Single-turnover and multiple-turnover measurement of phytoplankton photosynthesis parameters using variable light pulse induced fluorescence

Chaoyi Shi (石朝毅)[1], Xianhe Gao (高先和)[1,*], Gaofang Yin (殷高方)[2], Zhisong Qin (覃志松)[2], Jingbo Duan (段静波)[2], Shuang Chen (陈 双)[2], and Jun Lu (卢 军)[1]

[1]*Department of Electronic Information and Electrical Engineering, Hefei University, Hefei 230601, China*

[2]*State Environmental Protection Key Laboratory of Optical Monitoring Technology, Anhui Institute of Optics and Fine Mechanics, Chinese Academy of Sciences, Hefei 230031, China*

**Corresponding author: gaoxh@hfuu.edu.cn*

Received November 14, 2017; accepted November 23, 2017; posted online December 6, 2017

Using a measurement system based on fluorescence induced by variable pulse light, photosynthesis parameters of *chlorella pyrenoidosa* are obtained, employing single-turnover and multiple-turnover protocols under dark-adapted and light-adapted conditions. Under the light-adapted condition, σ'_{PSII} is larger, and $F'_v/F'_{m(\mathrm{ST})}$ and $F'_v/F'_{m(\mathrm{MT})}$ are smaller than those of the dark-adapted condition, but the corresponding parameters possess good linear correlations. $F_{m(\mathrm{MT})}$, $F'_{m(\mathrm{MT})}$, $F_v/F_{m(\mathrm{MT})}$, and $F'_v/F'_{m(\mathrm{MT})}$, which are measured using the multiple-turnover protocol, are larger than those of the single-turnover protocol. The linear correlation coefficient between $F_{m(\mathrm{ST})}$ and $F_{m(\mathrm{MT})}$ is 0.984, and $F_v/F_{m(\mathrm{MT})} = 1.18F_v/F_{m(\mathrm{ST})}$. The linear correlation coefficient between $F'_{m(\mathrm{ST})}$ and $F'_{m(\mathrm{MT})}$ is 0.995, and $F'_v/F'_{m(\mathrm{MT})} = 1.36F'_v/F'_{m(\mathrm{ST})}$.

OCIS codes: 300.6280, 010.4450, 120.0120, 280.4788.
doi: 10.3788/COL201816.013001.

Phytoplankton chlorophyll fluorescence is closely related to the photosynthetic process[1–3]. When excited, the photosystem II (PSII) reaction center pigment P680 is oxidized and releases an electron. The electron then reduces the primary electron acceptor Q_A to Q_A^-, leading to the closure of the PSII reaction center and, consequently, an increase in the fluorescence yield. After Q_A^- transfers an electron to plastoquinone (PQ) and reoxidizes to Q_A, the reaction center reopens, and the fluorescence yield declines. Thus, the fluorescence yield reflects the electron transport state, which is the essence of photosynthesis, and photosynthetic parameters can be obtained by analyzing the chlorophyll fluorescence yield[4–6]. Based on this, different photosynthesis measurement techniques have been developed. Strasser[7] put forward JIP-test technique, in which the PSII photochemical reaction is reflected by fluorescence induced by continuous excitation light. While this technique is susceptible to ambient light, Schreiber[8] proposed the pulse amplitude modulation (PAM) technique based on the multiple-turnover measuring protocol. In this technique, saturation pulse light is employed to reduce all of the PQ, and modulated measurement light is used to record the induced fluorescence, from which the photosynthetic parameters can be obtained. This technique is unable to get the PSII functional absorption cross section σ_{PSII} for the low frequency of its modulated measurement light. Kolber[9] presented fast repetition rate (FRR) technology based on the single-turnover measuring protocol. This technique reduces all the Q_A in their single-turnover period using a single pulse light, and the fluorescence yield curve is analyzed to obtain σ_{PSII} and other photosynthetic parameters. Shi *et al.*[10] established a phytoplankton photosynthetic parameter measurement system based on fluorescence induced by variable light pulse, which incorporates single-turnover, relaxation, and multiple-turnover measuring protocols. This system takes Q_A and PQ as nodes to measure the photosynthetic process in sections, and more photosynthetic detail parameters can be obtained. Qin *et al.*[11] further studied the photosynthetic parameter inversion method of fast phase and relaxation fluorescence kinetics.

