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Analysis of MDA-MB-231 Cancer Cell Dielectrophoretic Response

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ABSTRACT

Dielectrophoresis (DEP) is a phenomenon where a neutral particle can be either attracted to (pDEP) or repelled away from (nDEP) specific regions with highest electric field gradient. In previous work, we demonstrated the simulation and experimental work with yeast cell suspended in deionized water, where the result showed that yeast cells experience linear and rotational motion under DEP. In this work, we extend our work into the study of cancer cells, specifically MDA-MB-231 cell. While no DEP response is predicted at higher DMEM concentration, for MDA-MB-231 cells suspended in diluted DMEM (1%), the simulation shows that within frequency range of 1 kHz to 10 GHz, the cross-over frequency located at 80 kHz and 120 MHz, with cell demonstrates pDEP at frequency range between those two, while nDEP is at both frequency ranges less than 80 kHz and higher than 120 MHz. The following experimental work justify the simulation result, with the cell exhibits nDEP at 50 kHz, while pDEP is observed at 100, 200 and 500 kHz, among those selected frequencies. Due to substantial difference between the cell mass, the response time is hugely different with cancer cell shown significant reduction, however, the quantification is expected at future work.

Keywords: Dielectrophoresis, Dielectrophoretic Force, Cancer Cells, MDA-MB-231.

1. INTRODUCTION

DEP has been applied to various biological particles, implemented on the study of large bioparticles such as model organisms down to the smallest bioparticle known, that is, proteins [1]. This proved its applicability for the wide range of bioparticles, thus, advantageous into application for bio-clinical devices.

We performed study on a model organism, i.e. yeast, which reported in previous works [2], [3]. Yeast demonstrated exciting responses including linear motion as well as rotational motion [4]. In this work, we furthered our study onto a model of cancer cells, which is MDA-MB-231 breast cancer cells [5]–[9]. A DEP microdevice with 40μm-width slit gap Al microelectrode was fabricated. Here, we report the simulation and experimental work results, including the analysis and comparison with our previous work on yeast cells. It should be noted that cancer cell is a single shell bioparticle, consists of cytoplasm enveloped with plasma membrane. This is of distinguished structure from yeast cells, which possesses cell wall at the outermost layer.

The simulation demonstrates that the low frequency transition of pDEP-nDEP response of MDA-MB-231 cells and yeast cells occurs at close frequencies, i.e. 80 and 90 kHz, respectively, however, the pDEP-nDEP transition at high frequency is predicted at hugely different frequencies, i.e. 120 and 22 MHz [2], respectively. As the experimental setup suits for testing frequencies between 1 kHz to 2 MHz, we managed to justify the simulation result, where pDEP-nDEP transition at low frequency occurs between 50 and 100 kHz. It should be noted that MDA-MB-231 cells response

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time is significantly low, compare to yeast cells, perhaps due to substantial difference between cancer cells and yeast cells, including cell mass and shells structure.

2. METHODOLOGY

2.1 Real Part of Clausius-Mossoti Factor Calculation

Numerical simulations of the real part of Clausius-Mossoti Factor, Re[f_{CM}] was performed using Octave 4.0 (Figure 1). The calculation is essential to predict the dielectrophoretic response of cells, either pDEP or nDEP. It was found that cross-over frequency is predicted at f_{xo} = 80 kHz and 120 MHz.

