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Photoacoustic correlation technique for low-speed flow measurement

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Abstract

A photoacoustic correlation spectroscopy (PACS) technique was proposed for the first time. This technique is inspired by its optical counterpart—the fluorescence correlation spectroscopy (FCS), which is widely used in the characterization of the dynamics of fluorescent species. The fluorescence intensity is measured in FCS while the acoustic signals are detected in PACS. To proof of concept, we demonstrated the flow measurement of light-absorbing beads probed by a pulsed laser. A PACS system with temporal resolution of 0.8 sec was built. Polymer microring resonators were used to detect the photoacoustic signals, which were then signal processed and used to obtain the autocorrelation curves. Flow speeds ranging from 249 to 15.1 $\mu\text{m/s}$ with corresponding flow time from 4.42 to 72.5 sec were measured. The capability of low-speed flow measurement can potentially be used for detecting blood flow in relatively deep capillaries in biological tissues. Moreover, similar to FCS, PACS may have many potential applications in studying the dynamics of photoacoustic beads.

Keywords

Photoacoustic correlation spectroscopy; photoacoustic imaging; flow measurement; blood flow; polymer microring resonators; fluorescence correlation spectroscopy; functional imaging; imaging depth

1. INTRODUCTION

Fluorescence correlation spectroscopy¹ (FCS) is a common technique used in analytical chemistry and biological research. The FCS is able to extract a wealth of molecular and environmental information from weak fluorescence signals from a very small observation volume even with similar level of background noise. In FCS, molecular diffusion, physical

processes, and chemical reactions of a few fluorescent molecules result in fluorescence intensity fluctuation, which is analyzed using temporal correlation. Various FCS applications from diffusion and flow in solutions to chemical reaction kinetics,¹ molecular interactions,² and pharmaceutical screening method³ have been demonstrated.

Photoacoustic (PA) imaging, a non-invasive technique for imaging optical properties in biological tissues, can not only provide the high optical contrast between various intrinsic contrast origins, such as oxygenated and deoxygenated haemoglobin, but also circumvent the diffusive light scattering in tissues, preserving the high spatial resolution and/or deep imaging depth of pure ultrasound imaging. Photoacoustic correlation spectroscopy (PACS) is proposed for the first time to utilize both the benefits of PA signals and the powerful ability of FCS. By studying the dynamics of PA species, PACS could bring a range of new applications in chemical analysis and medical diagnosis. One example is to investigate the microcirculation system.

Studying microcirculation, the smallest functional unit of the cardiovascular system, sheds light on learning disease processes such as cancer, atherosclerosis, and diabetes.⁴ However, the study remains a challenge due to small microvessels, low blood flow speed,⁵ and microvascular structure depth. Using PA signals from the red blood cells (RBCs) excited by a pulsed laser is one way to overcome these limitations. First, slow flow speed can be measured by a PACS system with a proper temporal resolution and probe beam size. Second, high imaging depth is achieved by detecting low-scattering sound signals. Third, a small probe volume can be designed using photoacoustic microscopy scheme.⁶ Besides, for some particular optical wavelength, the absorption coefficient of RBCs can be obviously higher than that of its surrounding tissues, which facilitates a label-free measurement. In this paper, we conduct experiments on flow measurement to demonstrate the PACS feasibility and its potential to become an important tool for non-invasive assessment of microvascular blood flow.

2. METHODS

2.1 Principles of photoacoustic correlation spectroscopy

When beads absorb laser energy, a temperature rise results in thermal expansion and generate PA pressure waves. In PACS, the probe volume is defined by the laser beam used. When the beads move inside the probe volume, the temporal PA signals can be detected. We used PACS strength as the counterpart of fluorescence intensity in FCS and expressed it as

$$P(t) \equiv \int I(r) n(r, t) d^3r, \quad (1)$$

where $I(r)$ is the normalized spatial fluence distribution of the laser beam, and $n(r, t)$ is the bead concentration at position r and time t . In FCS, the fluorescence intensity is proportional to the photon counts detected by photodetectors, while in PACS, the $P(t)$ needs to be extracted by proper signal processing from the measured temporal PA signals. With the fluctuation of $P(t)$, the normalized autocorrelation function can then be calculated as

$$G(\tau) = \frac{\langle \delta P(t) \delta P(t+\tau) \rangle}{\langle P(t) \rangle^2}, \quad (2)$$

where $\delta P(t) = P(t) - \langle P(t) \rangle$ is the fluctuation of $P(t)$ and $\langle \rangle$ denotes ensemble average. The autocorrelation curves, $G(\tau)$, are usually a decay profile, providing the information of dwell time and number density of the beads in the probe volume.

