Cerebrovascular imaging in vivo by non-contact photoacoustic remote sensing with a laser diode for interrogation

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Photoacoustic microscopy (PAM) is a unique tool for biomedical applications because it can visualize optical absorption contrast in vivo. Recently, non-contact PAM based on non-interferometric photoacoustic remote sensing (PARS), termed PARS microscopy, has shown promise for selected imaging applications. A variety of superluminescent diodes (SLDs) have been employed in the PARS microscopy system as the interrogation light source. Here, we investigate the use of a low-cost laser diode (LD) as the interrogation light source in PARS microscopy, termed PARS-LD. A side-by-side comparison of PARS-LD and a PARS microscopy system using an SLD was conducted that showed comparable performance in terms of resolution and signal-to-noise ratio. More importantly, for the first time to our knowledge, in vivo PAM imaging of mouse brain vessels was conducted in a non-contact manner, and the results show that PARS-LD provides great performance.

Photoacoustic (PA) imaging is a rapidly developing and appealing imaging technique in the biomedical field [1,2]. Typically, a nanosecond pulsed laser is used to illuminate objects, which induces thermal expansion and then produces PA waves. By using a focused light beam for illumination, PA microscopy (PAM) can provide micrometer-scale resolution for visualizing absorption contrast in biological tissue. PAM is useful for biomedical and, potentially, clinical applications such as brain imaging [3] and cell imaging [4], to name a few.

PA brain imaging is an effective tool to monitor vessels, neurons, and tumors [5]. In functional PA brain imaging, the oxygen extraction fraction and cerebral metabolic rate of oxygen were obtained to analyze brain metabolism [6]. Gliosarcoma and its surrounding microvasculature morphology were detected by dual-wavelength PAM [7]. PA molecular imaging represents a potential solutions to brain diseases such as Alzheimer’s disease, stroke, and epilepsy [8]. In all those works, piezoelectric transducers were used, and coupling agents (e.g., gel and water) had to be applied to tissue.

Recently, a new non-interferometric PA remote-sensing (PARS) technique that enables non-contact PAM imaging with high sensitivity was developed [9–15]. In PARS, a pulsed laser is used to illuminate objects. Due to the PA effect, initial acoustic pressure is produced in the absorber. Then, due to the elastooptic effect, the refractive index of the absorber changes, which can be detected by an interrogation light beam by monitoring the changes in the reflected light power [12]. Similar to conventional PA imaging, PARS employs a nanosecond pulsed laser for efficient PA excitation. On the other hand, PARS typically adopts a continuous-wave (CW) laser as the interrogation light beam to facilitate the monitoring of reflected light power changes. To enable sensitive PARS microscopy with micrometer-scale resolution (the same as PAM), confocal alignment of the excitation and interrogation light beams is important. There have been technological advances in PARS microscopy, such as depth-resolved imaging [10], real-time two-dimensional imaging (C-scan) [9], and dual-modal imaging [14,15]. Potential clinical applications of PARS microscopy include the histology of human tissue [11] and ophthalmic imaging [13]. In all those works (i.e., [9–15]), the superluminescent diodes (SLDs) featured a short coherence length to intentionally eliminate sensitivity to phase oscillations [12]. If different layers within the coherence length along the axial direction have different refractive indices, multiple reflections occur that coherently interfere with each other, which leads to phase oscillations.

In conventional PAM, an ultrasound transducer is typically used, and acoustic coupling agents must be applied to the tissue, which complicates the imaging procedure and cause issues such as infection and contamination of the tissue [13]. For example, conventional PAM for brain imaging in vivo may lead to contamination, infection, and injury (e.g., bleeding) of the brain tissue. In another example, for PA brain imaging, liquid water is commonly used as the coupling agent. Light absorption by ordinary water at 1064 nm affects the maximum light that can be delivered, so deuterium oxide must be used as the coupling agent [16]. In addition, to keep the liquid water stable and maintain good coupling efficiency, a horizontal working plane is required. This causes inconvenience, especially for selected applications, e.g., when the patient is on a surgical bed [17,18]. By contrast, PARS...
microscopy circumvents the above challenges and is supposed to significantly facilitate brain imaging because no coupling agents are needed.

In the work reported here, PARS using a low-cost laser diode (LD) for interrogation, termed PARS-LD, was investigated. The LD used in this work costs less than 200 US dollars. To evaluate the performance of PARS-LD, a side-by-side comparison of PARS-LD and a PARS microscopy system using an SLD [15], termed PARS-SLD, was conducted, and the two systems were found to provide comparable performance in terms of resolution and signal-to-noise ratio (SNR). More importantly, for the first time to our knowledge, PARS-LD was employed to image mouse brain vessels in a non-contact manner, and good image quality was obtained. Our work opens up new opportunities for non-contact PA cerebrovascular imaging in vivo.

