Cell-free layer analysis in a polydimethysiloxane microchannel: a global approach

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Abstract: The cell-free layer (CFL) is a hemodynamic phenomenon that has an important contribution to the rheological properties of blood flowing in microvessels. The present work aims to find the closest function describing RBCs flowing around the cell depleted layer in a polydimethysiloxane (PDMS) microchannel with a diverging and a converging bifurcation. The flow behaviour of the CFL was investigated by using a high-speed video microscopy system where special attention was devoted to its behaviour before the bifurcation and after the confluence of the microchannel. The numerical data was first obtained by using a manual tracking plugin and then analysed using the genetic algorithm approach. The results show that for the majority of the cases the function that more closely resembles the CFL boundary is the sum of trigonometric functions.

Keywords: red blood cells; RBCs; cell-free layer; CFL; nonlinear optimisation; global optimisation.

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1 Introduction

Blood is a complex fluid composed mainly of suspended red blood cells (RBCs) within plasma where RBCs are responsible for the supply of oxygen and nutrients to the body and removal of carbon dioxide and metabolic wastes from tissues. Throughout the years, several experimental methods were performed in both *in vivo* (Maeda, 1996; Pries and Secomb, 1994; Suzuki et al., 1996; Kim et al., 2009) and *in vitro* (Faustino et al., 2014; Goldsmith and Turitto, 1986; Lima et al., 2006, 2008, 2009a, 2009b; Rodrigues et al., 2014) environments, in an attempt to understand the flow behaviour of RBCs in microchannels and microvessels. These studies have produced significant findings on the blood rheological properties at a micro-scale level. A hemodynamic phenomenon observed in both *in vivo* and *in vitro* studies is the formation of a marginal cell-free layer (CFL) at regions adjacent to wall due to the tendency of RBCs to migrate toward the centre of the microtube (Caro et al., 1978; Garcia et al., 2012; Maeda, 1996). The existence of a cell depleted layer in microvessels, tend to reduce the apparent viscosity of blood and by increasing this layer the blood viscosity tend to decrease in both microchannels and microvessels. Hence, it is important to understand the behaviour of the CFL in microcirculation as it contributes to the rheological properties of blood flowing in microvessels, modulates the nitric oxide scavenging effects by RBCs and may lead to heterogeneous distribution of blood cells in microvascular networks (Fedosov et

al., 2010; Kim et al., 2009). Additionally, several research studies have developed microfluidic systems able to perform blood separation using the advantage of the CFL formation in PDMS microchannels with dimensions smaller than 300 μm. Faivre et al. (2006), Sollier et al. (2010), Yaginuma et al. (2013) and Pinho et al. (2013b) have demonstrated that the presence of a constriction increases the CFL and as a result they were able to perform the separation of RBCs from plasma. Therefore, it is also important to improve our understanding regarding the CFL phenomenon happening in constriction geometries in order to improve the performance of blood separation microfluidic devices.

Although *in vivo* and *in vitro* experiments gives more realistic information on the flow properties of blood, once validated, physical models and their numerical results are extremely valuable tools to obtain more insight on the blood rheological properties at a micro-scale level. Recently due to the advances of the computational techniques and computing power, several numerical models have been proposed based on a multiphase approach, in which the blood is considered as a multiphase suspension of deformable particles and where levels of submodelling for the blood cells behaviour are also taken into account. Some examples for this type of approach are the boundary element method (Omori et al., 2011), the immersed boundary method (Bagchi, 2007; Eggleton and Popel, 1998), the lattice Boltzmann method (Dupin et al. 2007) the dissipative particle dynamics method (Fedosov et al., 2010), the moving particle semi-implicit (MPS) method (Imai et al., 2010; Tsubota et al., 2006a, 2006b; Gambaruto, 2015) and spring-network model based on the minimum energy concept (Lima et al., 2009a, 2009b; Nakamura et al., 2013). Reviews on these numerical methods can be found at Liu et al. (2006), Yamaguchi et al. (2006) and Lima et al. (2012). Although multiphase approaches are promising methods, it is still extremely complex to consider the CFL in their numerical models, so optimisation can be also an important field of study to help in the development of numerical simulations. In recent years, optimisation algorithms have become increasingly robust and as a result several researchers have applied this methodology to study phenomena happening in microfluidic devices. For instance, Bento et al. (2015) have measured the CFL in a network containing multiple bifurcations and confluences and they have shown that the function that best fits the CFL was the sum of trigonometric functions.

