In vivo optical imaging of human retinal capillary networks using speckle variance optical coherence tomography with quantitative clinico-histological correlation


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Abstract: Retinal capillary networks are critically linked to neuronal health and disease. The ability to perform accurate in vivo examination of human retinal capillary networks is therefore valuable for studying mechanisms that govern retinal homeostasis and retinal vascular diseases. Speckle variance optical coherence tomography (svOCT) is a non-invasive imaging technique that has the capacity to provide angiographic information about the retinal circulation. The capability of this technology with respect to quantifying capillary network information has however not been validated. This report utilizes a custom-built svOCT device to qualitatively and quantitatively study the various capillary networks in the human perifovea. A prototype svOCT system based on a wavelength-swept source engine, with real time processing of OCT intensity data, was used to generate en face images of retinal capillary networks. Capillary networks corresponding to the nerve fibre layer (NFL), the retinal ganglion cell/superficial inner plexiform layer (RGC/sIPL), the deep inner plexiform layer/superficial inner nuclear layer (dIPL/sINL) and the deep inner nuclear layer (dINL) were imaged in 9 normal human subjects. Measurements of capillary diameter and capillary density measurements were made from each of these networks and results were compared to our previously published histological data. We found that svOCT images of capillary networks were morphologically comparable to our previous histological data. Using svOCT we found that capillaries in the NFL network ran parallel to the direction of RGC axons while capillaries in the dINL network demonstrated a planar configuration with multiple closed loops. Capillaries in remaining networks were convoluted with a complex three-dimensional architecture. For all networks, capillary diameter was significantly greater in svOCT images compared to histology. We did not find a statistical difference in capillary density measurements between svOCT and histology for all networks. The results of this study suggest that in vivo svOCT imaging allows accurate morphometric assessment of capillary networks in the human perifovea. Therefore, svOCT may have broad clinical applications in ophthalmic practice. Further work is required to validate the utility of this device for managing patients with retinal microvascular diseases such as diabetic retinopathy.

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Dear Editor

Please consider this manuscript “Validating the utility of speckle variance optical coherence tomography for quantitatively analysing human perifoveal capillary networks” for publication in Experimental Eye Research.

I can confirm that this manuscript is not under consideration by any other journal.

Yours sincerely,

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Highlights:

- Perifoveal capillary network imaging was compared between an svOCT device and histology
- In vivo svOCT allows accurate morphometric assessment of capillary networks
- svOCT provides real-time angiographic information about the retinal circulation
Title: Validating the utility of speckle variance optical coherence tomography for quantitatively analysing human perifoveal capillary networks

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Abstract

Retinal capillary networks are critically linked to neuronal health and disease. The ability to perform accurate in vivo examination of human retinal capillary networks is therefore valuable for studying mechanisms that govern retinal homeostasis and retinal vascular diseases. Speckle variance optical coherence tomography (svOCT) is a non-invasive imaging technique that has the capacity to provide angiographic information about the retinal circulation. The capability of this technology with respect to quantifying capillary network information has however not been validated. This report utilizes a custom-built svOCT device to qualitatively and quantitatively study the various capillary networks in the human perifovea. A prototype svOCT system based on a wavelength-swept source engine, with real time processing of OCT intensity data, was used to generate en face images of retinal capillary networks. Capillary networks corresponding to the nerve fibre layer (NFL), the retinal ganglion cell/superficial inner plexiform layer (RGC/sIPL), the deep inner plexiform layer/superficial inner nuclear layer (dIPL/sINL) and the deep inner nuclear layer (dINL) were imaged in 9 normal human subjects. Measurements of capillary diameter and capillary density measurements were made from each of these networks and results were compared to our previously published histological data. We found that svOCT images of capillary networks were morphologically comparable to our previous histological data. Using svOCT we found that capillaries in the NFL network ran parallel to the direction of RGC axons while capillaries in the dINL network demonstrated a planar configuration with multiple closed loops. Capillaries in remaining networks were convoluted with a complex three-dimensional architecture. For all networks, capillary diameter was significantly greater in svOCT images compared to histology. We did not find a statistical difference in capillary density measurements between svOCT and histology for all networks. The results of this study suggest that in vivo svOCT imaging allows accurate morphometric assessment of capillary
networks in the human perifovea. Therefore, svOCT may have broad clinical applications in ophthalmic practice. Further work is required to validate the utility of this device for managing patients with retinal microvascular diseases such as diabetic retinopathy.

