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Hyperspectral Image Mapping Spectrometry for Retinal Oximetry Measurements in Four Diseased Eyes

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Abstract

We describe the hyperspectral Image Mapping Spectrometer (IMS) which is able to acquire spectral images of the retina with no scanning components. We then utilize this technology to obtain absolute oxygen saturation measurements in four patients with retinal diseases. The IMS acquires spatial and spectral information of a scene in a single camera exposure time. The IMS is attached to the port of a Topcon TRC-50EX fundusoscopic camera in order to acquire images. The IMS has high spatial (350×350) and spectral sampling (40), and avoids motion artifacts associated with eye movements. Oximetry values are obtained by measuring the absorption of retinal blood. Four patients with different retinal diseases were imaged via the IMS in this study. Oxygen saturation is calculated using a least squares fit applied to the whole blood oximetry equation. Oxygen saturation was found to be 88.6% (±1.9) and 34.1% (±7.0) for the artery and vein, respectively, in a patient with non-exudative age-related macular degeneration (AMD) with borderline glaucoma, 100.0% (±0.0) and 50.4% (±8.2) in a patient with exudative AMD, 100.0% (±0.0) and 45.2% (±7.0) in a patient with retinitis pigmentosa, and 94.1% (±2.5) and 44.9% (±5.6) in a patient with chronic iridocyclitis. These values are consistent with previously published data. Based on spatial and spectral data, we created an oxygen saturation map with oxygenation data overlying retinal vasculature. Hyperspectral imaging may be a promising way to measure retinal oxygen saturation. IMS technology is advantageous in its ability to obtain images free of motion artifacts.

Keywords

retina; oximetry; hyperspectral; snapshot; age-related macular degeneration; glaucoma; retinitis pigmentosa; chronic iridocyclitis; spectrometry; retinal oxygenation

Introduction

Inadequate oxygenation of the eye perturbs retinal function and processes, and can lead to visual loss [1]. The difference in oxygen saturation between the retinal arteries and veins

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correlates with oxygen metabolism. When light interacts with the retina, spectral reflectance is generated; this conveys information about the state and composition of retinal tissue, including oxygen saturation [2, 3]. Light has been used to estimate oxygen saturation levels non-invasively based on the unique spectral signatures of oxyhemoglobin and deoxyhemoglobin in the retinal blood vessels. Notable publications in the past have acquired oximetry data using only two- or three-wavelength measurements of the eye [2, 4, 5]. However, the acquisition of oxygen saturation values is complicated by undesired scattering and absorption effects due to heterogeneous tissue properties; extensive calibration procedures are required to account for these effects. Significant work has been done to address the challenges in obtaining accurate oxygen saturation measurements posed by vessel diameter differences, variations in pigment, and scattering and absorption effects of blood [6–10].

Two commercial retinal oximeters based on two-wavelength oximetry, the Oxymap T1 [11, 12] and the Imedos oximeter [10], have been utilized in a significant portion of the clinical research done regarding saturation measurements in retinal diseases. These systems produce automatic oxygen saturation maps, but are limited to retinal vessels. Oximetry of choroidal vessels with the Oxymap T1 can be done for lightly pigmented individuals, but its accuracy needs refinement [13]. Geirsdottir, et al. used the Oxymap T1 to demonstrate that oxygen saturation in retinal veins decreases with age in healthy eyes [12]. Several studies have found reduced oxygen saturation values in occluded arteries and veins [14–17] and increased venous oxygen saturation in patients with exudative age-related macular degeneration (AMD) [18], advanced primary open-angle glaucoma [19–21], diabetic retinopathy [22–24], and retinitis pigmentosa [25, 26]. For patients with retinitis pigmentosa, increased arterial oxygenation saturation and increased arteriovenous oxygen saturation differences have been observed, with age seemingly affecting these values [27, 28]. Another study on primary open-angle glaucoma [29] indicated no change in venous oxygen saturation. Oxygen metabolism is clearly altered in many diseases and trends can be seen in published work, but more formal studies are needed to explore the variability amongst studies and better understand the physiological mechanism for altered oxygen metabolism.

