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Study of Skin Phantoms by Photothermal Radiometry in Frequency Domain and Multivariate Methods

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Abstract In this paper the use of the photothermal radiometry technique in the frequency domain (PRTF) and the use of multivariate methods in the study of two types of skin phantoms: (a) one in which skin pigmentation was simulated dyeing the gel phantom and (b) the other consists of exposure of animal skin samples to different degrees of thermal damage. In experiment (a), gel phantoms were prepared with different concentrations of methylene blue (MB). The mean values of the radiometry signal (RS) show significant differences in only those cases in which changes in the concentration of MB were higher than 0.38 mM. This result was confirmed with a t test for independent samples of the data ($p < 0.05$). The mean values of the amplitude and phase signal do not permit discrimination between phantoms with changes in pigmentation equal to or lower than this value. However, principal component analysis (PCA) demonstrated that it is possible to discriminate between phantoms with changes in molar concentration equal to 0.38 mM (for the phase signal). In the case of experiment (b), the following four groups of pork skin samples were analyzed: one

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consists of samples of fresh skin, while the other three consist of samples exposed to thermal damage at 45 °C (the exposure time was 4 s) and 80 °C (exposure times were 4 s and 8 s, respectively). The mean values of the RS for each group of samples did not show a clear visual discrimination. However, the *t* test for independent samples applied to the data demonstrated significant differences only between fresh skin and skin exposure to thermal damage at 80 °C (with exposure times of 4 s and 8 s). PCA was used to discriminate between the four different skin samples.

Keywords Burned skin · Multivariate method · Photothermal technique · Radiometry · Skin

1 Introduction

It has been demonstrated that the optical properties of skin may be related to tissue pathologies. Moreover, noninvasive characterization of tissue pathologies by optical techniques is a challenging problem due to the structural heterogeneity of biological tissues which often consist of multiple layers with different optical and thermal properties [1]. In the special case of thermal injury of human skin that allows for infection, which is the most common cause of morbidity and mortality in victims, a noninvasive method of assessing burn depth would be beneficial to both the patient and surgeon [2–7].

The choice of a proper technique for the study of skin depends on the problem under investigation and also on the specific response of tissue to laser exposure (photochemical, photomechanical, or photothermal). In the case of a photothermal response, the relaxation of optical excitation results in inhomogeneous heat generation and its propagation by diverse mechanisms such as heat diffusion, IR radiation, perfusion, and blood flow.

For the case of inorganic materials such as metals, semiconductors, and polymers, the principal mechanism of heat transport is diffusion, which makes the study of heat transport straightforward. However, for living systems this study is a big challenge [8]. Photoacoustic (PA) and photothermal techniques (PT) have been used for many years in nondestructive evaluation of different materials. Among the PT, the photothermal radiometry technique (PRT) is often selected because it is a nondestructive and noncontact technique [9]. In this technique, laser absorption on the surface sample can be monitored remotely using an infrared (IR) sensor. Recently, the PRT has been used to study the skin under various circumstances, for example, (a) in skin pathologies [10], (b) in the study of over-heating damage in laser surgery [11], (c) in the case of topical skin penetration, and (d) in skin hydration [12]. The PRT can be used in either the frequency (sinusoidal modulation excitation) or the time domain (pulsed excitation). The first one involves narrow-band measurements of the amplitude and phase signal at the same frequency. The second is a wide-band measurement of the pulsed response signal [13].

The photothermal radiometry technique in the time domain (PRTT) has been applied to the evaluation of the thickness of surface coatings in industrial components, identification of sub-surface features, such as micro-cracks in aircraft structures, the

determination of the optical absorption coefficient in human arteries, and the characterization of vascular lesions and monitoring of laser damage during surgery [10–12].

