Hyperspectral Imaging for Measurement of Oxygen Saturation in the Optic Nerve Head

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Methods
The optic nerve head (ONH) and overlying vessels in cynomolgus monkey eyes were imaged with a fundus camera attached to a hyperspectral imaging system. Images were acquired with inspiration of room air and pure oxygen and at controlled intraocular pressures (IOP) of 15 mm Hg (normal) and 60 mm Hg (sustained for up to 5 minutes). Changes in relative blood oxygen saturation in the vessels and ONH were assessed from reflectance spectra. Saturation maps were derived from contributions of oxygenated and deoxygenated hemoglobin spectral signatures extracted from hyperspectral images. The results obtained with hyperspectral imaging were compared with known experimental outcomes.

Results
Pure oxygen markedly increased oxygen saturation in veins. Increases in arteries and the ONH were smaller. The results obtained with hyperspectral image analysis agreed with known changes in oxygen saturation from breathing experiments. Raising IOP reduced saturation in all structures and resulted in profound desaturation of arteries. During sustained high IOP, a rebound in saturation was observed in the ONH. Spatial maps clearly showed the saturation changes in arteries, veins, and surrounding tissues.

Conclusions
Hyperspectral imaging can be adapted to measure and map relative oxygen saturation in retinal structures and the ONH in nonhuman primate eyes. (Invest Ophthalmol Vis Sci. 2004;45:1464–1472) DOI:10.1167/iovs.03-1069

Pathologic conditions in the retina and optic nerve head (ONH) can cause vision loss and blindness. Both structures have a high demand for oxygen, and loss of the normal oxygen supply through vascular insufficiency is believed to play an important role in diseases affecting the retina and ONH. 1–5 Hypoxia of the retina and ONH is believed to be a factor in the development of ocular vascular disorders, such as diabetic retinopathy, 4,5 arteriovenous occlusion, 6 and glaucoma. 7 The ability to obtain relative measurements of oxygen saturation in the human ocular fundus could aid diagnosis and monitoring of these and other disorders. For example, measurement of changes in retinal and ONH oxygen saturation under controlled conditions could establish relationships between oxygen consumption, blood sugar levels, and vascular autoregulatory function in diabetic retinopathy. Assessment of oxygenation in the ONH may facilitate early detection of the onset of glaucoma, a disease in which timely diagnosis is crucial for effective treatment.

Measurements of oxygen tension (PO2) in the ONH have been performed using O2-sensitive microelectrodes inserted into the eye. 7–11 Although this technique is accurate and can determine PO2 distribution in three dimensions, its invasive nature limits its use to animal models and precludes clinical application. Another technique involving injection of a phosphorescent dye has been used to study PO2 in the retinal and choroidal vessels, as well as the microvasculature of the ONH. 9,12–14 However, this use of the dye in humans has yet to be approved. Imaging techniques based on spectral changes of oxygenated hemoglobin (HbO2) and reduced hemoglobin (Hb) have been used in humans to assess oxygen saturation in the ocular fundus 6 and in retinal artery/vein pairs. 15–22 These methods have been based most often on recordings at several discrete wavelengths chosen for their relative sensitivity to changes in oxygen saturation. 15–19,22 Full spectral methods, using a continuous range of wavelengths, were developed by Delori 23 and Delori and Phlipsen 24 to record the reflectance profile versus wavelength from the ocular fundus. Full spectral imaging technique has been used by Schweitzer et al. 20,21 to measure oxygen saturation in retinal arteries and veins under various conditions. Yoneya et al. 6 mapped oxygen saturation in the ocular fundus using Fourier transform spectral imaging. 6 The full spectral technique used most often, that of Schweitzer et al., 20,21 employs a high-resolution imaging spectrograph to collect the spectral information from a band of tissue in a single spatial dimension. The method acquires data rapidly and is applicable for use in human subjects.

