OPTI-SCIENCES INC.

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OS1p Chlorophyll Fluorometer

with F_M' correction according to Loriaux 2013

Pulse Modulated, field portable

Best in Class* - *reliable plant stress measurement using light and dark adapted tests.

Reliable* - *strict adherence to proven scientific protocols, and methods make the OS1p the instrument of choice

Affordable* - *Y(II) or $\Delta F/F_M$' and ETR measurements are included, as well as F_V/F_M, Rapid Light Curves, & Henrickson lake model quenching with NPQ

Fast* - *measure plant stress in a few seconds, allowing non-destruction evaluation of large plant populations

High Precision* - *F_M' correction according to Loriaux 2013 - Stable built-in actinic light source for more reliable measurements

Easy to use* - *menu driven with color graphic touch screen

While Fv/Fm is the most used fluorescent measurement parameter, *Y(II) or quantum yield of photosystem II, and ETR, are likely the more versatile measurements.* These two parameters have successfully demonstrated the ability to measure more types of plant stress than Fv/Fm, and in some cases, detect stress earlier.

Replacing the popular OS1-FL, the OS1p represents the next generation. This research grade instrument offers a number of new enhancements including: New measuring protocols for added flexibility, a color graphic touch screen for simple operation, a USB port, and MMC/SD data card technology that allows multiple instrument user management without compromise.

In addition, OSI has developed an innovative PAR clip for use with the OS1p that exceeds previous industry designs. The PAR Clip includes a cosine corrected PAR sensor that is located to provide measurement of ambient light irradiation or internal actinic LED irradiation. Calibrations are made for sensor location, and light source type. It also includes a calibrated and reliable leaf temperature sensor.

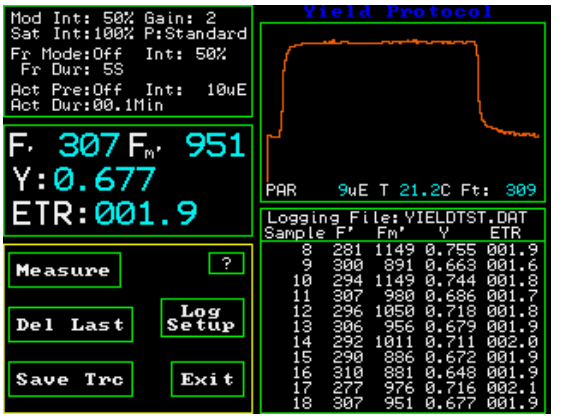
The mechanical design of the PAR Clip is also unique. This PAR clip is designed for one handed operation and it will not open at inappropriate times.

Better tests allow better science

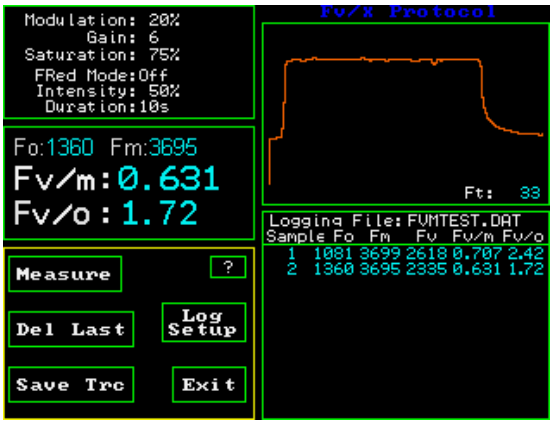
The Most Popular Tests:

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

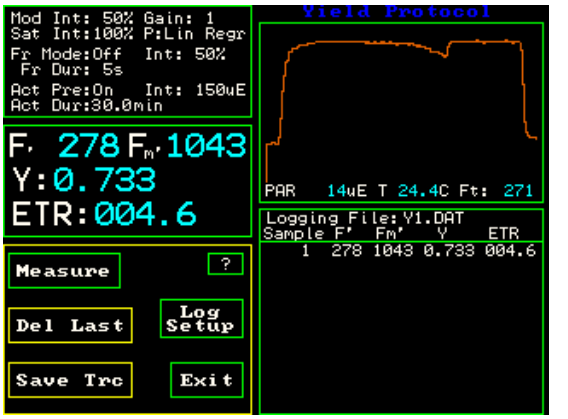
$\Delta F/F_M'$ or Y(II) with square topped flash



F_V/F_M & F_V/F_O



$\Delta F/F_M'$ or Y(II) with F_M' correction according to Loriaux 2013



Protocols:

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 OS1p provides curve fitting software and direct read out of* ***ETR_{MAX}, Ik Im, and*** α.

