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Interactive retinal blood flow analysis of the macular region

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ABSTRACT

The study of retinal hemodynamics plays an important role to understand the onset and progression of diabetic retinopathy. In this work, we developed an interactive retinal analysis tool to quantitatively measure the blood flow velocity (BFV) and blood flow rate (BFR) in the macular region using the Retinal Function Imager (RFI). By employing a high definition stroboscopic fundus camera, the RFI device is able to assess retinal blood flow characteristics in vivo. However, the measurements of BFV using a user-guided vessel segmentation tool may induce significant inter-observer differences and BFR is not provided in the built-in software. In this work, we have developed an interactive tool to assess the retinal BFV and BFR in the macular region. Optical coherence tomography data was registered with the RFI image to locate the fovea accurately. The boundaries of the vessels were delineated on a motion contrast enhanced image and BFV was computed by maximizing the cross-correlation of pixel intensities in a ratio video. Furthermore, we were able to calculate the BFR in absolute values (μl/s). Experiments were conducted on 122 vessels from 5 healthy and 5 mild non-proliferative diabetic retinopathy (NPDR) subjects. The Pearson's correlation of the vessel diameter measurements between our method and manual labeling on 40 vessels was 0.984. The intraclass correlation (ICC) of BFV between our proposed method and built-in software was 0.924 and 0.830 for vessels from healthy and NPDR subjects, respectively. The coefficient of variation between repeated sessions was reduced significantly from 22.5% to 15.9% in our proposed method ($p < 0.001$).

Introduction

As a leading cause of blindness in American adults (Mohamed et al., 2007), diabetic retinopathy (DR) is related to the damage of retinal blood vessels and retinal neuronal cell death (Barber, 2003; DeBuc, 2013; National Eye Institute, 2012). Early diagnosis of DR by retinal imaging plays an important role in arresting the progression of the disease and slowing the loss of vision. However, the disturbance of retinal hemodynamics involved in the onset and progression of DR is not yet fully understood (Kur et al., 2012; Pemp and Schmetterer, 2008).

Various techniques have been developed to assess the retinal circulation, including video fluorescein angiography (Arend et al., 1995), ultrasound flowmetry (Bohdanecka et al., 1999), laser speckle flowgraphy (Sugiyama et al., 2010), retinal vessel analyzer (Garhofer et al., 2010), color Doppler imaging (Harris et al., 1998; Stalmans et al., 2011), laser Doppler velocimetry (Riva et al., 1979) and scanning laser Doppler flowmetry (Petrig et al., 1999; Riva et al., 2001; Kimura et al., 2003; Nagaoka et al., 2004; Polska et al., 2003). However, these methods all suffered from the drawbacks of either being invasive, having high variability or only providing the measurement of the larger retinal vessels while the capillary hemodynamics play a vital role in tissue oxygenation.

By employing a high definition stroboscopic fundus camera, the Retinal Function Imager (RFI-3005, Optical Imaging, Rehovot, Israel) is able to assess retinal blood flow characteristics in vivo to the resolution of single red blood cells moving through capillaries (Nelson et al., 2005) using a non-invasive approach. Under red-free illumination, the retinal blood flow velocity (BFV) is calculated by using cross-correlation matching to determine the relative offset of path segments in sequential images that contain approximately the same pattern of moving blood cells (Izhaky et al., 2009). Moving red blood cells provide the contrast between adjacent frames and a non-invasive capillary perfusion map (nCPM) is generated without the need of injection of a contrast agent. The RFI built-in software provides a user-guided vessel tracking tool, which traces the mouse cursor movement controlled by the operator. With the input of user defined fovea location, the vessel type (artery or vein) is identified and the retinal BFV is calculated automatically. If the coefficient of variation (COV) of BFV value in multiple sessions is greater than 45%, the labeled vessel is invalid and is hence excluded from the final analysis (Izhaky et al., 2009; Optical Imaging, 2010).

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Several studies have been conducted using the RFI built-in software in DR (Burgansky-Eliash et al., 2010, 2013; Landa et al., 2011) and various other pathologies. (Barak et al., 2012; Beutelspacher et al., 2011; Böhni et al., 2015; Burgansky-Eliash et al., 2014; Landa and Rosen, 2010).

Despite the promising potential of the RFI, there are a few drawbacks in the built-in analysis software of our current research model (RFI 3005) that could impair the accuracy of the hemodynamic measurements. First, the segmentation of the vessel depends on the operator's cursor movement, which may, in our experience, induce significant inter-observer difference. Second, the location of the fovea is also depending on the user input, which is challenging to identify accurately. Third, the diameters of the vessels are not provided and thus blood flow rate information at each vessel is not available to the end users. Finally, there is a lack of widely accepted labeling systems for the retinal vessels and hence the results from different subjects are hard to compare. The BFV changes depend on the vessel type, length, hierarchy (e.g. secondary or tertiary vessel) and distance to the fovea. Without careful control of the vessel labeling task, the results of the particular clinical studies are hard to compare and reproduce.

