Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation

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1. Introduction

Chlorophylls are the most important pigments for photosynthesis (Evans, 1989; Yoder and Pettigrew-Crosby, 1995; Ninemets and Tenhunen, 1997). The amount of chlorophyll per unit leaf area in maize is an important indicator of the overall condition of the plant. Healthy plants capable of maximum growth are generally expected to have larger amounts of chlorophyll than unhealthy ones. Therefore, determination of the chlorophyll content of a leaf can be used to detect and study plant...
increased, stress, and nutritional state and consequently has important potential implications on crop stress and chlorosis detection, agricultural field management, and especially for precision agriculture practices (Zarco-Tejada et al., 2004).

Remote sensing plays a unique and essential role provided that we can relate remote sensing measurements to the biochemical at Earth surfaces in a reliable and operational way. However, the relationship between the surface measurement and satellite data strongly depends on the study area and the experimental conditions of the reflectance acquisition. Vegetation indices have been developed as an attempt to reduce spectral effects caused by external factors such as the atmosphere and the soil background (Demarez and Gastellu-Etchegorry, 2000).

The chlorophylls have strong reflectance peaks in the red and blue wavelength regions. Blue peak is not used to estimate the chlorophyll content because it overlaps with the absorbance of the carotenoids. Maximal absorbance in the red region occurs between 660 nm and 680 nm. However, it is unknown if reflectance at these wavelengths can be used to predict chlorophyll content as reflectance at slightly longer or shorter wavelengths. This is because absorption in the 660–680 nm tends to saturate at low chlorophyll content, thus reducing the sensitivity of the spectral indices based on these wavelength to high chlorophyll content (Sims and Gamon, 2002). Empirical models to predict chlorophyll content are largely based on reflectance around the 550 nm or 700 nm regions where the absorption is saturated at higher chlorophyll. Indices formulated with these bands would thus have higher accuracy in estimating chlorophyll content.

Increasing efforts have focused on understanding the relationships between vegetation optical properties and photosynthetic pigments concentrations within green leaf tissues such as chlorophylls and carotenoids. Obviously, different pigments have specific absorption features at different wavelengths which have promoted the development of various approaches, based on model inversion or the use of empirical and semi-empirical methods, to estimate the chlorophyll content both at the leaf and canopy scales (Daughtry et al., 2000; Demarez and Gastellu-Etchegorry, 2000; Zarco-Tejada et al., 2001). Sims and Gamon (2002) used a large experimental database composed of a vast range of functional types, leaf structure, and development stage (nearly 400 leaves). They compared their indices with commonly used ones and found that the indices mSR_{705} (simple ratio) and mND_{705} (normalized difference) had the best correlation with chlorophyll concentration. Zarco-Tejada et al. (1999) performed a study of hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops which showed that crown-derived relationships between hyperspectral indices and biochemical constituents cannot be readily applied to hyperspectral imagery of lower spatial resolutions due to large soil and shadow effects. Haboudane et al. (2002) developed an index, transformed chlorophyll absorption ratio index/optimized soil-adjusted vegetation index (TCARI/OSAVI) that integrates advantages of indices minimizing soil background effects and indices that are sensitive to chlorophyll concentration. Ground truth data proved that a good correlation can be acquired between the estimated and field measured chlorophyll content data with R^2 of 0.81. Le Maire et al. (2004) developed, tested, and compared several leaf chlorophyll indices using a larger simulated database (11,583 spectra) of a leaf-radiative transfer model (PROSPECT, Jacquemoud and Baret, 1990) and ground truth data to determine “universal” chlorophyll content indices applicable to a wide range of species and leaf structures.

In most of these studies, leaf area index (LAI) is considered as a disturbance to the vegetation indices because of its significant role in surface energy system and its scale concept nature. LAI is one of the key state variables in land surface models which is defined as half of the all-sided green leaf area per unit ground area. Vegetation LAI governs net radiation and its expenditure (energy balance), net primary production (carbon fixation), evapotranspiration and canopy interception (water budget) (Daughtry et al., 2000; Broge and Leblanc, 2000; Haboudane et al., 2002; Zarco-Tejada et al., 2004). LAI is also closely related to exposed area of living leaves which plays a key role in various biophysical processes such as plant transpiration and CO2 exchange. These functions are important to understand energy exchanges between the vegetation and the atmosphere.

The objective of this study is to compare the performance of a set of hyperspectral vegetation indices in chlorophyll content estimation based on different bands combination of the reflectance simulated by the PROSPECT and SAIL models (Verhoef, 1984). Four new vegetation indices are derived which have better linearity with chlorophyll content yet resistant to LAI saturations and background effects. Validation of these four indices is also conducted at canopy scale using ground truth data (reflectance and chlorophyll data) during the growth duration of winter wheat. Two integrated vegetation indices are proved to have the best potential in chlorophyll content estimation. To test the reliability of the two selected indices, the spaceborne hyperspectral Hyperion data is used in the application of real remote sensing to estimate the chlorophylls of two types of corns.

