Spectral Differentiation of β-Carotene Accumulation Patterns in Skin Tissues with Distinct Levels of Constitutive Pigmentation

Gladimir V. G. Baranoski[1]

***Abstract*— The accumulation of β-carotene in human skin has been connected to various forms of protection offered by this ubiquitous carotenoid, from the absorption of ultraviolet radiation to the neutralization of reactive oxygen species. These protective mechanisms, in turn, are likely to be associated with the accumulation pathways of this pigment, via the top (epidermal) and/or the bottom (dermal) cutaneous tissues. The differentiation of the distinct accumulation patterns of β-carotene through noninvasive methodologies is still an open problem, however, notably for skin specimens with relatively high levels of constitutive pigmentation. In this paper, we address this open problem by proposing and evaluating three spectral tests based on carotenemia-elicited variations in skin reflectance within the red end of the visible spectrum. Their efficacy is examined using a first-principles *in silico* experimental framework grounded on measured data for different skin specimens. The outcomes of our investigation indicate that the use of such a spectral testing approach can lead to reliable and cost-effective differentiation assessments of distinct β-carotene accumulation patterns in skin tissues.**

***Index Terms*— skin, pigmentation, β-carotene, carotenemia, spectral reflectance, predictive simulations.**

I. Introduction

The intake of carotenoids, through diet or supplements, has important implications for human health and appearance. The presence of these pigments in the cutaneous tissues can contribute to the prevention of a myriad of detrimental processes, from photoaging to photocarcinogenesis [1], [2]. They also contribute to the elasticity [2] and color [3] of human skin.

When ingested in large amounts, high concentrations of these pigments can be accumulated within the cutaneous tissues and the blood stream, a condition known a carotenemia [4]. This condition can alter skin chromatic attributes, notably in lightly pigmented individuals. The relative low amounts of eumelanin and pheomelanin, in both colloidal and granular (melanosome encapsulated) forms, present in their skin enables more visible light to be absorbed by the carotenoids, eliciting a yellowish tint [3], [5].

Two main pathways for the accumulation of carotenoids within the cutaneous tissues have been discussed in the related literature. The first corresponds to the diffusion from adipose tissue, blood and lymph nodes [1], [2], [6]. The second corresponds to the secretion via sweat glands and/or sebaceous glands onto the skin surface, and their subsequent penetration into the cutaneous tissues [1]. The first pathway is more likely to lead to a predominant accumulation of carotenoids in the dermal tissues and their network of blood vessels. The second pathway, on the other hand, is more likely to lead to a predominant accumulation of carotenoids in the stratum corneum and epidermal tissues [1], [3], [7]. These distinct accumulation patterns, in turn, are associated with the carotenoids' different protective roles.

The carotenoids are also absorbers of ultraviolet (UV) radiation [3], [6]. Accordingly, they can contribute to the mitigation of UV-induced harmful effects such as erythema and DNA damage [2], [8], particularly in skin sites more likely to be exposed to UV radiation such as those found in the face, dorsum of the hands, arms and legs. Moreover, this contribution tends to be more significant when these pigments are accumulated in the top skin layers (stratum corneum and epidermal tissues) since UV radiation has a relatively low penetration depth in human skin [9], [10]. The carotenoids have also anti-oxidant properties that can contribute to the neutralization of reactive oxygen species (ROS), whose elevated concentration in a given tissue may lead to cell damage and cancer [1], [11]. This contribution can be more effective when the carotenoids are being accumulated in the bottom skin layers (dermal tissues) and dispersed through their network of blood vessels, facilitating the scavenging of ROS [2].

The β-carotene pigment is among the most abundant carotenoids found in human tissues. It is also a provitamin A, a precursor of immunomodulatory vitamin A. Although it is a widely investigated carotenoid [3], [6], [7], [12], [13], a comprehensive understanding about its accumulation patterns within the cutaneous tissues is still lacking. Given their distinct connections with the protective roles of β-carotene, the reliable differentiation of these patterns, particularly through noninvasive methodologies posing minimum risks to human health, would not only be instrumental to reduce the current knowledge gap, but could also contribute to the development of new methodologies to enhance the different types of protection offered by this lipophilic pigment.