The present study tracks RBCs flowing around the CFL and calculates the most suitable function by using global optimisation technique. The measurements were performed in a polydimethysiloxane (PDMS) microchannel with a diverging and a converging bifurcation and all images were obtained by means of a high-speed video microscopy system.

The paper is organised as follows. Second section shows the materials used in this work and the methods that were applied in this study. The third section presents the numerical results and discussion. The last section presents the main conclusions and some future directions.

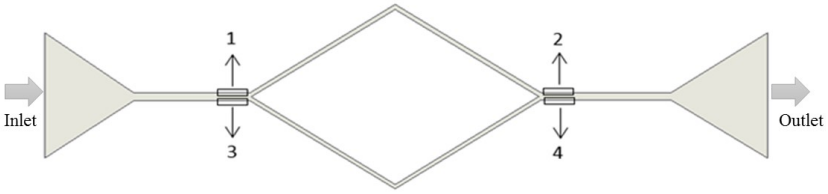
2 Materials and methods

2.1 Microchannel geometry

Microchannels were initially developed with a CAD software, where the geometries were selected taking into account a previous study about the blood flowing through

microchannels with bifurcations and confluences fabricated by a soft lithography technique (Leble et al., 2011). In this study, the parent microchannels have a width of 300, 500 and 1,000 μm and the two branches of the bifurcation and confluence correspond to 50% of the parent microchannel width. Figure 1 shows the configuration of the network and the regions where the CFL was measured, where RA and RB are the upper regions of the microchannel and RC and RD are the lower regions.

Figure 1 Schematic representation of the microchannel geometry and location of the sections where the images were collected and the CFL was measured



Notes: 1 – Region A ($R_{A(width)}$); 2 – Region B ($R_{B(width)}$); 3 – Region C ($R_{C(width)}$) and 4 – Region D ($R_{D(width)}$).

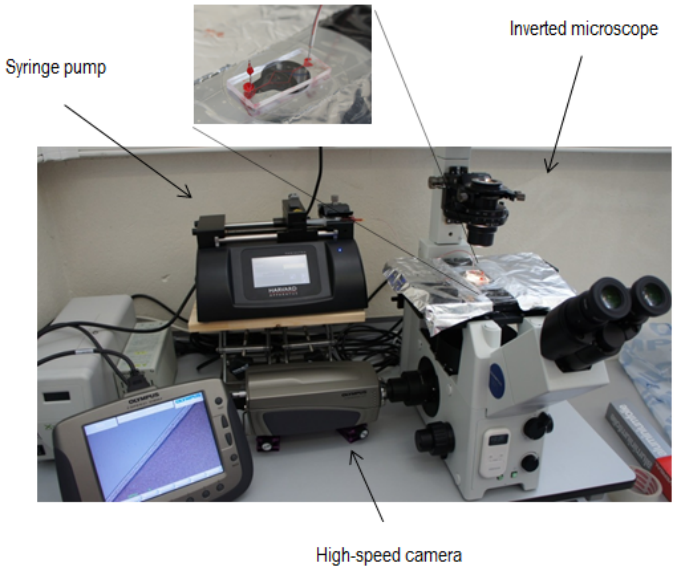
This geometry was used to fabricate the vinyl master moulds by using a soft xurography technique (Pinto et al., 2015). The moulds were used for the production of PDMS microchannels. Briefly, the PDMS was obtained by mixing a curing agent (10:1 ratio) with PDMS prepolymer. By using a spin coater, a residual amount of PDMS with a ratio 20:1 was dispersed on a slide glass. The PDMS was cured in an oven at 80°C for 20 minutes. Then, by using a blade the microchannels were cutted off and the inlet/outlet holes were done by using a fluid dispensing tip. Finally, to have a strong adhesion of the materials, the device was placed in the oven at 80°C for 24 hours. More detailed information about this process can be found at Pinto et al. (2015).

