

Design and Evaluation of a Novel Portable Erythema-Melanin-Meter

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Background and Objectives: Objective evaluation of the pigmentation index (PI) and the erythema index (EI) of human skin is a prerequisite for successful optimization of laser- and intense-pulsed-light (IPL)-based treatment modalities in dermatology.

Study Design/Materials and Methods: We describe a three-wavelength technique for determining PI and EI as well as its particular implementation using LEDs operating at wavelengths of 560, 650, and 710 nm and a large-area photodiode. The instrument has been evaluated both in vitro and in vivo.

Results: In vitro, good correlation between the measured indices and results obtained with commercially available techniques has been observed. In addition, linearity of the PI with melanin concentration in the phantom medium up to $7 \times 10^{-3} \text{ nm}^{-1}$ (defined as a slope of the optical density spectrum) has been established. In vivo, feasibility of using the technique for predicting the minimal erythema dose (MED), minimal phototoxic dose (MPD), and the threshold of epidermal damage in a photothermal treatment has been demonstrated.

Conclusions: The data suggest that the technique has a substantial potential as a method of pre-treatment diagnostics for photochemical and photothermal procedures. *Lasers Surg. Med.* 34:127–135, 2004.

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Key words: erythema; pigmentation; skin color; portable instrument

INTRODUCTION

Increasing use of light- and laser-based treatment modalities in dermatology instigates the need for objective techniques, which can be used for both planning the treatment and evaluating the results [1]. Such techniques have a significant potential for further minimizing risks of adverse side effects [2], reducing or eliminating patient's discomfort during treatment, and increasing efficacy. In addition, availability of these techniques can facilitate selection of the most suitable treatment approach for a given patient.

It is established that for photothermal treatments the risk and severity of side effects as well as treatment discomfort generally increase for darker and tanned skin [3]. Several studies (e.g., Ref. [4]) indicate that skin character-

ization according to Fitzpatrick scale may not be sufficient for accurate assessment of possible side-effects. One possibility to rectify the situation is to use objective measurements of skin melanin content as a predictive parameter for adjusting settings of a photothermal device.

For photochemical (e.g., UVB and PUVA) treatments, important parameters are the minimal erythema dose (MED) and the minimal phototoxic dose (MPD), which are used to determine the optimal treatment dose [5]. An objective technique for precise quantitative determination of MED and MPD is highly desirable for improving treatment outcome. Such a technique should be capable of determining an average concentration of hemoglobin (proportional to blood content) in skin at the treatment site. Moreover, a recent study [6] indicates that measurements of the melanin content can be used to individualize the initial treatment dose and may potentially replace the time-consuming MED testing.

Both quantities (i.e., concentrations of melanin and hemoglobin) can be evaluated from information contained in the optical signal diffusely reflected by human skin.

Epidermal melanin is a major target chromophore for photoinduced injury [8,9]. It effectively absorbs light of all wavelengths from 300 to 1,000 nm, but the strongest absorption occurs at shorter wavelengths, in near-UV spectral range [7,8]. Both oxy- and deoxyhemoglobin (the major blood chromophores) absorb light predominantly in several distinctive absorption bands and influence in vivo reflectance spectra accordingly [10,11]. Two forms of hemoglobin have the strongest absorption bands in 405–430 nm spectral range (the Soret band) and secondary absorption bands in 540–580 nm spectral range. Both hemoglobins exhibit the lowest absorption at wavelengths longer than 620 nm. Blood content in skin varies within a wide range depending on skin pathologies (inflammation, edema, burn, etc) and environmental factors (temperature, radiation, pressure, etc.).

Complete information on concentration and spatial distribution of skin chromophores can, in principle, be

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E-mail: iyarosla@palmed.com
 Accepted 17 September 2003
 Published online in Wiley InterScience
 (www.interscience.wiley.com).
 DOI 10.1002/lsm.10233

obtained from spectrally and spatially resolved measurements of skin reflectance. However, such level of detail involves sophisticated equipment as well as data-processing techniques and, moreover, is unnecessary for most practical applications.

In practice, chromophore content in skin is characterized by a parameter called “pigment index,” which is defined for a given chromophore as a quantity proportional to the average concentration of the chromophore in a sampled volume of tissue. Pigment indices are useful measures of cutaneous chromophore content, and can be used for quantitative characterization of pathological tissue conditions. The diffuse reflectance spectrum of skin includes spectral regions in which the measured *in vivo* skin reflectance is mainly determined by a specific chromophore only, and thus individual pigment indices can be calculated. Pigment indices have been introduced for melanin (also known as pigmentation index (PI)), bilirubin, and hemoglobin (also referred to as the erythema index (EI)) [5,7,12–20].

In recent decades, several academic and industry groups have developed optical instruments for *in vivo* non-invasive assessment of erythema and/or pigmentation indices.

All these instruments can be categorized as follows:

- (1) spectrophotometers for measuring skin diffuse reflectance spectra with subsequent computation of the erythema index (E) and the pigmentation index (M) [5,6,21];
- (2) spectrofluorimeters that use the skin autofluorescence for determining E and M [22,23];
- (3) chromameters for the analysis of white light skin reflection in suitable spectral ranges (blue, green, and red) [10,24,25];
- (4) specialized instruments for evaluating E and M in dermatological and cosmetologic applications.

Instruments of the last category typically use narrow-band illumination of the test area with one or several light-emitting diodes (LEDs) and compute the erythema and pigmentation indices from the measured value of diffuse reflectance [16,26,27].

Our previously reported *in vivo* investigations of the human skin by reflectance spectroscopy [23] enabled us to develop a method for determining the E and M indices based on a comparison of skin reflection coefficients at three wavelengths (one in green, and two in red spectral regions) [22]. Construction of the instrument, its performance, test measurements, and pilot clinical trials are discussed in this paper.

