Y(II) meter & Plant Stress Kit

User's Guide

Reliable Y(II) measurement

Leaf absorptance measurement

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Chapter 1 • Introduction

Overview

This chapter provides you with a list of the equipment that you should have received with your Y(II) meter or Plant Stress Kit, and information about chlorophyll fluorescence with scientific references.

To select the best chlorophyll fluorescence measuring parameter for your application, consult the Plant Stress Guide provided as a separate document

The Plant Stress Guide is available on the memory stick provided with this instrument or by visiting www.optisci.com under Stress Testing, and receive a Stress Guide with References.

Welcome !

Congratulations on your purchase of the Y(II) Modulated Fluorometer. Please check the carton for any visible external damage. If you notice any damage, notify the freight carrier immediately. Follow their procedures for reporting and filing a claim. The carton and all packing materials should be retained for inspection by the carrier or insurer.

List of Equipment

Carefully unpack the carton. You should have received the following items:

- Y(II) Meter with USB cord
- USB charger plug with cable
- USB lithium ion battery
- Storage and shipping case
- Leaf absorptance standard in plastic envelope
- Thumb drive with instrument manual, application notes, and Plant Stress Guide

If purchased with the F_V/F_M meter, the kit becomes known as the "Plant Stress Kit". The additional items are listed below

- F_V/F_M meter with USB cord
- Ten dark-adaptation clips
- A second USB lithium ion battery
- A second charger plug with cable.

If any item is missing, please contact your authorized Opti-Sciences agent.

Getting Started

The user interface consists of a black and white graphic screen LCD with an arrow keypad and a central green accept button.

For editing parameters and making measurements, menu options are presented as underlined words. To move the underline, use the blue arrows that run parallel to the measuring screen length. The perpendicular up and down arrows change parameter values. When done, use the parallel arrow closest to the screen to back out to the measuring screen or go back to the measuring screen by pressing the green button in the middle of the arrows.. Measuring tests and parameter adjustments are all menu driven.

The Y(II) meter and F_V/F_M meter have default settings that allow the unit to work for many applications, however, changing the settings are very easily done.

Data is stored in a 2 Gigabyte on-board flash memory, as a result, no data will be lost if the main battery is depleted. Stored data may be transferred to other systems through use of the USB port. The data is output in comma delimited, carriage return separated ASCII strings, easily importable to most spreadsheet programs such as Excel and Mat Lab.

Unpacking and Assembly

F_V/F_M meter

USB lithium ion batteries

Dark clips,

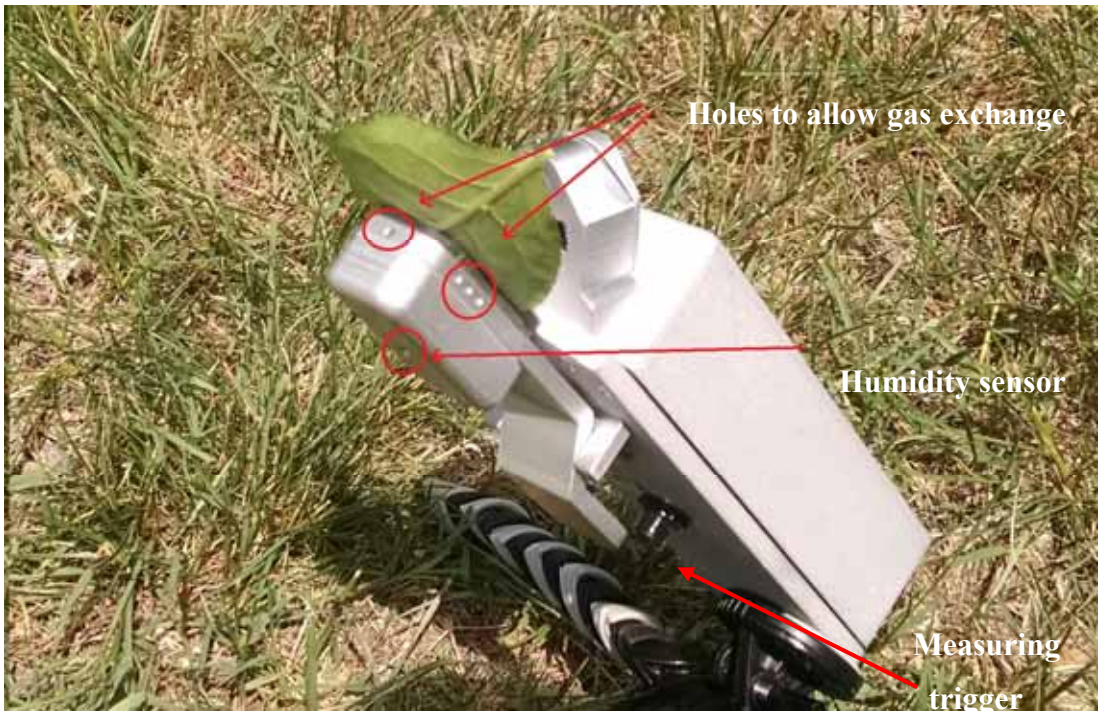
USB mains plug

Y(II) meter

Picture of open plant stress kit

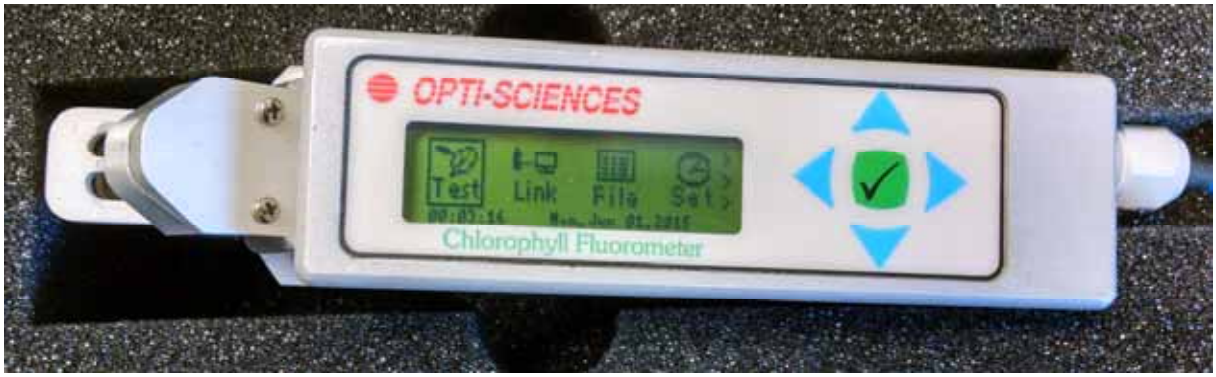
Assembled Y(II) meter

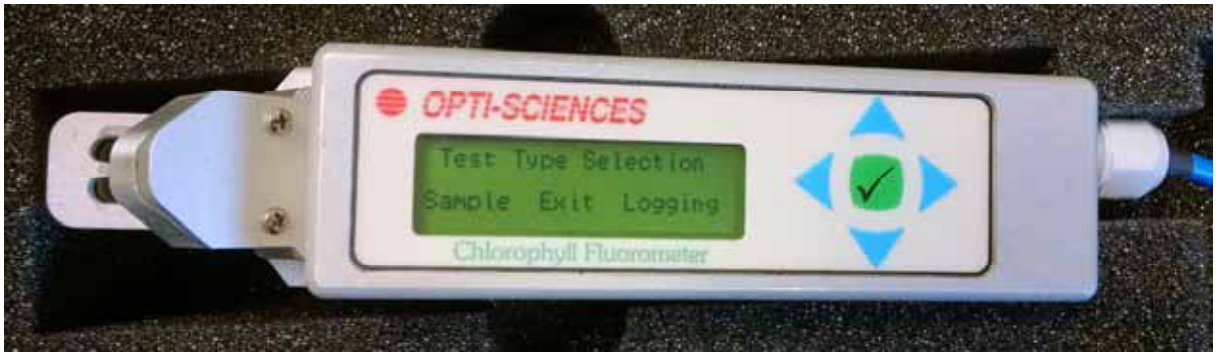
The Tripod is not standard.



After the Y(II) Meter has been assembled and connected to a charged battery, tap the green button in the middle of the blue arrows to turn on the instrument. A beep should occur.

A main menu with icons should appear. Choose “Set” to change date and time using the arrows that are parallel to the display. When the time and date icon have been highlighted, tap the green button to adjust the time and date. Use the parallel arrows to move around, and the perpendicular arrows to increase or decrease values. When done, tap the green button to back out of the main menu location.





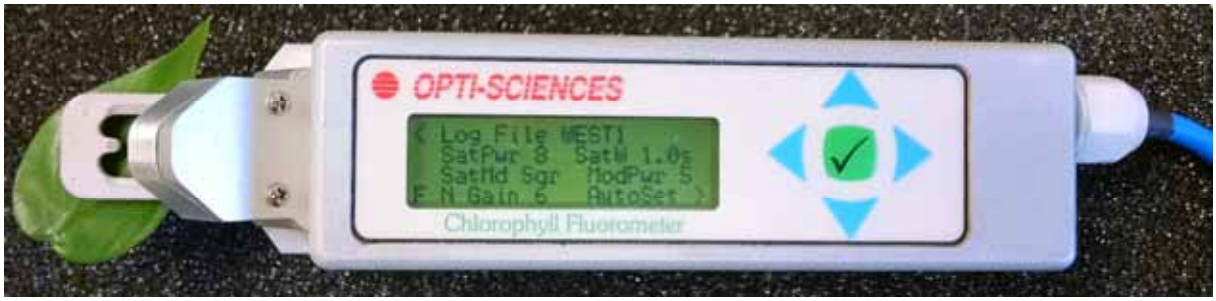
Main measuring screen

Measurement Set Up

From the main menu, select “Test”.

The main Y(II) measuring screen will appear as shown above, but without the graph and measured values. To adjust the settings, tap the blue arrow parallel and farthest from the display. Adjustments include:

Adjustments are reached using the arrows that are parallel to the length of the instrument from the measuring screen.



1 “Log File” – make sure that the flashing cursor line is under the Log file name using the arrows parallel to the length of the instrument. Use the arrow perpendicular to the long axis to select changing of the name and scrolling through the alphabet as well as numbers 0-9 for each character in the log file name. There is a blank between the Z and 0 value. Names can be up to eight alpha-numeric characters long. When done, press the green button to return to the previous screen. Existing files can be loaded by going to the main instrument screen and by moving the cursor to “Files”. Press the green button and select “View”. Use the perpendicular arrows to scroll through the list of existing Log files. The number of existing files and the file being viewed are shown on this screen. When done, press the green button three times to go back the main menu.

2 “SatPwr” is saturation light intensity. It can be set from 1-8. 8 is 7,000 µmols, and each step is approximately 1000 µmols lower than the last higher number. If F_M’ correction or “MLv” (multi-level – variable) is selected, SatPwr does not work. The saturation flash goes through a set routine using the protocol from Loriaux 2013. When done, move the cursor with the parallel arrows.

3 "SatW" is saturation flash width. It be adjusted from 0.5 seconds to 2.0 seconds; however, the Y(II) meter has a special 8 point 25ms rolling average algorithm that ensures the correct measurement of F_M' will occur by finding the highest average fluorescence value over a 25ms period, independent of saturation pulse NPQ, as long as the saturation flash is wide enough. For most plants that is 1.0 seconds. If F_M' correction or "MLv" (multi-level – variable) is selected, SatW does not work. The saturation flash goes through a set routine using the protocol from Loriaux 2013. When done, move the cursor with the parallel arrows.

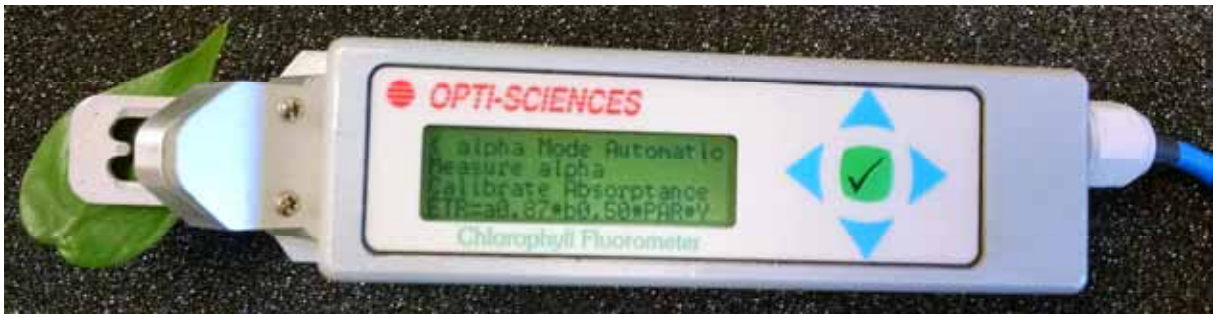
4 "SatMd" is saturation method. The choices are "Sqr" or square flash, and "MLv" or multi-level-variable uses for F_M' correction according to Loriaux 2013. Square flash provides the standard square topped saturation flash. F_M' correction provides a special protocol routine that follows Loriaux 2013 for F_M' correction. One of the Co-authors of the paper was Bernard Genty, the inventor of light adapted measurement back in 1989. For a number of years, it has been found that at high actinic light intensities, near light saturation, even the most intense saturation flash could not completely close or chemically reduce all PSII reaction centers (Earl 2004), (Loriaux 2008) (Loriaux 2013). This is a requirement for reliable Y(II) or $\Delta F/F_M$' measurement. If F_M' correction is used, it will not significantly change F_M' values at lower actinic light levels, but it will significantly change F_M' values at higher actinic light levels. The protocol starts at 7,000 µmols for 0.3 seconds, and then ramp down 20% in intensity while measuring the resulting fluorescence for another 0.5 seconds. Finally the value will become 7,000 µmols again for another 0.3 seconds. If square topped saturation values are chosen, the intensity may be adjusted from a maximum intensity of 7,000 µmols, in approximately 875 µmol steps down to a minimum of 875 µmols. Values should be at least 1,800 µmols for sun leaves and at least 500 µmols for indoor plants. Light adapted plants are not damaged by very intense saturation flashes even at 7,000 µmols. For more details, see the F_M' correction application note from Opti-Sciences, or read the Loriaux 2013 paper.

5 "ModPwr" & "Gain" are the *modulated light intensity setting and modulated light gain control*. ***This parameter is only used if the "Autoset" is not used.*** The combined intensity and gain must be set high enough to make a measurement but low enough so that it does not drive photosynthesis. ***First, put a leaf in the measuring chuck.*** Both values may also be adjusted manually from 1-6 by adjusting the "Gain" first and "ModPwr" second, until the instrument will measure. The perpendicular blue arrows adjust the value. When done, use the parallel arrows to go to the next parameter. If manual adjustment is used, one must watch the "Ft" value on the main measuring screen to see if it rises after one or two minutes with unchanged actinic lighting conditions. The intensity of the modulated light is in the range below 0.4 µmols. "Autoset" adjusts the electronic gain first.

6 "F" or electronic filtering. The choices here are "Y" for yes, and "N" for no. We recommend that the "Y" be used. It makes for a less noisy graphic display and a more reliable measurement.

7 "Autoset" automatically sets the modulation intensity to the correct setting. One that is not to high to drive photosynthesis, but high enough to allow measurement. It saves

time and eliminates errors. The gain is adjusted first and the intensity is adjusted second. *A leaf similar to the leaves to be measured should be in the measuring chuck before adjustment takes place!* Press one of the perpendicular arrows to enable "Autoset".



