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LETTER

In vivo imaging of a single erythrocyte with high-resolution photoacoustic microscopy

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Abstract In this letter, we reported a high-resolution photoacoustic microscopy (PAM) to image erythrocytes and blood vessels. The developed system had the ability to provide a lateral resolution of 1.0 μm at the wavelength of 532 nm with a × 10 objective. First, we used a sharp edge to measure the lateral resolution of the PAM and testified the stability with carbon fibers. Then, using this system, *in vivo* blood vessels and capillaries of a mouse ear, even a single erythrocyte can be clearly imaged. There was a pair of accompanying venule and arteriole, whose detailed and further complicated branches can be clearly identified. And likely red blood cells (RBCs) arrayed one by one in microvasculature was also shown. The experimental results demonstrate that the high-resolution PAM has potential clinical applications for imaging of erythrocytes and blood vessels.

Keywords *in vivo*, photoacoustic microscopy (PAM), erythrocyte, microvasculature

1 Introduction

Photoacoustic microscopy (PAM) has been recognized as a kind of rapid development of medical imaging technology [1–3]. PAM has been applied in brain functional imaging [4], detection of early breast tumor [5] and dynamic change of oxygen saturation [6,7], monitoring of cerebrovascular activity as an noninvasive manner in small animals, which can provide high sensitivity and high specificity [8], and detection of differentiate atherosclerotic plaques [9], monitoring of vascular damage during tumor photodynamic therapy [10]. In PAM imaging, laser pulses are delivered into biologic tissues. Some of the delivered energy has been absorbed and converted into heat, leading to photoacoustic (PA) effect, inducing transient thermoelastic expansion and thus releasing wideband ultrasonic. The generated ultrasonic waves, namely PA signals, are then detected by ultrasonic transducers to reconstruct images. Then, we can get the distribution of light absorption information in tissues [11–14]. With the characteristic of point source excitation, PAM imaging can be obtained by scanning the biologic tissues point by point. To achieve the above procedure, we can move the imaging tissue with two-dimensional stepper motor [15] or move the scanning excitation beam with two-dimensional galvanometer [16,17] and digital micromirror device [18,19]. Considering from the scanning speed and stability, PAM imaging generally adopts the method of moving the scanning excitation beam.

Here, we developed the high-resolution PAM for imaging erythrocytes and blood vessels. In this system, the laser was through a two-dimensional scanning galvanometer, the scanning lens was focused on intermediate image plane. Then, the PAM and optical microscopy simultaneously image the intermediate image plane. Thus, the focus of photoacoustic imaging and the interested imaging area can be quickly found by observation of optical image. The developed PAM has the ability to provide a lateral resolution of 1.0 μm at 532 nm with a × 10 objective. The PA image of blood vessels and capillaries in the mouse ear was reconstructed by the maximum amplitude projection algorithm, and in vitro red blood cells was clearly distinguished, which means it has the potential of clinical application for imaging of erythrocytes and blood vessels. What's more, our system was the reflection mode, so that optical excitation and ultrasonic detection were the same side of the sample. This configuration makes the proposed PAM much more flexible and convenient in imaging of some thick samples with different structure and function.

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2 Methods

The schematic of the experimental setup is shown in Fig. 1. An Nd:YAG laser (LS-2134, LOTIS TII, Belarus), working at the wavelength of 532 nm and this exciting laser has a full width at half maximum (FWHM) of 10 ns with a 15 Hz repetition. The laser, through a two-dimensional scanning galvanometer (6231H; Cambridge Technology, Inc., Lexington, MA, USA), and the scanning lens was focused on intermediate image plane. Then, the light was collimated with a tube lens and focused with the long working distance objective. The focused light passed through a custom-made hollow focused ultrasound transducer and irradiated the biologic tissues to generate photoacoustic signals. The hollow focused ultrasound transducer, which has a center frequency of 50 MHz, was used to receive the PA signals. The charge coupled device (CCD) was used to get the optical microscopy image of the sample. According to the reversibility principle of beam path, the PAM and optical microscopy simultaneously image the intermediate image plane. The focus of photoacoustic imaging and the interested imaging area can be quickly found by observation of optical image. Through the signal amplifier and a dual-channel data acquisition card (NI5124, National Instruments Corp., Austin, TX, USA), the computer recorded the PA signals from each point. Finally, the photoacoustic image was reconstructed by the software of Labview 2012. The sampling rate of the data acquisition card was 200 M samples/s.

