Measurement of pulsatile total blood flow in the human and rat retina with ultrahigh speed spectral/Fourier domain OCT

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Abstract: We present an approach to measure pulsatile total retinal arterial blood flow in humans and rats using ultrahigh speed Doppler OCT. The axial blood velocity is measured in an en face plane by raster scanning and the flow is calculated by integrating over the vessel area, without the need to measure the Doppler angle. By measuring flow at the central retinal artery, the scan area can be very small. Combined with ultrahigh speed, this approach enables high volume acquisition rates necessary for pulsatile total flow measurement without modification in the OCT system optics. A spectral domain OCT system at 840nm with an axial scan rate of 244kHz was used for this study. At 244kHz the nominal axial velocity range that could be measured without phase wrapping was ±37.7mm/s. By repeatedly scanning a small area centered at the central retinal artery with high volume acquisition rates, pulsatile flow characteristics, such as systolic, diastolic, and mean total flow values, were measured. Real-time Doppler C-scan preview is proposed as a guidance tool to enable quick and easy alignment necessary for large scale studies. Data processing for flow calculation can be entirely automatic using this approach because of the simple and robust algorithm. Due to the rapid volume acquisition rate and the fact that the measurement is independent of Doppler angle, this approach is inherently less sensitive to involuntary eye motion. This method should be useful for investigation of small animal models of ocular diseases as well as total blood flow measurements in human patients in the clinic.

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1. Introduction

Assessing ocular blood flow in vivo is an important research area because many ocular diseases, such as diabetic retinopathy, glaucoma and age-related macular degeneration, are associated with alterations in retinal blood flow. Therefore, the measurement of total retinal blood flow and pulsatility is important for investigating pathophysiology in small animal models of ocular diseases and may also be useful for the diagnosis of these diseases in humans [1–6].

Conventionally, techniques such as color Doppler imaging, scanning laser Doppler flowmetry, retinal vessel analyzer, laser speckle flowgraphy, digital scanning laser ophthalmoscope angiography and laser Doppler velocimetry have been used to characterize hemodynamics in the normal and diseased eyes [2,5]. However, many of these techniques...
measure only velocity and not total flow, as in color Doppler imaging and laser Doppler velocimetry, or vessel diameter as in retinal vessel analyzer. One of the main limitations of scanning laser Doppler flowmetry is that it does not provide depth-resolved measurements, thereby making quantitative comparison among different subjects challenging. Other techniques, such as laser speckle flowgraphy or scanning laser ophthalmoscope angiography, also have the limitation that they require intravenous dye injection and/or are difficult to convert into actual flow. Therefore, there has been a considerable demand for new techniques that can reliably assess retinal blood flow in vivo [7,8].

Since structural optical coherence tomography (OCT) imaging has become a clinical standard in many areas of ophthalmic clinical diagnosis and medical research [9], it is desirable to use OCT for functional measurement of total retinal blood flow. Fourier-domain Doppler OCT methods enable direct access to phase and can measure the speed of moving scatterers, such as red blood cells and also enable a dramatic increase in sensitivity and speed compared with time-domain OCT [8,10,11]. However, since Doppler OCT measures only the axial component of velocity that is parallel to the probe beam direction, conventional Doppler techniques require information on blood vessel angles in addition to speed for quantitative assessment of retinal blood flow [7,12–15]. Another method is to detect Doppler shifts in two different directions using two beams impinging at different angles without explicit extraction of blood vessel angles, but this requires hardware scan modifications and is therefore more difficult to implement with standard commercial OCT patient interfaces [16]. While blood flow has been measured in both humans and small animals using these conventional methods, flow measurement was usually based on an individual arterial or venous branch, rather than the total retina [17].

Total retinal blood flow can be measured using a novel technique which acquires multiple concentric circumpapillary scans with different diameters centered at the optic nerve to intercept all of the retinal vessels while also measuring the vessel angles [7,15]. However, angle measurement is sensitive to eye motion and errors in detected angle can cause flow measurement error. In order to overcome this limitation, a software approach to register circumpapillary scans, which is used to extract speed information, to motion-corrected two-dimensional raster scan, which is used to extract blood vessel angles, has been developed to reduce measurement variability [18]. It has been recently shown that total retinal flow measurements using circumpapillary scans generated by Fourier-domain Doppler OCT exhibit statistically significant differences between normal and diseased eyes [19].

