Attenuated total reflection Fourier transform infrared and polarization spectroscopy of *in vivo* human skin ablated, layer by layer, by erbium:YAG laser

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Abstract. The results of an experimental study of the possibilities of monitoring erbium yttrium aluminum garnet laser-mediated ablation of human epidermis with the use of Fourier transform infrared (FTIR) spectroscopy and spectral polarization techniques are presented. The attenuated total reflection (ATR) method was used for FTIR spectroscopic measurements. Spectral polarization monitoring of the ablation was carried out by analyzing the spectra of the degree of residual linear polarization of a probe light diffusely reflected from the laser-treated region of skin. It was found that the analysis of FTIR spectra allows monitoring of the water and protein contents in the subsurface layers of the treated skin, while the degree of residual polarization measured at the wavelengths of maximal absorption of hemoglobin is sensitive to changes in the epidermis thickness and the blood content in the dermal layer (the degree of erythema). © *2004 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.1755719]

1 Introduction

The optical properties of human skin are now studied[1–3] in relation to the development of novel techniques for monitoring normal and pathogenic tissue. In perticular, the problem of real-time functional diagnostics of human skin arises with the implementation of modern laser technologies (such as, skin resurfacing[4,5]) in clinical practice. In the case of laser ablation of skin, it is important to have precise control of the thickness of the epidermis and upper layer of derma removed. If the depth of laser treatment is too shallow, it will result in low efficiency of the laser resurfacing procedure. If the ablation is too deep, these may be undesirable collateral effects such as long-term erythema on skin reddening, scarring, and hypo- or hyperpigmentation. In order to determine the exposure necessary for skin resurfacing procedures. the physician usually uses his practical experience. That is why the development of quasi-real-time, noninvasive optical technologies for monitoring human skin will provide novel opportunities for further use of laser methods in dermatology and cosmetology.

Laser ablation of the epidermis and partial damage of the upper layers of the derma is accompanied by expansion of the microcapillaries and an increase in the blood perfusion level in the treatment zone. This is manifested as reddening and swelling of the skin and can be characterized by the degree of

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erythema[6] which depends strongly on the thickness of the removed layer. Typical changes in the coloration and fluorescence spectra of *in vivo* human skin were studied in Refs. 6 and 7 for layer-by-layer removal of the epidermis; using adhesive tape (the so-called skin-stripping technique).

The optical properties of human skin depend strongly on its melanin and hemoglobin content. The universally adopted methods for noninvasive determination of the values of the melanin and hemoglobin concentration in the skin are reflection or fluorescence spectroscopy.[7,8] Well-developed techniques and instrumentation allow estimates of the erythema and melanin pigmentation indices of the skin with high efficiency and accuracy. Typically, these estimates are based on measurements of the intensity of polychromatic light diffusely reflected by the probed tissue in the characteristic spectral regions.[9,10] Another approach to monitoring tissues using the absorption expressed in the visible range, which is induced by presence of the above-mentioned chromophores, can be based on the use of polarization and infrared (FTIR) spectroscopy techniques because of their high sensitivity to changes in the optical properties of the probed tissues.[11,12]

In particular, the possibility of using polarization analysis of multiply scattered light for noninvasive diagnostics of weakly ordered media, including biological tissues, was considered in Refs. 13 and 14. The specific case typical for biomedical diagnostic applications is the detection of radiation diffusely reflected from the probed tissue. For these scattering and detection conditions, the degree of residual polarization of the diffusely reflected light depends strongly on the average penetration depth of the probe light into the tissue and the relation between the reduced scattering coefficient and the absorption coefficient of the probed tissue. For human skin in the visible region (400 to 700 nm), the degree of residual polarization for diffusely reflected light with an initial linear polarization is controlled by the tissue absorption coefficient, i.e., it depends on the concentration of natural chromophores such as hemoglobin and melanin. Thus, we should expect noticeable changes in the degree of residual polarization of light diffusely reflected by human skin in the course of a laser ablation procedure. Therefore this parameter can be considered as the diagnostic parameter for evaluating the efficiency of the laser treatment. Note that the heat degradation of laser-treated tissue may affect the spectra and polarization degree of diffusely reflected light only from a very thin layer because of the almost complete removal of thermally damaged tissue in ablation by an erbium:yttrium aluminum garnet (Er:YAG) laser.[15] A residue of burnt tissue remains on the surface after laser ablation and may influence the measured spectra.

