FIELD APPLICATION REPORT

Materials Characterization: UV/Vis/NIR Spectroscopy

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Visible Reflectance Spectroscopy of Human Skin: the use of CIE L*a*b* Color Analysis for *In Vivo* Ethnic Skin Characterization

Introduction

There have been numerous attempts to scientifically characterize ethnic human skin types via the use of color criteria. Most of these methods are based on an empirical eyesight evaluation of the skin's coloration. The most widely used is the Fitzpatrick Scale, which is a numerical classification schema developed in 1975 by Harvard dermatologist Thomas B. Fitzpatrick. It groups skin types into six categories. This schema remains a recognized tool for dermatological research in classifying the response of human skin to visible light for the health and skin care industry. The Fitzpatrick Scale is a modernization of the older Von Luschan's Chromatic Scale which uses a series of 36 opaque glass tiles to characterize skin color. Figure 1 shows the range of colors for human skin as described by Von Luschan's tiles. The Fitzpatrick scale then groups these into six skin types: albino, fair, beige, Mediterranean brown, dark brown, and black.

The subject's skin is visually compared to the colored tile plates for the closest match. This method of visual inspection and comparison is now considered problematic, since it was very inconsistent and in many instances different investigators would get different measurements for the same subject.

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Variations in the color sensitivity of the human eye as well as differences in ambient lighting conditions are the main factors for visual inspection variation. Visual techniques have largely been replaced by reflectance spectrophotometry methods that do not suffer from human and environmental fluctuations.

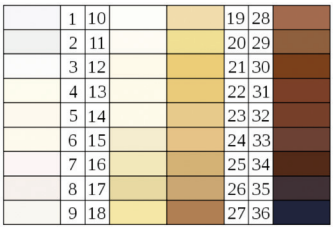

Figure 1. Von Luschan's color scale.

Modern spectrophotometers equipped with integrating spheres are capable of making accurate reflectance measurements on human skin. There are also numerous mathematical color analysis methods that make the evaluation of skin color a rigorous, reproducible, and accurate technique. Mathematical color analysis utilizes the sample's percent reflectance spectra, along with the spectral response curve of a given illuminant, in conjunction with the spectral sensitivities of the three visual pigments of the human eye to yield a quantitative metric that represents the sample's visual color. Thus, modern spectral color analysis permits one to calculate three numbers that correspond to what the human eye sees as color under defined lighting conditions.

Reflectance spectroscopy measures data that is directly related to the chemistry of the sample. Human skin consists of three sub layers; 1) the stratum corneum is a thin layer of dead cells employed for protection, 2) the epidermis, and 3) the dermis. Reflection spectroscopy penetrates and contains chemical information from all three of these skin sub layers. The epidermis is made up of only cells. The bottom of the epidermis contains specialized melanin containing cells called melanocytes. The major epidermal chemistry originates from proteins (the amino acids tryptophan, phenylalanine, and tyrosine), DNA, melanin, and urocanic acid. The dermis contains nerves and blood capillaries as well as cells. Additional dermal chemical constituents include oxygenated hemoglobin, deoxygenated hemoglobin, and bilirubin.

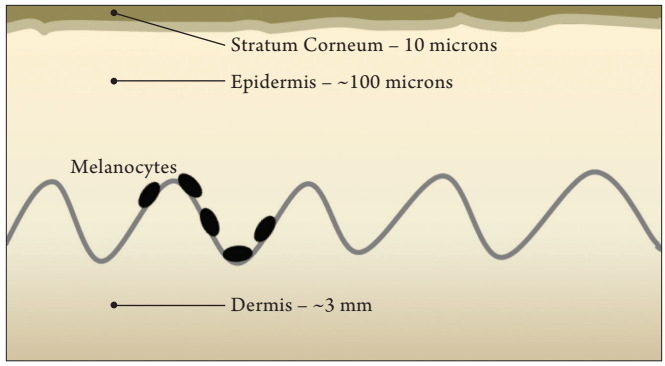

Figure 2. Layers of human skin involved in reflectance spectroscopy.