Recently, an en face Doppler OCT approach which does not require blood vessel angle information, became feasible due to an increase in OCT imaging speed [20,21]. This en face Doppler technique simplifies total blood flow measurement by using the fact that total flow in a vessel is given by the integral of the product of a differential area and velocity component perpendicular to the area over the cross-section of the blood vessel. In Doppler OCT, total blood flow can be calculated simply by summing all axial velocity components in an en face plane generated from an OCT raster scan centered at the optic nerve at a given depth location and multiplying the sum with an area calibration factor, which is readily measurable. Our group demonstrated that at a high enough imaging speed, this en face Doppler can be applied to the human retina to calculate total retinal blood flow [22]. This method combined with swept source technology had several advantages including negligible fringe washout compared with spectral-domain OCT [23,24], simple flow calculation without the need for vessel angle information, and compatibility with existing standard OCT patient interfaces. However, pulsatility still remained a challenge as in all other retinal blood flow measurement techniques, potentially causing up to 50% or higher variation in measured flow values [22,25].

In this paper, we present a novel approach to measure total retinal arterial blood flow and pulsatility in rats as well as in humans. As demonstrated previously, the axial blood velocity is measured in an en face plane by scanning a raster pattern to avoid the need to measure the Doppler angle and to achieve a simple and robust measurement technique. In addition, total blood flow is measured at or near the central retinal artery in this approach, and therefore, the scan area can be extremely small. Combined with ultrahigh speed Fourier domain OCT, this
acquisition scheme enables high repeated volume acquisition rates necessary for pulsatile total blood flow characterization. The total acquisition time required for pulsatile blood flow measurement with this approach is only a few seconds for both humans and small animals because blood flow is continuously recorded in time without gating. Moreover, because of the short acquisition time required per volume, this approach is inherently less sensitive to involuntary patient eye motion, which is highly advantageous for implementation in the clinic. Due to the simplicity and robustness of data processing algorithm, completely automatic total blood flow calculation was achieved in this study.

2. Methodology

2.1. Ultrahigh speed spectral/Fourier domain OCT system

A sketch of the OCT system used in this experiment is shown in Fig. 1. A commercially available superluminescent diode (Superlum) and line scan camera (Basler Sprint spL4096-140 km) were used to develop an ultrahigh speed spectral/Fourier domain OCT system at 840 nm with an imaging speed of 244,000 A-scans per second. The full width at half maximum (FWHM) bandwidth of the superluminescent diode light source was 55 nm. The spectrometer used a collimating lens with an effective focal length of 100 mm, a 1200 lines/mm transmission holographic grating and an 80 mm scan lens. The line scan camera had 10 µm square pixels in two rows and was 4096 pixels wide. However for this design only 832 pixels were illuminated and the camera was read at a line rate of 244 kHz where the exposure time was 2.8 µs, accounting for the reading time. The spectrometer was calibrated by using an approach similar to that described in reference [26]. The output was processed by spline interpolation, followed by numerical dispersion compensation and Fourier transformation.

Fig. 1. Schematic of the ultrahigh speed spectral domain OCT system. PC: polarization controller, DC: dispersion compensation glass, RM: reference mirror, GS: galvanometer scanner pair, DG: diffraction grating, SLD: superluminescent diode, CMOS: line scan camera. A similar OCT system with reduced power at the cornea was used for human imaging.

The total imaging range was 1.5 mm in tissue with a measured axial resolution of 5.7 µm in tissue. The power of the OCT beam at the cornea was 2.5 mW for rat imaging, and the system sensitivity was 99 dB. A non-contact scanning configuration was used to avoid pressure on the cornea which may change intraocular pressure and retinal blood flow. The scan interface for rat imaging consisted of a pair of galvanometer scanners (Cambridge Technology 6210H, 3 mm mirrors), a 50 mm scan lens, and an ocular lens with an effective focal length of 12.5 mm. The incident beam diameter on the cornea was 0.5 mm. A similar system was used for human eye imaging, but the output power at the cornea was reduced to 750 µW, consistent with American National Standards Institute (ANSI) standards safe exposure limits, resulting in a sensitivity of 94 dB. The human imaging interface consisted of a pair of galvanometer
scanners (Cambridge Technology 6215H, 5mm mirrors), a 80mm scan lens, and an ocular lens with an effective focal length of 30mm, resulting in a beam diameter of 1.8mm incident on the cornea. Theoretical spot sizes on the retina calculated with ZEMAX using standard eye models were ~15μm for the small animal interface and ~20μm for the human imaging interface. The phase stability of the spectrometer measured with a common path cover slip interferometer was 1.1mrad (standard deviation of measured phase). The superior phase stability of spectral/Fourier domain OCT enabled robustness necessary for large scale studies without the need for sophisticated trigger fluctuation compensation algorithms as required in swept source OCT. However, the approach proposed here is in principle compatible with any high speed Fourier domain OCT system, including swept source OCT.