Infrared (IR) spectroscopic techniques have been shown to be an adequately effective tool for analyzing the structure and phase properties of lipids,[16] the structure of proteins in tissues,[17] the transformation of proteins under the effect of various physical and chemical factors,[18–20] and the concentration and spatial orientation of the polypeptide chains of biopolymers.[21] However, practically all these studies have so far been performed for *in vitro* isolated and dehydrated tissue samples. *In vivo* studies of Fourier transform infrared (FTIR) spectra of biological tissues have not been made until now because of the unsuitable design of the sample holder in a conventional attenuated total reflection (ATR) system.

The development of IR fiber-optic probes operating on the principle of the attenuated total internal reflection has provided the possibility of locally monitoring various components for *in vivo* tissues. This technique allows one to obtain an analysis of the changes in the IR transmission spectra in the skin's shallow layer with a high performance and high enough spatial resolution.[22] Another feature is the local character of such analysis provided by the small size of the fiber-optic probe.

We have studied the possibility of using both ATR FTIR spectroscopy and polarization spectroscopy to monitor human skin undergoing ablation of the epidermis and part of the derma with a pulsed Er:YAG laser.

2 Materials and Methods

We used a pulsed Er:YAG laser-based portable dermatological apparatus (''Dobry Svet'' type, Medical Laser Technologies, Ltd., Russia) for the skin treatment procedure. The wavelength of laser radiation is 2.94 μm, and the pulse duration is 300 μs. The treatment conditions were similar to those usually applied in laser cosmetology.[4,5] In our case, the energy of the laser pulse was equal to 5 J; the diameter of the light spot on the skin's surface was approximately 7 mm; and the pulse repetition rate was about 1 pulse per second.

The changes in optical properties of the treated skin were analyzed for a local area on the forearms of healthy volunteers (two men, aged 45 and 48, with normal skin types I and II after Fitzpatrick[5]). With the use of a specially prepared opaque screen, a skin area 12 mm in diameter was irradiated by laser light. About 5 min before laser treatment, the skin was sponged with a slightly heated 0.9% physiological solution to remove surface debris and some oil. We avoided the use of alcohol because of its drying effect. This was no sponging of the skin between laser scans. The treated region was scanned by the light spot with partial overlapping of the exposed zones corresponding to each laser pulse. The degree of overlap was about 10 to 20% of the total area of the light spot. Typically, four laser pulses are needed to completely cover the selected skin region in a single scan. After each single scan, the FTIR spectra and the degree of residual linear polarization of the diffusely reflected probe light were measured for the laser-treated skin region.

FTIR spectra were measured using the attenuated total reflection. A spectrophotometer (Vector 20 model, Bruker, Germany) was arranged with a couple-in-couple-out fiber-optic device (Infrared Fiber Sensors, Germany). The probe was designed as a piece of bared AgBr/AgI IR light guide 0.7 mm in diameter.[22] The probe sensing section was bent with a radius approximately equal to 3.5 mm. The scheme of measuring the ATR FTIR spectra and the image of the probe are shown in Fig. 1. When obtaining the spectral measurements, the sensor part of the probe was attached to the surface of the skin with a constant force of about 0.2 N. Such a probe allowed us to use an uncooled deuterated triglycine sulfate (DTGS) detector in the spectrophotometer without the noticeable deterioration of skin spectra that occurs with mercuric cadmium telluride (MCT)-cooled detectors.[22] For each spectral measurement the IR spectra obtained were averaged over 32 consecutive data scans. The spectral resolution was 4 cm^{-1}. Figure 2 presents a typical spectrum of human forearm skin with respect to the
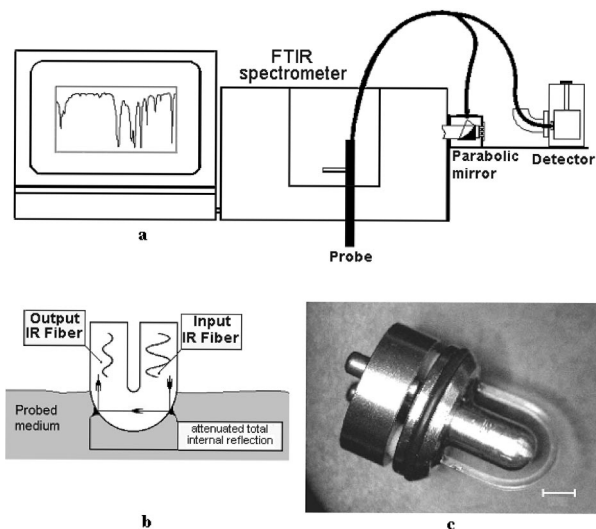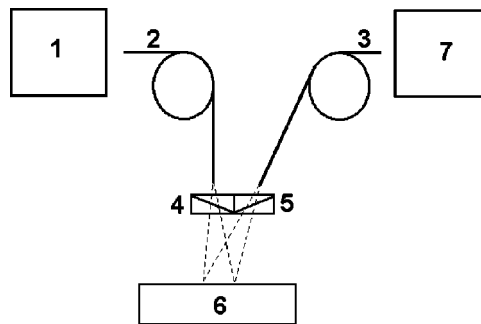
Fig. 1 (a) Schematic diagram of the experimental setup for ATR FTIR spectra measurements. (b) Attenuated total reflection scheme for fiber probe. (c) The probe. The white bar corresponds to 2 mm.

