Design of a Dielectrophoretic Based Micropipette for Gene Expression Applications Using COMSOL

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Abstract: This research uses COMSOL Multiphysics® to design a micropipette for single -cell gene expression profiling. The micropipette design allows us to insert it into a single-cell to extract genes through dielectrophoresis. As the dielectriphoretic force depends on the applied electric field ($\boldsymbol{E}$) and its gradient (∇E^2), we have successfully used COMSOL to calculate $\boldsymbol{E}$ and ∇E^2 in the vicinity of the tip of the micropipette. Our design is capable of generating the highest ∇E^2 ever reported which enables a high-throughput gene extraction. Through a series of COMSOL calculations, we optimized the tip diameter of the micropipette for high-throughput gene extraction. COMSOL calculations were further used to determine the volume of the cytosol region where micropipette is capable of extracting molecules through dielectrophoresis for gene expression analysis.

Keywords: Dielectrophoresis, gene expression analysis, micropipette, COMSOL

1. Introduction

Tumor is a system of cells that consists of various cell-types including cancer stem cells, indolent cells, and stromal cells [1]. During development, these cells undergo several genetic/epigenetic changes. Therefore, to develop therapy for elimination, a full understand of those molecular changes is required. Currently, most of the molecular profiling is performed through gene expression assuming that all cells in the tumor are genetically identical. However, current literature reports indicate that this assumption is incorrect in the context of tumors [1]. To address this critical need, we have developed a technology that is capable of analyzing extremely subtle genetic changes in single-cell. Our technique is based on using dielectrophoresis (DEP) implemented on a micropipette tip.

Micropipettes have been used in various biological applications such as cell manipulation, detection and trapping of biomolecules, and detection of enzymatic reactions in single-cells [2-5]. In our single-cell gene expression profiling technique, the micropipette with fabricated electrodes is inserted into a single-cell to extract mRNA molecules (representative of genes) through DEP (Figure 1). A positive DEP is generated on mRNA molecules forcing them to move towards the tip of the micropipette. Finally, the micropipette with mRNA molecules is withdrawn from the cell, and mRNA molecules are collected into a micro-well by applying a negative DEP. The negative DEP are repelled the extracted mRNA molecules from the micropipette. Extracted molecules are quantified using traditional RT-PCR techniques [6].

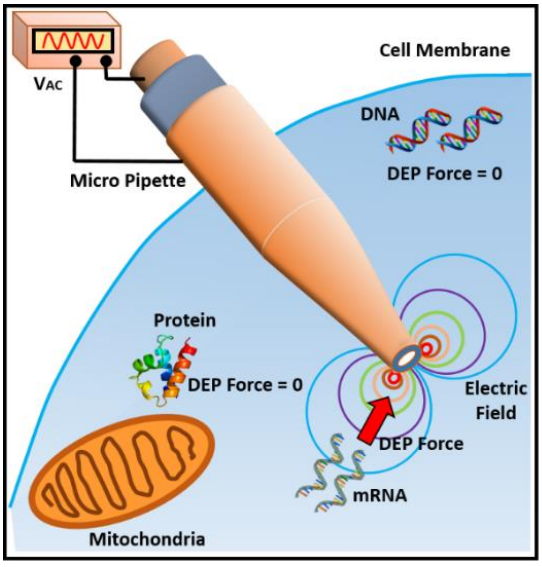

Figure 1. Schematic representation of DEP based single-cell mRNA extraction technique (not to scale).

2. Theory

2.1 Dielectrophoresis (DEP)

When a dielectric particle such as cell, mRNA and DNA is subjected to a non-uniform electric field, a force is exerted on the particle due to polarization effects and local electric field gradients [7-12]. This phenomenon is called dielectrophoresis [7-12]. The DEP force acting on a molecule can be written as,

$$F_{DEP} = \frac{1}{2}\alpha\nabla(E^2) \quad (1)$$

,where α is the total effective polarizability of the molecule, $\boldsymbol{E}$ is the magnitude of the AC electric field, and $\nabla(\mathrm{E}^2)$ is the gradient of E^2 [6, 9]. Since α is frequency dependent, frequency of the electric field can be tuned so that the polarizability of the molecule is higher or lower than the polarizability of the medium. According to the definition of DEP, if the suspended particle has a higher polarizability than medium, DEP will push the particle towards regions of high electric field (positive DEP) [10, 11]. In the other hand, if the suspended particle has a lower polarizability than medium, DEP will push the particle towards low field regions (negative DEP) [10, 11]. Therefore utilizing a suitable frequency, mRNA molecules can be selectively move onto the apex of the micropipette when the micropipette is inside the cell. After that, using negative DEP, mRNA molecules are deposited on micro-wells for quantification.

