Motions of Trace Particles and Red Blood Cells in a PDMS Microchannel with a Converging Bifurcation

Vladimir Leble[1], Ricardo Dias[1),2)], Rui Lima[*1),2)], Carla Fernandes[1)], Takuji Ishikawa[3)], Yohsuke Imai[3)], and Takami Yamaguchi[4)]

1) ESTiG/IPB, Polytechnic Institute of Bragança, Bragança, Portugal.
2) CEFT, Faculdade de Engenharia da Universidade do Porto (FEUP), Porto, Portugal.
3) Depertment of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai, Japan.
4) Depertment of Biomedical Engineering, Graduate School of Engineering, Tohoku University, Sendai, Japan.
E-mail: ruimec@ipb.pt

Abstract

This paper presents microflow visualizations of both pure water and red blood cells (RBCs) in a converging bifurcation. The visualizations are a combination of results obtained from a confocal micro-PTV system and an image analysis technique known as "Zproject" from ImageJ. Our preliminary qualitative results suggest that the cell-free layer (CFL) that is formed in middle of the microchannel (after confluence apex) may play an important role on RBCs trajectories.

1. Introduction

Along the years, various experimental techniques have been performed in both *in vivo* and *in vitro* environments in an effort to understand the blood flow behavior in microcirculation [1-4]. However, several studies on blood flow in glass capillaries and in microvessels have yielded conflicting results with respect on the rheological properties of blood [3, 4]. The main potential causes for the observed in *vivo/in vitro* discrepancies may be due to the endothelial surface layer and complex microvascular networks composed by diverging and converging bifurcations [2]. In order to clarify the causes for the observed discrepancies we need to better understand the effect of both diverging and converging bifurcations on the rheological properties of blood. Hence, the main purpose of this paper is to analyse the motion of both trace particles suspended in pure water and *in vitro* blood in a converging bifurcation. To accomplish it experimental flow studies performed with a confocal micro-PTV system will be used. Additionally, image analysis techniques will be also performed to visualize flow phenomena happening at the confluence.

2. Materials and Methods

2.1. Working fluids

The working fluids used in this study were: pure water (PW) with fluorescent trace particles of 1μm and Dextran 40 (Dx-40) containing about 14% (14Hct) of human RBCs. The washed RBCs were fluorescently labeled with a lipophilic carbocyanine derivative dye, chloromethylbenzamido (CM-Dil, Molecular Probes), using a previously described procedure [5].

2.2. Microchannel geometry

The microchannels tested in this study were fabricated using common soft lithography technique [6] and consist of a confluence with 84.3 μm and 83.1 μm wide for daughter vessels and 159.3 μm wide for the parent vessel. The microchannel height was measured by a profilometer to be 50 μm. Figure 1 shows the confluence with the correspondent dimensions.

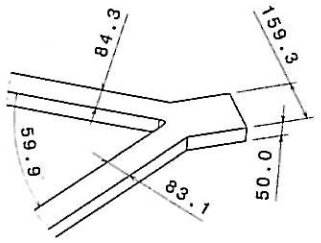

Fig. 1. Dimensions of the confluence used in this study. The channel dimensions are in μm.

2.3. Experimental set-up

The confocal micro-PIV/PTV system used in the present study consists of an inverted microscope combined with a confocal scanning and a diode-pumped solid state (DPSS) laser with an excitation wavelength of 532 nm and a high-speed camera. The microchannel was placed on the microscope stage where the flow rate of the working fluids was kept constant (Q = 0.18μL/min) by means of a syringe pump. By using a thermo plate controller, the microscope stage was set to 37°C. Detailed information about the experimental set-up, microchannel fabrication and RBC labeling used in the present study, has already been described previously [1, 5, 6].

