Preliminary 2021 edition OS5p+ operators guide

Advanced Pulse Modulated Portable Chlorophyll Fluorometer.

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CONTENTS

This modulated fluorometer is a versatile measuring instrument designed to precisely measure chlorophyll fluorescence. It uses a pulse modulated detection principle to allow for a variety of tests to be conducted under greatly varying environmental conditions. The system is simple to use, light in weight, and battery powered, making it an excellent choice for field studies.

OVERVIEW

Light energy utilized in photosynthesis by higher plants and algae cells is absorbed by a number of photosynthetic pigments with absorption spectra covering a large range of the available light energy. The most prominent pigments that absorb this energy are chlorophyll-a and chlorophyll-b. The light energy absorbed by the chloroplast first excites pigment molecules of the light harvesting chlorophyll proteins (LHC). These LHC proteins transfer their energy to either Photosystem I (PSI) or Photosystem II (PSII). These photosystems contain the reaction center pigments for the conversion of absorbed light energy to oxidation and reduction potential to drive dark electron transport. Light energy absorbed initially by the LHC and transferred to the reaction centers is lost by a number of different mechanisms. Approximately 3%-9% of the light energy absorbed by chlorophyll pigments is re-emitted from the first excited state as fluorescence. The emission peak is of a longer wavelength than the excitation energy. This effect was first observed more than 100 years ago, when N.J.C. Müller (1874) by visually using colored glass filters. He also noted that fluorescence changes that occur in green leaves are correlated with photosynthetic assimilation. Lack of appropriate technical equipment prevented a more detailed investigation of this phenomena. The light energy absorbed by the reaction center drives photosynthetic electron transport through PSII and PSI leading to the oxidation of water, oxygen evolution, the reduction of NADP+ to NADPH, membrane proton transport and eventually to ATP synthesis.

The loss of light energy from the reaction center as fluorescence comes primarily from the PS II reaction. When the chloroplast or leaves have been dark-adapted, the pools of oxidation or reduction intermediates for the electron transport pathway return to a common level. Upon illumination of a dark-adapted leaf, there is a rapid rise in light emission from PS II fluorescence followed by a series of slow oscillations. This is referred to as the "Kautsky Effect". Named after the researcher who did detailed studies on the phenomena (Kautsky and Hirsch 1931). Fig x shows the usual onset kinetics of fluorescence emission from a typical dark-adapted leaf. Changes in the intensity of the fluorescence emission from dark-adapted leaves are sensitive to changes in the photosynthetic apparatus. Following many years of study of chlorophyll fluorescence to analyze its relationship to photosynthesis and characterize photosynthesis, it has been shown (see reference list) that any unusual change in the overall bioenergetic status of the plant can be detected by a change in chlorophyll fluorescence. This includes all the reactions from the oxidation of water through electron transport, development of the electrochemical gradient, ATP synthesis, and eventually the series of enzymatic reactions for CO2 reduction to carbohydrate in the leaf. Even changes in the plant that affect stoma opening and gas exchange with the atmosphere are reflected by changes in the fluorescence characteristics of a leaf.

PRINCIPAL OF OPERATION

Chlorophyll fluorescence is excited by a 660 nm solid-state light source with filters blocking radiation longer than 690 nm. The average intensity of this modulated light is adjustable from 0 to 1 uE. The source output is optically stabilized for improved accuracy over a wide operating temperature range.

Detection is in the 700 to 750 nm range using a PIN silicon photodiode with appropriate filtering to remove extraneous light. The photodiode is directly connected to a high performance, low-noise preamplifier mounted directly in the detector assembly. The detection method is a high-performance lock-in amplifier design which allows the use of a very low modulated light energy. This allows monitoring of the fluorescence level without exciting any noticeable variable fluorescence. In effect, the system measures the change in fluorescence due to effects other than the measuring beam. The detection rate is determined by the instrument for maximum signal to

noise ratios and accurate sensing of rapidly changing signals. An added benefit of this design is its ability to disregard extraneous ambient light.

Saturation of the photosystem being measured is provided by a stable high intensity white light LED. The duration of the saturation pulse may be adjusted to both ensure maximum saturation, and to prevent over excitation of the photosystem.

