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基于光脉冲诱导快相与弛豫荧光的光合作用参数测量技术

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摘 要:为了更准确获取反映植物生理状态的荧光动力学曲线,基于光合作用电子传递过程研究了植物光合作用参数测量技术。采用可变光脉冲技术将植物光合作用过程分段为快相与弛豫过程,并测量激发光诱导产生的荧光动力学曲线.对激发光带宽与响应时间进行了定量分析;对 I-V 转换单元与 MFB 滤波器进行了设计与仿真分析,获取快相荧光动力学信息;采用同步脉冲采样积分技术,对微弱弛豫荧光进行积分,实现了快相与弛豫荧光动力学曲线的完整测量,并结合非线性拟合算法获取光合作用参数.测试结果表明,系统信噪比达到 23.8 dB;暗适应与光适应下,本系统所测 F_v/F_m 与 Water-PAM 测量结果的线性相关系数分别达到 0.980 和 0.997.该研究结果为植物光合作用研究及过程参数测量提供了一种测量手段.

关键词:快相和弛豫荧光;叶绿素荧光动力学;同步采样积分;光合作用参数测量

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Photosynthesis Parameters Measurement Technology Based on Fast Phase and Relaxation Fluorescence Induced by Optical Pulses

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Abstract: The photosynthetic parameters can reflect the physiological state of plants. In order to obtain a more accurate fluorescence kinetic curve, a technique for measuring plant photosynthetic parameters

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based on the electron transfer process was proposed in this paper. Variable excitation light pulses was employed to devide the plant photosynthesis process into two stages: fast phase and relaxation phase, and corresponding fluorescence curves were induced for each stage. The excitation bandwidth and response time were analyzed using the TINA simulation tool. The *I-V* conversion and MFB filter were designed and simulated quantitatively. Synchronous sampling integral technique was employed to improve the SNR of weak relaxation phase fluorescence. As a result, a complete fluorescence kinetics curve that consist of fast phase and relaxation phase was obtained, from which the plant photosynthesis parameters can be calculated using nonlinear fitting algorithm. Experiment results showed that the system SNR reached 23.8 dB, and the correlation coefficients of measured F_v/F_m that obtained by this system were respectively 0.980 and 0.997 for dark and light adaptation conditions.

Key words: Fast phase and relaxation fluorescence; Chlorophyll fluorescence kinetics; Synchronous sampling integral technique; Photosynthetic parameters measurement

OCIS Codes: 300.2530; 300.6280; 130.6622; 160.2540; 260.2510

0 Introduction

Chlorophyll fluorescence is a quick and efficient probe for plant photosynthesis measurement [1]. For the purpose of studying photosynthetic parameters, several models and methods such as the Pump and Probe(P&P)[2](Mauzerall D, 1972), the Pulse Amplitude Modulation(PAM)[3](Schreiber U, 1986), the Fast Repetition Rate(FRR)[4-5](FALKOWSKI P G, 1995 and Kolber Z S, 1998) were proposed in the last ten years. In recent years, Vredenberg W.[6](in 2011) and Stirbet A.[7](in 2014) established photochemical electron transfer process and photoelectron-chemical model. In 2011, Tongra T.[8] calculated and analyzed fluorescence kinetics under different PH conditions, including the fluorescence kinetics of relaxation.

In the study of measuring methods, in 2006, An L.[9] measured fast fluorescence induced by strong light. In 2011, Stirbet A. [10] proposed OJIP-test method to measure the fast phase fluorescence. In 2012, Vredenberg W.[11] proposed a method consists of single and multiple turn-over as the supplement of fast fluorescence kinetics. In 2014, SHI Chaoyi[12] established a VLPF measurement method and system. In 2015, Fernandez-Jaramillo A.[13] designed FGPA arbitrary waveform generator based on the excitation light research results, providing a wide range of plant photosynthesis measuring excitation.

However, the study of photosynthesis parameter measurement is mainly concentrated in the photosynthetic model and analysis methods. Except for Refs.[12][13], there are few reports about the measurement techniques that analyze the fast and relaxation fluorescence signal characteristics, excitation bandwidth requirements, and the SNR enhancement of weak fluorescence kinetics curves. Based on the biofilm energy flow theory, a new measurement technology is proposed to measure and verify the parameters and fluorescence kinetics curve of photosynthesis in this paper.