2.2. Assessment of Doppler flow velocities

Doppler flow velocities were calculated as [8]

$$v_z = \frac{\lambda \Delta \Phi}{4\pi T n}$$

where $\lambda$ is the light source center wavelength, $\Delta \Phi$ is the phase difference between two consecutive A-scans, $T$ is the time between two A-scans and $n$ is the effective refractive index of the tissue.

Using an acquisition rate of 244,000 A-scans per second, the maximum axial velocity range that could be measured without phase wrapping was ±37.7mm/s in tissue. However, the maximum axial velocity that could be practically unwrapped without ambiguity was ~75.4mm/s in tissue, since only arteries near the central retinal artery were summed in the en face plane for the Doppler OCT flow measurement and the direction of blood flow in the arteries is the same in all eyes. No software bulk motion correction was necessary for this study because the velocity of typical bulk motion of the retina encountered during imaging was negligible compared to the axial velocity of blood near the central retinal artery.

2.3. Total retinal blood flow measurement using en face Doppler OCT

Total retinal blood flow measurement was based on a method demonstrated by Srinivasan et al. in the small animal brain [20]. This method was later applied by Jenkins et al. for measuring total flow in developmental biology specimens and by Baumann et al. for measuring total flow in the human retina [21,22]. Flow may be calculated as

$$F = \int_S v \cdot dA = \int_S v \cos \theta dA$$

where $v$ is the velocity vector of a moving scatterer and $dA$ is the differential area with the vector normal to the surface area. This concept is illustrated in Fig. 2.

Fig. 2. (A) In conventional Doppler methods, calculation of blood flow involves extraction of the Doppler angle $\theta$, which is the angle between the probe beam and blood vessel. (B) In en face Doppler, simply integrating the axial velocity components over an en face cross-section that intercepts the vessel provides blood flow.

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In Doppler OCT, the axial velocity component of a moving scatter can be readily measured without information on the vessel angle. However, using conventional Doppler methods (Fig. 2(A)), blood vessel angles are needed in addition to the axial velocity in order to calculate flow because the measurement is performed in a cross-sectional plane which is along the direction of the OCT beam. Raster scanning over an en face plane intercepting the blood vessel (Fig. 2(B)) requires a higher imaging speed because of the larger number of A-scans necessary compared with scanning a cross-sectional plane. On the other hand, the z-component or axial component of the velocity is $v_\cos \theta = v_z$ and blood flow in a vessel can also be calculated by simply integrating the axial velocity components in an OCT en face plane that intercepts the vessel (Fig. 2(B)). Therefore, total retinal blood flow can be measured by scanning an area that intercepts all retinal vessels.

2.4. Pulsatile total blood flow measurement in the human and rat retina

One method to intercept all retinal vessels with an en face plane is to scan a large area around the optic nerve head, as in reference [22]. Although this approach measured all retinal vessels, it was not possible to characterize pulsatility in blood flow with this approach because of the limited OCT acquisition speed. Another method to measure pulsatile blood flow with en face Doppler OCT is to use gating to reconstruct pulsatile blood flow as demonstrated by Jenkins et al. in the quail embryo heart tube [21]. However, depending on the method used gating may require additional hardware and/or software for synchronization. Gating typically also requires a longer total measurement time, which limits its use for large scale studies in humans or rodent models. This paper describes an approach which specifically measures the central retinal artery to rapidly and repeatedly scan a small area, thereby achieving sufficient volume acquisition rate to characterize blood flow pulsatility.

In the rat retina, the central retinal artery is readily accessible by the OCT probe beam, and rapid, repeated scanning of a small area is possible to achieve rapid volume acquisition rate. In the human retina, the central retinal artery cannot always reliably be imaged at 840nm wavelengths due to limited image penetration depth. However, by choosing an en face plane which intercepts all retinal arteries just after they branch from the central retinal artery, the scan area can still be small enough for rapid, repeated volume acquisition necessary for pulsatile blood flow imaging in humans.

