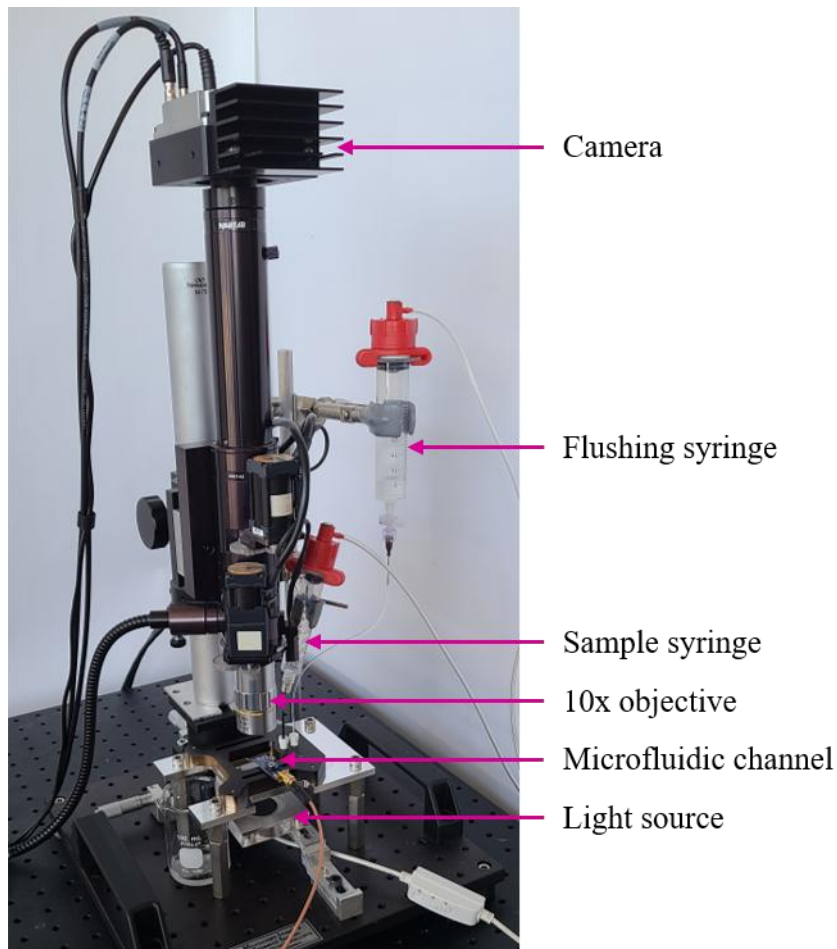


Figure 3-1: Experimental system diagram of the video cytometer.

3.1.1 Dark Field Imaging System

The imaging system is responsible for capturing images for particle morphology and trajectory extraction. As seen in Figure 3-1, the camera sits on top of the microscope tube with a 10x objective (Mitutoyo, Edmund Optics, USA). This system utilizes the monochromatic version of the Basler Boost CXP-12 camera (boA2832-190cm, Basler, Germany) for its high speed and high resolution. This camera can capture a maximum frame rate of 190 fps and was operated at 150.8 fps. The resolution is 2832×2840 px with a pixel size of 2.74×2.74 μm . When the camera is attached to the microscope tube with a 10x lens, the field of view was measured to be 1395×1399 μm using a micrometer scale reticle. This results in a spatial resolution of 0.49 $\mu\text{m}/\text{px}$.

Dark field illumination is employed using a custom light fixture that was redesigned from the Illume 4" light (I-CCMDPN4, Illume, Canada). These LEDs produce 750 lumens and were shortened to fit in a ring with a 55 mm diameter inside the fixture, with the diffuser trimmed to a 2-inch circle placed in the centre of the ring. The fixture was designed with a 41 mm hole to allow light to pass through and hold the diffuser in place with a small O-ring under the lip. A light stop is placed in the centre of the diffuser with a 26 mm diameter. The backplate is attached using 4 screws and features a small hole to allow the electronic attachments from the LED strip to pass through. The entire width of the fixture is 60×60 mm allowing it to fit underneath the microfluidic channel with the light stop centred to the field of view to ensure no uninterrupted light enters the objective.

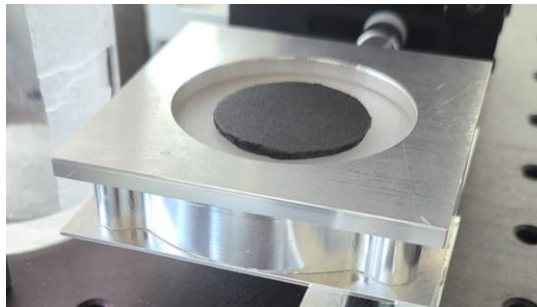


Figure 3-2: Custom light fixture for dark field illumination.

3.1.2 Fluid Delivery System and Microfluidic Channel

The fluid delivery system transports the sample from either the sample or flushing syringe, through a microfluidic channel with integrated DEP electrodes and into a disposal reservoir. This design was largely based on the microfluidic system seen in Emerich Kovacs III's M.Sc. thesis [42]. A 10 ml syringe attached to a 28-gauge needle guides the fluid into the sample port on the microfluidic channel. This sample syringe is used to deliver the sample with particles or cells into the microfluidic channel by gravity and a syringe pump is attached for preparing and cleaning the channel. A 30 ml syringe attached to an 18-gauge needle guides the fluid through the flushing port 10 mm behind the sample port. This flushing syringe is used with a syringe pump to prepare the channel before experiments and to clean the channel after experiments are completed. Once the fluid has gone through the channel, it is guided from the outlet into a beaker using capillary action with a Kim wipe.

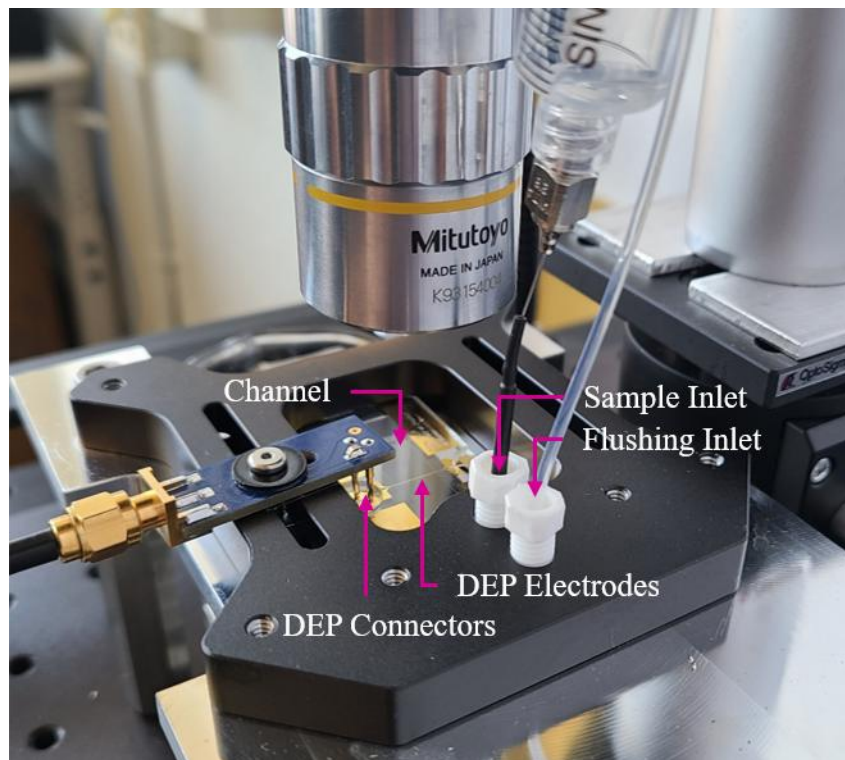


Figure 3-3: Attachments to the microfluidic channel with integrated DEP electrodes.

3.1 Hardware Design

The microfluidic channel is designed using an adhesive polymer sandwiched between two glass slides as seen in Figure 3-4. The top glass slide features two inlets as the sample and flushing ports that were drilled using 0.8 mm diameter carbide burs (Delta Kits, USA). Two side notches were ground using 3/8-inch 120 grit sanding drums, enabling access to the electrodes to apply a signal. The middle layer is a double-sided adhesive polymer (3M, DigiKey, USA) that is cut using a Cricut Maker® 4 to create a channel that is 8 mm wide and 50 μm high as well as the two side notches matching the top glass slide. The bottom glass slide is fabricated using gold titanium electrodes patterned through a lithography process to create two coplanar electrodes that are 35 μm wide with a 25 μm gap between. The lithography process uses a negative photoresist (AZ1518, Merck Performance Materials, Germany) that is exposed using a maskless aligner (μMLA Maskless Aligner, Heidelberg Instruments, Germany) and developed with AZ400K (AZ400K, Merck Performance Materials, Germany). The 100 nm layer of gold is etched using Potassium Iodide (KI) made from a 4:1:40 ratio of KI (J.T. Baker™, Thermo Fisher Scientific, USA), Iodine (EMD Chemicals, USA), and deionized water. This is followed by a buffered oxide etchant (BOE 10:1, Sigma-Aldrich, USA) to etch the 40 nm layer of titanium. The electrode attachment points were redesigned from previous iterations in the Biosensors Research Lab. These were optimized to be able to easily connect to the custom pogo pin adapters designed by Emerich Kovacs III to apply the DEP signal to the electrodes seen in Figure 3-3.

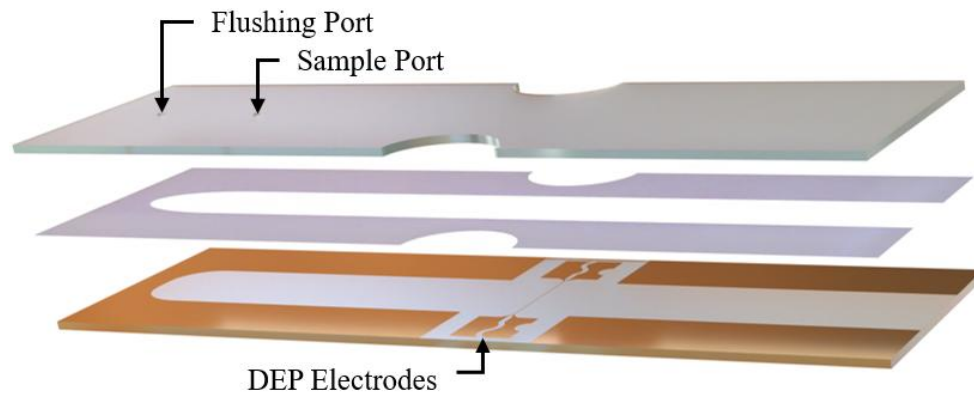


Figure 3-4: Rendered model of the three layers making up the microfluidic channel.



Figure 3-5: Fabricated microfluidic channel with integrated DEP electrodes.

3.2 Particle Analysis Software

The software system captures images of particles in flow during experiments, then utilizes a post-processing algorithm to detect and identify particles. Images are filtered to highlight moving particles where features are detected and appearances on subsequent frames are linked together to find trajectory and morphology data.

3.2.1 Data Capture

Images are captured at a frame rate of 150.8 fps in 30 second intervals. At the start of each experiment, a shorter 5-second video is captured to check the velocity range. The gain and exposure time are optimized for the particle type in the channel. Images with PS samples use an exposure time of 900 μ s and 0.01 dB gain. CHO cells have a refractive index very close to the media they are in, making them very difficult to see. For this reason, the exposure time and

gain are higher at 5000 μs and 25 dB, respectively. A sample image for each of these cases can be seen in Figure 3-6, with a cropped image highlighting the visualization of a particle.

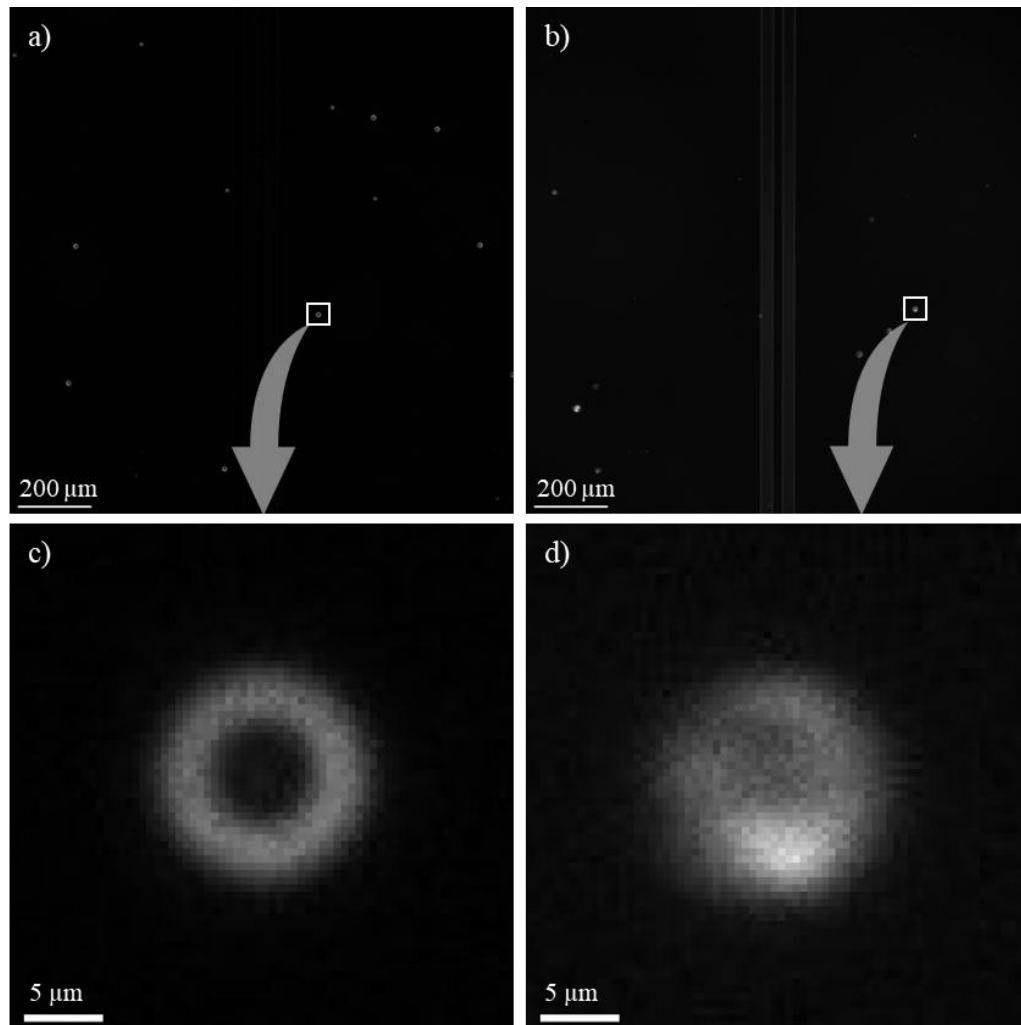


Figure 3-6: Sample frame with a) PS microspheres with an exposure time of 900 μs and gain of 0.01 dB b) CHO cells with an exposure time of 5000 μs and gain of 25 dB c) cropped frame highlighting a 15.7 μm PS microsphere d) cropped frame highlighting a CHO cell.

3.2.2 Image Processing and Feature Detection

The collected images are loaded sequentially into an image processing algorithm, written in python, that filters each frame and detects features to identify particle trajectories and morphology. The algorithm initially converts any coloured images into greyscale images then applies an adaptive background subtraction to find objects in motion on each frame. The first 20

frames are used to create a background model before beginning to track particles. The background in the frames is dark with minimal noise variation, allowing particles to have a high contrast due to the dark field illumination. The number of frames used to initialize the background primarily allows for particles in motion that begin in the field of view to move past their original location, so that the remaining area becomes a part of the background model. Different background subtraction methods are used for each of the different particle types to account for visual differences from the contrast, noise, and light sensitivity from the particle types used. Since PS microparticles have a higher contrast than cells due to a higher difference in refractive index compared to the medium, openCV's mixture of Gaussian (MOG) background subtraction method is used. This is a well known algorithm that models each pixel as a Gaussian distribution and determines whether a pixel is considered foreground or background based on whether it matches the distributions from the collective previous frames [50]. OpenCV's k-nearest neighbours (KNN) background subtraction method is used for CHO cells, since it is generally more sensitive to changes in light intensity and shadows. This method works similarly to MOG but instead of using Gaussian distributions, it works by comparing the red, green, and blue (RGB) or greyscale values of the pixels within a set number (k) of previous frames to determine whether it is within the threshold to be considered foreground or background [51]. Next, a small kernel is used to close any small holes that resulted from noise during the background subtraction. Each frame is then binarized for objects to be detected.

The feature detection uses a combination of sci-kit-image's regionprops and openCV's contours modules. The features for each frame are first found using regionprops. Then for each item found, a series of parameters are found including centroid, area, frame number, major and minor axes, eccentricity, and other parameters that are not utilized. Any particles with areas less than 50 pixels are ignored as this would equate to a circular particle less than 2 μm in diameter, which may also result from noise. When calculating the perimeter, regionprops uses an estimation rather than counting the number of pixels. For this reason, openCV's contours is

used on each particle to find the perimeter based on the outer pixels. A list of other available parameters can be found at [52], [53].

Once features are detected for each frame, trackpy is used to link all the particles together to identify their trajectories. Particles with small trajectories that are less than 10 frames are ignored. An identification number is assigned to each particle enabling sorting and analysis for individual particles.

