The Application of Photoacoustic Absorption Spectral Data to the Modeling of Leaf Optical Properties in the Visible Range

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***Abstract*—Due to the importance of plants in the Earth's ecosystem, their photobiological responses have become the subject of extensive research in life sciences. Leaf optical models have been developed to assist in the analysis of remotely sensed data to derive information on leaf biochemistry and anatomy from foliar spectral curves (transmittance and reflectance). In this paper, we investigate the implications of using *in vitro* pigment absorption spectra to model foliar optical properties in the visible domain. Typically, pigment absorption spectra have been determined using light absorption spectroscopy (AS) or by applying a data fitting approach. Alternatively, we propose the use of photoacoustic pigment AS, which, despite being available in the literature, has not been used in the modeling of foliar optical properties before. We also perform computational experiments in which foliar modeled spectral curves generated using these different absorption data sets are compared with actual measured data. Our findings indicate that the proposed alternative not only allows key pigments to be individually incorporated into the models, which, in turn, increases the predictability of the simulations, but also enables the generation of modeled foliar spectral curves that are more accurate than those obtained using absorption data derived from standard AS procedures.**

***Index Terms*—Leaf, photoacoustic absorption spectroscopy (PAS), pigments, reflectance, transmittance.**

I. Introduction

PLANTS play a vital role in the Earth's ecosystem by converting light energy, water, and carbon dioxide into organic compounds and oxygen. These byproducts fuel the food chain on which all life depends on and contribute to the exchange of gases that influence our planet's atmosphere and climate. Consequently, plants have become an important subject of theoretical and applied biological research. In areas such as forestry [1], agriculture [2], [3], and ecology [4], remote sensing has been used to monitor the health and development of crops and trees as well as the prediction of fire danger in live vegetation [5].

The development of predictive models to simulate the interaction of light with foliar tissue has improved our use of remote sensing data in these applications. Modeling leaf optical properties allow researchers to investigate links between the biophysical characteristics of plants and their spectral responses [6]. Based on these relationships, valuable information on leaf biochemistry and anatomy can be obtained from their measured reflectance and transmittance spectra. We believe that the use of physically measured data to test and validate such models strengthens their predictability as well as the reliability of their results. For a review of current leaf optical models, we refer the reader to [7] and [8].

In the visible range (400–700 nm), the reflectance and transmittance of fresh green leaves are influenced primarily by photosynthetic pigments, specifically chlorophylls and carotenoids. Therefore, it is necessary to take their absorption characteristics into consideration when modeling leaf optical properties in this spectral domain. To accomplish this, one needs to determine the absorption spectra of individual pigments. While this may appear to be a straightforward task, past studies have shown that it is not. Although many researchers have measured the spectra of pigments using light absorption spectroscopy (AS), comparison of published curves shows a lack of agreement between them [9].

The differences among published pigment absorption spectra can often be attributed to the impact of the preparative process on the purity of the pigment and the influence of the solvent used during the measurement process. For example, pigments should be extracted in the dark and measured soon after separation to avoid deterioration [10]. In addition, it has been shown that the same pigment sample measured in different solvents produced absorption spectra that differed in shape and wavelength position of their absorption maxima [11]. Consequently, it is difficult to determine the absorption spectra that are "best" for characterizing a particular pigment. However, more importantly, pigments in a plant leaf (*in vivo*) absorb light differently than those that have been extracted (*in vitro*). This is due to the optical effects associated with the distribution and molecular state of pigments under *in vivo* conditions as well as the leaf tissue itself (Fig. 1). After extraction, these optical effects disappear, altering the pigments' absorption spectrum. Hence, the measured absorption spectrum of a given pigment no longer reflects its *in vivo* absorptive properties. Therefore, regardless of which absorption spectrum is used, these optical effects should be taken into account since they may introduce undue bias into comparisons between modeled and measured foliar spectral data.

Leaf optical models have dealt with these issues in a variety of ways. Some focus on optical behavior outside of the visible

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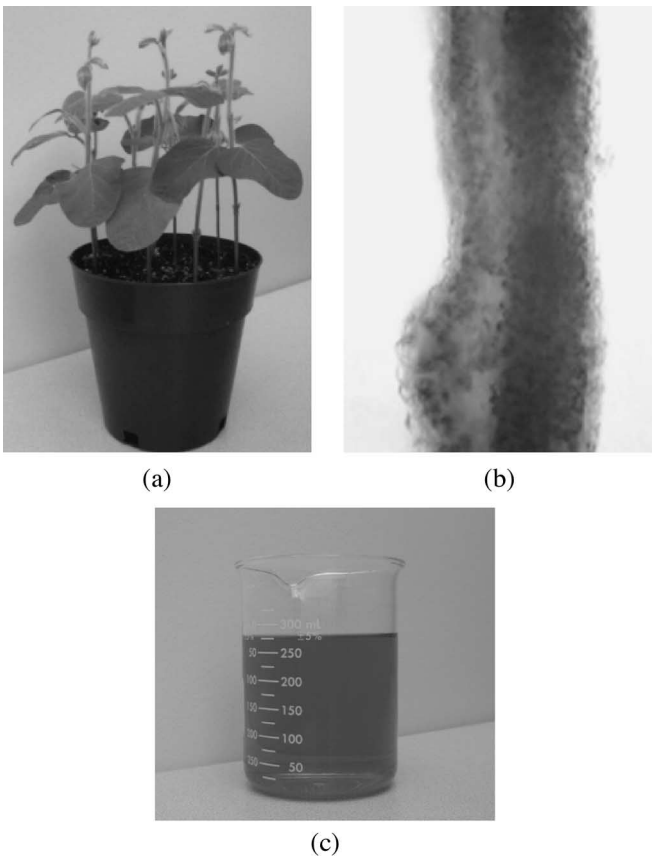

