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Automated segmentation and fractal analysis of high-resolution non-invasive capillary perfusion maps of the human retina

Hong Jiang, MD, PhD^{1,2,*}, Delia Cabrera DeBuc, PhD¹, Tatjana Rundek, MD PhD², Byron L Lam, MD¹, Clinton B. Wright, MD², Meixiao Shen, PhD³, Aizhu Tao, MD^{1,3}, and Jianhua Wang, MD, PhD¹

¹Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

²Department of Neurology, University of Miami, Miami, FL, USA

³School of Ophthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang, China

Abstract

The retina provides a window to study the pathophysiology of cerebrovascular diseases. Pathological retinal microvascular changes may reflect microangiopathic processes in the brain. Recent advances in optical imaging techniques have enabled imaging of the retinal microvasculature at the capillary level, and the generation of high-resolution, non-invasive capillary perfusion maps (nCPMs) with the Retinal Function Imager (RFI). However, the lack of quantitative analyses of the nCPMs may limit the wider application of the method in clinical research. The goal of this project was to demonstrate the feasibility of automated segmentation and fractal analysis of nCPMs. We took two nCPMs of each subject in a group of 6 healthy volunteers and used our segmentation algorithm to do the automated segmentation for monofractal and multifractal analyses. The monofractal dimension was 1.885 ± 0.020 , and the multifractal dimension was 1.876 ± 0.010 ($P = 0.108$). The coefficient of repeatability was 0.070 for monofractal analysis and 0.026 for multifractal analysis. This study demonstrated that the automatic segmentation of nCPMs is feasible for fractal analyses. Both monofractal and multifractal analyses yielded similar results. The quantitative analyses of microvasculature at the capillary level may open up a new era for studying the microvascular diseases such as cerebral small vessel disease.

Keywords

retina; capillary perfusion; fractal analysis; segmentation; image processing; in vivo; Non-invasive capillary perfusion map

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***Corresponding Author:** Hong Jiang, MD, PhD, Mailing address: Bascom Palmer Eye Institute, University of Miami, Miller School of Medicine, 1638 NW 10th Avenue, McKnight Building - Room 202A, Miami, FL, 33136. Tel: (305) 482-5010, hjiang@med.miami.edu.

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Introduction

In biology and medicine, the shapes of structures, such as molecules, cells, tissues and organs, have been found to have fractal characteristics (Goldberger and West, 1987). A fractal is a mathematical set with a fractal dimension that differs from its topological dimension. Fractal analysis has been used to assess fractal characteristics, and fractal dimensions are capable of revealing the differences and irregularities in these structures (Mandelbrot BB, 1983). Quantitative measurements of the fractal dimension could be an effective approach to discriminate diseased tissues from healthy tissue (Talu, 2011). Moreover, the utilization of various types of medical imaging techniques promotes the application of fractal analysis in biology and medicine. Abnormal tissue can be detected by performing a fractal analysis for particular biological structures in medical images (Lim et al., 2009). Thus, fractal analysis with medical imaging techniques may provide an effective diagnostic methodology to detect diseases.

Fractal analysis has been extensively used to investigate changes in the retinal vascular complexity and branching density (MacGillivray and Patton, 2006; Avakian et al., 2002; Azemin et al., 2011; Cheung et al., 2010; Liew et al., 2008). It has been used to analyze retinal vascular trees in fundus photos and fluorescein angiography to quantitatively measure the microvascular damage in diabetes (Lim et al., 2009; Cheung et al., 2009; Avakian et al., 2002) and lacunar infarcts (Doubal et al., 2010; Cheung et al., 2010). However, these studies were based on the analysis of large vessels in the fundus images. Recent advances in optical imaging techniques have enabled the generation of nCPMs of the retinal capillary vessels using the Retinal Function Imager (RFI, Optical Imaging Ltd, Rehovot, Israel) (Burgansky-Eliash et al., 2010). The vessel network, as shown in perfusion maps, extends far beyond the fourth branches, and the spider web-like capillary map can be visualized. Based on the nCPMs, one could realize that the network of the vasculature is very dense, with the perfusion area covering almost the entire foveal region except for the avascular zone. Thus, nCPMs reveals more information about small vessels than standard fundus images. Although the microvascular network can be visualized in great detail, the lack of quantitative analyses on nCPMs may limit its application. The goal of this study was to demonstrate the feasibility of automated segmentation and fractal analysis applied to the retinal microvascular network observed in the nCPMs from the RFI.