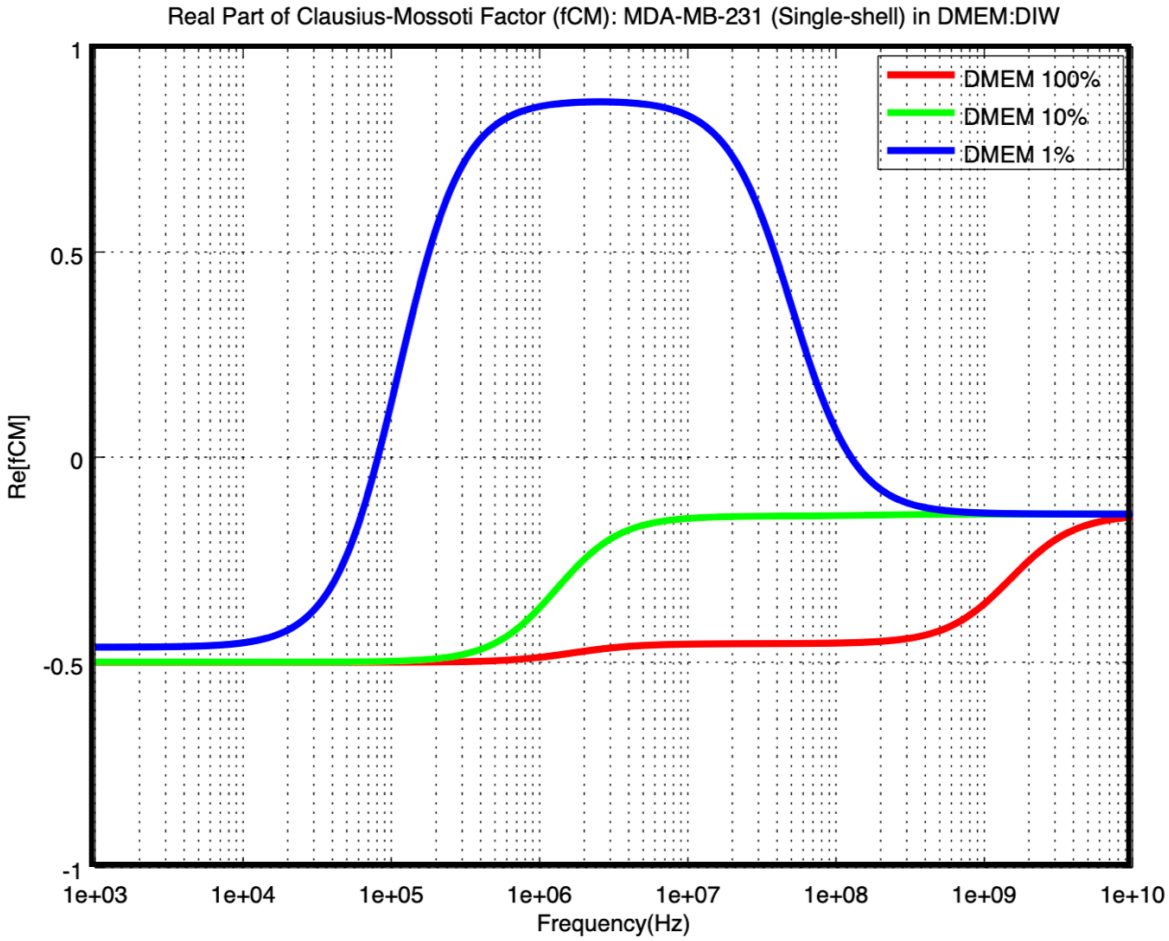
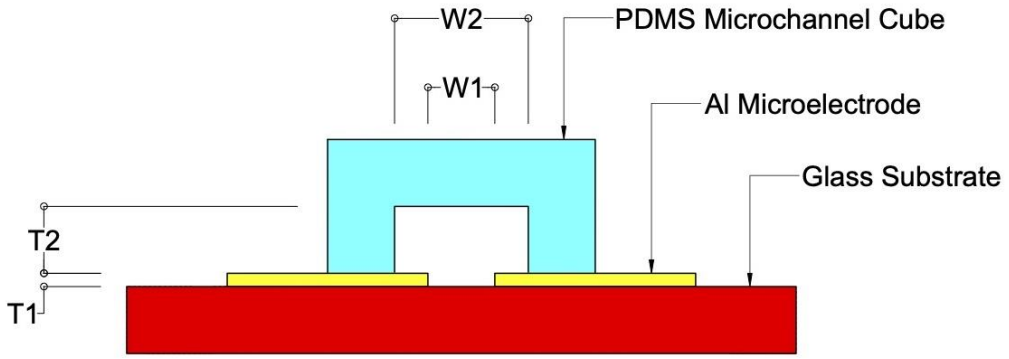