Hyperspectral imaging (HSI) allows for the collection of multiple spectral bands in a single image, and has been increasingly utilized in measuring ocular oximetry [21, 30–36]. HSI is advantageous over two- or three-wavelength oximetry in multiple ways. There is a reduced need for system calibration due to the increased number of spectral channels which reduces the level of spectral noise [37]. Given its greater number of spectral samples, HSI allows one to solve more complex oximetry models with multiple unknown parameters [38]. Traditional HSI requires scanning which has several drawbacks. Scanning times require eye fixation and image registration to reduce motion artifacts [30]. Additionally, the total amount of light collected is reduced by a factor proportional to the number of scanned frames required, compromising overall light collection efficiency [39]. Snapshot hyperspectral systems acquire spatial and spectral information of an object simultaneously, and due to the lack of scanning, allow for visualization of dynamic processes free of motion artifact. Previous work has been done utilizing snapshot retinal imaging technology in a system called Computed Tomography Imaging Spectroscopy (CTIS) [31–33]. Unfortunately, CTIS is significantly affected by the unfocused background commonly present in tissue imaging and

also requires long computational times to analyze results. Fiber bundles have also been used for snapshot spectral imaging, but the limited amount of fibers gives low spatial sampling of the fundus image on the order of 20×20 spatial values [35, 36].

This study focuses on the Image Mapping Spectrometer (IMS), a snapshot HSI with high spatial sampling density (350×350) and 40 spectral channels, which acquires images at the frame rate of the CCD camera [40]. This system overcomes previous snapshot limitations associated with CTIS and fibers. We investigate absolute oxygen saturation values without any prior calibration for a patient with exudative AMD, a patient with non-exudative AMD and borderline glaucoma, a patient with retinitis pigmentosa, and a patient with chronic iridocyclitis to demonstrate applicability in various retinal milieu. Furthermore, we discuss upcoming enhancements of the technology and its algorithms as well potential future applications.

Materials and Methods

Image Mapping Spectrometry

The Image Mapping Spectrometer (IMS) uses a custom-fabricated mirror array constructed from aluminum to divide an imaged scene into 350 strips. These strips are mapped onto a monochromatic CCD pixel array (Imperx Inc. ICL-B4820M) with unused pixel space, called void space, separating the individual strips. Using a prism array, mapped strips are dispersed across the void space. The dispersed light contains the spectral signature of the imaged object being mapped by the strip. A pixel on the detector corresponds to a unique location in the hyperspectral datacube (x, y, λ). The present spectral range is 470 to 670 nm, and is limited by the amount of void space separating mapped strips. Additional information about the IMS system can be found in our previous publications [41–44].

The IMS was integrated with a Topcon TRC-50EX camera using the top port (Fig. 1). The use of the IMS did not require any physical alteration to the fundus camera besides a frame that mounted the system to the camera's body. The installation of the IMS did not affect the fundus camera's operation by the clinician. A model eye was used to align the imaging system. All images were captured with a 50° field of view which allowed visualization of both the optic nerve and macula in a single image. The IMS's electronic shutter was synchronized with the Topcon's xenon flash using the TTL signal from the joystick. The xenon flash's brief duration avoided patient discomfort while providing enough light intensity for the IMS's full dynamic range. Due to a variable delay between the flash and TTL signal, the IMS's exposure duration was 200 ms once triggered. The flash intensity was controlled through 21 different flash settings on the dashboard of the TRC-50EX to adjust for different levels of fundus reflectivity. The discrete settings range from 12 Ws (Watt-seconds) to 300 Ws and increase logarithmically. Typical flash settings used on patients were between 12 Ws and 50 Ws. To prevent saturation of the IMS camera at 12 Ws, a neutral density filter with an optical density of 0.3 was inserted into the aperture stop of the IMS system. The IMS camera was triggered by a dedicated flash button integrated with the fundus camera joystick. Acquired raw data was analyzed off-site in Matlab.

