Prediction of SPAD chlorophyll meter readings using remote sensing technique
(Ramalan bacaan meter klorofil SPAD menggunakan teknik penderiaan jauh)

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Keywords: rice plant, SPAD reading prediction, unmanned airborne vehicle, remote sensing

Abstract
A method using unmanned airborne vehicle (UAV) and image processing technique to enable prediction of SPAD chlorophyll meter readings was developed. Relationships between SPAD readings and R, G, B, R/(R+G+B), G/(R+G+B), and B/(R+G+B) values were analysed. The R/(R+G+B) values indicate the highest correlation with SPAD readings with $r^2$ value of -0.9695 and a SPAD reading prediction model was developed from the relationship analysis. The prediction model is capable to predict SPAD reading with average accuracy value of 89%. A SPAD reading map was generated by converting the spectral reflectance values into SPAD readings using the prediction model. This SPAD reading map was classified into high, medium and low levels of SPAD values for easy identification of N stress levels in the paddy fields.

Introduction
Rice is a staple food for more than 3 billion people in the world. In 2007 the rice self-sufficiency level for Malaysia was 72% (Liew et al. 2010). Total area of rice cultivation in Malaysia in 2009 was 676,034 hectares with total production of about 2.19 million tonnes (MOA 2010). In the past few years, rice growers were facing higher production costs especially fertilizer.

Crop yield is directly related to the amount of nutrients taken up by the crop, and fertilizers supply a significant portion of the nutrients required to achieve a high and profitable yield. Insufficient fertilizer inputs will affect plant growth while over application will acidify soils and water resources. Optimization of fertilizer inputs is important for minimizing production cost, maximizing profitable yields and decreasing environmental impact.

Precision farming (PF) is a solution for minimizing fertilizer input and maximizing yields. According to Patil (2009), PF is a management strategy that employs detailed site specific information to precisely manage production inputs. PF can contribute in many ways to long term sustainability of agriculture. The idea is to know the soil and crop characteristics unique to each part of the field, and to optimize the production inputs within small portions of the field.

Currently, the farmer's usual practice is to apply fertilizers at one rate throughout the farming area. Such practice can lead to wastage of resources and maximum yields cannot be achieved, since spatial variability is altogether ignored in the management...
option (Patil 2009). This blanket or package fertilizer recommendations over large areas is not efficient because indigenous nutrient supply varies widely among rice fields (Dobermann and White 1999; Olk et al. 1999).

Generally, nitrogen (N) is required in large quantities, and it is the most mobile and dynamic nutrient in soil systems. It is well documented that soil physical and chemical properties are spatially variable and affect N dynamics and the mechanisms for its losses (Khosla et al. 2002). N application often dramatically increases crop yields, but N needs vary spatially across fields and landscapes (Scharf et al. 2002). Variable-rate N management is one of the most important activities in PF for applying precise amount of fertilizer required to maximize crop yields.

According to Scharf et al. (2002) the soil spectral property (i.e. colour) is related to soil organic matter and soil moisture levels, factors that influence the N-supplying ability of the soil. Plant spectral properties reflect crop N status and soil N availability that are useful for directing in-season variable-rate N applications. Plant colour may also be useful for assessing the adequacy of crop N supply achieved with a given N management practice. Various strategies of variable-rate N management system based on plant spectral properties are being developed for optimizing fertilizer usage in rice fields.

Holland and Schepers (2010) developed a production-based in-season N recommendation model for use with crop canopy sensors and remote sensing data. This approach is based on the general shape of a N fertilizer response function (sensor index vs. N rate) and the relationship between N rate and in-season crop vegetation index data. Transformation and substitution techniques were used to generate a simple function that offers an N fertilizer recommendation based on spatially variable in-season remote sensing data and established local crop production information such as the economic optimum N rate or producer defined optimum N rate.

Gholizadeh et al. (2011) described a conceptual framework for applying principles and technology of PF to understand and control the system of agriculture towards a low input, high efficiency and sustainable agriculture in a paddy field. The experiment concluded that SPAD (Soil Plant Analysis Development) readings can be used to predict leaves’ total N amount and future crop N needs. The adaptation of the SPAD meter is needed in PF for Malaysian paddy fields under double cultivation to assess crop N status and determine the plant’s needs.

