Vascular morphology and blood flow signatures for differential artery-vein analysis in optical coherence tomography of the retina

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Abstract: Differential artery-vein (AV) analysis is essential for retinal study, disease detection, and treatment assessment. This study is to characterize vascular reflectance profiles and blood flow patterns of retinal artery and vein systems in optical coherence tomography (OCT) and OCT angiography (OCTA), and establish them as robust signatures for objective AV classification. A custom designed OCT was employed for three-dimensional (3D) imaging of mouse retina, and corresponding OCTA was reconstructed. Radially resliced OCT B-scans revealed two, i.e. top and bottom, hyperreflective wall boundaries in retinal arteries, while these wall boundaries were absent in OCT of retinal veins. Additional OCTA analysis consistently displayed a layered speckle distribution in the vein, which may indicate the venous laminar flow. These OCT and OCTA differences offer unique signatures for objective AV classification in OCT and OCTA.

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1. Introduction

As a functional extension of optical coherence tomography (OCT), OCT angiography (OCTA) allows noninvasive label-free imaging of retinal vasculature at the capillary level resolution [1,2]. Because vascular changes are often involved in the symptoms of various retinal diseases, such as glaucoma [3], diabetic retinopathy (DR) [4], age-related macular degeneration (AMD) [5], and retinitis pigmentosa (RP) [6], the analysis of retinal vasculature in OCTA has been widely implemented in clinics and preclinical research for the detection of early pathologies and the development of treatment strategies. However, a vessel map of OCTA is inherently limited to provide information on the vessel types. Artery-vein (AV) classification is of high interest since abnormal features on specific vessel types can be valuable inputs for detecting systemic and retinal diseases [7,8]. Kromer et al. showed altered retinal veins in Parkinson’s disease patients [9]. Abdelhak et al. found that the wall-to-lumen ratio of the retinal artery was correlated with the volume of white matter hyperintensities, microglial activation, and neuroaxonal damage in cerebral small vessel disease [10]. Reagan et al. reported that aging caused a significant reduction of vascular smooth muscle cells (SMCs) in focal patches along retinal arterioles [11]. In addition, venous beading has been shown in patients with hypertension and diabetes. AV caliber ratio has been also demonstrated as a predictor of retinal diseases [12].

To achieve AV classification in OCTA in clinical practice, color fundus image–guided method has been reported [13]. However, using two separate imaging modalities is a less effective strategy. Thus, recent approaches have been made to leverage features solely acquired from OCT and OCTA. Alam et al. demonstrated OCT intensity feature based analysis to guide AV classification in OCTA. A blood vessel tracking algorithm was employed to automatically generate the AV vessel maps [14]. Furthermore, Alam et al. have recently established a fully convolutional network AV-Net for AV classification in OCTA [15]. Xu et al. introduced a method that can
differentiate retinal veins from arteries in OCTA by identifying venous origin in the deep capillary plexus (DCP) [16]. Son et al. demonstrated the feasibility of using near infrared OCT oximetry to guide AV classification in OCTA [17].

