Video Article

Doppler Optical Coherence Tomography of Retinal Circulation

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URL: https://www.jove.com/video/3524
DOI: doi:10.3791/3524

Keywords: Medicine, Issue 67, Ophthalmology, Physics, Doppler optical coherence tomography, total retinal blood flow, dual circular scan pattern, image analysis, semi-automated grading software, optic disc

Date Published: 9/18/2012

Citation: Tan, O., Wang, Y., Konduru, R.K., Zhang, X., Sadda, S.R., Huang, D. Doppler Optical Coherence Tomography of Retinal Circulation. *J. Vis. Exp.* (67), e3524, doi:10.3791/3524 (2012).

Abstract

Noncontact retinal blood flow measurements are performed with a Fourier domain optical coherence tomography (OCT) system using a circumpapillary double circular scan (CDCS) that scans around the optic nerve head at 3.40 mm and 3.75 mm diameters. The double concentric circles are performed 6 times consecutively over 2 sec. The CDCS scan is saved with Doppler shift information from which flow can be calculated. The standard clinical protocol calls for 3 CDCS scans made with the OCT beam passing through the superonasal edge of the pupil and 3 CDCS scan through the inferonal pupil. This double-angle protocol ensures that acceptable Doppler angle is obtained on each retinal branch vessel in at least 1 scan. The CDCS scan data, a 3-dimensional volumetric OCT scan of the optic disc scan, and a color photograph of the optic disc are used together to obtain retinal blood flow measurement on an eye. We have developed a blood flow measurement software called "Doppler optical coherence tomography of retinal circulation" (DOCTORC). This semi-automated software is used to measure total retinal blood flow, vessel cross section area, and average blood velocity. The flow of each vessel is calculated from the Doppler shift in the vessel cross-sectional area and the Doppler angle between the vessel and the OCT beam. Total retinal blood flow measurement is summed from the veins around the optic disc. The results obtained at our Doppler OCT reading center showed good reproducibility between graders and methods (<10%). Total retinal blood flow could be useful in the management of glaucoma, other retinal diseases, and retinal diseases. In glaucoma patients, OCT retinal blood flow measurement was highly correlated with visual field loss (R^2>0.57 with visual field pattern deviation). Doppler OCT is a new method to perform rapid, noncontact, and repeatable measurement of total retinal blood flow using widely available Fourier-domain OCT instrumentation. This new technology may improve the practicality of making these measurements in clinical studies and routine clinical practice.

Video Link

The video component of this article can be found at https://www.jove.com/video/3524/

Protocol

1. Protocol Text

1. Patients are scanned by RTVue Fourier-domain optical coherence tomography (OCT) system (Optovue Inc., Fremont, CA, USA) using the circumpapillary double-circular scan (CDCS) and the 3D optic disc scan.
    1. The CDCS pattern consists of two concentric circles around the optic nerve head. The inner ring diameter is 3.40 mm and the outer ring diameter is 3.75 mm. This pattern transects all branch retinal arteries and veins emanating from the optic nerve head. The double circles are performed 6 times in a single scan to cover about 2 cardiac cycles. To calculate Flow velocity, Doppler shift and Doppler will be estimated on vessel detected in the OCT image. (**Figure 1a, Figure 1b**)
    2. A "dual angle" protocol is used to acquire Doppler OCT scans. In the "dual-angle" protocol, 3 scans are obtained with the OCT beam passing through the superonasal portion of the pupil and 3 scans through the inferonasal portion.
    3. The quality of each scan is evaluated for signal strength, motion error, and Doppler angle. The technician is required to re-do any scan that did not pass the quality check. A total of 6 acceptable CDCS scans are performed for each eye. The "dual-angle" protocol ensures that, for each vessel, at least half of the scans provide good Doppler angles.
    4. The 3D disc scan pattern is a raster scan covering a 6x6 mm region around the optic disc. It is only done once and provides a detailed en face image of this area. (**Figure 1b**)
    5. A color photograph of the optic disc is also imported to help distinguish arteries and veins.

