Cardiac-Gated En Face Doppler Measurement of Retinal Blood Flow Using Swept-Source Optical Coherence Tomography at 100,000 Axial Scans per Second

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PURPOSE. To develop and demonstrate a cardiac gating method for repeatable in vivo measurement of total retinal blood flow (TRBF) in humans using en face Doppler optical coherence tomography (OCT) at commercially available imaging speeds.

METHODS. A prototype swept-source OCT system operating at 100-kHz axial scan rate was developed and interfaced with a pulse oximeter. Using the plethysmogram measured from the earlobe, Doppler OCT imaging of a 1.5- × 2-mm area at the optic disc at 1.8 volumes/s was synchronized to cardiac cycle to improve sampling of pulsatile blood flow. Postprocessing algorithms were developed to achieve fully automatic calculation of TRBF. We evaluated the repeatability of en face Doppler OCT measurement of TRBF in 10 healthy young subjects using three methods: measurement at 100 kHz with asynchronous acquisition, measurement at 100 kHz with cardiac-gated acquisition, and a control measurement using a 400-kHz instrument with asynchronous acquisition.

RESULTS. The median intrasubject coefficients of variation (COV) of the three methods were 8.0%, 4.9%, and 6.1%, respectively. All three methods correlated well, without a significant bias. Mean TRBF measured at 100 kHz with cardiac-gated acquisition was $40.5 \pm 8.2 \mu\text{L}/\text{min}$, and the range was from 26.6 to 55.8 $\mu\text{L}/\text{min}$.

CONCLUSIONS. Cardiac-gated en face Doppler OCT can achieve smaller measurement variability than previously reported methods. Although further validation in older subjects and diseased subjects is required, precise measurement of TRBF using cardiac-gated en face Doppler OCT at commercially available imaging speeds should be feasible.

Keywords: optical coherence tomography, retinal blood flow, Doppler OCT, en face OCT, cardiac gating

Measurement of retinal blood flow in vivo is an important research area in ophthalmic imaging since alterations in retinal perfusion are suggested to play a role in pathogenesis of ocular diseases such as glaucoma^{1–4} and diabetic retinopathy.^{5–8} Quantitative assessment of total retinal blood flow (TRBF) enables objective comparison of retinal circulation among different subjects and simplifies longitudinal tracking of changes associated with disease progression. Total retinal blood flow measurement can provide useful information for investigating ocular pathophysiology as well as for identifying potential early diagnostic markers.

Multiple imaging modalities such as video fluorescein angiography, laser speckle flowgraphy, laser Doppler velocimetry, scanning laser Doppler flowmetry, and ultrasound color Doppler imaging can be used to investigate quantitative retinal blood flow.^{9–16} However, objective measurement of TRBF remains challenging. Video fluorescein angiography and laser speckle flowgraphy cannot directly measure blood flow velocity but yield indirect metrics such as arteriovenous

passage time and mean blur rate. Scanning laser Doppler flowmetry (e.g., Heidelberg Retina Flowmeter; Heidelberg Engineering, Heidelberg, Germany) can map the flow velocity component along the probe beam direction over a two-dimensional scan area; however, the absolute flow velocity is required for quantitative flow rate measurement. Ultrasound color Doppler imaging can perform cross-sectional imaging and volumetric imaging of blood flow velocity but has $\sim 200\text{-}\mu\text{m}$ spatial resolution, which is insufficient to visualize detailed structure of the central retinal vasculature, complicating accurate blood flow calculation.

Among these techniques, bidirectional laser Doppler velocimetry (BLDV) combined with fundus photography is one of the best-suited methods for quantitative TRBF measurement. This technique measures the maximum absolute flow velocity in a vessel by detecting the Doppler frequency shifts of the light backscattered in two different directions. The blood flow in the vessel is calculated as the product of the maximum absolute flow velocity and the effective vessel cross-sectional area,

estimated from the vessel diameter measured in a fundus photograph. Total retinal blood flow is then calculated as the sum of the blood flow in major retinal vessels near the optic disc. Birectional laser Doppler velocimetry measurement of TRBF was validated in healthy subjects^{17–20} and used for investigating TRBF in diabetic patients.^{21–24} However, the calculation of effective vessel cross-sectional area relies on the assumption that the vessel shapes are circular and the blood flow velocity follows a parabolic profile. This assumption can be inaccurate in some subjects and may result in flow measurement errors. Moreover, measurement times can be preclusive because individual major retinal vessels must be measured separately, requiring 20 to 55 minutes per eye.²⁵ Due to this limitation, the number of patients in most TRBF studies using BLDV has been limited.

