Journal of Biomedical Optics

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Abstract. Background and aim: One source of error with near-infrared spectroscopy (NIRS) is the assumption that the measured tissue is optically homogeneous. This is not always the case. Our aim is to assess the impact of tissue homogeneity (TH) on the precision of NIRS measurements in neonates. Methods: On 36 term and 27 preterm neonates at least five 1-min measurements are performed on each subject using the OxiplexTS. The sensor position is slightly changed before each measurement while assessing TH. The precision for cerebral tissue oxygenation saturation (StO_2) and total hemoglobin concentration (tHb) are calculated by repeated measures analysis of variance. Results: The mean StO_2 is not significantly different between term and preterm infants. The mean tHb is significantly lower in preterm infants ($p < 0.01$). With increasing TH, the precision of StO_2 increase from 5.6 to 4.6% for preterm and from 11.0 to 2.0% for term infants; the precision of tHb increases from 10.1 to 7.5μM for preterm and from 16.4 to 3.5μM for term infants. The precision for StO_2 is higher in term than in preterm infants. The precision for tHb shows no significant difference between the two groups. Conclusions: The precision of NIRS measurements correlates with tissue homogeneity. [DOI: 10.1117/1.3570303]

Keywords: within-infant variations; between-infant variations; term infants; preterm infants; reproducibility.

Paper 10150RR received Mar. 22, 2010; revised manuscript received Feb. 28, 2011; accepted for publication Mar. 4, 2011; published online Apr. 21, 2011.

1 Background

Neonatal brain injury as a result of perinatal hypoxia-ischemia remains a common cause of morbidity and mortality in infants.[1,2] To allow prevention or early intervention, continuous cerebral monitoring is important to detect cerebral aberrations in term and preterm neonates undergoing intensive care.[3–5]

Near-infrared spectroscopy (NIRS) is a continuous and also noninvasive method to measure regional changes in cerebral tissue oxygenation and perfusion at the bedside.[6,7] Thus, NIRS is suitable for cerebral monitoring. But until now it has rarely been used in clinical settings. This could be due to the partially challenging application and to the lack of sufficient precision necessary to distinguish normal from pathological conditions.[8,9]

In a previous study performed by Soerensen and Greisen,[10] the within-infant precision of tissue oxygenation saturation (StO_2) for single measurements was judged to be insufficient. Using the NIRO 300 (Hamamatsu Photonics, Hamamatsu, Japan) on 37 preterm infants (mean gestational age 28 weeks), they performed between three and eight measurements on each infant. The sensor was replaced before each measurement, presuming that there are no regional differences in cerebral oxygenation. The precision of the measurements, defined as the square root of the within-subject variation, was found to be 5.2%. Because of this high value Soerensen and Greisen[10] concluded that it is unfeasible to perform StO_2 measurements clinically.

One cause for the dissatisfying precision could have been the NIRS device used for this study. The NIRO 300 is a continuous-wave spectroscopy device. To obtain absolute StO_2 and total hemoglobin concentration (tHb) values, a multidistance model with three detectors and one light source is applied. The tissue below the sensor is assumed to be flat, semi-infinite, and homogeneous, and the light scattering to be at least ten times larger than absorption. The first two assumptions can be fulfilled by carefully choosing the measurement area. However, inhomogeneity may occur originating from superficial factors, such as hair, moles, blood vessels and dirt, or from deeper structures (e.g., larger blood vessels and gyri). These latter can be impossible to see by eye. The assumption that scattering is much higher than absorption is fulfilled for most tissues in the near-infrared range. In the case that the tissue underneath the sensor is inhomogeneous, the measurement may be inaccurate.[11] Therefore, tissue homogeneity needs to be checked for each measurement. There is no feature provided for that by the NIRO 300.

