Finally, …an inexpensive *light adapted* Y(II) & ETR measuring instrument based on hard science.

Y(II) or $\Delta F/F_M$' or ($F_M' - F_S$) / F_M') is a time tested *light adapted* parameter that is more sensitive to more types of plant stress than F_V/F_M. A survey of existing research strongly supports this statement. While F_V/F_M is an excellent way to test for many kinds of plant stress, and the health of Photosystem II, ***Quantum Yield of PSII or Y(II) is a test that allows the measurement of the efficiency of photosystem II under actual light adapted environmental and physiological conditions.***

The new Y(II) meter includes:

1. ***Y(II) measurement & ETR measurement corrected for leaf absorptance.***
2. ***Leaf absorptance measurement including leaf transmittance using RGB sensors. (It may be used on larger Arabidopsis leaves.)***
3. ***PAR measurement (photosynthetically active radiation near the leaf)***
4. ***Leaf temperature measurement averaged over a large area.***
5. ***F_M' correction option at high actinic light levels, according to Loriaux 2013.***
6. ***A long term automated "monitor fluorometer mode" that allows measurement day and night. Useful for laboratory work. There are holes in the base of the chamber to allow photosynthesis.***
7. ***Relative humidity sensor***

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Affordable

Y(II) meter was designed to provide an inexpensive way to measure Y(II) and to make it available to more people that need it. Opti-Sciences developed a system that does not require expensive fiber optics, but still follows the proven methods needed for reliable plant stress measurement. Y(II) is measured from the top of the leaf along with PAR or photosynthetically active radiation. Furthermore, leaf temperature is measured from the bottom over a large area, allowing a more reliable value.

Leaf absorptance is normally measured using an integrating sphere; however, this new instrument provides a reliable measuring estimate of leaf absorbtance and absorptance change due to different field conditions. Results correlate well to integrating sphere values.

According to Eichelman (2004) leaf absorptance can vary from 0.7 to 0.9 in healthy leaves. The values vary not only by species, but they can also vary by the plant type, and the amount of some types of plant stress (Carter 1993). Absorptance also changes if plants are subjected to different lighting conditions or some types of plant stress while growing (Baker 2008). It was recently found that leaf absorptance also changed with light intensity. At near saturating light intensities, chloroplasts migrate from the top of plant cells to the sides of the cell, increasing leaf transmittance, and decreasing leaf absorptance (Cazzaniga 2014, (Dall'Osta 2014). As a result, it may be used in conjunction with other instruments to provide a more reliable ETR, or "*J*" value (electron transport rate).

The Y(II) meter also allows the option to use F_M' correction according to Loriaux & Genty 2013.

The new Y(II) meter can also be purchased with an inexpensive F_V/F_M meter for dark adapted measurement.

The meter (shown below) follows proven methods to make reliable measurements. It may also be used with dark adaption clips for measuring large sample populations quikly.

F_V/F_M is a measurement made at a known, common dark adapted point for comparing samples. Values from 0.79 to 0.84 can indicate no measureable plant stress, while lower values indicate that plant stress is affecting the F_V/F_M measurement and photosystem II.

FV/FM meter used with dark clips.
The inset shows the graphic measuring screen in bright sun light.

F_V/F_M Meter:

1. Works with <u>dark clips</u> to allow measurement of large populations quickly.
2. Screen visible in bright sun light
3. Graphic F_V/F_M trace display
4. 2 GB of memory allow storage of tens of thousands of measurements
5. USB transfer of comma-delineated files to Excel or other spread sheets.

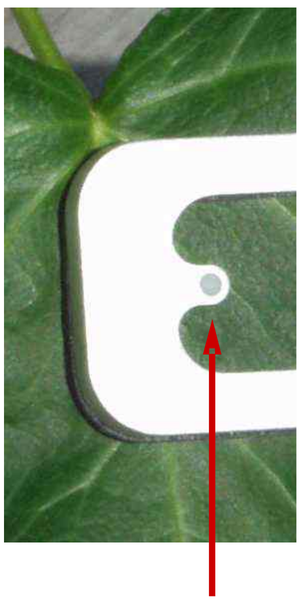
Innovative Y(II) meter design

Technology Advances

The Opti-Science Y(II) meter - PAR Clip was created to improve upon previous industry designs and maintain an affordable price.

