Title: **Retinal angiography with real-time speckle variance optical coherence tomography**

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Summary

This report describes a novel, non-invasive and label-free optical imaging technique, speckle variance optical coherence tomography (svOCT), for visualising blood flow within human retinal capillary networks. This imaging system utilizes a custom-built swept source OCT system operating at a line rate of 100 kHz. Real-time processing and visualization is implemented on a consumer grade Graphics Processing Unit (GPU). To investigate the quality of microvascular detail acquired with this device we compared images of human capillary networks acquired with svOCT and fluorescein angiography (FA). We found that the density of capillary microvasculature acquired with this svOCT device was visibly greater than FA. We also found that this svOCT device had the capacity to generate *en-face* images of distinct capillary networks that are morphologically comparable to previously published histological studies. Finally, we found that this svOCT device has the ability to non-invasively illustrate the common manifestations of diabetic retinopathy. The results of this study suggest that GPU accelerated svOCT has the potential to non-invasively provide useful quantitative information about human retinal capillary networks. Speckle variance OCT may therefore have clinical and research applications for the management of retinal microvascular diseases, a major cause of visual morbidity worldwide.

Introduction

Retinal vascular diseases remain a major cause of visual morbidity worldwide. Optical coherence tomography (OCT) and fluorescence angiography (FA) are invaluable tools in clinical ophthalmology that are used for the diagnosis and management of retinovascular conditions such as diabetic retinopathy and retinal vascular occlusions. Current OCT technology has the capacity to provide high-resolution histology-like anatomical information of different retinal layers. In contrast, FA provides wide-angle information of the retinal circulation and, in particular, is useful for identifying areas of blood-retina-barrier compromise. One of the limitations of FA is the need to inject fluorescein dye which is associated with minor side effects. There is also a small but significant risk of anaphylaxis and death with FA (estimated at 1 in 222,000) [1]. Furthermore, only limited information concerning depth along the z-axis can be acquired with FA.

Circulatory disturbances within regional capillary beds are an important and early pathogenic event in many retinal vascular diseases. ref The ability to acquire *en face* images of distinct capillary beds with current FA and OCT technology is however limited. Recent reports have described adaptations to OCT technology that permit *in vivo* examination of the human microvasculature.[2–6] However, generating images of vascular networks is computationally intensive and usually requires off-line image processing. The time to image patients is limited and acquiring usable data without real-time feedback of the vasculature network can pose a great challenge.

We present a clinical prototype speckle variance OCT (svOCT) device with hardware accelerated processing for real-time visualization of human microvascular networks. In this study we illustrate that this device has the capacity to non-invasively provide anatomical information of retinal capillary beds that is greater than what is acquired

using standard fluorescein angiography. The device presented in this report may therefore have broad clinical application for the management of retinal vascular diseases.

Methods

This study was approved by the human ethics committee at the University of British Columbia. All subjects were imaged at the Eye Car Centre in Vancouver. The clinical prototype svOCT used in this report is based on a 1060nm swept-source OCT system with 100 kHz A-scan rate. The axial resolution is ~6 μm in tissue and the lateral resolution is ~17 μm. 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, which required ~2.7 seconds for image acquisition. The real-time svOCT processing and display was performed using our open source software [7, 8].

The following qualitative assessments were performed in this study:

1. To determine if there was a difference in the morphology and density of capillary networks represented in svOCT and FA images (Fig. 1). Comparisons were made between macular images from a healthy human subject that was imaged with the two modalities.
2. To determine if svOCT has the capacity to isolate and image distinct capillary networks within the human retina (Fig. 2). The perimacular region of a healthy human subject was imaged and manual segmentation of B Scan images was used to generate *en face* images of different retinal capillary networks. Images were acquired of histologically documented capillary networks including the nerve fiber layer (NFL) network, ganglion cell layer

(GCL)/ inner plexiform layer (IPL) network and outer plexiform layer (OPL) network.

3. To determine if the common and serious complications of diabetic retinopathy could be identified using svOCT. Areas of retinal pathology, identified on FA were imaged using svOCT (Fig. 3). Comparisons were made between FA and svOCT images.

Results

In the macula we observed greater capillary density in svOCT images compared to FA (Fig. 1). We also found that svOCT was able to identify with greater precision the terminal capillaries around the foveal avascular zone.

