Retinal Oximetry With a Scanning Laser Ophthalmoscope

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Purpose. The purpose of the study was to assess if a scanning laser ophthalmoscope (SLO), Optomap 200Tx, could be used for measurements of hemoglobin oxygen saturation in retinal blood vessels.

Methods. Optomap 200Tx uses two lasers for image acquisition, 532 and 633 nm. Retinal images of healthy individuals and patients with retinal vein occlusion were analyzed with modified Oxymap Analyzer software, which tracks retinal vessels and calculates relative hemoglobin oxygen saturation.

Results. Oxygen saturation in healthy individuals was measured as 92% ± 13% for arterioles and 57% ± 12% for venules (mean ± SD, $n = 11$, $P = 0.0001$). Standard deviation for repeated measurements of the same eye was 3.5% for arterioles and 4.4% for venules. In patients with confirmed venular hypoxia, central retinal vein occlusion (CRVO) or hemivein occlusion, the average venular oxygen saturation was measured as 23% ± 3% in the affected eyes and 59% ± 3% in the fellow eyes ($n = 4$, $P = 0.0009$).

Conclusions. Technically, it is possible to derive information on retinal oxygen saturation from an SLO with a 2-wavelength oximetry algorithm. The system produced both sensitive and repeatable results. The remaining challenges include decreasing variability between vessels of the same eye and variability between individuals. Given the advantages that SLO imaging has over conventional fundus camera optics in retinal oximetry, further development of SLO oximetry may provide the optimal approach to retinal oximetry.

Keywords: hyperoxia, hypoxia, oxygen, hemoglobin, retina

Measurement of hemoglobin oxygen saturation in blood vessels is based on different light absorption of oxyhemoglobin and deoxyhemoglobin. Current conventional approach to retinal oximetry consists of white light illumination of the fundus where the image is subsequently filtered with light filters for spectrophotometric analysis. Two such retinal oximetry systems are currently available commercially (Oxymap ehf., Reykjavík, Iceland, and Imedos, Jena, Germany).[1,2] Both use conventional fundus camera optics and dual wavelength analysis. Other researchers in retinal oximetry have used various optical approaches with either two wavelengths or multispectral analysis.[3–9]

Scanning laser ophthalmoscope (SLO) can also be used to measure retinal hemoglobin oxygen saturation. Studies on SLO oximetry have been published mainly by three other research groups, Smith and Denninghoff et al.,[10] Ashman et al.,[11] and Li et al.[12] In 1998, Smith et al.[12] reported on a 2-wavelength scanning laser Eye Oximeter (EOX) prototype, which was used for retinal oxygen saturation measurements in anesthetized swine during blood loss. Strong correlations were found between retinal arterial and femoral arterial oxygen saturation, and retinal venous saturation and blood loss. In 1999, this same research group[13] reported on a second generation of the EOX for studies on retinal oxygen saturation in human subjects. The EOX was mounted on a slit-lamp base and four diode lasers at wavelengths 629, 678, 821, and 899 nm used to image the retina. Oxygen saturation was found to be 101% to 102% and 65% in the retinal arteries and veins, respectively, suggesting that the EOX is sensitive to retinal oxygen saturation. In 2011, Denninghoff et al.[14] reported on the use of a new modified confocal SLO (ROx-3) for blue-green oximetry in both swine and human subjects. Results on retinal oxygen saturation were in the same range as reported by other research groups. Ashman et al.[10] used a prototype SLO to measure the retinal oxygen saturation under different percentages of oxygen breathing mixtures; 10%, room air, and 100%. Lasers used for imaging were 633 and 815 nm. A difference was seen between retinal arterioles and venules but no difference was measureable between different oxygen breathing mixtures. Li et al.[11] measured the oxygen saturation in small retinal vessels (diameter < 50 μm) using adaptive optics confocal SLO with two wavelengths, 680 and 796 nm. A difference between arterioles and venules was detected.

