

Validation Of NIRS In Measuring Tissue Hemoglobin Concentration And Oxygen Saturation on Ex Vivo and Isolated Limb Models

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ABSTRACT

Photonify's tissue spectrometer uses Near-Infrared Spectroscopy for real-time, noninvasive measurement of hemoglobin concentration and oxygen saturation [SO₂] of biological tissues. The technology was validated by a series of ex vivo and animal studies. In the ex vivo experiment, a close loop blood circulation system was built, precisely controlling the oxygen saturation and the hemoglobin concentration of a liquid phantom. Photonify's tissue spectrometer was placed on the surface of the liquid phantom for real time measurement and compared with a gas analyzer, considered the gold standard to measure oxygen saturation and hemoglobin concentration. In the animal experiment, the right hind limb of each dog accepted onto the study was surgically removed. The limb was kept viable by connecting the femoral vein and artery to a blood-primed extracorporeal circuit. Different concentrations of hemoglobin were obtained by adding designated amount of saline solution into the perfusion circuit. Photonify's tissue spectrometers measured oxygen saturation and hemoglobin concentration at various locations on the limb and compared with gas analyzer results. The test results demonstrated that Photonify's tissue spectrometers were able to detect the relative changes in tissue oxygen saturation and hemoglobin concentration with a high linear correlation compared to the gas analyzer

Keywords: hemoglobin concentration, tissue oxygen saturation, NIR spectroscopy, isolated limb model.

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1. INTRODUCTION

In biological tissue, near infrared light is highly scattered and lowly absorbed. The scattering is caused by the ultrastructure of the tissue whose heterogeneity leads to fluctuations in optical properties. The absorption is caused by various forms of tissue chromophores. In the wavelength range of 700- 900 nm, the oxygenated hemoglobin and the deoxygenated hemoglobin dominate the optical absorption. The light in this wavelength range can penetrate as deep as several centimeters into the tissue, which provides a useful tool for quantification of local tissue parameters such as hemoglobin concentration ($[Hgb] = [Hb] + [HbO_2]$) and tissue oxygen saturation ($[SO_2]$). Due to its real time, non-invasive feature, the NIR technology has gained widespread use in clinical applications such as functional brain imaging, PVD diagnosis, breast cancer diagnosis, plastic surgery monitoring, neonatology, trans-abdominal fetal oximetry, etc. [1-7].

Three common NIR methodologies have been developed for monitoring deep interior tissue volumes from the surface: continuous wave (CW), time-domain photon migration, and frequency-domain photon migration. They differ in the time dependence of the source intensity. Time-domain photon migration, and frequency-domain photon migration tap the temporal information associated with light propagation through tissue, the former discretely and the later continuously. While the temporal propagation information is potentially useful, the cost of goods and the extracting information are major barriers to clinical utility. Moreover, most linear reconstruction algorithms need baseline measurements from normal tissue and only report deviations from the normal tissue sample, not absolute measurements.

Photonify's tissue spectrometer is a CW device designed for non-invasive, quantitative characterization of tissue hemoglobin concentration $[Hgb]$ and oxygen saturation $[SO_2]$ [6]. The advanced algorithm was developed based on the diffuse equation to calculate the tissue scattering and absorption coefficients at different wavelengths. The self-calibration algorithm and the system hardware design minimizes the error caused by the opto-electrical component variation and the improper contact at the tissue-probe interface. The technology was validated quantitatively through a series of bench-top and animal tests conducted within the Photonify facilities and at contract laboratories. The test results indicate that Photonify's tissue spectrometer prototype is an effective tool for non-invasive measurement of tissue hemoglobin concentration and oxygen saturation.

2. MATERIALS AND METHODS

The Photonify's tissue spectrometer system consists of a computer console (Figure 1) and multiple optical probes (Figure 2). This research model console has embedded PIC controller and LCD display, and integrates source /detector control, data acquisition, data analysis and image display functions. The optical probe is made of multiple fiber bundles housed by an Aluminum probe head with designated source-detector geometry.



Figure 1 . Photonify's tissue spectrometer prototype.



Figure 2 . Close view of the spectrometer probe.

2.1. Ex vivo Validation

A close loop blood circulation system was developed for the ex vivo validation of Photonify's tissue spectrometer. Major control parameters of the study include the blood oxygenation level, the hemoglobin concentration, the PH value, the partial pressure of the dissolved oxygen in the solution, the phantom temperature, etc. An OSM3 hemoximeter and an ABL505 gas analyzer were used as standards for the measurement of oxygen partial pressure, oxygen saturation and hemoglobin concentration.

