Optical coherence tomography angiography of retinal vascular occlusions produced by imaging-guided laser photocoagulation

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Abstract: Retinal vascular occlusive diseases represent a major form of vision loss worldwide. Rodent models of these diseases have traditionally relied upon a slit-lamp biomicroscope to help visualize the fundus and subsequently aid delivery of high-power laser shots to a target vessel. Here we describe a multimodal imaging system that can produce, image, and monitor retinal vascular occlusions in rodents. The system combines a spectral-domain optical coherence tomography system for cross-sectional structural imaging and three-dimensional angiography, and a fluorescence scanning laser ophthalmoscope for Rose Bengal monitoring and high-power laser delivery to a target vessel. This multimodal system facilitates the precise production of occlusions in the branched retinal veins, central retinal vein, and branched retinal arteries. Additionally, changes in the retinal morphology and retinal vasculature can be longitudinally documented. With our device, retinal vascular occlusions can be easily and consistently created, which paves the way for futures studies on their pathophysiology and therapeutic targets.

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1. Introduction

Retinal vascular occlusive diseases represent the most common cause of visual disability in the elderly population [1]. Two important vascular occlusive diseases are retinal vein occlusions (RVO), and retinal artery occlusions (RAO), with each having different etiologies, pathogenesis, and visual outcomes. Generally speaking, these diseases present with painless, sudden vision loss or blurring in a patient greater than 50 years of age. A unifying feature of these diseases is reduced blood flow, which occurs either in a retinal vein (RVO), or in a retinal artery (RAO). Causes for the ischemia include vessel blockage by either thrombi or emboli, among many others. For RVO, the central vein or a branch thereof can be affected, which are termed central RVO (CRVO) and branch RVO (BRVO), respectively. Similarly, RAO can also be classified into central RAO (CRAO) and branch RAO (BRAO).

Importantly, BRVO is the second most common retinal vascular disease after diabetic retinopathy, affecting approximately 16.4 million people worldwide [2].

Two major techniques have been developed to create rodent models of retinal vascular occlusions. In the first technique, the fundus is visualized with a modified slit-lamp biomicroscope using a high-power laser delivery system (~100-200 mW) [3–5]. A skilled operator then manually delivers high energy laser shots to a target retinal vessel until vascular occlusion appears to have occurred. The high-power laser shots may damage the retina, leading to interstitial edema. In turn, the edema eventually compresses the target vessel, resulting in vascular occlusion. In the case of RVO’s, the retina appears swollen and pale, and retinal vessels may appear tortuous and white. In the second technique, a photoreactive dye, such as fluorescein or Rose Bengal (RB), is injected intravenously [6–9]. Similar to the first technique, high-power laser shots are delivered to a target vessel. When exposed to high-power light, the intravascular RB (or fluorescein) releases singlet oxygen, which in turn, react with proteins and fatty acids on the blood vessel wall. This oxidation process results in the recruitment of platelets and activation of the coagulation cascade, which altogether lead to the formation of an intravascular thrombus at the targeted site. Compared to the first technique, the second technique creates occlusions which better resemble the pathophysiology of human retinal vascular occlusions; therefore, in this paper, we sought to improve upon the second technique.

Unfortunately, using a slit-lamp biomicroscope to observe and initiate occlusion has several drawbacks. First, extensive training and expertise are required to maneuver the slit-lamp and deliver the laser shots [10], especially when dealing with the small dimensions of the mouse eye and the fast clearance of RB from the circulation (~5 minutes). This steep learning curve also introduces variations among different operators. Second, the spot size of the high-power laser on the retina is difficult to control visually; therefore, a large area around the vessel may be illuminated with high-power, resulting in unwanted tissue damage. Finally, the retinal vascular occlusion is not monitored using the direct evidence from examining the blood flow within the vessel. Instead, vascular occlusion is inferred when the retinal vessel, or the surrounding area, appears pale.