This study will evaluate reflectance spectra, obtained from both the Von Luschan's color schema tiles and a range of ethnic human skin subjects, by color analysis methods. While spectroscopic color analysis is far more reliable than visual inspection, it will still have its shortcomings for understanding the chemistry of human skin coloration. This is because more than one distribution of spectra data can give rise to the similar color values. While color analysis does an excellent job of measuring what the eye sees, it is limited to evaluating only the visible, qualitative color properties of samples.

Experimental

Reflection spectra of samples were measured with a LAMBDA 1050+ UV/Vis/NIR spectrophotometer equipped with a 150 mm high performance Polytetrafluoroethylene (PTFE) integrating sphere. The specular port was in place so that total reflectance was obtained. Skin sample spectra were collected by having the subject place the inside forearm of the arm against the diffuse reflectance port of the sphere. Von Luschan's tile plates were measured at the diffuse reflectance port as well. Figure 3 shows the sample beam path in a 150 mm sphere configured in total reflectance mode. Both the specular and diffuse reflectance components are collected. In order to fully investigate the range of human skin types, subjects from a wide range of ethnic types were measured for this study. The subjects ranged from very light albino to black African American.

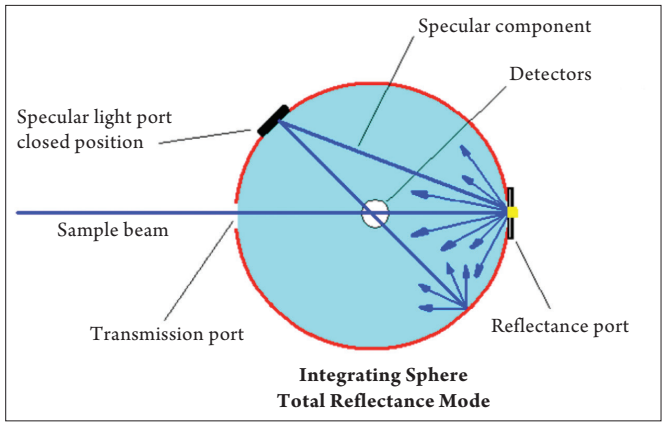

Figure 3. Lightpath in a 150 mm integrating sphere.

Spectra were displayed and processed using PerkinElmer's UV WinLab™ V.6 spectral analysis software. Color analysis employed PerkinElmer's color software and was performed according to the CIE 1976 L*a*b* protocol for the standard wavelength range of 400 nm to 700 nm. This analysis was performed using a CIE 1964 10 degree standard observer in conjunction with standard illuminant C, representing an average daylight illumination with a correlated color temperature of 6774 degrees Kelvin.

The CIE L*a*b* color space was selected for the color analysis process in this study for several reasons. L*a*b* color spaces separate the hue color component from the lightness/darkness component. This separation permits the independent investigation of hue (color) and lightness intensity of a sample.

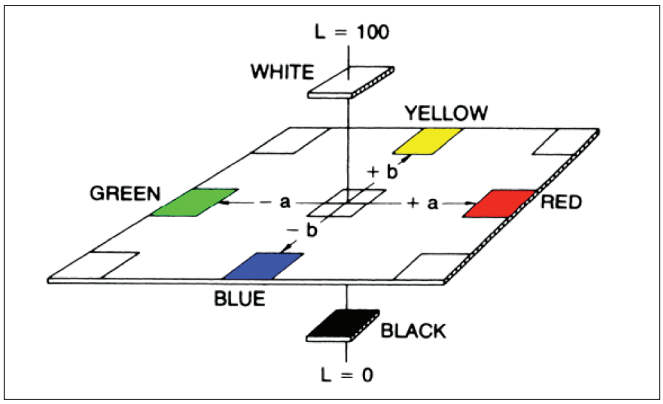

Figure 4. The L*a*b* color space.

