Three-beam Doppler optical coherence tomography using a facet prism telescope and MEMS mirror for improved transversal resolution

R. Haindl*, W. Trasischker, B. Baumann, M. Pircher and C.K. Hitzenberger

Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria

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An improved three-beam Doppler optical coherence tomography system was developed. It utilizes a custom-made three-facet prism telescope to improve the transversal resolution at the sample. Furthermore, a two-axis gimbal-less MEMS mirror is used to minimize off-pivot beam movement at the pupil of the eye, enabling circular scanning for in vivo retinal measurements. We demonstrate the system’s abilities for in vitro circular scanning to measure absolute flow and to reconstruct the full velocity vector on a bifurcation flow phantom. Moreover, in vivo retinal measurements using circular scanning around vessel bifurcations of healthy human volunteers were performed. Measurements of the absolute mean flow and its orientation are in good agreement with the expected values for in vitro measurements. For in vivo measurements, the in- and outflow of blood for retinal vessel bifurcations show an excellent agreement, demonstrating the reliability of the technique.

Keywords: optical coherence tomography; Doppler; flow measurement; multibeam; retina

1. Introduction

Since its development in the 1990s optical coherence tomography (OCT) [1] became particularly important in the field of ophthalmology. The transition from time domain to Fourier domain (FD-) OCT led to a considerable improvement of sensitivity and acquisition speed [2–4], aiding clinicians in diagnosis and monitoring major ocular diseases. Conventional FD-OCT generates images based just on backscattering intensity, which limits its use to structural images with sometimes insufficient tissue contrast and does not provide information on flow. Several functional extension, like polarization sensitive (PS-) OCT [5,6] and Doppler OCT (DOCT) [7–9] were developed to overcome these limitations.

Since major eye diseases such as glaucoma [10–12], age-related macular degeneration [13,14], and diabetic retinopathy [14–16] have been shown to be related to alterations in the retinal blood flow, its quantification is of particular interest. However, the quantification of absolute retinal blood flow still remains a challenge. Several approaches were published regarding the extraction of absolute flow. These approaches can roughly be divided into phase-sensitive and non-phase-sensitive methods.

Non-phase-sensitive techniques make use of time varying speckle [17] providing quantitative velocity information, with the restriction of not being able to determine the direction of the flow. Autocorrelation techniques [18,19] use statistical intensity fluctuations of backscattered light caused by moving particles to detect motion. In addition, dynamic light-scattering OCT [20] can deduce the axial and transverse components of the flow velocity, but requires long measurement times and high computational effort.

DOCT is a phase-sensitive approach providing additional contrast by exploiting the phase shift introduced by moving scatterers, such as blood cells, to calculate velocity and flow. Since DOCT only allows to measure the velocity parallel to the sampling beam, further information is necessary to reconstruct the absolute velocity vector of a moving scatterer and therefore gain access to absolute flow measurements. Both single and multibeam (MB-) DOCT approaches were reported, capable to measure absolute flow under certain assumptions.

Single-beam DOCT approaches often rely on the extraction of the 3-D vessel geometry to reconstruct the velocity vector. This can be achieved using the local vessel gradient at adjacent vessel cross-sections. The gradient can be obtained using circular scans with different radii [21] or high-speed FD-DOCT where volume scans can be used to extract vessel orientation, position, and diameter [22–24]. Other approaches divide the sample beam into several path length-encoded components with different group delays and Doppler angles by the insertion of a thickness-sectored glass plate into the OCT probe beam [25,26]. Furthermore, transversal DOCT utilizing a four-channel quadrant detector was reported to calculate the velocity vector [27]. However, the techniques using a
single Doppler angle acquire the vessel geometry from consecutive images; therefore, they are sensitive to sample motions in case of in vivo measurements. The path length-encoded methods depend on calibration measurements and suffer from reduced imaging depth.

Several MB-DOCT approaches, using two or more measurement beams, were also reported. Dave and Milner [28] illuminated an in vitro sample from two angles with orthogonally polarized light. Ittimia et al. [29] used two sample beams and two spectrometer units to image zebrafish larvae and acquired absolute flow rates in larger vessels. Werkmeister et al. [30,31] reported a dual beam DOCT system capable to perform human retinal blood flow measurements as long as the orthogonal vector of the plane, spanned by the two incident beams, and the vessel is close to 90°. Blatter et al. [32] utilized a rotating Dove prism in a dual beam setup to control the relative angle between illumination plane and vessel to assess absolute flow in retinal vessels in vivo using a circular scan pattern. Doblhoff-Dier et al. [33] used a dual beam DOCT system with an integrated Dynamic Vessel Analyzer and rotatable beams for vertical and horizontal line scan positions. However, an optimal orientation of the incidence plane with respect to the vessel’s angle cannot be guaranteed for dual beam setups without additional information on the vessel geometry. As of yet, none of these methods have found its way into clinical praxis [34].

