tvst

DOI: 10.1167/tvst.3.5.2

Review

Pathway to Retinal Oximetry

James Beach

CytoViva, Inc., Auburn, Alabama

Correspondence: James M. Beach, PhD, CytoViva, Inc., 570 Devall Drive, Ste 301, Auburn, AL 36832, USA; e-mail: eadeae@gmail.com

Received: 27 March 2014
Accepted: 16 June 2014
Published: 3 September 2014

Keywords: oximetry; in vivo imaging; historical review

Citation: Beach J. Pathway to Retinal Oximetry. Tran Vis Sci Tech. 2014; 3(5):2, http://tvstjournal.org/doi/full/10.1167/tvst.3.5.2, doi:10.1167/tvst.3.5.2

Events and discoveries in oxygen monitoring over the past two centuries are presented as the background from which oximetry of the human retina evolved. Achievements and the people behind them are discussed, showing parallels between the work in tissue measurements and later in the eye. Developments in the two-wavelength technique for oxygen saturation measurements in retinal vessels are shown to exploit the forms of imaging technology available over time. The last section provides a short summary of the recent research in retinal diseases using vessel oximetry.

Introduction

The quest to measure blood oxygenation in the human body, which started over a century ago with discovery of the oxygen carrier hemoglobin, laid the groundwork for present day studies of the role of oxygen in retinal diseases. Using increasingly sophisticated techniques, workers from diverse areas of applied science and medicine made advances leading to the revolutionary pulse oximetry monitor, and ultimately to new instruments for oxygen measurements in the eye. Here, we look back at significant developments and interesting anecdotes from early and contemporary work that have led to the present form of two-wavelength retinal oximetry. Other important forms of retinal oximetry are touched on, but here the story of two wavelengths is told, which is becoming an important tool for clinical research worldwide.

The Path Toward Tissue Oximetry

In the mid-19th century the German biochemist Hoppe[1] noted that Hematin, which could be crystallized from blood pigment, came from an iron-containing protein found in red blood cells.[2] He called this pigment hemoglobin and described its spectrum in the oxygen-bound form of oxyhemoglobin. This discovery was quickly noted by the eminent British physicist George G. Stokes,[3] who showed that hemoglobin bound reversibly with oxygen, with distinct spectra for the two forms of oxyhemoglobin and deoxyghemoglobin. In 1935, the ensuing work with blood analysis, particularly in skin, led to an oxygen sensor when German physicists Matthes and Gross[4] produced the first continuous in vivo oxygen saturation monitor, using transmission of red and infrared light in the ear lobe. Their technique was the first to employ a second wavelength to compensate for variation in tissue and instrumental factors. Then in 1940, working in London, J. R. Squire[5] recognized that absolute oxygen saturations could be determined using differential changes in red and infrared light transmission that were caused by tissue compression. By squeezing the blood from tissue between the thumb and first finger, he could zero the blood reading to obtain a calibration.

The need for a portable blood oxygenation monitor became urgent at the start of the Second World War. In 1941, Glenn Millikan,[6] an American scientist, produced a portable oxygen sensor as a tool for training pilots and to study effects of higher altitude flight. His sensor was combined with the pilot's breathing apparatus, a system designed to keep pilots from blacking out in high-altitude dog fights,[7] however, the device was heavy and was not used in combat. Millikan called his device an ear oximeter,

coining the term oximetry, and although it was not designed to measure oxygen saturation, it was able to control the delivery of oxygen to the pilot's mask in response to light signals that sensed blood saturation. His device also used the red and infrared wavelengths that were more readily transmitted through tissue, and a photoelectric sensor to signal the electronic controls feeding the pilot's mask. Although the sensor did not selectively sense the arterial saturation, the method worked because pilots breathed pure oxygen, which tends to equalize oxyhemoglobin levels across the blood supply. In 1948, Earl Wood and his student J. E. Geraci,[8] working at the Mayo Clinic, refined the optical and mechanical techniques of Millikan and Squire. Wood[9] applied the idea of using tissue compression in the ear lobe, using a tissue compression membrane to stop blood flow and then allow reperfusion. With four light signals from red and infrared light transmitted alternately through the compressed and decompressed ear lobe, Wood devised a self-calibrating, continuous reading oximeter that worked across subjects with variable-tissue background. Then in the mid 1960's Robert Shaw,[10] a San Francisco surgeon, invented an eight-wavelength ear oximeter, which could determine relative amounts of the hemoglobin derivatives oxy- deoxy-, carboxy-, and methemoglobin. Knowing the light absorptions at each wavelength permitted calculation of their concentrations using a simultaneous set of Beer-law equations. The Shaw system, commercialized by Hewlett-Packard, heated the skin to 45°C to arterialize blood in a manner similar the transcutaneous oxygen electrode measurement of tissue PO_2.[11] Unfortunately the multiwave oximeter was expensive and did not achieve wide-spread clinical acceptance since blood gas analysis was also available.

