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Comparative analysis of PAM fluorometer detector circuits

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Abstract

Measuring the quantum yield's efficiency of photosystem II (Φ_{PSII}) allows to noninvasively monitor the photosynthetic activity in plants and indicate their health or stress. However, measuring Φ_{PSII} with the pulse amplitude modulation (PAM) technique faces different challenges. The main challenge copes with finding the balance between sensitivity and dynamic range in the chlorophyll fluorescence detector circuit. Because when measurements take place in the field, the detector should reveal fluorescence changes by modulated light in a wide range of light intensities. This contribution presents two different electronic read-out circuits for the fluorescence detection: (i) transimpedance amplifiers, and (ii) switched integrators. Φ_{PSII} is calculated for the leaves of a houseplant Ficus benjamina in dark and ambient light up to 300 $\mu\mathrm{mol}/\mathrm{m}^2\mathrm{s}$ with a laboratory setup. The Φ_{PSII} values exhibit more than 81 % match with those reported by a commercial PAM fluorometer. Further developments of the sensor for measuring in a wide range of ambient light with these two approaches are also discussed.

Keywords: Chlorophyll fluorescence, Quantum yield's efficiency, PAM fluorometer, Pulse amplitude modulation, Transimpedance amplifier, Switching integrator, PIN photodiode.

Introduction

Plants use light to convert carbon dioxide into carbohydrates through photosynthesis. This process happens in two main stages: (i) the light reaction centers capture and convert light energy into chemical energy and (ii) this energy is used to transform carbon dioxide into carbohydrates, which serve as crucial sources for growth and metabolic functions within the plants [1]. However, a portion of the absorbed energy dissipates as heat or re-emits as chlorophyll a fluorescence. Measuring chlorophyll a fluorescence provides detailed information on processes inside a photosynthetic organism and how efficiently it can convert the energy. This helps biologists to draw conclusions about plant's health or stress [2]. The intensity of chlorophyll a fluorescence corresponds to the reaction centers of photosystem II (PSII). The excitation energy from a photon absorbed by PSII releases an electron in its reaction center and the energy from a second photon will move the electron through photosystem I [3]. Based on this phenomenon, the quantum yield efficiency of photosystem II is used to quantify the plants photo physiological state and is given by

$$\Phi_{\mathrm{PSII}} = \frac{F'_{\mathrm{m}} - F}{F'_{\mathrm{m}}}, \qquad (1)$$

where F'_{m} is the maximum fluorescence and F is the variable fluorescence in the light exposed leaves [4].

To separate the ambient light from the plant's fluorescence light, the fluorescence excitation light should be modulated [5]. The maximum fluorescence F'_{m}, is achieved by applying a strong light pulse, referred to as the saturation pulse, which can close all the reaction centers of the leaf and lead to a maximum fluorescence level. To measure the variable fluorescence, PAM fluorometers use short and weak pulses of light called measuring light, so as not to influence the physiological state of the photosystem. The F value donates the difference between the fluorescence light with the measuring light and without it.

The challenge of measuring Φ_{PSII} is the acquisition of a fluorescence signal under different levels of the ambient light, which requires a fast and sensitive detector circuit. In recent years, several studies have been investigating the development of a low-cost device to achieve this goal. Reimer et al, developed a low cost, small size and energy efficient chlorophyll fluorescence sensor based on pulse amplitude modulation method [6]. Bates et al. used a programmable gain transimpedance amplifier for an Open-JIP chlorophyll fluorometer based on the Kautsky induction curve [7]. When using the

transimpedance amplifier with a very high gain, the unstable state of the operational amplifier needs to be addressed by placing a capacitor parallel to the gain resistor. The drawback of using this capacitor is an increase in the rise time proportional to the multiplication of the gain and the capacitor value. For a specific range of light intensities, the gain and damping capacitor value can be optimized. However, once the gain changes, an adjustment of the damping capacitor is required and an analog to digital conversion shall only take place once the signal is sufficiently settled which affects the overall evaluation time. Haidekker et al. used switching integrators in combination with an avalanche photodiode for measuring fluorescence changes in a PAM fluorometer with a laser diode as the light source [8]. They could measure the fluorescence changes during the measuring light and within 3 milliseconds after the termination of saturation pulse, which resulted in an underreporting F_m' value.
Within this contribution, we apply these two circuits for converting the fluorescence light into voltage and evaluating their performance for further developments of the sensor application in the field.

