Design of microfluidic platform for white blood cell separation from serum

Venkatesh Kadbur Prabhakar Rao[♣] and Sahil Desai[♠]

[♣] Department of Mechanical Engineering, Birla Institute of Technology and Science, Pilani, Rajasthan, India — 333 031

[♠]Department of Chemical Engineering, Birla Institute of Technology and Science, Pilani, Rajasthan, India — 333 031

E-mail: venkateshkp.rao@pilani.bits-pilani.ac.in

Abstract. Blood provides major information about the working of the tissues and organs in the body. Plasma, Red Blood Cells (RBCs), White Blood Cells (WBCs), and platelets comprise of the majority of the components of the blood of which WBCs are of utmost importance since they are an integral part of the defense mechanism of the body against germ-cells. Non-conventional methods of separation of WBCs involve centrifugation and are costly and time consuming categorized as off-chip methods. Microfluidics finds its vast applications in the field of immunoassay, clinical diagnostic, drug delivery and material synthesis. This work is to compare the theoretical results of magnetophoretic separation of the white blood cells from the dielectrophoretic separation in a lab-on-chip application. The efficiency comparison is made on the basis of the volume of the blood sample that is treated per unit time.

1. Introduction

Blood provides major information about the working of the tissues and the organs in the body. Plasma, Red Blood Cells, White Blood Cells, Platelets comprise of the majority of the components of the blood of which WBCs are of utmost importance since they are an integral part of the defense mechanism of the body against germ-cells. Non-Conventional methods of separation of WBCs involve centrifugation and are costly and time consuming, often termed as off-chip methods. Microfluidics finds its vast applications in the field of immunoassay, clinical diagnostic, drug delivery and material synthesis.

Magnetophoresis is the manipulation of magnetic particles using an externally applied magnetic field. Many applications of magnetophoresis are developed by tagging the target substance/particles with superparamagnetic microbeads. A non-uniform magnetic field is created by an array of soft magnetic components in an external applied biasing field. The magnetic field generated, applies a force on the magnetic dipole of the biological particles causing a separation.

In dielectrophoresis, particles move in a non-uniform electric field because of the interaction of the external electric field with the electric dipole moment of the biological particles. The force experience by particles in an external electric field is given by:

$$F_{\text{ext}} = 2\pi r_p^3 \epsilon_0 \,\text{Re}(\epsilon_f^*)\,\text{Re}\left(\frac{\epsilon_p^* - \epsilon_f^*}{\epsilon_p^* + \epsilon_f^*}\right) \nabla |E_{\text{rms}}|^2$$

2. Modeling

2.1. Magnetophoretic Separation

A continuous separation of White Blood Cells (WBCs) from Red blood Cells (RBCs) in plasma is carried out. An array of integrated soft magnetic elements are embedded adjacent to the microfluidics channel. The biasing field is activated which produces a non-uniform external magnetic field.

The basis of separation is the magnetic behavior of WBCs and RBCs in external magnetic field. WBCs have a diamagnetic behavior while RBCs possess magnetic property based on oxygenation of the hemoglobin. Oxygenated RBCs are paramagnetic while deoxygenated RBCs are diamagnetic.

The forces experienced by the cell consists of magnetic force, fluid force and buoyant forces. The biasing in the magnetic field of the soft-embedded magnetic elements is done by using a permanent magnet. The fluid flows parallel to the direction of the gravitational forces. This is because gravitational and fluidic forces are greater than magnetic forces in magnitude and vertical direction makes them not interfere with magnetic force. Cells are separated in a direction perpendicular to the flow where WBCs experience a force in an opposite direction than that of the RBCs.