F_M' correction for more reliable Y(II) and ETR measurements under high light conditions. It has been known for some time that not all PSII reaction centers can be closed with an intense standard square saturation flash, a requirement for accurate measurement. Opti-Sciences now provides the Loriaux (2013) (2006) F_M' correction protocol for more reliable Y(II) and ETR measurement. See the page on Multi-Flash for more details.

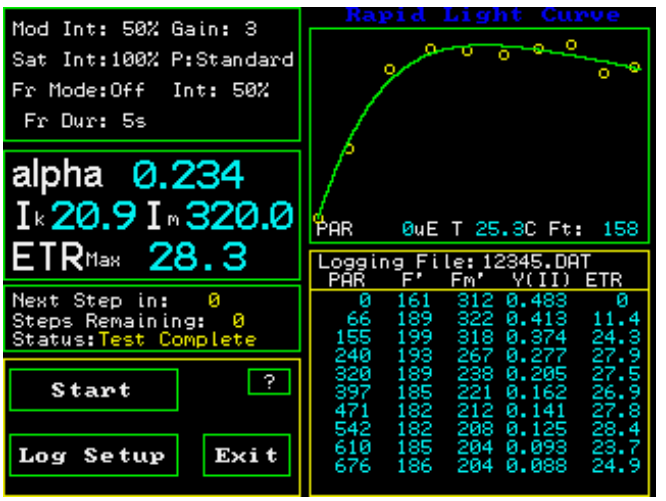
Quenching tests – Hendrickson simplified lake model parameters with NPQ, resurrected from the puddle model by Klughammer, are provided as standard. When used with the optional PAR clip, the OS1p provides a built-in stable actinic light source that is recommended for field and lab quenching measurements, for more reliable work.

Automated modulation light intensity 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 the chemical reduction of Q_A. A new automated routine ensures the proper setting for all samples, eliminates errors, and simplifies the process.

Rapid light Curves

Y(II) and ETR are designed to be used under steady state photosynthetic lighting conditions. Under variable lighting conditions, Y(II) and ETR overstate 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.



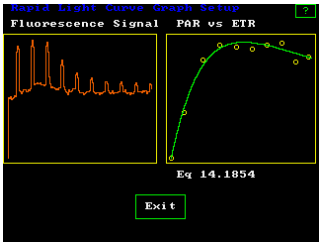
RLC cardinal points & fitted curve for ETR vs. PAR light intensity

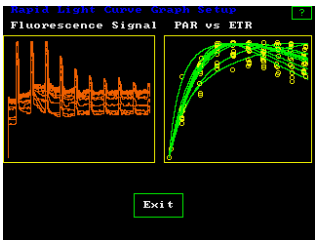
RLC

When the graphic display is touched, a secondary screen is displayed that shows the actual fluorescence trace side by side with the resulting curve fitting ETR vs intensity graph.

The lower screen shows multiple RLCs taken at different times of day. Light history changes many of the RLC parameters but ETR_{MAX} changes little.

The time duration for actinic light steps is adjustable from 5 seconds to more than 90 seconds.





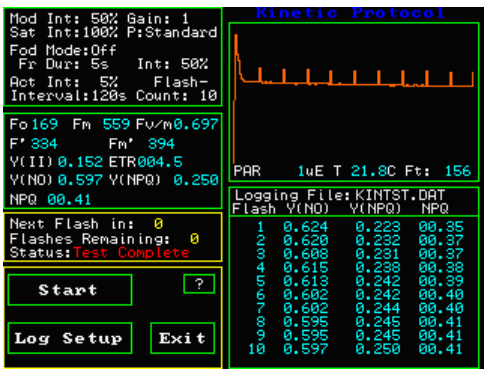
Eilers and Peeters curve fitting formulas are used with this system

Quenching measurements – Hendrickson lake model parameters with NPQ are provided as standard.

