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In vitro comparisons of near-infrared spectroscopy oximeters: Impact of slow changes in scattering of liquid phantoms

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Abstract

Several cerebral oximeters based on near-infrared spectroscopy (NIRS) are commercially available that determine tissue oxygen saturation (StO_2). One problem is an inconsistency of StO_2 readings between different brands of instruments. Liquid blood phantoms mimicking optical properties of the neonatal head enable quantitative device comparisons. However, occasionally, the reduced scattering coefficient (μ_s') of these phantoms decreases over time. Aim: To investigate whether this decrease in μ_s' affects the validity of comparison of these devices. StO_2 was measured by several NIRS oximeters simultaneously on a phantom, which exhibited a particularly strong decrease in μ_s'. We found that a decrease in μ_s' by $\leq 16\%$ from baseline led to deviations in StO_2 of $\leq 3\%$.

1 Introduction

The number of commercial oximeters to measure the absolute values of tissue haemoglobin oxygen saturation (StO_2) continues to rise [1, 2]. Unfortunately, these oximeters do not display comparable StO_2 values when applied over the same media, neither in vivo nor *in vitro* [3]. To address this problem, Kleiser et al. [3] designed a liquid phantom simulating optical properties of tissue and enabling simultaneous measurements of four oximeters; this work resulted in a table enabling conversion of StO_2 for different oximeter brands.

One potential problem of these liquid phantoms was an occasional slow decrease in their reduced scattering coefficient (μ_s') during measurements lasting several hours.

Therefore, the aim of the present study was to investigate the effect of decreasing μ_s' on oximeter cross-comparisons and to define the maximally allowable decrease of μ_s' with respect to the baseline value as a quality criterion for the phantom.

2 Materials and Methods

The current phantom setup is described in detail in [3, 4]. We employed the OxiplexTS (ISS Inc., Champaign, IL, USA) frequency-domain near-infrared spectroscopy (NIRS) instrument with a rigid sensor as a reference device for StO_2. The OxiplexTS measures absolute values of μ_a (absorption coefficient) and μ_s'. We compared the neonatal sensor of INVOS 5100C (INVOS neo; Medtronic, Inc., Minneapolis, MN, USA), the small reusable sensor of NIRO 200NX (NIRO-200NX small RU; Hamamatsu Photonics K.K., Hamamatsu, Japan) and the small sensor of FORE-SIGHT Elite (FORE-SIGHT small; CAS Medical Systems, Inc., Branford, CT, USA) to the OxiplexTS. In brief, the phantom consists of a black container with four windows to simultaneously attach four sensors. The windows are made of silicone layers optically mimicking the neonatal skull of 2.5 mm thickness. These also enable realistic optical coupling of the sensors to the liquid mixture inside the container (Table 1).

The phantom was oxygenated by supplying O_2 at a flow rate of 1 L/min. Deoxygenation was achieved by addition of 4.5 g of yeast, which was activated by 9 mL of glucose (50%) in total (three steps of 3 mL). The activated yeast cells metabolize glucose and O_2 carried by oxyhaemoglobin (O_2Hb) converting it to deoxyhaemoglobin (HHb), thereby producing water and carbon dioxide. The OxiplexTS readings revealed a slow decrease of μ_s' over time. Possible explanations for this effect are discussed below.

Table 1 Ingredients of the liquid phantom: 55 mL erythrocyte concentrate leads to 85 µM haemoglobin in the phantom. Quantities are the accumulated values after multiple injections

Ingredient	Quantity	Producer
PBS (pH = 7.4)	2500 mL	Kantonsapotheke Zürich, Switzerland
Erythrocyte concentrate	55 mL	Blood bank, USZ, Switzerland, c_{tHb} = 266.3 g/l, htc = 79.4%
SBB (8.4% = 1 M)	25 mL	B. Braun, Sempach, Switzerland
IL	74 mL	Fresenius Kabi Gmbh, Germany
Glucose	9 mL	AlleMan Pharma, Germany
Yeast	4.5 g	Frische Wieninger Hefe, Aldi, Germany

PBS phosphate-buffered saline, *IL* intralipid, *SBB* sodium bicarbonate buffer, c_{tHb} haemoglobin concentration, *htc* haematocrit

3 Results

The reference device OxiplexTS measured StO_2 and μ_s' throughout the course of five oxygenation and deoxygenation cycles (Fig. 1a). Although μ_s' was relatively stable during the first 0.5 h, it decreased linearly between 0.5 and 2 h, and faster after 2 h. The liquid phantom's temperature and pH were kept constant between 36.5–37 °C and 7.18–6.94, respectively.

