Microfluidics Blood Separations through Optical Sorting and Deterministic Lateral Displacement

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***Abstract*—This paper presents an analysis and parametrical modeling of blood plasma separation devices with optical sorting and deterministic lateral displacement mechanisms. The new device for optical sorting is offered. The modeling with the novel device shows that this device can separate red blood cells of different sizes from whole blood. Special numerical technique with periodical boundary conditions was applied for simulation of deterministic lateral displacement. Comparison of optical sorting and deterministic lateral displacement is performed. Unexplored optical and mechanical properties of blood components and indispensability of additional researches are also discussed.**

Keywords-blood separation; optical sorting; deterministic lateral displacemenet

I. INTRODUCTION

Separation of the cellular component of human blood is of essential interest for medicine and research. In general outline human blood consists of plasma, erythrocytes or red blood cells (RBCs), reticulocytes, platelets, and leukocytes or white blood cells (WBCs). About 55% of whole blood is blood plasma. The proportion of blood volume that is occupied by erythrocytes is normally about 48% for men and 38% for women. This proportion is a hematocrit. Normally leukocytes take up approximately 1% of blood volume in a healthy adult human. The number of WBCs in the blood is a very sensitive indicator of diseases. Thus it is especially important to remove all RBCs for further analysis of WBCs. There are diverse classes of WBCs. A major separation of leukocytes includes five types, neutrophils, lymphocytes, monocytes, eosinophils, and basophils [1].

Usually, human blood is separated by large volume centrifugation [2]. With modern lab equipment such as lab-on-a-chip-type devices this approach is not so effective. This has lead to an interest in adopting new microscopic separation techniques. Two of these promising methods are optical sorting [3][4] and deterministic lateral displacement [5] methods.

In the present paper, we first consider the new design for optical sorting and then we compare it to the deterministic lateral displacement. Also we discuss unexplored optical and mechanical properties of blood components.

II. OPTICAL AND MECHANICAL PROPERTIES OF BLOOD

The analysis of the reviews devoted to optical sorting and deterministic lateral displacement shows that only separation of colloidal particles of spherical shape in special mixture is well investigated [6-12]. Fractionation of real human blood requires essential efforts and more complicated devices [13].

Major problem here is that we have multi-component mixture of composite particles in non-Newtonian liquid.

Not all of the physical properties of these particles and liquid are well known.

A. Refractive Index and Absorption Coefficient

Mostly examined are the refractive index of whole blood, blood plasma, and RBCs. We collected some of measured refractive indexes and description of measuring methods in Table I.

If λ is the wavelength in nm then according to [26] the spectral dependence of refractive index of blood plasma is:

$$n_p=1.3254+8405.3/\lambda^2-3.9572\cdot10^8/\lambda^4-2.3617\cdot10^{13}/\lambda^6. \quad (1)$$

We managed to find in the literature only one mention of platelet [22] and WBC [25] refractive index.

Absorption and scattering coefficients of whole blood at different oxygenation and different wavelength of incident light is widely published [16][27-29]. These coefficients for blood components are studied not completely. Absorption coefficient is very important for estimation of heating of plasma and particles during optical sorting.

B. Mechanical Properties of Blood

In a first approximation the blood plasma can be considered as a Newtonian liquid, but whole blood is a non-Newtonian.

At a normal physiological hematocrit of 45%, the viscosity of blood is $\eta = 4\cdot10^{-2}$ poise, which is roughly 4 times that of water. Plasma alone (zero hematocrit) has a viscosity of $\eta = 1.1\cdot10^{-2}$ to $1.6\cdot10^{-2}$ poise, depending upon the concentration of plasma proteins [30]. Variation of viscosity with shear rate at different haematocrits is well studied [31].

