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Carlos Cuartas-Vélez, Colin Veenstra, Saskia Kruitwagen, Wilma Petersen, Nienke Bosschaart, "Quantification of total haemoglobin concentrations in human whole-blood based on the optical density with spectroscopic optical coherence tomography," Proc. SPIE 11924, Optical Coherence Imaging Techniques and Imaging in Scattering Media IV, 119240R (9 December 2021); doi: 10.1117/12.2616058

SPIE.

Event: European Conferences on Biomedical Optics, 2021, Online Only

Quantification of total haemoglobin concentrations in human whole-blood based on the optical density with spectroscopic optical coherence tomography

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Abstract: We present a novel processing technique to retrieve total haemoglobin concentrations with spectroscopic optical coherence tomography data based on numerical optimization of the optical density. We validated our method with *ex-vivo* human whole blood.

1. Introduction

The diagnosis of haematologic disorders such as anaemia and polycyathemia requires the quantification of total haemoglobin concentrations [tHb] in blood. Haemoglobin is a protein contained by red blood cells (RBCs) and its main function is to transport oxygen molecules through the body. According to the oxygen content, haemoglobin is classified as oxy-haemoglobin (HbO_2) if saturated with oxygen and deoxy-haemoglobin (Hb) if unsaturated. Together, Hb and HbO_2 represent the [tHb] of blood. Standard measurements to quantify [tHb] are invasive, since they require samples extracted from the patient. However, this process is a high risk factor for vulnerable groups such as premature infants and patients in intensive care units. Therefore, non-invasive approaches to determine the [tHb] from blood have been developed. Among those, spectroscopic optical coherence tomography (sOCT) has recently shown great potential to quantify [tHb].

In a recent work, we demonstrated the feasibility of [tHb] measurements with sOCT in *ex-vivo* human whole blood in the clinical range (7–23 g/dL) [1]. In that work, the retrieved [tHb] based on conventional sOCT achieved low precision (9.1 g/dL with a bias of 1.5 g/dL) compared to a commercial blood analyser. The precision was improved to 3.80 g/dL with a bias of 1.50 g/dL by performing measurements with an alternative experimental approach, in which data was acquired with a combination of focus tracking and zero-delay acquisition (FZA). Despite those results, sOCT still has low precision when compared to other non-invasive clinically relevant techniques, whose precision is ~ 2 g/dL. In this work, we aimed to improve the precision of sOCT and developed a procedure to quantify [tHb] by means of a numerical optimization of the optical density (OD) considering the absorption flattening effect in whole-blood. Our method leverages on the wavelength and depth dependence of the OD and simplifies the fitting procedure into a single step compared to traditional Lambert-Beer fitting that requires two fitting steps. This simplification reduces the variability of [tHb] estimations, thereby improving the precision and bias of sOCT. We validated our technique by processing *ex-vivo* human whole blood data with conventional sOCT and its extension with FZA.

2. Methods

2.1. sOCT system and human whole-blood samples

We used a custom-built sOCT described in our previous work [1, 2] and depicted in Fig. 1. Briefly, the light from a supercontinuum broadband source was expanded and collimated by a set of three lenses. Wavelengths above 700 nm were filtered out by a short-pass filter. A 10:90 beam splitter guided the light towards the reference and sample arms. In the reference arm, a variable neutral density filter controlled the intensity on the reference mirror. For our FZA method, a motorized stage displaced the reference lens and mirror. In the sample arm, a variable neutral density filter adjusted the intensity of light focused into a glass capillary with an inner and outer diameter of 1.2 mm and 1.8 mm respectively. For our FZA method, a motorized stage controlled the sample lens. Light reflected at the reference mirror and backscattered by blood combined at the beam splitter and was guided into a custom-built spectrometer that had a spectral resolution $\delta\lambda = 0.1$ nm in the range 460 – 650 nm.

