In vivo volumetric imaging of subcutaneous microvasculature by photoacoustic microscopy

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Abstract: Photoacoustic microscopy was developed to achieve volumetric imaging of the anatomy and functions of the subcutaneous microvasculature in both small animals and humans *in vivo* with high spatial resolution and high signal-to-background ratio. By following the skin contour in raster scanning, the ultrasonic transducer maintains focusing in the region of interest. Furthermore, off-focus lateral resolution is improved by using a synthetic-aperture focusing technique based on the virtual point detector concept. Structural images are acquired in both rats and humans, whereas functional images representing hemoglobin oxygen saturation are acquired in rats. After multiscale vesselness filtering, arterioles and venules in the image are separated based on the imaged oxygen saturation levels. Detailed structural information, such as vessel depth and spatial orientation, are revealed by volume rendering.

OCIS codes: (170.3880) Medical and biological imaging; (170.5120) Photoacoustic imaging; (180.5810) Scanning microscopy.

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#72153 - $15.00 USD Received 20 June 2006; revised 14 August 2006; accepted 14 August 2006

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1. Introduction

High-resolution imaging of subcutaneous blood microvessels in three dimensions can be of great benefit to dermatology, cancer research [1], and plastic surgery [2]. However, accurate noninvasive imaging of the anatomy and functions of the subcutaneous microvasculature remains a challenge. First, the imaging modality employed must have high sensitivity so that small-diameter (tens of μm) blood vessels can be detected. Second, the imaging modality employed must have high spatial resolution so that the vessel network can be resolved. Third, it must have enough penetration depth so that the major vasculature in the dermal layer (1-3 mm below the skin surface in humans and small animals) can be measured. Fourth, it must be able to acquire volumetric data with good spatial resolution in both the lateral and axial directions. Fifth, it must image appropriate intrinsic contrast so that physiological functions can be measured.

In blood vessel imaging, the intrinsic contrast comes from either the strong optical absorption of hemoglobin [3] or the velocity of the blood flow [4,5]. Because the relative optical absorption contrast between blood and dermal tissue can be up to 100, depending on the optical wavelength used [6], an optical absorption-based high-resolution imaging modality is well suited for microvascular imaging. Moreover, since optical absorption is associated with both forms of hemoglobin in blood, optical absorption-based imaging can quantify functional parameters such as the total hemoglobin concentration and hemoglobin oxygen concentration (SO_2).

The ratio of the maximum imaging depth to the depth (axial) resolution, which gives the number of effective pixels, is used to evaluate the spatial resolution and image quality of an imaging modality. A depth-to-resolution ratio greater than 100 has been achieved by several optical modalities, such as confocal microscopy, two-photon microscopy, and optical coherence tomography (OCT). However, all are not directly dependent on optical absorption contrast, and none can reach a penetration depth of more than ~1 mm (related to one transport mean free path) in biological tissue owing to strong optical scattering. Hence, existing high-resolution optical imaging modalities are not ideal for imaging subcutaneous microvasculatures.