The essential difference between single-turnover and multiple-turnover measuring protocols is that the sites regulated by the excitation light are different. Comparisons of the photosynthetic parameters obtained, respectively by single-turnover and multiple-turnover measuring protocols have been reported. But, these studies were carried out using two different instruments, respectively, based on the single-turnover protocol (FRR technique) and the multiple-turnover measuring protocol (PAM technique)[12–15], or intrusively executed with the aid of electron transfer inhibitor diuron(3-[3,4-dichlorophenyl]-1,1-dimethylurea: DCMU)[16]. In this Letter, we non-intrusively measured the phytoplankton photosynthetic parameters under dark-adapted and light-adapted conditions using the same measurement system that incorporates single-turnover, relaxation, and multiple-turnover measuring protocols, and the measured parameters were compared and analyzed. The instrument that is used is established based on fluorescence induced by variable pulse light.

Hereafter, subscripts ST and MT are used to represent the parameters measured, respectively, by single-turnover and multiple-turnover measuring protocols. The parameters measured under the light-adapted condition was marked by "'" to distinguish from those of the dark-adapted condition.

For the single-turnover measuring protocol, all of the Q_A are reduced in their single-turnover period, and all the reaction centers are closed, leading the fluorescence yield increases to a maximum $F_{m(\mathrm{ST})}$, and the discrete fluorescence yield curve f_n that is sampled with a sampling period of Δt can be fitted by Eqs. (1)–(3) to invert σ_{PSII}, $F_{m(\mathrm{ST})}$, the minimal fluorescence yield F_o, and the maximum PSII photochemistry quantum yield $F_v/F_{m(\mathrm{ST})}$[10,17]:

$$f_n = F_o + (F_m - F_o)C_n \frac{1-p}{1-C_n p}, \tag{1}$$

$$C_n = C_{n-1}A_n + I_n \sigma_{PSII} \frac{1-C_{n-1}A_n}{1-pC_{n-1}A_n}, \tag{2}$$

$$A_n = A_{n-1} + C_{n-1}/\sigma_{PSII}. \tag{3}$$

Following the single-turnover measuring protocol, the relaxation measuring protocol is used to record the relaxation fluorescence that is caused by electron transport from Q_A^- to PQ. The average reoxidation time constant τ_{QA} can be obtained by fitting the relaxation fluorescence using Eq. (4)[10,17]:

$$f_n = F_o + (F_{m(\mathrm{ST})} - F_o)\exp(-t/\tau_{\mathrm{QA}}). \tag{4}$$

The multiple-turnover measuring protocol reduces all the PQ, and the maximum fluorescence yield $F_{m(\mathrm{MT})}$ can be obtained by fitting the induced fluorescence yield curve using Eqs. (1), (2), and (5), as well as σ_{PSII}, F_o, and τ_{QA}, which are obtained in the single-turnover and relaxation measuring protocols. Consequently, the maximum PSII photochemistry quantum yield $F_v/F_{m(\mathrm{MT})}$ can be calculated[17]:

$$A_n = (A_{n-1} + C_{n-1}/\sigma_{PSII})\exp(-\Delta t/\tau_{Q_A}). \tag{5}$$

The principle applies to both the dark-adapted and light-adapted conditions.

The measurement system was described in detail in Ref. [18]. The high brightness blue LED array controlled by a microcontroller unit (MCU) is employed as an excitation light[19,20]. The single-turnover measuring protocol uses a 100 μs single light pulse with an intensity of $30,000\ \mu\mathrm{mol\,quanta/m^2/s}$. The multiple-turnover measuring protocol employs a series of light pulses with 5 μs duration at 100 μs intervals, possessing an average intensity of $2000\ \mu\mathrm{mol\,quanta/m^2/s}$, and the excitation stays at 200 ms. The relaxation measuring protocol is composed of a series of light pulses with 0.3 μs duration at 60 μs intervals, and the excitation keeps 500 ms with an average intensity of $3\ \mu\mathrm{mol\,quanta/m^2/s}$. The ambient light intensity for the light-adapted condition is $35\ \mu\mathrm{mol\,quanta/m^2/s}$.

