Received: 27 July 2011 ■ *Revised: 23 November 2011* ■ *Accepted: 23 November 2011* ■ *Publication: 31 January 2012*

JNIRS JOURNAL OF NEAR INFRARED SPECTROSCOPY

Special Issue on Medical Applications

Review

Near infrared brain and muscle oximetry: from the discovery to current applications

Marco Ferrari* **and Valentina Quaresima**

Department of Health Sciences, University of L'Aquila, L'Aquila, Italy. E-mail: marco.ferrari@univaq.it

In the early 1960s, Norris introduced near infrared (NIR) spectroscopy (700–2500 nm) as an analytical technique for agricultural products. In 1977, Jöbsis founded *in vivo* medical NIR spectroscopy, reporting that the relatively high degree of transparency of brain tissue in the NIR spectral window (700–1000 nm) enables safe real-time non-invasive detection of regional haemoglobin oxygenation using trans-illumination spectroscopy. In order to place current medical NIR spectroscopy in its proper perspective, this review provides a snapshot of the roots of the discovery and the early years of medical NIR spectroscopy research and development. Starting in 1992, the opportunity of measuring quantitatively, by different NIR spectroscopy techniques, regional oxy-haemoglobin saturation by NIR oximetry, it is possible to monitor brain/muscle reserve capacity following tissue oxygen extraction in different pathophysiological conditions. This review reports the status of the current commercial oximeters (including wireless instrumentation) and their main clinical and physiological applications. In the last decade, NIR spectroscopy brain oximetry has obtained significant clinical relevance as suggested by the more than 10,000 instruments sold and the high number of related scientific publications. The most relevant clinical application is represented by the evaluation of cerebral oxy-haemoglobin saturation during adult cardiac surgery and cardiopulmonary bypass. Many commercial oximeters are presently available. However, their relatively poor precision and the lack of standardisation amongst the different instruments suggest that further technological advances are required before NIR spectroscopy oximetry can be adopted more widely under the "guidelines" of regulatory authorities.

Keywords: near infrared spectroscopy, oximetry, brain oxygenation, muscle oxygenation, spatially resolved spectroscopy

Introduction

In the early 1960s, Karl Norris and co-workers of the Agricultural Research Service of the USA Department of Agriculture introduced NIR spectroscopy (700–2500 nm) as an analytical technique for agricultural products. They recognised the potential of the diffuse reflectance measurement for rapid analysis of grains that exhibit specific absorption bands due to proteins and moisture in the region. In the following years, Norris and co-workers introduced the use of NIR instruments for measuring different agricultural products. Since the 1970s, commercial companies have started to market NIR spectroscopy instrumentation for use in different fields of application. *In vivo* medical NIR spectroscopy, using NIR light in the 700–1000 nm wavelength range, goes back to 1977. In order to place current medical NIR spectroscopy in its proper perspective, this review provides a snapshot of the roots of the discovery and the early years of medical NIR spectroscopy research and development. The status of the current commercial oximeters and their more relevant medical applications are discussed.

ISSN: 0967-0335
doi: 10.1255/jnirs.973

Methods

Papers were retrieved by the authors through different strategies. First, the databases PubMed, Scopus and Web of Science were searched using the keywords: "near-infrared", "near-infrared oximetry", "cerebral oximetry", "muscle oxygenation", and/or "instrument". In addition, newborn NIR spectroscopy, functional NIR spectroscopy and NIR imaging were excluded because these topics are reported in other papers of this special issue of the *Journal Near Infrared Spectroscopy* dedicated to medical applications of NIR spectroscopy. Research groups known to be active in a specific field were contacted to gather further information. The websites of commercial NIR systems manufacturers were visited and searched to obtain the specifications of the instruments.

The roots of medical NIR spectroscopy discovery and the early years of research and development of the technique

Medical applications of optical methods are very old. The major advantages of using optical methods include biochemical specificity, temporal resolution in the millisecond range, the potential of measuring intracellular/intravascular events simultaneously, and the easy transportability of optical devices. The effect of oxygenation to change the colour of haemoglobin (Hb) was observed in a human hand in 1875 by Karl von Vierordt, a German physician who developed techniques for monitoring oxy-haemoglobin (O_2Hb) saturation of circulating blood. O_2Hb and de-oxygenated haemoglobin (HHb) preferentially absorb visible light and NIR of different wavelengths, as evidenced by the more reddish colour of oxygenated blood and the more bluish colour of de-oxygenated blood. The spectra of O_2Hb and HHb in the visible and NIR range are shown in Figure 1. The extent of absorption by each type of haemoglobin provides information about their relative concentrations and indirectly provides information about the vascularisation and metabolic status of tissue.

In 1927, Millikan began studying blood oxygenation *in vitro* using visible light (400–650 nm) in Cambridge (UK). In 1937, he demonstrated muscle deoxygenation upon electrical stimulation in laboratory animals. This application was limited by the poor penetration depth of the visible light (few millimetres) in tissues. Early in World War II, there were reports of black-outs in fighter pilots at high altitude. Then, Millikan developed the first portable human ear oximeter using red and NIR light at the University of Pennsylvania (Philadelphia, PA, USA).[1,2] This ear oximeter was lightweight and, with later modifications, was used in many pulmonary function laboratories for about 30 years. It is worth noting that the word "oximeter" was coined by Millikan.[1]

NIR light was also utilised in pulse oximetry, which was discovered by Takuo Aoyagi at the Research Division of Nihon Kohden Corporation (Tokyo, Japan) in 1972. Pulse oximetry is an extremely simplistic version of reflectance spectroscopy. From the red light signal he subtracted a NIR wavelength signal which contained the same arterial pulses. Then, he discovered that the cancellation failed if O_2Hb saturation was

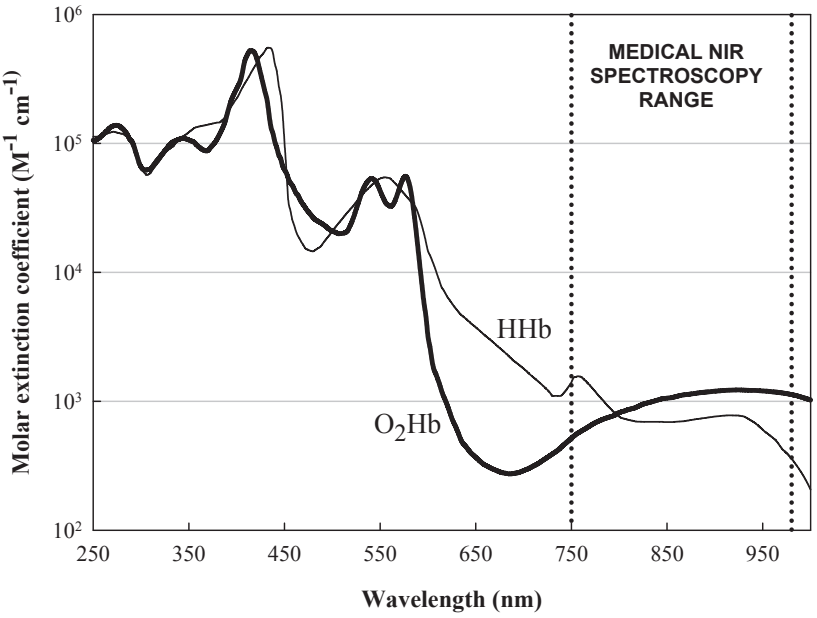

Figure 1. Absorption spectra of oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin. Most commercial oximeters currently utilise wavelengths in the 750–850 nm range to maximise the separation between O_2Hb and HHb and minimise the overlap with the water peak absorption centred around 980 nm.

changing. In addition, he realised that this method could be used to measure O_2Hb saturation because the ratio of the pulsatile fractions of the red and NIR lights was a unique function of arterial O_2Hb saturation, independent of Hb concentration, subject to skin colour, tissue thickness or other pigments. In 1978, Minolta (Japan) marketed the first commercial pulse oximeter. In 1995, fingertip oximeters, which are small enough to accommodate a finger, first appeared on the market. Typically, pulse oximeters have a pair of small light-emitting diodes (LEDs) facing a photodiode through a translucent part of the patient's body, usually a fingertip or an earlobe. One LED is red, with a wavelength of 660 nm and the other is infrared, 905 nm, 910 nm or 940 nm. Absorption at these wavelengths differs significantly between O_2Hb and HHb. Pulse oximetry, continuously measuring arterial O_2Hb saturation in the index finger or in other vascular districts, is considered the most significant technological advance ever made in monitoring the safety of patients during anaesthesia and critical care. The introduction of pulse oximetry has reduced 90% of anaesthesia-related fatalities.

