Visualizing the vasculature of the entire human eye posterior hemisphere without a contrast agent

MIRCEA MUJAT,* YANG LU, GOPI MAGULURI, YOUBO ZHAO, NICUSOR IFTIMIA, AND R. DANIEL FERGUSON

Physical Sciences, Inc., 20 New England Business Center, Andover, MA 01810, USA
*mujat@psicorp.com

Abstract: The platform described here combines the non-invasive measurement of the retina/choroid structure and ocular blood flow based on optical coherence tomography (OCT) and wide-field semi-quantitative global flow visualization using line-scanning Doppler flowmetry (LSDF). The combination of these two imaging modalities within the same platform enables comprehensive assessment of blood flow in the retina and choroid in animals and human subjects for diagnostic purposes. Ultra-widefield vasculature visualization is demonstrated here for the first time without injecting additional contrast agents and based only on the motion of particles within the vasculature.

1. Introduction

The leading causes of blindness worldwide including age-related macular degeneration (AMD), diabetic retinopathy (DR), and glaucoma exhibit vascular alterations that have been investigated for many years. The total number of cases for these three eye diseases in the U.S. (age 50 and older) grew from 8.03 million in 2000 to 12.48 million in 2010 and is expected to rise above 25 million by 2050 [1], with a devastating impact on the quality of life and a staggering economic burden on society [2]. The retina is a highly vascularized tissues and it represents the only part of the central nervous system where blood flow is visible and can be measured non-invasively. Examination of the retinal vasculature plays an important role in diagnosing eye diseases. However, a critical barrier in understanding the in vivo vascular involvement in these diseases is the lack of non-invasive methods for precise quantification of the vascular flow within the ocular structures.

Existing technologies, such as dye angiography and laser Doppler velocimetry (LDV) have a series of limitations. Although dye angiography is a powerful tool for global visualization of retinal vessel topology, occlusions and, uniquely, leakage, it provides no information on flow velocity. Fluorescein, generally used in ophthalmology to visualize retinal vasculature, and indocyanine green (ICG) used to visualize the choroid, are considered safe drugs; however, there are health risks associated with injecting contrast agents and both dyes can have adverse reactions, including nausea, vomiting, skin rash, or even death although very rare [3–6].

Riva et al. [7,8] were the first to introduced Doppler methods to retinal blood flow diagnostics. Initially, a laser beam focused on the retina was used to measure blood flow using laser Doppler flowmetry. Later, CCD fundus imaging (laser speckle imaging or flowgraphy) [9,10] and flying-spot confocal scanning laser Doppler flowmetry (e.g. Heidelberg Retinal Flowmeter, HRF) [11,12] were introduced as imaging modalities. At about the same time, time-domain Doppler optical coherence tomography (DOCT), also called optical Doppler tomography (ODT), was introduced to quantify the axial component of the blood flow [13–15]. Soon after that, the remarkable speed increase provided by spectral-domain OCT lead to the introduction of spectral-domain ODT (SDODT) for blood flow
measurement [16] which mostly replaced the slow time-domain version. Several useful strategies such as dual-beam DOCT [17–20] and narrow bandwidth phase-reference OCT [21] were reported to improve the sensitivity of DOCT. All of these methods are phase-sensitive, and therefore a phase-stable system is necessary for obtaining high-contrast images.

DOCT is highly dependent on the scanning beam angle, as flow cannot be detected in vessels perpendicular to the incident light beam, and small variation in the incident angle has a profound impact on the measured flow [22]. Furthermore, this method is also very sensitive to motion artifacts [23]. Two recently developed high-contrast in vivo 2-D/3-D microcirculation imaging techniques, with various implementations by multiple research groups, are speckle-variance (SV) and phase-variance (PV) OCT. SV-OCT images microvasculature by calculating the interline or interframe speckle variance of the intensity-based structural OCT images [24]. The PV-OCT method identifies the phase difference between consecutive B-scans of the same transverse position and allows for mapping the microvasculature [25,26]. A more recent implementation combining phase and intensity variance, OCT angiography (OCTA) was introduced into clinical practice [27,28]. However, the information provided by these maps is binarized into moving vs. non-moving locations, with no information on flow velocity or dynamics within vessels. Slow flow below the “moving” threshold and very rapid flow above the maximum threshold is marked as no flow, while any flow within the threshold range is marked as a vessel. Considering that metabolic demand of the tissue impacts vessel size and flow [29], precise quantification of flow is paramount to gauging in vivo tissue functionality, determining disease mechanism, progression, and response to treatment. Computational complexity, slow frame-rate, blood vessel shadowing, phase stability, and bulk tissue motion have been considered drawbacks for these techniques.

