Quantifying the effect of adipose tissue in muscle oximetry by near infrared spectroscopy

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Abstract: Change of muscle tissue oxygen saturation (StO2), due to exercise, measured by near infrared spectroscopy (NIRS) is known to be lower for subjects with higher adipose tissue thickness. This is most likely not physiological but caused by the superficial fat and adipose tissue. In this paper we assessed, in vitro, the influence of adipose tissue thickness on muscle StO2, measured by NIRS oximeters. We measured StO2 of a liquid phantom by 3 continuous wave (CW) oximeters (Sensmart Model X-100 Universal Oximetry System, INVOS 5100C, and OxyPrem v1.3), as well as a frequency-domain oximeter, OxiplexTS, through superficial layers with 4 different thicknesses. Later, we employed the results to calibrate OxyPrem v1.3 for adipose tissue thickness in-vivo.

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The state of muscle oxygenation after specific interventions may be a valuable indicator of different medical conditions. For example, in [1], $\text{StO}_2$ has been measured on thenar eminence muscle of trauma patients with hemorrhagic shock. The results have shown that $\text{StO}_2$ was different in patients with unchanged conditions compared to those with worsened condition, after 72 hours of admission. Moreover, muscle oxygenation at the onset of muscle activity has been employed as a diagnostic tool for mitochondrial diseases. A review on the application of near infrared spectroscopy (NIRS) for diagnosis of mitochondrial dysfunction is available in [2]. Response of muscle oxygenation to interventions such as movement, treadmill-walking, arterial and venous occlusion, and plantar reflection has also been applied for diagnosis of peripheral arterial disease, acute and chronic compartment syndrome of the lower extremity, deep vein thrombosis, and buttock or lower extremity ischemia [3]. As a result, monitoring the
state of muscle oxygenation seems to have high clinical relevance. However, measuring muscle oxygenation may be difficult if NIRS oximeters are affected by superficial adipose layer. It is, hence, needed to measure the influence of adipose tissue layer on the StO$_2$ measured by NIRS oximeters.

In a previous study, the multi-distance approach and a frequency domain (FD) NIRS oximeter were applied on several blocks with known optical properties while they were covered with thin layers with different optical properties. It was concluded that superficial layers with thickness of less than 6mm have negligible effects on measurement of absorption ($\mu_a$) and reduced scattering ($\mu'_s$) coefficients of the deep layer [4]. But these results may not be transferable to continuous-wave (CW) NIRS oximeters which are more widely used in clinics and research. Most of these instruments have only two different source-detector separations (SDS) and do not allow for custom algorithms based on detected raw light intensities, rather only have a single StO$_2$ value as output. Hence it is not clear if CW NIRS oximetry is applicable in case of high adipose tissue thickness (ATT).

Determining the effect of ATT on muscle StO$_2$ in-vivo is not possible. StO$_2$ corresponds to the ratio of oxyhemoglobin and total hemoglobin concentrations ($C_{O_2Hb}$ / ($C_{O_2Hb} + C_{HHb}$), with $C_{O_2Hb}$ = oxyhemoglobin concentration and $C_{HHb}$ = deoxyhemoglobin concentration). NIRS measures the average $C_{O_2Hb}$ and $C_{HHb}$ in the light path and is most sensitive to the hemoglobin (Hb) in small blood vessels, i.e. arterioles, capillaries and venules. Often a contribution of 30% arterial and 70% venous blood to the cerebral and muscle StO$_2$ is assumed. But this proportion cannot easily be measured and is likely not constant over time. It may change in patients with arteriovascular disease and also differs between tissues and subjects [5, 6]. This is not only the case for cerebral oximetry but also for muscle oximetry as blood from one muscle may drain into several veins and one vein may on the other hand carry blood from several muscles [7]. Therefore, it is not currently possible to establish the effect of the ATT on muscle StO$_2$ in-vivo from blood sample co-oximetry.

Phantoms are practical for evaluating oximeters and assessing their stability and performance. To test absorption measurement, solid or liquid phantoms containing a dye and a scattering agent at known concentrations may be applied [8, 9]. Phantoms may model several layers of tissue with different thicknesses. Hb has a very distinctive absorption spectrum. As a result, phantoms are desirable which contain real Hb whose oxygenation can be changed by yeast [10, 11] or gas exchange [12].