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

Y(II) Quantum photosynthetic 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)

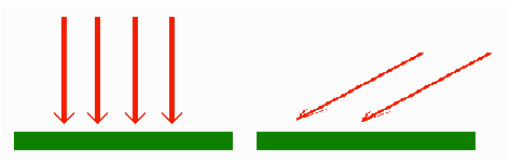
The PAR Clip is recommended for quenching measurements.
It is an option that allows the built in actinic light source to remain at a fixed light intensity over time, a requirement for reliable measurement.
This combination allow both field and lab measurement.



Hendrickson simplified lake model protocol with NPQ

Innovative PAR Clip

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.

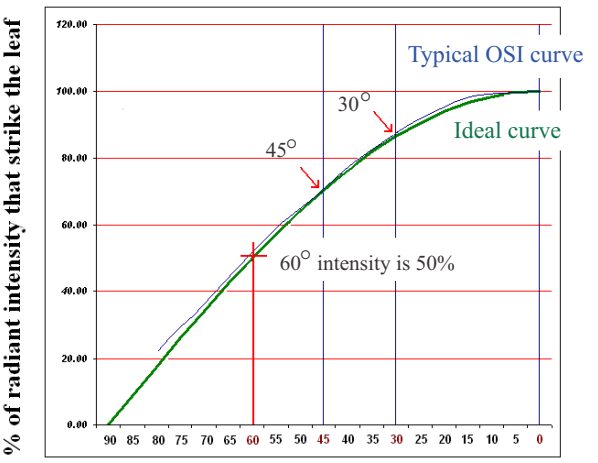
By developing a *bottom opening* PAR Clip, this new model prevents inappropriate opening when measuring leaves above the operators head, or when mounted on a tripod that occurs with some industry designs. As a result, the Opti-Science PAR Clip allows one handed operation, and eliminates two handed operation.

This PAR light sensor is positioned to allow measurement of ambient PAR as well as PAR from internal actinic light sources. Special care must be taken when using internal light sources for actinic illumination measurement. For reliable measurement, with internal light sources, cosine correction, spectral error, PAR sensor location error, lamp and instrument heat must all be take in to account, as is done with Opti-Sciences PAR Clip.

Leaf temperature is measured reliably to +/- 0.1 °C over the instrument operating range and during all measuring protocol conditions.

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 the 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

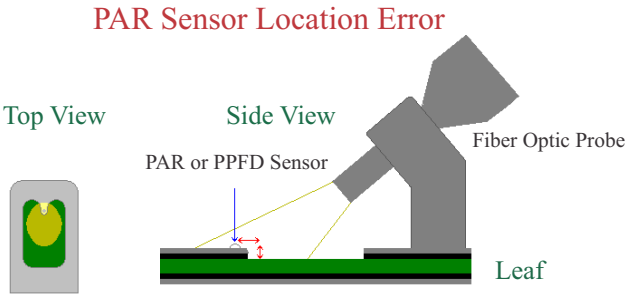


Angle variation from perpendicular (or normal)
As the angle of irradiation increases from perpendicular, the irradiation per unit area per second decreases.

Innovative PAR Clip

Sensor Location error In 2000, a researcher found that having a PAR sensor at a different plane from the leaf plane, and that is also laterally displaced from the center of measuring field, can produce an error in PAR measurement of up to 10%.

To correct for this error, Opti-Sciences calibrates the PAR at the leaf plane in the center of the measuring field.



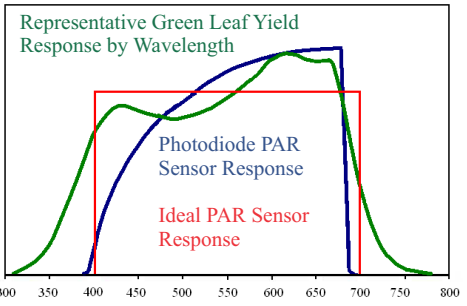
There is no significant error when measuring sun light. However, when internal actinic illuminators are used to drive photosynthesis at an angle, and through a fiber optic bundle, the error can be as much as 10% (Rascher 2000).

When making light curves, rapid light curves, non-sequential light curves, quenching measurements, and Yield measurements with internal illuminators, one should correct for this error. Opti-Sciences does the correction automatically.