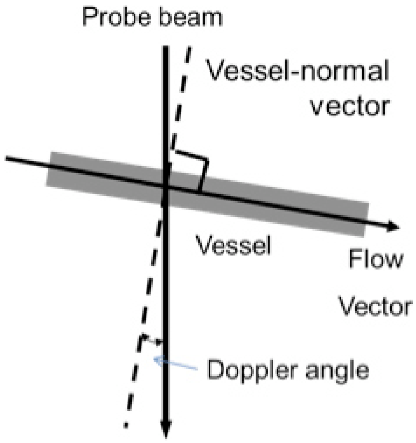

Figure 1a. Measuring total blood flow with the circumpapillary double-circular scan and the 3D disk scan using DOCTORC.

a. The Doppler angle is the angle between probe beam and normal vector of the vessel, and the Doppler signal is the Doppler frequency shift proportional to the flow velocity component parallel to the axis of the probe beam. Thus flow velocity can be estimated from Doppler angle and Doppler signal. However, both Doppler angle and Doppler signal become unreliable when they are below the noise level. On the other hand, when Doppler angle is large, The Doppler shift will be out of the measureable range. When the Doppler angle is appropriate, about 5-15 degrees for the FD-OCT system used in this study, the flow velocity can be correctly estimated by Doppler shift and angle.

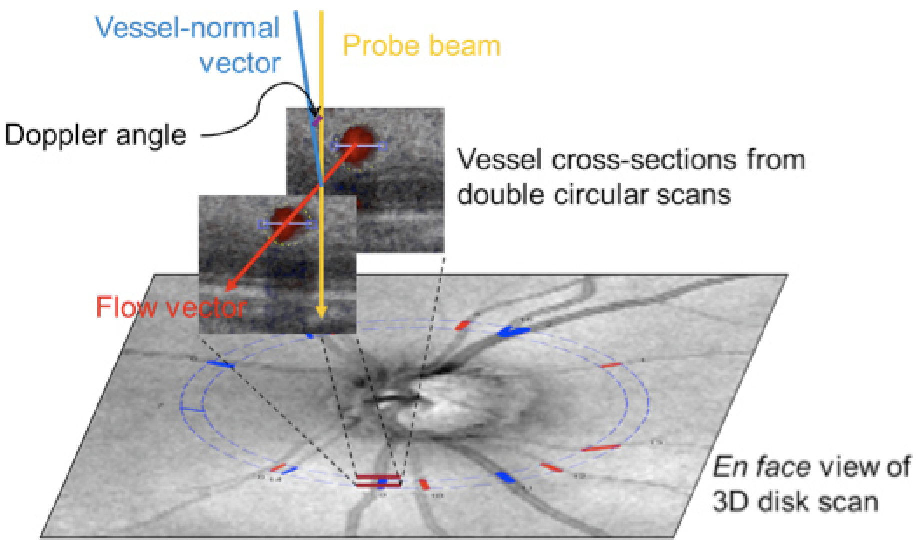

Figure 1b.

2. The Doppler angle in each vessel is measured by the relative positions of the vessel lumina in the two concentric circular scans. The axial component of the flow velocity is obtained by the Doppler shift measured from the relative phase of adjacent OCT axial scans. The total flow velocity is calculated from the axial flow component and the Doppler angle. The flow in each vessel is then calculated by integrating the total velocity profile over vessel cross-sectional area. The vessel pattern is matched to the en face image of the 3D disk scan and fundus photograph to identify each vessel as either a vein or an artery. The total retinal flow is calculated by summing flow in the major retinal veins, *i.e.*, those with luminal diameters above 33 μm.

