

Red Blood Cells Motion in a Glass Microchannel

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Abstract. The motion of the red blood cells (RBCs) flowing in microvessels and microchannels depend on several effects, such as hematocrit (Hct), geometry, and temperature. According to our knowledge, the effect of the temperature on RBC motion was never investigated at a microscale level. Hence, the aim of the present work is to determine the effect of the temperature on the RBC's trajectories and to investigate the best approximation of the trajectories through a nonlinear optimization. In vitro human blood was pumped through a $100\ \mu\text{m}$ circular microchannel and by using a confocal micro-PTV system the RBC's trajectories were measured at different temperatures, i.e., 25°C and 37°C . In this study we measured the motion of forty cells flowing in the middle of the microchannel and applied different functions to approximate its behavior.

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INTRODUCTION

Blood is a fluid composed of a suspension of cells, proteins and ions in plasma. In normal blood, three types of cells comprise about 46% of its volume. These cells are the red blood cells (also known as erythrocytes), white blood cells (also known as leukocytes) and platelets (also known as thrombocytes). Figure 1 shows different kinds of human blood cells.



FIGURE 1. Scanning electron microscope image of human blood cells (adapted from [13]).

In the microcirculation, the flow behavior of RBCs plays a crucial role in many physiological and pathological phenomena. For example, the random-like transverse motion and rotation of RBCs in shear flow is believed to play an important role in thrombogenesis [3, 10]. As a consequence, many rheological studies have been performed on both microvessels and microchannels to investigate the effect of the hematocrit (Hct), geometry, and temperature on the RBCs flowing behavior [2, 3, 10]. Recently Lima and his colleagues measured the red blood cells (RBCs) radial dispersion (Dyy) in both glass [6, 7] and polydimethylsiloxane (PDMS) [8] microchannels by using a confocal micro-PTV system. By comparing Lima et al. results with the measurements performed by Goldsmith and his colleagues [3] several quantitative deviations were observed between both experimental results. One possible reason for observed discrepancies may be due to the different temperatures used in the two cases, i.e., Lima et al. used body temperatures (37°C) whereas Goldsmith et al. used room temperatures. Hence, it is important to investigate the effect of the temperature on the RBC motion. The experiments were performed in the middle of $100\ \mu\text{m}$ glass capillaries at temperatures of 25°C and 37°C , by using a confocal micro-PTV system. In the present study, the trajectories of forty

RBCs were measured and different functions were applied with the purpose to find the one that best approximate to its flow behavior.

MATERIALS AND METHODS

In the present study we used Dextran 40 (Dx-40; Otsuka Medicine, Tokyo, Japan) containing $12 \pm 2\%$ (12Hct) of human RBCs. The RBCs were labeled with a lipophilic carbocyanine derivative, chloromethylbenzamido (CM-Dil, C-7000, Molecular Probes, Eugene, OR, USA). A detailed description about the procedure for labeling the human RBCs can be found elsewhere [7]. Additionally, we used a $100\mu\text{m}$ circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes, NJ, USA). The microchannel was mounted on a slide glass with a thickness of $80 \pm 20\mu\text{m}$ and was immersed in glycerol to minimize the refraction from the walls.

The confocal micro-PTV system used in this study consists of an inverted microscope (IX71; Olympus, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Japan), a diode-pumped solid-state (DPSS) laser (Laser Quantum, UK) with an excitation wavelength of 532nm and a high-speed camera (Phantom v7.1; Vision Research, USA) (see Figure2). The microchannels were placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific, USA) a pressure-driven flow was kept constant ($\text{Re} \approx 0.008$). Additionally, by using a thermo plate controller (Tokai Hit) it was possible to apply different temperatures to the surrounding environment, i.e., $25^\circ\text{C} \pm 1$ and $37^\circ\text{C} \pm 1$. More detailed information about this system can be found elsewhere [4, 5, 7].

FIGURE 2. Confocal micro-PTV experimental set-up and trajectory of labeled RBC through a circular glass microchannel.

The confocal images were captured in middle of the capillary through a dry $40\times$ objective lens at a rate of 100 frames/s. A manual tracking plugin (MTrackJ) [11] of an image analysis software (Image J, NIH) [1] was used to track individual RBC. By using MTrackJ plugin, the bright centroid of the selected RBC was automatically computed through successive images for an interval of time of 10 ms. After obtaining series of x and y positions, data were exported for the determination of each individual RBC trajectory and to analyse the best mathematical function that approximates to the RBCs experimental flow behavior.

In this study it was observed and analyzed forty cells: twenty at temperature 25°C and other twenty cells at the temperature 37°C . For each cell i , and using MTrackJ plugin system, we obtain $\{(x_j, y_j), j = 1, \dots, k_i\}$ data. In this experiences k_i can assume values between 23 and 195. The aim of this work is to obtain a better approximation for the data using nonlinear optimization [12]. For that we consider three different functions (polynomials, sum of trigonometric functions and a sum of exponential functions) defined as:

$$f_1(x, p) = \sum_{i=0}^9 p_i x^i; \quad f_2(x, a, b) = a_0 + \sum_{i=1}^8 a_i \cos(ix) + b_i \sin(ix); \quad \text{and} \quad f_3(x, c, d, g) = \sum_{i=1}^8 g_i e^{-\left(\frac{(x-c_i)}{d_i}\right)^2},$$

where $p \in \mathbb{R}^{10}$, $a \in \mathbb{R}^9$, $b, c, d, g \in \mathbb{R}^8$ are the function parameters and the vector $x \in \mathbb{R}^{k_i}$, where i represents the cell number. To identify the functions parameters it was used the tool `cfTool` present in Curve Fitting Toolbox from Matlab [9].

