Absolute Retinal Blood Flow Measurement With a Dual-Beam Doppler Optical Coherence Tomography

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Purpose. To test the capability of a novel dual-beam Doppler optical coherence tomography (OCT) technique for simultaneous in vivo measurement of the Doppler angle and, thus, the absolute retinal blood velocity and the retinal flow rate, without the influence of motion artifacts.

Methods. A novel dual-beam Doppler spectral domain OCT (SD-OCT) was developed. The two probing beams are separated with a controllable distance along an arbitrary direction, both of which are controlled by two independent 2D optical scanners. Two sets of optical Doppler tomography (ODT) images are acquired simultaneously. The Doppler angle of each blood vessel segment is calculated from the relative coordinates of the centers of the blood vessel in the two corresponding ODT images. The absolute blood flow velocity and the volumetric blood flow rate can then be calculated. To measure the total retinal blood flow, we used a circular scan pattern centered at the optic disc to obtain two sets of concentric OCT/ODT images simultaneously.

Results. We imaged two normal human subjects at ages of 48 and 34 years. The total retinal blood flow rates of the two human subjects were calculated to be 47.01 μL/min (older subject) and 51.37 μL/min (younger subject), respectively. Results showed that the performance of this imaging system is immune to eye movement, since the two sets of ODT images were acquired simultaneously.

Conclusions. The dual-beam OCT/ODT system is successful in measuring the absolute retinal blood velocity and the volumetric flow rate. The advantage of the technique is that the two sets of ODT images used for the calculation are acquired simultaneously, which eliminates the influence of eye motion and ensures the accuracy of the calculated hemodynamic parameters.

Keywords: optical coherence tomography, Doppler angle, absolute retinal blood velocity, retinal volumetric flow rate

Optical Doppler tomography (ODT) is a branch of optical coherence tomography (OCT) for imaging the spatially resolved speed of blood flow in a blood vessel. Optical Doppler tomography has been widely demonstrated in imaging retinal blood flow velocity in both human and animals. It is potentially a powerful tool for the early diagnosis of diseases that have a vascular etiology, such as glaucoma and diabetic retinopathy.[1,2]

Optical Doppler tomography is based on measuring the Doppler shift caused by the moving blood cells to the probing light in the sample arm of a Michelson interferometer.[3–5] The measured Doppler shift (f_d) is related to the velocity by

$$f_d = \frac{2nv_a}{\lambda_0} \cos \alpha \qquad (1)$$

where v_a is the absolute velocity of the moving blood cells in a blood vessel, λ_0 is the center wavelength of the light source, n is the refractive index of the sample, and α is the Doppler angle (the angle of the probing light beam with respect to the flow vector). From the above equation we can see that the calculated velocity from the measured Doppler shift is a projection of the absolute velocity on the direction of the incident probing light. As a result, to calculate the absolute velocity from the measured Doppler shift we need to know the Doppler angle α, especially in imaging the retinal blood flow because α is close to 90°.

Different techniques have been developed to measure the Doppler angle and further to calculate the absolute velocity and volumetric flow rate, such as using the Doppler broadening,[6] dual incident angle with polarization,[7–10] and path length encoding.[11] In recent years, with the development of segmentation techniques and spectral-domain OCT (SD-OCT), retinal vessel orientations have been successfully obtained from 3D OCT data or dual-scan-pattern ODT images by processing a straight portion of the vessel.[4,12–15] However, these techniques suffer from motion artifacts, and 3D data processing is time-consuming.

