# Dielectrophoresis Characterization of Particles and Cells using Imaging Flow Cytometry

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***Abstract*— In this report, we present a microfluidic flow cytometer capable of dual-modality imaging/dielectric characterization of single biological cells. A high-speed camera captures and tracks multiple particles as they pass through a microfluidic channel and over a coplanar electrode array that applies a non-uniform electric field and induces dielectrophoresis (DEP) translation. Imaging and particle tracking provides particle morphology and velocity information. Multiphysics dielectric-fluid dynamics simulation is used to determine cell dielectric properties from velocity changes. The system is evaluated using polystyrene microspheres and demonstrated using Chinese hamster ovary (CHO) cells.**

***Keywords*– biological cell, dielectric-microfluidic simulation, dielectric spectroscopy, dielectrophoresis, microfluidics**

## I. Introduction

For clinical and diagnostic applications, rapid characterization of single cells in a large heterogeneous population is desired. Microfluidic devices are promising tools for rapid and real-time single-cell analysis [1]. One powerful technique for cellular separation, identification and manipulation is dielectrophoresis (DEP) [2], which is a label-free and non-invasive method based on the dielectric properties of biological cells [3]. Video flow cytometry uses a high-speed camera to capture cell images as they pass through a detection window and provide their optical properties, such as size and circularity [4, 5]. Both dielectric and optical properties are key indicators of a healthy or diseased cell.

We present a dual modality in-flow imaging/DEP particle analysis microfluidic system that provides information on both the morphology and the dielectric properties of every particle in a heterogeneous sample population. Particle imaging velocimetry of in-flow particles has previously been used, but with imaging employed only after DEP application [6]. We use a differential velocity DEP approach coupled with computational fluid dynamics for accurate particle analysis. We demonstrate measurement of polystyrene microspheres (PSS) and Chinese hamster ovary cells.

## II. Device Operation

The in-flow imaging cytometer is illustrated in Fig. 1. The device includes a microfluidics channel, light source and camera. A 50 µm deep, 8 mm wide channel, fabricated using double-sided tape sandwiched between glass slides. Two gold coplanar DEP electrodes, 35 µm wide with a 25 µm gap are patterned on the bottom slide. Two fluid ports are drilled into the top slide. Cells enter at the port and are imaged as they flow over the DEP electrodes.

Analysis samples with concentration of $5\text{x}10^4$ particles/ml are pumped through the channel using a syringe pump. An average fluid velocity 2000 µm/s, corresponding to a flow rate of 50 µl/min, is typically used. A FLIR Blackfly camera with a frame rate of 226 and 1.6 MP sensor is used to capture images over a 670 x 894 $\mu m^2$ field of view. An image processing and particle tracking algorithm, based on Track.py, uses a static background subtraction to locate moving particles, and an application defined threshold to ignore small objects, such as debris. Information, including velocity, diameter, circularity, and surface roughness for each particle is analysed. Differential velocity is then used for dielectric analysis.

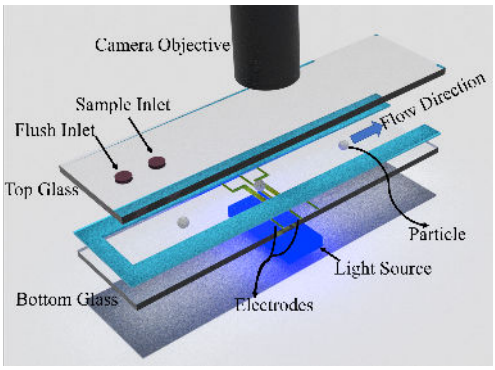


Fig. 1. Schematic diagram of the imaging-DEP flow cytometer, comprising camera, light source and microfluidic channel sandwiched between glass slides with fluid inlet ports.

## III. Dielectrophoresis Analysis

To determine the dielectric properties of cells, we use in-flow dielectrophoresis (DEP). When a polarizable particle is placed in a non-uniform electric field it will experience a DEP force. The magnitude and direction of the DEP force depend on the particle's polarizability and the medium surrounding it. For biological cells, this depends on the structure and dielectric properties of their compartments as shown in Fig. 2. For the spherical particles, the time-averaged DEP force on the particles is given by [7]

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_{rm}r_p^3 Re\{K_{cm}(\omega)\}\nabla(E_{rms}^{DEP})^2 \quad . \tag{1}$$

Here $r_p$ is the particle radius, $E_{RMS}^{DEP}$ is the rms value of the applied electric field at the particle's location. $Re\{K_{cm}(\omega)\}$ is the real part of the Clausius-Mossotti factor, given as

$$K_{cm} = \frac{\tilde{\varepsilon}_p - \tilde{\varepsilon}_m}{\tilde{\varepsilon}_p + 2\tilde{\varepsilon}_m} \quad , \tag{2}$$

where $\tilde{\varepsilon}_p$ and $\tilde{\varepsilon}_m$ are the complex permittivity of the particle and medium.

The real part of $K_{cm}$ is frequency dependent and exhibits different dispersive behavior based on the properties of the cell. We use a double-shell model involving eight dielectric and four geometric parameters. Fig. 2 shows the $\text{Re}\{K_{cm}\}$ spectrum for CHO as well as a polystyrene microsphere. If the

DEP frequency is chosen appropriately, the sign of $Re\{K_{cm}\}$ can be used to identify viable and non-viable cells.

