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Modulation of retinal image vasculature analysis to extend utility and provide secondary value from optical coherence tomography imaging

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Abstract. Retinal image analysis is emerging as a key source of biomarkers of chronic systemic conditions affecting the cardiovascular system and brain. The rapid development and increasing diversity of commercial retinal imaging systems present a challenge to image analysis software providers. In addition, clinicians are looking to extract maximum value from the clinical imaging taking place. We describe how existing and well-established retinal vasculature segmentation and measurement software for fundus camera images has been modulated to analyze scanning laser ophthalmoscope retinal images generated by the dual-modality Heidelberg SPECTRALIS® instrument, which also features optical coherence tomography. [DOI: 10.1117/1.JMI.3.2.020501]

Keywords: ophthalmology; retina; image analysis; scanning laser ophthalmoscope.

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1 Introduction

Retinal image analysis is a key component in translational medical imaging research, providing new insights into chronic systemic diseases, as well as emerging as a source of important biomarkers of disease diagnosis, severity, monitoring, and response to treatment.[1,2] The morphometric properties of the retinal vessels have assisted in the new understandings of stroke pathology[3] as well as in diseases of the central nervous system, such as Alzheimer's disease[4] and cerebral malaria.[5]

Vascular Assessment and Measurement Platform for Images of the REtina (VAMPIRE) is a semiautomatic bespoke software platform, developed under an academic collaboration among the University of Edinburgh, University of Dundee, Universita degli Studi Di Palermo, and Universita degli Studi di Verona.[6–8] Analysis of conventional digital color fundus photographs provides efficient quantification of standard retinal vascular parameters as well as the complexity of the visible vascular network (through fractal analysis). Validation of image analysis algorithms is essential if the results are to be clinically meaningful.[9] VAMPIRE has undergone continuous evaluation with each extension and study, such as with automatic optic disc and fovea detection,[10,11] artery and vein classification,[12] and measurement of vessel caliber.[13,14] VAMPIRE was also the first tool to be used in assessing fundus camera images held in the UK Biobank—the largest retinal image repository in a prospective population-based medical data resource—to deliver computational quantification of retinal vascular parameters in relation to cardiovascular disease.[15]

Modulation of the software to be able to analyze other types of retinal images, such as those produced by a scanning laser ophthalmoscope (SLO), to provide automated vascular measures as required, is also a goal of the VAMPIRE project. A recent report described the process of adaptation to images captured by the ultrawidefield Optos P200C SLO device.[13] This method involved the development and validation of a new vessel detection algorithm incorporating multiscale matched filters, a neural network classifier, and hysteresis thresholding.

The Heidelberg SPECTRALIS® OCT is a popular imaging device in clinical ophthalmology, leading the current generation of spectral-domain optical coherence tomography (OCT) devices, with 4 μm axial resolution and inbuilt automatic quantitative segmentation of the retinal layers on the cross-sectional OCT image. It is used increasingly in specialties with ophthalmology as evidence emerges of its utility in informing on cardiovascular and neurodegenerative disease, particularly multiple sclerosis.[16]

The instrument also acquires an SLO image simultaneously with the OCT image. This is a sharp, high-contrast confocal SLO, with a viewing angle of 35 deg, utilizing a laser light of 785 nm and generating an image of 1536×1536 pixels. It is primarily used for guiding the location of the OCT imaging and enabling image registration for follow-up scans, ensuring the same precise location is reimaged. However, there is potential to evaluate the retinal vessels appearing in these SLO images

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in much the same way as previous work featuring fundus camera photographs. This would add value to the already acquired patient imaging and provide a unique opportunity for the development of dual-mode image analysis derived from a single instrument and a single patient imaging event. Apart from efficiency and patient convenience, this development brings additional advantages: it allows direct point-to-point correlation between the OCT and SLO image, and also the SLO-generated fundus image is of high contrast, potentially facilitating more accurate measures of the retinal vessels. This will ultimately provide additional retinal vascular data to inform on studies utilizing this device to investigate brain and systemic diseases.

The SLO images from SPECTRALIS® are very different from those produced by the Optos SLO device—a different field of view, image resolution, and illumination source (SPECTRALIS® utilizes near-infrared light and requires no visible light flash). Therefore, the previous modulation of VAMPIRE for Optos ultra-widefield imaging described above could not be used.

We, therefore, sought to modulate the original color fundus VAMPIRE software to accept and process SPECTRALIS® SLO images, and provide accurate and repeatable measurements of the retinal vasculature.