OCTA has been also widely used in preclinical animal research [18–23]. Transgenic mice are commonly used for the study of retinal vascular pathology and development of treatment protocols of eye diseases. OCTA analysis of mouse models exhibiting vascular abnormalities on a specific vessel type, such as retinal artery and vein occlusion [24,25], hyperglycemia [26,27], neovascular disease [28], and neurodegenerative disease [29,30], would benefit from AV classification; however, characterization of arteries and veins in OCTA has not been well explored. Methodologically there are relatively more options available in animal study to differentiate the vessel types in OCTA. Fluorescein angiography (FA) is a standard method for classifying retinal arteries and veins [31]. However, it is rarely employed just for the purpose of AV classification due to an invasive nature. FA also requires the selection of adjacent arteries and veins for measurement to avoid misclassification [31]. Another gold standard is ex vivo fluorescent histochemical analysis [11]. However, this method does not allow long-term observation of the same animal at different time points and has limited capabilities to study retinal physiology and function. Functional OCT can be also utilized to guide AV classification in OCTA. Visible OCT oximetry enables accurate identification of the vessel type by quantifying oxygen level [32]. However, photoreceptor bleaching is unavoidable, limiting its usage to light-induced functional examinations [33]. Also, standard OCT is mostly built on near infrared light. Doppler OCT has been used to measure and compare the blood flow velocities in AV systems [34,35]. However, this technique requires extra scanning protocols with high sampling density to extract velocity components. Thus, it is inconvenient to be readily used for AV classification in typical OCTA. In some instances, descriptive features on en face OCT images can aid AV classification. For example, the retinal arteries are generally smaller than the veins, and the arteries have more branch points than the veins in the superficial vascular plexus (SVP) [36,37]. However, these descriptive features become complicated when the two vessel types reveal similar structures or patterns depending on retinal regions. In addition, there are always variations in the vessel distribution and arrangement in both the human and mouse retina [38]. Mutant mice often exhibited altered patterning of retinal vessels relative to wild-type mice [39]. Therefore, none of the descriptive features can be used as a global standard.

In this article, we demonstrate the feasibility of using radially resliced OCT for AV classification in OCTA of mouse retinas. Near infrared OCT and OCTA volumes, originally acquired by a standard raster scan, were resliced in polar coordinates, centered at the optic nerve head (ONH). This method allows the pseudo scanning lines to be aligned as much as parallel to the main vessels radially emanating from the ONH, disclosing continuous cross-sectional images of arteries and veins. Our approach provides decisive information for AV classification, i.e., different vessel wall boundary signals and intensity distributions inside the lumen. In addition, we found a unique pattern of laminar flow in the vein on radial OCTA B-scans.

2. Methods

2.1. Experimental setup

A custom-built OCT system was used in this study. The system has been used in our previous functional OCT and OCTA studies [40–42]. Briefly, a broadband near-infrared superluminescent diode ($\lambda = 810$ nm and $\Delta\lambda = 100$ nm; D-810-HP, Superlum, Carrigtwohill, County Cork, Ireland) was used as the OCT light source with 1 mW power illuminated on the mouse cornea. A linear CCD camera with 2048 pixels (AViiVA EM4; e2v Technologies, Chelmsford, UK) was used for recording OCT spectra in the custom-built OCT spectrometer. The frame speed of the camera was set to a 50-kHz A-scan rate. The axial and lateral resolutions were estimated as 3 and 11 $\mu$m, respectively.
2.2. Experimental procedure

B6SJLF1/J and C57BL/6J (The Jackson Laboratory, Bar Harbor, ME) mice were used in this study. For the imaging, the mouse was first anesthetized by a mixture of 100 mg/kg ketamine and 5 mg/kg xylazine. A drop of 1% tropicamide ophthalmic solution (Akorn, Lake Forest, IL) was applied to the imaging eye, and a cover glass (12-545-80; Microscope cover glass, Fisherbrand, Waltham, MA) with a drop of lubricant eye gel (Severe; GenTeal, Novartis, Basel, Switzerland) was placed. Once the mouse was fully anesthetized, the head was fixed by using a bite bar and ear bars. All animal care and experiments adhered to the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research. All experiments were performed following the protocols approved by the Animal Care Committee at the University of Illinois at Chicago.

2.3. Data acquisition and radial B-scan preparation

Volumetric raster scans were acquired over 1.2 × 1.2 mm retinal region. Each volume consisted of 500 A-lines for each B-scan, and four repeated B-scans at each slow scan position were collected. Thus, 4 × 500 × 500 A-scans were acquired for each OCT and OCTA volume. Speckle variance processing was used for OCTA volume reconstruction [43], and the repeated 4 B-scans were averaged for OCT volume construction. OCT and OCTA volumes were then resliced on the polar coordinate system (Fig. 1(A)). The center of the circle was specified at the ONH with radius 300 pixels, and the step angle was 1 degree to sweep the OCT and OCTA volumes, creating 359 radial B-scans. To achieve better contrast, each frame was averaged with adjacent 2 frames.