1 Theory

The substance of photosynthesis is the electron transport. The key steps of linear electron transport of "Z" diagram[14] are presented in Fig.1. Following light irradiation, the primary charge separation takes

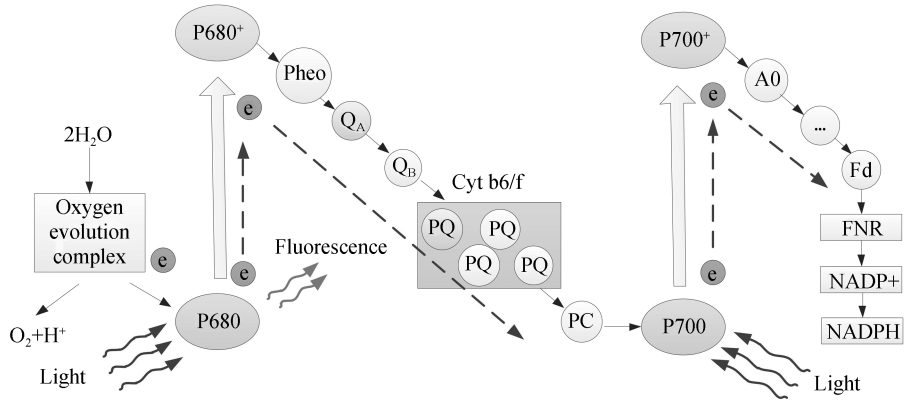

Fig.1 Photosynthesis electron transfer reactions of "Z" diagram

place in Reaction Center (RC), producing strong oxidant $P680^+$ and electron. The electron transfers to Q_A, leading to a relatively stable deoxidizer Q_A^-. The charge separation produces a stable reducing agent (Ferrdoxin, Fd) and a weak oxidant ($P700^+$) in PSII. The $P680^+$ with a strong oxidation potential can trigger the water to release an electron, and Q_A^- possesses a reduction potential to drive electron transmit to $P700^+$. In the mechanism of electron transport, proton gradient that drives ATP synthesis is set up on both sides of the photosynthetic membrane. There are two significant time nodes in this electron transport mechanism: Q_A to PQ taking about 100-200μs, PQ to PSI taking from hundreds of microseconds to tens of milliseconds.

When triggered by artificial modulation single-pulse intense light, a good amount of electrons well be hampered at Q_A. The RC is closed and any following energy is unavailable for the photochemical reaction. Most of the energy is dissipated in the form of fluorescence and heat, leading to a rise of fluorescence from the static minimum fluorescence yield F_o to the maximum fluorescence yield F_m. The chlorophyll fluorescence yield is closely related to the excitation energy, PSII functional absorption cross section σ_{PSII} and redox state of Q_A. Under an intense light excitation, the fluorescence yield can be described by Eq.1[14]

$$F(t)=F_o+(F_m-F_o)(1-\exp(-\sigma_{PSII}\int_0^t E\,dt))=F_o+F_v(1-\exp(-\sigma_{PSII}\int_0^t E\,dt)) \quad (1)$$

Where F_o is the minimum fluorescence yield; F_m is the maximum fluorescence yield after the reaction center is completely closed, the difference F_v(eq. F_m-F_o) is the maximum variable fluorescence yield; and σ_{PSII} is the absorption cross section for the PSII function. E is excitation intensity, which is a constant in single exciting cycle. The t is the excitation duration time. Using Eq.1, the photochemical efficiency yield F_v/F_m, functional absorption cross-section σ_{PSII} and other photosynthetic parameters can be inverted.

Once external excitation stops, Q_A^- is re-oxidized and the reaction center opens again. As a result the fluorescence yield reduces. This phenomenon is the relaxation process. The chlorophyll fluorescence yield of this mechanism is related to the excitation energy and PQ reduction state. The fluorescence yield can be described by Eq. 2[14]

$$f_2(x)=F_o+(F_m-F_o)\exp(-x/\tau_{QA})=F_o+F_v\exp(-x/\tau_{QA}) \quad (2)$$

Where F_m, F_o andF_v represent the same meaning with the above description, τ_{QA} is the PQ average reduction time. If F_o and F_v are determined by the fast phase fluorescence process, τ_{QA} can be measured by Eq.2.