Materials and methods

Optical setup

The schematic of the optical setup used for our experiments is shown in Fig. 1. The chlorophyll a fluorescence absorbs red and blue spectrums of light and emits fluorescence between 650 nm and 750 nm. we have chosen a red LED (LED635L, Thorlabs) with a peak wavelength of 635 nm and a half viewing angle of 7° as a light source. This LED provides both the measuring light and saturation pulse.

Since the fluorescence excitation and emission are in the same spectrum, optical elements are needed to separate them. We have utilized a short pass filter on top of the LED, which filters wavelengths greater than 660 nm. Since the path of excitation light is the same as the path of emission light in our optical setup, a dichroic mirror reflects the wavelengths smaller than 685 nm and passes the wavelengths greater than this value. A PIN photodiode (BPW24R, Vishay), which has a high photosensitivity, fast response time and large linear range is applied as the photodetector. The photodiode is also protected by a long pass filter with a cutoff wavelength of 700 nm to ensure that it only receives the fluorescence light. In addition to the filter set, a Plano-Convex lens with a focal point of 25 mm is utilized to focus the excitation light on the leaf surface and one more to focus the fluorescence light on the photodiode surface.

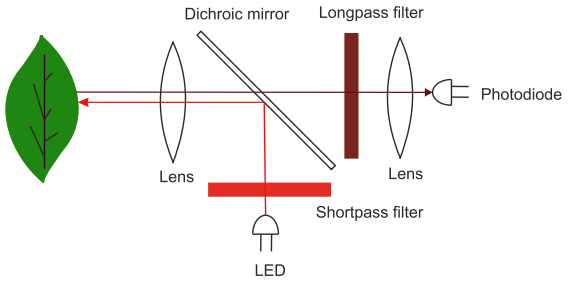

Figure 1: Optical setup for measuring chlorophyll a fluorescence

Pulse control

The fluorescent excitation light needs to be modulated to allow for the distinction between the excitation fluorescence itself and the ambient light along with the fluorescence caused by the ambient light. When a fluorescence measurement is initiated, the microcontroller (Apollo 3 blue) will send the signal to the LED driver to switch the LED on and off for the measuring light and the saturation pulse. The measuring light is applied for 4 s, followed by a saturation pulse of 0.5 s to measure variable fluorescence and maximum fluorescence in succession. In our experiments, measuring light comprises 50 µs flashes of light applied at a 10 Hz interval, which yields an intensity equal to 0.1 $\mu mol/m^2\,s$. The saturation pulse is a high amplitude modulated pulse with a frequency of 100 Hz and a duty cycle of 95 % that results in an intensity equal to 5000 $\mu mol/m^2\,s$. The same microcontroller controls the accurate timing for the Hold, Reset and Select Switch of the switching integrator.

Chlorophyll a fluorescence readout circuitry

Two different approaches, (i) transimpedance amplifiers (TIA) and (ii) switching integrators are used as the analog front end in the current conversion of near-infrared fluorescence light. The analog circuitry of the TIA configuration is shown in Fig. 2.

To amplify the chlorophyll a fluorescence signal, an operational amplifier (AD8615, Analog Devices) is chosen due to its low input bias, low current noise, and high bandwidth. The feedback resistor R_1 determines the amplifier gain and the capacitor C_1 guarantees the stability of the operational amplifier. The photodiode is used in photoconductive mode with a -5 V bias voltage for a fast response and

linear output with respect to illumination. Due to the sensitivity of the detector circuit, a 9 V battery with linear voltage regulators supplies ± 5 V. To ensure that the operational amplifier operates in its linear range, a small bias voltage derived from the positive supply, is applied to the non-inverting input. This will prevent the output from saturating at the negative supply rail when there is no current.

