

Wavelength-dependent penetration depths of ultraviolet radiation in human skin

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Abstract. The wavelength-dependent penetration depth of ultraviolet radiation in human skin is a fundamental parameter for the estimation of the possible photobiological impact of ultraviolet (UV) radiation. We have determined the absorption spectra of human skin *in vivo* in the wavelength range from 290 to 341 nm in 3-nm steps using laser optoacoustics and calculated the respective penetration depths. Data were analyzed with respect to different skin regions and skin phototype of the 20 subjects in the study (phototype I: $n=3$; II: $n=7$; III: $n=5$; IV: $n=5$), revealing large variability between individuals. The penetration depth of UV radiation in human skin is highly dependent on wavelength and skin area, but no significant dependence on skin phototype could be found. © 2008 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2957970]

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1 Introduction

The wavelength-dependent penetration depth of light into human skin is an important mark for photobiologists helping to estimate ultraviolet (UV)-induced processes in the different skin layers. For example, knowing how much radiation of what wavelength is available at a certain depth in living human skin is one prerequisite for a valid risk assessment for skin cancer based on *in vitro* and animal models.

Thus far, photobiologists had to rely mainly on *in vitro* and *ex vivo* data on optical properties and penetration depths because of the lack of an appropriate *in vivo* technique.¹⁻⁴ Especially in recent years, available *in vivo* data on optical properties and relevant chromophores have been advanced by reflectance spectroscopy, fluorescence, and optical coherence tomography for the visible and infrared wavelength range.⁵⁻⁸ However, measurements in the UV range suffer from the strong increase of absorption toward shorter wavelengths, making purely optical techniques less and less favorable. This challenge could be met by the development of UV optoacoustics, a hybrid technique allowing noninvasive, depth-resolved determination of optical properties of human skin even in the UV.⁹⁻¹¹ Thus far, optoacoustics has been successfully applied as a tomographic technique tracing especially chromophores, such as melanin, oxy- and deoxyhemoglobin, for image contrast.¹²⁻¹⁴

The mechanism behind optoacoustics is the absorption of radiation energy in matter, its transfer to heat and pressure, and the release of a resulting transient stress wave—thermo-optical excitation of ultrasound. If the incident light pulses are sufficiently short—typically in the low, one-digit nanosecond range—the profile of the stress transient repro-

duces the distribution of heat sources in the sample and, consequently, the light distribution in the sample can be deduced allowing one to calculate the depth-dependent optical properties of the sample. Depending on the cumulative dose of UV exposure and the UV sensitivity of the subject, this technique may be completely noninvasive or induce a (mild) erythema at worst.

Here, we present the first *in vivo* data on the wavelength-dependent penetration depths of UV radiation at different skin sites which were derived from absorption coefficients of human skin measured by optoacoustics.

2 Materials and Methods

2.1 Volunteers

The optical properties of human skin *in vivo* were determined on the volar and dorsal aspect of the forearm as well as on the thenar in a subject study ($n=20$, 14 women, 6 men) using UV optoacoustics. The study design was approved by the local ethics committees, and all volunteers gave informed consent.

2.2 Phototype/MED

Subjects with different phototypes (I: $n=3$, II: $n=7$, III: $n=5$, IV: $n=5$) were tested for their individual minimal erythema dose (MED) at the volar side of the forearm. The subject's individual MED at the volar side of the forearm was determined by chromameter measurements (Minolta CR200, Minolta, Osaka, Japan) according to COLIPA recommendations, defining the erythema threshold as a change in a^* of +2.5 units 24 h after irradiation with a solar simulator (M.U.T. GmbH, Hamburg, Germany). For subjects with Fitzpatrick phototype characteristics in-between two types, the phototype classification was decided with the help of their

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MED at the volar side of the forearm, resulting in a corresponding MED range for each phototype: phototype I 200–230 mJ/cm², phototype II ca. 250 mJ/cm², phototype III 260–280 mJ/cm², and phototype IV 300–400 mJ/cm².

2.3 Optoacoustics

Optoacoustic measurements were carried out in reflection mode. Laser radiation is delivered via a UV-enhanced optical fiber that illuminates a ca. 4 mm² large area of the skin through UV-transparent ultrasound gel, which also ensures acoustic coupling between the sample and a poly(vinylidene fluoride) ultrasound detector above the skin. More details of the optoacoustic sensor have been described elsewhere.^{15,16}

A pulsed UV laser system (NL303G+PG122/UV, Ek-spla, Lithuania) provided wavelength tunable radiation in the range from 290 to 341 nm with pulse duration of 5 ns and pulse energies in the range of 200 to 700 μJ. The energy of each pulse was recorded by an energy monitor at the UV fiber. For one optoacoustic wavelength scan, the subject's skin was exposed to an erythemally weighted UV dose on the order of 50 mJ/cm².