We describe herein a new technique for spatially mapping the relative oxygen saturation in retinal structures based on hyperspectral imaging, a method for recording the optical spectrum at each pixel of a conventional image. With constant illumination, our method can be used to collect spectral information over the surface of the retina. Because sequential one-dimensional images are obtained, image collection can take several seconds. Therefore, the present implementation of our hyperspectral imaging technique applies only to the immobilized eye. We report relative oxygen saturation measurements from primate retinal vessels and ONH in response to controlled changes in inspired oxygen and intraocular pressure (IOP).

Methods

Animals

The use of animals in this study was approved by the LSU Health Sciences Center Institutional Animal Care and Use Committee and confirmed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Two normal cynomolgus monkeys with normal eyes were used. The monkeys were anesthetized and their eyes dilated. The initial ophthalmologic examination included fluorescein angiography, color
and red-free fundus photography, and slit lamp examination of the fundus. To measure oxygen saturation of the ONH and paired retinal vessels, we placed a contact lens on the cornea to prevent drying and obtained reflectance hyperspectral imaging measurements in one eye of each monkey. During imaging, pure oxygen was administered to one monkey to control blood oxygen saturation directly, and IOP was controlled in the other monkey using methods described later.

**Systemic Oxygen Saturation.** An ear oximeter probe (model 3700; Ohmeda, Wallingford, CT) was placed on the monkey’s earlobe to measure systemic oxygenation. A tracheal tube was positioned at the trachea and connected to a small-animal breathing chamber (Quantiflex; MDS Matrx Co., New York, NY). The oxygen chamber was supplied through a pressure regulator from an oxygen tank at a rate of 3 L/min at atmospheric pressure. This procedure brought the oximeter reading to 100% saturation, and hyperspectral images were obtained while the monkey breathed room air and during inspiration of pure oxygen.

**Intraocular Pressure.** To raise IOP, we inserted a 27-gauge needle into the anterior chamber under slit lamp examination. The needle was connected to a 500-mL reservoir containing saline solution with 0.1 mL gentamicin (40 mg/mL), 0.03 mL clindamycin (150 mg/mL), and 4 mL dexamethasone (4 mg/mL). IOP was raised by elevating the reservoir. IOP was monitored by means of a tonometer (Tonopen XL; Medtronic, Jacksonville, FL). Imaging was performed at normal (15 ± 2 mm Hg) and high (60 ± 2 mm Hg) IOPs.

**Fundus Camera**

Images were acquired with a fundus camera (TRC-50vt; Topcon, Tokyo, Japan), with a lens and a c-mount through the vertical path of the camera. Hyperspectral images were obtained through the vertical viewing port using an imaging spectrograph and digital camera, as described later. Figure 1 shows the components and position of the hyperspectral imager on the fundus camera. Vertical mounting facilitated image scanning by maintaining the center of gravity of the moving components over the line of travel. A sleeve held the system at the proper height to sample the focused image. The entrance slit of the spectrograph was placed at a conjugated image plane of the eye fundus. To measure oxygen saturation of the ONH and paired retinal vessels, we placed a contact lens on the cornea to prevent drying and obtained reflectance hyperspectral imaging measurements in one eye of each monkey. During imaging, pure oxygen was administered to one monkey to control blood oxygen saturation directly, and IOP was controlled in the other monkey using methods described later.

**Hyperspectral Imaging**

The hyperspectral images were obtained by translating an imaging spectrometer and charge-coupled device (CCD) camera (model VNIR 100; Photon Industries Inc., Stennis Space Center, MS) across the fundus image, as described later (Fig. 2). The spectrograph used a prism-grating-prism (PGP) architecture with 2.5 nm spectral resolution (25-μm slit) and a range of 410 to 950 nm. Images of the back of the eye were acquired using the 35° viewing mode of the fundus camera. The image from the vertical camera port was focused onto the entrance slit of the spectrograph. The output spectrum was in turn focused onto the CCD image sensor. This arrangement caused the spectrum of all points along a line in the fundus image to be recorded in a single CCD frame. Frames contained a maximum of 1024 points per line and 1024 points per spectrum.

