Photoacoustic Doppler flow measurement in optically scattering media

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We recently observed the photoacoustic Doppler effect from flowing small light-absorbing particles. Here, we apply the effect to measure blood-mimicking fluid flow in an optically scattering medium. The light scattering in the medium decreases the amplitude of the photoacoustic Doppler signal but does not affect either the magnitude or the directional discrimination of the photoacoustic Doppler shift. This technology may hold promise for a new Doppler method for measuring blood flow in microcirculation with high sensitivity. © *2007 American Institute of Physics*.
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Microcirculation is the smallest functional unit of the cardiovascular system. Its study can provide a unique perspective on the process of several major diseases such as diabetes and cancer.[1] However, because of its small vessel size (~5–100 μm), low blood flow speed (<50 mm/s), and microvascular structure depth (up to ~5 mm), the study remains a challenge.[2]

Several techniques based on the Doppler principle have been developed for measuring blood flow in tissues, but they all have their own limitations in microcirculation study. Doppler ultrasound, the principal clinical tool for examining flow above 10 cm/s in large vessels, has difficulty detecting flow with speed less than 1 mm/s. Since the backscattered ultrasound signal from the small amount of red blood cells (RBC) is much weaker (~100 dB) than that from the surrounding tissue,[2,3] the Doppler shift from the slowly moving RBC is masked by that from the tissue with slow motion. Laser Doppler flowmetry[4] can measure very low speed flow in microcirculation owing to the short optical wavelength[5] but it suffers from multiple light scattering, which randomizes the optical phase rapidly [scattering coefficient in tissue μ_S ~100 cm^{-1} (Ref. 6)]. It usually can measure only the averaged flow speed in a tissue volume of ~1 mm^3 with no information about the flow direction.[5] Optical Doppler tomography[7] alleviates the multiple light scattering problem by combining the principles of laser Doppler and of coherent gating to detect singly backscattered light from RBC. It can detect flow as slow as ~100 μm/s with a spatial resolution of ~$5\times5\times15$ μm^3.[7] However, multiple scattered light dominates the signal at increased tissue depth, thus, its imaging depth is usually less than 1 mm.[8]

Recently, we described the photoacoustic Doppler (PAD) effect from flowing small light-absorbing particles.[9] There, the experiment of measuring fluid flow in clear media (distilled water) was reported. In this letter, we report the PAD flow measurement of a blood-mimicking fluid flow in optically scattering media and discuss the potential advantages of PAD over other aforementioned Doppler techniques when applied to studying microcirculation.

The PAD effect derives from the photoacoustic effect generated by small particles,[10,11] where the particles absorb intensity-modulated (also referred to as amplitude-modulated) light, experience heating, and produce acoustic waves. When the particles are in motion, the acoustic waves have a PAD-shifted frequency. We have demonstrated[9] that the PAD shift in a clear medium can be expressed as

$$f_{\text{PAD}} = f_O \frac{V}{c_A} \cos\theta \equiv \frac{V}{\lambda_A} \cos\theta, \tag{1}$$

where f_O denotes the optical modulation frequency, V the flow speed of the particles, c_A the sound speed in the medium, λ_A the acoustic wavelength, and θ the angle between the flow direction and the acoustic wave propagation direction. The Doppler shift in the light received by the flowing particles can be neglected in Eq. (1) because the PAD effect is directly related to the photon density wave instead of the optical wave and the photon density wave has a much longer wavelength than the acoustic wave. When the light enters the scattering medium, a diffusive photon density wave is formed.[12] Although the diffusive photon density wave has a phase velocity of approximately one to two orders of magnitude slower than the speed of light,[12] its wavelength is still much longer than the acoustic wave. Therefore, the PAD shift in scattering media is accurately described by Eq. (1).