2. Method

2.1. The study area

Two sites are selected in this paper. The first is located at National Experiment Station for Precision Agriculture (40°10.6’N, 116°26.3’E) 20 km northeast of Beijing, China. This experiment station has been operational since 2001 and used for precision agriculture research. The site is within the warm temperate zone with a mean annual rainfall of 507.7 mm and a mean annual temperate of 13 °C. The plant selected in this site is winter wheat (Triticum aestivum L.) which is one of the most important crops in China. The second site is located near the city of Zhangye (38°50.26’N, 100°27.36’E) in the western area of China. Two types of corns (eared-corn and the no-eared corn) are measured in this site (Fig. 1).

2.2. The ground data

2.2.1. Canopy data acquisition

Six types of winter wheat that can be classified into three leaf structural types were selected in this experiment (Table 1). Each winter wheat type was cultivated in an area of about 4000 m² (about 200 m × 20 m). Wheat is planted in a silt clay
soil with sufficient water supply. The nutrients of surface soil (0–30 cm) were as follows: the organic matter, 1.42–1.48%; total nitrogen, 0.081–0.100; alkali-hydrolysis nitrogen, 58.6–68.0 mg/kg available phosphorus, 20.1–55.4 mg/kg and available potassium, 7.6–29.1 mg/kg. The data collections were carried out in four clear days during a typical winter wheat growth season: 17 April, 28 April, 16 May and 29 May in 2007.

Canopy radiance data were collected at 380–2500 nm using a portable spectroradiometer (FS-FR2500, ASD, USA) normal to the canopy to obtain hyperspectral indices with field of view of 25° and a distance of about 130 cm above the ground surface. Reflectance spectra were derived through calibration with the 99% white reference board (Labsphere, Inc., North Sutton, New Hampshire, USA). Ten spots for each type of wheat were measured and averaged for more accurate analysis.

2.2.2. Leaf chlorophyll content acquisition
An easy method for determining the chlorophyll content is using the portable Chlorophyll Meter SPAD-502 (Minolta Corporation, New Jersey, USA). However, the SPAD-502 meter provides the data only in arbitrary units rather than the actual amounts of chlorophyll per unit area of leaf tissue. In this paper, a standard method was used to determine the amount of chlorophyll in a wheat and corn leaf sample. We first homogenized the leaf tissue in 80% acetone, and then measured the absorbance at 663 nm and 645 nm. The chlorophyll concentration was then calculated using the specific absorption coefficients for chlorophyll a and b provided by Arnon (1949).

Twenty-six sites were selected to calculate the corn chlorophylls and each sample site was big enough (90 m × 90 m) to ensure the existence of a pure pixel in the image. The corn chlorophyll contents were collected at three positions randomly distributed inside the sample site and averaged to represent the ground truth data for a single pixel in the image. Finally, central position of the three samples was georegistered to the Hyperion image with GPS measurements.

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Structural type</th>
<th>Leaf color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laizhou</td>
<td>Erective</td>
<td>Dark green</td>
</tr>
<tr>
<td>2</td>
<td>Chaoyou</td>
<td>Middle</td>
<td>Dark green</td>
</tr>
<tr>
<td>3</td>
<td>Linkang 2</td>
<td>Loose</td>
<td>Dark green</td>
</tr>
<tr>
<td>4</td>
<td>Jing 8</td>
<td>Middle</td>
<td>Dark green</td>
</tr>
<tr>
<td>5</td>
<td>Jing 411</td>
<td>Erective</td>
<td>Light green</td>
</tr>
<tr>
<td>6</td>
<td>9507</td>
<td>Loose</td>
<td>Light green</td>
</tr>
</tbody>
</table>
2.2.3. Hyperspectral image acquisition

Hyperion is the first civilian spaceborne hyperspectral image and acquires data in 242 10-nm bands covering the visible, near, and shortwave-infrared ranges. The spatial resolution is 30 m and each pixel is processed using cubic convolution resampling kernel. Each Hyperion scene is collected in a narrow strip, covering a ground area approximately 7.7 km in the across-track direction, and 42 km or 185 km in the along-track direction. While the Hyperion instrument collects a total of 242 channels, not all of the acquired bands are calibrated primarily due to diminishing detector response at less optimum wavelengths, in addition to an area of overlap between the two spectrometers. Therefore, out of the 242 collected channels, bands 1–7 (356–417 nm) and bands 225–242 (2406–2578 nm) are not calibrated. Bands 58–70 (collected by the VNIR instrument) and bands 71–76 (collected by the SWIR instrument) are also not calibrated. Thus, the final data product will provide a total of 198 bands representing a spectral range from 427 nm to 2395 nm.

