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Pulsed laser diode photoacoustic tomography (PLD-PAT) system for fast *in vivo* imaging of small animal brain

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ABSTRACT

In recent years, high-repetition rate pulsed laser diode (PLD) was used as an alternative to the Nd:YAG lasers for photoacoustic tomography (PAT). The use of PLD makes the overall PAT system, a low-cost, portable, and high frame rate imaging tool for preclinical applications. In this work, we will present a portable *in vivo* pulsed laser diode based photoacoustic tomography (PLD-PAT) system. The PLD is integrated inside a circular scanning geometry. The PLD can provide near-infrared (~803 nm) pulses with pulse duration ~136 ns, and pulse energy ~1.4 mJ / pulse at 7 kHz repetition rate. The system will be demonstrated for *in vivo* fast imaging of small animal brain. To enhance the contrast of brain imaging, experiments will be carried out using contrast agents which have strong absorption around laser excitation wavelength. This low-cost, portable small animal brain imaging system could be very useful for brain tumor imaging and therapy.

Keyword: Pulsed laser diode, Photoacoustic tomography, Brain imaging, Small animal imaging

1. INTRODUCTION

Monitoring brain functional activity is important for behavioral and pathological studies.^{1,2} For such studies, imaging system with high spatial resolution, temporal resolution, and deep imaging facility is required. Non-optical methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), diffuse optical tomography (DOT) etc., are used for deep brain imaging. Here fMRI, PET, and DOT have low spatial resolution (~millimeters), and fMRI and PET have low temporal resolution (~seconds). Multi-photon microscopy (MPM)³ can provide high spatial (~micrometers) and high temporal (~milliseconds) resolution, but its imaging depth is limited to ~1 mm. Therefore, an imaging technique with high spatial and temporal resolution that can image brain activity is necessary. Photoacoustic tomography (PAT) is widely used in biomedical applications. In PAT, nanosecond laser pulses irradiate the tissue sample.⁴ Due to absorption of light energy by the chromophores (such as melanin, red blood cells, etc.), there is a local temperature rise, which in turn produces pressure waves emitted in the form of sound waves. An ultrasound transducer receives the photoacoustic (also known as optoacoustic) signal outside the sample boundary. PA/OA signals are recorded at different positions around the sample. All the collected A-lines are then used to reconstruct the initial pressure rise within the tissue (brain, tumor, etc.).⁵⁻⁷ It can provide multi-contrast and multi-scalable imaging. Using exogenous contrast agents, imaging depth ~12 cm is achieved in biological tissues, which is much larger than that provided by pure optical methods such as white light microscopy, two-photon microscopy, optical coherence tomography etc.⁸⁻¹⁸ Multiple-wavelength PAT can provide functional changes along with structural information.

Several small animal models have been established for various human diseases. To study the diseases in small animals, few small animal *in vivo* imaging modalities have developed. Photoacoustic tomography has gained great attention due to its merits aforementioned. It has been successfully applied to small animal brain imaging, structural, functional, and molecular imaging.^{6, 19-24} Since PA signals are generated due to optical absorption, chromophores with different absorption characteristics can be spectrally separated. This enables PAT to provide label-free functional brain images of oxygen saturation (SO₂) and total hemoglobin concentration (HbT) based on the absorption spectra of oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb).^{25, 26} Nanoparticles (metallic, inorganic, organic, semiconducting,

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etc.) are used to enhance the contrast of the brain vasculature/neurovascular structures.^{18, 27-30} Brain information at genetic and molecular levels can be obtained using PAT, which will help in understanding tumor biology and brain diseases.^{31, 32}

PAT systems with Nd:YAG/OPO or dye-based laser as excitation source^{33, 34} and single-element ultrasound transducer (UST) as a detector have been widely used for small animal *in vivo* imaging. Such PAT systems can provide multiple wavelength NIR pulses with high energy. The limitations with such systems are: expensive, bulky, and cannot achieve high-speed imaging with single-detector due to the low-repetition rate of the laser. Typically, the data acquisition duration in such systems is ~5-10 min per cross-section. The lengthy measurement time creates great challenges for the control of small animal physiological parameters for time-resolved functional imaging or whole-body imaging. Therefore, high-speed or real-time PAT imaging is necessary, which may be realized either by using array transducers or high-repetition rate lasers. Laser technology has advanced to generate high repetition rate lasers such as pulsed laser diode (PLD). In recent years, PA imaging was successfully demonstrated using PLD. A fiber based illumination with cylindrical scanning geometry was reported using pulsed NIR PLD based PAT.³⁵ *In vivo* imaging of superficial human blood vessels at a depth of ~1 mm below the skin was demonstrated.³⁶ PLD based optical resolution-photoacoustic microscopy was reported.³⁷ Imaging depth ~1.5 cm was shown in phantoms at a frame rate of 0.43 Hz.³⁸ Very recently, portable, a low-cost, high-speed PLD-PAT system was reported. It can provide high-speed (~3 sec) imaging and relatively deeper imaging (~2 cm).^{33, 39, 40} This PLD-PAT system can provide high-speed imaging even with single-element UST circular scanning. The main purpose of this paper is to demonstrate the PLD-PAT system for small animal *in vivo* brain imaging. To enhance the contrast of the brain vasculature exogenous contrast agent, indocyanine green (ICG), was used. *In vivo* brain images obtained in as short as 5 sec scan time are presented.