The photothermal radiometry technique in the frequency domain (PRTF) is used to characterize the surface and sub-surface of opaque homogeneous systems. One of the principal advantages of PRTF is for imaging sub-surface details of the sample due to the transport of coherent thermal waves [8–13]. A theoretical analysis of the PRTF or PRTT is well understood for the case of homogeneous inorganic materials. However, for living systems the interpretations of the photothermal radiometry (PR) signal are not well understood. A theoretical analysis of heat transport in this type of system is a complex problem.

On the other hand, multivariate methods (MM) have been used to analyze multiparametric systems [14–17]. These statistical methods can extract qualitative and quantitative information of these systems with multi-factorial information such as the case for biological systems.

In this work, we report the results of the study of gel phantoms, which were pigmented with methylene blue to obtain small variations in the optical and thermal properties, which simulate skin abnormalities. The study was focused on the analysis of the amplitude and phase signal of the PRTF by principal component analysis (PCA) in order to identify each pigmented phantom. Also, using the same methodology, the radiometry signal (RS) of healthy and burned samples of skin extracted from pig were studied to determine which signal corresponded to the respective group of samples.

2 Multivariate Method

Multivariate methods (MM) have been developed to deal with complex information, in which two or more variables have to be analyzed simultaneously. MM are used in different fields from neurosciences to computer graphics. PCA is a classical MM widely used in appearance-based approaches for data reduction and feature extraction. It is generally believed that when it comes to solving problems of pattern classification, PCA can help to discriminate the samples.

2.1 Principal Component Analysis

Principal component analysis (PCA) is a multivariate technique that acts in an unsupervised manner and is used to analyze the inherent structure of the data. PCA reduces the dimensionality of the data set by finding an alternative set of coordinates, the principal components (PCs) [14–17]. Mathematically, PCA is a linear transformation,

$$PC = XW \tag{1}$$

where the rows of the matrix X represent each radiometric signal and the columns of the matrix transformation W are the loading vectors, while the columns of PC represent the new set of variables called "scores." PCs are a linear combination of the original variables, which are orthogonal to each other and designed in such a way that each one successively accounts for the maximum variability of the data set. In other

words, when radiometric data are analyzed, each spectrum contains a large number of variables, in this case, the modulation frequencies. The principal goal of PCA is to obtain information about the structure of the spectroscopic data, looking for differences between samples in such a way that it is possible to divide the data into groups. In fact, when PC scores are plotted, for example, PC1 versus PC2 or any combination of the PCs, they can reveal relationships between samples (grouping). It is important to remember that PCA does not act in a supervised manner, which means that PCA does not know *a priori* the number of kinds (groups) of samples under study. PCA provides insight into how much variance is explained by each PC, and how many PCs should be kept in order to maintain the maximum information from the original data without adding noise to the given information [17].

3 Experimental Setup

A schematic diagram of the experimental setup is shown in Fig. 1. The light excitation consists of a laser beam (488 nm, 100 mW) provided by an argon ion laser (Innova 170C-512); the laser beam was expanded with a negative lens ($f = -20$ cm) to illuminate samples on an area of 1.5 cm^2. The collimated laser beam impinges on the surface of the phantom. The phantom consists of a gel which is pigmented with methylene blue (MB) (Sigma-Aldrich) at different concentrations. The infrared radiation emitted from the sample by modulated heating is collected by two parabolic mirrors (Oriel, Model 45347) and sent to an infrared (IR) detector (Judson HgCdTe, Model PN: 250005-7). Finally, the signal of the IR detector is discriminated according to amplitude and phase by a lock-in amplifier (SR-830 Stanford Research System). The laser beam is modulated using a mechanical chopper. Before preparing each phantom and recording the experimental data, the optical absorption spectrum of methylene blue was obtained using a Perkin–Elmer Uv-Vis Spectrometer λ25. The optical spectrum of MB shows maximum peak absorption at 666.0 nm. This information helped us to verify that the laser with excitation at 488 nm is a good choice to avoid heating damage in the case of phantoms, and also for the case of the skin.