As described, the improvement in retinal flow visualization has been considerable, however, there is currently no imaging technique that can provide rapid ultra-wide area imaging of retinal and choroidal vasculature non-invasively (without dyes). In spite of decades of research and introduction of several commercially available instruments, retinal blood flow Doppler imaging did not achieve widespread clinical adoption due to critical issues in interpretability, reproducibility, and imaging speed.

The purpose of this research is to demonstrate a new approach for retinal and choroidal blood flow visualization and quantification that enables hemodynamic studies in animals (not shown here) and human subjects. The platform described here combines non-invasive measurement of retina/choroid structure and ocular blood flow based on OCT and wide-field semi-quantitative global flow visualization using line-scanning Doppler flowmetry (LSDF). OCT provides 3D structural information and precise local flow parameters while semi-quantitative LSDF flow visualization aids in characterizing global blood flow patterns.

The paper is organized as follows: the specifics of the imaging system are presented followed by data processing and results illustrating the multiple visualizations of the ocular vasculature provided by this multi-modal instrument. Additional comments are then presented pointing to unique features of this technique followed by conclusions.

2. The imaging system

The confocal line-scanning ophthalmoscope (LSO) and its semi-quantitative flow visualization variant LSDF have been previously described [30,31]. LSO uses line illumination and a linear detector for confocal retinal imaging. LSDF involves acquisition of multiple images of a stationary line of deeply penetrating coherent light on the retina. Temporal fluctuations arising from the motion (flow) of particles (erythrocytes) are detected with a confocal line detector at high line rates from 20 kHz to 92 kHz depending on maximum desired flow speed, while the dwell time on each line sets the minimum flow speed. As the line beam is slowly swept across the fundus, the motion of blood itself acts as the contrast agent, allowing the visualization of the vessels without dye. The fluctuations are
converted to frequencies via Fourier transformation to form a representation of the Doppler power spectrum, which in turn is related to the flow speed in the vessels.

Given the parallel nature of line scanning, LSDF is a significantly improved version of the commercially available technology based on point scanning laser Doppler flowmetry (e.g., Heidelberg Retinal Flowmeter, HRF [11,12]). With this novel imaging approach the retinal vasculature is visualized in a dye-free angiography mode. The accessible Doppler frequency range for blood flow speeds has been significantly expanded. The LSDF technique introduces a new, fast, and efficient approach to Doppler image capture. Our previous study [30] demonstrated significant improvements as compared to the commercially available HRF: 16 times the spatial resolution, and 4 times the temporal resolution, up to 10 times the accessible peak Doppler frequency, and detectable low frequency down to 14 Hz.

The LSDF imager uses a fixed cylindrical lens (top-right of Fig. 1) to generate a line of light which is scanned with a single galvanometer scanner and de-scanned to a line camera. The OCT imager uses a pair of galvanometer scanners to steer the OCT raster independently from LSDF. The LSDF and OCT optical paths are then combined at a dichroic beamsplitter D1. The common path proceeds to the scan lens group S and to an ophthalmoscopic lens (up to 66 D) which together with the eye optics relays the image plane to the retina. The LSDF galvanometer is a dichroic (D2) which reflects the LSDF light (850 nm) and transmits visible light for the pupil camera and the fixation display.

The LSDF/OCT imaging system is based on two main optical paths: LSDF and OCT imaging paths. The additional paths for pupil alignment camera and for fixation target are not discussed here for simplicity. These paths have been independently modeled in ZEMAX and exported as STP files for all subsequent optomechanical engineering steps. The LSDF illumination path is more difficult to visualize from the ZEMAX model line drawings, and is not included here; the layout is more easily grasped from the SolidWorks model on the left and top-right of Fig. 1. The LSDF path begins with the LSDF collimator (located on the back of the plate shown in Fig. 1) which produces a 10 mm beam. The LSDF beam is then passed with a turning mirror to the central section optical plate where a rotary mount holds a 15 mm focal length cylindrical lens which focuses the beam to a line near the aperture-separating strip mirror SM as shown in the top-right side of Fig. 1. This line is scanned by the LSDF galvo, reflected by the LSDF/OCT combining dichroic D1, and relayed by the scanning/imaging optics (and the eye optics) to the retina.

