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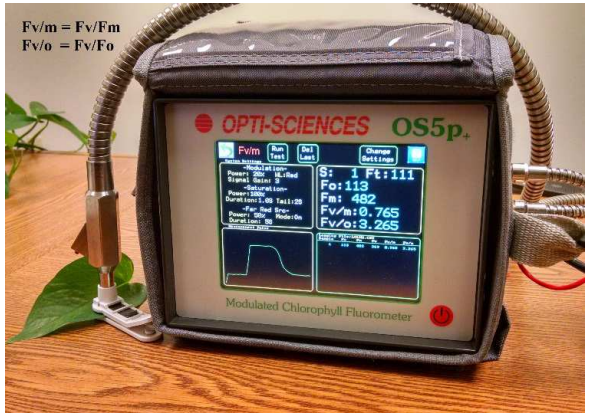
# Using the OS5p+ Chlorophyll Fluorometer effectively

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# Measuring Protocols and their value:

1. Fv/Fm – dark adaptation protocol – plant stress
2. Y(II) & ETR – light adaptation protocol – plant stress
3. Quenching measurement – photoprotection …&
4. Rapid Light Curves- for variable light conditions
5. Strasser OJIP- another plant stress protocol
6. Vredenburg OJIP – for the study of Photosystem II

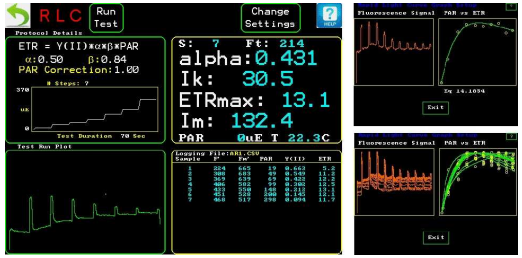
Application notes available for all measuring protocols at www.optisci.com or www.adc.co.uk

“Cookbook checklists” are available for several protocols.

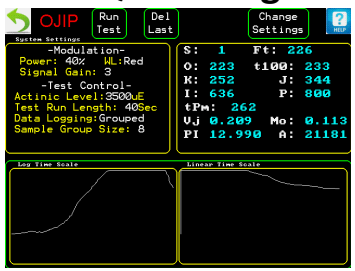

$F_V/F_M$


Y(II) & ETR

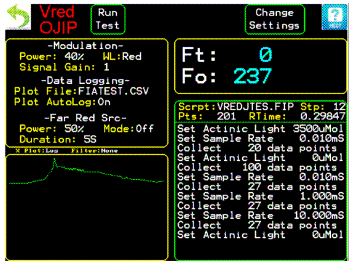

Quenching




Rapid Light Curves




Strasser OJIP




Vredenburg OJIP

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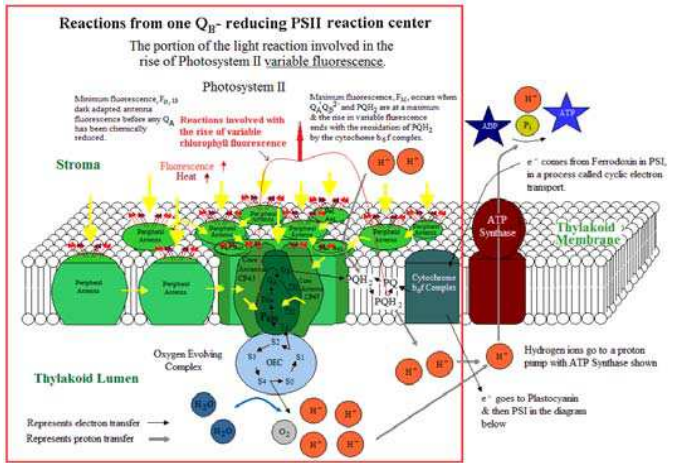
Photosynthesis is comprised of a **light reaction**, and a **dark reaction.** **Chlorophyll fluorescence measures the light reaction and gas exchange measures the dark reaction. Variable chlorophyll fluorescence only occurs in photosystem II in chlorophyll "a".**




1. In **$C_4$ plants** the relationship between chlorophyll fluorescence and gas-exchange measurements is ***linear*** *(Baker 2004)* ***for both Fv/Fm & Y(II).***

2. In **$C_3$ plants**, the relationship is <u>generally</u> ***curve-linear.*** Because in $C_3$ plants, Rubisco will combine with either carbon dioxide, or various forms of oxygen under oxidative stress conditions, <u>sometimes</u>, delaying the response of chlorophyll fluorescence for plant stress detection (Baker 2004*)* ***for both Fv/Fm & Y(II).***

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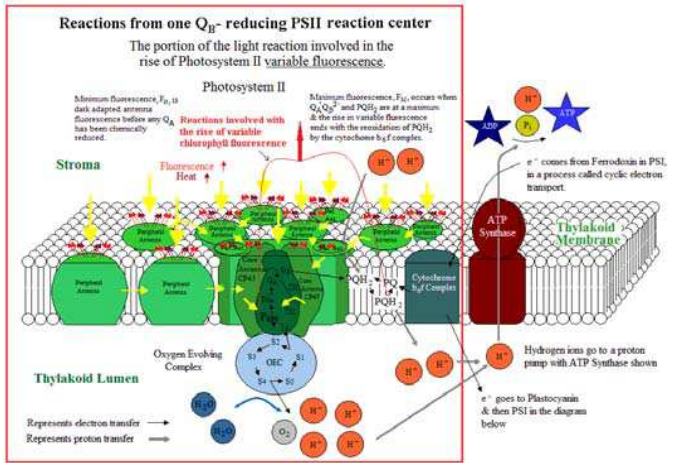
Photosynthesis is comprised of a **light reaction**, and a **dark reaction.** **Chlorophyll fluorescence measures the light reaction and gas exchange measures the dark reaction. Variable chlorophyll fluorescence only occurs in photosystem II in chlorophyll "a".**




**3. CAM plants**

a. **Obligate CAM plants** also have a $CO_2$ concentrating mechanism and so Chlorophyll Fluorescence will also provide a linear correlation to gas exchange measurements, **like $C_4$ plants**.

b. **Some types of CAM plants** revert to either **$C_3$** or **$C_4$** plant operation **during the rainy season**. As a result, plant stress sensitivity for different types of plant stress will be **like either $C_3$ or $C_4$ type plants during this period**.