To obtain reference StO$_2$ values, co-oximetry may be applied. Co-oximetry in phantoms is appropriate for set-ups containing undiluted blood [13]. However, it is difficult to apply co-oximetry in phantoms with diluted blood [12]. As a result we employed an in-house made oximeter (OxyVLS) which functions based on visible light spectroscopy (VLS) as the reference oximeter. A good agreement between the values measured by this oximeter and those measured by OxiplexTS has been previously reported [14]. To compensate for small differences between the values measured by OxyVLS and those measured by OxiplexTS, we applied the calibration equations derived in [14] to the values measured by OxyVLS. This conversion also allows other research groups to replicate our results by employing OxiplexTS because OxyVLS is still not available in the market.

We employed a liquid phantom similar to [10] which simulates the optical properties of human muscle with a layer of adipose tissue. The aim of this paper is to quantify the effect of superficial layers with different thicknesses on the sensitivity of different CW NIRS oximeters. An additional aim is to correct the results obtained by our in-house made NIRS oximeter, OxyPrem v1.3, for this effect in-vivo.
2. Materials and methods

2.1. Experimental set-up

The set-up consisted of a phantom container which had previously been described in detail [14] and is shown schematically in Fig. 1. The geometry of the container enables placing 4 NIRS sensors on the windows. The windows are the interface between the sensors and the liquid phantom. They are 90mm wide and 50mm high and are placed centered on each wide side of the container at the height of $\approx 70mm$.

To investigate the effect of ATT on the sensitivity of oximeters, we produced 4 windows with optical properties similar to those of adipose tissue of adult human calf muscle. These optical properties have previously been measured in-vivo [15]. Table 1 shows the optical properties of these windows. These windows were cast with 4 different window thicknesses ($d_{\text{window}}$) (2.5mm, 5mm, 9mm, and 16mm), reflecting the variety of ATT in human. Windows were manufactured from Silpuran 2420 silicone (Wacker Chemie AG, Munich, Germany) which was dyed with 0.69ml/L white Elastosil pigment paste FL RAL 9010 (Wacker) and 1.08mg/L carbon black powder (Alfa Aesar, Thermo Fisher (Kandel) GmbH, Karlsruhe, Germany). The windows were placed on each wide face of the container and formed the interface between the NIRS oximeters and the liquid phantom. Although in reality adipose tissue is multi-layered, we neglected the very superficial skin, containing much more hemoglobin, because all NIRS oximeters employed in this study claim to reduce the effect of superficial tissue in their algorithms. We think that the single-layered windows, not containing hemoglobin, are a good estimation of the remaining adipose tissue which consists mainly of fat with very little hemoglobin.

Fig. 1. The experimental set-up is shown schematically. The cap of the container effectively prevented oxygen and light entering into the phantom. NIRS sensors were placed in the middle of each window.

The liquid phantom consisted of phosphate-buffered saline "Kreis" (PBS, $pH = 7.4$, volume in phantom = 2500mL, Kantonsapotheke Zurich, Zurich, Switzerland), human erythrocyte concentrate from expired bags (expiry date < 2 months) (total hemoglobin concentration: $tHb = 220g/L$, hematocrite ($htc$) = 67%, volume in phantom = 53.5mL), intralipid 20% (IL, Fresenius Kabi AG, Bad Homburg, Germany, volume in phantom = 74mL), sodium bicarbonate
buffer (SBB, 8.4%(1mmol/ml), B. Braun Medical AG, Sempach, Switzerland, volume in phantom = 55mL), and glucose 50% (AlleMan Pharma GmbH, Reutlingen, Germany, volume in phantom = 9mL). The optical properties of the liquid phantom simulated the calf muscle [15]. Table 1 indicates the optical properties of the deoxygenated liquid phantom.

Table 1. Optical properties of the deoxygenated liquid phantom and the windows.

|                  | 834 nm |       | 692 nm |
|------------------|--------|-------|--------|
|                  | \(\mu_a\) (cm\(^{-1}\)) | \(\mu'_a\) (cm\(^{-1}\)) | \(\mu_a\) (cm\(^{-1}\)) | \(\mu'_a\) (cm\(^{-1}\)) |
| windows          | 0.057  | 4.4   | 0.059  | 5.0   |
| liquid phantom   | 0.13   | 4.9   | 0.26   | 5.9   |

