

Absolute Quantification of Oxygenated Hemoglobin within the Visual Cortex with Functional Near Infrared Spectroscopy (fNIRS)

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PURPOSE. To examine absolute changes in oxygenated (HbO) and deoxygenated (Hb) hemoglobin concentrations over the visual cortex in response to visual stimulation. Before this study, only relative changes have been reported at the visual cortex.

METHODS. Near infrared spectroscopy (NIRS) can be used to assess changes in hemoglobin concentration in tissue. A frequency domain oximeter with a specially designed probe was placed over the visual cortex while the participant viewed a checkerboard stimulus. The stimulus was alternated at 7.5 Hz for 30 seconds before being replaced by a control gray screen.

RESULTS. The mean HbO concentration when the stimulus was presented was $26.8 \pm 3.9 \mu\text{M}$, as opposed to $25.9 \pm 3.9 \mu\text{M}$ for the control condition, and the mean Hb concentration was $18.8 \pm 2.3 \mu\text{M}$ during stimulation and $19.1 \pm 2.3 \mu\text{M}$ during the control condition. The greatest change in HbO concentration occurred within the first 10 seconds. It did not increase significantly ($P > 0.01$) after that time.

CONCLUSIONS. This study is the first to demonstrate absolute quantification of HbO and Hb concentrations in the visual cortex with functional changes in hemoglobin concentrations in response to a visual stimulus. NIRS has the potential to be a valuable clinical tool in assessing the hemodynamics of the visual system in a quantitative and localized manner. (*Invest Ophthalmol Vis Sci.* 2010;51:4856–4860) DOI:10.1167/iovs.09-4940

Near infrared spectroscopy (NIRS) is a noninvasive, nonionizing imaging modality that can be used to measure oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (Hb) concentrations within a tissue. Since first being reported by Jobsis¹ in 1977 for noninvasive monitoring of cerebral and myocardial oxygen sufficiency, this technique has developed to the point that it enables absolute quantification of cerebral hemoglobin concentrations.

It has been known since the early 1900s² that when a particular area of the brain is activated, it receives an increase in blood flow. The increase provides a focal increase in HbO,

as the volume of the provided HbO is greater than the increase in oxygen consumption.³ It is this hemodynamic change that is of interest and can be used to provide information regarding brain activation by visual stimuli.

There is a unique optical window between 650 and 950 nm where visible and near-infrared (NIR) light can be used to image the brain.⁴ Light with a wavelength lower than 650 nm is strongly absorbed by hemoglobin, whereas light with a wavelength higher than 950 nm is mainly absorbed by water. When NIR light is passed through a tissue, it can be absorbed, reflected, or scattered. The amount of scattered light must be greater than the amount of light absorbed by the tissue, to allow for the signal to be detected.

One method of obtaining absolute measurements of Hb and HbO concentrations within a tissue is to use a frequency domain (FD) oximeter and to employ the frequency-domain multidistance (FDMD) method. An FD oximeter provides measurements of the intensity and the phase of the emergent light at two selected wavelengths, allowing for differentiation between Hb and HbO concentrations, as the absorption spectra of these two compounds is markedly different. By using multiple emitters at set distances from a detector, different depths of the tissue can be probed. As the emitter/detector distances are known and the intensity and phase of the emergent signal can be measured, the absolute absorption (μ_a) and reduced scattering coefficient (μ_s') can therefore be calculated. It is the absolute absorption coefficient that makes this method different from many others and allows for the quantification of absolute Hb and HbO concentrations. Other systems make assumptions regarding the path length of the light and can therefore not quantify Hb and HbO. An example of this would be the continuous wave (CW) oximeter, which measures changes in intensity but does not measure phase. As phase is not measured, assumptions have to be made regarding the path of the light within the tissue, which allows only relative changes to be measured.