**Key words:** Retina, capillary, perifovea, vasculature, speckle variance OCT, confocal microscopy
1. **Introduction**

The human retinal capillary circulation is an architecturally complex collection of vascular networks that are responsible for satisfying the non-uniform energy demands of layered neuronal populations (Chan et al., 2012; Chan et al., 2013; Snodderly et al., 1992). The specific organization and density of these networks permit instantaneous delivery of energy substrates to metabolically intense structures without significantly altering the optical properties of the eye (Nelson et al., 2011; Tan et al., 2012). Our previous histological report quantified and documented the morphological features of different capillary networks in the perifovea (Chan et al., 2012). We demonstrated significant anatomic heterogeneity between capillary networks implicating important relationships with respect to neurovascular co-patterning and functional crosstalk in the human retina.

As the retina is supplied by an end-arterial system without anastomoses, it is especially vulnerable to ischemic injury (Bek, 2009; Kaur et al., 2008). Experimental studies by Hayreh and colleagues have shown that the tolerance of retinal neurons to ischemic injury is significantly less than neurons in other central nervous system compartments (Hayreh and Weingeist, 1980). In the presence of cardiovascular disease we have shown that the perifoveal circulation is altered in a non-uniform manner with selective capillary networks being more susceptible to hemodynamic perturbation than others (Chan et al., 2013). Similar mechanisms may be pathogenically relevant to conditions such as diabetic retinopathy and retinal vascular occlusion –diseases that are major causes of visual morbidity worldwide.

Fluorescein angiography (FA) has broad clinical application in the management of retinal vascular diseases (Novotny and Alvis, 1961). A major drawback of FA however includes its invasive nature necessitating the administration of fluorescein dye. Intravenous fluorescein administration is associated with a large number of minor side effects and also a very small
but significant risk of anaphylaxis and death (Bearelly et al., 2009; Yannuzzi et al., 1986). Furthermore, a clinico-histological correlation study performed in our laboratory demonstrated that FA has only limited ability to resolve and provide quantitative information about retinal capillary networks (Mendis et al., 2010). Speckle variance optical coherence tomography (svOCT) is an imaging modality that is gaining increasing popularity for the study of retinal vascular anatomy and disease (Mariampillai et al., 2010), along with other label-free OCT based flow contrast techniques (Wang et al., 2010; Fingler et al., 2009; Jia et al., 2012; Braaf et al., 2013). svOCT is a non-invasive technique based on red blood cell movement that is capable of providing real-time angiographic information about retinal capillary networks without the administration of intravenous dye (Mahmud et al., 2013; Xu et al., 2014). For these reasons, svOCT is potentially an attractive tool with a myriad of clinical applications. In this report, we use a prototype svOCT device to quantitatively and qualitatively study perifoveal capillary networks in healthy human eyes. We first illustrate how this device has the capacity to image and isolate distinct capillary networks in the perifovea. Quantitative measurements derived from this device are then validated by making comparisons between svOCT and age-matched histology data. These results are used to illustrate the advantages and limitations of this device for studying retinal vascular diseases.

2. Materials and Methods

This study was approved by the human research ethics committee at The University of Western Australia and The University of British Columbia. All live-patient imaging was performed at the Eye Care Centre in Vancouver. All human tissue was handled according to the tenets of the Declaration of Helsinki.

2.1. Live Human Subjects
Seventeen eyes from 9 healthy human subjects, aged between 28 and 60, were imaged using speckle variance OCT. In this study, the group from which speckle variance OCT measurements were made are referred to as the 'svOCT' group and eyes from the confocal microscopy images as the 'histology' group.

**Human Donor Eyes**

Eleven human eyes from 8 donors, aged between 22 and 66, were used for this study. Donor eyes used for this research had no documented history of eye disease. All eyes were obtained from the Lions Eye Bank of Western Australia (Lions Eye Institute, Western Australia) after removal of corneal buttons for transplantation. The donor eyes used in this report were also used in our previous publication (Chan et al., 2012). The demographic data and medical co-morbidities of human subjects used for svOCT imaging and human eye donors for confocal microscopy are presented in Table 1.

