Retina

Evaluation of Retinal Circulation Using Segmental-Scanning Doppler Optical Coherence Tomography in Anesthetized Cats

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Purpose. To study retinal blood flow (RBF) measurement reproducibility using segmental-scanning Doppler optical coherence tomography (DOCT) in vitro in glass capillaries and in vivo in anesthetized cats.

Methods. As a preliminary study, the flow rates of human blood through glass capillaries were changed by using an infusion pump and measured at 13 preset velocities by DOCT. For in vivo measurement, the cats were anesthetized using sevoflurane. The flow in the parent vessel was compared with the sum of the flow values in the two daughter vessels. The RBF was measured using two different instruments: bidirectional laser Doppler velocimetry (LDV) and DOCT. The reproducibility of the measurements was assessed by calculating the coefficients of variation (CVs) for repeated measurements of RBF at the superior retinal arterioles and venules.

Results. In vitro, the flow velocities measured by DOCT agreed well with the preset velocities. In vivo, the flow in the parent vessel agreed with the sum of the flow values in the two daughter vessels. In addition, there were no significant differences in the mean averaged CVs of the RBF in both the arterioles and venules between LDV and DOCT.

Conclusions. The newly developed segmental-scanning DOCT revealed the accuracy of the measurement in in vitro glass capillaries and reproducibility of the measurements of blood velocity in both the retinal arterioles and venules in anesthetized cats.

Keywords: retinal microcirculation, Doppler optical coherence tomography, retinal blood flow measurement

Investigative Ophthalmology & Visual Science

Numerous clinical studies have reported that measuring the retinal blood flow (RBF) is very important for diagnosing and monitoring ocular vascular diseases.[1] We reported previously that RBF is impaired in patients with type 2 diabetes mellitus with no and mild diabetic retinopathy (DR) using laser Doppler velocimetry (LDV), suggesting that impaired RBF may contribute to the pathogenesis of DR.[2] In addition, LDV has been used previously to evaluate the RBF in patients with glaucoma,[3] branch retinal vein occlusion,[4] AMD,[5] and retinitis pigmentosa.[6] Because the advantage of the LDV is that it can calculate the absolute RBF values by measuring the absolute blood velocity in humans,[7] it has been used clinically to compare the RBF values obtained from patients with retinal vascular disorders with those from healthy subjects.

However, the LDV technique has some shortcomings. In this method, the blood flow in the retinal vessels should be considered as parabolic to calculate the absolute values of blood velocity.[7] It is generally accepted that it is impossible to measure the bifurcation or the margin of the optic nerve head because of uncertainty about whether the flow is parabolic or turbulent in those areas. Therefore, a novel technique is needed to measure the absolute RBF values.

Recently, some research groups have reported such a novel technique using optical coherence tomography (OCT) technology, that is, Doppler OCT (DOCT),[8–10] which enables measurement of the absolute values of RBF in humans with good repeatability. However, those researchers reported that DOCT can measure the retinal venous blood flow velocity but not the arterial velocity. The blood flow measurement must be continued for at least one cardiac cycle to obtain blood velocity in the pulsatile retinal arterioles. Recently, we developed a DOCT instrument with novel software, referred to as a segmental-scanning method (Yoshida A, et al., *IOVS* 2013;54:ARVO E-Abstract 4664). This method is expected to enable simultaneous measurement of the RBF in the retinal arterioles and adjacent venules during one cardiac cycle. In the current study, we evaluated the accuracy of the measurements in vitro in glass capillaries and the reproducibility of the RBF measurements in retinal arterioles and venules in vivo using segmental-scanning DOCT in anesthetized cats.

Methods

Animal Preparation

The Animal Care Committee of Asahikawa Medical University approved the protocols involving the use of cats, and the study adhered to the ARVO Statement for the Use of Animals in