By using en face Doppler OCT, knowledge of the angle between the blood vessel and OCT beam, i.e. the Doppler angle, is not required, which dramatically simplifies total retinal blood flow measurement. Moreover, because the scanned area is very small and has high sampling density, blood vessel detection and segmentation becomes highly robust and simple, thereby enabling entirely automatic processing algorithms for pulsatile blood flow calculation. Completely automatic processing is especially useful for large scale studies, which are necessary in the investigation of small animal models of ocular diseases as well as in clinical retinal blood flow measurements in human patients with ocular disease. The fact that Doppler angle information is not required also implies that this approach is highly robust to segmentation error which might otherwise lead to large variations in calculated flow from inaccurate Doppler angle measurement.

2.5. Real-time Doppler C-scan preview

Because of variations in the three-dimensional retinal anatomy in both humans and rats, it is not always obvious which scan area will intercept all the blood vessels necessary to measure total retinal blood flow. One approach is to scan a large area near the optic nerve head as shown in reference [22]. However, a rapid volume acquisition rate is required to characterize pulsatility in blood flow so it is necessary to limit the scan area in order to achieve rapid scan rates. Because of the vessel geometry and lack of contrast, conventional intensity B-scan previews are not sufficient to reliably locate the central retinal artery or blood vessels of interest. Although OCT intensity fundus projection can sometimes provide a rough location of the central retinal artery, it is still insufficient for defining a small scan area because fundus
projection images do not preserve depth-resolved information. This depth-resolved information is critical since blood vessels are not necessarily parallel to the OCT probe beam. An OCT intensity \textit{en face} cross-section, or C-scan, preserves this depth-resolved information, but the inherent lack of contrast between blood vessels and neighboring tissue limits its practical use for locating blood vessels.

Since reliability and ease of alignment are of key importance for large scale studies involving small animals and humans, we developed a real-time \textit{en face} Doppler cross-sectional preview, or Doppler C-scan preview. The Doppler C-scan preview can provide an accurate location of the blood vessels of interest as well as preview the quality of the Doppler images that will be acquired.

Because Doppler processing requires oversampling in at least one scan direction and Doppler C-scan preview is useful only if it scans an area larger than the actual area of interest, it requires a large number of A-scans per \textit{en face} frame. However, it is important to note that the full discrete Fourier transform (DFT) is not required for Doppler C-scan preview because only the phase at a single depth is required from the A-scan. For small animal Doppler imaging, the Doppler C-scan preview consisted of 400 $\times$ 100 A-scans over a 0.5mm $\times$ 0.5mm area, which resulted in a 12$\times$ oversampling in the fast scan direction and 3$\times$ oversampling in the slow scan direction. Although the number of A-scans is 100 times larger than that is typically required for an intensity B-scan preview consisting of 400 A-scans, the total number of computations required for DFT in the preview is even smaller for the Doppler C-scan preview, as long as the number of samples per A-scan is larger than the number of B-scans.

Although fast Fourier transform (FFT) algorithms cannot be used for Doppler C-scan preview because only the Fourier transform at a single depth is computed for a given A-scan, it is still possible to reduce the number of multiplications required by a factor of roughly two by using complex conjugate symmetry ($e^{-jk\omega(N-\omega)} = e^{j\omega(N-\omega)}$ where $k$ and $n$ are integers) as in the following [27]:

$$X[k] = \sum_{n=0}^{N/2-1} x[n] e^{-j2\pi kn / N} = \sum_{n=0}^{N/2-1} x[n] \left[ \cos(2\pi kn / N) - j \sin(2\pi kn / N) \right]$$

Without further optimization, a Doppler C-scan preview frame rate of >1fps was achieved, which was fast enough for small animal imaging. Although this frame rate was still useful for human imaging, further optimization in computer hardware and the use of recursive algorithms, such as the Goertzel algorithm [27], should improve the frame rate. These increases in preview speed would be important for imaging patients in the clinic. Figure 3 shows a flow chart of how Doppler C-scan preview can be used for alignment and an example of a Doppler C-scan preview image.

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**Fig. 3.** (A) Flow chart for alignment/acquisition procedure for pulsatile total retinal blood flow imaging. (B) An example of a Doppler C-scan preview image. The red square denotes the scan area of interest, which can be adjusted by moving the crosshair. Velocity wrapping can be observed at the center of the central retinal artery, which can be unwrapped for flow calculation during post-processing.
2.6. Automatic flow calculation

Flow calculation could be performed entirely automatically for both humans and small animals because of the robustness and simplicity of the algorithm. This is a very useful feature for large scale studies where a large number of subjects are involved and/or longitudinal measurements are required. The flow chart for the automatic flow calculation scheme is illustrated in Fig. 4.