THREE-WAVELENGTHS METHOD FOR DETERMINING ERYTHEMA AND PIGMENTATION INDICES

A simple model for analysis of skin reflectance is based on the assumption that the skin consists of three or four layers, each of them homogeneously transmits and scatters light [5,6,13,14,21]. The relationship between the simplified

three-layer model (Fig. 1) and the anatomic structure of skin is apparent. The stratum corneum (included in layer 1) is responsible for only 5–7% of the reflectance (including the Fresnel’s reflectance) and mainly induces diffuse forward scattering and allows penetration of light into epidermis. In the epidermis (layer 1), light is strongly absorbed by melanin and the non-absorbed part reaches the hemoglobin-rich papillary dermis (layer 2). The remaining part of the light is then diffusely reflected by dermal collagen (layer 3). Thus, light diffusely reflected by dermis reaches the skin surface after passing twice through the hemoglobin- and melanin-rich layers.

For a turbid medium such as human skin, the diffuse reflectance R_d determines the apparent optical density OD of the medium [3,21],

$$OD = -\lg R_d. \quad (1)$$

Assuming that the contribution of the first two layers in the total skin diffuse reflectance is significantly lower than that of the third layer, the apparent optical density of skin is given by the sum of optical densities of the first two layers, containing melanin (OD_1^{mel}) and blood (OD_2^{blood}),

$$OD = OD_1^{\text{mel}} + OD_2^{\text{blood}} - \lg (R_{d,3}^{\text{coll}}), \quad (2)$$

where $R_{d,3}^{\text{coll}}$ is the diffuse reflectance of dermal collagen (third layer).

Due to the strong absorption of melanin in the UV spectral range the melanin index M may be defined as a slope of the *in vivo* reflectance spectrum between 365 and 395 nm, which correlates with melanin content [1,28]. But this spectral range is not very suitable for a portable instrument design.

Due to minimal hemoglobin influence, the near-infrared spectral range is an alternative region for determining melanin index. Kollias and Baqer [7,29] reported that the slope of the skin OD in the spectral range 620–720 nm is directly proportional to melanin content in the epidermis. Specifically, the melanin index is defined by the slope of OD (λ) in the region above 620–640 nm (Fig. 2):

$$M = 100 \left(\overline{OD}_{650} - \overline{OD}_{700} \right), \quad (3)$$

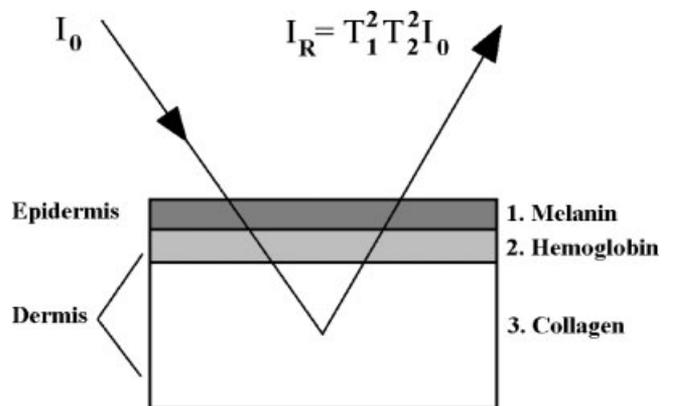


Fig. 1. Simplified three-layer skin model.

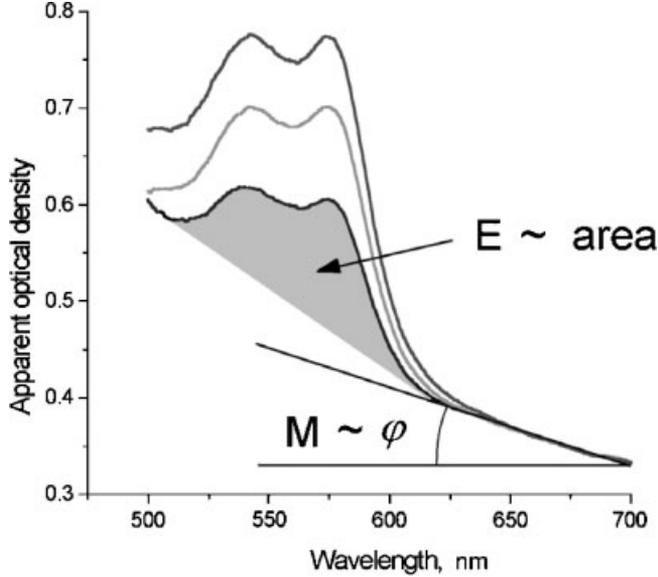


Fig. 2. Definition of erythema and pigmentation indices.

where line denotes the mean value of OD measured near 650 and 700 nm.

Changes in hemoglobin content in the dermis (or changes in hemoglobin oxygenation) lead to absorption changes in the 535–585 nm spectral range (oxygenated hemoglobin has a double-peak maximum at 545 and 575 nm, whereas deoxygenated hemoglobin has a single peak at 560 nm). Therefore, skin reflectance in this spectral range can be used to obtain information about blood content and hemoglobin oxygenation.

Several formulas for determining the erythema index E using in vivo reflectance spectroscopy have been suggested. The most widely used definition for the erythema index is given by the area under the spectral curve OD (λ) in the region 510–610 nm [21] (see Fig. 2)

$$E = 100[\text{OD}_{560} + 1.5(\text{OD}_{545} + \text{OD}_{575}) - 2.0(\text{OD}_{510} + \text{OD}_{610})], \quad (4)$$

where subscripts denote wavelength in nm.

