

Design and Simulation of Two-Stage Dielectrophoretic Separation for Various Biomolecules in Lab-on-Chip Applications

S Vinurajkumar¹, S Ramya², P Mohanapriya³, S Santhosh⁴

Department of Medical electronics Engineering,
Saveetha Engineering College, Chennai

Abstract - Nowadays, there is a burgeoning development of microfluidic platforms catering to point-of-care diagnostics within the biomedical realm. The segregation of blood cells and plasma stands as a crucial aspect of medical diagnosis, particularly in the analysis of diseases associated with thrombocytopenia, anemia, and leukopenia. To address this need, a novel Double-microfluidic device has been introduced, focused on the continuous separation of platelets (PLTs), red blood cells (RBCs), white blood cells (WBCs), and plasma utilizing the negative dielectrophoresis (DEP) force principle. The device design, explored using COMSOL Multiphysics 5.5, features triangular micro-tip electrodes generating a nonuniform electrical field with minimal AC voltage. Through negative DEP force, blood cells are deflected towards their respective outlets, thereby enhancing separation purity and efficiency. Experimental results indicate optimal conditions at a blood sample flow velocity of 50 $\mu\text{m/s}$ and buffer solution flow velocity of 109 $\mu\text{m/s}$ with a 1 Vpp electrode voltage. This innovative approach holds promise for advancing biomedical diagnostics in a range of clinical settings.

Keywords: blood cell separation, dielectrophoresis, double microfluidic device, RBC, WBC, Platelets.

I. Introduction

Human blood, consisting of platelets (PLTs), red blood cells (RBCs), white blood cells (WBCs), and plasma, serves as a vital indicator of overall health. Abnormalities such as thrombocytopenia (low PLT count), viral infections (low WBC count), or leukemia (excessive WBC count) can be detected through blood analysis. Microfluidic technology offers a promising avenue for blood component separation, minimizing complexities associated with conventional methods like gradient centrifugation. Lab-on-a-chip devices leverage microfluidics, employing both active and passive mechanisms for efficient separation. Active techniques such as acoustophoresis, magnetophoresis, and dielectrophoresis (DEP) manipulate particles based on physical properties like size and electrical conductivity. Passive methods, including hydrodynamics and filtration, also contribute to effective cell separation. Previous studies [1] have explored various microfluidic designs, achieving notable success in plasma separation or individual cell isolation. However, comprehensive blood analysis necessitates simultaneous separation of blood particles and plasma. To address this gap, this paper proposes a double-microfluidic device integrating negative DEP force for separating PLTs, RBCs, and WBCs while continuously diluting plasma. Through simulations using COMSOL Multiphysics 5.5, we optimized design parameters such as electrode configurations, voltages, and flow velocities to ensure

efficient blood cell and plasma separation. This innovative device aims to streamline blood analysis, facilitating comprehensive diagnostic insights with minimal complexity.

II. Materials and Methods

In our study, we focus on the separation of three distinct cell types within a two-stage Y-channel microfluidic device. These cell types include white blood cells (WBCs), platelets (PLTs) and red blood cells (RBCs) are separated. The physical and electrical properties of these target cells are crucial for modeling and simulation purposes, as summarized in Table 1, drawing upon relevant literature sources [2]. This comprehensive understanding of cell properties facilitates accurate modeling and simulation, enabling the optimization of the separation process within the microfluidic device.

2.1 Targeting cell types

In this study, three distinct cell types are under consideration following of red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs). Essential physical and electrical properties of these targeted cells are detailed in Table 1, drawing upon pertinent sources for reference [3]. This comprehensive understanding of cell characteristics serves as a crucial foundation for modeling and simulation, facilitating accurate analysis and optimization of the lab-on-chip (LOC) device for

efficient separation.

2.2 Modeling and Simulation Setup

For our research, we have opted to utilize COMSOL Multiphysics® version 5.5 as our simulation software of choice. This selection stems from the software's exceptional flexibility in integrating the necessary multiphysics capabilities required for modeling the lab-on-chip (LOC) device. With COMSOL Multiphysics®, we can seamlessly combine fluid flow modeling, electric current analysis, and particle tracing functionalities. This comprehensive suite of capabilities enables us to accurately simulate and analyze the behavior of the LOC device, ensuring robust performance in separating the three cell species: red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs).