In this paper, the Hyperion data was acquired at the noon of 10 September and the chlorophyll content of corn was collected from 2 September to 12 September. Radiometric and geometric corrections were performed before analysis for Hyperion data.

2.3. Sensitivity study

2.3.1. Leaf reflectance simulation with the PROSPECT model

The PROSPECT model was used for leaf reflectance simulation because it is a widely accepted model in simulating leaf reflectance of different biochemical components (Jacquemoud and Baret, 1990). It can simulate upward and downward hemispherical radiation fluxes from 400 nm to 2500 nm, and link foliar biochemistry and scattering parameters to leaf reflectance and transmittance spectra.

To examine the linearity of different indices to chlorophyll content, the chlorophyll content increased from 10 µg cm⁻² to 100 µg cm⁻² at a step of 10 µg cm⁻² while the other parameters were assigned with normal values (Table 2). The selection of parameters values were based on observed during the LOPEX’93 (leaf optical properties experiment) experiment on fresh and dry leaves.

2.3.2. Canopy reflectance simulation with SAIL model

Canopy reflectance spectra were simulated using the scattering by arbitrary inclined leaves (SAIL) model (Verhoef, 1984). The revised form of SAIL, called SAILH, was adapted to take into account the hotspot effect or the multiple scattering in the canopy by Kuusk (1985). The SAIL model is based on a four-stream approximation of the radiative transfer equation with two direct fluxes (incident solar flux and radiance in the viewing direction) and two diffuse fluxes (upward and downward hemispherical flux). The interactions of these fluxes with the canopy were described by a system of four linear differential equations that can be analytically solved. Discussions and mathematical formalisms of SAIL and SAILH were provided by Goel (1988, 1989), Verhoef (1984, 1998), and Zarco-Tejada (2000). Here we used this model to simulate canopy reflectance in aiming to find indices which can overcome the saturation limits and thus to improve the linearity with LAI variations. LAI was changed from 0.2 to 10 and other parameters were set with normal values (Table 3).

2.4. Vegetation indices used in this analysis

2.4.1. Indices of normalized difference

The most well known and widely used vegetation index is the normalized difference vegetation index (NDVI) developed by Rouse et al. (1974). It is based on the contrast between the maximum absorption in the red due to chlorophyll pigments and the maximum reflection in the infrared caused by leaf cellular structure. Using hyperspectral narrow wavebands, this index is quantified by the following equation, where \( R_\text{n} \) is the reflectance at the given wavelength (nm):

\[
\text{NDVI}[670, 800] = \frac{R_{670} - R_{800}}{R_{670} + R_{800}}
\]

Despite its intensive use, NDVI saturates at dense and multi-layered canopy and shows a non-linear relationship with biophysical parameters such as LAI (Baret and Guyot, 1991; Lillesæter, 1982). Two indices (NDVI [670,800] and NDVI [705,750]) were tested in this study.

2.4.2. Indices of simple ratio

Simple ratio vegetation indices directly compare signals between the reflection and absorption peak of chlorophyll pigments which mean they are sensitive to changes in chlorophyll content changes. However, compared to NDVI, simple ratio indices are more influenced by environmental factors, such as cloud and soil (Slater and Jackson, 1982). In this analysis, we used the modified simple ratio (MSR) instead of SR to avoid these disturbances.

\[
\text{MSR}[670, 800] = \frac{(R_{680}/R_{670}) - 1}{\sqrt{(R_{680}/R_{670}) + 1}}
\]
2.4.3. Indices of three wavebands and integrated form

Indices incorporating bands in the green- and red-edge parts of the solar spectrum were developed to measure the light absorption by chlorophyll in the red region (670 nm). Kim et al. (1994) developed the chlorophyll absorption ratio index (CARI) which measures the depth of chlorophyll absorption at 670 nm relative to the green reflectance peak at 550 nm and the reflectance at 700 nm. CARI was designed to reduce the variability of the photosynthetically active radiation due to the presence of diverse nonphotosynthetic materials. It uses bands corresponding to the minimum absorption of the photosynthetic pigments, centered at 550 nm and 700 nm, in conjunction with the chlorophyll a maximum absorption band, around 670 nm. Detailed description of the CARI mechanism can be found in Kim et al. (1994) and Haboudane et al. (2002). Here we tested the modified chlorophyll absorption ratio index (MCARI) which is simplified by Daughtry et al. (2000) as the following equation:

\[
\text{MCARI}[670,700] = \frac{R_{700} - R_{670}}{R_{700} - R_{550}} \times \frac{R_{700}}{C_{138}}
\] (3)

However, previous studies demonstrated that MCARI was still sensitive to background reflectance properties and it was difficult to interpret the values at low LAI (Daughtry et al., 2000).

