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# Area between Peaks Feature in the Derivative Reflectance Curve as a Sensitive Indicator of Change in Chlorophyll Concentration

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**Abstract:** Vegetation spectral features can detect chlorophyll concentrations. Two key spectral features evident in the first derivative (FD) of reflectance constitute the two main peaks: one located around 685–705 nm and the other near 710–725 nm. We propose that the area between peaks (ABP) can be used as a sensitive indicator of changes in the photosynthetic pigments at leaf level and demonstrate it using a high-spectral-resolution dataset of maize leaves collected by Gitelson and coworkers (2005). We find significant high positive correlations ( $r^2 > 0.90$ ) between chlorophyll concentrations and both the ABP and its continuum length feature.

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## INTRODUCTION

The most promising results for detecting the presence of plant stress are obtained by studying the sharp increase in reflectance of green vegetation normally between the range 670 and 780 nm, known as the vegetation red-edge, as mentioned by Horler et al. (1983). To resolve the red-edge feature, relatively narrow spectral bands are required. Filella and Peñuelas (1994) pointed out that high-spectral-resolution spectrometry offers the potential to estimate the relationship between the red-edge and the chlorophyll concentration for vegetation. Spectroscopy can detect many features in the reflected spectrum, such as the large increase in reflectance at the red-edge, the broad absorption features caused by liquid water in the leaf, and perhaps much finer absorption features caused by biochemicals within the leaf. As Clark (1999) observed, it gives finer details over a broader wavelength range and with greater precision.

In spectroscopy, derivative methods are a familiar term for spectral discrimination. It is a qualitative and quantitative technique to accentuate and assess small structural differences between spectra as defined by O'Haver et al. (1982). Researchers thus far have addressed applications of spectral derivatives in remote sensing (Demetriades-Shah et al., 1990; Philpot, 1991; Peñuelas et al., 1994). In the case of vegetation spectra, the first derivative curve reveals the red-edge inflection points

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(REIP) through the occurrence of peaks and troughs that may explain physiological changes of the vegetation. It provides important information about the spectral features (Bruce and Li, 2001, p. 1542).

Horler et al. (1983) and Curran et al. (1990) previously demonstrated an explicit relation between the wavelength of the REIP and the chlorophyll content of leaf samples. Laboratory studies showed a strong correlation between red-edge position and leaf chlorophyll content, such as the result from Gitelson et al. (1996). Moreover, the position of the REIP, as shown in modeling by Guyot et al. (1992), is controlled by factors such as canopy Leaf Area Index (LAI), leaf chlorophyll content, and leaf inclination angle.

A more in-depth analysis was conducted by Guyot et al. (1992) to compare spectral shifts characterized by the wavelength position of the REIP with varying chlorophyll concentration of maple and horse chestnut leaves. Results showed the presence of four inflection points, shown in four distinguishable peaks at 685–705, near 710, 720, and 740 nm. The same peaks define a vegetation feature termed the “double-peak” around the red-edge boundaries as found by Zarco-Tejada et al. (2002, p. 1438; 2003, p. 291). Gitelson et al. (1996) related the positions of the each individual peak to variations of chlorophyll concentrations.

In a study by Kruse et al. (1988), a technique was introduced for extracting spectral features using the continuum. A continuum in a spectrum is defined by finding the high points and fitting a straight line segment between these points. Applying this technique to the first derivative spectral peaks can reveal another important vegetation feature such as the area between two peaks. Salas (2004) used this technique, dealing with the first two pronounced peaks of the derivative spectra, and saw that the changing (deepening or narrowing) of the “double-peak” had caused the shape of the curve within the red-edge range to vary.

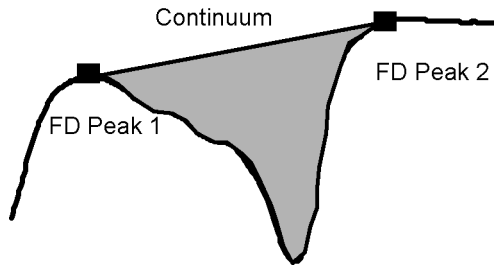
By integrating the previous findings of Gitelson and Merzlyak (1994), which made it possible to create sensitive indices directly related to Chl, with the behaviors of the area between and under the red-edge double-peak curve, a different view of the vegetation pigment responses can be obtained. This view may address index limitations (Stark et al., 2000, p. 242) and explain the movement of spectral peaks at longer wavelengths, which Gitelson et al. (1996) mentioned were not often completely resolved.

