Retinal blood flow detection in diabetic patients by Doppler Fourier domain optical coherence tomography

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Abstract

We present human retinal blood flow investigation for diabetic patients using Doppler Fourier domain optical coherence tomography (FD-OCT). The scanning pattern consisted of two concentric circles around the optic nerve head. The blood flow in one patient with diabetes and no retinopathy and another patient with treated proliferative diabetic retinopathy were measured. The patient without retinopathy showed a total blood flow value at the lower level of the normal range. The flow distribution between superior and inferior retina was balanced. The patient with diabetic retinopathy had a flow value lower than the normal people. Our study shows that Doppler FD-OCT can be used to evaluate the total retinal blood flow in patients with retinal diseases.

1. Introduction

Diabetic retinopathy is the most common cause of blindness in working age American. The underlying pathophysiology in diabetic retinopathy involves thickening of the retinal blood vessel wall, microvascular occlusion, retinal tissue ischemia and finally growth of abnormal new blood vessels, leading to severe complications and loss of functional vision [1]. This has led many investigators to use several approaches to study retinal blood flow as a surrogate marker for retinal function [2-6]. However, none of the blood flow imaging modalities studied previously have found their way to wide-spread clinical application, aside from fluorescein angiography (FA), which only provides qualitative imaging of retinal microvascular occlusion.

Optical coherence tomography (OCT) [7] provides high-resolution cross-sectional imaging and is commonly used in the diagnosis and management of retinal diseases [8-12]. In addition to obtain morphological images, OCT can also detect a Doppler frequency shift of reflected light, which provides information on flow and movement [13-15]. Several investigators have studied the visualization of blood flow and flow dynamics using Doppler OCT [16-20], but the measurement of total flow velocity and volume requires additional information on the incident angle between OCT probe beam and the blood vessel, which is not available within a single cross-sectional OCT image. Recently, in vivo measurements of volume retinal blood flow were reported by sequentially scanning each of retinal branch vessels that surrounded the optic nerve head utilizing Doppler Fourier Domain OCT (FD-OCT) [21-23]. Phantom flow measurement [24] showed the flow difference was less than 10% between the measured flow and flow setting. Through 3D circular scanning, Wehbe and co-workers showed blood flow measurement in two retinal vessels [25]. However, there is no report of employing Doppler FD-OCT for clinical study up to now.

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In this paper, we present the patient case study with Doppler FD-OCT. Using a Doppler FD-OCT system, we investigated the retinal venous blood flow in diabetic patients with and without retinopathy. The patient's retinal blood flow was also compared with the result from a normal study. This new technology may improve the early diagnosis and treatment of diabetic retinopathy.

2. Study population

The research adhered to the tenets of the declaration of Helsinki in the treatment of human subjects. Protocol was approved by human subjects committee in University of Southern California (USC). Two diabetic patients were recruited to participate in the study at Doheny Eye Institute in USC. The first patient was a 26 year old Hispanic female with Type 2 Diabetes of eight years duration, with no retinopathy. The second patient was a 37 year old Hispanic female, with Type 1 DM of 34 years duration, with quiescent proliferative diabetic retinopathy (PDR), status post vitrectomy and endolaser panretinal photocoagulation. Informed consents were obtained for both subjects. For each subject, one eye was measured in this study.

3. Methods

3.1 Theory

In Doppler OCT, light reflected by moving blood incurs a Doppler frequency shift ($\Delta f$) which is proportional to the flow velocity component parallel to the axis of the probe beam. Given the angle direction, the Doppler shift is simplified to $\Delta f = -2nV\cos\alpha/\lambda_0$, where $n$ is the refractive index of the medium, $V$ is the flow velocity, $\alpha$ is the angle between the OCT beam and flow, and $\lambda_0$ is the center wavelength of the light. In Doppler FD-OCT [19,20], this frequency shift $\Delta f$ introduces a phase shift in the spectral interference pattern that is captured by the line camera. With fast Fourier transform (FFT), the transformed result is a complex function $f(z) = A(z)\exp(i\phi(z))$ characterized by amplitude $A(z)$ and phase $\phi(z)$. The phase difference $\Delta \phi(z) = \phi_2(z) - \phi_1(z)$ between sequential axial scans at each pixel is calculated to determine the Doppler shift using the following equation [15]:

$$\Delta f = \frac{\Delta \phi(z)}{2\pi T} = \frac{1}{2\pi T} \tan^{-1} \left[ \frac{\text{Im} \left( \sum_{j=1}^{N} f_j(z) \cdot f^*_j(z) \right)}{\text{Re} \left( \sum_{j=1}^{N} f_j(z) \cdot f^*_j(z) \right)} \right]$$

