Non-invasive measurement of blood and tissue parameters based on VIS-NIR spectroscopy

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ABSTRACT

Currently, invasive methods are used to measure the hemoglobin concentration and the most hemoglobin-derivatives, whereby blood is taken from the patient and subsequently analyzed. The noninvasive method presented here allows pain free continuous on-line patient monitoring with minimum risk of infection and facilitates real time data monitoring allowing immediate clinical reaction to the measured data. Visible and near infrared (VIS-NIR) spectroscopy in combination with photo-plethysmography (PPG) is used for a detection of human tissue properties and the measurement of hemoglobin concentration in whole blood and hemoglobin derivatives. The absorption, scattering and the anisotropy of blood and tissue is a function of the irradiated wavelengths. This fact is used to calculate the optical absorbability characteristic of blood and tissue which is yielding information about blood components like hemoglobin-concentration (cHb), carboxyhemoglobin (COHb) and arterial oxygen saturation (SaO2). The ratio between the PPG peak to peak pulse amplitudes for each wavelength is used in combination with a dynamic spectrum extraction. The prediction of the blood- and tissue-parameters is based on a Principal Component Regression (PCR) method. The non-invasive sensor system is calibrated with a lab based artificial blood circulatory system and with data from clinical studies.

Keywords: hemoglobin, noninvasive, carboxyhemoglobin, spectroscopy, photo-plethysmography

1. INTRODUCTION

This paper reports about fundamentals, simulations and measurements of optical absorption characteristics of whole blood. The human circulatory system is a fast regulatory transport system. This closed circuit consists of parallel and serially connected blood vessels for which the heart acts as a circulation pump. The mandatory blood circulation is maintained by the contraction of the muscular cardiac wall. The heart is made up of a left and right cardiac half, each of them being a muscular hollow organ. In functional and morphologic terms the circulatory system consists of two serial sections. The heart also can be considered as two serial pumps. The right cardiac side absorbs the deoxygenated blood from the body and transports it to the lungs (lung circuit), where it is re-oxygenated. The oxygenated blood arrives at the left cardiac side, wherefrom the dispensation to various organs takes place (body circuit). The oxygenated blood in the body circuit is pumped from the left ventricle to the aorta and main arteries whose sub branches lead to the tissue and organs. Following the next stage of branching into arteriole and capillary, the oxygen and nutrients and the ingestion of carbon monoxide and intermediate catabolic products are distributed to the tissue and organs of the body. Finally the capillaries terminate into venules and veins which transport the deoxygenated blood back to the right atrium. A sensor system to measure blood components like hemoglobin is presented and corresponding results of in-vitro and in-vivo measurements. As basic technology NIR-spectroscopy and Photoplethysmography (PPG) is used for these non-invasive optical measurements. The characteristic absorption coefficient of blood in the visible and NIR region is well known and is mainly influenced by the different hemoglobin derivate [1]. This fact is used to calculate the optical absorbability characteristics of blood which is yielding information about blood components as arterial oxygen saturation (SaO2), hemoglobin concentration (cHb), carboxyhemoglobin (COHb) and met-hemoglobin (MetHb). The measured spectra signals and the ratio between the peak to peak pulse amplitudes are used for a calculation of these parameters. Hemoglobin is the main component of red blood cells. The primary function of Hemoglobin is the transport of oxygen from the lungs to the tissue and carbon dioxide back to the lungs. The Hemoglobin concentration in human blood is an important parameter in evaluating the physiological status of an individual and an essential parameter in every blood count.

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In currently standards, invasive methods are used to measure the hemoglobin concentration and the most hemoglobin derivate, whereby blood is taken from the patient and subsequently analyzed. Apart from the discomfort of drawing blood samples, an added disadvantage of this method is the delay between the blood collection and its analysis, which does not allow real time patient monitoring in critical situations. A non-invasive method allows pain free continuous online patient monitoring with minimum risk of infection and facilitates real time data monitoring allowing immediate clinical reaction to the measured data. The non-invasive multi-spectral measurement method was tested and analyzed with spectrometers and prototype-devices based on radiation of monochromatic light emitted by laser diodes and by using light emitting diodes (LED) through an area of skin on the finger. The sensors assembled in this investigation are fully integrated into wearable finger clips.