2.2. Comparison of the liquid phantom to previous studies

The liquid phantom described here has been previously employed and in detail described for in-vitro comparison of cerebral oximeters [14]. The no. 3 mixture of phantom 2 [14] with \(C_{\text{Hb}} = 70\mu\text{M}\) and the same concentration of all other ingredients has been used with the only difference of setting the concentration of SBB to 55mL this time. The windows assembled in this study had different thicknesses (\(d_{\text{window}}\)) and optical properties when compared to set-up in [14] to reflect adipose tissue with different thicknesses. Temperature in this measurement (30.50\(^\circ\text{C} < \text{temperature} < 34.02\(^\circ\text{C}\)) was lower than the one reported in [14] (37.10\(^\circ\text{C} < \text{temperature} < 38.40\(^\circ\text{C}\)). This deviation does not falsify our results because reference oxygenation values are not obtained as described in [14] from oxygen-hemoglobin dissociation curve. The mixture no. 3 from [14] is in good agreement with the current mixture (Table 2) in the sense of pH quantities (7.06 < \(pH < 7.27\)) and \(pCO_2\) quantities (7.07kPa < \(pCO_2 < 14.96kPa\)).

Table 2. pH, temperature, and \(pCO_2\) range in 4 cycles of measurement.

|       | pH | temperature (\(^\circ\text{C}\)) | \(pCO_2\) (kPa) |
|-------|----|---------------------------------|-----------------|
|       | min | mean | max | min | mean | max | min | mean | max |
| cycle 1 | 7.14 | 7.17 | 7.21 | 30.50 | 31.24 | 32.00 | 11.96 | 13.41 | 15.15 |
| cycle 2 | 7.15 | 7.20 | 7.22 | 32.02 | 32.58 | 33.20 | 12.87 | 13.93 | 16.35 |
| cycle 3 | 7.18 | 7.23 | 7.27 | 32.80 | 33.21 | 33.60 | 11.32 | 12.22 | 14.59 |
| cycle 4 | 7.25 | 7.31 | 7.35 | 33.21 | 33.60 | 34.02 | 10.38 | 11.51 | 13.43 |

2.3. NIRS oximeters

In this paper we employed OxiplexTS with rigid sensor (ISS, Inc., Champaign, IL, USA), INVOS 5100C with adult SomaSensor SAFB-SM (Medtronic, Inc., Minneapolis, MN, USA/INVOS adult), Sensmart Model X-100 Universal Oximetry System with adult 8004CA (Nonin Medical, Inc., Plymouth, MN, USA/Nonin adult) and OxyPrem v1.3 (in-house made NIRS oximeter, University Hospital Zurich, Zurich, Switzerland). All oximeters measure absolute \(StO_2\). Table 3 shows wavelengths employed by each oximeter, SDS, number of light paths, and the average penetration depth (APD) that each oximeter achieves in a semi-infinite medium with the same optical properties as the windows. We calculated the mean APD based on Eq. (1) [16], including the longest SDS, assuming \(\mu'_a\) and \(\mu'_s\) as measured by OxiplexTS on a solid block phantom with window optical properties, \(d_{\text{window}} = 55mm\), and linearly interpolated over wavelength. For each oximeter we calculated the APD for each wavelength, the oximeter employs and then reported the average of these APD values.
\[ APD = \frac{1}{2} \left[ \frac{< SDS >}{(3\mu_a\mu'_s)^{1/2}} \right]^{1/2} \]  

(1)

Table 3. Technical information on NIRS oximeters, INVOS adult, Nonin adult, OxyPrem v1.3, OxiplexTS.

| Oximeter       | peak wavelength (nm) | SDS (mm) | APD (mm) | no. of light paths |
|----------------|----------------------|----------|----------|-------------------|
| INVOS adult    | \( \lambda_1 \) \ 730 | 30       | 10.6     | 2                 |
|                | \( \lambda_2 \) \ 810 | 40       |          |                   |
| Nonin adult    | \( \lambda_3 \) \ 730 | 20       | 10.6     | 4                 |
|                | \( \lambda_4 \) \ 760 | 40       |          |                   |
| OxyPrem v1.3   | \( p_1 \) \ 690      | 15       | 9.9      | 8                 |
|                | \( p_2 \) \ 760      | 20       |          |                   |
|                | \( p_3 \) \ 810      | 30       |          |                   |
|                | \( p_4 \) \ 870      | 35       |          |                   |
| OxiplexTS      | \( p_1 \) \ 692      | 25       | 10.5     | 4                 |
|                | \( p_2 \) \ 834      | 30       |          |                   |

2.4. In-vitro measurement

We conducted 4 cycles of oxygenation-deoxygenation of hemoglobin in the liquid phantom. At the end of each cycle we shifted the NIRS oximeters to the next window. Hence after 4 repetitions, each oximeter had measured through 4 different \( d_{\text{window}} \).

