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Dynamic in vivo imaging of small animal brain using pulsed laser diode-based photoacoustic tomography system

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Abstract. We demonstrate dynamic in vivo imaging using a low-cost portable pulsed laser diode (PLD)-based photoacoustic tomography system. The system takes advantage of an 803-nm PLD having high-repetition rate ~7000 Hz combined with a fast-scanning single-element ultrasound transducer leading to a 5 s cross-sectional imaging. Cortical vasculature is imaged in scan time of 5 s with high signal-to-noise ratio ~48. To examine the ability for dynamic imaging, we monitored the fast uptake and clearance process of indocyanine green in the rat brain. The system will find applications to study neurofunctional activities, characterization of pharmacokinetic, and biodistribution profiles in the development process of drugs or imaging agents. © 2017 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.22.9.090501]

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1 Introduction

Small animals are important models for preclinical studies and play a key role in guiding the study of human diseases and in seeking effective treatment. The ideal small animal imaging technique should provide noninvasive, high spatiotemporal resolution, deep penetration, anatomical and functional contrasts. The ability to directly visualize dynamics in small animal models at the full-body scale provides insights into biological processes at the whole-organism level. Optical imaging techniques suffer from either shallow penetration or a poor depth-to-resolution ratio; on the other hand, nonoptical imaging techniques for full-body imaging of small animals lack either spatiotemporal resolution or functional contrast.

Photoacoustic tomography (PAT) is an emerging noninvasive, nonionizing, hybrid imaging modality that has found many demanding applications in both clinical and preclinical studies. PAT combines the advantages of both optical and ultrasound imaging modalities: high optical contrast and scalable ultrasound resolution and imaging depth. In PAT, short laser pulses (nanosecond) are absorbed by tissue chromophores and produce an acoustic wave [also known as photoacoustic (PA) wave]. Images are formed by reconstructing the PA signals acquired at various positions around the tissue. Over the past couple of decades, PAT has been successfully used for animal and human imaging. PAT has shown its potential for imaging various organs (heart, lungs, liver, eyes, spleen, brain, skin, spinal cord, kidney, etc.) in small animals. Multiple-wavelength PAT allows mapping of HbT—total hemoglobin concentration and SO2—oxygen saturation. PAT can provide information at molecular and genetic levels for better understanding of brain health. Photoacoustic microscopy (PAM) provides higher resolution imaging with sacrificed imaging depth and may also require scalp removal (minimally invasive). Whereas, PAT can provide few cm imaging depth, and it is a complete noninvasive technique. Therefore, PAT systems were widely used for whole field brain imaging.

A Q-switched Nd:YAG-pumped optical parametric oscillator is the most commonly used excitation sources for PAT, as they provide sufficient pulse energies (tens-milliJoules) with ~5 ns pulse widths at ~10 to 100 Hz repetition rate. If single ultrasound transducer (UST) scanning is used, the temporal resolution of a Nd:YAG-based PAT system is limited by the low repetition rate of the laser and scanning/rotation speed of the UST. Typically, few minutes or longer are normally required to acquire one cross-sectional in vivo PAT image. A PAT system with such lengthy measurement time is not ideal for small animal imaging because it is difficult to control the physiological parameters for whole body imaging, time-resolved functional imaging, etc. Small animal neurofunctional imaging, oxygen saturation imaging, blood velocity imaging, etc. do require dynamic imaging capability. Monitoring the pharmacokinetic, circulation time of drugs/contrast agents is another important area where dynamic imaging is required. If only one single-element UST is used to collect all the PA signals around the sample, it will limit the imaging speed of the system. Nd:YAG/PAT combined with multiple single-element USTs or array-bases USTs could achieve high-speed imaging. A multi-transducer PAT was demonstrated based on the electrical slip ring. The lateral and axial resolution of the system was ~0.129 mm and ~1.49 mm, respectively. The scanning speed was 1.5 min/frame. In this system, electromagnetic shielding was a challenge for transmission of weak PA signals. A linear array-based PAT was demonstrated for full-view imaging. The imaging resolution was ~60 μm, and the imaging speed was limited by the low repetition rate (20 Hz) of the laser and the slow manual rotation scan. A linear array-based PAT system was demonstrated for whole mouse brain imaging. Imaging resolution of the system was ~70 μm and temporal resolution was 1 s. Several array-based USTs such as linear, semicircular, circular, and volumetric array are successfully used for real-time imaging. These array-based USTs will increase the imaging speed, but they are expensive, require custom made electronics, and will reduce the measurement sensitivity. Moreover, the imaging speed of a PAT system that uses array-based USTs is still limited by the repetition rate of the excitation laser.