(1)

where $N$ is the number of A-lines used for averaging, $T$ is time interval between two adjacent A-lines. One limitation of phase resolved flow measurement is an aliasing phenomenon caused by $2\pi$ ambiguity in the arctangent function. This phenomenon limits the maximum determinable Doppler shift to $\Delta f_{\text{max}} = 1/(2T)$. Thus, the maximum detectable speed is $V = \lambda_0/(4nT\cos\alpha)$. The minimum detectable flow velocity is determined by the phase noise of the FD-OCT system.

Detection of the relative angle $\alpha$ between probe beam and flow direction is required in order to determine the real flow speed. We used a double circular scanning pattern (DCSP) in our flow measurement [26]. A three-dimensional diagram of DCSP method is shown in Fig. 1, where the retina is scanned circularly by the probe beam at radii $r_1$ and $r_2$. A small radius difference $\Delta r = r_2 - r_1$ is chosen so that the blood vessel (VE) between the scanning circles has an approximately linear shape. The relative positions of the blood vessel in the two Doppler
OCT images $P_1$ and $P_2$ can help us determine the angle between probe beam NB and blood flow $r_b$.

### 3.2 Experiment setup

The spectrometer-based Doppler FD-DOCT system employed in this experiment [27] contained a superluminescent diode with a center wavelength of 841 nm and a bandwidth of 49 nm. The measured axial resolution was 7.5 μm in air. Considering the refractive index of tissue, the axial resolution would be 5.6 μm in tissue. The transverse resolution was about 20 μm as limited by optical diffraction of the eye. The sample arm contained a slit-lamp biomicroscope base that was adapted with OCT scanning optics. Power incident on the cornea was 500 μW, which was well below the ANSI limits for beam exposure. The interference signal was detected by a custom spectrometer. The OCT imaging depth was 2.2 mm. The measured system sensitivity was 107 dB at 200 μm from the zero-path length difference location. The depth-dependent sensitivity falloff was about 15 dB over the entire imaging depth. Time interval between two sequential A-lines was 56 μs. The maximum determinable Doppler shift was 8.9 KHz, yielding a maximum velocity component in the eye of 2.8 mm/s. The measured system phase noise was 51Hz using a mirror reflex without scanning [19].

### 3.3 Image sampling and processing

A green cross fixation target was used to direct the scanning position and reduce the subject's eye movements. The FD-OCT probe beam was scanned on the retina around the optic nerve head at radii $r_1 = 1.8$ mm and $r_2 = 2.0$ mm repeatedly. There were 2700 and 3000 A-lines sampled for radii $r_1$ and $r_2$ separately to keep the same lateral space ratio. Phase differences for every three A-lines were calculated to get the Doppler frequency shift. So each frame consisted of 900 vertical lines for images sampled at $r_1$ and 1000 vertical lines for images sampled at $r_2$. The frame rate for Doppler imaging was 4.2 frames per second for real time display. There were four pairs of (total of eight) Doppler FD-OCT images sampled in each flow measurement for a total recording time of approximately 2 seconds.

Sampled Doppler FD-OCT images were saved for data processing. Retinal blood flow was calculated in a semi-automatic fashion [26]. Locations of blood vessels were selected by a human operator and then blood flows were automatically calculated. The speed profiles of a single vessel in the eight Doppler images were analyzed. Eight flow velocities from the analysis were normalized to the maximum one and plotted against time to show the flow pulsation. This curve was integrated as the pulsation factor. Doppler image with the maximum speed in the eight Doppler images was chosen for space integration to calculate the volume blood flow (F). For some veins, Doppler flow signal was too weak for accurate reading at diastole, the pulsation factor in the adjacent venules was used for flow calculation.