**2.2. svOCT Imaging technique**

SvOCT images of human subjects were acquired from a GPU-accelerated svOCT clinical prototype (Xu et al., 2014). The details of the acquisition system have previously been published (Young et al., 2011). In short, the OCT system was based on a 1060nm swept-source with 100 kHz A-scan rate. Real-time processing of the OCT intensity image data was performed using our open source GPU algorithm (Jian et al., 2013). The retinal region that was imaged was ~ 3mm nasal to the fovea, evaluated based on real-time volumetric OCT images (Fig. 1B). For the speckle variance calculation, three repeat acquisitions at each B-scan location were acquired. The scan area was sampled in a 300x300(x3) grid with a ~ 1x1 mm field of view in 3.15 seconds. **En face** visualization of the retinal microvasculature was processed and displayed in real-time using our open source GPU algorithm for svOCT (Xu et al., 2014). Scan dimensions were calibrated based on the eye length of each participant,
measured using the Zeiss IOLMaster 500 (IOL master 500, Carl Zeiss, Jena, Germany). Images were acquired from both eyes of 9 patients. svOCT images from one eye was not included in the study due to the presence of significant movement artefact.

2.3. Tissue Preparation for Confocal Microscopy

Our method of micro-cannulation and targeted perfusion based labelling techniques was utilized to label the retinal microvasculature in human donor eyes (Yu et al., 2010). In brief, the central retinal artery was cannulated and the retinal circulation perfused for 20 minutes with a mixture of oxygenated Ringer’s solution and 1% bovine serum albumin. Fixation with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer was followed by permeabilisation of endothelial cell membranes by a 0.1%Triton-X-100 in 0.1 M phosphate-buffered solution. Detergent was removed from the retinal circulation by perfusion with 0.1M phosphate buffer solution. Endothelial microfilaments were labelled over 2 hours by perfusion with a solution comprising of either phalloidin conjugated to Alexa Fluor 546 (30 U; A22283; Invitrogen, Carlsbad, CA) or Lectin-TRITC (Sigma L5266, 1:40). Nuclei were labelled with bisbenzimide (1.2 µg/mL; Sigma-Aldrich, St. Louis, MO). Residual label was cleared from the vasculature by further perfusion with 0.1 M phosphate buffer. Labelled specimens were immersion fixed in 4% paraformaldehyde overnight prior to dissection and flat mounting.

2.4. Confocal Microscopy techniques

Images were captured from the retinal eccentricity that was located 2mm nasal to the center of the fovea (Fig. 1A). Confocal images were captured using a Nikon C1 Confocal Microscope equipped with three lasers (wavelengths 408 nm, 488 nm and 561 nm) and EZ-C1 (v. 3.20) image acquisition software. Images of different wavelengths were acquired by sequential laser excitation. A 20x dry objective lens (NA 0.4) was used for all scans.
capturing a 636.5 by 636.5 µm region of interest. Using a motorised stage, a series of z-stacks were captured for each specimen beginning from the vitreal surface, at the level of the inner limiting membrane, to the outer retina. Each z-stack consisted of a depth of optical sections collected at 0.35 µm increments along the z-plane.

2.5. Image Preparation

ImagePro Plus (Media Cybernetics, Version 7.1) software was used to quantify confocal microscope images. All images for the manuscript were prepared using Adobe Photoshop (version 12.1, Adobe Systems Inc.) and Adobe Illustrator CS5 (version 15.1.0, Adobe Systems Inc.).

