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Retinal and choroidal imaging *in vivo* using integrated photoacoustic microscopy and optical coherence tomography

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Abstract

Most reported photoacoustic ocular imaging work to date uses small animals, such as mice and rats, the eyes of which are small and less than one-third the size of a human eye, which poses a challenge for clinical translation. Here we achieved chorioretinal imaging of larger animals, i.e. rabbits, using a dual-modality photoacoustic microscopy (PAM) and optical coherence tomography (OCT) system. Preliminary experimental results in living rabbits demonstrate that the PAM can noninvasively visualize depth-resolved retinal and choroidal vessels using a safe laser exposure dose; and the OCT can finely distinguish different retinal layers, the choroid, and the sclera. This reported work might be a major step forward in clinical translation of photoacoustic microscopy.

Keywords

Ophthalmology; photoacoustic microscopy; PAM; optical coherence tomography; ophthalmic optics and devices; chorioretinal imaging; retinal imaging

1. INTRODUCTION

Numerous causes of visual impairment and blindness are preventable through earlier diagnosis and improved monitoring of patients, which can be achieved through improvements in eye imaging. Current ophthalmic clinical imaging techniques include fundus photography [1], fluorescein angiography (FA) [1], indocyanine green angiography (ICGA) [1], and optical coherence tomography (OCT) [1, 2]. Fundus photography can

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capture color fundus images with excellent contrast but only provides structural information. FA permits visualization of retinal circulation in detail, but its role in studying choroidal circulation is limited since fluorescein is freely permeable in choroidal vessels. Using a larger fluorescence molecule with 98% bound to plasma, ICGA can reveal choroidal circulation, but the images are difficult to interpret. In addition, both FA and ICGA are invasive, require injection of an exogenous dye, provide limited physiologic information, and may cause complications such as nausea, emesis, and anaphylactic reactions which can lead to death [3]. Without the need of an exogenous dye injection, OCT and OCT angiography can provide structural and functional information of retina, but they often have limited visualization of the choroid and molecular imaging capabilities remain rudimentary [4, 5].

As a novel technology based on energy conversion from nanosecond pulse duration laser light to ultrasound, photoacoustic imaging is an emerging, noninvasive modality being extensively explored in various biomedical applications [6–14]. Photoacoustic imaging can not only provide structural information, but also can reveal functional and molecular details of biological tissue and is well-suited for ophthalmic applications. First described in 2010, photoacoustic ocular imaging has been an active field and continues to arouse interests of scientists and clinicians of the optics and ophthalmic communities [15–22].

However, most reported work in photoacoustic ocular imaging so far uses mice and rats as animal models [23, 24], the eyeball of which is small and less than one-third the size of a human eye (axial length of mice eyeball ~3 mm, rats ~6 mm, humans ~23mm). Using animals like rabbits with larger eyes [25] may accelerate its clinical translation. In this work, we report label-free chorioretinal imaging in living rabbits based on a novel custom-built photoacoustic microscopy (PAM) and spectral-domain OCT (SD-OCT) imaging platform [26, 27].

2. METHODS

2.1 Imaging system

The custom-developed, high-speed, high-resolution, multimodal retinal imaging system (Fig. 1) integrates two modalities: photoacoustic microscopy (PAM) and spectral domain optical coherence tomography (SD-OCT). The PAM system employs a pulsed laser (Ekspla NT-242, Lithuania) with tunable wavelengths (405–2,600 nm) as the illumination source, a two-dimensional galvanometer as the scanning mechanism, an achromatic scan lens (LSM 03-BB, Thorlabs) as the objective, an ophthalmic lens (AC080–010, Thorlabs) to relay the laser beam to the retina, and a transducer (center frequency: 35 MHz, –6 dB bandwidth: 50%) as the photoacoustic signal sensor. The Thorlabs Ganymede-II-HR SD-OCT system, center wavelength: 905 nm, was coaxially aligned with the PAM.

High-speed imaging is achieved by taking advantage of the high pulse repetition rate (1 kHz, pulse energy: 2.6 mJ @ 532 nm) of the laser and fast scanning rate (36 kHz) of the two-dimensional galvanometer. High lateral resolution (4.1 μm in PAM and 3.8 μm in OCT at the focal plane of the scan lens) is achieved by taking advantage of the well-compensated scan lens. High axial resolution (37.0 μm in PAM and 4.0 μm in OCT) is achieved by taking

advantage of the broad bandwidth of the ultrasonic transducer in PAM and broad bandwidth of the superluminescent diode in OCT.

2.2 Animal preparation

All rabbit experiments were performed in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research, after approval of the laboratory animal protocol by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan (Protocol PRO00006486, PI Yannis Paulus).

