Original Investigation

Retinal Vascular Layers Imaged by Fluorescein Angiography and Optical Coherence Tomography Angiography

Richard F. Spaide, MD; James M. Klancnik Jr, MD; Michael J. Cooney, MD

Related article page 66

IMPORTANCE The retinal vasculature is involved in many ocular diseases that cause visual loss. Although fluorescein angiography is the criterion standard for evaluating the retina vasculature, it has risks of adverse effects and known defects in imaging all the layers of the retinal vasculature. Optical coherence tomography (OCT) angiography can image vessels based on flow characteristics and may provide improved information.

OBJECTIVE To investigate the ability of OCT angiography to image the vascular layers within the retina compared with conventional fluorescein angiography.

DESIGN, SETTING, AND PARTICIPANTS In this study, performed from March 14, 2014, through June 24, 2014, a total of 5 consecutive, overlapping B-scan OCT angiography images composed of 216 A-scans were obtained at 216 discrete positions within a region of interest, typically a 2 × 2-mm area of the retina. The flow imaging was based on split-spectrum amplitude decorrelation angiography (SSADA), which can dissect layers of vessels in the retina. These distinct layers were compared with the fluorescein angiograms in 12 healthy eyes from patients at a private practice retina clinic to evaluate the ability to visualize the radial peripapillary capillary network. The proportion of the inner vs outer retinal vascular layers was estimated by 3 masked readers and compared with conventional fluorescein angiograms of the same eyes.

MAIN OUTCOMES AND MEASURES Outcome measures were visualization of the radial peripapillary capillary network in the fluorescein and SSADA scans and the proportion of the inner retinal vascular plexus vs the outer retinal capillary plexus as seen in SSADA scans that would match the fluorescein angiogram.

RESULTS In none of the 12 eyes could the radial peripapillary capillary network be visualized completely around the nerve head by fluorescein angiography, whereas the network was readily visualized in the SSADA scans. The fluorescein angiograms were matched, with a mean proportion of the inner vascular plexus being 95.3% (95% CI, 92.2%-97.8%) vs 4.7% (95% CI, 2.6%-5.7%) for the outer capillary plexus from the SSADA scans.

CONCLUSIONS AND RELEVANCE Fluorescein angiography does not image the radial peripapillary or the deep capillary networks well. However, OCT angiography can image all layers of the retinal vasculature without dye injection. Therefore, OCT angiography, and the findings generated, have the potential to affect clinical evaluation of the retina in healthy patients and patients with disease.

JAMA Ophthalmol. 2015;133(1):45-50. doi:10.1001/jamaophthalmol.2014.3616
Published online October 9, 2014.

Author Affiliations: Vitreous, Retina, Macula Consultants of New York, New York.

Corresponding Author: Richard F. Spaide, MD, Vitreous, Retina, Macula Consultants of New York, 460 Park Ave, Fifth Floor, New York, NY 10022 (rickspaide@gmail.com).

Fluorescein sodium is a fluorophore with high absorptivity, excellent fluorescence quantum yield, and a long history of being used in ophthalmology. In 1930, Kikai[1] published a report describing injection of intravenous fluorescein and noted that the dye could be seen in the retinal vessels. In 1959, Flocks et al[2] published a report that documented the retinal blood flow in cats with the use of a motion picture camera. They injected the fluorescein intravenously, excited the dye with blue light with the aid of a cobalt filter, and photographically recorded the fluorescein filling sequence of the retinal vasculature in the cat. In the same year, MacLean and Maumenee[3] described how fluorescein angioscopy could be performed using slit-lamp biomicroscopy to diagnose a choroidal hemangioma. Novotny and Alvis[4] published sequential still frames of fluorescein angiography in humans in the journal *Circulation* in 1961. Fluorescein angiography was adopted by early pioneers in medical retina research studying the retinal vasculature in health and disease because the architecture of the blood vessels, the blood flow, and leakage from damaged or diseased retinal vessels could be evaluated with a simple technique.