2.6. Qualitative Differentiation of Capillary Networks

Our previously defined morphometric criteria, that has been validated by inter-observer correlation studies, was used to partition the retinal circulation into separate capillary networks (Chan et al., 2012; Less et al., 1991; Richard et al., 2010; Snodderly et al., 1992). Capillary branching patterns, trajectory and position respective to nuclear layers were used to stratify the retinal circulation into 4 separate networks corresponding to the following regions within the retina:

1. The nerve fibre layer (NFL)
2. The retinal ganglion cell/superficial inner plexiform layer (RGC/sIPL)
3. The deep inner plexiform layer/superficial inner nuclear layer (dIPL/sINL)
4. The deep inner nuclear layer (dINL)

The svOCT images were processed using the GPU-accelerated program (Xu et al., 2014), and the retinal layers were automatically segmented using a 3D Graph Cut based segmentation
tool implemented in Matlab (Lee et al., 2012). *En face* retinal network images were generated from the same 4 capillary networks using the morphometric criteria described above. The *en face* retinal network images were generated by summing the svOCT data along the depth direction within each segmented retinal network. A region of interest corresponding to 636.5 µm by 636.5 µm was cropped from *en face* images permitting direct comparison between svOCT and histology images.

### 2.7. Morphometric Quantification of Capillary Networks

Our previously published methods were used to quantify the morphometric features of capillary networks (Chan et al., 2012; Chan et al., 2013). Quantitative data from capillary networks were attained following z projection of all images between the first and final image slice for each network. ImagePro Plus (Media Cybernetics, Version 7.1) was employed to attain the following quantitative measurements from each capillary network (Fig. 2):

- **Capillary diameter**- Defined as the perpendicular distance across the maximum chord axis of each vessel. Each confocal image was partitioned into 9 equal regions (Fig. 2A) and measurements were obtained from each region to ensure representative sampling. An average of 45 measurements was obtained from each image.

- **Capillary density**- Reflected by measurements including number of vessel intersections per 100 µm and intercapillary distance. A 3x3 grid consisting of two equally spaced horizontal and vertical perpendicular line segments was superimposed over images. Manual counts of capillary intersections across the 4 line segments covering a distance of 636.5 µm were recorded (Fig. 2B) and expressed as an average number of capillary intersections per 100 µm. Intercapillary distance was based on an average of 4 inter capillary distance measurements calculated by dividing line segment distance by number of vessel intersections.
2.8. Statistical Analysis

All data is expressed in terms of mean and standard error which were calculated using Sigmastat (Sigmastat, ver. 3.5; SPSS, Chicago, IL). The svOCT cohort data was initially analyzed followed by comparisons with histology groups. For these comparisons, T-testing was performed with a P value of < 0.050 considered significant. Similarly, Mann-Whitney rank sum test was utilized with a significant value of P < 0.050 where normality failed. SigmaPlot (Sigmaplot, ver. 12.0; Systat Software, Inc., San Jose, CA) was used to create the bar graphs that are presented in this manuscript.

3. Results

3.1. Demographics

The mean age of subjects in the svOCT group was 43.11 ± 4.46 years. We examined 9 right eyes and 8 left eyes from a total of 9 subjects (4 male and 5 female). One eye was excluded due to significant motion artefact.

The mean age of donors in the histology group was 40.12 ± 6.05 years. We examined 3 right and 8 left eyes from a total of 8 male donors. There was no statistically significant difference between the mean age of svOCT and histology eyes (P = 0.630).

3.2. Morphometric Comparisons Between svOCT and Confocal Eyes

All four retinal microvascular networks were visualized with the svOCT imaging technique. Morphometric characteristics of the 4 different capillary networks observed using svOCT and histology techniques are shown in Figure 3. Quantitative comparisons between svOCT and histology images are displayed as bar-charts in Figure 4.
Although retinal ganglion cell axons could not be distinguished by this technique, NFL capillaries in the svOCT images were seen to be predominantly oriented parallel to one another as in our previous descriptions of this network (Chan et al., 2012; Chan et al., 2013). The svOCT-imaged capillaries of the RGC/sIPL network were closely approximated to the larger vessels and comprised a dense meshwork. The svOCT-imaged capillaries of the dIPL/sINL network were associated with a prominent homogeneous back-scattered signal and ran a tortuous trajectory. In contrast, the dINL was the most easily discernible network, comprised of a flat network of capillaries with large sloping capillaries in loop configurations.

In general, the svOCT retinal layer images closely approximated those derived from histological techniques. Across all svOCT-imaged retinal networks, background was noisier and capillary margins were less well defined.

3.3. Quantitative Analysis of Capillary Diameter

A total of 1760 capillary diameter measurements were made in histology eyes and 3060 capillary diameter measurements were performed in svOCT eyes.