Simplified methods for measuring erythema index are based on comparison of the skin reflectance in the green (~ 560 nm) (high hemoglobin absorption) and red (~ 650 nm) (low hemoglobin absorption) spectral ranges [26]

$$E = 100 \left(\frac{R_d^G}{R_d^R} \right), \quad (5)$$

where R_d^G and R_d^R are reflectance coefficients in the green and red spectral ranges, respectively. Alternatively, difference of the skin optical density in the green and red spectral ranges [16] can be used:

$$E = 100(\text{OD}_{560} - \text{OD}_{650}). \quad (6)$$

A drawback of these simplified methods is that they ignore the contribution of melanin absorption in the green spectral range.

The three-wavelengths method [23,30] developed by us is based on the following:

- (1) PI is determined from the difference in the apparent optical densities between normal and vitiligo skin measured at two wavelengths in the red spectral range above 620 nm. The melanin level corresponds to the slope of this difference between 620 and 710 nm [31]. Moreover, as shown by Kollias and Bager [31,32] as well as in our measurements, values of reflectance coefficients at 650 and 710 nm for non-pigmented (vitiligo) skin have very small interpersonal variability (in our case the variability was 5% for $n=10$), and depend predominantly on the experimental setup. These values were included in the algorithm of the PI computation as factory-set calibration parameters;
- (2) the method incorporates corrections of the measured erythema index as a function of melanin absorption.

Resulting formulas for computing the pigmentation and erythema indices are:

$$M = k \left(\frac{\text{OD}_2 - \text{OD}_3}{\Delta\lambda_{23}} \right), \quad (7)$$

$$E = 100 \left[\text{OD}_1 - \text{OD}_2 - \frac{M}{k} \Delta\lambda_{12} \right], \quad (8)$$

where $\Delta\lambda_{12} = \lambda_2 - \lambda_1$, $\Delta\lambda_{23} = \lambda_3 - \lambda_2$, $\lambda_1 = 560$ nm, $\lambda_2 = 650$ nm, $\lambda_3 = 710$ nm, and k is a calibration factor.

DESIGN OF THE INSTRUMENT

The EMM-01 erythema/melanin-meter has three principal components: the optical head, the control/data processing unit, and the power supply unit. Schematic of the instrument is presented in Figure 3. The optical head contains 12 light emitting diodes of three spectral ranges with the central wavelengths at 560, 650, and 710 nm (Fig. 4) and the photodiode for the collection of the reflected light.

The control and data processing unit provides switching of the LEDs, analog-to-digital conversion of the measured signals as well as computation and display of pigmentation and erythema indices.

Relatively high combined incident irradiance and large detection area of the photodiode ensure wide dynamic range and satisfactory signal-to-noise ratio for majority of subjects of I through VI Fitzpatrick skin types.

MATERIALS AND METHODS

Phantom Measurements

Purpose of the phantom measurements was twofold: first, to compare the E and M values obtained using the proposed three-wavelengths technique with those reported by commercially available devices; and, second, to deter-

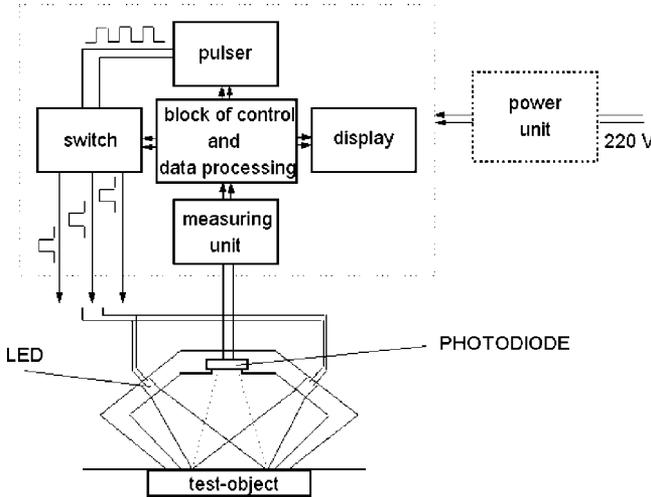


Fig. 3. Schematics of the EMM-01 erythema-melanin-meter.

mine the linearity range of the EMM-01 device with special emphasis on the usability of the instrument for characterizing skin types V and VI [33].

In the first set of measurements, pieces of blotting paper colored by red and brown inks (Pelikan 4001, Hanover, Germany; 0–30% volume concentration) were employed as “standards” of red and brown colors. The absorption spectra of the red ink and hemoglobin as well as, respectively, the brown ink and melanin are quite similar in the relevant spectral ranges. This is illustrated by Figure 5. The ink absorption spectra have been obtained from direct measurements with the Lambda16 spectrophotometer (Perkin Elmer, Beaconsfield, UK). Thus, the ink/paper phantoms were used for imitating various degrees of erythema and tanning.

Comparative measurements of *E* and *M* indices with the EMM-01 and Mexameter MX-16 (Courage + Khazaka electronic GmbH, Cologne, Germany) devices as well as color measurements with the Minolta chromameter CR-200b (Higashi-Ku, Japan) were performed.

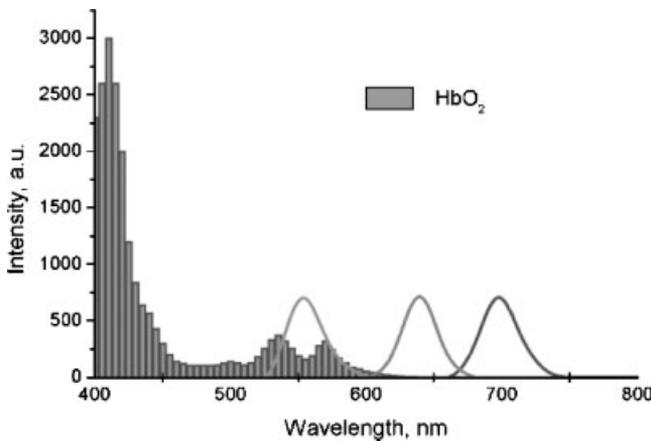


Fig. 4. Spectral ranges of the LEDs used and the hemoglobin absorption spectrum.