2.3 Computational Model

The primary force driving cell manipulation within the system is the dielectrophoretic force, induced by the electric field. This force is pivotal in directing the movement of cells based on their dielectric properties. The relationship between electric potential and electric field can be expressed as follows:

Parameter	WBC	RBC	Platelets
Cell diameter	14µm	6µm	1.8µm
Intracellular conductivity	1 S/m	0.18 S/m-1	0.25 S/M
Cell relative permittivity	50	59	50
Cell electrical conductivity	7e-7 S/m	9e-6 S/m	1e-6 S/m
Cell relative permittivity	12.5	10	6

Table 1: Targeting cells values

$$E = -\nabla V \quad (1)$$

Where:

- E represents the electric field vector.
- V denotes the electric potential.

This relation highlights how changes in electric potential across space generate an electric field, which in turn influences the movement of particles, such as cells, within the system.

The current within the system is determined by Ohm's law, which relates the electric field (E), conductivity (σ), and current density (J) as follows:

$$J = \sigma E \quad (2)$$

Where:

- J represents the current density vector.
- σ denotes the conductivity of the medium.
- E signifies the electric field vector.

This expression elucidates how the current density is influenced by both the electric field and the conductivity of the medium.

The fluid flow within the microchannels of the LOC is modeled using creeping flow physics, which employs the Navier-Stokes equations without considering the inertia term. This type of flow, often referred to as Stokes flow, is characterized by low Reynolds numbers. The primary equations governing this flow are:

$$0 = \nabla \cdot [-pI + K] + F, \text{ and,} \quad (3)$$

$$\rho \nabla \cdot u = 0. \quad (4)$$

The Di electrophoresis (DEP) force is mathematically described by:

It shows if a particle is drawn toward or away from the electrodes. The following expression represents the real part of CMF, where σ_p and σ_m stand for the particle and medium electrical conductivities, respectively, and ϵ_p is the particle permittivity. The previously outlined physics are solved in the following order using the COMSOL Multiphysics software package:

1. Handle the creeping flow;
 2. Adjust for currents in the electrical system.
 3. Tracking particles using the two physics from earlier.
- It should be mentioned that this model only takes into account the fluid-cell interaction and ignores the two-way cell-cell contact. A common drawback in particle trajectory.

Boundary Conditions

The boundaries of the Lab-on-a-Chip (LOC) enforce a no-slip Dirichlet condition, ensuring the confined flow within the channels. Flow rates at the buffer inlet are specified at constant 109µm/s, while the blood inlet provides options at 50µm/s. Simultaneously, a static pressure condition, consistently set at 0 Pa, is imposed at the outputs [4].

Here, pI represents the pressure multiplied by the identity matrix, K denotes the viscous stress tensor, F is the volume force vector, ρ is the fluid density, and u signifies the fluid velocity field. The dielectrophoretic (DEP) force arises from the net polarization forces induced in a nonuniform electric field (NUEF). When electrically neutral particles are exposed to such a field, they become asymmetrically polarized, leading to the generation of DEP force. In uniform electric fields, this

force is null, whereas in NUEF, it can be either positive (pDEP) or negative (nDEP), depending on the arrangement of electrodes, as depicted in Figure 1. The equation governing the DEP force is provided by COMSOL software properties.

$$F_{DEP} = 2\pi\epsilon m R_p^3 \quad (5)$$

In the case of both the two- and four-electrode versions of the LOC, electrodes experience alternating fixed voltages, oscillating between $\pm V_a$. V_a undergoes incremental changes of 1V, initiating from ± 1 V. The applied electric field, generated by these voltage variations, induces the Dielectrophoresis (DEP) force. This force significantly influences cell trajectories, ultimately leading to their isolation from one another. Throughout the simulation period, [5][6] these cells are assigned to be released simultaneously from the blood inlet. Di-electrophoretic forces and internal drag forces both have an impact on the discharged particles. This approach's primary drawback is that it solely relies on a fluid-cell connection; particle-particle collaboration modeling is absent.