In addition, double circular scanning around the ONH with radii of 0.5 mm and 0.7 mm was implemented for Doppler OCT (DOCT). Each circular scan had 1500 A-lines. The phase differences of adjacent A-lines were calculated to draw the Doppler frequency shift, and the Doppler angle was calculated by relative position of each blood vessel in the two circular scans. The blood flow velocity was then calculated from the Doppler shift and Doppler angle [44–46]. When the peak flow had opposite direction at the center of the vessel compared to the edge of the vessel, phase unwrapping was applied to correct flow measurement. Total 10 DOCT images were averaged to mitigate phase noise.

2.4. Data analysis

In OCT and OCTA volumes, each major vessel consisted of several consecutive radial B-scans, thus the B-scans belonging to the same vessel were merged by maximum intensity projection, returning a single vessel image. The vessel area was then manually segmented and flattened by using the “Straighten” function in Fiji software. A-lines in each vessel image were then averaged in lateral direction, resulting in a line intensity profile per vessel, followed by profile length rescaling and intensity normalization. We first cropped the vessel region in the line intensity profile including extra 5 pixels above (vitreous side) and below (inner retinal side) the vessel wall boundaries, and each cropped intensity profile was then rescaled into 30 pixels using bilinear interpolation for comparison of intensity profiles between the vessels; thus, the vessel region, smaller or larger than 30 pixels, were upsampled or downsampled, respectively. 30 pixels were chosen considering the average pixel number along the vessel width and 10 extra pixels near the vascular boundaries. The intensity normalization was then performed by dividing the intensity of all pixels by the maximum pixel intensity. The normalization was independently processed in each line intensity profile. This process was applied to all major vessel branches (Fig. 2(C1) and (2D1)). Based on all the normalized vascular intensity profiles, an averaged intensity profile was further constructed and compared between arteries and veins via the two-sample Kolmogorov-Smirnov (K-S) test. The vessel width was manually measured on the flattened OCT images. Since mouse retinal vessels are exposed at the retinal surface, the posterior border of the vitreous was chosen as the upper boundary of the vessels, and the bottom lines of hyperreflective
Fig. 1. Representative en face OCT (A) and OCTA (B) volumes acquired by a raster scan protocol. To reconstruct radial B-scans, the center of circle with radius $r$ and step angle $\theta$ was specified at the ONH in polar coordinates. Radial OCT B-scans of an artery (C1), a vein (C2), and an avascular region (C3), and radial OCTA B-scans of an artery (D1), a vein (D2), and an avascular region (D3). White arrows in inset figures (C1 and C2) indicate the boundaries between the vessel and surrounding tissues. ONH: optic nerve head; IPL: inner plexiform layer. Scale bars: 100 $\mu$m.

vessel wall and hyperreflective zone were chosen as the lower boundary of arteries and veins, respectively, in the width measurement (inset figures in Fig. 1(C1) and 1(C2)). The two-sample Welch’s t-test was performed for the comparison between arteries and veins. P-value < 0.05 was considered statistically significant. Image reconstruction and processing were done by MATLAB R2016a (MathWorks, Natick, MA), and statistical analysis was done by Origin 2020b (OriginLab, Northampton, MA).
Fig. 2. Representative radial OCT B-scan images of 3 arteries and 3 veins. Arteries reveal hyperreflective boundaries along the vessel walls (yellow arrows), while veins lacking boundary signals show a hyperreflective zone at the bottom half of the vessel (blue arrow). Normalized intensity maps constructed from 39 arteries (C1) and 35 veins (D1). Each column represents single vessel information. Corresponding average intensity plots of artery (C2) and vein (D2). Solid curves represent the average values, and the shaded areas represent the standard deviations. Data were collected from 7 different mice. All images were processed and normalized on a linear scale. (E) En face OCT image of a mouse retina used in DOCT processing. Arteries and veins were independently classified by the radial OCT B-scans (F) and Doppler angle (G). Circular scanned OCT image (G1) and circular scanned DOCT image (G2) with blood flow velocity information. Red color indicates the artery, and blue color indicates the vein. ONH: optic nerve head. Scale bars: 200 µm.

