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Short communication

Modification of the Hansatech FMS fluorometer to facilitate measurements with microalgal cultures

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Abstract

This paper describes a simple modification to the Hansatech FMS1 fluorometer for fluorescence measurements in diluted microalgal cultures that increased sensitivity four-fold and allowed measurements with 0.015 mg Chl $a\,l^{-1}$. The modified FMS1 fluorometer was used to measure the variability of fluorescence yield, calculate the electron transport rate (ETR), and plot curves for ETR versus irradiance (ETR/E) in the microalga *Dunaliella tertiolecta* cultured at two irradiances (75 and 350 μmol photon $m^{-2}\,s^{-1}$). Curves were also plotted for oxygenic photosynthesis versus irradiance (P/E), using a Clark-type electrode. High-irradiance acclimation resulted in an increased saturation point (E_k), and maximal photosynthetic rate (P_{max}). The same result was obtained in fluorescence measurements, as the maximal electron transport rate (ETR_{max}) increased in the same proportion as P_{max} (42 and 53%, respectively). The fluorescence saturation point (ETR E_k) also increased, but in a lower proportion than oxymetric-calculated E_k (49 and 60%, respectively). The modified FMS1 fluorometer proved its reliability, allowing comparison of photosynthesis estimated either by oxygen evolution or fluorescence measurements. The relation between the two methods was not always linear, illustrating the strong effects of the cuvette geometry in fluorescence measurements, and the difficulty of converting fluorescence data into oxygen evolution rates.

Keywords: Modulated fluorescence; Oxygen measurement; *Dunaliella tertiolecta*; Microalga

1. Introduction

Chlorophyll fluorescence is one of the most convenient and powerful tools for the study of photosynthesis. Fluorescence measurements provide rapid, non-intrusive, sensitive and

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reproducible data on in vivo photosynthetic mechanisms. Like most modulated fluorometers, the FMS1 (Hansatech Ltd., UK), designed for higher plants, is equipped with an actinic light source of variable intensity (halogen bulb) for pulses and photosynthesis activation, as well as a modulated analytic light source (GaInN blue light-emitting diode (LED)) for fluorescence measurement. An induced modulated fluorescence signal is detected and analysed by a photodiode in phase with the modulated beam. A far-red LED is used in some experiments to excite PSI preferentially and thus fully oxidise the PQ pool and the PSII acceptors. These elements are built-in, and light beams are driven via an optical fibre floppy tunnel to the measurement cell. Incident and emergent beams are thus parallel, which is convenient in the case of higher plants for which the FMS1 fluorometer was originally designed. However, this arrangement, together with the length of the optical tunnel, results in low sensitivity when the fluorometer is used on microalgal suspensions. To overcome this limitation, the microalgal culture needs to be concentrated and the sample end of the optical fibre cable positioned directly on one side or at the open top of the glass spectrophotometer cuvette (Mouget et al., 1999; Tremblin et al., 2000; Mouget and Tremblin, 2002). Emergent signals need to be increased when using the fluorometer with unconcentrated microalgal cultures. A simple solution consists in using perpendicular incident and emergent beams together with short optical tunnels. This requires modifications in the arrangement of both emitting and receiving elements so that they can be positioned near the measurement cuvette. The present paper describes an improvement in the arrangement of these elements, which provides greater sensitivity, thereby avoiding any need for cell concentration steps, and allows direct use of the FMS1 on algal cultures. After various tests with different microalgal species, the modified FMS1 was used to estimate the photosynthetic characteristics of *Dunaliella tertiolecta*, cultured at two different irradiances (75 and 350 μmol photon m^{-2} s^{-1}). Comparison was made with gross oxygen response measurements.

2. Materials and methods

2.1. Algal culture conditions

D. tertiolecta (Chlorophyceae), *Porphyridium cruentum* (Rhodophyceae) and *Skeletonema costatum* (Bacillariophyceae) were grown in Provasoli 33% (v/v) medium at 15 °C in 500 ml Erlenmeyer flasks with a 14 h photoperiod under two different light conditions: 75 (low) and 350 μmol photon m^{-2} s^{-1} (high). Irradiance was measured with a Walz US-SQS 4π light probe (Walz GmBH, Germany). The cultures were maintained in exponential growth phase by dilution with fresh medium every 2 (high irradiance) or 3 days (low irradiance).