baseline of the probe in a position of no contact. The measured spectra were corrected with the baseline procedure that provides an ATR transmittance equal to unity in a wave number region of 1800 to 2500 cm^{-1}, where no absorption bands are expected.

The functional diagnostics of the laser-mediated skin in the course of layer-by-layer removal of the epidermis was carried out by measuring the degree of residual linear polarization of the diffusely reflected light with the setup shown schematically in Fig. 3. An illuminating unit with a halogen incandescent lamp was used as the source of the probe light. In the course of polarization diagnostics, the spectra of two linearly polarized components of diffusely reflected light were analyzed in the visible region. One of these components was the so-called co-polarized component with the same polarization azimuth as the incident light, and the other one was the cross-polarized component with an orthogonal direction of polariza-
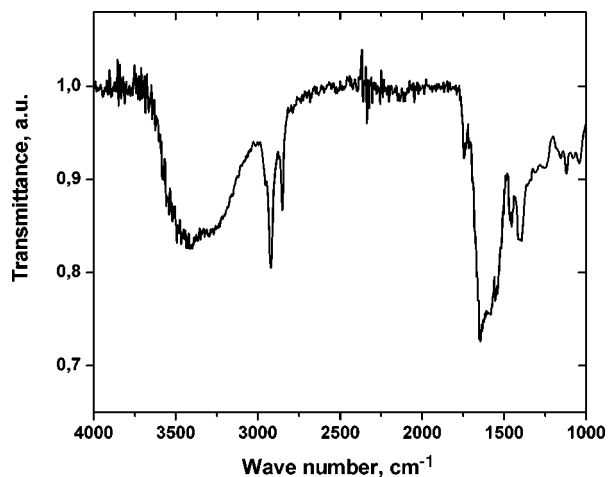
Fig. 2 The ATR FTIR spectrum of human forearm skin measured with an infrared fiber probe (baseline is the spectra of the probe with no skin touching).


Fig. 3 Schematic diagram of the experimental setup used for spectral analysis of the degree of residual polarization of diffusely reflected light. 1, nonmonochromatic light source (halogen lamp); 2, light-delivering fiber-optic bundle; 3, light-collecting fiber-optic bundle; 4 and 5, polarization filters; 6, object under study; 7, optical multichannel analyzer.

tion with respect to the incident light. The spectral measurements of the degree of residual polarization were carried out with a specially designed probe consisting of two fiber-optic light-delivering and light-collecting bundles 5 mm in diameter. The numerical aperture of both bundles was equal to 0.4. In order to illuminate the probed tissue with linearly polarized light and obtain polarization selection of diffusely reflected radiation, the polarization filters were placed immediately behind the output tip of the light-delivering bundle and in front of the input tip of the light-collecting bundle. The polarization filter placed on the input tip of the light-collecting bundle consisted of two pieces of polarization film with orthogonally directed optical axes. The polarization state of the detected light was switched by manually shifting the polarization filter in the transverse direction with respect to the light-collecting bundle; thus it allowed either the co- or cross-polarized component of diffusely reflected light to be detected.