We deoxygenated the phantom by adding respiring yeast into the phantom and reoxygenated it by providing an oxygen in-flow to the phantom, as described in [14]. We preferred this approach over gas exchange [12] because of less set-up complexity, higher speed of deoxygenating and more homogeneity in the phantom [14]. Table 4 shows the location of each sensor in different cycles of the measurement and Table 2 shows the ranges of pH, temperature and \( pCO_2 \) in each cycle.

Table 4. Sensor placement during 4 different cycles of oxygenation-deoxygenation.

| \( d_{\text{window}} \) | cycle 1 | cycle 2 | cycle 3 | cycle 4 |
|-------------------------|---------|---------|---------|---------|
| 2.5mm                   | OxyplexTS | OxyPrem v1.3 | INVOS adult | Nonin adult |
| 5mm                     | Nonin adult | OxyplexTS | OxyPrem v1.3 | INVOS adult |
| 9mm                     | INVOS adult | Nonin adult | OxyplexTS | OxyPrem v1.3 |
| 16mm                    | OxyPrem v1.3 | INVOS adult | Nonin adult | OxyplexTS |

2.4.1. Oximetry by visible light spectroscopy (OxyVLS)

For oximetry by visible light spectroscopy we employed our in-house made oximeter, OxyVLS (Biomedical Optics Research Laboratory (BORL), University Hospital of Zurich, Zurich, Switzerland) [14]. This oximeter measures \( S_tO_2 \) based on the shape of the absorption spectrum of hemoglobin in the range \( 520nm < \lambda < 600nm \). Figure 2 shows how the shape of the absorption spectrum of hemoglobin changes based on its oxygenation state. We have provided the technical details of this oximeter in [14]. Previously, we set the FD NIRS oximeter, OxiplexTS, as the reference oximeter because it measures independent from \( C_{tHb} \) and in a robust way and because it is available in the market for other researchers. Because of the same reasons, we applied the equation we calculated previously to convert the results obtained by OxyVLS to OxiplexTS for \( C_{tHb} = 70\mu M \) and reported these values as reference \( S_tO_2 \) values [14].
2.4.2. Data processing

We applied a moving average filter over 3 samples on the OxyVLS dataset. The data is dynamic, but the deoxygenation occurs in approximately a linear manner and averaging over 3 samples, therefore, does not introduce an error in comparison. For all other oximeters, raw $StO_2$ values were recorded. The different oximeters with different sampling rates were connected to different computers with slightly different clocks. Event marks were not available in all oximeters. Thus, data were synchronized manually based on the $StO_2$ rising point as soon as we started reoxygenating. We resampled the data from all oximeters to $1/12$ Hz and applied a kolmogorov zurbenko filter with 3 iterations on them. Obvious artifacts were removed, i.e. data with saturated detector for the OxiplexTS. We visually inspected data for correct alignment of the time-series within one sample. We applied 1$^{st}$ degree polynomial fits to calculate the relation between $StO_2$ measured by each oximeter vs. reference during deoxygenations in the range of $80\% \geq StO_2 \geq 30\%$ and at 4 different $d_{\text{window}}$.

For sensitivity analysis, we applied sigmoid functions to model the loss of sensitivity to the change of oxygenation as $d_{\text{window}}$ increases. We normalized the sensitivity of each oximeter to its sensitivity at $d_{\text{window}} = 0mm$ and call this relative sensitivity (RS). We introduced a scaling factor in the sigmoid equations. We then divided all sensitivity values by this factor to produce Fig. 5. This way, as expected, we reach relative sensitivity of 1 when there is no adipose tissue ($ATT = 0$). The sigmoid function estimates the relative sensitivity of the oximeter to muscle oxygenation when it is applied on tissue with known $ATT$. In case of peripheral tissue, this thickness can be measured by a caliper and may be applied for calibration. Such sigmoid functions have previously been employed to model the superficial layer thickness dependence of $\mu_a$ and $\mu'_s$ measurements by FD NIRS oximeters [4]. Extrapolation until 35mm and the specific form of the sigmoid functions have been chosen for the purpose of comparison to [4].

2.5. In-vivo measurement

We recruited a 32 years old, male subject with a $BMI$ of 29.6 and blood pressure of 120/80mmHg at the time of the measurement. We employed OxyPrem v1.3 and conducted 3 measurements on the subject, one on the belly and the other two, on different arm muscles. We chose extensor carpi ulnaris and brachioradialis muscles, as they are easily accessible and
their oxygenation can be varied by pneumatic pressure cuff occlusion on the upper arm. Prior to the NIRS measurements, we measured the \( ATT \) by a caliper over these two muscles, as well as on the belly. At each location we measured 5 times and took the average value as \( ATT \) (Table 5). We chose a caliper for this purpose because its results are in good agreement with those from CT [18].

