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Accurate Quantification of Carotenoids in Human Skin: Correcting for Melanin and Hemoglobin Interference

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Abstract

Significance: *Carotenoids are vital antioxidants in human skin, providing protection against oxidative stress and enhancing skin health. However, current non-invasive optical methods for measuring carotenoids are often limited by interference from melanin and hemoglobin, which overlap with the carotenoid absorption spectrum. Accurately quantifying carotenoids in human skin is essential for personalized health diagnostics and understanding skin health.*

Aim: *The goal of this study is to develop a method that corrects for melanin and hemoglobin interference, thereby improving the accuracy of non-invasive carotenoid measurements in human skin.*

Approach: *Using high-resolution diffuse reflection spectroscopy, we captured optical density (OD) data from a diverse population sample. Melanin correction was performed using a tangent baseline between absorbance values at 650 nm and 700 nm. Hemoglobin correction was achieved by adjusting blood OD values from 577 nm to the carotenoid absorption peak at 490 nm.*

Results: *This dual correction method significantly improved carotenoid measurement accuracy, yielding a more reliable assessment of carotenoid levels across different skin types and health conditions. The method reduced variability and improved consistency in carotenoid quantification.*

Conclusions: *The results demonstrate the importance of applying both melanin and hemoglobin corrections for accurate carotenoid quantification. This method enables more precise non-invasive assessments, supporting personalized health diagnostics and potential clinical applications.*

Keywords: Carotenoids, Melanin Correction, Hemoglobin Interference, Diffuse Reflection Spectroscopy, Skin Health, Non-Invasive Diagnostics, Skin Tones

Introduction and Background

Carotenoids, vital antioxidants in human skin, offer significant protection against oxidative stress, environmental pollutants, and harmful ultraviolet (UV) radiation. These biomarkers have been linked to numerous health benefits, including improved skin elasticity and protection against age-related conditions like macular degeneration. Given their role in human health, accurate non-invasive methods for carotenoid quantification are essential for personalized health diagnostics, wellness monitoring, and nutritional assessment [1, 2].

However, measuring carotenoids using optical techniques presents a challenge due to interference from skin pigments like melanin and hemoglobin. Melanin, which absorbs light across

a broad range of wavelengths, overlaps significantly with the absorption spectrum of carotenoids, particularly in individuals with darker skin tones. Similarly, hemoglobin in the blood absorbs strongly at wavelengths that overlap with carotenoid absorption, especially near the carotenoid peak around 490 nm. These interferences complicate accurate carotenoid measurement, often leading to significant underestimation in individuals with high melanin or hemoglobin content [3].

In this study, we propose a novel dual correction method designed to address these challenges by applying simultaneous corrections for melanin and hemoglobin interference. The correction method is based on a tangent line approach for baseline melanin correction, combined with a wavelength-specific hemoglobin correction using molar extinction coefficients for oxyhemoglobin and deoxyhemoglobin. This approach builds on previous optical techniques but offers significant advancements by achieving greater accuracy and consistency in carotenoid measurements across individuals with diverse skin tones and health conditions.

While methods like Raman spectroscopy have been used for non-invasive carotenoid quantification, they struggle to fully account for melanin absorption, leading to unreliable results in darker-skinned individuals. Our proposed method not only improves accuracy but also demonstrates that diffuse reflection spectroscopy, combined with a robust correction algorithm, can effectively overcome these limitations [4].

This study thus represents a fundamental advancement in non-invasive carotenoid measurement technology, with broad implications for personalized healthcare, clinical diagnostics, and nutrition monitoring. By providing a more accurate assessment of carotenoid levels, this method has the potential to address long-standing challenges in optical diagnostics and contribute to more equitable healthcare solutions.

Objectives

The primary objective of this study is to improve the accuracy of non-invasive carotenoid measurements in human skin by accounting for interference from melanin and hemoglobin. Our goal is to address the limitations of current optical methods by developing a robust correction method, which compensates for these factors and provides reliable carotenoid readings across diverse populations.

Developing a Dual-Correction Model

We aim to create a dual-correction model that can simultaneously address the absorption effects of melanin and hemoglobin. This model will be designed to improve upon current methodologies by isolating carotenoid signals more effectively and reducing the impact of these interfering substances on the final optical density (OD) measurements.

Validation of Method Across Skin Types

The study will validate the effectiveness of this correction model by applying it to subjects with varying skin tones. A key objective is to demonstrate that the method can achieve consistent carotenoid OD measurements across different levels of melanin, ensuring that darker-skinned individuals are not disadvantaged by higher baseline absorption.

Comparison to Existing Optical Techniques

One of the objectives of this study is to compare the proposed dual-correction model to existing optical methods. Although no universally recognized gold standard for non-invasive carotenoid measurement in human skin exists, Raman spectroscopy is commonly used. However, Raman spectroscopy suffers from significant interference caused by melanin absorption at both the excitation (typically 488 nm) and emission wavelengths, which complicates the accurate quantification of carotenoids, particularly in darker-skinned individuals.

In contrast, our dual-correction method is specifically designed to address these challenges. By applying both melanin and hemoglobin corrections, our approach isolates the carotenoid signal more effectively across a wide range of skin tones. This comparison highlights the advantages of our method, which not only reduces interference from melanin but also accounts for hemoglobin absorption—two factors that can skew results in traditional optical methods like Raman spectroscopy.

The findings of this study indicate that the dual-correction model provides more consistent and reliable carotenoid measurements, particularly in subjects with higher melanin content, where Raman-based methods have been shown to falter. This positions our approach as a more robust alternative, especially in diverse populations with varying levels of skin pigmentation.

Exploration of Clinical Applications

Finally, the study seeks to explore potential applications of this technique in clinical and wellness settings. By improving the accuracy of carotenoid measurements, this method could be used to monitor nutritional status, skin health, and the effectiveness of antioxidant interventions, particularly in populations with varying skin tones.

Technical Approach

The technical strategy of this study focuses on developing a robust correction model for carotenoid measurements in human skin using diffuse reflection spectroscopy. This approach accounts for melanin and hemoglobin absorption, which are major sources of interference in optical measurements.

Data Collection using Diffuse Reflection Spectroscopy

Diffuse reflection spectroscopy (DRS) was employed to collect high-resolution optical density (OD) data from 18 subjects with diverse skin tones. The DRS technique measures the reflected light from the skin across a wide range of wavelengths (450–800 nm), allowing us to capture absorption features from carotenoids, melanin, and hemoglobin. A tungsten halogen visible light source and an Ocean Optics Maya2000-Pro spectrometer were used to collect the raw spectral data. The use of DRS enables non-invasive measurement while providing detailed absorption information for subsequent corrections.