Optical Coherence Imaging Techniques and Imaging in Scattering Media IV, edited by Benjamin J. Vakoc, Maciej Wojtkowski, Yoshiaki Yasuno, Proc. of SPIE-OSA Vol. 11924, 119240R · © 2021 OSA-SPIE
CCC code: 1605-7422/21/$21 · doi: 10.1117/12.2616058

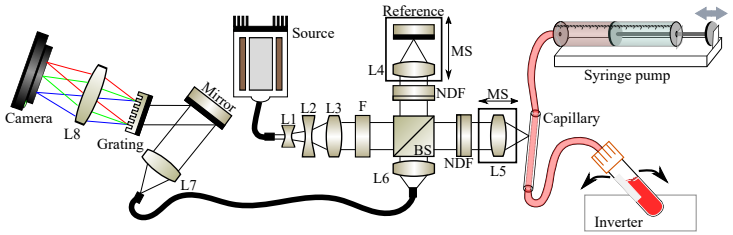

Fig. 1. Schematic of the sOCT system to measure *ex-vivo* [tHb] in human whole-blood. L1–L8: lenses; F: short-pass filter; BS: beamsplitter; NDF: neutral density filter; MS: motorised stage.

We analysed *ex-vivo* human whole-blood as in our previous work [1]. Blood samples were prepared from six healthy donors of the Experimental Centre for Technical Medicine of the University of Twente. The local Medical Research Ethics Committee (METC Twente now part of MEC-U) approved the blood collection process. All volunteers gave written informed consent for data usage. We mixed the blood with PBS to reduce [tHb] or removed blood plasma to increase it, creating a set of samples with varying [tHb]. Blood in falcon tubes were placed on an inverter, and a syringe pump established a blood flow of 0.3 mL/min. We created a set of 17 blood samples with a varying concentration in the range 6.0 – 23.0 g/dL. The 17 samples were measured with FZA and 15 of those samples measured with conventional sOCT. We obtained validation values for expected [tHb] from three measurements on each sample with a blood analyser (Avoximeter 1000E, Instrumentation Laboratory, USA) and calculated the mean and standard deviation (SD) in each case. By using sOCT, we also estimated [tHb] for three measurements per sample with respective mean and SD.

2.2. *Total haemoglobin concentration*

2.2.1. Conventional sOCT

We acquired 2500 measurements per blood sample with an exposure time of 50 μs and a line rate of 16.7 kHz. Short-time Fourier transforms were taken with a Gaussian window of 20 nm overlapping 70%. We corrected the signal losses due to the system roll-off as described by Rubinoff *et al.* [3] and the axial point spread function of the sample lens with the model introduced by Almasian *et al.* [4]. Then, we estimated the experimental OD in a region of interest (ROI) with a length of 40 μm. To use the same ROI for all wavelengths, we equalized the number of data points by over-sampling the intensity values to 2048 points across the spectral range and retrieved the experimental OD for each measurement. In order to quantify [tHb], we computed a numerical estimate $\widehat{\mathrm{OD}}(z,\lambda)$ of the experimental OD defined as

$$\widehat{\mathrm{OD}}(z,\lambda) = -2\,(z-z_0)\left[a\lambda^{-b} + \frac{2-\exp\left[-C_{Hb}\mu_{a,Hb}(\lambda)d_{RBC}\right]-\exp\left[-C_{HbO_2}\mu_{a,HbO_2}(\lambda)d_{RBC}\right]}{d_{RBC}}\right]+\alpha, \qquad (1)$$

where $z-z_0$ is the depth interval, λ is wavelength, a is a scaling factor, b is the scattering power, $\mu_{a,Hb}$ and μ_{a,HbO_2} are the absotion spectra of Hb and HbO_2 respectively, C_{HbO_2} and C_{Hb} are the concentrations of HbO_2 and Hb respectively, α is a background term and d_{RBC} is the width of an RBC. The unknown set of parameters $\mathbf{u} = [C_{HbO_2}, C_{Hb}, a, b, \alpha]$ was determined with a non-linear gradient-based optimisation method by minimizing an error function $f(\mathbf{u})$ defined as

$$\min_{\mathbf{u}} f(\mathbf{u}) = \sum_z \sum_\lambda \left| \ln\left[\frac{I(z,\lambda)}{I_0(\lambda)}\right] - \widehat{\mathrm{OD}}(z,\lambda)\right|^2. \qquad (2)$$

We set a seed of zeros for all parameters $\mathbf{u} = 0$ with boundaries between zero and infinity $0 \leq \mathbf{u} < \infty$. We optimized the error function with the Matlab solver *fmincon*, and used as stop criteria a function tolerance of 10^{-10}, a tolerance on the current point of 10^{-10} and a limit of 2×10^4 iterations.

