Physiological and structural characterization of human skin in vivo using combined photothermal radiometry and diffuse reflectance spectroscopy

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Abstract: In this proof-of-concept study we combine two optical techniques to enable assessment of structure and composition of human skin in vivo: Pulsed photothermal radiometry (PPTR), which involves measurements of transient dynamics in mid-infrared emission from sample surface after exposure to a light pulse, and diffuse reflectance spectroscopy (DRS) in visible part of the spectrum. The analysis involves simultaneous fitting of measured PPTR signals and DRS with corresponding predictions of a Monte Carlo model of light-tissue interaction. By using a four-layer optical model of skin we obtain a good match between the experimental and model data when scattering properties of the epidermis and dermis are also optimized on an individual basis. The assessed parameter values correlate well with literature data and demonstrate the expected trends in controlled tests involving temporary obstruction of peripheral blood circulation using a pressure cuff, and acute as well as seasonal sun tanning.

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1. Introduction

Pulsed photothermal radiometry (PPTR) involves measurement of transient dynamics in mid-infrared (IR) emission from sample surface after exposure to a short light pulse. In addition to assessment of optical and thermal properties of homogenous samples, light-induced temperature depth profiles in layered structures can also be determined from such radiometric signals [1–4]. However, this technique doesn't allow for unique extraction of physiological characteristics of human skin, which contains multiple absorbing substances (e.g., melanin, oxy- and deoxyhemoglobin) [5].

Diffuse reflectance spectroscopy (DRS) in visible and near-infrared part of the spectrum is a popular experimental technique, which enables differentiation between different chromophores. Thus, comparisons of measured DRS with model-based predictions are often applied for non-invasive assessment of composition in various tissues and organs [6]. Nevertheless, quantitative assessment of the chromophore contents requires knowledge about their depth distribution, i.e., determination of the thicknesses of the characteristic skin layers at the investigated site using a separate experimental approach [7,8].

In the following, we introduce and test an innovative methodology for noninvasive assessment of physiological characteristics of human skin in vivo, which combines PPTR and DRS in order to overcome their individual weaknesses by exploiting the respective strengths [7]. More specifically, the stated goal is achieved by matching measured PPTR signals and DRS spectra with corresponding predictions of a numerical model of light-tissue interaction in an iterative optimization process, (a.k.a. inverse Monte Carlo, IMC). Based on our preliminary analyses [9] we apply here a four-layer optical model of skin, representing the
epidermis, papillary and reticular dermis, and subcutis. This provides a sufficiently detailed description of the light interaction with human skin to enable a good match between the model predictions and experimental data, while maintaining the number of free parameters in the inverse analysis at a manageable level.

The described approach allows simultaneous assessment of several physiologically relevant parameters (e.g., the contents and oxygen saturation levels of dermal blood in the papillary and reticular compartments), as well as structural properties of the selected test site (i.e., the epidermal and dermal thickness). The match between the experimental and best-fitting model data is significantly improved upon allowing for individual adjustment of the scattering properties of both epidermis and dermis, which indicates that the presented technique is also sensitive to their inter- and intra-personal variations. Using such an approach, the assessed skin properties conform very well with literature data and exhibit the anticipated trends in controlled tests involving temporary obstruction of peripheral blood circulation using a pressurized arm cuff, acute sun tanning, and monitoring of seasonal changes in human volunteers.

2. Methodology

2.1 Experimental protocol

The presented examples involve three healthy volunteers with fair skin (Fitzpatrick type II, age 27–28), who have provided an informed consent. The entire protocol conforms to the principles expressed in the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Republic of Slovenia.

Selected test sites on volunteers’ arms were shaved as necessary and the dehydrated superficial layer (stratum corneum) was removed by tape stripping. This should ensure unimpaired heat diffusion all the way to the skin surface, as assumed in our mathematical model of the PPTR procedure (see Sect. 2.3). The site was then cleaned with medical-grade ethanol, rehydrated using physiological solution, and left to dry.

The test sites were irradiated with individual 1 ms pulses at \( \lambda = 532 \text{ nm} \) emitted from a medical laser (DualisVP by Fotona, Ljubljana, Slovenia). The applied light is absorbed primarily by epidermal melanin and hemoglobin in dermal vasculature. At the effective spot size of ~5 mm, the radiant exposure was around 0.30 J/cm² [5].

Mid-IR emission from the tissue surface was recorded by a fast IR camera (FLIR Systems, model SC7500; \( \lambda = 3.5–5.1 \mu \text{m} \)) with a 50 mm objective, at a rate of 1000 frames per second. PPTR signals were obtained by lateral averaging of the radiometric data over a suitable area of interest (1–3 mm², with no visible veins) and subtracting the pre-pulse baseline value [5,10]. The manufacturer-provided calibration system (Hypercal™) was used for nonlinear conversion of the signal amplitudes to radiometric temperature values.

Diffuse reflectance spectra in visible spectral range were measured using an integrating sphere (IS) with an internal light source and sample opening diameter of 10.3 mm (ISP-REF by Ocean Optics, Dunedin, FL). Spectral response of the spectrometer (USB4000, Ocean Optics) was calibrated using a white standard (Spectralon© by Labsphere, North Sutton, NH). The signal-to-noise ratio was improved by averaging 100 subsequently acquired spectra (at integration time of 15 ms).

