

# Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity

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## INTRODUCTION

- It is necessary to determine maximal fluorescence ( $F_m'$ ) in order to estimate the effective quantum efficiency of Photosystem II ( $\Phi_{PSII}$ ) and the rate of electron transport (ETR) from chlorophyll fluorescence measurements (Genty *et al.*, 1989).
- $F_m'$  is commonly measured using a single saturating, multi-turnover flash of 400-1200 ms duration to reduce the  $Q_A$ -PQ pool. In many conditions, full reduction of the  $Q_A$ -PQ pool cannot be achieved using such a protocol, especially in field plants.
- Here we present a 'multiphase' single flash method (MSF) to estimate the true maximal fluorescence level in these conditions.

## MATERIALS & METHODS

- With light emitting diode (LED) technology, "saturating" light can be supplied in several different ways, which are described in Figure 1.
- LI-6400 Portable Photosynthesis Systems with 6400-40 Leaf Chamber Fluorometers (LI-COR, Lincoln, NE, USA) were used to compare the three methods. Chamber conditions were maintained at ambient levels of  $CO_2$ , relative humidity, and temperature.
- $\Phi_{PSII}$  was calculated as:  $1 - F_s/F_m'$ , where  $F_s$  is steady state fluorescence and  $F_m'$  corresponds to maximal fluorescence. ETR was calculated as:  $\Phi_{PSII} \cdot P \cdot Q \cdot \alpha_{Lef}$ . The fraction of absorbed quanta used by PSII,  $f$ , was assumed to be 0.5; incident photon flux density,  $Q$ , was measured with an internal PAR sensor; and maize leaf absorbance,  $\alpha$ , was measured as 0.85.

## THE MULTIPLE FLASH TRAIN SOLUTION

- Markgraf & Berry, 1990 and Earl & Ennahli, 2004, proposed an approach using a multiple flash train (FT) of variable light intensities ( $Q$ ) to estimate the true  $F_m'$  (Figure 1).
- Table 1 shows the rectangular single flash (RSF) method under-estimates  $F_m'$  compared to the FT method at all light intensities in field-grown maize leaves.
- However, the FT method requires multiple fluorescence measurements at different light pulse intensities, with wait times between each measurement, to obtain one valid estimate of  $F_m'$ ,  $\Phi_{PSII}$  and ETR, which takes about 10 minutes. This is time-consuming and laborious if many measurements are to be made, and is especially difficult under rapidly changing environmental and physiological conditions.

## THE NEW "MULTIPHASE" SINGLE FLASH SOLUTION

- The MSF method uses a ramp of rapidly varying light intensity to generate  $F_m'$  values over a range of light intensities (B, Figure 1).
- Total duration of one MSF measurement is only about one second.

### Validation with DBMIB

- As a first step to evaluate the MSF method, we vacuum infiltrated leaves of *P. sativum* with 150  $\mu M$  DBMIB to restrict turnover of the PQ pool and provide conditions in which nearly full saturation could be assured.

- The fluorescence response during the ramp phase (Panel II Inset, Figure 2) has a hyperbolic shape. The y-intercept of a plot of the fluorescence response as a function of  $1/Q$  (Panel II, Figure 2) is used to estimate the maximal fluorescence at infinite  $Q$ .
- $F_m'$  measured by the RSF method was within 1% of the extrapolated  $F_m'$  value measured by the MSF method (Panel II, Figure 2). Thus it should be possible to use the MSF method to derive  $F_m'$  in conditions where saturation by the traditional RSF method cannot be achieved.

### Validation in field-grown *Z. mays*

- Figure 3 illustrates the new MSF method in field-grown maize. In this case, the traditional RSF method gave a 17% lower estimate of  $F_m'$  than the extrapolated value predicted by the MSF method.
- Table 2 and Figure 4 show that the FT and MSF methods gave similar estimates of  $F_m'$ ,  $\Phi_{PSII}$  and ETR at all light levels.
- Figure 5 shows that ETR calculated by both the new MSF and FT methods are proportional to gross  $CO_2$  assimilation at all irradiances, as expected for  $C_4$  plant metabolism; however, ETR estimated using the traditional RSF method increasingly underestimates results from gas exchange as irradiance increases.
- In maize, under-estimation of  $F_m'$  by the traditional RSF method can lead to miscalculation of  $\Phi_{PSII}$  and ETR by as much as -41% at high light intensities. The new multiphase single flash (MSF) method resolves this problem.

