OPTI-SCIENCES, INC.

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OS5p+

Pulse Modulated Chlorophyll Fluorometer

The Most Advanced Portable System Available

- **Detection and Measurement of Most Types of Plant Stress**
- **New Technology – Designed to allow & measure chloroplast migration q_M**
- **Stable Internal Actinic Light Source – for reliable field and laboratory quenching tests.**
- **Loriaux (2013) F_M' Correction Option or Square Topped Saturation Flash.**
- **Wider Range of Programmable Testing Protocols – More than any other portable fluorometer**
- **Rugged Field Portable – Designed for extended field studies and remote locations.**

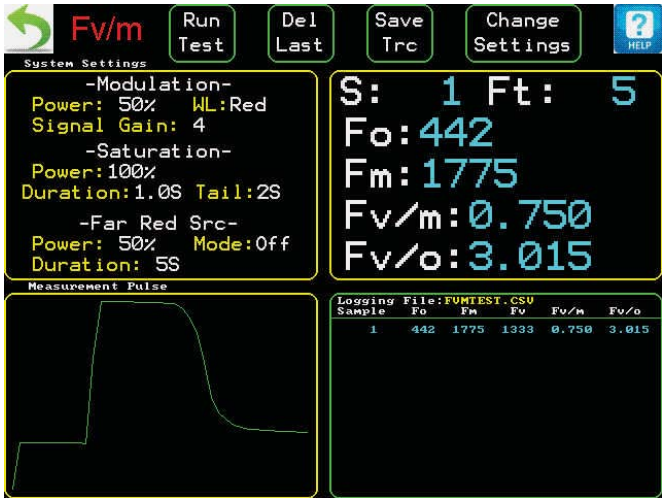
Better tests allow better science

The Most Popular Tests:

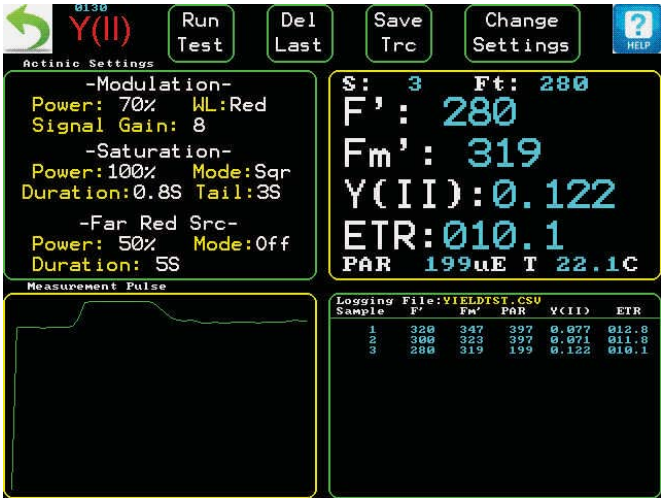
Y(II): or $\Delta F/F_M'$ quantum photosynthetic yield of PSII
F_V/F_M: Maximum quantum yield - Photochemical efficiency of PSII
F_V/F_O: A more sensitive stress detector than Fv/Fm.
ETR: Electron Transport Rate (with standard PAR clip included)
PAR: Photosynthetically Active Radiation value
(with standard PAR clip included)
T: Leaf temperature (with standard PAR clip included)

Chloroplast migration is responsible for up to about 30% of NPQ at high actinic light levels (Cazzaniga 2013)

Chloroplast migration, as it occurs in nature, requires a white actinic light source or an intense blue spectrum for reliable measurement of all light adapted measuring parameters as well as q_M and q_I



F_V/F_M & F_V/F_O



$\Delta F/F_M'$ or Y(II)

Advanced Protocols:

Quenching tests – *The widest range available.* ***Used with a stable internal actinic illuminator****:*
Hendrickson lake model protocol parameters with NPQ, resurrected from the puddle model by Klughammer,
Kramer lake model protocol parameters,
Puddle model protocol parameters
Quenching relaxation protocol with q_E, **q_M**, q_Z, q_T, and q_I

OJIP research protocol for quenching - designed by Wim Vredenberg. Intended for basic research and maximum flexibility. Ideal for the study nonphotochemical quenching related to different steps of the OJIP fluorescence rise with high time resolution. Values reported as F_t/F_O

OJIP stress measuring protocol – with all of Strasser's measuring parameters.

RLC - rapid light curves are used to study the light saturation characteristics of samples. They are primarily used in under canopy work, and aquatic work, where the light irradiation level is constantly changing. It has been found that RLCs provide a more reliable measure of Rubisco activity than standard light curves, or Y(II) under these conditions.
The OS5p+ provides Eilers and Peeters curve fitting software and direct read out of ***ETR_{MAX}, Ik, and*** **α.**

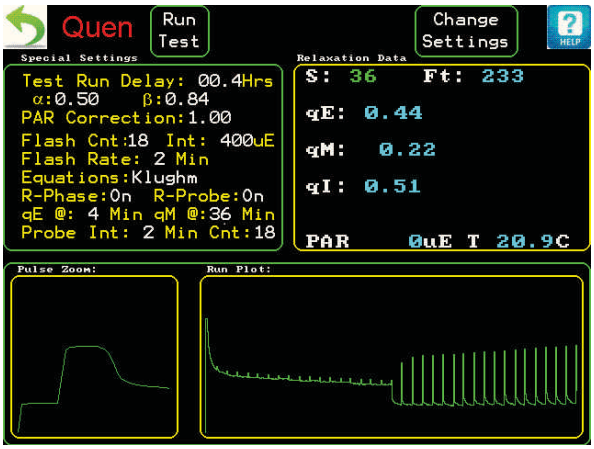
F_M' correction option adapted from Loriaux 2013 - for more reliable Y(II) and ETR measurements under high light stress conditions. It has been known for some time that an error exists, and it is greater under high actinic light conditions. More recently, the scientist that invented this protocol has gotten behind the use of a single multiple phased saturation Flash, used with linear regression analysis, to correct the error. Opti-Sciences includes this standard option for correction of F_M'.

