

# **Chlorophyll fluorescence, an indicator of plant physiology disorder**

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## **Abstract**

Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists and ecophysiologicalists. This review aims to provide an introduction for the novice into the methodology and applications of chlorophyll fluorescence. After a brief introduction into the theoretical background of the technique, the methodology and some of the technical pitfalls that can be encountered are explained. A selection of examples is then used to illustrate the types of information that fluorescence can provide. In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data.

**Keywords:** chlorophyll fluorescence, fluorometer, photochemical quantum yield

## **1 Introduction**

A number of excellent reviews exist that discuss the theoretical background of both chlorophyll fluorescence measurement and analysis, however, these are typically written from a biophysicist's or a molecular plant physiologist's point of view (Horton and Bowyer, 1990; Krause and Weis, 1991; Govindjee, 1995). The aim of this review is to provide a simple, practical guide to chlorophyll fluorescence for those beginners who are interested in applying the technique in both field and laboratory situations. Whilst the principles behind the measurements will be discussed briefly, the emphasis will be on the applications and limitations of this technique in plant ecophysiology. (Kate Maxwell<sup>1</sup> and Giles N. Johnson *Journal of Experimental Botany*)

## **2 Chlorophyll fluorescence and photosynthesis**

In principle, chlorophyll fluorescence can function as an indicator at all of these levels of the photosynthesis process. Chlorophyll is the major antenna pigment, funneling the absorbed light energy into the reaction centers, where photochemical conversion of the excitation energy takes place.

The indicator function of chlorophyll fluorescence arises from the fact that fluorescence emission is complementary to the alternative pathways of de-excitation, which are photochemistry and heat dissipation. Generally speaking, fluorescence yield is highest when the yields of photochemistry and heat dissipation are lowest. Hence, changes in fluorescence

yield reflect changes in photochemical efficiency and heat dissipation. In practice, the variable part of chlorophyll fluorescence originates mainly in photosystem II and excitation transfer to photosystem I may be considered an additional competitive pathway of de-excitation.

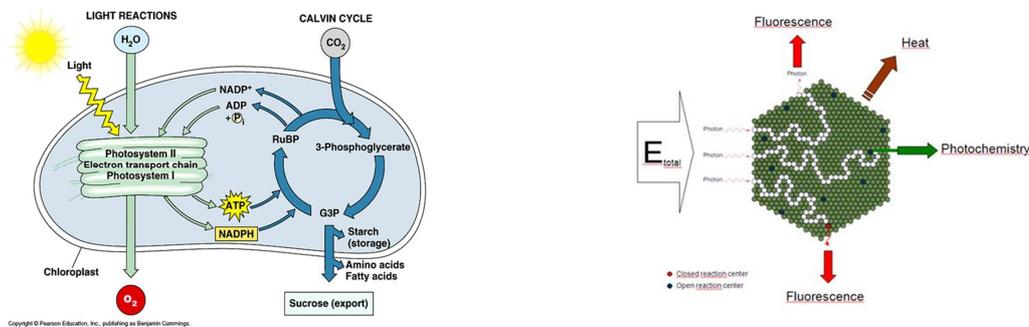


Figure 1: Photosynthesis (source Pearson Education Inc.)

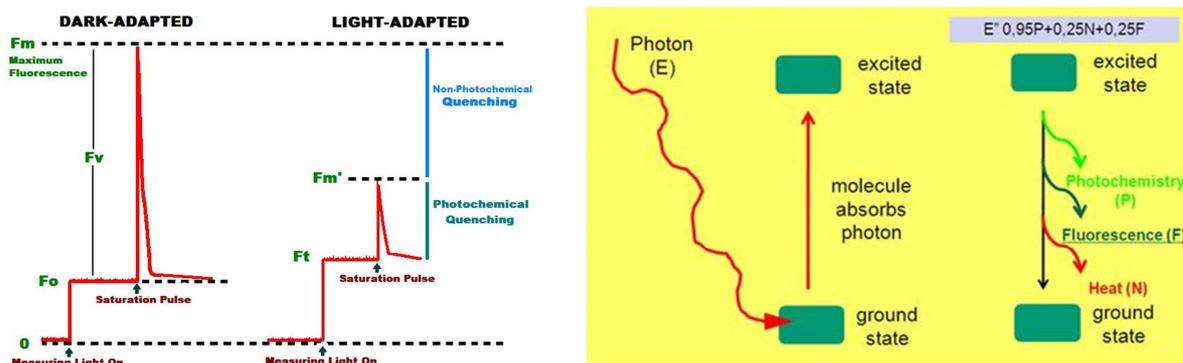


Figure 2: Fluorescence analysis and the three fates of absorbed light energy (source: Horváth)

To use measurements of chlorophyll fluorescence to analyse photosynthesis, researchers must distinguish between photochemical quenching and non-photochemical quenching (heat dissipation). This is achieved by stopping photochemistry, which allows researchers to measure fluorescence in the presence of non-photochemical quenching alone. To reduce photochemical quenching to negligible levels, a high intensity, short flash of light is applied to the leaf. This transiently closes all PSII reaction centres, which prevents energy of PSII being passed to downstream electron carriers. Non-photochemical quenching will not be effected if the flash is short. During the flash, the fluorescence reaches the level reached in the absence of any photochemical quenching, known as maximum fluorescence  $F_m$ .

