

***In Vitro* Blood Flow Behaviour in Microchannels with Simple and Complex Geometries**

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1. Introduction

Over the years, various experimental methods have been applied in an effort to understand the blood flow behaviour in microcirculation. The development of optical experimental techniques has contributed to obtain possible explanations on the way the blood flows through microvessels. In recent years, due to advances in computers, optics, and digital 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. The present review outlines our most relevant studies on the flow properties of blood at a microscale level by using current micro-PIV and confocal micro-PIV techniques.

Blood flow in both microvessels and microchannels has been measured by several measurements techniques such as: double-slit photometric (Nash & Meiselman, 1983), laser-Doppler anemometer (Uijtewaal et al., 1994), video-based methods (Parthasarathi et al., 1999). Although the past research findings 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 (Santiago et al., 1998; Koutsiaris et al., 1999). This system, known as micro-PIV (see Fig.1), has been applied to study the flow behaviour in several research fields in science and engineering. In the biomedical field, Sugii and his co-workers, by using a conventional micro-PIV system, they have used red blood cells (RBCs) as tracer markers to measure their velocities in straight (Sugii et al., 2002) and they found that the velocity profiles were markedly blunt in the central region. However, later they measured both tracer particles and RBCs through a 100 μm glass capillary and they reported that by using in vitro blood

with about 20% Hct the velocity profiles were parabolic (Sugii et al., 2005). By using a microchannel close to a rectangular shape, Bitsch and his co-workers (Bitsch et al., 2005) have reported blunt profiles. More recently, by using liposomes tracer particles the blood-plasma velocity was measured in the beating heart of a chicken embryo (Vennemann et al., 2006). Kim and Lee (2006) have analysed the flow behaviour of blood through a circular opaque microchannel by using an X-ray PIV technique. Their measurements have shown typical non-Newtonian flow characteristics of blood such as yield stress and shear-thinning effects. In addition, Chiu et al. (2003) have also applied the conventional micro-PIV system to analyse the effect of flow in monocyte adhesion to endothelial cells cultured in a vertical step flow chamber. 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 (Meinhart et al., 2000). These errors can be partially removed by using a spinning disk confocal microscope (SDCM), i. e., by combining a SDCM with a laser, the emitted light intensity is significantly improved and as a result, it is possible to obtain adequate signal to noise ratio to detect the motion of the RBCs in both diluted and concentrated suspensions (Tanaami et al., 2002; Park et al., 2004, 2006; Lima et al., 2006, 2007, 2008). Moreover, in contrast to the conventional microscope where the entire flow region is illuminated, the confocal systems have the ability to obtain in-focus images with optical thickness less than 1 μm (optical sectioning effect). As a result, confocal systems due to its spatial filtering technique and multiple point light illumination system, confocal micro-PIV has become accepted as a reliable method for measuring in vitro blood flow through microchannels.

In this chapter, our recent studies about in vitro blood flow behaviour in microchannels both in straight and with complex geometries are presented. In straight microchannels we present some phenomena such as Fahraeus effect and Fahraeus-Lindqvist effect, the flow of particles and red blood cells (RBCs) in diluted suspensions, the flow of RBCs in concentrated suspensions, the cell-free layer and sedimentations effects. The most recent studies in blood flow through complex geometries such as bifurcations, confluences and stenosis are also reviewed. By using a chromatographic method, the flow of RBCs through a network of microcapillaries is presented.

2. Conventional micro-PIV and confocal micro-PIV/PTV

The main components of a conventional micro-PIV system consists of a microscope, a high resolution objective lens, optical filters, a high power light source for flow illumination and a high speed camera. Briefly, the light enters the inverted microscope and is reflected by a dichromatic mirror to be transmitted through the objective lens which illuminates the entire flow volume. The light emitted from fluorescent trace particles travels back to the dichromatic mirror and filters out all reflected light only allowing to pass the emitted light from the particles. Finally, the signals from the trace particles are recorded by a high speed camera and then by using a PIV cross-correlation method it is possible to obtain velocity fields of the working fluid (see schematic illustration of a conventional micro-PIV in Figure 1). The resolution of a micro-PIV system is influenced by many factors such as out-of-focus particle images from volume

illumination, density and size of the tracer particles, size and optical characteristics of the microchannel and image quality (Lima, 2007).

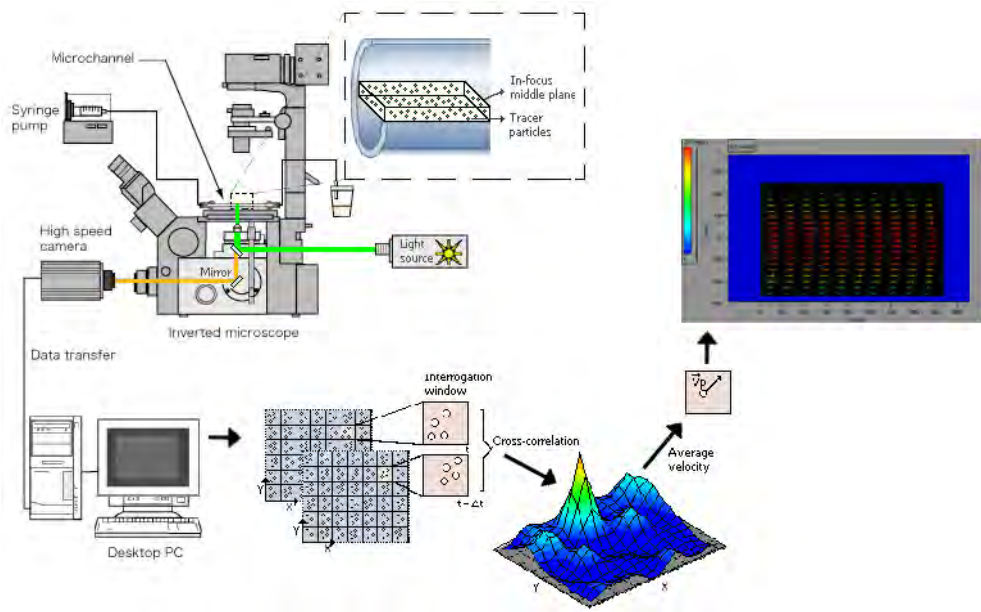


Fig. 1. Experimental setup of a typical conventional micro-PIV system and PIV cross-correlation method.

For the case of a confocal micro-PIV system, the main components of consists of a microscope combined with a confocal scanning unit (CSU), a high resolution objective lens, a high power light source for flow illumination and a high speed camera. In brief, the light emitted by the laser enters the CSU and then is conducted to the microscope to illuminate the microchannel from below the microscope stage. The light emitted from fluorescent particles travels back into the CSU, where a dichromatic mirror reflects it onto a high-speed camera to record the confocal images and by using a PIV cross-correlation method to obtain the velocity fields of the flowing trace particles (see Figure 2).

The main advantages of using a confocal spinning disk (CSD) are: the ability to obtain thin in-focus images, improve image definition and contrast of the trace particles (see Figure 3). As a result confocal micro-PIV systems have potential to obtain three-dimensional information about the fluid flow and also to obtain accurate flow-field measurements. In this way it is possible to study complex blood flow phenomena that often occur in two-phase flows (Lima, 2007).