Daughtry et al. (2000) showed that MCARI was influenced by various parameters such as: LAI, chlorophyll, LAI–chlorophyll interaction, and the background reflectance. Moreover, Haboudane et al. (2002) pointed out that MCARI was still sensitive to nonphotosynthetic elements effects, mainly at low chlorophyll concentrations. Therefore, to compensate for the variations of reflectance characteristics of background materials (soil and nonphotosynthetic components) and to increase the sensitivity at low chlorophyll values, the transformed chlorophyll absorption ratio index (TCARI) can be defined as follows:

\[
\text{TCARI}[670,700] = 3 \left( \frac{R_{700} - R_{670}}{R_{700} - R_{550}} \right) \times \frac{R_{700}}{C_{138}}
\] (4)

Daughtry et al. (2000) proved that when MCARI combined with a soil line vegetation index like optimized soil-adjusted vegetation index (OSAVI; Rondeaux et al., 1996), the sensitivity to the underlying soil reflectance properties can be reduced. OSAVI belongs to the soil-adjusted vegetation index (SAVI; Huete, 1988) family and is defined by the following equation:

\[
\text{OSAVI}[670,800] = \frac{(1 + 0.16)(R_{800} - R_{670})}{(R_{800} + R_{670} + 0.16)}
\] (5)

Therefore, the two integrated forms of these three wavebands reflectance indices can be defined as:

\[
\text{TCARI}_{\text{OSAVI}}[670,800] = \frac{3(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})(R_{700}/R_{670})}{(1 + 0.16)(R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)}
\] (6)

\[
\text{MCARI}_{\text{OSAVI}}[670,800] = \frac{((R_{700} - R_{670}) - 0.2(R_{700} - R_{550}))(R_{700}/R_{670})}{(1 + 0.16)(R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)}
\] (7)

2.5. Bands used in this paper

Note that the aim of this paper was to find indices that can be used to predict chlorophyll content with high precision (better linearity) and at meanwhile also resistant to LAI variations. Since most of vegetation indices become saturated with increasing chlorophyll content or LAI, in this sensitivity study we were faced with two problems: band selection (chlorophyll prediction) and improvement of saturation limits (both chlorophyll content and LAI).

Spectral reflectance of vegetation from 400 nm to 700 nm region is primarily governed by the abundance of chlorophyll and other pigments absorbing most of the incident radiation (Thomas and Gausman, 1977). In this paper, bands at 550 nm, 670 nm, 700 nm, 705 nm, 750 nm, and 800 nm were selected. Reflectance of 670 nm is the maximum absorption in the red region. Sims and Gamon (2002) found that there were reliable relationships between leaf total chlorophylls and reflectance at 680 nm and 705 nm with correlation coefficients $r^2$ of 0.84 and 0.92, respectively. They also found a strong correlation between different combinations of reflectance at 700 nm, 705 nm, 750 nm, and 800 nm and leaf chlorophyll content. Therefore, reflectance of these bands was selected to formulate vegetation indices which may have potentials in estimating chlorophyll content. Specifically, reflectance at 800 nm and 670 nm were replaced by that of 705 nm and 705 nm to form the revised indices. Ten indices (Table 4) were tested in this study.

As previously mentioned, Hyperion data covers a long spectrum from 350 nm to 2580 nm with 242 bands. In the bands selection of Hyperion data, six bands were considered to be appropriate in this paper (Table 5). Since no Hyperion band centered at 705 nm, two relative bands (B035 and B036) are chosen to calculate the reflectance of $R_{705}$ with the formula below:

\[
R_{705} = 0.6R_{803} + 0.4R_{803}
\] (8)

3. Results and analysis

3.1. Sensitivity to chlorophylls effects and saturation limits

The sensitivity study was based on models rather than on the ground truth data. In sensitivity comparison, indices that can estimate chlorophyll content more accurately (better linearity with increasing chlorophyll content) will be the best choice especially for high content of chlorophylls. Since most of vegetation indices become saturated with increasing chlorophylls, they will have low sensitivity to high chlorophylls. The main purpose of this sensitivity test is to explore the performance of different indices and band combinations and to find indices which can overcome the saturation limits. To compare with other indices, we scaled the sensitivity results between 0 and 1 as shown in Fig. 2.