Recently, we reported a three-beam phase-sensitive DOCT system [35] which enabled the reconstruction of the absolute velocity vector of moving scatterers without a priori knowledge of the vessel geometry. The method is based on illumination of the sample at three different angles with three independent measurement beams focused on one spot. While the basic functionality and promising first results could be demonstrated, the system had several shortcomings. Two major issues were the low transversal resolution of ~60 µm, due to the small beam diameter, and beam motion at the pupil of the eye, caused by off-pivot axis scanning with a galvo scanner pair with separate mirrors for X and Y scanning.

The purpose of this work is to present solutions to the above-mentioned shortcomings. We use a custom-made three-facet prism telescope (FPT) to reduce the beam separation at the scanning mirror, while maintaining the initial diameter of the beams. This provides a higher numerical aperture of the beams at the sample, which yields a better transversal resolution for retinal imaging. Additionally, a two-axis gimbal-less MEMS scanning mirror is used to considerably reduce the beam motion at the pupil of the eye. Consequently, the phase shift in the Doppler image was reduced, which makes circular scan patterns more feasible. We demonstrate the performance of the system by circular scan measurements on an in house designed in vitro flow bifurcation phantom of well-defined flow and in vivo for circular scans around vessel bifurcations in the retina of healthy human eyes.

2. Methods
Our three-beam DOCT system (Figure 1) is based on the spectral-domain (SD-) OCT principle [36–38]. Individual 840-nm SLD (Exalos Inc.) sources were used for each of the three channels. Hence, the system comprises three independent DOCT systems. The beams were collimated after exiting the source fiber and aligned in parallel to each other, with respect to a triangle geometry, setting the three beams on the corners of the triangle. When focused in one spot, the system provides sample illumination at three different angles, which enables the reconstruction of the absolute velocity/flow vector without a priori knowledge of the vessel orientation. A description of the interferometer part of the three-beam DOCT system and a detailed explanation on the alignment and the beam geometry to reconstruct the absolute velocity/flow vector can be found elsewhere [35]. In our setup, a 70/30 (R/T) bulk optics beam splitter (BS) was used for both, in vitro and in vivo measurements.

2.1 Interferometer setup
The first telescope in the sample arm of the original setup was replaced by an FPT (Figure 2) which consists

![Figure 1. Three-beam DOCT system. C – Miniature Fiber Collimator, FC – Fiber Collimator, L – Lens, G – Grating, BS – Beamsplitter, M – Mirror, NF – Neutral Density Filter, FPT – Facet Prism Telescope, T – Telescope, LS – Linear Stage, FL – Focusing Lens for in vitro measurements, SLD – Superluminescent Light Emitting Diode, CAM – Line Scan Camera. (The color version of this figure is included in the online version of the journal.)](image-url)
of a pair of custom-made three-facet prisms (FP) (Figure 3). The facets are designed as a pyramid with an equilateral triangle as a base. The angle of inclination between the base and the hypotenuses was 4.26°. The prisms were mounted in opposite directions with respect to each other, the tips of the pyramids coinciding with the common optical axis and pointed against each other, while one prism is rotated by 180° along this axis. The material used was N-BK7, coated with an anti-reflection coating to reduce losses. The beams enter the prism system on the flat surface of FP1, while the centroid of the beam geometry coincides with the optical axis. After passing the first surface unaltered, the beams are refracted on the second inclined surface of FP1. They remain collimated and intersect each other after a pseudo focal length \( f_p \). After the pseudo focus, the beams diverge until they are inversely refracted to the initial parallel traveling path by the inclined surfaces of FP2. Thus, the FPT does not change the basic parallel beam alignment according to the corners of the triangle nor the beam diameter. However, if the distance between the two FPs is not equal to \( 2f_p \), the beam spacing is altered. An FP distance smaller than \( 2f_p \) leads to decreased beam distance and vice versa. Maintaining the beam diameter provides a better resolution for sampling as compared to the original lens telescope which reduces the beam diameter proportional to the separation distance between the beams.