According to Percival et al. (2008), the SPAD meter is a commercially available portable piece of equipment that is used to measure leaf greenness (SPAD readings) based on optical responses when a leaf is exposed to light. These responses are then used to estimate foliar chlorophyll concentrations. The meter makes instantaneous and non-destructive readings on a plant based on the quantification of light intensity.

The SPAD meter has been used by Peng et al. (1996) to monitor plant N status in situ in the fields and to determine the right time of N topdressing in rice.

Balasubramanian et al. (1999) reported that the SPAD threshold value of 35 is good for transplanted rice in dry season. The threshold has to be reduced to 32 for wet-seeded rice in dry season and for all rice during wet season with cloudy weather and low radiation. Thus, when calibrated with local cultivar groups and crop conditions, it can be used to accurately monitor crop N status and to advise farmers on N topdressing for rice. It can also be used effectively to verify the adequacy of existing N fertilizer recommendations to rice by the in situ monitoring of foliar N status of crops fertilized with current recommendations and to refine them to further improve N fertilization of rice.
Nguyen et al. (2007) mentioned that N management at the panicle initiation stage (PI) should be fine-tuned for securing a concurrent high yield and high quality rice production. Hence an experiment for calibration and testing of the recommendation models of N topdressing rates at PI for target grain yield and protein content of rice was conducted. Data from the first two years of experiments were used to calibrate models to predict grain yield and milled rice protein content using shoot fresh weight (FW), SPAD reading, and the N topdressing rate (Npi) at PI by stepwise multiple regression. Results showed that the recommended N rate treatments for the target protein content of 6.8% and grain yield were highly dependent on FW and SPAD reading at PI.

Recently, optical sensing of crop canopy spectral reflectance from ground to aircraft and satellite-based platforms has been introduced to estimate the crop N deficient portions of the whole fields, and directing site-specific fertilizer applications for improving N use efficiency in the fields (Raun et al. 2002). Remote sensing collects spatially dense information that may contribute to, or provide feedback about N management decisions. There is potential to accurately predict N fertilizer need at each point in the field. This would reduce surplus N in the crop production system without reducing crop yield, which would in turn reduce N losses to surface and ground waters (Scharf et al. 2002).

Shigeto and Makoto (1998) developed a low-cost diagnostic method that is easy to use to assess the nutrient status of plants, based on the estimation of chlorophyll content of leaves using a portable colour video camera and a personal computer. The method analysed the relationships between chlorophyll content and various functions derived from red, green and blue wavelengths. Results showed that although red-blue and green-blue wavelengths present the highest correlation with chlorophyll content under a limited range of meteorological conditions, the normalized difference (red-blue)/(red-blue) is the most applicable function which can use data collected under different meteorological conditions. The accuracy in estimating chlorophyll content from video images could be improved by correcting the solar radiation data.

Yoder and Pettigrew-Crosby (1995) investigated that the spectral characteristics associated with crop N status rely on the variation in strength of chlorophyll absorbance in the visible (450–690 nm) region of the electromagnetic spectrum. Results indicated that shortwave infrared bands were best predictors for nitrogen, visible bands best for chlorophyll. In the shortwave infrared region, however, the absolute differences in reflectance at critical bands were extremely small, and the bands of high correlation were narrow. High spectral and radiance resolution are required to resolve these differences accurately.

Daughtry et al. (2000) conducted an experiment to select wavelengths sensitive to leaf chlorophyll concentration, to simulate canopy reflectance using a radiative transfer model, and to propose a strategy for detecting leaf chlorophyll status of plants using remotely sensed data. In the study, a wide range of leaf chlorophyll levels was established in field-grown corn (*Zea mays* L.) with different rates of N application. Crop canopy reflectance was simulated using the SAIL (Scattering by Arbitrarily Inclined Leaves) canopy reflectance model. Spectral vegetation indices that combined near-infrared reflectance and red reflectance (e.g., OSAVI and NIR/Red) and near-infrared and other visible bands (MCARI and NIR/Green) when plotted together produced isolines of leaf chlorophyll concentrations. The slopes of these isolines were linearly related to leaf chlorophyll concentration.