The simulation results showed that the normalized difference indices saturated with chlorophylls increasing from 10 $\mu$g cm$^{-2}$ to 100 $\mu$g cm$^{-2}$ (Fig. 2). The curve of NDVI[670,800]...
Table 4 – All hyperspectral vegetation indices used in the sensitivity analysis

<table>
<thead>
<tr>
<th>Indices</th>
<th>Wavebands (nm)</th>
<th>Formula</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original indices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDVI[670,800]</td>
<td>670, 800</td>
<td>(\text{NDVI} = \frac{\text{R}<em>{670} - \text{R}</em>{800}}{\text{R}<em>{670} + \text{R}</em>{800}})</td>
<td>Rouse et al., 1974</td>
</tr>
<tr>
<td>NDVI[705,750]</td>
<td>705, 750</td>
<td>(\text{NDVI} = \frac{\text{R}<em>{705} - \text{R}</em>{750}}{\text{R}<em>{705} + \text{R}</em>{750}})</td>
<td>Gitelson and Merzlyak, 1994</td>
</tr>
<tr>
<td>MSR[670,800]</td>
<td>670, 800</td>
<td>(\text{MSR} = \frac{(R_{670}/R_{705}) - 1}{\sqrt{(R_{670}/R_{705}) + 1}})</td>
<td>Chen, 1996</td>
</tr>
<tr>
<td>MCARI[670,700]</td>
<td>550, 670, and 700</td>
<td>(\text{MCARI} = \frac{(R_{550} - R_{670}) - 0.2 \times (R_{550} - R_{705})}{R_{550}/R_{705}})</td>
<td>Daughtry et al., 2000</td>
</tr>
<tr>
<td>TCARI/OSAVI[670,800]</td>
<td>550, 670, 700, and 800</td>
<td>(\text{TCARI} = \frac{3(R_{550} - R_{670}) - 0.2 \times (R_{550} - R_{705})(R_{550}/R_{705})}{(1 - 0.16)(R_{670} - R_{705})(R_{670} + R_{705} + 0.16)})</td>
<td>Daughtry et al., 2000; Rondeaux et al., 1996</td>
</tr>
<tr>
<td>MCARI/OSAVI[670,800]</td>
<td>550, 670, 700, and 800</td>
<td>(\text{MCARI} = \frac{(R_{550} - R_{670}) - 0.2 \times (R_{550} - R_{705})(R_{550}/R_{705})}{(1 - 0.16)(R_{670} - R_{705})(R_{670} + R_{705} + 0.16)})</td>
<td>Daughtry et al., 2000; Rondeaux et al., 1996</td>
</tr>
<tr>
<td>Revised indices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSR[705,750]</td>
<td>705, 750</td>
<td>(\text{MSR} = \frac{(R_{705}/R_{750}) - 1}{\sqrt{(R_{705}/R_{750}) + 1}})</td>
<td>–</td>
</tr>
<tr>
<td>MCARI[705,750]</td>
<td>550, 705, and 750</td>
<td>(\text{MCARI} = \frac{(R_{550} - R_{705}) - 0.2 \times (R_{550} - R_{750})}{R_{550}/R_{750}})</td>
<td>–</td>
</tr>
<tr>
<td>TCARI/OSAVI[705,750]</td>
<td>550, 705, and 750</td>
<td>(\text{TCARI} = \frac{3(R_{550} - R_{705}) - 0.2 \times (R_{550} - R_{750})(R_{550}/R_{750})}{(1 - 0.16)(R_{705} - R_{750})(R_{705} + R_{750} + 0.16)})</td>
<td>–</td>
</tr>
<tr>
<td>MCARI/OSAVI[705,750]</td>
<td>550, 705, and 750</td>
<td>(\text{MCARI} = \frac{(R_{550} - R_{705}) - 0.2 \times (R_{550} - R_{750})(R_{550}/R_{750})}{(1 - 0.16)(R_{705} - R_{750})(R_{705} + R_{750} + 0.16)})</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 5 – Information of the selected bands of Hyperion data**

<table>
<thead>
<tr>
<th>Hyperion band</th>
<th>Average wavelength (nm)</th>
<th>FWHM (nm)</th>
<th>Spatial resolution (m)</th>
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</thead>
<tbody>
<tr>
<td>B020</td>
<td>548.92</td>
<td>11.0245</td>
<td>30</td>
</tr>
<tr>
<td>B032</td>
<td>671.02</td>
<td>10.2980</td>
<td>30</td>
</tr>
<tr>
<td>B035</td>
<td>701.55</td>
<td>10.4592</td>
<td>30</td>
</tr>
<tr>
<td>B036</td>
<td>711.72</td>
<td>10.5322</td>
<td>30</td>
</tr>
<tr>
<td>B040</td>
<td>752.43</td>
<td>10.7058</td>
<td>30</td>
</tr>
<tr>
<td>B045</td>
<td>803.30</td>
<td>11.1044</td>
<td>30</td>
</tr>
</tbody>
</table>

saturated when chlorophyll content exceeded 60 \(\mu\)g cm\(^{-2}\). Compared to NDVI[670,800] and NDVI[705,750], the saturation problem is to some extent alleviated. The reason is that relatively low chlorophyll content is sufficient to saturate absorption in 670 nm region. Therefore, different wavelength, such as 550 nm, 705 nm, and 750 nm are more suitable for the prediction of chlorophyll content. Similar situation occurs for indices MSR[670,800] and MSR[705,750].