2.2 Capture Volume

When micropipette is inserted into a cell and an AC electric field is applied between the electrodes, the mRNA molecules in the cell will experience a positive DEP force. But, the DEP must be sufficiently large to move mRNA molecules toward the tip by overcoming other forces such as viscos drag, Brownian motion and electrostatic forces [11]. Since DEP decreases with the distance from the micropipette tip, there must be a cell volume where DEP is large enough to exert a sufficient force to overcome other forces—called capture volume. The capture volume is defined as the region that satisfies,

$$\frac{1}{2}\alpha E^2 \geq KT \quad (2).$$

K is the Boltzman constant and T is the absolute temperature [6].

3. Use of COMSOL Multiphysics

We have used COMSOL Multiphysics® to calculate electric fields and electric field gradients around the micropipette tip. In addition, we have also used COMSOL calculations to design the critical dimensions of the micropipette for efficient capture of mRNA molecules from cells.

3.1 Geometry and Materials

A two dimensional axis-symmetric model was developed using COMSOL Multiphysics® 5.0. The geometry used in the calculations are indicated in Figure 2(a) and the modeling parameters are given in Table 1. Materials properties were assigned to the geometry including cell (cytoplasm) and micropipette [12]. The materials properties used in the model were indicated in Table 2.

3.2 Physics and Study

The electric circuit physics under AC/DC module was employed to calculate the electric field and its gradient. It has been demonstrated that 120 kHz is the proper frequency for efficient capture of mRNA molecules from single-cells [6]. Therefore, we have used 120 kHz in our simulations. Briefly, an AC voltage (1Vpp, 120 kHz) was applied between electrodes, and the model was meshed using a user-defined dense mesh. The meshed model is shown in Figure 2(b). Finally, a frequency domain analysis was performed at 120 kHz.

First, the variation of electric potential was calculated. Using the potential, we have also calculated the variation of E and $\nabla(E^2)$ in the vicinity of the micropipette tip assuming micropipette was inserted into a cell. The calculated variations of E and $\nabla(E^2)$ are shown in Figure 3. Moreover, we used COMSOL model to optimize the tip diameter, so that the micropipette can generate a high DEP. A series of calculations were performed by varying the diameter of the apex to observe the variations of $\nabla(E^2)$ and E with tip diameter (Figure 4). In addition, using these results, we also calculated the capture volume. Figure 5 illustrates the calculated capture volume when the micropipette is in a cell.

