MODELING OF DIELECTRPHORETIC SEPARATION PLATELETS FROM RED BLOOD CELLS

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Abstract

The cell separation has been playing a major role in many biological and medical applications. In this paper, modeling and simulation of dielectrophoresishas been carried out using COMSOL Multiphysics v5.0. Dielectrophoresisis a unique approach to separate platelets from red blood cells (RBCs). It occurs when the force is exerted on a dielectric particle under a non-uniform electric field. It has significant impact on the size, shape and dielectric properties of the particles. The theoretical cell direction has been determined by numerical simulations of flow speed and electrical field. This permits dielectrophoresis to be utilized to isolate various types of particles, for example, different sorts of cells from a mixture. The red blood cell partitionfrom blood using dielectrophoresis has been modeled and simulated by means of applying different electric fields/voltages, velocities for inlet & outlets, frequency and amplitude. Itshows how red platelets can be specifically shifted from a blood test so as to segregate red platelets. Dielectrophoresishas many applications in the field of biomedical devices used for biosensors, diagnostics, filtration, particle assembly, particle manipulation and etc.

Keywords: Red blood cell separation, platelets, dielectrophoresis, electric field, microfluidics.

1. Introduction:

There has been growing demand for the investigations on new biomedical techniques and subsequently development of ultrasensitive medical devices.More specifically,numerous devices are being used to separate platelets from blood cells with good sensitivity. Various techniques have been utilized for separation, mainly between platelets (PLTs) and red blood cells (RBCs), including centrifugation [1], mechanical filtering [2], and antibody recognition [2]. Although, these techniques are macro-scale techniques, and have the following disadvantages: (1) The process of non-continuous separation which takes more time for analysis, [3] The requirements of a large size and adjust the samples, and [4] the requirement of well-trained staff to work with enormous and expensive tools [5-9]. Traditionally, micro-scale cell separation techniques take advantage of the variation in the intrinsic characteristics of the population of the different cell to accomplish separation. Considering above mentioned disadvantages, a typical approach Dielectrophoretic effect is chosen for the efficient separation of platelets from redblood cells. Dielectrophoresis (DEP) happens when a force is applied on a dielectric particle as it is exposed to a non-uniform electric field. The DEP force is delicate to the size, shape, and dielectric properties of the particles.The proposed system is able to separate platelets from blood utilizing a single-phase and low-voltage system adding micro-channels in the composition of the H-filter with DEP separation. The system is modeled using COMSOL Multiphysics v 5.0 to get optimized results for high sensitivity based separation of platelets by means of applying different electric fields/voltages, velocities for inlet & outlets, frequency and amplitude.

2. Materials and Methods:

The modeling of any device using COMSOL Multiphysics consists of i. Design of geometry, ii. Addition of Materials, iii. Addition of suitable physical interfaces and iv. Simulation.

2.1. Design of Geometry

The proposed device consists of two rectangular inlets, two rectangular outlets, and a separation region. In the region of separation, there is an arrangement of alternating polarity electrodes which control the trajectories of the particles. The square shape electrodes were added to the top of the separation region so, the non-uniform electric field needed for utilizing the dielectrophoretic effect. The geometry has been constructed using two rectangles, two mirrors, one square, array, and fillet. The details of dimensions of different rectangles, square, array and fillet for the proposed model geometry are tabulated below. The fig.1 shows the proposed geometry.