The spectra of the co-polarized and cross-polarized components of light diffusely reflected from a probed tissue were analyzed in the wavelength range from 450 to 700 nm with the use of an optical multichannel analyzer (LESA-6m model, "Biospek," Russia). The measuring system was calibrated using model samples with well-established values of the degree of residual linear polarization measured at fixed wavelengths (the measurement procedure is described in Ref. 13). Based on the obtained spectra of the co-polarized and cross-polarized components [$R_{\Pi}(\lambda)$ and $R_{\perp}(\lambda)$, respectively], the spectra of the degree of residual polarization were calculated as

$$P(\lambda) = \frac{R(\lambda)_{\Pi} - R(\lambda)_{\perp}}{R(\lambda)_{\Pi} + R(\lambda)_{\perp}}. \tag{1}$$

The applicability of this technique for functional diagnostics of *in vivo* human skin was recently demonstrated by studying the polarized reflection spectra of skin with erythema,[23] which was artificially induced by using the skin-stripping technique. The relation between the increasing degree of residual linear polarization for wavelengths corresponding to hemoglobin absorption maxima and the increasing level of the capillary blood flow, which is manifested as an increasing degree of erythema, is obvious.
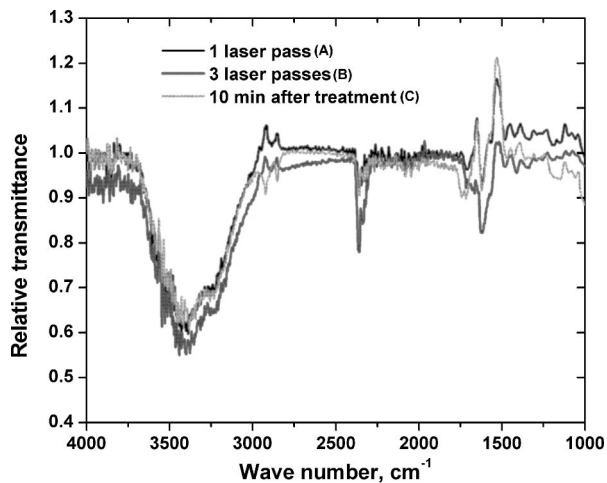
Fig. 4 The ATR FTIR spectra of the transmittance of human skin *in vivo* during gradual removal of epidermis by a pulsed Er:YAG laser with respect to the intact skin. Curves (A) and (B) refer to one and three laser scans, respectively; the curve (C) shows the same skin 10 min after treatment and sponging with a tampon wetted in 0.9% physiological solution.

Fig. 5 The curves (A) and (B) of Fig. 4 extended in the region of 1050 to 1750 cm^{-1} for a detailed view, presented as curves 1 and 2, respectively.

In our experiments the changes in blood perfusion level and, correspondingly, the degree of erythema were quantitatively characterized by the so-called "erythema index" E, which was evaluated with the use of the technique and instrumentation (erythema-melanin-meter, EMM-01 model) described in Refs. 9 and 10.

The most characteristic changes in the spectra $P(\lambda)$ are observed for the absorption bands of oxygenated hemoglobin (near 545 and 575 nm) with an increasing degree of erythema. Far from the absorption bands of human blood, the value of the degree of residual linear polarization $P(\lambda)$ diminishes with the increasing wavelength of the probe light. This behavior can be interpreted as demonstrating the spectral properties of melanin as one of the basic components of human skin.

