Cell-Free Layer (CFL) Analysis in a Glass Capillary: Comparison Between a Manual and Automatic Method

David Bento*, Diana Pinho***, Ana I. Pereira*§ and Rui Lima***

* Polytechnic Institute of Bragança, ESTiG/IPB, Bragança, Portugal.
** CEFT, Faculdade de Engenharia da Universidade do Porto (FEUP), Porto, Portugal.
§ ALGORITMI, University of Minho, Braga, Portugal.

Abstract. In this study, *in vitro* blood flowing through a 100 μm glass capillary was studied. The images were captured using a confocal system and post-processed using Image J and MatLab. The aim of the present work, was to measure the trajectories of the cell-free layer (CFL) by using two different methods, i. e., a manual method (MM) and an automatic method (AM). For the MM we have used a manual tracking plugin (MTrackJ) from Image J to track labeled red blood cells (RBCs) flowing around the boundary of the RBCs core. For the AM we have used a MatLab scripts to measure automatically the CFL trajectories. The preliminary numerical results suggest that the CFL trajectories follow a polynomial function for both methods.

Keywords: Cell-free layer. Automatic Method. Red Blood Cells. Nonlinear Optimization.

PACS: 02.60.Pn

INTRODUCTION

A well known hemodynamic phenomenon observed in both *in vivo* and *in vitro* studies states that for narrow microtubes (<300 μm) both hematocrit and apparent blood viscosity decreases as the tube diameter is reduced [1, 2]. The physical reason behind this phenomenon, known as Fahraeus-Lindqvist effect, is the tendency of RBCs to migrate toward the centre of the microtube resulting in a marginal cell-free layer (CFL) at regions adjacent to wall [3]. Recently, studies have shown strong evidence that the formation of the CFL is affected by both the geometry of the microchannel [4, 5, 6, 7] and the physiological conditions of the working fluid [8, 9]. Although there have been several studies on the measurement of CFL thickness [10, 11], according to our knowledge there have been very few studies on the determination of CFL trajectory. The main purpose of the present work is to measure several trajectories of the CFL by means of a manual method (MM) and an automatic method (AM) in a 100 μm glass capillary. This experimental study was performed using a confocal microscopy system together with image analysis techniques. Additionally, nonlinear optimization techniques were used to compare automatic and manual methods.

MATERIALS AND METHODS

Working fluids and microchannel geometry

The working fluid used in this study was Dextran 40 (Dx-40; Otsuka Medicine) containing 12 ± 2% (12Hct) of human RBCs. The Hcts corresponded to the feed reservoir Hct and were measured using a hematocrit centrifuge (Kubota 3220) immediately before each experiment. The RBCs were labeled with a lipophilic carbocyanine derivative, chloromethylbenzamido (CM-Dil, C-7000, Molecular Probes). A detailed description about the procedure for labeling the human RBCs can be found elsewhere [12]. The capillaries tested in this study were 100-μm circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes). The capillary was mounted on a slide glass was immersed in glycerol to minimize the refraction from the walls.
Experimental set-up

The confocal micro-PTV system used in this study consists of an inverted microscope (IX71; Olympus) combined with a confocal scanning unit (CSU22; Yokogawa), a diode-pumped solid-state (DPSS) laser (Laser Quantum) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research). The glass capillary was placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific) a pressure-driven flow was kept constant. Additionally, by using a thermo plate controller (Tokai Hit) the temperature was set to 37°C±1. More detailed information about this system can be found elsewhere [5, 12, 13]. Figure 1 shows the blood samples preparation and experimental set-up used in this study.

![Experimental set-up diagram](image)

**FIGURE 1.** Blood samples preparation and experimental set-up.

The confocal images were captured around the middle of the capillary with a resolution of 640×480 pixel at a rate of 100 frames/s. Two image analyses methods were used in this study: manual method (MM) and automatic method (AM).