The average capillary diameter for all networks in the svOCT and histology techniques was 10.05 ± 0.05 µm and 8.31 ± 0.06 µm respectively. Mean capillary diameters for each retinal network for both svOCT and histology techniques are provided in Table 2. There was no difference in retinal network capillary diameter within the histology cohort (P >0.216). Within svOCT images, there was a significant difference between capillary network diameters between all networks (all P < 0.033) except the RGC/sIPL and dIPL/sINL network (P = 0.565). Comparisons between svOCT and histology showed a significantly increased capillary diameter for all retinal networks (all P < 0.001) as demonstrated in Figure 4A.

3.4. Quantitative Analysis of Capillary Density
The average number of capillary intersections per 100 µm for all networks in the svOCT and histology techniques was $1.54 \pm 0.05 \mu m$ and $1.51 \pm 0.09 \mu m$ respectively. Mean capillary intersections per 100µm for each retinal network for both svOCT and histology techniques are provided in Table 2. Capillary intersections per 100 µm was largest in the dIPL/sINL network on svOCT and was significantly larger than the NFL network ($P = 0.038$). There was no difference in capillary intersections per 100 µm between the NFL, RGC/sIPL and dINL networks (all $P > 0.155$). There were no differences in capillary intersections per 100µm between capillary networks in the histology specimens (all $P > 0.196$). The number of capillary intersections per 100 µm comparisons between svOCT and histology did not show a significant difference for all retinal networks (all $P > 0.157$).

The intercapillary distances for all networks in the svOCT and histology techniques was 68.69 ± 2.67 µm and 71.30 ± 5.17 µm respectively. Mean intercapillary distances for each retinal network for both svOCT and histology techniques are provided in Table 2. Intercapillary distance was largest in the NFL network on svOCT and was significantly larger than the dIPL/sINL network ($P = 0.029$). There was no difference in intercapillary distance between the NFL, RGC/sIPL and dINL networks (all $P >0.080$). There were no differences in intercapillary distance between capillary networks in the histology specimens (all $P > 0.177$). Intercapillary distance comparisons between svOCT and histology did not show a significant difference for all retinal networks (all $P > 0.094$).

4. Discussion

The major findings from this study are as follows: (1) svOCT has the capacity to delineate the morphometric characteristics of different capillary networks in the human perifovea. (2) The morphological characteristics of capillary networks presented in svOCT images are highly comparable to age-matched histology images. (3) Capillary diameter measurements in
svOCT images are significantly greater than histological data for all networks. (4) Capillary density measurements are not significantly different between svOCT and histological data for all networks.

Retinal neurons in the human perifovea are nourished by four morphologically distinct capillary networks (Chan et al., 2012). We have previously shown that the anatomical configuration of each capillary network is altered in accordance with the unique metabolic demands of the regional neuronal milieu. The vulnerability of these networks to systemic cardiovascular disease is not uniform. Our prior work has provided evidence that hypertension and other cardiovascular co-morbidities induce selective alteration to capillary networks in the perifovea (Chan et al., 2013). Therefore, in addition to providing vital information about neurovascular coupling mechanisms that regulate retinal homeostasis, the study of capillary networks is important for our understanding of vascular patho-biology.

SvOCT is a non-invasive imaging technique that is gaining greater application in clinical medicine (Mahmud et al., 2013; Mariampillai et al., 2008). In ophthalmology, svOCT has been used to acquire real-time, flow contrast images of the macula and optic nerve head (Xu et al., 2014). A major advantage of svOCT is the ability to perform angiography without contrast administration. This avoids many potential adverse effects that are commonly seen with fluorescein angiography. The capability of svOCT to overcome Doppler angle also makes it less user-dependent than Doppler OCT techniques (Larina et al., 2011; Mariampillai et al., 2008). The manner in which the svOCT data is collected and processed, (repeat B-scans at the same location), also makes it more sensitive to detecting flow contrast in regions of low flow. Finally, the relatively fast image acquisition and processing time and the capacity to provide real-time images that are immediately available to the clinician are other major advantages of svOCT.
The application of svOCT technology to image the clinical manifestations of age related macular degeneration have previously been described, however to our knowledge, this is the first report to validate and quantitatively compare svOCT to histological data (Ellabban et al., 2012). Using our prototype svOCT device we were able to reliably image the various capillary networks in the perifovea. Specifically, we were able to image the NFL network, the RGC/sIPL network, the dIPL/sINL network and the dINL network. Capillaries in the NFL network were seen to run parallel to the direction of axons while capillaries in the dINL network demonstrated a planar configuration with multiple closed loops. Capillaries in RGC/sIPL and dIPL/sINL networks were observed to have a tortuous trajectory and displayed a complex three-dimensional configuration. We found that the morphological characteristics of networks in svOCT images were highly comparable to our histological images.