Fourier-domain optical coherence tomography (OCT) has enabled phase-sensitive imaging methods such as Doppler OCT, which promises simpler and more robust measurements of TRBF using depth-resolved information on vascular structure and Doppler velocity.²⁶ The first demonstration of TRBF measurement with Fourier-domain Doppler OCT used multiple circumpapillary scans intercepting all of the retinal vessels around the optic disc in order to measure the flow velocity component along the OCT probe beam direction and the Doppler angle between the velocity vector and the OCT beam.^{27–30} However, the flow measurement was highly sensitive to errors in the Doppler angle, especially when the vessel is oriented near 90° to the OCT beam as in the optic disc margin. Eye motion artifacts as well as varying vessel geometries in different subjects can make angle measurements challenging and require reader assistance to analyze data. Another method, bidirectional Doppler OCT, is analogous to BLDV. This approach measures Doppler velocity in two probe beam directions and uses trigonometric relations to calculate the absolute flow velocity³¹ and blood flow.³² This method addresses some of the limitations in the circumpapillary method, but cannot be performed with commercial OCT instruments because a major hardware modification is required for dual-beam scanning.

Recent improvements in OCT imaging speed enabled a new Doppler OCT approach, which avoids the need to measure the Doppler angle by analyzing blood flow in the en face plane. This method, en face Doppler OCT, was first demonstrated in brain sciences for cerebral blood flow measurement in small animals.³³ In ophthalmology, TRBF can be measured by integrating the Doppler velocity over an en face plane in volumetric data of the optic disc. En face Doppler OCT does not require Doppler angle measurement because the detected flow velocity component is always perpendicular to the en face plane. This simplicity enables fully automatic analysis of TRBF. High imaging speed is crucial for en face Doppler OCT because a densely sampled volumetric image is required to measure TRBF and multiple volumetric images per cardiac cycle are necessary to accurately assess pulsatile blood flow. Our group has demonstrated en face Doppler measurements of human TRBF using swept-source/Fourier domain OCT (SS-OCT) at 200-kHz axial scan (A-scan) rate,³⁴ spectral/Fourier domain OCT (SD-OCT) at 244-kHz A-scan rate,³⁵ and SS-OCT at 400-kHz A-scan rate.³⁶

Current commercial OCT instruments operate at A-scan rates of 100 kHz or less, which yield low volume rates, limiting the ability to sample the cardiac cycle. Therefore, in order to perform accurate pulsatile TRBF measurements with en face Doppler OCT, data acquisition should be cardiac gated so that the measurements at different cardiac phases can be distributed across multiple cardiac cycles. Cardiac gating is commonly used in other imaging modalities such as computed tomography³⁷ and magnetic resonance imaging.³⁸ In OCT,

pulsatile blood flow measurements using cross-sectional Doppler with pulse oximetry cardiac gating were previously performed in a single choroidal vessel.³⁹ Another demonstration of en face Doppler OCT partitioned multiple volumes based on the cardiac phase to reconstruct cardiac phase-coherent volumes and measure pulsatile blood flow in individual retinal vessels and TRBF.⁴⁰ However, this implementation used long imaging sessions with 10 to 20 OCT acquisitions.

In this paper, we present a new method for measuring TRBF using pulse oximetry-gated en face Doppler with SS-OCT at 100-kHz A-scan rate. Doppler SS-OCT at 1050-nm wavelength has several advantages over SD-OCT at 840 nm, such as larger measurable velocity ranges, improved visibility of deeper blood vessels, and absence of fringe washout. Pulsatile TRBF was measured using three OCT acquisitions and fully automatic postprocessing methods. Cardiac pulse information obtained from the pulse oximetry plethysmogram was used to trigger the OCT acquisition during imaging as well as to accurately integrate the pulsatile TRBF in post processing. Since commercial SS-OCT instruments operating at 100-kHz A-scan rates have been recently released (DRI OCT-1 Atlantis; Topcon Medical Systems, Oakland, NJ, USA), this technique can be applied in the clinic with a pulse oximeter and relatively simple instrument modifications. If sufficient reproducibility can be achieved, the short measurement time and the ability to automatically process data and obtain quantitative measurements should facilitate larger-scale studies of TRBF.

