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Ultrasound Array Photoacoustic Microscopy for Dynamic *In Vivo* 3-D Imaging

Liang Song[1], Konstantin Maslov[1], K. Kirk Shung[2], and Lihong V. Wang[1]

[1]Optical Imaging Laboratory
Department of Biomedical Engineering
Washington University in St. Louis, St. Louis, Missouri 63130
[2]Department of Biomedical Engineering
University of Southern California, Los Angeles, California 90089

ABSTRACT

Using realtime ultrasound array photoacoustic microscopy (UA-PAM), we demonstrated the feasibility of noninvasive *in vivo* imaging of human pulsatile dynamics, as well as 3-D dynamic imaging of sentinel lymph nodes (SLNs) in a murine model. The system, capable of realtime B-scan imaging at 50 Hz and high-speed 3-D imaging, was validated by imaging the subcutaneous microvasculature in rats and humans. After the validation, a human superficial palmar was imaged, and its pulsatile dynamics monitored, with 20-ms B-scan imaging temporal resolution. In addition, noninvasive photoacoustic sentinel lymph node (SLN) mapping with high spatial resolution has the potential to reduce the false negative rate and eliminate the use of radioactive tracers. Upon intra-dermal injection of Evans blue, the system maps SLNs accurately in mice and rats. Furthermore, the ~6 s 3-D imaging temporal resolution offers the capability to quantitatively and noninvasively monitor the dye dynamics in SLNs *in vivo* through sequential 3-D imaging. The demonstrated capability suggests that high-speed 3-D photoacoustic imaging should facilitate the understanding of the dynamics of various dyes in SLNs, and potentially help identify SLNs with high accuracy. With the results shown in this study, we believe that UA-PAM can potentially enable many new possibilities for studying functional and physiological dynamics in both preclinical and clinical imaging settings.

Keywords: photoacoustic microscopy, ultrasound array, dynamic *in vivo* imaging, three-dimensional imaging, microvascular imaging

1. INTRODUCTION

Owing to both the clinical needs and the demand for biomedical study of physiological dynamics, high-speed *in vivo* functional imaging has become highly desirable. Although MRI provides good functional imaging capability, it usually cannot perform real-time imaging [1]. Ultrasound offers real-time imaging capability, but the mechanical contrast does not provide much physiological information besides flow [2]. Previously available high-resolution optical microscopy modalities—including confocal microscopy [3], two-photon microscopy [4], and optical coherence tomography [5]—are capable of real-time imaging. However, as none of them sense optical absorption directly, their sensitivity for functional imaging is relatively low. Moreover, they rely on the detection of ballistic photons, and thus cannot image beyond one optical transport mean free path in highly scattering biological tissue.

Photoacoustic imaging is a noninvasive biomedical imaging technology that provides excellent optical absorption contrast—endogenous contrast for many physiological phenomena—with high ultrasonic resolution at super-depths—depths beyond the optical transport mean free path [6]. It has been used to

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study whisker stimulation [7], single vessel oxygenation [8], and tumor hypoxia [9]. It is also a high-speed imaging modality by nature, with its speed fundamentally limited only by the photoacoustic wave propagation time. In principle, A-lines (i.e., depth-resolved 1-D images) can be acquired at 100 kHz with a depth of 1.5 cm.

Using a 30-MHz ultrasound array and a kHz repetition laser system, we developed photoacoustic microscopy capable of real-time B-scan imaging at 50 Hz and high-speed 3-D imaging, offering the feasibility to image physiological dynamics [10-12]. In addition, this ultrasound array photoacoustic microscopy (UA-PAM) system, with axial, lateral, and elevational resolutions of 25, 70, and 200 μm, respectively, provides ~3 mm imaging depth in scattering biological tissue [10]. In this study, by imaging human pulsatile dynamics and murine sentinel lymph node dynamics, we demonstrated the system's dynamic functional imaging capability.

2. IMAGING SYSTEM

A schematic of the system is shown in Fig. 1. The key components include a diode-pumped Q-switched Nd:YLF laser, a tunable dye laser, a 30-MHz ultrasound array, an 8-channel PCI data acquisition (DAQ) card, a multi-core PC, and a custom designed light delivery system. Details of the system can be found in our previous publications [10, 11, 13].