Fig. 1. Photographs illustrating chlorophyll occurrence in plant leaves. (a) Intact soybean (*G. max*, *S. hispida*) leaves in their natural state. (b) Microscope cross section of a soybean leaf showing the heterogeneous *in vivo* distribution of chloroplasts (cellular granules containing chlorophyll). (c) Homogeneous *in vitro* distribution of chlorophyll obtained by immersing crushed soybean leaf in acetone.

domain [12]–[15]. Others treat the whole leaf tissue as the absorber rather than individual pigments [16]–[19]. There are also models that employ indirectly determined pigment absorption spectra. For example, the model described by Yamada and Fujimura [20] expresses absorption as a linear function of pigment content, which uses a constant that must be determined using a data fitting approach (DFA). Another example is the leaf optical Properties Spectra (PROSPECT) model [21], which calculates foliar reflectance and transmittance curves using the specific absorption coefficient (s.a.c.) of biochemical constituents, including chlorophylls a and b, whose absorption spectra are combined into a single curve $a + b$. The s.a.c. of chlorophyll $a + b$ is determined using PROSPECT in conjunction with a DFA, whose description appears in a subsequent publication by Jacquemoud *et al.* [22]. Similarly, the Monte Carlo ray-tracing model [23] also includes chlorophyll $a + b$ in its formulation.

At present, only the following three models use the physically measured absorption spectra of individual pigments to simulate leaf optics in the visible range: 1) stochastic leaf radiation model (LFMOD1) [24], [25]; 2) leaf incorporating biochemistry exhibiting reflectance and transmittance yields (LIBERTY) [26]; and 3) stochastic model for leaf optical properties (SLOP) [27]. LFMOD1 uses the shifted absorption spectra of chlorophylls and carotenoids in ethanol and acetone to handle absorption by pigments. Testing was performed for a maple leaf using parameters drawn from literature, and a comparison of LFMOD1 results against measured reflectance data showed, as stated by Tucker and Garrat [24], that the modeled results did not account for the high degree of absorption, which occurs in the visible region of the light spectrum for green leaves. LIBERTY, which is an optical model designed for pine needles, uses the shifted absorption spectra of extracted leaf pigments in acetone. Biophysical and spectral measurements were used to parameterize and validate LIBERTY. As stated by Dawson *et al.* [26], the modeled results showed an excessive absorption level in the 400–470-nm region when compared to measured data for fresh leaves, and a poor agreement between modeled and measured "lobes" around the reflectance peak at 520 nm. Likewise according to Dawson *et al.* [26], the excessive absorption observed in the modeled results obtained using absorption coefficients for *in vitro* pigments in acetone can be accounted for by *in vivo* absorption effects, and the fact that, within the leaf, chlorophyll does not exist in free solution. Instead, it occurs as a pigment–protein complex (Section II-A). It is worth noting that the incorporation of absorption coefficients for combined chlorophyll and carotenoids determined by the model inversion procedures improved the quantitative agreement between the results provided by LIBERTY in the visible region of the light spectrum and the measured data [26]. SLOP, like LFMOD1, simulates absorption by chlorophylls and carotenoids. To characterize absorption by chlorophylls, Maier *et al.* [27] measured the absorption spectra of chlorophyll a and chlorophyll b in dimethyl sulfoxide, and then modified the resulting curves to reflect the influence of *in vivo* conditions. In contrast, absorption by carotenoids was determined by the unadjusted absorption spectrum of extracted β-carotene. Using test parameters derived from biophysical measurements, SLOP showed good quantitative agreement with the measured spectral data [27].

In this paper, we examine the underlying factors involved in the propagation of visible light in plant tissue under *in vivo* conditions, and how the use of *in vitro* data can affect the accuracy of models simulating these phenomena. Furthermore, we investigate the use of photoacoustic AS (PAS) [28] to improve the accuracy and reliability of foliar modeled spectral curves. We introduce a simple conversion technique for deriving the s.a.c. of individual pigments from their photoacoustic absorption spectrum, one that does not alter the measured curves significantly, and demonstrate the use of these spectra in the modeling of leaf optical properties in the visible range. Comparison between modeled and measured foliar spectral curves (reflectance and transmittance) indicate that the employment of pigment absorption spectra obtained through PAS not only mitigates the introduction of bias and mathematical inaccuracies in the simulations but also strengthens their predictability.

II. Data and Methods

In this section, we discuss issues related to the measurement of pigment absorption spectra and highlight effects that should be considered when using *in vitro* data to model the optical

properties of intact leaves. In addition, we describe direct and indirect methods normally employed for obtaining the absorption spectra of individual pigments such as chlorophylls and carotenoids. Finally, details on the model used to investigate the influence of absorption spectra on leaf reflectance and transmittance are given.