In an effort to fulfill this assumption, a different NIRS device, the OxiplexTS (ISS Inc., Champaign, Illinois) has been used in our study. It displays slope graphs (Fig. 1) showing the measures mean intensity (DC), modulation amplitude (AC) and phase shift (PH) at four distances. If the measured DC, AC, and PH at the four distances are all precisely on one line, that means that the tissue is very likely to be homogeneous. In addition, this setup

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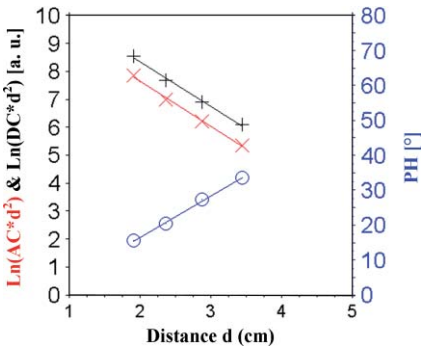

Fig. 1 Slope graph for homogeneous tissue: The mean intensity $\ln[d^2*\mathrm{DC}(d)]$, the modulation amplitude $\ln[d^2*\mathrm{AC}(d)]$, and the PH are linear with respect to the source-detector separation distance d.

has the advantage that it theoretically enables one to identify an outlier (as long as there is only one), remove it, and obtain correct data. Please note that in this study we did not take advantage of this procedure. This does not work for three distances and is not provided by the NIRO 300, where slope graphs are not displayed. In addition the OxiplexTS measures the scattering coefficient directly.

The aim of our study was to first, to determine the precision of the NIRS measurements in term and preterm infants and second, to assess the impact of tissue homogeneity on the precision of NIRS measurements.

2 Patients and Methods

Thirty-six term and twenty-seven preterm infants admitted to the maternity wards of the Department of Obstetrics and Gynecology and to the Division of Neonatology at the University Hospital Zurich were enrolled in our study. The demographic data of the neonates are shown in Tables 1 and 2. Infants with severe birth asphyxia, neurological abnormalities, or severe malformations were excluded. Parental consent was obtained in all cases prior to enrollment.

Near-infrared spectroscopy was performed using the commercially available OxiplexTS (ISS Inc., Champaign, Illinois) was provided at first use. This device is based on reflectance mode multidistance frequency-domain spectroscopy (fd-NIRS) and features two photomultiplier tubes as detectors and 16 laser diodes as light sources, eight at 692 nm and eight at 834 nm. Multidistance fd-NIRS was introduced by Fantini *et al.* and is based on the diffusion approximation to the radiative transport equation,[12] which is solved for photons travelling in semi-infinite, homogenously turbid media.[13] The emitted light intensity is sinusoidally modulated at 110 MHz, the time resolution is 50 Hz. The light is coupled to fibers (400 μm diam) that guide the light to the tissue. After penetrating the tissue, the light is received and guided back to the instrument by another fiber (3 mm diam), where it is detected by a photomultiplier tube. There are four source-detector distances (d) of 2, 2.5, 3, and 3.5 cm that implement multidistance geometry. The light sources are turned on one at a time in sequence. At the photomultiplier, the light that has passed through the tissue is detected and demodulated (i.e., DC, AC and PH), with reference to the incident sinusoidally modulated light, is determined. The main optical properties of the tissue in the near-infrared range are a high scattering and a much lower absorption coefficient. Because of the scattering, the path of the light is deflected and totally disordered after 1 cm of tissue (diffusion process). The scattering and absorption lead to a decrease in DC and AC with increased source detector distance. The PH, which corresponds to the photons' mean time-of-flight increases with the distance traveled and, hence, the source-detector distance. The decrease in DC or AC is linear when DC or AC are multiplied with d^2 and logarithmized {i.e., $\ln[d^2*\mathrm{DC}(d)]$, $\ln[d^2*\mathrm{AC}(d)]$}. The increase in PH(d) is linear as well. Therefore, a line as a function of d is fitted into the measured parameters and the resulting slope is used to calculate the scattering and absorption coefficient of the tissue. From the absorption coefficient at the two mentioned wavelength oxyhemoglobin concentration (O_2Hb), deoxy-hemoglobin concentration (HHb), tHb, and StO_2, are calculated. These calculations are correct, provided that the medium is homogeneous. In this case, the functions $\ln[d^2*\mathrm{DC}(d)]$, $\ln[d^2*\mathrm{AC}(d)]$, and PH(d) are linear with respect to d. In order to check for tissue homogeneity, the instrument displays this linear fitting as slope graphs. Figure 1 demonstrates the case of a homogeneous medium where the measured points at the four distances are all on the regression line. An inhomogeneity is displayed in Fig. 2. At all four distances, the parameters are not well predicted by the best-fitted line. This leads to a larger error in the calculated slope and thus may lead to erroneous values. When an inhomogeneity is detected, the sensor can be repositioned to find a homogeneous location and thus perform accurate measurements.