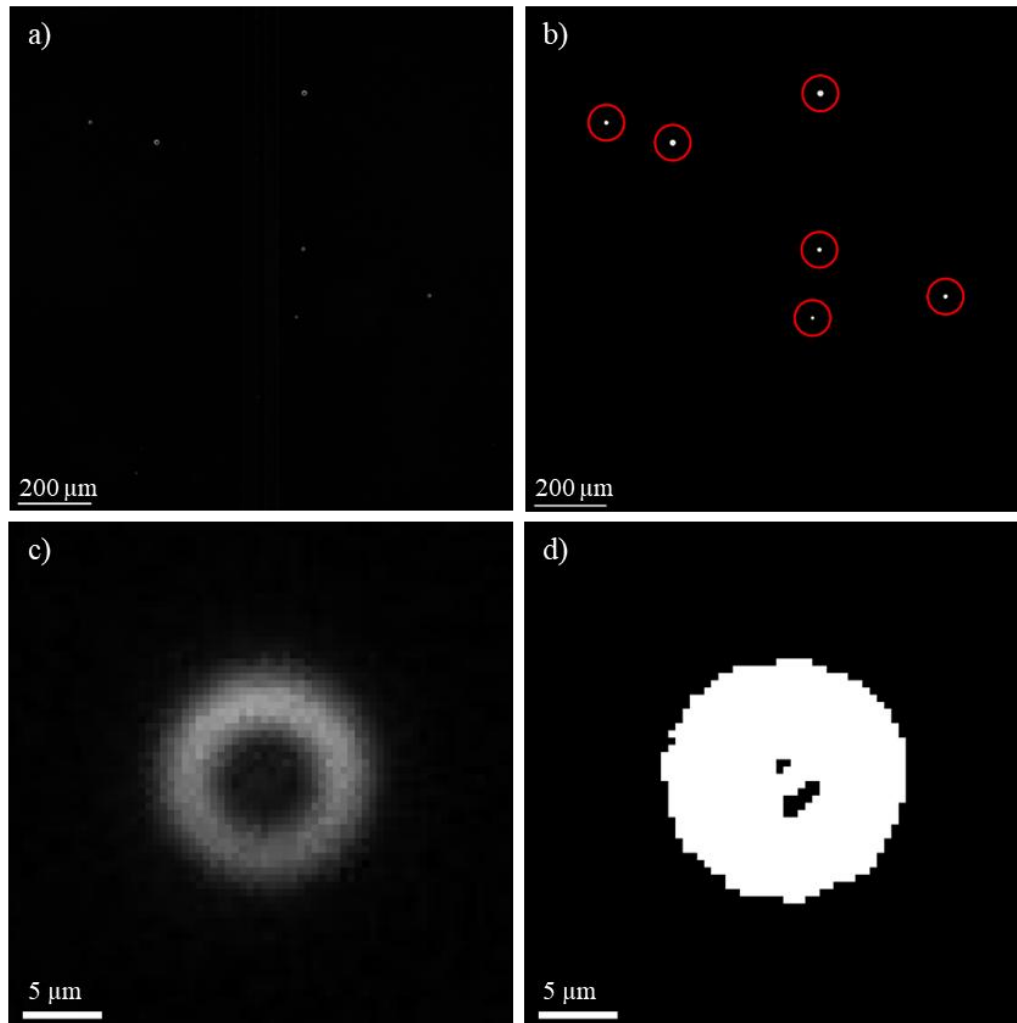


Figure 3-7: Image processing showing a) the original frame b) the filtered frame with detected particles circled in red c) a 15.7 μm PS microsphere on the original frame d) the filtered image of the same 15.7 μm PS microsphere.

3.2.3 Particle Data Extraction

For each tracked particle, morphological data is extracted from each of the frames it is associated with. The median is used to represent the morphological features to reduce tracking errors and fluctuations between frames. The size is characterized using the diameter of each particle and is calculated as an average between the median of the major and minor axes across the frames a particle is tracked.

$$diameter = \frac{major + minor}{2} \quad (3.1)$$

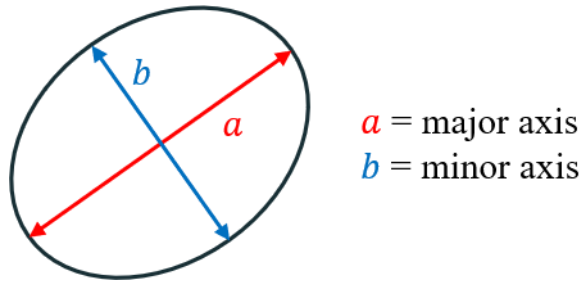


Figure 3-8: Major and minor axes of an ellipse.

Similarly, the median is used to calculate other morphological data including eccentricity, area, perimeter, and more. The median of the area and perimeter are used to calculate the circularity, which quantifies how closely a particle resembles a perfect circle.

$$circularity = \frac{4\pi * area}{perimeter^2} \quad (3.2)$$

A circularity of 1 corresponds with a perfect circle, while lower values indicate irregular, elongated, or angular shapes. Another parameter that can be used to measure deviations from a perfect circle is eccentricity which is calculated as,

$$eccentricity = \sqrt{1 - \frac{minor^2}{major^2}} \quad (3.3)$$

An eccentricity of 0 corresponds with a perfect circle, while higher values indicate elongated shapes, where a value of 1 is considered a line. While other morphological data can be captured, the above are the morphological features used in this thesis for particle analysis.

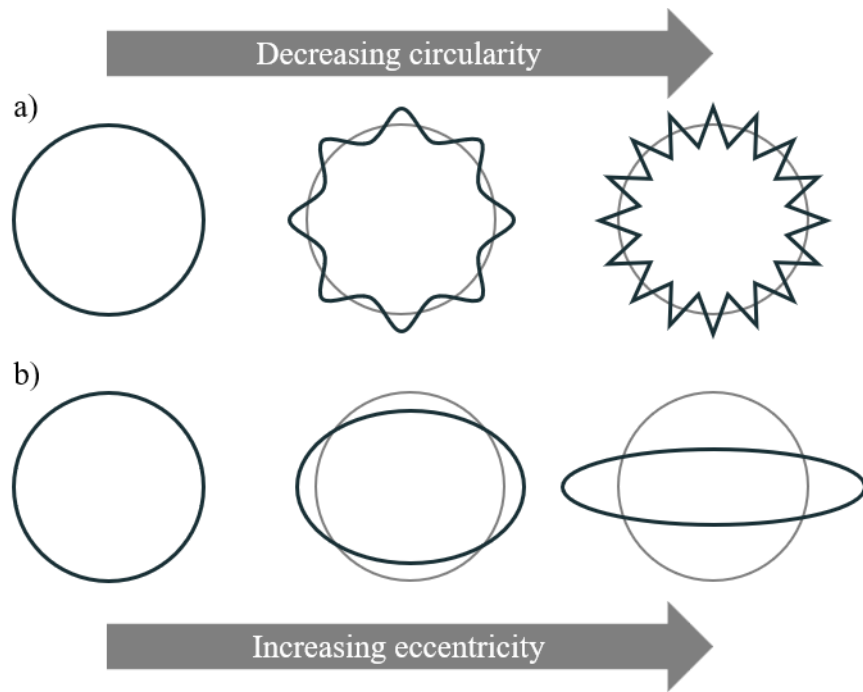


Figure 3-9: Shape evolutions showing deviations from a perfect circle with a) decreasing circularity and b) increasing eccentricity.

In addition to morphological data, the velocity is calculated to characterize particle motion in the channel which serves as an indicator of the influence from the DEP force. The velocity is tracked based on the change in position using centroids between two consecutive frames. Regions are defined to separate the input and output velocity before and after the electrodes. Any particles that do not have a velocity successfully tracked before and after the electrodes are discarded. The velocity is calculated as,

$$velocity \left[\frac{\mu m}{s} \right] = \sqrt{(\Delta x)^2 + (\Delta y)^2} * spatial\ resolution \left[\frac{\mu m}{px} \right] * \frac{framerate [fps]}{\Delta frame} \quad (3.4)$$

Where Δx and Δy are the difference between consecutive tracked centroids in the x and y directions and $\Delta frame$ is the difference between frame numbers to account for any skipped frames. The electrodes block light from illuminating particles as they pass over top, resulting in frames skipped in the trajectory data. The trajectory for each particle is divided into regions

before and after the electrodes, where the mean value of the calculated velocity corresponds to the input and output velocity. The differential velocity is calculated as,

$$dv = v_{in} - v_{out} \quad (3.5)$$

The differential velocity relates to the KCM and will indicate nDEP or pDEP. The morphological features and velocity data are compiled into a dataset to be further analyzed and compared to theoretical results or data from other modalities. The features can be visualized and plotted to investigate statistical DEP and morphological data to help identify and differentiate particles.

Chapter 4 – Numerical Modelling of Particles

in Flow

4.1 Channel Geometry

Fluid dynamic simulations are created to model the channel using COMSOL Multiphysics 6.3. These simulations are performed to investigate the behaviour of particles flowing through the electric field when an AC signal is applied to the coplanar DEP electrodes. The trajectories of particles are numerically solved to find the change in velocity that occurs from the influence of the DEP signal.

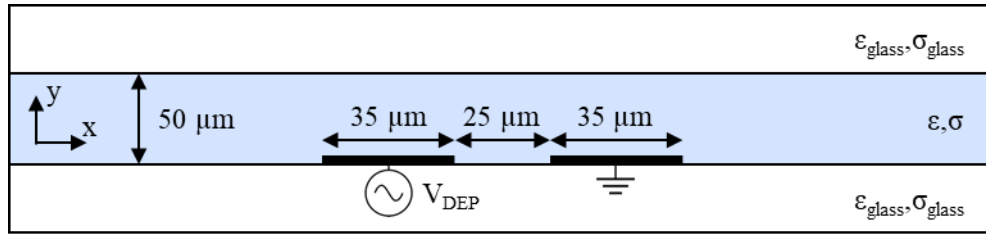


Figure 4-1: Channel geometry used in numerical modelling with COMSOL Multiphysics.

The channel is modelled using a 2D cross section through the length of the channel with two 35 μm wide electrodes running parallel across the width of the channel with a 25 μm gap between. The DEP signal is applied to the left electrode while the right electrode is grounded, as particles pass from left to right along the x-axis. Glass is modelled above and below the 50 μm high microfluidic channel. This geometry is seen in Figure 4-1 and uses the parameters in Table 4-1. The length is arbitrarily set to 1 mm and mimics the field of view in experiments while simplifying the computational analysis. The flow is laminar in the channel and thus has a parabolic flow profile relative to the height in the channel which can be seen in Figure 4-2.

4.1 Channel Geometry

Table 4-1: Parameters for modelling PS microspheres and CHO cells in COMSOL

Multiphysics simulations			
Parameter	Symbol	Polystyrene	CHO
Channel Height [μm]	h	50	50
Channel Length [μm]	l	1000	1000
Particle Radius [μm]	r	5-7.85	5-6.25
Particle Density [g/cm^3]	ρ_p	1.050	1.050
Medium Dynamic Viscosity	η	10^{-3}	10^{-3}
Medium Density [g/cm^3]	ρ_m	1.000	1.026
Medium Permittivity[F/m]	ϵ_m	$76.4\epsilon_0$	$78\epsilon_0$
Medium Conductivity [S/m]	σ_m	5×10^{-4}	0.17
Average Flow Velocity [$\mu\text{m}/\text{s}$]	$\langle v \rangle$	1300	1050
Lift Coefficient	C	0.031	0.031
Frequency [MHz]	f_{dep}	1	6
Voltage [V_{pp}]	V_{pp}	6	6

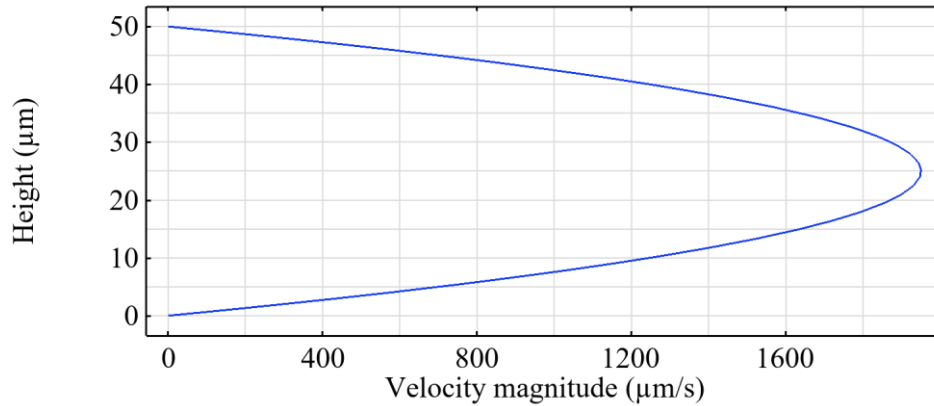


Figure 4-2: Cross-sectional flow velocity relative to height in the microfluidic channel.

An electric potential is applied to the left electrode using the electric currents module while the right electrode is grounded. This creates the non-uniform electric field that enables DEP analysis. The electric field intensity demonstrates the strength of the electric field at different heights in the channel. This is shown in Figure 4-3 and highlights that the electric field strength is stronger at lower heights and weaker at heights further away from the electrodes.

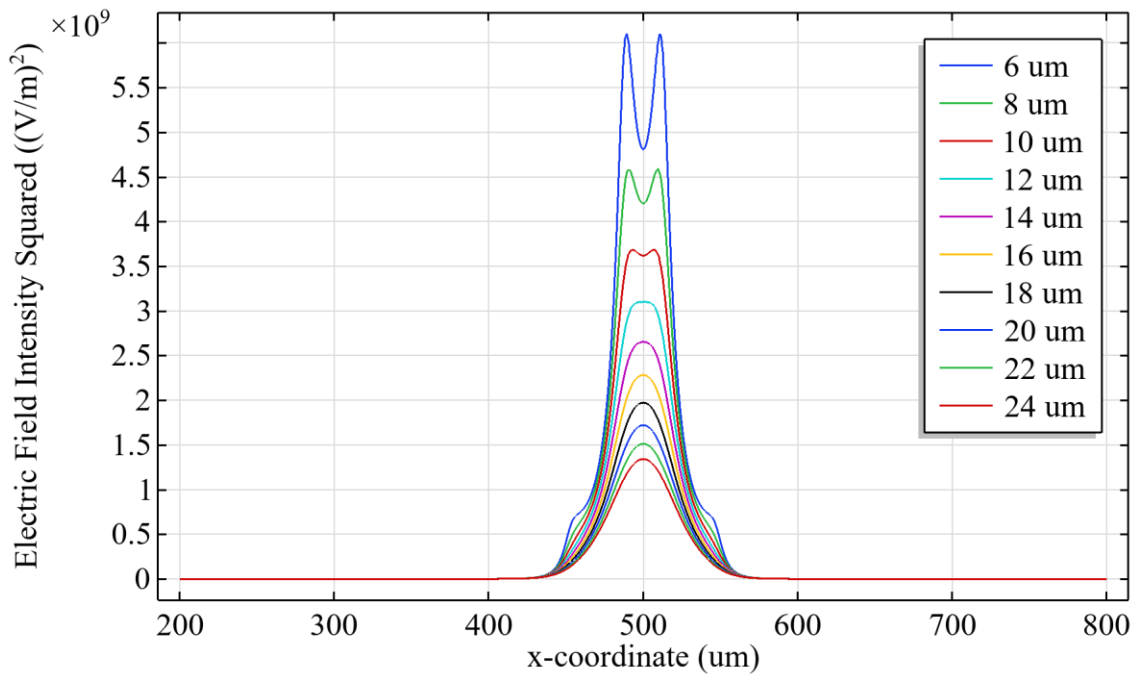


Figure 4-3: Electric field intensity squared across the length of the channel at various heights.

Particles are modelled using the particle trajectories module for particles on the bottom half of the channel. Gravity and buoyancy forces are applied as well as custom forces for the drag and lift forces based on Equation 2.6 and Equation 2.7.

4.2 Polystyrene Microspheres

Particle trajectories for PS microspheres are modelled after the fluid profile and electric field have been established for a 1 MHz 6 Vpp signal to effectively analyze particle responses to the electric field. At this frequency, these particles will experience a nDEP force as seen in Figure 2-3. For a 10 μm PS microsphere, the real part of the KCM is calculated to be $\text{Re}\{\text{KCM}\} = -0.4744$ and for 15.7 μm $\text{Re}\{\text{KCM}\} = -0.4751$. As the particles flow over the electrodes, they will be pushed away from the area of high electric field intensity towards the centre of the channel and will consequently speed up. An example trajectory can be seen in Figure 4-4 demonstrating this phenomenon where the x-axis shows the particle position along the length of the channel and the y-axis shows the position of the particle along the height of the channel.

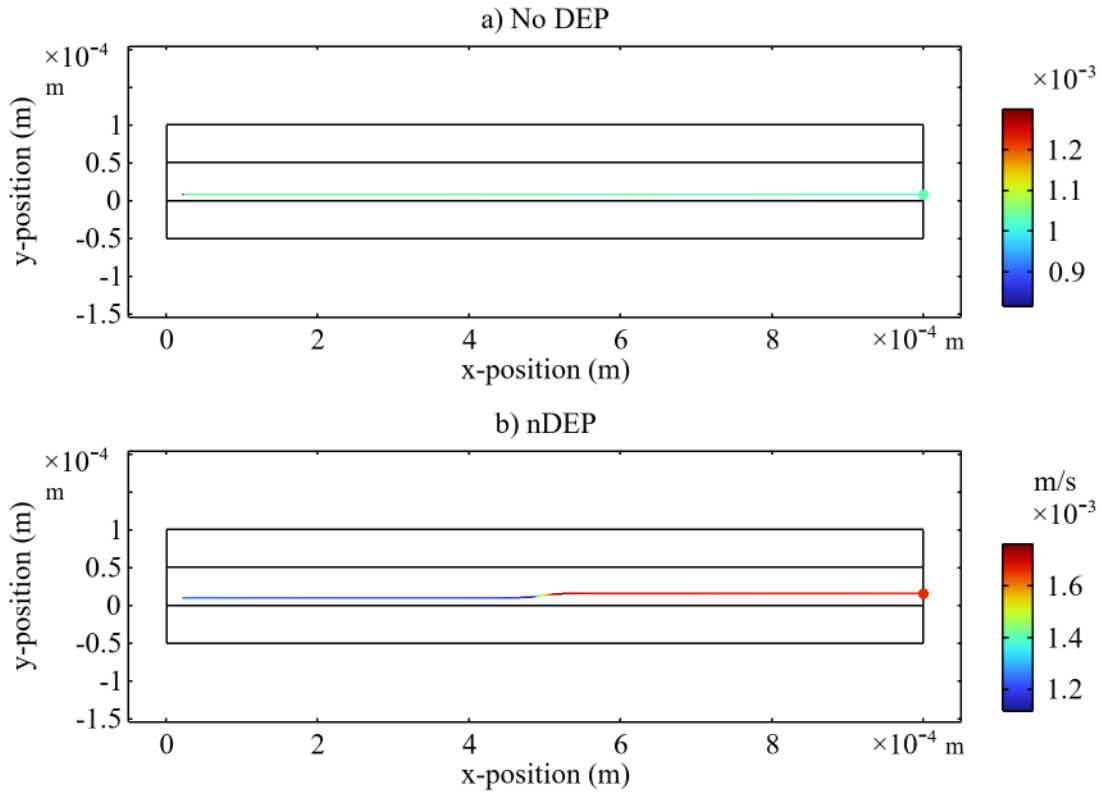


Figure 4-4: Theoretical particle trajectories for a 10 μm PS microsphere experiencing a) no DEP and b) nDEP starting at a height of 10 μm from the bottom of the channel with an applied DEP signal that is 6 Vpp and 1 MHz.

