Skin Melanin, Hemoglobin, and Light Scattering Properties can be Quantitatively Assessed *In Vivo* Using Diffuse Reflectance Spectroscopy

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Noninvasive and real-time analysis of skin properties is useful in a wide variety of applications. In particular, the quantitative assessment of skin in terms of hemoglobin and melanin content, as well as in terms of its light scattering properties, is a challenging problem in dermatology. We present here a technique for examining human skin, based on the *in vivo* measurement of diffuse reflectance spectra in the visible and near-infrared ranges of the electromagnetic spectrum. Spectra were measured by means of a fiber optic probe, and they were analyzed using an analytical model of light diffusion in the skin. The results of the analysis indicate that it is possible to obtain quantitative information about hemoglobin and melanin content, as well as basic information regarding the scattering properties of the skin. *Key words: diffusion/hemoglobin/melanin/ optical properties/reflectance/scattering. J Invest Dermatol 117:1452–1457, 2001*

The optical properties of human skin have been the subject of numerous investigations (Edwards and Duntley, 1939; Anderson and Parrish, 1981; van Gemert *et al*, 1989; Graaff *et al*, 1993). Interest in skin optics stems from the fact that useful information about the skin physiology, morphology, and composition can be obtained in a noninvasive manner and in real time using an optical method of analysis. One such optical method is diffuse reflectance spectroscopy, which has already been widely used in the study of skin, especially in the study of hemodynamics and inflammation (Kollias *et al*, 1994; Merschbrock *et al*, 1994; Svaasand *et al*, 1995) and skin pigmentation (Kollias and Baqer, 1986; Marchesini *et al*, 1991). In this work, we examine human skin *in vivo* using an analytical model of diffuse reflectance that provides quantitative information in a more direct and potentially more accurate and reliable manner (Zonios *et al*, 1999).

Diffuse reflectance probes the scattering and absorption properties of skin. Light is absorbed by the various skin chromophores (Edwards and Duntley, 1939; Young, 1997) and scattering arises because of the refractive index fluctuations on a microscopic level (Schmitt and Kumar, 1996). In the visible range, the main chromophores of human skin are hemoglobin and melanin. Hemoglobin is found in the microvascular network of the dermis, typically 50–500 μm below the skin surface. In contrast, melanin is located in the epidermis, which occupies the top 50–100 μm. Human skin is characterized by variable concentration in melanin, ranging from very low in light complexioned Caucasian skin (type I), to very high in black African skin (type VI) (Young, 1997). Melanin and hemoglobin strongly absorb light in the ultraviolet (UV) and visible ranges and they present low absorption in the near-infrared range. Melanin in particular has a photo-protective action because of its light absorption properties (Kollias *et al*, 1991).

We have measured diffuse reflectance spectra from skin sites located on the forearm and on the back of healthy volunteers by means of a fiber optic probe. The goal of this work was to investigate the potential to obtain quantitative information regarding the absorption and scattering properties through the use of an analytical model of diffuse reflectance. The model has been previously extensively tested and successfully applied to the analysis of diffuse reflectance from human colon tissue *in vivo* (Zonios *et al*, 1999).

In this study, the model was extended and applied to the diffuse reflectance from skin. To perform this task, melanin was introduced as an absorber in addition to hemoglobin. Furthermore, it was assumed that the reduced scattering coefficient of skin exhibits a linear dependence on the wavelength of light. This approximation is well supported by scattering theory as will be discussed further below.

The ability of the model to measure the absorption and scattering properties of tissue has already been established on tissue phantoms (Zonios *et al*, 1999). To evaluate the utility of the model for measuring melanin and hemoglobin content in skin, we studied skin sites with variable melanin and hemoglobin content. Skin sites with variable melanin content were studied on individuals with different skin types and, in addition, UVB irradiation was used to produce skin pigmentation at various levels. Variable hemoglobin content was studied by blocking blood flow in the arm of human volunteers with a pressure cuff and by inducing reactive hyperemia immediately after the release of pressure.

