Optical Techniques for Blood and Tissue Oxygenation

Chapter · January 2018
DOI: 10.1016/B978-0-12-801238-3.10886-4

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Optical Techniques for Blood and Tissue Oxygenation

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Glossary

Arterial (venous) oxygen saturation Amount of oxygen, expressed in percentage, measured from the arterial (venous) blood.
Hemoglobin Protein present in red blood cells responsible for the transport of oxygen in the blood. When the molecule is fully bound with oxygen, its form is known as oxyhemoglobin; otherwise, it is defined deoxyhemoglobin.
Light absorption Phenomenon in which photons coming in contact with a matter are absorbed by its molecules. An absorption event can cause the transformation of energy into heat or the re-emission of a photon at a lower energy.
Light scattering Phenomenon related to the refractive properties of molecules, causing photons to change the direction of motion when in contact with a matter's molecules. Depending if the photon has lost energy during the interaction, scattering can be divided in "inelastic" or "elastic."
Microcirculation Term used to define the blood flow (volume) in the capillaries, where the exchange of oxygen, nutrients, and waste products between blood and tissues takes place.
Oxygen saturation Amount of oxygen, usually in percentage, present in blood or tissue. It is the ratio of oxyhemoglobin and total hemoglobin (oxyhemoglobin + deoxyhemoglobin).
Photons In the quantum model of light, photons are the smallest energy packets in which light is constituted. The energy of the photons is proportional to the wavelength (frequency).
Wavelength Light can be defined as comprising oscillating electromagnetic waves. As the name suggests, the wavelength is the length of a light wave.

Introduction

The human body is composed of trillions of cells, each of which requires the continuous supply of oxygen (O_2) and the excretion of carbon dioxide (CO_2) for metabolism and survival. Since measuring the amount of oxygen present in blood or tissues could help assessing a person's health and overall well-being, this has constituted one of the main challenges in Biomedical Engineering.

Blood oxygenation is the measure of oxygen present in arterial or venous blood, while the measure of oxygen present in the vascular tissue or microvascular bed is referred to as tissue oxygenation. Blood oxygenation assesses how well oxygen binds to hemoglobin, indicating adequacy of pulmonary gas exchange. Tissue oxygenation indicates adequacy of tissue perfusion and is primarily determined by local delivery of oxygenated blood. Oxygen levels in the blood or tissue can be measured using chemical, optical, or colourimetric techniques. Optical methods have several advantages over chemical or calorimetric techniques as light is virtually harmless, and can easily be generated and detected by optoelectronic components (e.g., light emitting diodes (LEDs) or photodiodes). Also, with the availability of lasers and optical fibers, the measurement of oxygenation from within the body cavities is easily possible with great accuracy and precision. "Oximetry" is the general term used to refer to optical methods of measuring oxygen levels, and is widely used in clinical environments. In oximetry, the level of oxygen in the arterial and venous blood is indirectly determined by measuring the arterial oxygen saturation (SaO_2) and the venous oxygen saturation (SvO_2). Oxygen levels measured in tissue are determined by measuring the tissue oxygen saturation, generally known as StO_2, rSO_2, or TO.

From the time the physical principles of light transmission in vascular tissue and absorption of light by blood chromophores were devised, there has been a constant effort to develop optical techniques for measuring blood and tissue oxygenation. Currently, there are a vast number of optical techniques utilizing light to measure oxygen content either in tissues or blood. Spectrophotometry forms the basis of all oximetry techniques, which include spectrophotometry, Cooximetry, pulse oximetry, optical fiber venous oximetry, Near-infrared spectroscopy (NIRS), Resonance Raman Spectroscopy, etc. It would be rather challenging to cover all these in a single document, and therefore this article will only focus in providing an overview of the main in vivo techniques used for the continuous measurement of blood and tissue oxygenation.

It is also worth mentioning that several clinical and research instruments use light to measure parameters related to blood or tissue oxygenation, without measuring the oxygen content (or saturation) directly. For instance, laser Doppler flowmetry uses coherent laser light to assess the flow of red blood cells (RBCs). Since RBCs transport oxygen in the blood, assessing the flow in normal physiological conditions may also provide an indirect measure of the oxygenation state in the sample volume of tissue. Similarly, indocyanine green (ICG) imaging uses fluorescence principles to visualize the microcirculation in a tissue bed. This allows clinicians to identify any areas of the tissue in which blood (oxygen) is not supplied, thus preventing the onset of ischemia. For conciseness, techniques that do not strictly measure oxygenation, such as laser Doppler flowmetry or ICG imaging, will not be included in this review, but we refer the reader to some sources in the "further reading" section as a good starting point for familiarizing with some of these techniques.

Pulse Oximetry

Pulse oximetry is a noninvasive optical technique used for the continuous assessment of arterial oxygen saturation (SpO_2). Pulse oximetry is arguably the greatest advancement in patient monitoring in recent years. Spurred on by operational simplicity, decreasing cost, and the ability to quickly detect hypoxemia, pulse oximetry became the standard for monitoring oxygen saturation during anesthesia as well as in the recovery room and intensive care units across the world (Kelleher, 1989). Since its widespread availability in the 1980's, pulse oximetry usage in critical care has increased rapidly, the global market for pulse oximetry is projected to reach US $2.4 billion by 2024. Prior to pulse oximetry, there was no direct and simple method of continuously measuring oxygen saturation. The only readily available indicator of oxygen status was the measurement of partial pressure of oxygen (PO_2) in arterial blood as measured by a blood gas analyzer. However, due to the complex oxygen–hemoglobin reaction, the relationship between PO_2 and percentage oxygen saturation is nonlinear, making the interpretation rather difficult. Clinicians used to also look for signs of cyanosis (blue coloration of the skin), but this method could be unreliable as it is subjective and can depend on external factors related to the observer and the environment. In modern anesthesia and critical care setting, SpO_2 measured continuously by pulse oximetry and intermittent PO_2 measurements, measured by a blood gas analyzer, complement one another and provide a comprehensive assessment of a patient's oxygen saturation.