Automated modulation light intensity setup routine – In order to make reliable measurements, the intensity of the modulated light source must be set high enough to make measurements, but it must also be low enough so that it does not drive photosynthesis. A new automated routine ensures the proper setting for all samples, eliminates errors, and simplifies

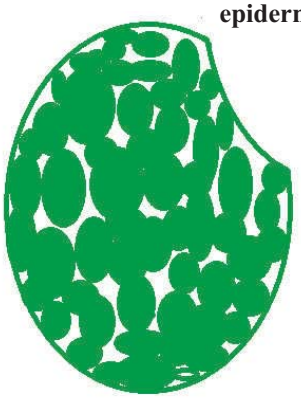
Unique - Stable, White actinic light source

Recently it was found that chloroplast migration was responsible for up to 30% of all NPQ at high actinic light levels. (Cazzaniga 2013), (Dall'Osto 2014)

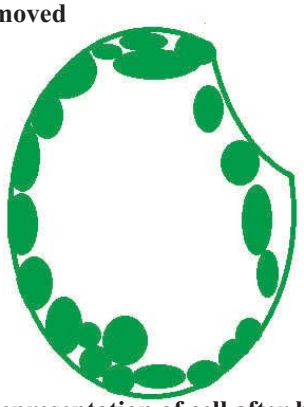
It was also found that chloroplast migration, a high light avoidance mechanism, responded to **white light**, and to **intense blue light**, but not significantly to red light. Futhermore, the process takes between 20 to 35 minutes to reach equilibrium or to relax. While the original work regarding fluorescence NPQ and chloroplast migration was done with dicots (Cazzaniga 2013), chloroplast migration in response to blue light has also been shown in monocots (Maii 2011). *The end result is that significant errors can result in all chlorophyll fluorescence measurements and measuring parameters, at steady state photosynthesis, if white actinic light or an intense blue actinic light are not used to irradiate samples at higher light levels.* Chloroplast migration is known as q_M. An application note called "q_M the game changing parameter" is available at www.optisci.com



Cells - Top of leaf view
epidermis removed



Representation of chloroplasts in a cell after dark adaptation



Representation of cell after high white actinic light treatment for 35 min.

When the OS5p+ is used with the PAR Clip, the white actinic light intensity stability is maintained with less than +-3 µmols variation during all tests. This is unique. With non-stabilized light sources, the light intensity drops significantly for longer tests due to heat, and can be a source of significant error. Accurate quenching measurements, Rapid light curves, pre-illuminated Y(II) and ETR tests require a stable light source.

Light intensity output changes with lamp temperature and instrument temperature. Normally, the longer that an instrument is on, the greater the heat, and the lower the actinic light intensity. When the OS5p+ is used with the PAR clip, the PAR clip monitors PAR level and maintains that level for the length of the test. This ensures that samples are at steady state photosynthesis, a process that takes between 20 to 35 minutes for a specific light level (Cazzaniga 2013). There is sufficient reserve light intensity to maintain an actinic intensity of greater than 1,850 µmols for extended periods of time.

Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) "Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photo oxidative stress in Arabidopsis", The Plant Journal, Volume 76, Issue 4, pages568–579, November 2013 DOI: 10.1111/tpj.12314

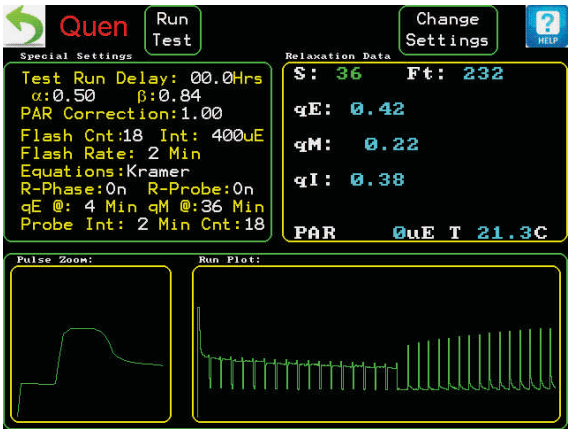
Dall'Osto L, Cazzaniga S, Wada M, Bassi R. (2014) On the origin of a slowly reversible fluorescence decay component in the Arabidopsis npq4 mutant. Phil. Trans. R. Soc. B 369: 20130221. http://dx.doi.org/10.1098/rstb.2013.0221

Maai E., Shimada S., Yamada M.,, Sugiyama T., Miyake H., and Taniguchi M., (2011) The avoidance and aggregative movements of mesophyll chloroplasts in C4 monocots in response to blue light and abscisic acid Journal of Experimental Botany, Vol. 62, No. 9, pp. 3213–3221, 2011, doi:10.1093/jxb/err008 Advance Access publication 21 February, 2011

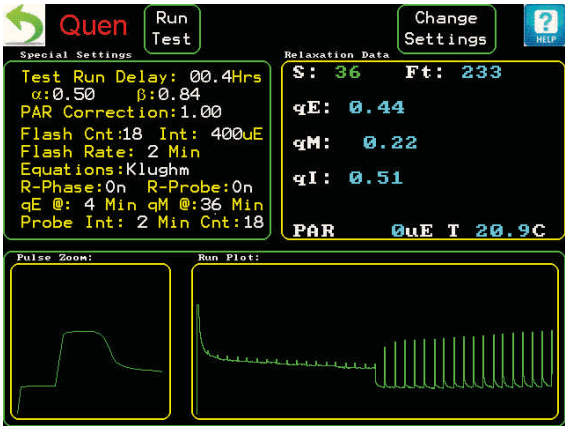
Quenching New - q_M

Chloroplast migration is be responsible for up to about 30% of NPQ at high actinic light levels (Cazzaniga 2013)