2.2 Mesh Refinement

Three different mesh element sizes can be referred to as "Coarse," "Normal," and "Fine" meshes are assessed in a grid independence test based on the average element size

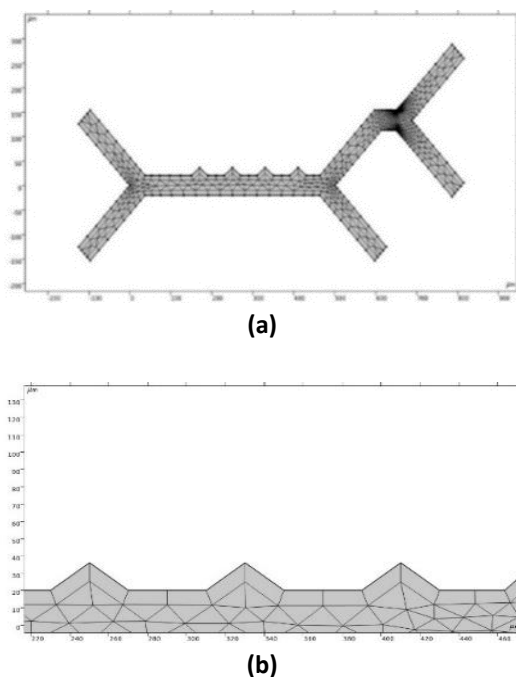


Figure 1: Fine Mesh Element Size Visualization: (a) Entire Geometry Overview; (b) Detailed View of Y-Channel and Electrodes

III. List of Particles to Isolate

White blood cells, also known as leukocytes, are cancer cells that are present in the bloodstream and offer a unique opportunity for liquid biopsies. The list of separated cells also includes the significance of the particles. They also function as abnormalities biomarkers for early detection. Therefore, it is paramount to separate WBCs from other blood constituents to optimize cancer diagnosis, prognosis, and treatment monitoring.

The ability to separate WBCs originating from different elements is particularly Advantageous for several reasons. First off, due to their unique characteristics, WBCs can be used to pinpoint the specific characteristics of a cancer and assist in determining its stage and kind. This information is crucial for developing effective treatment plans. Second, early detection—a crucial component of cancer screening—is made possible by the isolation of WBCs. Early bloodstream detection of WBCs allows for timely intervention prior to tumor dissemination and metastasis onset.

In the context of health and illness, platelets, also known as thrombocytes, are important for a number of reasons. They are essential to the blood clotting process. It has a significant impact on Blood clotting, for diagnosis and monitoring, cancer treatment, cardiovascular health, and surgical procedures. In general, seeking advice and an appropriate assessment from a healthcare professional is advised. Red blood cell (RBC): RBC particles have a diameter of 6.5 to 8.7 μm , however in this study, the size was measured at 5 μm to determine whether sickle cell identification is possible. Can also aid in conducting research based on the existence of any foreign particles in cells, which make up a significant portion of blood. The three species (WBCs, RBCs, and platelets (PLTs)) can be separated well enough from the single blood sample entering inlet 1. The simulation should be run at low voltages to minimize any possible damage to the cell membrane.

IV. Model and Function of Channel

Measurements of channel

Four triangle structure electrodes mounted sequentially in positive and negative positions at its top. Then, for the outlet 1, consequences platelets, in outlet 2 provides RBC, such as in outlet 3, upshot the WBC.

Set the voltage to +1V to prevent cell death by applying

an electric field to the particles. Subsequently, adjust the creeping flow for Inlet 2 (also known as V_{in} buffer; $109\mu\text{m/s}$) and Inlet 1 (also known as V_{in} cell; $50\mu\text{m/s}$). Add the total number of random particles found in the blood sample, for example, to obtain the output of a particular particle.

V Proposed Design

The proposed design, depicted in Figure 2, features two cascaded Y-junctions in series. Within this design, electrodes are strategically positioned within the first stage, while the second stage operates passively. To minimize the microchannel's footprint for packaging purposes, channel lengths (CL1 and CL2) are optimized to be as short as possible. Additionally, the channel width (CW) is varied parametrically to examine its impact on cell species separation, while the channel depth (CD) remains fixed at $100\mu\text{m}$.