2.2 Working fluids and experimental set-up

The fabricated microchannels were used to study *in vitro* blood flow with Dextran 40 containing 10% of RBCs. The blood was collected from a healthy sheep and heparin was added to prevent clotting. Additionally, the cells were separated from blood by centrifugation.

A syringe pump (Harvard Apparatus PHD ULTRATM) was used to control the flow rate of the working fluid. To visualise and measure the flow we have used an inverted microscope (IX71, Olympus) combined with a high-speed camera (i-SPEED LT). Figure 2 shows the experimental apparatus used to control the flow and to visualise the CFL within the microchannels. The microfluidic device containing the microchannels was placed on the stage of the inverted microscope and a pressure-driven flow was kept constant by means of a syringe pump. All images have a resolution of 800 × 600 pixels and were recorded at a frame rate of 200 frames/s.

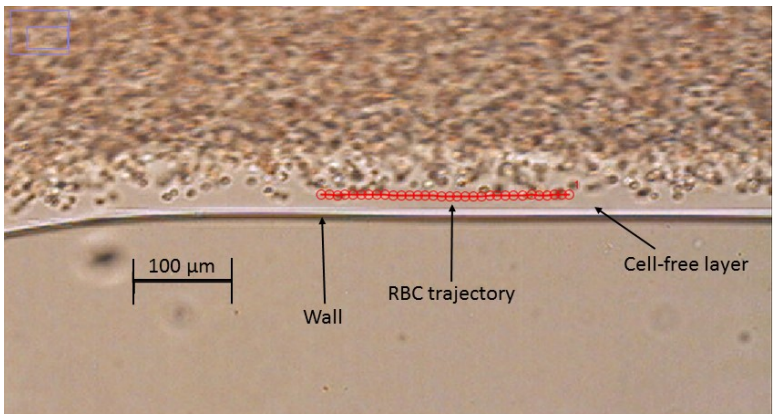
Figure 2 Experimental apparatus to control and visualise the flow in microchannels produced by xurography (see online version for colours)



2.3 Image analysis

A manual tracking plugin (MTrackJ), of the image analysis software Image J (NIH), was used to track individual RBC flowing around the boundary of the RBCs core. By using MTrackJ plugin, the centroid of the selected RBC was automatically computed. After obtaining x and y coordinates of the RBC centroids, the data were exported for the determination of each individual RBC trajectory (Lima et al., 2008; Pinho et al., 2013a). Figure 3 shows a trajectory of a RBC flowing around the boundary region between the CFL and RBCs core.

Figure 3 A trajectory of a RBC flowing around the boundary region between the CFL and RBCs core (see online version for colours)



2.4 Global optimisation method: genetic algorithm

Genetic algorithms are based on theory of evolution of species from Darwin. This method allows to find a global minimum in a large search space (Holland, 1975). The genetic algorithm starts with a set of solutions called population, where the solution is represented by an individual and the population size is preserved through each generation. The objective function is evaluated in each individual. Then individuals are selected according to their objective value. Those selected will be reproduced up randomly, by using genetic operators such as mutation and crossover. Individuals with less value have a high probability of being selected whereas the new generation of individuals may have a minor objective value than the previous generation. The evolution process is repeated until the stopping criterion is satisfied (Bento et al., 2013, 2015; Catlin et al., 2011; Kumar et al., 2010). In this work it was implemented the genetic algorithm proposed by Bento et al. (2013, 2015) using Matlab software.