In this paper we aim to describe the change of the shape of the double-peak through the use of the area between peaks (ABP). Moreover, we evaluate the hypothesis that the area between peaks and the continuum line (Fig. 1) are sensitive to changes in chlorophyll concentrations, and assess how the movement of peak locations relates to vegetation status.

## MATERIALS AND METHODS

### Photosynthetic Pigments

The method in the determination of the leaf level chlorophyll content of maize follows that in Gitelson et al. (2005), as we used the same dataset. Reflectance measurements were collected in the range 400 to 900 nm using a black plastic polyvinyl chloride leaf clip, with a 2.3 mm diameter bifurcated fiber-optic attached to both an



**Fig. 1.** The first derivative (FD) curve of reflectance with the double-peak, area between peaks (ABP) (shaded), and the continuum connecting the peaks.

Ocean Optics USB2000 spectroradiometer and to an Ocean Optics LS-1 tungsten halogen light source. The system has a sampling interval of 0.3 nm and a spectral resolution of around 1.5 nm (details by Viña et al., 2004).

With the leaf clip, individual leaves are held with a 60° angle relative to the bifurcated fiber optic. A Spectralon reflectance standard (99% reflectance) was scanned for each leaf sample. The reflectance factor at each wavelength was calculated as the ratio of the upwelling leaf radiance to the upwelling radiance of the standard, and averaged across 10 separate scans made for each leaf. All scans were corrected for the instrument's dark current. After spectral readings, the measured areas of leaves were punched and total Chl (a and b) was determined analytically.

#### Area between Peaks and the Continuum

The ABP computation started by filtering the noise using a linear (mixture) combination of means of the field spectra measured from seven points, as used by Datt et al. (2003). The shapes and features that we expected to find in the hyperspectral data were thus preserved in the fitted model.

The Pythagorean Theorem proved as an efficient way to compute the length of the continuum. We applied equation 1, where  $\lambda_1$  is the wavelength of the first peak,  $\lambda_2$  is the wavelength of the second peak,  $R_1$  is the FD reflectance of the first peak, and  $R_2$  is the FD reflectance of the second peak.

$$\text{Continuum} = \sqrt{(\lambda_1 - \lambda_2)^2 + (R_1 - R_2)^2}. \quad (1)$$

Location of the peaks and the FD values were the data needed for this step. For ease of calculation, the SAMS software (Spectral Analysis and Management System, developed by and downloaded from the Center for Spatial Technologies and Remote Sensing at the University of California, Davis; <http://cstars.ucdavis.edu>) was used to analyze the absorption feature. Basically, SAMS employs the same principle as the composite-area analysis discussed by Meriam (1966) to calculate areas. The composite-area method integrates smaller areas, usually rectangular, into a bigger area. Integration starts at the first peak, then moves along the wavelength range, and ends at the location of the second peak. Once areas are computed, a ratio between the area under

the function (in a specified spectrum interval) and the area under the straight line connecting the maxima (the continuum line) is performed. Equation 2 shows the absorption feature equation:

$$a = 1 - \frac{Au}{Ac}, \quad (2)$$

where  $a$  is the absorption feature,  $Au$  is the area under the curve, and  $Ac$  is the area under the continuum line.

The method by SAMS may be confirmed manually by applying composite-area analysis, with few integral calculus concepts, integration of the sub-parabolic areas, and utilization of the equation of the sub-parabolic curve.

### Bootstrapping Regression

In bootstrapping statistics, multiple samples are developed by random sampling with replacement from the sample domain as mentioned by Efron and Tibshirani (1993). Applying the bootstrap regression is similar to running a regression model for each bootstrap data set and computing the beta coefficients ( $\beta$ ) that are used to predict the value of the dependent variable, in this case the Chl. In a nutshell, the bootstrap analogy is applied to test the bootstrapped difference in regression coefficients ( $\beta_0$  and  $\beta_1$  in a regression equation). We also used the method to check other properties of the general linear regression's estimators, such as the bias and standard error.