The so-called single-beam substitution error, which can be significant when using such small IS, was removed by numerical pre-processing based on prior analysis of the system performance [11], thus yielding artifact-free DRS values.

2.2. Optical model of human skin

Based on our previous experience [7,9,12], we apply a model of human skin with four optically homogenous layers, representing the epidermis, papillary and reticular dermis, and subcutis (Table 1).
Absorption coefficient of the epidermis, $\mu_{\text{a,_epi}}$, is calculated using the customary relations [13]:

$$\mu_{\text{a,_epi}} = m \mu_{\text{a, mel}} + b_{\text{epi}} \mu_{\text{a, bl}} + (1-m-b_{\text{epi}}) \mu_{\text{a, base}}$$

(1)

$$\mu_{\text{a, mel}}(\lambda) = 6.6 \times 10^{10} \text{mm}^{-1} \left(\frac{\lambda}{\text{nm}}\right)^{-3.33}$$

(2)

$$\mu_{\text{a, base}}(\lambda) = 0.0244 \text{mm}^{-1} + 8.53 \text{mm}^{-1} \exp \left(-\frac{\lambda-154 \text{nm}}{66.2 \text{nm}}\right)$$

(3)

where $\mu_{\text{a, mel}}$ represents the melanin absorption coefficient and $\mu_{\text{a, base}}$ is baseline absorption of bloodless skin. The melanin volume fraction ($m$) is constant throughout the epidermis. In order to account for pronounced undulation of the actual epidermal-dermal boundary (i.e., dermal papillae), we allow also for a small amount of blood within the epidermal layer ($b_{\text{epi}}$).

The absorption coefficient of blood ($\mu_{\text{a, bl}}$) is calculated as a linear combination of the values for oxygenated and deoxygenated whole blood [14], according to the respective oxygen saturation level in each layer. Papillary and reticular dermis have independent blood contents and oxygen saturation levels ($b_{\text{pap}}$ and $S_{\text{pap}}$ vs. $b_{\text{ret}}$ and $S_{\text{ret}}$). We also account for optical screening in larger blood vessels by using the customary correction factor and typical vessel size distribution [2,15]. The absorption coefficient for dermis is then obtained by combining the obtained effective blood absorption coefficient with the baseline absorption spectrum (Eq. (3)), in a manner analogous to Eq. (1).

The absorption spectrum for subcutaneous adipose layer is taken from Simpson et al. [16].

| Table 1. Overview of our four-layer optical model and its parameters |
|---|---|---|---|
| thickness | absorption | scattering |
| approach | approach | approach |
| param. | param. | param. |
| epidermis | fitted | $d_{\text{epi}}$ | Jacques [13] | m | fitted | $a_{\text{epi}}$, $p_{\text{epi}}$ |
| papillary dermis | 0.10 mm | fitted | Jacques [13], $b_{\text{pap}}$, $S_{\text{pap}}$ | Jacques [13], $b_{\text{ret}}$, $S_{\text{ret}}$ | fitted | $a_{\text{der}}$, $p_{\text{der}}$ |
| reticular dermis | fitted | $d_{\text{der}}$ | Meinke [17] | | | |
| subcutis | infinite | – | Simpson [16] | – | Salomatina [18], Naglič [8] | A |

In the first version of our optical model, the reduced scattering coefficient of the epidermis and dermis is described as a combination of Rayleigh and Mie contributions [18]:

$$\mu'_{\text{e}}(\lambda) = a \left[f_{\text{Ray}} \left(\frac{\lambda}{500 \text{nm}}\right)^{-4} + (1-f_{\text{Ray}}) \left(\frac{\lambda}{500 \text{nm}}\right)^{-p_{\text{Mie}}}\right].$$

(4)

Here, the amplitude $a$ represents the value of $\mu'_{\text{e}}$ at wavelength $\lambda = 500 \text{nm}$, $f_{\text{Ray}}$ indicates the fraction of Rayleigh scattering relative to Mie contribution, and $p_{\text{Mie}}$ is the characteristic power of the latter. The parameter values, representing average properties of human skin, are taken from Jacques [13]: $a = 4.80 \text{mm}^{-1}$, $f_{\text{Ray}} = 0.409$, and $p_{\text{Mie}} = 0.702$.

In the subsequent step, we will augment the analysis by allowing individual optimization of $\mu'_{\text{e}}(\lambda)$ according to the customary ansatz, suitable for the relatively narrow spectral range used in this study:

$$\mu'_{\text{e}}(\lambda) = a \left(\frac{\lambda}{500 \text{nm}}\right)^{-p}. $$

(5)

This step is justified because scattering properties of skin can vary between different subjects and different anatomical sites within the same subject [13]. We will consider the approach...
where the same parameter values \((a \text{ and } p)\) are applied to both epidermis and dermis, as well as the case where they are adjusted independently for each layer.