Figure 4 graphically shows this separation of the L* and a*b* components of a typical L*a*b* color plot. This three dimensional space depicts green/red on the x axis (a*), blue/yellow on the y axis (b*), and darkness/lightness on the z axis (L*). This separation of hue and lightness is important in skin color analysis. The L* component for human skin color would largely be derived from the amount of melanin present in the lower epidermal layer; however, the hue component would be dominated by the influence of hemoglobin and bilirubin in the dermal layer.

Now that we can put a metric on the measurement of color, we can evaluate human skin in comparison to the Von Luschan's tile plates. The key question is how close a match to human skin are the plates used in the Fitzpatrick scale? This is a concern due to the fact that the underlying chemistry that gives rise to the color of skin and the plates is very different and could be an issue.

Results

In human skin spectroscopy, the wavelengths of visible light are able to penetrate the relatively transparent epidermis to interact with the diverse and complex chemistry of the heavily vascularized dermis. Figure 5 shows a percent transmission spectrum for isolated porcine epidermis. The epidermis is relatively transparent for wavelengths of visible light with only scattering processes attenuating the visible light reaching the dermis (above 400 nanometers). Ultra violet light below 400 nanometers is absorbed by cellular compounds such as urocanic acid, the nucleic acid rings of DNA, and the aromatic amino acids of proteins. The result of this generalized epidermal absorption can be seen in the inverse %T peak around 280 nm.

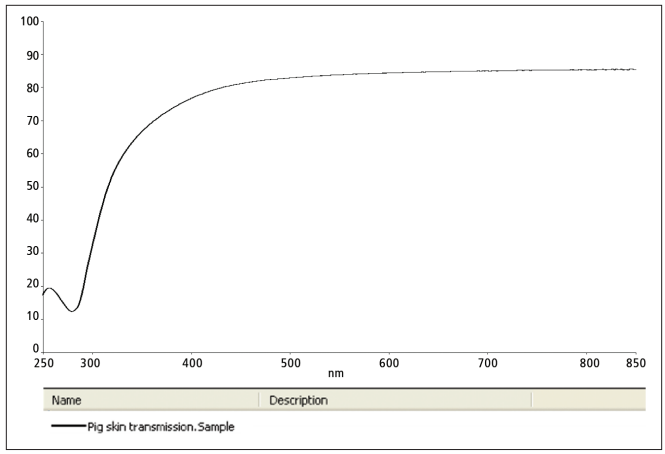

Figure 5. Porcine epidermal percent transmission spectrum.

The reflectance of skin is dominated by the chemistry of the dermis and is varied for the different human ethnic skin types. Changing amounts of hemoglobin and melanin lead to drastically different spectral shapes, as seen in Figure 6.

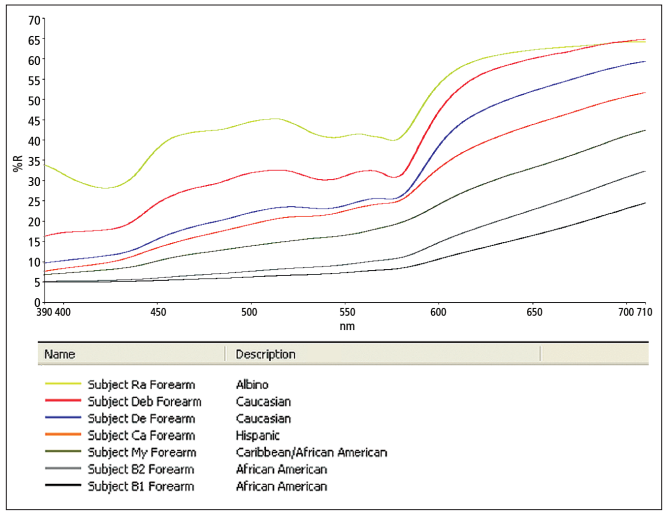

Figure 6. Percent reflectance spectra of different ethnic skin types.