Our experimental system is based on the intensity-modulated continuous-wave (cw) photoacoustic microscopy as detailed in (Refs. 13 and 9). Here, Fig. 1 plots the schematic of the detection geometry. A particle suspension flows with velocity **V** inside a small tube (inner diameter: 0.51 mm and outer diameter: 1.53 mm). An intensity-modulated cw diode laser beam (f_O=2.4550 MHz, center wavelength: 780 nm, and average power: 120 mW) is focused into the

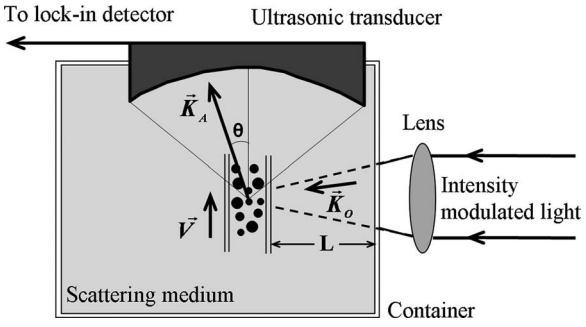

FIG. 1. Geometry of the photoacoustic Doppler flow measurement system.

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scattering medium in a container. If the medium were clear, the laser beam would propagate along the dashed lines onto the tube with a spot size of ~1 mm. In the scattering medium, the laser beam is smeared, forming a diffusive photon density wave with the wave vector $\mathbf{K}_O$. The produced acoustic wave with the wave vector $\mathbf{K}_A$ is detected by an ultrasonic transducer which is homemade with the following properties:[13] the diameter of the active element is 38 mm, the radius of curvature is 22 mm, the numerical aperture is $NA = \sin(60°)$, the center frequency is 2.455 MHz, and the −6 dB bandwidth is 0.1 MHz. The acoustic signal is then quadrature demodulated by a lock-in detector, after which a pair of X and Y signals [Eq. (3)] are obtained. Finally, the spectral analysis on the X and Y provides the PAD shift. Because of the distribution of θ (from 0° to 60°), according to [Eq. (1)], the PAD shift will be distributed as well and the mean shift can be calculated as

$$\bar{f}_{\mathrm{PAD}} = \frac{\int_{0°}^{60°} f_{\mathrm{PAD}} \times 2\pi R \sin\theta d\theta}{\int_{0°}^{60°} 2\pi R \sin\theta d\theta} = f_O \frac{V}{c_A} \frac{(1+\cos 60°)}{2}. \tag{2}$$

The particle suspension includes carbon glassy particles with sizes ranging ~2–12 μm and has a particle volume fraction of 40%. Both the particle size and the volume fraction mimic the situation of RBC in normal whole blood. The optical absorption coefficient μ_a of the suspension, however, is estimated to be ten times higher than that of whole blood [$\mu_a \sim 0.5\ \mathrm{mm}^{-1}$ at the laser wavelength of 780 nm (Ref. 6)]. The scattering medium is a 0.04% intralipid solution, which has an optical scattering coefficient $\mu_S \approx 1.2\ \mathrm{cm}^{-1}$ and an optical reduced scattering coefficient $\mu_S' \approx 0.4\ \mathrm{cm}^{-1}$ at the laser wavelength.[14] In the current study, we used a diluted intralipid solution with low μ_S not only because of the long measuring depth $L \approx 3.0$ cm limited by the transducer's diameter but also because of the low signal level limited by the laser power.

Figure 2 shows the PAD shifts observed at various average flow speeds within 0.027–1.100 mm/s. Figures 2(a)–2(f) plot the PAD spectra, and Fig. 2(g) plots the mean and the standard deviation of each shift calculated from the spectra. Figures 2(a)–2(f) show that when the speed decreases, the spectrum moves to the lower frequency end and becomes narrower. As can be seen in Fig. 2(g), the measured mean shifts agree well with the theoretical values predicted by Eq. (2) with $c_A = 1.5 \times 10^3$ m/s. Each standard deviation equals approximately half of the corresponding mean shift. The spectra and, thus, the standard deviations can be described by a complex model.[15,9] The cause of the spikes in the spectra [Figs. 2(a)–2(f)] is unclear and subject to further investigations.

