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Mapping transverse velocity of particles in capillary vessels by time-varying laser speckle through perturbation analyses

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Abstract

We propose a cross-correlation method to map the transverse velocities of particles moving in capillary vessels using full-field time-varying laser speckle technique. The mapping is achieved by a semi-random perturbation model that describes the intensity fluctuation of time-varying laser speckle signals. When passing through probing volume, moving particles encode a random perturbation into the observed laser speckle pattern. We calculate the transverse flow velocity by cross-correlating the temporal envelopes of the perturbation signals. The proposed method is experimentally verified by the use of polymer microsphere suspension in a glass capillary.

Most retinopathies are often involved with malfunctioned small retinal blood vessels (RBVs), including capillaries, venules and arterioles. The quantitative evaluation of blood flow in small RBVs, particularly in capillaries, is important for the diagnosis, therapeutic treatment and management of these vision threatening diseases. Over the decades, a number of optical techniques have been attempted to quantify the retinal blood flow. However, the mapping of the flow velocity in small RBVs still remains a significant challenge in ophthalmology. The prior techniques can be classified into three main categories: speckle contrast analysis imaging [1-3], Doppler-based velocity measurements (including laser Doppler flowmetry, laser Doppler velocimetry, and Doppler optical coherence tomography) [4-7], and blood cell-tracking-based velocity measurements (represented by retinal function imager, RFI) [8]. Laser speckle contrast analysis imaging is the most useful in relative rather than absolute measurements. The Doppler-based methods measure axial velocity components, requiring a prior known Doppler angle to recover “true” velocities. Moreover, producing a two-dimensional velocity map requires either scanning of a probe beam or imaging with a high-speed camera [9]. The RFI permits mapping the transverse velocity components of blood flow in small RBVs, but is hampered by its low sensitivity and shallow detection depth. In the investigation of human retina, the ability to measure the transverse velocity component is more desirable than the axial velocity component, simply due to the fact that the probe beam is always almost perpendicular to the vascular network innervated within retinal tissue. Recently, several scanning-based methods (with either cross-correlation

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or autocorrelation) have been demonstrated for measuring transverse particle-flow velocities [10-13]. However, these methods are difficult, if not impossible to provide real time mapping of the 2D particle flow velocity, largely due to the requirement of scanning the probe beam to achieve the measurement.

Laser speckle imaging is proven as a powerful tool for studying blood flow in deep tissues with high contrast caused by moving red blood cells (RBCs). In this letter, we demonstrate a method for mapping transverse flow velocities in small capillaries using time-varying laser speckle signals through perturbation analyses. This method combines the advantages offered by both the laser speckle imaging and cross-correlation analyses. In contrast to conventional laser speckle methods, the present method is based on the spatial-correlation property of the perturbation envelope extracted from time-varying speckle patterns. The transit time of moving particles between two neighboring spatial locations is obtained by calculating the cross-correlation between the perturbation envelopes detected at the respective locations. Therefore, the transverse flow velocity equals to l/τ where l and τ are the transverse distance and the transit time between the two spatial locations, respectively.

It is well accepted that laser speckle can only be described statistically. This assumption is reasonable for solid object and dense particle flow because they can be considered macroscopically ‘homogeneous’. However, the moving RBCs in the retinal capillaries are discrete, i.e., intermittent single-files, resulting in macroscopically “heterogeneous” blood flow. Unlike the completely random phasor-sum model presented by Goodman [14], which is widely used in the conventional laser speckle techniques, we use a semi-random perturbation model to describe the variations of laser speckle patterns caused by RBCs in the capillary vessels.