In retinal OCT imaging, the Doppler information contained artifacts from motions of the eye and OCT system. Doppler noise due to these movements would have influenced the measurement result if uncorrected. In our data processing, background axial motion of the retina was evaluated by the Doppler shift of the tissue between the inner retinal boundary and the vessel wall. This background signal was subtracted from the whole A-line to obtain the net Doppler shift induced by blood flow. Eye movements during image acquisition can cause error in the measurement of retinal vessel location due to the limited imaging speed. In our study, the standard error of Doppler angle measurement was 1.4°. If the incidence angle was less than 2.8° from perpendicular, the data was rejected to prevent excessive amplification of angle error from the cosine calculation [26]. For each vessel, its diameter was determined from Doppler image in the region between upper and lower boundary of the flow signal. In the eight sampled Doppler images, the Doppler image which contained the maximum Doppler signal for each vessel was used for vessel diameter calculation. In our Doppler image, the length ratio in the
axial dimension was 3.7 μm/pixel. So the measurement accuracy for vessel diameter was 3.7 μm.

4. Statistical analysis

Correlations between vessel diameter and flow for all study subjects were analyzed using SPSS 15.0 software.

5. Results

In vivo retinal blood flow measurements were performed on the right eye of the first patient and left eye of the second PDR patient. Figure 2 shows the fundus images of the two subjects. The identified retinal branch veins are labeled. The dashed circles show the scanning positions on the retina.

Figures 3 and 4 show the sampled Doppler and intensity images of the subjects. In Figs. 3(a) and 4(a), the grey signals show the Doppler frequency shift induced by the blood flow within major blood vessels distributed around the optic nerve head. Retinal branch veins are labeled for each subject in both intensity and Doppler images which matching with Fig. 2.

Due to signal fading in the arterial vessels induced by high flow speed, such as the artery vessel between veins 1 and 2 in Fig. 3, the data analysis was performed on retinal branch veins. The identification of veins among the other vessels distributed around the optic nerve head was based on the flow direction derived from the recorded Doppler frequency shift and the calculated angle between the probe beam and blood vessel [21]. Knowing the direction of flow can help separate veins from retinal arteries because arteries have a direction of flow towards the retinal periphery from the nerve head, and veins have a direction of flow towards the nerve head from the peripheral retina.

There were eight measurements performed for each subject. Venous blood flow was calculated for each measurement separately. During data processing, if the calculated incidence angle for a single vessel was less than 2.8° from perpendicular, the dataset for that measurement would be rejected to prevent angle error [26]. Finally, we could get retinal blood flow from five measurements for each subject. The sampled Doppler and OCT images can be seen in the view links in Figs. 3 and 4, in which every eight consequent images are from one measurement. There are total 40 Doppler and 40 intensity images for each subject saved in the OSA ISP file. For images sampled at inner circle r₁, the image size is 900 (horizontal) × 512 (vertical). For images sampled at outer circle r₂, the image size is 1000 (horizontal) × 512 (vertical). The length units in the horizontal and vertical dimensions are 12.56 and 3.7 μm/pixel separately. In Doppler OCT images, the phase value ϕ versus grey scale I is ϕ = (I/128.-1.)*π.

In order to get total retinal volumetric flow rate, flows from individual branch veins were summed. The calculated total retinal blood flows of each subject were averaged and the coefficient of variation (CV) was calculated. Table 1 shows the result for the two patients. The first subject (#1) had diabetes but no retinopathy. Her measured total retinal blood flow had a range from 37.4 to 49.2 μl/min. The mean value was 43.3 μl/min, and the standard deviation was 4.4 μl/min. The CV was 10.2%. For the second patient (#2) with PDR, the measured total retinal blood flow had a range from 30.1 to 39.6 μl/min. The mean value was 33.2 μl/min, the standard deviation was 3.7 μl/min and the CV was 11.3%. In Table 1, flow data from a normal human group study are also listed as a comparison [28].

