Traking Red Blood Cells in a Circular Pdms Microchannel using a Confocal Micro-Piv System

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Abstract

The blood flow in microcirculation is characterized mainly by the flow of red blood cells (RBCs), which may be normal or pathological. This paper presents the application of a confocal micro-PIV system to track RBCs through a circular polydimethysiloxane (PDMS) microchannel. This technique, consists of a spinning disk confocal microscope, high speed camera and a diode-pumped solid state (DPSS) laser combined with a single particle tracking (SPT) software (MtrackJ). To show the ability of this system detailed motions of individual RBCs were measured at different haematocrits (Hct): 3%, 14% and 37%. Our results show clearly that this technique can provide detailed information about micro-scale disturbance effects caused by RBCs to the blood flow.

1. Introduction

Detailed knowledge on the motion of individual RBCs flowing in microchannels is essential to provide a better understanding on the blood rheological properties and disorders in microvessels. Several studies on both individual and concentrated RBCs have already been performed in the past [1, 2]. However, all studies used conventional microscopes and also ghost cells to obtain visible trace RBCs through the microchannel. Recently, considerable progress in the development of confocal microscopy and consequent advantages of this microscope over the conventional microscopes have led to a new technique known as confocal micro-PIV [3-7]. This technique combines the conventional PIV system with a spinning disk confocal microscope (SDCM). Due to its outstanding spatial filtering technique together with the multiple point light illumination system, this kind of microscope has the ability to obtain in-focus images with optical thickness less than 1 μm, a task extremely difficult to be achieved by using a conventional microscope.

The main purpose of this paper is to investigate the ability of the confocal micro-PIV system to measure individual RBCs at different Hct through a 75μm circular PDMS microchannel.

2. Materials and methods

2.1. *Working fluids and microchannel*

Three working fluids were used in this study: dextran 40 (Dx40) containing about 3%(3Hct) 14% (14Hct) and 37% (37Hct) of human red blood cells (RBCs). The blood was collected from a healthy adult volunteer, where ethylenediaminetetraacetic acid (EDTA) was added to prevent coagulation. The RBCs where separated from the bulk blood by centrifugation (1500 RPM for 5 minutes) and aspiration of the plasma and buffy coat and then washed twice with physiological saline (PS). The washed RBCs were labeled with a fluorescent cell tracker (CM-Dil, C-7000, Molecular Probes) and then diluted with Dx40 to make up the required RBCs concentration by volume. All blood samples were stored hermetical at 4°C until the experiment was performed at controlled temperature of about 37°C.

The microchannel used in this study was a PDMS circular microchannel (75μm in diameter) fabricated by a wire casting technique.

2.2. *Confocal micro-PIV experimental set-up*

The confocal micro-PIV system used in our experiment consists of an inverted microscope (IX71, Olympus, Japan) combined with a confocal scanning unit (CSU22, Yokogawa, Japan) and a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd, England) with an excitation wavelength of 532 nm. Moreover, a high-speed camera (Phantom v7.1, U.S.A.) was connected into the outlet port of the CSU22. The microchannel was placed on the stage of the inverted microscope where the flow rate of the working fluids was kept constant (Re = 0.004) by means of a syringe pump (KD Scientific Inc., U.S.A.). A thermo plate controller (Tokai Hit) was set to 37°C.

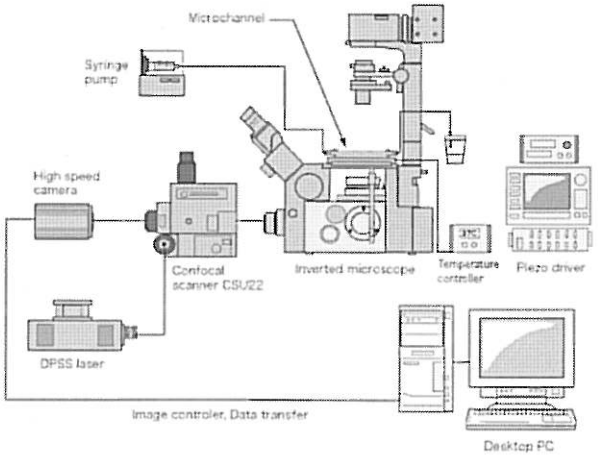

Fig. 1. Confocal micro-PIV experimental set-up.