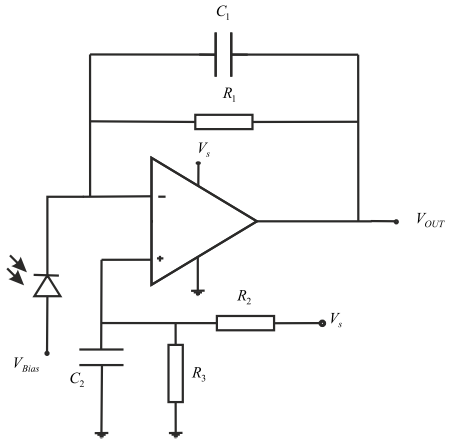

Figure 2: Transimpedance amplifier as fluorescence pre-amplifier.

In the second approach for converting the fluorescence current signal into a voltage signal, a switching integrator is utilized. Compared to transimpedance amplifiers, integration circuits are more complex. The Burr-Brown ACF2101 IC provides two integrators in one IC which fits our application the best. Fig. 3 shows the schematic of the switching integrator's circuitry for integrating chlorophyll fluorescence current signal. Each integrator includes a Hold Switch to let the input current flow through the integrator, a Reset Switch to discharge the integration capacitor before the start of a new integration period, and two Select Switches to multiplex the outputs when multiple integrators are connected to a common bus. All switches are controlled by the microcontroller with timer-controlled intervals. The integrator's output voltage is determined by

$$V_{\mathrm{OUT}} = \frac{I_{\mathrm{IN}} \Delta T}{C_{\mathrm{INT}}}, \qquad (2)$$

where I_{IN} is the input current, ΔT is the integration time and C_{INT} is the integration capacitance. The IC includes a precise internal 100 pF capacitor, and operates from supplies 5 V and -15 V and the output voltage integrates negatively toward -10 V. Compared to the transimpedance amplifiers where the feedback resistor determines the gain and the sensitivity, switching integrators have innate gain proportional to the integration time and integrator's capacitor. Therefore, changing the integration duration in the microcontroller, enables us to easily adjust the gain and extend the dynamic range. Since the IC provides two integrators and we are interested in fluorescence changes, one part of the IC can integrate the area under the curve when there is no excitation pulse and the other part will integrate when an excitation pulse exists in a rapid succession. This provides us with the area under the curve for constant fluorescence in one output and constant fluorescence plus modulated fluorescence in the other output. Subtracting these values results in the fluorescence changes with respect to the modulated light.

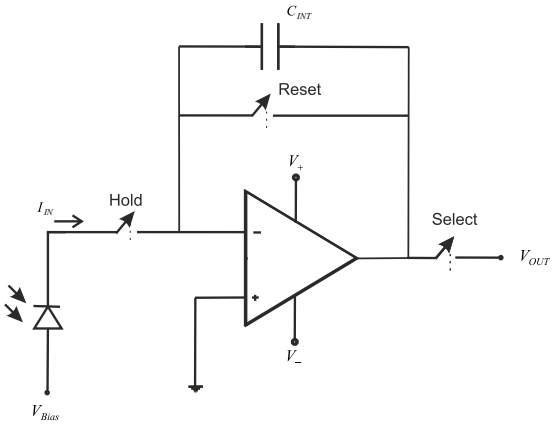

Figure 3: Schematic of the switching integrator's circuitry.

Results

Fig. 4 (a) shows the response of a Ficus benjamina leaf to emitting 4 s measuring light and 0.5 s saturation pulse in a constant ambient light equal to 300 $\mu\mathrm{mol}/\mathrm{m}^2\mathrm{s}$ with a transimpedance amplifier. Figure 4 (b) and (c) zoom in how fluorescence changes with respect to the measuring and saturation pulses. As shown, plants are quite fast in responding to the light changes. Ambient light causes an offset value proportional to its intensity and when a measuring pulse switches on and off, fluorescence value will change accordingly. Variable fluorescence F denotes the change in fluorescence, between the state where no measuring light is applied to a leaf and the one where it is applied. Meanwhile, a saturation pulse is a strong pulse, which closes all the reaction centers of a leaf for a short time and leads to the maximum fluorescence changes F'_{m}. We are interested in fluorescence changes with respect to the measuring light and saturation pulse to calculate Φ_{PSII}.

