



Retrieval of foliar information about plant pigment systems from high resolution spectroscopy

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ABSTRACT

Life on Earth depends on photosynthesis. Photosynthetic systems evolved early in Earth history and have been stable for 2.5 billion years, providing prima facie evidence for the significance of pigments in plant functions. Photosynthetic pigments fill multiple roles from increasing the range of energy captured for photosynthesis to protective functions. Given the importance of pigments to leaf functioning, greater effort is needed to determine whether individual pigments can be identified and quantified *in vivo* using high fidelity spectroscopy. We review recent advances in detecting plant pigments at the leaf level and discuss successes and reasons why challenges remain for robust remote observation and quantification. New methods to identify and quantify individual pigments in the presence of overlapping absorption features would provide a major advance in understanding their biological functions, quantifying net carbon exchange, and identifying plant stresses.

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1. Introduction

Life on Earth is driven by photosynthesis, producing both oxygen and organic matter (Nelson & Yocum, 2006). Photosynthesis is one of the earliest biological processes to evolve and the pigment systems in modern photosynthetic bacteria, algae, and higher plants appeared at least 2.5 billion years ago (Olson & Blankenship, 2004; Kiang et al., 2007). Photosynthetic pigments including chlorophylls a, b, and several carotenoid pigments date from this period. The length and stability of this record, and the various roles of pigments in photosynthesis, protection and defense, demonstrate the functional importance of their composition, associated protein complexes, and chloroplast structure. Their significance for life on Earth provides the rationale for improving our capability to remotely measure them. Furthermore, the stability of chlorophyll molecules makes them a

target, along with water, in the search for extraterrestrial life (Arnold et al., 2002; Wolstencroft & Raven, 2002; Seager et al., 2005). The range of characteristic reflectance patterns varies among different taxonomic groups of photosynthetic organisms in the visible and near-infrared spectrum as illustrated in Fig. 1 (Kiang et al., 2007). Although terrestrial and aquatic plants and mosses share a common photosynthetic apparatus, in contrast to some algae, lichens, and bacteria, reflectance differences among different taxa exist across the range of wavelengths that relate to both structure and biochemistry.

Developing methods to quantify pigment content and composition from remotely sensed data would clearly provide a capability that could advance understanding of photosynthetic processes (e.g., light regulation, photooxidation, chlorophyll fluorescence) and provide insight into detection and monitoring of foliar condition (e.g., environmental stressors). This review provides a brief summary of the major plant pigments and their ecophysiological functions and the most widely used spectroscopic methods for retrieving information from high spectral resolution remote sensing. This paper is dedicated to Professor Alexander F.H. Goetz, who led a major investigation into the potential for measuring plant biochemistry from narrow-band imaging spectrometry in the early 1990s. This activity stimulated

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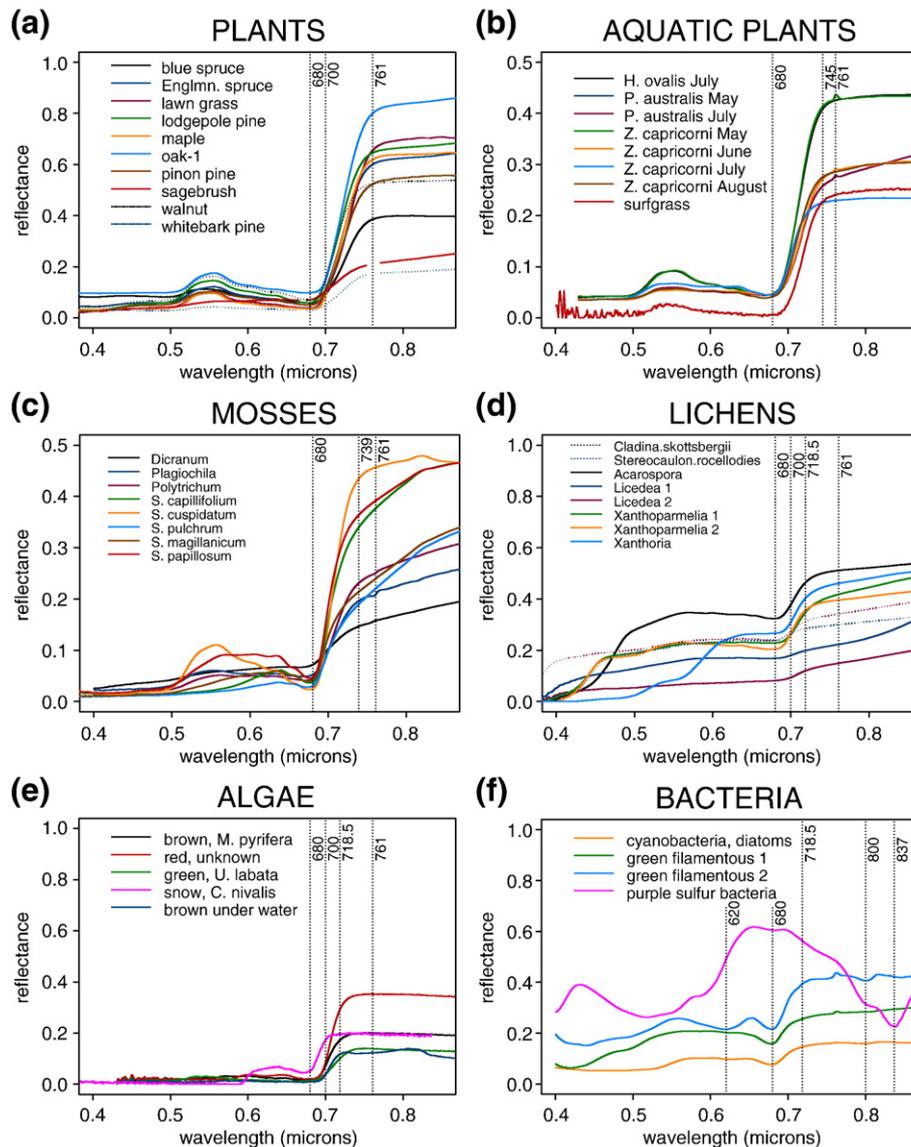


Fig. 1. Characteristic reflectance patterns among major groups of photosynthetic organisms in the visible and near-infrared spectrum. The oxygen absorption band, included for reference, is the vertical bar at 761 nm on the near-infrared plateau, shorter wavelength bars indicate the primary chlorophyll band at 680 nm and the region of the red-edge inflection point (reproduced from Kiang et al., 2007).

international research in the detection of plant biochemicals and many advances in measuring leaf pigments from reflectance data originate from this effort. To narrow the scope of this review, we focus primarily on reflectance measurements, at the leaf level, emphasizing advances in the past 15–20 years, and examining two types of quantitative approaches: (1) empirical methods and (2) physically based radiative transfer models and quantitative methods.