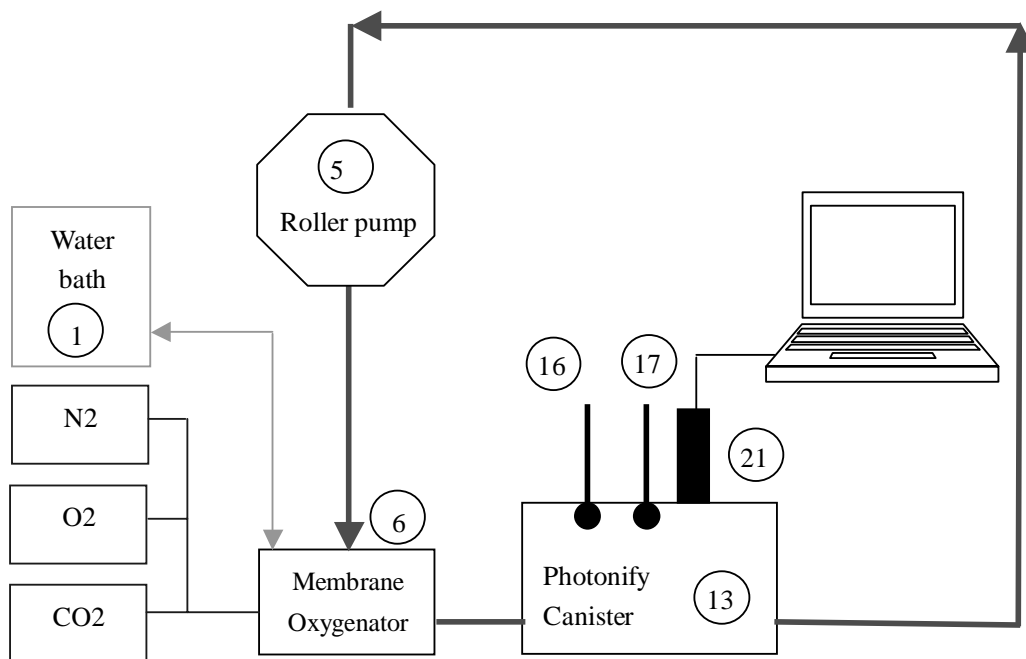


Figure 3 Sketch of the layout for ex vivo validation of Photonify's tissue spectrometer.

Figure 3 shows the schematic layout of the blood circuit. A canister (13) was filled with blood-intralipid solution and sealed on the top. A transparent window was opened on the lid of the canister for Photonify's tissue spectrometer probe (21). An oxygen partial pressure meter (16) and a PH meter(17) were placed into the liquid phantom for real time monitoring. A sampling port was opened next to Photonify's probe in order to collect phantom samples for gas analyzer measurement. A roller pump (5) pumped the liquid phantom through a membrane oxygenator (6) and then back to the canister. A mixture of $\text{CO}_2/\text{O}_2/\text{N}_2$ at a certain ratio was used to control the oxygenation and pH level of the phantom solution flowing through the oxygenator. The phantom solution temperature was maintained by a water bath connected to the oxygenator. The picture of the benchtop setup is shown in Figure 4.

The ex-vivo validation consisted of two tests: a stepped deoxygenation test and stepped hemoglobin concentration test. The stepped deoxygenation test quantifies the linear correlation between Photonify's tissue spectrometer and the gold standard gas analyzer through the entire oxygenation range. The stepped hemoglobin concentration test quantifies the linearity of Photonify's tissue spectrometer in measuring hemoglobin concentrations of different levels.

