

# Simultaneous assessment of pulsating and total blood in inflammatory skin lesions using functional diffuse reflectance spectroscopy in the visible range

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**Abstract.** We present a simple and cost-effective optical technique for the simultaneous assessment of pulsating and total blood noninvasively in an inflammatory skin lesion. Acquisitions of diffuse reflectance spectra in the visible range at 6 Hz are used to trace the oscillating components of reflectance. Measurements on erythematous lesions from a UV insult show slow changing signal at about 0.1 Hz and heart-driven regular oscillations at about 1 Hz simultaneously. The results demonstrate the potential of the technique in monitoring both pulsating and steady components of the blood in inflammatory lesions of the skin. © 2010 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3524191]

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There has been heightened interest in noninvasive assessment of blood flow in skin tissues using optical techniques.<sup>1</sup> The assessment relies mostly on the use of photoplethysmography<sup>2,3</sup> (PPG) or laser Doppler, using red to near-infrared (NIR) light to probe the dermal vasculature at depths greater than 1 mm and exploiting the pulsatile nature of arterial blood. When a single wavelength is used, a measure of blood flow or perfusion (velocity times concentration of red blood cells) can be obtained from the amplitude of an oscillating signal ( $ac$ ) that is synchronous to the heart rate or the time-averaged signal that can be associated with total blood volume. Blood oxygenation ( $SpO_2$ ) can be obtained from measurements of plethysmographic pulses at multiple wavelengths based on empirical scaling. The readings of  $SpO_2$  are accurate in a limited range.<sup>4</sup>

An accurate assessment of cutaneous microcirculation is still challenging, because probing small volumes of skin with variable content of melanin precludes the use of models based on diffusion approximation to quantify oxy- and deoxyhemoglobin that are pulsating or steady in the same volume of interrogation. In addition, from two independent *in-vivo* studies for as-

sessing UV-induced erythema, different dose-response relations for blood concentration measured from time-averaged diffuse reflectance spectroscopy (DRS)<sup>5</sup> and blood perfusion measured from laser Doppler velocimeters<sup>6</sup> have been found. In this work, we present a simple and cost-effective functional diffuse reflectance spectroscopy (fDRS) system that enables us to study hemodynamics in the papillary dermal vasculature, where the contribution to the signal at the “superficial” vascular plexus is dominant.

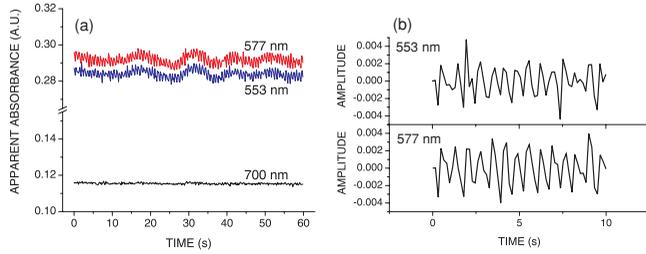
The configuration of the fDRS system is identical to that of the conventional DRS used in many other studies,<sup>7,8</sup> except that the spectra are acquired sequentially at 6 Hz. Briefly, one leg of a bifurcated optical fiber probe (600 randomly mixed fibers, 50  $\mu$ m diameter) was coupled to a Tungsten-halogen lamp (HL2000, Ocean Optics, Dunedin, Florida), and the other leg was coupled to a spectrometer (BTC613, B and W TeK Inc., Newark, Delaware). To optimize speed (needed for consecutive spectra acquisition) and noise level, temporal and spectral resolutions were set at 160 ms and 1 nm, respectively. The acquired signal was then normalized by the reflectance from a standard ( $I_0$ ) to convert the remitted intensity  $I$  into a time trace of apparent absorbance  $A(t)$  calculated from  $A(t) = -\log_{10} [I(t)/I_0]$ .

To demonstrate the performance of the technique, six healthy individuals with skin phototypes I–III were recruited for a UV sensitivity phototest, and their backs were exposed to an increasing dose of solar-simulated radiation (COLIPA standard) with a maximum of two times the minimum erythema dose (MED).<sup>9</sup> This study was conducted according to the Declaration of Helsinki Principles and approved by the Institutional Review Board. Evaluation and measurements were conducted at 24-h postirradiation. Prior to the measurement, each subject was allowed to rest for 15 min to acclimatize to room temperature. The probe was gently attached on the site with tape throughout the measurement.