NIR spectroscopy (NIRS) has been used to assess relative changes in hemoglobin concentration within the visual cortex.^{5–7} However, there are no reports of the use of the quantitative FDMD method to measure absolute levels and functional changes over the visual cortex in adults. Although relative shifts in Hb and HbO levels may be useful, they do not give information about the baseline status, the magnitude of a change, or the potential clinical significance. From a clinician's viewpoint, baseline measures may indicate whether there is ischemia within a tissue and whether the level of ischemia changes over time. The use of absolute values also indicates the magnitude of a change and potential importance of a change. An increase of 1 unit would have a profoundly different meaning if the starting point were 1 compared with 100. Thus, absolute values of Hb and HbO allow for this comparison between measurements and can give valuable clinic input to the level of oxygen within a tissue.

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In this study, our purpose was to use NIRS to quantify functional changes over the visual cortex. In doing so, we assessed the feasibility of using a commercially available FD oximeter for quantification of the absolute values and detection of the subtle changes in hemoglobin concentrations over the visual cortex during the presentation of a visual stimulus.

MATERIALS AND METHODS

Instrumentation

The oximeter used to measure cerebral tissue hemoglobin concentrations (OxiplexTS; ISS Inc., Champaign, IL) is a dual-wavelength (690 and 830 nm), frequency-domain oximeter, modulated at a radio frequency of 110 MHz with a cross-correlation frequency of 4 kHz. It measures the emergent light from the emitters, after it has passed through the tissue and converts the light intensity values into the absorption and reduced scattering coefficients of the tissue. Its probe consists of a flat, flexible plastic sensor in which small prisms are placed to refract light toward the scalp from incoming perpendicular fiber optics. The fibers are linearly arranged with a 3-mm detector fiber optic bundle at one end and four pairs of 400-μm core diameter fiber optics arranged at multiple distances from the detector. The distance between the sources and the detector ranges between 1.93 and 3.51 cm. Each emitting source contains a pair of fiber optics emitting NIR light of 690 and 830 nm (Fig. 1).

Knowing the absorption coefficient and using the FDMD method,⁸ absolute levels of Hb, HbO, and total hemoglobin (tHb) and the percentage of oxygen saturation (StO₂%) can be obtained. Below is a brief description of the FDMD method (for comprehensive coverage see Hueber et al.⁹ and Fantini et al.¹⁰).

FDMD Method

The three measurements recorded by the oximeter are the amplitude of the light intensity (AC), the average light intensity (DC), and the phase (φ). With increasing distance from a point source of light, AC and DC decrease, whereas the phase increases. The rate of change in AC, DC, and phase with respect to distance is an optical property of the medium.⁹ Knowing these values at different source-detector separations allows for the slopes of AC, DC, and phase to be calculated (S_{AC}, S_{DC}, and S_φ). Absorption (μ_a) and reduced scattering (μ_s), coefficients can be calculated from the slope of S_φ and either S_{AC} or S_{DC}. As S_{AC} is less affected by room lighting, S_{AC} and S_φ are used. S_{AC} corresponds to the slope of ln(r²AC) plotted against r, and S_φ denotes the slope of phase against r, where r is the source/detector separation. The following equations can be used to calculate the absorption and reduced scattering coefficients¹⁰:

$$\mu_a = \frac{\omega}{2\nu} \left(\frac{S_\phi}{S_{ac}} - \frac{S_{dc}}{S_\phi} \right) \quad \mu_s = \frac{S_{ac}^2 - S_\phi^2}{3\mu_a}$$

In these equations, ν is the speed of light in the tissue and ω is the angular modulation frequency. The concentration of Hb and HbO can be calculated, by using the absorption coefficient and the principles of the Beer-Lambert law:

$$[\text{oxy-Hb}] = \frac{\epsilon_{\text{deoxy-Hb}}(\lambda_2)[\mu_a(\lambda_1) - B(\lambda_1)] - \epsilon_{\text{deoxy-Hb}}(\lambda_1)[\mu_a(\lambda_2) - B(\lambda_2)]}{\ln(10)[\epsilon_{\text{deoxy-Hb}}(\lambda_2)\epsilon_{\text{oxy-Hb}}(\lambda_1) - \epsilon_{\text{deoxy-Hb}}(\lambda_1)\epsilon_{\text{oxy-Hb}}(\lambda_2)]}$$