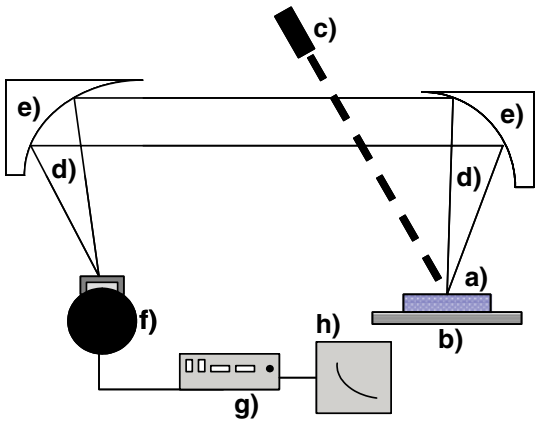

Fig. 1 Photothermal radiometry experimental system: (**a**) sample, (**b**) Peltier cooling system, (**c**) laser, (**d**) IR radiation, (**e**) parabolic mirrors, (**f**) HgCdTe detector, (**g**) lock-in amplifier, and (**h**) computer

4 Sample Preparation

4.1 Agar Phantoms

Each phantom was prepared in the following way: 20 ml of distilled water was heated to its boiling point, after that, 0.78 g of agar (Bioxon, Cat 211900) was added under continuous agitation and different concentrations of MB (Sigma-Aldrich).

4.2 Skin

Fresh skin of pig was obtained from the slaughter; the skin was maintained in refrigeration at 4 °C until the samples were used. Different pieces of skin were selected and cut out, with an area of 9 cm^2. In order to avoid dehydration during the experiment, the sample was placed on the surface of a Peltier cooling system, which maintains the temperature at 4 °C. The Peltier system not only maintains the hydration of the sample but also reduces the heating damage due to laser exposure. Samples were divided into two groups: (a) the first consists only of healthy skin and (b) the second group consists of several samples with different degrees of thermal damage, which was produced when skin is maintained in contact with a hot metal plate at different temperatures and durations of contact.

5 Results and Discussion

5.1 Gel Phantom

The PR signal of pigmented gel phantoms was recorded in terms of the amplitude and phase as a function of the modulation frequency to explore if PRT can be useful to discriminate the pigmented phantoms. For this study, six different groups of phantoms were prepared with different molar concentrations of MB dye, and the following molar concentrations were used (1.60 mM, 3.00 mM, 3.38 mM, 3.55 mM, 6.60 mM, and 9.91 mM).

As can be seen, some of these phantoms simulate very large changes of skin pigmentation, for example, those phantoms at 1.60 mM, 6.60 mM, and 9.91 mM, while samples with 3.00 mM, 3.38 mM, and 3.55 mM simulate skin phantoms with small changes of pigmentation. Figure 2a and b shows the mean values of the phase and amplitude signal for each kind of phantom. The amplitude and phase RS do not need a sophisticated analysis to discriminate the phantoms with very large changes in pigmentation. The discrimination is possible, because the error bars never overlapped among the mean values of the radiometry signal. However, those groups corresponding to samples with concentrations of 3.00 mM, 3.38 mM, and 3.55 mM do not show a clear visual discrimination. In this case, the error bars overlap in all frequency ranges, and it is hard to discriminate the phantoms based on only the mean value analysis. In fact, after we analyzed the data carefully using commercial software (StatSoft, Inc. (2005), Statistica), we found that the data are normally distributed, and then a *t* test for independent samples was used to determine if there are significant differences between

