Quantification of cerebral hemoglobin as a function of oxygenation using near-infrared time-resolved spectroscopy in a piglet model of hypoxia

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Abstract. Near-infrared spectroscopy (NIRS) has been used for measurement of cerebral hemoglobin (Hb) concentrations in neonates to study cerebral oxygenation and hemodynamics. We perform measurements by portable three-wavelength NIR time-resolved spectroscopy (TRS) in a piglet hypoxia model with various degrees of oxygenation to estimate the absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$) of the head. Measurements of absolute values of $\mu_a$ at three wavelengths enable estimation of Hb concentration and Hb oxygen saturation in the head ($SO_2$). However, there is a problem concerning which background absorption should be used to estimate Hb concentration in the head derived from $\mu_a$ at three wavelengths because it is different from a simple in vitro model. Therefore, we use two different background absorption values with the assumption that background absorption is due only to 85% (by volume) water or that background absorption is equal to absorption of the piglet head with blood exchange transfusion by fluorocarbon (FC), and we compared $SO_2$ measured by TRS with arterial Hb oxygen saturation ($SaO_2$) and sagittal sinus venous Hb oxygen saturation ($SvO_2$) measured by a co-oximeter at several inspired fractional $O_2$ ($FIO_2$) concentrations.

A number of approaches for measuring cerebral Hb concentration and oxygen saturation using several types of NIRS systems have been proposed. These approaches include (1) continuous wave spectroscopy (CWS), by which only the relative value of Hb can be estimated;6 (2) full spectral spectroscopy (FSS), by which the NIRS full spectrum of the NIR range can be measured;9–12 (3) time-resolved spectroscopy (TRS), by which the transit time of each photon through the tissue of interest can be measured;13–18 (4) phase-modulated spectroscopy (PMS), by which amplitude signals for phase,

1 Introduction

Near-IR spectroscopy (NIRS), which uses light in the NIR range (700 to 900 nm), enables detection of changes in the oxygenation state of hemoglobin (Hb) and water in biological tissues. Several studies have shown the usefulness of NIRS for noninvasive measurement of cerebral blood volume and oxygenation in infants.1–7

Key words: background absorption; cerebral hemoglobin concentration; cerebral hemoglobin oxygen saturation; fluorocarbon; hypoxia; near-infrared spectroscopy; newborn piglets; time-resolved spectroscopy.
intensity and depth of modulation after passage can be measured, and spatially resolved spectroscopy, by which the slope of light attenuation versus distance is determined at a distant point from the source.

A new TRS system that is portable and has a high data acquisition rate was recently reported. A TRS device enables quantitative analysis of light absorption and scattering in tissue using the photon diffusion theory. The absorption coefficient ($\mu_a$) represents the physiological state, particularly the Hb concentration and oxygen saturation, and the reduced scattering coefficient ($\mu_s'$) represents the structural change in tissue. However, for application to tissue measurements, there is the problem concerning how to determine the background absorption for estimation of Hb in the head. Hube et al. reported the use of fixed weighted averages of SaO$_2$ and SvO$_2$ for estimation of background tissue absorption using PMS. However, an exact background absorption has not been reported.

In this paper, we first investigate whether our TRS measurements could be used to accurately measure Hb concentration and oxygen saturation in an in vitro model such as the brain. Next, we apply this method to newborn piglets during hypoxic loading. In this experiment, we use two different methods to determine Hb concentration and oxygen saturation in the head using $\mu_a$ values measured at three wavelengths. One method uses a water-only background absorption to determine the Hb concentration, and we investigate the relationships of cerebral Hb oxygenation with arterial and sagittal sinus venous Hb oxygen saturation at various concentrations of inspired oxygen using a piglet hypoxic model. Another method uses background absorption from the piglet head with blood exchange by fluorocarbon (FC), and we reestimate cerebral Hb concentration for comparison to that with water-only background absorption calculations. Then the reliability of the instruments is evaluated. The results of optical path length obtained in this study were previously published.