3. Results

3.1. Radially sliced OCT and OCTA of retinal blood vessels

Volumetric raster scans were first obtained in the mouse retina. Figure 1(A) and 1(B) show the original en face OCT and OCTA volumes, respectively, and the corresponding B-scan fly-through movies were provided as Visualization 1. The radial slicing was performed through the entire volume with 1-degree step angle (Fig. 1(A)), creating a new set of B-scans parallel...
to the vessels. Since the first branches of the mouse retinal vessels radially emanate from the ONH, the radial B-scans can visualize continuous cross-sectional vessel images. While the OCT reveals reflectance intensity correlated with vascular morphology; the OCTA reflects reflectance variance due to blood flow activity. In other words, the OCT can be used for imaging vascular morphology, and OCTA can be used for mapping blood flow activity. In radial OCT B-scans, arteries surrounded by a thick layer of SMCs exhibited hyperreflective boundaries along the vessel wall (Fig. 1(C1)), lacking in veins surrounded by a thin membrane (Fig. 1(C2)). In addition, in both OCT and OCTA B-scans, arterial lumens showed a homogeneous intensity distribution (Fig. 1(C1) and (1D1)), while a layered intensity distribution was observed in the venous lumens (Fig. 1(C2) and (1D2)), which may indicate different blood flow signatures in arteries and veins. Using these vascular morphology and blood flow signatures in OCT and OCTA, AV classification was manually performed in the following sections.

3.2. Comparative analysis of reflectance profiles in retinal arteries and veins

Representative radial OCT B-scans in Fig. 2(A) and 2(B) illustrate the different reflectance profiles between artery and vein. To quantitatively analyze the OCT reflectance profiles across the vessel, normalized vascular intensity maps were constructed from all the first-branch vessels in 7 different B6SJLF1/J mice (Fig. 2(C1) and (2D1)). Figure 2(C2) and (2D2) show the average normalized intensity profiles from the intensity maps of arteries and veins, respectively. Two local maxima were observed in the arterial profile while the intensity profiles in veins were skewed towards the bottom half of the lumen. Difference of the two distributions was also confirmed by the two-sample K-S test ($P = .035$). In order to further verify the reflectance profile-based classification, we implemented dual-scan DOCT for comparative validation. Figure 2(E) shows en face OCT image of a mouse retina with 12 main vessel branches. TK classified the artery and vein based on the reflectance profiles of the radial OCT B-scans (Fig. 2(F)), and TS classified the artery and vein based on DOCT measurement independently. The identification of vessel types in DOCT was based on the calculated Doppler angle and Doppler frequency shift. Red color represents positive Doppler shift in arteries, i.e., blood flow moving away from the ONH, and blue color represent negative Doppler shift in veins, i.e., blood flow moving toward the ONH (Fig. 2(G2)). We found that these two classification results were entirely consistent (Fig. 2(E-G)), demonstrating a high reliability of AV classification by referring to the vascular morphology and blood flow signatures.

3.3. AV classification and characterization

We next checked the strain dependence of the reflectance profiles in two different mouse strains (B6SJLF1/J and C57BL/6J). These are popular mouse models widely used in ocular research. Figure 3(A) and 3(B) show representative en face OCT images and vascular images acquired by the pseudo radial scans, and radial B-scan fly-through movies were also provided as Visualization 2.

We confirmed that both strains unambiguously revealed the two types of reflectance profiles demonstrated in Fig. 2(A)–2(D). We next characterized the retinal vessels after AV classification. Figure 3(C) shows original en face OCTA images obtained from 4 different mice, and Fig. 3(D) shows corresponding AV classified images. Generally, for the 1st order branching, i.e. originating from the ONH, an alternating pattern between arteries and veins is observed; examples of this pattern are shown in Fig. 3(D). It is also known that 5–6 arteries and 5–6 veins are typically present in the mouse retina [47], and we confirmed that number of arteries and veins were comparable (B6SJLF1/J: 5.57 ± 0.53 for artery and 5 ± 0.82 for vein ($P = .15$); C57BL/6J: 5.86 ± 0.38 for artery and 5.57 ± 0.53 for vein, $P = .27$). Since the radial slicing method can be implemented in any OCT volumes containing the major vessel branch regardless of the imaging area, we successfully classified the first branches of arteries and veins in OCTA of peripheral retinal
Fig. 3. 