2.1.1. Governing Equations All the governing equations for magnetophoretic separation are adapted from [1]. The governing equations for the separation are given by:

Equation of motion

$$m_c \frac{dv_c}{dt} = F_m + F_f + F_g$$

Where, F_m: Magnetic forces F_f: Fluidic forces F_g: Gravitational forces

Gravitational Forces

$$F_g = V_c(\rho_c - \rho_f)gx$$

Where, V_c: Volume of cell ρ_c: Density of cell ρ_f: Density of fluid g: acceleration due to gravity

Magnetic Forces

Applied magnetic field He due to each magnetic element can be expressed in x and y components as: Total external applied magnetic field H_a can be expressed as sum of magnetic field from each element as:

$$F_m = \mu_o V_c(\chi_c - \chi_f)(H_a.\nabla)H_a$$

Applied magnetic field H_e due to each magnetic element can be expressed in x and y components as:

$$H_{ex}^{(0)}(x,y) = \frac{M_e}{4\pi}\left\{\ln\left[\frac{(x+w)^2+(y-h)^2}{(x+w)^2+(y+h)^2}\right] - \ln\left[\frac{(x-w)^2+(y-h)^2}{(x-w)^2+(y+h)^2}\right]\right\}$$

$$H_{ey}^{(0)}(x,y) = \frac{M_e}{2\pi}\left\{\tan^{-1}\left[\frac{2h(x+w)}{(x+w)^2+y^2+h^2}\right] - \tan^{-1}\left[\frac{2h(x-w)}{(x-w)^2+y^2+h^2}\right]\right\}$$

Total external applied magnetic field H_a can be expressed as sum of magnetic field from each element as:

$$H_{ex}^{(0)}(x,y) = \sum_{n=0}^{Ne-1} H_{ex}^{(0)}(x-s_n,y)$$

$$H_{ey}^{(0)}(x,y) = \sum_{n=0}^{Ne-1} H_{ey}^{(0)}(x-s_n,y)$$

The total force F_m on any cell can be expressed in x and y component as:

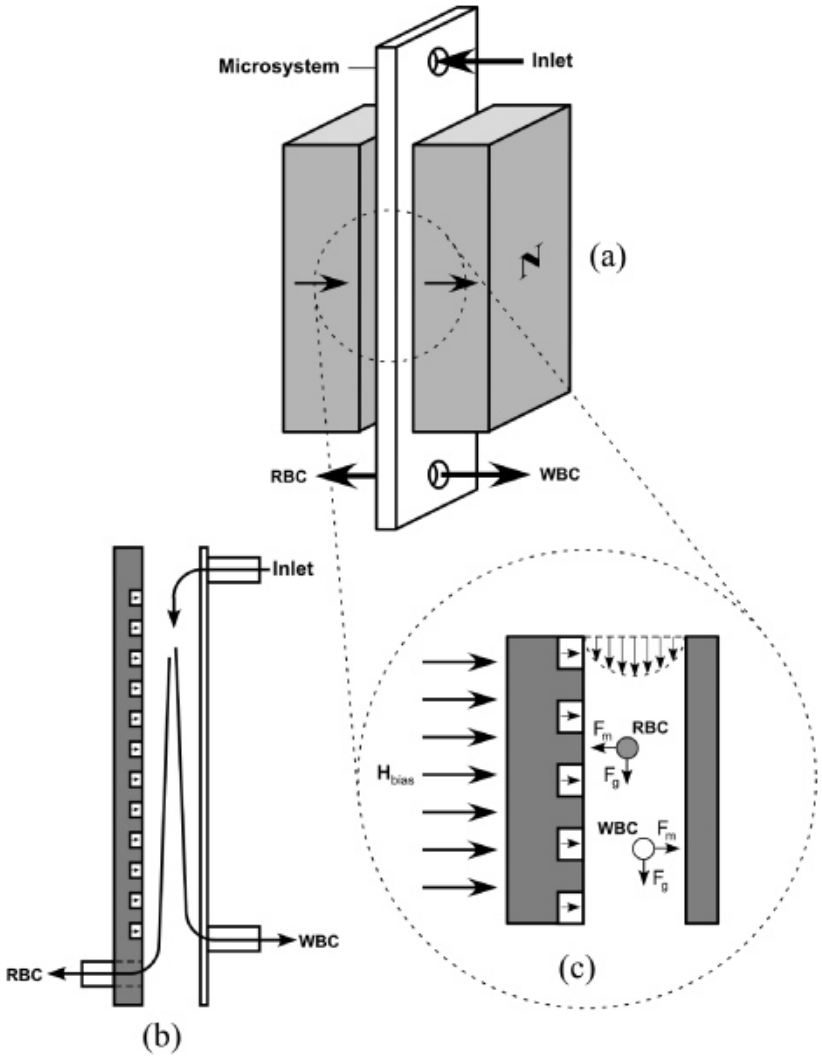

Figure 1. a) Biasing magnetic field b) Cross view of the separation system c) zoomed view of microsystem cross-section.

$$F_{mx} = \mu_o V_c(\chi_c - \chi_f)\left[H_{ex}^{(0)}(x,y)\frac{\partial H_{ex}^{(0)}}{\partial x} + \left(H_{\text{bias}} + H_{ey}^{(0)}(x,y)\right)\frac{\partial H_{ex}^{(0)}(x,y)}{\partial y}\right]$$

$$F_{my} = \mu_o V_c(\chi_c - \chi_f)\left[H_{ex}^{(0)}(x,y)\frac{\partial H_{ey}^{(0)}}{\partial y} + \left(H_{\text{bias}} + H_{ey}^{(0)}(x,y)\right)\frac{\partial H_{ey}^{(0)}(x,y)}{\partial y}\right]$$

Fluidic Forces

$$F_f = -6\pi\eta R_c(V_c - V_f)$$

Blood flow profile is calculated as:

$$V_f(y) = \frac{3V_f}{2}\left\{1 - \left(\frac{y - (h + h_c + t_b)}{hc}\right)^2\right\}$$