The example trajectory for a 10 μm particle experiencing nDEP changed from a height of 10 μm to 15.5 μm and sped up from 1233 $\mu\text{m/s}$ to 1675 $\mu\text{m/s}$. The trajectory data is captured at a range of heights from 6 μm to 24 μm for 10 μm particles and from 8 μm to 24 μm for 15.7 μm particles. Particles released at this range of heights ensures they are on the bottom half of the channel and start from heights slightly larger than the radius of the particles. Each trajectory is captured to gather the velocity before and after the electrodes. The expected differential velocity is then calculated for both 10 and 15.7 μm particles at each released height in the channel and compared to the input velocity to observe the effect from the DEP actuation. At

4.3 CHO Cells

lower starting heights, particles experience a stronger force and have a larger change in velocity, whereas particles further from the electrodes will exhibit a smaller translational motion.

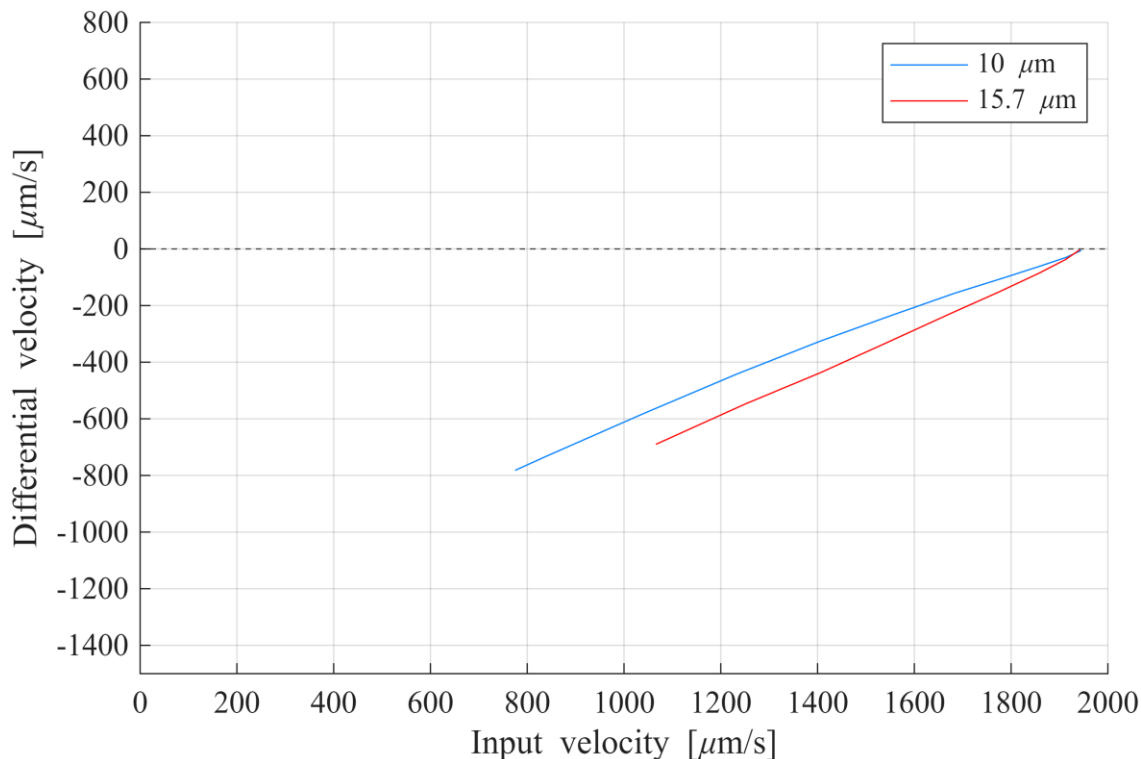


Figure 4-5: Theoretical differential velocities compared to input velocities for 10 and 15.7 μm PS microspheres at a range of starting heights from 6 to 24 μm and 8 to 24 μm respectively with a 1 MHz 6 Vpp DEP signal.

4.3 CHO Cells

Particle trajectories were calculated for viable and non-viable CHO cells with an applied signal at 6 MHz and 6 Vpp. The real part of the KCM at this frequency and voltage is shown in Figure 2-5 with a medium conductivity of 0.17 S/m. Viable CHO cells experience pDEP with $\text{Re}\{\text{KCM}\} = 0.3404$, while non-viable CHO cells experience nDEP with a calculated $\text{Re}\{\text{KCM}\} = -0.2043$. Analysis at this frequency allows for CHO cells to be differentiated based on their positive and negative differential velocities. An example trajectory of each of the particles showing positive, negative, and no DEP is shown in Figure 4-6.

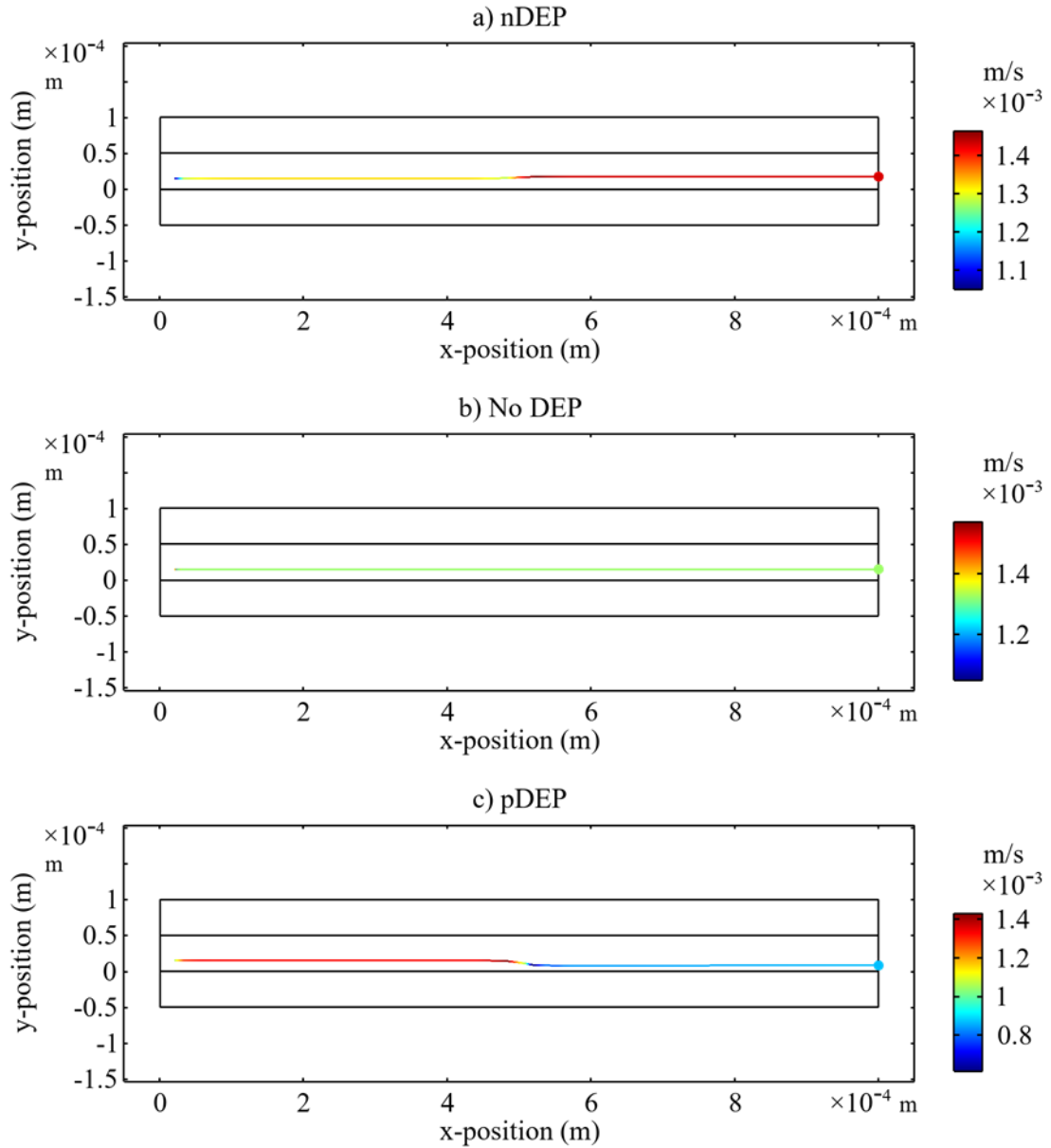


Figure 4-6: Theoretical particle trajectories starting at a height of $15 \mu\text{m}$ for a) a non-viable CHO cell experiencing nDEP b) a viable CHO cell experiencing no DEP and c) a viable CHO cell experiencing pDEP with an applied DEP signal that is 6 Vpp and 6 MHz.

The example trajectories demonstrate the ability to differentiate particles based on their response to the DEP signal. The positive and negative DEP forces translate to a positive and negative change in velocity. Trajectories are simulated for viable and non-viable cells with

4.3 CHO Cells

starting heights from 7 to 24 μm from the bottom of the channel. The differential velocity is calculated at each of these heights and is plotted in Figure 4-7. Similar to PS microspheres, non-viable CHO cells experience a nDEP force. At lower starting heights and velocities, particles experience a larger change in velocity. At starting heights near the center of the channel, the DEP force is weaker and thus there is a smaller magnitude in the differential velocity. Conversely, viable CHO cells experience a pDEP force. At low starting heights and low velocity, particles are pulled to the minimum height in the channel, which appears as a minimal change in velocity. As these particles begin at higher starting velocities, there is a larger distance to travel before reaching the minimum height in the channel, resulting in larger differential velocities. The maximum differential velocity for viable CHO cells in this configuration is reached at a starting height around 14 μm from the bottom of the channel and an incoming velocity around 1270 $\mu\text{m/s}$. Above this starting velocity, the parabolic flow profile, shown in Figure 4-2, becomes more curved, meaning a particle experiencing the same change in height in this region compared to below will appear as a smaller change in velocity. As particles are incoming closer to the center of the channel, the electric field becomes weaker and the magnitude of the differential velocity will decrease.

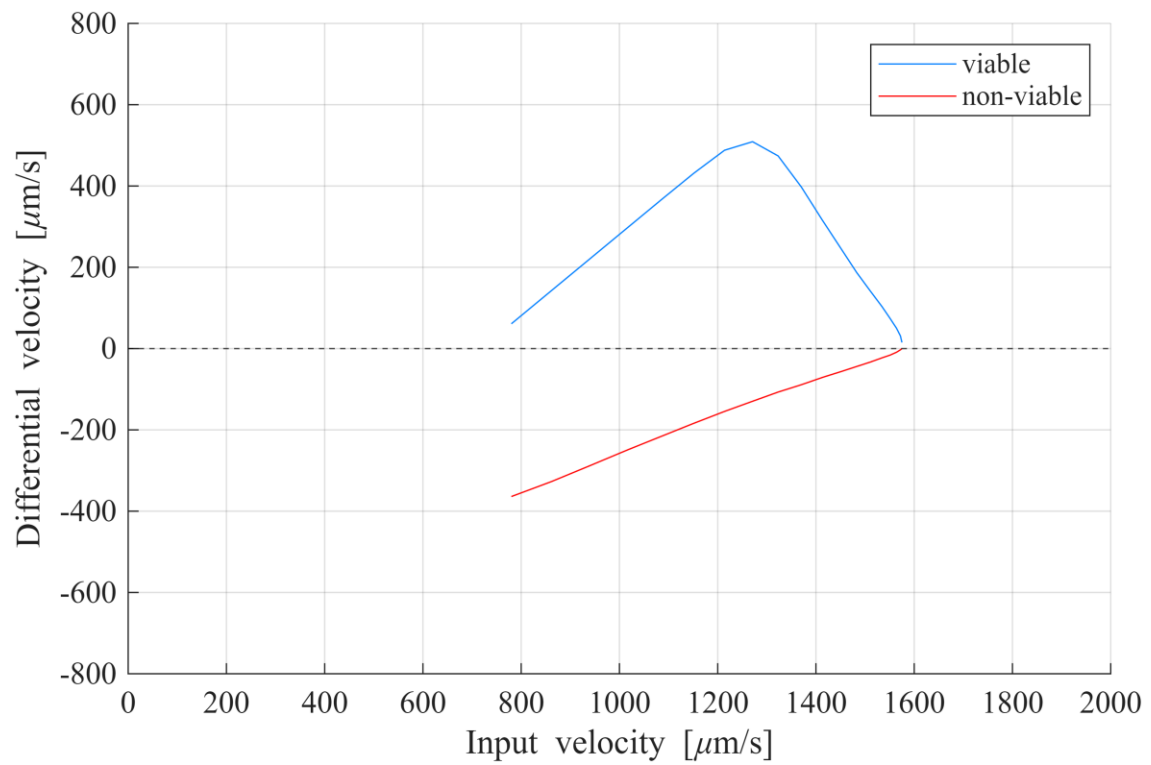


Figure 4-7: Theoretical differential velocities compared to input velocities for viable and non-viable CHO cells with a range of starting heights from 7 to 24 μm with a 6 Vpp and 6 MHz DEP signal.

Chapter 5 – Mixed Polystyrene Experiments

Polystyrene microspheres are initially used as verification and validation of the device. Experiments were performed with polystyrene particles at a range of voltages. Two samples were analyzed to investigate the DEP response and morphological data. The two sizes of known microspheres are also further analyzed and differentiated.

5.1 Sample Preparation

Polystyrene microspheres (Polybead® Microspheres, Polysciences, USA) with 10 and 15.7 μm diameter were chosen due to their consistent size, density, and dielectric properties. These have similar sizes to many biological cells including CHO cells which are further discussed in chapter 6. The coefficient of variance (CV) for both the 10 and 15.7 μm PS microspheres is 9%, giving them a consistent size range. The standard deviation (STD) is calculated using $\text{STD} = \text{CV} * \text{mean}$, which equates to 0.9 and 1.413 μm for the 10 and 15.7 μm microspheres respectively. A cell counter (Countess™ 3 FL, Thermo Fisher Scientific, USA) is used to cross-reference the morphological data for these PS samples.

The background medium is a 0.1% Tween® 20 (Sigma Aldrich, USA) solution with de-ionized (DI) water. This solution helps coat the particles to remain suspended and to avoid clumping or sticking to the channel. The density of the background medium is 1000 kg/m^3 while polystyrene has a density of 1050 kg/m^3 allowing for particles to settle at a slow rate and remain suspended while flowing through the channel. The background medium has a high dielectric contrast compared to PS particles allowing for effective manipulation from the DEP force.

In addition to analyzing the PS microspheres, non-spherical microplastics are fabricated to investigate a range of sizes and shapes. Polystyrene was ground using 220-grit sandpaper and was suspended in the same 0.1% Tween 20 solution. The particles in the suspended solution

settled through a 30 μm filter. The 10 and 15.7 μm PS microspheres were then added to the mixture and measured using the same parameters as the previous sample.

5.2 Voltage Relationship with DEP

The DEP force a particle will experience while passing through the non-uniform electric field is largely dependent on the magnitude of the applied voltage. The DEP force equation $\vec{F}_{DEP} = 2\pi r^3 \epsilon_m \text{Re}\{K_{CM}(f_{DEP})\} \nabla |\vec{E}_{RMS}^{DEP}|^2$ uses the gradient of the magnitude of the electric field squared. This system maintains a constant geometry and medium throughout experiments. Under these conditions, the magnitude of the electric field is expected to increase proportionally with the applied voltage when the frequency is constant as $\nabla |E|^2 \propto V^2$. The resulting change in force will consequently increase the change in velocity a particle will experience when passing through the electric field.

To investigate the influence of the voltage on the strength of the electric field, particle motion is analyzed with an applied voltage of 0, 2, 4, and 6 Vpp at a constant frequency of 1 MHz. Polystyrene microplastics have consistent dielectric properties between sizes at this frequency as seen in Figure 2-3 and will experience a strong nDEP force. As the applied voltage increases, the force becomes larger and causes a corresponding increase in the magnitude of the differential velocity for PS particles in this configuration. The differential velocities compared to the incoming velocities from this experiment are plotted in Figure 5-1 for both samples. Sample 1 was used initially and consists of 10 and 15.7 μm PS microspheres in 0.1% Tween 20 and sample 2 uses the same PS microspheres with the addition non-spherical PS particles. While the size range and shapes are broader for sample 2, the relationship between the applied voltage and the DEP force remains apparent.

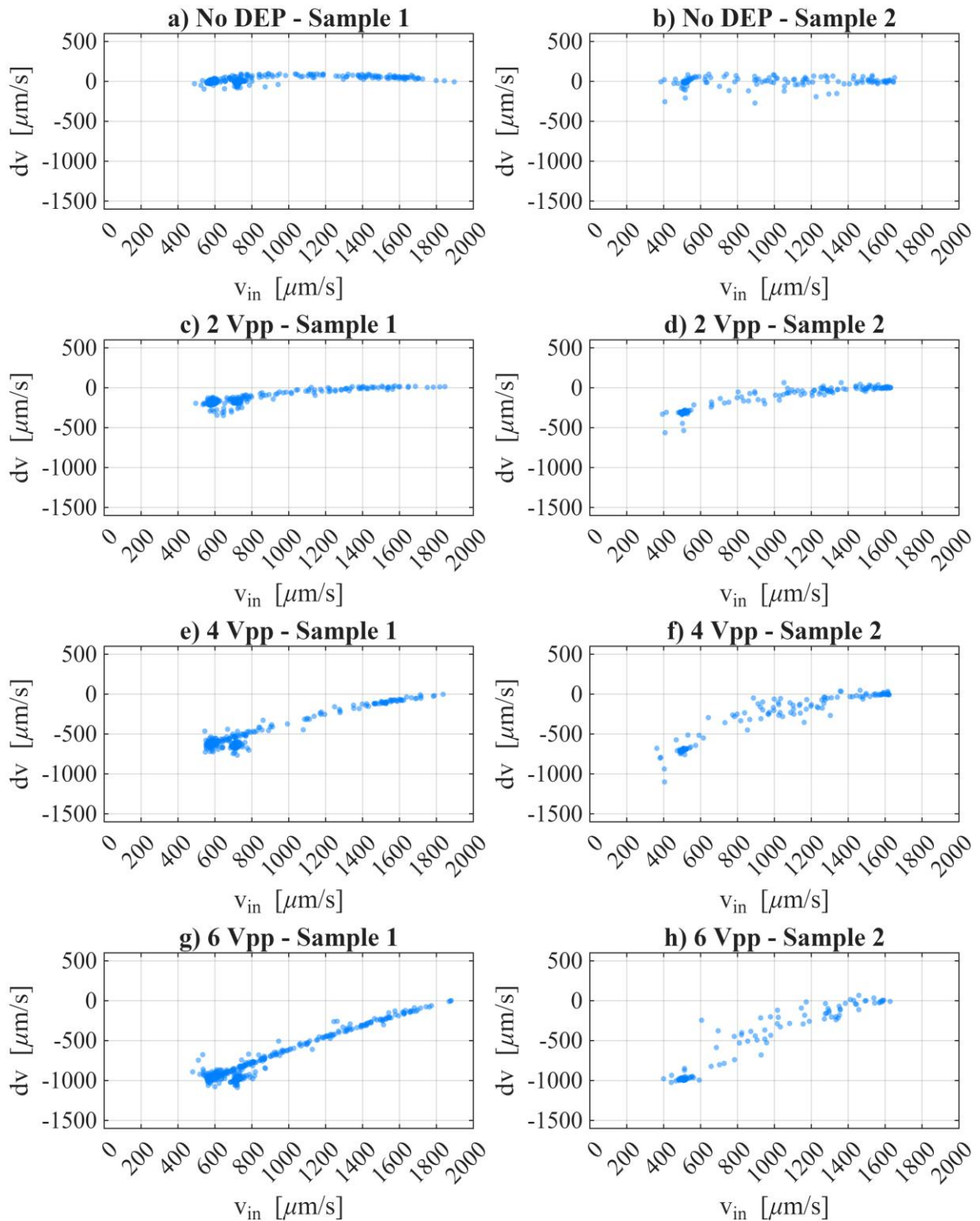


Figure 5-1: The differential velocity compared to the input velocity where sample 1 is a mixture of 10 and 15.7 μm PS microspheres and sample 2 is a mixture of 10 and 15.7 μm PS microspheres and non-spherical PS microplastics in a solution of 0.1% Tween 20.

