1 Introduction

Microcirculation, a complex network of small vessels consisting of arterioles, capillaries, and venules, with a typical diameter ranging from 5 to 50 μm, delivers blood, nutrients, and signaling molecules to tissues and organs, playing a crucial role in their maintenance and hemodynamics. Moreover, microcirculation impairments and dysfunctions are involved in a variety of pathological processes, including cardiovascular diseases, diabetes, excessive angiogenesis, and tumors.

It is, therefore, clear that the characterization of the blood flow velocity, in parallel to the high-spatial-resolution imaging of the tissue, is fundamental to provide critical and essential information regarding healthy and pathological conditions, disease diagnosis, and develop and monitor the response to treatments.

Many different blood flow measurement techniques have been proposed. Our specific focus here is to review noninvasive optical methods to determine the blood flow velocity in microcirculation, giving emphasis to the most recent developments in this field and to the possibility to routinely apply these methods in most research laboratories.

Methods covered in this Spotlight are based both on whole-field and scanning optical imaging techniques, and rely on endogenous or exogenous (fluorescent) contrast agents for plasma and red blood cell (RBCs) visualization. Optical techniques based on dynamic light scattering—such as laser speckle contrast imaging (LSCI, Section 2), laser Doppler optical coherence tomography (D-OCT, Section 3), and diffuse correlation spectroscopy (DCS, Section 4)—are the most common methods for monitoring blood flow. Whereas LSCI and D-OCT probe superficial tissues (~1 mm), since they exploit single or only a few scattering events, DCS takes advantage of photon diffusion theory and probes deep tissues up to several centimeters. Although these techniques differ in their measurement geometry and analysis, each relies on the statistics of the laser speckles: DCS uses light intensity autocorrelation, and LSCI uses the spatiotemporal blurring of the speckles. Recently, LSCI was combined with optical tomography or with DCS, allowing one to determine blood flow information in regions deeper than 1 mm.

Moreover, in Sections 5 and 6, we will present cross-correlation-based techniques in which the signal is primed by single- or two-photon processes. In these cases, the blood flow velocity is measured at the single-vessel level by taking advantage of whole-field or scanning-based acquisition.

In particular, in Section 5, the dual-spot method is presented and extended to multiplexed and wide-field excitation. Moreover, two optical whole-field techniques, particle image velocimetry (PIV) and the recently developed spatiotemporal image correlation spectroscopy, will be discussed.

In Section 6, we will review, in particular, techniques based on raster scanning acquisition and point-by-point detection processes: line-scan-based methods

and the recently developed single raster-scanned *xy*-image technique [flow image correlation spectroscopy (FLICS)] that allows the quantitative measurement of the blood flow velocity in the whole vessel pattern within the field of view, while simultaneously maintaining the morphological information related to the explored circulatory system. In parallel, we will also review methods that can be applied to space–time diagrams obtained not only from line-scan acquisitions but that can also be reconstructed starting from subsequent video frames. The acquired signal can originate directly from RBCs (scattering or fluorescence), or exogenous agents can be injected, so that flowing erythrocytes appear dark against a bright plasma background. Since the excitation process is due to visible or two-photon laser systems, the penetration depth in tissue is limited to ~1 mm, limiting the applicability of these techniques to superficial flow measurement.

By means of these methods, (blood) flow velocity can be recovered with a temporal resolution that depends on the camera acquisition frame rate or on the line-scan frequency, and a spatial resolution related to the dimension of the region of interest (RoI) considered for the cross-correlation computation [up to the point spread function (PSF) limit].

The basic operational principles, the advantages, and drawbacks of all these methods and their technical issues will be discussed and compared, and their applications in biomedical research will be presented.

The characteristics of these techniques are summarized in Fig. 1 and Table 1.

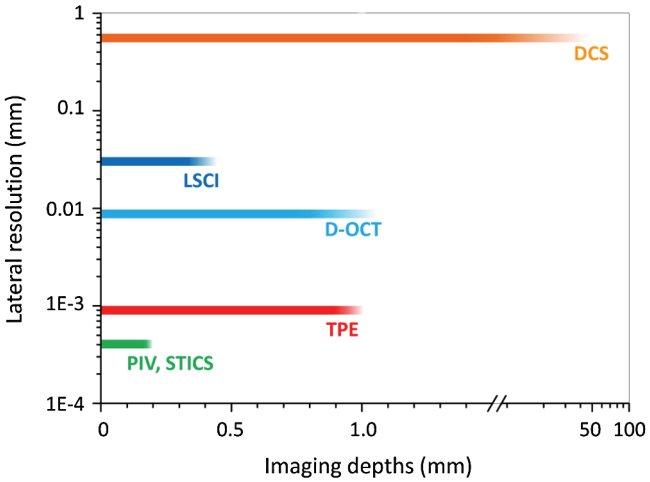

Figure 1 Comparison of lateral resolution and penetration depth of several state-of-the-art blood flow optical imaging techniques (PIV, particle imaging velocimetry; STICS, spatiotemporal imaging correlation spectroscopy; TPE, two-photon excitation; OPE, one-photon excitation; D-OCT, Doppler optical coherence tomography; LSCI, laser speckle contrast imaging; and DCS, diffuse correlation spectroscopy).