Here we developed a multimodal imaging system for producing laser-induced vascular occlusions with RB. Multimodal imaging systems are capable of both producing, imaging, and monitoring an animal model. These systems may include combinations of fundus photography [11], scanning laser ophthalmoscopy (SLO) [12–15], autofluorescence imaging [16], or photoacoustic ophthalmoscopy [17], with optical coherence tomography (OCT). Certain systems, especially in neuroscience [18, 19], include directed laser delivery, which enables researchers to consistently reproduce animal models with reduced training time. Recently, such approaches have been developed for ophthalmology research. For example, laser-induced choroidal neovascularization (CNV) in rodents, which is a model of wet age-related macular degeneration, can sometimes be difficult to produce due to inconsistent laser burns administered by a slit-lamp protocol. As a potential solution, a recent multimodal
device was able to consistently produce this animal model [10]. In this paper, we have created a system for producing animal models of retinal vascular occlusive diseases.

The aims of our system were four-fold: (1) to visualize the three-dimensional (3D) retinal structure before and after vascular occlusion; (2) to visualize the retinal microvasculature before and after vascular occlusion; (3) to precisely direct actinic laser light to a specific vessel location with minimal laser power; and (4) to visualize vascular occlusions in real-time. To achieve these goals, we developed an integrated optical coherence tomography (OCT) and fluorescent scanning laser ophthalmoscope (SLO) system. The OCT sub-system performed high-resolution cross-sectional imaging of the retina and granted us the ability to perform OCT angiography, which can obtain high-contrast images of the microvasculature. The SLO sub-system enabled us to perform RB angiograms, ensuring RB within the retinal vasculature. Subsequently, the SLO also enabled us to direct actinic light precisely to a selected vessel location, while also visualizing the occlusion process in real-time. Ultimately, with our multimodal imaging system, we demonstrate BRVO, CRVO, and BRAO patterns in the murine retina.

2. Materials and methods

2.1 System setup

In this section, we describe the combined OCT and SLO system as shown in Fig. 1. The light source in the spectral-domain OCT (SD-OCT) sub-system was a superluminescent light emitting diode (SLED) (IPSDW0825C-0314, InPhenix) with a center wavelength of 840 nm and a bandwidth of 95 nm (full width at half maximum). A 50/50 fiber coupler (FUSED-22-850, OZ Optics) collected the light from the SLED and split it into sample and reference arms. The beam at each arm was collimated by an aspheric fiberport (FP 1 and FP 2; PAF-X-11-PC-B, Thorlabs). The sample arm beam was combined with the SLO illumination beam by a short pass dichroic mirror (DC 1; FF746, Semrock). The x-y scanning galvanometer mirrors (QS-7, Nutfield Technology) deflected the combined beam for raster scanning. A 5/1 Keplerian telescope consisting of two achromatic lenses (L 1 and L 2; VIS-NIR coated, 75 mm and 15 mm focal lengths, Edmund Optics) created a point conjugate to the scanning mirrors, which was aligned at the pupil plane of the mouse eye. The reference arm beam was reflected back by a silver mirror (M 1) after passing through several BK7 glass plates, which were used for dispersion compensation. The back-reflected sample beam recombined and interfered with the backscattered light from the sample. A homemade spectrometer detected and digitized the interference signals.

Fig. 1. Schematic of the combined OCT and SLO system. CW Laser: continuous wave laser. DC: dichroic mirror. FP: fiber port collimator. M: mirror. ND: neutral density filter wheel. PC: polarization controller. SLED: superluminescent light emitting diode. Focal lengths of lenses L 1, L 2, L 3, L 4, L 5 were 75 mm, 15 mm, 40 mm, 50 mm, and 30 mm, respectively.

The SLO and laser occlusion sub-system used a continuous wave (CW) diode-pumped solid-state laser (532 nm, 100 mW). For coarse control of the laser power, the SLO
illumination beam passed through a manual neutral density filter wheel (ND₁; Thorlabs, FW1AND). For fine control of the laser power, the SLO illumination also passed through a continuous neutral density filter wheel (ND₂; Thorlabs, NDC-50C-2M-A). A long pass dichroic mirror (DC₂; FF560, Semrock) served to reflect the illumination beam and pass RB fluorescence (peak emission: 571 nm). A Keplerian telescope (L₃ and L₄) resized the illumination beam and passed it to the dichroic mirror (DC₁) shared with the OCT sub-system. The SLO and OCT illumination beams were coaxially aligned, such that they shared the same relay optics and scanning mirrors to reach the subject’s pupil plane. After passing through the long pass dichroic mirror (DC₂), the RB fluorescence was focused by a lens (L₅) and spatially filtered by a pinhole (PH; 50 microns, Thorlabs). A photomultiplier tube (PMT; Hamamatsu) captured the fluorescence signal, which was subsequently converted from current to voltage and digitally acquired.

