A practical optical-resolution photoacoustic microscopy prototype using a 300 mW visible laser diode

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ABSTRACT

Optical-resolution photoacoustic microscopy (OR-PAM) is an emerging technique for microvasculature imaging at high spatial resolution and contrast. In this work, we present a practical visible laser-diode-based OR-PAM (LD-OR-PAM) prototype for vasculature imaging, which has the desirable properties of being portable, low-cost, and label-free. The prototype employs a 300 mW pulsed laser diode in a 3.8 mm diameter package, emitting 174 ns pulses at 405 ± 5 nm wavelength and a pulse energy of 52 nJ. An aspheric objective with a numerical aperture of 0.60 is used to achieve microscope optical illumination. The laser diode excitation has a compact size of 4.5 × 1.8 × 1.8 cm^3 assembled with a cooling block. The lateral resolution was tested to be 0.95 μm on ~7 μm carbon fibers. The subcutaneous microvasculature on a mouse back was label-free imaged *ex vivo*, which demonstrates the potential of the LD-OR-PAM prototype for *in vivo* imaging skin chromosphores such as hemoglobin. Our ultimate aim is to provide a practical and affordable OR-PAM system as a routine instrument for standard clinical applications.

Keywords: photoacoustic microscopy, laser diode, label-free, microvasculature imaging

1. INTRODUCTION

Photoacoustic (thermoacoustic, optoacoustic) imaging is a hybrid non-invasive biomedical imaging modality that combines the contrast and sensitivity of optical imaging with the resolution and depth of ultrasound imaging [1-4]. It can obtain multiple-scale structural, functional and molecular images of biological structures from organelles to organs *in vivo*. Currently, photoacoustic microscopy (PAM) is considered probably the fastest growing branch in photoacoustic field, which can be further classified into two major forms: acoustic-resolution PAM (AR-PAM) and optical-resolution PAM (OR-PAM) [5-8]. In OR-PAM, the laser beam is focused by microscope objective to a diffraction-limited spot for excitation in the tissue. In comparison with AR-PAM, it can provide a higher lateral resolution at subcellular or cellular scale with a lower pulse optical energy, ranging up to hundreds of micrometers in depth. As of yet, most OR-PAM systems use bulky and expensive solid-state lasers for photoacoustic excitation, which make the systems large, costly, and impracticable. Another alternative is semiconductor laser [9-19]. In this paper, a practical visible (VIS) OR-PAM prototype with a compact and inexpensive pulsed laser diode excitation has been developed and tested.

2. MATERIALS AND METHODS

Figure 1 shows the diagram of the VIS laser-diode-based OR-PAM (LD-OR-PAM) system, whose details were described in our previous work [20]. A pulsed laser diode at 405 nm (SLD3237VFR, Sony) was used as the light source, which was mounted in a 3.8 mm diameter package. The laser diode provides an optical peak power of 300 mW, allowing a pulse width of 174 ns at a pulse repetition rate of 1 KHz. The inset of Fig. 1 gives the photograph of the total laser diode excitation assembled with a cooling block, which has a compact size of 4.5 × 1.8 × 1.8 cm^3. The custom-built driving circuit of the laser diode excitation also has a compact size of 7 × 4 cm^2. The collimated laser has a beam diameter of ~4 mm and a full-angle divergence of 2.0 mrad. After optically collimating, an aspheric objective lens (C671TME-405, Thorlabs) was employed to focus onto the sample with a numerical aperture of 0.60. An ultrasonic transducer (V382-SU, Olympus) was used as a forward-mode sensor with a center frequency of 3.6 MHz and a -6 dB bandwidth of 61.8%. Once the photoacoustic signal was detected by the transducer, the signal was first pre-amplified by a low-noise amplifier (ZFL-500LN, Mini-Circuits) and amplified by a pulser/receiver (5073PR, Panametrics). Then the

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voltage signal was filtered by a low-passing filter (BLP-7-75, Mini-Circuits) to optimizing the SNR, and finally digitized by a 12-bit A/D card (ATS-9350, AlazarTech) for a series of data acquisitions. A three-dimensional motorized stage (CONEX-MFACC, Newport) was used to scan the total laser diode excitation to obtain the raster images along the horizontal *x*-*y* plane. In the experiments, the ultrasound velocity is assumed to be 1.5 μm/ns for imaging reconstruction. The sample was placed on a plastic tube, which was filled with ultrasound gel to couple the sound.

