# Retinal Blood Flow Response to Hyperoxia Measured with *En Face* Doppler Optical Coherence Tomography

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**Conflict of Interest Disclosures**

Oregon Health & Science University (OHSU), Yali Jia, Ou Tan, and David Huang have a significant financial interest in Optovue, Inc., a company that may have a commercial interest in the results of this research and technology. These potential conflicts of interest have been reviewed and managed by OHSU. David Huang and Ou Tan receive patent royalties from Carl Zeiss Meditec, Inc. Other authors do not have financial interest in the subject of this article.

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## Abstract

**Purpose** Use multi-plane *en face* Doppler optical coherence tomography (OCT) to measure the change in total retinal blood flow (TRBF) in response to hyperoxia.

**Methods** One eye of each healthy human participant (8) was scanned with a commercial high-speed (70 kHz) spectral OCT system. Three repeated scans were captured at baseline and after 10 minutes of oxygen (hyperoxia) by open nasal mask. The procedure was performed twice on day one and once more on day two. Blood flow of each vein was estimated using Doppler OCT at an optimized *en face* plane. The TRBF was summed from all veins at the optic disc. The TRBF hyperoxic response was calculated as the TRBF percent change from baseline.

**Results** Participants experienced a 23.6% ± 10.7% (mean ± standard deviation [SD]) decrease ($p < 0.001$, paired t-test) in TRBF during hyperoxia. The within-day repeatability of baseline TRBF was 4.1% and the between-day reproducibility was 10.9% coefficient of variation (CV). Between-grader reproducibility was 3.9% CV. The repeatability and reproducibility (pooled SD) of hyperoxic response was 6.1% and 6.4%, respectively.

**Conclusion** The multi-plane *en face* Doppler OCT algorithm was able to detect, in all participants, a decreased TRBF in response to hyperoxia. The response magnitude for each participant varied among repeated trials and the averaging of multiple trials was helpful in establishing the individual response. This technique shows good potential for the clinical investigation of vascular autoregulation.

## Introduction

Retinal tissue has an intrinsic ability to maintain a relatively constant blood supply through a range of hemodynamic conditions.[1] This homeostatic mechanism, known as autoregulation, is independent of systemic hormonal or sympathetic control. [2, 3] Instead, autoregulatory control involves local metabolic, myogenic and endothelium-related mechanisms.[2, 4] A deficit in retinal autoregulation is a likely indicator of retinal tissue distress and is thought to have a role in the pathogenesis of such eye diseases as age-related macular degeneration and diabetic retinopathy.[5-8] Therefore, measuring the retinal vascular autoregulatory response may be useful diagnostically.

A common approach to induce autoregulatory response is by changing blood gas concentration through alterations in inhaled oxygen level. Since the retina seeks to maintain a constant oxygen tension, blood flow is altered in order to compensate for fluctuations in blood oxygen concentrations. Several methods have been used to study changes in vessel diameter and blood flow during increases of inhaled oxygen concentration (hyperoxia).[9] Laser Doppler velocimetry showed a decrease in diameter and blood flow among both small and large retinal vessels in response to hyperoxic conditions.[10] Laser Doppler flowmetry (LDF) and blue field entoptic technique also detected a decrease in optic disc and macular flow when breathing increased oxygen.[11-13] Dual-beam bidirectional Doppler OCT has also been used to show reduced total retinal blood flow during hyperoxia.[14] The application of these methods, however, are limited by the rarity of these instrumentations.

Optical coherence tomography (OCT) angiography was also recently used to show a decrease in flow index and vessel density in the peripapillary retinal

68 microvasculature during hyperoxia.[15] However, because OCT angiography primarily
69 measures vascular area and is relatively insensitive to velocity changes, the measured
70 response magnitudes were small.

71 We recently developed an *en face* flow measurement algorithm with Doppler
72 OCT to improve the measurement of total retinal blood flow (TRBF).[16] The advantage of
73 this method is that it provides reliable volumetric measurement of the entire retinal
74 circulation in a scan of only a few seconds using a commonly available commercial
75 instrument. The purpose of this study is to utilize this measurement to investigate the
76 change in retinal blood flow in healthy human participants during hyperoxia.

77 **Methods**

78 *Study population*

79 The study was conducted at Casey Eye Institute at the Oregon Health & Science
80 University (OHSU). The tenets of the Declaration of Helskini in the treatment of human
81 participants were adhered to in this experiment. The protocol was approved by the
82 Institutional Review Board (IRB). Healthy adult volunteer participants with no history or
83 evidence of ocular disease were recruited at the Casey Eye Institute. Informed consent
84 was obtained from each subject following an explanation of the nature of the study. One
85 eye from each participant was selected and examined throughout the study.