2.2 Scanning protocols for OCT and SLO imaging

Both OCT and SLO used raster scanning to acquire images. The galvanometer mirrors deflected the illumination beams, which changed the angle of the illumination beam at the pupil plane, to achieve raster scanning of the retina. OCT detected backscattered photons by low-coherence interferometry, while SLO collected RB fluorescence in retinal blood flow. We used custom Labview software (2015 SP1, 64 bit, National Instruments) to control the imaging systems.

2.2.1 OCT imaging protocols

We designed two OCT imaging protocols: a preview OCT protocol and a high-density OCT/OCTA protocol. For both protocols, the A-line acquisition rate was 70 kHz; the scanning area was 2.5 mm x 2.5 mm; and the illumination at the pupil plane was 1 mW. The preview OCT protocol allowed us to quickly position the eye and preview the image quality. The image contained 64 B-scans with 128 A-lines in each B-scan. To preview the volumetric OCT image in real-time, we composed a CUDA C program and ran the CUDA-accelerated parallel functions on a graphics card (GeForce GTX 750Ti, NVIDIA Corporation). The real-time preview has a frame rate of 5.9 frames per second (FPS), which was limited by the galvanometer mirrors. We generated en face images by calculating the maximum amplitude projection (MAP) along the axial direction of the 3D volume data. Alongside the MAP, a selected B-scan from the 3D volume was displayed, helping the operators to better position the eye.

Once the initial alignment is completed, we captured images using the high-density OCT/OCTA protocol, where 400 x 512 A-lines were recorded per image. Additionally, at each of the 512 B-scan position, we sequentially acquired additional five co-localized B-scans for OCTA, making the total acquired B-scans equal to 2560. The total acquisition time for this protocol was 20.5 seconds. In this protocol, the data was processed offline with a custom MATLAB program. Due to limited field of view in each acquisition, we repeated the high-density protocol at different fundus locations and montaged those images later.

OCT images from the high-density protocol were constructed from an average of the five repeated B-scans. The OCT angiogram was constructed from the five repeated B-scans using an amplitude-based OCTA algorithm [20]. B-scans were correlated and shifted to adjust for global and lateral phase fluctuations [21]. For visualization, the 3D OCTA volumes were converted to depth color coded MAP, as described previously [22]. When possible, OCTA images were automatically montaged together using i2k Retina software (DualAlign LLC, Clifton Park, NY). If the automatic montaging failed, images were manually montaged in Adobe Photoshop (Creative Cloud, Adobe Systems Incorporated, San Jose, CA).
2.2.2 SLO imaging

The SLO sub-system also had two imaging protocols: an angiography protocol and an actinic protocol. The purpose of the angiography protocol was to obtain RB fluorescence angiograms of the retinal vessels. The scanning area was 2.5 mm by 2.5 mm (same as OCT) with a scanning density of 128 × 128 pixels; the illumination power at the pupil was 400 μW; and acquisition rate was 1.9 FPS. The purpose of the actinic protocol was to deliver high-power actinic light to a target vessel. Here, by manipulating the deflection angle of the x-y scanning galvanometers, the scanning area was reduced and shifted to cover only the diameter of the target vessel. The scanning density was 64 × 64 pixels; the illumination power at the pupil was 25 mW to 35 mW; and the acquisition rate was 5.9 FPS.

2.3 Retinal vascular occlusion protocol

Wildtype C57BL/6 mice were anesthetized with an intraperitoneal injection (10 ml/kg body weight) of a ketamine/xylazine cocktail (ketamine: 11.45 mg/ml; xylazine: 1.7 mg/ml, in saline). A drop of 0.5% tetracaine hydrochloride ophthalmic solution (Bausch & Lomb, Rochester, NY) was administered on the cornea for topical anesthesia. For pupil dilation, a drop of 1% tropicamide ophthalmic solution (Akorn, Lake Forest, IL) was also administered. The mice were placed on a custom-made animal holder, which allowed for translation and rotation. In between image acquisitions, we administered artificial tears (Henry Schein, Melville, NY, USA) to prevent corneal desiccation. We followed the guidelines established by the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research as well as those set by the Northwestern University Institutional Animal Care and Use Committee.