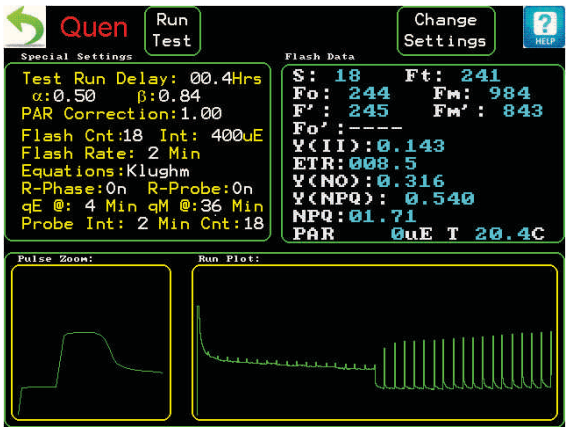
A stable, internal, white actinic light source with an intense blue spectrum, is used to ensure proper chloroplast migration, as it occurs in nature.



Kramer lake model quenching protocol



Hendrickson lake model protocol

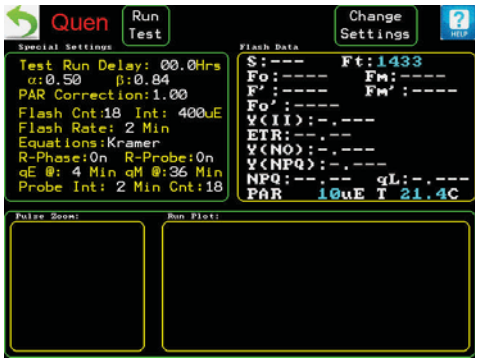


The OS5p+ includes the widest range of quenching protocols available. Protocols may be used with the standard square top saturation flash, or the Loriaux 2013 F_M' correction protocol. All measurements may be made with the stable internal white actinic light source.

Hendrickson lake model parameters with NPQ resurrected by Klughammer from the puddle model (2008)

Y(II) Quantum yield of PSII
Y(NPQ) Photoprotective non-photochemical quenching
Y(NO) All other non-photo-protective non-photochemical quenching
NPQ: Non-photochemical quenching NPQ=Y(NPQ)/Y(NO)

Kramer lake model quenching parameters (2004)



Y(II) Quantum yield of PSII
q_L Photochemical quenching
Y(NPQ) Photoprotective non-photochemical quenching
Y(NO) All other non-photo-protective non-photochemical quenching

Puddle model quenching

Y(II) Quantum photosynthetic yield
q_P: Photochemical quenching
q_N: Non-photochemical quenching
NPQ: Non-photochemical quenching

Quenching relaxation - for measuring the Δph of the thylakoid lumen and xanthophyll cycle, state transitions, chloroplast migrations, state transitions, and photoinhibition.

q_E Photoprotective non-photochemical quenching, - seconds to several minutes
q_M Chloroplast migration – chloroplast light avoidance mechanism at high actinic light levels - 20 -35 minutes
q_Z A proposed longer term xanthophyll mechanism – 20 – 30 minutes
q_T The portion of NPQ related to state transitions – 15 to 20 minutes
q_I The portion of NPQ due to photo-inhibition and photodamage. Starts to relax at 40 minutes and may take up to 30 or 60 hours

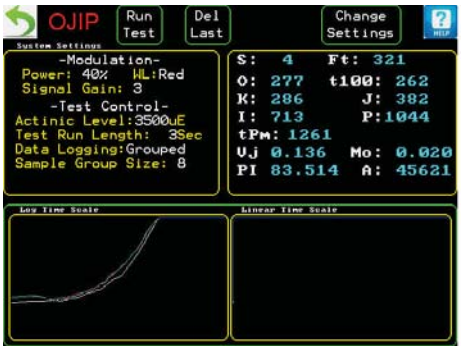
OJIP - The “JIP” Test

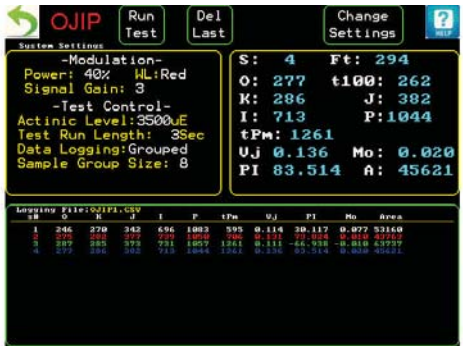
OJIP or the “JIP” test is another dark adapted test that has been used for detecting and measuring plant stress. It was first described in some detail by Kautsky (1957). Since that time, it has been found that if the rise in fluorescence, caused by illumination after dark adaptation, was analyzed, at high time resolution, there was a distinct curve shape with multiple steps. Using this approach, plant stress that affects PSII can be measured.

It has also been shown that some types of plant stress affect specific parts of the OJIP curve. For example, nitrogen stress, at higher levels, has been shown to display a K step at 300 µs (Strasser 2004). In addition, special measuring parameters have been developed as sensitive stress detectors such as PI_{ABS} or performance index.

Viewing OJIP graphic results can now be quickly and easily done in the field. The OS5p+ provides a color graphic display of the OJIP curve with a logarithmic time scale. It is common for researchers that use this technique to overlay measuring graph traces to study the effects of plant stress, and to use the special parameters that have been created, to quantify plant stress. Up to 32 traces may be overlaid on the graphic color instrument screen.