The scattering properties of the subcutaneous adipose tissue are also adjusted individually, according to

\[
\mu'_{sub}(\lambda) = A \left[ 16.43 \text{ cm}^{-1} + 303.8 \text{ cm}^{-1} \exp \left( -\frac{\lambda}{180.3 \text{ nm}} \right) \right],
\]

where inter- and intra-personal variations are accounted for by the varying amplitude \(A\). This function matches well the scattering spectra reported by Salomatina \textit{et al.} [19] at values of \(A = 0.64\) and \(1.5\), respectively.

The refractive index is set to 1.45 for the epidermis, 1.37 for dermis, and 1.34 for subcutis [20].

2.3. Numerical modeling of light-tissue interaction

Light transport and energy deposition in skin during our measurements are simulated using the weighted-photon multilayer Monte Carlo technique (MCML) [21]. Each simulation run involves launching of \(10^7\) energy packets (“photons”).

By considering also thermal properties of the involved tissues, the energy deposition profiles obtained for irradiation at 532 nm are then converted into predictions of the temperature depth profiles induced by our pulsed laser, \(\Delta T(z, t = 0)\). The corresponding radiometric transient \(\Delta S(t)\) is computed from the latter as [1,3]

\[
\Delta S(t) = \int_{z=0}^{\infty} K(z, t) \Delta T(z, t=0) d\lambda
\]

where the kernel function \(K(z, t)\) accounts for thermal properties of the skin (contained in the Green's function, \(G\)), absorption coefficient within the mid-IR acquisition band \((\lambda_1 \text{ to } \lambda_2)\), spectral sensitivity of the IR camera, \(R(\lambda)\), and temperature derivative of the Planck's law of radiation, \(B(\lambda)\) [3,5]:

\[
K(z, t) = C' \int_{\lambda_1}^{\lambda_2} R(\lambda) B_0'(T_b) \mu_t(\lambda) \int_{z=0}^{\infty} \frac{G(z', z, t)}{G(z, z, t)} e^{-\frac{\mu_t(\lambda) z'}{\mu_t(\lambda) z'}} dz' d\lambda
\]

Upon representing the PPTR signal and laser-induced temperature profile as vectors (\(S\) and \(T\), respectively), Eq. (8) becomes a matrix multiplication:

\[
S = KT; \quad K_{ij} = K(z_j, t) \Delta z.
\]

We use the same MCML model also to derive the DRS spectra for the same skin structure and composition. In doing so, we take into account the finite sample opening of our IS (see Sect. 2.1), which reduces the perceived sample reflectance at longer wavelengths [8].

2.4. Assessment of skin properties

The structure and composition of selected test sites are assessed by matching the acquired PPTR signals and DRS spectra with the respective predictions of our numerical model of light-tissue interaction (see Fig. 1). Specifically, this is attempted by varying the following skin model parameters: epidermal and dermal thickness \((d_{epi} \text{ and } d_{der})\), epidermal melanin content \((m)\), epidermal blood content \((b_{epi})\), papillary and reticular dermal blood content \((b_{pap} \text{ and } b_{der})\), papillary and reticular blood oxygenation level \((S_{pap} \text{ and } S_{der})\), subcutis scattering amplitude \((A; \text{ Eq. (6)})\), and ultimately the scattering amplitude and power \((a \text{ and } p; \text{ Eq. (5)})\), which may or may not differ between the epidermis and dermis.
The described task is performed by objective multi-dimensional minimization of the residual norm, using the nonlinear least-squares algorithm, implemented as function *lsqnonlin* in Matlab Optimization Toolbox (Mathworks Inc., Natick, MA, USA).

When performing simultaneous fitting of PPTR and DRS data, we anticipate that their respective contributions to the common residual norm may not be comparable due to different numbers of data points, selected units, etc. Specifically, the initial 1.5 seconds of the PPTR signals are used in our analyses, amounting to 1500 data points. Meanwhile, the DRS values are compared at only 14 wavelengths between 410 and 620 nm (see Fig. 2(b)), selected by considering the absorption characteristics of melanin, oxy- and deoxy-hemoglobin. This was dictated by the considerable computational load of the described IMC procedure.

Consequently, sensitivity of our inverse analysis to information provided by both techniques is ensured by introducing a merging factor $M$ in the combined residual norm ($E$):

$$E = E_{DRS} + ME_{PPTR}.$$  

In all presented examples, we use the value of $M = 10$, which was found earlier to be near-optimal for the problem at hand [7,12].

The optimization problem is typically solved in 20–30 iteration steps, which takes around 1 hour on a personal computer (Intel i7 processor, 16 GB RAM) equipped with a high-performance graphics card (Nvidia GTX 1080, 2560 cores). In order to control the influence of numerical noise inherent to the MC technique and increase the chance of finding the global minimum, each analysis is repeated 5 times with randomized initial parameter values.