Figure 2 illustrates the major steps of the retinal vascular occlusion protocol. After centering the optic nerve head on the OCT en face image using the preview protocol, we acquired baseline OCT/OCTA volumes using high-density protocol. Using the en face preview, we also selected either an artery or vein for vascular occlusion. Arteries could be distinguished from veins on en face OCT by their smaller diameter and more frequent branch points in the nerve fiber layer [5]. Next, RB (Sigma Aldrich, Milwaukee, WI) was prepared in physiological saline (5 mg/ml of saline), and 0.2 ml was injected into one of the lateral tail veins. RB was preferred over fluorescein because of its higher quantum yield (~25x) for producing reactive single oxygen [23]. Immediately after successful tail injection, we started RB angiography using the SLO angiography imaging protocol, enabling real-time visualization of RB in the retinal vasculature. We then selected a location along the target vessel for occlusion. Previous studies of BRVO in mice have noted that the distance from the optic nerve head (ONH) appeared to correlate with severity of the vascular occlusion. Specifically, vein occlusions close to the ONH tend to result in a CRVO pattern, while vein occlusions farther away from the ONH result in a BRVO pattern [7].

Once the occlusion site was determined, we initiated the SLO actinic protocol. We reduced the scanning area to span the vessel diameter and increased the laser power to 25 mW by adjusting the controllable ND filters. We then monitored the occlusion process using SLO. If the occlusion was successful, the intravascular fluorescence signal dramatically fell within several seconds. If the vessel appeared to be only partially occluded, we increased the laser

![Fig. 2. Key steps of the retinal vascular occlusion protocol.](image-url)
power up to a maximum of 35 mW to finish the occlusion process. Previous techniques to produce vascular occlusions used laser powers of 100-200 mW. Using OCT, we found that these powers often led to vessel hemorrhage, rupture of Bruch’s membrane, or severe edema immediately following laser delivery. Therefore, we opted to reduce the power to 25-35 mW to minimize these effects.

After completing the vascular occlusion, we reduced the SLO illumination power back to 400 μW and obtained a post-occlusion RB angiogram (SLO angiography protocol). We also collected post-occlusion OCT/OCTA volumes for comparison with baseline images (high-density OCT imaging protocol). After imaging, we placed the mice in a recovery area with a heat lamp. OCTA images were then processed offline.

3. Results

To demonstrate our technique for producing retinal vascular occlusions in the mouse retina, we created a BRVO pattern at the 10 o’clock vein in a healthy mouse eye (Fig. 3). To increase the probability of creating a BRVO pattern, we targeted a vessel location greater than four ONH diameters away from the ONH. To aid this process, we aligned the mouse eye, using the preview OCT protocol for guidance, such that the ONH was at the bottom right corner of the FOV. This alignment step placed a longer length of the vein within the FOV, thus enabling us to select an appropriate target location away from the ONH. Next, we acquired a pre-occlusion baseline OCTA image, showing the vein of interest, v, in Fig. 3(A). We administered RB via tail injection and immediately acquired a co-localized pre-occlusion RB angiogram (Fig. 3(A)) using the SLO angiography protocol. RB was seen flowing with the retinal arteries and veins. We selected a specific location on the vein for occlusion, indicated by the red box in Fig. 3(A).

To begin the occlusion procedure (actinic protocol), the SLO FOV was decreased until the target vein spanned the FOV. Continuous SLO scanning at high laser power (25 mW) was then initiated. From the collected SLO images, we calculated the mean RB fluorescence signal versus time, as shown in Fig. 3(B). In this experiment, the occlusion procedure lasted 12 seconds. Four selected frames of the vessel are shown as insets in Fig. 3(B, 1-4). Frame 1 shows the first acquired SLO image, where the vessel spans most of the FOV. At 2 s (frame 2), diminished SLO signal near the vessel wall was observed (yellow arrow), which represents the nidus for platelet aggregation [24, 25]. By 6 s (frame 3), RB fluorescence within the FOV was diminishing, which implied decreasing intravascular blood flow. At 9 s (frame 4), the RB SLO signal diminished abruptly, which indicated that blood flow was completely stopped at the target site. To ensure stable thrombus formation, we waited for an additional 3 seconds after observing diminished intravascular RB SLO signal, before we shut off the high-power scanning.

