35 Blood Analysis by NIR Spectroscopy

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35.1 INTRODUCTION

The physics of near-infrared (NIR) spectroscopy are favorable for biological applications, especially for in situ measurements. The low absorptivity inherent in NIR allows the radiation to penetrate deeper, resulting in longer pathlengths. The sources are intense, giving more radiation to work with; detectors are sensitive and nearly noise-free, giving a more precise and accurate spectrum. These physical realities allow for measurements through tissue, muscle, fat, and body fluids with great precision. Fiber-optic probes and, more recently, wearable systems complete the picture and make NIR spectroscopy a technique adaptable to any lab or clinic. This chapter will focus on the analysis of blood by NIR spectroscopy, specifically glucose and oxygenation.

35.2 BLOOD GLUCOSE

One of the most publicized and pursued uses of near-infrared in the life sciences is for in situ glucose measurements. Approximately 30,300,000 patients are believed to suffer from diabetes in the United States alone in 2015, with approximately 1.5 million new cases diagnosed every year. With the diagnostic market estimated in the multibillion-dollar range, the number of scientists seeking a spectroscopic solution to this analytical problem is significant. A large number of patents for blood-glucose-measuring devices relying on near-infrared spectroscopy have been issued. Examples of the patented technology are as follows:

- The patented device developed by Ham and Cohen [1]. In this transmission device, the light is passed through the finger. A neural network (NN) algorithm recognizes the spectral areas of greatest correlation with glucose concentration and builds the calibration equation automatically. It is hypothesized that because of the complex nature of blood chemistry, complex algorithms will likely be needed for determination of any component of the blood.

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The patented device developed by Acosta et al. [2]. In this reflectance/transflectance device, the glucose content is correlated with redistribution of fluids between vascular and extravascular and intra- and extracellular compartments.

The patented device developed by Acosta et al. [3]. In this reflectance device, the glucose content is directly modeled from the collected spectra. Reference values come from glucose testers available to diabetic patients.

The patented methodology developed by Chung et al. [4]. The proposed method uses a ratio of absorbance measurements at select wavelengths to develop a model that correlates directly the glucose content with the spectral information.

These few examples of patented technologies and methodologies point to the various approaches that have been selected by researchers to predict blood-glucose content: direct approaches that try to directly relate the glucose content to the NIR spectral information and indirect approaches that use a biological response to various glucose levels and attempt to relate these responses (more readily measurable by NIR) with glucose content. Along with the two types of approaches, significant work on chemometrics has been performed to enhance models’ performance and robustness.

Modeling of the blood-glucose system has been an ongoing project for numerous researchers for several decades. In 1999, Khalil [5] stated in a review document that to date, no experiment proved that the signal measured by near-infrared spectroscopy was the actual blood-glucose concentration. Gary Small and Mark Arnold (University of Iowa), in particular, have published widely on the subject. In a 2005 review article [6], they surveyed the literature and discussed the merit of direct and indirect approaches to glucose modeling. Indirect glucose measurement is commonly based on the effect of glucose on the scattering properties of the tissue [7,8]. Arnold and Small note that in indirect measurements, the major challenge is to ensure the selectivity of the measurement. Selectivity, along with sensitivity, is also an issue with direct measurements. Glucose is present at millimolar concentration levels, and while it does possess a unique vibrational spectrum compared to the other major blood components, existing devices may not have the sufficient sensitivity to the analyte to develop robust tools.

Nevertheless, many authors have published on methods to predict blood glucose. In 1993 [9], Arnold and Small published a paper modeling the NIR measurement of glucose in a protein-containing matrix. The region from 2000 to 2500 nm was used for analysis of a series of glucose solutions ranging from 1.2 to 20.0 mM in a phosphate buffer closely resembling blood. The buffers contained such materials as bovine serum albumin (BSA). They found that a Gaussian-shaped Fourier filter combined with a partial least squares (PLS) regression delivered a reasonable standard error of calibration. In all cases, the glucose absorbance at 2270 nm gave the optimal correlation, with a standard error of 0.24 mM. In 1994, the same authors reported work on a temperature-insensitive glucose-measuring method [10]. In an approach similar to that just detailed [9], using a Fourier filter/PLS combination, a temperature range between 32°C and 41°C was investigated. The temperature fluctuations caused relatively large variations in the spectra due to the water band shifts. Fourier filtering effectively eliminated the differences, providing a standard error that was even better than for their previous work (0.14 versus 0.24 mM). The necessity of spectral pretreatment is clearly seen in this study. Such temperature correction is necessary should measurements be taken from someone with a fever or who is in shock. This particular point is emphasized in many of the patents for analytical devices. A system to ensure consistency of the temperature of the skin at the sampling site is often included so that interferences due to temperature are limited.