2. PLD-PAT SYSTEM

The *in vivo* PLD-PAT system shown in Fig.1 (described in detail in Ref⁴⁹) was used for rat brain imaging. A pulsed laser diode (Quantel, France) was integrated inside a single-element UST circular scanner. PLD generated ~136 ns pulses at a NIR wavelength of ~803 nm, and pulse energy of ~1.42 mJ at 7000 Hz repetition rate. The PLD was mounted at the center of the circular scanner. Output of PLD was a rectangular beam with stripes. An optical diffuser was used to make the laser beam more homogenous in front of the laser window. A focused ultrasound transducer (V306-SU-NK-CF1.9IN Olympus NDT) 2.25 MHz central frequency and focal length 1.9 inch was used to detect PA signal from the animal brain. The UST was driven by a step motor, M, (Silverpak 23C) from Lin Engineering. A custom-made circular scanner was used to rotate the UST around the sample. The UST received signals were subsequently band pass filtered, and amplified by ultrasound pulser/receiver unit (Olympus-NDT, 5072PR), and then digitized and acquired by a data acquisition (DAQ) card (compuscope 4227) from GaGe. The DAQ card was operated at 25 MS/s sampling frequency. The computer has a LabVIEW program to control the motor and collect the A-lines, and MATLAB to load the A-lines and reconstruct the brain image using delay-and-sum algorithm.^{5, 41}

3. ANIMAL PREPARATION FOR BRAIN IMAGING

The PLD-PAT system described above was used for imaging rat brain. The system can provide ~1 mJ pulse energy in ~12.6 cm² area. Therefore, the laser fluence on the brain was 0.08 mJ/cm², it is quite close to the American National Standard Institute (ANSI) safety limit 0.07 mJ/cm² for 5 sec scan.³³ Female healthy rat (NTac: Sprague Dawley, SD, ~90 gm) from InVivos Pte. Ltd., Singapore was studied in this experiment. All animal experiments were performed in accordance with the guidelines and regulations, approved by the institutional Animal Care and Use committee of Nanyang Technological University, Singapore (Animal Protocol Number ARF-SBS/NIE-A0263). To prepare the animal (rat/mouse) for brain imaging following steps need to be followed: (1) Prepare anesthetic cocktail containing a mixture of 2 ml of Ketamine, 2 ml of xylazine and 1 ml of saline, (2) weigh the rat to be imaged using weighing machine, (3) Carefully inject 0.2 ml per 100 g of body weight intraperitoneally, (4) remove the hair on scalp of the rat using hair clipper, (5) apply hair removal cream on the scalp and carefully remove it after 3-5 minutes of application, (6) apply artificial tear ointment on the eyes to prevent dryness, (7) place the animal on animal holder in sitting position and secure the body with surgical tapes, (8) cover the mouth and nose of the rat with breathing mask to deliver anesthesia mixture, and (9) apply colourless ultrasound gel on the scalp of the brain.

Fig. 1. Schematic of the PLD-PAT system: PLD - pulsed laser diode, M – stepper motor, A-Amplifier, UST - Ultrasound transducer, DAQ-Data acquisition card.

