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2016

Upputuri, P. K., & Pramanik, M. (2016). High-speed pre-clinical brain imaging using pulsed laser diode based photoacoustic tomography (PLD-PAT) system. Proceedings of SPIE 9708, Photons Plus Ultrasound: Imaging and Sensing 2016, 9708, 97084R-.

<https://hdl.handle.net/10356/83647>

<https://doi.org/10.1117/12.2211891>

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High-speed pre-clinical brain imaging using pulsed laser diode based photoacoustic tomography (PLD-PAT) system

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ABSTRACT

Photoacoustic tomography (PAT) is a promising biomedical imaging modality for small animal imaging, breast cancer imaging, monitoring of vascularisation, tumor angiogenesis, blood oxygenation, total haemoglobin concentration etc. The existing PAT systems that uses Q-switched Nd:YAG and OPO nanosecond lasers have limitations in clinical applications because they are expensive, non-portable and not suitable for real-time imaging due to their low pulse repetition rate. Low-energy pulsed near-infrared diode laser which are low-cost, compact, and light-weight (<200 grams), can be used as an alternate. In this work, we present a photoacoustic tomography system with a pulsed laser diode (PLD) that can nanosecond pulses with pulse energy 1.3 mJ/pulse at ~803 nm wavelength and 7000 Hz repetition rate. The PLD is integrated inside a single-detector circular scanning geometric system. To verify the high speed imaging capabilities of the PLD-PAT system, we performed *in vivo* experimental results on small animal brain imaging using this system. The proposed system is portable, low-cost and can provide real-time imaging.

Keyword: Photoacoustic tomography, Small animal imaging, real-time imaging, Pulsed laser diode.

1. INTRODUCTION

In photoacoustic tomography (PAT) a short (nanosecond) laser pulses irradiate the tissue. Due to absorption of incident energy by the tissue 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 acoustics waves. A wideband ultrasound transducer receives the photoacoustic (PA) signal outside the tissue boundary. PA waves are acquired at various positions around the tissue boundary. Reconstruction techniques are used to map the initial pressure rise within the tissue from the measured PA signals.^{1,2} PAT has emerged as a promising hybrid imaging tool for many biomedical applications. It can provide images with high ultrasound-resolution and high optical contrast. Its imaging depth is ~8 cm in biological tissues, which is much larger than that provided by other popular optical methods such as confocal microscopy, multi-photon CARS microscopy, optical coherence tomography etc.³⁻¹³ Besides structural information, functional changes can also be obtained with multiple-wavelength PAT. In recent years several small animal models have been established for many human diseases. Hence, several *in vivo* small animal imaging modalities have developed. Out of all the modalities, PAT has gained great attention due to its merits aforementioned. It has been successfully applied to small animal brain imaging, structural, functional, and molecular imaging.^{1, 14-18}

Several PAT modalities have been demonstrated for brain imaging. 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).^{19, 20} Contrast agents, such as organic dyes and nanoparticles, can also be used to improve the contrast of the neurovascular structures.^{7, 21} PAT can also provide brain information at genetic and molecular levels, which will help in understanding tumor biology and brain diseases.^{22, 23}

PAT systems that integrate Nd:YAG pumped OPO or dye-based laser source and single-element ultrasound transducer (UST) have been widely used for *in vivo* small animal imaging. Typical Nd:YAG laser source delivers 5-10 ns pulses with pulse energy tens of millijoules at repetition rate ~10-50 Hz. The drawbacks associated with these PAT systems are, they are expensive, bulky, and are not suitable for high-speed imaging with single-detector due to the low-repetition rate. Typically, the data acquisition duration in such is ~5-10 min per cross-section. The lengthy

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measurement time creates great challenges for the control of small animal physiological parameters for time-resolved functional imaging or whole-body imaging. Therefore, real-time or near real-time PAT imaging is necessary, which may be realized either by using array transducers or high-repetition rate lasers. To achieve high-speed imaging, several scanning or array geometries were demonstrated. In circular scanning geometry, PA signals are collected while the single-element ultrasound transducer (UST) rotates around the sample in full circle. Since one detector rotates around the sample the imaging speed is rather slow. Therefore, PAT systems based on linear, semi-circular, circular array of USTs have been used for high speed imaging. They do not require scanning and hence can improve imaging speed (still limited to maximum imaging speed of 10 frames per second as the laser is operating at that frequency). However, such array transducers are very expensive and not readily available.