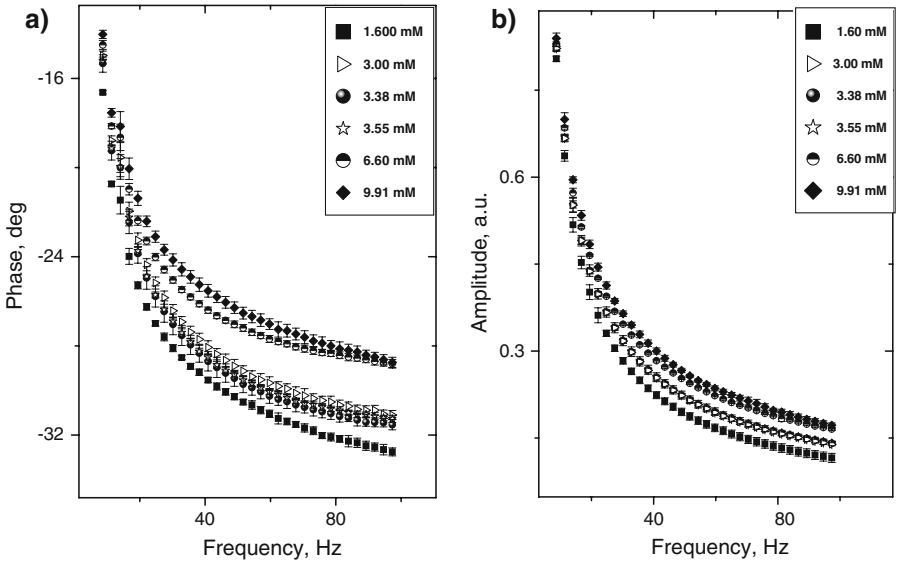

Fig. 2 Each scatter plot represents the average of 20 photothermal radiometric signals: (**a**) phase and (**b**) amplitude

these groups. However, the p values were greater than 0.05 for all frequencies. This result was confirmed for both the amplitude and phase signals, which means that it is not possible to discriminate between the phantoms based only on the simple mean data analysis. This fact makes it impossible to determine the discrimination of the signal and to define differences between phantoms just by a simple visual comparison or just by using the mean values of the RS. For these samples, it was useful to carry out an additional statistical analysis in order to discriminate the phantoms.

So the principal objective of this work was to use PRTF to identify significant differences between gel phantoms with different concentrations of pigmentation. To solve the problem we proposed to analyze the experimental data by using the well known method of principal component analysis (PCA), which is explained elsewhere [10,11]. To carry out PCA, each phantom was measured 12 times at different points. Experimental data were arranged in the form of column vectors to obtain an arrangement of 35 columns and 72 row vectors by group, where each row represents the radiometry signal.

On the other hand, the theory of photothermal radiometry for thermally thick and optically opaque samples predicts that the amplitude signal has information on both the thermal and optical properties of the sample, while the phase signal is independent of the optical properties. But in the case of thermally thick and optically transparent samples, both the amplitude and phase are functions of both thermal and optical parameters. However, the phase signal in both cases is independent of the power excitation. For this reason the amplitude of the signal was normalized before the analysis.

The PCA was carried out on six groups of samples; the results show that only two principal components describe the maximum variability of the data, and the scatter