Since scanning is performed in an off-pivot condition for all three beams, a small beam separation centered around the pivot point considerably reduces the introduced phase shift in the Doppler image, caused by the scanning motion of the mirror. Therefore, it was necessary to reduce the beam separation to approximately one-fourth of the initial separation distance of 4.4 mm. The initial separation depends on the size of the used collimators C1–3 and on the in house designed brass monoblock mount. Since the dimensions of these components were fixed, the reduction was achieved introducing the FPT.

A two-axis gimbal-less MEMS mirror with 4.2 mm in diameter (Mirrorcle Inc., S2708) was used for scanning. The mirror was driven with high voltage ranging from 0 to 136 V, allowing maximal optical scanning angles of 16°. By providing different voltage inputs to the \( X \)- and \( Y \)-axis, linear, rastered volume, circular or resonant scan patterns can be realized. The usage of a MEMS mirror significantly reduces the effect of off-pivot scanning in comparison with a pair of galvo scanners.

The second telescope features an 80/30 magnification to set the final beam diameter to 1.3 mm, allowing a calculated lateral resolution of ~20 µm for retinal imaging. The separation distance of the three beams after the second telescope was approximately 3 mm. This provides a substantially different inclination when the beams are focused on the sample (~4° for retinal imaging), while it still allows to penetrate an undilated human pupil with all three beams. The system was used for in vitro measurements on a bifurcation flow phantom and for in vivo retinal imaging of healthy human eyes. An additional focusing lens was used for in vitro measurements.

### 2.2 Scan pattern and signal processing

Details of the signal processing routine for our three-beam DOCT system were reported in our previous paper \[35\]. Since a circular scan pattern was chosen and all beams are offset from the pivot point, the scanning motion of the MEMS mirror will introduce a phase shift in the Doppler data. For a circular scan, the \( X \)- and \( Y \)-axis of the 2-D gimbal-less MEMS mirror are actuated with a sine pattern of a given amplitude and a phase shift of 90° between the axes. Such motion will introduce a non-constant sine-like phase shift in the final circular B-scan. This phase shift needs to be subtracted before the Doppler data of the sample can be evaluated. To do so, a histogram-based method was used to determine the introduced phase shift within...
every A-scan. For in vitro measurements, a sheet of paper was imaged with the three-beam DOCT system using the same scanning parameters as for the actual measurements. The recorded phase offset caused by the scanner movement was evaluated for every A-scan. Thereby, it was possible to subtract the introduced phase offset for the following in vitro measurements. For in vivo measurements, the vessel-free region between the anterior boundary of the vessel and the inner limiting membrane was evaluated with the histogram-based method for every A-scan. The obtained reference pattern was then subtracted from the recorded in vivo phase data [22]. This method also corrects for additional phase shifts introduced by the motion of the subject. The phase shift was recorded and subtracted for the three channels individually and the velocities parallel to the axis of each incident beam were calculated from the corrected phase data [35].

The images of all circular B-scans obtained by the different channels need to be correlated with respect to their axial and transversal position to account for sample motion artifacts between B-scans. In order to calculate the absolute velocity vector, a rectangular region of interest (ROI) around the respective vessel/bifurcation branch was defined and extracted from the image. The extracted ROIs of all B-scans of all channels were correlated using a cross-correlation algorithm. This procedure was repeated for all vessels/bifurcation branches of interest. After signal processing, correlation and averaging, the absolute velocity vector was calculated [35]. The velocity components in the x, y, z directions were used to calculate the velocity magnitudes forming the velocity profile. This total velocity distribution was then averaged over the cross-section to obtain the mean total velocity value, which was then multiplied by the cross-sectional area of the vessel to obtain the total flow.

3. Results
To demonstrate the feasibility of the system using the FPT and a MEMS mirror to perform circular scans to determine total in-, out-, and mean flow from and to a vessel bifurcation, circular scans around an in house designed in vitro bifurcation flow phantom were performed. In a next step, in vivo circular scans around human retinal blood vessel bifurcations were conducted.

