Experimental Eye Research 146 (2016) 330–340

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

Preliminary investigation of multispectral retinal tissue oximetry mapping using a hyperspectral retinal camera

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A R T I C L E I N F O

Article history:
Received 7 November 2015
Received in revised form
31 March 2016
Accepted in revised form 1 April 2016
Available online 6 April 2016

Keywords:
Spectral imaging
Retinal oximetry
Total hemoglobin content
Optic nerve head
Optic disc
Hypoxia

A B S T R A C T

Oximetry measurement of principal retinal vessels represents a first step towards understanding retinal metabolism, but the technique could be significantly enhanced by spectral imaging of the fundus outside of main vessels. In this study, a recently developed Hyperspectral Retinal Camera was used to measure relative oximetric ($SatO_2$) and total hemoglobin (HbT) maps of the retina, outside of large vessels, in healthy volunteers at baseline (N = 7) and during systemic hypoxia (N = 11), as well as in patients with glaucoma (N = 2). Images of the retina, on a field of view of ~30°, were acquired between 500 and 600 nm with 2 and 5 nm steps, in under 3 s. The reflectance spectrum from each pixel was fitted to a model having oxy- and deoxyhemoglobin as the main absorbers and scattering modeled by a power law, yielding estimates of relative $SatO_2$ and HbT over the fundus. Average optic nerve head (ONH) saturation over 8 eyes was 68 ± 5%. During systemic hypoxia, mean ONH saturation decreased by 12.5% on average. Upon further development and validation, the relative $SatO_2$ and HbT maps of microvasculature obtained with this imaging system could ultimately contribute to the diagnostic and management of diseases affecting the ONH and retina.

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1. Introduction

Retinal oxygenation is a potential marker of several diseases such as retinal vessel occlusions (Williamson et al., 2009; Yoneya et al., 2002), diabetic retinopathy (Hammer et al., 2009; Holekamp et al., 2006; Lange et al., 2011; Linsenmeier et al., 1998) and glaucoma (Ito et al., 2008; Michelson and Scibor, 2006; Olafsdottir et al., 2014; Olafsdottir et al., 2011; Tezel and Wax, 2004). Measuring retinal oximetry non-invasively in humans has been a topic of interest since the 1960's (Hickam et al., 1959). A widely used technique consists of measuring retinal images at two wavelengths of light to compute oximetry values using certain assumptions (J. M. Beach et al., 1999; Hardarson et al., 2006). However, this technique can only be applied in large retinal vessels because it requires definition of background intensity around the vessel, and it depends on a calibration by means of the manual selection and assumption of *a priori* values in arterioles and venules to quantify oxygen saturation ($SatO_2$). Thus, absolute saturation values measured using two-wavelength models depend directly on the values used for calibration, e.g. 96% and 54% in arterioles and venules (Hardarson et al., 2006).

Approaches based on multispectral retinal images may allow the measurement of absolute oxygen saturation without calibration, by using spectral models to recognize the spectral signature of molecules of known (measured) absorption spectra, in this case hemoglobin. Previous studies have used multispectral (>2 wavelengths) images to provide physiologically plausible saturation values in large retinal vessels in humans (Mordant et al., 2011;

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http://dx.doi.org/10.1016/j.exer.2016.04.001
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Ramella-Roman et al., 2008), however, with low spectral resolution (6–8 wavelengths measured) or high acquisition time (10–15 min).

Mostly ignored in the above studies, retinal oximetry outside of large vessels may be more closely related to tissue state, as capillaries are responsible for a significant portion of oxygen and nutrients exchanges with cells. Using multispectral models, retinal oximetry was studied in a small region of retinal tissue in humans (D. Schweitzer et al., 2001) as well as in ONH tissue of animal models (J. Beach et al., 2007; Khoobehi et al., 2004). Previous measurements in retina, outside of large vessels, have also been reported using Fourier-transform based spectral retinal imaging (Yoneya et al., 2002) in patients with central retinal vein occlusion. Using the same instrument in glaucoma patients, Ito et al. (2008) showed that oxygen saturation in the juxta-papillary retina was significantly lower in glaucoma patients than in controls.

Manipulating systemic oxygen saturation provides a means to vary oximetry to explore wider ranges of oxymetric measurements and thereby provocatively validate new methodologies. For instance, in (Kisilevsky et al., 2008), systemic hypercapnia and hyperoxia were combined during measurements of retinal arteriolar blood flow; the vasoconstrictive effect of hyperoxia was found to counteract the vasodilation induced by hypercapnia. Recently, using a two-wavelengths model in large retinal vessels, Choudhary et al. (2013) found that systemic hypoxia (9% decrease in peripheral $SatO_2$) decreased arteriolar and venular oxygenation by ~8%, while the arterio-venous difference was unaffected. Hyperoxia, achieved during 100% oxygen breathing, resulted in a significant increase of the retinal arterioles and venules oxygen saturation relative to normoxia (Olafsdottir et al., 2015). The net arteriovenous difference decreased significantly during hyperoxia because the oxygen saturation increase was greater in the venules.