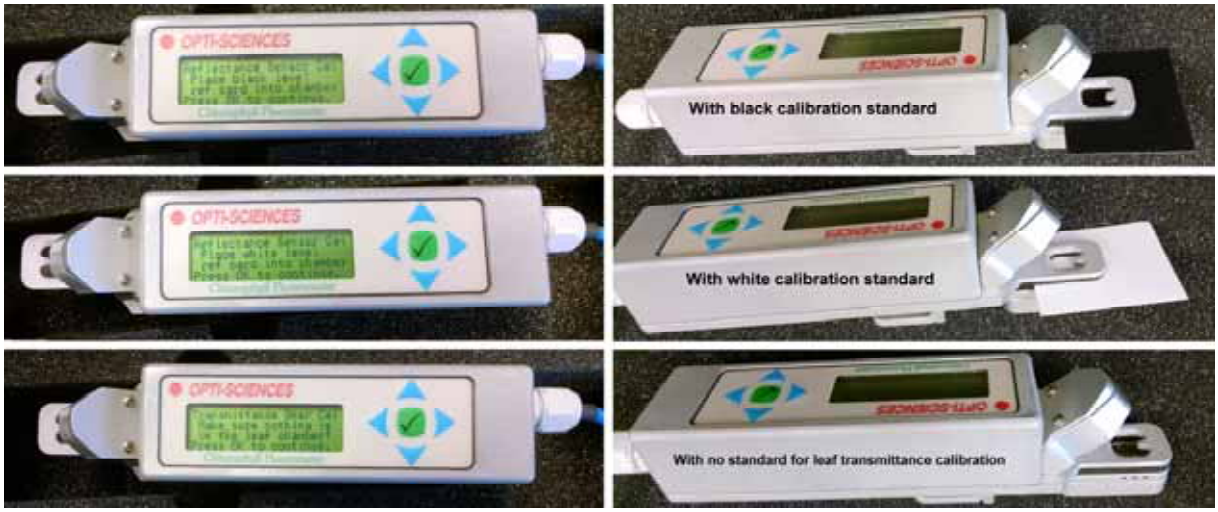
8 "alpha Mode" allows absorptance measurement or turns it off. The selections are "Automatic" or "Manual". On manual, the value at the bottom of the screen can be changed and added to the formula to determining ETR or electron transport rate. The default value is 0.84. When the "Automatic" mode is used the absorptance feature needs to be calibrated with black and white standard supplied. Select "Automatic" with the perpendicular arrows. Move the cursor to "Calibrate Aborptance" and use the perpendicular arrows to initiate calibration. A calibration rectangle is supplied that is black on one side and white on the other. When asked by the instrument, insert the black side up, inside the chuck, completely covering the open aperture for 0% calibration. Press the green button. Next, repeat the process with the white side up for 100% calibration. Press the green button. For transmitted calibration, remove the standard and anything else from the aperture, to calibrate 100% transmittance. Press the green button. Calibration is now complete. The instrument flashes a 1,000 µmol white light during calibration to ensure an adequate minimum measuring value. However, to keep the error small relative to the light intensity used, it is recommended that the calibration be done near the highest actinic light intensity to be use for measurement. This minimizes measurement variation. *Do not calibrate quickly changing light conditions, or an error will occur*.

Calibration of Absorptance

From the main screen, select "Test" using the arrows parallel to the length of the instrument. Push the green button. Use the parallel arrows to select "Sample" and push the green button. You are now on the main measuring screen. Tap the right parallel arrow, and a second set up screen appears. Continue to use the right hand parallel arrow to move the black line cursor through all of the options on this page, and then on to the next page. Continue using the same arrow to highlight "Calibrate Absorptance". Next, press the up perpendicular arrow until the "Reflectance Sensor Cal" appears. Take the Black and White card in the clear envelope out of the envelope and place it into the measuring chuck completely covering the measuring aperture with the black side facing up. Push the green button. Next the screen says to place the white side up in the chuck completely filling the measuring aperture. Press the green

button. Next, make sure that the black and white card has been removed with nothing in the measuring aperture. Press the green button. A screen will appear that says calibration complete. Using the left hand parallel arrow back out to the main measuring screen. The instrument flashes a 1,000 µmol white actinic light during calibration to ensure an adequate minimum measuring value. However, to keep the error small relative to the light intensity used, it is recommended that the calibration be done near the highest actinic light intensity to be use for measurement. This minimizes measurement variation. *Do not calibrate under quickly changing light conditions, or an error will occur.*

\Calibration screens: Black side up, white side up, and nothing in the chuck.



Page 1 of the measuring screen shows Y(II), ETR, F_S, F_M' & the graph of Y(II)

Page 2 of the measuring screen displays alpha or leaf absorptance, leaf temperature, cosign corrected PAR (photosynthetically active radiation) & Relative humidity.

Cook Book check list for reliable Y(II) measurements

Put together an experimental design that takes these issues into account.

1. Leaves must be at steady state photosynthesis. Above canopy leaves on a clear day, in the field, are considered to be at steady state photosynthesis. (Maxwell and Johnson 2000). This takes between twenty minutes *and thirty five minutes* due to chloroplast migration, a light avoidance mechanism that changes fluorescence parameters by changing leaf absorptance (Cazzaniga S. 2013). Wind may also be a factor, as it changes the angle of the leaf to the sun at a given light level.

2. It is dangerous to make Y(II) measurements on below canopy leaves in the field. The shade from higher leaves and wind can interrupt a plant's adjustment to steady state under ambient conditions. The xanthophylls cycle, and Δph of the thylakoid lumen adjust in about four to seven minutes in the field (Lichtenthaler 2004) (Baker 2004) (Baker 2008), lab grown plants and green house grown plants adjust in shorter time scales. State Transitions take between fifteen and twenty minutes to completely adjust. It has been found that state transitions are a big factor at lower light intensities, but they are not a factor at high light intensities. At near saturation light intensities, chloroplast migration, a light avoidance mechanism, takes between 20 minutes and thirty five minutes to adjust to steady state photosynthesis. 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 twenty to thirty five minutes, to reach steady state, and then make a measurement. Wind can also a problem, as it can change lighting conditions and ambient steady state is no longer possible.

3. Y(II) values vary with light level and with temperature. 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, near leaf level and leaf temperature. Comparing Y(II) values taken at different light levels and different temperature levels introduces a significant error, unless it is the change, at different light levels and heat levels, that is of interest. This is commonly done with a PAR sensor in the Y(II) meter, measuring PAR near the leaf to be

measured. (Genty 1989), (Genty 1990). Furthermore, light intensity varies inversely with the square of the distance. Since the sun is about 93,000,000 miles away, a few inches or feet have little affect on measurement; however, when using artificial lighting a few inches can make a significant difference.

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

5. 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)

6. Leaf orientation. When making a yield measurement, *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.

7. It is common to use the youngest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997). In addition, using below canopy leaves can be more difficult due to wind or partial shading conditions.

9. On some plants, such as wheat, one should select similar positions on leaves for comparison purposes, because measurements can vary by leaf measurement location. For example; the tip of the leaf will likely be different than the mid section or the base. (Discussions with Wayne Loescher at MSU)

10. 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. Times outside these ranges increase the error in Y(II) measurements with most chlorophyll fluorometers. 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). Some fluorometers allow adjustment of this parameter, and others are preset at the factory. The OS1p, the OS5p, the OS5p+ the iFL and the Y(II) meter have a special built-in algorithm that uses a rolling eight point average capability to detect the highest eight point, 25 msec. rolling average. This prevents saturation pulse NPQ from being a problem if the duration is long enough. If one wants to measure the same point again, one must wait for about 120 seconds because it takes that long for saturation pulse NPQ to fully dissipate.

11. Saturation pulse intensity. Saturation pulse intensity is more of an issue with Y(II) than with Fv/Fm. When dark adapting, shade leaves will saturate at a few hundred µmols, and sun leaves will usually saturate below 1,500µmols. Indoor plants and under canopy plants saturate at much lower light intensities. However, a problem has been found when measuring Y(II) at high 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 and required for reliable measurement. Even with a 7,000 µmol saturation pulse, or higher, 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, a protocol developed by Loriaux 2013 uses a multiple phased saturation flash. The first 0.3 seconds of the flash is at 7,000 µmols, followed by a steady reduction of intensity by 20%

over 0.5 seconds. The final 0.3 seconds of the flash is at 7,000 µmols again to detect saturation pulse NPQ. The protocol then uses least squares linear regression analysis to determine what the fluorescence intensity would be with an infinite saturation flash. At lower actinic light intensities there is no significant difference from square topped saturation flashes, but at near saturation light levels the difference is dramatic. One of the co-authors of the Loriaux 2013 paper is Bernard Genty, the man that invented the Y(II) test back in 1989. FM' Correction according to Loriaux 2013 is provided as an included option on the Y(II) meter, the OS1p, the OS5p+, and the iFL. (For more information on this topic, see the Multi-flash application note for more details www.optisci.com), (Loriaux 2013), (Earl 2004) (a poster by Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. Genty B. 2006) (Markgraf, T. and Berry J. 1990).

12. PSI fluorescence - Part of the fluorescence signal contains PSI fluorescence as well as PSII fluorescence. With Y(II), one is trying to measure variable fluorescence of PSII in a light adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces a small error but it is not a problem for comparing similar samples, because PSI fluorescence does not change with light intensity temperature or plant stress. (Baker, Oxborough 2004)

13. "Super-saturating flash" error is produced by using a very intense saturation light source that is longer that 2ms causing multiple turnovers of primary PSII receptor Q_A and the reduction of plasotoquinone to plastoquinol. This raises Fm' and can cause an overestimate of Yield by less than 10% (Baker and Oxborough 2004), (Schreiber 2004). *Use of a super-saturation flash is by far the most common method of measuring yield in higher plants. As long as one is interested in plant stress and not exact correlation to CO_2 carbon assimilation this is not an issue.*

14. Cold stress can produce a non-linear correlation with CO_2 assimilation. Electron transport of PSII in cold stressed corn far exceeds the requirements for CO_2 assimilation by more than three to one, indicating that under these conditions, other electron sinks are at work. The ratio of ETR (a product of Y(II), PAR, leaf absorption ratio, and PSII absorption ratio) to CO_2 assimilation, under cold stress, can be diagnostic for cold stress. (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998)

15. The ratio of ETR to CO_2 assimilation can be diagnostic for drought stress in C_3 plants. C_3 plants exhibit strong electron transport rates for early and moderate levels of water stress even when CO_2 assimilation has decreased due to water stress. This indicates that there are other electron sinks for electron transport. (Ohashi 2005). *This problem of early water stress measurement and detection may be overcome by using a special assay discussed in Burke 2007 and Burke 2010.*

16. 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)

17. While linear correlation occurs between Y(II) and ETR with CO_2 assimilation in C_4 plants and curvilinear correlation between Y(II) and ETR with CO_2 assimilation in C_3 plants, (Genty 1989), (Genty 1990), (Baker Oxborough 2004), *exact* correlation between fluorescence ETR and gas exchange carbon assimilation is not possible due to the fact that

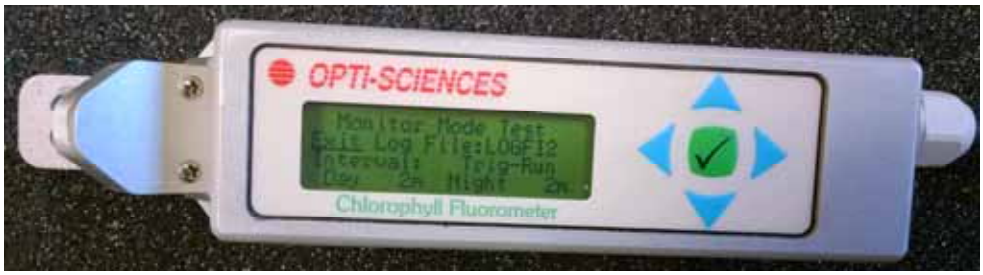
fluorescence comes from only the upper most layers of the leaf while gas exchange measurements measure lower layers as well (Schreiber 2004).

18. 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 CO_2 stress (low CO_2 levels as found in A/C_i curves), it is best to take multiple leaf measurements and average the values (Baker 2008). Chlorophyll fluorescence can also be heterogeneous under biotic stress or disease. Under these conditions it is best to take a few measurements on a leaf and average the values, for a more reliable measurement (Discussions with Clause Buschmann 2008).

19. Light history – It takes between forty minutes and sixty 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).

Monitor or Logging Mode

After Selecting “Test” from the main menu, select “Logging from the next menu.



The “Monitor Mode” allows long term measurement of a single sample over time. It is designed only for growth change use or laboratory use where the angle of light relative to the leaf does not change a great deal. The measuring chuck size is the limiting factor here. If the unit is used outside in the sun, edge effects will affect measurement when the sun is low in the sky. The Y(II) meter will measure reliably when the sun is about 35^{o} above the horizon if the open measuring aperture is facing south and the overhanging LED – sensor head is in a position not to shade the measuring surface. This would be the equivalent to the time between about 8:30 AM to about 5:30 PM on June 21st in Hudson NH. The time between measurements can be set separately for day and night. It is recommended that measurements be at least 2 minutes apart or longer during the day due to saturation pulse NPQ relaxation requirements. Time intervals may be set from 1 minute to 255 minutes. The time interval for night time or dark measurement should be longer. The literature recommends at least 1 hour between measurements. The reason is that while intense saturation flashes do not damage light adapted plants even if they are close together. They can damage dark adapted plants if less than 1 hour apart. This method also allows for predawn measurement of F_V/F_M and therefore quenching parameters can be determined using Excel, after the fact. There is no direct readout of quenching parameters on the measuring screen or in the measuring file. They must be computed using standard equations available under definitions below. The PAR sensor determines when the day time interval is used and the night time interval is used. When the PAR value gets down to a few µmols, it automatically switches to the night time measuring interval. When the PAR sensor senses more than a few µmoles, it switches back to the daylight interval again.

Using the data file, quenching values may also be calculated from the data provided. An example of the data file is shown under the Data section.

You may want to use AC power or a different USB battery for long term use.

Definitions:

Actinic light source – This is any light source that drives photosynthesis. It may be the Sun, or an artificial light. Higher end fluorometers contain one or more built-in artificial actinic light sources for experimentation with specific repeatable radiation (or light) levels. The OS1p uses a high intensity 660 nm red light LED.

Alpha or α is leaf absorptance measured by calibrated RGB sensors above and below the leaf. White actinic light at 1,000 µmols is turned on for a short period after the Y(II) measurement to determine leaf absorptance

ΔF/Fm' see Y(II).

Dark-adapted or Dark Adaptation – This is a term that means that an area of a plant, or the entire plant, to be measured has been in the dark for an extended period of time before measurement. Dark adaption requirements may vary for dark-adapted tests. Dark adaption times of twenty minutes to sixty minutes are common, and some researchers use only pre-dawn values. Dark-adapted measurements include Fv/Fm, and non-photochemical quenching parameters. Longer dark adaption times are common for quenching measurements. In this case, it is common to use times of eight to twelve hours, or overnight. For a detailed discussion of dark adaptation, refer to the section on dark adaptation, or the application note on dark adaptation.

Modulated light source This is the light source that makes light adapted quantum photosynthetic yield measurements possible along with direct measurements of Fo, Fs, and Fo'. The modulated light source is used at an intensity range that is too low to drive photosynthesis and yet allows fluorescence measurement of pre-photosynthetic Fo, and its quenched version Fo'. This light source is turned off and on at a particular frequency. The frequency is adjusted automatically for optimal application usage. Intensities are adjusted between 0 to 0.4 µmols. They Y(II) meter provides and Auto adjustment feature that eliminates errors. The algorithm adjusts gain first, and intensity last. The intensity must be set differently for light and dark adapted methods. It is critical to adjust the intensity of this lamp correctly in dark adapted protocols to prevent driving photosynthesis and introducing a measuring error in Fv/Fm, and quenching measurements. For more details see the application note on dark adaptation.

Saturation pulse is a short pulse of intense light designed to fully reduce a leaf's PSII system. For higher plants, the optimal duration of the saturation pulse is between 0.5 seconds and 1 second (Rosenqvist and van Kooten 2006). A high intensity 660 nm red light LED that has to be high enough to close all PSII reaction centers. This duration is adjustable from 0.5 to 2.0 seconds. For Algae the ideal saturation pulse duration is from 25 to 50 ms (Schreiber 1999). The maximum intensity of the saturation plus is 7,000 µmols. The Y(II) meter has a special algorithm that is also used in other Opti-Science instruments. It uses an 8 point rolling 25 ms average to determine the highest F_M, and F_M' independent of saturation pulse duration. As a result, as long as the duration is long enough, the Y(II) meter will provide the optimal maximum fluorescence F_M or F_M' value.