A major development occurred in 1972. Takuo Aoyagi,[12] a Japanese engineer working for the Nihon Kohden company, had been using an ear piece of Millikan's design to measure cardiac output from the washout curve of the perfusion cardio green dye, using an infrared ear sensor. Pulsations in light from the cardiac cycle were interfering with the measurement, but Aoyagi realized that during each of the pulses the blood supply became arterialized as fresh blood replaced oxygen-depleted blood. By also sensing the pulses at a second wavelength, Aoyagi and co-worker Michio Kishi[13] produced a pulse oximeter that did not require a bloodless calibration, and could provide a reproducible reading of the arterial saturation.[14,15] Their invention led to rapid development of pulse oximetry; however, Aoyagi did not continue to work on the new invention, and other groups pursued its commercialization with fingertip sensors. Severinghaus' Special Article,[16] "*Takuo Aoyagi: Discovery of Pulse Oximetry*" tells the interesting story of this period. An important concept came from the new pulse oximetry called the "ratio of ratios." By normalizing the pulsatile component of blood signals using the constant background absorption from venous blood and other tissue sources, a "first" ratio enabled an arterial signal to be isolated. This also removed any variation caused by differences in subject pigmentation and tissue structure. Then, by electronically extracting the time-varying and static components from each of the light signals, and taking the ratio of these components, a signal corresponding to arterialized blood was created. This "second" ratio was proportional to oxygen saturation, and could be converted to percent saturation using calibration coefficients determined empirically. Aoyagi's technique has been recognized as the first to employ the "ratio of ratios" idea to extract a desired signal, in this case an important vital sign, from a more complex optical measurement. Although only those main developments related to oximetry are described here, several excellent historic reviews of oxygen measurement from perspectives of physiology and medicine are available.[17–19] Over a period lasting a century, oximetry was conceived, developed and refined to where it had a critical role in anesthesia, infant monitoring and later, wound healing. Approaching the mid-20th century and following the path already paved, a decades-long development of oximetry in the eye began.

Two-Wavelength Retinal Vessel Oximetry

Development of oximetry for the retina used many of the same optical ideas that led to tissue oximetry. Here are the developments leading to a two-wavelength form of retinal oximetry. In 1959, working out of Indiana University (Indianapolis, IN) and Duke University (Durham, NC), John Hickam, Herbert Sieker, and Regina Frayser[20] reported the first measurement of oxygen saturation in human retinal vessels. Ten years prior at Duke, Hickam and Frayser[21] had shown that a spectrophotometric determination of blood oxygen saturation could replace time consuming gasometric blood analysis that was required for measurement of cardiac output and oxygen consumption. Over the next decade their pioneering work in the eye, using photographic film to record images of vessels on the optic disc, resulted in detailed studies of the retinal venous saturation, arteriovenous saturation

difference, and responses to vasoactive drugs.[22,23] They were first to show that a linear relationship existed between vessel oxygen saturation and a ratio of vessel optical densities (ODs) at two wavelengths, one being relatively insensitive to change in saturation and the other sensitive to this change. Hickam's measurements were calibrated by having subjects breathe mixtures of oxygen and nitrogen. One advantage of film recording was that it allowed the vessel OD to be determined directly from the film's negative. This work preceded the application of ratio measurements in pulse oximetry by over a decade, possibly following the approaches adopted earlier, although Hickam's references do not mention the precedent for ratio analysis.

