Bulk optical parameters of porcine skin dermis at eight wavelengths from 325 to 1557 nm

Xiaoyan Ma, Jun Qing Lu, Huafeng Ding, and Xin-Hua Hu

Department of Physics, East Carolina University, Greenville, North Carolina 27858

Received September 10, 2004

We introduce a method with which to obtain accurately the bulk optical parameters of fresh biological tissues *in vitro* by combining measurements of the sample surface profiles, reflectance, and transmittances with Monte Carlo-based inverse calculations. The bulk optical parameters of fresh porcine dermis tissue were determined at eight wavelengths from 325 to 1557 nm and were found to be much different from those determined without consideration of surface roughness. © 2005 Optical Society of America

OCIS codes: 170.3660, 170.4730, 290.5880.

Accurate knowledge of the optical properties of biological tissues is important in the development of photomedicine and has attracted extensive research interest. Based on the radiative transfer theory, three parameters are used to model tissue optics in bulk: scattering coefficient μ_s, absorption coefficient μ_a, and single-scattering phase function $p(\mathbf{s}, \mathbf{s}')$ that describes the probability of light being scattered from direction $\mathbf{s}$ to direction $\mathbf{s}'$.[1] Henyey–Greenstein function $p(\theta)$ has been widely used as a phase function to describe the scattering pattern averaged over different types of cell and scatterer within a tissue and over the azimuthal angle of scattering.[2,3] As a single-parameter function, $p(\theta)$ is fully characterized by its first moment, often called anisotropy factor g. Because of the insolubility of the radiative-transfer equation for all but simple geometries, a statistical method of Monte Carlo (MC) simulations is often used for numerical solution of radiative transfer problems with realistic boundaries.[4,5] Others prefer the use of approximations to obtain analytical solutions to the equation.[6] For example, the first-order diffusion approximation to the radiative-transfer equation has been applied extensively for modeling of tissue optics. The accuracy of the diffusion approximation, however, depends on how well the condition $\mu_a \ll \mu_s'$ is satisfied, where $\mu_s' = \mu_s(1 - g)$ is the reduced scattering coefficient.[7] In both cases, the correct values of bulk tissue optical parameters at relevant wavelengths must be known for validation of the model and accurate interpretation of experimental data.

Previous *in vitro* measurements of μ_s, μ_a, and g were carried out by use of slab tissue samples without consideration of the inherently rough surfaces of the samples (see Ref. 8 and references therein). We have demonstrated by MC simulations that surface roughness can significantly affect the accuracy of bulk optical parameters inversely determined from turbid samples.[8] In this Letter we present a method with which to determine the bulk optical parameters, with surface roughness taken into consideration, of fresh porcine dermis tissue samples at eight wavelengths from 325 to 1557 nm.

To model the effect of surface roughness accurately, one must know the real refractive-index and surface-profile parameters of the fresh porcine skin tissue. We investigated a coherent reflectance curve method for obtaining this information and determined the complex refractive indices of fresh porcine skin tissues at the eight wavelengths; the results for the porcine dermis are listed in Table 1 together with those of the sample holder glasses.[9] The surface profiles of two fresh porcine dermis samples were measured by use of a noncontact method of confocal imaging, and the roughness parameters were extracted based on a statistical analysis of the profiles.[10,11]

The surface-profile measurements were carried out with a confocal laser scanning microscope (LSM 510, Zeiss) with an infinity-corrected Plano-Neofluar objective (40×, 1.3 N.A.) and a wavelength of 488 nm. The fresh dermis samples were prepared in the form of a slab 10 mm square and ~0.5 mm thick and were sandwiched between two microscope cover glasses without staining. A stack of reflectance images of the sample in the format of 512 × 512 pixels (12 bits) was acquired in the x–y plane with a 230-μm field of view at the sample, each at a different z, until the top sample surface was translated through the focal

Table 1. Sample Thickness d and Real Index n_r of Fresh Porcine Dermis Tissue and Sample Holder Glass Adopted in MC Simulations

		n_r	
Wavelength (nm)	d (mm)[a]	Porcine Dermis[b]	Glass[c]
325	0.44 ± 0.090	1.393	1.482 (UV fused silica)
442	0.31 ± 0.021	1.376	1.466 (UV fused silica)
532	0.37 ± 0.10	1.359	1.520 (BK7)
633	0.53 ± 0.095	1.354	1.515 (BK7)
850	0.47 ± 0.79	1.364	1.510 (BK7)
1064	0.65 ± 0.13	1.360	1.507 (BK7)
1310	0.73 ± 0.087	1.357	1.504 (BK7)
1557	0.84 ± 0.076	1.361	1.501 (BK7)

[a]Mean value plus or minus standard deviation of five dermis samples.
[b]From Ref. 9.
[c]From the Melles–Griot catalog.