The strip mirror SM is a 2 mm width section of a 1 in. plane mirror that reflects the focused LSDF line beam and passes the returning reflection from retinal focal plane over the whole aperture. The LSDF scanner is conjugate to the ocular pupil (approximately 3 to 5 mm from the corneal surface) while the strip mirror is nearly conjugate to the corneal surface. This feature ensures that reflections from cornea are efficiently stopped by the strip mirror leaving as much as 80% of the collection aperture for gathering the reflected light from the retina, and therefore requiring no other means of eliminating unwanted reflections (e.g., polarizers). This arrangement requires that the incident beams were properly positioned with regard to the ocular entrance pupil: if not, bright interfering reflections are present in the LSDF image. In combination with the pupil camera, this lateral and axial guidance ensures uniform entry and exit condition for the beams with optimally flat LSDF and OCT scans.

The LSDF optical detection path shown schematically in the bottom-right of Fig. 1 begins from the eye model at right and proceeds through the VOLK 66D ophthalmic lens; through the front scan lens group S (achromat and negative meniscus); reflects from the LSDF/OCT beam-combining dichroic D1 and the LSDF scanning dichroic D2; passes the strip mirror SM to the line-scan focusing lens LS; and reflects from the turning mirror M to the line-scan camera (CCD).

The OCT imaging path consists of a triplet collimator C (Thorlabs - 25 mm focal length and ~5 mm beam diameter) and a pair of x-y galvo scanners SC (OCT H and V). The collimated beam passes through the LSDF/OCT beam-combining dichroic D1 and then to the
retina through the imaging optics common to the LSDF path. Triplet collimators were selected to maximize the back coupling over the OCT bandwidth. The OCT collimator C and the compound lens S define the focal plane to which the imaging path of the LSDF channel needs to be focused during instrument alignment to ensure that the depth range of the LSDF and the OCT channels overlap in the retina.

Fig. 1. Left: Central Section Plate - imaging, scanning, fixation display units; top right – cylindrical lens/strip mirror assembly; bottom right - LSDF Detection Path / OCT imaging optical path: S – scan lens, D1 – OCT/LSDF combining dichroic, D2 – LSDF scanner (dichroic), SM – strip mirror, LS – line-scan focusing lens, M – turning mirror, CCD – linear detector array (line-scan camera), C – OCT collimator, SC – OCT scanner assembly (OCT H, OCT V).

2.1 Human subjects and the imaging procedure

The LSDF/OCT imager developed by PSI has been tested on ten normal volunteers. A human subject protocol was approved by New England IRB and NASA IRB prior to imaging and all subjects gave informed consent to be imaged. The imaging sessions follow a set protocol including several stationary scans at different eccentricities combining LSDF imaging and OCT raster scans with multiple B-scan repetition and OCT double-circular scans around the optic disc for Doppler analysis. Only raster scans are analyzed and discussed here. Data from two healthy volunteers are shown in this paper: volunteer A – a 48 years old male, and volunteer B – a 27 year old male.

LSDF (850 nm laser diode) data cubes consisting of 450 lines repeated 128 times were acquired at a line rate of 60 kHz. These sampling parameters provide a minimum measurable frequency and the step in frequency sampling (Rayleigh frequency) of 0.47 kHz and a maximum measurable frequency (Nyquist frequency) of 30 kHz. Visualization 1 and Visualization 2 show the raw data for the two data cubes analyzed in Figs. 2 and 3. The data was rearranged to show an en-face visualization of the data cube considering the depth axis as time. Acquisition of the LSDF data cubes takes 0.96 s and each data cube was repeated ten times for post-processing averaging. The LSDF image covers an area of approximately 44.5°x35°. Nine image locations are typically selected in a 3x3 pattern with ~50% overlap between adjacent locations for building a large area mosaic. OCT (1060 nm) raster scans consisting of 200 B-scan locations (repeated 5 times) of 500 A-lines (cropped in processing to 437 A-lines) were acquired at 70 kHz line rate. OCT raster scans cover approximately 35°x35° area. Acquisition of the OCT raster takes ~7 s, slightly shorter than the acquisition of
the ten LSDF data cubes. The LSDF and the OCT data were acquired quasi-simultaneously and they have a significant spatial overlap. Average of repeated B-scans provides noise reduction, while inter-frame SV provides 3D visualization of the blood vessels (typical OCTA). Layer segmentation can be used to generate thickness maps for retinal nerve fiber layer (RNFL), retina, and choroid.