Recently, pulsed laser diodes (PLDs) and light emitting diodes have been reported as compact and less expensive alternatives for Nd:YAG lasers. High-frame rate (7000 fps) B-scan PA imaging was shown with PLDs using clinical ultrasound platform. PLD with high-repetition rate can improve...
In vivo imaging of blood vessels at ~1-mm-depth below the human skin was demonstrated using low-energy PLDs. A PLD-based optical resolution photoacoustic microscopy (ORPAM) was reported. Using PLDs, ~1.5-cm-deep imaging at a frame rate of 0.43 Hz was demonstrated. A low-cost photoacoustic computer tomography (LC-PACT) system was demonstrated for ex vivo imaging. The system uses 6 W 905-nm PLD, emitting 55 ns pulses at 20 kHz with 0.3 mJ pulse energy. The image of black tape acquired using LC-PACT in 3 s showed 5.4 dB signal-to-noise ratio (SNR). We reported a low-cost portable photoacoustic tomography (PLD-PAT) system for high-speed imaging. The system provided hair imaging at a rate of 0.43 Hz. The PLD was operated at 7000 Hz. In 5-s scan time, total 35000 (i.e., 5 × 7000) pulses were delivered to the sample, so per pulse the MPE is 0.074 mJ/cm². The pulse energy was ~1.4 mJ at laser window when the laser was operated at maximum power 10 W and repetition rate 7000 Hz. For in vivo imaging, pulse energy achieved on the tissue surface was ~0.86 mJ distributed over 12-cm² area. So, the energy density is ~0.071 mJ/cm² (0.86 mJ/12 cm²), which was within the ANSI limit 0.074 mJ/cm² for 5 s scan time.

For in vivo animal experiments, NIH: Sprague Dawley SD® female healthy rats of body weight ~95 ± 3 gm, procured from InVivos Pte. Ltd., Singapore, were used. Experiments were performed in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee of Nanyang Technological University, Singapore (ARF-SBS/NIE-A0263). The rat was anesthetized with a mixture of 2 ml of Ketamine (100 mg/ml), 2 ml of xylazine (20 mg/ml), and 1 ml of saline. Before going for imaging, the hair on the scalp was depilated. The animal was mounted in the system as shown in Fig. 1. A custom-designed animal holder was used to mount the animal. The animal was placed in sitting position, on its abdomen, and the body of the animal was secured to the mount with surgical tapes to provide grip for the animal. The mouth and nose of the animal were covered with a breathing mask to deliver anesthesia mixture during the experiment. The anesthesia was achieved by the continuous inhalation of a mixture of 1.0 L/min oxygen and 0.75% isoflurane. The animal brain was aligned to the center of the CS geometry. After completing the DAQ for imaging, the animal was euthanized by an intraperitoneal injection of pentobarbital of concentration 300 mg/ml.

