

PAM MICROVASCULATURE IMAGE OF A MOUSE EAR, LIHONG V. WANG

# Photoacoustic Microscopy

**Superdepth, superresolution,  
and superb contrast.**

By Junjie Yao, Liang Song, and Lihong V. Wang

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Since its invention in the 17th century, optical microscopy has revolutionized biomedical studies by scrutinizing the biological realm on cellular levels, taking advantage of its excellent light-focusing capability. However, most biological tissues scatter light highly. As light travels in tissue, cumulative scattering events cause the photons to lose their original propagation direction and, thus, their ability to be focused, which has largely limited the penetration depth of optical microscopy. Conventional planar optical microscopy can provide penetration of only ~100  $\mu\text{m}$  before photons begin to be scattered. The penetration of modern optical microscopy, such as confocal microscopy and multiphoton

microscopy, is still limited to approximately the optical diffusion limit (~1 mm in the skin as approximated by one optical transport mean free path), where scattered photons retain a strong memory of the original propagation direction. So far, it still remains a challenge for pure optical methods to achieve high-resolution *in vivo* imaging beyond the diffusion limit (i.e., superdepth imaging).

In contrast, photoacoustic microscopy (PAM) can readily provide penetration beyond the optical diffusion limit while maintaining high resolution; hence, PAM has achieved superdepth imaging. In PAM, the tissue is usually irradiated by a short-pulsed laser beam. The absorbed photon energy is partially or completely converted into heat, which induces wideband ultrasound waves that propagate in tissue. These ultrasound waves are detected outside the tissue by an ultrasonic transducer or transducer array to form an image that maps the original optical energy deposition in the tissue. The key difference between PAM and pure optical imaging modalities lies in its conversion of electromagnetic energy (light) into mechanical energy (ultrasound), which allows PAM to seamlessly combine the rich optical absorption contrast of biological tissue with high acoustic resolution at greater depths. In the optical excitation phase, a given percentage change in the optical absorption coefficient of the tissue yields the same percentage change in the linear photoacoustic (PA) signal amplitude; hence, PAM has 100% sensitivity to optical absorption contrast. In the acoustic detection phase, the ultrasound waves undergo only weak scattering in tissue, which enables high-resolution PA imaging at depths beyond the diffusion limit.

Besides its superb penetration depth, PAM complements other high-resolution optical imaging modalities in several additional aspects. In comparison to conventional planar microscopy, PAM provides acoustically determined depth resolution and much greater absorption based imaging contrast. In comparison to confocal and multiphoton microscopy, PAM does not need exogenously delivered fluorescent contrast agents and can potentially image more molecules, fluorescent or nonfluorescent. In addition, it is not necessary for PAM to possess depth scanning for three-dimensional imaging due to its acoustically resolved depth profiling. In comparison to optical coherence tomography (OCT), PAM can quantify oxygenation of the tissue with much greater sensitivity. Unlike OCT, PAM is speckle-free.

### State-of-the-Art PAM

PAM is highly scalable in both the optical and acoustic domains. Over the years, multiscale PAM has provided high-resolution images of biological structures ranging from organelles to organs, taking advantage of optical resolution within the diffusion limit (Figure 1) and fine acoustic resolution at depths beyond the limit [1]. Furthermore, miniaturized PA probes have enabled endoscopic and intravascular applications, providing functional information at unprecedented depths [2]. The unique scalability

of PAM and its transition into macroscopic imaging enables biological research at multiple length scales with consistent imaging contrast and can accelerate the translation of microscopic lab discoveries to macroscopic clinical practice.

PAM is also making progress on exciting new fronts. The wavefront of PAM's excitation light can potentially be optimized through iterative algorithms to confine the light to an optically determined region in deep tissue far beyond the optical diffusion limit [3]. This new ability will have a tremendous impact on both imaging and treatment.

Optical diffraction has long limited optical microscopy to a lateral resolution of ~200 nm, leaving the observation of ultrastructural cellular features to electron microscopy. Breaking the optical diffraction limit, which is defined by the size of the Airy disk, leads to superresolution imaging. PAM has recently achieved superresolution imaging by using various nonlinear mechanisms and extended its applications into nanodimensions [4], [5]. In particular, superresolution PAM using the optical absorption saturation effect has provided a lateral resolution of ~80 nm, enabling single-mitochondrion imaging [5].