A. Measurement Issues

The primary pigments that affect the reflectance and transmittance of plants in the visible range are chlorophylls and carotenoids [29], of which chlorophyll a, chlorophyll b, and β-carotene are the most common forms found in green plants [30], [31]. To measure these pigments, an extract is prepared using a sample of leaf tissue mixed with an organic solvent, such as acetone, and then individual pigments are separated using a chromatographic procedure [32] before being measured. However, due to differences in surrounding environment, distribution, and state, the absorption of pigments under *in vitro* conditions differs from that of pigments under *in vivo* conditions. *In vivo* chlorophylls and carotenoids occur as pigment–protein complexes, inhomogeneously distributed throughout the leaf tissue, which is an intensely scattering medium [33]. These factors affect the passage of light through the leaf and the probability that an incident light ray will be absorbed. Because the extraction process changes the environment, distribution, and complexing of pigments, the probability that an incident ray will be absorbed by chlorophylls or carotenoids *in vitro* is not the same as *in vivo*. Consequently, models that use *in vitro* data to characterize pigments must account for these *in vivo* optical effects; otherwise, the modeled spectral curves will not accurately reflect that of an intact leaf.

The cross section of a typical leaf (Fig. 1) can be concisely described as follows. The two outermost layers consist of a waxy cuticle over a layer of close fitting epidermal cells. In between is the mesophyll layer, which may be differentiated between cylindrical densely packed palisade cells and ovoid more loosely packed spongy cells. The chloroplasts (cellular granules containing chlorophyll and nitrogen [34]) are located in the mesophyll cells.

When an incident light ray passes through the leaf's surface layers into the mesophyll tissue, differences between the refractive indexes of intercellular air spaces and cell walls causes the ray to reflect or refract [35]. Multiple internal reflections and refractions of the light ray will lengthen its optical path length and increase the probability that it will encounter absorbers, which are nonuniformly distributed within the leaf [36]. This lengthening of the optical path is referred to as the detour effect, and it leads to a higher or an increased rise in *in vivo* absorption values [37], [38], which is more noticeable in bands of absorption minima as discussed by Garlaschi *et al.* [39]. In contrast, inhomogeneous distribution of pigments throughout the leaf tissue (Fig. 1) can lead to a situation where an incident light ray passes through the leaf without encountering any pigment at all [40]. This is referred to as the sieve effect, and it results in lower or a decreased rise in *in vivo* absorption values [37], which is more noticeable in bands of absorption maxima as discussed by Rabinowitch [33].

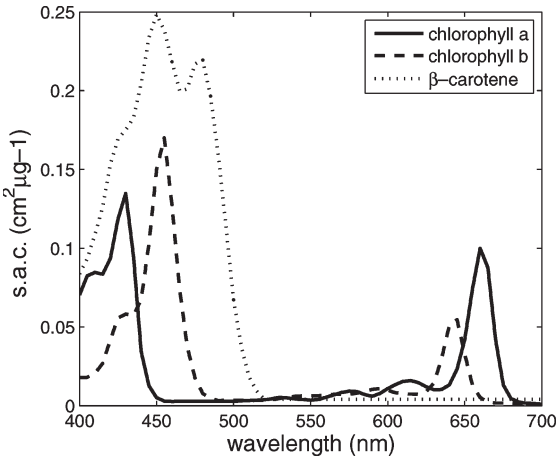

Fig. 2. s.a.c. of chlorophylls in ethyl ether [10] and β-carotene in hexane [48]. Note that the similar absorption behaviors of chlorophylls a and b are associated with their similar molecular structure [53]–[55].

To account for changes in the lengthening of the optical path under *in vivo* conditions when using *in vitro* pigment absorption curves, several researchers choose to employ an adjustment parameter known as the factor of intensification β [41], to scale the curves. Values for β have been determined by Rühle and Wild [42] and McClendon and Fukshansky [43] for several plant species using a statistical methodology.

Another issue that must be considered when interpreting *in vitro* absorption spectra is the occurrence of *in vivo* chlorophylls and carotenoids as pigment–protein complexes [44]. The organic solvent used to prepare leaf extracts destroys pigment–protein bonds and breaks down the complexed form of pigments under *in vitro* conditions. This results in a band shift toward the shorter wavelengths and a flattening of the absorption spectrum [33], [45], [46].

B. Measurement Methods

In light AS, pigments are isolated and purified from prepared leaf extract before being mixed with a solvent. An integrating sphere is used to measure how much incident light is reflected and transmitted by the pigment–solvent sample to determine its s.a.c. Although many researchers have measured the absorption spectra of pigments, disagreements between studies exist primarily due to the impact of the extraction and separation process on pigment purity, as well as the influence of the solvent on scattering [9]. From the available literature, we selected the absorption curves for chlorophylls a and b in ether solution provided by Zscheile and Comar [10], [47] and the absorption curve for β-carotene in hexane solution provided by Zscheile *et al.* [48] to be used in our experiments. These curves are presented in Fig. 2. It is worth noting that although the reliability of the seminal work done by Zscheile's group has been confirmed by several authors [32], [49], [50], the AS of photosynthetic pigments continues to be an active area of investigation [51], [52].