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The movement of a particle in the viscous medium is characterized by Reynolds number $Re = \rho v R/\eta$, where, ρ is the fluid density, v is the particle velocity, R is a characteristic dimension (for example, radius of spherical particle), and η is the fluid viscosity. In our case for very low Reynolds numbers ($Re \ll 1$) the drag force on a sphere is described by Stokes' law:

$$F_D = -6\pi\eta v R, \quad (2)$$

For a 10 μm diameter spherical particle at a speed of 20.0 μm/s the drag force is changed from 2.1 to 3.0 pN depending on plasma viscosity.

TABLE I. SIZE AND REFRACTIVE INDEX OF BLOOD COMPONENTS AND METHODS OF MEASURING

Samples	Size (μm)	Refractive index	Wave-length (nm)	Method	Ref.
Lymphocytes (rats)		1.3545 - -1.3580		Abbé refracto-meter	[14]
Cytoplasm (rats)		1.3583 - -1.3584			
RBC	5.5	1.40	842	Scattering	[15]
Whole blood		1.38	488 633		[16]
RBC	6.3	1.41	632.8	Scanning flow cytometer	[17]
RBC	7.5	1.4	440 1000	Scattering	[18]
Plasma		1.335			
RBC		1.405	820	Optical coherence tomography	[19]
RBC	4.82- -5.6	1.385 - - 1.405	632.8	Scattering	[20]
Haemoglobin cytoplasm		1.3871 1.3800	532 632.8	Total internal reflection	[21]
Blood plasma		1.3515 - 1.3479	532 632.8		
Lecithin		1.4852 1.4838	532 632.8		
Platelet	2-4	1.3999	660	Scanning Flow Cytometer	[22]
RBC	≈8	1.3999		Tomographic phase microscopy	[23]
RBC	7.7	1.418	663	Digital holographic microscopy	[24]
RBC		1.399	1064		[25]
WBC		1.360			

Elastic properties of blood cells are very important in collision between particles or between particles and mechanical hindrance. To the best of our knowledge, mainly works have been devoted to the coefficient of elasticity of RBCs. For example, in [32], the homogeneous Young's modulus for spherical red blood cells was measured as E = 11.0 ± 0.5 Pa. In all probability the elastic properties of over blood cells are still unknown.

III. OPTICAL SORTING

According to investigations of continuous flow separation [4, 33-38] optical fractionation may offer unparalleled selectivity for particle size and refractive index. WBCs have larger size than RBCs and smaller refractive index. Hence WBCs are undergone to greater drag force and smaller optical force than RBCs. It allows us to propose a device of optical sorting which separates only RBCs from whole blood.

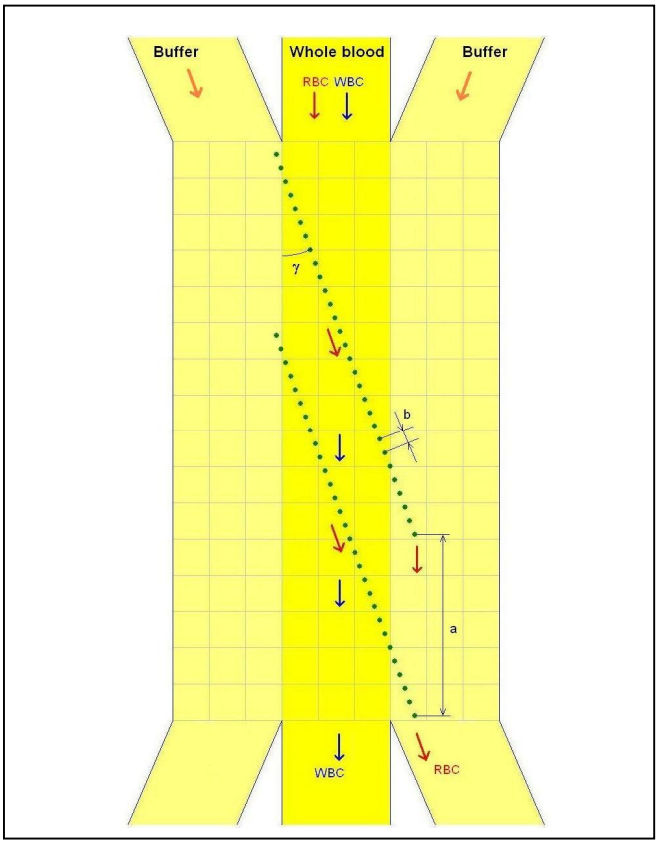

Figure 1. Schematic drawing of possible device for optical separation.