Scanning laser ophthalmoscope offers some advantages for retinal oximetry. It uses lasers to create monochromatic images at two or more wavelengths which minimizes unnecessary light exposure to the fundus. Scanning laser ophthalmoscope can easily be used with undilated pupils and penetrates cataracts and other optical opacities in the eye better than conventional spectrophotometric fundus camera. It also allows wide-field scanning of almost the entire fundus, whereas conventional fundus cameras are limited to relatively narrow images of the posterior pole. Given these advantages

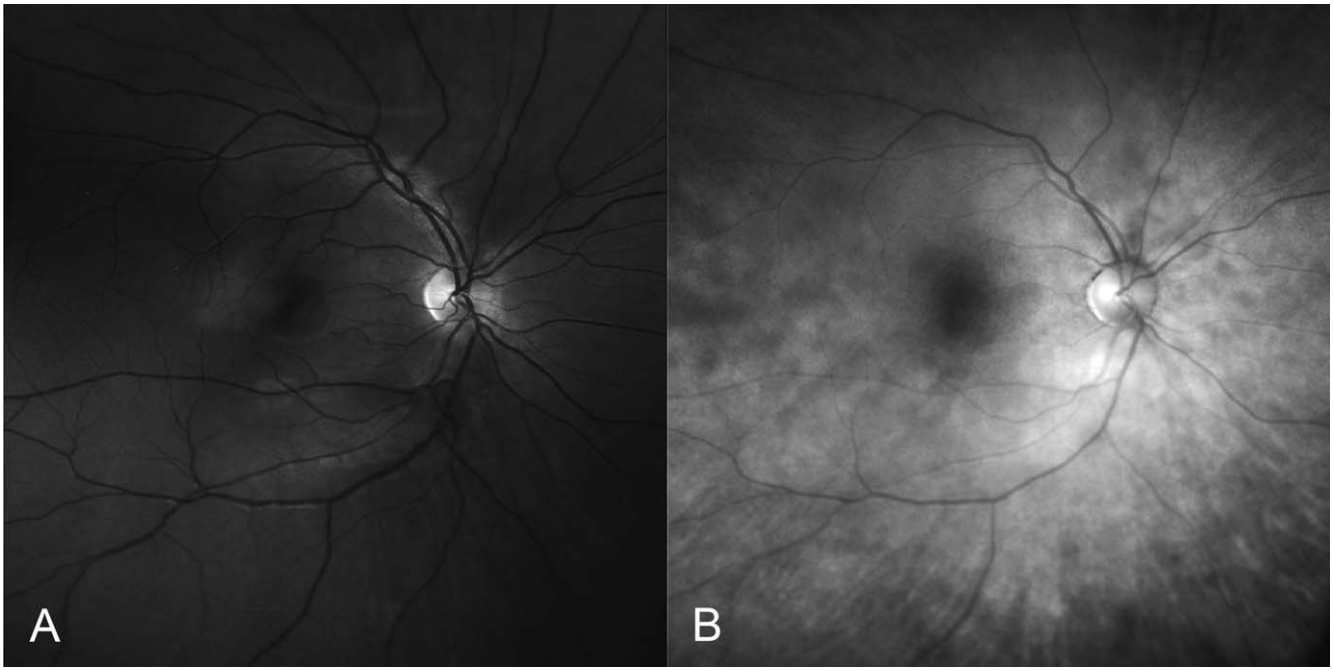

FIGURE 1. The scanning laser ophthalmoscope, Optomap 200Tx, uses two lasers to acquire fundus images (**A**) 532 nm (green) and (**B**) 633 nm (red).

of SLO over the conventional approach we set out to develop a system for retinal SLO oximetry. We combined the use of SLO, Optomap 200Tx, developed by Optos plc. (Dunfermline, Scotland, UK) and oximetry image analysis software developed by Oxymap ehf. The system was developed and tested in healthy human subjects breathing either room air or 100% oxygen and in patients with confirmed retinal venous hypoxia.

METHODS

Study Protocol

The study was approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority. All participants signed an informed consent before participation in the study. The study adhered to the tenets of the Declaration of Helsinki.

Scanning Laser Oximetry. The SLO Optomap 200Tx uses two lasers, 532 nm (green) and 633 nm (red), to capture fundus images (Fig. 1). The images from the Optomap 200Tx can, in principle, be used for dual wavelength retinal oximetry; the 633-nm image is sensitive to hemoglobin oxygen saturation, while the 532-nm image is close to being insensitive and can be used as a reference. The relative difference between light absorptivity of oxy- and deoxyhemoglobin at 532 nm is considerably less than the relative difference at 633 nm. Therefore, these wavelengths should work for dual-wavelength oximetry. For 532 nm, the ratio between light absorptivity of deoxyhemoglobin (Hb) and oxyhemoglobin (HbO_2) is 0.7, while the ratio for 633 nm is 8.1 (i.e., the Hb absorptivity at 633 nm is 8 times what it is for HbO_2, and the absorptivity of Hb at 532 nm is only 0.7 times that of HbO_2). These light absorptivity values of Hb and HbO_2 are according to results by Zijlstra et al.[15] Sensitivity of optical density ratio (ODR) to change in saturation is outlined in the Supplementary Material.