Typical fDRS data obtained from a UV exposed site with clear evidence of erythema are shown in Fig. 1(a). Of the full spectra measured in the visible, the apparent absorbance values at 553, 577, and 700 nm are displayed as a function of time to trace the reflectance changes modulated by blood flow. The time traces provide several notable features. First, the two traces selected at the Q-band of hemoglobin show oscillations in phase at the same frequencies: one from heart pulsations at around 1.2 cycles per second and the other irregular oscillation showing the vasomotor waves with a period of approximately 10 s. Second, the trace at 700 nm, in which the molar extinction coefficients of both forms of hemoglobin are relatively low, displays no evident oscillations. Finally, the constant level of the trace at 700 nm confirms that there is no effect of the respiratory motion on the measured signal during the measurement.

To extract the oscillatory component, particularly at the frequency of heart rate, from the time-varying apparent absorbance, we used a moving average method along the time trace of each absorbance with a 1.2-s window. The window size was optimized to reduce the effect of the slowly changing steady signal due to vasomotor changes. Deviations from the moving average value are stored as  $ac$  values, and Fig. 1(b) presents the  $ac$  signals obtained from the time traces at 553 and 577 nm. The

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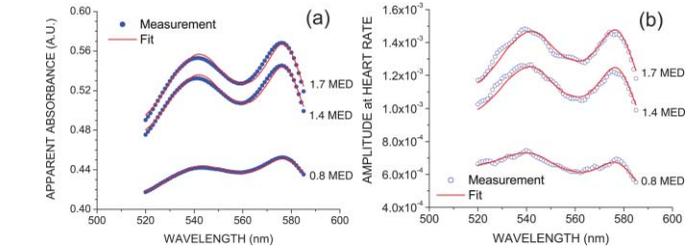
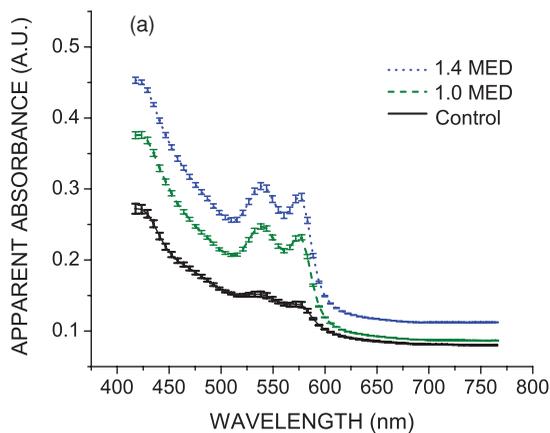


**Fig. 1** (a) Time traces of oscillating apparent absorption in absorbance units measured from an inflammatory lesion using the fDRS system. (b) The traces were processed to split oscillations at heart frequency from steady signals that change slowly. In the spectral range studied, traces at 553 and 577 nm were selected to demonstrate blood pulsations.

pulsations are in phase at the two wavelengths and the signal amplitudes are around two orders of magnitude smaller than the dc values. To display magnitude of the lower-frequency-induced absorbance change, time-averaged spectra with their lower and upper bounds over 60 s are presented in Fig. 2(a) for different UV doses. The variability of the absorbance spectra can be observed even in the control site, and the changes of absorbance are evident at wavelengths lower than 600 nm.

In Fig. 2(b), root-mean-squared (rms) values of the ac signal component from all of the 60-s interval measurements are plotted against wavelength. The amplitude of the curve represents the change of absorbance modulated by the heart-driven pulsations. In the higher UV doses, the pulsation is greater in amplitude than the control site, which indicates increase of blood flow as a result of vasodilation in the sites with erythema.

The fDRS system enables us to detect blood pulsations in the visible spectrum to identify pulsating chromophores. To quantify the content of blood in the tissue from the spectra acquired in both heart rate (1 Hz) and the steady component, we applied an algorithm made of two steps employing extinction coefficients of oxy- ( $\epsilon_{\text{HbO}_2}$ ) and deoxyhemoglobin ( $\epsilon_{\text{Hb}}$ ) in the Q-band. It is assumed in the algorithm that throughout the narrow wavelength range of the Q-band, the ratio of the absorption



**Fig. 3** Comparison of measured spectra with the predictions obtained from the algorithm for quantification of oxy- and deoxyhemoglobin. (a) Lower bounds of apparent absorbance, and (b) spectra of rms value obtained from each skin site exposed to solar-simulated radiation.