2 Materials and Methods

2.1 In Vitro Experimental Protocol

To provide a model for in vivo measurement of Hb in the piglet brain, we filled a 1.000-mL glass beaker with 0.1-M phosphate buffer (pH 7.4) containing 0.7% (w/vol) dry yeast in 1% intralipid and glucose (2% w/vol) to produce a solution with a light scattering condition equivalent to that of blood-perfused brain tissue. A magnetic stirrer was used to prevent particle settling. The measurement receiver fiber was set at a distance of 30 mm from the light source fiber, and the fibers were immersed directly in the suspension. The oxygen content was also monitored at the same time with a conventional gas analyzer (ABL5, Radiometer, Copenhagen, Denmark). In the first stage of the experiment, human blood was added gradually to the beaker to achieve blood volume ranging from 0 to 100 $\mu$L, and the resulting changes in the reemission profiles were recorded. Each model was bubbled with oxygen. In the second stage of the experiment, we used a model solution with a 80-$\mu$L Hb suspension, and the oxygen concentration in the solution was reduced to simulate anoxia in the brain. A mixture of O$_2$ and N$_2$ gases was prepared and bubbled through the suspension to manipulate the oxygen saturation of Hb from 0 to 100%.

Once $\mu_a$ had been determined at three wavelengths, the oxyHb and deoxyHb concentrations were calculated from the absorption coefficients of oxyHb and deoxyHb using the same law as that described in Sec. 2.4 with the assumption that background absorption is due to only 100% (by volume) water and that the refractive index of water is 1.3.

2.2 Animal Study Protocol

Eleven newborn piglets, less than 48 h old and weighing 1.9 to 2.4 kg, were each anesthetized with an intramuscular injection of sodium pentobarbital (2 mg/kg). The umbilical vein and artery of each piglet were cannulated. After cannulation, each piglet was paralyzed with pancuronium bromide at an initial dose of 0.1 mg/kg followed by infusion at 0.1 mg/kg h$^{-1}$ and then anesthetized with fentanyl citrate at an initial dose of 10 $\mu$g/kg followed by infusion at 5 $\mu$g/kg h$^{-1}$. The animals were then intubated and mechanically ventilated with an infant ventilator. A 24-gauge cannula was inserted into the sagittal sinus through a burr hole in the anterior fontanel for venous blood sampling.

The umbilical artery was used for blood pressure monitoring and arterial blood sampling. Infusion of maintenance solution was continued at a rate of 4 mL/kg h$^{-1}$ via the umbilical vein. Respiration and acid-base balance were checked by arterial blood gas analysis at each step of FIO$_2$. A negative base excess lower than 5.0 mmol/L, which was caused by hypoxia, was corrected as much as possible by sodium bicarbonate infusion to maintain pH between 7.3 and 7.5, thus minimizing the shift in the Hb dissociation curve. The hematocrit of arterial blood was in the normal range, and mean arterial pressure was maintained above 60 mm Hg except near zero FIO$_2$. Arterial Hb oxygen saturation (SaO$_2$) and sagittal sinus venous Hb oxygen saturation (SvO$_2$) were measured using a blood co-oximeter (OSM-3, Radiometer, Copenhagen, Denmark). To obtain aerobic and anaerobic conditions, FIO$_2$ was changed by mixing pure nitrogen and oxygen gases with continuous monitoring by an oxygen electrode. We first set FIO$_2$ at 27% and then at 100% to obtain hyperaerobic conditions. Then, to obtain anaerobic conditions, FIO$_2$ was decreased in steps from 100 to 4%. The hair on the scalp of the parietal region was removed with an epilating agent. Two optical fibers were brought into contact with the skin on the head of each piglet. Throughout the experiment, rectal temperature was monitored and maintained between 38.0 and 39.0 °C using a heated-water mattress. At the end of the experiment, animals were sacrificed with intravenous pentobarbital (100 mg/kg). The protocols for animal care were in compliance with institutional guidelines.