To overcome these limitations, we developed a dual-beam OCT technique that can measure the Doppler angle and the absolute blood flow velocity of retinal vessels by using two simultaneously acquired B-scan images covering the same vessel with a predefined distance. Since the two OCT images are acquired simultaneously the measurement is immune to eye

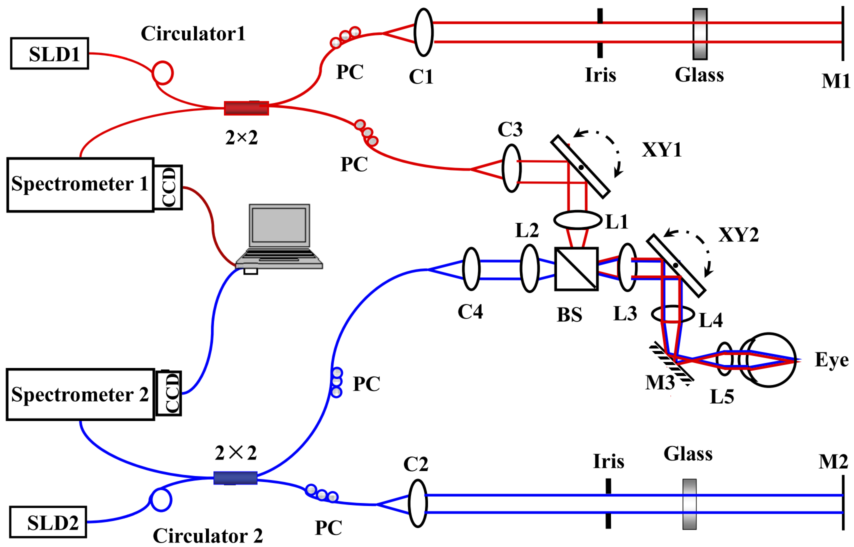

FIGURE 1. Schematic of the dual-beam OCT system. The two OCT subsystems are colored in *red* and *blue* (*red*: OCT1; *blue*: OCT2). The x-scanning mirror of XY1 is imaged onto the x-scanning mirror of XY2 by a 4f system consisting of L1 and L3. The probing beam of OCT1 is scanned by XY1 before it is coupled with the probing beam of OCT2 by a BS The probing beam of OCT2 passes through another 4f system consisting of L2 and L3. The coupled probing beams were then scanned by XY2 and delivered onto the retina by the same optical systems. BS, beam splitter cube; C1-C4, collimator; L1-L5, lens; M1-M3, mirror; PC, polarization controller; XY1 and XY2, 2D galvanometer scanners.

motion. The system was applied to imaging the blood flow of human retina in vivo.

Materials and Methods

Experimental System

A schematic of the experimental system is shown in Figure 1. Two independent fiber-based SD-OCT systems (OCT1 and OCT2) were integrated and two sets of X-Y galvanometer scanners (XY1 and XY2) were used in the sample arm to scan the probing light beams with predefined scan patterns. Owing to the availability issues and to avoid extra cost we used two different OCT systems with different light sources. In OCT1, a superluminescent diode (SLD; Superlum Diodes Ltd., Moscow, Russia) with a full-width-at-half-maximum (FWHM) bandwidth of 50 nm and a center wavelength of 840 nm was used. In OCT2, a three-module SLD (T-840 Broadlighter; Superlum Diodes Ltd.) with a center wavelength of 840 nm and FWHM bandwidth of 100 nm was used.

In the sample arm, the x-scanning mirror of XY1 is imaged onto the x-scanning mirror of XY2 by a 4f (f = 50 mm) system consisting of lenses (L) L1 and L3. The probing beam of OCT1 is scanned by XY1 before it is coupled with the probing beam of OCT2 by a beam splitter cube. The probing beam of OCT2 passes through another 4f (f = 50 mm) system consisting of L2 and L3. The coupled probing beams were then scanned by XY2 and delivered onto the retina by the same optical systems. In experiments, the two probing beams were separated at a preset distance on the retina at a direction controlled by the XY1 scanner.