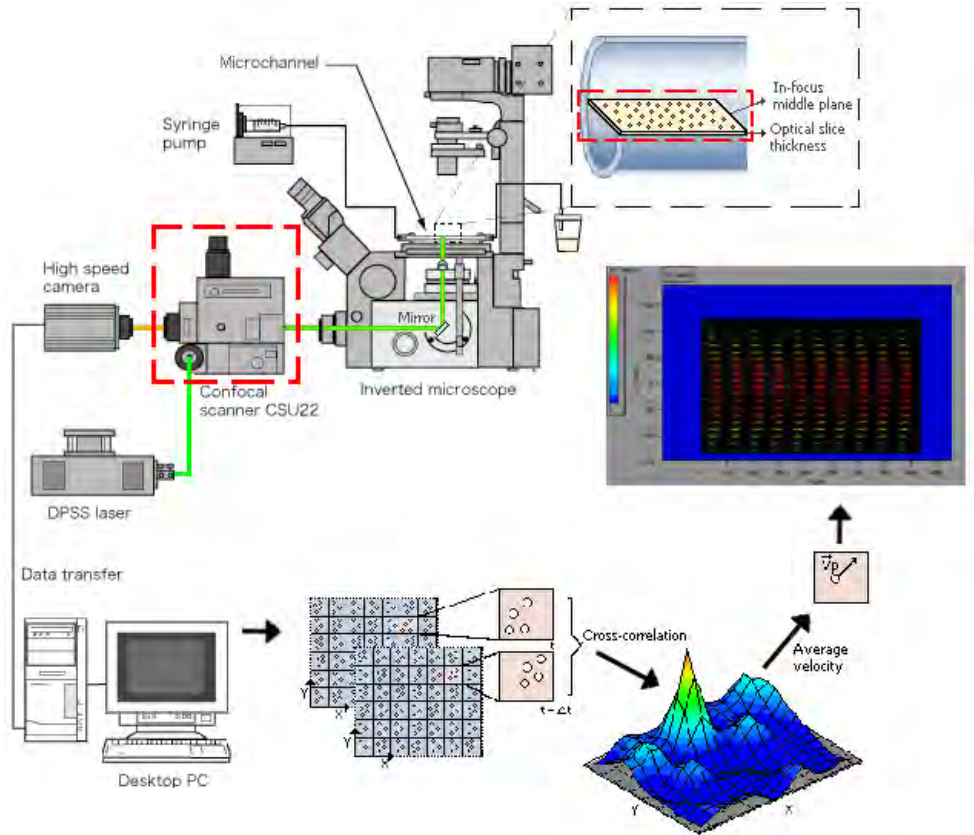


Fig. 2. Experimental setup of a confocal micro-PIV system and PIV cross-correlation method.

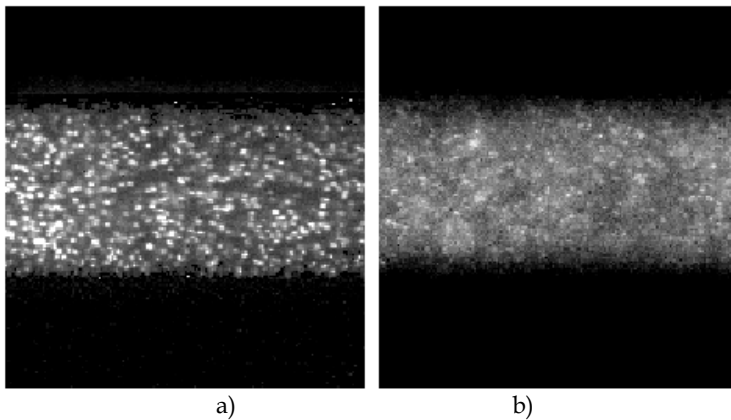


Fig. 3. Comparison of trace particles images from both confocal (a) and conventional micro-PIV (b) for pure water.

The density of particles in the recorded images determines the most adequate PIV methodology to obtain the velocity fields. When the concentration of particles is high enough that every interrogation window contains at least three particles, this method is called high-image-density PIV mode (Adrian, 1991). However, for the case of physiological fluids with high concentrations of cells, the amount of tracer particles captured within the fluid is often very low. Hence, if the number of particles within the interrogation area is small, it is recommended to measure the displacements by tracking individual particles in a Lagrangian way. This low-image-density PIV method is often referred as particle tracking velocimetry (PTV) (Adrian, 1991). The main advantage of PTV method is the ability to obtain detailed quantitative information on the motion of particles and cells flowing within the working fluid. This method is becoming essential in several biomedical fields such as cell biology and microcirculation. The present review will show several examples using a micro-PTV method to investigate *in vitro* blood flow behaviour in microchannels with both simple and complex geometries. A schematic illustration of a confocal micro-PTV is shown in Figure 4.

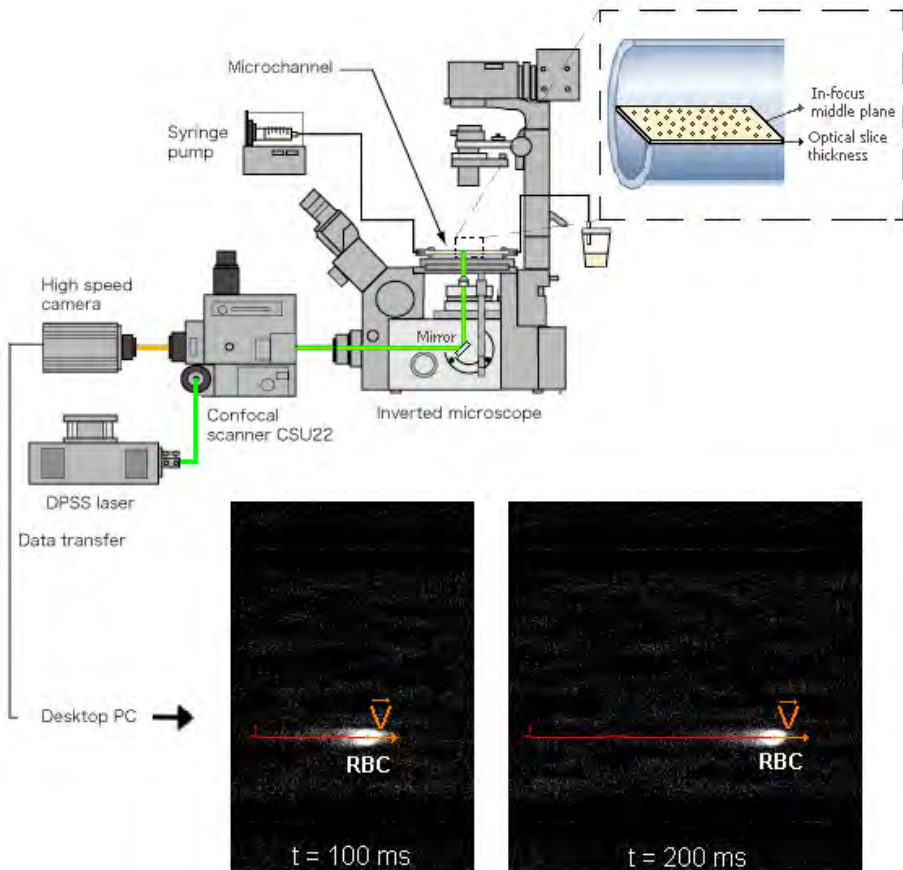


Fig. 4. Experimental setup of a confocal micro-PTV system with a labelled RBC trajectory and correspondent velocity at different times obtained by means of a particle tracking method.

3. Blood composition

In microcirculation, which comprises the smallest arteries and veins, the flow behaviour of individual blood cells and their interactions provide the microrheological basis of flow properties of blood at a macroscopic level. As a result, in microcirculation it is fundamental to study the flow behaviour of blood at cellular level. Thus, blood is not a homogeneous fluid, but one composed of a suspension of cells, proteins and ions in plasma. In normal blood, three types of cells comprise about 46% of its volume. These cells are the red blood cells (RBCs) (also known as erythrocytes) representing 45% of volume, white blood cells (WBCs) (also known as leukocytes) and platelets (also known as thrombocytes) (see Figure 5).