MSR was expected better than NDVI in terms of sensitivity to leaf biophysical parameters due to its combination with the simple ratio (SR = NIR/Red; Jordan, 1969). Our results supported the expectation (Fig. 2). MSR[670] becomes saturated when chlorophyll content exceeds 80 \(\mu\)g cm\(^{-2}\). When reflectance at 705 nm was introduced, as MSR[705,750], a better linear relationship can be obtained with the increase of chlorophyll content.

MCARI[670,700] was first introduced by Daughtry et al. (2000) to characterize chlorophyll variation. In this paper we replaced reflectance of 670 nm and 700 nm with that of 705 nm and 750 nm, respectively. Fig. 2 shows the replacement helped to improve the linearity. As shown in Fig. 3, MCARI[670,700] reached its maximum value at chlorophyll content near 30 \(\mu\)g cm\(^{-2}\). This is because reflectance of 670 nm dropped rapidly at chlorophyll content less than 30 \(\mu\)g cm\(^{-2}\) and then saturated quickly at higher values.

For the two integrated indices, Broge and Mortensen (2002) found that there existed an exponential relationship between the chlorophyll content and index of TCARI/OSAVI[670,800]. This exponential relationship was also found in our sensitivity study. However, we can also obtain a nearly linear relationship when wave bands selection was introduced. Results of our sensitivity study show that indices composed of reflectance at 750 nm, 705 nm, and 550 nm have better linearity than that of 800 nm, 670 nm, and 550 nm. Similar relationships can also be observed in the comparison between indices of MCARI/OSAVI[670,800] and MCARI/OSAVI[705,750].

Generally, reflectance of 705 nm and 750 nm are more suitable for chlorophyll content estimation than that of 800 nm and 670 nm. Therefore, the four indices (MSR[705,750], MCARI[705,750], TCARI/OSAVI[705,750], and MCARI/OSAVI[705,750]) that have better linearity with chlorophyll content were selected as shown in Fig. 4 because they are all better than their counterparts.

### 3.2. Sensitivity to LAI effect

A lot of vegetation indices are influenced by the background materials such as underlying soil reflectance properties. Therefore, the OSAVI is integrated with other indices to reduce this effect. Fig. 5 is the results of sensitivity to LAI variations based on reflectance simulated by SAIL model with a range of LAI ranging from 0.2 to 9. The aim of this sensitivity study is to find indices that can have better linearity with LAI variation and resistant to the background disturbances especially the soil effect at low LAI. Thus, these indices may have better potential capabilities in estimating canopy chlorophyll content.
All vegetation indices tested are insensitive to high values of LAI because they all saturated when LAI exceeds certain levels. The normalized difference indices are most affected by high values of LAI. For example, NDVI[670,800], and NDVI[705,750] get saturated at LAI = 3. However, MSR[670,800] and MSR[705,750] exhibit a similar but better resistance than normalized difference indices to LAI variations without a clear saturation at high LAI (up to 5) (Fig. 5). Index MCARI[705,750] seems to be a better indicator of LAI because it has the better linearity at LAI < 6.

The integrated indices TCARI/OSAVI[670,800] and TCARI/OSAVI[705,750] have a similar relationship with LAI variations. Both of these two indices increase at a low LAI, reach the maximum value at LAI = 0.5, then decrease at higher LAI values. Index MCARI/OSAVI[670,800] has the same trend with TCARI/OSAVI[670,800] and TCARI/OSAVI[705,750] only differing in the point of reaching the maximum value (LAI = 2). Nevertheless, index MCARI/OSAVI[705,750] increases progressively as LAI increases from 0.2 to 9 and reaches the saturation at a LAI = 8. Same sensitivity to LAI patterns can be found with indices MCARI[705,750], MSR[670,800], and MSR[705,750].

The integrated indices, such as TVARI/OSAVI, are primarily used to reduce the effects of background materials, especially for the nonphotosynthetic components and soil reflectance at

Fig. 2 – Relationships between different vegetation indices and chlorophyll content. Chlorophyll content changes from 10 to 100 μg cm⁻² in steps of 10 μg cm⁻².