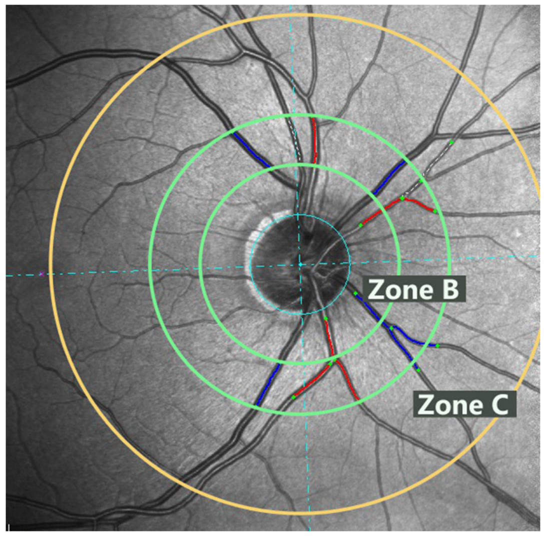

Fig. 1 Typical SLO image with optic disc boundary that was determined manually. The standard set of circular measurement zones commonly used in the analysis of fundus camera images is also shown—zone B, which is the ring 0.5 to 1 optic disc diameters away from the center, and zone C, which is the ring extending from optic disc boundary to 2 optic disc diameters away. VAMPIRE automatically detects and selects the six widest arterioles (red) and venules (blue) crossing zone B to calculate AVR, CRAE, and CRVE. The vessels in zone C (not marked in this example) were used to calculate arteriolar and venular tortuosity.

2 Methods

The VAMPIRE software was modulated by retraining certain software processing algorithms, in conjunction with the partner "manual annotation tool" and a set of training images.

This research involved images from volunteers and was approved by the South-East Scotland Research Ethics Committee, and observed the principles of the Declaration of Helsinki. Informed consent was obtained from all the participants.

2.1 *Software Modulation*

Starting with the current VAMPIRE software (version 3.0) used for color fundus images, the algorithms were adapted to the SPECTRALIS® OCT images. All algorithms were implemented in MATLAB® (The Mathworks Inc., Natick, Massachusetts).

For automatic detection of vessels, a two-dimensional Gabor wavelet approach for fundus camera images was adapted to emphasize the appearance of vessels captured with SLO, followed by supervised pixel classification with a Bayesian classifier.[17] This vascular detection algorithm was retrained to work on the SLO images by manually delineating vessels in 16 images (randomly selected from a study using SPECTRALIS® OCT), taking 1,000,000 samples of pixels with six features (i.e., original grayscale intensity and response to Gabor filters of size 2 to 6 pixels) to create a supervised classifier, which is applied to new images to automatically create pixel-by-pixel maps of the vessels.

Further postprocessing based on mathematical morphology was also adapted to vessels in SLO images, where the central reflex is more evident than in images acquired by a fundus camera. This caused misclassified gaps in vessels with the supervised classification technique, and this effect was lessened by removing such regions or holes with sizes <200 pixels to create an improved map of the vessel. This size was chosen following experimental investigation.

Using this vessel map, VAMPIRE creates a tree-like representation of the vasculature as a preprocessing step for performing vascular measurements.[7,8] From the vessel tree, the software automatically selects the six widest arterioles and venules crossing zone B (Fig. 1) and measures vessel calibers using a supervised algorithm[14] that was retrained on SLO images by manually annotating widths at 200 locations in five images.

These measurements were used to calculate the well-recognized summary parameters—central retinal arteriole equivalent (CRAE) and central retinal venule equivalent (CRVE)—yielding the arteriole to venule width ratio (AVR).[18] Similarly, for tortuosity, VAMPIRE selects the six widest arterioles and venules crossing zone C, evaluates the tortuosity for each using an established technique,[19,20] and calculates the median values (plus standard deviation and range).

Multiple images can be processed in parallel with the estimated automatic processing time for a single image being ~7 min. Once an image was processed, an operator manually inspects the selections made by the software and uses his own judgment to determine whether a vessel was an arteriole or venule. The user can change the classification of vessels (arteriole or venule) with a single click. If the operator was unsure of a vessel's classification or believed the vessel to have been detected incorrectly, it could be deselected. In such cases, the software provides a replacement vessel that was the next widest in caliber. This manual review process takes 1 to 2 min per image.

2.2 *Evaluation*

The modulated software was evaluated by assessing interoperator reliability.

We obtained optic disc centered images from 78 participants—48 males, 30 females, all white Caucasian, and an age range of 39 to 69 years (mean 52)—using the SPECTRALIS® OCT device.