Fm - is maximal fluorescence measured during the first saturation pulse after dark adaption. Fm represents multiple turnovers of Q_A with all available reaction centers closed. All available energy is channeled to fluorescence.

Fs also known as F' is the fluorescence level created by the actinic light. Initially the value is high and then decreases over time to steady state values due to the initiation of electron transport, carboxylation, and nonphotochemical quenching. Fs has also been used to designate steady state F' conditions.

Fm' is the saturation pulse value that is not dark-adapted. They are at a lowered values from Fm due to NPQ or non-photochemical quenching. When this parameter has reached steady state, it is used to calculate Quantum Yield of photosystem II - Y(II) or ΔF/Fm' along with Fs. Fm' at steady state is also used as a component to calculate q_N, NPQ, q_P, q_L, Y(NPQ), Y(NO), q_E, q_T, and q_I.

Fo is minimal fluorescence after dark adaptation. It is measured with a modulated light intensity too dim to drive photosynthesis and yet bright enough to detect "pre-photosynthetic" antennae fluorescence.

Ft – is the current instantaneous fluorescent signal shown on the fluorometer measuring screen. It is used to set the modulated light source intensity. See setting the modulated light source intensity.

Fv/Fm = (Fm – Fo) / Fm. This is a dark adapted normalized test used to determine Maximum quantum yield of PSII. This ratio is an estimate of the maximum portion of absorbed quanta used in PSII reaction centers (Kitajima and Butler, 1975). Another way to look at Fv/Fm is a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. 0.79 to 0.84 is the approximate optimal value range for most land plant species with lowered values indicating plant stress. It is important to dark-adapt samples properly for reliable test results. Since dark adaption requirement can vary with species and light history, testing should be done to ensure proper dark adaption, (See the section on dark adaptation). This test is a normalized ratio that allows comparison of samples that are dark adapted to the same known state.

Fv/Fo = (Fm-Fo)/Fo. This is a dark adapted test used to slightly improve the stress detection of Fv/Fm. While this number may run from zero to infinity, in reality, it normally runs from zero to about four. The lower the number, the greater the plant stress. It is important to dark-adapt samples properly for reliable test results. Since dark adaption requirement can vary with species and light history, testing should be done to ensure proper dark adaption, (See the section on dark adaptation). This test is a normalized ratio.

PAR – Photosynthetically Active Radiation between 400nm and 700nm. Measured in either µmols or µE. PAR can be measured in different dimensions such as Watts per meter or in micro- Einsteins or micro-moles. When using a PAR Clip, dimensions will always be in the equivalent terms, micro-Einsteins, or micro-moles PAR or PPFD and Leaf Temperature along are measured with the Y(II) meter along with Y or Y(II) measurements. Since Y(II) changes with PAR radiation (or light) levels and temperature levels as well as plant stress, the ability to record Yield values with these parameters provide control over important variables. It allows the calculation of relative ETR or Electron Transport Rate.

PPFD - Photosynthetic Photon Flux Density is the photon flux density of PAR. Measured in either umls or uE., PPFD, or "photosynthetic photon flux density", is the number of PAR photons incident on a surface in time and area dimensions (per meter squared per second). These terms are equivalent for PAR Clip leaf radiation measurements. Furthermore, both can be presented in either of the equivalent dimensions, micro-moles (µmols) or micro-Einsteins (µE).

μ_E – is a **micro Einstein**. This is a dimension that involves both time and area. It is equivalent to the µmol. Both terms have been used extensively in biology and radiation measurements. u_E tends to be used more in physics while µmol tends to be used more in biology.

µmls - is a **micro mole** (also abbreviated µmol, or µmol $m^{-2}s^{-1}$) . This is a dimension that involves both time and area (per meter squared per second) . It is equivalent to the micro Einstein. u_E tends to be used more in physics while µmol tends to be used more in biology.

µmol – or micro mole (also abbreviated µml, or µmol $m^{-2}s^{-1}$) . This is a dimension that involves both time and area (per meter squared per second) . It is equivalent to the micro Einstein. u_E tends to be used more in physics while µmol tends to be used more in biology.

$$1\mu_E = \quad 1\ \mu mol\ m^{-2}\ s^{-1} \quad = \quad 6.022 \times 10^{17}\ photons\ m^{-2}\ s^{-1}$$

Y(II) or ΔF/Fm' or Y, is the Quantum Yield of PSII= (Fm' – Fs) / Fm'. The quantum yield of PSII is a fast light adapted test taken at steady state photosynthesis levels. It is a normalized ratio that provides a measure of actual or effective quantum yield. This ratio is an estimate of the effective portion of absorbed quanta used in PSII reaction centers. (Genty, 1989) It is affected by closure of reaction centers, caused by light and heat dissipation involved in non-photochemical quenching mechanisms and is therefore sensitive to many types of plant stress. Y(II) allows investigation of the photosynthetic process while it is happening. No dark adaption is required. The previous view, according to Maxwell and Johnson (2000), states that it takes between fifteen to twenty minutes for a plant to reach steady state photosynthesis at a specific light level. However, recent research by Cazzaniga & Bassi (2013) and Dall"Osta (2014) shows that what was thought to be fluorescence change due to state transitions, and acute photoinhibition, at high light levels, is actually due to chloroplast migration, a light avoidance mechanism. *This means that it takes from 20 to 30 minutes to reach steady state.* To obtain a reliable Y(II) measurement, photosynthesis must reach steady state.

This is usually not a concern when using ambient sunlight unless winds are high, however, clouds and light flecks and high winds below a canopy level can cause problems. Under artificial light, steady state is reached after 20 to 35 minutes at one light intensity, If one uses a built in fluorometer actinic illuminator to measure yield, make sure that steady state photosynthesis has been reached (See the discussion on Yield for more information). Remember that ambient Sun light contains FAR red illumination for activation of PSI. It is something to consider when using an internal illuminator for Yield measurements. Far Red illumination is an option when using internal actinic illumination for yield measurements. See the section regarding an in depth discussion on quantum photosynthetic yield.

Y(II) has been found to be more sensitive to more types of plant stress than Fv/Fm, however one must only compare measurements at the same light level as the value changes at different light levels. A PAR clip should be used with the fluorometer to measure Yield in all field applications. This allows for proper comparisons of values and the determination of ETR or electron transport rate, a parameter that includes both yield and actinic light level. See the Stress guide for more details.

Quenching definitions:

Y(NPQ) is a lake model quenching parameter that represents heat dissipation related to all photo-protective mechanisms also called regulated heat dissipation. (Klughammer and Schreiber 2008). A low Y(NPQ) at high light levels is an indication of sub-optimal photo-protective mechanisms. (Klughammer and Schreiber 2008).

Y(NO) is a lake model quenching parameter that represents all other components of non-photochemical quenching that are not photo-protective. They include non-radiative decay, and fluorescence. Part of Y(NO) includes photoinhibition. Klughammer and Schrieber define Y(NO) as the "fraction of energy that is passively dissipated in the form of heat and fluorescence mainly due to closed PSII reaction centers". Hendrickson calls Y(NO) constitutive heat dissipation. A high Y(NO) value after dark adaptation is an indication of photo-damage. (Klughammer and Schreiber 2008). According to Klughammer (2008), Y(NO) is the only quenching parameter that does not need to be taken as steady state photosynthesis.

NPQ = Y(NPQ)/Y(NO) or NPQ= (F_M-F_M')/F_M' Klughammer and Schreiber reconcile NPQ with the lake model using simplified parameters. NPQ (resurrected puddle model parameter valid in Klughammer simplified Lake model equations. See above.) is non-photochemical quenching and is a measure of heat dissipation. NPQ is an alternate expression of non-photochemical quenching. It provides an estimate of quenching without knowledge of F_O'. The range of NPQ is affected by Δph of the thylakoid lumen, which is an important aspect of photosynthetic regulation, state transitions and photoinhibition. Numbers range from zero to infinity with typical samples measuring in the 0.5 to 3.5 range (Maxwell and Johnson 2000). (Bilger & Björkman, 1990), (Muller P., Xiao-Ping L., Niyogi K. 2001).
Quenching equations:

Hendrickson's equations & NPQ resurrected to the lake model from the puddle model by Klughammer and Schreiber 2008 (The measurement of F_o' is not required)

Y(II) = (F_M' -F_S)/F_M' or ΔF_M'/ F_M'
Y(NO) = F_S/F_M or F'/F_M Fs is fluorescence at steady state in the light. F_M is the highest F_M value pre-dawn in the dark
Y(NPQ) =(F_S/F_M')-Y(NO) or (F'/F_M') - Y(NO)
NPQ= Y(NPQ)/Y(NO) or
NPQ = (F_M-F_M')/F_M' Puddle model or Hendrickson lake model definition
F_M is the pre-dawn highest value of F_M in the measuring file. F_M' saturation flash value in the light. NPQ will vary with light level during the day.

These equations above can be applied to data in the monitor mode measuring file to determine quenching values after the data has been collected.

Chapter 2 – Y(II) - Quantum yield of PSII – an in depth discussion of its value and limitations.

Y(II) (or ΔF/Fm' or (Fm' – Fs) / Fm') is a time tested light adapted parameter that is more sensitive to more types of plant stress than Fv/Fm according to a survey of existing research. While Fv/Fm is an excellent way to test for some types of stress and the health of Photosystem II in a dark adapted state, Quantum Photochemical Yield is a test that allows the measurement of the efficiency of the overall process under actual environmental and physiological conditions. It has also been found to be more sensitive to more types of plants stress. See the Plant Stress Guide on this disc or contact Opti-Sciences at www.optisci.com for details.

Quantum Photochemical Yield of PSII is a normalized measurement ratio that represents achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions. (Genty 1989), (Maxwell K., Johnson G. N. 2000), (Rascher 2000) It is affected by closure of reaction centers and heat dissipation caused by non-photochemical quenching (Schreiber 2004).

As ambient light irradiates a leaf, about an average of 84% of the light is absorbed by the leaf, and an average of 50% of that light is absorbed by the antennae associated with PSII and transferred to PSII (Photosystem II) reaction centers. (Leaf Absorption can range from 70% to 90% (Eichelman H. 2004) and the ratio of PSII to PSI reaction centers can range from 40% to 64% (Edwards GE 1993) (Laisk A. 1996)). Under normal non-stressed conditions, most light energy is channeled into photochemistry with smaller amounts of energy channeled into heat and fluorescence. In photosystem II, this process is competitive so that as plant stress occurs, mechanisms that dissipate heat, photo-protect the leaf, and balance light between photosytem II and photosystem I, change the output of fluorescence and heat. In other words, conditions that maximize photochemistry minimize fluorescence and heat dissipation and conditions that maximize fluorescence minimize photochemistry and heat dissipation.

Once these mechanisms have achieved an equilibrium at a specific light level and temperature, steady state photosynthesis has been achieved. This is a process that takes twenty to thirty five minutes due to chloroplast migration (Cazzaniga 2013, and Dall O'sta 2014). Once at steady state photosynthesis, a very intense short light pulse, called a saturation pulse, is used to momentarily close or chemically reduce all capable PSII reaction centers. Apart from the known exceptions listed under "Correlation to Carbon Assimilation" later in this discussion, quantum photochemical yield will reflect changes in the function levels of PSII antennae, PSII reaction centers, electron transport, carbon assimilation, and regulatory feedback mechanisms.

Quantum yield of PSII or Y(II) is measured only at steady state photosynthesis. Fs is the fluorescence level at steady state photosynthesis, and Fm' maximum fluorescence value measured during a saturation pulse, and is taken to mean that all PSII reaction centers are closed. In a high light environment, this may not be true and the multi-flash method may be required. See the F_M' correction section for more details.

Graphic display of a single Y(II) measurement taken with a Y(II) meter.

Y(II) will change at different light levels and temperatures so the Y(II) meter measures PAR and leaf temperature. The cosign corrected PAR sensor measures Photosynthetically Active Radiation between the wavelengths of 400 nm and 700nm. When the dimensions per square meter per second in micro-mols or micro-einsteins are added, this parameter becomes Photosynthetic Photon Flux Density (or PPFD) (micromoles and micro-einsteins are equivalent, and when using a PAR Clip, PAR and PPFD are equivalent).

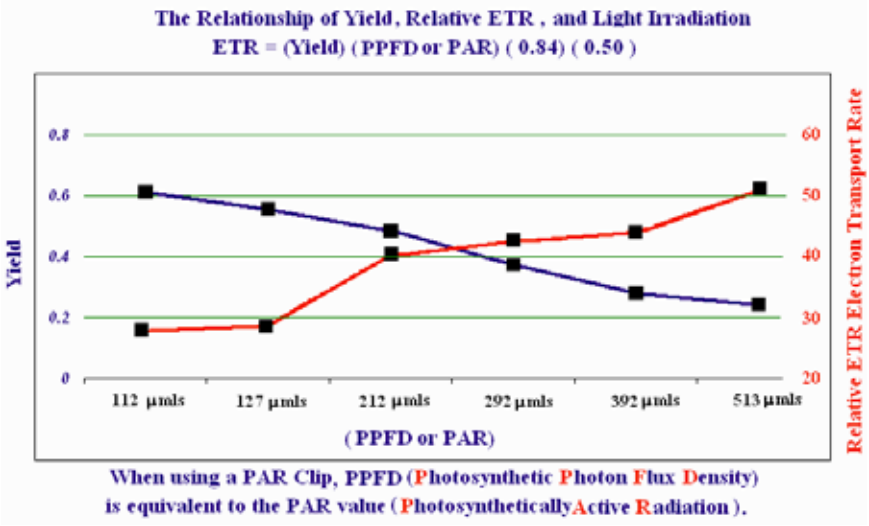
NOTE: It is possible to misinterpret results if PAR and temperature changes are not taken into account. One leaf may appear to be stressed compared to another when the only difference is light irradiation level. PPFD or PAR must be measured very close to the sample or errors can result.

In addition, it is important not to change the orientation of a leaf and to avoid shading the sample measuring area with the PAR clip or by other means. Extraneous reflections and breathing on the sample should also be avoided (Rosenqvist and van Kooten 2006).

PAR Clips also allow measurement of relative ETR or relative Electron Transport Rate. ETR is a parameter designed to measure the electron transport of PSII. It has also been found to correlate well with CO_2 assimilation. More advanced fluorometers provide built-in illuminators for greater experimental control of light irradiation intensity. This allows pre-illumination with a controlled predetermined intensity value for sample comparison.

For reliable Y(II) and ETR measurements, photosynthesis must be at steady state and with illumination on the same side of the leaf that is being measured (see number eight under correlation to carbon assimilation). Steady state photosynthesis is an equilibrium condition reached after a several minutes of exposure to existing light radiation conditions. It occurred in twenty minutes to thirty minutes in the plants measured, due to chloroplast migration (Cazzaniga 2013, Dall'Osta 2014). Chloroplast migration occurs, changing leaf absorptance and leaf transmittance. This process takes between twenty to thirty minutes in non-mutant plants. Measurements taken under variable lighting conditions will not provide reliable Y(II) results (Rascher 2000). No dark adaptation is required for Y(II) measurements.

Steady state is difficult to achieve during partly cloudy days, or during high wind days, because the light is constantly changing. It can also difficult "under canopy conditions" where the light can be constantly changing.



Correlation to Carbon assimilation:

In 1989, Genty developed the yield measurement and provided strong evidence of a linear correlation between Yield measurements, Electron Transport Rate, and CO_2 assimilation for C_4 plants (Baker and Oxborough 2004) and many others have confirmed the relationship (Edwards and Baker 1993), (Krall and Edwards 1990, 1991), (Siebke 1997). It was found that a curve-linear correlation between Yield and CO2 assimilation exists for C_3 species where photorespiration can also use significant products of electron transport (Genty 1990), (Harbinson 1990), (Baker and Oxborough 2004). Psydo-cyclic electron transport and other electron sinks may also be involved.