The objective of this paper was to develop a SPAD reading prediction model for generating N fertilizer treatment map using image processing technique.
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**Materials and methods**

*Figure 1* shows the flow of the methodology consisting of field-based data collection, images acquisition, spectral reflectance value extraction, statistical analysis and SPAD reading map generation.

**Field-based SPAD data collection**

An experiment on plant growth was conducted with application of 6 different fertilizer treatments in the paddy field at FELCRA Seberang Perak on 13th April 2009. The experiment was carried out by planting variety MR 219 seeds in four fertilizer treatment plots and each plot was subdivided into 6 equal size small plots. Each subdivided treatment plot (STP) with an area of 20 m X 10 m was applied a specific amount of N fertilizer as follows: STP1 = 0 kg, STP2 = 1 kg, STP3 = 2 kg, STP4 = 3 kg, STP 5 = 3.5 kg and STP6 = 4 kg. A field-based SPAD data was collected using SPAD chlorophyll meter on 3 June 2009 (at 51 days growing stage). The data collection was performed by taking SPAD reading at the four corner points and centre point of a 25 cm² aluminium frame square at the centre of each subdivided treatment plot.

**Images acquisition**

A total of 100 red (R), green (G) and blue (B) images of the study area were obtained below a cloud canopy by a CropCam unmanned airborne vehicle (UAV) at 9.30 a.m, 3 June 2009 with 280 m flying height. The CropCam is a radio controlled model glider plane equipped with a Trimble 1Hz update rate, Lassen IQ (P/N 46240-25), a miniature autopilot and digital camera. The weight of the miniature CropCam aircraft is about 2.7 kg, has a 2.44 m wingspan and an overall length of 1.22 m. The engine runs on four 2100 mah lithium polymer batteries. A set of full charged batteries provides about 30 min running time. The average speed of the CropCam is 60 km/h and can fly in winds up to 30 km/h. The CropCam can fly at an altitude of 122–640 m (depending on the country’s flying circular or regulation). The MP2028P Autopilot is installed and pre-programmed with capability to program user flight plans. Hand launched and automatically flown from take off to landing, the CropCam provides high resolution GPS based images on demand. Both the CropCam and the camera perform automatically to take GPS based digital images.

**Spectral reflectance value extraction**

All captured images with latitude, longitude and altitude information were mosaic and processed using a commercial PCI image processing software. After processing, the image spatial resolution was 6.25 cm. The locations of SPAD readings taken at each centre of the subdivided treatment plot were identified. A 4 x 4 pixels mask equal size with 25 cm² aluminium frame square was
marked at the centre of each subdivided treatment plot on the mosaic image for R, G, and B spectral reflectance values extraction.

**Statistical analysis**
The mean values of SPAD readings and R, G, and B spectral reflectance values were calculated. Relationships between SPAD readings and R, G, B values were analysed to establish the calibration curve to predict the SPAD readings. The equations, R/(R+G+B), G/(R+G+B) and B/(R+G+B), which have been used by Shigeto and Makoto (1998), were used to estimate chlorophyll content of leaves. The best coefficient of determination ($r^2$) of the calibration curve was selected to predict SPAD readings.

**Generation of SPAD reading map**
SPAD reading map was generated by converting the spectral reflectance values into SPAD readings using the selected calibration curve. The SPAD reading map was classified into low, medium and high value clusters using Iterative Self-Organizing Data Analysis (ISODATA) image processing technique. ISODATA is an unsupervised classification method that uses an iterative approach incorporating a number of heuristic procedures to compute clusters. This clustering method uses minimum spectral distance formula, which is based on Euclidean distance equation to form clusters (Swain and Davis 1978). The equation is given below:

$$SD_{xyc} = \sqrt{\sum_{i=1}^{n} \left( \mu_{ci} - X_{xyi} \right)^2}$$

where,
- $n =$ The number of bands
- $i =$ The band number
- $c =$ A particular class
- $X_{xyi} =$ The data file value of pixel $x$, $y$ in band $i$
- $\mu_{ci} =$ The mean of data file values (digital numbers) in band $i$ for the sample for class $c$
- $SD_{xyc} =$ The spectral distance from pixel $x$, $y$ to mean of class $c$

**Results and discussion**
*Figure 2* shows the mosaic image for the study area. *Table 1* shows the average SPAD readings, R, G and B spectral reflectance values for all experimental plots with different N fertilizer applications. Means and standard deviations for the average SPAD readings, R, G and B spectral reflectance values were 31.99 and 3.49, 91.88 and 8.2, 112.75 and 5.06, and 62.75 and 2.75 respectively.