Continuing in the mid-70s with film photography, Robert Laing, Allen Cohen, and Ephraim Friedman[24] at the University of Massachusetts (Boston, MA) showed in their "falling rabbit" study that this relationship was linear over both physiologic and hypoxic ranges. Pulse oximetry had also been checked to be sure it accurately reported oxygen saturations. However, deaths from hypoxia during surgeries began after the introduction of barbiturate anesthesia in the 1930s, which impaired ventilation, and continued even after clinics started using the pulse oximeter. The problem was quickly corrected in 1980 when Frank Sarnquist and colleagues[25] at Stanford Medical School showed that pulse oximetry had been significantly overestimating hypoxic blood saturation. After incorporating their results into the saturation calculation, pulse oximetry was able to greatly reduce hypoxia-related deaths in anesthetized patients. Retinal vessel measurements did not share this problem for two reasons. The measurement was made on homogeneous samples of blood in vessels appearing separately in images. Thus, measurements in vessels could distinguish arterial and venous saturation. Secondly, one of the wavelengths is chosen to be insensitive to changes in saturation. When both wavelengths are sensitive, the relationship between the OD ratio (termed the ODR) and oxygen saturation departs from linearity, however, when one of the wavelengths is insensitive, the relationship becomes linear. But still, there were potential inaccuracies. Stability of the vessel saturation during the cardiac cycle has been called into question, and thus far, no careful studies have reported an effect of the pulse on measured saturations, although Knudtson and colleagues[26] have shown cardiac-synchronized pulses in the diameters of retinal arteries and veins. A correlation between the pulse and vessel saturation could be exploited to detect vascular disease. The size of the vessel was shown to influence the two-wavelength saturation measurement,[27] however, this systematic error has been investigated and a correction proposed.[28] The linear relationship shown previously by Hickam[20] and Laing[24], which is widely assumed for two-wavelength measurements, strictly holds only if one of the measurements is made precisely at a wavelength where light absorption is the same for both oxy- and deoxyhemoglobin. This requirement is not met in most oximetry measurements with two wavelengths, and would not be required if an internal calibration existed to provide an operating curve to translate blood light absorption into saturation.

A significant development came in the mid-1980s from Francois Delori[29] at the Schepens Eye Research Institute in Boston. Delori developed a three-wavelength photoelectric retinal oximetry method that could operate continuously. Vessel saturations were found by scanning a focused point of light across the vessel, allowing both the vessel diameter and the optical density of the blood to be calculated from the profile of the reflected light. Unlike in film recordings, now the optical density needed to be calculated from its defining relationship, where $OD = \log_{10}$ (incident light/transmitted light). An assumption was made that the light returned just outside the vessel represents the incident value, while the drop in brightness inside the vessel is accounted for by absorption in the blood. These estimates, particularly for incident light, are subject to the variations in pigment density that occur across the retina and between individuals, and thus reduce the accuracy of saturation measurements. In fact, light returned is influenced by both absorption and scattering processes.[30,31] Delori eliminated most of the problem with two insensitive wavelengths to establish local baselines around which measurements at one-third oxygen-sensitive wavelength could be determined. Using light extinction coefficients that were corrected for the finite spectral resolution in his recordings, the three-wavelength technique gave an internally calibrated measurement of the saturation. In 1989, Sebag and Delori[32] published the first application of oximetry in retinal disease. Delori replaced the instantaneous film recordings with those of a continuous photoelectric sensor and built eye-tracking into his system; however, the method was not designed to easily capture measurements across the retina.

Near the end of the next decade Dietrich Schweitzer and Martin Hammer at the University of Jena (Jena, Thuringia, Germany) began publishing a series

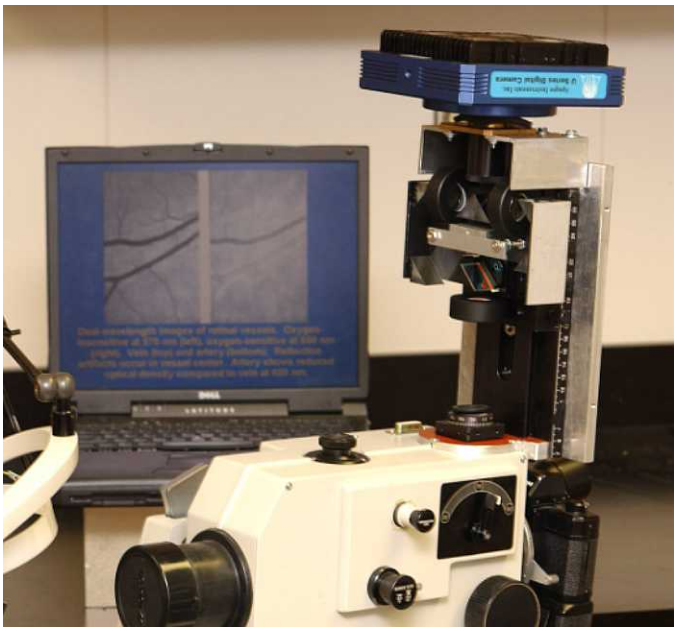

Figure 1. The 2003 prototype of a two-wavelength retinal oximeter at the Louisiana State University Lion's Eye Center. The light shield is removed showing the optical section, with dual optical paths directed by front surface and dichroic mirrors, similar to that of the first system built at the University of Virginia. Images were recorded by an astronomy CCD camera. In the background is a dual-wavelength recording of a primary artery and vein (reproduced with permission from Bahram Khoobehi).