Dimensions (µm)	**Rectangle-1**	**Rectangle-2**
Width	280	40
Length	40	200

Table 1: Dimensions of Rectangles

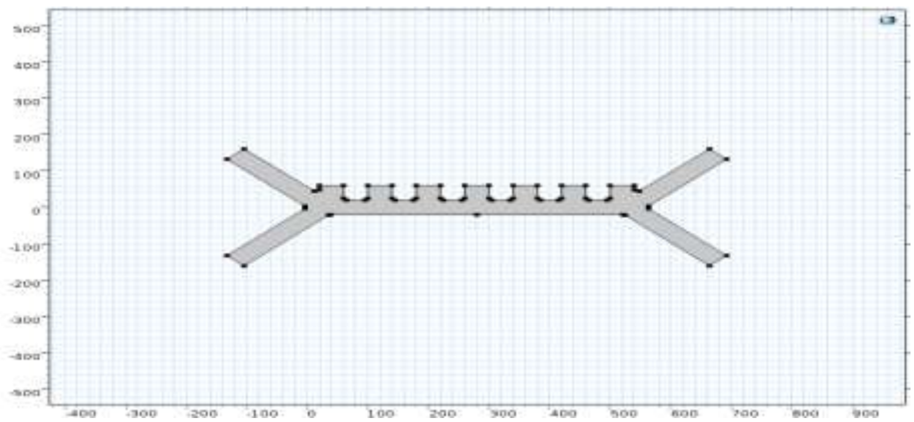

Fig. 1: 2D Geometry of proposed dielectrophoresis model

This device having two inlets and outlets. The geometry of the device's channels and electrodes were defined in the graphics section of the COMSOL package. Sample and buffer inlets with channel widths of 40 µm and length 200 µm respectively, merge into the 560 µm width and 40 µm duration separation tube. The collection outlets have the same size as two inlets, then pick the correct materials for each domain and set the electrical potential at the limit of each electrode.The dielectrophoretic voltage is applied on the "liquid electrode" which is placed on the left side of the top of the channel in the seperation region.Dielectrophoretic force repels the larger white blood cells (WBCs) and red blood cells (RBCs) on the right channel, while the smaller platelets (PLTs) are not adequately deviated and deposited on the left channel.

2.2 Materials:

From the built-in materials library of COMSOL software, required materials are selected and added to the required parts of the model in order to convert virtual design into solid design. Silicon, Germanium, Copper and so many combinations of alloys are available in the software. Mostly silicon is used to manufacturing the MEMS based technological devices. The physical properties such as electrical conductivity, relative permittivity, density&dynamic viscosity of all the materials [10]are considered.

2.3Physical Interfaces:

This model is carried out in particle tracing which analyzes the continuous separation of platelets from red-blood cells using dielectrophoresis. Here, we are using three different physical interfaces; electrical currents, particle tracing and creeping flow for fluid-flow. The Electric potential +5v and -5v are applied to the positive and negative electrodes respectively using proper boundaries. The two inlets and one outlet is used in creeping flow.

3.Simulation and Results:

Using DEP-field flow fractionation(FFF), the system proposed is used to isolate platelets from the blood. The sample is injected together with phosphate-buffered saline(PBS) in to the inlets. Thepressures is applied at the inlets to achieve required cells on the left side of the channel. The dielectrophoretic voltage is then applied between the two liquid electrodes as shown in below Fig.3(a).The 10 V_{pp} voltage at 100 kHz is applied to validate the operation of the device. Fig. 2 shows the trajectory of the RBCs and PLTs in the system, obtained by overlaying consecutive frames of a video taken in the separation region at 20 frames per second with the microscopically mounted CCD camera. Due to negative dielectrophoresis, the model shows a clear repulsion of the RBCs away from the electrodes and making the big cells exit the separation region in the right channel. The separation region on the left collection channel. The concentration of PLTs used in this experiment is high, rather than the concentration of RBCs to help visualize the trajectory of the platelet, otherwise it is difficult to see because of the small size of PLT. Due to their low concentration no WBC has been observed.The flow speeds used at the top and bottom of the inletsare 134 and 853 μm/s, respectively. Since of the parabolic flow pattern, cells are focused towards the middle of the inlet.

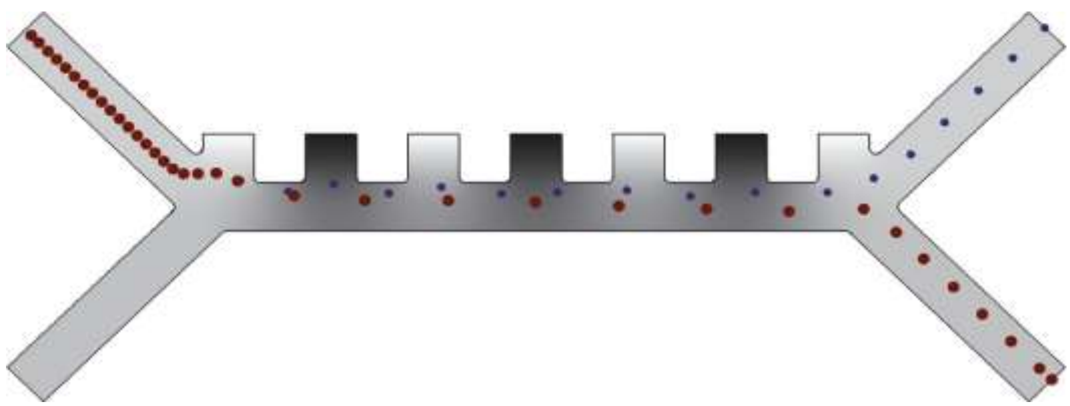

Fig 2: Visualization of the PLTs and RBCs trajectory.