If the highest spatial or spectral resolution is not needed, greater light sensitivity can be obtained by binning CCD pixels. For this study, two spatial and four spectral pixels were binned together to give spectral images containing 512 spatial points and 256 spectral bands. This resulted in sufficient light sensitivity of individual picture elements and sufficient spatial resolution to enable us to monitor oxygen-dependent spectral changes in vessels. The second spatial dimension was obtained by translating the imaging system at constant velocity in the direction transverse to the orientation of the slit. The translation system comprised two mounts attached respectively to the fundus camera and the spectrograph and a servocontrolled actuator that provided linear motion between these parts. Relative motion of this system caused the slit to remain in focus with the fundus image throughout the scan. This component is termed the focal plane scanner (FPS). The number of rows obtained in each hyperspectral image

![Figure 1. Hyperspectral imaging system in relation to the fundus camera. The image normally forms at the film camera port. During hyperspectral imaging, the image is redirected upward by a mirror. The imaging system is translated over the camera port by a linear actuator mounted below the imaging spectrograph and CCD camera. FPS, focal plane scanner.](image1)

![Figure 2. Optical diagram of the retinal hyperspectral imager. The area of interest on the retina is imaged with a fundus camera (FC). Dotted lines: light collection path only. The intermediate image (IM) is formed at the slit (S) of an imaging spectrograph (IS). The spectrograph is drawn above the image for clarity. The output spectrum is focused on the sensor of a CCD camera (C). As the spectrograph and camera are translated along the y axis, the spectrum from points on consecutive lines of the image is recorded in a series of frames. Motion is controlled to create a 1:1 aspect ratio between adjacent pixels in the x direction and lines in the y direction.](image2)
was equal to the number of frames acquired as the system was translated. The velocity of motion and the interval between frames was carefully adjusted so that adjacent pixels and adjacent rows of the image had the same spatial interval. Typically, 100 rows were obtained for this study.

Figure 3 shows the data structure of the recorded spectral images. Each frame holds the spatial (x, y) and spectral (λ) axis for each line of the acquired hyperspectral image, with successive lines forming the z-axis in the stack of frames. A "band-sequential" hyperspectral image is obtained by rotation of the stack of images, interchanging the z and λ axes. After rotation, each frame contains a two-dimensional spatial image at a distinct wavelength in which intact structures are recognizable.

**Extraction of Spectral Curves**

Band-sequential image sets were saved from the image-acquisition software (HyperVisual; Photon Industries, Stennis Space Center, MS) in ENVI image-processor format (Research Systems, Boulder, CO). Images were corrected for dark values by subtracting an image obtained after blocking illumination. Spectral curves were obtained by scanning the intensity profile along the z-axis of selected image pixels within the ONH border, corresponding to artery, vein, and surrounding ONH. For spectral curves, a five-point moving average filter was applied to individual curves of each time point, and the smoothed data were then averaged to obtain final curves that represent the spectral signatures obtained before application of high oxygen, after application of high oxygen, and before high IOP. Time points during high IOP were not averaged.

### Mapping Relative Oxygen Saturation

Relative saturation was assessed from amplitudes of the hemoglobin spectral signatures that were contained in the reflectance spectra from retinal blood. As saturation decreased from high to low, spectral minima at 542 and 577 nm from oxygenated hemoglobin (HbO2 spectral signature) were converted to a single minimum at 555 nm from deoxyhemoglobin (Hb spectral signature). No changes occurred at wavelengths where HbO2 and Hb spectral curves crossed (isosbestic points). These spectral features from reflectance recordings at high and low saturation are shown in Figure 4. Although the sloping baseline produces a slight blue shift of spectral minima, only the areas under curves are used in this method.

