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Transvaginal fast-scanning optical-resolution photoacoustic endoscopy

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Abstract. Photoacoustic endoscopy offers in vivo examination of the visceral tissue using endogenous optical absorption contrast, but its typical B-scan rate is ∼10 Hz, restricted by the speed of the scanning unit and the laser pulse repetition rate. Here, we present a transvaginal fast-scanning optical-resolution photoacoustic endoscope with a 250-Hz B-scan rate over a 3-mm scanning range. Using this modality, we not only illustrated the morphological differences of vasculatures among the human ectocervix, uterine body, and sublingual mucosa but also showed the longitudinal and cross-sectional differences of cervical vasculatures in pregnant women. This technology is promising for screening the visceral pathological changes associated with angiogenesis. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.23.12.121617]

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single-mode optical fiber (Thorlabs, S405-XP). The fiber guides the light to the fsOR-PAE probe with an insertion tube 20 cm in length and 20 mm in diameter [Fig. 1(b)].

In the probe, the laser beam from the single-mode fiber is focused by a set of doublets (Thorlabs, AC064-015-A) and then transmitted through the center of a custom-designed focused ultrasonic ring transducer (40-MHz central frequency), achieving an acoustic-optical coaxial confocal alignment [Fig. 1(c)]. To optimize the optical and acoustic transmittances through the imaging window, a polymethylpentene membrane (CS Hyde, 33-3F-24) seals the imaging window, preventing leakage when the chamber of the probe is filled with distilled water for ultrasound coupling. A MEMS scanning mirror drives the azimuth scanning. (c) Schematic of the acoustic-optical coaxial confocal alignment in the probe. MEMS drives the scanning parallel to the cylindrical axis. AW, acoustic wave; LB, laser beam; MEMS, microelectromechanical system scanning mirror; SMF, single-mode fiber; UT, ultrasonic transducer.

Fig. 1 Schematic of the fsOR-PAE probe and its peripheral systems. (a) Setup of fsOR-PAE. CS, control system; GGD, ground glass diffruser; NDF, variable neutral density filter; PD, photodetector. (b) Photograph of the fsOR-PAE probe. A linear actuator in the white housing drives the azimuth scanning. (c) Schematic of the scanning mechanism of the fsOR-PAE probe (Video 1, MP4, 3.78 MB [URL: https://doi.org/10.1117/1.JBO.23.12.121617.1]).

edge spread function, the line spread function was computed and found to have a full width at half maximum of 3.1 μm, which represents the lateral resolution [Fig. 3(a)]. The axial resolution was estimated to be 46.5 μm, based on the photoacoustic signal detected from a tungsten wire (diameter: 14 μm) [Fig. 3(b)]. A metal grid (127-μm pitch and 37-μm bar width) [Fig. 3(c)] was imaged [Fig. 3(d)], and the average signal-to-noise ratio (SNR) was 33.2 dB. Figure 3(e) is a B-scan image in the plane highlighted by the dashed line in Fig. 3(d). Because the angular scanning of fsOR-PAE maps the detected photoacoustic signal in polar coordinates, we transform the data to Cartesian coordinates in image reconstruction. These results suggest that the fsOR-PAE system is capable of imaging structures on the micrometer scale.

We then imaged various human tissues to demonstrate the imaging capability of fsOR-PAE. All the human experiments followed protocols approved by the Institutional Review Board administered by the Human Research Protection Office at Washington University in St. Louis.

In an ex vivo demonstration, we imaged a uterus obtained from hysterectomy. Figures 4(a) and (b) show the vascular networks in the ectocervix and the serosal layers of the uterine body, respectively. A volume-rendered image is shown in Fig. 5 (Video 2). Viewed as a projection on the coronal plane, the blood vessels in the ectocervix are more likely to have a small aspect ratio and to be oriented toward the sagittal plane. In addition, the morphology of the vascular network clearly varies from one tissue to another. For example, blood vessels longer than 2 mm are absent in the ectocervix [Fig. 4(a)], but these long blood vessels can be easily found in the human aspect ratio.
sublingual mucosa [Fig. 4(c)]. Additionally, we carefully investigated the imaged tissue to demonstrate the safety of fsOR-PAE. Standard hematoxylin and eosin stain on the imaged area after fsOR-PAE imaging showed no evidence of tissue damage, necrosis, or heat injury [Fig. 4(d)].

After we validated the imaging capability and safety of fsOR-PAE, we tested this imaging modality in vivo on human subjects. Previous studies found that cervical remodeling during pregnancy was associated with increased vascularity.18,19 We enrolled (n = 2) pregnant women and imaged the anterior surface of the ectocervix for our study. The first pregnant woman was imaged at 32 weeks of gestation [Fig. 6(a)] and again at 36 weeks of gestation [Fig. 6(b)]. In this subject, we did not observe a perceptible change of vascular aspect ratio or blood vessel orientation over this time frame. This patient is 30 years old, had two prior deliveries, and labored at 38 weeks of gestation in our study.