A two buffer streams of plasma and a central stream of whole blood move at equal speed (Fig. 1.). The optical traps are shown in Fig. 1 by green dots. RBCs are deviating by light (red arrows); WBCs are practically not at same conditions (blue arrows).

For our simulation, we assume that RBCs and WBCs are spherical balls. The radii of RBCs are R_{RBC} = 2.5, 3.0, 3.5, and 4.0 μm. The refractive index of plasma is n_p = 1.335, refractive indexes of RBCs and WBCs are n_{RBC} = 1.40 and n_{WBC} = 1.36 respectively. The distance between light spots is b = 4 and 5 μm. The angle of trap layout is γ = 15° and 20°. The laser power per trap is P =5.0, 6.5 and 8.3 mW/trap. The speed of plasma is 20 μm/s for all presented calculations. Trajectories of RBCs (red lines) and WBCs (blue lines) for

distance b = 4 μm and angle γ = 20° are shown in Fig. 2. Trajectories of RBCs are deviated on angle γ while WBCs practically freely pass through tweezers array.

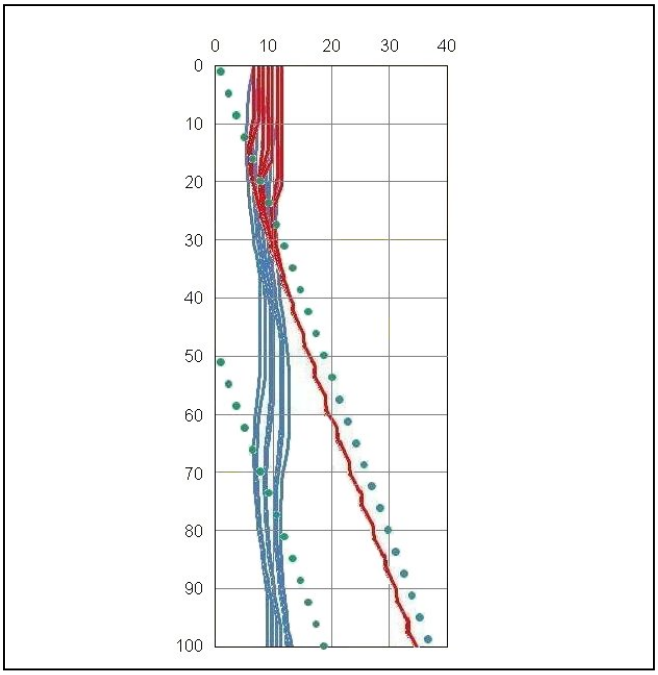

Figure 2. Caclulated trajectories of RBCs (red lines) and WBCs (blue lines).

TABLE II. VELOSITY OF RBC AT SMALL DISTANACE BETWEEN LIGHT POSTS

R, nm	Distance between light posts, b = 4 μm					
	P = 5 mW/trap		P =6.5 mW/trap		P = 8 mW/trap	
	γ = 15°	*γ = 20°*	*γ = 15°*	*γ = 20°*	*γ = 15°*	*γ = 20°*
2.5	18.67	17.56	18.32	17.49	17.93	17.18
3.0	18.33	17.94	17.34	17.09	15.86	15.69
3.5	18.23	17.81	16.87	16.46	14.70	14.28
4.0	17.97	17.71	16,53	16.15	14.32	14.01

TABLE III. VELOSITY OF RBC AT LARGE DISTANACE BETWEEN LIGHT POSTS

R, nm	Distance between light posts, b = 5 μm					
	P = 5 mW/trap		P =6.5 mW/trap		P = 8 mW/trap	
	γ = 15°	*γ = 20°*	*γ = 15°*	*γ = 20°*	*γ = 15°*	*γ = 20°*
2.5	16.96	16.64	13.69	14.20	Stop	Stop
3.0	18.48	17.41	18.05	16.96	17.95	16.82
3.5	18.77	18.13	18.31	17.70	17.64	17.20
4.0	18.56	18.06	17.94	17.51	16.52	16.64

To calculate the optical force we followed [4, 33], the drag force was evaluated by (2).