Computer software Oxymap Analyzer (Oxymap ehf.) processes the two spectral images acquired by Optomap, tracks retinal vessels, and measures light intensity inside vessels (I) and outside vessels (I_0) (see an example of measurement points used for oxygen saturation calculation in Fig. 2). From light intensity measurements the optical density (OD) (Equation 1) can be estimated at each point along the vessels. Optical density ratio (Equation 2) is the ratio between ODs at each wavelength and has an

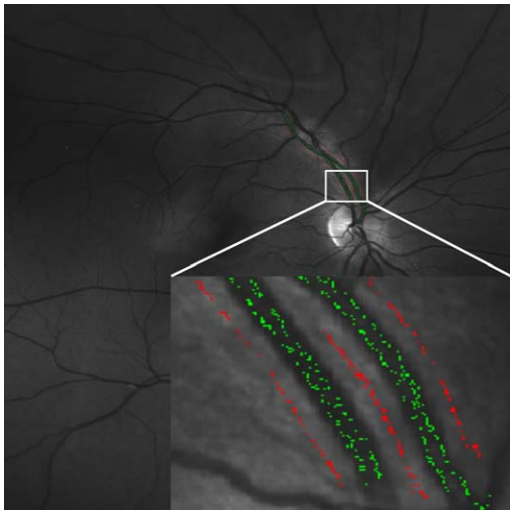

FIGURE 2. Oximetry image from one healthy subject showing measurement points inside and outside of vessels used for oxygen saturation calculation. *Green dots* denote measurement points inside vessels (i.e., light intensity I [Equation 1] and *red dots* denote measurements of light intensity outside vessels or I_0). A *white box* shows measurement area for all healthy subjects, superotemporal vessel pair (retinal arteriole and venule), both for normoxia and hyperoxia. Measurement area (*white box*) enlarged in lower right corner

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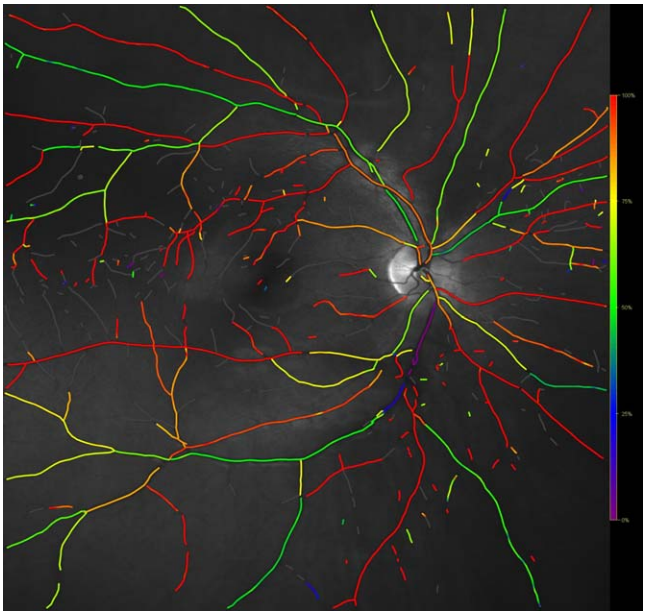

FIGURE 3. The software Oxymap Analyzer processes the two spectral images and calculates hemoglobin oxygen saturation of the retinal blood vessels. *Red color* represents 100% oxygen saturation and *violet color* 0% oxygen saturation (see *scale* on *right side*).

approximately linear relationship to hemoglobin oxygen saturation.

$$OD = log\frac{I_0}{I} \quad (1)$$

$$ODR = \frac{OD_{633}}{OD_{532}} \quad (2)$$

After calibration, the oxygen saturation ($SatO_2$) can be calculated according to following:

$$SatO_2 = (a^*ODR + b)^*100\% \quad (3)$$

The constants a and b were found by matching the average arteriolar and venular ODRs from the healthy subjects, of this study, with previously published mean retinal oxygen saturation values in healthy individuals, obtained with a calibrated device in a separate study by Schweitzer et al.[16] The previous study found that retinal arteriolar saturation was 92.2% and venular saturation was 57.9%. Matching resulted in following values: $a = -2.4733$ and $b = 1.4388$.