One of the main difficulties in this area is associated with the presence of the melanins and hemoglobins in the cutaneous tissues. The strong absorptive capabilities of these pigments can mask the impact that β-carotene may have on the spectral signatures of skin specimens. This aspect, in turn, makes the noninvasive assessment of β-carotene accumulation patterns challenging, notably in specimens with relative high level of constitutive pigmentation (determined by genetic factors instead of environmental stimuli such as

*This work was supported in part by the Natural Sciences and Research Council of Canada (NSERC) under Grant 238337.

[1] Gladimir V. G. Baranoski is with the Natural Phenomena Simulation Group, School of Computer Science, University of Waterloo, 200 University Avenue, Waterloo, Ontario, N2L 3G1, Canada. gvgbaran@uwaterloo.ca

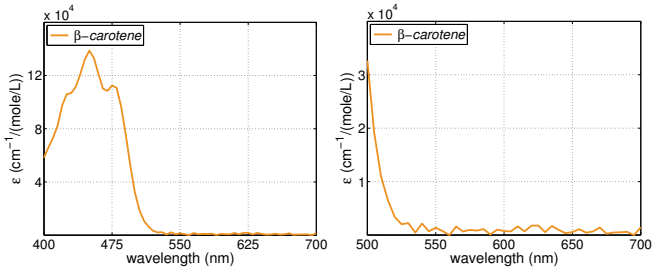

Fig. 1: Molar extinction coefficient of β-carotene [15], [16]. (a) Visible domain. (b) Zoom-in of the green and red regions.

those responsible for tanning [14]).

In our previous work in this area [5], we conducted computational (*in silico)* experiments whose outcomes pointed out to a possible viable alternative for the noninvasive assessment of β-carotene accumulation patterns. In short, they indicated that the impact of this absorber on the spectral responses of human skin tend to be more noticeable in the red region (600-700 nm) of the visible spectrum where the masking effects of the melanins and hemoglobins are lower. Although the absorption spectrum of β-carotene is also lower in this region (Fig. 1), it can still be sufficient to bring forward spectral features that can be employed in the noninvasive differentiation of its accumulation patterns, notably for individuals with high levels of constitutive pigmentation.

In this paper, we assess this hypothesis from a practical perspective. More precisely, we propose spectral tests for the differentiation of the distinct β-carotene accumulation patterns within the cutaneous tissues. The proposed tests are based on carotenemia-induced reflectance variations within the target region (600-700 nm). We evaluate their efficacy through controlled *in silico* experiments supported by measured data obtained from skin specimens with markedly distinct levels of constitutive pigmentation.

II. Materials and Methods

As mentioned earlier, our investigation builds on the research described in our previous work in this area [5]. For completeness and reproducibility purposes, we concisely provide below the data employed by both studies. Also, for brevity, we will use the following acronyms while referring to the skin tissues considered in this investigation, namely SC (stratum corneum), SG (stratum granulosum), SS (stratum spinosum), SB (stratum basale), PD (papillary dermis) and RD (reticular dermis).

The parameter values used in the characterization of the three distinct representative specimens, lightly pigmented (LP), moderately pigmented (MP) and darkly pigmented (DP), considered in our *in silico* experiments are provided in Tables I and II. We remark that the selection of these values was based on biophysically valid ranges provided in the literature, whose sources are listed elsewhere [17], [18] for conciseness, and used reflectance curves measured from real specimens [19], [20] as references. The reader interested in comparisons of modeled and measured reflectance curves for an assessment of the fidelity of our simulations is referred to our previous work in this area [5].

TABLE I: Parameters employed in characterization of the selected LP, MP and DP skin specimens.