3 Results and discussion

All videos captured were recorded in four different regions, i.e., region RA and RC correspond to locations before the bifurcation whereas region RB and RD correspond to locations after the confluence (see Figure 1). Moreover, this study investigated the CFL behaviour in three kinds of parent microchannels having widths of 300, 500 and 1,000 μm. For all the cases, the flow rate was constant (10 μl/min) and the working fluid had always a hematocrit (Hct) of 10 %, i.e., containing 10 % of ovine RBCs in the solution. By using 10 % Hct we were able to track several individual RBCs. In fact 10% Hct is not far from the real Hct in microcirculation. It is known that, when the size of vessels becomes smaller the Hct tends to decrease. Additionally, dextran 40 was used to avoid the sedimentation of the cells.

A manual tracking plugin from Image J was used to track individual RBC flowing around the boundary region between the CFL and RBCs core. All the selected RBCs have good enough quality images to track the trajectory of the cells flowing nearby the RBCs core (see Figure 3). Figures 4, 5 and 6 show representative RBC trajectories in the different cases under study, i.e., parent microchannels with widths of 300, 500 and 1,000 μm at two different regions (region before the bifurcation and after the confluence).

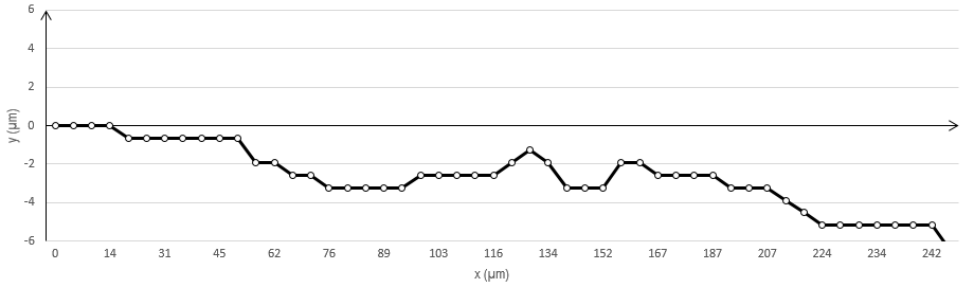
To obtain the numerical data a nonlinear least squares theory was used. In each region R_{Aw}, R_{Bw}, R_{Cw}, and R_{Dw} for w = 300, 500 and 1,000, we have applied the nonlinear optimisation problem defined as follows:

$$\begin{aligned} &\min\ f(y) = \sum_{k=1}^{N_R} \left(M_k - g_h\left(y, x_k\right)\right)^2 \\ &\text{s.t.}\quad g_h\left(y, x_k\right) \geq 0 \quad \forall k = 1,\ldots,N_R \end{aligned} \tag{1}$$

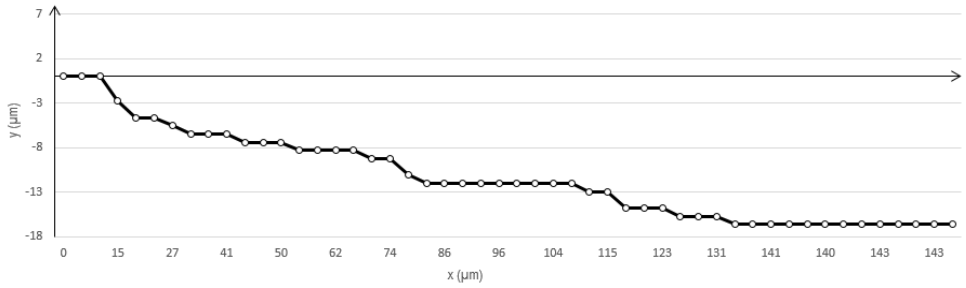
where (x_k, M_k), for $k = 1,\ldots,N_R$ are the CFL measurement of region R (defined as R_{Aw}, R_{Bw}, R_{Cw}, and R_{Dw} for w = 300, 500 and 1,000). The function g_h, for $h = 1,\ldots,3$, are defined as follows:

$$\begin{aligned} &g_1(y, x) = y_1 x^2 + y_2 x + y_3, \\ &g_2(y, x) = y_1 x + y_2, \\ &g_3(y, x) = \sin\left(y_1 x\right) + \cos\left(y_2 x\right) + y_3. \end{aligned} \tag{2}$$

