

Design and Simulation of a Novel Dielectrophoresis based Device to Separate Breast Normal and Cancerous Cells

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Abstract. Dielectrophoresis (DEP) is the motion of particles toward or away from the electrodes which are induced by AC voltage or current. Live cells have different electromagnetic and dielectric properties which can be used for separation. In this paper, we present a novel device to separate breast cancerous cells from normal ones based on dielectrophoresis (DEP). The device uses a matrix of electrodes on a silicon substrate where cancerous cells remain on the electrodes and normal cells move away from electrodes. For the condition of electric field frequency of 1GHz and the medium conductivity of 5 μ S/cm, the simulated device shows a complete separation. First, we discuss about principles of DEP, and then electromagnetic and dielectric properties of breast normal and cancerous cells are discussed in order to understand separation mechanism. Finally, simulation results of the device will be presented.

Keywords: dielectrophoresis, cell separation, electrode matrix, COMSOL Multiphysics 3.5a

1. Introduction

The integrated biological analysis systems have received an increased attention in the areas of point-of-care (POC) diagnostics, food pathogen screening, environmental monitoring and biomedical research for drug discovery by providing automated and potentially portable solutions to a wide range of fluid-base solutions [1].

Dielectrophoresis (DEP) has been extensively studied for cell separation because it does not require a labeling process and DEP devices can be fabricated easily using solid-state technologies. Moreover, cell separation using DEP has many applications since it can separate cells on the basis of physical changes occurring inside the cells such as a variation in cytoplasmic conductivity or the presence of an extra membrane [2]. For example, cell separation can make possible life-saving procedures such as autologous bone marrow transplantation for the remediation of advanced cancers where the removal of cancer-causing metastatic cells from a patient's marrow is necessitated [8]. DEP is an extremely effective means of manipulating particles of diameters between 100 nm and 10 μ m and offers an excellent choice for separation, isolation, and concentration of cells [7]. Most implementations of dielectrophoresis utilize micro electrodes to produce the necessary electric-field gradients.

In the previous DEP devices [3, 4], cell mixture was injected into the separation channel and the electric field was applied. After separation, hydrodynamic force washes out the negative DEP cells. Then, the electric field was turned off and remaining cells were collected. Markx and Pethig [5] tried to automate the above-mentioned process using valves for the separation of positive and negative DEP cells. This process used the discontinuous cell mixture flow controlled by valves and it required the larger electrode area for a high throughput cell separation.

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In this work, we propose a fast and high throughput device to separate breast normal and cancerous cells. The device uses only DEP forces to separate cells. By the means of a matrix of electrodes –instead of a row of electrodes—performance of the device significantly increased compared to previous works.

2. Theory

An electric field with a DC value produces electroosmosis and electrophoresis in microchannel which is filled with a conductive liquid. Electroosmosis is flow produced by electric field acting on the net mobile charge in the fluid within the electric double layer surrounding the charged surface of the microchannel [7]. Similarly, electrophoresis is the transport of mobile charges because of an electric field. Both electroosmotic and electrophoretic transport are linearly proportional to the local electric field, and the superposition of these can be termed as electrokinetic flow [7]. Ideal electrokinetic flow is a special limiting case of the combined transport in which the electrokinetic velocity field (u_{ek}) is everywhere proportional to the local electric field (E), as given by [7]:

$$u_{ek} = m_{ek} E = u_{eo} + u_{ep} = (u_{eo} - u_{ep}) E. \quad (1)$$

In (1), u_{ek} , u_{eo} , and u_{ep} are the electrokinetic, electroosmotic, and electrophoretic mobility, respectively. In ideal electrokinetic flow, particles flow along *electric field lines* [7]. Ideal electrokinetic flow can't concentrate or deplete particles in a uniform electric field distribution.

Fig. 1 illustrates the basic principle of the dielectrophoresis process. The AC signals applied to electrodes generate the DEP force which makes cells move in the mixture flow in the direction where the DEP forces and directions are dependent on cell properties. Due to the different DEP force directions, the cells with different DEP responses move continuously to the different locations.

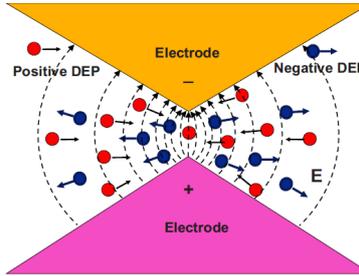


Fig. 1: Basic principle of DEP.

DEP is the movement of cells in the non-uniform electric field. When cells are subjected to non-uniform electric field, charges are induced at the interfaces resulting in electrical polarization along the direction of electric field.