METHODS

Study Population

Ten eyes from 10 healthy young subjects (mean $\pm$ standard deviation [SD] age, 28.2 ± 3.0 years; range, 24–34 years) with no history of retinal disease were imaged at the Massachusetts Institute of Technology (MIT) under a protocol approved by the MIT Committee on the Use of Humans as Experimental Subjects (COUHES). The research protocol complied with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to the study. Blood pressure was monitored during the imaging sessions (mean and SD 87.6 ± 5.6 mm Hg). Axial eye lengths (25.2 ± 0.9 mm), intraocular pressures (12.1 ± 3.1 mm Hg), and fundus photographs of all study eyes and fellow eyes were obtained at New England Eye Center at Tufts Medical Center.

Doppler OCT Instrumentation

100-kHz A-Scan Rate SS-OCT With Pulse Oximetry Cardiac Gating. We developed a research prototype SS-OCT instrument based on a commercial short-cavity laser operating at 1050-nm center wavelength and 100-kHz sweep rate (Axsun Technologies, Billerica, MA, USA). A phase shift of π at 1050 nm corresponds to a $\sim 194\text{-}\mu\text{m}$ displacement in tissue ($n = 1.35$), making the phase wrapping-free Doppler velocity range ± 19.4 mm/s. The wavelength sweep range was ~ 100 nm, corresponding to a $6.3\text{-}\mu\text{m}$ full-width-at-half-maximum (FWHM) axial resolution in tissue ($n = 1.35$). A high-speed digitizer (ATS9360; AlazarTech, Pointe-Claire, QC, Canada) externally clocked using the optical clock output from the laser was used for data acquisition. The maximum clock frequency was ~ 340 MHz, and the imaging range was ~ 2.8 mm in tissue. The measured 3-dB sensitivity roll-off range was ~ 2.1 mm in tissue. Timing jitter between the laser sweep and the A-scan was corrected using a fiber Bragg grating (990.0 nm) in one arm of the balanced photodetector.³⁶ The incident OCT beam was ~ 1

mm FWHM with ~ 1.87 mW at the cornea, and the measured system sensitivity was 102.8 dB. Phase stability was ± 2.4 mrad at 55.2 dB signal-to-noise ratio. A pulse oximeter (Nellcor N-395; Covidien, Mansfield, MA, USA) was used to acquire the plethysmogram using an ear clip probe. The plethysmogram was digitized by the OCT instrument to synchronize the Doppler OCT acquisitions to the subject's cardiac pulse. The recorded plethysmogram was used both in real time for triggering OCT scans and also retrospectively in post processing.

400-kHz A-Scan Rate SS-OCT for Control Measurements. A high-speed SS-OCT instrument based on a micro-electromechanical system (MEMS)-tunable vertical cavity surface-emitting laser (VCSEL) operating at ~ 1060 -nm center wavelength and 400-kHz sweep rate was used for control TRBF measurements without cardiac gating. This instrument was similar to the one previously reported by our group.³⁶ At 400-kHz A-scan rate, the phase wrapping-free Doppler velocity range was ± 78.5 mm/s, approximately four times larger than the range at 100 kHz. The wavelength sweep range was ~ 84 nm, corresponding to a ~ 9.0 - μ m FWHM axial resolution in tissue. The OCT signal was sampled at uniform wavenumber intervals by clocking the high-speed digitizer (ATS9360; AlazarTech) with a Mach-Zehnder interferometer. The maximum clock frequency was 1.0 GHz, and the imaging range was 1.9 mm in tissue. A-scan sampling timing jitter was corrected using a fiber Bragg grating (1087 nm) in one arm of the balanced photodetector. The incident power on the cornea was ~ 1.84 mW, and the measured sensitivity was 97.8 dB. Phase stability was ± 2.3 mrad at 55.9-dB signal-to-noise ratio. This instrument enabled en face Doppler imaging at a volumetric sampling rate high enough to resolve pulsatile fluctuation of retinal blood flow, and the TRBF measurements served as the control for evaluating the accuracy of the measurements performed at 100-kHz A-scan rate.