The measurement system was employed to measure the photosynthetic parameters of *chlorella pyrenoidosa* that were cultured in mediums with different nutrient concentrations. The nutrient concentration of the original medium was marked as one, while the nutrient concentrations of the 1000, 200, 100, 20, and 10 times diluted medium were marked as 0.001, 0.005, 0.01, 0.05, and 0.1, respectively. After 21 days in the culture, the photosynthetic parameters of the dark-adapted and light-adapted conditions were measured.

Under the light-adapted condition, the PSII functional absorption cross section (σ'_{PSII}) was larger than that of the dark-adapted condition (σ_{PSII}) [Fig. 1(a)], and the PSII photochemistry quantum yield ($F'_v/F'_{m(\mathrm{ST})}$) was smaller than that of the dark-adapted condition ($F_v/F_{m(\mathrm{ST})}$) [Fig. 1(b)]. The linear correlation coefficient of σ'_{PSII} and σ_{PSII} was 0.999, and that of $F'_v/F'_{m(\mathrm{ST})}$ and $F_v/F_{m(\mathrm{ST})}$ was 0.992, indicating good linear correlation.

The increase of the PSII functional absorption cross section under the light-adapted condition is caused by the energy transfer between PSII reaction centers, which is described by $p(0 < p < 1)$. Under the light-adapted condition with stable ambient light, the light energy capture and Q_A^- reoxidation reaches a balance, and the ratio of the open and closed reaction centers reaches a stable state. Under ambient light intensity i_o, the proportion of open reaction centers $q(i_o)$ $(0 < q(i_o) < 1)$ can be described by Eq. (6):

$$q(i_o) = \frac{\sigma'_{PSII}(i_o)i_o}{\sigma'_{PSII}(i_o)i_o + \frac{1}{\tau_{Q_A}(i_o)}}, \tag{6}$$

$$\sigma'_{PSII}(i_o) = \frac{\sigma_{PSII}}{1-p+pq(i_o)}, \tag{7}$$

where $\sigma'_{PSII}(i_o)$ and $\tau_{Q_A}(i_o)$ are, respectively, the PSII functional absorption cross section and Q_A^- reoxidation time constant under ambient light i_o. Equation (7) indicates that σ'_{PSII} is larger than σ_{PSII} due to the presence of energy transfer between PSII reaction centers p.

The decrease of $F'_v/F'_{m(\mathrm{ST})}$ is mainly affected by the minimal fluorescence yield and maximal fluorescence yield[21]. The minimal fluorescence yield F'_o is larger than F_o [Fig. 1(c)], because the ambient light closes part of the PSII reaction centers. Whereas, the maximal fluorescence yield $F'_{m(\mathrm{ST})}$ is smaller than $F_{m(\mathrm{ST})}$ [Fig. 1(d)] because of the increase of non-photochemical quenching. Finally, the increase of F'_o and decrease of $F'_{m(\mathrm{ST})}$ cause the decrease of $F'_v/F'_{m(\mathrm{ST})}$.

Under the light-adapted condition, the PSII photochemistry quantum yield ($F'_v/F'_{m(\mathrm{MT})}$) was smaller than that of the dark-adapted condition ($F_v/F_{m(\mathrm{MT})}$) [Fig. 2(a)], and the two parameters possessed a good linear correlation with a linear correlation coefficient of 0.996.

The maximal fluorescence yield $F'_{m(\mathrm{MT})}$ is smaller than $F_{m(\mathrm{MT})}$ [Fig. 2(b)], because the non-photochemical processes are activated under the light-adapted condition[22]. Meanwhile, the minimal fluorescence yield F'_o is larger than F_o, as analyzed before, thus, the PSII photochemistry quantum yield $F'_v/F'_{m(\mathrm{MT})}$ is smaller than $F_v/F_{m(\mathrm{MT})}$.