LIGHT SOURCES

MODULATED LIGHT SOURCE

This is a 660nm light source that makes light adapted quantum photosynthetic yield measurements possible along with direct measurements of F_o and F_o'. It is used at an intensity range that is too low to drive photosynthesis. Its intensity may be varied to adjust for different species and conditions in dark adapted samples and for use with the PAR clip on light and dark adapted samples. This source can also be adjusted automatically by the instrument for optimal performance.

SATURATION LIGHT SOURCE

A saturation pulse is a short pulse of intense light designed to fully reduce a leaf's PSII system. The duration can be set between 0.4 and 2.0 seconds. For higher plants, the optimal duration of the saturation pulse is between 0.5 and 1.5 seconds (Rosenqvist and van Kooten 2006). For Algae and cyanobacteria, the duration must be shorter to provide accurate measurements 25 ms to 50 ms. (Schreiber 1999). 0.8 seconds has been set as the default value for land plants.

An 8 point 25 ms rolling detection average is used to determine the highest F_M, and F_M' independent of saturation pulse duration. This ensures that as long as the duration is long enough, the optimal measurement will be made. The fluorometer uses a white light LED with a maximum intensity of 15,000 umols when used with the dark adaptation clip.

ACTINIC LIGHT SOURCE

Light source (high intensity white light LED) that drives photosynthesis. When used in conjunction with the PAR clip, the light intensity at the leaf can be set and maintained at a constant level for long periods of time.

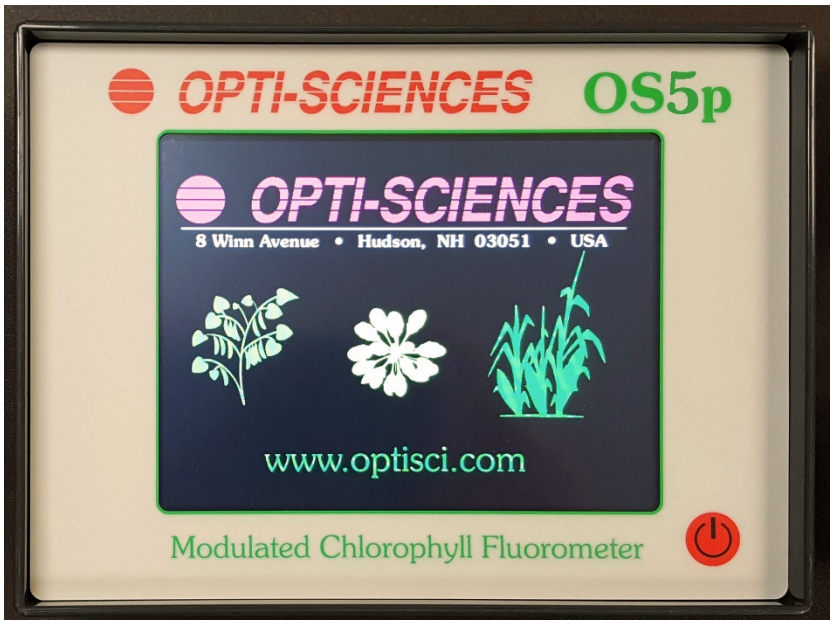
FAR RED SOURCE

Far red light drives PSI without driving PSII. This has the effect of draining the remaining electrons from PSII quickly to provide an optimal oxidized state in PSII. Solid state source with a peak wavelength ~735nm. It is commonly used for determination of F_o'.

HARDWARE

The fluorometer includes built in actinic, saturation, far red, measuring light sources, GPS, data storage, graphical color touch screen, rechargeable battery pack with battery management system and accessory port. Shipped with the instrument are the PAR clip, fiber optic light guide, dark adaptation clips, open body clip, charger, cloth carry bag, and transport case.

TOP



Color touch screen. Power switch.

MEASURING PROBE PANEL

Accessory port
Connection for PAR clip. Additional accessories may be available in the future.

Fiber-optic light guide
Connectors for the three fiber optic connectors. Any of the fiber connectors may be attached to any of the panel connectors.