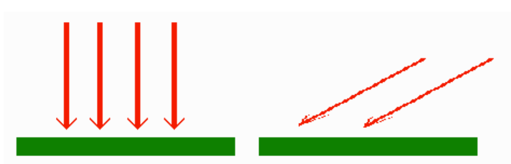
By developing a _bottom opening_ design, this new model prevents inappropriate opening when measuring leaves above the operators head, or when mounted on a tripod that occurs with some industry designs. As a result, the Opti-Science Y(II) meter allows one handed operation, and eliminates two handed operation.

This PAR light sensor is positioned to allow measurement of ambient PAR very close to the leaf and at the same angle as the leaf.



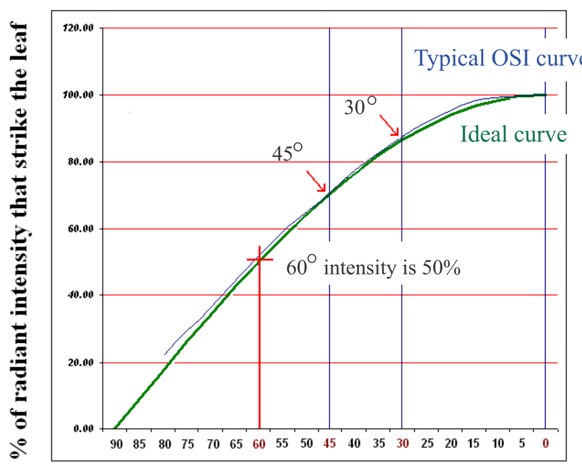
Cosine Corrected PAR Sensor

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.



Less light strikes the leaf at steeper angles

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.

F_M' correction - **based on Loriaux (2006), & Loriaux, (2013)**

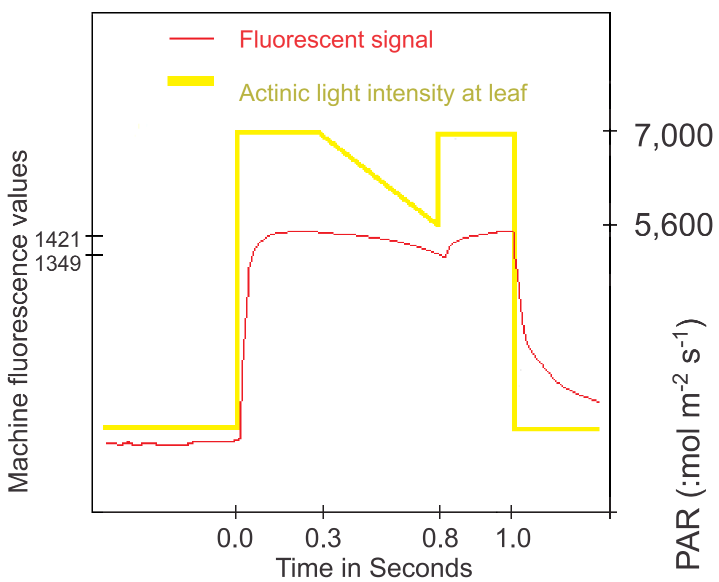
Saturation pulses used with modulated fluorometers are designed to close all PSII reaction centers.
The maximum fluorescence intensity value, of the saturation flash, F_M', is used in most measurements including, quantum yield of PSII M_{PSII} (also called Y(II) or)F /F_M'), *J* (or ETR), and in all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