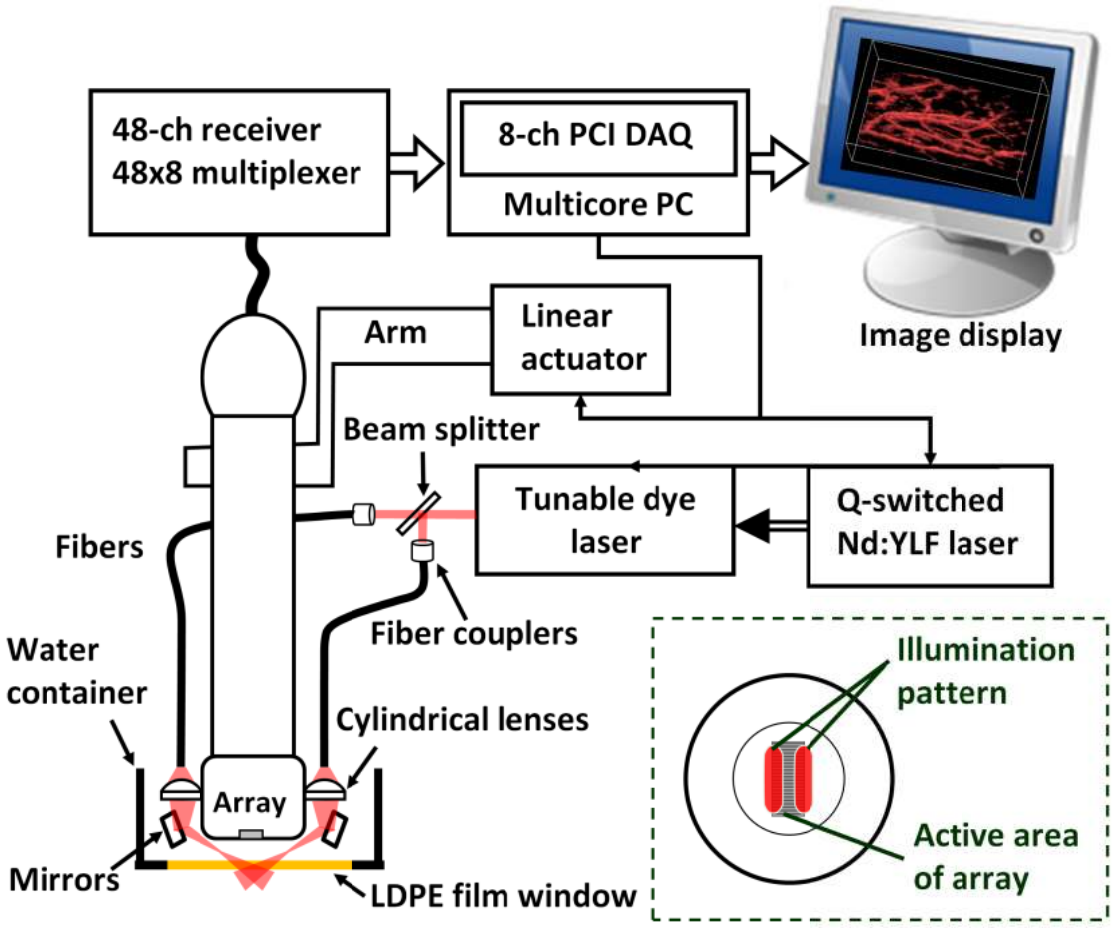

Fig. 1. Schematic of the ultrasound array photoacoustic microscopy system. Inset A, the dark-field illumination pattern on the skin surface.

3. RESULTS

To validate the system's performance of *in vivo* microvascular imaging in both small animals and humans, we imaged the upper dorsal region of a Sprague Dawley rat (Harlan Sprague Dawley, Inc., USA), as well as a human palm. All the imaging procedures were conducted in compliance with the experimental protocols approved by Washington University in St. Louis.