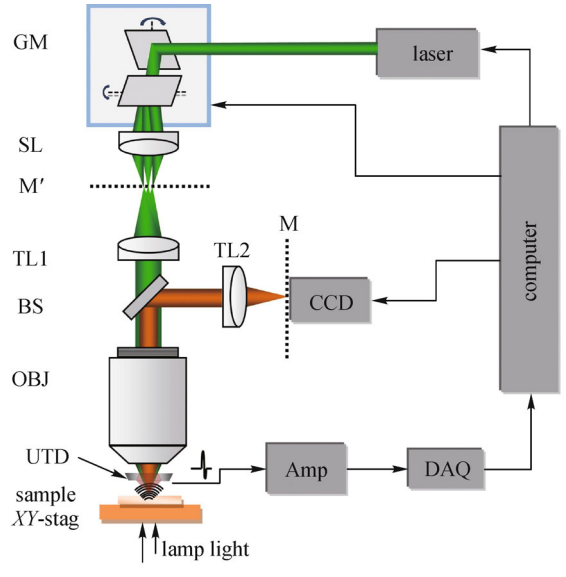

Fig. 1 Schematic of high-resolution PAM. GM = Galvo scanner; SL = scanning lens; TL1 = tube lens 1, TL2 = tube lens 2, M = intermediate image plane; BS = beam splitter; OBJ = objective (× 10, NA = 0.3; × 20, NA = 0.5; × 40, NA = 0.65); UTD = ultrasonic transducer; Amp = amplifier; DAQ = data acquisition

3 Experiments and results

3.1 Lateral resolution of the system

Lateral resolution of the high-resolution PAM is principally depended on numerical aperture (NA) of the objective, which determines optical focal diameter of the nearly diffraction limit [15,16]. To evaluate the lateral resolution at the laser wavelength of 532 nm, a sharp edge submerged in water was imaged with the PAM system. After scanning across the edge of a surgical knife blade at a 0.2 μm steps, the PA signal values were fitted by a sigmoidal-shaped function, then we can get the fitted edge spread function (ESF) of the system. The optical image and the PAM image of the edge are shown in Figs. 2(a) and 2(b), respectively. Figure 2(c) shows the photoacoustic signal of the white solid line in Fig. 2(b), the black square is the experimental measurement and the red solid line is the fitted ESF. The line spread function (LSF) shown in Fig. 2(d) is the derivative with respect to the ESF. So the lateral resolution of the system, which was evaluated by the FWHM of the LSF, was 1.0 μm with a × 10 objective, approached to the theoretical value $0.51\lambda/\mathrm{NA} \approx 0.9$ μm. The experimental results showed that the transverse spatial resolution of the PAM system can reach the nearly diffraction-limit of the objective.

3.2 Testifying the lateral resolution of the system

We first testified the resolution of our high-resolution PAM system by imaging of carbon fibers with different magnification objectives. The carbon fibers we used has a diameter of 7 μm. Figures 3(a), 3(b) and 3(c) present the photoacoustic image of the carbon fibers with the objective magnifications of × 10, × 20, × 40, using the scanning steps of 5, 3 and 1 μm, respectively. And the top right corner of each image is the guided optical image. From this experiment, the carbon fiber was clear imaged. So we can quickly find the focus of photoacoustic imaging and the interested imaging area through the observation of the optical image.

3.3 *In vitro* imaging of a single erythrocyte

Furthermore, we demonstrated the high-resolution ability of PAM system by imaging a single erythrocyte. The *in vitro* red blood cells (RBCs) were drawn from the rabbit ear arteries containing anticoagulant. As shown in Fig. 4(a), the PA imaging of a single erythrocyte can be clearly distinguished with high contrast. The diameter of the erythrocyte is about 5 – 6 μm. An optical image of a rabbit blood sample at the same place is shown in Fig. 4(b) for comparison. Each erythrocyte in photoacoustic image can be perfectly corresponded to the optical image. It is worth mentioning that the optical image in Fig. 4(b) is quite