The results of the analysis indicate that the model can be successfully used for the analysis of diffuse reflectance spectra from skin, as well as for the quantitative assessment of the melanin and hemoglobin content. This model-based approach to the analysis of diffuse reflectance spectra of skin presents a potential improvement

over previously employed empirical or semiempirical diffuse reflectance models for data analysis.

MATERIALS AND METHODS

Volunteers   Diffuse reflectance spectra were measured on skin sites (2.5 cm^2 area) located on the back of 10 volunteers, with a range of skin types, from very light Caucasian (type II), to black African (type V). In five out of 10 volunteers, skin sites were irradiated with UVB radiation (280–320 nm) to produce pigmentation at various levels and therefore obtain a wider and more continuous range of melanin concentration. Spectra were measured on these skin sites in 10 d following UVB irradiation. Irradiation was performed on a total of 12 skin sites on every volunteer at six different UVB doses (two sites per dose) with the starting dose set at 18 mJ per cm^2 and with subsequent dose increments of approximately 40%. UVB radiation was provided at a fluence rate of 0.5 mW per cm^2 by a lamp (UVB-HO-90, Elder Pharmaceuticals, Bryan, OH) located 30 cm away from the skin.

In a separate group of seven volunteers with skin type II or III, a pressure cuff was used to block completely arterial and venous blood flow (occlusion) in the arm by inflating at a pressure 50 mmHg higher than the systolic arterial pressure. This pressure was maintained for 4 min and then the pressure cuff was released. The arm of each volunteer was placed on an armrest such that the pressure cuff was not in contact with the body when inflated. The volunteers were allowed to equilibrate at ambient conditions for 20 min before measurements were made. The fiber optic probe was attached to the ventral forearm with adhesive tape prior to the inflation of the pressure cuff. Diffuse reflectance spectra were measured as a function of time, before, during, and after occlusion.

Prior to the study, IRB approval was obtained from the Subcommittee for Human Studies of the Massachusetts General Hospital and informed consent was obtained from all volunteers.

Instrumentation   The instrumentation employed for measurement of diffuse reflectance has been described in detail elsewhere (Knoefel *et al*, 1996). Briefly, a spectrophotometer (HP-8452, Hewlett-Packard, Palo Alto, CA) was used consisting of a CW white light source, a spectrograph, and a diode array detector. Light delivery and collection was by means of a fiber optic probe consisting of multiple, tightly packed together, randomly arranged optical fibers with 30 μm core diameter providing a total delivery/collection spot diameter of 2.5 mm. Diffuse reflectance spectra were measured in the range 460–820 nm. Prior to data acquisition, a reference spectrum was measured on a BaSO$_4$ diffuse reflectance standard and all skin spectra were subsequently divided by this reference spectrum to ensure proper data calibration.

Skin color measurements were made with a chromameter device (CR-200, Minolta, Japan) and skin color parameters were recorded in the Commission International d'Eclairage L*a*b* colorimetric system (Billmeyer and Saltzman, 1981; Weatherall and Coombs, 1992).

Diffuse reflectance model   Data were analyzed by means of an analytical diffuse reflectance model that has been described in detail previously, including testing and calibration on tissue phantoms (Farrell *et al*, 1992; Zonios *et al*, 1999). Data analysis produced a set of five parameters for every skin spectrum: three absorption parameters (total hemoglobin concentration $c_{Hb}{}^*$, hemoglobin oxygen saturation α, melanin concentration c_{mel}) and two scattering parameters [the maximum value of the reduced scattering coefficient $\mu_s'(\lambda_{min})$ and the effective scatterer size d_s]. Technical details regarding the implementation of the model can be found in the appendix.