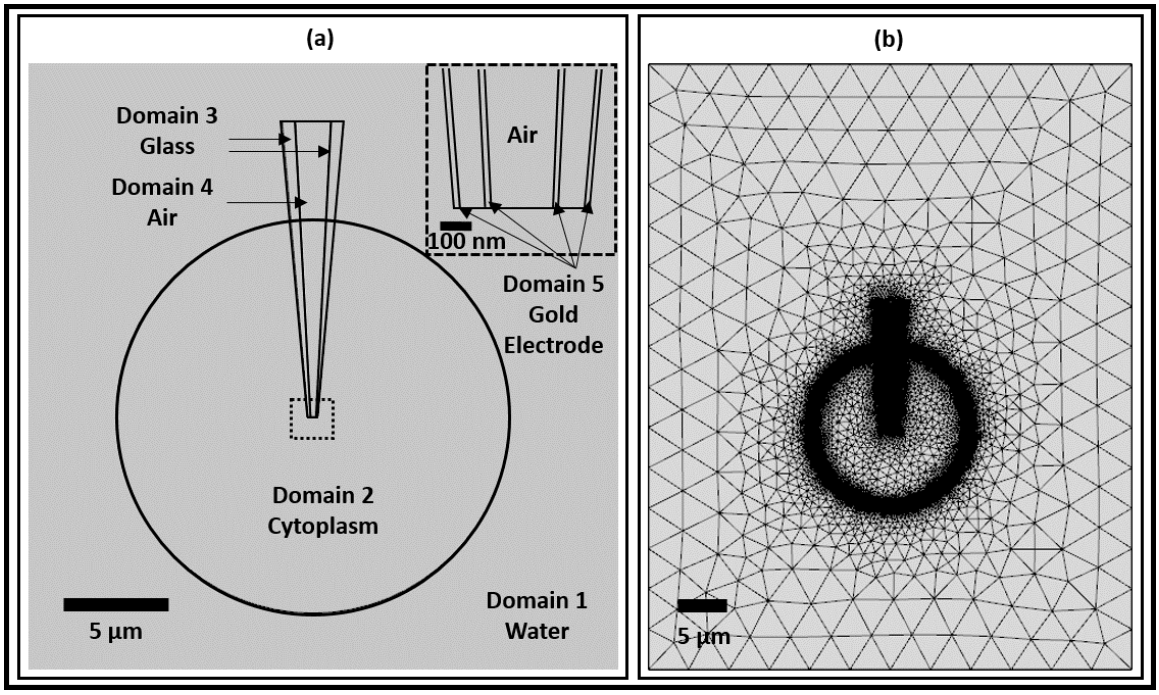

Figure 2. COMSOL Mutiphysics® was used to simulate the micropipette inside the cell. This was performed using a user defined free triangular dense mesh generated for finite domain analysis. (a) The geometry of the model; inset provides details at the apex of the micropipette. (b) User defined highly dense mesh of the system

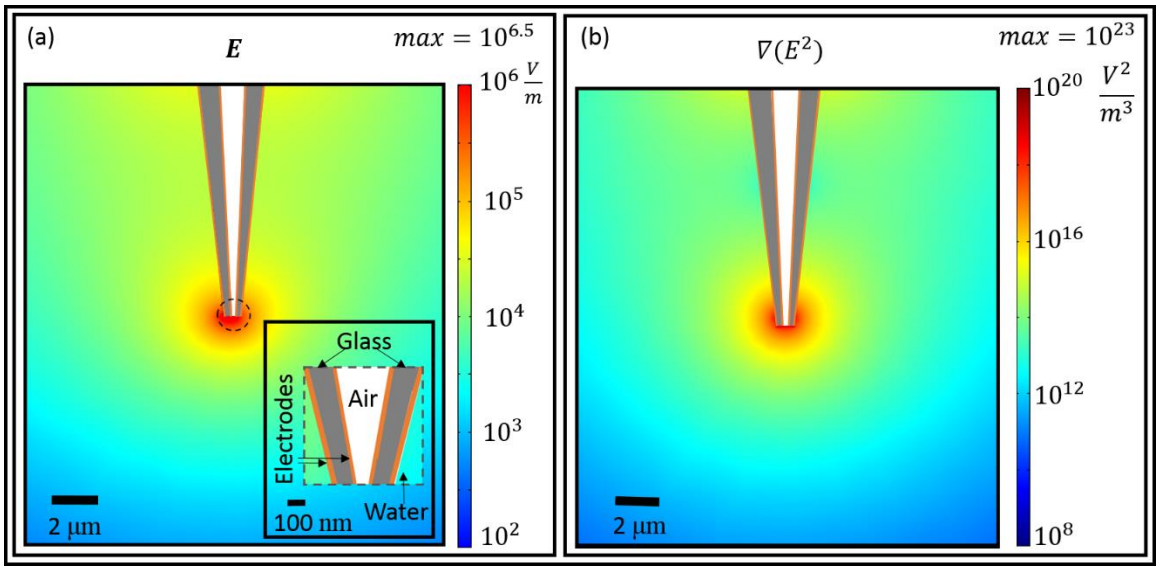

Figure 3. Frequency domain analysis performed at 120 kHz, revealed a higher $\boldsymbol{E}$ and $\nabla(E^2)$ in the vicinity of the micropipette. (a) Variation of $\boldsymbol{E}$ (b) variation of $\nabla(E^2)$ near the micropipette assuming micropipette was placed inside a cell.