Small, Arnold, et al. performed further work in 1996, this time using physiological levels of glucose in the presence of protein and triglycerides [11]. The solutions used in the study contained between 1 and 20 mM of glucose. The interferences were varied within each level of glucose. It was seen that multivariate algorithms compensated for the chemical variations in the blood where the glucose level remained unchanged. The same researchers also performed some published work on
the use of quadratic PLS and digital filtering techniques to account for non-glucose-related changes in the spectra [12]. They concluded that pretreatment helped reduce interferences and resulted in more robust equations. The instrumentation (either single-beam or double-beam) is made less important as interferences are accounted for in the NIR equation.

Two interesting papers by Arnold et al. were published in 1998 [13,14]. They were devoted to the calculations used in noninvasive blood-glucose measurements. The authors investigated neural networks and partial least squares as calibration approaches and examined such things as signal-to-noise enhancements through an understanding of how light is attenuated as it passes through tissue. The companion articles go into detail about the scattering effects of blood and tissue. Compensation schema are identified and proposed to alleviate some of these interferences. It is strongly recommended that fat, water, and tissue be compensated for in any model considered. This was further discussed by Uwadaira et al. [15] who investigated the effects of the interfering factors on the accuracy of noninvasive blood-glucose measurements.

A device developed in Germany by Schrader, where a laser illuminates the humor of the eye and measures the resulting absorbance spectrum was used to measure the amount of blood glucose in the patient [16]. The device is based on a patent developed by Backhaus et al. [17]. He found that the glucose levels in the anterior chamber of the eye closely follow changes in blood glucose with a latency of approximately 20 min. The equations developed by this instrumentation allow for noninvasive monitoring of physiological glucose levels with an error of ±30 mg dL⁻¹. The basis for using NIR for determination of blood glucose by scanning through skin and muscle is that the blood-glucose level in the blood is similar, if not identical, to the glucose level in tissues. This was claimed by Fischer et al. in 1994 and later demonstrated through a series of measurements [18].

However, contradicting this work was a paper by Sternberg et al. [19]. This group claimed that tissue contained only 75% of the glucose level found in blood. Fortunately, NIR measurements are inclusive of blood and tissue. The calibration was based on a point of contact for each individual patient; thus, the ratio of tissue to venous/arterial blood will be a constant. Correlation of the spectra with blood-glucose readings is then acceptable.

In order to model more correctly the in vivo realities of human body chemistry, “phantoms” (simulated biological conditions, containing fat, blood, skin, etc.) were built for simulated in vivo testing. Arnold et al. built phantoms of water, fat, and muscle tissue to mimic the skin of a patient [20]. They found that in vivo overtone spectra collected across human webbing tissue with a thickness of 6.7 mm could be simulated with water layer thickness from 5.0 to 6.4 mm combined with fat layer thickness from 1.4 to 4.2 mm. For the purposes of this study, animal tissue and fat were used; there is little difference in composition between human and animal materials. They concluded that these phantom studies would help researchers develop patient-applicable methods.

This phantom work was continued by Arnold et al. in a later publication [21]. This is a negatively designed study, used as an object lesson to “warn” the inexperienced user about the pitfalls of chemometrics. They used an in vitro model of blood-simulated samples to build a model for blood-glucose determination. In this case, however, they carefully omitted any glucose from the samples. The samples were randomly assigned glucose values and a PLS regression developed. As with any PLS model, an equation was created to provide reasonable standard errors, regression coefficients, etc. Since there was no glucose present, this equation could not predict glucose when samples containing the sugar were tested. This paper discussed potential errors to avoid during calibration development and analysis.