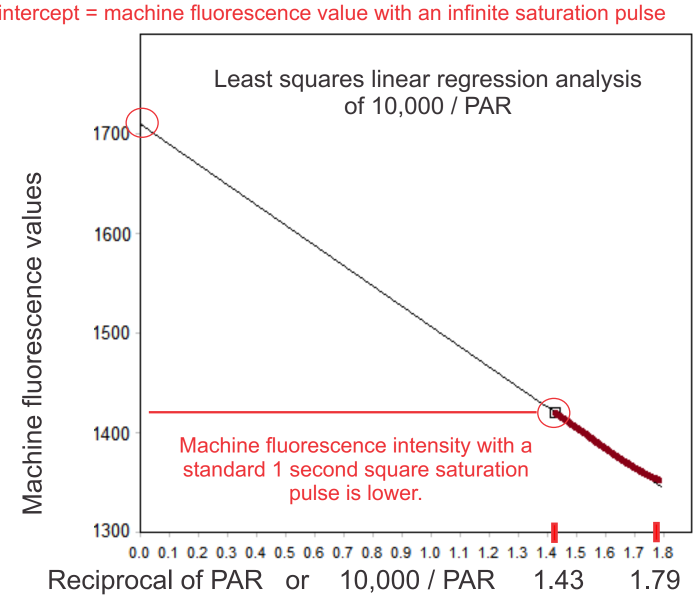
This standard option is provided on the Y(II) meter, OS5p+, the i*FL*, and OS1p instruments. It is *available for all Light adapted and quenching protocols,* and it can be turned off or on. The method described in a poster by the Loriaux, Burns, Welles, McDermitt, & Genty (2006) and expanded by Loriaux, Avenson, Welles, McDermitt, Eckles, Riensche, & Genty (2013), provides the most accepted method currently available. According to the science, the Y(II) meter provides the optimal saturation intensity of 7,000 :mols, 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 :mols 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 :mols. The ramping rate is less than 0.01 mol photons $m^{-2}s^{-2}$. The final phase is at 7,000 :mols 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

Based on hard science.

The original light adapted chlorophyll fluorescence test, developed by Bernard Genty, is designed to correlate with photosynthesis gas exchange measurements (Genty 1989, 1990). It provides a measurement of the light adapted, light reaction of photosynthesis under different conditions, and it can be more sensitive than F_V/F_M for some types of plant stress. (See the section on plant stress measurement).

For reliable measurement, samples must be at steady state photosynthesis, and measurement of Y(II) and PAR should be from the same side of the leaf as the light or photosynthetically active radiation (PAR) measurement. Most chlorophyll fluorescence happens in the upper layers of the leaf, and measuring from the top, significantly reduces leaf thickness and re-fluorescence as significant issues. Since Y(II) also varies with PAR and leaf temperature, both must be measured for reliable comparison of samples.

Measurement of leaf absorptance and leaf transmittance. ETR = Y(II) x 0.84 x 0.5
The average value for leaf absorptance, 0.84, and the ratio of PSII reaction centers to PSI reaction centers, 0.5, are shown as the default values normally used to determine Electron Transport Rate. The actual leaf absorptance varies for 0.7 to 0.9 in healthy plants (Eichelman 2004). This value varies with species, plant type, growth light conditions, growth plant stress conditions, current plant stress conditions (Baker 2008), and *light intensity* (Cazzaniga 2013, Dall'Osta 2014). While the most accepted way to measure leaf aborptance involves using and integrating sphere, the YII meter very closely approximates these measurements. The Y(II) meter uses RGB sensors to measure the PAR visible spectrum above and below the leaf. Calibration standards are included.

The ratio of PSII/PSI reaction centers varies by species, plant type (C_3 or C_4) light growing conditions and state transitions (Laisk 2014). Values of 0.4 to 0.6 have been measured (Edwards 1993, Laisk 1996). At this time, there is no reliable non-destructive way to measure PSII/PSI ratio in light adapted conditions. The PSII/PSI ratio value may be changed, if desired, in the software menu. Changing values will affect ETR.

Automated modulated light intensity adjustment -
The Y(II) meter provides an automated method to set the modulated light intensity correctly. It starts low and adjusts the detector gain control first 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. The intensity is less than 0.1 :mols.

Algorithm that prevents saturation pulse NPQ issue. 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. It also eliminates any electronic noise from being a factor.

More reliable leaf temperature measurement. By using an infrared, non contact sensor, the Y(II) meter measures leaf area over most of the measuring aperture and provides an average value over that area.
It is completely non-destructive, extremely durable, and provides more reliable measurement (Pons 2009) Measurements are made to +- 0.5^{o}C.

Humidity measurement - A solid state sensor that has been used in gas exchange measurement has been included in the Y(II) meter. Relative humidity is shown as a percent.

Reduction of leaf shading - the beveled edge of the measuring aperture helps prevent shading of the leaf by the instrument. However, the leaf measuring area has been reduced to allow absorptance measurement of smaller leaves like larger Arabidopsis leaves. Many smaller leaves can also be measured with the aid of custom measuring standards,

USB port for comma delineated measuring files that may be opened directly in Excel or Matlab .

2 gigabytes of data storage - ***for almost unlimited data collection.***

Red modulated light source.