The calibration procedure was carried out as described in the manual.[14] Before each measurement the sensor was placed on a calibration phantom, which was supplied by ISS Inc. The aim is to calibrate for instrumental factors (e.g., the difference in brightness between light sources). These phantoms have known and highly homogeneous optical properties. They are made of

Table 1 Demographic data of term infants.

$N = 36$	Median	Range
Birth weight (g)	3340	2130–4070
Actual weight (g)	3160	2280–3960
Gestational age (weeks)	38.7	37.1–41.3
Postnatal age (days)	4	2–18

Table 2 Demographic data of preterm infants.

$N = 27$	Median	Range
Birth weight (g)	1680	690–2540
Actual weight (g)	1790	730–2700
Gestational age (weeks)	30.9	25.6–35.7
Postnatal age (days)	15	4–60

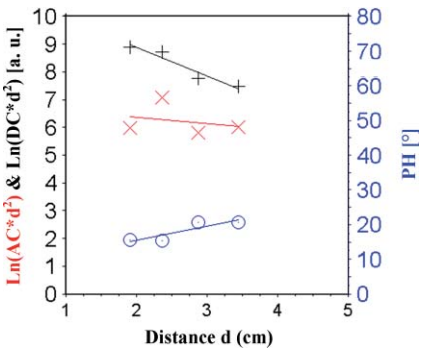

Fig. 2 Slope graph for non-homogeneous tissue: Outliers from a linear function of d in the mean intensity $\ln[d^2*\mathrm{DC}(d)]$, the modulation amplitude $\ln[d^2*\mathrm{AC}(d)]$, and the PH can be observed. The lines do not represent the points sufficiently.

solid silicone rubber containing titanium oxide (TiO_2) particles causing scattering and carbon powder causing absorption. In advance, light sources and detectors warmed up for at least 10 min. Afterward, the sensor was pressed firmly and evenly on the surface of the calibration block by hand. For correct calibration, it was essential to assure direct contact for all eight emitters and the detector bundle with the block surface. A calibration measurement was started, and the software of the instrument was calibrated for the instrumental factors.

In a pilot study on a solid calibration phantom, the standard deviation of StO_2 during 20 min (i.e., the time a measurement on an infant would maximally take) was 0.13%. This corresponds to the component of instrumental noise.

The sensor was embedded into a soft, medical-grade silicone casing. In order to fulfill the assumptions of semi-infinite boundary condition and to minimize the amount of variation in signal due to placement, the sensor was positioned over the flattest area of the tempoparietal head. Afterward, the sensor was fixed with an elastic bandage and positioning and pressure were controlled visually and by palpating. If the sensor was not placed accurately, then it was repositioned and newly controlled. In some cases, if the bandage could not be placed adequately, then the sensor was held in position by hand. Between each measurement, the sensor was repositioned and the slope graphs were inspected in order to assess the tissue homogeneity below the sensor. For each neonate, between 5 and 15 1-min measurements were performed. All measurements were performed by a single examiner.