2.3 Near-IR TRS System and Analysis

We used a portable three-wavelength TRS system (TRS-10, Hamamatsu Photonics K.K., Japan) and attached a probe to the head of each piglet with a light source fiber and light detector fiber separation of 30 mm. In the TRS system, a time-correlated single-photon-counting technique is used for detection. The system is controlled by a computer through a digital I/O interface consisting of a three-wavelength (761, 795, and 835 nm) picosecond light pulser (PLP) as the pulsed light source, a photon-counting head for single photon detec-
tion, and signal-processing circuits for time-resolved measurement. The PLP emits NIR light with a pulse duration of about 100 ps and an average power of at least 150 μW at each wavelength at a repetition of 5 MHz, and the input light power to the subject was around 20 μW because of the loss in an incident optical system of the TRS system.

The light from the PLP is sent to a subject by a source fiber with a length of 3 m, and the photon reemitted from the subject is collected simultaneously by a detector fiber bundle with a length of 3 m. The light source fiber was a graded index (GI) single fiber (GC200/250L, Fujikura, Japan) with a numerical aperture (NA) of 0.25 and a core diameter of 200 μm, and the light detector fiber was a bundle fiber with a diameter of 3 mm and NA of 0.21. Finally, a set of histograms of the photon flight time, which is called a reemission profile, is recorded. One temporal reemission profile includes 1024 time channels spanning about 10 ns with a time step of about 10 ps. In this study, the emerging light was collected over a period of 3 s to exceed at least 1000 photon counts at the peak channel of the reemission profiles, and the procedure was repeated 30 times for each measurement.

The instrumental response was measured with the input fiber placed opposite the receiving fiber through a neutral density filter. The instrumental response of the TRS system was assumed to be the background absorption value. The values at 761, 795, and 835 nm were estimated to be 0.1190, 0.1008, and 0.1260, respectively.

### Table 1 Absorption coefficients for oxyHb, deoxyHb, water, and absorption of the piglet head with blood exchange transfusion by FC.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>oxyHb (mM$^{-1}$ cm$^{-1}$)</th>
<th>deoxyHb (mM$^{-1}$ cm$^{-1}$)</th>
<th>Water (cm$^{-1}$)</th>
<th>Piglet Head with Blood Exchange Transfusion by FC (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>761</td>
<td>1.418</td>
<td>3.841</td>
<td>0.0272</td>
<td>0.1190</td>
</tr>
<tr>
<td>795</td>
<td>1.919</td>
<td>2.016</td>
<td>0.0214</td>
<td>0.1008</td>
</tr>
<tr>
<td>835</td>
<td>2.487</td>
<td>1.798</td>
<td>0.0358</td>
<td>0.1260</td>
</tr>
</tbody>
</table>

In these equations, $\varepsilon_\lambda$ is the extinction coefficient at $\lambda$ nm, and [oxyHb] and [deoxyHb] are the concentration.

First, the water absorption was subtracted from $\mu_\alpha$ at each of the three wavelengths, and then the values of oxyHb and deoxyHb were estimated using the least-squares fitting method. The absorption coefficients for oxyHb, deoxyHb, and water shown in Table 1 were used.

The cerebral total hemoglobin (total Hb) concentration and cerebral Hb oxygen saturation (SO$_2$) were calculated as follows:

$$[\text{total Hb}] = [\text{oxyHb}] + [\text{deoxyHb}],$$

$$\text{SO}_2 \ (\%) = \frac{[\text{oxyHb}]/([\text{oxyHb}] + [\text{deoxyHb}]) \times 100,}$$

where the square brackets indicate concentration.

### 2.5 Estimation of Absolute Values of oxyHb and deoxyHb with Absorption of the Piglet Head with Blood Exchange Transfusion by FC