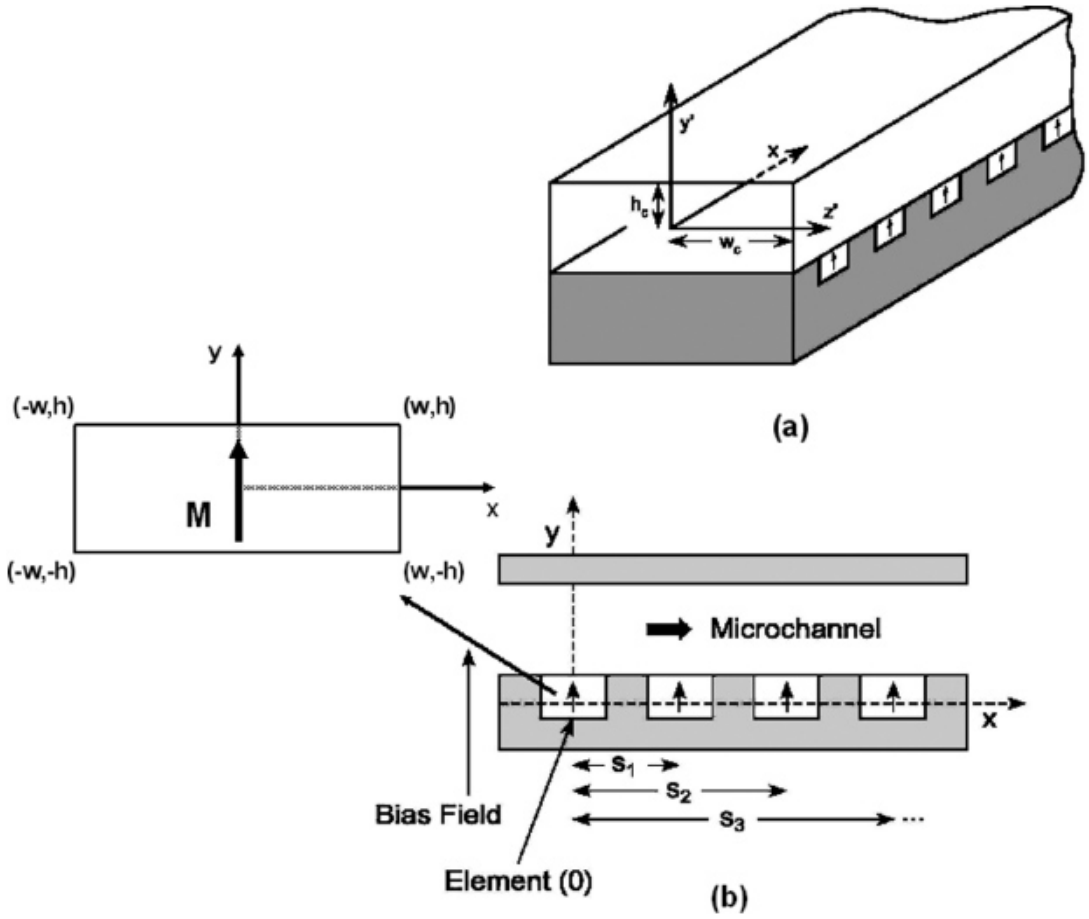

Figure 2. a) Separation channel b) Side view of separation microsystem.

Where, h_c: half height of microchannel t_b: Thickness of base of channel w_c: Half width of microchannel h: Height above the base V_f : Average blood flow velocity

Total fluidic forces Ff on cell can be expressed in x and y components as:

$$F_{fx} = -6\pi\eta R_c \left[V_{cx} - \frac{3V_f}{2} \left\{ 1 - \left(\frac{y - (h + h_c + t_b)}{hc} \right)^2 \right\} \right]$$

$$F_{fy} = 6\pi\eta R_c V_{cy}$$

As described in [1], the fluid channel is 120 μm high, 1 mm wide and 30 mm long and there are 45 permalloy elements embedded immediately beneath it. Each element is 300 um high and 300 μm wide, and they are spaced 300 μm apart (edge to edge). Magnetic element array spans a distance of 26.7 mm along the bottom of the microchannel. The cells enter the microchannel to the left of the first element at various initial heights. Average fluid velocity is 0.25 mm/s and the cells enter the channel with this velocity.

2.2. Dielectrophoretic Separation

A particle suspended in a non-uniform electric field polarizes and the poles then experience a force along the field lines, which can either be attractive or repulsive according to the orientation of the dipole.

For a homogenous sphere, surrounded by conducting medium:

$$F_{ext} = 2\pi r_p^3 \,\mathrm{Re}(\epsilon_f^*)\,\mathrm{Re}\left(\frac{\epsilon_p^* - \epsilon_f^*}{\epsilon_p^* + 2\epsilon_f^*}\right)\nabla|E_{\mathrm{rms}}|^2$$

Where, ϵ_f^*: Clausius-Mossotti factor is estimated based on complex relative permittivity for particle and medium

Dielectrophoretic separation relies on the fact that the diameter of the WBC is smaller than the diameter of the RBCs which creates a difference in the equivalent complex relative permittivity of a homogenous particle.

$$\epsilon_{eq}^* = \epsilon_s^* \frac{\left(\frac{r_0}{r_i}\right)^3 + 2\left(\frac{\epsilon_p^* - \epsilon_s^*}{\epsilon_p^* + 2\epsilon_s^*}\right)}{\left(\frac{r_0}{r_i}\right)^3 - \left(\frac{\epsilon_p^* - \epsilon_s^*}{\epsilon_p^* + 2\epsilon_s^*}\right)}$$

A difference in the equivalent complex relative permittivity of RBC and WBC is observed which allows to separate them in presence of an external electric field since they behave differently. Figures for dielectrophoretic separation are obtained by simulation results in COMSOL 5.3.

For dielectrophoresis a carrying/transport medium is required for carrying the mixture of blood cells. A fluid comprising properties similar to that of water (dynamic viscosity and density).