To explore what physiological features can be quantified from the fsOR-PAE images, we extrapolate from the two vascular parameters5 that could have close relationships with cervical remodeling: (1) the microvessel density (the number of vessels per unit area) and (2) total microvascular area (the percentage of area occupied by blood vessels) as shown in Fig. 7. Each parameter was calculated from five images measured from different areas. In the analysis, the blood vessels were segmented in three-dimensional (3-D) space, using a threshold set at three times the noise level, estimated as the standard deviation of the background signal outside the imaged region. The segmented outcomes were visually inspected and corrected if necessary. Our results show that the microvessel density is the more promising parameter for identifying the progress of cervical remodeling [Fig. 7(a)]. The total microvascular area, however, is a more discriminatory parameter for classifying the type of tissue [Fig. 7(b)]. Of course, these conclusions require validation in larger, blinded preclinical studies.

In summary, we have developed an fsOR-PAE system that can achieve a 250-Hz B-scan rate over a 3-mm scanning range. This research presents the first high-resolution in vivo imaging of the vascular network in the human cervix, and its gestational age (36 weeks) [Fig. 6(c)]. This patient is 24 years old, had one prior delivery, and labored at 38 weeks of gestation in our study.
capillary-level spatial resolution is beyond the scope of current clinical methods. Further improvements could include minimizing the size of the probe to reach smaller cavities in the human body and exploiting a dual-wavelength light source to quantify oxygen metabolism. Furthermore, with the development of artificial intelligence, emerging classification models may divulge latent information which is beyond human recognition, but more valuable for diagnosis than the conventional histomorphological quantities in the fsOR-PAE images.21,22

Disclosures
K. Maslov has a financial interest in Microphotoacoustics, Inc. L. V. Wang has a financial interest in Microphotoacoustics, Inc., CalPACT, LLC, and Union Photoacoustic Technologies, Ltd., which, however, did not support this work.

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References
1. M. Leslie, “Tumors’ do-it-yourself blood vessels,” Science 352, 1381–1383 (2016).
2. L. Li et al., “Single-impulse panoramic photoacoustic computed tomography of small-animal whole-body dynamics at high spatiotemporal resolution,” Nat. Biomed. Eng. 1, 0071 (2017).
3. Y. He et al., “In vivo label-free photoacoustic flow cytography and on-the-spot laser killing of single circulating melanoma cells,” Sci. Rep. 6, 39616 (2016).
4. P. Carmeliet and R. K. Jain, “Molecular mechanisms and clinical applications of angiogenesis,” Nature 473, 298–307 (2011).
5. S. Sharma, M. C. Sharma, and C. Sarkar, “Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis,” Histopathology 46, 481–489 (2005).
6. E. Crowley et al., “Liquid biopsy: monitoring cancer-genetics in the blood,” Nat. Rev. Clin. Oncol. 10, 472–484 (2013).
7. D. M. McDonald and P. L. Choyke, “Imaging of angiogenesis: from microscope to clinic,” Nat. Med. 9, 713–725 (2003).
8. L. Lin et al., “Handheld optical-resolution photoacoustic microscopy,” J. Biomed. Opt. 22, 041002 (2017).
9. L. Li et al., “Fully motorized optical-resolution photoacoustic microscopy,” Opt. Lett. 39, 2117 (2014).
10. S. Oladipupo et al., “VEGF is essential for hypoxia-inducible factor-mediated neovascularization but dispensable for endothelial sprouting,” Proc. Natl. Acad. Sci. U. S. A. 108, 13264–13269 (2011).
11. J. Yao et al., “Label-free oxygen-metabolic photoacoustic microscopy in vivo,” J. Biomed. Opt. 16, 076003 (2011).
12. J. Yang et al., “Simultaneous functional photoacoustic and ultrasonic endoscopy of internal organs in vivo,” Nat. Med. 18, 1297–1302 (2012).
13. C. Li et al., “Urogenital photoacoustic endoscopy,” Opt. Lett. 39, 1473–1476 (2014).
14. J. Yang et al., “Optical-resolution photoacoustic endoscopy microscopy in vivo,” Biomed. Opt. Express 6, 918–932 (2015).
15. M. Strathman et al., “MEMS scanning micromirror for optical coherence tomography,” Biomed. Opt. Express 6, 211–224 (2015).
16. J. Yao et al., “Wide-field fast-scanning photoacoustic microscopy based on a water-immersible MEMS scanning mirror,” J. Biomed. Opt. 17, 080505 (2012).
17. American National Standards Institute, American National Standard for the safe use of lasers, American National Standards Institute, New York (2000).
18. R. A. Word et al., “Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concept,” Sem. Reprod. Med. 25, 69–79 (2007).
19. C. M. O’Brien et al., “In vivo Raman spectroscopy for biochemical monitoring of the human cervix throughout pregnancy,” Am. J. Obstet. Gynecol. 218, 8528 (2018).
20. Y. Liang et al., “2 MHz multi-wavelength pulsed laser for functional photoacoustic microscopy,” Opt. Lett. 42, 1452–1455 (2017).
21. K. Yu et al., “Predicting non-small cell lung cancer prognosis by fully automated microscopic pathology image features,” Nat. Commun. 7, 12474 (2016).
22. A. Esteva et al., “Dermatologist-level classification of skin cancer with deep neural networks,” Nature 542, 115–118 (2017).