Table 5. Adipose tissue thickness on different body regions of the subject who was recruited for the in-vivo measurement.

| region                        | \( ATT \) (mm) |
|-------------------------------|----------------|
| extensor carpi ulnaris muscle | 3.6            |
| brachioradialis muscle        | 4.65           |
| belly                         | 33.40          |

3. Results

Figure 3 shows the time-series of the 4 cycles, measured by 4 different NIRS oximeters and at 4 different \( d_{\text{window}} \). It is observable that the dynamic range of \( StO_2 \) varies between oximeters and decreases when the oximeters measure through windows with higher \( d_{\text{window}} \).

Figure 4 shows scatter plots of Nonin adult, OxyPrem v1.3, OxiplexTS and INVOS adult, respectively vs. the reference. We limited our analysis to \( 30\% \leq StO_2 \leq 80\% \) in which the relation between OxyVLS and the NIRS oximeters was linear \((r^2 > 0.97 \text{ in all cases})\) and is physiologically relevant (gray square) [19, 20]. The results of cycle 1 for Nonin adult (Nonin adult on 5mm window) was implausible. Thus we reported this value but excluded it from further analysis. In addition to the data, we provided linear fits of all samples in the range \( 30\% \leq StO_2 \leq 80\% \) which is indicated by the gray background in Fig. 4. Here, we define sensitivity of each oximeter at each \( d_{\text{window}} \), to be the slope of its corresponding linear fit. As depicted in Fig. 4 the sensitivity of all oximeters decreases as \( d_{\text{window}} \) increases. Figure 5 shows the trend of the relative sensitivity of each oximeter as \( d_{\text{window}} \) increases. Figure 6 shows a comparison of the trend of relative sensitivity vs. \( \frac{d_{\text{window}}}{APD} \), between the CW oximeters and the FD oximeter. It is visible that as \( \frac{d_{\text{window}}}{APD} \) increases, the FD oximeter retains higher relative sensitivity.

3.1. Calibration in-vivo

We assume that at \( ATT = 2.5mm \) the influence of the superficial layers is negligible and we calibrate \( StO_2 \) with thicker and known \( ATT \) to \( StO_2 \) values with \( ATT = 2.5mm \). As shown in Fig. 4(a)-(d), linear fits at different \( d_{\text{window}} \) cross at different points for each oximeter. This crossing point is for each oximeter a function of the thickness of the window. There is a narrow region which is almost one point for OxyPrem v1.3 that all the linear fits cross. For OxyPrem v1.3, the y-value at this point corresponds to \( StO_2 \) superficial which is the \( StO_2 \) value that the oximeter measures on a block phantom with \( d_{\text{window}} = 55mm \) and the same optical properties as those of the windows. In in-vivo measurements, \( StO_2 \) superficial can be measured on a region with high \( ATT \), i.e. on the belly.

For in-vivo calibration by OxyPrem v1.3, we measured \( StO_2 \) superficial = 79% ± 1% on the belly of the subject. \( StO_2 \) superficial is to be measured for each single oximeter and each single subject on the belly, prior to the measurement on the muscle. However, it has to be noted that this method only works for oximeters like OxyPrem v1.3 which, as depicted in Fig. 4, have a single crossing point. RS of OxyPrem v1.3 at \( ATT = 3.6mm \) is 0.96 and at \( ATT = 4.65mm \) is 0.91 (Table 5 and Fig. 5(b)). Equation (2) depicts the general calibration and Fig. 7 shows \( StO_2 \)
of extensor carpi ulnaris as well as the brachioradialis muscles of the subject, before and after calibration, measured by OxyPrem v1.3.

$$StO_2_{\text{calibrated}} = \frac{StO_2_{\text{not calibrated}} + (RS - 1)StO_2_{\text{superficial}}}{RS}$$ (2)