Isosbestic points at 530, 545, 570, and 584 nm were selected from recorded spectra. As seen in Figure 4, the curve of saturated blood passes above the line that connects the points at 545 and 570 nm (region II). The curve moves toward the line and can pass below the line as the blood becomes more desaturated. This area between the curve and line (Fig. 4, all) is largest for 100% saturation and decreases, eventually changing sign, as the blood becomes desaturated. Changes in the total reflectance from different recordings were compensated for by dividing this saturation-sensitive area by the total area under the line (Fig. 4, All). This total area is proportional to the intensity of reflected light in the recorded spectrum and is not affected by saturation changes. A partial-signature map of relative oxygen saturation was found from the ratio of these saturation-dependent and saturation-independent areas (Fig. 4, all/All). The term partial signature refers to the use of only the region of the spectrum between the second pair of isosbestic points.

We produced a second map that includes the regions between the first and third pairs of points (Fig. 4, I, III), to determine whether a
significant reduction in noise and increase in sensitivity could be obtained by using the entire signature. Areas between the curve and the line in regions I and III were negative at high saturation and moved toward zero for low saturation. The second map, referred to as a full-signature map, was found by subtracting areas I and III from area II (after each area was compensated for total reflectance differences).

The use of the full signature gave a larger range of values for the same change in saturation and tended to average noise to a greater extent. For each type of map, values representing low to high saturation were color-coded as blue, green, yellow, and red. Because spectral changes were referenced to isosbestic points, this method should minimize errors contributed by variation in the slope of the spectral baseline from different recording sites.

Relative Saturation Indices
An index of the relative oxygen saturation (RSI) was determined from separate regions of the hyperspectral image containing artery, vein, and selected areas of the ONH (Fig. 5). This RSI was evaluated over each collection of image pixels by the same method described earlier for individual pixels of saturation maps. Plots of RSI versus time points for oxygen and IOP experiments are described in the Results section.

RESULTS

Figure 5 shows the area of the ONH obtained from the 570-nm band in the hyperspectral image for the oxygen concentration images (Fig. 5, top left) and the variable IOP images (Fig. 5, bottom left). Confirmation of vessel type was done by fluorescein angiography (images at right from the venous phase).

Spectral Signatures
Figures 6 and 7 show a portion of the reflectance spectra between 450 and 600 nm containing the hemoglobin signature from retinal artery and vein and nasal and temporal ONH under various experimental conditions. Increased oxygen saturation is indicated in these plots when the experimental spectrum changes to match more closely the HbO₂ signature of Figure 4, with stronger minima at 542 and 577 nm. Desaturation is indicated when the curve more closely resembles the Hb signature having a single spectral minimum. Higher reflectances at the longer wavelengths result mainly from weaker light absorption at these wavelengths by choroidal pigments in the fundus.

Oxygen Breathing. The effect of inspired O₂ concentration is shown in Figure 6. The artery (top left) showed a small increase in the HbO₂ signature with pure O₂, relative to room air. In the vein (top right), this increase was markedly larger. Inspiration of pure O₂ raised total reflectance, as shown by the greater spectral amplitude. In the nasal and temporal ONH (bottom left and right), pure O₂ increased the HbO₂ signatures, but not to the degree observed in the vessels. The larger increase was in the nasal ONH spectrum. Pure O₂ also increased total reflectance from the ONH. All spectra from the ONH showed an increased baseline slope because of higher reflectance at red wavelengths. As expected, the overall results show increased oxygen saturation in both the large vessels and the ONH microcirculation with increased concentration of inspired O₂.

Intraocular Pressure. The effect of increased IOP on oxygen saturation is shown in Figure 7. In the artery (top left), high IOP sustained for 5 minutes gradually converted the HbO₂ signature to an Hb signature. The data indicate that a deep level of desaturation occurred in the artery. In the vein (top right), the normal IOP curve shows a weak HbO₂ signature. Within 1 minute after the onset of high IOP, however, the curve was converted to a strong Hb signature. These results suggest that high IOP causes desaturation of the retinal blood supply in both arteries and veins. Increased IOP resulted in only modest increases in total reflectance.

