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Optical coherence photoacoustic microscopy for *in vivo* multimodal retinal imaging

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Abstract

We developed an optical coherence photoacoustic microscopy (OC-PAM) system, which can accomplish optical coherence tomography (OCT) and photoacoustic microscopy (PAM) simultaneously by using a single pulsed broadband light source. With a center wavelength of 800 nm and a bandwidth of 30 nm, the system is suitable for imaging the retina. Generated from the same group of photons, the OCT and PAM images are intrinsically registered in the lateral directions. To test the capabilities of the system on multimodal ophthalmic imaging, we imaged the retina of pigmented rats. The OCT images showed the retinal structures with quality similar to conventional OCT, while the PAM images revealed the distribution of absorbers in the retina. Since the absorption of hemoglobin is relatively weak at around 800 nm, the NIR PAM signals are generated mainly from melanin in the posterior segment of the eye, thus providing melanin-specific imaging of the retina.

Keywords

(110.4500) Optical coherence tomography; (110.5120) Photoacoustic imaging; (170.4470) Ophthalmology

Optical coherence tomography (OCT) [1,2] is a low-coherence interferometry-based three-dimensional imaging modality, which has been widely used in ophthalmology and animal research for high-resolution imaging of the retina. In OCT, the contrasts are provided by the photons backscattered from the biological tissues, which carry information about mainly their scattering properties. OCT can thus provide imaging of the micro-structures of

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biological tissues like the layered structures of the retina. The development of OCT has greatly changed the landscape of diagnosis and patient care in ophthalmology clinics.

Photoacoustic microscopy (PAM) [3–7] is a novel microscopic three-dimensional noninvasive imaging modality that is used for imaging the microvasculature and the associated blood oxygenation of biological tissues [8]. PAM is based on the optical-absorption properties of biological tissues. When irradiated by a short laser pulse, the optical energy absorbed by a substance, such as hemoglobin or melanin in tissue, is converted to heat, which induces localized thermo-elastic expansion and leads to the generation of wideband ultrasonic waves. The ultrasonic waves can be detected with an ultrasonic transducer to reconstruct an image mapping the location and absorption strength of the absorbers.

Multimodal imaging with combined OCT and PAM is able to provide both the optical scattering and optical absorption contrasts of a sample. Previous studies about integrated OCT and PAM imaging have demonstrated the complementary nature of the contrasts provided by the two imaging technologies. The combined OCT and PAM is thus potentially able to provide more comprehensive imaging of a subject [9,10]. As an example, combined OCT and PAM is able to provide simultaneous imaging of the retinal structures, retinal vasculature, and melanin in the retinal pigment epithelium [5,11]. By combining Doppler OCT and multi-spectral PAM, it is also possible to quantify the metabolism of oxygen in biological tissues *in vivo* [12,13].

The two technologies, however, have different requirements for their light sources. OCT needs a broadband light source to achieve depth resolution, whereas PAM requires a pulsed laser to deposit the light energy in the absorber in a time scale shorter than the requirements of thermal and stress confinement [14]. Thus, two different light sources are usually used to achieve simultaneous OCT and PAM imaging.

Using a single light source to achieve simultaneous OCT and PAM imaging was first reported in 2012, in which a dye-laser-based pulsed broadband light source centered at 580 nm was used [15]. The technique was termed optical coherence photoacoustic microscopy (OC-PAM) because OCT and PAM are an integral part of the technology. To demonstrate the feasibility of imaging biological tissues, the system was successfully used to image a mouse ear in a transmission mode. Lee et al. described an OC-PAM system in the near infrared (NIR) spectral range [16]. However, no *in vivo* imaging of biological samples was presented in their report.

Because of its better penetration depth and better tolerance by the eye of an imaging subject, NIR light is more suitable for imaging the retina. Most ophthalmic OCT systems are in the NIR and have a center wavelength of ~830 nm [1,17,18]. As a result, we believe an OC-PAM system at a center wavelength of around 830 nm will be more suitable for retinal imaging. Since the optical absorption coefficient of hemoglobin in the NIR is much smaller than that within the visible spectrum, a PAM in NIR would not be expected to provide good contrast for imaging the retinal vasculature. In contrast to hemoglobin, melanin in the RPE cells and in the choroid has broad absorption spectrum extending from the visible to the NIR

[19,20]. As a result, we expect that melanin will be the major contributor to the signals of the NIR-PAM imaging mode.