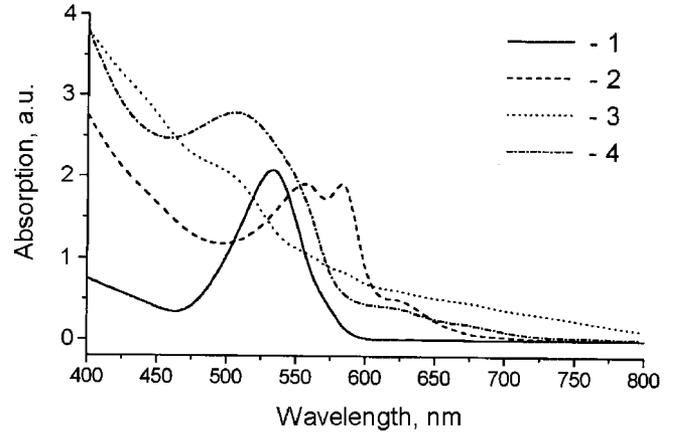


Fig. 5. Absorption spectra of the red (1) and brown (2) ink, hemoglobin (3), and melanin (4). Ink concentration $C \approx 1\%$.

In the second set of measurements, we used press tablets of whitening powder with varying quantity of melanin-containing Foundation Cream (Melasyn, Vian Pharmaceuticals, Inc., New Haven, CT). Pigmentation indices measured with the EMM-01 instrument were compared with the values of the slope of the apparent optical density versus wavelength above 620 nm. The apparent optical density spectra of the phantom were measured with an optical fiber probe and an optical multichannel analyzer (OMA)-based spectrophotometer [6,22].

Phototesting of Human Skin In Vivo

The EMM-01 was evaluated for human skin phototesting in vivo in four separate studies.

Study 1 (n = 15). Dynamics of UV-induced erythema and pigmentation was monitored in 15 subjects of skin types I and II. The dose of UV radiation (irradiation was performed with UFO-L source, Electronica, Russia) was equivalent to four MEDs. Erythema and pigmentation indices of the irradiated skin sites (diameter ≈ 3 cm) of the inner surface of forearm were determined 1, 3, and 6 hours and 1, 3, 5, and 45 days after UV exposure. The results obtained were compared with the results of computing the *E* and *M* indices from the diffuse reflectance spectra data as had been described earlier [6,23].

Study 2 (n = 16). The EMM-01 was used for an objective determination of MED. Standard procedure of human skin phototesting in UV spectral range was performed at increments of 5 J/cm^2 in the range $5\text{--}30 \text{ J/cm}^2$ using the Dermolum UM/W source (Mueller Electronic-Optik, Moosinning, Germany). Sixteen healthy volunteers (6 men and 10 women) of skin types II, III, and IV were exposed to UV radiation. Measurements were performed at several sites before UV-irradiation, immediately after UV-irradiation, and 8 and 24 h after UV exposure. Results of the EMM-01 measurements were compared with results obtained by the Minolta chromameter, the MX-16 erythema-meter MX-16, and the scanning laser Doppler flowmeter

moorLDI[®] (Moor Instruments, Ltd., Devon, Great Britain). The latter measurements allowed determination of the volumetric skin blood content.

Study 3 (n = 39). The EMM-01 has been used for determining the MPD of UV-A radiation prior to PUVA therapy. Phototests were made on 39 patients with psoriasis. PI was measured at 5–6 sites of a lateral region of the back. Immediately, phototests were made prior to 8-MOP plus UVA therapy at the same sites of skin. Skin sites were exposed to doses of 1.0 (0.5 for skin type I) to 9.0 J/cm² in steps of 2.0 J/cm². MPD was assessed visually for each volunteer 8 and 24 hours after exposure.

Study 4 (n = 6). Feasibility of employing the *E* and *M* indices as predictive parameters for planning photothermal treatments has been assessed, the hypothesis being that measurements of either *E* or *M* could be used for determining safe fluence setting prior to treatment. Subjects with skin types of I through VI were enrolled in the

study. A Xe-flashlamp system (EsteLux prototype, LuxY handpiece, Palomar Medical Technologies, Inc., Burlington, MA) with the spectral output range between 525 and 1,200 nm was used. Nine test spots were demarcated on each subject's back. The test treatment consisted of a single exposure (one pulse per spot) with fluences ranging between 4.4 and 13.2 J/cm². The *E* and *M* indices were measured at each spot before and after treatment. Epidermal damage was assessed immediately (30–60 minutes) after treatment and at 1-day follow-up.

RESULTS AND DISCUSSION

Phantom Measurements

Results of the phantom measurements with the EMM-01 instrument, the Minolta chromameter, and the MX-16 Meaxameter are shown in Figure 6a–d.