PAS is based on the photothermal effect, which was discovered by Alexander Graham Bell in 1880. Details on the theoretical foundations of the photothermal effect, experimental

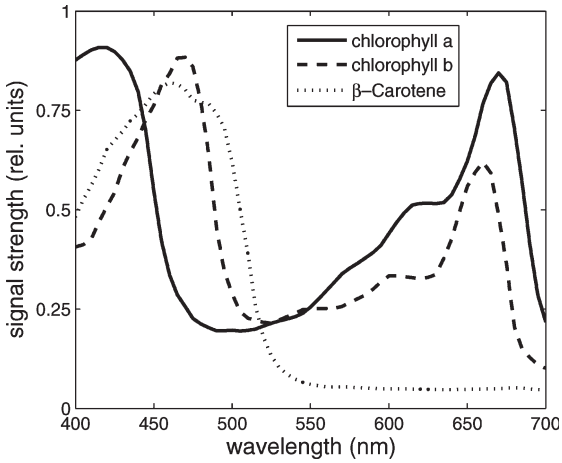

Fig. 3. Photoacoustic absorption spectra of individual pigments chlorophyll a, chlorophyll b, and β-carotene. Relative signal strength is determined by referencing the pigments' measured photoacoustic signal strength against the measured photoacoustic signal of carbon black [62].

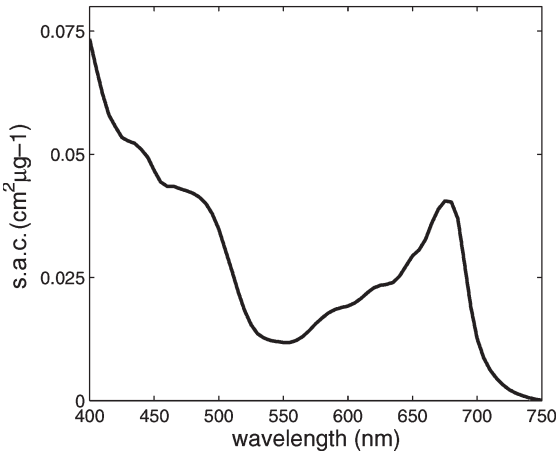

Fig. 4. s.a.c. of chlorophyll $a + b$ determined using PROSPECT model in conjunction with a DFA [22].

setup, and applications of PAS in fields such as medicine and biology can be found in [28] and [56]. Briefly, this technique uses pulsed light to illuminate a sample in an enclosed gas-filled cell that is referred to as a photoacoustic cell. The incident light is absorbed, which causes the sample to enter into an excited state. Deexcitation of the sample can take place in a number of different ways including the reradiation of the absorbed energy as thermal energy or heat. When the sample radiates heat, it also raises the temperature of the surrounding gas and, correspondingly, the pressure inside of the photoacoustic cell. The pulsed nature of the incident light causes the pressure to change in a similar manner, generating waves that can be picked up with a detector, such as a microphone. Plotting the relative signal strength generated by the sample at different wavelengths of incident light produces a photoacoustic absorption spectrum, which qualitatively resembles that of an absorption spectrum.

PAS has many advantages over other forms of spectroscopy including the ability to obtain the optical and thermal properties of highly scattering solid and semisolid materials such as powders, gels, suspensions, and tissues. The Rosencwaig–Gersho theory [56] provides a formulation relating the depth of the material from which the photoacoustic signal is detected, i.e., the thermally active layer, to the rate at which the incident light is pulsed. Due to damping effects, the lower the pulse rate, the deeper the thermally active layer is located. Thus, this method allows us to conduct nondestructive *in vivo* studies at varying subsurface layers of the sample (depth profile analysis). These advantages make PAS well suited to the study of plants, particularly photosynthesis research [57]–[61]. For our investigations, we will be using the photoacoustic absorption spectra of chlorophyll a, chlorophyll b, and β-carotene (Fig. 3). To the best of our knowledge, these are the only photoacoustic absorption curves for individual pigments available in the literature.

In contrast to physical measurements, many researchers have run simulations in conjunction with DFAs to determine absorption coefficients from measured spectral data. Although many candidates exist, we select without loss of generality the curve for chlorophyll $a + b$ provided by Jacquemoud *et al.* [22] (Fig. 4) since they have been the subject of several works relating foliar optical properties to biochemical constituents [6].

C. Modeling Leaf Optical Properties

To model the leaf optical properties of plant leaves, we will be using the algorithmic bidirectional scattering distribution function model for bifacial leaves (ABM-B) [63], [64]. ABM-B uses Monte Carlo methods to simulate the passage of light photons through plant tissue. Probability distributions calculated from parameters relating to leaf physiology and biochemical constituents are used to randomly determine whether a photon is absorbed, reflected, or refracted. For a detailed description of these models, we refer the interested reader to recent publications by Baranoski [63] and Baranoski and Eng [64]. This model was selected due to the ease with which the absorption spectra of individual pigments could be incorporated into the simulation. Furthermore, its predictability has been evaluated against measured data [63].

The ABM-B was originally employed to simulate the interaction of infrared radiation (750–2500 nm) with plant leaves. To allow its use to simulate the interaction of visible light (400–700 nm) with foliar tissue, its parameter space required several modifications. Since absorption in the visible range is dominated by pigments, the relatively low absorption by other leaf constituents, such as protein and cellulose + lignin, were not taken into account in our experiments. The specific absorption spectrum of water was obtained from data measurements performed by Pope and Fry [65]. In addition, its refractive index was set to an average value of 1.33, since it does not vary significantly in the visible range [66]. Finally, with the incorporation of pigments in the modeling of leaf optical properties, the calculation of the effective absorption coefficient was modified to include the s.a.c. and the concentrations of chlorophyll a, chlorophyll b, and carotenoids.