Within this wavelength range, optoacoustic measurements were carried out in 3-nm steps at the volar and dorsal aspect of the forearm as well as on the thenar. Three spectra were recorded for averaging within a circular area of <2.5 cm² on the relevant skin areas of each subject. On the volar aspect of the forearm, these measurements were carried out on both arms and again 72 h after the first measurements on the right arm (no indication of significant differences between these three measurement triples). If necessary, the skin area was shaved at the dorsal aspect of the forearm to minimize the influence of hair on the optoacoustic measurements. In any case, measurement spots were aimed to be in the hair-free space between follicles.

For signal processing and analysis, we used a self-developed simulation software based on the equations for thermo-optical excitation of sound in a dissipative medium with inhomogenous optical properties. The program takes into account the actual geometry of our experimental setup and was calibrated using melanin dyed tissue phantoms,¹⁶ thus also incorporating sound attenuation, influences of the electronic setup, etc. For analysis, the complete optoacoustic signal is simulated. Even though the optical properties of the skin are expected to change considerably with depth—especially from stratum corneum to viable epidermis—we could not resolve a depth dependency in our measured data. Student's t-test was used for statistical analysis of significant differences between the UV penetration depths at different skin sites.

3 Results

The absorption coefficients of human skin *in vivo* were measured at the volar and dorsal aspect of the forearm as well as at the thenar (three measurements at each site per subject) in a study on 20 subjects of different UV sensitivity (phototype I: *n*=3, II: *n*=7, III: *n*=5, IV: *n*=5). Penetration depth was calculated on the basis of these absorption coefficients.

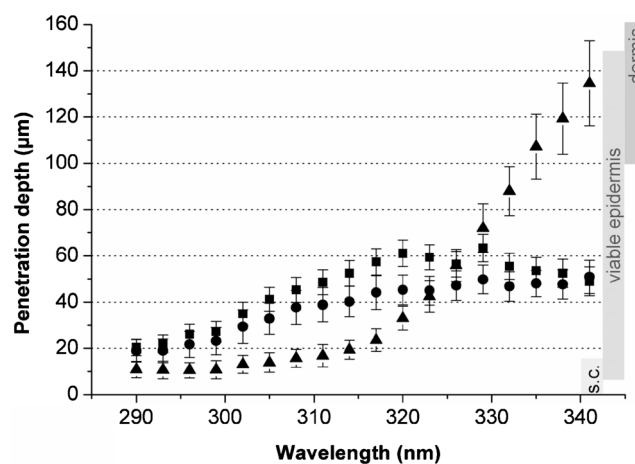


Fig. 1 Penetration depths (1/e-level) of UV radiation in human skin. Error bars mark 90% confidence intervals. The wavelength dependence of the penetration depth is similar at the volar (■) and dorsal (●) aspect of the forearm. It is increasing from 290 nm (volar: 20.4 ± 3.5 90%-CI μm; dorsal: 18.9 ± 4.8 μm) toward longer wavelengths. In the UVA, penetration depths are fairly constant with a maximum of 61.1 ± 5.7 μm at 329 nm at the volar and a maximum of 50.9 ± 7.2 μm at 341 nm at the dorsal aspect. Spectral characteristics are different at the thenar (▲). Here, the penetration depth is only very slowly rising in the UVB (10.9 ± 3.5 to 19.4 ± 4.1 μm at 290–314 nm) and increases steeply in the UVA (up to 134.6 ± 18.4 μm at 341 nm). The bar scheme at the side indicates the ranges of important skin layers in normal skin (not thenar) for orientation (s.c.: stratum corneum).