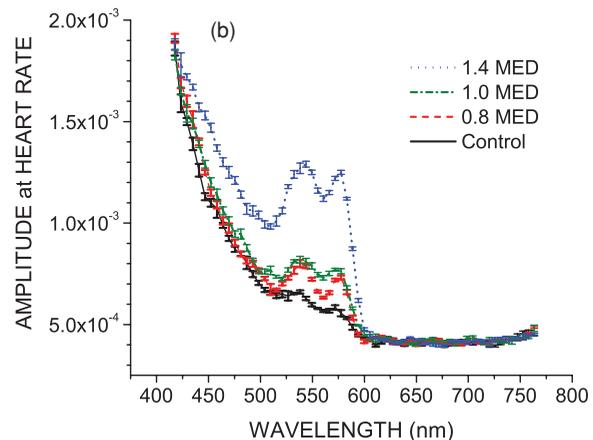
to scattering coefficients does not change dramatically, hence the optical penetration depth is somewhat similar. This limits the blood plexus being measured under the same effect of probe geometry. The spectrum from 520 to 585 nm is then assumed to contain the contribution from oxy- and deoxyhemoglobin, plus a straight line that represents a modulation by the reduced scattering coefficients and melanin. The effect of scattering and melanin is treated as a lumped parameter that linearly alters the spectrum. In the algorithm each absorbance spectrum ( $A$ ) in the Q-band region is adjusted for the effect of scattering and melanin absorption by fitting four isosbestic points at 529, 545, 570, and 584 nm to

$$A = a + [\lambda_{529}, \lambda_{545}, \lambda_{570}, \lambda_{584}] \cdot b + f_{\text{blood}} \cdot \epsilon_{\text{HbO}_2}(\lambda_{529}, \lambda_{545}, \lambda_{570}, \lambda_{584}), \quad (1)$$

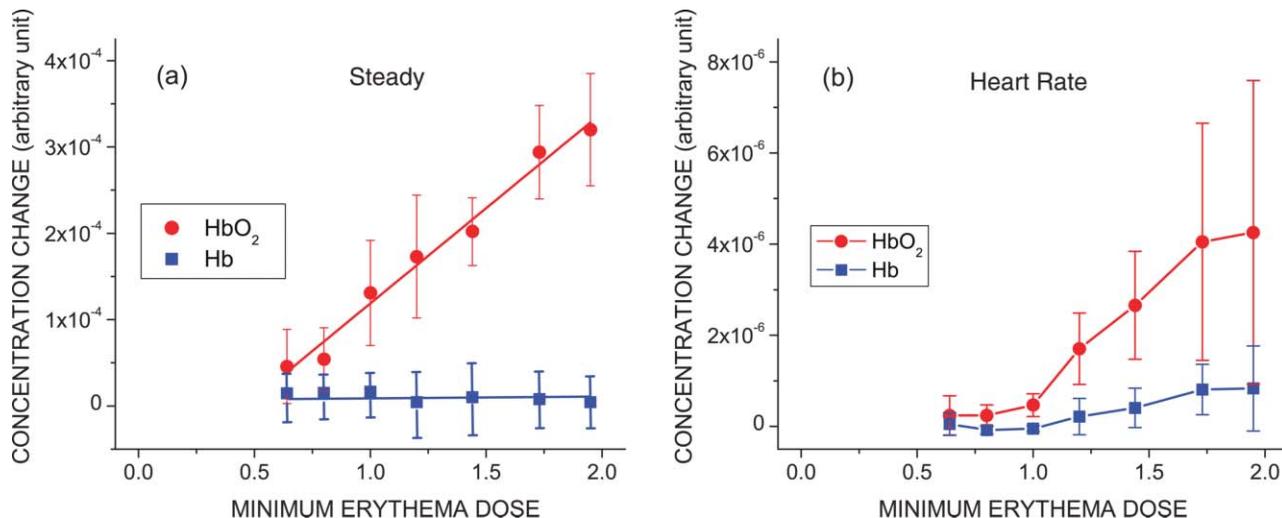
where  $a$  is the line intercept,  $b$  is the slope, and  $f_{\text{blood}}$  is the apparent blood content. The parameters  $a$  and  $b$  are then used to define a line in the range of 520 to 585 nm that is subtracted from  $A$  to find the adjusted spectrum ( $S$ ), which is fitted to

$$S = m \cdot \epsilon_{\text{HbO}_2} + n \cdot \epsilon_{\text{Hb}}, \quad (2)$$

where  $m$  and  $n$  are the apparent concentrations of oxy- and deoxyhemoglobin, respectively. Fittings were performed using a constrained Levenberg-Marquardt (MATLAB, Mathworks Inc.,



**Fig. 2** (a) Apparent absorbance spectra obtained from skin sites exposed to 1.0 and 1.4 MED of solar simulated radiation after 24 h of UV radiation on the lower back. The upper and lower bounds of the slow fluctuations of the spectra ( $<1$  Hz) are shown in error bars. The upper and lower bounds were determined by monitoring the fDRS signal for 60 s. (b) Spectra of rms values of oscillations at heart frequency obtained from 60 s of fDRS at each tested skin site. Error bars represent standard deviation of the rms during the entire measurement period of 2 min.