Some results of our simulations for average velocity of RBCs are summarized in Table II and III. At some set of parameters it is possible to keep RBC at the trap. At the same time WBCs practically do not change speed.

IV. DETERMINISTIC LATERAL DISPLACEMENT

Deterministic lateral displacement has been applied to the size separation of particles and DNA molecules by pumping through an array of obstacles [5, 13, 39-41]. The array consisted of rows of micrometer-scale posts with a consistent gap between the posts in each row. Each row of obstacles is shifted horizontally with respect to the previous row (Fig. 3).

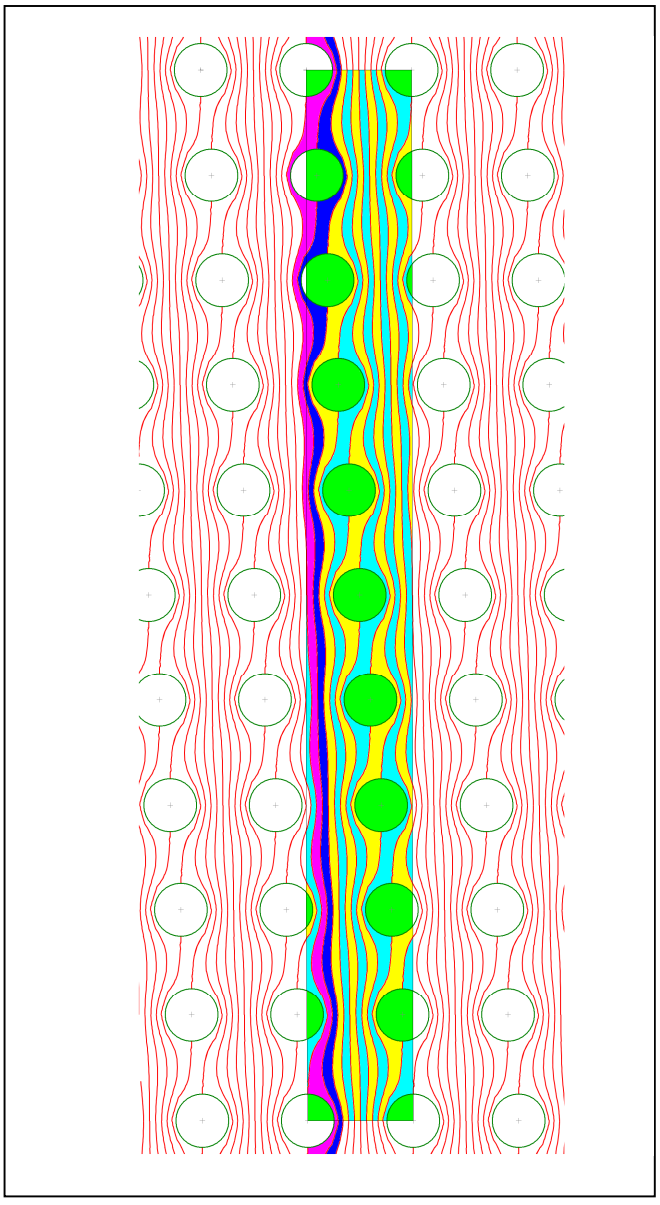

Figure 3. Streamlines in low Reynolds number flow through a periodic array of micrometer-scale obstacles.

