Optical microangiography of retina and choroid and measurement of total retinal blood flow in mice

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Abstract: We present a novel application of optical microangiography (OMAG) imaging technique for visualization of depth-resolved vascular network within retina and choroid as well as measurement of total retinal blood flow in mice. A fast speed spectral domain OCT imaging system at 820 nm with a line scan rate of 140 kHz was developed to image the posterior segment of eyes in mice. By applying an OMAG algorithm to extract the moving blood flow signals out of the background tissue, we are able to provide true capillary level imaging of the retinal and choroidal vasculature. The microvascular patterns within different retinal layers are presented. An en face Doppler OCT approach [Srinivasan et al., Opt Express 18, 2477 (2010)] was adopted for retinal blood flow measurement. The flow is calculated by integrating the axial blood flow velocity over the vessel area measured in an en face plane without knowing the blood vessel angle. Total retinal blood flow can be measured from both retinal arteries and veins. The results indicate that OMAG has the potential for qualitative and quantitative evaluation of the microcirculation in posterior eye compartments in mouse models of retinopathy and neovascularization.

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OCIS codes: (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging.

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1. Introduction

Ocular blood flow plays an important role in the normal function of vision. Abnormal ocular blood flow has been shown to be related to many ocular diseases, such as age-related macular degeneration, diabetic retinopathy, and glaucoma. Hence, visualization and quantification of ocular blood flow, especially the measurement of total retinal blood flow, is important to the diagnosis and management of different type of ocular diseases [1–3].

Optical coherence tomography (OCT) is a non-contact and non-invasive imaging modality that can provide high resolution, depth-resolved imaging of internal microstructures within the scanned tissue volume. Due to easy optical access to the posterior segments of the eye, OCT has become a widely used imaging technique in ophthalmology. Currently, structural OCT imaging has become a clinical standard in many areas of ophthalmic clinical diagnosis, and considerable attention in ophthalmic OCT has been paid to the functional imaging of blood flow. The development of Fourier domain optical coherence tomography (FDOCT) which provides markedly increased imaging speed and sensitivity [4] has enabled in vivo imaging of blood flow. Currently, functional imaging of blood flow using FDOCT can be divided into two categories: Doppler OCT and OCT angiography. By analyzing the Doppler effect of a flowing particle on light frequency, Doppler OCT allows the quantification of the blood flow speed. The most frequently used OCT technique to measure blood flow speed is the phase resolved Doppler OCT (PRDOCT) [5,6]. On the other hand, in order to visualize the microcirculation network, a number of strategies to enable better contrast of microvasculature components, which we termed OCT angiography, have been introduced during recent years. These OCT angiography methods generate contrast for blood flow based on either the phase [7–13] or intensity [14–16] or complex information [17–20] of FDOCT signals.

In addition to the visualization of ocular microvasculature, the quantitative assessment of retinal blood flow by imaging modalities may be important for the diagnosis and management of different ocular diseases. Retinal blood flow has been measured in both humans and small animals using the conventional Doppler OCT method; however, flow measurement was usually based on individual arterial or venous branches [21,22]. Wang et al have applied Doppler OCT to measure the total human retinal blood flow using circumpapillary scans with different scanning diameters centered at the optic nerve to intercept all of the retinal vessels for measuring the vessel Doppler angles [23,24]. All of these conventional Doppler OCT
techniques for quantitative assessment of retinal blood flow require the Doppler angle information to calculate the absolute blood flow velocity. However, angle measurement is sensitive to motion and prone to errors.

Recently, an *en face* Doppler OCT approach [25] which does not require blood vessel angle information, was proposed for the measurement of blood flow. This *en face* Doppler technique simplifies total blood flow measurement based on the fact that total flow in a vessel can be determined by the integral of the product of a differential area and velocity component perpendicular to the area over the cross-section of the blood vessel. Total blood flow can be calculated simply by summing all axial velocity components in an *en face* plane generated by an OCT raster scan centered at the optic nerve at a given depth location and multiplying the sum with an area calibration factor, which is readily measurable [26,27].

Rat and mouse models of ocular diseases are becoming increasingly utilized for systematic analysis and characterization of disease pathogenesis and responses to various treatment approaches [28,29]. Among these two, mouse models are especially valuable with the availability of the genome database and ease of genetic manipulation in this species. Mouse models are expected to play a vital role in research to understand mechanisms of disease and to develop new treatment methods such as gene therapy and stem cell therapy for glaucoma [30], retinal degeneration [31], and retinal vascular diseases [32].