Each image was uploaded into the modulated VAMPIRE software. The boundary of the optic disc was selected manually by the operator who clicks two points (on the boundary and directly opposite each other). The user further manually identifies the location of the center of the fovea (a single click). This creates a circular approximation to the optic disc outline and also places the standard set of measurement zones used in conventional analysis of fundus camera pictures[18]—zone B is a ring 0.5 to 1 optic disc diameters away from the center, and zone C is the ring extending from the optic disc boundary to 2 optic disc diameters away (Fig. 1).

The two operators were blinded to each other's use of the software and a comparison between their results was assessed as an outcome measure of the successful modulation of VAMPIRE to these SLO retinal images. Interoperator reliability was assessed using intraclass correlation coefficients (ICCs) and a Bland–Altman approach, to display the extent of agreements. Statistical analyses were performed using MedCalc for Windows, version 15.11 (MedCalc Software, Ostend, Belgium).

3 Results

Of the 78 images available, two were not analyzed due to insufficient image quality. A further two participants' images were not included due to insufficient vessel selection (a selection of <3 of either arterioles or venules was deemed insufficient for accurate analysis).

Each operator analyzed the images independently, recording values for AVR, CRAE, CRVE, arteriolar tortuosity, and venular tortuosity. The total manual operator time for these 78 images was ~3 h.

The ICCs were >0.9 for all metrics (Table 1), demonstrating very high reliability and repeatability of these measurements with the modulated software. The Bland–Altman analysis (only AVR and arteriolar tortuosity are reproduced here) demonstrated a high level of consistency between the operators (Fig. 2).

Table 1 ICCs [and 95% confidence intervals (CIs)] for absolute agreement between two operators of the retinal parameters.

	AVR	CRAE	CRVE	Arteriolar tortuosity	Venular tortuosity
ICC	0.961	0.936	0.961	0.955	0.958
95% CI	0.939 to 0.975	0.900 to 0.959	0.938 to 0.975	0.930 to 0.971	0.934 to 0.973

4 Discussion

We have successfully modulated the VAMPIRE software to accept and analyze the SLO retinal images acquired by the SPECTRALIS® machine. In addition, our initial evaluation has demonstrated a high reliability of the vascular measurements that can be made on these images. The low level of image rejection is reassuring, given the challenge of adapting the software to a new fundal image representation that, while recognizably similar to the human viewer, represents a greater challenge to the software interpretation of vessel and background.

At this stage, the software still requires manual supervision, as the operator is asked to make decisions about which vessels are arteries and veins, and to manually select the optic disc, or correct incorrect vessel detection. VAMPIRE for analysis of fundus camera pictures features automatic detection of these features. In part, this is facilitated by the color information inherently contained in a fundus photograph, particularly for vessel classification where there are distinct and measurable differences in color features.[21] Additional modulation of VAMPIRE to work with SPECTRALIS® SLO would, therefore, benefit from implementation of these automatic processes as this would further enhance the efficiency and usability of the software.

A further limitation of this study is the low number of images assessed thus far. However, as in previous developments of the VAMPIRE software, evaluation remains essential and will continue with each new study and dataset of images analyzed.

This development of retinal image analysis holds tremendous potential for use as part of a multimodal retinal analysis, from one single patient acquisition, using the patient-friendly SPECTRALIS® device. The possibility to now truly integrate neuroretinal metrics with vasculature analysis, from the imaging acquired in a single device, invites potential in multiple medical research domains, where OCT imaging, with SLO image acquisition, is already well established.

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References

1. N. Patton et al., "Retinal image analysis: concepts, applications and potential," *Prog. Retinal Eye Res.* **25**(1), 99–127 (2006).

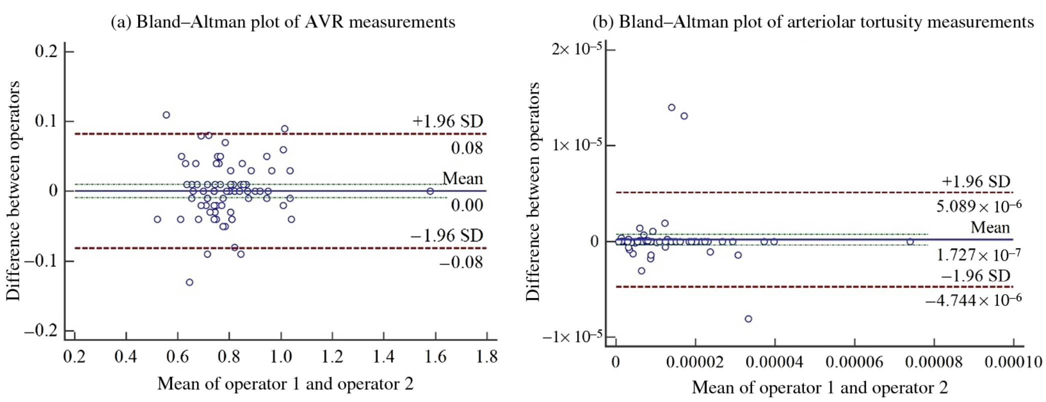

Fig. 2 Bland–Altman plots of agreement between two operators (with 95% confidence intervals for limits of agreement) for (a) AVR and (b) arteriolar tortuosity.