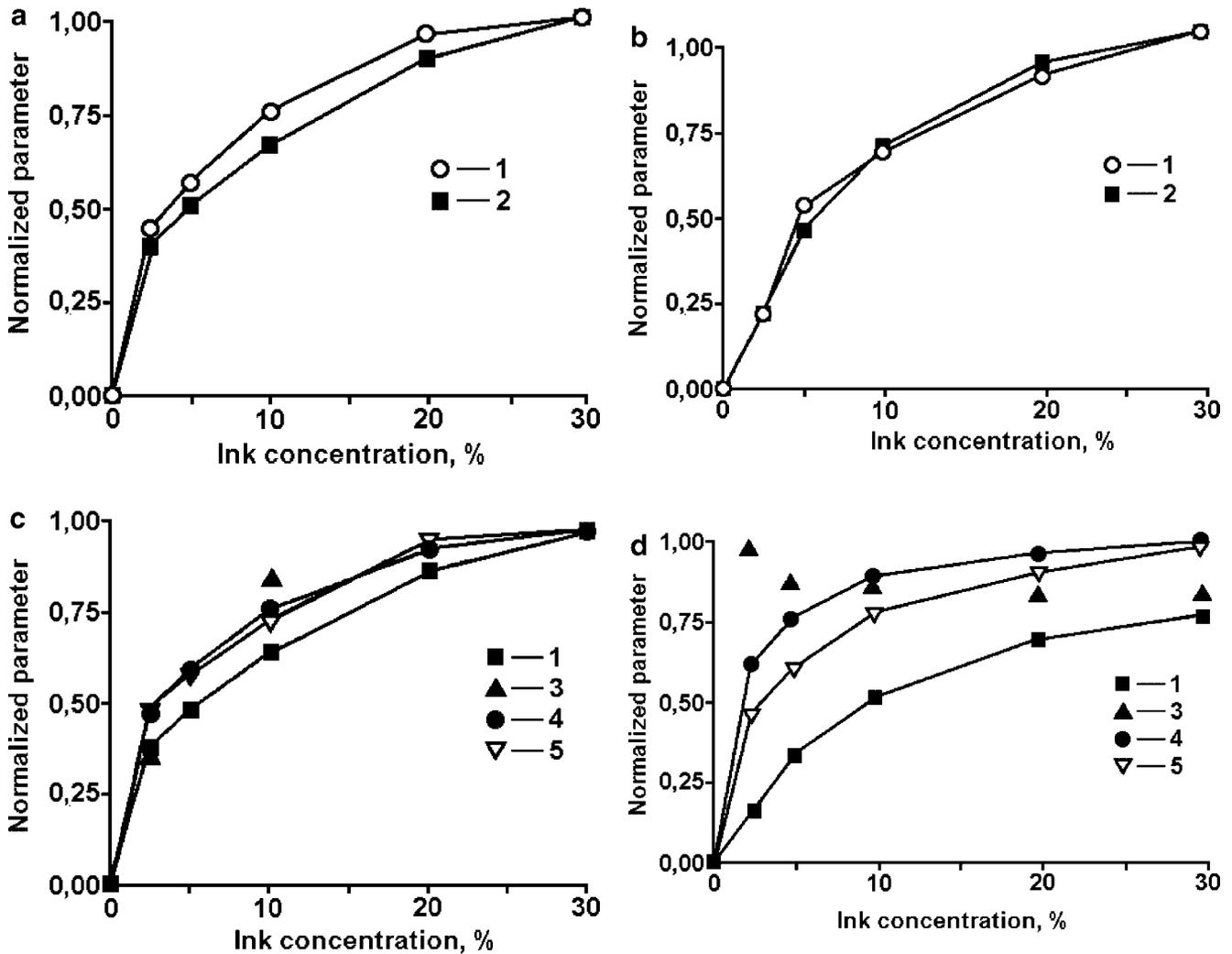


Fig. 6. Normalized parameters for the red (a, c) and brown (b, d) ink as functions of ink concentration. 1, indices (EMM-01); 2, indices (Mexameter MX-16); 3, hue (Minolta[®]); 4, chroma (Minolta); 5- ΔE (Minolta).

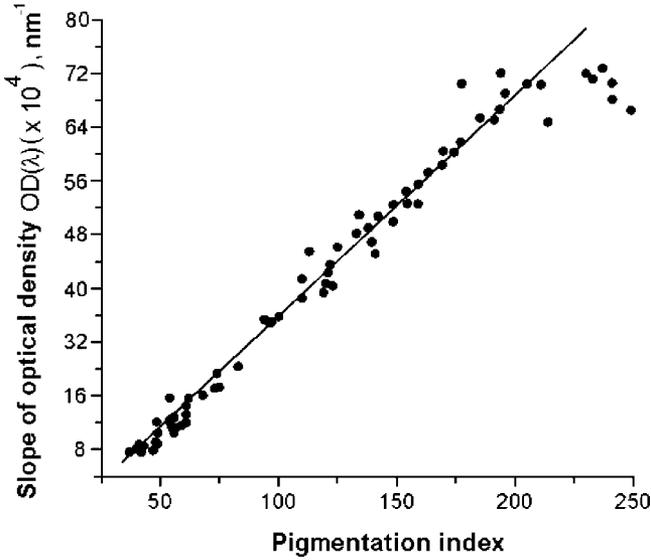


Fig. 7. Correlation between pigmentation index (PI) (EMM-01) and OD (λ) slope in 630–710 nm spectral range (reflectance spectroscopy).