Chapter 3 - Relative Electron Transport Rate

Relative Electron Transport Rate - ETR μmols = (Y(II)) (PAR) (.84) (.5)

ETR or Relative Electron Transport Rate is a parameter that is measured with a PAR Clip. ETR is a relative measurement that provides comparative electron transport rates for PSII at different light or radiation levels. It is considered relative because chlorophyll fluorescence does not correlate exactly with absolute gas exchange measurements. While most fluorescence occurs in the upper most layers of the leaf, gas exchange measures the effects of photosynthesis in deeper layers as well. (Schreiber 2004).

Even with this in mind, relative ETR can be valuable. While exact correlation to gas exchange carbon assimilation is not possible, linear correlation is possible with C_4 plants (Genty 1989) and a curvilinear correlation is possible with C_3 plants (Genty 1990). While four electrons must be transported for every CO_2 molecule assimilated, or O_2 molecule evolved, differences from gas exchange measurements can occur under conditions that promote of photorespiration, cyclic electron transport, and nitrate reduction (Schreiber 2004) (Baker, Oxborough 2004). For more detailed information concerning the relationship between fluorescence and gas exchange measurements again refer to Opti-Sciences application note #0509 on Yield measurements.

The equation for Relative ETR is ETR = (YII or ΔF/Fm') (0.84) (0.50) (PPFD or PAR)

In this equation, Yield represents overall PSI and PSII yield. It assumes an average leaf light absorbance to be 84%, and the portion of light provided to PSII to be 50%. PPFD is PAR irradiation measured very near the leaf in micro-moles or micro-einsteins (equivalent units). The end result is a close approximation of PSII ETR that can be used for relative evaluation of different samples. With the Y(II) meter, the actual values for leaf light absorbance can be measured, and the portion used by PSII can be input into the actual formula to provide more accurate results.

The absolute amounts for leaf absorptance can vary at steady state conditions with species, growing conditions, previous or current plant stress, light level, and water content (Cazzaniga 2013). Terrestrial leaf absorbance has been found to vary between 70% to 90% (Eichelman H., Oja V., Rasulov B., Padu E., Bichele I., Pettai H., Niinemets O., Laisk A. 2004), and the percentage of light absorbed by PSII has been found to range from 40% to 60% (Laisk A. and Loreto F. 1996), (Edwards GE and Baker NR 1993). Land plants have a ratio of PSII to PSI reaction centers between 0.4 and 0.62 (Edwards GE and Baker 1993), (Laisk A. and Loreto F. 1996). The ratio of PSII to PSI in measured forms of green algae was found to be between 0.58 and 0.75 depending on light stress (Smith B.1990). In Red Algae and Cyanobacteria, about 15% of the reaction centers are PSII (Allen J. 2004). In addition, fluorescence is measured from only the top leaf layers as compared to gas exchange measurements that measure the lower layers as well (Schreiber 2004).

Relative electron transport rate provides an estimate of CO_2 assimilation under most stress conditions. C_4 plants have been found to correlate in a linear manner with CO_2 assimilation.

(Genty 1989, 1990). In C_3 plants, Correlation with CO_2 assimilation is curvilinear due to photorespiration, pseudocyclic electron transport or other electron sinks (Fryer 1998),(Genty 1990). Under some forms of stress, such as cold stress, and water stress, this relationship can be diagnostic for these two types of stress in C_3 plants (Fryer 1998).

ETR is provided using average values for PSII absorption and measured values for leaf absorptance. Even so, ETR provides highly useful comparative data (U. Schreiber 2004). By plotting ETR vs. PAR, potential ETR rates at maximal quantum yield, photosynthetic capacity, and ETR rate limitations at a given radiation level (light intensity) can be determined. When ETR is graphed vs. PAR at specific leaf temperatures a significant amount of information regarding photosynthesis is obtained. Note: Four electrons must be transported for every CO_2 molecule assimilated or O_2 molecule evolved.

Absolute electron transport rate is measured by gas exchange measurements. While linear and curve linear correlation with CO_2 assimilation is possible, relative ETR does not correlate exactly because while most of radiation is absorbed in the upper layers and provide fluorescent information, some radiation does enter lower layers and the information is not captured in fluorometry. CO_2 and H_2O gas exchange carbon assimilation includes information from all layers. (U. Schreiber 2004).

Y(II) can vary significantly with light level and with temperature. Without controlling irradiation and temperature, it is possible to misinterpret results. In fieldwork, where both light and temperature can vary, a lower Yield measurement on one plant as compared to another could be misdiagnosed as stress, when it may only be an increase in irradiation or a change in temperature on the leaf. When a PAR Clip is used to take Y(II) measurements, the combination can be formidable. *Only samples at similar light levels should be compared for plant stress using Y(II).*

The actual optical characteristics of plants can vary substantially with species and growing conditions (Baker 2008). Land plants have been found to have a leaf light absorption range of between 0.7 and 0.9 (Eichelman H. 2004). Algae have an absorption range of between 0.36 and 0.96 (Nielsen H. D. 2008). The amount of light that is channeled to PSII is dependant on the ratio of PSII reaction centers to PSI reaction centers as well as state transitions. At steady state photosynthesis, state transitions are regarded as stable. However, for shorter periods of time and at lower light levels they are not stable. (Schreiber 2004).

While ETR is a relative measurement, it is very useful when comparing different samples, and a single sample at different parameter values. *(More information on ETR our Yield are available in the Yield and PAR measurement application notes from OSI.) Optical characteristics of leaves can be determined with a spectral radiometer or quantum sensor used with and integrating sphere to measure absorbance.*

Measuring leaf absorptance

Measuring leaf absorptance is now possible over the entire PAR range. Leaf transmittance is also measured for the most reliable absorptance measurement.

ETR or electron transport rate, should never be used for comparing different leaves without measuring leaf absorptance (Baker N. 2008) for the following reasons: absorptance can vary with plant stress level, and it can vary by species, leaf age, chlorophyll content (In addition, electrons can flow to other electron sinks other that photosynthesis like photorespiration. This has been found in plants under chilled conditions (Fryer 1998) and in C_3 plants under drought conditions (Flexas 1999, 2000). Eichelman (2004) reports leaf absorptance variations from 0.7 to 0.9 on samples tested. *Recently Cazzaniga 2013 found the chloroplast migration changed leaf transmittance at high actinic light levels.*

Using papers done by J.R. EVANS & H.POORTER (2001) and C. J. BERNACCHI, C. PIMENTEL, & S. P. LONG (2003) as a guide for measuring absorptance, we used an integrated sphere, the white actinic light source used in the iFL, and a scanning spectrophotometer to measure leaf reflectance, leaf transmittance and leaf absorptance. This was then compared to measurements made in the iFL leaf chamber. After calibration, differences between the two methods were in the range of electronic noise.

Red, green, and blue sensors, located above the leaf, are used to measure reflectance. Red, green and blue sensors are also located under the leaf to measure leaf transmission for the most reliable leaf absorptance measurement. *The change in leaf transmittance after chloroplast migration can be measured.*

$$ETR = Y(II) \times PAR \times \alpha \times \beta$$

$$\alpha = ((\alpha_r)(B_r)+(\alpha_b)(B_b)+(\alpha_g(B_g) - (T_r/I_r + T_b/I_b + T_g/I_g))$$

ETR = electron transport, **Y(II)** or yield of PSII (F '- Fs / F '), **PAR** is photosynthetically active radiation at the leaf, α is leaf absorptance using the equation shown above. β is the ratio of PSII reaction centers to PSI reaction centers in the leaf.

α is derived in the following way: The amount of red, blue and green-yellow radiation incident on the leaf is measured. $\mathbf{B_r}$, $\mathbf{B_g}$ & $\mathbf{B_b}$ are the fractions of each spectral range incident on the leaf, determined at the factory; α_r, α_g, α_b, or absorptance in each spectral range, are determined by measuring the amount of light reflected from the leaf in each spectral range, and subtracting the amount from the light incident in each spectral range on the leaf. Then the amount of light transmitted through the leaf, in each spectral range, is measured. Those values are represented by $\mathbf{T_r}$, $\mathbf{T_g}$, & $\mathbf{T_b}$. The values $\mathbf{I_r}$, $\mathbf{I_g}$, and $\mathbf{I_b}$ are the raw red, green, and blue radiation incident on the leaf. The light transmitted through the leaf is then subtracted from the equation. The result is more reliable ETR measurements.
The ratio of PSII reaction centers to PSI reaction centers varies from 0.4 in some C_4 plants to 0.6 in some C_3 plants (Edwards 1993, Laisk 1996,). While 0.5 is sometimes used for an average value, the most used method for measuring the ratio of PSII to PSI, involves the use of spectral analysis of samples at 77 K (Anderson 1999), (Zell 2010); This ratio varies by type of plant, C_3 or C_4 , by plant species, by sun grown leaves vs. shade leaves, and in carbon deficient leaves.

F_M' correction - based on Loriaux (2013), a detailed description

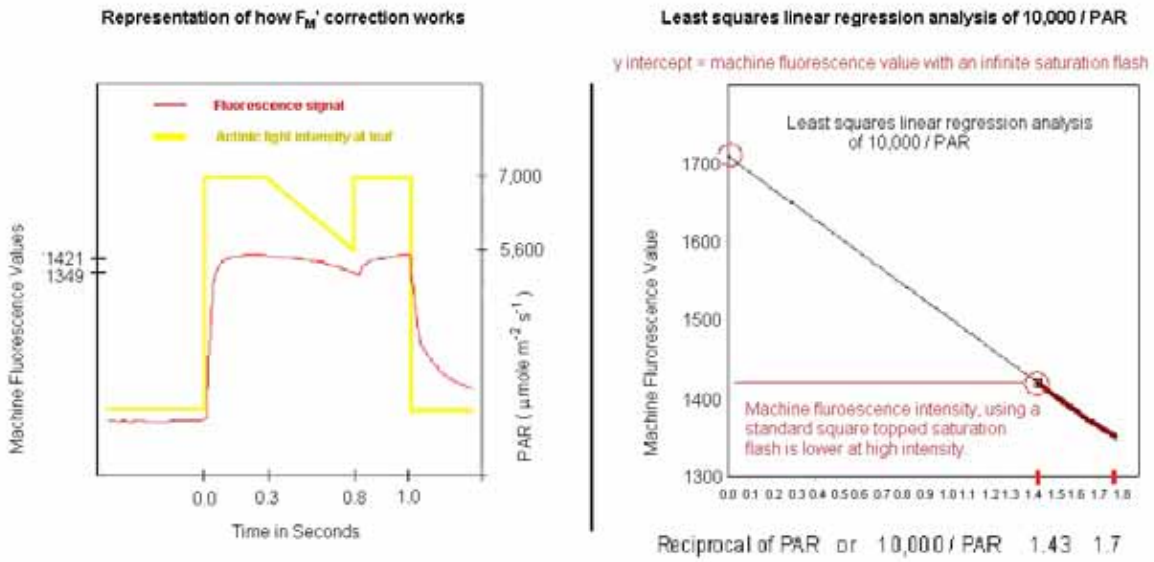
Saturation pulses used with modulated fluorometers are designed to close or chemically reduce all PSII reaction centers. Reliable measurement requires this to happen.

The maximum fluorescence intensity value, of the saturation flash, F_M', is used in measurements of Y(II) or $\Delta F'/F_M'$) and ETR parameters.

While it is possible to chemically reduce or close all PSII 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 significantly underestimated. In a poster in 2006, and later in a paper published in 2013, researchers that included Bernard Genty, the developer of quantum yield of PSII (the Y(II) test) verified the issue. They also developed a method for F_M' correction. It involved a multiple phased single saturation pulse with multiple light intensities, and the use of least squares linear regression analysis of the reciprocal of PAR (Photosynthetically Active Radiation). This group used this method to determine what the F_M' fluorescence level would be, if an infinitely intense saturation pulse is used. Furthermore, it can be done 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 Y(II)) and ETR measurements. The method was refined in Loriaux 2013.

This standard option is provided on the Y(II) meter, 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 is described by the Loriaux, Avenson, Welles, McDermitt, Eckles, Riensche, & Bernard Genty (2013). 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 (Loriaux, Burns, Welles, McDermitt, & Genty 2006). The Loriaux 2013 paper improves on the 2006 poster method. All current Opti-Science light adapted instruments, including the Y(II) meter, uses the Loriaux 2013 protocol. It provides the optimal saturation intensity of 7,000 µmols for 0.3 seconds, the optimal light down ramping of 20% for 0.5 seconds, and a ramping rate less than 0.01 mols $m^{-2}s\text{-}^{2}$. It ends with a 0.3 second rise again, to test for saturation pulse NPQ.



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 also 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) allows determination of the "y" intercept, which represents the machine fluorescence value with an infinite saturation flash.

The final period at 7,000 µmols is used to determine if saturation flash NPQ occurs with the sample. Bernard Genty recommends not using the method if F_M' is lower for the second measurement than the first, as this indicates that saturation duration is too long for the species being measured.

Chapter 4 - Using the F_V/F_M meter

F_V/F_M meter used with a dark adaptation clip

Graphic measuring screen in bright sun light

Fvm = F_V/F_M & Fvo is = F_V/F_O

The F_V/F_M meter is designed to provide an inexpensive companion tool to the Y(II) meter based on hard science, and capable of being used with dark adaptation clips to allow fast measurement of large plant populations. While Y(II) is more sensitive to some types of plant stress than F_V/F_M (Example: Heat stress. Y(II) will detect heat stress at about 35^{o}C and F_V/F_M not until 45^{o}C (Haldiman P, & Feller U. 2004). F_V/F_M can be used without measuring PAR or leaf temperature. It can be also be used in windy conditions, or below canopy conditions successfully. The biggest advantage to F_V/F_M is that it allows comparison of samples that have been dark adapted to the same known state.

The F_V/F_M meter includes a lithium ion battery that should be connected to the instrument using the USB cable that is attached to one end. After assemble, the battery may be placed in a pocket, allowing one hand operation.

Getting started

1. Turn on the unit by pressing the green button on the top of the instrument. A menu will appear.
2. Use the black arrows that run parallel to the instrument length to move the cursor from one icon to another and from one parameter to another.
3. Select time and date first by pushing the green button after the icon has been high lighted.

4. Move from one parameter to another using the parallel arrows, and change the values using the arrows perpendicular to the length of the instrument.
5. When finished, press the green button to back out of the menu to the main screen.
6. Use the parallel arrows to go to "Test" and press the green button. A screen will appear that says "Please remove sample material from probe". Remove leaves or other things from in front of them measuring probe, and then press the green button. The instrument then self calibrates the light intensity. Next, the main measuring screen appears.
7. "Log File" -To change the name of the measuring file use the blue arrow parallel to the instrument length, farthest from the viewing screen. Use the perpendicular arrows to go to the naming screen. This is an alpha numeric screen that allows an eight character name. Use the perpendicular arrows to scroll through the alphabet, a blank, and then numbers 0-9. When the correct character is in place, use the parallel arrow to move to the next character. When done, press the green button. To go back to the previous screen.
8. "SatPwr" – This is saturation pulse intensity. The values range from 1-8. 8 is 6,000 µmols. Each step down is approximately 857 µmols less.
9. "SatW" – This is saturation pulse width. The values range from 0.5 seconds to 2.0 seconds. Usually 1.0 seconds is adequate for most samples. In the past, correct setting and testing of the leave in question was required due to saturation pulse NPQ; however, the F_V/F_M meter has a special algorithm that finds the highest 8 point 25 ms rolling average of F_M. This eliminates saturation pulse NPQ from being a problem. As long as the saturation pulse is wide enough to saturate the leaf, one can make reliable measurements. (See the cook book check list for F_V/F_M measurements for more information.)
10. "ModPwr" & "Gain" These parameters are used if one wants to set the modulated light intensity manually. *It is recommended that "AutoSet" be used instead.* Autoset automatically adjusts the modulated light intensity and gain quickly and correctly so that measurement can be made, and that the modulated light intensity does not drive photosynthesis or is actinic. It adjusts gain first and intensity second. If one wants to set the value manually. Place a leaf of the species that will be used in testing, in a dark adaptation clip, place the F_V/F_M meter black probe all the way into the cylinder of the dark adaptation clip and open the black shutter. Watch to see if the "Ft" value on the measuring screen, increases over a two minute time frame, after the initial jump upward. If it does, the "ModPwr" must be decreased, until the increase no longer occurs. Try a second dark adapted sample at the new setting, if there is no slow increase after the initial jump in signal, take a measurement using the black measuring button on the bottom of the instrument. If it makes a measurement, it is properly set. If it says "Ft signal too low", the "Gain must be increased until measurement is possible.
11. "AutoSet" - Use the parallel arrows to find "AutoSet". Place a leaf of the species that will be used in testing, in a dark adaptation clip, place the F_V/F_M meter black probe all the way into the cylinder of the dark adaptation clip and open the black shutter of the dark adaptation clip. Use one of the perpendicular arrows to adjust the "AutoSet". The

modulated light intensity and gain are now properly set. It is likely that the "ModPwr" or "Gain" numbers have also changed. This is an algorithm that allows automatic optimal setting to the modulated light intensity. It adjusts gain before it adjusts intensity. For error free measurement, the modulated light intensity must be high enough for the instrument to detect the fluorescence from the leaf caused by the low modulated light intensity, but not high enough to drive photosynthesis. Push the green button to go back to the main measuring screen. Follow the directions on the screen, and the main measuring screen will appear. The setting may also be manually set.