2.2. Technical modifications of the FMS1

Neither the actinic nor the far-red light source was modified, as these elements are not strictly necessary for fluorescence measurements and their location has no effect on sensitivity. The emitting gallium blue LED and the receiving photodiode were placed in

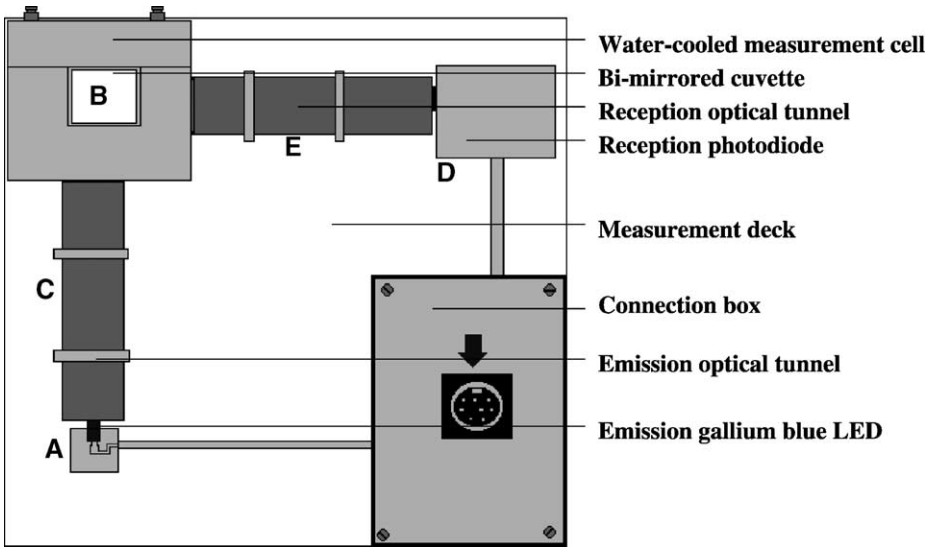

Fig. 1. Element disposition on measurement deck. Modulated excitation beam produced by the gallium blue LED (A) is driven to sample in square bi-mirrored measurement cuvette (B) through emission optical tunnel (C). Emergent beam is received by the photodiode (D) through reception optical tunnel (E).

separate compartments outside the fluorometer case, but near the measurement cuvette. The electronic components used (supplied by Hansatech Ltd., UK) were similar to those built into the FMS1. Internal connections were modified to drain electrical signals to external elements via a shielded connector at the rear. The photodiode and its preamplifier were sealed in a shielded case, and the blue LED was placed on a home-made shielded support. Each element was linked to a connection case via a shielded cable.

Measurement elements were located on a measurement deck (Fig. 1), which held the shielded connection case and the emitting-receiving elements. Perspex optical guides (10 mm × 10 mm × 100 mm) housed in opaque PVC tubes ensured light beam conduction to the measurement cuvette, which was located in a thermostated home-made aluminium element. Samples were placed in a square bi-mirrored spectrophotometer measurement cuvette (Hellma 101R-QS) with a 10 mm light path. Stirring was performed by a magnetic micro-follower dropped into the cuvette and driven by a stirrer placed under the measurement deck. Actinic and far-red beams were conducted to the upper side of the cuvette through the standard optical fibre cable.

Video quality coaxial wire (Matsushita Electrics) used to link the FMS1 to the measurement deck allowed a large frequency range. All connections and cases were shielded and linked to the fluorometer metallic ground, which was itself connected to the earth. The impedance of the modified system (MS) was strictly the same as that of the original (OS) one. Due to extreme shielding, all external electronic components were well protected from ambient magnetic or electrostatic disturbances, as tested with an Opélec OP-500 (Longjumeau, France) heterodyne field-meter.

2.3. Oxygen evolution

Oxymetric measurements were performed using concentrated algal solutions in order to plot photosynthesis versus irradiance (P/E) curves. Algae were collected by centrifugation at 900 × g for 15 min, using slow rotor acceleration and deceleration. Cell density was estimated using a Neubauer haematocytometer, and an aliquot of concentrated culture was filtered for Chl *a* determination by spectrophotometry after extraction in dimethylformamide (DMF), according to Speziale et al. (1984).