Materials and Methods

This protocol was approved by the institutional review board for human research at the University of Miami; informed consent was obtained from each subject; and all subjects were treated in accordance with the tenets of the Declaration of Helsinki. In this prospective study, there were 6 healthy subjects (3 males and 3 females, mean age: 28.5 ± 6.2 years, range: 19 – 41 years) without history of cerebral small vessel disease, hypertension, diabetes or kidney disease. The nCPMs were acquired using RFI. As described elsewhere in details (Nelson et al., 2011; Nelson et al., 2005; Beutelspacher et al., 2011; Landa et al., 2012; Landa and Rosen, 2010). The device has a specific digital camera with 1024×1024 camera pixels and is attached to a standard Topcon fundus camera (Topcon Medical Systems, Inc, Oakland, NJ, USA). Stroboscopic illumination with wavelengths between 530 and 590 nm was used to capture eight consecutive images with flashes. Multiple image sessions were acquired for the creation of nCPMs using the proprietary software in the device. The motion of the hemoglobin in the red blood cells serving as a contrast agent was detected by the proprietary software (Nelson et al., 2011; Izhak et al., 2009). This software aligns sequential images with one another, and a signal-averaged map is generated, resulting in the nCPM (Figure 1A). One eye of each subject was imaged after pupil dilation. Two nCPMs were obtained at the same visit.

Image segmentation was custom-developed in Matlab (The Mathworks, Inc, Natick, MA, USA). The processing procedures were similar to those reported previously for segmenting the retinal vascular tree in fundus images (MacGillivray et al., 2007). The order of gray scale in the nCPMs (image size of 1024×1024 pixels) was inverted, and non-uniform illumination in the images was corrected by applying adaptive histogram equalization, which enhanced the contrast between the vessel and the background (Figure 1). The background noise was removed, and non-vessel structures were eliminated by morphological opening operations, which consisted of erosion followed by dilation. First, the supremum of the openings with a linear structuring element [$se=strel('line',10,0)$] at 18 rotations each 10° apart were taken as the vessel images where the vessel was enhanced along the vessel direction. The infima of the openings were then taken as the background. Subtracting the background image from the enhanced vessel image enabled the removal of the background noise and non-vessel structures. The processed image was then converted to a binary image using thresholding. Image J (Wayne Rasband, National Institutes of Health, Bethesda, MD) was used to skeletonize the segmented vascular network before the fractal analysis was run (Figure 1). In addition, an area of a diameter of 2.3 mm (512 pixels, half of the full size of the nCPM) centered on the fovea was extracted from the nCPMs, representing the macular area for fractal analysis (Figure 1).

The fractal dimension can be analyzed using monofractal and multifractal methods. In the present study, we used the fractal analysis toolbox from Benoit (TruSoft Benoit Pro 2.0, TruSoft International Inc, St. Petersburg, FL, USA) to analyze the fractal dimension of the segmented vascular network images. Compared to monofractal analysis, multifractal analysis may be more sensitive to subtle changes in the retinal vasculature because the retina appeared to have multifractal properties (Doubal et al., 2010; Talu, 2013). We used both monofractal and multifractal analyses to process nCPMs and compare the results. We measured the monofractal dimension (D_{box}) using the box-counting technique with a max size of 104 and a rotation of 15 in the monofractal analysis settings. We used the dimension D_0 as the measure of multifractal analysis; D_0 has been suggested as the most appropriate parameter of the retinal vasculature (Doubal et al., 2010).

A statistical software package (Statistic, StatSoft, Inc, Tulsa, OK) was used for descriptive statistics and data analysis. All data were presented as the mean $\pm$ standard deviation. A paired t-test was used to test the differences between these repeated measurements of each subject. $P < 0.05$ was considered significantly different between the two sets of images. The coefficient of repeatability (CoR) was calculated as 2 standard deviations (SDs) of difference between two measurements. CoR% was defined as the percentage of the CoR over the mean of two measurements.

Results

Our results showed that the methodology evaluated for quantifying nCPMs from RFI produced promising segmentation results (Figure 1) for further fractal analysis. A visual inspection of the segmented results indicates that the algorithm successfully detects vessels of all shapes and sizes, including the small capillaries that were not well defined in the original nCPMs because of a non-homogeneous textured background. Of note, no incorporation of the foveal contour detection stage was required to avoid the algorithm's false classification of the fovea as a capillary perfusion region. For the full-sized nCPMs, the mean D_{box} was 1.885 ± 0.020 , and the mean D_0 was 1.876 ± 0.010 when the two measurements were averaged ($P = 0.108$). The CoR of D_{box} was 0.070 (CoR%: 3.7%), and the CoR of D_0 was 0.026 (1.3%). For the macular area analyzed in the cropped image, the mean D_{box} was 1.732 ± 0.034 , and the mean D_0 was 1.773 ± 0.020 when the two

measurements were averaged ($P = 0.001$). The CoR of Dbox was 0.076 (4.3%), and the CoR of D0 was 0.052 (2.9%).

Discussion

In the present work, we demonstrated for the first time the feasibility of using an unsupervised algorithm for automatically segmenting nCPMs from the RFI. The retina provides a window to study the pathophysiology of cerebrovascular diseases. Pathological retinal vascular changes may reflect microangiopathic processes in the brain (Wong et al., 2002; Wong, 2004). Increased retinal venule diameters and decreased arteriovenous ratios have been found to be significantly associated with lacunar, rather than cortical ischemic strokes (Doubal et al., 2009; Bettermann et al., 2012). The causes of these changes are not clear. These structural changes in the larger vessels usually do not present in the early stages of microvascular disease, and therefore they may not be useful as markers to evaluate therapeutic interventions or to identify subclinical stroke patients. The study of the microvasculature of smaller vessels, especially at the capillary level, may hold the key to unlocking the mysteries of the enlarged venules in central nerve system vascular diseases, such as lacunar strokes.