Regional flow distributions of the two patients are shown in table two. The averaged superior (F_{sup}) and inferior flow (F_{inf}) for the first patient was 21.9 and 21.4 μl/min separately. The flow difference ΔF between superior and inferior was 0.5 μl/min. This value showed there was
no big difference comparing the blood flow between superior and inferior retina and her flow distribution was balanced. For the PDR patient, her superior flow was 20.4 μl/min while her inferior flow was 12.8 μl/min. The flow difference between superior and inferior was 7.6 μl/min. Our earlier study [28] showed a normal value for the blood flow in superior and inferior retina was 23.5 and 22.2 μl/min separately (the last row in Table 2). The inferior blood flow of the second patient was obviously lower than that of normal.

For the first patient, measured vessel diameter ranged from 59.2 to 133.2 μm. For the second patient, measured vessel diameter ranged from 51.8 to 122.1 μm. To determine the relationship between flow value and vessel diameter, we plotted F as function of D in log-log scale for the two patients. Figure 5 shows the linear regression result, where the black squares and solid line show the measurement and fitting result from the first patient. The white circles and dashed line show the result for the patient with diabetic retinopathy. For the first patient, the linear regression had a slope of 1.69. The correlation coefficient between vessel diameter and flow was $r^2=0.97$. For the second patient, the slope of the linear regression was 1.39, which was smaller than that of the first patient. The correlation coefficient between vessel diameter and flow was $r^2=0.46$.

To compare the flow condition in different vessels, we analyzed the averaged flow velocity $V_{ave}$ for each vein. $V_{ave}$ was calculated with $F/S$, where $S$ was the vessel cross section area size, $S=\pi(D/2)^2$. Table 3 shows the result. For the first patient, $V_{ave}$ had a range from 15.4 to 22.6 mm/s. The mean value was 18.0 mm/s, at a standard deviation of 2.6 mm/s. For the second patient, the averaged velocity had a range from 8.6 to 25.1 mm/s. The mean value was 16.2 mm/s, at a standard deviation of 5.5 mm/s. For the second patient, its averaged flow speed was lower than that of the first one.

For each vessel, blood flow velocities were different in the 8 sampled Doppler images due to flow pulsatility. For a single vessel, sampled eight flow velocities were normalized to the maximum one and plotted against time to show the flow pulsation. In Fig. 6, the solid curve shows the flow pulsation for vessel 2 (in Fig. 3; Diameter 85.1 μm) of the first patient, and the dashed curve shows the flow pulsation for vessel 5 (in Fig. 4; Diameter 74 μm) of the second patient. There was no big difference observed for the pulsatility for those vessels with similar diameter from two patients.

The PDR patient was further examined with fundus photograph and OCT setup (Rtvue; Optovue Inc.). Her fundus image is shown in Fig. 7. Averaged flow velocities for branch veins are labeled. Grey marks in her retina show the surgery treatment history due to retinal damage. It can be seen that more treatments have been done in the lower rim of her inferior retina. This means more damage happened in that area. These damages can correlate with the low blood flow observed in her inferior retinal hemisphere with OCT.

Macular scan was done with Rtvue OCT setup for the PDR patient. Figure 8(a) shows a three-dimensional image of her macular area. Three-dimensional image set can be found in the view link in Fig. 8(a). Figure 8(b) shows her macular thickness measurement result (unit: μm). Based on her macular OCT thickness map, her inferior retina was thinner than her superior retina. In the scanning window, retinal thickness difference between bottom area at inferior side and top area at superior side was more than 20 μm. This means her inferior retina was affected more by retinal disease. Her inferior retinal thickness thinning also matched with flow measurement result, in which her inferior flow is much lower than that of normal people.

### 6. Discussion

By using the DCSP method, our study showed that patient retinal blood flow could be determined when a fast data acquisition method was utilized. We targeted the major branches
of central retinal veins because their size and velocities were within the dynamic range of our
Doppler FD-OCT system. Because the total venous flow volume is identical to that of arteries
in the retina, as shown by Riva and colleagues [29], measuring the total venous flow alone is
sufficient to quantify the total retinal blood flow.