### Red-Edge Chlorophyll Index

The red-edge chlorophyll index ( $CI_{\text{red-edge}}$ ) was suggested by [Gitelson et al. \(2006\)](#) for Chl determination in leaves. The relationship between analytical Chl and the  $CI_{\text{red-edge}}$  measured with a linear best-fit function was  $r^2 > 0.94$ . We chose  $CI_{\text{red-edge}}$  to contrast results we derived from ABP. The detail of the index is shown in equation 3:

$$CI_{\text{red-edge}} = (NIR/Red-edge) - 1, \quad (3)$$

where  $NIR$  is the average reflectance from 770 nm to 800 nm and  $Red-edge$  is the average reflectance in the range 720–730 nm.

## RESULTS

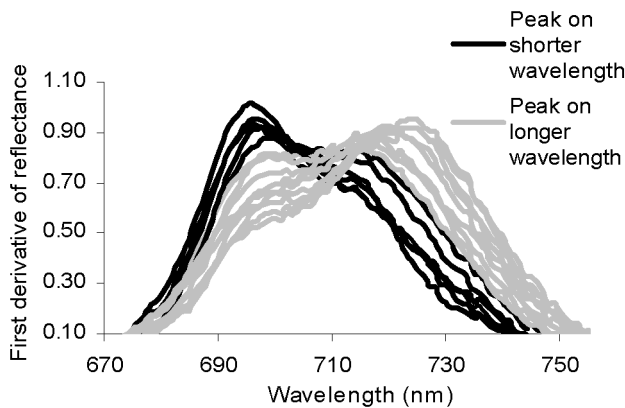
The basic statistics of the samples are presented in Table 1. Laboratory analytical extraction of pigments varied widely, with a broad range of total Chl values from 200.69 to 804.04 mg m<sup>-2</sup>.

The first derivative of the reflectance of maize is shown in Figure 2, where two groups are observed. The first group corresponds to the six samples with maximum peaks occurring at a shorter wavelength, around 685–705 nm, while the second group has the remaining nine samples with their maximum values at a longer wavelength, near 710–725 nm. Maximum peaks on a shorter wavelength have range values for

**Table 1.** Chlorophyll Content Statistics Used in This Study<sup>a</sup>

Pigment	Chlorophyll ( $\text{mg m}^{-2}$ )			
	Min	Max	Mean	Median
Chl a	160.93	651.13	363.95	346.13
Chl b	39.77	152.92	86.08	86.51
Total Chl	200.69	804.04	450.03	432.64

<sup>a</sup>Total number of samples used is 15.



**Fig. 2.** First derivative of reflectance of maize showing the double-peak, one around 685–705 nm and the other near 710–725 nm. No third or fourth peaks were evident in the vegetation considered.

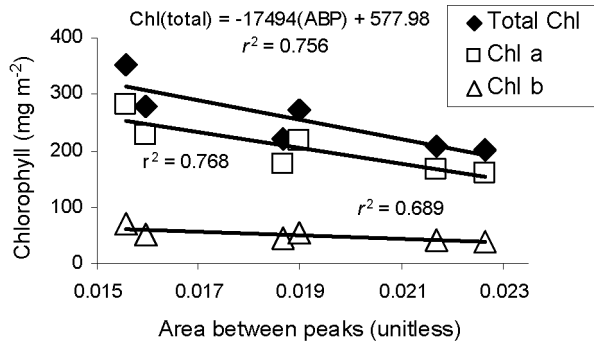
Chl from 270 to 350  $\text{mg m}^{-2}$ . Higher ranges for Chl are observed for those maximum peaks falling at longer wavelengths, 360–800  $\text{mg m}^{-2}$  compared to the first group.

### Area between Peaks

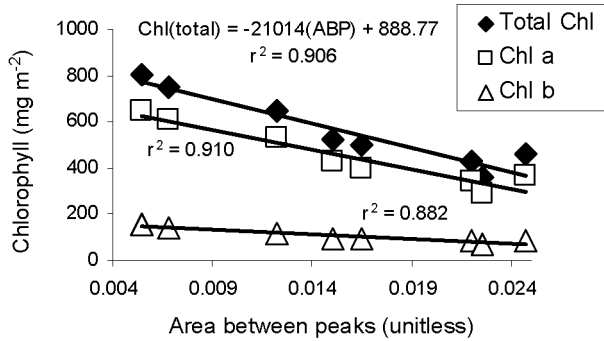
No distinct differences are seen in the range of values of the ABP for the two groups. The first has a range of 0.015 to 0.022, while the second spans 0.005 to 0.025. Nonetheless, the first group has a narrower range (0.007) than the second group (0.02). This disparity can be explained (details below) when the movement between peaks and the continuum line are taken into consideration.

