Design of a Simple and Low-cost Fluorescence-based Chlorophyll Meter

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ABSTRACT
Accurate, non-destructive prediction of chlorophyll content in leaves is crucial to guaranteeing an optimum plant's growth and productivity. This is due to the fact that chlorophyll is an important photosynthetic pigment for plants, determining photosynthetic capacity and hence plant growth. In this paper, efforts to design, calibrate, and test a preliminary prototype of a low-cost chlorophyll meter based on the quantification of fluorescence signals measured from a plant's leaf will be described. The results of the measurements obtained using this developed preliminary prototype were validated using the SPAD 502 Chlorophyll meter.

Keywords: *chlorophyll meter, fluorescence, plant's leaves, low-cost*

1. INTRODUCTION

Chlorophyll is an important pigment for photosynthesis of plants, and a sufficient amount of this pigment is required to maintain the optimum and healthy status of the plant's growth, as well as fertilizer requirements in order to produce a good crop yield [1]. In this context, continuous and regular monitoring of chlorophyll content in plants is necessary to be performed [2].

Traditionally, chlorophyll content in plants' leaves is measured *ex-situ* using a spectrophotometric technique in a laboratory from the destructively extracted chlorophyll content in leaves by using polar solvents such as ethanol or acetone. Then the chlorophyll content is calculated from spectral absorption intensities at 646 nm and 665 nm using an equation proposed by [3], as a modification of a previously well-known equation, which was proposed by Arnon [4]. This measurement technique requires tedious sample preparation in addition to the need for a bulky spectrophotometer, which is normally possessed only by analytical laboratories in universities or research centres in big cities.

Simpler and in situ non-destructive ways to accurately predict the chlorophyll content in plants' leaves have been developed in the last decade. These techniques use several quantification modes, such as colour [5] and ratio of percent transmission at two different wavelengths in the red and NIR regions, such as SPAD-502 (from Konica Minolta Sensing, Inc., Sakai, Osaka, Japan), CCM-200 from Opti-Sciences, Inc., Hudson, NH, USA), and Dualex 4 Scientific (from FORCE-A, Orsay, France) [6]. Unfortunately, these systems are quite expensive and difficult to purchase for most Indonesian traditional farmers, who live in poverty. As a result, efforts to provide accurate but low-cost equipment to monitor a plant's chlorophyll content would be beneficial to our traditional farmers while also indirectly supporting national efforts to maintain food resilience programs.

Fluorescence is light that is emitted when stimulated electrons in molecules return to their original resting state levels. According to Jablonski, a Polish physicist, most of the fluorescence emissions are radiated as stimulated electrons undergo radiative deactivation from the zeroth vibrational level of the first excited singlet to their originally populated levels in the zeroth (ground) singlet. Meanwhile, the deactivation pathways from the last attained excitation levels to the zeroth vibrational level of the first excited singlet are achieved non-radiatively [7]. The fluorescence emission is normally red-shifted, which means that its photon's energy content is lower than the excitation photons. In order to

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better quantify this fluorescence emission, the stronger excitation light must be correctly blocked using a suitable optical filter [8].

Chlorophyll fluorescence analysis has been recently used as an alternative method and has become a significant technique for monitoring plant growth, illuminating the basic mechanisms of photosynthesis, the plant's response to changes in environmental conditions, as well as quantifying biochemical properties of plants [9] [10][11].

In this reported work, research attention is devoted to how to quantify the fluorescence signals generated from stimulated chlorophyll molecules in leaves. This current reported work is a continuation of previous developmental research efforts in creating simple, low-cost but accurate in situ leaf chlorophyll content prediction tools [12][13].

2. MATERIALS AND METHODS

This section describes the steps and approaches that were implemented in developing the proposed optical system. The descriptions can be straightforwardly grouped into the following sub-sections:

2.1. Chlorophyll's Fluorescence Spectra

Chlorophyll pigments in plants' leaves are Chlorophyll A and Chlorophyll B, and both of them are involved in photosynthesis with different roles. Chlorophyll A is the primary pigment collecting light for photosynthesis, while Chlorophyll B functions as an extra pigment that gathers the sunlight to be further passed to the Chlorophyll A, particularly when the availability of light is low. In this low light condition, plants tend to produce more chlorophyll B to increase their photosynthetic capability. A comparison of the absorption spectra of these two types of chlorophyll can be given in Figure 1a).

