Laser-scanning photoacoustic microscopy with ultrasonic phased array transducer

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Abstract: In this paper, we report our latest progress on proving the concept that ultrasonic phased array can improve the detection sensitivity and field of view (FOV) in laser-scanning photoacoustic microscopy (LS-PAM). A LS-PAM system with a one-dimensional (1D) ultrasonic phased array was built for the experiments. The 1D phased array transducer consists of 64 active elements with an overall active dimension of 3.2 mm × 2 mm. The system was tested on imaging phantom and mouse ear in vivo. Experiments showed a 15 dB increase of the signal-to-noise ratio (SNR) when beamforming was employed compared to the images acquired with each single element. The experimental results demonstrated that ultrasonic phased array can be a better candidate for LS-PAM in high sensitivity applications like ophthalmic imaging.

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1. Introduction

Photoacoustic microscopy (PAM) [1–5] is an emerging high resolution three-dimensional imaging technology based on optical-absorption contrast. The signal-to-noise ratio (SNR) of PAM depends on the laser pulse energy and sensitivity of the ultrasonic transducer. Due to laser safety considerations the laser pulse energy cannot exceed a certain level defined by laser safety standards when imaging biological tissues in vivo. As a result, the ultrasonic transducer plays a key role in determining the SNR of PAM and a high sensitivity ultrasonic transducer is desired [6,7].

Laser-scanning PAM (LS-PAM) is a branch of PAM [8–10] which can accomplish high speed in vivo imaging of biological tissues without scanning the ultrasonic transducer or the imaging subject. To achieve high speed imaging especially for ophthalmic applications, where scanning the ultrasonic transducer mechanically on the surface of the imaging subject is forbidden, LS-PAM uses a stationary ultrasonic transducer and only scans the illuminating laser light with an X-Y galvanometer scanner. To achieve a large field of view (FOV) while keeping the ultrasonic transducer stationary, LS-PAM usually uses unfocused ultrasonic transducer. In conventional PAM, a focused ultrasonic transducer is used in a confocal configuration with the illuminating laser light. Since with the same piezoelectric material focused ultrasonic transducer has better sensitivity, using the concept of ultrasonic focusing may increase the SNR for LS-PAM. It would be ideal to have a focused ultrasonic transducer whose focal point can be scanned together with the scanning laser focus without mechanically scan the transducer. One of the candidates for achieving this goal is an ultrasonic phased array.

An ultrasonic phased array is a transducer array consisting of multiple small transducer elements. These elements usually are rectangular in shape and can be arranged on a straight line in one dimension. The signals from each element of the array can be acquired independently. By applying the different time delays on the received signals from each channel and summing all the delayed signals (so-called delay-sum beamforming technique), the beam can be steered to and focused at different spatial positions. By performing beamforming the desired signals will be enhanced and the noise will be suppressed simultaneously, thereby the SNR will be improved. Ultrasonic array transducer has been successfully used in mechanical scanning PAM system with multifocal optical illumination to improve the imaging speed significantly [11].

In the paper, we report our first effort to build and test a LS-PAM system with an ultrasonic phased array transducer. The images of a resolution target and the vascular images of a mouse ear in vivo were acquired to demonstrate the feasibility of the system. The current study is a proof of concept for the idea to apply ultrasonic phased array technique in LS-PAM.

2. Experimental system

Figure 1 shows a schematic of the experimental system. A Q-switched Nd:YAG laser (SPOT-10-100-532, Elforlight Ltd, UK: 532 nm; 10 µJ/pulse; 2 ns pulse duration; 30 kHz pulse repetition rate) was used as the illumination source. The output light was attenuated by a neutral density filter, and then was reflected into the imaging system. The beam was scanned by an X-Y galvanometer scanner and was focused on the sample by an achromatic lens (f = 30 mm).

The induced photoacoustic signals from the illuminating target were detected by a custom-built 64-element linear phased array ultrasonic transducer fabricated with traditional array technology [12]. It has approximately 30 MHz center frequency and 60% −6dB bandwidth, as shown in Fig. 2(a). The size of each element was about 40 µm × 2 mm. The pitch (distance between the centers of the adjacent elements) was about 50 µm. In addition, the array
Fig. 1. Schematic of the experimental laser-scanning PAM system with an ultrasonic phased array. ND: neutral density filter; M: mirror; L: lens; PAUT: phased array ultrasonic transducer; DSP: digital signal processor. x: lateral direction; y: elevation direction; z: axial direction.

has an elevation lens focusing at about 7 mm. The detected 64-channel photoacoustic signals were first amplified (40 dB) by 16 4-channel low noise amplifiers (AD8334, Analog Devices Inc.), then digitized by a customized 64-channel high speed digitizer with 12-bit analog-to-digital converter (AD9230, Analog Devices Inc.). The sampling rate was 130 MS/s. The digitized data was further transferred to a computer by a digital signal processor (DSP) module with a gigabit Ethernet interface.

