OS1p User's Guide

(Preliminary Advanced Version with RLCs for Algae and Higher Plants)

The New Standard in Portable Chlorophyll Fluorometers

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OS1p040111Advanced

Chapter 1 • Introduction

Overview

This chapter provides you with a list of the equipment that you should have received with your OS1p, 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 CD provided with the OS1p or by visiting www.optisci.com under Stress Testing, and Receive a Stress Guide with References.

Welcome !

Congratulations on your purchase of the OS1p 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:

- OS1p Fluorometer
- Universal Voltage battery charger
- Trifurcated fiber optic light guide with built-in trigger switch
- Ten dark-adaptation cuvettes
- Open body cuvette
- OS1p owner's manual (this document)
- 1 GB MMC/SD data card
- USB cable
- USB SD card reader
- Nylon field bag

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

Getting Started

Throughout this manual, you will be shown setup options and response messages. When an example of a program screen is given, you may assume that this is shown, as it will appear on the OS1p.

The user interface consists of a high-resolution color graphic touch screen LCD.

For editing parameters and making measurements, menu options are presented as icons or text legends. Measuring tests and parameter adjustments are all menu driven. Certain keywords are used to identify common functions. For example, the word or icon Exit will always step you to the previous program screen, ultimately ending in the "Main Menu".

The OS1p has default settings that allow the unit to work for many applications, however, changing the settings are very easily done.

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

Chlorophyll Fluorescence

A generalized description as it relates to chlorophyll fluorometry.

The following is a generalized description of the photosynthesis light reaction and the value of chlorophyll fluorescence for investigation of plant health, plant function and plant stress.

Changes in photosystem II (PSII) fluorescence have been shown to be a sensitive test for most types of plant stress, and reflect measurable changes of many plant functions including photochemistry, photoprotective mechanisms, low light survival state transition mechanisms, and heat dissipation photoinhibition mechanisms. PSII fluorescence measurements have been found to correlate well with changes in CO_2 fixation under most conditions (for more detailed correlation information refer to the section on "quantum photosynthetic yield of PSII").

Reaction centers are of two types, Photosystem II (PSII), and Photosystem I (PSI). Both are located in the thylakoid membrane of chloroplasts in higher plants. In bacteria, they are in a membrane surrounding the cytoplasm or in more intricate constructs. All plants that produce oxygen have both types of reaction centers.

While Photosystem I goes through a somewhat similar process to PSII, PSI fluorescence does not vary with plant stress nor does it change as changes occur with various photosynthetic mechanisms. Therefore PSII fluorescence is used for investigation into these areas.

Light energy utilized in photosynthesis by higher plants and algae cells is collected first by an antenna pigment system and transferred to reaction centers where light quanta are converted to chemical energy by chlorophylls in a protein environment. Electron transfer starts in the reaction center when a chlorophyll molecule transfers an electron to a neighboring pigment molecule. Pigments and protein involved in this primary electron transfer define the reaction center. This initial electron transfer is also called charge separation.

Competing models of energy capture and transfer have existed. In the puddle model, each reaction center possesses its own independent antenna system. In the lake model, reaction centers share antenna. The "lake model" is considered more realistic for terrestrial plants.

PSII and PSI reaction centers also share antenna during a process called state transitions. This process takes between ten and twenty minutes as a subset of PSII antenna detach from PSII reaction centers and migrate to PSI reaction centers (Ruban, Johnson 2009). They can also move back to PSII reaction centers. The process is governed by the oxidation-reduction state of the plastoquinine pool, and it is thought to be a survival mechanism for plants to subsist in low light conditions by balancing light levels between the two types of reaction centers (Allen, Mullineau 2004). Light level changes and changing light quality can trigger transitions.

During dark adaptation, higher plants and algae shift toward state 1 conditions and cyanobacteria to state 2 conditions. (Papageorgiou G.C. Tismmilli-Michael M. Stamatakis K. 2007). State transitions should be considered when deciding on dark adaptation times and for determination of steady state photosynthesis for quantum photosynthetic yield measurements, and quenching measurements. State transitions affect measurements more at low light levels than at high light levels (Lichtenthaler 1999).

It is in PSII that oxygen evolution and the splitting of water occurs. In the presence of light energy, an electron is pulled from a water-splitting complex and is used to reduce a PSII reaction center.

Charge separation occurs as Q_A, the primary plastoquinone receptor, is reduced to Q_{A-}. Electrons flow from there to other nearby plastoquinone molecules in the thylakoid membrane by oxidation-reduction reactions. They act as energy transfer molecules in an electron transport chain.

The next stop is a cytochrome b_6f complex where a proton is supplied to the thylakoid lumen. These protons and those supplied by the splitting of water are used by an ATP pump in the thylakoid membrane, in the presence of ATP synthase, to create ATP from ADP. Eventually the cytochrome b_6f complex also supplies an electron to PSI.

PSI then goes through a somewhat similar process to PSII, and eventually produces NADPH at the end of PSI. Both ATP and NADPH are used as energy sources to drive the dark reaction, called the Calvin Benson Cycle of carbon fixation.

Factors such as light levels, light quality, water availability, nutrient availability, heat, cold, herbicides, pesticides, pollution, disease, and genetic make up can all have an impact on CO_2 assimilation, plant health and condition. When these factors are not provided at optimal levels plant stress occurs, and most of these types of plant stress are also reflected in the fluorescence signal from PSII (For more detailed information about the best test for specific types of plant stress, consult the Plant Stress guide supplied on this disc or visit www.optisci.com under stress testing).

In 1989, Bernard Genty found that there is a linear correlation between fluorescent quantum photosynthetic yield measurements in C_4 plants and CO_2 assimilation. In 1990, Genty found a curve linear correlation between fluorescent quantum photosynthetic yield and CO_2 assimilation in C_3 plants, where photorespiration can also use significant products of electron transport. Psydo-cyclic electron transport and other electron sinks may also be involved.

Different types of plant stress affect PSII differently, therefore one should consult the Plant Stress Guide on this disc or contact Opti-Sciences at www.optisci.com to determine the best measuring protocol or special assay before working with a specific type of plant stress. Research referenced in the Plant Stress guide shows that while some types of plant stress affect chlorophyll fluorescence of a plant in a dark adapted state (Fv/Fm), measuring some types of plant stress, at a sensitive useful level, requires the light adapted Y(II) or $\Delta F/Fm'$. The Plant Stress guide is updates on a regular basis. For an updated version contact Opt-Sciences Inc at www.optisci.com.

The most prominent antenna pigment that absorbs energy is usually chlorophyll a. Other accessory pigments can also be involved, such as chlorophyll b., carotenoids, or phycobilins in cyanobacteria, or bacteriochlorophyll in some bacteria. We are primarily concerned with chlorophyll a fluorescence.

Light energy absorbed initially by the antenna and transferred to the reaction centers is channeled to competitive, different plant processes that include photochemistry, photo-protective heat dissipation, and other heat dissipation. Normally a healthy plant will channel

about 3%-9% (Govindgee 2004) of the light energy absorbed by chlorophyll pigments as chlorophyll fluorescence. Healthy plants in a healthy environment will use most light energy for photochemistry. More stressful environments will channel additional energy to heat dissipation and fluorescence.

This effect was first observed more than 100 years ago, when N.J.C. Müller (1874), using colored glass filters, studied the phenomenon. He also noted that fluorescence changes that occur in green leaves was related to photosynthetic assimilation. Lack of appropriate technical equipment prevented a more detailed investigation.

The basic OS1p is equipped to make several different kinds of tests including: Dark-adapted Fv/Fm, Light adapted Yield (Y) or ΔF/Fm' or Y(II), Fo, Fm, Fms (or Fm'), Fs (or F').It will also measure fluorescence quenching, and rapid light curves.

PAR Clips are sold separately, and are required for ETR, PAR, and Leaf temperature.

PAR Clips are highly recommended for field measurement of Y(II) and ETR. Because Y(II) values vary not only with plant stress, but also with light level and temperature, only samples at very similar light levels and light histories should be compared. Sun leaves will respond differently than shade leaves to different light levels.

The ability to use Rapid light curves will be provided in 2011 without additional charge for those that buy the advanced version of the OS1p. The advanced version also allows the user to select quenching protocols of interest when the unit is purchased. Other Protocols may also be purchased during or after the initial purchase. Fod (or Fo') are provided in the Kramer, and puddle model quenching protocols. The default quenching protocol that is offered on the advanced version of the OS1p is the Luke Hendrickson – Klughammer simplified lake model. This provides the most versatile quenching solution because it includes NPQ that has been resurrected from the puddle model. Other protocols are available for an additional charge. One may replace the Hendrickson protocol with either the Kramer protocol or the puddle model protocol at the time of purchase for no additional charge.

The quenching options include:

1. Dave Kramer's lake model parameters (Kramer 2004) - Y(II), qL, Y(NPQ), and Y(NO)

2. Luke Hendrickson's lake model parameters with Klughammer's resurrection of NPQ from the puddle model are included in this protocol - Y(II), Y(NPQ),Y(NO), and NPQ.

3. Puddle model quenching parameters qN, qP, and NPQ.

4. A relaxation protocol for subdividing NPQ into q_E, q_T, and q_I. This protocol may be used with NPQ in either the puddle model, or the Hendrickson lake model. The test runs at the same time as the protocols mentioned above. To use this protocol either the Hendrickson or puddle model protocols must also be purchased.

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 white light LED.

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.

Far red light – is a light source that provides light above 700 nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It is used extensively for the determination of quenching parameters in Quenching protocols, and for pre-illumination and rapid re-oxidation of PSII in Fv/Fm measurements. Fo' or Fod is used in determining Kramer's quenching parameters, as well as puddle model, qN. They require the use of far red light to determine quenched Fo' or Fod.

Modulated light source This is the light source that makes light adapted quantum photosynthetic yield measurements possible along with direct measurements of Fo and Fo' or Fod. 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 post photosynthesis Fo' or Fod. 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. 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 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 0.1 seconds and 1.5 seconds (Rosenqvist and van Kooten 2006). In algae and Cyanobacteria, the optimal saturation pulse duration is between 25ms. and 50ms. It is typically a white light that has to be high enough to close all PSII reaction centers. On the OS1p an LED light source is used. Opti-Sciences uses 0.8 seconds as the default saturation pulse duration for higher plants. This duration is adjustable from 0.025 to 2.0 seconds.

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

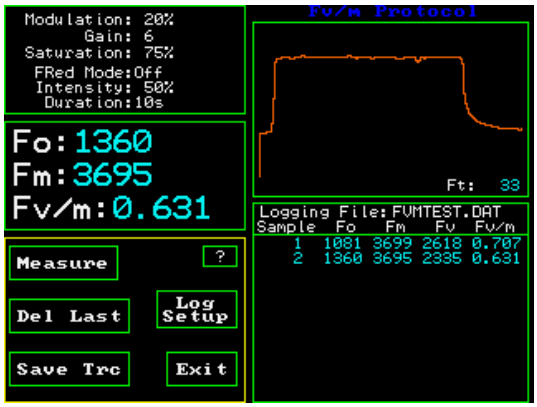
Fs also known as F' is the fluorescence level created by the actinic light . Initially the value is high and then decreases over time to steady state values due to the initiation of electron transport, carboxilation, and nonphotochemical quenching. Fs has also been used to designate steady state F' conditions.

Fms – also known as Fm' is the saturation pulse value that is not dark-adapted. They are at a lowered values due to NPQ or non-photochemical quenching. When this parameter has reached steady state, it is used to calculate photosynthetic Yield - Y(II) or ΔF/Fm' along with Fs. Fms at steady state is also used to calculate qN, NPQ, qP, qL, Y(NPQ), Y(NO), qE, qT, and qI.

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.

Fod – also known as Fo', is the minimal value after the actinic light has been turned off and after a far red light is turned on for several seconds. It represents Fo with non-photochemical quenching. It may also be described as minimum chlorophyll fluorescence yield with maximal opening of all PS II reaction center traps in a light-acclimated state.

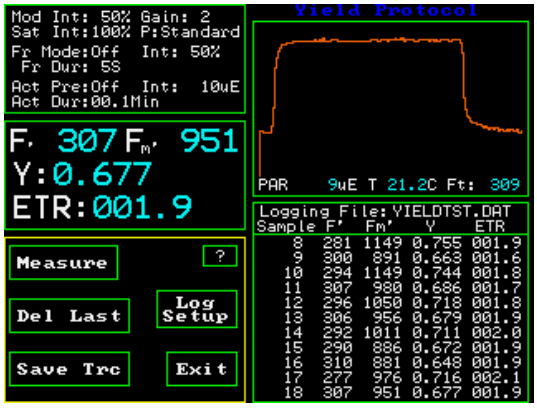
Ft – is the current instantaneous fluorescent signal shown on the fluorometer measuring screen. It is used to set the modulated light source intensity. See setting the modulated light source intensity.



FV/Fm measuring Screen

***Fast* -Dark Adapted Fv/Fm**
Usually less than 2 seconds

Fv/Fm = (Fm – Fo) / Fm This is a dark adapted test used to determine Maximum quantum yield. 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.



Y(II) or Yield Measuring Screen

***Fast* -Light Adapted Y(II)**
Yield Test - *Usually less than 2 seconds*

Yield or quantum photosynthetic yield of PSII= (Fm' – Fs) / Fm' -This test is also known as ΔF/Fm' or Y(II). Yield of PSII is a fast light adapted test taken at steady state photosynthesis levels. It 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 and heat dissipation caused by non-photochemical quenching. Y(II) allows investigation of the photosynthetic process while it is happening. No dark adaption is required. According to Maxwell and Johnson (2000) it takes between fifteen to twenty minutes for a plant to reach steady state photosynthesis at a specific light level. To obtain a reliable Y(II) measurement, photosynthesis must reach steady state.

This is usually not a concern when using ambient sunlight or artificial greenhouse light, however, clouds and light flecks below a canopy level can cause problems. 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.

Yield 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.

Light adapted measurements include Yield of PSII or **Δ**F/Fm' or Y(II), ETR, PAR (or PPFD), and Leaf Temp. With RLC (rapid light curves) The OS1p measures ETRmax, lk, and α.

µE – is a **micro Einstein**. This a dimension that involves both time and area. It is equivalent to the micro mol. Both terms have been used extensively in biology and radiation measurements.

µmls - is a **micro mole**(also abbreviated µmol, or µmol $m^{-2}s^{-1}$) . This a dimension that involves both time and area (per meter squared per second) . It is equivalent to the micro Einstein. Both terms have been used extensively in biology and radiation measurements.

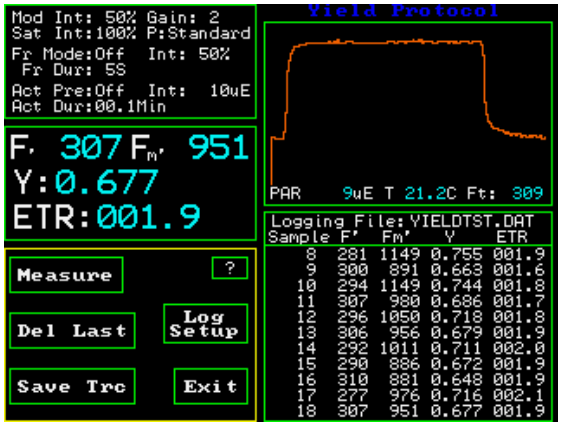
µmol – or micro mole(also abbreviated µml, or µmol $m^{-2}s^{-1}$) . This a dimension that involves both time and area (per meter squared per second) . It is equivalent to the micro Einstein. Both terms have been used extensively in biology and radiation measurements.

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

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 Clip – This is a fluorometer accessory that allows the measurement of PAR or PPFD and Leaf Temperature along with Yield or Y(II) measurements. Since Yield 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. A PAR Clip allows the calculation of relative ETR or Electron Transport Rate. It will also work with the internal fluorometer actinic illuminator, to measure reproducible and repeatable controlled values. PAR clips are recommended for field use with quantum photosynthetic yield measurements. See the section on quantum photosynthetic yield for an in depth discussion.

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).



Y(II) or Yield Measuring Screen

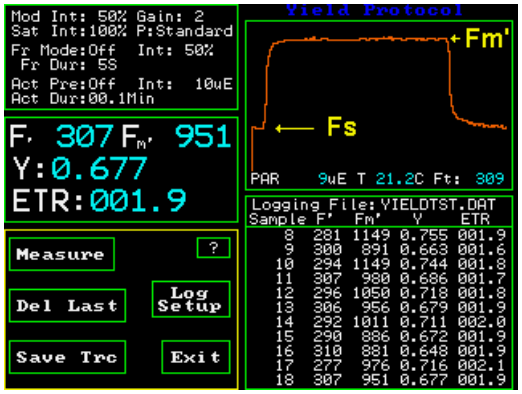
Quantum photosynthetic yield of PSII – an in depth discussion of its value and limitations.

Yield (or ΔF/Fm' or (Fm' – Fs) / Fm') or Y(II) 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 PSII absorption 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 fifteen to twenty minutes (Maxwell and Johnson 2000). 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.



$$Y(II) = (Fm' - Fs) / Fm'$$

Quantum photosynthetic yield 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 multi-flash section for more details.

Graphic display of a single Yield measurement taken with a PAR Clip. Yield measurements may also be taken with an Open Body Clip (without PAR or temperature measurement).

Yield Y(II) will change at different light levels and temperatures so it can be of great value to use a heavily recommended accessory called a PAR Clip that measures Y(II) relative to light intensity, or irradiation level, and temperature. PAR Clips measure 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).

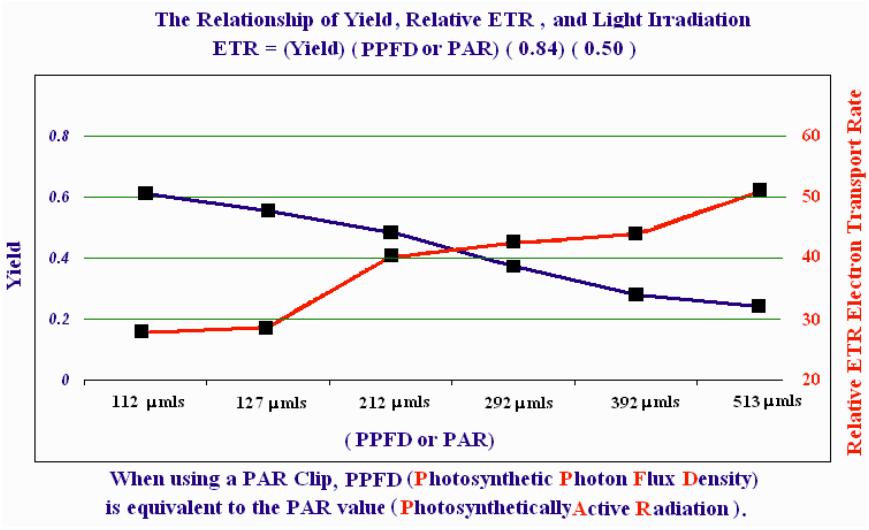
NOTE: It is possible to misinterpret results if PAR and temperature changes are not taken into account. One leaf may appear to be stressed compared to another when the only difference is light irradiation level. PPFD or PAR must be measured very close to the sample or errors can result.

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 CO2 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 Yield 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. Maxwell and Johnson (2000) tested 22 different species of British plant and found that steady state occurred in fifteen to twenty minutes in the plants measured. Measurements taken under variable

lighting conditions may not provide reliable Yield results (Rascher 2000). No dark adaptation is required for Yield measurements.



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 CO2 assimilation for C4 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 C3 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.

Limitations of Y(II) or ΔF/Fm'

The strong relationship between Yield and CO2 assimilation correlation has been reaffirmed repeatedly by many researchers with the following caveats:

1. There is a small percentage of chlorophyll fluorescence that comes from photosystem I that does not change with light intensity (PPFD) or plant stress. Therefore, the error is greatest at very high light levels when yield is minimized and PSI fluorescence remaining constant. This error is not large (Baker Oxborough 2004).

2. "Super-saturating flash" error is produced by using a very intense saturation light source that is longer that 2 milliseconds causing multiple turnovers of primary PSII receptor Q_A and the reduction of plasotoquinone to plastoquinol. This raises Fms (or 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.

3. 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. This indicates, that under these conditions, other electron sinks are at work. The ratio of ETR 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)

4. The ratio of ETR to CO_2 assimilation can be diagnostic for water 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 the Burke assay (Burke 2010). Yield can be used to measure very early water stress (Burke 2007 and Burke 2010).

5. 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)

6. Measurements not taken at steady state photosynthesis can lead to non-linearity caused by state transitions. This error can be in the range of 10% to 30% depending on the organism (Allen and Mullineau 2004). The error can be avoided by allowing plant samples to reach steady state photosynthesis, a process that takes between fifteen and twenty minutes (Maxwell and Johnson 2000).

7. At very high light stress levels, the correlation between ETR and CO_2 assimilation breaks down. It is thought by some to be caused by the inability of the most intense saturation light sources to completely close all PSII reaction centers under high light stress conditions. To compensate for this issue, Earl (2004) uses saturation pulses at various levels and extrapolates the saturation pulse fluorescence intensity at infinity using linear regression analysis. This method restores the correlation of ETR and CO2 assimilation and it is an option that is offered on the Opti-Sciences OS1p and the OS5p.

8. While linear correlation and curvilinear correlation are possible (Genty 1989), (Genty 1990), (Baker Oxborough 2004), exact correlation between fluorescence ETR and gas exchange ETR 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).

9. In CAM plants, gas exchange measurements are not possible during daylight hours so Yield measurements can provide insights into daytime light reactions (Rosenqvist and van Kooten 2006).

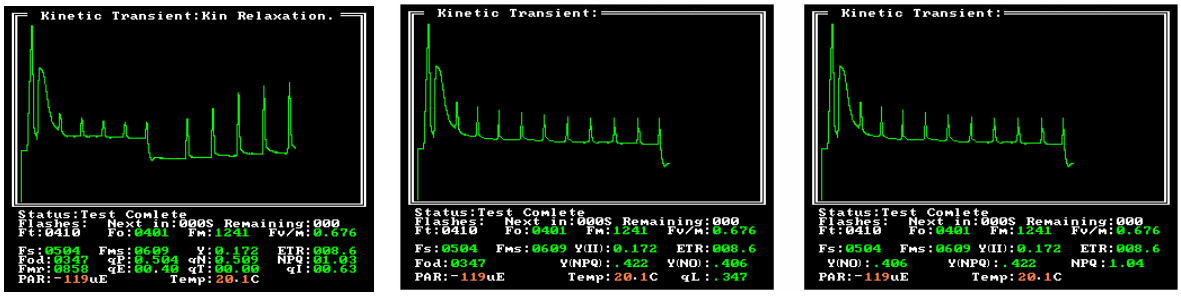
As illustrated by the exceptions listed above, in some cases the relationship between light reactions and dark reactions is not straightforward. The energy molecules ATP and NADPH can be used for carbon fixation and for photorespiration (Rosenqvist and van Kooten 2006), or light reaction electrons may flow to other electron sinks (Ohashi 2005), (Baker Oxborough 2004), (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998). For this reason, it is not uncommon for authors to differentiate between work done under non-photorespiratory conditions and under photorespiratory conditions (e.g. Earl 2004), (e.g. Genty B, Harbinson J., Baker N.R. 1990).

The Opti-Sciences chlorophyll fluorometer models OS5p and OS1p can be used to make Quantum Photosynthetic Yield Y(II) measurements. Both units accommodate optional digital PAR Clips.

Yield,Y(II), is the more versatile fluorescence measuring parameter, but it is best to use a system that offers multiple test parameters for diverse stress applications. While systems that provide true yield measurements tend to cost more than ones that provide just Fv/Fm measurements, they offer greater capability.

Quenching Measurements, an Overview

(Images from the OS5p)



Puddle Model | Kramer Lake Mode Hendrickson | Klughammer Lake Model

Dark–Light Pulse modulated Chlorophyll Fluorescence Trace – Screens are taken from the OS5p.

Parameter read outs from the OS1p will be in a separate window but on the same screen, and only one of the protocols shown, come as standard with the system. Others may be purchased for an additional price.

Introduction:

This article is an overview of the value and limitations of kinetic traces and provides a basic understanding of photochemical and non-photochemical quenching measurements. The puddle model, the Kramer lake model, and the Hendrickson /Klughammer & Schreiber lake model parameters will be reviewed and practical considerations are added to the discussion.

Kinetic traces are used in measuring photoprotective mechanisms, state transitions, photoinhibition, passive energy dissipation and creating light curves. These measurements are usually determined when a plant reaches steady state photosynthesis, however, Klughammer states that Y(NO) is not limited to steady state measurement if his simplified equations are used. Without a good understanding of the mechanisms that affect the light trace, machine artifacts and user errors can be included in measurements.

The Fluorescence signal

Fluorescence has been found to provide significant information regarding photosynthetic processes, plant health, and plant stress measurement. Fluorescence from light that is absorbed by PSII has been shown to vary with these conditions and allow measurement, where as PSI fluorescence is low, and does not vary in intensity. The fluorescence signal that comes from PSII is the result of a competitive process with photochemistry and heat dissipation (heat dissipation can further be divided into non-radiation decay and photoprotective regulated heat dissipation). For example, when most of the light is used by photochemistry, less is given off as fluorescence and heat. PSII has also been found to be sensitive to most types of plant stress (Baker 2008).

Proper dark adaptation is very important for quenching measurements

Normally the Dark-Light Kinetic curve begins with dark adaption of the sample leaf. It is common when making quenching measurements to dark-adapt for a full night, or even 24 hours (Maxwell and Johnson 2000).

In some cases, longer times may be appropriate. Lichtenthaler (2004) found that it could take up to 60 hours for complete relaxation of NPQ in plants subjected to chronic photoinhibition conditions. *All non photochemical quenching measurements use Fm as a reference* and some parameters also use Fo (Schreiber 2004). For this reason it is important to ensure that one starts with a properly dark adapted sample because all measurements are made relative to these values. In the field, pre-dawn quenching measurements can provide a way to measure plant stress, however it is important to understand that these values may be still be affected by recent light history (Maxwell and Johnson 2000). Leaves with dissimilar light histories, different Fv/Fm, or of different species should not be compared using non-photochemical quenching parameters. (Maxwell & Johnson 2000). Baker (2008) states that only leaves with similar Fv/Fm values should be compared. (for more details on dark adaptation please request the Opti-Sciences dark adaptation application note).

Actinic Light Source

Most built-in fluorometer light sources used as actinic sources for quenching measurements and light curves decline in intensity during these measurements. This is due to the fact that heat from the internal light sources reduces light output. It can happen to halogen light sources and to LED light sources. When this happens, the photosynthetic sample may never really reach steady state photosynthesis, a process that takes between fifteen and twenty minutes (Maxwell and Johnson 2000). Such light sources can produce errors in all quenching and quantum photosynthetic yield values. It is recommended that the PAR clip be used for quenching measurements and for the creation of quenching tests and rapid light curves.

Lake model and puddle model quenching parameters.

Understanding of the organization of antennae and reaction centers has changed over the years. It is now understood that a single antennae does not link only to a single reaction center as was previously described in the puddle model. Current evidence indicates that reaction centers are connected with shared antennae in terrestrial plants. qP, the parameter that has been used in the past to represent the fraction of PSII reaction centers that are open, is a puddle model parameter. Dave Kramer (2004) has come up with a set of fluorescence parameters that represent the newer shared antennae paradigm called the lake model. Others have also come up with more simplified equations that eliminate the need for the measurement of Fo and Fo' and approximate the measurements made by Kramer.

Hendrickson's (2004) work offered such a solution with Y(NPQ) measurements that are consistently and only marginally lower values than Kramer's work, and Y(NO) measurements that are consistently and marginally lower except at high light levels and low temperatures than Kramer's work. He speculates that the differences in values between Kramer and his own were possibly due to the difficulties in making Fo'(or Fod) measurements. Furthermore, Hendrickson does not provide a parameter like qL to estimate the fraction of open PSII centers.

From Hendrickson's work, and earlier works by Cailly (1996) and Genty (1989, 1996), Klughammer and Schreiber derive simplified equations that allow for Hendrickson's parameters, and also allow users to reconcile NPQ measurements with the lake model.

The Luke Hendrickson simplified lake model parameter were chosen as the default quenching protocol for the OS1p.This was done because they are lake model parameters that allow the resurrection of NPQ from the puddle model by Klughammer, and because relaxation protocols, available in the puddle model, still work for the Hendrickson lake model. Other protocols may be selected at the time of purchase, or they may be purchased at a later date. They include Kramer parameters, puddle model parameters and a quenching relaxation protocol that works with either the Hendrickson lake model or the puddle model protocols. The quenching relaxation protocol has been retained because it allows for the separation of state transition measurements, and photo-inhibition in quenching relaxation protocols. In standard lake model parameters, they are both part of Y(NO). There is also a significant volume of work done using the older puddle model parameters that may be valuable for comparison especially in regard to NPQ.

The loss of light energy from the reaction center as fluorescence comes primarily from the PSII reaction. When leaves have been dark-adapted, the pools of oxidation-reduction intermediates in the electron transport pathway return to a oxidized state and quenching mechanisms relax. At this point a low intensity modulated light turns on and off and the minimal fluorescence signal, Fo, is measured. The modulate light source is at an intensity too low to drive photosynthesis but high enough to provide a weak fluorescence signal so it is ideal for measurement. Upon saturation illumination of a dark-adapted leaf, there is a rapid rise in fluorescent light emission from PSII as all reaction centers are closed and all the maximum amount of light is channeled to fluorescence. Multiple turn-overs of the Q_A molecule occur before maximal fluorescence, Fm, is reached in a healthy leaf. If an Fv/Fm measurement is being made then this is where the process ends.

Quenching equations:

Kramer, and Hendrickson / Klughammer &Schreiber's, lake model parameters account for all light that is absorbed by PSII. All parties agree with the following equation.

Kramer's equation is 1 = Y(II)+Y(NPQ)+Y(NO)

Y(II) is quantum yield of photochemical energy also known as ΔF/Fm' or (Fm'- Fs')/ Fm'

For comparison purposes, the differences in the equations are listed below.

Kramer's equations

Y(II) = (Fm' -Fs)/Fm' or ΔFm'/ Fm'

$q_L = q_P(Fod/Fs)$ or $q_L = q_P(Fo'/F)$

$Y(NO) = 1/(NPQ+1+q_L(Fm/Fo -1))$

Y(NPQ) = 1- Y(II)-Y(NO)

Hendrickson - Klughammer and Schreiber's simplified equations

Y(II) = (Fm' -Fs)/Fm' or ΔFm'/ Fm'

Y(NO) = Fs/Fm or F/Fm

Y(NPQ) =(Fs/Fm')-Y(NO) or (F/Fm') - Y(NO)

NPQ= Y(NPQ)/Y(NO) or NPQ = (Fm-Fm')/Fm'

Puddle model parameters

q_P = (Fm' - Fs)/(Fm' - Fo) Above 0.4, Fo' or Fod should replace Fo

q_N = 1- ((Fm'-Fo) /(Fm-Fo)) Above 0.4, Fo' or Fod should replace Fo

NPQ = (Fm-Fm')/Fm'

$NPQ=q_E+q_T+q_I$

q_E = ((Fme-Fm')/(Fm-Fm')) is the relaxation saturation value

at four minutes in the dark. (Values can be changed in Excel).

q_T = ((Fmt-Fme)/ (Fm-Fm')) is the relaxation saturation value at

twenty minutes in the dark. (Values can be changed in Excel).

q_I = ((Fm-Fmt) / Fm-Fm')) Relaxation of q_I starts at about forty minutes and can take up to sixty hours. q_I can be determined from the dark adapted Fm measurement and the saturation pulse after 20 minutes used for q_T or Fmt. (Values can be changed in Excel).

Puddle model parameters notes.

Puddle model event times for q_E, q_T and q_I were taken from Lichtenthaler (1999). Descriptions of q_E, and q_I are taken from Muller P., Xiao-Ping L., Niyogi K. (2001), q_T is taken from(Rubin, Johnson 2009) (Allen, Mullineau 2004), and Lichtenthaler (1999)

According to Baker (2008), the event times such as the time it takes for q_E, used in the quenching relaxation test, can change under field conditions.

Definitions - lake model parameters

Y(NPQ) is a lake model quenching parameter that represents heat dissipation related to all photo-protective mechanisms also called regulated heat dissipation. (David M. Kramer, Giles Johnson, Olavi Kiirats & Gerald E. Edwards 2004) (Klughammer and Schreiber 2008).A low Y(NPQ) at high light levels is an indication of sub-optimal photoprotective 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 (David M. Kramer, Giles Johnson, Olavi Kiirats & Gerald E. Edwards 2004). Klughammer and Schreiber 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 photodamage. (Klughammer and Schreiber 2008).

q_L is the lake model quenching parameter that represents photochemical quenching. It is a measure of the fraction of still open PSII reaction centers. (David M. Kramer, Giles Johnson, Olavi Kiirats & Gerald E. Edwards 2004).

Y(II) = quantum yield of photosynthetic energy in PSII. The equation is ΔF/Fm' or (Fm' – Fs) / Fm'.

Puddle model parameter reconciled with the lake model
(Klughammer and Schreiber 2008)

NPQ = Y(NPQ)/Y(NO) or NPQ= (Fm-Fm')/Fm' 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 Fo' or Fod. The advantage of NPQ over qN depends on the specific application. NPQ is more heavily affected by non-photochemical quenching that reflects heat-dissipation of excitation energy in the antenna system. So, it may be thought of as an indicator of 'excess excitation energy'. Alternatively, NPQ is relatively insensitive to the part of nonphotochemical quenching associated with qN values lower than 0.6. The range of NPQ is affected by Δph of the thylakoid lumen, and the xanthophyll cycle, 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). (Bilge & Bookman, 1990), (Muller P., Xiao-Ping L., Niyogi K. 2001).

Definitions - puddle model parameters

NPQ (puddle model parameter) 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 Fo' or Fod. The advantage of NPQ over q_N depends on the specific application. NPQ is more heavily affected by non-photochemical quenching that reflects heat-dissipation of excitation energy in the antenna system. So, it may be thought of as an indicator of 'excess excitation energy'. Alternatively, NPQ is relatively insensitive to the part of nonphotochemical quenching associated with q_N values lower than 0.6. NPQ is affected by Δph of the thylakoid lumen, the xanthophyll cycle, 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). (Bilge & Bookman, 1990), (Muller P., Xiao-Ping L., Niyogi K. 2001).

q_N (puddle model parameter) is similar to NPQ but requires Fod or Fo' in the calculation. qN is defined as the coefficient of non-photochemical fluorescence quenching. The original definition of this term implied that fluorescence quenching affects primarily the 'variable fluorescence' (Fv) and not the minimal fluorescence (Fo). In cases where qN is greater than 0.4 this may not be a good assumption. When q_N is above 0.4, Fo' (or Fod) should replace Fo in q_P equations. q_N is less sensitive than NPQ at higher values (Maxwell and Johnson 2000). By using the Far-Red source after actinic illumination is turned off, the PSII acceptors re-oxidized and PSI is reduced. An Fo' value is measured and used for corrections to the quenching coefficients. Numbers range from zero to one. (puddle model) (Van Kooten & Snell, 1990)

q_P (puddle model parameter) is the quenching parameter that represents photochemical quenching. It is a measure of the fraction of still open PSII reaction centers. q_P is defined as the coefficients of photochemical fluorescence quenching. The original definition of this term implied that fluorescence quenching affects primarily the 'variable fluorescence' (Fv) and not the minimal fluorescence (Fo). In cases where qN is greater than 0.4 this may not be a good assumption. When qN is above 0.4, Fo' (or Fod) should replace Fo in qP equations. By using the Far-Red source for post illumination, the PSII acceptors may be re-oxidized through the illumination affect on PSI. A new Fod value may be measured and used for corrections to the quenching coefficients. This assumes the PSI acceptors are properly activated, which may not be the case in a dark adapted sample. Therefore, the Fod determination should be done after induction of photosynthesis has been done for several minutes. Numbers range from zero to one. (Puddle model) (Van Kooten & Snell, 1990)

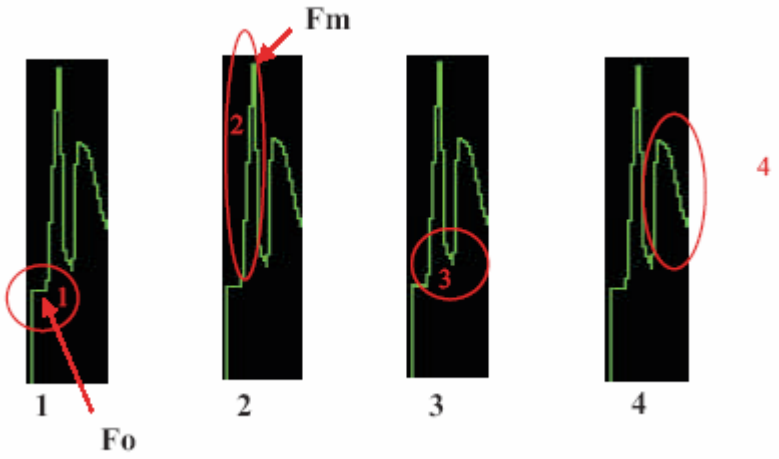
q_E (puddle model parameter) is the quenching parameter that represents the photo-protective mechanisms in the leaf that allow rapid compensation for changes in light levels due to cloud cover and increased light intensity. It is directly related to Δph of the thylakoid lumen and the xanthophyll cycle. (Muller P., Xiao-Ping L., Niyogi K. 2001) This process is completed in two to four minutes after an actinic light is turned on. (Lichtenthaler 1999). It is delineated as a percent of NPQ by using a quenching relaxation method. Some researchers in the past have also divided qN into q_E, q_T, and q_I instead of NPQ (Lichtenthaler 1999) The relaxation characteristics of field plants can vary with changing environmental conditions (Baker 2008). NPQ is used to determine relaxation coefficients because it has been resurrected for Klughammer's lake model parameters. Use of q_N has been discredited in the lake model.

q_T (puddle model parameter) is not true quenching. Instead, the parameter represents state 1 and state 2 transitions. This value is negligible in higher plants at high light levels but may be substantial at low light levels (Lichtenthaler 1999) (Baker 2008). According to Ruban (2008) state transitions require between fifteen and twenty minutes to complete. It can be delineated as a percent of NPQ by using a quenching relaxation method (Muller P., Xiao-Ping L., Niyogi K. 2001),(Lichtenthaler 1999). For more information on state transitions, and how they affect fluorescence measurement contact Opti- Sciences for the application note on state transitions. The relaxation characteristics of field plants can vary with changing environmental conditions (Baker 2008). NPQ is used to determine relaxation coefficients because it has been resurrected for Klughammer's lake model parameters. Use of q_N has been discredited in the lake model.

q_I (puddle model parameter) is the quenching parameter that represents photo-inhibition and photo-damage. (Puddle model) (Muller P., Xiao-Ping L., Niyogi K. 2001) According to Lichtenthaler (1999, 2004) chronic photoinhibition starts to relax after forty minutes in the dark and may take up to sixty hours. It can be delineated from NPQ by using a quenching relaxation method. The relaxation characteristics of field plants can vary with

changing environmental conditions (Baker 2008). NPQ is used to determine relaxation coefficients because it has been resurrected for Klughammer's lake model parameters. Use of q_N has been discredited in the lake model.