2. Photosynthesis and other functions of leaf pigments

Absorption of light in the visible spectrum by plant pigments produces a unique spectral reflectance signature. Light is captured by the process of photosynthesis (Govindjee & Krogmann, 2004) and the light energy is stored as carbohydrate, through a series of electron transfers that occur on the thylakoid membranes in chloroplasts. In the intact chloroplast, pigment–protein complexes are organized into two photosystems that harvest light and transfer energy to the reaction centers. Besides chlorophyll a and b, the photosynthetic antenna (the organized association of pigments that capture photons and transfer energy to the reaction centers) contain other mem-

brane-bound accessory pigments that include β -carotene, lutein and xanthophyll cycle pigments (Lichtenthaler, 1987). Fig. 2 shows the absorption spectra of chlorophyll a and b and β -carotene. Accessory pigments increase the spectrum over which light can be absorbed and also perform other functions, not all of which are fully understood, but which alter the efficiency of photon capture and/or provide protective and defensive functions, e.g., avoiding damage to the reaction centers under excess UV light or freezing temperatures. For example, under high illumination conditions, light intensity may exceed the capacity for electron transfer between the photosystems and some carotenoid pigments, such as xanthophylls, are involved with protection from photooxidation and photoinhibition (Demmig-Adams & Adams, 1996) and with release of excess solar energy through leaf fluorescence.

3. Measuring the absorption spectra of foliar pigments from high resolution spectroscopy

One factor that has limited our ability to quantify individual pigments from reflectance data is that we do not precisely know their

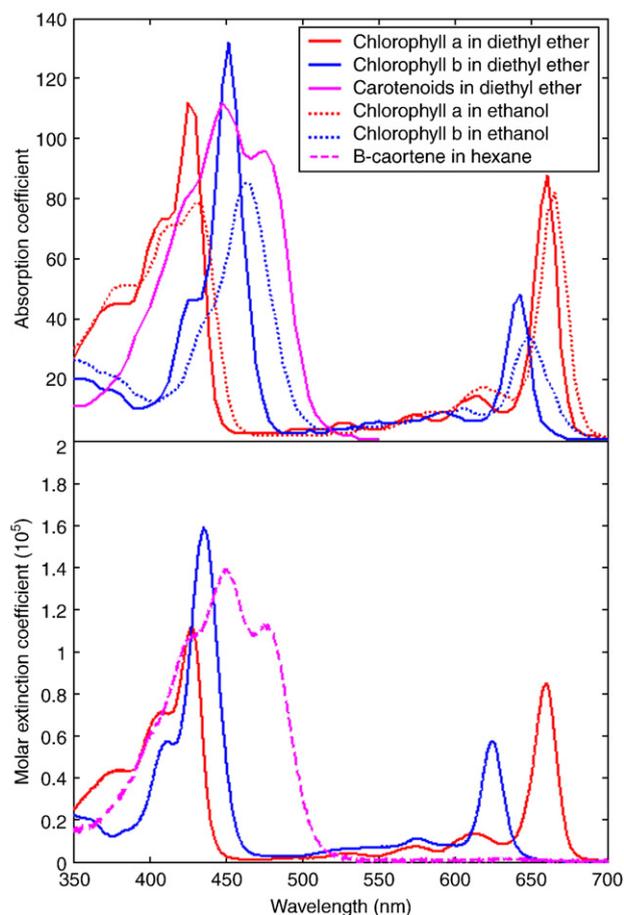


Fig. 2. (upper) Differences in absorption spectra of chlorophyll a, chlorophyll b and β -carotene in diethyl ether and chlorophyll a and b in ethanol. (lower) Molar extinction coefficient of chlorophyll a and b dissolved in diethyl ether and β -carotene dissolved in hexane (reproduced from Lichtenthaler, 1987 and Du et al., 1998).

absorption characteristics. It has long been noted (Rühle & Wild, 1979) that extracted chlorophyll absorption peaks are shifted about 20 nm to shorter wavelengths than observed in reflectance from intact leaves. The wavelength positions of maximum absorption vary with the extraction solvent used, due to differences in polarity, and the loss of pigment–protein interactions (Porra, 2002). The absorption maxima of chlorophyll a when extracted in diethyl ether are at 430 and 662 nm, and chlorophyll b has peaks located at 453 and 642 nm (Fig. 2). β -carotene extracted in hexane absorbs at 451 and 470 nm (Du et al., 1998). In the presence of multiple accessory pigments and non-photosynthetic cytoplasmic pigments like anthocyanin, it becomes clear why identification and quantification of individual pigments in the intact leaf has been difficult to obtain from their spectral absorbance signatures at specific bands (Buschmann et al., 1994). Multiple scattering from within the leaf further complicates pigment retrievals making remote detection even more challenging, as further discussed below.

A large number of narrow-band spectral methods have been proposed to detect plant pigments, ranging from simple band ratios to radiative transfer methods. Indexes currently used for estimating chlorophyll from leaf optical properties exploit the differences in reflectance between healthy and stressed vegetation in the visible and the red edge (Horler et al., 1983; Vogelmann et al., 1993; Carter, 1994; Carter & Spiering, 2002; Zarco-Tejada et al., 2001; Sims & Gamon, 2002; Malenovsky et al., 2006). These indexes are classified into red/NIR ratios, green and red edge indexes, and derivative

indexes. Table 1 provides examples of these indexes; see more comprehensive reviews of chlorophyll indexes in Blackburn (1998, 2007), le Maire et al. (2004) and Zarco-Tejada et al. (2005a,b).

Grossman et al. (1996) tested various regression methods on several leaf datasets and concluded that maximum correlations were produced at different wavelengths, which depended on the index and whether the pigment was expressed on an area or mass basis. Blackburn (1998) reviewed the ability of several ratio-based indexes, $RARS_{a,b}$, $PSSR_{a,b}$ and $PSND_{a,b}$ (Table 1) and first and second derivatives of the reflectance and the log 1/R (Yoder & Pettigrew-Crosby, 1995) to predict contents of chlorophyll a, b and carotenoids, and found best performance from PSSR and PSND indexes. Blackburn (1998, 2007) found strong but non-linear relationships, typically either power or exponential fits, could be developed at either leaf or canopy scales. He concluded that maximum correlations varied with wavelengths, index, and whether the analysis was on an area or mass basis.