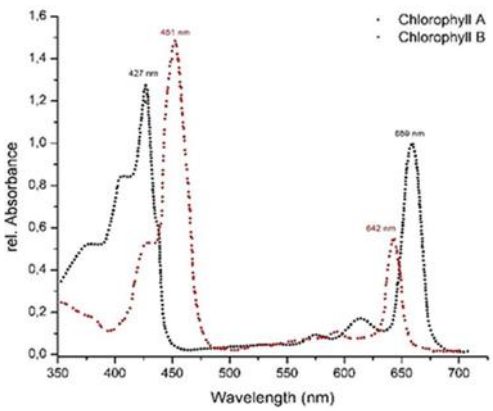
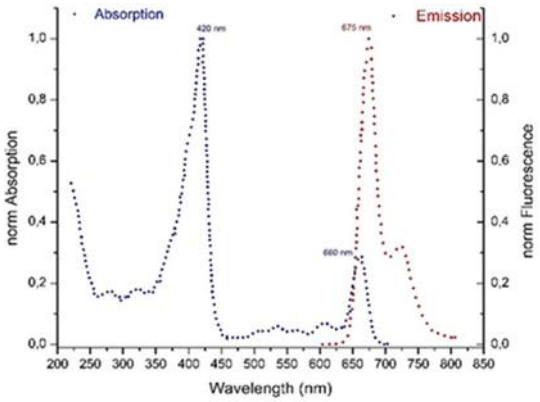


Figure 1 a) Difference profile of typical absorption spectra between Chlorophyll A and B



b) Typical absorption and emission spectra of Chlorophyll A

Because Chlorophyll A is the most important pigment in plant photosynthesis, a thorough understanding of the spectral behaviour of both absorption and emission is required to quantify the chlorophyll content in plant leaves using the fluorescence quantification technique. Peak of Chlorophyll A is located at around 420 nm, while its maximum fluorescence intensity is emitted at a wavelength of 675 nm, which slightly overlaps with the absorption of spectra in the red region, as being depicted in Figure 1b).

These wavelengths were used to choose the suitable light source and detector that are required for measuring fluorescence signals from plants' leaves, as their characteristics will be discussed in the next section.

2.2. Light Source, Photodetector, Electronics Data Acquisition & Display System

As a light source for exciting fluorescence of plant's leaves, a Picotronic 70115505 Blue Class 2 Laser from Picotronic GmbH Germany has been chosen, which has a center wavelength of 405 nm, while for detecting the fluorescence signal, a phototransistor from Vishay Intertechnology, Inc. The spectral sensitivity response of this chosen phototransistor is quite good compared with the emission spectra of chlorophyll-A. A dedicated Arduino-based electronic signal acquisition and display system was also developed to measure the signals from the phototransistor, amplify, process, and display the quantified fluorescence signals generated after excitation using a laser source. Figure 2 shows the laser and spectral sensitivity response of the phototransistor, as well as the dedicated Arduino-based electronics.

2.3. Calibration Steps

To provide good measurement accuracy and reliability, several calibration steps need to be accomplished for the electronics and optical detection system. These steps include:

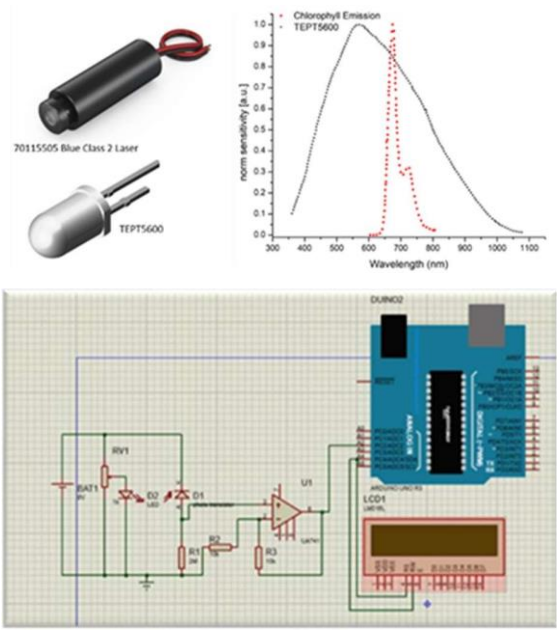


Figure 2 (above) Laser diode for excitation and phototransistor TEPT5600 and the spectral sensitivity response of the phototransistor; (below) dedicated Arduino-based electronics

