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High-speed video capillaroscopy method for imaging and evaluation of moving red blood cells

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ABSTRACT

The video capillaroscopy system with high image recording rate to resolve moving red blood cells with velocity up to 5 mm/s into a capillary is considered. Proposed procedures of the recorded video sequence processing allow evaluating spatial capillary area, capillary diameter and central line with high accuracy and reliability independently on properties of individual capillary. Two-dimensional inter frame procedure is applied to find lateral shift of neighbor images in the blood flow area with moving red blood cells and to measure directly the blood flow velocity along a capillary central line. The developed method opens new opportunities for biomedical diagnostics, particularly, due to long-time continuous monitoring of red blood cells velocity into capillary. Spatio-temporal representation of capillary blood flow is considered. Experimental results of direct measurement of blood flow velocity into separate capillary as well as capillary net are presented and discussed.

1. Introduction

Evaluation of blood microcirculation is of high importance in biomedical diagnostics. The well-known Laser Doppler Flowmetry (LDF) method is widely used, however, the high spatial variability in blood flow across tissue such as the skin limits the clinical utility of single point LDF measurements [1]. The full-field Laser Doppler Perfusion Imaging (LDPI) allows overcoming this limit, but does not provide high enough signal-to-noise ratio and, consequently, high evaluation speed that can be achieved in the Laser Speckle Contrast Imaging (LSCI) [1,2], however at the expense of limited resolution caused by spatial averaging [1,2]. It is worth noting that the both mentioned above methods are not focused to measure the red blood cells (RBCs) speed directly giving estimates of blood perfusion, i.e. product of the RBCs local speed and concentration [2].

At the same time direct measurement of blood flow velocity in capillaries is very important for medical diagnostics of many diseases such as diabetes, system sclerosis, Raynaud syndrome, coronary heart disease and others [1–4]. These diseases lead to specific changes of a capillary loop at the border of arterial and venous parts and capillary diameter [5,6]. Along with evaluation of morphological features and density of a capillary net, estimate of capillary blood flow velocity is of high importance [6,7].

Evaluation of RBCs velocity is possible to carry out using coherent or incoherent imaging methods. Coherent methods as, e.g., Digital Holographic Microscopy (DHM), are able to image directly each single RBC in a blood flow, from which not only ensemble information but even single cell analysis is allowed over a wide field of view (FoV) and with decent resolution. Recently, RBCs moving inside capillaries have been imaged using DHM, and tomography results of the RBCs and the capillary itself have been demonstrated [8].

In coherent methods, a signal acquired from a highly multiple scattering medium suffers speckle noise influence, phase wrapping, and interferometric fringe washout effects causing measurement ambiguities making it difficult to perform holographic imaging in vivo [8]. In spite of effective processing algorithms developed in [8] like the scattering reduction based on the optical field subtraction of stationary signals, RBCs flow has been evaluated in comparably thin tissue layer in the light transmission mode with fixed spatial position of the investigated (animal) object and small capillary diameter (about 6 μm), where RBCs velocity did not exceed 1 mm/s. As mentioned in [2], methods which would allow the routine clinical assessment and monitoring at the microcirculatory level could greatly improve patient recovery rate. The DHM methods do incompletely meet this condition yet and are considered as the basis of perspective techniques especially for 3D imaging and evaluation in vivo. It should be noted one of the promising techniques based on spatio-temporal synthesis of holograms [9,10] that allows exploiting natural object movements to obtain phase-shifted interferograms with extended FoV and increased resolution. However, this method was applied to evaluate a micro flow in fixed direction [10] that is not the case for arbitrary capillary net.

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Incoherent imaging methods are based on recording of intensity distribution and do not suffer undesirable coherent effects mentioned above being insensitive to light field phase. In spite of this limit, the incoherent methods are applicable to solving the problem of RBC velocity measurement. When evaluating the RBCs flow into a capillary using incoherent imaging in nailfold video capillaroscopy (VCS) [3–6], the following typical procedures are applied [11]: recording of video frames series, intensity binarization and segmentation of individual capillary shape, extraction of capillary central line, calculation of blood flow plasma gaps displacement along the capillary central line and estimation of blood flow velocity by dividing the plasma gaps displacement value to the time interval related to the video frames corresponding to initial and subsequent position of plasma gaps. It has been demonstrated in [11] experimentally applicability of this approach to evaluate the RBCs speed in arbitrary capillary. However this method is based on the assumption that RBCs flow velocity is equal to velocity of plasma gaps that is valid for thin capillaries only, and any lateral RBC displacements are neglected. Movement of RBCs aggregates into capillaries with diameter 10… 20 μm is not considered. In practice, the plasma gaps can absent in a capillary during some part of observation interval that presents a barrier of the method [11] application for continuous RBCs velocity monitoring. In addition, this is especially significant when one has to make measures on a capillary net, in order to increase the repeatability and reliability of the experiment for real world diagnostic needs. This problem is not considered in [11].