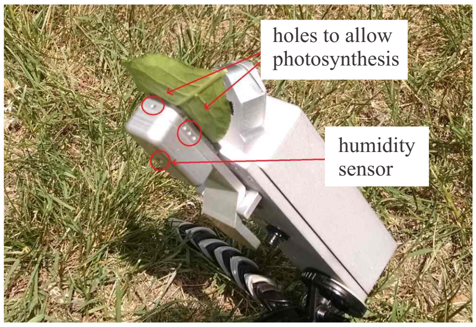
Attention to Detail

Data Management

The Y(II) meter provides a two gigabytes of non-volatile flash memory designed to prevent data loss due to power interruption.

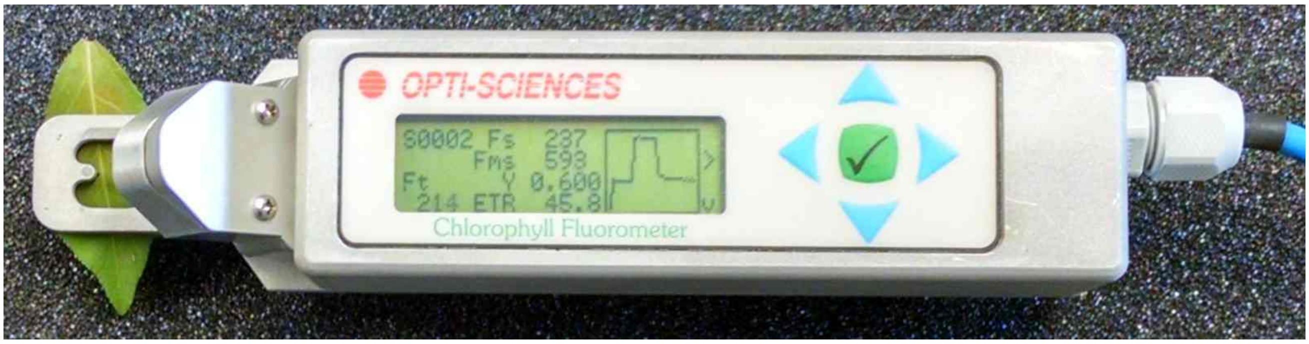
USB

A USB cable is provided on the back of the Y(II) meter. When connected to a PC, the Y(II) meter becomes a hard drive for a computer, allowing the transfer of data measuring files, and software upgrades. No special software is required. Files may be opened with Excel , or any other program that takes comma delineated information. *Any USB battery may be used*. The one shown below will provide more than 8 hours of use without recharging, connect to mains current, and ***can be put in a pocket.***

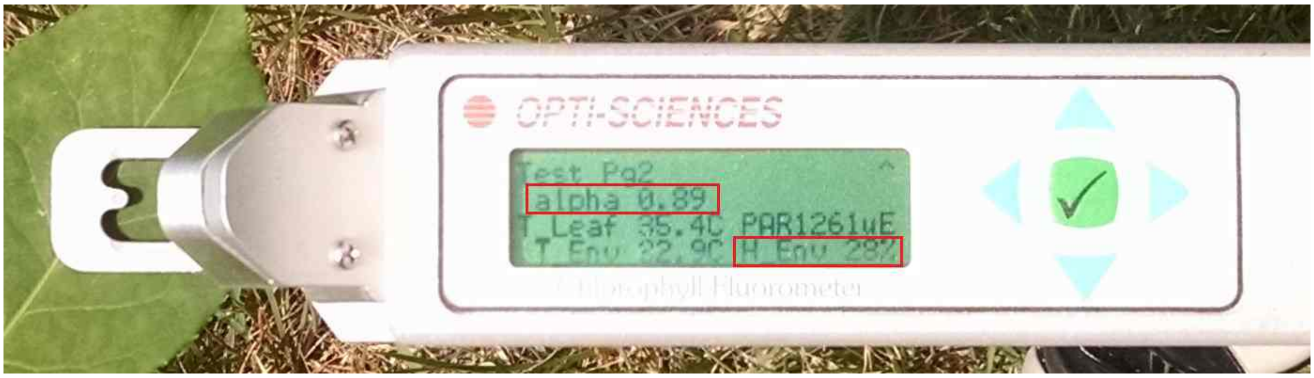


Holes allow CO_2, O_2, and H_2O to enter and leave the leaf.

Humidity sensor allows relative humidity measurements to be related to other measurement parameters.