The morphological characteristics of capillary networks in *en face* images correlated closely with the results of previous histological studies performed on the human retina (Fig. 2) [9, 10]. We observed that capillaries in the NFL network were longitudinally orientated with a trajectory that was predominantly parallel to the direction of retinal ganglion cell axons in the NFL. In contrast, the capillaries in the GCL/IPL network demonstrated a complex three-dimensional organization. The capillary network in the OPL layer was found to be planar with multiple closed loops. Colour-coded projection of various capillary network images permitted us to explore important spatial vascular relationships within the retina and also allowed us to identify the change in capillary topography relative to retinal depth.

Figure 3 illustrates the FA appearance of a patient with proliferative diabetic retinopathy. Insets within the image demonstrate in great detail the morphological appearance of these eccentricities examined with svOCT imaging. Optic disk

neovascularization is clearly seen using svOCT as are areas of capillary drop out within and outside the macula.

Discussion and conclusion

Capillary networks are inherently linked to retinal homeostasis and disease. This study highlights the utility of our prototype svOCT device for non-invasively studying the human retinal vasculature in real-time. The results presented in this report suggest that svOCT is complementary to FA, and may be superior in providing retinal capillary detail. Current limitations of svOCT technology is that it cannot assess pooling/staining and low flow aneurysms. Previous work has shown that the anatomical information captured on FA is predominantly that of the inner most retinal capillary networks. ref The *en face* images of distinct capillary networks illustrated in this report demonstrate that this device has the capability for providing information about all networks in the retina.

Speckle variance OCT also has the potential to non-invasively identify the important pathological manifestations of diabetic retinopathy, ischemia and proliferation. In patients with compromised renal function, where the administration of fluorescein dye may be contraindicated, this device may be particularly advantageous. In this report we present images with a field of view ranging from 5x5mm to 1x1mm. It is possible to acquire images with a wider field of view using a higher acquisition speed laser, hardware motion tracking and image mosaicking. Further quantitative work is required to define the role of svOCT in clinical practice.

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Ethics approval This study was approved by the human ethics committee at the University of British Columbia.

Legends

Figure 1 – Human macula vasculature. Comparison between fluorescein angiography (A) and speckle variance OCT (B) images captured from a healthy subject demonstrates an observable increase in the density of capillary detail in the svOCT image (scale bar = 200 μm). The FA image was acquired with 40^{o} field of view and cropped to correspond to the region acquired with the svOCT

Figure 2 – Isolation of distinct human capillary networks with speckle variance OCT. (A) Representative B-scan image of the human retina (A) demonstrates various inner retinal layers including nerve fibre layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL). Manual segmentation of B-scan images (red, green and blue lines) allows generation of *en-face* OCT images of different capillary networks within, and between, these retinal layers. The morphology of capillary networks within the NFL (B) and networks located between GCL and INL (C) and INL and ONL (D) bear close morphological correlations to previous histologic studies of these networks. Superimposing *en-face* images (E) also allows study of spatial relationships between various networks (scale bar = 200 μm).

Figure 3 – Application of speckle variance OCT to investigate diabetic retinopathy. Fluorescein angiography (A) of a diabetic patient demonstrates proliferative disease with marked retinal non-perfusion. En-face images of the optic disk (Inset I) acquired with svOCT clearly illustrates neovascularization of the optic disk (arrows). Speckle variance OCT images of the peri-macular (Inset II) and macular (Inset III) eccentricities also demonstrates marked capillary dropout (arrows) within these regions.