2 System design

The kinetic curve was divided into two stages: fast phase and relaxation. For the fast phase fluorescence excitation process, the excitation light pulse intensity is as high as several 10^4 μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}. The relaxation excitation light is composed of a series of weak light pulses, and the average intensity was about several μmol quanta $\cdot$ m^{-2} $\cdot$ s^{-1}. The light pulse with resolution of 10 ns is assumed in relaxation excitation, which implies that the bandwidth was about 35.0 MHz.

A measurement system is shown in Fig.2. A 3×3 LEDs array (CL-P10WB35R, EVERIGHT) at 465 nm was used as the excitation light source , which may provide a maximum instantaneous intensity of

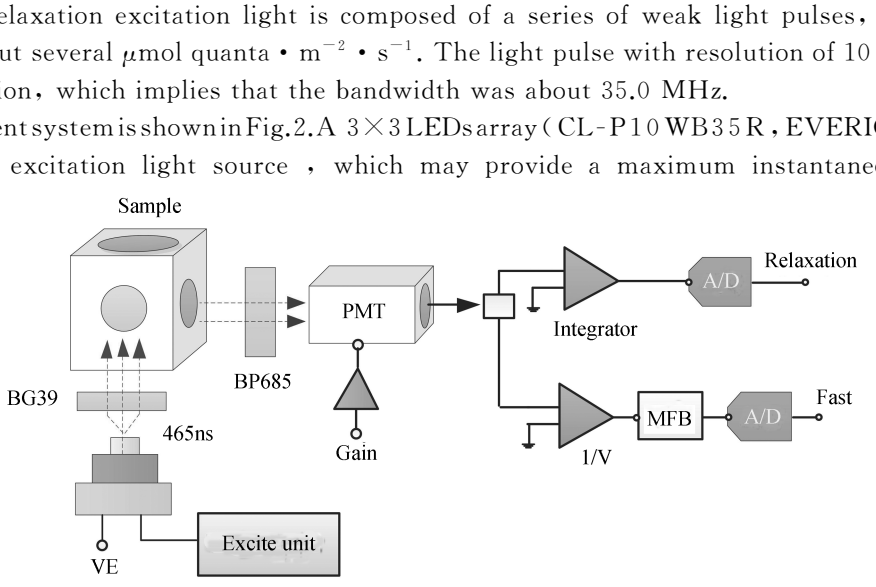

Fig.2 Exciting circuit of relaxation phase

80 000 μmol quanta • m^{-2} • s^{-1}. The exciting light passes through a glass filter BG39 to cut off lights with wavelength above 650 nm. The fluorescence at 685 nm was detected in 90 degree in order to avoid the stray light from the excitation light, and a band-pass filter BP685 was employed before the fluorescence sensor. The photomultiplier tube (H6779-1, HAMAMATSU) with a current gain of almost 60 dB was employed for the detection of the fluorescence at 685 nm.

A 210MH high speed MCU was employed to generate excitation pulse sequence for fast phase and relaxation phase. The fast phase pulse was a single pulse with 190μs duration time, and it drove the CMOS power transistor IRF540 and the LED array to generate a saturated light pulse. By changing the excitation voltage, excitation light intensity resolution of fast phase can reach of 120μmol quanta • m^{-2} • s^{-1}. The relaxation light was triggered by a sequence of 2μs pulse with a period of 50 μs. The relaxation light intensity resolution can reach 0.1 μmol quanta • m^{-2} • s^{-1}. In relaxation excitation, the desired duration time of each light pulse is only 2 μs, thus, it is critical to generate high time resolution pulse signal. The exciting circuit is discussed as follows.

The exciting circuit of relaxation was designed by TINA software as shown in Fig.3. LED Symbol was replaced by LNG992CFB, but the simulation parameters were CL-P10WB35R. The current that passed through LED1 was controlled by a high-frequency NPN transistor (2N4401, FAIRCHILD) to generate light pulses, and the NPN transistor was controlled by the RE signal, which is a pulse sequence with 2 μs duration time for each pulse. The simulation of LED1 current and RE voltage pulse was showed in Fig.4. The bandwidth of excitation signal may be calculated as : B_R=0.35/148.18 ns≈2.36 MHz.