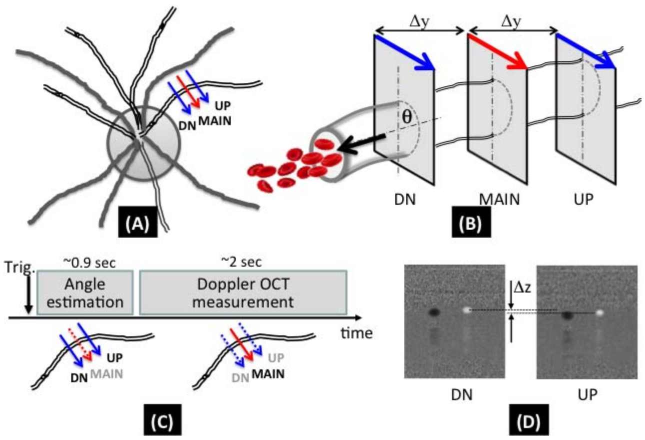

Figure 1. Illustration of a Doppler angle and velocity measurement using the segmental-scanning DOCT method. (**A**–**C**) The Doppler angle is measured with two alternative scans (UP and DN) in separation of $2\Delta y$ followed by main scan (MAIN) for 2 seconds. (**D**) Using the phase image from two alternative scans (UP and DN), the centers of the vessels are identified to calculate the incident Doppler angle.

Ophthalmic and Vision Research. Five adult cats (Shiraishi Animal Laboratory Co., Ltd., Saitama, Japan) of either sex were used (weight, 2.8–3.8 kg) in the in vivo study. As described previously,[11] anesthesia was induced with sevoflurane, oxygen, and nitrous oxide with the animals in a closed box followed by an intraperitoneal injection of atropine (0.04 mg/kg). The animals then were tracheostomized and ventilated mechanically with 1.5% to 2.0% sevoflurane and room air during the experiment. Catheters were placed in the femoral arteries and the left femoral vein. Pancuronium bromide (0.1 mg/kg/h; Daiichi Sankyo Co., Tokyo, Japan) was infused continuously. A blood gas analyzer (Model ABL5; Radiometer, Copenhagen, Denmark) intermittently measured the arterial pH, arterial partial carbon dioxide tension, and arterial partial oxygen tension. The mean arterial blood pressure (MABP) and heart rate were monitored continuously. A heated blanket was used to maintain each cat's rectal temperature between 37°C and 38°C. The pupils were dilated with 0.5% tropicamide (Santen Pharmaceutical Co., Osaka, Japan); a 0-diopter contact lens was placed on the cornea, and a drop of sodium hyaluronate (Healon; Abbott Medical Optics, Inc., Santa Ana, CA, USA) was instilled. A 26-gauge butterfly needle connected to a bottle of balanced saline solution (BSS Plus; Alcon Laboratories, Fort Worth, TX, USA) was inserted into the anterior chamber and connected to a pressure transducer. The IOP was monitored continuously.

Retinal Blood Flow Measurement

Laser Doppler Velocimetry. We used an LDV system (Canon Laser Blood Flowmeter CLBF; Canon Co., Tokyo, Japan) customized for feline use to measure the RBF in cats. The instrument simultaneously measures blood vessel diameter and blood flow velocity in the retinal vessels and automatically calculates the RBF, as described previously.[12] Laser Doppler velocimetry measurements were obtained from either the arteriole or venule in one eye of each animal. The retinal vessels chosen for measurement had relatively straight segments that were sufficiently distant from the adjacent vessels. The RBF was calculated according to the formula RBF $= S \times V_{\text{mean}}$, where S is the cross-sectional area of the retinal artery at the LDV measurement site, assuming a circular cross-section, and V_{mean} is the mean blood velocity calculated as $V_{\text{mean}} = \text{V}_{\text{max}}/2$.[12]

Doppler OCT. Our system is based on a commercially available spectral-domain OCT system (3D OCT-2000 FA; Topcon Corp., Tokyo, Japan) operated at an 800-nm wavelength range (Yoshida A, et al., *IOVS* 2013;54:ARVO E-Abstract 4664). Image capturing software was modified for Doppler imaging, and image processing software was newly developed for the blood flow measurement.

In DOCT, the flow velocity $v(z)$ can be derived from the Doppler shift incurred by the moving blood:

$$v(z) = \frac{\Delta\Phi(z,\tau)\cdot\lambda_0}{4\pi\cdot n\cdot\tau}\cdot\frac{1}{\cos\theta} \qquad (1)$$

where z is the depth location, $\Delta\Phi$ is the phase difference at the same depth location between the adjacent profiles after Fourier transform, λ_0 is the center wavelength, n is the refractive index of blood, τ is the time interval between the adjacent profiles, and θ is the Doppler angle between the flow vector and the incident probe beam.