Afterwards, we returned to the SLO angiography protocol (Fig. 3(C)), which revealed vessel discontinuity at the target location and absence of RB fluorescence upstream of the occlusion site, indicating a successful occlusion. Moreover, co-localized post-occlusion OCTA showed diminished OCTA signal within the target vein (Fig. 3(D)), which also indicates reduced blood flow. Comparing the SLO image with OCTA image, OCTA showed higher contrast and more details of the deeper capillary network. The most striking difference between the pre-occlusion and post-occlusion OCTA images is an area of capillary non-perfusion in a sector surrounding the occluded vein (white dashed region in Fig. 3(E)). Pre-occlusion cross-sectional OCT revealed intact retinal layers and a vessel shadow corresponding to the target vessel (Fig. 3(F)). After the occlusion, the vessel shadow disappeared indicating less absorption by hemoglobin from the reduced blood flow (Fig. 3(G)). The Bruch’s membrane and RPE were intact. There was no evidence of retinal edema or swelling, and retinal layers were intact even in the areas with capillary non-perfusion on OCTA.
Fig. 3. Producing a branched retinal vein occlusion. (A) Pre-occlusion RB angiogram. Red box indicates scanning area for occlusion. (B) Mean RB fluorescence signal during occlusion corresponding to red box in A. Insets 1 to 4 show frames at points 1 to 4 on the curve. Laser power was 25 mW at the pupil. (C) Post-occlusion RB angiogram. (D) Pre-occlusion OCTA of a retinal vein. (E) Post-occlusion OCTA. White solid circles in D and E indicate the site of occlusion. White dashed region indicates area of capillary non-perfusion. (F) Pre-occlusion OCT B-scan at the white dashed line in D. (G) Post-occlusion OCT B-scan at the white dashed line in E. Yellow arrows in F and G indicate vessel location. Horizontal scale bars are 500 µm. Vertical scale bars are 100 µm. a: artery; v: vein.

We performed another BRVO experiment where we longitudinally monitored the retinal microvasculature before and after the occlusion. A BRVO was created at the 9 o’ clock position, indicated by the yellow circles on the pre-occlusion and post-occlusion RB angiograms (Fig. 4(A) & Fig. 4(B)). The post-occlusion RB angiogram showed increased dilation and tortuosity before reaching the occlusion site (yellow arrow). Additionally, we also observed leakage of the RB dye in the peripheral area (magenta arrow). Pre-occlusion (Fig. 4(C), post-occlusion (Fig. 4(D)) and day 1 (Fig. 4(E)) OCTA montages were performed. The montages consisted of nine images and covered a larger FOV of approximately 3.5 mm². In the pre-occlusion depth-colored OCTA (Fig. 4(C)), three healthy vascular networks can be seen: one near the inner limiting membrane (ILM) in red, a second near the inner plexiform layer (IPL) in orange-yellow, and a third near the outer plexiform layer (OPL) in green [5]. Labeled arteries, a, and veins, v, alternate in the typical mouse retinal pattern. The BRVO location is denoted by the white solid circle at the 9 o’ clock position.

The white dashed region in Fig. 4(D) shows the sectorial area of capillary non-perfusion associated with the vein occlusion. At day 1, the area of capillary non-perfusion enlarged but stopped at the nearest adjacent veins (white dashed region in Fig. 4(E)). This progression of
capillary non-perfusion has been observed in both monkey and rat models of RVO [26, 27]. Slow blood flow may predispose to increased clot formation in the capillary bed, and the interstitial edema may create enough interstitial pressure to close capillaries [27].

Previous investigations of BRVO mouse models have observed interstitial edema and increased retinal thickness by day 3 post-occlusion [6, 7]. We observed similar results in our animal model. A pre-occlusion OCT B-scan through the ONH showed normal intact retinal layers (Fig. 4(F)). On the post-occlusion B-scan (Fig. 4(G)), the retinal layers were still distinguishable on both the sides of the ONH. On day 1, the OCT B-scan showed evident increase in retinal thickness on the side of the retina with the occlusion (Fig. 4(H)). On the side with occlusion, we observed increased scattering in the GCL, IPL, and INL layers, and the retinal layer boundaries became hard to distinguish. The retinal layers on the side without occlusion appeared intact. Retinal thickness measurements on the side of the occlusion were 265 µm, 273 µm, and 389 µm pre-occlusion, post-occlusion, and day 1 post-occlusion, respectively. On the opposing side, retinal thickness measurements were 257 µm, 250 µm, and 266 µm pre-occlusion, post-occlusion, and day 1 post-occlusion, respectively. Measurements were performed at a radial distance of 800 µm from the ONH.

