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4 Low-Cost Chlorophyll Meter (LCCM) : Portable Measuring Device for Leaf Chlorophyll

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ABSTRACT

Portable leaf chlorophyll meter, named low-cost chlorophyll meter (LCCM), has been created. This device was created to help farmer determining the health condition of plant based on the greenness level of leaf surface. According to previous studies, leaf greenness with a certain amount of chlorophyll level has a direct correlation with the amount of nitrogen in the leaf that indicates health of the plant and this fact needed to provide an estimate of further measures to keep the plants healthy. Device that enables to measure the leaf color change is soil plant analysis development (SPAD) meter 502 from Konica Minolta but it is relatively expensive. To answer the need of low-cost chlorophyll scanner device, this research conducted experiment using light reflectance as the base mechanism. Reflectance system from LCCM consists of near-infrared light emitting diode (LED) and red LED as light resources and photodiode. The output from both of light resources calculated using normalized difference vegetation index (NDVI) formula as the results fetched and displayed on the smartphone application using Bluetooth communication protocol. Finally, the scanner has been made as well as the Android application named NDVI Reader. The LCCM system which has been tested on 20 sample of cassava leaf with SPAD meter as a variable control showed coefficient of determination 0.9681 and root-mean-square error (RMSE) 0.014.

Keywords: Reflectance, NDVI value, regression analysis, low-cost device

1. INTRODUCTION

Chlorophyll is an important visible parameter to monitor the physiological status of plants. Greenness or the chlorophyll content in plants is controlled by the availability of nutrients such as nitrogen, magnesium, iron, calcium, manganese and zinc^{1,2}. Shortages of these elements can affect chlorophyll formation, thus causing leaf discoloration and impacting on photosynthesis efficiency and yield^{2,3}. On the other hand, an excessive level of nutrient like nitrogen by fertilizer can leach through the soil beyond the root system potentially polluting groundwater resources⁴. The loss of greenness can also be used to monitor leaf senescence as well as biotic and abiotic stresses such as pathogen infection and water limitation⁵.

To check the plant health status, i.e. the level of nitrogen, different tools such as the N-Kjeldahl and leaf color charts have been used. The Kjeldahl test is most accurate, but a destructive, time consuming and high costs method. The leaf color chart method is a fast, non-destructive and cheap method, but uses the principle of subjective qualitative comparison by eye. Recently, a method has been developed to estimate nitrogen content using two-dimensional data of the color image from the leaf⁶.

This technique has been implemented to estimate SPAD values from images acquired by a smartphone app named SmartSPAD⁷. The SmartSPAD application uses image data acquisition technique which implements dark green

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color index (DGCI) to estimate nitrogen content on the leaf surface. Nitrogen, in some exposure studies, has been verified to show a correlation to chlorophyll⁸. If the nitrogen content is high, chlorophyll content is also high, and vice versa. In order to get DGCI index, SmartSPAD uses contact imaging method where image acquisition is taken by attaching the camera to the leaf. The advantage of this technique is that it does not need segmentation technique anymore in the separation of the background reference to the object which is being acquired. But it has some drawbacks when the android application is installed on another smartphone which varies in the camera specification. The constraint of every camera specification is different and there is no difference between blur or focus acquisition data, this is a drawback of this method.

Currently, there is already a Konica Minolta SPAD 502 Chlorophyll Meter in the market which uses two light-emitting diodes (650 and 940 nm) and a photodiode detector to sequentially measure transmission through leaves of red and near infrared light⁹. In comparison to the smartphone data acquisition, Minolta SPAD 502 Chlorophyll Meter has a clip mechanism that enables this tool impermeable of ambient light and only acquires one dimension data. However, Minolta SPAD 502 Chlorophyll Meter has the limitation of insufficient storage to save the image data acquisition. Relating with the cost, Minolta SPAD 502 Chlorophyll Meter is rather expensive for farmers.

When two different techniques of the SmartSPAD and Minolta SPAD 502 Chlorophyll Meter are combined, it gives an idea to create a system tool that provides a simple, fast, and non-destructive way for evaluating the chlorophyll level. This idea consists of a simple multispectral scanner, an Android application as a viewer and data storage for data acquisition. Here, we created a device that can save data image into a micro SD card which can save more data than the SPAD meter. Moreover, the data can also be processed in several formats, i.e. .csv and .txt, and its can be transferred easily to computer for further processing. Our device has slip-on design which is focusing on one point scan and to suppress the ambient interference of light. Then data acquisition processed into NDVI value, transferred through Bluetooth communication protocol and can be directly displayed and stored on a smartphone in the form of a .csv format.