Understanding the quenching mode trace



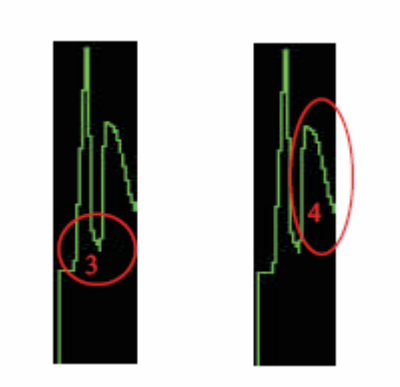
#1 represents a condition that is normally dark-adapted (proper dark adaption is required when making nonphotochemical quenching measurements) with the sample shielded from any actinic light that would drive photosynthesis. The only light on at this time is the modulated measuring light at about 0.1umols intensity. This is not enough to drive photosynthesis but it is enough to detect and measure minimum fluorescence from the leaf antennae. In most commercial fluorometers, 30% of the intensity of Fo in C_3 plants is the result of fluorescence from PSI. In C_4 plants, 50% of the intensity of Fo is the result of fluorescence from PSI. This contributes to a small error in Fv/Fm measurements and creates an underestimation of maximum quantum efficiency

#2 shows the first saturation pulse flash. This is a very intense short lasting flash of light that is designed to saturate PSII and close all available reaction centers. For higher plants, the optimal time duration for a saturation pulse has been found to be between 0.5 seconds to 1.0 seconds (Rosenqvist and van Kooten 2005). For Algae and cyanobacteria the optimal duration of the saturation pulse is shorter, 25 to 50 ms (Schreiber 1995).

This allows complete saturation without causing NPQ to underestimate Fm. Saturation pulse durations that are longer than recommended create a rounding of the top back of the saturation pulse caused by a form of NPQ. However, this is not true of the OS1p. It seeks to find the highest eight point rolling average of intensity points from the saturation pulse and reports the value. With dark adapted samples complete closure of all PSII reaction centers can be accomplished with minimal saturation intensity. PAR Light values of 3,000 µmols or higher are commonly used. Certainly values of 3000 µmols or higher will fully saturate any properly dark adapted sample.

The rise from Fo to Fm represents multiple turn-overs of Q_A, the primary electron transport receptor. PSII becomes fully reduced at Fm with all PSII reaction centers closed. All absorbed

light at this point is given off as fluorescence and is not involved in photochemistry or heat dissipation (Baker 2008). (Baker 2008).

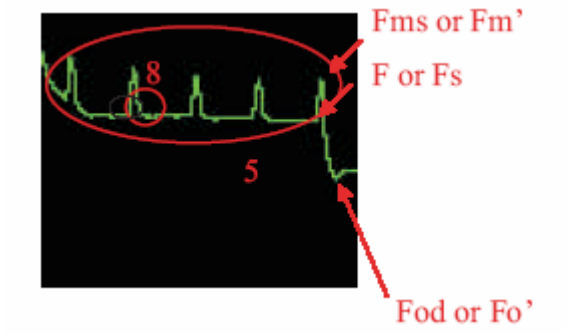


#3

All light sources except the modulated measuring light source are turned off after the saturation flash. The fluorescence graph tails off until it reaches bottom exhibiting non-photochemical quenching related to the saturation flash. During this period Q_A is re-oxidizing but it is a partial condition because there is not enough time for full re-oxidation.

#4

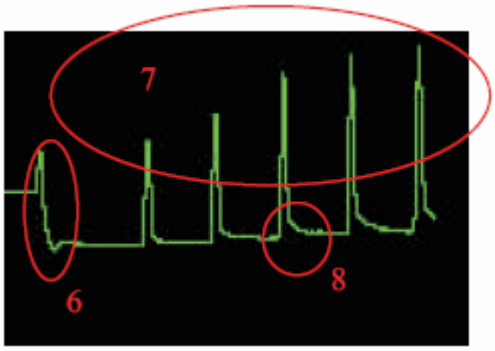
The fluorescence signal rises again due to the turning on of an actinic light source to drive photosynthesis. The fluorescence signal competes for energy with photochemistry and heat dissipation. Since photochemistry and heat dissipation mechanisms have just been initiated, most of the energy goes to fluorescence. The fluorescence signal starts to fall as electron transport and carboxylation begins (Schreiber 2004). Heat dissipation mechanisms are also beginning to affect the signal to drive down the intensity values of both the saturation flashes and the fluorescence signal itself at higher light intensities.



#5

The fluorescence signal continues to drop as full activation of rubisco continues and state transitions occur. Full activation of rubisco in both algae and higher plants takes between three to four minutes plus intensity values are driven down by non-photochemical quenching. Nonphotochemical quenching is involved in photo-protection, state transitions, and photoinhibition. The Δph of the thylakoid lumen and the xanthophyll cycle take between two to four minutes to adjust to a new light level (Lichtenthaler 1999). State transitions take between fifteen and twenty minutes to adjusts to a light level, and therefore, steady state photosynthesis takes between fifteen and twenty minutes (Maxwell and Johnson 2000). Under photo inhibitory conditions, D1 protein degradation found in PSII reaction centers, close PSII reaction centers. Other mechanisms have also been suggested for being involved in photoinhibition.

It is common to take quenching measurements at steady state photosynthesis after the leaf has fully adapted to a specific light level. However Klughammer claims that Y(NO) can be measured at other times. Graphing quenching values at non steady state conditions as well as at steady state can be done to understand the process. (MacIntyre, Sharkey, Geider 1997).



6

At this point the actinic light source is turned off, and a far red light is turned on for several seconds to activate PSI and drain all remaining electrons from PSII. This results in a quenched measurement of Fo called Fo' or Fod, the minimum value measured. Fod is used in the quenching parameters q_P, q_L, q_N, and Kramer's Y(NPQ), and Y(NO). It has also been used in q_E , q_T , and q_I when q_N is used in place of NPQ. After five to ten seconds, the far red light is turned off. The Far red light at 735 nm is too long to drive PSII, but it will drive PSI.

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This section of the graph is used for non-photochemical quenching relaxation measurements required in the puddle model for separation of q_E, q_T, and q_I. During this phase of the graph, the actinic light is automatically turned off and the sample is in the dark. Only the modulated light and saturation pulses are used here. The increase in the peak height is a result of the relaxation of non- photochemical mechanism including; photo-protective mechanisms, state transitions, and eventually photoinhibition. Lichtenthaler found that the relaxation of photo protective mechanisms that involve Δph of the thylakoid lumen and the xanthophyll cycle takes between two and four minutes. A saturation pulse at the end of this period can be used to measure q_E (puddle model) photoprotective mechanisms (Lichtenthaler 1999). The relaxation characteristics of field plants can vary with changing environmental conditions (Baker 2008).

The relaxation of state transitions takes between fifteen and twenty minutes, so a saturation pulse after twenty minutes in the dark can provide a measurement of q_T. q_I or photoinhibition can also be determined with this peak, because Fm is known from the dark adapted first pulse and the difference is considered to be photo inhibition. Chronic photoinhibition starts to relax at about forty minutes in the dark and Lichtenthaler (1999, 2004) found that it could take up to 60 hours for complete relaxation of photoinhibition.

It is common for researchers to dark adapt for 24 hours or overnight (Maxwell and Johnson 2000) when making quenching measurements.

It should be noted that NPQ should only be used to compare plants of the same species and with similar Fv/Fm values (Baker 2008), a (Maxwell and Johnson 2000).

Quenching relaxation measurements are still valuable because there is a significant volume of literature using puddle model parameters and that literature can be helpful for future research. One can still delineate state transitions and photoinhibition separately as well. In addition, with the reconciliation of NPQ to lake model parameters, there is interest in separating state transitions and photo-inhibition related measurements. There is some overlap between photoprotective mechanisms and state transitions. Lichtenthaler (1999).

It is valuable to use a PAR Clip and a shroud to measure all quenching parameters. This ensures that all measurements are made at steady state photosynthesis at a specific light level. The relaxation characteristics of field plants can vary with changing environmental conditions (Baker 2008).

8

After a saturation flash, the tailing off of the signal is the result of NPQ caused by the saturation flash. According to Rosenqvist and van Kooten (2006) it takes between one and two minutes for complete dissipation of saturation pulse NPQ. With this in mind, saturation pulses should be spaced to avoid build up of NPQ. It is also mentioned that photo-damage can occur to samples when saturation flash intensities are too high and there is no actinic light. There is evidence that damage does not occur on samples in the light. (Rosenqvist 2006)

More helpful hints for setting test variable in quenching protocols.

Saturation intensity

The saturation pulse should be intense enough to completely close all PSII reaction centers. To test this, one can try different saturation intensities and examine the saturation peak. This can be done in the Y(II) protocol. After a measurement is made a partial graph of the saturation pulse is viewed.

By touching the graph with a finger, the whole trace becomes visible. One must adjust the intensity high enough so that the most of the top of the saturation pulse is flat. If it is rounded at the middle of the top, more intensity is needed. According to studies done on the subject, even the most intense saturation pulses do not damage plant tissue in a light adapted environment. It has been found that damage can occur with night measurements if the saturation pulses are made too frequently (Rosenqvist and van Kooten 2006) (Albert Porcar-Castell 2008). With this in mind, one can use maximum intensity for saturation pulses in a light adapted environment.

Saturation pulse frequency

By using the Stepped Actinic Test in the Kinetic Protocol, one can check to see if there is enough time between pulses to allow complete relaxation of saturation pulse NPQ. If not, then saturation pulse related NPQ would cause an error. Rosenqvist and van Kooten (2006) state that a time of between one and two minutes is required for complete relaxation of saturation pulse NPQ. When in doubt use two minutes.

If one wants to test for the minimum time required to eliminate saturation pulse NPQ, then using the kinetic test with different times between saturation pulses will provide the answer.

The test should allow about twenty minutes for the plant to reach steady state photosynthesis (Maxwell and Johnson 2000). If the Fm' values continue to decline and the F' values continue to change, then the pulses are too close together. If you are looking for a safe time to use, two minutes is suggested as the longest safe time by Rosenqvist and van Kooten (2006).

Saturation pulse duration

On the OS1p, the saturation pulse duration is set at the default value of 0.8 seconds. This is generally ideal for higher land plants, however, times of 0.5 to 1.5 seconds have been used Rosenqvist and van Kooten (2006). The ideal saturation duration is determined as the value that completely saturates PSII without causing saturation pulse curvature at the trailing edge of the saturation pulse. This is a form of saturation pulse NPQ, and it lowers the average value of Fm, or Fm' used in Fv/Fm quenching, and Y(II) measurements. The saturation pulse duration can be set on the OS1p from 0.10 seconds to 2.00 seconds When in doubt, use 0.8 seconds. With modulated fluorometry, the value measured at Fm, or Fm' represents multiple

turn-overs of Q_A According to Schreiber (1995). The measuring file only displays ten points for a one second saturation pulse duration. The machine actually measures 320 points in one second, and gives the highest eight point rolling average as an Fm' measurement value. This automation feature, ensures that the optimal duration is always provided as long as the duration is set long enough.

Algae and cyanobacteria saturation pulse duration - new feature:

The ideal saturation pulse duration for algae and cyanobacteria range from 0.025 seconds and 0.050 seconds (Schreiber 1995). When using the OS1p for algae work, the OS1p uses a special algorithm that finds the highest eight point rolling average at the top of the saturation pulse. The eight point average represents a time of 25ms. Therefore, finding the optimal saturation duration is always achieved for algae without testing. With a saturation pulse duration of 0.1 seconds, an algorithm that averages only the highest eight points over a 0.025 second time period, saturation pulse NPQ will not reduce the optimal measurement, even if the duration is set for longer periods of time.

Length of quenching test.

The leaf must be at steady state photosynthesis before most quenching measurements are made. This means that the leaf is exposed to a specific light level for between fifteen and twenty minutes. Due to the fact the fluorometer light intensity changes due to increased heat while the actinic light source is on, we recommend that quenching tests be taken after twenty five to thirty minutes of actinic light exposure. The light intensity change is most significant during the first five minutes as the fluorometer warms up due to the actinic light source.

By extending the test measuring time, the remaining intensity change becomes insignificant and steady state is reached. The actinic light level can be measured if a PAR Clip is used with a shroud or it is used in a darkened room.

Dark Adaptation – How Long is Long Enough?

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 Fv/Fm values, decisions need to be made for control and test measurements. The plant mechanisms listed below will lower Fm, and possibly raise Fo, changing Fv/Fm 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.

Fv/Fm 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 light levels it represents about 6% of total quenching. State transition quenching relaxes in ten to twenty minutes in terrestrial plants. (Lichtenthaler H. Burkart S 1999)

It has been shown that the effects of acute photo-inhibition caused by exposure to high light intensities can be reversed with 20 to 30 minutes of dark adaption (Theile, Krause & Winter 1998). The reversal of chronic photo-inhibition caused by several hours of 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 longer quenching and quenching relaxation parameter 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 Fm and Fo under chronic photo-inhibition conditions (components of non-photochemical quenching parameters) it is common to dark adapt for a full night, or 24 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 60 hours. Never compare NPQ values with samples that have different Fv/Fm readings. Fv/Fm is the yardstick for the individual plant NPQ values (Baker 2008). Comparing NPQ values of plants with different Fv/Fm values is like measuring samples with a ruler and dimensions that change

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 is designed to rapidly re-oxidize PSII by activating PSI. This can be valuable in field work, (Maxwell and Johnson 2000), but it does not affect the relaxation of non-photo-chemical quenching mechanisms. Consalvey (2004).

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 or darkened rooms may 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 Fv/Fm 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.

Relative Electron Transport Rate

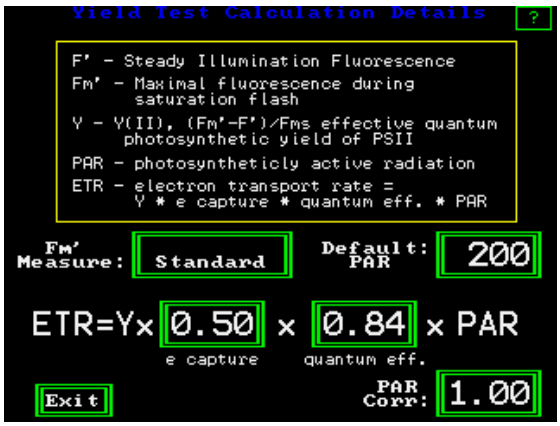
Relative Electron Transport Rate - ETR µmols = (Y) (PAR) (.84) (.5)

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 extremely 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 = (Yield or F/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 micromoles 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 OS1p, the actual values for leaf light absorbance and the portion used by PSII can be input into the actual formula to provide more accurate results.



The values for leaf absorption and PSII absorption can be changed by touching the green box surrounding the value. For recommended measured values found for different types of plants, refer to the papers listed below.

The PAR Correction factor allows for PAR measurement correction of different types of light sources other than sun light, and the internal actinic light source. Under most conditions, it should be set at 1.00.

It also allows for distance and location error correction capability. When using an artificial light source, the location of the PAR sensor may cause an error relative to the location of the leaf. This correction factor allows following the correction procedure in a paper by Rascher (2000) listed below.

The default values of ETR= Y (0.50) (0.84) (PAR) are average plant values used for relative comparisons.

ETR absorption setting screen

This screen can be reached by touching the box with ETR in it on the Y(II) measuring screen.

The absolute amounts for leaf and PSII light absorption can vary at steady state with species, and water content. 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). (quantum yield of PSII) (measured photosynthetically active radiation measured in μmols.) (leaf absorption coefficient) (fraction of absorbed light by PSII antennae).

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 leaf absorption and PSII absorption. 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).

Yield 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 Yield measurements, the combination can be formidable. Only samples at similar light levels should be compared for plant stress using Y(II).

A PAR Clip is a leaf cuvette that allows the holding of the sample leaf at a repeatable angle and distance from the measuring probe while measuring Yield Y(II), ETR, PAR, and leaf temperature. These values are contained in same data file with a time and date stamp. PAR (Photosynthetically Active Radiation) is irradiated light between the wavelengths of 400 nm and 700nm and it is measured very near the sample measuring area. When PAR is measured using a PAR Clip and the dimensions per square meter per second are used, the value becomes PPFD (Photosynthetic Photon Flux Density), (When using a PAR Clip the terms PAR and PPFD are interchangeable).

Some PAR Clips offer the option of using a predetermined light intensity level from an internal fluorometer light source to measure Yield and ETR. In this case, irradiation levels can be forced to be consistent for each sample. Note: When using internal illumination it is important to allow time for a plant to reach steady state photosynthesis before measurement or errors will result. Even small changes in irradiance level can change the measurement of both yield and ETR. Maxwell and Johnson (2000) found that it takes between 15 minutes and 20 minutes for plants to reach steady state. (For more information on this topic see Opti-Sciences App note # 0509, "Yield value & Limitations".)

Some PAR Clips, such as the digital PAR clip for Opti-Sciences OS1p, allow PAR measurement while an internal light source is being used, thus providing a reliable PAR or PPFD values over time. Of course in this case, the PAR sensor should be calibrated to the internal actinic illumination as well as to sun light. It should also be calibrated for the location difference between the PAR sensor and the leaf sample (Rascher 2000).

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Chapter 2 • The OS1p

Introduction

The OS1p Modulated Fluorometer is a multipurpose portable measuring instrument designed to precisely measure chlorophyll fluorescence. The system is simple to use, light in weight, and battery powered, making it an excellent choice for field studies. It represents culmination of several years of testing and development and was engineered to give many years of reliable service. This chapter provides general information about the OS1p Modulated Fluorometer.

Key Features

The OS1p includes the following features:

A modulated detection system which allows measurements to be made in variable test conditions, even in bright daylight.

A wide range of modulation intensity and gain settings, allowing for measurement in plant species with low chlorophyll content.

Option for automated modulated light setup

On-board fully adjustable actinic and far red illumination sources.

USB, MMC/SD data card outputs

Light weight design excellent for field studies.

Fully portable Nickel Metal Hydride battery operation. It can also be operated from a AC outlet. A Charge will last up to ten hours.

Full measurement control from on-board computer system.

Graphic touch screen.

1 Gbyte of non volatile flash memory

Versatile software, optimized for variations in testing procedures.

One modulated measuring light source is included. Red is standard but blue may be ordered in its place.

One set of quenching protocols comes as standard on the advanced version of the OS1p. Various quenching protocols can be added for an additional price including quenching relaxation tests, with both puddle and lake model readouts.

Rapid light Curves are included as standard on the advanced version of the OS1p and will be available in early 2011.

Options Include:

PAR clip with PAR sensor and thermistor for leaf temperature.

Extended life battery belt

Tripods

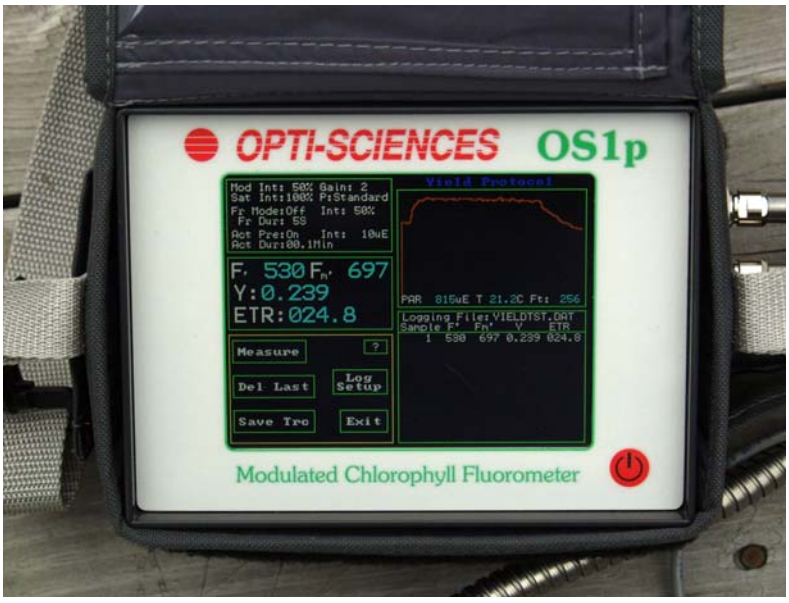
Transport – Storage Case

Additional quenching protocol

Physical Features Panel Photographs

Top Panel:

Figure 2-1 • Front Panel of OS1p



Front Panel of OS1p Touch screen showing Y(II) measuring screen.

The Red round button is for OFF and ON

Left Side Panel:

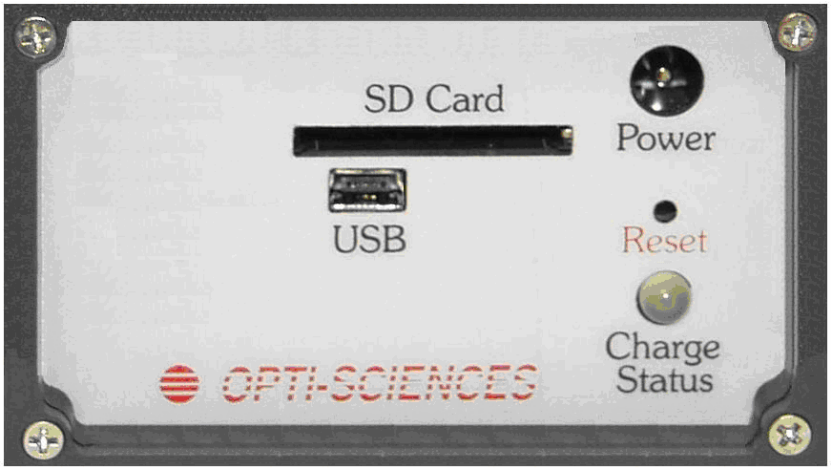


Figure 2-2 • OS1p left side panel

SD Card Slot – used for MMC/SD format data cards up to one GByte. The port can bee used for data transfer to other computers or other OS5p fluorometers and it can be used for software updates.

USB port –used for data output

Power – used for the battery charger. The charger also doubles as a mains connector. The Charge status light is green when the unit is fully charged. It is red when it is charging and not fully charged. The OS1p only charges when the unit is turned off.

Reset hole – By inserting a pin or paper clip, the system can be rebooted if the system should lock up.

Charge Status Light – The light turns green when the Nickel Metal Hydride battery is fully charged. It will be red when it is not, and yellow when it is partially charged. It only turns on when the OS1p is turned off and the unit is plugged in. If the light is flashing, check the charger connection. Make sure that it is properly inserted and the wall outlet is working. If there is still a problem contact OSI

Right Side Panel:

Figure 2-3 • OS1p right side panel

Accessory port – connection for PAR clip.

Fiber-optic light guide connectors – male connectors for the tree randomized fiber optic bundle connectors. Any of the fiber optic female connectors may be attached to any of the right side panel connectors.

Remote Connector – This is used for the trigger switch wire connector attached to the fiber optic bundle, and it is used to trigger measurements from a button on the other end of the fiber optic bundle.

Picture of optional PAR Clip on articulating arm stand

This picture shows the correct placement of the fiber optic bundle on the PAR Clip. The fiber bundle should be moved in as close as possible to the leaf. PAR Clips are highly recommended for field light adapted use. Y(II) varies not only with plant stress but also with light level. Only samples at the same light level and with the same light history should be compared. Sun leaves respond differently to light levels than shade leaves.

Hardware Overview and Measurement Principles

The OS1p is a portable pulse modulated chlorophyll fluorescence fluorometer It is completely self-contained, eliminating the need for any external hardware or computing device (palmtop computer, etc.). All detection and data logging components are on-board.

Fluorescence is measured by a modulated light source in the OS1p. The standard and more commonly used red modulated source is a diode that peaks at 660 nm with filters blocking radiation longer than 690 nm. The average intensity of this modulated light is adjustable from 0 to 1 $\mu mol/m^2/s$. It is adjusted to samples so that it does not drive photosynthesis in dark adapted tests. The source output is optically monitored inside its assembly to correct for variations in output due to changes in ambient temperature. The optional, less commonly used, blue modulated diode peaks at 450nm and can be used for algae work or for work with higher plants as well. Detection is done in the 700 to 750 nm range using a sensitive PIN silicon photodiode with appropriate filtering to narrow the optical response. A low-noise preamplifier is mounted in the detector assembly as well. The detection method is a modified lock-in amplifier design which allows the use of a very low average modulated light energy. It is the modulated light source that allows actual measurement of Fo, and Fo' as well at the measurement of light adapted quantum photosynthetic yield. The gain control is adjustable for optimum signal to noise ratio. The capture rate is automatically set for the test that is selected. An added benefit of this system is its ability to disregard extraneous ambient light.

Light sources

There are multiple standard light sources present in the OS1p:

Modulated light source This is the light source that makes light adapted quantum photosynthetic yield measurements possible along with direct measurements of Fo and Fo' or Fod. The modulated light source is used at an intensity range that is too low to drive photosynthesis and yet allows fluorescence measurement of pre-photosynthesis Fo, and post photosynthesis Fo' or Fod. This light source is turned off and on at a particular frequency. Opti-Sciences adjusts the frequency automatically.

1. The standard modulation source is a 660nm output used exclusively for measuring of the fluorescence signal. It's intensity may be varied to adjust for dark adapted samples and for use with the PAR clip on light adapted samples.

Optional:

2. The second modulation source is a 450nm LED and is also used exclusively for measuring of the fluorescence signal. The red source can be replaced by a blue source at time of order. It's intensity may be varied to adjust for dark clips, Par Clips, and sample differences as well. Because of absorption differences, using the blue modulated light source will change ETR values. In the equation used for blue modulated light, leaf absorption should be changed to 0.94, on average, from 0.84. for best correlation to red modulated light values. This can be done in Excel using the measuring file. ETR= Yield X PAR X 0.94 X 0.5 (for blue modulated light).

Saturation pulse light source 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 1.5 seconds (Rosenqvist and van Kooten 2006). For Algae and cynobacteria, the duration must be shorter to provide accurate measurements 0.025 to 0.050 seconds. (Schreiber 1995). Opti-Sciences uses 0.8 seconds as the default value for land plants however the duration can be set between 0.1 seconds and 2.0 seconds by pressing the Y(II) graph on the Y(II) measuring screen and pressing the duration button. The Saturation pulse light source is a high intensity white light LED. The Saturation light source is 11,000 µmols at 100% intensity when used with a dark clip, and about 6600 µmols when used with the PAR Clip at 100% intensity. The machine actually measures 320 points in one second, and gives the highest eight point rolling average as an Fm' measurement value.

Algae and cyanobacteria saturation pulse duration - new feature:

The ideal saturation pulse duration for algae and cyanobacteria range from 0.025 seconds and 0.050 seconds (Schreiber 1995). When using the OS1p for algae work, the OS1p uses a special algorithm that finds the highest eight point rolling average at the top of the saturation pulse. The eight point average represents a time of 25ms. Therefore, finding the optimal saturation duration is always achieved for algae without testing. With a saturation pulse

duration of 0.1 seconds, an algorithm that averages only the highest eight points over a 0.025 second time period, saturation pulse NPQ will not reduce the optimal measurement, even if the duration is set for longer periods of time.

The stepped intensity linear regression mode allows one to determine values with an infinitely bright light for high light sample conditions. See multi-flash for more details.

The LED actinic light source

An actinic source is a light source that drives photosynthesis. It may be the sun or an artificial light source. Using an artificial light source has the advantage that it can eliminate the variability of sun light shading, and partly cloud day cover. The OS1p has a high intensity white light LED that is used as an actinic light source. This same light source is used for saturation flashes.

The far-red light source

The peak wavelength for the Far Red source is ~735nm. Far-red light is a light source that drives PSI without driving PSII. This has the effect of draining the remaining electrons from PSII quickly to provide a completely oxidized state in PSII. It is used for determination of Fod or Fo'. Light at these wavelengths allows electron transfer from PSII to PSI, and it allows the fast re-oxidation of PSII. The far red source may be used in the Fv/Fm protocol to rapidly re-oxidize PSII before measurement.

During the yield test when artificial internal illumination is used, far red light is filtered out of both internal illuminators. Therefore, to closely replicate solar radiation that has both PAR radiation and far-red radiation to drive both PSII and PSI, the far red light should be on during internal illuminator actinic illumination. Both intensity and duration are adjustable.

The Fiber Optic Light Guide

The optical signals are transferred to and from the sample by a custom-designed trifurcated fiber optic light guide, with randomly mixed fibers. One end of this fiber bundle has three BNC (twist-lock) connectors for the three BNC male connectors on the OS1p right side panel. Any fiber optic BNC connector can be attached to any OS1p BNC connector. In addition there is a trigger switch on the far end of the bundle from the BNC Connectors. This switch can be used to take a measurement, or one can press the enter button on the OS1p front panel, The trigger switch is connected electronically by a wire running down the fiber optic cable, and attaches to the "remote" port on the right side panel. The other end provides a comfortable fiber bundle grip and an interface section designed to fit into the many cuvette options.

Electronics

The system has 32 bit microprocessor with one Gigabyte of flash memory.
No data will be lost due to loss of power.

Housing and Carrying Case

The OS1p is housed in a durable wear resistant ABS plastic case. This is the same material that is used in the manufacture of motorcycle helmets. It also comes with a nylon field bag with a pocket to carry accessories, and a shoulder strap for ergonomic field use

Chapter 3 • Operating the OS1p

Introduction

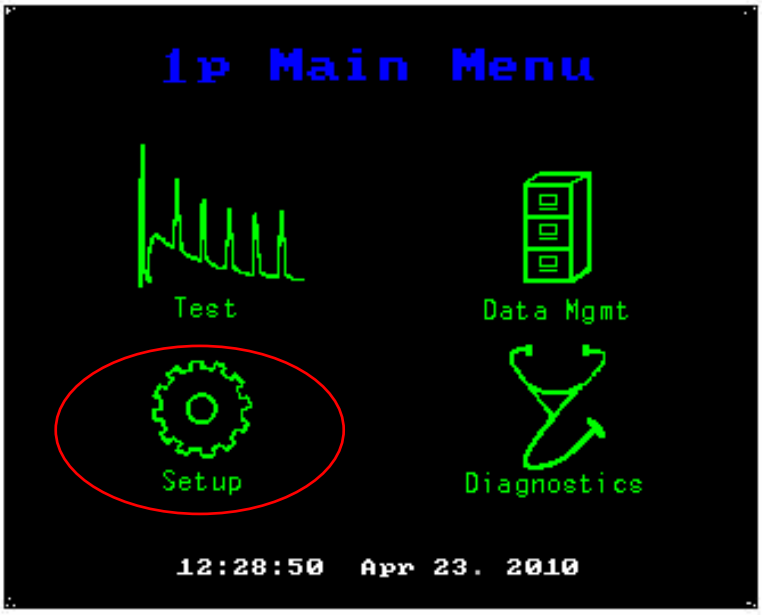
The OS1p is shipped with the battery connected. It is a good idea to give the battery a full overnight charge before the first use.

Connect the three fiber optic light guide BNC ends to any of the BNC ports on the right side panel of OS1p. Make sure that all three of the BNC connectors are locked in place. The remote trigger switch (the 1/16" red wire on the fiber) connects to the jack labeled "remote" on the right side panel. (see figure 2-3 Right Panel).

Introduction to running tests

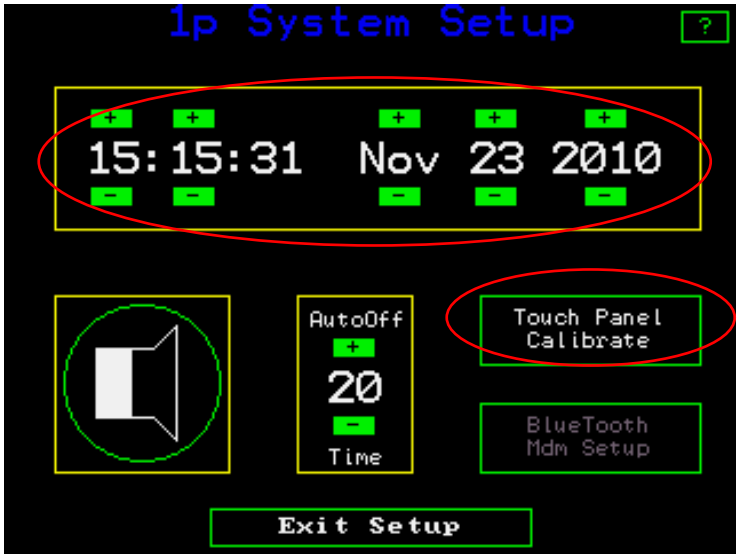
As with any electronic measuring tool, it is important to turn on the tool before use and let the electronic temperatures reach operating range. This takes about 15 to 20 minutes for best results.

Press the red main power button on the front panel. At first a screen appears that shows a graph and the OS1p name. Then the main menu screen shown below appears.



Main Menu

Press the touch screen over the "Setup" Menu button and a second screen seen below will appear.



Setup Menu

Set up menu

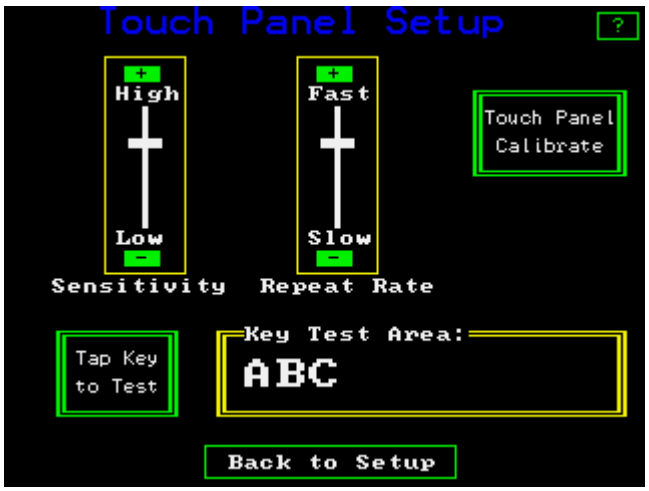
The default time is set for US eastern standard time (24 hour format). On this screen you can adjust the time and date. Press the + or – buttons to change the values. The speaker button allows an audible beep when measurements are made and provides other audible indications as well. This may be turned off or on by pressing the button with the speaker.

The "autoOff " button allows the instrument to turn off automatically when not in use. The time is adjustable between zero minutes and twenty minutes by using the + and – buttons. At the zero setting the instrument will not turn off by itself, you must turn it off.

The "Blue Tooth Mdm set up" is for the blue tooth option. It is not part of the standard system and must be ordered as an option. It allows data transfer to hand held computers after every measurement. The set up menu connects the instrument with the blue tooth device through a hand shake routine.

Adjust screen touch sensitivity

To adjust the sensitivity of the touch screen press “Touch Panel Calibrate” and an adjustment screen will appear as pictured on the next page.