In this study, we exploit an improved version of a prototype retinal camera based on a tuneable light source (Patel et al., 2013a; Shahidi et al., 2013) that can provide high spectral resolution multispectral fundus images. Compared to the previously described prototype, the acquisition time is now under 3 s and the image quality has been improved by removing a major artifact. We also introduce a simple linear spectral model to analyze the images. This allows the non-invasive derivation of oximetric maps of the fundus from capillaries and optic nerve head tissue in human subjects. The method is demonstrated in healthy subjects during systemic gas manipulations and a potential application is proposed in preliminary scans of glaucoma patients.

The Metabolic Hyperspectral Retinal Camera (MHRC) (Optina Diagnostics, Montreal, Canada) enables spectrally resolved imaging on a 30° field of view of the fundus with an acquisition time of 1–3 s, which limits eye movements and artifacts. Moreover, the analysis model yields relative $SatO_2$ measurements independent of any *a priori* values, and provides an additional measurement with potential clinical value, the relative total (oxy and deoxygenated) hemoglobin concentration (HbT). HbT is proportional to the local blood content and thus indirectly related to tissue perfusion. Finally, the rapid automatic preprocessing software and linear model used allow for automatic generation of relative $SatO_2$ and HbT over the entire retina in under 30 s, which opens the door to onsite oximetry mapping in patients in the clinic.

2. Methods

2.1. Hyperspectral retinal camera

The Metabolic Hyperspectral Retinal Camera (MHRC, see Fig. 1(a)-(c)) developed by Optina Diagnostics (Montreal, Canada) is based on a custom-built mydriatic fundus camera incorporating a Tunable Light Source (TLS) able to transmit safe light levels within a spectral range covering the visible to near infrared with a narrow bandwidth (~2 nm). The instrument is capable of imaging the retina on a >30° field-of-view at high resolution (1.3 Megapixels) at several wavelengths (>30) in a few seconds (up to 27 images obtained at different wavelengths per second). Images of the retina are sequentially obtained for different monochromatic illumination wavelengths in order to build a hyperspectral cube of data. In each pixel of the fundus, spectral signatures can be used to localize and quantify biomolecules of known absorption spectra (Fig. 1(d)), such as oxy (HbO) and deoxyhomoglobin (HbR). In data presented below, images were captured between 500 and 650 nm in steps of 2, 5 or 10 nm (see details below).

2.2. Data preprocessing and analysis

The raw reflectance images 'I' were preprocessed using in-house Matlab (The Mathworks, Natick, MA) code. The images were first normalized for spatial and spectral variations in light source intensity and system optics (procedure described in (Patel et al., 2013a)). The resulting hyperspectral cube I_{norm} is theoretically normalized by incident intensity and corrected for parasitic reflections, for temporal and spatial inhomogeneities of incident light, and for the spectral and spatial intensity response of the camera. Then, each image of the hyperspectral cube was spatially registered with the previous one in order to correct for eye movement. In the resulting 3D image cube, a reflectance spectrum is generated at each pixel which can be analyzed to recognize the known spectral signatures of the main chromophores in the visible wavelengths, HbO and HbR.

The main large retinal vessels as well as the optic nerve head (ONH) were segmented automatically and semi-automatically, respectively. The automatic vessel segmentation was done using Fiji (Schindelin et al., 2012) and in-house Matlab code to implement filtration and morphological operations (top-hat transformation, thresholding). For the ONH, Matlab code was written to allow manual selection of the ONH region and adjustment of the border based on intensity thresholding of the reflectance image.

At each pixel outside of large vessels, preprocessed reflectance measurements $I_{norm}(\lambda)$ were fitted to a model based on the Modified Beer-Lambert law (Delpy et al., 1988) and an exponential term modelling diffusion (Roggan et al., 1999; Dietrich Schweitzer et al., 1995):

$$\log\left(\frac{1}{I_{norm}(\lambda)}\right) = OD(\lambda) = (S_{HbO}(\lambda)\cdot p_1 + S_{HbR}(\lambda)\cdot p_2) + p_3 + p_4 \log\left(\frac{1}{\lambda}\right), \tag{1}$$

In the above model equation, $I_{norm}(\lambda)$ is the normalized and registered (preprocessed) reflectance, the inverse log of which yields the optical density $OD(\lambda)$; the p_i's are the unknown parameters estimated at each pixel; S_{HbO} and S_{HbR} are the known absorption spectra of oxy/reduced hemoglobin (HbO/HbR) (Scott A. Prahl, 2010). Appendix A details the derivation of this equation.

The model yields oxygen saturation $SatO_2$ and relative total hemoglobin concentration (HbT = HbO + HbR) at each pixel (i.e. 2D maps), computed from the estimated parameters by Eq. (2). Being conservative, without direct validation of the model (e.g. against *ex vivo* measurements in phantom or Monte-Carlo simulations), we cannot claim to measure absolute $SatO_2$. Therefore, we present relative $SatO_2$ ($rSatO_2$) maps, which we believe could also approximate absolute $SatO_2$ once validated. Relative $SatO_2$ is in fact what is measured when employing standard calibrated models (such as the two-wavelength model), since $SatO_2$ from these

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