### ABP Relationship to Chlorophyll Concentrations

The ABP exhibits sensitivity to chlorophyll (Chl) concentrations, including Chl a, Chl b, and total Chl, in samples with a maximum peak at shorter wavelengths (Fig. 3). High negative correlations are observed due to the rise of the first peak caused by



**Fig. 3.** Linear relationships between Chl versus the ABP for maize samples with a maximum peak on the shorter wavelength. Best-fit functions for each pair are represented by the regression lines, significant at  $p = 0.05$ .



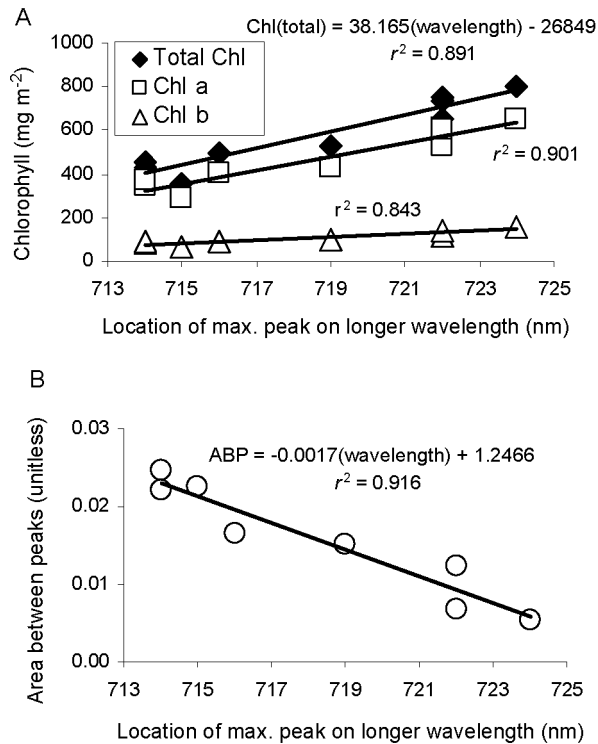
**Fig. 4.** Linear relationships between Chl versus the ABP for maize samples with a maximum peak on the longer wavelength. Best-fit functions for each pair are represented by the regression lines, significant at  $p = 0.05$ .

vegetation maturity as Chl increases, which in turn decreases the ABP. The rise of the peak ceases when Chl concentration exceeds  $45\mu\text{g cm}^{-2}$  (François et al., 2004, p. 13).

The ABP is more sensitive to Chl concentrations in samples with a maximum peak at longer wavelengths (Fig. 4). In contrast to the samples with a maximum peak at a shorter wavelength (first group), the samples show much higher coefficients of determination ( $r^2 > 0.90$ ) for total Chl when fitted against the area between peaks.

Gitelson et al. (1996) found that the second peak is related to the increased Chl concentration; hence, it becomes the main peak of the first derivative curve (Fig. 5A). The graph also confirms the already established concepts of the blue-shift and red-shift of the location of the maximum peak as previously investigated by Rock et al. (1988) and Clevers and Buker (1991).

Since ABP is sensitive to the movement of the second peak (Fig. 5B), the decrease of the ABP is instigated by Chl increases (Fig. 4). As Chl increases, the ABP decreases in response as the location of the maximum peak moves further to a longer



**Fig. 5.** Linear relationships between Chl (A) and ABP (B) versus the location of the maximum peak on the longer wavelength (second group) for maize. Best-fit functions for each pair are represented by the regression lines, significant at  $p = 0.05$ .

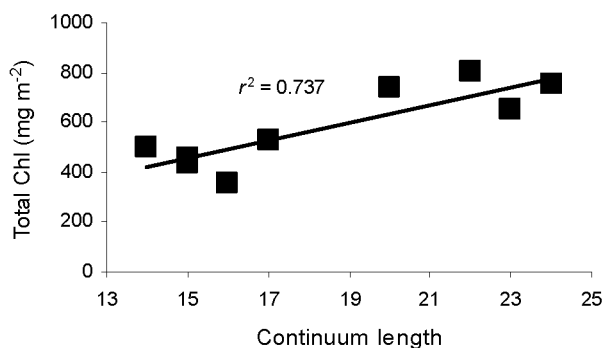
wavelength. François et al. (2004) also pointed to the emergence of the second peak as the concentration of chlorophyll increases.