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Value for measuring plant stress:

Use Chlorophyll Content Meter

## *Fast tests - $C_3$ plants*

| Protocol | Drought | Heat | Light | Nitrogen | Sulfur | Flooding | Chemical | Other Nutrients | Other Chemical |
|---|---|---|---|---|---|---|---|---|---|
| Fv/Fm | **After 1 day Above 26° C** | At 45° C & above in $C_3$ plants | **Very sensitive** | Not sensitive | Not sensitive | **Very sensitive** | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |
| Y(II) & ETR | After 7 days | **At 35°C or above in $C_3$ plants** | **Very sensitive** | Not sensitive | Not sensitive | **Very sensitive** | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |
| Strasser OJIP | After 7 days | At 44° C & above in $C_3$ plants | **Very sensitive** | Not sensitive | Not sensitive | **Very sensitive** | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |

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Value for measuring plant stress:

Use Chlorophyll Content Meter

***Fast tests – $C_4$ plants***

| | Drought | Heat | Light | Nitrogen | Sulfur | Flooding | Chemical | Other Nutrients | Other Chemical |
|---|---|---|---|---|---|---|---|---|---|
| Fv/Fm | Very sensitive | Sensitive | Very sensitive | Not sensitive | Not sensitive | Very sensitive | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |
| Y(II) & ETR | Very sensitive | Very sensitive | Very sensitive | Not sensitive | Not sensitive | Very sensitive | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |
| Strasser OJIP | Very sensitive | Sensitive | Very sensitive | Not sensitive | Not sensitive | Very sensitive | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide | See Desk Top Plant Stress Guide |

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Value for measuring plant stress:

***Fast tests – $C_3$ plants***

| Protocol | Cold | Herbicide | Pesticide | Biotic | Herbivory | Radiation |
|---|---|---|---|---|---|---|
| Fv/Fm | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Very sensitive | Very sensitive |
| Y(II) & ETR | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Very sensitive | Very sensitive |
| Strasser OJIP | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Lack of data | Very sensitive |

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Value for measuring plant stress:

***Fast tests – $C_4$ plants***

| Protocol | Cold | Herbicide | Pesticide | Biotic | Herbivory | Radiation |
|---|---|---|---|---|---|---|
| Fv/Fm | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Very sensitive | Very sensitive |
| Y(II) & ETR | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Very sensitive | Very sensitive |
| Strasser OJIP | Very sensitive | See Plant Stress Guide | See Plant Stress Guide | See Plant Stress Guide | Lack of data | Very sensitive |

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## *Other tests for $C_3$, $C_4$ & CAM plants*

| Protocol | Measuring time | Advantages | Other Advantages |
|---|---|---|---|
| Quenching | Overnight dark adaption or equivalent, and at least 30 minutes of light adaption. *Plant mechanism relaxation time requires another 30 minutes.* | **Allows investigation into plant photoprotective mechanisms** such as the **xanthophyll cycle, delta pH of the thylakoid lumen and chloroplast migration .** | Allows measurement of **photodamage** or **photoinhibition, and recovery**. Puddle model, and Lake model quenching parameters included. … state transitions. |
| Rapid Light curves | Variable times but at least 80 seconds. | ***Designed to work reliably under variable light conditions*** *like: Under canopy leaves, partly cloudy days, & underwater.* | **Provides *light saturation characteristics of leaves*** including $ETR_{MAX}$, light intensity for $ETR_{MAX}$ , minimum saturation, & quantum efficiency. |
| Vredenburg OJIP | Similar to Fv/Fm | Free form investigation of Photosystem II mechanisms. | Designed for basic research into photosystem II. |

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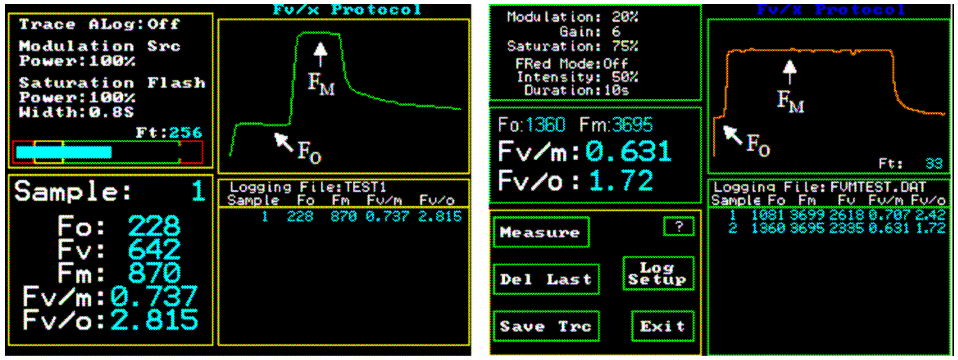
# $F_V/F_M$ measurements are considered the "gold standard" of chlorophyll fluorescence measurements because:

They allow comparison of samples at the same known dark adapted state with a normalized measuring ratio (Fm – Fo)/Fm

Maximum fluorescence - minimum fluorescence, divided by maximum fluorescence.



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$F_V/F_M$ measurements:

However, to get reliable measurements, there are guidelines to follow from previous research.

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# $F_V/F_M$ measurements:

## Dark adaption times – how long is long enough?

To reach the known dark adaption state, plant mechanisms must relax and photosystem II (a series of oxidation reduction reactions) must become fully oxidized.

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FV/FM measurements: Dark adaption times – how long is long enough?

For indoor plants, the photoprotective xanthophyll cycle and Δ pH of the thylakoid lumen can take from seconds to a few minutes to relax whereas for field plants, 4 to 7 minutes are required for relaxation to fully occur (Baker 2008).

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FV/FM measurements: Dark adaption times – how long is long enough?

It takes state transitions between 15 to 20 minutes to relax (Ruban 2009).

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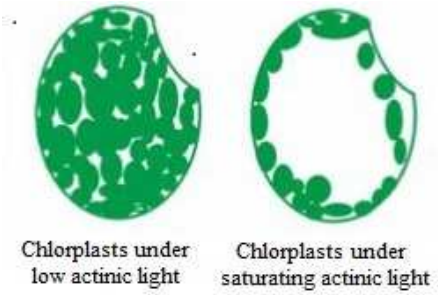
FV/FM measurements: Dark adaption times – how long is long enough?

# It takes chloroplast migration between 20 to 30 minutes to relax (Cazzaniga 2013)



Chlorplasts under low actinic light

Chlorplasts under saturating actinic light

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FV/FM measurements: Dark adaption times – how long is long enough?

Photoinhibition starts to repair after 40 minutes in the dark and can take between 30 to 60 hours to repair. (Lichtenthaler 2004)

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FV/FM measurements: Dark adaption times – how long is long enough?

For these reasons, 30 minutes could be used for dark adaption. However…

There is a point of view, among some researchers, that only the equivalent of overnight dark adaption with predawn measurement is acceptable dark adaptation.

For this reason, we recommend that you check with potential research reviewers for their points of view before experimental design.

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Low intensity modulated light

## $F_V/F_M$ measurements: Modulation light intensity setting Fv/Fm = (Fm-Fo)/Fm.

Minimum fluorescence **Fo**, is a "pre-photosynthetic" dark adapted value measured by exposing the leaf antennae to a very low intensity modulated light. **The intensity must be set properly to allow detection, but not high enough to drive photosynthesis**.

If it is set too high, it will drive photosynthesis and provide an Fo value that is too high and artificially reduce Fv/Fm causing a measuring error. **When setting the modulating light intensity, the Ft value or fluorescence signal should not rise over a 30 second period when a leaf is used.** If it does, the intensity must be lowered.

An automated modulated light set up routine is now available for:
OS5p+, OS1p, OS30p+ and PSK chlorophyll fluorometers.

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# $F_V/F_M$ measurements:

**Maximum Fv/Fm values vary with species.** The average maximum Fv/Fm value is between 0.79 - 0.84 (Maxwell and Johnson 2000).