Data Acquisition

In order to measure pulsatile TRBF, three multiple-volume OCT acquisitions were separately triggered at specific phases in the cardiac cycle. We marked the systolic peaks in the plethysmographic waveform and defined one cardiac cycle as the time period between two subsequent systoles. The term "cardiac phase" refers to a time variable between zero and one indicating the relative time position in the current cardiac cycle:

$$\begin{aligned} (\text{Cardiac Phase}) &= \frac{(\text{Time Elapsed in the Current Cardiac Cycle})}{(\text{Total Duration of the Current Cardiac Cycle})} \\ &= \frac{t - t_1}{t_2 - t_1} \end{aligned} \quad (1)$$

where t is the current time, t_1 and t_2 are two subsequent systolic time points, and $t_1 < t < t_2$.

Our prospective cardiac gating algorithm computed a real-time estimate of the subject's cardiac phase because the true value of the cardiac phase is unknown before the next systole. Each OCT acquisition comprised four repeated volumes, each with 600×80 A-scans covering a 1.5×2 -mm area of the optic disc, requiring a total of 2.2 seconds acquisition time. Rather than adjusting the volume rate according to the subject's heart rate, the four OCT volumes were acquired at the maximum 1.8 volumes/s fixed rate in order to minimize the total scan time. Shorter scan times are easier to tolerate for patients and minimize artifacts from blinking or eye motion. However, scanning at fixed volume rate is disadvantageous in that the cardiac cycle may be inadequately sampled depending on the relationship between the OCT scan rate and the heart rate—if

FIGURE 1. Timing diagram showing how the cardiac cycle is sampled for different constant heart rates. Three repeated OCT acquisitions (shown by different symbols), of four OCT volumes each, sample the cardiac cycle as shown by the distribution of symbols on the horizontal lines. Different heart rates are shown by offset horizontal lines. The phase of the OCT acquisitions is chosen to improve sampling: For heart rates < 85 bpm, the three acquisitions were triggered at 1/6 multiples of the cardiac cycle, while for heart rates > 85 bpm, the three acquisitions were triggered at 1/3 multiples of the cardiac cycle.

the OCT volumes repeatedly sample the same cardiac phase, measurements will be redundant. For example, at a 1.8 volumes/s scan rate (110 volumes/min), continuous scanning of a subject with a heart rate of 55 beats per minute (bpm) can sample only two cardiac phases separated by one-half the cardiac cycle. In order to improve sampling of the entire cardiac cycle for a range of heart rates, we used a cardiac gating algorithm that triggers three repeated multiple-volume OCT acquisitions at three distinct cardiac phases optimized for adequate sampling of the cardiac cycle. If the heart rate was less than 85 bpm, the three acquisitions were triggered at three different cardiac phases separated by 1/6 of the cardiac cycle. However, if the heart rate was greater than 85 bpm, the three acquisitions were triggered at three different cardiac phases separated by 1/3 of the cardiac cycle. Figure 1 illustrates how the cardiac cycle is sampled for different heart rates.

Post Processing

Volumetric images of intensity and Doppler phase were generated from the raw OCT data. Each A-scan consisted of 1408 samples, resulting in 704 pixels after Fourier transformation. The pixel spacing was ~ 4 μ m in tissue.

Tilted Plane Analysis. The 12 OCT volumes obtained from the three acquisitions were individually processed to automatically measure TRBF. The algorithm searched for arteries in en face planes, beginning from a superficial plane and proceeding into deeper planes. Because the optic disc often appear as tilted in an OCT B-scan, a tilted en face plane that follows the general contour of the vitreoretinal interface was used for initial segmentation of vessel areas. The blood vessel area was segmented in three steps. First, areas with positive Doppler velocity were roughly segmented in the tilted plane and the centroids of the areas were calculated. Then, a "mosaic en face plane" was constructed based on the Voronoi diagram⁴¹ associated with the centroids. The mosaic en face plane is a compilation of nontilted en face plane fragments containing the initially segmented vessel areas. Each fragment

