Sinusoidal Amplitude Modulation and Coherent Demodulation Technique for Chlorophyll Fluorescence Measurement

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Abstract. As a quick and non-intrusive probe of photosynthesis, the chlorophyll fluorescence has been widely studied. Extracting the weak fluorescence signal from the strong noise is necessary to the chlorophyll fluorescence measurement and analysis. In this paper, the time-domain and frequency-domain characteristics of the chlorophyll fluorescence signal and noise caused by the actinic light and the saturation pulse light were analyzed, and a new fluorescence measurement technique, sinusoidal amplitude modulating and coherent demodulating the fluorescence signal, was presented. Using the new technique, the measurement light was modulated by a *1KHz* sinusoid, the modulated noisy fluorescence was demodulated using the coherent method with several different cut off frequencies, and signal to noise ratios and measurement errors were calculated and analyzed. The results indicated that the new measurement technique can be employed to measure accurately and efficiently the chlorophyll fluorescence in situ.

Introduction

When the dark adapted chlorophyll in vivo is illuminated, it induces the time varying fluorescence^[1]. Firstly, the fluorescence intensity increases rapidly to the maximum, then decrease slowly, and finally stabilized. As shown in Fig. 1 (A), the chlorophyll fluorescence curve contains many parameters, such as the minimal fluorescence, *Fo*, the maximal fluorescence, *Fm* and *Fm*', the actual fluorescence at any time, *Ft*, the fluorescence in stable state, *Fs*. In addition, using the parameters mentioned above, other parameters can be obtained, such as the variable fluorescence, *Fv=Fm-Fo*, the maximal photochemical efficiency of photo-system II in the dark, *Fv/Fm*, the actual photochemical efficiency of photo-system II in the light, (*Fm-Ft*)/*Fm*'. The parameters can be employed to research the absorption, transmission, dissipation, distribution of the light energy in chlorophyll photo-systems. The chlorophyll fluorescence technique has been referred to as a quick and non-intrusive probe in the studies of plant photochemical reaction^[2-4].

Usually, in order to avoid affecting photochemical reaction of chlorophyll in vivo, the measurement light is sufficiently weak. As a result, the fluorescence signal induced by the measurement light is too weak to detect easily. In addition, the fluorescence noise most induced by the actinic light and the saturation pulse light, which are employed to change photochemical states of the chlorophyll in vivo, is usual very strong. How to extract the weak fluorescence signal from the strong noise has presented a challenge to researchers^[4].

A lot of methodologies have recently been used to measure chlorophyll fluorescence. The pulse amplitude modulated fluorescence technique used widely can measure steady fluorescence parameters in situ, because the measurement light is modulated by the pulse, and the modulated fluorescence is demodulated by using the lock-in amplifier^[5, 6]. The fast repetition rate (FRR) fluorescence technique is very efficient in measuring variable fluorescence parameters ^[7, 8]. Due to using the direct-current amplifier, it is usually used in dark.

In order to measure the steady and variable fluorescence parameters in situ, a new technique of the fluorescence modulation and demodulation is presented in this paper.

New Methodology in Measuring Chlorophyll Fluorescence

Simulated Continuous Fluorescence Signal and Noise. The simulated fluorescence signal induced by the continuous measurement light, FS(t) (t:0-320s), and the simulated noise caused by the actinic light and the saturation pulse light, FN(t) (t:0-320s), are shown in Fig. 1.



Fig. 1. Simulated continuous chlorophyll fluorescence signal, *FS*, (A) and simulated noise induced by the actinic light and the saturation pulse light, *FN*, (B).



Fig. 2 shows the frequency spectrum of FS and FN by using the fast Fourier transform.

Fig. 2. The frequency spectrum of the continuous fluorescence signal, *FS*, (A) and noise, *FN*, (B) by using the fast Fourier transform.

From Fig. 1 and Fig. 2, it can be seen clearly that the shapes of *FS* and *FN* are very similar, and their frequency spectra mostly overlap. As a result, it is very difficult to extract directly the signal, *FS*, from the noise, *FN*. In order to measure accurately and efficiently fluorescence signal, the modulation and demodulation of the signal *FS* is necessary.

Sampled Signal and Noise. In order to process data, FS(t) and FN(t) shown in Fig. 1 are sampled. $fs(i) = FS(t)|_{t=(i-1)/f}$, (i = 1, 2, ..., 16000001) and $fn(j) = FN(t)|_{t=(j-1)/f}$, (j = 1, 2, ..., 16000001)are respectively the sampled FS(t) and the sampled FN(t), where the sampling rate, f, is 5*KHz*.