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# $F_V/F_M$ measurements:

Compare samples with a similar light history.
Field plants should only be compared to field plants and green house plants should be compared to green houseplants.
Due to the fact that it can take up to 60 hours for chronic photoinhibition to relax, photoinhibition can be involved in some measurements more than others (Lichtenthaler 2004).
*Results after a sunny day in the summer may be different that measurements on the same plant after a few days of overcast,* again because it takes a long time for photoinhibition to relax or repair.

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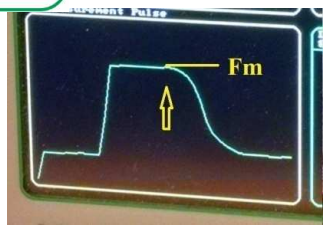
Correct duration

# $F_V/F_M$ measurements:




Duration too long

The duration of the saturation pulse should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria (Schreiber 1997).

Times outside these ranges increase the error in Fv/Fm measurements. Shorter durations prevent complete saturation of PSII regardless of the light intensity. Longer durations create a form of saturation pulse NPQ that rounds the tail end of the pulse maximum value, and reduces the average maximum saturation pulse value.

*OS5p+ provides a rolling or moving 8 point 25 ms average to determine the highest Fm. This ensures that a reliable value will be measured even if the saturation pulse width or duration is too long.*

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# $F_V/F_M$ measurements:

**Saturation pulse intensity** It may take a **few hundred µmols to saturate shade leaves** and **sun leaves will saturate below 1,500 µmol.** Lower values may not fully saturate PSII, and provide an error. Higher values always work with dark adapted samples (Ralph 2005).

Requirements are different for Y(II).

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# $F_V/F_M$ measurements:

Some Fv/Fm fluorometers have the ability to pre-illuminate dark-adapted leaves with far-red light, usually in the range of 735 nm. When this feature is used for five to ten seconds before an Fv/Fm measurement takes place, it activates PSI, and ensures that all electrons have been drained from PSII before the measurement of Fo. It also allows the measurement of Fo'. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, or photoinhibition.
Time is still required in a darkened environment to relax all forms of NPQ and to obtain a reliable Fv/Fm measurement (Maxwell and Johnson 2000).

If comparing results between fluorometers with and without far red light, turn off the far red light before measurement.

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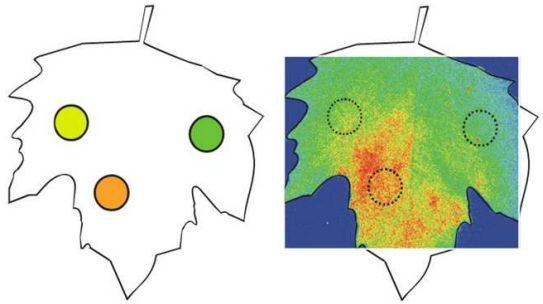

# $F_V/F_M$ measurements:

Fluorescence heterogeneity presents itself as different Fv/Fm measurements on different parts of the leaf. It has been found to occur under cold stress conditions, with biotic stress, and under water stress conditions.

“By using multiple measurements and a sampling plan, heterogeneity can be overcome” (Claus Buschmann- in correspondence by e-mail 2008).

Imaging fluorescence can also be used.

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# $F_V/F_M$ measurements:

Part of the minimum fluorescence, the Fo parameter, in Fv/Fm (Fm – Fo)/Fm), contains **PSI fluorescence** as well as PSII fluorescence.

With Fv/Fm, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error.

In $C_3$ plants, about 30% of Fo fluorescence is due to PSI, and in $C_4$ plants about 50% of Fo fluorescence is due to PSI fluorescence.

PSI produces about 6% of the fluorescence found in Fm in $C_3$ plants, and about 12% of Fm in $C_4$ plants (Pfundle 1999).

This not a problem when comparing Fv/Fm measurements for plant stress because **PSI fluorescence does not change. It remains constant.**

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# $F_V/F_M$ measurements:

**Wind is a variable that should be accounted for in field plants.** In a study from 2000 it was found that wind improved photosynthesis in one species tested , decreased chlorophyll fluorescence in another species and it had no affect on a third species tested(Strasser 2000).

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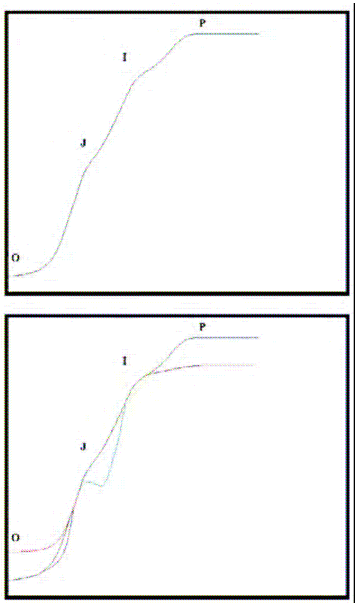
# Strasser OJIP measurements:

OJIP is a high time resolution protocol that analyses the rise from a dark adapted state to a fully light saturated Photosystem II condition.

- High time resolution in microseconds
- For plant stress use **3,500 µmol** of light because results change with different light intensities
- For **nitrogen and sulfur stress use a chlorophyll content meter** not OJIP. While the K step has been shown to be somewhat more sensitive, it will not be at acceptable levels for nutrient management
- OJIP is popular in Europe but it is not as highly regarded in other locations
- The OS5p+ uses a modulated light to measure Fo. *It is not estimated*
- See the Plant Stress Guide for specific plant stress recommendations



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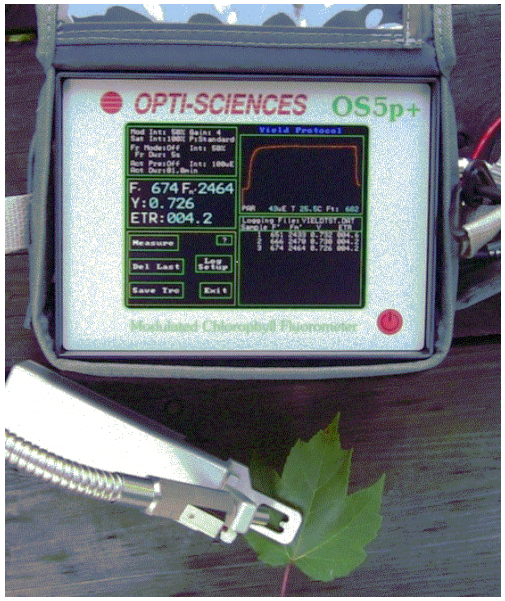
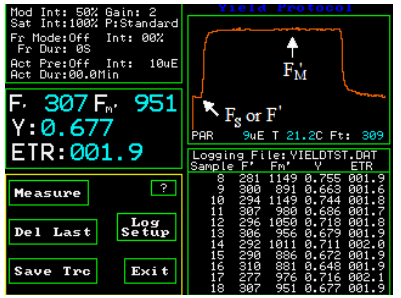



# Y(II) or ΔF'/Fm' measurements

(Fm' – Fs) / Fm'




(maximum fluorescence – fluorescence at steady state conditions) / maximum fluorescence

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# Y(II) or ΔF'/Fm' measurements:

Y(II) is the same normalized ratio as Fv/Fm. However, measurements occur under steady state lighting conditions instead of under dark adaption conditions.

$$(Fm'-Fs)/Fm'$$

Fm' = maximum fluorescence

Fs = fluorescence at steady state under actinic light conditions.