An interesting observation was made by Maier et al. where it was observed that there exists a correlation between blood-glucose concentration and the reduced NIR scattering coefficient of tissue [22]. Using a frequency-domain NIR spectrometer, the scattering coefficient of tissue was measured with adequate precision to detect changes in glucose. The work was based on the theory that as the glucose concentration increased, the refractive index of the blood also increased in a predictable manner. This increased refractive index would then decrease the scattering coefficient of the blood and give an indication of the concentration. Some questions of the applicability of this
work to in vivo measurements must be asked, but it does demonstrate one of the novel approaches being investigated in the field.

One other technique [23] employed a fiber-optic light pipe to measure blood glucose through the skin of a finger. The device uses a portion of the fiber, stripped of its cladding, as a virtual attenuated total reflectance (ATR) device positioned against the skin of the thumb. Because so much radiation is lost into the skin, white light was used with a monochromator situated postsample. This configuration gave better sensitivity than when first resolving the wavelengths. Postsampling indicates that “white” light is impinged on the skin and that the resultant emerging light is collected and submitted to a monochromator. A study by the team of Dr. Ozaki [24] investigated the use of a particular illumination and detection geometry with fiber optics that allowed them to successfully interrogate dermis tissue and reduce other interferences. Using partial least squares, they were able to obtain prediction error for all the subjects involved in the study of 23.7 mg dL\(^{-1}\), thus showing similar results to other studies and showing the possibility to use the same model for different patients.

One “nonskin” application was published in a paper by Heise et al. [25], where a procedure for measuring blood glucose through the lip was described. They used the 1100–1800 nm wavelength range, and partial least squares was the algorithm of choice. The mean-square prediction error was estimated between 45 and 55 mg dL\(^{-1}\). A lag time of approximately 10 min exists between the drawn blood values and the values derived from the lip tissue. The authors recommend using fiber optics for further developments in this field. An update to the study was presented in 2009 (Heise, Lampen, and Marbach [26]).

An example of indirect measurement of blood glucose was provided by Small and Arnold in a study investigating the change in blood scattering with respect to the change in the concentration of glucose [27]. The authors showed prediction errors in the 1–2.5 mM range depending on the part of the NIR signal considered.

A lot of work has been put into the development of chemometric methods that would extract the most relevant signal to allow an improved prediction performance and robustness of the glucose models. Besides the work done with artificial neural networks, literature exists on the use of the net analyte signal (NAS). Initially developed by Lorber as a means to calculate multivariate figures of merit [28], the NAS corresponds to the net signal of the analyte of interest. It can be used as a preprocessing step to focus the analysis on only the relevant signal. An example of the use of NAS for blood glucose can be found in Ren and Arnold [29]. On the same topic of removing interfering signal, techniques based on eliminating the signal that is not related to the signal of glucose have been published. Thus, Ozaki proposed to use a local orthogonal signal correction method to remove interferences from the main blood components in addition to searching for the best suited wavelength regions with a moving-window partial least squares [30,31]. Another technique utilizing the uninformative variable elimination combined with successive projections was intended by Li et al. [32]. A non-linear regression version of the uninformative variable elimination was later proposed by the same authors [33]. Approaches using Beer’s law have also been investigated through the use of classical least squares. In an example by Maruo and Yamada [34], the pure component matrix was augmented by a constructed spectrum to correct for baseline drifts as a function of time. Researchers working at the University of Krakow have published several papers and given numerous talks and posters devoted to the math treatments of the complex spectra produced from the NIR examination of blood through the skin and muscle [35–37]. They have been working with neural networks in particular and have made some interesting observations.

To improve model robustness and performance, several authors have worked at optimizing the samples included in calibration. So et al. [38] used a Monte Carlo approach to determine the best set of samples that should be used. The use of simulated spectra has also been tested with results in in vivo experiments in the 12.3 mg dL\(^{-1}\) range [39].