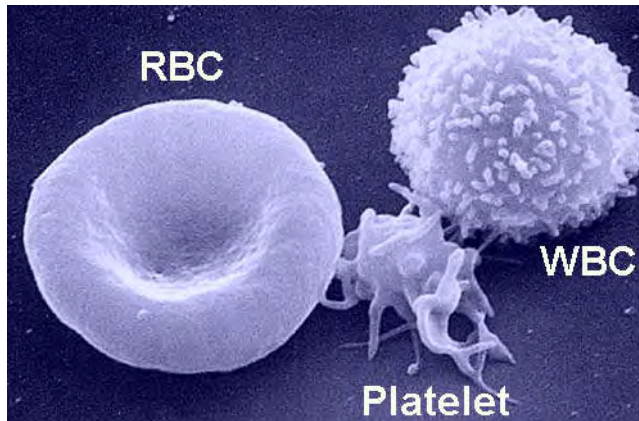


Fig. 5. Scanning electron micrograph of a white blood cells (WBC), a platelet and a red blood cell (RBC) (adapted from NCI-Frederick, 2005).

In vitro blood flow behaviour in microchannels is strongly influenced by the RBCs, since they occupy almost half of whole blood volume. RBCs are formed in the bone marrow and during maturation they lose their nuclei before entering the circulatory system. When suspended in an isotonic medium RBCs has a biconcave discoid shape, with a major diameter of about 8 μm . The density of a RBC is about $1.08 \times 10^3 \text{ kg.m}^{-3}$ and its major cellular components are the cytoplasm and a thin membrane composed of lipid bilayer and protein molecules. There is experimental evidence that healthy RBCs are extremely deformable into a variety of shapes in flowing blood in response to hydrodynamic stresses acting on them. Figure 6 shows a RBC deformation in a capillary (Lima et al., 2012).

The WBCs are nucleated cells that represent the major defence mechanism against infections. Generally, their shape is roughly spherical but their surface is not normally smooth (see Figure 5). The diameter of WBCs ranges from about 7 up to 22 μm , depending on its type. Healthy blood contains normally less than 1% of WBCs of the total volume of blood cells (Lima et al., 2012). Little is known about the effect of the WBCs on the blood flow behaviour in microcirculation. The blood flow under pathological conditions may increase amount of WBCs within the flow and consequently they may disturb the flow behaviour in microvessels (see Figure 7).



Fig. 6. RBCs deformation *in vivo* capillary (Minamiyama, 2000).

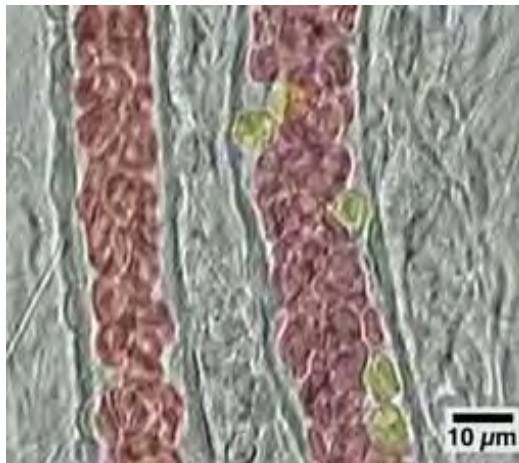


Fig. 7. Rolling of WBCs (yellow colour) in arterioles (Minamiyama, 2000).

Platelets are cells with no nuclei, round or oval discoid shape, in general, and with diameters from about 1 to 2 μm (see Figure 5). The number of platelets is usually less than the WBCs and they may have little effect on the blood flow behaviour. Although platelets play an important role in blood coagulation and thrombus formation, this topic is beyond the scope of the present review. Plasma is a yellowish fluid which contains 90% of water by volume and 10% of proteins, inorganic substances, vitamins, dissolved gases, etc. The proteins within the plasma flow, due to their large molecular size, usually do not pass through the capillary wall, thus generating an osmotic pressure. In *in vitro* experiments the osmotic pressure is an important parameter that needs special attention (Lima et al., 2012).

4. In vitro blood flow behaviour in straight microchannels

4.1 Fahraeus effect and Fahraeus-Lindqvist effect

In large arteries, where the diameter of the blood vessels is large enough compared to individual cells, it has been proved adequate to consider blood as a single-phase fluid (Caro et al., 1978). Accordingly, blood in large arteries may be treated as a homogeneous fluid where its particulate nature is ignored. Moreover, due to the large Reynolds number (Re) in arteries, blood flow is governed by inertial forces. However, arteries divide into successive smaller arteries and consequently the cross-sectional area of the vascular bed increases. As a result both pressure and velocity decrease as the blood flows into the smaller vessels. When the blood reaches the arterioles and capillaries the Re became less than 1, where viscous force dominates over inertial forces. At this microscale it is fundamental to take into account the effects of the multiphase properties of the blood on its flow behaviour (Caro et al., 1978). A clear example of the multiphase nature of the blood is the formation of a plasma layer at microvessels less than $300\ \mu\text{m}$, known as Fahraeus-Lindqvist effect (Fahraeus & Lindqvist, 1931).

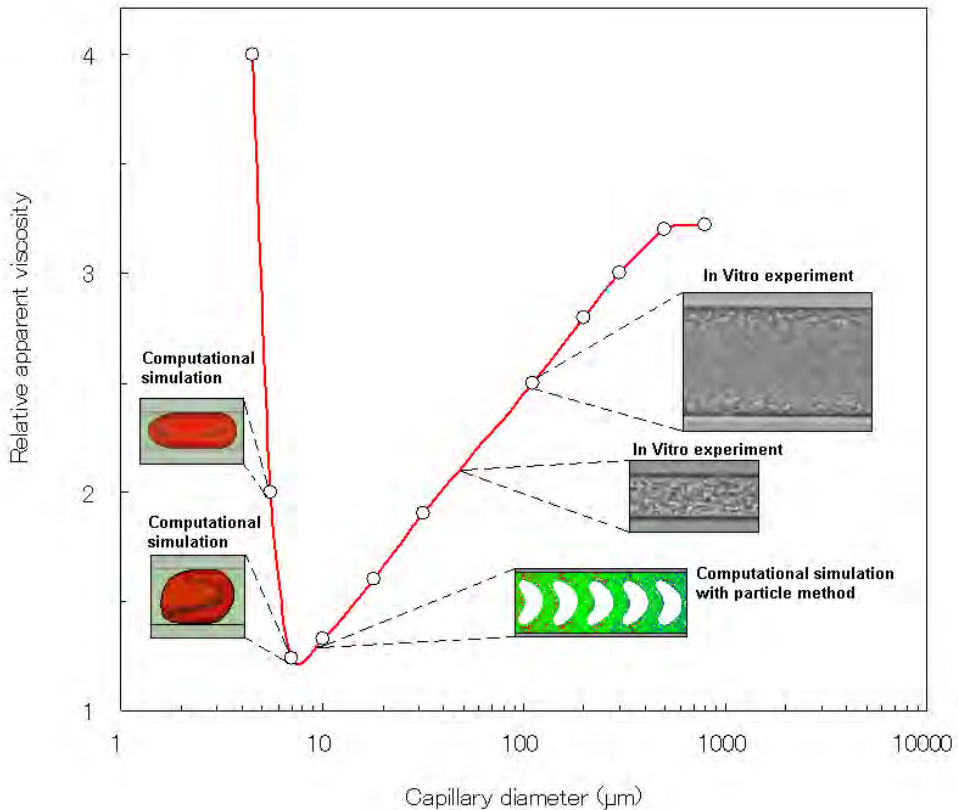


Fig. 8. Relative apparent viscosity of *in vitro* blood through glass capillaries (adapted from Pries et al., 1992; Wada & Kobayashi, 2002; Tsubota et al., 2006).