2. All scans of the same eye are exported as raw data using the RTVue software. The raw OCT images, including both Doppler and intensity images, are first tested for image quality using "Doppler optical coherence tomography of retinal circulation" (DOCTORC) software.
    1. Eye movements are measured by the pooled standard deviation of the inner limiting membrane and the maximum difference between two frames that is estimated by the bulk motion.
    2. Signal strength (reflectance and estimation of average Doppler angle) are also calculated.
    3. According to the average eye movement and signal strength of the repeated scans, the data is classified as either "good" or "poor" in quality. Only good quality data are graded.

3. For acceptable scans, an automated segmentation algorithm is applied to each OCT image for vessel detection.
    1. An automated algorithm that matches vessels is used to locate the same vessel in each frame. The frames on the same circle are registered, and two averaged frames of the inner ring and outer ring are created. Both reflectance and Doppler images are averaged.
    2. For an overview, the detected vessel is projected as a line segment onto the en face fundus image calculated from the 3D disc scan.

4. Graders review each vessel on a small portion of the averaged inner and outer ring frames overlaid with a circle that represents the automated segmentation result.

   1. The grader judges if the location, vessel diameter, and vessel type (vein/artery) matching the vessel on the two rings are correct according to the OCT image, en face OCT image, and disc photograph of the same eye.
   2. The grader is allowed to change any of the above values if he thinks it is necessary. The grader also judges the quality of the Doppler signal in the vessel and gives a subjective confidence score for the vessel label on each scan.
   3. A confidence score of 0-5 is automatically given to each vessel based on Doppler signal strength in the vessel area. It is then manually corrected by the grader based on the strength of the vessel Doppler signal, regularity of vessel boundary, agreement between inner and outer rings of vessel size, and sign agreement of Doppler shift between inner and outer rings.

5. After all vessels are verified and corrected, an automated algorithm is applied to calculate the blood flow of each vein with a method adapted from our previous publication.[1]
   1. The Doppler signal is integrated over the vessel area and then averaged among all frames. Then the Doppler flow is calculated as the summed Doppler signal divided by the Doppler angle.
   2. For each vessel, 6 scans are evaluated on subjective confidence scores, Doppler angles, and Coefficient of variation of the Doppler angles. The flow of a vein is considered valid only if more than 1 scan passes the quality check. For vessels that pass the quality check, the flow is averaged among valid scans.
   3. For vessel with invalid results, the flow is estimated using the vessel area and the average flow speed from valid veins. The average flow speed is calculated by summing the flows in the valid veins and dividing by the summed areas of those veins. The estimate of flow starts from the average flow speed and is then corrected for the dependence of flow velocity on vessel area. Larger vessels have a higher average flow velocity. Thus the correction is made based upon the slope of average velocity versus vessel diameter, 2.13, that we previously reported.[2]
   4. The calculated flow of valid veins and the estimated flow of invalid veins are added to determine the total retinal blood flow.
   5. The total retinal blood flow result is evaluated based on the valid venous area percentage, eye movement, and signal strength.
   6. Venous area and total arterial area are also obtained by adding the vessel areas. Assuming the total retinal blood flow is same in arteries and veins, the arterial and venous velocities are calculated by dividing total blood flow with arterial area and venous area.

2. Representative Results

Normal and glaucomatous eyes are selected from the Advanced Imaging for Glaucoma Study (AIGS, www.aigstudy.net). 48 eyes were scanned by the "dual-angle" protocol and produced scans that passed the image quality check. Using the DOCTORC software, valid flow measurements could be obtained from 83% of the eyes.

To evaluate the reproducibility of the DOCTORC system, another small dataset with 20 eyes was graded by 3 graders. This dataset was also used to train and test graders. 2 graders used the semi-automated DOCTORC software and 1 used an earlier totally manual software employed in previous publications.[2,3] The total retinal blood flows (**Table 1**) determined by the two graders using DOCTORC software are similar to one another and to flow rates determined by the other grader using the manual software. Only 65% of the eyes had valid results because some of the data were not based on dual angle protocol, but single angle protocol.[2] The single angle protocol includes 5 Doppler scans obtained with the OCT beam passing through the center of the pupil. Therefore the Doppler angle is more often small and therefore a greater portion of vessels are usually not gradable.