For all three oximeters there was a tendency to higher StO_2 values with decreasing μ_s', especially for StO_2 < 30% (Fig. 1b–d). The agreement was good between deoxygenations 1 and 4. In contrast, deoxygenation 5 (Fig. 1b–d) clearly deviates. Within deoxygenation 5, μ_s' dropped by 32.9% from its baseline value (5.65 cm^{-1}).

The linear fits of StO_2 within 15–85% between OxiplexTS and the three other oximeters had correlation coefficients of $r^2 \geq 0.99$. Table 2 presents the absolute differences in StO_2

between the first deoxygenation (fresh phantom) and the following deoxygenations for all oximeters. Table 3 shows the quantitative reduction in μ_s' over time.

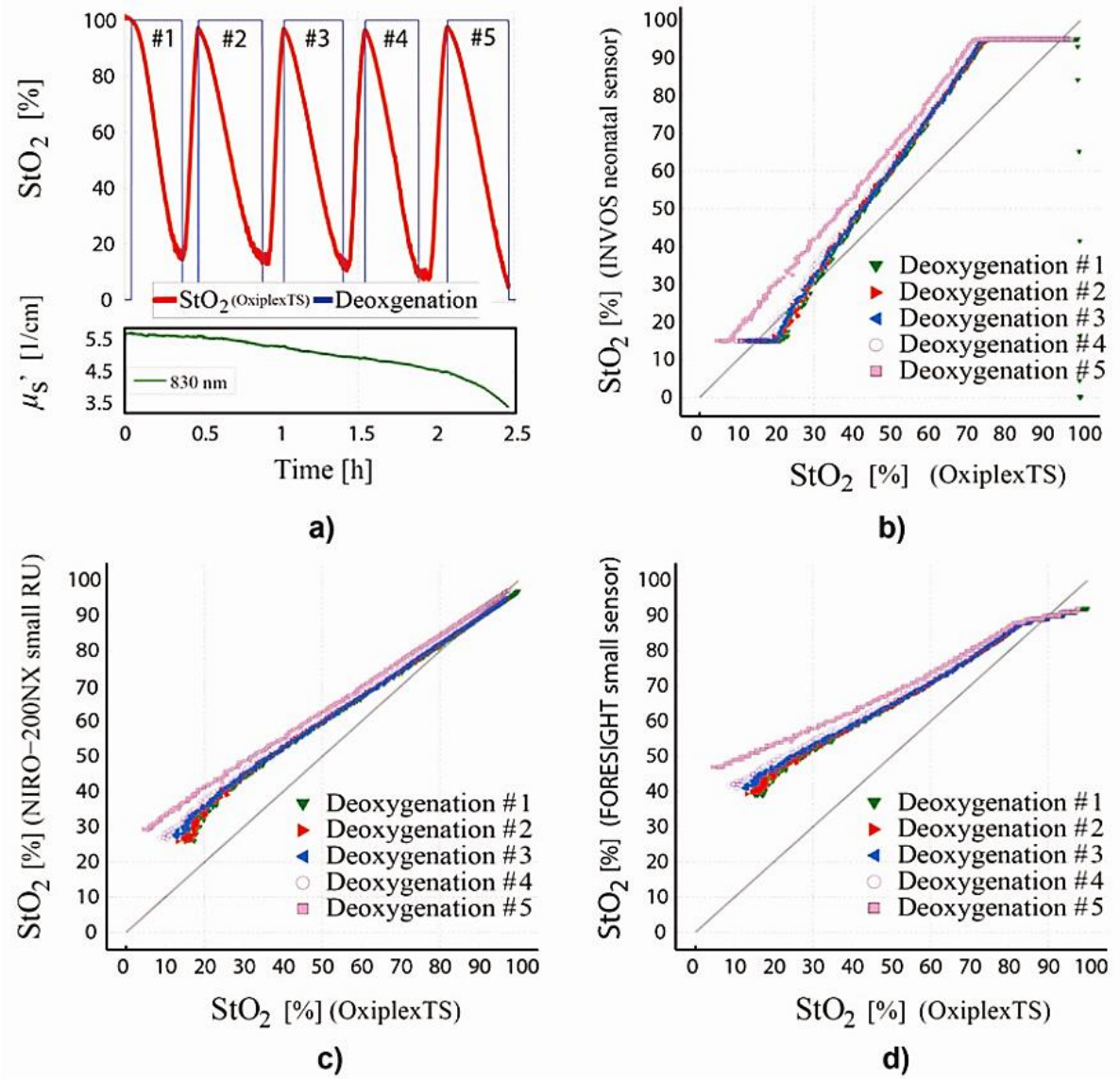

Fig 1. Fig. 1 (**a**) StO_2 and μ_s' during deoxygenation and oxygenation cycles #1–5. (**b**) StO_2 values of INVOS (clips at 15% and 95%) on the y-axis with respective OxiplexTS values on the *x*-axis. The same applies for NIRO-200NX (**c**) and FORESIGHT (**d**). StO_2 values for deoxygenations 1–4 agree well, but clearly deviate for deoxygenation 5

Table 2 Absolute difference in StO_2 between the deoxygenation cycles 2–5 and the first deoxygenation cycle (fresh phantom) for three devices. Mean difference [%] represents the mean value of absolute StO_2 difference for one specific cycle, which remains below 3% for the first four deoxygenation cycles

		ΔStO_2 [%] for reference StO_2 [%] of						
Deoxygenation cycle		30	40	47	50	60	77	Mean difference [%]
INVOS neo	#2	1.17	1.08	1.01	0.98	0.89	0.73	● (0.9)
	#3	1.58	1.24	0.99	0.89	0.55	−0.03	● (0.9)
	#4	3.92	3.23	2.75	2.55	1.86	0.69	●●● (2.5)
	#5	11.6	9.24	7.56	6.84	4.43	0.35	●●●●●●●● (6.7)
NIRO-200NX small RU	#2	0.75	0.63	0.54	0.51	0.39	0.18	● (0.5)