Vital parameters, such as heart rate and blood pressure, were not monitored during the measurements. All term infants were placed in the maternity ward and were therefore without any monitoring. The included preterm infants were circulatory and respiratory stable. Measurements were only performed on silent and content or sleeping infants; additionally, the total measurement period was <20 min. We therefore assume that vital parameters were stable and it was the same for all measurements. Potential fluctuations would lead to a falsely lower precision (i.e., our precision values are conservative).

As a measure for tissue homogeneity, a self-designed Matlab script calculated the square of the Pearson product-moment correlation coefficient r^2 of the slope graphs. The correlation coefficient (r^2 ranging from 0 to 1) is a widely applied statistical measure, indicating how well two variables correlate. Specifically, it correlates the source-detector distance d to the mean of the measured values (AD, DC, PH): $r^2 = 1$, indicating an entirely linear dependence (i.e., a high tissue homogeneity); $r^2 = 0$ indicates that there is no linear dependence (i.e., a low tissue homogeneity).

In order to investigate how the inhomogeneity of the tissue affects the precision of the measurements, the results were grouped by r^2 values, calculated for each measurement. Four categories were defined: $r^2 \leq 0.925$, $0.925 < r^2 < 0.95$, $0.95 \leq r^2 < 0.975$, and $r^2 \geq 0.975$. If one infant had only one measurement within a certain r^2 group, then this single measurement did not contribute to the within variance of this group. However, there were enough infants with several measurements per r^2 group. Measurements with negative StO_2 or negative tHb, and tHb > 500 μmol/l were excluded.

The precision of StO_2 measurement was defined as the within-infant standard deviation as estimated by the repeated measures analysis of variance (ANOVA), which considered the individual subject as a random effect.[15] The same algorithm also yielded the standard deviation of the between-subject variability. The measurement precision was calculated for every r^2 group by repeated measures ANOVA (IBM SPSS Statistics-Software Advanced-Models 12.0 and 15.0). Furthermore, a repeated measures ANOVA was applied to check for statistically significant differences in the mean StO_2 and tHb values between term and preterm infants.

3 Results

There was no significant difference in the mean StO_2 between term and preterm infants ($p = 0.12$). However, we found a significant lower mean tHb in preterm than in term infants ($p < 0.01$) (Tables 3 and 4).

Table 3 Results for four categories of tissue homogeneity expressed as r^2 in term infants.

r^2	N 214	Mean StO_2 (%)	Precision StO_2 (%)	Between StO_2 (%)	Mean tHb (μM)	Precision tHb (μM)	Between tHb (μM)
<0.925	52/53	74.4	11.0	4.2	60.7	16.4	31.2
0.925 to 0.95	32	72.8	3.1	4.4	57.1	4.0	20.6
0.95 to 0.975	73	75.3	3.1	5.6	57.9	8.2	17.9
>0.975	48	73.1	2.0	6.9	50.0	3.5	16.6

N = total number of measurements, StO_2 = tissue oxygenation saturation, Between StO_2 = variation between infants, tHb = total hemoglobin

Table 4 Results for four categories of tissue homogeneity expressed as r^2 in preterm infants.

r^2	N 161	Mean StO_2 (%)	Precision StO_2 (%)	Between StO_2 (%)	Mean tHb (μM)	Precision tHb (μM)	Between tHb (μM)
<0.925	31	70.5	5.6	10.1	41.1	4.6	15.2
0.925 to 0.95	24	67.3	5.7	13.4	38.1	4.1	11.3
0.95 to 0.975	42	73.5	4.2	7.6	37.9	6.9	12.0
>0.975	54	72.4	4.6	7.5	34.2	3.1	9.2

N = total number of measurements, StO_2 = tissue oxygenation saturation, Between StO_2 = variation between infants, tHb = total haemoglobin

Tissue homogeneity, quantified by r^2, was assessed to investigate the effect on the precision of NIRS measurements. As expected, categories with a higher r^2 had lower within-infant and between-infant variability for StO_2 and tHb. Only the between-infant variability for StO_2 in term neonates increased with rising r^2. A higher tissue homogeneity improved the precision for StO_2 and tHb significantly in term infants ($p = 0.001$, respectively, $p = 0.001$). No significant difference was demonstrated in preterm infants (Tables 3 and 4). The absorption and scattering coefficients of the infants' head for different gestational ages are given in Table 5.