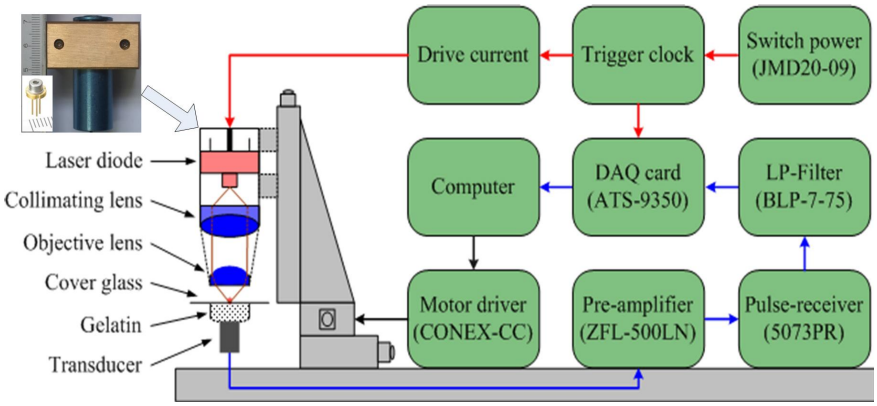
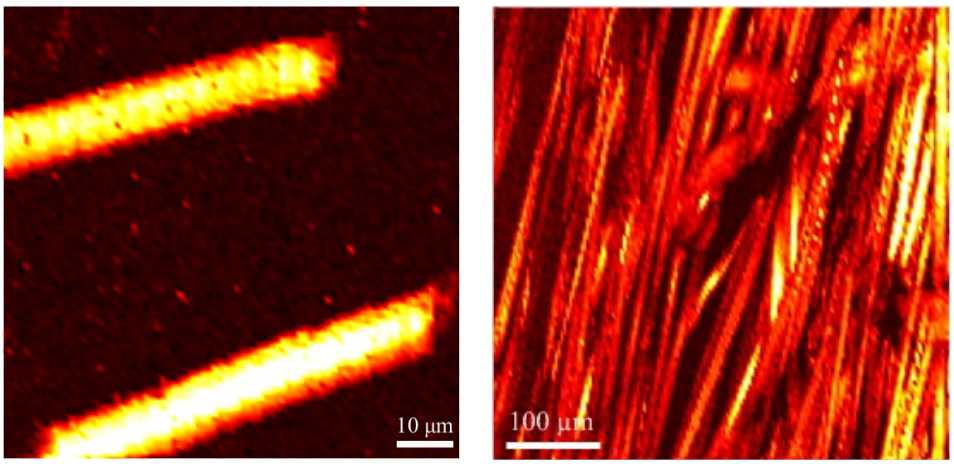


Figure 1. Schematic diagram of the VIS LD-OR-APM system; inset: the photo of the laser diode excitation.

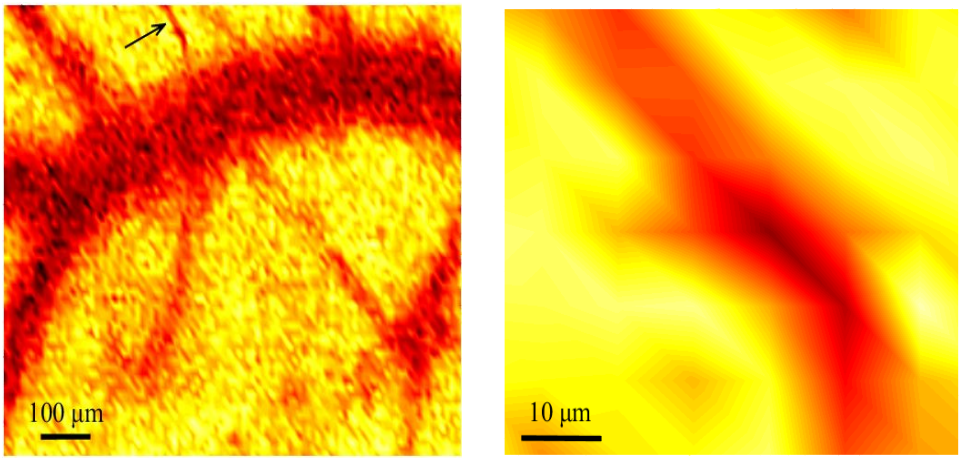
3. EXPERIMENTAL RESULTS

The performance of the VIS LD-OR-PAM system was demonstrated by imaging carbon fibers (diameter, ~7 μm) as small as capillary sized blood vessels. Figure 2 shows the maximum amplitude projection (MAP) images of carbon fibers with different field-of-view (FOV). An imaging FOV of 80 × 80 μm^2 was scanned in Fig. 2(a) with a step size of 1 μm. The relative position of the two carbon fibers was clearly displayed, and the lateral resolution was estimated to be ~0.95 μm [20]. Figure 2(b) gives a MAP image of a milled carbon fiber network with a step size of 5 μm, and a signal averaging of 16 times was implemented to improve the detectable signal-to-noise ratio. In order to further validate the label-free imaging feasibility of the system, we imaged a subcutaneous microvasculature on a mouse back *ex vivo*. The MAP image of the microvasculature is shown in Figure 3. An imaging FOV of 990 × 330 μm^2 was scanned in Fig. 3(a) with a step size of 5 μm and a signal averaging of 512 times. The branches of the major blood vessel (diameter, ~100 μm) are clearly resolved, and some blood vessels are degraded due to the blood coagulation. Figure 3(b) gives an enlarged MAP image (FOV, 60 × 30 μm^2) of a blood vessel marked in Fig. 3(a) with a black arrow. It has a diameter of ~15 μm and hence is most probably single capillary. Once the VIS LD-OR-PAM system is improved to operate in reflection mode, it can potentially be applied to more anatomical sites *in vivo*. Therefore, the VIS LD-OR-PAM is able to image single capillaries with endogenous contrast owing to the optical absorption of hemoglobin.



(a) (b)

Figure 2. MAP images of carbon fibers with different field-of-view.



(a) (b)

Figure 3. *Ex vivo* MAP image of subcutaneous microvasculature.

4. CONCLUSION

In summary, we have successfully developed a VIS LD-OR-PAM system for biomedical imaging. The lateral resolution was estimated to be ~0.95 μm by imaging carbon fibers. The subcutaneous microvasculature on a mouse back was *ex vivo* imaged without exogenous contrast agent. The compact and inexpensive properties would accelerate the research and development of clinical LD-OR-PAM production.

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