### Changes of the Continuum Length in Relation to Chl

The minimum-distance straight line that connects the double-peak is referred to as “the continuum length” (see Fig. 1). It provides a metric to track the variation of the ABP and the movement of the maximum peaks. The decrease of the ABP is a result of the shifting of the peaks of the second group to a longer wavelength (due to Chl increase) and not because of the changes of the peak positions of the first group. Figure 6A illustrates how the length of the continuum varies directly with the increase of Chl content for the second group, in contrast to Figure 6B, which shows weaker correspondence. The continuum is seen to be sensitive to the shifting of the maximum peak located on the longer wavelength.

François et al. (2004) found a reduced shift (715–720 nm) for the second group compared to the shift of the first group (705–715 nm). The same results are observed in this study, with the first group having a shift from 685 to 705 nm and a reduced shift for the second group, 710 to 725 nm. However, even for a diminished shift, the





**Fig. 6.** Variation of the continuum length for samples with the maximum peak on the longer wavelength, second group, in relation to the increasing Chl content.

shifting to a longer wavelength of the second group still captures the increase in chlorophyll concentrations.

#### **ABP in Relation to Both Peaks**

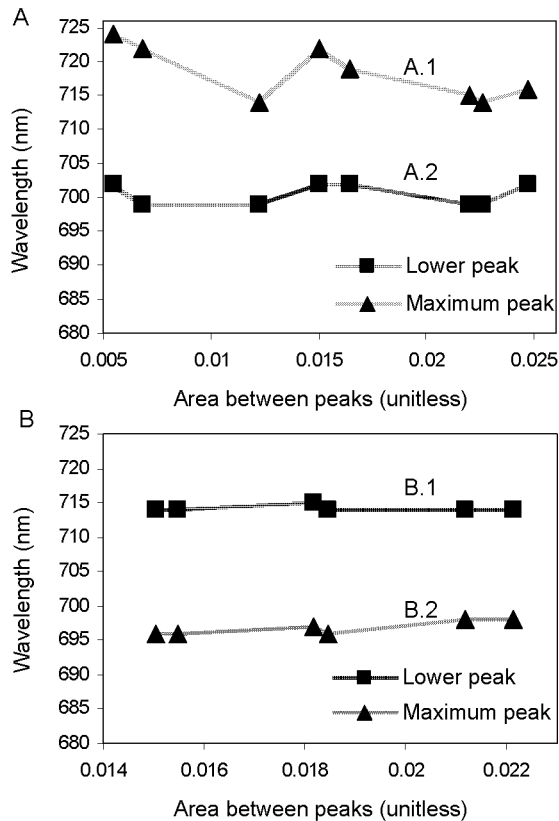
Figure 7 further illustrates the movement of all peaks, wavelength-wise, in relation to the ABP. Results from the second group (Fig. 7A) support that the maximum peak (A.1) is most affected by shifts to longer wavelengths because of the decrease of the ABP. The other peaks, regardless of where they are located (first or second group), do not appear to be affected by the increase of the ABP, with a pattern maintaining the same wavelength peak locations (A.1, B.1, and B.2).

#### **Red-Edge Chlorophyll Index**

The resulting linear best-fit function between CI and Chl is shown in Figure 8. The CI explains 95% of the total Chl variability. This high  $r^2$  is manifested when all samples, without regard of the location of the peaks, are taken all together in the analysis. However, the coefficient of determination decreased to 0.87 when samples that have peaks on shorter wavelengths are taken out of the equation. We note that samples with peaks on shorter wavelength show a stronger linearity for CI vs. Chl, with a higher  $r^2 = 0.96$ .