Although fluorescence of the injected dye enabled improved visualization of retinal capillaries, it became evident that not all retinal capillaries were being visualized. The radial peripapillary capillaries were described from histologic analysis by Michaelson,[5] but good visualization with fluorescein angiography proved elusive. Alterman and Henkind[6] and Henkind[7,8] published articles concerning the radial peripapillary capillary network based on india ink injection, even though fluorescein angiography was readily available. The radial peripapillary capillary network could be demonstrated in vivo using adaptive optics with fluorescein dye or with adaptive optics, an offset pinhole, and subsequent motion contrast processing techniques.[9,10] Custom dental impressions with a bite bar were used to obtain sufficient stabilization of the head. Articles by Weinhaus et al[11] and Snodderly et al[12] found that the fluorescein angiographic images of the retina corresponded to the anatomical arraignment of the superficial retinal vessels, whereas the deeper retinal capillaries were not visualized in the angiogram. Comparable histopathologic correlations in humans have not been reported, but comparative findings suggest the deeper capillary network in the retina is not visualized well by fluorescein angiography, possibly because of light scattering in the retina.[13]

Fluorescein angiography is the criterion standard for in vivo evaluation of the retinal circulation, but paradoxically 2 of the 3 major capillary networks do not appear to be imaged well despite the retina being a nearly transparent structure. Optical coherence tomography (OCT) is a noninvasive technique that provides high-resolution images of the retina. Rapidly performed scans of areas of retinal tissue can be analyzed for variation in some measure of reflectivity, phase shift, or phase variance to construct microvascular flow maps, which are collectively referred to as OCT angiography.[14-21] In this study, the ability to visualize the retinal capillary layers was evaluated in healthy eyes with conventional fluorescein angiography and OCT angiography.

Methods

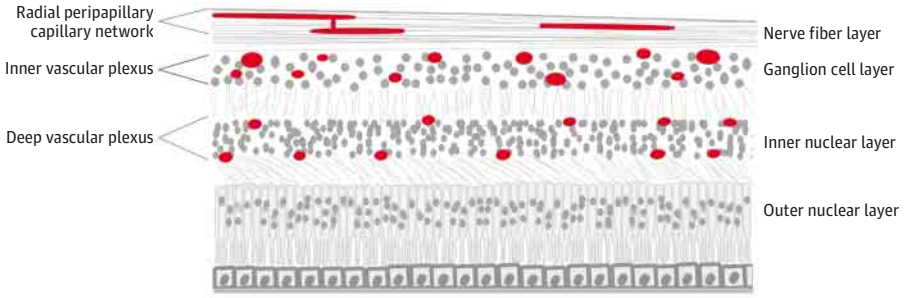
The study was approved by the Western Institutional Review Board, and patients signed written informed consent forms. The study was performed at a private practice retinal clinic from March 14, 2014, through June 24, 2014, and included 2 volunteers with no significant medical history and no signs or symptoms of ocular disease. Seven patients had ocular problems that were unilateral or, in 1 patient, not involving the macula. These patients all had normal macular OCT evaluation results (Spectralis HRA+OCT; Heidelberg Engineering GmbH). The fluorescein angiograms uniformly were well focused, with adequate exposure and sharpness to visualize the perifoveal capillaries. Fluorescein angiography was performed with the Spectralis HRA+OCT in 1 eye and the IMAGEnet system (Topcon Medical Systems Inc) in 11 eyes. Patients were given a 2-mL intravenous injection of 25% fluorescein solution (Akorn Inc).

OCT Angiography

The instrument used for the OCT images was based on the Optovue RTVue XR Avanti (Optovue Inc) and was used to obtain split-spectrum amplitude decorrelation angiography (SSADA) images. This instrument has an A-scan rate of 70 000 scans per second, using a light source centered on 840 nm and a bandwidth of 45 nm. The tissue resolution is 5 µm axially, and there is a 22-µm beam width. Each B-scan contained 216 A-scans. Five consecutive B-scans (M-B frames) were captured at a fixed position before proceeding to the next sampling location. A total of 216 locations (B-scans) along the slow transverse direction were sampled to form a 3-dimensional data cube. With a B-scan frame rate of 270 frames per second, the 1080 B-scans in each scan sequence were acquired in approximately 4 seconds. Four volumetric raster scans, including 2 horizontal priority fast transverse (x-fast) scans and 2 vertical priority fast transverse (y-fast) scans, were obtained consecutively in 1 session. The best x-fast and y-fast scans were registered using the contained software (ReVue, version 2014.2.0.15; Optovue Inc), which has the ability to correct some motion artifacts, including residual axial motion and transverse saccadic motion.