For measurement of background absorption of the head without Hb, we used a piglet for blood exchange transfusion by FC. First, partial blood exchange was done by saline and then blood was replaced with perfluorobutylamine emulsion (FC43 Emulsion, Green Cross Corp., Japan) as an oxygen carrier. We used 500 mL of FC and the hematocrit was lowered to 0.5%. After replacing the blood with FC, oxygenated head TRS measurements were done while the piglet breathed 100% O$_2$. The EEG showed normal activity during the period of ventilation with 100% O$_2$. We used the $\mu_\alpha$ of piglet head with blood exchange transfusion by FC at three wavelengths as the background absorption value. The values at 761, 795, and 835 nm were estimated to be 0.1190, 0.1008, and 0.1260,
respectively (Table 1). The following equations were used for estimation of oxyHb and deoxyHb concentrations:

\[
\mu_a(761\text{ nm}) = \varepsilon_{761\text{ nm}}^{\text{oxyHb}} [\text{oxyHb}] + \varepsilon_{761\text{ nm}}^{\text{deoxyHb}} [\text{deoxyHb}] + \mu_a(761\text{ nm}),
\]

\[
\mu_a(795\text{ nm}) = \varepsilon_{795\text{ nm}}^{\text{oxyHb}} [\text{oxyHb}] + \varepsilon_{795\text{ nm}}^{\text{deoxyHb}} [\text{deoxyHb}] + \mu_a(795\text{ nm}),
\]

\[
\mu_a(835\text{ nm}) = \varepsilon_{835\text{ nm}}^{\text{oxyHb}} [\text{oxyHb}] + \varepsilon_{835\text{ nm}}^{\text{deoxyHb}} [\text{deoxyHb}] + \mu_a(835\text{ nm}).
\]

In these equations, \(\mu_a(\lambda, \text{nm})\) is the background absorption derived from the piglet head with blood exchange transfusion by FC. First, the absorption of the head with blood exchange transfusion by FC (abs of the head with BET by FC) was subtracted from \(\mu_a\) at each of the three wavelengths, and then the values of oxyHb and deoxyHb were estimated using the least-squares fitting method.

2.6 Statistical Analysis

A Statview J 4.5 package for the Macintosh computer was used for statistical analysis. Values obtained at different levels of \(\mathrm{FiO}_2\) were compared from values at \(\mathrm{FiO}_2\) of 21% by Wilcoxon's signed-rank test. The level of statistical significance was set at a probability of \(p<0.05\) for all tests. Results at \(\mathrm{FiO}_2\) levels in the range 10 to 100% were obtained from measurements in 11 piglets, results at \(\mathrm{FiO}_2\) of 8 and 6% were obtained from 10 piglets and results at \(\mathrm{FiO}_2\) of 4% were obtained from 8 piglets. Values are expressed as means ± standard deviation (SD).

3 Results

3.1 In Vitro Experiment Results

Figure 1(a) shows the relationship between the actual blood volume and total Hb concentration of the model as measured by TRS. The relationship between the actual blood volume \((X)\) and Hb concentration measured by TRS \((Y)\) was \(Y=0.843X+2.21\) \((r=0.998, P<0.001)\). We can see that the relationship between these two parameters is linear over the experimental range of Hb concentrations. Figure 1(b) shows the relationship between the values of Hb oxygen saturation in the 80-\(\mu\)M Hb suspension measured by a conventional gas analyzer and by TRS. The relationship between values of Hb oxygen saturation measured by a conventional gas analyzer \((X)\) and by TRS \((Y)\) was \(Y=0.960X+0.889\) \((r=0.991, P<0.001)\).

In Fig. 2, the values of \(\mu_a'\) measured concurrently at corresponding \(\mathrm{FiO}_2\) levels are plotted against \(\mathrm{FiO}_2\). At \(\mathrm{FiO}_2\) of 21%, the values of \(\mu_a'\) of the newborn piglet brain at 761, 795, and 835 nm were estimated to be 8.70±1.23, 8.33±0.91, and 8.24±1.11/cm, respectively. There were no significant differences between the values of \(\mu_a'\) for each \(\mathrm{FiO}_2\) level in the range of 4 to 100% at all three wavelengths.