Principle of Operation

Pulse oximeters shine light at two different wavelengths into vascular tissue and detect the changes in transmitted or reflected light energy. This process takes place by connecting a transducer containing light emitters and a photodetector to a highly perfused area such as the finger, the toes, or the ear lobe. The transmitted or reflected light energy detected by the photodetector is converted into a voltage signal called the photoplethysmogram (PPG) (Kelleher, 1989). As shown in Fig. 1, the PPG signal consists of a pulsatile alternating part (AC) component and a slowly varying DC component with a magnitude that is determined by the nature of the matter through which the light passes. The amplitude of the pulsatile component varies cyclically and in synchrony with the pumping action of the heart. As the volume of blood in arteries and arterioles starts to increase during systole, the proportion of incident light absorbed also increases. This is possible because the arterial walls are elastic so their diameter changes in response to variations in transmural pressure. Conversely, during diastole, the light absorption decreases with a decrease in blood volume, creating a rapidly alternating signal (Kyriacou, 2006). These excursions typically account for 1%–2% of the total light absorption. This AC allows for differentiation between the absorbance due to nonpulsatile components (skin, venous blood, and a constant amount of arterial blood) from the absorbance due to the pulsatile arterial blood.

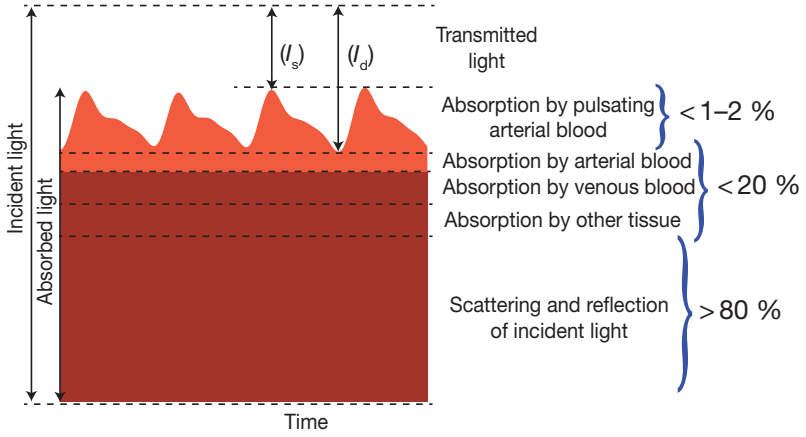

Fig. 1 Light absorption profile in pulse oximetry. In the figure, the photoplethysmogram (i.e., the signal used to estimate arterial oxygen saturation in pulse oximetry) is decomposed into its absorbing components. An alternating signal (AC) originates from the pulsating arterial blood and rides at the top, corresponding to 1%–2% of the total absorption. The rest of the signal is composed by the absorption of quasi-static components such as venous blood and skin. The light intensities I_s and I_d, corresponding, respectively, to the systolic and diastolic phases of the cardiac cycle are then measured at two different wavelengths (red and infrared) to estimate the arterial oxygen saturation SpO_2. Modified from Webster, J. G., ed. Design of pulse oximeters. CRC Press, 1997.

The absorbance of light energy by the pulsatile arterial blood is determined using the Beer-Lambert law, which states that the light absorption through a medium is proportional to the concentration of the light absorbers present in the substance, the optical properties of the light absorber, and the optical pathlength traveled by the light beam.

$$A_\lambda = \varepsilon_{(\lambda)}.C.L \tag{1}$$

where A_λ is the light absorbance at a given wavelength, $\varepsilon_{(\lambda)}$is the molar absorptivity of the absorber at wavelength λ, C is the concentration of the absorber, and L is the optical pathlength traveled by the light. Rewriting Eq. (1) to include the baseline transmittance (I_d) during diastole and the pulsatile transmittance (I_s) during systole (Fig. 1), the light absorbance of the arterial blood is given as

$$A_\lambda = \varepsilon_{(\lambda)}.C.L = \ln(T) = \ln\left(\frac{I_s}{I_d}\right) \tag{2}$$

"T" here represents the transmittance of light through the medium. Since the AC component of the transmitted signal represents the difference between I_s and I_d at the wavelength λ ($AC_{(\lambda)} = I_s - I_d$), Eq. (2) can be rewritten as:

$$A_\lambda = \ln\left(\frac{I_d + AC_\lambda}{I_d}\right) = \ln\left(1 + \frac{AC_\lambda}{I_d}\right) \simeq \left(\frac{AC_\lambda}{I_d}\right) \tag{3}$$

As mentioned earlier, the AC component typically accounts for 1%–2% of the total light absorption. The consequence of AC being so small is that the slowly varying DC component of the signal may be considered roughly equal to I_s or I_d; hence Eq. (3) can be written as:

$$A_\lambda = \left(\frac{AC_\lambda}{DC_\lambda}\right) \tag{4}$$

The ratio of light absorption at the two wavelengths as in Eq. (5), along with an empirically derived calibration curve, is used to provide an instantaneous in vivo measurement of oxygen saturation (SpO_2) by the pulse oximeter.

$$R = \left(\frac{A_{\lambda 1}}{A_{\lambda 2}}\right) = \left(\frac{{}^{AC}/_{DC})_{\lambda 1}}{({}^{AC}/_{DC})_{\lambda 2}}\right. \tag{5}$$

Hemoglobin Absorption and Choice of Wavelengths

As mentioned earlier, hemoglobin is a protein responsible for the transport of oxygen in blood. In its oxygenated form, the protein is known as oxyhemoglobin (HbO_2) and in its reduced form, it is known as deoxyhemoglobin (HHb).

The two wavelengths chosen in pulse oximetry are typically 660 nm (in the red part of the visible spectrum) and 940 nm (in the infrared part of the spectrum), though other wavelengths could be used. The choice of wavelengths is based on three main criteria.

First, the selected wavelengths ($\lambda_1$and λ_2) should each be sensitive to one of the main absorbers in blood, the HbO_2, and HHb. Second, the difference in absorption between HHb and HbO_2 at the chosen wavelengths should be the greatest. Finally, the absorption spectra in the region of the chosen wavelengths should be relatively flat (Bowes et al., 1989).

