Melanosome Distribution Patterns Affecting Skin Reflectance: Implications for the In Vivo Estimation of Epidermal Melanin Content

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***Abstract*— Several techniques employed in the *in vivo* estimation of epidermal melanin content rely on the assumption that the effects of different distribution patterns of aggregated melanin (clustered within the melanosomes) on skin spectral responses, particularly across the 600-1350 nm range, can be ignored. Accordingly, for all practical purposes, only the non-aggregated (colloidal) form of melanin is taken into account by these techniques. In this paper, however, we demonstrate through predictive computer simulations that these responses are directly influenced by the occurrence of both forms of melanin. Our *in silico* findings, in turn, indicate that such an assumption may lead to inaccurate estimations of epidermal melanin content.**

***Index Terms*— skin, melanin, melanosome, reflectance, sieve and detour effects, simulation.**

I. Introduction

The correct interpretation of skin spectral responses is essential for a wide range of biomedical applications, from noninvasive health-monitoring programs (*e.g.*, [1], [2]) to the screening (*e.g.*, [3], [4]) and treatment of medical conditions (*e.g.*, [5]). Moreover, its use in inversion procedures aimed at the estimation of skin biophysical parameters, such as the content and distribution patterns of its main light attenuation agents, has a pivotal relevance for medical and cosmetics research. For example, ultraviolet (UV) and visible skin responses can be used in the assessment of the intrinsic photoprotective properties of a skin specimen [6], while infrared (IR) responses can be used in the assessment of skin hydration [7], a key factor contributing to the protective properties of this complex organ. These research efforts, in turn, contribute to the scientific foundation required to increase the efficacy of products (*e.g.*, sunscreens) designed to mitigate the harmful effects of light overexposure such as photoaging [8] and skin cancer [9].

Light impinging on the skin surface can be reflected back to the environment or transmitted into its internal tissues (stratum corneum, epidermis and dermis). Once light is transmitted into the skin tissues, it can be attenuated (absorbed and/or scattered) by their constituent materials. This investigation is centered on one of the main light attenuation agents acting within the cutaneous tissues: melanin. This pigment is synthesized by melanocyte cells in the stratum basale (the innermost epidermal layer), where it is preferentially concentrated [10]. During this process, termed melanogenesis, it is distributed (dispersed) throughout the full thickness of the upper layers as the epidermal cells move upward [11].

The two types of melanin found in human skin tissues, namely the dominant brown-black eumelanin and the yellow-red pheomelanin, may occur in a colloidal form or clustered within the melanosomes [9]. These melanin-containing organelles can be described as particles with the shape of a prolate spheroid [12]. In lightly pigmented specimens, they can occur in groups surrounded by a transparent membrane forming melanosome complexes [12], [13], which are characterized to be approximately spherical in shape [11], [13]. In darkly pigmented specimens, however, melanosomes occur as denser and individually dispersed particles [12], [13].

Although it has been established [9], [14], [15] that the effects of melanin on skin appearance attributes (*e.g.*, color) and UV light attenuation must be related not only to melanin content, but also to where it is found and how it is dispersed within the cutaneous tissues, several techniques employed in *in vivo* estimation of melanin content do not explicitly account for the particle nature and dispersion patterns of the melanosomes. For example, Kolias and Baqer [16] proposed a technique for the estimation of melanin content based on the measurement of skin reflectance values in the 600-720 nm range. It was later modified [17] to account for reflectance values in the 600-850 nm range. This technique, which is often employed in skin pigmentation research (*e.g.*, [18], [19]), explicitly assumes melanin occurring only in colloidal form [16].

There are also inversion procedures [20] in which the interpretation of measured skin reflectance values is based on the analysis of results derived from models of light interactions with cutaneous tissues (*e.g.*, [21], [22]). Although, the models employed in these procedures account for the epidermal melanin content in terms of average epidermal volume occupied by melanosomes, they do not explicitly account for the particle nature and dispersion patterns of the melanosomes either.

In this paper, we investigate the impact of the different forms of melanin and their distribution patterns on human skin reflectance using a novel hyperspectral light transport model, henceforth referred to as HyLIoS (*H*yperspectral *L*ight *I*mpingement on *S*kin) [23]. In the following section, we concisely describe the *in silico* framework used in our investigation, and in Section III we present our findings and discuss their practical implications. Finally, in Section IV, we outline directions for future research.