Parameter	LP	MP	DP
Ratio of Skin Surface Folds	0.10	0.25	0.45
SC Thickness (cm)	0.0010	0.0010	0.0002
SG Thickness (cm)	0.0011	0.0033	0.0009
SS Thickness (cm)	0.0011	0.0033	0.0009
SB Thickness (cm)	0.0011	0.0033	0.0009
PD Thickness (cm)	0.04	0.01	0.03
RD Thickness (cm)	0.1	0.1	0.2
SG Melanosome Cont. (%)	0.75	0.0	7.5
SS Melanosome Cont. (%)	0.75	0.22	7.5
SB Melanosome Cont. (%)	0.75	3.75	7.5
SG Colloidal Melanin Cont. (%)	3.75	1.25	12.5
SS Colloidal Melanin Cont. (%)	3.75	1.25	12.5
SB Colloidal Melanin Cont. (%)	3.75	1.25	12.5
Melanosome Dim. ($\mu m \times \mu m$)	0.36×0.14	0.41×0.17	0.69×0.28
Eumelanin Conc. (g/L)	50.0	50.0	50.0
Pheomelanin Conc. (g/L)	4.0	2.0	1.0
PD Blood Cont. (%)	0.18	1.2	2.5
RD Blood Cont. (%)	0.18	0.8	2.5
Oxy. Hemoglobin Frac. (%)	85.0	90.0	85.0
Hemoglobin Conc. (g/L)	140.0	130.0	147.0
Radius of PD Scatterers (nm)	100.0	80.0	100.0

TABLE II: Parameters kept fixed during the simulations.

Parameter	Value
SC Melanosome Cont. (%)	0.0
SG Colloidal Melanin Cont. (%)	0.0
Melanosome Complex Diam./Axis	2.0
Melanin Refractive Index	1.7
Methemoglobin Conc. in Blood (g/L)	1.5
Carboxyhemoglobin Conc. in Blood (g/L)	1.5
Sulfhemoglobin Conc. in Blood (g/L)	0.0
Bilirubin Conc. in Blood (g/L)	0.003
SC Water Cont. (%)	35.0
Epidermis Water Cont. (%)	90.0
PD Water Cont. (%)	65.0
RD Water Cont. (%)	75.0
SC Lipid Cont. (%)	10.0
Epidermis Lipid Cont. (%)	1.0
PD Lipid Cont. (%)	5.0
RD Lipid Cont. (%)	5.0
SC Keratin Cont. (%)	55.0
SC Urocanic Acid Density (mol/L)	0.01
Skin DNA Density (g/L)	0.185
Melanin Refractive Index	1.7
SC Refractive Index	1.55
Epidermis Refractive Index	1.4
PD Refractive Index	1.39
RD Refractive Index	1.41
PD Scatterers Refractive Index	1.5
PD Fraction Occupied by Scatterers (%)	22.0

In this investigation, the main pathways for the accumulation of β-carotene in human skin are associated with distinct groups of cutaneous tissues: *epidermal* (comprising SC as well as SG, SS and SB), *dermal* (comprising PD, RD as well as their blood vessels) and *all* (comprising all the aforementioned tissues). The anomalous β-carotene concentrations considered in our simulations were classified as mild and accentuated. Their respective values are depicted in Table III. We remark that these values are also based on biophysically valid ranges reported in the literature [4], [21].

TABLE III: Normal and anomalous (mild and accentuated) concentrations (in g/L) of β-carotene considered in this work. For the mild and accentuated cases, we account for the anomalous presence of β-carotene in the *epidermal* (E), *dermal* (D) and in *all* (A) tissues.

		Mild			Accentuated		
Tissue	Normal	E	D	A	E	D	A
SC	2.1E-4	0.5E-2	2.1E-4	0.5E-2	1.5E-2	2.1E-4	1.5E-2
SG	2.1E-4	0.5E-2	2.1E-4	0.5E-2	1.5E-2	2.1E-4	1.5E-2
SS	2.1E-4	0.5E-2	2.1E-4	0.5E-2	1.5E-2	2.1E-4	1.5E-2
SB	2.1E-4	0.5E-2	2.1E-4	0.5E-2	1.5E-2	2.1E-4	1.5E-2
PD	7.5E-5	7.5E-5	0.5E-2	0.5E-2	7.5E-5	1.5E-2	1.5E-2
RD	7.5E-5	7.5E-5	0.5E-2	0.5E-2	7.5E-5	1.5E-2	1.5E-2
Blood	7.5E-5	7.5E-5	0.5E-2	0.5E-2	7.5E-5	1.5E-2	1.5E-2

The proposed spectral differentiation tests employ directional-hemispherical reflectance values obtained at selected wavelengths. In our simulations, these values are computed for each selected specimen using the first-principles model of light and skin interactions known as HyLIoS (*H*yperspectral *L*ight *I*mpingement on *S*kin) [17], which has been effectively employed in a wide range of biomedical investigations (*e.g.*, [14], [22], [23], [24]), and a virtual spectrophotometer [25].