$$[\text{deoxy-Hb}] = \frac{\epsilon_{\text{oxy-Hb}}(\lambda_1)[\mu_a(\lambda_2) - B(\lambda_2)] - \epsilon_{\text{oxy-Hb}}(\lambda_2)[\mu_a(\lambda_1) - B(\lambda_1)]}{\ln(10)[\epsilon_{\text{deoxy-Hb}}(\lambda_2)\epsilon_{\text{oxy-Hb}}(\lambda_1) - \epsilon_{\text{deoxy-Hb}}(\lambda_1)\epsilon_{\text{oxy-Hb}}(\lambda_2)]}$$

where ε is the extinction coefficient, λ represents the wavelength of light used, and B denotes the background absorption.⁹ Two other values that the oximeter computes are the total hemoglobin concentration (THC) and the percentage of oxygen saturation in tissue (StO₂), where THC = Hb + HbO, and StO₂ = HbO/THC × 100%.

Participants

There were seven participants (one man and six women) with an age range between 20 and 53 years. Each participant had light-pigmented hair or no hair in the relevant scalp areas. All had a monocular visual acuity of 6/6 Snellen or better, with no known ocular disease or abnormalities. This study was conducted in the Vision Sciences department of Glasgow Caledonian University, was approved by the institutional ethics committee, and complied with the Declaration of Helsinki. The details of the study were outlined and explained to each participant before written consent was obtained.

Visual Stimulation

We had performed pilot studies to select a visual stimulus that would strongly activate the visual cortex. Visually evoked potentials (VEPs) were recorded from three locations on the occipital scalp in 10 participants by using a range of temporal frequencies to both flicker and pattern-reversal stimuli. High-contrast pattern-reversal checkerboards with a temporal frequency of 7.5 Hz (15 reversals per second) elicited optimal VEP magnitudes, consistent with previous stimulus parameter studies.¹¹ VEP magnitudes in response to unpatterned flicker were more variable across participants and were not significantly larger than those in response to pattern reversal.

Procedure

The participant was comfortably seated 1 m from a computer screen. The oximeter's probe was attached over the right occipital cortex with the detector placed 1 cm above and 1 cm to the right of theinion. The probe

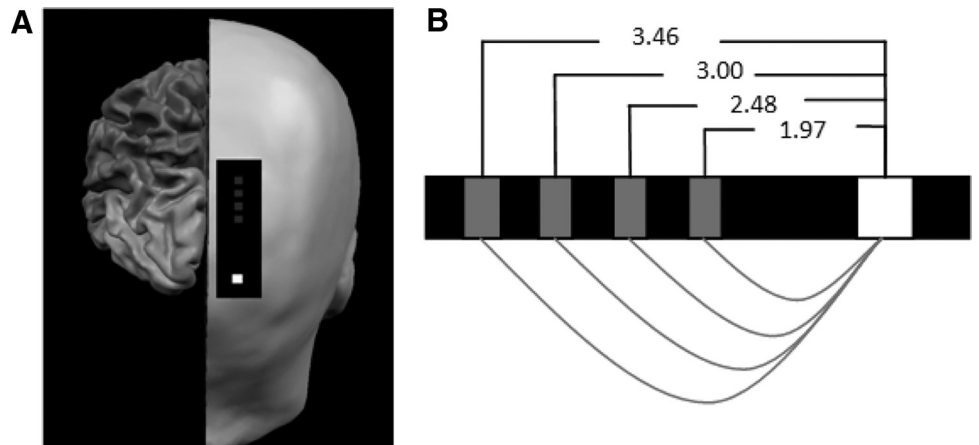


FIGURE 1. (A) Schematic of the oximeter probe, showing its placement in relation to the skull and occipital cortex. *White square:* the detector; *gray squares:* the four pairs of emitters. (B) A cross section of the probe showing the distances in centimeters from the center of the pairs of emitter to the center of the detector.