Fig. 3 – Effects of different chlorophyll content on reflectance of 550 nm, 670 nm, and 700 nm.
low LAI (Haboudane et al., 2002). However, the TCARI/
OSAVI[670,800] integrated index still seems largely affected
by the background materials as it quickly reaches the maximum
value at a LAI of 0.5 in this sensitivity study. This phenomenon
indicates a possible overestimation of the amount of chlor-
ophylls at low LAI. Index MCARI/OSAVI[670,800] exhibits
a similar character as shown in Fig. 5. However, the new
indices (TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750])
using reflectance at 750 nm, 705 nm, and 550 nm are not such
affected by the background disturbances at low LAI (LAI < 1).
Therefore, four vegetation indices (Fig. 6) that are relatively
resistant to LAI variations and background disturbances were
selected in this paper to explore their potentials in canopy
chlorophyll content estimation.

3.3. Ground validation

3.3.1. Regression between chlorophyll content and vegetation
indices at canopy scale
As analyzed above, the four new derived vegetation indices
(MSR[705,750], MCARI[705,750], TCARI/OSAVI[705,750], and

Fig. 4 – Indices of MSR[705,750], MCARI[705,750], TCARI/
OSAVI[705,750], and MCARI/OSAVI[705,750] with
chlorophyll content varying from 10 to 100 µg cm⁻² in
steps of 10 µg cm⁻².

Fig. 5 – Relationships between different vegetation indices and a range of LAI variations.

Fig. 6 – Relationships between different vegetation indices and a range of LAI variations.
MCARI/OSAVI[705,750] have the better linearity with chlorophylls variations and low sensitivity to nonphotosynthetic element effects, mainly at low chlorophyll concentrations and low LAI. In canopy validation study, both the original indices (based on 670 nm band) and four new proposed vegetation indices are validated with the ground truth data because it is very important to show differences between them which can testify the improvement of the new derived ones. Table 6 shows the correlation coefficients for the original indices, ranging from 0.4086 for MSR[670,800] to 0.5673 for MCARI/OSAVI[670,800]. Fig. 7 is the results of relationship between the new derived hyperspectral vegetation indices and the ground measured chlorophyll content on the 4 days. These results indicated that the new indices are better for chlorophyll content estimation because both of them have higher correlation coefficients than its counterparts.

As shown in Fig. 7, MSR[705,750] is not a reliable index for chlorophylls estimation. MSR[705,750] is affected by the soil reflectance and has low sensitivity to high LAI values. MCARI[705,750] (a revision of MCARI[670,700]) was initially developed responsible for chlorophyll content variations yet is largely affected by the underlying soil reflectance and the nonphotosynthetic materials such as stalks, heads, and senescent leaves. The new derived indices TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] that were modified from TCARI/OSAVI[670,800] and MCARI/OSAVI[670,800] are reliable in chlorophyll content estimation ($R^2$ of 0.8808 and 0.9406, respectively). This strong correlation existed for all the data collected in the four different days. The reason is that the integrated indices cancel out the effect of disturbances. For example, bands in the green- and red-edge wavelength of the spectrum were used to measure chlorophyll absorption (originally is 670 nm, substituted with 705 nm here). Kim et al. (1994) introduced the ratio ($R_{750}/R_{670}$, replaced with $R_{705}/R_{705}$ in this paper) to minimize the combined effects of the underlying soil reflectance and the canopy nonphotosynthetic materials. The index OSAVI was incorporated to reduce the

<table>
<thead>
<tr>
<th>Vegetation indices</th>
<th>$R^2$</th>
</tr>
</thead>
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<tr>
<td>MSR[670,800]</td>
<td>0.4086</td>
</tr>
<tr>
<td>MCARI[670,700]</td>
<td>0.4682</td>
</tr>
<tr>
<td>TCARI/OSAVI[670,800]</td>
<td>0.4984</td>
</tr>
<tr>
<td>MCARI/OSAVI[670,800]</td>
<td>0.5673</td>
</tr>
</tbody>
</table>

Table 6 – Correlation coefficients between the original indices and chlorophyll content of wheat

Fig. 6 – The relationship between four selected vegetation indices and LAI.

Fig. 7 – Relationship between measured chlorophyll content and the four different vegetation indices: MSR[705,750], MCARI[705,750], TCARI/OSAVI[705,750], and MCARI/OSAVI[705,750] ($R^2$ = 0.6742, 0.6968, 0.8808, and 0.9406, respectively).
background effect which was confirmed by Haboudane et al. (2002). The bands replacement is also consistent with the results of sensitivity analysis by Gitelson and Merzlyak (1996).