After adding physical interfaces and selective meshing, then the simulation carried out. Here, we are applying electric currents to the micro-channel. If the device having alternating polarity of electrodes, then this model will have initial frequency 100 kHz and the electric field is applied to this device such that whole device will have electric field for the total time. After applying, the dielectrophoretic force (n-Dep), both the inlets and outlets having high voltages (red color) as shown in below fig.3.If we apply the DEP force, then the electric filed is maximum in the inlets, outlets and positive electrodes, which were shown in red color. But, in the separation region, under the (+) ve electrode, reduced electric field is seen because field appliedat (n-Dep). The negative electrodes will have low electric field, since it helps to move the particles away from high field regions, which is known as negative DEP (or nDEP). It will be shown in blue color. The logical cell path was derived from the modeling results demonstrated in this paper. By generating a non-uniform electric field and arranging the alternating polarity of electrodes, the particle paths were altered. Simulations results were carried out in COMSOL finite element simulation package.

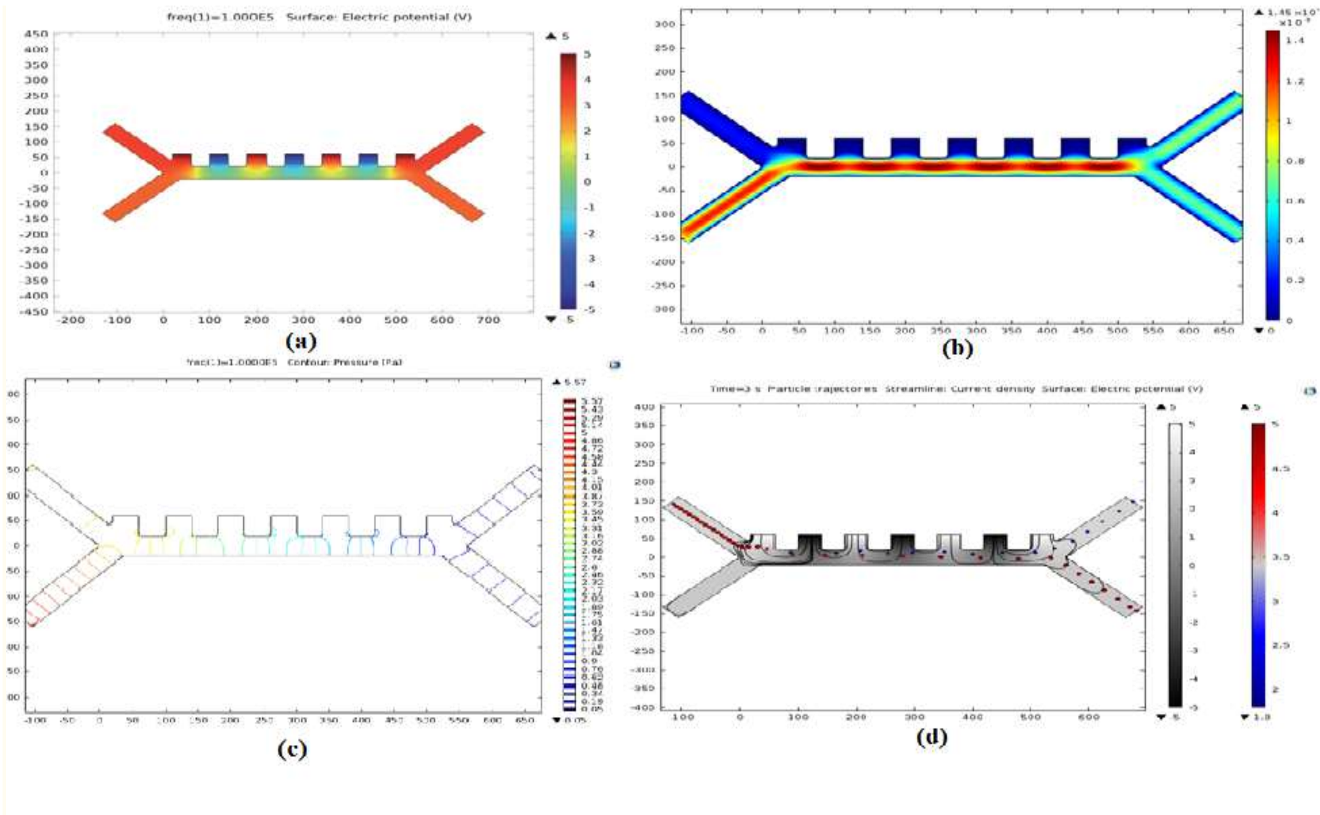

Fig.3.The microfluidic channel by applying(a)ElectricPotential,(b)Velocity,(c)Pressure and(d)pressure and velocity.