3. Results

3.1. Skin scattering properties adopted from literature

Figure 2 presents the PPTR signal and DRS spectrum acquired from the inner forearm in a 28 years old woman (orange solid lines). The best-fitting predictions of our four-layer skin model with scattering properties adopted from literature (Eq. (4)), resulting from iterative optimization of its eight free parameters, are presented by dashed lines. For both applied techniques, the match between the former and the latter is reasonably good.
Fig. 2. PPTR signal (a) and DRS spectrum (b) as measured in a healthy volunteer (solid orange lines), compared with the best-fitting model predictions when using skin scattering properties from literature [18] (dashed). The arrows indicate the wavelengths considered in the optimization process.

Table 2. The permitted ranges for fitted parameters of our four-layer model of skin with scattering properties adopted from literature, and the results from analysis of a healthy skin site in vivo (see Fig. 2). The results are presented as average values from five independent IMC runs and the respective standard deviations. The last line shows the achieved value of the residual norm ($\varepsilon$).

| Parameter | Permitted range | IMC result (Fig. 3) |
|-----------|----------------|---------------------|
| $d_{epi}$ [mm] | 0.05–0.20 | 0.085 ± 0.005 |
| $d_{der}$ [mm] | 0.70–4.00 | 0.79 ± 0.24 |
| $m$ [%] | 0.1–15 | 1.4 ± 0.1 |
| $b_{epi}$ [%] | 0–5.0 | 0.08 ± 0.04 |
| $b_{pap}$ [%] | 0.1–20 | 3.3 ± 0.1 |
| $b_{ret}$ [%] | 0.1–20 | 0.5 ± 0.1 |
| $S_{pap}$ [%] | 10–100 | 47 ± 34 |
| $S_{ret}$ [%] | 10–100 | 30 ± 8 |
| $A$ | 0.1–2.0 | 0.9 ± 0.1 |
| $\varepsilon$ | | 46.0 ± 1.5 |

The corresponding parameter values are listed in Table 2 (right column). Most of them lie within anatomically and physiologically plausible ranges for human skin. E.g., the assessed epidermal thickness matches rather well the spread of values reported for inner forearm in women ($d_{epi} = 0.074 ± 0.009$ mm) [22]. In addition, the melanin volume fraction is consistent with earlier reported values for fair Caucasian skin ($m = 1.3–1.9\%$) [23]. The indicated dermal blood contents ($b_{pap}$ and $b_{ret}$) also fall within the range of values reported for healthy adults (0.2–7\%) [24–26], while the epidermal blood content is appropriately small ($b_{epi} = 0.1\%$). Finally, the adipose scattering amplitude ($A = 0.9$) corresponds to a value of $\mu_s^\prime_{sub}(500 nm) = 2.9$ mm$^{-1}$ (see Eq. (6)), which falls between the values obtained by Salomatina et al. for two different samples of adipose [8,19].

However, the oxygen saturation levels indicated for the dermal layers (especially $S_{ret} = 30 ± 8\%$) are suspiciously low. This deficiency, combined with the unsatisfying match between the experimental data and model predictions, calls for improvement of our model-based analysis.

3.2. Individually optimized scattering properties of skin

In the following, we analyze the same experimental data as above while allowing also individual optimization of the scattering properties of skin according to Eq. (5). We consider first the approach where the same parameter values ($a$ and $p$) are used for epidermis and dermis.
As is evident from Fig. 3, such augmentation leads to remarkable improvement of the match between the experimental data and best-fitting model predictions in comparison with the previous result (see Fig. 2). This is reflected also in the substantial reduction of the achieved residual norm, from $\varepsilon \approx 46$ to 2.0 (Table 3, left column). The only remaining mismatch, i.e., the overshoot of model-predicted diffuse reflectance values at $\lambda = 475–520$ nm in Fig. 3(b), can be attributed to presence of skin chromophores such as beta-carotene, lycopene, and/or bilirubin [27], which are not included in the discussed optical model.

| Table 3. The assessed model parameter values when assuming the same scattering properties for epidermis and dermis (left column), and optimized separately for epidermis and dermis (right column). |
|-----------------|-----------------|-----------------|-----------------|
|                | Scattering properties assessed for | epidermis, dermis | epidermis, dermis |
| $d_{ep}$ [mm]  | 0.093 ± 0.001    | 0.085 ± 0.001    |
| $d_{der}$ [mm] | 0.70 ± 0.01      | 0.72 ± 0.01      |
| $m$ [%]        | 1.4 ± 0.1        | 1.4 ± 0.1        |
| $b_{ep}$ [%]   | 0.04 ± 0.04      | 0.07 ± 0.04      |
| $b_{pap}$ [%]  | 1.9 ± 0.1        | 2.5 ± 0.1        |
| $b_{ret}$ [%]  | 1.3 ± 0.1        | 1.4 ± 0.1        |
| $S_{pap}$ [%]  | 60 ± 1           | 58 ± 3           |
| $S_{ret}$ [%]  | 75 ± 3           | 75 ± 4           |
| $a_{ep}$ [mm$^{-1}$] | 8.7 ± 0.2       | 11.7 ± 0.3       |
| $a_{der}$ [mm$^{-1}$] |              | 6.6 ± 0.2        |
| $p_{ep}$       | 2.2 ± 0.1        | 1.6 ± 0.1        |
| $p_{der}$      | 1.1 ± 0.1        | 0.7 ± 0.2        |
| $A$            | 2.0 ± 0.1        | 1.7 ± 0.1        |

Moreover, the values of $S_{pap}$ and $S_{ret}$ (60% and 75%, respectively) now fall within the earlier reported ranges of 65% ± 12% [28] and 73% ± 12% [29].