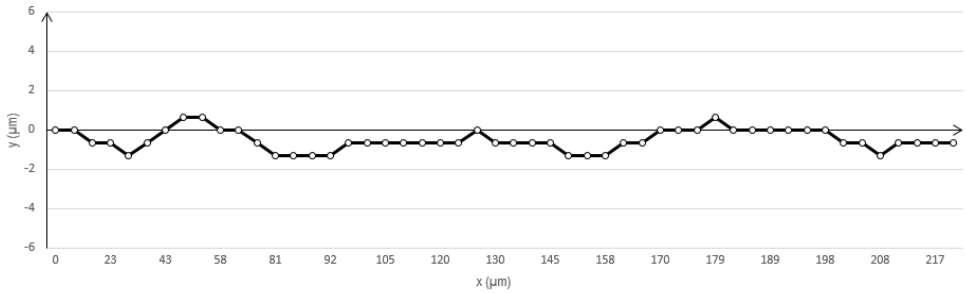
Figure 4 Trajectories of individual RBCs flowing through a microchannel with the width of 300 µm, around the CFL regions, (a) R_{A300} (b) R_{B300} (c) R_{C300} (d) R_{D300}



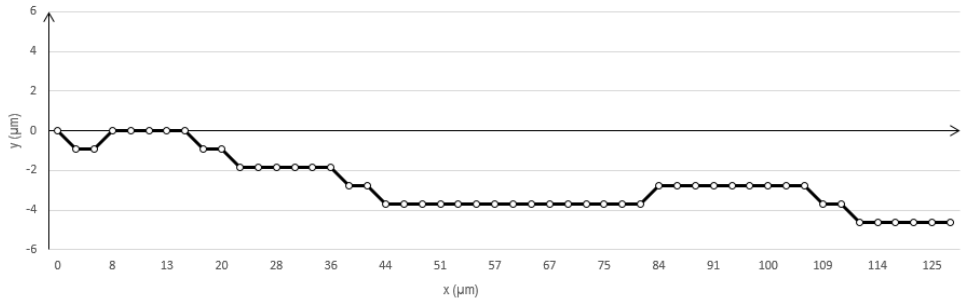
(a)



(b)

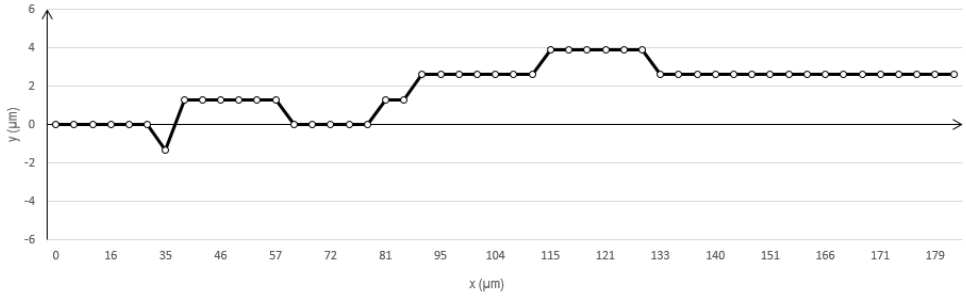


(c)

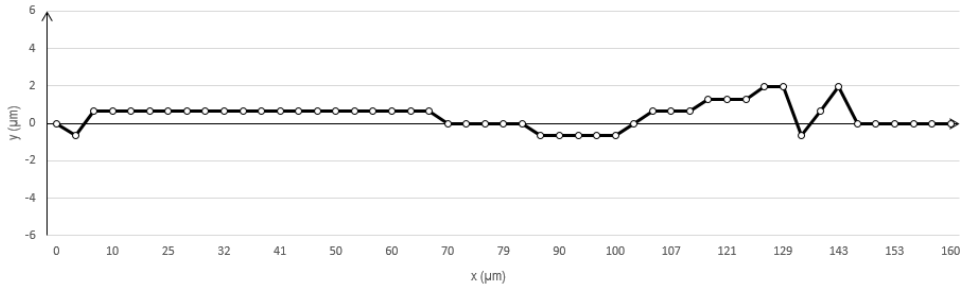


(d)