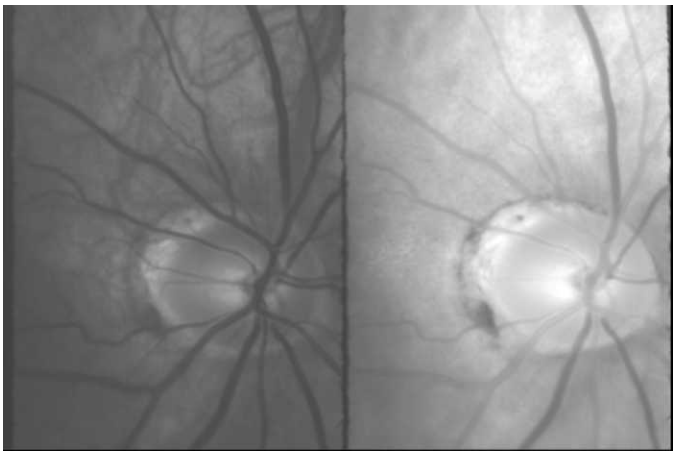

Figure 2. The optic disc and surrounding vessels side-by-side in a single camera image with the 2003 prototype oximeter (Hiroyuki Kawano, Lion's Eye Center). *Left*: reference image (570 nm) is insensitive to oxygen saturation. *Right*: measurement image (600 nm) was recorded simultaneously with the reference and is sensitive to saturation, causing arteries to appear light and veins are dark. The retinal tissue background is lighter due to decreased light absorption by retinal pigments at the red wavelength.

of reports on retinal oxygen saturation using spectral recordings.[33,34] They replaced the light scan with the entrance slit of an imaging spectrograph, which gave them a continuous spectrum of the light reflected from inside and outside a short section of vessel. By fitting their spectral curves with the known absorption for oxy- and deoxyhemoglobin, and the baseline retinal pigment, they could obtain a calibrated measurement. The Jena imaging spectrograph was also used to obtain optical properties from the complex tissue environment comprising the retinal vessel and fundus backround.[35,36] These recordings with multiple wavelengths, at single-vessel sites, produced a benchmark for the normal values of vessel saturation in the retina as well as changes in disease. Around the same time, two biomedical engineers, James Beach and Sankar Srinivas, working with retinal specialist James Tiedeman at the University of Virginia (Charlottesville, VA), were trying to adapt Delori's three wavelengths to digital imaging in order to obtain wider retinal coverage in diabetic subjects. They found that their filter wheel was too slow to keep up with eye motion and so this approach was abandoned. However, Srinivas noted that the arteries always showed up lighter than veins in red images. Beach had just done a series of intravital membrane potential recordings in microvessels with a voltage dye, which used the ratio of fluorescence at two wavelengths to compensate for vessel motion,[37] and so they tried a ratio method for retinal oxygen recordings. Digital cameras were quite expensive at that time, so a way to record two images at the same time with a single camera was needed. After building an image splitter from right angle prisms (Fig. 1) and choosing red and green wavelengths for oxygen-sensitive and insensitive images, they succeeded in recording pairs of retinal images side-by-side on a digital camera (Fig. 2). The green wavelength was set where the oxy- and deoxyhemoglobin curves crossed with equal and opposite slopes, which kept the reference image insensitive to saturation as the filter passed light over a finite spectral width. The red wavelength was set near the maximum of the oxy-deoxyhemoglobin difference spectrum for high sensitivity, but not so far to the red that the artery image disappeared. The new system was employed by Tiedeman et al.[38] to explore oxygen saturation changes during acute hyperglycemia in diabetic patients. His study showed that blood flow autoregulation in retinal vessels was impaired in preretinopathy to a degree that correlated with the disease duration. One year later the digital imaging method was reported after rigorously establishing the external