However, the assessed skin scattering amplitude ($a = 8.7$ mm$^{-1}$) is rather high when compared to the range of earlier assessed values for human dermis (3.0–6.9 mm$^{-1}$) [13]. This can be attributed to the fact that the $\mu_s'$ in the visible spectral range was often found to be higher in epidermis than in dermis (typically by a factor of ~2) [19,30–32], although the values for the former remain sparse and inconsistent [33]. We thus interpret this result as a weighted average of the values for epidermis and dermis, which are larger and lower, respectively.
3.3. Scattering properties optimized independently for epidermis and dermis

Based on the rationale presented just above, we further augment our approach by allowing independent optimization of the scattering parameters ($a$ and $p$) for epidermis and dermis.

As can be seen in Table 3 (right column), the resulting values of most model parameters change only marginally in comparison with the previous approach (left column), and remain within the anatomically and physiologically plausible ranges. The match between the corresponding model predictions and experimental data has also improved, as evidenced by the further drop of the residual norm to $\varepsilon = 1.7$.

The obtained blood content in papillary dermis ($b_{pap} = 2.5\%$) is now significantly higher than in reticular dermis ($b_{ret} = 1.4\%$). This is consistent with the well-known anatomical feature of the papillary dermis, the superficial vascular network (plexus) and capillary mesh responsible for delivering oxygen and nutrients to the epidermal cells.

Most notably, thus assessed scattering amplitude for the epidermis ($a_{epi} = 11.7\;\text{mm}^{-1}$) is indeed considerably larger than that for dermis ($6.6\;\text{mm}^{-1}$), while the effective value obtained using the previous approach lies appropriately between them. This is illustrated in more detail in Fig. 4(a), where we compare the obtained spectral dependencies $\mu_s'(\lambda)$ for epidermis and dermis (blue solid lines) with the result corresponding to the effective (lumped) description of skin scattering (Sect. 3.1; dashed line), and our initial assumption (Eq. (4); dotted).

The assessed result for dermis is also in good agreement with data from literature, as demonstrated in Fig. 4(b). The included experimental results [13,16,19,34-36] feature lower scattering amplitudes compared to our result, which could be attributed to the fact that they were assessed ex vivo. The samples were thus processed in different ways (e.g., storage, rinsing), which likely resulted in complete loss of strongly scattering blood cells [33]. In addition, none of those samples was acquired from the inner forearm, and the age and gender of the small number of donors were not standardized. Meanwhile, Altshuler et al. [36] obtained realistic modeling results by assuming significantly higher scattering amplitudes.

![Fig. 4. Spectral dependence of the reduced scattering coefficient of epidermis and dermis as assessed by our analysis (solid blue lines; right column in Table 3) compared with the result corresponding to the effective behavior of entire skin (dashed) and our initial assumption (Eq. (4); dotted) (a). Our result for dermis (solid line) compared with data from refs [13,16,19,34–36]. (b).](image)

3.4. Temporary obstruction of blood circulation

In order to test the reliability and robustness of the presented methodology, we present first a customary test involving temporary obstruction of peripheral blood circulation using a pressurized cuff. Specifically, PPTR signals and DRS were acquired as described above from the dorsal side of the forearm, before (Fig. 5, orange lines) and 3 minutes after application of a blood-pressure cuff inflated to 100 mm Hg (red). The test was performed in one female (NV, 28 years old) and two male volunteers (ML and JR, both 27 years). In all subjects, the stated cuff pressure fell between their respective systolic and diastolic blood pressure values.
The data obtained in such a test feature a significant increase of the PPTR signal amplitude (Fig. 5(a)) and considerable reduction of diffuse reflectance across the involved spectral range (Fig. 5(b)) upon application of the blood-pressure cuff [37]. This points to increased blood content in skin, leading to enhanced absorption of incident laser pulse and consequent subsurface heating. At the same time, the double absorption peak characteristic for oxyhemoglobin, which is evident in intact skin (at 541 nm and 577 nm; orange line in Fig. 5(b)) transforms into a single absorption band at 555 nm, indicative of deoxyhemoglobin.

The match between the measured (solid lines) and best-fitting model predictions (dashed lines) is very good, both before and after the application of the pressurized cuff.

The skin model parameters extracted from both data sets are presented in Table 4. Of most relevance in this test is evidently the substantial rise of the dermal blood contents \( b_{pap} \) and \( b_{ret} \) upon application of the pressure cuff (Fig. 6(a)). This is consistent with the fact that the applied cuff pressure of 100 mm Hg completely obstructs blood flow through the veins but not also through the arteries (i.e., venous occlusion), which results in pronounced blood containment inside the skin. Moreover, the relative increase of the blood content is much higher in the papillary dermis (by a factor of 3–4) compared with the reticular layer, in agreement with an earlier report by Douven and Lucassen [37].