FIGURE 2. Description of tilted plane analysis. (A–D) Tilted plane marked in intensity and Doppler phase images of cross sections along the fast scan direction (A, B) and the slow scan direction (C, D). (E) Fundus projection image of the optic disc. (F) Initial vessel segmentation on the tilted plane. (G) Final vessel segmentation in each fragment of the mosaic plane. (H) Phase-unwrapped and noise-filtered en face Doppler phase map of the arterial blood flow. Scale bars: 500 μm .

was extracted at the depth of the centroid on the tilted plane, so that the true en face Doppler velocity profiles of the vessels could be represented. Lastly, accurate blood vessel areas were segmented in the mosaic en face plane. Because high arterial flow velocities exceeded the measurable range and caused Doppler phase to wrap from $+\pi$ to $-\pi$, the true Doppler velocity was recovered using an iterative phase unwrapping algorithm. The depth positions of the mosaic en face plane relative to the vitreoretinal interface were then constrained to be within a $\sim 50\text{-}\mu\text{m}$ range across the 12 volumes. The common optimum depth was automatically selected by plotting the TRBF in each volume as a function of depth and finding the peak of the simple gross sum of depth-dependent TRBF in all volumes. After this process was completed, each volume was reduced into an optimum-depth en face Doppler velocity map. Key steps of tilted plane analysis are illustrated in Figure 2.

Retrospective Cardiac Gating. The last key step in our automatic TRBF calculation algorithm is retrospective cardiac gating, where the mean TRBF is calculated by integrating blood flow with respect to the cardiac phase calculated from the recorded plethysmogram. As shown in Figure 1, the cardiac phase intervals between the volumes are nonuniform. Therefore, a simple average of TRBF in the 12 volumes may not accurately measure the mean TRBF because densely sampled portions of the cardiac cycle are overrepresented while sparsely sampled portions are underrepresented. Instead of simple averaging, the plethysmogram recorded with OCT can be used to time-stamp the OCT data, enabling a more accurate integration of flow over the cardiac cycle. Since the raster scan time per volume (0.55 seconds) was itself comparable to a cardiac cycle, individual B-scan lines at all raster positions on the en face Doppler velocity map were labeled with instantaneous cardiac phase. This method guarantees that all blood vessels are labeled with the correct cardiac phases. If each volume is labeled with only a single cardiac phase, perturbations such as saccadic eye motion and small fluctuations in heart rate may introduce cardiac gating errors that affect TRBF measurement.

Figure 3 schematically shows the process of integrating the blood flow in one B-scan position in order to obtain the mean flow. First, the individual B-scan lines in the 12 en face Doppler velocity maps were grouped based on their raster positions in the scan area. At each raster position, the amounts of blood flow intercepted by the 12 B-scan lines were used to generate an approximation of the flow over a cardiac cycle as shown in Figure 3B. Then, the mean flow over the cardiac cycle was measured by integrating the flow with respect to cardiac phase using the trapezoidal rule. This technique essentially integrates the linearly interpolated flow and is well known for its high accuracy when integrating periodic functions.⁴² For the B-scan lines at the m th row in the raster scan, the mean blood flow F_m was calculated as

$$F_m = \sum_{n=1}^{12} f_{m,n} \frac{\phi_{m,n+1} - \phi_{m,n-1}}{2}$$

where

$$\begin{aligned} \phi_{m,0} &= \phi_{m,12} - 1, \\ \phi_{m,13} &= \phi_{m,1} + 1. \end{aligned} \quad (2)$$

In this equation and Figure 3, $f_{m,n}$ represents amounts of blood flow intercepted by the B-scan lines and $\phi_{m,n}$ represents their associated cardiac phases, where n is indexed from the smallest to the largest value of cardiac phase. It is important to mention that the integration of flow requires only the relative cardiac phase, and the absolute cardiac phase is not necessary. Lastly, the mean TRBF was calculated by aggregating the mean blood flow in all 80 B-scan positions:

$$(\text{Mean TRBF}) = \sum_{m=1}^{80} F_m. \quad (3)$$

The resulting mean TRBF gives an accurate measurement because potential causes of measurement errors such as nonuniform sampling of the cardiac cycle, saccadic eye