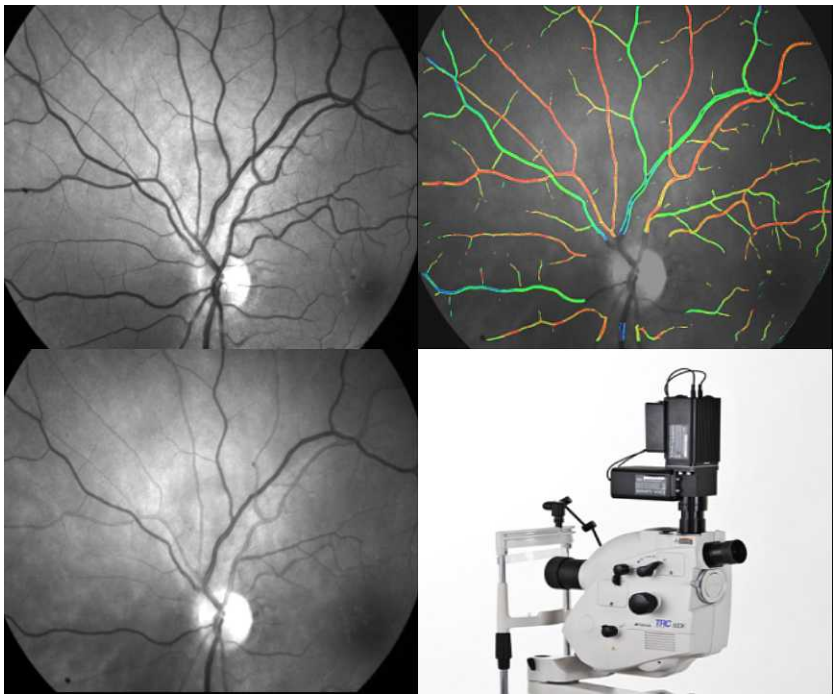

Figure 3. Retinal vessels imaged over a wider field of view (12 × 12 mm) with two synchronized cameras on the Oxymap oximeter in 2009 (Reykjavik). Arteries and veins show similar density in the reference image (*top left*), while arteries are less dense than veins in the corresponding measurement image (*bottom left*). *Top right*: Color saturation map by the author. Arteries and veins appear *red* and *green*, respectively, denoting higher and lower saturation. Higher saturation seen in smaller branching veins is an artifact caused by the fundus background. A correction for this effect has been proposed.[28] The mapping process ignores vessels near the optic disc area. *Bottom right*: Oxymap T1 oximeter with dual cameras mounted on an ophthalmoscope (reproduced with permission from Oxymap ehf).

calibration with breathing experiments using oxygen–nitrogen mixtures, and after assessing influences of the vessel diameter and retinal pigment density.[27] By estimating the amount of light falling on vessels using only the red light, where pigment absorption is reduced, variation of the saturation measurement was also reduced as the correction made the measurement less susceptible to variation in retinal background. Recording the two images simultaneously with a single (CCD) charge-coupled device camera precluded full retinal coverage (Figs. 1 and 2), however, it was possible in single recordings to measure along several millimeters of artery and vein and determine the arterio-venous saturation difference. This digital two-wavelength method turned out to be serendipitously similar to Hickam's first photographic measurements, but the author did not know this until work was well along. The retinal two-wavelength method also relies on a "ratio of ratios" idea. The "first" ratio occurs in the logarithmic definition for OD. In the "second" ratio, both the sensitive and insensitive ODs are dependent on similar factors, such as vessel diameter and image focus. However, the sensitive OD is dependent on saturation while the insensitive OD is not. Therefore, the ratio of the two ODs, which has been termed the ODR, is sensitive to saturation, while other factors tend to cancel out. This "ODR" is converted to saturation values from an external calibration.

Just before the end of the decade Beach began a collaboration with medical faculty at the University of Iceland (Reykjavik, Iceland) and shortly after, with Bahram Khoobehi at the Louisiana State University (Baton Rouge, LA), where improvements to the two-wavelength camera were tested. Einar Stefansson, an early advocate of altered oxygen in retinal diseases, and Thor Eysteinsson, a retinal electrophysiologist, were interested in the new technology for clinical research. Together with signal processing expert Jon Atli Benediktsson, the four cofounded the Icelandic company that would produce the first commercial two-wavelength retinal oximeter. Still working with single cameras, a series of advanced oximeter prototypes were developed and saturation maps were added to show the oxygen transport in the vessels. This work stimulated new image processing methods that were needed for automated oximetry, and improved accuracy and reproducibility of the satura-