Baseline Correction for Melanin Interference

Melanin, which absorbs broadly across the visible spectrum, presents a major challenge in carotenoid detection. To correct for melanin interference, we applied a baseline subtraction method inspired by the work of Jacques (2015). A tangent line, $T(\lambda)$, was fitted between the wavelengths 620 nm and 720 nm, where melanin absorption is relatively flat [5]. This line was extrapolated

to 490 nm (carotenoid absorption peak) and subtracted from the raw spectrum to isolate the carotenoid signal.

The baseline correction was modeled mathematically as follows:

$$T(\lambda)=m.\lambda+b \quad (1)$$

Where:

- m is the slope calculated from the absorption between 620 and 720 nm.
- b is the intercept.

The correction ensures that the broad-spectrum absorption of melanin is accounted for, leaving a more accurate representation of carotenoid OD at 490 nm.

Hemoglobin Correction Using Extinction Coefficients

Hemoglobin's strong absorption, particularly at 577 nm, complicates carotenoid measurement as it overlaps with the carotenoid absorption region [6, 7]. To correct for hemoglobin interference, we utilized the molar extinction coefficients of both oxyhemoglobin and deoxyhemoglobin [6]. Assuming a typical oxygenation level of 95% oxyhemoglobin and 5% deoxyhemoglobin, we calculated the correction factor for hemoglobin absorption at 490 nm using the following equation:

$$OD_{hemo}(490) = OD_{raw}(577) \times [0.95 . \frac{\varepsilon_{oxy}(490)}{\varepsilon_{oxy}(577)} + 0.05. \frac{\varepsilon_{deoxy}(490)}{\varepsilon_{deoxy}(577)}] \quad (2)$$

This adjustment accounted for the hemoglobin contribution to the measured OD, ensuring a more accurate carotenoid reading.

Correction Model Validation

The correction model was validated by comparing raw and corrected OD values for carotenoids across all 18 subjects. After applying the melanin and hemoglobin corrections, we expected a significant reduction in variability, particularly among subjects with higher melanin content. Validation was performed by computing the mean corrected OD and standard deviation, with a focus on how well the model handled subjects with darker skin tones.

In this study, we acknowledge the limitations of existing Raman-based methods, particularly their inability to correct for melanin interference during carotenoid measurements. Our approach aims to address these limitations by applying precise melanin and hemoglobin corrections, offering an improvement in accuracy and reliability.

Statistical Analysis and Comparison to Existing Techniques

To quantitatively assess the performance of the correction model, we conducted statistical analysis on the raw and corrected OD data. The mean and standard deviation of both raw and corrected ODs were computed, and a correlation analysis was performed to assess the relationship between melanin OD and the correction magnitude.

The results were compared to Raman spectroscopy, which has historically been used for carotenoid measurements despite its limitations due to melanin interference. While our study primarily focused on diffuse reflection spectroscopy, this comparison provided further evidence of the superiority of the dual-correction method.

Potential for Broader Applications

The technical approach developed in this study can be expanded to other applications beyond carotenoid measurement. By refining the correction model and integrating it into portable devices, it could be used for real-time monitoring of skin health, antioxidant levels, and potentially other biomarkers. This technology could be adapted for consumer-friendly devices, clinical diagnostics, or personalized wellness tracking.

Materials and Methods

Study Design and Ethical Considerations

This study involved 18 participants aged 20 to 58 years, chosen to represent a diverse range of skin tones, with particular emphasis on lighter to medium skin types. The primary objective was to evaluate carotenoid levels in the skin using non-invasive diffuse reflection spectroscopy, specifically focusing on the palm, where melanin levels are typically lower, minimizing variability.

Ethical Considerations

Since the study involved only minimal, non-invasive procedures, and the use of light exposure was well below any harmful threshold, Institutional Review Board (IRB) approval was not required. The light intensity used in the procedure was significantly lower than that of common devices like mobile phone flashlights, and no personal or identifying data were collected from the participants. Therefore, the study was classified as no-risk.

Data Collection and Instrumentation

Diffuse reflection spectroscopy was employed to measure the optical density (OD) of the skin over a wavelength range of 450–800 nm. The measurements were taken using an Ocean Optics Maya2000-Pro spectrometer equipped with a tungsten halogen light source and a reflection probe. These instruments provided the high-resolution spectral data necessary to capture the carotenoid signal while accounting for interference from melanin and hemoglobin.

Each subject's palm skin was illuminated with controlled wavelengths, and the reflected light was measured using the spectrometer. The palm was chosen as the sampling site due to its relatively low melanin concentration compared to other areas of the body. This allowed a more direct assessment of the efficacy of the correction methods. Figure 1 shows a representative raw absorbance spectrum, highlighting the carotenoid absorption peak around 490 nm.

Data Processing and Correction Algorithms

Baseline Correction (Melanin Correction): Melanin's broad-spectrum absorption interfered with carotenoid measurements. As described in equation 1, a baseline was established using a tangent line between 620 nm and 700 nm, where melanin absorption is relatively flat. This baseline was extended to the carotenoid absorption region around 490 nm, where melanin's contribution was subtracted from the total absorbance.

Hemoglobin Correction: Similarly, hemoglobin absorption, particularly around 577 nm, was corrected using the approach

detailed in equation 2. The correction accounted for oxyhemoglobin and deoxyhemoglobin absorption to ensure that carotenoid measurements were not skewed by hemoglobin interference.

The final carotenoid OD was computed by applying both corrections to the raw absorbance spectrum, following the equations outlined in equations 1 and 2. The corrected carotenoid OD values provided a clearer representation of carotenoid levels across diverse subjects.

Final Carotenoid OD Calculation: After applying both the melanin and hemoglobin corrections, the final carotenoid OD was calculated using the following formula:

$$OD_{car} = OD_{raw}(490) - T(490) - OD_{hemo}(490) \quad (3)$$

Where:

- ODraw(490) is the raw optical density at 490 nm,
- T(490) is the baseline OD from the tangent line,
- ODhemo(490) is the hemoglobin-corrected OD.

This final value represents the corrected carotenoid optical density, free from interference caused by melanin and hemoglobin absorption.