Figure 3 compares the measurements of the same flow in the scattering medium and in a clear medium (average flow speed: 0.11 mm/s). The X and Y signals in the scattering medium decrease about ten times from those in the clear medium. The corresponding power spectral density decreases by about 100 times. However, the value of the PAD shift remains the same, as manifested by the similar oscillation periods (~10 s) of the X and Y signals and the frequency peaks (~0.1 Hz) of the spectra.

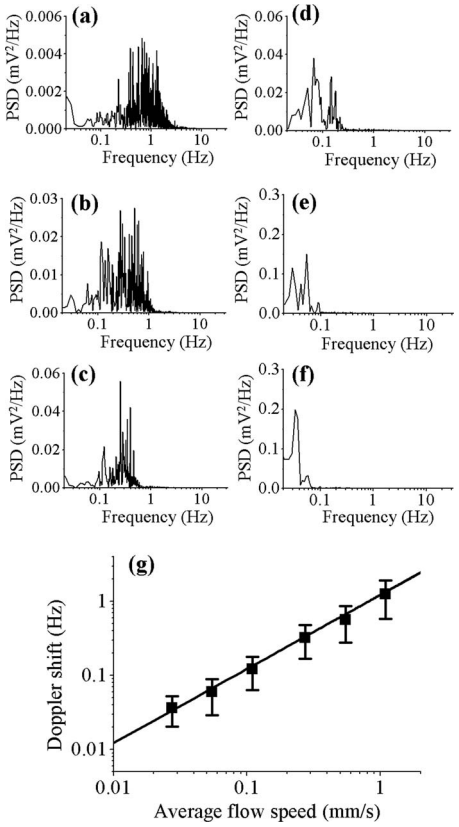

FIG. 2. PAD measurement of the fluid flow in a scattering medium with various speeds. [(a)–(f)] PAD spectra for the average flow speeds from 1.100 to 0.027 mm/s. PSD: power spectral density. (g) PAD shifts calculated from the spectra in (a)–(f). The squared dots plot the mean shifts, the error bars plot the standard deviations of the shifts, and the solid line plots the theoretical mean shifts calculated from Eq. (2).

Also, not changed is the information included in the X and Y signals about the flow direction. In Figs. 3(a) and 3(b), as can be seen, both Y lag the paired X by a phase of $\pi/2$. This lag indicates a positive PAD shift $\bar{f}_{\mathrm{PAD}}$ because the flow is toward the transducer. This observation can be understood from a simplified model,[9] where the ac components of the X and Y signals are expressed as

$$X_{\mathrm{ac}} = \bar{A}_D \cos(2\pi \bar{f}_{\mathrm{PAD}} t + \psi_D),$$

$$Y_{\mathrm{ac}} = \bar{A}_D \cos(2\pi \bar{f}_{\mathrm{PAD}} t + \psi_D - \pi/2). \tag{3}$$

Here, $\bar{A}_D$ and ψ_D denote the amplitude and initial phase, respectively, of the PAD-shifted acoustic signal. When $\bar{f}_{\mathrm{PAD}}$ is negative, Y leads X by $\pi/2$. A negative $\bar{f}_{\mathrm{PAD}}$ has been measured with an inversed flow (with the direction away from the transducer). Figure 4 shows the ac signals measured in the scattering medium for the average flow speed of 0.27 mm/s. Similar results measured in a clear medium can be found in Ref. 9.

Therefore, in PAD fluid flow measurement, the illumination laser provides a sufficient, time-varying photon density wave to produce detectable, PAD-shifted acoustic signals. The multiple light scattering in the medium decreases the photon density. As a result, the PAD signal amplitude is dampened. However, neither the value nor the directional discrimination of the PAD shift is affected. The consequence of a reduced photon density in deeper tissue limits both the