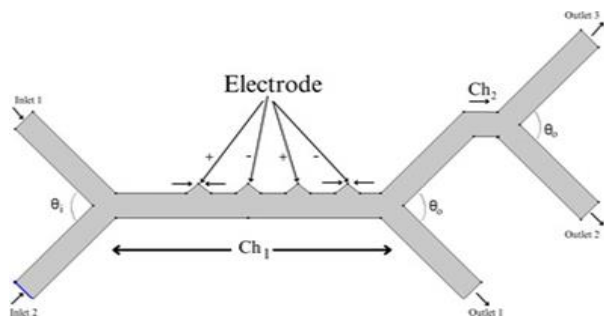


Figure 2: Designed channel

Figure (2) Separator microchannel structure and terminology for the design dimensions. The overall size is about $919\mu\text{m} \times 440\mu\text{m} \times 100\mu\text{m}$, and all inlets and outlets have the same length [7]. Notably, the electrodes in the first stage are deliberately offset from the center of the microchannel. This positioning facilitates flow focusing, bringing cells closer to the electrodes before separation attempts occur. This optimization ensures enhanced efficacy of the dielectrophoretic (DEP) force, as its effectiveness diminishes beyond approximately $30\mu\text{m}$ from the electrodes. Furthermore, the proposed LOC design adopts a two-dimensional, planar configuration. Planar geometries offer advantages in terms of fabrication simplicity and ease of implementation, particularly in scenarios requiring multiple devices or stages within the same LOC setup in Figure (2).

VI Validation of Numerical Models

The accuracy of the model is confirmed by contrasting it with the experimental findings from the configuration

used by Piacentini et al. The resulting model's characteristics, which include an applied voltage of $\pm 1\text{V}$, a constant cell inlet velocity (V_{in} , cells) of $50\mu\text{m/s}$, and a buffer inlet velocity (V_{in} , buffer) of $109\mu\text{m/s}$, exactly match those of the experimental arrangement. By superimposing the experimental cell positions onto the simulated cell trajectories, the model's predictions and the experimental results are shown to be consistent. The precision and dependability of the model are validated by this agreement.

VII Results

Di-electrophoretic force (DEP) and flow focusing are used in a two-stage micro channel device simulation to separate RBCs, PLTs, and WBCs according to their size and electrical characteristics. With low voltage values, this design effectively isolates cells to reduce the possibility of cell damage. The LOC is cascaded with cell counters and parametrically optimized to achieve high separation efficiency. Impact analysis reveals that changing the number of electrodes, voltage intensity, channel geometry, and buffer inlet velocities can change the separation efficiency and cell trajectories [8]. Target cell trajectories may be jeopardized by increasing buffer inlet velocity, v_{in} , buffer, which is going to force the cells toward the electrodes and lower separation efficiency overall. When wider micro channels are used, higher applied voltages are required to achieve similar efficiency levels; • Four electrodes allow lower voltage values to be used when two electrodes are used; Stronger DEP forces produced by higher voltage levels push cells away from the electrodes. In order to minimize damage to cell membranes, the suggested design aims for total separation at a comparatively low voltage of $\pm 1\text{V}$. The separation of other blood cell types could be the subject of future research. In addition, integrating cell counters improves the LOC's functionality[9][10]. It is also possible to take into account more realistic modeling of non-spherical cells. In the end, simulating particle-particle interactions rather than just fluid-particle interaction is necessary to increase the simulation's reliability.

A common step in evaluating throughput—a critical indicator of separation speed—is figuring out the volumetric flow rate at the intended outlet. However, in some cases, sample dilution can be incorporated by multiplying the flow rate by the cell density per volume. Reporting the actual number of sorted cells is another

strategy. Notably, throughput may become less important in microfluidic applications because parallel micro-channels can be used to increase it.