12. For recommended dark adaptation times, see the section on dark adaptation.
13. You can now make measurements. (See the Cook-book Checklist for F_V/F_M measurements for measuring recommendations.)

A Cookbook Checklist before making reliable F_V/F_M measurements

Put together an experimental design that takes these issues into account.

Accuracy is the ability to hit the bull's eye.
In many types of measurements, accuracy is determined by calibrating to a measuring a standard that is traceable to the National Agency. With such measurements, tolerances are always involved.

Repeatability is the ability to achieve the same measurement again and again to a certain tolerance level.

A **Reliable** measurement is one that is accurate and repeatable**. With Chlorophyll fluorometers, accuracy is determined in a different way.** In this application note, will provide a cookbook style checklist of issues that musts be considered to get reliable F_V/F_M measurements.

First –F_V/F_M
The biggest advantage of F_V/F_M is that it is a measure of PSII performance that puts all samples in the same known dark adapted state before measurement. F_V/F_M is a normalized ratio that does not use a traceable standard. Instead, it's accuracy is determined by properly using the instrument and following the lessons learned about plant physiology by several great researchers. For most species, the optimal F_V/F_M reading for stress free plants is in the range of 0.79 to 0.84 (Maxwell and Johnson 2004). Lower values indicate plant stress.

To get a reliable measurement, one has to follow tested guidelines.

1. Dark-adapt properly knowing the plant's light history. It takes only a few minutes for the xanthophyll cycle and the Δph of the thylakoid lumen to return to a dark-adapted state. It can take up to 4 minutes with indoor and green house plants, and it can take up to 7 minutes in field plants (Baker 2008). Recently Cazzaniga (2013) found that the intermediate fluorescence change at high light intensity, previously attributed to state transitions, and acute phtoinhibition, were in fact due to chloroplast migration in C_3 plants. Maai (2011) found that chloroplast migration was also a mechanism present in C_4 plants. It was found that it takes from 20 minutes to 35 minutes for complete chloroplast migration to occur. At low light intensities, and in lower plants, there is evidence that state transitions occur. (State transitions however, take between fifteen to twenty minutes (Ruban 2009) (Lichtenthaler 1999). These times can vary somewhat in field plants, and can take slightly longer. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton (MacIntyre 1997). In addition, field plants and other plants that have been exposed to photoinhibition conditions for a number of hours, will retain a certain amount of NPQ for up to 60 hours (Lichtenthaler 2004).This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into most summer field measurements of F_V/F_M. This is all right if one is measuring "light stress" and comparing results, but when measuring other types of plant stress, light history should be taken into account when comparing samples. It is common for researchers to choose dark adaptation times anywhere from twenty minutes to overnight, using pre-dawn values. Shorter times may be used to study the effects of plant protective mechanisms. For more information, see the section on dark adaptation times. If possible, testing should be done to find the time required to reach a **stable steady dark adapted state.** If not, then 35 minutes is safe for F_V/F_M measurements on samples with a similar light history (Different dark adaption times are recommended for quenching measurements and Rapid light curves). ***However, it is recommended that you check with your projected research reviewer, for acceptable dark adaptation times, as some will only accept the equivalent of pre-dawn dark adaptation. Sometimes, belief is part of science.***

2. Modulation light intensity setting $F_V/F_M = (F_M-F_O)/F_M$. Minimum fluorescence , is a "pre-photosynthetic" a 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 F_O value that is too high 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. *Opti-Sciences now has an automated set up routine that allows fast easy and reliable adjustment of the modulated light intensity. On the FV/FM meter, it is called "AutoSet".*

3. Shade leaves vs. Sun leaves. – The F_V/F_M ratio will be slightly higher on sun leaves than on shade leaves (Lichtenthaler 2004).

4. F_V/F_M will be higher with a white saturation pulse than a red saturation pulse. Some fluorometers use a red saturation pulse. This is not an issue for comparative measurements of plant stress with similar instruments, but values measured on a fluorometer with a white saturation pulse should not be directly compared to measurements of a

fluorometer with a red saturation pulse. There is evidence to show that systems with a red saturation pulse correlate but measure consistently lower than systems with white light saturation lights. (Cessna 2010)

5. Maximum F_V/F_M values vary with species. The average maximum F_V/F_M value is between 0.79 - 0.84 (Maxwell and Johnson 2000).

6. 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 than measurements on the same plant after a few days of overcast, again because it takes a long time for photoinhibition to relax or repair.

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

8. 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 1995). 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. *Opti-Sciences provides an 8 point moving 25 ms average to determine the highest F_M. This ensures that a reliable value will be measured even if the saturation pulse width or duration is too long. This algorithm ensures that one less error is possible.*

9. Saturation pulse intensity. Dark adapted leaves saturate easily with lower saturation pulse intensities. It may take a few hundred µmols to saturate shade leaves and sun leaves will saturate below 1,500 µmols. 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).)

10. Some F_V/F_M fluorometers have the ability to pre-illuminate dark adapted leaves with far-red light. When this feature is used, for five to ten seconds before an F_V/F_M measurement takes place, it activates PSI, and ensures that all electrons have been drained from PSII before the measurement of F_O. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, chloroplast migration or photoinhibition. *Time is still required in a darkened environment to relax all forms of NPQ and to obtain a reliable F_V/F_M measurement.* (Maxwell and Johnson 2000). The F_V/F_M meter does not have far red light.

11. Fluorescence heterogeneity presents itself as different Y(II) or F_V/F_M measurements on different parts of the leaf. It has been found to occur under cold stress conditions, with biotic stress, low CO_2 levels, and under drought stress conditions. By using multiple measurements on the same leaf and using a sampling plan, heterogeneity can be over come (Buschmann C. in correspondence by e-mail 2008).

12. Part of the minimum fluorescence, the F_O and F_M parameter, in F_V/F_M ($F_M - F_O)/F_M$), contains PSI fluorescence as well as PSII fluorescence. With F_V/F_M, 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 F_O fluorescence is due to PSI, and in C_4 plants about 50% of F_O fluorescence is due to PSI fluorescence. PSI produces about 6% of the

fluorescence found in F_M in C_3 plants, and about 12% of F_M in C_4 plants (Pfundle 1998). This not a problem when comparing F_V/F_M measurements for plant stress because PSI fluorescence does not change. It remains constant.

13. On some plants, such as wheat, with long leaves, one should select similar positions on leaves for comparison purposes, because measurements can vary by leaf measurement location. For example; the tip of the leaf will likely be different than the mid section or the base. (Discussions with Wayne Loescher at MSU 2015).

There are fluorescence solutions and assays available that are sensitive to most types of plant stress. F_V/F_M is not as sensitive as Y(II) for some types of plant stress.

F_V/F_M is not a sensitive test for drought stress, heat stress, nitrogen stress, nickel stress, sulfur stress, zinc stress, some herbicides and salt stress in some types of plants (Opti-Sciences Plant Stress Guide 2010). It can be used effectively in most other types of plant stress. *For specific research results on specific types of plant stress, see the Plant Stress Guide offered by Opti-Sciences Inc.*

Dark Adaptation – How Long is Long Enough?

Check with your research reviewer for acceptable dark adaption times. Some reviews only believe in pre-dawn measurement after overnight dark adaptation. While the information below is up to date, belief is still sometimes part of science.

Dark adaptation is a technique used in some chlorophyll fluorescence measurements to fix a non-stressed reference point relative to various measurements (Maxwell and Johnson 2000). Deciding where to put that reference is based on an understanding of plant mechanisms that can affect measurements, and what one wants to measure.

Dark adaption times of twenty minutes, thirty minutes, forty minutes and sixty minutes are common for terrestrial plants, and some researchers use pre-dawn values.

To obtain reliable modulated F_V/F_M values, decisions need to be made for control and test measurements. The plant mechanisms listed below will lower Fm, and possibly raise F_O, changing F_V/F_M measurements downward like other types of plant stress. One must decide which mechanisms are of concern for specific types of plant stress measurement and dark adapt accordingly.

F_V/F_M is affected by both photochemical and non-photochemical factors. If a leaf is dark adapted and measured, then subjected to high light levels, then dark adapted and re-measured, the first measurement will be higher then the second measurement. The decline in Fv/Fm measurement may be due to a decrease in reaction centers capable of photochemistry or un-reversed non-photochemical quenching. (Baker N.R., Oxborough K. 2004)

Papageorgiou reports that results may vary greatly depending on how long dark adaptation is done. A few minutes of dark adaptation is enough to re-oxidize the plastoquinone pool and the $CaMn_4OxCly$ cluster, while longer periods deplete respiratory substrates through respiration in cyanobacteria and chlororespiration in higher plants and algae. Longer times will also deplete ATP pools, and trans-membrane ion concentration gradients. Dark adaptation also shifts higher plants and algae toward state 1 conditions and cyanobacteria to state 2 conditions. (Papageorgiou G.C. Tismmilli-Michael M. Stamatakis K. 2007)

Rapid acting photo-protective mechanisms activated by exposure to variable light intensities (designated in the parameters q_E and Y(NPQ) are the xanthophyll cycle and thylakoid lumen Δph. They relax in a few minutes during dark adaptation. (Muller, Niyogi 2001),(Kramer D. M., Johnson G., Kiirats O., Edwards G. (2004). According to Lichtenthaler (1999) this time is 4-6 minutes. According to Baker(2008) this time can be longer in the field.

State I – State 2 transition quenching (called q_T) is most significant at lower light levels in terrestrial plants and can represent more than 60% of quenching at low light levels. At high actinic light levels, the effects that were thought to be due to state transitions and acute photoinhibition have been replaced by chloroplast migration (Cazzaniga 2013) (Dall'Osta 2014). State transition quenching relaxes in ten to twenty minutes in terrestrial plants. (Lichtenthaler H. Burkart S 1999).

Recently it was discovered that chloroplast migration was responsible for the intermediate fluorescence adjustment at high actinic light levels. As stated above, previously, it was thought to be due to state transitions, and acute photoinhibition (Cazzaniga 2013, and Dall O'sta 2014). It takes 20 to 30 minutes of dark adaption to reverse the affects of chloroplast migration in non-mutant plants, and up to 35 minutes in some mutants. The reversal of chronic photo-inhibition caused by several hours of high light exposure starts to relax at about 40 minutes and may take 30 to 60 hours to fully relax under dark adaptation (Lichtenthaler H. & Babani F. 2004) (Theile, Krause & Winter 1998)

When making measurements related to photo-inhibition and photo damage mechanisms that are common in chronic high light stress, high heat stress, cold stress and over wintering stress, one should understand that it could take days for full relaxation or repair of the non-photochemical quenching parameters, q_I and Y(NO), to pre-stress conditions. To get an accurate control value for F_M and F_O under chronic photo-inhibition conditions (components of non-photochemical quenching parameters) it is common to dark adapt for a full night, hours. (Maxwell and Johnson 2000) In some cases longer times may be appropriate. Lichtenthaler (2004) One must assume that there is some residual NPQ in all field measurement taken with plants exposed to chronic photo-inhibition conditions, and dark adaptation times that are shorter than 30 to 60 hours. For these reasons, when developing an experimental design, it is important to compare samples with the same light history, unless one is measuring changes caused by light history differences.

In Aquatic Plants Gorbunov (2001) is a good source for corals, and Consalvey (2004) is a good source for Algae. For information regarding dark adaption for rapid light curves Rascher 2000 is a good source. Ralph (2004) describes momentary dark adaptation for Rapid Light Curves.

The use of far-red pre-illumination that is available on some fluorometers. It is designed to rapidly re-oxidize PSII by activating PSI. (Maxwell and Johnson 2000), but it does not affect the relaxation of non-photo-chemical quenching mechanisms (Consalvey 2004) and chloroplast migration still takes 20 to 30 minutes with or without far-red light.

Dark adaptation can be accomplished by using dark adaptation leaf clips or cuvettes. Some researchers use hundreds of inexpensive clips to make measurements on larger populations quickly. Shrouds, darkened rooms, or growth chambers can also be used.

In review, it is important to take a few things into account. Reliable dark adaptation times can vary by species, plant light history, the fluorescence parameter of interest, and the type of stress that needs to be measured. When dealing with a new species or an unknown photo-history it is best to test for maximum and stable F_V/F_M at different dark adapted times for best results. When testing for optimal dark adapting times it is important to use samples that have been exposed to the maximum light conditions that will occur during the experiment. Remember, some research reviewers will only accept the equivalent of pre-dawn dark adaptation values.

Fv/Fm Protocol (or Fv/m on the screen) – *Background*

Dark adapted test - a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. 0.79 to 0.85 is the approximate optimal value for most plant species with lowered values indicating plant stress. (Maxwell K., Johnson G. N. 2000) (Kitajima and Butler, 1975). Fv/Fm has a photochemical component and a non-photochemical component (Baker 2004). Fv/Fm is a fast test that usually takes less than two seconds. (see the section on dark adaptation.)

Fv/Fm allows comparison of samples at the same known dark adapted state. While it is not as sensitive as the light adapted Y(II) measurement for some types of plant stress measurement, (See the plant stress guide on the thumb drive sent with the instrument), PAR and temperature do not have to be measured, and the test can be more successful difficult to measure samples. For example: Under canopy leaves or in windy conditions where light intensity can be constantly changing, Fv/Fm, doesn't care. However, Y(II) requires steady state photosynthesis.

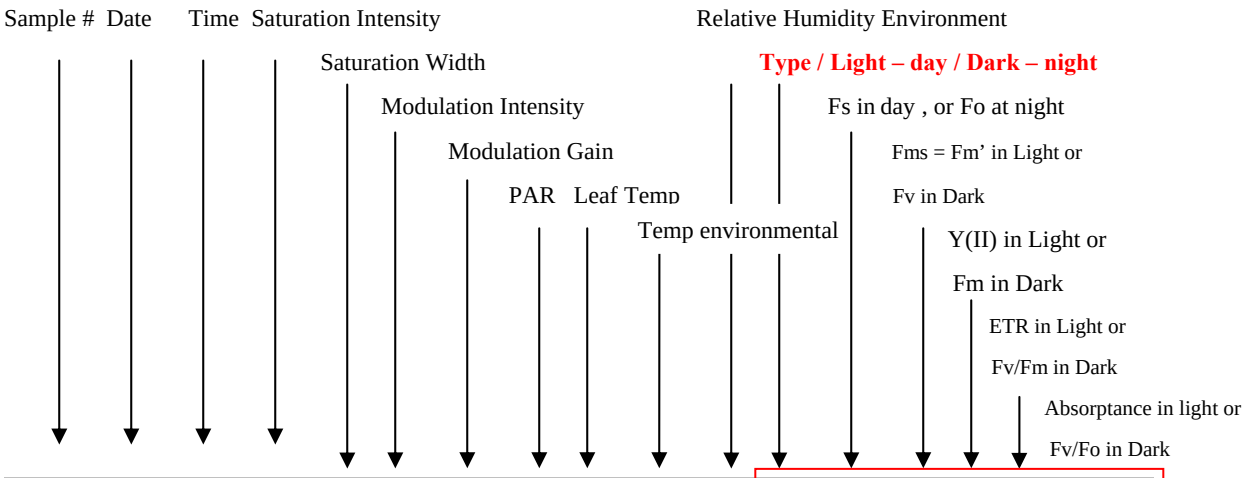
The assumptions are that before the test, PSII is fully oxidized and quenching mechanisms have relaxed to a know state. With the Fv/Fm test, it is important to dark adapt the sample being tested to get reliable measurements. Fv/Fm dark adaption times can vary due to light history. Times of twenty minutes to thirty five minutes are common. *Some scientists only work with pre-dawn dark-adapted samples. (Check with potential scientific reviewers for their views before designing your experiment. They can have strong feeling on the subject).* Experiments should be done on plants to be tested to find the appropriate dark adaption times for best results.