Graphic display of Y(II) called Y here. Fms is F_M', and F_S is the light adapted fluorescence signal



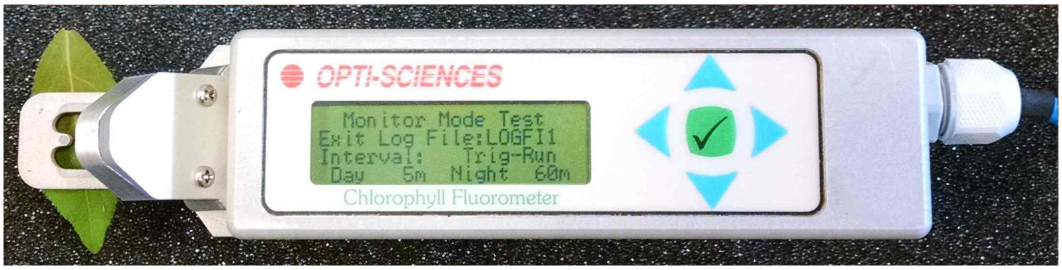
Alpha is leaf absorptance, PAR, leaf temperature, environmental temperature, and relative humidity readout are shown above **in the brightest sunlight**.

New - Monitor Mode

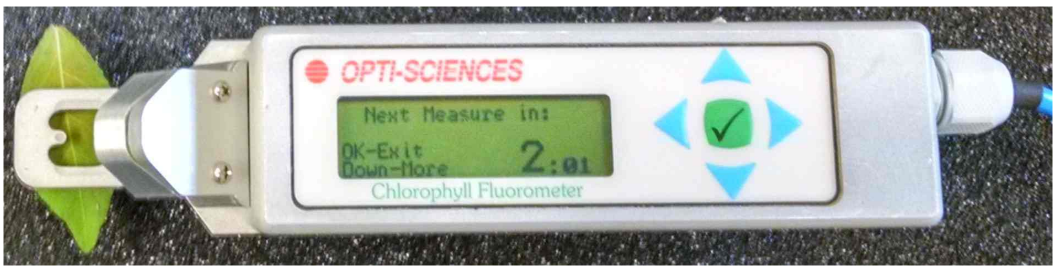
This measuring mode is designed to measure leaves, day and night, over long periods of time, during growth periods, or even season long periods. Due to its design, it can be used in growth chambers or for laboratory use, with controlled lighting. While the Y(II) meter is perfect for field use, the monitor mode is not designed for field use, due the edge effects that can occur with a smaller measuring aperture when the sun is low in the sky. Y(II) = F_V/F_M in the dark at steady state. Furthermore, this data can be used to calculate NPQ and other quenching parameters.

The times between measurement can be set differently for day and night measurement. While frequent intense saturation flashes do not damage plants in the day time, they can damage dark adapted plants. Both times can be set from 1 minute to 255 minutes. Each instrument will run as long as it has power (It will work with any USB power source including AC) or until measurements fills up the huge 2 gigabytes of memory.

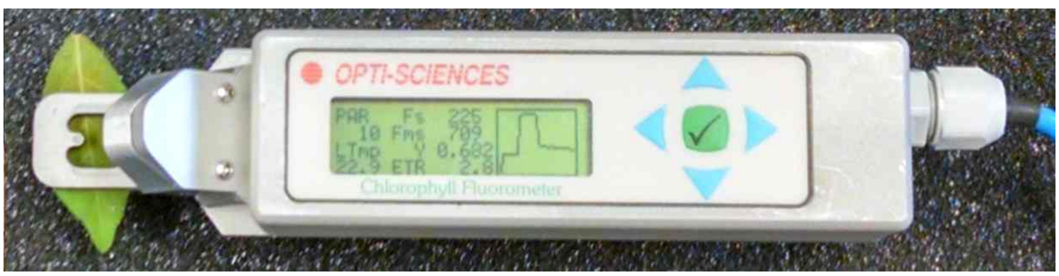
The White light saturation flash occurs just before the leaf absorptance measurement.



During a measuring cycle, the screen looks like the one shown below. It shows 2 minutes and 1 second until the next measurement.



By pressing the down arrow, after a measurement, the following screen appears. The Y(II) (or Y here) is shown with a PAR value, ETR, leaf temperature, F_M' (or Fms), and Fs or the light adapted fluorescence measurement. Leaf absorptance is also measured day and night; however, the values are only displayed in the data file. The graphic trace from the last measurement is also shown. Relative humidity is measured, but it is only available in the data file.