3 Results and Discussion

3.1 FTIR Spectra Study

Figure 4 illustrates the specific changes in IR Fourier spectra of the relative transmittance appearing for an *in vivo* skin sample (type II) after one and three Er:YAG laser scans. These spectra were obtained as the relation of the FTIR transmittance spectra of the laser-treated skin to the FTIR transmittance spectra of the intact skin. Such a presentation of the spectra is suitable for comparing the spectra of the treated skin with that of the intact skin. The lower the value of the ordinate (y-axis) for a particular wave number, the less light transmission and, respectively, the more concentration of absorbing components in the treated skin. The specific changes in FTIR spectra induced by laser treatment are obvious. Note that the changes in FTIR spectra for treated skin of type I in general are similar to those for type II skin. The difference between two sets of FTIR spectra appears only in the relations between the heights of the main absorption peaks. In particular, one of the most indicative factors is the behavior of the absorption bands of water in the vicinity of 3450 and 1640 cm^{-1}. It can be presumably concluded from the
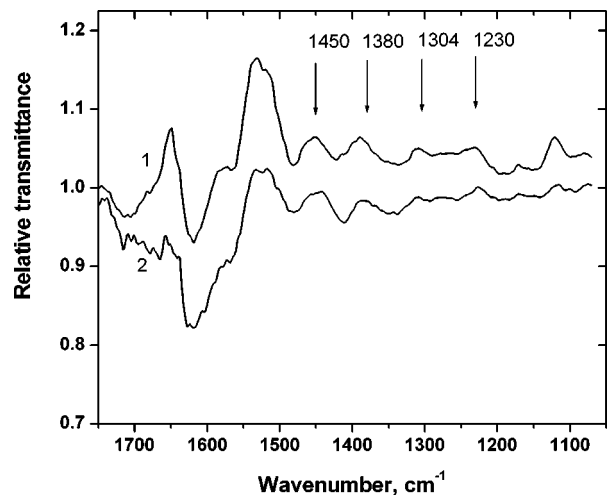
changes in absorption of the treated skin for these spectral regions that even after the first laser scan the water content in the subsurface skin layers increases substantially. This result is not obvious because the thermal action of the laser can cause a partial drying of skin within the treatment zone.

The absorption peak in the vicinity of 2360 cm^{-1} is attributed to carbon dioxide (CO_2). It may be connected with the CO_2 convection from the air in the spectrophotometer or with the carbonization of tissue in the superficial layer of the skin as a result of explosive laser burning. Usually this high-amplitude peak appeared just after laser treatment of the skin, indicating that it is associated with tissue carbonization. The proof of this statement may be the almost complete disappearance of the carbon dioxide peak after removal of the superficial layer of the skin by sponging it with 0.9% physiological solution (see the curve (C) in Fig. 4).

Another feature is the behavior of the absorption peaks near 1304, 1380, and 1450 nm (see Fig. 5). These absorption bands can be related to various types of vibrations of CH_2 and CH_3 groups. The intensities of these peaks change insignificantly and that presumably indicates that the content of the organic components in the superficial layers of the treated skin remains practically unchanged in the course of laser treatment.

The absorption peak between 1610 and 1690 cm^{-1}, which is typical for practically all types of biological tissues, is associated with the stretching vibrations of the C═O bond in the amide group of proteins (Amid I-type vibrations).[24] For the FTIR spectra presented in Fig. 5, this peak is substantially distorted because of the partial overlap with other absorption peaks localized in the vicinity of 1640 cm^{-1} and associated with one of the absorption bands of tissue water. Another feature consists of the Amid II-type polypeptide vibrations in the spectral region around 1550 cm^{-1}. These vibrations are the result of a combination of stretching vibrations of the C—N bond and deformation vibrations of the N—H bond. The peak caused by an Amid II-type is substantially separated from the above mentioned peak of water absorption, but it also appears distorted by the long-wavelength part of the wa-

Fig. 6 An image of human forearm skin (type II) after three passes with the beam of an Er:YAG laser. A slight erythema (reddening) and edema could be seen.

Fig. 7 Laser ablation-induced changes in spectra of the degree of residual linear polarization of a probe light diffusely reflected by the skin surface. 1, normal skin ($E=77$); 2, after a single laser scan ($E=99$); 3, after three laser scans ($E=112$).

ter absorption band. In our case, the peculiarity in behavior of this peak is seen when its increasing height is compared with the spectrum of the intact skin. This peculiarity occurs after the first laser scan, but after the subsequent scans its height decreases gradually. The peak of Amid I-type polypeptide vibrations exhibits similar behavior.

An absorption peak of small amplitude, which appears in the vicinity of $1230\,cm^{-1}$, can be associated with the Amid III-type polypeptide vibrations. The Amid III-type vibration is a combination of the stretching vibrations of the C—N bond, deformation vibrations of the N—H bond, and stretching vibrations of the C—C bond. Consequently, this peak is very sensitive to the conformational changes in protein structures, especially in the case of their denaturation.[19] In our case, the change in the shape of the Amid III-type peak is observed with an increasing exposure dose and presumably indicates a partial denaturation of proteins in the near-surface zone of the skin, but owing to the low intensity of this peak, these changes cannot be reliably identified.