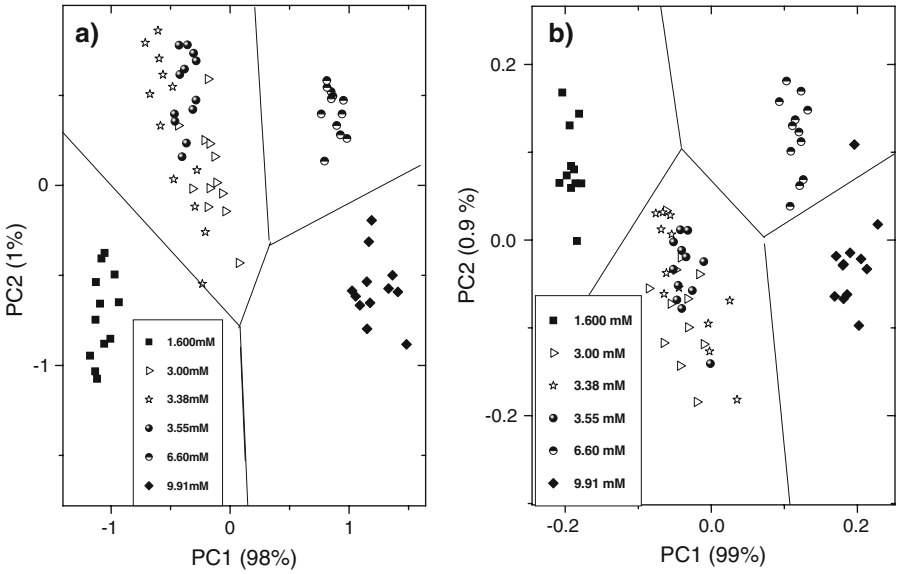

Fig. 3 Scatter plots of PCs show a perfect discrimination for all RS corresponding to those phantoms with a change in concentration equal to or higher than 1.4 mM: (**a**) phase and (**b**) amplitude (continuous lines delimit the regions which show the best discrimination)

plots of PC1 versus PC2 show that it is possible to discriminate the RS of these phantoms only when a change in molar concentration is greater or equal to 0.38 mM. The corresponding results are shown in Fig. 3a and b for the phase and amplitude, respectively. Figure 3a and b show the border decision lines; they are represented by continuous lines; and the intersections of these lines delimit the regions where each group is well discriminated. All these calculations were obtained considering that samples for 3.00 mM, 3.38 mM, and 3.55 mM belong to only one group, and each border decision line was calculated using linear discriminant analysis (LDA) [8,9] on the two principal components.

As can be seen in Fig. 3a and b, these three groups (3.00 mM, 3.38 mM, and 3.55 mM) show a partial discrimination for the case of the phase signal, while Fig. 4 shows the border decision line where phantoms with 3.0 mM of MB were well discriminated from the rest of the samples. This result demonstrates that a change in the concentration of MB of 0.38 mM is the limit to discriminate RS using PCA, as can be seen using the groups of 3.38 mM and 3.55 mM, which were not well discriminated. For the case of the amplitude signal, the border decision lines were not calculated because the RS represented on the space of the PCs is not well discriminated, such as demonstrated by the t test.

5.2 Pork Skin at Different Experimental Conditions

For a more realistic study of the skin with different degrees of pigmentation changes, pork skin was studied in the following conditions: samples of healthy skin were

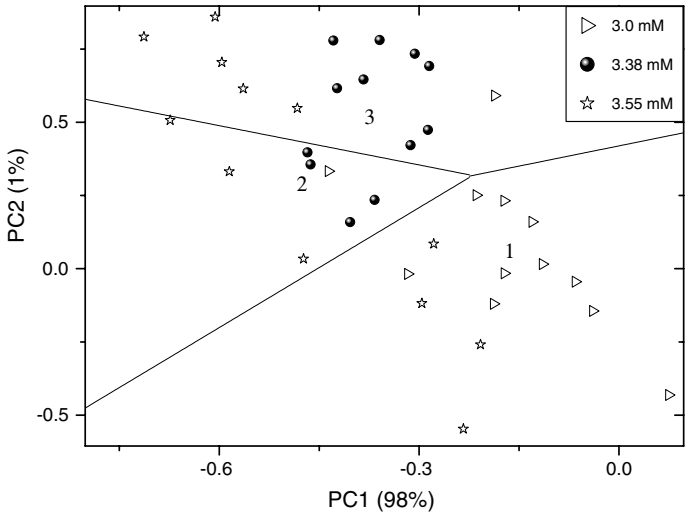

Fig. 4 Scatter plot of PCs shows the border line decisions for those phantoms (phase signal) for which the MB concentration difference is 0.17 mM, 0.38 mM, and 0.55 mM. This plot shows that samples with concentration differences equal to 0.38 mM and 0.55 mM can be discriminated using PCA analysis, but not for the case of 0.17 mM

compared with skin samples which were exposed to different degrees of thermal damage. The thermal damage of the skin samples was produced by exposing the skin to contact with a hot metal plate at different temperatures and using different lengths of exposure. The phase and amplitude of the RS for fresh skin and skin with thermal damage are shown in Fig. 5a and b, respectively.