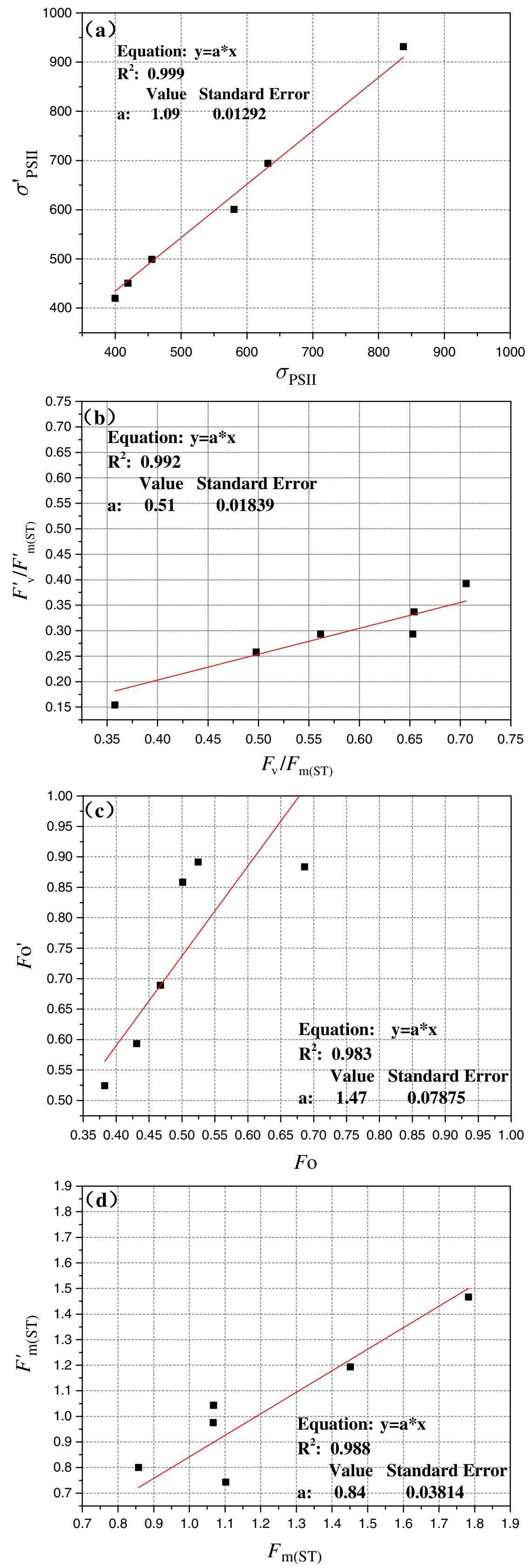

Fig. 1. (a) PSII functional absorption cross section, (b) PSII photochemistry quantum yield, (c) minimal fluorescence yield, and (d) maximal fluorescence yield measured by the single-turnover protocol, respectively, under dark-adapted and light-adapted conditions.