0146-9592/05/040412-03$15.00/0

plane in 0.20-μm steps in air with an accuracy of 0.05 μm. We used the stack of reflectance images $I_z(x, y)$ to determine the dependence of the reflected light intensity on z at a fixed transverse position of (x, y), i.e., $I_{x,y}(z)$. Based on the diffraction theory of confocal microscopy,[12] $I_{x,y}(z)$ reaches a maximum at $z = \zeta$ when the focal plane crosses the sample surface at (x, y) because the maximum index mismatch then occurs at the surface. By repeating this process for each pixel of (x, y) along a line parallel to the x axis, we determined a linear surface-profile function, $z = \zeta(x, y) = \zeta(x)$, in which the surface height is plotted as a function of x at a fixed y. A typical linear surface-profile function for one porcine dermis sample is presented in Fig. 1, in which the top of the curve indicates the surface of the cover glass.

The accuracy of this method for determining the surface profiles of fresh tissue samples was evaluated with polystyrene microspheres suspended in deionized water between two cover glasses under light pressure. For microspheres with nominal diameters of 9.6 $\pm$ 1.9 μm (7510A, Duke Scientific) we found the distance between the two water–glass interfaces to be 10.6 μm, whereas for microspheres of 25 $\pm$ 4.0 μm the distance was 33.2 μm. These results demonstrate that our surface-profile measurements have an error of less than 2 μm in the z axis if a normal distribution of microsphere diameters is assumed. We determined the accuracy in the x or y axis to be $\sim$1 μm, using a microscope scale of 10 μm/line pair. The statistical treatment of the linear surface-profile functions used for extracting surface-profile parameters has been well established.[10] On each of the two dermis samples, nine linear surface profiles at different values of y were measured and combined into three extended lines $\sim$690 μm long to make parameter calculations statistically significant. The rms surface height and lateral correlation length were $\delta = 8.17 \pm 3.0$ and $a = 8.96 \pm 5.6$ μm, respectively, where the mean values and standard deviations were obtained from the values of δ and a of six extended lines from the two dermis samples.

Fresh porcine skin patches were obtained from the departments of surgery and comparative medicine, Brody School of Medicine, East Carolina University, stored in an ice bucket at $\sim$2 °C, and kept hydrated with saline drops. Fresh porcine dermis samples were prepared by procedures identical to those used in the surface-profile measurements and placed between two optical windows. We measured diffuse reflectance R_d and diffuse transmittance T_c by using an integrating sphere and collimated transmittance T_e by using a spatial filtering setup with the sample *in situ*.[8,13] For each of the eight wavelengths, five dermis samples of different thicknesses d from different pigs were used for measurements of R_d, T_d, and T_c. All the measurements were carried out with a lock-in amplifier at a room temperature of $\sim$22 °C and were completed no later than 30 h postmortem.

At each wavelength the R_d, T_d, and T_c data from each sample were inverted to yield the bulk value of optical parameters μ_s, μ_a, g by an iteration process based on the least-squares method.[8] We used a previously developed MC code[5,8] to obtain $(R_d)_{\text{cal}}$, $(T_d)_{\text{cal}}$, and $(T_c)_{\text{cal}}$ for a slab sample, using rough surface parameters δ and a. Other input parameters included the real refractive indices of the porcine dermis sample and the glass holder at the wavelengths listed in Table 1, and their diameters and thicknesses. With the accurate description of the configurations of the integrating sphere and spatial filtering setup in the MC code, we used the squared error function

$$\Sigma = \left[\frac{(R_d)_{\text{cal}} - R_d}{R_d}\right]^2 + \left[\frac{(T_d)_{\text{cal}} - T_d}{T_d}\right]^2 + \left[\frac{(T_c)_{\text{cal}} - T_c}{T_c}\right]^2 \quad (1)$$

to guide the iteration process. The iteration was terminated when $\Sigma \le \Sigma_c$ $(= 4 \times 10^{-4})$, and the uniqueness of the inverse solution has been verified numerically for selected samples and wavelengths.[8] For comparison when smooth surfaces are assumed, we conducted similar inverse calculations, using the same set of experimental data (R_d, T_d, T_c) to obtain μ_s, μ_a, and g with $\delta = 0$. All the MC simulations were carried out on our parallel computing network with eight nodes of 3.06-GHz dual CPUs. In Fig. 2 we compare the inversely determined bulk parameters μ_s, μ_a, and g for porcine dermis under the two assumptions.