For transparent skin types, such as albino, the incoming light penetrates deeply into the dermal tissue. In the mid-visible region of the spectra the twin peak structure (540 nm and 580 nm) of oxygenated hemoglobin is clearly seen. The oxygen free form of hemoglobin, with a single peak at 560 nm, is buried under the oxygenated hemoglobin peaks. A broad combined oxygenated and deoxygenate hemoglobin peak is found at 425 nm. A small peak for bilirubin appears at 540 nm. All of the compounds are present because of the generous blood supply of the dermal tissue layer.

Melanin plays a significant role in the spectroscopy of ethnic skin types. Melanin is a black polymer made from variations of the amino acid tyrosine. The melanin polymer has a very broad absorption from 200 nm all the way out to over 1000 nm in the near infrared. Melanin is concentrated in special cells called melanocytes that can be found in the basal cell layers at the bottom of the epidermis. The darker the skin, the more melanocytes populate this bottom layer just above the dermis (Figure 2 – Page 2). As these melanocytes increase in number, the more they

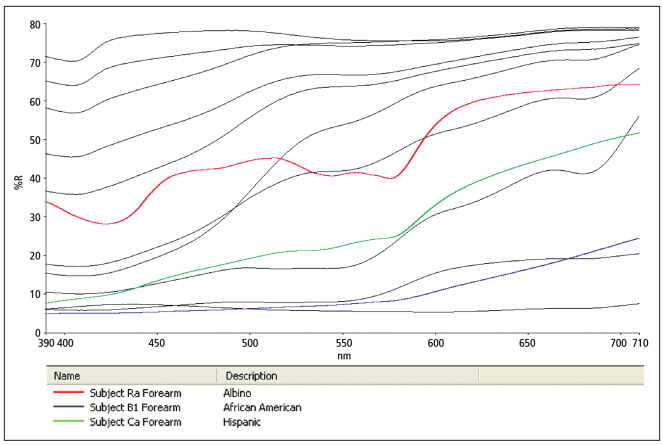

Figure 7. Spectral comparison of Von Luschan's plates and ethnic skin types.

make an effective "screen" to block harmful UV light from reaching the vulnerable dermis. If the light is absorbed by the melanocytes, it is prohibited from reaching the dermis and interacting with the compounds there. The darker skin types start to lose the spectral features associated with the dermal chemistry. Black African skin spectra consist solely of features related to melanin reflectance. The gradual wavelength dependent increase in the percent reflectance of black skin types is due primarily to the decrease in the melanin extinction coefficient with wavelength.

We can now consider how the reflectance spectroscopy of human skin compares to the Von Luschan's plates used for ethnic characterization. Figure 7 shows three representative ethnic skin spectra (red spectrum-Albino, green spectrum – Hispanic, and blue spectrum – African) overlaid with the range of Von Luschan's plate spectra (black spectra). There is little correlation of the peak structure between the two groups. This is not surprising when you consider that the coloration of the plates is due to the properties of inorganic pigments, but skin color arises from biological aromatic compounds. The electronic bonding signatures for these two groups of compounds are considerably different. Many plate spectra display a cut-off feature typical of inorganic compounds; whereas, the skin spectra are have numerous peak like features.

The Von Luschan's plate reflectance spectra were analyzed by the CIE L*a b* color methodology and the values collected in Table 1. Also the L* values for lightness/ darkness are correlated in the table with the Fitzpatrick Scale skin types.

