Retinal Oximetry Images Must Be Standardized: A Methodological Analysis

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Purpose. Retinal vessel oximetry is a new technology and needs detailed methodological scrutiny. We determine (1) the repeatability of retinal vessel oxygen saturation measurements, (2) whether measured saturation is different between retinal quadrants, and (3) whether the angle of gaze changes measurements of the same vessels.

Methods. Fundus oximetry images were obtained from 26 healthy individuals, 18 to 30 years old, using the Oxymap retinal oximeter. Oxygen saturation in the same vessel segments was compared between two similar images of each individual to determine repeatability. Vessel oxygen saturation was also compared between different quadrants of the retina in the same oximetry image. Finally, oxygen saturation measurements were made on the same vessel segments at different angles of gaze.

Results. Mean and standard deviation of saturation measurements was 93.1% ± 2.3% in arterioles and 64.9% ± 3.3% in venules. Standard deviation of repeated saturation measurements on the same vessel segment was 1.0% in arterioles and 1.4% in venules. Significant differences were seen between retinal quadrants. When angle of gaze was altered, measured saturation was lower in the same vessels when they were located in the inferior portion compared with other parts of the image (−1.3% ± 1.7%, $P = 0.0004$ in arterioles and −1.9 ± 2.4%, $P = 0.0007$ in venules).

Conclusions. Retinal vessel oxygen saturation measurements are repeatable with a small standard deviation. When oximetry results are compared between time points or eyes, the imaging must be standardized and similar parts of the images analyzed. (*Invest Ophthalmol Vis Sci.* 2012;53:1729–1733) DOI:10.1167/iovs.11-8621

There is evidence that oxygen metabolism plays a role in retinal disease, such as diabetic retinopathy,[1–4] venular[5–7] and arteriolar[8,9] occlusions, glaucoma,[10–12] and retinal detachment.[13–16] Noninvasive retinal oximetry allows study of retinal oxygen metabolism in human patients.[17] The retinal oximeter, used in the present study, relies on spectrophotometric measurements to determine oxygen saturation in the retina. A previous version of the oximeter, which is based on the same principles, has been described in detail.[18] Like any new investigational device, the oximeter needs to be tested for reliability, and technical limitations must be explored. In this study, the repeatability of measurements was determined. Secondly, variations between retinal quadrants and whether the measurement is affected by the direction of gaze were explored.

Methods

The Oxymap Retinal Oximeter T1 (Oxymap ehf, Reykjavik, Iceland) utilizes the fact that oxyhemoglobin and deoxyhemoglobin have different light absorbance spectra (color). It takes two images at the same moment with 570- and 600-nm light and compares the light absorbance of retinal vessels to determine oxygen saturation. Oxygen saturation is measured automatically with the Oxymap Analyzer software 2.2.1 (version 3847) in vessel segments that are selected by the user.

Fundus images were obtained from 26 healthy individuals, 18 to 30 years old. Left or right eye was chosen randomly for each subject. Five images were obtained from each individual (eye) at different angles of gaze and of these, two images were at a similar angle (Fig. 1).

According to previous measurements and analyses, there is an artifactual decrease in measured saturation values with increased vessel diameter (Gottfredsdottir MS, et al. *IOVS* 2011;52:ARVO E-Abstract 5668). Therefore, all saturation measurements were corrected by adding 0.7% to the saturation value for each pixel above the mean diameter for arterioles and 1.3% for each pixel above the mean diameter for venules (the oximeter measures vessel diameter in pixels). Similarly, 0.7% (arterioles) or 1.3% (venules) were subtracted from the saturation value for each pixel by which the diameter was below average diameter.

The major retinal vessels were measured close to the optic disc. Some of the data were for measurements of a single arteriole or venule in each eye, in which case the major superotemporal vessel was chosen. Some of the data were for average saturation in an eye, in which case all vessels around the optic disc above 8 pixels (approximately 80 μm) in diameter were measured.

All statistical analyses were performed using GraphPad Prism version 5.00 (Graphpad Software, La Jolla, CA). The study was approved by The National Bioethics Committee of Iceland and The Icelandic Data Protection Authority and adhered to the tenets of the Declaration of Helsinki. All participants signed informed consent.