3.1. In vitro measurement
A custom in vitro bifurcation flow phantom (Figure 4) was fabricated out of a PMMA block. Separated by 120° the three branches of the bifurcation with a rectangular flow channel cross-section of 0.09 mm² were machined into the PMMA using a CNC mill. Finally, the open top was sealed with a thin N-BK7 glass window to guarantee a high optical quality for the OCT measurement. The phantom bifurcation was perfused with a scattering fluid (1:2 milk diluted with water) at a constant flow. An injection pump for medical applications was used (MGVG Combimat 2000, flow range 0.028–52.8 µl/s) for constant and accurate flow injection. To increase stability and homogeneity of the flow distribution, two branches of the bifurcation were used as an input and one branch as an output.

Circular scans were performed around the bifurcation phantom to provide a velocity vector component in every spatial direction of an orthogonal coordinate system. The flow was varied between 0.1 and 0.83 µl/s for each input branch resulting in mean velocities between 1.6 and 11.8 mm/s. For a circular B-scan, 4096 A-scans were recorded for every channel. Eight circular B-scans were averaged to generate noise-reduced images. Five of those
noise-reduced B-scans were used to analyze reproducibility and to calculate mean and standard deviation.

The absolute velocity vector profile was calculated for all branches of the bifurcation. The mean velocity was calculated from the velocity profile and multiplied with the branch area (measured using the OCT intensity image) to determine the absolute mean flow of the scattering fluid through every branch of the phantom.

The total measured in- and outflow was compared with the applied total in- and outflow to and from the bifurcation for various input flows (Figure 5). The equation of the linear fit was $y = 1.0488x - 0.2013$ with an $R^2$ of 0.9948. The measured flow agreed well with the applied flow for inflows greater than 0.22 µl/s (corresponding to a mean flow velocity of ~3 mm/s). The total flow was overestimated by approximately 20% for an input flow of 0.11 µl/s. This might be due to instabilities of the pump at low flow rates. Furthermore, as averaged component magnitudes were used to calculate total velocity profiles and the total flow, the phase noise of the system might have contributed to an overestimation of the total flow at lower flow velocities.

Because the full geometry of the 3-D flow was calculated, the calculated branch geometry was also compared to the physical branch geometry for different total applied flows (Figure 6). The measured branch geometry agreed very well with the physical branch geometry over a range of applied input flows.

Figure 6. Measured and physical branch geometry of the bifurcation flow phantom at different total applied flows. Dotted lines: Physical branch geometry, dots: Measured branch geometry. (The color version of this figure is included in the online version of the journal.)

Figure 7. (a) Color fundus photo. Black circular arrow indicates the direction and the path of the circular scan. White numbers: Vessel numbering. Black numbers: calculated vessel orientation from three-beam DOCT data. (b) Single circular B-scan for every channel, consisting of 4096 A-scans each. (c) Phase difference images from all three channels of vessel 1 after phase offset subtraction, phase unwrapping, and averaging over 10 B-scans. These images are overlaid to reconstruct the absolute velocity vector/profile (Figure 8). (The color version of this figure is included in the online version of the journal.)
3.2. In vivo measurement

For in vivo measurement, circular scans were performed around bifurcations of retinal vessels in healthy human eyes. During in vivo scanning, the combined power of the beams was set to 700 µW to agree with the laser safety standards [39,40]. Informed consent was obtained from all participating volunteers. The measured eye was aligned using a standard chin/headrest.

Four thousand and ninety-six A-scans were recorded for one circular B-scan. Ten B-scans were used for averaging over two cardiac cycles. After the ROIs for all B-scans around the vessels of the bifurcation obtained by the different channels were properly correlated with respect to each other, the absolute velocity vector profiles and the mean absolute velocities were calculated. The absolute mean flow was computed by extracting the vessel area from the intensity OCT image.

Figure 7(a) shows a fundus image of a healthy retina with vasculature around the optic nerve head (ONH). The black circular arrow indicates the direction of the circular scan. The tail of the arrow indicates the starting position of the B-scan. Two vessel bifurcations are covered by the scan: one bifurcation comprises vessel number 1, 3, and 4, the other comprises vessels number 2, 5, and 6. In Figure 7(b), the OCT intensity images of all three channels, imaged by the three-beam DOCT, are

Figure 8. From left to right: Reconstructed velocity profile inside vessel 2, 5, 6. (The color version of this figure is included in the online version of the journal.)