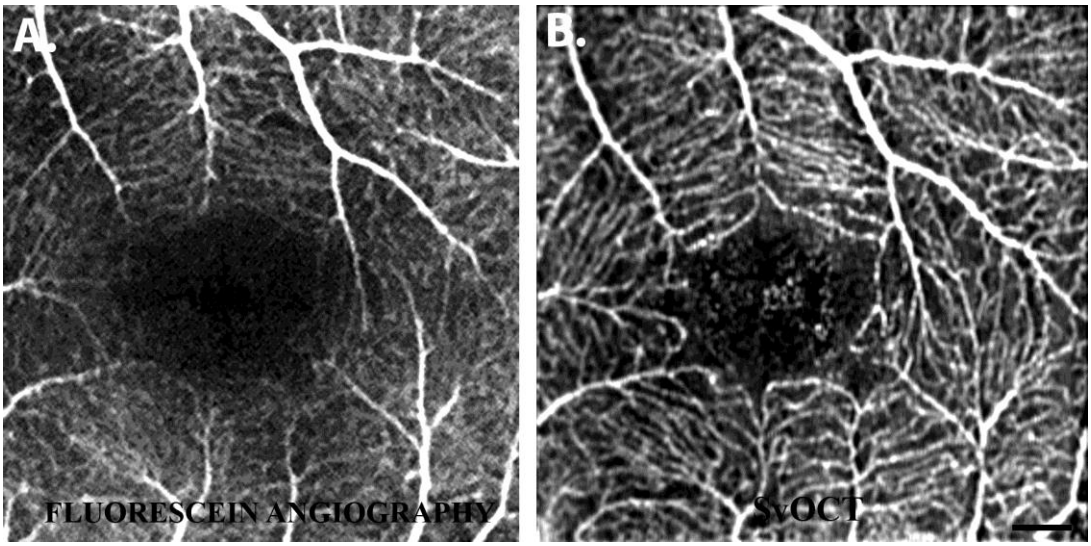

Figure 1

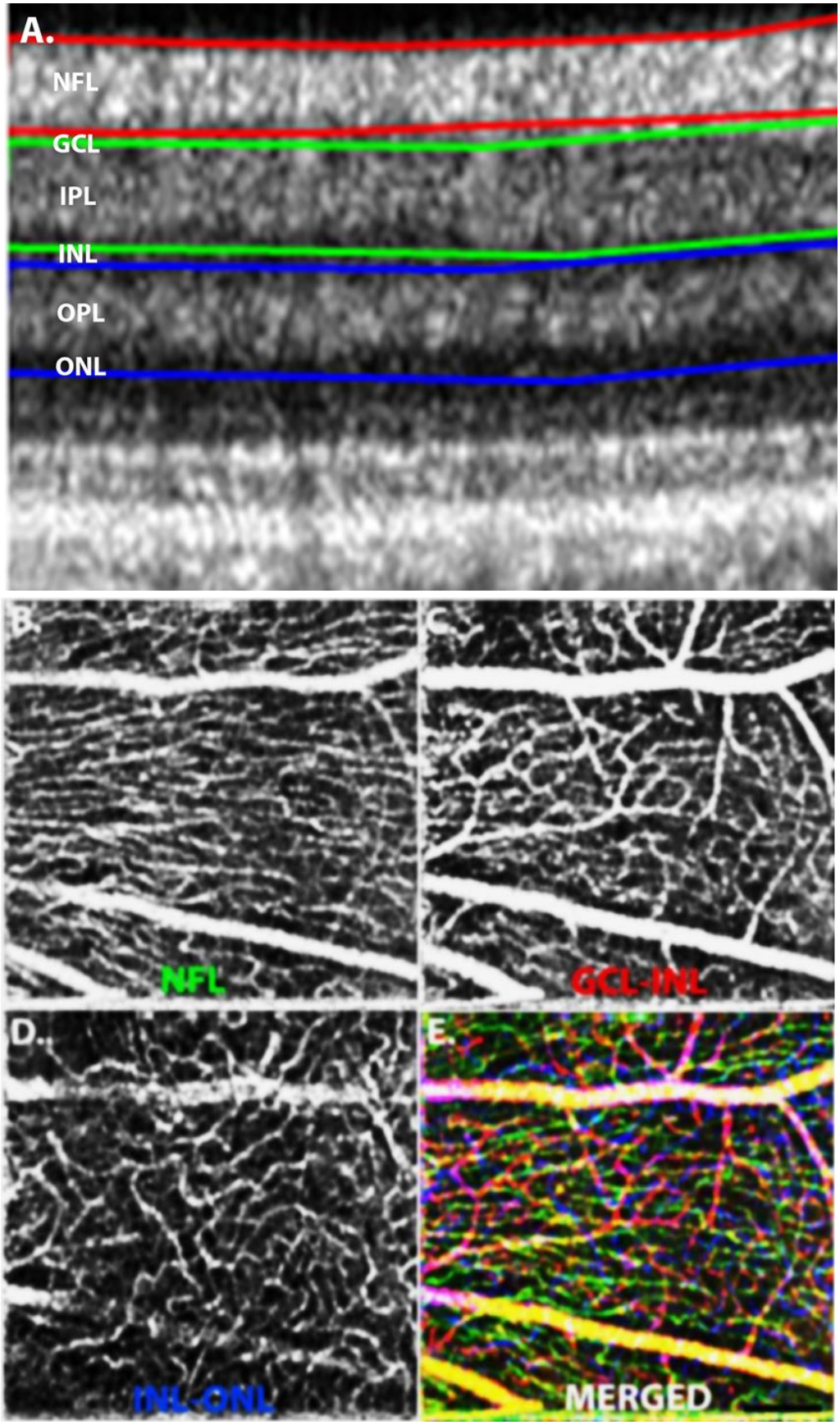

Figure 2