Repeatability

Two images of the same eye, with the optic disc in the center, were analyzed to determine repeatability. All vessels were measured, in a standardized manner, in images 2 and 5 in the imaging protocol (Fig. 1). Mean and standard deviations between repeated measurements were

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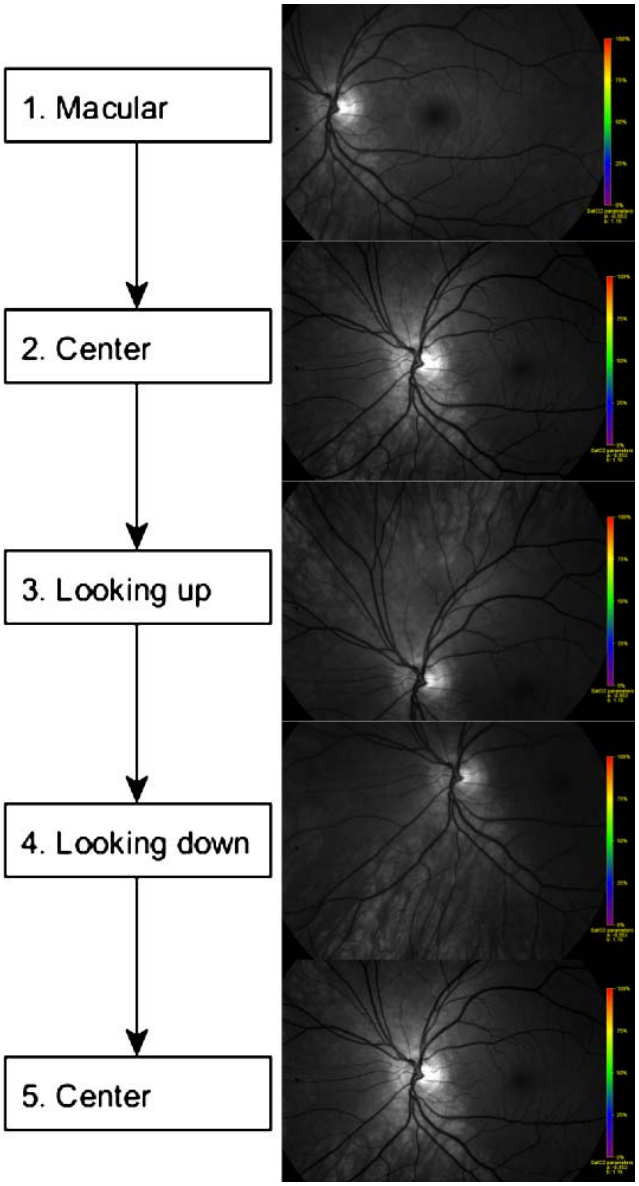

FIGURE 1. Protocol for fundus image acquisition. First, a fundus image centered on the macula was obtained (**1**), then an image centered on the optic disc (**2**), followed by images where the subject looked up (**3**), then down (**4**), and finally a repeat of image 2 with the optic nerve head in the center (**5**).

determined, both for the average retinal vessel saturation in the whole retina and, in a separate analysis, for repeated measurements of one vessel in each subject. For measurements of single vessels, a large vessel in the superotemporal quadrant was chosen.

Paired *t*-tests were used to test the difference between the repeated measurements. One-way analysis of variance (ANOVA) was used to estimate the standard deviation between repeated measurements as recommended by Bland.[19] One-way ANOVA partitions the variability between all measurements into variability between subjects and variability between repeated measurements on the same subjects. More specifically, the square root of the mean square within subjects is an estimate of the standard deviation between repeated measurements.[19]

Retinal Quadrants

For comparison of retinal quadrants, only one image was used for each individual. Image number 2, with the optic disc in the center (Fig. 1), was analyzed, and vessels were classified according to which retinal quadrant they belonged to (Fig. 2). For comparison of quadrants, only subjects with a measured arteriole or venule in each quadrant were included in the analysis, which left 21 subjects for comparison of arteriolar saturation and 19 subjects for comparison of venular saturation. Measurements were evaluated with ANOVA and Tukey's test.