RESULTS

Figure 1 shows representative model fits to three diffuse reflectance spectra measured on the back of three volunteers with three different skin types, prior to UVB irradiation (normal unirradiated skin sites). The model described the reflectance spectra with very good accuracy over the entire wavelength range, for all three skin types. The agreement between the model and the experimental data was particularly good in the 600–800 nm range where the reflectance spectral features were mainly affected by scattering. At wavelengths shorter than 600 nm, absorption by melanin and hemoglobin played a key role. Effects of light absorption by these two chromophores were increasingly evident by the lower intensity of the spectra in the short wavelength range, especially for darker skin types.

Table I summarizes the model parameter values obtained by analyzing the reflectance spectra measured on the back of the 10 volunteers before UVB irradiation. Hemoglobin and scattering parameters exhibited small variations among volunteers, with no
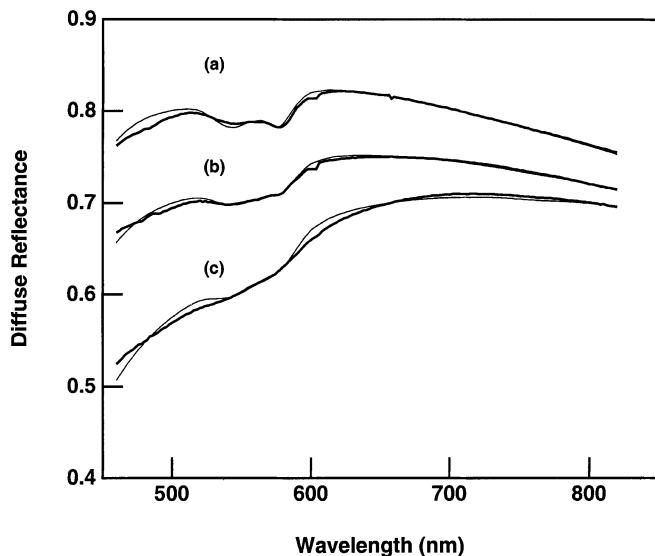

Figure 1. Diffuse reflectance spectra from skin sites on the back of three volunteers with different skin types. (*a*) White Caucasian (type II), (*b*) Japanese (type III), and (*c*) black African (type V). The presence of melanin is most evident by the larger intensity variation in the 460–560 nm region of the spectrum. Reflectance spectra (*thick lines*) and model fits (*thin lines*).

Table I. Model parameters

Volunteer number (skin type)	Melanin content c_{mel} ($\times 10^{-7}$ mmol per dl)	Hemoglobin content $c_{Hb}{}^*$ (mg per dl)	Hemoglobin oxygen saturation α	Reduced scattering coefficient $\mu_s'(\lambda_{min})$ (per mm)	Scatterer size d_s (μm)
1 (type V)	118	2.8	0.66	1.27	0.78
2 (type IV)	67	3.5	0.76	1.31	0.88
3 (type IV)	47	2.2	0.78	1.82	0.62
4 (type IV)	35	3.1	0.65	1.77	0.63
5 (type III)	24	3.7	0.78	1.65	0.61
6 (type III)	17	3.3	0.76	1.31	0.59
7 (type III)	15	2.9	0.64	1.57	0.44
8 (type III)	14	3.9	0.68	1.61	0.47
9 (type II)	4.5	4.1	0.71	1.44	0.51
10 (type II)	2.7	2.5	0.74	1.69	0.44

1993). This information could be useful in the assessment of burn damage or in the examination of skin resurfacing conditions.

We have found that the melanin content of the skin, as evaluated through the model, shows a good correlation with independent measurements performed using a standard chromameter device. This is an important finding, as it opens the way for direct, quantitative, noninvasive, assessment of the melanin content in human skin.

The hemoglobin content as measured by the model on the back of the volunteers was found to be consistent among different volunteers, and the average concentration was lower than in previous studies that measured the average concentration of hemoglobin in the capillary network of the colon mucosa (Zonios et al, 1999). This is reasonable, because the skin of the volunteers studied was under normal conditions and no inflammation or other abnormality related to increased hemoglobin content was clinically observed. In the case of forearm arterial and venous blood flow occlusion, the typical hemodynamic response of the anticipated reactive hyperemia was observed, immediately after the release of the pressure cuff. These results are in good agreement with previous studies (Hampson and Piantadosi, 1988).