Another step in the usefulness of using NIR light in the medical field is represented by the discovery of medical NIR spectroscopy. The NIR spectroscopy technique, which non-invasively monitors brain/muscle oxygenation, exploits the changes in the optical properties of haemoglobin at the microcirculation level associated with tissue activity (either in physiological or pathological conditions).

Frans Jöbsis, the founder of *in vivo* medical NIR spectroscopy, was Professor of Physiology at the Department of Physiology of Duke University (NC, USA) and, in 1977, reported that the relatively high degree of transparency of brain tissue in the NIR range ("optical window") enables real-time non-invasive detection of haemoglobin oxygenation using trans-illumination spectroscopy.[3] NIR light scattering is a function of tissue composition and the number of tissue interfaces, while absorption is determined by the molecular properties of substances within the light path, mainly haemoglobin. Above 1300 nm, water absorbs all photons over a pathlength of a few millimetres with a secondary peak between 950 nm and 1050 nm whereas, below 700 nm, the increase of light scattering and the more intense haemoglobin absorption bands prevent effective light transmission. The details of the discovery have been reviewed.[4] In his article, Jöbsis also described how it is possible to monitor the redox state of the copper band of cytochrome *c* oxidase, the terminal enzyme of the mithocondrial respiratory chain, responsible for about 90% of the cellular oxygen consumption. Oxidised cytochrome *c* oxidase has a broad peak at 820–840 nm. Cytochrome *c* oxidase was the target of several investigators during the first years of medical NIR spectroscopy research. After almost 10 years of studies performed on the brain of haemoglobin-free anaesthetised laboratory animals, it was concluded that the redox state of cytochrome *c* oxidase does not affect the haemoglobin oxygenation measurements because its absorption is less than 10% of haemoglobin absorption and it only starts to reduce at very low levels of tissue oxygenation. In recent years, much work has been done on the refinement of NIR spectroscopy hardware and algorithms (utilised to deconvolute the light absorption signal) for monitoring the cytochrome *c* oxidase redox state that might be a potential intracellular marker of tissue oxygenation. To improve the accuracy of the measurement of this NIR spectroscopy parameter, most of the recent animal and human NIR spectroscopy studies have been performed using broadband. At the present time, no commercial system includes the cytochrome *c* oxidase redox state measurement. Boxes 1 and 2 summarise the features, strengths, advantages, and limitations of medical NIR spectroscopy instruments for non-invasive monitoring of tissue oxygenation according to present knowledge.

Interestingly, the first NIR absorption spectrum of human muscle was measured by Norris and co-workers in the same year as Jöbsis' discovery.[5] The spectral features of haemoglobin, water and fat were identified by Norris and, later, a chemometric algorithm was developed to quantify body fat.[6] The possibility of measuring haemoglobin, cholesterol, total proteins, albumin and urea concentrations in whole human blood using NIR spectroscopy was demonstrated in various papers by Kuensner and colleagues.[7–10] Unfortunately, these interesting methods have never found application in commercial clinical chemistry instrumentation. In 2008, a non-invasive spectrophotometry-based technology (Radical-7; Masimo Corp., Irvine, CA, USA), providing continuous online fingertip haemoglobin concentration measurement, was introduced. When compared with laboratory reference values, haemoglobin content measured by pulse oximetry has absolute and trending accuracy similar to that measured by the more extensively used invasive methods.[11]

Following several demonstration studies about the applicability of NIR spectroscopy on laboratory animals,[12] Jöbsis and his colleagues used the technique for studying cerebral oxygenation in newborn sick infants.[13] In 1980, Marco Ferrari, at that time working at the "Istituto Superiore di Sanità" (Rome, Italy) (before moving in 1988 to the University of L'Aquila, Italy), started to utilise NIR spectroscopy instrumentation prototypes for measuring brain/muscle oxygenation changes in experimental animal models[14,15] and human adults.[16,17] In 1985, data on the effects of carotid artery compression tests on regional cerebral haemoglobin oxygenation and volume of cerebrovascular patients were presented at the 13th Meeting of the International Society on Oxygen Transport to Tissue.[18] Moreover, data collected on the brains of newborns were presented at the same meeting.[19]

In 1984, Professor David Delpy (University College London, UK), and colleagues, started to develop several NIR spectroscopy instruments and, three years later, reported the first quantitative measurements on sick newborn infants of various oxygenation and haemodynamic parameters [including changes in O_2Hb, HHb and total haemoglobin (tHb; tHb = O_2Hb + Hb) concentrations], cerebral blood volume, and cerebral blood flow.[20,21] A four-wavelength system, described by Cope and Delpy,[22] was used as the basis for the single-channel continuous wave (CW) NIRO-1000, the first

- Medical NIR spectroscopy is a non-invasive and safe optical technique which uses light emitting diodes or laser diodes as light source, and different NIR detectors to measure regional tissue oxygenation. In the case of the adult brain, the frontal lobes are the monitored area.
- Human tissues are relatively transparent to light in the NIR spectral window (650–1000 nm).
- The NIR light is either absorbed by pigmented compounds (chromophores) or scattered in tissues. NIR light is able to penetrate human tissues because the dominant factor in its transport to tissue is represented by scattering, which is typically about 100 times more probable than absorption.
- The relatively high attenuation of NIR light in tissue is due to the main chromophore haemoglobin (the oxygen transport red blood cell protein) located in small vessels (< 1 mm diameter) such as the capillary, arteriolar and venular bed of the microcirculation. Blood vessels > 1 mm completely absorb light. Given the fact that 70–80% of the blood in the tissues is in the venous compartment, NIR spectroscopy technique offers information mainly about the oxygenation changes occurring at the venous blood level (NIR spectroscopy signal from venous, capillary and arterial side being approximately 70:20:10, respectively).
- Haemoglobin absorption spectrum depends on its oxygenation status.
- As a consequence of the complex light scattering effect by different tissue layers, the pathlength of NIR light through tissue (optical pathlength) is longer than the physical distance between the source and the detector. The spatial distribution of NIR light through the different tissue layers is a banana-shaped region.
- Adequate NIR light penetration depth (almost half of the source–detector distance) can be achieved using a source–detector distance of 2–3 cm and 4–5 cm on an infant's and adult's head, respectively, or 4–5 cm on muscle. The selection of the optimal source–detector separation depends on NIR light intensity, the subject's head/muscle region and age, in the case of the brain.
- NIR spectroscopy allows for semiquantitative/quantitative monitoring of important physiological measures: (i) oxyhaemoglobin (O_2Hb); (ii) deoxyhaemoglobin (HHb); (iii) total haemoglobin (tHb), (tHb = O_2Hb + HHb) (tHb is strictly related to blood volume); (iv) O_2Hb saturation (measurable only by NIR tissue oximeters). Parameters quantification depends on the adopted NIR spectroscopy technology. NIR spectroscopy measurements are repeatable and reproducible.
- NIR spectroscopy is characterised by a relatively high temporal resolution (typically between 1 Hz and 10 Hz) enabling to measure the time course of cortical or muscle oxygenation changes. NIR spectroscopy instrumentation is relatively low/moderate costly, transportable or portable or wearable. NIR spectroscopy systems can be miniaturised and even made wireless.

Box 1. Features, strengths and advantages of medical near infrared spectroscopy.

- Optode–skin coupling/sliding. A stable contact between light source and skin is critical for continuous wave-based NIR spectroscopy instrumentation. The layering and dark colour of hair attenuate NIR light.
- NIR spectroscopy measurements are restricted to outer cortex or superficial muscle(s). Depth sensitivity of NIR spectroscopy signal depends on many factors (i.e. source–detector separation, source power, detector sensitivity, optical properties of the skin/skull layers, degree of white matter myelination, adipose tissue thickness etc.). Typical depth sensitivity of most NIR spectroscopy systems is ~1.5 cm.
- The separation of NIR spectroscopy signals originating either from cerebral tissue or extracerebral tissues/structures (scalp, temporal muscle, skull, frontal sinus, cerebrospinal fluid and dura) is difficult.
- The determination of NIR spectroscopy parameters is influenced by skin blood flow/volume changes.
- Continuous wave-based systems cannot measure optical pathlength, then do not provide absolute quantification value of NIR spectroscopy parameters. Optical pathlength differences across diverse head/muscle regions should be taken into consideration for interpreting NIR spectroscopy data. Technological developments of time-domain and frequency-domain-based instrumentations (being able to measure the spatial and temporal variations in optical pathlength) would improve the NIR spectroscopy sensitivity and the quantification of NIR spectroscopy parameters.
- No standardisation is available for NIR spectroscopy instrumentations/signal processing/data analysis. Regulatory authorities or network of research laboratories should provide "guidelines".
- NIR spectroscopy signal artefacts (due to head/muscle motion or reduction of the grip of the light source on the skin) are not automatically corrected by software.
- Most of the commercial brain/muscle oximeters are not approved by the USA Food and Drug Administration.