The vessel detection algorithm of Oxymap Analyzer software was modified in order to process the larger images (100°) of the SLO and take into account the different magnification compared with conventional Oxymap T1 fundus camera–based system (50°). Oxymap Analyzer processes the two spectral images from the SLO and produces 100° oximetry fundus images (Fig. 3). For further explanation of the Oxymap T1 system and Oxymap Analyzer software, see Geirsdottir et al.[1]

Feasibility and Repeatability. For initial adaption and testing of the Optomap 200Tx SLO as a retinal oximeter, two 100° (ResMax setting) fundus images were acquired from 11 healthy volunteers (6 males and 5 females), age 34 ± 10 years (mean ± SD). Images were acquired of an undilated right eye of each subject. The Optomap 200Tx was set to store unscaled 12-bit images and red and green sensors set at gain 2 (for lightly pigmented iris). The first image from each subject was used for initial assessment of the device as a retinal oximeter, to test if there was a clear difference between arterioles and venules. Two images were acquired in a row, approximately 15 seconds apart, of the right eye of each subject to test the repeatability of measurements between images.

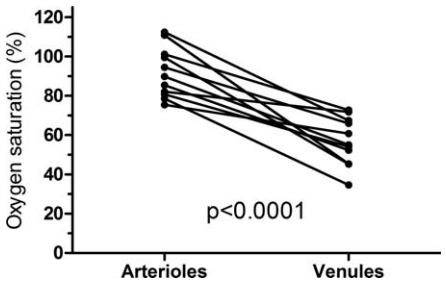

FIGURE 4. The graph shows oxygen saturation of the main superotemporal vessel pair (arteriole and venule) for 11 healthy subjects. The *lines* connect vessels in the same eye. According to a paired *t*-test the difference between arterioles and venules is statistically significant ($P < 0.0001$).

Sensitivity. Two different methods were used to investigate the sensitivity of the device, (1) 100% oxygen inhalation, and (2) measurements of patients with confirmed retinal venous hypoxia due to either central retinal vein occlusion (CRVO) or hemivein occlusion:

1. Two healthy subjects were measured before and after inhalation of 100% oxygen for 10 minutes (10 L/min), mask covering both mouth and nose. Images were acquired before oxygen inhalation started (baseline image), when oxygen inhalation ended (time = 0 seconds) and every 5 seconds for the next 135 seconds during recovery and then finally after 10 minutes (600 seconds) of recovery; and
2. Three patients with CRVO and one patient with hemivein occlusion were also measured. Hypoxia had already been confirmed with the Oxymap T1 oximeter (Eliasdottir T, et al. *IOVS* 2013;54:ARVO E-Abstract 46). Central retinal vein occlusion affected eye was compared with the fellow eye in the same patient. For the patient with hemivein occlusion the affected area (inferior fundus) was compared with the same area in the fellow eye.

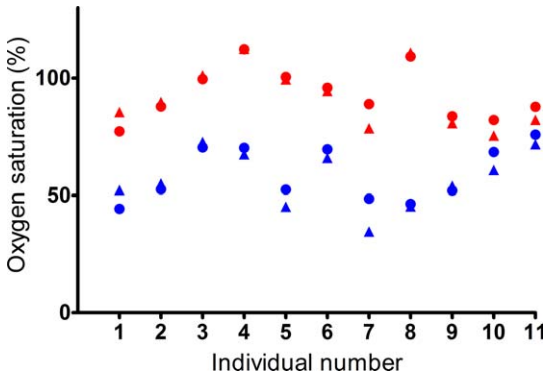

FIGURE 5. The graph shows oxygen saturation of the main superotemporal vessel pair (arteriole and venule) from 11 healthy subjects. Two images were analyzed for each subject. The *triangle* denotes the first image for each individual and the *circle* denotes the second image. (*Red*, arterioles and *blue*, venules).