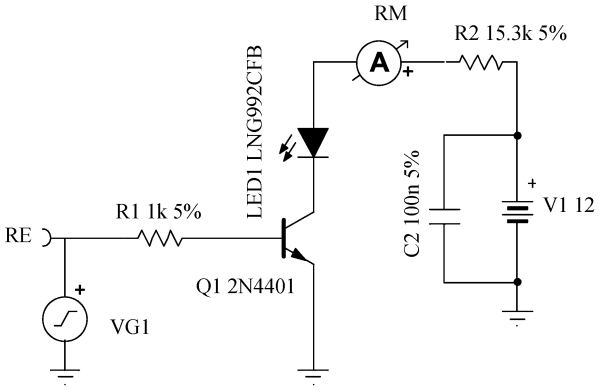

Fig.3 Exciting circuit of relaxation phase

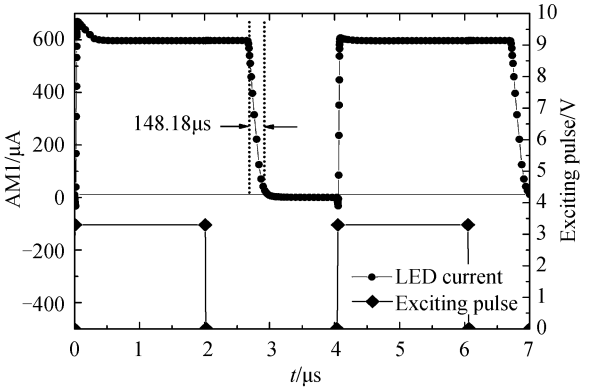

Fig.4 Transient simulation result

The fast phase and relaxation fluorescence signals were selected by a multiplexer (ADG1608, Analog Device). A broadband amplifier (OPA655, Texas Instrument) with low bias current (I_b=1 pA) and low voltage noise was employed in I/V conversion circuit as is showed in Fig.5.

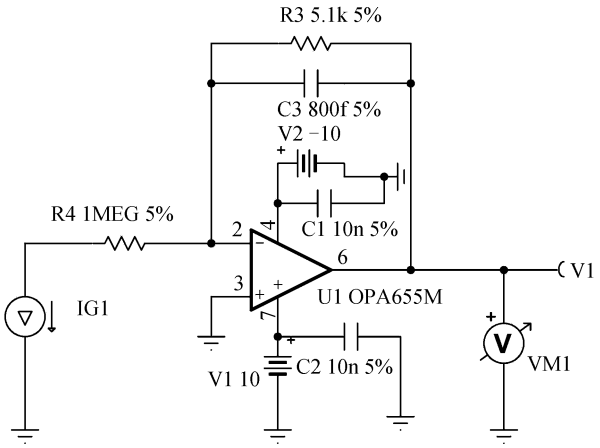

Fig.5 I/V conversion design

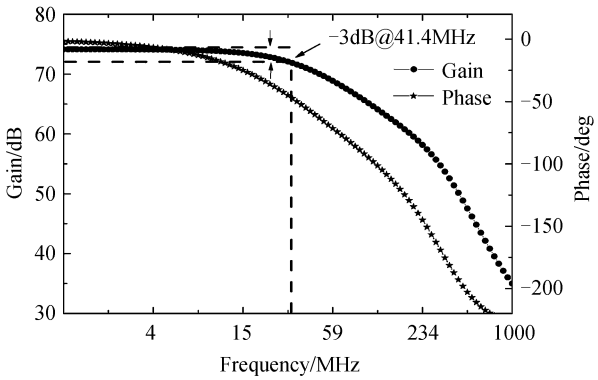

Fig.6 I/V bandwidth simulation result

A two-order MFB filter shown in Fig.7 was designed to cut down high frequency noise after I-V conversion. The simulation bandwidth of this filter was 1.0MHz. The gain was −43.5 dB at 10 MHz.