3. Data processing and results

3.1 LSDF

It is important to notice in Visualization 1 and Visualization 2 that the intensity/speckle fluctuations vary across the image and strong fluctuations seem to occur along the apparent vasculature as well outside of it. Looking at areas that seem to have no structure in still images, one can notice certain patterns in the movies that suggest local motion corresponding to the choroidal flow as it will be explained below in more details. In Visualization 2, the strongest fluctuations occur in the top central region that corresponds to an important choroidal structure, a vortex vein as detailed below.

The LSDF data is analyzed following the standard Doppler flowmetry procedure that outputs images of Flow, Velocity, and Volume as defined in the literature [32,33]. In standard LDV, a time trace of intensity fluctuations in each pixel of the image is recorded and processed. A Doppler power spectrum is obtained by Fourier transformation and the Velocity of the blood flow is estimated from the power spectrum as the mean frequency (in Hz) within the measured frequency range. Volume is calculated as the total power of the intensity fluctuations (area under the power spectrum curve not including the DC (the first point of the spectrum) normalized to the DC value) and is generally assumed to be proportional to the number of moving particles within the imaged pixel. Flow is simply the product of Velocity and Volume. Flow and Volume are given in arbitrary units. The measurements seem to depend on a lot of uncontrolled factors, are operator dependent, and cannot reliably support longitudinal studies on the same eye or comparisons among individuals [34–37]. For these reasons, maps of Velocity, Volume, and Flow should be regarded as relative, semi-quantitative visualization methods of retinal vasculature without a contrast agent. Even if our line-scanning version of LDV is significantly faster, covers a larger area, and enables measurement of larger velocities, it still suffers from some of the disadvantages of the standard LDV. However, our LSDF technique, due to its advantages over standard LDV, provides a retinal and choroidal vasculature visualization method of comparable quality to ICG angiography but without injection of the contrast agent.

The average of the intensity fluctuations in each pixel provides the overall reflectivity of the tissue at that location, i.e. a typical LSO image. The two examples of the reflectivity image shown in the top row of Fig. 2 were obtained from two retinal locations of volunteer A. The left column in Fig. 2 shows images centered on the optic disc and the right column illustrates a vortex vein area close to the eye equator. The center row in Fig. 2 shows the Velocity map and the bottom row shows the Volume map. The Flow map which is just the multiplication of Velocity and Volume is not shown since it brings no new information in terms of vasculature visualization.

In an alternative visualization method, the power spectrum is split in three frequency ranges, the power is integrated in these ranges, and three images associated to slow, medium, and fast flow are generated [30]. Figure 3 shows these three images for the same retinal locations as in Fig. 2, which were actually obtained from the same scan data but with this alternative processing method. The advantage of this visualization method is that it can selectively highlight flow in a specific frequency/velocity range.
3.2. OCT

The standard steps in OCT processing are initially performed: zero-padding, interpolation, dispersion compensation, and discrete Fourier transform (DFT). The modulus and the argument of the complex DFT are extracted and used for different purposes: the modulus is used for the intensity image; the argument is used for phase processing in circular scans for Doppler analysis. Each group of 5 B-scans is processed by aligning the 5 images through cross-correlation to remove motion artifacts. The average and the standard deviation images are calculated for these 5 images in the overlap region. Several algorithms have been published over the last few years for OCTA processing [38–41]. The average B-scans are combined in a 3D volume which is further processed.
We used retinal layers. This step removes the curvature of the retina and corrects motion artifacts. Several images are then generated based on this flattened data set. The top surface of the retina is identified and the retina thickness (defined from the top of the RNFL to the RPE) map is generated (Fig. 4(F)). Four reflectivity maps are generated by integrating the OCT signal: as overall reflectivity (Fig. 4(A)), above the RPE (basically RNFL reflectivity, Fig. 4(B)), at the RPE level (a shadowgram of the retinal blood vessels, Fig. 4(C)), and below RPE (choroid reflectivity, Fig. 4(D)). The standard deviation for the repeated B-scans was calculated followed by integration above the RPE (basically including only the RNFL vasculature) generating a 2D map. A threshold set at the mean plus one standard deviation of the entire
map was used to binarize this 2D map and produce the OCTA image shown in Fig. 4(E). The OCT results shown in Fig. 4 were obtained at the same location as the LSDF scan analyzed in the left column of Figs. 2 and 3.