F_M' correction according to Loriaux 2013 is also possible.

Comparison of Y(II) and F_V/F_M for stress measurement

For more detailed information request the Opti-Sciences Desktop Stress Guide.

Stress	Yield of PSII – Y(II)	F_V/F_M
Drought Stress	Y(II) Is not sensitive to drought stress in C_3 plants until the drought is severe due to photorespiration (Flexas 1999, 2000). *The only reliable fluorescence test is using the Burke assay with Y(II). It is very* sensitive to *very early water stress in* C_3 *plants.* (Burke 2007, 2010)	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008) It can be used for severe drought stress in plants like trees that survive severe drought stress. It will not detect drought stress until about 7 days after irrigation.
Drought Stress	In $\mathbf{C_4}$ plants, Y(II) and ETR can be used for reliably for drought stress. The ratio of ETR to carbon assimilation is consistent. It does not work in C_3 plants. ETR = (Yield) (PAR) (0.84)(0.50) (Cavender-Bares & Bazzaz 2004) Y(II) is sensitive to drought stress in C_4 plants (da Silva J. A. & Arrabaca M.C. 2008).	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008). Fv/Fm is not sensitive to water stress in C4 plants, grasses are tested (da Silva J. A. & Arrabaca M.C. 2008).
Drought Stress	Fs a component of Y(II) is sensitive to *moderate drought stress* at saturation light levels. Fs/Fo is a normalized ratio that uses predawn dark adaptation and steady state fluorescence measurement at high light levels for moderate drought stress. While adequate for plants such as grapes, it is does not work well for most plants. (Flexas 1999), (Flexas 2000), (Flexas 2002).	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (Zivcak M., Brestic M, Olsovska K. Slamka P. 2008)

Continued on next page

references at the end

For more details request the free Plant Stress Guide from Opti-Sciences

Stress	Yield of PSII – Y(II)	F_V/F_M
Nitrogen Stress	Y(II) can be used for *early* nitrogen stress by adding *intense light* (Cheng 2001). *It is common to use chlorophyll content meters for nitrogen stress.*	F_V/F_M is not sensitive to nitrogen stress until very low levels are reached. (Baker 2004) *It is common to use chlorophyll content meters for nitrogen stress*
Light stress	Y(II) can also be used for light stress in steady state sensitive to light stress. (Cavender-Bares & Bazzaz 2004)	F_V/F_M can be used to detect light stress (Adams & Demming-Adams 2004)
Heat stress	Y(II) can also be used for moderate heat stress from 35ºC and higher in oak. (Haldimann P, & Feller U. 2004)	F_V/F_M can be used to detect severe heat stress in cotton above 45ºC. (Crafts-Brander and Law 2000)
Sulfur Stress	Y(II) is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004) *It is common to use chlorophyll content meters for sulfur stress.*	F_V/F_M is not sensitive to sulfur stress until starvation levels are reached. (Baker 2004) *It is common to use chlorophyll content meters for sulfur stress.*
CO_2 Stress	Not sensitive to early or moderate CO_2 stress. (Siffel & Braunova 1999)	F_V/F_M is sensitive to early or moderate CO_2 stress. (Siffel & Braunova 1999)
Salt Stress	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)
Nickel	ETR, a parameter derived from Yield at a known light level, is sensitive to nickel stress (Joshi & Mohanty 2004), (Tripathy 1981)	Not sensitive to nickel stress. (Joshi & Mohanty2004)
Zinc	Fs in Y(II) is a good indicator of zinc stress. (Joshi & Mohanty 2004) (Tripathy & Mohanty 1980) (Krupa 1993)	Not sensitive to zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980) (Krupa 1993)
Cold	Y(II) is sensitive to Cold stress(Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).	F_V/F_M is sensitive to Cold stress(Oquist and Huner 1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).
Herbicide	Sensitive to most types of herbicides. See the Opti-Sciences Stress guide for specific information.	Sensitive to most types of herbicides Not sensitive to DCMU (Nedbal & Whitmarsh 2004). See the Opti-Sciences Stress guide for specific information
Pesticides	Is sensitive to pesticides tested *including Trimax.* See the Opti-Sciences Stress guide for specific information	Is sensitive to pesticides tested but not Trimax. See the Opti-Sciences Stress guide for specific information
Chemical Stress	See the Opti-Sciences Stress guide for specific information	See the Opti-Sciences Stress guide for specific information
Other Stress	See the Opti-Sciences Stress guide for specific information	See the Opti-Sciences Stress guide for specific information