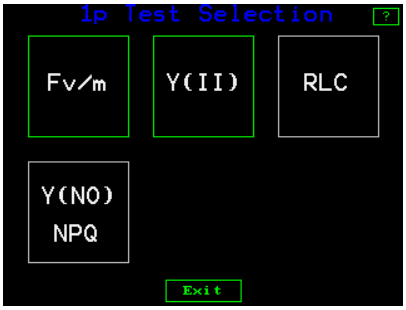


Touch screen adjustment screen

If it is necessary, to adjust the sensitivity of the touch screen, press the – or + buttons for increased or decreased sensitivity, and the repeat rate keys for speed of response to touch. After adjustment, tap the “Tap Key to Test” button. Letters will appear in the “Key Test Area”. Adjust the sensitivity and the repeat rate for personal preferences. When done, press back to Setup.

Touch Panel Calibrate

Do not make this adjustment unless necessary, it comes pre-calibrated. This feature allows the calibration of touch sensitivity to X-Y location. The instrument will run through a menu driven routine that requires touching the center of + signs in different locations using a *ball point pen with the ball retracted* on the screen. If the ball is not retracted, ink marks will appear on the screen and they can be hard to remove. When done, press “Exit”.



Test selection menu found by pushing “Test”

Test menu

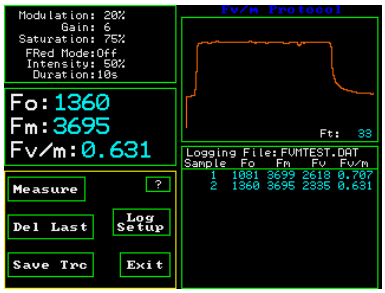
The OS1p has four different test protocols. Each mode providing the necessary setup and data logging for each type of test.

Press “Test Menu” using the touch screen and the measuring protocols will appear. Press the button for the measuring protocol that is desired.

Fv/Fm Protocol — Dark adapted Fv/Fm test for maximum quantum yield.

1. Y(II) – Quantum Photochemical Yield Protocol — Light adapted test of (PSII) for effective quantum photochemical yield - Y(II) or ΔF/Fm’
2. Y(NO) NPQ – This represents the Luke Hendrickson simplified lake model quenching equations along with NPQ resurrected from the puddle model by Klughammer for the lake model. Parameters include Y(NPQ), Y(NO), Y(II), and NPQ. When used with a PAR Clip, PAR, leaf temperature, and ETR are also reported.
3. RLC – This is the protocol for rapid light curves. It will be available in 2011. Parameters include ETRmax, Ik, and α are provided.

* Other quenching protocols may be delivered in place of the Luke Hendrickson model upon customer request. Other protocols may also be added for an additional price. Optional quenching protocols include: Dave Kramer’s lake model parameters, puddle model parameters, and a quenching relaxation protocol that may be added to the Hendrickson or puddle model protocols.



Fv/Fm measuring Screen

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 in chapter 1)

The assumptions are that before the test, PSII is fully oxidized and quenching mechanisms have relaxed. 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 an hour are common. Some scientists only work with pre-dawn dark-adapted samples. Experiments should be done on plants to be tested to find the appropriate dark adaption times for best results. Fv/Fm values will be at their highest amount, and longer dark adaptation times should not increase the value.

A total of 10 dark adaption white clips are provided with the system, to be used for dark adaption measurement. A clip should be placed on the leaf with the black slider covering the cylindrical opening. After dark adaption, the end of the fiber optic bundle should be placed in the cylindrical opening and the dark slide of the clip should be opened allowing the sample to be exposed to the fiber optic bundle. Dark shrouds can also be used for dark adaption or lights can be turned off in a windowless environment.

Cookbook check list before making Fv/Fm measurements

Fv/Fm is a normalized ratio that does not use a traceable standard. Instead, its accuracy is determined by properly using the instrument and following the lessons learned about plant physiology by several great researchers.

To get an accurate 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. State transitions, however; take between fifteen to twenty minutes. These times can vary somewhat in field plants and can take slightly longer. 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 summer field measurements of Fv/Fm. For this reason it is important to only compare samples with a similar light history. 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)

2. Modulation light intensity setting Fv/Fm is (Fm-Fo)/Fm. Fo, or minimum fluorescence is a dark adapted value made by exposing the leaf antennae to a very low intensity modulated measuring light, that is not set high enough to drive photosynthesis. The modulation light intensity must be set correctly for best accuracy and repeatability. If it is set too high, it will drive photosynthesis and provide an Fo value that is too high. The modulated light allows the measurement of pre-photosynthetic antennae fluorescence.

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

4. **White light vs. red light saturation pulse.** Fv/Fm will be higher with a white saturation pulse than a red saturation pulse. Some fluorometers use a red saturation pulse. This not an issue for comparative measurements of plant stress, but values measured on a fluorometer with a white saturation pulse should not be compared to measurements of a fluorometer with a red saturation pulse.

3. Maximum Fv/Fm values vary with species. The average maximum Fv/Fm value is 0.83 (Schreiber 2004)

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

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

6. 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 2006). 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. The default value is 0.8 seconds, a figure that works well for almost all higher plants.

7. 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.

8. Far-red pre-illumination. Some Fv/Fm 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 Fv/Fm measurement takes place, it activates PSI, and ensures that all electrons have been drained from PSII before the measurement of Fo. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, or photoinhibition. Time is still required in a darkened environment to relax all forms of NPQ and to obtain a reliable Fv/Fm measurement.

9. Overlap of PSI fluorescence -Part of the minimum fluorescence, the Fo parameter, in Fv/Fm or (Fm – Fo)/Fm, contains PSI fluorescence as well as PSII fluorescence. With Fv/Fm, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error. In C_3 plants, about 30% of Fo fluorescence is due to PSI, and in C_4 plants about 50% of Fo fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in Fm in C_3 plants, and about 12% of Fm in C_4 plants (Pfundle 1998). This not a problem when comparing Fv/Fm measurements for plant stress because PSI fluorescence does not change. It remains constant.

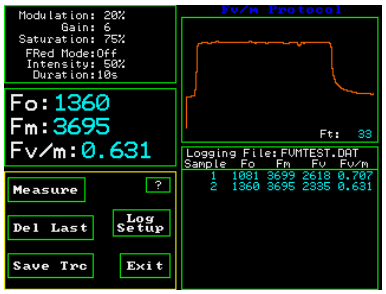
The best experiments are ones that take these issues into account. PSI fluorescence is involved in all measurements. It does not vary with light level or plant stress (Schreiber 2004). With this in mind, comparing samples with similar light histories allows comparison of many types of plant stress. The Plant Stress guide provided by Opti-Sciences references papers that deal with specific types of plant stress and limitations of different chlorophyll fluorescence parameters for measuring plant stress.

Measurement parameter selection for different types of plant stress. Fv/ Fm is not a sensitive test for water 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 may 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. If the

stress that you want to measure is on the list for not being sensitive to Fv/Fm, it is suggested that Quantum photosynthetic yield of PSII be considered. It is sensitive to most types of stress listed

Running the Fv/Fm test

The last file used for this type of measurement will open up and a small screen will appear within the larger screen. It will show the file name and ask if the file is to be used and if one wants to proceed to measure with this existing file, and the parameters that have been set in the existing file. The other option is to create a new file by pressing Change.



Fv/Fm measuring Screen

Press “proceed” to use the existing file and “change” to use a different file.

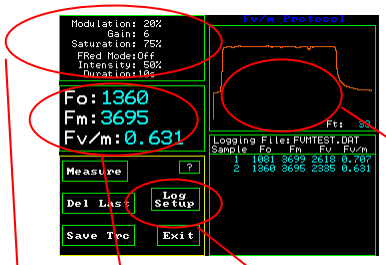
A second box appears and asks if you want to add measurements or “append” the loaded file, “cancel” and go back to the main menu, or “overt” overwrite the last file entirely.

To make a measurement with the default settings programmed into the OS1p at the factory, place the end of the fiber optic bundle into a dark clip that is being used to dark adapt a leaf.

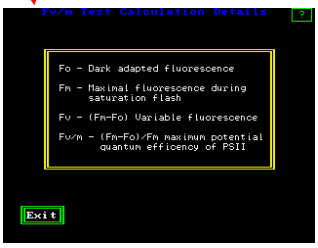
Slide open the black slider, and press the red button near the end of the fiber optic bundle. This will trigger a measurement. Measurements may also be triggered by pressing the measure button on the measuring touch screen. (The PAR clip is not used for Fv/Fm measurements). If a warning appears that says the fluorescent signal is too low, the modulated light intensity must be adjusted. (see the next section).

Press windows to go to other screens

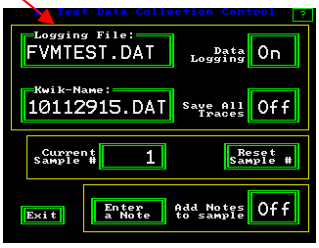
Drill down view of Fv/Fm measuring screens



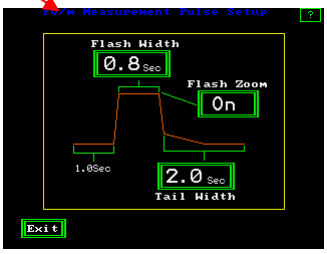
Fv/Fm Measuring Screen



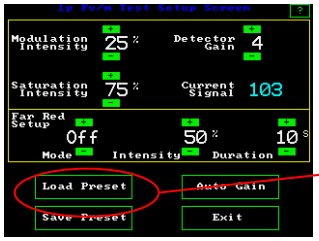
Fv/Fm Test Calculations screen



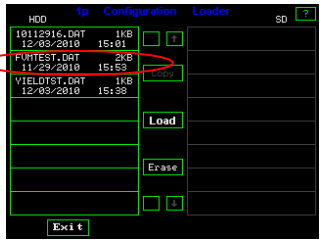
Fv/Fm Test Data Collection screen

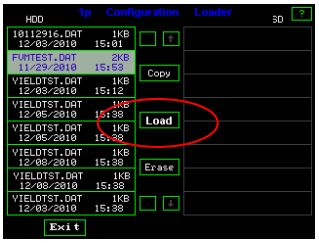


Fv/Fm Measurement Pulse Screen



Fv/Fm Test Setup Screen





1p Configuration Loader Screens

Loading measuring parameter preset files –

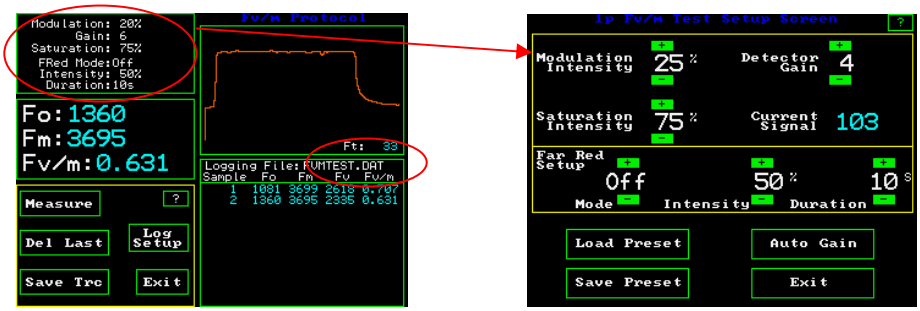
Highlight test by touch and press load.

Arrows appear when there are more tests than viewed on the screen.

Copy only works when an SD card is inserted.

Changing Fv/Fm measuring parameters -

One can change the test parameters by pressing the box in the upper left hand corner of the measuring screen.



Fv/Fm measuring Screen

1p Fv/Fm Test Setup Screen

Setting the modulation light source intensity- It is adjustable between 0.1µmol and 1 µmol when using a dark clip. adjustments are made in 5% increments between 5% and 100%. The correct setting depends upon the distance from the sample , and nature of the sample.

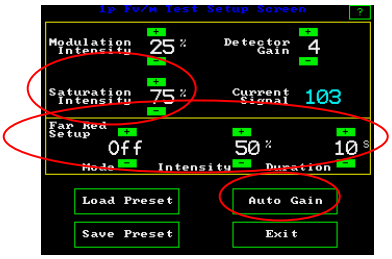
The modulation source should be optimally set to allow pre-photosynthetic antennae fluorescence measured as the Fo value. It must be set at a value that too low to drive photosynthesis. If it too high, it will cause an error in measured Fv/Fm values

If the intensity is too high, it can be seen as a slow rise in “fluorescence signal” visible on the set up screen or the “Ft” value visible on the Fv/Fm measuring screen. After setting the modulation intensity, watch the Ft value. If the average value increases over a 30 second period, the intensity should be reduced.

For correct testing of the modulation intensity setting, put a leaf, of the type to be measured in a study, in a dark clip, and dark adapt. Place the fiber optic probe all the way into the dark clip, and expose the dark adapted area of the leaf to the modulated light. The modulated light is always coming out of the end of the fiber bundle when a measuring screen is viewed. The modulated light is set correctly if it is high enough to get a fluorescence measurement when the measuring button is pressed, but not too high so that it is driving photosynthesis. A setting that is too high will give an incorrect reading of Fo, a value that should be a pre-photosynthetic measurement. The setting is linear. The “Gain” should be as low as possible for the best signal to noise ratio.

New -automated modulation light set up

“Auto Gain” – On the set up screen, this option automatically adjusts the modulated light setting to a detection range that will work for the sample. It is known from experience that this level does not drive photosynthesis on tested samples. Dark adapt a sample to be tested in either a dark clip or with a shrouded PAR clip. Press “Auto Gain” with the leaf sample in place, and the instrument automatically goes through a routine that sets the modulation intensity correctly.

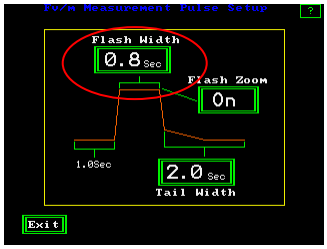


1p Fv/Fm Test Setup Screen

To use the automated modulation light setup mode, press "Auto Gain" . The system then adjusts the modulation light source into an acceptable minimum reading range for the PIN diode detector.

Far red light allows the reduction of PSI and the rapid re-oxidation PSII. Options are offered for OFF, ON, and PRE. Off turns the far red light off during the test. The pre setting allows illumination of the sample with far red light before the test, and the on position keeps the far red light on during the test. Intensity is adjustable between 5% and 100% with 5% increments. The duration time for pre-illuminating samples with far-red light can be set from one second to sixty seconds using the + and – keys above and below the values. It has been found that five seconds works well, and that ten seconds provides optimal results. (Maxwell and Johnson 2000). Using far red light pre-illumination does not relax NPQ (Consalvey (2004).(See the Dark adaptation section for more details) It is safe to use the pre-far-red light at 100%.)

Setting the saturation pulse intensity - Values for saturation pulse intensity range from 5% to 100% in 5% increments. At 100%, the intensity of the saturation pulse is approximately 11,000 umols when used with a dark clip. Saturation intensity is approximately 6,200 umols when used with a PAR Clip.

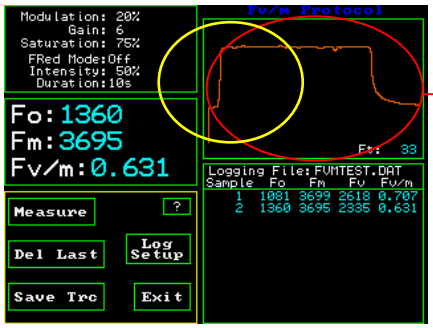


Fv/Fm Measurement Pulse Screen

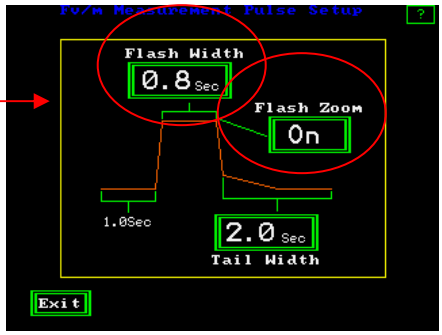
Setting saturation pulse duration and graphic screen display of the measuring trace. Duration is the time that the saturation flash is turned on. For higher plants, times of 0.5 to 1.5 seconds have been used. The optimal saturation duration for algae and cyanobacteria is between 25 and 50 msec. (Schreiber 1995). If the duration is not long enough, then PSII is not fully saturated, even at the highest intensity, and Fm values are reduced. If the Duration is too long, on most chlorophyll fluorometers, the saturation pulse peak becomes subject to a type of NPQ that rounds the tailing edge of the saturation peak and also reduces the average Fm value. The Ideal saturation pulse peak is flat on the top with the trailing edge rounding minimized (Roseqvist and van Kooten 2006).The OS1p provides an automation feature that uses a rolling continuous eight point average to find the highest average value on the top of the saturation pulse. These eight

points represent a time of 25 msec. As a result, the optimal saturation duration is automatically provided for higher plants, algae and cyanobacteria if the duration is long enough. 0.8 seconds is adequate for most plants. The saturation pulse duration is adjustable between 0.1 and 2.0 seconds.

To adjust the duration, touch the upper right graphic display window of the Fv/Fm measuring window.



Fv/Fm Measuring Screen



Fv/Fm Measurement Pulse Screen

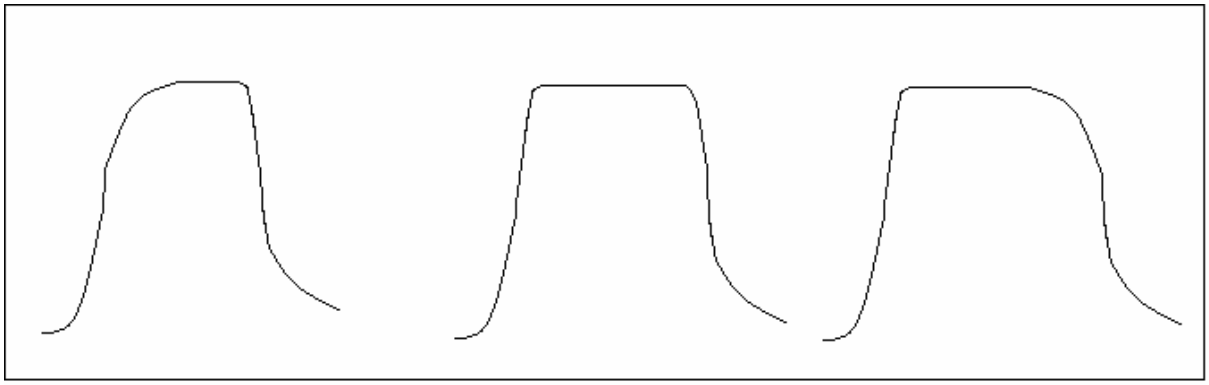
By pressing the "Flash Width" button one goes to a keyboard screen and allows the saturation pulse duration to be adjusted between 0.1 and 2.0 seconds. Press "Ent"

Flash Zoom – This button allows a choice between displaying the full Fv/Fm trace on a partial basis or displaying the full saturation pulse trace and the subsequent trailing fluorescence signal after the saturation pulse. The default setting is to display the partial trace for reasons of speed. Viewing the trailing edge of the saturation pulse adds time to the measurement. The full trace is displayed on the top left side screen. This is what is seen when the "Flash Zoom" is turned "On'. The portion circled in yellow represents the amount of the trace that is displayed when the Flash Zoom is turned "Off".

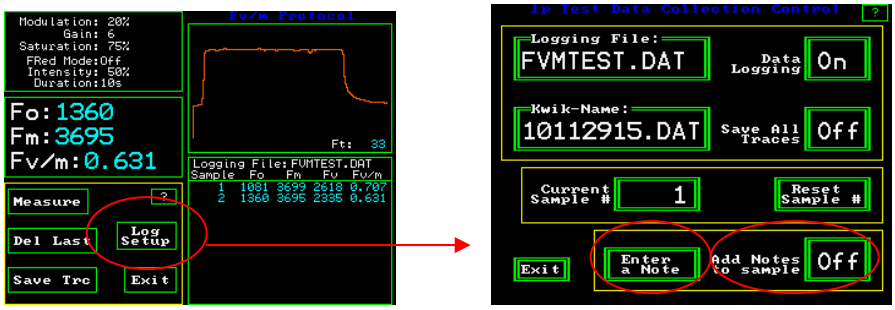
The "Tail Width" button allows one to select the amount of trailing fluorescence trace to be displayed. By pressing the "Tail Width" button, a key board will be displayed that allows adjustment from zero to 9.9 seconds after the saturation pulse is turned off. Press "Ent" enter after typing the value.

One can also test for the proper saturation flash width by using the flash zoom and trying various saturation flash widths. The diagram below explains how to evaluate the flash.

With the OS1p either the center or the right peak will provide optimal saturation pulse settings.



Entering a note with a measurement



Fv/Fm measuring Screen 1p Test Data Collection Control Screen

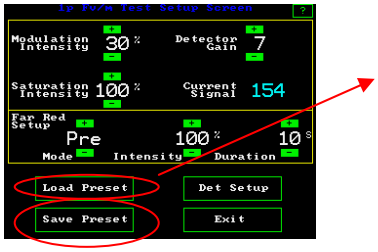
A new feature included with the OS1p makes it possible to enter a note with any measurement, or to require a note with every measurement. To enter a note, press the "Log Setup: button. Next press "Enter a Note". A touch keyboard will appear and one can enter up to a 38 characters note with each measurement.

To require the note screen to appear with each measurement, press the "Add Notes to sample" button and turn the feature ON. The keyboard screen will appear after the measurement is complete. If the occasional note is needed, turn the feature off by touching the "Add Notes to sample" button.

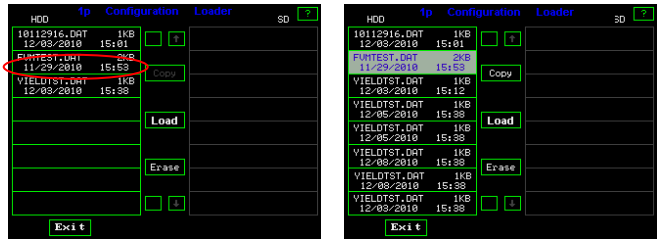
Loading and saving preset measuring routines

When the parameters have been adjusted for a specific set of conditions, the test parameter setup can be saved by pressing “Save preset” while on the Test Setup Screen. A key board screen will appear, and the test set up can be named, and it is saved, when “Ent” is pressed. In Fact, changes that are made to the “Test Collection Control” screen, the “Fv/Fm Measurement Pulse Setup” screen, and the “Fv/Fm Test Setup” screen can all be saved in a single measuring file by pressing “Save Preset” on the Fv/Fm Test Set up screen. The changes from other screens may be done in any order, and saved from this screen when complete. If parameters are changed, and a new file name is not given to this new set of parameters, then the changes will be made to the existing preset file. Follow the directions listed above to save measuring parameters in files.

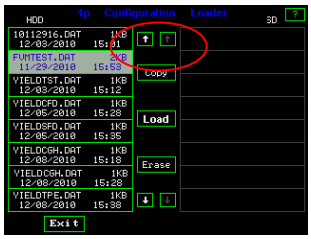
To load previously created tests, press “Load preset”. Press the test of interest. After it is highlighted in light blue, press load. To erase a test, highlight the test and press “Erase”. The number of tests that can be saved is almost unlimited. Use the up and down arrows to scroll through the tests if there are more that are not shown on the screen. Press “Exit” if there is no action.



Fv/Fm Test Setup Screen



1p Configuration Loader Screens



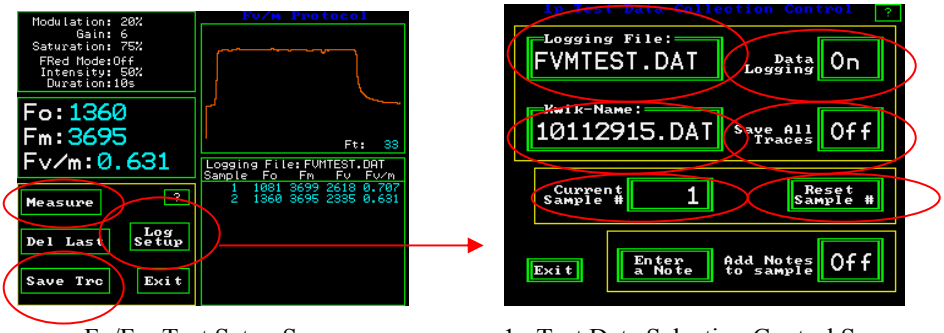
Loading measuring parameter preset files –

Highlight test by touch and press load.

Arrows appear when there are more tests than can be viewed at one time on the screen.

Copy only works when an SD card is inserted into the side of the OS1p.

Other Fv/Fm function buttons



Fv/Fm Test Setup Screen 1p Test Data Selection Control Screen

Data logging retains all measured values when turned on.

Save All Traces for all up coming measurements. Turn "On" the "Save All Traces" button and all future traces will be saved to the measuring file. Turn "Off" the "Save All Traces" button and a single trace is only saved by pressing the "Save Trc" button from the test screen after the measurement is made.

Kwik-Name – The "logging file name" is the file name. Kwik-Name allows one to quickly create a new file name without typing in the characters. The Code represents the year -10, the month -11, the date 29, and the hour – 15 (24 hour clock). This is especially valuable for people making measurements in high volume that want to change the file name for a second field or a sub set. The measuring parameters stay the same as the existing file and the code allows easy and fast differentiation of different measured values where appropriate. Press the "Kwik-Name" button and the Kwik name becomes the "logging file name" moving forward.

Current Sample # – Press the "Current Sample #" button to change the measurement number before you measure. The number that you enter will appear on the next measurement. A key pad will appear. Enter the new number, and press "Ent".

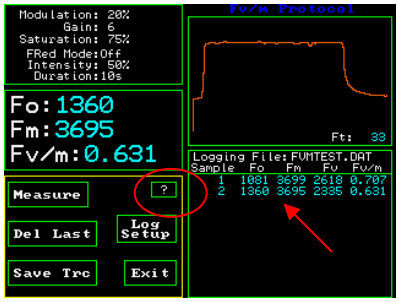
Reset Sample # - resets the sample number to 1 for the next measurement.

Save Trc – Saves the trace displayed on the measurement screen, or the last trace.

Del Last – Deletes the last measurement.

Measure – measurements can be made by pressing the measuring button on the Fv/Fm measuring screen, the red button at the tip of the fiber optic cable, or the black button on the bottom of the PAR Clip.

Measurement review



Fv/Fm Test Setup Screen

On the main Fv/Fm measuring screen, the lower right hand side window logs all of the measurements made in the current measuring session for the current file name. One can scroll through measurements by touching the screen. If one touches the top of the review area window, the earliest measurements may be reviewed. If one touches the bottom of the review area window, then the latest measurement can be reviewed. The session remains current as long as the file name remains the same, the instrument is not turned off, or one does not leave the Fv/Fm protocol. It will stay intact if one goes to different screens within the Fv/Fm protocol.

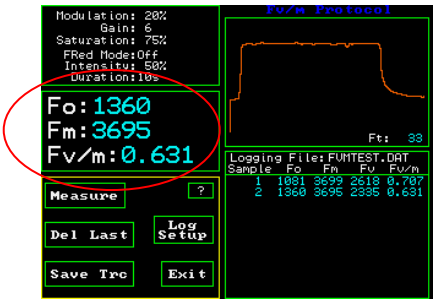
After a session is ended, data, and traces may still be viewed by going to the main menu, selecting “Data Management”, highlighting the file of interest with your finger, and pressing view. If a trace is saved with the measurement, a low-resolution trace will appear in the lower left hand corner of the screen. For more information, go to the “Data Management” section of the manual.

All measuring data is retained with the current file name in the measuring file, and it may be viewed in Excel on a computer. It does not disappear like the measurement review session data.

Help screen

The Question mark button is a help screen that provides basic instructions for the screen shown. Screen shots of the existing display screen , may also be saved by pressing “capture” that appears after the “?” button is pressed. Naming the screen shot is also possible with a keyboard that appears.

Basic definitions of parameters may be viewed by touching the Fv/m section of the Fv/Fm measuring screen.



Fv/Fm Test Setup Screen

Error messages common to the test modes:

There are several common errors that can occur in the test modes.

If the battery voltage is 10.5 or less, the warning message "!! Battery Low !!" will appear and a long beep will be sounded.

If the amount of in-band IR radiation is above set limits thereby saturating the pre-detector the error message "!! IR to High !!" will be displayed.

"Fluorescent signal too low" appears if there is not enough signal getting to the detector. This may be due to the fiber optic being too far from the sample, the intensity of the modulated source being too low or the gain control set too low.

"FL > 3600 pos det pin out" appears if the combination of the modulated light intensity and modulated light gain are set too high. To fix the problem go to Auto Gain on the setup screen, put a leaf in the PAR Clip, and press Auto Gain. The modulation intensity will adjust automatically.

Y(II) Protocol: Quantum Photosynthetic Yield of PSII Y(II) or ΔF/Fm'

Yield (or ΔF/Fm', or Fm'-Fs /Fm') (Genty 1989) is a fast (it normally takes about two seconds) light adapted, steady-state photosynthetic test, that provides a measure of the proportion of the amount of light used in photochemistry in PSII to the amount of light absorbed by chlorophyll associated with PSII. (Maxwell and Johnson 2000). It can also be viewed as achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions with quenching mechanisms at steady-state photosynthesis. It is the easiest test to take because the test sample does not have to be dark adapted. Modulated fluorometer design allows this measurement.

This is different from the Fv/Fm measurement that measures maximum photosynthetic efficiency in a dark adapted state. Fv/Fm does not measure photosynthetic efficiency while photosynthesis is taking place.

Y(II) provides a ratio that is a related to electron transport and achieved photosynthesis efficiency. Under laboratory conditions, and most stress conditions, Y(II) provides a measure directly related to linear electron transport. It has been found to correlate to carbon assimilation in a linear manner for C_4 plants, and in a curve linear manner for C_3 plants. (Genty 1989, 1990). Results are different for C_3 plants than for C_4 plants due to photorespiration, pseudo-cyclic electron transport, and possibly other electron sinks. For more information, review the Plant Stress Guide provided at www.optsci.com .

In the Y(II) test mode, it is assumed that the sample is being illuminated by an actinic light source to drive photosynthesis. Either the sun or an artificial light can be used. The OS1p has a white light diode that can be used as an actinic light source for Y(II) measurements.

Yield Y(II) has been shown to measure plant stress more successfully than Fv/Fm for some types of plant stress. For example it is more effective than Fv/Fm for heat stress and for water stress. For details about other types of plant stress, please review the Opti-Sciences Plant stress guide. www.optsci.com

Y(II) varies with light level and temperature. For that reason, it is imperative that the light level is measured and controlled. Only samples with very similar PAR values should be compared, and only sun leaves should be compared with sun leaves. The photosynthetic make up of sun leaves and shade leaves are different. With this in mind, a PAR Clip is highly recommended for all field measurements.

Steady State photosynthesis is an equilibrium of various plant mechanisms at a specific light level. Factors that determine the time required for steady state photosynthesis to be reached include the xanthophyll cycle, Δph of the thylakoid lumen, and state transitions. The xanthophyll cycle, Δph of the thylakoid lumen typically reach steady state in a few minutes, and state transitions can take fifteen to twenty minutes. Maxwell and Johnson (2000) found that it took fifteen to twenty minutes for 22 British terrestrial plants to reach steady state photosynthesis.

As with the "Fv/Fm" test, far-red pre-illumination is an option. Far –red illumination may also be turned off or turned on during the entire measurement. If ambient sun light is used for yield measurement, far-red light is provided by sunlight. It can be added in the set up menu if internal artificial illumination is used to drive PSI . Internal actinic illuminators filter out far red light.

When the test is triggered, Fs (or F') the measure of steady state fluorescence is determined and then the saturation pulse is applied to close all capable PSII reaction centers for determination of Fms (or Fm'). In steady state photosynthesis, Fms is depressed from Fm by non-photochemical quenching,

The resultant data is then processed to find Yield Y(II), (or ΔF/Fm'). A graph of the measured data is displayed along with measured and calculated parameters. ETR or relative electron transport rate and leaf temperature are also displayed if the measurement is made with a PAR clip. PAR Clips are optional accessories.

"Default" PAR values can be put in the measuring file as well as measured values. This is done on the Yield test calculations screen. "Default" PAR is an estimate of actual PAR values.

When the optional PAR Clip is not used, this number sets the PAR reading. It has a range from 0 to 2496 uE in steps of 1 uE (1 uml), and it allows estimated ETR. If the PAR Clip is used, then the measured PAR value is always automatically reported to the measuring file.

Cookbook checklist before making Y(II) measurements.

Fm' is maximum fluorescence in a light adapted environment at steady state photosynthesis. Fs' is the fluorescence signal in a light adapted environment at steady state photosynthesis.

Y(II) is = (Fm' – Fs') / Fm' = ΔF/Fm'

1. Leaves must be at steady state photosynthesis. This takes between fifteen and twenty minutes at a new light level. Above canopy leaves on a clear day, in the field, are considered to be at steady state photosynthesis. (Maxwell and Johnson 2000).

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. The xanthophylls cycle, and Δph of the thylakoid lumen adjust in about four minutes although it takes longer in the field. (Lichtenthaler 2004) State Transitions take between fifteen and twenty minutes to completely adjust. State transitions are a big factor at lower light intensities, but they are not much of a factor at high light intensities. Rapid light curves and Fv/Fm may be better solutions for below canopy work where appropriate.

3. Y(II) values vary with light level and with temperature. The higher the light level, the lower the Y(II) value. When measuring Y(II) in the field, it is extremely important to measure leaf irradiation or light level at the leaf and leaf temperature. Comparing Y(II) values taken at different light levels and different heat 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 Clip.

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

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. Times outside these ranges increase the error in Y(II) measurements. 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 at either. 0.8 seconds, or 1.0 seconds for higher plants. 0.8 seconds is the default value on the OS1p and it will work well with almost all higher plants.

9. 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. However, a problem has been found when measuring Y(II) at high light levels. It has been discovered that at high actinic or sun light levels, leaves resist the complete closure of all PSII reaction centers that is expected when using a saturation pulse. Even with a 6,400 μmol saturation pulse, some reaction centers remain open. Up to a 41% error was found in Y(II) measurements using standard techniques at high actinic light levels. To correct for this issue, multiple saturation flashes are used, and the measured maximum fluorescence value for each flash is entered into a linear regression analysis formula to determine the maximum fluorescence intensity with an infinite saturation flash. The multiple saturation pulse approach has been shown to work in multiple papers and posters. The resulting value has been shown to correlate well with gas exchange carbon assimilation values. This multi-flash method is available on the OS5p and OS1p fluorometers. (see the Multi-flash section for more details).

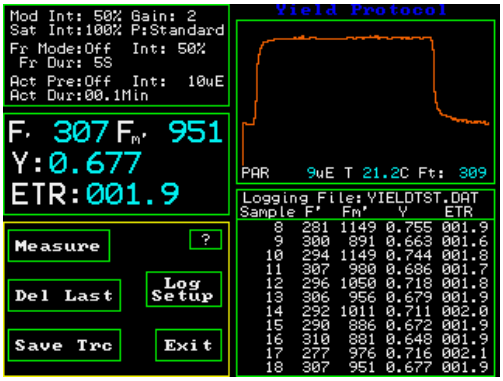
10. PSI fluorescence - Part of the fluorescence signal contains PSI fluorescence as well as PSII fluorescence. With Y(II), one is trying to measure variable fluorescence of PSII in a light adapted state.

PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an a small error. This is not a problem for comparing similar samples, because PSI fluorescence does not change with light intensity temperature or plant stress.

PAR is photosynthetically active radiation. Radiation on the leaf is measured between the wavelengths of 400nm to 700nm. PAR sensors and thermisters for measuring temperature are calibrated to other instruments that are traceable to the NIST. It is recommended that recalibration should occur every two years. Most modern sensors are solid state, so drift is minimal.

Running the Yield of PSII Y(II) Test

From the main screen select Test Menu by pressing the touch screen. The Test Selector screen will appear. Press the “Y(II)” button.



Y(II) Measuring Screen

small screen

second small screen

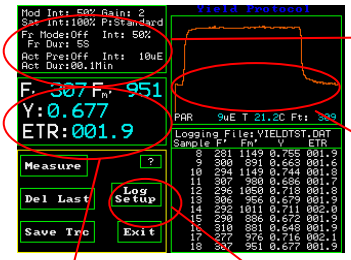
As with the Fv/Fm test, the last file used for this type of measurement will open up and a small screen will appear within the larger screen. It will show the file name and ask if the if one wants to “Proceed” to measuring with this file and the parameters that have been set. The other option is to create a new file with “Change”.

When “Proceed” is pressed, a second small window appears with three choices. “Appnd” adds measurements to the existing file, “Cncel” brings one back to the main menu, and “OvrRt” will overwrite the previous measuring file with existing measuring parameter settings.

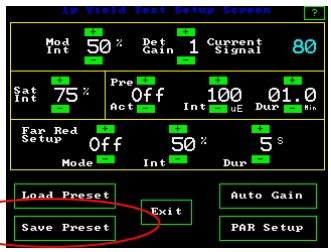
To make a measurement with the default setting programmed into the OS1p at the factory, place the single end of the fiber optic bundle into either the open body clip or the PAR Clip (The PAR Clip is an Optional accessory but it is highly recommended). Make sure that the fiber bundle is inserted all the way into the hole provided, and lock the locking screw. If the PAR clip is being used, then the measurement may be triggered by pressing the black button on the bottom of the PAR Clip, the measure button on the touch screen, or the red button on the end of the fiber optic probe. Do not change the orientation of the leaf relative to the light source, and place the clip in such a way that the part of the leaf to be measured is not shaded by the PAR Clip or the operator. Y(II) varies with most types of plant stress, but it also varies with light level and temperature. For that reason, the PAR Clip measures both variables and records the information in the measuring file with Y(II) and ETR.