3.1 Skin Sites

The skin on the inner and outer sides of the arm is very much alike; it may be a little thicker and more strongly pigmented on the outer side of the arm. Figure 1 shows the average wavelength-dependent penetration depths (290–341 nm) at the three sites together with their 90% confidence intervals. As expected, the penetration depths at the volar and dorsal forearm show fairly similar characteristics. Ultraviolet radiation can penetrate deeper into the skin at the volar side throughout the spectrum ($p < 0.05$ for 335–305 and 296 nm, $p < 0.005$ for 329–311 and 305 nm; one-tailed paired t-test). This is in accordance with the consideration that skin on the dorsal forearm of the same subject should display the same pigmentation as on the volar side, plus possibly some amount of extra facultative pigmentation because the outer side of the forearm is more strongly exposed to the sun than the inner side. Equal or stronger pigmentation should result in equal or shallower penetration depths comparing these two sites for the same subject. Minimal penetration depths of ca. 20 μm are found at 290 nm for both sites. Toward longer wavelengths, the penetration depths are slowly increasing in both spectra (up to 50–60 μm, see Fig. 1 for details). At the dorsal side, maximal penetration depths are found at 320 nm followed by a weak decrease toward 341 nm. At the volar side, the penetration depths do not show a maximum but reach a plateau in the UVA (315–499 nm).

The spectrum of penetration depths at the thenar differs significantly from those at the forearm (volar/thenar: $p < 0.005$ for 341–332 and 323–290 nm, one-tailed paired t-test; dorsal/thenar: $p < 0.05$ for 341–329 and 320–290 nm,

$p < 0.005$ for 341–332 and 317–299 nm, one-tailed paired t-test): whereas in the UVB (290–315 nm) the penetration depth only slowly rises from well above $10 \mu\text{m}$, it increases steeply in the UVA so that radiation even penetrates deeper at the thenar than at the forearm sites at wavelengths longer than ca. 326 nm.

As already indicated when comparing volar and dorsal forearm spectra, a one-tailed paired t-test was used because we expect a change of optical properties from skin site to skin site caused by UV adaptation and/or a thicker horny layer to go in the same direction for each subject. Intraindividual comparison of the site spectra supports this assumption. However, even if this is not assumed and a two-sided test is used, the results substantially stay the same: for the difference between volar and dorsal forearm spectra, p is then slightly larger than 0.05 for 335 and 296 nm and drops from highly significant ($p < 0.005$) to significant ($p < 0.05$) for 326 nm; no changes in the level of significance occur for the t-test of volar/thenar, and for dorsal/thenar, p only turns from highly significant to significant at 299 nm.

3.2 Phototypes

Figure 2 takes a closer look at the phototype dependence of the penetration depths at the three sites. This time the average penetration depth was calculated separately for the different phototypes. Of course, the 90% confidence intervals are fairly large here because of the small number of subjects: $n=3$ for phototype I, $n=7$ for phototype II, $n=5$ for phototype III, and $n=5$ for phototype IV. Still, the breakdown of the data into the four phototype groups allows an interesting perspective.

At the volar side of the forearm [Fig. 2(a)], penetration depths of phototypes I–III are very close in the UVB. Penetration depth is minimal at 290 nm ($20 \mu\text{m}$) and rises constantly up to ca. $55 \mu\text{m}$ at 314 nm. It is maximal in the range from 317 to 329 nm and decreases again toward longer wavelengths. In the UVA, the close agreement between the three phototypes is lost to some degree and the curves are less smooth. Penetration depths are now higher (approximately $60\text{--}80 \mu\text{m}$) in phototypes I compared to phototypes II and III (well above $40 \mu\text{m}$ to ca. $60 \mu\text{m}$). Phototype IV behaves a little differently from the other three types. Penetration depth stays at a low level of $<20 \mu\text{m}$ from 290 nm up to 299 nm. Then it rises to another plateau at $60 \pm 5 \mu\text{m}$ from 332 nm onward. This implicates higher penetration depths in phototypes I and IV in the UVA range above ca. 330 nm than in phototypes II or III.

At the dorsal side of the forearm [Fig. 2(b)], phototypes I and II as well as III and IV seem to be grouped throughout the UVB and up to ~ 320 nm. Penetration depth is again rising from shorter to longer wavelengths, and it is higher in the light skin types than in the darker ones. Phototypes behave differently toward longer wavelengths. In type IV, the penetration depth is constantly rising from $\sim 20 \mu\text{m}$ at 290 nm up to above $45 \mu\text{m}$ at 341 nm. Types III and II reach a kind of plateau in the UVA. However, this level is reached earlier in type II (at ca. 318 nm) than in type III (at ca. 326 nm) and it is as high as $50 \mu\text{m}$ in skin type II and $<45 \mu\text{m}$ in type III. In phototype I, penetration depths reach maximal levels of $50\text{--}55 \mu\text{m}$ in the range of 318–329 nm and then decrease toward longer wavelengths down to $40 \mu\text{m}$.