Within the HyLIoS' ray-optics formulation, a (light) ray interacting with a given skin tissue can be associated with any wavelength (λ) within a spectral domain of interest. Accordingly, the modeled reflectance values, denoted by $\rho(\lambda)$, were obtained considering an angle of incidence of 0° and a spectral resolution of 5 nm, with 10^6 incident rays per sampled λ. This number of rays (cast by the virtual spectrophotometer) was selected to ensure asymptotically convergent reflectance values with a confidence of 0.1% [26].

To further facilitate the reproduction of the outcomes of this work, we have made HyLIoS accessible for online use [27]. Also, all supporting datasets (*e.g.,* molar extinction coefficient of β-carotene (Fig. 1)) employed in this work are openly available in a dedicated data repository [28].

We remark that proposed spectral differentiation tests are based on spectral features that become more prominent in the 600-700 nm region of visible domain, less subject to masking effects by the melanins and hemoglobins, as larger contents of β-carotene are accumulated in the dermal tissues. More specifically, the reflectance curves start to depict noticeable spectral variations associated with the absorption spectrum of β-carotene in this region (Fig. 1(b)). The proposed tests aim to detect such variations in order to identify the distinct β-carotene accumulation patterns.

More precisely, each proposed test is based on a specific condition. If this condition is satisfied, then the accumulation pattern is predominantly *dermal*. Otherwise, it is either predominantly *epidermal* or the concentration of β-carotene in the cutaneous tissues is within *normal* limits. The conditions employed by each test are presented below:

- T1: $\rho(670) < (\rho(645) + \rho(695))/2$,
- T2: $\rho(700) < \rho(695)$, and

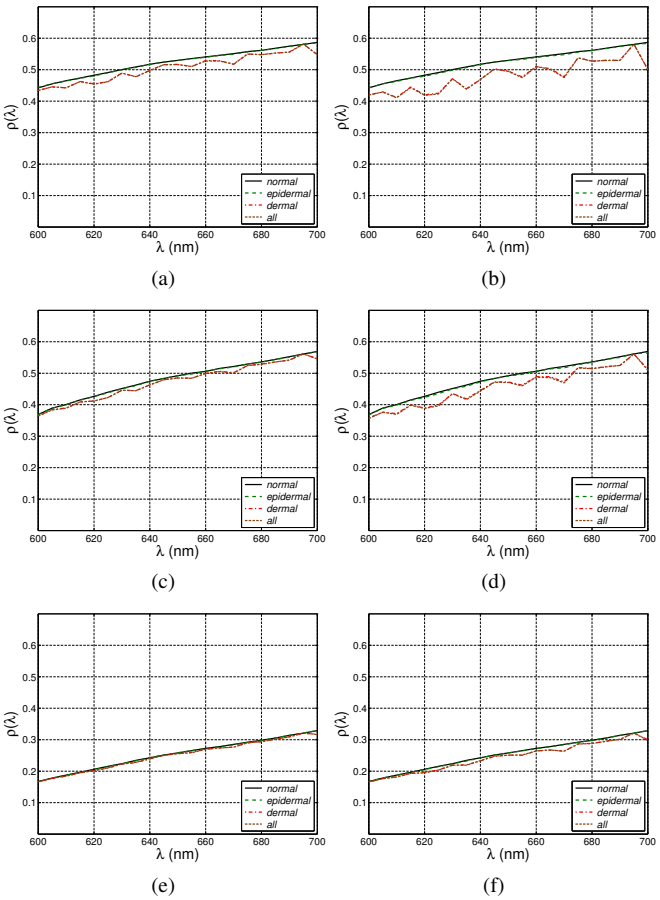

Fig. 2: Effects of mild (left column) and accentuated (right column) β-carotene accumulations in the affected tissues (as indicated in Table III) on the reflectance ($\rho(\lambda)$) of the LP (top row), MP (middle row) and DP (bottom row) specimens within the 600-700 nm spectral region. Each depicted curve is associated with a group of affected tissues: *epidermal*, *dermal* and *all* tissues. For comparison purposes, the control curves obtained considering *normal* amounts of β-carotene (Table III) are also provided.