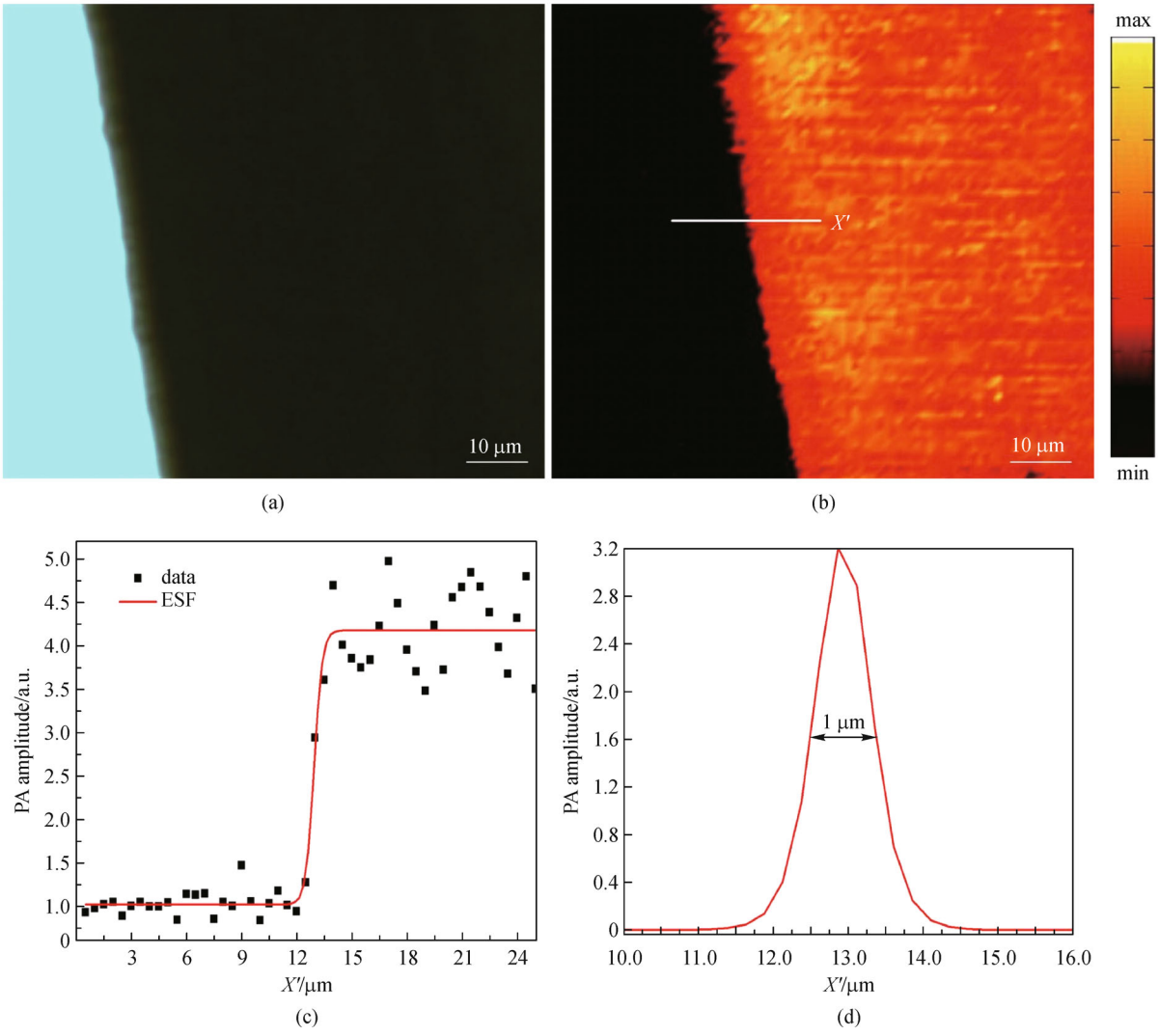

Fig. 2 Lateral resolution of the high-resolution PAM. (a) Optical image of a knife; (b) photoacoustic image of the knife; (c) photoacoustic signal of the white solid line in (b), black square = experimental measurement, ESF = edge spread function, red solid line = theoretical fit; (d) LSF derived from the fitted ESF. LSF = line spread function