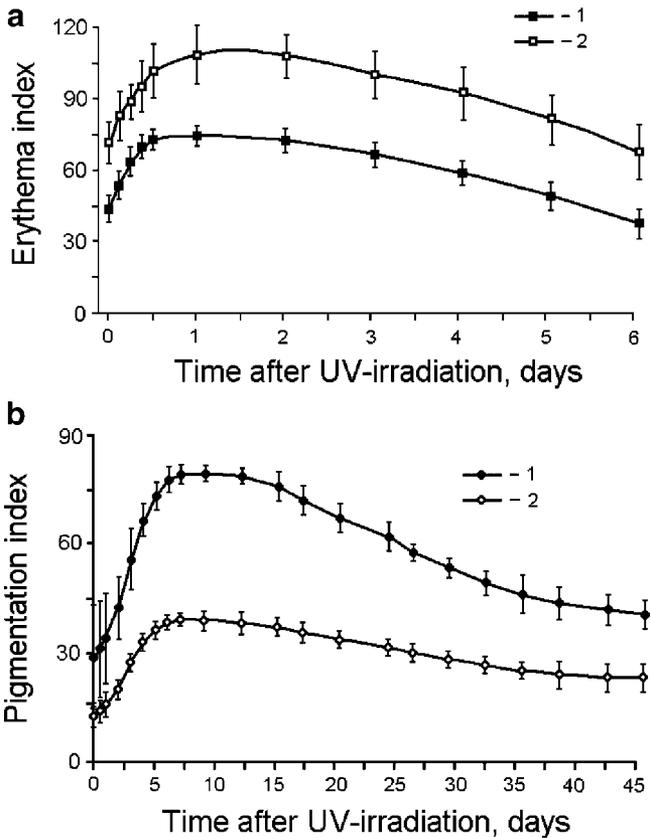
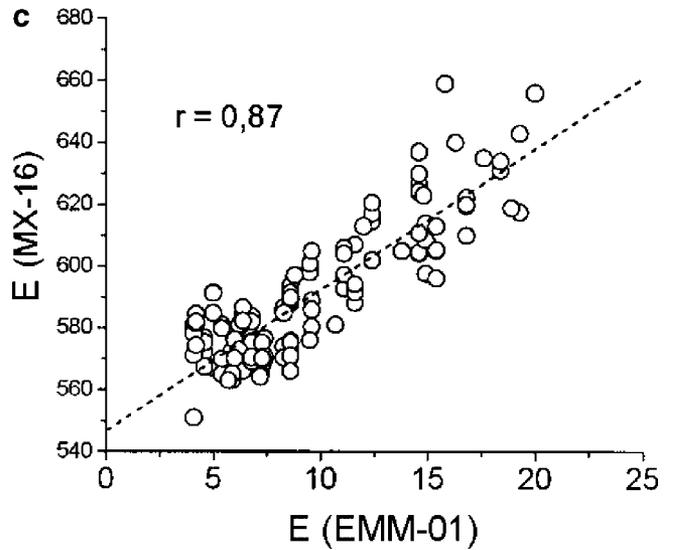
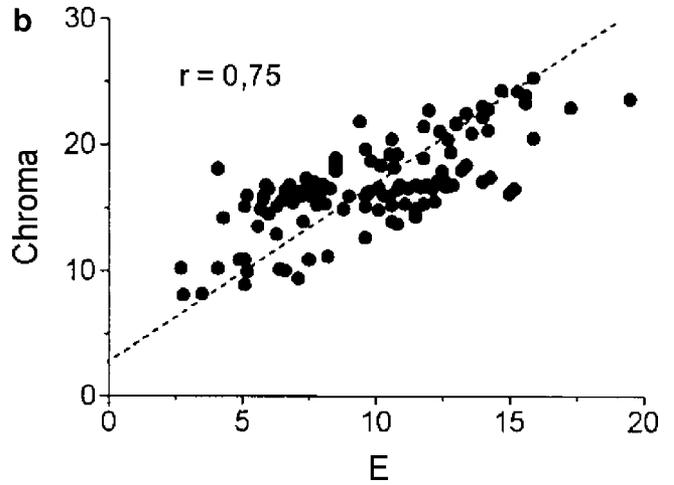
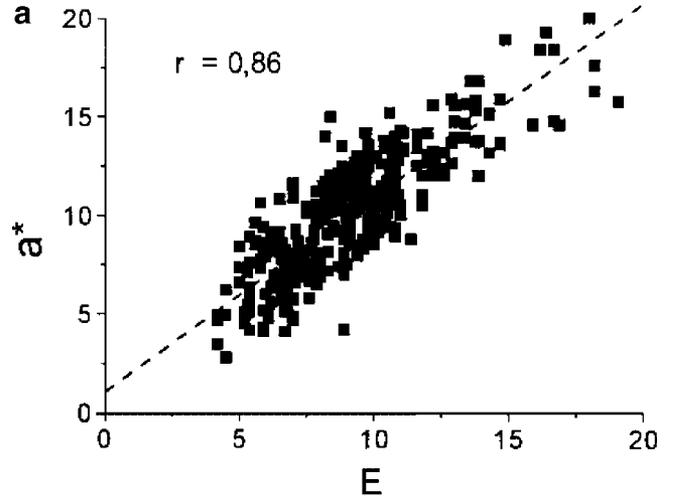


Fig. 8. Dynamics of erythema (a) and pigmentation (b) during UV-erythema formation. 1, EMM-01; 2, “classic” reflectance spectroscopy.

Fig. 9. Correlation between erythema and pigmentation indices measured by EMM-01 and parameters measured by Minolta (a, b) and MX-16 (c).

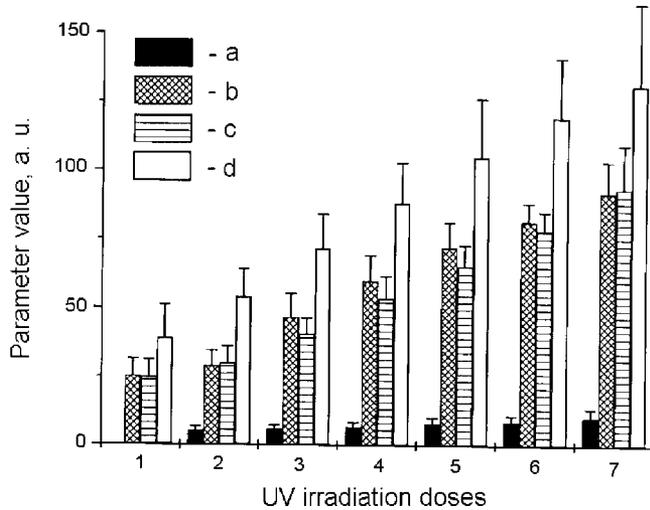


Fig. 10. Evaluation of phototesting results (24 hour erythema). UV-radiation dose: 1, control (0 mJ/cm²); 2–5 J/cm²; 3–10 mJ/cm²; 4–15 mJ/cm²; 5–20 mJ/cm²; 6–25 mJ/cm²; 7–30 mJ/cm². Measured parameter: **a**: ΔE (Minolta); **b**: erythema index (Mexameter MX-16); **c**: erythema index (EMM-01); **d**: skin volume flowblood (moor LDITM).