- T3: $\rho(670) < \rho^*(670)$, where the reference $\rho^*(670)$ is calculated using Lagrangian interpolation [29]:

$$\rho^*(670) = 0.2631\ \rho(600) + 0.7368\ \rho(695). \quad (1)$$

Lastly, we note that the expressions for tests T1 and T2 originally suggested in our previous work [5] presented typos that were detected after its final submission to publication. Those typos have been corrected in this work.

III. Results and Discussion

In Fig. 2, we present the graphs depicting the reflectance curves (in the 600-700 nm region) obtained for the selected specimens considering the distinct β-carotene concentrations and accumulation patterns. As it can be observed in these graphs, higher β-carotene contents lead to larger variations in the reflectance curves. Conversely, higher levels of constitutive pigmentation, lead to smaller variations. These variations are more prominently associated with specific

TABLE IV: Reflectance ($\rho(\lambda)$) values employed by the proposed tests for the differentiation of the distinct accumulation patterns of normal and anomalous (mild and accentuated) concentrations of β-carotene in the LP, MP and DP specimens. The letters E, D, and A refer to the anomalous presence of β-carotene only in the *epidermal* tissues, only in the *dermal* tissues and in *all* tissues, respectively.

	LP	Mild			Accentuated		
$\lambda(nm)$	Normal	E	D	A	E	D	A
600	0.442	0.444	0.434	0.434	0.442	0.420	0.420
645	0.524	0.524	0.516	0.516	0.525	0.503	0.502
670	0.551	0.549	0.517	0.518	0.548	0.478	0.475
695	0.580	0.581	0.582	0.582	0.581	0.581	0.581
700	0.587	0.585	0.548	0.548	0.583	0.501	0.500
	MP	Mild			Accentuated		
$\lambda(nm)$	Normal	E	D	A	E	D	A
600	0.369	0.369	0.364	0.365	0.368	0.358	0.356
645	0.483	0.484	0.479	0.479	0.483	0.473	0.471
670	0.522	0.520	0.502	0.500	0.517	0.472	0.475
695	0.561	0.561	0.562	0.561	0.562	0.561	0.562
700	0.569	0.568	0.546	0.546	0.565	0.513	0.509
	DP	Mild			Accentuated		
$\lambda(nm)$	Normal	E	D	A	E	D	A
600	0.167	0.167	0.166	0.167	0.167	0.165	0.166
645	0.251	0.251	0.251	0.248	0.252	0.247	0.248
670	0.285	0.284	0.276	0.276	0.284	0.264	0.264
695	0.321	0.321	0.320	0.321	0.321	0.322	0.321
700	0.329	0.329	0.318	0.317	0.329	0.300	0.298

spectral features considered by the proposed tests, namely the reflectance drops at 670 nm and 700 nm elicited by a predominantly dermal accumulation pattern.

In Table IV, we list the reflectance values obtained throughout our simulations and employed by the proposed tests T1, T2 and T3. Similarly, in Table V, we list the reference reflectance values calculated using Eq. 1 and employed by test T3. These steps are aimed to enable the independent verification of our evaluation of the proposed tests.

It is worth noting that reflectance values measured using real spectrophotometers may be subjected to uncertainties, *i.e.*, small, random differences among readings. These differences may result from a combination of many small fluctuations due to independent variations of different components of the instrument, different factors in the environment and how the specimen is handled [26]. Measurements obtained with a high precision device will have an uncertainty of ± 0.001 or 0.1% [30], [31]. A low precision device may lead to an uncertainty close to 1% [30], [32]. In order to implicitly account for these random differences, all reflectance values obtained in our simulations and used in the proposed tests were rounded to three decimal places as depicted in Tables IV and V.