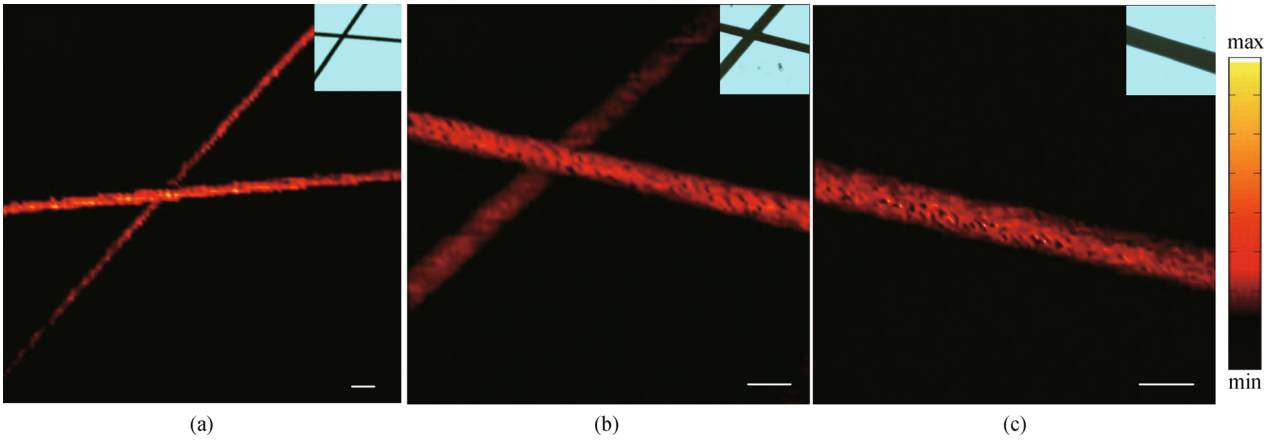

Fig. 3 PAM imaging of the carbon fibers. (a), (b), (c) present the photoacoustic image of the carbon fibers with the objective magnification of × 10, × 20, × 40, respectively. The top right corner of each image is the guided optical image. The bar in each picture was 10 μm

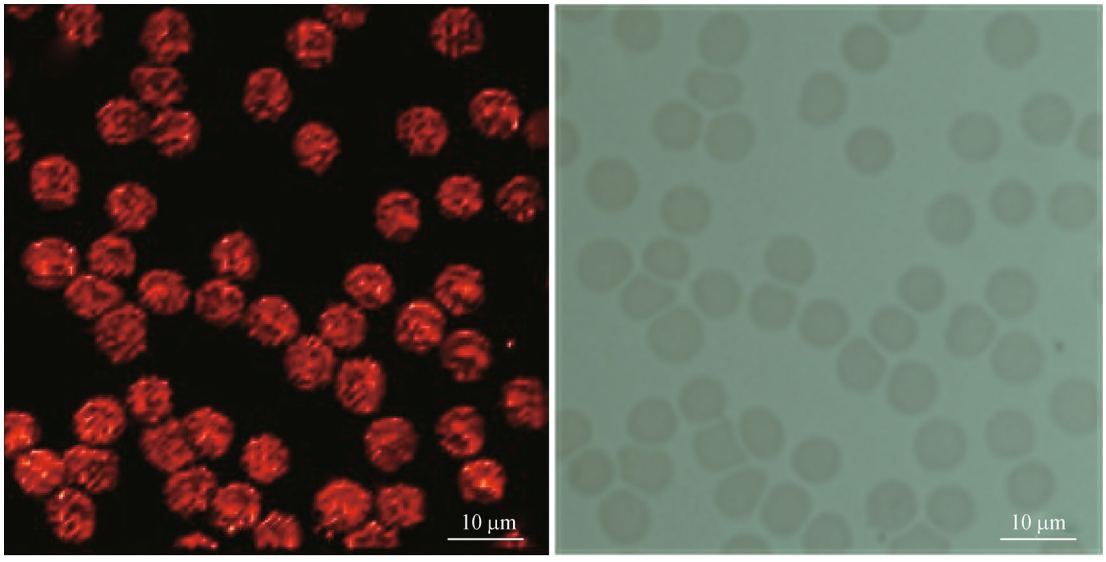

Fig. 4 Red blood cells imaged *in vitro* using our high-resolution PAM. (a) Photoacoustic image of red blood cells; (b) optical image corresponding to (a)

ambiguous. This is mainly because we have covered 500 μm depth of silicone oil on the blood smear. The experimental result proves that the fabricated high-resolution PAM microscopy system is able to image single erythrocyte.