In the detection arms, the combined reflected beams from the sample and reference arms were collimated and detected by two spectrometers (spectrometer 1 and spectrometer 2), respectively. Spectrometer 1 consists of an 1800 line/mm transmission grating, a multi-element imaging lens (f = 100 mm), and a line scan charge-coupled device (CCD) camera (Sprint spL2048-70k, 2048 pixels with 10-μm pixel size; Basler, Ahrensburg, Germany); spectrometer 2 consists of a 1200 line/mm transmission grating, a multi-element imaging lens (f = 150 mm), and a line scan CCD camera (AVIIVA EM4 2k 4 × 12bits, 2048 pixels with 14-μm pixel size; e2V, Saint Egreve, France). The linear CCD cameras were synchronized and operated at a line rate of 30k lines per second. Two image acquisition boards (NI PCI-1429) acquired the images captured by the cameras and transferred them to a computer for signal processing and image display.

The two OCT systems have different performances. The sensitivities of OCT1 and OCT2 are 105 dB and 96 dB, respectively. The actual detectable imaging depth ranges were measured to be 4.978 and 2.684 mm for OCT1 and OCT2, respectively. Theoretical and measured depth resolutions of OCT1 are 6.2 μm and 6.9 μm in air. The theoretical and measured depth resolutions of OCT2 are 3.1 μm and 4.2 μm in air. Since we used exactly the same optical components in the sample arm of the two OCT systems, and the two probing light beams had the same beam diameter, the lateral resolutions of the two OCT systems are the same, which were mainly limited by the optical properties of the eye.

The system was adjusted so that the two probing beams were coaxial when the driving voltages of the two scanners were set to zero volts. For compensating the residual small misalignment between the two scanners we made a coordinate transformation between the two sets of scan data. By using a 2D raster scan the constructed projection images (OCT fundus image) of a model eye were compared quantitatively. A coordinate transformation matrix between the two sets of scan data was constructed to ensure that the scaled images accurately matched with each other. The imaging depth ranges of the two OCT channels were calibrated by using an optical flat as a sample together with a high-precision translational stage. The power of the probing light was 1.3 mW in OCT1 and 0.7 mW in OCT2. Since the probing light beams illuminated different locations in the retina, the light intensity is well below the American National Standards Institute (ANSI) standard for laser safety.

Methods

In the dual-beam OCT system, the simultaneously acquired OCT B-scans are separated by a predetermined distance in the

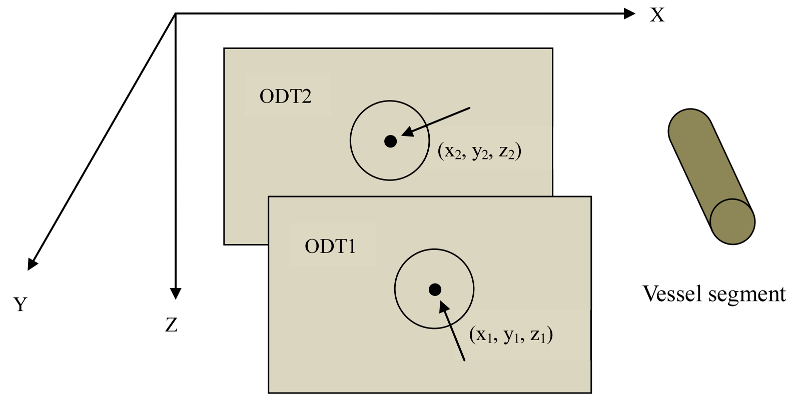

Figure 2. Illustration for Doppler angle calculation. A vessel segment appears on the two ODT images (ODT1 and ODT2) as a circular area. The centers of the circular areas have coordinates (x_1, y_1, z_1) and (x_2, y_2, z_2). For a small segment the blood vessel can be treated as a straight line. The Doppler angle can be calculated from the two sets of coordinates.

selected direction by controlling the two scanners. As illustrated in Figure 2, a vessel segment appears on an OCT/ODT image as a circular area. For a small segment the blood vessel can be treated as a straight line. If coordinates of the center of a vessel in the ODT images are defined as (x_1, y_1, z_1) and (x_2, y_2, z_2) (where x, y lie in the lateral scanning plane, and z is the direction of the probe light), the Doppler angle α can be calculated from the formula below:

$$\cos\alpha = \frac{(z_1 - z_2)}{\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}} = \frac{\Delta z}{\sqrt{\Delta x^2 + \Delta y^2 + \Delta z^2}}, \quad (2)$$

where the (x, y) coordinate relationships between two images of the same vessel is preset by the probing beams simultaneously incident on the retina.