*Figure 3* shows the relationship between measured SPAD readings and N fertilizer treatments. The chlorophyll
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Table 1. Average of SPAD readings and R, G and B spectral reflectance values of the experimental plots with different N fertilizer applications

<table>
<thead>
<tr>
<th>Subdivided treatment plot</th>
<th>N fertilizer treatment (kg)</th>
<th>Average SPAD reading</th>
<th>Average spectral reflectance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>STP1</td>
<td>0</td>
<td>25.99</td>
<td>106.75</td>
</tr>
<tr>
<td>STP2</td>
<td>1</td>
<td>30.49</td>
<td>91.75</td>
</tr>
<tr>
<td>STP3</td>
<td>2</td>
<td>32.71</td>
<td>92.75</td>
</tr>
<tr>
<td>STP4</td>
<td>3</td>
<td>32.65</td>
<td>89.50</td>
</tr>
<tr>
<td>STP5</td>
<td>3.5</td>
<td>33.86</td>
<td>88.50</td>
</tr>
<tr>
<td>STP6</td>
<td>4</td>
<td>36.25</td>
<td>82.00</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>31.99</td>
<td>91.88</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td>3.49</td>
<td>8.20</td>
</tr>
</tbody>
</table>

content in the plant leaves measured by SPAD meter is closely related to the N applied in the field for plant growth. The SPAD readings increased as the amount of N application increased due to sufficient amounts of available nutrients taken up by the plants to develop a healthy green colour. SPAD reading depends on the amount of chlorophyll content (i.e. green pigment) found in the plant.

Figure 4a indicates the relationship between measured SPAD readings and R, G and B spectral reflectance values. Figure 4b presents the relationship...
between measured SPAD readings and R/(R+G+B), G/(R+G+B) and B/(R+G+B) values. The negative $r^2$ values indicated that as the values for spectral reflectance increased, values for SPAD readings decreased while the positive values showed a linear relationship between the two variables such that an increase in spectral reflectance also increased the SPAD readings. Results showed that the highest and lowest relationships were found in R/(R+G+B) and B with $r^2$ values of -0.9695 and -0.175 respectively. Comparison of $r^2$ values indicated that the first two highest values were present in R/(R+G+B) and R. The regression models describing the dependency between (i) SPAD readings and R spectral reflectance values (Figure 4a) and (ii) SPAD readings and R/(R+G+B) values (Figure 4b) are shown in the equations below:

$$\text{SPAD reading} = -0.4108 \times [\text{R spectral reflectance value}] + 69.737 \quad \text{Equation (1)}$$

$$\text{SPAD reading} = -277.05 \times \left[ \frac{\text{R}}{\text{R}+\text{G}+\text{B}} \right] + 127.05 \quad \text{Equation (2)}$$

Figures 5a and 5b indicate 95% confidence and prediction intervals result. The prediction bands are wider than the corresponding confidence bands to allow the linear model to predict the value of a random variable rather than estimating a parameter. As a result, the linear models give a good confidence for prediction of SPAD reading based on R spectral reflectance values, and R/(R+G+B) values. The 95% confident intervals for intercept and slope of linear model for R spectral reflectance values and R/(R+G+B) values were 55.86 to 83.62 and -0.5614 to -0.2602, and 103.6 to -150.5 and -345 to -209 respectively.