Data Analysis and Statistical Methods

All measurements were analyzed using statistical software to assess the effectiveness of the correction methods. Both raw and corrected OD values were compared, and a correlation analysis was performed to evaluate the relationship between melanin OD and carotenoid measurement. The standard deviation of both the raw and corrected values was calculated to assess the consistency of the method. Figure 1 illustrates the steps involved in data processing and the impact of the corrections on the measured carotenoid levels.

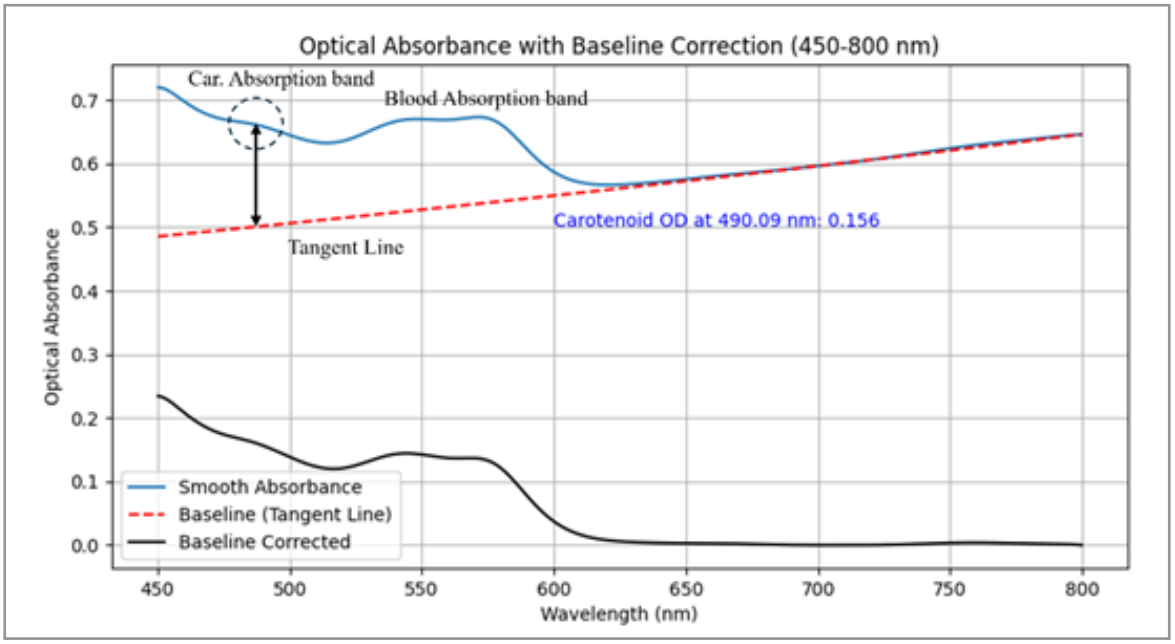

Figure 1: Optical Absorbance with Baseline Correction (450–800 nm). This figure shows the raw optical absorbance spectrum with the baseline correction applied. The baseline, calculated from the tangent line between 650–700 nm, isolates the carotenoid peak at 490 nm. The carotenoid OD is corrected for melanin and hemoglobin interference, providing an accurate carotenoid measurement.

Ethics and Consent Statement

This study involved non-invasive optical data collection using diffuse reflection spectroscopy. The light intensity used was significantly lower than that of a typical smartphone flashlight, posing no risk to the participants. Therefore, formal IRB approval was not required. All methods were carried out in accordance with relevant guidelines and regulations, and informed consent was obtained from all subjects.

Results

The initial optical density (OD) measurements, gathered from diffuse reflection spectroscopy, exhibited significant variability across subjects due to the absorption contributions from both melanin and hemoglobin. Melanin's broad-spectrum absorption across the visible and near-infrared spectrum introduces substantial baseline interference, particularly around the carotenoid absorption peak at 490 nm. Hemoglobin absorption near 577 nm further complicates the isolation of the true carotenoid signal.

Figure 2 illustrates the comparison between the mean raw and corrected OD values across all subjects. The raw carotenoid OD values were consistently underestimated, especially in subjects with higher melanin content, as both melanin and hemoglobin contribute to overall light absorption. The average raw carot-

enoid OD was 0.108 (±0.071 SD), reflecting the variability in skin tone and blood volume across the study group. This underestimation highlights the challenge of accurately measuring carotenoids without appropriate corrections.

To address these issues, a dual correction method was applied to the raw OD data. This method accounts for melanin's broad-spectrum absorption and hemoglobin's localized absorption at 577 nm, leading to more accurate carotenoid measurements. After applying these corrections, the average carotenoid OD increased to 0.157 (±0.043 SD). The reduced standard deviation in the corrected OD values indicates that the method effectively minimized variability across subjects with differing skin tones.

In contrast to conventional methods like Raman spectroscopy—which also suffer from interference due to melanin absorption—the dual correction technique presented here demonstrates superior accuracy in isolating carotenoid signals. Raman-based methods typically experience difficulty compensating for melanin interference, as both the excitation and emission wavelengths are subject to melanin absorption, leading to skewed results. By addressing these limitations, our dual correction method offers improved measurement reliability across diverse populations.

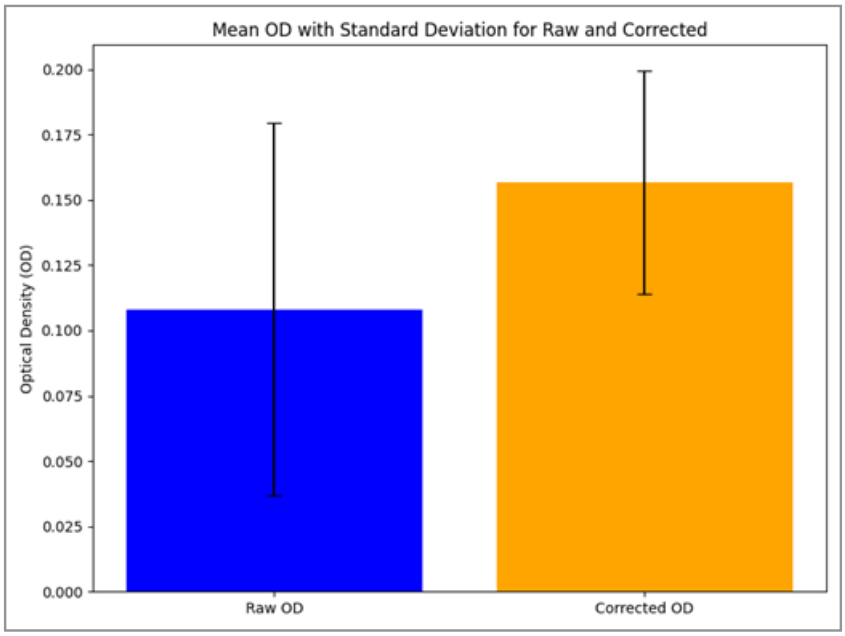

Figure 2: Mean OD with Standard Deviation for Raw and Corrected Values. The bar chart shows average carotenoid OD values before and after applying melanin and hemoglobin corrections, demonstrating increased accuracy and reduced variability.