Oximetry

The calculation of oxygen saturation is based on the equation for the absorbance of whole blood.

$$A_{\lambda} \cong S(\lambda) + s * c * d \left[OS * \left(\epsilon_{HbO_2}(\lambda) - \epsilon_{Hb}(\lambda) \right) + \epsilon_{Hb}(\lambda) \right]$$

Where c is the total hemoglobin concentration, d is the path length, OS is the oxygen saturation, and s and $S(\lambda)$ are scattering terms caused by red blood cells [2]. When $S = 0$ and $s = 1$, the equation reduces to the absorption for hemolyzed blood. $\epsilon_{HbO_2}(\lambda)$ is the extinction coefficient of oxy-hemoglobin, and $\epsilon_{Hb}(\lambda)$ is the extinction coefficient of deoxyhemoglobin. The values of these coefficients have been previously published [45]. Absorbance is related to the transmittance of the vessel in accordance with the Beer-Lambert law by

$$A_{\lambda} = -\log(T_{\lambda})$$

Since transmission measurements of the vessels are not accessible in the eye, the transmittance is calculated as

$$T_{\lambda} = \frac{V_{\lambda}}{B_{\lambda}}$$

Where V_{λ} is vessel reflectance and B_{λ} is the fundus background reflectance adjacent to the vessel. This relationship gives approximated transmittance values. The normalization of vessel reflectance by adjacent fundus background reflectance removes spectral components associated with the background. This approximation does not account for all scattering effects, such as lateral diffusion from adjacent tissue and may affect the estimate's accuracy. For the purposes of simplicity, the calculation of OS is reduced to the calculation of OS for hemolyzed blood. The parameters c , d , and OS are solved by a best least squares fit (LSQ) to the measured absorbance of the vessels using the IMS's 40 spectral values. The term, cd , is bound from 0 to 1, and the oxygen saturation, OS , is bound from 0% to 100%. Oxygen saturation is found by averaging the absorbance measurements using every pixel within a certain region in the vessels.

To account for the difference in spectral resolution between the IMS and the spectrometers used to acquire published extinction coefficient data, the published values [45] were mathematically convolved with the spectral response of the IMS. The IMS's spectral response was modeled by measuring the spectrum of 5 filters with a 1-nm bandwidth at different positions in the spectral range and interpolating the response at all wavelengths (Fig. 2).

Oximetry Maps

Oximetry maps overlay oximetry values along the lengths of retinal vessels in a pseudo-colored fundus image to demonstrate the change in OS with respect to position. Oximetry

maps allow researchers to observe oxygen change in diseased eyes and have elucidated oxygen metabolism for a variety of conditions [11, 46]. To construct oximetry maps with the IMS, the vessels were separated from the fundus background by manually drawing a mask in Matlab. The mask was then segmented into separate 100-pixel areas. The reflectance spectrum was averaged for each 100-pixel segment. To calculate transmittance, the averaged reflectance spectrum was divided by the mean background reflectance spectrum defined as a 100-pixel sampling of the fundus background adjacent to the vessel segment.

Patients

This study included 4 patients with different retinal pathologies (exudative AMD, non-exudative AMD with borderline glaucoma, retinitis pigmentosa, chronic iridocyclitis) with the aim of obtaining their absolute oximetry measurements with the IMS. Patients were identified during routine clinical visits at the Alkek Eye Clinic at the Baylor College of Medicine in Houston, TX; all patients were from the panel of a single provider [REC]. Patients were formally consented for this HIPAA-compliant study which was approved by the Baylor College of Medicine Institutional Review Board. Images were acquired by a trained clinician. Images with poor quality were excluded from analysis.

Results

Hyperspectral Images

Hyperspectral image stacks were acquired for each patient. Each hyperspectral image stack has a datacube size of 350×350×40. We selected 8 spectral channels ranging from 470 nm to 670 nm for display in Figure 3. The channels were pseudocolored using a wavelength-to-RGB conversion algorithm [47]. The field-of-view (FOV) of the images was centered on the optic nerve. Contrast between retinal vessels and the fundus background is greatest between 500 nm and 600 nm due to the high absorption of blood at these wavelengths. At the 670 nm wavelength, the contrast between the vessel and the fundus background is very poor. Visualization of choroidal structures is enhanced at wavelengths greater than 600 nm (Fig. 3). Composite images were formed by combining all 8 spectral channels for each patient (Fig. 4).

