Institute of Physics Publishing — Physics in Medicine and Biology
Phys. Med. Biol. **47** (2002) N233–N238 — PII: S0031-9155(02)36059-7

NOTE

A simple algorithm for *in vivo* ocular fundus oximetry compensating for non-haemoglobin absorption and scattering

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Received 22 April 2002
Published 21 August 2002
Online at stacks.iop.org/PMB/47/N233

Abstract
An algorithm is introduced for the compensation of the influence of non-haemoglobin absorption as well as tissue scattering on blood spectra used in optical oximetry at the ocular fundus. The *in vivo* measured spectra were corrected by a linear transformation in order to match the reference spectra of fully oxygenated and reduced blood, respectively, at three isosbestic points (522 nm, 569 nm and 586 nm). The oxygen saturation can then be determined at a wavelength showing a high contrast between oxygenated and reduced haemoglobin (e.g., 560 nm). Reflection measurements at blood flowing through cuvettes were used to validate the algorithm. The oxygen saturation values were compared to measurements of the same samples at a laboratory haemoximeter. The mean deviation was found to be 2.65%.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Optical oximetry, i.e. the determination of oxygen saturation of the blood from transmission or reflection spectra obtained *in vivo*, is widely used in clinical diagnostics and patient monitoring (Pittman and Duling 1975, Reynolds *et al* 1976, Schmitt *et al* 1986, Steinke and Shepherd 1988, Benaron *et al* 1992, Chance 1994). Because of the transparency of the anterior ocular media, it can be applied to measurements at the ocular fundus (Hickham *et al* 1963, Laing *et al* 1975, Delori 1988, Schweitzer *et al* 1995, 1999).

It is well known that the absorption spectrum of haemoglobin changes with attachment to oxygen (van Assendelft 1970). An application to retinal oximetry on the basis of Beer's law has recently been published by Smith *et al* (2000). *In vivo*, however, the haemoglobin is encapsulated in the erythrocytes and the extinction spectrum of whole blood is greatly affected by light scattering at these cells. Fortunately, the contributions of absorption and scattering

0031-9155/02/170233+06$30.00 © 2002 IOP Publishing Ltd Printed in the UK

to the blood spectrum can be separated according to the multiple scattering theory (Twersky 1970). This is used in a method described by Pittman and Duling (1975) and applied to retinal vessel oximetry by Delori (1988).

However, a crucial point of this technique is the absorption by other chromophores, e.g. melanin, as well as light losses by tissue scattering. As we showed earlier (Hammer *et al* 2001), spectra measured at retinal vessels are not only the result of haemoglobin absorption and scattering at blood cells and in the surrounding tissue, but are influenced by melanin in the retinal pigment epithelium and in the choroid as well. The same holds for the skin and other pigmented organs. Several authors described the compensation of melanin absorption by referring the spectrum measured at the vessel to a spectrum besides the vessel (Delori 1988, Schweitzer *et al* 1999, Smith *et al* 1999). However, the complicated radiation propagation in and around a vessel (Hammer *et al* 2001) is not allowed for by this approach. Therefore, we introduce a simple compensation for any corruption of haemoglobin spectra, which can be approximated by an exponential dependence on the wavelength (as holds for melanin and scattering).

2. Methods

The oxygen saturation is determined by a comparison of the spectrum measured from the ocular fundus with the reference spectra of fully oxygenated and deoxygenated blood (figure 1(a)). The algorithm to be described uses data at the three isosbestic wavelengths 522 nm, 569 nm and 586 nm, as well as at 560 nm: M_{522}, M_{560}, M_{569} and M_{586} from the measurement and R_{522}, $R_{560}^{0\%}$, $R_{560}^{100\%}$, R_{569} and R_{586} from the reference data. The superscripts 0% and 100% denote deoxygenated and oxygenated blood, respectively. The algorithm comprises the following six steps:

(1) The logarithms of the measured and reference data were taken as M_λ and R_λ, respectively.
(2) A linear function $g(\lambda)$ with $g(522) = R_{522}$ and $g(586) = R_{586}$ is determined.
(3) A linear function $f(\lambda)$ with $f(522) = M_{522}$ and $f(586) = M_{586}$ is determined.
(4) The measured data are added by the difference between $g(\lambda)$ and $f(\lambda)$:

$$M'_\lambda = M_\lambda + g(\lambda) - f(\lambda). \quad (1)$$

This correction forces the values of the measurement to be identical with that of the reference data at the isosbestic wavelengths 522 nm and 586 nm (figure 1(b)). Thus, it compensates for any extinction (by absorption or scattering) other than haemoglobin absorption provided that this extinction is linear with the wavelength in a logarithmic scale (i.e. an exponential function of the wavelength).