Alternative way to improve the imaging speed is to use single ultrasound detector based PAT with high repetition rate lasers such as pulsed laser diode (PLD). The use of a single detector is inexpensive and can provide an image with good signal-to-noise ratio (SNR). In recent years, photoacoustic imaging was successfully demonstrated using PLD as an excitation source. A fiber based illumination with cylindrical scanning geometry was demonstrated on phantoms using pulsed near infrared laser diode based PAT.²⁴ *In vivo* PA imaging of superficial human blood vessels at a depth of ~1 mm below the skin was achieved.²⁵ Optical resolution-photoacoustic microscopy (OR-PAM) with PLD was reported.²⁶ Imaging depth of up to 15 mm was demonstrated in phantoms for a frame rate of 0.43 Hz.²⁷ Very recently, we reported a low-cost, high-speed PLD-PAT system which can provide high-speed (~3 sec) imaging and relatively deeper imaging (~2 cm).²⁸⁻³⁰ This PLD-PAT system is less expensive, portable, can provide high-speed and deeper imaging with good SNR (due to single-element detector) and hence suitable for translating into clinical use. The main purpose of this paper is to demonstrate that our PLD-PAT system is capable of providing good *in vivo* data on small animal brains for pre-clinical applications. So, testing and demonstrate its capability is the first step for more complex brain structural and functional detection in the future. The *in vivo* results suggest that our system can perform PAT of small animal brains. The experimental set-up and *in vivo* mouse brain images at different scan speeds are presented.

2. PLD-PAT SYSTEM

The schematic of our PLD-PAT system is shown in Fig. 1. The PLD (Quantel, France) provides ~136 ns pulses at a near-infrared wavelength of ~803 nm, and pulse energy of ~1.4 mJ at maximum 7 kHz repetition rate. The laser head is mounted in such a way that the laser window is always at the center of the circular scanning system. The PLD generates a rectangular beam which diverges with an angle of ~11.5, and ~0.65 degree along slow and fast axes, respectively. To make the laser beam more homogenous an engineered diffuser (ED) is used in front of the PLD window. The PLD is controlled by the laser driver unit (LDU) which consists of a temperature controller (LaridTech, MTTC1410), a 12 V power supply (Votcraft, PPS-11810), a variable power supply (to change the laser output power), and a function generator (to control the laser repetition rate). The pulse energy (0.2-1.4 mJ) and repetition rate (up to 7 kHz) can be controlled independently with variable power supply (BASETech, BT-153), and function generator (FG250D, Funktionsgenerator), respectively. The function generator provides a TTL (Transistor-Transistor Logic) signal to synchronize the data acquisition (DAQ) with the laser excitation.

The animal was mounted as shown in Fig. 1. The UST was immersed in water for coupling of PA signal to UST. Here, two non-focus transducers (V323-SU/2.25 MHz Olympus NDT) with 13 mm active area and ~70% nominal bandwidth were used. The UST is driven by a step motor (M) (Lin Engineering, Silverpak 23C) to scan circularly around the sample. The UST holder is mounted on a circular scanning plate (CSP) as shown in Fig. 1. The motion of the circular plate or UST is controlled by the computer-controlled stepper motor (M). A mechanical scanning system was built which was driven by the stepper motor. Appropriate gear box, pulley and belts were used to translate the stepper motor motion into the motion of the UST circularly around the sample. The UST detected signals are subsequently band pass filtered, and amplified by ultrasound signal receiver amplifier (A) unit (Olympus-NDT, 5072PR), and then digitized and recorded by the PC with DAQ card (GaGe, compuscope 4227) installed in it. Usually, low-frequency ultrasound detectors (1-5 MHz) are used in PAT, so the DAQ card was operated at a sampling frequency of 25 Ms/s.

Fig. 1. Schematic of *in vivo* PLD-PAT system, where PLD - pulsed laser diode, ED - Engineered diffuser, CSP - circular scanning plate, M - Motor, LDU - Laser driver unit, A - Amplifier, AM -Anesthetic Machine, UST - Ultrasound transducer, F - 100 μm thick polythene film, WT - Acrylic water tank for *in vivo* imaging, MPS - motor pulley system, DAQ - Data acquisition system.

3. PLD-PAT *IN VIVO* IMAGING OF MOUSE BRAIN

In our current *in vivo* animal experiments, 2 male and 2 female healthy mice of body weight 28 ± 3 gm and aged 6 weeks, procured from InVivos Pte. Ltd., Singapore, were used. All experiments were performed in accordance with the approved guidelines and regulations, and were approved by the institutional Animal Care and Use committee of Nanyang Technological University, Singapore (Animal Protocol Number ARF-SBS/NIE-A0263). For *in vivo* imaging the mouse was anesthetized. The anaesthetic cocktail contains Ketamine and Xylazine in the dosage of 120 mg/kg and 16 mg/kg respectively. 0.1 ml per 10 g of mouse body weight was injected intraperitoneally. Before imaging experiments, the hair on the head of the small animal was depilated using hair removal cream. The mouth and nose of the animal were covered with a breathing mask to deliver anaesthesia mixture. The anaesthesia was achieved by the inhalation of a mixture of O₂ and isoflurane. A custom-designed animal holder was used to mount the animal in the way shown in Fig. 1. 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 the animal. During experiments, the animal and the animal holder were mounted on a translation stage to align the brain to the center of the circular scanning geometry. After the data acquisition for PAT, the animal was euthanized by the intraperitoneal injection of pentobarbital of concentration 300 mg/ml.