Until now, most published studies using OCT to image the posterior eye in mice have been concentrated on imaging retinal micro-structures [33–35]. Since OCT allows high resolution cross-sectional visualization of retinal structures, it has proven to be useful for studying mouse models of retinal degeneration [36,37]. However, visualization of microvasculature and quantification of retinal blood flow in mouse eyes using OCT has yet to be accomplished.

As one of the earliest proposed OCT angiography methods, optical microangiography (OMAG) [17], is capable of generating 3D images of dynamic blood perfusion distribution within microcirculatory tissue beds by high-pass filtering the complex spectrum. Most recently, OMAG has been further developed into ultra-high sensitive OMAG (UHS-OMAG) [38] that dramatically improves the detection sensitivity of blood flow to a level that enables imaging of true capillary vessels within the scanned tissue volume. Unlike the phase-based approaches, OMAG directly analyzes the complex spectral interferogram to produce the imaging contrast, which essentially minimizes the instability coming from the phase noise. UHS-OMAG has been successfully applied to image microvasculature at the capillary level within human retina and choroid [39], and was extended to small animal studies, including imaging microvasculature within the meninges and brain cortex [40] and kidney [41] in mice.

In this work, we first developed a high speed SDOCT/OMAG system for imaging the posterior eye of mice. Then we applied an OMAG scanning protocol and post processing algorithm to achieve high quality imaging of microvasculature within the retina and choroid. We show different microvascular patterns within different retinal layers and choroid after segmentation. We also applied an existing *en face* Doppler method [26,27] to measure the total retinal blood flow in mice, and the total retinal blood flow values were measured through integration of retinal arteries and veins, respectively.

2. Experimental methods

2.1. Animal preparation

22 week-old adult female Black and Tan Brachyuric (BTBR) mice (~30 ± 1g weight) were used in this study. The mice were anesthetized with inhalational isoflurane (1.5%) mixed with 80% air and 20% oxygen, and positioned for OCT/OMAG imaging in a custom-made head holder. Before OCT imaging, the pupil was dilated with eye-drops (10% phenylephrine hydrochloride ophthalmic solution, USP, AK-Dilate) to allow OCT beam access to the
posterior segment. The laboratory animal protocol was approved by the Animal Care and Use Committee of University of Washington.

2.2. Spectral domain OCT/OMAG system

In this study, we built a high speed spectral domain OCT/OMAG system as shown in Fig. 1 to image the posterior eye of mice. The system used a superluminescent diode (SLD, Superlum, Ireland) with a central wavelength of 840 nm and a bandwidth of 42 nm, which provides a theoretical axial resolution of ~7 μm. The light from the SLD was split into two paths with an 80:20 fiber based beam splitter (Thorlabs, Inc. USA) where the 80% power path goes to the sample arm while the 20% power path goes to the reference mirror. The sample arm beam was scanned by a galvo-mirror (Cambridge Technology, USA) in both x and y directions. After the scanner, an objective lens with a 75 mm focal length and an ocular lens (Ocular Instrument, Maxlight OI-HM 90D) were employed to adjust the beam size and enable large field of view. With this design, the sample beam size at the cornea was measured to be ~0.5 mm, which allows for the entire beam to pass through the dilated mouse pupil and reach the retinal and optic nerve surface of the eye. The theoretical lateral resolution was estimated to be ~12 μm using existing mouse eye models.

![Fig. 1. Schematic of the high speed spectral domain OCT/OMAG system. CMOS: line scan camera, PC: polarization controller, SLD: superluminescent diode.](image)

The custom-built high speed spectrometer was consisted of a collimating lens with an effective focal length of 100mm, a 1200 lines/mm transmission holographic grating, a 100mm scan lens and a CMOS line scan camera (Basler Sprint spL2048-140 km). The line scan camera had 10 μm square pixels in two rows and was 2048 pixels wide. However in order to increase the camera speed, only 896 pixels were illuminated and the camera was read at an exposure time of 5.7μs which provided a line scan rate of 140 kHz. The total depth range was measured to be ~2.5 mm in air. The power of the OCT beam at the cornea of mouse eye was ~1.2 mW. The measured signal to noise ratio (SNR) was ~100 dB at the focus spot of the sampling beam, which was ~0.5 mm below the zero delay line. The system had a 6 dB sensitivity roll-off at the depth of ~1.5 mm.