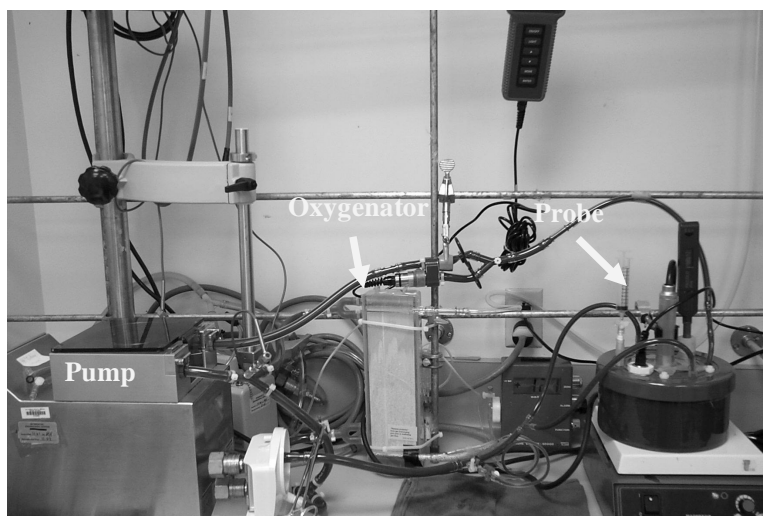


Figure 4 Picture of Photonify's tissue spectrometer benchtop setup

The blood circulation system shown in Figure 4 was first rinsed with saline. Then a designated amount of blood intralipid solution was slowly primed into the circuit, filling the canister. The temperature was controlled at 37°C and the pH level within a range of 7.35 – 7.45. The flow rate of the roller pump was adjusted to 3 LPM and the magnetic stirrer maintained on “low”. In the stepped deoxygenation procedure, the flow rates of N_2 , O_2 and CO_2 were adjusted to a stabilized ratio so that the oxygen saturation of the blood solution was controlled at a designated level ranging from above 95% to below 5%. After equilibrium had

been reached, Photonify's tissue spectrometer collected data while 2cc of blood sample was drawn from the sampling port of the canister for standard measurements using ABL505 gas analyzer and OSM3 hemoximeter, respectively. In the stepped hemoglobin concentration procedure, the oxygenation level was maintained to an intermediate level while a designated amount of blood was primed into the circuit to successively increase the hemoglobin concentration level. After the solution was completely mixed and stabilized, the blood solution [Hgb] level was measured simultaneously by Photonify's tissue spectrometer and the gas analyzer.

2.2. Animal Validation

The objective of the animal study was to validate Photonify's tissue oximetry technology through a controlled study on isolated canine limbs. The right hind limb of each dog accepted into this study was surgically removed. The limb was kept viable by cannulating the femoral vein and artery and connecting to a blood-primed extracorporeal circuit with blood pump and oxygenator, as shown in Figure 5. Each dog was euthanized after collecting blood for use in the perfusion circuit. A total of four probes, two probes from each of two of Photonify's Tissue Spectrometer systems, were used for data collection. These four probes were placed at four different locations on the limb. The positioning of four probes considered avoiding major vessels, tubes and bony areas. Figure 6 sketches the limb model and the probe layout. The local tissue oxygenation and hemoglobin concentration at four positions was captured by four probes simultaneously. After baseline readings were taken, the stepped deoxygenation and the stepped hemoglobin concentration tests were performed.



Figure 5 System setup for the animal study

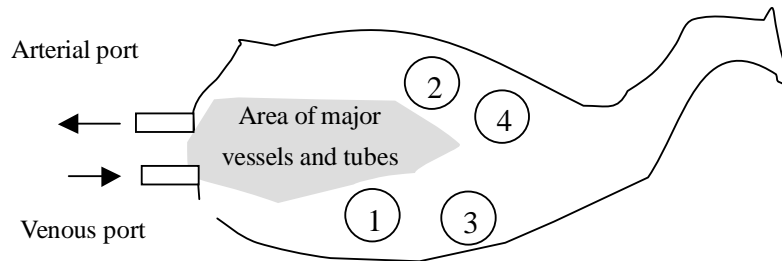


Figure 6 Sketch of the limb model and the probe layout

In the stepped deoxygenation process, the tissue oxygenation was controlled to stabilize at different levels ranging from above 95% to below 5% by adjusting the flow rate of oxygen, carbon dioxide and nitrogen delivered to the oxygenator. Photonify's measurements of tissue oxygen saturation at different positions were compared with the OSM3 gas analyzer measurement for correlation.

In the stepped hemoglobin concentration test, decreasing concentrations of hemoglobin ([Hgb]) were obtained by adding a designated amount of saline solution into the perfusion circuit. Photonify's measurement of [Hgb] was compared with the OSM3 measurement.

3. RESULTS AND CONCLUSIONS

3.1. Ex vivo test results

In the stepped deoxygenation test, the bovine whole blood was diluted to 3g/dL in saline (no lipid was added) in order to match the measurement range of the OSM3 hemoximeter. The results for 3 runs of tests were plotted in Figure 7. From the figure, one can see a linear correlation between Photonify's measurement and OSM3 measurement, with a high correlation factor of 0.9883. The Photonify and OSM3 reading reached 1:1 correlation as shown in Figure 8, after a calibration slope of 1.1404 and a calibration offset of -21.976 were applied.