TABLE I
MEASURED (LOPEX) BIOPHYSICAL DATA USED TO COMPUTE THE MODELED SOYBEAN SPECTRAL SIGNATURES

Biophysical measurement	Value
Area (cm^2)	4.10
Thickness (cm)	0.0166
Fresh weight (g)	0.0494
Dry weight (g)	0.0119
Fresh chlorophyll a content (mg/g)	2.74
Fresh chlorophyll b content (mg/g)	0.80
Fresh carotenoid content (mg/g)	0.78

TABLE II
BIOPHYSICAL PARAMETERS USED TO COMPUTE THE MODELED SOYBEAN SPECTRAL SIGNATURES

Symbol	Biophysical parameter	Value
t_m	Thickness of mesophyll (cm)	0.00830
C_a	Concentration of chlorophyll a (g/cm^3)	0.003978
C_b	Concentration of chlorophyll b (g/cm^3)	0.001161
C_c	Concentration of carotenoids (g/cm^3)	0.001132
δ_c	Aspect ratio of cuticle	5.0
δ_e	Aspect ratio of epidermis	5.0
δ_p	Aspect ratio of palisade mesophyll	1.0
δ_s	Aspect ratio of spongy mesophyll	5.0

D. Biophysical Data and Experimental Setup

Measured biophysical data for testing and spectral data for validation was obtained from the Leaf Optical Properties Experiment (LOPEX) data set [67]. We selected, without loss of generality, soybean (*Glycine max*, *Soja hispida*) because of its standard foliar characteristics and the large variety of experimental data available for comparison [68], [69]. For soybean reflectance and transmittance, we used LOPEX spectral files 0219 and 0220. A virtual spectrophotometer was used to generate the modeled spectra for comparison [70].

Table I presents the LOPEX biophysical data used to determine the concentration of constituents. Pigment concentration was calculated by multiplying the fresh weight content of each pigment by the fresh weight of the leaf, and dividing that result by the mesophyll volume. The mesophyll volume was chosen, as opposed to leaf volume, since pigments are located in chloroplasts, almost all of which are found in the mesophyll tissue [71]. The mesophyll volume was determined by multiplying leaf area by mesophyll thickness, which we estimate to be 50% (based on morphological characteristics of bifacial leaves [35], [72]–[74]) of the total leaf thickness.

In addition to the concentration of constituents, aspect ratios used to characterize the shape of cells in the cuticular, epidermal, and mesophyll layers of the leaf were specified [63]. Table II presents a summary of the parameter values used to model the soybean specimens used in our experiments.

III. METHOD TO CONVERT PHOTOACOUSTIC ABSORPTION SPECTRA

Because photoacoustic signals are generated by the nonradiative deexcitation of absorbed energy, photoacoustic absorption spectra closely resemble that of light absorption spectra. However, it is difficult to make direct quantitative comparisons because of other deexcitation processes that can take place but do not result in the production of heat. For example, in addition to heat emission, pigments can channel absorbed energy into fluorescence emission or photosynthesis [55], [57]. However, if either of these processes become blocked or interrupted, a larger percentage of the absorbed energy is emitted as heat, and stronger photoacoustic signals are observed. Buschmann and Prehn [75] demonstrated this by comparing the photoacoustic absorption spectra of a healthy leaf with that treated with 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (DCMU), which is a chemical that inhibits the electron transport chain used in photosynthesis. The photoacoustic signals observed for the DCMU-poisoned leaf were higher than those of the healthy one, as a decrease in photosynthesis led to an increase in heat. Consequently, the photoacoustic absorption spectra come closer to quantitatively approximating its light absorption spectra.

As described in Section II-A, under *in vivo* conditions pigments occur as pigment–protein complexes. Two complexes of particular importance for photosynthesis studies are the chlorophyll a–protein complex referred to as P700, and the chlorophyll a/b–protein complex referred to as P680 [55]. Both P700 and P680 are responsible for performing the photochemical reactions that drive photosynthesis. The role of other pigments, including chlorophyll b and β-carotene, are to collect and transfer energy to these chlorophyll a complexes. The photoacoustic absorption spectra presented earlier (Fig. 3) were that of pigments separated from leaf extract using thin layer chromatography. Due to their separation, chlorophyll b and β-carotene are prevented from transferring their captured light energy to P700 and P680, and thus a larger percentage of the absorbed energy is emitted as heat [76]. Furthermore, the organic solvent used to prepare the extract destroys the bonds of the photosynthetically active chlorophyll a complexes. This decrease in photosynthetic ability should also lead to an increase in heat. Our assumption is supported by Veeranjaneyulu and Das [77], who compared the photoacoustic absorption spectra of an intact leaf and its extract in acetone and observed that the extract exhibited predominately higher photoacoustic signals than that of the leaf. Consequently, we expect that under *in vitro* conditions, separated pigments channel a larger percentage of their absorbed energy into heat emission than any other deexcitation process. This give us reasonable confidence that the photoacoustic absorption spectra of the separated *in vitro* pigments presented in Fig. 3 will closely correspond to their light absorption spectra.

Several guidelines were adopted to assist in the development of a conversion method for determining s.a.c. values from measured photoacoustic signals.

First, s.a.c. values are equated to corresponding photoacoustic signals at wavelengths of weak light absorption. In this paper, we are dealing primarily with green plants so wavelengths of weak absorption are those in the green region of the visible light spectrum, which is approximately 500–560 nm. By focusing on wavelengths of weak light absorption, it is less likely that the critical energy levels necessary to trigger a fluorescent or photosynthetic reaction will be reached. Consequently, this increases the probability that absorbed energy will be channeled into heat emission and the likelihood

that a photoacoustic signal will reflect the total amount of absorbed light energy.