The schematic of the PARS-LD microscopy system is shown in Fig. 1. A 532 nm pulsed laser (FDSS532-Q3, CryLas, Germany) with a repetition frequency of 1 kHz was employed to provide the excitation light beam. The laser was first split into two paths by a 9:1 beam splitter. 10% of the light power was detected by a photodetector to provide trigger signals. The other 90% of the light power was attenuated by neutral density filters, shaped by a lens set consisting of two convex lenses (L1 and L2 in Fig. 1) with an iris in between them, and coupled into a single-mode fiber (SMF) by another lens (L3 in Fig. 1). The light exiting from the SMF was collimated by a homemade collimator (C1 in Fig. 1; the fabrication of the homemade collimator is described in Section 1 of Supplement 1) and then reflected by a dichroic mirror (DM). Finally, the light was focused by a doublet lens (L4 in Fig. 1) (AC127-019-C, Thorlabs) onto the sample for PA excitation.

An LD with a wavelength of 1310 nm was used to provide the interrogation light beam (more information on the LD is detailed in Section 2 of Supplement 1). The interrogation light beam was linearly polarized, and its linewidth was 2.2 nm. For efficient collection of the reflected light power, the interrogation light beam was transmitted through a polarized beam splitter (PBS) and passed through a quarter-wave plate (QWP), which changed the light to be circularly polarized. Then, the interrogation light beam was transmitted through the DM and focused by L4 onto the sample. Note that careful adjustment was conducted to achieve confocal alignment between the excitation and interrogation light beams. The interrogation light beam was reflected by the sample and transmitted through the DM and the QWP, which changed the light to be linearly polarized perpendicular to the original polarization of the light emitted from the LD. Thus, the returned interrogation light beam was reflected by the PBS. Then, the interrogation light beam was coupled into a multi-mode fiber by another collimator (C2 in Fig. 1) and received by a photoreceiver (1811-FS, New Focus). After the photoreceiver, a pulse receiver (5073PR, Olympus) was used as an amplifier with a gain of 30 dB. The pulse receiver also served as an analog low-pass filter with a cutoff frequency of 20 MHz. The signals were finally acquired by a digitizer (CSE1422, GaGe) with a sampling rate of 200 MS/s and saved in a computer. During image acquisition, the sample was placed on a two-dimensional motorized stage (M-404, Physik Instrumente [PI], Karlsruhe, Germany) for sample scanning. PARS-SLD employed a 1310 nm SLD (IPSDM1302C, INPHENIX) for interrogation, which was also used in our previous study [15]. To facilitate a side-by-side comparison and to ensure a fair comparison between PARS-SLD and PARS-LD, the switch between the SLD and the LD was facilitated by an FC adaptor (FCA1 in Fig. 1), and all the other parts of the PARS microscopy system were kept the same, which led to a similar confocal alignment (i.e., a similar overlap of the focused excitation and interrogation light beams) for PARS-SLD and PARS-LD.

The resolutions of PARS-SLD and PARS-LD were measured. The lateral resolution measurement is detailed in Section 3 of Supplement 1. The measured lateral resolution was \( \sim 2.1 \mu m \) for both PARS-SLD and PARS-LD [Figs. 2(a) and 2(b)]. The numerical aperture of the focused excitation light beam was 0.18. Therefore, the theoretical resolution was calculated as \( 1.51 \mu m \times \frac{0.51 \times 0.532/0.18}{2 \pi} \), which is smaller than the measured resolution. This discrepancy may be due to the imperfect Gaussian profile of the focused excitation light beam. The axial resolution measurement is detailed in Section 4 of Supplement 1. The approximate measured axial resolution was 45 \( \mu m \) and 47 \( \mu m \) for PARS-SLD and PARS-LD, respectively [Figs. 2(c) and 2(d)]. Assuming that the focused excitation light beam was a Gaussian beam, the axial full width at half maximum (FWHM) can be estimated as \( \sim 38 \) (\( = \frac{2 \pi \times (2.1 \times 0.85)}{0.532} \)) \( \mu m \), which is close to the measured axial resolution. Note that the factor of 0.85 is the ratio of the beam radius to the lateral FWHM, which is \( \sim 2.1 \mu m \) [Figs. 2(a) and 2(b)]. Because a similar confocal alignment was achieved for both PARS-SLD and PARS-LD, similar lateral and axial resolutions were obtained for both PARS-SLD and PARS-LD.

We compared the sensitivities of PARS-LD and PARS-SLD by imaging a 30 \( \mu m \) tungsten wire. The excitation light energy was kept the same. The interrogation light power (on the samples) was 1–5 mW for the LD and 2 mW for the SLD. The sensitivity comparison measurement is detailed in Section 5 of Supplement 1. Figure 3 shows the measured results. For the same interrogation light power of 2 mW, comparable SNRs were achieved with PARS-LD and PARS-SLD [Fig. 3(c)]. For PARS-LD, the PARS peak signal increased with the interrogation light power [Fig. 3(a)], as expected [12], while the noise did not

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**Fig. 1.** Schematic of PARS-LD and PSRS-SLD. BS, beam splitter; ND, neutral density filter; L, convex lens; C, collimator; FCA, FC adaptor; MMF, multi-mode fiber.
Resolution measurements for PARS-SLD and PARS-LD. Lateral resolution of PARS-SLD (a) and PARS-LD (b). Axial resolution of PARS-SLD (c) and PARS-LD (d).