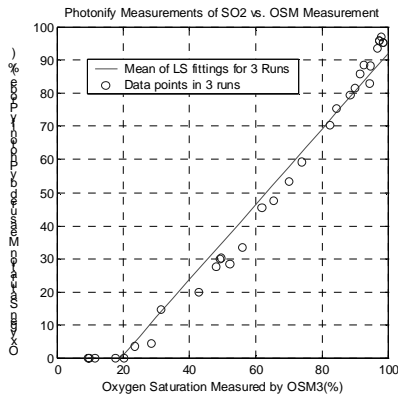


Figure 7 Correlation for [SO₂] measurements by PTS and OSM3.

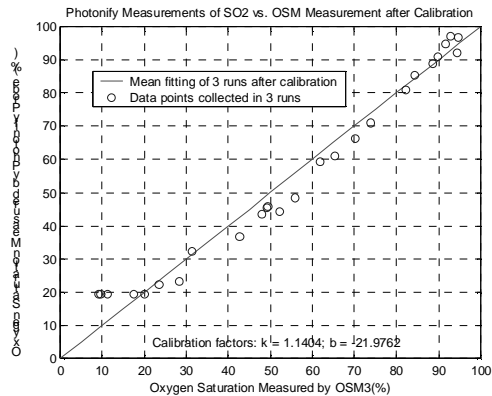


Figure 8 Correlation for [SO₂] measurements after calibration factors were applied.

The oxygen dissociation curve relates oxygen saturation (SO₂) and oxygen partial pressure in the blood (PO₂), and is determined by the hemoglobin's affinity for oxygen, that is, the hemoglobin's capacity to carry and release oxygen molecules from its surrounding tissue. The earliest attempt to analyze hemoglobin's sigmoid O₂ dissociation curve was formulated by the following Hill equation, where n is called the Hill constant [9]:

$$\% S_{O_2} = \frac{100\% \cdot (P_{O_2})^n}{(P_{50})^n + (P_{O_2})^n}$$

The simultaneous measurement of the oxygen saturation (by Photonify's tissue spectrometer and OSM3 hemoximeter) and the oxygen partial pressure (by ABL505 gas analyzer) enables the comparison of the oxygen dissociation curves obtained by different devices. In Figure 9, the oxygen dissociation curve plotted according to Photonify's readings shows significant similarity to that based on OSM readings. After applying the same calibration factors shown in Figure 8, Photonify's oxygen dissociation curve correlates with the OSM. This, on other hand, demonstrates the accuracy of Photonify's tissue spectrometer in measuring the oxygen saturation level.

The stepped hemoglobin tests were performed on a liquid phantom with 10% lipid and the blood percentage ranging from 1% to 6.7%. Photonify's tissue spectrometer readings show extremely high linear correlation (correlation coefficient: 0.9999) as plotted in figure 11.

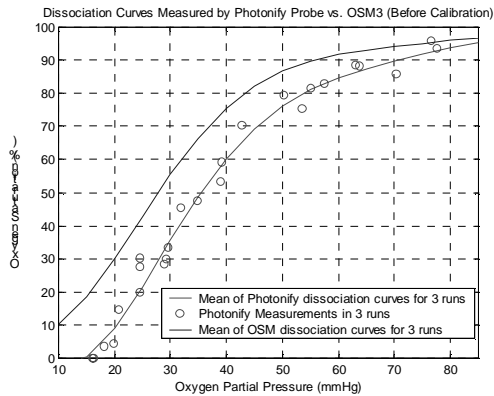


Figure 9 Dissociation curves based on $[SO_2]$ readings from PTS and OSM3.

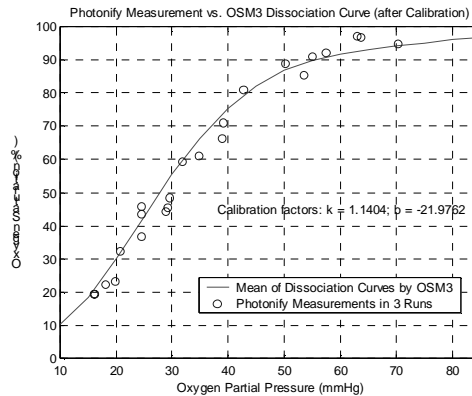


Figure 10 Dissociation curves after calibration factors were applied.