was then covered with a dark, light-excluding material and secured with an elastic headband. The participant was asked to maintain fixation on a small central dot and blink naturally throughout the experiment. An initial recording of approximately 1 minute was made while the viewer fixated a gray screen, to ensure that the hemoglobin concentration levels had reached a steady baseline. The time taken to turn each of the eight emitters on and off (source cycle time) was 20 ms. A reading was taken every second. Thus, each second of recorded data constituted an average of 50 cycles. The stimulus (reversing checkerboard, temporal frequency of 7.5-Hz, check width 15 minutes of arc, field size of $20.7^\circ \times 15.4^\circ$, and 90% contrast) was presented for 30 seconds and then replaced with a gray screen with the same mean luminance profile as the checkerboard, for a further 30 seconds. This cycle was repeated 10 times in a stimulus block. Each participant completed two stimulus blocks.

Analysis

The data recorded with the oximeter were exported in an ASCII text file and block averaged in a specially written macro (Excel 2007; Microsoft, Redmond, WA). The last 15 seconds of each 30-second window was analyzed, comparing the hemoglobin concentration levels when the stimulus was on and when it was off. The data were first assessed for each trial individually and then combined (statistical analysis performed in Excel 2003 and 2007; Microsoft).

RESULTS

All participants showed a significant change in each of the four measurement parameters, Hb, HbO, THC, and StO₂, when viewing the alternating checkerboard pattern compared with the levels when viewing the gray control screen. When the participant viewed the stimulus, HbO concentration increased ($P < 0.001$, $df = 13$, $t = 5.404$), Hb concentration decreased ($P < 0.001$, $df = 13$, $t = -4.637$), THC increased ($P < 0.001$, $df = 13$, $t = 5.758$), and StO₂ increased ($P < 0.001$, $df = 13$, $t = 5.701$).

When the alternating checkerboard was presented, there was an increase in HbO and a decrease in Hb, compared with the response to a control gray screen. This result was true of HbO for both individual stimulus blocks for each participant ($P < 0.05$). For Hb, 2 of the 14 blocks did not show a significant difference when the viewer examined single blocks but when averaged over the two blocks per participant a significant difference was seen in every participant ($P < 0.01$). Means, standard deviations, and the range of data for the HbO and Hb concentration levels are shown in Table 1.

The average HbO concentration, while the participant viewed the reversing checkerboard pattern, was $26.8 \pm 3.9 \mu\text{M}$ compared with $25.9 \pm 3.9 \mu\text{M}$ when the gray screen was shown. This result equated to an HbO concentration increase of 3.36%. Hb concentration decreased by 1.6% when the reversing checkerboard was viewed.

On visual inspection of the data, it was apparent that there was a definite pattern within the HbO data. For 64% of blocks, the stimulus presentation was always followed by an increase

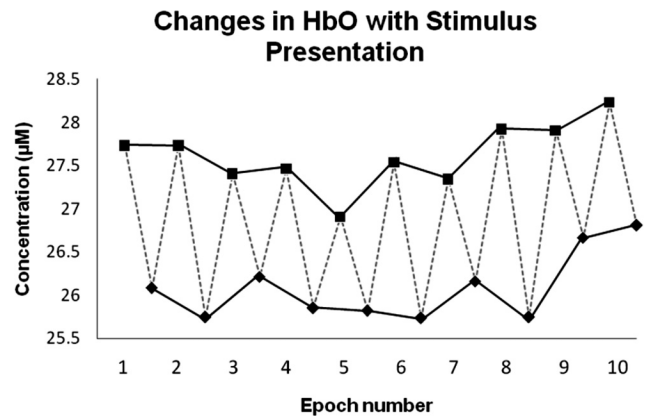


FIGURE 2. Changes in HbO with stimulus presentation for an individual participant are illustrated for one block. (■) The mean value for the final 15 seconds of each epoch during the pattern reversal checkerboard stimulation; (◆) the mean value for the final 15 seconds of each epoch containing a gray screen. *Dashed gray line:* change over time.

in HbO for each of the 10 epochs, and when the control gray screen was observed, there was a decrease from the previous level for every epoch. The remaining 34% of blocks required the results to be averaged over two blocks before this pattern was observed. This is pattern is illustrated in Figure 2.