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# Y(II) or ΔF'/Fm' measurements:

**Leaves must be at steady state photosynthesis**. Under lower and medium light levels this takes between 15 and 20 minutes at a given light level.

Above canopy leaves on a clear day, in the field, are considered to be at steady state photosynthesis (Maxwell & Johnson 2000, P 77).

**At high light levels steady state may take between 20 and 30 minutes due to chloroplast migration,** a light avoidance mechanism that changes fluorescence parameters by changing leaf absorptance (Cazzaniga S. 2013).

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# Y(II) or ΔF'/Fm' measurements:

It can be **problematic to make Y(II) measurements on below canopy leaves** in the field. The shade from higher leaves clouds and wind can interrupt a plant's adjustment to steady state under ambient conditions. **Rapid light curves and Fv/Fm may be better solutions for below canopy work, where appropriate**.

The alternative is to use an **internal fluorometer actinic light source**, **under a shroud**, expose the sample to light for up to thirty minutes, to reach steady state, and then make a measurement.

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# Y(II) or ΔF'/Fm' measurements:

The OS5p+ has a *unique actinic light closed-loop feed-back system* that maintains actinic light intensity as the illuminator heats up or as the chlorophyll fluorometer heats up. It works in combination with the PAR Clip PAR sensor to main a *programmable constant and exact actinic light intensity over time.*

Normally, according to the laws of physics, ***light output goes down as a light source heats up.*** This causes a declining actinic light intensity over time and can cause measuring errors.

It is ideal for use with quenching protocols, Rapid Light Curves, and using the internal actinic illuminator for Y(II) and ETR measurements. A dark towel may be used for dark adaption and quenching measurement as shown in the photos.

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# Y(II) or ΔF'/Fm' measurements:

## Y(II) values vary with light level

The higher the light level, the lower the YII) value. When measuring Y(II) in the field, it is extremely important to measure leaf irradiation or light level, at the leaf Comparing Y(II) values taken at different light levels and unless it is the change, at different light levels and, that is of interest. This is commonly done with a PAR Clip (Genty 1989), (Genty 1990).

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# Y(II) or ΔF'/Fm' measurements:

**Shade leaves vs. Sun leaves.** – The Y(II) ratio will be higher on sun leaves than on shade leaves (Lichtenthaler 2004).

**Field plants should only be compared to field plants and green house plants should be compared to green houseplants due to light history** (Lichtenthaler 2004).

**Leaf orientation. When making a yield measurement, with or without a PAR Clip, it is important not to change the orientation of the leaf.** The leaf is at steady state photosynthesis in its current orientation. Changing the orientation changes the amount of light falling on the leaf, and the leaf will no longer be at steady state photosynthesis.

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# Y(II) or ΔF'/Fm' measurements:

It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997).

The duration of the saturation pulse should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria.

Shorter durations prevent complete saturation of PSII regardless of the light intensity (Roseqvist & van Kooten 2006).

Longer durations create a form of saturation pulse NPQ that rounds the tail end of the pulse maximum value, and reduces the average maximum saturation pulse value (Roseqvist & van Kooten 2006).

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# Y(II) or ΔF'/Fm' measurements:

Saturation pulse intensity. Saturation pulse intensity is more of an issue with Y(II) than with Fv/Fm. However, a problem has been found when measuring Y(II) at near saturating actinic light levels. It has been discovered that at high actinic or sun light levels, leaves resist the complete closure of all PSII reaction centers that is expected when using a saturation pulse.

Even with a 7,000 µmol saturation pulse, some reaction centers remain open. Up to a 41% error was found in Y(II) measurements using standard techniques at high actinic light levels. To correct for this issue, multiple saturation flashes are used, to estimate an infinite saturation flash (Earl 2004), (Loriaux et al. 2013), (Markgraf & Berry 1990).

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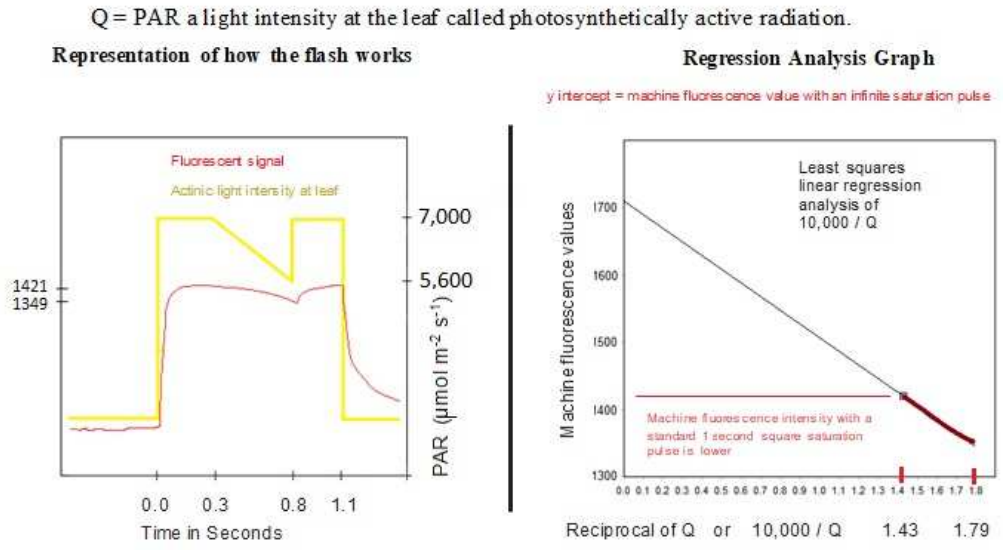
# Y(II) or ΔF'/Fm' measurements:

# Fm' correction from Loriaux 2013




The Loriaux 2013 Fm' correction protocol is available on all Opti-Sciences chlorophyll fluorometers that measure Y(II)

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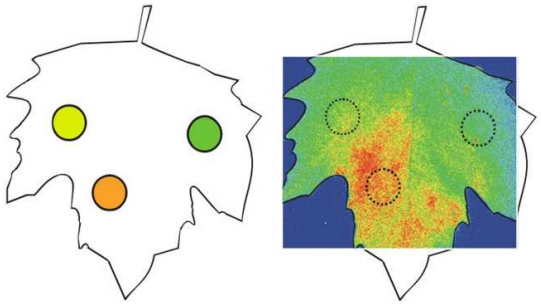

## Y(II) or ΔF'/Fm' measurements:

**Chlorophyll fluorescence Heterogeneity** – Chlorophyll fluorescence can vary from one part of a leaf to another and become patchy under certain circumstance. Under drought stress, cold stress, or biotic stress or low $CO_2$ levels it is best to take multiple leaf measurements and average the values (Baker 2008) *(Claus Buschmann- in correspondence by e-mail 2008).*

**Mangrove leaves growing in the tropics. Here again electron transport rate is more that three times that of $CO_2$ assimilation**. It is believed that this is mostly due to reactive oxygen species as an electron sink (Baker Oxborough 2004), (Cheeseman 1997).