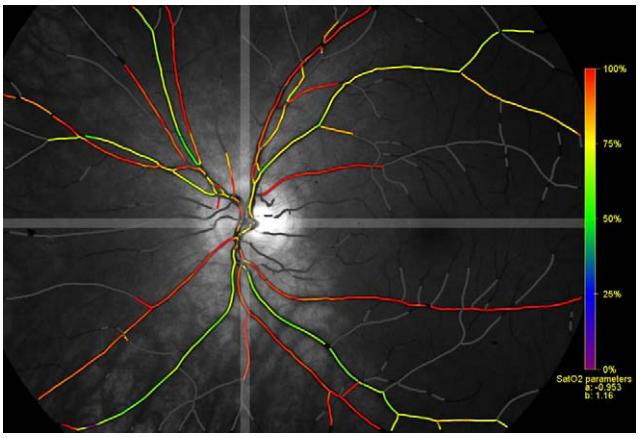

FIGURE 2. Pseudo-color image from the Oxymap oximeter. The color code on the right side shows the oxygen saturation of hemoglobin in retinal blood vessels. Note the slightly different color of the inferotemporal venule indicating lower oxygen saturation compared with the superotemporal venule.

Angle of Gaze

To evaluate the effect of the angle of gaze on oxygen saturation measurements, the same vessel segments were measured twice; once in an image with the optic disc in the center of the image and once in an image in which the optic disc was moved up or down (subject gazed down or up, images 2, 3, and 4 in Fig. 1). That is, location of the same vessel segment was changed with gaze. Vessels below the optic disc were measured in images 2 and 4 (Fig. 1, vessel segments move upwards between image 2 and 4). Vessels above the optic disc were measured in images 2 and 3 (vessel segments move downwards between image 2 and 3). Measurements on the same vessel segments at different angles of gaze were compared with a paired *t*-test.

RESULTS

Repeatability

The results of repeatability measurements on two images of the same area (same angle of gaze) of the same retina can be seen in Table 1. Two types of analyses were made separately; one in which the data were only from one main superotemporal vessel in each eye and one in which the data were average saturation values for each retina. In the case of the averages, the saturation was slightly higher in the latter image (0.5%, $n = 26$, $P = 0.017$ in arterioles and 1.1%, $P = 0.0009$ in venules, image 5 compared with image 2 in Fig. 1, paired *t*-test). The results of measurements of a single vessel in each eye were not dependent on order of measurements ($P > 0.23$, paired *t*-test). Figure 3 shows more detailed results of repeated measurements of single vessels.

Retinal Quadrants

Table 2 shows the results of intraretinal quadrant comparisons, which specifically demonstrated that the inferotemporal quadrant had lower saturation measurements compared with the other three for both arterioles and venules. Measurements

Table 1. Repeatability of Retinal Vessel Oxygen Saturation Measurements from 26 Eyes in 26 Subjects

	Mean ± SD*	Mean of Differences between Repeated (2) Measurements†	SD of Repeated Measurement‡
All arterioles, average for an eye	94.1 ± 2.3	−0.5	0.8
All venules, average for an eye	64.9 ± 3.3	−1.1	1.3
Main superotemporal arteriole	93.1 ± 2.8	−0.3	1.0
Main superotemporal venule	66.7 ± 4.5	−0.3	1.4

All values are hemoglobin oxygen saturation percentage (%). Each measurement was performed on the same vessel segments in two images acquired in the same visit, with the same angle of gaze.

* Mean ± SD was determined from an average of the two repeated measurements of retinal vessel oxygen saturation.

† The measurement values in image 5 (see Fig. 1) are subtracted from values in the earlier image 2.

‡ Standard deviation is derived from a one-way analysis of variance.[19]

of different quadrants were made on the same image (image 2 in Fig. 1).