The scattering properties of skin were found to be in general agreement with existing reports, with the average scatterer size being in the range $0.4 < d_s < 1.5$ μm (Mourant et al, 1998; Zonios et al, 1999). A very weak correlation was observed between the scattering properties of skin and the tissue type, with the average scatterer size being slightly larger in volunteers with higher melanin content. This could be an indication that scattering from melanin, which has not been explicitly taken into account in the model, possibly contributes to the skin scattering properties as already reported (Jacques and McAuliffe, 1991; Rajadhyaksha et al, 1995). Nevertheless, this is an observation that requires further investigation, in which we are currently engaged.

Another point requiring additional investigation is the multi-layered structure of skin. The model employed in this work assumes a one-layer skin geometry, but the two major absorbers found in skin, hemoglobin and melanin, reside in different skin layers, with the melanin found in the top layer (epidermis) and the hemoglobin found in the capillary network of the second layer (dermis). For skin that is not very dark, our results show that the single-layer approximation is applicable and yields reasonable results. For very dark skin types (type V and above), however, which we did not study in detail, the applicability of the model remains to be investigated.

Finally, in this work, hemoglobin and melanin were assumed to be the only skin chromophores. Other chromophores may be present in the skin though, especially in pathologic conditions. In such a case, the present model could be enhanced to include these chromophores. One such example is bilirubin, which has a broad absorption band at 460 nm (Hannemann et al, 1979) and gives skin the characteristic yellow color of jaundice.

REFERENCES

Anderson RR, Parrish JA: The optics of human skin. *J Invest Dermatol* 77:13–19, 1981

van Assendelft OW: *Spectrophotometry of Haemoglobin Derivatives*. Springfield, IL: C C Thomas, 1970: pp 355–359

Billmeyer FW, Saltzman M: *Principles of Color Technology*, 2nd edn. New York: Wiley-Interscience, 1981

Born M, Wolf E: *Principles of Optics*. New York: Pergamon Press, 1975

Chardon A, Cretois I, Hourseau C: Skin colour typology and suntanning pathways. *Int J Cosm Sci* 13:191–208, 1991

Crippa RP, Christofoletti V, Romeo N: A band model for the melanin deduced from optical absorption and photoconductivity experiments. *Biochim Biophys Acta* 538:164–170, 1978

Edwards EA, Duntley SQ: The pigment and color of human skin. *Am J Anat* 65:1–33, 1939

Farrell TJ, Patterson MS, Wilson BC: A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the non-invasive determination of tissue optical properties. *Med Phys* 19:879–888, 1992

Feather JW, Ellis DJ, Leslie G: A portable reflectometer for the rapid quantification of cutaneous hemoglobin and melanin. *Phys Med Biol* 33:711–722, 1988

Ferdman AG, Yannas IV: Scattering of light from histologic sections: a new method for the analysis of connective tissue. *J Invest Dermatol* 100:710–716, 1993

van Gemert MJC, Jacques SL, Sterenborg HJCM, Star WM: Skin optics. *IEEE Trans Biomed Eng* 36:1146–1154, 1989

Gniadecka M, Wulf HC, Mortensen NN, Poulsen T: Photoprotection in vitiligo and normal skin. A quantitative assessment of the role of stratum corneum, viable epidermis and pigmentation. *Acta Derm Venereol* 76:429–432, 1996

Graaff R, Dassel ACM, Koelink MH, de Mul FFM, Aarnoudse JG, Zijlstra WG: Optical properties of human dermis *in vitro* and *in vivo*. *Appl Opt* 32:435–447, 1993

Hampson NB, Piantadosi CA: Near infrared monitoring of human skeletal muscle during forearm ischemia. *J Appl Physiol* 64:2449–2457, 1988