Finally, with the development of wearable technologies, much work has been done on the development of new sensors. While not NIR-based, Verily, a Google subsidiary, first announced in 2014...
the development of contact lenses before providing an update at the end of 2018 on their halt of the program (https://blog.verily.com/2018/11/update-on-our-smart-lens-program-with.html). Another recent article showed promising results prior to clinical trials (Park et al. [40]).

Other indirect measurements of the effect of glucose in the body have been used to measure glucose. Rachim and Chung [41] used the reflected optical signal of the change in arterial blood volume pulsation on a wristband as an indirect indication of glucose content. A more direct measurement of glucose using the first overtone band region (1500–1800 nm) was also recently proposed by Bae et al. [42].

### 35.3 BLOOD OXYGENATION

Venial and cranial blood oxygenation is simultaneously a (relatively) simple and yet nontrivial measurement. Early reports of NIR for diagnostic applications came from researchers such as Jobsis in 1977 [43]. He used NIR to monitor the degree of oxygenation of certain metabolites. Later, Ozaki et al. [44] examined venal blood to determine the level of deoxyhemoglobin. The back of the hand was illuminated, and the diffusely reflected light was captured by a miniature integrating sphere equipped with a lead sulfide (PbS) detector. The spectra were correlated with results from a CIBA Corning 278 blood-gas analyzer. The 760-nm band in the spectrum was seen to correlate quite well with deoxyhemoglobin, and a negative correlation exists with oxygenated hemoglobin.

Michael Sowa and his group [45] used NIR imaging as a noninvasive technique to monitor regional and temporal variations in tissue oxygenation. The purpose was to ascertain the effects of restricted blood outflow (venous outflow restriction) and interrupted blood inflow (ischemia). In this work, the software was the heart of the paper. Multivariate analyses of image and spectral data time courses were used to identify correlated spectral and regional domains. Fuzzy C-means clustering of image time courses revealed finer regional heterogeneity in the response of stressed tissues. The wavelength region from 400 to 1100 nm was monitored from 0 to 30 min, and a plot of these data was developed to produce a “topographical” representation of the phenomenon. Peaks and valleys were apparent where blood became oxygenated and deoxygenated. These standard wavelength-based values correlated well with the images developed by the 512 × 512 back-illuminated charge-coupled device (CCD) element.

Clustering results clearly showed areas of both low and high oxygenation. These results have important implications in the assessment of transplanted tissue viability. Mancini et al. [46] estimated skeletal muscle oxygenation by using the differential absorption properties of hemoglobin. Oxygenated and deoxygenated hemoglobin have identical absorptivities at 800 nm, while deoxygenated hemoglobin predominates at 760 nm. The effects of myoglobin on the readings were also investigated, and it was found that the readings’ correlations were due to hemoglobin oxygenation. Venous oxygen saturation and absorption between 760 and 800 nm were correlated. Mancini and colleagues reached several conclusions: (1) Hypoxia in KCl-arrested hearts results in only moderate activation of anaerobic glycolysis; (2) oxygenation of the epicardial and midmural LV layers is similar; and (3) a large pO2 gradient exists between vascular and intercellular space in beating and arrested crystalloid-perfused hearts.

Lin et al. examined the influence of fat layers on the determination of blood oxygenation in 1998 [47]. The phantom experiments showed fat influences patient-to-patient measurements. This is more easily compensated for in any individual patient. Yamamoto addressed the issue of fat interference with an oximeter that corrected for the influence of subcutaneous fat [48]. The wavelengths, again, were the key, as was the algorithm.

The effect of water on NIR determination of hemoglobin concentration in a tissue-like phantom was studied by Franceschini et al. in 1996 [49]. Their in vitro studies consisted of aqueous suspensions containing Liposyn, bovine blood, and yeast, buffered at pH 7.2. The optical coefficients of the mixture matched those of biological tissue in the NIR, and the hemoglobin concentration (23 μM) was also similar to that found in tissues. They oxygenated and deoxygenated the hemoglobin by
sparging the mixture with either oxygen or nitrogen. They determined that water concentration must be taken into account to obtain accurate results of hemoglobin concentrations.