Remote
Fiber optic trigger switch. Used to start a measurement.

DATA AND CHARGING PANEL

SD card
Accepts SD format data cards up to 2GB.

Power
Power supply and battery charger port. Accepts up to 19VDC (3A).

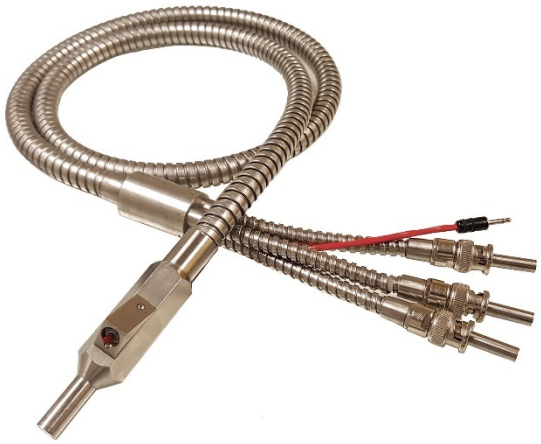
Reset
The system can be rebooted by inserting a small diameter wire (paper clip).

CHARGE STATUS LED

Off	Not charging
Yellow	Pre-charge for deeply discharged batteries
Red	High current charging
Blinking red	Battery out of specification, or is hot
Blinking red/green	Low current charge
Green	Charge complete

USB port
Supports data exchange with a host computer. Files are comma delineated for direct opening into Excel, Mat Lab, or other analysis programs. USB storage devices may be used with an adaptor cable (not included). The port is barcode reader capable. Scanned information is added to as a note.

FIBER

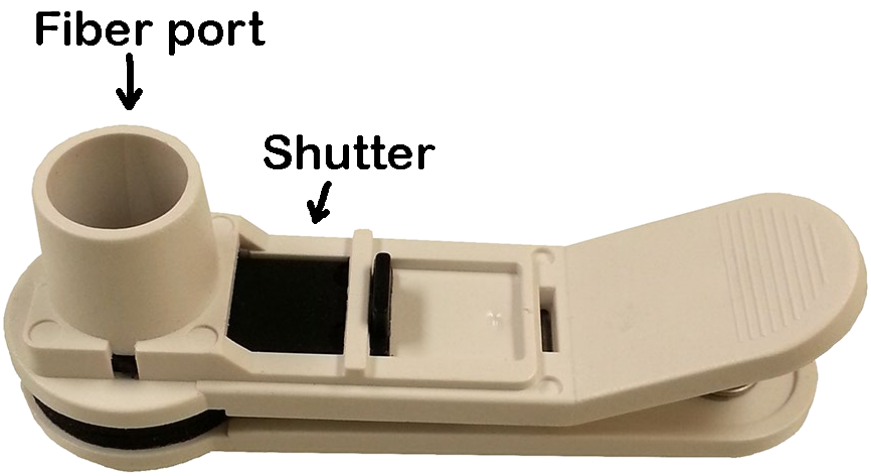


Optical signals are transferred to and from the sample by a custom-designed trifurcated fiber optic light guide, with randomly mixed fibers. One end of this fiber bundle has three BNC (twist-lock) connectors that attach to the fluorometer. The optical connectors can attach to any port. The trigger switch is used to initiate a measurement.

PAR CLIP

Measures PAR (photosynthetically active radiation measured between 400nm and 700nm) and leaf temperature. This data is used to calculate relative ETR. Changes in Y(II) can be due to changes in plant stress or light level, so it is important to control and measure light level when comparing values.

DARK ADAPTATION CLIPS



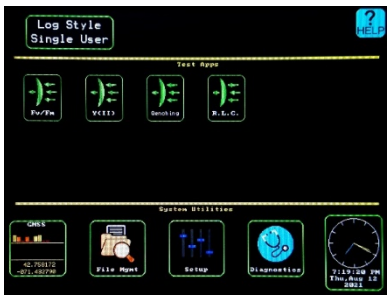
Ten dark adaption clips are provided with the system to be used for dark adaption. A Clip is placed on the leaf with the black shutter covering the opening. After dark adaption, the end of the fiber optic bundle is placed in the port and the shutter opened, allowing the sample to be exposed to the fiber optic bundle. Dark shrouds can also be used for dark adaption or lights can be turned off in a windowless environment.