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TABLE I

HyLIoS parameters employed to characterize a lightly (LP) and a darkly (DP) pigmented skin specimen. The acronyms SC, SG, SS, SB, PD and RD refer to the skin layers considered by HyLIoS, namely, stratum corneum, stratum granulosum, stratum spinosum, stratum basale, papillary dermis and reticular dermis, respectively. Also, the abbreviations Eu. and Pheo. refer to eumelanin and pheomelanin, respectively.

Parameter	LP	DP
Surface Fold Aspect Ratio	0.1	0.45
SC Thickness (cm)	0.0004	0.0002
SG Thickness (cm)	0.0033	0.0007
SS Thickness (cm)	0.0033	0.0007
SB Thickness (cm)	0.0033	0.0007
PD Thickness (cm)	0.02	0.023
RD Thickness (cm)	0.125	0.2
SG Melanosome Content (%)	0.0	0.0
SS Melanosome Content (%)	0.0	0.0
SB Melanosome Content (%)	3.0	30.0
SG Colloidal Melanin Content (%)	1.35	15.0
SS Colloidal Melanin Content (%)	1.35	15.0
SB Colloidal Melanin Content (%)	1.35	15.0
Melanosome Eu. Concentration (mg/mL)	32.0	50.0
Melanosome Pheo. Concentration (mg/mL)	2.0	4.0
PD Blood Content (%)	0.3	2.5
RD Blood Content (%)	0.3	2.5

II. Investigation Framework

HyLIoS employs a first principles modeling approach that explicitly accounts for the particle nature and the different dispersion patterns of the melanosomes within the epidermal layers (stratum granulosum, spinosum and basale). Hence, it enables the computation of skin reflectance considering melanin occuring in colloidal form (colloidal case) as well as clustered within melanosomes, which may be located in the stratum basale (baseline case) or dispersed throughout the epidermal layers (dispersion case).

Using HyLIoS, we generated directional-hemispherical reflectance curves for skin specimens with different levels of pigmentation (light and dark) considering an angle of incidence of 10° and using the subset of model parameters provided in Table I. Furthermore, during the computation of these curves, which correspond to the baseline case of our investigation, the melanosomes were distributed as complexes in the lightly pigmented specimen, and as individually dispersed particles in the darkly pigmented specimen as indicated in the related literature [12], [13].

Regarding the dispersion case, the melanosomes were considered dispersed in equal percentages in the stratum spinosum, granulosum and basale. In order to account for melanosome degradation in the upper epidermal layers [15], the axes of the melanosomes located in the stratum spinosum and stratum granulosum were set to be, respectively, 50% and 25% of the values (in $\mu m \times \mu m$) provided by Olson *et al.* [12], namely 0.40×0.17 and 0.69×0.28 for lightly and darkly pigmented specimens, respectively.

We then compared the baseline and dispersion (case) reflectance curves with curves obtained considering the occurrence of melanin only in colloidal form (colloidal case). These were computed with melanosome percentages set to zero and their corresponding melanin content values transferred to the colloidal melanin percentages in the respective epidermal layers depicted in Table I.

In order to enable the full reproduction of our investigation results, we made HyLIoS available online [24] through a model distribution system, known as Natural Phenomena Simulation Group Distributed (NPSGD) [25], along with the complete set of model parameters, which includes those that have been assigned the same value for both specimens (*e.g.*, water content and the oxygenated blood fraction). Although the reflectance curves provided by HyLIoS cover the 250-2500 nm range, our analysis is focused on the 600-1300 nm region often employed in melanin content estimations (*e.g.*, [16], [17], [22], [19]).

For completeness, we also provide root mean square error (RMSE) values computed for the reflectance curves associated with the baseline and dispersion cases with respect to reflectance curves associated with the colloidal case considering the entire 250-1400 nm region notably affected by melanin [14]. These values were computed using the following expression:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\rho_c(\lambda_i) - \rho_a(\lambda_i))^2}, \qquad (1)$$

where ρ_c and ρ_a correspond to the reflectances associated with the colloidal and aggregated melanin cases, respectively, and N is the total number of wavelengths sampled with a 5 nm resolution within a selected spectral region.