Jiang et al. presented a device that allowed noninvasive measurement of cerebral tissue oxygenation to be performed [50]. Again based on fiber optics, shorter NIR wavelengths were used. Another device allowing diffuse reflectance measurements of the skin was developed by Marbach and Heise [51]. The device presented has an on-axis ellipsoidal collecting mirror with efficient illumination for small sampling areas of bulky body specimens. The actual schematic is too complex to describe in this chapter. The researchers supported the optical design with a Monte Carlo simulation study of the reflective characteristics of skin tissue. While their work was centered on the 1600-nm peak associated with glucose (using the lip as the point of entry), the work is applicable to other tissue research.

Keiko Miyasaka presented some of his work [52] at a meeting in Toronto. As a worker in the field of critical care for children, he introduced what he calls a “Niroscope” for near-infrared spectroscopy. His work was performed during pediatric anesthesia and intensive care. Dr. Miyasaka found the Beer’s law relationship not followed rigorously when the signal was passed through the cranium. Considering the massive scattering absorbed light, this was understandable. It did give, however, a semiquantitative or indicating equation. What Miyasaka was measuring was the intercranial levels of oxygenated hemoglobin (HbO₂), deoxygenated or reduced hemoglobin (Hb), and cytochrome redox status. Two methods were used: photon counting and a micro-type pulse laser. The photon-counting method is necessary because of the extreme attenuation of the incident radiation when traversing the cranium. The pulse laser was used to enhance the amount of light introduced into the brain.

Three conclusions may be reached from the NIR data: (1) Changes in HbO₂ levels reflect changes in arterial blood, (2) Hb changes are due to venous blood, and (3) total hemoglobin reflects changes in cerebral blood volume or intercranial pressure. This tool will be invaluable for emergency and operating room situations both for children and, someday, for adults. Van Huffel et al. used NIR to monitor brain oxygenation. They used the information to correlate with behavioral states of preterm infants and to understand the development of brain hemodynamic autoregulation [53]. The concentrations of HbO₂, Hb, and cytochrome aa₃ (Cytaa₃) are used to monitor the oxygenation level in infant brain blood. Some novel chemometrics were involved as well; windowed fast Fourier transform (WFFT) and wavelet analyses were employed. The purpose of the work was to see relationships between the computed chromophore concentrations and heart rate, breathing, and peripheral oxygen saturation. They presented similar work in 1998 as well [54].

Chris Cooper et al. [55] performed another study; this one aimed at the adult brain. In this work, NIR was used to determine the effects of changes in the rate of oxygen delivery on adult rat brain chemistry. Absolute levels of oxyhemoglobin, deoxyhemoglobin, and the redox state of the CuA center in mitochondrial cytochrome oxidase were determined. An interesting finding was that as the mean arterial blood pressure reached 100 mmHg, hemoglobin oxygenation began to fall, but the oxidized CuA levels only fell when cerebral blood volume autoregulation mechanisms failed at 50 mmHg. Hemoglobin oxygenation fell linearly with decreases in the rate of oxygen delivery to the brain, but the oxidized CuA concentration did not start to fall until this rate was 50% of normal. The results suggested that the brain maintained more-than-adequate oxygen delivery to mitochondria. Their conclusion was that NIR is a good measure of oxygen insufficiency in vivo.

A related study on human infants was performed by Wyatt et al. [56]. They used NIR to quantify the cerebral blood volume in human infants. Similar difficulties were encountered with the amount of light actually penetrating the cranial cavity, but useful equations were generated.

Kupriyanov et al. determined intracellular pO₂ in cardiac muscle by the balance between its diffusion from vascular to intercellular space and its uptake by mitochondria [57]. They reasoned that cessation of mechanical work decreased O₂ demand and should have reduced the O₂ gradient between vascular and intercellular spaces. For their research, they compared the effects of
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arterial pO2 on myoglobin (Mb) oxygenation, O2 uptake, and lactate formation rates in beating and KCl-arrested pig hearts.