The validity of the results of the linear regression analysis requires fulfilment of certain assumptions about the data. Particularly, the study assumes that the linear model is appropriate, the residuals are independent and normally distributed with the same variance everywhere (Chiparus and Chen 2003). An analysis of the residuals was used to detect violations of assumptions. Figures 6a and 6b present the plots of residuals versus independent values. It can be seen that the plots show homogeneous error variances and does not show any pattern. This gave confidence regarding the normal distribution of the data and suggests that assumptions are fulfilled. The results from the plots also showed that there are no extremely large residuals (and hence no apparent outliers) and that there is no trend in the residuals to indicate the two linear models are inappropriate.

![Figure 6a. The residuals (error) distribution for R spectral reflectance value](image1.png)

![Figure 6b. The residuals (error) distribution for R/(R+G+B) value](image2.png)
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The two SPAD reading prediction models were verified by 8 random SPAD reading samples that were measured from outside the experimental plots using SPAD meter. Table 2 shows the measured SPAD readings and predicted SPAD readings using the two SPAD reading prediction models. Mean and standard deviation for the measured SPAD readings were 34.79 and 1.33 respectively while for predicted SPAD readings using linear models based on R spectral reflectance values and R/(R+G+B) values were 33.07 and 4.8, and 31.78 and 3.02 respectively. Comparison between measured and predicted SPAD values showed that the R spectral reflectance values, and R/(R+G+B) values are capable to predict SPAD readings with average accuracy values of 87% and 89% respectively. The prediction accuracy by the R/(R+G+B) values is slightly higher than R spectral reflectance values due to the normalizing correction of the distortions caused by solar radiation and shadows in the image. Figure 7 shows the SPAD reading map that is generated by converting the spectral reflectance values into SPAD readings using prediction model developed from the R/(R+G+B) equation. The SPAD readings ranged from 28 to 61 with mean and standard deviation values of 48.07 and 3.22 respectively. Figure 8

Table 2. Measured SPAD readings and predicted SPAD readings

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Measured average SPAD readings</th>
<th>Predicted SPAD readings by R</th>
<th>Predicted SPAD readings by R/(R+G+B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.78</td>
<td>29.89</td>
<td>30.73</td>
</tr>
<tr>
<td>2</td>
<td>35.58</td>
<td>26.19</td>
<td>30.13</td>
</tr>
<tr>
<td>3</td>
<td>35.58</td>
<td>31.12</td>
<td>28.4</td>
</tr>
<tr>
<td>4</td>
<td>33.28</td>
<td>41.8</td>
<td>36.04</td>
</tr>
<tr>
<td>5</td>
<td>36.54</td>
<td>36.46</td>
<td>35.83</td>
</tr>
<tr>
<td>6</td>
<td>35.36</td>
<td>33.59</td>
<td>32.55</td>
</tr>
<tr>
<td>7</td>
<td>35.46</td>
<td>30.3</td>
<td>28.18</td>
</tr>
<tr>
<td>8</td>
<td>33.78</td>
<td>35.23</td>
<td>32.45</td>
</tr>
<tr>
<td>Mean</td>
<td>34.79</td>
<td>33.07</td>
<td>31.78</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.33</td>
<td>4.8</td>
<td>3.02</td>
</tr>
<tr>
<td>Average accuracy</td>
<td>87%</td>
<td>89%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. SPAD reading map

Figure 8. Classified SPAD reading map
presents the classified SPAD reading map using ISODATA unsupervised classifier to classify the map into high, medium and low SPAD readings classes. This classified map is useful in identifying N stress levels based on SPAD readings for more precise N management in the field.

Conclusion
Relationships between SPAD readings and R, G, B, R/(R+G+B), G/(R+G+B) and B/(R+G+B) values have been analysed. The R/(R+G+B) values indicate the highest $r^2$ with a value of -0.9695. A SPAD reading prediction model that is capable to predict SPAD readings with an average accuracy value of 89% has been developed. A SPAD reading map classified into high, medium and low levels of SPAD values was generated using the developed prediction model for easy identification of N stress levels in the field. The classified SPAD reading map in conjunction with other field information such as soil fertility and yield maps can be used to develop variable-rate N application system for site-specific farming operations. The present work is still preliminary using RGB images obtained from UAV system with limited ground sampling points. Further research emphasis will be on development of SPAD reading prediction method using RGB and near infrared images and more ground sampling points will be collected to improve the accuracy of SPAD reading prediction model.

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