After the processing of the volume scans, the decorrelation in the images, which is essentially 1 minus the correlation, was calculated. Stationary tissue shows a high correlation in imaging characteristics from one frame to the next. Blood flowing through vessels causes a changing reflectance over time and localized areas of low correlation between frames (or conversely a high decorrelation). This method does not use phase information from the OCT signal. The correlated frames were evaluated and statistical outliers were removed from the averaging process to reduce the possibility of tissue motion being present. The spectrum of the light source was split into 4 component parts to decrease the noise present in the image; each was used to perform the decorrelation step, and the results of all 4 were averaged. This split-spectrum strategy trades axial resolution for decreased noise. After this step, a block of information exists that contains levels of decorrelation that range from 0 to 1. In any given region of tissue, the maximal projection image can be viewed to obtain an image

Figure 1. Vascular Layers in the Retina



The vessels are shown in red. The radial peripapillary capillaries course parallel to the nerve fibers.

of the contained blood flow. Because the retina is a laminar structure with a corresponding stratification of the blood supply, segmentation of the retina in specific layers allows simple en-face visualization of the corresponding vascular supply for that layer.

Segmentation of the OCT Angiographic Image

Three main regions were evaluated for the purposes of this study. The radial peripapillary capillary network was visualized in scans centered on the optic nerve head by performing a 3 × 3-mm scan over the nerve. The internal limiting membrane was used as a plane of reference, and a slab thickness sufficient to contain the full thickness of the nerve fiber layer in the surrounding retina was selected. In adjacent retinal areas, separate scans were obtained in 2 × 2-mm sections, and the nerve fiber layer was segmented to obtain the appropriate section of tissue. The thickness of the nerve fiber layer becomes thinner with increasing distance, but the software reveals increasing noise with thinner sections. Therefore, the thinnest sections used were 55 µm. The aggregate images of the radial peripapillary capillary network were merged after elastic warping of the images was performed. The larger vessels were segmented and given a blue color and the radial peripapillary capillaries a red color.

For the purposes of this study, the multiple retinal vascular planes as described by Weinhaus et al[11] were simplified into 2 main layers as was done by Snodderly et al.[12] The ganglion cell layer is invested with one or more layers of capillaries, and these were termed the *inner vascular plexus* (**Figure 1**). The inner capillary layers were imaged by starting with the internal limiting membrane in the macular region and selecting sufficient thickness to include the ganglion cell layer. The inner retina at the fovea and its margin were included in this scan. The inner nuclear layer is ordinarily bracketed by a layer of capillaries on either side.[11,12] The en-face image was segmented with the inner boundary at the outer inner plexiform layer and the outer boundary was set at the midpoint of the outer plexiform layer to obtain images of the outer layers of capillaries.

Image Preparation

The fluorescein image was selected from the earliest frames that showed venous filling that had the sharpest imaging of the retinal capillaries. The central portion of the angiographic image was scaled in size to match the SSADA scan. Because the OCT angiography encompasses a very limited field of view, the corresponding angiographic image had to be substantially enlarged. A composite image structure was made to set up each test image using Photoshop CS6 (Adobe Systems Inc). The fluorescein angiographic image was placed on the right side, and a stack that contained the inner and outer capillary plexuses was placed on the left. With the use of a slider that could be set from 0% to 100%, the mixture of the inner and outer retinal vasculature images of the SSADA scan could be varied by the user and then compared with the conventional fluorescein angiographic image.