In Table VI, we present the outcomes of our evaluation of the proposed tests for the differentiation of the distinct β-carotene accumulation patterns in the selected specimens. More specifically, test T1 failed in 9 cases, while tests T2 and T3 passed by a small margin (relative difference between the compared values $<$ 1%) in four and two cases, respectively. Also, for the cases considered in this investigation, test T3 demonstrated slightly more reliability than test T2, particularly for specimens with higher levels of skin pigmentation, which can make the detection of spectral signature changes more difficult. On the other hand, we note that test T3 is more costly than test T2 since the former requires three reflectance values, instead of two required by the latter, as well as the calculation of a reference value.

As previously discussed [5], the masking effects of relatively large melanin and hemoglobin contents present in darkly pigmented specimens preclude the reliable detection of anomalous β-carotene contents, let alone the differentiation of its distinct accumulation patterns, using visual or chromatic methodologies. Although our findings, as with any *in silico* study, are still subject to *in vivo* confirmation, they

TABLE V: Reference reflectance ($\rho^*(\lambda)$) values calculated using Eq. 1 and employed in the proposed test T3 for the differentiation of the distinct accumulation patterns of normal and anomalous (mild and accentuated) concentrations of β-carotene in the LP, MP and DP specimens. The letters E, D, and A refer to the anomalous presence of β-carotene only in the *epidermal* tissues, only in the *dermal* tissues and in *all* tissues, respectively.

		Mild			Accentuated		
	Normal	E	D	A	E	D	A
LP	0.544	0.545	0.543	0.530	0.544	0.539	0.539
MP	0.510	0.510	0.510	0.509	0.511	0.508	0.508
DP	0.281	0.281	0.280	0.281	0.281	0.281	0.280

TABLE VI: Results of the proposed tests for the differentiation of the distinct accumulation patterns of normal and anomalous (mild and accentuated) concentrations of β-carotene in the LP, MP and DP specimens. The symbols $\star$, $\mathcal{F}$, $\triangle$, $\mathcal{I}$ indicate pass, fail, pass by a small margin (relative difference between compared values $<$ 1%) and fail by a small margin, respectively. The letters E, D, and A refer to the anomalous presence of β-carotene only in the *epidermal* tissues, only in the *dermal* tissues and in *all* tissues, respectively.

	LP	Mild			Accentuated		
Test	Normal	E	D	A	E	D	A
T1	$\mathcal{I}$	$\mathcal{I}$	$\star$	$\star$	$\mathcal{I}$	$\star$	$\star$
T2	$\star$	$\triangle$	$\star$	$\star$	$\triangle$	$\star$	$\star$
T3	$\star$	$\triangle$	$\star$	$\star$	$\triangle$	$\star$	$\star$
	MP	Mild			Accentuated		
Test	Normal	E	D	A	E	D	A
T1	$\mathcal{I}$	$\mathcal{I}$	$\star$	$\star$	$\mathcal{F}$	$\star$	$\star$
T2	$\star$	$\star$	$\star$	$\star$	$\triangle$	$\star$	$\star$
T3	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$
	DP	Mild			Accentuated		
Test	Normal	E	D	A	E	D	A
T1	$\mathcal{I}$	$\mathcal{I}$	$\star$	$\star$	$\mathcal{I}$	$\star$	$\star$
T2	$\star$	$\star$	$\triangle$	$\star$	$\star$	$\star$	$\star$
T3	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$	$\star$

indicate that spectral tests can represent a viable alternative for the differentiation of distinct β-carotene accumulation patterns, particularly for skin specimens with relatively high levels of constitutive pigmentation. They also suggest that these tests can be employed to support the noninvasive screening of pathologies that may bring forward carotenemia.

IV. Conclusion

The noninvasive differentiation of β-carotene distribution patterns in the cutaneous tissues is a difficult problem due to the masking effects of other pigments on skin spectral responses. We examined how this problem can be tackled by carefully sampling these responses in a spectral region where this carotenoid's absorption spectrum tends to have a more detectable impact on those responses as its accumulation increases, notably in the dermal tissues. Although future *in vivo* analyses are required to fully assess this approach's repeatability and applicability range, the obtained *in silico* results showed a promising high-fidelity to cost ratio.