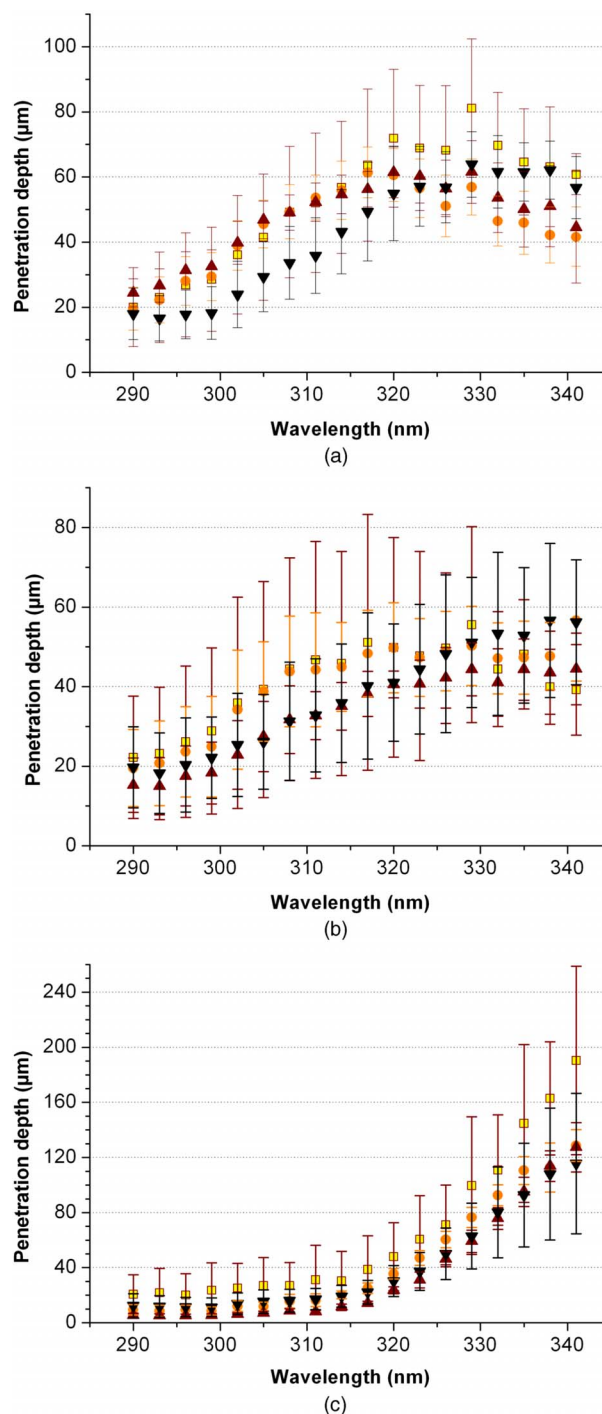


Fig. 2 Average spectra of the penetration depths at different skin sites: (a) volar aspect of the forearm, (b) dorsal aspect of the forearm, and (c) thenar (ball of the thumb). Mean spectra and 90% confidence intervals are shown for phototypes I–IV; phototype I ($n=3$): ■, phototype II ($n=7$): ●, phototype III ($n=5$): ▲; and phototype IV ($n=5$): ▼.

The phototype-dependent penetration depths calculated for the thenar [Fig. 2(c)] show an interesting trend: the penetration depths in the horny layer are considerably higher in phototype I than in the other skin types throughout the measured spectral range. The confidence intervals of phototype I data are relatively large due to the small number of subjects in this

group; thus, this difference is not significant. If, however, the variance in a larger group of phototype I subjects turned out to be equally small, as that of the group containing phototypes II–IV, the difference between the thenar absorption spectra would become significant for wavelengths other than 335–323 and 314 nm.

4 Discussion and Conclusions

Our new data show the natural variance of *in vivo* properties between individuals and between different skin sites and provide enhanced wavelength resolution. Thus, these first *in vivo* results add important new information to the *ex vivo* results^{1–4} and answer the call for an *in vivo* validation of earlier *ex vivo* data. Anderson and Parrish⁴ estimate the penetration depth of UV radiation in fair Caucasian skin to be 1.5 μm at 280 nm, 6 μm at 300 nm, and 60 μm at 350 nm. According to Jacques,² penetration depths for a moderately pigmented adult with a 10% volume fraction of melanosomes can be expected to rise from ca. 13 μm at 290 nm to 23 μm at 341 nm. Our results are in the same order of magnitude and also agree with respect to the wavelength dependency of the penetration depths but provide a more differentiated view.