### 3.2. In-vitro evaluation of calibration

To evaluate the calibration procedure proposed in section 3.1, we applied the calibration equation (Eq. 2) on the OxyPrem v1.3 in-vitro dataset, obtained in the phantom, for $d_{\text{window}} = 5, 9, 16mm$. We calculated RS based on Fig. 4(b) and measured $StO_2_{\text{superficial}}$ by applying OxyPrem v1.3 on a block phantom ($d_{\text{window}} = 55mm$) which had the same optical properties as those of the windows, prior to the measurement ($StO_2_{\text{superficial}} = 68\%$). Table 6 shows the value each oximeter measured on this block phantom. We interpolated all datasets ([reference $StO_2_{\text{OxyPrem,d_{window}=1}}$]) during deoxygenation, in the range $80\% \geq StO_2_{OxyVLS} \geq 30\%$, to 1% steps (corresponding to $78.1\% \geq StO_2_{OxyPrem,2.5mm} \geq 42.6\%$). We calculated the maximum error ($e_{\text{max}}$) and additionally we applied Eq. (3) to calculate the RMS error ($e_{\text{RMS}}$) where, $n$ is the number of data points ($n = 51$). Table 7 shows $e_{\text{max}}$ and $e_{\text{RMS}}$ of the in-vitro measurement by OxyPrem v1.3 due to window thickness and also indicates the relative reduction of this error due to the calibration.

Table 6. $StO_2$ value measured by different oximeters on a solid block phantom with the same optical properties as those of the windows ($StO_2_{\text{superficial}}$).

| oximeter   | OxyPrem v1.3 | OxiplexTS | INVOS | Nonin |
|------------|--------------|-----------|-------|-------|
| $StO_2_{\text{superficial}}$ | 68%          | 13%       | 53%   | 78%   |

$$e_{\text{RMS}} = \sqrt{\frac{1}{n} \sum_{i=1}^{n}(StO_2_{\text{calibrated}} - StO_2_{2.5mm})^2}$$ (3)
Fig. 4. The StO2 values obtained by NIRS oximeters vs. reference at different window thicknesses: (a) Nonin adult vs. OxyVLS, (b) OxyPrem v1.3 vs. OxyVLS, (c) OxiplexTS vs. OxyVLS, (d) INVOS adult vs. OxyVLS.
Fig. 5. Relative sensitivity of NIRS oximeters measured on superficial layers with different thicknesses and the corresponding trend lines: (a) Nonin adult: \( y = \frac{411.2x^{2.724}}{1+411.2x^{2.724}}, r_{adj}^2 = 1.000 \), the gray point was excluded from fitting because of implausibility, (b) OxyPrem v1.3: \( y = \frac{1732x^{2.346}}{1+1732x^{2.346}}, r_{adj}^2 = 0.9997 \), (c) OxiplexTS: \( y = \frac{260.2x^{2.188}}{1+260.2x^{2.188}}, r_{adj}^2 = 0.9999 \), (d) INVOS adult: \( y = \frac{449.1x^{2.767}}{1+449.1x^{2.767}}, r_{adj}^2 = 1.000 \).

Fig. 6. Trend of the relative sensitivity of oximeters vs. \( \frac{\text{window thickness}}{\text{average penetration depth}} \). The trend lines follow the equations \( y = \frac{0.7052x^{-2.977}}{1+0.7052x^{-2.977}}, r_{adj}^2 = 0.9945 \) (CW oximeters) and \( y = \frac{1.517x^{-2.188}}{1+1.517x^{-2.188}}, r_{adj}^2 = 0.9999 \) (FD oximeter). The gray point was excluded from fitting because of implausibility.
**4. Discussion**

4.1. **Reference oximetry**

OxyVLS was the only oximeter which was directly immersed into the liquid phantom and its output was independent of $d_{window}$. We calibrated the results obtained by OxyVLS to the results obtained by OxiplexTS based on the equations we reported in [14] for $C_{Hb} = 70 \mu M$ and then employed them as the reference $StO_2$ values. This allows other research groups to reproduce our results.

4.2. **Initial sensitivity of oximeters and the influence of superficial layers on it**

It has been reported that different cerebral oximeters show different absolute values of tissue oxygenation on the same tissue. These values depend on the assumptions they make and algorithms they apply [5,21–28]. Similar results have been reported for oximetry on the muscles of lower arm by different NIRS oximeters [29]. As a result the exact relation between the values measured by different oximeters are not known. The aim of this study was to solely quantify the effect of adipose tissue on measurements by a specific oximeter. A separate calibration step is

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**Table 7. Maximum and RMS error of $StO_2$ measurement for uncalibrated and calibrated in-vitro data**, measured by OxyPrem v1.3.

| ATT  | $\epsilon_{max}$ uncalibrated | $\epsilon_{max}$ calibrated | relative reduction |
|------|-------------------------------|-------------------------------|--------------------|
| 5mm  | 4.9%                          | 4.1%                          | 16%                |
| 9mm  | 16.8%                         | 9.1%                          | 46%                |
| 16mm | 23.3%                         | 10.9%                         | 53%                |

| ATT  | $\epsilon_{RMS}$ uncalibrated | $\epsilon_{RMS}$ calibrated | relative reduction |
|------|-------------------------------|-------------------------------|--------------------|
| 5mm  | 2.9%                          | 2.1%                          | 28%                |
| 9mm  | 8.5%                          | 6.2%                          | 27%                |
| 16mm | 11.9%                         | 8.4%                          | 29%                |
needed to be able to compare the results obtained by different oximeters as shown in [14].