FIGURE 3. Schematic of the retrospective cardiac gating for integration of blood flow at each B-scan position. (A) Blood flow measurements in the 12 B-scan lines at the m th row of the raster scan area were grouped together and their associated cardiac phases were obtained from pulse oximetry plethysmogram. A hypothetical TRBF waveform is presented as the red curve to aid interpretation. (B) In order to account for the nonuniform cardiac phase sampling, the mean blood flow at a given B-scan position was calculated by integrating the flow waveform over the cardiac cycle using the trapezoidal rule. Scale bars: 500 μm .

motion, and fluctuation in heart rate are effectively handled by time-stamping individual B-scans.

Repeatability Study

We evaluated pulse oximetry-gated en face Doppler OCT on 10 healthy young subjects to quantify the impact of cardiac gating on TRBF measurement repeatability. We measured TRBF in all 10 subjects using three different approaches: (1) asynchronous acquisition using 100-kHz SS-OCT, (2) cardiac-gated acquisition using 100-kHz SS-OCT, and (3) asynchronous acquisition using 400-kHz high-speed SS-OCT as a control. When processing asynchronously acquired data, mean TRBF was calculated as the simple average of blood flow in all volumes. Imaging procedures for asynchronous acquisition and cardiac-gated acquisition with 100-kHz SS-OCT were identical except for the use of pulse oximetry cardiac gating. Asynchronous 400-kHz SS-OCT used the same scan pattern as reported previously,³⁶ comprising 750×60 A-scans covering a 1.5×1.5 -mm area. Since the volume scan rate of 7.6 volumes/s improves the sampling of pulsatile blood flow, this method was used as a control to test the accuracy of pulse oximetry cardiac gating. Three measurements were repeated for each method in each subject to calculate mean, SD, and intrasubject coefficient of variation (COV) to assess the measurement repeatability.

RESULTS

Results show that at 100-kHz A-scan rate, pulse oximetry cardiac gating can reduce measurement variability compared to asynchronous acquisition at similar as well as at higher imaging speeds (400 kHz). Figure 4 shows correlation plots and Bland-Altman mean difference plots between the averages of three repeated measurements from the same subjects using our pulse oximetry cardiac gating approach versus asynchronous acquisition, as well as a box plot comparing COV of the three methods. The correlation plots and Bland-Altman plots confirm that the measurements from the three methods are consistent since there is a high degree of linear correlation without a significant bias. At 100 kHz axial scan rate, the COV of three measurements in the 10 healthy young subjects was 1.9% to 8.6% (median 4.9%) with cardiac gating and 0.7% to 17.5% (median 8.0%) without cardiac gating. The control measurement at 400 kHz axial scan rate recorded 0.8% to 10.9% (median 6.1%) COV. The maximum COV for all three methods was observed in the same subject, who had a high pulsatility index (~ 0.9) in the central retinal artery. The COV in this subject showed a large improvement, 8.6% with cardiac gating versus 17.5% without cardiac gating, suggesting that pulse oximetry cardiac gating can enable reliable measurements of mean TRBF even in subjects with high flow pulsatility. Encouragingly, the box plot also shows that 100-kHz SS-OCT with cardiac gating can achieve similar or higher repeatability compared to high-speed, 400-kHz SS-OCT with asynchronous acquisition. This suggests that pulse oximetry cardiac gating can be used for reliable measurement of TRBF using commercially available OCT imaging speeds and that higher imaging speeds may not be required.

DISCUSSION

Comparing the measured COV of the cardiac gating method (1.9%–8.6%, median 4.9%) with previous repeatability studies using conventional techniques suggests that TRBF measurement using gated en face Doppler OCT is potentially more precise. An early study reported the COV of five TRBF measurements using the Canon Laser Blood Flowmeter in 20 healthy subjects ranging from 4.8% to 37.3% (median 19.3%).²⁰ The high COV may have resulted from a combination of daily