Box 2. Limitations of medical near infrared spectroscopy.

commercial system built in 1989 by Hamamatsu Photonics K.K. (Hamamatsu City, Japan). This instrument represented the first results of a long-lasting collaboration between the University College London and Hamamatsu Photonics.

From 1980 to 1995, another nine companies were involved in the development of NIR spectroscopy prototypes:

- American Edwards Laboratories in collaboration with Duke University (NC, USA)
- Critikon (UK) and Johnson & Johnson (Bridgewater, NJ, USA) with Zurich University (Switzerland)
- ISS Inc. (Champaign, IL, USA) with the University of Illinois at Urbana-Champaign (Urbana, IL, USA)
- Near Infrared Imaging Inc. with the University of Pennsylvania (both in Philadelphia, PA, USA)
- NIRSystems, Inc. (Laurel, MD, USA) and Edwards Lifesciences Corp (Irvine, CA, USA) with Johns Hopkins University (Baltimore, MD, USA)
- Radiometer (Copenhagen, Denmark) with Copenhagen University (Denmark)
- Sclavo (Siena, Italy) with the "Istituto Superiore di Sanità" (Rome, Italy)
- Shimadzu Co. (Kyoto, Japan) with Hokkaido University (Sapporo, Japan)
- Somanetics Corporation (Troy, MI, USA)

Only four of these companies (Hamamatsu, ISS, Shimadzu and Somanetics) are still producing NIR spectroscopy instrumentations in 2011 (Table 1). Wahrt *et al.*[23] reviewed the technical and historical development of the instruments utilised up to 1995 (including experimental and medical applications). In the case of the adult brain, the frontal lobes, due to the lack of hair, represent the most convenient region to be monitored by NIR spectroscopy.

Interestingly, in 1987–1990, Ferrari (during his stay at the Johns Hopkins University) collaborated with Norris (consultant of NIRSystems company) in the construction of a prediction algorithm of cerebral venous O_2Hb saturation.[24] The four medical NIR spectroscopy pioneers (Delpy, Chance, Norris and Ferrari) during the 32nd Annual Eastern Analytical Symposium held in 1993 at Somerset (NJ, USA) are shown in Figure 2.

In general, spectroscopy using the NIR region of the spectrum can be performed in a variety of ways. Although two wavelengths are required to resolve the spectroscopic equations with two chromophores (O_2Hb and HHb), some NIR spectroscopy instruments utilise more wavelengths to improve the accuracy of the algorithms. The wavelengths of NIR light used in the commercial devices are selected to be sensitive to haemoglobin and generally utilise wavelengths between 700 nm and 850 nm where the absorption spectra of O_2Hb and HHb are maximally separated and there is a minimal overlap with H_2O. The isobestic point (wavelength at which O_2Hb and HHb have the same molar absorptivity) for HHb/O_2Hb is around 810 nm. The isobestic wavelength has been utilised in some of the early instrumentation used for measuring tHb.

Most of the first generation instruments used laser diodes and flexible fibre-optic bundles to carry the light to (source) and from (detector) the monitored area. The laser diodes were switched on in sequence and, thus, a single detector could have been used. Most of the present commercial systems use LEDs, each providing a small range of wavelengths.

Near infrared brain/muscle oximetry

The first generation of medical NIR spectroscopy instruments used a CW-based NIR spectroscopy approach which only provided concentration changes in O_2Hb and HHb (with respect to an initial value arbitrarily set equal to zero) calculated by using the modified Lambert–Beer law and expressed in micromolar × centimetres.[25] Somanetics (Troy, MI, USA) was the first company to market a single-channel NIR spectroscopy brain oximeter (INVOS 3100) using two LEDs (725 nm and 797 nm) and two diode detectors (in 1992). In May 1993, the INVOS 3100 was the first cerebral oximetry device to be approved by the USA Food and Drug Administration (FDA). The oximeters provide a continuous measure of regional O_2Hb saturation on a scale from 0% to 100%. Unlike conventional pulse oximetry, NIR spectroscopy tissue oximetry does not rely on a pulsating flow and measures a weighted average of arterial, capillary and venous blood oxygenation. Therefore, NIR spectroscopy tissue oximeters provide information on the balance between oxygen supply and demand in capillary, arteriolar and venular beds of tissue (mainly at brain cortex and superficial muscle levels). Regional O_2Hb saturation represents the tissue reserve capacity following tissue oxygen extraction.

The principles of the operation of the NIR spectroscopy tissue oximeters differ among instruments. The three main principles exploited in making the oximeters are: spatially resolved spectroscopy (SRS), phase modulation spectroscopy (PMS) and time resolved spectroscopy (TRS).[25] SRS, also called multi-distance spectroscopy, is based on light intensity being measured at several different source–detector distances. SRS techniques assume that the coupling is the same for the different source–detector distances and, by measuring the intensity as a function of the distance, determine a parameter which is independent of the coupling. This allows the determination of ratios of O_2Hb to tHb and, thus, tissue O_2Hb saturation. Moreover, a scaled absolute value for tHb can also be obtained by the SRS method. The application of cerebral NIR spectroscopy in adults has been hampered by concerns about contamination from extra-cerebral tissues. Using SRS, the brain cortex was identified as the anatomic source of the signal on the forehead of adult patients undergoing carotid endarterectomy. In Figure 3, an example of the changes in prefrontal cortex O_2Hb saturation (measured as tissue oxygenation index (TOI %) by the brain oximeter NIRO-300 (Hamamatsu Photonics, Japan) during elective carotid surgery is reported. The compression of the external carotid artery provoked a decrease in frontal cutaneous blood flow

Table 1. Main current commercial near infrared spectroscopy brain and/or muscle oximeters.

Instrument	Technique	Year of release	N° and type of sources, N° of channels	Company	Website
Astem Hb-1[2c, a–d]	Multi-distance CW	2010	2 LEDs , 2	Astem, Japan	www.astem-jp.com
CerOx 3210F[e–g]	Multi-distance CW	2007	3 LDs, 2	Ornim, Inc., USA	www.ornim.com
EQUANOX 7600[e,f]	Multi-distance CW	2009	2 LEDs, 2	Nonin, USA	www.nonin.com
EQUANOX 8004CA[e,f]	Multi-distance CW	2011	4 LEDs, 4	Nonin, USA	www.nonin.com
Fore-sight 2040[e,f]	Multi-distance CW	2006	4 LDs, 2	Casmed, USA	www.casmed.com
InSpectra StO_2 650[e,h]	Multi-distance CW	2003	4 LEDs, 4	Hutchinson, USA	www.htibiomeasurement.com
INVOS 5100C[e,f]	Multi-distance CW	2006	2 LEDs, 1–4	Somanetics, USA	www.somanetics.com
NIMO[c]	Multi-distance CW	2008	3 LD, 2	Nirox, Italy	www.nimoworld.com
NIRO-200NX	Multi-distance CW	2010	3 LDs, 2	Hamamatsu, Japan	www.hamamatsu.com
Oxymon-II A	Multi-distance CW	2003	2-6 LDs, 2	Artinis, Netherlands	www.artinis.com
OxiplexTS	Multi-distance FD	1998	2 LDs, 2	ISS, USA	www.iss.com
PortaLite[b,f]	Multi-distance CW	2011	2 LEDs, 1	Artinis, Netherlands	www.artinis.com
PortaMon[a,b,i]	Multi-distance CW	2006	2 LEDs, 1	Artinis, Netherlands	www.artinis.com
TRS-20[d]	TD	2009	3 LDs, 2	Hamamatsu, Japan	www.hamamatsu.com

[a]Oximeter for muscle studies only;
[b]wireless system;
[c]oximeter with fat-layer compensation in real time;
[d]commercially available only in Japan;
[e]USA Food and Drug Administration's approval;
[f]oximeter for brain studies only;
[g]capable of microcirculation blood flow measurement by ultrasounds;
[h]for thenar muscle only;
[i]an accelerometer is available on request
CW, continuous-wave spectroscopy;
FD, frequency-domain spectroscopy;
LD, laser diode;
LED, light-emitting diode;
TD, time-domain spectroscopy.