4.3. Effect of superficial layers on continuous-wave and frequency-domain NIRS oximeters

In Fig. 5(a)-(d) it is observable that when $d_{\text{window}} \leq 5\, \text{mm}$ all oximeters have a relative sensitivity of more than 84% with OxiplexTS having the highest relative sensitivity at this thickness and also at other thicknesses. OxiplexTS is an FD oximeter and its high relative sensitivity at $d_{\text{window}} = 16\, \text{mm}$ may be due to the fact that its phase measurement contributes to having more information from the deep tissue [30].

Relative sensitivity $= 50\%$ is reached for CW oximeters at $d_{\text{window}}/\text{APD} \approx 0.9$ and FD oximeter at $d_{\text{window}}/\text{APD} \approx 1.2$. This means that the penetration depth of the oximeters is dominated by their largest SDS and that the FD oximeter performs better at higher superficial layer thickness than the CW oximeters.

4.4. In-vivo calibration of OxyPrem v1.3

For in-vivo calibration of $\text{ATT}$, a calibration point is required. For calibration of OxyPrem v1.3 we proposed to measure this point ($\text{StO}_2_{\text{superficial}}$) on the belly of the subject, where $\text{ATT}$ is high and the oximeter only measures the adipose tissue. This value corresponds to the point all curves cross one another in Fig. 4(b).

Figure 7 shows the result of an arterial occlusion on extensor carpi ulnaris and brachioradialis muscles of a subject, before and after calibration, by OxyPrem v1.3. The two muscles have different initial $\text{StO}_2$. The difference between calibrated and not calibrated $\text{StO}_2$ is bigger for brachioradialis muscle as this muscle had a higher $\text{ATT}$ compared to extensor carpi ulnaris. After calibration and with time passing by from the moment of occlusion, the two curves converge. This is expected, as for very long complete occlusions $\lim_{t \to \infty} (\text{StO}_2) = 0\%$. Table 7 shows that the calibration procedure reduced $\varepsilon_{\text{RMS}}$ at $\text{ATT} > 2.5\, \text{mm}$ by $\approx 28\%$ and $\varepsilon_{\text{max}}$ by $16 - 53\%$ for the in-vitro data which is also transferable to the in-vivo data.

4.5. Applicability of in-vivo calibration to other NIRS oximeters

By comparing Fig. 4(a) and (b) to Table 6, it is observable that all curves of OxyPrem v1.3 and 3 curves of Nonin adult ($5\, \text{mm}$ was excluded due to implausibility) cross at about $\text{StO}_2_{\text{superficial}}$. OxiplexTS is an FD oximeter and it measures absolute $\mu_a$ and $\mu'_a$. $\mu_a$ and $\mu'_a$ may have different penetration depths [4] and as a result its crossing point does not correspond to $\text{StO}_2_{\text{superficial}}$. The location of the crossing point for the INVOS adult is strongly dependent on the window thickness. In our calibration procedure we assume that all the linear fits in Fig. 4 cross one another at a single point which corresponds to the value the oximeter measures on a solid block phantom with the same optical properties as those of windows. Our results show that this assumption is appropriate for OxyPrem v1.3 and may be appropriate for Nonin adult too but not for other oximeters employed in this measurement. Slight deviation of $\text{StO}_2_{\text{superficial}}$ from the crossing point in Fig. 4(b) creates the $\varepsilon_{\text{RMS}}$. As a result absolute calibration for $\text{ATT}$ was only possible for OxyPrem v1.3. However, in case of $\Delta\text{StO}_2$ measurement, for which only the relative sensitivity is needed (Eq. (2)), our procedure is still applicable. This point has to be noted because $\Delta\text{StO}_2$ is the main parameter which has been previously clinically monitored in muscle oximetry [1–3]. In case of correcting $\Delta\text{StO}_2$, we expect lower errors than reported in Table 3 because no $\text{StO}_2_{\text{superficial}}$ is required but only the equations for relative sensitivity and a measure of $\Delta\text{ATT}$ ($\Delta\text{StO}_2_{\text{calibrated}} = \frac{\Delta\text{StO}_2_{\text{not calibrated}}}{\varepsilon_{\text{RS}}}$).
4.6. **Suitability of the optical properties of the windows**