5.2 Voltage Relationship with DEP

To further understand the relationship between the force and the voltage, the collected values using sample 1 can be correlated with the velocity flow profile. The average values of the differential velocity were taken from the particles incoming between 560 and 600 $\mu\text{m/s}$ from the results at each voltage. These values are then correlated with the corresponding height in the channel to calculate the change in height using an average incoming velocity of 580 $\mu\text{m/s}$ as seen in Figure 5-2. The force acting on each particle is expected to increase with the squared amplitude of the voltage, resulting in an expected proportional increase of 1, 4, and 9 for a signal that is 2, 4, and 6 Vpp. The experimental change in heights were calculated to be approximately 1.44, 5.44, and 9.47 μm for an applied signal of 2, 4, and 6 Vpp and correlated with differential velocities of -182, -619, and -958 $\mu\text{m/s}$. At particle heights further from the electrodes, the electric field becomes weaker, resulting in a smaller change in height and velocity. Since the flow profile is parabolic, there are more noticeable changes in the differential velocities when particles are closer to the edges rather than near the center due to the curvature near the center of the channel. The results using the differential velocities to show the DEP response for PS particles align with the expected relationship between the voltage and electric field.

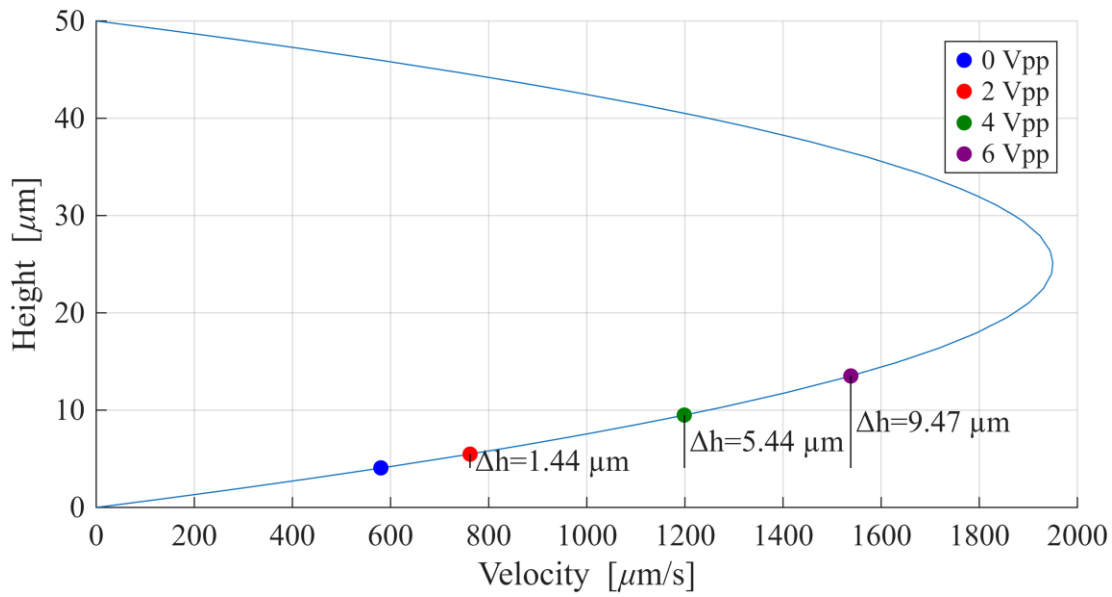


Figure 5-2: Velocity profile in the microfluidic channel and the average change in position and velocity of particles with an incoming velocity of 580 $\mu\text{m/s}$ at 0, 2, 4, and 6 Vpp.

5.3 Morphological Data Extraction

In addition to gathering velocity data that is representative of the DEP force acting on particles, the morphological features are extracted from the detected particles in both samples. The size and shape are important features that can be quantified using the major and minor axes, diameter, area, perimeter, circularity, and eccentricity. Images of detected polystyrene microspheres and non-spherical particles can be seen in Figure 5-3.

Analyzing each of the example particles' average data in Table 5-1 demonstrates some of the capabilities of the morphological data extraction. This data uses the median value across all appearances of each particle. The size is indicated using an average between the major and minor axes. The PS microspheres have higher circularity values indicating how round the object is and how closely it resembles a perfect circle, whereas the non-spherical particles with edges appearing more jagged have lower circularity values. Using eccentricity as a secondary measure of how closely particles resemble a circle indicated how elongated the particles are. For microspheres, these values are much lower than those of the non-spherical microplastics.

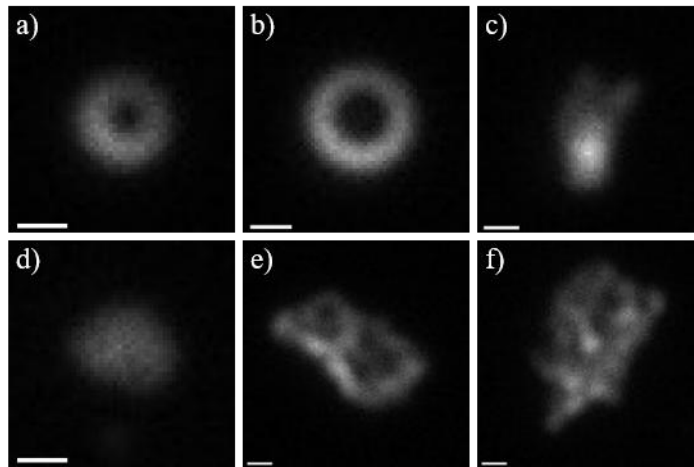


Figure 5-3: Detected PS microplastics with a scale bar of 5 μm of a) a 10 μm microsphere
b) a 15.7 μm microsphere c)-f) non-spherical particles.

Table 5-1: Calculated data corresponding with the detected particles from Figure 5-3

Label	Major axis [px]	Minor axis [px]	Diameter [μm]	Area [px]	Perimeter [px]	Circularity	Eccentricity
a	21.75	20.24	10.34	331	68.18	0.8947	0.3838
b	32.53	31.46	15.76	714	100.57	0.8871	0.2580
c	31.85	15.58	11.68	339.5	87.86	0.5527	0.8694
d	21.86	14.92	9.06	241	64.89	0.7192	0.7273
e	67.512	34.34	25.09	1734	197.97	0.5560	0.8655
f	68.19	50.41	29.21	2338	271.07	0.3998	0.6832

The collected data for the size, circularity, and eccentricity are plotted in Figure 5-4 and compared for sample 1 containing PS microspheres and sample 2 containing both PS microspheres and non-spherical particles. While the previous section demonstrating the DEP response at a range of voltages remained closely aligned regardless of shape, this data demonstrates a wider variety in the morphological data that is quantified using circularity and eccentricity. While these values demonstrate quantifiable differences between spherical and non-spherical particles, there are some noticeable deviations due to pixelation.

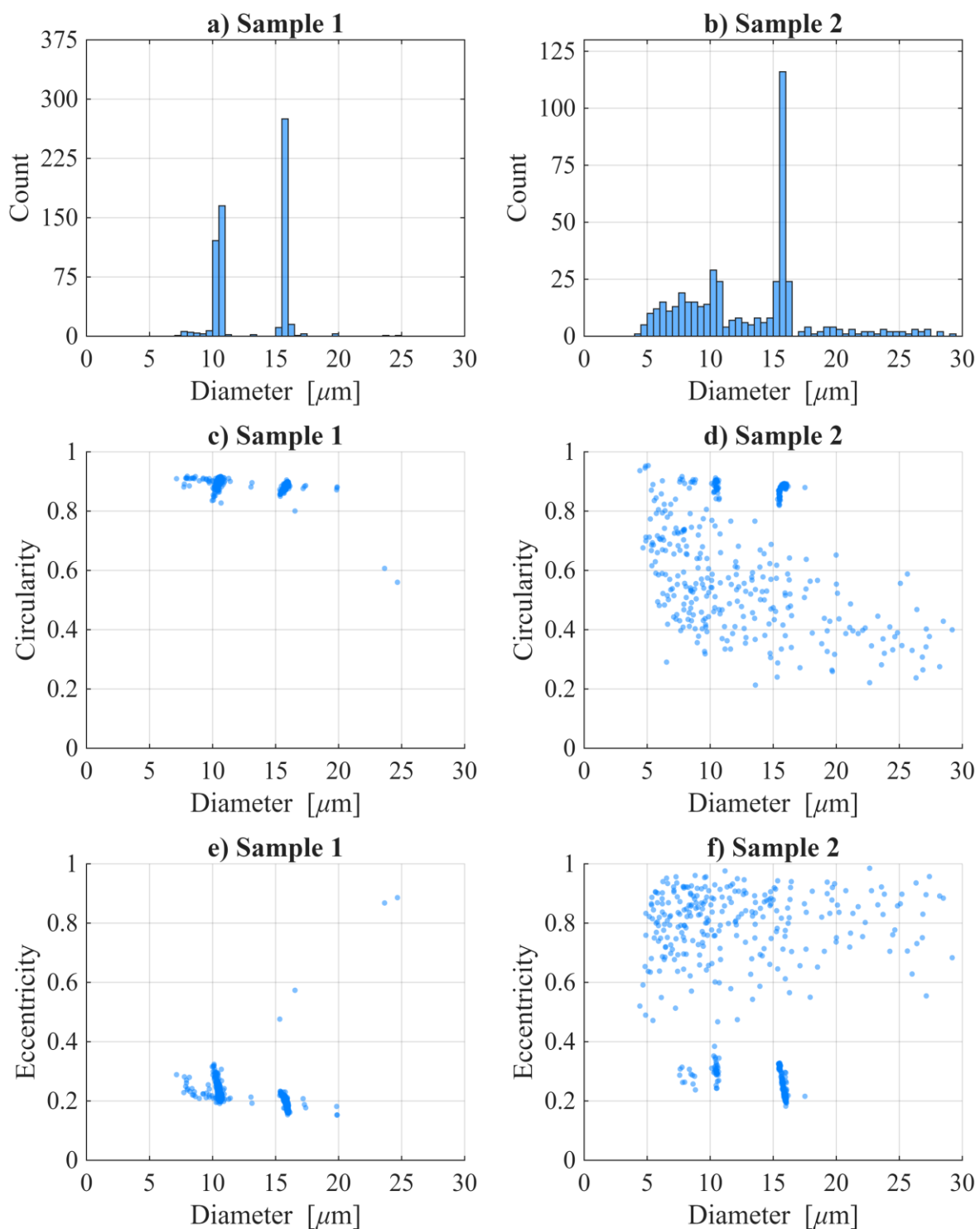


Figure 5-4: The average morphological data from detected particles where sample 1 is a mixture of 10 and 15.7 μm PS microspheres and sample 2 is a mixture of 10 and 15.7 μm PS microspheres and non-spherical PS microplastics in a solution of 0.1% Tween 20.

5.4 Size Differentiation

The mixture of 10 and 15.7 μm PS microspheres was further analyzed and compared to additional measured data with an automated cell counter. Plotting the diameter of the sample as a histogram has two distinct peaks representative of each population. These particles were differentiated based on the two sizes of PS microspheres and were plotted and compared to other collected data. Curves were fitted according to the mean and standard deviation.

Separate samples were prepared for the Countess cell counter with 10 and 15.7 μm PS microspheres in the optimal concentration range at 1×10^6 cells/ml and 3×10^5 cells/ml respectively. The measured size is plotted in comparison to the data collected by the video cytometer in Figure 5-5. This collected size information was fitted with normal distributions and both datasets were compared to the distribution derived from the manufacturer's specifications.

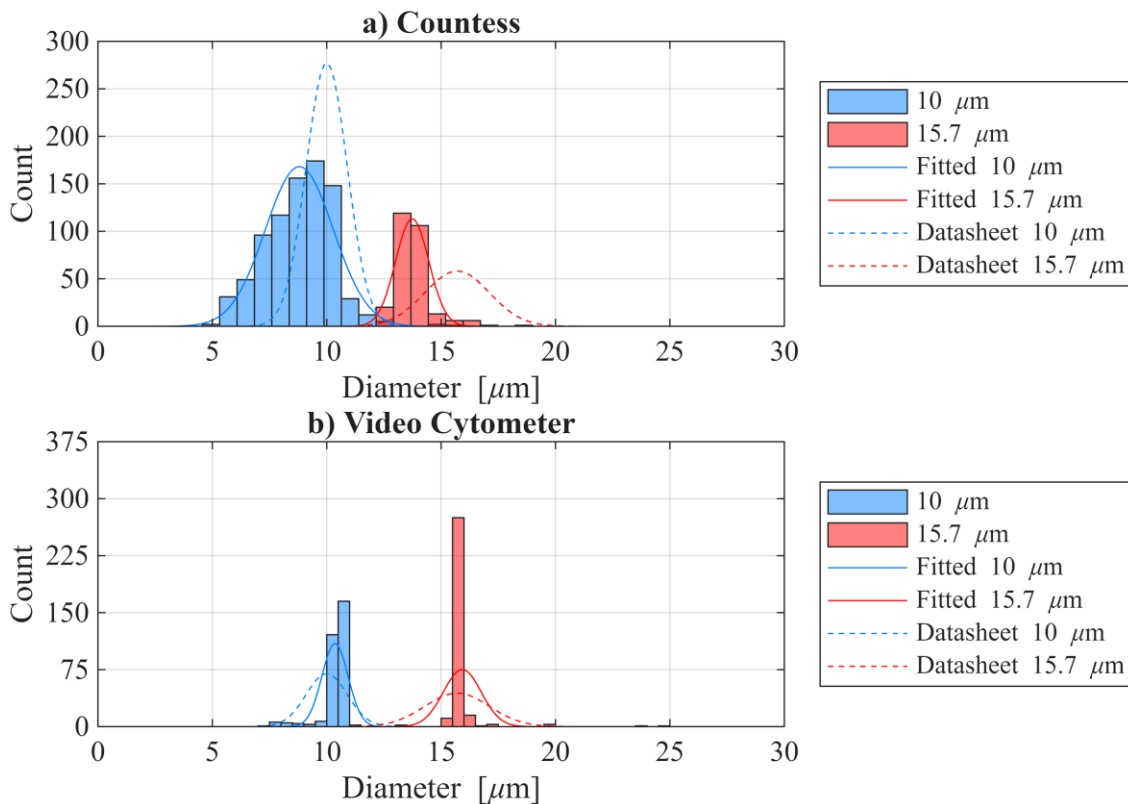


Figure 5-5: Comparison of size data collected with a) Countess and b) video cytometer along with the curves based on the data sheet specifications from Polysciences.

5.4 Size Differentiation

The mean and standard deviation for the fitted distribution from the data collected with the Countess and video cytometer were compared to the values collected from the datasheet provided by Polysciences in Table 5-2. A total of 1094 particles were measured with the Countess, while 778 particles were measured using the video cytometer. This data demonstrates the ability of the video cytometer to classify particles based on size for 10 and 15.7 μm PS microspheres with minimal deviation from the expected size range. Measurements from the Countess demonstrated larger variations from the expected size range. The Countess is optimized for mammalian cells and may have some error when measuring microplastics due to feature differences such as high contrast and larger refractive indices.

Table 5-2: Measured statistical size data for 10 and 15.7 μm PS microspheres from the Countess and video cytometer

Size	Statistic	Countess	Video Cytometer	Polysciences Datasheet
10 μm	Mean [μm]	8.8039	10.3633	10
	STD [μm]	1.4838	0.5705	0.9
15.7 μm	Mean [μm]	13.7360	15.9159	15.7
	STD [μm]	0.7243	0.7604	1.413

The Countess also captures other morphological data including circularity, brightness, and the major and minor axes of detected particles. The major and minor axes can be used to calculate the eccentricity using Equation 3.3. This data is compared to the data collected from the video cytometer in Figure 5-6. This demonstrates both systems' ability to measure morphological features. The video cytometer has a denser population in measuring both the circularity and eccentricity compared to the data collected from the cell counter.

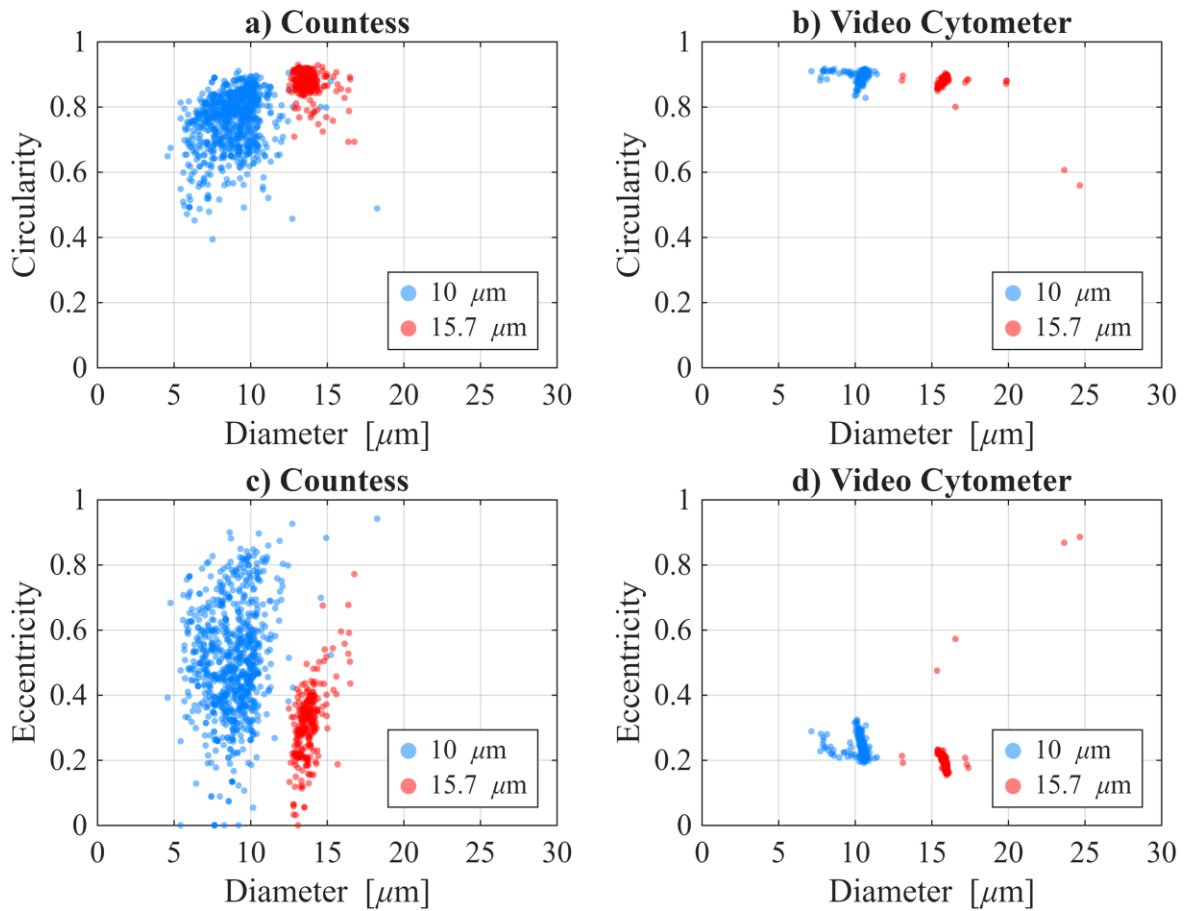


Figure 5-6: Comparison of circularity, eccentricity and size between measured data from the Countess and video cytometer.