2. T. J. MacGillivray et al., "Retinal imaging as a source of biomarkers for diagnosis, characterization and prognosis of chronic illness or long-term conditions," *Br. J. Radiol.* **87**(1040), 20130832 (2014).
3. F. Doubal et al., "Differences in retinal vessels support a distinct vasculopathy causing lacunar stroke," *Neurology* **72**(20), 1773–1778 (2009).
4. C. Y. Cheung et al., "Retinal microvasculature in Alzheimer's disease," *J. Alzheimers Dis.* **42**(Suppl. 4), S339–S352 (2014).
5. I. J. MacCormick et al., "Cerebral malaria in children: using the retina to study the brain," *Brain* **137**(8), 2119–2142 (2014).
6. A. Perez-Rovira et al., "VAMPIRE: Vessel Assessment and Measurement Platform for Images of the Retina," in *33th Int. IEEE EMBS Conf.*, pp. 3391–3394 (2011).
7. E. Trucco et al., "Novel VAMPIRE algorithms for quantitative analysis of the retinal vasculature," in *Biosignals and Biorobotics Conf.*, pp. 1–4 (2013).
8. E. Trucco et al., "Morphometric measurements of the retinal vasculature in fundus images with vampire," in *Biomedical Image Understanding*, J.-H. Lim, S.-H. Ong, and W. Xiong, Eds., pp. 91–111, John Wiley & Sons Inc., Hoboken, NJ (2015).
9. E. Trucco et al., "Validating retinal fundus image analysis algorithms: issues and a proposal," *Invest. Ophthalmol. Vis. Sci.* **54**(5), 3546–3559 (2013).
10. A. Giachetti, L. Ballerini, and E. Trucco, "Accurate and reliable segmentation of the optic disc in digital fundus images," *J. Med. Imaging* **1**(2), 024001 (2014).
11. A. Giachetti et al., "The use of radial symmetry to localize retinal landmarks," *Comput. Med. Imaging Graph* **37**(5–6), 369–376 (2013).
12. D. Relan et al., "Automatic retinal vessel classification using a least square-support vector machine in VAMPIRE," in *36th Annual Int. Conf. of the IEEE Engineering in Medicine and Biology Society*, pp. 142–145 (2014).
13. E. Pellegrini et al., "Blood vessel segmentation and width estimation in ultra-wide field scanning laser ophthalmoscopy," *Biomed. Opt. Express* **5**(12), 4329–4337 (2014).
14. C. Lupascu, D. Tegolo, and E. Trucco, "Accurate estimation of retinal vessel width using bagged decision trees and an extended multiresolution Hermite model," *Med. Image Anal.* **17**(8), 1164–1180 (2013).
15. T. J. MacGillivray et al., "Suitability of UK Biobank retinal images for automatic analysis of morphometric properties of the vasculature," *PLoS One* **10**(5), e0127914 (2015).
16. J. R. Cameron and A. J. Tatham, "A window to beyond the orbit: the value of optical coherence tomography in non-ocular disease," *Acta Ophthalmol.* (2016).
17. J. V. B. Soares et al., "Retinal vessel segmentation using the 2-D Gabor wavelet and supervised classification," *IEEE Trans. Med. Imaging* **25**, 1214–1222 (2006).
18. M. D. Knudtson et al., "Revised formulas for summarizing retinal vessel diameters," *Curr. Eye Res.* **27**(3), 143–149 (2013).
19. R. Annunziata et al., "Tortuosity classification of corneal nerves images using a multiple-scale-multiple-window approach," in *Proc. of the Ophthalmic Medical Image Analysis First Int. Workshop*, pp. 113–120 (2014).
20. A. Lisowska et al., "An experimental assessment of five indices of retinal vessel tortuosity with the RET-TORT public dataset," in *36th Annual Int. Conf. of the IEEE Engineering in Medicine and Biology Society*, pp. 5414–5417 (2014).
21. D. Relan et al., "Retinal vessel classification: sorting arteries and veins," in *35th Annual Int. Conf. of the IEEE Engineering in Medicine and Biology Society*, pp. 7396–7399 (2013).

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