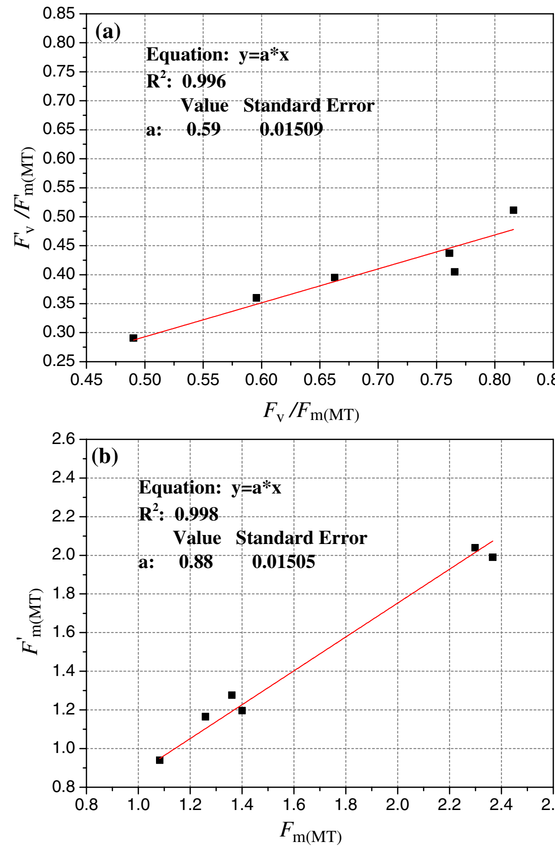

Fig. 2. (a) PSII photochemistry quantum yield and (b) maximal fluorescence yield measured by the multiple-turnover protocol, respectively, under dark-adapted and light-adapted conditions.

The maximal fluorescence yield and PSII photochemistry quantum yield measured by the multiple-turnover protocol were larger than those of the single-turnover protocol, as shown in Figs. 3 ($F_{m(\mathrm{ST})} < F_{m(\mathrm{MT})}$ and $F'_{m(\mathrm{ST})} < F'_{m(\mathrm{MT})}$) and 4 ($F_v/F_{m(\mathrm{ST})} < F_v/F_{m(\mathrm{MT})}$ and $F'_v/F'_{m(\mathrm{ST})} < F'_v/F'_{m(\mathrm{MT})}$).

The increase of the maximal fluorescence yield in the multiple-turnover protocol is due to the prolonged electron occupation of site Q_B in Q_A. During the photosynthetic process, the fluorescence yield is also affected by the electron occupation of the site Q_B in Q_A, which is mainly dependent on the PQ pool size and the balance between the PQ reduction rate and reoxidation rate. In the multiple-turnover protocol, as the PQ pool becomes progressively reduced, the electron occupation of the site Q_B in Q_A is prolonged, thus leading to a larger maximal fluorescence yield[17]. Meanwhile, because the minimal fluorescence yield used in the multiple-turnover protocol is the same as that of the single-turnover protocol, the calculated PSII photochemistry quantum yield is larger than that of the single-turnover protocol.

Under the dark-adapted condition, the linear correlation coefficients for $F_{m(\mathrm{ST})}$ and $F_{m(\mathrm{MT})}$, as well as $F_v/F_{m(\mathrm{ST})}$ and $F_v/F_{m(\mathrm{MT})}$, were, respectively, 0.984 and 0.998, indicating good linear correlation. The linear

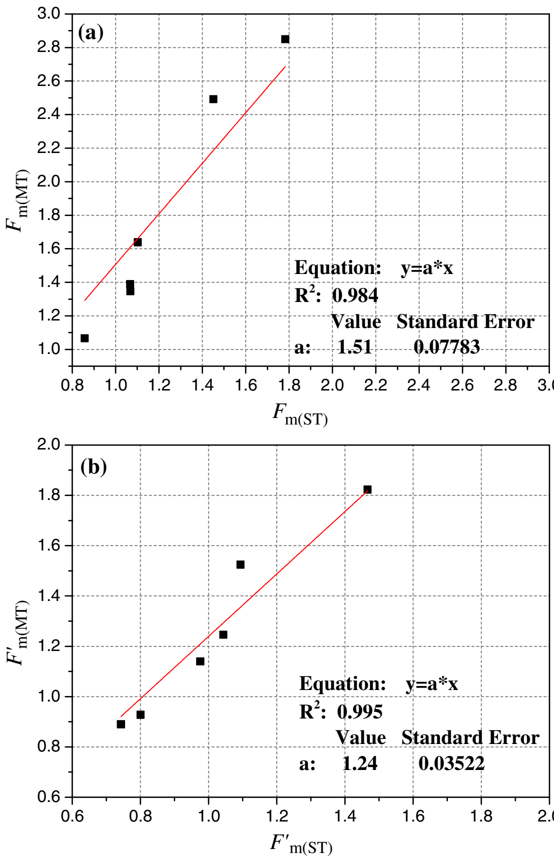

Fig. 3. Maximum fluorescence yield measured by single-turnover and multiple-turnover protocols under (a) the dark-adapted condition and (b) the light-adapted condition.