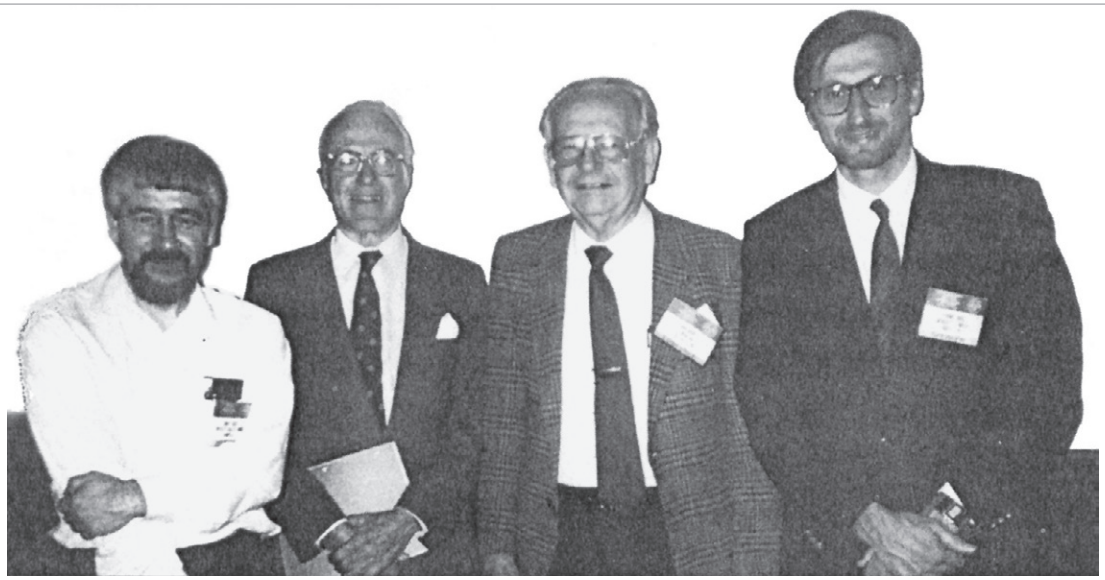

Figure 2. The medical NIR spectroscopy pioneers at the 32nd Annual Eastern Analytical Symposium, 16 November 1993, Somerset, NJ, USA. From the left: David Delpy (UK), Britton Chance (USA), Karl Norris (USA), Marco Ferrari (Italy).

(measured by a skin laser Doppler flowmeter) without changes in TOI. Conversely, the compression of the internal carotid artery provoked a prompt decrease in mean ipsilateral middle cerebral artery blood flow velocity (measured by intracranial laser Doppler) accompanied by a concomitant decrease of TOI. After insertion of the shunt, TOI promptly started to recover. Therefore, a change in brain O_2Hb saturation was associated only with the internal carotid artery clamping. The reason is that using the SRS approach, the superficial layers of tissue have an effect on all the light bundles similarly. Therefore, their influence is cancelled out. Only deeper tissue layers have an effect on the values.

TRS, also known as time-domain spectroscopy, is a technique that measures the time of flight in addition to the light intensity. It does so by emitting a short (~100 ps) pulse of light into the tissue and measuring the time point spread function of the light after it has passed through the tissue. Due to the scattering process, the pulse will broaden and, due to absorption, the intensity will be reduced. The result of such a measurement is a histogram of the number of photons on the *y*-axis and their arrival times on the *x*-axis. The histogram also contains information about the depth of the photonic path, because photons that arrive later have a higher probability of having travelled deeper. The absorption and the reduced scattering coefficients are calculated from the histogram and the absorption coefficients are utilised to calculate the absolute values of O_2Hb and HHb. The NIR TRS-based approach requires sophisticated and expensive instrumentation; so far, only one system has been commercially available (Table 1).

PMS is also called frequency-domain spectroscopy. This technique is, in principle, equivalent to TRS, except that it operates in the Fourier domain. This means that the light sources are intensity modulated at radio frequencies (50 MHz to 1 GHz). After passing through the tissue, the mean intensity, amplitude and phase of the emerging wave are measured. The phase contains information about the time of flight. Most of the instruments are single frequency instruments and use multi-distance or SRS geometry. It has been shown that the latter type of instrument is, technically, much simpler than the one using the TRS-based approach, and provides measurements with a good signal-to-noise ratio and a high time resolution. NIR PMS-based approach requires sophisticated instrumentation; so far, one system has been commercially available (Table 1). In ascending order of cost and technological complexity are the CW-, PMS- and TRS-based instruments. On the other hand, only the PMS and TRS techniques offer absolute characterisation of the tissue optical properties (absorption and reduced scattering coefficients) from which it is possible to retrieve absolute concentration values of O_2Hb and HHb.

The different types of NIR spectroscopy instruments, with the related key features, advantages and disadvantages and all the parameters measurable by using different NIR spectroscopy techniques, have been reviewed in detail.[25–29] In addition, some accompanying articles will cover the most recent technical aspects of medical NIR spectroscopy instrumentation.

The first commercial single-channel SRS oximeter was introduced in 1997 (the OM-200, Shimadzu Co., Japan). In 1998, the two-channel SRS oximeters (the NIRO-300 by Hamamatsu, Japan, and the INVOS-4100 by Somanetics) were introduced onto the market. In 1999, the Shimadzu Company introduced the two-channel SRS oximeter OM-220. Table 1 shows the main commercial brain and/or muscle oximeters which are currently available. The details of each system can be found on the websites of the related companies. Commercial NIR