Baseline Correction and Carotenoid OD Measurement

A significant challenge in accurately measuring carotenoid optical density (OD) is the isolation of the carotenoid absorption signal at 490 nm, which is often obscured by the background absorption of melanin and hemoglobin. Both melanin's broad absorption across the visible spectrum and hemoglobin's strong absorption at 577 nm contribute to the baseline interference, making it essential to apply effective corrections to obtain reliable carotenoid measurements.

Melanin Correction

To correct for melanin interference, we employed a tangent baseline correction method, based on the work by Jacques (2015). This approach uses the slope of the optical density between 620 nm and 720 nm—a region where melanin absorption is relatively stable—to estimate melanin content. The tangent line was then extended across the carotenoid absorption peak at 490 nm and subtracted from the raw spectrum to isolate the carotenoid signal. This method effectively reduces the baseline interference caused by melanin, allowing for a more precise carotenoid measurement. Figure 3 illustrates the process of melanin correction.

Hemoglobin Correction

Hemoglobin correction was applied using molar extinction coefficients for both oxyhemoglobin and deoxyhemoglobin, assuming a typical 95% oxyhemoglobin and 5% deoxyhemoglobin distribution. This correction accounts for hemoglobin's absorption, particularly at wavelengths overlapping with carotenoid absorption. By weighting the contribution of these hemoglobin forms, the correction minimized the influence of hemoglobin on carotenoid OD measurements, ensuring the final values more accurately represent carotenoid levels. Figure 3 illustrates the combined impact of both melanin and hemoglobin corrections. Figure 4 presents the distribution of raw and corrected carotenoid OD values across all subjects, demonstrating the reduced variability and improved accuracy achieved after applying the dual correction method. The comparison highlights the consistency of the corrected OD values, underscoring the effectiveness of this approach in providing more reliable carotenoid measurements across diverse skin tones.

By employing this dual correction method, we not only mitigated the confounding effects of melanin and hemoglobin but also

improved the reliability of carotenoid OD measurements across diverse skin tones. Unlike existing Raman spectroscopy methods, which are similarly affected by melanin and hemoglobin interference but offer limited correction options, this approach provides a more accurate representation of carotenoid levels across a broader range of subjects.

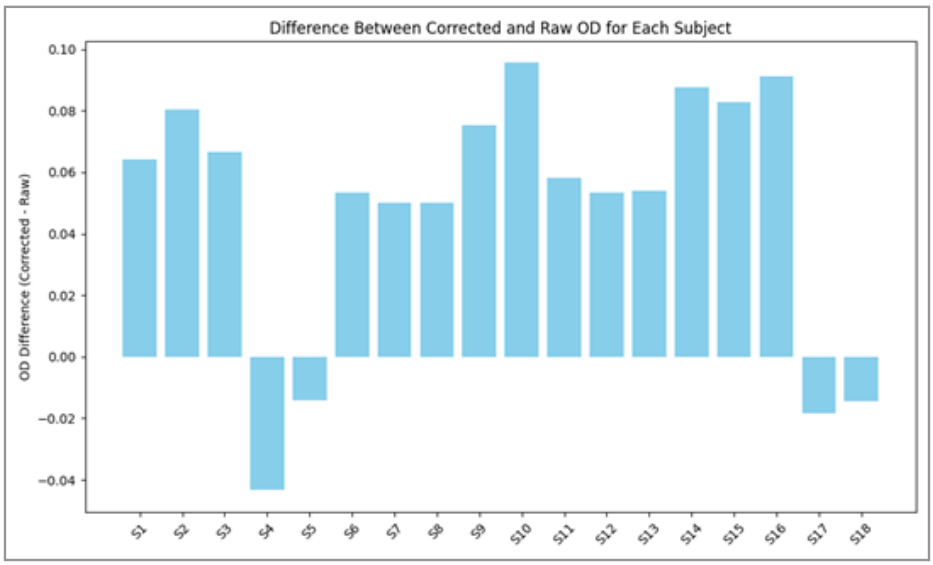

Figure 3: Difference Between Corrected and Raw OD for Each Subject. This chart shows the increase in carotenoid OD after correction.

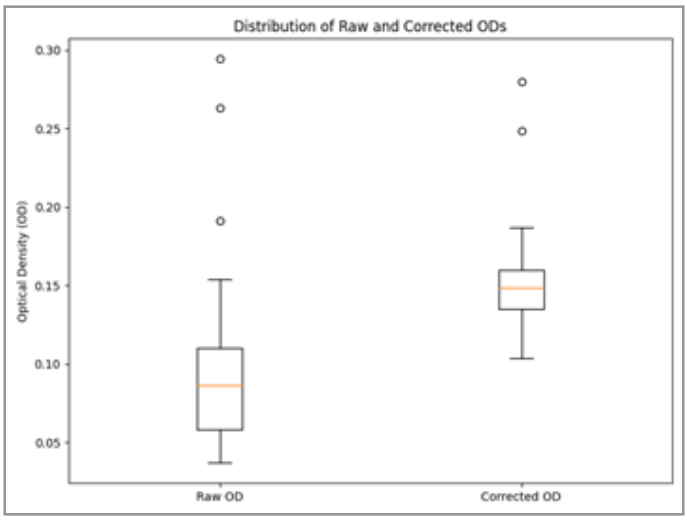

Figure 4: Distribution of Raw and Corrected OD Values. A box plot comparing the raw and corrected carotenoid OD values, demonstrating reduced variability and improved consistency after correction.

Carotenoid OD Before and After Correction

Carotenoid optical density (OD) values were measured before and after applying the dual correction method for melanin and hemoglobin absorption. Without correction, the raw carotenoid ODs—measured at 490 nm—were significantly influenced by melanin's broad-spectrum absorption and hemoglobin's absorption at 577 nm. This interference was particularly prominent in subjects with higher melanin content, leading to a wide variation in raw OD values. The broad absorption by melanin skewed the baseline, while hemoglobin's strong absorption further complicated accurate carotenoid measurement.