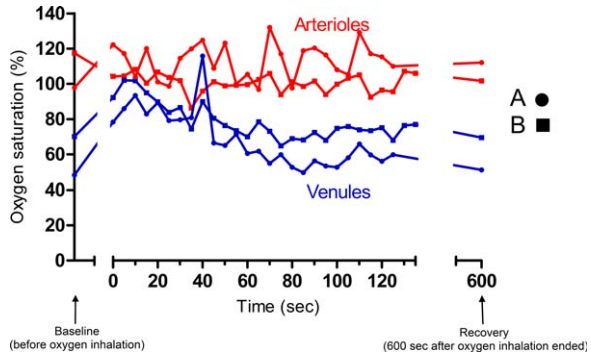

Figure 6. Oxygen saturation over time for retinal arterioles and venules for two healthy subjects (**A**, **B**). Subjects inhaled 100% oxygen for 10 minutes (10 L/min) and fundus images were acquired with the Optomap 200Tx before inhalation (baseline) after the inhalation ended (time = 0) and every 5 seconds for the next 135 seconds during breathing of room air. Final image was acquired after 10 minutes of recovery (time = 600 seconds). Oxygen saturation was analyzed using Oxymap Analyzer software.

Image Analysis. All oximetry images were analyzed in a standardized manner. For healthy volunteers, oxygen saturation was measured in the main superotemporal vessel pair (retinal arteriole and venule; Fig. 2). For CRVO patients, oxygen saturation of all retinal arterioles and venules above 6 pixels in diameter (6 pixels ~ 60 μm) were measured and averaged for one eye, all vessel segments were between 50 and 200 pixels in length. Retinal vessels with diameter smaller than 6 pixels were excluded from analysis because the saturation measurement can be unreliable.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA). A paired *t*-test was applied to test the difference between arterioles and venules and between affected eyes and healthy fellow eyes for CRVO/hemivein occlusion patients. Standard deviation between two repeated measurements of the same vessel was calculated using the square root of the within subjects mean square from ANOVA as recommended by Bland.[17]

Results

Oxygen saturation was measured as 92% ± 13% ($n = 11$, mean ± SD) for arterioles and 57% ± 12% for venules (the mean saturation values are a direct result of calibration). The difference between arterioles and venules was statistically significant according to a paired *t*-test ($P = 0.0001$). Standard deviation for repeated measurements of the same vessel was 3.5% for arterioles and 4.4% for venules. Raw data for retinal vessel oxygen saturation calculation for the 11 healthy subjects and variability in ODs along the measured vessel segments can be found in Supplementary Tables S2 through S5. Figure 4 highlights the difference between arteriolar and venular saturation in each individual. Figure 5 shows the repeatability, while Figure 6 shows results of oxygen breathing experiments for test of sensitivity. Figure 7 shows an example of how the pseudo color map of retinal oxygen saturation changes with breathing of 100% oxygen. The Table and Figures 8 and 9 show the results of measurements on patients with CRVO or hemivein occlusion.

Discussion

The combination of Optomap 200Tx SLO imaging and Oxymap Analyzer software was successful in the development of an SLO based retinal oximetry system. The SLO oximetry system provides oxygen saturation measurements in retinal arterioles and venules and the difference between arterioles and venules is statistically significant (Fig. 4), which is the first confirmation that the technique is working.

The short-term repeatability of the measurement is good as indicated by the SD for repeated measures (Fig. 5), 3.5% for arterioles and 4.4% for venules. This is acceptable considering the early stage in the development. The more developed fundus camera–based Oxymap T1 has SD for repeated measures of 1.0% for arterioles and 1.4% for venules.[18]

The sensitivity of the SLO oximetry system to different oxygen saturations can, first of all, be seen from clear difference measured between arterioles and venules, and second of all as the response to breathing either room air or 100% oxygen (Fig. 7), which also demonstrates the ability to detect changes in venular oxygen saturation over time (Fig. 6). From Figure 6, we can see that the oxygen saturation for retinal