Comparison of the SNRs of PARS-LD and PARS-SLD. (a) PARS peak signal. (b) Noise. (c) SNR.

Fig. 3.

change much with the interrogation light power [Fig. 3(b)], leading to a higher SNR with increasing interrogation light power. Note that PARS-SLD is expected to show an enhanced SNR with increasing interrogation light power, but this was not seen in the experiment. The limit of the interrogation light power is discussed in Section 6 of Supplement 1.

To compare the imaging capabilities of PARS-LD and PARS-SLD, phantom imaging was conducted. For a fair comparison, the same pulsed laser energy was used for excitation in both PARS-SLD and PARS-LD. Several 6 μm carbon fibers were imaged. The surfaces of the carbon fibers were kept moist to improve the stability of the PA signal. As shown in Figs. 4(a) and 4(b), a carbon fiber diameter of 6 μm can be extracted from the PA images. Another phantom of leaf veins was prepared and imaged. The leaf veins were dyed with black ink. Figures 4(c) and 4(d) show that the PA images are of good quality. Note that Fig. 4 shows the enhanced images processed by (i) applying a digital low-pass filter with a cutoff frequency of 6 MHz to improve the SNR of raw PA time-domain signals and (ii) employing a 3 × 3 median filter to further improve the image quality. In Fig. 4, comparable SNRs of PARS-SLD and PARS-LD are observed.

In vivo mouse brain imaging was also conducted. A 6-week-old BALB/c mouse was used. The mouse was anesthetized by an isoflurane anesthesia machine (R500IP, RWD Life Science, China) with a gas mixture of 1% isoflurane and oxygen. The scalp and the skull of the mouse were removed to avoid refraction and attenuation of the excitation and interrogation light beams. Afterward, 1 ml of phosphate buffer saline was applied to the brain every 20 min to maintain its salt ion concentration. To facilitate image acquisition, the mouse was fixed on a homemade animal platform whose angle was adjustable so that the imaged brain surface could be well aligned with the scanning plane. All experimental animal procedures were carried out in conformity with the laboratory animal protocol approved by the Laboratory Animal Care Committee at Shanghai Jiao Tong University. A pulsed laser energy of 250 nJ/pulse was used for excitation. Potential approaches that use a lower pulsed laser energy for in vivo imaging are discussed in Section 7 of Supplement 1.

Figure 5 shows the imaging results from PARS-SLD and PARS-LD. Figure 5(a) shows a photo of the mouse brain after removing the scalp and the skull. Figures 5(b) and 5(c) show PA images from PARS-SLD and PARS-LD, respectively, corresponding to the dashed boxes in Fig. 5(a). As can be seen, mouse cerebral vessels can be visualized by non-contact PAM with good contrast. The vessel pattern images from PARS-SLD (Fig. 5(b)) and PARS-LD [Fig. 5(c)] are generally consistent. Small features can be observed as small as approximately 12 μm. The vessel pattern seen in PA images [Figs. 5(b) and 5(c)] is also consistent with that in the photo [Fig. 5(a)]. Similar to Fig. 4, comparable SNRs of PARS-SLD and PARS-LD are observed in Fig. 5. Note that, except for the different interrogation light
power of the LD used in the sensitivity comparison (Fig. 3), the same power (2 mW on the samples) from the SLD and the LD was used in all measurements and imaging demonstrations (Figs. 2–5).

The SLD featured a low coherence compared with lasers. The linewidths of the SLD and the LD used in this work were 45 nm and 2.2 nm, respectively, corresponding to coherence lengths of 38 µm \((= 1.31 \mu \text{m} / (0.045))\) and 780 µm \((= 1.31 \mu \text{m} / (0.0022))\), respectively. The specifications of the SLD and the LD are described in Section 8 of Supplement 1. In this work, a PARS-LD microscopy system using a low-cost LD for interrogation was investigated. PARS-LD was experimentally demonstrated to possess high resolution and sensitivity, comparable to those of PARS-SLD. More importantly, non-contact PAM of mouse cerebrovascular imaging in vivo was demonstrated. The promising results show the potential of PARS microscopy for brain research.

In conclusion, a PARS-LD microscopy system using a low-cost LD was investigated. PARS-LD was experimentally demonstrated to possess high resolution and sensitivity, comparable to those of PARS-SLD. More importantly, non-contact PAM of mouse cerebrovascular imaging in vivo was demonstrated. The promising results show the potential of PARS microscopy for brain research.

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Data availability. Data underlying the results presented in this Letter are available upon request.

Supplemental document. See Supplement 1 for supporting content.

REFERENCES