In situations where the maximum velocity produces one phase wrap, unwrapping can be performed reliably by adding \( \pi \) when phase differences cross a negative threshold value. If the velocity is high and produces multiple phase wrapping, unwrapping is more difficult. The first volume from a repeated volumetric data set is used to calculate flow as a function of depth of the \textit{en face} plane by detecting the central retinal artery by velocity thresholding median-filtered \textit{en face} Doppler images. For the rodent data sets, because the central retinal artery is accessible with the OCT probe beam, only the largest connected area above a velocity threshold was summed for flow calculation. At shallow depths, an initial rise in flow occurs as multiple retinal arteries enter the field of view. Analyzing progressively deeper depths, eventually the OCT signal is lost due to limited imaging depth and the measured flow decreases. Between these two depth limits, there is a \( \sim 30 \mu m \) depth range in case of the rodent retina, where measurements are free of these two counteracting effects and the measured flow is independent of depth as expected. The flow measured within this depth range corresponds to the true total retinal arterial flow value at the time the volume was acquired. Since repeated volumes are rapidly acquired, for the next volume in the data set it is only necessary to analyze a depth window near the depth where flow was maximal in the previous volume. By iterating this process through the entire data set, a pulsatile total retinal arterial blood flow waveform can be obtained. The length of this depth range where measured flow is constant depends on the scanned area because image penetration depth is usually fixed for a given wavelength. This depth range where constant flow is measured can vary between animals depending on the window size, and must be chosen so that it can intercept all retinal arteries in most animals. Similar effects can also be observed in the human retina where the depth range for measuring constant flow was \( \sim 70 \mu m \). One major difference between the automatic flow calculation algorithms for the human and rat data comes from the fact that the human central retinal artery cannot always be imaged by the OCT probe beam. Therefore, for the human data, several connected areas above a velocity threshold as well as above an area threshold were summed together for flow calculation. Area thresholding was employed in order to exclude small random high velocity regions arising from Doppler noise. It should be noted that shadowing from blood vessels may make Doppler images noisy at deeper depths due to high scattering as in other Doppler OCT methods, and care must be taken in determining the correct depth where flow is calculated.

![Flow chart for automatic flow calculation](image)

**Fig. 4.** Flow chart for automatic flow calculation. Because of the robustness of the algorithm, essentially no user input is necessary and entirely automatic processing becomes feasible. The plot shows flow as a function of depth is generated from a data set acquired from a normal rat.
2.7. Animal protocol

Animal procedures were performed under an approved protocol by the Committee on Animal Care (CAC) at MIT. For all experiments involving animals, male Sprague Dawley rats weighing 250-500g were used. Immediately before OCT imaging, animals were anesthetized with ketamine/xylazine (ketamine 80mg/kg, xylazine 8mg/kg) or isoflurane (2%) and xylazine (8mg/kg), and dilated with 1% tropicamide. Hypothermia in animals was prevented by using heat pads. The animals were then imaged with the ultrahigh speed OCT system. During imaging, the animals were closely monitored for their heart rate, breathing rate, and oxygen saturation to assess depth of anesthesia and check for signs of distress.

3. Results and discussion

3.1. Structural and functional blood flow imaging in the rat retina

Imaging at a rate of 244,000 A-scans per second, a volumetric data set of 700 × 700 A-scans over 1.5mm × 1.5mm can be acquired in only 2.4 seconds. Figure 5(A) shows an example of a cross-sectional image from the volumetric data set. Properly anesthetized rats have very stable eyes with minimal eye movement. Therefore, at this speed, 10 neighboring B-scans spanning a lateral distance of only ~20μm can be averaged without requiring registration and have negligible motion artifacts. This enables high quality structural imaging with suppressed speckle as well as Doppler imaging with a single system.

The availability of high-quality volumetric imaging and functional blood flow imaging with a single system makes OCT a powerful tool for studies of disease progression in small animal models. For example, high speed volumetric imaging can enable visualization of capillary network as shown in Fig. 5(B). This image was generated from a densely scanned volumetric dataset.