Drill down menu for Y(II)

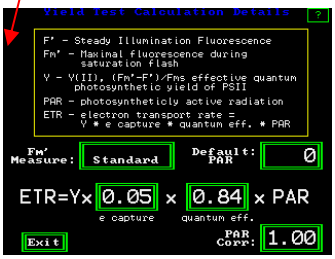
Press windows to go to other screens



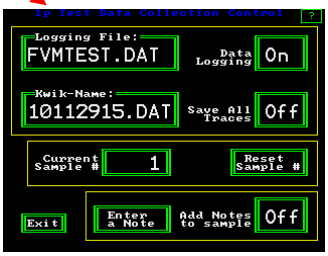
Y(II) Measuring Screen



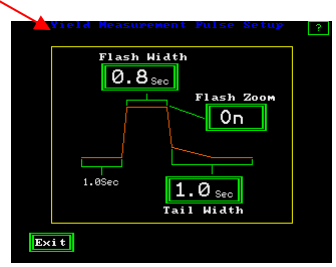
Yield Test Setup Screen



Y(II) Test Calculation Screen



Y(II) Test Data Collection Control Screen

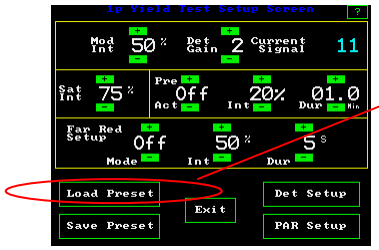


Y(II) Measurement Pulse Setup Screen

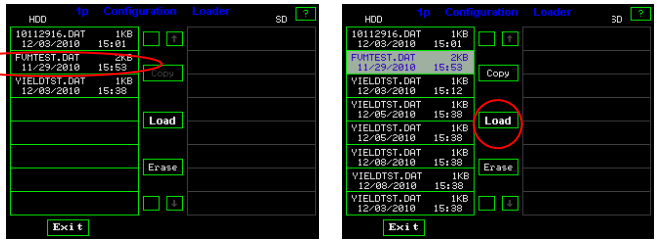
Changes that are made to the "Y(II) Collection Control" screen, the "Y(II) Measurement Pulse Setup" screen, and the "Y(II) Test Setup" screen can all be saved in a single measuring file by pressing "Save Preset" on the Fv/Fm Test Set up screen. The changes from other screens may be done in any order, and saved from this screen when complete. Follow the directions listed above to save measuring files.

Loading and saving preset measuring routines

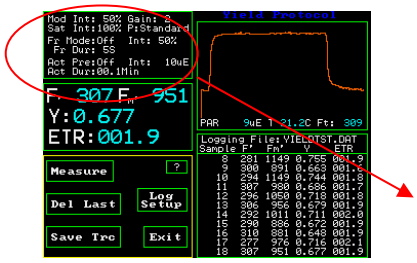
To load previously developed parameter routines, follow the directions listed below.



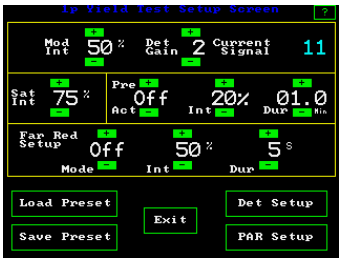
Yield Test Setup Screen



Configuration Loader Screens



Y(II) Measuring Screen



Yield Test Setup Screen

Loading preset measuring parameter files –

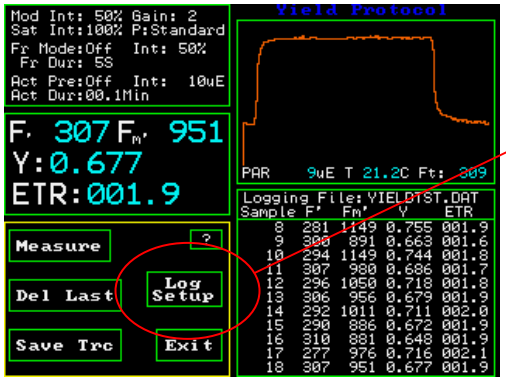
Highlight test by touch, and press load.

Arrows for scrolling appear when there are more tests than can be viewed on the screen.

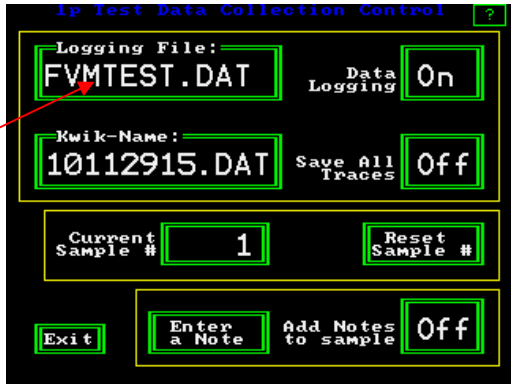
Copy only works when an SD card is inserted into the side of the OS1p.

When the parameters have been adjusted for a specific set of conditions, the test setup can be saved by pressing “Save preset” while in the Test setup screen. A pop up appears asking if you want to make a new file or use an existing one. Next a key board screen will appear if the new option is selected, and the test set up can be named and it is saved when “Ent” is pressed. To load previously created tests, press “Load preset”. Press the test of interest. After it is highlighted in light blue, press load. To erase a test, highlight the test and press “Erase”. The number of tests that can be saved is almost unlimited. Use the up and down arrows to scroll through the tests if there are more than are shown on the screen. Exit if there is no action.

Copy presets parameters into measuring file – Insert the SD data card into the SD card port on the OS1p, and press copy while the 1p Configuration Loader screen in open. Presets can be stored on the SD card for future use or transferred to a PC. A preset is different than a measuring file. A Measuring file, or Data file, contains presets, data and saved traces. A preset file only contain parameter presets and no data. To store the preset in a new measuring file, load the preset from the configuration loader screen or create the "Preset". Press "log setup" from the main measuring screen and change the "logging file name". The presets will be logged to the new file name.

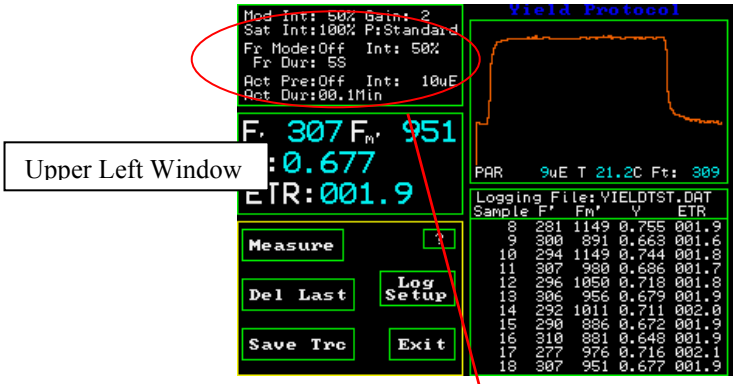


Y(II) Measuring Screen



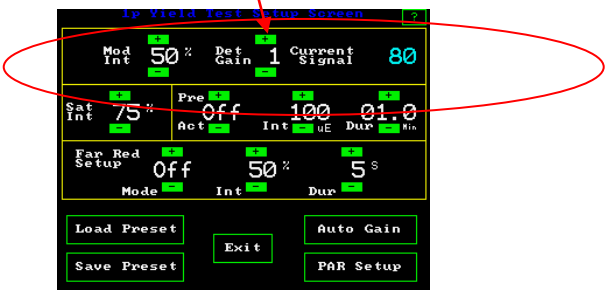
1p Test Data Collection Control Screen

Change Y(II) measuring parameters -



Y(II) Measuring Screen

One can change many of test parameters by pressing the upper left window on the screen.



Yield Test Setup Screen

Screen revealed by pressing the upper left hand window.

Setting modulation light source intensity. It is adjustable between 0.1 µmol and 1 µmol when using an open body clip or a PAR Clip. The settings range from 5% to 100% in 5% increments. The correct setting depends upon the distance from the sample and the nature of the sample. The optimum setting for Y(II) is not as critical as it is for Fv/Fm and quenching measurements. It must, however, be high enough to make a measurement and prevent the Fluorescence signal too low error.

Put a leaf, of the type to be measured in a study, into the PAR Clip or open body clip, and place the fiber optic probe all the way into the PAR Clip or Open body Clip.

The modulated light is always on when a measuring screen is viewed. The modulated light is set correctly, if it is set high enough to get a reading when the measuring button is pressed. The setting is linear. The “Gain” should be as low as possible for the best signal to noise ratio.

New -automated modulation light set up

Auto Gain – On the set up screen. This option automatically adjusts the modulated light setting to a detection range that will work for the sample. Press "Auto Gain" and the instrument automatically goes through a routine that sets the modulation intensity correctly. A leaf must be in the PAR Clip or open body clip when this routine is running.

Yield Test Setup Screen

To use the automated modulation light setup mode, press "Auto Gain". The system then adjusts the modulation light source into an acceptable minimum reading range for the PIN diode detector.

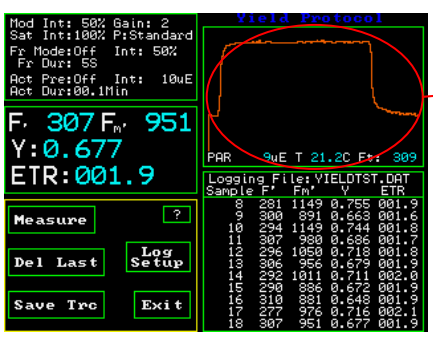
Setting the saturation pulse intensity - Values can be set from 5% to 100% in 5% increments. At 100%, the intensity of the saturation pulse is approximately 11,000 umols when used with a dark clip. Saturation intensity is approximately 6,200 umols when used with a PAR Clip.

Setting saturation pulse duration

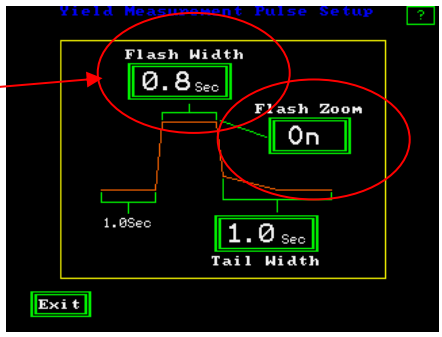
Press the graphic screen display of the measuring trace. Duration is the time that the saturation flash is turned on. For higher plants, times of 0.5 to 1.5 seconds have been used. The optimal saturation duration for algae and cyanobacteria is between 25 and 50 msec. (Schreiber 1995). If the duration is not long enough, then PSII is not fully saturated, even at the highest intensity, and Fm values are reduced. If the Duration is too long, on most chlorophyll fluorometers, the saturation pulse peak becomes subject to a type of NPQ that rounds the tailing edge of the saturation peak and also reduces the average Fm value. The Ideal saturation pulse peak is flat on the top with the trailing edge rounding minimized (Roseqvist and van Kooten 2006).The OS1p provides an automation feature that uses a rolling continuous eight point average to find the highest average value on the top of the saturation pulse. These eight points represent a time of 25 msec. As a result, the optimal saturation duration is automatically provided for higher plants, algae and cyanobacteria if the duration is long enough. 0.8 seconds is adequate for most plants. The saturation pulse duration is adjustable between 0.1 and 2.0 seconds.

To adjust the duration, touch the upper right graphic display window of the Y(II) measuring window.

To adjust the duration, touch the upper right graphic display window of the Yield protocol measuring window.



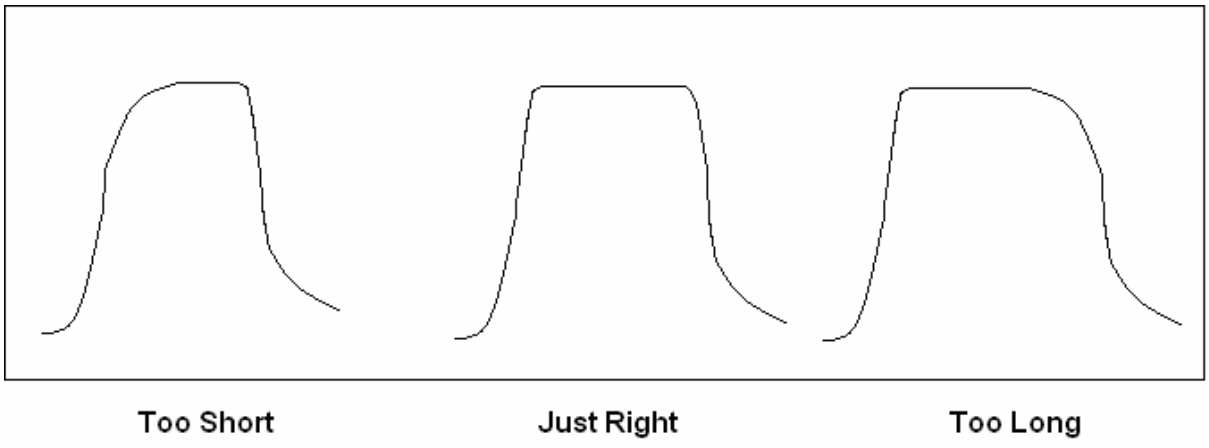
Y(II) Measuring Screen



Y(II) Measurement Pulse Setup Screen

By pressing the **Flash Width** button one goes to a keyboard screen and allows the saturation pulse duration to be adjusted between 0.1 and 2.0 seconds. Press "Ent"

Either the center or right image below, will provide optimal results with the OS1p.



Flash Zoom – This button allow a choice between displaying the Y(II) trace on a partial basis or displaying the full saturation pulse trace and the subsequent trailing fluorescence data after the saturation pulse. The default setting is to display the partial trace for reasons of speed. To view the trailing edge of the saturation pulse adds time to the measurement.

In the "Off" position, one sees only the leading edge and part of the top of the trace. By turning the Flash Zoom "On", it allows the viewing of each graphic measurement trace to be displayed completely. The "**Tail Width**" button allows one to select the amount of trailing fluorescence data to be displayed. By pressing the "Tail Width" button, a key board will be displayed that allows adjustment from zero to 9.9 seconds after the saturation pulse is turned off. Press "Ent" enter value.

One can also test for the proper saturation flash width by using the flash zoom and trying various saturation flash widths. The diagram below explains how to evaluate the flash.

Multi-flash vs. standard single flash saturation pulse

Multi-flash ("Step Int Lin Regr") -*measuring yield at high actinic light levels.*

The multi-flash option has been added to correct for the inability of very intense saturation light pulses to completely close reaction centers under high actinic light conditions. It is thought to be caused by mechanisms responsible for non-photochemical quenching. Research has found that under high actinic light conditions, measured electron transport rates (ETR) and yield values did not correlate well with carbon assimilation measurements. The inability of very intense light saturation pulses, of any intensity, to close all reaction centers, was thought to be the culprit.

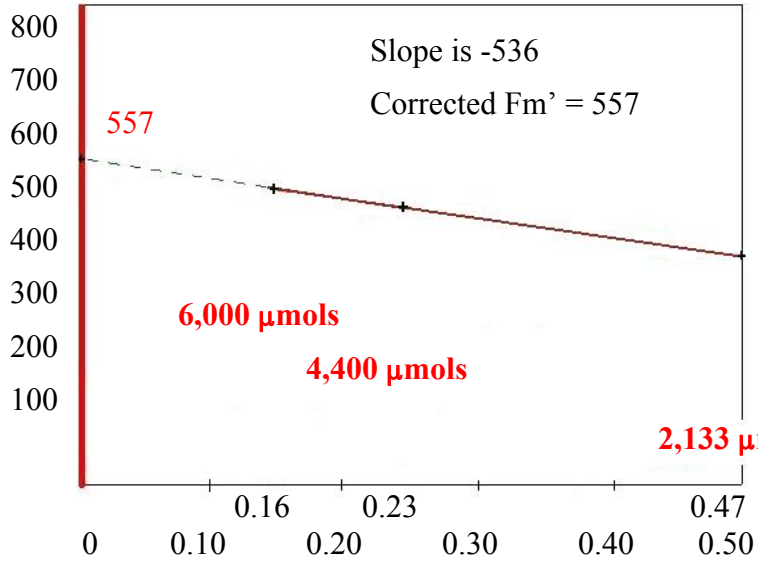
By using multiple saturation light pulses of varying intensity, and linear regression analysis, an infinite saturation pulse intensity can be estimated. It was found by Earl (2004) that by using multiple intense saturation pulses at varying intensity levels, one could use regression analysis to estimate the intensity of an infinite saturation pulse. Testing was done on corn (C_4 plants), and on cotton plants (C_3 plants) and it was found that in non-photo-respiratory conditions, the linear relationship between ETR and gross CO_2 assimilation was restored by using the Fm' or saturation pulse correction method.

This option should be considered for yield and ETR measurements when actinic light levels are high. When used, The Fm' value displayed on the graphic screen represents Fm' at infinity. The R^2 value is also displayed on the graphic screen. Saturation pulse intensities used in the multi-flash mode are preset at the factory. When used with a PAR Clip, they are at 2,133 µmols, 4,400µmols, and 6,000 µmols. The total flash duration that includes all three values is 1.0 second.

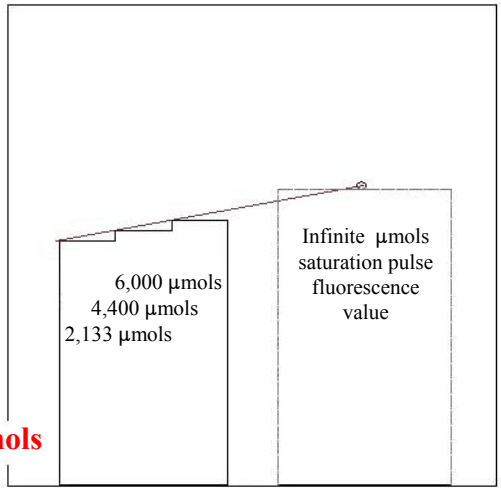
Since the Earl work, Bernard Genty the man that developed quantum photosynthetic Yield of PSII, back in 1989, has co-authored a poster that supports the work of Earl and the use of regression analysis for correction of yield and ETR values. A Licor research team of team of Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. that also included Bernard Genty of Cadarache DEVM-Laboratoire d'Ecophysiologie Moleculaire des Plantes of the University of Marseille, studied the issue. They found that in Z. mays, with the standard saturation pulse method, in high light field conditions, measurements produced up to a -41% error in ETR values, and up to a -22% error in Y(II) values . The research recommends the Multi-phase flash process for field measuring work. The research is titled "Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity". (Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. Genty B. (2006) "Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity". Abstract # P13011 August 1996. American Society of Plant Biologists Annual Meetings, Boston MA).

Multi-flash Method

Regression Analysis Graph



Representation of how the flash works



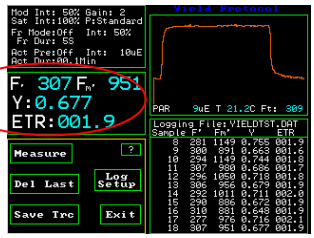
Values represent (1/PAR) x 1000

Linear regression analysis is used to estimate true Fm' using an infinitely intense saturation pulse according to Earl (2004) y=mx+b

Three saturation pulse flashes are fired in succession for 0.33 seconds. The firsts is at 2,133 µmols, the second is at 4,400 µmols and the third is at 6,000 µmols

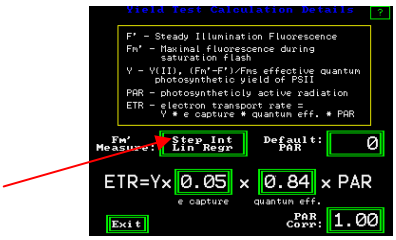
Hugh Earl 2004 – At high actinic light levels, Fm', the fluorescence value measured by a modulated chlorophyll fluorometer to determine saturation level, is underestimated due to nonphotochemical quenching effects. To saturate PSII, and achieve the expected correlation between carbon fixation and Y(II) measurements, a linear regression analysis is performed. Saturation pulse fluorescence intensity values for one third of full intensity, two thirds of full intensity, and at full intensity, along with the corresponding PAR values, represented as (1/PAR) x 1000, are into the equation y=mx+b. The "y "intercept represents the saturation fluorescence value with an infinite PAR saturation flash.

The Multi-flash option may be turned on or off in the Y(II) Protocol by pressing the second window down from the top on the left had side of the Y(II) measuring screen.

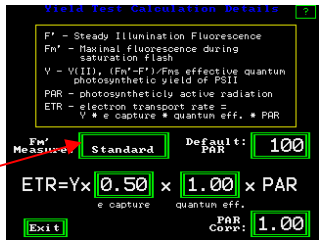


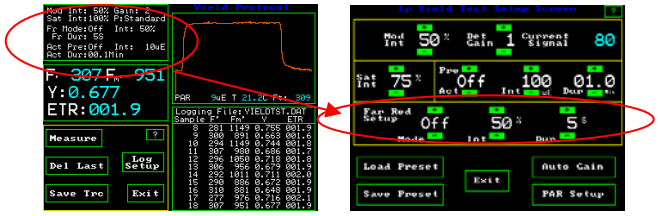
Y(II) Measuring Screen

Under "Fm' Measure" select **"**Step Int Lin Regr". For using the standard single saturation pulse method select "Standard".



Y(II) Test Calculation Details Screens



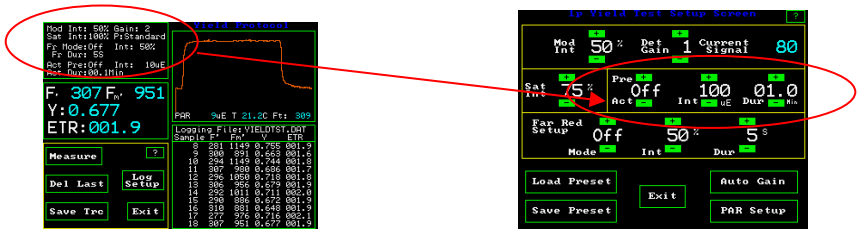


Y(II) Measuring Screen Y(II) Test Setup Screen

Far red light

Far red light can be used with the internal illuminator for the Y(II) test to allow the continued reduction of PSI and the re-oxidation PSII. Options are offered for off, on, and pre. "Off" turns the far red light off during the test. The "Pre" setting allows illumination of the sample with far red light before the test, and the "On" position keeps the far red light on during the test. Intensity is again adjustable between 5% and 100% with 5% increments. The duration setting is used with the pre setting to allow pre illumination with far red light from one second to sixty seconds. Sun light contains far red light, but the LED actinic light source filters out far red light when used as a pre-actinic source.

Pre-actinic light

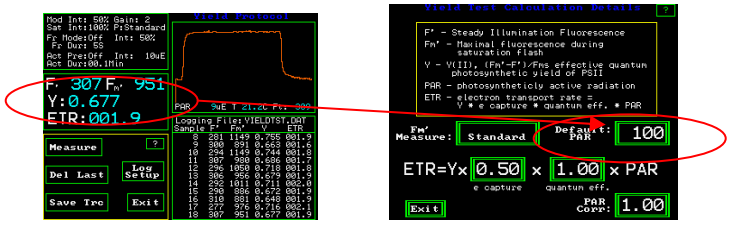


Y(II) Measuring Screen 1p Yield Test Setup Screen

This feature allows the illumination of the sample leaf with a predetermined amount of actinic white light. Y(II) requires leaf samples to be at steady state photosynthesis before measurement. When using pre-actinic lighting, it takes between fifteen to twenty minutes at a new light level for a leaf to reach steady state photosynthesis (Maxwell and Johnson 2000).

When the PAR Clip is used-

The instrument default setting is off for this feature. By using the + or – button, the feature can be turned on. The "Int" can be set between from 0% to 100% in 5% increments 100% is approximately 1500 µmols with a PAR Clip. The actual intensity is recorded when the PAR Clip is used. For correct Y(II) measurements, the "Dur" (duration) should be set so that the leaf reaches steady state photosynthesis before measurement. When done, one can exit or press save preset. The intensity of the pre-actinic light is held constant with in a few µmols by the PAR Clip to ensure steady state photosynthesis. This feature is great for the Lab.



Y(II) Measuring Screen Yield Test Calculation Details screen

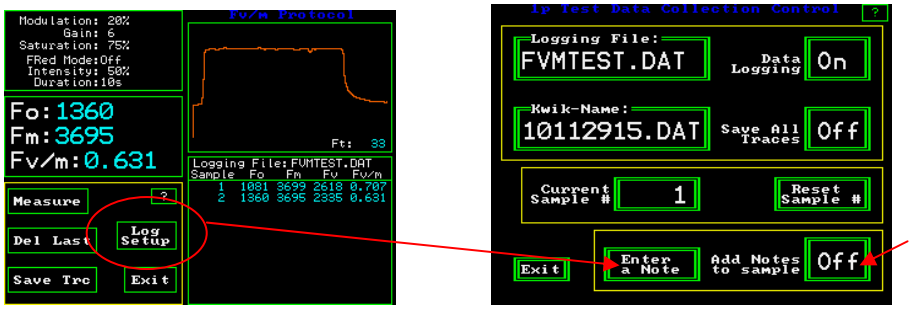
"Default PAR" When a PAR Clip is not used –

When a PAR Clip is not attached to the OS1p, the user may select the approximate pre-illumination intensity. From the Y(II) measuring screen, press the second window down from the top on the left had side to get to the Yield Test Calculation Details screen shown above.

On the Yield Test Calculation Details screen, press “Default PAR”, and a key board screen will appear. “Default PAR” can be set from Zero to 2465 µmols. OSI makes no claims about the accuracy of “Default PAR” values. The actual value should be read with an independent PAR meter at the leaf plane and leaf angle. When “Default PAR” is used, all measurements of ETR appear using the setting selected. When a PAR clip is connected to the OS1p, the “Default PAR” setting is automatically deactivated.

Default PAR can be used even when Pre-actinic illumination is not used. In this case, when ambient illumination is used, all ETR values and PAR values found in the measuring file will reflect the preset “Default PAR” value selected.

Entering a note with a measurement

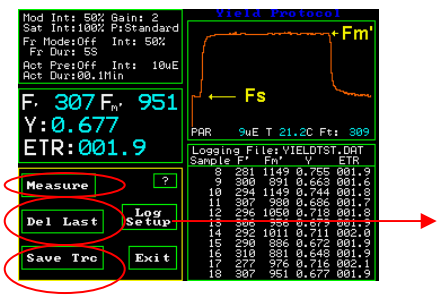


Y(II) Measuring Screen 1p Test Data Collection Control

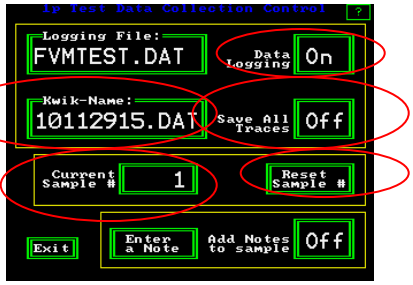
It is now possible to enter a note with any measurement, or to require a note with every measurement. To enter a note, press the “Log Setup”: button. Next, press “Enter a Note”. A touch keyboard will appear, and one can enter up to a 38 characters note with each measurement.

To require the note screen to appear with each measurement, press the ‘Add Notes to sample’ button and turn the feature "On". The keyboard screen will appear after the measurement is complete. If the occasional note is needed, turn the feature off by touching the “Add Notes to sample” button until it reads "Off".

Other Y(II) or yield function buttons



Y(II) Measuring Screen



1p Test Data Collection Control

Data Logging - retains all measured values when turned on.

Save All Traces -To save all trace data for all up coming measurements, the “Save All Traces” button must be turned on from the “Log Setup” screen. The instrument default setting is "Off".

Kwik-Name – the “Logging File” name is the file name. “Kwik-Name” allows one to quickly create a new file name without typing in the characters. The name is a code that represents the year -10, the month -11, the date 29, and the hour – 15 (24 hour clock). This is especially valuable for people making measurements in high volume that want to change the file name for a second field or sub set. The parameters stay the same as the existing file, and the code allows easy tracking where appropriate. Press the “Kwik-Name” button and the Kwik name becomes the “logging file name” moving forward.

Current Sample – Press the “Current Sample #” button to change the measurement number before you measure. The number that you enter will appear on the next measurement. A key pad will appear. Enter the new number, and press “Ent” (enter).

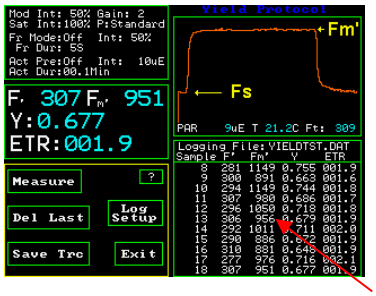
Reset Sample # - resets the sample number to 1 for the next measurement.

Save Trc on the Y(II) measuring screen, saves the trace displayed on the measurement screen, or the last trace. It is not saved normally, unless it is turned on in the Data logging collection screen shown above.

Del Last – Deletes the last measurement.

Measure – Measurements can be made by pressing the measuring button on the Y(II) measuring screen, or the red button at the tip of the fiber optic cable, or the black button on the bottom of the PAR Clip.

Measurement review



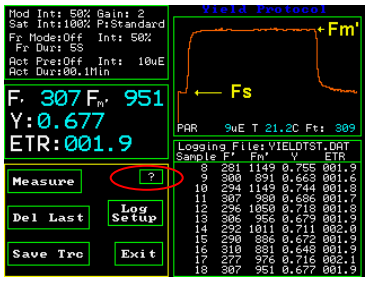
Y(II) Measuring Screen

On the main Y(II) measuring screen, the lower right hand side window logs all of the measurements made, in the current measuring session, for the current file name. One can scroll through measurements by touching the screen. If one touches the top of the review area window, the earliest measurements may be reviewed. If one touches the bottom of the review area window, then the latest measurement can be reviewed. The session remains current as long as the file name remains the same, and one does not leave the Y(II) protocol. It will stay intact if one goes to different screens within the Y(II) protocol. If one turns off the instrument, changes the file name, or goes to a different protocol, then the session is ended.

After a session is ended, data, and traces may still be viewed by going to the main menu, selecting “Data Management”, highlighting the file of interest with your finger, and pressing view. If a trace is saved with the measurement, a low-resolution trace will appear in the lower left hand corner of the screen. For more information, go to the “Data Management” section of the manual.

All measuring data is retained with the current file name in the measuring file, and it may be viewed in Excel on a PC computer. It does not disappear like the measurement review session data in the window.

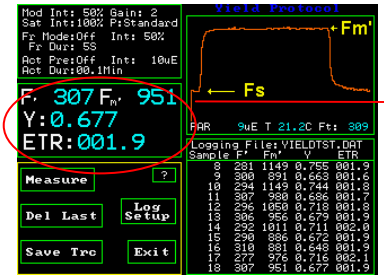
Help screen



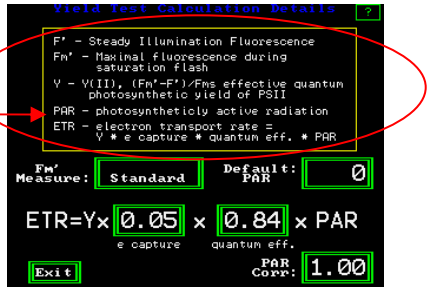
Y(II) Measuring Screen

The Question mark button is a help screen that provides basic instructions for the screen shown. It also allows one to capture the existing screen image in memory for later retrieval on a PC.

Basic definitions of Parameters may be viewed by touching the window shown below in the left hand picture. Of the Y(II) measuring screen.



Y(II) Measuring Screen



Y(II) Test Calculation Details Screen

Relative Electron Transport Rate

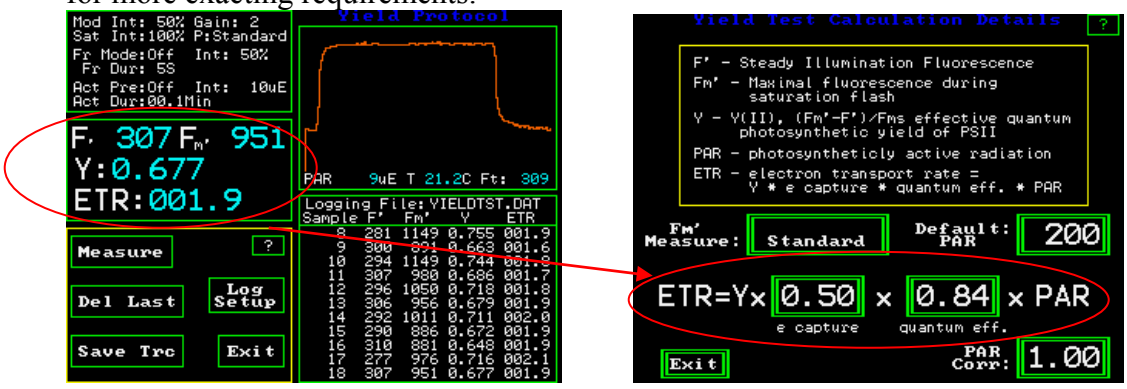
Relative Electron Transport Rate - ETR µmols = (Yield) (PAR) (.84) (.5)

(quantum yield of PSII) (measured Photosynthetically Active Radiation measured in µmols quanta m^{-2} s^{-1}.)(leaf absorption coefficient)(fraction of absorbed light by PSII antennae)

Average plant values are used in the standard equation. 0.84 is a good average value for many species of plants (Bjorkman and Demming, 1987). Research has shown that the leaf absorption coefficient can vary between 0.7 and 0.9 (Eichelman H. 2004), with species, chlorophyll content, and water content. Research has also shown that the fraction of light that is absorbed by PSII varies by species and can range from at least .40 to .60 (Laisk and Loreto, 1996). Even if the default average values are used, ETR can provide useful relative comparative information between different samples and the same sample under different conditions.

e capture and quantum eff – setting leaf absorption & PSII ratio

As stated above, the default settings in the ETR equation are considered to be average plant values for relative comparison of measurements. “e capture” is the ratio of PSII reaction centers to PSI reaction centers, and “quantum eff” is the ratio of light absorbed by the leaf to the amount irradiated on the leaf, at leaf level. The actual values for these parameters can be inserted into the ETR if they are wanted. The ratio of PSII reaction centers can be changed in the equation by pressing “e capture” and using the key board screen. Similarly, the over all leaf absorption can be changed by pressing “quantum eff”. Baker (2008) recommends using a integrating sphere to measure leaf absorption values for more exacting requirements.



Y(II) Measuring Screen

Y(II) Test Calculation Details Screen

For more exact ETR values, refer to papers listed above. The ratio of PSII reaction centers to PSI reaction centers change with plant species and type. The number of PSII reaction centers tends to be lower in C_4 plants than in C_3 plants (Laisk and Loreto,1996).

This correction is not needed for most relative comparison ETR applications; however, it has been made available for people that need it.

For a more in depth discussion on this topic, see Quantum photosynthetic yield of PSII – an in depth discussion of its value and limitations in chapter one.

"PAR Corr" is for correcting PAR sensor location error according to Rascher (2000). When some artificial light sources are used, Rascher found that the location the PAR sensor relative to the leaf surface can cause an error of up to 10%. This error is insignificant if sun light is used due to the much greater distance from the light source. Rascher used an independent PAR sensor and measured the intensity at the leaf plane. He then made corrections due to PAR Clip sensor location, by comparing the differences between the PAR clip values, and the leaf plane values. This correction may not be needed for most relative comparison ETR applications, however it has been made available for more exacting work when necessary.

Notes: When using the blue modulated light it was found that the average absorption value should be set to 0.94 instead of 0.84 as is used by the red modulated light.

By plotting ETR vs. PAR, potential ETR rates, photosynthetic capacity, and ETR rate limitations, at given light intensities, can be determined. (U. Schreiber 2004). Note: Four electrons must be transported for every CO_2 molecule assimilated or O_2 molecule evolved.

It has been found that Rapid Light Curves are a better solution in a variable light environment. Light saturation rate, as measured by rapid light curves, highly correlates with the concentration and maximum activity of Rubisco (Macintyre 1997), (Macintyre 1996). Measured Steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997).

Error messages common to the test modes:

There are several common errors that can occur in the test modes.

If the battery voltage is 10.5 or less, the warning message "!! Battery Low !!" will appear and a long beep will be sounded.

If the amount of in-band IR radiation is above set limits thereby saturating the pre-detector the error message "!! IR to High !!" will be displayed.

"Fluorescent signal too low" appears if there is not enough signal getting to the detector. This may be due to the fiber optic being too far from the sample, the intensity of the modulated source being too low or the gain control is set too low.

"FL > 3600 pos det pin out" appears if the combination of the modulated light intensity and modulated light gain are set too high. To fix the problem go to Auto Gain on the setup screen, put a leaf in the PAR Clip, and press Auto Gain. The modulation intensity will adjust automatically.