Fig. 2 depicts the absorption of HHb and HbO_2 across a portion of the visible and near-infrared electromagnetic spectrum. The HHb and HbO_2 have different absorption across the entire portion of the spectrum except at 805 nm, termed the isosbestic point. Below this point, absorption is principally due to HHb, while above the isosbestic point most absorption is due to HbO_2. The absorption between HHb and HbO_2 is greatest at 660 nm; thus, absorption measured at this wavelength will be largely proportional to the concentration of HHb. At any wavelengths below 600 nm, skin pigment melanin sharply absorbs most of the light. Above the isosbestic point, the difference between HbO_2 and HHb absorption is greatest above 970 nm. However, from 950 nm and upward, water in the tissue absorbs light significantly, making use of these wavelengths unviable. At 940 nm, absorption of water is sufficiently low, and both the HHb and HbO_2 absorption curves are relatively flat. Another compelling reason for the choice of 660 and 940 nm is the common availability of light emitters at these wavelengths.

Estimation of Oxygen Saturation

Pulse oximeters estimate SpO_2 from empirically calibrated curves, derived by correlating the measured ratio of absorbencies at red (660 nm) and infrared (940 nm) wavelengths to arterial oxygen saturation (SaO_2) measured from in vitro oximeters such as the Cooximeters. The calibration procedure involves desaturating healthy volunteers by asking them to breathe hypoxic gas mixtures (range: 100%–80%) and collecting optical measurements of blood samples at different steady-state oxygenation levels (Aoyagi, 2003). At each oxygenation level, the measured SaO_2 value is correlated to the "R" value measured by the pulse oximeter. The same procedure is repeated in a large group of volunteers and a mean calibration curve is then obtained. This curve is programmed into the digital microprocessor within the pulse oximeter and during subsequent use, it is used to estimate the arterial oxygen saturation (SpO_2). The percentage saturation of oxygen measured by a pulse oximeter in healthy individuals ranges between 95% and 100%.

Pulse Oximetry Probes

A conventional pulse oximetry probe consists of a pair of light emitters and a highly sensitive photodetector mounted inside a reusable spring-loaded clip. The most commonly used light sources are LED. The photodetectors usually used are silicon photodiodes, but other devices such as photocells and phototransistors are also used. The arrangement of the LEDs and the photodiode inside the sensor defines the configuration of the probe and its possible measurement site application. In a transmission probe, the LEDs and the photodiode are placed on opposite sides of the clips, where the light emitted by the LEDs transmits through one side of the vascular tissue to the photodiode on the other side, as shown in Fig. 3. Transmission probes are the most commonly used probes in the clinical settings and are usually placed on the patient's finger, earlobe, or toe. In reflectance probes, both the LEDs are aligned a few millimeters away from the photodiode so that the light emitted by the LEDs transilluminates the tissue and the backscattered light is detected by the photodiode. As the LEDs and the photodiode are placed adjacent to each other, reflection pulse oximetry probes can be used to measure arterial oxygen saturation at virtually any place on the skin. The most common surfaces for monitoring oxygen saturation with a reflectance pulse oximetry probe are the forehead and the temple.

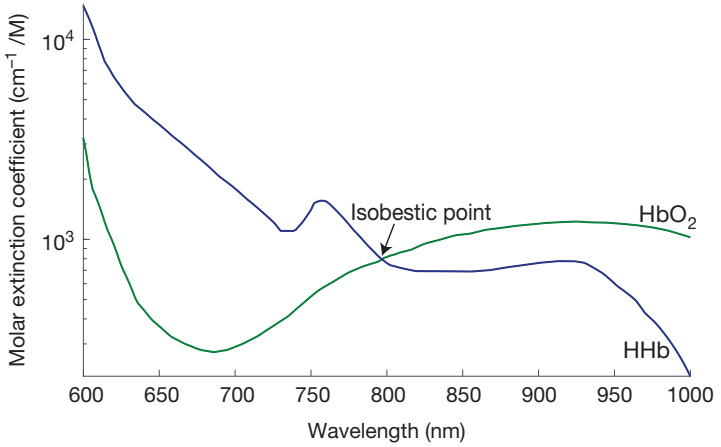

Fig. 2 Absorption spectrum of oxyhemoglobin and deoxyhemoglobin in the range between 600 and 1000 nm (infrared). As depicted in the graph, the two hemoglobin species have different absorption characteristics. At the isosbestic point (approximately 800 nm), oxyhemoglobin and deoxyhemoglobin absorb light identically.

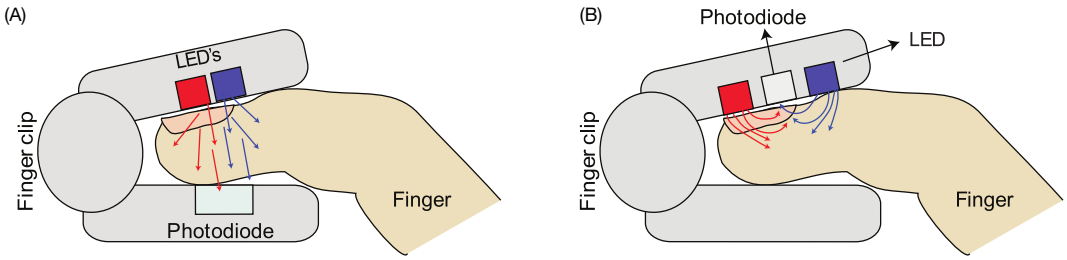

Fig. 3 Transmission and reflectance pulse oximetry probes. In the transmission probes (A), the LEDs and the photodiode are placed on opposite sides of the clip. In reflectance probes (B), the LEDs and photodiode are placed on the same side of the clip, few millimeters adjacent to each other.

Applications and Limitations of Pulse Oximetry

Pulse oximeters provide an estimate of arterial oxygen saturation (SpO_2), a quantity that provides a real-time indication of hypoxaemia. The device is noninvasive, easy to use, and is regularly used in anesthetic rooms, operating theaters, intensive care units, ambulances, and in routine monitoring of all neonates. Recent advancements in technology have seen the device to not only estimate SpO_2 but also calculate heart rate, respiration rate, perfusion index (PI), Pleth variability index (PVI), oxygen reserve index (ORI), and saturation of dysfunctional hemoglobins such as carboxyhemoglobin (SpCO %) and methemoglobin (SpMet %). Although some of these features are only available in pulse oximeters manufactured by Masimo Corp, a real-time measure of these parameters will help early diagnosis and prediction of fluid responsiveness, sleep apnoea, hypoxaemia, occult hemorrhage, hypovolaemia, hyperoxia, peripheral circulatory strength, and carbon monoxide poisoning (Nitzan et al., 2014). Plausible uses of pulse oximeters and photoplethysmography extend to vascular assessment and autonomic function. In vascular assessment, the technology is used to assess microcirculation, arterial compliance and aging, and endothelial function. Pulse oximeters are also used in the assessment of vasomotor function. Assessment of vasculature and vasomotor function by pulse oximetry is still in an early research stage. Another area in which pulse oximeters have gained immense interest is in the development of unique sensor technology utilizing either miniaturized optoelectronic components or optical fibers, to measure the volumetric changes directly from organs such as the esophagus, liver, bowel, brain, ear canal, and other vascular tissues such as free flaps (Budidha and Kyriacou, 2014; Kyriacou, 2013).