The windows do not have the same absorption spectrum as adipose tissue. However, if we assume adipose tissue consists of 70% fat [31] and 30% water with very small \( C_{tHb} = 0.043 < \mu_a < 0.063 \text{ cm}^{-1} \); for 690 nm < \( \lambda < 870 \text{ nm} \), \( \mu'_s = 5 \text{ cm}^{-1} \), and SDS = 40 mm, this leads to 10.1 mm < APD < 11.2 mm. For our windows we determined 10.5 mm < APD < 10.6 mm for the oximeters with SDS = 40 mm (Table 3), which is in good agreement with expected APD in-vivo. Therefore, the loss in sensitivity to muscle will be similar in-vitro and in vivo, but the absolute \( StO_2 \) differs on the block phantom and on the belly. Our in-vivo calibration procedure meliorates this by measuring \( StO_2\text{superficial} \) on the belly.

The variation in the optical properties of the adipose tissue as observed in [15], in the worst case produces 21% variation in the APD of the oximeters, assuming SDS = 40 mm. However, in most realistic in-vivo measurements we expect a much lower variation (≈ 11%) due to the inter-quartile range of adipose tissue optical properties reported in [15] with small intra-subject variation.

4.7. **Comparison to [14], presenting data from the same phantom**

This study was conducted by employing mixture no. 3 of phantom 2 which is described in [14]. We compared the results reported here to those reported in [14]. In the present measurement we converted OxVLS raw values to those which would have been measured by OxiplexTS at \( C_{tHb} = 70 \mu M \) based on the equation in [14]. In Fig. 4(c) we would therefore expect a linear equation of \( y = x + 0 \) on the 2.5 mm window and have found \( y = 1.005 \times x - 3.5 \) which in our opinion is in a good agreement with our previous findings. The OxiplexTS to OxyPrem v1.3 equation for \( C_{tHb} = 70 \mu M \) was \( y = 0.77x + 17.2 \) in [14]. In the present experiment the comparison of linear fits for the 2.5 mm window yields \( y = 0.71x + 23.9 \). This results in an average difference of 3.3% for 30% < \( StO_2 < 80\% \) between the two experiments. For the OxiplexTS to INVOS adult equation this difference is in average 6.4% in the same range of \( StO_2 \). As a result there was a good agreement between the results reported here and our previous findings. Small differences between the phantom employed here and the phantom in [14], as described in section 2.2, may explain the small differences in the results we reported.

4.8. **Applicability to cerebral oximetry**

The calibration method demonstrated in the current manuscript reduces the effect of the superficial adipose tissue layer in muscle oximetry. This is only feasible if a measurement can be conducted on a region in which the thickness of the adipose tissue is high (> 3 APD). Thus, this method is not applicable in absolute brain oximetry to remove the influence of the skull.

4.9. **Contribution of myoglobin**

Boushel et al. state in their review on muscle oximetry that the spectra of hemoglobin and myoglobin are indistinguishable, e.g. the absorption peak at 755 nm of hemoglobin is shifted by only ≈ 5 nm to 760 nm due to presence of myoglobin [33,34]. Since most NIRS oximeters apply LEDs with broad wavelength distribution of ≈ 30 nm (Full width at half maximum (FWHM)), they cannot distinguish between presence and absence of myoglobin. As a result our phantom containing only hemoglobin was a realistic model of human muscle.

5. **Conclusion**

We conclude that all oximeters employed in this study were seriously affected by the thickness of the superficial layer. At 16 mm window thickness, sensitivity was only a small fraction of the sensitivity at 2.5 mm window thickness, but all oximeters still measured the changes in phantom...
oxygenation. We modeled the trend of the relative sensitivity of the oximeters, measured in-vitro, in relation to window thickness with sigmoid functions. Generally, relative sensitivity decreased to \( \approx 50\% \) when the window thickness was equal to the average penetration depth of each oximeter in the windows. OxiplexTS performed better than the continuous-wave oximeters, but was still seriously affected by the thickness of the superficial layer. Our OxyPrem v1.3 in-vivo calibration procedure significantly reduced the maximum measurement error due to adipose tissue thickness but its practical value has to be further studied because it depends on a number of assumptions and involves a multiplication of any error of estimation. A similar calibration procedure for \( \Delta S\!rO_2 \) measurement can be applied to all NIRS oximeters employed in this study.

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