Although there was a decrease in mean Hb with the checkerboard stimulus, the result was not a mirror image of the HbO response. Only 29% of blocks displayed a decrease in Hb during each epoch within a block. When the two blocks were averaged, only 43% followed this pattern.

The time course for the change in HbO was also assessed. From the onset of the checkerboard pattern, the HbO concentration steeply increased before becoming significantly different ($P = 0.01$, $df = 13$, $t = -2.89$) from the onset concentration at 7 seconds. After 10 seconds, the concentration increased more gradually, to reach a maximum of $27.0 \mu\text{M}$ after 26 seconds. The small increase between 10 and 26 seconds did not reach significance ($P > 0.01$, $df = 13$, $t = -2.50$). When the checkerboard was replaced with the gray screen, it took 8 seconds before there was a significant decline in hemoglobin concentration ($P = 0.002$, $df = 13$, $t = 3.74$); a more rapid decrease then began.

Although the Hb response was smaller in amplitude, the time course approximately mirrored that of the HbO. After onset of the stimuli, it took 7 seconds for Hb to significantly decrease ($P = 0.01$, $df = 13$, $t = 3.10$) from the starting level, with a minimum of $18.62 \mu\text{M}$ at 14 seconds. After stimulus offset, a constant upward trend was observed in the first 7 seconds.

The mean change with time in HbO and Hb concentrations is illustrated for all epochs, trials, and participants. The data have been normalized to allow the relationship between Hb and HbO to be viewed (Fig. 3).

TABLE 1. Concentration of HbO and Hb in the Right Occipital Cortex with and without Visual Stimulation

	HbO		Hb	
	Pattern Reversal (15–30 s after Onset)	Gray Screen (15–30 s after Offset)	Pattern Reversal (15–30 s after Onset)	Gray Screen (15–30 s after Offset)
Trial 1	26.8 ± 4.3 (19.9–30.8)	25.9 ± 4.3 (19.2–30.5)	19.0 ± 2.6 (14.8–22.3)	19.3 ± 2.6 (15.0–22.3)
Trial 2	26.7 ± 3.8 (20.8–29.9)	26.0 ± 3.8 (20.1–29.7)	18.5 ± 2.0 (15.5–21.4)	18.8 ± 2.0 (15.8–21.5)
Trials 1 and 2	26.8 ± 3.9	25.9 ± 3.9	18.8 ± 2.3	19.1 ± 2.3

Data are expressed as mean micromolar \pm SD (range).

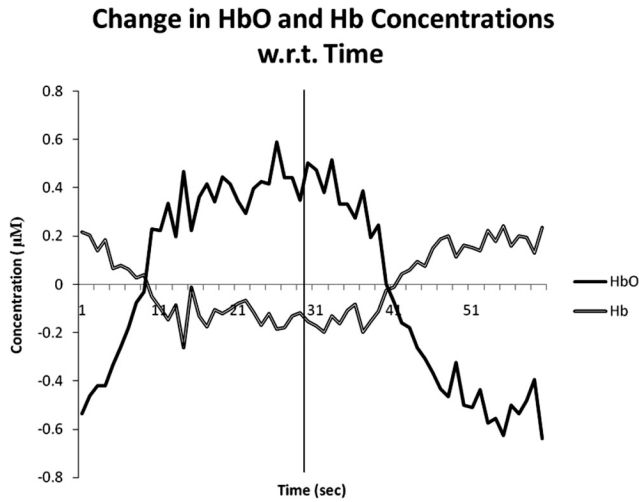


FIGURE 3. Normalized data demonstrating the changes in Hb and HbO over time. The onset of alternating checkerboards was at time 0. Offset, replaced by a uniform gray screen, was at 30 seconds (vertical line).