The efficiency of photochemical quenching (which is a proxy of the efficiency of PSII) can be estimated by comparing  $F_m$  to the steady yield of fluorescence in the light  $F_t$  and the yield of fluorescence in the absence of photosynthetic light  $F_0$ . The efficiency of non-photochemical quenching is altered by various internal and external factors. Alterations in heat dissipation mean changes in  $F_m$ . Heat dissipation cannot be totally stopped, so the yield of chlorophyll fluorescence in the absence of non-photochemical quenching cannot be measured. Therefore,

researchers use a dark-adapted point ( $F_m^0$ ) with which to compare estimations of non-photochemical quenching.

### 3 Common fluorescence parameters

$F_0$ : Minimal fluorescence (arbitrary units). Fluorescence level when all antenna pigment complexes are associated with the photosystem are assumed to be open (dark adapted).

$F_m$ : Maximal fluorescence (arbitrary units). Fluorescence level when a high intensity flash has been applied. All antenna sites are assumed to be closed.

$F_{tr}$ : Terminal fluorescence (arbitrary units). Fluorescence quenching value at the end of the test.

$T_{1/2}$ : Half rise time from  $F_0$  to  $F_m$ .

### Calculated parameters

$F_v$  is variable fluorescence. Calculated as  $F_v = F_m - F_0$ .

$\frac{F_v}{F_m}$  is the ratio of variable fluorescence to maximal fluorescence. Calculated as  $\frac{F_m - F_0}{F_m}$ . This is a measure of the maximum efficiency of PSII (the efficiency if all PSII centres were open).  $\frac{F_v}{F_m}$  can be used to estimate the potential efficiency of PSII by taking dark-adapted measurements.

$\Phi_{PSII}$  measures the efficiency of Photosystem II. Calculated as  $\frac{F_m - F_{tr}}{F_m}$ . This parameter measures the proportion of light absorbed by PSII that is used in photochemistry. As such, it can give a measure of the rate of linear electron transport and so indicates overall photosynthesis.

$qP$  (photochemical quenching). Calculated as  $\frac{F_m - F_{tr}}{F_m - F_0}$ . This parameter approximates the proportion of PSII reaction centres that are open.

Whilst  $\Phi_{PSII}$  gives an estimation of the efficiency,  $qP$  and  $\frac{F_v}{F_m}$  tell us which processes which have altered the efficiency. Closure of reaction centers as a result of a high intensity light will alter the value of  $qP$ . Changes in the efficiency of non-photochemical quenching will alter the ratio  $\frac{F_v}{F_m}$ .

## 4 MINI-PAM fluorometer

The MINI-PAM, like all PAM Fluorometers, applies pulse-modulated measuring light for selective detection of chlorophyll fluorescence yield. Numerous studies with the previously introduced PAM Fluorometers have proven a close correlation between the thus determined YIELD-parameter ( $\Delta F/F_m'$ ) and the effective quantum yield of photosynthesis in leaves, algae and isolated chloroplasts. With the help of the optional Leaf-Clip Holder 2030-B also the photosynthetic active radiation (PAR) can be determined at the site of fluorescence measurement, such that an apparent electron transport rate (ETR) is calculated. In addition to this central information, the MINI-PAM also provides the possibility of measuring fluorescence quenching coefficients (qP, qN, NPQ), applying continuous actinic light for measurement of induction curves (Kautsky-effect) and automatic recordings of light-saturation curves with quenching analysis.



Figure 4: MINI-PAM Photosynthesis yield analyzer and the schematic view of the PAM measuring principle (source: <http://www.odt.com.tw/>)



Figure 6: Dark adapted leaves and the measurement process (photo Baglyas)

## 5 Conclusions

The Photosynthesis Yield Analyzer MINI-PAM has been developed with special attention to the quick and reliable assessment of the effective quantum yield of photochemical energy conversion in photosynthesis. The most relevant information is obtained by a single key operation within a second and up to 4000 data sets can be stored for later analysis. Due to a

novel opto-electronic design and modern microprocessor technology, the MINI-PAM is extremely compact and at the same time highly sensitive and selective. It is ideally suited for rapid screening of photosynthetic activity in the field, green house and laboratory and due to its robust, waterproof housing it can be used even in extreme environments.

The MINI-PAM, like all PAM Fluorometers, applies pulse-modulated measuring light for selective detection of chlorophyll fluorescence yield. The actual measurement of the photosynthetic yield is carried out by application of just one saturating light pulse which briefly suppresses photochemical yield to zero and induces maximal fluorescence yield. The given photochemical yield then immediately is calculated, displayed and stored. Numerous studies with the previously introduced PAM Fluorometers have proven a close correlation between the thus determined YIELD-parameter ( $\Delta F/F_m'$ ) and the effective quantum yield of photosynthesis in leaves, algae and isolated chloroplasts. With the help of the optional Leaf-Clip Holder 2030-B also the photosynthetic active radiation (PAR) can be determined at the site of fluorescence measurement, such that an apparent electron transport rate (ETR) is calculated. In addition to this central information, the MINI-PAM also provides the possibility of measuring fluorescence quenching coefficients (qP, qN, NPQ), applying continuous actinic light for measurement of induction curves (Kautsky-effect) and automatic recordings of light-saturation curves with quenching analysis.

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