Despite problems in identifying specific wavebands for individual pigments, the earliest attempts to use spectroscopy focused on estimating the content of total photosynthetic pigments using ratios of different spectral bands (e.g., simple ratio as R_{NIR}/R_{red}) or the normalized difference vegetation indexes (Table 1). As interest in narrow-band spectrometry increased, other ratio-based indexes or simple transforms of band combinations were developed as practical methods of analysis. Similarly, the use of first and second derivatives, which were applied across the full spectrum, came into common use in the 1990s. Derivative analyses, discussed in the following section, are primarily focused on the red edge, which is the long wavelength edge of the chlorophyll absorption, in the wavelengths between 700 and 750 nm.

From the beginning of systematic earth observation, remote sensing has focused on measuring plant “greenness”, often described as synonymous with chlorophyll content. There is a close relationship between photosynthetic capacity, estimated by pigment contents, and net primary production that is captured to first approximation by the greenness indicators measured by broad-band multispectral instruments (Gates et al., 1965; Monteith, 1976; Sellers, 1985, 1987; Asrar et al., 1984). Many band combinations in the visible spectrum have been used to estimate total pigments as illustrated by the examples shown in Table 1. This list is not exhaustive but it does cover the commonly used methods for leaf analysis, while also including more recent examples.

The empirical nature of the early studies, although based on a physiological understanding of absorption spectra of photosynthetic pigments, produced variable results when applied to new conditions due to the range of analytical methods used, experimental conditions, and characteristics of the species. At the leaf scale, when applied to a limited number of species or phenologic conditions, such as in agricultural fields or grasslands, indexes have provided good results despite lack of agreement on optimal methods, as attested to by the extensive literature. Despite the search for a universal method, no one method has been adopted as satisfactory under all growth and environmental conditions.

4. Measuring photosynthetic capacity in chlorotic and healthy leaves

Within a species, a variety of factors, including growth stage, irradiance, and various environmental stress conditions, can change the total pigment content and the chlorophyll a:b ratio (Anderson et al., 1988). Changes in the wavelength of the red edge inflection point position (IPP) have been observed for different species (Kiang et al., 2007), time during the growing season (Gates et al., 1965; Horler et al., 1983; Belanger, 1990) and from environmental stresses (Chang & Collins, 1983; Milton et al., 1983; Ustin & Curtiss, 1990; Hoque & Hutzler, 1992). Chlorosis increases reflectance across the visible spectrum and causes a shift to shorter wavelengths (blue-

Table 1
Spectral indexes developed as chlorophyll indicators.

Index	Short	Formula	Source
<i>Single wavelengths</i>			
Reciprocal Reflectance	RR	R_{700}^{-1}	Gitelson et al. (1999)
Logarithm of Reciprocal Reflectance	LRR	$\text{Log } R_{737}^{-1}$	Yoder and Pettigrew-Crosby (1995)
<i>Combinations of wavelengths</i>			
Waveband Ratios	Ratios	$R_{NIR}/R_B, R_{NIR}/R_G, R_{NIR}/R_R, R_{NIR}/R_{RE}, R_B/R_G, R_B/R_R, R_R/R_G$	Aoki et al. (1980, 1986), Vogelmann et al. (1993), Carter (1994), Gitelson and Merzlyak (1994, 1996, 1997), Datt (1998), Maccioni et al. (2001), Sims and Gamon (2002)
Modified Red-edge Ratio	mSR	$mSR = (R_{750} - R_{445}) / (R_{705} - R_{445})$	Sims and Gamon (2002)
Pigment Specific Simple Ratio	PSSR	$PSSR_a = R_{800} / R_{675}$ $PSSR_b = R_{800} / R_{650}$	Blackburn (1998, 1999), Sims and Gamon (2002)
Ratio Analysis of Reflectance Spectra	RARS	$RARS_a = R_{675} / R_{700}$ $RARS_b = R_{675} / (R_{650} \times R_{700})$	Chappelle et al. (1992), Blackburn (1999)
Normalized Difference Vegetation Index	NDVI	$NDVI = (R_{NIR} - R_R) / (R_{NIR} + R_R)$	Deering (1978), Datt (1998)
Red-edge NDVI	mNDVI	$mNDVI = (R_{750} - R_{705}) / (R_{750} + R_{705})$	Gitelson and Merzlyak (1994), Gamon and Surfus (1999), Datt (1999), Sims and Gamon (2002))
Modified Red-edge Normalized Difference Vegetation Index	mNDI	$mNDI = (R_{750} - R_{705}) / (R_{750} + R_{705} - 2R_{445})$	Sims and Gamon (2002)
Green NDVI	gNDVI	$gNDVI = (R_{750} - R_G) / (R_{750} + R_G)$	Gitelson et al. (1996), Datt (1998, 1999)
Pigment Specific Normalized Difference	PSND	$PSND_a = (R_{800} - R_{675}) / (R_{800} + R_{675})$ $PSND_b = (R_{800} - R_{650}) / (R_{800} + R_{650})$	Blackburn (1998)
Eucalyptus Pigment Indexes	EPI	$Chl_{a,b} = \alpha_{a,b} \times (R_{672} / (R_{550} \times R_{708}))^{b_{a,b}}$	Datt (1998)
Summed Reflectance Index	SRI	$S_1 = \int_{700}^{750} (R_\lambda / R_{555} - 1) d\lambda$ $S_2 = \int_{700}^{750} (R_\lambda / R_{705} - 1) d\lambda$	Gitelson and Merzlyak (1994)
<i>Red edge position</i>			
Red Edge Inflection Point Position	IPP	$R''(\lambda_i) = 0$	Horler et al. (1983), Curran et al. (1995), Gitelson et al. (1996), Kochubey and Kazantsev (2007)

shift) of the red-edge IPP, due to narrowing of the chlorophyll absorption feature and a reduction in depth (Ustin & Curtiss, 1990). Gates et al. (1965) and Collins (1978) provided early observations of a blue shift of the red edge which was attributed to the loss of chlorophyll. Rock et al. (1988) noted a disproportional loss of chlorophyll b accompanied by a blue shift of the IPP for foliage exposed to air pollution. In contrast, under increased chlorophyll content, the chlorophyll absorption feature deepens and broadens (e.g., Buschmann & Nagel, 1993) causing a red-shift of IPP (Collins, 1978). Kiang et al. (2007) note that the red-edge shifts of IPP from shorter to longer wavelengths for species ranging from algae, lichens, mosses, to aquatic and terrestrial plants. Thus, this index is best applied to variation within a vegetation type to detect stress and between types when it is used to identify taxa. Several index methods have been used to detect red-edge shifts in narrow band spectra (Table 1).