Spectral Error Different light sources produce different spectrums. Opti-Sciences calibrates it's PAR sensors to different light sources to minimize the error at the leaf plane.

The Opti-Sciences PAR clip is calibrated to sunlight, the and the internal LED actinic light source. Correction factors may also be added for external light sources found in laboratories.

The correct calibration is automatically selected when a light source is chosen.



Unique - Stable light source

When used with the PAR Clip, the OS1p+ maintains a stable actinic light intensity to +- 2 % 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 OS1p+ 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 15 to 20 minutes for a specific light level. There is sufficient reserve light intensity to maintain an actinic intensity of greater than 2,000 µmols for extended periods of time.

F_M' correction – based on Loriaux, (2013)

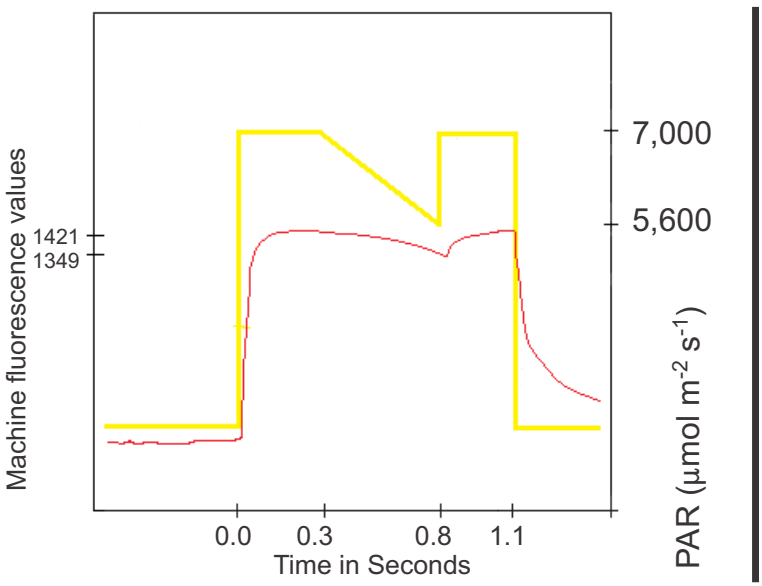
Saturation pulses used with modulated fluorometers are designed to close all PSII reaction centers. The maximum fluorescence intensity value, of the saturation flash, F_M', is used in most measurements including, quantum yield of PSII Φ_{PSII} (also called Y(II) or $\Delta F / F_M$'), *J* (or ETR), and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

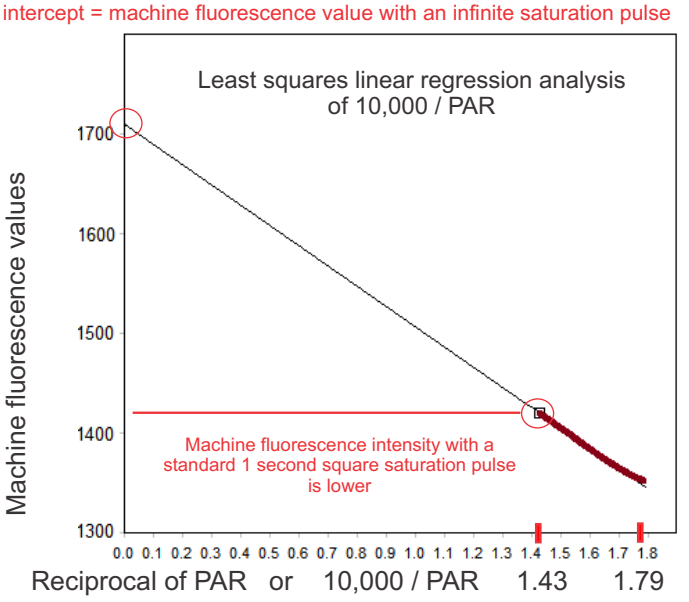
This standard option is provided on the OS5p+, the i*FL*, and OS1p instruments. It is *available for all Light adapted and quenching protocols,* and it can be turned off or on. The method described by the Loriaux, Burns, Welles, McDermitt, & Genty (2006) and expanded by Loriaux, Avenson, Welles, McDermitt, 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 F_M' correction works