Ischemia in the forearm was studied by Mansfield et al. in 1997 [58]. In this study, the workers used fuzzy C-means clustering and principal component analysis (PCA) of time series from the NIR imaging of volunteers’ forearms. They attempted predictions of blood depletion and increase without a priori values for calibration. For those with a mathematical bent, this paper does a very nice job describing the theory behind the PCA and fuzzy C-means algorithms.

Another interesting paper was published by Wolf et al. in 1996 [59] where they used NIR and laser Doppler flowmetry (LDF) to study the effect of systemic nitric oxide synthase (NOS) inhibition on brain oxygenation. The study, performed on rats, demonstrated no effects on brain oxygenation during cortical spreading depression (CSD).

Doppler ultrasound was combined with NIR in another study [60]. Liem et al. used NIR and ultrasound to follow the cerebral oxygenation and hemodynamics in preterm infants treated with repeated doses of indomethacin. In addition to the normal concentrations of oxyhemoglobin, deoxyhemoglobin, and oxidized cytochrome aa3 measured by NIR, transcutaneous pO2 and pCO2, arterial O2 saturation, and blood pressure were measured. Along with the cerebral blood volume, they were all used for diagnosis and research. Low oxygenation was then thought to be a possible contraindication for indomethacin treatment for preterm infants.

The physical placement of detectors on the scalp for brain blood oxygenation was studied by Germon et al. in a 1998 study [61]. Detectors placed 2.7 and 5.5 cm from a NIR emitter were compared for the determination of HHb, O2Hb, oxidized cytochrome C oxidase, and total hemoglobin. The biological portion of the experiment was to measure the chemical changes with an induced reduction of the mean decrease in middle cerebral artery blood flow. The signal change per unit photon pathlength detected at 5.5 cm, for HHb, was significantly greater than at 2.7 cm. On the other hand, the increases in all chromophores detected at 5.5 cm during scalp hyperemia were significantly less than those detected at 2.7 cm. More work is indicated before meaningful applications can be designed from this work.

Using similar instrumentation, Henson et al. determined the accuracy of their cerebral oximeter under conditions of isocapnic hypoxia [62]. In healthy volunteers, dynamic end-tidal forcing was used to produce step changes in PET O2, resulting in arterial saturation ranging from ~70% to 100% under conditions of controlled normocapnia (resting PTET O2) or hypercapnia (resting plus 7–10 mmHg). Using standard methods, the O2 concentrations for each patient under each condition were determined. Excellent correlation resulted in the rSO2 and [S\textcircled{\text{c}}]\text{bar}\text{\text{O}}2 for each individual patient. However, wide variability between patients was discovered. They concluded that under the current limitations of the equipment, the device was good for tracking trends in O2 but could not be used as an absolute measure for different patients.

Numerous and disparate studies have been published or presented regarding the effect of various normal and pathologic conditions on blood oxygen:

1. Hoshi et al. investigated the neuronal activity, oxidative metabolism, and blood supply during mental tasks [63].
2. Okada presented work on impaired interhemispheric integration in brain oxygenation and hemodynamics in schizophrenia [64].
3. Hoshi looked into the features of hemodynamic and metabolic changes in the human brain during all-night sleep [65].
4. Akına et al. studied the clinical application of NIR in migraine patients [66]. They assessed the transient changes in brain tissue oxygenation during the aura and headache phases of a migraine attack.

Surgeons are concerned with brain blood flow to patients undergoing cardiopulmonary bypass surgery. An intensive study by Chow et al. was conducted where blood flows were restricted to patients...
from age 2 weeks to over 20 years [67]. Near-infrared was used to correlate blood flow rate with NIR spectra of the brain. Flows of 0.6, 1.2, and 2.4 L m\(^{-2}\) min\(^{-1}\) were used. Their results showed that flow was related to mean arterial pressure, but did not correspond to pulsatility. This was interesting in that pulse rate is often used as a diagnostic to assure sufficient blood flow to the brain during surgery.

Totaro et al. published a detailed paper on the factors affecting measurement of cerebrovascular reactivity when measured by NIR [68]. Some of the points covered were the relative transparency of the skin, skull, and brain in the 700–1100 nm region and the oxygen-dependent tissue absorption changes of hemoglobin. Their study covered all relevant factors, such as age, sex, reproducibility, and venous return. The test was based on a 3-min baseline, a 3-min hypercapnia (5% CO\(_2\) in air), and a 2-min recovery period.