Figure 1. Re[f_{CM}] simulation result for MDA-MB-231 cell suspended in DMEM with various concentration (DMEM 100,10,1%). Cross-over frequency is located at f_{xo} = 80 kHz and 120 MHz. Cells potentially exhibit response to dielectrophoretic field at DMEM concentration of 1% and below, while at higher concentration, no response would be predicted.

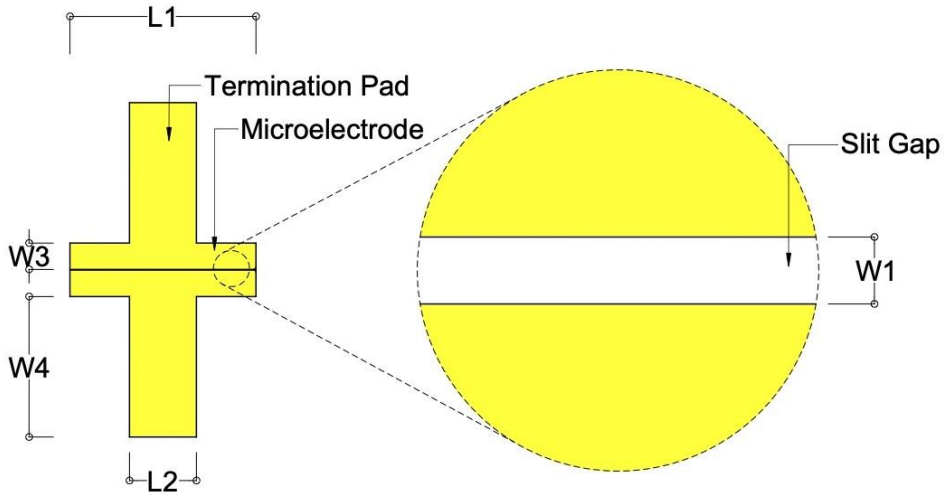
2.2 Device Fabrication

A dielectrophoretic microdevice with 150 nm-thick Aluminum microelectrodes was fabricated using standard photolithography process (Figure 2). The microelectrode with 40 μm-width slit gap configuration was intended to project highest DEP concentration region at the electrode edges, while lowest DEP region located at the center line parallel to the electrode edge (Figure 3). A PDMS cube forming a microchannel with 100x50μm (width x height) was attached on top of the electrode after plasma surface treatment to the PDMS interfacial surface. Inlet and outlet were

constructed with 0.5 mm-diameter holes connected with plastic tube. Copper wires were connected to termination pad using epoxy-based Silver adhesive paste for signal input.



Microdevice Cross Section View



Microelectrode Layout

Legend

T1: microelectrode thickness,	150nm	W1: microelectrode slit gap,	40μm
T2: microchannel height,	50μm	W2: microchannel width,	100μm
L1: microelectrode length,	14mm	W3: microelectrode width,	2mm
L2: termination pad length,	5mm	W4: termination pad width,	11mm

Figure 2. (Above) Cross sectional view of Microdevice. (Below) Layout of Microelectrode. Inset: Zoom view of the slit gap.

2.3 Sample Preparation

MDA-MB-231 cancer cells suspended in Dulbecco's Modified Eagle Medium (DMEM) 1% media were prepared by IMR researcher according to standard biological procedure.