Figure 5 Trajectories of individual RBCs flowing around the CFL in the microchannel with the width of 500 µm at the regions, (a) R_{A500} (b) R_{B500} (c) R_{C500} (d) R_{D500}



(a)



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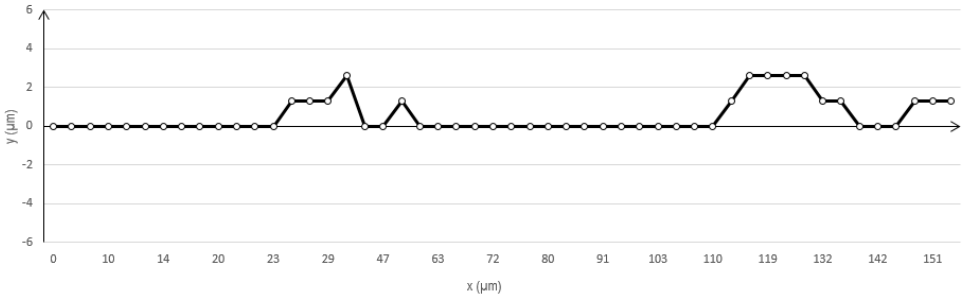
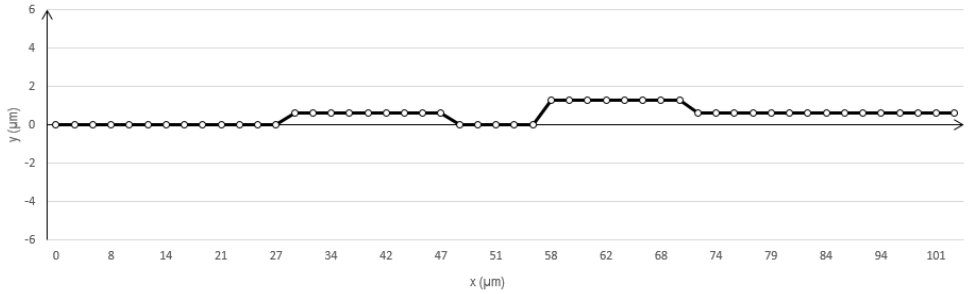


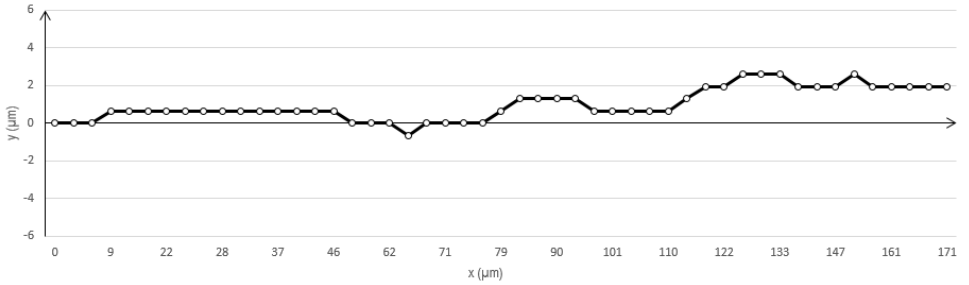
(c)

(d)

Figure 6 Trajectories of individual RBCs flowing around the CFL for the regions, (a) R_{A1000} (b) R_{B1000} (c) R_{C1000} (d) R_{D1000}



(a)



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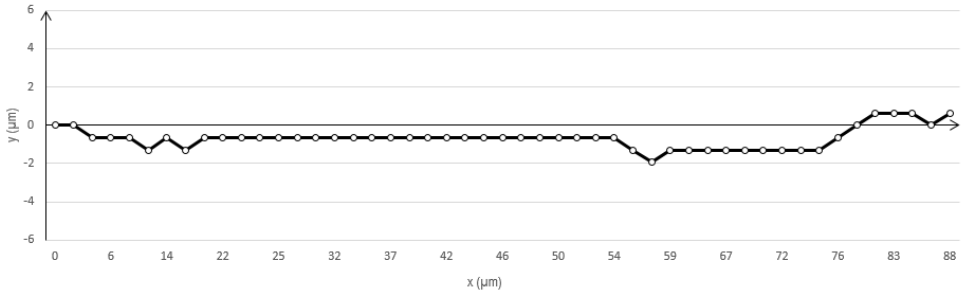