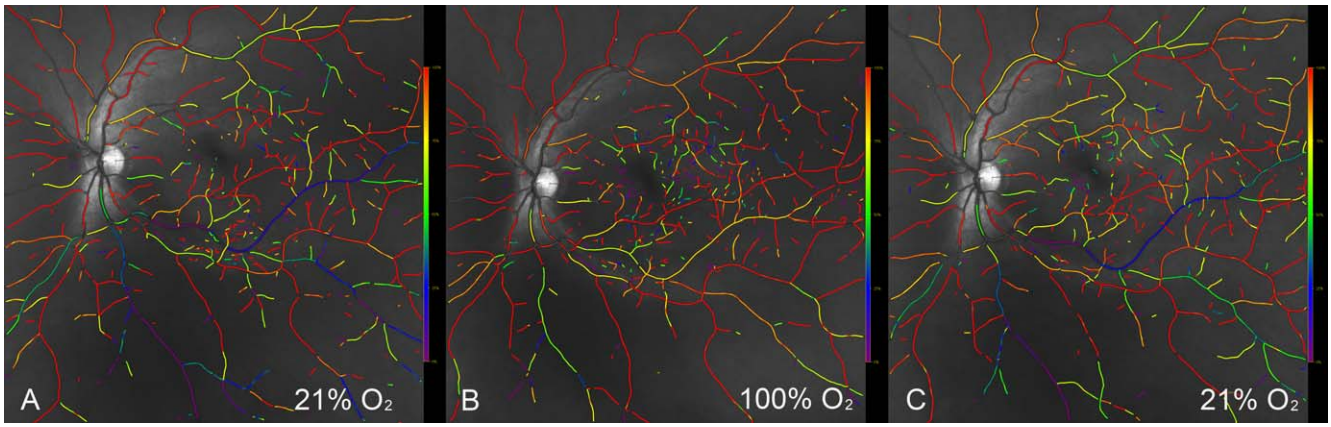

Figure 7. Oximetry fundus images from one healthy subject acquired with the SLO Optomap 200Tx and analyzed with Oxymap Analyzer software (**A**) before oxygen inhalation (baseline), (**B**) after inhalation of O_2 for 10 minutes (10 L/min), and (**C**) after 10 minutes (600 seconds) of recovery. The *color scale* is optimized for saturation values below 100%. Variability above 100% can be seen in Figure 6. The apparent hypoxia in some inferior veins is most likely a technical artifact. However, the saturation in these vessel segments changes in the correct direction during 100% oxygen inhalation and recovery.

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TABLE. Oxygen Saturation (Mean ± SD) of Four Patients With Either CRVO (Patients 1–3) or Hemivein Occlusion (Patient 4)

	Oxygen Saturation (%) in Retinal Vessels (Mean ± SD)			
	CRVO Affected Eye		Fellow Eye	
Patient Number	Arterioles	Venules	Arterioles	Venules
1	109 ± 21	25 ± 42	110 ± 13	56 ± 5
2	108 ± 9	26 ± 26	101 ± 14	60 ± 22
3	115 ± 11	19 ± 13	116 ± 8	59 ± 24
4	95 ± 10	20 ± 28	109 ± 15	63 ± 7
Mean	107 ± 9	23 ± 3	109 ± 6	59 ± 3

The affected eye is compared with healthy fellow eye. Difference between retinal arterioles in affected eyes and fellow eyes was not statistically significant ($n = 4$, $P = 0.64$, paired t-test); difference between retinal venules in affected eyes and fellow eyes was statistically significant ($n = 4$, $P = 0.0009$, paired t-test).

arterioles stays relatively stable over time, while the saturation for retinal venules decreases for both subjects (A and B). These results are in good agreement with previously published results using Oxymap T1 system (Olafsdottir OB, et al. *IOVS* 2012;270:ARVO E-Abstract 2166). The sensitivity of the measurement can also be seen from the change in the color code in Figure 7, especially for venules. Even though some vessel segments show abnormally low saturation, caused by intra-eye variability of the measurement, the color code changes when oxygen saturation increases (image B) and goes back to the same color as before when oxygen saturation gets back to normal (image C).