Second, minimum s.a.c. values are equated to their corresponding photoacoustic signals. As described previously, total absorbed light energy is channeled into photosynthesis, fluorescence, and heat emission. Therefore, to minimize the amount of absorbed energy missed by a photoacoustic signal due to photosynthesis and fluorescence, we minimize the total absorbed light energy, i.e., focus on minimum s.a.c. values. Again, this increases the likelihood that a photoacoustic signal will come closer to reflecting the total amount of absorbed light energy.

Using these guidelines, a simple approach for converting a photoacoustic absorption spectrum, which is expressed in relative units, to a light absorption spectrum is to multiply the photoacoustic signals by a selected scaling value. This allows one to obtain the specific absorption spectrum for each pigment that we need to account for in the simulations. Furthermore, if each photoacoustic absorption spectrum is scaled by the same value, the independent nature of the physically measured data is preserved since the shape of each curve is maintained as well as their positions relative to one another. To find such a value for all three pigments, we look at the intersection points of their light absorption curves (Fig. 2) and photoacoustic absorption curves (Fig. 3).

Although both sets of curves do not intersect at a single point in the green region of the visible light spectrum, they get closer within small sectors centered at approximately the same wavelength, namely 520 nm for the light absorption curves and 517.5 nm for the photoacoustic absorption curves. We consider this difference (2.5 nm) acceptable since it is smaller than the spectral resolution of the data (5 nm). Averaging the absorption coefficient data points (for the three pigments) within the first sector, we obtained an average absorption coefficient of 3781.06 cm^2/g. Averaging the photoacoustic signal data points (for the three pigments) within the second sector, we obtained an average photoacoustic signal of 0.213. Finally, dividing the average absorption coefficient by the average photoacoustic signal, we obtained the scaling value (17 751.46 cm^2/g) used in our experiments.

IV. Results and Discussion

Modeled spectral curves for soybean were generated using absorption spectra obtained through light AS, conversion from photoacoustic absorption spectra, and calculation using a DFA (Figs. 2–4). The AS and PAS absorption spectra correspond to individual pigments, whereas the DFA absorption spectra corresponds to the combined absorption of chlorophylls a and b. Consequently, when using this curve as an input to the leaf optical model, it was treated as the absorption spectrum of chlorophyll a. The concentration of chlorophyll a was set to include the concentration of chlorophyll b, while the concentration of both chlorophyll b and β-carotene were set to zero.

The graphs presented in Fig. 5 show good quantitative and qualitative agreement using the DFA absorption spectra. In contrast, modeled results using the PAS absorption spectra are qualitatively similar, but both reflectance and transmittance

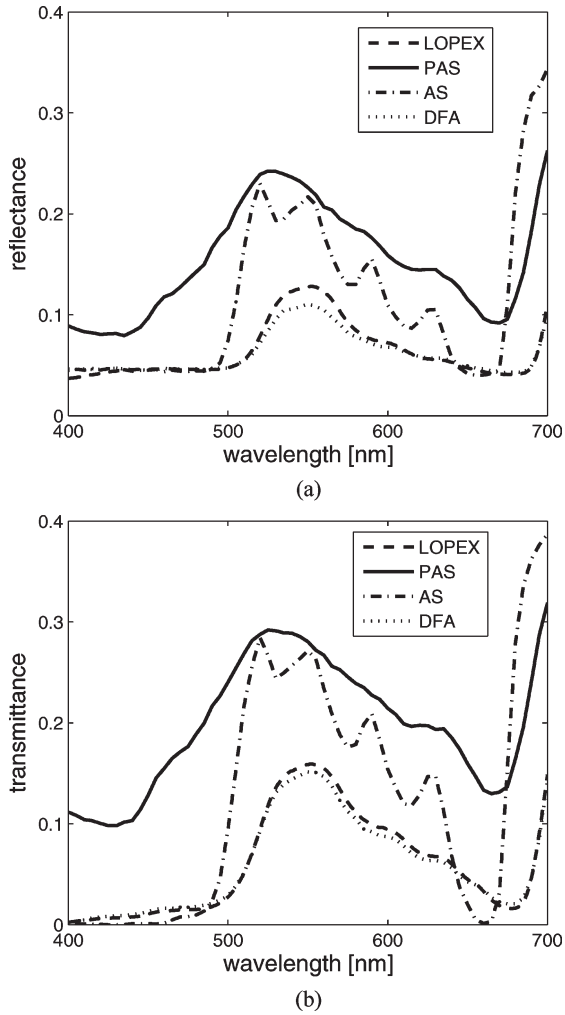

Fig. 5. Comparison between measured (LOPEX) and modeled (ABM-B) (a) reflectance and (b) transmittance curves of soybean leaf (*G. max*, *S. hispida*). Modeled curves were generated using pigment spectra obtained through AS, PAS, and DFA.

values are higher than the measured data. The AS spectral curves show the greatest difference, both qualitatively and quantitatively, when compared with the LOPEX curves. From 400 to 500 nm, there is close quantitative agreement before the curve sharply rises. Between 500 and 640 nm, the modeled values are higher than the measured values, and three minor minima occur at 530, 575, and 615 nm. In Fig. 5(b), the curve also exhibits distinct minima at 660 nm that drops transmittance values below the measured values.