Changes in NIR spectra and transcranial Doppler sonography parameters were significantly correlated with variations of end-tidal CO\(_2\) (\(P<0.005\)). In addition, a significant correlation between the reactivity indexes (approximately, absorptivities) of NIR spectrometry parameters and flow velocity was found (\(P<0.01\)). High reproducibility was also found for deoxyhemoglobin (\(r=0.76\)), oxyhemoglobin (\(r=0.68\)), and flow velocity (\(r=0.60\)) reactivity indexes. No significant differences between the reactivity indexes of different body positions were found (\(P<0.05\)). The reactivity indexes of oxyhemoglobin decreased (\(P>0.05\)) and of deoxyhemoglobin increased (\(P<0.01\)) with age. Their overall conclusion was that NIR is a viable technique for evaluation of cerebrovascular reactivity for patients with cerebrovascular disease.

Some exciting work was reported by Hitachi [69]. The research, conducted at the Tokyo Metropolitan Police Hospital, used NIR to detect blood flow changes in the brain to determine sites of epileptic activity. The location of blood flow increases corresponded well with conventional methods such as intercranial electroencephalogram (EEG) or single-photon emission computed tomography (SPECT). The technique was able to determine the side of the brain where the episode was taking place in all the patients on whom it was tried. This technique could replace the intrusive electrodes currently in use.

Near-infrared spectroscopy has been used to determine the activity of the brain using oxygenation levels. More particularly, a series of articles by Boas et al. [70,71] discusses the limitations that single-point NIR has in the analysis of focal brain activation compared to diffuse activation. Researchers found that it is possible using NIR spectroscopy to determine regions of the brain responsible for simple motor tasks such as moving the finger. However, the local nature of the focal activation is a challenge to NIR measurements. The main source of error was determined to be the cross talk between the pathlength of the entire area illuminated and the partial pathlength of the activated region, thus making it difficult to perform accurate measurements of the focal change. The authors determined that some wavelengths were more prone to cross talk (780- and 830-nm pairing compared to 690- or 760-nm pairing with 830 nm).

On the same topic, a significant amount of work has been done to study the level of oxygenation of the brain during exercise at sub-maximal [72] and maximal [73,74] levels. In a review by Cherie et al. [75], some very interesting results provided by NIR showed that oxygenation of the brain increases during exercise to hit a plateau and decline toward a baseline level at very hard exercise levels. The response was modified as a function of the training level with lower oxygenation levels attained by less fit people. Interestingly, the review ends by stating that improvements of NIR should allow a better understanding of local focal changes, a task undertaken a decade earlier by Boas et al. [70].

Cerebral oxygenation has been used in a multitude of other applications and helped develop the field of functional near-infrared spectroscopy. In addition to the topics already mentioned, fNIRS has found application in speech analysis and disorder [76], psychiatry and disease state [77], cerebral ischemia and hypoxia [78,79], brain injuries [80,81], and cardiac surgery [82,83], to name only a few. However, in a review, Highton et al. [84] noted that fNIRS is limited by the contamination of the signal by extracranial tissues. In addition, the large variability between individuals makes it
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It is difficult to set thresholds over or under which a problem is detected, thus making fNIRS ideal for trending and monitoring, but limits decision making to the individual level.

Improvement in the specificity of the measurements is now the focus of the research. Examples of measurements of nerve oxygenation have been presented [85].

Finally, a significant amount of work has been done with optoacoustic systems to image the vascular system. Using short pulses of near-infrared light, strong absorbers such as hemoglobin can be used to provide detailed images of blood vessels and are well suited for diagnosing and monitoring tissue pathologies such as those induced by tumors [86–89]. By modulating the wavelengths used, it is then possible to model the blood oxygen saturation and provide cancer research with the possibility to monitor tumor oxygenation [90].

In summary, oxygenation in general has been a major focal point for NIR medical research [91–117]. It continues to be a successful application for NIR.

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Blood Glucose


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**BLOOD OXYGENATION**


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