Fig. 4. OCT en-face maps: A – integrated OCT, B – RNFL reflectivity, C – RPE reflectivity, D - choroid reflectivity, E - RNFL OCTA, F – retina thickness (colormap in microns). Image size: 34.3°x35° which for a normal eye (~290 μm²) corresponds to 9.95x10.15 mm².

Figure 5 shows a large area montage of nine individual scans obtained non-mydriatic from volunteer B. The top right image illustrates the segmentation results for the top of the retina and the choroid-sclera interface. The RPE (top left) and choroid (center right) reflectivity and
RNFL OCT (bottom right) maps are also shown in Fig. 5. A typical ~35° B-scan crossing through the optic disc and fovea is illustrated in the center left image. The bottom left image in Fig. 5 shows the OCT scan area with respect to the larger montage of the Doppler flowmetry Velocity map. The OCT montage covers approximately 70°x70° area while the LSDF scan covers approximately 80°x80°.

![Fig. 5. Large area montage of nine individual scans illustrating various display modalities: top-left - RPE map, top-right – B-scan illustrating specific landmarks (BV – blood vessels), center-left – typical large size B-scan, center-right – choroid map, bottom-left – Velocity map highlighting the OCT scan area, bottom-right – OCTA map.](image)

The data shown in Figs. 2 and 3 represent two patches from a larger data set. Figures 6 and 7 illustrate ultra-widefield montages of the retinal and choroidal vasculature covering almost the entire posterior hemisphere of both eyes of volunteer A. The image size in Fig. 6 is 130°x115° and in Fig. 7 is 130°x111°.
Fig. 6. Ultra-widefield Velocity montage of the left eye of volunteer A illustrating visualization of vortex veins without injecting contrast agent. Frequency range: 0.47 – 14 kHz.

Fig. 7. Ultra-widefield Velocity montage of the right eye of volunteer A.
4. Discussions

The retinal circulation originates from the central retinal artery that passes through the optic nerve head (ONH) before branching into superior, inferior, nasal, and temporal arteries, into many smaller vessels, and ultimately, into capillary networks. The returning retinal blood is collected through a network of veins back into the central retinal vein passing through the ONH. The retinal vasculature is visualized and easily identified in Figs. 4 and 5 with the help of OCT raster scans. Registration of the OCT and LSDF scans as shown in Fig. 5 allows for identification of the retinal vasculature in the LSDF visualization and its separation from the choroid vasculature.

The underlying choroidal vessels and choriocapillaris beneath the RPE account for approximately 90% of the blood flow nourishing the retina [43]. All the incoming choroid blood comes in through the short posterior ciliary arteries (SPCA) located around the ONH and two long posterior arteries (LPA). These arteries are difficult to image with OCT because of their proximity to ONH (for SPCA’s) where the tissue is thick and the depths of their scleral branches (for LPA’s). However, LSDF can image these arteries as seen in Figs. 2-3 and 6−7. Many vessels can be seen in these imaging originating right at the edge of the ONH. These are SPCA’s. They come into the eye along the surface of the optic nerve (perpendicular to the image) and then turn about 90° and spread in the choroid (roughly parallel to image plane). On the surface of the optic nerve they are too deep in the tissue to be seen and they become visible in the LSDF images as they turn into the choroid. One can also see to the left and the right of the ONH some bright clusters of vessels that seem to originate from nowhere. They are branches of the LPA’s that penetrate from the sclera into the choroid and spread across the choroid.

The outgoing blood is collected by four or more vortex (or vorticose) veins. The vortex veins are farther apart (nearly equatorial in the globe) and also difficult to access. The feeding trees of the vortex veins have been visualized with ICG angiography (~800 nm) [44] without information of flow characteristics; however, the method proposed here uses no additional contrast agent and is only based on the motion of flowing particles. The squid-like structures seen in the right column of Figs. 2-3, top-right corner of the Velocity map in Fig. 5 and at the top and bottom of Figs. 6-7 are vortex veins. The back of the “head” (opposite to the “tentacles”) blurs out in these images as the vortex vein goes deeper into the sclera. Three of the four vortex veins in Fig. 6 (one on top-left and the two at the bottom) and one on the bottom-left in Fig. 7 seem to consist of two “squids”. They are actually branches of the same vortex vein that connect deeper into the sclera.