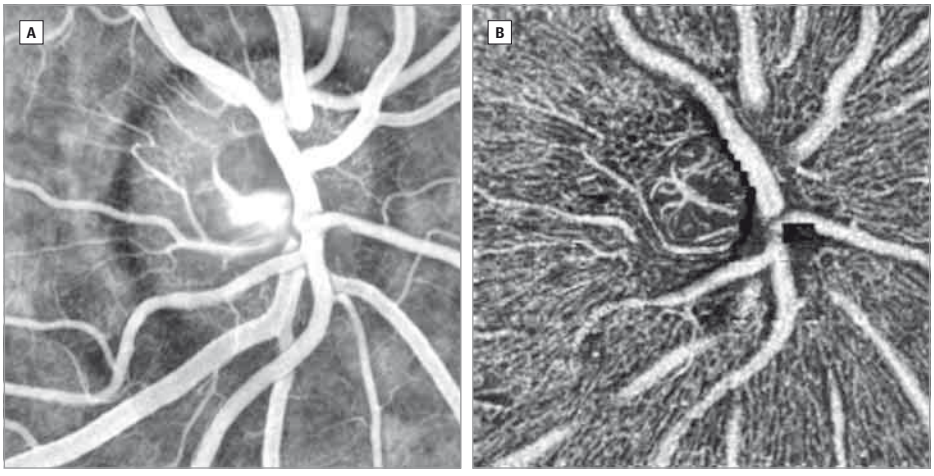
Image Test

Each grader (R.F.S., J.M.K., M.J.C.) was given a short tutorial on how to use the grading system followed by 2 illustrative cases that were not part of the test series. The test images were loaded into Photoshop CS6, and each grader adjusted the slider so that the details in the mixed inner and outer retinal plexus images as visualized by the SSADA scan matched the details seen on the fluorescein angiogram. Each grader performed the assessment independently and was masked to the results of the others. The proportion of the inner to the outer capillary layers was recorded, and the results were analyzed using a 2-way analysis of variance test (general linear model; IBM SPSS Statistics 20, IBM).

Results

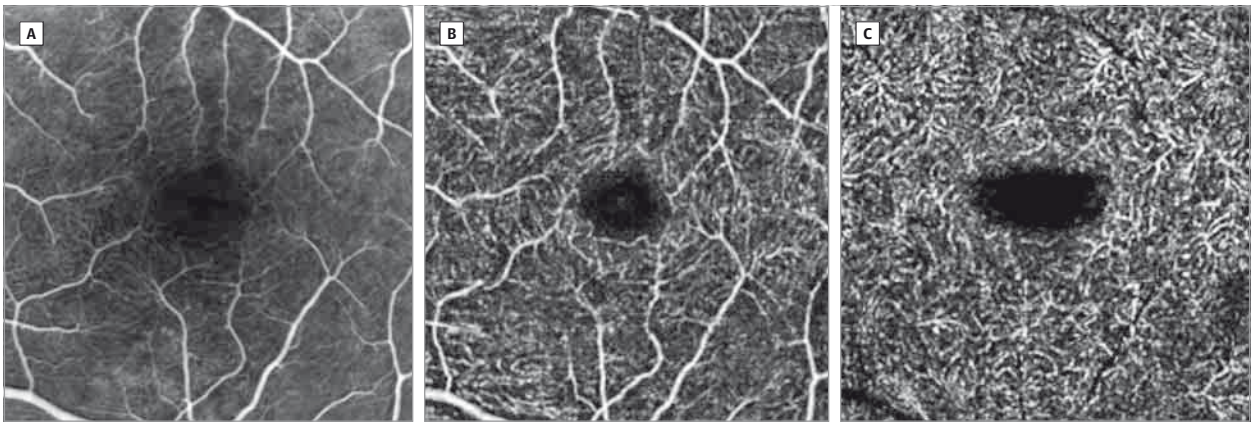
There were 12 eyes, with the patients ranging in age from 24 to 60 years (median, 52 years). In some eyes, radial arranged capillaries, potentially consistent with the radial peripapillary capillary network, could be imaged in the fluorescein angiogram superotemporal or inferotemporal to the disc, but in no eye could the capillaries be clearly identified elsewhere (**Figure 2**). The radial peripapillary capillary network was readily visible around the disc in all eyes in the SSADA scans. The fluorescein angiograms were matched, with a mean proportion of the inner vascular plexus being 95.3% (95% CI, 92.2%-97.8%) vs 4.7% (95% CI, 2.6%-5.7%) for the outer capillary plexus from the SSADA scans. The 2-way analysis of variance analysis revealed no significant difference among the graders (P = .12) and no significant difference in any of the images graded (P = .73) in terms of proportion of inner vs outer vas-

Figure 2. Optic Nerve Head and Surrounding Region in the Right Eye



A, The fluorescein angiographic image. B, The optical coherence tomography angiography image of the same region in which the radial peripapillary capillary network is readily visible.