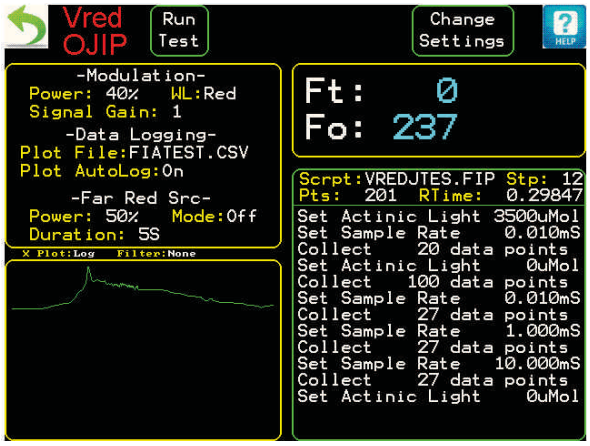
The Strasser protocol parameters O, J, I, P, t100us, t300us (or K), M_O (or RC/ABS), PI_{ABS} (or Performance Index), A (or Area above the curve), and tF_M (or time to F_M) are all displayed, All other Strasser protocol parameters are reported to the data file.





OJIP research quenching protocol

designed by Wim Vredenberg



Modulated light allows measurement of mechanisms that are difficult to measure. On the left an actinic light of 3,500 µmols is turned on for 0.2 ms, and then turned off.

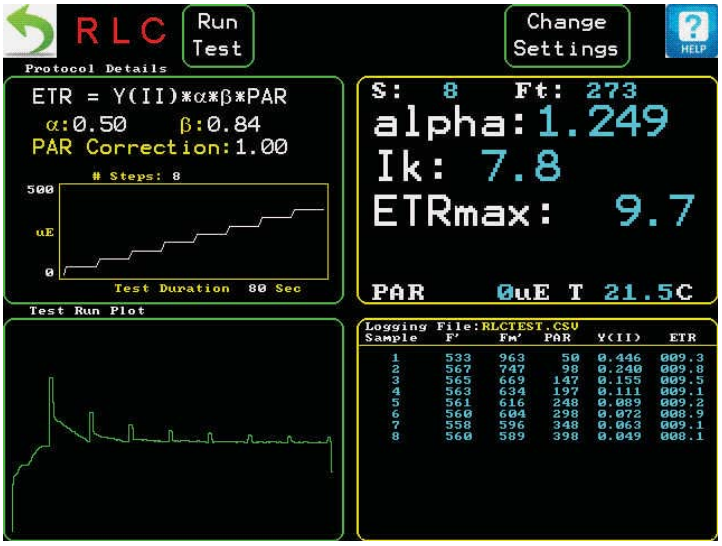
A scripting capability, shown on the right side, allow complete control of time resolution, the number of measuring points (up to 65,000), and actinic light intensities. The light may be turned on or off at any point. Far Red light may be turned off or on as well.

This protocol allow investigation of NPQ as it relates to the OJIP fluorescence rise, using high time resolution. A special step sequence generator allows great flexibility, and the pulse modulated design allows measurement of related NPQ after the actinic light has been turned off. Actinic light intensities up to 5,800 µmols are possible, and the unit may be ordered with a white or red actinic light source.

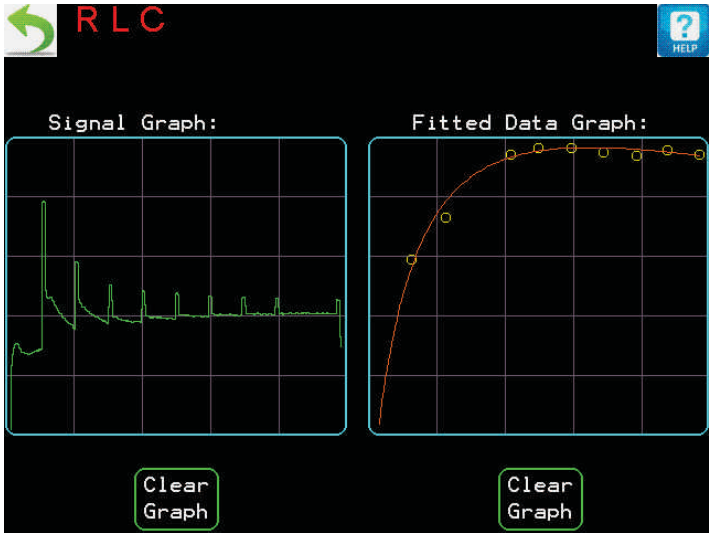
Rapid light Curves

Y(II) and ETR are designed to be used under steady state photosynthetic lighting conditions. Under *variable lighting* conditions, Y(II) understates and ETR overstates the real condition.

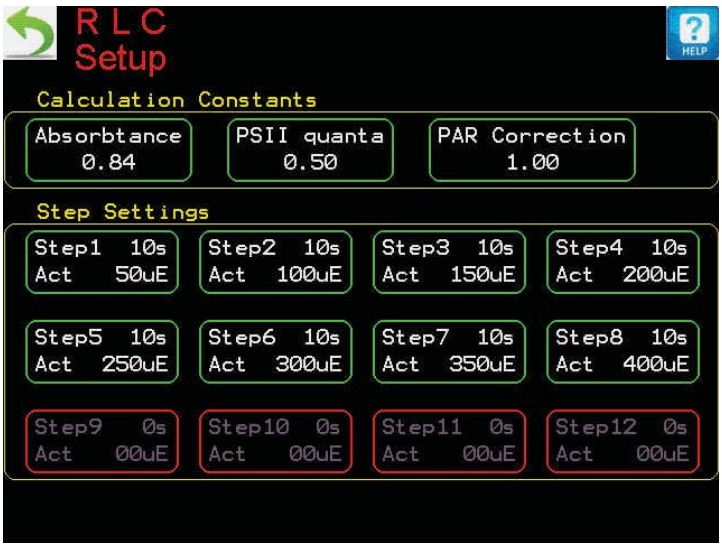
It has been shown by various researchers, that Rapid light Curves provide a more realistic estimate of Rubisco activity under variable light conditions that are found under canopy, and in aquatic environments. For more information contact Opti-Sciences for the RLC application note.