Accordingly, the proposed spectral tests can be seen as a proof of concept for the exploration of specific features of the absorption spectra of carotenoids to expand the scope of spectrophotometric investigations in this area, notably those involving the interconnections between their cutaneous accumulation patterns and their protective roles. We note that current related studies seldom account for high levels of constitutive skin pigmentation due to the technical constraints outlined earlier. We hope that the proposed tests can represent a step toward alleviating such constraints.

References

[1] M.E. Darwin, W. Sterry, J. Lademann, and T. Vergou, "The role of carotenoids in human skin," *Molecules*, vol. 16, pp. 10491–10506, 2011.

[2] S. Zerres and W. Stahl, "Carotenoids in human skin," *BBA - Molecular and Cell Biology of Lipids*, vol. 1865, pp. 158588:1–6, 2020.

[3] S. Alaluf, U. Heinrich, W. Stahl, H. Tronnier, and S. Wiseman, "Dietary carotenoids contribute to normal human skin color and UV photosensitivity," *J. Nutr.*, vol. 132, no. 3, pp. 399–403, 2002.

[4] A.D. Lascari, "Carotenemia a review," *Nutrition*, vol. 20, no. 1, pp. 25–29, 1981.

[5] G.V.G Baranoski, "On the pathways leading to carotenemia-elicited changes in the spectral responses and chromatic attributes of human skin," in *46th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Orlando, FL, USA, July 2024, pp. 10782225:1–7.

[6] P.S. Bernstein and W. Gellermann, "Noninvasive assessment of carotenoids in the human eye and skin," in *Carotenoids in Health and Disease*, N.I. Krinsky, S.T. Mayne, and H. Sies, Eds., pp. 53–84. Marcel Deck, New York, USA, 2004.

[7] R. Lee, M. Mathews-Roth, M.A. Pathak, and J.A. Parrish, "The detection of carotenoid pigments in human skin," *J. Investig. Dermatol.*, vol. 64, no. 3, pp. 175–177, 1975.

[8] G.J. Clydesdale, G.W. Dandie, and H.K. Muller Konrad, "Ultraviolet light induced injury: immunological and inflammatory effects," *Immunol. Cell. Biol.*, vol. 79, no. 6, pp. 547– 568, 2001.

[9] R.R. Anderson and J.A. Parrish, "The optics of human skin," *J. Investig. Dermatol.*, vol. 77, no. 1, pp. 13–9, 1981.

[10] G.V.G Baranoski, P. Alencar, Spencer R. Van Leeuwen, and Tenn F. Chen, "Tanning-elicited variations in the ultraviolet absorption spectra of the cutaneous tissues: Skin photobiology and photomedicine implications," in *43rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Virtual Conference, October 31 - November 4 2021, pp. 4268–4273.

[11] Y. Sharoni, W. Stahl, M. Danilenko, and J. Levy, "Anticancer activity of carotenoids: from human studies to cellular processes and gene regulation," in *Carotenoids in Health and Disease*, N.I. Krinsky, S.T. Mayne, and H. Sies, Eds., pp. 165–196. Marcel Deck, New York, USA, 2004.

[12] S. Piña-Oviedo, C. Ortiz-Hidalgo, and A.G. Ayala, "Human colors - the rainbow garden of pathology, What gives normal and pathologic tissues their color?," *Acta Pathol. Lab. Med.*, vol. 141, pp. 445–462, 2017.

[13] H.D. Sesso and J.M. Gaziano, "Heart and vascular tissues," in *Carotenoids in Health and Disease*, N.I. Krinsky, S.T. Mayne, and H. Sies, Eds., pp. 473–490. Marcel Deck, New York, USA, 2004.

[14] T.F. Chen and G.V.G. Baranoski, "A physiologically-based framework for the simulation of skin tanning dynamics," in *Proc. of SPIE, Vol. 10877, Dynamics and Fluctuations in Biomedical Photonics XVI: Tissue and Cell Dynamics, SPIE Photonics West - BiOS*, M.J. Leahy V.V. Tuchin and R.W. Wang, Eds., San Francisco, CA, USA, March 2019, pp. 108770H–1–20, Supplemental video: https://www.youtube.com/watch?v=I1quYM34wWw.