Thus, IR Fourier spectroscopy can be used to monitor changes in the water and protein content in the shallow layers of skin in the course of a laser-mediated ablation procedure. The spectra obtained indicate that the concentration of tissue water in the treatment zone increases rapidly and approaches to a stable level with increasing number of laser scans. In contrast, the contrary, the protein content decreases relative to that of the intact skin after the first scan, but the subsequent scans cause an increase in the protein content of the treated skin.

3.2 Analysis of Spectra of the Residual Polarization Degree

Figure 6 shows a visual image of the Er:YAG laser-treated zone of the skin. Only slight erythema and edema could be seen. Ordinary physicians estimate the degree of ablation using a similar visual view and their own experience. Such estimation is very subjective and may produce an error. For objective qualitative estimation of the degree of skin ablation, the method using polarization spectroscopy may be useful.
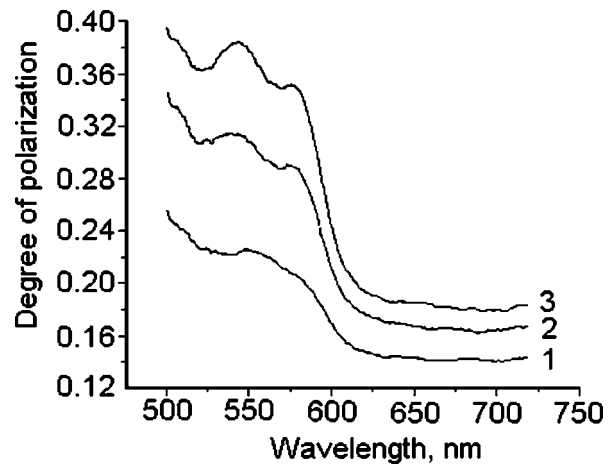
Figure 7 shows the spectral dependencies of the degree of residual polarization, $P(\lambda)$, of the diffusely reflected light for intact skin (1) and laser-ablated skin (2 and 3). Laser treatment increases the erythema index from 77 units (intact skin) to 112 units (skin after three consequent laser scans). The increase in the erythema index reflects an increase in the blood content of the papillary layer of the derma, which is induced by the laser procedure. A similar effect was observed in removal of the epidermis with the skin-stripping technique.[23] An erythema of a considerable degree appeared after the removal of an epidermal layer of 30 to 40-μm thickness.

The increase in the blood concentration in the papillary dermal layer reduces the penetration depth of the probe light, with the wavelengths corresponding to the hemoglobin absorption regions, so the fraction of the co-polarized component in the detected diffusely reflected light becomes more significant than if there is no selective absorption by hemoglobin. In turn, this increases the degree of residual polarization $P(\lambda)$ in the regions of selective absorption.[25]

The depth of the epidermal layer removed in the course of laser ablation can be estimated by comparing the values of the degree of residual polarization and the coefficient of the diffuse reflection for the laser-treated zone with those for intact skin at the wavelengths of the probe light that correspond to maximal selective absorption and are far from the selective absorption bands. This approach requires a preliminary calibration with the use of a two-layered phantom scattering media with different values for the thickness of the upper nonabsorbing layer and controllable absorption of the second underlying layer.

A theoretical analysis of the degree of residual linear polarization influenced by the content of the selective absorber can be carried out in terms of the phenomenological model, that describes the decay of polarization of the probe light in multiple-scattering media.[12] With this approach, the degree of residual polarization in the case of noticeable absorption of the probe light is proportional to

$$\int_0^\infty \exp(-\mu_a s - s/\xi)\rho(s)\,ds \Big/ \int_0^\infty \exp(-\mu_a s)\rho(s)\,ds,$$

and the diffuse reflection coefficient is proportional to

$$\int_0^\infty \exp(-\mu_a s)\rho(s)\,ds \Big/ \int_0^\infty \rho(s)\,ds.$$

In these expressions $\rho(s)$ is the probability density function of the Feynman paths that correspond to partial waves propagating in the scattering medium and forming the detected optical signal; μ_a is the absorption coefficient of the scattering medium, and ξ is the depolarization length, which characterizes the degradation rate of the initial polarization state of the probe light.