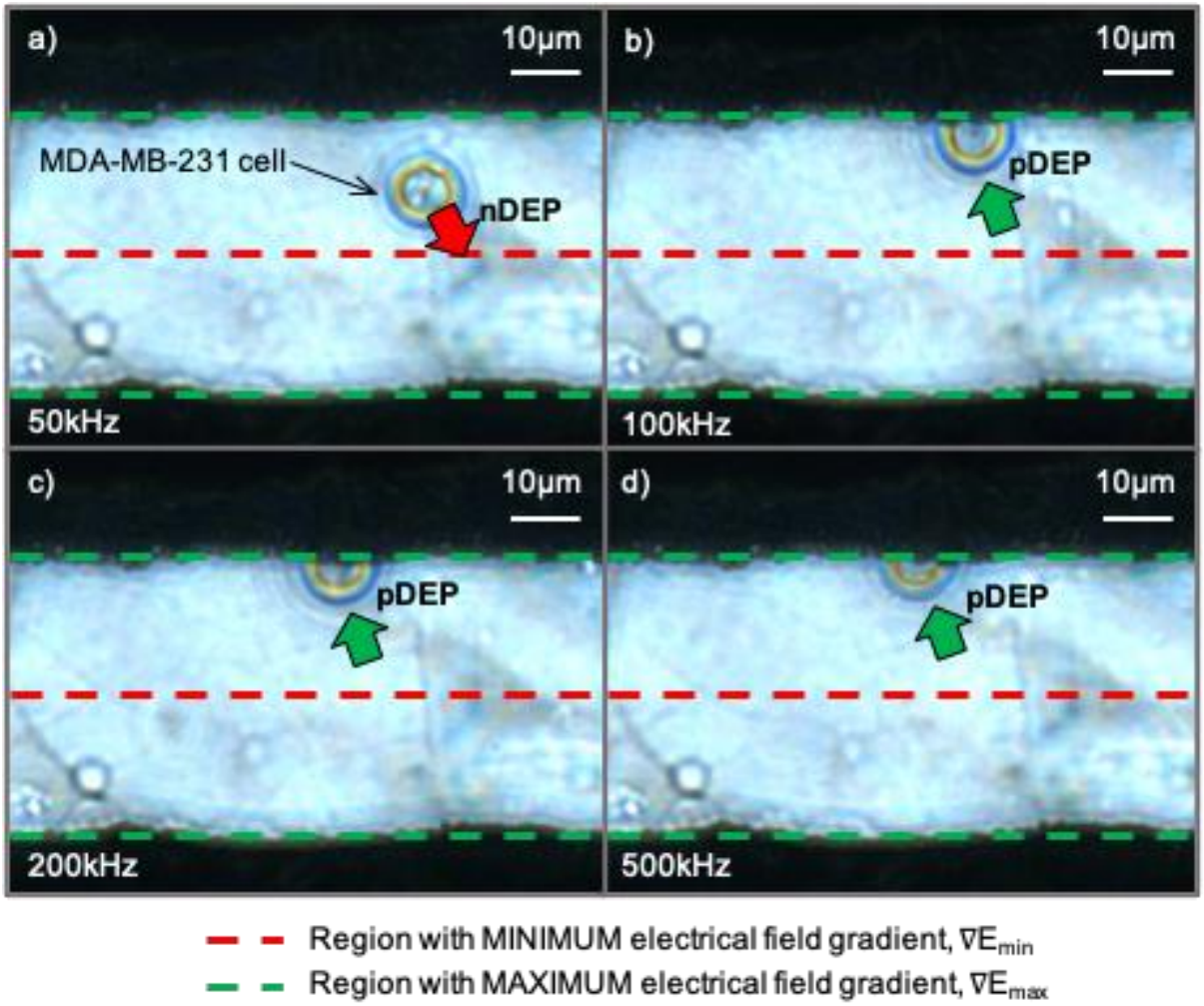


Figure 3. Dieletrophoretic response of MDA-MB-231 cancer cell. Negative DEP (nDEP) is exhibited at frequency a) 50 kHz (a), while Positive DEP (pDEP) is observed at frequencies b) 100 kHz, c) 200 kHz, and d) 500 kHz.