2.3. Visualization of ocular microcirculation with UHS-OMAG

Ultrahigh sensitivity OMAG (UHS-OMAG) has been demonstrated to be able to achieve:4 μm/s low sensitivity by switching the conventional OMAG algorithm onto the slow scanning axis. The underlying mechanism of this markedly increased sensitivity relies on the fact that a
longer time-interval was used to sample slow blood movement. Hence, in order to achieve high quality 3D imaging of microvasculature within the mouse retina and choroid, we adopted UHS-OMAG scanning protocol and data processing algorithm.

The data acquisition was achieved by the scanning protocol similar to that of reported in [41]. We captured 512 A-lines for each B-frame and 400 cross-sections covering a region of 2x2 mm² around the optic nerve head of mouse posterior eye. At each cross-section 5 B-frames were acquired to extract flow signals out of the background tissue. The line scan camera employed in the spectrometer was run at an A-line scan rate of 140 KHz and 80% duty cycle, providing an imaging speed of 220 frames per second (fps) with 512 A-lines in each frame. Acquisition of the whole 3D data volume took ~9 seconds, which is acceptable for anesthetized animal imaging. A faster A-line scan rate camera would allow us to shorten this time and minimize the motion artifacts.

The acquired data was then processed with the UHS-OMAG algorithm. The essential principle of UHS-OMAG is the same as the traditional OMAG [17], except that UHS-OMAG applied high pass filtering along the slow scanning direction, rather than the fast scanning direction. Before the high pass filtering was applied to extract flow image, a high order phase compensation method [42] was applied to remove the motion artifact due to mouse head movement.

2.4. Measurement of total retinal blood flow rate

Total retinal blood flow measurement was performed using an elegant en face Doppler method demonstrated by Srinivasan et al [25]. Unlike conventional Doppler OCT methods, the knowledge of the blood vessel Doppler angle is not required. This method was later applied for measuring total flow in the human retina [26] and further developed to evaluate the pulsatile total blood flow in the human and rat retinas [27].

![Fig. 2. Schematic of en face Doppler approach for flow measurement. (A) In conventional Doppler OCT methods, the blood vessel angle $\Theta$ is required to compute absolute velocity values $V_{abs}$. Total blood flow is calculated by multiplying with $V_{abs}$ the cross-sectional area of the vessel $S$. (B) In En face Doppler method, total blood flow is computed by simply integrating the axial velocity components $V_z$ over the en face cross-section $S$ that intercepts the vessel.](image)

In conventional Doppler OCT methods (Fig. 2(A)), the axial velocity component of the blood vessel is first measured using phase resolved analysis. Absolute blood flow velocity is then calculated form the axial component corrected by the blood vessel Doppler angle $\Theta$. The area of the vessel in the en face (xy) plane is inversely proportional to $\cos (\Theta)$, whereas the axial velocity $V_z$ is proportional to $\cos (\Theta)$. Therefore, these two effects cancel when the integration is performed in the en face plane, meaning that the blood flow in a vessel can also be calculated by simply integrating the axial velocity components in an OCT en face plane that intercepts the vessel (Fig. 2(B)).

Measurement of axial flow velocity within a three-dimensional volume is required before the total retinal blood flow can be calculated. A three-dimensional data set was acquired...
consisting of 200 images with 1024 axial scans per image over an 800 μm square area in approximately 2 seconds. The axial flow velocity can be derived from phase differences between adjacent A-scans introduced by the motion of blood cells using PRDOCT. The relationship between them is:

\[ V_z = \frac{\Delta \phi \cdot \lambda_0}{4\pi n \Delta t} \]  

where \( \Delta \phi \) is the phase difference between adjacent A-scans; \( \lambda_0 \) is the central wavelength of the light source; \( n \) is the refractive index of tissue (~1.35); \( \Delta t \) is the time interval between adjacent A-scans as mentioned above.

Phase wrapping happens if \( |\Delta \phi| \geq \pi \), hence, the maximal axial velocity component which can be unambiguously measured with a given time interval \( \Delta t \) would be:

\[ V_{z_{\max}} = \pm \frac{\lambda_0}{4n \Delta t} \]  

With an acquisition rate of 140,000 A-scans per second which means \( \Delta t \) equals 7.1μs, the maximum axial velocity range that could be measured without phase wrapping was ± 20.6 mm/s in tissue. Phase wrapping occurred in the blood flow velocity measurement for some of the big retinal arteries, meaning that the phase unwrapping is required in order to obtain the true total retinal blood flow rate. The phase unwrapping method we used here has been discussed in detail in [43].