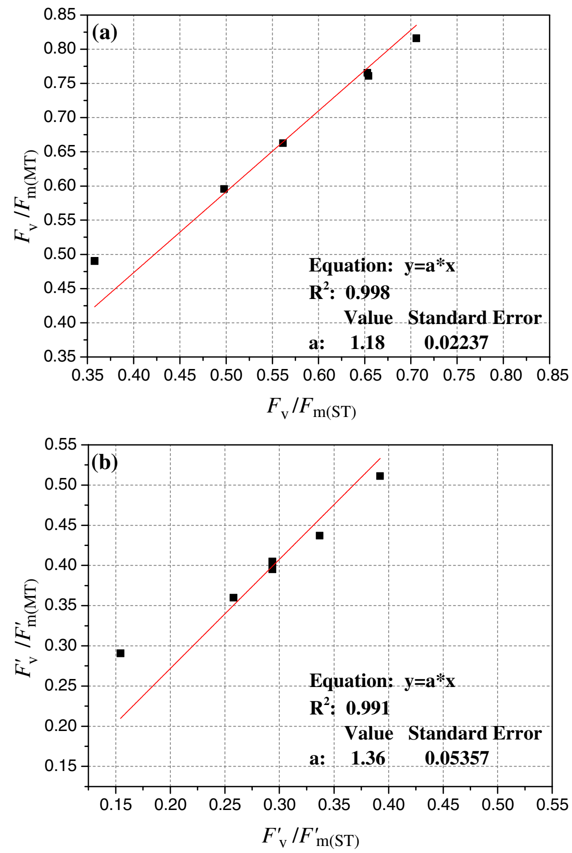

Fig. 4. PSII photochemistry quantum yield measured by single-turnover and multiple-turnover protocols under (a) the dark-adapted condition and (b) the light-adapted condition.

relationship between $F_v/F_{m(\mathrm{ST})}$ and $F_v/F_{m(\mathrm{MT})}$ was described as $F_v/F_{m(\mathrm{MT})} = 1.18F_v/F_{m(\mathrm{ST})}$, which was basically consistent with the results reported by Rottgers[15] ($F_v/F_{m(\mathrm{MT})} = 1.21F_v/F_{m(\mathrm{ST})}$). Under the light-adapted condition, the linear correlation coefficients for $F'_{m(\mathrm{ST})}$ and $F'_{m(\mathrm{MT})}$, as well as $F'_v/F'_{m(\mathrm{ST})}$ and $F'_v/F'_{m(\mathrm{MT})}$, were, respectively, 0.995 and 0.991, and $F'_v/F'_{m(\mathrm{MT})} = 1.36F'_v/F'_{m(\mathrm{ST})}$. While Rottgers reported that there was a non-linear relationship between $F'_v/F'_{m(\mathrm{ST})}$ and $F'_v/F'_{m(\mathrm{MT})}$, the reason for the inconsistency remains to be revealed. The wavelength and intensity of the ambient light, the excitation light source, the phytoplankton species, and other elements should be considered.

In conclusion, based on the variable optical pulse induced fluorescence technique, the photosynthetic parameters of *chlorella pyrenoidosa* are measured by single-turnover and multiple-turnover protocols under dark-adapted and light-adapted conditions, and the obtained parameters are analyzed and discussed. Compared with the parameters measured under the dark-adaptation condition, the PSII functional absorption cross section measured under the light-adapted condition is larger because of the energy transfer between PSII reaction centers, and the PSII photochemistry quantum yield is smaller because the ambient light closes part of reaction centers, and the non-photochemical quenching increases. Compared with the parameters measured in the single-turnover protocol, the maximum fluorescence yield measured in the multiple-turnover protocol is larger because of the prolonged electron occupation of the site Q_B in Q_A, consequently leading to a larger PSII photochemistry quantum yield, which is calculated using the multiple-turnover measured maximum fluorescence yield and the minimal fluorescence yield measured in the single-turnover protocol. The results and discussion in this Letter provide an important reference for the analysis and application of the photosynthetic parameters measured by single-turnover and multiple-turnover protocols.