Fig. 5. (A) An OCT cross-sectional image acquired at 244,000 A-scans per second centered at the optic nerve head (ONH). An average of 10 neighboring B-scans from a volumetric data of 700 × 700 A-scans over 1.5mm × 1.5mm is shown. (B) Visualization of the capillary network. (C) An OCT fundus projection view over an area of 0.5mm × 0.5mm at ONH. (D) A Doppler B-scan image showing vasculature located at the red dotted line in the fundus projection view. (E) A Doppler B-scan image showing the vasculature located at the blue dotted line in the fundus projection view. The blue arrow indicates the central retinal artery cross-section. The axial velocity range of ±15mm/s was chosen for display purposes in order to optimize image contrast. Scale bar: 100μm in all images.
volumetric data set using a method similar to speckle correlation mapping approach demonstrated in [28]. Volumetric structural imaging with minimum motion artifact is also useful for accurate thickness mapping. Therefore, combined with pulsatile total retinal blood flow imaging, high speed OCT is capable of both functional and structural imaging and promises to be a powerful tool for investigation of small animal models of retinal diseases.

Figure 5(C) shows an OCT fundus view over a 0.5mm × 0.5mm area. Figures 5(D) and 5(E) show Doppler B-scan images of the vasculature located at the red and blue dotted lines, respectively, in the fundus projection view. As shown by the blue arrow in Fig. 5(E), the central retinal artery for this eye is approximately located near the center of the optic nerve. However, the location of an appropriate cross-section for central retinal artery does not necessarily coincide with the center of the optic nerve head in many eyes. Moreover, in many cases, it is not straightforward to define where the center of the optic nerve head is, which necessitates real-time Doppler C-scan preview for pulsatile total retinal blood flow imaging as described previously.

Total Doppler flow measurements in the small animal eye are challenging because the rapid heartbeat requires high speed volumetric imaging. By scanning a smaller area of 200μm × 200μm centered at the central retinal artery repeatedly, a volume acquisition rate of up to 140Hz was achieved. At 244kHz axial scan rates, increasing the volume acquisition rate requires smaller Doppler oversampling, which eventually results in loss of phase correlation. In principle, this limit could be overcome with higher imaging speed. For small animal imaging at an A-scan rate of 244kHz, a volume acquisition rate of 55Hz (150 × 25 A-scans in each volume with a fast axis scan duty cycle of 85%) provided the most stable pulsatile total blood flow waveform without compromising the number of volume samples per cardiac cycle. Figures 6(A) and 6(B) show the pulsatile total blood flow measured in the central artery of a Sprague Dawley rat anesthetized with isoflurane/xylazine at a volume acquisition rate of

![A]. Pulsatile Total Arterial Flow

![B]. Plethysmographic Pulse Waveform

![C]. En face Doppler images at time points indicated by the red arrows in (A). The arrows from left to right in (A) correspond to Doppler images 1 to 4 in (C). 200μm × 200μm.
55Hz and a simultaneously acquired plethysmographic pulse waveform from a pulse oximeter. The heart rate was ~300 beats per minute. Figure 6(C) shows en face Doppler images at 4 different time points indicated by the arrows in Fig. 6(A). Pulsatile changes in axial velocity can be clearly seen. However, parabolic flow profile is not clearly observed because the en face cross-section chosen by the automatic processing software for the calculation of total blood flow was located where the central retinal artery branched into multiple retinal arteries. The black vessel boundaries, determined by the automatic processing software, in Fig. 6(C) look slightly different from each other because of the vessel segmentation algorithm. With improved automatic blood flow computation software, variations in the segmentation of the vessel cross sections should be reduced. Waveforms in Figs. 6(A) and 6(B) do not coincide perfectly in phase due to imperfect synchronization between the two acquisition channels and/or phase delay between the paw measured by the pulse oximeter and the retina.

The flow characteristics of the pulsatile total retinal blood flow shown in Fig. 6 are summarized in Table 1, where the coefficients of variation are indicated in parentheses. The systolic, diastolic and mean flow values were calculated from the 9 pulse cycles shown in Fig. 6(A). Pulsatility (PI) and resistivity (RI) indices were defined as:

\[
PI = \frac{(F_{sys} - F_{dia})}{F_{mean}} \\
RI = \frac{(F_{sys} - F_{dia})}{F_{sys}}
\]

where \(F_{sys}\) is peak total systolic flow rate, \(F_{dia}\) is end total diastolic flow rate, and \(F_{mean}\) is mean total flow rate. Note that the PI and RI indices used here are different from indices which are conventionally defined in terms of velocities. The coefficients of variation for all flow characteristics were less than 10%, and it was only 2% for mean flow, demonstrating excellent short-term stability of the measurement technique.