Net oxygen evolution was measured with a CB1D oxymeter (Hansatech Ltd., UK) coupled to a DW2 measuring cuvette (Hansatech Ltd., UK). Measurements were performed at culture growth temperature. After a 3 ml aliquot of homogenised algal suspension was transferred into the measuring chamber, the level of dissolved O_2 was reduced to 50% saturation by N_2 bubbling. Prior to the start of measurements, the medium was CO_2 saturated (2 mM $NaHCO_3$, pH 8.3). White actinic light was provided through the optical fibre cable to the cuvette by an Intralux 5000-1 cold light source (Volpi AG, Switzerland). Irradiance was varied using a crescent-shaped diaphragm that preserved the light spectrum. Gross oxygen production was calculated by correcting the net oxygen evolution rate by the dark respiration rate measured after the light period.

2.4. Fluorescence measurement and electron transport rate determination

Fluorescence was measured at growth chamber temperature on algae maintained in suspension by magnetic stirring. A 2 ml sample of algal culture was pipeted into the cuvette located on the water-thermostated measurement deck. Algae were acclimated to the dark for 30 min, and the following fluorescence parameters were then measured:

(i) $F_v = F_m - F_0$, where F_0 is the minimal fluorescence yield and F_m the maximal one, corresponding to fully open or closed PSII centers, respectively;
(ii) F_v/F_m, the ratio of variable fluorescence to maximal fluorescence, interpreted as an estimation of the maximal quantum efficiency of PSII;
(iii) $\varphi\text{PSII} = (F'_m - F_s)/F'_m = \Delta F/F'_m$, the effective quantum efficiency of PSII for a light-acclimated sample, according to Genty et al. (1989), where F_s is the steady-state fluorescence yield.

The modified FMS1 fluorometer was used to plot curves for the electron transport rate versus irradiance (ETR/E). ETR values at a given actinic irradiance (E) were calculated as: $\text{ETR} = \varphi\text{PSII} \times E \times 0.5 \times \Delta_{\text{ETR}}$, where φPSII is the effective quantum efficiency of PSII in light; E the incident irradiance (in μmol photon m^{-2} s^{-1}); 0.5 a multiplication factor, as electron transport requires the absorption of two photons (one per photosystem); and Δ_{ETR} the absorbance factor of the studied species, estimated according to Beer and Björk (2000). Light absorbed by *D. tertiolecta* cultures was measured behind a glass spectrophotometer cuvette (Hellma 104-OS) filled either with culture medium or with algae in suspension. Light reflection was not taken into account. The fraction of light absorbed by algal cultures of different concentrations was plotted against the respective Chl *a* concentration, and the average Δ_{ETR}, calculated from linear regression, was 0.94 ± 0.01 (mean ± S.E., $n = 3$). After the F_v/F_m ratio of dark-acclimated cells was measured, the sample was exposed to

increasing actinic light levels delivered by the built-in actinic source. Selected irradiances to carry out a complete curve ranged from 0 to 900 μmol photon m^{-2} s^{-1}, as measured with a Walz US-SQS 4π light probe (Walz GmBH, Germany). After acclimation to each irradiance, a 0.7 s saturating pulse was applied to determine F'_m and calculate φPSII.

Parameters for fluorescence and gross oxygen evolution (P_{max}, ETR_{max}, α, E_k and ETR E_k) were determined by fitting P versus E or ETR versus E curves, using the differential equations of Eilers and Peeters (1988) or a hyperbolic tangent function (Jasby and Platt, 1976).

3. Results

3.1. Sensitivity gain due to FMS1 modifications

Two sets of experiments were performed to estimate sensitivity gain, first using the original and then the modified system. As shown in Table 1, sensitivity was about four times higher with MS than OS, with no significant alteration of the F_v/F_m ratio (indicating that the modifications did not alter FMS1 response). The increase in sensitivity with MS was sufficient to allow fluorescence measurement for unconcentrated cultures of microalgae.

To determine the efficiency of the modified instrument, other tests were performed using more diluted cultures. Dilution was repeated until F_s reached approximately 50 bits after 30 min of acclimation to the dark (the maximum signal allowed by the DA converter is 4096 bits). The 50 bit value corresponded to Chl *a* concentrations ranging from 0.045 to 0.027 mg l^{-1}, depending on the algal species. For each dilution, after 30 min of acclimation to the dark, F_v and F_m were measured and F_v/F_m was calculated. The F_v/F_m ratio and fluorometer response were constant throughout the Chl *a* concentration range tested. Moreover, 0.027 mg Chl *a* l^{-1} was not the lowest limit of sensitivity. Experiments run with the green alga *D. tertiolecta* showed that measurement was reliable down to 0.015 mg Chl *a* l^{-1}, despite a very low fluorescence signal (25 bits, data not shown). As the background signal was never greater than 10 bits, the 25 bit value was considered as a sensitivity limit for steady-state in all measurements.