In the experiment, the computer first sent a start trigger signal to the DSP module, which then provided a trigger signal for a digital delay generator (DG645, Stanford Research Systems). The output of the digital delay generator was used as clock for a high-speed analog output board (AO, PCI-6731, National Instruments), which was used to control the galvanometer. The output of the sample clock from the AO board was used to trigger the laser. Meanwhile the DSP module triggered the 64-channel high speed digitizer to acquire data simultaneously and transferred the data to a computer.

3. Results and discussion

3.1. Performance of the experimental system

Beamforming is a technique of combining the signals detected at all the elements of an array transducer to achieve constructive interference. Since the sound wave generated at each laser-scanning point arrives at different array elements at different time, a proper time delay needs to be applied to each of the 64 active elements before their signals can be summed. Figure 2(b) shows the measured A-line signals of the array transducer at one laser scan position by...
using a black tape as the imaging target. Figure 2(c) shows 64-channel post-beamforming A-line signal in Fig. 2(b), which is the sum of 64-channel signal with proper time delays.

During imaging the ultrasonic phased array transducer was immersed into a water tank, which had a window on the bottom covered with plastic film. The water tank was placed on the imaging subject and coupled by ultrasound gel. The distance between the ultrasonic phased array and the imaging subject was about 7 mm.

![Mean SNR of the each element and the mean SNR with 64-channel beamforming.](image)

Figure 3 shows the SNR of all 64 channels (scatter points) and the SNR of the phased array after beamforming (red line). To measure the SNR, we acquired 64-channel photoacoustic signals repeatedly from a single illuminating point on a black tape with laser pulse energy of 70 nJ. Mean SNR of the 64 elements varied from 21 dB to 30 dB with a standard deviation from 1.14 to 1.97, and the mean SNR after beamforming was about 41 dB with a standard deviation of 1.47.

To test the performance of the ultrasonic phased array on improving the imaging quality, the system was first used to image a resolution target (USAF 1951 T21, Applied Image Inc.). Figure 4 shows a typical imaging result where both images are displayed with a dynamic range of 30dB. Figure 4(a) shows the maximum amplitude projection (MAP) of the data acquired by one of the array elements (#32). Due to the small element size, the sensitivity and SNR of each element of the phased array ultrasonic transducer are so low that the bars in the resolution target can be barely detected. (b) shows the MAP image after 64-channel beamforming. From Fig. 4(b) we can see that with 64-channel delay-sum beamforming the SNR is improved significantly; as a result, the image is able to resolve the details of the resolution target. We also

![Images of the resolution target (both images are displayed with a dynamic range of 30 dB).](image)

(a) Maximum amplitude projection (MAP) image acquired by one of the array elements (#32); (b) MAP image after 64-channel beamforming. Bar: 200 μm.
noticed that the field of view at the elevation direction (y direction) is increased significantly after beamforming, which is remarkable considering that beamforming is performed only in the lateral direction. For the current configuration, the field of view was about 3 mm in the lateral direction (x direction) and 1.5 mm in the elevation direction (y direction).

3.2. In vivo imaging

To test for biological imaging, we imaged an ear of a Swiss Webster mouse (body weight: 45 g) in vivo. The hairs on the ear were gently removed with commercial non-irritating hair-removing lotion. The mouse was anesthetized 5 minutes before the experiments by intraperitoneal (IP) injection of a cocktail containing Ketamine (54 mg/kg body weight) and Xylazine (6 mg/kg body weight). All experiments were performed in compliance with the guidelines of the University of Southern California’s Institutional Animal Care and Use Committee.

Figure 5(a) shows the MAP image of the data acquired by one of the array center elements (#32). Figure 5(b) shows the MAP image after performing 64-channel beamforming. The dynamic ranges of Fig. 5(a) and Fig. 5(b) are both 30 dB. We can clearly see the improvement of the image quality by the beamforming. With a single element the SNR is not good enough to clearly recognize the blood vessels in the sample. In contrast, with delay-sum beamforming the SNR is greatly improved and more small vessels along the lateral direction (horizontal direction in the figure) can be recognized. In the experiment, the laser scanning range was set to 3 mm × 1.5 mm.

4. Conclusion

We have successfully demonstrated the feasibility of LS-PAM with a phased array ultrasonic transducer for the first time. We have tested the system on imaging a USAF 1951 resolution target and a mouse ear in vivo. The imaging results showed that the sensitivity and SNR can be significantly improved by performing delay-sum beamforming. Since the ultrasonic phased array transducer used in the experiment was not designed for photoacoustic microscopy, the element parameters are far from optimized for our applications. The sensitivity of each element in the phased array ultrasonic transducer is much lower than that of the transducers we used in our previous publications [8–10]. As a result, we are confident that by optimizing the parameters the performance of the array transducer will be much better than that of the single element transducer. Future development will be focused on (1) optimizing the design of the ultrasonic phased array transducer to meet the requirements of ophthalmic application; (2) increasing sensitivity of each element by adjusting the element size (in a certain range) and selecting a more suitable piezoelectric material; (3) increasing the field of view by building a 2D phased array ultrasonic transducer with more array elements.
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