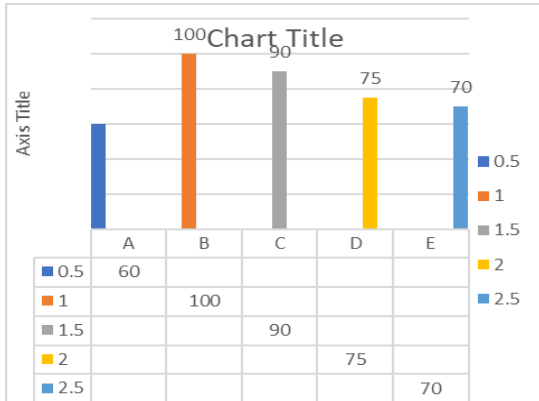
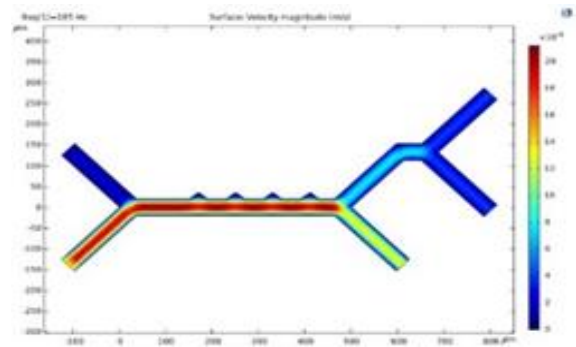


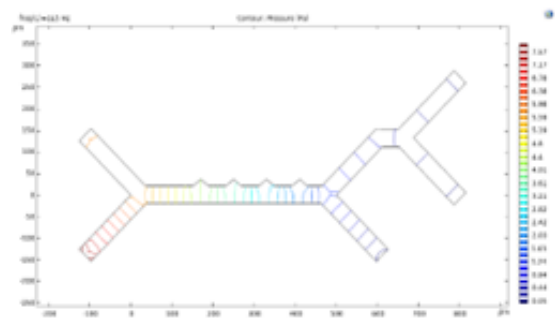
CHART 1: Analysis view

S.no	volt	inlet 1	inlet 2	current	No of electrodes	separation efficiency
A	0.5	50	109	60	4	60%
A	1	50	109	60	4	100%
A	1.5	50	109	60	4	90%
A	2	50	109	60	4	75%

Table 2 analysis of different input voltages

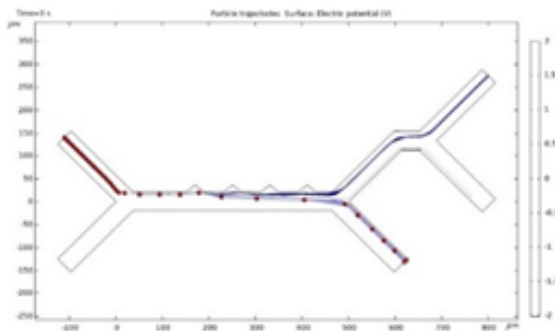


(iii)

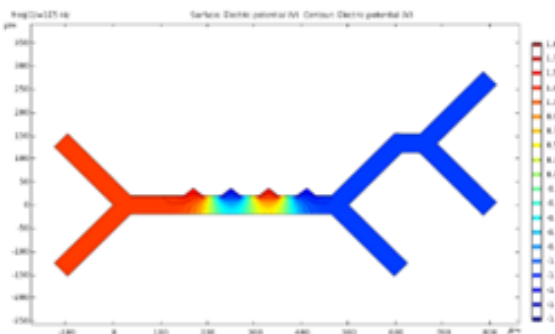


(iv)

Figure 4. Authentication of numerical model showing: (i) cell trajectories, (ii) pressure, (iii) electric potential, (iv) Velocity



(i)



(ii)

VIII Conclusion

A highly developed and simulated microfluidic Lab-on-a-Chip (LOC) with a two-stage design has been created to enable the accurate separation of platelets (PLTs), Red Blood Cells (RBC) and white blood cells (WBCs). Dielectrophoretic force (DEP) and flow focusing are combined in this creative design to selectively separate each type of cell according to its electrical properties and size. The process makes sure that WBCs are effectively separated from other blood cells. Because of its versatility and low voltage operation, which reduces the possibility of potential cell damage, the LOC can be integrated with other devices such as cell counters [11][12][13]. The goal of the methodical LOC geometry optimization is to achieve high separation efficiency for each of the three target cell types. Utilizing dielectrophoretic, this lab-on-chip enables rapid detection of diverse biomolecules in blood, from 5micrometers in size of particles to 7micrometre. It ensures a quick analysis within seconds, offering approximate outputs without compromising cell viability [14][15]. The applied electric field through dielectrophoresis plays a pivotal role in this non-

invasive approach to biomolecule detection.