Although pulse oximeters are mostly accurate, numerous factors have been shown to affect negatively the absorbance characteristics, the signal-to-noise ratio of PPG signals, and estimated SpO_2 values. The main limitations are motion artifacts, calibration assumptions, and inadequate peripheral perfusion (Sinex, 1999).

Near-Infrared Spectroscopy

NIRS is an optical technique, which can provide a noninvasive and continuous measure of tissue oxygenation (Jobsis, 1977). In NIRS, near-infrared light (700–1000 nm) at two or more discrete wavelengths is shone into the tissue, where it undergoes scattering and absorption. Appropriate photodetectors collect the backscattered light and transform it into a voltage signal for further processing.

The Modified Beer-Lambert Law for NIRS

As mentioned previously, the Beer-Lambert law defines that the light attenuation through a medium (A_λ) is proportional to the concentration of the light absorbers present in the substance (C_K), the optical properties of the light absorber ($\varepsilon_{K(\lambda)}$), and the optical pathlength traveled by the light beam (L). From this, it is clear that the concentrations of the light absorbers in tissue can be determined by processing the light attenuations. NIRS uses this principle to determine the concentration of oxyhemoglobin (C_{HbO2}) and deoxyhemoglobin (C_{HHb}), assuming these are the two main light absorbers in the tissue (Rolfe, 2000). Following this concept and using the additive property of the Beer-Lambert law, the equation above can be transformed into:

$$A_\lambda = \left(\varepsilon_{HHb(\lambda)} C_{HHb} + \varepsilon_{HbO_2(\lambda)} C_{HbO_2}\right) \cdot d \cdot DPF + G$$

This equation is generally known as Modified Beer-Lambert law for NIRS and it features the additional parameters *DPF* and *G*. The term $d \cdot DPF$ represents the optical pathlength, where d is the distance between the light emitter and detector, while the constant *DPF* is defined as differential pathlength factor and represents the increase in the light pathlength due to scattering (Rolfe, 2000). The final term *G* is intended to represent the scattering properties of the tissue, which were not considered in the original Beer-Lambert law.

NIRS Instruments and Tissue Oxygenation

The drawback of the modified Beer-Lambert law is that for quantifying the absolute concentrations of C_{HbO2} and C_{HHb}, the optical properties of the tissue (absorption and scattering) and the optical path traveled by light should be known or determined. The simplest and early NIRS devices, known as continuous wave (CW), are unable to provide this information and can only express changes in C_{HbO2} and C_{HHb} from an arbitrary baseline point. Therefore, for the absolute quantification of C_{HbO2} and C_{HHb}, more sophisticated instruments have been developed. Time-resolved spectroscopy (TRS) and phase-resolved spectroscopy (PRS) are capable of fully quantifying C_{HbO2} and C_{HHb}, thus providing an absolute measure of tissue oxygenation (Rolfe, 2000; Pellicer and del Carmen Bravo, 2011; Ferrari and Quaresima, 2012).

Time-resolved spectroscopes determine the optical pathlengths by shining a pulse of light of a few picoseconds into the tissue. The photons that travel within the tissue are then collected by photomultiplier tubes and ordered in their arrival time. The profile of the photons' arrival sequence is known as temporal point spread function (TPSF) and it resembles a bell shape. The delay between the emitted light pulse and the detected TPSF represents the time necessary for the photons to travel inside the tissue. Once the travel time is known, the optical pathlength can be determined from the relationship of space and velocity (space = velocity × time) (Rolfe, 2000). In addition, by analyzing the attenuation and spread of the TPSF acquired at different wavelengths, the absorption and scattering properties of the tissue could be obtained (Wolf et al., 2007). Once the pathlength traveled by light and the optical properties is estimated, the C_{HbO2} and C_{HHb} can be fully quantified.

Phase-resolved spectroscopes allow the determination of the optical pathlength and optical properties of tissues from the phase shift (or frequency shift) of a modulated light beam passing through a tissue (Rolfe, 2000). For this, the light emitted is frequency modulated before being shown into the tissue. When the light passes through the tissue, it undergoes a shift in frequency which can be related to the optical pathlength. In addition to the shift in frequency, the amplitude modulation of the light beam could also be used to extrapolate information on the absorption and scattering properties of the tissue, which are vital for the quantification of C_{HbO2} and C_{HHb}.

Although extremely useful, time-resolved and phase-resolved instruments require powerful light sources, high voltages, and complex instrumentation (Ferrari and Quaresima, 2012). This results in cumbersome instruments that are not easily transportable and therefore restricted to research purposes (Wolf et al., 2007). However, in the recent years, advancements in the technology have allowed the miniaturization of these devices and it is hoped that these instruments would be soon used at the patient's bedside.

Once the concentrations of oxyhemoglobin and deoxyhemoglobin are determined, the oxygen content in the tissue, that is tissue oxygenation, can be calculated from the ratio of oxyhemoglobin and the total hemoglobin (Rolfe, 2000).

$$TOI = \frac{C_{HbO_2}}{C_{HbO_2} + C_{HHb}}$$

Even though this concept originates from the definition of functional oxygen saturation employed in oximetry to estimate the arterial saturation, it is important to highlight some important differences. NIRS measures light attenuations originating from the tissue's microcirculation, composed of arterioles, capillaries, and venules (Ferrari and Quaresima, 2012). Since the technique does not differentiate the contribution from each of these vessels, the oxygenation measured by tissue oxygenation index (TOI) represents a mixed oxygenation, with values close to the venous saturation (Ferrari and Quaresima, 2012). The main reason for this is the ratio in which most tissues are partitioned (venous to arterial ratio of 70:30) (Ferrari and Quaresima, 2012). From this, it can be deduced that the oxygenation measured by NIRS structurally differs from the one measured by pulse oximetry, since the latter appositely separates the contribution of arterial blood for calculating SpO_2.