In the ONH (Fig. 7, bottom left and right), HbO₂ spectral signatures were present at low IOP. One minute after IOP was increased to 60 mm Hg, the amplitude of the signature de-
creased. At 5 minutes, the nasal ONH curve was nearly parallel to that at 1 minute, whereas the temporal ONH curve showed some small restoration of the HbO\(_2\) signature. These results show that high IOP reduced saturation in the ONH microcirculation but to lesser degree than in the retinal circulation and suggest that saturation was partially restored in some regions.

**Responses to Oxygen Breathing**

Figure 8 shows spatial changes in the relative saturation of ONH structures during room air breathing (left) and 2 minutes after switching to pure oxygen (right). The upper and lower panels compare results from the partial- and full-signature methods. The partial-signature maps (Fig. 8A) reveal saturation differences; however, structures such as the large vein are more clearly delineated during high saturation in the full-signature maps (Fig. 8B, right). These results show that better definition of the changes is revealed in the full-signature maps. Accordingly, the full-signature method was used to map IOP saturation changes and to determine the RSIs from vessel and ONH areas.

Under room air conditions, high saturation areas included outlines of arteries out to the ONH boundary. These vessels continued outside the ONH with a different saturation code. During pure oxygen breathing, saturation increased in the arteries, and new areas of high saturation appeared where veins were located. The ONH tissue surrounding the vessels, particularly on the nasal side, showed smaller increases in saturation. These results agree with the spectral changes shown in Figure 6. All structures showed significant increases \((P < 0.05)\) in the RSI during pure oxygen breathing (Table 1). The increase in the veins was nearly twice (factor of 1.9) that found in the artery, whereas smaller increases in the ONH (averaged over the cup and rim) were approximately half (factor of 0.52) that of the artery. A slow decrease in the saturation over time occurred in the vein and ONH RSIs but not in the artery RSIs (Fig. 9).
Responses to High IOP

Hyperspectral imaging showed good repeatability, as is evident in the saturation maps (Fig. 10A, top row) from repeated recordings during low IOP (room air). High saturation appears at artery locations within the border of the ONH and in the ONH tissue surrounding the vessels. Changes in saturation at 1-minute intervals after switching to high IOP are shown in Figure 10A, bottom row. The high saturation of the arteries and most of the ONH disappeared after 1 minute. A gradual return of saturation over the temporal ONH cup was observed from 2 through 4 minutes after IOP elevation. Figure 10B shows these changes during high IOP using a compressed scale for greater resolution of saturation differences.

Relative saturation indices are shown in Figure 11 and Table 2 for the IOP experiment. High IOP resulted in significant reduction \((P < 0.05)\) of the RSI for each structure. After 3 minutes of high IOP, the RSIs from artery and vein were not significantly different from one another. In the temporal ONH cup, the RSI decreased initially, but then recovered 24% of its original normal IOP within the 4-minute high-IOP period. This phenomenon was not observed in other areas of the ONH.

DISCUSSION

Pure Oxygen Breathing Experiment

The spectral curves showed the expected HbO₂ signature in arteries and the mixed Hb-HbO₂ signature in veins under room air conditions. Switching to pure O₂ strengthened the HbO₂ signature of both types of vessels. If retinal arterial saturation is closely matched to the systemic saturation (95%–97%), observed increases in the HbO₂ signature in the artery represent only a 3% to 5% increase in saturation. A lower arterial saturation of 92% has been reported using a spectral technique (Scibor M, et al. IOVS 2002;43:ARVO E-Abstract 3305). Oxygen leakage from the ophthalmic artery could cause the retinal artery saturation to be lower than systemic levels. In that case, the response seen in the artery may represent up to an 8%
increase in saturation. Because a fixed leakage rate would result in more or less arterial saturation depending on the flow rate, evaluation of retinal arterial saturation could effectively probe changes in blood flow at the major vessels supplying blood to the inner retina. The proportionately stronger HbO2 signature observed in veins corresponds to significantly larger increases in venous saturation. This effect was noted previously by Hickam and Frayser20 and Beach et al., 15 using multispectral recordings, and is attributed to inhibition of the desaturation of capillary blood in the presence of high plasma PO2.