86 *System setup*

87 The experiment was conducted using a commercial spectral OCT system (RTVue-XR,
88 Optovue, Inc., Fremont, CA). This instrument performs 70,000 A-lines per second, and
89 uses a light source centered on 840 nm wavelength. It has a 2.3 mm scan depth with a

5 μm full-width-half-maximum depth resolution in tissue. The time interval between 2 consecutive axial line scans (A-lines) is 14.3 μs, which corresponds to a phase wrapping velocity limit of 11.1 mm/sec. The exposure power at the pupil of 750μW was within the safety limits set by the American National Standards Institute.[17]

The TRBF scan pattern contained five consecutive volumes covering a 2 mm by 2 mm area centered at the optic disc. Each volume scan contained 80 B-frames with each B-frame containing 500 A-lines. This created a three-dimensional volumetric data set containing 195 *en face* planes. The TRBF scan was obtained in three seconds. Three repeated scans, containing a combined 15 volumes, were performed consecutively during each scan session.

*Data acquisition*

Each experimental trial consisted of one baseline and one hyperoxia scan sessions. In order to establish a baseline, participants were asked to sit and breathe normally for 10 minutes. Baseline blood pressure and heart rate were then recorded. Participants were then fitted with a nasal mask (OxyMask, Southmedic, Barrie, Ontario, Canada) and given supplemental oxygen for ten minutes at a rate of 15 liters per minute. This flow rate should create a mean fraction of inspired oxygen ($FiO_{2)}$ of 80% inhaled oxygen concentration according to Paul et al [18]. Following the ten minutes, hyperoxia was maintained while blood pressure and heart rate were recorded and the scan session was completed.

On day one, participants completed two trials separated by 10 minutes of breathing normally, in order to re-establish baseline. The difference between the 2 trials was used to established within-day repeatability. On day two, participants completed a

third trial, in order to establish a between-day reproducibility of baseline TRBF and hyperoxic response.

*Data processing*

In this experiment, we used multi-plane *en face* Doppler OCT to detect blood flow, in which the flow is equal to integration of the axial velocity derived from Doppler phase shift in an *en face* plane. This method eliminates the need to determine the angle between the vessel and OCT beam.[19, 20] However, a high scan speed is required to obtain three dimensional volumetric scans and avoid artifacts. In this study, because scans were taken on a relatively slow, 70kHz spectral OCT system, fringe washout and phase wrapping artifacts make it difficult to find a single *en face* plane to include all vessels without artifacts. Instead, we determined the flow values of branch retinal veins at different optimized *en face* planes.[16] Since phase wrapping and fringe washout artifacts generally decrease the measured flow, the optimal *en face* plane was established by searching for the plane of maximum integrated flow in the relevant vein.

The raw spectral data was exported from OCT. A custom MatLab software was used to calculate the Doppler OCT image, detect vessels, and compute TRBF. A phase-resolved technique was used to calculate Doppler phase shift (*D*) which is proportional to the axial component of blood flow velocity by,

$$V = \frac{\lambda_0 \cdot D}{4\pi n T}$$

where $\lambda_0$ is the central wavelength of the laser, *n* is the refractive index of water, and *T* is the time interval between consecutive axial scans.[16]

The split-spectrum method was used to divide the 3D OCT data into multiple image cubes, corresponding to separate spectral bands. Averaging of the Doppler

phase shift in these bands helps to reduce the noise in the Doppler OCT image.[16] The averaged Doppler phase shift of static retinal tissue was then used to remove the bulk motion artifact caused by eye movement. An automated phase unwrapping algorithm was applied to correct Doppler phase shift in each *en face* plane. Then vessel trees were then detected as voxels with Doppler phase shift above a threshold set above the noise level. For each vessel segment, the flow is calculated on the *en face* plane which maximizes the integration of Doppler phase shift in the vessel area. Veins were distinguished from arteries by the sign of the Doppler shift, based on the knowledge that veins in the optic nerve head flows away from the OCT beam source. Finally, flow of all vein segments were summated for TRBF. The TRBF was estimated from only veins because retinal arteries have more artifacts due to the higher velocity and larger pulsatile variation compared to veins.[16]

To reduce measurement variability due to pulsatility during the cardiac cycle, the TRBF measurements from all volumes within scan were averaged. Lastly, TRBF was corrected for magnification variations due to axial eye length differences.