	#3	1.89	1.48	1.19	1.06	0.65	−0.5	● (1.0)
	#4	3.19	2.63	2.24	2.07	1.52	0.57	●● (2.0)
	#5	6.03	5.18	4.58	4.33	3.48	4.27	●●●●● (4.3)
ForeSight small	#2	0.63	0.43	0.29	0.23	0.03	−0.31	● (0.2)
	#3	1.79	1.29	0.94	0.79	0.28	−0.57	● (0.8)
	#4	3.28	2.57	2.07	1.86	1.14	−0.07	●● (1.8)
	#5	6.98	5.65	4.71	4.31	2.97	0.7	●●●●● (4.2)

Table 3 Relative change of μ_s' in percent compared to its initial value of $\mu_s'_{,0}$ = 5.65 cm^{-1} for all cycles and StO_2 values

Reference StO_2 [%]	Deoxygenation #1	Deoxygenation #2	Deoxygenation #3	Deoxygenation #4	Deoxygenation #5
77	−0.0	−1.9	−9.0	−14.5	−25.3
60	−0.0	−2.3	−9.6	−15.4	−27.4
50	−0.4	−3.0	−9.6	−15.8	−29.0
47	−0.5	−2.8	−9.9	−15.6	−29.4
40	−0.5	−3.2	−10.1	−16.1	−30.8
30	−0.4	−3.7	−10.6	−16.3	−32.9

4 Discussion and Conclusions

Liquid blood phantoms provide excellent means for assessing performance and comparing devices in tissue oximetry, which is necessary due to large variability of readings between the different sensors [3]. These phantoms have several advantages compared to in vivo methods summarized as: (i) known and accurate reference values for haemoglobin concentrations (*c*tHb), (ii) low levels of StO_2 achievable, (iii) good homogeneity within the phantom (iv) adaptability of optical properties to mimic the population or condition of interest (v) capability to support simultaneous measurements with multiple devices, (vi) known StO_2 within field of view of the sensor, and (vii) no ethical concerns. Thus, phantom experiments may supplant the in vivo methods of performance assessment in tissue oximetry. However, there are no recommendations on the boundaries of acceptable variations of optical properties of the phantom.