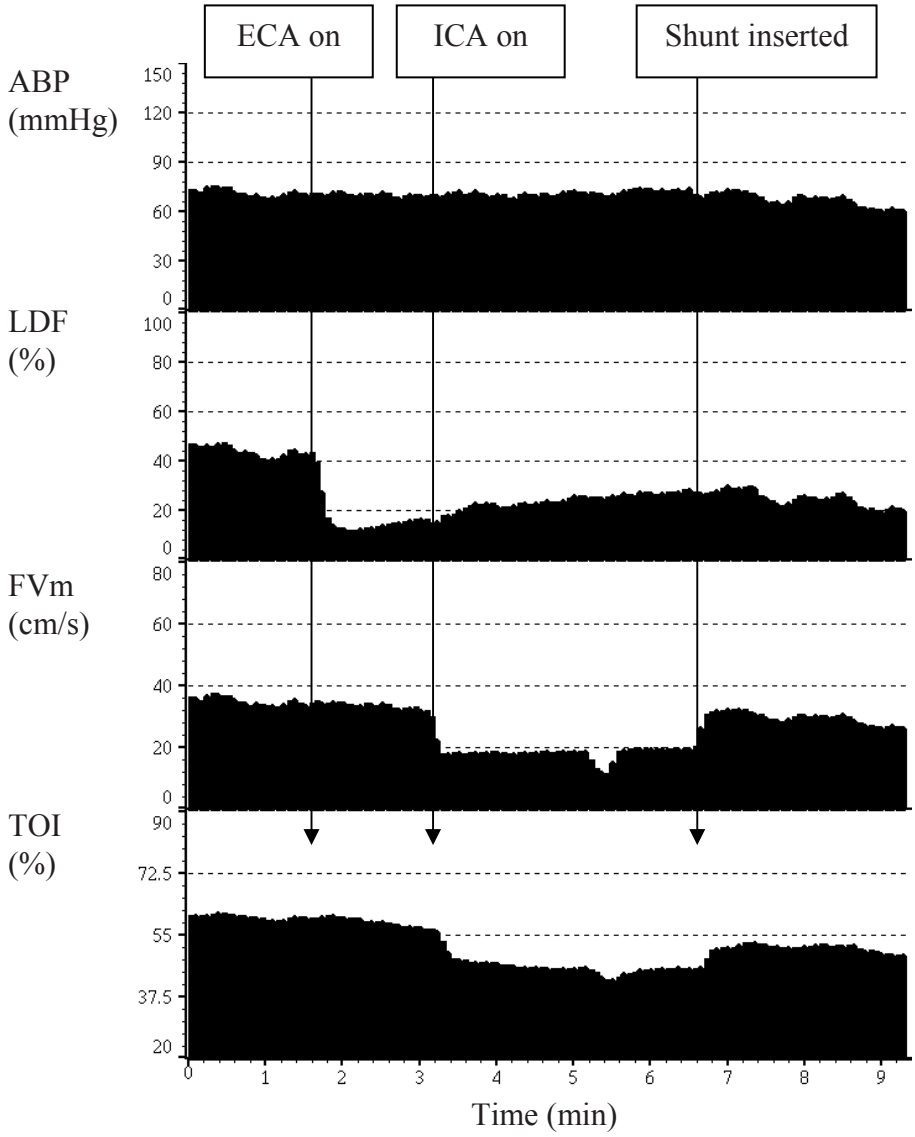

Figure 3. Graphic display of data obtained from a patient during elective carotid endarterectomy. The vertical lines demonstrate time of application of vascular clamps. The NIR spectroscopy oximeter NIRO-300 (Hamamatsu Photonics, Japan) provided a continuous online measurement of the changes in prefrontal cortex O_2Hb saturation, measured as tissue oxygen index (TOI) using spatially resolved spectroscopy. ECA, external carotid artery; ICA, internal carotid artery; ABP, mean arterial blood pressure; LDF, frontal cutaneous laser Doppler flow (Moor Instruments, UK); FVm, mean ipsilateral middle cerebral artery flow velocity (Scimed, UK), TOI, tissue oxygenation index. (Unpublished data from Professor P.G. Al-Rawi, University Department of Neurosurgery, Addenbrooke's Hospital, Cambridge, UK.).

spectroscopy oximeters with USA FDA approval are shown in Figure 4. Adhesive pads are used to fix the optical probes to the hairless skin of the forehead or the skin over the monitored muscle group. The overall chronology of the introduction of the main brain and muscle commercial NIR spectroscopy-based oximeters is reported in Table 2.

Since it was first launched in 1992, the INVOS oximeter has constantly been evolving and improving. Now in its sixth generation, the four-channel INVOS 5100C (Figure 4) is a commercially available cerebral/somatic oximeter indicated also for use on neonates. There are currently four FDA approved devices that are marketed to assess cortical oxygenation (Figure 4, Table 1). It is worth mentioning that Ornim's oximeter only provides a simultaneous, yet independent, measure of HbO_2 saturation and blood flow measured by a small ultrasound transducer. Miniturised oximeters are now being manufactrured. Wireless oximetry is particularly useful for measuring muscle oxygenation during exercise.[30,31] It is estimated that approximately 10,000 brain oximeters (almost two-thirds made by Somanetics) have been utilised worldwide, mostly for adult medical applications. In addition, several more quantitative oximeter prototypes are

Figure 4. Commercial brain NIR oximeters with USA Food and Drug Administration approval: CerOx 3210F (Ornim, Inc., USA); EQUANOX 7600 (Nonin, USA); Fore-sight 2040 (Casmed, USA); INVOS 5100C (Somanetics, USA). The oximeters are equipped with two or four optical probes for monitoring the tissue oxygenation over the forehead.

under development in several university research groups.[25] Because each oximeter uses a unique processing algorithm to calculate O_2Hb saturation, it is not possible to compare the clinical results obtained by different instrumentations. However, in the majority of publications, the Somanetics brain oximeters have been used.

The importance of the introduction of the NIR spectroscopy oximeters is also witnessed by the fact that the Japanese Society on Medical NIR Spectroscopy, founded in 1994, is holding regular annual meetings.

Laboratory and clinical validation studies are required to demonstrate the utility of any monitoring modality. While arterial O_2Hb saturation, measured by pulse oximetry, can be easily validated by collecting arterial blood samples, tissue O_2Hb saturation, measured by NIR spectroscopy, is a new parameter for which no "gold standard" method, invasive or non-invasive, is available. Therefore, the accuracy of NIR spectroscopy tissue oximetry cannot be assessed. So far, tissue NIR spectroscopy oximetry has been compared with the O_2Hb saturation measured in jugular vein blood considering a weighted average of venous and arterial blood. The weighted factor is impossible to determine precisely and it changes in pathological conditions.

Unfortunately, the optical probes and the algorithms of commercial NIR spectroscopy oximeters are different and no standardisation is available yet. In particular, (i) the operating range (i.e. the interval within which the instrument works reliably) of the oximeters should be recognised and indicated, and (ii) the comparison between oximeters at rest and during physiological changes such as muscle exercise should be performed. Commercial NIR spectroscopy-based oximeters can also measure a brain blood flow index by measuring the time course of the NIR dye indocyanine green injected into a peripheral vein.

Table 2. Overall chronology of the introduction of the main brain and muscle commercial near infrared spectroscopy-based oximeters.

Year	Major events
1977	Jöbsis demonstrates the possibility to detect changes of adult cortical oxygenation during hyperventilation by NIR spectroscopy
1985	First NIR spectroscopy clinical studies on newborns and adult cerebrovascular patients
1989	First commercial continuous wave one-channel clinical instrument: NIRO-1000 by Hamamatsu Photonics, Japan
1992	First commercial continuous wave one-channel brain oximeter: INVOS 3100 by Somanetics (USA)
1993	The oximeter INVOS 3100 gets the Food and Drug Administration approval
1994	First commercial continuous wave one-channel portable instrument for muscle studies: HEO-100 by Omron (Japan)
1997	First commercial continuous wave one-channel spatially resolved spectroscopy brain oximeter: OM-200 by Shimadzu (Japan)
1998	First commercial continuous wave two-channel spatially resolved spectroscopy brain oximeter: NIRO-300 by Hamamatsu Photonics (Japan)
	First commercial frequency-domain spectroscopy one-channel brain/muscle oximeter: OxiplexTS by ISS (USA)
2003	First commercial time-resolved spectroscopy one-channel brain/muscle oximeter: TRS-10 by Hamamatsu Photonics (Japan)
	First commercial continuous wave one-channel oximeter for thenar muscle studies: InSpectra StO_2 650 by Hutchinson (USA)
2006	First commercial continuous wave four-channel brain/somatic oximeter: INVOS 5100C by Somanetics (USA)
	First commercial continuous wave spatially resolved spectroscopy wireless and portable one-channel muscle oximeter: PortaMon by Artinis (Netherlands)
2009	First commercial time-resolved spectroscopy two-channel brain/muscle oximeter: TRS-20 by Hamamatsu Photonics (Japan)
2011	First commercial continuous wave spatially resolved spectroscopy wireless and portable one-channel brain oximeter: PortaLite by Artinis (Netherlands)

Main applications of near infrared brain/muscle oximetry

Brain oximetry

The application of NIR spectroscopy to the preterm and newborn infant brain and its clinical value has been previously reviewed[32–34] and is also reviewed by Wolf *et al*. in this special issue of *Journal of Near Infrared Spectroscopy*.[35]

The measurement of tissue O_2Hb saturation by NIR oximeters offers the great advantage of detecting clinical situations in which the oxygenation status of the adult brain can change dangerously. Continuous real-time monitoring of the adequacy of cerebral oxygenation/perfusion can provide important therapeutic information in a variety of clinical settings. The current clinical availability of several brain oximeters represents a potentially important development for the detection of cerebral ischaemia. The most relevant applications of adult brain oximetry are in: (i) surgery (for evaluating cerebral oxygenation during cardiac, renal or carotid artery surgery, as an indicator of shunting in carotid endarterectomy, balloon test occlusion, aortic surgery, cardiopulmonary bypass, venous endovascular trombolysis, neurosurgical procedures etc.); (ii) anaesthesia (for evaluating cerebral oxygenation during induction of general anaesthesia, drugs infusion, tracheal extubation etc.); and (iii) stroke (stroke occurrence in intensive care, to follow effects of drugs, to evaluate cerebrovascular reactivity, to evaluate the post-stroke functional recovery etc.). Different studies have demonstrated a close correlation between changes in cerebral oxygenation by NIR spectroscopy and other monitoring modalities under different conditions. Increasing evidence suggests that a decrease in O_2Hb saturation during surgery is associated with unwarranted neurological effects and postoperative complications.[36–38]

One retrospective[39] and three randomised controlled trials[40–42] have examined the clinical value of cerebral oximetry during cardiac surgery and cardiopulmonary bypass. A relationship between cerebral hypoxia and post-operative complications was found, suggesting that brain oximetry–guided brain protection protocols might lead to reduced neurological complications.[37,43]

The American Society of Thoracic Surgeons began collecting cerebral oximetry data in its Adult Cardiac Surgery Database in January 2008. In January 2010, the same society began collecting cerebral and somatic oximetry data, including those for operating room and intensive care unit applications, in its Congenital Heart Surgery Database.