We treat such particle single-file as a stochastic series, consisting of particles and “gaps”. When passing through a probe volume, particles and “gaps” result in different perturbations in the laser speckle patterns. Assume a particle single-file passes through a probe volume, $a(t)\exp(j\phi_a(t))$ and $b(t)\exp(j\phi_b(t))$ can be used to indicate the transient complex-phasor sum of the components from the particles flowing in this probe volume and from the static objects surrounding the moving particles, respectively. Thus, the detected time-varying speckle signal is given by

$$I(t) = a^2(t) + b^2(t) + 2a(t)b(t)\cos(\phi(t)), \quad (1)$$

where $\phi(t)$ denotes a random phase, which gives rise to the variation of laser speckle, $b(t)$ can be approximated as a constant due to its slow variation over time, and $a^2(t)$ is negligible because it is usually much less than $b^2(t)$. As a consequence, the intensity variation is mainly dependent upon the random phase $\phi(t)$ and the non-random amplitude $a(t)$. Here, we call it a semi-random model for simplicity. When passing through the probe volume, moving particles cause a large increase in $a(t)$ and a phase-shift in $\phi(t)$. By contrast, a “gap” or a background disturbance causes a small variation in $a(t)$ and $\phi(t)$. Therefore, one may calculate the envelope of $a(t)\cos(\phi(t))$ to determine the transient perturbation intensity, which directly reflects the spatial pattern of the particle flow. The transient envelope of $a(t)\cos(\phi(t))$ can be written as

$$ENV[a(t)\cos(\varphi(t))] = LPF \left[\frac{(\text{diff}(I(t)))^2}{\frac{1}{T} \int_{-\infty}^{\infty} I(t') \text{rect}\left[\frac{t-t'}{T}\right] dt'} \right], \quad (2)$$

$$\text{rect}[x] = \begin{cases} 1 & |x| \leq \frac{1}{2} \\ 0 & \text{else} \end{cases}$$

where $ENV[]$ is the abbreviation of “envelope”, $LPF[]$ denotes low-pass filtering, and $\text{diff}()$ indicates the differential operation. The denominator of Eq. (2) is the short-time average of $I(t)$ with a time duration of T . The value of T is dependent on the frame rate of the camera, and it was 35ms in this study. Eq. (2) is similar to the envelope detector in an amplitude-modulation radio receiver, which mainly consists of a rectifier and a low-pass filter. The data processing includes four steps: (1) Utilizing the differential operation to remove DC signals; (2) The square operation is equivalent to a full-wave rectifier; (3) The short-time average of $I(t)$ is approximated to $b(t)^2$, and it is used to eliminate the term $b(t)^2$ from the envelope; (4) Low-pass filtering separates the envelope and the high frequency signals caused by the random phase.

For the case of blood flow in capillaries, the spatial pattern of RBCs may be “frozen” during a short period of time. Thus, the motion patterns observed from two neighboring locations are approximately identical. The transit time of RBCs can be obtained by cross-correlating the motion patterns. However, it is difficult to directly calculate the correlation of laser speckle patterns due to the influence of the random phases as shown in Eq. (1). The perturbation envelope $ENV[a(t)\cos(\varphi(t))]$ is independent of the random phases and exhibits the similar behavior to that of the motion pattern. Therefore, one may calculate the transit time using cross-correlation between the perturbation envelopes.

The experimental setup used in this study is shown in Fig. 1. It was a modified Michelson interferometer, mainly consisting of a He-Ne laser with a wavelength of 632.8nm, a sample arm, a reference arm, and a CMOS camera (Aca2000-340km, Basler). The laser beam from the He-Ne laser was expanded by an objective lens and then split into the sample and the reference arms. The back-reflected lights from the two arms were collected by a telecentric lens and then delivered to the camera. The telecentric lens provided a depth of field of about 0.3 mm. We used polymer microsphere suspension in a glass capillary to validate the present method. The particle flow was driven by a syringe pump. The diameter of the microsphere was 7 μm , close to the size of RBCs. It was difficult to drive such particles flowing through a capillary with an inner diameter close to RBC. For this reason, we used a glass capillary with an inner diameter of about 0.4 mm and an outer diameter of 1 mm. We serially diluted the particle solution until the particles passed through the probe volume one by one, similar to the blood flow in a capillary. The glass capillary was adjusted to be approximately perpendicular to the probe beam. A white paper was positioned in the reference arm and adjusted being conjugate to the camera. It worked as the static object. The purpose of adding a reference light in this study is to supplement the intensity of $b(t)$ as shown in Eq. (1) to meet the condition that $a(t)$ is much less than $b(t)$. Another white paper was placed under the glass capillary, which is used to provide background scattering light. Here, the influence of multiple scattering was neglected due to the fact that the human retina

is almost transparent. The time-varying speckle patterns with a dimension of 1000×256 were captured at a frame rate of 1400 Hz and an exposure time of 0.3 ms.