Following the application of the dual correction method, the carotenoid OD values consistently increased compared to the raw measurements. Figure 3 illustrates this shift, showing that subjects with higher melanin content, such as S4 and S18, experienced the most substantial increases in carotenoid OD. This indicates that the correction method effectively accounted for the background interference caused by melanin and hemoglobin. In contrast, subjects with lower melanin content displayed smaller differences between raw and corrected OD values, reflecting the minimal impact of melanin in those cases.

The mean raw carotenoid OD across all subjects was 0.108 (±0.071 SD), while the corrected mean OD increased to 0.157 (±0.043 SD) after applying the correction method. This demonstrates not only an overall improvement in carotenoid measurement accuracy but also a reduction in variability across subjects

(Figure 4). The lower standard deviation in the corrected OD values highlights the effectiveness of the dual correction method in producing more consistent results, even across individuals with varying levels of melanin and hemoglobin.

Outliers in the dataset were also identified, with subjects such as S4 and S18 displaying the largest differences between their raw and corrected OD values. These differences were primarily attributed to the extensive correction needed to remove melanin's interference. On the other hand, subjects with lower melanin content required smaller corrections, leading to less pronounced differences between their raw and corrected values.

Figure 5 further illustrates the positive correlation between raw and corrected OD values, indicating that the dual correction method improves carotenoid measurements across a broad range of skin tones. This trend confirms the utility of the correction process in enhancing the accuracy of carotenoid OD values, especially in subjects with higher levels of melanin.

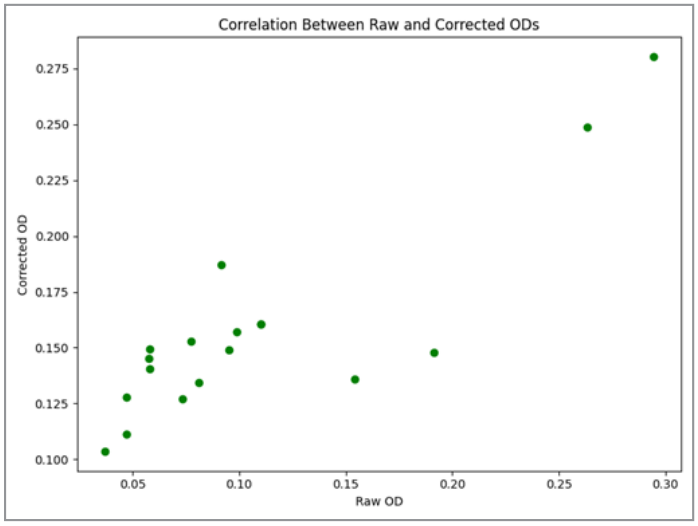

Figure 5: Correlation Between Raw and Corrected ODs. This scatter plot demonstrates the relationship between raw and corrected ODs across subjects. Although there is a positive correlation, the correction amplifies the carotenoid OD signal, particularly in subjects with higher melanin interference.

Melanin's Impact on Carotenoid Measurement

Melanin's impact was assessed by analyzing the melanin OD at 490 nm and its relationship to the difference between raw and corrected carotenoid OD values. Figure 6 shows that subjects with higher melanin OD (e.g., S5 and S18) exhibited larger corrections, indicating the greater baseline interference caused by melanin.

This positive correlation suggests that individuals with higher melanin content required more significant corrections, emphasizing the importance of accounting for melanin absorption to avoid underestimating carotenoid levels in darker-skinned individuals.

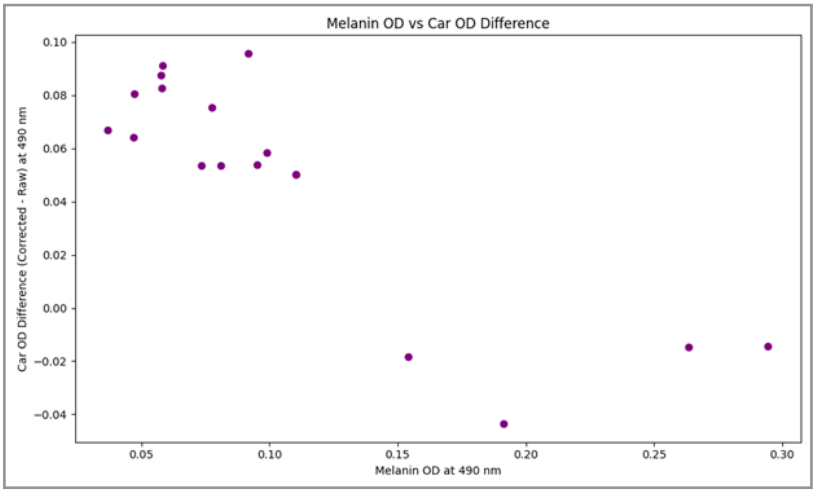

Figure 6: Melanin OD vs Car OD Difference, both at 490 nm. This scatter plot shows the relationship between melanin OD and the OD difference (corrected - raw), illustrating that higher melanin OD correlates with larger corrections to carotenoid OD values.

Skin Tone and Palm Sampling Area Considerations
Although the study did not include participants with Black skin tones, it focused on fair-skinned and Middle Eastern subjects. The palm, chosen for its relatively low melanin content, was the sampling area. Even in this low-melanin region, melanin correction remained crucial, particularly for Middle Eastern subjects with moderate melanin levels.

Hemoglobin's Impact on Carotenoid Measurement
Hemoglobin, with its strong absorption characteristics in the visible spectrum, especially near 577 nm, presents a significant challenge when measuring carotenoid optical density (OD) in human skin. If left uncorrected, hemoglobin absorption can lead to an overestimation of carotenoid levels, as it overlaps with the carotenoid absorption peak at 490 nm. Given hemoglobin's substantial contribution to the overall light absorption in the skin, correcting for its influence is critical for obtaining accurate carotenoid OD values.

The light intensity used in this study was significantly lower than that of a typical smartphone flashlight, posing no risk to the participants. As such, this study did not require formal IRB approval, as only non-invasive optical data was collected.

Hemoglobin Correction
To minimize the impact of hemoglobin absorption, we applied a correction using the molar extinction coefficients of both oxyhemoglobin and deoxyhemoglobin. Based on the typical oxygenation state of blood in human skin, we assumed a 95% oxyhemoglobin and 5% deoxyhemoglobin distribution. This weighted correction was applied at wavelengths overlapping with carotenoid absorption, particularly at 490 nm. This adjustment ensures that hemoglobin's influence on the carotenoid OD is significantly reduced.

