Flow Behaviour of Labeled Red Blood Cells in Microchannels : a confocal micro-PTV assessment

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***Abstract*—** The development of optical experimental techniques has contributed to obtain explanations on the blood flow behaviour through microvessels. Although the past results have been valuable, detailed studies on the flow properties of blood in the microcirculation have been limited by several technical factors such as poor spatial resolution and difficulty to obtain quantitative detailed measurements at such small scales. In recent years, due to advances in computers, optics, and digital image processing techniques, it has become possible to combine particle tracking methods with confocal microscopes. As a result, this combination has greatly increased the resolution of the conventional PIV systems and consequently provided additional detailed description on the blood cells motion not obtainable by traditional methods. In this work, we will show an overview of the most recent results on the blood flow behaviour in microchannels obtained by state-of-the-art confocal micro-PIV/PTV system.

Keywords— Microcirculation, red blood cells, microchannels, confocal micro-PTV, radial dispersion

I. Introduction

Blood flow in both microvessels and microchannels has been measured by several measurements techniques such as: double-slit photometric [1], laser-Doppler anemometer [2, 3], video-based methods [4, 5]. Although the past research fondinds have been encouraging, detailed studies on the way blood flow behaves at a microscopic level have been limited by several factors such as poor spatial resolution, difficulty to obtain accurate measurements at such small scales, optical errors arisen from walls of the microvessels, high concentration of blood cells, and difficulty in visualization of results due to insufficient computing power and absence of reliable image analysis techniques. In recent years, due to advances in computers, optics, high-speed imaging and image processing techniques, it has become possible to combine a conventional particle image velocimetry (PIV) system with an inverted microscope and consequently improve both spatial and temporal resolution [6-10]. This system, known as micro-PIV (see Fig.1), has been applied to study the flow properties of blood in both microvessels and microchannels [6, 7, 9, 10]. Although, micro-PIV systems are gaining widespread among the biomicrofluidics community due to its high spatial and temporal resolution, the employment of conventional microscope leads to the entire illumination of the flow field resulting in high levels of background noise and consequently errors on the flow measurements [11]. These errors can be partially removed by using a spinning disk confocal microscope (SDCM) [12, 13]. Due to its spatial filtering technique and multiple point light illumination system, confocal micro-PIV [14 - 16] has become accepted as a reliable method for measuring in vitro blood flow through microchannels. The current mini review will show a short overview of the most relevant results on the blood flow behaviour in microchannels obtained by our confocal micro-particle tracking velocimetry (PTV) system.

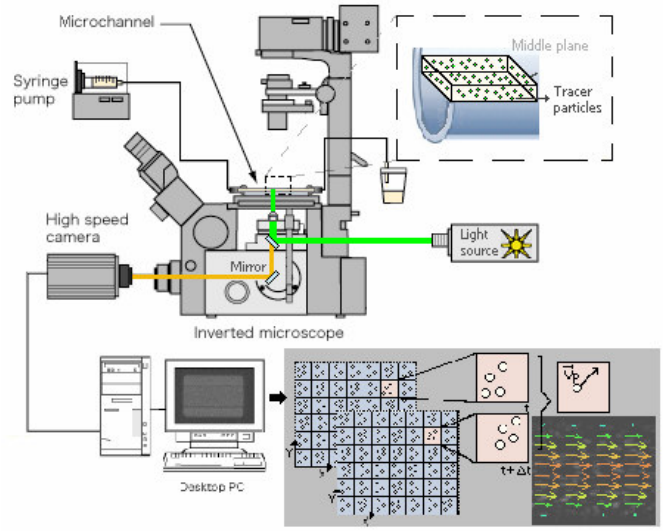


Fig. 1 Conventional micro-PIV system (adapted from [19]).