We noninvasively imaged the brains of healthy rats using the high-speed in vivo PLD-PAT system. The rat was placed at the center of the laser illumination area and CS area. The A-lines signals from the rat brain area were acquired by continuously scanning the UST at predefined speed. Multiple PA signals were collected by the UST, the collected signals were preamplified with gain of 50 dB, and finally the amplified A-lines were saved once the rotation is complete. PA signals can be averaged later if needed. We acquired the PA signals from brain area in different scan times. The photograph of the brain taken before and after opening the scalp is shown in Figs. 2(a) and 2(b), respectively. An open scalp anatomical photograph of the cortex vasculature was taken after removing the scalp for comparison. PAT imaging was done noninvasively, i.e., with skin and skull intact. The reconstructed PAT cross-sectional images of the rat brain are shown in Fig. 1. The PLD (Quantel, France) provides 55 ns pulses at 20 kHz with 0.3 mJ pulse energy. The image of black tape acquired using LC-PACT in 3 s showed 5.4 dB signal-to-noise ratio (SNR). We reported a low-cost portable photoacoustic tomography (PLD-PAT) system for high-speed imaging.
Fig. 2 Noninvasive images of brain vasculature in 95 gm female rat at different scan times: photograph of rat brain before (a) and after (b) removing the scalp. In vivo brain images at (c) 5-s, (d) 10-s, (e) 20-s, and (f) 30-s scan time. (g) SNR of in vivo images as a function of scan time. Here, SS, sagittal sinus; TS, transverse sinus; and CV, cerebral veins.

In vivo imaging was demonstrated by monitoring a fast uptake and clearance process of ICG in the cortex vasculature. The in vivo brain images were acquired in scan time as short as 5 s with high SNR. The increase in the PA values indicates the increase in the dye concentration within the cortex vessels. To generate the plot in Fig. 3(g), images were not acquired continuously. One PAT image in every ~30 s or so was acquired, that means the laser was ON for 5 s and OFF for the rest 25 s.

In this letter, we demonstrated an affordable and portable PLD-PAT system for high-speed and high-quality in vivo imaging on small animals. The potentiality of the system for dynamic in vivo imaging was demonstrated by monitoring a fast uptake and clearance process of ICG in the cortex vasculature. The in vivo brain images were acquired in scan time as short as 5 s with high SNR ~48 using a single-element UST scanner. The temporal resolution can be improved using multiple UST and instead of rotating a full circle of 360 deg, one can rotate only 360/N deg (where, N is the number of USTs used). For example, if eight USTs are used, then imaging can be done at one frame per ~0.5 s. The spatial resolution of the current PLD-PAT system is ~384 μm (for 2.25 MHz transducer) and ~185 μm (for 5 MHz transducers). The spatial resolution can be further improved using UST with higher central frequency and bandwidth. Although ring PA systems can provide real-time PA imaging, they are custom made and very expensive. The portability, low-cost, image quality promises that the proposed system will find applications in neurofunctional activities (such as epilepsy), characterization of pharmacokinetic, biodistribution profiles in the development process of drugs or imaging agents in small animal study.

Disclosures
Authors have no relevant financial interests in this work and no other potential conflicts of interest to disclose.