In CRVO, SLO oximetry clearly detects the difference between the venular oxygen saturation in CRVO affected eyes and the healthy fellow eyes (Table, Figs. 8, 9), the difference is statistically different. The venular hypoxia had already been confirmed in all of these subjects with the use of the more developed Oxymap T1 oximeter (Eliasdottir T, et al. *IOVS*

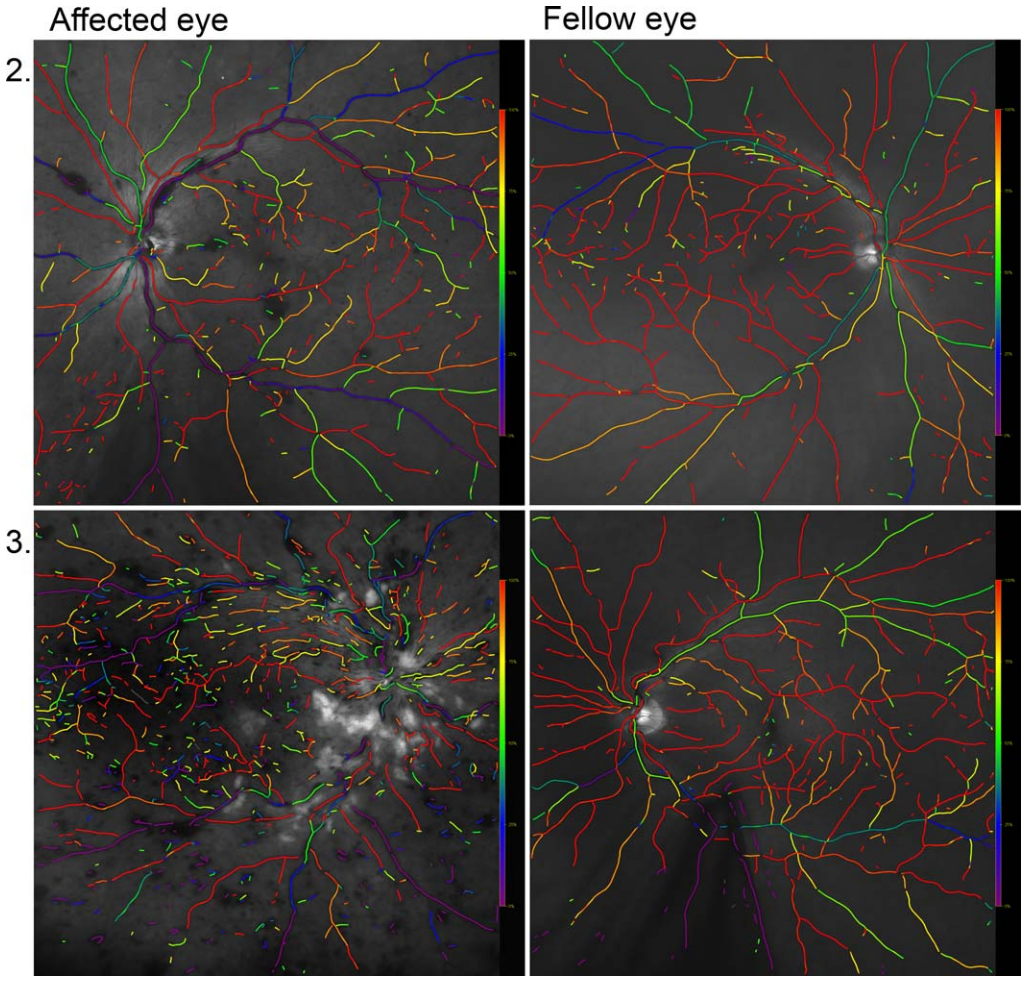

FIGURE 8. Oximetry fundus images from two patients with CRVO (patients 2 and 3, see Table), acquired with Optomap 200Tx and analyzed with Oxymap Analyzer software. A clear difference is seen in venular oxygen saturation between the CRVO affected eye and the fellow eye (*blue color* denotes approximately 25% oxygen saturation, see *scale* on *right side*).