In the case of negligibly small absorption, the degree of residual polarization is approximately proportional to

$$\int_0^\infty \exp(-s/\xi)\rho(s)\,ds \Big/ \int_0^\infty \rho(s)\,ds,$$

and the diffuse reflection coefficient is proportional to

$$\int_0^\infty \rho(s)\,ds.$$

Monte Carlo (MC) simulation can be used to evaluate the path-length distribution $\rho(s)$, which characterizes the propagation of the probe light in a layered scattering media with variable thickness of the upper nonabsorbing layer, variable absorption of the underlying layers, and optical parameters corresponding to human skin. Then the theoretical predictions for $P(\lambda)$ and the diffuse reflection coefficient obtained with the use of the above-presented expressions can be applied to fit the experimentally obtained dependencies of corresponding values on the wavelength of the probe light.

The effects of the thickness of the epidermal layer and increasing blood content in the dermal layer on the degree of residual polarization of the detected probe light were theoretically analyzed using the above-described approach. A two-layer model of human skin was used for Monte Carlo simulation of the path-length distributions $\rho(s)$ of diffusely reflected radiation. The optical parameters of the model, such as the absorption coefficient μ_a, the scattering coefficient μ_s, and the anisotropy parameter g for both layers were taken from Ref. 26 for a wavelength of 575 nm (in the vicinity of the hemoglobin absorption peak). The upper layer corresponding to an epidermis with zero blood content was characterized by the values of $\mu_a^1 = 1.0$ mm^{-1}, $\mu_s^1 = 20.0$ mm^{-1}, and $g^1 = 0.79$. The thickness of this layer L_1 was varied from 100 to 20 μm in the course of the simulation procedure.

The optical parameters of the second layer (''derma'') were considered to be dependent on the blood volume content f in the dermal layer in accordance with the following relations:

$$\mu_a^2 = \mu_a^d(1-f) + \mu_a^b f, \quad \mu_s^2 = \mu_s^d(1-f) + \mu_s^b f,$$
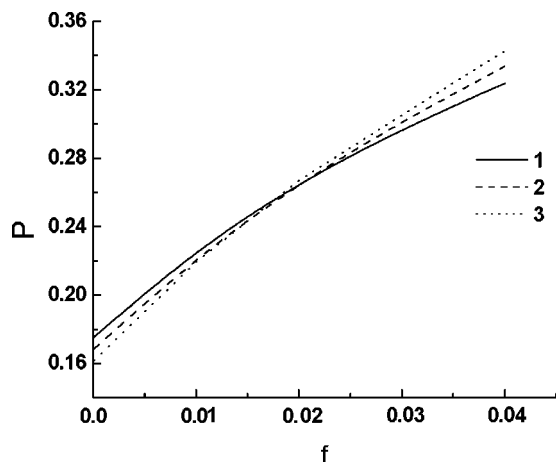
where μ_a^d, μ_s^d are the optical parameters of the bloodless derma ($\mu_a^d = 0.28$ mm^{-1} and $\mu_s^d = 21.5$ mm^{-1} at 575 nm),[24] and μ_a^b, μ_s^b are similar parameters for blood (μ_a^b

$=0.28$ mm^{-1} and $\mu_s^b = 21.5$ mm^{-1} at 575 nm).[26] The anisotropy parameter g^2 for the second layer was taken equal to g^1 and independent of the blood content f, which was varied from 0 to 0.04 in our simulation procedure. The second layer was considered a semi-infinite random medium. Despite the obvious simplicity of this tissue model, which does not take into account the inhomogeneous (stratified) structure and finite thickness of real dermal tissue, it has allowed us to analyze the effects of epidermis thickness and blood content in skin on the degree of residual polarization of diffusely reflected light and to obtain a satisfactory agreement between the simulation results and experimental data for reasonable values of the model's parameters.

Our MC algorithm for simulating the photon random walk in the layered scattering medium is basically similar to that of Boas and Yodh.[27,28] The photon propagates from one interaction event to another, and a scattering length and an absorption length are calculated for each step from the values of μ_s and μ_a chosen according to the photon position inside the medium. Calculation of the absorption length and the scattering length is based on the exponential distributions derived from the scattering and the absorption coefficients, respectively. If the absorption length l_a is shorter than the scattering length l_s, then the photon is considered as absorbed at the current step and is terminated. A new photon is launched from the source position. In the opposite case of $l_s < l_a$, the photon propagates the scattering length, and then the scattering angle, as well as the azimuth angle, are calculated. The former is obtained with the help of the commonly used Henyey-Greenstein phase function,[29] and the second angle is simulated as a random value uniformly distributed in the range from 0 to 2π. In addition, a new scattering length and an absorption length are calculated, and the current position of the photon within the scattering slab is derived. This procedure continues until the photon is absorbed or leaves the medium, or until the number of interaction events exceeds a maximal value, set in our case by 10^8.