Besides measurement of the phase difference, the Doppler angle θ must be known to calculate the velocity using Equation 1. In the retina, most blood vessels run nearly parallel to the retinal surface except at the area around the optic nerve head. It has been noted that as θ approaches 90 degrees, the measured velocity becomes very sensitive to the accuracy of θ. To minimize this potential velocity measurement error, our Doppler blood flow measurements were performed where the Doppler angle θ is considerably less than 90 degrees (i.e., approximately 80 degrees). In this experiment, the incident beam was shifted intentionally from the pupillary center where the probe beam is away from the right angle to the vessel in most cases.

To calculate the Doppler angle, we developed the following scanning protocol and algorithms. The Doppler angle θ was measured by performing two OCT scans (UP and DN) from the DOCT image location (MAIN) (Figs. 1A, 1B). The operator designated the scanning position and its direction for blood velocity analysis such that the scanning direction was perpendicular to the blood vessel and the Doppler angle θ was considerably less than 90 degrees. Meanwhile, the UP and DN scans were positioned on the opposite sides of MAIN with an equal distance of Δy. Once imaging started, the OCT images were acquired at the UP and DN positions alternatively with a time interval of B-scan frame (approximately10 ms); the MAIN scan then was performed at the same position for at least one cardiac cycle. Based on the OCT and phase images acquired at the UP and DN positions, the vessels were identified automatically on the OCT and phase images. The axial distance Δz between the blood vessel centers for corresponding vessels can be calculated to derive the Doppler angle θ according to the formula: $\theta = \tan^{-1}\Delta z/(2\Delta y)$. In this case, Δy was set to 100 μm.

Unlike laser Doppler velocimetry, DOCT provides a two-dimensional cross-sectional imaging feature that enables depth-resolved velocity profiling. Therefore, the blood flow rate can be calculated easily by integrating the velocity distribution within the blood vessel. Because the bulky motion caused by involuntary ocular movement can introduce artifacts into the DOCT images, the software corrected the motion automatically. The blood flow rate can be calculated by integrating the velocity in the blood vessel. A mask is generated based on the intensity threshold in the DOCT image. The flow rate is the sum of the velocity inside the mask multiplied by the corresponding spatial area for each pixel, which is determined by the sampling resolution of the OCT instrument. The analysis was automated so that no further manual operation was

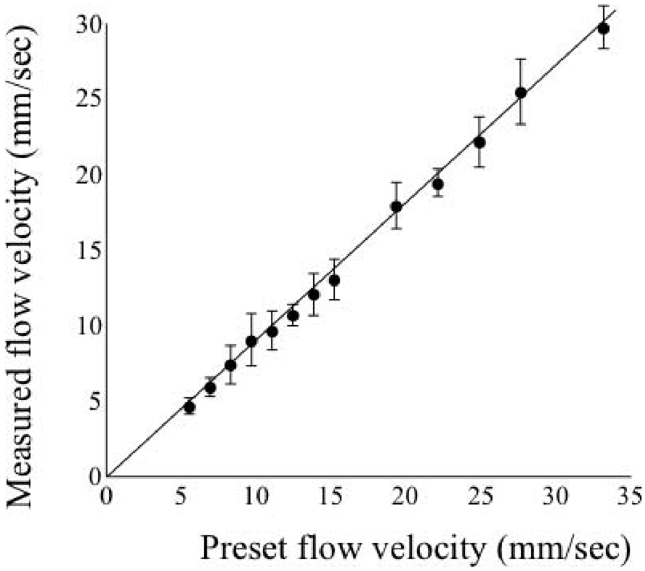

Figure 2. The relation between the preset flow rate and measured flow rate by segmental-scanning DOCT in vitro for three measurements in three glass capillaries. Data were expressed as the mean ± SD ($n = 3$). mm/sec, millimeters/second.

necessary. In our segmental-scanning DOCT system, the axial and transverse sampling pitches were 2.6 and 1.8 µm, respectively. For example, if the vessel diameter is 100 µm, the numbers of pixels to determine the axial and transverse directions were 38 and 57 pixels, respectively, which comprised approximately 2200 pixels for calculating the blood vessel velocity.