In our normal group study finished recently, we observed a normal total retinal blood flow
ranged from 40.7 to 52.9 μl/min [28]. The mean value was 45.6 μl/min, and the standard
deviation was 3.8 μl/min. No flow difference was observed between superior and inferior retina
in normal group. In this study, the measured total blood flow from the first diabetic patient,
43.3 μl/min, was within the normal flow range, close to the lower limit. The balanced flow
distribution between superior and inferior retina agreed with our normal result. However, for
the second patient with PDR, her total retinal blood flow was 32.3 μl/min. This was lower than
that of our normal result due to the low flow, 12.8 μl/min, in her inferior retina. The analysis
of averaged flow speed in her branch veins showed a large standard deviation. There were three
vessels which showed low flow speed (vessels 4, 6, 7) compared with other vessels. Two of
them (vessels 6, and 7) were mainly related with inferior vascular circulation, as shown in Fig.
7.

For the PDR patient, fundus photograph showed retinal damage in her inferior retina rim. With
OCT measurement, her inferior retina thickness thinning was also observed. These tests
showed that her inferior retina was more affected by retinal disease. Blood flow in branch
retinal veins related with affected inferior retinal area had low value compared with other
vessels.

In our normal study, volumetric flow rate varied with the vessel diameter at a power coefficient
of 1.97, which indicated that flow speed was not affected by vein diameter. This logarithmic
slope value previously reported for LDF was 2.76~3.35, [29,30] which indicated faster flows
in larger veins. The difference might be due to the vessel diameter measurement methods. In
our study, the inner diameters of retinal veins were measured from the Doppler shift image.
For LDF, vessel diameters were measured from a fundus camera image and might be slightly
larger. For the first patient, the linear regression between blood flow and vessel diameter
showed a slope of 1.69. This slope was within the slope range in our normal study, from 1.52
to 2.54 [28]. For the second patient, the slope of 1.39 was smaller less than that of our normal
result. This means it is possible to give an evaluation about retinal vascular condition from the
relationship between vessel diameter and blood flow.

Ophthalmologists currently do not have a standardized applicable tool to measure retinal blood
flow in clinic. FA is used in clinical practice to evaluate retinal blood flow. It is a relatively
invasive test that involves the injection of an intravenous dye to show areas of capillary
occlusion, new blood vessel formation, and arteriovenous shunting. It does not allow for
quantitative measurements of retinal blood flow. Using ultrasound color Doppler imaging
(CDI), previous authors had shown decreased blood velocity in diabetic patients with PDR
[2], as well as patients with non proliferative disease [3,4]. In contrast, other authors identified
higher blood flow velocity in the central retinal vein than in the artery in a group of patients
with non-PDR [5]. These controversies showed that CDI were limited to larger retrobulbar
blood vessels, and are unable to quantify total retinal blood flow.

LDF is another optical method used to measure retinal hemodynamics. In a group of well
controlled type 1 diabetic patients, Lorenzi and co-workers showed normal retinal blood flow,
compared to controls [6]. Our measurement result from the first patient agreed with them.
Unlike LDF which cannot spatially resolve the origin of the Doppler flow profile in retinal
vessels, Doppler OCT permits depth localization with unprecedented axial resolution.
The DCSP method used in this paper can be used to determine the angle of a blood vessel relative to a probe beam. This method produces an absolute flow measurement through integrating the flow profile over the blood vessel cross-section without resorting to any assumptions on the anatomic or flow parameters. For most of the reported techniques for blood flow measurement, data sampling was time consuming. With the data sampled within 2 seconds by DCSP method, total retinal blood flow could be calculated. For each patient, total data sampling time was less than ten minutes. This greatly reduced the chair time for the photographer and patient in the clinic. As a non-invasive technique, our study shows that Doppler FD-OCT is helpful for clinical diagnosis. With validation of our results in a larger group of patients, Doppler OCT may be able to provide quantitative blood flow evaluation, in contrast to FA, which currently is the standard method for clinical monitoring of microvascular compromise in diabetic retinopathy, but mainly provides qualitative information.