Sinusoidal amplitude modulation of the fluorescence signal. The measurement light is modulated by a 1*KHz* sinusoid. $FS_M(t) = FS(t) \times [\cos(2000 \times \pi \times t) + 1], (t:0-320s)$ denotes the modulated fluorescence signal induced by the modulated measurement light. $fS_M(i) = FS_M(t)|_{t=(i-1)/f}, (i = 1, 2,, 16000001)$ denotes the sampled $FS_M(t)$.

Coherent demodulation of the fluorescence signal. The noisy output signal of photoelectric device is multiplied by the 1*KHz* sinusoid. f_{S_M} "(*i*) = f_{S_M} '(*i*) + f_n '(*i*), (*i*=1,2,....,16000001) represents the sampled noisy output signal of the multiplying unit, where f_{S_M} '(*i*) = $f_{S_M}(i) \times \cos(2000 \times \pi \times t)|_{t=(i-1)/f}$,

and
$$fn'(i) = fn(i) \times \cos(2000 \times \pi \times t)|_{t=(i-1)/f}$$
.

 fs_M "(*i*) was low pass filtered with cut off frequencies of 0.1*Hz*, 0.2*Hz*, 0.5*Hz*, 1*Hz*, 1.5*Hz*, 2*Hz*, 5*Hz*, 10*Hz*, 20*Hz*, 500*Hz*, 500*Hz*. $fs_D(i)$ denotes the low pass filtered fs_M "(*i*).

Measurement Error and Signal to Noise Ratio. As are expressed as Eq. (1) and (2), the measurement error, \triangle , and the signal to noise ratio, *SNR*, are calculated by using the normalized fs(i) and $fs_D(i)$.

$$\Delta = \left| f_{S}(i) - f_{S_{D}}(i) \right|, \quad (j = 1, 2, \dots, 16000001).$$
⁽¹⁾

$$SNR = 20 \times \log_{10} \left| \sum_{i=1}^{16000001} \left| f_S(i) \right| \right| \left| \sum_{j=1}^{16000001} \left| f_S(j) - f_{S_D}(j) \right|.$$
(2)

Results and Analysis

Signal to Noise Ratios. Table 1 shows the signal to noise ratios, SNRs, with different cut off frequencies by using the coherent demodulation method.

Cut off frequency [Hz]	SNR [dB]	Cut off frequency [Hz]	SNR [dB]	Cut off frequency [Hz]	SNR [dB]
0.1	5.08367	2	23.9779	20	15.68999
0.2	5.64729	3	23.31227	50	9.55546
0.5	9.4503	4	23.30094	100	3.06981
1	19.78716	5	22.79781	200	1.2928
1.5	24.15073	10	19.964	500	0.43577

Table 1. Signal to noise ratios, SNRs, with different cut off frequencies.

In terms of Table 1, it is clear that the measurement technique of sinusoidal amplitude modulating and coherent demodulating the fluorescence is usually accurate and efficient in reducing the noise.

Fig. 3 shows the frequency spectrum of the signal, $fs_M'(i)$, and the noise, fn'(i), by using the fast Fourier transform.



Fig. 3. The frequency spectrum of the signal, $fs_M'(i)$, (A) and the noise, fn'(i), (B).

From Fig. 2 and 3, it can be seen that the low frequency component of $f_{S_M}'(i)$ is similar to the fluorescence signal, *FS*, but distinct from that of $f_n'(i)$.

Therefore, by low pass filtering the noisy signal, $fs_M''(i)$, and using the appropriate cut off frequency, the fluorescence signal can been obtained, and the noise can been efficiently reduced.

Measurement Errors. The measurement errors with the low pass filter's cut off frequency of 1.5Hz is shown in Fig. 4.



Fig. 4. The measurement errors, \triangle , with cut off frequency 1.5*Hz*.

From Fig. 4, it can be seen clearly that the low frequency noise can be reduced much more efficiently than the high frequency noise. The reason may be that the modulation frequency of 1KHz is too low to separate effectively the fluorescence signal from the noise.

Conclusions

By using the appropriate modulation frequency of measurement light and cut off frequency of the low pass filter, the new technique, sinusoidal amplitude modulating and coherent demodulating the fluorescence signal, can efficiently reduce the noise caused by the actinic light and the saturation pulse light, and accurately measure the chlorophyll fluorescence curve in situ.

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