1. The calibration for the current-voltage response, which are measured using calibrated digital multimeter, for both the laser driver and phototransistor output signals ports.
2. Meanwhile, the emitted laser intensities for varying the driving currents were also measured using a Red Tide USB650 Fiber Optic Spectrometer from the output port of the FOIS-1 Integrating Sphere, both are from Ocean Optics, Inc. The results of the measurements were then used as calibration constants to be programmed into the Arduino-based measurement electronics.
3. Main chlorophyll solution extracted from 5 mg of crushed Kale (*kangkung*) and Spinach (*bayam*) vegetable leaves were dissolved in 50 mL ethanol solvent, i.e. initial concentration of 10-4 gr/mL. This calibration solution in several concentration values was created by dripping 25 mL of main chlorophyll solution into a 2.5 mL (or 2500 mL) ethanol solvent. Before the measuring process, these created varying concentrations of solutions were homogenized using an ultrasonic water bath shaker. These solutions were then excited using the blue laser and measuring the generated fluorescence signals using the calibrated phototransistor, and parallelly, these fluorescence signals were also measured using the spectrometer. These two measurements will be used as a correlation constant for the chlorophyll concentration measurements. A schematic for these measurements can be shown in Figure-3.

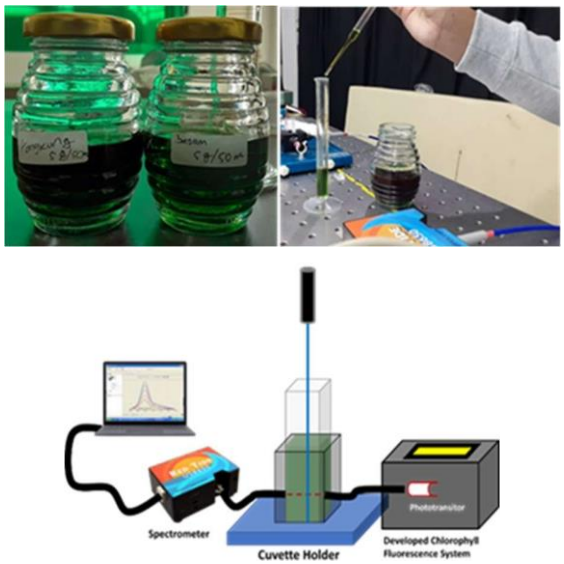


Figure 3 Calibrating fluorescence measurements using extracted chlorophyll solution using ethanol solvent from spinach and kale leaves.

2.4. Measurement Validation

Doing all the previously above-mentioned calibration steps, then the developed fluorescence-based Chlorophyll meter were used to measure chlorophyll contents in plant's leaves directly, which are parallelly also validated using SPAD 502 Chlorophyll meter measurements. Nine pieces of each spinach and kale leaf were used for these measurements, and measurements were carried out at several points along the length of each leaf. For each measurement point along the leaf, three measurements were carried out and averaged. Results from both measurements will be compared to get insight into the measuring performance of the developed chlorophyll fluorometer.

3. RESULTS AND DISCUSSION

A stable output of the laser's electronic driving circuit is important to generate repeatable laser output. This driving circuit was tested for both increasing and decreasing voltage changes to observe its hysteresis behaviour. Results can be depicted in Figure 4. The measurements confirmed that the driving circuit for the laser shows good performance in terms of linearity and negligible hysteresis.

Meanwhile, a linear and stable response of the phototransistor detector is also required. Figure 5 (left) shows a calibration correlation between the measured output voltage of the TEPT-5600 phototransistor and the ones measured and displayed on the LCD of the developed system.

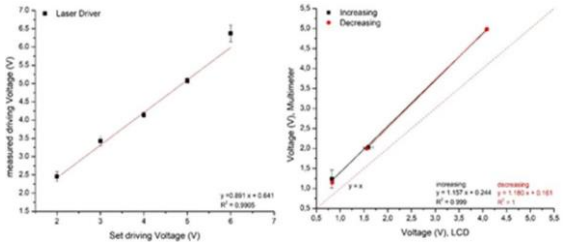


Figure 4 (above) Calibration of laser driving voltage; (below) calibration of phototransistor's voltage