2. MATERIAL AND METHODS

2.1 Data collection

The data were collected from cassava leaf (*Manihot utilissima* Pohl) at Ma Chung University MRCPP field, Malang, East Java, Indonesia on January 2016 growing season. All fresh cassava leaves were taken on the site without harming the leaves with both SPAD meter and LCCM device at 10.00 AM – 12.00 PM with 5 scan iterations, 20 total samples mixed with greenish and yellowish cassava leaf (Figure 1). Main reason using cassava leaf as material testing tools is because the foliar surface of cassava leaf has a flat surface and relatively has wider foliar size. The selected cassava leaf are the cassava leaf which has same color distribution on its foliar surface. It aims to reduce a big difference of standard deviation on LCCM and SPAD dataset.



Figure 1. Some of cassava leaf samples with normalized background ordered from yellowish (upper left) to greenish color (bottom right)

2.2 LCCM software development

The development stage was divided into two parts: android application development and Arduino microcontroller scripts. Android which has roles as data storage and data viewer was picked prior to large

penetration of market acceptance and affordable development price in comparison to the iPhone iOS¹⁰. The base language used in developing LCCM android application is Oracle Java version 8 Update 45, Android SDK (Software Development Kit) Build tools 22, Android 5.1.1 Lollipop, integrated development environment (IDE) Android Studio 1.5 and Smartphone Asus Zenfone 5 A500CG with v4.0 Bluetooth as deployment tool.

First of all, it was necessary to design a workflow of an android application within activity which was the basic component to specifies an interaction with a user¹¹. The real appearance of the activity was a full-screen window of the mobile application. The activities that would be made are Bluetooth activity list, the data viewer activity, and the data gatherer activity.

LCCM Scanner would compute both near infrared and red led reflectance input in order to generate NDVI value as an output. The overcome this issue, a script program would be needed. Arduino is an open-source hardware and software based on C/C++ function. LCCM NDVI script was built on Arduino IDE 1.6.6.

2.3 LCCM-hardware development

LCCM scanner was built with Arduino mini pro 3.3V, Bluetooth module HC-05, one red LED (peak of arb. Unit = 654.36), one near infrared LED (peak of arb. Unit = 944.12), one TSL250 photodiode, variable resistors, a set of three-dimensional printed (3D printed) casing design made by own, one switch, one 9V battery, and some wired jumper. All electronic devices are assembled and powered by 9V battery. The most important thing is the Bluetooth data communication component and reflectance data processing. Bluetooth module HC-05 was used to connect the LCCM scanner with its application in Android. This module has a role in listening to android application command and sending the output of NDVI back to it. The reflectance data processing was setup on both light resources which assembled in a row with the photodiode in LCCM scanner reflectance chamber.

2.4 SPAD meter

Chlorophyll meter SPAD-502Plus (Konica Minolta Osaka, Japan) was used to take one-dimensional absorbance data of cassava leaves. Sample dimension was 2 mm x 3 mm approximately and maximum thickness was 1.2 mm. This device uses absorbance to determine the amount of chlorophyll with index accuracy ± 1.0 SPAD unit for SPAD value between 0.0 and 50.0¹². With handheld size dimension and clipper design for leaf scanning, SPAD meter could scan amount of chlorophyll on the leaf plant briefly.

2.5 Data analysis

In model evaluation and prediction study, root means square error (RMSE) and regression analysis was used as a standard statistical modeling to measure the performance and relation in between the data model. These formulas were used frequently for assessing water quality^{13,14}, assessing water-stressed leaf¹⁵, even assessing for relation between leaf chlorophyll content with spectral reflectance¹⁶. In this research, these would be used in order to evaluate NDVI value from LCCM with SPAD value. The reason behind this is to evaluate an error possibility that will be occurred relating to LCCM which is using in situ detection mechanism. Regression analysis would be used to an observed relation in between both of this value and presented in coefficient of determination value. In a predictive assessment of LCCM, the authors used root mean squared error (RMSE) shown in Eqs. (1):