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# Y(II) or ΔF'/Fm' measurement

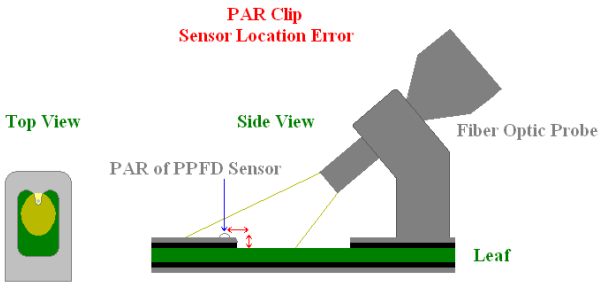
**Light history: It takes between 40 minutes and 60 HOURS for chronic photoinhibition to relax or repair in a leaf**. Since photoinhibition reduces chlorophyll fluorescence measuring parameters, it is important to compare samples that have a similar recent light history. **There will be some residual photoinhibition after a bright summer day and there may be no residual photoinhibition after a few over cast days** (Lichtenthaler 2004).

**Wind is a variable that should be accounted for in field plants.** In a study from 2000 it was found that wind improved photosynthesis in one species tested , decreased chlorophyll fluorescence in another species and it had no affect on a third species tested (Clark & Strasser 2000).

# Y(II) or ΔF'/Fm' measurements:

**Light intensity varies inversely with the square of the distance.** For this reason, **PAR sensor location**, relative to the leaf location, becomes important when using artificial actinic light to drive photosynthesis. Since the sun is about 93,000,000 miles from earth, PAR sensor location is not an issue when measuring Y(II) and ETR for solar irradiated photosynthesis. However, since artificial light sources are much closer, a few millimeters can make a significant difference in PAR measurement (Rascher 2000)

PAR sensor error of up to 10%
OSI instruments provide correction factor adjustment



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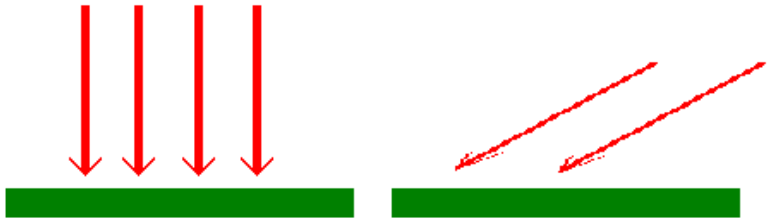
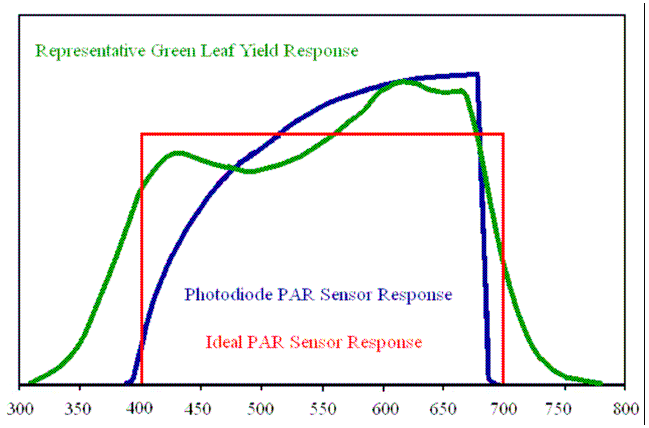
# Y(II) or ΔF'/Fm' measurements:

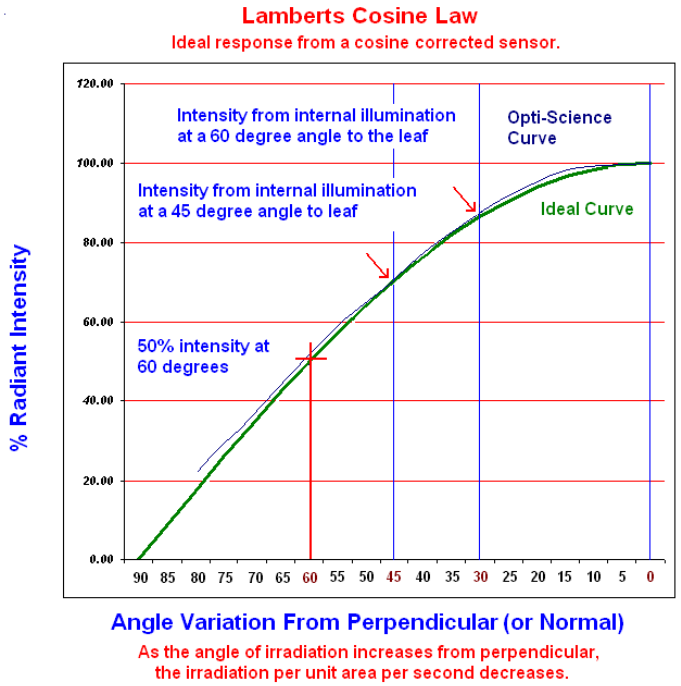
Light sensor issues and corrections. For ETR ETR = Y(II) x .84 x 0.5 x PAR