The same effect is evidenced also by the remarkable decrease of blood oxygenation in the papillary layer, which drops from 58% in intact skin to ~26% (Fig. 6(b)), as well as in the reticular dermis (from 75% to ~17%). The same trends are observed also in the results obtained from the other two subjects (Fig. 6(c–f)).

![Fig. 5. Comparison of the PPTR signal (a) and DRS spectrum as measured in intact skin (orange solid lines) and during the blood-pressure cuff test (red solid lines) in subject NV, with the best-fitting model predictions (dashed).](image)

**Table 4. Skin model parameters assessed from the blood-pressure cuff test performed in subject NV (Fig. 5).**

|                  | intact skin | venous occlusion |
|------------------|-------------|------------------|
| \( d_{epi} \) [mm] | 0.085 ± 0.001 | 0.087 ± 0.002   |
| \( d_{der} \) [mm] | 0.72 ± 0.01  | 0.70 ± 0.01     |
| \( m \) [%]      | 1.4 ± 0.1    | 1.5 ± 0.1       |
| \( b_{epi} \) [%] | 0.07 ± 0.04  | 0.09 ± 0.09     |
| \( b_{pap} \) [%] | 2.5 ± 0.1    | 8.9 ± 0.7       |
| \( b_{ret} \) [%] | 1.4 ± 0.1    | 2.3 ± 0.5       |
| \( S_{epi} \) [%] | 58 ± 3       | 26 ± 11         |
| \( S_{ret} \) [%] | 75 ± 4       | 17 ± 15         |
| \( \alpha_{epi} \) [mm\(^{-1}\)] | 11.7 ± 0.3   | 10.0 ± 0.6      |
| \( \alpha_{pap} \) [mm\(^{-1}\)] | 6.6 ± 0.2    | 8.2 ± 1.0       |
| \( \rho_{epi} \) | 1.6 ± 0.1    | 2.3 ± 0.3       |
| \( \rho_{pap} \) | 0.7 ± 0.2    | 1.2 ± 0.4       |
| \( A \)           | 0.6 ± 0.1    | 0.2 ± 0.1       |
It is also important to note that, although the experimental data obtained from intact and partially occluded skin were analyzed independently, the resulting values for $d_{\text{epi}}$, $d_{\text{der}}$ and $m$ are the same within the respective error margins (Table 4). This demonstrates the robustness of our inverse analysis, since the related skin properties are indeed not expected to change during the pressure-cuff test [38,39].

Fig. 6. Blood contents and oxygenation levels before and during temporary obstruction of blood circulation in volunteers NV (a), (b), JR (c), (d), and ML (e), (f).

Finally, Fig. 7 illustrates the scattering amplitudes $a_{\text{epi}}$ and $a_{\text{der}}$, which represent the respective values of $\mu_s' (\lambda)$ at $\lambda = 500$ nm (5). Upon venous occlusion the value for dermis exhibits a considerable rise, which can be linked directly to increased presence of strongly scattering blood cells, especially in the papillary dermis (Fig. 6(a)). This effect is absent in the epidermal layer, which contains a negligible amount of blood.

Fig. 7. Reduced scattering coefficient of the epidermis and dermis at $\lambda = 500$ nm before (orange) and during (blue) temporary obstruction of blood circulation in subject NV.
3.5. Comparison of acutely tanned and untanned skin

The second test of our methodology involves a comparison between two nearby skin sites on the upper arm of subject NV (28 y.). One site was acutely tanned by extensive sun exposure during a two-week long vacation, while the other was protected by a short sleeve (Fig. 8(a)).

Just as anticipated, the assessed skin properties indicate a pronounced difference in the melanin contents between the acutely tanned and nearby untanned skin site ($m = 3.7\%$ vs. $2.0\%$, Fig. 8(b)). In addition, the dermal scattering amplitude ($a_{\text{der}}$) is significantly higher in tanned skin (Fig. 8(c)). This could be attributed to increased number of scattering blood cells due to erythema. Meanwhile, the two epidermal scattering amplitudes are equal within the experimental error.

![Fig. 8.](image)

The associated analysis of the blood status is presented in Fig. 9. The most evident difference between the two test sites is indeed the increased blood content in the papillary dermis of the acutely tanned skin compared to the untanned site (Fig. 9(a)). The same trend, albeit not significant, is indicated also in the underlying reticular layer. This observation matches nicely earlier reports, which demonstrated elevated cutaneous blood content for 3 weeks after UV irradiation, thus importantly contributing to the characteristic appearance of sun-tanned skin [38,40].

Our results also indicate a small decrease of the oxygen saturation levels (Fig. 9(b)), although this may not be significant in view of the uncertainties in the related parameter values. These are large especially in the tanned skin site with more pronounced absorption and scattering of the probing light. Such a trend would be consistent with enhanced oxygen consumption by the cells in the epidermis and upper dermis, recovering from (or actively repairing) the damage caused by extensive UV exposure.

