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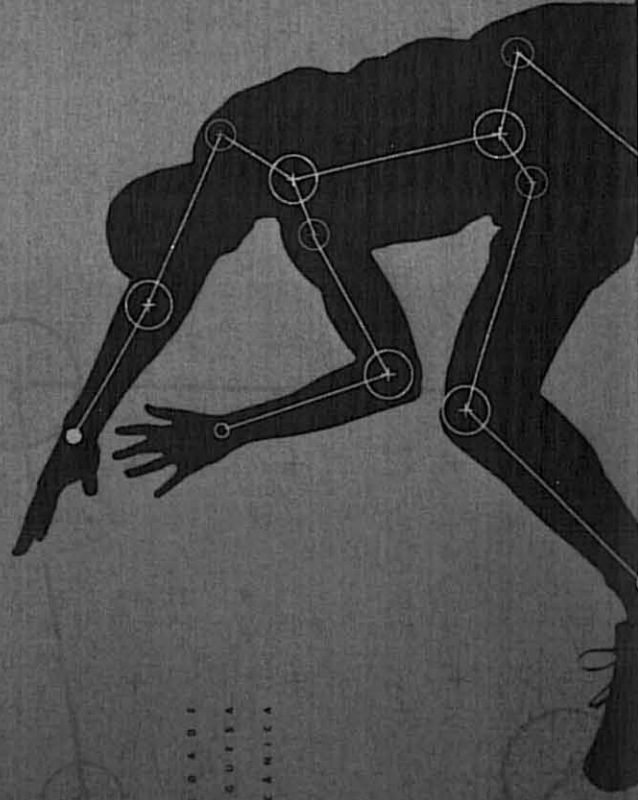
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CELL-FREE LAYER MEASUREMENTS IN A BIFURCATION MICROCHANNEL: COMPARISON BETWEEN A MANUAL AND AUTOMATIC METHODS

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ABSTRACT: *In the present work in vitro blood flowing through a bifurcation microchannel was studied. The aim was to measure the trajectories of the cell-free layer (CFL) by using different methods, i. e., a manual method and two automatic methods.*

1 Introduction

Blood flow in microcirculation shows several hemodynamic phenomena, *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 [8, 9]. For these studies image analysis is extremely important to obtain crucial information [10]. The main purpose of the present work is to measure several trajectories of the CFL in a microchannel with a diverging and converging bifurcation by means of a manual method and two automatic methods developed in MatLab.

2 Experimental setup

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 by using a syringe pump (PHD ULTRA) a pressure-driven flow was kept constant.

2.1 Manual Method

A manual tracking plugin (MTrackJ) [6] of an image analysis software (Image J, NIH) [7] was used to track individual RBC. By using MTrackJ plugin, the bright centroid of the selected RBC was automatically computed.

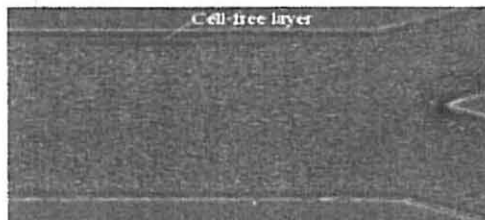


Fig. 1 Manual method showing a trajectory of a labeled RBC.

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

2.2 Automatic Methods

All image sequences were processed using image processing techniques provided by MatLab [11]. Firstly, a median filter was applied to each frame to remove the noise from the images, by using a mask 3×3 pixel. 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.

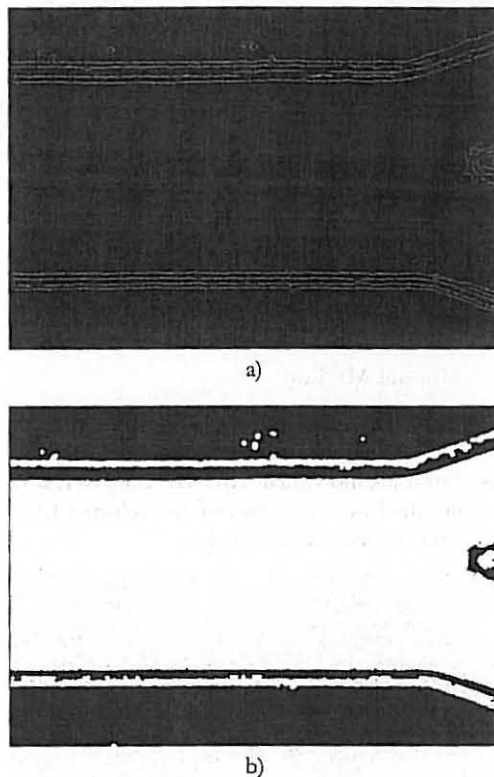


Fig. 2: Automatic Methods: a) Method A; b) Method B.

3 Results and discussion

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)
Input	5%	16.0249	11.6508	16.8523
	10%	11.7096	8.2707	12.9645
Output	5%	14.4679	6.7014	20.2241
	10%	10.7721	3.7087	17.0205

The data obtained from the input of the Method B presents very close results to the one obtained manually. However, the results obtained in the output have some discrepancy. The Method A may not be a satisfying method, because the difference between this method and the manual method is high.

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