(5) The data M' are stretched or compressed around the line $g(\lambda)$ in order to match the reference data at the isosbestic wavelength 569 nm ($M''_{569} = R_{569}$), applying

$$M''_\lambda = g(\lambda) + \frac{(M'_\lambda - g(\lambda))(R_{569} - g(569))}{M'_{569} - g(569)}. \quad (2)$$

This step, illustrated in figure 1(c), compensates for different absolute values of measured and reference data resulting from different conditions of illumination and measurement.

(6) The oxygen saturation OS is indicated by M''_{560} on a linear scale between $R_{560}^{0\%}$ and $R_{560}^{100\%}$:

$$\mathrm{OS} = \frac{M''_{560} - R_{560}^{0\%}}{R_{560}^{100\%} - R_{560}^{0\%}} \times 100\%. \quad (3)$$

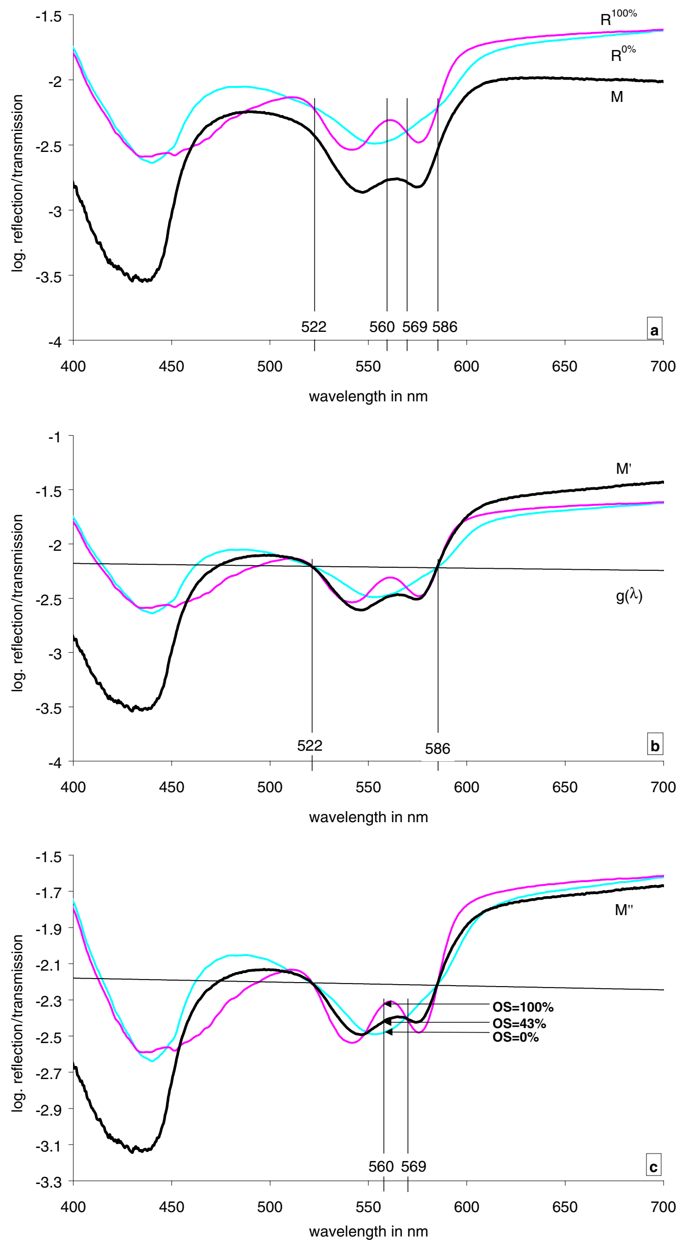

Figure 1. Reflection spectrum of blood in a cuvette with frosted glass background (M) along with the reference spectra (R) measured in transmission (a). Reflection spectrum (M') transformed to match the reference spectra at the isosbestic wavelengths 522 nm and 586 nm according to step 4 of the algorithm (b). Reflection spectrum (M'') compressed to match the reference spectra at the isosbestic wavelength 569 nm according to step 5 of the algorithm and reading of the oxygen saturation OS according to step 6 (c).

For experimental validation of the algorithm, we measured reflection spectra of whole fresh blood samples drawn from a healthy volunteer on EDTA. The oxygenation of the blood was

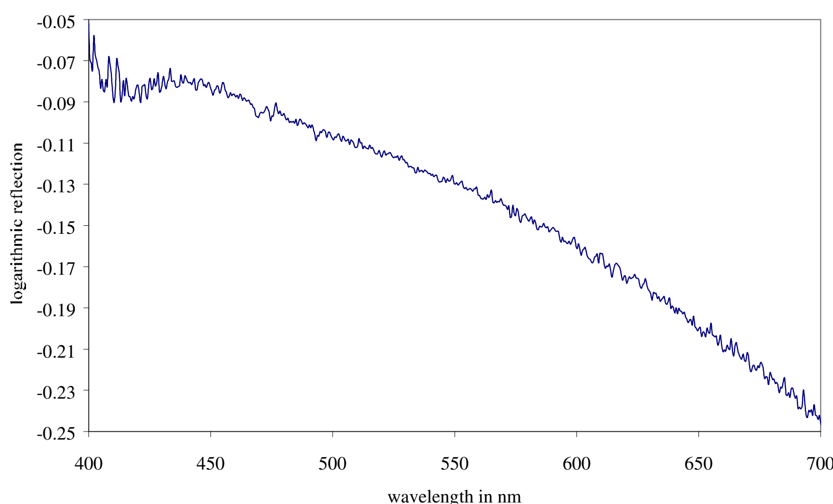

Figure 2. Logarithmic reflection spectrum of the empty cuvette.