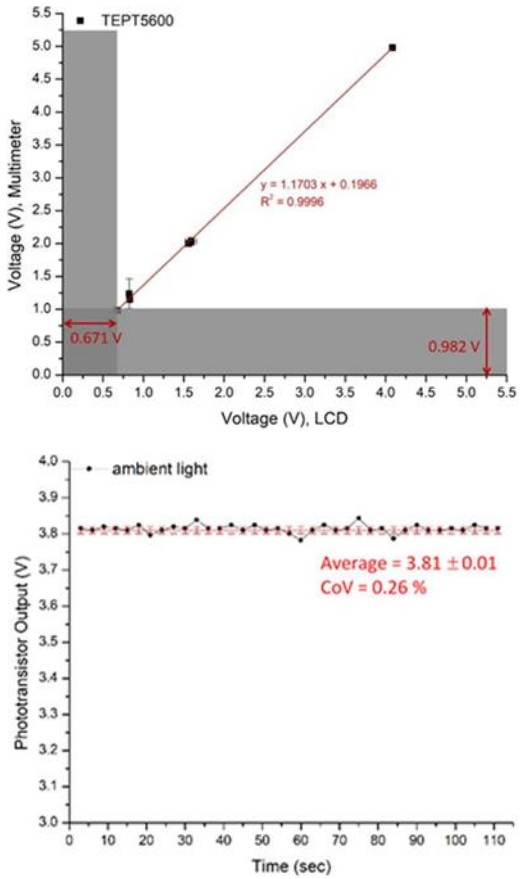


Figure 5 (Above): Calibration Correlation for the output voltage of TEPT 5600, (Below): Stability response of TEPT 5600 Phototransistor under laboratory's ambient lighting.

Fluorescence spectral measurements on an extracted varying concentration of leaf extract chlorophyll solution using a spectrometer can be shown in Figure 6, while Figure 7 shows the results of quantified signals as measured by both the spectrometer and the phototransistor.

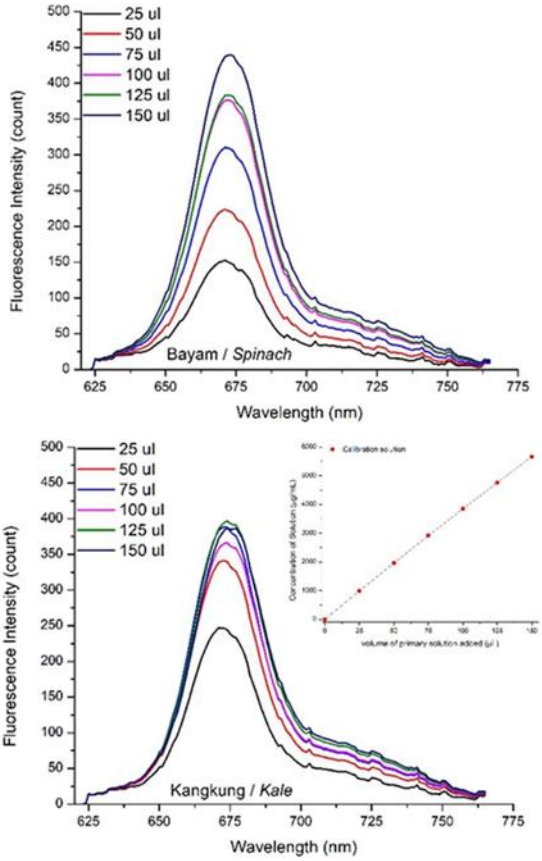


Figure 6 Fluorescence spectral measurements for varying concentration of solution extracted from Spinach (above) and Kale (below). Inset is corresponding solution's concentration in mg/µL

Changing concentrations were set at 25 mL up to 150 mL (step 25 mL), which corresponded to the solution concentrations as indicated in the inset graph in Figure 6a, upper right. The detected fluorescence spectral range is between 625 nm and 765 nm, with the peaks centred around 671–674 nm for both spinach and kale.

Quantifying these spectra usually can be based on maximum peaks intensity or integrated area under the curve for each of the spectrum. Both of the approach shows similar trend as being shown in Figure 7 left. Fluorescence intensities from the Kale show steeper increasing in comparison to the ones of Spinach with the increasing solution's concentrations, which then shows a faster decreasing trend as the concentration being further increased. This changing trend was observed up to 100 µL additional main solution for Kale (i.e. corresponds to 3846.15 µg/mL) and up to 125 µL for Spinach (i.e. corresponds to 4761.90 µg/mL). It seems that the fluorescence from Kale extract was stronger than the one from Spinach for the same concentration, and it showed faster quenching (i.e. diminishing intensity) If we see the changing spectral intensities from Kale's chlorophyll in more detail, it shows higher intensities for the first three concentration values in

comparison to the ones of Spinach. Then the intensities tend to be saturated as the solution's concentration getting higher, i.e. starting the fourth concentration values, while the saturation onset of the Spinach is after the sixth concentration values. Further higher concentrations will tend to saturate the signals, as can be confirmed by measurements using spectrometer and phototransistor detector of the developed fluorometer, as can be seen in Figure 7.

These saturations are contributed by the quenching of fluorescence signals, and it suggests that the best measurement range will be up to concentrations of 4761.90 mg/mL for spinach and 2912.62 mg/mL for kale.