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad (1)$$

3. RESULT AND DISCUSSION

3.1 Workflow of LCCM scanner and mobile application

Figure 2 shows the workflow of the proposed LCCM scanner with LCCM's android mobile application. First of all, the Bluetooth communicator in both android apps and LCCM scanner are turned on. The leaf sample was placed in the existing gap of LCCM device. In the same time, "SCAN" button on the mobile apps was tapped. The mobile apps then triggered LCCM scanner to commence a reflectance mechanism to collect reflectance values on both red led and near-infrared led. These values are processed to generate NDVI value. Furthermore, NDVI value would be sent back to LCCM mobile apps and showed it in the mobile apps directly. These data also has been saved into the local database for processed further.

Bluetooth as the primary communication protocol was used to communicate between LCCM apps with LCCM scanner. LCCM has been deployed with minimal requirement of android Bluetooth library on android KitKat (v4.4.2) and has been tried on several smartphone vendors, such as Asus Zenfone 5, Lenovo A6000, and the Google Nexus 4 and it was running properly. The generated data from this application consisted of NDVI value, the date was taken, latitude and longitude data. This Bluetooth was made on separate activity and was setup on search mode. On search mode, this app would search for another device to connect with. When it success to find the LCCM scanner (appear as HC-05) the state would be changed into connected mode and it would ready to use. This early version of LCCM android application used external memory to save LCCM data scanning packed in CSV file in favor of easiness to decode and encode the data.

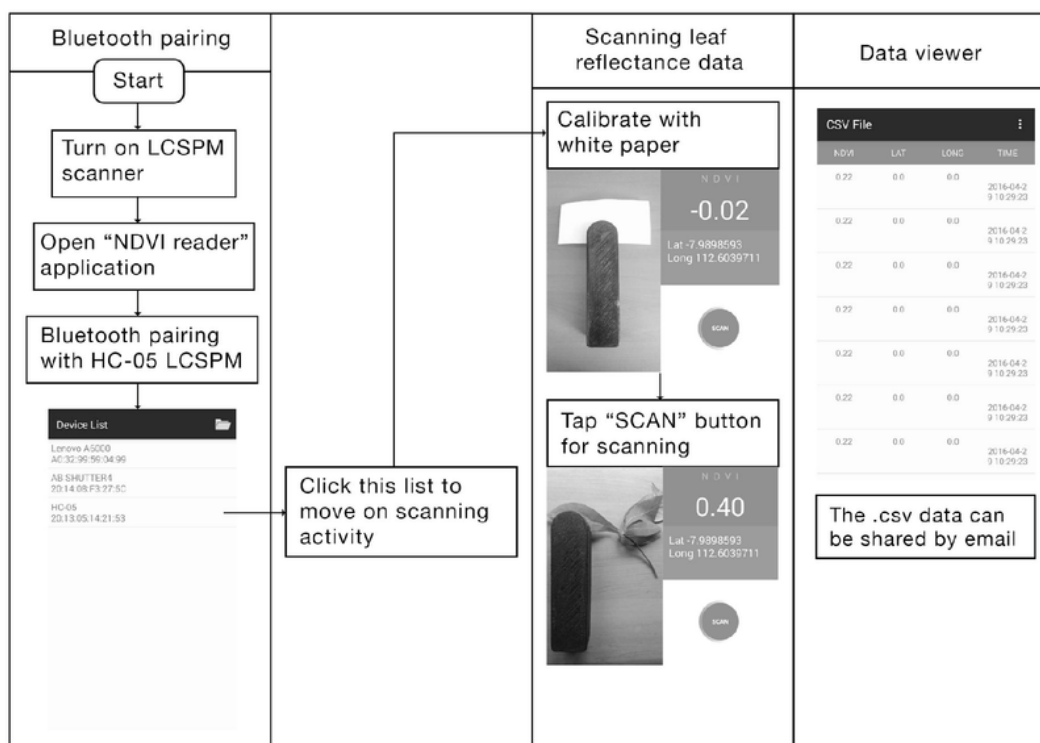


Figure 2. Workflow of proposed LCCM scanner with its android mobile application

3.2 LCCM Scanner device

After constructing a mobile app finished, the next phase was to construct electronic parts (subsection 2.3) into propose LCCM scanner along with Arduino program script data input processing. Designing a three-dimensional (3D) model of LCCM scanner casing was also conducted. The dimension of LCCM scanner is 16.5 cm × 4.0 cm × 3.5 cm (Figure 3) while the material was made using black filament. Black material of the case was used to minimize reflectance of ambient light on the photodiode. The 3D scanner model designed in openSCAD open source software.