5. Using the red edge to detect stress

Most early studies of the red edge used a first or second derivative or the amplitude of the derivative to identify the wavelength at the inflection point, which was then correlated with chlorophyll content (Horler et al., 1983; Wessman, 1990). Fig. 3 illustrates how environmental stressors, in this case oil contamination, in a grassland site with relatively little variation in leaf area index can affect the wavelength position of the inflection point (Fig. 3a) and how the IPP can be used to map spatial variation in chlorophyll content due to the oil contamination (Fig. 3b).

Despite 30 years of research defining the relationships between pigment content and the red edge using various spectral indexes, the red edge remains an area of active research (e.g., Gitelson et al., 1996; le Maire et al., 2004; Asner et al., 2005; Zarco-Tejada et al., 2005a; Sims et al., 2006). Although the use of derivatives to estimate the red edge position is widely used, because of their sensitivity to noise, other methods e.g., the inverted Gaussian of Miller et al. (1990) are now preferred. Recently, Cho and Skidmore (2006)

proposed an improved method to estimate the red-edge inflection wavelength using intersecting lines originating from the shoulders of the derivative. More research is needed to understand precisely what controls the position of long wavelength side of the chlorophyll absorption band. For example, Curtiss and Ustin (1989) observed a broadening of the chlorophyll absorption band in ponderosa pine needles following exposure to atmospheric ozone, which they interpreted as a red-shift that mimicked increased chlorophyll content rather than a blue-shift which is expected under lower chlorophyll content. This effect was hypothesized to be due to increasing disorder in the chloroplast with ozone exposure, a pattern consistent with observations that an early sign of ozone injury is granulation of the thylakoid membranes. Recently Noomen et al. (2006) reported that exposure to natural ethane gas caused a small red shift in corn reflectance and a significant decrease in absorption in the 550–750 nm region.

6. Leaf fluorescence and stress detection

Use of remotely sensed fluorescence to estimate photosynthetic activity began in the oceanography community to detect phytoplankton productivity (e.g., Kim, 1973). For both marine and terrestrial applications, two types of systems have been used, active and passive lasers. Marine systems usually use lasers in the blue spectral region to excite photosystem II activity, with chlorophyll fluorescence being observed in the 730 nm region. Passive systems rely on measuring natural fluorescence stimulated by sunlight. Although the signal is small, as spectral resolution in the visible spectrum and signal/noise in spectrometers have increased, interest in using this technology to directly observe photosynthetic functioning has become of more interest to plant physiologists and ecologists (Lichtenthaler, 1988). Fig. 4 shows the wavelength specific absorption of light excitation by a UV laser and corresponding chlorophyll fluorescence emission for a typical leaf.

Several authors have noted a double peak in the first derivative of leaf reflectance around 700–725 nm (Horler et al., 1983; Boochs et al.,

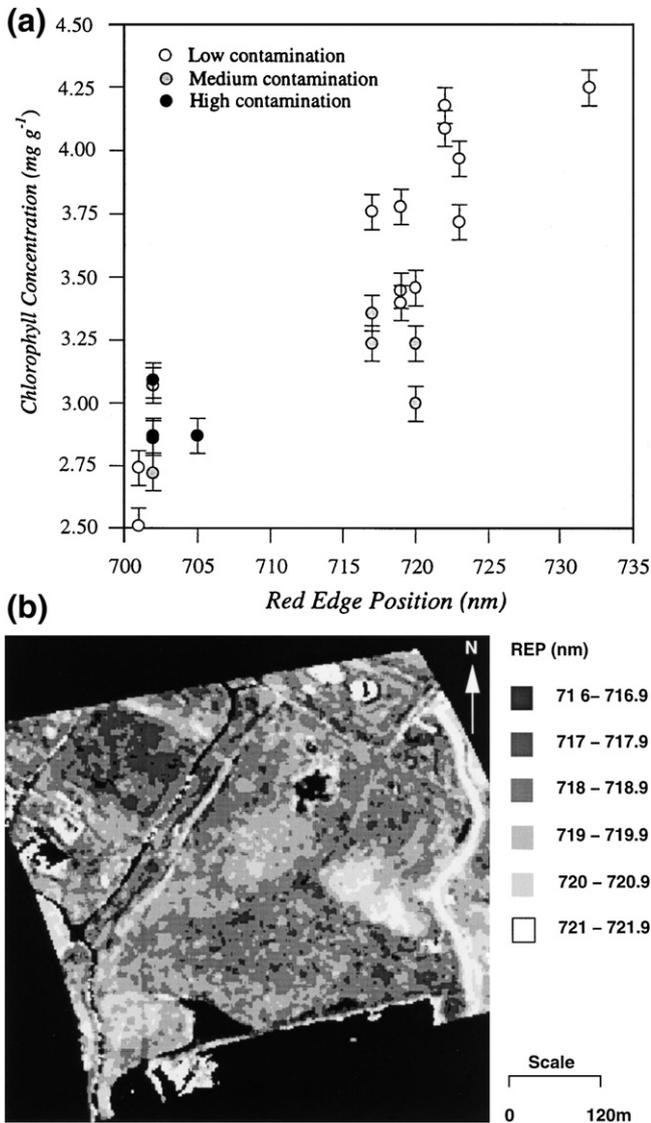


Fig. 3. Correlation between the red-edge position (REP) and chlorophyll concentration (upper panel) of a grass canopy, Isle of Grain, Kent, United Kingdom for plot averages ($r = 0.84$, $n = 23$). Error bars show the standard deviation of the chlorophyll determination. The airborne CASI hyperspectral image (lower panel) of the site illustrates spatial variation in REP response to oil contamination at the site. The grassland is reported to be relatively uniform with little variation in leaf area index, however REP indicates substantial differences in chlorophyll concentration (reproduced from Jago et al., 1999).

1990). It was later shown that the reflectance derivative could exhibit several local maxima in the red edge area in measured (Gitelson et al., 1996) and simulated (le Maire et al., 2004) spectra. The nature of this multiple-peak feature is not well understood. Zarco-Tejada et al. (2003) suggested that the double-peak is due to steady-state fluorescence emission. Le Maire et al. (2004) showed that the double-peak can be simulated by the PROSPECT model by increasing the chlorophyll content alone, regardless of the values of other parameters. It can be also simulated by increasing the structure parameter alone if the total chlorophyll content exceeds 400 mg cm^{-2} .