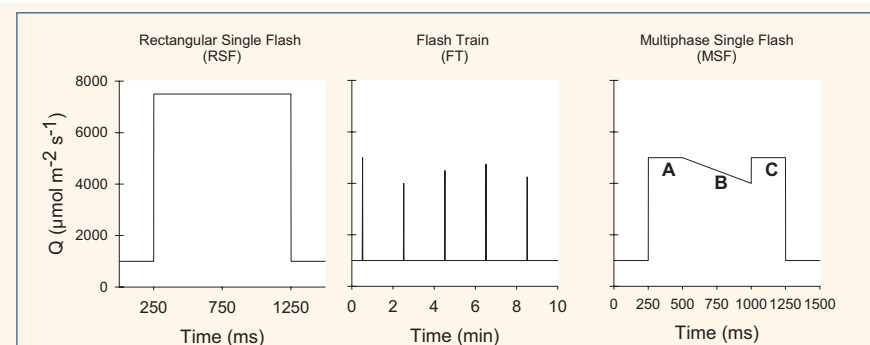


Figure 1. Methods to determine maximal fluorescence ( $F_m'$ ).

**Rectangular Single Flash method (RSF):** a saturating multi-turnover flash ( $Q$ ) of 400-1200 ms duration. **Flash Train method (FT):** applies five rectangular flashes of various  $Q$  in random order, separated by two minutes. **Multiphase Single Flash method (MSF):** (A) high, nearly saturating  $Q$  for 250 ms to reduce  $Q_A$ -PQ pool; (B) ramp of declining  $Q$  for 500 ms; (C) return to the initial high  $Q$  for 250 ms to check for flash-induced non-photochemical quenching (qN).  $F_m'$  values from phase B of the MSF method and each flash of the FT method are regressed against  $10^4/Q$  and extrapolated to estimate the maximal fluorescence at infinite flash intensity.

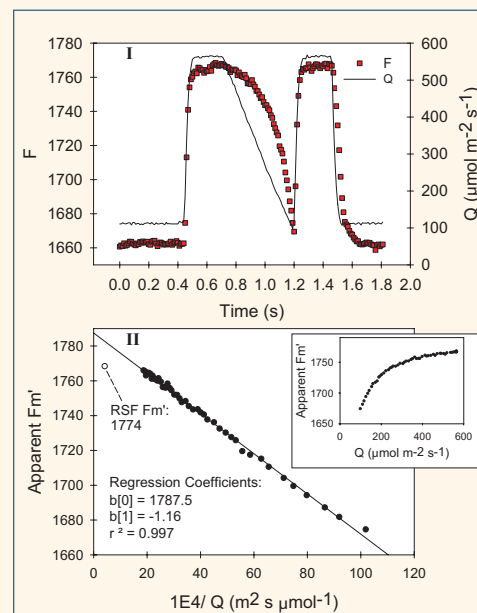


Figure 2. Panel I. Fluorescence response to the MSF protocol in a typical leaf of greenhouse-grown *P. sativum* vacuum infiltrated with DBMIB.

Panel II. Linear regression of  $F_m'$  versus  $10^4/Q$  obtained during phase B, Panel I.

Inset: Fluorescence response to  $Q$  during the ramp.

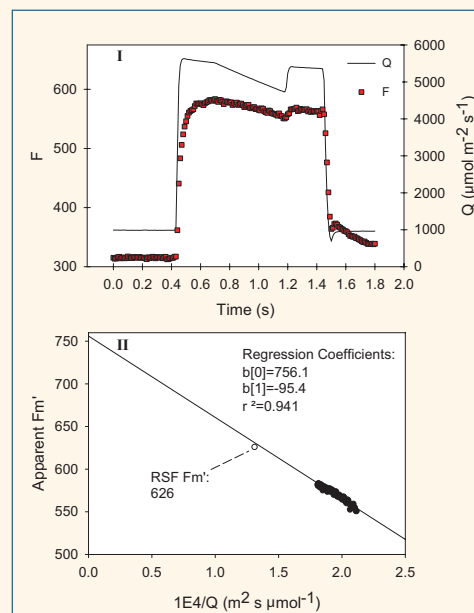


Figure 3. Panel I. Typical fluorescence response to the MSF protocol in a field-grown maize leaf at an incident photon flux density of  $1,000 \mu mol m^{-2} s^{-1}$ . Panel II. Linear regression of  $F_m'$  versus  $10^4/Q$  using the ramp data.