In the classical work of Robin Fahraeus, he observed that blood flow behaviour and its hematocrit are strongly affected by microtubes diameters less than 300 μm . The Fahraeus effect indicates that the Hct in the glass capillaries ($< 300 \mu\text{m}$) is lower than the feed Hct, which suggests that the Hct decreases as the blood proceeds through narrower microvessels. This phenomenon results from the axial migration of the RBCs to the centre of the microtube and consequent faster motion of the cells when compared with the suspending medium, such as plasma or dextran (Fahraeus & Lindqvist, 1931). The Fahraeus-Lindqvist effect is somehow related to the above phenomenon. Again for microtubes diameters less than 300 μm , Fahraeus and Lindqvist observed that the apparent blood viscosity decreases as the microtube diameter became smaller (Fahraeus & Lindqvist, 1931). After them, several works have extended their experiment down to diameters of about 3 μm and they have observed that the decrease of the apparent viscosity continues down to diameters of about 10 μm . However, the Fahraeus-Lindqvist effect is reversed at diameters 5 to 7 μm (see Figure 8) (Pries et al., 1992).

4.2 Particles and RBCs in diluted suspensions

In Poiseuille flow, the behaviour of suspended particles depends on several factors such as shear rate, particle deformability, size and shape. Generally, at low shear rates and diluted suspensions, rigid spherical particles and hardened RBCs (HRBCs) tend to move axially without any radial migration. On the other hand, deformable bodies, such as healthy RBCs tend to migrate towards the tube axis due to a radial hydrodynamic force. For higher (>1) particle Reynolds number (Re_p) where the inertial forces become important, both deformable bodies and rigid spheres have axial migration, however the spheres not always migrate toward the centre. The spheres near the wall moves towards the centre whereas the ones in the centre moves towards the wall. At the end they reach an equilibrium radial position of $0.6R$, where R is the tube radius. This effect is known as tubular pinch effect (see Figure 9) (Caro et al., 1978).

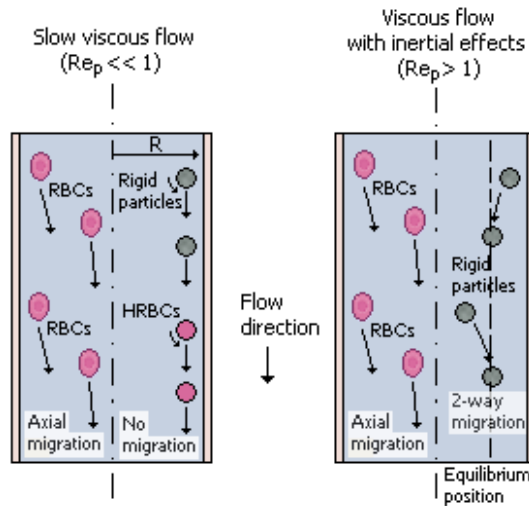


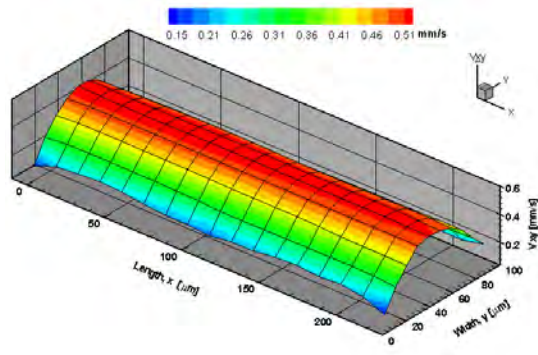
Fig. 9. Schematic representation of migration differences of rigid, healthy RBCs and hardened RBCs (HRBCs) in the centre of a capillary. Re_p corresponds to the particle Reynolds number (adapted from Goldsmith, 1971a, 1971b).

4.3 RBCs in concentrated suspensions

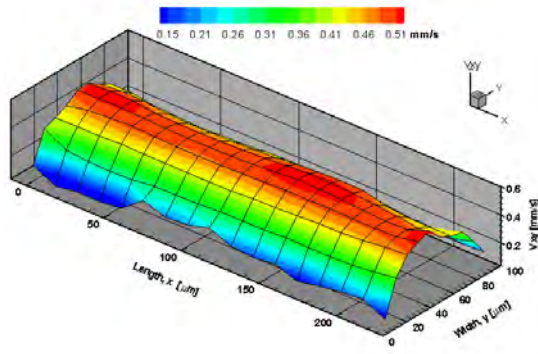
Although the flow properties of RBCs in diluted suspensions were extensively studied for many years, such is not the case when the RBCs flow within a crowded environment. The reason is mainly related to technical limitations of optical systems to obtain reliable measurements at Hct bigger than 10%. However, Goldsmith and coworkers (Goldsmith & Turitto, 1986) have overcome this technical difficulty by using transparent RBCs (known as ghost cells) as the suspension medium. By using ghost cells they were able to study the behaviour of individual RBC flowing in concentrated suspension of RBC ghosts. The motion of RBCs in concentrated suspensions is appreciably different from those observed in very diluted suspensions (Hct < 1 %). At concentrated suspensions the motion of RBCs is disturbed not only by the collisions with neighbouring cells but also by the plasma layer near the wall. In this way, the cell paths exhibit continuous erratic displacements with the largest ones occurring in the region between 0.5 and 0.8 of the tube radius from the axis. At a given microtube, the magnitude of radial displacements tends to increase with the concentration of RBC ghost cells. However, at concentrations bigger than 50%, the displacement decreases. At Hct > 50%, although the crowded environment leads to an increase of the cell deformation, it also limits the magnitude of the RBC radial dispersion (Goldsmith & Turitto, 1986).

Recently, Lima et al. demonstrated the ability of confocal micro-PIV not only to measure both pure water and suspensions of RBCs through a square glass microchannel (Lima et al., 2006) but also to measure the velocity profiles of both physiological saline (PS) and *in vitro* blood (20% Hct) in a rectangular polydimethylsiloxane (PDMS) microchannel (Lima et al., 2008). Good agreement between the measured velocity profiles of pure water and an established analytical solution was obtained for both studies. Further work was also performed by the Lima et al. but this time to measure both ensemble and instantaneous velocity profiles for *in vitro* blood with Hcts up to 17% (Lima et al., 2007). Although the ensemble velocity profiles were markedly parabolic, some fluctuations in the instantaneous velocity profiles were found to be closely related to the Hct increase. Hence, those results have suggested that the presence of RBCs within the plasma flow influences the measurements of the instantaneous velocity fields (see Figure 10).

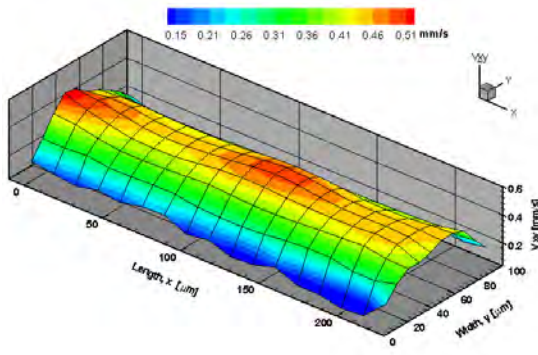
Lima and his colleagues have also observed that by using a confocal micro-PIV system (see Figure 2) it was possible to measure with good accuracy blood plasma containing trace particles with Hct up to 9%. For Hct bigger than 9%, the light absorbed by the RBCs has contributed significantly to diminish the concentration of fluorescent tracer particles in the acquired confocal images. This low density images become evident for Hct bigger than 20 %, which generates errors in the velocity fields. Hence, Lima et al. (Lima et al., 2008, 2009) have developed a new approach to track individual tracer cells at high concentration suspensions of RBCs. The confocal micro-PTV system (see Figure 4) was employed, for the first time, in an effort to obtain detailed quantitative measurements on the motion of blood cells at both diluted and high suspensions of RBCs in simple straight microchannels. Lima et al. have successfully labelled both RBCs and WBCs and have measured their motions through microchannels. The ability of the confocal system to generate thin in-focus planes has allowed measurements in flowing blood at concentrated suspensions of: cell-cell hydrodynamic interaction, RBC orientation and RBC radial dispersion at different depths (Lima et al., 2008, 2009). To analyse the ability of the confocal micro-PTV system to track RBCs, the motions of labelled RBCs were followed at several Hcts (3% to 37%).



a)



b)



c)

Fig. 10. Time series of the instantaneous velocity profiles of (a) pure water (b) *in vitro* blood with a 9% Hct and (c) *in vitro* blood with a 17% Hct, in the central plane of the microchannel with a $\Delta t = 10$ ms (adapted from Lima et al., 2007).