TRANSPORT CASE

The instrument and accessories are housed in a durable transport case

SYSTEM OPERATION

MAIN SCREEN



When turned on, available test applications and system utilities will be displayed. A context sensitive help icon is located in the upper right.

LOG STYLE

Selects the default storage device for data and system preferences.

Single user
Data and system settings are saved to internal storage.

Multi user
When set to multi user, all data files and system preferences are stored on a removable SD card. This allows the fluorometer to be used by multiple researchers without having to manually reset test settings.

AVAILABLE TEST APPS

The fluorometer has several different test applications (protocols), each with necessary setup and data logging options.

FV/FM

The F_V/F_M Protocol — Dark adapted test for maximum quantum yield.

A measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open. 0.79 to 0.83 is the approximate optimal value for most plant species with lower values indicating plant stress. It is a fast test that usually takes less than two seconds.

Y(II)

Light adapted test of PSII for effective quantum photochemical yield Y(II) and ETR (electron transport rate).

Y(II) (also known as $\Delta F/F_M'$ and $F_M' – Fs / F_M'$) is a time-tested light adapted, normalized ratio parameter that is more sensitive to more types of plant stress than F_V/F_M according to a survey of existing research. Y(II) allows

measurement of efficiency of the overall process under actual environmental and physiological conditions. It represents achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions.

QUENCHING

Several different quenching protocol options are available.

Kramer lake model quenching parameters, including Y(II), Y(NPQ), Y(NO), and qL at steady state photosynthesis. Kramer uses F_O' in his parameters.

Hendrickson lake model quenching parameters with NPQ resurrected from the puddle model by Klughammer. It includes parameters NPQ, Y(II), Y(NPQ), Y(NO), PAR and leaf temperature (using the PAR Clip). Hendrickson uses F_O instead of F_O' in his parameters.

Puddle model quenching parameters include: Y(II), NPQ, qN, qP. Parameters qN and qP use F_O' in their formulas while Y(II) and NPQ do not.

Protocol measurement parameters are provided along with the quenching relaxation parameters qE, qM, qZ, qT, and qI.

RLC

Rapid light Curves. Provides for the stepped change of actinic light during a test. This test is commonly used to study saturation characteristics of plants especially for under canopy work or for aquatic plants. While almost all chlorophyll fluorescence measuring parameters require steady state photosynthesis for reliable measurement, this protocol allows measurement of PSII under changing light conditions.

OJIP

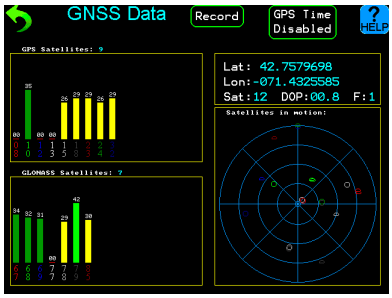
The Strasser OJIP protocol used for plant stress testing. The most used Strasser parameters are directly displayed. All of the Strasser parameters are saved to the data file.

F. I. A.

Fast induction analysis. A special OJIP quenching protocol designed in conjunction with Wim Vredenberg. It is designed to allow investigation of quenching phenomenon with high-speed time resolution, and measurement of saturation pulse NPQ.

GPS

Displays the current GPS information. Tap to view details.



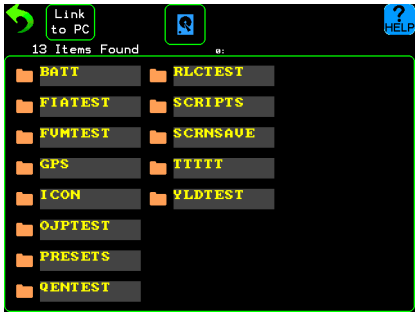
Satellites currently in view and location data are shown. Tapping record will record location information to a file until stop is pressed. When enabled, GPS time will set the date and time from GPS data.