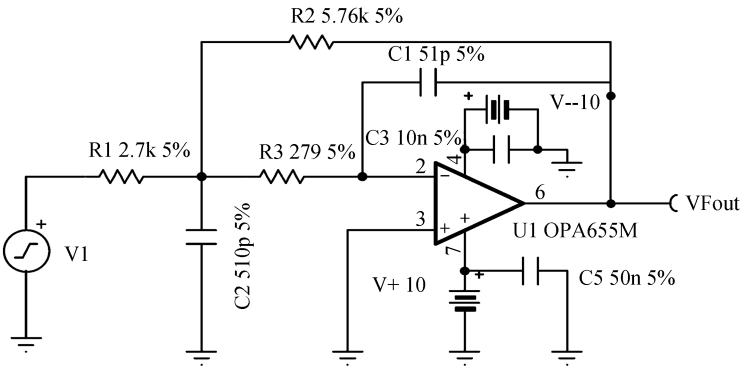

Fig.7 Two-order MFB Filter

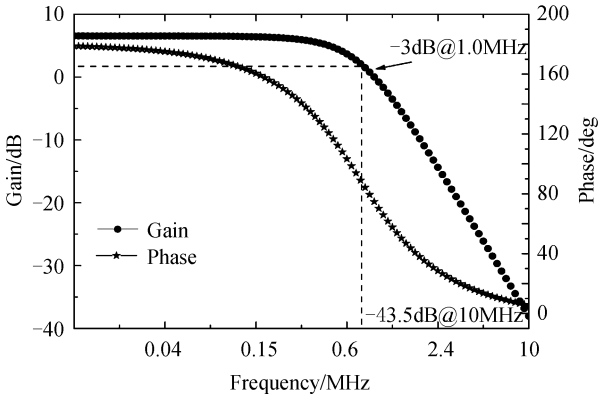

Fig.8 MFB Filter simulation result

Synchronous pulse integration (IVC102, Texas Instruments) has the advantages of high gain and low voltage output error, which may make the white noises (et. sensor noise and circuit noise) converge to zero according the sample theory[15]. The three integrating capacitors: 10, 30 and 60pF were used by any combination. The V_{out} is determined by input current I_{IN}, integrating capacitance C_{INT} and integral time T_{INT} according to Eq.3

$$V_{Out} = \frac{-1}{C_{INT}} \int I_{IN}(t)\,dt \tag{3}$$

A 16 bits SAR A/D device (AD8330, Texas Instruments) with a sampling rate of 1.0MSPS was used to acquire digital fluorescence signal. The excitation light intensity was measured by Water-PAM. The digital fluorescence signal was transmitted to MCU or PC for photosynthetic parameters calculation.

After fast and relaxation phase fluorescence and light intensity were attained, analysis procedures including background light removal, fluorescence signal normalization and fluorescence kinetics curve were needed for the inversion of the photosynthetic parameters. Multivariate nonlinear regression[16] was employed to fit the fluorescence kinetics curve according to Eq.1 and Eq.2.

3 Results and discussion

Algae are ideal objects for photosynthetic research. Chlorella pyrenoidosa are cultured in laboratory at 25℃ and 200 μmol quanta • m^{-2} • s^{-1} illumination. In dark and light adaption, the fluorescence kinetic curves consist of fast and relaxation phase curve was obtained, and then F_v/F_m, σ_{PSII} and τ_{QA} were inverted. The inverted F_v/F_m were compared with the F_v/F_m measured by Water-PAM.

3.1 Fluorescence kinetic measurement of chlorella pyrenoidosa

At First, in order to investigate the performance of the designed system, two experiments were setup. Chlorella pyrenoidosa at logarithmic phase was used in the experiments. The ratio of fluorescence and stray light was evaluated. Two different concentrations of chlorophyll were set for measurement. One was a blank sample of deionized water, the other was chlorella pyrenoidosa in a concentration of 2.50 μg/L. The excitation light of 2.5×10^4 μmol quanta • m^{-2} • s^{-1} and system gain were the same for the two samples. The measured fluorescence kinetics curves of two groups were showed in Fig.9.

Due to the characteristic of LEDs and glass filter, tiny stray lightwas collected as "fluorescence". The average stray light intensity of blank sample was 154, while the F_m of 2.50 μg/L chlorella pyrenoidosa was 2 389. Thus, the ratio of fluorescent and stray light was 23.8 dB in fast phase. Similarly, fluorescence and stray light ratio was 12.3 dB in relaxation phase. The stray light intensity can be removed as black ground.