Some articles have raised questions regarding the clinical utility of cerebral oximetry monitoring.[44–47] However, a number of clinical studies and case reports have demonstrated that, despite some limitations, the ability of cerebral oximetry monitoring to detect otherwise clinically silent episodes of cerebral ischaemia in a variety of clinical settings renders it an important safeguard for cerebral function.

The proper management of brain oxygenation is one of the principal endpoints of all anaesthesia procedures. However, the brain remains one of the least monitored organs during clinical anaesthesiology. There are some medical procedures where iatrogenic brain ischaemia is present, including carotid endarterectomy in patients with high-grade carotid artery stenosis, temporary clipping in brain aneurysm surgery, hypothermic circulatory arrest for aortic arch procedures and others in which the pathology itself generates brain ischaemia, such as traumatic brain injury and stroke. One of the most common limitations seen in studies assessing the impact of cerebral oximetry monitoring has been the absence of a defined protocol based on physiologically derived interventions to treat decreases in O_2Hb saturation.

Considering the poor reliability of absolute O_2Hb baseline values (coefficient of variation of about 10%), some researchers suggest using O_2Hb saturation trends instead of absolute values. Moreover, no reliable absolute O_2Hb saturation threshold for inadequate cerebral perfusion has been determined to date. In addition, it is not clear how changes in cortical oxygenation can be interpreted. This suggests that oximetry should be accompanied by other neuromonitoring tools.

Although there are some limitations, the clinical results and the ongoing technological advances suggest that NIR spectroscopy oximeters could represent a unique, very useful and non-invasive tool for diagnosing neural and vascular diseases and for monitoring the effects of therapy/rehabilitation on the brain.

Muscle oximetry

Since the end of the 1980s, NIR spectroscopy has been utilised to investigate local muscle oxidative metabolism at rest and during different exercise modalities. The unique advantage of using NIR spectroscopy is that, when proper care is taken to minimise movement artefacts, it can yield acceptable signal-to-noise ratios during exercise. Several review articles reported the methodological issues of NIR spectroscopy for monitoring muscle oxygenation and haemodynamics in healthy and diseased humans.[48–52] The first low-cost CW-NIR spectroscopy instrument dedicated to muscle studies was made by Chance (Pennsylvania University, USA) in 1990. In 1994, Nakase and Shiga (Omron Institute of Life Science Co. Ltd., Kyoto, Japan) developed, in collaboration with Chance, the first single-channel portable NIR spectroscopy instrument for muscle studies (HEO-100, Omron Ltd. Inc., Japan) that was commercialised worldwide up to 2003.[53,54]

Different NIR spectroscopy techniques (for example, CW, SRS, TRS and PMS) have been utilised for measuring muscle oxygenation during exercise. In the last decade, approximately 300 articles relating to muscle NIR spectroscopy have been published on different physiological aspects (primarily muscle oxygenation and haemodynamics) of several upper- and lower-limb muscle groups investigated by using mainly two-channel CW and SRS commercial instruments. A recently published review provides a snapshot of muscle NIR spectroscopy at the end of 2010 summarising the recent literature, offering the present status and perspectives of NIR spectroscopy instrumentation and methods, describing the main NIR spectroscopy studies on skeletal muscle physiology, posing open questions and outlining future directions.[55] So far, only about 20 of the ~600 skeletal muscles have been investigated by NIR spectroscopy. Specifically, lower limb muscles (i.e. biceps femoris, gastrocnemius, rectus femoris, tibialis anterior, vastus lateralis, vastus medialis) have been studied during diverse conditions (cycling, upon electrical stimulation, during knee extension exercise, leg press exercise, plantar flexion exercise, running, squatting, Wingate test) and upper limb muscles (i.e. biceps brachii, brachioradialis, deltoid, forearm flexors, triceps brachii) during diverse conditions (arm abduction exercise, bench press exercise, cycling, elbow flexion exercise, upon electrical stimulation, handgrip, rowing, Wingate test). The trunk muscles (i.e. erector spinae, intercostal, multifidus, paravertebral, serratus anterior) were also investigated during cycling, back extension and bending forward.

NIR spectroscopy has also been utilised as a non-invasive method to assess O_2 delivery, O_2 consumption, blood flow, O_2 re-saturation and recovery times in diverse muscle groups at rest, during exercise and in the recovery phase in peripheral vascular disease patients and neuromuscular disease patients.[25,51]

So far, NIR spectroscopy has been applied for studying exercise-induced muscle damage,[56,57] ergonomics/biomechanics,[58] heterogeneity of muscle O_2 supply/demand,[59,60] muscle activation,[57,61] priming exercise,[62,63] respiratory muscle blood flow/fatigue,[64,65] the role of the brain in muscle fatigue,[66,67] the time course of oxidative metabolism[68,69] and the effect of exercise training.[70] Unfortunately, NIR spectroscopy has rarely been utilised in clinics.[46] There are still several open questions in the application of NIR spectroscopy in muscle studies: (i) whether NIR spectroscopy can be used in subjects with a thick fat layer and (ii) the effect on NIR spectroscopy measurements due to scattering which may vary during exercise.[55]

The most exciting prospect of muscle oximetry studies is the full understanding of skeletal muscle biochemistry/physiology/pathology for improving human health care, athletic performance and rehabilitation monitoring.

Conclusions

In the last decade, NIR brain and muscle oximetry has obtained significant patho-physiological relevance as suggested by the high number of instruments sold and the high number of scientific publications. The most relevant clinical application is the evaluation of cerebral oxygenation

during adult cardiac surgery and cardiopulmonary bypass. Many commercial oximeters are available. However, their relatively poor precision and the lack of standardisation amongst the different instruments suggest that further technological advances are required before NIR spectroscopy oximetry can be adopted more widely under the "guidelines" of regulatory authorities.

Acknowledgements

The authors wish to thank all the companies mentioned throughout the text for providing the data (including the market) about their instruments. The authors would like to thank Rosanna De Carolis for the technical support.

References

1. G.A. Millikan, "The oximeter, an instrument for measuring continuously the oxygen saturation of arterial blood in man", *Rev. Sci. Instrum.* **13,** 434 (1942). doi: 10.1063/1.1769941

2. J.W. Severinghaus, "Takuo Aoyagi: discovery of pulse oximetry", *Anesth. Analg.* **105(6 Suppl.),** S1 (2007). doi: 10.1213/01.ane.0000269514.31660.09

3. F.F. Jöbsis, "Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters", *Science* **198(4323),** 1264 (1977). doi: 10.1126/science.929199

4. C.A. Piantadosi, "Early development of near-infrared spectroscopy at Duke University", *J. Biomed. Opt.* **12(6),** 062102 (2007). doi: 10.1117/1.2804925

5. K.C. Smith "New topics in photobiology", in *The Science of Photobiology*, Ed by K.C. Smith. Plenum Press, New York, USA, p. 397 (1977).

6. J.M. Conway, K.H. Norris and C.E. Bodwell, "A new approach for the estimation of body composition: Infrared interactance", *Am. J. Clin. Nutr.* **40,** 1123 (1984).

7. J.T. Kuenstner and K.H. Norris, "Near infrared haemoglobinometry", *J. Near Infrared Spectrosc.* **3(1),** 11 (1995). doi: 10.1255/jnirs.50

8 J.T. Kuenstner and K.H. Norris, "Spectrophotometry of human haemoglobin in the near infrared region from 1000 to 2500 nm", *J. Near Infrared Spectrosc.* **2(2),** 59 (1994). doi: 10.1255/jnirs.32

9. J.T. Kuenstner, K.H. Norris and W.F. McCarthy, "Measurement of hemoglobin in unlysed blood by near-infrared spectroscopy", *Appl. Spectrosc.* **48(4),** 484 (1994). doi: 10.1366/000370294775269036

10. K.H. Norris and J.T. Kuenstner, "Rapid measurement of analytes in whole blood with NIR transmittance", in *Leaping Ahead with Near Infrared Spectroscopy*, Ed by G.D. Batten, P.C. Flinn, L.A. Welsh and A.B. Blakeney. *Proceedings of the 6th International Conference on NIRS*. p. 431 (1995).