variation and measurement error, since the five measurements were distributed on five different visits within a month period, because of the long measurement time. In another BLDV study in which TRBF in 16 healthy participants was monitored every 3 hours over a 12-hour time span from 8 AM to 8 PM, the average COV of TRBF was 18.5%.⁴³ Although this variation may be a mixture of diurnal fluctuation and measurement error, the lack of clear time dependence in the average TRBF suggested that the primary source of variation was measurement error. A later study using cross-sectional Doppler OCT with double circumpapillary scans demonstrated improvements in repeatability compared with BLDV. Wang et al.³⁰ reported that the average COV of five TRBF measurements was 10.9% in 20 normal eyes and 14.3% in 28 eyes with diseases such as glaucoma, nonarteritic ischemic optic neuropathy, and proliferative diabetic retinopathy. However, recent work by Rose et al.⁴⁴ showed a systematic increase in TRBF measured by the same reader before and after training, suggesting a potential risk of reader-dependent or training-dependent variation of TRBF measured from the same dataset. Our measurements in healthy young subjects demonstrate that cardiac-gated en face Doppler OCT with fully automatic post processing enables higher measurement repeatability than existing methods.

The B-scan-based retrospective cardiac gating method substantially reduces the impact of eye motion on TRBF measurement. Eye motion in the horizontal direction does not affect the flow measurement because it causes only a displacement of the vessel along the B-scan direction. Eye motion in the vertical direction that occurs between successive volumes or within a volume also does not affect the measurement, provided that the motion does not cause part of a vessel to be missed by B-scans or to be scanned twice. It is important to mention that Figure 3B shows a specific example of flow waveform with respect to cardiac phase when vertical eye motion is small and the vessel always appears in the same B-scan position. There are many B-scan positions that do not intercept a vessel and would have zero flow. In other cases, vertical eye motion can change the relative position of a vessel in the OCT raster volume and may move the blood flow out of one B-scan position. Then, the flow waveform for a single B-scan position will not reflect the true flow pulsatility, but the blood flow will be measured at another B-scan position and will be labeled with the correct cardiac phase. Therefore, the mean TRBF will not change substantially because it is the sum of the integrals of the flow over a cardiac cycle at 80 B-scan positions. Vertical saccades that cause a major vessel to be missed or scanned twice will cause oversampling or undersampling of blood vessels, resulting in an error in TRBF measurement. Therefore, the operator must exclude OCT data with excessive vertical eye motion from the measurement and repeat the acquisition. The quality criteria for data selection are similar to those used for any OCT acquisition.

It is important to note that current commercial SS-OCT instruments are not equipped with A-scan timing jitter compensation and thus the optical phase may not be stable from one A-scan to another. Phase-sensitive OCT imaging methods such as Doppler or phase variance angiography require phase stabilization, which can be achieved by using a fiber Bragg grating in one arm of the balanced photodetector or triggering the A-scan acquisition using a fiber Bragg grating signal. The fiber Bragg grating generates a sharp fiducial edge at a fixed wavenumber in the interference fringes, enabling timing jitter to be compensated and stabilizing the phase across all A-scans. Although this is a simple modification, it requires additional hardware not present in the installed base of existing SS-OCT instruments.

Commercial SD-OCT instruments have stable A-scan phase because they use spectrometers to measure the interference

FIGURE 4. (A–D) Correlation and Bland-Altman plots between the averages of three repeated total retinal blood flow measurements using different methods. (A) Correlation plot between 100 kHz with cardiac gating and 100 kHz with asynchronous acquisition. (B) Correlation plot between 100 kHz with cardiac gating and 400 kHz with asynchronous acquisition. (C) Bland-Altman plot between 100 kHz with cardiac gating and 100 kHz with asynchronous acquisition. (D) Bland-Altman plot between 100 kHz with cardiac gating and 400 kHz with asynchronous acquisition. (E) Box plot comparing the intrasubject coefficient of variation of the three methods.

fringes rather than high-speed digitizers. However, SD-OCT has several limitations. Commercial SD-OCT instruments operate at ~ 840 -nm center wavelength, shorter than the 1050 nm used in SS-OCT, and the highest commercially available SD-OCT imaging speed is currently 70 kHz, slower than the 100 kHz of SS-OCT. These differences result in a smaller phase wrapping-free Doppler velocity range, which spans only

between ± 10.9 mm/s. Spectral-domain OCT also suffers from signal loss due to fringe washout at high flow velocities because each fringe is integrated over the entire A-scan time.⁴⁵ Therefore, en face Doppler imaging of arterial blood flow is difficult with SD-OCT. Although venous flow velocity can be detected more reliably, it is still challenging to calculate TRBF because large veins inside the optic disc margin are frequently

located below the arteries. Therefore, en face Doppler OCT measurements of TRBF with currently commercial SD-OCT instruments may be challenging.