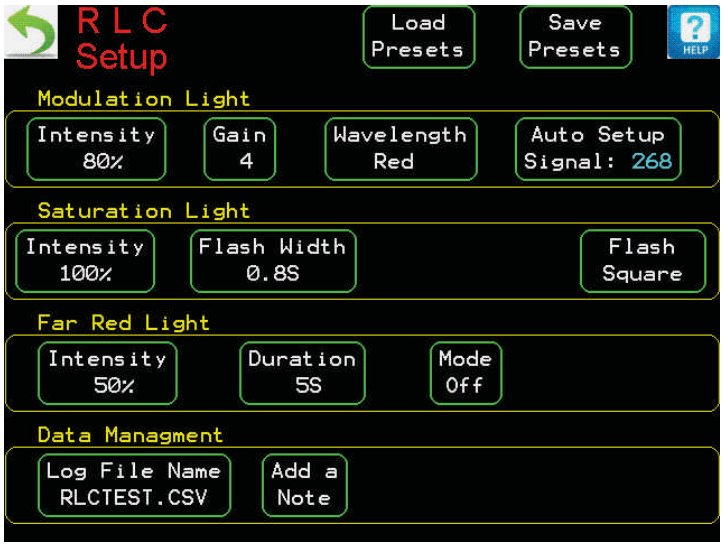


RLC cardinal points & fitted curve for ETR vs. PAR light intensity



Eilers and Peeters curve fitting formulas are used with this system



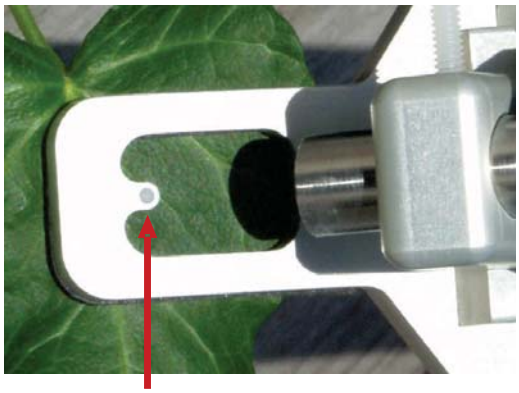


RLC

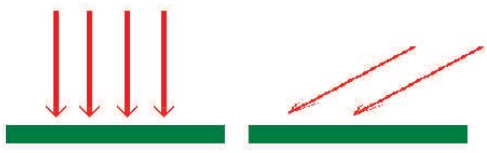
The time duration for actinic light steps is adjustable from 5 seconds to more than 90 seconds. The number of steps and the intensity of each step may be set according to preference.

This protocol has an auto-modulated light intensity set up routine, and multiple notes can be made for each measuring curve.

Innovative PAR Clip - ***now standard equipment !***



Cosine Corrected PAR Sensor



Less light strikes the leaf at steeper angles

Technology Advances

The Opti-Science PAR Clip was created to improve upon previous industry designs.

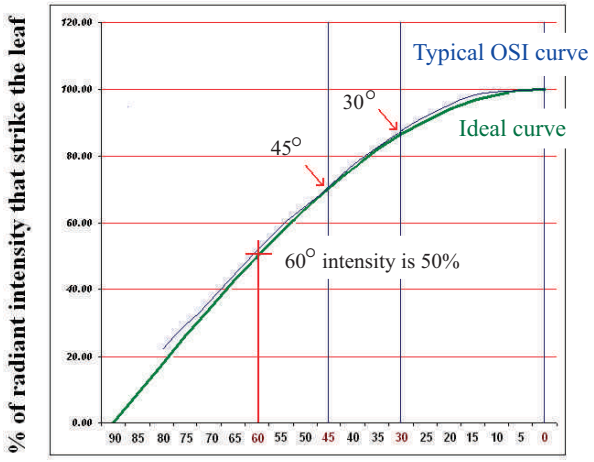
By developing a *bottom opening* PAR Clip, this new model prevents inappropriate opening when measuring leaves above the operators head, or when mounted on a tripod that occurs with some industry designs. As a result, the Opti-Science PAR Clip allows one handed operation, and eliminates two handed operation.

This PAR light sensor is positioned to allow measurement of ambient PAR as well as PAR from internal actinic light sources.

Leaf temperature is measured reliably to +/- 0.5 ^{o}C over the instrument operating range during all measuring protocol conditions. New thermistors provide improved measuring accuracy over older thermocouple designs. In addition, they rarely have to be replaced and they do not damage leaf samples.

Cosine correction When measuring PAR in ambient light or with internal illumination, one must not change the orientation of the leaf to make a measurement. Yield is a always measured at steady state photosynthesis so a change in orientation to a light source will cause an error. Cosign correction insures that leaves that are oriented at different angles to actinic light sources will be measured reliably.

Lambert's Cosine Law
Comparison of an ideal response from a cosign corrected sensor and an OSI sensor



Angle variation from perpendicular (or normal)
As the angle of irradiation increases from perpendicular, the irradiation per unit area per second decreases.

