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In Vitro Determination of Concentration-Dependent Optical Properties of Hemoglobin

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Abstract: The interest of this paper is the determination of the optical properties of oxygenated (saturation above 97 %) hemoglobin in clinical relevant concentrations (ranging from 5 to 15 g/dl), dependent on the layer thickness. Furthermore the generation of a high rate data set for training with machine learning approaches was intended. With a double integrating sphere setup (laser diodes from 780 to 1310 nm) - as a well referenced method - and flow through optical cuvettes ranging from 1 to 3 mm layer thickness, the transmission (M_T) and reflection (M_R) values of the samples were acquired. From those the layer thickness independent absorption (μ_a) and reduced scattering coefficients (μ_s') were calculated by the means of the Inverse Adding Doubling (IAD) algorithm. For each sample the same coefficients should result correspondingly for all cuvette thicknesses in test. This relationship serves as an internal standard in the evaluation of the collected data sets. In parallel a spectrophotometer in the range from 690 to 1000 nm recorded transmission spectra for all samples as a second reference. First, the IAD algorithm provided optical coefficients (μ_a , μ_s') in all measurements, with few exceptions at low hemoglobin concentrations. The resulting coefficients match independently of the layer thickness. As a main second result, a high rate data set was generated which serves for further analysis - for example with machine learning approaches.

Keywords: optical interaction coefficients, optical properties, blood, hemoglobin concentration

1 Introduction

Hemoglobin - bound in the erythrocytes of human blood - is the main light absorber in the tissue. Parallel to this effect, the phenomenon of diffuse reflection - generated by the special shape of the erythrocytes - should be considered. The relatively strong absorption of hemoglobin results in the principle for the determination of the hemoglobin concentration

on the basis of optical non-invasive transmission approaches [1]. Other methods work on the principle of reflection [2], or - to generate a change in "optical layer thickness" - additionally with occlusion [3]. An elimination of the layer thickness as in the principle of pulse oximetry (ratio of ratios of the pulsatile signal) fails due to the often inadequate signal quality, clinically attributed to reduced perfusion in the sensor area [4]. For the development of non-invasive sensors in the measurement of total hemoglobin concentration (tHb in g/dL) it is imperative to know the exact optical properties of hemoglobin under clinically acceptable conditions. The standard setup for measuring the optical properties of transmission and reflection is based on the principle of using two integrating spheres. These variables are strongly dependent on the hemoglobin concentration on the one hand and the layer thickness on the other hand. For the standardized laboratory measurement, it is essential to record the deflection of both parameters - adapted to the clinical marginal limits - in the entire width. The Inverse Adding Doubling (IAD) algorithm [5] additionally allows the determination of absorption coefficients (μ_a) and reduced scattering coefficients (μ_s') in the samples. These are layer thickness independent and can thus be used for a plausibility check of the measurements. The test setup [6] presented here basically meets these requirements. In parallel the validation of the generated data sets - as a second optical system - is based on continuous recording by a spectrophotometer. In this context the following goals should be achieved:

1. To measure the optical properties under the clinically relevant conditions characterized by the requirements (tHb, layer thicknesses, selected wavelengths).
2. Establish conditions with flow through optical cuvettes that meet clinical conditions.
3. Generate a sufficiently sized data set to enable training on "machine learning" approaches.

2 Material and Methods

2.1 Measurement setup

The tISM (transportable Integrating Sphere Setup for Standardized Measurements [6]) and a spectrophotometer setup -

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Fig. 1: Measurement setup (double integrating spheres and spectrophotometer). A peristaltic pump generates a flow through in the optical cuvette, alternately placed in the systems.

using an Epp2000-UVN-100 (StellarNet Inc, USA) - are combined with a peristaltic pump as specified in Figure 1 and Table 1. Three self-manufactured flow through optical cuvettes with different layer thicknesses (1, 2, 3 mm) - to simulate different vessel diameters - were measured alternately in both setups.

2.1.1 tISM

For the presented measurements tISM - equipped with lasers of the wavelengths 780 nm, 808 nm, 850 nm, 980 nm as well as 1310 nm - acquired the reflection and transmission values of the samples. The reflection (M_R) is defined as the amount of light reflected from the sample into the reflection sphere, compared to a sample with total reflection. Whereas the transmission (M_T) is defined as the amount of light transiting through the sample into the transmission sphere, in relation to the light intensity without sample. Both values are given as a ratio, where 1 equals total reflection/transmission and 0 equals no reflection/transmission. In tISM the reflection and transmission are calculated by a microcontroller - taking into account the shunt light - averaging over ten measurements (in 500 ms). tISM is calibrated with an internal reference, based on the findings from previous investigations [6]. A suspension prepared from Intralipid (ClinOleic 20%, Baxter, USA), India ink (Fount India, Pelikan, Germany) and distilled water serve as a precise reference for adjustment [6]. From the measured values (M_R , M_T) and the system parameters - including the layer thickness - the absorption coefficient μ_a and the reduced scattering coefficient μ_s' are calculated with the IAD algorithm (version: 3-9-12) [5]. Both the calculated absorption coefficient

Tab. 1: Measurement parameters of tISM and the EPP2000.

	tISM	Epp2000
Measurements	Transmission (M_T)	Transmission
	Reflection (M_R)	
Calculations	Absorption (μ_a)	
	reduced Scattering (μ_s')	
Wavelengths	780, 808, 850, 980, 1310 nm	690...1000 nm
Layer Thickness		1, 2, 3 mm
Concentrations		5, 6, ..., 15 g/dl
Oxygenation		$\geq 97\%$
Test Series		4
Repetitions	3	1
Data Sets	396	132

cient (μ_a), representing the absorption and the reduced scattering coefficient (μ_s'), representing scattering in the samples, are values independent from the layer thickness.