The dynamic of the fluorescence signal shows that when the ambient light has higher intensity, the fluorescence changes will be smaller and

we need a more sensitive detector. On the other hand, increasing the gain of the amplifier to improve its sensitivity results in a large DC offset, which cannot be removed fast by a high pass filter and increases the settling time of the output. The equivalent current of this DC offset value can reach up to $100\,\mu\mathrm{A}$ while the fluorescence changes are in nA range. Finding a balance between sensitivity and fast response time is a challenge in chlorophyll fluorescence detection based on the PAM method.

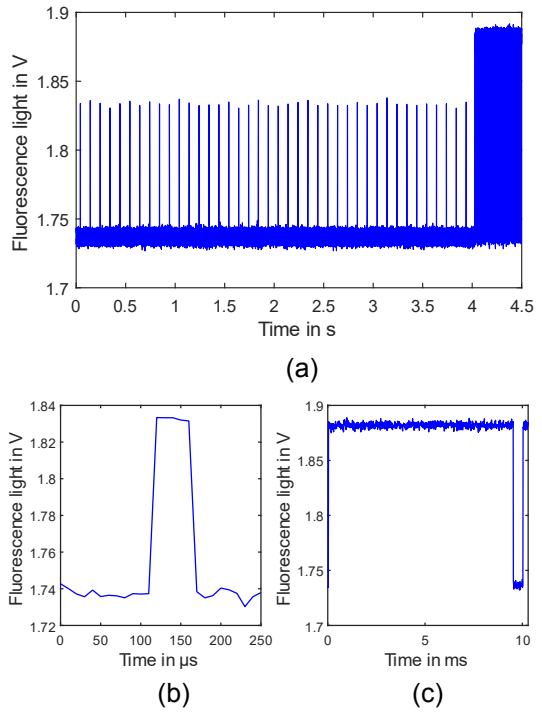

Figure 4: (a) Chlorophyll a fluorescence measured with a transimpedance amplifier. (b) Fluorescence changes during the measuring light. (c) Fluorescence changes during the saturation pulse.

Fig. 5 (a) shows the results of measuring chlorophyll a fluorescence with the switching integrator for the same leaf and the same ambient light. Fig. 5 (b) depicts the integration of ambient light for 50 µs before applying a measuring light and 50 µs duration of the measuring light. In both cases, the output holds the integration value for a few microseconds and then resets the integrator output. Subtracting these two values yields the variable fluorescence F. The same integration timing repeats for the whole duration of measuring light. Figure 5 (c), shows the integration result for the saturation pulse. Due to the high intensity and large duty cycle (95 %) of the saturation pulse, integrating its induced fluorescence for the duration of the entire pulse is not possible. We have decreased the integration time to avoid saturating the integrator to its negative rail. Controlling the gain with the integration time results in an extension of the dynamic range.
For each saturation pulse, we integrate from the fluorescence light 50 µs at the end of the pulse to ensure it has reached its maximum value and integrate ambient light 100 µs after the saturation pulse turns off to ensure that the fluorescence value has decreased to the ambient light fluorescence. The integration time of ambient light is also 50 µs in this case. Subtracting these two values yields the maximum fluorescence F'_{m}. The same procedure repeats for the whole duration of the saturation pulse.