A total of 10 dark adaption white clips are provided with the system, to be used for dark adaption measurement. (Some scientists use hundreds of dark adaptation clips for fast measurement of large plant populations). A clip should be placed on the leaf with the black slider covering the cylindrical opening. After dark adaption, the end of the optic probe, on the Fv/Fm meter, should be placed all the way into the cylindrical opening, and the dark slide of the clip should be opened. This allows the sample to be exposed to the probe. At this point, press the black measuring button to make a measurement. Dark shrouds can also be used for dark adaption, growth chambers can duplicate pre-dawn dark adaptation, or lights can also be turned off in a windowless environment.

Chapter - Data Management

1. To transfer data files to a computer, Unplug the USB connector from the lithium ion battery or mains plug and insert it into a USB port on your computer.
2. Go to the main menu on the Y(II) meter or the FV/FM meter and use the arrows parallel to the length of the instrument to move the outlining cursor to link and press the green button. A message saying: “PC Link Active, Use the PC Drive Eject to Stop” appears.
3. Go to your computer and use Windows Explorer to find the “Y(II) drive”. The instrument becomes a drive for your computer and it is listed as the “Y(II) drive”.
4. Files are comma delineated and shown as Excel files. By tapping on the desired Excel file name the data will open directly in Excel. If other spread sheet programs are used, highlight the file name, right click the mouse, choose open with, select the desired spread sheet program and open the file.

The data file for the Y(II) meter is shown below on the next page:



Sample Number	Date	Time	SatI	SatW	ModI	Gain	PAR	T_Leaf	T_Env	H_Env	Type L_D	Fs_Fo	Fms_Fv	Y_Fm	ETR_Fv/Fm	alpha_Fv/Fo
1	Jul/01/2015	15:51:46	8	1	6	5	723	27.6	27.5	53	L	167	505	0.669	210.3	0.87
2	Jul/01/2015	15:56:46	8	1	6	5	140	26.7	26.7	58	L	154	524	0.706	42.9	0.87
3	Jul/01/2015	16:01:46	8	1	6	5	61	25.8	25.7	58	L	162	543	0.701	18.5	0.87
4	Jul/01/2015	16:06:46	8	1	6	5	70	25.6	25.1	58	L	177	508	0.651	19.8	0.87
5	Jul/01/2015	16:11:46	8	1	6	5	192	25.9	25.3	59	L	167	497	0.663	55.3	0.87
6	Jul/01/2015	16:16:46	8	1	6	5	161	27.2	26.1	58	L	174	307	0.433	29.6	0.85
7	Jul/01/2015	16:21:46	8	1	6	5	507	28.6	27.6	55	L	163	368	0.557	120	0.85
8	Jul/01/2015	16:26:46	8	1	6	5	387	28.9	28.3	53	L	173	440	0.606	100.8	0.86
9	Jul/01/2015	16:31:46	8	1	6	5	307	28	28.1	50	L	178	500	0.644	85.9	0.87
10	Jul/01/2015	16:36:47	8	1	6	5	217	28.9	28	53	L	176	380	0.536	50.5	0.87
11	Jul/01/2015	16:41:47	8	1	6	5	416	28.3	28.2	51	L	176	510	0.654	116.9	0.86
12	Jul/01/2015	16:46:47	8	1	6	5	199	27.4	27.5	52	L	174	510	0.658	56.2	0.86
13	Jul/01/2015	16:51:47	8	1	6	5	202	27.4	27.3	52	L	173	510	0.66	57.3	0.86
14	Jul/01/2015	16:56:47	8	1	6	5	199	27.3	27.1	55	L	174	510	0.658	56.2	0.86
15	Jul/01/2015	17:01:47	8	1	6	5	187	27.3	27	54	L	176	504	0.65	52.8	0.87
16	Jul/01/2015	17:06:47	8	1	6	5	201	27.2	26.9	54	L	170	510	0.666	57.5	0.86
17	Jul/01/2015	17:11:47	8	1	6	5	182	27	26.7	55	L	169	511	0.669	52.3	0.86
18	Jul/01/2015	17:16:47	8	1	6	5	179	26.9	26.5	55	L	180	513	0.649	49.9	0.86
19	Jul/01/2015	17:21:47	8	1	6	5	176	26.5	26.4	53	L	172	512	0.664	50.2	0.86
20	Jul/01/2015	17:26:47	8	1	6	5	174	25.9	25.9	50	L	168	516	0.674	50.9	0.87
21	Jul/01/2015	17:31:47	8	1	6	5	169	25.8	25.6	53	L	167	517	0.676	49.1	0.86
22	Jul/01/2015	17:36:47	8	1	6	5	151	25.6	25.4	53	L	161	503	0.679	44	0.86
23	Jul/01/2015	17:41:47	8	1	6	5	141	25.4	25.3	54	L	166	513	0.676	41.4	0.87
24	Jul/01/2015	17:46:47	8	1	6	5	138	25.2	24.9	52	L	163	513	0.682	40.4	0.86
25	Jul/01/2015	17:51:48	8	1	6	5	123	25.2	24.6	53	L	160	506	0.683	36.1	0.86
26	Jul/01/2015	17:56:48	8	1	6	5	118	25.1	24.4	55	L	160	509	0.685	34.7	0.86
27	Jul/01/2015	18:01:48	8	1	6	5	108	25	24.3	55	L	158	508	0.688	31.9	0.86
28	Jul/01/2015	18:06:48	8	1	6	5	101	24.8	24.3	54	L	158	510	0.69	29.9	0.86
29	Jul/01/2015	18:11:48	8	1	6	5	94	24.8	24.2	56	L	160	511	0.686	27.7	0.86
30	Jul/01/2015	18:16:48	8	1	6	5	88	24.6	24.1	58	L	157	509	0.691	26.1	0.86
31	Jul/01/2015	10:21:40	0	1	6	5	02	24.4	24	55	L	154	507	0.696	24.5	0.00
32	Jul/01/2015	18:26:48	8	1	6	5	79	24.2	24	55	L	156	510	0.694	23.5	0.86
33	Jul/01/2015	18:31:48	8	1	6	5	75	24.1	23.9	57	L	158	514	0.692	22.3	0.86
34	Jul/01/2015	18:36:48	8	1	6	5	72	23.9	23.8	59	L	153	509	0.699	21.6	0.86
35	Jul/01/2015	18:41:48	8	1	6	5	53	23.6	23.7	58	L	151	515	0.706	16	0.86
36	Jul/01/2015	18:46:48	8	1	6	5	31	23.3	23.5	59	L	153	525	0.708	9.4	0.86
37	Jul/01/2015	18:51:48	8	1	6	5	31	23.3	23.3	60	L	151	521	0.71	9.4	0.86
38	Jul/01/2015	18:56:48	8	1	6	5	20	23.1	23.1	61	L	147	526	0.72	6.1	0.86
39	Jul/01/2015	19:01:48	8	1	6	5	4	20.9	21.9	64	L	105	393	0.732	1.3	0.9
Sample Number	Date	Time	SatI	SatW	ModI	Gain	PAR	T_Leaf	T_Env	H_Env	Type L_D	Fs_Fo	Fms_Fv	Y_Fm	ETR_Fv/Fm	alpha_Fv/Fo
1	Jul/02/2015	10:45:58	8	1	6	5	114	21.4	21.3	58	L	163	504	0.676	32.7	0.85
2	Jul/02/2015	10:50:58	8	1	6	5	130	21.3	21.4	55	L	161	498	0.676	37.3	0.85
3	Jul/02/2015	10:55:58	8	1	6	5	151	21.8	21.6	55	L	167	498	0.664	42.6	0.85
4	Jul/02/2015	11:00:58	8	1	6	5	162	22	21.8	55	L	167	494	0.661	45.5	0.85
5	Jul/02/2015	11:05:58	8	1	6	5	158	22.3	22	55	L	173	494	0.649	43.5	0.85
6	Jul/02/2015	11:10:58	8	1	6	5	202	22.2	22.1	59	L	147	492	0.701	60.1	0.85
7	Jul/02/2015	11:15:58	8	1	6	5	12	22.2	22.1	59	L	147	546	0.73	3.7	0.85
8	Jul/02/2015	11:20:58	8	1	6	5	2	22	21.9	63	L	150	552	0.728	0.6	0.85
9	Jul/02/2015	11:25:58	8	1	6	5	2	21.9	21.8	65	L	148	552	0.731	0.6	0.85
10	Jul/02/2015	11:30:58	8	1	6	5	2	21.9	21.8	66	L	150	559	0.731	0.6	0.85
11	Jul/02/2015	11:35:59	8	1	6	5	4	22.1	21.9	67	L	149	553	0.73	1.2	0.85
12	Jul/02/2015	11:40:59	8	1	6	5	8	22.2	22	70	L	149	562	0.734	2.4	0.85
13	Jul/02/2015	11:45:59	8	1	6	5	9	22.9	22.7	64	L	155	569	0.727	2.7	0.85
14	Jul/02/2015	11:50:59	8	1	6	5	18	23.4	23.5	62	L	153	566	0.729	5.5	0.85
15	Jul/02/2015	11:55:59	8	1	6	5	18	24	23.8	61	L	152	562	0.729	5.5	0.85
16	Jul/02/2015	12:00:59	8	1	6	5	17	24	23.8	66	L	152	563	0.73	5.2	0.85
17	Jul/02/2015	12:05:59	8	1	6	5	13	24	23.8	57	L	234	400	0.415	2.2	0.85
18	Jul/02/2015	12:10:59	8	1	6	5	163	23.7	23.7	53	L	187	445	0.579	40	0.85
19	Jul/02/2015	12:15:59	8	1	6	5	240	24.2	23.7	53	L	191	437	0.562	57.3	0.85
20	Jul/02/2015	12:20:59	8	1	6	5	303	24.7	24	53	L	188	462	0.593	76.3	0.85
21	Jul/02/2015	12:25:59	8	1	6	5	295	24.6	24.1	47	L	198	462	0.571	71.5	0.85
22	Jul/02/2015	12:30:59	8	1	6	5	319	24.9	24.2	50	L	191	438	0.563	76.3	0.85

The L or D under "Type L D" stand for measurement in the light or dark. If an L is in the column, the following columns highlighted in red represent the first parameter listed. If a D is in the column, the following columns highlighted in red represent the second parameter listed.

The data file for the F_V/F_M meter is shown below:

Sample Number	Date	Time	SatI	SatW	ModI	Gain	Fo	Fv	Fm	Fv/Fm	Fv/Fo
1	May/20/20	0:33:58	5	1	2	2	204	50	254	0.196	0.245
2	May/20/20	0:34:05	5	1	2	2	219	33	252	0.13	0.15
3	May/20/20	0:34:10	5	1	2	2	222	31	253	0.122	0.139
Sample Number	Date	Time	SatI	SatW	ModI	Gain	Fo	Fv	Fm	Fv/Fm	Fv/Fo
1	May/20/20	13:05:23	8	1	3	3	203	3	206	0.014	0.014
2	May/20/20	13:05:37	8	1	2	3	549	1599	2148	0.744	2.912
3	May/20/20	13:06:10	8	1	2	3	344	816	1160	0.703	2.372
4	May/20/20	13:06:26	8	1	2	3	383	775	1158	0.669	2.023

Appendix A • Maintenance

Note: opening the Y(II) meter case or the F_V/F_M meter case voids the warranty!

Your Y(II) meter and F_V/FM meter contains no user serviceable components. Please contact your closest authorized agent for periodic preventative maintenance information. The only component that requires calibration every two years, is the PAR Clip. Fluorometers by themselves, do not require calibration

The Lithium ion battery, is a very reliable type of battery. It exhibits no memory effects whatsoever. It is recommended that when the system is not in use, even for very long periods of time, that the charger should be plugged into the Y(II) meter battery and the FV/FM meter battery and into a wall outlet for continuous charging. Prolonged periods of discharge will significantly decrease it's life span. Please contact Opti-Sciences if you have any questions concerning proper long term storage.

Any USB battery may be used with the instrument.

Cleaning

These instruments are made from durable materials, but some organic solvents can damage the surface finishes. Use a damp cloth with a mild detergent to clean the outside. The display window is made of a special, low reflectance glass, the keypad legend is lexan, while the case is aluminum. Should dirt get into the optical ports, uses s vacuum cleaner to remove the dirt. clean the optical probes with Windex, a clean cloth, or lens paper. If the unit becomes submerged in water, return it to the factory for maintenance (do not plug it in).

Battery

The is a USB Lithium ion battery that will provide about eight hours of average use. The typical recharging period is 8 hrs. Leaving the charger plugged in for longer than this will not damage the battery.

WARNING: only use USB batteries and chargers with this instrument.

As the battery ages, it will lose its ability to hold a charge. When this gets to four hours, buy a replacement.

Note: you can run these instruments from the battery charger alone, however it will not recharge while the instrument is on.

It is recommended that when the system is not in use, even for very long periods of time, that the charger should be plugged into the battery and into a wall outlet for continuous charging. Prolonged periods of discharge will significantly decrease it's life span. Please contact Opti-Sciences if you have any questions concerning proper long term storage.

Light sources

All Light sources are LEDs rated for thousands of hours. Replacement must be done at the factory.

Trouble shooting power problems

A majority of the problems encountered over the years with portable fluorometers are directly related to the battery, the circuit breaker, and the battery charger. The various sources (especially the saturation source) require a significant amount of power to operate properly.

The charging plug and cable should be plugged in the Y(II) meter battery and the F_V/F_M meter battery when not in use. Prolonged periods of discharge will significantly decrease it's life span. Both instruments use either portable USB lithium ion batteries or mains current.

Trouble shooting tables

Symptom	Probable Cause	Cure
Unit will not turn on	Dead battery	Charge battery or use AC power.
Unit resets when a test is run	Battery insufficiently charged Battery has become "weak" and needs replacement	Charge battery Contact factory
Display dims or changes contrast during a test	Battery low	Charge battery Contact Factory
Battery does not charge	Charger connector loose Charger is defective Dead battery	Check connections Replace charger Replace battery Contact factory
Trigger does not initiate a test	Bad battery connection Not in measuring screen Fluorescence signal is too low There is no leaf in the measuring chuck	Check connections Go to measuring screen Raise modulation intensity using "AutoSet" with a leaf in the measuring chuck

Saturation light does not trigger	Fluorescence signal (Ft) Is too low.	Increase the modulation intensity
	There is no leaf in the measuring chuck	Put a leaf in the measuring chuck.
	If display also blanks, battery may be low	Contact factory
	Modulation light or gain too low.	Use “AutoSet” with leaf in the chuck.