3.4 *In vivo* imaging of RBCs and microvasculature in a mouse ear

The high-resolution PAM system was further applied in *in vivo* imaging blood vessels of a BALB/c mouse. The hair of the mouse ear was chemically depilated before imaging and the mouse were intraperitoneally anesthetized with a mixture of a-chloralose and urethane to keep the mouse with no movement during the experiment. At the edge of a mouse ear, an interesting area with a size of 2 mm × 2 mm was chosen to image. Then using our high-resolution PAM, this selected area was scanned with ~ 5 μm steps. Figure 5(a) is blood vessels in a mouse ear imaged *in vivo* using our high-resolution PAM. As shown in Fig. 5(a),

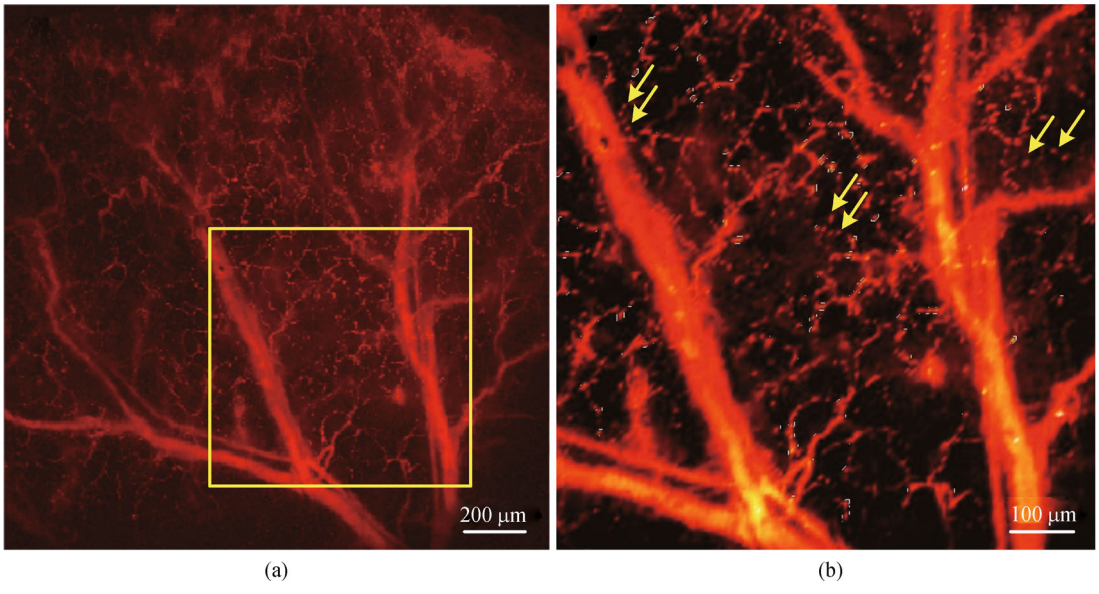

Fig. 5 Blood vessels in a mouse ear imaged *in vivo* using our high-resolution PAM. (a) PA image of the microvasculature in a mouse ear with large field; (b) local amplification of the square area in (a)

there is a pair of accompanying venule and arteriole, whose detailed and further complicated branches can be clearly identified. And some smaller microvasculature also can be distinguished in the square. Figure 5(b) is the local amplification of the square area in Fig. 5(a). That one by one point labeled with arrows is likely red blood cell arrayed one by one in microvasculature. The experimental results show that the high resolution PAM system is able to image blood vessels and red blood cells *in vivo*, which illustrates this system has clinical potential for imaging of blood vessels and red blood cells.

4 Discussion

The experimental results suggest that the PA images of cellular and vascular can be clearly reconstructed by using the high resolution PAM system. There is a pair of accompanying venule and arteriole, whose detailed and further complicated branches can be clearly identified. Some smaller microvasculature also can be distinguished. And likely red blood cells arrayed one by one in microvasculature was also shown. In our experiment, the repetition rate of the pulsed laser influenced the speed of imaging. For this reason, the system cannot accomplish real-time dynamic imaging and monitoring, which means that a laser with higher repetition frequency is required for future work. In addition, an improved transducer with low-resistance electronic devices as well as higher sensitivity would help to reduce the excitation laser power in the future.

5 Conclusions

This paper reported a high-resolution PAM system to image erythrocytes and blood vessels with high contrast. In the present study, the developed system has the ability to provide lateral resolution of 1.0 μm at 532 nm with a × 10 objective. We used a sharp edge to measure the lateral resolution of the PAM and testify the stability with carbon fibers. Also, using this system, blood vessels and capillaries in the mouse ear even a single erythrocyte can be clearly imaged. Therefore, the high-resolution PAM can accomplish imaging of microvasculature and a single erythrocyte *in vivo,* demonstrating its great potential of clinical applications for imaging of erythrocytes and blood vessels.

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