tion measurement.[39–42] Coverage of the retinal vessels was increased after switching to two synchronized cameras, which by then cost about the same, and in 2009 a commercial retinal oximeter (Fig. 3) was available for research from Oxymap ehf in Reykjavik.[43] Near the same time, Hammer and colleague Walthard Vilser[28] also produced a retinal oximeter using a color camera to capture the two-wavelength image. In their version, the eye was illuminated solely by measurement and reference wavelengths which were obtained with a custom optical filter placed in the illumination path. This reduced the amount of light reaching the subject's retina, lessening the possibility for light-induced vascular responses that could alter saturations in subsequent measurements, and also the degree of discomfort to the subject from the flash. After this, both oximeters were equipped with light filters for patient comfort. The Hammer-Vilser[28] oximeter also incorporated the "red" correction for pigment variation as well as improved methods to compensate for variations in pigment density and vessel size. Soon after, a commercial oximeter was produced by Imedos, also in Jena, as a companion to the retinal vessel analyzer. Both the Oxymap and Imedos oximeters were deployed with commercial fundus cameras, and in a short-time, clinical research papers began to appear.

Study of Retinal Disease

Following in the steps of pioneering studies of retinal oxygen and disease, the next efforts began mainly to gain new understanding of retinal disease and to determine if the new technique could detect or predict the presence of specific disorders. Results reported using both versions of the oximeter have been largely consistent. In arterial and venous occlusions involving central and branching vessels, oxygen saturation was found to be reduced in the affected eye, with a trend toward partial normalization after treatment.[44–46] In diabetes, a significant elevation of the saturation in retinal veins, with degree dependent on severity of retinopathy, has been consistently reported.[47–50] These findings were at first surprising since capillary dropout and proliferative retinopathy are believed to result from hypoxia. However, they are consistent with known diabetic complications of capillary wall thickening and development of vascular shunts, which decrease oxygen extraction from the blood and reduce the surface for gas exchange. Tiedeman et al.'s[38] earlier finding for impaired vessel autoregulation may point to an additional mechanism. These pathogenic mechanisms could thus reduce oxygen tension in retinal tissue. A test that can predict increased risk for progression of retinopathy should be forthcoming from this work. In glaucoma the results so far have been less conclusive. Studies combining blood flow and oximetry measurements suggested that treatment with carbonic anhydrase inhibitors raises the oxygen delivery to the retina,[51,52] while almost no effect of glaucoma filtration surgery on retina oxygenation was found.[53] Stimulation of oxygen consumption by light flicker caused venous saturations to be raised to a lesser degree in glaucoma patients than in normals, suggesting that oxygen supply or consumption is reduced in glaucoma.[54] In one early study carried out before commercial oximetry, normal-tension glaucoma was associated with lower arterial saturation.[55] Together, these studies suggest an altered oxygen use in glaucoma that requires further analysis. In age-related macular degeneration, the normal reduction in saturation of veins with age was found to be reversed, suggesting an altered oxygen metabolism.[56] Finally, a modest yet significant venous increase was reported following pars plana vitrectomy, presumably because the source of oxygen to the retina is increased.[57] Nearly all of the work to date points to abnormal venous saturation in retinal disease. But the work is just beginning and the findings, although preliminary, suggest a rich path for future research. A more complete review of oximetry and retinal disease is available in Sveinn Hardarson's doctoral thesis.[58]

Conclusion

The path leading to clinical retinal oximetry is grounded in the tissue measurements that came before. In the human retina the work began with imaging technology that was available at the time, and has made a wide circle back to imaging with digital methods. Along the way highly innovative work added electronic sensing and spectral analysis in ways that could be incorporated into a modern imaging format. The strategy continues to exploit the ratiometric measurement concepts that were successful for tissue oximetry; however, there are problems still to be solved and we may look back again at tissue optics for solutions. We are starting to understand the role of oxygen in different retinal diseases from the perspective of clinical measurements. The path now should lead to improved patient care in the diagnosis and treatments for many of the retinal disorders.

Acknowledgments

The author thanks Sveinn Hakon Hardarson for his critical reading of the manuscript. Robert Bryant, Brian Duling, Gisli Hreinn Haldorsson, Robert Karlsson, Herbert Kaufman, Hiroyuki Kawano, Daniel Kim, and Jinfeng Ning have also made valuable contributions.

Support by the National Institutes of Health Grants EY-12606 and EY-14776.

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