Fig. 2 shows that blood vessels down to ~100 μm size were clearly imaged by the system. The acquisition time for each 3-D photoacoustic image was only 2 s. Limited by the data transfer speed, the temporal resolution of sequential 3-D imaging was ~6 s.

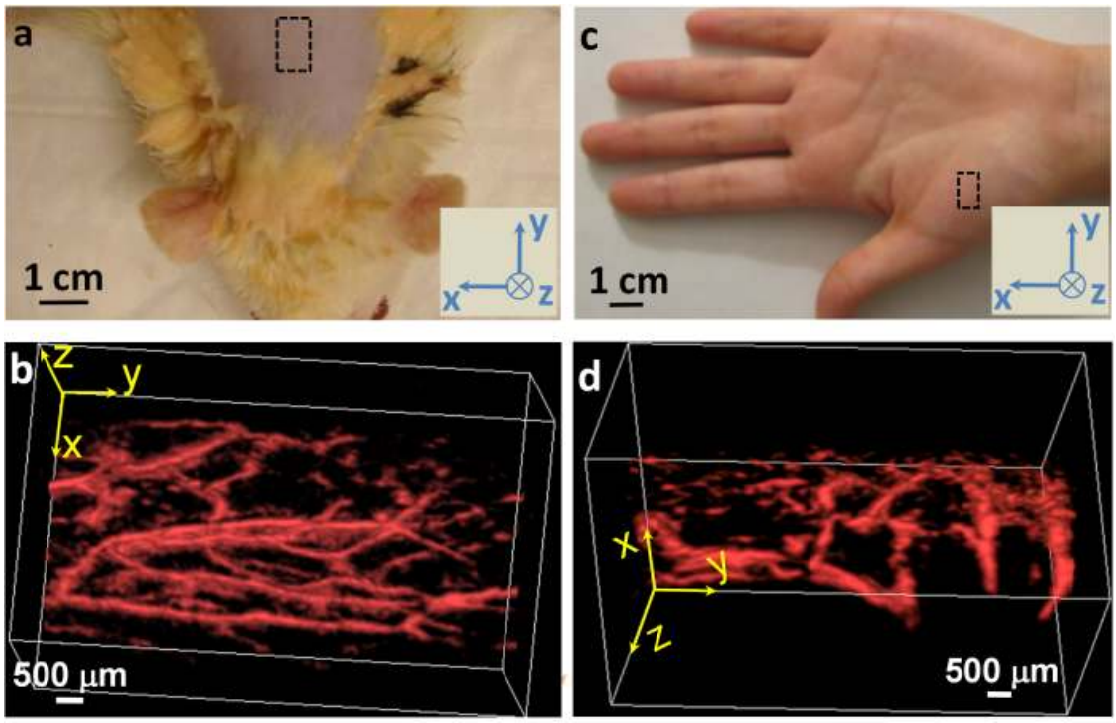

Fig. 2. (a) Photograph of the Sprague Dawley rat with hair removed before photoacoustic imaging. (b) *In vivo* photoacoustic 3-D image corresponding to the dashed rectangle area in (a). (c) Photograph of the imaged human palm. (d) *In vivo* photoacoustic 3-D image corresponding to the dashed rectangle area in (c).

In order to study the pulsatile dynamics, we scanned a region of the palm near the wrist, where the superficial palmar, ~1 mm in diameter and over 1 mm deep, was imaged (Fig. 3). After acquiring the initial 3-D image, we fixed the scanning probe and continuously monitored the pulsation using B-scan (Video 1). The pulsatile rate, estimated from the image, was 66 per minute, consistent with the 65 ± 2 per-minute rate measured from a pulse oximeter.