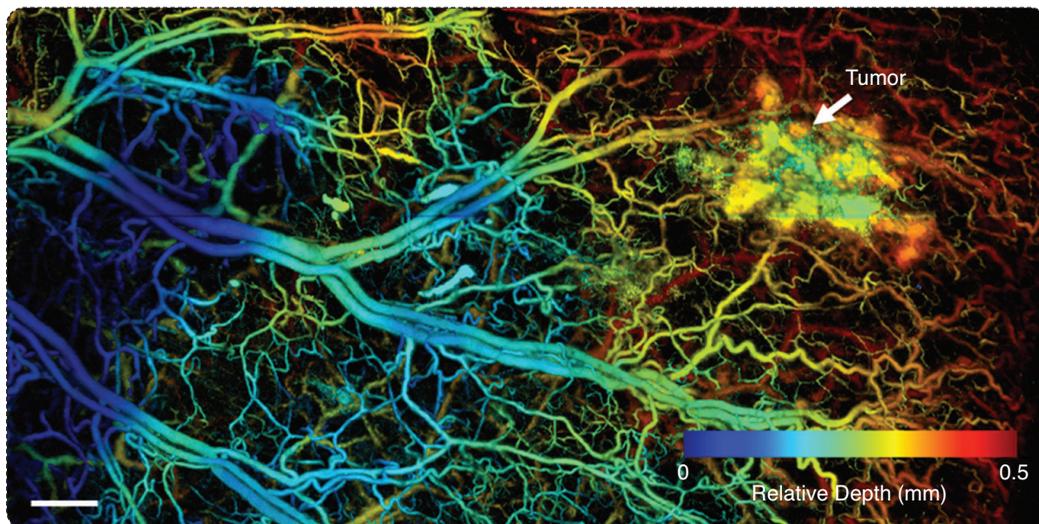
Pure ultrasonic imaging provides good spatial resolution in deep tissue, but has strong speckle artifacts and lacks molecular contrast. PAM, on the other hand, takes advantage of the rich optical absorption contrast of tissue due to molecular absorption and provides speckle-free images with superb contrast. For example, when PAM operates with visible light, the high image contrast of blood vessels (Figure 1) comes from the absorption of hemoglobin in red blood cells (RBCs), which overpowers the absorption of water and lipids outside the blood vessels by more than four orders of magnitude. Furthermore, the variety of contrasts available for PAM is much greater than for fluorescence microscopy, because all molecules, fluorescent or nonfluorescent, have their own absorption wavelengths, and, hence, they can be imaged by PAM [6]. The primary endogenous absorbers in tissue for PA imaging include DNA/RNA, cytochrome, hemoglobin, melanin, lipids, and water. For PA molecular imaging, various exogenous contrast agents have been explored, including organic dyes, nanoparticles, fluorescent proteins, and reporter gene products. These contrast agents, especially nanoparticles, can be specifically engineered for different PA applications [7]–[9].

Using endogenous or exogenous contrast, PAM has measured a number of important functional parameters of absorbers and their microenvironments, such as the total hemoglobin concentration, oxygen saturation of hemoglobin (Figure 2), partial oxygen pressure, temperature, blood flow speed, metabolic rate of oxygen, and pH [10]. The inherent functional imaging capability of PAM can provide a more comprehensive understanding of disease states, thus benefiting the diagnosis and treatment.

### The Future and the Promise

We expect PAM to experience continued breakthroughs. Fully integrated multiscale PAM, with a fast scanning mechanism and

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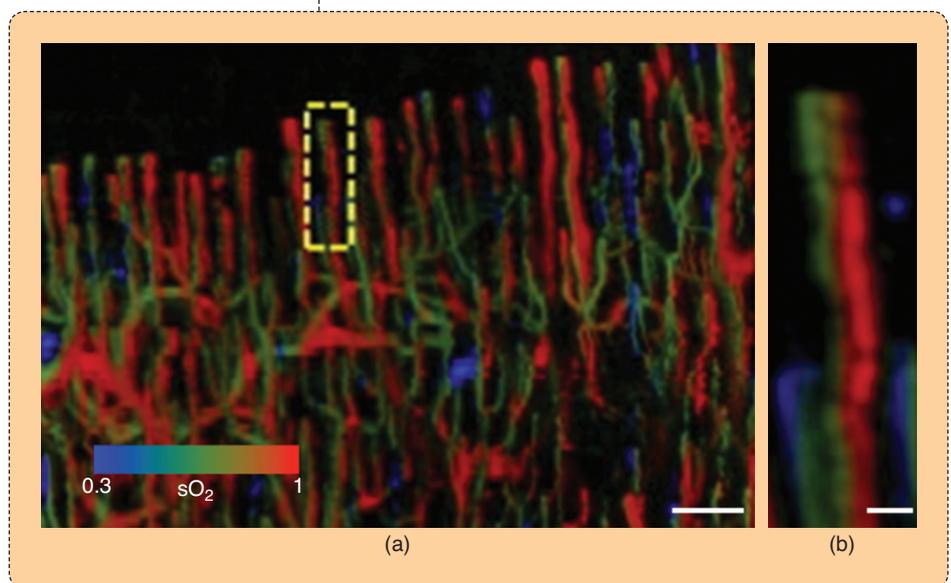
**FIGURE 1** A PAM microvasculature image of a mouse ear bearing a xenotransplanted B16 melanoma tumor. Note the principal artery-vein pair feeding and draining the tumor region. The depth is coded from blue (superficial) to red (deep). Scale bar: 1 mm. (Image courtesy of Lihong V. Wang.)