RESULTS AND DISCUSSION

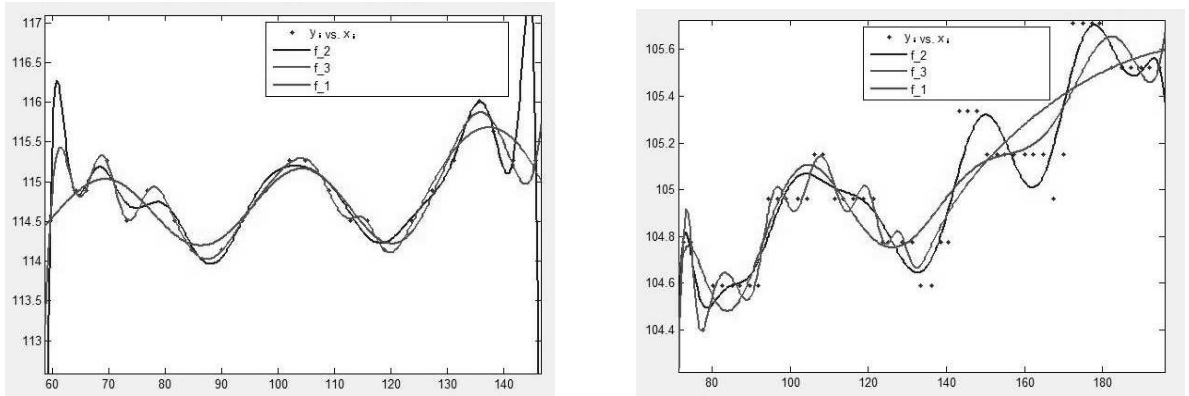
The error of nonlinear least squares approximation of the selected cells are listed in the Table 1, where **Cell** refers to the cell number, $F_i = \sum_{j=1}^{k_i} (y_j - f_i(x_j))^2$ is the nonlinear least squares approximation error of the function $f_i(x)$, with $i = 1, 2, 3$, **Av** refers the error average, and **s** corresponds to the standard deviation of the errors.

The results, from Table 1, indicate that the function $f_2(x, a, b)$ (sum of trigonometric functions) was the best approximation to the motion of the cells in the microchannel. Only in five cells (three in the with temperature 25°C and two with the temperature 37°C) we have obtained a better performance with the function $f_3(x, a, b, c)$. This is confirmed by the value of the error average. Another important aspect is the fact that the standard deviation of the

TABLE 1. Numerical Results obtained using `cftool`.

Cells with temperature 25°C				Cells with temperature 37°C			
Cell	F_1	F_2	F_3	Cell	F_1	F_2	F_3
1	3.453	2.537	2.701	21	0.792	0.199	0.485
2	1.707	0.834	2.363	22	0.994	0.497	0.985
3	3.258	2.282	1.644	23	2.898	1.664	1.230
4	1.484	0.720	1.066	24	0.222	0.099	0.217
5	1.146	0.117	1.121	25	0.660	0.328	0.409
6	3.937	0.938	3.677	26	0.876	0.331	0.513
7	1.756	1.256	1.565	27	2.072	0.569	1.243
8	2.521	1.005	1.343	28	0.175	0.116	0.157
9	0.237	0.056	0.189	29	1.999	1.030	1.769
10	1.757	0.820	1.028	30	1.572	0.849	0.629
11	1.868	0.865	0.941	31	7.461	3.477	4.180
12	1.400	0.482	0.631	32	1.703	0.789	1.070
13	1.574	0.899	1.342	33	7.486	2.832	7.572
14	3.684	2.421	2.772	34	2.855	0.569	2.123
15	0.514	0.222	0.255	35	1.089	0.659	1.667
16	1.321	0.597	0.808	36	5.204	1.949	2.004
17	4.010	3.342	2.169	37	3.223	0.712	9.552
18	2.426	1.782	2.017	38	1.729	0.585	0.761
19	0.650	0.354	1.061	39	1.318	0.605	0.828
20	4.353	2.391	1.95	40	1.963	0.862	1.864
Av	2.153	1.196	1.532	Av	2.315	0.936	1.963
s	1.240	0.936	0.891	s	2.114	0.892	2.454

errors is small when we use the function f_2 . Two examples of the calculated functions, f_1 , f_2 and f_3 can be observed in the Figure 3.

**FIGURE 3.** Cell number 15 at $T=25^\circ\text{C}$ and cell number 2 at $T=37^\circ\text{C}$.

The preliminary results, suggest that the movement of RBCs along the microchannel follow a behavior equivalent to the sum of trigonometric functions.

CONCLUSIONS

In this work we measure the motion of RBCs along the middle of a microchannel. The results were approximated by three different types of functions applying nonlinear least squares theory.

The results obtained from this preliminary study, indicates that the function that has a better approximation to the data is based on trigonometric functions. In future work we will consider different type of functions and a bigger amount of cells with different temperatures and Hcts.

An on going study to obtain more detailed quantitative measurements of the blood flow behavior through a glass microchannel is currently under way.

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