altered by bubbling the blood with oxygen or nitrogen. The spectra were recorded by a home-made imaging ocular fundus reflectometer described elsewhere (Hammer *et al* 1997). The blood was kept in a laminar flow (velocity: 12.8 mm s^{-1}) through a cuvettes with a thickness of 50 μm placed in the focal plane of a model eye (focal length: 20 mm). The rear panel of the cuvette behind the blood layer was a frosted glass slide the logarithmic spectrum of which, given in figure 2, was nearly linear between 522 nm and 586 nm. The oxygen saturation of the samples was calculated by the algorithm described above and compared with measurements of the same samples at a laboratory haemoximeter (OSM3, Radiometer Copenhagen, Denmark).

The reference spectra were measured at flowing erythrocyte suspensions (velocity: 12.8 mm s^{-1}, haematocrit: 0.45) fully oxygenated by bubbling with pure oxygen or fully deoxygenated by the application of sodium dithionite (30 mmol l^{-1}). The reference measurements where carried out using the same technique as in the sample measurements but replacing the rear panel of the cuvette by a quartz glass and replacing the illumination of the ocular fundus reflectometer by a collimated illumination of the rear surface of the cuvette by a tungsten halogen lamp.

3. Results and discussion

The reference spectra and an example of a reflection measurement are given in figure 1(a), whereas the algorithm for the determination of the oxygen saturation is illustrated in figures 1(b) and (c). The oxygen saturations calculated from the reflection measurements are given in figure 3 in comparison with the values for the same samples determined at the laboratory haemoximeter OSM3. The error bars indicate the standard deviation of ten measurements taken simultaneously by the imaging ocular fundus reflectometer. The mean deviation between the OSM3 readings and our measurements was 2.65% oxygen saturation.

The method proposed here is suitable for the calculation of blood oxygenation from reflection or transmission measurements at four wavelengths. It compensates for any perturbation of the spectra obeying an exponential dependence on the wavelength between 522 nm and 586 nm. This enables the correction of melanin absorption in measurements at the skin or at the ocular fundus *in vivo*. In retinal oximetry, the absorption of the anterior ocular

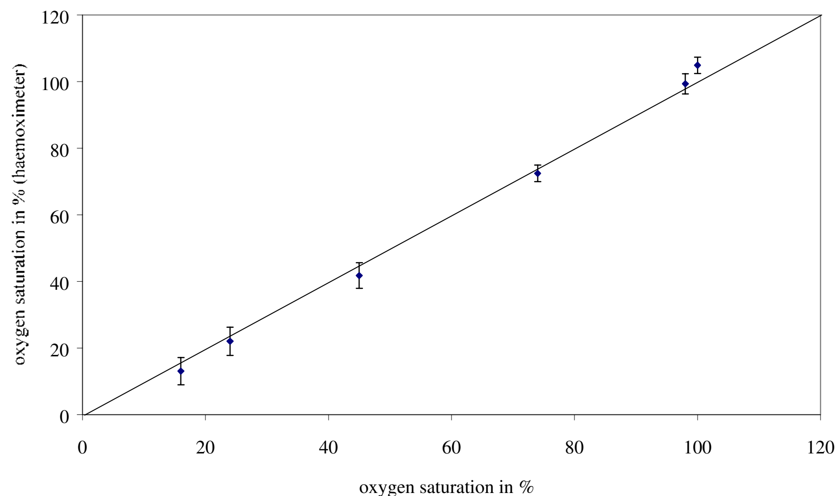

Figure 3. Oxygen saturation of blood streaming through the cuvette determined from reflection spectra versus haemoximeter readings.

media can be corrected additionally. Furthermore, tissue scattering can often be approximated by an exponential function of the wavelength (Hammer *et al* 1995). Thus, the algorithm is suitable for the compensation of scattering losses too. However, the scattering at the erythrocytes shows a more complicated spectral behaviour in the region of the haemoglobin absorption bands (Hammer *et al* 1998) and, therefore, scattering inside the blood cannot be compensated. This is the reason why we had to use spectra of whole blood as reference instead of haemoglobin spectra.

Besides the use of only four wavelengths, a great advantage of the method is its restriction to linear transformations and the abdication of any regression analysis. This makes calculation very fast and, therefore, suitable for image analysis and the two-dimensional determination of the oxygen saturation which is demanded by clinicians.

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