Figure 6a,b demonstrate a good agreement between the E and M values obtained with the EMM-01 and the MX-16 for the varying ink concentration.

Human skin with erythema or pigmentation is usually perceived as a surface with more red or, respectively, brown color hue than the normal one. The Minolta chromameter allows to evaluate the skin color as a set of three parameters L^* , a^* , and b^* in the color space of CIE1976 ($L^*a^*b^*$)-system [34]. Analysis of the skin color based on the combination of two parameters, hue and chroma, is more conventional.

Figure 6c,d shows the measured values of the phantom color parameters (Minolta) and the E and M values (EMM-01) as a function of the ink concentration. Changes in the hue parameter correlate well with the results obtained using the EMM-01 for both kinds of ink. However, behavior of the chroma parameter was substantially different for the two kinds of ink: it did not change significantly in case of the brown ink, but reproduced the concentration dependence of the hue alterations in case of the red ink. Concentration dependence of the color difference ΔE also corresponded to changes of E and M indices (Fig. 6c,d).

Figure 7 shows the high degree of correlation ($r = 0.98$, $P < 0.0001$) between the PI and the tangent of the OD (λ) slope angle in a range of slope angle variation up to $7,000 \times 10^{-6} \text{ nm}^{-1}$. According to Jacques [2], the difference in the OD (λ) slopes for pigmented and vitiligo skin approximately ranges from $100 \times 10^{-6} \text{ nm}^{-1}$ (skin type I) to $1,500 \times 10^{-6} \text{ nm}^{-1}$ (skin type IV), and can reach values about $6,000 \times 10^{-6} \text{ nm}^{-1}$ for skin of type VI. Results, shown in Figure 7, illustrate the ability of the EMM-01 possibility to measure PI for any Fitzpatrick skin type in the range I through VI.

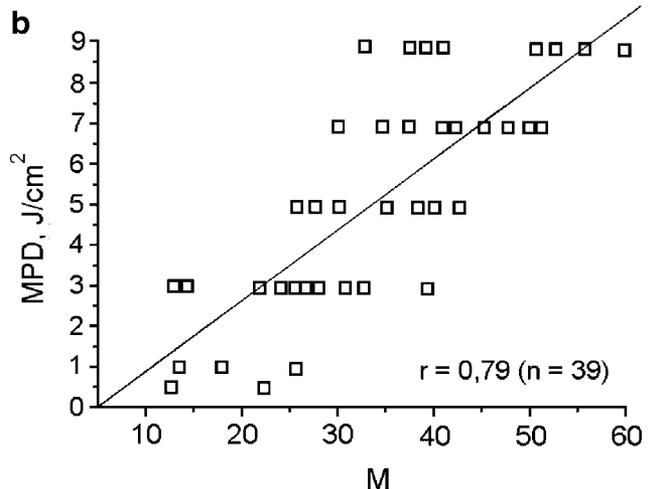
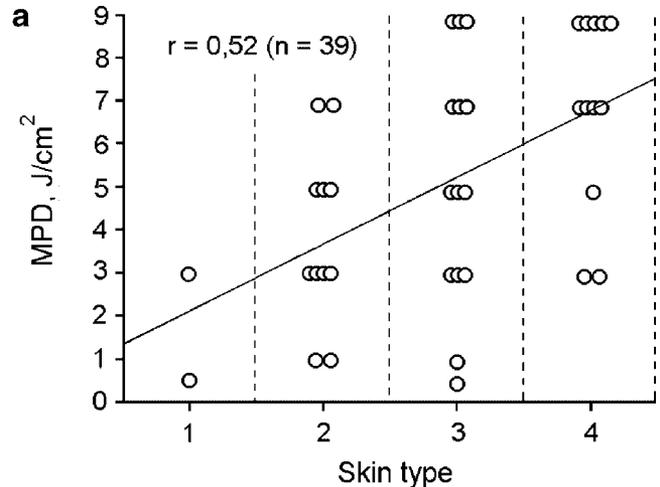


Fig. 11. Correlation between the minimal phototoxic dose (MPD) and Fitzpatrick skin type (a), the MPD and PI (b).

In Vivo Measurements

Study 1. Study of UV-erythema dynamics using EMM-01 and the spectrometer has demonstrated a good agreement between results obtained by a “classic” method (reflectance spectroscopy) and the EMM-01 (Fig. 8). These results suggest that the spectral information collected using the three-wavelength technique is sufficient for reliable determination of the absorption contributions by different chromophores.

Study 2. The comparative analysis of in vivo measurements of E and M indices shows a considerable correlation ($r = 0.66\text{--}0.87$, $P < 0.05$) between the EMM-01 data and the respective parameters measured by MX-16 and Minolta[®] (Fig. 9).

Monitoring of the basic photobiological phenomena after a UV irradiation (24-hour erythema) clearly demonstrates sufficiently high sensitivity of EMM-01. Figure 10 shows results of phototesting the volunteers ($n = 16$) evaluated by EMM-01, MX-16, Minolta, and laser Doppler’s