In both cases, the error bars were overlapping and it was not clear that the capability to distinguish between both kinds of samples existed. Visual analysis cannot offer sufficient information to discriminate the signals. Also, for this case the data were analyzed carefully using the commercial software (StatSoft, Inc. (2005), Statistica). The analysis demonstrated that these data had a normal distribution. So a t test for independent samples was carried out, comparing pairs of groups. Significant differences were observed only for fresh skin and skin with damage at 80 °C at 4 s and 8 s of exposure time, but significant differences were not observed in the other cases. Again, PCA was performed in order to demonstrate if there is discrimination between all skin samples. The results of PCA are shown in Figs. 6 and 7, which suggested that three principal components describe the maximum variability of the data. For this reason, three-dimensional (3D)-scatter plots were considered to show the discrimination. In both cases, for the phase and the amplitude signal, good discrimination was obtained. However, better discrimination was observed for the phase of the signal.

6 Conclusions

In this work, we presented the utility of PRTF and PCA statistical analysis in the study of phantoms with different degrees of pigmentation, and in the study of pork skin

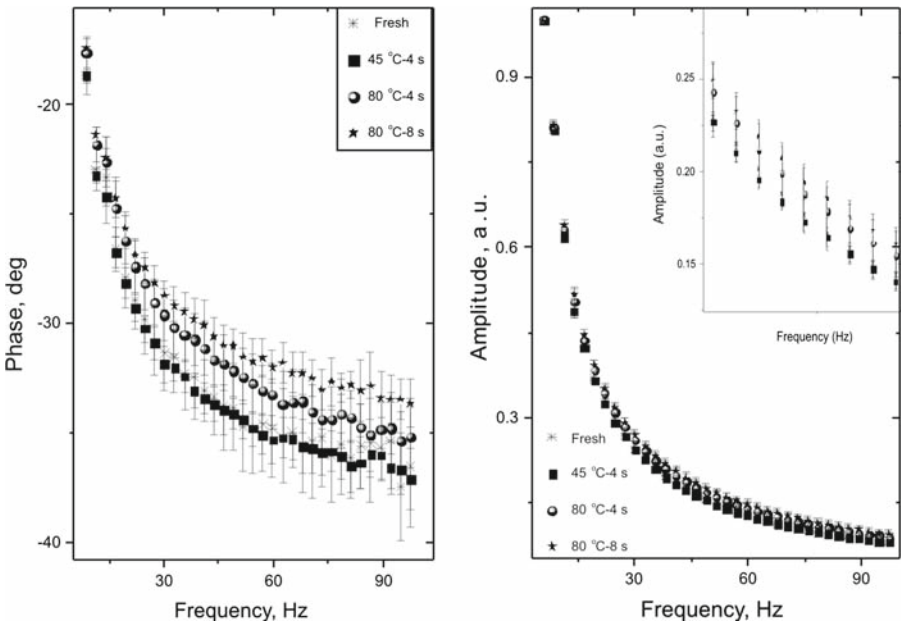

Fig. 5 Each scatter plot represents the mean value of several photothermal radiometry signals; as can be seen, a simple visual analysis of the mean values can not be used to make discriminations: (**a**) phase and (**b**) amplitude