![Fig. 9.](image)
3.6. Monitoring of seasonal changes in skin

Our final test involves longitudinal monitoring of seasonal changes in human skin. PPTR and DRS data were acquired from the same test spot on the inner forearm of a healthy subject (NV, 28 years old) at 5 time points, between late May and mid-November.

Figure 10(a) shows the obtained variations of the melanin content. A pronounced increase is evident at the end of the summer (September 6; day 249 of the year), followed by a significant decline in the fall. Meanwhile, the epidermal thickness values remain essentially constant throughout the measurement window (Fig. 10(b)).

![Fig. 10. Seasonal changes of the melanin content show a pronounced increase over the summer months (a), while the epidermal thickness stays practically constant (b).](image)

The corresponding blood contents in reticular dermis are also quite constant throughout the season, \( h_{\text{ret}} = 0.7–1.2\% \) (Fig. 11(a), closed triangles), while the papillary values fluctuate quite a bit between individual measurement sessions. Nevertheless, the latter are systematically higher than the former, in agreement with anatomy of human skin (Sect. 3.3).

![Fig. 11. Variations of the blood contents (a) and oxygen saturation values in the papillary (dark red) and reticular dermis (red) (b), as indicated by our analysis of DRS spectra and PPTR signals acquired from inner side of the forearm in a healthy volunteer (NV).](image)

Similarly, the oxygen saturation values (Fig. 11(b)), while varying a little between different time points, always assume significantly lower values in the papillary dermis \( S_{\text{pap}} = 54–81\% \) than in the underlying reticular layer \( (78–100\%) \). Oxygen saturation of dermal blood is known to exhibit such fluctuations [41], e.g., due to uncontrolled physiological processes such as thermoregulation.

The highest oxygen saturation values indicated on Day 249 coincide with the peak of the melanin content in Fig. 10(a), although we don't have a definitive explanation for such correlation. Stamatas and Kollias reported a dramatic increase of the oxyhemoglobin content on Day 1 after UV exposure (by \( \approx 100\% \)), which was not accompanied by a matching increase of deoxyhemoglobin and subsided by Day 7 post irradiation [38]. However, the dermal blood contents (Fig. 11(a)) don't show any evident increase at the same time point. We tentatively attribute this difference from the previous result for acutely tanned skin (Fig. 9(a)) to the fact that this test involves gradual accumulation of sun exposure over the summer season.
Consequently, the associated blood pooling may have been less pronounced and/or has largely subsided before the measurement session on Day 249.

4. Discussion

Multi-dimensional optimization of our four-layer optical model of skin, coupled with Monte Carlo modeling of light-tissue interaction as presented above, enables excellent matching of the PPTR signals and DRS obtained from healthy human skin in vivo, especially when scattering properties of the epidermis and dermis are also adjusted on individual basis (Fig. 3).

Some spectral properties assumed in our optical model (e.g., absorption in bloodless skin, blood, and subcutis) are not consistent in literature [13,33,35], so different combinations of the above spectra could in principle be applied. The specific selection made for this study (see Table 1) is based on a critical review of published reports, combined with gradual development of the model over the past years (often by trial-and-error) until good correlations with various experimental data were obtained. The core of our model was described in an earlier report [42], in which DRS values, fluence thresholds for epidermal damage, and those for successful treatment of port wine stain at different visible wavelengths were correlated with actual data. In addition, our current model includes explicit description of the subcutaneous adipose layer, which was found imperative to match the DRS of skin up to $\lambda = 650$ nm [8]. Thus, the combination of basic spectra is similar to the one proposed recently by Mignon et al. [33], but with scattering in the epidermis, dermis, and subcutis allowed to vary according to Eqs. (5) and (6).

The small residual mismatch in the DRS data at $\lambda = 475$–520 nm can be attributed to presence of skin chromophores such as beta-carotene and/or bilirubin [27], which are not accounted for in the presented optical model. In an augmented version of the same analysis approach, individual optimization of the beta-carotene content ensures a near-perfect match of DRS also in this spectral range without compromising the robustness of our inverse analysis or adversely affecting the assessed parameter values [43].

As demonstrated by the presented results, the parameter values assessed for healthy human skin lie within anatomically and physiologically plausible ranges. This is our general experience with the same or very similar analyses, which involved also other subjects [43,44].

The epidermal thickness values obtained for our female subject NV ($d_{epi} = 85 \pm 1$ $\mu$m, Table 3; $\approx 88$ $\mu$m in Fig. 10) lie at the high end of the distribution for the inner forearm in women ($74 \pm 9$ $\mu$m) assessed using optical coherence tomography (OCT) [22]. Note, however, that our recent objective testing of these values, using the approach presented in Sect. 3.2 of this study and co-registration with multi-photon microscopy (MPM) [44,45], demonstrated that our results ($d_{epi}$) correspond to the maximal epidermal thickness within the investigated area. In contrast, most literature values represent the mean values, which are much less sensitive for the relatively sparse epidermal projections into dermis (rete ridges).

In that study, our results for two test sites in a 54 years old man ($d_{epi} = 0.10$ and 0.11 mm, respectively) matched the maximal epidermal thicknesses seen in the MPM cross-sectional images of the same sites, where highly cellular epidermis is clearly delineated from the collagen-rich dermis [44]. At the same time, the difference between these values and those obtained for subject NV in present study demonstrates that the presented technique is sensitive for inter- and intra-personal variations of the epidermal thickness.