An elegant development of CW NIRS instruments allows the estimation of the TOI without fully quantifying C_{HbO2} and C_{HHb} or knowing the optical properties of the tissue (Wolf et al., 2007). This method, known as spatially resolved spectroscopy (SRS), measures light intensities from two photodiodes placed close to each other (Wolf et al., 2007), as illustrated in Fig. 4. From the light attenuations measured at the two photodiodes, the slope of the attenuations with respect to the distance is determined. Using the diffusion approximation, it is then possible to relate the slopes of the light attenuations, obtained at different wavelengths, with the hemoglobin concentrations and the tissue oxygenation TOI (Suzuki et al., 1999). This concept has revolutionized NIRS because it allowed the use of CW NIRS, without the need for complex instrumentations as in TRS and PRS. Because of its advantages, SRS algorithms are nowadays employed in commercial NIRS devices from various manufacturers.

Applications of NIRS

Due to the capability of near-infrared light to penetrate the skull, the main application of NIRS is the continuous and noninvasive monitoring of cerebral perfusion and oxygenation. By using NIRS, clinicians have a unique tool to measure cerebral oxygenation and detect complications of this vital organ (Scheeren et al., 2012; Ferrari and Quaresima, 2012). During surgery, NIRS can be used to monitor the cerebral oxygenation in operations such as carotid endarterectomy, where the perfusion to the brain is compromised due to the temporary occlusion of the carotid arteries (Scheeren et al., 2012).

NIRS can also be used to assess oxidative metabolism in muscles at rest and/or exercise. Using vascular occlusions tests, muscle NIRS can be used to assess the physiological status of the tissue (Ferrari and Quaresima, 2012; Abay and Kyriacou, 2016). Also, the dynamic responses in the signals during occlusions can be used to differentiate between healthy and diseased subjects, as well as to assess the oxygen consumption of muscles (Hamaoka et al., 2011). NIRS measurements from other organs such as the liver can help

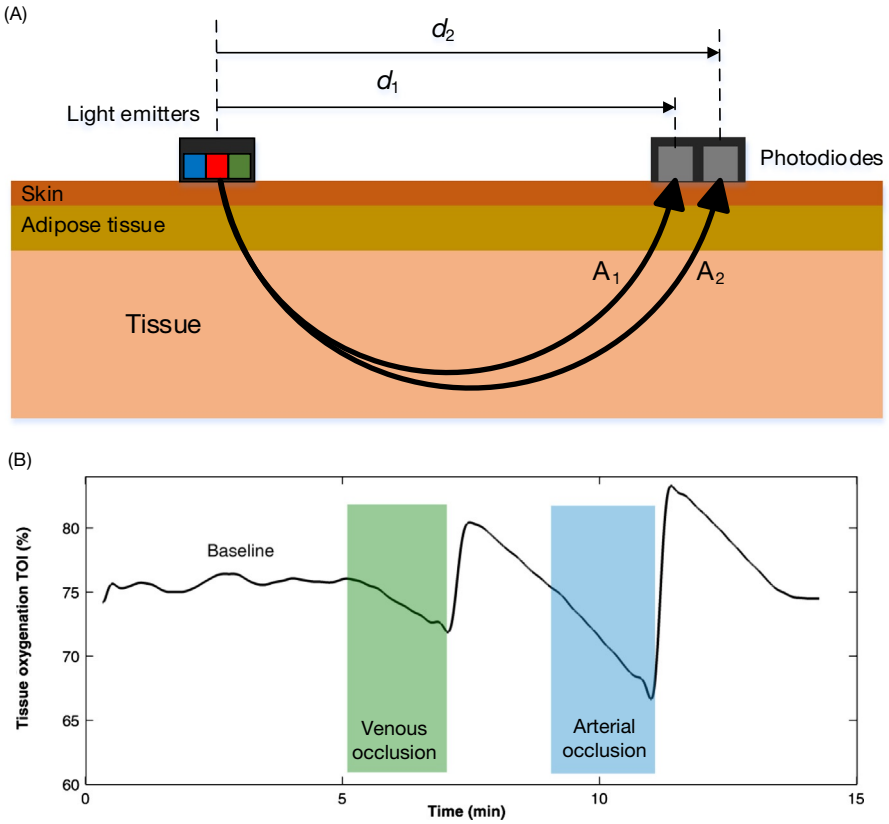

Fig. 4 Diagram of spatially resolved near-infrared spectroscopy (SRS) and TOI measurements. (A) In spatially resolved spectroscopy, two photodiodes placed close to each other detect the light attenuations A_1 and A_2 at two different distances d_1 and d_2. With some mathematical steps, it is then possible to relate the slope of the attenuations (with respect to the distances) to the concentration of oxy- and deoxyhemoglobin. From these concentrations, the TOI can be estimated as explained in the text. (B) Measurements of SRS TOI acquired at the forearm during venous occlusion (*green region*) and arterial occlusion (*blue region*). TOI offers the capability to monitor the tissue oxygenation continuously and detect any change in oxygenation induced by vascular occlusions.

clinicians in monitoring noninvasively the splanchnic perfusion of critically ill neonates. In intensive care, NIRS can be used for resuscitation or for detecting regional oxygen imbalances that may eventually lead to anaerobic metabolism or hemodynamic shock (Scheeren et al., 2012).

An emerging application of NIRS is functional NIRS (fNIRS), in which an array of light emitters and photodetectors are deployed to reconstruct a map of C_{HbO2} and C_{HHb} in functional activation areas within the cerebral cortex. fNIRS can provide a mapping of blood–oxygen-level-dependent areas within the brain, representing regions where oxygen is highly utilized for performing a task.