As seen on the Fig.3, the velocity for the lower inlet is significantly higher (853 µm/s) than the upper inlet (134 µm/s) in order to focus all the injected particles towards the upper outlet. The velocity difference among separation region (maximum velocity) and collection region (minimum velocity), particles easily approach towards collection region subject to applied electric field with initial frequency 100 KHz, It is observed that the maximum potential is found to be 1.45 x 10^{-3} mV respectively.

The dielectrophoretic force is then realized between adjacent liquid electrodes, as shown in Fig.4. The inlets having high pressure in the injection region and there should be gradual decrease of pressure in the separation region that enables accurate focusing of cells. The reduction of pressure (pa) in the collection region is taking place in following steps.

1. In the inlet, it varies from 5.57 to 4.0
2. In the separation region varies from 4.0 to 1.04
3. In the collection region is totally reduces its having <1.0.

If the pressure is applied to the inlets, gradually decreases in the micro-channel and finally the zero pressure in the outlets.The frequency used for this initial model is 100 kHz, with negative DEP (n-DEP) effect on each platelet and red blood cell. Nevertheless, due to the difference in size, the dielectrophoretic force is tougher on red blood cells than on platelets.It shows three variations as mentioned below.

1) Separation of Blood Cells w.r.t frequency Change
2) Separation of Blood Cells w.r.t amplitude Change
3) Separation of Blood Cells w.r.t flow speed Change

This model used different studies like stationary, frequency and time dependent domains. This plot is a time-dependent solver which varies a time-step and reciprocal of step-size.

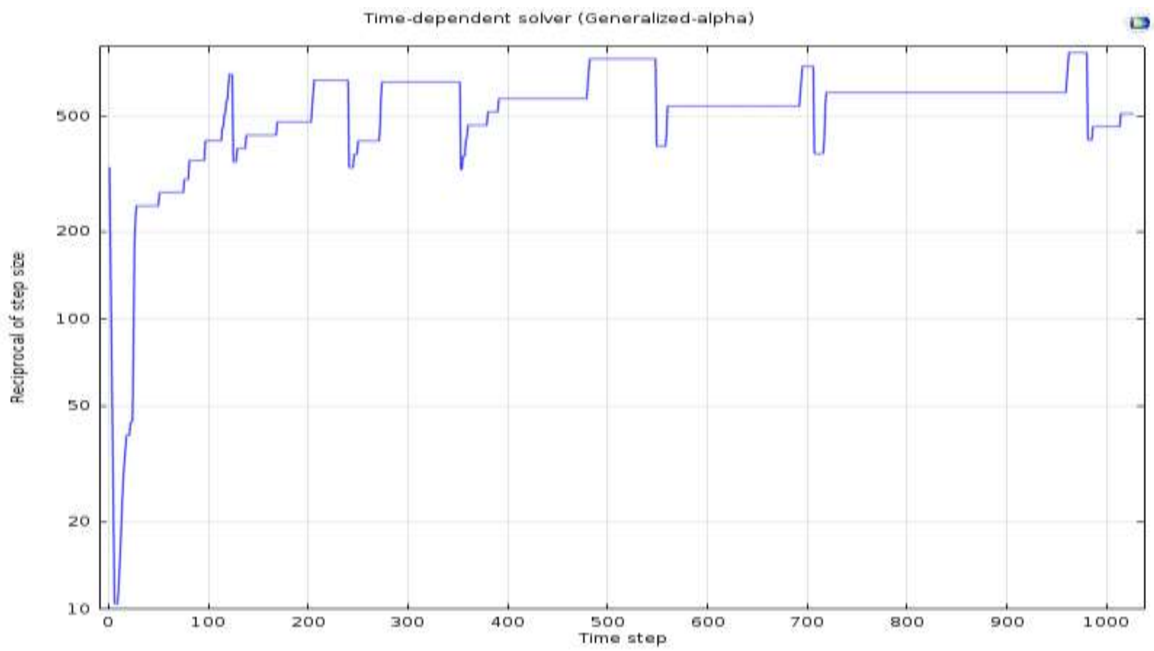

Fig.4. Time-dependent plot w.r.to 100 kHz frequency.

i. Separation of Blood Cells w.r.t frequency Change

Under constant applied voltage of 10Vpp, flow speeds of inlets and various frequencies ranging from 100 kHz to 1MHz with 200 kHz interval, separation of blood cells is modeled. The proposed system could differentiate the platelets from other blood cells. Separation efficiency was found to be 98.8% at 100 kHz and 97% at 1MHz. If the frequency issmaller than 100 kHz or the frequency increased from 10MHz to 1GHz, the particles were not isolated and followed a similar path with significant separation efficiency as shown in Table2.

