AN OPTIMIZED TECHNIQUE FOR RED BLOOD CELLS VELOCITY MEASUREMENT IN MICROVESSELS

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Introduction

Oxygen and nutrient delivery to living tissues, as well as metabolic waste removal, are essentially determined by the dynamics of blood flow in microvascular networks. In these vessels, measuring the velocity distribution of red blood cells (RBCs) is still challenging. One of the most popular techniques used for that purpose is the *Dual-Slit* (DS), a temporal correlation technique, first introduced by Wayland and Johnson (1967): the vessel under study is trans-illuminated and two photo-sensors (slits) are positioned, separated by a known distance, L_s, along the vessel axis. The time modulation of light is recorded at both positions. A cross-correlation velocity, $V_{ds}=L_s/T_d$, is obtained, where T_d is the time delay for which the cross-correlation between the two signals is maximum. However, RBCs are positioned at different depths within the channel and thus move at different velocities. Baker and Wayland (1974) suggested that V_{ds} is related to a dynamic averaged velocity, but this has never been proved.

The aim of this work is to determine the relationship between the measured velocity V_{ds} and the actual velocity scales of the flow. For that purpose, the DS technique is first optimized using sequences of synthetic images representing RBCs flow. By this way, all the parameters characterizing the RBCs flow, including the shape of their velocity profile in the direction parallel to the incident light beam, which is inaccessible to the observer in real experiments, are controlled. The DS is then applied to *in vitro* RBCs flows in microchannels.

Methods

Synthetic image sequences are computer generated by simulating the flow of elliptical particles representing RBCs for various flow parameters: channel depth D, velocity V_{max} at the centre of the channel, shape of the velocity profile (from flat to parabolic) and RBCs volume fraction H. The parameters characterizing the technique are: spatial resolution (δ), frame rate (F), number of images in the sequence (M), slits width (w) and height (h), and distance between the slits (L_s).

***In vitro* RBCS flows** image sequences are recorded using square and rectangular PDMS micro-channels and human RBCs washed suspensions.

Results

The parametric study performed on synthetic images shows that, when L_s increases, the measured velocity V_{ds} approaches V_{max}, whatever the flow parameters. Thus, in optimal conditions (see Tab. 1), the measured velocity corresponds to V_{max}. The DS is then applied on *in vitro* image sequences for every position in the width of the channel, which leads to velocity profiles where each point represents the maximal velocity in the depth of the channel, see Fig. 1.

V_{im} *(pixels/frame)*	M	w and h*(pixels)*	L_s *(RBCs size)*
$V_{im}=\frac{V_{\max}\delta}{F}<0.15$	$V_{im}M$>9000	w=h=1	5 to10

Table 1: Operational parameters for an optimal use of the DS. V_{im} represents the maximal displacement of RBCs from one image to the other.

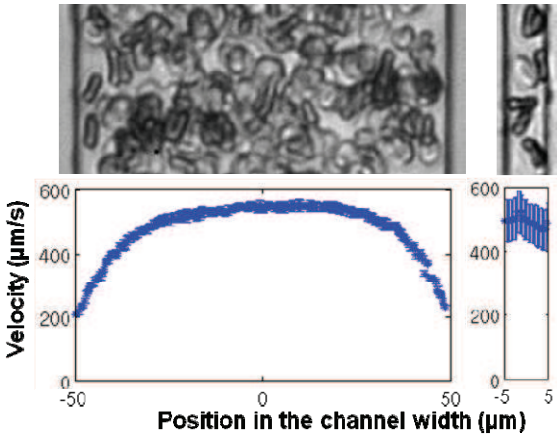

Figure 1: Typical images (bottom) and transverse velocity profiles (top) obtained for RBCs flow in a 20x100µm^2 (left) and a 10x10µm^2 (right) channel.

Discussion

We showed that, contrarily to previous thinking, the velocity measured by the *Dual-Slit* technique is the maximal velocity in the depth of the channel, provided that suitable conditions (Tab. 1) are applied. Therefore, many previous experimental results obtained by this technique might need to be revisited, with potential consequences on the phenomenology of microvascular flows.