II. Confocal micro-PTV system

Working fluids and RBC labeling

Experiments were carried out with different working fluids: dextran 40 (Dx-40; Otsuka Medicine, Tokyo, Japan) containing about 3% hematocrit (Hct) up to 35% Hct of human red blood cells (RBCs). The RBCs were labeled with a lipophilic carbocyanime derivative, chloromethylben-

zamido (CM-Dil, C-7000, Molecular Probes, Eugene, OR, USA). This cell tracker was selected due to its strong photostable fluorescence, excellent cellular retention, and minimal cytotoxicity. A full description about the procedure for labeling the human RBCs can be found at Lima et al. 2009 [17].

Microchannels

A 100-µm and 50-µm circular borosilicate glass microchannel was fabricated by Vitrocom (Mountain Lakes, NJ, USA). The microchannel was mounted on a slide glass approximately 80 µm thick and immersed in glycerol that had the same refractive index to minimize refraction from the walls. Additionally, by using a wire casting technique [18] it was possible to fabricate 75-µm circular polydimensiloxane (PDMS) microchannels.

Experimental setup

The confocal micro-PTV system consisted of an inverted microscope (IX71; Olympus, Tokyo, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Tokyo, Japan), a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd., Stockport, UK) and a high-speed camera (Phantom v7.1;Vision Research, NJ, USA). The microchannels were placed on the stage of the inverted microscope where the flow rate of the working fluids was kept constant using a syringe pump (KD Scientific Inc., Holliston, MA, USA). A thermo-plate controller (Tokai Hit, Shizuoka, Japan) was employed to apply a temperature around the microchannel of about 37ºC. More detailed information about this system can be found elsewhere [14, 17, 19].

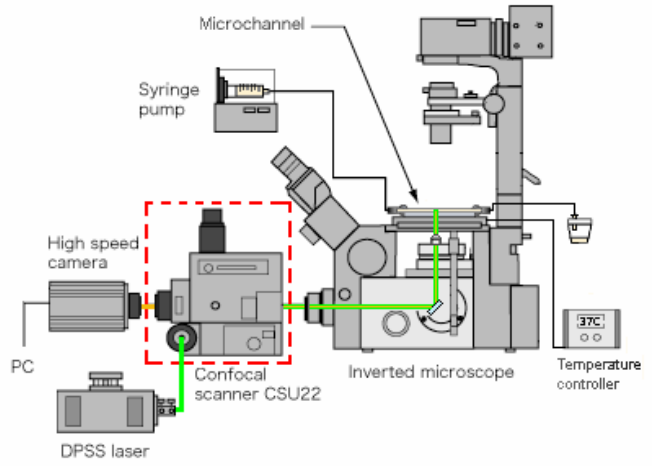


Fig. 2 Confocal micro-PTV experimental set-up (adapted from 17).

Tracking RBC trajectory

The recorded series of *xy* confocal images were evaluated in Image J [20] (NIH, Bethesda, MD, USA) using the manual tracking MtrackJ [21] plug-in. Generally, most of RBCs were followed for periods of time from 0.34s up to 1s, with temporal resolution of 10 ms. The motion of the labeled RBCs, they were manually tracked through successive images using the bright centroid criteria available at the MtrackJ. Using this method, it was possible to track labeled RBCs even when two cells were in near proximity. After obtaining series of *x* and *y* positions, data were exported for the determination of physical quantities such as velocity, radial displacement, and dispersion coefficient.

III. Confocal Micro-PTV Results & Discussion

First confocal microscopy experiments with in vitro blood were carried out in both glass [14, 15] and PDMS [16] microchannels. In these research woks measurements of both ensemble and instantaneous velocity profiles for *in vitro* blood with Hcts up to 20% were performed. Although the ensemble velocity profiles were in close agreement with a theoretical model, some fluctuations in the instantaneous velocity profiles were found to be closely related to the increase in the Hct implying that the presence of RBCs within the plasma flow influences the measurements of the instantaneous velocity fields. Moreover, for the case of glass microchannels and for Hct bigger than 9%, the light absorbed by the RBCs contributes to diminish the concentration of tracer particles in the recorded confocal images. This low density images become more evident for Hct bigger than 20 %, which generates spurious errors in the velocity fields. As a result, Lima and his colleagues [17, 19, 22] have applied a new approach, known as confocal micro-PTV, to track the trajectories of individual labeled RBCs at high Hcts. Figures 3 and 4 show the ability of this confocal method to measure the motion of blood cells at both diluted and high suspensions of RBCs, respectively. Figure 5 shows trajectories of labeled RBCs in a PDMS microcannel.