In this work, we introduce an interactive tool to assess the retinal BFV as well as blood flow rate (BFR) in the macular region. The boundaries of the vessels are delineated by using Dijkstra's algorithm (Dijkstra, 1959) on a motion contrast enhanced image and BFV is computed by maximizing the cross-correlation of pixel intensities in a ratio video. Furthermore, we are able to calculate the blood flow rate (BFR) in absolute values (μl/s) which other currently available devices targeting the retinal microcirculation are not yet capable of. In addition, Optical coherence tomography (OCT) data from Cirrus HD-OCT 5000 (Zeiss Meditec, Dublin, CA) or Spectralis SD-OCT (Heidelberg Engineering, Germany) are registered with the RFI image to locate the fovea accurately with the following benefits:

- Accurate vessel type identification: if the starting point of a vessel is closer to the fovea, then the vessel is a vein and vice versa.
- Precise tracking of functional changes: vessels can be separated into different grids or rings in the macular region to track the functional changes more precisely;
- Multimodal image analysis advantage: a link between structural features (e.g. total retinal layer thickness in map format) with the functional features, i.e. BFV and BFR may add another dimension for the understanding of retinal pathologies.

We report measurements of BFV and BFR in normal healthy as well as in pathological subjects and demonstrate how retinal hemodynamic measurements are combined with OCT measurements to yield a quantitative multimodal platform for measuring the retinal disturbances in diabetic complications. These multimodal measures in the normal retina may serve as standards against which altered retinal hemodynamics in disease states can be evaluated.

Materials and methods

Subjects

This study was approved by an Institutional Review Board at the University of Miami. Prior to enrollment informed consents were obtained according to the tenets of the Declaration of Helsinki. All patients underwent RFI and OCT scanning at the Bascom Palmer Eye Institute, University of Miami, FL, USA. Five eyes from four healthy control subjects and five eyes from four mild non-proliferative diabetic retinopathy (NPDR) subjects were included in this study. All study subjects underwent routine ophthalmic examination with indirect ophthalmoscopy. Healthy control subjects had no history of retinal diseases, primary or secondary glaucoma, intraocular inflammation, intraocular surgery, diabetes mellitus or uncontrolled arterial hypertension. The diagnosis of the subjects in the mild NPDR group was based on indirect fundus examination by an expert retina specialist. For both groups, ophthalmic exclusion criteria were ocular media opacity, any previous intraocular surgery except uneventful cataract extraction at least 6 months prior to enrollment and myopia of more than 6 diopters. The demographic and clinical data are shown in Table 1.

Image acquisition

The RFI system is deployed based on a standard fundus camera extended by a customized stroboscopic flash lamp system. A green ("red-free") light with a spectrum of 548 ± 75 nm is used for illumination and the interval between consecutive flashes is typically 17.5 ms. One session of RFI data consists of 8 images with a resolution of 1024×1024 pixels in an area of 4.3×4.3 mm or 7.2×7.2 mm depending on the choice of field of view (20° or 35°) during the imaging acquisition. The setting of 50° field of view is also available in the device but is unsuitable for the BFV measurement assessment as the retinal blood cells are hardly visible due to the limited resolution. In this study, all of the images were captured with the setting of 35° field of view. The heartbeats of the patient were monitored with a nail probe sensor and the image acquisition was synchronized with the cardiac cycle to neutralize the effects of pulsation on arterial blood flow velocity. After the image acquisition, the RFI built-in software generated (a) the flow movie (a.k.a. "ratio video") through differential processing so that the motion of individual clusters of red blood cells can be followed by the human eye; and (b) the non-invasive capillary perfusion map (nCPM) was generated through analyzing the difference of pixel intensities in adjacent frames. (Izhaky et al., 2009; Nelson et al., 2005).

A good quality scanning session is characterized by sharp vessel borders on the raw fundus images, clear red blood cell movement along the vessels on ratio videos and a visible capillary network on the nCPM. Each subject was scanned repeatedly for 8–10 times and five good quality sessions were chosen for our proposed algorithm. In this work, each input RFI data session consisted of (a) the first frame of raw image (or key frame) used for registration, (b) ratio video used for BFV calculation and (c) nCPM image used for vessel tracking, as illustrated in Fig. 1.

Besides the RFI assessment, each patient was also scanned by Cirrus HD-OCT 5000 (Carl Zeiss Meditec Inc., Dublin, CA) or Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) after dilation. The volumetric data from Cirrus HD-OCT 5000 consisted of 200×200 A-scans on a 6×6 mm area while each scanning session for Spectralis SD-OCT consisted of 61×768 A-scans on an 8.9×8.9 mm area with the setting of Automatic Real Time (ART) equal to 10. The input OCT data were both the SLO image and the video with marked boundaries which were used for registration and thickness map calculation, respectively.

Image analysis

The proposed image analysis software was implemented using Matlab 2014a consisting of four main blocks, which are (a) registration, (b) fovea detection, (c) vessel tracking and (d) BFV and BFR calculations. The overview of the algorithm is illustrated in Fig. 2. For each analyzed vessel, we could automatically get the vessel type (artery or vein), vessel diameters, BFV, BFR, and coefficient of variation (COV) of BFV among sessions.

The details of each block are explained in the following subsection.

(a) Registration
First, the input RFI data sessions were registered to align the vessel structure so that the BFV and BFR could be easily analyzed

Table 1
Demographic information of the study subjects. Note that all healthy subjects had a visual acuity of 1.0.

Group	OD/OS	Age	Gender	Mean visual acuity (range)
Healthy	4/1	46–53	5 Female	1.0
Mild NPDR	3/2	56–68	4 Male, 1 female	0.56 (0.1–1.0)