Rabbits were anesthetized with a mixture of ketamine (40 mg/kg) and xylazine (4 mg/kg) by intramuscular injection. Pupillary dilation was achieved by tropicamide 1% and phenylephrine hydrochloride 2.5%. Topical tetracaine 0.5% was instilled in the treated eyes before imaging. After anesthesia and pupil dilation, rabbit fundi were first examined using a fundus camera (TRC 50EX, Topcon Corporation, Tokyo, Japan), and then imaged using the dual-modality system. A water-circulating blanket (TP-700, Stryker Corporation, Kalamazoo, MI) was used to keep the body temperature of the rabbits. During experiments, eyewash (Altaire Pharmaceuticals, Inc., Aquebogue, NY) was applied to rabbit cornea frequently to prevent corneal dehydration. Vitals, including mucous membrane color, heart rate, respiratory rate, and rectal temperature, were monitored and recorded every 15 minutes. Post experiment, the rabbit fundi were re-examined using the Topcon TRC 50EX fundus camera to note any morphological changes of blood vessels due to laser exposure.

3. RESULTS

A total of three New Zealand albino rabbits (~6 months old, ~3 kg body weight) were used in the imaging experiments [Fig. 2(a)]. Figure 2(b) shows a photograph of a rabbit fundus, demonstrating that retinal blood vessels are confined to the medullary ray and the remaining retina is avascular.

Figure 2(c) shows the MIP of a PAM image of blood vessels within the medullary ray, where single retinal and choroidal vessels are discernible. The sizes of major retinal vessels are estimated from 70 to 100 μm , which are in good agreement with previously reported anatomical data [28]. Three-dimensional (3D) images of the fundus were reconstructed and rendered (Amira, FEI, Hillsboro, Oregon) according to the time-of-flight (TOF) information of the photoacoustic signals, as shown in Fig. 2(d). Figure 2(e) illustrates three orthogonal slices of the volume, showing the sections of depth-resolved retinal and choroidal vessels. The estimated thickness between retinal vessels and choroidal vessels in the medullary ray region is about 200 – 300 μm , which is within the resolving ability (37.0 μm axial resolution) of the PAM system and in agreement with anatomic data. The finding also agrees with the OCT image shown in Fig. 2(f), in which retinal vessels and the NFL are easily seen.

Figure 3(a) displays a photograph of the rabbit fundus inferior to the optic nerve, showing numerous, dense choroidal vessels. Figure 3(b) shows the MIP of a typical PAM image acquired in the experiment. A similar region was also scanned by the OCT system. The result illustrated in Fig. 3(c) confirms the presence of blood vessels in the choroid.

In the PAM experiments presented above, the raster-scan regions on the retina have a typical field of view (FOV) of about 3×3 mm (limited by the FOV of the ultrasonic transducer) with 256×256 pixels. The laser energy used in PAM is about 80 nJ at 570nm [26], which is well below the American National Standards Institute (ANSI) safety limit of 160 nJ [29].

4. CONCLUSION

We demonstrate the first label-free chorioretinal imaging in living rabbits based on a custom-developed photoacoustic microscopy (PAM) and spectral-domain OCT (SD-OCT) imaging platform. Preliminary results in rabbits show that the PAM can noninvasively resolve retinal and choroidal blood vessels with single blood-vessel discernible resolution using a laser exposure dose (80 nJ) well below the ANSI safety limit (160 nJ) at 570 nm; and the OCT could finely distinguish different retinal layers, the choroid, and the sclera. Since rabbits have an eye closer in size to that of humans, the presented work may have a major impact on the clinical translation of photoacoustic microscopy of the eye.

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Fig. 1.

In vivo retinal and choroidal imaging using integrated photoacoustic microscopy (PAM) and spectral domain optical coherence tomography (SD-OCT). OPO: Optical parametric oscillator; SLD: superluminescent diode; BS: beam splitter; DM: dichroic mirror; DCG: dispersion compensation glass; OL: ophthalmic lens.

Fig. 2.

PAM and OCT dual-modality imaging of retinal blood vessels in rabbits. (a) Experimental photograph showing the rabbit, the ophthalmic lens, and the transducer. (b) Fundus photograph showing rabbit retinal vessels (RVs). (c) Maximum intensity projection (MIP) of PAM signals of RVs and choroidal vessels (CVs). (d) 3D volumetric rendering of the PAM image. (e) 2D orthogonal slices of the PAM image showing RVs and CVs at different depths. (f) OCT image showing RVs, the nerve fiber layer (NFL), and different retinal layers.

Fig. 3. PAM and OCT dual-modality imaging of choroidal vessels (CVs) in living rabbits. (a) Fundus photograph demonstrating the distribution of CVs in living rabbits. (b) MIP of PAM signals of CVs. (c) OCT image showing different retinal layers, the choroid, and the sclera.