The comparison of penetration depths at different skin sites hint at keratin as an important natural UVB filter apart from the skin pigment melanin. At the forearm, the keratin-containing stratum corneum at the skin surface is only ca. 10 μm thick, whereas it is several 100 μm thick at the thenar. Keratin is a potent short-range UVB absorber and a strong scatterer throughout the spectrum, whereas melanin is a broadband UV absorber.^{17,18} Melanin also acts as a scatterer, but its scattering coefficient only contributes a few percent of the total attenuation coefficient in the UV.¹⁹ Thus, the thenar absorption spectrum is dominated by the characteristics of keratin, whose absorption coefficient is rapidly decreasing toward longer wavelengths. This is not compensated for by melanin absorption at the palm. In contrast to this, the much higher melanin concentration at the forearm limits UV penetration throughout the spectrum.

The subdivision of the skin site spectra according to the subjects' phototypes shows interesting trends, even though the relatively small number of subjects per phototype impedes significant results. Apart from different values for the penetration depth at the same wavelength, the spectral course may be different for different phototypes as well. At the volar side of the arm, for example, the penetration depth reaches its maximum at 317 nm in phototype II, whereas maximal penetration depths for phototype IV are found at 299 nm and above. Comparing volar forearm spectra and the horny layer spectra from the thenar suggests that the comparably small penetrations depths and the shift of maximal penetration depth toward longer UV wavelengths found at the volar forearm of phototype IV subjects might be due to a stronger influence of the horny layer on the optical properties, possibly because of a thicker horny layer. A thicker horny layer could also provide an explanation for phototype IV showing a trend to higher penetration depths at the volar forearm than phototype II and III for wavelengths longer than 330 nm.

Melanin is not distributed equally in the epidermis, but for white subjects, 56% is contained in the lower (basal) layer, 30% in the middle (spinous) layer, and only 14% in the upper

(granulosum) layer.²⁰ We were not able to differentiate optically different layers of the epidermis by our optoacoustic measurements. Thus, if the upper layer of phototype IV skin is thickened, the measured optoacoustic data are more strongly influenced by the properties of the rather weakly pigmented upper skin layers, showing in comparably low measured absorption coefficients in the UVA and consequently higher penetration depths. However, this would have to be seen in the perspective of a thicker horny layer and thus a thicker epidermis (i.e., the viable epidermis may still be well shielded as would be expected for phototype IV). The decrease of penetration depth for wavelengths above ca. 330 nm, which is seen at the volar side for at least phototypes I–III and at the dorsal side for phototype I, could possibly be due to a maximum in the absorption of constitutive melanin.¹⁷ The spectral characteristics of this pigment could play a role at sites with low UV exposure or for subjects with a very weak ability to tan. If the trend of horny layer spectra splitting up in phototype I and phototypes II–IV can be confirmed in measurements on more subjects, then it will give way to some interesting speculations. The measurements at the thenar are representative for pure horny layer data. The differences between phototypes I and II–IV indicate that the horny layer of type I is more transparent for UV radiation than that of the other three. There is no reason why this should be a characteristic unique to very thick horny layers only. Consequently, a horny layer of the same thickness would provide much less UV shielding in type I than in the other three. Taking into account that the significance of the stratum corneum in photoprotection is higher in fair-skinned individuals than in pigmented individuals,²¹ this deficiency would represent an important cause for the high UV sensitivity of type I.