The size distribution from the video cytometer creates two distinct populations and allows for analysis on how size affects the DEP force. The DEP force is dependent on the volume of the particle and is expected to increase with larger volumes. Simulations created in COMSOL Multiphysics are used as a reference for the theoretical trajectories. The differential velocity comparison is shown in Figure 5-7 and shows alignment with the expected results, with a population of slower incoming particles than those simulated. This discrepancy is likely due to the idealized forces in the fluid dynamic simulations that may underestimate drag force or not account for wall interactions and friction for particles close to the bottom of the channel.

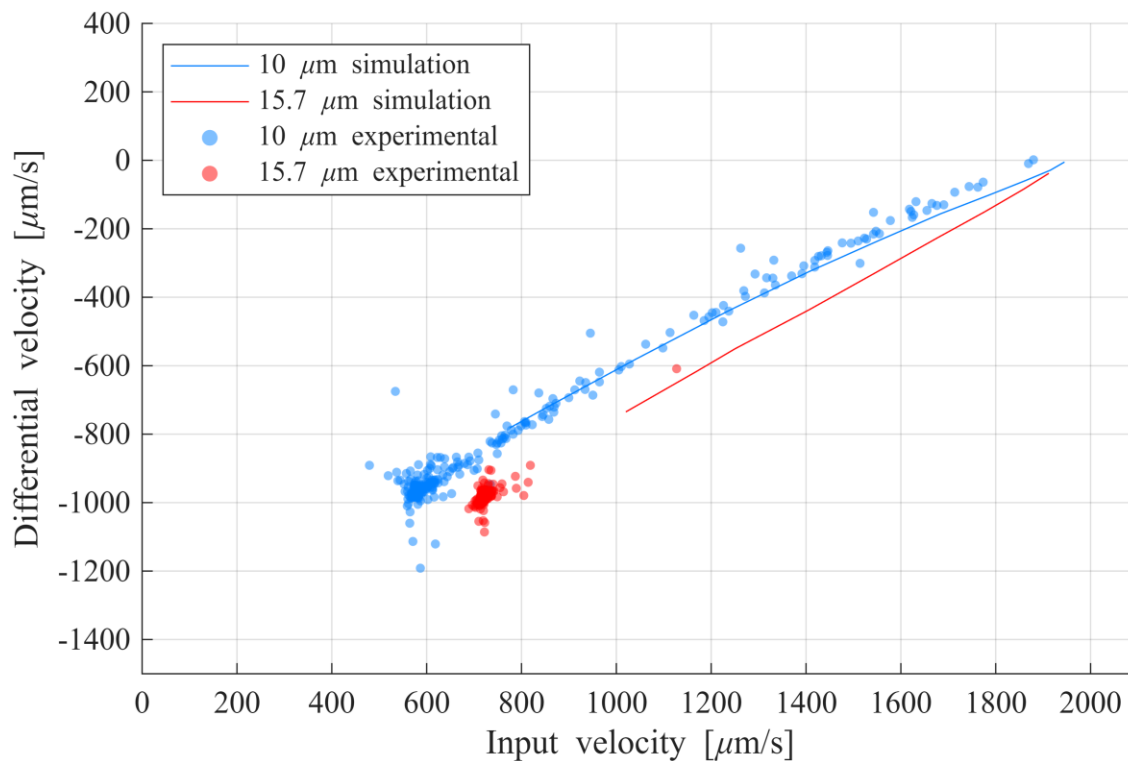


Figure 5-7: Comparison of differential velocity of experimental and simulated data for 10 and 15.7 μm PS microspheres with an applied 1 MHz and 6 Vpp DEP signal.

5.5 Morphological Differentiation

Experiments performed with a mixture of spherical and non-spherical particles showed distinct differences in morphological shape from their circularity and eccentricity data, as seen in Figure 5-4. Three populations can be differentiated based on these plots for 10 μm microspheres, 15.7 μm microspheres, and non-spherical microplastics. Three regions are defined using the circularity, eccentricity, and diameter from collected morphological data. The region for a particle to be considered a 10 μm microsphere is a circularity greater than 0.8, an eccentricity less than 0.4, and a size range from 7.5 to 12.5 μm . For a 15.7 μm microsphere, the circularity is greater than 0.8, the eccentricity is less than 0.4, and the size range is between 12.5 and 18 μm . Particles that are in the size range for the 10 or 15.7 μm microspheres and meet only one of the criteria for either the eccentricity or circularity are considered unknown.

Any particles with morphological values outside of these ranges are categorized as non-spherical. The circularity and eccentricity data for the differentiated particles are shown in Figure 5-8.

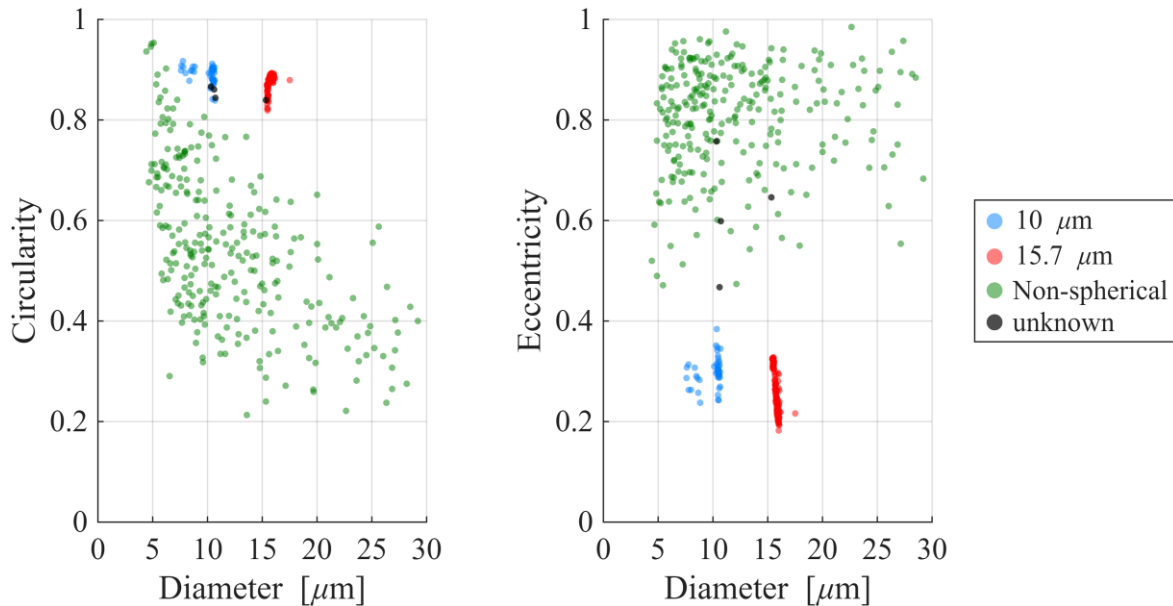


Figure 5-8: Circularity and eccentricity data for a mixture of 10 and 15.7 μm PS microspheres with non-spherical PS microplastics.

The differentiated particles can be further analyzed to explore the DEP response. The differential velocities compared to the incoming velocities are shown in Figure 5-9. The microspheres appear to primarily begin lower in the channel from the low incoming velocities with a denser region of 15.7 μm microspheres. The non-spherical particles are more spread out at various incoming and differential velocities. There is a wider range of sizes, circularities, and eccentricities for the non-spherical particles. A broader range of sizes results in a wider spread of the magnitude of the differential velocities due to the volume dependency of the DEP force. The drag force fluctuates with the orientation and surface roughness, affecting particles with a lower circularity and higher eccentricity, and may appear as deviations from the expected DEP response.

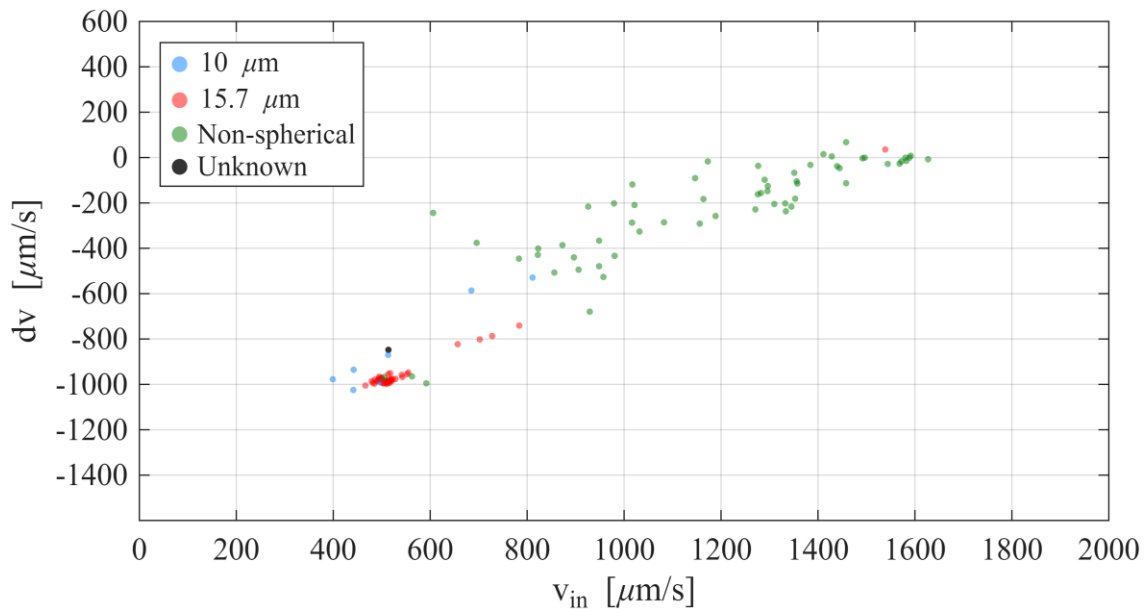


Figure 5-9: Differential velocity data for a mixture of 10 and 15.7 μm PS microspheres with non-spherical PS microplastics.

5.6 Tracking Efficiency

As an evaluation of the device's capability, particles' trajectories were analyzed to identify outliers. Initially, the differential velocity was used as an indicator for physically impossible cases where the magnitude of the differential velocity was well above the maximum flow velocity (at least 2 times). Then trajectories for suspicious particles were checked manually for cases such as misidentification of two particles with trajectories close together in the channel and particles that get stuck to the bottom of the channel or lift off from being previously stuck. These particles were excluded and recorded to find the number of anomalous tracked particles found throughout the videos taken. The total number of anomalous tracked particles for the videos taken with samples 1 and 2 respectively are shown in Table 5-3. This system demonstrated a high reliability between the videos taken using microplastics with 96% of the trajectory data being usable for analysis from the image processing algorithm.

5.6 Tracking Efficiency

Table 5-3: The outliers in the trajectory data compared to the total number of particles across multiple videos to find the tracking efficiency, where sample 1 is a mixture of 10 and 15.7 μm PS microspheres and sample 2 is a mixture of 10 and 15.7 μm PS microspheres and non-spherical particles

	Sample 1	Sample 2
Anomalous Tracked Particles	69	21
Total Particles Tracked	1914	573
Tracking Efficiency	96.39%	96.34%

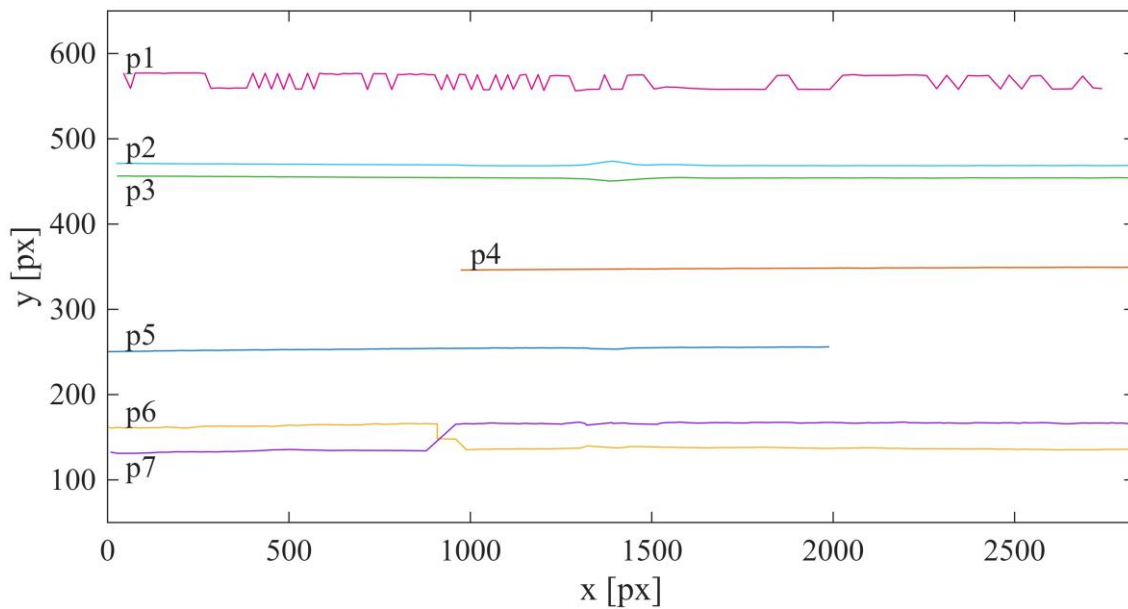


Figure 5-10: Example trajectories of anomalous trajectories that are not included in analysis.

Example trajectories excluded from calculations shown in Figure 5-10 are representative of particles that are removed automatically in the algorithm or manually in post-processing. P1 is an example of three particles stuck together vertically that are rolling. The trajectories are based on the centroid, thus the rolling effect causes a vertical deviation due to the direction of rotation. P2 and p3 demonstrate particles that have deviations in their paths from moving around a particle that is stuck on the bottom of the channel. These deviations can affect the velocity calculation since they slow when moving around the stationary particle and thus are removed. P4 is a particle that was previously stuck on the bottom of the channel and was lifted

back into flow. P5 is a particle that settled to the bottom of the channel and got stuck. P6 and p7 were particles that were close together in flow and the trajectories were misidentified and swapped for each respective path.

5.7 Summary

This chapter presented the experimental evaluation of the video cytometer using polystyrene microplastics. The uniform dielectric properties of PS microplastics were utilized to assess the relationship between the applied voltage and the DEP force. As expected, the applied force increased proportionally with the applied voltage. Analysis of the collected morphology highlighted the differences between spherical and non-spherical PS particles. However, the results in measuring the size, circularity, and eccentricity demonstrated some error due to pixelation and noise.

Polystyrene microspheres with 10 and 15.7 μm diameters were differentiated based on size and were compared to measurements taken with a cell counter. Deviations between measurements occurred and were compared to the expected values from the datasheet. This comparison demonstrated a small amount of overestimation in the measurements from the video cytometer while the cell counter exhibited a more significant underestimation. The morphological data for circularity and eccentricity also demonstrated more consistent results than the results from the cell counter. Analyzing the DEP reaction of the classified 10 and 15.7 μm PS microspheres showed a strong correlation between the simulated and experimental data and confirmed the expected increase in the magnitude of the DEP force for particles with a larger volume.

Chapter 6 – Biological CHO Cell Experiments

Chinese hamster ovary cells at a range of applied frequencies were measured to see the transition of DEP force acting on viable and non-viable CHO cells. Morphological features were detected and compared to data collected with the Countess. Further analysis of CHO cells was done to differentiate populations based on their DEP data and an investigation was done to analyze the differences in morphological data between viable and non-viable cells.

6.1 Sample Preparation

Chinese hamster ovary cells (CHODG44-EG2-hFc/clone 1A7), provided by Yves Du-rocher of the National Research Council were used in experiments. Cells were grown by Paige Adams, a technician in the Biosensors Research Lab, at 37 °C with 10% CO₂ in a 125ml baffled shaker flask (318 rpm, 19 mm orbital) with growth media consisting of BioGro-CHO™ serum-free medium (BioGro Technologies, Canada) that is supplemented with 0.5 g/L yeast extract (Becton Dickinson, Canada), 1 mM glutamine (Sigma-Aldrich, USA), and 4 mM GlutaMax™ (Gibco, Thermo Fisher Scientific, USA). Cells were passaged every 2-3 days with a seeding density of 2×10^5 cells/ml. The osmolarity was measured with an osmometer (The Advanced™ Micro Osmometer Model 3300, Advanced Instruments Inc, USA) to be 326 mOsm and the conductivity was measured with a conductivity meter (Orion Star A212 Conductivity Meter, Thermo Fisher Scientific, USA) to be 1.44 S/m at 21.6 °C. Samples were removed at 96 and 120 hours to perform DEP measurements to analyze a mixture of viable and non-viable populations at different viabilities.