To assist in the evaluation of the quantitative differences between the modeled and measured spectra, the corresponding root-mean-square errors (RMSE) have been computed (Table III) according to the following expression:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=0}^{60} \left(S_{\mathrm{lopex}}(\lambda) - S_{\mathrm{abm}}(\lambda)\right)^2}{61}} \tag{1}$$

where λ is the wavelength ($400\ \mathrm{nm} + i * 5\ \mathrm{nm}$), S_{lopex} is the value of the LOPEX spectral curve (reflectance or transmittance) at wavelength λ, and S_{abm} is the value of the ABM modeled spectral curve at wavelength λ.

TABLE III
COMPUTED RMSE VALUES FOR THE MODELED SOYBEAN SPECTRA USING RAW ABSORPTION SPECTRA

	AS	DFA	PAS
reflectance	0.0846	0.0074	0.0961
transmittance	0.1081	0.0049	0.1419

TABLE IV
FACTORS OF INTENSIFICATION SELECTED FOR THE PIGMENTS CHLOROPHYLL a (β_a), CHLOROPHYLL b (β_b), AND β-CAROTENE (β_c) BASED ON PUBLISHED VALUES [42]

Factor of Intensification	Value
β_a	2.0
β_b	4.1
β_c	4.1

The RMSE values for the spectra generated using the DFA absorption spectra are less than 0.03, which according to Jacquemoud *et al.* [22] indicates good spectral reconstruction for both reflectance and transmittance. The RMSE values for the spectra generated using the AS and PAS absorption curves are quite high, i.e., between 0.0846 and 0.1419, highlighting the poor quantitative agreement with the actual measured data as observed in Fig. 5(b).

A second set of modeled curves was generated using adjusted AS and PAS absorption spectra. Adjustments were made to account for optical effects associated with *in vivo* pigments. No adjustments are necessary for the DFA curves since they are obtained using a process that computes absorption values from whole leaf values. Hence, they implicitly account for *in vivo* effects (see Section II-A).

The first adjustment involved correcting for the detour effect. As previously discussed in Section II-A, the detour effect is caused by the leaf tissue, which is a highly scattering medium. The optical path length of incident rays is lengthened, and this increases the probability of absorption under *in vivo* conditions. Therefore, to account for the detour effect, the *in vitro* s.a.c. values are scaled by a factor of intensification β. According to Rühle and Wild [42], the factor of intensification is most closely related to the pigment concentration, and McClendon and Fukshansky [43] observed that β values are remarkably uniform across species. Consequently, in the absence of specific data (factor of intensification) measured for the specific species under consideration in our experiments, we use the best possible data in the available literature. Although this may not be an optimal procedure, we believe that the benefits that come from its use outweigh the negative effects that one may get by neglecting the lengthening of the optical path length. Based on factors of intensification β computed for different plant species with respect to different pigment concentrations [42], we have selected the β values presented in Table IV to scale the absorption curves (AS and PAS) employed in our second set of experiments. For details on how these values were selected, we refer the interested reader to the first authors' thesis [78].

The second adjustment involved a shift of the pigment absorption spectra toward the red to account for *in vivo* pigment–protein complexes. Several research works consider

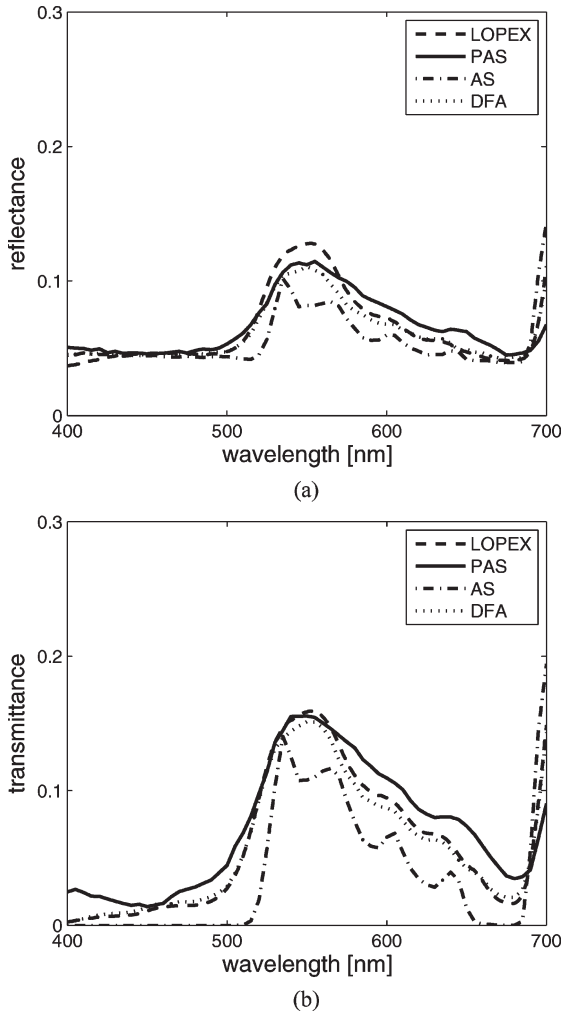

Fig. 6. Comparison between measured (LOPEX) and modeled (ABM-B) (a) reflectance and (b) transmittance curves of soybean leaf (*G. max*, *S. hispida*). Modeled curves were generated using pigment spectra obtained through AS, PAS, and DFA. The AS and PAS pigment spectra were multiplied by factors of intensification β (Table IV) and shifted 15 nm to account for *in vivo* optical effects.

the spectral shift of chlorophyll a to be approximately 15 nm [9], [79]. Although it has been established that chlorophyll b and carotenoids also undergo a similar *in vivo* to *in vitro* spectral shift, it has been more difficult to determine since the more strongly absorbing chlorophyll a masks their *in vivo* absorption bands [9]. Therefore, to avoid any undue bias, a uniform shift of 15 nm has been applied to all pigment spectra.