The potential to use fluorescence spectroscopy as a non-destructive method to detect plant stress was recognized in several early studies (e.g., Buschmann & Schrey, 1981; Chappelle et al., 1984; Lichtenthaler & Rinderle, 1988). Fluorescence emission maxima were observed at 440–450, 525–535, 680–685, and 735–740 nm (e.g., Richards et al., 2003) and related to components of photosynthesis and leaf pigments (e.g., Buschmann et al., 2000). Gitelson et al. (1999) used a ratio of fluorescence at 735/700 nm which provided an accurate

prediction of chlorophyll content in intact leaves. Recent authors have continued to explore active (e.g., laser-induced) fluorescence (Corp et al., 2006; Richards et al., 2003) and solar-induced fluorescence (Zarco-Tejada et al., 2003; Dobrowski et al., 2005) to detect environmental stresses. Carter et al. (2004) measured solar-induced fluorescence in 10 nm bands at 690 and 760 nm using the Fraunhofer Line Depth Principle to detect stress, and more recently, Meroni and Colombo (2006) used very high resolution (0.06 nm) spectrometry at 687 and 760 nm to detect chlorophyll fluorescence in an oxygen band where solar irradiance is reduced.

7. Detecting and quantifying foliar pigment composition in relation to ecological condition

The concept of ecological convergence expresses the “economy of form” or the efficiency that particular sets of traits provide in exploiting limited environmental resources (Field et al., 1992). This concept implies that the cost of biochemical investments should be related to growth potential and thus better measurements of pigment distributions and concentrations could provide a basis for monitoring physiological and ecological processes. It is known that pigment composition varies with species and environmental conditions (e.g., Peñuelas et al., 1995a; Carter & Knapp, 1991; Asner et al., 2008). Deciduous species have leaves that are generally adapted for faster growth rates and higher photosynthetic capacity (e.g., higher chlorophyll and nitrogen concentrations) than needles in evergreen species where the cost of producing lower photosynthetic capacity foliage can be amortized over several years (Wright et al., 2004). Fig. 5 illustrates differences in the amount and composition of photosynthetic pigments in leaves from a deciduous oak grown in the higher light environment of the savanna compared to leaves from the more closed canopy of the evergreen oak community (Ustin et al., 1993). Reflectance from the evergreen oak leaves is lower across the visible spectrum and the spectral shape is different. The total amount of pigments is lower in leaves from the evergreen oak and their relative proportions differ from the deciduous species. Note that small absorption features (at this presentation scale) are observed in these leaves near 585 nm, 620 nm, and 650 nm, suggesting that it may be possible to identify the basis for these features, particularly at the canopy level where absorption features are enhanced by transmission through multiple leaf layers (Allen & Richardson, 1968; Knipling, 1970; Curran, 1980; Styliniski et al., 2001; Roberts et al., 2004).

8. Detection of foliar stress using the Photochemical Reflectance Index (PRI)

Under high light, it is well established that xanthophyll cycle pigments function to prevent photooxidation of the reaction centers

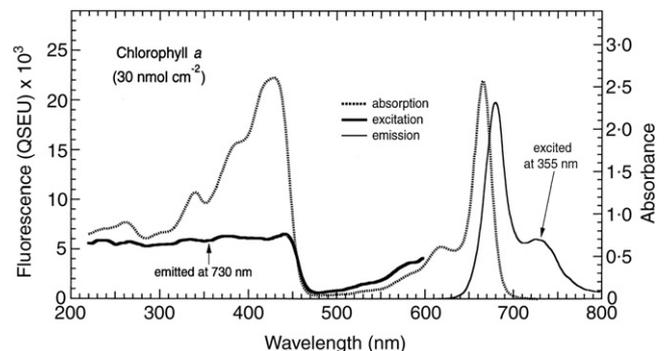


Fig. 4. Comparison of absorption spectrum for pure chlorophyll a, its active fluorescence excitation and its emission spectrum (reproduced from Cerovic et al., 2002).

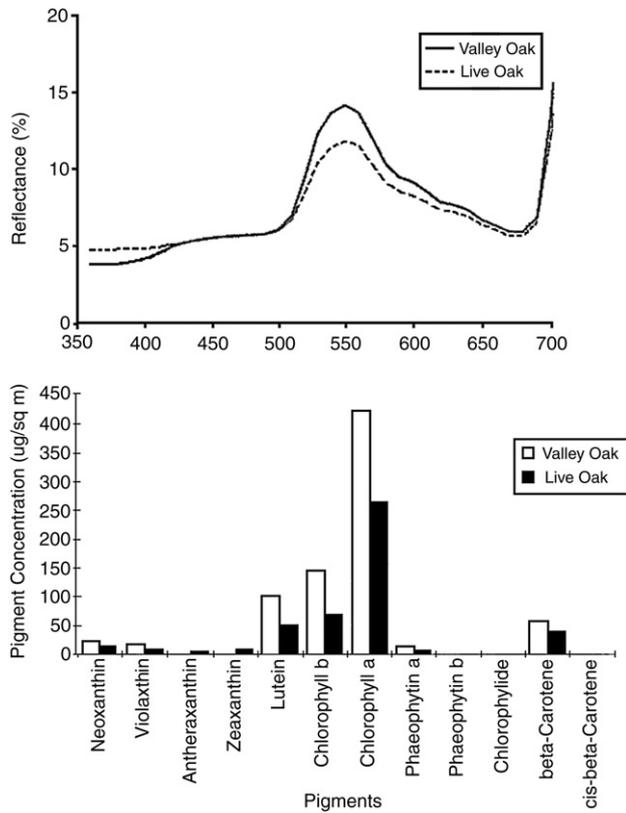


Fig. 5. (left) Mean reflectance spectra of leaves from two species of California oaks the winter deciduous *Quercus lobata* (Valley Oak) and evergreen *Q. agrifolia* (Live Oak), measured in midsummer at Jasper Ridge Biological Preserve. (top) Mean pigment concentration and composition for these species (bottom). (reproduced from Ustin et al., 1993).

(Demmig-Adams & Adams, 1996). Short-term changes in reflectance in response to the light environment are observed at 531 nm that detect reversible changes in the distribution of xanthophyll cycle pigments (violaxanthin is converted to zeaxanthin through the intermediate antheraxanthin under high light and reverts to violaxanthin under low light; Demmig-Adams, 1990). This is the basis for the photochemical reflectance index (PRI; Table 2), a normalized ratio of 531 nm to 570 nm developed by Gamon and colleagues (Gamon et al., 1990, 1992, 1993, 1997; Peñuelas et al., 1995a, 1997). The PRI, at the time of the NASA Accelerated Canopy Chemistry Program (ACCP), was shown to detect the transition to

zeaxanthin in foliage exposed to high light intensities and this response is closely tied to photosynthetic activity (Gamon et al., 1990, 1992, 1993; Peñuelas et al., 1995a). The relationship between PRI and photosynthetic light-use efficiency has been explored at multiple scales (leaf to stand-level) in the years since this index was developed (e.g., Nichol et al., 2000; Gamon et al., 2001; Drolet et al., 2005; Nakaji et al., 2005). When the PRI is measured over longer time spans (seasons, years) or across species, variation appears to track relative composition of chlorophylls and carotenoids (e.g. Styliński et al., 2001; Sims & Gamon, 2002).