For measuring the major retinal vessels around the optic disc a circular scan pattern centered at the optic disc should be selected. The two probing beams form two concentric circles with different radii (r_1 and r_2) as illustrated in Figure 3a. After data processing, the across sectional ODT images were displayed in a coordinate system defined by (θ, z), as shown in Figure 3b, where the horizontal axis corresponds to the scanning angle θ ($0° \leq \theta \leq 360°$) and the vertical axis z corresponds to the depth dimension along the axis of beam propagation. In the two ODT images, the blood vessel segment has coordinates of (θ_1, z_1) and (θ_1, z_2). The dimensions of the blood vessel segment can be expressed as ($\Delta x = r_1 \cos\theta_1 - r_2 \cos\theta_2$, $\Delta y = r_1 \sin\theta_1 - r_2 \sin\theta_2$, $\Delta z = z_1 - z_2$). The Doppler angle can then be calculated with Equation 2.

The projected flow speed on the direction of the incident sample light can be calculated from the phase difference among the adjacent A-line $\Delta\varphi_i = \varphi_{i+1} - \varphi_i$,[4,5,12] where i is the A-line number:

$$v_p = \frac{\Delta\varphi_i \lambda_0 f_{A-line}}{4\pi n}, \quad (3)$$

where v_p is the projection of the absolute velocity v_a along the depth direction, λ_0 is the center wavelength of the light source, f_{A-line} is the axial scan frequency (A-line rate), and n is the index of refraction of the sample.

The averaged $\bar{v}_p$ across the vessel is then calculated. The absolute mean velocity of the blood $\bar{v}_a$ can be calculated accordingly:

$$\bar{v}_a = \frac{\bar{v}_p}{\cos\alpha}, \quad (4)$$

Knowing the $\bar{v}_a$ and the vessel diameter (d), the blood flow rate (R) can be calculated as

$$R = \frac{1}{4}\bar{v}_a \pi d^2, \quad (5)$$

Results and Discussion

Validation of the Angle Calculation

A flat plate mounted on a rotational stage together with a translational stage was used as a sample to validate the angle calculation. In the experiments, linear scan along either the X or Y directions were chosen. When scan along the X direction was chosen, the OCT images were separated by 0.45 mm in the

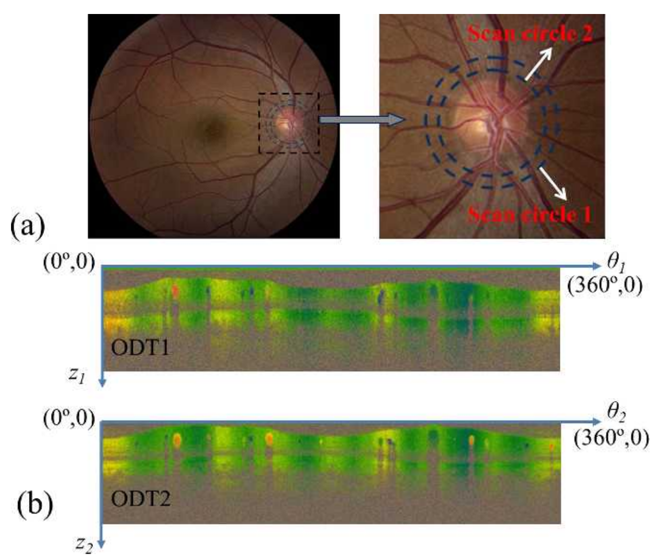

Figure 3. (**a**) Circular scan pattern centered on the optic disc in the dual-beam ODT system. (**b**) The ODT images simultaneously acquired by using the circular scan. The circular ODT images are displayed in the (θ, z) plane, where the *horizontal axis* (θ) corresponds to the scanning angle from 0° to 360°; the *vertical axis* (z) corresponds to the depth dimension along the axis of beam propagation.