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References

1. F. A. Duck, *Physical Properties of Tissue*, Academic Press, London (1990).
2. S. L. Jacques, <http://omlc.ogi.edu/news/jan98/skinoptics.html> (1998).
3. M. J. C. van Gemert, S. L. Jacques, H. J. C. M. Sterenborg, and W. M. Star, "Skin optics," *IEEE Trans. Biomed. Eng.* **36**(12), 1146–1154 (1989).
4. R. R. Anderson and J. A. Parrish, "The optics of human skin," *J. Invest. Dermatol.* **77**, 13–19 (1981).
5. T. Gambichler, S. Boms, M. Stücker, G. Moussa, A. Kreuer, M. Sand, D. Sand, P. Altmeyer, and K. Hoffmann, "Acute skin alterations following ultraviolet radiation investigated by optical coherence tomography and histology," *Arch. Dermatol. Res.* **297**, 218–225 (2005).
6. R. Zhang, W. Verkruijse, B. Choi, J. A. Viator, B. Jung, L. O. Svaasand, G. Aguilar, and J. S. Nelson, "Determination of human skin optical properties from spectrophotometric measurements based on optimization by genetic algorithms," *J. Biomed. Opt.* **10**(2), 024030 (2005).
7. M. Larsson, H. Nilsson, and T. Strömberg, "In vivo determination of local skin optical properties and photon path length by use of spatially resolved diffuse reflectance with applications in laser Doppler flowmetry," *Appl. Opt.* **42**(1), 124–134 (2003).
8. R. M. P. Doornbos, R. Lang, M. C. Aalders, F. W. Cross, and H. J. C. M. Sterenborg, "The determination of *in vivo* human tissue optical properties and absolute chromophore concentrations using spatially resolved steady-state diffuse reflectance spectroscopy," *Phys. Med. Biol.* **44**, 967–981 (1999).
9. A. A. Karabutov, E. V. Savateeva, and A. A. Oraevsky, "Imaging of

- layered structures in biological tissues with opto-acoustic front surface transducer," *Proc. SPIE* **3601**, 284–295 (1999).
10. A. A. Oraevsky, S. L. Jacques, and F. K. Tittel, "Determination of tissue optical properties by piezoelectric detection of laser-induced stress waves," *Proc. SPIE* **1882**, 86–101 (1993).
 11. B. P. Payne, V. Venugopalan, B. B. Mikić, and N. S. Nishioka, "Optoacoustic determination of optical attenuation depth using interferometric detection," *J. Biomed. Opt.* **8**(2), 264–272 (2003).
 12. J. Oh, M. Li, H. F. Zhang, K. Maslov, G. Stoica, and L. V. Wang, "Three-dimensional imaging of skin melanoma *in vivo* by dual-wavelength photoacoustic microscopy," *J. Biomed. Opt.* **11**(3), 034032 (2006).
 13. U. Oberheide, I. Bruder, H. Welling, W. Ertmer, and H. Lubatschowski, "Optoacoustic imaging for optimization of laser cyclophotocoagulation," *J. Biomed. Opt.* **8**(2), 281–287 (2003).
 14. H. F. Zhang, K. Maslov, G. Stoica, and L. V. Wang, "Functional photoacoustic microscopy for high-resolution and noninvasive *in vivo* imaging," *Nat. Biotechnol.* **24**, 848–851 (2006).
 15. R. Krebs, M. Bartels, M. Meinhardt, H. Lubatschowski, and A. Anders, "Characterization of optical structures in biological tissue by means of UV-optoacoustics," *Laser Phys.* **13**, 1–6 (2003).
 16. M. Meinhardt, R. Krebs, A. Anders, U. Heinrich, and H. Tronnier, "Effect of UV adaptation on the UV absorption spectra of human skin *in vivo*," *Photodermatol. Photoimmunol. Photomed.* **24**(2), 76–82 (2008).
 17. N. Kollias, "The spectroscopy of human melanin pigmentation," in *Melanin: Its Role in Human Photoprotection*, L. Zeise, M. R. Chedekel, and T. B. Fitzpatrick, Eds., pp. 31–38, Valdenmar, Overland Park, KS (1995).
 18. E. Bendit and D. Ross, "A technique for obtaining the ultraviolet absorption spectrum of solid keratin," *Appl. Spectrosc.* **15**(4), 103–105 (1961).
 19. J. Riesz, J. Gilmore, and P. Meredith, "Quantitative scattering of melanin solutions," *Biophys. J.* **90**, 4137–4144 (2006).
 20. T. Tadokoro, Y. Yamaguchi, J. Batzer, S. G. Coelho, B. Z. Zmudzka, S. A. Miller, R. Wolber, J. Z. Beer, and V. J. Hearing, "Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation," *J. Invest. Dermatol.* **124**, 1326–1332 (2005).
 21. F. de Fine Olivarius, H. C. Wulf, P. Therkildsen, T. Poulsen, J. Crosby, and M. Norval, "Urocanic acid isomers: relation to body site, pigmentation, stratum corneum thickness and photosensitivity," *Arch. Dermatol. Res.* **289**, 501–505 (1997).