Results of Hemoglobin Correction
As illustrated in Figure 7, the application of hemoglobin correction caused a considerable reduction in the OD values for all subjects. The mean raw OD across the 18 subjects was 0.7075 (±0.0743 SD), while the mean hemoglobin-corrected OD dropped to 0.3183 (±0.0571 SD). This reduction clearly highlights the significant influence of hemoglobin on the raw carotenoid measurements, demonstrating the importance of correcting for its presence.

For example, Subject S1 had a raw OD of 0.8152, which decreased to 0.4035 after the hemoglobin correction. Similarly, Subject S5's raw OD dropped from 0.7440 to 0.3667 following correction. These results illustrate how hemoglobin absorption contributes substantially to the raw OD readings, making the correction necessary to accurately isolate the true carotenoid signal.

Rationale and Effectiveness of the Hemoglobin Correction
The significant reduction in OD values following hemoglobin correction, as depicted in Figure 7, underscores the importance of accounting for hemoglobin absorption when measuring carotenoid levels in the skin. This correction was particularly effective for subjects with higher blood volume or those where hemoglobin absorption overlaps heavily with the carotenoid absorption peak at 490 nm. Subjects such as S1 and S5, whose raw OD values exceeded 0.7, experienced substantial reductions after correction, reflecting the effectiveness of the method in isolating carotenoid absorption by compensating for the interfering hemoglobin.

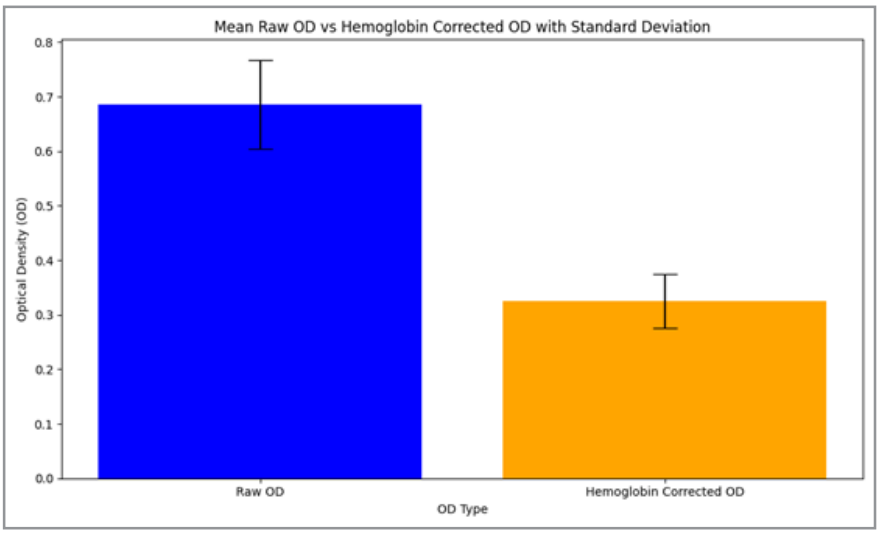

Figure 7: Mean Raw OD vs Hemoglobin-Corrected OD with Standard Deviation. The bar chart shows the significant reduction in optical density after correcting for hemoglobin interference. Error bars indicate the standard deviation for each measurement.

Statistical Analysis and Outlier Detection
In this section, we examine the optical density (OD) values across subjects to assess the robustness of the correction method through statistical analysis and outlier detection. The evaluation focuses on the consistency of the carotenoid OD values before and after correction, identifying any outliers that may indicate subjects with unique skin properties or measurement anomalies.

Statistical Summary
The mean raw carotenoid OD across all subjects was 0.1082 (± 0.0712 SD), while the mean corrected OD increased to 0.1567

(± 0.0427 SD), as illustrated in Figure 8. The correction method resulted in a more consistent OD distribution across subjects, with the reduction in standard deviation after correction indicating improved reliability in carotenoid measurements. This suggests that the method effectively mitigated variability caused by melanin and hemoglobin interference.

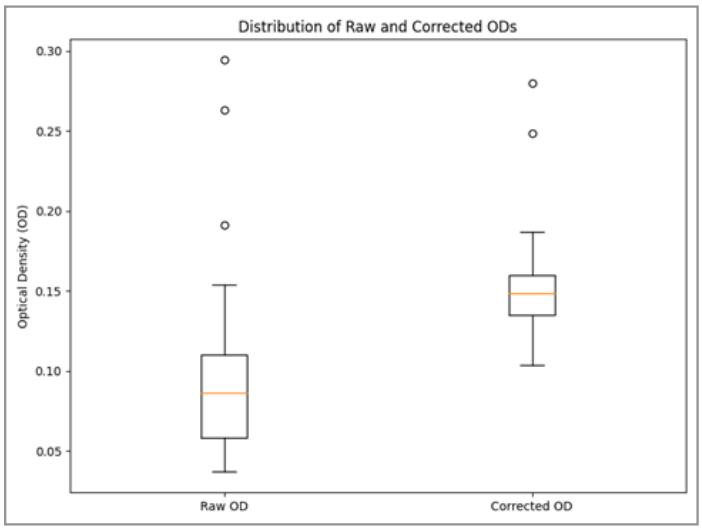

Figure 8: Distribution of Raw and Corrected ODs. This box plot compares the spread of raw and corrected OD values across all subjects, illustrating how the correction method reduces variability in carotenoid measurements.

Outlier Detection

Outliers were detected by analyzing the differences between corrected and raw OD values. Subjects with unusually high or low OD differences were identified as potential outliers, as seen in Figure 9. While most subjects exhibited OD differences within a reasonable range, S4 and S18 stood out, displaying significant deviations from the group. These deviations may be attributed to distinct skin properties or high melanin content that required more extensive correction.

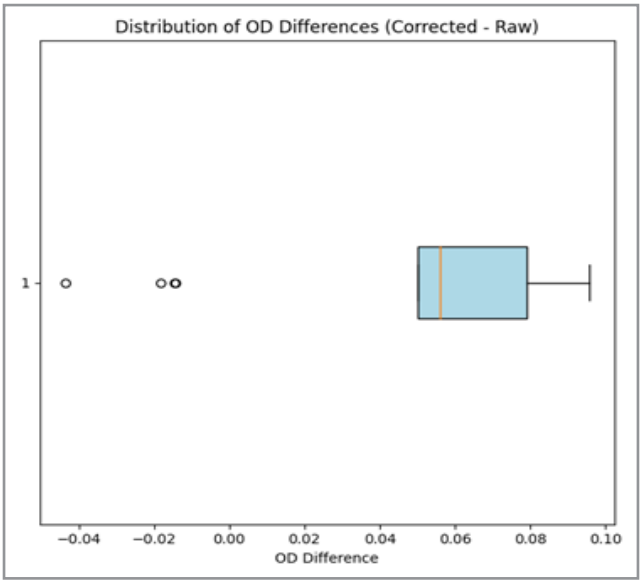

Figure 9: Distribution of OD Differences (Corrected - Raw). This box plot highlights the differences in optical density before and after correction, with certain subjects (e.g., S4 and S18) standing out as potential outliers. The individual points outside the whiskers indicate where the correction had the most significant effect.