The (μ_s, μ_a, g) data presented in Fig. 2 clearly show that the surface roughness of sectioned tissue samples can significantly affect the values of bulk optical parameters. Marked decreases in μ_s and g were observed when surface roughness was considered, in agreement with the previous studies of phantom sample thickness of 0.2 mm and $\delta/a < 0.3$.[8] At the three wavelengths longer than 850 nm, the values of μ_a were larger when rough surfaces were assumed than for smooth surfaces. Through MC simulations on both thin and thick samples, we found that this variation in the direction of μ_a from that presented in Ref. 8 can be attributed to two factors, the large thickness of the samples, as given in Table 1, and the large ratio of δ/a. For example, as δ/a increases, photons exiting a tissue surface have an augmented chance to reenter the tissue; thus μ_a needs to be increased. It

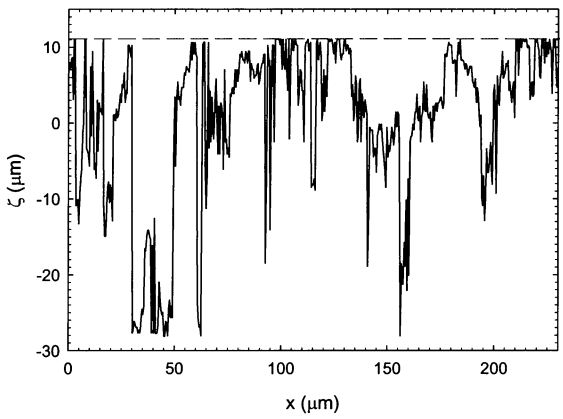

Fig. 1. Typical linear surface-profile function along one of the lines sampled across a porcine dermis sample. Dashed line, cover glass surface.

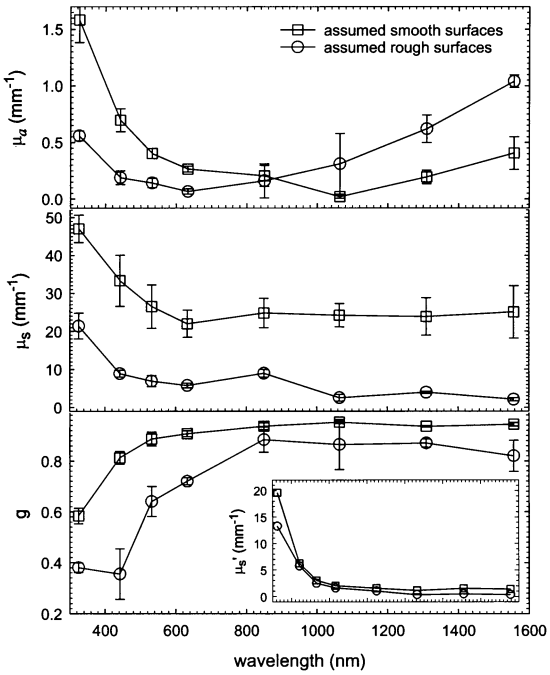

Fig. 2. Wavelength dependence of the optical parameters of porcine dermis samples inversely determined with assumptions of rough surfaces and of smooth surfaces. Error bars, standard deviations obtained from five samples of different thicknesses at each wavelength; solid curves are provided as a visual aid. Inset, wavelength dependence of reduced scattering coefficient $\mu_s' = \mu_s(1 - g)$.

should also be pointed out that the blood component was almost absent and the concentration of melanin pigments was low in the porcine dermis samples. The effect of these chromophores on μ_a should be considered when one is comparing our data with *in vivo* results for wavelengths below 800 nm.[2,3]