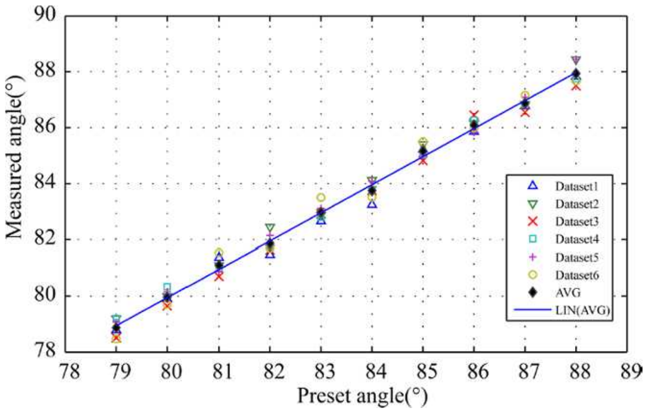

Figure 4. Validation of the angle calculation, using a flat plate as the sample.

Y direction ($\Delta y = 0.45$ mm). Similarly, when scan along the Y direction was chosen the OCT images were separated 0.45 mm in the X direction ($\Delta x = 0.45$ mm). We tested the system at 10 different angles from 88° to 79° (rotation about y-axis). At each set angle 6 pairs of OCT images were acquired (3 pairs in X-scanning direction and 3 pairs in Y-scanning direction). The angles calculated from the simultaneously obtained OCT images are shown in Figure 4. We can see from the figure that the angles measured are in good agreement with the preset angle. Results showed that using the dual-beam OCT system we can calculate the Doppler angle accurately.

Evaluation of the Flow Velocity Determination

We built a phantom to simulate the blood flow by using milk flowing in a glass capillary with a diameter of 100 μm. The flow was controlled by a perfusion pump. The capillary was mounted on a rotational stage. We preset the flow rate with the perfusion pump and the Doppler angles by rotating the stage around the y-axis. At each flow velocity, the OCT scan was set along the Y direction while the two ODT images were separated in the X direction ($\Delta x = 0.3$ mm). For each flow rate the capillary was rotated at three different angles, and 18 measurements were taken.

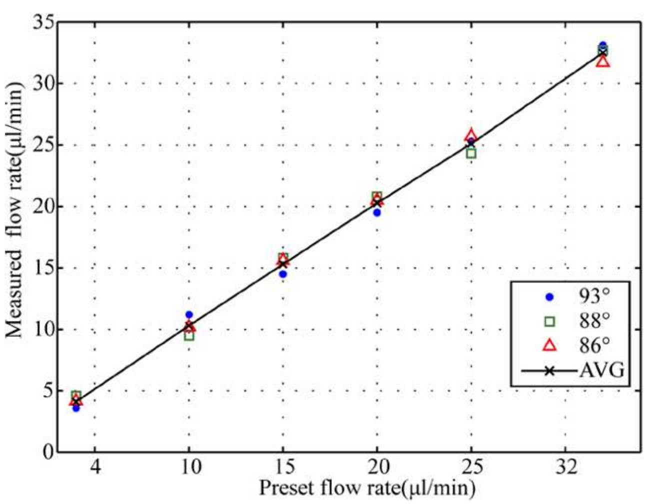

Figure 5. Calculation of the flow rate in a glass capillary filled with milk. The flow was controlled by a perfusion pump. The preset flow rates are 32 μL/min, 25 μL/min, 20 μL/min, 15 μL/min, 10 μL/min, and 4 μL/min at Doppler angles 93°, 88°, and 86°.