Since the near infrared light was found to penetrate a great depth into biological tissues, near infrared spectroscopy has been developed into a non-invasive method for biomedical sensing and clinical diagnosis [2]. Oximetry is well known as typical example of a near-infrared application in clinic and can be used to non-invasive measure the functional arterial oxygen saturation (SpO2) of human blood in-vivo [3]. The hemoglobin concentration in human blood is also an important parameter to evaluate the physiological condition and the capability of oxygen transportation in blood. With this information anemia (a low hemoglobin level) and polycythemia vera (a high hemoglobin level) can be diagnosed and monitored. It is also possible to observe imminent postoperative bleedings and autologous retransfusions. It is well known that pulsatile changes of blood volume in tissue can be observed by measuring the transmission or reflection of light through it [4]. This diagnostic method is called Photoplethysmography (PPG). The newly developed multiwave-sensors system operates in the range of 500nm to 1400nm for the measurement of the hemoglobin concentration and hemoglobin derivate. For in-vitro tests and measurements a hemodynamic blood flow model (BFM) has been developed which allows spectrometer measurements and the validation of the new sensors. The absorption and scattering of blood in the visible and near infrared range is dominated by the red blood cells, the different hemoglobin derivates and the blood plasma that consists mainly of water. From on optical point of view, the human tissue is a turbid media. The radiation transport equation (1) can be regarded as the mathematical basis of tissue optics for the description of light-tissue interaction and the related phenomena [5].

\[
\frac{1}{c_n} \frac{\partial}{\partial t} + \mathbf{s}(\mathbf{n}) \mathbf{L}(\mathbf{r}, \mathbf{s}, t) = - (\mu_a + \mu_s) \mathbf{L}(\mathbf{r}, \mathbf{s}, t) + \mu_s \int \mathbf{P}(\mathbf{s}, \mathbf{s}') \mathbf{L}(\mathbf{r}, \mathbf{s}', t) d\Omega' + Q(\mathbf{r}, \mathbf{s}, t)
\]

(1)

Where \( \mathbf{L}(\mathbf{r}, \mathbf{s}, t) \) is the radiance intensity of light at position \( \mathbf{r} \) in direction \( \mathbf{s} \) at time \( t \), \( c_n = \frac{c_0}{n} \) is the velocity of light in tissue with index-of-refraction \( n \), \( \mathbf{s}(\mathbf{n}) \) is the unit vector direction \( \mathbf{s} \) and scattered direction \( \mathbf{s}' \), \( d\Omega' = \sin \nu' \partial\nu' \partial\phi' \) is the element of the solid angle, \( S(\mathbf{r}, t) \) is the energy intensity of the light source, \( Q(\mathbf{r}, \mathbf{s}, t) = \frac{\partial S(\mathbf{r}, t)}{\partial \Omega} \) is a source term.

Description of light-tissue interactions using the transport theory requires first to solve Eq.1. Because of difficulties to obtain exact solutions for biological tissues, several approximations have been made for the representations of \( P(\mathbf{s}, \mathbf{s}') \) and \( \mathbf{L}(\mathbf{r}, \mathbf{s}, t) \). Virtual modeling of turbid media, like biological tissue, and the description of photon transport in this media can lead to a better understanding of photonic processes and enables an effective development and optimization of optical systems.

In contrast to the analytical description of light-tissue-interaction with the radiation transport equation a numerical method like Monte Carlo simulation or Monte Carlo ray tracing, provides considerable advantages like greater flexibility during model development and higher accuracy of the results [6]. With the Monte-Carlo simulation (MC) it is possible to trace photons step by step through a turbid medium. A large quantity of the traced photons with their resulting paths in the medium results in a realistic mapping of the propagation of light in the target tissue [7]. The medium itself is described by the optical parameters scattering coefficient \( \mu_s \), absorption coefficient \( \mu_a \), the anisotropy coefficient \( g \) and the index of refraction \( n \). The Henyey-Greenstein phase function describes the probability of the polar deflection angle after each scattering event [8]. The simulations of light-tissue interactions were performed with the optic software ASAP® (Breault Research). ASAP® includes functions for simulations of geometric- and physical-optical-properties and delivers complete 3D-models of optical and mechanical systems. In addition the Monte-Carlo simulation is already...
implemented in the software. Besides the simulation of multi-layered tissue models, ASAP® was used to simulate cuvettes and blood transporting tubing’s of a hemodynamic blood flow model which has been developed. The results of the simulations provide important information such as optical path lengths, penetration depths and internal distributions of the energy in the target tissues. Furthermore the simulations supplement the analysis of real measurements.