Quenching Protocol – Hendrickson – Klughammer Lake Model:

The OS1p can be ordered with various quenching protocols, but the advanced version comes with the Hendrickson – Klughammer lake model protocol as standard. Other options include, Dave Kramer lake model protocol, puddle model protocol, and quenching relaxation protocol that may be used with either the Hendrickson or puddle model protocols. For an in depth discussion of the value and differences between the protocols go to page quenching section in the first chapter. The Hendrickson – Klughammer protocol was chosen because NPQ has been resurrected by Klughammer for the lake model of antennae – reaction center interaction using the Hendrickson equations. There are very few reference papers available that use the Kramer lake model at this time, however, NPQ is well understood by most, and there is a wealth of reference literature available for the many uses of NPQ.

One can choose Kramer protocol, Hendrickson – Klughammer protocol, or puddle model protocol at the time of purchase. If no selection is made, then the Hendrickson – Klughammer lake model protocol will be supplied. Other protocols may be added for an additional price, or they may be added at a later date for an additional price.

The OS5p offers all of these protocols as standard.

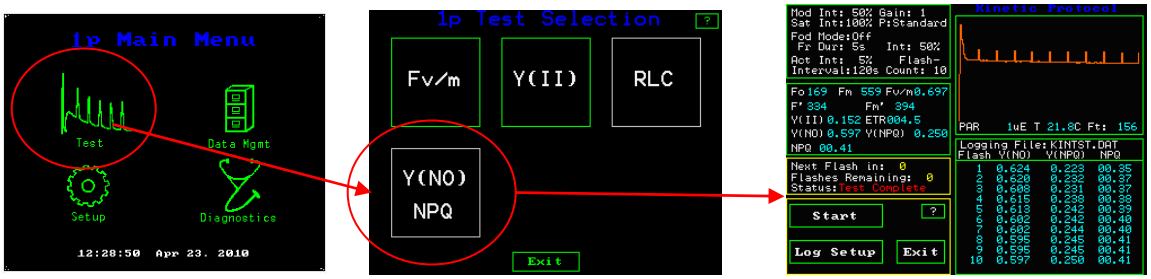
Hendrickson - simplified equations

Y(II) = (Fm' -Fs)/Fm' or)Fm'/ Fm'
Y(NO) = Fs/Fm or F/Fm
Y(NPQ) =(Fs/Fm')-Y(NO) or (F/Fm') - Y(NO)

Klughammer -NPQ was resurrected from the puddle model for the lake model using Hendrickson's equations.

NPQ= Y(NPQ)/Y(NO) or NPQ = (Fm-Fm')/Fm'

Using the Hendrickson Klughammer protocol.



Main Menu Test Menu Kinetic Quenching Measuring Screen

Cookbook checklist before making quenching measurements.

NPQ and other calculated parameters such as Fv/Fm, Y(II), Y(NPQ), Y(NO) are all normalized ratios that do not use a traceable standard. Instead, their accuracy is determined by properly using the instrument and following the lessons learned about their use with plant physiology by several great researchers.

To get an accurate 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. State transitions, however, take between fifteen to twenty minutes. These times can vary somewhat in field plants and can take slightly longer (Baker 2004). 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 30 to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into summer field measurements of Fv/Fm, and other displayed quenching parameters. For this reason, it is important to only compare samples with a similar light history. It is common for researchers to choose dark adaptation times anywhere from overnight, using pre-dawn values, to twenty-four hours, Shorter times are not normally used to study quenching (For more information, see the section on quenching measurements in chapter one.)

2. Samples that are compared must have the same Fv/Fm values. Quenching measurements of different samples with different Fv/Fm values should not be compared (Baker 2008). Fv/Fm is used as the measuring standard for non-photochemical quenching measurements, and if the measuring standard is different, the quenching values are meaningless. Comparing values from samples with different Fv/Fm values is like measuring items with a ruler that has dimensions that change.

3. Modulation light intensity setting Fv/Fm is (Fm-Fo)/Fm. Fo, or minimum fluorescence is a dark-adapted value made by exposing the leaf antennae to a very low intensity modulated measuring light, that is not set high enough to drive photosynthesis. The modulation light intensity must be set correctly for best accuracy and repeatability. If it is set too high, it will drive photosynthesis and provide an Fo value that is too high. The modulated light allows the measurement of pre-photosynthetic antennae fluorescence. Maximum fluorescence is measured when exposing a leaf to a saturation flash with light intense enough to close all PSII reaction centers.

4. Leaves must be at steady state photosynthesis. This takes between fifteen and twenty minutes at a new light level. (Maxwell and Johnson 2000). This is accomplished by setting the number of saturation pulses and the time between saturation pulses. For example, if there are ten saturation pulses spaced 120 seconds apart, the leaf will be exposed to the actinic light for twenty minutes after dark adaptation. Since an internal artificial light source is used, the test allows one to compare below canopy leaves as long as the Fv/Fm values are the same. According to Klughammer (2008), the only non-photochemical parameter that does not have to be taken at steady state photosynthesis is Y(NO) from Hendrickson.

5. Proper test length. OSI recommends that the actinic light should be on for thirty minutes when using the OS1p for quenching measurements. The intensity of the actinic LED light source output changes as the heat from the lamp changes the lamp temperature. The drop in intensity can be up to 13% of the initial lamp intensity over several minutes. since most of the drop happens in the first ten minutes, the change is minimized by extending the test to thirty minutes, allowing steady state photosynthesis.

6. Y(II) values vary with light level and with temperature. The higher the light level, the lower the Y(II) value. When measuring Y(II) in the field, it is extremely important to measure leaf irradiation or light level at the leaf, 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 Clip.

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

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

9. Leaf orientation is not important because an artificial actinic light source is used.

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

11. 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) and quenching 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 saturation pulse maximum value, and reduces the average maximum saturation pulse value. (Schreiber 2006). Some fluorometers allow adjustment of this parameter, and others are preset at the factory at either. 0.8 seconds, or 1.0 seconds for higher plants. 0.8 seconds is the default value on the OS1p and it will work well with almost all higher plants.

12. Saturation pulse intensity. Saturation pulse intensity is more of an issue with Y(II) than with Fv/Fm. While shade leaves will saturate at a few hundred μmols, sun leaves will usually saturate below 1,500μmols. However, a problem has been found when measuring yield at high light levels. It has been discovered that at high actinic or sun light levels, leaves resist the complete closure of all PSII reaction centers that is expected when using a saturation pulse. Even with a 6,400 μmol saturation pulse, some reaction centers remain open. Up to a 41% error was found in yield measurements using standard techniques at high actinic light levels. To correct for this issue, multiple saturation flashes are used, and the measured maximum fluorescence value for each flash is entered into a linear regression analysis formula to determine the maximum fluorescence intensity with an infinite saturation flash. The multiple saturation pulse approach has been shown to work in multiple papers and posters. The resulting value has been shown to correlate well with gas exchange carbon assimilation values. This multi-flash method is available on the OS5p and OS1p fluorometers. (see the Multi-flash section in chapter 3 for more details).

13. The time between saturation pulses. Rosenqvist and van Kooten (2006) state that a between one to two minutes is required for complete relaxation of saturation pulse NPQ. If saturation pulses are not separated by this distance range, then an error caused by this type of saturation pulse NPQ will result. It will accumulate with each saturation pulse. When in doubt, space saturation pulses 120 seconds apart or more.

14. Overlap of PSI fluorescence -Part of the minimum fluorescence, the Fo parameter, in Fv/Fm ((Fm – Fo)/Fm), contains PSI fluorescence as well as PSII fluorescence. With Fv/Fm, one is trying to measure the maximum variable fluorescence of PSII in a dark-adapted state. PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap with PSII. This produces an error. In C_3 plants, about 30% of Fo fluorescence is due to PSI, and in C_4 plants about 50% of Fo fluorescence is due to PSI fluorescence. PSI produces about 6% of the fluorescence found in Fm in C_3 plants, and about 12% in C4 plants. (Pfundle 1998). This not a problem when comparing quenching measurements for plant stress because, PSI fluorescence does not change with light level or plant stress.

15. PAR is photosynthetically active radiation. Radiation on the leaf is measured Between the wavelengths of 400nm to 700 nm. PAR sensors and thermisters for measuring temperature are calibrated to other instruments that are traceable to the NIST. It is recommended that recalibration should occur every two years. Most modern sensors are solid state, so drift is minimal. Since Y(II) and quenching parameter change with light and temperature, as well as plant stress levels, there are advantages to using a shrouded leaf and PAR Clip when making quenching measurements.

16. Far-red pre-illumination. Some 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 Fv/Fm measurement takes place. It activates PSI, and ensures that all electrons have been drained from PSII before the measurement of Fo. While this feature ensures that PSII is completely re-oxidized, it does not relax the xanthophyll cycle, state transitions, or photoinhibition. Time is still required in a darkened environment to relax all forms of NPQ and to obtain reliable quenching values.

17. **Far-red illumination.** Ambient actinic sunlight contains far-red light to activate PSI. The internal LED actinic light filters out far-red light. To approximate sun light, the far-red light should be turned on for the entire quenching measurement.

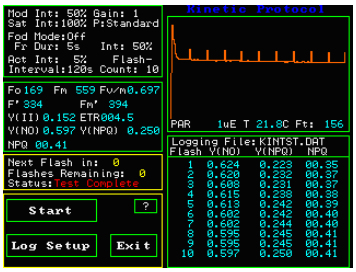
The best experiments are ones that take these issues into account. PSI fluorescence is involved in all measurements. It does not vary with light level or plant stress (Schreiber 2004). With this in mind, comparing samples with similar light histories allows comparison of many types of plant stress.

The Plant Stress Guide provided by Opti-Sciences www.optisci.com , references papers that deal with specific types of plant stress and limitations of different chlorophyll fluorescence parameters for measuring plant stress.

Running the quenching test

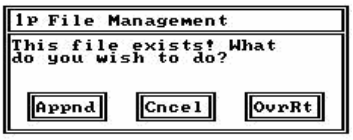
Press the “Test icon”, and the Test menu appears. Press “Y(NO) NPQ” and the quenching measuring screen appears.

The last file used for this type of measurement will open up, and a small screen will appear within the larger screen. It will show the file name and ask if the file is to be used and if one wants to proceed to measuring with this file and the parameters that have been set. The other option is to create a new file.



Quenching Measuring Screen

small screen

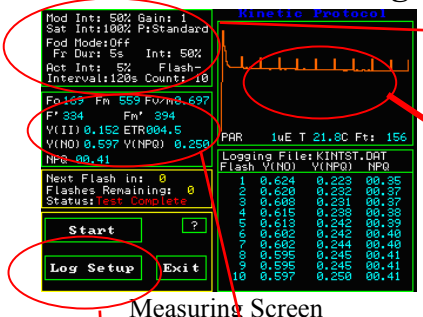


second small screen

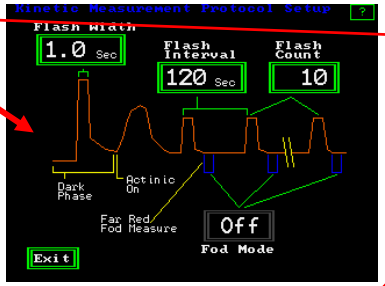
Press “Proceed” to use the existing file and “Change” to use a different file. A second box appears and asks if you want to add measurements or “Append” the loaded file, “Cancel” and go back to the main menu, or “OveRt” overwrite the entire measuring file.

The quenching test may be used with the instrument default settings or they may be changed to meet your needs. The various set-up screens are shown below. To get to the drill down screens, press the window indicated by the arrow.

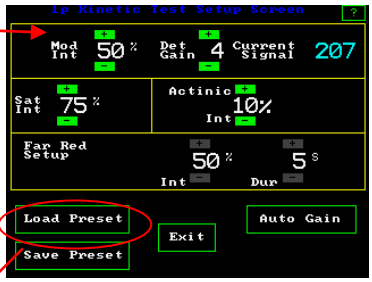
Screen drill down diagram for quenching



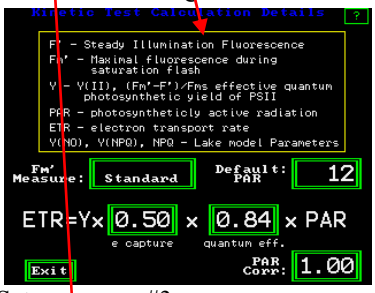
Measuring Screen



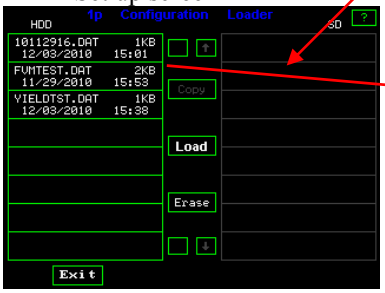
Set up screen #1



Set up screen #2

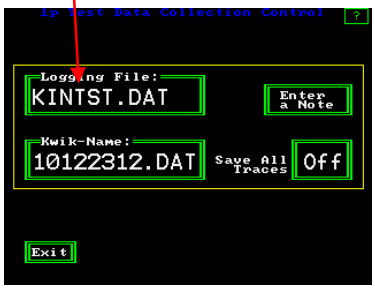


Set up screen #3



Stored test preset file screen

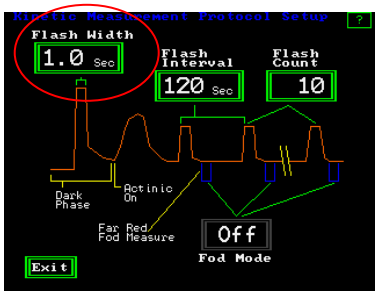
Preset values are selected by touch



Log set up screen

Arrow appear when there are more preset files than can be displayed

Saturation pulse duration



Set up screen #1

The saturation "Flash Width" may be set from 0.1 seconds to two seconds. The flash width is commonly set between 0.5 seconds and 1.5 seconds for optimal work. The width must be long enough to fully saturate PSII but narrow enough to prevent saturation flash NPQ from reducing the average value of Fm', the saturation intensity value. 0.8 seconds is a good default setting for most plants, however, if you would like to test your plant, use the following method.

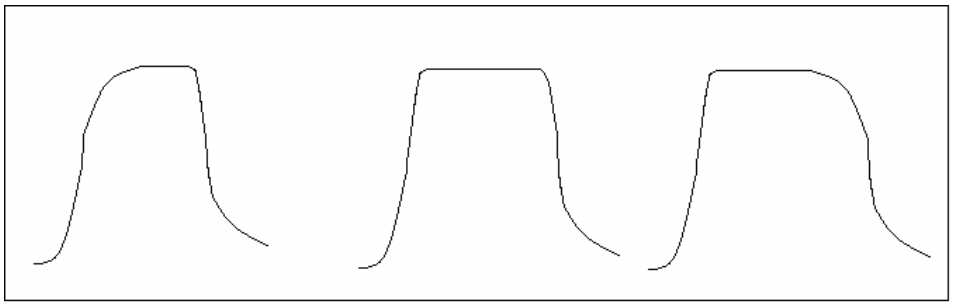
Setting saturation pulse duration

Duration is the time that the saturation flash is turned on. For higher plants, times of 0.5 to 1.5 seconds have been used. The optimal saturation duration for algae and cyanobacteria is between 25 and 50 msec. (Schreiber 1995). If the duration is not long enough, then PSII is not fully saturated, even at the highest intensity, and Fm values are reduced. If the Duration is too long, on most chlorophyll fluorometers, the saturation pulse peak becomes subject to a type of NPQ that rounds the tailing edge of the saturation peak and also reduces the average Fm value. The Ideal saturation pulse peak is flat on the top with the trailing edge rounding minimized (Roseqvist and van Kooten 2006).The OS1p provides an automation feature that uses a rolling continuous eight point average to find the highest average value on the top of the saturation pulse. These eight points represent a time of 25 msec. As a result, the optimal saturation duration is automatically provided for higher plants, algae and cyanobacteria if the duration is long enough. 0.8 seconds is adequate for most plants. The saturation pulse duration is adjustable between 0.1 and 2.0 seconds.

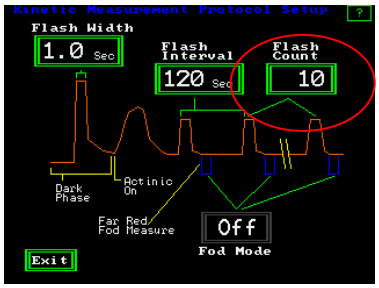
To adjust the duration, touch the upper right graphic display window of the quenching measuring window, and then, touch the "Flash Width" button.

It is best to use the Y(II) protocol or the Fv/Fm protocol to test the correct width of the saturation pulse because only one saturation pulse is displayed and it is magnified on the trace screen. The width may be adjusted and viewed by touching the trace display screen in these two protocols. Select the full view, adjust the width of the saturation pulse, until the trace looks like the trace in the middle picture or the one on the right. If the saturation pulse is too narrow, it does not matter how high the saturation flash is set, it will not fully saturate PSII. If the saturation pulse is too wide, the automated optimal measuring routine will still provide the optimal measurement. On other chlorophyll fluorometers, the average Fm' value is reduced by the curvature of the trailing edge of the saturation pulse caused by a form of NPQ (Roseqvist and van Kooten 2006).

Setting saturation pulse width



Saturation pulse count

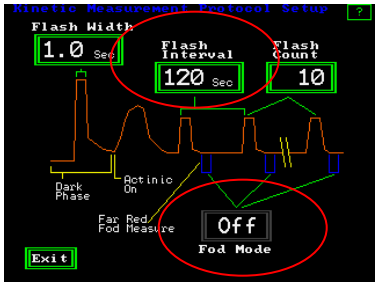


Set up screen #1

Flash Count has been set at 10. To get reliable quenching measurements, the leaf must be at steady state photosynthesis, a process that takes between fifteen and twenty minutes at a constant actinic light level. Ten flashes with an interval of 120 seconds allows twenty minutes at a set light level before the quenching measurement is made. For extra safety, changing the flash count to 15 may improve results. The LED actinic light source output declines up to thirteen percent as it heats up.

Most of the change in light output occurs over the first five minutes. By adding the extra flashes, the change in the intensity is minimized and becomes insignificant. If the PAR Clip is used with a shroud, or the measurement is made in a darkened room with a PAR Clip, or pre-Dawn measurements are made with a PAR Clip, the intensity change is recorded with the measurement.

Saturation pulse interval



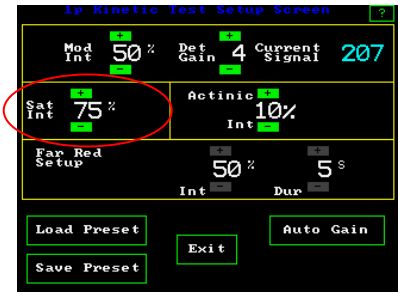
Set up screen #1

"Flash Interval" is the time between flashes. According to Roseqvist and van Kooten (2006) saturation pulses must be 60 to 120 seconds apart, in higher plants, to ensure that saturation flash NPQ is fully dissipated. If it is not, the Fm' peaks will continue to decline from one flash to the next as the saturation flash NPQ builds up. F may also rise. To prevent this, one can do test the sample, or use 120 seconds to be safe.

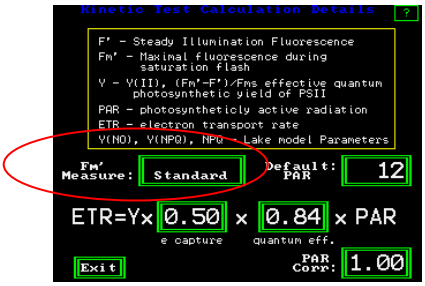
Fo' or Fod mode

Fod Mode – Fod mode or Fo' mode is used with the Kramer and puddle model protocols only. It is not used with the Hendrickson –Klughammer protocol. When used, it allows the measurement of Fo' at the end of the trace or after every saturation pulse. Knowledge of Fo' (or Fod) is required for most Kramer parameters, and for qN in the puddle model.

Saturation pulse intensity



Set up screen #2

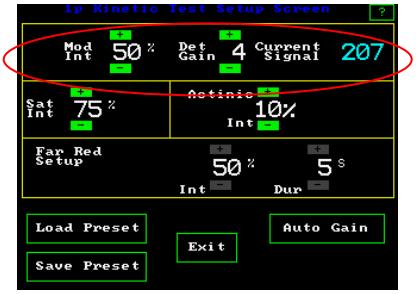


Kinetic Test Calculation Details Screen

The default "Sat Int" (saturation intensity) is set at 100% or about 11,000 umols with a dark clip and about 6600 umols with a PAR Clip. Evidence has shown that there is no damage to the leaf if saturation pulse intensities are very high in a light adapted environment. Damage to plants can occur if the saturation flash is too high, and too frequent in a dark environment (Roseqvist and van Kooten 2006). For this reason, we recommend maximum saturation pulse intensity for most quenching measurements. Quenching relaxation measurements, available as an option, are made in the dark, so the number of saturation flashes may be an issue with this test. The saturation pulse is adjustable from 5% to 100% in 5% increments.

It has been discovered that at high actinic or sun light levels, leaves resist the complete closure of all PSII reaction centers that is normally expected when using a saturation pulse. Even with a 6,400 µmol saturation pulse, some reaction centers remain open. Up to a 41% error was found in Y(II) measurements using standard techniques at high actinic light levels. To correct for this issue, multiple saturation flashes are used, and the measured maximum fluorescence value for each flash is entered into a linear regression analysis formula to determine the maximum fluorescence intensity with an infinite saturation flash. The multiple saturation pulse approach has been shown to work in multiple papers and posters. The resulting value has been shown to correlate well with gas exchange carbon assimilation values. This multi-flash method is available on the OS5p and OS1p fluorometers. (see the Multi-flash section for more details). To use the multi-flash correction, go to the "Kinetic Test Calculation Details Screen" shown above. Press the "Fm' measure:" button and change from standard to **"**Step Int Lin Regr".

Modulation light source intensity

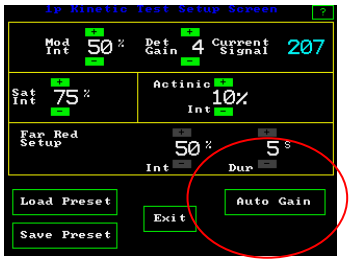


Set up screen #2

Modulation intensity should be critically set when doing quenching measurements. It must be high enough to prevent a fluorescence signal too low error message, but not high enough to drive photosynthesis.

The modulation light source intensity is adjustable between 0.1 µmol and 1 µmol. Settings between 5% to 100% can be made in 5% increments. The correct value depends upon the distance from the fiber optic to the sample, and nature of the sample.

Put a leaf, of the type to be measured in a study, in a dark clip or in a PAR Clip and dark adapt. Place the fiber optic probe all the way into the dark clip or PAR Clip, and expose the dark adapted area of the leaf to the modulated light that is always coming out of the end of the fiber bundle when a measuring screen is viewed. If the intensity is too high, it can be seen as a slow rise in "Current Signal" visible on the set up screen or the "Ft" value visible on the Fv/Fm measuring screen. After setting the modulation intensity, watch either value. If the average value increases over a 30 second period, the intensity should be reduced because it is driving photosynthesis. The modulated light is set correctly if it is high enough to get a reading when the measuring button is pressed, but not too high so that it is driving photosynthesis. The setting is linear. *The "Gain" should be as low as possible for the best signal to noise ratio.*

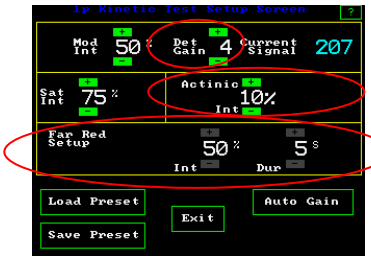


Set up screen #2

To use the automated modulation light setup mode, press "Auto Gain". The system then adjusts the modulation light source into an acceptable minimum reading range for the PIN diode detector.

New -automated modulation light set up

"Auto Gain" – On the set up screen, this option automatically adjusts the modulated light setting to a detection range that will work for the sample. It is known from samples tested, that this automated modulated light level does not drive photosynthesis. Place a sample leaf, of the type to be tested, into either a dark clip or with a shrouded PAR clip. Dark adapt for five minutes and press "Auto Gain". The instrument automatically goes through a routine that sets the modulation intensity correctly.



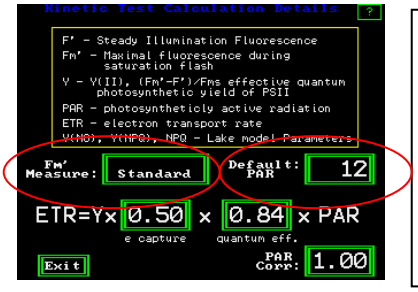
1p Kinetic Test Setup Screen

Det Gain

This is detector electronic gain control. It should only be raised if the modulation intensity can not be raised high enough to prevent a "signal too low" message. Raising this value lowers the signal to noise ratio, and can produce a noisier trace.

Far red intensity and duration are set at the default settings of 50% and a duration of 5 seconds. This feature is only used with optional Kramer lake model and puddle model protocols. Actinic int.

Actinic intensity is adjusted from zero to 100% intensity with 5% increments, using the plus and minus buttons. The value may be measured with the PAR Clip if it is used for measurements. The actinic intensity is about 1,500 umols at 100% intensity when using the PAR Clip and about 2500 umols with a dark clip. The scale is almost linear from 0 to 100%, however, for exact measurements, the PAR Clip or an external PAR meter should be used to measure the light irradiation on the leaf. Shade leaves will saturate at much lower levels than sun leaves.



Set up screen #3

"Fm' Measure" - On the third set up screen, the user can change from standard saturation flashes to multi-flash saturation pulses. This feature is recommended for high light levels. It estimates the fluorescence intensity with an infinitely intense saturation flash.

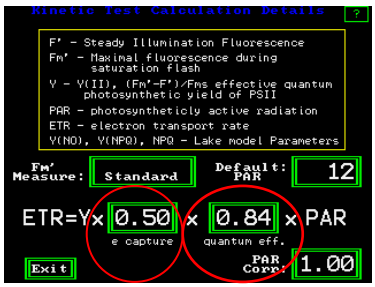
For detailed information, see the section on Multi-Flash in chapter 3.

Using Default PAR

This allows one to enter a PAR light level into measuring files, when PAR is measured with a separate instrument, and if the PAR Clip is not used. The PAR CLIP can be used to measure the PAR (light) intensity before the test or a separate PAR meter can be used. When making this measurement, it is recommended that the light level be tested just after the actinic light is turned on, after five minutes, and at twenty minutes. As the actinic light heats up, the intensity output drops somewhat. The greatest amount of change occurs in the first five minutes. The change after five minutes is within a just a few percent of the light intensity. For this reason, one may want to run quenching tests for twenty-five or thirty minutes to insure steady state photosynthesis at a specific light level.

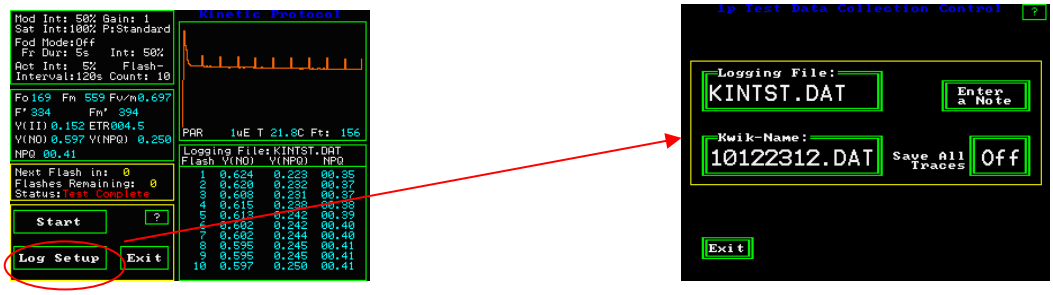
“Default PAR” does not work when the PAR Clip is attached to the OS1p. The PAR Clip over rides “Default PAR”.

e capture and quantum eff – setting leaf absorption & PSII ratio



The default settings in the ETR equation are considered to be average plant values for relative comparison of measurements. “e capture” is the ratio of PSII reaction centers to PSI reaction centers, and “quantum eff” is the ratio of light absorbed by the leaf to the amount irradiated on the leaf at leaf level. The actual values for these parameters can be inserted into the ETR equation for more exacting requirements. Sources for the ratio of PSII to PSI reaction centers can be found in Chapter one. Baker (2008) recommends using a integrating sphere to measure leaf absorption values for more exacting requirements. For a more in depth discussion on this topic, see Quantum photosynthetic yield of PSII – an in depth discussion of its value and limitations in chapter one.

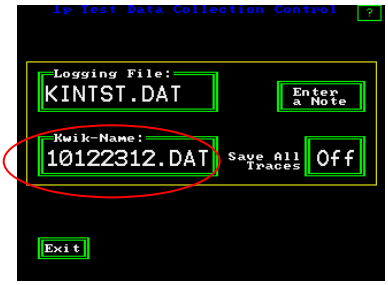
Log Set Up



Measuring screen

Log setup screen

Logging file is the name of the current data file being used for measurement. It includes the last set of measuring parameters that were set up, traces, and data. It is possible to use more than one set of measuring parameters in the same data file, however, one has to make a conscious choice to do so. The OS1p will ask if you want to change the file name when parameters are changed. When one proceeds to the measuring screen, the file that was last used will load automatically



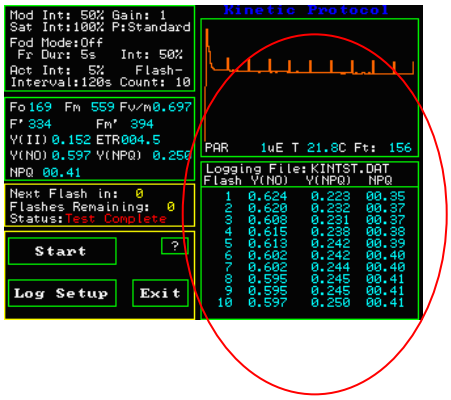
Kwik-Name is a feature that allows the user to use today's date and time as file name 10 is the year, 12 is the month 23 is the day and 12 is the hour. When it is pressed the number moves to the "Logging file". This feature has been added as a time saver.

Save all traces This allows traces to be saved in the same data file as the measured data. Each time resolved intensity value of the trace has its own row when transferred to Excel or other spread sheets. The time resolution for quenching tests is 0.1 seconds per data point. This means that a twenty minute trace has 12,000 data points. With this in mind, one may have to limit the number of traces per file because spread sheets usually have a limited number of rows. This can also vary with the version of spread sheet software. For example, some versions of Excel have more that 65,000 rows while the most up to date version has more than 1,000,000 rows. For this reason, you may want to have separate data files for each trace. The Kwik-Name feature makes this easy and fast.

Enter a note

It is now possible to enter a note with any measurement. To enter a note, press "Enter a Note". A touch keyboard will appear, and one can enter up to 38 characters per note with each measurement. Capitals, and symbols from a normal keyboard can be selected. When done, press "Enter", and then "Esc".

Measurement review



On the main quenching measuring screen, the lower right hand side window logs all of the measurements made, in the current measuring session, for the current file name. One can scroll through measurements by touching the screen. If one touches the top of the review area window, the earliest measurements may be reviewed. If one touches the bottom of the review area window, then the latest measurement can be reviewed. The session remains current as long as the file name remains the same, and one does not leave the quenching protocol. It will stay intact if one goes to different screens within the quenching protocol. If one turns off the instrument, changes the file name, or goes to a different protocol, then the session is ended.

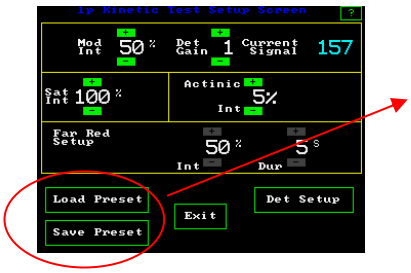
After a session is ended, data, and traces may still be viewed by going to the main menu, selecting “Data Management”, highlighting the file of interest with your finger, and pressing view. If a trace is saved with the quenching data, the trace may be seen by using the “Line up” and “Line down” arrow keys to go to the last measurement of a quenching trace. It there is a trace saved, a low resolution trace will appear in the lower left hand corner of the screen. For more information, go to the “Data Management” section of the manual.

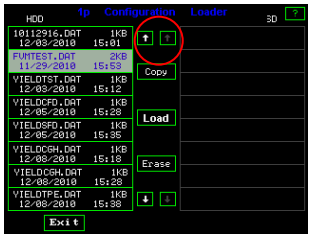
Measuring data and traces are also retained with the current file name in the measuring file, and it may be viewed with Excel on a PC computer. It does not disappear like the measurement review session data in the window.

Test pre-set files. Saving and loading test parameters

To save a test set of parameters for future use, press “Save presets” and name the new file. A key board will appear and allow naming.

To load a previously saved set of parameter presets press “Load presets”





Loading preset measuring parameter files –

Highlight the test of interest by touch, and press load.

Arrows appear when there are more tests than can be viewed on the screen at one time, and they are used for scrolling.

Copy only works when an SD card is inserted into the side of the OS1p.

Copy - To copy Preset parameters, inset the SD data card into the slot provided on the side of the fluorometer. Highlight the file name of interest by touch, and then press “Copy”. The files are copied to the SD card where they can be stored for future use or transferred to a PC. A preset is different than a measuring file. A specific preset can be part of a measuring file. To store the preset in a new measuring file, load the preset from the configuration loader screen. “Exit” this screen and press “log setup” from the main measuring screen. Change the “logging file name”. The preset and all measurements will be logged to the new file name, and all data, traces and the presets can be used for measurement, or transferred to a computer in the Data Management section by SD card, or USB port.

Erase – To erase files, highlight the file of interest by touch and press “Erase”.

Error messages common to the test modes:

There are several common errors that can occur in the test modes.

If the battery voltage is 10.5 or less, the warning message “!! Battery Low !!” will appear and a long beep will be sounded.

If the amount of in-band IR radiation is above set limits thereby saturating the pre-detector the error message “!! IR to High !!” will be displayed.

“Fluorescent signal too low” appears if there is not enough signal getting to the detector. This may be due to the fiber optic being too far from the sample, the intensity of the modulated source being too low or the gain control set too low.

“FL > 3600 pos det pin out” appears if the combination of the modulated light intensity and modulated light gain are set too high. To fix the problem go to Auto Gain on the setup screen, put a leaf in the PAR Clip, and press Auto Gain. The modulation intensity will adjust automatically.

Rapid Light Curves (RCL)

Rapid light curves have been heavily used by researchers to study aquatic plants, and for under canopy research on land. Some traditional fluorescence parameters and methods run into difficulty when the light level changes rapidly, or when one is studying the effects of sun flecks. While the measurement of photosynthetic yield only takes a couple of seconds, it is defined as a measurement taken during steady state photosynthesis, a process that takes between fifteen and twenty minutes at given light level (Maxwell and Johnson 2000). Under canopy leaves are exposed to sun flecks and variable illumination. Traditional photosynthetic yield, ETR, and standard light response curves run into problems. Wave action, changes in water column depth, tides, currents, clouds and turbidity constantly change the amount of light that is received by aquatic plants. Of course, Fv/Fm may be used to measure the health of PSII with some of these situations; however, the need to study and measure the reaction of plants under changing ambient conditions and the need to study light saturation characteristics has driven research into methods such as rapid light curves.

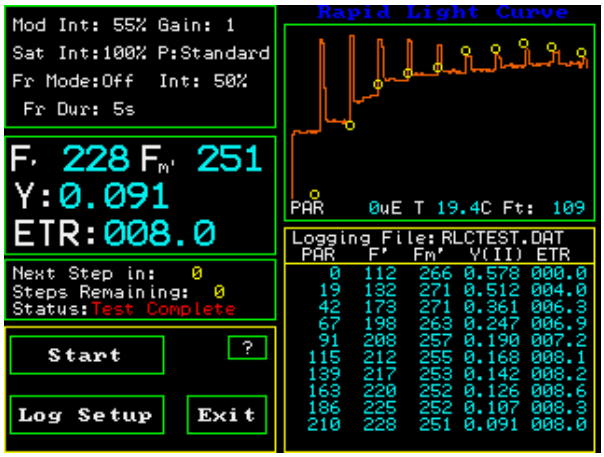
Rapid light curves provide relevant information on the saturation characteristics of electron transport. (Schreiber 2004). When working with Aquatic plants, and water column productivity, saturation characteristics are among the most important determinates. Marra (1978), (Banse and Yong 1990).

Light saturation rate as measured by rapid light curves highly correlates with the concentration and maximum activity of Rubisco (Macintyre 1997), (Macintyre 1996). Measured Steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997).

There are many claims about the value of rapid light curves (or RLCs), and some are controversial. It has been mentioned by Ralph (2005) that a possible reason for rapid light curves not being more highly used is that researchers are not sure what the data means, and how reliable the data is. We will provide an overview of the Rapid light curve and the research regarding RLC.

How they work:

Rapid light curves are created by dark adapting samples for a specific period of time and stepping a photosynthesis driving actinic light source for short specific periods of time at specific intensities. The light source is usually built into the fluorometer and the sample is shrouded to allow only the actinic light from the fluorometer to hit the sample. Steps may be up or down. Typically, after a short period of time at a specific actinic light level, a single saturation pulse is triggered and the internal light.