For the calculation of the radial dispersion coefficient (D_{yy}), measurements were performed at middle plane of the microchannels. However to investigate complex microrheological events in flowing blood (such as interaction and orientation of blood cells) all measurements were performed near the wall of the microchannel ($z = 20 \mu\text{m}$) with $\text{Hct} \sim 20\%$ and $\text{Re} \sim 0.007$. Figure 11 shows the trajectories of two-RBC interactions near the wall and within the plasma layer. This figure shows the radial disturbance effect enhanced by the collision of a neighbouring RBC. The hemodynamic interaction effect of WBC on the motion of RBCs was also possible to be measured. Figure 12a shows a RBC interacting with a WBC. In Figure 12a it is possible to observe that the transversal displacement increases due to the collision with a flowing WBC. An interesting measurement of both RBC translational and rotational motion was also possible by adjusting the image contrast (see Figure 12b). The translational motion was measured at the centre of the RBC whereas the rotational was measured along the RBC membrane.

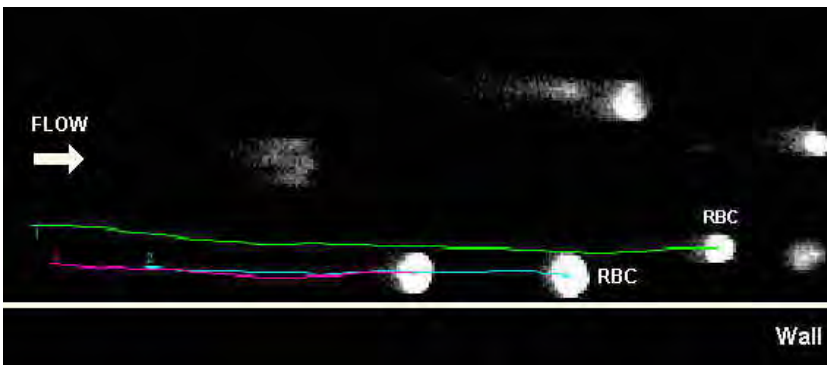


Fig. 11. Two-RBC interactions in a straight microchannel.

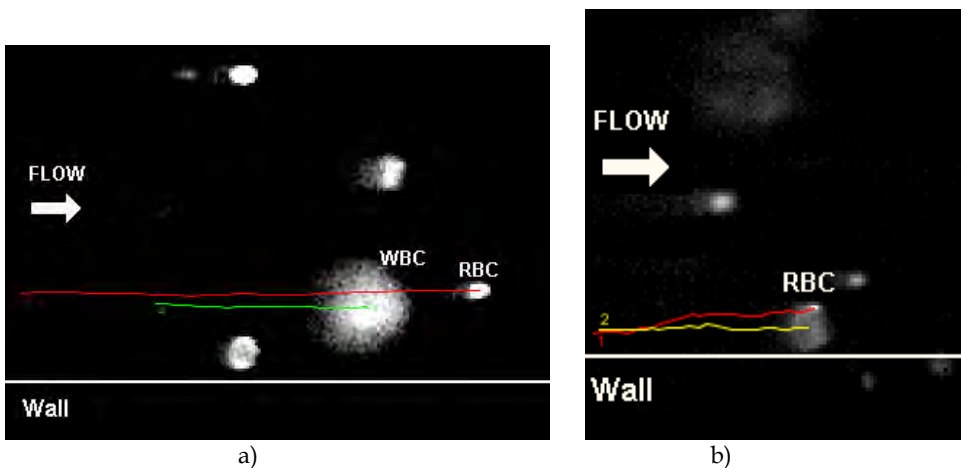


Fig. 12. (a) RBC-WBC interaction in a straight microchannel; (b) Translational and rotational motion of a RBC.

Further research was carried by using in vitro blood with several Hcts and low Reynolds numbers ($Re \sim 0.005$). The paths of hundreds labeled RBCs were measured in the centre plane of both straight glass and PDMS circular microchannel (Lima et al., 2008, 2009, 2009). The RBC dispersion coefficient (D_{yy}) for two different diameters (75 μm and 100 μm) and for several Hcts is shown in Figure 13. The results show that RBC D_{yy} rises with the increase of the Hct and that RBC D_{yy} tends to decrease with the diameter.

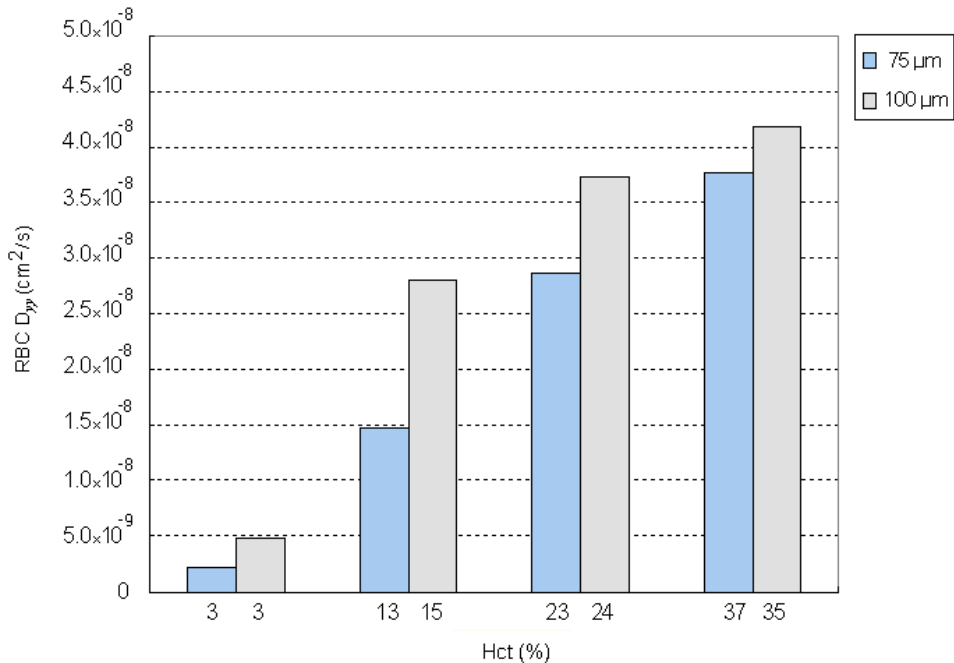


Fig. 13. Effect of the Hct on the RBC D_{yy} at 75 μm PDMS microchannel and 100 μm glass capillary (adapted from Lima et al., 2008, 2009).

4.4 Cell-free layer (CFL)

In microcirculation the cell-free layer is believed to reduce the friction between red blood cells (RBCs) and endothelial cells and consequently reduce blood flow resistance. However, the complex formation of the cell-free layer has not yet been convincingly described mainly due to multi-physical and hemorheological factors that affect this phenomenon. Recent studies have measured the effect of hematocrit (Hct) on the thickness of the cell-free layer in straight circular polydimethylsiloxane (PDMS) microchannels. The formation of a cell-free layer is clearly visible in the images captured (see Figure 14) and by using a combination of image analysis techniques we are able to detect an increase in the cell-free layer thickness as Hct decreases.