References

1. Altay, R.; Yapici, M.K.; Koşar, A. A Hybrid Spiral Microfluidic Platform Coupled with Surface Acoustic Waves for Circulating Tumor Cell Sorting and Separation: A Numerical Study. *Biosensors* 2022, *12*, 171. [Google Scholar] [CrossRef]
2. Ali Othman, N.T.; Sahul Hameed, H.; Rosli, M.I. Simulation on Red Blood Cell's Separation in Microchannel by Using COMSOL® Multiphysics. *J. Teknol.* 2022, *84*, 103–112. [Google Scholar] [CrossRef]
3. Zeynep Çağlayan^{1,2}, Yağmur Demircan Yalçın^{1,3,†} and Haluk Külâh A Prominent Cell Manipulation Technique in BioMEMS: Dielectrophoresis, *Micromachines* 2020, *11*(11), 990; <https://doi.org/10.3390/mi11110990>
4. Villarruel Mendoza, L.A.; Scilletta, N.A.; Bellino, M.G.; Desimone, M.F.; Catalano, P.N. Recent Advances in Micro-Electro-Mechanical Devices for Controlled Drug Release Applications. *Front. Bioeng. Biotechnol.* 2020, *8*, 1–28. [Google Scholar] [CrossRef]
5. Azizpour, N.; Avazpour, R.; Rosenzweig, D.H.; Sawan, M.; Ajji, A. Evolution of biochip technology: A review from lab-on-a-chip to organ-on-a-chip. *Micromachines* 2020, *11*, 599. [Google Scholar] [CrossRef]
6. Mohankumar, P.; Ajayan, J.; Mohanraj, T.; Yasodharan, R. Recent developments in biosensors for healthcare and biomedical applications: A review. *Measurement* 2020, *167*, 108293. [Google Scholar] [CrossRef]
7. Zhu, H.; Fohlerová, Z.; Pekárek, J.; Basova, E.; Neužil, P. Recent advances in lab-on-a-chip technologies for viral diagnosis. *Biosens. Bioelectron.* 2020, *153*. [Google Scholar] [CrossRef]
8. Keçili, R.; Büyüktiryaki, S.; Hussain, C.M. Micro total analysis systems with nanomaterials. In *Handbook of Nanomaterials in Analytical Chemistry: Modern Trends in Analysis*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 185–198. ISBN 9780128166994. [Google Scholar]
9. Bayareh, M. An updated review on particle separation in passive microfluidic devices. *Chem. Eng. Process. Process Intensif.* 2020, *153*. [Google Scholar] [CrossRef]
10. Zhang, S.; Wang, Y.; Onck, P.; den Toonder, J. A concise review of microfluidic particle manipulation methods. *Microfluid. Nanofluidics* 2020, *24*, 1–20. [Google Scholar] [CrossRef]
11. Abt, V.; Gringel, F.; Han, A.; Neubauer, P.; Birkholz, M. Separation, characterization, and handling of microalgae by dielectrophoresis. *Microorganisms* 2020, *8*, 540. [Google Scholar] [CrossRef] [PubMed] [Green Version]
12. Shokouhmand, H.; Abdollahi, A. Detection of cell-free DNA nanoparticles in insulator based dielectrophoresis systems. *J. Chromatogr. A* 2020, *1626*. [Google Scholar] [CrossRef]
13. Kawai, S.; Suzuki, M.; Arimoto, S.; Korenaga, T.; Yasukawa, T. Determination of membrane capacitance and cytoplasm conductivity by simultaneous electrorotation. *Analyst* 2020, *145*, 4188–4195. [Google Scholar] [CrossRef]
14. Çağlayan, Z.; Demircan Yalçın, Y.; Külâh, H. Examination of the dielectrophoretic spectra of MCF7 breast cancer cells and leukocytes. *Electrophoresis* 2020, *41*, 345–352. [Google Scholar] [CrossRef] [PubMed]
15. Çağlayan, Z.; Demircan Yalçın, Y.; Külâh, H. Examination of the dielectrophoretic spectra of MCF7 breast cancer cells and leukocytes. *Electrophoresis* 2020, *41*, 345–352. [Google Scholar] [CrossRef] [PubMed]