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The green circles represent the array of obstacles; the red lines are the streamlines in Fig. 3. The shift of each next row equals to tenth of the horizontal distance between obstacles in our illustration. The total fluid flux through each gap between two obstacles is divided into 10 flow streams in Fig. 3. After 10 horizontal rows the picture is repeated. Rectangular periodical part of obstacle array is colored in Fig. 3.

According to deterministic lateral displacement conception, small particles shall move in zigzag mode (blue and pink paths in Fig. 3) along a streamline from top to bottom. Large particles should jump from one post to another and deviate to the right from horizontal direction.

Apparently the flow in infinite periodic array of obstacles is presented by the primitive cell shown in Fig. 4. Obstacles are painted in green color; the cell is marked by pink color. We applied the finite element method and used special numerical technique to take into account periodical boundary conditions for simulation of deterministic lateral displacement in primitive cell. This reception allows to reduce number of nodes essentially.

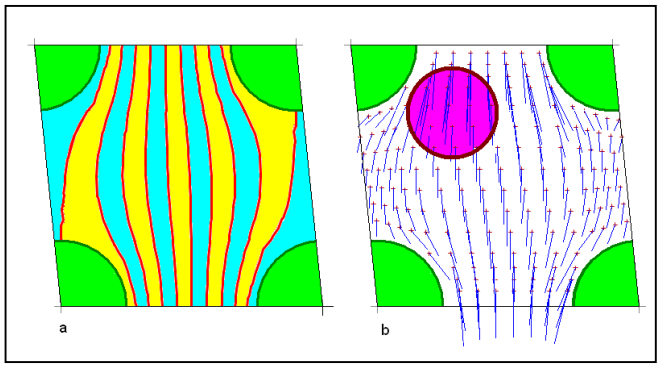

Figure 4. Streamlines (a) and velosity distribution (b) for primitive cell of obstacle array.

For simulation which shown in Figs. 3 and 4 the diameter of cylindrical obstacles was 10 μm, Distance between centers of cylinder in horizontal direction and period of obstacle rows in vertical direction were 20 μm.

The large problem is a trajectory analysis of individual blood cell:

- Application of the Stokes' law (2) for evaluation of the drag force is incorrect;
- The size of a particle is comparable with distance between posts as shown in Fig. 4b. Such particle has to perturb the fluid flow;
- Parameters of collision between a blood cell and obstacles are unknown. Conditions of sticking of a cell to an obstacle are unknown too;
- Density of blood cells is quite high therefore RBCs undergo impacts at passing through an array.

Let's note also that boundary conditions between plasma and obstacles as well as between plasma and blood cells are insufficiently studied.

V. CONCLUSION

The review of measured optical and mechanical properties of blood is presented. It is noted, that physical properties of many blood components are studied not fully. Thus it is essential to investigate refractive indexes and absorption coefficients of reticulocytes, platelets, and white blood cells (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). Also elastic properties of all blood cells except RBCs are practically unknown.

We offered a new device for optical sorting of blood cells. The modeling with the novel device shows that this device can separate RBCs of different sizes from whole blood. For simulation of deterministic lateral displacement we developed special finite-element technique which allows to take into account periodical boundary conditions for primitive cell and to reduce number of nodes.

We can conclude that optical separation possesses certain advantages in comparison with deterministic lateral displacement:

- Smaller distance of separation;
- Tunable distance between light spots and tunable power per trap;
- Absence of mechanical interaction and hence absence of mechanical damage of cells.

Additionally we note that the gaps between mechanical posts in deterministic lateral displacement method can be littered by the stuck cells.

At the same time optical separation has the certain drawbacks:

- Heating of plasma and blood cells;
- Optical traps can hold small blood particles and obstruct to extraction of RBC;
- Speed of the RBC guided by optical traps is smaller than speed of a free RBC thus it is possible a collision between RBCs.

The large benefit of the deterministic lateral displacement is that the method is very simple and devices are very cheap. Although the optical sorting still are on a development stage the deterministic lateral displacement are already close to clinical tests.

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