*Manual validation*

A grader validated the vein decision and optimized *en face* plane selection generated by the automated algorithm. Volumes were excluded by the grader when motion artifacts interfered with vein identification or flow calculation. Scan session data was included if at least 8 of 15 volumes from each OCT scan were gradable. Fundus photos (Fig. 1A) and *en face* structural OCT images (Fig. 1B) were used to confirm retinal veins automatically identified on the *en face* Doppler phase shift images (Figure 1C). The grader ensured each vein was selected once, at either the root or branch (depending on

which was larger) but never both. The TRBF is the summation of maximum blood flow for each identified vein (Fig. 1D-G).

*Data analysis*

Averages of baseline and hyperoxic measurements for superior, inferior, and TRBF were calculated from all three trials of all participants and compared using a paired t-test. The hyperoxic response was calculated as the TRBF percent change (average ± standard deviation) from baseline of all participants from all three trials. Analysis of variance (ANOVA) was used to compare the response magnitude between the three trials. Pooled standard deviation was used to measure repeatability and reproducibility of the hyperoxic response. Coefficient of variation (CV) was used to determine within-day repeatability and between day-reproducibility for baseline measurements. Two graders separately graded a randomly selected data subset (33% of data set) in order to determine between-grader reproducibility CV. Within-session repeatability CV was determined for baseline and hyperoxia scan sessions.

## Results

*Participant Characteristics*

Eight participants, 7 male and 1 female, were recruited. Their age ranged from 23 to 50 years (34 ± 10 years, average ± standard deviation [SD]). The participants were emmetropic or myopic, with axial eye lengths of 25.3 ± 0.72 mm as measured by partial coherence biometer.

*Quality of total retinal blood flow measurements*

The multi-plane *en face* Doppler TRBF measurement was applied to each of 15 volumes acquired in three 3-second scans during each scan session. An average of 13 volumes per session was gradable. All of the sessions met the minimum of 8 gradable volumes, therefore usable TRBF averages were obtained for all sessions in all participants. Repeated scans acquired during baseline and hyperoxia sessions showed good within-session TRBF repeatability CV of 6.6% and 8.1%, respectively. Average TRBF measurements from the 2 baseline sessions on the same day showed a good repeatability CV of 4.1%. Between-day baseline sessions had a reproducibility CV of 10.9%. The between-grader reproducibility CV was 3.9%.

*Hyperoxic response*

There was no significant decrease in heart rate due to hyperoxia (Table 1). There was a slight reduction in mean arterial pressure of borderline statistical significance. Total and hemispheric retinal blood flow during hyperoxia were significantly reduced compare to baseline (Table 2). Hyperoxic reductions in TRBF was noted in all trials in all participants (Fig. 2). The response magnitude for each participant varied among repeated trials conducted within-day and between-day (Table 3). However, there was no systematic difference in response magnitude between any of the three trials (ANOVA, $p = 0.69$).

## Discussion

The average baseline TRBF found in this study is within the range of healthy blood flow reported in previous investigations. Bidirectional LDV and Doppler OCT have quantified overall retinal flow profiles ranging from 34 ± 6.3 μL/min to 54.7 μL/min.[21-24] In a previous publication on multi-plane *en face* Doppler OCT, a TRBF of 45.4 ± 6.7 μl/min

was found in a healthy population.[16] The within-session TRBF repeatability for baseline and hyperoxia in the current study is slightly better than the previous report of 8.6% CV.[16] We have further demonstrated that this method of TRBF measurement is reproducible within a day, between days, and between graders.

Although a deficit in retinal vascular autoregulatory response to hyperoxia has been detected in the diseased eye, clinical application requires widely available instrumentation that can detect this deficit with high sensitivity relative to the normal population. Because OCT is widely used in ophthalmology and the new generation of commercial OCT retinal scanners has sufficient speed to utilize the multi-plane Doppler technique, our method has the potential to be used for clinical investigation. The remaining obstacle is the relatively large population standard deviation of 10.7% in hyperoxic response, which is nearly half of the average response of 23.6% in our present study. This variability is similar to previous results with dual-beam bidirectional Doppler OCT (13.2%), and LDV (11.9%).[14] This variability may be inherent to the complex autoregulatory mechanism,[9] which is known to be sensitive to variations in a person's baseline physiologic state such as heart rate, blood pressure, and intraocular pressure.[19, 25-27]