**Fig. 4** Changes in oxy- and deoxyhemoglobin concentration versus applied UV doses in MED units obtained from the fit of (a) steady component absorption spectra and (b) the pulsating component at heart rate. The data represent the average values with standard deviation from six individuals.

Natick, Massachusetts) algorithm that seeks to minimize a sum of squares between the predicted equation and the data.

The representative measured spectra for the lower bounds of absorbance and the ac components at different UV doses, along with the predictions given by Eq. (2) are displayed in Figs. 3(a) and 3(b), respectively. The amounts of oxy- and deoxyhemoglobin that best fit the spectra are summarized relative to the control site in Fig. 4 against the UV doses in MED units; from Fig. 4(a) the average of steady components and Fig. 4(b) the heart pulsation. As reported in other studies,<sup>5,10</sup> the erythema (redness) response with the applied UV dose resulted in a linear increase of total hemoglobin that is oxygenated on average 85% up to 2 MED. In contrast, the pulsating blood at heart rate showed a biphasic increase, as presented in Fig. 4(b), and confirmed the results in a study using the laser Doppler system.<sup>6</sup> The amount of pulsating blood at heart rate remained at lower levels until 1 MED and showed a steep increase of oxyhemoglobin at doses greater than 1.2 MED. The gradually increasing error bars with applied UV doses indicate the variability of pulsating blood amounts of each individual, and do not affect the overall biphasic response of blood flow due to the UV insult. Comparison of the two behaviors between the total blood and pulsatile component may implicate the recruitment of additional capillaries contributing to the blood flow in activity at an early stage of inflammation, while larger venules and arterioles of the dermal plexus are actively involved in vasodilation resulting in evident heart rate signal at doses greater than 1.2 MED.

In summary, we present a simple approach to assess pulsating blood at heart frequency and total blood simultaneously in superficial dermal plexus of the skin by using fDRS in the visible spectrum. This technique can introduce a better way of documenting a sequence of events to an inflammatory response

of skin by providing an additional dimension of measurement to the common DRS system.

#### References

1. M.-Z. Poh, D. J. McDuff, and R. W. Picard, "Non-contact, automated cardiac pulse measurements using video imaging and blind source separation," *Opt. Express* **18**(10), 19762–10774 (2010).
2. J. Spigulis, "Optical noninvasive monitoring of skin blood pulsations," *Appl. Opt.* **44**(10), 1850–1857 (2005).
3. J. Spigulis, L. Gailite, A. Lihachev, and R. Ertz, "Simultaneous recording of skin blood pulsations at different vascular depths by multiwavelength photoplethysmography," *Appl. Opt.* **46**(10), 1754–1759 (2007).
4. R. K. Webb, A. C. Ralston, and W. B. Runciman, "Potential errors in pulse oximetry: II. Effects of changes in saturation and signal quality," *Anaesth.* **46**(3), 207–212 (1991).
5. N. Kollias, I. Seo, and P. R. Bargo, "Interpreting diffuse reflectance for in vivo skin reactions in terms of chromophores," *J. Biophoton.* **3**(1–2), 15–24 (2010).
6. A. R. Young, R. H. Guy, and H. I. Maibach, "Laser Doppler velocimetry to quantify UV-B induced increase in human skin blood flow," *Photochem. Photobiol.* **42**(4), 385–390 (1985).
7. G. N. Stamatias, B. Z. Zmudzka, N. Kollias, and J. Z. Beer, "In vivo measurement of skin erythema and pigmentation: new means of implementation of diffuse reflectance spectroscopy with a commercial instrument," *Br. J. Dermatol.* **159**, 683–690 (2008).
8. G. Zonios, J. Bykowski, and N. Kollias, "Skin melanin, hemoglobin, and light scattering properties can be quantitatively assessed in vivo using diffuse reflectance spectroscopy," *J. Invest. Dermatol.* **117**, 1452–1457 (2001).
9. B. E. Johnson, F. J. Daniels, and I. A. Magnus, "Response of human skin to ultraviolet light," in *Photophysiology: Current Topics in Photochemistry and Photochemistry*, A. C. Giese, Ed., pp. 139–202, Academic Press, New York (1968).
10. G. N. Stamatias and N. Kollias, "Blood stasis contributions to the perception of skin pigmentation," *J. Biomed. Opt.* **9**(2), 315–322 (2004).