Typical trace of a rapid light curve (Shade leaf) The yield and ETR values for each step are reported on the same screen and in the measuring file. The orange trace represents the actual fluorescence intensity. The spikes represent the saturation flashes and Fm' values, while the lower values, at each step, represents the fluorescence output at each actinic light step F'. Y(II) or yield is (Fm'-F)/Fm'. Y(II) declines as actinic light step intensity increases. The Yellow circles represent relative electron transport rate or relative ETR. The first circle on the lower left is a dark-adapted zero value. The F' value for the first measurement shown in blue, in the lower right hand window, is the modulated light intensity, not the actinic light intensity.

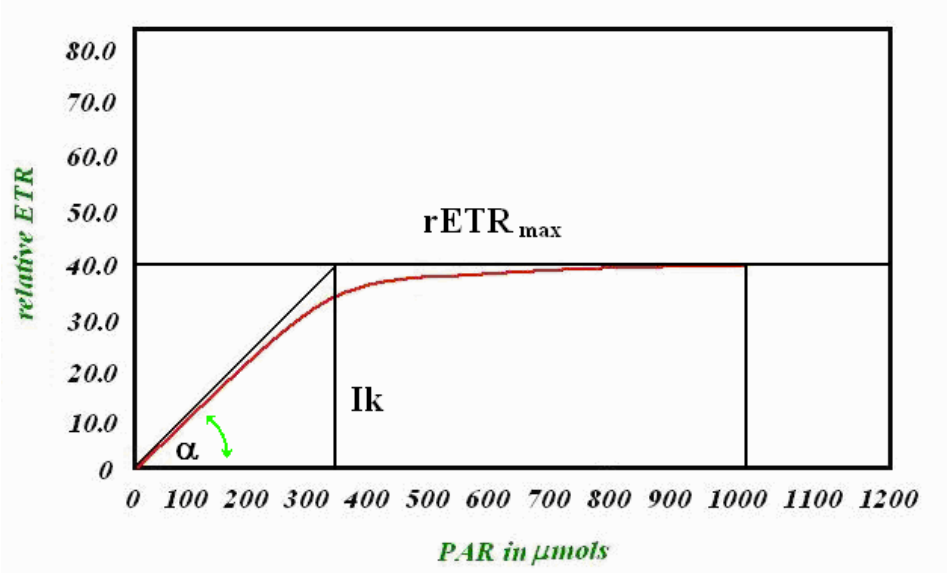
Relative ETR is calculated using a Quantum photosynthetic yield of PSII measurement, taken at a given light level, and using the equation: rETR = (Quantum photosynthetic yield of PSII) x (PAR - the light level) x (0.84) x (0.5). rETR is scaled on the Y axis, and PAR (photosynthetically active radiation per meter squared per second) is on the X axis. It is common for the first measurement to be made in the dark and the second step at a low PAR level. It is also common for successive steps to be measured at higher light levels with the last step or two steps being measured at or above the leaf light saturation levels. Intensity values are commonly equally spaced.

Curve fitting software

The data can be put into Sigmaplot software where a regression wizard can use a curve fitting model to determine Cardinal points for analysis. (Ralph 2005) Web address for Sigmaplot software:

http://www.sigmaplot.com/products/sigmaplot/sigmaplot-details.php .

Cardinal points are then derived from equations determined by the curve model of the researcher's choice. Jassby and Platt (1976), Platt (1980)and Eilers and Peeters (1988) have been used.



Cardinal points description

α is the initial slope of line at low PAR values created by relating ETR to PAR. It provides a measure of quantum efficiency(Schreiber 2004)
ETR_{max} is a measure of a leaf's photosynthetic capacity or maximum electron transport rate (Schreiber 2004).
$I_k = \alpha / ETR_{max}$ is a measurement of the point where light saturation dominates, or the minimum saturation level (Schreiber 2004). (Ik is also called Ek in some literature (Ralph 2005)) According to Ralph (2005), the initial slope of the curve " is proportional to efficiency of light capture. While photochemical quenching predominates before Ik, non-photochemical quenching dominates after Ik.

After the RLC reaches the peak value, any decline in ETR is related to down regulation and not photoinhibition (Ralph 2005).

The first measurement is taken with no actinic illumination and either dark adaptation or "quasi-dark adaptation". While the number of steps, the intensity of each step, the length of time for actinic illumination at each step, and whether one steps up or down can be variables in RLCs. Some fluorometers offer only a programmed routine that limit choices. The second measurement is usually low in the 10 to 50 umol range, and the last two steps are usually above saturation levels. Ralph (2005) recommends that saturation should be at or above 1000 umols. Low light leaves require a different intensity range for measurement than high light leaves. Intensities above 100 umols will commonly saturate low light leaves.

The length of actinic illumination is also programmable in some fluorometers. Times of 5 seconds, 10 seconds, 30 seconds, 40 seconds, 50 seconds and 60 seconds have been used. Ralph (2004) indicates that Rapid light curves should provide the shortest actinic light time possible to prevent plants from reaching steady-state or causing photoacclimation. The minimum length for actinic time at each step is governed by the time it takes for the saturation pulse fluorescence signal to relax according to Ralph (2005). High light leaves tend to relax faster than low light leaves according to Ralph. Ralph finds that 10 seconds works for the samples he has measured.

Ralph also shows that all RLC cardinal points, including ETR_{max}, change substantially with different actinic step times.

Since RLC vary significantly at different times of day because of different light history (Rascher 2000)., it is recommended that the data from several light curves, taken at different times of day, should be added together and then subjected to light curve fitting software (Rascher 2000).

Saturation Pulse Duration

The ideal saturation pulse duration is different for higher plants than for algae and cyanobacteria. The OS1p has a special software routine that provides the optimal duration value if the saturation flash duration is long enough to fully saturate PSII reaction centers. In previous generations, it was important to more closely match saturation duration with the plant type. For example, land plants require a duration of between 0.5 seconds to 1.5 seconds for correct measurement. Algae and Cyanobacteria require a duration time of 25 to 50 msec (Schreiber 1995). In the past, times that were too short would not allow the full saturation of PSII, even at the highest light intensities. Saturation pulses that were too long, would include a form of saturation pulse NPQ, that would lower the average saturation pulse intensity value, and produce an error. (Roseqvist & van Kooten 2006)

The OS1p has a software routine that provides the highest eight-point rolling average intensity value on the top of the saturation pulse peak. This means that it looks only at the

highest average eight points over a 25msec time frame. It also means that one setting can work well for higher plants, algae, or cyanobacteria.

What are the limitations of RLC?

The rapid light curve is affected by immediate light history and longer term light history (Rascher 2000, (Ralph 2005). It is also affected by time required to dark adapt (Rascher 2000), NPQ related to the previous light step (Herlory 2007), and in many cases the NPQ from the previous saturation pulse (Roseqvist and van Kooten 2005). These factors will be discussed below.

Rascher (2000) explored the value and limitations of Rapid Light Curves in detail and found that not only did the results from RLC and ETR_{max} change dramatically depending on the time of day that they were measured, but that they also provided different " and Ik information with different dark adaptation times. The slopes were found to be steeper with 30-second dark adaption than with 30-minute dark adaption. ETR_{max} was found to be the same with both dark adaption times. Since light history changes the results of RLCs, Rascher recommends making measurements at different times of day, combining the data, and then feeding it into light curve fitting software.

Ralph (2005) recommends a 5-10 second dark adaption time to measure the effects with no actinic light to drive photosynthesis. This allows for rapid reoxidation of Q_A without significant relaxation of non-photochemical quenching. Ralph also recommends the 5-10 second dark adaptation to prevent the deactivation of rubisco and to prevent a rubisco reactivation induction effect.

Related information in this area includes the following: Full rubisco deactivation takes between 9 to 18 minutes in Algae and up to 28 minutes in land plants (MacIntyre 1997). Full reactivation of Rubisco takes between 3 to 4 minutes for both algae and land plants (MacIntyre 1997). Consalvey (2004) found that far red light illumination used to activate PSI was very helpful in the complete reoxidizing QA in a short period of time, whereas dark adaptation took much longer on his samples.

Herlory (2007) found that the time of actinic illumination impacts results. Each successive RLC step adds non-photochemical quenching to the next step. He also found that the time used for actinic illumination at each step affects the repeatability of the results. The most repeatable results were achieved with actinic step times of 50 seconds or longer, and the lowest precision was found with 10 second times.

Roseqvist and van Kooten (2005) found that saturation pulses create a short lived NPQ that takes between 60 seconds and 120 seconds to fully dissipate, so if the actinic steps are shorter than that time frame, then each saturation pulse in the RLC will reduce the yield and ETR values as well.

Conclusion:

Even with these limitations, the literature supports that Rapid Light Curves offer a tool to investigate the saturation characteristics of plants. One must be careful in drawing additional conclusions.

Cookbook checklist before making Rapid Light Curve measurements.

Fm' is maximum fluorescence in a light adapted environment. F' is the fluorescence signal in a light adapted environment.

Y(II) is = (Fm' – Fs') / Fm' = ΔF/Fm'

relative ETR = Y(II) x PAR x 0.84 x 0.5

1. The first value is zero. Dark adaption times vary from momentary dark adaption times of five to ten seconds (Ralph 2005) to longer dark adaptation times (Rascher 2000). RLCs can provide different cardinal values depending on the time of day, and the dark adaptation time used. In other words, the results vary with light history.

2. Light history correction. Some researchers recommend taking RLC at different times of day, and overlaying the results to reduce the effects of light history (Rascher 2000)

3. RLC can be used on below canopy leaves in the field, algae and cyanobacteria. Light saturation rate, as measured by rapid light curves, highly correlates with the concentration and maximum activity of Rubisco (Macintyre 1997), (Macintyre 1996). Measured Steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997).

4. Y(II) and relative ETR values vary with light level and with temperature. The higher the light level, the lower the Y(II) value. When measuring Y(II) in the field, it is extremely important to measure leaf irradiation or light level at the leaf and leaf temperature. Comparing Y(II) values taken at different light levels and different heat 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 Clip. If a PAR clip is used, then a shroud must also be used.

5. Shade leaves vs. Sun leaves. – The Y(II) and cardinal RLC values will be different on sun leaves than on shade leaves (Ralph 2005). Most shade leaves will saturate at about 300 μmols, and most sun leaves will saturate below 1,500 μmols. (Ralph 2005).

6. When setting the steps in a RLC. Most should be at an intensity level below saturation level. At least one intensity value should be above saturation level. (Ralph 2005).

7. Steps can go up or down. (Rascher 2000).

8. Actinic light step time. These range from ten seconds, to about two minutes. Ralph (2005) uses ten seconds, while Rascher (2000) and Herlory (2007) found greater repeatability with longer actinic step times.

9. Residual saturation pulse NPQ. It takes one to two minutes for saturation pulse NPQ to fully dissipate. This means that there is some build up of saturation pulse NPQ with each actinic step that is shorter than one to two minutes. Saturation pulse NPQ reduces the Fm' value.

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. *The OS1p will automatically provide the optimal measurements for higher plants, algae and cyanobacteria as long as the duration is long enough.* Times outside these ranges increase the error in Y(II) measurements. 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 at either. 0.8 seconds, or 1.0 seconds for higher plants. 0.8 seconds is the default value on the OS1p and it will work well with almost all higher plants.

11. Saturation pulse intensity. Set it to 100% it will not damage the plant.

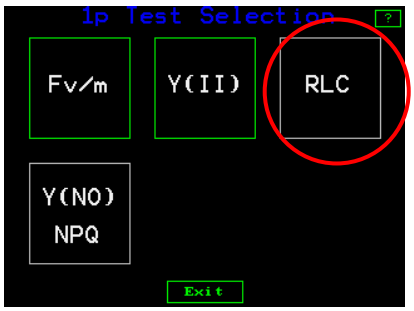
12. PSI fluorescence - Part of the fluorescence signal contains PSI fluorescence as well as PSII fluorescence. With RLC Y(II) and ETR, one is trying to measure variable fluorescence of PSII in non-steady state conditions.

PSI fluorescence is not variable, but the low fluorescent signal from PSI does overlap somewhat with PSII. This produces an a small error. This is not a problem for comparing similar samples, because PSI fluorescence does not change with light intensity temperature or plant stress.

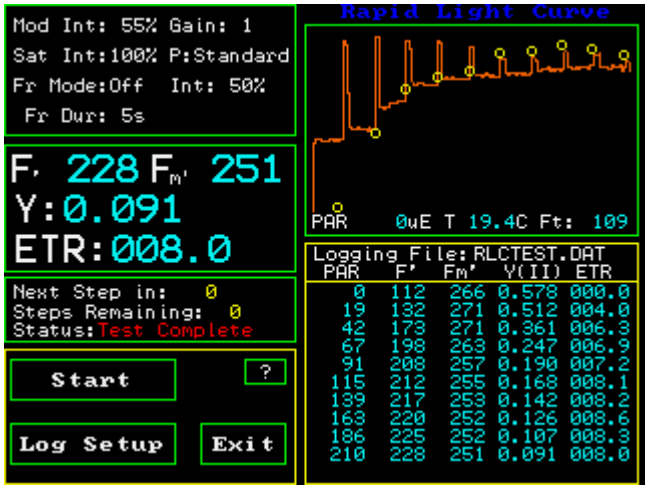
PAR is photosynthetically active radiation. Radiation on the leaf is measured between the wavelengths of 400nm to 700nm. PAR sensors and thermisters for measuring temperature are calibrated to other instruments that are traceable to the NIST. It is recommended that recalibration should occur every two years. Most modern sensors are solid state, so drift is minimal. Measuring PAR is better than assuming intensity values for different steps. ETR values can change dramatically for different PAR values.

Running the RLC Test

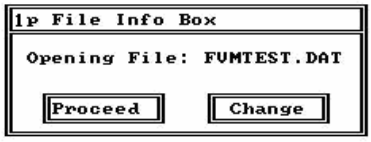
From the main screen select Test Menu by pressing the touch screen. The Test Selector screen will appear. Press the “RCL” button.



The “RLC” measuring screen will appear



RLC Measuring Screen



small screen

second small screen

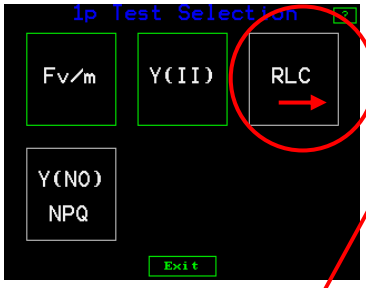
As with the Fv/Fm test, the last file used for this type of measurement will open up, and a small screen will appear within the larger screen. It will show the file name and ask if the if one wants to “Proceed” to measuring with this file, and the parameters that have been set. The other option is to create a new file with “Change”.

When “Proceed” is pressed, a second small window appears with three choices. “Appnd” adds measurements to the existing file, “Cncel” brings one back to the main menu, and “OvrRt” will overwrite the previous measuring file with existing measuring parameter settings.

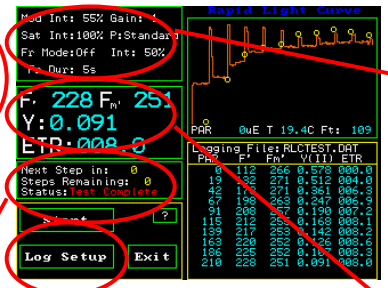
Due to variations in RLC measuring protocols, as well as variations in sun and shade leaves, it is not recommended that you use default settings for RLC measurements.

Drill down menu for RLC

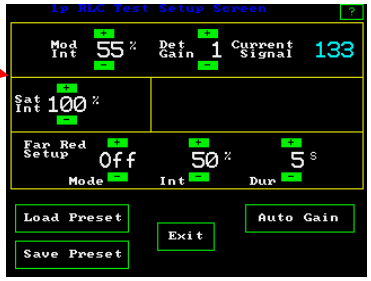
Press windows to go to other screens



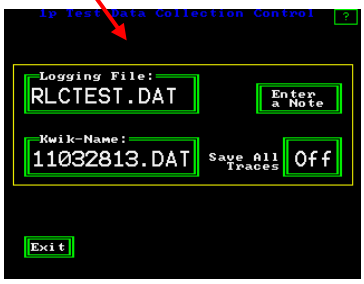
Test Menu



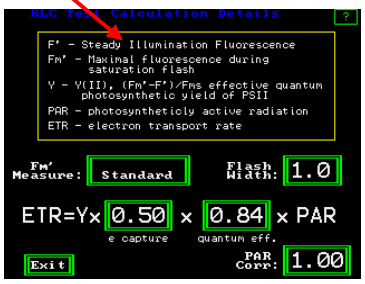
RLC Measuring Screen



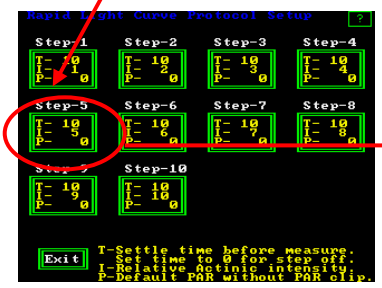
Test Setup Screen



Log Set Up Screen



ETR Test Calculation Screen



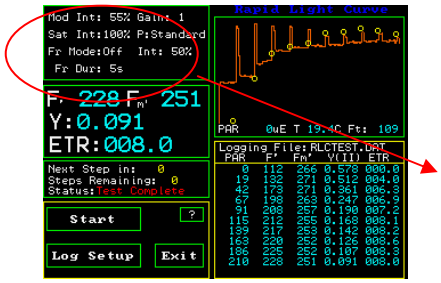
Step Set Up Screen

Individual Step Set Up Screen

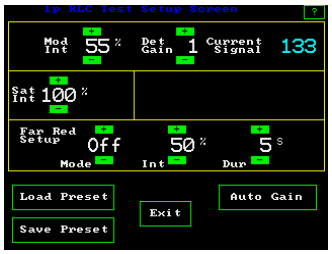
Saving measuring parameters

Changes that are made to the “RLC Test Set Up” screen, the “RCL ETR Test Calculation” setup screen, and the “Step Set Up” screens can all be saved in a single measuring file by pressing “Save Preset” on the RCL Test Set up screen. The changes from other screens may be done in any order, and saved from this screen when complete. Follow the directions listed above to save measuring files.

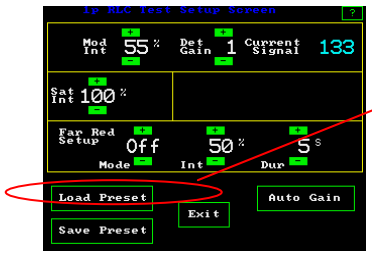
To up load previously created parameter sets and measuring files, follow the directions listed below.



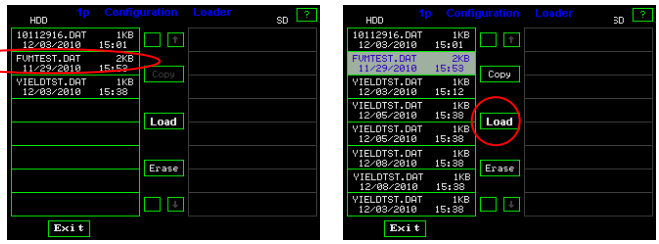
RLC Measuring Screen



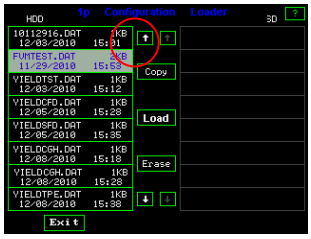
RLC Test Setup Screen



RLC Test Setup Screen



Configuration Loader Screens



Loading preset measuring parameter files –

Highlight test by touch, and press load.

Arrows for scrolling appear when there are more tests than can be viewed on the screen.

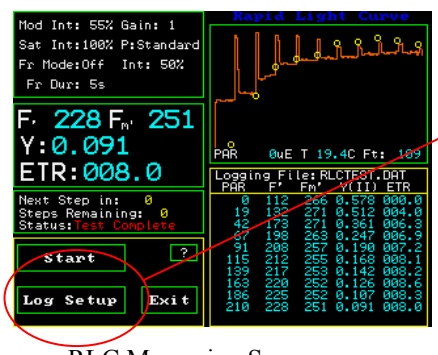
Copy only works when an SD card is inserted into the side of the OS1p.

Loading and saving preset measuring routines

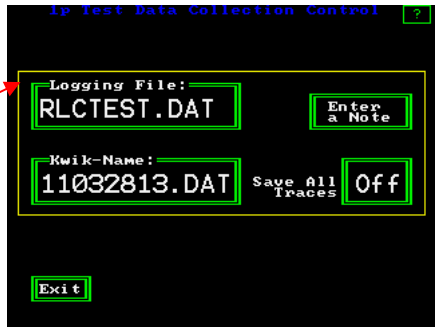
When the parameters have been adjusted for a specific set of conditions, the test setup can be saved by pressing “Save preset” while in the “Test setup” screen. A pop up appears asking if you want to make a new file or use an existing one. Next a key board screen will appear if the new option is selected, and the test set up can be named and it is saved when “Ent” is pressed. To load previously created tests, press “Load preset”. Press the test of interest. After it is highlighted in light blue, press load. To erase a test, highlight the test and press “Erase”. The number of tests that can be saved is almost unlimited. Use the up and down arrows to scroll through the tests if there are more than are shown on the screen. “Exit” if done, or “Exit” if there is no action.

Copy presets parameters into a new measuring file

Insert the SD data card into the SD card port on the OS1p, and press copy while the “1p Configuration Loader” screen is open. Presets can be stored on the SD card for future use or transferred to a PC. A preset is different than a measuring file. A Measuring file, or Data file, contains presets, data and saved traces. A preset file only contain parameter presets and no data. To store the preset in a new measuring file, load the preset from the configuration loader screen or create the “Preset”. Press “log setup” from the main measuring screen and change the “logging file name”. The presets will be logged to the new file name.

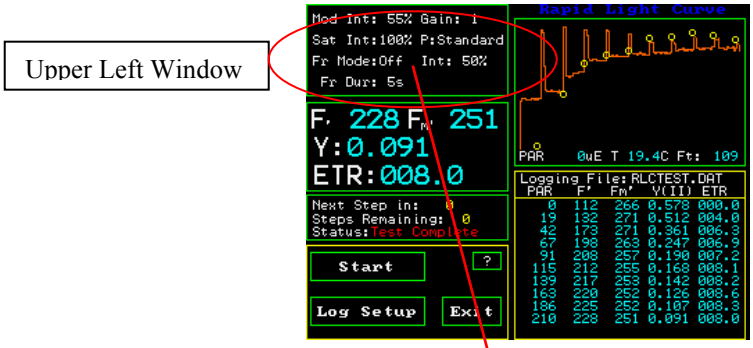


RLC Measuring Screen



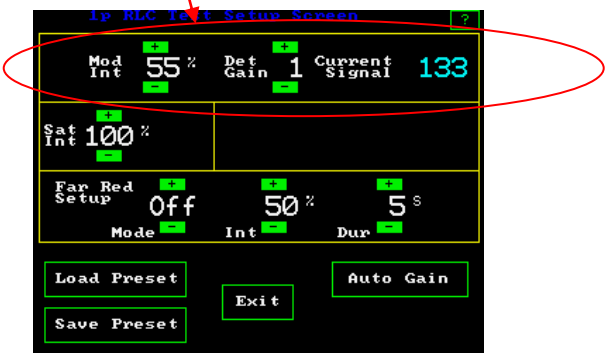
RLC Log set up Screen

Change RLC measuring parameters -



RLC Measuring Screen

One can change many of test parameters by pressing the upper left window on the screen.



RLC Test Setup Screen

Screen revealed by pressing the upper left hand window.

Setting modulation light source intensity. It is adjustable between 0.1 µmol and 1 µmol when using an open body clip, a PAR Clip, or a dark clip. The settings range from 5% to 100% in 5% increments. The correct setting depends upon the distance from the sample and the nature of the sample. The optimum setting for RLC is not as critical as it is for Fv/Fm and quenching measurements if momentary dark adaptation is used as in Ralph (2005), but it is important if true dark adaptation is required, as in Rascher (2000). It must, however, be high enough to make a measurement and prevent the "Fluorescence signal too low" error.

Put a leaf, of the type to be measured in a study, into the PAR Clip or open body clip, or dark clip, and place the fiber optic probe all the way into the PAR Clip or Open body Clip, or dark clip.

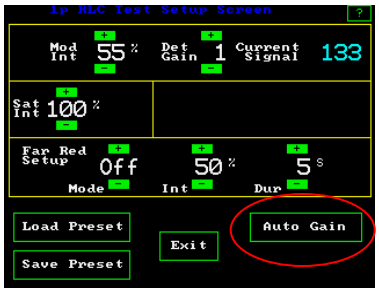
The modulated light is always on when a measuring screen is viewed.

When adjusting the modulated light for momentary dark adaptation, the modulated light is set correctly, if it is set high enough to get a reading when the measuring button is pressed. The setting is linear. *The "Gain" should be as low as possible for the best signal to noise ratio.*

When adjusting the modulated light for longer dark adaptation, use the following procedure. Put a leaf, of the type to be measured in a study, in a dark clip, and dark adapt. Place the fiber optic probe all the way into the dark clip, and expose the dark adapted area of the leaf to the modulated light. The modulated light is always coming out of the end of the fiber bundle when a measuring screen is viewed. The modulated light is set correctly if it is high enough to get a fluorescence measurement when the measuring button is pressed, but not too high so that it is driving photosynthesis. A setting that is too high will give an incorrect reading of Fo, a value that should be a pre-photosynthetic measurement. The setting is linear. The "Gain" should be as low as possible for the best signal to noise ratio.

New -automated modulation light set up

"**Auto Gain**" – On the set up screen. This option automatically adjusts the modulated light setting to a detection range that will work for the sample. Press "Auto Gain" and the instrument automatically goes through a routine that sets the modulation intensity correctly. A leaf must be in the PAR Clip, open body clip, or dark clip when this routine is running.



To use the automated modulation light setup mode, press "Auto Gain". The system then adjusts the modulation light source into an acceptable minimum reading range for the PIN diode detector.

Yield Test Setup Screen

Setting the saturation pulse intensity

Values can be set from 5% to 100% in 5% increments. At 100%, the intensity of the saturation pulse is approximately 11,000 umols when used with a dark clip. Saturation intensity is approximately 6,200 umols when used with a PAR Clip or open body clip.

Setting saturation pulse duration

Duration is the time that the saturation flash is turned on. For higher plants, times of 0.5 to 1.5 seconds have been used. For algae and cyanobacteria, the optimal saturation pulse duration is 25 to 50 msec (Schreiber 1995).

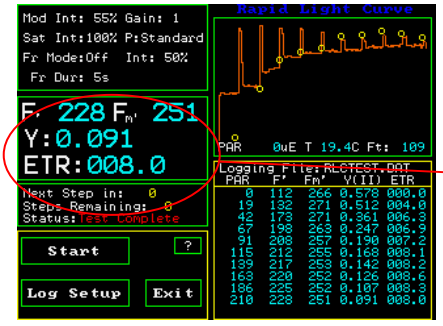
Automatic routine for setting of the saturation pulse duration

The OS1p will always measure the optimal duration time as long as the duration is set long enough for a particular type of plant. The instrument has an automated software routine that always takes a rolling average of the top continuous eight points on the top of the saturation pulse. This represents a time duration of twenty five milliseconds. As a result, this automated routine works for higher plants, algae, and cyanobacteria if the duration is set long enough.

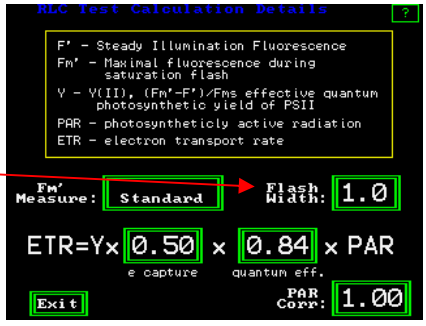
Manual setting of the saturation pulse duration

of the saturation pulse. The default value is 0.8 seconds and works well for most higher plants tested. If the duration is not long enough, then PSII will not be fully saturated at any light intensity, and Fm values are reduced. On most modulated chlorophyll fluorometers (but not the OS1p), if the duration is too long, the saturation pulse top becomes subject to a type of NPQ that rounds the tailing edge of the saturation peak and also reduces the average Fm value. The Ideal saturation pulse top is flat on the top with the trailing edge rounding minimized (Roseqvist and van Kooten 2006).

The saturation pulse duration is adjustable between 0.1 and 2.0 seconds. To adjust the duration, touch the upper right graphic display window of the Yield protocol measuring window. Since the automated routine is always running on the OS1p, it also works for algae and cynanobacteria, even when the duration is set longer.



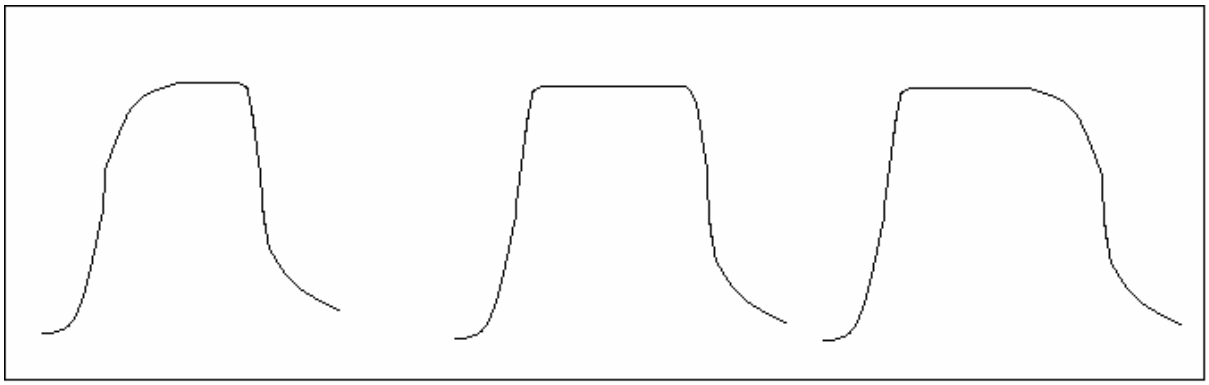
RLC Measuring Screen

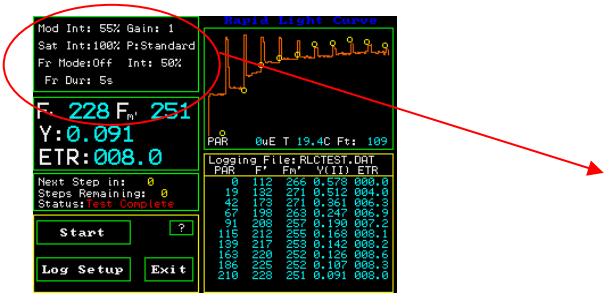


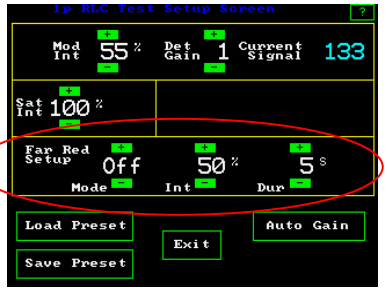
ETR Test Calculation Screen

By pressing the **Flash Width** button one goes to a keyboard screen and allows the saturation pulse duration to be adjusted between 0.1 and 2.0 seconds. Press “Ent”

With the OS1p, the curve in the center or on the right will provide optimal results.







RLC Measuring Screen

RLCTest Setup Screen

Far red light

can be used with the internal illuminator for the RLC test to allow the continued reduction of PSI and the re-oxidation PSII. Options are offered for off, on, and pre. “Off” turns the far red light off during the test. The “Pre” setting allows illumination of the sample with far red light before the test, and the “On” position keeps the far red light on during the test. Intensity is again adjustable between 5% and 100% with 5% increments. The duration setting is used with the pre setting, to allow pre illumination with far red light from one second to sixty seconds. Sun light contains far red light, but the LED actinic light source filters out far red light when used as an actinic source.

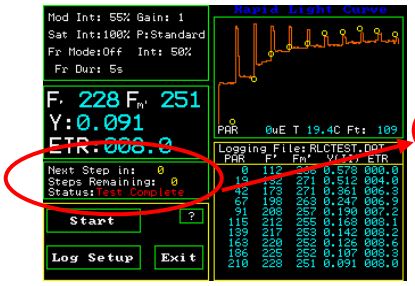
Default PAR values can be entered into each step by pressing the third window on the left of the screen shown above. Next press the “step window” of interest, and enter the PAR value for “Step Default PAR Value”. (Default PAR measurements are estimates of PAR value, or measurements made without the use of a PAR Clip) When a PAR Clip is attached to the OS1p it overrides “Default PAR”.

When the PAR Clip is used

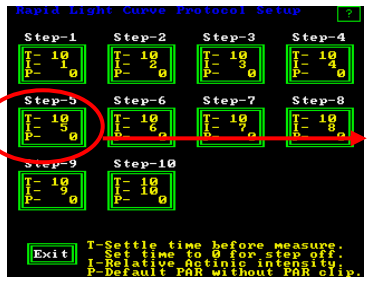
The PAR Clip is an Optional accessory but it is highly recommended. Make sure that the fiber bundle is inserted all the way into the hole provided on the PAR Clip, and lock the locking screw. If the PAR clip is being used, then the measurement may be triggered by pressing the black button on the bottom of the PAR Clip, the measure button on the touch screen, or the red button on the end of the fiber optic probe. Y(II) varies with most types of plant stress, but it also varies with light level and temperature. For that reason, the PAR Clip measures both variables and records the information in the measuring file with Y(II) and ETR. When the PAR clip is attached to the fluorometer, it overrides "default PAR" values. A shroud must be used to dark adapt the sample when a PAR Clip is used.

The instrument default setting is off for this feature. By using the + or – button, the feature can be turned on. The "Step Actinic Intensity" can be set between from 0% to 100% in 1% increments 100% is approximately 1,500 µmols with a PAR Clip. The actual intensity is recorded when the PAR Clip is used. Shade leaves usually saturate at about 300 µmols, while most sun leaves saturate below 1,500 umols. Most steps should be lower than saturation with one step at or above saturation levels(Ralph 2005). It is common for "Step Actinic Intensities" to be set at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 for shade leaves. Higher values are required for sun leaves.

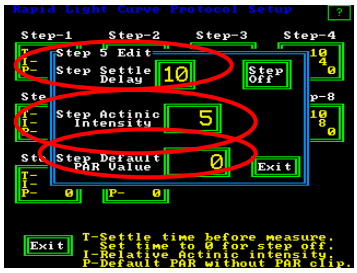
For Y(II) and ETR measurements, the "Step Settle Delay", or the time the actinic light is on for each step, should be set from ten seconds to two minutes before measurement. When done, one can exit or press save preset. The intensity of the pre-actinic light is held constant with in a few µmols by the PAR Clip to ensure steady state photosynthesis. This feature is great for the Lab.



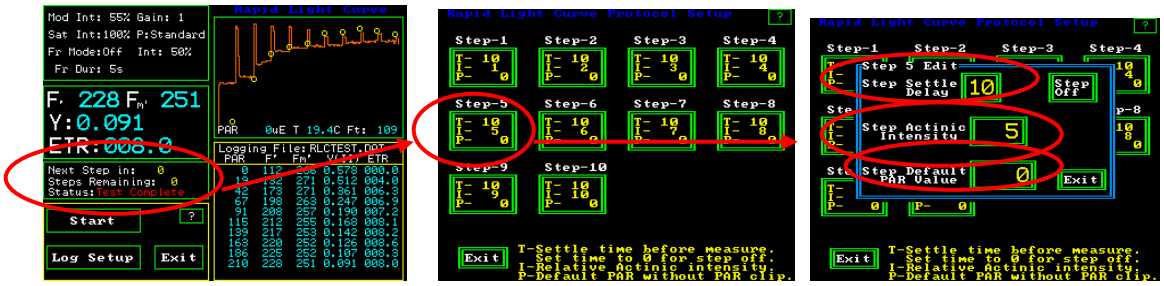
RLC Measuring Screen



Step Set Up Screen



Individual Step Set Up Screen



RLC Measuring Screen Step Set Up Screen Individual Step Set Up Screen

When a PAR Clip is not used “Default PAR”

When a PAR Clip is not attached to the OS1p, the user may select the approximate pre-illumination intensity. From the RLC measuring screen, press the third window down from the top on the left had side to get to the “Step Set Up Screen” shown above. Next, press each step button that will be used in the RLC and enter a measured “Step Default PAR Value”. When the “Step Default PAR Value” is pressed, a key board screen will appear. “Default PAR” can be set from Zero to 2465 µmols. OSI makes no claims about the accuracy of “Default PAR” values. The actual value should be read with an independent PAR meter at the leaf plane and leaf angle. When “Default PAR” is used, all measurements of ETR appear using the setting selected. When a PAR clip is connected to the OS1p, the “Default PAR” setting is automatically deactivated.

Multi-flash vs. standard single flash saturation pulse

Multi-flash ("Step Int Lin Regr") -*measuring yield at high actinic light levels.*

The multi-flash option has been added to correct for the inability of very intense saturation light pulses to completely close reaction centers under high actinic light conditions. It is thought to be caused by mechanisms responsible for non-photochemical quenching. Research has found that under high actinic light conditions, measured electron transport rates (ETR) and yield values did not correlate well with carbon assimilation measurements. The inability of very intense light saturation pulses, of any intensity, to close all reaction centers, was thought to be the culprit.