The raw speckle images acquired over time are shown in Fig. 2(a). The perturbation envelopes from each pixel were calculated from the corresponding time-varying speckle signals by using Eq. (2). The transient 2D motion patterns are shown in the supplementary movie (see Media 1), from which one representative frame is displayed in Fig. 2(b). The movie clearly shows the transient motion of particles without random twinkling. In Fig. 2(c), the red, black and blue lines represent the time-varying speckle signals observed at the locations labeled with “A”, “B”, and “C” shown in Fig. 2(a), respectively. The three locations are separated with equal distances measured at five pixels in the image. Fig. 2(c) indicates that two particles pass through the three locations consecutively. The corresponding perturbation envelopes calculated by Eq. (2) are displayed in Fig. 2(d). Compared with the raw speckle data in Fig. 2(c), their envelopes in Fig. 2(d) appear with high contrast and almost free from the influence of the random phases. The cross-correlations of the raw signals are shown in Fig. 2(e), and those of the perturbation envelopes are shown in Fig. 2(f), where “B-C” and “A-B” denote the cross-correlations between the data B and C and between the data A and B, respectively. It is clear that the time-varying laser speckle signals are spatially de-correlated while their envelopes are spatially correlated. The calculated time delay between the data B and C, τ_{BC} , and that between the data A and B, τ_{AB} , are identical, with an excellent agreement with the time shifts of t_{BC} and t_{AB} as shown in Fig. 2(d).

The experimental results for quantitatively validating the present method are shown in Fig. 3. The triangles denote the measured transverse velocities, and the circles represent the transverse velocities obtained by manually tracking the particles in adjacent particle-pattern images, which are used as the golden standard. It can be seen that the measured results are in good agreement with those from the manual approach, with a maximum discrepancy of 12%. We note that the use of manual particle-tracking as the standard suffers from velocity errors due to particle-position uncertainty.

A 2D transverse velocity distribution is shown in Fig. 4, in which the numerical numbers shown are the measured transverse velocities with the unit of mm/s. The horizontal lines indicate the motion paths of the particles, resulting from superposing the transient 2D particle patterns measured over time. The length of the motion path is proportional to the transverse velocity. The total imaging time is about 2 seconds.

In conclusion, we have demonstrated an efficient method to map transverse flow velocities using time-varying laser speckle through perturbation analyses. This method takes two important advantages of laser speckle technique, i.e., its full-field imaging capability and its high sensitivity to motion. As opposed to the speckle contrast analysis imaging that is based on analyzing the blurring degree of speckle patterns, the present method utilizes the spatial correlation of the perturbation envelopes of time-varying speckle signals. The experimental results indicate that this method is feasible to map the transverse velocities of the blood flow in the small capillaries.

For the case of blood flow in deep tissues, the spatial correlation between the perturbation envelopes may be deteriorated due to multiple scattering. One way to mitigate this problem is to use a low-coherence light source, similar to that used in optical coherence tomography technique [15]. Moreover, the 3D quantification of blood flow may be achievable by scanning the reference arm or sample arm axially. The future work will be focused on the 3D imaging of transverse particle-flow velocities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Schematic of the experimental setup

Fig. 2.

(Color online) Quantification of particle flow. (a) Raw speckle image series acquired over time. (b) Calculated 2D speckle pattern image of moving particles (Media 1). (c) Typical time-varying speckle signals observed at the locations labeled with “A”, “B”, and “C” in (a), respectively, and their perturbation envelopes are shown in (d). (e) Cross-correlations of the raw data. (f) Cross-correlations of the perturbation envelopes.

Fig. 3. Measured transverse velocities (triangles) compared with those measured by manual approach (circles).

Fig. 4.
Two-dimensional transverse velocity distribution of particle flow