### 3.3.2. Validation of two integrated indices with Hyperion image and ground data

Two types of corns were selected in the validation of spaceborne Hyperion data. Due to the difference in the canopy component and structure, differences in the canopy, the spectroscopy varies over the Hyperion spectral range, especially in the green and near-infrared ranges (see Fig. 8).

As previously explained, only two indices and their original forms were tested in the chlorophyll content estimation with spaceborne remote sensing data. For the two types of corns, the two indices have shown to be reliable in the estimation of chlorophyll content with spaceborne hyperspectral data. Compared to indices based on 670 nm, the revised indices prove to be more feasible in chlorophyll content estimation as the clear improvement on correlation coefficients. Correlation coefficients $R^2$ for TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] are 0.6798 and 0.7618, respectively (Fig. 9).

### 3.3.3. Possibility of deriving a unique relationship in estimating chlorophylls

Much effort has been conducted to retrieve the biochemical components of leaf or canopy with remote sensing technology aiming to derive a unique relationship between the biochemical components and certain indices. However, our analysis shows it is still very difficult to establish a unique relationship relating the two parameters.

First, different slopes and intercepts were obtained in the validation of winter wheat and the predictive functions for wheat were also not reliable in the chlorophyll content estimation of corns with Hyperion data (data not shown here). Second, almost all the previous studies were on a single experiment vegetation species which means the specific formula derived from one plant species may not fit to other species. This may partly explain the existence of a number of "most appropriate indices" in the retrieval of chlorophyll content. Third, chlorophyll content estimation from spaceborne data became more complicated because more uncertainty related to scaling issues such as mixed pixel emerge. All these effects make estimation of chlorophyll content more challenging. Where a pixel covers more than one single species, the pixel reflectance is a combination of the reflectance of canopy and the type and amount of understory observed by the sensor (Robinove et al., 1981). In this situation, there is no single chlorophyll content versus vegetation index curve, but rather a family of curves. Each curve represents a...
function of canopy closure and the species reflectance characteristics. Therefore, the feasibility of certain indices depends on the situation such as species, developmental stages, stress, and nutritional state. It is of much more meaning of finding an appropriate index rather than establishing the unique relationship between the leaf biochemical or canopy parameters and remote sensing observations.

4. Conclusion

In this paper, 10 hyperspectral vegetation indices from three classes were tested to explore their potentials in the chlorophyll content estimation. The PROSPECT and SAIL models were used for leaf and canopy reflectance simulation in the sensitivity study with a wide range of chlorophyll content and LAI variations. As reflectance at 670 nm will quickly become saturated with relatively low chlorophyll content, different bands combinations were applied to derive the new indices that may have better linearity with the chlorophyll content. Index that is composed of reflectance at 750 nm and 705 nm (NDVI[705,750]) proves to have better linearity than index composed of reflectance at 800 nm and 670 nm (NDVI[670,800]), especially at high chlorophyll content values. The method of reflectance replacement also proves to be successful for other two classes of indices (MSR indices, indices of three bands, and the integrated forms). The result of LAI sensitivity study demonstrated that the four new derived vegetation indices (MSR[705,750], MCARI[705,750], TCARI/OSAVI[705,750], and MCARI/OSAVI[705,750]) are appropriate candidates for canopy chlorophyll content estimation. With the incorporation of the index OSAVI, the two integrated indices (TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750]) are reliable in reducing the background effects which is consistent with the finding of Haboudane et al. (2002).

Validation study was also conducted with the ground truth data in 4 days of 2007 (17 April, 28 April, 16 May and 29 May). The two integrated vegetation indices TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] were proved to be the best for chlorophyll content estimation with correlation coefficients $R^2$ of 0.8808 and 0.9406, respectively. This is because these two integrated indices accounts more effects of disturbances, such as the soil reflectance and nonphotosynthetic materials. This finding agrees well with the results of Haboudane et al. (2002).

The assessment of the feasibility of certain indices in estimating chlorophyll content at canopy level with ground truth data can be viewed as a first step of remote estimation of chlorophyll content from satellite data (Weiss et al., 2001). The validation of the indices TCARI/OSAVI[705,750] and MCARI/OSAVI[705,750] with Hyperion data indicates that these indices are feasible across species in chlorophylls estimation with spaceborne hyperspectral Hyperion data. Haboudane et al. (2002) pointed out that one thing of particular importance in chlorophyll content estimation was the analysis of right spectral bands and combination which could enhance sensitivity to chlorophyll content variations and reduce responsivity to background and canopy structure effects. In this paper, two specific bands at 705 nm and 750 nm were introduced to propose new indices (MSR[705,750], MCARI[705,750], TCARI/OSAVI[705,750], and MCARI/OSAVI[705,750]) to explore the potentials in chlorophyll. The primarily results of this study may provide some reference to the further research of chlorophyll content estimation.

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