**En face OCT images and corresponding vascular images of a B6SJLF1/J mouse (A) and a C57BL/6J (B).** Red color for arteries and blue color for veins. Original en face OCTA images (C) and the same en face images with pseudo-colors (red for arteries and blue for veins) after AV classification (D). (E) AV classifications on peripheral retinal OCTA on different cardinal axes: Nasal (E1), dorsal (E2), ventral (E3), and temporal (E4) area. (F) Relative axial positions of artery (F1) and vein (F2) based on the ONH. (G) Box-scatter plot of vessel width measurement from seven B6SJLF1/J mice ($N = 39$ for arteries and $N = 35$ for veins) and seven C57BL/6J mice ($N = 41$ for arteries and $N = 39$ for veins). Scale bars: 200 µm.
areas (Fig. 3(E)) and confirmed that arteries branched into precapillary arterioles and distributed predominantly within the SVP. The radial B-scans also showed relative axial positions of arteries and veins. Arteries with outgoing flows were located above the major veins with incoming flows (Fig. 3(F)). Based on the cross-sectional vessel images, retinal vessel width was also measured (Fig. 3(G)), and we found that the venous size exhibited higher variations compared to the arterial size (F-test, \( P = .0014 \)). The thickest vessel in each mouse retina was vein, while arteries show relatively consistent sizes. The mean vessel width was comparable between arteries and veins in B6SJLF1/J (27.42 ± 3.10 µm for artery and 27.96 ± 5.33 µm for vein; \( P = .60 \)) as well as C57BL/6J (26.43 ± 3.09 µm for artery and 27.12 ± 5.52 µm for vein; \( P = .49 \)).

### 3.4. Blood flow patterns in retinal arteries and veins

A serendipitous observation in this study was the layered intensity distribution in the venous lumen (Fig. 2(B)). To better understand these intensity patterns, radial OCTA B-scans were reconstructed and overlaid on the OCT B-scans (Fig. 4(A) and 4(B)). Intriguingly the hyperreflective bottom half of veins on OCT was exactly matched with high speckle variations, presumably caused by moving blood cells in OCTA (Fig. 4(B3)), which may indicate a unique laminar flow in retinal veins. Likewise, the intensity pattern of the arteries in OCT were well matched with the arterial OCTA images that present homogeneous intensity distribution (Fig. 4(A3)). Real-time OCT and OCTA recordings (Visualization 3) further confirmed the unique speckle patterns inside the lumens of arteries and veins. Figure 4(C) shows 1000 frames averaged OCTA images, clearly displaying the difference between arteries and veins.
Fig. 4. Different blood flow patterns between artery (A) and vein (B). Artery in OCT (A1) and OCTA (A2) and composite of OCT and OCTA (A3). Highlighted red color indicates OCTA information. Vein in OCT (B1) and OCTA (B2) and composite of OCT and OCTA (B3). Highlighted blue color indicates OCTA information. (C) Averaged OCTA B-scan containing both artery and vein. The artery reveals an alternating hyperreflective and hyporeflective zones (green dotted box). The vein displays a hyporeflective zone (blue arrow) and a hyperreflective zone (white arrow). Scale bars: 200 µm.

4. Discussion

In this study, we implemented the pseudo radial scanning in OCT volumes to characterize reflectance profiles of arteries and veins for AV classification. Radial OCT B-scans showed 1) two hyperreflective boundary signals along the vessel wall of arteries and 2) different intensity distributions in the vessel lumen between arteries and veins. These reflectance profiles provided decisive information for AV classification, enabling the differentiation in OCTA at various retinal regions. Moreover, the radial OCTA displayed discernible laminar flow patterns in arteries and veins.