2.2.2. Focus tracking and zero-delay acquisition

We also processed data acquired with FZA, as introduced in our previous work [1,2,5]. A periodic displacement of the reference mirror induced a Doppler shift with frequency $f_D = 2v_R/\lambda_0$, where $v_R = 0.85$ mm/s is the velocity of the mirror and $\lambda_0 = 550$ nm is the central wavelength of the spectrum. The motorized stages displaced the sample lens and reference mirror in steps of 2 μm across 100 μm inside the blood sample. At each step, we acquired 2500 spectra, with an exposure time of 50 μs and a line rate of 16.7 kHz. A band-pass filter filtered out the complex conjugate and the negatively shifted frequencies while maintaining those between 0.4 and 7.3 kHz. We performed

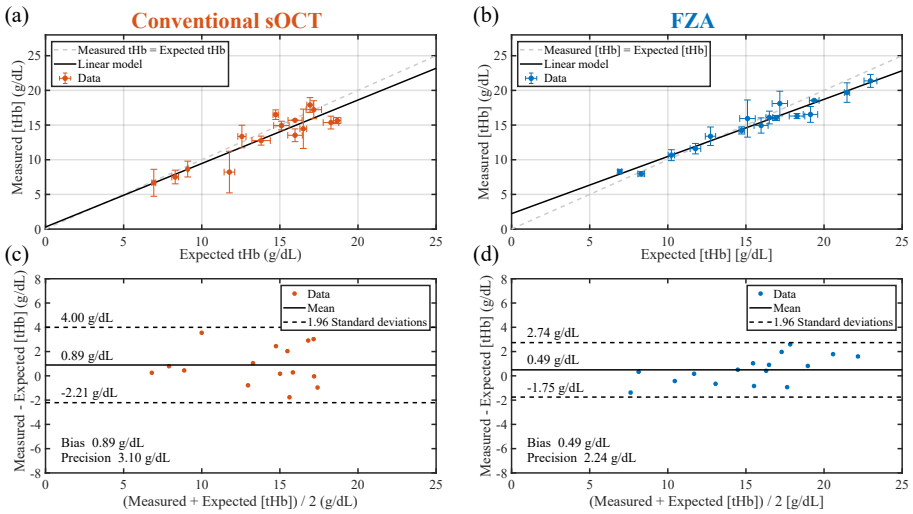

Fig. 2. Total haemoglobin concentrations retrieved after processing all data sets. (a) Measured [tHb] as a function of expected [tHb] with conventional sOCT (mean value $\pm$ SD) and (b) Bland-Altman plot for the same data. (c) Measured [tHb] as a function of expected [tHb] with FZA and (d) Bland-Altman plot for the same data.

the spectral analysis at each step with a Gaussian spectral window of 5 nm without overlapping. We retrieved the experimental OD within a depth interval of 40 μm and derived the [tHb] by numerical optimization of equation (2) with the same parameters as for conventional sOCT.

3. Results

Figure 2 presents the measured [tHb] as a function of expected [tHb] . Conventional sOCT is shown in Fig. 2-(a) for which a linear regression yields $y = 0.92x + 0.30$ with Pearson correlation coefficient $\rho = 0.91$ and $p < 0.001$. From the Bland-Altman plot [Fig. 2-(b)], we obtained a bias of 0.89 g/dL and a precision of 3.10 g/dL (defined as 1.96 SDs). For FZA, the measured [tHb] as a function of expected [tHb] is presented in Fig. 2-(c), leading to a linear regression $y = 0.82x + 2.23$ and Pearson correlation coefficient $\rho = 0.98$ with $p < 0.001$. Figure 2-(d) is the Bland-Altman plot that yields a bias of 0.49 g/dL and a precision of 2.25 g/dL (1.96 SDs).

4. Conclusion

In conclusion, we developed and applied a novel processing scheme to obtain [tHb] concentrations with sOCT. Our approach leverages on numerical optimisation of the OD considering the blood flattening effect. Our method directly relates the [tHb] to the OD, thus estimating the [tHb] in a single step. We validated our methodology with *ex-vivo* human whole-blood data under two acquisition schemes. For conventional sOCT, we obtained a precision of 3.10 g/dL and a bias of 0.89 g/dL, representing an improvement with a factor of 2.9 and 1.7 respectively compared to previous works. We achieved a precision of 2.25 g/dL with a bias of 0.49 g/dL for data acquired with FZA, improving in a factor of 1.7 and 3.1 for the precision and the bias, respectively.

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