For all graders, the inter-grader reproducibility, as measured by the coefficient of variation, is similar for both glaucomatous and normal eyes (**Table 2**). Likewise, the reproducibility measurements for the two methods, DOCTORC and the Manual software,[1-5] are similar (**Table 2**). For three graders, a good correlation exists between total blood flow and the pattern standard deviation from visual field tests (**Figure 2**) for glaucomatous eyes.

	DOCTORC software Manual software[3]		
Condition	Grader 1	Grader 2	
Normal	47.0±9.1	48.7±7.2	48.0±6.5
Glaucoma	36.5±5.5	36.7±5.9	34.9±5.1

Table 1. Total Retinal Blood Flow using 2 Different Software.

	Coefficient of Variation	
Glaucoma (7 eyes)		
Grader 1 vs. Grader 2(DOCTORC)	9.58%	
DOCTORC vs. manual method[3]		
Grader 1	8.00%	
Grader 2	9.74%	
Normal (6 eyes)		
Grader 1 vs. Grader 2 (DOCTORC)	5.99%	
DOCTORC vs. manual method		
Grader 1	8.87%	

Grader 2	9.98%	

Table 2. Reproducibility of Total Retinal Blood Flow Measurements.

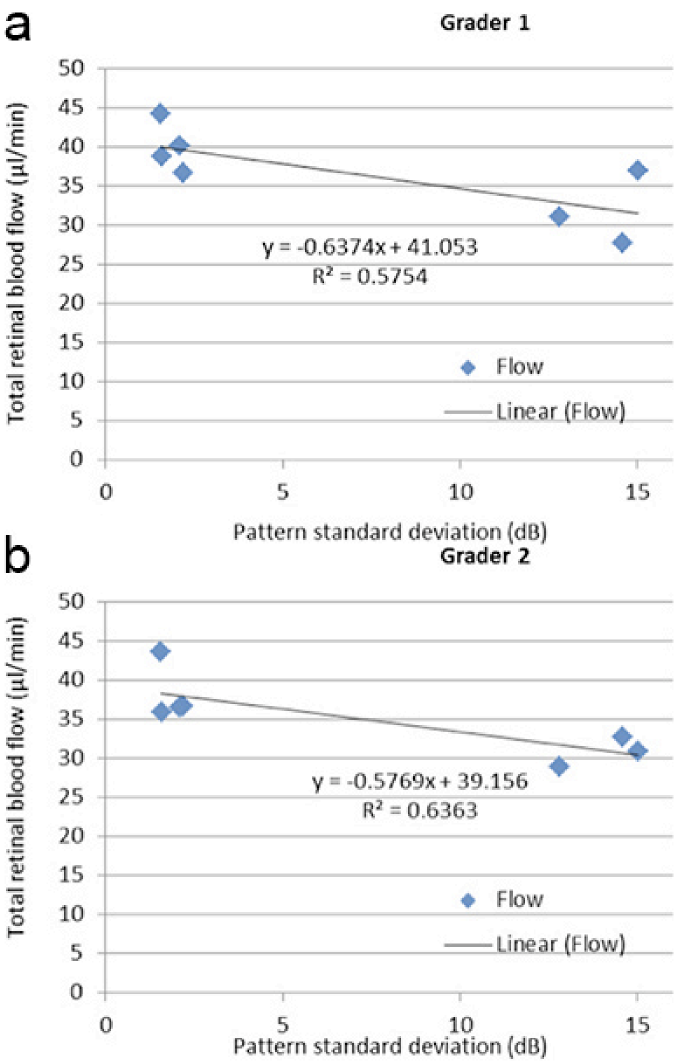

Figure 2. Correlation between Total Retinal Blood Flow and Visual Field in Glaucoma. **a.** Grader 1 using DOCTORC software. Visual field loss is summarized by pattern standard deviation (p=0.048). **b.** Grader 2 using DOCTORC software. Visual field loss is summarized by pattern standard deviation (p=0.032).