2.4. Image analysis

All the confocal images were captured around the middle of the microchannel with a resolution of 640×480 pixels, at a rate of 100 frames/s. The recorded images were transferred to the computer and then evaluated in the image processing program ImageJ (NIH) [7] by using the manual tracking MtrackJ plugin

[8] and automatic ParticleTracker 2D plugin [9] to detect and track particles in pure water and RBCs in Dx40 respectively. The captured videos were converted to a sequence of static images (stack) where each pixel's maximum intensity of all images in the stack was selected by using "Z project" function (ImageJ).

3. Results and Discussion

In this section we present the results of flow visualizations and evaluate the effect of the confluence on the both trace particles in pure water (see Fig. 2 and 3) and labeled RBCs (see Fig. 4 and 5).

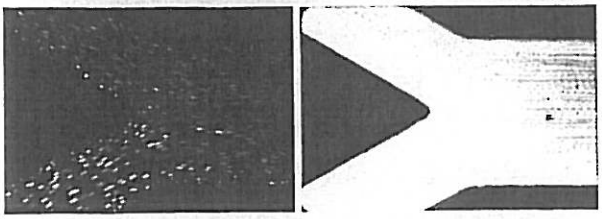

Fig. 2. Original image of pure water captured by high-speed camera (left side). Image obtained after "Zproject" (maximum intensity function) (right side).

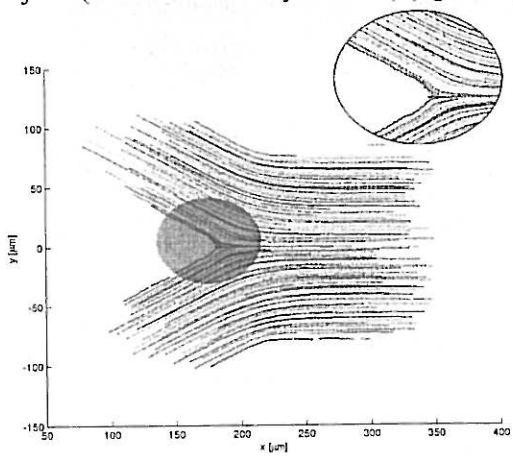

Fig. 3. Trajectories of fluorescent particles in PW.

From Fig. 4 we can clearly observe a cell-free layer (CFL) in middle of the microchannel after the apex of the confluence. This flow phenomenon is not observed with pure water (see Fig. 2). Fig. 5 suggests that RBCs tend to undergo lateral deviations just before the confluence apex. Although, the CFL may be the main cause for observed flow phenomenon a detailed quantitative study to clarify the observed deviations is currently under way.

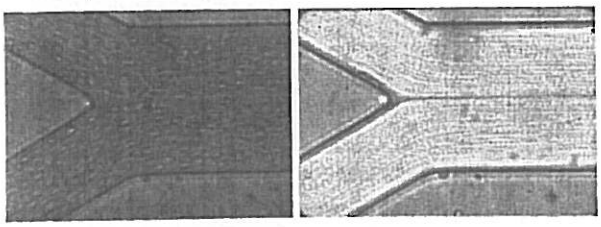

Fig. 4. Original image of *in vitro* blood captured by high-speed camera (left side). Image obtained after "Zproject" (maximum intensity function) (right side).

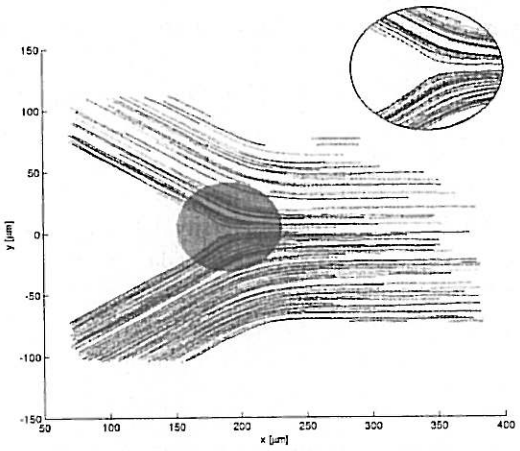

Fig. 5. Trajectories of RBCs in Dx40.

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