Secondly, fluorescence kinetic curves of two samples with the same chlorophyll concentration of 2.50 μg/L were investigated. One was the normal growth chlorella pyrenoidosa, which means its

photosynthesis performed normally. Another was the extracted chlorophyll solution that the membrane of the cell has been destroyed and can not be used for photosynthesis. The measured fluorescence kinetic curves of the two samples were measured and showed in Fig.10.

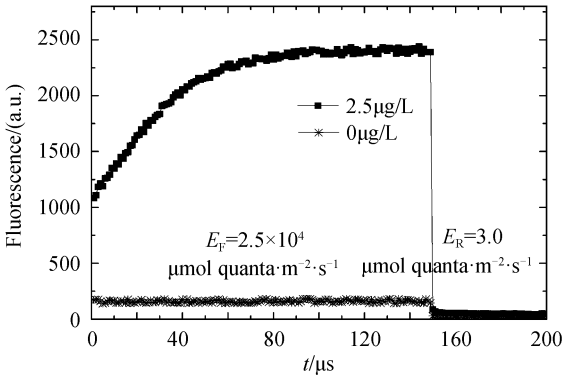

Fig.9 Fluorescence kinetic curve comparison with blank sample

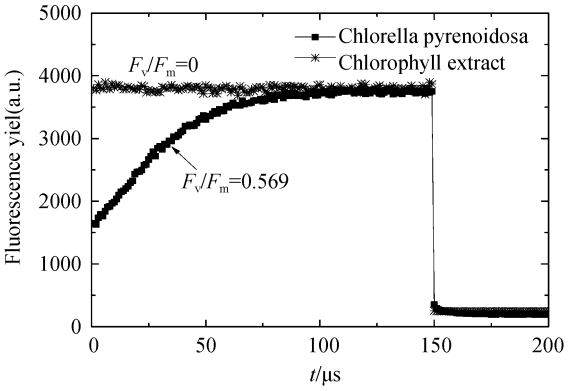

Fig.10 Fluorescence kinetic curves of normal chlorella pyrenoidosa and extracted chlorophyll

The two fluorescence kinetic curves were obviously different, indicating that the measurement results were closely related to the internal state of the sample itself. The F_v/F_m of normal chlorella pyrenoidosa was 0.569, while the F_v/F_m of extracted chlorophyll was zero due to disability of photosynthesis. This experiment indicates that the variety of fluorescence yield can reflect the details of plant photosynthesis.

3.2 Parameters measurement under different nutrients condition

After cultured 7 days in laboratory, fluorescence kinetic curves of 9 different nutrition groups of the chlorella pyrenoidosa of 200 μg/L were measured and the photosynthetic parameters were inverted. The F_v/F_m shown in Fig.11 was compared with that of the Water-PAM instrument in dark adaptation. The linear correlation coefficient of the values of F_v/F_m calculated by two methods was 0.980, as shown in Fig.12.