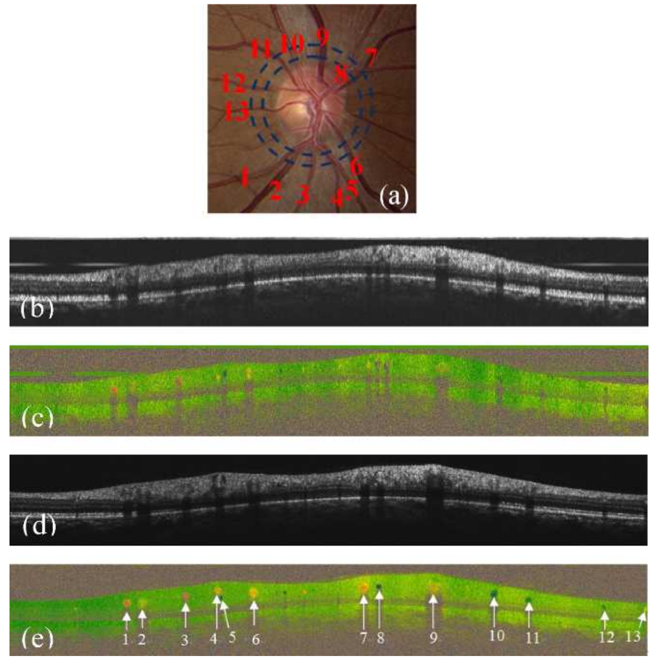

Figure 6. Retinal OCT/ODT images of the right eye of a normal human subject. (**a**) Fundus photograph where the positions of the major vessels are marked. (**b**, **c**) OCT and ODT images of OCT1 with a circular scan of 1.638 mm in radius. (**d**, **e**) OCT and ODT images of OCT2 with a circular scan of 1.365 mm in radius.

To calculate the Doppler angle the center of the capillary in the ODT images was selected manually. The average projected flow speed on the direction of the incident probing light and the absolute flow speed were calculated by using Equation 3 and Equation 4, respectively. The flow rate can then be calculated with Equation 5.

Figure 5 shows the experimental results. As we can see from Figure 5, the calculated flow rates were in good agreement with the set values. This experiment proved that we can accurately measure the flow rate, using this system.

Retinal Volumetric Flow Measurement

In human retinal imaging, a circular scan pattern consisting of 8192 sampling points centered at the optic disc was used. The scan radii were $r_1 = 1.638$ mm and $r_2 = 1.365$ mm for OCT1 and OCT2, respectively. During alignment we set the scan pattern as a horizontal line, which scans the probing beams across the center of the optic disc. When the alignment is satisfactory the acquisition mode is activated, which switches the scan pattern into a circle and acquires the OCT signals. The acquired OCT images were compared with the fundus photograph or OCT fundus image of the same eye (use vessel positions as landmarks) to ensure the location of the circular scan is correct. The total image acquisition time was 0.27 seconds.

The in vivo retinal flow measurement was performed on the right eye of a normal human subject (male, 48 years old). All the experiments were in compliance with the Declaration of Helsinki. The simultaneously acquired ODT images are shown in Figure 6. After bulk motion correction by using a histogram-based method,[12] the relative positions of the center of the blood vessels in the two ODT images were measured manually and used to determine the angle between the probing beam and the vessel. Then the measured Doppler angle, the average

Table 1. Measured Blood Vessel Parameters of the Major Retinal Arteries for Human Subject No. 1

Vessel No.	Doppler Angle, °	Diameter, μm	Velocity, mm/s	Flow Rate, μL/min
1	87.78	99	24.53	11.32
3	85.25	95	22.17	9.42
5	95.66	73	15.79	3.96
8	N/A	N/A	N/A	N/A
10	92.59	102	18.41	8.91
12	91.01	65	26.38	5.24

N/A, not applicable.