III. Results and Discussion

As it can be observed in the comparisons between the baseline and the colloidal cases depicted in Figure 1, reflectance values may be underestimated when one considers the occurrence of melanin only in the colloidal form, particularly in the 600-900 nm region. Although the same trend applies to both specimens, the RMSE values presented in Figure 2 indicate a larger impact for the lightly pigmented specimen in the visible region, and a larger impact for the darkly pigmented specimen in the near-infrared (NIR) region.

The comparisons between the dispersion and the baseline cases depicted in Figure 3 show a similar trend for the lightly pigmented specimen. For the darkly pigmented specimen, however, the reflectance values are slightly overestimated in the 600-900 nm region when one considers the occurrence of melanin only in colloidal form. Accordingly, the RMSE values presented in Figure 4 indicate a larger impact for the lightly pigmented specimen across the entire UV-NIR region of interest.

The reflectance curves for the darkly pigmented specimen presented in Figures 1 and 3 also show lower reflectance values for the dispersion case in comparison with the baseline case. Such reflectance decrease is observed when an individual's facultative pigmentation (determined by environmental stimuli [14]) is enhanced by UV light exposure. It

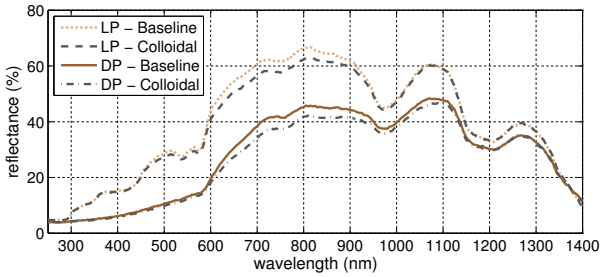

Fig. 1. Reflectance curves for a lightly (LP) and a darkly (DP) pigmented specimen considering the baseline and the colloidal case.

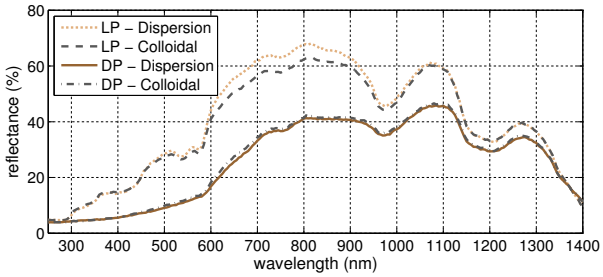

Fig. 3. Reflectance curves for a lightly (LP) and a darkly (DP) pigmented specimen considering the dispersion and the colloidal case.

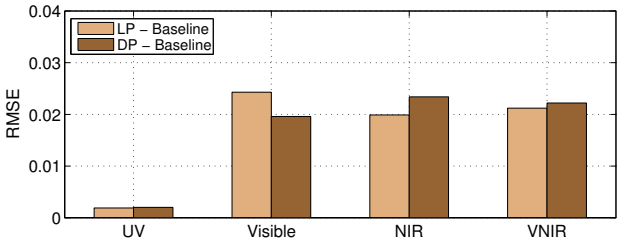

Fig. 2. RMSE values for the baseline (case) reflectance curves of a lightly (LP) and a darkly (DP) pigmented specimen with respect to their corresponding colloidal (case) reflectance curves. Selected spectral ranges: UV (250-400 nm), Visible (400-700 nm), NIR (700-1400 nm) and VNIR (400-1400 nm).

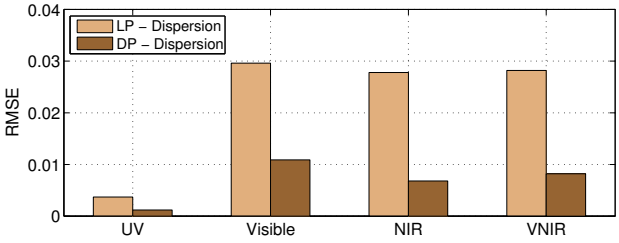

Fig. 4. RMSE values for the dispersion (case) reflectance curves of a lightly (LP) and a darkly (DP) pigmented specimen with respect to their corresponding colloidal (case) reflectance curves. Selected spectral ranges: UV (250-400 nm), Visible (400-700 nm), NIR (700-1400 nm) and VNIR (400-1400 nm).

has been stated that this reflectance decrease (resulting in an increasingly darker skin tone in the visible domain) is primarily caused by an increase in melanosome dispersion (accompanied by a degradation into smaller particles) toward the surface of the skin [26]. Moreover, such reflectance variations are more noticeable in individuals characterized by a higher level of constitutive pigmentation (genetically determined, uninfluenced by UV light exposure [14]). As further illustrated by the plot presented in Figure 5, this reflectance decrease cannot be predictively reproduced when one considers the occurrence of melanin only in colloidal form.