Inlets Flow Speed (μm/s) (Upper-Lower)	**Frequency**	**Separation (Efficiency)**
134-853	<100kHz	Non- Separated
	100kHz	Separated (98.8%)
	>100kHz to1MHz	Separated (98.8%to 97%)
	10MHz to 1GHz	Non- Separated

Table 2: Effect of frequency change with amplitude 10 V_{PP}

ii. Separation of Blood Cells w.r.t amplitude Change:

On the unit a second variation was produced using various electrode voltages at a frequency of 100 kHz. The simulations showed that the system was not able to distinguish platelets from other blood cells when applying voltages below 10 VPP and above 15 VPP and followed a similar path, but it was capable of separating the PLTs using only 10 VPP and 15VPP.

Amplitude(V_{PP})	**Inlets Flow Speed(µm/s) (Upper- Lower)**	**Separation (Efficiency)**
5	134-853	Non-Separated
15		Separated (99.6%)
20		Separated but non-uniform (96.1%)
24		Non-Separated

Table 3: Effect of amplitude with 100 kHzfrequency

iii.Separation of Blood Cells w.r.t flow speed Change:

A flow rate is varied at the upper inlet of 134 to 300µm/s and increasing the flow rate by 1300µm/s lower inlet, the proposed system was still capable of cell separation, but was unable to separate platelets when the flow rate increased from 400µm/s and above.The best cell separator should have 15v peak to peak(PP),100 kHz frequency and 150µm/s (upper inlet) and 850µm/s (lower inlet) flow speed 15 V_{PP} voltage used by adjacent electrodes at 100 kHz, 150 and 850 µm/s flow rate at the upper and lower inlets, respectively. These optimized parameters would improve the device's separation efficiency with resultant separation efficiency of 99.8% for the modified design parameters.

Amplitude(V_{PP})	**Inlets Flow Speed (µm/s)(Upper- Lower)**	**Separation (Efficiency)**
10	150 - 850	Separated (98.9%)
10	134-1300	Separated (97.3%)
10	500-1200	Separated (96.3%)
10	400-1300	Non- Separated
10	500-1500	Non- Separated
15	150-850	Separated (99.8%)

Table 4: Effect ofchange of flow speed at 100 kHzfrequency

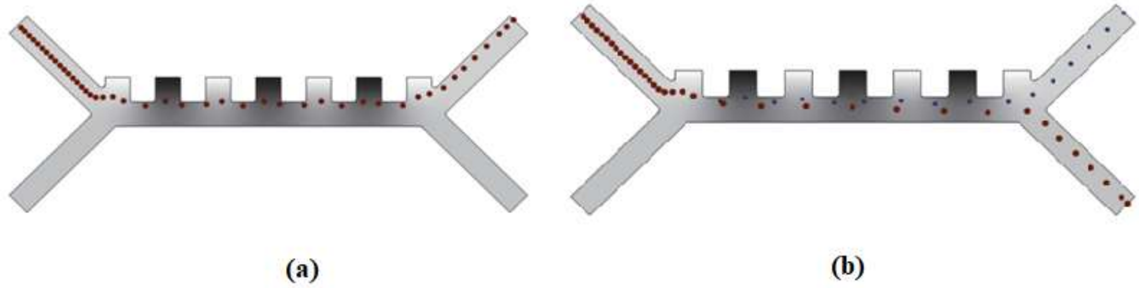

Fig.5: (a)Without DEP Force (b) With DEP Force

4. Conclusion:

The design of MEMS based "Dielectrophoreticseperation of platelets" was made by using COMSOL multiphysics. Here, the platelet gets divided with the applied electric field (n-DEP) in turn separation taken place. In this method, a modified solution is applied to separate platelets from blood utilizing dielectrophoresis field flow fractionation. The micro-fluid device utilized adding fractionation functions and pre-focusing to trap cells into distinct places relying on their size specifically. The particle paths were altered by creating a non-uniform electric field and by arranging the electrodes of alternating polarity. This sorting principle could also be integrating with a system sorting cells based on their "opacity." The 2D finite element model was exploited to test variations of the design parameters, including the applied separation voltage, frequency, and flow inlet speeds. An electric voltage of 15V is extremely suitable for this modified design to efficiently separate red blood cells and platelets. This modified design can achieve separation with higher efficiency of platelets from RBCs, getting increased by 99.8%.

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