### Table 1. Pulsatile total blood flow characteristics in a normal rat

| Systolic flow [µl/min] | Diastolic flow [µl/min] | Mean flow [µl/min] | Pulsatility index | Resistivity index |
|------------------------|-------------------------|--------------------|-------------------|------------------|
| 9.42 ± 0.2 (0.02)      | 4.44 ± 0.3 (0.06)       | 6.40 ± 0.1 (0.02)  | 0.78 ± 0.06 (0.08)| 0.53 ± 0.03 (0.06) |

It is important to point out that shadowing of the blood vessels by other ocular structures in front of the vessels can occur and may result in an underestimate in the measured blood flow. However, if present, this shadowing can usually be significantly reduced by carefully tilting the retina to avoid the ocular structures that cast shadows. This alignment can be performed in real-time with the Doppler C-scan preview.

### 3.2. Effect of anesthesia on retinal blood flow

Total retinal blood flow is dependent on anesthesia. Figure 7 shows an example of pulsatile total retinal blood flow of a Sprague Dawley rat anesthetized with ketamine/xylazine. As seen from Figs. 6 and 7, the mean total retinal flow value is higher by a factor of ~2 for the rat anesthetized with isoflurane/xylazine compared to that with ketamine/xylazine. This is not surprising since isoflurane is a known vasodilator when used at a high concentration. Although the rats imaged in Figs. 6 and 7 are different animals, similar differences in blood flow were consistently measured in other rats. This indicates that a careful choice of anesthesia is required for total retinal blood flow measurement in small animals, depending on the study objectives.
Pulsatile retinal blood flow imaging was also performed in the human retina to demonstrate that the technique can be extended to human retinal imaging. In many cases, the human central retinal artery is located too deep in the optic nerve head for OCT to image. Therefore, it was necessary to scan a relatively larger area of 0.8mm × 0.8mm in order to intercept all retinal arteries necessary and measure total retinal blood flow. The Doppler C-scan preview was again a useful tool for locating the blood vessels. However, since the scan area was larger for human imaging, it was less important to determine the exact location of the blood vessels. To maintain a high enough oversampling factor for Doppler processing, a 400 × 50 A-scan pattern was used. Although this scan pattern contains significantly larger number of A-scans per en face frame, which makes the frame rate slower than in small animal imaging, the human heart rate is also significantly lower and the resultant number of samples per cardiac cycle is roughly the same. For this experiment, at 244kHz axial scan rate, a scan pattern of 400 × 50 A-scans provided an en face frame rate of ~10Hz. This frame rate corresponds to ~10 sampling points per cardiac cycle assuming a resting heart rate of 60 beats per minute. A total data acquisition time of ~2 seconds will capture at least one continuous cardiac cycle in most subjects. Aside from the differences mentioned above, en face Doppler OCT successfully measured pulsatile total blood flow in the human retina as well as in the rodent retina.

Figure 8 shows OCT images of the optic nerve head of a normal subject. The yellow square in the wide-field en face projection image in Fig. 8(A) indicates the size and approximate location of the scanned area used for repeated volumetric data acquisition for pulsatile total arterial blood flow measurements. Although an 0.8mm × 0.8mm area was chosen in this study with the spectral OCT system, increased imaging speeds provided by future camera and swept source technologies will enable the scanned area to be increased without sacrificing volume acquisition rate, which will facilitate alignment in patients as well as in normal subjects. Figures 8(B) and 8(C) demonstrate the quality of structural images that can be obtained with the high speed spectral domain OCT system used in this study.

Figure 9 and Table 2 summarizes pulsatile total arterial blood flow measurements in the same normal subject. Figures 9(A) and 9(B) show examples of an intensity B-scan and corresponding Doppler B-scan from one of the volumetric data sets used for pulsatile blood flow measurement. Figure 9(C) shows the pulsatile total arterial blood flow measured in the

| Systolic Flow  [μl/min] | Diastolic Flow [μl/min] | Mean Flow  [μl/min] | PI   | RI   |
|------------------------|------------------------|---------------------|------|------|
| 5.35±0.2 (0.04)        | 2.06±0.2 (0.10)        | 3.28±0.1 (0.04)     | 0.99±0.07 (0.07) | 0.61±0.08 (0.08) |

Fig. 7. (A) Pulsatile total flow measured at the central artery of a Sprague Dawley rat anesthetized with ketamine/xylazine and simultaneous acquisition of plethysmographic pulse waveform from a pulse oximeter. (B) En face Doppler images at time points indicated by the red arrows in (A). The arrows from left to right in (A) correspond to Doppler images 1 to 4 in (B). 150μm × 150μm. (C) Systolic, diastolic and mean total flow values. PI: pulsatility index, RI: resistance index. Numbers in parentheses are coefficients of variation.
Fig. 8. (A) An intensity en face projection image of the human optic nerve head of a normal subject. The yellow square indicates the size and approximate location of the area of scanning used for repeated volumetric acquisition for pulsatile total blood flow measurements. 600 × 600 A-scans over 6mm × 6mm acquired within 1.7 seconds. Scale bar: 500μm. (B, C)Intensity B-scan images extracted from the locations indicated by the blue and tan dotted lines in (A). Averages of 4 neighboring B-scans displayed in logarithmic scale. Scale bar: 250μm.