HbO2 signatures were also obtained from areas between vessels within the border of the ONH. Because our light probe is in the green-red spectral range, we interpret these readings to be signatures of blood carried by the microcirculation near the surface nerve fiber layer. It is also possible that some of this signal results from light first passing through surface vessels and then returning through the microcirculation of the surrounding tissue. Pure O2 strengthened the HbO2 signature in the ONH, but to a lesser degree than that observed in the vein, as expected if this signature represents the averaged blood saturation in the microcirculation. To the best of our knowledge, these results are the first report of measurements of oxygen saturation changes in the ONH microcirculation using noninvasive reflectance imaging.

FIGURE 8. Saturation maps of the oxygen breathing experiment during (left) room air and (right) pure O2 breathing. (A) Partial-signature maps; (B) full-signature maps. Increasing saturation is indicated by the progression from blue to green to yellow to red. Temporal-to-nasal orientation in each map is top to bottom.

FIGURE 9. RSIs from retinal vessels and ONH during an oxygen breathing experiment. RSIs were determined from vessel segments inside the ONH and from rim and cup regions, as denoted in Figure 5. Vessel segments (large symbols): artery (filled diamonds), vein (filled rectangles). ONH regions (small symbols): temporal (open circles), nasal (open triangles), average over ONH (×). Breathing pure oxygen began immediately after the second data point (time 0).

Under pure O2 conditions, the ONH and vessel reflectance at the hemoglobin absorption wavelengths was consistently greater than under room air conditions. This effect may be the result of vasoconstriction under high O2 that reduces the luminal blood volume in the surface vessels and, correspondingly, the perfusion of the microcirculation. The features of the spectral profiles of vessels and tissue are thus in agreement with changes anticipated when the vascular supply of O2 is increased.

Because metabolic changes associated with progression of retinal disorders presumably alter the oxygen utilization in the tissues, venous saturation maps should be a sensitive probe for disease states. Saturation maps determined by assessment of the Hb and HbO2 spectral signatures, in particular the relative contributions of the Hb and HbO2 spectral peaks between isosbestic points, were able to monitor the venous saturation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Artery</th>
<th>Vein</th>
<th>Optic Nerve Head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air*</td>
<td>0.261 ± 0.007</td>
<td>0.159 ± 0.001</td>
<td>0.137 ± 0.007</td>
</tr>
<tr>
<td>Pure oxygen†</td>
<td>0.312 ± 0.002</td>
<td>0.256 ± 0.002</td>
<td>0.151 ± 0.004</td>
</tr>
<tr>
<td>Difference‡</td>
<td>0.051 ± 0.009</td>
<td>0.097 ± 0.010</td>
<td>0.014 ± 0.011</td>
</tr>
</tbody>
</table>

Unpaired samples of equal variance.
* Average over two time points during room air breathing.
† Average over five time points at high O2.
‡ All differences are significant (P < 0.05). Data are the means ± S.D.
increases in response to breathing pure O₂. Previous work estimated these increases in the range of 8% to 23%. If changes of similar size are present during the state of hypoxia, maps similar to ours should be able to isolate hypoxic areas when the scale is set to operate over the lower venous saturation range. Calibration for different saturation ranges would make our maps more sensitive in low- and high-saturation regions.