When developing methods for RBCs velocity monitoring into a capillary net, it is important to take into account the following properties. A capillary net generally has *a priori* unknown individual morphology with arbitrary position, length, width and orientation of each separate capillary. The nailfold area is randomly moving with respect to fixed FoV of the optical system with limited, but arbitrary velocity vector. RBCs flow can contain or not plasma gaps and RBCs aggregates within limited time interval, and separate RBC moves within a capillary with slightly variable velocity relatively to other neighbor RBCs. Due to high scattering and absorption, the observed intensity distribution is essentially non-uniform and variable depending on an observation area. Thus, the object under evaluation is generally variable by nature introducing uncertainty in measurement results.

These properties define the following requirements to measurement of RBC velocity. The method has to provide accurate spatial matching of images within video sequence, detect and evaluate geometry of each individual capillary with high noise-immunity and resolution. It is necessary to find reliably borders of each capillary and its central line. It has to be used high-speed video recording to provide high enough time resolution of moving RBCs taking into account typical velocity variation range within a cardiac cycle.

In this paper, we consider a method that meets the requirements mentioned above. The method implementation is based on high-speed (up to 2000 fps) incoherent VCS system and noise-immune image processing procedures applied to recorded video sequence that provide robust estimates of individual capillary geometry and direct measurement of RBCs velocity in wide range (up to 5 mm/s and higher) irrespectively from particular morphology of a capillary net. The reliable estimates of RBCs velocity allow continuous monitoring of blood flow during long time interval (up to a few minutes) that opens new modalities in medical diagnostics. The experimental results of direct RBCs velocity measurement in a capillary net are presented and discussed below. The considered VCS system allows the routine clinical assessment and monitoring of RBCs velocity in separate capillaries as well as into a capillary net.

2. Material and methods

2.1. Optical system configuration

The blood flow velocity into a capillary may reach 10 mm/s [1], and taking into account small size of a separate RBC (typically about a few micrometers [2]) the frame rate up to 1000 fps [11] or even higher is needed to resolve in time a separate moving RBC. This means comparably short exposure time for a video frame. Thus, to evaluate RBCs velocity by VCS method, appropriate light source, high resolving and high sensitive light sensitive array are needed as well as high performance processing of recorded video sequence.

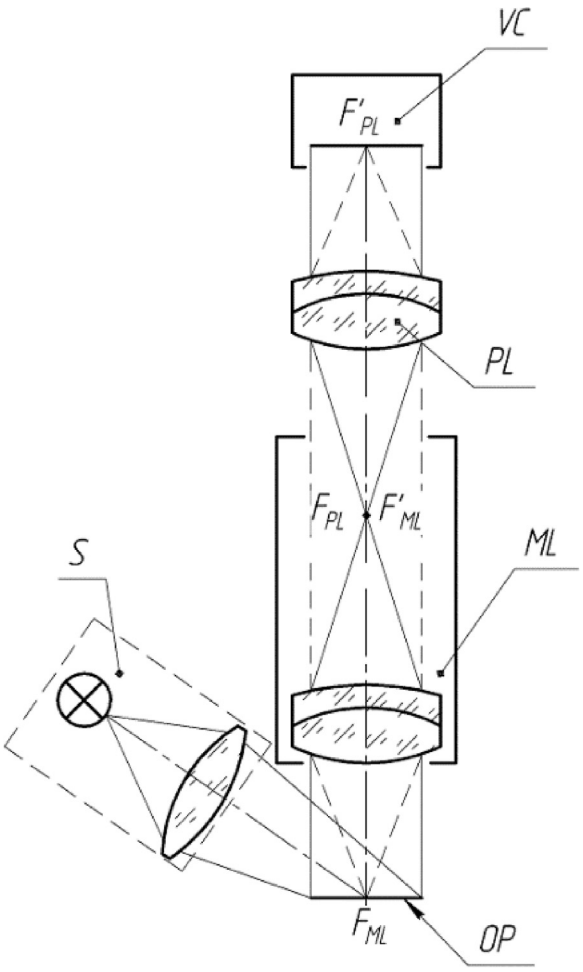

Fig. 1. Optical scheme of the video capillaroscope. *S*: light source, *VC*: video camera, *ML*: microscope lens with focal points F_{ML} and F'_{ML}, *PL*: projection lens with focal points F_{PL} and F'_{PL}, *OP*: object plane.

To observe capillary blood flow within a finger nail fold bed area, the VCS system based on microscope with side illumination was used. Optical scheme of the system represented in Fig. 1. White light from light emitting diode (LED) source S (model Cree XM-L T6, Jetbeam Technology Co.) with light power about 50 mW in 450–750 nm spectral range illuminates a finger distal phalanx at the area of nailfold bed under angle about 45°. Microscope lens ML (Mitutoyo GmbH) with aperture 0.14 and projection lens PL image an object observation plane OP to video camera VC sensitive matrix (CMOS camera model UI–3060CP, IDS GmbH). Optical elements are mounted utilizing cage system (Thorlabs, Inc.) with ability of displacement and focusing at an object area under investigation. Overall view of the system is shown in Fig. 2.

The working distance from objective to skin surface is about 34 mm that enables to change easily and set suitable illumination angle to avoid local specular light reflection component from skin surface. A microscope lens with 5× magnification and depth of focus 14 μm forms sharp capillary image with resolution about 2 μm that is high enough to allocate separate RBC. Linear (paraxial) magnification of the optical system is defined as $m = f_P/f_M$, where f_P and f_M are focal lengths of the projection lens and microscope lens, accordingly. It is possible to vary