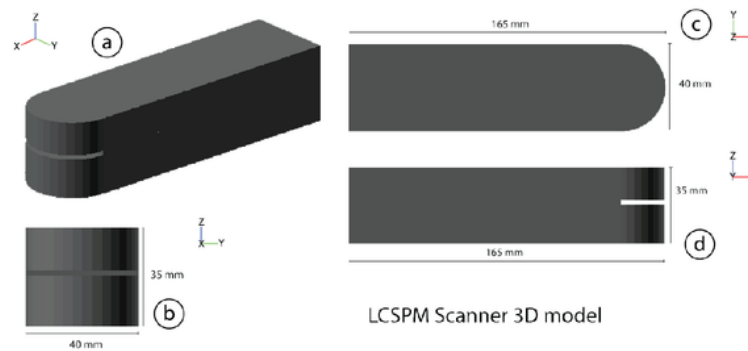


Figure 3. (a) LCCM scanner 3D model, (b) front view, (c) top view, and (d) side view

3.3 Data processing

Data in LCCM android application has been processed through several steps that occurred in Arduino mini pro microcontroller board. The steps consisted of scanning the leaf surface to get reflectance value, forming NDVI value based on red and near-infrared, and sent the data back to android application.

The first phase was scanning the leaf surface which could be described as turn on both LEDs alternately. Because it turned alternately, each of the LED would pass sleep state that around 1000 millisecond after each of these LED blinks. This flip-flop mechanism was intended to retrieve the data from each band reflectance of light bounced from leaf samples. If both of the light sources are turned on simultaneously, those light sources will make the photodiode unable to distinguish which one is the reflectance values of red LED and near infrared. The function of photodiode type TSL250 was to change light intensity into a voltage output. NDVI value is built by reading the digital number from both converted light source. Below is a formula used for calculating NDVI index^{16,17}:

$$NDVI = \frac{(NIR - RED)}{(NIR + RED)} \quad (2)$$

Description:

NIR : digital number of near infrared

RED : digital number of red LED

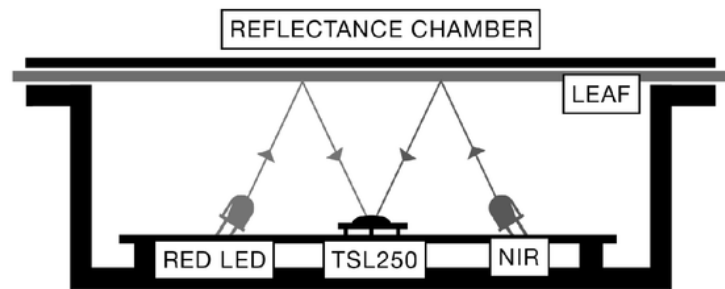


Figure 4. LCCM scanner reflectance mechanism chamber

Figure 4 shows the chamber design for LCCM using reflectance mechanism as its primary mechanism corresponding to measure chlorophyll content. The calibration process was performed on a slipping on white paper into reflectance chamber. If the value was around zero, the scanner was ready to use. Meanwhile, if the deviation value was far from zero it is necessary to recalibrate the Arduino code and hardware tuning if needed. Calibration was tested on two types of leaves, i.e. leaf with a strong green color and leaf with strong yellow color. Green leaf absorbs most of the visible light in which in this case is red led and reflects more in near infrared. It would vice versa if it is applied on yellow leaf. NDVI had a value range from -1 to 1. If the value was close to -1 in which indicating the leaf had a strong yellow color significantly less chlorophyll content occurred and vice versa.