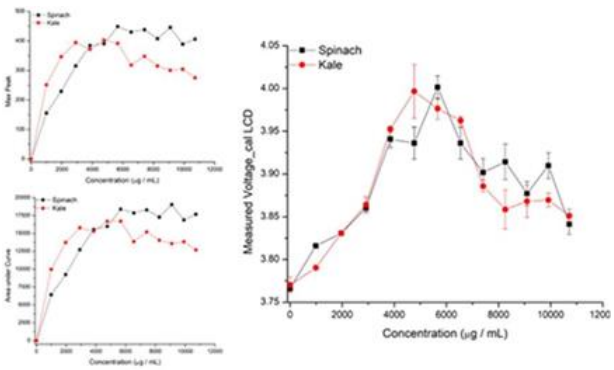


Figure 7 Quantification of measured fluorescence spectra using spectrometer (left) and using phototransistor of the developed chlorophyll fluorometer (right).

Linearly fitting these two graphs shows good coefficient of determination (COD) values, which determine the goodness of the fit, i.e., 0.959 and 0.983 for spinach and kale, respectively. These conditions will be added as additional calibration factors in determining the chlorophyll concentration values using the proposed developed chlorophyll meter.

After the above-mentioned calibration steps, the developed chlorophyll fluorometer was then tested to measure the chlorophyll content in leaves directly. As test objects, nine (9) pieces of spinach and kale leaves were used. For each leaf, a minimum of five measurements were made at arbitrary measurement points along the area of the leaf, and similar measurements were also made by using SPAD 502 Plus from Minolta. The results can be seen in Figure 8.

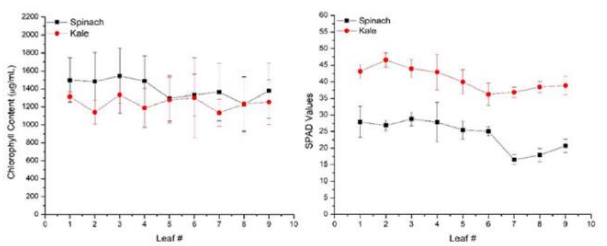


Figure 8 Results of measurements of chlorophyll content directly on leaf (left) and of parallel measurements using SPAD 502 Plus from Minolta.

Average measurements on spinach and kale leaves using the developed chlorophyll fluorometer yield predicted values of (1402.12 ± 289.02) mg/mL and (1241.24 ± 246.72) mg/mL, with coefficient of variation (CoV) values ranging from 3.9% to 34.4%. Unfortunately, often the predicted chlorophyll values were seeming a bit overlapped as it was unable to better separated between the two. A higher amplification of the sensed fluorescence signals is planned to be implemented to boost the sensitivity of the measurement. Meanwhile, the average measurements using SPAD-502 are (24.11 ± 3.06) mg/mL and (40.79 ± 3.05) mg/mL, respectively, with the Coefficient of Variation (CoV) values spread between 4.0 and 21.4%. From Figure 8 (right), we can see that the results were in accordance with the measured spectra as shown in Figure 6.

4. CONCLUSION AND FUTURE WORK

Several concluding points can be deduced from the previously mentioned steps, i.e.,

1. A preliminary prototype of a low-cost chlorophyll meter can be developed, which is based on the quantification of fluorescence signals measured from a plant's leaf. It was also calibrated and tested for direct measurements of chlorophyll content in leaves quite well. The accuracy and reliability of its measuring performance still needs to be further improved.
2. Better design of the measurement chamber conditions, i.e., one that can prevent any influence of ambient light on the measured signals, is necessary to be implemented for the coming prototype.
3. Stronger signal amplification factor is also required to produce more sensitive measurements and better SNR measurements.
4. Better, more scrutinizing, and well-controlled calibration procedures are required to produce better, more accurate and reliable measurements. Ongoing work is devoted to improving these steps by incorporating multi-modal measurement techniques.

AUTHORS' CONTRIBUTIONS

Conceptualization, A.N.; methodology, A.N., S.N.P.; software, M.R.D; validation, A.N., S.N.P., D.Y.P., I.C.S., formal analysis, A.N.; investigation, A.N., M.R.D.P., S.N.P, F.F., resources, A.N., S.N.P.; data curation, A.N.; writing—original draft preparation,

A.N., writing—review and editing, A.N., S.N.P.; visualization, A.N., M.R.D.P.; supervision, A.N.; project administration, M.R.D.P.; funding acquisition, A.N.

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