Calculations

The MABP was determined using the formula MABP = diastolic BP + (systolic BP − diastolic BP)/3. Because the cats were in the prone position during the experiments, the ocular perfusion pressure (OPP) was calculated using the formula OPP = MABP − IOP.[11]

Procedures

In Vitro. The velocities of human blood through glass capillaries were measured at 13 different preset velocities (range, 5.5–33.2 mm/s) using segmental-scanning DOCT. A fine glass tube with an inner diameter of 140 µm (AS ONE, Osaka, Japan) was connected to an infusion pump to circulate heparinized human blood collected from a healthy volunteer who was one of the authors (AI). The preset blood flow velocity in the glass tube was varied between 5 and 35 mm/s to be within normal flow velocity ranges, as we reported previously using LDV in healthy eyes.[7]

In Vivo. To compare the reproducibility of the RBF measurements obtained using segmental-scanning DOCT with those obtained using LDV, we calculated the coefficient of variation (CV) (100 [SD/mean]; mean ± SD). We obtained five values from the same vessel every minute for 5 minutes. The average value of the CV for each vessel was calculated for each cat to evaluate the reproducibility with both techniques. To demonstrate the conservation of flow in the retinal vessels using segmental-scanning DOCT, we examined the relation of the RBF between a first-order (parent) vessel and the sum of two second-order (daughter) vessels.

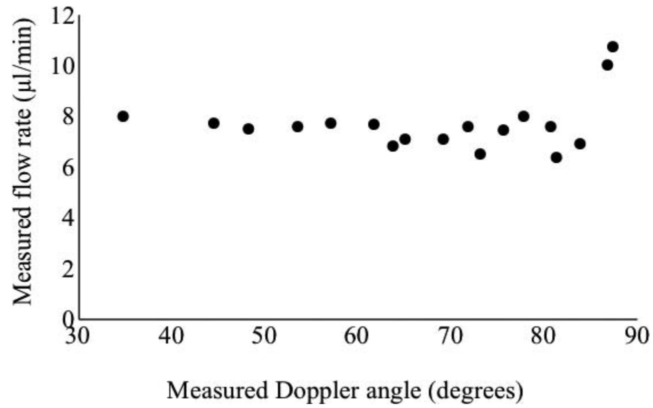

Figure 3. The flow rates measured with different preset Doppler angles. The Doppler angle is changed at the various angles of the glass capillary with constant preset flow. The measured flow rate seems stable under 85 degrees but is overestimated over 86 degrees of the Doppler angle measured by segmental-scanning DOCT.

Statistical Analysis

All data are expressed as the mean ± SD. The Student's paired *t*-test was used to compare the paired data. Linear correlation analysis was used to compare the preset values and the actual measured values using DOCT. The reproducibility of the blood flow measurement was evaluated by calculating the CV. Two-way ANOVA was used to compare the results measured by LDV and DOCT; P less than 0.05 was considered significant.

Results

In Vitro

With increases of the preset flow rate in a glass capillary with human blood at the Doppler angle of 80 degrees, the flow rate was measured using segmental-scanning DOCT in vitro in a glass capillary. The measured blood flow velocity agreed well with the preset flow velocity for three measurements of three glass capillaries (Fig. 2). The Doppler angle was set against the various angles of the glass capillaries with the constant preset flow. The flow rate measured by segmental-scanning DOCT was nearly constant if the Doppler angle was less than 85 degrees. However, it seems to be overestimated when set to over 86 degrees (Fig. 3).

In Vivo

Figure 4 shows the imaging result of a cat using our DOCT. The OCT intensity and phase images are shown in Figures 4A and 4B, where the phase image was represented as a pseudo-color code. We confirmed that the blood velocity profiles of a superior retinal arteriole and venule were obtained simultaneously by segmental-scanning DOCT in an anesthetized cat (Figs. 4C, 4D). Figure 5 shows the measured flow rates at various Doppler angles in the in vivo cat model. To examine the range of the Doppler angle for accurate measurement of the RBF in the in vivo cat model, we changed the Doppler angle by tilting the head of the cat against the incident beam. The measured flow rates in the arterioles and venules seemed stable under 85 degrees but overestimated over 86 degrees of the Doppler angle measured by segmental-scanning DOCT. This experiment proved that the results of measuring the RBF in both retinal arterioles and venules are reliable at less than 86 degrees of the measured Doppler angle using our DOCT system (Fig. 5).