Table 1. **CIE L*a*b* color analysis of Von Luschan's plates.**

Von Luschan's Plate #	L	a	b	Fitzpatrick Scale
Blank	90.19	-0.40	-1.21	
Number 1	88.59	-0.04	-2.17	
Number 2	87.46	-0.64	-0.78	
Number 3	90.07	-0.69	-0.46	
Number 4	89.62	-1.75	6.72	Type I (scores 0-7)
Number 5	88.73	-0.49	5.17	L = 100 - 85
Number 6	88.68	-0.88	5.91	
Number 7	88.12	1.45	-0.18	
Number 8	86.98	0.56	0.47	
Number 9	87.94	-0.58	0.92	
Number 10	90.18	-0.90	1.48	
Number 11	89.12	-0.57	2.95	Type II (scores 8-14)
Number 12	88.56	-0.79	7.22	L = 100 - 86
Number 13	88.99	-1.60	8.24	
Number 14	89.00	-1.63	8.32	
Number 15	85.06	-1.05	12.32	
Number 16	83.08	-1.12	19.49	
Number 17	80.09	-1.84	25.02	
Number 18	82.82	-0.32	29.05	
Number 19	80.93	0.77	21.72	
Number 20	80.36	-0.26	35.82	
Number 21	77.10	2.46	41.80	Type III (scores 14-26)
Number 22	76.56	2.81	39.00	L = 85 - 60
Number 23	75.60	3.60	26.83	
Number 24	75.77	3.61	27.16	
Number 25	71.32	2.99	27.96	
Number 26	68.47	4.02	24.32	
Number 27	57.44	10.53	21.54	Type IV (scores 27-29)
Number 28	53.02	14.02	17.75	L = 59 - 40
Number 29	46.33	10.86	16.55	
Number 30	39.61	11.97	13.22	
Number 31	38.41	12.62	11.93	Type V (scores over 30)
Number 32	37.78	11.37	11.09	L = 40 - 35
Number 33	36.61	8.70	7.64	
Number 34	30.60	5.95	5.78	
Number 35	30.83	2.37	-0.36	Type VI
Number 36	28.94	1.22	-5.40	L = 35 - 0

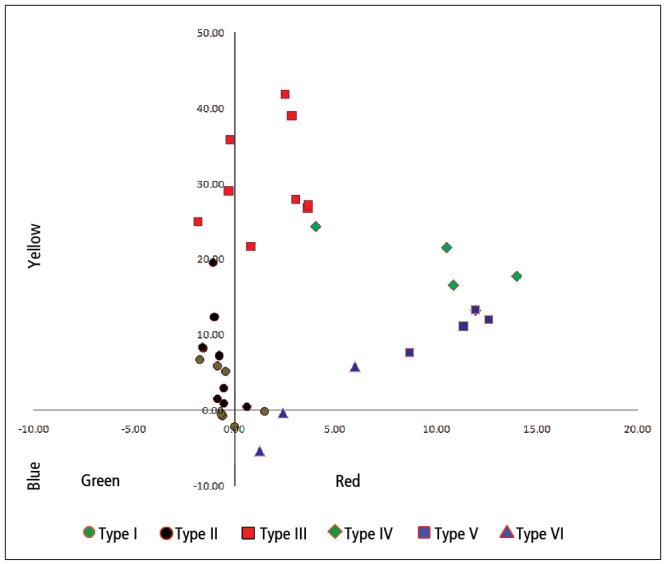

Figure 8. Plotted CIE hue values a*b* for Von Luschan's plates.

The L* values for the plates cover a range from 89 (light) to 29 (dark), for a total L* range of 60 L* units. When the hue values are assessed, the possible artificiality of the Von Luschan's plate colors start to become apparent. Figure 8 plots the a* vs. b* hue values, where the x axis represents the red/green component and the y axis represents the blue/yellow component. The hues for the lightest plates are greenish-yellow changing to more reddish values for the darker plate colors. The darkest plates are a bluish-red. Blue and green are hues that are not normally found in healthy human skin.

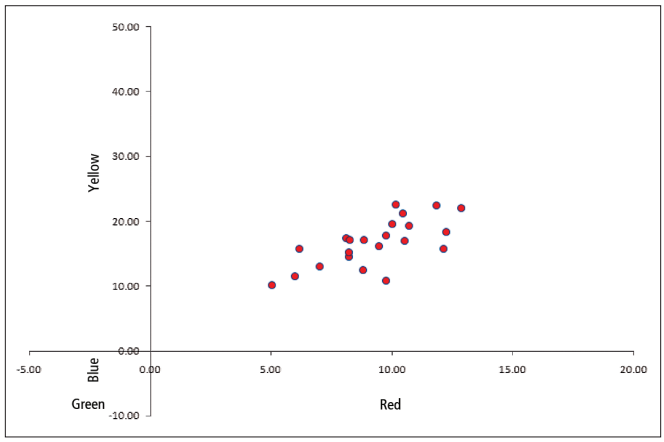

Figure 9. Plotted CIE hue values a*b* for forearm reflectance ethnic human subjects.