One main difference between the left/right columns in Figs. 2-3 and between the center/periphery in Figs. 6-7 is that there are more RNFL vessels in the left and center regions vs. right and periphery and it looks like that those images are sharper. In fact, choroidal vessels are blurrier than the RNFL vessels in all images/regions. The RPE and the upper layers of the retina add significant scattering (like looking through a shower curtain) and therefore imaging the choroid suffers from this unavoidable blurring due to additional light scattering in tissue not present in imaging the RNFL vessels.

Both OCT and LSDF as used here should be viewed as imaging platforms. By changing the scanning pattern and the data processing and display modality, one can obtain different imaging characteristics with no hardware modifications. For example, in the line-scanning configuration (typical LSO), large area confocal reflectance images of the retina are obtained by rapidly scanning the illuminating line over the retina. Flow data is obtained in the same configuration by switching the fast scan to a slow scan modality in which the line is stationary for a set period of time before is shifted to the next position. In this way, the temporal evolution of intensity fluctuations is recorded at each line position. A simple change in scanner control, data processing, and display provides complementary information.

Similarly, rapid scanning in a raster pattern of the OCT beam provides a 3D structural representation of the retina and choroid. If the scanning pattern is changed such that multiple
B-scans are recorded at the same location, speckle variance at each pixel along the depth profile can be calculated for these multiple B-scans. Stationary tissue exhibits little speckle changes, while the speckle corresponding to blood flow regions varies rapidly depending on the flow speed, generating a large SV. This is the principle of the SV technique or OCTA. SV was developed as a simple and robust depth resolved visualization technique for blood flow and is in fact a simple change in scanning pattern from the standard OCT configuration. One drawback of SV is that it does not provide the direction or the velocity of the flow. This can be resolved however by another simple data processing technique: phase-sensitive OCT. Standard OCT processing provides both amplitude and phase through the Fourier transform process. The amplitude is used for generating the reflectivity profile while the phase can be used for Doppler analysis. The phase difference between pairs of A-lines can be used for calculating the depth-resolved axial flow velocity component. The main requirement for this technique to work is that enough overlap is provided for the pair of A-lines, which is actually satisfied in the SV technique. However, for some modalities and flow ranges, B-scan Doppler may be preferred, depending on required speed and area coverage.

It should be noted that various tradeoffs must be made between different imaging configurations. Rapid scanning for large area structural information does not provide enough overlap for SV and phase-sensitive Doppler analysis, while these last two techniques are slower and can only be done over limited areas. What we propose here is to use large area scanning LSDF to visualize and identify the main retinal landmarks and vasculature structure and large OCT raster scans to collect 3D structural information. Based on these maps, more sensitive and specific techniques such as DOCT can be used at selected locations for flow quantification. All these can be done by selecting the appropriate scan patterns, data processing, and display algorithms. Essential for this procedure is the complementarity provided by a common OCT and LSDF optical path with quasi-simultaneous and independent scan control.

LSDF uses coherent illumination and interference between many scattered photons along the entire depth of the beam illumination. Flowing particles induce Doppler shifts with frequencies that depend on the speed of the moving particles. Isotropic scattering and multiple scattering tend to randomize the light field. Single backscattering and forward scattering (most probable for blood) back-reflected by other stationary structures can preserve some relationship between the scalar velocity of an ensemble of particles moving in a certain direction and the measured speckle intensity fluctuation frequencies (Doppler power spectra). However, given the long coherence length of the laser diode used for illumination there is no depth selectivity in LSDF.

LSDF has a number of important advantages for flow visualization: a) inter-modulation relatively insensitive to bulk motion; b) the motion of blood itself acts as the contrast agent, allowing the visualization of the vessels without dye injection; c) simple data analysis – Fourier analysis of temporal speckle fluctuations; d) relatively independent of flow direction and therefore sensitive to flow orthogonal to the laser beam (OCT cannot detect flow orthogonal to the laser beam). The main disadvantages of LSDF are: i) it is a statistical method and therefore semi-quantitative, and ii) it has no depth selectivity. However, both of these disadvantages can be complemented by OCT in a dual-platform instrument as discussed here.

5. Conclusion

Retinal and choroidal blood flow can be identified and quantified using these two complementary imaging modalities (LSDF and OCT). An advanced diagnostic imaging system empowered by this combined modality is fundamental to understanding hemodynamic processes in the eye.