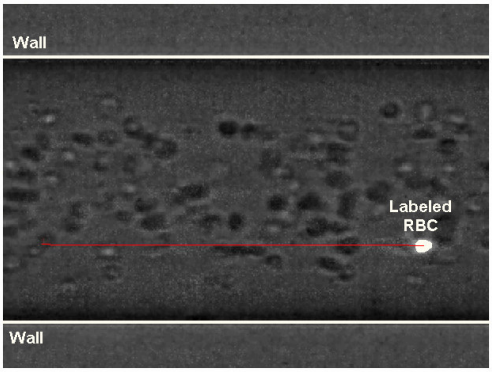


Fig. 3 Trajectories of labeled RBCs at diluted suspensions of cells obtained by the proposed confocal micro-PTV system (adapted from [22]).

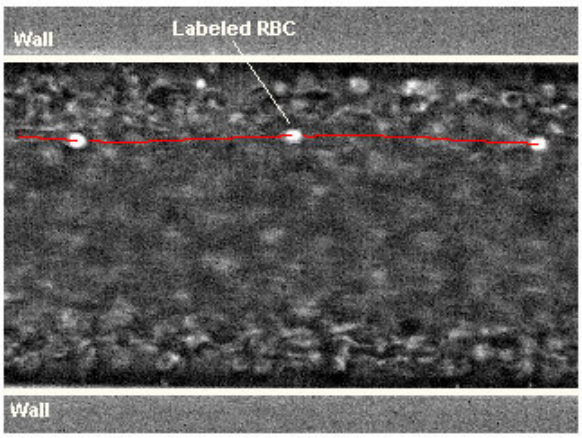
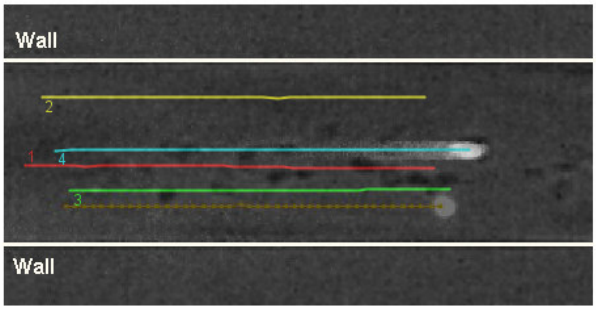
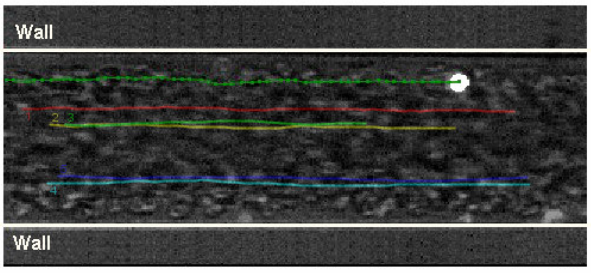


Fig. 4 Trajectories of labeled RBCs at high suspensions of cells obtained by the proposed confocal micro-PTV system. Experiments were carried out in glass microchannels (adapted from [22]).



a)



b)

Fig. 5 Trajectories of labeled RBCs at a) diluted and b) high suspensions of cells obtained by the proposed confocal micro-PTV system. Experiments were carried out in PDMS circular microchannels (adapted from [18]).