A low conductivity media was made using 229 mM sucrose (Sigma-Aldrich, USA), 16 mM glucose (Thermo Fisher Scientific, USA), 1 mM calcium chloride (CaCl₂, Thermo Fisher Scientific, USA), and 5 mM sodium phosphate dibasic (Na₂HPO₄, Sigma-Aldrich, USA). This was measured to be 0.081 S/m at 21.9 °C with an osmolarity of 274 mOsm. The sample media was created by spiking the low conductivity media with growth media to get a

final conductivity of 0.17 S/m. For each experiment, a 15 ml sample was prepared with a concentration of 5×10^4 cells/ml and a measured conductivity of 0.17 S/m at 21.6 °C. Experiments were performed within 1 hour of placing the cells into the sample media to ensure viability remains constant. Trypan blue (Gibco, Thermo Fisher Scientific, USA) was used to determine the viability and concentration of the cells using the Countess Cell Counter (CountessTM 3 FL, Thermo Fisher Scientific, USA).

6.2 Frequency Relationship with DEP

The real part of the KCM for viable and non-viable CHO cells has a strong frequency dependency as seen in Figure 2-5. This dependence becomes apparent through analysis of the differential velocities at different frequencies. With a medium conductivity of 0.17 S/m and in the frequency range from 100kHz to 10MHz, the real part of the KCM for viable CHO cells increases from negative to positive, whereas non-viable CHO cells remain negative but decrease in magnitude. Figure 6-1 demonstrates the measured differential velocities compared to input velocities at 300 kHz, 600 kHz, and 6 MHz, with an applied voltage of 6 Vpp. At 300 kHz, the real part of the KCM for both viable and non-viable CHO cells is negative. Increasing to 600 kHz, the real part of the KCM is close to the zero crossing and is expected to behave similarly to a reaction with no DEP force, whereas the differential velocity of non-viable CHO cells remains negative. When a frequency of 6 MHz is applied, the differential velocity of viable CHO cells is positive as cells are pulled towards the bottom of the channel. There is also a measured sample with no DEP signal applied in Figure 6-1. Experiments were performed with cells at 96 hours and 120 hours, where the viability measured in the Countess with trypan blue was 95% and 73% respectively. These samples demonstrate an increase in the population of non-viable CHO cells in the sample measured at 120 hours and their DEP response.

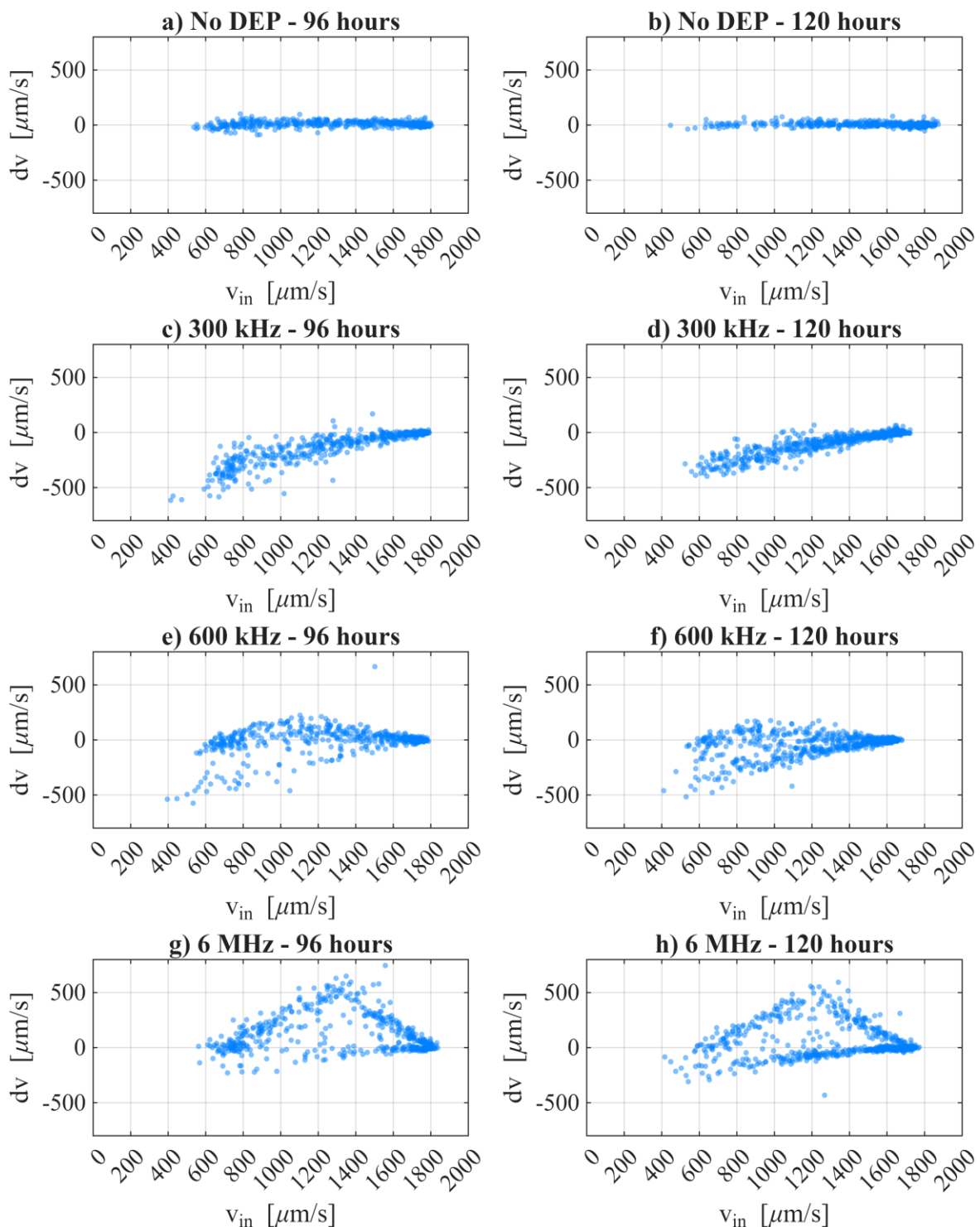


Figure 6-1: The differential velocity compared to the input velocity at a range of frequencies demonstrating the change in DEP response for a mixture of viable and non-viable CHO cells.

6.2 Frequency Relationship with DEP

To further explore the frequency dependency of the KCM, the real part of the KCM can be compared to the mean values of differential velocity, representing the DEP response. Experiments are performed with an applied voltage of 6 Vpp and a frequency range from 100 kHz to 10 MHz. The mean differential velocity is calculated for the particles in each of the two 30 second videos taken at each frequency for the CHO cell sample measured at 96 hours. This sample had a measured viability of 95% with trypan blue. An effective curve is calculated for the real part of the KCM using a weighted average with 95% viable CHO cells and 5% non-viable CHO cells. Figure 6-2 compares the mean differential velocity from each video with the effective curve for a population with 95% viability. The magnitude and sign of differential velocity align with the effective KCM and demonstrate a crossover frequency in the same region.

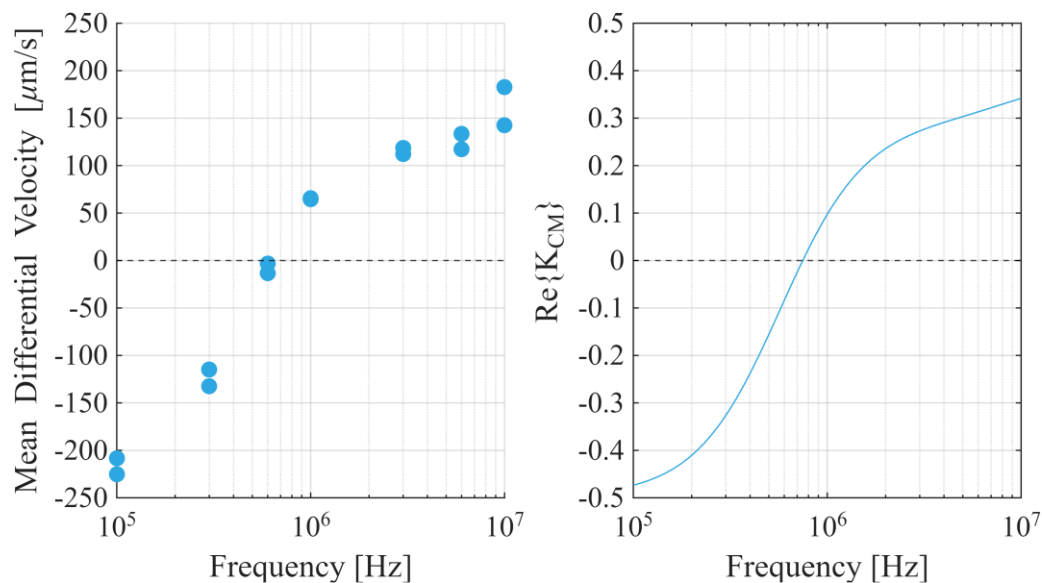


Figure 6-2: a) Mean differential velocity from 30 second videos at a range of frequencies of viable CHO cells b) real part of the Clausius-Mossotti factor for 95% viable CHO cells from 100 kHz to 10 MHz.

6.3 Morphological Feature Extraction

A representative set of videos was chosen to demonstrate the morphological features detected in CHO samples from 96 and 120 hours. A higher percentage of non-viable CHO cells is expected to appear in the sample from 120 hours. Non-viable CHO cells are expected to appear smaller in size when compared to viable CHO cells and may have some blebbing as they undergo apoptosis. Blebbing may appear smaller than the resolution of the camera and would not be visible.

Figure 6-3 shows the morphological data collected from 670 cells at 96 hours and 660 cells at 120 hours. As expected, the estimated size shows an increase in smaller particles at 120 hours compared to the data collected at 96 hours. The circularity and eccentricity have a much wider range than seen when using PS microspheres, with some potential error due to the increased gain and exposure time. These results demonstrate the difficulty of differentiating viable and non-viable CHO cells solely on their morphological features and thus further investigation is required.

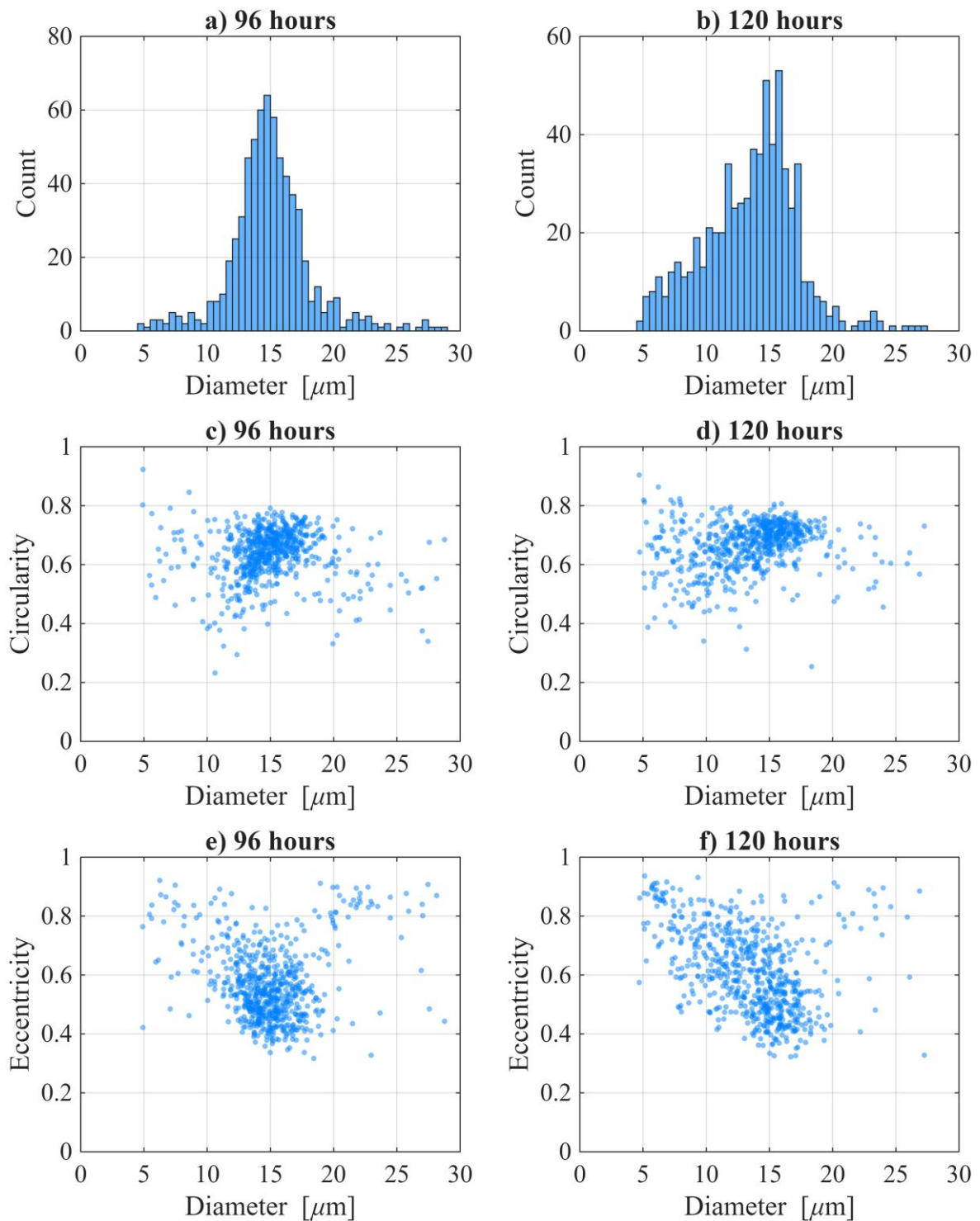


Figure 6-3: The average morphological data from detected particles of a mixture of viable and non-viable CHO cells at 96 and 120 hours.

6.4 Differentiation of Viable and Non-viable CHO cells

The morphological data provided by the system is challenging to use as a method to differentiate between viable and non-viable CHO cells. Instead, the DEP response is used as the differentiation method while the morphological data can be analyzed for observation of any physical differences. An applied signal at 6 MHz and 6 Vpp allows for differentiation based on their differential velocity. Non-viable CHO cells experience nDEP and have a negative differential velocity from moving towards the centre of channel while viable CHO cells experience pDEP and have a positive differential velocity from being pulled down towards the bottom of the channel. When viable cells are already at a low height in the channel, there is a limited region that they can move down and they will exhibit a small change in velocity. As starting heights increase, the magnitude of the change in velocity increases with the space a particle can move down. The magnitude decreases towards the middle of the channel as the electric field becomes weaker. When particles are close to the centre of the channel, it becomes difficult to differentiate particles due to their velocities having minimal changes before and after the electrodes. For this reason, a region is defined to account for particles that cannot be differentiated.

When particles are flowing in the channel with no DEP signal applied, there will still be some variation in the differential velocity. This is used to define a region where particles cannot be differentiated based on their DEP response. The differential velocity for 738 particles was plotted in Figure 6-4 without a DEP signal applied. The histogram is plotted to demonstrate the standard deviation that occurs for CHO cells in the channel. The standard deviation was calculated to be ± 19.8 . This is used as the region where particles cannot be differentiated between positive and negative differential velocities at 6 MHz.

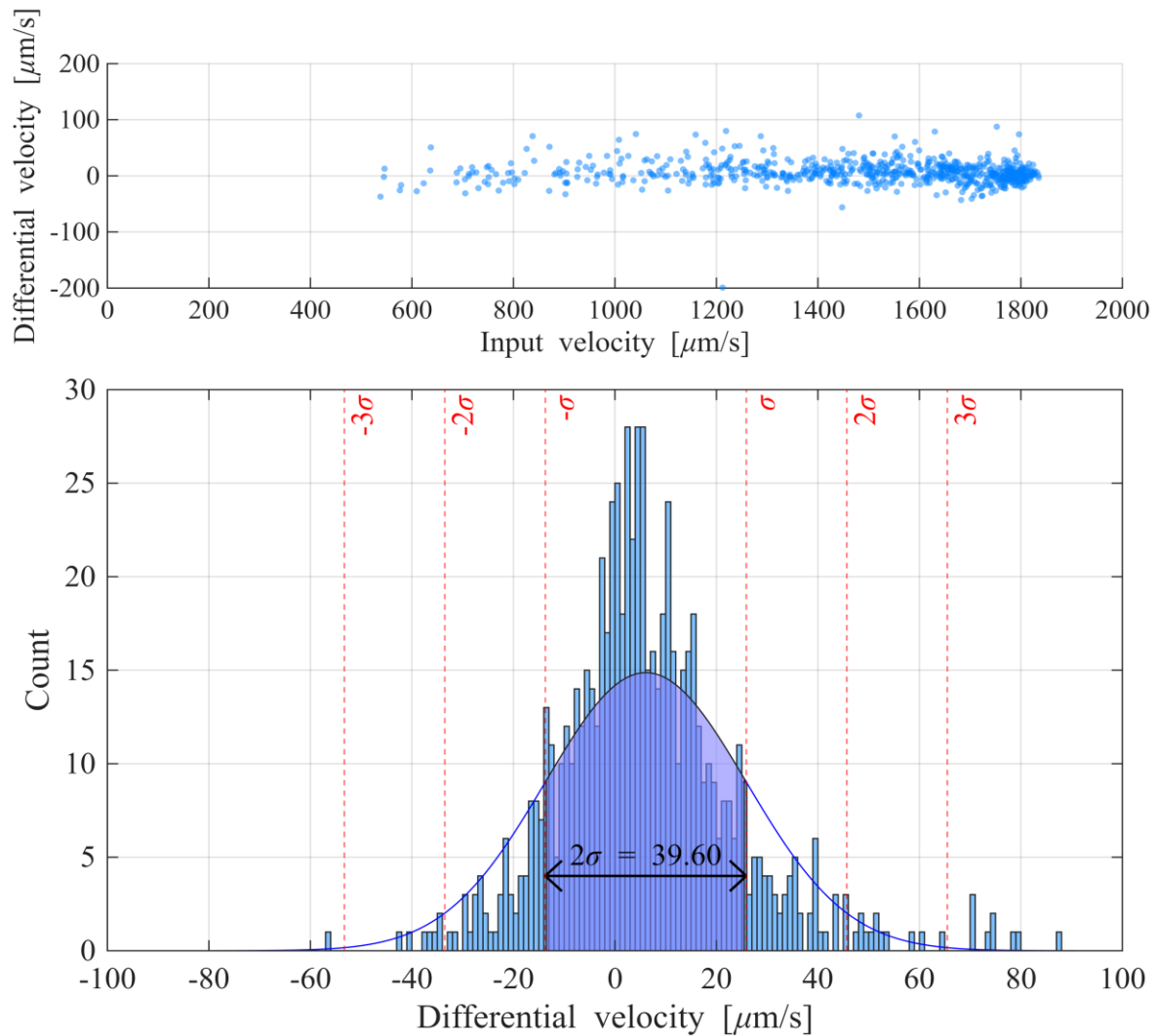


Figure 6-4: Standard deviation calculation from the differential velocity of CHO cells with no DEP signal applied.