The mixed blood cells mixture can be injected with only a certain inlet velocity since a greater velocity caan caause greater amount of friction between the layers of blood stream and cause the sample to deteriorate. Hence, to carry the mixed blood cell mixture through out the length of the separating apparatus, another fluid is injected from the bottom inlet at a greater inlet velocity. An assumption of creeping flow is made for the flow of fluid and blood cells inside the microfluidic channel.

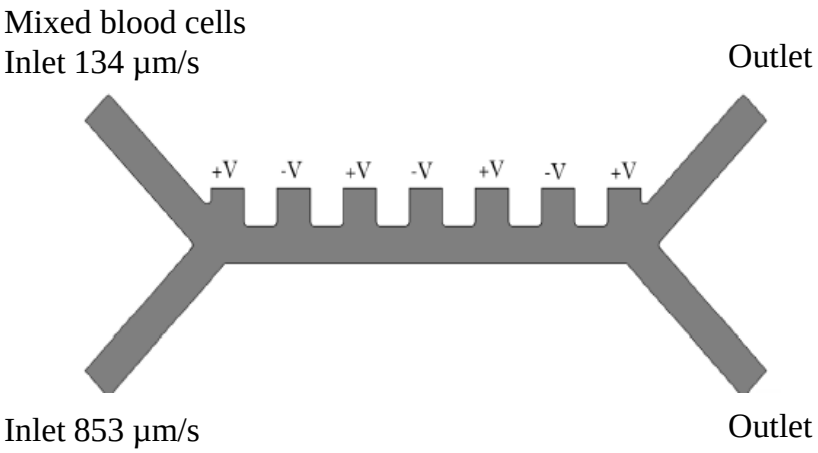

Figure 3. Numerical model of dielectrophoresis system

3. Results and Discussion

Typically magnetophoretic separation takes about 80 Sec for separation RBC and 60 sec for separation of WBC in 300 μL of fluid while 326 μL of fluid containing RBCs and WBCs can be separated in 48 sec using a continuous dielectrophoretic separation device.

Table 1. Parameters used for evaluation of results of dielectrophoretic separation.

Parameter	Value
Frequency of Electric Field	100 kHz
Fluid medium conductivity	55 mS/m
Fluid relative permittivity	80
Fluid density	1000 kg/m^3
Fluid dynamic viscosity	0.001 Pa.s
Particle density (RBCs and WBCs)	1050 kg/m^3
Particle diameter: WBCs	1.8 μm
Particle diameter: RBCs	5 μm
Particle conductivity: WBCs	0.25 S/m
Particle conductivity: RBCs	0.31 S/m
Particle relative permittivity: WBCs	50
Particle relative permittivity: RBCs	59
Shell electrical conductivity: WBCs	10^{-6} S/m
Shell electrical conductivity: RBCs	10^{-6} S/m
Shell relative permittivity: WBCs	6
Shell relative permittivity: RBCs	4.44
Shell thickness: WBCs	8 nm
Shell thickness: RBCs	9 nm