If the variability of the autoregulatory response cannot be reduced, another way to increase the contrast would be to enhance the size of the response. Previous studies using the blue field entoptic technique, LDV, and LDF had detected reductions of 37% to 61% in retinal blood flow during exposure to 100% oxygen.[11-13, 28] The TRBF decrease found in our study (23.6%) fell below this range. Since retinal autoregulation acts to maintain blood gas concentrations, it is likely that the lower inhaled oxygen level

that we were able to achieve with nasal mask (estimate 80%) may have induced a weaker response compared to exposure to 100% oxygen.[13] Furthermore, the open nasal mask we used (OxyMask) allowed open venting and access to normal atmospheric carbon dioxide. The presence of CO2 has been found to attenuate the vasoconstriction of retinal vessels during hyperoxia, and may further explain the smaller response magnitude observed in this study.[29] Thus a potential way to increase the hyperoxic response would be to increase oxygen delivery using a nonrebreather face mask with reservoir bag.

OCT angiography is another technique to measure the retinal autoregulatory response. In a recent study, we used OCT angiography to detect a reduction in peripapillary flow index (8.87 ± 3.09) during the same oxygen exposure method described here.[15] Because the OCT angiography signal (decorrelation between consecutive B-frames) reaches a plateau value at low velocity values,[30] the flow index is a composite value that mainly measures vascular constriction, but has some sensitivity to velocity reduction in capillaries. Therefore the hyperoxic response measured by OCT angiography was smaller (8.87% mean). Fortunately, the response variability was also smaller (3.09 SD) in the normal population. The ratio between the average response magnitude and population SD was 2.9 for OCT angiography peripapillary flow index and 2.2 for *en face* Doppler OCT TRBF in the present study. These values cannot be directly compared because the populations were not the same. Both methods may be viable approaches.

In summary, we have demonstrated that multi-plane *en face* Doppler OCT can reproducibly measure TRBF and detect a reduction in blood flow in response to

hyperoxia. This method may be useful in an investigation of the retinal vascular autoregulation.

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326 **Tables**

327

**Table 1**. Hemodynamic Parameters

| Parameters | Baseline | Hyperoxia | P-value |
|---|---|---|---|
| Heart Rate | 69.3 ± 4.8 | 67.7 ± 6.7 | 0.63 |
| Mean Arterial Pressure | 88.7 ± 6.8 | 84.8 ± 6.9 | 0.055 |

Combined mean ± standard deviation for all three trials. P-values, based on paired t-tests.

**Table 2**. Retinal Blood Flow Measured by Doppler OCT

| Parameters | Baseline (μL/min) | Hyperoxia (μL/min) | P-value |
|---|---|---|---|
| Superior retinal blood flow | 26.2 ± 5.06 | 19.1 ± 3.63 | <0.001 |
| Inferior retinal blood flow | 18.6 ± 2.71 | 14.3 ± 2.20 | <0.001 |
| Total retinal blood flow | 43.9 ± 4.18 | 33.4 ± 4.60 | <0.001 |

Baseline and hyperoxia values are mean ± population standard deviation from all 3 trials. P-values, based on paired t-tests.

**Table 3.** Response of total retinal blood flow under hyperoxia

| | Average change in total retinal blood flow | Within-day repeatability (Pooled SD) | Between-day reproducibility (Pooled SD) |
|---|---|---|---|
| **Hyperoxic response** | -23.6 ± 10.7 % | 6.1 % | 6.4 % |

Change in total retinal blood flow relative to baseline (mean ± population standard deviation from all 3 trials). Within-day repeatability between Day 1, Trail 1 and 2. Between-day reproducibility is between Day 1, Trial 1 and Day 2, Trial 1.