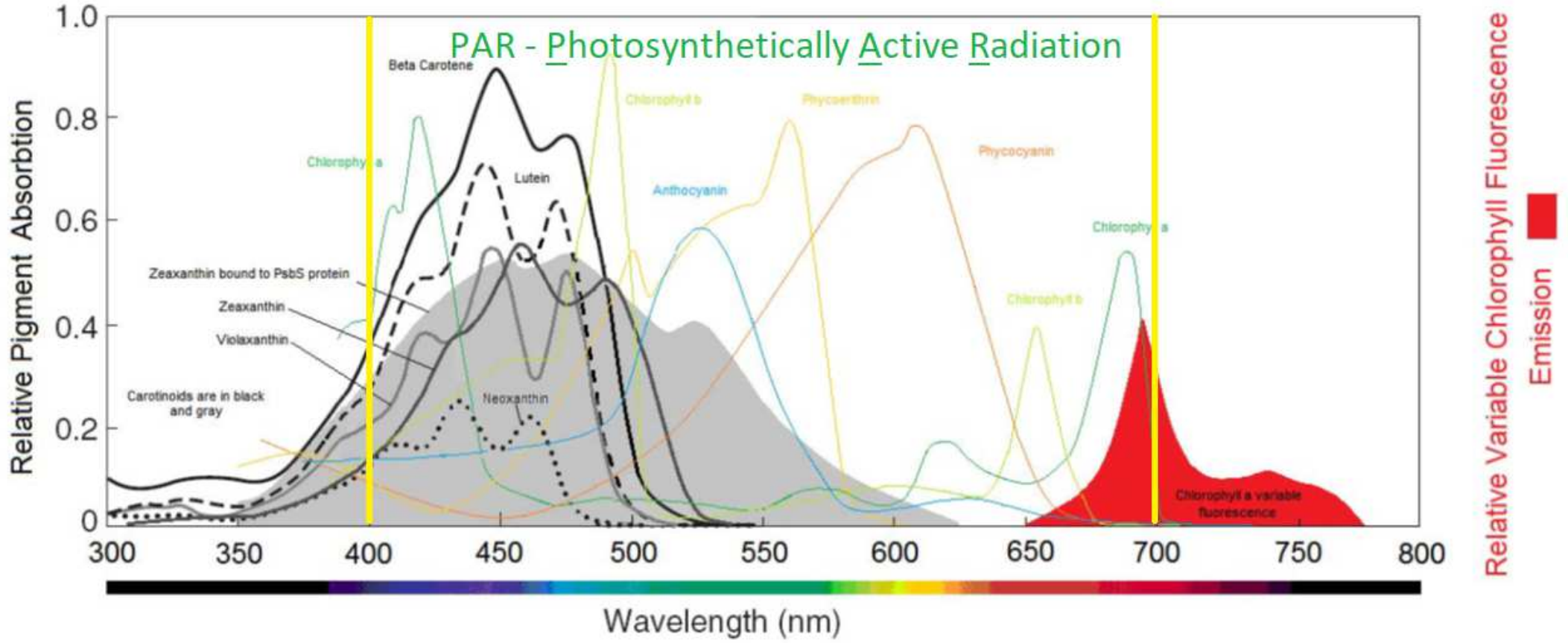


**Less light strikes the leaf surface at steeper angles**




PAR sensor issues






Relative absorbance of plant pigments and variable chlorophyll a fluorescence.

Relative absorbtion of Zeaxanthin, and Zeazanthin bound to PSbS protien are adapted from Aspinall-O'dea M.(2002) Chlorophyll a, & b absorbtion spectra, chlorophyll a emission spectra, anthocyanin absorbtion spectra are adapted from Papageorgiou & Govinjee (2004). Relative absorption of Lutein, Beta Carotene, Neoxanthin, and Violaxanthin adapted from Lichtenthaler 2001

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# Y(II) or ΔF'/Fm' measurements:

Areas where Y(II) performs better than Fv/Fm:

Heat stress in $C_3$ plants:

Gas exchange detects heat stress at 30°C (Baker 2008)

Y(II) detects heat stress at 35°C and (Baker 2008)

Fv/Fm detects heat stress at 45°C (Baker 2008)

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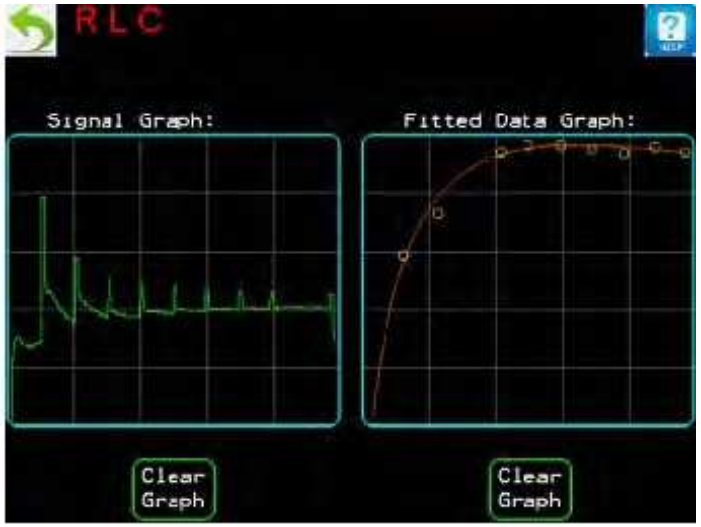
# Rapid Light Curves – A method to measure photosystem II under changing light conditions


Under canopy leaves, partly cloudy conditions, windy conditions, underwater.

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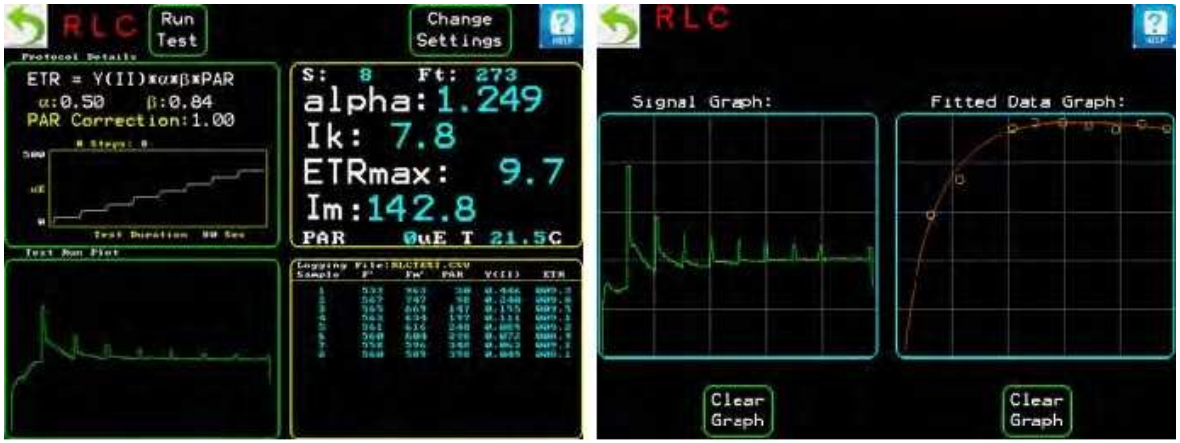
# Rapid Light Curves – A method to measure photosystem II under changing light conditions




Under canopy leaves, partly cloudy conditions, windy conditions, underwater.

**Alpha** or α– initial graph slope a measure of light capture efficiency.

**Ik** – ETRmax /α - measurement of the point where light saturation dominates, or the minimum saturation level.

**Im** – Intensity where $ETR_{MAX}$ occurs.

**$ETR_{MAX}$** - Calculated maximum electron transport rate.

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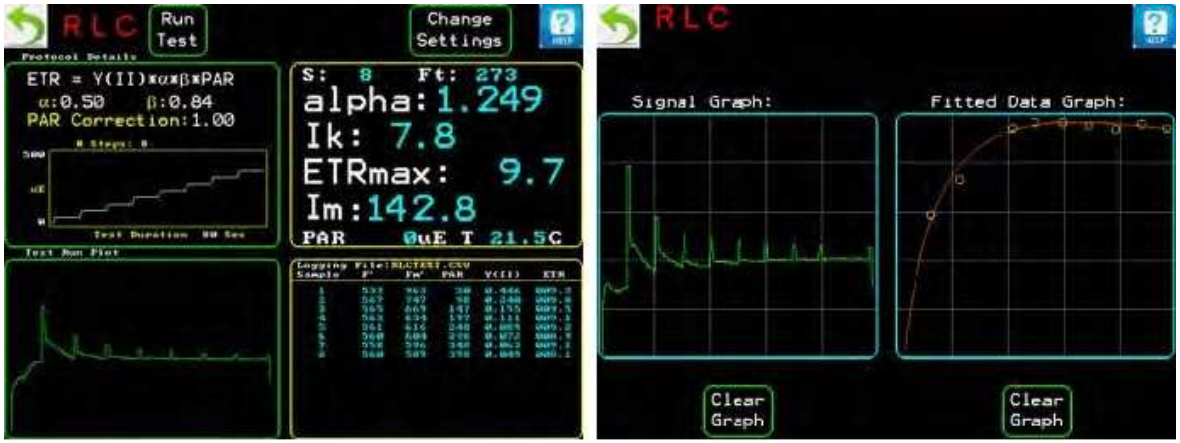
# Rapid Light Curves – A method to measure photosystem II under changing light conditions




Under canopy leaves, partly cloudy conditions, windy conditions, underwater.