2. HEMODYNAMIC BLOOD FLOW MODEL

A hemodynamic blood flow model (BFM) based on the human circulatory system was developed to allow a controlled variation of the blood parameters like hemoglobin concentration (cHb), oxyhemoglobin (O2Hb resp. SaO2), carboxyhemoglobin (COHb) and methemoglobin (MetHb) [9]. For this reason the optical properties of the blood were observed continuously by spectrometer measurements to determine the absorption, transmission and scattering properties of human whole blood in a wavelength range from 500 to 1000 nm and to test new noninvasive measurement systems.

The following blood parameters were varied by using the blood flow model:

- hemoglobin concentration (cHb)
- oxyhemoglobin (O2Hb)
- carboxyhemoglobin (COHb)
- deoxyhemoglobin ((HHb)
- temperature (T)
- flowrate (Q)
- kind of fumigation (N2-CO, N2, compressed air)
- flowrate of fumigation

The model enables a defined circulation of app. 250 ml of human blood. The peristaltic blood pump has a maximum rotation speed of 250 rpm (Q: 0 to 200 ml/min). To determine the optical properties of the blood components two grid spectrometer were used (Avantes AvaSpec 2048x14, Jeti VS140). With these spectrometers it is feasible to cover an overall range of 400nm to 1700 nm to obtain the absorption spectra of the blood. Both spectrometers are connected with optical cuvettes integrated in the BFM. All the measurements taken noninvasively were compared with sample results obtained by a Blood Gas Analyzer (BGA device) to validate the measurements. Fresh erythrocyte concentrate from human donors were centrifuged three times and washed in phosphate buffer solution (300 mmol/l, pH 7.4). By doing this the blood samples contained no plasma, proteins, leukocytes or thrombocytes. Light microscopy control of the samples ensured that they were neither hemolyzed nor were the cells deformed. Concentration of erythrocytes as well as hemoglobin was changed by adding plasma with different intervals. Blood circulation and predetermined oxygen states were adjusted with an extra-corporal circulation unit (Stöckert Instruments GmbH, Germany). The blood was gently stirred and kept flowing through the model blood tubes and the specially designed turbulence free cuvettes (for the

![Figure 1. Structure of the hemodynamic blood flow model (BFM) and photos during system operation.](http://proceedings.spiedigitallibrary.org/ss/rightslogin.aspx?doi=10.1117/12.2308510)
spectrometers) with a laminar flow. Oxygen saturation was adjusted by continuous flow of O₂, air and N₂ through a neonatal hollow fiber membrane oxygenator. Important parameters as Temp (°C), cHb (g/l), pH, pCO₂ (mmHg), pO₂ (mmHg), HbO₂ (%) were also measured by Blood Gas Analyzers. All investigations were derived with different oxygenation and hemoglobin levels. The blood temperature was kept constant at 37°C via a water heating mechanism.

3. IN-VITRO BLOOD SPECTRA ANALYSIS

The blood absorption spectra are obtained continuously for various oxygenation levels, hemoglobin contents and for variations of carboxyhemoglobin. Figure 2A shows the typical blood spectra (650nm-1000nm) from our measurements where the hemoglobin concentration remains constant (80g/l) and the oxygen saturation (HbO₂ resp. SaO₂) in the blood varies from 100% to 5%.

Figure 2. Typical blood absorption spectra (A) and the Second-Derivative-Spectra (B) with different SaO₂.

The principle of measurement is based on the fact of a substantial absorption/transmission difference of light in red and near infrared region between oxygenated hemoglobin (HbO₂), deoxygenated hemoglobin (HHb) and blood plasma (optical mainly water). As shown in Figure 2A, deoxygenated hemoglobin (HbO₂=5%) is optically much denser to the red light (650 nm to 750 nm) than oxygenated hemoglobin (HbO₂=100%), whereas the reverse is true in the near infrared region (900 nm to 1000 nm), even to a lesser degree. Note that there is an isosbestic wavelength band in the range of 800 nm to 810 nm within which the transmission of oxyhemoglobin and deoxyhemoglobin is nearly equal. The water absorption is very distinct in the blood near infrared spectrum for the wavelength of above 960 nm. Water has a weak absorption peak around 965nm. If the hemoglobin/hematocrit concentration of blood decreases, the plasma concentration in blood increases, and subsequently the water absorption should be relatively advanced. In figure 2A an absorption peak of deoxyhemoglobin around 760nm is apparently shown for the hypoxia blood but the water absorption at 965nm is not distinguishable.