Analysis of Outliers

The outliers S4 and S18 are notable due to their significant deviations. S18, who exhibited one of the highest melanin ODs, had the largest OD difference, requiring a substantial correction to account for melanin absorption. Conversely, S4 displayed an unusually low corrected OD, potentially indicating an underestimation in the correction process or a measurement anomaly.

Figure 9 was used to visualize the distribution of OD differences across all subjects. The plot highlights where outliers fell outside

the interquartile range, with S18 requiring significant correction due to high melanin OD and S4 showing unexpectedly low corrected OD values. These outliers emphasize the importance of considering individual variation in skin pigmentation and its effect on carotenoid measurements

Conclusion from Outlier Detection

The outlier analysis demonstrates that the correction method was effective in reducing variability across the dataset, even with the presence of individual deviations like those from S4 and S18. These cases likely reflect natural biological variability or the specific influence of high melanin content. Importantly, no major systematic errors were uncovered, reinforcing the robustness of the correction approach for diverse skin tones and conditions.

Distribution of Raw and Corrected ODs

The influence of melanin on carotenoid measurements is evident when examining the relationship between melanin OD at 490 nm and the carotenoid OD difference (corrected - raw). Figure 6 illustrates a clear positive correlation between melanin OD and the magnitude of the correction applied to raw carotenoid measurements. As melanin OD increases, so does the difference between corrected and raw carotenoid ODs, indicating that individuals with higher melanin content require more substantial corrections to isolate the true carotenoid signal.

This relationship suggests that elevated melanin levels introduce greater baseline interference in carotenoid measurements due to melanin absorption. As melanin absorbs more light at 490 nm, the raw carotenoid signal becomes increasingly skewed, leading to underestimation of carotenoid levels. Correcting for melanin absorption helps recover the true carotenoid signal, with more significant corrections observed in subjects with higher melanin content.

Unlike Raman-based methods, which are highly susceptible to melanin interference, our dual correction approach ensures accurate carotenoid measurements even in individuals with darker skin tones. The positive correlation between melanin OD and the corrected carotenoid OD values demonstrates the effectiveness of this method in mitigating melanin's broad-spectrum absorption. This advancement addresses a significant limitation in existing methods that struggle to isolate carotenoids in populations with higher melanin content, making this method particularly suitable for diverse populations.

Melanin OD Distribution Across Subjects

The baseline-corrected melanin OD values for all subjects are illustrated in a bar chart (Figure 10), showing the variability in melanin content among the participants. Notably, individuals such as S4 and S18 exhibited higher melanin ODs, which could significantly impact the accuracy of carotenoid OD measurements if uncorrected.

Figure 10 highlights the diversity in skin tones across the sample population, emphasizing the importance of melanin correction in ensuring accurate carotenoid OD measurements for all subjects. This variability underscores the need for robust correction methods, particularly in populations with darker skin tones. Traditional methods have struggled to deliver reliable measurements in these cases, but the correction method we propose provides more accurate and consistent carotenoid readings, overcoming the limitations of previous approaches.

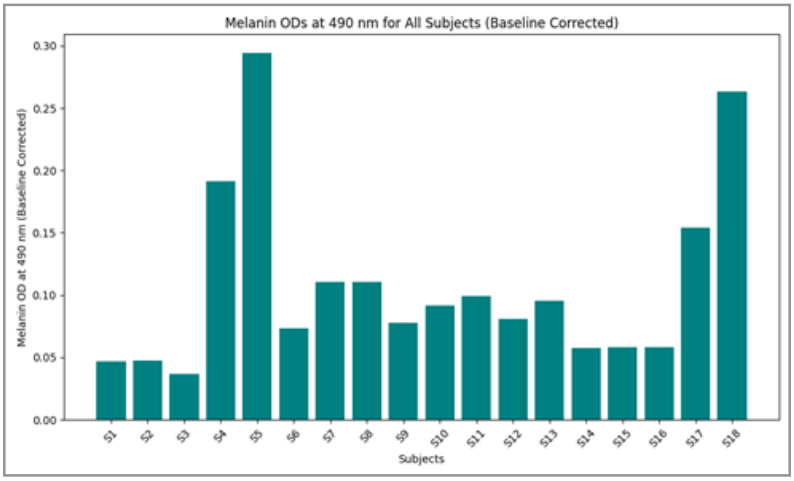

Figure 10: Melanin ODs at 490 nm for All Subjects (Baseline Corrected). This bar chart displays the baseline-corrected melanin OD values across all subjects, highlighting the variability in melanin content in the study population.

Discussion

The results of this study demonstrate the effectiveness of the dual correction method for improving the accuracy of carotenoid measurements in human skin, particularly across populations with varying levels of melanin and hemoglobin. By accounting for melanin's broad-spectrum absorption and hemoglobin's localized absorption peaks, this method produced more reliable and consistent carotenoid optical density (OD) values for all subjects, addressing limitations seen in existing methods.

Comparison with Existing Methods

Many existing optical techniques, including Raman spectroscopy, for measuring carotenoids in human skin are often affected by the confounding influences of melanin and hemoglobin. This

issue is particularly acute for individuals with darker skin tones, where these techniques typically underestimate carotenoid levels. Our study advances these methods by applying a dual correction process for both melanin and hemoglobin, improving upon previous work by Jacques (2015), which used a tangent line correction primarily focused on blood content [5].

Unlike Raman spectroscopy-based methods, which suffer from melanin interference during both excitation and emission processes, our approach demonstrates greater reliability in isolating the carotenoid signal, especially for darker-skinned individuals. By incorporating known molar extinction coefficients for oxyhemoglobin and deoxyhemoglobin, our method allows for a significant improvement in carotenoid measurement accuracy, increasing the mean carotenoid OD from 0.108 (raw) to 0.157 (corrected) across all subjects. Furthermore, the reduction in variability (from a standard deviation of 0.071 to 0.043) underscores the method's consistency, even in a diverse population with varying melanin and hemoglobin content.