In Fig. 3, the values of \(\mu_a\) measured concurrently at corresponding \(\mathrm{FiO}_2\) levels are plotted against \(\mathrm{FiO}_2\). The values of \(\mu_a\) at 761 and 795 nm at \(\mathrm{FiO}_2\) levels below 15% were higher than the values at \(\mathrm{FiO}_2\) of 21% at each wavelength. When \(\mathrm{FiO}_2\) was decreased from 21 to 15%, the values of \(\mu_a\) at 761 and 795 nm increased from 0.227±0.021 to 0.259±0.024/cm and from 0.189±0.016 to 0.203±0.013/cm, respectively. The values of \(\mu_a\) at 835 nm at \(\mathrm{FiO}_2\) levels below 10% were higher than those at an \(\mathrm{FiO}_2\) level of 21%. When \(\mathrm{FiO}_2\) was decreased from 21 to 10%, the value of \(\mu_a\) at 835 nm increased from 0.223±0.020 to 0.251±0.025/cm.

3.2 Values of \(\mu_a'\) and \(\mu_a\)

3.3 Concentration of oxyHb and deoxyHb with Water-Only Background Absorption

As shown in Fig. 4(a), the relationship between oxyHb and deoxyHb changed reciprocally in a mirror-image manner.
when \( F_{\text{I O}_2} \) was changed. Total Hb showed an increase of 31.3\% when \( F_{\text{I O}_2} \) was decreased from 21 to 6\%, indicating that cerebral blood volume increased during hypoxia. The values of \( \text{oxyHb}, \text{deoxyHb}, \) and \( \text{total Hb} \) at \( F_{\text{I O}_2} \) of 21\% were 53.5\pm8.1, 33.4\pm5.8, and 86.9\pm8.1 \( \mu \text{M} \), respectively.

In Fig. 5(a), levels of \( \text{SO}_2, \text{SaO}_2, \) and \( \text{SvO}_2 \), which were measured concurrently at corresponding \( F_{\text{I O}_2} \) levels, are plotted against \( F_{\text{I O}_2} \). At \( F_{\text{I O}_2} \) of 21\%, the mean \( \text{SO}_2 \) of newborn piglets was calculated to be 61.5\pm6.3\%. The values of \( \text{SaO}_2 \) and \( \text{SvO}_2 \) were simultaneously determined to be 92.1\pm3.76\% and 40.1\pm6.4\%, respectively. The contributions of \( \text{SaO}_2 \) and \( \text{SvO}_2 \) to \( \text{SO}_2 \) were 41.1\% and 58.9\%, respectively. However, values of \( \text{SO}_2 \) at \( F_{\text{I O}_2} \) in the range of 10 to 4\% were higher than those of \( \text{SaO}_2 \).

The \( \text{SaO}_2 \) values also showed a positive linear relationship with \( \text{SO}_2 \) [Fig. 6(a)]. The relationship between \( \text{SaO}_2 \) and \( \text{SO}_2 \) was \( \text{SO}_2 = 37.9 \times 10^{-2} \times \text{SaO}_2 + 27.5 \) \( (r = 0.862, P < 0.001) \). From this regression equation, the values of \( \text{SaO}_2 \) and \( \text{SO}_2 \) were estimated to be 25.5\pm7.3, 18.6\pm5.8, and 44.1\pm7.4 \( \mu \text{M} \), respectively. Here, #, \( P < 0.001 \); *, \( P < 0.01 \); and $, \( P < 0.05 \), indicate significant differences from values at \( F_{\text{I O}_2} \) of 21\% using Wilcoxon’s signed-rank test.
and \( \text{SO}_2 \) was \( \text{SO}_2 = 69.3 \times 10^{-2} \times \text{SvO}_2 + 31.8 \) \((r=0.841, \ P < 0.001)\).

### 3.4 Concentration of oxyHb and deoxyHb with Absorption of the Piglet Head with Blood Exchange Transfusion by Fluorocarbon

As shown in Fig. 4(b), the relationship between oxyHb and deoxyHb changed reciprocally in a mirror-image manner when \( \text{FiO}_2 \) was changed. The values of oxyHb, deoxyHb, and total Hb at \( \text{FiO}_2 \) of 21% were 25.5±7.3, 18.6±5.8, and 44.1±7.4 \( \mu \text{M} \), respectively. The values of oxyHb, deoxyHb, and total Hb are significantly lower at each \( \text{FiO}_2 \) level than those in the case of water-only background absorption.