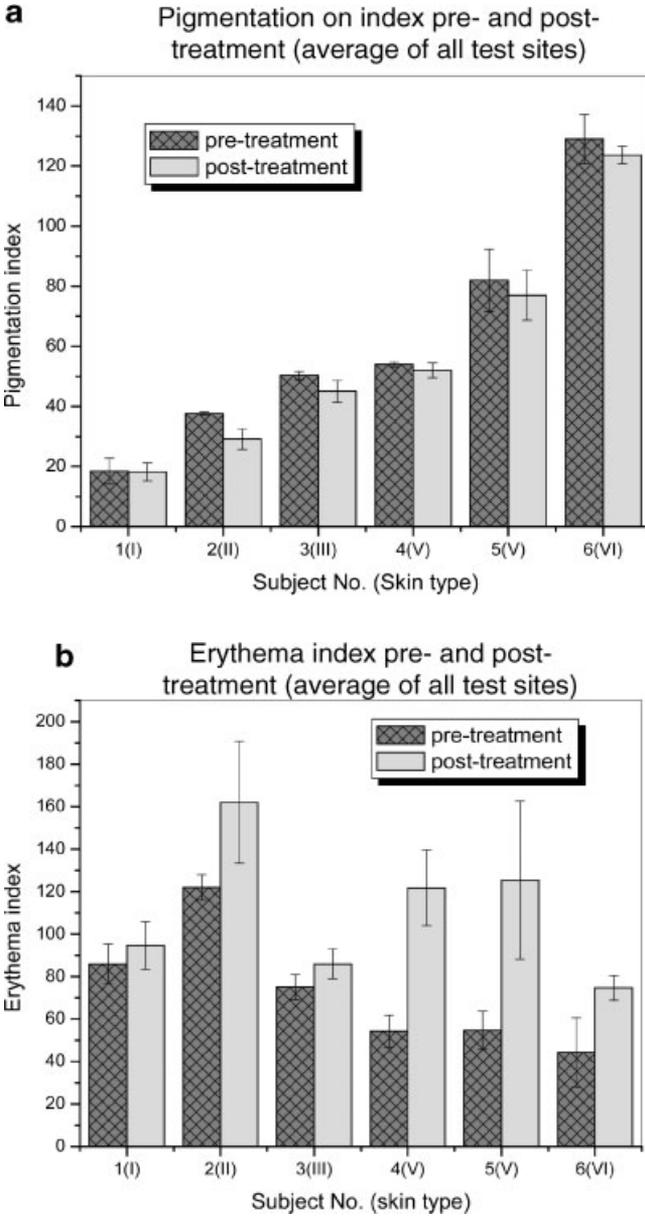


Fig. 12. Pigmentation (a) and erythema (b) indices pre- and post-treatment with Palomar EsteLux LuxY.

flowmeter. The data indicate that the increase in the *E* index is directly proportional ($r = 0.89, P < 0.01$) to increase of the skin volumetric blood index.

Study 3. Figure 11a,b illustrate the dependence of the MPD on the *M* index and skin type. The correlation between the MPD and the PI was expressed much more strongly than that between the MPD and the Fitzpatrick skin type ($r = 0.79$ and $r = 0.52$, accordingly, $P < 0.05$).

According to our calculations, MPD (J/cm^2) could be determined as:

$$MPD = -0.92 + 0.18 \times M. \quad (9)$$

The initial dose (ID) of UV-A radiation for the photochemotherapy of psoriasis could be computed as:

$$ID_{UVA} = (-0.92 + 0.18 \times M) - 0.35. \quad (10)$$

Not unexpectedly, the erythema and pigmentation indices were typically higher for skin sites exposed to UV irradiation (forehead, checks, neck).

The analysis of interrelation between a PI of exposed sites and the skin type has demonstrated considerable correlation, whereas for covered sites of skin the correlation level was lower. Our observations are consistent with results obtained by other groups [35–38], who have been trying to verify objectively the procedure of the skin type separation offered by the Fitzpatrick scale [35,37, 39–41].

Study 4. Measurements of the *M* and *E* indices pre- and post-treatment are exemplified by Figure 12a,b. As can be seen from the data, the PI did not change significantly as a result of the treatment, whereas the erythema index clearly reflected the increased blood content after the treatment. The *M* index measured pre-treatment appeared to be a good prognostic parameter for predicting the incidence of epidermal side effects (see Fig. 13). The *E* index revealed no correlation with the incidence of epidermal side effects. The maximal tolerable fluence F_{MT} could be approximated as a linear function of the *M* index. Obviously, the slope of the line will depend on the parameters (wavelength range, pulsewidth, etc.) of the particular photothermal system. These data suggest that a “smart” photothermal treatment procedure is feasible. Such a procedure would involve the determination of the F_{MT} as a function of the *M* index on the system development stage. This information can then be stored in the system. The *M* index will be measured on the treatment planning stage, and the safe fluence will be determined as a percentage (e.g., 80%, see Fig. 13) of the corresponding F_{MT} value.

In conclusion, the EMM-01 instrument described in this paper has been in testing for over 4 years during which it

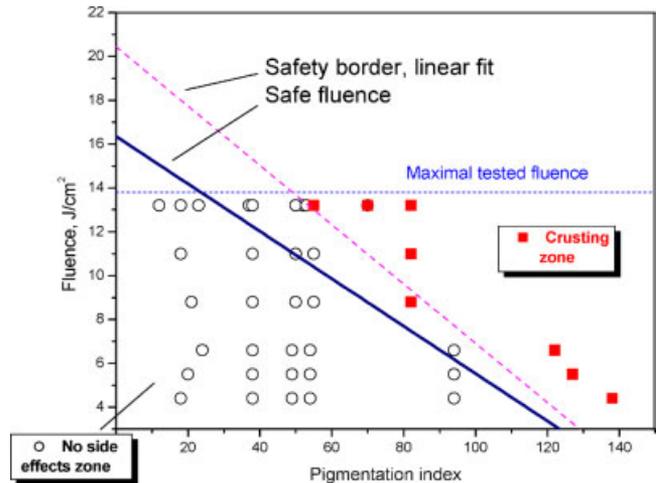


Fig. 13. Incidence of epidermal side effects as a function of the PI and fluence.

has proved its capability to obtain objective and reproducible data on the blood and melanin content in the human skin. High discrimination between hemoglobin and melanin contributions, high instrument accuracy (an error of measuring reflectance coefficients $\leq 5\%$), repeatability ($\sim 95\%$), short acquisition time (~ 1 second), portability, and simplicity and ease of use suggest that the technique has a high potential as a valuable pre-treatment diagnostic modality in dermatology.

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