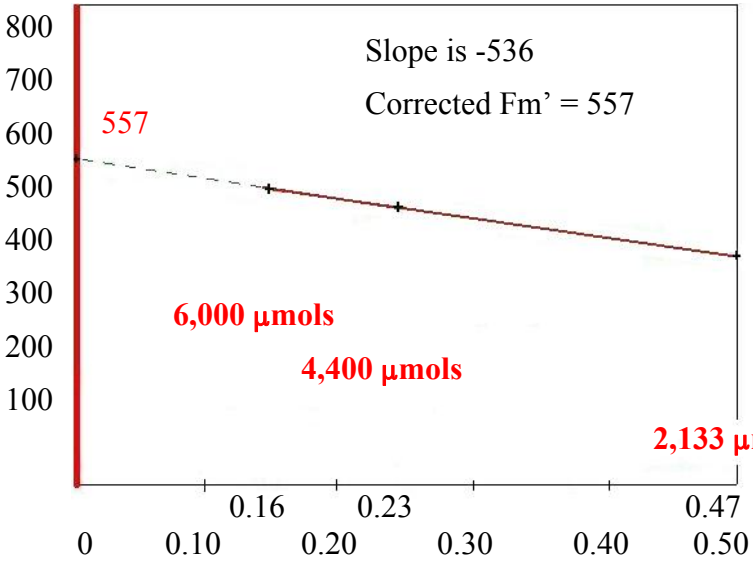
By using multiple saturation light pulses of varying intensity, and linear regression analysis, an infinite saturation pulse intensity can be estimated. It was found by Earl (2004) that by using multiple intense saturation pulses at varying intensity levels, one could use regression analysis to estimate the intensity of an infinite saturation pulse. Testing was done on corn (C_4 plants), and on cotton plants (C_3 plants) and it was found that in non-photo-respiratory conditions, the linear relationship between ETR and gross CO_2 assimilation was restored by using the Fm' or saturation pulse correction method.

This option should be considered for yield and ETR measurements when actinic light levels are high. When used, The Fm' value displayed on the graphic screen represents Fm' at infinity. The R^2 value is also displayed on the graphic screen. Saturation pulse intensities used in the multi-flash mode are preset at the factory. When used with a PAR Clip, they are at 2,133 µmols, 4,400µmols, and 6,000 µmols. The total flash duration that includes all three values is 1.0 second.

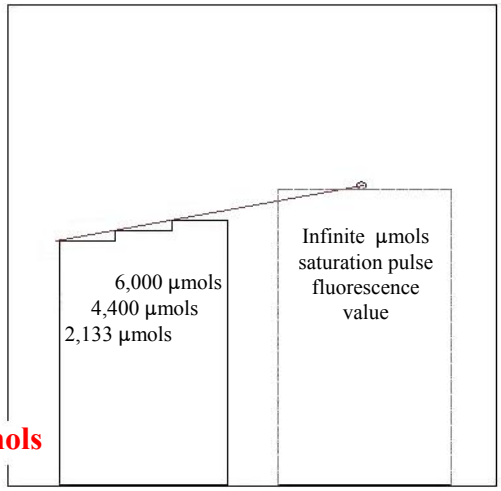
Since the Earl work, Bernard Genty the man that developed quantum photosynthetic Yield of PSII, back in 1989, has co-authored a poster that supports the work of Earl and the use of regression analysis for correction of yield and ETR values. A Licor research team of team of Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. that also included Bernard Genty of Cadarache DEVM-Laboratoire d'Ecophysiologie Moleculaire des Plantes of the University of Marseille, studied the issue. They found that in Z. mays, with the standard saturation pulse method, in high light field conditions, measurements produced up to a -41% error in ETR values, and up to a -22% error in Y(II) values . The research recommends the Multi-phase flash process for field measuring work. The research is titled "Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity". (Loriaux S.D., R.A Burns, Welles J.M., McDermitt D.K. Genty B. (2006) "Determination of Maximal Chlorophyll Fluorescence Using A Multiphase Single Flash of Sub-Saturating Intensity". Abstract # P13011 August 1996. American Society of Plant Biologists Annual Meetings, Boston MA).

Multi-flash Method

Regression Analysis Graph



Representation of how the flash works



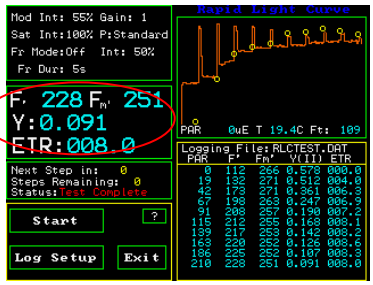
Values represent (1/PAR) x 1000

Linear regression analysis is used to estimate true Fm' using an infinitely intense saturation pulse according to Earl (2004) y=mx+b

Three saturation pulse flashes are fired in succession for 0.33 seconds. The firsts is at 2,133 µmols, the second is at 4,400 µmols and the third is at 6,000 µmols

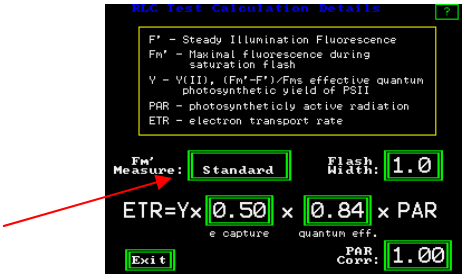
Hugh Earl 2004 – At high actinic light levels, Fm', the fluorescence value measured by a modulated chlorophyll fluorometer to determine saturation level, is underestimated due to nonphotochemical quenching effects. To saturate PSII, and achieve the expected correlation between carbon fixation and Y(II) measurements, a linear regression analysis is performed. Saturation pulse fluorescence intensity values for one third of full intensity, two thirds of full intensity, and at full intensity, along with the corresponding PAR values, represented as (1/PAR) x 1000, are into the equation y=mx+b. The "y "intercept represents the saturation fluorescence value with an infinite PAR saturation flash.

The Multi-flash option may be turned on or off in the RLC Protocol by pressing the second window down from the top on the left had side of the RLC measuring screen.



RLC Measuring Screen

Under "Fm' Measure" select **"**Step Int Lin Regr". For using the standard single saturation pulse method select "Standard".



ETR Test Calculation Details Screen

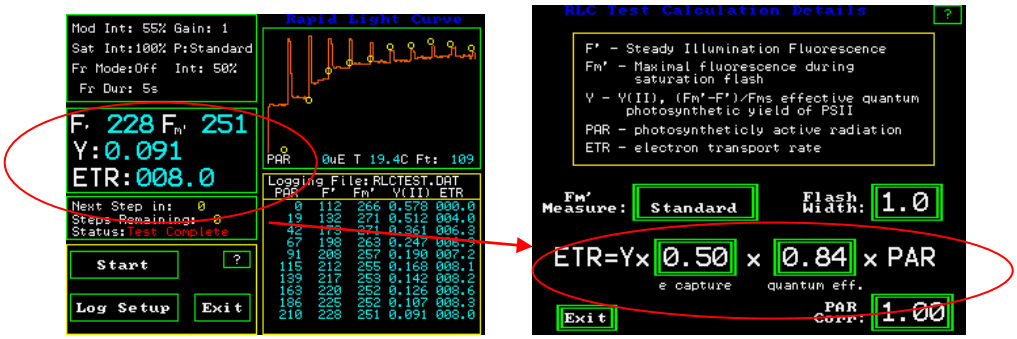
ETR Test Calculation Screen

Relative Electron Transport Rate

Relative Electron Transport Rate - ETR µmols = (Yield of PSII) (PAR) (.84) (.5)

(quantum photosynthetic yield of PSII) (measured Photosynthetically Active Radiation measured in µmols quanta m^{-2} s^{-1}.)(leaf absorption coefficient)(fraction of absorbed light by PSII antennae)

Average plant values are used in the standard equation. 0.84 is a good average value for many species of plants (Bjorkman and Demming, 1987). Research has shown that the leaf absorption coefficient can vary between 0.7 and 0.9 (Eichelman H. 2004), with species, chlorophyll content, and water content. Research has also shown that the fraction of light that is absorbed by PSII varies by species and can range from at least .40 to .60 (Laisk and Loreto,1996). Even if the default average values are used, ETR can provide useful relative comparative information between different samples and the same sample under different conditions.



RLC Measuring Screen RLC ETR Calculation Details Screen

For more exact ETR values, the user can refer to papers by (Eichelman H. 2004) (Laisk and Loreto,1996) (Edwards & Baker 2002). The ratio of PSII reaction centers to PSI reaction centers change with plant species and type. The number of PSII reaction centers tends to be lower in C_4 plants than in C_3 plants (Laisk and Loreto,1996).

The ratio of PSII reaction centers can be changed in the equation, on the OS1p, by pressing “e capture” and using the key board screen. Similarly, the over all leaf absorption can be changed by pressing “quantum eff”. This correction is not needed for most relative comparison ETR applications, however, it has been made available for more exacting work when necessary.

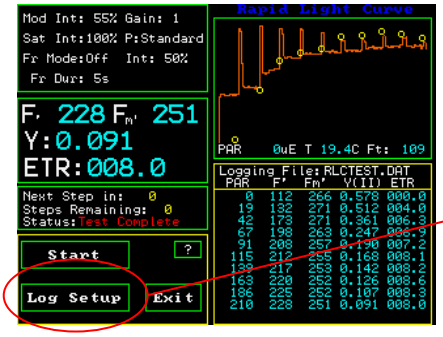
“PAR Corr” is for correcting PAR sensor location error according to Rascher (2000). When some artificial light sources are used, Rascher found that the location the PAR sensor relative to the leaf surface can cause an error of up to 10%. This error is insignificant if sun light is used due to the much greater distance from the light source. Rascher used an independent PAR sensor and measured the intensity at the leaf plane. He then made corrections due to PAR Clip sensor location, by comparing the differences between the PAR clip values, and the leaf plane values. This correction may not be needed for most relative comparison ETR applications, however it has been made available for more exacting work when necessary.

Notes: When using the blue modulated light it was found that the average absorption value should be set to 0.94 instead of 0.84 as is used by the red modulated light.

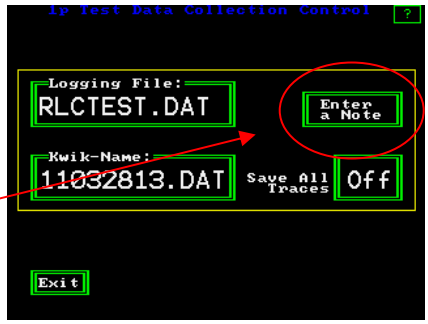
By plotting ETR vs. PAR, potential ETR rates, photosynthetic capacity, and ETR rate limitations, can be determined at given light intensities. (U. Schreiber 2004). Note: Four electrons must be transported for every CO_2 molecule assimilated or O_2 molecule evolved.

It has been found that Rapid Light Curves are a better solution in a variable light environment. Light saturation rate, as measured by rapid light curves, highly correlates with the concentration and maximum activity of Rubisco (Macintyre 1997), (Macintyre 1996). Measured Steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997).

Entering a note with a measurement



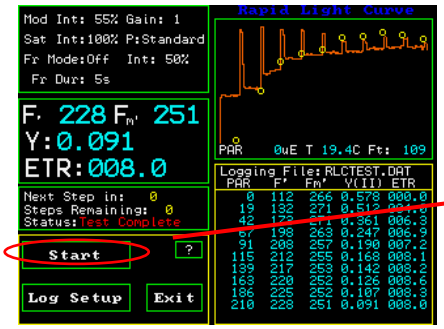
RLC Measuring Screen



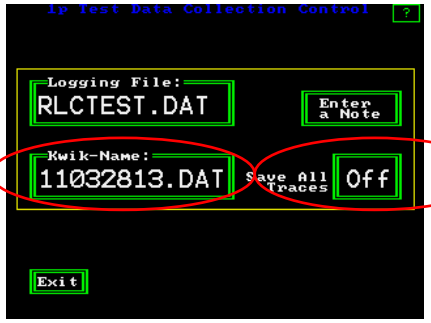
RLC Log set up screen

It is now possible to enter a note with any RLC. To enter a note, press the “Log Setup”: button. Next, press “Enter a Note”. A touch keyboard will appear, and one can enter up to a 38 characters note with each measurement.

Other RLC function buttons



Y(II) Measuring Screen



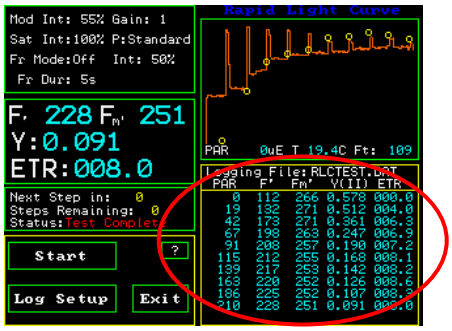
RLC Data Logging Screen

Kwik-Name – the “Logging File” name is the file name. “Kwik-Name” allows one to quickly create a new file name without typing in the characters. The name is a code that represents the year -10, the month -11, the date 29, and the hour – 15 (24 hour clock). This is especially valuable for people making measurements in high volume that want to change the file name for a second field or sub set. The parameters stay the same as the existing file, and the code allows easy tracking where appropriate. Press the “Kwik-Name” button and the Kwik name becomes the “logging file name” moving forward.

Save all Traces on the RLC Data logging screen, saves all trace with the data file. It is not saved normally, unless it is turned on.

Start– Measurements can be made by pressing the start button on the RLC measuring screen, or the red button at the tip of the fiber optic cable, or the black button on the bottom of the PAR Clip.

Measurement review



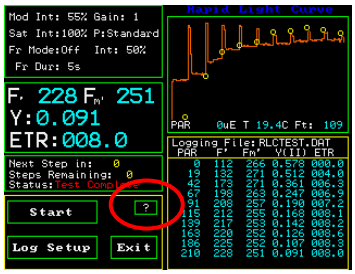
RLC Measuring Screen

On the main RLC measuring screen, the lower right hand side window logs all of the measurements made, in the current measuring session, for the current file name. One can scroll through measurements by touching the screen. If one touches the top of the review area window, the earliest measurements may be reviewed. If one touches the bottom of the review area window, then the latest measurement can be reviewed. The session remains current as long as the file name remains the same, and one does not leave the Y(II) protocol. It will stay intact if one goes to different screens within the Y(II) protocol. If one turns off the instrument, changes the file name, or goes to a different protocol, then the session is ended.

After a session is ended, data, and traces may still be viewed by going to the main menu, selecting “Data Management”, highlighting the file of interest with your finger, and pressing view. If a trace is saved with the RLC, the trace may be seen by using the Line up and down arrow keys to go to the last measurement of a RLC. It there is a trace saved, a low resolution trace will appear in the lower left hand corner of the screen. For more information, go to the “Data Management” section of the manual.

Measuring data and traces are also retained with the current file name in the measuring file, and it may be viewed with Excel on a PC computer. It does not disappear like the measurement review session data in the window.

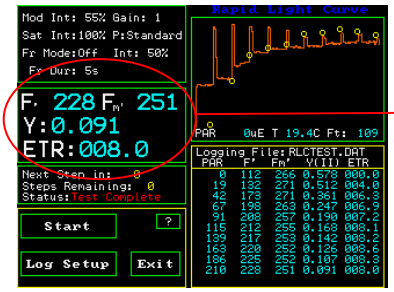
Help screen



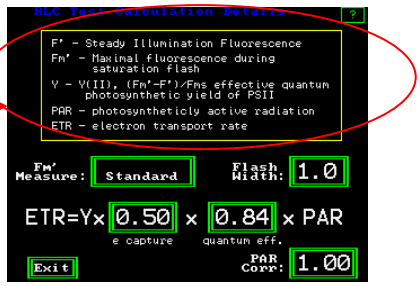
Y(II) Measuring Screen

The Question mark button is a help screen that provides basic instructions for the screen shown. It also allows one to capture the existing screen image in memory for later retrieval on a PC.

Basic definitions of Parameters may be viewed by touching the window shown below in the left hand picture. Of the RLC measuring screen.



RLC Measuring Screen



RLC ETR Calculation Details Screen

Error messages common to the test modes:

There are several common errors that can occur in the test modes.

If the battery voltage is 10.5 or less, the warning message “!! Battery Low !!” will appear and a long beep will be sounded.

If the amount of in-band IR radiation is above set limits thereby saturating the pre-detector the error message “!! IR to High !!” will be displayed.

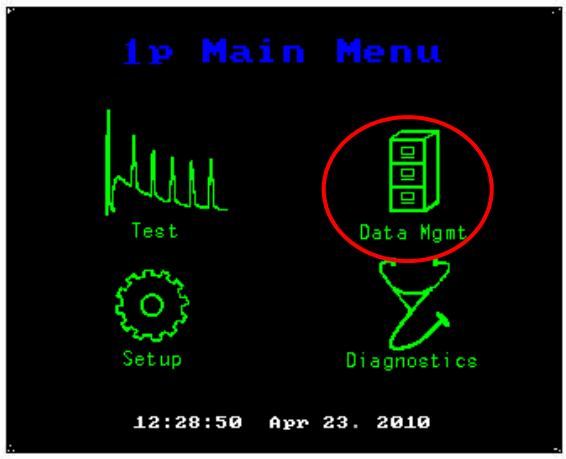
“Fluorescent signal too low” appears if there is not enough signal getting to the detector. This may be due to the fiber optic being too far from the sample, the intensity of the modulated source being too low or the gain control is set too low.

“FL > 3600 pos det pin out” appears if the combination of the modulated light intensity and modulated light gain are set too high. To fix the problem go to Auto Gain on the setup screen, put a leaf in the PAR Clip, and press Auto Gain. The modulation intensity will adjust automatically.

Chapter 4 • OS5p Data Management

USB data transfer

Plug the USB cable into the OS1p and into your computer. It may be off, or on. Go to the Main Menu and press the “Data Mgmt” image.



Main Menu

A Screen will appear that says:

USB Remote PC Access Mode V1.0

The OS1p is now connected to your host PC. Ready to transfer data or update files. Use the PS eject feature to stop drive access. Do not disconnect the USB cable when data is being transferred or the file may be lost or corrupted

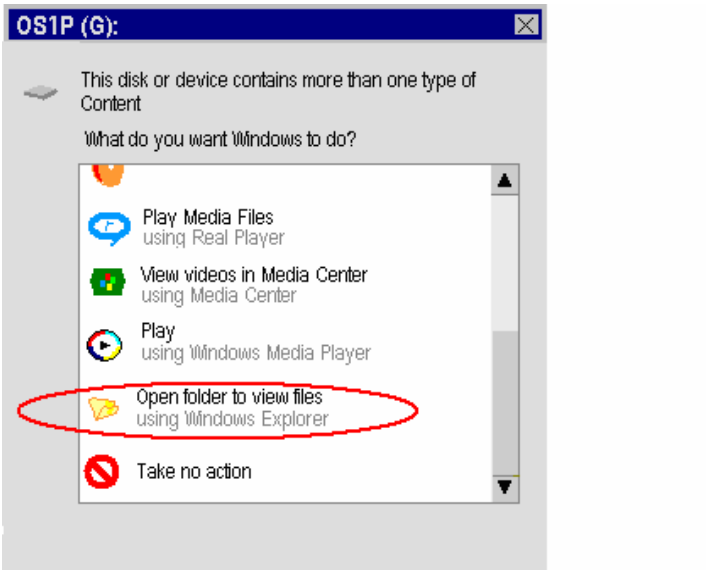
File transfer by USB cable

Turn on the OS1p and press “Data Mgnt”. Next, plug in the USB cable provided to a PC and the OS1p.

When the PC and the fluorometer are connected, the fluorometer screen will display a message titled: USB Remote PC Access Mode V1.0 and a green colored message.

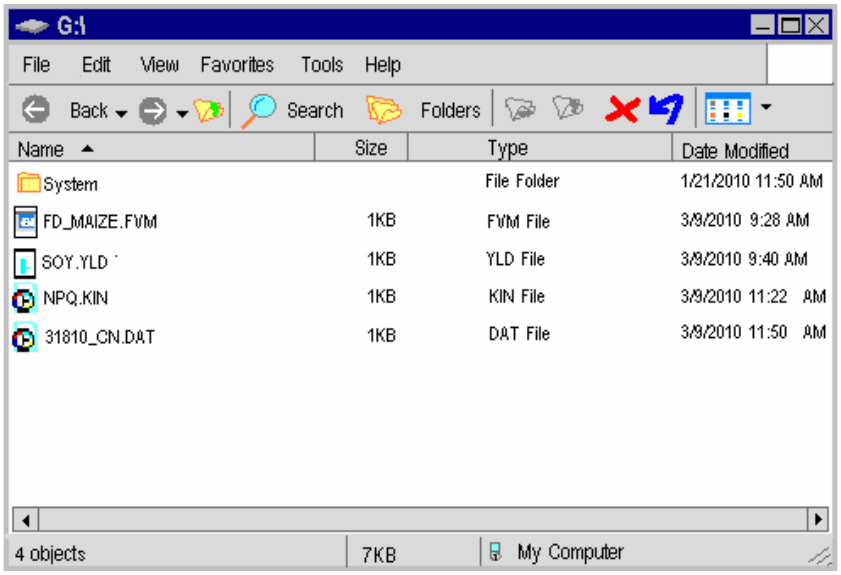
Under these conditions, the fluorometer becomes a removable drive for the PC. The PC screen is now used to transfer files, not the OS1p.With many versions of the Windows operating system, a small window appears on the PC screen that should look something like the one pictured below. If it does not appear, then go to the page after the next one..

Select the “Open folder to view files” option.



Microsoft Windows Screen on a PC

After clicking on “Open folder to view files”, the following window will appear.



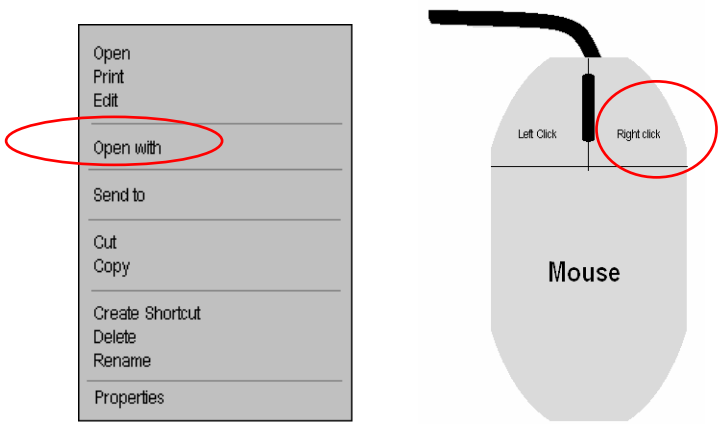
Microsoft Windows Screen on a PC

From this window, the various files may be opened using Excel for viewing, analysis or graphing. (The "System" folder is the software that is used to run the fluorometer. If updates become available this window can also be used to update software with an e-mailed link. *Special instructions are available Update software. Call 603-883-4400)*

Do not manipulate or change the system file or the system file name.

Files that end in .DAT are data measuring files. Files the end in FVM, YLD, KIN, or some other suffix are measuring recipes.

If the file will not open in Excel, highlight the file of interest using a mouse, and right click the file. A small window will appear like the one below. Select the option "Open with…", and select Excel (It is also recommended that the file be saved on the PC as an Excel file).



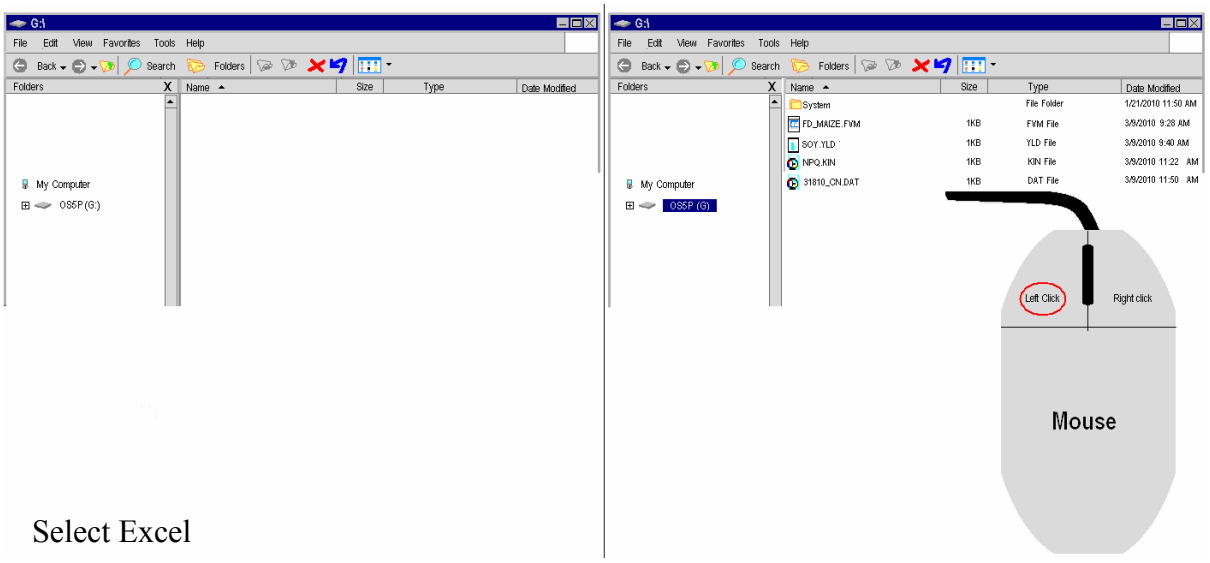
Microsoft Windows Screen on a PC

If the removable drive window shown above does not appear on your screen, then follow the directions listed below.

Turn on the OS1p and press “Data Mgnt”. Next, plug in the USB cable provided to a PC and the OS1p.

When the PC and the fluorometer are connected, the fluorometer screen will display a message titled: USB Remote PC Access Mode V1.0 and a green colored message. Under these conditions, the fluorometer becomes a removable drive for the PC. The PC screen is now used to transfer files, not the OS1p.

Open Windows Explorer, and click on “My Computer”. The image should look something like the one shown below. The OS1p drive may be listed as OS1P and the appropriate new drive letter designation. Below the drive is listed as G, but it may be any letter. In some cases, a new drive will appear without the OS1p designation. In this case, left click on the new drive and the files that are currently present on the OS1p will appear as shown below. These files may be opened in Excel for analysis and graphing. If they do not automatically open in Excel when clicked, right click the file and select “Open with…” and choose Excel (see the next page).



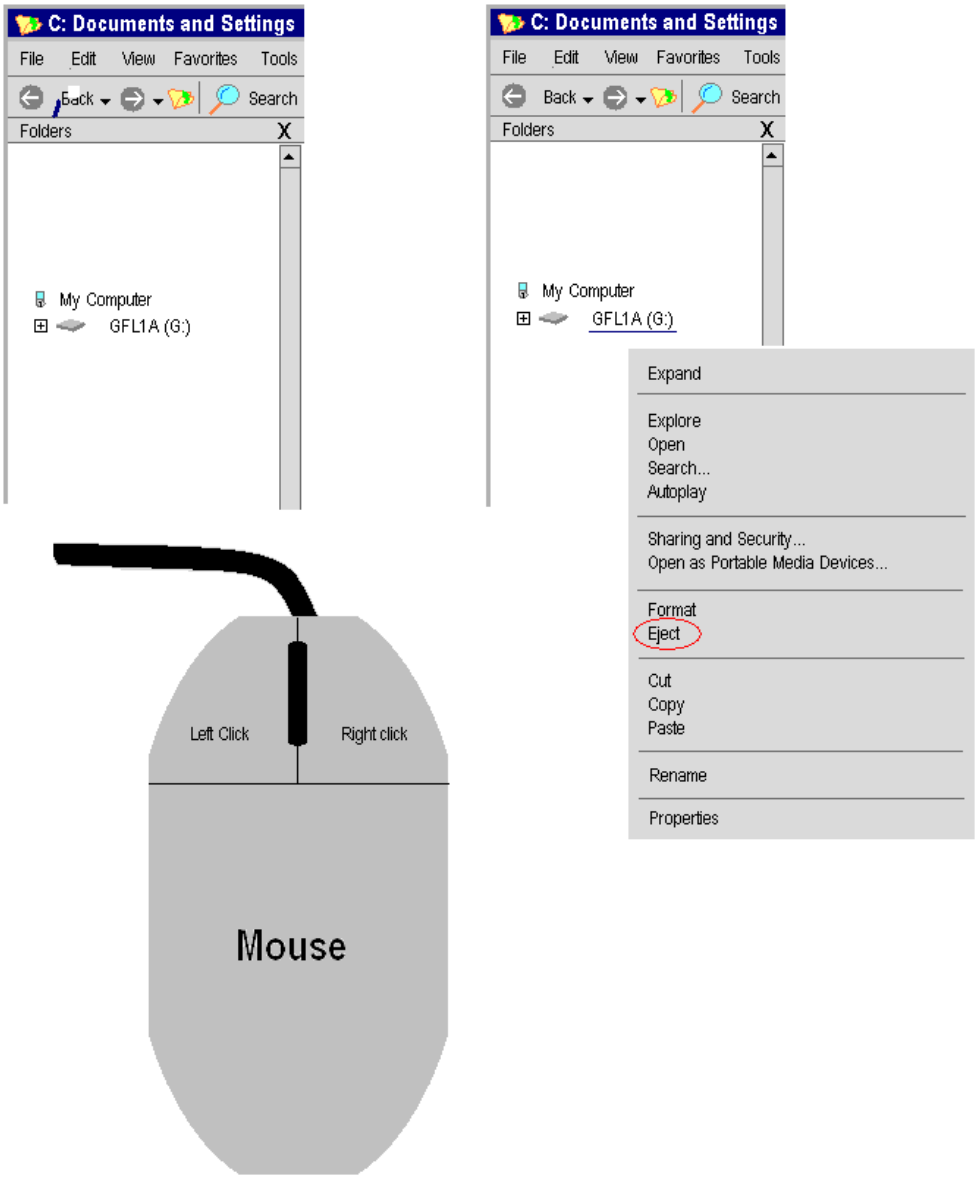
Microsoft Windows Screen on a PC

Microsoft Windows Screen on a PC

Ejection process

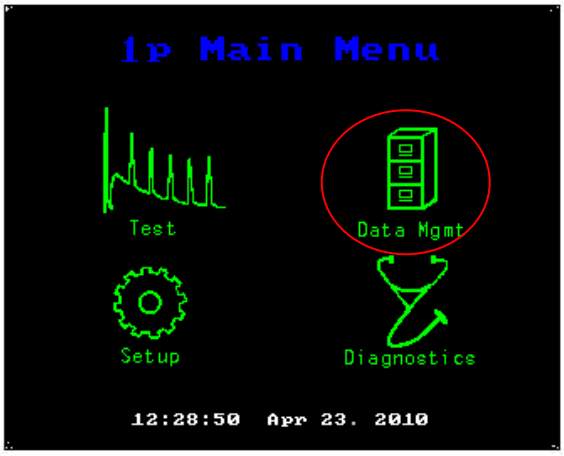
The Eject option may be used as listed below, however, in our experience it is not necessary as long as the file has been completely transferred. Do not disconnect the cable until file transfer is complete.

Microsoft Windows Screen on a PC

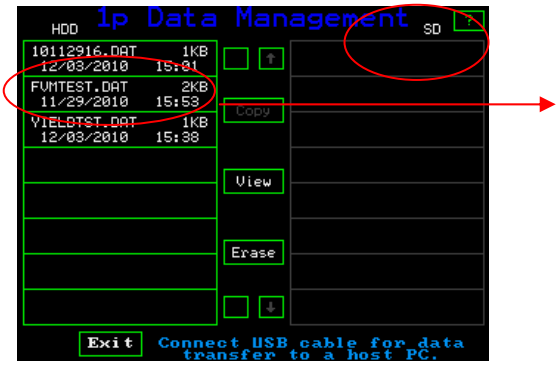


When done, there is usually a double beep sound from the OS1p, and a message appears on the OS1p screen that says that “Disconnect is successful. Unplug the USB Cable to continue.

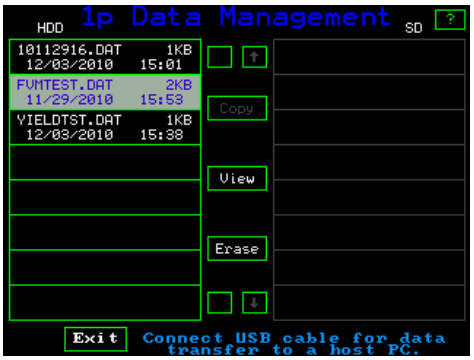
File transfer by SD Card and Data Management Utilities



Main Menu



Data Management Screen



File selected by touch.

The Main Menu appears a few seconds after the OS1p is turned on. Press the "Data Management", an the **Data Management Screen** appears.

"HDD:" is for the non-volatile 1 GByte of internal flash memory. Flash memory retains data even when power is lost. KB indicates the amount of memory space that is used. The date and time of file creation are also displayed.

"SD Card :" provides the same information from an inserted 1Gbyte data card.

"View" : loads the file of interest into the active memory and allows viewing of parameter settings from the measuring screens.

"Copy": allows copying files from the HDD drive to the SD Card and back. The SD card must be inserted before the Copy function can be activated.

"Erase" permits erasing files that have been highlighted by touch.

MMC/SD Data Card use and file transfer.

Insert an SD card into the OS1p slot on the side of the instrument.

“Copy” allows the coping of files to the SD Card. Highlight the file to be transferred with your finger and press “Copy”

A “Pick Data File” screen will appear. Pick the file of interest and press “Enter”. The file will be transferred to the SD card.

Notes: The SD card slot is on the left side panel. Push the card all the way in. It can be put in or removed while the OS1p is on or off. Do not remove the card when transferring data. To remove the card from the OS1p, push in the card and it will pop out. Remove the card.

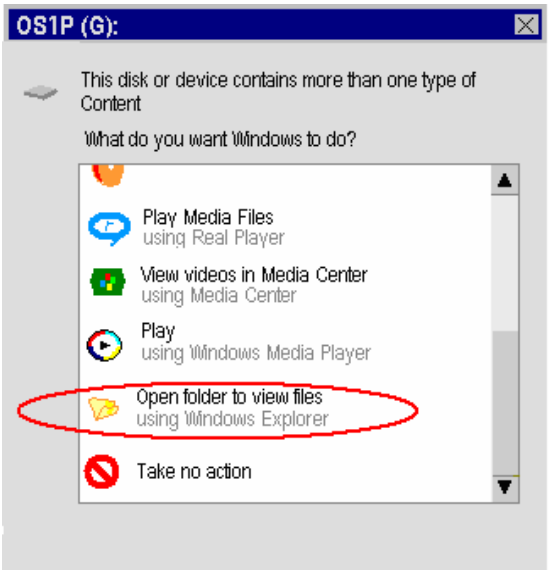
If one does not have an MMC/SD data card port on their computer, take the MMC/SD adapter provided with the OS1p, and insert it into a USB port while the computer is on.

The data card should be inserted into the other end of the port adaptor and the cover should be closed.

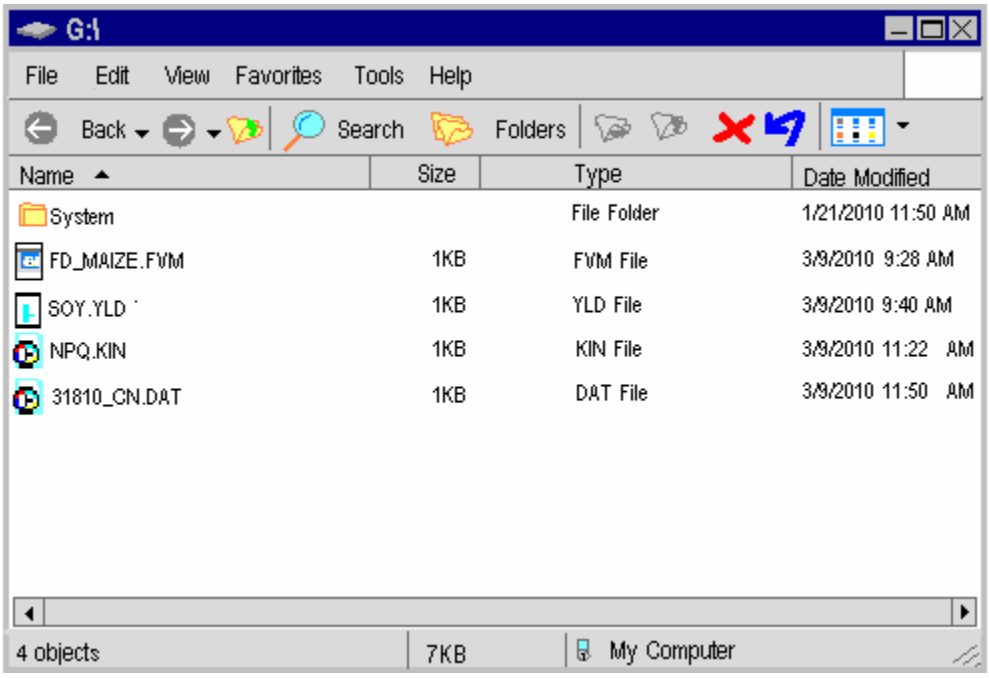
Data can be accessed by opening the new removable drive that appears under “My Computer” in Windows Explorer, on your PC. It may also be viewed by clicking “Open folder to view files” in a window that may appear on your PC screen (The appearance of the window is dependent on the Windows operating system that you have, and the drivers available on your computer.) The next few pages help with this process. It is recommended that the file be opened in Excel, MatLab or some other spread sheet program. The format is ASCII.