We noninvasively imaged the brains of healthy mice using our PLD-PAT system shown in Fig. 1. The mouse, prepared as disused in section 3, was placed at the center of the circular scanning area and laser illumination area. The A-lines signals from the mouse brain were collected using a circular scanning single-element 2.25 MHz UST. In PAT system that uses single-element detector scanning, the A-line acquisition can be done by following two different ways: (a) stop-and-go scan, (b) continuous scan. In our experiments we have used continuous scanning to collect the A-lines. In continuous scan, the stepper motor rotates the UST continuously at predefined speed, collects multiple PA signals as transducer is moving, save the A-lines once the rotation is complete. Signal averaging can also be done later if needed. Continuous scan is faster than the stop-and-go data acquisition method.

For the fastest possible rotational speed available in the current setup, 3 seconds is required for a full 360 degree rotation. We will show the *in vivo* images acquired at different scan speeds using the PLD-PAT system. The PAT reconstructed images of mouse brain obtained by collecting PA signals in 30, 20, 10, 5 and 3 sec scan time using PLD-PAT are shown in Figs. 2(a-e). In all the images the superior sagittal sinus (SS) and transverse sinuses (TS) of the mouse brain are clearly visible. The resolution of our PLD-PAT system with 2.25 MHz is $\sim 380 \mu\text{m}$, hence the superficial/bridging veins whose diameter is $<200 \mu\text{m}$ are not clearly visible in the PAT images. To study the effect of scan speed on the quality of the *in vivo* images, we calculated the signal-to-noise ratio (SNR) of images acquired at different scan speeds. The SNR was defined as the peak-to-peak amplitude of the PA signal divided by the standard deviation of the noise, $\text{SNR} = V/n$, here V is the peak-to-peak PA signal amplitude, and n is the standard deviation of the background noise. The SNR as a function of scan time is plotted for the images [Figs. 2(a-e)] and is shown in Fig. 2(f).

Fig. 2. *In vivo* PAT imaging of mouse brain in lateral plane at different scanning speeds acquired using a 2.25 MHz UST. Scan speed: (a) 30 s, (b) 20 s, (c) 10 s, (d) 5 s, (e) 3 s. (f) SNR as a function of scan time. SS - Superior sagittal sinus, TS - Transverse Sinus.

4. LASER SAFETY FOR *IN VIVO* IMAGING

For PAT *in vivo* imaging, the maximum permissible pulse energy and the maximum permissible pulse repetition rate are governed by the ANSI laser safety standards.³¹ The safety limits for the skin depend on the optical wavelength, pulse duration, exposure duration, and exposure aperture. In the spectral region of 700-1050 nm, the maximum permissible exposure (MPE) on the skin surface by any single laser pulse should not exceed $20 \times 10^{2(\lambda-700)/1000} \text{ mJ/cm}^2$ (λ is the wavelength in nm). Therefore, at 803 nm the MPE is $\sim 31 \text{ mJ/cm}^2$. The MPE for exposure time $t = 3 \text{ sec}$ is $1.1 \times 10^{2(\lambda-700)/1000} \times t^{0.25} \text{ J/cm}^2$ ($= 2.3 \text{ J/cm}^2$). Since the PLD is operating at 7 kHz (total number of laser pulses = $3 \times 7000 = 21000$), the MPE becomes 0.11 mJ/cm^2 per pulse. In our imaging system, the pulsed diode laser provides $\sim 1.28 \text{ mJ}$ pulse energy and the laser beam spread over an area $\sim 7 \text{ cm}^2$. Therefore, the laser fluence is $\sim 0.18 \text{ mJ/cm}^2$. At present, the fluence is slightly above the MPE for 3 sec scanning time. For higher scanning time the fluence is within the ANSI safety limit. In future, we will optimize the system by tuning the system parameters such way that the fluence is within the ANSI standards. This can be achieved by spreading the beam over a larger area or reducing the pulse repetition rate or reducing the laser power output. For example, the MPE can be made 0.18 mJ/cm^2 by operating the PLD at $\sim 4000 \text{ Hz}$ and scan time still 3 sec. Another way is to make the scan faster by using multiple UST and instead of rotating a full circle of 360 degrees we can rotate only $360/N$ degrees (where, N is the number of USTs used). Thus reducing the total scan time will change the MPE limit of the system.

5. CONCLUSIONS

In this work, we demonstrated an affordable, and portable PLD-PAT system for high-speed *in vivo* imaging. The potentiality of the system for high-speed *in vivo* imaging was demonstrated on mouse brain. The *in vivo* brain images acquired at different scan speeds are presented. The A-line data collected in scan time of 3 s could provide the reconstructed 2D image of lateral view of the brain. Due to the low-energy pulses, the SNR of the *in vivo* images at high speed is low. In future we will optimize the parameters of the system for high-quality high speed *in vivo* imaging. We will use optical contrast agent to improve the image contrast and imaging depth in our PLD-PAT system. The imaging speed can further be improved by using multiple ultrasound transducers at the same time. The portability, low-cost, image quality promises that the proposed system will find near-real time *in vivo* imaging applications in biomedical imaging areas.

ACKNOWLEDGMENT

The authors would like to acknowledge the financial support from Tier 1 grant funded by the Ministry of Education in Singapore (RG31/14: M4011276).

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