### **DISCUSSION**

The spectral derivatives in the neighborhood of the red-edge have an informative structure. The red-edge inflection point, easily seen as a peak, is a wavelength position that is affected by the changing of chlorophyll content as concluded by Clevers and Buker (1991) and Gitelson et al. (1996). A multiple-peak feature may be revealed: four to six peaks according to François et al. (2004) for simulated spectra, four peaks according to Gitelson et al. (1996), and more than one peak from other authors: Zarco-Tejada et al. (2003), Clevers et al. (2004), and Kooistra et al. (2004). The presence of multiple peaks creates a dilemma as to the decision of selecting the

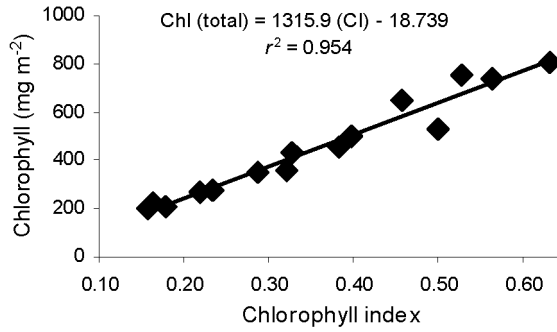


**Fig. 7.** Locations of the peaks for samples with the maximum peak on the longer wavelength, second group (A), and for samples with the maximum peak on shorter wavelength, first group (B) in relation to increasing ABP.

“real” maximum peak on the first derivative of the reflectance to serve as an REIP index for vegetation studies. Despite the possibility that several local peaks observed, two main and prominent peaks, or double-peak, are widely recognized. This study addressed the vegetation feature as first peak on a shorter wavelength and second peak on a longer wavelength.

It is seen that the maximum peak on a longer wavelength (second peak) is considered the main peak of the first derivative curve; its movement is found to be highly correlated with the ABP. Changes of the pigments are also seen to have caused the shifting, causing evidently high negative correlations between ABP vs. Chl.

In the situation when the first peak is dominant, an increase in magnitude of the ABP does not necessarily follow an increase of the photosynthetic pigment. Although the dominance of the first peak is related to the yellowing of leaves ([Gitelson et al., 1996, p. 503](#)) or lower chlorophyll content ([François et al., 2004, p. 14](#)), it is also related to the vegetation maturity. When the first peak goes up to a maximum, up to  $45 \mu\text{g Chl}/\text{cm}^2$  ([ibid., p. 13](#)), the ABP diminishes in response.



**Fig. 8.** Linear best-fit function for Chl (total) versus red-edge chlorophyll index (CI red-edge) combining all samples.

**Table 2.** Bootstrap Regression Statistics Using Observation Resampling (Correlation Model) to Obtain Properties of  $\beta_0$  and  $\beta_1$ <sup>a</sup>

	A. Peak on longer wavelength				B. Peak on shorter wavelength			
	Least square		Bootstrap		Least square		Bootstrap	
	$\beta_0$	$\beta_1$	$\beta_0$	$\beta_1$	$\beta_0$	$\beta_1$	$\beta_0$	$\beta_1$
Bias	0	0	-3.10	-62.88	0	0	-8.91	349.88
Standard error	47.01	2756	47.43	3,214	92.44	4,969	216.02	12,277
	95% confidence interval							
Lower bound	773.73	-27,758	791.54	-26,969	321.33	-3,1291	343.17	-27,461
Upper bound	1,003.80	-14,269	948.53	-15,363	834.63	-3,697	737.52	-5,381

<sup>a</sup>Using dataset for ABP and Chl from maize samples with a maximum peak on the longer wavelength (A) and maximum peak on the shorter wavelength (B). The statistics are based on 500 replications for each sample from the original dataset.

From the assessment of the ABP against the photosynthetic pigment, it is clear that the new vegetation feature plays a role in detecting small changes of the pigment concentrations, e.g. total Chl (all  $r^2 > 0.75$ ). Validation using bootstrap regression produces the results shown in Table 2, with comparable values of  $\beta_0$  and  $\beta_1$  when we resample the observations (correlation model). Bootstrap does a good job in estimating the properties of the beta coefficients, because the results from the method are not very different from the classical method. The resulting biases for the bootstrap are small (e.g., -3.10 and -62.88 for  $\beta_0$  and  $\beta_1$ , respectively) and the standard errors are comparable, especially for data samples from peaks on longer wavelengths. Results indicate that the ABP approach could supplement other methods to quantify Chl concentrations in plants.

The distance between peaks is a component of the ABP. Variations of the magnitude of the continuum length is related to the movement of the peak on the longer

wavelength. François et al. (2004) stated that the maximum of the peaks slowly shifts toward higher wavelengths, whether the maximum peak is at the shorter wavelength or the longer wavelength. However, we find that the shifting of the peak located on the shorter wavelength is not associated with the changing of the continuum length.