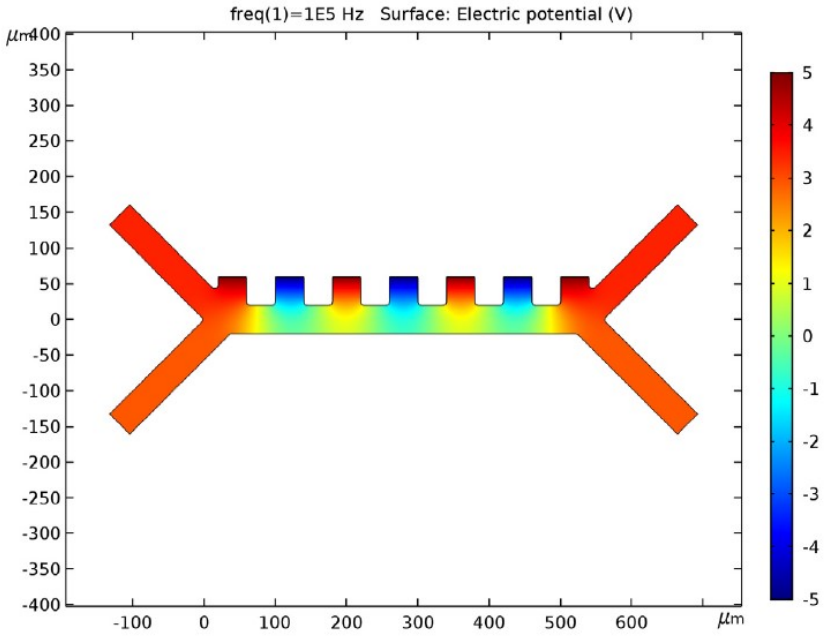

Figure 4. Electric field representation for separation design

4. Conclusions

The greater time for magnetophoretic separation is accounted by the weak magnetic properties of the biological materials compared to their response to electrical changes (good electrical