Hannemann RE, Dewitt DP, Hanley EJ, Schreiner RL, Bonderman P: Determination of serum bilirubin by skin reflectance: effect of pigmentation. *Pediatr Res* 13:1326–1329, 1979

Jacques SL, McAuliffe DJ: The melanosome: threshold temperature for explosive vaporization and internal absorption coefficient during pulsed laser irradiation. *Photochem Photobiol* 53:769–775, 1991

Knoefel WT, Kollias N, Rattner DW, Nishioka NS, Warshaw AL: Reflectance spectroscopy of pancreatic microcirculation. *J Appl Physiol* 80:116–123, 1996

Kollias N, Baqer A: On the assessment of melanin in human skin *in vivo*. *Photochem Photobiol* 43:49–54, 1986

Kollias N, Sayre RM, Zeise L, Chedekel MR: Photoprotection by melanin. *J Photochem Photobiol B* 9:135–160, 1991

Kollias N, Baqer A, Sadiq I: Minimum erythema dose determination in individuals of skin type V and VI with diffuse reflectance spectroscopy. *Photodermatol Photoimmunol Photomed* 10:249–254, 1994

Marchesini R, Brambilla M, Clemente C, et al: *In vivo* spectrophotometric evaluation of neoplastic and non-neoplastic skin pigmented lesions – I. Reflectance measurements. *Photochem Photobiol* 53:77–84, 1991

Merschbrock U, Hoffman J, Caspary L, Huber J, Schmickaly U, Lubbers DW: Fast wavelength scanning reflectance spectrophotometer for noninvasive determination of hemoglobin oxygenation in human skin. *Int J Microcirc* 14:274–281, 1994

Mourant JR, Freyer JP, Hielscher AH, Eick AA, Shen D, Johnson TM: Mechanisms of light scattering from biological cells relevant to noninvasive optical-tissue diagnostics. *Appl Opt* 37:3586–3593, 1998

Perelman LT, Backman V, Wallace M, et al: Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution. *Phys Rev Lett* 80:627–630, 1998

Press WH, Teukolsky SA, Vetterling WT, Flannery BP: *Numerical Recipes in C*, 2nd edn. Cambridge University Press, 1992:pp 681–699

Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR: *In vivo* confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol* 104:946–952, 1995

Schmitt JM, Kumar G: Turbulent nature of refractive-index variations in biological tissue. *Opt Lett* 21:1310–1312, 1996

Svaasand LO, Norvang LT, Fiskerstrand EJ, Stopps EKS, Berns MW, Nelson JS: Tissue parameters determining the visual appearance of normal skin and port-wine stains. *Lasers Med Sci* 10:55–65, 1995

Weatherall IL, Coombs BD: Skin color measurements in terms of CIELAB color space values. *J Invest Dermatol* 99:468–473, 1992

Wiscombe WJ: *NCAR Technical Note, NCAR/TN-140+STR*, National Center for Atmospheric Research, Boulder, Colorado, 1979

Young AR: Chromophores in human skin. *Phys Med Biol* 42:789–802, 1997

Zonios G, Perelman LT, Backman V, Manoharan R, Fitzmaurice M, Van Dam J, Feld MS: Diffuse reflectance spectroscopy of human adenomatous colon polyps *in vivo*. *Appl Opt* 38:6628–6637, 1999

APPENDIX

According to the model, the diffuse reflectance $R(\lambda)$ is given by

$$R(\lambda) = R_0 \frac{\mu_s'}{\mu_s' + \mu_a} \left\{ \exp(\mu z_0) + \exp[-(1 + (4A/3))\mu z_0] \right.$$

$$\left. -z_0 \frac{\exp(-\mu r_1')}{r_1'} - (1 + (4A/3))z_0 \frac{\exp(-\mu r_2')}{r_2'} \right\} \qquad (A.1)$$