In Fig. 5(b), levels of \( \text{SO}_2 \), \( \text{SaO}_2 \), and \( \text{SvO}_2 \), which were measured concurrently at corresponding \( \text{FiO}_2 \) levels, are plotted against \( \text{FiO}_2 \). At \( \text{FiO}_2 \) of 21%, the mean \( \text{SO}_2 \) of newborn piglets was calculated to be 57.6±12.3%. The contributions of \( \text{SaO}_2 \) and \( \text{SvO}_2 \) to \( \text{SO}_2 \) were 33.7 and 66.3%, respectively. \( \text{SO}_2 \) values were between those of \( \text{SaO}_2 \) and \( \text{SvO}_2 \) at all levels of \( \text{FiO}_2 \); \( \text{SO}_2 \) values are not significantly different from \( \text{SO}_2 \) values using the water-only background at \( \text{FiO}_2 \) in the range of 15 to 100%, but the values are lower than the values using the water-only background at \( \text{FiO}_2 \) in the range of 4 to 12%.

The \( \text{SaO}_2 \) values also showed a positive linear relationship with \( \text{SO}_2 \) [Fig. 6(b)]. The relationship between \( \text{SaO}_2 \) and \( \text{SO}_2 \) was \( \text{SO}_2 = 59.0 \times 10^{-2} \times \text{SaO}_2 + 4.9 \) \((r=0.843, \ P < 0.001)\).

### 4 Discussion

Cerebral Hb content and Hb oxygen saturation in infants have already been estimated using NIRS in several studies. However, the values estimated in those studies cannot be compared easily because the same phantom models, animal models, and experimental protocols were not used in those studies. Although the ultimate purpose of this study was to estimate \( \text{SO}_2 \) and Hb content in infants, cerebral Hb content and \( \text{SO}_2 \) have already been estimated by NIRS in previous studies using an experimental animal model, i.e., the newborn piglet, which approximates infants. The NIRS used in those studies can be divided into three categories: (1) FSS (Refs. 11 and 12), (2) PMS (Refs. 20, 22, 23, 25, and 31), and (3) TRS (Ref. 17).
Therefore, we used newborn piglets with hypoxia for our study to investigate the validity of NIR TRS. This model consists of multilayers including the scalp, skull, cerebrospinal fluid (CSF) layer, gray matter, and white matter. The optical properties are not those of only the brain but the average of those of the multiple layers.

Kienle et al.\textsuperscript{32,33} investigated analytical solutions to the two-layered optical phantom model using the PMS method. They demonstrated that a superficial layer of 4 mm in thickness (or less) will have only a small influence on the accuracy of the measured optical properties of an underlying thick layer. In the piglets used in our study, the thickness of the scalp and skull layer measured postmortem were about 1.5 and 1.5 mm, respectively, similar to values obtained in an earlier study.\textsuperscript{24,25} Huber et al.\textsuperscript{24} and Fantini et al.\textsuperscript{25} concluded that in the presence of a relatively thin ($\leq 4$ mm) scalp/skull layer (as in the case of the neonatal piglet), those absolute values of Hb concentration and oxygen saturation should be actually representative of the brain. In contrast, the significantly thicker tissue inhomogeneities found in the adult human head have an effect on the absolute optical measurements.