Angle of Gaze

Table 3 shows the results of measurements on the same vessel segment in images taken during different angle of gaze. In the vessels below the optic disc, measured retinal oxygen saturation was higher when the subject was looking down and the vessel was close to the center of the image, compared with the image in which the optic disc was in the center and the same vessel was in the inferior part of the image. This applies to both arterioles (1.3% ± 1.7% higher, $P = 0.0004$) and venules (1.9% ± 2.4% higher, $P = 0.0007$). No significant difference was noted in the vessels above the optic disc when the subject looked up, compared with the image in which the optic disc was in the center; however, arterioles showed a trend toward lower saturation with an upwards gaze (0.4% ± 1.1% lower than when the optic disc was in the center, $P = 0.052$).

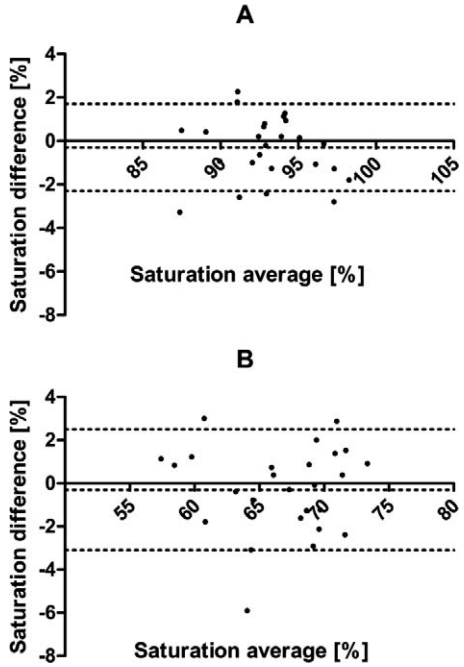

Figure 3. Analysis of repeatability of retinal vessel oximetry results for 26 healthy subjects, age 18 to 30 years. Two images, both taken under the same angle of gaze (images 2 and 5 in Fig. 1) and the same single vessel segments measured in both. (**A**) arterioles; (**B**) venules. The vertical axis shows the difference between two saturation measurements on the same vessel segment (image 2 minus image 5) and the horizontal axis shows their mean. The broken line in the middle shows the mean difference and the other two broken lines show the ±2 standard deviations for repeated measurements (derived from analysis of variance, not the standard deviation of the difference).

Discussion

Repeated measurements of the same vessels in similar images (same angle of gaze) showed that the latest version of the Oxymap Retinal Oximeter (the Oxymap T1) has good repeatability, although the latter image (image 5 in the imaging protocol, Fig. 1) showed slightly but statistically significantly higher mean saturation than the earlier image (image 2). The difference was less than 1%, which is unimportant in most clinical research applications. There was a significant difference in measured oxygen saturation between different quadrants of the retina compared within the same image. This was most pronounced in the inferotemporal quadrant, which had a lower saturation than the other three quadrants. As explained below, the difference between measured saturation in different quadrants may be a technical artifact.

Repeatability

The standard deviation between repeated measurements (same angle of gaze) of average oxygen saturation in the retina was 0.8% and 1.3% in arterioles and venules, respectively, which is sufficient for most clinical measurements. For repeated measurements of single vessels (main superotemporal vessel), the standard deviation was 1.0% and 1.4%. Hammer et al.[20] demonstrated variability with a mean standard deviation for single vessel measurements of 2.52% for arterioles and 3.25% for venules. Earlier, Hardarson et al.[18] presented standard deviations for repeated measurements of single vessels of 3.7% for arterioles and 5.3% for venules with a previous version of the Oxymap oximeter. This shows that the current Oxymap oximeter has good repeatability, slightly better than the earlier version of the instrument[18] as well as other oximeters.[20] However, the latter image (image 5, Fig. 1) showed slightly higher saturation than an earlier image, taken in the same manner (image 2, Fig. 1). This difference was small and unlikely to have an effect in clinical research. The reason for an increased saturation in the latter image is unclear.