9. Detection of carotenoid pigments and anthocyanin pigments

The red and yellow colors of autumn foliage are seen because of the change in photoperiod (toward short days/long nights) and/or low temperatures that initiate a senescence response in which chlorophyll pigments breakdown before the carotenoid pigments (e.g., lutein and β-carotene). Table 2 lists several wavelength specific indexes that have been used to estimate carotenoid pigment contents in foliage. Anthocyanins are red flavonoid pigments that are cytoplasmic and not associated with the chloroplast but they are often observed during environmental stresses (e.g., low or high temperatures) and during senescence (Schaberg et al., 2008). Anthocyanins are also common during the earliest stages leaf development before the photosystems are fully functional (Gamon & Surfus, 1999). Like carotenoids, anthocyanins protect the photosynthetic system from excess light, particularly excess UV radiation (Merzlyak & Chivkunova, 2000; Gitelson et al., 2001), and may provide other functions, e.g., protection from herbivory and fungal pathogens (Close & Beadle, 2003). These pigments have a single absorption maximum around 529 nm and can be detected by reflectance changes in the green region (Table 3); reflectance in the red-edge region does not vary with anthocyanin content and so the red edge can be used as a reference against which anthocyanin is determined (Curran et al., 1991; Neill & Gould, 1999; Gitelson et al., 2001, 2006).

10. Development of spectral vegetation indexes for individual pigments

Detection of individual pigments from reflectance data has been given less attention by the remote sensing community than total pigment measures, despite the importance of accessory pigments in light capture, photosystem protection, and in various growth and development functions. This lack is due to the difficulty in resolving the overlapping absorptions of individual pigments and their high degree of autocorrelation (Chappelle et al., 1992; Ramsey & Rangoonwala, 1995; Blackburn, 1998, Grossman et al., 1996). Nonetheless, because pigments have distinctive absorption spectra depending on their molecular structure and local chemical environment, there is a potential to measure these properties using reflectance spectroscopy. The challenge is to properly account for the multiple factors influencing the retrieved signal. While many models relate chlorophyll content to reflectance (e.g., Gitelson & Merzlyak, 1994, Sims & Gamon, 2002; Richardson et al., 2002; Gitelson et al., 2003; le Maire et al., 2004) and some are robust in chlorophyll prediction, only few models support retrieval of

Table 2
Spectral indexes developed as carotenoid indicators.

Index	Short	Formula	Source
Ratio Analysis of Reflectance Spectra	RARS	$RARS_c = R_{760} / R_{500}$	Chappelle et al. (1992)
Structure Insensitive Pigment Index	SIPI	$PSND_c = (R_{800} - R_{445}) / (R_{800} - R_{680})$	Peñuelas et al. (1995a), Sims and Gamon (2002)
Pigment Specific Simple Ratio	PSSR	$PSSR_c = R_{800} / R_{500}$	Blackburn (1998)
Carotenoid Reflectance Index	CRI	$CRI_{550} = R_{510}^{-1} - R_{550}^{-1}$ $CRI_{700} = R_{510}^{-1} - R_{700}^{-1}$	Gitelson et al. (2002)
Modified Carotenoid Reflectance Index	mCRI	$mCRI_c = (R_{510}^{-1} - R_{520}^{-1}) \times R_{NIR}$ $mCRI_{EE} = (R_{510}^{-1} - R_{520}^{-1}) \times R_{NIR} - (R_{690}^{-1} - R_{710}^{-1}) \times R_{NIR}$	Gitelson et al. (2006)
Photochemical Reflectance Index	PRI	$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$	Gamon et al. (1990, 1992, 1997)
Eucalyptus Pigment Indexes	EPI	$Car = \alpha \times (R_{672} / (R_{550} \times R_{708}))^\beta$	Datt (1998)

Table 3
Spectral indexes developed as anthocyanin indicators.

Index	Short	Formula	Source
Anthocyanin Reflectance Index	ARI	$ARI = R_{550}^{-1} - R_{700}^{-1}$	Gitelson et al. (2001)
Modified Anthocyanin Reflectance Index	mARI	$mARI = (R_{530}^{-1} - R_{570}^{-1}) \times R_{NIR}$ $R_{690}^{-1} - R_{710}^{-1}) \times R_{NIR}$	Gitelson et al. (2006)
Red:Green Ratio	RGR	$RGR = R_R / R_G$	Gamon and Surfus (1999), Sims and Gamon (2002)

anthocyanins and carotenoids (e.g., Gitelson et al., 2001, 2002; Sims & Gamon, 2002).

There are a few examples in the literature where chlorophyll a and chlorophyll b have been separately assessed from reflectance data using empirical models. Among the first, Chappelle et al. (1992) and Blackburn (1999) used band ratios to quantify chlorophyll b. Curran et al. (2001) demonstrated retrieval of foliar chlorophyll b using the continuum removal method of Kokaly and Clark (1999). Pinzón et al. (1998) used a hierarchical singular value decomposition method to quantify biochemical constituents in intact leaf samples, where total sample variance was broken down into smaller ranges of variation using a series of weighting vectors.

One explanation for past inconsistencies in separating and quantifying different pigments is that their absorption spectra overlap, thus simple methods do not account for the interacting effects of multiple pigments and fail across a range of taxa (e.g., Fig. 1) or where the conditions are outside the range that these empirical methods were tested and calibrated. In other cases, indexes that seem to provide consistent results at the leaf level fail or become inconsistent at the canopy or stand levels, in part due to the complexities of the three-dimensional structure and multiple scattering in intact leaves. Additionally, despite progress at the leaf level, no analytical model today has accounted for the quantification of anthocyanin and carotenoid contents at the canopy or stand scales.