Limitations and Future Directions in NIRS

Research on NIRS has however highlighted some limitations that should be taken into consideration in the context of this review. The algorithms used in NIRS assume that the only chromophores changing in concentration in tissue are oxy- and deoxyhemoglobin, which may not hold in some circumstances. Due to the overlapping spectra of myoglobin and hemoglobin, the absorption of the first cannot be distinguished from the latter in muscle NIRS measurements (Scheeren et al., 2012; Hamaoka et al., 2011). The contribution of extra-cerebral or extra-muscle tissues to the signals is still under debate. While many contrasting results have been reported on this, multidistance NIRS devices seem to be able to solve the issue by subtracting the absorption of shallow tissues (Wolf et al., 2007). Also, several NIRS monitors based on CW or SRS assume the same DPF at the different wavelengths employed. This may introduce inaccuracies since scattering is wavelength-dependent and it may cause differences in the optical paths. Due to the mixed nature of the tissue oxygenation estimated by NIRS and the different venous contribution among subjects, the comparison between oxygenation values is rather difficult (Scheeren and Bendjelid, 2015). Therefore, the NIRS TOIs should be used as trend indicators of oxygenation rather than absolute values like SpO_2 in pulse oximetry. Several researchers in the field have also underlined the difficulty in comparing different devices due to the lack of standardization in the algorithms, wavelengths, and sensor configurations (Ferrari and Quaresima, 2012).

With the recent developments in portable technologies, NIRS could play a major role in the wearable biomedical technology industry, with cognitive assessment and sports sciences being some of the exciting applications. However, several challenges such as standardization still need to be addressed.

White Light Reflectance Spectrophotometry

White light reflectance spectrophotometry (WLRS), also known as "micro-lightguide spectrophotometry" or "reflectance spectroscopy," is a technique that exploits the reflection and absorption properties of oxyhemoglobin and deoxyhemoglobin for the calculation of tissue oxygenation.

White Light Spectra Measurements

Contrarily to NIRS or pulse oximetry, in which only a few discrete number of wavelengths are emitted, WLRS shines white light into the tissue. White light is defined as the combination of wavelengths from a broad range of the spectrum, spanning from visible to near-infrared light. For WLRS, the range of irradiation can vary depending on the tasks or the specific device, but it often spans between 400 and 700 nm (Buise et al., 2003; Wallace et al., 2009).

Commonly, the light source employed in WLRS is white-light lamps with a high emission power, whereas fiber optics are used to transport the light to the tissue (Wallace et al., 2009). The reflected white light from the tissue is also collected by fiber optics and transported to the processing instrumentation to be split in the respective wavelengths (Wallace et al., 2009). The separation is usually performed by rotating optical filters, CCD chips, or other appropriate detectors and it aims to reconstruct a reflected spectrum in a specific region of interest or wavelengths range (Wallace et al., 2009). The resolution of the separation into discrete wavelengths may vary between instruments, but a resolution of 1 nm can be achieved in advanced devices. This splitting procedure is performed at elevated speeds (approximately 100 spectra per second) to ensure the reconstruction of a reflected spectrum for real-time measurements (Buise et al., 2003).

Spectra Fitting and Hemoglobin Oxygenation Index

WLRS estimates the oxygenation of a tissue, by assessing the content of oxyhemoglobin and deoxyhemoglobin in the tissue. Similar to NIRS, WLRS assumes that oxyhemoglobin and deoxyhemoglobin are the main absorbers present in the tissue and the reflected spectrum represents the wavelength-dependent light absorption of these hemoglobin species within the tissue or organ. To extract the concentrations of oxyhemoglobin (C_{HbO2}) and deoxyhemoglobin (C_{HHb}) from the reflected spectra, mathematical fittings are performed between the reflected spectrum from the tissue and reference spectra of oxyhemoglobin and deoxyhemoglobin (Buise et al., 2003).

Several algorithms in the literature aim at estimating the tissue oxygenation from the reflected spectra. The oxygenation can be directly obtained from the main features of the oxy- and deoxyhemoglobin spectra, such as absorption peaks or isosbestic points (i.e., wavelengths where oxy- and deoxyhemoglobin have the same absorption), or by deriving the C_{HbO2} and C_{HHb} from the reflected spectra (Buise et al., 2003). This process, illustrated in Fig. 4, can be achieved by some mathematical processes and by knowing the basic absorption and scattering properties of hemoglobin and the tissue under examination. Once C_{HbO2} and C_{HHb} have been estimated, a hemoglobin oxygenation index (HOI) can be calculated from their ratio similarly to NIRS (Buise et al., 2003).

$$HOI = \frac{C_{HbO_2}}{C_{HbO_2} + C_{HHb}}$$

As mentioned above, this method of quantifying oxygenation requires the fitting of the reflected spectra acquired from the tissue and reference spectra of oxyhemoglobin and deoxyhemoglobin. In addition, WLRS instruments require calibration with reference spectra from black and white samples in order to correct for other absorbers and noise. Early WLRS devices relied on these regular calibrations, but modern instruments have been precalibrated and they are ready to use.

Applications of WLRS

Since WLRS uses fiber optics for the transportation of light to and from the tissue, the technology can be easily implemented into catheters for the measurement from hollow organs. For this reason, the technique has been extensively used to measure oxygenation from internal tissues such as the mucosa of the gastrointestinal tract or from the liver (Benaron et al., 2004). Obtaining oxygenation from these organs in critically ill patients could offer an important indicator for the identification of septic shock in multiorgan failure.

Transcutaneous reflectance sensors have also shown good results for assessing vascular diseases and for the perioperative monitoring of free flaps in plastic surgery (Fox et al., 2013). The technique can also be combined with other modalities such as laser Doppler flowmetry for the measure of multiple physiological parameters from a single optical sensor.

Unlike NIRS, which can penetrate deep into tissue, WLRS offer a limited penetration depth. The shallow penetration of white light into tissue and the short separation distance between the fiber optics restrict the sampled volume of WLRS. It is estimated that WLRS can penetrate up to 1–2 mm, corresponding to the skin microvasculature for transcutaneous sensors, or to the mucosal layer for gastrointestinal applications.

Continuous Venous Saturation

Fiber optic venous oximetry is an in vivo catheter-based technique used to measure continuously venous blood oxygen saturation in critically ill patients. This technique measures oxygen levels in venous blood instead of arterial blood. When the oxygenated arterial blood reaches the tissues, the oxygen is released through the microcirculation, where it is used for the metabolic activity of the cells. Therefore, the oxygen saturation measured from the arterial blood indicates how well the body is perfused with oxygenated blood (Frazier, n.d.). Once oxygen is diffused from the capillaries through the tissue, it enters the venous circulation and the oxygen saturation measured from mixed venous blood reflects the amount of O_2 left in the blood, after the O_2 uptake. Measuring SvO_2 will provide a measure of the balance between oxygen delivery and consumption. This can be deduced from the relationship between venous saturation, oxygen supply, and oxygen consumption.

$$SvO_2 = SaO_2 - vO_2$$

where SaO_2 is the arterial oxygen saturation (oxygen supply) and vO_2 is the venous oxygen saturation (oxygen consumption).