We speculate that the hyperreflective boundary signals in arteries result from the arterial wall morphology. The blood vessel wall can be divided in three layers: the tunica intima, the tunica media, and the tunica adventitia. Retinal arteries have a well-developed tunica media, formed by a few layers of SMCs [48], and the number of SMCs gradually decreases when arteries branch in precapillary arterioles (Fig. 5). In contrast, the venous wall is very thin, consisting of a single layer of endothelial cells and only a few SMCs (Fig. 5) [49]. Zhu et al. reported that the retinal arteriole had a significantly thicker vessel wall compared with the retinal venule [50], and Chui et al. demonstrated that the venular wall was relatively thinner compared to the arterioles due to
their different structure [51]. Motte et al. further demonstrated by OCT that the arterial walls generally have higher reflectivity compared with the venous walls [37]. Unlike previous studies examining vascular features through transverse scanning by either circular or raster B-scans [9,10,37,50,52–54], the proposed radial sectioning along the vessel can reveal a continuous longitudinal vasculature, offering a better opportunity to classify AV as well as to analyze the vascular morphology and pathologies.

![Diagram of retinal vascular tree](image)

**Fig. 5.** (A) Illustration of the retinal vascular tree of the rat. (B) Light micrographs of first-order arterioles (1°), second-order arteriole (2°), capillary, and venule. Scale bars represent 10 µm. This is adapted from Curtis, T. M., McLaughlin, D., O’Hare, M., Kur, J., Barabas, P., Revolta, G., Scholfield, C. N., McGeown, J. G., McGahon, M. K. Isolation of Retinal Arterioles for Ex Vivo Cell Physiology Studies. *J. Vis. Exp.* (137), e57944, doi:10.3791/57944 (2018) [55].

In addition, the radial OCTA B-scans showed different blood flow patterns between arteries and veins. Especially, the veins revealed a distinct hyperreflective zone at the bottom half of the lumen in which moving blood cells might be predominant, observed by a sequence of OCTA images (Visualization 3). This hyperreflective zone can be explained by venous laminar flow [56]. The retinal veins drain blood from the DCP; thus, erythrocytes move upward to the SVP. Due to laminar flow characteristics limiting lateral and axial mixing, most erythrocytes would follow the basal bloodstream in the lumen. Willerslev et al. also suggested different laminar flow profiles of arteries and veins in OCT [57]. Liang et al. observed the retinal venous laminar flow at the beginning of FA in human subjects. They suggested that venous flows do not mix immediately after entering the upper branches, which might be a permanent phenomenon [58]. Similarly, López-Herrero et al. observed thin columns of fluorescein along the venous walls due to laminar flow [59]. To the best of our knowledge, this is the first OCT visualization of the layered venous laminar flow in the mouse retina, and further analysis on the radial B-scans using mutant animal models would provide useful information in the study of hemorheology, hemodynamics, and oxygenation in the retina. We could also observe alternating hyper-hypo-hyper reflective zones in the artery (green dotted box in Fig. 4(C)). This observed OCTA pattern in the arterial lumen might reflect an hourglass pattern due to shear flow-induced orientation change of erythrocytes within the vessel lumen. The hyporeflective zone in the middle of the artery has been attributed to the high shear flow-induced elongated erythrocytes, causing low backscattering of light [60]. Bernucci et al. demonstrated that intralipid injection can eliminate the hourglass pattern artifact.
in OCTA images of retinal vessels [61]. Further analysis of the intravascular OCT and OCTA intensity patterns through the radial scans would be useful for a better understanding of such flow characteristics.

5. Conclusions

In conclusion, the radially sliced OCT/OCTA revealed distinct reflectance profiles associated with vascular morphology and blood flow signatures for robust artery-vein differentiation. Future integration of automated vessel tracking will improve the usability of the proposed approach not only in the preclinical animal study but also in clinical practice.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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