Oximetry

A plot of measured absorbance from the patient with AMD and borderline glaucoma for a 50-pixel region in the retinal artery and a 50-pixel region in the retinal vein is shown (Fig. 5a). An LSQ fit is also plotted with estimated oxygen saturation (Fig. 5b, c). Table 1 shows the average oxygen saturation values for all patients. Each oxygen saturation value is derived from a constant region in the patient's eye averaged over several image stacks. Oxygen saturation was found to be 88.6% (± 1.9) and 34.1% (± 7.0) for the retinal artery and vein of the patient with non-exudative AMD and borderline glaucoma, 100.0% (± 0.0) and 50.4% (± 8.2) for the patient with exudative AMD, 100.0% (± 0.0) and 45.2% (± 7.0) for the patient with retinitis pigmentosa, and 94.1% (± 2.5) and 44.9% (± 5.6) for the patient with chronic iridocyclitis. The arteriovenous differences in oxygen saturation were 54.5% (non-exudative AMD w/ borderline glaucoma), 49.6% (exudative AMD), 54.8% (retinitis pigmentosa), and 49.2% (chronic iridocyclitis).

Oximetry map

An oximetry map was generated for the patient with AMD and borderline glaucoma (Fig. 6). Each segment is 100 pixels in area and color-coded based on oxygen saturation.

Discussion

The results of this study demonstrate the capability of the Image Mapping Spectrometer to perform oxygen saturation calculations of the retinal vessels in different fundus conditions. The incorporation of the IMS with the TRC-50EX camera did not impede the clinician's ability to observe the fundus and acquire images, although the configuration can potentially be improved. For example, the present CCD camera (Imperx Inc. ICL-B4820M) can be replaced with an sCMOS camera (pco.edge 5.5) to improve detector array performance. The sCMOS detector array offers a faster maximum frame rate (100 fps vs 7.2 fps), wider dynamic range (88.6 dB vs 65 dB), diminished readout noise ($1.0 e^-$ at 30 fps vs $16.0 e^-$ at 3.2 fps), and better quantum efficiency when compared to the present CCD detector array [48, 49]. The quantum efficiency is greatly improved in the red and near-infrared region of the electromagnetic spectrum. This region is useful for visualizing the choroidal vasculature due to the deeper penetration of longer wavelengths. Despite the IMS system's high light throughput efficiency of 58% [43], the level of halogen illumination sufficient for image acquisition often caused the patient discomfort. The improved detector array performance of the sCMOS would enable image acquisition with the lower intensity halogen settings, preventing patient discomfort. Thus, hyperspectral images of the eye could be acquired at the frame rate of the camera since halogen illumination is emitted continuously versus the xenon flash which is emitted intermittently. This allows for videos of oxygen dynamics in the eye. Another potential area for improvement concerns real-time construction of the hyperspectral data cubes. Currently, this is occurring post-image acquisition, but real-time reconstruction is possible with the IMS [40] and would allow the clinician to correct any field-of-view or alignment issues while the patient was still positioned in the machine.

Oximetry calculations performed in this study were based on previous publications using the hemolyzed version of the whole blood oximetry equation. An LSQ solution was used for each measured absorbance spectrum for the pixels sampling a region of retinal vessels (Fig. 5). Average oxygen saturation was calculated for 4 patients (Table 1). Over 4 patients and with 18 total measurements, the average arterial oxygen saturation was 95.8% (± 5.1) and the average venous oxygen saturation was 43.5% (± 8.9) with an arteriovenous difference of 52%. The variance of oxygen saturation values was small between patients despite different disease states. Using a scanning hyperspectral system and measured mean oxygen saturation values for healthy patients, Schweitzer [38, 50] suggested a normal oxygen saturation of 92.2% (± 4.1) for arteries and 57.9% (± 9.9) for veins with a mean arteriovenous difference of 35%. It is reasonable to assume that different disease states are associated with different levels of oxygen saturation. Elevated oxygen saturation levels have been found in diseased eyes [18, 19, 22, 25], but reliable data for healthy controls does not yet exist in order to validate this trend. Another current challenge in interpreting retinal oxygenation saturation values is that different oximetry systems and techniques tend to produce different measures. Further experiments are needed to validate the oxygen saturation measurements obtained

here. The oxygen gradient maps show clear distinction between retinal arteries and veins as expected, but the variation of oxygen saturation values seen with vessel location warrants further investigation.