Venous Oximetry Measurements

SvO_2 oximetry sensors are composed of a multilumen tube (catheter) made of flexible plastic with a number of channels. The distal end of the catheter consists of a small inflatable balloon and a thermistor, used to measure cardiac output. Running along the distal end of the catheter is a pair of optical fibers that are employed to measure the venous oxygen saturation. The technology used to measure SvO_2 is based on reflection spectrophotometry. This involves transmitting light of selected wavelengths through one of the fiber-optic filaments to the blood flowing past the catheter tip and detecting the reflected light back using the other fiber-optic filament. The detected light is then converted into an electrical signal using the photodetector located in the optical module. Similar to pulse oximetry and NIRS, the different absorption properties of oxyhemoglobin and deoxyhemoglobin can be exploited to determine the percentage SvO_2 after light reflection from the venous blood (van Beest et al., 2011).

The placement of the oximetry sensor, however, plays an important role for the accurate measurement of venous blood saturation. As shown in Fig. 5, the preferred location for SvO_2 measurements is the pulmonary artery, since this location allows for adequate mixing of venous blood returning to the right side of the heart from the superior and inferior venae cavae, as well as from the coronary sinus. The term "mixed venous saturation" is usually employed for describing these SvO_2 measurements, since the right side of the heart collects venous blood from the different body organs, causing the "mixing" of this blood (Fig. 6).

Applications and Limitations of SvO_2

The normal range for SvO_2 is 60%–80% and it will indicate a sufficient oxygen supply available to the tissues. However, a low value of SvO_2 would highlight a situation where either the oxygen supply is insufficient or the oxygen demand (consumption) is elevated. Regardless of the cause, a decrease in SvO_2 in critically ill patients could indicate that the body has called upon its last line of defense to preserve oxygen balance, and in these cases therapeutic interventions may be appropriate (Frazier, n.d.).

Because of its strong relations with oxygen consumption, venous oximetry is considered the gold-standard technique for the continuous assessment of oxygen imbalances. This measure can be very useful in hemodynamically unstable patients for detecting the onset of metabolic complications (abnormal oxygen consumption) such as in shock or multiple organs failures. Specially designed catheters can also be used to measure regional venous oxygenation from specific regions such as the head and upper extremities, as well as peripheral and hepatic regions.

However, due to the measurement of mixed venous blood, the technique is considered a global hemodynamic measure, without the ability to provide a precise regional assessment. Due to the delicate positioning of the sensor, venous oximetry is only performed in critically ill patients who have been anesthetized and the invasive procedure could result in numerous complications.

Fluorescence Quenching and Phosphorescence

In fluorescence, a physical principle relating to certain properties of materials, the absorption of photons during exposure to light, causes a molecular effect that leads to the reemission of photons from the object. Since the absorption of photons implies an intrinsic loss of energy, the fluorescent light is reemitted at a different (usually longer) wavelength than the light originally absorbed. Similarly, phosphorescence is a fluorescent process that takes place at a lower rate, causing the reemission of photons to last for longer periods of time.

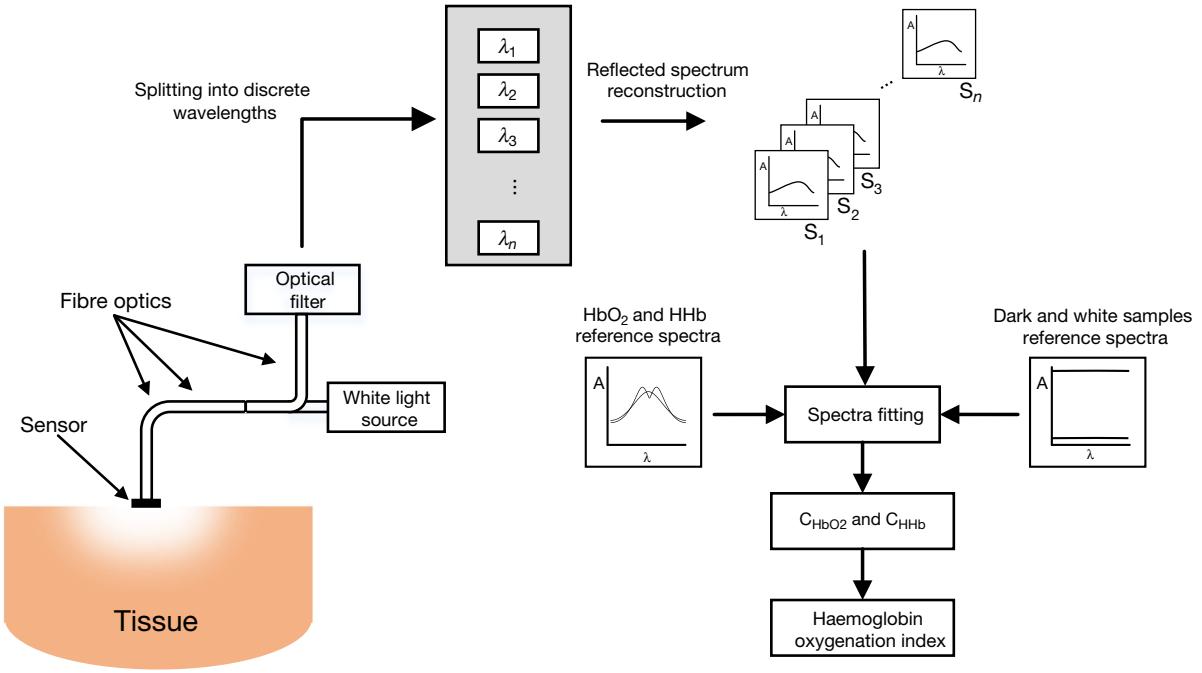

Fig. 5 Principles of white light spectroscopy. The white light is generated by a light source and transported to the tissue by fiber optics. The reflected light from the tissue is then collected by other fiber optics and directed toward an optical filter, which will be responsible for splitting the reflected white light (i.e., mix of wavelengths) into discrete wavelengths. Once the separation into wavelengths is achieved, spectra of the reflected absorption in the tissue are continuously generated (S_1, S_2,. . ., S_n). The reconstructed spectra are then input to fitting algorithms to estimate the concentrations of oxy- and deoxyhemoglobin (C_{Hb02} and C_{HHb}) in tissue, which are then employed to calculate the hemoglobin oxygenation index. Reference spectra of oxy- and deoxyhemoglobin absorptions, as well as black and white samples, are used to extract C_{Hb02} and C_{HHb} from the obtained spectra, as well as to correct for other absorbers and noise.