Early diagnosis and treatment in diabetic retinopathy is essential for vision recovery and preventing blindness. Our study shows that Doppler FD-OCT can be used to detect blood flow change in patients with diabetic retinopathy. The measurement of total retinal blood flow is also important for the treatment of other eye diseases. Other causes of blindness in the U.S. [31-35], such as age-related macular degeneration and glaucoma, are also associated with blood flow abnormalities. In glaucoma, poor circulation of the retina and optic nerve is thought to be a risk factor for disease progression [33-35]. Central retinal vein occlusion and branch retinal vein occlusions are also common retinal diseases characterized by decreased retinal blood flow. An accurate measurement of total blood flow could enhance our understanding of the pathophysiology of various eye diseases, as well as provide an objective tool to monitor treatment approaches targeting retinal vascular conditions.

7. Conclusion

In summary, we present in vivo measurement of retinal blood flow in diabetic patients using Doppler FD-OCT. Total retinal blood flow was measured in two diabetic patients with and without retinopathy. The patient without retinopathy showed a normal total retinal blood flow. Her regional flow distribution was balanced between superior and inferior retina. For the patient with PDR, her total retinal blood flow was lower than that of normal subjects due to low flow in her inferior retina. Linear regression between blood flow and vessel diameter showed a slope less than that of normal controls. Fundus photograph and OCT diagnosis showed retinal damage and thickness thinning in her inferior retina, which matched with low flow area determined with Doppler FD-OCT. Our result showed that Doppler FD-OCT could be used to determine retinal blood flow difference between patients and normal people.

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**Fig. 1.**
Three-dimensional diagram of DCSP method: OCT beam scans circularly across the retina. Two scanning radii $r_1$ and $r_2$ are shown in this diagram. $P_1$ and $P_2$ are the vessel positions on the two scanning cones.
Fig. 2.
Fundus images of the patients: (a) diabetic patient without retinopathy; (b) PDR patient. Branch retinal veins are labeled.
Fig. 3.
(a) Doppler (View_1) and (b) intensity image (View_2) of the diabetic patient. Retinal branch veins are labeled as 1 to 6.
Fig. 4.
(a) Doppler (View_3) and (b) intensity image (View_4) of the PDR patient. Retinal branch veins are labeled as 1 to 7.
Fig. 5.
Relationship between vessel diameter and blood flow for the two patients: #1: diabetic patient; #2: PDR patient
Fig. 6.
Flow pulsatility from the two patients: #1: diabetic patient without retinopathy; #2: patient with PDR.
Fig. 7.
Fundus image of the patient with PDR. Averaged flow velocities for retinal branch veins are labeled (unit, mm/s).
Fig. 8.
OCT test result. (a) Three-dimensional OCT image (View_5); (b) retinal macular thickness map.
| Subjects | Vessel Number | Flow Range (μl/min) | Mean | STD  | CV(%) |
|----------|--------------|---------------------|------|------|-------|
| #1       | 6            | 37.4–49.2           | 43.3 | 4.4  | 10.2  |
| #2       | 7            | 30.1–39.6           | 33.2 | 3.7  | 11.3  |
| Normal   | --           | 40.8–52.9           | 45.6 | 4.8  | 10.5  |
## Table 2

Regional Blood Flow in Diabetic Patients

| Subjects | $F_{\text{sup}}$ (μl/min) | $F_{\text{inf}}$ (μl/min) | Δ$F$ (μl/min) |
|----------|--------------------------|------------------------|---------------|
| #1       | 21.9                     | 21.4                   | 0.5           |
| #2       | 20.4                     | 12.8                   | 7.6           |
| Normal   | 23.5                     | 22.2                   | 1.3           |
Table 3

Average Flow Velocity in Branch Veins (unit: mm/s)

| Patients | 1  | 2  | 3  | 4  | 5  | 6  | 7  | Mean | STD |
|----------|----|----|----|----|----|----|----|------|-----|
| #1       | 22.6 | 15.6 | 15.4 | 17.4 | 18.5 | 18.3 | -- | 18.0 | 2.6 |
| #2       | 17.7 | 25.1 | 20.4 | 8.6 | 16.4 | 12.5 | 12.7 | 16.2 | 5.5 |