The parameters of μ_s and g presented in this Letter are substantially smaller than the published values of human and porcine dermis[2,14,15] that have been widely cited. Because our *in vitro* method takes into account surface roughness for the first time to our knowledge, the comparison of μ_s and g should be made against the *in vivo* results with a minimized surface effect. Because of a lack of μ_s and g data in the literature, reduced scattering coefficient μ_s' was chosen for comparison with published *in vivo* results of measurements made with contact fiber detectors from breast tissues (including the skin). With frequency-domain[16] and time-resolved methods based on diffusion models,[17] μ_s' has been found to vary from 1.1 to 0.6 mm^{-1} for wavelengths of 674–956 nm (Ref. 16) and 0.6–1.1 mm^{-1} at a wavelength of 753 nm.[17] These values agree better with our μ_s' determined by assuming rough surfaces ranging from 1.6 to 0.4 mm^{-1} than by assuming smooth surfaces ranging from 2.0 to 1.1 mm^{-1} for wavelengths of 632–1064 nm. It is interesting to note that the ratios of μ_s' to μ_a at the three wavelengths of 1064, 1310, and 1557 nm were found to be ~1 or less, and therefore the application of diffusion models at these wavelengths has to be carefully validated.

In summary, we have developed a Monte Carlo-based method with which to determine accurately the bulk optical parameters of fresh tissue samples *in vitro*. The measurements of refractive-index and surface-profile parameters of the slab samples allowed us to take into account the inherent surface roughness of the tissue samples, which proved to be significant. The bulk optical parameters of the fresh porcine dermis tissue were found to be significantly different from those determined without consideration of surface roughness.

X.-H. Hu's e-mail address is hux@mail.ecu.edu.

References

1. S. Chandrasekhar, *Radiative Transfer* (Dover, New York, 1960).
2. M. J. van Gemert, S. L. Jacques, H. J. Sterenborg, and W. M. Star, IEEE Trans. Biomed. Eng. **36,** 1146 (1989).
3. J. Q. Lu, P. Yang, and X. H. Hu, "Simulations of light scattering from a biconcave red blood cell using the FDTD method," J. Biomed. Opt. (to be published).
4. B. C. Wilson and G. Adam, Med. Phys. **10,** 824 (1983).
5. J. Q. Lu, X. H. Hu, and K. Dong, Appl. Opt. **39,** 5890 (2000).
6. A. Kienle and M. S. Patterson, J. Opt. Soc. Am. A **14,** 246 (1997).
7. D. A. Boas, Opt. Express **1,** 404 (1997), http://www.opticsexpress.org.
8. X. Ma, J. Q. Lu, and X. H. Hu, Opt. Lett. **28,** 2204 (2003).
9. H. Ding, J. Q. Lu, K. M. Jacobs, and X. H. Hu, "Determination of refractive indices of porcine skin tissues and Intralipid at eight wavelengths between 325 and 1557 nm," J. Opt. Soc. Am. A (to be published).
10. J. M. Bennett and L. Mattsson, *Introduction to Surface Roughness and Scattering*, 2nd ed. (Optical Society of America, Washington, D.C., 1999).
11. X. Ma, "Study of optical properties of skin tissues from ultraviolet to short-wave infrared," Ph.D. dissertation (East Carolina University, Greenville, N.C., 2004).
12. J. B. Pawley, *Handbook of Biological Confocal Microscopy*, 2nd ed. (Plenum, New York, 1995).
13. X. Ma, J. Q. Lu, R. S. Brock, K. M. Jacobs, P. Yang, and X. H. Hu, Phys. Med. Biol. **48,** 4165 (2003).
14. R. Graaff, A. C. M. Dassel, M. H. Koelink, F. F. M. de Mul, J. G. Aarnoudse, and W. G. Zijlstra, Appl. Opt. **32,** 435 (1993).
15. Y. Du, X. H. Hu, M. Cariveau, X. Ma, G. W. Kalmus, and J. Q. Lu, Phys. Med. Biol. **46,** 167 (2001).
16. B. J. Tromberg, O. Coquoz, J. B. Fishkin, T. Pham, E. R. Anderson, J. Butler, M. Cahn, J. D. Gross, V. Venugopalan, and D. Pham, Philos. Trans. R. Soc. London Ser. B **352,** 661 (1997).
17. K. Suzuki, Y. Yamashita, K. Ohta, M. Kaneko, M. Yoshida, and B. Chance, J. Biomed. Opt. **1,** 330 (1996).