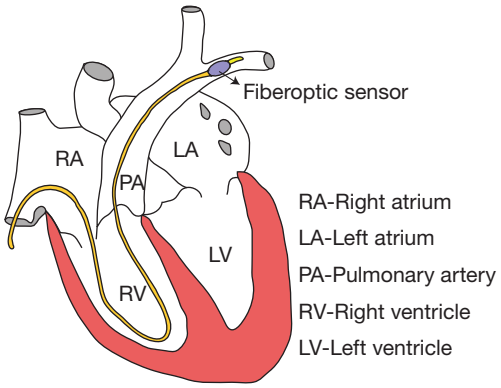

Fig. 6 Placement of venous oximetry catheter (fiber optic sensor) into the pulmonary artery (PA). The catheter is inserted through the venous circulation, passing from the right atrium and right ventricle of the heart before being kept in place in the PA for measuring the venous oxygen saturation.

Quenching relates the intensity of the fluorescence emission to the concentrations (or pressure) of certain ions present in the solution. The quenching properties of some substances can be used as indicators of the ions content surrounding a material. By placing these indicators (also known as tracers or dyes) in an environment with ions and shining light onto them, the reemitted fluorescence intensity can be measured and directly related to the concentration of the ions in contact with the dye.

Fluorescence, phosphorescence, and quenching can be exploited to determine the oxygen content present in blood and tissues. An indirect approach to assess the oxygen content may also involve determining the increase of certain waste substances such as carbon dioxide and nicotinamide adenine dinucleotide that can accumulate in blood and tissues in cases of oxygen deprivation.

Sublingual Capnometry

Sublingual capnometry (SC) is a noninvasive measurement used for the global assessment of oxygenation. Generally, it is believed that a higher sublingual presence of CO_2 can be considered a global measure of tissue hypoxia or oxygen imbalance in the body (anaerobic metabolism). An SC sensor comprises a permeable membrane, in which a dye is constantly illuminated by a light beam. When the CO_2 diffuses from the sublingual environment across the membrane, the dye binds with CO_2 and produces a quenching fluorescence. The reemitted fluorescent light is detected by appropriate instrumentation and the quenching intensity is proportionally related to the concentration of CO_2 (Marik and Bankov, 2003). A similar approach to SC can be implemented in catheters for the measure of O_2 and CO_2 in hollow organs. In these sensors, the tip of an optical sensor is coated with a dye, which produces quenching fluorescence directly proportional to the O_2 (CO_2) content.

SC can help in the identification and classification of states of circulatory failure (shock) and as an endpoint for goal-directed therapies (Marik and Bankov, 2003). Although the technique is easy to use and noninvasive, it has the main limitation of being a global indicator of anaerobic metabolism, without the capability of representing a specific regional oxygen imbalance.

NADH Fluorescence

Nicotinamide adenine dinucleotide (NADH) is a molecule involved in the cellular metabolism of the mitochondria. In conditions of normal oxygen distribution in the tissue, NADH is oxidized to NAD^+, but in situations of low oxygen delivery, the molecule fails to oxidize, with a consequent accumulation of NADH. The fluorescence properties of NADH (i.e., the molecule absorbs light at 320–380 nm and emits fluorescence at 420–480 nm) can then be exploited for relating the fluorescence intensity to the NADH accumulation (Mayevsky and Rogatsky, 2007). This principle can be either implemented in a video recording system or the fluorescence intensity can be converted into a signal (Mayevsky and Rogatsky, 2007). Using video recordings offers the capability to monitor moving organs such as the heart, while the conversion into signals allows following trends in NADH concentrations (Mayevsky and Rogatsky, 2007).

Palladium—Porphyrin Phosphorescence

Palladium (Pd)-porphyrin is a water-soluble compound that can be injected intravenously and confined into vascular compartments. The optical properties of the Pd-porphyrin can be exploited for the determination of the oxygen concentration in vascular beds. When a molecule of Pd-porphyrin is excited by a light beam, it produces phosphorescence. The decay in this phosphorescence intensity can then be measured and directly related to the oxygen tension (concentration) by appropriate photochemical calibration equations. Generally, longer decays of Pd-porphyrin phosphorescence represent less oxygen present in solution and vice versa (Siegemund et al., 1999).

Once Pd-porphyrin has been injected intravenously and has thus bound with oxygen, a pulse of light excites the molecule, creating phosphorescence. The decay in phosphorescence is then measured by appropriate instruments such as phosphorimeters. This process can be integrated into microscopes for the measure of oxygen in blood vessels or it can be implemented in fiber optics for the measure of oxygen content from hollow/internal organs such as intestine, kidneys, and heart (Siegemund et al., 1999).

Conclusion

Optical technologies have become an integral component in the assessment of oxygen in both blood and tissue and have had a significant impact in current clinical practice. As well as improving patient treatment and supporting clinical decision-making, the techniques described in this review have provided researchers the means for a deeper understanding of human physiology and oxygen metabolism.

Although some limitations persist, the future of optical technologies applied to healthcare is bright. Advances in medical optics could make life easier and safer for both patients and medical staff by minimizing invasive tests and providing continuous and noninvasive monitoring. With the continuous exponential growth in optoelectronic components and advanced signal processing techniques, the development of novel intelligent multiparametric noninvasive optical technologies, both wearable and noncontact, will contribute greatly in how healthcare will be delivered in the near future.

The vision and future efforts of scientists, engineers, and healthcare experts should be in the strengthening of interdisciplinary research collaborations with a promise to deliver methodologies and technologies that are expected to lead to clinical adoption within the next 10 years. Such technological achievements are likely to be a key step toward achieving the goals of effective, safe, reliable, and affordable optical monitoring of patients in the hospital, home, or other remote areas.

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