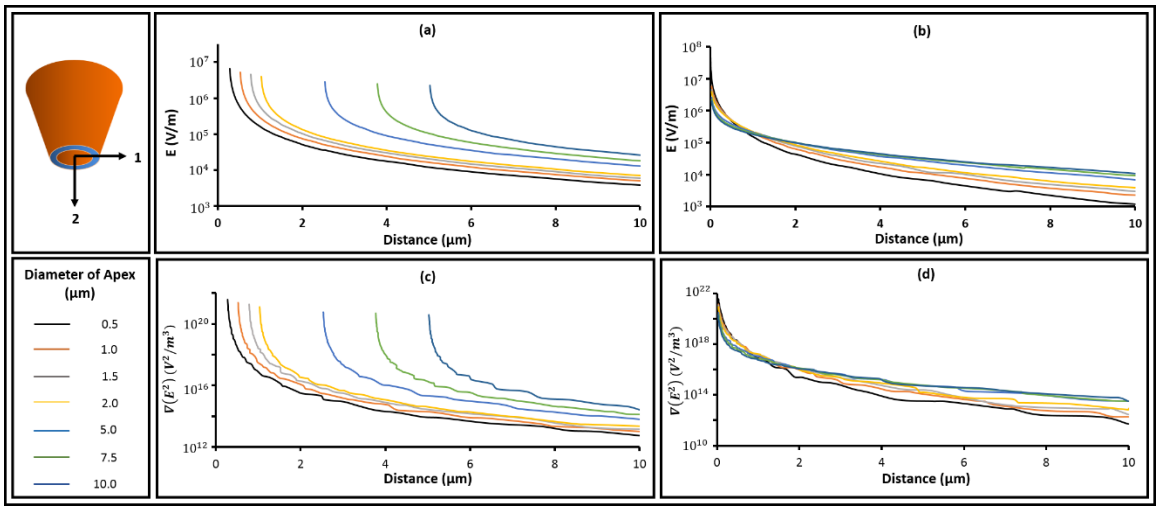

Figure 4. Through COMSOL calculations, a larger $\boldsymbol{E}$ and $\nabla(E^2)$ were observed in micropipettes with smaller diameters. (a) Variation of E along horizontal direction (line 1). (b) Variation of E along vertical direction (line 2). (c) Variation of $\nabla(E^2)$ along horizontal direction (line 1). (d) Variation of $\nabla(E^2)$ along vertical direction (line 2).

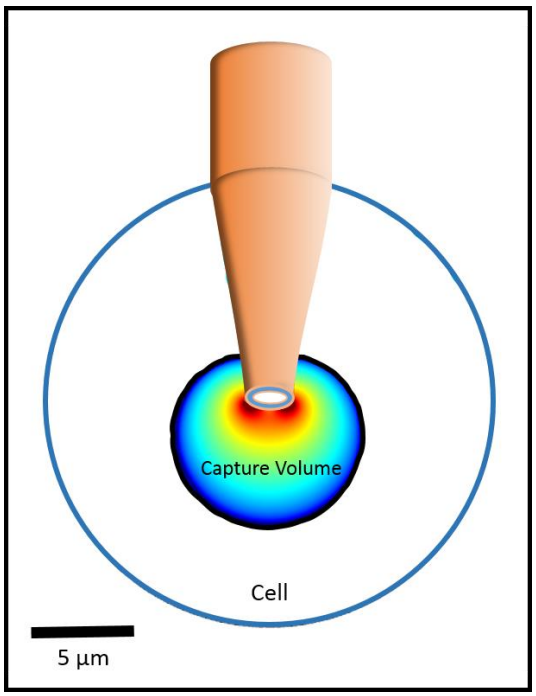

Figure 5. Capture volume of the micropipette. The micropipette will effectively capture mRNA molecules from the capture volume. The capture volume is indicated in the rainbow colors and the outer boundary of the cells is indicated in blue.

Parameter	Value
Radius of the cell	10 μm
Outer diameter of the micropipette at the apex	0.5 μm
Outer diameter/inner diameter ratio	1.724
Angle of the outer surface	5^0
Thickness of the gold layer	20 nm
Height of the micropipette	15 μm

Table 1: The model parameters used in the COMSOL Multiphysics® design

Domain	σ at 120 kHz (S/m)	ε_r at 120 kHz
Domain 1: Water	5×10^{-3}	80.3572
Domain 2: Cytoplasm	4.8×10^{-3}	60-j719
Domain 3: Glass	1×10^{-14}	5.3-j0.02756
Domain 4: Air	0	1
Domain 5: Gold Electrode	4.10×10^{7}	1

Table 2: Properties of the materials used in the each domain of the COMSOL Multiphysics® design. Properties of the cytoplasm was taken from [13].

4. Results

Our COMSOL model analysis indicates that a large $\nabla(E^2)$ can be expected to produce a large DEP on mRNA molecules. To the best of our knowledge, this is the largest $\nabla(E^2)$ ever reported and it is very important to generate a large $\nabla(E^2)$ in order to have a high-throughput gene expression profiling. The analysis of E vs. diameter of the micropipette and $\nabla(E^2)$ vs. the diameter of the micropipette suggests that an outer diameter should be around 0.5-2.0 µm to capture mRNA molecules efficiently. In addition, calculated capture volume is about 22% of the total cytosol volume.

5. Conclusion

We have successfully utilized AC/DC module to calculate $\nabla(E^2)$ and E in the vicinity of a micropipette tip when it is inserted into a single-cell. Our calculations indicate that a larger DEP force can be expected on mRNA molecules forcing them towards the tip of the micropipette. Further, an optimized range of the diameter at the tip of the micropipette was calculated. We have also calculated the capture volume of the micropipette. Currently, experiments being performed to find other important parameters such as range, throughput and sensitivity of the technique.

6. References

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