2.1.2 Spectrophotometer

With an Epp2000-UVN-100 (StellarNet Inc, USA) spectrophotometer the transmission spectra in the range from 690 to 1000 nm were recorded with a step size of 0.75 nm. The range results from the intersection of the theoretical spectrometer range and the light source used (SL1 Tungsten Halogen Lamp, StellarNet Inc, USA). The data were acquired with SpectraWiz (version: 5.33, StellarNet Inc, USA) automatically averaging out of ten spectra per data set. The transmission is given as a total value in counts per ms.

2.2 Hemoglobin dilution

The erythrocyte concentrates (EC) provided from the Institute for Transfusion Medicine (University Medical Center Lübeck, Germany) were no longer applicable for patient treatment.

Tab. 2: Schematic representation of the generated data sets.

Sample			Cuvette		
No.	tHb [g/dl]		1 mm	2 mm	3 mm
EC 1	1	5	N1-3	N34-36	N67-69
	:	:	:	:	:
	11	15	N31-33	N64-66	N97-99
:	:	:	:	:	:
EC 4	34	5	N298-300	N331-333	N364-366
	:	:	:	:	:
	44	15	N328-330	N361-363	N394-396

Heparin and sodium bicarbonate were supplemented to prevent from coagulation and to buffer the pH-value. From each EC eleven samples with uniformly distributed hemoglobin concentrations were prepared to target the range from 5 to 15 g/dl. The dilution was performed with saline and referenced with an IL-682 CO-Oximeter (Instrumentation Laboratory, USA). The oxygen saturation was monitored, to be above 97 %. A total of 4 ECs were processed on separate days, resulting in 396 data sets acquired with tISM (Table 2) and another 132 data sets with the spectrophotometer.

3 Results

According to goal 1 and 2 with tISM and the spectrophotometer measurements could be recorded for all samples at all layer thicknesses. The calculation of the optical parameters (μ_a , μ_s') by means of IAD was achievable for almost all values (97,1 %). The calculations with the 1 mm optical flow through cuvette from the measurements N1-9 (EC 1, tHb ≤ 7 g/dl) at 780 nm failed, and those from all samples with a hemoglobin concentration less equal to 8 g/dl at 1310 nm were labeled with a warning, due to extreme transmission values in this area.

3.1 tISM

As expected, the dependence of the transmission values on the layer thickness is clearly confirmed. This relationship could be represented for all wavelengths. In contrast the reflection values of the measurements with layer thicknesses of 1 to 3 mm are nearly identically - demonstrating, that these data seem to be independent of the layer thickness (Figure 3).

3.2 Spectrophotometer

An exemplary presentation of the spectra from EC 1 at 1 mm layer thickness is given in Figure 4 - with inverted hemoglobin concentration axis for clear presentation. The peak transmission is located at 790 nm. A nonlinear relationship between transmission and hemoglobin concentration was detected.

4 Discussion

4.1 tISM

For all datasets the variations in the test setup are smaller than the differences between the optical properties of the four ECs.

Fig. 2: Averaged transmission values, dependent on the hemoglobin concentration and the wavelength for the 1, 2 and 3 mm optical flow through cuvettes.

Fig. 3: Averaged reflection values.

Both the calculated absorption and reduced scattering coefficients from the different cuvettes are similar (see Figure 5 and 6). The results of the 2 and 3 mm cuvettes are nearly identical, while the calculations from the 1 mm cuvette yielded slightly higher average values. The calculated coefficients of the 1 mm cuvette at 980 nm are additionally slightly increased.

4.2 Spectrophotometer

The data generated by the spectrophotometer serves as a second reference in the range between 690 and 1000 nm. In the Data no systematic deviations could be detected. The results of the measurements with different erythrocyte concentrates coincide with small deviations. The spectra are strongly analog to the transmission data from the measurements in tISM. The ratio of the transmissions in the different layer thicknesses is comparable to those in tISM (in contrast to Figure 2 the spectrum in Figure 4 is inverted due to the more clearly representation of the data).

Fig. 4: Spectrophotometer data (transmission) of EC 1 at 1 mm dependent on the hemoglobin concentration and the wavelength. The hemoglobin concentration was inverted for clear presentation.

Fig. 5: Averaged absorption coefficient μ_a .

5 Conclusion

The optical properties of hemoglobin in clinically relevant concentrations for layer thicknesses of 1 to 3 mm were determined with tISM and the spectrophotometer. The calculation by the IAD - which is absolutely sensitive for minor variations - was successful with slight limitations at low hemoglobin concentrations. Comparisons between the results of different layer thicknesses fit with these minor differences. The original hypotheses of goal 1 and 2 can be accepted according to the results. With respect to the goal number 3, a high rate data set could be generated, serving as basis for further analysis with machine learning approaches.

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Fig. 6: Averaged reduced scattering coefficient μ_s' .

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