After above processing, a three-dimensional map of the axial velocity component \( V_z(x,y,z) \) was obtained, from which an en face plane bisecting all the retinal arteries and veins could be chosen. We can compute the absolute flow values by integrating the axial flow components over the vessel cross-section in the en face plane using the following equation:

\[ F = \iiint_{\text{xy-plane}} V_z(x,y)dx\,dy \]  

where \( V_z(x,y) \) is the axial flow velocity measured for different pixels and \( dx\,dy \) represents the actual size of one pixel in the en face plane.

3. Results and Discussion

3.1. Depth-resolved volumetric perfusion imaging of retina and choroid in mice

The captured three dimensional OCT data provides a fundus image as well as cross-sectional images of the structure in the posterior eye. The fundus image was obtained using Amira 3D software (Visage Imaging, Inc.) by integrating the structural signals along the depth direction (z axis). From the OCT fundus image, as shown in Fig. 3(A), we were able to visualize specific anatomy of the mouse posterior eye, including the optic nerve head, some large retinal vessels and optic nerve fiber bundles as indicated by the arrows. The cross-sectional layer structure of the retina and choroid in mouse eye is shown in Figs. 3B and 3(C). All the cross-sectional structure images were averaged for 5 times to reduce the speckle noise. From the images, we were able to recognize all the retinal layers as well as the choroid layer. Figure 3(D) shows one typical OCT image across the central retina, containing the optic nerve head (*). By processing the data with the UHS-OMAG algorithm, the corresponding blood flow image can be obtained that contains only the blood flow signal as shown in Fig. 3(E). Besides the microstructures of tissue, OMAG has the capability to provide detailed images of the vascular networks that support the tissue beds. There are two sources of blood supply to the retina: the central retinal artery and the choroidal blood vessels. The central retinal artery enters through the optic nerve head into the retina to nourish the inner retinal layers and the choroid receives the greatest ocular blood flow which is vital for the maintenance of the outer
Fig. 3. OCT imaging of morphology in mouse posterior eye. A) OCT fundus image of mouse posterior eye, arrows point to optic nerve fiber bundles. B) Recognition of all the mouse retinal layers and choroid layer. NFL: nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, ELM: external limiting membrane, IS/OS: junction between the inner and outer segment of the photoreceptors, RPE: retinal pigment epithelium layer, CH: choroid. C) OCT section across the periphery retina. D) OCT section across the central retina (optic nerve head *) and E) corresponding blood flow image. Scale bar = 200 µm.

retina (particularly the photoreceptors). Both vascular networks are important for the normal function of vision.

The UHS-OMAG technique can provide depth-resolved imaging (Fig. 4(A)) of the ocular microvasculature within different depths. The mouse retina consists of three layers of vascular networks [44]: retinal arteries and veins along with the radial peripapillary capillaries within the nerve fiber layer (NFL) and ganglion cell layer (GCL), capillary networks within the inner plexiform layer (IPL), and capillary networks within the outer plexiform layer (OPL). To better show the retinal vessel distribution and pattern in different retinal layers, we segmented these layers in order to render microvascular maps in different layers as shown in Figs. 4(B, 4(C), and 4(D).

The retinal arteries (red arrows) and veins (green arrows) within the NFL and GCL (Fig. 4(B)) are arranged alternately and radiate in spoke-like fashion towards the retinal periphery. The arteriole branches are mostly visible in NFL and GCL whereas the precapillary venules drain into veins are located in the bottom layers as pointed by the yellow arrows in Fig. 4(D). Dense capillary networks were well distributed and observed in IPL (Fig. 4(C)) and OPL (Fig. 4(D)). These capillary vessels are thought to supply the nutrients for the ganglion cells and nuclear cells [44]. We also noticed that the shadow effect of superficial vessels (artery and vein) is not so obvious that enables us to see connected capillary network at deeper layers (Figs. 4(C) and 4(D)).