Figure 3. Comparison Between the Fluorescein Angiographic Image and the Layers Seen in Optical Coherence Tomography Angiography of the Macula



A, Fluorescein angiographic image of the central macular region. B, Optical coherence tomography angiography image of the inner retinal vascular plexus. C, Optical coherence tomography angiography image of the outer plexus. The capillaries around the foveal avascular zone are included in the segmentation of the inner layer.

cular plexus contribution to the fluorescein image (**Figure 3**). With the use of multiple montaged images, a map of the radial peripapillary network could be achieved (**Figure 4**).

Discussion

It seems axiomatic that fluorescein, an efficient fluorophore injected intravenously and then imaged with a high-resolution fundus camera, should be able to show the vessels embedded in a nearly transparent structure only hundreds of micrometers thick. Early investigators examining the radial peripapillary capillary network used histologic techniques and did not find correlative fluorescein angiographic images.[6-8] One fluorescein angiographic image that showed a section of the radial peripapillary capillary network was published, but this eye had venous-to-venous collaterals that suggested past veno-occlusive disease.[22] Ueno[23] imaged eyes with curved sector-shaped loss of ground glass fluorescence from the inner retina and ascribed this to loss of the radial peripapillary capillary network. The actual capillaries could not be seen outside the affected areas.[23] Hamanaka et al[24] evaluated the fluorescein angiographic results of a diabetic patient and assumed that lack of sectors of fluorescence necessarily meant the radial peripapillary capillaries were not perfused. It seems likely that multiple scattering by the adjacent nerve fiber bundles may lead to a diffuse glow of fluorescence instead of a sharply defined capillary image.

Using animal models, Weinhaus et al[11] and Snodderly et al[12] found that fluorescein angiography does not image the deeper capillary plexus well in animal models. They thought that scattering from the deeper layers obliterated the specific image of the capillaries. It is common to see the retinal capillaries in a fluorescein angiogram over a backdrop of low-level, poorly defined fluorescence. The diffuse fluorescence is often ascribed to background choroidal fluorescence, but it may be derived, in part, from the deeper capillary plexus. Using the SSADA technique, we found the radial peripapillary capillary network and the inner and outer retinal plexuses were readily imaged. The fluorescein image corresponded to the in-

ner retinal vascular plexus in the present series of human eyes, which seems to confirm the histologic findings of experimental animal models.

These findings may portend important consequences in our understanding of the associations between retinal vasculature and ocular diseases. The relative importance of the radial peripapillary capillary network in diseases as wide ranging as glaucoma[6,25] to diabetic retinopathy[24] has been proposed but has not been studied in detail because of imaging difficulties. Likewise, the relative importance of the outer retinal plexus in retinal diseases has been difficult to ascertain. It is possible that for many disease states the outer plexus is affected in parallel to the inner plexus and fluorescein angiography is sufficient to grade the amount of vascular compromise. It is possible that some conditions, particularly those that involve perfusion, could affect one layer differently from the other. Appreciation that the deeper layer may be selectively involved in vein occlusions and other diseases has come to the fore, not because of unequivocal primary evidence supplied by fluorescein angiography but because of the need to explain opacification in middle layers of OCT B-scans of affected patients.[26,27] More than 50 years after the publication of fluorescein angiography findings, potential circulatory changes in the outer retinal capillary plexus were deduced principally by the combination of conventional OCT imaging and logic, not by assessment of the outer capillary plexus by fluorescein angiography.