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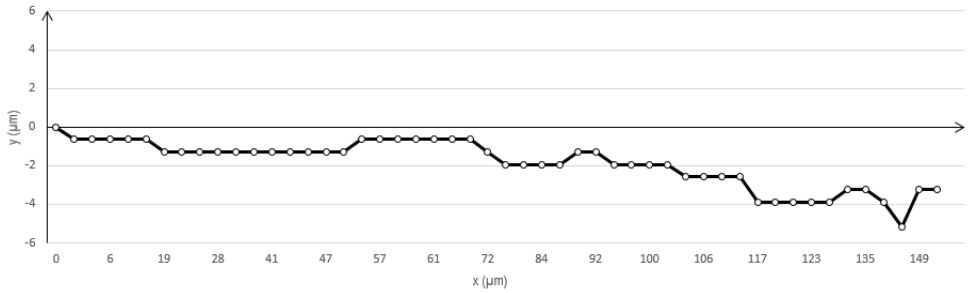


(d)

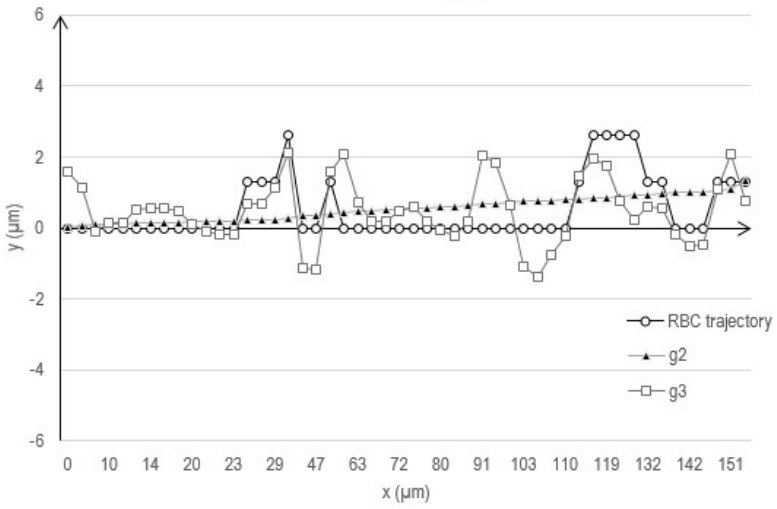
Table 1 Numerical results obtained using a genetic algorithm

Region	*Function*	*Average*	*Minimun*
R_{A300}	g_1	4.90E+05	52.18
	g_2	7.54E+01	31.90
	g_3	*7.39E+01*	26.89
R_{A500}	g_1	1.29E+05	59.82
	g_2	*6.24E+01*	38.76
	g_3	7.00E+01	45.07
R_{A1000}	g_1	2.00E+04	20.8
	g_2	*1.23E+01*	6.46
	g_3	2.09E+01	8.89
R_{B300}	g_1	8.94E+04	97.17
	g_2	*6.93E+01*	30.42
	g_3	7,86E+02	621.43
R_{B500}	g_1	1.37E+05	54.71
	g_2	3.99E+01	20.63
	g_3	*3.25E+01*	19.64
R_{B1000}	g_1	1.58E+05	50.98
	g_2	*2.73E+01*	14.33
	g_3	3.24E+01	11.09
R_{C300}	g_1	2.89E+05	42.35
	g_2	4.88E+01	13.86
	g_3	*2.45E+01*	10.50
R_{C500}	g_1	7.30E+04	57.04
	g_2	4.66E+01	32.60
	g_3	*3.93E+01*	26.63
R_{C1000}	g_1	1.37E+04	11.36
	g_2	2.16E+01	14.82
	g_3	*2.06E+01*	6.42
R_{D300}	g_1	4.20E+04	31.76
	g_2	5.42E+01	35.33
	g_3	*5.23E+01*	14.36
R_{D500}	g_1	2.87E+04	13.32
	g_2	2.96E+01	19.96
	g_3	*2.18E+01*	6.78
R_{D1000}	g_1	6.92E+04	58.85
	g_2	4.32E+01	18.71
	g_3	*3.50E+01*	13.60