IMS technology offers multiple advantages over two-wavelength systems. The Oxymap T1 and other two-wavelength systems provide reliable oxygen saturation maps; however, these systems are limited to retinal vessels and do not provide choroidal oximetry for all patients. The IMS's spectral range can be modified to span from green to near-infrared wavelengths, which would also enable the simultaneous visualization of the choroidal and retinal vessels for oximetry. Two-wavelength systems require somewhat arbitrary calibration procedures [10] due to the limited number of wavelength channels. In contrast, hyperspectral systems use more complex oximetry models, mitigating the need for calibration. Additionally, the multi-band spectral signatures of the IMS allow for the probing of more biomolecular signatures, such as different drusen types in AMD [51]. The IMS may offer the benefit of lower cost (\$35,000) compared to other similar technologies (\$72,000 for the Oxymap T1) [52]. The IMS has high spatial sampling compared to other snapshot modalities. In this study, low magnification settings were chosen in order to maximize the FOV, but it might be insightful to evaluate smaller regions of the posterior segment with higher vasculature resolution in future experiments. Additionally, the IMS avoids heavy computational times, and is minimally affected by unfocused background light. Further improvements to the current setup and algorithms are being planned. Additionally, video rate oximetry maps of retinal vasculature would provide useful information about oxygen dynamics.

We are still learning about the clinical significance of retinal oxygenation saturation levels, but they may play a key role in better understanding disease states and guiding treatment. IMS might be able to provide clinicians with this potentially important data. While the technology still requires validation studies and further investigation into the interpretation of its data output, it holds promise to become a novel and valuable imaging modality in the future.

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Fig. 1.

The IMS was mounted onto a TRC-50EX fundus camera. (a) The IMS provides a 1.25 $\times$ magnification of the top port image. (b) The mapper splits the image into 350 strips and images these strips onto the detector (Imperx Inc. ICL-B4820M). These strips are dispersed onto the detector via a prism and lenslet array. The hyperspectral datacube size is 350 $\times$ 350 $\times$ 40 once the unprocessed images are reconstructed in Matlab.

Fig. 2.
The normalized extinction coefficients of oxygenated and deoxygenated blood after convolution with the spectral response of the IMS.

Fig. 3. Selected spectral channels from an image stack acquired from the patient with AMD and borderline glaucoma. Each spectral channel has been pseudocolored with an RGB value. Notice the artifact from stray light seen in the upper left corner of the image captured at 481 nm; this occurred when the top port of the imaging system was not shielded from ambient light in the room.

Fig. 4.
Composite images of 8 spectral channels from a patient with non-exudative AMD and borderline glaucoma (**a**), a patient with retinitis pigmentosa (**b**), a patient with exudative AMD (**c**), and a patient with chronic iridocyclitis (**d**).

Fig. 5. Oxygen saturation calculations for two 50-pixel regions of an eye with non-exudative AMD and borderline glaucoma (a). Using LSQ fitting, the oxygen saturation is estimated for a retinal vein and artery. The fitted curve against the measured absorbance is shown for the retinal vein (b) and the retinal artery (c).

Fig. 6.

Oxygen saturation map of the patient with AMD and borderline glaucoma. Oxygen saturation values are overlaid onto the map in proximity to vessel segments. Vessel segments are color-coded based on oxygen saturation.

Table 1

Average oxygen saturation for each patient

Patient condition (n = # of measurements)	Artery (%)	Vein (%)	Arteriovenous difference of means (%)
Non-exudative AMD w/ borderline glaucoma (n=5)	88.6 (± 1.9)	34.1 (± 7.0)	54.5
Exudative AMD (n=5)	100.0 (± 0.0)	50.4 (± 8.2)	49.6
Retinitis pigmentosa (n=5)	100.0 (± 0.0)	45.2 (± 7.0)	54.8
Chronic iridocyclitis (n=3)	94.1 (± 2.5)	44.9 (± 5.6)	49.2
Mean (n=18)	95.8 (± 5.1)	43.5 (± 8.9)	52.3