Figure 1 shows a schematic of the experimental system. A commercial ultrafast Ti:sapphire laser amplifier (Legend Elite HE+ USX-10K-I, Coherent Inc.) operating under an unseeded mode was used as the light source. The light source is able to provide light pulses with the following parameters: pulse energy >0.1 mJ; center wavelength, 800 nm; bandwidth, 30 nm; pulse duration, 3 ns; pulse repetition rate (PRR), 10 kHz. The output laser pulses were first attenuated to the desired energy with a series of neutral density filters. The beam size was reduced with a beam reducer to fit the aperture of the optical components. The laser pulses were then coupled into the source arm of a 2×2 single-mode optical fiber coupler, which forms the basis of a fiber-based Michelson interferometer. The bare-fiber tips of the output arms of the 2×2 fiber coupler were polished to 8° to reduce back reflection from the glass-air boundaries. In the sample arm, the light output from the fiber was collimated and scanned by an x - y galvanometer scanner. The light was delivered to the eye through the combination of an achromatic lens L1 ($f = 75$ mm) and an ocular lens L2 ($f = 20$ mm, 49322INK, Edmund Optics). The light pulse energy was measured to be 400 nJ at the surface of the eye.

For the OCT imaging mode, the combined reflected light from the sample and reference arms of the interferometer was detected in the detection arm with a spectrometer, i.e., accomplish the OCT function in the spectral domain. The spectrometer consisted of a 1200-line/mm transmission grating, an imaging lens ($f = 150$ mm), and a line scan CCD camera (AVIIVA EM2 $2k \times 14\mu\text{m}$ pixel size, e2V). The theoretical depth resolution of the OCT mode was $9.4 \mu\text{m}$.

For PAM imaging mode, the induced photoacoustic waves from the sample were detected by a custom-built needle ultrasonic transducer (30 MHz; bandwidth: 50%; active element diameter: 0.4 mm). When imaging the retina, the ultrasonic transducer was placed in contact with the eyelid coupled with ultrasound coupling gel. The detected photoacoustic (PA) signals were first amplified by 40 dB, and then digitized and streamed to the computer by a high-speed 14-bit digitizer (PCI-5122, National Instruments) at a sampling rate of 100 MS/s. Synchronization among the output of the laser pulse, scanning of the galvanometer, acquisition of the OCT interfering spectrum, and acquisition of PAM data was achieved by a multi-channel digital delay generator (DG645, Stanford Research Systems). A synchronization signal from the light source was used to trigger the delay generator. The outputs of the delay generator served as the sample clock of an analog output board (PCI-6731, National Instruments), which controlled the x - y galvanometer scanner, and triggered the image acquisition board for the CCD camera of the spectrometer and digitizer for acquisition of the PAM signals.

Figure 2 shows the measured spectrum of the light source and the corresponding point-spread function (PSF) of the OCT system. The PSF was measured with a mirror as the sample and the path length difference was set at 1 mm. The measured depth resolution is $9.9 \mu\text{m}$ in air, which well agreed with the theoretical prediction.

To test the capabilities of the system for imaging biological tissues *in vivo*, we imaged the eyes of Long Evans rats (body weight: 600 g, Charles Rivers). The animals were anesthetized by intraperitoneal (IP) injection of a cocktail containing Ketamine (54 mg/kg body weight) and Xylazine (6 mg/kg body weight). Then we dilated the rats' pupils with 0.5% tropicamide ophthalmic solution. Artificial tears were applied to the animals' eyes every 2 min to prevent dehydration of the cornea and cataract formation. After anesthetization, the rats were restrained in an animal mount, which was fixed on a five-axis platform. All experiments were performed in compliance with the guidelines of the Florida International University's Institutional Animal Care and Use Committee.

Figure 3 shows the simultaneously acquired OCT and PAM images of a rat eye. Figure 3(a) shows the OCT fundus images generated from the acquired 3D OCT dataset [18]. Figure 3(b) shows the maximum-amplitude projection (MAP) of the photoacoustic dataset. Both 3D datasets consist of 256×256 A-lines (depth scans) covering a retinal area of $1.7 \text{ mm} \times 1.7 \text{ mm}$. Since both OCT and PAM images are generated from the same group of photons, they are automatically and precisely registered in the lateral directions, which is evidenced in the figure. The lateral positions of each pixel of OCT and PAM image are determined by the scanning of the light pulse. Each light pulse contributes to both one A-line of OCT and one A-line of PAM.

We can see that the projected OCT and PAM images show significantly different features of the retina, although the corresponding signals were generated from the same group of photons. The OCT fundus image shows clearly the structure of the major retinal blood vessels, while the contrast of these blood vessels to the background of the PAM image is so small that they are barely recognizable. In the projected PAM image, we also see thinner blood vessel-like shadows in areas between the major retinal blood vessels, and these shadows are absent in the OCT fundus image. Our previous retinal imaging results of pigmented rats with visible light PAM have shown that except the retinal blood vessels, the photoacoustic signals also come from melanin in the RPE cells. In the current PAM image, if the photoacoustic signals come from the RPE layer as in the case of visible light PAM, one would expect that the shadows are casted by the retinal vessels because only the retinal vessels are in front of the RPE layer and can block the illuminating light. However, in the wavelength range of the light source, the light absorption by hemoglobin in the retinal vessels is weak. As a result, although the major light absorbers are melanin either in the RPE cells or in the choroid, the shadows were unlikely casted by the small retinal vessels.