Furthermore, with regard to the effect of the CSF layer on photon migration, Fukui et al.\textsuperscript{34} estimated light properties from results of Monte Carlo simulation using the neonatal head model. In their model, the thicknesses of the scalp, skull, and CSF layer were about 2, 2, and 1 mm, respectively, almost the same as the values in our piglet model. A photon that has penetrated into the white matter can be identified by a detector 10 mm from the source. The partial DPF for the white matter proportionally increased with the increase in source-detector spacing, whereas that for the gray matter was almost constant at source-detector spacing greater than 30 mm. At source-detector spacing of 30 mm, the DPF was almost 4.3 and the values of partial DPF of gray matter and white matter were 1.3 and 0.7, respectively. Fukui et al.\textsuperscript{34} reported that the intensity sensitive region in the neonatal brain is confined to the gray matter, but the spatial sensitivity profile penetrates into the deeper region of the white matter. Therefore, our TRS measurements of the piglet head enable estimation of Hb of the brain, which is a multilayer structure, and the contribution of the brain to the measurement signal is estimated to be approximately 50%. If the contributions of the scalp, skull, and CSF layers of the measurement signal are assumed to be 10, 20, and 20\%, respectively,\textsuperscript{35} and $\mu_a$ values of the scalp, skull, and CSF at 795 nm are assumed to be 0.18, 0.16, and 0.04/cm, respectively,\textsuperscript{34} the $\mu_a$ of the brain is estimated to be 0.24/cm. This value is lower than that of the human neonatal brain gray or white matter.\textsuperscript{32}

There were no significant differences between the values of $\mu'_s$ at each wavelength for $F_{O_2}$ levels in the range of 4 to 100%. These results are similar to those obtained in a study by Zhang et al.,\textsuperscript{36} showing that scattering changes detected by a frequency-domain oximeter were associated only with asphyxia and death. Yamashita et al.\textsuperscript{37} reported the results of a preliminary study on light scattering in the piglet brain by using TRS. Their results showed that the values of $\mu'_s$ in piglet brains were around 1.3 mm$^{-1}$ in a state of normoxemia and that the value of $\mu'_s$ showed a notable decrease after death. Tissue edema and structural changes occur during severe hypoxia, particularly at and after death, and values of $\mu'_s$ are thought to change only during structural changes in tissue due to cerebral energy failure. Therefore, there were no sig-

![Fig. 7](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics)
significant differences between the values of $\mu'_s$ during hypoxia in this study.

The values $\mu'_s$ at 761 and 795 nm at $F_{I2O}$ levels below 15% were significantly higher than those at $F_{I2O}$ of 21% at each wavelength because deoxyHb increased during hypoxia. The values of $\mu'_s$ at 835 nm at $F_{I2O}$ levels below 10% were higher than those at an $F_{I2O}$ of 21%. This is because of an increase in both oxyHb and deoxyHb absorption, indicating an increase in cerebral blood volume during hypoxia.

In this paper, at levels of $F_{I2O}$ in the range of 10 to 4%, the values of $SO_2$ using a water-only background were higher than those of $SaO_2$ [Fig. 4(a)]. These results are thought to be mainly due to the effect of background absorption in tissue. In the brain, Hb, water, and cytochrome $c$ oxidase are known chromophores in the NIR region, but the concentrations vary between individuals and the in vivo absorption spectra of cytochromes are difficult to measure precisely. It is also likely that there are other important chromophores, such as fats, that make a significant contribution to non-Hb absorption. Indeed, Hueber et al. reported that using the frequency-domain multidistance method in the piglet head, $SO_2$ is greater than $SaO_2$ under the condition of extreme hypoxia with the assumption of a water-only background.

The estimated background tissue absorption with the assumption that $SO_2$ is equivalent to a mixture of 50% $SaO_2$ and 50% $SvO_2$ during normoxia and mild hypoxia to improve the estimation of brain $Hb$.

In this paper, we estimated the background absorption from the piglet head with blood exchange transfusion by FC. Using this absorption, the Hb values were lower than those using a water-only background, especially at a low level of $F_{I2O}$. The $SO_2$ values obtained using abs of the head with BET by FC were between the values of $SaO_2$ and $SvO_2$. These values were more accepted from a physiological point of view.

The mean $SO_2$ in 11 newborn piglets at $F_{I2O}$ of 21% was calculated to be 58% using FC $F_{I2O}$ exchange piglet head absorption. The values of $SaO_2$ and $SvO_2$ were simultaneously determined to be 92 and 40%, respectively. The contributions from arterial blood and venous blood were estimated to be 34 and 66%, respectively. The ratio of the contribution of arterial blood to that of venous blood is almost the same as the ratios reported by Brun et al. and Kusaka et al. Thus, the validity of measurement in vivo can be verified, even in an inhomogenous piglet head.