**IOP Perturbation Experiment**

Raising IOP to 60 mm Hg had essentially the opposite effect on blood saturation. At this IOP, the perfusion pressure is very low. Arterial desaturation could have resulted from a slowing or stoppage of flow caused by collapse of the vessel under pressure, during which time oxygen diffused from the vessel. The more rapid appearance of the Hb signature in the veins was probably due to lower initial saturation of venous blood. An interesting feature of the high IOP response was partial recovery of saturation in the ONH microcirculation while the pressure remained high. Saturation recovery was seen near the cup of the ONH, which was temporal with respect to the origin of the vessels. The full-signature map reduced noise enough to allow good visualization of this recovery. Because the high IOP effectively occluded the surface vessels, the source of oxygen is most probably from deeper levels of the circulation, which includes the retrolaminar layer. Increased reflectance during high IOP can be explained by low blood volume, since high IOP would partially occlude the major surface vessels and vessels feeding the outer ONH microcirculation, causing this area to blanch.

**Hyperspectral Imaging**

These results demonstrate the ability of hyperspectral imaging to measure relative changes in oxygen saturation of the retinal macro- and microcirculation. The usefulness of relative measurements of the oxygen saturation for assessing the vascular response to controlled changes in oxygen supply and utilization is evident from these data.

At present, it takes 8 seconds to scan a rectangle encompassing the optic nerve. Eye gaze can be maintained in human subjects for longer than this. However, involuntary eye movement present during fundus examination with bright light and in elderly patients would cause gaps in image data. The ability to scan the image at a faster rate, on the order of 1 second per 100-line scan, would be needed to routinely acquire distortion-free images during fixed gaze in clinical subjects. Acquisition speeds can be significantly improved with multi-tapped frame-transfer CCD cameras and higher speed bus interfaces between the camera and frame buffer.

The usefulness of spectral information for quantification of oxygen saturation has been demonstrated. The present hyperspectral imaging technique enables spectral quantitation to be performed on the ONH over two dimensions, allowing regional changes in saturation to be identified. We obtained different saturation color codes from retina outside the ONH. This difference may reflect disparate amounts of light being scattered into vessels from the pigment-free ONH and pigmented retina. Future work will determine appropriate ranges of the RSI for retinal saturation mapping.

In addition to our method of curve integration, other spectral quantitation methods, such as curve fitting, can be used with our system. Significantly faster recording techniques are needed to achieve a clinically acceptable method for mapping spectral information on the ocular fundus. At that point, hyperspectral imaging should provide a much needed diagnostic tool for prevention and treatment of retinal disorders. The desired goal is the successful application of therapeutic interventions before irreversible damage occurs. One potential gain for detecting abnormalities in the oxygen saturation response is significantly earlier diagnosis of glaucoma. It is presently...
believed that autoregulation is impaired in glaucoma,1–3,7,25,26 possibly as a result of anatomic vascular impairment of the retina and the ONH. It would be of considerable interest to determine whether the threshold for autoregulation impairment is affected during the pre-onset stages of early phase glaucoma.

Acknowledgments

The authors thank Mark Lanoue for invaluable technical assistance in the development of the hyperspectral imaging system and Jinjeng Ning for assistance with analysis of data and production of saturation maps.

References


TABLE 2. Relative Saturation Indices from the IOP Experiment

<table>
<thead>
<tr>
<th>Condition</th>
<th>Artery</th>
<th>Vein</th>
<th>Averaged ONH</th>
<th>Temporal Cup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IOP*</td>
<td>0.210 ± 0.008</td>
<td>0.139 ± 0.005</td>
<td>0.101 ± 0.001</td>
<td>0.081 ± 0.001</td>
</tr>
<tr>
<td>High IOP†</td>
<td>0.030 ± 0.010</td>
<td>0.029 ± 0.010</td>
<td>0.041 ± 0.002</td>
<td>0.054 ± 0.001</td>
</tr>
<tr>
<td>Difference</td>
<td>0.180 ± 0.018</td>
<td>0.110 ± 0.015</td>
<td>0.060 ± 0.003</td>
<td>0.027 ± 0.002</td>
</tr>
</tbody>
</table>

Unpaired samples of equal variance.
* Average over three time points at normal IOP.
† Average over last two time points at high IOP.
‡ All differences are significant (P < 0.05). Data are the means ± S.D.