11. D. Frasca, D. Dahyot-Fizelier, K. Catherine, Q. Levrat, B. Debaene and O. Mimoz. "Accuracy of a continuous noninvasive hemoglobin monitor in intensive care unit patients", *Crit. Care Med.* **39(10),** 2277 (2011). doi: 10.1097/CCM.0b013e3182227e2d

12. F.F. Jöbsis-vander Vliet, "Discovery of the near-infrared window into the body and the early development of near-infrared spectroscopy", *J. Biomed. Opt.* **4(4),** 392 (1999). doi: 10.1117/1.429952

13. J.E. Brazy, D.V. Lewis, M.H. Mitnick and F.F. Jöbsis vander Vliet, "Noninvasive monitoring of cerebral oxygenation in preterm infants: Preliminary observations", *Pediatrics* **75(2),** 217 (1985).

14. M. Ferrari, I. Giannini, A. Carpi, P. Fasella, C. Fieschi and E. Zanette, "Non invasive infrared monitoring of tissue oxygenation and circulatory parameters", in XII World Congress of Angiology, Athens, September 7–12, abs. 663 (1980).

15. I. Giannini, M. Ferrari, A. Carpi and P. Fasella, "Rat brain monitoring by near-infrared spectroscopy: an assessment of possible clinical significance", *Physiol. Chem. Phys.* **14(3),** 295 (1982).

16. M. Ferrari, I. Giannini, A. Carpi and P. Fasella, "Near I.R. spectroscopy in non invasive monitoring of cerebral function", in *Proceedings of the World Congress on Medical Physics and Biomedical Engineering 1982, Hamburg, September 5–11*, Ed by W. Bleifeld, D. Harder, H.K. Leetz and M. Schaldach, MPBE 1982 e. V., Hamburg, abs. 22.17 (1982).

17. M. Ferrari, I. Giannini, G. Sideri and E. Zanette, "Continuous non invasive monitoring of human brain by near infrared spectroscopy", *Adv. Exp. Med. Biol.* **191,** 873 (1985).

18. M. Ferrari, E. Zanette, I. Giannini, G. Sideri, C. Fieschi and A. Carpi, "Effects of carotid artery compression test on regional cerebral blood volume, hemoglobin oxygen saturation and cytochrome-*c*-oxidase redox level in cerebrovascular patients", *Adv. Exp. Med. Biol.* **200,** 213 (1986).

19. M. Ferrari, C. De Marchis, I. Giannini, A. Di Nicola, R. Agostino, S. Nodari and G. Bucci, "Cerebral blood volume and hemoglobin oxygen saturation monitoring in neonatal brain by near IR spectroscopy". *Adv. Exp. Med. Biol.* **200,** 203 (1986).

20. E.O. Reynolds, J.S. Wyatt, D. Azzopardi, D.T. Delpy, E.B. Cady, M. Cope and S. Wray, "New non-invasive methods for assessing brain oxygenation and haemodynamics", *Br. Med. Bull.* **44(4),** 1052 (1988).

21. J.S. Wyatt, M. Cope, D.T. Delpy, S. Wray and E.O. Reynolds, "Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infra-red spectrophotometry", *Lancet* **2(8515),** 1063 (1986). doi: 10.1016/S0140-6736(86)90467-8

22. M. Cope and D.T. Delpy, "System for long-term measurement of cerebral blood and tissue oxygenation on newborn infants by near infra-red transillumination",

Med. Biol. Eng. Comput. **26(3),** 289 (1988). doi: 10.1007/BF02447083

23. J.A. Wahrt, K.K Tremper, S. Samra and D.T. Delpy, "Near-infrared spectroscopy: Theory and applications", *J. Cardiothorac. Vasc. Anesth.* **10(3),** 406 (1996). doi: 10.1016/S1053-0770(96)80107-8

24. M. Ferrari, D.A. Wilson, D.F. Hanley, J.F. Hartmann, M.C. Rogers and R.J. Traystman, "Noninvasive determination of hemoglobin saturation in dogs by derivative near-infrared spectroscopy", *Am. J. Physiol.* **256(5 Pt 2),** H1493 (1989).

25. M. Wolf, M. Ferrari and V. Quaresima, "Progress of near-infrared spectroscopy and topography for brain and muscle clinical applications", *J. Biomed. Opt.* **12(6),** 062104 (2007). doi: 10.1117/1.2804899

26. M. Calderon-Arnulphi, A. Alaraj and K.V. Slavin, "Near infrared technology in neuroscience: Past, present and future", *Neurol. Res.* **31(6),** 605 (2009). doi: 10.1179/174313209X383286

27. D.T. Delpy and M. Cope, "Quantification in tissue near-infrared spectroscopy", *Philos. Trans. R. Soc. London. Ser. B. Biol. Sci.* **352(1354),** 649 (1997). doi: 10.1098/rstb.1997.0046

28. M. Ferrari, L. Mottola and V. Quaresima, "Principles, techniques, and limitations of near infrared spectroscopy", *Can. J. Appl. Physiol.* **29(4),** 463 (2004). doi: 10.1139/h04-031

29. A. Pellicer and M. del C. Bravo, "Near-infrared spectroscopy: A methodology-focused review", *Semin. Fetal Neonatal Med.* **16(1),** 42 (2011). doi: 10.1016/j.siny.2010.05.003

30. M.A. McNarry, J.R. Welsman and A.M. Jones, "Influence of training and maturity status on the cardiopulmonary responses to ramp incremental cycle and upper body exercise in girls", *J. Appl. Physiol.* **110(2),** 375 (2011). doi: 10.1152/japplphysiol.00988.2010

31. Q. Zhang, X. Yan and G.E. Strangman, "Development of motion resistant instrumentation for ambulatory near-infrared spectroscopy", *J. Biomed. Opt.* **16(8),** 087008 (2011). doi: 10.1117/1.3615248

32. G. Greisen, T. Leung and M. Wolf, "Has the time come to use near-infrared spectroscopy as a routine clinical tool in preterm infants undergoing intensive care?" *Philos. Trans. R. Soc. London Ser. A* **369(1955),** 4440 (2011). doi: 10.1098/rsta.2011.0261

33. M. Wolf, G. Morren, D. Haensse, T. Karen, U. Wolf, J.C. Fauchre and H.U. Bucher, "Near infrared spectroscopy to study the brain: an overview", *Opto-Electron. Rev.* **16(4),** 413 (2008). doi: 10.2478/s11772-008-0042-z

34. M. Wolf and G. Greisen, "Advances in near-infrared spectroscopy to study the brain of the preterm and term neonate", *Clin. Perinatol.* **36(4),** 807 (2009). doi: 10.1016/j.clp.2009.07.007

35. M. Wolf, G. Naulærs, F. van Bel, S. Kleiser, G. and G. Greisen, "A review of near infrared spectroscopy for term and preterm newborns", *J. Near Infrared Spectrosc.* **20,** 43 (2012). doi: 10.1255/jnirs.972

36. P.C. Drayna, T.J. Abramo and C. Estrada, "Near-infrared spectroscopy in the critical setting", *Pediatr. Emerg. Care* **27(5),** 432 (2011). doi: 10.1097/PEC.0b013e3182188442

37. J.M. Murkin, "NIRS: A standard of care for CPB vs. an evolving standard for selective cerebral perfusion?" *J. Extra Corpor. Technol.* **41(1),** 11 (2009).

38. M.C. Taillefer and A.Y. Denault, "Cerebral near-infrared spectroscopy in adult heart surgery: systematic review of its clinical efficacy", *Can. J. Anaesth.* **52(1),** 79 (2005). doi: 10.1007/BF03018586

39. S. Goldman, F. Sutter, F. Ferdinand and C. Trace, "Optimizing intraoperative cerebral oxygen delivery using noninvasive cerebral oximetry decreases the incidence of stroke for cardiac surgical patients", *Heart Surg. Forum* **7(5),** E376 (2004). doi: 10.1532/HSF98.20041062

40. A. Casati, G. Fanelli, P. Pietropaoli, R. Proietti, R.Tufano, G. Danelli, G. Fierro, G. De Cosmo and G. Servillo, "Continuous monitoring of cerebral oxygen saturation in elderly patients undergoing major abdominal surgery minimizes brain exposure to potential hypoxia", *Anesth. Analg.* **101(3),** 740 (2005). doi: 10.1213/01.ane.0000166974.96219.cd

41. J.M. Murkin, S.J. Novick, R.J. Adams, M. Quantz, D. Bainbridge, I. Iglesias, A. Cleland, B. Schaefer, B. Irwin and S. Fox, "Monitoring brain oxygen saturation during coronary bypass surgery: A randomized, prospective study", *Anesth. Analg.* **104(1),** 51 (2007). doi: 10.1213/01.ane.0000246814.29362.f4