If the electric field is uniform, then the electrostatic forces acting on opposite ends of the dipole are equal and there is no net movement, unless cells carry a net charge and the electric field frequency is equal to zero. However, if the field is non-uniform, then the forces on either side of cells will be different, and the net DEP force can induce movement of cells [2].

If electrical polarizability of cells exceeds that of the suspending medium, DEP force is the same direction as the gradient of electric fields (Fig. 1). In this case, cells move to the strong electric field region (positive dielectrophoresis or pDEP). On the contrary, when the electrical polarizability of cells is less than that of the medium, the direction of DEP force is reverse to the gradient of electric fields (Fig. 1) and cells move to the weak electric field region (negative dielectrophoresis or nDEP). The polarizability of cells depends strongly on their composition, morphology, phenotype and the electric field frequency [1]; therefore, the cells of different types or physiological states including viability can be discriminated by the DEP. The time-averaged DEP force is given by [4, 6]:

$$F_{DEP} = 2\pi\epsilon_m r^3 \text{Re} [f_{CM}] \cdot \nabla |E_{rms}|^2. \quad (2)$$

In (2), r is a radius of cell; ϵ_m the permittivity of the medium; f_{CM} the Clausius–Mossoti factor and E_{rms} is the root mean square value of an electric field. $\text{Re}[f_{CM}]$ means a real part of the f_{CM} which can be represented as follows [4, 6]:

$$f_{CM} = (\epsilon_p^* - \epsilon_m^*) / (\epsilon_p^* + 2\epsilon_m^*). \quad (3)$$

In (3), ϵ^* is the complex permittivity ($\epsilon^* = \epsilon - j\sigma/\omega$); σ the conductivity and ω is the electric field frequency. Subscripts p and m mean cells and the medium, respectively. $\text{Re}[f_{CM}] > 0$ means that cells show pDEP response while $\text{Re}[f_{CM}] < 0$ means nDEP response.

As illustrated in Fig. 2, except for viruses, all living materials consists of cells that have a similar structure consisting of cytoplasm and a membrane. In many cases (plants and most microorganisms), the cell is further enveloped by a negatively charged cell wall [9] which tries to collect positive ions from the surrounding medium.

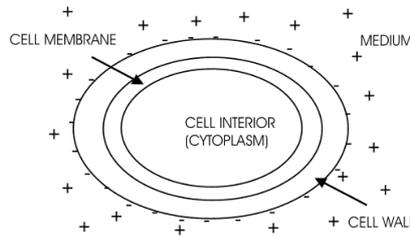


Fig.2: Basic structure of a living cell.

As (2) showed earlier, DEP force depends on size of the cell. So, big cells would experience larger DEP force while small cells would experience smaller DEP force. Also, DEP force depends on frequency of applied voltage or current. To understand this correlation, consider that two different cells have similar characteristics but different sizes. The larger cell needs more time to collect positive ions from the medium. In the context of frequency ($f = 1/T$), this means that larger cell would experience pDEP in lower frequencies. Similarly, the smaller cell needs less time to collect positive ions, so it would experience pDEP at higher frequencies. Note that both cells (large and small) at the start of the process are naturally negatively charged and would experience nDEP until whole wall of the cell is covered by positive charges.

In a research [8], above explanations were tested practically on normal and cancerous human breast cells. Research result is shown in Fig. 3 below. Obviously, cancerous cells had an experience of pDEP sooner than normal cells. Considering that cancerous cells are larger than normal breast cells, the results agreed with previous paragraph's statements. Also, note that both normal and cancerous cells experience nDEP at the start of the process.

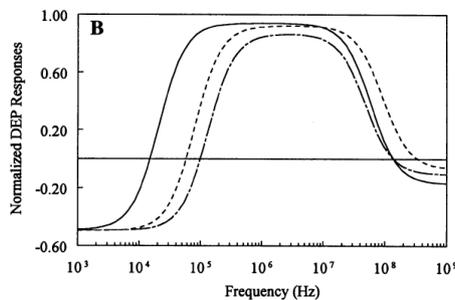


Fig. 3: DEP response for cancerous (—) and normal (- - - and — —) breast cells [8].

3. Simulation

Fig. 4 shows final design of the device. A matrix of 6×6 squares which had diameters of $100 \times 100 \mu\text{m}^2$ was used to simulate the electrodes. Design of top right and bottom left electrodes was changed to obtain

maximum DEP force efficiency. The biological fluid (fluid which consists of cells) was supposed to have a 5×10^{-6} S/m electric conductivity (σ) while electrodes' conductivity was 4.52×10^7 S/m.