2.2 Experimental setup of flow measurement

To demonstrate the PACS, a flow experiment is chosen because of easier design of various dwell times. The experimental setup is shown in Fig. 1. A syringe pump (Model 100, Kd Scientific, Holliston, MA) with 1 cc syringe was used to make beads flow in a PTFE tubing (inner diameter: 0.8 mm; outer diameter: 1.6 mm; Cole-Parmer). We added 1% volume of Tween-20 to minimize aggregation of the 49 μm black polystyrene beads (BK050, Microgenics Corp., Fremont, CA). Flow speeds from 200 to 14 $\mu\text{m/s}$ were calibrated using a microscope. A polymer microring resonator was used to detect PA signals⁷ and was positioned in the x-z plane, (x,0,z), as shown in Fig. 1. The signal traces were recorded by a digital oscilloscope (WaveSurfer 432, LeCroy, Chestnut Ridge, NY) and then saved to computers for data analysis. A 532 nm pulsed laser (Surelite I-20, Continuum, Santa Clara, CA) generating 6-ns pulses with a 20 Hz repetition rate was used as the probing light source. The laser fluence used is $\sim 70 \text{ mJ/cm}^2$. We used a slit aperture to define a beam width of $\sim 1.1 \text{ mm}$. The one-dimensional step excitation profile can be expressed as $I(x) = 1$ at $|x| \leq w/2$ and $I(x) = 0$ otherwise, where w is a width of the probe laser beam. The corresponding autocorrelation function⁸ takes the following form:

$$G(\tau)/G(0) = \begin{cases} 1 - \tau/\tau_0, & \text{for } \tau \leq \tau_0 \\ 0, & \text{for } \tau > \tau_0 \end{cases}, \quad (3)$$

where $\tau_0 = w/V$ and V is flow speed of the beads. The average number of beads in the probe volume can also be calculated by taking the inverse of $G(0)$.⁹

3. RESULTS

A low-pass filter with a cutoff frequency of 50 MHz was applied to measured PA signals. Fig. 2 (a) shows the complete PA signals collected as a function of elapsed time at a calibrated flow speed of 33 $\mu\text{m/s}$, and one example of temporal PA waveform at elapsed time 316.0 sec is shown in Fig. 2 (b). From Fig. 2 (a), we can determine the flow direction is away from the detector. Considering less-than-1 average number of beads in probe volume, the normalized PACS strength $P(t)$ was extracted by taking the root-mean-square value of the measured PA signals. Fig. 2 (c) shows the PACS strength for four calibrated speeds with 0.8 sec temporal resolution, that is, one sample of $P(t)$ per 0.8 sec. Note that the different time scales are used for the four plots. Longer dwell times in the probe volume for slower flow speeds can be observed in Fig. 2 (c). Device's sensitivity is different in the four cases due to different operating wavelength of microring resonators used. To have more accurate estimation of $G(0)$,¹⁰ noise of $P(t)$ estimate was offset to zero.

Fig. 2 (d) shows the PACS curves for the flow measurement. From Eq. (2), we can calculate the autocorrelation curves, as shown in the dotted points. The solid curves are the fits of data using Eq. (3). The mean dwell times, τ_0 , obtained from PACS curves were 4.42–72.5 sec. The measured flow speeds were 249–15.1 $\mu\text{m/s}$, which was calculated from the fitted τ_0 and the relation $V = w/\tau_0$. In Fig. 2 (e), we show the comparisons of calibrated and measured flow speeds, which is represented as the dotted points. The solid line plots the direct measurement. We can see excellent agreement between the flow speeds obtained by the two different methods. Due to limited fitting points at faster flow speed, there is a little discrepancy between PACS measured and calibrated flow speeds. From the PACS fitted curves in Fig. 2 (d), the $G(0)$ values were 2.9, 1.5, 1.4, and 2.6, and the average bead number in probe volume is estimated as 0.48 $[=4/(2.9+1.5+1.4+2.6)]$. Thus, the concentration can be calculated as $0.87 \text{ mm}^{-3} [=0.48/(1.1 \times \pi \times (0.8/2)^2)]$.

Similar to FCS, it is better to have the average number in probe volume less than 1 to enhance the fluctuation signals. That is, to measure higher concentration, we need a PACS system with smaller probe volume, which can be achieved using photoacoustic microscopy scheme.⁶ The maximum measurable flow speeds are limited by the temporal resolution of the PACS system. As V increases, the flow time τ_0 decreases and eventually approaches the limit of system's temporal resolution. The issue can be addressed by using faster data acquisition methods and faster repetition rate of a pulsed laser, which is our ongoing work. The minimum measurable V has no theoretical limitation in PACS flowmetry. Small probe volume is helpful to reduce dwell times in low-speed flow measurement. Our results infer that the PACS technique is potentially useful to study low-speed blood flow in microcirculation, which has a flow speed of a fraction of mm/s in capillaries.

4. CONCLUSIONS

We developed a photoacoustic correlation technique for low-speed flow measurement. The PACS technique can accurately measure bead flow speeds as slow as 15.1 $\mu\text{m/s}$. The technique has abilities to measure the concentration of solution and to discriminate flow direction. With a small probe volume and higher temporal resolution, PACS techniques can be further engineered for clinical applications.

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Figure 1.

Experimental setup for PACS flow measurement. PS stand for polystyrene. Beads flow in the x-direction. Pulsed laser illuminates in the y-direction. The PA signals were detected using a microring resonator positioned in the x-z plane. The probe volume is at the origin. The shape of the pulsed laser at probe volume is illustrated.

Figure 2.

(a) Detected PA signals as a function of elapsed time, measured at the calibrated speed of 33 $\mu\text{m/s}$. (b) PA signal from (a) at elapsed time = 316.0 sec. (c) PACS strength fluctuation as a function of elapsed time at calibrated speeds of 200, 90, 33, and 14 $\mu\text{m/s}$. (d) PACS autocorrelation curves of the four designed flow speeds. (e) The measured flow speeds by the PACS technique, shown in stars, versus the calibrated flow speeds. The solid line represents the results from direct measurement.