RFI was designed to directly and non-invasively image the capillary network of the retina while providing a great deal of detail regarding capillary perfusion (Izhaky et al., 2009; Witkin et al., 2012). This information can be used to measure the microvascular dysfunction at the capillary level due to its unique ability to enhance the images by utilizing red blood cells as contrast agents. Based on the quantitative analysis of the capillary network and perfusion demonstrated in our work, the fractal analysis of nCPMs could be well suited for large-scale clinical studies. Ultimately, this method can be used in the clinical routine. Adding quantitative analysis of the nCPMs will extend the usefulness of this new imaging modality.

The normal retina has a vascular branching network obtained from fundus photos that provides an ideal vascular distribution of the large vessels with a fractal dimensional value of ~ 1.7 (MacGillivray et al., 2007; Talu, 2013). In patients with lacunar stroke, the fractal dimension was performed on wide-field fundus images and was found to be decreased, indicating the loss of vascular branching and complexity (Doubal et al., 2010). Controversially, the fractal dimension was found to be increased in another study investigating the fractal features in a small field centered by the optic nerve head (Cheung et al., 2010). The diameters of the smallest vessels in those previous methods were reported to be up to 50 μm , which represent the third to fourth branches of the retinal vessels (Avakian et al., 2002). The current fractal analysis of fundus photos and fluorescein angiograms may lack of the ability to visualize smaller vessels, such as capillaries, with widths as small as 4 μm . The capillary perfusion can be non-invasively visualized by RFI, which reveals the capillary network in great detail. As expected, the fractal dimension from both monofractal and multifractal analyses showed high scores, indicating a greater density and complexity in the capillary perfusion compared to the retinal vascular tree obtained from fundus photos. Interestingly, we found that the results from both monofractal and multifractal analyses are similar to those of the full-sized nCPMs. This phenomenon may indicate that nCPMs exhibit both mono- and multiple-fractal characterizations, which may differ from those of the retinal vessel tree (Doubal et al., 2010; Talu, 2013). On the other hand, when the macular area was analyzed, multifractal analysis showed higher score than that by monofractal analysis, which may indicate the multifractal features in the macular capillary perfusion. We noted that the score of the cropped image in the macula was smaller than that of the full-sized image, possibly due to the avascular zone in the fovea, which comprised a large portion of the image.

The nCPMs showed the details of capillary perfusion, making it almost impossible to manually segment the capillary network, as it often appears to be intricate or ill-defined. A fully automatic segmentation approach will be essential for applying the RFI in future clinical studies. As we demonstrated in the present work, this approach may add diagnostic value to advanced optical imaging modalities and could open up a new era for studying retinal microvasculature. The repeatability is good for both monofractal and multifractal analyses. It appears that the repeatability was better with multifractal analysis in both full-sized and cropped nCPMs. This may constitute a piece of evidence that the nCPMs are more suitable for multifractal analysis (Talu, 2013; Doulal et al., 2010).

In conclusion, this study demonstrated that the automatic segmentation of nCPMs is feasible for fractal analysis. The quantitative analysis may open a new field of quantitative analysis for the monitoring of microvascular changes in various ocular and systemic diseases, such as cerebral small vessel disease, lacunar stroke, hypertension and diabetic retinopathy.

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Highlights

- Retinal microvascular changes reflect microangiopathic processes in the brain.
- Retina is easily accessible for in vivo studying of cerebral small vessel disease.
- Non-invasive capillary perfusion maps (nCPMs) are generated.
- The nCPMs were automatically segmented and measured by fractal analysis.
- Quantitative in vivo analyses of nCPMs are feasible with high repeatability.

Figure 1. Automated segmentation results of a nCPM

A healthy subject was imaged with RFI, and an nCPM was created (A). To segment the capillary perfusion pattern, the order of gray scale in the image was inverted (B), and non-uniform illumination was corrected (C). After the removal of the background noise and non-vessel structures using a morphological opening operation the vessel network was segmented (D). The image was then skeletonized (E) for fractal analysis. The macular area (in the yellow circle with a diameter of 2.3 mm) was extracted (F) for fractal analysis. Bar = 1 mm.

Table 1Fractal analyses of nCPMs (n=6) using monofractal analysis (D_{box}) and multiple fractal analysis (D_0)

	First	Second	Difference	P-value*
Full image size (1024×1024)				
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
D_{box}	1.889 ± 0.017	1.881 ± 0.034	0.008 ± 0.035	0.108
D_0	1.877 ± 0.009	1.875 ± 0.014	0.002 ± 0.013	
Cropped image size centered on the fovea				
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
D_{box}	1.739 ± 0.029	1.725 ± 0.047	0.014 ± 0.038	0.001
D_0	1.777 ± 0.017	1.769 ± 0.029	0.008 ± 0.026	

* paired t-test between monofractal and multifractal analyses using averaged results of the two repeated measurements.