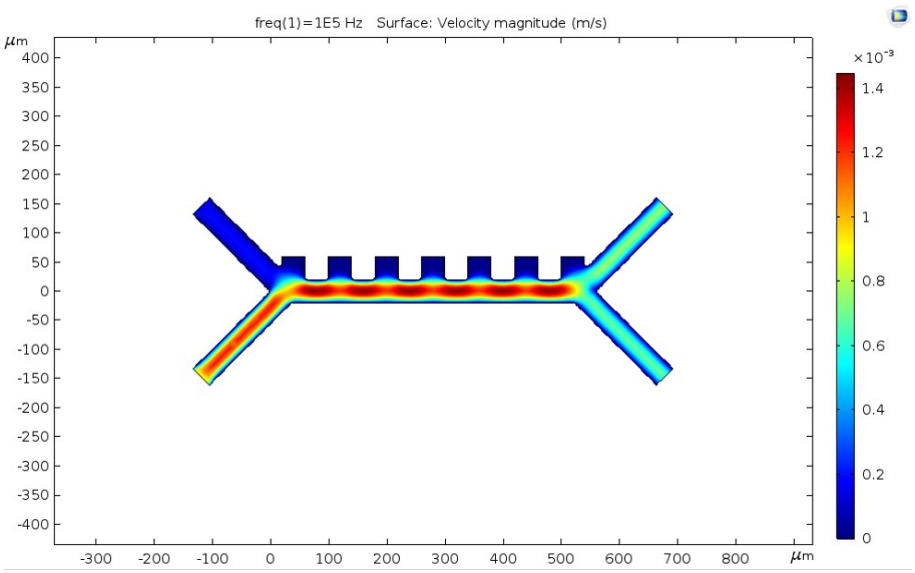

Figure 5. Fluid velocity distribution

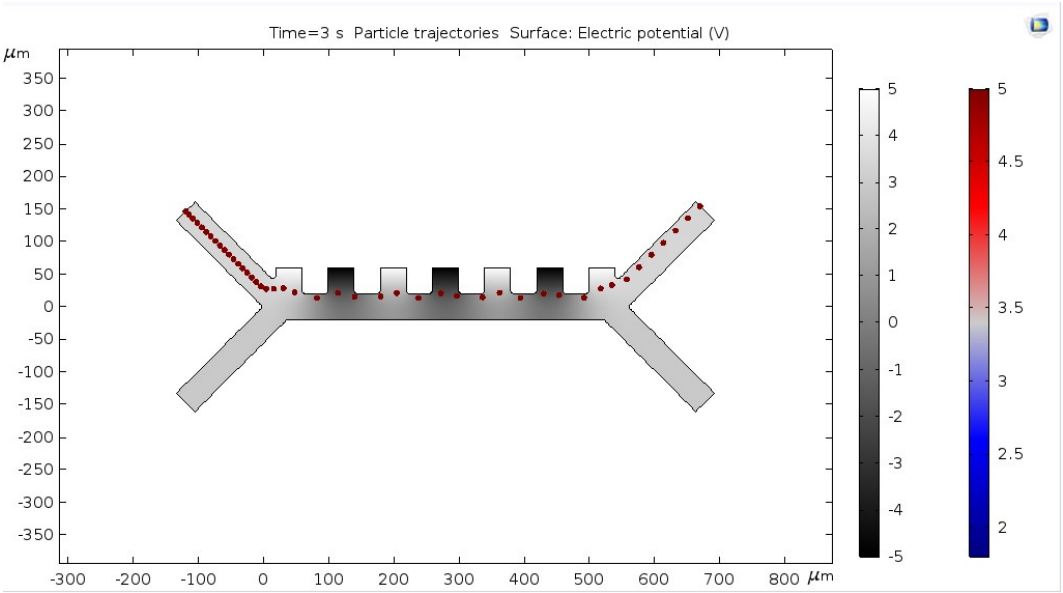

Figure 6. Combined movement of RBCs and WBCs in absence of applied electric field.

properties). Also, the magnetophoretic separation accounts that the WBC and RBC exhibit different magnetic property: WBC are diamagnetic and deoxygenated RBC are paramagnetic. But for application on lab-on-chip separation, if the blood sample is contaminated by the presence of oxygenated RBC, they exhibiting diamagnetic properties, will also separate out with the WBC and hence a greater number of WBC count will be observed. One improvement in the magnetophoretic system can be achieved by magnetically tagging the WBCs in the blood before sending them in the array of soft magnetized elements which cam increase the separation efficiency.

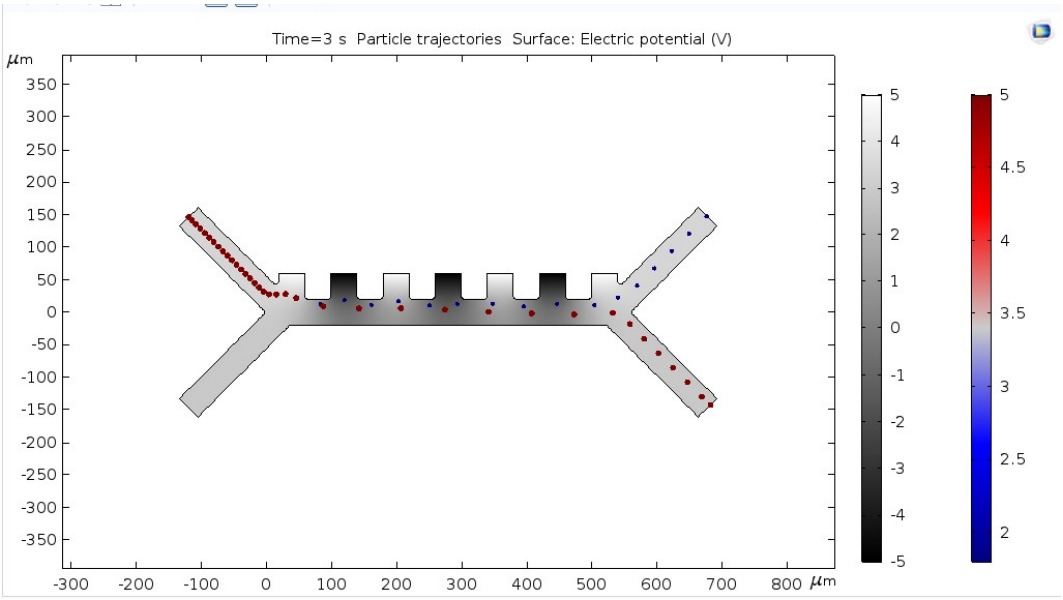

Figure 7. Combined movement of RBCs and WBCs in presence of applied electric field.

References