Previously, there were methods and attempts that have been conducted to measuring chlorophyll content on an intact leaf, for an instance low-cost chlorophyll determination device which is applying light reflectance mechanism¹⁸, smartSPAD which is applying image processing⁷, leaf color analyzer which is upgrading used of leaf color chart (LCC) using image processing⁶, and rapid nitrogen determination of soybean leaf which is applying image processing too¹⁹. These four types of research can be concluded into two mechanisms, i.e. reflectance mechanism and image processing mechanism in which cannot be denied that each mechanism has its own advantages and drawback. The advantages of reflectance mechanism are from its measurement accuracy owing to a reflectance chamber in the front of a scanner, mostly made to reduce the interference from ambient light outside signal into the scanner. Several electronics doodads were assembled and connected to give power resources. But, this additional device could be a drawback because it will need more cost and time to produce. It would need a basic electronic fundamental knowledge and some programming technique to build it.

In comparison to the reflectance mechanism, image processing mechanism was designed to process image input for overcoming a problem and it relies heavily on programming algorithms. According to previous report, image processing was used to extracting the greenness-color of the leaf surface and it was deployed quite simply in the form of android application. But, it does not mean this approach is flawless, a random environment lux which reflecting leaf surface could contribute a reading error. Hence, an idea to combine both advantages of most reflectance mechanism device with image processing mechanism was proposed.

3.4 Evaluation of LCCM

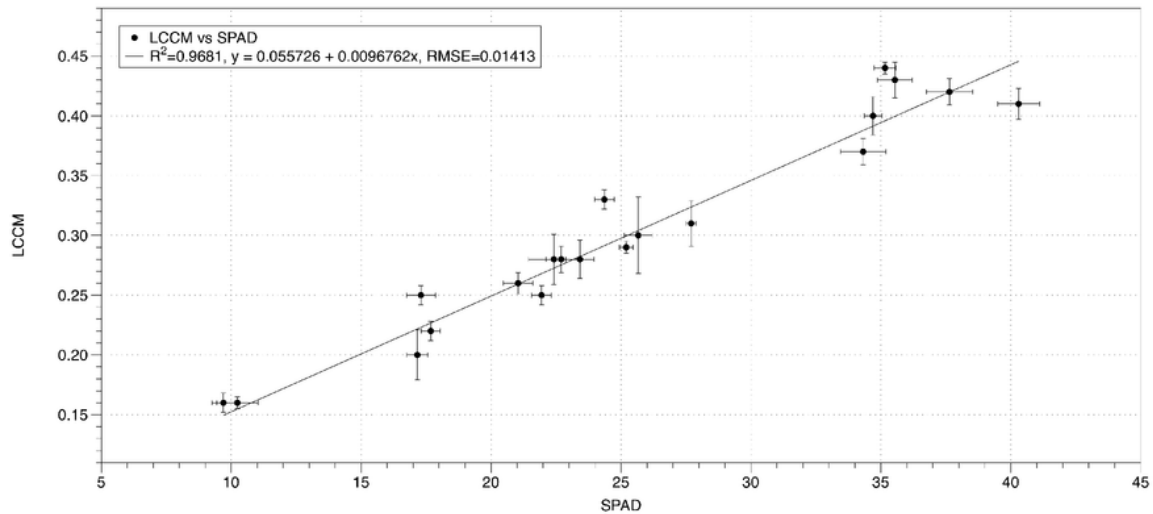


Figure 5. Correlation between LCCM with SPAD

LCCM proposed device and mobile application have been successfully assembled. The accuracy has been compared with commercial SPAD meter. LCCM data acquisition from leaf samples was carried out in January 2016, in hot weather conditions, 20 samples were collected at 10.00 AM – 12.00 PM. and already analyzed using regression analysis in order to find the correlation value between LCCM value and SPAD meter value. As shown in Figure 5 above, the correlation between NDVI values from LCCM with SPAD value from SPAD meter Minolta 502 as control variable was 0.9681 with RMSE 0.014, indicating that both values have a strong correlation. It can be read in Figure 5 there is enough span of error bars occurred which owing to the spotted color of leaf surface.

CONCLUSION

LCCM device was successfully developed and was able to detect chlorophyll with coefficient of determination value 0.9681 in correlation with SPAD value Minolta 502. Prototype casing of LCCM was also successfully created with volume dimension of $\pm 231 \text{ cm}^3$ (1/3 smaller than the size of its counterpart, SPAD meter Minolta which reaches number $\pm 626.8 \text{ cm}^3$). Total production costs to build LCCM tool (excluding android application) was approximately 10 times lower than the price of SPAD Minolta 502.

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