4 Discussion

In modern neonatology, a noninvasive method for continuous cerebral monitoring in term and preterm neonates undergoing intensive care is a tremendous demand. Currently, there exists no established method to provide quantitative and qualitative information on cerebral parameters in a clinical routine and hence enable us to detect cerebral pathologies more easily and sooner.

NIRS is a noninvasive method to measure continuously regional changes in cerebral tissue oxygenation and perfusion.[6,7] There is even the possibility to perform functional brain activity measurements.[16] In a clinical setting, for NIRS devices it is important to feature a sufficient precision allowing distinguishing normal from pathological conditions. There are several NIRS devices commercially available based on different technologies, with individual strengths and limitations.

Menke *et al.* were the first to provide data on the precision[17] of NIRS measurements. Using the Critikon 2020 (Johnson and Johnson Medical, Newport, United Kingdom) for StO_2, they maintained a very low within-infant and between-infant variation (1.7%, respectively, 4.1%) in neonates. However, this device has been shown to underestimate true variability of StO_2 measurements,[18,19] which probably explains the low values.

The mentioned study performed by Soerensen and Greisen,[10] using the NIRO 300 (Hamamatsu), revealed for StO_2 a within-infant precision of 5.2% for single measurements. By averaging multiple measurements, the error improved by 2.3%. However, averaging is not very practical in a clinical setting. The need of multiple measurements prolongs the intervention time and therefore, increases the stress on the fragile and maybe instable infants undergoing intensive care.

Our results showed no significant difference between the 74.5% mean StO_2 in term infants and the 71.8% in preterm infants ($p = 0.12$). Van Bel *et al.*[20] summarized nicely normal values of StO_2 in adults, term and preterm infants ranging from 58 to 75%, respectively, 49 to 75% and 54 to 85%. In accordance with our results, they reported no significant difference between the different groups.

In contrary, the mean tHb differs with high significance between term (55.1 μmol/l) and preterm (39.6 μmol/l) infants ($p < 0.01$). These results are clearly lower than the 98.8 μmol/l mean tHb obtained in 20 term infants by our group earlier.[21] This is probably due to the quite different algorithms. Whereas the 66% mean StO_2 differs only little from our current results.

Table 5 Mean ± standard deviation absorption and scattering coefficients and respective differential pathlength factors (DPF) of the infant's head for different gestational ages.

GA	N	μ_a 692 nm	μ_s' 692 nm	DPF 692 nm	μ_a 834 nm	μ_s' 834 nm	DPF 834 nm
30–31	2	0.064 ± 0.008	5.0 ± 0.9	5.6 ± 0.9	0.079 ± 0.002	4.0 ± 0.9	4.5 ± 0.7
32–33	7	0.080 ± 0.020	4.1 ± 1.8	4.4 ± 1.2	0.104 ± 0.021	3.3 ± 1.5	3.5 ± 1.0
34–35	13	0.088 ± 0.027	4.3 ± 1.3	4.5 ± 0.8	0.106 ± 0.027	3.3 ± 1.0	3.5 ± 0.7
36–37	3	0.094 ± 0.028	5.9 ± 2.7	5.2 ± 1.3	0.102 ± 0.025	4.6 ± 2.1	4.3 ± 1.0
38–39	16	0.118 ± 0.036	5.3 ± 2.3	4.4 ± 1.3	0.148 ± 0.042	4.0 ± 1.7	3.4 ± 1.1
40–41	12	0.131 ± 0.027	5.7 ± 1.7	4.5 ± 0.6	0.161 ± 0.030	4.1 ± 0.9	3.5 ± 0.4