The capacity of the confocal system to generate thin in-focus planes has allowed both qualitative and quantitative measurements in flowing blood at concentrated suspensions of: cell-cell hydrodynamic interaction, RBC orientation and RBC radial dispersion at different depths [17, 22]. Furthermore RBCs radial dispersion coefficient (D_{yy}) were determined in the middle plane of 100 µm capillaries [22]. The results demonstrated that RBCs D_{yy} tends to increase with the Hct. For instance Fig.6 shows clear evidence that RBCs D_{yy} at Hct of 24% is almost one order magnitude bigger than D_{yy} with 3% Hct. Similar qualitative results were also obtained in a 75µm PDMS circular microchannel (see Fig.7). These research findings show evidence that the RBCs flowing in a crowded environment tend to undergo multi-body collisions which increases the amplitude of the RBC's lateral motion and consequently RBC D_{yy}. Hence, RBCs at high concentrations tend to exhibit higher erratic radial displacement compared to dilute suspensions of RBCs.

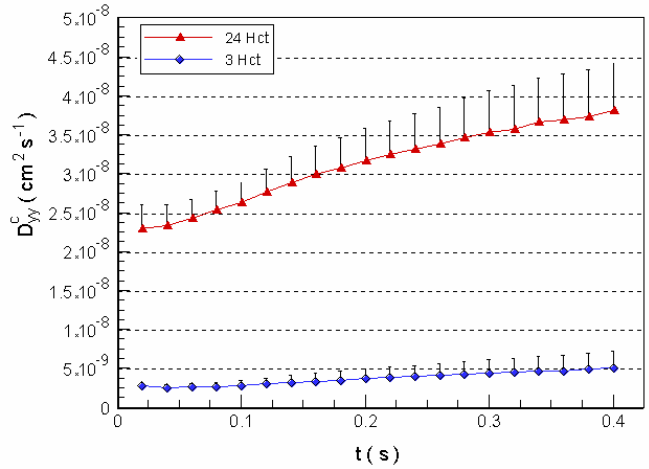


Fig. 6 RBCs radial dispersion coefficient (D_{yy}) of 100µm glass microchannel for 3% Hct and 24%Hct. (adapted from [22]).

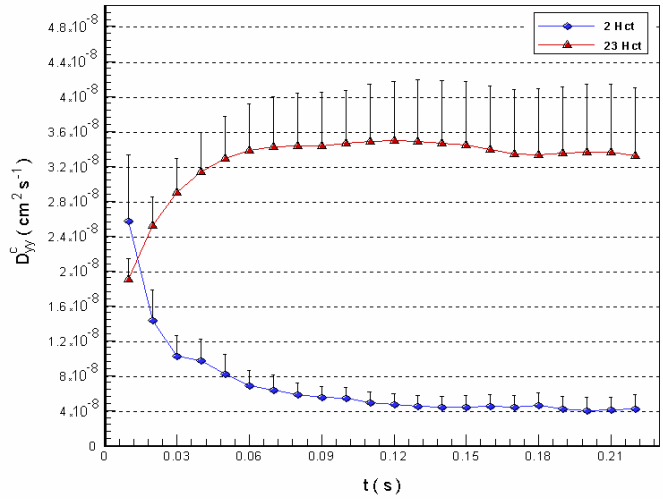


Fig. 7 RBCs radial dispersion coefficient (D_{yy}) of 75µm PDMS circular microchannel for 2% Hct and 23%Hct. (adapted from [18]).

IV. Conclusion & Future Directios

Owing to its optical sectioning ability and consequent improvement of the image contrast and definition, the proposed confocal micro-PTV system has proved to be a powerful technique to provide detailed quantitative measure-

ments on the motion of RBCs cells at both diluted and high suspensions of cells. The results suggest that the RBC paths are strongly dependent on the Hct and as a result the RBC dispersion coefficient tend to increase with the Hct.

The results obtained from the presented study were performed in straight microchannels. In the near future, we expect to measure the RBC´s motions in more complex geometries such as bifurcations, contractions and expansions. Moreover, by culturing endothelial cells within the PDMS microfluidic device, we expect to develop a flow system device that closely mimics the *in vivo* microvessel environment.

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