Y(II) meter Parameters Measured and Protocols included:

Y(II): Quantum Photosynthetic Yield of PSII (or)F/F_M' or Y)
ETR: Electron transport rate
PAR: Photosynthetically Active Radiation value
T: Leaf temperature
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)
Loriaux 2013 correction of ETR, and F_M' option included for Y(II) mode and monitor mode.
α, or alpha, - leaf absorptance.
Monitor mode: allows long term measurement day, and night. Allows F_V/F_M, YII, ETR, leaf absorptance, PAR, leaf temperature, relative humidity, and calculation of NPQ. (*Mode is designed for growth chamber & lab work only*)
Relative Humidity: 5% to 95% to +- 2% over the range.

Will work with *any* USB power supply or AC.

Has tripod mount (tripod not included).

With F_V/F_M meter

F_V/F_M: Maximum Photochemical efficiency of PSII
F_V/F_O: A more sensitive detector of stress than F_V/F_M, but it does not measure plant efficiency.
F_O: Minimum fluorescence
F_M: Maximal fluorescence
F_V: Variable fluorescence

Automated modulated light set-up option included.

Will work with any USB power supply or AC.

USB data file output in a comma delineated format can be opened directly in Excel or other spread sheet products without additional software.

The size of the battery supplied allows easy insertion into clothing pockets.

Opti-Sciences Inc.
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Light Sources:
Saturation pulse White LED with 7,000 :mols with PAR clip
Modulated light
Red: 660 nm LED with 690 nm short pass filter.

Actinic light source: Ambient light only

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.

F_M' correction according to Loriaux 2013, for all light adapted modes . It may be turned on or off.

Test Duration: About 3 seconds for fast tests and may be run for several months in monitor mode.

Storage Capacity: 2 gigabyte of non-volatile flash memory, supporting almost unlimited data sets and traces.

Special Algorithms:
8 point rolling 25 ms average to determine F_M, F_M', F_O, & F_S eliminates saturation pulse NPQ & any electronic noise as an issue.

Automated modulation light intensity set up.
(manual may also be used)

Output: USB comma delineated files may be opened in Excel . No special software is required.

User Interface:
Display: Graphic black and white display
Menu driven with arrows. 132 x 32 pixels.

Power Supply: 8 hour USB lithium ion battery is standard, *but any USB battery can be used*. mains current may also be used. Mains plug is also supplied.
2 batteries are supplied if both the Y(II) & the F_V/F_M meter are purchased. USB chargers included.

Dimensions:
Both the Y(II) meter and the F_V/F_M meter are 9 in long with a USB cable that is 65 inches long
The case is 14"x 11"x 6" - included.

Weight:
Y(II) meter w/battery & USB cable- 1 lbs. or 0.45 kg.
F_V/F_M meter w/bat. & USB cable- 0.8 lbs. or 0.36 kg.
Complete w/case & accessories- 4.3 lbs. or 1.95 kg.

Operating temperature range $0^{O}C$ to $50^{O}C$

Dark adaptation clips - 10 supplied with case

Absorptance measuring standard - 2 included.

Accessories

Standard Storage Shipping and Transport Case.

This durable abrasion resistant water tight plastic case allows storage of the Y(II) meter and accessories There is also room F_V/F_M meter as well along with charger and dark adapting leaf cuvettes.

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Accessories included

- Battery Charger with mains plug
- Built in USB Cable
- Storage and Transport Case
- Ten dark adaptation clips when the F_V/F_M meter is purchased.
- Two absorptance standards.
- Manual on USB memory stick
- Lithium ion battery

Optional features & accessories:

- Tripods
- F_V/F_M meter
- Dark adaptation clips 10 are provided when the F_V/F_M meter is purchased.
- A second Lithium ion battery is included when the F_V/F_M meter is purchased.
- A case is included if the F_V/F_M meter is purchased separately.
- It is not uncommon for researchers to buy additional large quantities of dark adaptation clips for large plant populations. They are relatively inexpensive.

Y(II) meter

Is designed as an affordable & reliable light adapted chlorophyll fluorescence system.

OPTI-SCIENCES
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Email: sales@optisci.com Website: www.optisci.com

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