FILE MANAGEMENT


View, copy and delete stored data. Provides communication to a host PC for data transfer.

Files are stored in test protocol specific folders by default.

Tap on a folder to see its contents.



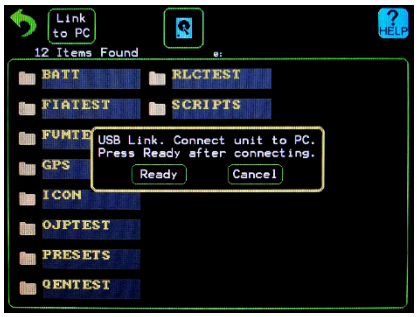
Tap on a file to copy, delete or view it.

Menu bar showing available options.

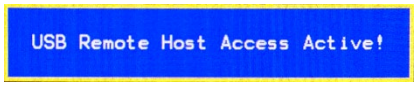
Tap on link to pc to activate the USB file transfer mode.

Once the USB cable is connected to a PC, tap ready. The host PC will show the instrument as a portable storage device called OSI_FL.

Some PCs may take a while to recognize the instrument.



The instrument will display a message indicating an active link.



From the host PC, you may copy, move, and delete files as usual.

To disconnect the instrument from the host PC, right mouse click over the drive and select “Eject” from the pop up menu. The instrument will confirm the action (see message). Normal operation will return once the USB cable is disconnected. Data loss or storage corruption may occur if this process is not followed.

Move up one folder (back). This icon is only available if you are currently viewing a subdirectory.

Indicates the type of storage currently being viewed. Tap this icon to select a different storage device.

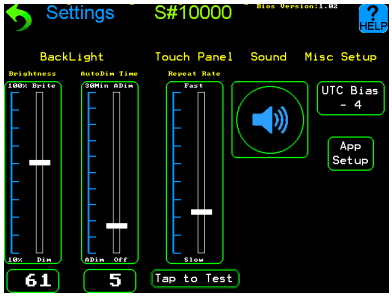
Options include Internal storage (Int HDD), SD Card, USB drive (required adaptor cable not included) and system memory (Sys HDD).

Copy contents of folder to selected location.

Delete contents of folder.

SETUP

System settings.



BACKLIGHT

Brightness
Sets the intensity of the backlight.

Auto dim time
Time allowed to pass before the screen dims. If it is set to zero it is turned off.

TOUCH PANEL

Repeat rate
Sets the repeat rate for long button presses.

SOUND

Enables or disables system sounds.

UTC BIAS

Set to your time zone measured from UTC.

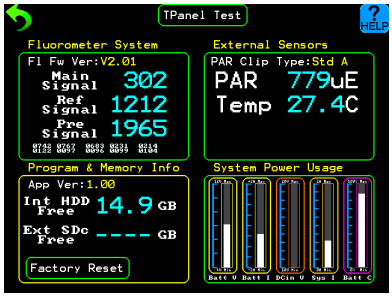
APP SETUP

Reserved for future use

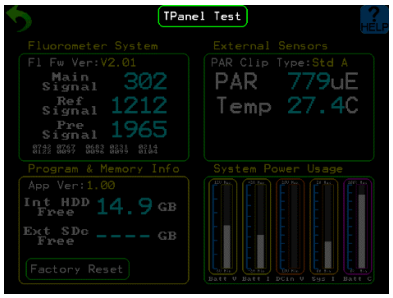
DIAGNOSTICS

Diagnostics information.

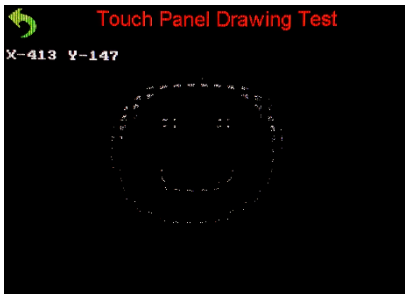
Tap the diagnostic icon to view detailed information.



Various system values are displayed.