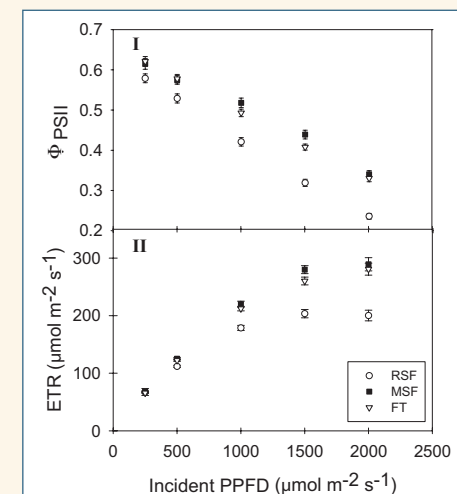


Figure 4. Panel I.  $\Phi_{PSII}$  measured in field-grown maize at growth stage V-5 as a function of light intensity. Panel II. PSII electron transport rate (ETR) computed from the data in Panel I versus light intensity. Each data point is the mean of 9 to 14 observations. Error bars indicate standard errors of the means.

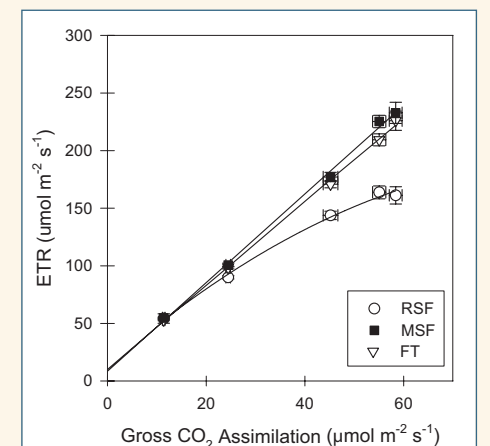


Figure 5. Comparison of ETR calculated using the RSF, FT and MSF methods to gross  $CO_2$  assimilation rate measured from gas exchange in field-grown maize at growth stage V-5.

Table 1. Comparison of  $F_m'$ ,  $\Phi_{PSII}$ , and ETR in field-grown maize leaves measured by RSF and FT methods. Data are computed as  $[(RSF - FT)/FT] \times 100\%$  for each of the variables on each of  $n$  leaves.

Incident PPFD ( $\mu mol m^{-2} s^{-1}$ )	n	$F_m'$	$\Phi_{PSII}$ , ETR
		---% difference---	
250	9	-19**	-8**
500	11	-16**	-10**
1000	14	-20**	-19**
1500	13	-22**	-28**
2000	14	-16**	-41**

\*\* Significant at  $\alpha 0.01$

Table 2. Comparison of  $F_m'$ ,  $\Phi_{PSII}$ , and ETR in field-grown maize leaves measured by FT and MSF methods. Data are computed as  $[(MSF - FT)/FT] \times 100\%$  for each of the variables on each of  $n$  leaves.

Incident PPFD ( $\mu mol m^{-2} s^{-1}$ )	n	$F_m'$	$\Phi_{PSII}$ , ETR
		---% difference---	
250	9	2 <sup>ns</sup>	3 <sup>ns</sup>
500	11	6 <sup>ns</sup>	2 <sup>ns</sup>
1000	14	4 <sup>ns</sup>	4 <sup>ns</sup>
1500	13	4*	8*
2000	14	6 <sup>ns</sup>	4 <sup>ns</sup>

<sup>ns</sup> Significant at  $\alpha 0.05$

\*Significant at  $\alpha 0.05$

## REFERENCES

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## CONCLUSIONS

- The new multiphase single flash method can be used to derive true estimates of  $F_m'$ ,  $\Phi_{PSII}$  and ETR in a flash time duration of approximately 1s.
- The traditional rectangular saturating flash method leads to large under-estimates of  $F_m'$ ,  $\Phi_{PSII}$  and ETR, especially at higher light intensities.
- For the first time, this new approach allows both rapid and valid determination of the rate of photosynthetic electron transport in conditions where  $F_m'$  is difficult to saturate, or when leaf physiological and environmental status is changing rapidly (e.g. field conditions).

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