CELL-FREE LAYER MEASUREMENTS IN A BIFURCATION MICROCHANNEL: COMPARISON BETWEEN A MANUAL AND AUTOMATIC METHODS

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ABSTRACT: *In the present work, in vitro blood flowing through a bifurcation microchannel was studied. The aim was to develop different automatic methods capable to measure the cell-free layer (CFL) thickness in the input and output of the microchannel. The results were compared with the trajectories of the CFL obtained manually. The method with the best results was the method that uses the binarization.*

1 INTRODUCTION

Blood flow in microcirculation shows several hemodynamic phenomenons, *in vivo* and *in vitro*. Hence, over the years *in vitro* blood studies in microchannels have been extensively performed in order to obtain an understanding of blood rheology and its flow dynamics [1, 2]. The Fahraeus-Lindqvist effect is one of the physical reasons for the tendency of RBCs to migrate toward the centre of the microtube resulting in a marginal cell-free layer (CFL) at regions adjacent to the wall [3]. Recently several studies showed strong evidence that the formation of the CFL is affected by the geometry of the microchannel [4-7] and the physiological conditions of the working fluid, such as the haematocrit (Hct) [8, 9]. Until now, most of the experimental data resulted from the studies have been analysed manually. The manual methods can be highly reliable but they are relatively time consuming and can also introduce user errors into the data. So, as an outcome it is essential to develop image analysis methods able to process the data automatically. In this point, image analysis plays an extremely important role to obtain information about the blood rheology [10].

In this study, the experimental phase was performed using a confocal microscopy system. It was used the image analysis techniques to measure several trajectories of the CFL in a microchannel with a diverging and converging bifurcation. In this study it is compare one manual method and two automatic methods developed in MatLab

The paper is organized as follows. The Section 2 presents the experimental set-up. In Section 3 is described the manual method and the two proposed automatic methods. In Section 4 is presented and discussed the results, following the Section

5 and 6, Conclusion and Future Work respectively.

2 EXPERIMENTAL SET-UP

The confocal micro-PIV system used in this study consists of an inverted microscope (IX71; Olympus) combined with a high-speed camera (*i*-SPEED LT). The microchannel was placed on the stage of the inverted microscope and a pressure-driven flow was kept constant based on a syringe pump (PHD ULTRA). The confocal images have the resolution of 800×600 pixel at a frame rate of 100 frames/s.

The working fluid used in this study was Dextran 40 (Dx-40; Otsuka Medicine) containing 5 and 10% (± 2%) i.e., Hct = 5 and 10 by volume of RBCs. The blood was collected from a healthy sheep and was added heparin to prevent clotting. Further, the cells were separated from blood by centrifugation.

3 IMAGE ANALYSIS

3.1 MANUAL METHOD

A manual tracking plugin (MTrackJ) [6] of an image analysis software (Image J, NIH) [7] 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.

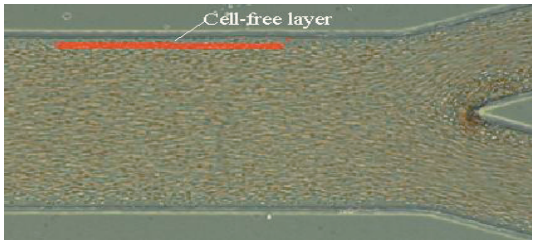


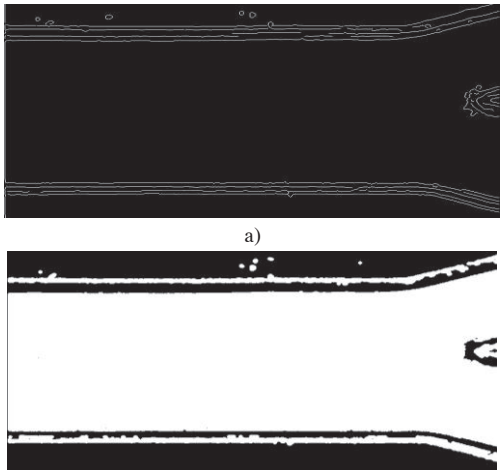
Fig. 1 Manual method showing a trajectory of a labeled RBC.

After obtaining *x* and *y* positions, the data were exported for the determination of each individual RBC trajectory.

3.2 AUTOMATIC METHODS

All image sequences were processed using image processing techniques provide by MatLab [11].

Firstly, a median filter was applied to each frame to remove the noise from the images, by using a 3×3 mask. Then the intensity of each pixel in the frame sequences was evaluated to obtain an image with the maximum intensity. In the Method A, we used the previous image, and found the edges of the channels by using the Canny algorithm [11] (Fig. 2 a)). After selected the region of interest, upper and lower CFL trajectories were automatically measured. In the case of the Method B, the image with the maximum intensity was converted into a binary image (Fig. 2 b)), and the upper and lower CFL trajectories were automatically measured.



a)

b)

Fig. 2: Automatic Methods: a) Method A; b) Method B.

4 RESULTS AND DISCUSSION

The results were taken in the inflow and outflow of the microchannels, Fig. 3. The flow rate used was a 500nl/min and two fluids were studied, respectively with 5 and 10% of Hct.

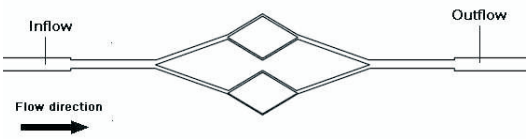


Fig. 3 Geometry used in the study, and the representation of where is taken the results.

In Table 1, the results from the three methods are presented.

Table 1 - Comparison between the different methods.

	Hct	Manual (μm)	Automatic A (μm)	Automatic B (μm)
Inflow	5%	16.0249	11.6508	22.5815
	10%	11.7096	8.2707	12.2182
Outflow	5%	14.4679	6.7014	21.321
	10%	10.7721	3.7087	15.2006

The data obtained from both automatic methods presents some different results from the manual data. However, the results obtained from the Method B, as shown in the Fig. 4, present best results because they have a similar behavior between both.

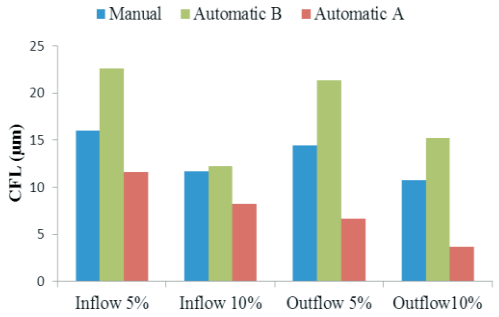


Fig. 4 Comparison between the manual and the two automatic methods.

The Method A, by applying the Canny filter along the channel, have a constant decreases of the CFL thickness and this behavior is not observed in the case of the manual method. Though, in the case of the Method B that use the binarization, presents the same comportment of the manual method. Other result analysis that is possible to observe for the three methods is the CFL decreasing from the inflow to the outflow and that the hematocrit has a considerable influence in the CFL thickness, increasing the hematocrit the CFL decreases.

5 Conclusions

The present study indicates that the proposed automatic Method B have the best agreement to data obtained from the manual method. This method uses the binarization of the image with the maximum intensity. From the inflow to the outflow is possible to observe a decreasing of the CFL thickness and the CFL tend to decrease as the Hct increases. Hence, the Method B may be a promising way to carry on this study.

6 Future Work

In this type of study the quality of the image sequence plays a crucial rule and as result we plan to obtain sequence of images with more quality and to use an objective lens with better resolution. Additionally, we also plan to improve the current automatic method to obtain similar results to those obtained manually.

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