with

$$r_1' = (z_0^2 + r_c^2)^{1/2}$$

$$r_2' = [z_0^2(1 + (4A/3))^2 + r_c^2]^{1/2}$$

$$\mu = [3\mu_a(\mu_a + \mu_s')]^{1/2}, z_0 = \frac{1}{\mu_s'}.$$

$\mu_s'(\lambda)$ and $\mu_a(\lambda)$ are the reduced scattering and absorption coefficients, respectively, and A is a parameter that depends on the refractive index of skin. We have assumed $n = 1.4$ for the skin refractive index, which yields $A = 2.8$ (Zonios *et al*, 1999). The parameters R_0 and r_c are empirically determined by measuring the diffuse reflectance spectra of polystyrene bead suspensions as explained further below and elsewhere (Zonios *et al*, 1999).

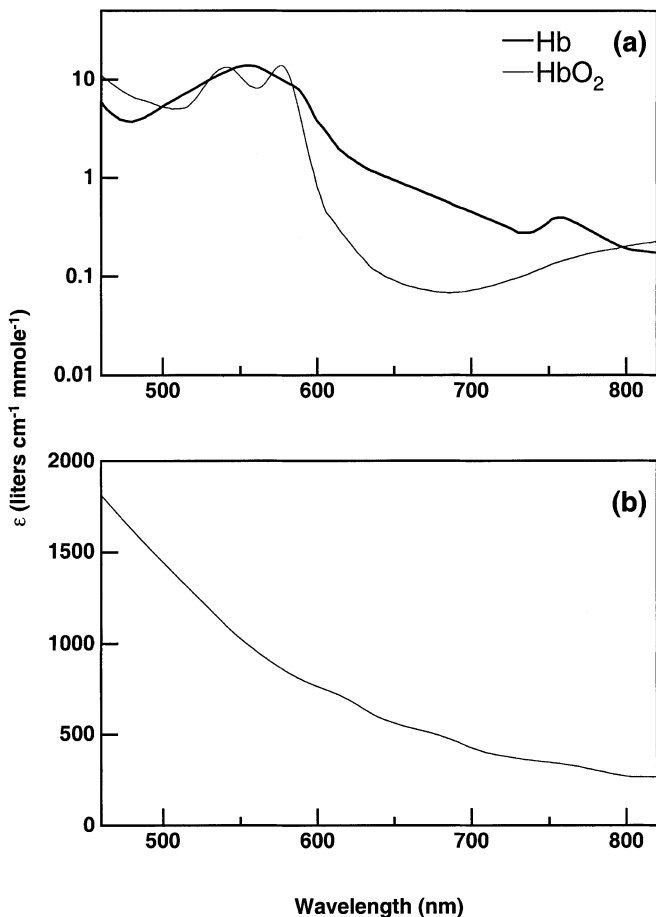Absorption in skin was assumed to be due to hemoglobin and melanin. Thus, the absorption coefficient $\mu_a(\lambda)$ was written as the sum of the oxy/deoxy hemoglobin (van Assendelft, 1970) and melanin (Crippa *et al*, 1978) absorptions as follows:

$$\mu_a(\lambda) = c_{Hb}^*[\alpha\varepsilon_{HbO_2}(\lambda) + (1 - \alpha)\varepsilon_{Hb}(\lambda)] + c_{mel}\varepsilon_{mel}(\lambda) \quad (A.2)$$

where

$$\alpha = \frac{c_{HbO_2}}{c_{HbO_2} + c_{Hb}}$$

is the hemoglobin oxygen saturation parameter, c_{HbO_2} and c_{Hb} are the concentrations of oxyhemoglobin and deoxyhemoglobin, respectively, and $c_{Hb}^* = c_{HbO_2} + c_{Hb}$ is the total concentration of hemoglobin. The molecular extinction coefficient spectra of oxyhemoglobin, deoxyhemoglobin, and melanin are $\varepsilon_{HbO_2}(\lambda)$, $\varepsilon_{Hb}(\lambda)$, and $\varepsilon_{mel}(\lambda)$, respectively, and are shown in **Fig A1**. Note the characteristic absorption bands of oxyhemoglobin (415, 542, and 577 nm) and deoxyhemoglobin (430 and 555 nm), as well as the relatively smooth and featureless absorption spectrum of melanin.