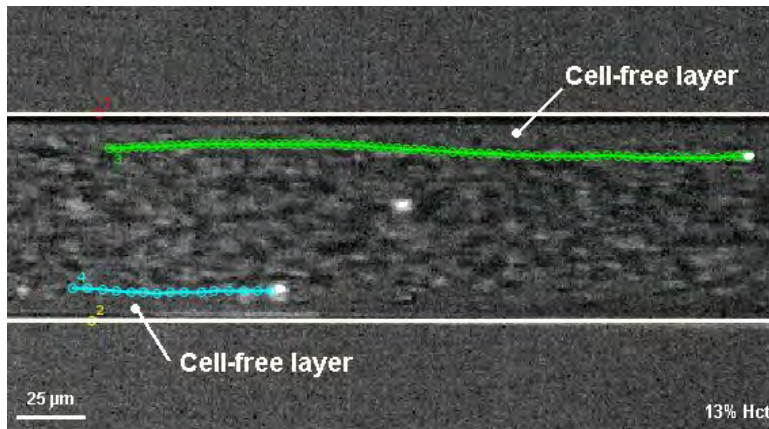


Fig. 14. Labelled RBCs flowing around the boundary region of the cell-free layer (Lima et al., 2009).

Labelled RBCs flowing around the boundary region of the cell-free layer were able to track manually by using the MtrackJ plugin from Image J. Figure 14 shows an example of the trajectories of two labelled RBCs flowing close to the boundary of the cell-free layer. The radial position of the tracked RBCs is then determined and the corresponding thickness of the cell-free layer is calculated. Finally, the data is time-averaged. When the number of labelled RBCs flowing close to the boundary region is not significant, additional analysis is performed by manually measuring the distance from the boundary region to the wall for several points along the microchannel (Lima et al., 2009; Lima et al., 2009). Examination of Figure 15 shows an overall reduction of the thickness of the cell-free layer as Hct is increased. In particular, the thickness decreases almost four fold as Hct is increased from 3% to 37% (Lima et al., 2009; Lima et al., 2009).

4.5 Sedimentation effects

Recently Garcia and his colleagues (Garcia et al., 2010, 2011) have investigated the flow behaviour of two different physiological fluids frequently used in biomedical microdevices. The working fluids used in this study were physiological saline (PS) and dextran 40 (Dx40) containing about 6% of sheep red blood cells (RBCs), respectively. By using a syringe pump and a video camera it was possible to measure qualitatively the flow behaviour within a horizontal capillary. To analyze the dynamic sedimentation of PS and Dx40 containing RBCs they decided to use flow rates close to the one observed in vivo, i.e., 10 $\mu\text{l}/\text{min}$. During the experiment we made flow qualitative visualizations measurements in glass capillaries with diameters of about 1,2 mm. The visualizations were captured by a camera for about 15 minutes. Figure 16 shows the flow qualitative measurements for 0 minutes and 15 minutes. This image shows clearly that for a period of 15 minutes the RBC tend to settle down in the fluid with PS whereas using Dx40 we did not observe any RBC sedimentation. Although not shown in Figure 16, for the case of PS fluid we did not observe any RBC sedimentation for the first 10 minutes. According to our visualization the RBC tend to settle down for period of time superior to 10 minutes.

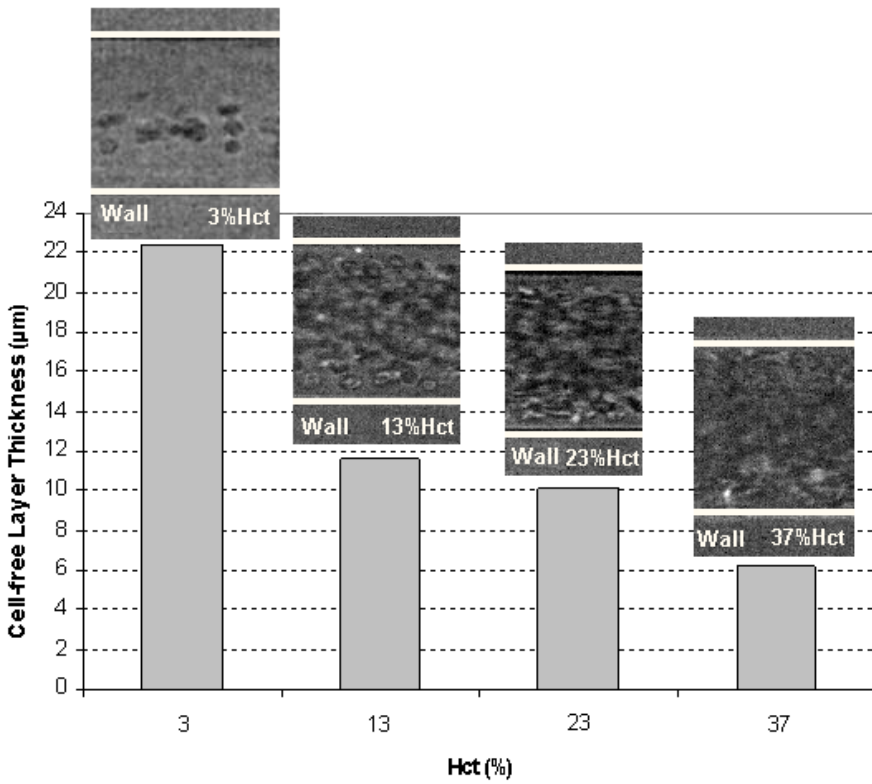


Fig. 15. Average thickness of the cell-free layer at several Hct layers (Lima et al., 2009; Lima et al., 2009).

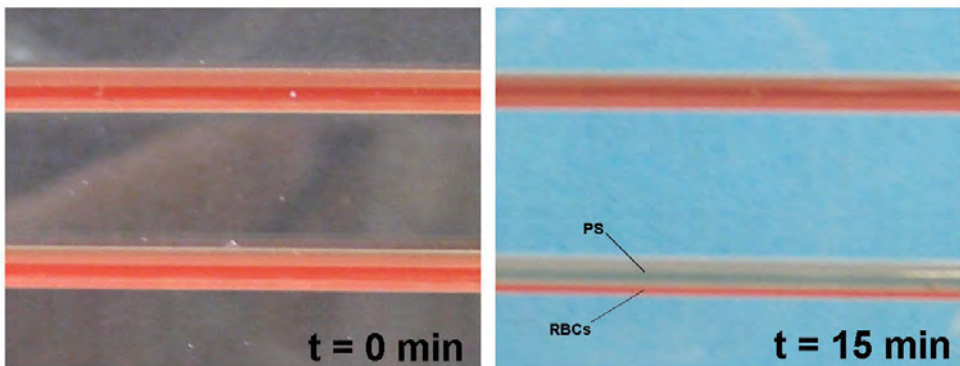


Fig. 16. Dynamic sedimentation measurements for two different time periods of PS and Dx40 containing RBCs, with a flow rate of 10 μl/min (Garcia et al., 2010, 2011).

Additionally, flow visualization measurements were also performed in glass microchannels (see Fig.17) and compared with *in vivo* blood flow (see Fig.17c). Figure 17 shows that for the case of Dx40 there is a clear formation of cell-free layer adjacent to the walls of microchannels. However, in the fluid with PS the RBCs do not exhibit a clear tendency to migrate into the microtube axis. The *in vivo* visualization measurements (Fig. 17c) have shown a clear tendency for the formation of a plasma layer in microvessels (Kim et al., 2006; Minamiyama, 2000). These results indicate that *in vitro* blood containing Dx40 has a flow behaviour closer to the one observed *in vivo* microvessels.