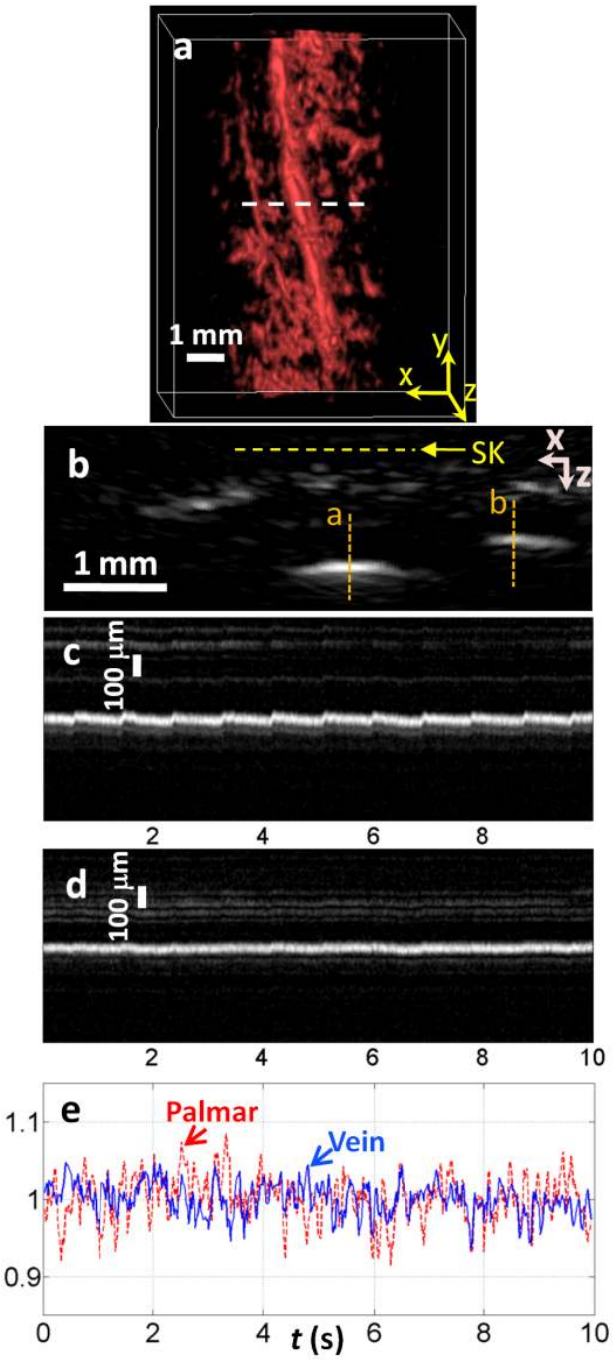

Fig. 3. *In vivo* photoacoustic imaging of human pulsatile dynamics. (a) 3-D photoacoustic image of the superficial palmar. The dashed line indicates the cross-section monitored by real-time B-scan imaging. (b) One B-scan slice from the real-time photoacoustic B-scan movie (http://dx.doi.org/10.1117/12.840582.1). SK, skin surface.
(c) M-mode image corresponding to the dotted vertical line *a* in (b), showing the superficial palmar's pulsatile motion (A-line) as a function of time.
(d) M-mode image of a vein corresponding to the dotted vertical line *b* in (b).
(e) Normalized integrated signal over the superficial palmar (red) and the vein (blue) regions as a function of time.

Using a murine model, we also demonstrated the system's capability to accurately identify SLNs and quantitatively image the dye uptake and clearance dynamics [13].

4. CONCLUSIONS AND DISCUSSION

Our UA-PAM system was capable of high-speed *in vivo* imaging of microvasculature details in both small animals and humans. Using UA-PAM, human pulsatile dynamics was captured in real time. Ideally, to better characterize the pulsatile hemodynamics, oxygen saturation should be measured within each cardiac cycle. However, accurate computation of the blood oxygenation in real-time requires photoacoustic imaging with laser-wavelength tunability in real time (because oxygenation quantification requires multi-wavelength measurements), which is currently not available in our laser system.

In addition, UA-PAM demonstrated accurate *in vivo* SLN mapping in a murine model. The dynamics of dye accumulation and clearance in murine SLNs were quantitatively monitored with a high temporal resolution, up to 6 s. This capability should facilitate further studies to understand the dynamics of different dyes in SLNs, and potentially help identify SLNs with high accuracy.

With the promising results shown in this study, we believe that UA-PAM will open up many new possibilities for studying functional and physiological dynamics in both preclinical and clinical imaging settings.

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