Contrast this with the hue values plotted for the reflectance spectra of human skin from different ethnic subjects displayed in Figure 9. Note that the data is arranged in a single group located in the yellow-red quadrant of the graph. As ethnic skin tones darken from albino to black African the shades (hue components) of yellow-red generally increase. This is no doubt due to the color of human skin being dominated by hemoglobin and bilirubin chemistry located in the dermis.

Table 2. **CIE L*a*b* color analysis for ethnic human skin (forearm).**

Forearm Spectra	Ethnic Group	L	a	b
Subject Ra	Albino	72.86	5.02	10.24
Subject TRA	Albino	71.67	5.98	11.61
Subject Ta	Light Caucasian	70.45	7.00	13.09
Subject TJA	Light Caucasian	69.89	8.21	14.60
Subject Ja	Light Caucasian	69.31	9.45	16.19
Subject Ji1	Caucasian	67.55	9.77	17.89
Subject Ch	Caucasian	67.38	6.17	15.80
Subject Gr	Caucasian	66.46	8.83	17.25
Subject Ne	Caucasian	66.32	8.82	12.62
Subject Ji2	Caucasian	66.03	8.11	17.45
Subject Deb	Caucasian	66.00	10.52	17.08
Subject St2	Caucasian	65.37	8.27	17.23
Subject Je	Tanned Caucasian	64.01	8.21	15.25
Subject Ka	Tanned Caucasian	63.43	10.45	21.23
Subject Ki	Tanned Caucasian	59.55	10.72	19.43
Subject De	Tanned Caucasian	59.44	12.88	22.14
Subject Ca	Hispanic	56.76	10.17	22.66
Subject Ci	African American	54.37	11.84	22.58
Subject St1	Caribbean	52.43	12.25	18.42
Subject My	Caribbean	49.87	10.01	19.66
Subject B2	African American	39.07	12.15	15.82
Subject B1	African American	34.46	9.77	10.93

Complete color L*a*b* data for ethnic human skin is compiled in Table 2. From this table we see the L* values for the skin measurements range from 73 (light) to 34 (dark). When the ranges for the L* values from the Von Luschan's plates and human skin are compared we note that the skin L* values span the smaller range of 39 L* units; whereas, the Von Luschan's plate's range is 60 L* units. The primary reason for the more compact range in human skin is most likely due to the surface structure of the reflecting cells. The surface texture of skin is rougher than the plates. Surface texture plays an important role in diffuse reflectance spectroscopy. The smoother the surface of a diffuse reflecting sample, the brighter it will appear to the eye. This is because a 'rough" surface is composed of tiny holes and crevices that can trap incoming light and prevent its reflection. The more numerous and larger these surface irregularities become, the darker the surface appears to the eye. This means that subjects with dry, rough, skin will appear darker than equally pigmented subjects with smoother skin.

Conclusion

A UV/Visible spectrophotometer equipped with a 150 mm standard color sphere using CIE L*a*b* color analysis is a viable method for the analysis of skin pigmentation due to human ethnicity. It is both a qualitative and reproducible technique, superior to earlier empirical visual based methodologies. There appear to be significant differences when the L*a*b* data is compared with the Von Luschan's plate tiles and actual human skin subjects. Analysis of the Von Luschan's plates, used as a basis for the Fitzpatrick Scale, indicated the presence of color artifacts due to the chromophore chemistry and surface texture employed in the tiles. The Fitzpatrick Scale could easily be re-evaluated in terms of a library of L*a*b* data obtained from the range of ethnic skin types.

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