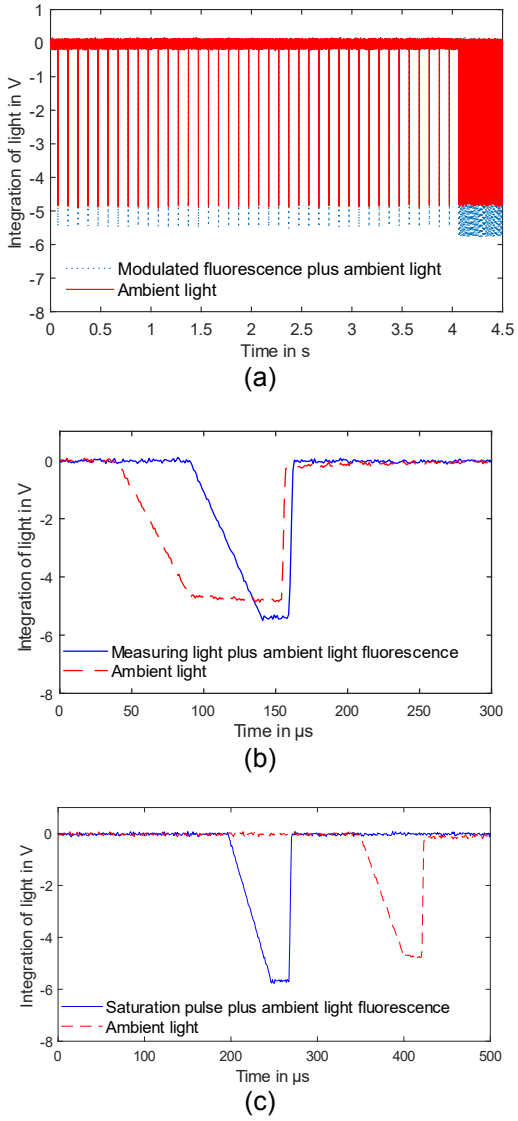

Figure 5: (a) Integration of Chlorophyll a fluorescence light. (b) Integration of light before and during the measuring light. (c) Integration of light during and after saturation pulse.

Fig. 6 shows the calculated Φ_{PSII} values with both approaches and reported values of a commercial device for Ficus benjamina leaf under dark-adapted conditions and ambient light intensities equal to 150 $\mu\mathrm{mol}/\mathrm{m}^2\mathrm{s}$ and 300 $\mu\mathrm{mol}/\mathrm{m}^2\mathrm{s}$. Results indicate that the measurements with both circuitries match the reported Φ_{PSII} by the commercial device more than 81 %.

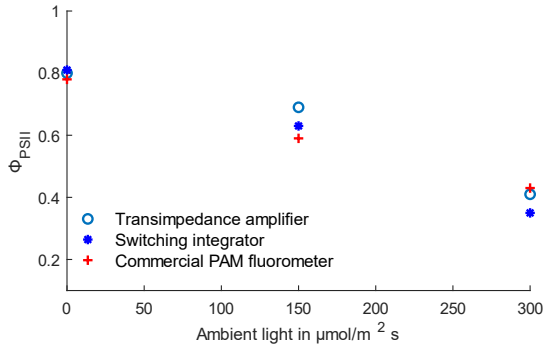

Figure 6: Reported Φ_{PSII} by detector circuits and the commercial device.

Conclusion

We measured chlorophyll a fluorescence with two different circuits, transimpedance amplifiers and switching integrators. Calculated Φ_{PSII} for both have accuracy greater than 81 % compared to the measurements of a commercial PAM fluorometer. To develop the sensor further for autonomous measuring in the field, iterative design optimizations should be considered for each method to ensure sensitivity and response time of detector. In detectors based on transimpedance amplifiers, the feedback resistor and capacitor should be adjusted in different levels of ambient light intensity. The DC level of the ambient light should be measured in advance and be subtracted from the measured fluorescence in an analog stage. To ensure response time and sensitivity, amplifying the fluorescence changes is preferred over amplifying the entire signal with a large gain. On the other hand, the switching integrator BURR-BROWN ACF2101 IC features a dual integrator, which allows a direct analog subtraction by feeding the outputs of the two integrators into a differential amplifier and canceling the DC offset. Meanwhile, the fluorescence changes can be amplified without the risk of saturating the amplifier and extending the dynamic range of the detector circuitry. Developing the sensor with this IC will need less electronic components while the gain can be modified in software by changing integration time or using an external capacitor.

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