The inherent advantages of OCT angiography appear to be the ability to optically dissect and visualize the flow in various layers of the retina, the high resolution obtainable, and the freedom and safety of not having to use an injected dye. Fluorescein angiography provides flow information in that the speed of filling can be roughly compared in patients, physiologic information concerning the health of vessels can be assessed by looking for leakage, and the field of view is much larger. In addition, OCT angiography requires that the patient fixate precisely for several seconds, whereas a useful fluorescein angiographic frame can be obtained in a fraction of a second. Fluorescein angiography involves injection of a dye, which has a small probability of serious complications but a common incidence of minor adverse effects, such as nausea and hives.[28] Adaptive optics methods of visualizing the retinal vasculature require much more time and effort but have the potential to have higher resolution. A dedicated adaptive optics instrument is used to acquire a large number of images, and this is followed by image averaging, which in turn is followed by montaging separate images to obtain a field of view large enough to have clinical utility. Adaptive optics imaging has a limited depth of focus, so each layer of circulation being evaluated has to be specifically targeted at the time of acquisition. With OCT angiography, the layers can be dissected arbitrarily any time after acquisition to obtain flow-based images.

Figure 4. Wide-Field Pseudocolor Montage of the Right Eye of a 58-Year-Old

The larger retinal vessels are shown in blue, and the radial peripapillary capillary network is mapped in red. Partially disconnected segments of larger retinal vessels appear in the image if they course superficially into the inner retina.

This study has the potential for numerous weaknesses inherent in any study of limited sample size. Although it has been recognized for years that fluorescein angiography has problems imaging some layers of capillaries, there is no criterion standard with which to compare OCT angiography. Matching the results of OCT angiography to the results of histologic analysis in animal models would greatly enhance our concepts of this new form of vascular imaging. Many strategies could be used to extract flow information from OCT data sets, and the methods used in the present instrument may not be the best. Future developments in OCT angiography could include being able to have a more continuous monitoring of flow, imaging a larger area of the fundus, and shortening the acquisition time.

ARTICLE INFORMATION

Submitted for Publication: June 27, 2014; final revision received August 1, 2014; accepted August 3, 2014.

Published Online: October 9, 2014. doi:10.1001/jamaophthalmol.2014.3616.

Author Contributions: Dr Spaide had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Spaide.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Spaide.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Spaide.
Administrative, technical, or material support: Spaide, Klancnik.
Study supervision: Spaide.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Spaide reported receiving consulting and royalty payments from Topcon Medical Systems Inc and working as a consultant to Bausch and Lomb Inc, Teva Pharmaceuticals Industries Ltd, and ThromboGenics Inc. No other disclosures were reported.

Funding/Support: This study was supported in part by the Macula Foundation.

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study;

collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Kikai K. Uber die vitalfarbung des hinteren bulbussabschnittes. *Arch Augenheilkd*. 1930;103:541-553.

2. Flocks M, Miller J, Chao P. Retinal circulation time with the aid of fundus cinephotography. *Am J Ophthalmol*. 1959;48(1, pt 2):3-10.

3. Maclean AL, Maumenee AE. Hemangioma of the choroid. *Trans Am Ophthalmol Soc*. 1959;57:171-194.

4. Novotny HR, Alvis DL. A method of photographing fluorescence in circulating blood in the human retina. *Circulation*. 1961;24:82-86.

5. Michaelson I. *Retinal Circulation in Man and Animals*. Springfield, IL: Charles C Thomas; 1954.

6. Alterman M, Henkind P. Radial peripapillary capillaries of the retina, II: possible role in Bjerrum scotoma. *Br J Ophthalmol*. 1968;52(1):26-31.

7. Henkind P. Radial peripapillary capillaries of the retina, I: anatomy: human and comparative. *Br J Ophthalmol*. 1967;51(2):115-123.

8. Henkind P. Symposium on glaucoma: joint meeting with the National Society for the Prevention of Blindness: new observations on the radial peripapillary capillaries. *Invest Ophthalmol*. 1967;6(2):103-108.

9. Pinhas A, Dubow M, Shah N, et al. In vivo imaging of human retinal microvasculature using adaptive optics scanning light ophthalmoscope fluorescein angiography. *Biomed Opt Express*. 2013;4(8):1305-1317.