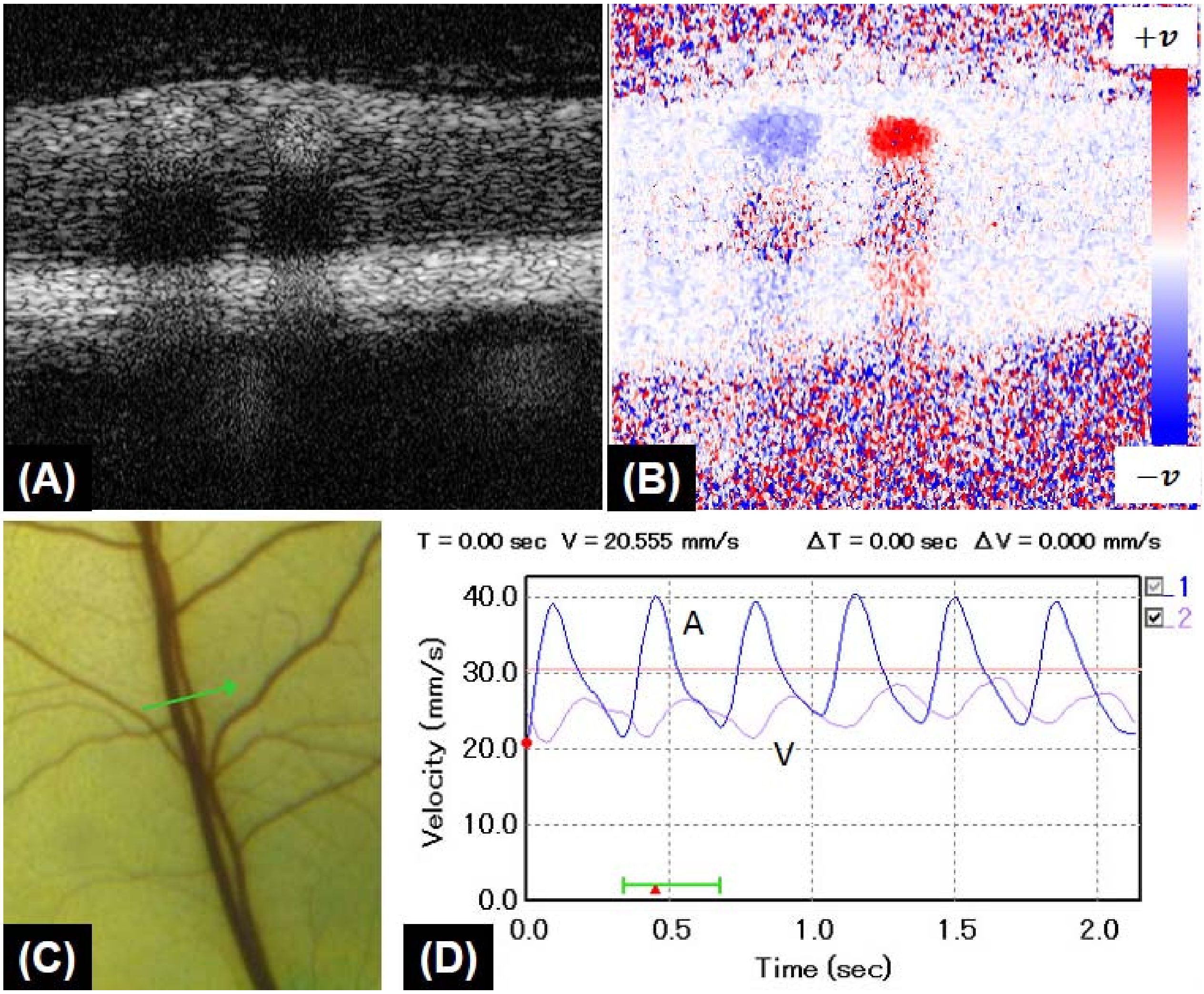

FIGURE 4. Simultaneous measurement of the RBF in the retinal arterioles and venules in an anesthetized cat. (**A**) An OCT image. (**B**) A phase image with color coding. (**C**) A cat fundus image. The *green bar* indicates the measured location. (**D**) Blood velocity profiles of the retinal arterioles and venules measured simultaneously by segmental-scanning DOCT. sec, seconds.

There was no significant difference between the flow in the parent vessel and the sum of the flow values in the two daughter vessels in anesthetized cats (Table 1). Table 2 shows the CVs (%) for the measurements of the RBF in the retinal arterioles and venules measured by LDV and DOCT in five cats. The averaged CVs (mean ± SD) of the RBF were 7.7% ± 6.4% and 9.1% ± 4.4% in the arterioles and 9.5% ± 4.9% and 12.4% ± 5.4% in the venules using LDV and DOCT, respectively ($P = 0.34$ and $P = 0.18$).