Discussion

Blood flow abnormalities occur in glaucoma and vascular diseases of the retina such as diabetic retinopathy.[6-10] Volumetric measurement of retinal blood flow gives valuable information about the disease process.[4-6,11,12] DOCTORC provides a practical way to estimate total retinal blood flow based on the measurements in individual vessels determined by Doppler OCT using the double circle scan pattern.[1-5]

The mean total retinal blood flow measured by Doppler OCT in normal eyes is 47-49 µl/min, comparable to literature values of 34-65 µl/min obtained using laser Doppler techniques.[13,14] Doppler OCT measurements made with the newer semi-automated DOCTORC software agreed closely with the results of manual measurements that we published previously.[1-5] The difference between DOCTORC measurements and manual measurements in individual cases, as measured by CV, is similar to inter-grader differences. This indicates that the difference was primarily associated with subjective portion of the grading process, and not the difference between software. With the manual method and DOCTORC, we measure only veins with diameter larger than 33 µm. Veins with diameters less than 33 µm were usually not detectable using DOCTORC. These veins constitute only a very small portion of the total venous area (0.2%), and they contribute even less to total retinal blood flow because the flow speed in these vessels is less than in larger vessel.[2] Thus, the difference between including and excluding very small vessels is not significant for the determination of total retinal blood flow. The high correlation between visual field tests and total retinal blood flows agrees with our previous result, indicating a close link between perfusion and visual function. Glaucoma eyes also have significantly lower blood flow than normal groups, which agrees with other studies.[15-17] Thus total retinal blood flow determined by DOCTORC will be useful in the diagnosis and monitoring of the progression of glaucoma. In addition to blood flow measurements, DOCTORC also provides vessel area and vessel velocity measurements, which may also be useful in the clinic.

Other techniques are also available for measuring retinal blood flow; however each has some limitations. Laser Doppler techniques need many measurements over a long session as it tests only one vessel at a time. Ultrasound color Doppler evaluates only velocity in larger retrobulbar vessels, and it cannot determine volumetric blood flow. Ultrasound Doppler results vary with operator and subject anatomy. The variability makes it problematic to compare results between subjects and study centers.[18] These instruments are also expensive and only available in major research centers. Other techniques such as fluorescein and indocyanine green angiography require intravenous injection, and they do not provide quantitative results. Fourier domain (or spectral domain) OCT is popular in ophthalmology and only software upgrade is required to enable Doppler blood flow measurement in these equipments. Our Doppler OCT method is the only means to measure blood flow with clinically available FD-OCT instruments. The prevalence and relatively low cost of this instrumentation make possible large multicenter studies of retinal blood flow in health and disease.

There are several limitations for the current version of DOCTORC. The grading process is still not fully automated, and the grading time of one eye is up to 30 min. This grading time is acceptable for large scale clinical studies but not fast enough for daily clinical usage. Direct arterial flow measurement is not available for DOCTORC because the high flow rate in artery is beyond the measurement range of the selected OCT system with speed of 26,000 a-scans/sec. Faster OCT systems would enable the measurement of arterial flow. About 17% of eyes scanned did not yield valid blood flow measurement due to poor Doppler angles on major vessels.

In summary, we provide a practical method to measure total retinal blood flow with a commercially available Fourier-domain OCT instrument. It will have wide applications for optic nerve and retinal diseases, such as glaucoma, diabetic retinopathy, and non-arteritic ischemic optic neuropathy.

Disclosures

Dr. Huang receives grant support, patent royalty, stock options, travel support and lecture fees from Optovue, Inc.; Dr. Tan and Dr. Wang receives patent royalty and grant support from Optovue, Inc.; Dr. Koduru and Dr. Sadda received grant support from Optovue.

Acknowledgements

This study is supported by NIH grant RO1 013516 and a grant form Optovue.

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