The shortcomings of overlooking the particle nature and distribution patterns of the melanonomes can be associated with changes in cutaneous light absorption profiles elicited by detour and sieve effects. When light traverses a turbid medium, refractive index differences between pigment-containing structures and the surrounding medium may cause multiple interactions that increase the light optical pathlength, a phenomenon known as detour effect [27]. Conversely, the traversing light may undergo a sieve effect, *i.e.*, it may not encounter a pigment-containing structure [28]. The net result of these effects depends on the absorption spectra of the pigments as well as on the distribution and volume fraction of these structures [27], [28]. In the case of human skin, these structures are represented by melanosomes and melanosome complexes [12], and the pigment of interest, melanin, may also be present in the surrounding medium in colloidal form [9], further influencing the net result of detour and sieve effects. Hence, by considering the occurrence of melanin only in colloidal form and ignoring detour and sieve effects associated with the particle nature and distribution patterns of these melanin-containing organelles, one cannot establish a correct correlation between reflectance values and epidermal melanin content.

In order to to take into account sieve and detour effects, one may consider adjusting the *in vitro* absorption spectra of the pigments of interest (eumelanin and pheomelanin) according to the lengthening of the optical path verified in the skin tissues [20], referred as differential pathlength in biomedical investigations [29]. In plant sciences, a similar adjustment is performed using a quantity called ratio of intensification [27] or factor of intensification [30]. The main difficulty in employing this correction factor is the scarcity of available measured data to allow its accurate quantification for different *in vivo* conditions.

Although light transport algorithms based on first principle approaches also depend on data availability, notably with respect to specimen characterization data, such a dependence is less restrictive than the dependence associated with the measurement of differential pathlength. We remark that the latter involves a larger number of unknowns, including not only specimen characterization data, but also different illumination geometries as well as spectral and temporal dimensions associated with the actual measurement conditions. Such conditions, on the other hand, can be reproduced through computational experiments in a straightforward manner. Hence, we believe that, by explicitly accounting for the particle nature and distribution patterns of the melanosomes, predictive simulation framework supported by first principles

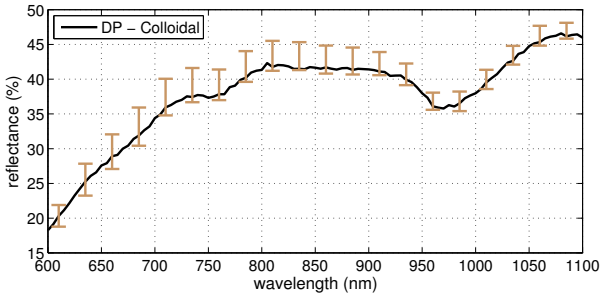

Fig. 5. Variations (bars) in the reflectance values of a darkly pigmented specimen considering melanosomes located in the stratum basale and dispersed throughout the epidermis. The corresponding colloidal (case) reflectance curve is included for comparison.

light interaction models, such as HyLIoS [23], can provide effective contributions for the enhancement of melanin content estimations based on reflectance inversion procedures.

IV. Conclusion

Melanin has been object of extensive studies across different disciplines not only due its impact on skin appearance, but more importantly its central role on the photoprotection of this complex organ. Its *in vivo* identification (different forms and types) and quantification remains an elusive task, however. Although recent advances in the capture of hyperspectral skin reflectance data offer a myriad of opportunities for improving the accuracy of these procedures, we believe that the scientific community has to take a synergistic and comprehensive view of the biological and optical processes taking place within the cutaneous tissues, without overlooking important phenomena affecting their interactions with light, in order to achieve a tangible progress in this area. Such an approach will be essential for enhancing the qualitative and quantitative predictions regarding the occurrence and photobiological functions performed by this fundamental material.

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