DISCUSSION

In the current study, we confirmed for the first time that the newly developed segmental-scanning DOCT showed the accuracy of measurements in vitro in glass capillaries and reproducibility of the blood velocity measurements in retinal arterioles and venules in anesthetized cats.

It has been generally accepted that the velocity cannot be measured accurately using DOCT when the Doppler angle is close to 90 degrees (i.e., with the optical axis and flow vector perpendicular), because the Doppler shift or the phase difference becomes 0.[9] In our segmental-scanning DOCT, the blood velocity is determined by the combination of the phase shift $\Delta\phi(x,\tau)$ and Doppler angle θ. We mimicked the blood flow in the major retinal vessels using fine glass tubes with an inner diameter of 140 μm through which blood samples were pumped at different flow velocities. We scanned the glass tube with DOCT and confirmed that the measured blood flow using our segmental-scanning DOCT corresponded to the preset blood flow in vitro in the glass capillaries (Fig. 2). In addition, in our in vivo cat model, we also found that the sums of the absolute values of the RBF in the daughter vessels were similar to that in one parent vessel (Table 1) using our DOCT. These in vitro and in vivo data indicated that the accuracy of the calculation of the RBF by our DOCT is warranted.

Because the detected velocity is sensitive to the Doppler angle, it is important to measure the Doppler angle accurately. We confirmed that the measurement of the Doppler angle was reliable in in vitro (Fig. 3) and in vivo (Fig. 5) experiments by using our segmental-scanning DOCT. In another Doppler frequency-domain OCT system[8] (RTVue System; Optovue, Inc., Fremont, CA, USA), the vessel identifications were graded manually based on their information about the Doppler signal (i.e., vessel size, location between the inner and outer circles,

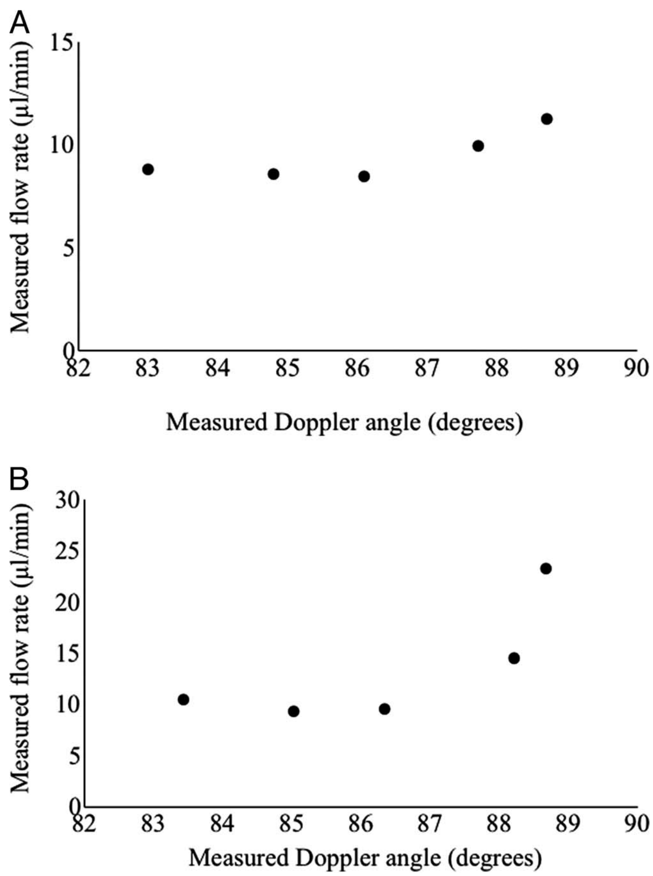

FIGURE 5. The measured flow rates at various Doppler angles by tilting the head of the cat. The measured flow rates in the arterioles (**A**) and venules (**B**) seem stable under 85 degrees but overestimated over 86 degrees of the Doppler angle measured by segmental-scanning DOCT.

clarity of the vessel boundary, and vessel type).[8] In that system, the grader was required to adjust the Doppler signal, size, and vessel location based on the DOCT image to determine the flow; however, these modifications are subjective and time-consuming. In contrast, our segmental-scanning DOCT system enables automatic calculation of the Doppler angle using the combination of OCT intensity and phase images. This fully automated calculation facilitates objective evaluation of the results in a clinic setting.