DISCUSSION

Using the FDMD method of NIRS optical brain imaging with a stimulus known to produce strong responses in the visual cortex, we found an increase in the absolute concentration of cerebral HbO and a decrease in cerebral Hb, compared with levels recorded when the participants viewed a control gray screen.

The absolute HbO concentration of $26.8 \pm 3.9 \mu\text{M}$ during checkerboard stimulation decreased to $25.9 \pm 3.9 \mu\text{M}$ when the gray screen was viewed. There are no similar studies of the physiologic changes in HbO in the visual cortex. However, Gatto et al.¹² used the FDMD method on the forehead and reported an HbO concentration of $24.9 \pm 9.1 \mu\text{M}$. This finding is similar to the mean HbO concentration found in our study when the control gray screen was viewed.

The lag and duration of the hemodynamic response depends on many factors, such as the length of the stimulation and the interstimulus interval.¹³ Takahashi et al.,¹⁴ using a continuous-wave system, presented an alternating checkerboard for 20 seconds followed by a 50-second rest. They reported that the relative increase in HbO concentration reached a plateau after 10 seconds, which is a time course similar to that in the present results.

When neurons in the visual cortex respond to visual stimulation, there is a resultant increased demand for oxygen. The relationship between the neuronal and hemodynamic response is known as neurovascular coupling. Increases in oxygen demand trigger a resultant increase in cerebral blood flow (CBF) giving a local increase in HbO concentration.¹⁵ As a greater volume of HbO is flushed into the area, the concentration of Hb decreases.

In the present study, the Hb levels decrease with visual stimulation, demonstrating that the increases in blood flow overcompensates for the increase in oxygen consumption by the activated cortex. The Hb decrease is smaller, however, than the HbO increase in response to checkerboard stimulation. (The average HbO increase was 3.4%, whereas Hb decreased by only 1.6% during visual stimulation.) Similar results were found by Wolf et al.¹⁶ who reported that Hb decreased less than HbO increased. They used a one-compartment model to describe their results, in which increased neuronal activity leads to an increase in the oxygen consumption. Their results

also showed that the increase in cerebral blood flow is instantaneous, with no preceding increase in Hb, which also matches our results.

Evaluation of the integrity of the hemodynamic response in the brain may be a valuable tool for quantitative assessment and monitoring of conditions that affect the vasculature of the occipital cortex, such as stroke, trauma, and migraine, to name but a few. It has been shown in an animal model that the neurovascular coupling relationship can change in regions such as the penumbra of ischemic stroke, giving an abnormal vascular response to neuronal activity.¹⁷ The present study reports the hemodynamic response in a healthy human cohort and provides a standard response for clinical comparisons.

Pigmentation within the hair and also in the hair follicle strongly absorbs NIR wavelengths, resulting in less light transmission. It has been reported that hair pigmentation can affect NIRS.^{18,19} Our data support these reports. Comparing the pair of emitters closest to the detector for a blond-haired participant with those in one with brown hair, the blond-haired participant gave AC levels of 450 to 500, whereas the brown-haired subject had an AC level of between 70 and 120. Therefore, more gain was needed to amplify the light detected from brown-haired participants. Participants who exceeded the acceptable detector sensitivity range (30%–90%)²⁰ were excluded from the study; thus, only the participants with blond hair could be included.

We also observed that hair density, as reflected by the width of the participants' hair parting influenced the quality of the signal. Comparing two participants of similar age and hair coloring, we noted that one participant with lower hair density gave a clear, noise-free recording, while readings from the other participant with dense hair were outside the acceptable limits. Therefore, hair density also appears to be a contributory factor to the recording of the functional hemodynamic response, probably because it is more difficult with dense hair to avoid having the hair cross the emitter or detector windows. Future work will entail the design and testing of a probe that uses the FDMD principles that can accommodate a greater range of hair density and pigmentation.