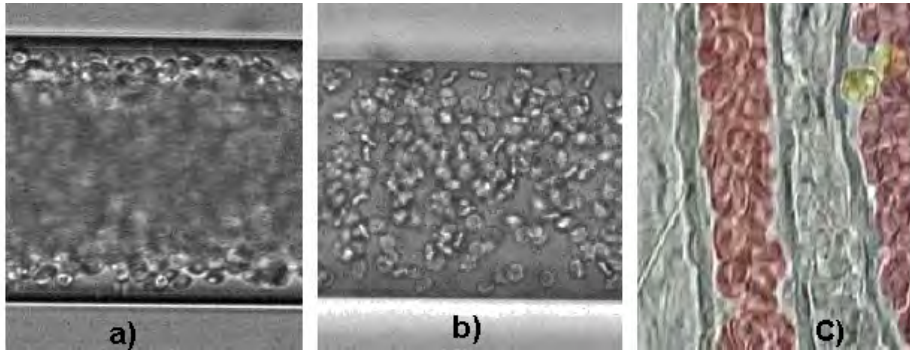


Fig. 17. *In vitro* flow visualization in glass microchannels for a period time bigger than 10 minutes a) Dx40 containing RBCs; b) PS containing RBCs. c) *In vivo* flow visualization in a microvessel (Lima et al., 2009a, 2009b; Minamiyama, 2000).

5. In vitro blood flow behaviour in microchannels with complex geometries

5.1 Bifurcation and confluence

By using a soft lithographic technique it is possible to fabricate polydimethylsiloxane (PDMS) microchannels with complex geometries similar to human blood arterioles and capillary networks (Lima et al., 2011). In this section we show the application of a confocal micro-PTV system to track RBCs through a rectangular polydimethylsiloxane (PDMS) microchannel with a diverging bifurcation and a confluence (see Figure 18).

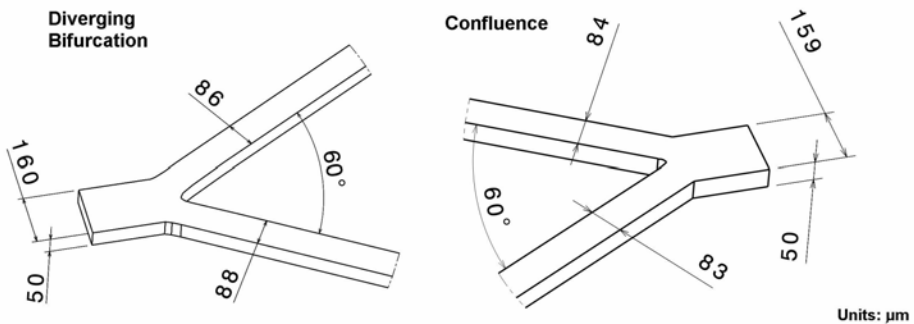


Fig. 18. Dimensions of the a) diverging and b) confluence used in this study (Leble et al., 2011a, 2011b; Lima et al., 2011).

By using a confocal system, we have measured the effect of both bifurcation and confluence on the flow behaviour of both fluorescent particles suspended in pure water (PW) and RBCs in concentrated suspensions. For the case of trace particles in PW we have observed that the trajectories were almost symmetric and do not present so many fluctuations for both geometries. These results are consistent with the Stokes flow regime. In contrast, for the case of labelled RBCs the trajectories are more asymmetric when compared with PW trajectories. Additionally, we can also observe several fluctuations on their trajectories possibly due to cell interactions enhanced by the high local Hct originated at this region (Lima et al., 2011a, 2011b).

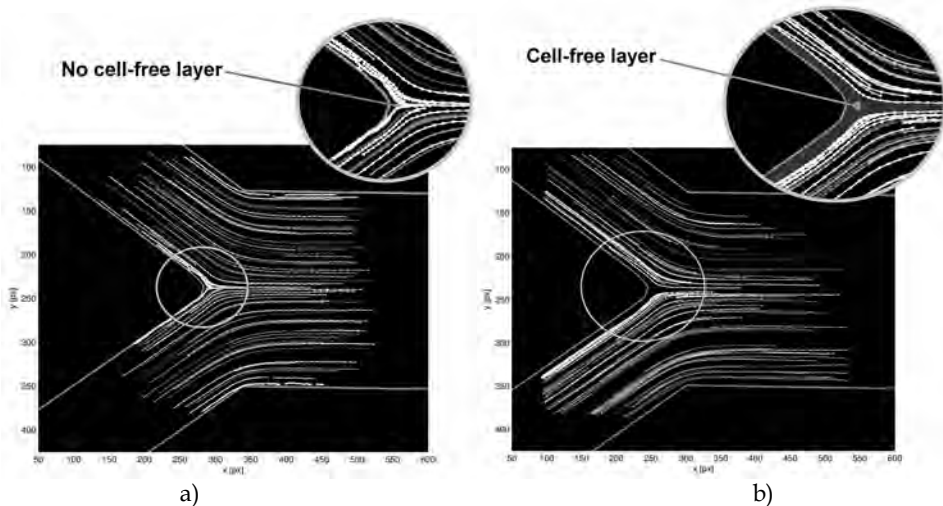


Fig. 19. Trajectories in a confluence of a) fluorescent particles in PW and b) labelled RBCs in Dx40 (Leble et al., 2011a, 2011b; Lima et al., 2011).

In the confluence, it is possible to observe that the trace particles tend to flow very close to the inner walls and as a result they tend to flow in the centre of the microchannel, just downstream of the confluence apex (see Fig.19a). However, for the case of labelled RBCs we could not measure any trajectory passing in this centre region (see Fig.19b). This is due to the existence of a cell-free layer (CFL) in both inner walls and a consequent formation of a triangular CFL in the region of the confluence apex (see Fig.19b) (Leble et al., 2011a, 2011b; Lima et al., 2011). As this triangular CFL seems to play an important role on the *in vitro* blood flow characteristics, a detailed quantitative study, to clarify the CFL effect in the velocity profiles, is currently under way and it will be published in due time (Leble et al., 2011).

5.2 Stenosis

The behaviour of RBCs in a microchannel with stenosis was also investigated using a confocal micro-PTV system. Individual trajectories of RBCs in a concentrated suspension of 10% hematocrit (Hct) were measured successfully. Results indicated that the trajectories of healthy RBCs became asymmetric before and after the stenosis, while the trajectories of tracer particles in pure water were almost symmetric. The effect of deformability of RBCs on the cell-free layer thickness, by hardening RBCs using a glutaraldehyde treatment, was also investigated.

Fujiwara et al. have found that deformability has a considerable effect on the asymmetry of the cell-free layer thickness and as result they concluded that the motions of RBCs are influenced strongly by the Hct, the deformability, and the channel geometry (Fujiwara et al., 2009).

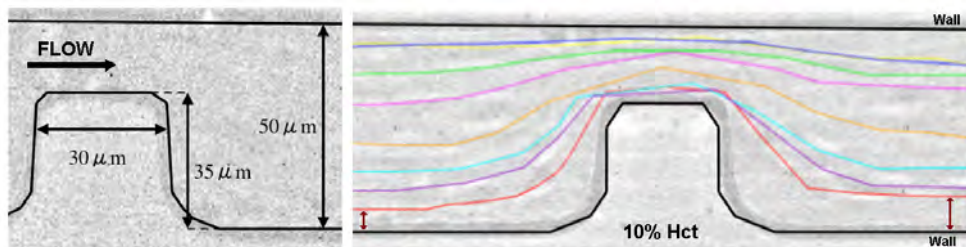


Fig. 20. Geometry of the stenosis and Flow of blood with trajectories of labelled RBCs with 10% Hct (Fujiwara et al., 2008, 2009).

5.3 Capillary networks

The flow of red blood cells through a column packed with soda lime glass spheres was recently studied by Couto and her colleagues (Couto et al., 2011). Since the diameter of the glass spheres was $337.5 \mu\text{m}$ and the packing porosity 0.4 it was easy to determine (Dias et al., 2006, 2007, 2008; Dias, 2007) that the average capillary diameter of the porous network was $150 \mu\text{m}$, this diameter being within the range of the typical dimensions observed in human blood arterioles.