A technical strength of the current study was the use of fully automated grading and calculation techniques for the RBF measurements in the retinal arterioles and venules. In contrast,

TABLE 1. Measurements of RBF (μL/min) at the Bifurcation in the Retinal Vasculature in Anesthetized Cats ($n = 4$)

Cat No.	Vessel	Portion 1	Portion 2	Portion 3	Portions 2+3
Cat 1	Arteriole 1	12.7	7.7	4.6	12.3
Cat 1	Arteriole 2	7.4	4.6	2.9	7.5
Cat 1	Arteriole 3	3.1	1.9	1.1	3.1
Cat 1	Venule 1	14.6	12.7	3.3	16.0
Cat 1	Venule 2	22.9	19.1	3.6	22.7
Cat 2	Venule	8.8	3.5	4.3	7.8
Cat 3	Arteriole	7.9	4.2	2.7	6.9
Cat 4	Venule	9.7	7.2	3.2	10.4

Portion 1, first-order (parent) vessel; Portions 2 and 3, two second-order (daughter) vessels.

TABLE 2. Comparison of CVs (%) for Measurement of RBF in Retinal Arterioles and Venules Measured by LDV and DOCT ($n = 5$)

	LDV		DOCT	
	Arterioles	Venules	Arterioles	Venules
Cat 1	10.0	12.8	7.2	15.9
Cat 2	1.8	5.4	14.2	13.9
Cat 3	4.6	16.2	5.6	6.0
Cat 4	17.7	16.8	5.1	18.8
Cat 5	4.3	6.3	13.4	7.5
Average	7.7 ± 6.4	9.5 ± 4.9	9.1 ± 4.4	12.4 ± 5.4

as an alternative technique, bidirectional two-beam systems have been recently introduced to measure absolute blood flow velocities in not only humans,[13] but also animals.[14] The dual-beam bidirectional technique is a reliable method that does not require the Doppler angle. This technique is suitable for the detection when the vessel is nearly parallel to the fundus. However, the technique requires complex optical settings using two-beam and two-spectrometer configuration with polarization optics. To achieve a relatively reasonable Doppler angle difference between the two beams, beam separation of approximately 4 mm at the pupil also requires a relatively large pupil. In contrast, our single-beam–based segmental-scanning DOCT was incorporated into the commercially available spectral-domain OCT system with modified software. Although it is easy to use, we found that the measured phase change is sensitive to the Doppler angle. Because the Doppler shift is dependent on the Doppler angle θ between the incident beam and flow direction, such angle dependency needs to be compensated by the factor of $1/\cos(\theta)$. Based on our preliminary experiment, the Doppler angle should be set at less than 85 degrees for reliable measurements, which requires the clinician to operate the instrument at the proper position. To make operation easier, real-time Doppler angle monitor function is integrated for the alignment. Such user-interface makes the system practical for use in clinical settings.

The detected velocity is the product of the phase signal and Doppler angle, meaning that the phase signal is compensated by the Doppler angle. Increasing the Doppler angle makes the phase signal weak from Equation 1. We observed that the RBF tended to be overestimated at Doppler angles above 85 degrees in vitro (Fig. 3) and in vivo (Fig. 5). The current results suggested that a blood velocity may be sensitive to the Doppler angle above 86 degrees using our segmental-scanning DOCT. Based on our findings, we determined that the RBF result cannot be used automatically if the calculated Doppler angle exceeded 86 degrees.

We compared the ability to detect the changes in RBF by our segmental-scanning DOCT with the LDV. We found that the individual CVs for RBF obtained using DOCT were similar to those of LDV (Table 2). We believe that our DOCT system enables accurate and reliable measurement of RBF during rest and in response to physiologic stimuli, which can change the RBF at least in an in vivo cat model.

The current study had some limitations. First, unlike the double-circle–based calculation methods,[8] it was impossible to measure all segments of the retinal vessels using our segmental-scanning DOCT for one single measurement. Multiple measurements are needed to calculate the total RBF using our DOCT. When measuring the RBF using this system in humans, we need to confirm that an appropriate Doppler angle is warranted to prevent overestimation of the blood velocity. Second, it is possible that some very low speeds may not be detected because of the phase noise using velocity profiles.