For each propagating photon, the propagation path is accumulated by summation of the values of l_s calculated for each step. Interaction of the photon with the media boundary is characterized by the probability P_C for the photon to cross the boundary and thus to escape. The value of P_C is determined by the boundary transmittance for nonpolarized light. The transmission coefficient is calculated with the Fresnel formula[30] for the given angle of incidence of the photon onto the boundary. The simulation of the photon random walk continues in case of reflection of the photon from the boundary with a probability of $1 - P_C$. The path-length histograms are obtained for the photons leaving the slab and scored by a circular detector positioned on the media boundary. The aperture angle of the detector and its area are chosen according to the light-collection conditions used in our polarization experiment. The photon launching conditions on the boundary correspond to the irradiation conditions (i.e., the diameter of the light spot and the angle of incidence of a probe beam onto the surface of the skin). We used at least 10^5 scored photons to obtain the path-length histograms. The simulation procedure takes ~ 20 min (run on a 466-MHz Pentium-II).

The values of the degree of residual linear polarization are

Fig. 8 Theoretical dependencies of the degree of residual polarization on the blood volume content in the "derma" layer for a two-layer skin model. The optical parameters of both layers correspond to the mean values of those for human skin at 575 nm. 1, $L_1 = 100$ μm; 2, $L_1 = 60$ μm; 3, $L_1 = 20$ μm.

calculated from discrete sets of normalized path-length density data $\tilde{\rho}(s_i)$ using the following relation:

$$P \approx \sum_{i=1}^{N} \exp\left(-\frac{s_i}{\xi}\right) \tilde{\rho}(s_i),\qquad(2)$$

where $\tilde{\rho}(s_i)$ was obtained as $N_i / \Sigma_{i=0}^{N} N_i$ (N_i is the number of scored photons for the ith bin of the path-length histogram and N is the total number of bins). The depolarization length ξ was set equal to $\approx 1.5 l^* \approx 380$ μm (l^* is the transport mean-free path) in accordance with the results of an analysis of polarization decay in random media characterized by the scattering anisotropy.[12,13] Note that both layers of the model are characterized by close values of l^*.

Figure 8 shows the theoretical dependencies of the degree of residual polarization P on the blood volume content f in the "derma" layer for different values of L_1. The satisfactory agreement between theoretical and experimental values of P at 575 nm (see Fig. 7) is obvious; this diagnostic parameter appears much more sensitive to changes in the blood volume content f in the "derma" layer than to changes in thickness of the epidermal layer. The opposite tendencies in the behavior of theoretical values of P in the dependence on L_1 for small and large values of f should be mentioned. The degree of residual polarization decreases slightly with decreasing "epidermis" thickness for $f=0$ ("bloodless derma" with relatively small absorption at 575 nm) and increases for large values of f ("derma with high perfusion level" and, correspondingly, with the expressed absorption).

The results obtained allow us to suggest that the increasing degree of residual polarization of diffusely reflected light as the result of laser-mediated skin resurfacing of skin is mainly caused by the increasing blood content in the dermal layer.

4 Conclusions

Infrared Fourier spectroscopy of the attenuated total reflection can be used for *in vivo* monitoring of the water and protein content in subsurface layers of human skin during a laser-mediated skin resurfacing procedure. The concentration of tissue water in the treatment zone increases rapidly and approaches a stable level with an increasing number of laser scans. The protein content decreases relative to that of the intact skin after the first scan, but the subsequent scans cause an increase in the protein content of the treated skin.

The spectra of the degree of residual linear polarization of a probe light diffusely reflected by laser-treated *in vivo* human skin show substantial changes in the visible range in the course of epidermal ablation by pulsed laser radiation. The increase in the degree of residual linear polarization of the diffusely reflected probe light measured at the wavelength of maximal absorption of hemoglobin correlates with an increase in the erythema index for the treated skin region.

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