**5-10 second dark adaptation times** *allow rapid re-oxidation of $Q_A$ without significant relaxation of non-photochemical quenching at a given actinic light level & prevents the deactivation of rubisco and a rubisco reactivation induction effect.*

***Rapid Light Curves* offer a tool to investigate the saturation characteristics of plants.**

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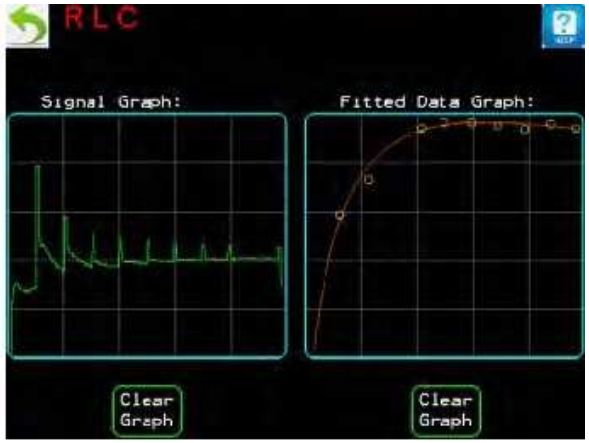
# Rapid Light Curves – A method to measure photosystem II under changing light conditions


**RLCs provide a measure of actinic light saturation characteristics of plants.**

It is affected by **immediate light history** as well as **longer term light history** or photoinhibition.

Under canopy leaves, partly cloudy conditions, windy conditions, underwater.

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# Quenching protocols – A method to measure plant photoprotective mechanisms heat dissipation and state transitions

## 1= all light absorbed by photosystem II

$$1 = Y(II)+Y(NPQ)+Y(NO)$$

Y(II) is the portion of light used drive photosynthesis in photosystem II

Y(NPQ) is regulated heat dissipation of the xanthophyll cycle and $\Delta$pH of the thylakoid lumen

Y(NO) is unregulated heat dissipation.

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# Quenching protocols

**Puddle model parameters**

qP = $(F_M' - F_S)/(F_M' - F_O)$ Above 0.4, Fo' should replace Fo **Photochemical quenching –open PSII reactions centers**

qN = $1- ((F_M'-F_O) /(F_M-F_O))$ or qN = $1- ((F_M'-F_O) / (F_M-F_O))$ Above 0.4, Fo' should replace Fo **Nonphotochemical quenching**

NPQ = $(F_M-F_M')/F_M'$ **Simplified Nonphotochemical quenching**

**Hendrickson's equations & NPQ resurrected to the lake model** from the puddle model by Klughammer and Schreiber

Y(II) = $(F_M' - F_S)/F_M'$ or $\Delta F_M'/ F_M'$ **Yield of PSII**

Y(NO) = $F_S/F_M$ or $F'/F_M$ **Nonregulated Nonphotochemical quenching**

Y(NPQ) =( $F_S/F_M'$)-Y(NO) or $\Delta F'/F_M'$) - Y(NO) **Regulated Nonphotochemical quenching**

NPQ= Y(NPQ)/Y(NO) or NPQ = $(F_M-F_M')/F_M'$ Simplified Nonphotochemical quenching

**Kramer's equations**

Y(II) = $(F_M' - F_S)/F_M'$ or $\Delta F'/ F_M'$ **Yield of PSII**

qL = qP (Fo'/Fs) **Photochemical Quenching measure of the fraction of still open PSII reaction centers**

Y(NO) = $1/(NPQ+1+qL(F_M/F_O -1))$ **Nonregulated Nonphotochemical quenching**

Y(NPQ) = 1- Y(II)-Y(NO) **Regulated Nonphotochemical quenching**

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# Quenching protocols

Quenching relaxation valid with Hendrickson as well as puddle model equations.

NPQ=qE+qT+qI or NPQ =qE+qM+qI

**Xanthophyll Cycle & Delta pH of the thylakoid lumen**

**qE** = ((FME-FM')/(FM-FM')) is the relaxation saturation value at four minutes to ten minutes in the dark. (Time is adjustable).

**Chloroplast Migration**

**qM** = ((FMM-FME)/ (FM-FM')) is the relaxation saturation value at twenty to thirty five minutes in the dark. (Time is adjustable) thirty five minutes is the default value.

**State Transitions**

**qT** = ((FMT-FME)/ (FM-FM')) is the relaxation saturation value at fifteen to twenty minutes in the dark. (Time is adjustable) twenty minutes is the default value.

**Photoinhibition**

**qI** = ((FM-FMM) / FM-FM')) Relaxation of qI starts at about forty minutes and can take up to sixty hours. qI can be determined from the dark adapted FM measurement and the saturation pulse at thirty five minutes used for qM .

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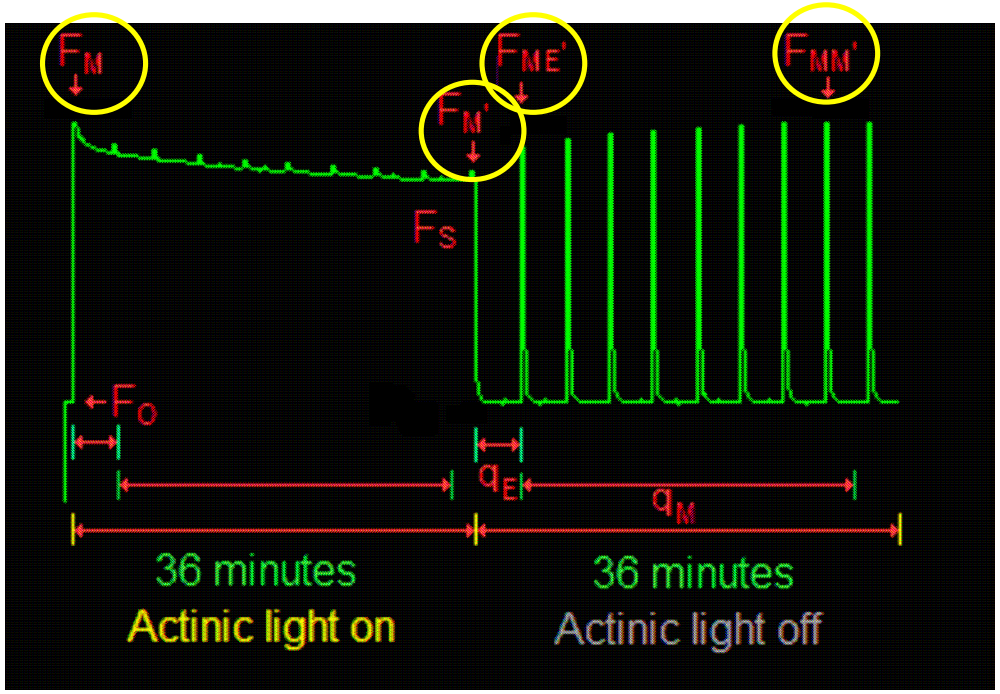
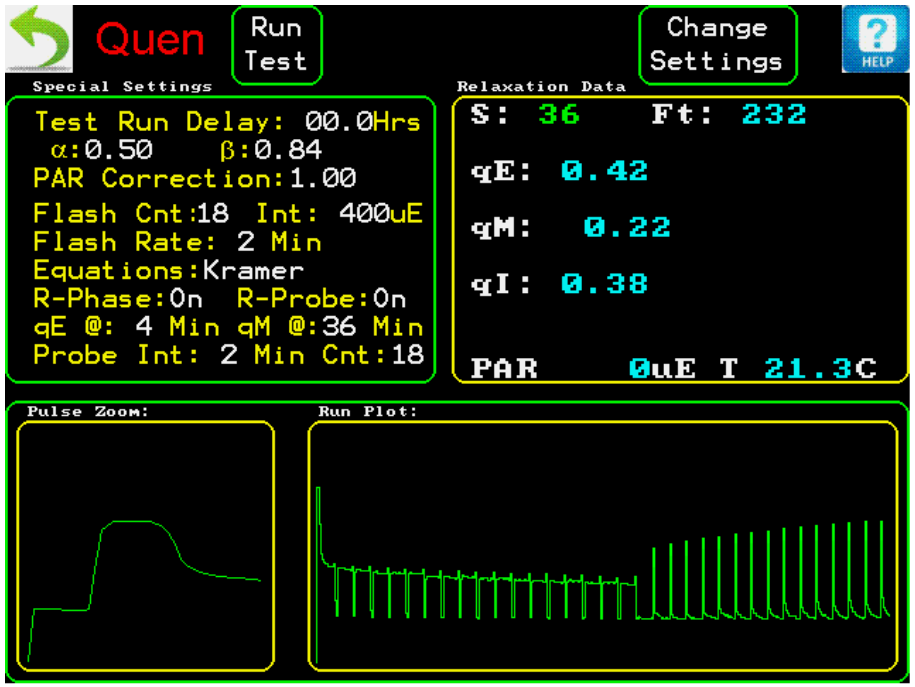
# Quenching protocols –