All the confocal images were performed in the middle of the microchannels, using a piezo driver system and RT3D software from the Yokogawa corporation. The series of xy confocal images were captured with a resolution of 640×480 pixels, 12-bit grayscale, at a rate of 100 frames/s with an exposure time of 9.4 ms. The recorded images were transfered to the computer and then evaluated in the Image J (NIH) [8] by using the manual tracking MTrackJ [9] plugin. As a result it was possible to track single RBCs through the middle plane of the microchannel. Deatailed information about the experimental set-up, used in the present study, has already been described previously [6].

3. Results and discussion

3.1. Tracking displacement of RBCs at different Hct

By using the optical sectioning ability of the confocal system it was possible to obtain series of optical sectioned images at the middle of microchannel. Figures 2 to 4 show images with both RBCs (halogen illumination) and labeled RBCs (laser-emitted light) at different Hcts together with the correspondent time position tracking of individual RBCs.

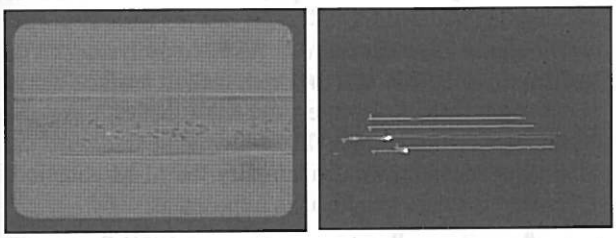

Fig. 2. Both normal and labeled RBCs (bright spot form laser-emitted light) with 3% Hct (20×, 1.6 zoom objective lens) and correspondent RBC paths displacement.

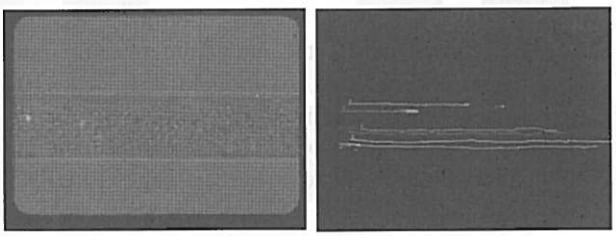

Fig. 3. Both normal and labeled RBCs (bright spots from laser-emitted light) with 14% Hct (20×, 1.6 zoom objective lens) and correspondent RBC paths displacement.

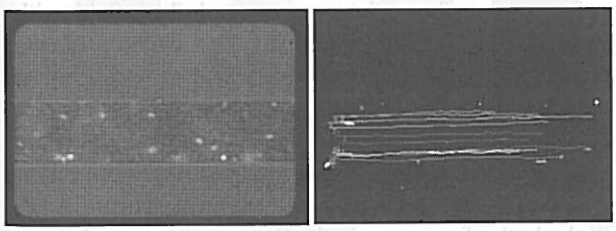

Fig. 4. Both normal and labeled RBCs (bright spots from laser-emitted light) with 37% Hct (20×, 1.6 zoom objective lens) and correspondent RBC paths displacement.

Figures 2 to 4 show the ability of the confocal system to track single RBCs at the middle plane with Hct up to 37%. From Figure 2, it is possible to observe that at 3%Hct the RBC paths are almost parallel to the flow direction without any appreciable fluctuations on the radial direction. By contrast, Figures 3 and 4 shows clearly that the RBC paths exhibit erratic radial displacements due the high-concentration of RBCs on the adjacent streamlines. As a result, this preliminary observations suggests that the RBC paths are strongly dependent on the Hct. An ongoing detailed study to quantify the radial displacement of the tracer RBCs is currently under way.

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