10. Chui TY, Dubow M, Pinhas A, et al. Comparison of adaptive optics scanning light ophthalmoscopic fluorescein angiography and offset pinhole imaging. *Biomed Opt Express*. 2014;5(4):1173-1189.

11. Weinhaus RS, Burke JM, Delori FC, Snodderly DM. Comparison of fluorescein angiography with microvascular anatomy of macaque retinas. *Exp Eye Res*. 1995;61(1):1-16.

12. Snodderly DM, Weinhaus RS, Choi JC. Neural-vascular relationships in central retina of macaque monkeys (*Macaca fascicularis*). *J Neurosci*. 1992;12(4):1169-1193.

13. Mendis KR, Balaratnasingam C, Yu P, et al. Correlation of histologic and clinical images to determine the diagnostic value of fluorescein angiography for studying retinal capillary detail. *Invest Ophthalmol Vis Sci*. 2010;51(11):5864-5869.

14. Mariampillai A, Standish BA, Moriyama EH, et al. Speckle variance detection of microvasculature using swept-source optical coherence tomography. *Opt Lett*. 2008;33(13):1530-1532.

15. Enfield J, Jonathan E, Leahy M. In vivo imaging of the microcirculation of the volar forearm using correlation mapping optical coherence tomography (cmOCT). *Biomed Opt Express*. 2011;2(5):1184-1193.

16. Jonathan E, Enfield J, Leahy MJ. Correlation mapping method for generating microcirculation morphology from optical coherence tomography (OCT) intensity images. *J Biophotonics*. 2011;4(9):583-587.

17. Chui TY, Vannasdale DA, Burns SA. The use of forward scatter to improve retinal vascular imaging with an adaptive optics scanning laser ophthalmoscope. *Biomed Opt Express*. 2012;3(10):2537-2549.

18. Tokayer J, Jia Y, Dhalla AH, Huang D. Blood flow velocity quantification using split-spectrum amplitude-decorrelation angiography with optical coherence tomography. *Biomed Opt Express*. 2013;4(10):1909-1924.

19. Subhash HM, Leahy MJ. Microcirculation imaging based on full-range high-speed spectral domain correlation mapping optical coherence tomography. *J Biomed Opt*. 2014;19(2):21103.

20. Jia Y, Wei E, Wang X, et al. Optical coherence tomography angiography of optic disc perfusion in glaucoma. *Ophthalmology*. 2014;121(7):1322-1332.

21. Jia Y, Bailey ST, Wilson DJ, et al. Quantitative optical coherence tomography angiography of choroidal neovascularization in age-related macular degeneration. *Ophthalmology*. 2014;121(7):1435-1444.

22. Hogan M, Alvarado J, Weddell J. *Histology of the Human Eye—An Atlas and Textbook*. Philadelphia, PA: WB Saunders; 1971.

23. Ueno H. Studies on the radial peripapillary capillaries (RPCs): (1) clinical features on fluorescein fundus angiography [in Japanese]. *Nihon Ganka Gakkai Zasshi*. 1976;80(5):267-280.

24. Hamanaka T, Akabane N, Yajima T, Takahashi T, Tanabe A. Retinal ischemia and angle neovascularization in proliferative diabetic retinopathy. *Am J Ophthalmol*. 2001;132(5):648-658.

25. Kornzweig AL, Eliasoph I, Feldstein M. Selective atrophy of the radial peripapillary capillaries in chronic glaucoma. *Arch Ophthalmol*. 1968;80(6):696-702.

26. Sarraf D, Rahimy E, Fawzi AA, et al. Paracentral acute middle maculopathy: a new variant of acute macular neuroretinopathy associated with retinal capillary ischemia. *JAMA Ophthalmol*. 2013;131(10):1275-1287.

27. Rahimy E, Sarraf D. Paracentral acute middle maculopathy spectral-domain optical coherence tomography feature of deep capillary ischemia. *Curr Opin Ophthalmol*. 2014;25(3):207-212.

28. Yannuzzi LA, Rohrer KT, Tindel LJ, et al. Fluorescein angiography complication survey. *Ophthalmology*. 1986;93(5):611-617.