With many versions of the Windows operating system, a small window appears on the PC screen that should look something like the one pictured below. If it does not appear then go to the page section shown below the computer mouse

Select the “Open folder to view files” option.



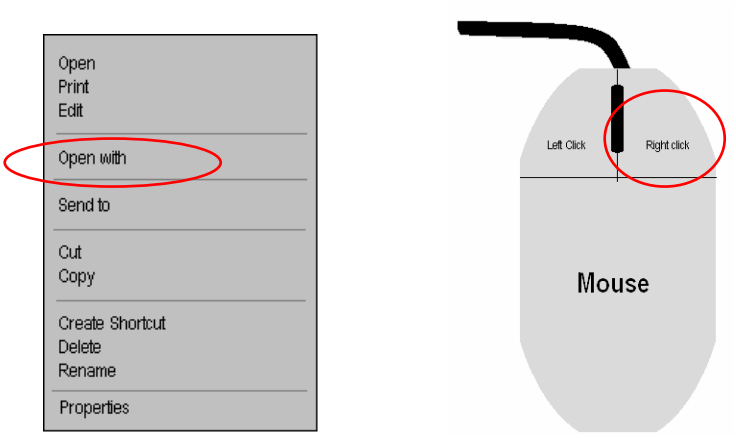
After clicking on “Open folder to view files”, the following window will appear.



Microsoft Windows Screen on a PC

From this window, the various files may be opened using Excel for viewing, analysis or graphing. Files that end in .DAT are data measuring files. Files the end in FVM,YLD,KIN, or some other suffix are measuring recipes.

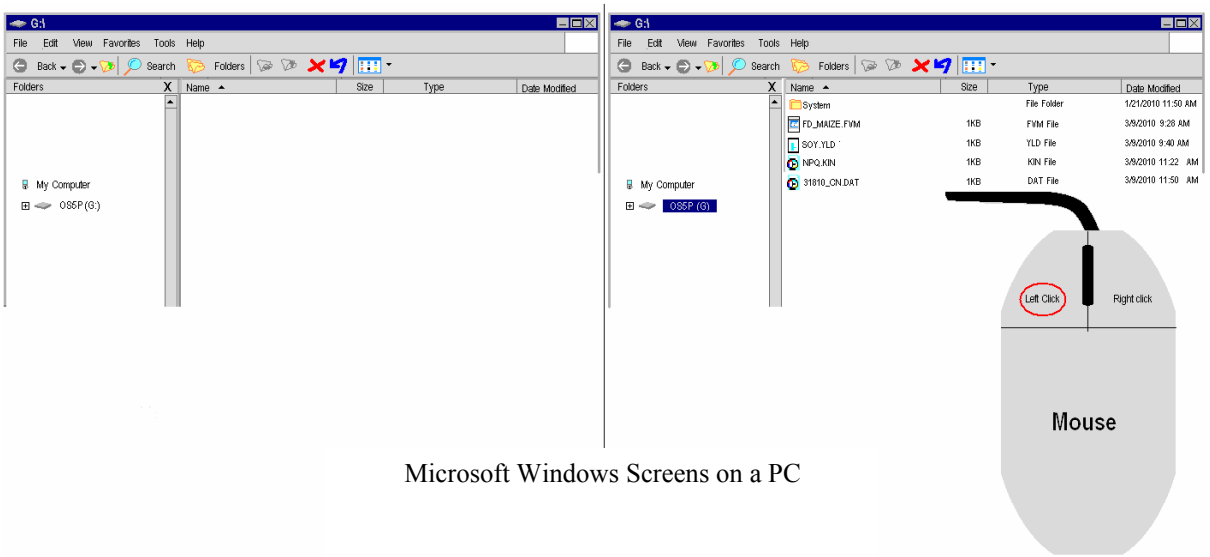
If the file will not open in Excel, highlight the file of interest using a mouse, and right click the file. A small window will appear like the one below. Select the option "Open with", and select Excel.



Microsoft Windows Screen on a PC

If the removable drive window shown above does not appear on your screen, then follow the directions listed below.

Open Windows Explorer, and click on "My Computer". The image should look something like the one shown below. The OS1p drive may be listed as OS1P and the appropriate new drive letter designation. Below, the drive is listed as G, but it may be any letter. In some cases, a new drive will appear without the OS1p designation. In this case, left click on the new drive and the files that are currently present on the MMC/SD Data Card will appear as shown below. These files may be opened in Excel for analysis and graphing. If they do not automatically open in Excel when clicked, right click the file and select "Open with…" and choose Excel.



Microsoft Windows Screens on a PC

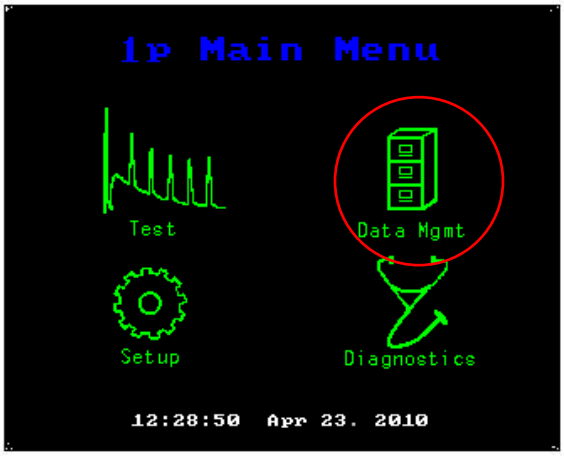
The Data card may be removed from your PS when done. Do not remove the data card until file transfer is complete.

“Erase” allows the erasure of files in the flash memory, or on the inserted SD data card. Select HDD to erase a file from the internal flash memory, or SD to erase a file from an SD card. The file will be erased.

Data Viewer

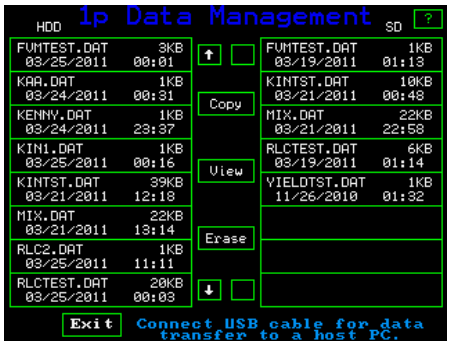
The data management section has a data viewer that allows the review of all previous measurements in a measuring file, and saved graphs, without exporting the file to Excel.

To use this feature go to the Main Menu screen and press “Data Mgmt”

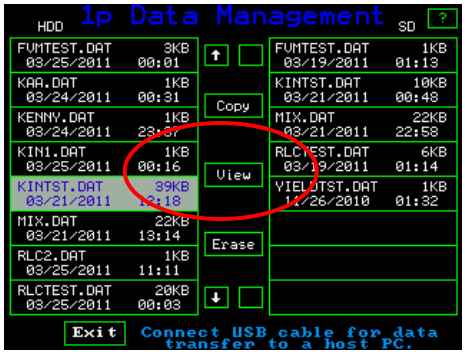


Main Menu

The following data management screen will appear. Touch the file that you want to view, and it will become highlighted as shown below. Then press “View”

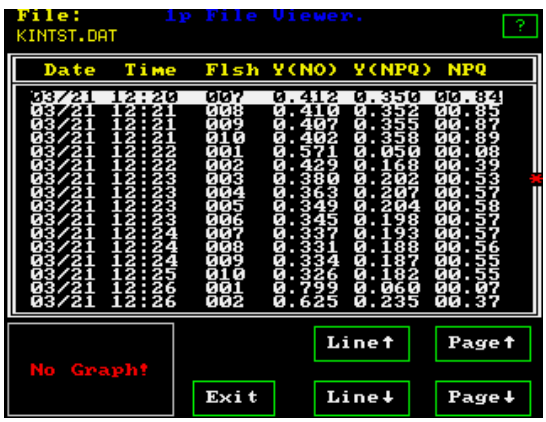


Data management File screen

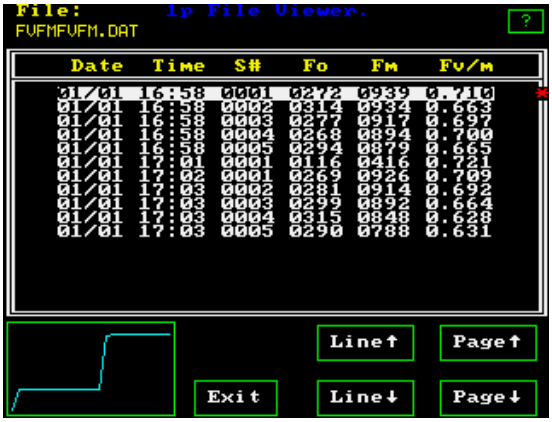


Highlighted file

The two slides shown below, show the data in a measuring file. The one on the left is for quenching measurements, and the one on the right is for Fv/Fm measurements.

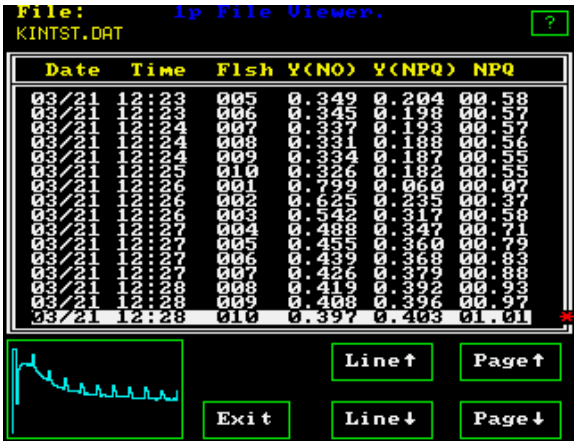


File viewer with no graph



File viewer with graph

By using the “Line” arrows, you can select the measurement of interest. It will become highlighted and if there is a graph associated with the data, it will be displayed in the lower left hand corner of the screen, as shown on the right hand screen. By using the “Page” arrow keys, you can scroll up or down in the measuring file. The little red asterisk on the right side of the screen indicates where you are in the file. If it is at the top, then you are at the beginning of the file. If you are lower, as shown on the left had screen, the asterisk is lower.



File viewer with quenching graph

To display quenching graphs, use the “Page” arrow buttons, and the “Line” arrow buttons to highlight the last row of data. This will bring up the graph. If there are two or more quenching measurements in a file, then when you highlight the last row of each quenching measurement, the graph will appear if it has been saved.

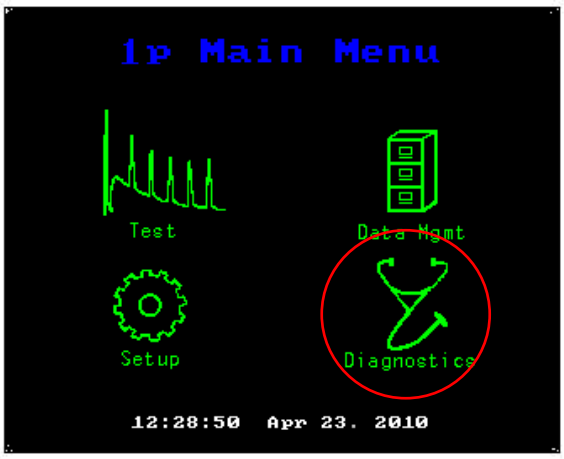
It is recommended that each quenching measurement have its own file name if graphs are being saved. When the file is downloaded into Excel, the number of rows in Excel, are limited, and quenching graphs use a large number of rows in Excel. Having too many rows can cause some results to be cut off in Excel.

Software Updates

From time to time, software updates may be available for improvement of existing functionality or to increase instrument functionality. To update software versions., contact Opti-Sciences Inc. 603-883-4400 www.optisci.com, or support@optisci.com , 8 Winn Ave. Hudson, NH 03051.

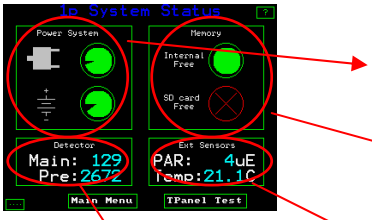
Chapter 5 ● Diagnostics

The Diagnostic screens are used to trouble shoot the system. It is recommended the you contact Opti-Sciences for trouble shooting help if you have a problem. These screens will help OSI diagnose machine issues and recommend a solutions.

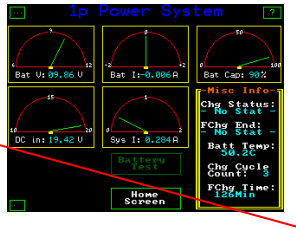


Main Screen

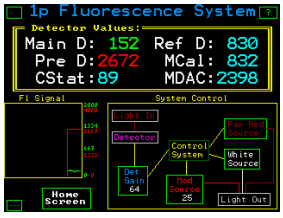
Drill down view of diagnostic screens



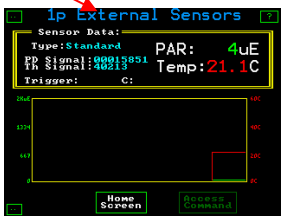
Main diagnostic Screen



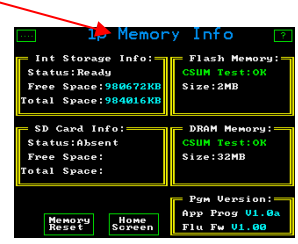
Battery and Power test screen



Test screen for fluorometer function



Test screen for PAR Clip Test details



Screen for memory & Software Version

Notes:

The Battery test and Access command require a special password from Opti-Sciences and they can not be accessed without contacting the factory.

The Fluorometer will not charge unless it is turned off. However, It may be used with AC power when it is plugged into an AC outlet.

Appendix A • Maintenance

Note: opening the OS1p case voids the warranty!

Your OS1p 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 Nickel Metal Hydride battery that is supplied with the OS1p, 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 OS1p 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.

Cleaning

The OS1p is 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 an ABS plastic. Should dirt get into the optical ports, uses s vacuum cleaner to remove the dirt. clean the fiber optic surfaces and glass surfaces 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).

Miscellaneous Maintenance

Battery

The Sealed Nickel Metal Hydride Battery will provide about eight to twenty hours of average use, depending primarily on use of the saturation source lamp.

The typical recharging period is 10 hrs. Leaving the charger plugged in for longer than this will not damage the battery.

> ***WARNING: Do not connect anything other than the provided charger to the charger jack, or you may damage the OS1p!***

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

Note: you can run the OS1p off the battery charger alone, however it will not recharge while the instrument is on.

This is a very reliable type of battery that 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 OS1p 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.

Circuit breaker

The OS1p uses a circuit breaker. If the system is not working, reset the circuit breaker with a pin, or paper clip, inserted into the reset port on the left side panel.

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 large amount of power to operate properly.

Current models are supplied with a Nickel Metal Hydride Batteries. The charge life of the NMH battery is eight and twelve hours between charges, under most conditions. The charger should be plugged in the OS1p and a wall outlet when not in use. Prolonged periods of discharge will significantly decrease it's life span.

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	Charge battery
	Battery has become "weak" and needs replacement	Contact factory
Display dims or changes contrast during a test	Battery low	Charge battery
		Contact Factory
Battery does not charge	Charger connector loose	Check connections
	Charger is defective	Replace charger
	Dead battery	Contact factory
Remote trigger does not initiate a test	Remote trigger wire is not plugged in correctly	Check connections
	OS5p is not in proper test mode	Be sure that a cursor is not blinking in the test mode box. If it is, the test mode has not been entered. Press the test mode key to enter the test mode.
	Fluorescence signal is too low	Raise modulation intensity
Saturation light does not trigger	Fluorescence signal (Ft) Is too low.	Increase the modulation intensity
	If display also blanks, battery may be low	Contact factory

Symptom	Probable Cause	Cure
Cannot output data to computer - or - Output to computer garbled	Cable is loose Hardware handshake protocol on host	Secure cable connections See data management section See data management section
Unit consistently loses data - or - Odd characters appear on various screens	Flash memory defective	Contact nearest authorized agent for service
The main screen does not appear when the instrument is turned on, Instead, another screen appears.	A software bug that has been corrected in the latest version of software occasionally causes this problem	Contact Opti-Sciences Inc to reload the latest version of software.
Fluorescence signal is noisy	Battery is insufficiently charged External actinic light source is heavily modulated (pulsed xenon, or fluorescent light) External illumination source has too much IR radiation The PIN diode detector gain is set too high. Fiber not steady on sample	Charge battery Use actinic illuminator with a continuous output that is not modulated. Do not use fluorescent lights!!! Filter out excessive IR Raise the Modulated light intensity and reduce the gain control setting Use open body clip or a PAR Clip

Screen flickers.	Battery is insufficiently charged	Charge battery Use battery charger to run the system.
Incorrect PAR reading with optional PAR cuvette attached	PAR sensor not connected	Secure the connection
Incorrect temperature reading with optional PAR cuvette attached	Temperature sensor not connected	Secure the connection
Charge Status Light It only turns on when the OS1p is turned off and the unit is plugged in. Green - Yellow - Red - Flashing -	 The light turns green when the Nickel Metal Hydride battery is fully charged. It is yellow momentarily when it is partially charged. It will be red when it is not fully charged. If the light is flashing, check the charger connection.	 Nothing is required Nothing is required Turn on the OS1p and go to diagnostics. Check battery capacity for the battery charging level. If it is low you may want to charge the system before use. Check the connection and the wall outlet for proper function.
!! Battery Low !!	Battery needs charging or the system must be used with the power cord	Charge the fluorometer or use a power cord.
!!IR to High!!	Far red light too high	Adjust it lower
"Fluorescent signal too low"	The modulated light intensity is too low	Adjust the modulated light intensity or set the intensity with Auto Gain
"FL > 3600 pos Det pin out"	The modulated light source intensity is too high	Adjust the modulated light source gain downward, and the intensity, or use Auto Gain.

Appendix B• PAR Clip

PAR Clip

The PAR leaf Clip is designed to provide data to the OS1p system on Photosynthetically Active Radiation, or PAR and leaf temperature conditions. This clip resembles the standard 45^0 angle open body clip provided with the OS1p, with two additional features. A thermistor for measuring leaf temperature and a PAR sensor for measuring light radiation on the leaf are included. The PAR sensor is mounted in a small support extending into the leaf measuring area. The thermistor is tethered below the support to measure leaf temperature.

Unlike other brands of PAR Clips, the OS1p PAR Clip measuring jaw opens from the bottom. This prevents the unwanted opening of the PAR Clip when measuring high leaves or when the PAR Clip is used on a tripod stand for longer quenching measurements.

The default equation for ETR when using the PAR Clip is:

ETR=Y(II) x PAR x 0.84 x 0.50. This equation uses average plant values for relative comparison between samples of the same species. For more exacting ETR values the leaf absorption and the ratio of PSII reaction centers may be changed in the Y(II) protocol. See the section on Quantum Photosynthetic Yield of PSII in chapter one and the section on ETR in chapter three for more details.

Connection

The fiber optic light guide is positioned in the PAR Clip in the same manner as the dark clip. Insert the single cylindrical barrel all the way into the PAR clip as shown below. A nylon locking screw secures the fiber end. The PAR clip is connected to the “accessory” port on the OS1p.It is recommended that the OS1p should be turned off when connecting the electronic PAR clip connector to the fluorometer.

Technical specs

Weight: 0.6 Lbs. or .27kg

Cable length: 1 m.

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

Temperature Sensor: Thermister

Temperature Range 5~60 ^oC.

Temperature Accuracy ± 1.0 ^oC (worst case).

Resolution ± 0.1 ^{o}C.

PAR Sensor: GaAs sensor, Cosine Corrected

Range 0 ~ 3000 µE or µmols

Resolution +/- 2 µE or µmols

Cosine Corrected to 80 degrees

The PAR Clip 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.

Using the PAR Clip

The PAR clip must be connected to the accessory port on the OS1p when the unit is turned off.

The PAR Clip will continuously read different light or irradiation values as PAR in the Y(II) and quenching kinetic tests, as it moves from one light condition to another before a measurement.

The PAR Clip is designed to measure actinic light intensity and record the average value just before the saturation pulse, in the Y(II), ETR, and quenching tests. During the quenching test, it may register up to 3000 uE during the saturation pulse, however, it is designed to record the actinic value just before the saturation pulse to the measuring file.

When using the PAR Clip, internal actinic light intensities are slightly more that half of the intensities found when using the dark clips.

When moving from a dark clip to a PAR clip, the modulated light source will require adjustment in the test protocol that is being used. You may read the "fluorescence value too low" when the measuring button is pressed. This is because the modulated light source intensity needs to be adjusted upward. Gain should be adjusted last. The lower the gain setting, the lower the signal to noise ratio. The modulation light source intensity should be set with a leaf in the leaf holder of the PAR clip. If it is too low, then the "too low" message appears during measurement.

The setting can sometimes vary with different samples, for instance, leaves with a low chlorophyll content may require more modulated light intensity.

When the PAR Clip is used in ambient light, it is important not to change the orientation of the leaf in relation to the sun or external light source. The leaf has reached steady state

at its current orientation and changing the angle of the leaf will cause plant mechanisms to start to adjust to the new actinic light level. Yield measurements are always done at steady state photosynthesis or errors will result. Reoriented leafs are not at steady state. According to Maxwell and Johnson (2000), it take between fifteen and twenty minutes for most leaves to reach steady state. (See the section on Yield in depth discussion)

In addition, the PAR clip should be oriented on the leaf to minimize sample measuring area shading by the PAR clip and the operator.

What is the value of a PAR clip in photosynthesis measurement?

PAR clips should be used for all field applications and where ambient light and temperature fluctuate. Since Y(II) measurements and other parameters, change with light levels and temperature, the ability to study measurements as a function of PAR value or temperature allow for reliable plant stress measurement.

PAR clips have also been used for fixed level illumination studies and predetermined variable illumination studies as well. In such cases, quenching studies, quenching relaxation studies, and plant performance at different light levels can be evaluated as a function of PAR and temperature. In order to measure ETR a PAR clip is necessary.

PAR Clips are also used for development of rapid light curves.

The Term PAR means photosynthetically active radiation in the wave band between 400-700 nm. PAR can be measured in different dimensions such as Watts per meter or in micro- Einsteins (μE)or micro-moles (μmols). When using a PAR Clip, dimensions will always be in the equivalent terms, micro-Einsteins, or micro-moles.

PPFD, or “photosynthetic photon flux density”, is the number of PAR photons incident on a surface in time and area dimensions. These terms are equivalent for PAR Clip leaf radiation measurements. Furthermore, both can be presented in either of the equivalent dimensions, micro-moles or micro-Einsteins.

Both the PAR and PPFD terms have used extensively in biology. Micro-Einsteins, and micro-moles have also been used extensively in biology.

Appendix C • Technical Specifications

Measured and calculated parameters

Optional

ETR, PAR, and leaf temperature are measured when the optional PAR Clip is purchased.

Standard Advanced OS1p parameters

Fo, Fm, Fv/Fm, Fm', Fs, Y(II) or ΔF/Fm')

Hendrickson - Klughammer and Schreiber's simplified lake model quenching equations

Y(II) = (Fms -Fs)/Fms or ΔFm'/ Fm'
Y(NO) = Fs/Fm or F/Fm
Y(NPQ) =(Fs/Fms)-Y(NO) or (F/Fm') - Y(NO)
NPQ= Y(NPQ)/Y(NO) or NPQ = (Fm-Fm')/Fm'

(Optional) (Available in 2011)

Kramer's equations

Y(II) = (Fm' -Fs)/Fm'
$q_L = q_P(Fod/Fs)$ or $q_L = q_P(Fo'/F)$
Y(NO) = 1/(NPQ+1+q_L(Fm/Fo -1))
Y(NPQ) = 1- Y(II)-Y(NO)

(Optional) (Available in 2011)

Puddle model parameters

q_P = (Fms - Fs)/(Fms -Fo) or q_P = Fm'-F)/(Fm'-Fo) Above 0.4, Fo' or Fod should replace Fo
q_N = 1- ((Fms-Fo) /(Fm-Fo)) or q_N = 1- ((Fm'-Fo) / (Fm-Fo)) Above 0.4, Fo' or Fod should replace Fo
NPQ = (Fm-Fms)/Fms or NPQ = (Fm-Fm')/Fm'
NPQ=q_E+q_T+q_I

(Optional) (Available in 2011)

Relaxation test for Hendrickson Klughammer and puddle model

q_E = ((Fme-Fms)/(Fm-Fms)) is the relaxation saturation value at four minutes in the dark. (Values can be changed in Excel).
q_T = ((Fmt-Fme)/ (Fm-Fms)) is the relaxation saturation value at twenty minutes in the dark. (Values can be changed in Excel).

q_I = ((Fm-Fmt) / Fm-Fms)) Relaxation of q_I starts at about forty minutes and can take up to sixty hours. q_I can be determined from the dark adapted Fm measurement and the saturation pulse after 20 minutes used for q_T . (Values can be changed in Excel).

Fod (or Fo') q_P, q_N, NPQ, q_E, q_T, q_I,

PAR, Leaf temperature, estimated ETR (using Standard PAR Clip).

Light Sources

Saturation pulse: White light LED with 690 nm short pass filter. Adjustable 0 ~ 11,000 uE, or 0-6600 uE with a PAR Clip

Modulated light: 660 nm LED with 690 nm short pass filter. Adjustable 0 ~ 1 µE

Actinic illumination: White light solid state source. Adjustable 0~ 1500 µE

Far red: 735 nm LED with far-red cut off filter (Fod or Fo' determination, and for re-oxidizing PSII before Fv/Fm measurements). Intensity and duration adjustable.

Detector and Filters

A PIN photodiode with a 700 ~ 750 nm band pass filter.

Modulation frequency

Selectable from 25 Hz to 1 MHz with auto switching based on phase of test.

Filter constants

Automated from 1µs to 1 S with auto switching based on phase of test.

Sampling Rate

Variable from 1 to 1,000,000 points per second with auto switching based on phase of test.

Test Duration

Adjustable from 2 seconds ~ 10 hours (Longer with adapter and AC source).

Processor

ARM 7

Storage Capacity

1 GByte Flash memory almost unlimited testing

Digital Output

USB, MMC/SD data card with up to 1 GByte data card size

User Interface
Display: 320 x 240 dot super-twist Color LCD, with LED back light., touch screen

Power Supply
Internal rechargeable sealed Nickel Metal Hydride battery, or AC current.

Battery Life
8 to 12 hours of continuous operation.

Dimensions
7.75 in x 6 in x 4.5 in. in carrying bag

7.25 in x 5.38 in x 3.25 in without bag

Weight

3.0 lbs. (including fiber-optic and battery)

6.0 lbs with Fiber optic and PAR Clip included

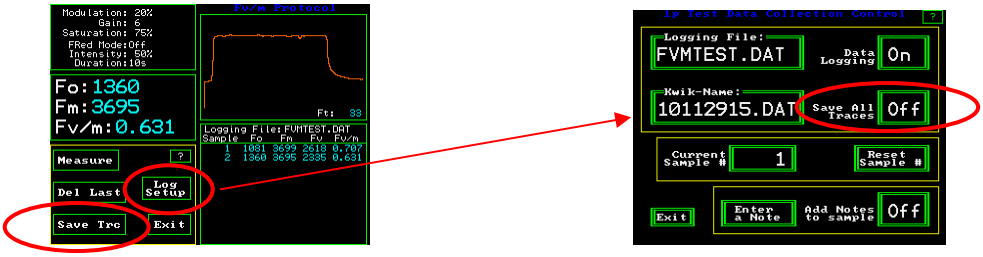
For best results, the instrument should be left plugged into an outlet when not in use.

Appendix D • Data Formats

Overview

Each test has a unique data output format, tailored to the changing measured parameters. The differences between the data groups are listed in this section. Measurement values are represented in a spread sheet format and intensity trace data is presented in a vertical sequential list of intensity values. Traces may be graphed in Excel or other spread sheet products.

Data exists in two forms in the OS1p. The first type is the fluorescence signal over the course of a test. This "raw data" is useful when visualizing what is happening dynamically in photosystem II, as it is exposed to the different illumination sources over the course of the test. The second kind of data is the tabular data that is generated around the saturating pulses. The specifics of each of these parameters are mentioned later in this section. Storage method varies between these two types of data. The “raw data” for a trace is allotted a unique storage or row number based on the next open location in the storage directory, as they are saved. The user can not specify which slot number a trace is stored to. The "Tabulated Data", on the other hand, is stored in logical groupings called file numbers. Unlike traces, the current file number is a user set-able option. When joint saving of trace and data is desired, press the “Save Trc” trace button on the measuring screen of each protocol that is being used. This will save the trace from the last measurement. To save a trace with each measurement, press “Log Setup”. Next press “Save All Traces” until the On value is displayed.



Fv/Fm measuring screen

1p Test Data Collection Control screen

This will give the file and trace the same identifying number. There can be almost an unlimited number of different files and traces stored simultaneously in the system depending on memory use. The limiting factor is usually the number of rows that Microsoft Excel software can support. Different versions have different maximum values. Consult Microsoft for details on your software. Information on free memory status is always available in the Diagnostics section, by looking at the screen for memory and software version. See the Diagnostics section to see an image of this screen. Traces and tabulated data are always saved together in quenching tests, There are specific formats for each type of data and trace. Each test mode will generate a slightly different data output (each test measures different parameters). The details of each type are listed at the end of this section.

All types of formats include a common header containing information about the date, time, machine number, and setup of the machine when each measurement was made.

Data format information:

Data formats for test protocols are listed below. All data types and output methods are presented in a ASCII format that is comma delimited with a character 13 (<CR.>) code at the end of each line. This type of data output format, while bulkier than binary coding, is compatible with most data analysis and spreadsheet programs "Import" feature. The following files have been copied from Microsoft Excel.

Fv/Fm data file format

Type	Date/Time	Sample #	Mod Int	Det Gain	Sat Flash Int	Sat PW	Far Red Mode	Far Red Int	Far Red Dur	Fo	Fm	Fv	Fv/m	Note
1p Fv/m	12/23/2010 16:13	1	25	4	75	1	Off	50	10	243	763	520	0.681	
1p Fv/m	12/23/2010 16:13	2	25	4	75	1	Off	50	10	263	768	505	0.657	
1p Fv/m	12/23/2010 16:13	3	25	4	75	1	Off	50	10	264	771	507	0.657	
1p Fv/m	12/23/2010 16:13	4	25	4	75	1	Off	50	10	262	774	512	0.661	
1p Fv/m	12/23/2010 16:13	5	25	4	75	1	Off	50	10	273	772	499	0.646	
1p Fv/m	12/23/2010 16:13	6	25	4	75	1	Off	50	10	278	770	492	0.638	
1p Fv/m	12/23/2010 16:13	7	25	4	75	1	Off	50	10	279	768	489	0.636	
1p Fv/m	12/23/2010 16:13	8	25	4	75	1	Off	50	10	281	764	483	0.632	
1p Fv/m	12/23/2010 16:13	9	25	4	75	1	Off	50	10	307	761	454	0.596	
1p Fv/m	12/23/2010 16:14	10	25	4	75	1	Off	50	10	278	760	482	0.634	

Y(II) data file format

Type	Date/Time	Sample #	Mod Int	Det Gain	Sat Flas	Sat PW	Flash M	Far Re	Far Red	Far Re	Pre-Actini	Actinic	Ke	Kq	PAR Cc	F'	Fm'	Y(II)	ETR	PAR	Temp	Note
1p Y(II)	12/23/2010 16:10	1	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	527	1513	0.651	4.3	16	21.8	
1p Y(II)	12/23/2010 16:11	2	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	548	1505	0.635	4.2	16	21.9	
1p Y(II)	12/23/2010 16:11	3	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	546	1497	0.635	4.2	16	22	
1p Y(II)	12/23/2010 16:11	4	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	561	1492	0.623	4.1	16	22	
1p Y(II)	12/23/2010 16:11	5	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	556	1487	0.626	4.2	16	22.1	
1p Y(II)	12/23/2010 16:11	6	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	555	1482	0.625	4.4	17	22.1	
1p Y(II)	12/23/2010 16:11	7	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	530	1472	0.639	4.2	16	22.2	
1p Y(II)	12/23/2010 16:11	8	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	546	1464	0.627	4.2	16	22.2	
1p Y(II)	12/23/2010 16:11	9	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	555	1459	0.619	4.6	18	22.2	
1p Y(II)	12/23/2010 16:11	10	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	549	1452	0.621	4.6	18	22.3	
1p Y(II)	12/23/2010 16:12	11	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	535	1445	0.629	4.7	18	22.3	
Curve:	Sat Tail:0.0	Zoom:Off																				
533																						
529																						
534																						
528																						
530																						
541																						
1433																						
1448																						
1436																						
1439																						
1440																						
1443																						
1454																						
1443																						
1440																						
1p Y(II)	12/23/2010 16:12	12	50	4	75	1	Standar	Off	50	5	Off	1	0.5	0.84	1	513	1439	0.643	4.5	17	22.3	

Quenching data file format – Hendrickson – Klughammer equations

Type	Date/Time	Flash #	Mod Int	Det Gain	Sat Flash	Sat PW	Flash Mod	Act Intens	Flash Inter	Ke	Kq	PAR Corr	Fm	F'	Fm'	Y(II)	ETR	Y(NO)	Y(NPQ)	NPQ	PAR	Temp
1p Kin	12/23/2010 15:28	1	50	1	100	1	Standard	6	120	0.5	0.84	1	721	447	544	0.178	10.9	0.619	0.202	0.32	146	21.4
1p Kin	12/23/2010 15:30	2	50	1	100	1	Standard	6	120	0.5	0.84	1	721	450	524	0.141	8.6	0.624	0.234	0.37	146	21.4
1p Kin	12/23/2010 15:32	3	50	1	100	1	Standard	6	120	0.5	0.84	1	721	435	521	0.165	10.1	0.603	0.231	0.38	146	21.4
1p Kin	12/23/2010 15:34	4	50	1	100	1	Standard	6	120	0.5	0.84	1	721	438	513	0.146	8.9	0.607	0.246	0.4	146	21.4
1p Kin	12/23/2010 15:36	5	50	1	100	1	Standard	6	120	0.5	0.84	1	721	434	504	0.138	8.4	0.601	0.26	0.43	146	21.4
1p Kin	12/23/2010 15:39	6	50	1	100	1	Standard	6	120	0.5	0.84	1	721	431	497	0.132	8	0.597	0.27	0.45	146	21.5
1p Kin	12/23/2010 15:41	7	50	1	100	1	Standard	6	120	0.5	0.84	1	721	425	492	0.136	8.3	0.589	0.274	0.46	146	21.5
1p Kin	12/23/2010 15:43	8	50	1	100	1	Standard	6	120	0.5	0.84	1	721	423	487	0.131	8	0.586	0.282	0.48	146	21.5
1p Kin	12/23/2010 15:45	9	50	1	100	1	Standard	6	120	0.5	0.84	1	721	421	482	0.126	7.7	0.583	0.29	0.49	146	21.5
1p Kin	12/23/2010 15:47	10	50	1	100	1	Standard	6	120	0.5	0.84	1	721	417	478	0.127	7.7	0.578	0.294	0.5	146	21.5
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RCL Rapid Light Curve data format

Type	Date/Time	Step #	Mod Int	Det Gain	Sat Flash	Sat PW	Flash Mode	Act Intensity	Step Duration	Ke	Kq	PAR Corr	F'	Fm'	Y(II)	ETR	PAR	Temp
1p RLC	3/31/2011 13:52	1	55	1	100	1	Standard	1	10	0.5	0.84	1	124	347	0.642	0	0	21.5
1p RLC	3/31/2011 13:52	2	55	1	100	1	Standard	2	10	0.5	0.84	1	197	346	0.43	2.7	15	21.6
1p RLC	3/31/2011 13:52	3	55	1	100	1	Standard	3	10	0.5	0.84	1	239	331	0.277	4	35	21.6
1p RLC	3/31/2011 13:52	4	55	1	100	1	Standard	4	10	0.5	0.84	1	250	319	0.216	5	56	21.6
1p RLC	3/31/2011 13:52	5	55	1	100	1	Standard	5	10	0.5	0.84	1	255	312	0.182	5.8	77	21.6
1p RLC	3/31/2011 13:52	6	55	1	100	1	Standard	6	10	0.5	0.84	1	260	307	0.153	6.3	99	21.6
1p RLC	3/31/2011 13:52	7	55	1	100	1	Standard	7	10	0.5	0.84	1	261	305	0.144	7.1	119	21.6
1p RLC	3/31/2011 13:52	8	55	1	100	1	Standard	8	10	0.5	0.84	1	264	304	0.131	7.7	140	21.6
1p RLC	3/31/2011 13:52	9	55	1	100	1	Standard	9	10	0.5	0.84	1	266	305	0.127	8.5	160	21.6
1p RLC	3/31/2011 13:52	10	55	1	100	1	Standard	10	10	0.5	0.84	1	274	309	0.113	8.5	181	21.7
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