The mean capillary diameter was significantly greater in svOCT images compared to age-matched histological images for all networks. This may be attributed to the difference in lateral resolution of the two imaging systems; the lateral point spread function (PSF) of the svOCT imaging beam can be modelled as a Gaussian with $1/e^2$ radius of 8.5 µm, artificially broadening the measured width of the capillaries. This difference may also have been due to the effects of fixation artefact and shrinkage in histology specimens (Rastogi et al., 2013). Other plausible explanations for increased capillary diameter in svOCT images may be due to the dynamic effects of physiological variables such as blood pressure, tissue interstitial pressure and vessel wall elasticity acting upon capillary beds (Tawia and Rogers, 1992). These variables have been shown to influence the anatomical dimensions of live tissue. Pericyte mediated reduction of capillary diameter has also been shown in the context of neuronal death to influence capillary diameter and this effect may also have resulted in
smaller diameters in histological images (Hamilton et al., 2010). Investigation of these potential physiological confounding factors with a high resolution svOCT system is required. In this report we used the number of capillary intersections per 100 μm and the inter-capillary distance as indices of capillary density. Inter-capillary distance measurements in the retina using svOCT closely approximated what has previously been reported in the human gray matter (Mintun et al., 2001). The mean value of 68.69 μm was significantly less than 123 μm which is the reported value of inter-capillary distance in human white matter (Yoshii and Sugiyama, 1988). Similar to our histology data, we found that capillary density in svOCT images was greatest in the network bordering the plexiform layer and least in the nerve fibre layer network. When comparisons were made between svOCT and histology data we did not find a difference in capillary intersections per 100 μm and inter-capillary distance for any network.

This report validates the utility of our prototype svOCT for studying human perifoveal capillary networks but we acknowledge several limitations of this device. Firstly, patient cooperation and steady fixation are essential for acquiring high quality images of capillary networks. Although the image acquisition time was only 2.7 seconds it may not be possible for patients with significant visual disease to fixate for this period of time. In these patients, motion artefact may obscure important capillary detail. Modifications to this device with shorter image acquisition times (Motaghiannazam and Fraser, 2012; van Velthoven et al., 2007) or the incorporation of motion tracking technology (Braaf et al., 2013; Vienola et al., 2012) may potentially overcome this limitation. Secondly, the resolving capability of the svOCT is not as precise as what is attainable histologically. Furthermore, the duration of an imaging session, speed of the OCT system, and lateral resolution combine to limit the field of view that can be acquired with this technique in a clinical setting.
4.1. Conclusion

The results of this study suggest that svOCT is a promising technique for the qualitative and quantitative assessment of capillary networks in the human retina. Further work is required to validate the utility of this device for managing patients with retinal microvascular disease such as diabetic retinopathy. Additionally, it will be important to determine if this device has the capacity to reliably provide dynamic information about blood flow in the human retina. Such *in vivo* information may be particularly useful for stratifying patients into high-risk groups for retinal disease progression, particularly in the context of conditions such as diabetic retinopathy.
**Legends**

**Fig. 1.** – Anatomy of the human perifovea. Speckle variance optical coherence tomography (A) and a corresponding transverse confocal microscopy retinal section (B) from a healthy subject illustrate the various retinal layers in the perifoveal region. On the confocal image, endothelial cells are labelled with Phalloidin and nuclei are labelled with Hoescht antibody. Retinal layers corresponding to the nerve fibre layer (yellow), ganglion cell layer (green), inner nuclear layer (light blue) and outer nuclear layer (red) are clearly demarcated in each image. Capillary networks were co-localised within these retinal layers. Scale bar = 50 µm.