42. J.P. Slater, T. Guarino, J. Stack, K. Vinod, R.T. Bustami, J.M. Brown, 3rd, A.L. Rodriguez, C.J. Magovern, T. Zaubler, K. Freundlich and G.V. Parr, "Cerebral oxygen desaturation predicts cognitive decline and longer hospital stay after cardiac surgery", *Ann. Thorac. Surg.* **87(1),** 36 (2009). doi: 10.1016/j.athoracsur.2008.08.070

43. G.M. Hoffman, "Pro: near-infrared spectroscopy should be used for all cardiopulmonary bypass", *J. Cardiothorac. Vasc. Anesth.* **20(4),** 606 (2006). doi: 10.1053/j.jvca.2006.05.019

44. D. Highton, C. Elwell and M. Smith, "Noninvasive cerebral oximetry: Is there light at the end of the tunnel?", *Curr. Opin. Anaesthesiol.* **23(5),** 576 (2010). doi: 10.1097/ACO.0b013e32833e1536

45. J.C. Hirsch, J.R. Charpie, R.G. Ohye and J.G. Gurney, "Near-infrared spectroscopy: what we know and what we need to know a systematic review of the congenital heart disease literature", *J. Thorac. Cardiovasc. Surg.* **137(1),** 154, 159 e1–12 (2009).

46. J.M. Kane and D.M. Steinhorn, "Lack of irrefutable validation does not negate clinical utility of near-infrared spectroscopy monitoring: learning to trust new technology", *J. Crit. Care* **24(3),** 472 e1–7. (2009).

47. N. Kasman and K. Brady, "Cerebral oximetry for pediatric anesthesia: Why do intelligent clinicians disagree"? *Paediatr. Anaesth.* **21(5),** 473 (2011). doi: 10.1111/j.1460-9592.2011.03549.x

48. Y.N. Bhambhani, "Muscle oxygenation trends during dynamic exercise measured by near infrared spectroscopy", *Can. J. Appl. Physiol.* **29,** 504 (2004). doi: 10.1139/h04-033

49. R. Boushel, H. Langberg, J. Olesen, J. Gonzales-Alonzo, J. Bulow and M. Kjaer, "Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease", *Scand. J. Med. Sci. Sports* **11,** 213 (2001). doi: 10.1034/j.1600-0838.2001.110404.x

50. M. Ferrari, T. Binzoni and V. Quaresima, "Oxidative metabolism in muscle", *Philos. Trans. R. Soc. London Ser. B. Biol. Sci.* **352,** 677 (1997). doi: 10.1098/rstb.1997.0049

51. T. Hamaoka, K.K. McCully, V. Quaresima, K. Yamamoto and B. Chance, "Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans", *J. Biomed. Opt.* **12,** 062105 (2007). doi: 10.1117/1.2805437

52. V. Quaresima, R. Lepanto and M. Ferrari, "The use of near infrared spectroscopy in sports medicine", *J. Sports Med. Phys. Fitness* **43(1),** 1 (2004).

53. T. Shiga, K. Tanabe, Y. Nakase, T. Shida and B. Chance, "Development of a portable tissue oximeter using near infrared spectroscopy", *Med. Biol. Eng. Comput.* **33,** 622 (1995). doi: 10.1007/BF02522525

54. T. Shiga, K. Yamamoto, K. Tanabe, Y. Nakase and B. Chance, "Study of an algorithm based on model experiments and diffusion theory for a portable tissue oximeter", *J. Biomed. Opt.* **2,** 154 (1997). doi: 10.1117/12.268963

55. M. Ferrari, M. Muthalib and V. Quaresima, "The use of near-infrared spectroscopy (NIRS) in under standing skeletal muscle physiology: recent developments", *Philos. Trans. R. Soc. London Ser. A* **369(1955),** 4577 (2011). doi: 10.1098/rsta.2011.0230

56. S. Ahmadi, P.J. Sinclair, N. Foroughi and G.M. Davis, "Monitoring muscle oxygenation after eccentric exercise-induced muscle damage using near-infrared spectroscopy", *Appl. Physiol. Nutr. Metab.* **33(4),** 743 (2008). doi: 10.1139/h08-048

57. M. Muthalib, H. Lee, G.Y. Millet, M. Ferrari and K. Nosaka, "Comparison between maximal lengthening and shortening contractions for biceps brachii muscle oxygenation and hemodynamics", *J. Appl. Physiol.* **109(3),** 710 (2010). doi: 10.1152/japplphysiol.01297.2009

58. R.V. Maikala and A.R. Hargens, "Application of near-infrared spectroscopy (NIRS) in ergonomics and exercise", *Int. J. Ind. Ergon.* **40,** 123 (2010). doi: 10.1016/j.ergon.2009.12.003

59. S. Koga, D.C. Poole, L.F. Ferreira, B.J. Whipp, N. Kondo, T. Saitoh, E. Ohmae and T.J. Barstow, "Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise", *J. Appl. Physiol.* **103,** 2049 (2007). doi: 10.1152/japplphysiol.00627.2007

60. T. Saitoh, L.F, Ferreira, T.J. Barstow, D.C. Poole, A. Ooue, N. Kondo and S. Koga, "Effects of prior heavy exercise on heterogeneity of muscle deoxygenation kinetics during subsequent heavy exercise", *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297(3),** 615 (2009). doi: 10.1152/ajpregu.00048.2009

61. R.D. Kooistra, C.J. de Ruiter and A. de Haan, "Knee angle-dependent oxygen consumption of human quadriceps muscles during maximal voluntary and electrically evoked contractions", *Eur. J. Appl. Physiol.* **102(2),** 233 (2008). doi: 10.1007/s00421-007-0573-x

62. M. Buchheit, P.B. Laursen and S. Ahmaidi, "Effect of prior exercise on pulmonary O_2 uptake and estimated muscle capillary blood flow kinetics during moderate-intensity field running in men", *J. Appl. Physiol.* **107(2),** 460 (2009). doi: 10.1152/japplphysiol.91625.2008

63. G. Layec, A. Bringard, Y. Le Fur, C. Vilmen, J.P. Micallef, S. Perrey, P.J. Cozzone and D. Bendahan, "Effects of a prior high-intensity knee-extension exercise on muscle recruitment and energy cost: a combined local and global investigation in humans", *Exp. Physiol.* **94(6),** 704 (2009). doi: 10.1113/expphysiol.2008.044651

64. J.A. Guenette, I. Vogiatzis, S. Zakynthinos, D. Athanasopoulos, M. Koskolou, S. Golemati, M. Vasilopoulou, H.E. Wagner, C. Roussos, P.D. Wagner and R. Boushel, "Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green", *J. Appl. Physiol.* **104(4),** 1202 (2008). doi: 10.1152/japplphysiol.01160.2007

65. I. Vogiatzis, D. Athanasopoulos, R. Boushel, J.A. Guenette, M. Koskolou, M. Vasilopoulou, H. Wagner, C. Roussos, P.D. Wagner and S. Zakynthinos, "Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists", *J. Physiol.* **586(Pt 22),** 5575 (2008). doi: 10.1113/jphysiol.2008.162768

66. Y. Bhambhani, R. Malik and S. Mookerjee," Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold", *Respir. Physiol. Neurobiol.* **156(2),** 196 (2007). doi: 10.1016/j.resp.2006.08.009

67. T. Rupp and S. Perrey, "Prefrontal cortex oxygenation and neuromuscular responses to exhaustive exercise", *Eur. J. Appl. Physiol.* **102(2),** 153 (2008).

68. V. Cettolo, M. Ferrari, V. Biasini and V. Quaresima, "Vastus lateralis O_2 desaturation in response to fast and short maximal contraction", *Med. Sci. Sports Exerc.* **39(11),** 1949 (2007). doi: 10.1249/mss.0b013e3181453476

69. G.R. duManoir, D.S. DeLorey, J.M. Kowalchuk and D.H. Paterson, "Kinetics of $\dot{V}o_2$ limb blood flow and regional muscle deoxygenation in young adults during moderate intensity, knee-extension exercise", *Eur. J. Appl. Physiol.* **108(3),** 607 (2010). doi: 10.1007/s00421-009-1263-7

70. B.R. McKay, D.H. Paterson and J.M. Kowalchuk, "Effect of short-term high-intensity interval training vs continuous training on O_2 uptake kinetics, muscle deoxygenation, and exercise performance", *J. Appl. Physiol.* **107(1),** 128 (2009). doi: 10.1152/japplphysiol.90828.2008