The samples used in the referred study (Couto et al., 2011) were suspensions of sheep RBCs (with major diameter close to $6.5 \mu\text{m}$) in physiological saline (PS) containing 0.1 to 1% Hct and the experimental apparatus was that shown in Fig. 21 and contains: a pump (1); injector (2); a chromatographic column packed with the above mentioned glass spheres (3); refractive index detector (4) and data acquisition system (5a and 5b). The mobile phase was PS and the flow rates varied between 0.2 ml/min and 1 ml/min .

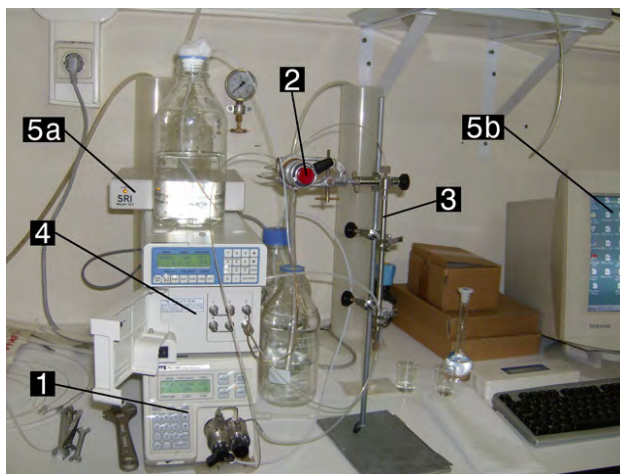


Fig. 21. Chromatographic apparatus (Couto et al., 2011).

An infinite small sized marker (sucrose) dissolved in PS was used in order to know the velocity of the carrier fluid (PS). The different experiments (different flow rates and Hcts) have shown that the RBCs migrate faster through the capillary network than the fluid (PS) that suspends the RBCs, as can be seen in Fig. 22. The authors (Couto et al., 2011) suggested that this behaviour can be explained by the theory that supports the hydrodynamic fractionation (Tijssen et al., 1986; Stegeman et al., 1993; Dias et al., 2008; Dias, 2007, 2008) and by the formation of a cell-free layer adjacent to the walls of the capillaries.

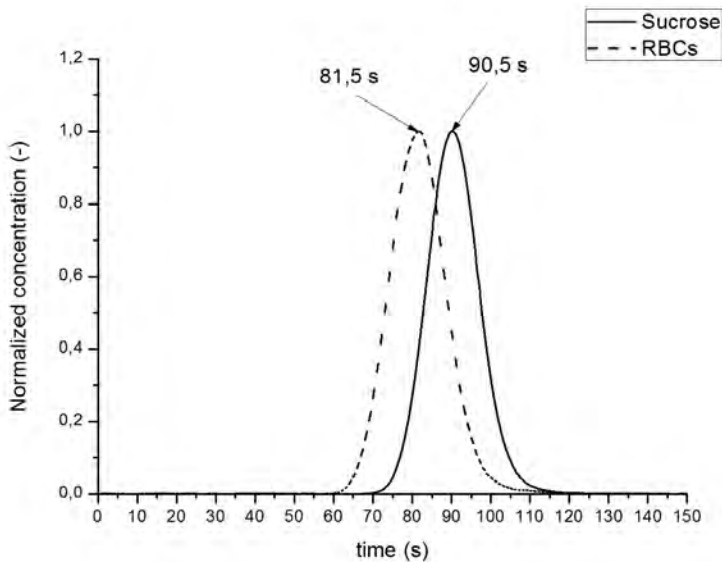


Fig. 22. Chromatogram for 1ml/min.

6. Conclusions

Advances in computers, image analysis, and optics have made it possible to combine both particle image velocimetry (PIV) and particle tracking velocimetry (PTV) system with a spinning disk confocal microscope. This revision summarise the most relevant studies of *in vitro* blood flow, through simple and complex microchannels, by means of a confocal micro-PIV/PTV system.

In vitro blood studies showed that the confocal micro-PIV system is able to measure with good accuracy blood plasma with Hct up to 9%, in a 100- μ m square microchannel. However, for Hct bigger than 9%, the light absorbed by the RBCs contributes to diminish the concentration fluorescent particles in the acquired confocal images. This low density images become more evident for Hct bigger than 20%, which generates spurious errors in the velocity profiles. Hence, a Langragian approach was proposed to the confocal system in order to track individual blood cells at high Hcts. Owing to its optical sectioning ability and consequent improvement of the image contrast and definition, the proposed confocal micro-PTV system eliminates several problems and concerns of the microvisualization systems

used in the past and provides additional detailed description on the RBC motion. Hence, the proposed confocal system has shown detailed RBC trajectories of RBC-RBC interaction at moderate and high Hcts (Hcts up to 35%) never obtainable by other conventional methods. As a result, by measuring hundreds of RBC trajectories, it was possible to conclude that RBC paths are strongly dependent on the Hct and as a result the RBC dispersion coefficient tends to increase with the Hct. Another unique measurement was the possibility to obtain both translational and rotational motion of RBCs flowing in highly concentrated suspensions. Hence, it was possible to compare in detail the motion of a RBC without any kind of interaction (rolling on the wall of the microchannel) with a RBC interacting with neighbouring blood cells. These remarkable results, have shown for the first time, that RBC rolling on the wall and without interaction tends to rotate in regular and periodically way whereas RBCs that interact with neighbouring cells tend to rotate in a rather erratic way. Very recently, our confocal system have shown also for the first time, a very astonishing blood flow phenomenon happening downstream of a confluence. Our results show the formation of a triangular cell-free layer (CFL) in the region of the confluence apex which consequently promotes a development of thin CFL in the middle of the microchannel. This finding suggests that in our microcirculatory system we may have CFLs not only on the walls but also in the middle of the microvessels. Additionally, the confocal system have also proved to be powerful tool to obtain further insight onto the flow behaviour of blood through other kinds of complex geometries such as diverging bifurcations, and stenosis.

The net of capillaries obtained by packing chromatographic columns with glass spheres may be used as a simple model in order to further understand the blood flow in complex microvascular networks.

7. Future directions

Past *in vitro* research performed with classical glass microchannels has revealed several amazing phenomena happening in microcirculation. However, recently several studies on the blood flow behaviour have shown quantitative difference between the results in microvessels and in glass microchannels. The reason for these differences still remains unclear mainly because glass microchannels differ from microvessels in several aspects, such as elasticity and geometry and biological effect by the endothelial inner surface. These limitations encountered in glass microchannels can be overcome by using PDMS microchannels. Hence, in the near future we are planning to develop a biochip to mimic the *in vivo* environment. By using an innovative cellular micropatterning technique based on electrochemical method, we expect to culture living cells on their surfaces and consequently to provide experimental evidence on several microcirculation mysteries, such as the role of the glycocalyx on the flow behaviour of blood and the thrombogenesis process.

Computational modelling is a powerful way to obtain detailed information on the mechanical behaviours of multiple RBCs. Recently, several numerical methods have been proposed to analyse microscale blood flows in microvessels such as the particle method and the elastic RBC flow model. We expect in the near future to compare the reported experimental results with the numerical methods mentioned above. This collaborative work between experimental and computational methods will contribute not only to improve the modelling of cell behaviour but also to provide better understanding on several microcirculation phenomena

Another area for further research is the improvement of the current particle tracking methods. In this work a manual tracking method was used due to the inability of the automatic methods to track RBCs when they experienced collisions. Hence, a more reliable automatic tracking method needs to be developed to reduce the time-consuming and also to avoid the tedious work performed during the post-processing procedure.

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