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Enhanced Signal Detection for Chlorophyll a Fluorescence Signal

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Summary: Monitoring the quantum yield efficiency of Photosystem II evaluates plant health and stress by estimating electron transport rate in the photosystem. This measurement is typically performed using Pulse Amplitude Modulation (PAM) in combination with saturation pulses. The derived fluorescence signal is weak, often buried in noise, and has a low duty cycle. This contribution proposes a hybrid signal processing approach that combines aspects of boxcar averaging and lock-in amplification to extract low duty cycle signals from below the noise floor. We were able to detect the signals with a duty cycle of 0.01 %, with signal to noise ratio (SNR) up to –30 dB.

Keywords: Chlorophyll a fluorescence, Quantum yield's efficiency, PAM fluorometer, Lock-in amplifier, boxcar averaging

Introduction

The quantum yield efficiency of Photosystem II not only reflects how effectively plants utilize absorbed light but also indicates the electron transport rate within the photosynthetic system in vivo [1]. This efficiency is described by

$$\Phi_{\text{PSII}} = \frac{F'_m - F}{F'_m}, \qquad (1)$$

where F'_m is the maximum fluorescence and F denotes the variable fluorescence in light-exposed leaves. To separate the ambient light from the plant's fluorescence light, the fluorescence excitation light should be modulated [2]. Variable fluorescence is obtained by applying weak and short flashes of light (in the µs-range) called measuring light, to the leaf surface, ensuring no impact on the physiological state of the photosystem. Maximum fluorescence, on the other hand, is achieved by applying the same measuring light along with strong pulses that saturate all reaction centers in the leaf [3]. The modulated fluorescence signal is prone to noise interference, and an appropriate signal processing method could help detect the fluorescence changes. A Fast Fourier Transform (FFT) combined with a Welch window is used to recover the chlorophyll fluorescence signal [4]. The FFT method is computationally expensive and it is unable to detect the signals buried in noise.

Lock-in amplifiers are used for a wide range of applications where weak signals are obscured by noise and the frequency of the desired signal is known [5, 6, 7]. Low duty cycle signals spread across multiple harmonics in the frequency domain. Since lock-in amplifiers focus on the fundamental frequency, they fail to capture the information contained in the higher-order harmonics. In addition, the shorter the active signal duration, the more noise tends to dominate the overall measurement. In low duty cycle signals, boxcar averaging allows for recording during the short duty cycle, when the signal is present, and excluding the remainder where no relevant information exists to improve the signal to noise ratio [8].

In this contribution, we use boxcar gating to isolate the signal during the excitation pulse and an equivalent duration afterward, effectively increasing the duty cycle to 50 %. This adjustment allows us to apply a lock-in amplifier to the gated signal, taking advantage of the lock-in amplifier's strong capability to detect signals below the noise floor, even when the original duty cycle is low.

Experimental Setup

Fig. 1 shows the block diagram of the experimental setup to capture the chlorophyll a fluorescence signal from the leaf surface. It consists of (i) a sensor head, (ii) fluorescence excitation and detection circuitry, and (iii) a microcontroller.

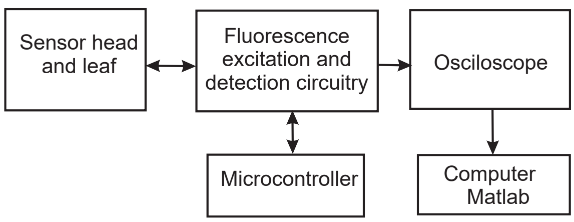

Fig. 1: Block diagram of the experimental setup

The sensor head includes a blue LED to excite the fluorescence and a PMMA plastic fiber to transfer the fluorescence light to the detector circuit. The LED is positioned at a 45 ° angle to the leaf surface at a distance of 3 mm, while the plastic fiber is aligned to sample fluorescence from the same excitation point.

The LED excitation circuitry drives the LED to produce both measuring light and saturation pulses. The measuring light consists of 10 µs flashes at a frequency of 10 Hz with a current amplitude of 25 mA for assessing variable fluorescence. To measure the maximum fluorescence, 10 µs flashes of light at the frequency of 100 Hz are followed by saturation pulses with a current amplitude of 80 mA. The fluorescence detection circuitry includes a PIN photodiode covered by a longpass optical filter, which suppresses LED reflections and ambient light below its cutoff wavelength, 650 nm. A transimpedance amplifier then converts the detected fluorescence light into a voltage signal and amplifies it for further processing. The microcontroller controls the precise timing of the LED excitation signal and performs digital signal processing. For this study, we acquired the fluorescence signal using an oscilloscope, and the signal processing approaches were implemented in MATLAB software.

Results

The fluorescence signal in our measurements has a 0.01 % duty cycle. Simulation results show that combining the boxcar technique with a lock-in amplifier can effectively detect weak and low duty cycle chlorophyll fluorescence signals with an SNR as low as −32 dB, while boxcar averaging alone cannot detect signal even when the SNR is −16 dB (Fig. 2).

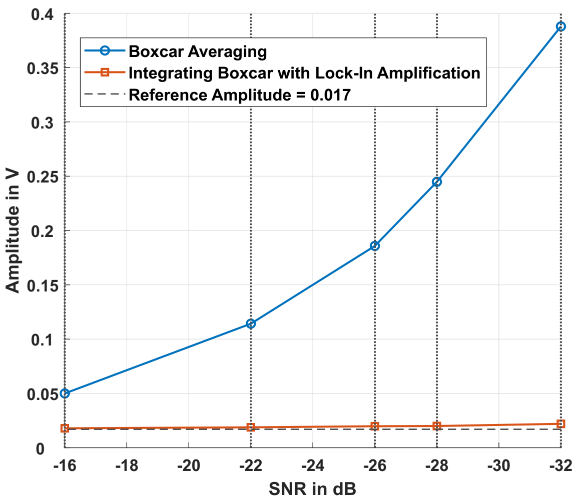

Fig. 2: Amplitude deviation across SNR levels for boxcar averaging and integrating boxcar with lock-in amplifier.

Conclusion

Lock-in amplifiers can detect signal amplitudes even when they are smaller than the noise floor. However, their efficacy will decrease when the duty cycle drops below 10 % due to energy distribution across harmonics. On the other hand, Boxcar averaging offers a high SNR value for very low duty cycle signals. While it effectively reduces noise, it mainly relies on averaging, which can struggle to extract the signal when it is deeply buried in noise. The proposed approach, which integrates boxcar and lock-in amplification, can detect the low duty cycle signals that are deeply buried in noise.

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