Multi-flash – based on Loriaux (2006), & Loriaux, (2013)

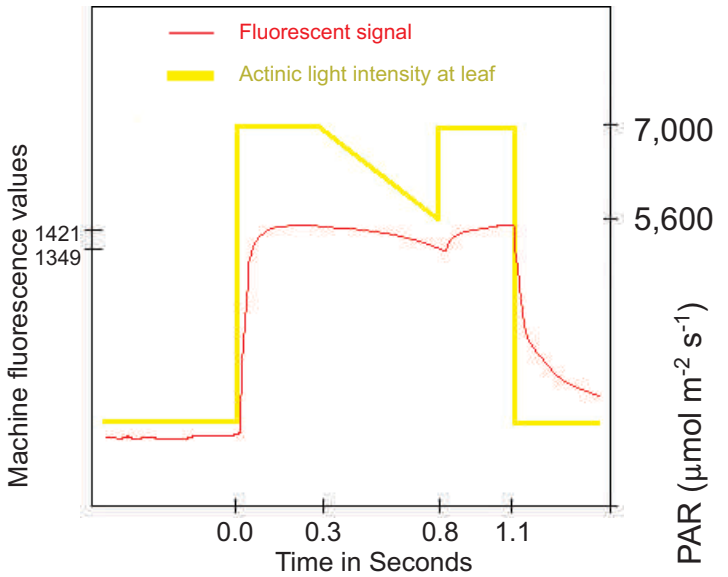
Saturation pulses used with modulated fluorometers are designed to close all PSII reaction centers. The maximum fluorescence intensity value, of the saturation flash, F_M', is used in most measurements including, quantum yield of PSII Φ_{PSII} (also called Y(II) or $\Delta F / F_M$'), *J* (or ETR), and in all quenching protocol parameters.

While it is possible to reduce or close all reaction centers in a properly dark adapted sample, with a relatively low amount of light, it has been found that in light adapted samples, with a high actinic light history, complete closure of all PSII reaction centers becomes problematic with even the highest amounts of saturation light. It is thought that complete reduction of Q_A is prevented by fast turnover of the plastoquinone pools. (Margraph 1990, Loriaux 2013). With this in mind, Y(II) and ETR measurements taken under these conditions, can be underestimated. In a poster, researchers that included Bernard Genty, the developer of quantum yield of PSII, verified the issue, and developed a method for F_M' correction. It involved a multiple phases single saturation pulse with multiple light intensities, and the use of least squares linear regression analysis of the reciprocal of PAR (Photosynthetically Active Radiation), to determine the F_M' fluorescence level using an infinitely intense saturation pulse, without causing damage to the plant and without closing all of the reaction centers.

Studies by Earl (2004), and Loriaux (2006), have compared chlorophyll fluorescence measurement results with gas exchange measurements and found that by using multiple saturation flashes, and regression analysis, an infinite fluorescent saturation light flash intensity can be determined and used to correct Φ_{PSII} or (Y(II)) and *J* (ETR) measurements. *Research has shown that Y(II) measurements, taken under high actinic light conditions, can be underestimated with up to a 22% error, and there can be up to a 41% error in ETR values if this method is not used.*

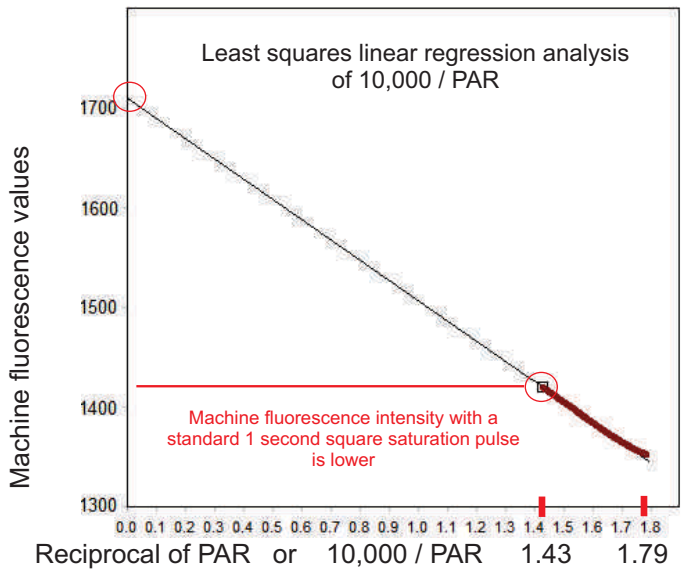
This standard option is provided on the OS5p+, the i*FL*, and OS1p instruments. It is *available for all Light adapted and quenching protocols,* and it can be turned off or on. The method described by the Loriaux, Burns, Welles, McDermitt, & Genty (2006) and expanded by Loriaux, Avenson, Welles, McDermitt, Eckles, Riensche, & Genty (2013), provides the most accepted method currently available. According to the science, the OS5p+ provides the optimal saturation intensity of 7,000 µmols, optimal light ramping of 20%, and a ramping rate less than 0.01 mol $m^{-2}s^{-2}$. While some adjustment is possible, the default protocol has been optimized for most applications.

Representation of how the Multiple Phased Flash works



Least squares linear regression of 10,000 / PAR values

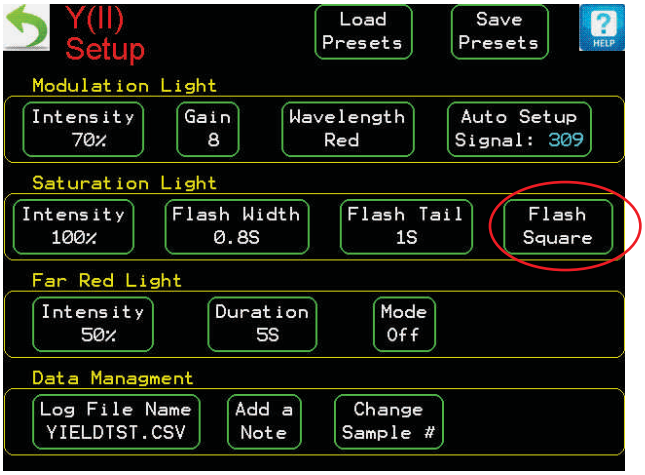
y intercept = machine fluorescence value with an infinite saturation pulse