Symptom	Probable Cause	Cure
Cannot output data to computer - or - Output to computer garbled	Cable is loose Improper method Hardware handshake protocol on host	Secure cable connections Select “Link” on the main instrument screen. Go to Windows explorer on your computer, look for either the Y(II) drive, or the FVFM drive. Open the file using Excel or “open with” other spread sheet program. See data management sec.
Unit consistently loses data - or - Odd characters appear on various screens	Flash memory defective	Contact nearest authorized agent for service
Fluorescence signal is noisy	Battery is insufficiently charged External actinic light source is heavily modulated (pulsed xenon, or fluorescent light) The PIN diode detector gain is set too high.	Charge battery Use actinic illuminator with a continuous output that is not modulated. Do not use fluorescent lights!!! Under “F” in the set up screen Select “Y” for yes. This turns on electronic noise filtering. Raise the Modulated light intensity and reduce the gain control setting
Screen flickers.	Battery is insufficiently charged	Charge battery Use battery charger to run the system.
Incorrect PAR reading	PAR sensor problem	Contact Opti-Sciences
Incorrect temperature reading with optional PAR cuvette attached	Temperature sensor problem	Contact Opti-Sciences

Appendix C • Technical Specifications

The Y(II) meter is a low cost portable field instrument that measures Bernard Genty's fast light adapted parameter- $\Delta F/F_M'$, ETR, PAR, leaf temperature, relative humidity, and leaf absorptance. By eliminating fiber optics, and using the latest LED technology, Opti-Sciences provides a durable instrument, based on hard science, without compromise. Y(II) is more sensitive to some types of plant stress than F_V/F_M. For example: Y(II) will detect heat stress at about 35°C while F_V/F_M will not detect heat stress until about 45°C. For more information, request the Opti-Sciences Plant Stress Guide on this website.

Y(II) Meter Specifications:

Fast measurements: Y(II) or $\Delta F/F_M'$ with the option for F_M' correction according to Loriaux 2013 included, ETR, PAR, leaf temperature, relative humidity, leaf absorptance. 1-3 seconds. It also provides values for F_s, F_M' and F_t.

In **"monitor mode"**, it can measure the same sample for months at a time, using USB batteries or mains current. The monitor is designed for growth chamber, or laboratory work but not field work. The instrument can be programmed to measure at different intervals during light and dark periods or day and night. The monitor mode allows measurement of Y(II) or $\Delta F/F_M'$, ETR, PAR, leaf temperature, relative humidity and leaf absorptance. From the dark, or night time data, F_V/F_M, NPQ, Y(NPQ), and Y(NO) can be calculated.

Lighting conditions:

Ambient light only at steady state photosynthesis

Light sources:

White LED Saturation flash to 7,000 μmoles.

Choice of red modulated light source or blue modulated light source at time of purchase.

Modulated frequency is set at the factory. The red LED peaks at 660nm with a cut off filter at 690nm. The blue modulated light is at 450nm.

Sensors:

2 RGB sensors for measurement of leaf reflectance, leaf transmittance, and leaf absorptance, calibrated with standard. From 0 to 100%, read as a decimal, +- 0.005

1 PAR sensor – from 400nm to 700 nm – cosine corrected to 80^O +- 2 μmols

Pin Photodiode with a 700nm to 750nm band pass filter.

Relative humidity 0% to 100% +-2%

Leaf temperature measurement: with IR sensor over 80% of measuring aperture.

Temperature Accuracy ± 1.0 °C (worst case).

Temperature Resolution ± 0.1 ^{o}C.

Battery:

Lithium ion battery with 8 hour charge life at room temperature. Connected by USB port with charging plug and cable. Design to be carried in a pocket. However, the unit may be used with any USB compatible battery of any size. The instrument will also run on mains current.

Size:

6.34" x 1.5" x 2.0", USB cable 32" long, Battery 4.5" x 1" x 1" Weight with battery 0.8 lbs

17.15cm x 3.81cm x 5.08cm, USB cable 81.28cm, Battery 11.43cm x 2.54cm x 2.54cm Weight with battery 0.36 kg.

Memory:

2 Gigabytes

Output:

USB port with comma delineated format. Files may be opened in Excel or other programs with spread sheet formats.

Graphic screen display:

132 dots by 30 dots LCD.

Tripod Thread Mount -¼ inch 20 thread (Standard 35mm camera mounts use the same thread)

F_V/F_M Meter Specifications:

The F_V/F_M meter is companion instrument to the Y(II) meter that allow F_V/F_M measurements. When purchased together as the plant stress kit, there is a substantial savings compared to buying the F_V/F_M meter separately. F_V/F_M is the most used chlorophyll fluorescence measuring protocol in the world because it allows comparison of samples in the same known dark adapted state. This allows comparison of samples where the light is changing. While it is not as sensitive to some types of plant stress as the Y(II) meter, samples can be compared under canopy or situations where steady state photosynthesis is hard to measure. The F_V/F_M meter has an optical probe that permits measurement with dark adaptation clips, allowing fast measurement of large plant populations.

Fast measurements:

F_V/ F_M , F_V/ F_O, F_O, & F_M

Lighting conditions:

Dark adapted conditions only

Light sources:

Red LED Saturation flash array up to 6,000 µmoles.

Red modulated light source

Modulated frequency is set at the factory. The red LEDs peak at 660nm with a cut off filter at 690nm.

Sensors:

Pin Photodiode with a 700nm to 750nm band pass filter.

Battery:

Lithium ion battery with 8 hour charge life. Connected by USB port with charging plug and cable. Design to be carried in a pocket. However, the unit may be used with any USB compatible battery of any size. The instrument will also run on mains current.

Size:

8.5” x 1.125” x 1.625”, USB cable 32” long, Battery 4.5” x 1” x 1” Weight with battery 0.8 lbs

21.59 cm x 2.86 cm x 4.13cm, USB cable 81.28cm, Battery 11.43cm x 2.54cm x 2.54cm Weight with battery 0.36 kg.

Memory:

2 Gigabytes

Output:

USB port with comma delineated format. Files may be opened in Excel or other programs with spread sheet formats.

Graphic screen display:

132 dots by 30 dots LCD.

The PAR is calibrated To a Licor L-190 Quantum Sensor at the factory. The L190 calibration is traceable to National Institute of Standards and Technology. They should be returned to the factory every two years for recalibration.

For best results, the instrument should be left plugged into an outlet when not in use.

Bibliography

Adams WW III, Demmig-Adams B., Winter K. (1990) Relative contributions of zeaxanthin-unrelated types of high-energy-state quenching of chlorophyll fluorescence in spinach leaves exposed to various environmental conditions. Plant Physiol. 92, 302-309.

Allen J. F., Mullineaux C.W., (2004) Probing the mechanism of State Transitions in Oxygenic Photosynthesis by Chlorophyll Fluorescence Spectroscopy, Kinetics and Imaging. From Chapter 17, “Chlorophyll a Fluorescence a Signature of Photosynthesis”, edited by George Papaqeorgiou and Govindjee, pages 447-460

Baker N.R., Bradbury M. (1981) Possible applications of chlorophyll fluorescence techniques for studying photosynthesis in vivo. In Plants and the Daylight Spectrum (ed. H. Smith), pp 355-373. Academic Press. London

Baker N.R., East T.M., Long S.P. (1983) Chilling damage to photosynthesis in young Zea mays. II. Photochemical function of thylakoids in vivo. Journal of Experimental Botany, 34, 189-197.

Baker N. R., Oxborough K., (2004) Chlorophyll fluorescence as a probe of photosynthetic productivity. From Chapter 3, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, pages 66-79

Baker NR (2008) Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu Rev Plant Biol 59: 89–113

Banse K and Yong M (1990) Sources of variability in satellite derived estimates of phytoplankton production in the eastern tropical Pacific. J Geophys Res 95: 7201–7215

Bilger W., Bjorkman O, (1990) Role of xanthophyll cycle in photo-protection elucidated by measurements of light induced absorbance changes , fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynthesis Research 10: 303-308

Bilger W, Johnsen T., Schreiber U. (2001) UV –excited chlorophyll fluorescence as a tool for assessment of UV-protection by the epidermis in plants. Journal of Experimental Botany, Vol 52, No 363, 2007-2014

Bilger W., Schreiber U., Lange O.L. (1984) Determination of leaf heat resistance: Comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia 63, 256-262.

Bilger W., Schreiber U. (1986) Energy dependent quenching of dark-level chlorophyll fluorescence in intact leaves. Photosynth. Res. 10, 303-308.

Blankenship R. (1996) Photosynthetic Antennas and Reaction Centers: Current Understanding and Prospects for Improvement. ASU workshop 1996

Bradbury M., Baker N.R. (1981) Analysis of the slow phase of the in vivo fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystems I and II. Biochim. boiphys. Acta 63, 542-551.

Bukhov & Carpentier 2004 – Effects of Water Stress on the Photosynthetic Efficiency of Plants, Bukhov NG., & Robert Carpentier, From Chapter 24, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 627-628

Burke J. (2007) Evaluation of Source Leaf Responses to Water-Deficit Stresses in Cotton Using a Novel Stress Bioassay, Plant Physiology, Jan. 2007, Vol 143, pp108-121

Buschmann C. (2008) in correspondence by e-mail, Dr. Buschmann recommended taking multiple measurements per leaf to find potential infection locations as a substitute for fluorescence leaf imaging: Dr. Claus Buschmann (Priv.Doz), Botanik 2, Universität Karlsruhe (TH), 76128 Karlsruhe (Germany).

Butler W.L. (1972) On the primary nature of fluorescence yield changes associated with photosynthesis. Proc. nain. Acad. Sci. U.S.A. 69, 3420-3422

Cavender-Bares J. & Fakhri A. Bazzaz F. A, (2004) – "From Leaves to Ecosystem: Using Chlorophyll Fluorescence to Assess Photosynthesis and Plant Function in Ecological Studies". Cavender-Bares J., Bazzaz F. A,, From Chapter 29, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 746-747

Cailly AL, Rizzal F, Genty B and Harbinson J (1996) Fate of excitation at PS II in leaves, the nonphotochemical side. Abstract book of 10th FESPP Meeting, September 9-13, 1996, Florence, Italy. Supplement of Plant Physiol Biochem p.86

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 photooxidative stress in Arabidopsis”, The Plant Journal, Volume 76, Issue 4, pages 568–579, November 2013 DOI: 10.1111/tpj.12314

Cerovic Z, Goulas Y. Gorbunov M, Britantais J-M, Camenen L, & Moya I., (1996) Fluoresensing of water stress in plants. Diurinal changes of mean lifetime and yield of chlorophyll fluorescence, measured simultaneously at distance with a Lidar and modified PAM-fluorometer, in maize, sugar beet and Kalanchoe. Remote Sense Environment 58:311-321

Cerovic Z. G., Samson G., Morales F.,Tremblay N., Maoya I.(1999), “Ultraviolet-induced fluorescence for plant monitoring: present state and prospects”, a Groupe photosynthèse et télédétection. LURE/CNRS, Bât 203, centre universitaire Paris-Sud, B.P. 34, 91898 Orsay cedex, France, Agronomi 19 (1999) 565-566bCe

Cessna S., Demmig-Adams B., and Adams W. (2009) “Exploring Photosynthesis and Plant Stress Using Inexpensive Chlorophyll Fluorometers” J. Nat. Resour. Life Sci. Educ. 39:x–x (2010). doi:10.4195/jnrlse.2009.0024u • http://www.JNRLSE.org © American Society of Agronomy 677 S. Segoe Road, WI 53711 USA

Critchley C., Smilie R.M. (1981) Leaf chlorophyll fluorescence as an indicator of photoinhibition in Cucumis sativus L. Aust J Plant Physiol 8, 133-141.

Consalvey M., Jesus B., Perkins R.G., Brotas V., Underwood G.J.C., Paterson D.M. (2004) Monitoring migration and measuring biomass in benthic biofilms: the effects of dark/far-red adaptation and vertical migration on fluorescence measurements, Photosynthesis Research 81: 91-101, 2004

Dall'Osto L., Cazzaniga S.,Wada M. and Bassi R. (2014) On the origin of a slowly reversible fluorescence decay component in the Arabidopsis npq4 mutant, Phil.Trans. R. Soc. B 2014 369, 20130221, published 3 March 2014, http://rstb.royalsocietypublishing.org/content/suppl/2014/02/25/rstb.2013.0221.DC1.html

Duysens L.N.M., Sweers H.E. (1963) Mechanisms of two photochemical reactions in algae as studied by means of fluorescence. In: Studies on Microalgae and Photosynthetic Bacteria. University of Tokyo Press, Tokyo, pp 353-372.

Demming B., Winter K. (1988) Characterization of three components of non-photochemical quenching and their response to photoinhibition. Aust J Plant Physiol 15, 163-177.

Earl H., Said Ennahli S., (2004) Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. Photosynthesis Research 82: 177186, 2004. Edwards GE and Baker NR (1993) Can CO2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynth Res 37: 89102

Edwards GE and Baker NR (1993) Can CO2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynth Res 37: 89–102

Eichelman H., Oja V., Rasulov B., Padu E., Bichele I., Pettai H., Niinemets O., Laisk A. (2004) Development of Leaf Photosynthetic Parameters in Betual pendula Roth Leaves: Correlation with Photosystem I Density, Plant Biology 6 (2004): 307-318

Eilers P.H.C., Peeters, J.C.H.A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecological Modelling, Volume 42, Issues 3-4, September 1988, Pages 199-215

Flexas 1999 – "Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines" J. FLEXAS, J. M. ESCALONA & H. MEDRANO Plant, Cell & Environment Volume 22 Issue 1 Page 39-48, January 1999

Flexas 2000 – "Steady-State and Maximum Chlorophyll Fluorescence Responses to Water Stress In Grape Vine Leaves: A New Remote Sensing System", J. Flexas, MJ Briantais, Z Cerovic, H Medrano, I Moya, Remote Sensing Environment 73:283-270

Genty B., Briantais J-M, Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochem Biophys Acta 990; 87-92.

Genty B., Briantais J-M, Baker N.R. (1989) Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-photorespiratory conditions. Plant Physiol. Biochem. 28, 1-10.

Genty B.,Harbinson J, Briantais J-M, Baker N.R. (1990) The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem II photochemistry in leaves. Photosynth. Res. 25, 249-257.

Gorbunov M.Y., Kolber Z S, Lesser M.P., Falkowski P. G. (2001) Photosynthesis and photoprotection in symbiotic corals. Limnol Oceanogr., 46(1), 2001, 75-85

Govindjee, Baker N., . DE Sturler E. Ort D., Long S. (2005) "Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II", Planta 2005 223:114-133.

Govindjee, Downton W.J.S., Fork D.C., Armond P.A. (1981) Chlorophyll a fluorescence transient as an indicator of water stress in maize plants. Plant Sci. Lett. 20, 191-194.

Harbinson J., Genty B., Baker N.R. (1990) The relationship between CO_2 assimilation and electron transport in leaves. Photosynth. Res. 25, 213-224.

Haldimann P, & Feller U. (2004) Inhibition of photosynthesis by high temperature in oak(Quercus pubescens L.) leaves grown under natural conditions closely correlates with a reversible heat dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant, Cell and Environment (2004) 27, 1169–1183

Havaux M., Lannoye R. (1983) Chlorophyll fluorescence induction: A sensitive indicator of water stress in maize plants. Irrig Sci. 4, 147-151.

Heber U., Neimanis S., Lange O.L. (1986) Stomatal aperture, photosynthesis and water fluxes in mesophyll cells as affected by the abscission of leaves. Simultaneous measurements of gas exchange, light scattering and chlorophyll fluorescence. Planta. 167, 554-562.

Hendrickson L., Furbank R., & Chow (2004) A simple alternative approach to assessing the fate of absorbed Light energy using chlorophyll fluorescence. Photosynthesis Research 82: 73-81

HERLORY O. , RICHARD P. , BLANCHARD G. F (2007) “Comparing rapid response light curves vs. Non –sequential light curves allowed to reach steady state. The information for RLC curves under or over estimates parameters!” Marine biology 2007, vol. 153, pp. 91-101

Hodges M, Cormic G, Briantais J-M (1989) Chlorophyll fluorescence from spinach leaves: resolution of non-photochemical quenching. Biochim. biophys. Acta 289-293.

Hunt E. R Jr., Rock B. N., Detection of Changes in Leaf Water Content Using Near and Middle-Infrared Reflectances. Remote Sensing of Environment (1989)

Hunt E. R. (2008) references to Hunt 2008 reflect e-mail consultation with Dr. Hunt in 2008. (Available on request.)