Table 1
Measurement of F_v/F_m ratio with original or modified system for FMS1 fluorometer, and estimation of the sensitivity gain between the two systems (average of gain for each parameter) for *D. tertiolecta*, *P. cruentum* and *S. costatum*

Species	System	F_0	F_m	F_v	F_v/F_m	Sensitivity gain
D. tertiolecta	Original	135 ± 1	441 ± 1	306 ± 12	0.694 ± 0.001	4.22
	Modified	640 ± 2	1877 ± 1	1312 ± 1	0.699 ± 0.003	
P. cruentum	Original	160 ± 1	245 ± 1	85 ± 1	0.347 ± 0.002	3.99
	Modified	509 ± 2	976 ± 2	336 ± 2	0.344 ± 0.005	
S. costatum	Original	126 ± 1	290 ± 2	164 ± 1	0.566 ± 0.002	4.07
	Modified	565 ± 2	1187 ± 2	678 ± 2	0.571 ± 0.004	

F_0, F_v, F_m (arbitrary units) and F_v/F_m are given as mean ± S.E. ($n = 3$).

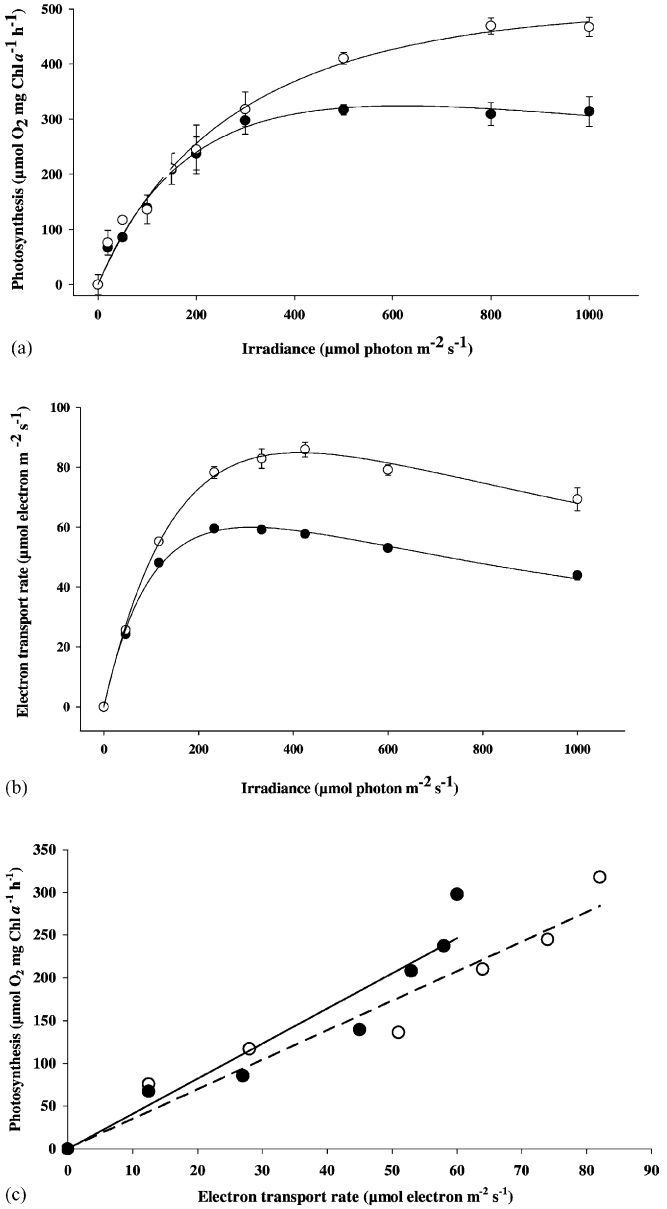

Fig. 2. Light response versus irradiance (a); electron transport rate versus irradiance (b) and photosynthesis versus electron transport rate in the non-photoinhibition zone (c) curves of *D. tertiolecta* cultivated under 75 (●) or 350 µmol photon m^{-2} s^{-1} (○); mean ± S.E. ($n = 3$). Photosynthesis versus electron transport rate curves were fitted using linear regression and are represented in full line (low irradiances, $R^2 = 0.91$) and dotted line (high irradiances, $R^2 = 0.93$).

