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 chromophores 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 beam 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
driver (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
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.

![Image of a schematic diagram of the VIS LD-OR-APM system; inset: the photo of the laser diode excitation.](http://www.example.com/image)

### 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 µm² 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 µm² 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 µm²) 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.

![Figure 2. MAP images of carbon fibers with different field-of-view.](http://www.example.com/image)
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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