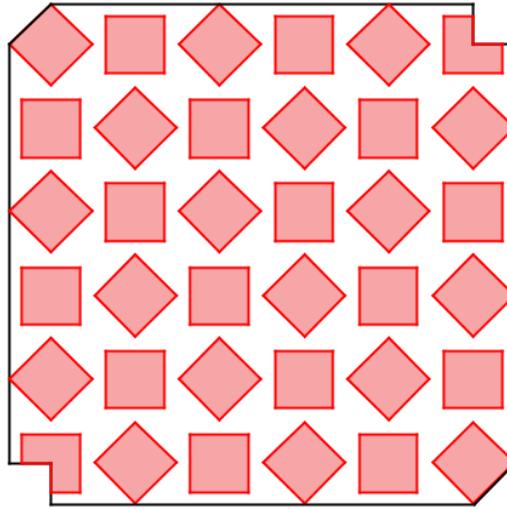


Fig. 4: Final Design of the device.

Fig. 5 shows simulation results; simulation was accomplished by COMSOL Multiphysics 3.5a. A $f_0 = 2\pi \times 1$ GHz was defined as a constant frequency. Then, using “AC/DC Module”, this frequency was introduced to the electrodes as a sinusoidal voltage. The amplitude of the applied signal was supposed to be ± 5 V. All side boundaries of the device were kept in electric insulation condition to achieve a real response.

Considering effect of DEP force on the cells with different sizes (Fig. 3), we can understand from Fig. 5 that cancerous cells move toward the electrodes while normal cells stay between spaces of the electrodes. By using an appropriate counting method, cancerous cells can be counted, so stage of the cancer can be estimated and necessary treatments would be accomplished to avoid tumor growth.

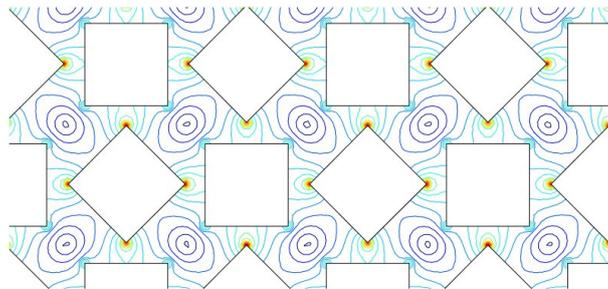


Fig. 5: Simulation result of the device.

It's interesting to mention again that the simulation was done in 1 GHz frequency. So, low frequency problems, such as high currents which can damage cells, do not exist in this device. Also, by increasing the frequency, amplitude of the applied voltage was decreased and compared to similar works [1, 3, 5], with same voltage amplitude stronger DEP forces were obtained. The electric potential changed from 9.443×10^6 to 2.927×10^8 V/m in this model. Compared to previous works [1, 3, 5] our device showed 1000 times stronger DEP forces. This means that by applying a constant voltage to electrodes, this device would separate cells faster and is more accurate. Also, instead of using a channel as conventional devices, a chamber was used. This means that there is no need to use other forces, such as hydrodynamic force, to achieve an acceptable separation result. DEP is the only involved force in this device, so extra equipments are unnecessary to attach to the device. Thereby, total fabrication cost would be much lower than similar devices.

4. Conclusion

Today, dielectrophoresis (DEP) is widely used in biological applications such as cell separation. Living cells, because of different electromagnetic and dielectric properties, respond differently to an AC non-uniform applied electric field. This means that by an appropriate design of electrodes, different cells can be separated, sorted, and counted. In this work, we presented a novel device for cell separation using dielectrophoresis. A ± 5 V sinusoidal wave form was applied to the electrodes and electric field distribution was studied as discussed in previous section. As illustrated before, cancerous cells move toward the electrodes, so by the means of a suitable counting mechanism, a general estimation of the cancerous cells can be obtained. Thereby, stage of the cancer can be specified and related treatments would be accomplished. Special design of electrodes resulted in a device 1000 times better than previous works. Also, there is no need to employ other forces, such as hydrodynamic force, to increase speed of this device i.e. only DEP is enough to thoroughly separate cells. So, instead of a channel, a chamber can be used for separation process and total fabrication costs would decrease compared to conventional separation devices.

The simulation was done in 1 GHz frequency which was higher than similar works and problems of low frequencies would not exist in such high frequency. Future work includes attaching a cell counter to the device, so real-time separation and counting of cells would be accessible.

5. References

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