As shown in Fig. 1a μ_s' slowly decreased from μ_s' = 5.65 cm−1 (start of 1st deoxygenation) to 3.80 cm^{-1} by −32.9% 2.5 h later (end of deoxygenation #5). The lipid emulsion is the main scattering agent. We observed that the small lipid droplets start to aggregate, which could explain the reduced μ_s'. This could be due to a reduced capacity of the emulsifier to stabilize the emulsion. In principle, the aggregation of IL may be triggered by excessive acidity (low pH) and inappropriate electrolyte content especially in the presence of divalent cations (Ca^{2+} and Mg^{2+}) [5]. We exclude low pH as reason, because pH values were kept within 6.98–7.18, i.e. well within the stated stability range of 6.00–8.9 for the IL emulsion [5]. Intracellular Ca^{2+} and Mg^{2+} cations of the red blood cells may enter the IL

emulsion in case of rupture of erythrocyte cell membranes, e.g. by mechanical stress from O_2 bubbling. The highest estimated cation concentrations are $[Ca^{2+}] = 1.05$ µM, $[Mg^{2+}] = 40.6$ µM originating from an erythrocyte concentrate amount (55 mL) in case of total cell membrane rupture are comfortably below the instability thresholds of IL, which according to [5] are defined at $[Ca^{2+}] = 2.5$ mM and $[Mg^{2+}] = 0.6–3.5$ mM per total mixture volume. The estimated intracellular $[Zn^{+}] = 2.8$ µM could affect the emulsion, since the threshold is between 0 and 70 µM. The sodium (threshold [5]: 20–80 mM) in SBB (Table 1) has a concentration of [Na] = 21 mM in the phantom and it thus may be one factor. However, the main factor is probably the cumulative phosphate (Na_2HPO_4, KH_2PO_2) of PBS (Table 1) of 29.2 mM concentration in the phantom, which substantially exceeds the 2.5–15 mM range of stability [5]. Reducing phosphate concentrations may thus extend the phantom's lifetime.

A further possible cause of instability is the locally increased temperature, near the heating plate, which maintains the overall temperature of the mixture at 36 °C. The heating plate's temperature has been set to regulate its own temperature to 39 °C on average. Microbiological contamination counts as another possible cause of instability, but the containers' surfaces were always disinfected.

The addition of yeast increased the μ_s' by 0.15 cm^{-1}. Similarly, the growth of yeast would be expected to increase the μ_s' over time. The yeast also needs adenosine triphosphate for aerobic and anaerobic respiration and is thus expected to reduce the concentration of phosphate. Both effects are counteracting a reduction in μ_s', but are obviously predominated by other factors. During desaturation cycle 5 the mean difference increases markedly (Table 2) and clearly exceeds 3% difference. According to Table 3, at the beginning of the 5th deoxygenation, μ_s' has decreased by 25.3% from the baseline value. Thus, we conclude, that the errors in StO_2 will remain <3% if the decrease in μ_s' is <16% from baseline. This also applies to the hypoxic StO_2 threshold of 47% [3, 6]. Thus, constant monitoring of μ_s' is important for correct measurements. Once μ_s' decreases by >16% from baseline we recommend to dispose of the phantom and prepare a fresh mixture to ensure validity of the experimental data.

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References

1. Wolf M, Naulaers G, van Bel F, Kleiser S, Greisen G (2012) A review of near infrared spectroscopy for term and preterm newborns. J Near Infrared Spec 20(1):43–55.
2. Scholkmann F, Kleiser S, Metz AJ, Zimmermann R, Pavia JM, Wolf U, Wolf M (2014) A review on continuous wave functional near-infrared spectroscopy and imaging instrumentation and methodology. NeurroImage 85(Part 1):6–27
3. Kleiser S, Nasseri N, Andresen B, Greisen G, Wolf M (2016) Comparison of tissue oximeters on a liquid phantom with adjustable optical properties. Biomed Opt Express 7(8):2973–2992.

4. Nasseri N, Kleiser S, Ostojic D, Karen T, Wolf M (2016) Quantifying the effect of adipose tissue in muscle oximetry by near infrared spectroscopy. Biomed Opt Express 7(11):4605–4619.
5. Fresenius Kabi Australia Pty Limited (2016) Product information on INTRALIPID 10%, 20% and 30%. Fresenius Kabi Australia Pty and New Zealand Limited, Mount Kuring-gai
6. Hyttel-Sorensen S, Pellicer A, Alderliesten T et al (2015) Cerebral near infrared spectroscopy oximetry in extremely preterm infants: phase II randomised clinical trial. BMJ 350:g7635.