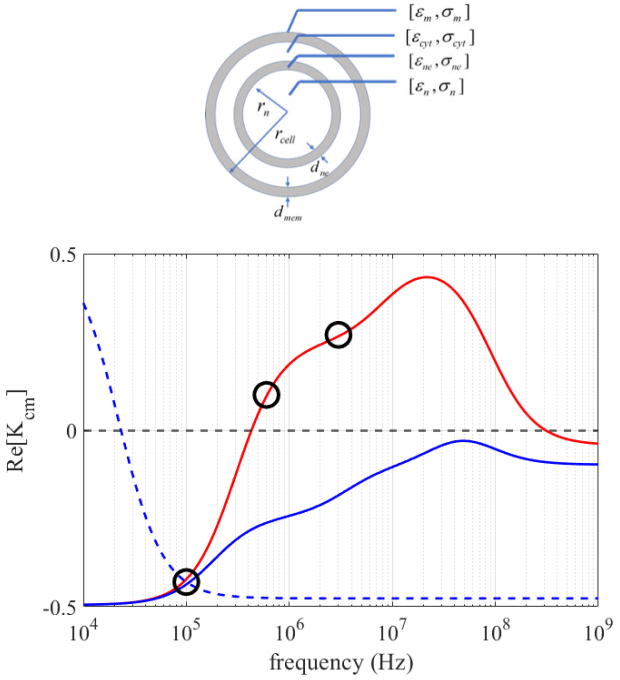


Fig. 2. Double-shell model of a CHO cell with radius, $r_{cell}$ = 6µm, nucleus radius, $r_n$ = 3µm , nuclear envelope, $d_n$ = 40nm , plasma membrane thickness, $d_{mem}$ = 5nm . $Re\{K_{cm}(\omega)\}$ spectra for viable (red), non-viable (blue) CHO cells and 15 µm diameter PSS with $\varepsilon_{rb}$ = 2.5 and surface conductivity, $\sigma_{surf}$ = 1 nS (dashed).

## IV. Results

The imaging region is centered over the electrode array so that particles can be tracked and analysed before and after DEP actuation, providing a differential velocity. As explained in the previous section, this is mapped to a differential force from which the sign and magnitude of $Re\{K_{cm}\}$ can be extracted. Results for a mixture of polystyrene microspheres of diameters 10 µm and 15 µm in deionized (DI) water is shown in Fig. 3. For an applied DEP frequency of 1 MHz, PSS has a $Re\{K_{cm}\} \sim$ -0.5 as shown in Fig. 2. The differential velocity can be used to distinguish the two sizes as the DEP force is a function of the cube of the radius (eqn. 1).

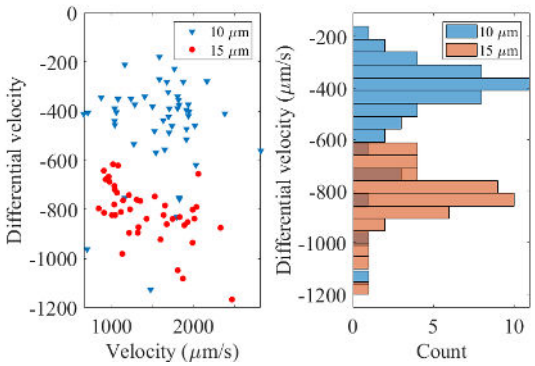


Fig. 3. Differential velocity versus incoming velocity for 10 µm (blue) and 15 µm (red) diameter PSS in DI water and differential velocity distribution. Blue and Red line are simulation results.

To demonstrate the ability to measure the DEP response of biological cells using the imaging cytometer, CHO cells were injected into the channel with an average flow velocity of 2000 µm/s. The differential velocity for DEP frequencies 100kHz, 600 kHz, 3 MHz and with $V_{pp}$ = 8 V are shown in Fig. 4. The differential velocity response matches the behavior of the spectrum predicted by the CHO cell dielectric model in Fig. 2. The distributions of the DEP responses in Fig. 4 are primarily due to differences in cell size. Unlike methods that employ only DEP analysis, the DEP imaging cytometer can provide information on individual cell size which enables a more accurate determination of $Re\{K_{cm}\}$.

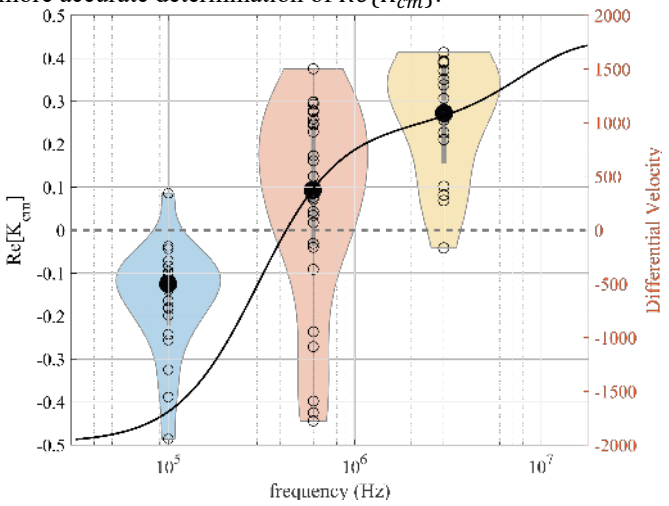


Fig. 4. Violin plots of the differential velocity for measured viable CHO cells at three different frequencies. The $Re\{K_{cm}\}$ spectrum from Fig. 2 is shown.

## V. Career Plan and Fellowship Impact

It is an honour to be one of the recipients of the IEEE Microwave Theory and Techniques Society (MTT-S) graduate fellowship award. This award has boosted my confidence and motivation to keep pursuing my research. Regarding my future career, I am planning to work in industry to gain some experience, and then return to academia, where I can contribute more to the Biomedical community.

## References


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