![Fig. 4. Longitudinal OCTA of branched vein occlusion.](image)

To demonstrate that our technique could also produce CRVO patterns of occlusion, we targeted a 12 o’clock retinal vein at a location approximately 4 ONH diameters away from the ONH. As reported previously, CRVO patterns are more likely when the target location along the vessel is positioned more proximal to the ONH [7]. Figure 5(A) shows the pre-occlusion RB angiogram with the target location indicated by the yellow circle. Figure 5(B) and Fig. 5(C) show the post-occlusion RB angiograms. RB flow was only observed past the occlusion site. The baseline OCTA image shows a healthy adult vascular network (Fig. 5(D)). However, unlike the BRVO-like post-occlusion OCTA montages, the CRVO-like post-occlusion OCTA showed widespread capillary non-perfusion extending beyond the adjacent retinal veins (Fig. 5(E)). On day 1, the capillary networks are markedly absent on OCTA, as shown in Fig. 5(F). The corresponding OCT B-scans pre-occlusion for each point in time are shown in Fig. 5(G,H,I). The pre-occlusion and post-occlusion OCT B-scans showed intact
retinal layers on both sides of the ONH. On day 1 post-occlusion, however, the retinal layer boundaries were obscured and there was increased scattering throughout the inner retinal layers. Diffuse swelling of the retina was observed on day 1 (Fig. 5(I)), which differed from the BRVO pattern in Fig. 4. The pre-occlusion retinal thickness measurements were 257 µm nasally and 246 µm temporally. The post-occlusion retinal thickness measurements were 273 µm nasally and 250 µm temporally. The retinal thickness measurements on day 1 were 524 µm nasally and 514 µm temporally.

Figure 5 demonstrates that our imaging-guided vascular occlusion technique could also produce animal models of artery occlusive disease. Figure 6(A) shows the pre-occlusion RB angiogram with the target location marked by the yellow circle. The white arrow indicates a branch point of the arterial tree. Figure 6(B) shows the post-occlusion RB angiogram. There was limited flow past the occlusion site. In pre-occlusion OCTA, shown in Fig. 6(C), the white solid circle indicates the target site of occlusion on an artery at the 12 o’clock position. In the post-occlusion OCTA, shown in Fig. 6(D), we observed a sector of non-perfusion, similar to the vein occlusions shown previously. The white arrows on Fig. 6(A-D) highlight a bifurcation of the retinal artery. Since the artery occlusion was positioned past the bifurcation, one of the branches had diminished OCTA signal, while the other branch continued to have OCTA signal, post-occlusion, which is expected for arterial blood flow. On day 1, the OCTA montage showed that region of non-perfusion increased in size, extending to the next adjacent artery (Fig. 6(C)).
Fig. 6. Longitudinal OCTA of branched artery occlusion (A) Pre-occlusion RB angiogram. Yellow circle indicates site of occlusion. (B) Post-occlusion RB angiogram. (C) Montage of 3 pre-occlusion OCTA images. White solid circle denotes the site of occlusion. White solid arrow indicates an arterial branch point. a: artery; v: vein; (D) Montage of 3 post-occlusion OCTA images. White dashed region denotes area of capillary non-perfusion. White solid arrow indicates an arterial branch point. (E) Montage of 3 OCTA images on day 1. White dashed region denotes area of capillary non-perfusion. Scale bars: 500 µm.

4. Discussion

We successfully developed an imaging system and experimental protocol for producing precise retinal occlusions in the murine inner retina (Figs. 1 & 2). To the best of our knowledge, this is the first demonstration of SLO guidance to produce retinal vascular occlusions for animal studies. To target a vessel for occlusion, we used SLO to ensure that RB fluorescence was observable within the retinal vasculature. After precisely choosing a target vessel location, we used the same SLO system at a higher laser power to deliver actinic light to the intravascular RB (Fig. 3). Using this imaging system and occlusion protocol, we demonstrated the production of BRVO (Fig. 4), CRVO (Fig. 5), and BRAO (Fig. 6) patterns in the mouse retina.