Table 2
Photosynthetic parameters of *D. tertiolecta* cultivated under a growth irradiance of 75 or 350 μmol photon m^{-2} s^{-1}, fitted with Jasby and Platt (1976) or with Eilers and Peeters (1988) model

Model	Growth irradiance	P_{max}	α-slope	E_k
Jasby and Platt	75	317 ± 8	1.48 ± 0.13	207 ± 18
	350	475 ± 15	1.16 ± 0.1	393 ± 39
Eilers and Peeters	75	319 ± 7	2.12 ± 0.06	151 ± 21
	350	489 ± 12	2.02 ± 0.08	242 ± 22

P_{max} (μmol O_2 mg Chl a^{-1} h^{-1}), α-slope [(μmol O_2 mg Chl a^{-1} h^{-1})(μmol photon m^{-2} s^{-1})$^{-1}$] and E_k (μmol photon m^{-2} s^{-1}) values are given as mean ± S.E. ($n = 3$).

3.2. Comparison of fluorimetric and oxymetric methods for the green alga D. tertiolecta

Oxymetric and fluorimetric methods were compared relative to the modified FMS1. Gilbert et al. (2000) have shown that this type of comparison implies the use of the same curve-fitting model to estimate photosynthesis-related parameters, as differences between models can reach 25%. As the Jasby and Platt model gave the best fittings of *P*/*E* curves in previous experiments (Tremblin and Robert, 1996), it was used to fit the ETR/*E* curves first. However, as the ETR/*E* curves showed clear photoinhibition trends that this model does not take into account, the Eilers and Peeters (1988) model was used to fit the same data sets. Comparison between the two models was performed for *P*/*E* (Table 2) and ETR/*E* (Table 3) curves. P_{max} and ETR_{max} values were found to be very similar for both irradiances, whereas α-slope and E_k values significantly differed from one model to another. Differences between photosynthesis or fluorescence parameters estimated with either model were very similar to those observed by Gilbert et al. (2000). Finally, the Eilers and Peeters model was selected, because it takes photoinhibition into account and allowed the best overall fittings for both methods.

The results indicate that photosynthesis and fluorescence parameters followed the same pattern in the green alga *D. tertiolecta*. As shown in Table 2, acclimation to high irradiances resulted in an increase of both P_{max} and E_k values (53 and 60%, respectively), together with a slight decrease (5%) of α-slope value. This trend is clearly apparent in Fig. 2a. The same phenomenon was observed with fluorescence measurement (Fig. 2b), as the maximal

Table 3
Fluorescence parameters of *D. tertiolecta* cultivated under a growth irradiance of 75 or 350 μmol photon m^{-2} s^{-1}, fitted with Jasby and Platt (1976) or with Eilers and Peeters (1988) model

Model	Growth irradiance	ETR_{max}	α-slope	ETR E_k
Jasby and Platt	75	55 ± 4	0.63 ± 0.05	87 ± 24
	350	80 ± 3	0.62 ± 0.02	129 ± 24
Eilers and Peeters	75	60 ± 5	0.72 ± 0.08	83 ± 12
	350	85 ± 6	0.69 ± 0.09	124 ± 19

ETR_{max} (μmol electron m^{-2} s^{-1}), α-slope [(μmol electron m^{-2} s^{-1})(μmol photon m^{-2} s^{-1})$^{-1}$] and ETR E_k (μmol photon m^{-2} s^{-1}) values are given as mean ± S.E. ($n = 3$).

electron transport rate (ETR_{max}) increased in the same proportion as P_{max} (42 and 53%, respectively), while the fluorescence saturation point (ETR E_k) also increased, but in a lower proportion than for oxymetric-calculated E_k (49 and 60%, respectively) when algae were cultured at high irradiance. Similarly, a slight decrease (5%) of ETR/E curve α-slope values was observed for algae cultured under high irradiance. Moreover, the decay of α-slope value was similar for both measurement methods (Tables 2 and 3).