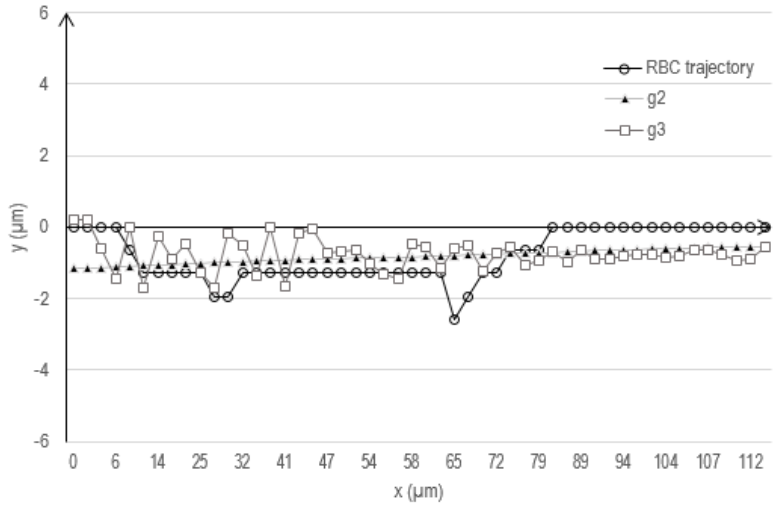
After developing a MatLab code and by applying the genetic algorithm to solve the optimisation problem (1), it was possible to obtain the numerical results shown in Table 1. Since the genetic algorithm is a stochastic method, each problem was solved 100 times. Table 1 presents the regions where the problem (1) was applied, the average of the optimum value and the minimum value obtained in the all 100 runs. The table shows that the minimum value for most of the cases corresponds to the function g_3.

Figure 7 shows an example of two RBCs trajectories flowing in region R_{A500} and R_{B500}, in a parent microchannel with a width of 500 μm, as well as the functions that have revealed a better approximation to the RBCs trajectories.

Figure 7 RBCs trajectories flowing in region (a) R_{A500} and (b) R_{B500}, with a width of 500 μm, as well as the functions that have showed a better approximation



(a)



(b)

Function g_1 was not displayed mainly because it was the worst approximation solution to the cells trajectories. Overall, the numerical results suggest that the trigonometric function (g_3) is the one that better resembles the RBCs trajectories and consequently the CFL boundaries, since for the majority of the cases studied the minimum value was obtained for this function. The trigonometric function (g_3) may be due to oscillations caused by collisions between neighbourhood cells flowing around the RBCs core. The only exception was verified in the regions R_{A500}, R_{A1000}, R_{B300} and R_{B1000} (Figures 4, 5, and 6), wherein these regions the best fit was obtained with the function g_2. Additionally, these results also show that the CFL boundary is size independent and its flow behaviour is not affected by complex geometries such bifurcations and confluences.

4 Conclusions and future directions

In this study, we present a method to measure individual RBCs trajectories flowing around the CFL region. These cells trajectories are believed to closely resemble the CFL boundary and they were fitted using three different functions. A genetic algorithm was used to solve the constrained optimisation problem and the best fit was obtained by using the function (g_3), i.e., a sum of trigonometric functions. This finding corroborates the results obtained by Taboada et al. (2013) and Bento et al. (2015) and where they have performed similar studies in microchannels networks and have found that the function (g_3) is the one that best fit to their CFL measurements. As a future work, we will test other functions and examine a bigger variety of physiological fluids used *in vitro* blood studies.

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