Fig. 6 shows an improved quantitative agreement obtained using the adjusted PAS absorption spectra. Both the modeled transmittance and reflectance values were lowered and more closely resemble the measured values. In contrast, the spectra modeled using the adjusted AS absorption spectra did not exhibit significant improvements. Although the reflectance and transmittance values produced by the AS absorption spectra were lowered, they were generally below the measured values. This difference is particularly noticeable in Fig. 6(b) between the wavelength ranges 400–510 and 660–690 nm, where transmittance values are close to zero. Furthermore, the adjusted AS spectral curves display the same minor minima

TABLE V
COMPUTED RMSE VALUES FOR THE MODELED SOYBEAN SPECTRA USING ADJUSTED ABSORPTION SPECTRA

	AS	DFA	PAS
reflectance	0.0182	0.0074	0.0102
transmittance	0.0315	0.0049	0.0172

observed in the nonadjusted AS spectral curves. These minor minima occur at approximately 545, 690, and 630 nm, which correspond to our shift of the pigment absorption spectra by 15 nm.

Table V presents the RMSE values for the curves shown in Fig. 6. With the exception of the transmittance curve generated using the AS absorption spectra, values are less than 0.03, which indicates good spectral reconstruction [22]. The RMSE values for the adjusted absorption spectra are smaller than those computed for the raw absorption spectra (Table III), demonstrating improved quantitative agreement using the adjusted absorption values.

Based on our experiments, the DFA and adjusted PAS absorption spectra produced the closest approximations with respect to the measured (LOPEX) spectral curves. We remark, however, that the use of PAS absorption spectra allows the incorporation of separate data for individual pigments in the simulations. In addition, the PAS absorption spectra are determined through direct physical measurement, i.e., independent of any particular model or process. These aspects illustrate the advantages of using PAS absorption spectra in the modeling of foliar optical properties.

An open issue related to the PAS absorption spectra is its reliance on the selected conversion method and, in particular, the selected scaling value used to convert the photoacoustic signal values to absorption coefficients. Although we have adopted a simple approach, one that avoids making any modifications to the shape or position of the original photoacoustic absorption spectra, alternatives may exist. This is a potential avenue for future work. However, regardless of the scaling value applied, it is evident that qualitatively the PAS absorption spectra more closely reflects the absorption of pigments, particularly when compared with results produced by the AS absorption spectra.

The AS absorption spectra generated spectral curves that exhibited three minor minima at 530, 575, and 615 nm. Similarly, the adjusted AS absorption spectra exhibited a shift in the minima, which is consistent with the 15-nm shift of the pigment spectra. Further investigation shows that the wavelength positions of these minima correspond to the wavelength positions of minima in the chlorophyll a absorption curve (Fig. 2). Comparison of the AS absorption spectra and the original photoacoustic spectra (Figs. 2 and 3) shows that in the wavelength range from 550–650 nm, the AS absorption spectra fluctuates, whereas the photoacoustic spectra is smooth. The smoothness of the photoacoustic curve in this region can be attributed to the increased sensitivity of PAS to low concentrations of pigments [62], [80], [81]. This feature gives PAS an advantage over light AS when obtaining the absorption of pigments for the modeling of leaf optical properties.

V. CONCLUSION

The physically based modeling of leaf optical properties in the visible range using individual pigments has clearly proven to be a difficult task. As outlined previously, many models focus on wavelengths outside of this spectral domain or treat absorption at the tissue level, which avoids having to address absorption by individual pigments.

Typical methods for determining the absorption properties of pigments are direct measurement using light AS or by applying a DFA to measured spectral data. In this paper, we introduced a third alternative, one that uses photoacoustic spectra to determine the absorption coefficients of leaf chlorophylls and carotenoids. To avoid significant modifications to the physically measured curve, a simple scaling procedure to convert photoacoustic signals to their corresponding absorption coefficients was proposed. This conversion method was evaluated by performing a comparison of the modeled results using the different absorption spectra. Our results showed that, once *in vivo* optical effects had been taken into account, the PAS absorption spectra produced results that were in good agreement with measured spectral curves.

To address the problem of obtaining accurate *in vivo* absorption spectra, many studies have adopted a DFA. To the best of our knowledge, this is the first work to introduce a measurement method, namely PAS, as a potential solution. The demonstrated applicability of photoacoustic absorption spectra in the modeling of leaf optical properties using individual pigments is a strong evidence that PAS is a competitive alternative to current methods. Furthermore, we have highlighted the importance of considering *in vivo* optical effects when using absorption spectra to model foliar optics. Our computational experiments showed that although the process used to obtain the DFA absorption spectra implicitly includes the influence of the detour effect and pigment–protein complexes, the AS and PAS absorption spectra do not and should be adjusted accordingly.

While PAS has primarily been used to investigate the properties of foliar tissue, we hope that this paper motivates further investigation into the application of photoacoustic absorption spectra in the modeling of leaf optical properties. As future work, we intend to explore alternative methods for converting photoacoustic absorption spectra and to expand the scope of our experiments to other plant species, particularly those with significant differences in structural characteristics such as unifacial leaves, and to other pigments such as anthocyanins, as absorption data for these pigments becomes available.

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