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References

1. L. Li et al., “Single-impulse panoramic photoacoustic computed tomography of small-animal whole-body dynamics at high spatiotemporal resolution,” *Nat. Biomed. Eng.*, 1, 0071 (2017).
2. P. K. Upputuri et al., “Recent developments in vascular imaging techniques in tissue engineering and regenerative medicine,” *BioMed Res. Int.* 2015, 1–9 (2015).
3. J. Xia and L. V. Wang, “Small-animal whole-body photoacoustic tomography: a review,” *IEEE Trans. Biomed. Eng.* 61(5), 1380–1389 (2014).
4. P. K. Upputuri and M. Pramanik, “Recent advances toward preclinical and clinical translation of photoacoustic tomography: a review,” *J. Biomed. Opt.* 22(4), 041006 (2017).
5. L. V. Wang and S. Hu, “Photoacoustic tomography: in vivo imaging from organelles to organs,” *Science* 335(6075), 1458–1462 (2012).
6. Z. Deng, W. Li, and C. Li, “Slip-ring-based multi-transducer photoacoustic tomography system,” *Opt. Lett.* 41(12), 2859–2862 (2016).
7. J. J. Yao et al., “Noninvasive photoacoustic computed tomography of mouse brain metabolism in vivo,” *NeuroImage* 64(1), 257–266 (2013).
8. J. Yao et al., “High-speed label-free functional photoacoustic microscopy of mouse brain in action,” *Nat. Methods* 12(5), 407–410 (2015).
9. J. Yao, J. Xia, and L. V. Wang, “Multiscale functional and molecular photoacoustic tomography,” *Ultras. Imaging* 38(1), 44–62 (2016).
10. I. Olefir et al., “Hybrid multispectral optoacoustic and ultrasound tomography for morphological and physiological brain imaging,” *J. Biomed. Opt.* 21(8), 086005 (2016).
11. P. K. Upputuri and M. Pramanik, “Performance characterization of low-cost, high-speed, portable pulsed laser diode photoacoustic tomography (PLD-PAT) system,” *Biomed. Opt. Express* 6(10), 4118–4129 (2015).
12. C. Xie et al., “Self-quenched semiconducting polymer nanoparticles for amplified in vivo photoacoustic imaging,” *Biomaterials* 119, 1–8 (2017).
13. Y. Jiang et al., “Broadband absorbing semiconducting polymer nanoparticles for photoacoustic imaging in second near-infrared window,” *Nano Lett.* 17(8), 4964–4969 (2017).
14. A. Taruttis et al., “Fast multispectral optoacoustic tomography (MSOT) for dynamic imaging of pharmacokinetics and biodistribution in multiple organs,” *PLoS One* 7(1), e30491 (2012).
15. G. Li et al., “Multiview Hilbert transformation for full-view photoacoustic computed tomography using a linear array,” *J. Biomed. Opt.* 20(6), 066010 (2015).
16. P. Zhang et al., “High-resolution deep functional imaging of the whole mouse brain by photoacoustic computed tomography in vivo,” *J. Biophotonics* 1–6 (2017).
17. X. Dai, H. Yang, and H. Jiang, “In vivo photoacoustic imaging of vasculature with a low-cost miniature light emitting diode excitation,” *Opt. Lett.* 42(7), 1456–1459 (2017).
18. J. T. Allen and C. P. Beard, “High power visible light emitting diodes as pulsed excitation sources for biomedical photoacoustics,” *Biomed. Opt. Express* 7(3), 1260–1270 (2016).
19. K. Sivasubramanian and M. Pramanik, “High frame rate photoacoustic imaging at 7000 frames per second using clinical ultrasound system,” *Biomed. Opt. Express* 7(2), 312–323 (2016).
20. J. S. Allen and P. Beard, “Pulsed near-infrared laser diode excitation system for biomedical photoacoustic imaging,” *Opt. Lett.* 31(23), 3462–3464 (2006).
21. R. G. M. Koelkman, W. Steenbergen, and T. G. van Leeuwen, “In vivo photoacoustic imaging of blood vessels with a pulsed laser diode,” *Lasers Med. Sci.* 21(3), 134–139 (2006).
22. K. Daoudi et al., “Handheld probe integrating laser diode and ultrasound transducer array for ultrasound/photoacoustic dual modality imaging,” *Opt. Express* 22(21), 26365–26374 (2014).
23. A. Hariri et al., “Development of low-cost photoacoustic imaging systems using very low-energy pulsed laser diodes,” *J. Biomed. Opt.* 22(7), 075001 (2017).
24. P. K. Upputuri and M. Pramanik, “Pulsed laser diode based photoacoustic imaging of biological tissues,” *Biomed. Phys. Eng. Express* 4(4), 045010 (2015).
25. American National Standard for Safe Use of Lasers, ANSI Standard Z136.1-2000, Laser Institute of America, New York (2000).
26. S. K. Kalva and M. Pramanik, “Experimental validation of tangential resolution improvement in photoacoustic tomography using a modified delay-and-sum reconstruction algorithm,” *J. Biomed. Opt.* 21(8), 086011 (2016).
27. M. Pramanik, “Improving tangential resolution with a modified delay-and-sum reconstruction algorithm in photoacoustic and thermoacoustic tomography,” *J. Opt. Soc. Am. A* 31(3), 621–627 (2014).
28. N. C. Burton et al., “Multispectral opto-acoustic tomography (MSOT) of the brain and glioblastoma characterization,” *NeuroImage* 65(2), 522–528 (2013).
29. C. Li et al., “Real-time photoacoustic tomography of cortical hemodynamics in small animals,” *J. Biomed. Opt.* 15(1), 010509 (2010).