GA = gestational age (weeks), N = total number of infants, μ_a = absorption coefficient (1/cm), μ_s' = scattering coefficient (1/cm)

Likewise Raj *et al.* showed that healthy children had a similar mean StO_2 (72%), whereas children with sickle cell disease and mild symptoms had a low mean StO_2 (62%) and even lower value (48%) with severe symptoms.[22] This could be expected, given that a low tHb increases oxygen extraction and thus leads to a lower StO_2. The lower mean tHb in preterm compared to term infants is in accordance with the well-known anemia of prematurity caused by physiologically and in a clinical setting iatrogenically reduced red blood cell mass and iron stores.[23–25]

Interestingly, Naulaers *et al.* revealed, depending on the postnatal age of the preterm infant, a significant rise in mean StO_2 from 57 to 66% ($p = 0.05$) between days 1 and 2 and from 66 to 76% ($p = 0.001$) between days 2 and 3.[26] The increase in StO_2, depending on the postnatal age, could be connected with the continuing increase in blood volume correlated to myelination changes during the postnatal cerebral development. This could be due to higher energy consumption during myelination or increased cerebral activity.

Franceschini *et al.*, however, demonstrated that StO_2 stayed constant in 47 term and preterm infants [range 27.0–41.5 weeks gestational age (GA)] within the first year of life. The average StO_2 was 64%. Their stable values for StO_2 might be due to a close interaction between oxygen delivery and oxygen consumption, shown by a significant increase of 84%, respectively, 50% in cerebral blood flow and tHb.[27]

In our study, the range for the postnatal age was wide with 2–18 days in term and especially with 4–60 days in preterm infants. The data showed no relation to gestational age or postnatal age. This could be due to lack of repeated measurements in the same infants; additionally, our protocol was not designed to detect such changes.

The precision of StO_2 measurement was defined as the within-infant variation. Depending on the correlation coefficient category, representing tissue homogeneity, the within-infant variation for StO_2 and tHb varied significantly in term infants ($p = 0.001$ for both). Indeed, a higher homogeneity leads to a significantly higher precision (i.e., lower within-infant variation). For preterm infants, although the values showed the same tendency, the difference in precision in relation to the tissue homogeneity was smaller and not significant. This probably is one of the particularities of our data set, and we do not have a reasonable explanation for this effect. Thus, a higher tissue homogeneity and optimization of the measurement condition leads to improved within-infant variation in term, but less so or not in preterm infants.

In parallel, the between-infant variation for StO_2 in preterm and the between-infant variation for tHb in term and preterm neonates decrease significantly ($p = 0.001$ for all three), depending on the correlation coefficient category. By comparison, Soerenson and Greisen[10] obtained a between-infant StO_2 variation of 6.9%. The within-infant StO_2 variation of 5.2% was almost as high, which makes it difficult to observe differences of measurements between different infants.[6]

To apply NIRS clinically [i.e., to distinguish subjects in a critical ill situation (e.g. hypoxia) from healthy subjects], two prerequisites must be fulfilled. First, measurements must be sufficiently precise, or the more precise measurements are, the better. Second, the values of subjects in a critically ill situation must be sufficiently different from the range of values in healthy infants, as expressed by the between-infant variation. The latter still must be established in clinical trials. In our data, the between-infant variation generally decreased with tissue homogeneity, probably as a result of the decrease in variability within infants. This means that higher tissue homogeneity increases the chances that the method yields clinically relevant results.

In conclusion, NIRS measurements performed in our study presented a higher precision in term than in preterm infants. The precision of NIRS measurements can be improved significantly in term infants by ascertaining high tissue homogeneity for each measurement. In preterm infants, the precision of the measurements was smaller and not significant. Considering that especially preterm infants often require cerebral monitoring, the future aim must be the improvement of NIRS devices to obtain higher precision, enabling us to monitor cerebral oxygenation in a routine clinical setting and thereby preventing cerebral pathology.

Acknowledgments

We gratefully acknowledge funding by the Swiss National Science Foundation.

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