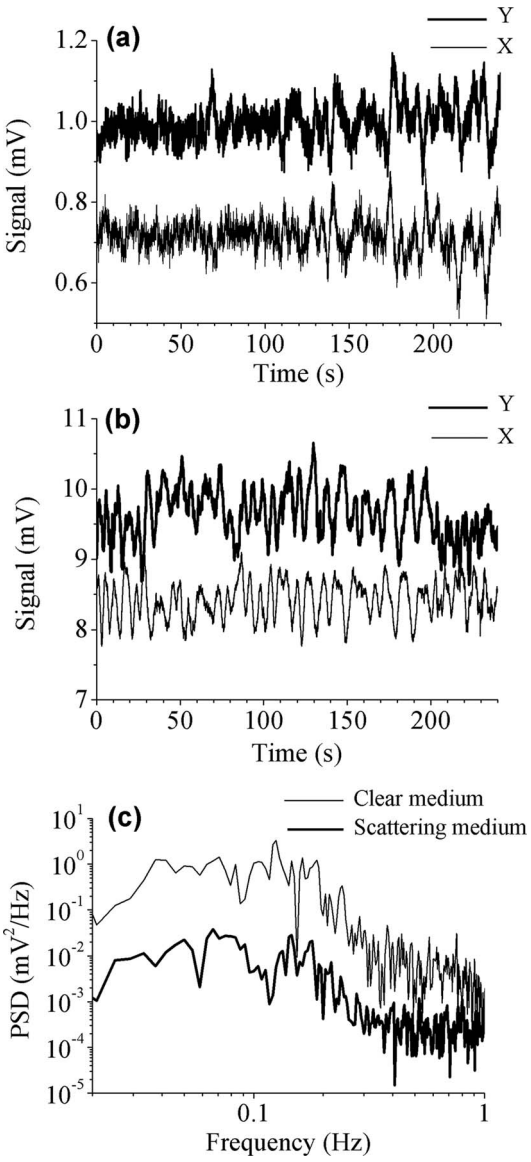

FIG. 3. PAD signals and spectra measured from the same flow in a scattering medium and in a clear medium. The flow has an average speed of 0.11 mm/s. (a) *X* and *Y* signals of the measurement in the scattering medium. (b) *X* and *Y* signals of the measurement in the clear medium. (c) PAD spectra obtained from the signals in (a) and (b). PSD: power spectral density.

measuring depth and the detectable maximum flow speed (~1 mm/s in the scattering medium compared to ~10 mm/s in the clear medium[9]), but they can both be extended by using a laser with higher power or a laser with a wavelength at which the particles have higher light absorption. Using the current system, we have only observed PAD shifts from blood flow in clear media, and we are working on upgrading the system to measure blood flow in scattering media. Remarkably, the detectable minimum flow speed is unaffected by multiple scattering.

For blood microcirculation studies, the PAD effect can potentially be used to measure the very low speed flow because the signal contrast comes from the light absorption of RBC instead of the ultrasound scattering of RBC. At some typical wavelengths (such as 532 nm), the light absorption of RBC can be 100 times higher than that of the surrounding tissue.[16] The PAD measurement could take advantage of photoacoustic imaging demonstrated in Refs. 16 and 17 to detect the microcirculation as deep as a few millimeters with preserved directional information at an ultrasonic spatial resolution of tens of micrometers.

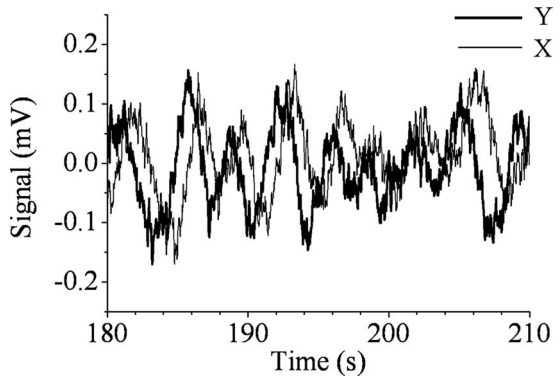

FIG. 4. Negative PAD-shifted ac signals of a flow measured in the scattering medium. The flow has an average speed of 0.27 mm/s. Its direction is reversed from the direction for the data plotted in Fig. 3 and is away from the transducer.

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