The combination of these two imaging modalities within the same imaging platform enables comprehensive assessment of blood flow in retina and choroid. By using LSDF as
global semi-quantitative (relative) flow visualization technique and OCT as a local quantitative probe with adaptable velocity scale, this combination has the potential to more efficiently characterize regional net flow.

**Funding**

National Aeronautics and Space Administration (NASA) (NNX16CC20C).

**Disclosures**

MM: PSI (E), YL: PSI (E), GM: PSI (E), YZ: PSI (E), NI: PSI (E), RDF: PSI (E, P).

**References**

1. “Statistics and data” (National Eye Institute, 2018), retrieved November 16, 2018, https://nei.nih.gov/eyedata.
2. D. B. Rein, P. Zhang, K. E. Wirth, P. P. Lee, T. J. Hoerger, N. McCall, R. Klein, J. M. Tielsch, S. Vijan, and J. Saudtine, “The economic burden of major adult visual disorders in the United States,” Arch. Ophthalmol. 124(12), 1754–1760 (2006).
3. R. Benya, J. Quintana, and B. Brundage, “Adverse reactions to indocyanine green: a case report and a review of the literature,” Cathet. Cardiovasc. Diagn. 17(4), 231–233 (1989).
4. J. Bjerregaard, M. P. Pandia, and R. A. Jaffe, “Occurrence of severe hypotension after indocyanine green injection during the intraoperative period,” A A Case Rep. 1(1), 26–30 (2013).
5. W. Chu, A. Chennamsetty, T. J. Hoerger, N. McCall, R. Klein, J. M. Tielsch, S. Vijan, and J. Saudtine, “The economic burden of major adult visual disorders in the United States,” Arch. Ophthalmol. 124(12), 1754–1760 (2006).
6. R. Benya, J. Quintana, and B. Brundage, “Adverse reactions to indocyanine green: a case report and a review of the literature,” Cathet. Cardiovasc. Diagn. 17(4), 231–233 (1989).
7. J. Bjerregaard, M. P. Pandia, and R. A. Jaffe, “Occurrence of severe hypotension after indocyanine green injection during the intraoperative period,” A A Case Rep. 1(1), 26–30 (2013).
8. W. Chu, A. Chennamsetty, T. J. Hoerger, N. McCall, R. Klein, J. M. Tielsch, S. Vijan, and J. Saudtine, “The economic burden of major adult visual disorders in the United States,” Arch. Ophthalmol. 124(12), 1754–1760 (2006).
9. R. Benya, J. Quintana, and B. Brundage, “Adverse reactions to indocyanine green: a case report and a review of the literature,” Cathet. Cardiovasc. Diagn. 17(4), 231–233 (1989).
10. J. Bjerregaard, M. P. Pandia, and R. A. Jaffe, “Occurrence of severe hypotension after indocyanine green injection during the intraoperative period,” A A Case Rep. 1(1), 26–30 (2013).
11. W. Chu, A. Chennamsetty, T. J. Hoerger, N. McCall, R. Klein, J. M. Tielsch, S. Vijan, and J. Saudtine, “The economic burden of major adult visual disorders in the United States,” Arch. Ophthalmol. 124(12), 1754–1760 (2006).
23. S. Makita, Y. Hong, M. Yamanari, T. Yatagai, and Y. Yasuno, “Optical coherence angiography,” Opt. Express 14(17), 7821–7840 (2006).
24. A. Mariampillai, B. A. Standish, E. H. Moriyama, M. Khurana, N. R. Munce, M. K. K. Leung, J. Jiang, A. Cable, B. C. Wilson, I. A. Vitkin, and V. X. D. Yang, “Speckle variance detection of microvasculature using swept-source optical coherence tomography,” Opt. Lett. 33(13), 1530–1532 (2008).
25. J. Fingler, C. Readhead, D. M. Schwartz, and S. E. Fraser, “Phase-contrast OCT imaging of transverse flows in the mouse retina and choroid,” Invest. Ophthalmol. Vis. Sci. 49(11), 5055–5059 (2008).
26. J. Fingler, R. J. Zawadzki, J. S. Werner, D. Schwartz, and S. E. Fraser, “Volumetric microvascular imaging of human retina using optical coherence tomography with a novel motion contrast technique,” Opt. Express 17(24), 22190–22200 (2009).