2.4 Experimental Setup

The DEP microdevice was mounted on optical microscope and the observation image was projected into a capturing computer software. A function generator was then used to apply electrical sinusoidal signal to the electrodes, which subsequently be monitored with an oscilloscope. Inlet tube was dipped into cell suspension in 1mL falcon tube, while outlet tube was connected to a syringe pump.

2.5 Observation

Syringe pump was operated to inject cells into microchannel from the cell suspension in falcon tube. The pump then stopped to provide non-flowing cells state, with buffer time 15min prior to signal application for cells to reach relative motional equilibrium. Electrical sinusoidal signal with voltage $20V_{pp}$ and frequency variant of 50, 100, 200 and 500kHz were applied to the microelectrode. Cells responses were then observed and recorded.

3. RESULTS AND DISCUSSION

Figure 1 shows the Re[f_{CM}] curve for MDA-MB-231 cancer cells in DMEM media with several concentration. It is well established that DMEM is the optimum suspending media for MDA-MB-231 cancer cells, however, by using the full concentration (100%) DMEM [8], which is shown by red color curve (bottom curve) in the figure, the cells might not response to the DEP field, due to high conductivity of the media, measured as approximately 8.4 S/m, contributed by high ionic contain. The cells perhaps hardly response to DEP field even at concentration as low as 10%, as dictated by the green color (middle) curve, where it doesn't cross with the x-axis line, i.e. Re[f_{CM}]=0. At DMEM concentration 1%, finally, there is potential response of the cells, with evident change or DEP response transition predicted at 80 kHz and 120 MHz, however, it should be noted that 120 MHz is beyond this work experimental setup.

Figure 2 shows our fabricated DEP microdevice for quick validation of the simulation result. This is crucial preliminary work before further research work upon this specific cell. It is worth to note that simple slit gap with proper microelectrode dimension (height) is sufficient to generate high electric field gradient at the electrode edge, against lowest region at the center line parallel to the electrode edge. Future work shall tailor the microelectrode design according to the desired configuration, for instance, to generate high DEP points, curves or surfaces etc.

Figure 3 presents the experimental work result. Aligned with our simulation, the nDEP response is demonstrated at frequency lower than 80 kHz, in particular, at 50 kHz in this work (Figure 3a). The cell is pushed away from the microelectrode edge (green dashed line) towards the lowest electric field gradient line (red dashed line), showing that the cells experience negative dielectrophoretic force. In contrast, at higher frequencies than 80 kHz, cells experience pDEP, where positive dielectrophoretic force attracted the cell towards the high electric field gradient region at the microelectrode edge (green dashed line). This is shown by experimental result at frequencies 100, 200 and 500 kHz (Figure 3b,c,d).

It is observed that the response time, i.e. the linear motion speed of cell is significantly low compare to yeast cell, perhaps due to their difference in cell mass, where the cancer cells volume is around 4-fold of the yeast cells. The cells diameter for cancer cell and yeast cell is around 10 and 5 μm, respectively. However, there may probably other factors that also might cause the distinction, which is worth to be studied in future work.

4. CONCLUSIONS

This work presented our extension study of DEP microdevice effect upon biological particles, in which yeast cell has been studied in previous work. We presented the current work, where MDA-MB-231, a model breast cancer cell has been selected. The simulation shows that rather than full concentration standard DMEM, diluted DMEM at 1% shall be used as suspending medium for the cells. Transitional response of pDEP-nDEP, and vice versa is predicted at frequencies 80 kHz and 120 MHz, though the latter is beyond our current setup. Experimental work with IMR collaboration demonstrated the capability of the application of DEP upon this cancer cell study, however, further modification to microdevice design and circuitry setup might be essential to improve the workability of the device, as well as to enhance the response time.

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