Using the sample of cells measured at 120 hours from seeding, cells with a differential velocity above 20 $\mu\text{m/s}$ were categorized as viable and non-viable below -20 $\mu\text{m/s}$. From two 30 second videos, 218 cells were categorized as viable, 186 were categorized as non-viable, and 238 cells were outside of the differentiation region. A considerable number of cells were categorized as unknown since they were incoming near the middle of the channel and thus had a weaker DEP force acting on them. The viability based on the successfully differentiated particles was calculated to be 53.96%. The differential velocity in Figure 6-5 demonstrates the

6.4 Differentiation of Viable and Non-viable CHO cells

classification of cells as well as the comparison to the expected differential velocities based on simulations created in COMSOL Multiphysics. There are some noticeable deviations between the experimental and simulated data, particularly for the non-viable CHO cells. This may be due to differences in the calculated DEP response from the dynamic viscosity, effective radius, or Clausius-Mossotti factor.

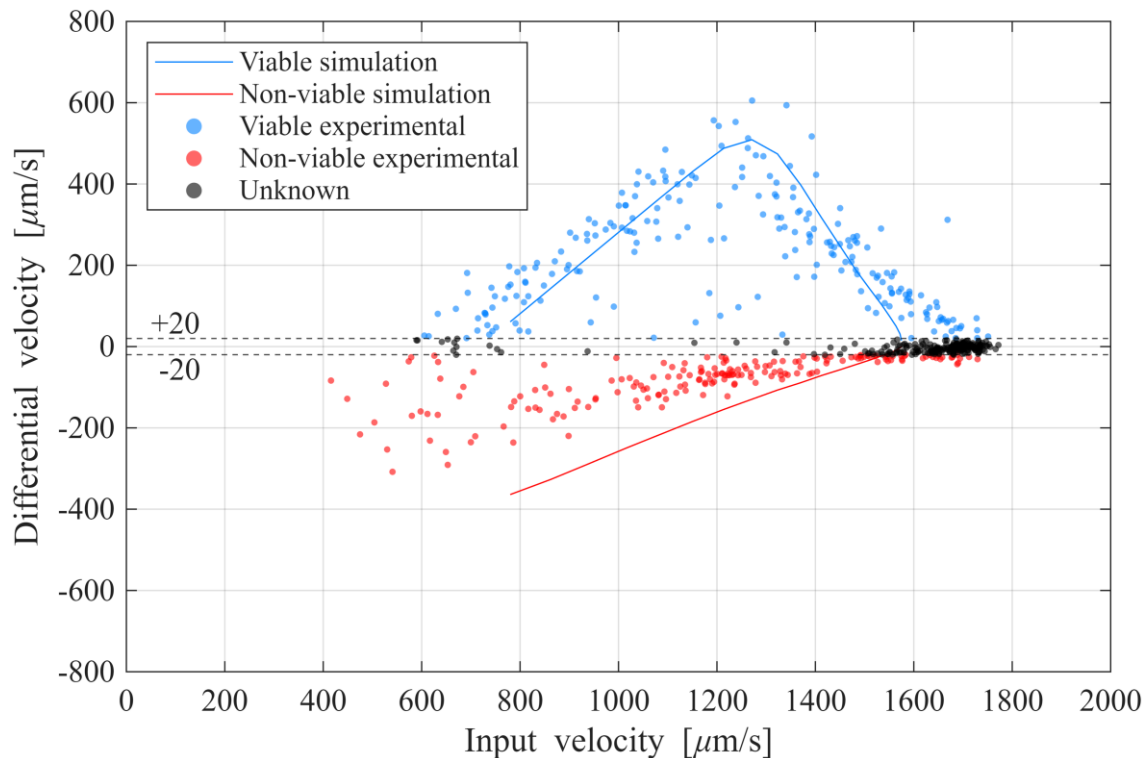


Figure 6-5: Differentiation of viable and non-viable CHO cells at 120 hours based on DEP response at 6 MHz and 6 Vpp with a calculated viability of 53.96%.

Differentiated viable and non-viable CHO cells were compared to measurements taken using CHO cells stained with trypan blue in the Countess. The total cell count recorded by the Countess was 609 cells with a viability of 73.4% compared to 409 cells with 53.96% viability from the video cytometer. This aligns with the anticipated overestimation in viability from trypan blue measurements compared to the DEP measurements. Trypan blue stains cells with compromised membranes which occurs in late apoptosis, while DEP measurements can provide insights on the internal properties of cells and indicate apoptosis earlier. The measured

6.4 Differentiation of Viable and Non-viable CHO cells

diameters of viable and non-viable CHO cells are plotted in Figure 6-6 with fitted curves for both devices. The statistical data from the curves are compared as well as the median value from each data set in Table 6-1. The mean diameters of cells measured with the Countess are smaller than the values calculated using the video cytometer which aligns with deviations seen using the PS microspheres.

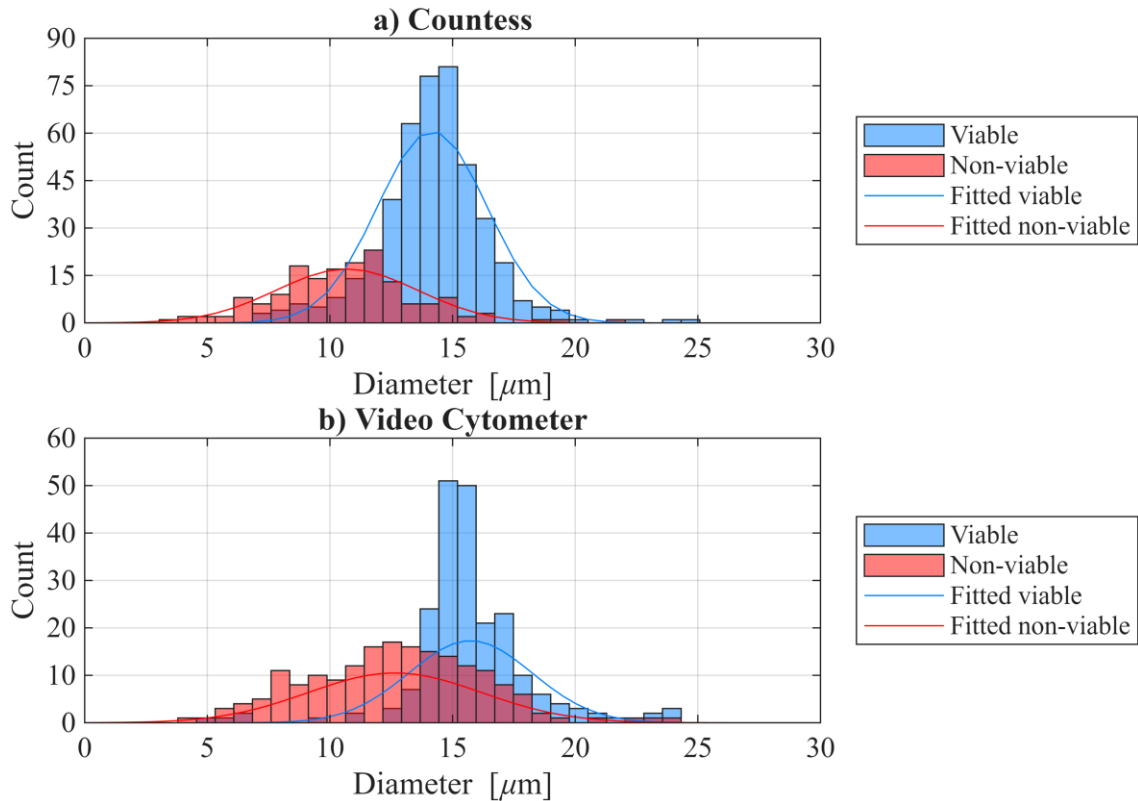


Figure 6-6: Comparison of measured diameter from differentiated viable and non-viable CHO cells at 120 hours from the Countess and video cytometer.

Table 6-1: Statistical data for viable and non-viable CHO cells from the Countess and video cytometer with viabilities of 73.4 and 53.96% respectively

	Countess		Video Cytometer	
	Viable	Non-viable	Viable	Non-viable
Mean [μm]	14.17	10.69	15.73	12.65
Median [μm]	14.32	10.80	15.54	12.63
STD [μm]	2.23	2.88	2.51	3.54

In addition to the comparison of the measured sizes using both devices, the eccentricity and circularity are calculated and compared in Figure 6-7. This is a representative dataset to demonstrate the capabilities of the video cytometer, but future studies should be done to analyze the quality of this information.

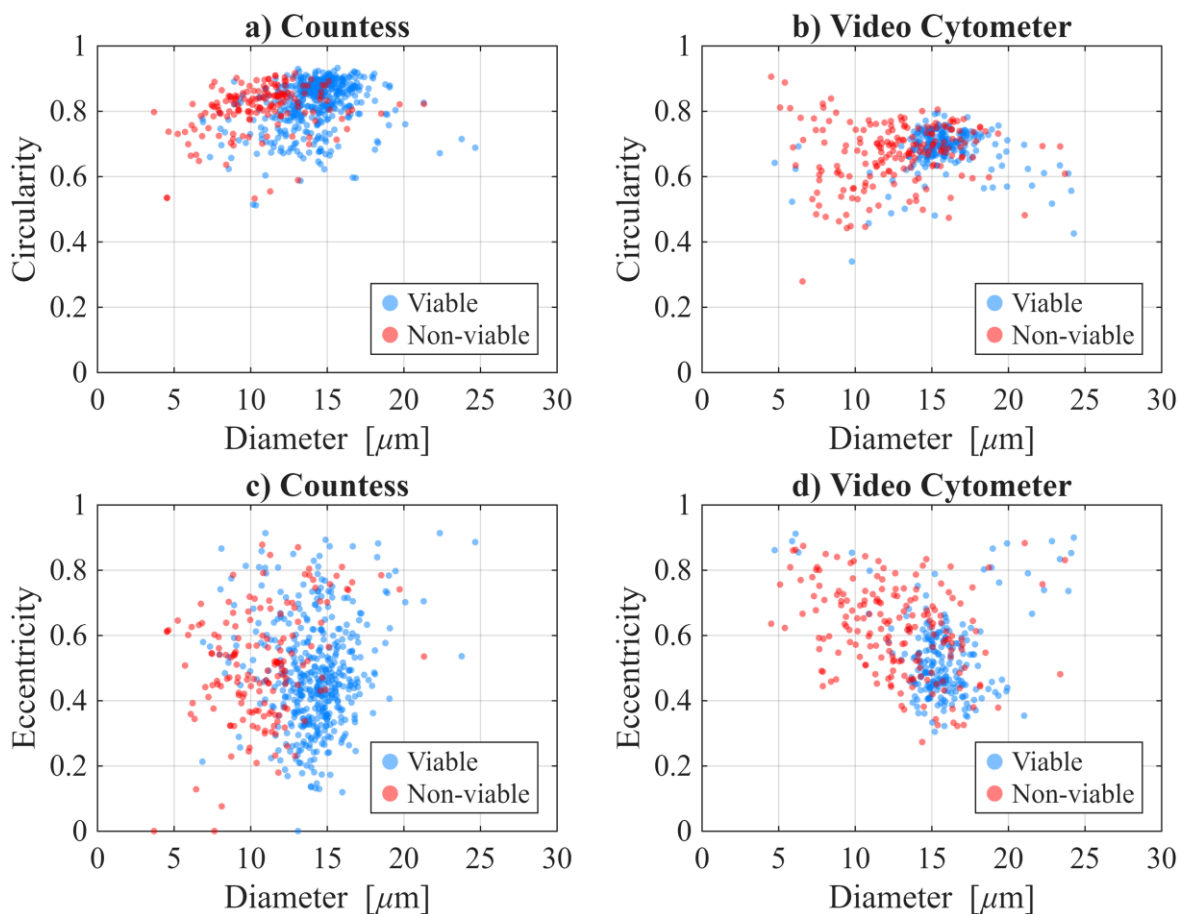


Figure 6-7: Eccentricity and circularity for viable and non-viable CHO cells.

6.5 Tracking Efficiency

The percent of trajectories from the CHO cell data included in analysis was calculated using the same method as the polystyrene particles. The trajectories with impossible velocities were immediately rejected, followed by a manual analysis of remaining trajectories. Trajectories were closely analyzed to ensure they were correctly linked to the same particle and to ensure trajectories successfully crossed the field of view without perturbations,

6.6 Summary

misidentifications, or interruptions from the flow. The number of particles with anomalous trajectories were tracked as well as the total number of particles identified to find the percent of particles and their trajectory data that fit the criteria for further analysis which was found to be 98% for the sample taken at 96 hours and 95% for the sample taken at 120 hours. This data is shown in Table 6-2 to demonstrate the high reliability of the system with an average of 96% tracking efficiency.

Table 6-2: Anomalous particles based on their trajectory data compared to the total number of particles across the same videos and tracking efficiency for CHO cells from samples measured at 96 and 120 hours

	96 hours	120 hours
Anomalous Tracked Particles	35	127
Total Particles Tracked	2002	2543
Tracking Efficiency	98.25%	95.01%

6.6 Summary

The experimental results presented in this chapter demonstrated the system's capability to differentiate viable and non-viable CHO cells. Analysis of the differential velocities reflecting the DEP response demonstrated the frequency relationship with the DEP response and the differences with an increased population density for the response of non-viable CHO cells from the sample measured at 120 hours compared to the sample measured at 96 hours with a higher viability. The response from the sample with a higher viability highlighted the proportionality relationship between the KCM and the applied frequencies.

The morphological differences measured for both samples illustrated the difficulty in differentiation using imaging alone due to overlapping data. Thus, differentiation was performed using the DEP response where viable and non-viable were separated based on their differential velocities, reflecting pDEP and nDEP reactions respectively at an applied frequency of 6 MHz. The differentiated viable and non-viable CHO cells were compared to the measured data using a cell counter and had similar deviations in size as seen with PS microspheres.

Chapter 7 – Conclusion and Future Work

7.1 Conclusion

The work presented in this thesis demonstrates the development, integration, and evaluation of a dual-modality platform to analyze particles using optical and dielectric analysis. Image analysis allowed for detection of both morphological features and particle trajectories, where the velocity of the particle trajectories indicated the dielectric response from particles using DEP analysis. The change in velocity reflects the translational motion a particle experiences from the DEP force employed by coplanar electrodes creating a non-uniform electric field. The experimental results using polystyrene microparticles and CHO cells exemplified the benefits of this multifaceted approach.

Experiments performed with PS samples validated the expected results, demonstrating the relationship between the applied voltage and the differential velocity. A larger voltage applied to the DEP electrodes caused an increase in the translational motion a particle experiences, represented by the differential velocity. The morphological features represented by the size, circularity, and eccentricity displayed quantifiable differences between spherical and non-spherical particles. Differentiation of 10 and 15.7 μm microspheres using measured size allowed for a comparison of morphological data with a cell counter, where the video cytometer displayed a closer alignment with the expected values based on the manufacturer's data sheet. The differentiation based on size also confirmed the increase in DEP response for particles of the same material with a larger volume.

Experimentation with biological CHO cells demonstrated the differences in dielectric responses at a range of frequencies and highlighted the populations of viable and non-viable CHO cells using two samples taken at 96 and 120 hours to capture populations before and after the viability decreases. Using the sample of CHO cells with a high viability also displayed the

relationship between the Clausius-Mossotti factor and the differential velocity at a range of applied frequencies from 100 kHz to 10 MHz. The morphological data displayed the populations at 96 and 120 hours. Analysis of the dielectric response with a 6 MHz signal allowed for differentiation of viable and non-viable cells based on their differential velocity and provided further insight into the morphological data based on the two populations of cells that were compared to results from a cell counter.

Particles analyzed during experimentation were successfully detected and tracked within the microfluidic system with an overall 96% tracking efficiency. The size, shape, circularity, eccentricity, and velocity data were all extracted from subsequent videos at a range of voltages and frequencies, providing both optical and dielectric information. The collected motion and morphological data supplied complementary information from the combined modalities. While this device demonstrates significant benefits to a variety of applications, challenges remain. The morphological feature detection is limited by the resolution of the camera, particularly for accurate edge detection of surface morphology. Objectives with a higher magnification may limit the field of view, causing an inadequate number of data points to extract particle velocity. On the other hand, a camera with a higher resolution while retaining a high frame rate may be costly and would require a significant amount of storage space and computational analysis. Overall, the developed device successfully integrated optical and dielectric analysis of particles and single cells and provides a platform for future advances in areas such as biological and environmental research, diagnostics, and drug development.

7.2 Future Work

Future work focuses on improving the system for enhanced particle analysis and a wider range of applications.

7.2.1 Hardware Improvements

Employing a higher-resolution camera would allow for higher accuracy in morphological feature detection. The ability to resolve smaller objects would allow for the analysis of smaller cells and features such as blebbing. A sensor with a higher sensitivity can improve the contrast and visibility of particles that are difficult to see but may result in additional noise. Employing a higher frame rate may reduce blurring issues and frame-to-frame error. This can also allow for an increase in throughput using a faster flow rate.

The light source in the current system requires higher exposure times and gain for samples that are close in refractive index to the media they are suspended in which leads to issues with blurring and uneven edges due to noise. This can result in excess error in the size and shape measurements. The light can be improved with a focused light source using a condenser with increased brightness without compromising excess heat as this can affect the channel conditions.

7.2.2 Software Improvements

The image processing algorithm to detect and track cells can be updated to gather more information and improve feature detection. The implementation of machine learning may aid the background subtraction method to discern blurring and noise effects to promote a higher accuracy of edge detection, thus improving the size measurement and the potential to distinguish surface features such as blebbing. Machine learning or an updated algorithm may aid in discerning particle trajectories to reduce tracking errors and anomalies.

Additional morphological features can be employed for further analysis such as orientation, granularity, solidity, brightness, surface roughness, and aggregation. Recent works explore the effect of the orientation of non-spherical objects factor described using the Clausius-Mossotti factor [35]. Further exploration into this phenomenon may yield propitious results.

7.2.3 Future Experimentation

This device has potential for a wide range of applications. Future experiments can investigate cellular properties to expand the utility of the device to areas such as diagnostics, biotechnology, and pharmaceutical studies. Different cell types possess unique morphological and dielectric responses which can provide a more comprehensive evaluation of the capabilities of this device. Additionally, investigation of microplastics and other small particles can provide insights into electrical properties and composition as well as the effects of altered surfaces from adsorption of chemicals. Exploration of other types of microplastics with dissimilar materials can provide a foundation to analyze collected samples to perform environmental analysis studies.