[15] S.A. Prahl, "PhotochemCAD spectra by category," Tech. Rep., Oregon Medical Laser Center, 2001.

[16] H. Du, R.A. Fuh, J. Li, A. Corkan, and J.S. Lindsey, "PhotochemCAD: A computer aided design and research tool in photochemistry," *Photochem. Photobiol.*, vol. 68, no. 2, pp. 142–142, 1998.

[17] T.F. Chen, G.V.G. Baranoski, B.W. Kimmel, and E. Miranda, "Hyperspectral modeling of skin appearance," *ACM Trans. Graph.*, vol. 34, no. 3, pp. 31:1–14, 2015, Supplemental video: https://www.youtube.com/watch?v=hKvIWS5H94s.

[18] G.V.G Baranoski, S.R. van Leeuwen, and T.F. Chen, "On the detection of peripheral cyanosis in individuals with distinct levels of cutaneous pigmentation," in *39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, Jeju Island, South Korea, August 2017, pp. 4260–4264.

[19] M.J. Vrhel, R. Gershon, and L.S. Iwan, "Measurement and analysis of object reflectance spectra," *Color Research and Application*, vol. 19, no. 1, pp. 4–9, 1994.

[20] J.A. Jacquez, J. Huss, W. McKeehan, J.M. Dimitroff, and H.F. Kuppenhein, "Spectral reflectance of human skin in the region 235-700 μ," *J. Appl. Physiol.*, vol. 8, pp. 212–214, 1955.

[21] Y. Takita, M. Ichimiya, Y. Hamamoto, and M. Muto, "A case of carotenemia associated with ingestion of nutrient supplements," *J. Dermatol.*, vol. 2, pp. 132–134, 2006.

[22] S. Askew and G.V.G Baranoski, "On the dysfunctional hemoglobins and cyanosis connection: Practical implications for the clinical detection and differentiation of methemoglobinemia and sulfhemoglobinemia," *Biomed. Opt. Express*, vol. 9, no. 1, pp. 3284–3305, 2018.

[23] S.R. van Leeuwen and G.V.G Baranoski, "Elucidating the contribution of Rayleigh scattering to the bluish appearance of veins," *J. Biomed. Opt.*, vol. 23, no. 2, pp. 025001–1–17, 2018.

[24] G.V.G Baranoski and P.M. Varsa, "Angiogenesis-elicited spectral responses of early invasive skin melanoma: Implications for the evaluation of lesion progression," *J.Biophotonics*, vol. 17, pp. 20240020840:1–13, 2024.

[25] G.V.G. Baranoski and A. Krishnaswamy, *Light & Skin Interactions: Simulations for Computer Graphics Applications*, Morgan Kaufmann/Elsevier, Burlington, MA, USA, 2010.

[26] G.V.G. Baranoski, J.G. Rokne, and G. Xu, "Virtual spectrophotometric measurements for biologically and physically-based rendering," *The Visual Computer*, vol. 17, no. 8, pp. 506–518, 2001.

[27] Natural Phenomena Simulation Group (NPSG), *Run HyLIoS Online*, School of Computer Science, University of Waterloo, Ontario, Canada, 2017, http://www.npsg.uwaterloo.ca/models/hyliosEx.php.

[28] Natural Phenomena Simulation Group (NPSG), *Human Skin Data*, School of Computer Science, University of Waterloo, Ontario, Canada, 2014, http://www.npsg.uwaterloo.ca/data/skin.php.

[29] R.L. Burden and J.D. Faires, *Numerical Analysis*, PWS-KENT Publishing Company, Boston, fifth edition, 1993.

[30] D.B. Judd and G. Wyszecki, *Color in Business, Science and Industry*, John Wiley & Sons, New York, NY, USA, third edition, 1975.

[31] D.L. MacAdam, *Color Measurements Theme and Variations*, Springer Verlag, Berlin, Germany, 1981.

[32] G.A. Zerlaut and T.E. Anderson, "Multiple-integrating sphere spectrophotometer for measuring absolute spectral reflectance and transmittance," *Applied Optics*, vol. 20, no. 21, pp. 3797–3804, November 1981.