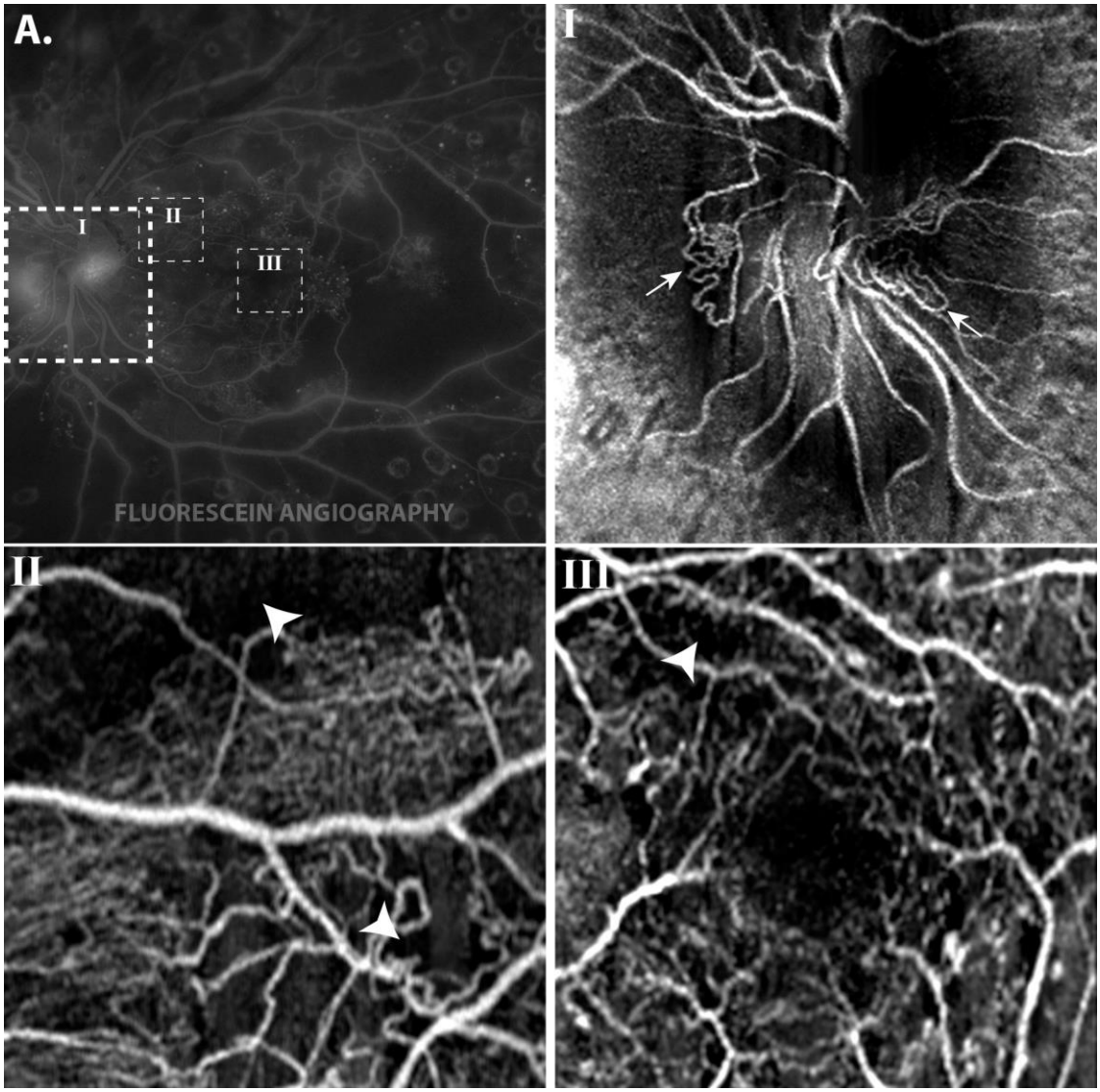

Figure 3

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