| Eye, Bifurcation | Outflow (µl/s) | Inflow (µl/s) | Inflow/Outflow |
|------------------|---------------|--------------|---------------|
| Eye 1, Bifurcation 1 | 0.51 | 0.20 | 0.32 |
| Total            | 0.51 | 0.52 | 1.02 |
| Eye 1, Bifurcation 2 | 0.38 | 0.23 | 0.15 |
| Total            | 0.38 | 0.38 | 1.00 |
| Eye 2            | 0.31 | 0.12 | 0.17 |
| Total            | 0.31 | 0.29 | 0.94 |
| Eye 3            | 0.12 | 0.07 | 0.06 |
| Total            | 0.12 | 0.13 | 1.08 |
shown. The white numbers link the vessels in the fundus image to the vessels in the intensity image. Figure 7(c) shows the averaged phase difference images of consecutive A-scans for every channel of the ROI for vessel 1. Figure 8 shows reconstructed absolute velocity profiles for vessel 2, 5, and 6 of the arterial bifurcation from Figure 7(a). The maximum detectable flow velocity was measured to be 55 mm/s, while the noise floor was measured at 2 mm/s.

Since the absolute 3-D velocity vector is calculated, the orientation of a vessel can easily be deducted from the spatial orientation of the vector. The black numbers and dotted arrows in Figure 7(a) show the calculated orientation and propagation direction of the flow. The calculated flow orientation was nearly identical to the vessel orientation in the fundus image.

Table 1 shows the measured mean absolute in- and outflow of retinal vessel bifurcations for four bifurcations of three healthy human eyes. The highest observed discrepancy between in- and outflow was 8%.

4. Discussion and conclusion
An improved three-beam DOCT system was developed. The system uses a novel FPT to improve the transversal resolution to approximately 20 μm and a two-axis gimbal-less MEMS scanning mirror to stabilize beam movement at the pupil of the eye, and thereby enables circular scan patterns. The functionality of the system was demonstrated in vitro and in vivo on an in house designed flow phantom bifurcation and on blood vessel bifurcations in healthy human eyes.

By performing circular scans around the flow phantom bifurcation, we demonstrated the reliability of the system to reproducibly measure absolute in- and outflow. Since the tube cross sections were extracted from the OCT data, the measurement also shows that no additional information on the vessel geometry, obtained by other imaging technologies (e.g. a fundus camera), is necessary to measure the absolute mean flow. Additionally we demonstrated that the reconstruction of the full 3-D velocity vector resembles the branch geometry and therefore the system’s capability to reliably reconstruct the flow orientation in 3 dimensions.

Applying the circular scan pattern to the human retina showed good agreement between in- and outflow of blood to and from vessel bifurcations in four bifurcations of three different healthy eyes. The measured mean absolute blood flow is well within the range of values reported in literature [22,30,35,41,42]. Furthermore, the orientation of the reconstructed absolute 3-D velocity vector agrees very well with the vessel orientation observed in the fundus photo. This indicates that the reconstruction of the absolute mean blood flow and the absolute velocity vector inside retinal vessels is reliable for circular scan patterns and that no a priori knowledge on the vessel geometry is necessary to measure these parameters.

The B-scans of the three channels need to be registered in an appropriate way to minimize errors both in the measured velocity/flow profiles and vessel orientations. Since the registration is performed automatically using a cross correlation algorithm operating on the intensity image, inaccurate registering might occur, influencing the accuracy of the resulting velocity profile. Deviations from correct registration can be caused by slightly different orientation of vessel cross sections in the different channels caused by the different angles of incidence of the probing beams at the sample. The different cross-section geometry can cause problems with image registration for certain vessel orientations, where the effect is pronounced. This problem may be accounted for by numerical compensation of the image distortion.

The final goal of this work is to perform circular scans around the ONH to measure total in- and outflow of all retinal vessels, a quantity important for glaucoma diagnostics. Currently, a circular scan around the ONH was not possible due to the limited scanning angle of the MEMS mirror, which needs to be replaced with a MEMS mirror allowing wider scan angles. Furthermore, the current cameras only allow an acquisition rate of 27 kHz which is too slow to measure the fast flow in the large arteries emerging from the ONH. In future work, they will be replaced by faster cameras. This will enable to resolve the higher flow velocity in large retinal arteries. After these changes, the three-beam DOCT system should be capable of measuring the total absolute in- and outflow from and to the ONH.

To summarize, we developed an improved three-beam DOCT system with increased resolution and the possibility to use circular scan patterns around retinal vessel bifurcations to measure absolute mean blood flow from and to the bifurcation. For all measurements, it was possible to reconstruct the 3-D absolute velocity vector and therefore, gain access to the vessel geometry measured by circular B-scans without an additional fundus image.

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