The low velocity values of the red blood cells flowing at the border of the vessel especially may lead to erroneously underestimated vessel diameters. Based on our spectral-domain OCT system, the detection limit of low flow speed is determined by the phase noise of the system. Phase noise is typically measured using a stationary mirror, and the SD of the phase recorded at all A-scans in a 512-line B scan was calculated. The minimal Doppler frequency calculated from the measured phase noise SD was 0.0174 radian, which was calculated to be approximately 250 μm/s when the Doppler angle was 80 degrees. In addition, an in vitro experiment using fine glass capillaries with an inner diameter of 140 μm with flowing human blood cells showed that the measured diameter was close to the specification of the fine glass capillaries (Supplementary Fig. S1). This result suggested that the accuracy of the measured diameter is within a couple of microns. Similarly, Muraoka et al.[15] reported that the measured vertical diameter of the glass capillary tube was not dependent on blood flow velocity when human blood was pumped through glass tubes at different flow velocities, which agreed with our finding. Further clinical study is warranted to confirm whether the flow velocity affects the measurement of the vessel diameter/cross-sectional area in human retinal vessels using DOCT.

In conclusion, the current findings indicated that the newly developed segmental-scanning DOCT enabled accurate and reproducible measurements of the blood flow in the retinal arterioles and venules in an in vivo cat model. We believe that DOCT looks promising for future clinical studies to elucidate the mechanisms of retinal vascular disorders in DR and retinal vein occlusions.

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References

1. Pournaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res*. 2008;27:284–330.
2. Nagaoka T, Sato E, Takahashi A, Yokota H, Sogawa K, Yoshida A. Impaired retinal circulation in patients with type 2 diabetes mellitus: retinal laser Doppler velocimetry study. *Invest Ophthalmol Vis Sci*. 2010;51:6729–6734.
3. Berisha F, Feke GT, Hirose T, McMeel WJ, Pasquale LR. Retinal blood flow and nerve fiber layer measurements in early-stage open-angle glaucoma. *Am J Ophthalmol*. 2008;146:466–472.
4. Nagaoka T, Sogawa K, Yoshida A. Changes in retinal blood flow in patients with macular edema secondary to branch retinal vein occlusion before and after intravitreal injection of bevacizumab. *Retina*. 2014;34:2037–2043.
5. Sato E, Feke GT, Menke MN, McMeel WJ. Retinal haemodynamics in patients with age-related macular degeneration. *Eye*. 2006;20:697–702.
6. Grunwald JE, Maguire AM, Dupont J. Retinal hemodynamics in retinitis pigmentosa. *Am J Ophthalmol*. 1996;122:502–508.
7. Nagaoka T, Yoshida A. Noninvasive evaluation of wall shear stress on retinal microcirculation in humans. *Invest Ophthalmol Vis Sci*. 2006;47:1113–1119.
8. Wang Y, Fawzi AA, Varma R, et al. Pilot study of optical coherence tomography measurement of retinal blood flow in retinal and optic nerve diseases. *Invest Ophthalmol Vis Sci*. 2011;52:840–845.
9. Leitgeb RA, Werkmeister RM, Blatter C, Schmetterer L. Doppler optical coherence tomography. *Prog Retin Eye Res*. 2014;41:26–43.
10. Wang Y, Bower BA, Izatt JA, Tan O, Huang D. Retinal blood flow measurement by circumpapillary Fourier domain Doppler optical coherence tomography. *J Biomed Opt*. 2008;13:064003.
11. Tani T, Nagaoka T, Nakabayashi S, Yoshioka T, Yoshida A. Autoregulation of retinal blood flow in response to decreased ocular perfusion pressure in cats: comparison of the effects of increased intraocular pressure and systemic hypotension. *Invest Ophthalmol Vis Sci*. 2014;55:360–367.
12. Nagaoka T, Sakamoto T, Mori F, Sato E, Yoshida A. The effect of nitric oxide on retinal blood flow during hypoxia in cats. *Invest Ophthalmol Vis Sci*. 2002;43:3037–3044.
13. Aschinger GC, Schmetterer L, Doblhoff-Dier V, et al. Blood flow velocity vector field reconstruction from dual-beam bidirectional Doppler OCT measurements in retinal veins. *Biomed Opt Express*. 2015;6:1599–1615.
14. Werkmeister RM, Vietauer M, Knopf C, et al. Measurement of retinal blood flow in the rat by combining Doppler Fourier-domain optical coherence tomography with fundus imaging. *J Biomed Opt*. 2014;19:106008.
15. Muraoka Y, Tsujikawa A, Kumagai K, et al. Age- and hypertension-dependent changes in retinal vessel diameter and wall thickness: an optical coherence tomography study. *Am J Ophthalmol*. 2013;156:706–714.