high-repetition-rate tunable laser, will be able to perform supercontrast imaging of biological tissue at different length scales in real time. Truly quantitative PAM, with advanced multispectral algorithms and optical fluence compensation methodologies, will be able to image the functional parameters of biological tissue at unprecedented depths. Two-photon or even multiphoton PAM, with novel methodologies for differentiating nonlinear PA signals from linear PA signals, will be able to penetrate even deeper by using longer wavelengths, and will achieve optically limited spatial resolutions in all dimensions. Reflection-mode PAM integrated with other optical imaging modalities with accessibility to many anatomical sites will be able to provide more comprehensive information of the tissue based on optical absorption, back-scattering, and various fluorescence contrasts.

Meanwhile, the translational potentials of PAM technologies will continue to be realized. First, it is just a matter of time before multicontrast PAM will enable simultaneous imaging of neural activities and functional vascular responses in the brain, providing a powerful tool for studying

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neurovascular coupling. Second, ultraviolet PAM can perform staining-free histology of DNA, RNA, and cytochromes, and may ultimately become a paradigm-shifting technology for daily operations in pathology. Third, high-speed, handheld PAM has demonstrated great promise for noninvasive label-free detection and staging of skin cancers (e.g., melanoma) and will continue to move toward clinical studies. Finally, endoscopic and intravascular PAM is expected to find a wide range of clinical applications in



**FIGURE 2** Label-free PAM of (a) oxygen saturation of hemoglobin ( $sO_2$ ) in a human finger cuticle in vivo. (b) A close-up view of the capillary loop indicated by the dashed box in (a) shows the sharp transition in  $sO_2$  at the tip of the loop, reflecting a marked oxygen release from hemoglobin. Scale bars: 200  $\mu\text{m}$  in (a) and 50  $\mu\text{m}$  in (b). (Image courtesy of Lihong V. Wang.)

gastroenterology, interventional cardiology, neurology, and obstetrics and gynecology.

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**Junjie Yao (yaoj@seas.wustl.edu)** received his B.E. and M.E. degrees in biomedical engineering from Tsinghua University, Beijing, China, in 2006 and 2008, respectively. He received his Ph.D. degree in biomedical engineering from Washington University, St. Louis (WUSTL), Missouri, in 2013, under the tutelage of Prof. Lihong Wang. He is currently a postdoctoral research associate at WUSTL. His research interests are novel photoacoustic, optical, and ultrasound imaging technologies in biomedicine. **Liang Song (liang.song@siat.ac.cn)** is currently a professor and the director of the Research Lab for Biomedical Optics and the Shenzhen Key Lab for Molecular Imaging at the Shenzhen Institutes of Advanced Technology (SIAT), Chinese Academy of Sciences. Prior to joining SIAT, he studied at Washington University in St. Louis and received his Ph.D. degree in biomedical engineering in 2010 under the tutelage of Lihong V. Wang. His current research at SIAT focuses on developing novel microscopic and endoscopic imaging systems and optical/ultrasonic multimodality imaging technologies for the diagnosis and therapy guidance of cancer and cardiovascular atherosclerosis. **Lihong V. Wang (lhwang@seas.wustl.edu)** earned his Ph.D. degree at Rice University, Houston, Texas, under the tutelage of Robert Curl, Richard Smalley, and Frank Tittel. He currently holds the Gene K. Beare Distinguished Professorship of Biomedical Engineering at Washington University in St. Louis. He is a Fellow of the American Institute for Medical and Biological Engineering, the Optical Society of America, the IEEE, and the Society of Photo-Optical Instrumentation Engineers. He is the editor-in-chief of *Journal of Biomedical Optics*. He chairs the annual SPIE conference on photons plus ultrasound.

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