**Fig. 2.** – Methodology for quantifying capillary network morphometry. Representative speckle variance optical coherence tomography *en face* images of the deep inner nuclear layer network are used to illustrate how capillary diameter (A) and capillary density measurements (B) were acquired. Each image was first partitioned into a 3x3 grid (solid yellow lines). Capillary diameter (red lines) was measured by determining the perpendicular distance across the maximum chord axis for each vessel. Capillary density was calculated by determining the number of vessel intersections (red squares) per 100µm of line segment. Intercapillary distance was also used as an indice of capillary density. Scale bar = 100 µm.

**Fig. 3.** – Morphometric comparisons between svOCT and histology eyes for NFL network (A and B), RGC/sIPL network (C and D), dIPL/sINL network (E and F) and dINL network (G and H) are presented. Scale bar = 100 µm.

**Fig. 4.** – Quantitative comparisons of capillary network morphometry. Comparisons between speckle variance optical coherence tomography and histology images for capillary diameter (A), capillary intersections per 100µm (B) and intercapillary distance (C) are presented. Mean ± standard error for each parameter is provided. *denotes a significant difference between svOCT and histology eyes ($P < 0.050$).


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<tr>
<td>O</td>
<td>M</td>
<td>27</td>
<td>L</td>
<td>Suicide</td>
</tr>
<tr>
<td>P</td>
<td>M</td>
<td>59</td>
<td>R+L</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Q</td>
<td>M</td>
<td>39</td>
<td>R+L</td>
<td>Bacterial Endocarditis</td>
</tr>
</tbody>
</table>

**Table 1.** – Donor demographic details. Age (years), sex (M = male or F = female), co-morbid conditions for each eye donor is provided. MVA = motor vehicle accident.
Table 2. Quantitative retinal capillary network data acquired using svOCT and histology imaging techniques. Mean capillary diameter, number of vessel intersections per grid, number of capillary intersections per 100µm and intercapillary distance (ICD) with corresponding standard errors for each network is provided. NFL = Nerve Fibre Layer, RGCL = Retinal Ganglion Cell Layer, sIPL = superficial Inner Plexiform Layer, dIPL = deep Inner Plexiform Layer, sINL = superficial Inner Nuclear Layer and dINL = deep Inner Nuclear Layer.

<table>
<thead>
<tr>
<th></th>
<th>Capillary Diameter (microns)</th>
<th>No. Vessel Intersections per grid</th>
<th>No. Capillary Intersections per 100µm</th>
<th>ICD (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>svOCT</td>
<td>NFL 9.86 ± 0.06</td>
<td>37.5 ± 1.32</td>
<td>1.47 ± 0.04</td>
<td>73.46 ± 3.61</td>
</tr>
<tr>
<td></td>
<td>RGCL/sIPL 10.01 ± 0.05</td>
<td>38.47 ± 1.49</td>
<td>1.51 ± 0.04</td>
<td>70.94 ± 3.43</td>
</tr>
<tr>
<td></td>
<td>dIPL/sINL 9.98 ± 0.05</td>
<td>41.12 ± 1.04</td>
<td>1.62 ± 0.03</td>
<td>64.31 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>dINL 10.34 ± 0.05</td>
<td>40.06 ± 1.22</td>
<td>1.57 ± 0.04</td>
<td>66.05 ± 1.96</td>
</tr>
<tr>
<td>Histology</td>
<td>NFL 8.32 ± 0.06</td>
<td>35.80 ± 2.97</td>
<td>1.41 ± 0.12</td>
<td>79.21 ± 8.73</td>
</tr>
<tr>
<td></td>
<td>RGCL/sIPL 8.28 ± 0.07</td>
<td>40.80 ± 1.93</td>
<td>1.60 ± 0.08</td>
<td>65.60 ± 2.89</td>
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<tr>
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<td>dIPL/sINL 8.33 ± 0.06</td>
<td>38.4 ± 2.46</td>
<td>1.51 ± 0.10</td>
<td>71.56 ± 5.09</td>
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<tr>
<td></td>
<td>dINL 8.30 ± 0.05</td>
<td>39.2 ± 2.24</td>
<td>1.57 ± 0.09</td>
<td>68.84 ± 3.97</td>
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</tbody>
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