11. Development of multiple pigment models

The first model to estimate multiple pigment contents was developed by Gitelson and colleagues (Gitelson et al., 2003, 2006). They proposed a conceptual model based on the relationship between the reciprocal of reflectance, a property closely related to the infinite reflectance of a leaf, and its inherent optical properties, absorption and backscattering coefficients. They then modeled pigment absorption using three narrow spectral bands, where reflectance in the first band (λ_1) is maximally sensitive to absorption by the pigment of interest but is also affected by absorptions from other pigments and variability in backscattering (Fig. 6). These extraneous effects are removed by identifying a second band λ_2 where the other pigments absorb but the pigment of interest has little effect and where backscattering is relatively unchanged from λ_1 . Then a third spectral band λ_3 is selected where backscattering controls the overall reflectance. Combining these three bands allows the pigment content (C_{pigment}) to be estimated:

$$C_{\text{pigment}} \propto [R^{-1}(\lambda_1) - R^{-1}(\lambda_2)] \times R(\lambda_3).$$

The spectral regions used in the model were tuned with respect to the pigment of interest and the optical characteristics of the leaves studied. The optimal bands for each pigment (chlorophyll, carotenoids and anthocyanins) retrieval are determined by performing the calibration for a continuous range from 400–800 nm, isolating one band at a time, and choosing each of the 3 bands according to a minimal root mean square error (RMSE) (Fig. 6). For total chlorophyll content retrieval optimum λ_1 was found in either the green (540–560 nm) or red edge (700–730 nm) range, and $\lambda_2 = \lambda_3$ was in the NIR beyond 760 nm (Fig. 6a, Gitelson et al., 2003, 2006).

Zur et al. (2000) and Gitelson et al. (2002) identified a spectral band near 510 nm that was sensitive to total content of carotenoids and used it in a three-band model to estimate the total carotenoid content (Fig. 6b, Gitelson et al., 2002, 2006). They applied this model to retrieve anthocyanin, developing an Anthocyanin Reflectance Index (Fig. 6c, Gitelson et al., 2001, 2006). In anthocyanin-free leaves, both the green and the red edge bands can be used as λ_1 for chlorophyll estimation and as λ_2 for carotenoid estimation (Fig. 6b). Thus, only four spectral bands are required to retrieve three pigment

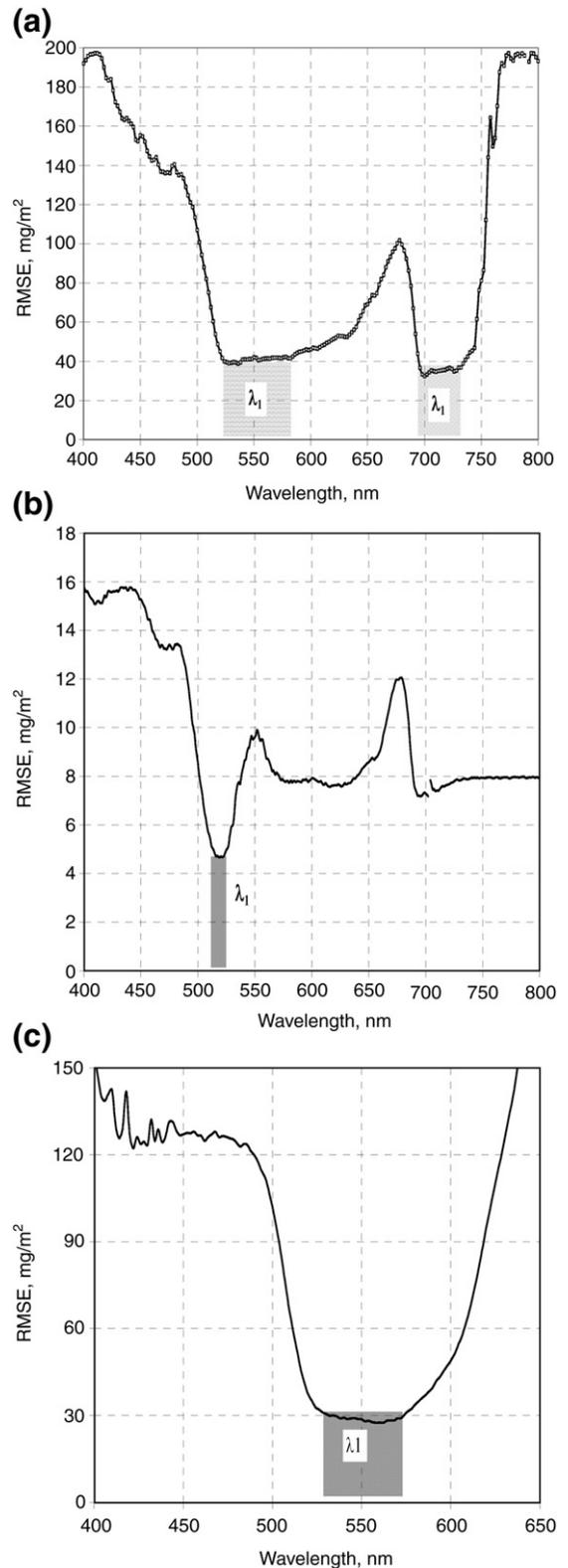


Fig. 6. Optimal positions of spectral band λ_1 of the model $[R(\lambda_1)^{-1} - R(\lambda_2)^{-1}] \times R(\lambda_3)$ for retrieval of (A) total chlorophyll, (B) carotenoid, and (C) anthocyanin content from reflectance spectra (reproduced from Gitelson et al., 2006).

contents: 510–520 nm (carotenoids), 540–560 nm (anthocyanins), 700–730 nm (total chlorophyll) and NIR in the range 760–800 nm. This model produced accurate estimates of the total chlorophylls, carotenoids, and anthocyanin contents, explaining more than 91%, 70%, and 93% of the variance, respectively (Gitelson et al., 2006).

However, the model for carotenoids retrieval was found to be species specific.

12. Leaf radiative transfer models

Physically based RT models have the potential to produce more accurate and consistent predictions of pigment interactions because they are based in physics and use the full spectrum rather than individual bands and therefore, do not require calibration each time they are used. However, RT model predictions of the optical properties of monocot or dicot leaves or needles depend on how well understood all processes affecting reflectance are and how they are accounted for in the models. Although they have potential to predict pigment content more consistently and accurately than empirical methods, they require more input parameters, which if wrong, result in poor model performance. Thus, empirical models can be more accurate than physical models if the components are improperly modeled or the input data is wrong.

Several leaf models, e.g., PROSPECT (*Leaf Optical Properties Spectra*, Jacquemoud and Baret, 1990), LIBERTY (*Leaf Incorporating Biochemistry Exhibiting Reflectance and Transmittance Yields*, Dawson et al., 1998), LEAFMOD (*Leaf Experimental Absorptivity Feasibility Model*, Ganapol et al., 1998), and SLOP (*Stochastic model for Leaf Optical Properties*, Maier et al., 1999; Maier, 2000), have been used since the 1990s to estimate total chlorophyll concentration (see review in Ustin et al., 2004). Instead of modeling multiple pigments, RT models currently assume that leaf pigments are entirely composed of chlorophyll and that the horizontal and vertical distribution of these absorbers is homogeneous within foliar tissues.