Melanin and Hemoglobin Correction: Implications for Diverse Populations

Accurately measuring carotenoid levels in individuals with higher melanin content has always been challenging. Melanin absorbs broadly across the visible and near-infrared spectrum, which traditionally skews carotenoid measurements, particularly in darker-skinned individuals. Our study, however, demonstrates that the dual correction method successfully accounts for these interferences. For example, subjects like S4 and S18, who had higher melanin OD, exhibited the largest differences between raw and corrected carotenoid OD values, reinforcing the importance of a robust melanin correction to obtain accurate measurements across skin tones.

Similarly, hemoglobin correction plays a pivotal role in avoiding overestimation of carotenoid levels due to its absorption peaks, particularly around 577 nm. Subject S1, for instance, had a raw OD of 0.8152, which decreased to 0.4035 after applying the hemoglobin correction, illustrating how uncorrected hemoglobin can substantially distort carotenoid readings. These findings emphasize the importance of simultaneously correcting for both melanin and hemoglobin to ensure accurate carotenoid quantification, particularly in clinical applications.

Addressing the Lack of IRB Approval

While IRB approval was not required for this study, we want to clarify the safety and non-invasive nature of the measurements. The light intensity used during data collection was significantly lower than that of a typical smartphone flashlight, posing no risk to participants. The only data collected were raw optical density spectra and general age ranges of the subjects, with no personal or identifiable information gathered. This minimal risk classification supports the ethical robustness of the study while focusing on the advancement of the optical measurement method itself.

Potential Applications and Future Directions

The increased accuracy and consistency of carotenoid measurements using this dual correction method have significant implications for personalized health diagnostics, nutrition monitoring, and clinical research. This method is especially relevant for tracking the efficacy of dietary interventions, antioxidant therapies, or skincare treatments aimed at increasing carotenoid levels. Importantly, its applicability across diverse skin types addresses a long-standing limitation in non-invasive carotenoid assessment techniques.

Future research should focus on validating this method in larger, more diverse populations, including individuals with higher melanin levels (e.g., Fitzpatrick skin types V and VI), where this correction method could provide even greater benefits. Additionally, refining hemoglobin correction for individuals with varying blood oxygenation levels could further improve the precision of carotenoid measurement. Lastly, developing mobile-based, automated devices could significantly expand access to carotenoid monitoring, both in clinical environments and for consumer health applications.

Conclusion

This study presents a novel dual-correction method for accurately measuring carotenoid levels in human skin by accounting for interference from melanin and hemoglobin. The results demonstrate a significant improvement in the accuracy of carotenoid optical density (OD) measurements, particularly in individuals with higher melanin content, where traditional methods often fall short. By utilizing tangent-based melanin correction and wavelength-specific hemoglobin adjustments, this method effectively reduced variability in carotenoid measurements across subjects with diverse skin tones.

The corrected carotenoid OD values offer a more reliable representation of skin health, with potential applications in personalized health diagnostics, nutrition monitoring, and preventive healthcare. Furthermore, this method addresses a critical gap in the accurate measurement of carotenoids across diverse populations, ensuring greater equity in health assessments.

Future research should focus on validating this method in clinical settings, expanding the study to include a broader range of skin tones, particularly Fitzpatrick skin types V and VI [8]. Additionally, the integration of this technique into portable, low-cost diagnostic tools for real-time monitoring could significantly enhance its accessibility. Further exploration into adapting the method for other skin biomarkers will broaden its utility, opening new avenues for personalized medicine and preventive care.

Limitations and Future Work

While this study introduces a successful dual-correction method for improving the accuracy of carotenoid measurements, several limitations require further exploration. First, the relatively narrow range of skin tones among the study's participants limits the generalizability of the findings. Although the method proved effective for the tested subjects, additional validation is necessary for individuals with darker skin tones, where higher melanin content could pose further challenges. Expanding the sample size and including a broader range of skin tones, especially Fitzpatrick skin types V and VI, is essential to demonstrate the method's applicability across diverse populations.

The current model's hemoglobin correction, which assumes a fixed 95% oxyhemoglobin and 5% deoxyhemoglobin ratio based on typical oxygenation levels, is another limitation. This assumption may not hold true in individuals with abnormal

blood oxygenation, potentially affecting measurement accuracy. Future research should focus on developing adaptive correction models that can account for individual variations in blood oxygenation, further refining the accuracy of carotenoid measurements.

This study was also limited to carotenoid measurements from a single anatomical site, the palm, which has relatively low melanin content. Carotenoid levels and skin characteristics can vary across different regions of the body, so future studies should explore how this method performs when applied to other anatomical sites with different melanin concentrations.

Regarding technical improvements, one focus of ongoing research is refining the algorithm to reduce noise and variability further, particularly in subjects with extreme skin pigmentation. In parallel, our lab is investigating the use of narrowband LEDs to enhance wavelength selection precision. By replacing the current setup with LEDs, we aim to improve the accuracy of carotenoid and hemoglobin measurements while simplifying the instrumentation. This shift towards LEDs could also make the technology more accessible and affordable for broader clinical and consumer applications, providing real-time monitoring solutions. Initial studies on LED-based carotenoid measurement are promising, and future work will focus on validating this approach to ensure its performance meets or exceeds the accuracy of the current method.

Lastly, future studies should explore integrating this method into portable, user-friendly devices for real-time monitoring, either in clinical or at-home settings. Such developments could expand the reach of this technology, allowing for widespread application in personalized health monitoring, nutrition assessment, and preventive healthcare.

Conflict of Interest Disclosure

The authors declare no conflict of interest related to this study. However, the method described for carotenoid measurement with melanin and hemoglobin correction is subject to a patent application by the primary author, titled "Method for Accurate Carotenoid Measurement in Human Skin by Correcting for Melanin and Hemoglobin Interference." This pending patent emphasizes the novel approach and commercial potential of the technique discussed in this manuscript.

Data Availability Statement

The data presented in this study are part of ongoing research under a pending patent application and are considered proprietary. As such, the raw and processed data cannot be shared publicly at this time. Researchers interested in specific aspects of the data may contact the corresponding author for potential collaboration or further information, subject to confidentiality agreements.

Disclosure Section

The author acknowledges the use of AI tools, specifically OpenAI's ChatGPT, for assistance in refining the language and grammar of the manuscript. No AI tools were used for data collection, analysis, or figure creation. All scientific content, methodologies, and results were developed by the author.

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