Quadrants

The topographical variability in the measurement within the same image was relatively large, up to 7.0% saturation difference between quadrants, with the inferotemporal quadrant most affected. The difference between quadrants was seen

Table 2. Difference between Quadrants in Retinal Vessel Oxygen Saturation

	Superonasal		Inferonasal		Inferotemporal	
	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
Arterioles						
Superotemporal	2.1 ± 3.7*	0.3 to 3.9	1.8 ± 2.3	−0.1 to 3.6	−2.4 ± 3.3*	−4.2 to −0.6
Superonasal			−0.3 ± 3.4	−2.1 to 1.5	−4.5 ± 2.9*	−6.3 to −2.7
Inferonasal					−4.2 ± 3.3*	−6.0 to −2.4
Venules						
Superotemporal	−3.6 ± 4.5	−7.6 to 0.5	−1.3 ± 5.5	5.4 to 2.7	−7.0 ± 8.0*	−11.0 to −2.9
Superonasal			2.2 ± 5.4	−1.8 to 6.3	−3.4 ± 8.1	−7.4 to 0.6
Inferonasal					−5.6 ± 7.4*	−9.7 to −1.6

The table shows the results of a Tukey post-test, the row is subtracted from the column and mean ± SD and 95% confidence interval (CI) are shown. All numbers are hemoglobin saturation percentages. There is a significant difference between quadrants ($P < 0.0001$ by one-way analysis of variance for both arterioles and venules [$n = 21$ for arterioles and $n = 19$ for venules]).

* Statistically significant difference ($P < 0.05$).

both in arterioles and venules, which suggests that the difference is an artifact rather than of physiological origin. Such a difference between branches of the central retinal artery seems very unlikely from a physiological consideration, since they are measured shortly after branching. It is unclear what caused this effect, but it is worthy of further investigation since it has a notable impact on clinical research. Clearly, retinal oximetry images must be standardized for comparison studies and the same parts of the retina must be compared.

Angle of Gaze

The difference in retinal vessel oxygen saturation with angle of gaze has to be kept in mind when images from different angles are compared. Our results emphasize the importance of standardization of imaging technique and image analysis so that similar images are compared. It is unlikely that this variation is of physiological origin, since it is observed in arterioles as well as in venules.

It seems that the variation between quadrants is related to the effect of the gaze (although both were tested separately here). Upwards gaze, for example, moves a vessel segment down in the image frame and leads to lower measured saturation. Inferior quadrants, which are of course also lower in the image than superior quadrants, show a lower measured saturation (compared within an image). For some reason measured saturation values are lower in the inferior part of the fundus image than in the superior part, regardless of whether this is in the same vessels, which are moved with gaze, or when different retinal quadrants are compared in the same image. This is most likely from optical or illumination aberrations, which we have not yet been able to characterize.

Conclusions

Repeatability in retinal vessel oxygenation saturation measurements is approximately 1% with the Oxymap Retinal Oximeter T1. The photographic flashes may have an effect on the measurement, but it is relatively small and unlikely to have an impact in clinical research. The angle of gaze as well as the quadrant of the measured vessel have a substantial effect. It is important to standardize image acquisition and analysis to have comparable oximetry data for comparison between eyes and quadrants of eyes.

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Table 3. Retinal Vessel Oxygen Saturation ($SatO_2$%) Difference between Images, Taken under Different Angles of Gaze for 26 Subjects

Angle of Gaze	Average Change in $SatO_2$% (Images 3 or 4 Subtracted from Image 2 with Optic Disc in the Center)* Mean ± SD	*P* Value†	95% CI
Arterioles above optic disc, upwards gaze (image 3 in Fig. 1)	0.4 ± 1.1	0.052	0.0 to 0.9
Venules above optic disc, upwards gaze (image 3 in Fig. 1)	0.1 ± 2.2	0.87	−0.8 to 1.0
Arterioles below optic disc, downwards gaze (image 4 in Fig. 1)	−1.3 ± 1.7	0.0004	−2.0 to −0.7
Venules below optic disc, downwards gaze (image 4 in Fig. 1)	−1.9 ± 2.4	0.0007	−2.8 to −0.9

The table shows average change in measurements compared to an image with the optic disc in the center.

* Image 2 has the optic disc in the center of the image, image 3 is of an eye gazing up and image 4 is of an eye gazing down (Fig. 1).

† Paired *t*-test

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