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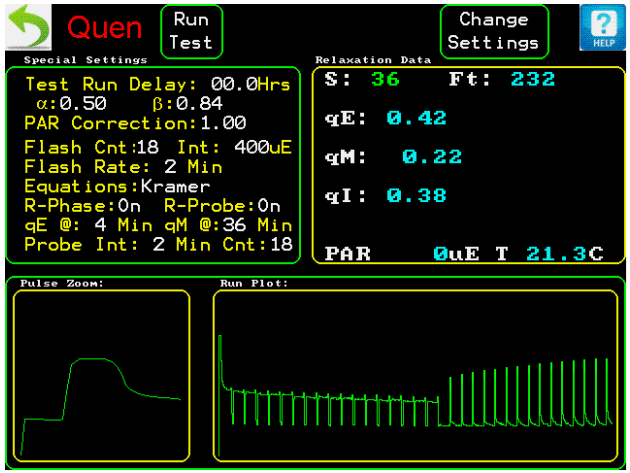
# Quenching protocols –




1. Saturation pulse NPQ can take up to 2 minutes to dissipate
2. qI is the difference between the peak at 30 minutes and predawn overnight dark adaptation.
3. Use a dark shroud on the PAR clip for dark adaption and actinic light measurement. – the OS5p+ has a closed loop feedback mechanism that maintains the programable light intensity.
4. Special algorithms prevent saturation pulse NPQ from creating an error in measurement. (20 ms 8 point rolling average to find the highest reliable Fm or Fm’ value).
5. Use the auto set up for modulated light set up.

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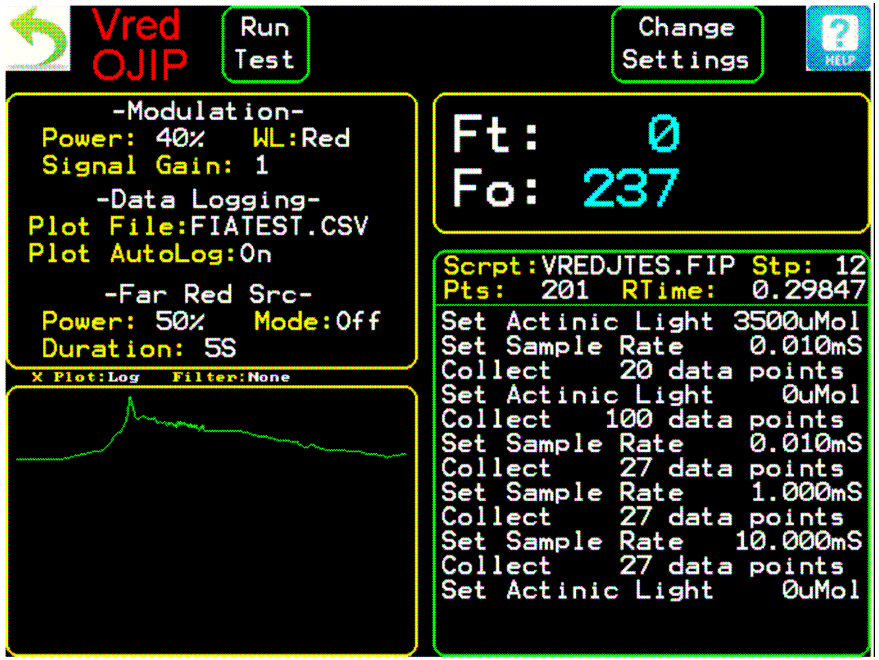
# Wim Vredenburg OJIP protocol




This protocol was designed by Wim Vredenburg for investigating Photosystem II.
It offers a free form protocol for measuring chlorophyll fluorescence values at specific times during the rise variable chlorophyll fluorescence in microseconds. Quenching from every point may also be evaluated.

Offered with either a red actinic light or a white actinic light.

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## OS5p+ Technical Specifications

**Excitation sources, Saturation pulse:**

White LED with 690nm filter.  0-15,000 µmol m-2 s-1. 7,500 µmol m-2 s-1 with PAR clip.

**Modulating light:** Two channel 660nm (red) and 450nm (blue) LED

**Actinic light:** White LED 0-5,800 µmol m-2 s-1, or 0-1,850 µmol m-2 s-1 with PAR clip

**Far red:** Intensity adjustable 740nm LED

**Detection method:** Pulse modulation

**Detector:** PIN photodiode with 700-750nm filter

**Sampling rate:** Auto-switching from 1 to 10,000 points per second, depending on test and phase of test

**Test duration:** Adjustable 0.1 seconds – 12 hours

**Data storage:** 1Gb internal memory for thousands of data sets and traces. Removable SD card.

**Digital output:** USB and 1Gb SD card

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**OS5p+ Technical Specifications**

**User interface:** Graphic, backlit, colour, touch screen display (114mm x 89mm)

**Battery:** Rechargeable nickel metal hydride providing up to 12 hours of continuous operation

**Operating temperature range:** 0-50˚C

**Dimensions:** 18cm x 14cm x 8cm

**Weight:** 1.6kg

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