With segmentation, we can also characterize the vascular map within the choroidal layer. Figure 5. shows the typical vascular perfusion map of choroid in mice eye. Notably, we were able to find two long posterior ciliary arteries entering the choroid from deeper retinal layers. Each of these then breaks up into fan-shaped lobules of smaller arterioles that supply localized regions of the choroidal capillaries. These choroidal capillaries are thought to nourish the outer retinal layers [44].
Fig. 4. (A) Color coded depth projection map of the retinal vascular network, blue: superficial, green: deep. (B-D) Vascular perfusion maps within different retinal layers in mouse eye after segmentation, where (B) is microvasculature within NFL and GCL, A: artery, V: vein, (C) within IPL, and (D) within OPL. Image size: 2x2 mm²

Fig. 5. Vascular perfusion map of choroid in mice eye. Arrows points to long posterior ciliary artery (LPCA) entering the choroid. ONH: optic nerve head.
3.2. Total retinal blood flow measurement in mice

Here, we present results for the total retinal blood flow measurement using the en face Doppler method. We first captured a 3D data using the UHS-OMAG scanning protocol to sharpen the angiographic appearance around the optic nerve head, which provides a map of the microvasculature as shown in Fig. 6(A). To measure the total retinal blood flow, a concurrent three-dimensional data set was captured using the raster scanning pattern described above (200 images with 1024 axial scans per image over an 800 µm square area) around the optic nerve head as well. The maximum projection view of axial flow velocity for the main retinal arteries and veins is shown in Fig. 6(B). As we can see from Figs. 6 (A) and 6(B), there are six main retinal arteries vein branches in this particular mouse retina. Bidirectional axial flow velocity was obtained and depicted colorimetrically: red denotes flow away from the optic disc (arteries) and green denotes flow towards the optic disc (veins). A potential confounding factor is that the retinal artery branches from ascending central retinal artery and first descends, which causes a change in the Doppler angle and results in the change of flow color as shown by the arrows in Fig. 6(B). However, this confounding factor can be obviated and will not affect the measurement of total retinal blood flow if an optimal en face plane that intercepts all vessels is selected for the measurement.

Figures 6(C) and 6(D) show en face plane view of axial blood velocity at one particular depth (~500 µm away from zero delay), where white squares denote the integration of arterial
flow and yellow squares denote the integration of venous flow. The measured blood flow rate in each single retinal artery or vein and the total retinal blood flow are shown in Table 1. For this particular mouse, the total retinal blood flow rate measured from the retinal arteries and veins are 2.82 (± 0.30) µl/min and 3.27 (± 0.28) µl/min as averaged by three independent measurements, respectively. These two values are close to each other, and the reason why the total arterial blood flow is lower is likely due to 1) the high pulsatility of flow in artery, 2) the error caused by the phase-unwrapping algorithm that might underestimate the arterial blood flow and 3) the imaging artifacts caused by optical aberration. Measurement of pulsatile total blood flow in the mouse retina could be accomplished with a faster camera speed OMAG system and proper scanning protocol. Notably, it is possible to measure the total retinal blood flow from en face planes at different depths. We present a video (Media 1) to better show how the axial blood flow velocity is distributed in the en face plane along different depths.

Table 1. Quantification of total retinal blood flow in mice

| Vessel No. | 1    | 2    | 3    | 4    | 5    | 6    | Total flow rate (µl/min) |
|------------|------|------|------|------|------|------|-------------------------|
| Artery     | 0.44 ± 0.04 | 0.55 ± 0.06 | 0.50 ± 0.05 | 0.48 ± 0.04 | 0.40 ± 0.06 | 0.45 ± 0.06 | 2.82 ± 0.30 |
| Vein       | 0.45 ± 0.04 | 0.62 ± 0.06 | 0.60 ± 0.04 | 0.59 ± 0.05 | 0.38 ± 0.03 | 0.63 ± 0.06 | 3.27 ± 0.28 |

4. Conclusions

In summary, we have presented high resolution images of the microcirculation within the retina and choroid, as well as the measurement of total retinal blood flow, in mouse eyes by the use of OMAG. The microvascular patterns within different retinal layers and choroid were visualized. An en face Doppler OCT approach without knowing Doppler angle was adopted for the measurement of total retinal blood flow. Total retinal blood flow could be measured from both retinal arteries, and the values match well. These results indicate that OMAG has the potential for qualitative and quantitative evaluation of the microcirculation of the posterior eye in disease models in mice. We are currently working on applying this technique to quantitatively evaluate changes in the microvasculature and the total retinal blood flow in mouse models of diabetes with early and potentially late diabetic retinopathy with neovascular proliferation.

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