A careful analysis of the B-scan images of OCT and PAM indicated that the shadows are corresponding to the locations of choroidal vessels, as shown in Fig. 4. The simultaneously acquired OCT [Fig. 4(a)] and PAM [Fig. 4(b)] B-scan images, each of 2048 A-lines, clearly show that the shadows in the PAM image correspond well with choroidal vessels in the OCT image [Fig. 4, vertical lines]. A shadow casted by a retinal blood vessel [Fig. 4(a), red arrow] also casted a shadow in the PAM image.

To further verify that many of the shadows in the PAM image were choroidal vessels, we performed histological analysis on one of the rat eyes. Cross-sections of the eye were obtained with a cryostat. From the histological image [Fig. 5(a)], it is clear that the

concentration of melanin in the choroid is much higher than that in the RPE, indicating that choroidal melanin contributes much more to the absorption contrast than the RPE melanin in PAM images. The absence of melanin in choroidal blood vessels and concentrated melanin in the surrounding tissue should produce high contrast images in PAM with weak absorption at the sites where the choroidal blood vessels locate and much stronger signal in surrounding tissue. These features are in good agreement with the weak signals of blood vessels in photoacoustic image. In addition, the choroidal blood vessels in the histological image [Fig. 5(a), red arrows] match well the choroidal vessels in the OCT cross-sectional image [Fig. 5(b), red arrows]. These results provide strong evidence that the shadows in PAM image are the distribution of choroidal blood vessels.

Studies by other groups have shown that differences exist between the melanin concentrations in the RPE between rodents and primates (results not published). The rat's RPE layer could not be imaged by PAM, whereas the RPE layers of monkey and primates could be. We haven't performed such a study yet, but we believe this is what we can further prove with this system in the future.

The maximum permissible exposure (MPE) at 800 nm for the eye is calculated to be 305 nJ for single pulse illumination according to the ANSI laser safety standard. When multiple light pulses overlap in the retina in the case of short scanning range, the MPE will be lower. As a result, the pulse energy of ~400 nJ in this experiment is higher than the MPE due to the sensitivity limitation of the ultrasonic transducer. The pulse energy can be lowered when a transducer with better sensitivity is available. Although the high pulse energy used in the experiments, we did not see any damage to the rat retina after at least ten times of imaging experiments in a period of 6 months.

Comparing with PAM using a monochromatic light source, the broader bandwidth in OC-PAM may have a negative effect on the signal intensity of PAM imaging depending on the absorption spectrum of the absorber. Because the absorption coefficient of RPE melanin decreases monotonically with wavelength in the visible and NIR spectral range, as long as the spectrum of the light source is symmetric, the bandwidth of the light source should not affect the signal-to-noise ratio. Achromatic aberration of the optical system including that of the eye may affect the lateral resolution of both the two imaging modes and thus the energy deposition in the absorbers. This may affect the signal intensity of PAM. In the current experiment, the bandwidth of the light source is only 30 nm, $\frac{\Delta\lambda}{\lambda} = 3.8\%$, thus the effect can be neglected.

The PRR of the light source is 10 kHz, i.e., the imaging speed is 10,000 lines/s, which is not as high as conventional spectral-domain OCT. Increasing the PRR can increase the imaging speed. However, increasing the PRR will also increase the average power of the light source, which may cause laser safety issues in practical applications when there is pulse overlapping in the retina, i.e., repetitive exposure in a single spot. Thus, in practical applications there is a tradeoff between pulse energy and PRR. In all situations, an ultrasonic transducer with high sensitivity is a key for reducing the laser pulse energy and thus makes it safe for eye imaging.

In conclusion, we have demonstrated the feasibility of an OC-PAM system working in the near-infrared. By using a single pulsed broadband NIR light source, OC-PAM can image the scattering and absorption contrasts simultaneously. This system can provide both deep-penetration depth as conventional OCT and melanin-specific absorption contrast, which is potentially suitable for human ophthalmic applications.

Acknowledgments

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Fig. 1. Schematic of the experimental system of a fiber-based OC-PAM. L1, L2, lens; PC, polarization controller; UT, ultrasonic transducer; M1, M2, mirror; C1, C2, collimator.

Fig. 2. Performance of the OCT mode. (a) The measured spectrum of the light source. (b) The calculated PSF of the OCT subsystem.

Fig. 3. Simultaneously acquired OCT and PAM images of a rat eye. (a) OCT fundus images generated from the acquired 3D OCT dataset. (b) The maximum-amplitude projection (MAP) of the PA dataset. Bar: 200 μm .

Fig. 4. Simultaneously acquired OCT and PAM B-scan images. The red arrow indicates retinal blood vessel. Bar: 100 μm .

Fig. 5.
Comparison of the histologic image (a) with the OCT cross-sectional image (b) of rat retina.
Bar: 100 μm .