In both cases, ETR saturation occurred before photosynthesis saturation. Fig. 2b shows that ETR saturation and inhibition occurred for higher irradiances ($E > 500$ μmol photon $m^{-2}\,s^{-1}$). Photoinhibition took place at a higher irradiance (between 400 and 500 μmol photon $m^{-2}\,s^{-1}$) in high-irradiance acclimated algae than in low-irradiance acclimated algae (between 200 and 300 μmol photon $m^{-2}\,s^{-1}$). However, Fig. 2a shows that no photosynthesis inhibition occurred up to 1000 μmol photon $m^{-2}\,s^{-1}$ for high-irradiance acclimated algae. A slight inhibition profile is visible above 700 μmol photon $m^{-2}\,s^{-1}$ for low-irradiance acclimated algae, but inhibition decay remained above the standard error value and might not have been significant.

To compare the two parameter sets more accurately, a photosynthesis versus fluorescence (P/ETR) curve (Fig. 2c) was plotted for each light culture condition according to the method of Beer and Björk (2000). Curves were fitted using linear regression method. The results were very similar for the two irradiance levels. Photosynthesis and ETR developed in the same way at lower irradiances (under 400 μmol photon $m^{-2}\,s^{-1}$). In this zone the fitting gave very good correlation factors ($R^2 = 0.91$ for low-irradiance curve and $R^2 = 0.93$ for high irradiance one).

4. Discussion

Gilbert et al. (2000) noted that cuvette geometry exerts a strong influence on fluorescence measurement. As our modifications involved no changes in electronic components, increased sensitivity resulted only from geometry-related variations. The use of a bi-mirrored cuvette allowed better distribution and enhancement of the excitation signal. Similarly, the reception signal was reflected to the photodiode by the opposite mirrored side of the cuvette: this is equivalent to a system in which two exciting diodes and two reception photodiodes are used. The theoretical gain is thus assumed to be a four-factor, which was the case in our measurements. The improved sensitivity is sufficient for applications to unconcentrated algal cultures containing as little as 0.027 mg Chl $a\,l^{-1}$, without resolution loss. This modification upgraded the efficiency of FMS1, making it suitable for monitoring the photosynthetic capacities of microalgae directly in unconcentrated cultures and for studies of how cells acclimate their photochemistry to saturating light conditions.

Results obtained with *D. tertiolecta* in our oxymetric measurements are in good agreement with those found by other workers (Sukenik et al., 1990; Geel et al., 1997). As regulation occurs directly at the stage of light capture by an effective light-harvesting complex, a good correlation between ETR and O_2 production should be found. In our measurements, the increase of P_{max} was found to be similar to that of ETR_{max} (53 and 42%, respectively), while the decrease in α-slope value (5%) was the same for both methods. This result is in agreement with the findings of Gilbert et al. (2000), who reported good correlation between

fluorescence-based calculated oxygen production and effective oxygen measurement for the green alga *Chlorella* grown at low irradiance (30 μmol photon m^{-2} s^{-1}), especially in the initial part of the curve.

Sukenik et al. (1990) and MacIntyre et al. (2002) did not observe inhibition of photosynthesis in *D. tertiolecta* at irradiances up to ca. 1400 μmol photon m^{-2} s^{-1}. Similarly, Geel et al. (1997), who measured fluorescence and oxygen evolution in a DW2 Hansatech chamber, failed to detect saturation until 1000 μmol photon m^{-2} s^{-1}, and as a consequence, correlation between both methods was linear up to this irradiance. In contrast to these last results, the correlation between ETR and oxygen in our study was linear only under 400 μmol photon m^{-2} s^{-1}, as ETR saturation and photoinhibition occurred before photosynthesis saturation. Moreover, Gilbert et al. (2000) demonstrated that fluorescence-calculated oxygen evolution curves tended to show inhibition above 600 μmol photon m^{-2} s^{-1}, and that both methods can deviate significantly from each other. Apart from specific algal history (i.e. acclimation status) depending on growth conditions, such discrepancy may result from the particular geometry of the DW2 cylinder-shaped cuvette, since fluorescence measurements in microalgae are closely related to the cuvette geometry, as evidenced by Mouget and Tremblin (2002).

In conclusion, the modified FMS1 is convenient to perform reliable fluorescence measurements on unconcentrated microalgal cultures, but this upgrade might not be sufficient in itself to help in converting fluorescence data into photosynthesis activity (O_2 evolution). In particular, further work is needed on the calculation of the real fraction of light absorbed by algae, and on the understanding of the non-linear relationship between oxygen evolution and ETR curves at high light intensity.

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