Scattering was modeled by assuming skin scatterers to be a homogeneous collection of microspheres (Zonios *et al*, 1999), and $\mu_s'(\lambda)$ was assumed to exhibit a linear dependence on wavelength. This is an approximation supported by Mie scattering theory (Born and Wolf, 1975; Wiscombe, 1979) as illustrated in **Fig A2(a)**, which shows $\mu_s'(\lambda)$ for a collection of homogeneous spherical scatterers with Gaussian distribution (with width σ) in the scatterer diameter. The reduced scattering coefficient for three different
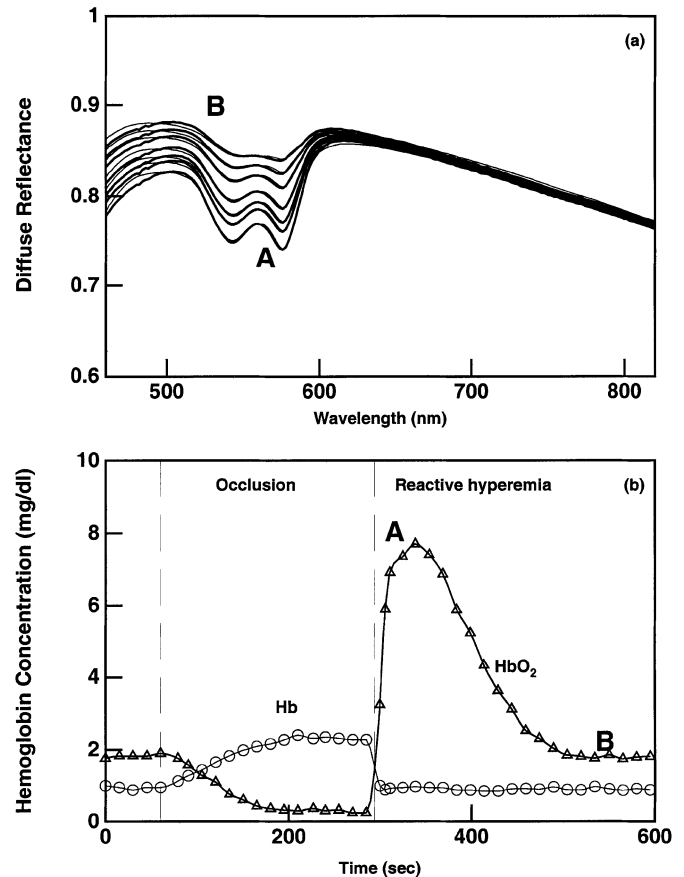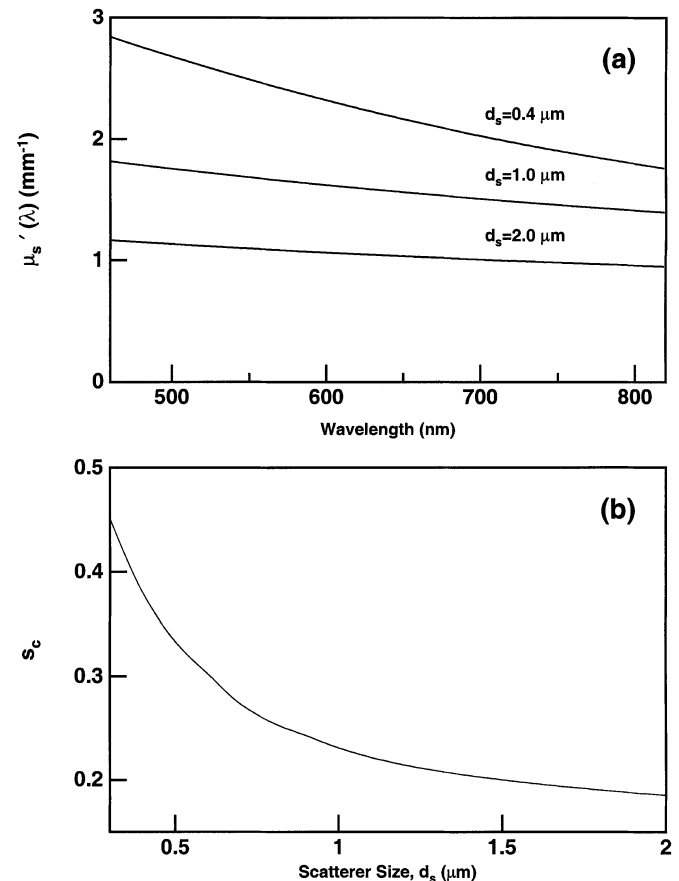