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Appendix A: Image Processing Code

```
import time
import cv2
import numpy as np
import pandas as pd
import trackpy as tp
import skimage as sk
import matplotlib.pyplot as plt
import os
import os.path

def tracker(inputFolder, folderName, frequency, voltage, ptype):

    print("START OF PROCESSING")
    startTime = time.time()

    #get video and properties
    video = [img for img in os.listdir(inputFolder) if img.endswith((".png",
".jpg", ".jpeg"))]
    video.sort() # Process frames in sequential order

    #read in first image to extract properties
    first_image_path = os.path.join(inputFolder, video[0])
    frame1 = cv2.imread(first_image_path)
    vidHeight, vidWidth, layers = frame1.shape #video dimensions
    vidDate = time.ctime(os.path.getctime(first_image_path)) #date and time
of first image in sequence

    #check if folder was given
    save=True
    if(len(folderName)<1):
        save=False
    if (save==True):
        if not os.path.exists(folderName):
            os.makedirs(folderName)

    #initialize parameters
    processedFrames=[]
    features = pd.DataFrame()
    data = pd.DataFrame()

    frameRate = 150.8 #frame rate in frames per second
```

```

ratio = 2.03 #basler #1.63 for flir #ratio of pixel/micron
stubLength = 10 #length for trajectory to be considered too short
maxSkippedFrames = 10 #number of frames to look for same particle (elec-
trode array width)
maxMovement = 400 #distance a particle can move in the max amount of
skipped frames (electrode array width)
lowV1 = vidWidth*1/9; upV1 = vidWidth*4/9; lowV2 = vidWidth*5/9; upV2 =
vidWidth*8/9 #range to get velocity before and after

#getting voltage and frequency to display on figures
if (voltage == '0' or frequency == "0"):
    titleName = '[No DEP]'
else:
    titleName = ' [%sVpp, %sHz]' %(voltage,frequency)

print('Processing frames and collecting features',end = '...')

#select background subtraction method for particle type
if ptype == 'CHO':
    fgbg = cv2.createBackgroundSubtractorKNN() #more sensitive background
subtraction
if ptype == "PS":
    fgbg = cv2.bgsegm.createBackgroundSubtractorMOG()

#initialize values
frameCount = 1
bkgFrames = 20 #number of frames to create background before tracking

for imageName in video:

    #read in next image in sequence
    imagePath = os.path.join(inputFolder, imageName)
    frame = cv2.imread(imagePath)

    #grayscale and filter frame
    gray = cv2.cvtColor(frame, cv2.COLOR_BGR2GRAY)
    gray = (255-gray)
    filteredFrame = fgbg.apply(gray)

    #fill small holes
    kernel = cv2.getStructuringElement(cv2.MORPH_ELLIPSE,(3,3))
    filteredFrame = cv2.morphologyEx(filteredFrame, cv2.MORPH_CLOSE, ker-
nel)

```

```

#apply threshold
filteredFrame[filteredFrame>10] = 1

if frameCount>bkgFrames:

    #find features on frames
    label_image, number_of_labels = sk.measure.label(filteredFrame,
background=0, return_num=True)

    for region in sk.measure.regionprops(label_image, intensity_im-
age=filteredFrame):

        # skip small areas
        if region.area < 50:
            continue

        #find centroid
        x0 = region.centroid[1]
        y0 = region.centroid[0]

        #get bounding box of region to calculate perimeter
        min_row, min_col, max_row, max_col = region.bbox

        #ignore particles travelling along edges
        if(min_row == 0 or max_row == vidHeight):
            continue

        # Crop the filtered frame to particle region
        cropped_particle = filteredFrame[min_row:max_row,
min_col:max_col]
        #find contour
        contours, _ = cv2.findContours(cropped_particle,
cv2.RETR_LIST, cv2.CHAIN_APPROX_NONE)

        # Calculate perimeter for the largest detected object in re-
gion

        if len(contours) > 0:
            largest_contour = max(contours, key=cv2.contourArea)
            perimeter = cv2.arcLength(largest_contour, closed=True)
        else:
            print(f"No object/contours detected {frameCount}")
            continue

        #collect features

```

```

        features = features._append([{'y': y0, 'x': x0, 'frame': frame-
Count, 'major':region.axis_major_length,'minor':region.axis_minor_length,
                                     'area':region.area_filled,'pe-
rimeter':perimeter, 'eccentricity':region.eccentricity,'orientation':re-
gion.orientation}])
        #example frame for tracking process
        if(frameCount==55):
            plt.imshow(frame)
            plt.show()
            plt.imshow(filteredFrame)
            plt.show()
            tp.annotate(features[features.frame==frameCount],fil-
teredFrame)

        processedFrames.append(filteredFrame)
        frameCount+=1

#Close video stream
cv2.destroyAllWindows()

print('done')

print('Linking data',end='...')
tp.quiet()

#link particles through frames
rawLinkData = tp.link_df(features,maxMovement, memory=maxSkippedFrames)

#filter out stubs
linkData = tp.filter_stubs(rawLinkData,stubLength)
print('done')

print('Calculating data',end='...')
for item in set(linkData.particle):

    #calculate average feature values for each particle
    sub = linkData[linkData.particle==item]
    xDis = np.ediff1d(sub.x)
    yDis = np.ediff1d(sub.y)
    frameDis = np.ediff1d(sub.frame)

    velocity = np.hypot(xDis,yDis)/ratio/frameDis*frameRate
    #(pix/pix*um/frame*frames/s)
    velocity=np.append(velocity,0)

```

```

        v1 = np.median(velocity[np.logical_and(lowV1<sub.x,sub.x<upV1,velocity>0)])
        v2 = np.median(velocity[np.logical_and(lowV2<sub.x,sub.x<upV2,velocity>0)])
        diffVelocity = v1-v2

        major = np.median(sub.major)
        minor = np.median(sub.minor)
        size = ((major+minor)/2)/ratio

        ecc = np.median(sub.eccentricity)
        eccSTD = np.std(sub.eccentricity)

        orientation = np.median(sub.orientation)
        oriSTD = np.std(sub.orientation)

        area1 = np.median(sub.area)
        perimeter1 = np.median(sub.perimeter)
        circularity = 4*np.pi*area1/(perimeter1*perimeter1)

        #remove anomalous particles
        #remove particles that dont make it across or are incorrectly tracked
        if ~(v1>0 and v2>0) or (any(abs(y)>50 for y in yDis)):
            linkData=linkData[~(linkData.particle==item)]
            continue

        #compile paricle data
        data = data._append([{'particle':item,'v1':v1,'v2':v2,'dV':diffVelocity,'major axis':major,'minor axis':minor,
                                'diameter':size,'area':area1,'perimeter':perimeter1,'circularity':circularity,
                                'eccentricity':ecc,'\u03C3eccentricity':eccSTD,'orientation':orientation,
                                '\u03C3orientation':oriSTD}])

        print('done')

        #renumber particles after removing anomalies
        count = 0
        for item in set(linkData.particle):
            linkData.particle[linkData.particle==item] = count
            data.particle[data.particle==item]=count
            count +=1
        print(str(count) + " particles found")
        print('Plotting data',end='...')

```

```

#plot average velocities for each particle
plot1 = plt.figure(figsize=(10,6))
width=0.3
plt.bar(data.particle-(width/2),data.v1,width,label='v1')
plt.bar(data.particle+(width/2),data.v2,width,label='v2')
plt.legend()
plt.xlabel('Particle number')
plt.ylabel('Velocity [\u03BCm/s]')
plt.title('Average Velocities\n%s \n%s' %(vidDate,titleName))
plt.ylim(0,2100)

#plot average diameter for each particle
plot2 = plt.figure(figsize=(10,6))
plt.hist(data.diameter,range=[0,30],bins = 60)
plt.legend()
plt.xlabel('Diameter [\u03BCm]')
plt.ylabel('Count')
plt.title('Average Diameter\n%s \n%s' %(vidDate,titleName))

#plot velocity in vs differential velocity for each particle
plot3 = plt.figure(figsize=(10,6))
plt.scatter(data.v1,(data.dV))
plt.xlabel('Velocity In [\u03BCm/s]')
plt.ylabel('Differential Velocity [\u03BCm/s]')
plt.ylim(-1000,1000) #limit if using full video
plt.xlim(0,2500)
plt.grid()
plt.title('%s\n%s' %(vidDate,titleName))

#histogram for velocity in
plot4 = plt.figure(figsize=(10,6))
# plt.suptitle('%s\n%s' %(vidDate,titleName))
plt.subplot(2,2,1)
plt.hist(data.v1, range=[0,2500], bins = 100)
plt.xlabel('Velocity [\u03BCm/s]')
plt.ylabel('Count')
plt.title('Velocity In')
plt.grid()

#histogram for velocity out
plt.subplot(2,2,2)
plt.hist(data.v2, range=[0,2500], bins = 100)
plt.xlabel('Velocity [\u03BCm/s]')
plt.ylabel('Count')
plt.title('Velocity Out')

```

```

plt.grid()

#histogram for differential velocity
plt.subplot(2,2,3)
plt.hist((data.dV),range=[-2500,2500], bins=120)
plt.xlabel('Velocity [\u03BCm/s]')
plt.ylabel('Count')
plt.title('Differential Velocity ')
plt.grid()

#add info to plots
plt.subplot(2,2,4)
plt.axis('off')
plt.text(0,1,'Number of Particles = %.0f ' %(len(data.particle)))
plt.text(0,0.8,'Number of Frames = %.0f, Duration = %.0f s' %(frame-
Count,frameCount/frameRate))
plt.text(0,0.6,'Velocity In: m = %.1f \u03BCm/s \u03BC = %.1f \u03BCm/s'
%(np.median(data.v1),np.mean(data.v1)))
plt.text(0,0.4,'Velocity Out: m = %.1f \u03BCm/s \u03BC = %.1f \u03BCm/s'
%(np.median(data.v2),np.mean(data.v2)))
plt.text(0,0.2,'Differential Velocity: m = %.1f \u03BCm/s \u03BC = %.1f
\u03BCm/s' %(np.median((data.dV)),np.mean((data.dV))))
plt.text(0,0,'STD Differential Velocity = %.1f \u03BCm/s'
%np.std((data.dV)))

plot4.tight_layout()

#plot trajectories
plot5 = plt.figure(figsize=(10,6))
plt.ylim(vidHeight,0) #limit if using full video
plt.xlim(0,vidWidth)
plt.title('Trajectories\n%s \n%s' %(vidDate,titleName))
tp.plot_traj(linkData,label=True) # label = True see particle identities

#plot circularity vs size
plot6 = plt.figure(figsize=(10,6))
plt.scatter(data.diameter,data.circularity)
plt.xlim(0,30)
plt.ylim(0,1)
plt.grid()
plt.xlabel('Diameter [\u03BCm]')
plt.ylabel('Circularity')
plt.title('Circularity \n%s \n%s' %(vidDate,titleName))

print('done')

```

```

if (save==True):
    print('Saving data',end='...')
    #save plots in folder
    plot1.savefig(folderName+'/AverageVelocityPlot.png')
    plot2.savefig(folderName+'/AverageDiameterPlot.png')
    plot3.savefig(folderName+'/V1vsDiffPlot.png')
    plot4.savefig(folderName+'/VelocityPlot.png')
    plot5.savefig(folderName+'/TrajectoryPlot.png')
    plot6.savefig(folderName+'/CircularitySize.png')

    #save data to excel sheets
    linkData.to_excel(folderName+"/TrajectoryDataOutput.xlsx")
    data.to_excel(folderName+"/AverageOutputData.xlsx")
    print('done')

#display processing time
print("END OF PROCESSING")
print("--- %s seconds ---" % (time.time() - startTime))
cv2.destroyAllWindows()

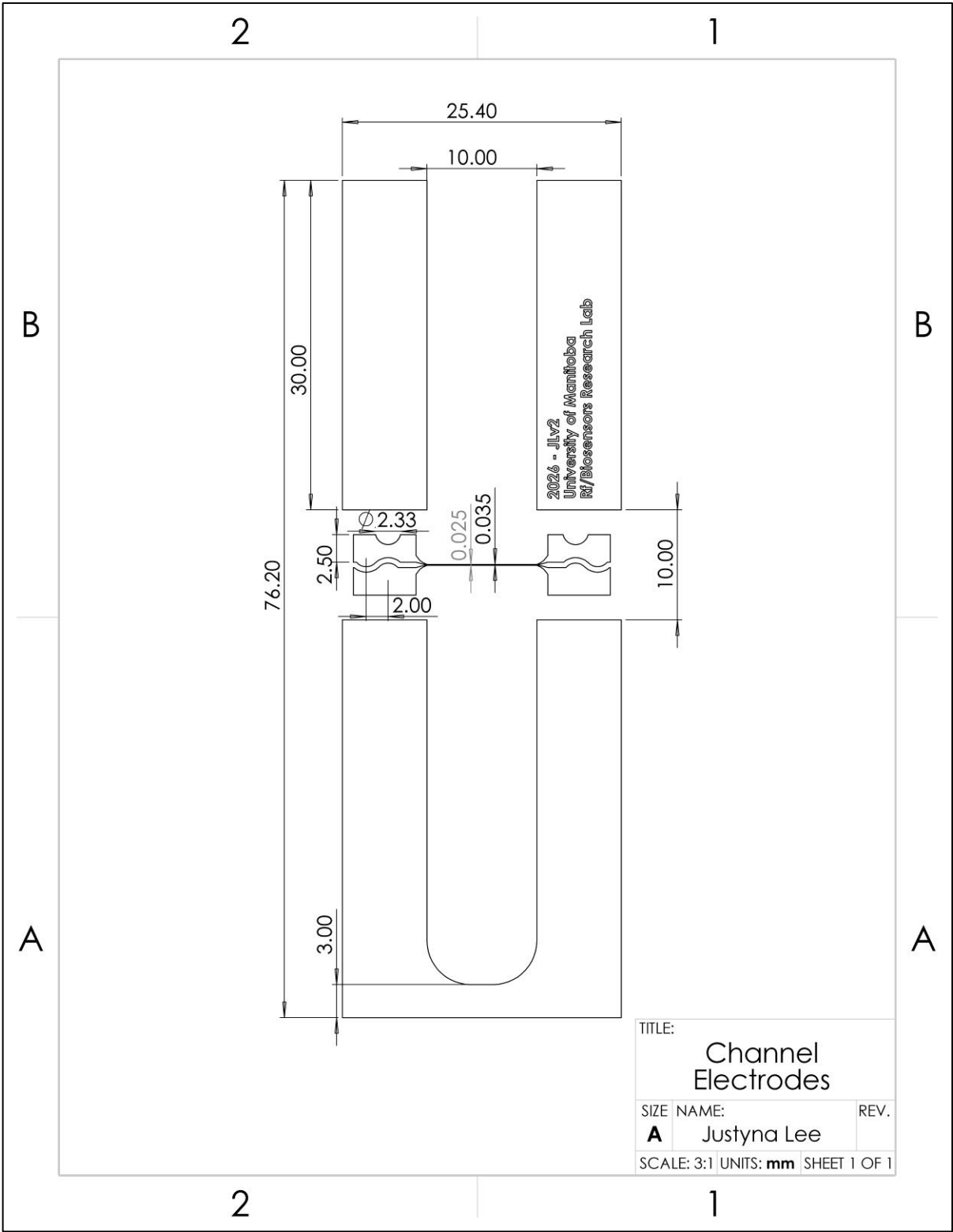
if __name__ == "__main__":

    video_file = ""
    save_folder = ""
    frequency = "1M"
    voltage = "6"
    ptype = 'PS' # 'PS' or 'CHO'

    tracker(video_file,save_folder,frequency,voltage,ptype)

```

Appendix B: Electrode Design



Appendix C: Drag Force Calculation

The figure from [48] is digitized to find the drag force acting on a sphere perpendicularly between two parallel planes with a constant particle size and channel height. In this figure, a is the particle radius, d is the channel height, and b is the height of the particle from the bottom of the channel.

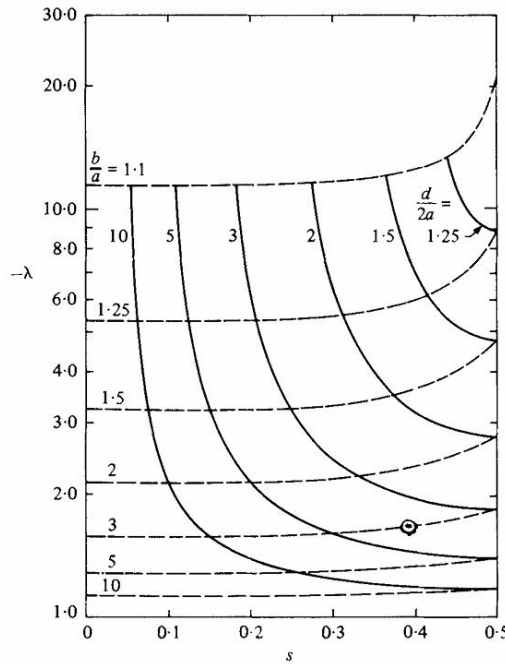


FIGURE 3. Drag on a sphere translating perpendicular to two plane parallel walls.
—, $d/2a = \text{constant}$; ---, $b/a = \text{constant}$.

(Reprinted from [48]. Copyright © © 1980 Cambridge University Press)

The digitized lines are used with the following code to find a fitted equation to be used in MATLAB with a particle diameter range from 10 to 16.667 μm in a 50 μm high channel. This process is based of the method performed by Behnam Arzhang.

```

%set particle size (nonviable = 11, viable = 12.5, PS = 10, PS = 15.7)
d = 15.7; %diameter
h = 50; %channel height
%import curves
data_r3=importdata('C:\Users\leej16\Documents\drag\r3.csv');
r3=data_r3.data;
data_r5=importdata("C:\Users\leej16\Documents\drag\r5.csv");
r5=data_r5.data;

%create mesh grid for interpolated lines
lines = 3:0.01:5;
l1 = [ones(length(r3),1).*3; ones(length(r5),1).*5];
x1 = [r3(:,1);r5(:,1)];
y1 = [r3(:,2);r5(:,2)];
[LI,YI]=meshgrid(lines, 1.4:0.001:11.3);
XI = griddata(l1,y1,x1,LI,YI);

%plot in 3d
figure
plot3(LI,YI,XI)
%plot in 2d
figure
plot(XI,YI)

%plot original lines
figure
plot(r3(:,1),r3(:,1),'b') %r3
hold on
plot(r5(:,1),r5(:,2),'r') %r5

%choose line from interpolated data
val = round((h/d)*100)/100;
idx = find(lines==val);
idx2 = find(~isnan(XI(:,idx)));
x11 = XI(idx2,idx);
y11 = YI(idx2,idx);

%fitting equation
p_11=fit(x11,y11,'rat32');
yp_11=p_11(x11);

%plot interpolated and fitted lines
plot(XI(:,idx),YI(:,idx),'g') %interpolated line
plot(x11,yp_11,'k') %fitted equation
legend('r3','r5','new curve','fitted curve')

%print equation
eq = formula(p_11);
c_names = coeffnames(p_11);
c_vals = coeffvalues(p_11);
for i = 1:length(c_names)
    eq = strrep(eq, c_names{i}, num2str(c_vals(i), 4));
end
newStr = replace(string(eq), "x", "(qy/hch)");
disp(newStr)

```