Cardiac gating may be valuable for en face Doppler OCT measurements of TRBF using high-speed instruments as well. Automatic blood vessel segmentation in en face Doppler OCT is susceptible to errors caused by timing jitter in the optical clock that determines the digitization of the OCT interference fringe data, as well as Doppler phase artifacts from highly reflective layers such as the retinal nerve fiber layer and retinal pigment epithelium. These noise sources and artifacts are often only partially suppressed by postprocessing methods and can cause errors in vessel segmentation—misinterpreting a reflection phase artifact as a blood vessel may lead to overestimation of blood flow, while failing to detect a blood vessel with low flow velocity may result in underestimation. The effect of these errors becomes more severe at higher imaging speeds because the same error in Doppler phase corresponds to a larger error in Doppler velocity given the shorter sampling time intervals. Conversely, the risk of errors can be reduced by increasing sampling time intervals or, equivalently, by calculating Doppler phase between nonneighboring A-scans. However, this will decrease the volumetric scan rate because the spatial sampling density must be increased to ensure that the two A-scans being compared still have a large spatial overlap and contain correlated speckle information. This implies that there is a tradeoff between volumetric scanning rate and sensitivity to low flow speeds. Therefore, we expect that cardiac gating may be helpful for accurate blood flow measurement even at high imaging speeds.

Among the 10 healthy young subjects in this study, the mean and the SD of the TRBFs were 40.5 ± 8.2 $\mu\text{L}/\text{min}$; and a wide range of TRBFs, from 26.6 $\mu\text{L}/\text{min}$ to 55.8 $\mu\text{L}/\text{min}$, was observed. The large intersubject variability within healthy subjects is consistent with the results from previous studies using other methods.^{25,30} However, this wide variation among healthy young subjects suggests that it will be difficult to use TRBF alone as an early diagnostic marker since there may be a large overlap between normal and diseased cohorts. However, measurement of changes in TRBF may potentially provide valuable information. Longitudinal measurements of TRBF can be investigated to assess disease progression or treatment response. Measuring changes in TRBF response to visual stimuli^{46–49} may enable early assessment of disease in spite of the basal TRBF variation and could potentially enable new diagnostic applications. Reduction of measurement variance is important for all of these studies.

In conclusion, we demonstrated measurement of TRBF using cardiac-gated en face Doppler OCT at commercially available SS-OCT imaging speeds. The slow volumetric scan rate and inability to time-resolve pulsatile fluctuation of blood flow were compensated by using pulse oximeter cardiac gating. Precise measurement of pulsatile blood flow was enabled by the combination of a prospectively cardiac-gated data acquisition scheme as well as postprocessing methods such as tilted plane analysis and retrospective cardiac gating. We believe that cardiac gating will be useful at higher imaging speeds as well, because there is a tradeoff between volumetric scanning rate and sensitivity to low flow speeds. Our TRBF study in small number of healthy young subjects showed that cardiac-gated en face Doppler OCT measurement of TRBF is possible at commercially available SS-OCT imaging speeds and is in good agreement with control measurements using asynchronous en face Doppler OCT at a high imaging speed, and that cardiac gating yields an improvement in measurement repeatability compared with ungated asynchronous measurement. We achieved a median COV lower than that reported by previous studies using conventional methods such as BLDV and cross-sectional Doppler OCT. Our study was limited in that it

involved only a small number of healthy young subjects and did not include older subjects or subjects with retinal disease. Further validation in older subjects and subjects with ocular pathology is important for comprehensive evaluation of this technique compared with conventional methods. However, cardiac-gated en face Doppler OCT has the advantage of fully automatic post processing, combined with a rapid and simple imaging procedure, which is important for conducting larger-scale clinical retinal blood flow studies.

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