Figure 3. Blood absorption spectra with different hemoglobin concentrations and identical HbO₂ of 98%.
Figure 2B shows the spectra of figure 2A after applying a second derivative transformation to the data of the original spectra. Second derivatives may swing with greater amplitude than the primary spectra. The more distinguishable second derivatives are especially useful for separating out weak peaks of overlapping absorption bands. In some cases derivative spectra can be a good noise filter since changes in base line have negligible effect on derivatives. In figure 2B the absorption peak of deoxyhemoglobin at 760nm and the water absorption peak at 965nm is clearly visible. Figure 3 shows the absorption spectra of blood with different hemoglobin concentrations and identical HbO2 of 98%. The water absorption is very distinct in the blood near infrared spectrum for the wavelength of above 960 nm. If the hemoglobin concentration (resp. the hematocrit) of blood decreases, the plasma concentration in blood increases, and subsequently the water absorption should be relatively advanced. In the measurement shown in Figure 3 it is obvious to see that the water absorption at 965nm becomes more pronounced for a decrease of the hemoglobin concentration in blood (conspicuous at cHb=46g/l). The variation of the blood hemoglobin (resp. hematocrit) induces the changes of both the absorption and the scattering coefficients of blood. With the BFM and the experimental results it was possible to perform the quantification of chromophore components in scattering media like blood by means of spectral multicomponent analysis which is based upon spectral correlation. The optical spectra of blood are analyzed by combination of this multilinear regression method with a Partial Least Squares Regression (PLSR) and Principal Components Regression (PCR). This both methods are used to model a response variable from the large number of variables (spectra points). Both methods construct new variables, known as components, as linear combinations of the original variables, but they construct those components in different ways. PCR creates components to explain the observed variability in the variables, without considering the response variable at all. PLSR takes the response variable into account and therefore often leads to models that are able to fit the response variable with fewer components. Generally the context can be expressed by the following matrix equation [10]

\[ A = \varepsilon CL + E \]  

(2)

where \( A = (A_j) \) expressed the measured absorption as a function of wavelength for \( j = 1 \) to \( n \) wavelength points. \( C = (C_i) \) is the concentration to be estimated by the multilinear regression, where the element \( C_i \) is the concentration of the \( i \)-th chromophore in the blood. \( E \) is the residual error in the multicomponent analysis in which multiple scattering effects are involved. The estimation in this form depends on the optical path length \( L \) and the term \( \varepsilon = (\varepsilon_{ij}) \) which expresses the specific absorption coefficients of the chromophores within the blood. During the estimation, a least squares routine is used to fit the measured data over the used spectral region.

Figure 4. Invasive reference chb measured by BGA vs. predicted noninvasive chb (A) and the derived scatter plot (B).

The figure 4 shows the result with the algorithm for the example of hemoglobin concentration in blood. A number of \( n=90 \) in-vitro measurements were made with a systematical variation of hemoglobin concentration and oxygen saturation separately or for both parameters together. The calibration model for chb is computed with measured blood absorption spectra over the region of 650nm to 1000nm with a number of 351 spectra points. The developed calibration models are applicable for oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, methemoglobin, hemoglobin concentration and hematocrit. The interaction of all these parameters is eliminated by a recursive algorithm. A second example in figure 5 shows the application for a measurement of carboxyhemoglobin in blood. Because of the spectral characteristics of carboxyhemoglobin the calibration model for COHb is based on absorption spectra in the region of 460nm to 590nm. The figure 5A shows a data set of spectra measured with the BFM with a variation of carboxyhemoglobin and oxyhemoglobin (functional). The Figure 5B shows a BFM measurement (2 hours) with a variation of carboxyhemoglobin in the blood. The figure shows the good correlation of the computed and predicted noninvasive...
values for carboxyhemoglobin (COHb) and the fractional oxygen saturation (HbO2) versus the invasive BGA reference values.

Figure 5. Blood absorption spectra with different levels of carboxyhemoglobin COHb and fractional oxygen saturation O2Hb (A) and measurement of COHb and HbO2 through a 0.5mm cuvette of the BFM (B).

4. **IN-VIVO BLOOD SPECTRA ANALYSIS**

After a good correspondence between in-vitro experimental results with cuvettes of the blood tube system (BFM) and our mathematic models the applicability of the method for in-vivo measurements was investigated. Figure 6 shows a measurement of the absorption spectra (650nm-1000nm) during a hypoxia study through the fingertip (index finger, dexter) of one subject. The arterial oxygen saturation during this breath down study was reduced to about 75%. Thereby the recorded data of the spectrometer was compared with the data of the blood-gas-analysis BGA from the A. radialis (arterial oxygenic saturation - SaO2 in %). In figure 6 the in-vivo absorption spectra of the finger with an arterial blood oxygenation SaO2=98%, SaO2=88% and SaO2=75% is represented. The hemoglobin concentration of the subject during the measurement was constant at 145 g/l.