Characterization of *in situ* absorption coefficients for xanthophylls, carotenes and even chlorophyll b need to be added to leaf RT models to estimate individual pigments. This will require defining *in vivo* absorption coefficients for all individual pigments but also better knowledge of the spectral variation of the refractive index of leaves, information that is not currently available and which may vary depending upon the chemical environment within the leaf. Maier et al. (1999) and Berdnik and Mukhamed'yarov (2001) followed this approach and utilized separate *in vitro* absorption spectra of chlorophyll a, b and several carotenoids in developing their leaf optical properties model. An advanced version of the PROSPECT model (PROSPECT 5) that can discriminate chlorophylls from total carotenoids has been developed (Feret et al., 2008). This required a long phase of calibration using datasets carefully selected to cover a wide range of leaf photosynthetic pigments. Feret et al. (2008) showed that some improvements in prediction of leaf reflectance, on the order of 5% in the visible, could be made by better characterization of pigment

contributions. Fig. 7 shows the comparison between measured and PROSPECT-5 predicted total chlorophyll (left) and total carotenoid (right) contents from leaves representing a wide range of ecological conditions and communities, from mesic and semiarid temperate forests, shrublands, and agriculture, to subtropical and tropical systems.

The fact that light can be propagated through leaves without encountering foliar pigments, for instance in veins, is a phenomenon known as the sieve effect which has been accounted for in RT models to better interpret absorption spectra of leaves (Latimer, 1983; McClendon & Fukshansky, 1990). Additionally, bifacial leaves with different chlorophyll content in the palisade and spongy mesophylls can be simulated using the Kubelka–Munk theory applied to two or more stacked layers (Yamada & Fujimura, 1991; Richter & Fukshansky, 1996). With this added level of physical detail being built into RT leaf reflectance models, it is likely that it will lead to better characterization of individual pigments.

A recent European program to develop methods to detect leaf and canopy fluorescence has produced a new model, FluorMOD, with linked leaf and canopy models to detect the effects of steady-state solar-induced chlorophyll fluorescence (Zarco-Tejada et al., 2006; Middleton et al., 2008).

13. Challenges in imaging spectroscopy of vegetation pigments

The application of narrow-band spectral methods for pigment detection to airborne and spaceborne spectrometers has been the intended goal of much of the leaf-level research since the time of the ACCP program that Dr. Goetz headed. Field- and laboratory-based spectroscopy of plant pigments has had a long period of development and today, a large number of studies have explored the detection of pigments in many leaf types originating from a wide range of ecosystems and using a wide range of methods. The use of airborne and space-based imaging spectrometers to detect and map foliar pigments is still relatively new (e.g., Collins, 1978; Rock et al., 1988; Zarco-Tejada & Miller, 1999). These examples highlight the potential contribution of imaging spectroscopy for detecting and quantifying foliar pigments from imaging spectroscopy to studies of canopy physiology and ecology. At this time, current methods have not delivered unambiguous results and operational methods for this level of biochemical retrieval. Advances in algorithm development as highlighted above indicate that numerous challenges remain to be solved before pigment concentrations can be routinely retrieved from space. Imaging spectrometer design is a key issue in the quest for more quantitative approaches to identification of pigments. This will require an instrument that has high spectral resolution, ~3–5 nm to measure pigment details but which has good signal to noise characteristics of up to 1000:1 in the visible and infrared region. The fidelity of the sensor, which includes signal-to-noise performance, uniformity of the image, and stability of the electronics all affect the outcome of a canopy chemical analysis. Instruments must be calibrated in terms of wavelength (i.e., avoiding keystone and smile) and have radiometric stability.

A good example of current technological capability is found by looking at the evolution of the Jet Propulsion Laboratory's Advanced Visible Infrared Imager Spectrometer (AVIRIS) program, which started under the leadership of Dr. A.F.H. Goetz in 1982. In the early 1990s, AVIRIS signal-to-noise ratio was in the 10–100 range depending upon wavelength region (Vane et al., 1993). Since then, AVIRIS has evolved, through major upgrades and constant hardware and software adjustment, to provide spectra with effective signal-to-noise performances of many 100 s to 1000 s (Green et al., 2003). The resolution, stability, and sensitivity of the sensor technology bears squarely upon the state of hyperspectral algorithm development. With the high fidelity of AVIRIS today and a few other sensors, it is possible to use physically-based methods, such as canopy RT models,

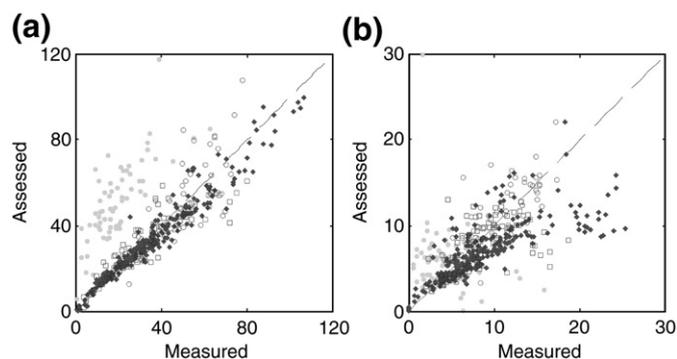


Fig. 7. Predicted and measured total chlorophyll (left) and total carotenoid (right) pigments based on an inversion of the PROSPECT 5 model (reproduced from Feret et al., 2008) using leaves from four sources: (●) LOPEX (Hosgood et al., 1994) □ CALMIT (Gitelson et al., 2002, 2003) ◆ ANGERS (INRA, France) ○ HAWAI (Asner & Martin, 2008).

to explore spectra in some detail. Thus, advances in modeling pigments at the leaf level can be incorporated into models suitable for imaging spectrometers. However, since most remote mapping of ecosystems will be at canopy level, solid coupling of leaf radiative transfer models with canopy scale models will be key to successful retrieval of multiple pigments from air- or spaceborne spectrometers. At canopy scale, the simultaneous assessment of canopy heterogeneity as well as pigments will require a better representation of clumped and sparse canopies in coupled RT models. Future sensors, having capabilities similar to the proposed NASA HypIRI mission and the German EnMap program (Kaufmann et al., 2006), will eventually deliver spectroscopic measurements of sufficiently high fidelity to advance the mapping of canopy pigments and other chemicals.

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