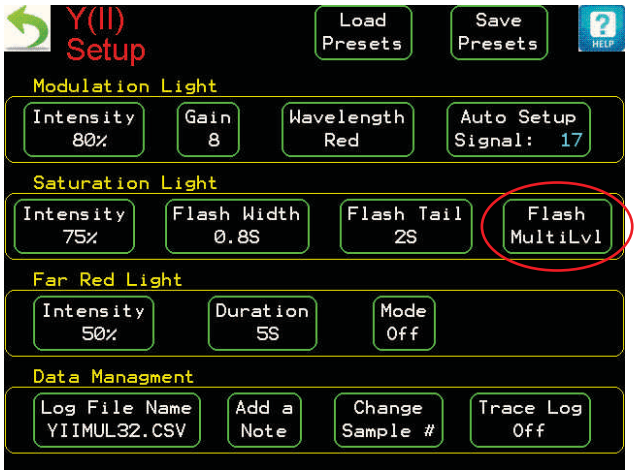
The first saturation flash step, shown on the left, is at 7,000 µmols for 0.30 seconds to saturate PSII. The saturation flash intensity is then ramped downward by 20%, making a large number of fluorescence measurements along the way, to 5,600 µmols. The ramping rate is less than 0.01 mol photons $m^{-2}s^{-2}$. The final phase is at 7,000 µmols to check for saturation pulse NPQ. Recent studies have shown that those setting provided optimal results for plants that have been tested. (Loriaux 2013). A rolling 25ms eight point average is used to determine maximum F_M'

The graph on the right represents the Loriaux, (2006) & Loriaux (2013) method for estimating Fm' with an infinitely intense saturation flash. Least squares linear regression analysis of the reciprocal of PAR (or 10,000 / PAR) allow determination of the y intercept, which represents the machine fluorescence value with an infinite saturation flash.

Attention to Detail

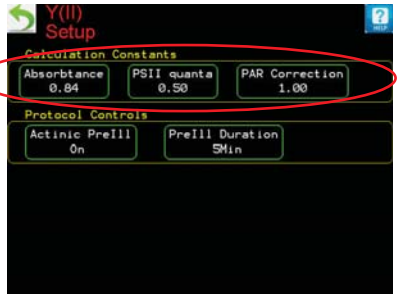


Square Saturation Flash type & width setting

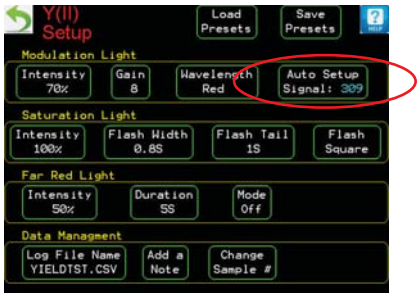


Multi-Flash - Single Multiple Phased Saturation Flash F_M' Correction

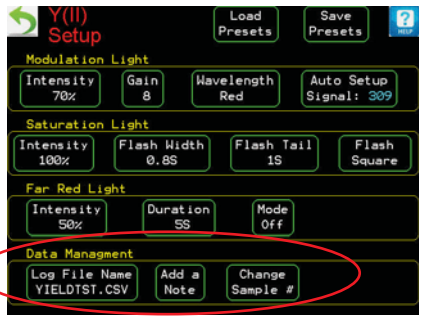
The F_M' correction protocol can also be chosen for use in other protocols such as quenching and light curves.



Input of actual values - The average values for leaf absorptance, 0.84, and the ratio of PSII reaction centers to PSI reaction centers, 0.5, are shown as the default values used to determine electron transport rate. The window on the left allows input of actual measured values for a more accurate electron transport rate. It also allow input of PAR measurement by other means when the PAR clip is not used.



Automated modulated light intensity adjustment - Det Setup
The OS5p+ provides an automated method to set the modulated light intensity correctly. It starts low and adjusts the light intensity and the detector gain control until the fluorescence signal is high enough for detection, but low enough so that there is no Q_A being reduced. While one can still adjust the modulated light intensity manually, the automated method saves time and helps prevent mistakes.



Kwik Name - To create a new file name with existing settings, Kwik Name allows rapid naming convenience with the touch of a button. It uses today's date and time to create a unique name. Of course more descriptive names may also be entered as well.

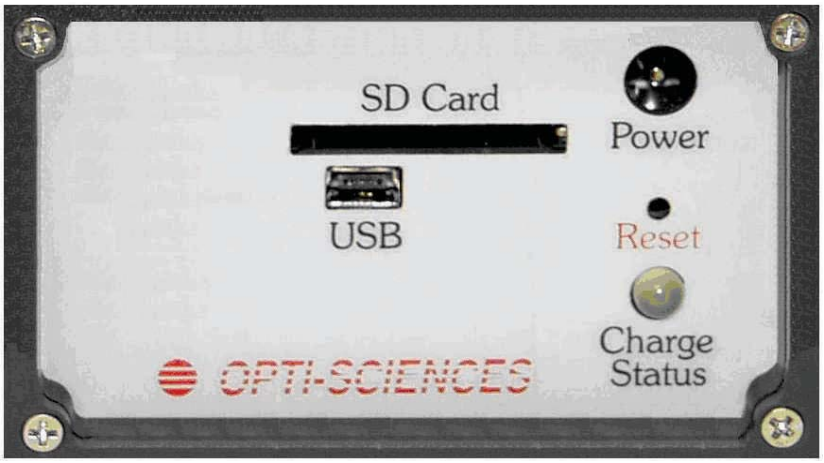
Notes - Notes that include up to 34 alpha-numeric characters may be created for each measurement. The note is transferred the comma delineated data file for future reference. Multiple notes can be made for each measurement.

The instrument measures the highest 25 ms. 8 point rolling average to determine F_M and F_M'. This prevents saturation pulse NPQ from being a problem for all samples, even if the Flash width is set too wide.

Attention to Detail

Data Management

The OS5p+ provides a gigabyte of non-volatile flash memory designed to prevent data loss due to power interruption.