To conclude, it is evident from this study that oximetry (OxiplexTS; ISS) can be used to measure absolute oxyhemoglobin concentration changes over the visual cortex in response to a standard visual stimulus. The results open the possibility for further application of this technique in a clinical setting to assess the integrity of the blood supply to the visual cortex with a noninvasive and portable instrument.

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References

1. Jobsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science*. 1977;198:1264–1267.
2. Fulton JF. Observations upon the vascularity of the human occipital lobe during visual activity. *Brain*. 1928;51:310–320.
3. Fox PT, Raichle ME, Mintun MA, Dence C. Nonoxidative glucose consumption during focal physiologic neural activity. *Science*. 1988;241:462–464.
4. Toga AW, Mazziotta JC. *Brain Mapping: The Methods*. 2nd ed. San Diego, CA: Academic Press; 2002:142.
5. Griffin M, Prior D, Cooper CE, Wilkins AJ, Elwell CE. Near infrared spectroscopy as a non-invasive assessment of cortical abnormality in migraine. *Adv Exp Med Biol*. 2006;578:203–209.
6. Meek JH, Elwell CE, Khan MJ, et al. Regional changes in cerebral haemodynamics as a result of a visual stimulus measured by near infrared spectroscopy. *Proc R Soc Lond*. 1995;261:351–356.

7. Okubo K, Kusaka T, Isobe K, et al. Hemodynamic changes in the neonatal visual cortex by using near-infrared topography. *Int Congress Series*. 2002;1232:593-597.
8. Fishkin JB, Gratton E. Propagation of photon-density waves in strongly scattering media containing an absorbing semi-infinite plane bonded by a straight edge. *J Opt Soc Am A*. 1993;10:127-140.
9. Hueber DM, Franceschini MA, Ma HY, et al. Non-invasive and quantitative near-infrared haemoglobin spectroscopy in the piglet brain during hypoxic stress, using a frequency-domain multidistance instrument. *Phys Med Biol*. 2001;46:41-62.
10. Fantini S, Franceschini MA, Fishkin JB, Barbieri B, Gratton E. Quantitative determination of the absorption spectra of chromophores in strongly scattering media: a light-emitting diode based technique. *Appl Opt*. 1994;33:5204-5213.
11. Regan D. Assessment of visual acuity by evoked potential recording: ambiguity caused by temporal dependence of spatial frequency selectivity. *Vision Res*. 1977;18:439-443.
12. Gatto R, Hoffman W, Mueller M, et al. Frequency domain near-infrared spectroscopy technique in the assessment of brain oxygenation: a validation study in live subjects and cadavers. *J Neurosci Methods*. 2006;157:274-277.
13. Miezin FM, Maccotta L, Ollinger JM, Petersen SE, Buckner RL. Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. *Neuroimage*. 2000;11:735-759.
14. Takahashi K, Ogata S, Atsumi Y, et al. Activation of the visual cortex imaged by 24-channel near-infrared spectroscopy. *J Biomed Opt*. 2000;5:93-x.
15. Fox PT, Raichle ME. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci USA*. 1986;83:1140-1144.
16. Wolf M, Wolf U, Toronov V, et al. Different time evolution of oxyhemoglobin and deoxyhemoglobin concentration changes in the visual and motor cortices during functional stimulation: a near-infrared spectroscopy study. *Neuroimage*. 2002;16:704-712.
17. Schmitz B, Hoehn-Berlage M, Kerskens CM, Böttiger BW, Hossmann KA. Recovery of the rodent brain after cardiac arrest: a functional MRI study. *Magn Reson Med*. 1998;39(5):783-738.
18. Pringle J, Roberts C, Kohl M, Lekeur P. Near infrared spectroscopy in large animals: optical pathlength and influence of hair covering and epidermal pigmentation. *Vet J*. 1999;158:48-52.
19. Strangman G, Boas DA, Sutton JP. Non-invasive neuroimaging using near infrared light. *Biol Psychiatry*. 2002;52:679-693.
20. Oxiplex TS. *Manual*. Champaign, IL: ISS Inc., document 96-055, Rev. 3.10; March 2005.