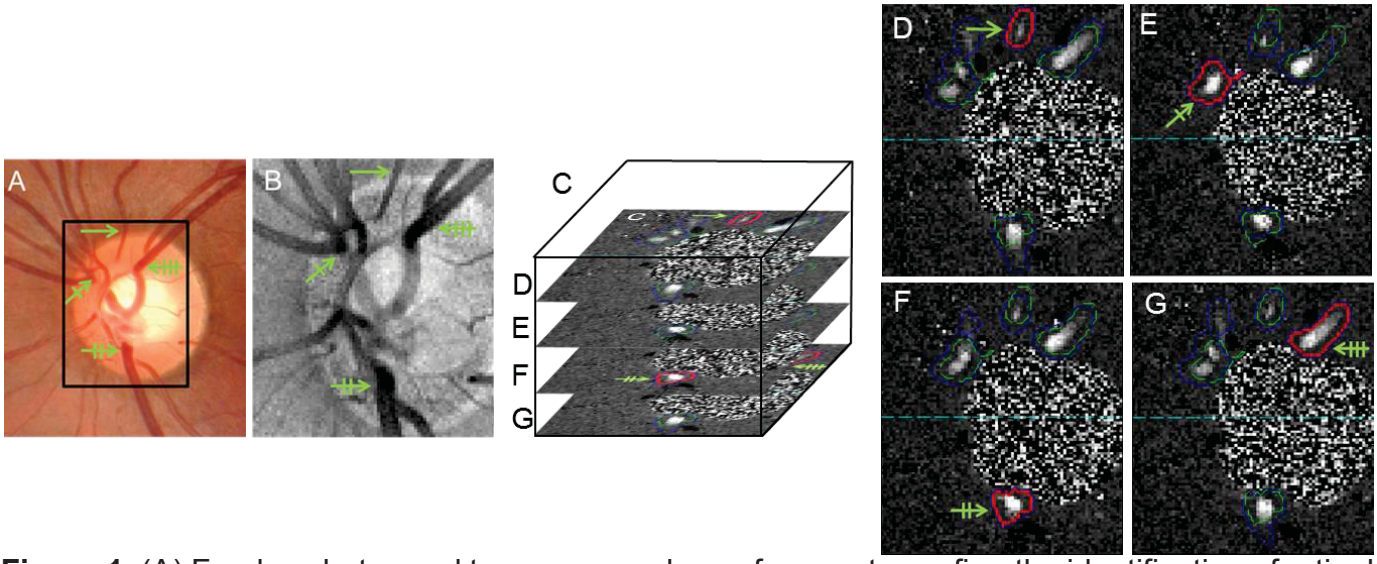


**Figure 1**. (A) Fundus photo used to as a secondary reference to confirm the identification of retinal veins (green arrows with unique tickmark pattern for each vein). Box shows OCT scan area. (B) *En face* structural OCT image. (C) Illustration showing that the 3D volumetric OCT data was divided into 195 *en face* planes of 15 µm thickness. (D-G) *En face* Doppler OCT images. The enclosures outline automatically identified veins. Red enclosures identify the plane of maximum Doppler signal at which flow is measured for the enclosed vein section. Green and blue enclosures outline venous sections that were not used for flow measurement. Each branch vein is measured on only one plane to avoid duplicative flow measurement. Horizontal dotted line separates blood circulation into superior and inferior hemispheres.

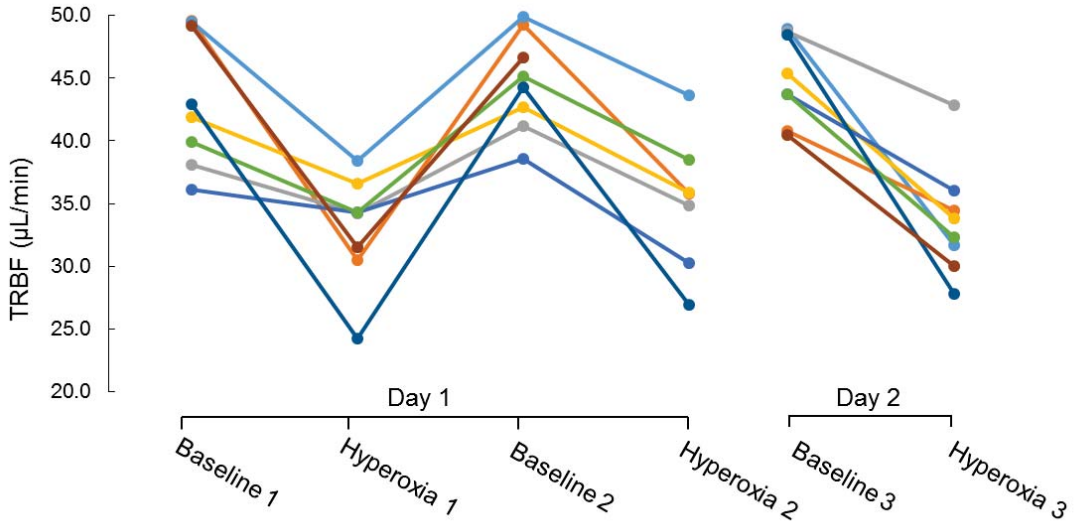


**Figure 2.** The average total retinal blood flow (TRFB) for participants (N=8) under each breathing condition (mean ± SD). Two-tailed t-tests show statistical significant differences between Baseline 1 and Hyperoxia 1 (P=0.05), Baseline 2 and Hyperoxia 2 (P=0.001), and Baseline 3 and Hyperoxia 3 (P=0.002).