Our system has permitted the characterization of retinal occlusions with OCT and OCTA. Acquired OCT volumes allowed us to observe the retinal edema associated with vascular occlusion. Whereas previous studies have used fluorescein angiography (FA) to study retinal vascular occlusions [7, 8], we used OCTA to monitor the longitudinal changes in the retinal microvasculature, pre-occlusion, immediately post-occlusion, and on day 1 post-occlusion. Unlike FA, OCTA provides 3D images of the vascular network, enabling us to use a depth color map on our en face OCTA montages. Additionally, no contrast agent was required to obtain the angiograms with OCTA, which was extremely desirable for longitudinal monitoring. Moreover, FA has difficulty visualizing the complete deep vascular network compared to OCTA [28]. With OCTA, we were able to monitor the changes in capillary non-perfusion cases of BRVO, CRVO, and BRAO.

Creating retinal occlusions has depended primarily upon the slit-lamp biomicroscope [3–9]. Our multimodal imaging system for producing retinal occlusions in animal models has several advantages over using a slit-lamp biomicroscope. First, the system is easy to use and requires little training to perform the procedure, other than learning how to perform tail injections. Second, the SLO enables the verification of RB within the retinal vessels of the eye. Since RB has a short half-life in the bloodstream of approximately 5 minutes [29], it is important to verify that RB is within the target retinal vessel. Third, instead of relying on non-specific signs of occlusion (e.g. whitening of the vein) to determine when vascular occlusion
occurs, SLO enables monitoring of the thrombus formation in real time. The real-time monitoring allows the operator to remove the high-power illumination after the occlusion is observed, which prevents excess laser dosage to the animal eye. On the other hand, real-time monitoring with SLO also enables the operator to tell if the occlusion is partially formed. At that point, the operator can choose to increase the illuminating laser power to ensure the full formation of the occlusion. Finally, compared with the slit-lamp biomicroscope, the spot size on the retina is better controlled in SLO because the same spot size used for imaging is also used to perform the occlusion. This makes the actinic laser delivery much more precise and minimizes damage to the surrounding retina.

There are some limitations to this work. First, producing retinal vascular occlusions using RB, although similar to producing a thrombus, is similar, but not equivalent, to the pathogenesis of human retinal occlusive diseases. For example, the pathogenesis of the disease for BRVO’s in humans is believed to result from arterial compression of venules by stiffened arteries at arterio-venous crossings [1]. Additionally, thrombus formations usually cause CRVO’s, but emboli usually account for BRAOs [1, 30]. Nevertheless, the RB method provides a reliable method to produce an animal model with similarities to human retinal vascular occlusions, such as the inner retinal edema. Another limitation of this study is that it is unknown why occlusions performed close to the ONH cause a CRVO pattern while occlusions further from the ONH result in a BRVO pattern. This phenomenon has also been observed by other researchers [7]. Explanations of this phenomenon remain to be explored in detail by future investigations, but they may include that the thrombus formed by RB travels further down towards the central vein, thereby affecting more branches. Alternatively, the retinal circulation in mice may be interconnected such that capillary closure close to the ONH affects a larger radius than closure farther away from the ONH.

Other limitations relate to the angiographic methods used in this study. We noticed that there was some discrepancy between OCTA and RB angiography in showing the flow in the major vessels, especially in areas near the occlusion site. In some cases, RB still showed flow, while OCTA did not. This could be because the OCTA takes some time to acquire after the occlusion, when the flow patterns are adjusting to the intervention. Another possible reason could be that the OCTA only detects blood movement, while RB angiography detects both moving and static dye. This could be modified in future studies by changing the time between successive B-scans [31]. Finally, another limitation is that RB angiography was unable to observe retinal capillaries in the deep retinal network, which is likely due to the low fluorescence quantum yield of RB (0.05 in ethanol) [32]. For comparison, fluorescein has a quantum yield of 0.79 [33]. Future work may incorporate other fluorescing dyes with higher quantum yields for better visualization of the deep capillary network.

In conclusion, we have outlined the development of an OCT and SLO based retinal vascular occlusion system. We have detailed the retinal vascular occlusion protocol when using this system. In addition, we have showed real-time monitoring of retinal vascular occlusions with RB and performed OCT and OCTA monitoring of the occlusions. Finally, we have discussed the benefits and limitations of the system as compared with traditional approaches. The demonstrated ability to precisely and consistently create vascular occlusions in the rodent retina will be an important step for animal studies of retinal vascular occlusive disease.

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