![Absorption spectra](image)

At a first appearance the absorption spectra of a finger is comparable with the absorption spectra of whole blood (figure 2A). The arterial and venous blood layer in the finger causes the main percentage of absorption. The deoxyhemoglobin absorption peak is distinct in the spectra (caused by venous blood). The water absorption peak at 965nm is much stronger than in whole blood (compare figure 2A). This is caused by the high percentage of water in the bloodless tissue. Figure 6B shows the spectra of figure 6A after applying a second derivative transformation to the data of the original spectra. The figure 6B shows besides the absorption peaks of the blood chromophores some weak absorption bands of bloodless tissue components like fat and collagen. Deduced from the spectrometer measurements we developed a new sensor device which is based on a multi-wavelength measurement and allows a continuous non-invasive in-vivo detection of hemoglobin concentration, oxygen saturation and pulse [11]. The arteries contain more blood during the systolic phase of the heart than during the diastolic phase, due to an increased diameter of the arteries during the systolic phase. This effect occurs only in arteries but not in veins. For this reason the absorbance of light in tissues with arteries increases during
systole because the amount of hemoglobin (absorber) is higher and the light passes through a longer optical path length in the arteries. These intensity changes are the so called Photoplethysmography-waves (PPG). The time varying part allows the differentiation between the absorbance due to venous blood (DC part) and that due to the pulsatile component of the total absorbance (AC part). This effect is used to separate the arterial blood absorption from absorption of the other tissue components in our application. The in-vivo measurement technique requires a pulse signal for the calculation of the relative attenuation coefficients. Vasocostriction at the extremities can be a problem, as it decreases the signal amplitude, and therefore the signal to noise ratio. Small signal amplitudes tend to give inaccurate results. This may be a limitation when using the system on various patient groups.

One of the newly developed sensor devices emits light in the range of wavelengths between 600 – 1000 nm (670, 808, 905 and 980 nm). This is the therapeutic window region, in which the blood absorption is dominated by the hemoglobin derivatives. Additionally a wavelength of 1300 nm is integrated. At this wavelength the absorption of water is dominant. The frontpanel of the online software is shown in figure 7A [12].

Figure 7. Frontpanel online-software for cHb measurement (A) and non-invasive cHb vs. invasive cHb (HemoCue™) (B).

A pre-study with n=43 adult healthy male and female volunteers (range 19 - 60 years, 15 women and 28 men) was already implemented to test the ability to measure the hemoglobin content non-invasive and in-vivo. The third or fourth finger of the left hand was connected with the new sensor. A HemoCue™ hemoglobin device (HemoCue AB, Sweden) was used for invasive reference measurements. This system provides reliable quantitative hemoglobin results with the same performance as a large hematology analyzer. A drop of capillary blood was taken from the same fingertip used for the photometric measurements for each volunteer, and analyzed with the HemoCue™ device. The figure 7B shows the results of the non-invasive photometric measurements vs. the invasive measured hemoglobin values (131 measurements). In this study, we achieved good results with our application for a non-invasive hemoglobin determination. The example is too small to represent the variability in the population. Therefore the whole data set of 43 volunteers is not sufficient by itself to further evaluate the effects of patient-to-patient variation on the measurement method. The potential of this photometric method to measure the non-invasive hemoglobin and other hemoglobin derivatives in-vivo were proved with good results. Future work will involve further clinical studies, which allows an extensive statistical analysis of these measurements.

5. CONCLUSIONS

In this paper a multi-wavelength photometric measurement method that provides non-invasive in-vitro and in-vivo spectral measurements in human blood and tissue has been described. The fact that the absorption-coefficients $\mu_a$ and scattering-coefficients $\mu_s$ for blood differ at different wavelengths has been exploited and is used for calculation of the optical absorbability characteristics of human blood yielding information on the blood composition. The results for non-invasive in-vitro determinations of hemoglobin and carboxyhemoglobin of whole blood are shown in the paper. For these measurements a hemodynamic bloodstream model BFM has been used which allows spectrometer measurements and the validation of sensor devices. The potential to measure the hemoglobin content of blood in-vivo were proved and demonstrated. What could not be determined so far in a first approach is a measurement with a large
group of patients and volunteers. Future work will involve further clinical studies with this measurement method. A main aspect is the test of an optimized hardware system and a further evaluation of suitable statistical on-line analysis algorithms for the blood components.

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