Fig. 9. (A) An intensity B-scan image of the human retina of a normal subject. (B) A Doppler B-scan image corresponding to (A) showing the vasculature. (C) Pulsatile total retinal arterial blood flow measured at near the center of the optic nerve head. (D) An example of intensity fundus projection of a volumetric scan. (E) En face Doppler images at time points indicated by red the arrows in (C). The arrows from left to right in (C) correspond to Doppler images 1 to 4 in (E). 800μm × 800μm.
subject. The heart rate measured by a pulse oximeter was 57 beats per minute. Over the
cardiac cycle centered in Fig. 9(C), the mean total arterial blood flow was 43.2μl/min. The
resistivity and pulsatility indices were calculated as 0.48 and 0.69, respectively. The resistivity
and pulsatility indices of the subject may appear lower than similarly defined indices based on
velocity measurements due to the low pass filtering effect of the technique, which arises from
the limited volume sampling rate of ~10Hz and Doppler velocity noise suppression effects,
which result from summing multiple pixels to calculate total flow. Therefore, care is required
in interpreting pulsatility and resistivity indices when comparing values measured by different
devices. Figure 9(D) shows an example of an OCT intensity fundus projection of a volumetric
data set. Figure 9(E) shows en face Doppler images at time points indicated by the red arrows
in Fig. 9(C). The black boundaries around the blood vessel are found by the automatic blood
flow computation software. The vessel cross-sections in Fig. 9(E) look slightly different from
each other because slightly different depth locations were chosen by the software for flow
measurement. Variations in the segmentation of the vessel cross sections could result in
differences in flow measurement. However with improved automatic blood flow computation
software, the boundaries should become smoother and more reproducible. Regardless of these
effects, pulsatile variation of flow speed can be clearly seen. It may appear that in Fig. 9(C),
the pulsatile total flow trace is not as consistent as in Fig. 6(A) due to the limited number of
cardiac cycles displayed in Fig. 9(C). However, the actual relative variations between cardiac
cycles for the two cases are not significantly different. These variations probably arise from
the limited sampling rate.

Another advantage of the technique presented in this paper is that it is inherently less
sensitive to involuntary eye motion compared with other Doppler measurements. This is
particularly attractive for human retinal imaging. Although a total acquisition time on the
order of a few seconds is required for imaging over at least one cardiac cycle, each volumetric
data set takes less than 0.1 second to acquire, which makes each volume virtually motion
artifact free. In principle, involuntary eye motion in the axial direction does not affect the flow
measurement, as long as the movement speed is negligible compared to the axial speed of
blood flow and the retina remains within the OCT imaging range. Involuntary eye motion in
the lateral direction does not affect the flow measurement either, as long as all the blood
vessels are intercepted by the scan area. This scan area can be increased using future OCT
instruments with higher imaging speed. This point is important because this approach scales
significantly better with OCT imaging speed, compared to conventional approaches used for
total blood flow measurement, which may not benefit from increased imaging speeds.

4. Conclusion

These results demonstrate the ability of en face Doppler OCT to characterize the pulsatile
total arterial blood flow in small animals as well as humans. The advantages of en face
Doppler include: (1) no hardware modification in OCT hardware is required, so that both
structural and functional blood flow imaging can be performed with a single device, (2)
knowledge of Doppler angle is not required for flow calculation, resulting in improved
accuracy and robustness, (3) high speed enables detection of velocities of up to 75mm/s and
rapid volume acquisition rates necessary to characterize pulsatility, (4) alignment can be easy
and simple due to real-time Doppler C-scan preview, (5) measurements can be entirely
automatic without requiring user input, and (6) repeated rapid volume acquisition is inherently
less sensitive to involuntary eye motion. This method promises to be useful for investigation
of small animal models of ocular diseases and can be extended to clinical pulsatile total retinal
blood flow measurement in patients.
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