Manual Method (MM)

A manual tracking plugin (MTrackJ) [14] of an image analysis software (Image J, NIH) [15] was used to track the label RBCs. By using MTrackJ plugin, the bright centroid of the selected RBC was used through successive images. After obtaining series of x and y positions, data were exported for MatLab and the cftool package was used to calculate the best function that fits the experimental data. Figure 2 shows a trajectory of a labeled RBC flowing around the boundary region between the CFL and RBCs core.

![RBC trajectory](image)

**FIGURE 2.** RBC trajectory, at different time intervals, flowing around the boundary region between CFL and RBCs core.
Automatic Method (AM)

The frames were processed using image processing techniques in MatLab [16]. Firstly, it was applied to each frame a median filter to remove the noise from the images, by using a mask 3×3 pixel. Then the intensity of each pixel in the frames sequence was evaluated to obtain an image with the maximum intensity. As a final step, the previously image (Fig. 3b) was converted into a binary image (Fig. 3c). Finally, the region of interest is selected and the upper and lower CFL trajectories are automatically measured. Figure 3 shows the image processing sequence to obtain the resulting binary image.

FIGURE 3. a) Original image; b) Maximum intensity image; c) Binary image.

RESULTS AND DISCUSSION

After obtaining series of x and y positions for both methods, data were exported for the determination of each individual CFL trajectory and to analyze the best mathematical function that approximates to the CFLs experimental flow behavior.

In this study it was measured and analyzed two CFLs trajectories at a temperature of 25 °C. For each CFL, by using the MTrackJ plugin (MM) form Image J and by using the proposed automatic method (AM), we have obtained \{\((x_j, y_j), j = 1, ..., k\}\) data. To compare the consistency of both methods we decided to determine the better approximation for the data by using the tool cftool existing in the Curve Fitting Toolbox from MatLab [17]. Hence, we consider seven different functions: polynomials, exponential, Fourier and power functions.

The error of nonlinear least squares approximation of two CFLs trajectories are listed in the Table 1, where \(F_i = \sum_{j=1}^k (y_j - f_i(x_j))^2\) is the nonlinear least squares approximation error of the function \(f_i(x)\), with \(l = 1, ..., 7\).

<table>
<thead>
<tr>
<th>Function</th>
<th>(F_i) - Manual</th>
<th>(F_i) - Automatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly5</td>
<td>18.0606 18.6327</td>
<td>17.3717 12.5877</td>
</tr>
<tr>
<td>Poly4</td>
<td>20.2669 22.9811</td>
<td>18.4294 15.7613</td>
</tr>
<tr>
<td>Poly3</td>
<td>24.1842 23.7068</td>
<td>20.0685 16.1108</td>
</tr>
<tr>
<td>Poly2</td>
<td>25.7616 23.7738</td>
<td>20.4083 16.1177</td>
</tr>
<tr>
<td>Exp2</td>
<td>24.6697 23.7505</td>
<td>20.2906 16.1024</td>
</tr>
<tr>
<td>Fourier1</td>
<td>20.2669 21.6219</td>
<td>23.1763 16.6979</td>
</tr>
<tr>
<td>Power2</td>
<td>51.3068 24.3397</td>
<td>20.5599 15.7486</td>
</tr>
</tbody>
</table>

The results from Table 1 suggest that the best mathematical function that fits the data is based on the polynomial of degree five.


CONCLUSIONS

The present study indicates that the CFL trajectories that exist in microchannels, follow a polynomial function of degree five. Additionally, the data obtained from the proposed automatic method (AM) is equivalent to data obtained from the manual method (MM). Hence, the proposed automatic method is a promising way to measure automatically the trajectory of the CFL in microchannels.

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by: PTDC/SAU-BEB/108728/2008, PTDC/SAU-BEB/105650/2008, PTDC/EME-MFE/099109/2008 and PTDC/SAU-ENB/116929/2010 from the FCT (Science and Technology Foundation) and COMPETE, Portugal.

REFERENCES