This work was supported by the Natural Science Foundation of Anhui Province (No. 1708085QD87), the Open Fund of Qingdao National Laboratory for Marine Science and Technology (No. QNLM2016ORP0312), the Natural Science Foundation of Higher Education Institutions of Anhui Province (Nos. KJ2017A530 and KJ2016A594), the National Key Research and Development Plan (No. 2016YFC1400602), and the Natural Science Foundation of China (No. 31400317)

References

1. R. Hill and F. Bendall, Nature **186**, 136 (1960).
2. G. Papageorgiou, “Chlorophyll fluorescence: an intrinsic probe of photosynthesis,” in *Bioenergetics of Photosynthesis* (Academic Press, 1975) p. 319.
3. Z. Kolber and P. G. Falkowski, Limnol. Oceanogr. **38**, 1646 (1993).
4. P. Falkowski and D. A. Kiefer, J. Plankton Res. **7**, 715 (1985).
5. A. K. Biswal, F. Dilnawaz, K. A. David, N. K. Ramaswamy, and A. N. Misra, Luminescence **16**, 309 (2001).
6. L. Fei, “Study of mechanism of electron transfer of the Q_B site in photosystem II of higher plants,” PhD. Thesis (Dalian University of Technology, 2012).
7. R. J. Strasser and A. Srivastava, Photochem. Photobiol. **61**, 32 (1995).
8. U. Schreiber, Photosynth. Res. **9**, 261 (1986).
9. Z. S. Kolber and P. G. Falkowski, in *Proceedings of the Oceans ‘92, Mastering the Oceans Through Technology* (1992), p. 637.
10. C. Shi, X. Gao, G. Yin, Z. Zhou, J. Lu, and X. Hu, Laser Optoelectron. Prog. **53**, 120002 (2016).
11. Z. Qin, N. Zhao, G. Yin, C. Shi, T. Gan, X. Xiao, J. Duan, X. Zhang, S. Chen, J. Liu, and W. Liu, Acta Opt. Sin. **37**, 350 (2017).
12. B. Osmond, W. S. Chow, R. Wyber, A. Zavafer, B. Keller, B. J. Pogson, and S. A. Robinson, Funct. Plant Biol. **44**, 985 (2017).
13. W. Vredenberg, M. Durchan, and O. Prášil, J. Photochem. Photobiol. B **107**, 45 (2012).
14. R. Röttgers, Deep Sea Res. Part I Oceanogr. Res. Pap. **54**, 437 (2007).
15. D. J. Suggett, K. Oxborough, N. R. Baker, H. L. MacIntyre, T. M. Kana, and R. J. Geider, Eur. J. Phycol. **38**, 371 (2003).
16. U. Schreiber and A. Krieger, FEBS Lett. **397**, 131 (1996).
17. Z. S. Kolber and P. G. Falkowski, “Multiple protocol fluorometer and method,” U.S. Patent 6,121,053 (September 19, 2000).
18. C. Y. Shi, Y. J. Zhang, G. F. Yin, N. J. Zhao, J. B. Duan, X. H. Qiu, L. Fang, X. Xiao, and W. Q. Liu, Acta Photon. Sinica **44**, 5 (2015).
19. Z. Li, H. Wang, B. Yu, X. Ding, and Y. Tang, Chin. Opt. Lett. **15**, 042301 (2017).
20. R. Zhao, Z. Huang, Y. Liu, and Y. Ji, Chin. Opt. Lett. **14**, 070601 (2016).
21. K. Oxborough, C. M. Moore, D. J. Suggett, T. Lawson, H. G. Chan, and R. J. Geider, Limnol. Oceanogr. Methods **10**, 142 (2012).
22. B. Han, Z. Han, and X. Fu, *Algal Photosynthesis: Mechanisms and Models* (Science Press, 2003).