4. *IN VIVO* RAT BRAIN IMAGING

The rat was prepared for brain imaging as discussed in Section-3. After applying the ultrasound gel on the scalp of the rat, flow the chart below to perform the brain imaging. In the imaging system, PLD delivers pulses having energy ~ 1.05 mJ per pulse and the laser beam expanded in ~ 12.6 cm² area. Hence the laser energy density is ~ 0.08 mJ/cm² on the brain area. We have prepared ICG (Sigma-Aldrich, 12633-25mg) solution with 323 μ M concentration to absorb 803 nm wavelength.¹⁸ The ICG solution (~ 0.5 ml) was injected into the circulatory system of the rat through the tail vein injection. To perform the *in vivo* animal (rat/mouse) for brain imaging with exogenous contrast agent following steps need to be followed: (1) After applying the gel, place the animal brain at the center of the laser beam and scan area, (2) clamp a pulse oximeter to rat's leg to monitor its physiological condition during imaging, (3) using lab-jack, the image plane of the brain was adjusted to the center of the UST, (4) set the scanning parameters in the LabVIEW program and by monitoring the PA signal in real-time accurately adjust the imaging plane of the brain, (5) rotate the UST around the brain and collect all the A-lines from the brain area, (6) export the stored A-line data to MATLAB to reconstruct the brain image i.e. pre-injection image, (7) carefully perform the tail vein injection to inject the contrast agent (e.g., ICG) in to the blood flow, (8) rotate the UST around the brain, collect the A-lines, and reconstruct the image i.e., post-injection image, (9) after completing the data acquisition, turn the laser off, unplug the pulse oximeter, and lower down the animal using the lab-jack, (10) turned the anesthesia system off, and took the breathing mask off, (11) unload the animal from the animal holder and clean the animal scalp with cotton wipes to remove the gel, (12) euthanize the animal by injecting 0.2 ml of Valbarb (pentobarbital sodium 120 mg/ml) intraperitoneally, and (13) make sure the animal is dead and dispose the animal in the animal facility center following the animal protocols.

5. RESULTS AND DISCUSSION

A female healthy rat (NTac: Sprague Dawley) from InVivos Pte. Ltd., Singapore was studied in this experiment. The animal was prepared by following the procedure discussed in Section-3 and *in vivo* imaging of brain [photograph shown in Fig. 3(a)] was done by following procedure discussed in Section-4. First we acquired A-lines by rotating the focused 2.25 MHz UST around the brain area for 5 sec. Total 35000 (= 5x7000) A-lines were acquired from the brain area, then these A-lines were averaged over 70 to reduce the A-lines to 500. The averaging will reduce the computational load while reconstructing the image. The 500 averaged A-lines were then used to reconstruct the PA image of the brain before

injecting the contrast agent. This is the pre-injection image of the rat brain vasculature shown in Fig. 3(b). A 0.0013 gm of Indocyanine green dye powder (12633-25MG, Sigma-Aldrich) was mixed in 5 ml of distilled water to prepare 323 μM concentration ICG solution. The ICG solution with 323 μM concentration shows strong absorption around 800 nm excitation wavelength.¹⁸ About 1 ml of ICG solution was injected in to the blood circulation system through the tail vein. Then similarly A-lines were acquired for 5 sec by rotating the UST around the brain area. The image obtained after 5 minutes of the injection is shown in Fig. 3(c). It is the post-injection image which shows the same brain vasculature with enhanced PA signal. The average PA signal in the blood vessel location in the post-injection image is about 66% higher than that in the pre-injection image. The enhancement in the PA signal is due to the ICG concentration in the brain blood veins. The PA signal enhancement depends mainly on the concentration of the contrast agent and the amount injected in to the blood stream. In the post-injection image, the superior sagittal sinus, transverse sinuses, and small vein branches are visible, whereas the small veins are not visible in the pre-injection image. These results suggest that PLD-PAT with good imaging quality and high imaging speed capabilities, it could become a potential tool for preclinical *in vivo* small animal imaging applications.

Fig. 2. *In vivo* PLD-PAT imaging of rat brain in lateral plane using a focused 2.25 MHz UST: (a) photograph of the rat brain, (b) pre-injection brain image, and (c) post-injection brain image. Image (c) was acquired 5 min after the injection of ICG solution through the tail vein of the rat.

6. CONCLUSIONS

In this paper, a high-performance portable PLD-PAT system was demonstrated for fast *in vivo* imaging. The potentiality of the system was demonstrated on rat brain. Good-quality images were obtained from the proposed system at high-scanning-speed. Brain images collected in as short as 5 sec were presented in this paper. The system was also demonstrated for contrast enhanced brain imaging using exogenous contrast agent. ICG solution with strong absorption around 800 nm wavelength was injected in to the rat tail vein. The post-injection images acquired at about 5 minutes showed 66% enhancement in the photoacoustic signal compared to the pre-injection image. Such good-quality imaging and 5 sec-imaging time promises that PLD-PAT system could be a useful tool for near-real time *in vivo* imaging applications in biomedical imaging areas.

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