Ikegami I. (1976) Fluorescence changes related to the primary photochemical reaction in the P-700 enriched particles isolated from spinach chloroplasts. Biochim. biophys. Acta 426, 559-574.

Kautsky H., Hirsch A. (1931) Neue Versuche zur Kohlenstoffassimilation. Naturwissenenschaften 19, 964

Kautsky H., Hirsch A. (1934) Das Fluoreszenzverhalten grUner Pflanzen. Biochem Z 274, 422-434

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochem Biophy Acta 376:105-115

Klughammer C., and Schreiber U. (2008) PAM Application notes 2008 1:27 -35

Kolb C.A., Schreiber U., Gadmann R. Pfundel E.E, (2005) UV-A screening in plans determined a new portable fluorometer.

Kramer D. M., Johnson G., Kiirats O., Edwards G. (2004) New fluorescence parameters for determination of QA redox state and excitation energy fluxes. Photosynthesis Research 79: 209-218

Krause G.H., Briantais J-M, Vernotte C. (1982) Photo induced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim biophys. Acta 679, 116-124.

Krause G.H., Weis E. (1984) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth. Res. 5, 139-157.

Laisk A and Loreto F (1996) Determining photosynthetic parameters from leaf CO_2 exchange and chlorophyll fluorescence. Ribulose-1,5-bisphosphate carboxylase / oxygenase specificity factor, dark respiration in the light, excitation distribution between photosystems, alternative electron transport rate, and mesophyll diffusion resistance. Plant Physiol 110: 903–912

Larcher W, Neuner G. (1989) Cold-induced sudden reversible lowering of in vivo chlorophyll fluorescence after saturating light pulses. A sensitive marker for chilling susceptibility. Plant Physiol. 136, 92-102.

Lichtenthaler H. K., Burkart S., (1999) Photosynthesis and high light stress. Bulg. J. Plant Physiol., 1999, 25 (3-4), 3-16

Lichtenthaler H. K., Babani F. (2004) Light Adaption and Senescence of the Photosynthetic Apparatus. Changes in Pigment Composition, Chlorophyll Fluorescence Parameters and Photosynthetic Activity. From Chapter 28, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George

Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 716

Loescher, Wayne at MSU Professor of Horticulture - discussions at ASPB meeting (2015)

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

MacIntyre HL and Geider RJ (1996) Regulation of Rubisco activity and its potential effect on photosynthesis during mixing in a turbid estuary. Mar Ecol Prog Ser 144: 247–264

MacIntyre HL, Geider RJ and McKay RM (1996) Photosynthesis and regulation of Rubisco activity in net phytoplankton from Delaware Bay. J Phycol 32: 718–732

MacIntyre H. L., Sharkey T.D., Geider R. (1997) Activation and deactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in three marine microalgae. Photosynthesis Research 51: 93–106, 1997

Markgraf, T. and J. Berry. 1990. Measurement of photochemical and non-photochemical quenching: correction for turnover of PS2during steady-state photosynthesis. In: M. Baltscheffsky (ed.), Curr. Res. Photosynth. IV:279-282.

Marra J (1978) Phytoplankton photosynthetic response to vertical movement in a mixed layer. Mar Biol 46: 203–208

Maxwell K., Johnson G. N, (2000) Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany Vol. 51, No. 345, pp.659-668- April 2000

Morales F., Abadia A., Abadia J. (1991) Chlorophyll fluorescence and photon yield of oxygen evolution in iron-deficient sugar beet (Beta vulgaris L.) leaves. Plant Physiol 97, 886-893.

Muller P., Xiao-Ping L., Niyogi K. (2001) Non-Photochemical Quenching. A Response to Excess Light Energy. Plant Physiology 125, 1558-1556

Newton B.A., Baker N.R., Long S.P., Lawlor D.W. (1981) In vivo photochemical function in water stressed leaves of Zea mays. In Photosynthesis VI. Photosynthesis and Productivity, Photosynthesis and the Environment (ed. G. Akoyunoglou),pp. 209-218, Balaban International Science Services, Philadelphia.

Nielsen H. D., Nielsen S., L., (2008) Evaluation of imaging and conventional PAM as a measure of photosynthesis in thin- and thick-leaved marine macro-algae AQUATIC BIOLOGY Aquat Biol 3: 121–131, 2008

Noctor G., Rees D., Young A, Horton P. (1991) The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence, and trans-thylakoid pH gradient in isolated chloroplasts. Biochim. boiphys. Acta 1057, 320-330.

Ogren E., Baker N.R. (1985) Evaluation of a technique for the measurement of chlorophyll fluorescence from leaves exposed to continuous white light. Plant, Cell & Environment 8, 539,547

OHASHI Y., NAKAYAMA N., SANEOKA H., FUJITA K., (2006) Effects of drought stress on photosynthetic gas exchange,chlorophyll fluorescence and stem diameter of soybean plants. BIOLOGIA PLANTARUM 50 (1): 138-141, 2006

Oberhuber W., Edwards G.E. (1993) Temperature Dependence of the Linkage of Quantum Yield of Photosystem II to CO_2 Fixation in C4 and C3 Plants. Plant Physiology 101; 507-512.

Opti-Sciences Plant Stress Guide www.optisci.com

Opti-Sciences Dark Adaptation Application Note www.optisci.com

Ouzounidou G, Moustakas M and Strasser RJ (1997) Sites of action of copper in the photosynthetic apparatus of maize leaves: Kinetic analysis of chlorophyll fluorescence, oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals. Aust J Plant Physiol 24: 81—90

Papageoriou G.C. Tismilli-Michael M. Stamatakis (2007) The fast and slow kinetics of chlorophyll a fluorescence induction in plants, algae and cyanobacteria: a viewpoint, Photosynth Res. (2007) 94:275-290

Peterson R.B., Sivak M.N., Walker D.A. (1988) Relationship between steady-state fluorescence yield and photosynthetic efficiency in spinach leaf tissue. Plant Physiol. 88, 158-163.

Pfundle E. (1998) Estimating the contribution of Photosystem I to total leaf fluorescence. Photosynth Res 56: 185-195

Pfundel E. E., Ghozlen N. B., Meyer, S, Cerovic Z.G. (2007) Investigating UV screening in leaves by two different types of portable UV florimeters reveals in vivo screening by anthrocyanins and carotenoids. Photosynthesis Research 10.1007/s11120-007-9135-7

Quick W.P., Horton P. (1984) Studies on the induction of chlorophyll fluorescence quenching by redox state and transthylokoid pH gradient. Proc R Soc Lond B 217, 405-416

Ralph P. J., Gademann R., (2005) Rapid light curves: A powerful tool to assess photosynthetic activity Aquatic Botany 82 (2005) 222–237

Rascher U (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field U. RASCHER, M. LIEBIG & U. LÜTTGE Plant, Cell and Environment (2000) 23, 1397–1405

Rees D., Noctor G.D., Horton P. (1990) The effect of high-energy state excitation quenching on maximum and dark level chlorophyll fluorescence yield. Photosynth. Res 25, 199-211.

Reuter D., Robinson JB., (1997) Plant Analysis: An Interpretation Manual Second Edition Chapter 3: Chapter 3 – Guidelines for Collecting, Handling and Analyzing Plant Material CSIROPUBLISHING ISBN: 0643059385 – AU 1997

Rosenqvist E., van Kooten O., (2006) Chlorophyll Fluorescence: A General Description and Nomenclature. From Chapter 2 “Practical Applications of Chlorophyll Fluorescence in Plant Biology”. by Jennifer R. DeEll (Editor), Peter M.A. Toivonen (Editor) Kluwer Academic Publishers group, P.O Box 322, 3300 A.H. Dordrecht, the Netherlands, pages 39-78

Ruban A.V., Johnson M.P., (2009) Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynthesis Research 2009 99:173-183

Samson G, Tremblay N., Dudelzak A.E., Babichenko S.M., Dextraze L., Wollring J.,Sampson (2000) NUTRIENT STRESS OF CORN PLANTS: EARLY DETECTION AND DISCRIMINATION USING A COMPACT MULTIWAVELENGTH FLUORESCENT LIDAR Proceedings of EARSeL-SIG-Workshop LIDAR, Dresden/FRG,
June 16 – 17

Schansker G. Toth S. Z., Strasser R. J. (2005), Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP, Bioenergetics Laboratory, University of Geneva, Chemin des Embrouchis 10, CH-1254 Jussy, Geneva, Switzerland, Biochimica et Biophysica Acta 1706 (2005) 250– 26

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62

Schreiber U., Neubauer C. (1987) The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. II. Partial control by the photosystem II donor side and possible ways of interpretation. Naturf. 42c, 1255-1264.

Schreiber U., Rientis K.G. (1987) ATP-induced photochemical quenching of chlorophyll fluorescence. FEBS Lett. 211, 99-104

Schreiber U., Endo T., Mi H., and Asada K. (1995) Quenching Analysis of Chlorophyll Fluorescence by the Saturation Pulse Method: Particular Aspects Relating to the Study of Eukaryotic Algae and Cyanobacteria Plant Cell Physiol. 36(5): 873 882 (1995)

Schreiber U, Tsuyoshi E., Hualing M., Kozi A. (1995) Quenching Analysis of Chlorophyll Fluorescence by the Saturation Pulse Method: Particular Aspects Relating to the Study of Eukaryotic Algae and Cyanobacteria, Plant and Cell Physiology Volume 36, Issue 5 pp 873-882

Schreiber U, (2004)Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation Pulse Method: An Overview From Chapter 11, “Chlorophyll a Fluorescence a Signature of Photosynthesis”, edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 279-319

Stern O., Volmer M., (1919) Uber die abklingungszeit der fluoreszenz. Physikalische Zeitsschrift 20: 183-1886

Simona Apostol 2006 - Leaf Fluorescence as Diagnostic Tool for Monitoring Vegetation Leaf Fluorescence as Diagnostic Tool for Monitoring Vegetation Book series:NATO Security through Science Series, Springer Netherlands, Volume 3/2006, From Cells to Proteins: Imaging Nature across Dimensions, DOI:10.1007/1-4020-3616-7, pages 423-430

Seaton CGR, Walker DA (1990) Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. Proc R Soc Lond B 242, 29-35.

Smilie R.M., Gibbons G.C. (1981) Heat tolerance and heat hardening in crop plants measures by chlorophyll fluorescence. Carlsburg Research Communications ,46, 395-403.

Smilie R.M., Nott R. (1982) Salt tolerance in crop plants monitored by chlorophyll fluorescence in vivo. Plant Physiology 70, 1049-1054

Smith B. M., Morrissey P. J., Guenther J.E., Nemson J. A., Harrison M. A., Allen J.F., and Melis A., (1990), Response of the Photosynthetic Apparatus in Dunaliella salina (Green Algae) to Irradiance Stress Plant Physiol. (1990) 93, 1433-1440

Srivastava A and Strasser RJ (1995) "How do land plants respond to stress temperature and stress light?" Archs Sci Genève 48: 135—145

Srivastava A and Strasser RJ (1996) "Stress and stress management of land plants during a regular day." J Plant Physiol 148: 445—455

Srivastava A. and Strasser RJ (1997) "Constructive and destructive actions of light on the photosynthetic apparatus." J Sci Ind Res 56: 133—148

Strasser BJ and Strasser RJ (1995) "Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: Mathis P (ed) Photosynthesis: from Light to Biosphere", Vol V, pp 977-980. Kluwer Academic Publishers, The Netherlands

Strasser RJ and Tsimilli-Michael M (2001) Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-Test. Chimie Nouvelle (SRC) (in press)

Strasser RJ, Srivastava A and Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U and Mohanty P (eds)Probing Photosynthesis: Mechanism, Regulation and Adaptation, Chapter 25, pp 443--480. Taylorand Francis, London, UK

Strasser R.J, Tsimilli-Michael M., and Srivastava A. (2004) - Analysis of Chlorophyll a Fluorescence Transient. From Chapter 12, "Chlorophyll a Fluorescence a Signature of Photosynthesis", edited by George Papaqeorgiou and Govindjee, published by Springer 2004, PO Box 17, 3300 AA Dordrecht, The Netherlands, page 340

Schansker G., Szilvia Z. Toth, Strasser R J. (2005) "Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP", Biochimica et Biophysica Acta 1706 250– 261

Schansker G. (2008)(http://come.to/bionrj) JIP Test assumptions. University of Geneva, Laboratoire de Bioenergetique et de Microbiologie.

Stryer, L (1988) Biochemistry, 2nd edition W.H. Freedman, San Francisco

Thach L. B., Shapcott A., Schmidt S. Critchley C. “The OJIP fast fluorescence rise characterizes Graptophyllum species and their stress responses”, Photosynth Res (2007) 94:423–436

Thiele A., Krause G.H., & Winter K. (1998) In situ study of photo-inhibition of photosynthesis and xanthophyll cycle activity in plants growing in natural gaps of the tropical forest. Australian Journal of Plant Physiology 25, 189-195

Tsimilli-Michael M and Strasser RJ (2001) “Mycorrhization as a stress adaptation procedure. In: Gianinazzi S, Haselwandter K, Schüepp H and Barea JM (eds) Mycorrhiza Technology in Agriculture: from Genes to Bioproducts.” Birkhauser Basel, (in press)

Tsimilli-Michael M and Strasser RJ (2001) “Fingerprints of climate changes on the photosynthetic apparatus behaviour, monitored by the JIP-test.” In: Walther G-R, Burga CA and Edwards PJ (eds) “Fingerprints” of Climate Changes – Adapted Behaviour and Shifting Species Ranges, pp 229--247. Kluwer Academic/Plenum Publishers, New York and London

Tsimilli-Michael M, Krüger GHJ and Strasser RJ (1995) “Suboptimality as driving force for adaptation: A study about the correlation of excitation light intensity and the dynamics of fluorescence emission in plants, In: Mathis P (ed) Photosynthesis: from Light to Biosphere”, Vol V, pp 981--984. Kluwer Academic Publishers, The Netherlands

Tsimilli-Michael M, Krüger GHJ and Strasser RJ (1996) “About the perpetual state changes in plants approaching harmony with their environment”. Archs Sci Genève 49: 173—203

Tsimilli-Michael M, Pêcheux M and Strasser RJ (1998) Vitality and stress adaptation of the symbionts of coral reef and temperate foraminifers probed in hospite by the fluorescence kinetics O-J-I-P. Archs. Sci. Genève 51 (2): 1—36

Tsimilli-Michael M, Pêcheux M and Strasser RJ (1999) “Light and heat stress adaptation of the symbionts of temperate and coral reef foraminifers probed in hospite by the chlorophyll a

fluorescence kinetics O-J-I-P”. Z Naturforsch 54C: 671—680

Tsimilli-Michael M, Eggenberg P, Biro B, Köves-Pechy K, Vörös I and Strasser RJ (2000) “Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and Azospirillum and Rhizobium nitrogenfixers on the photosynthetic activity of alfalfa, probed by the chlorophyll a polyphasic fluorescence transient O-J-I-P”. Applied Soil Ecology 15: 169—182

van Kooten O, Snel J (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25: 147–150.

Vermaas W.(1998) An introduction to Photosynthesis and its Applications. The World and I 158-`165

Vernotte C., Etienne A.L., Briantais J-M (1979) Quenching of the system II chlorophyll fluorescence by the plastoquinone pool. Biochim. biohys. Acta 545, 519-527

Weis E., Berry J. (1987) Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence. Biochim biophys. Acta 849, 198-208

Waterman P.G., Mole S., ,Waterman and Mole (1994) Analysis of Phenolic Plant Metabolites Blackwell Scientific Publications, Oxford, 1994.

Weis E., Lechtenberg D. (1989) Fluorescence analysis during steady-state photosynthesis. Phil Trans R Soc Lond B 323, 253-268.

Yilmas M.T. ,. Hunt E. R Jr., Jackson T.J, Remote sensing of vegetation water content from equivalent water thickness using satellite imagery. Remote Sensing of Environment (2008)

Yilmas M.T. ,. Hunt E. R Jr., Goins L.D., Ustin S. L., Vanderbuilt V. C., Jackson T.J, Vegetation water content during SMEX04 from ground data and Landsat 5 Thematic Mapper imagery. Remote Sensing of Environment (2007)