27. R. F. Spaide, J. G. Fujimoto, N. K. Waheed, S. R. Sadda, and G. Staurenghi, “Optical coherence tomography angiography,” Prog. Retin. Eye Res. 64, 1–55 (2018).
28. A. H. Kashani, C. L. Chen, J. K. Gahm, F. Zheng, G. M. Richter, P. J. Rosenfeld, Y. Shi, and R. K. Wang, “Optical coherence tomography angiography: A comprehensive review of current methods and clinical applications,” Prog. Retin. Eye Res. 60, 66–100 (2017).
29. J. Kiryu, S. Asrani, M. Shahidi, M. Mori, and R. Zeimer, “Local response of the primate retinal microcirculation to increased metabolic demand induced by flicker,” Invest. Ophthalmol. Vis. Sci. 36(7), 1240–1246 (1995).
30. R. Ferguson, D. Hammer, A. Elsner, R. Webb, S. Burns, and J. Weiter, “Wide-field retinal hemodynamic imaging with the tracking scanning laser ophthalmoscope,” Opt. Express 12(21), 5198–5208 (2004).
31. D. X. Hammer, R. D. Ferguson, T. E. Ustun, C. E. Bigelow, N. V. Ifimia, and R. H. Webb, “Line-scanning laser ophthalmoscope,” J. Biomed. Opt. 11(4), 041126 (2006).
32. C. E. Riva, M. Geiser, and B. L. Petrigr, “Ocular blood flow assessment using continuous laser Doppler flowmetry,” Acta Ophthalmol. 88(6), 622–629 (2010).
33. M. H. Geiser, U. Diermann, and C. E. Riva, “Compact laser Doppler choroidal flowmeter,” J. Biomed. Opt. 4(4), 459–464 (1999).
34. K. Gugleta, S. Orgül, I. Flammer, D. Gherghel, and J. Flammer, “Reliability of confocal choroidal laser Doppler flowmetry,” Invest. Ophthalmol. Vis. Sci. 43(3), 723–728 (2002).
35. E. Polska, K. Polak, A. Luksch, G. Fuchsberger-Mayrl, V. Petternel, O. Findl, and L. Schmetterer, “Twelve hour reproducibility of choroidal blood flow parameters in healthy subjects,” Br. J. Ophthalmol. 88(4), 533–537 (2004).
36. K. Strenn, R. Menapace, G. Rainer, O. Findl, M. Wolzt, and L. Schmetterer, “Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in PO2,” Br. J. Ophthalmol. 81(5), 360–364 (1997).
37. A. Wang, M. Yuan, M. J. Byrnes, and A. Ingerman, “Assessment of Choroidal Blood Flow Parameters Using Flom-S Laser Doppler Flowmeter: Reproducibility and Effect of Intense Light Exposure,” Invest. Ophthalmol. Vis. Sci. 51, 5038–5038 (2010).
38. A. Zhang, Q. Zhang, C.-L. Chen, and R. K. Wang, “Methods and algorithms for optical coherence tomography-based angiography: a review and comparison,” J. Biomed. Opt. 20(10), 109901 (2015).
39. A. S. Nam, I. Chico-Calero, and B. J. Vakoc, “Complex differential variance algorithm for optical coherence tomography angiography,” Biomed. Opt. Express 5(11), 3822–3832 (2014).
40. J. Barton and S. Stromski, “Flow measurement without phase information in optical coherence tomography images,” Opt. Express 13(14), 5234–5239 (2005).
41. S. S. Gao, Y. Jia, M. Zhang, J. P. Su, G. Liu, T. S. Hwang, S. T. Bailey, and D. Huang, “Optical Coherence Tomography Angiography,” Invest. Ophthalmol. Vis. Sci. 57(9), OCT27–OCT36 (2016).
42. M. Mujat, R. Chan, B. Cense, B. Park, C. Joo, T. Akkin, T. Chen, and J. de Boer, “Retinal nerve fiber layer thickness map determined from optical coherence tomography images,” Opt. Express 13(23), 9480–9491 (2005).
43. J. J. Weiter, R. A. Schachar, and J. T. Ernest, “Control of intraocular blood flow. I. Intraocular pressure,” Invest. Ophthalmol. 12(5), 327–331 (1973).
44. K. Ohno-Matsui, N. Morishima, T. Teramatsu, T. Tokoro, and T. Nakagawa, “The long-term follow-up of a highly myopic patient with a macular vortex vein,” Acta Ophthalmol. Scand. 75(3), 329–332 (1997).