Least squares linear regression of 10,000 / PAR values

y intercept = machine fluorescence value with an infinite saturation pulse



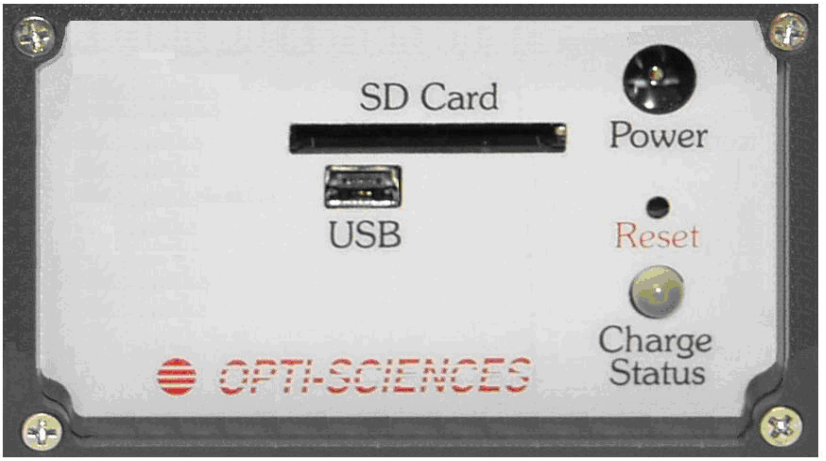
The first saturation flash step, shown on the left, is at 7,000 µmols for 0.30 seconds to saturate PSII. The saturation flash intensity is then ramped downward by 20%, making a large number of fluorescence measurements along the way, to 5,600 µmols. The ramping rate is less than 0.01 mol photons $m^{-2}s^{-2}$. 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 (2013) method for estimating F_M' 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

Data Management

The OS1p 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 OS1p. When connected to a PC, the OS1p 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 OS1p 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.

Parameters Measured and Protocols included:

Y: Quantum Photosynthetic Yield of PSII (or $\Delta F/F_M'$ or Y(II))
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
F_S (or F): Fluorescence under steady state conditions (prior to saturation pulse)
Multi-Flash with F_M' correction and ETR correction

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
$I_k = /\alpha$ is a measurement of the light intensity where light saturation dominates, or the minimum saturation level
I_m = PAR light intensity at ETR_{MAX}

Hendrickson lake model quenching protocol with NPQ, Y(NPQ), Y(NO), Y(II), NPQ, F_V/F_M

The Optional PAR Clip - provides PAR and leaf temperature. It is should be purchased for Y(II) and ETR measurements. The PAR Clip is also recommended for quenching measurements because it allows the built-in actinic light source to remain at a stable light intensity during longer measurements.

Light Sources:
Saturation pulse White LED with 690 nm short pass filter. 11,000 µmols
Modulated light
660 nm LED with 690 nm short pass filter.
Actinic light source: White LED to 3000 µmols
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 10 to 10,000 points per second, depending on phase of test.

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

Multi-Flash Fm' correction for all light adapted protocols. 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

Chlorophyll fluorescence is the method of choice for measuring most types of plant stress and monitoring plant health. The reasons for this are simple. Fluorometers are truly field portable, stress measurements only take a couple of seconds, and instrumentation is very cost effective.

An up to date compilation of papers related to plant stress measurement is available from Opti-Sciences free of charge. The compilation lists the value and limitations of the technology.

Opti-Sciences line of Chlorophyll Fluorometers are superb for field research, work, lab work, and even teaching. Instruments are available to meet most needs and budgets.

To receive a free plant stress guide that provides and overview of the value and limitations of chlorophyll fluorescence in stress measurement contact Opti-Sciences

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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 cuvetts.

Airline approved for carry -on luggage.

Accessories included:

- 1 Open Body Actinic Light Leaf Cuvette –light adapted work
- 10 Dark Adaption Cuvettes
- Fiber Optic Probe
- Battery Charger
- USB Cable
- Carrying bag with shoulder strap
- Data Card Reader and 1 GByte Data Card
- Storage and Transport Case

Optional features & accessories:

- PAR Clip - for Photosynthetically Active Radiation and leaf temp.
- Algae Cuvette
- 70 hour battery belt
- Tripods

OS1p - Best in Class

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