Figure A1. Molecular extinction coefficient spectra of the major chromophores found in human skin. (*a*) Oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb), and (*b*) melanin.

Figure A2. Reduced scattering spectra according to Mie scattering theory. (*a*) μ_s' for a Gaussian distribution of spherical particles with constant particle density. Scattering spectra are shown for three different particle diameters: $d_s = 0.4$, 1.0, and 2.0 μm. The width of the distribution is $\sigma = 0.1$ μm. (*b*) The scattering slope s_c of the same spectra, shown as a function of the scatterer diameter d_s.

distributions is shown with average scatterer sizes d_s = 0.4, 1.0, and 2.0 μm, and σ = 0.1 μm. As can be seen, $\mu_s'(\lambda)$ has an approximately linear dependence on wavelength in the 460–820 nm range, with a slope depending on the scatterer size. Furthermore, the slope of $\mu_s'(\lambda)$ was not found to have a significant dependence on the width of the Gaussian distribution. Thus, $\mu_s'(\lambda)$ was written as follows:

$$\mu_s'(\lambda) = \left(1 - s_c \frac{\lambda - \lambda_{min}}{\lambda_{max} - \lambda_{min}}\right)\mu_s'(\lambda_{min}) \qquad (A.3)$$

where s_c is the spectral slope, which is a function of the characteristic scatterer size d_s, and λ_{max} = 820 nm and λ_{min} = 460 nm are the maximum and minimum wavelengths, respectively. **Figure A2**(*b*) shows the dependence of s_c on d_s as calculated by Mie theory in the range 0.3 < d_s < 2.0 μm. In this range, this dependence can be expressed by

$$s_c \approx \frac{1}{4\sqrt{d_s^{1/2}}} \qquad (A.4)$$

with d_s in μm. An average "effective" scatterer size can thus be assigned to a given scattering spectrum, from the value of the scattering slope s_c. In the above Mie theory calculations, a refractive index of n_s = 1.40 was assumed for the scatterers, and n_0 = 1.36 for the surrounding material (Perelman *et al*, 1998; Zonios *et al*, 1999).

Determination of r_c and R_0 was performed as explained in detail elsewhere (Zonios *et al*, 1999). Briefly, the procedure followed is outlined here. A polystyrene bead suspension was used with bead diameter 1.0 μm and the bead density was adjusted such that the diffuse reflectance signal intensity was made comparable to that of skin. The reduced scattering spectrum of the beads was calculated using Mie theory and equation (A.1) was fitted to the actual measured bead reflectance spectrum to determine the optimal value of r_c. Fitting was performed by minimizing the χ^2 difference between the diffuse reflectance spectrum of the bead suspension and equation (A.1), using the Levenberg–Marquardt nonlinear iterative minimization method (Press *et al*, 1992). Once r_c = 4.0 mm and R_0 = 0.70 were determined in this way, they were kept fixed throughout the entire data analysis of the skin spectra.

Diffuse reflectance spectra from skin were analyzed by fitting to equation (A.1). Fitting was carried out by minimizing the χ^2 difference between the model and the experimental data for the entire wavelength range using the Levenberg–Marquardt minimization method (Press *et al*, 1992).