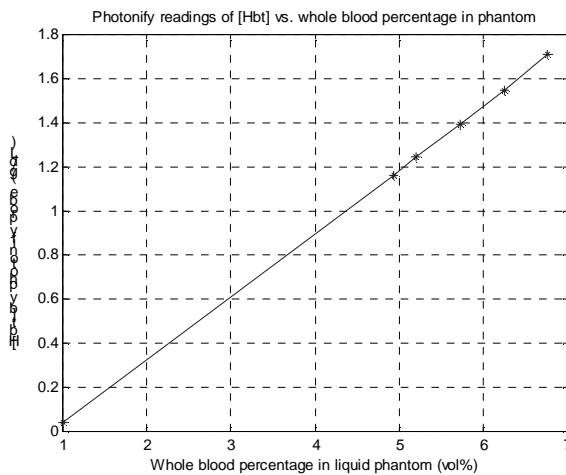


Figure 11 Linear correlation between photonify's reading of [Hgb] and the volume percentage of the blood in liquid phantom

3.2. Animal tests

The right hind limb of a male adult Mongrel canine was measured by Photonify's tissue spectrometer probes using the setup shown in Figure 4. Figure 11 shows the correlation between $[SO_2]$ readings by one of Photonify's probes (probe #4) and OSM3 readings in the stepped deoxygenation test. The [Hgb] correlations in the stepped hemoglobin test are plotted in figure 12.

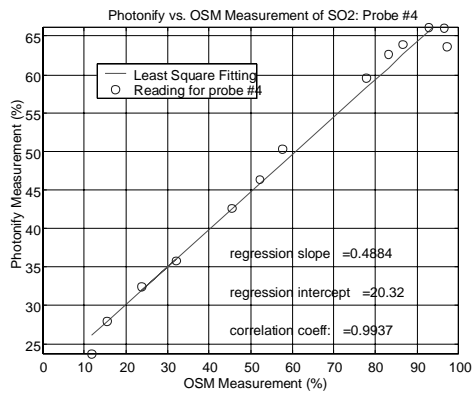


Figure 12 Correlation between Photonify's reading (probe #4) and OSM reading of [SO₂] during the stepped de-oxygenation test on an isolated limb model

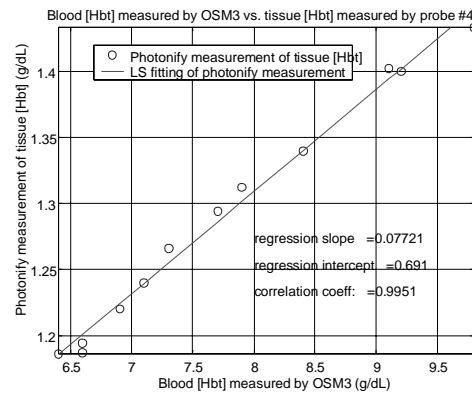


Figure 13 Correlation between Photonify's reading (probe #4) and OSM reading of [Hgb] during the stepped hemoglobin test on an isolated limb model.

The correlation coefficients for all the four Photonify spectrometer probes when compared with the standard readings in the stepped [SO₂] and [Hgb] tests are listed in Table 1 below.

Probe No.	[SO ₂] correlation between Photonify's spectrometer and OSM hemoximeter	[Hbt] correlation between Photonify's spectrometer and OSM hemoximeter
Probe #1	0.927	0.9914
Probe #2	0.994	0.9761
Probe #3	0.9943	0.3593
Probe #4	0.9937	0.9951

Table 1 Correlations between Photonify readings and OSM readings in stepped [SO₂] and [Hgb] tests

From the above table, one can observe high correlation for all four probes in [SO₂] measurements. As to [Hgb], all probes except probe #3 show high correlation. Investigation on probe #3 indicated that the probe was compressed deep against the tissue, which may block the local blood flow and cause inconsistency in the [Hgb] measurements.

3.3. Conclusion and future works

Both the ex vivo tests and the animal tests have demonstrated that Photonify's tissue spectrometers are able to detect the relative changes in tissue oxygen saturation and hemoglobin concentration with a high linear correlation when compared with the golden standard measurements of blood gas analyzer and hemoximeter. Clinical evaluation is in progress to confirm these results. The challenge for clinical validation will be to differentiate the instrument fluctuations from normal physiological variation. In addition, non-invasive devices are not available for direct comparison of [Hgb] and [SO₂] in human tissue.

4. REFERENCES

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