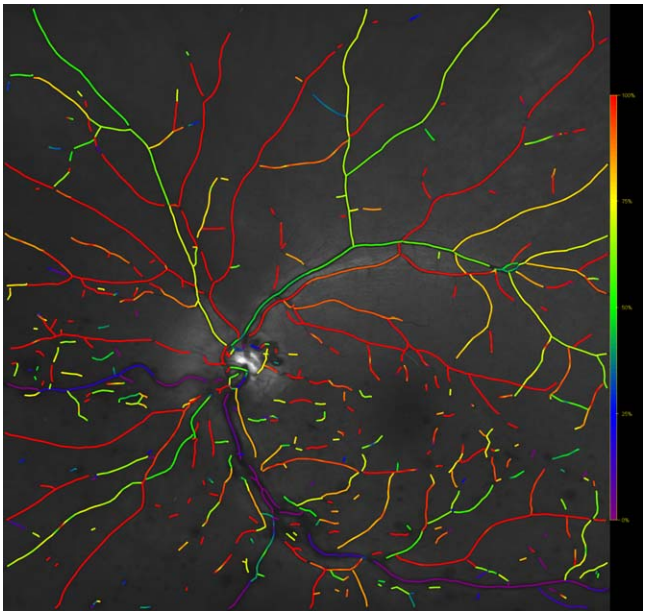

FIGURE 9. Oximetry fundus image from an individual with inferior hemivein occlusion. Images acquired with Optomap 200Tx and analyzed with Oxymap Analyzer software. A clear difference is seen in oxygen saturation between the superior and inferior retinal venules, inferior venules are occluded. See *color scale* on *right side*, *red color* indicates 100%, and *violet* indicates 0% oxygen saturation.

2013;54:ARVO E-Abstract 46). The SLO oximetry images also provide picturesque wide-field images of the variable hypoxia in CRVO or hemivein occlusion cases, which could be clinically useful.

The main difficulty of the SLO oximetry measurement is the considerable variability in oximetry measurements. There is both intra-eye (between vessels within an eye) and intereye (between eyes/individuals) variability. An example of intra-eye variability can be seen in the healthy eye in Figures 7A and 7C, where several vessel segments show abnormally low saturation. Intereye variability is also considerable as evidenced by the SDs for the group of 11 healthy individuals, 13% for arterioles and 12% venules (Fig. 5). Even though the variability is greater than seen with Oxymap T1 retinal oximetry system today,[1,18] it is similar to the variability seen in the early days of developing the Oxymap system.[19] Technical optimization, both hardware and software, proved successful in reducing the variability of oxygen saturation measurements with the Oxymap system. Similar technical improvements are needed for the SLO oximetry system to reduce both the intra- and inter eye variability. To improve the SLO oximetry system, technical changes to the Optomap must be made such as trying different laser wavelengths for imaging. Those kinds of tests require hardware changes to the Optomap and should be the work of future studies.

In the current study, the 100° ResMax image was used for SLO oximetry. However, retinal oximetry images could also be produced with the 200° wide-field SLO imaging, but to further test 200° oximetry images, more extensive software modification and peripheral image quality improvement is needed. Further development of the wide-field imaging for oximetry would allow oximetry analysis of the peripheral fundus, which could be useful in various eye diseases (e.g., diabetic retinopathy). Still, the SLO oximetry system produces an oxygen saturation map of the entire 100° fundus image, which is at much larger angle than the other published SLO oximetry studies have managed.

Wider field of view is one advantage of the SLO oximetry system over a conventional fundus camera system. Other advantages are that no dilation of the pupil is needed and imaging through cataracts and other ocular opacities is easier. It can be helpful to be able to image without being dependent on dilation in cases where patients pupils do not dilate enough (e.g., in some elderly people and people with diabetes). Elderly people do most often have cataracts and/or some other ocular opacity and they are mostly the group of interest for retinal oximetry, due to the age-dependent prevalence of many common and serious eye diseases.

One drawback to the SLO oximetry measurement is less sensitivity of ODR to change in oxygen saturation than Oxymap T1, the fundus camera–based system. The methods section explains that for every unit change in ODR, the calculated saturation decreases by 2.47 percentage points. This is approximately 1.93 times greater rate of change than for Oxymap T1 oximeter, according to default manufacturer calibration. A smaller change in ODR for each unit change in saturation suggests that the SLO oximeter is less sensitive to change in saturation (for more details see Supplementary Material: Sensitivity of ODR to change in saturation).

The SLO oximetry system has also been used to image patients with a variety of other eye diseases including diabetic retinopathy, AMD, and retinal detachment and the use of SLO oximetry in these diseases will be analyzed further in future studies.

In summary, the combination of SLO imaging and retinal oximetry software was successful in developing a system for SLO oximetry, which is both sensitive and gives repeatable results. The intra- and intersubject variability is still large and further hardware and software development is needed. Given the advantages that SLO imaging has over conventional spectrophotometric fundus cameras in retinal oximetry, further development of SLO oximetry may provide the optimal approach for retinal oximetry.

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