Data Card

The built-in MMC/SD data card system can be used with an unlimited number of fluorometer users to store individual measuring routines and individual data records. The data cards are very inexpensive and can store up to an additional gigabyte of information. Instrument settings, and experimental results can be automatically, an instantaneously set by each researcher, by loading the stored file from the data card.

USB

A small USB port is provided on the side of the OS5p+. When connected to a PC, the OS5p+ becomes a hard drive for a computer allowing the transfer of data, and measuring files, and allows software upgrades. No special software is required. Files may be opened with Excel, or any other program that takes comma delineated information.

Touch Screen Menu Driven Software

To ensure that the OS5p+ is easy to use in the field, a high degree of automation, a touch sensitive screen, and menu driven software are provided. Even custom measuring routine functions are easily changed.

OS5p+ Parameters Measured and Protocols included:

Y(II): Quantum Photosynthetic Yield of PSII (or ΔF/Fm' or Y
ETR: Electron transport rate (w/optional clip)
PAR: Photosynthetically Active Region value (with optional PAR clip)
T: Leaf temperature (with optional PAR clip)
F_V/F_M: Maximum Photochemical efficiency of PSII
F_V/F_O: A more sensitive detector of stress than Fv/Fm, but it does not measure plant efficiency.
F_O: Minimum fluorescence
F_M: Maximal fluorescence
F_V: Variable fluorescence
F_{MS} (or F_M'): Maximal fluorescence with actinic illumination at steady state fluorescence.
F_S (or F): Fluorescence under steady state conditions (prior to saturation pulse)

RLC: Rapid light curves.
$rETR_{MAX}$ - a measure of a leaf's photosynthetic capacity or maximum electron transport rate
α is the initial slope of line at low PAR values created by relating ETR to PAR. It provides a measure of quantum efficiency
I_k $=rETR_{MAX}/\alpha$ is a measurement of the light intensity where light saturation dominates, or the minimum saturation level

Hendrickson Quenching with NPQ (standard)
Y(NPQ), Y(NO), Y(II), NPQ, F_V/F_M

Kramer Quenching (standard)
q_L, Y(NPQ), Y(NO), Y(II), F_V/F_M

Puddle model parameters (standard)
NPQ, q_N, q_P, Y(II), F_V/F_M

Quenching relaxation protocol (standard)
q_E, q_M, q_Z, q_T, q_I along with either puddle model, or Hendrickson parameters. Adjustable times for each

OJIP - Vredenberg OJIP quenching protocol, F_t/F_O

OJIP - Strasser Protocol - Direct readout of: OJIP, t100μs, t300μs (or K step), **tF_M** (or time to P or F_M), **A** (or area above the curve), **M_O** (or RC/ABS), **PI_{ABS}** (or performance index),

Strasser OJIP parameters reported to the data file only: ABS/RC, TR_O/RC, DI_O/CS, ET_O/RC, TR_O/ABS, ET_O/TR_O, ET_O/CS,RC/CS_O, RC/CS_M , S, M, T

Light Sources:
Saturation pulse White LED with 690 nm short pass filter. 15,000 μmols, 7,500 μmols with PAR clip
Option: Red saturation light source
Modulated light
Red: 660 nm LED with 690 nm short pass filter.
Blue: 450 nm LED.
Actinic light source: white LED to 5,800 μmols for OJIP, and to 1,850 μmols in PAR Clip
Option: red actinic light source for OJIP
Far red light: above 740 nm

Detection method: Pulse modulation method.

Detector & Filters: A PIN photodiode with a 700 ~ 750 nm bandpass filter.

Sampling Rate: Auto-switching from 1 to 10,000 points per sec., depending on test & on phase of test.

Automated routine to optimally set the modulated light intensity. The modulated light may also be set manually.

Multi-Flash F_M' correction for all light adapted protocols & quenching. It may be turned on or off.

Test Duration: Adjustable from .1 seconds to 12 hours.

Storage Capacity: 1 Gigabyte of non-volitile flash memory, supporting unlimited data sets and traces

Digital Output: USB, SD/MMC 1 gigabyte data cards .

User Interface:
Display: Graphic color touch screen
Menu driven touch screen.

Power Supply: Internal 12V, rechargeable nickel metal hydride battery.

Battery Life: 8 to 12 hours of continuous operation.

Dimensions: 7 in x 5.5 in x 3.25 in. or 17.8 cm, x 14 cm, 8.3 cm.

Weight: with fiber optic probe - 3 lbs or 1.36 kgs. with fiber optic probe and PAR Clip- 3.6 lbs or 1.62 kg

Operating temperature range 0°C to 50°C

OJIP: Up to 32 traces can be overlaid on the graphic display screen. The data file is designed for easy overlay graphing and spider plot graphing. Thousands of measurement parameter sets can be stored in a single data file for spider graphing. The number of data files are only limited by machine memory limits. F_O is measured not estimated.

Accessories

Standard Storage Shipping and Transport Case.

This durable abrasion resistant water tight plastic case allows storage of the OS1p with the fiber optic sensor attached. There is also room for a PAR clip, charger and leaf cuvettes.

Airline approved for carry -on luggage.

Accessories included:

- 1 Open Body Actinic Light Leaf Cuvette –light adapted work
- 10 Dark Adaption Clips
- Fiber Optic Probe
- Battery Charger
- USB Cable
- Carrying bag with shoulder strap
- Data Card Reader and 1 GByte Data Card
- Storage and Transport Case
- PAR Clip - for Photosynthetically Active Radiation and leaf temp.

Optional features & accessories:

- Algae Cuvette
- 70 hour battery belt
- Tripods
- More Dark adaptation clips

OS5p+ - The most advanced portable chlorophyll fluorometer available

OPTI-SCIENCES

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