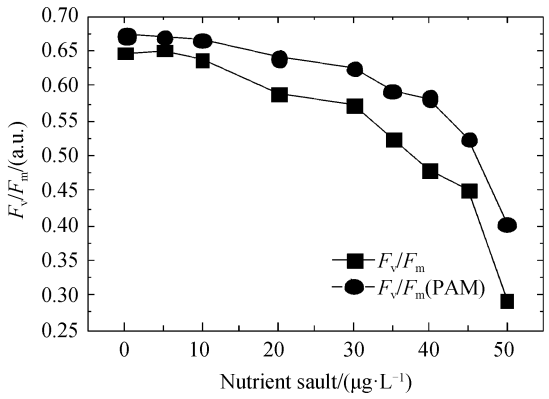

Fig.11 F_v/F_m comparison with Water-PAM

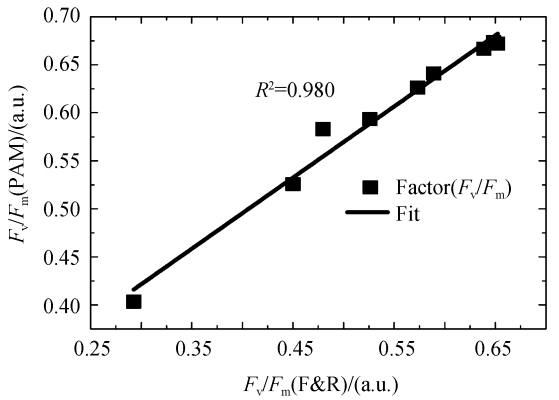

Fig.12 F_v/F_m correlation analysis under dark adaptation

The trend of F_v/F_m of chlorella pyrenoidosa under 118 and 249 μmol quanta • m^{-2} • s^{-1} light adaption conditions was measured and obtained parameters of photosynthesis are showed in Fig.13 and Fig.14.

Under different concentrations of nutrients salt, the correlation analysis between the two methods was showed in Fig.15. The measurement results under two light adaption conditions were in accordance with each other, and the correlation coefficients R^2 were 0.998 and 0.997.

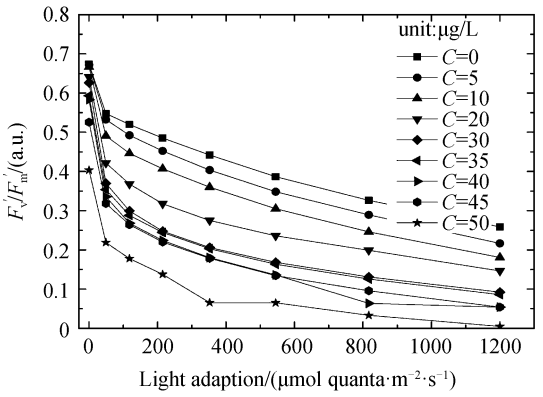

Fig.13 F_v/F_m measured by Water-PAM under light adaptation

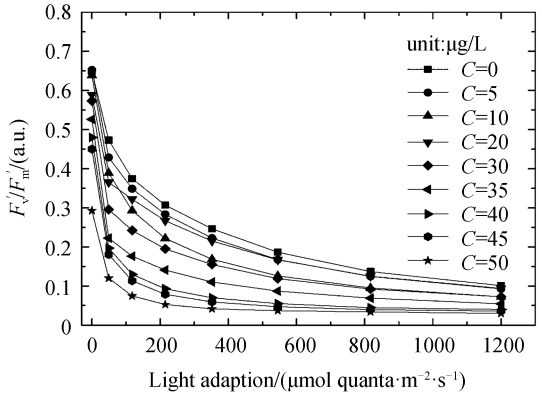

Fig.14 F_v/F_m measured by experimental system under light adaptation

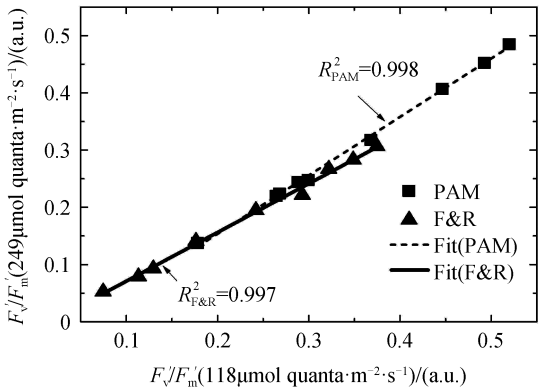

Fig.15 F_v/F_m correlation analysis under light adaptation

4 Conclusion

Based on the fluorescence kinetic model of plant photosynthesis and the characteristics of excitation light and fluorescence, a measurement process of the fast and relaxation phase was established. The bandwidth requirements, excitation control signal and fast pulse excitation circuit were designed and analyzed. The optical sensing system, *I-V* conversion, MFB filter and synchronous pulse integration technology were also applied. The fluorescence kinetic curve of low concentration chlorophyll of chlorella pyrenoidosa was compared with the blank sample to verify the result of fluorescence kinetics curve in fast and relaxation phase; the photosynthetic parameters of chlorella pyrenoidosa cultivated in nine concentration groups of nutrient sault were measured and inverted respectively under dark and light adaption conditions. The results obtained by the designed system were consistent with those that measured by Water-PAM. The results of this paper would provide a new way to develop instruments for photosynthetic parameters measurement.

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