The assessed dermal thicknesses can be compared with reported values for the entire skin thickness. In one recent study, the average value for inner forearm in women (age 20–29 years) was $1.03 \pm 0.15$ mm [46]. Our result for female subject NV ($d_{epi} + d_{der} = 0.81 \pm 0.01$ mm, Table 3) is thus slightly below the expected range, but still very plausible. Moreover, the skin thickness values obtained for our subjects JR (1.19 $\pm$ 0.04 mm) and ML (1.29 $\pm$ 0.17 mm) match nicely the reported range for men (age 20–29 years), 1.22 $\pm$ 0.16 mm [46].
Obviously, the assessment of $d_{\text{epi}}$ and $d_{\text{der}}$ is not the main goal of the discussed skin characterization using combined PPTR and DRS. Many more accurate and practical techniques are available for this task, such as OCT, MPM, confocal microscopy, photoacoustics, etc. Nevertheless, it is very important that our approach provides reasonable and individually optimized estimate values, because they can significantly affect the values of chromophore contents extracted from DRS [7,8]. Consequently, fixing them to average values adopted from literature may introduce uncontrolled errors in other parameter values, similarly to the effect demonstrated in present study for scattering properties of skin (Sect. 3.1).

The assessed dermal blood contents lie within the range of values reported earlier for healthy adults (0.2–7%) [24–26]. More specifically, in all included subjects and time points the blood contents in the papillary layer ($b_{\text{pap}} = 0.9–4%$ overall) assume higher values compared to reticular dermis (0.7–1.9%; see Figs. 6(a), 6(c), 6(e), 9(a), and 11(a)). This is consistent with the existence of extensive capillary network in the papillary dermis (see Sect. 3.3). We want to emphasize that this effect does not result from any biasing of the optimization process with prior knowledge, which is evidenced by the identical (and broad) ranges of permitted values for model parameters $b_{\text{pap}}$ and $b_{\text{ret}}$ (see Table 2).

The epidermal blood content is always much smaller in comparison, typically $b_{\text{epi}} < 0.1\%$ (Tables 3 and 4, Figs. 6 and 9), although it is allowed to reach 5% in the optimization process (Table 2). This correctly reflects the very small fractional volume occupied by the capillaries running vertically in the undulations of the epidermal-dermal junction, which are thus included in the epidermal layer of our skin model with a flat boundary with the dermis.

The assessed oxygen saturation levels in unperturbed skin match nicely earlier reported values for human forearm, 65 ± 12% [28] and 73 ± 12% [29]. In addition, the values for papillary dermis are consistently lower than in the underlying reticular layer (see Figs. 6(b), 6(d), 6(f), 9(b), and 11(b)). It is thus very appropriate that the former values are spread along the lower end, while the latter lie at the upper end of the ranges given in the literature.

Regarding the presented pressure-cuff tests, note that the moderate cuff pressure of 100 mm Hg was selected in order to cause venous occlusion (see Sect. 3.4). This results in maximal blood accumulation inside the skin, and obviously its deoxygenation (Fig. 6), as demonstrated in numerous earlier reports [37,38]. In contrast, application of cuff pressures above the subject’s systolic value will obstruct also arterial blood flow, but lead to a smaller increase of the dermal blood content relative to intact skin [39,47], while the oxygen saturation level can get even lower than upon venous occlusion (depending also on duration of the obstruction). Using the analysis approach very similar to that presented in current study, we have recently replicated these effects by applying incrementally higher cuff pressures (i.e., 80, 100 and 120 mm Hg) to induce either venous or arterial (a.k.a. total) occlusion [48].

Finally, the assessed scattering properties of the dermis in subject NV (age 28) are in excellent agreement with earlier published results (Fig. 4(b)) and consistent between measurements performed at different time points (Figs. 4(a), 7, and 8(c)). Moreover, approximately 2-times higher values are consistently indicated for the epidermis, also in good agreement with available literature data [19,30–32].

A recent study on a large volunteer cohort [49] reported a lower average value, $\mu'_s(500 \text{ nm}) = 2.8 \pm 0.6 \text{ mm}^{-1}$, compared with our result for subject NV (see Table 4, Fig. 8(c)). We attribute this difference primarily to the fact that their study involved much older subjects (ages 50–64 y.), based on the negative correlation between the scattering amplitude and subject age observed within the same study [49]. Our measurement in a 65 years old man, e.g., yielded $a_{\text{der}} = 2.3 \text{ mm}^{-1}$ (unpublished), in perfect agreement with this argument.

In conclusion, the presented approach, which combines PPTR and DRS measurements with IMC analysis, enables noninvasive characterization of human skin in terms of the epidermal and dermal thickness, blood content and oxygenation level, as well as scattering properties. Encouraged by the positive experience presented above, we are applying the
described approach in ongoing studies of traumatic bruises [43] and laser skin rejuvenation [50], which present further tests of its viability.

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