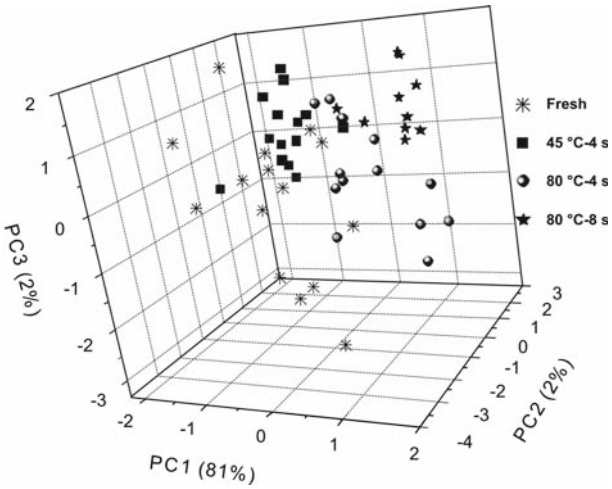

Fig. 6 3D scatter-plot shows the results of the analysis of PCA on the phase signal; three principal components describe the variability and can be used to make a good discrimination of the data

exposed to different degrees of thermal damage. The results demonstrated that the PR signal together with multivariate analysis is a reliable methodology to discriminate changes in pigmentation and also in the degree of skin damage. For the case of a phantom pigmented with MB, we demonstrated that the simple analysis of the mean

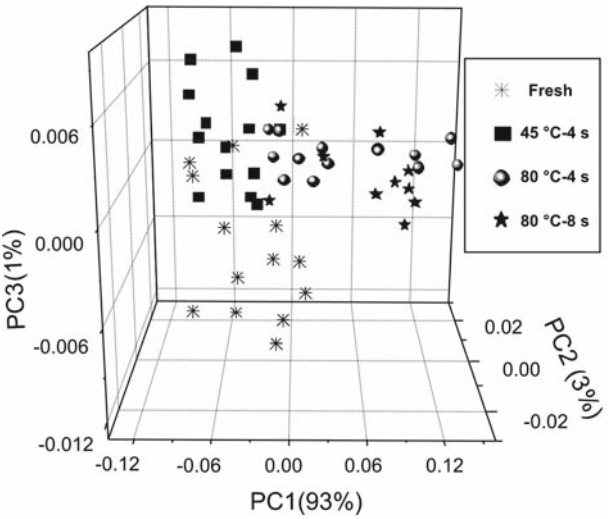

Fig. 7 3D scatter-plot shows the results of the analysis of PCA on the amplitude signal; three principal components describe the variability and can be used to make a good discrimination of the data

values of the RS with a *t* test for independent samples did not give enough information to determine significant differences between phantoms with changes of MB concentrations equal to or less than 0.38 mM. However, PCA can be used to discriminate pigmented gel phantoms with changes in concentration equal to 0.38 mM. Also in Fig. 2, we can see that the phantom's amplitude of the signals with concentrations of 6.60 mM and 9.91 mM (change in concentration equal to 3.31 mM) started to be saturated. This means that probably PCA will not be able to discriminate the phantom's amplitude of the signal when they are prepared with higher concentrations.

In the case of the study of fresh skin and skin exposure to thermal damage at 45 °C, we did not find significant differences comparing the mean values of the RS (*t* test for independent samples), or even when we compared the signals obtained from skin damaged at 80 °C for 4 s and 8 s of heat exposure. However, PCA statistical analysis discriminates all these cases. So, a thermal stimulus produces a gradual change in the skin's optical and thermal properties, but saturation is not observed. The phenomenon of optical saturation will be obtained only when skin can be nearly carbonized.

One of the important results of this work was to demonstrate that the statistical method of PCA offers in some cases better results in the discrimination of RS from pigmented samples and burning skin than the usual statistical methods. PRTF together with MM may be useful in many medical applications; for example, in the discrimination of human skin with secondary lesions which has a higher risk to be transformed to malignancy in the case of discoid lupus, in sun-burned skin, skin damaged for chronic use of corticoids, and other skin problems that predispose to the epidermal cancer; and also to discriminate between different skin malign and non-malign pathologies related with melanin.

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