Table 2. Measured Blood Vessel Parameters of the Major Retinal Veins for Human Subject No. 1

Vessel No.	Doppler Angle, °	Diameter, μm	Velocity, mm/s	Flow Rate, μL/min
2	92.67	132	13.82	11.34
4	93.28	75	11.46	3.04
6	95.39	125	13.3	9.78
7	98.30	95	14.87	6.32
9	96.43	168	8.02	10.66
11	86.58	81	12.06	3.73
13	87.3	70	9.31	2.14

Doppler shift, and the vessel diameter were used to compute blood flow parameters in the vessel.

In this experiment, we chose the major vessels to measure the blood velocity and calculate the retinal flow rate. The measured parameters of the major arteries and veins are shown in Table 1 and Table 2, respectively. As we can see from Table 1, an artery bifurcation occurred in vessel No. 8 between the two circles. Therefore, we chose the major veins to calculate the total retinal flow rate. In this subject, the total retinal venous flow was calculated to be approximately 47.01 μL/min, which is in agreement with that measured by using laser Doppler flowmeter.[16–18]

The total retinal flow rate was also measured in the left eye of another normal subject (female, 34 years old). Through similar data analysis, the total venous flow rate was calculated to be 51.37 μL/min, which is also in the range measured by other researchers.[18]

Discussion

As shown in the experiments the dual-beam arrangement can successfully measure the Doppler angle except when a blood vessel bifurcates between the two scanned circles. In this situation, as a solution a second measurement with a different scan diameter will be necessary to make sure the flow rates for all the blood vessels can be calculated.

To calculate the Doppler angle accurately, determining the coordinates of the center of a blood vessel in each of the paired OCT/ODT images is critical. In our experiments we determined the coordinates manually, which is practical but may not be suitable for high-throughput applications. Determining the coordinates automatically with software is possible and will be investigated in the next step.

Owing to limited resources we did not use two identical spectrometers and light sources in the system, which made calibration of the coordination system a bit more complicated. Using two identical OCT systems we can have more uniform quality images and make the system more practical for diagnostic and research applications.

One possible weakness of the current arrangement is that the backscattered light is attenuated by 3 dB before it is collected by the single-mode optical fiber in the sample arm owing to the beam splitter used to couple the two probing beams. However, from the experimental results we can see that the imaging quality was not significantly affected. A possible solution for this weakness is to use two OCT systems working in different bands, for example, one in the 830-nm band and the other in the 1050-nm band. Then, a dichroic mirror can be used to couple the two probing beams, which may avoid the 3-dB attenuation.

Interindividual eye geometry variation is another possible source of error in future applications. This variation cannot be compensated unless measured with instruments, which is also the limit for all quantitative retinal image evaluations. However, the angle between the two probe beams is known. As a result, the distance between the two OCT images may be compensated when the refractive power of the eye is known or measured.

We used two OCT subsystems that were available in our laboratory, the imaging speed of which is not comparable with the current high-speed swept-laser OCT systems. However, there is no limit on the speed of the OCT systems that can be used in the dual-beam technique. By using higher-speed OCTs, better performance of the system, for example, higher measurable flow speed, can be expected.[19]

Conclusions

In summary, we successfully developed a novel dual-beam OCT/ODT system to measure the absolute retinal blood velocity and volumetric flow rate. By using two simultaneously acquired ODT images to calculate the Doppler angle the effect of eye motion is minimized. As a result, the system does not require ultrahigh speed imaging for avoiding eye motion artifacts. The system has been successfully applied to imaging normal human subjects. The test results have shown that the system can work accurately and reliably. Although more tests are necessary for further improvement, the technology is promising to provide more accurate and reliable measurements of retinal blood flow and thus be a valuable imaging tool for the research and clinical diagnosis of retinal diseases.

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