An advantage of the ABP method employed in this study is that it is robust, as relatively little error due to the utilization of SAMS is induced during the whole double-peak analysis. The authors also conducted manual computations of the ABP that have resulted in slightly higher  $r^2$  (all  $r^2 > 0.80$ ) when assessed against Chl. However, the latter is only for substantiation purposes and not recommended as an approach for ABP calculation as it takes up much time and is computationally expensive.

The comparison between the capabilities of the ABP against the CI as an indicator of change in chlorophyll concentration is vital. While the CI yields a higher coefficient of determination than the ABP ( $r^2 = 0.95$  vs.  $r^2 = 0.60$ ) when samples are combined, it is a bit weaker ( $r^2 = 0.87$  vs.  $r^2 = 0.91$ ) when samples have a higher Chl concentration. The possible explanation for the differences lies in the varying Chl that may have stimulated the slight movement of the peaks, which the ABP is initially designed for. However, the CI may have missed the movements at higher concentrations. Although CI seems to be a robust method, an advantage of the ABP in revealing peak shifting is something the CI fails to demonstrate.

It should be noted that this new feature provides an opportunity to look further into the first derivative curve generated from hyperspectral data. Given that the ABP is only a small component of the FD curve, smoothing procedures for signal denoising must be approached with caution. How much filtering can be applied to the spectra before a relevant feature, such as the ABP, disappears from the FD curve? The answer may depend on the characteristics of the signature at hand. In this study, the linear combination of means was found to be an efficient way to retain the two peaks.

## CONCLUSIONS

Despite a limited number of samples, we are confident about the general implications made of our findings for two reasons: (1) each individual hyperspectral signature visibly defined the peaks in question; and (2) the Chl pigments were laboratory-extracted from a leaf level with high accuracy.

The results of this study signify close relationships between the ABP feature, including the continuum length, and chlorophyll concentrations in maize leaves. The area between the two peaks found in the first derivative curve of a vegetation spectrum exhibited high sensitivity to the movement of the maximum peak located on a longer wavelength. When vegetation is green or when Chl is high, reflectance increases, followed by the decrease of the ABP, forcing the shift of the maximum peak on the longer wavelength to even longer wavelength positions (red-shift). As soon as the vegetation starts to yellow and chlorophyll decreases, the maximum peak subsequently relocates to a shorter wavelength, as the results suggest. This condition may be reversed during early stages of vegetation, with the maximum peak located at a shorter wavelength before shifting to a longer wavelength when vegetation grows or Chl builds up.

It is evident that continued yellowing and decrease of the photosynthetic pigments could cause the ABP to drop in magnitude and almost certainly disappear.

Further, these metrics (ABP and continuum length) do not indicate when the maximum peak on the longer wavelength (second peak) ceases to be the maximum and when the maximum peak on the shorter wavelength (first peak) begins to prevail. There is no means to tell what maximum level of Chl concentrations corresponds to the dominance of the second peak or the shifting of the maximum peak from longer to shorter wavelength, despite the fact that François et al. (2004) assigned a threshold Chl value ( $45\mu\text{g}/\text{cm}^2$ ) to indicate the shifting of the peaks. Additional study is required to investigate this phenomenon and its significance for monitoring vegetation status.

The efficiency of the ABP is dependent on the presence of the double peak found around 685–725 nm of the FD curve. Although our findings are specific to a hyperspectral dataset, we believe that the methodology used in this study could also be applied to other visible peaks, without regard to wavelength locations. Additional research is essential to verify the ability of the ABP feature to gauge levels of Chl concentrations for other crops, not only maize.

Finally, the investigation of the ABP and the method employed in this study realize two goals that were set forth: (1) describe the characteristics of the double-peak, with the continuum line alongside and its movement in relation to Chl concentrations; (2) show through regression analysis that the ABP feature can become a good estimator of chlorophyll concentrations for maize.

### ACKNOWLEDGMENTS

The authors thank A. Gitelson for his insights and for use of his spectral data, S. Wacker and M. Schickel for numerous discussions and critical comments on the manuscript, and J. G. P. W. Clevers for remarks on previous work by E. Salas. The research was supported in part by a GIScCE graduate research fellowship.

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