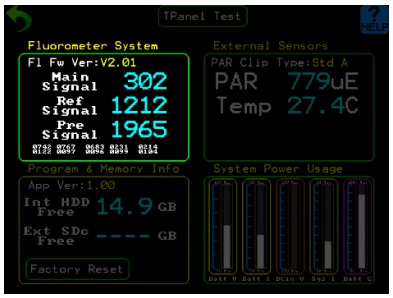
TPanel test



Displays the X,Y coordinates of a screen touch.

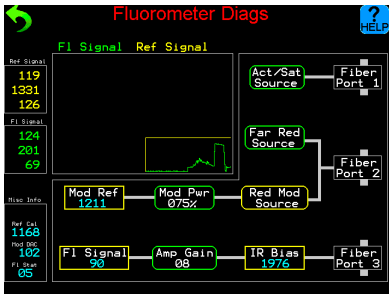
Touch panel test

Useful for testing the response of the touch panel.



Fluorometer System.

Displays current fluorometer signal values.

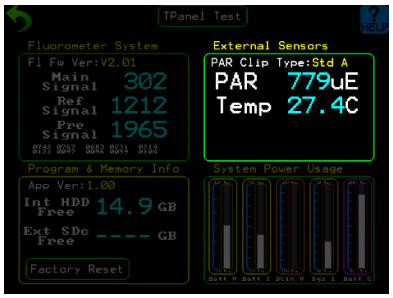


Here, individual sources may be turned on and off.

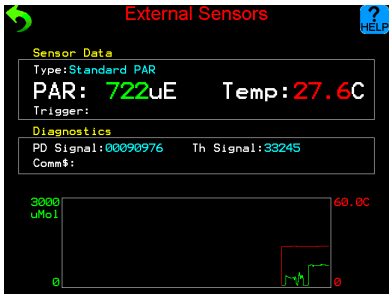
Amplifier gain and modulated source intensity can be

Tap this box to display detailed analysis and control of the various sources and detectors

changed. The resulting detected fluorescence is graphed in real time.

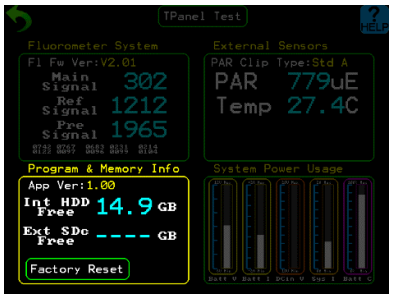


Eternal Sensors
Displays current values reported from external sensors.

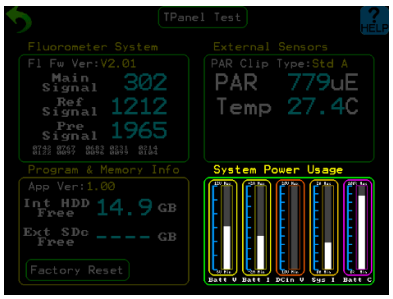


Sensor reading are continuously updated. The graph will show real time data.

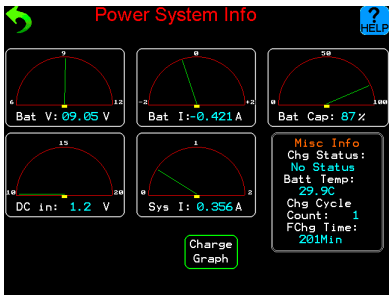
Tap this box to display detailed information.



Program and Memory Info
Display of memory usage. The Factory Reset button will reset all test settings back to factory default. This will not alter any data or settings stored in a file.



System Power Usage
Display of important system power measurements.



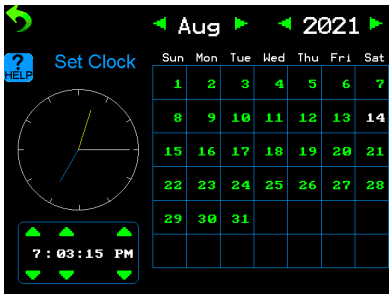
The charge graph button will display the most recent

Tap this box to show detailed information and battery charge status.

battery charge cycle. Loading the data to create the graph can take up to several minutes.

Battery capacity is an estimated value.

DATE AND TIME



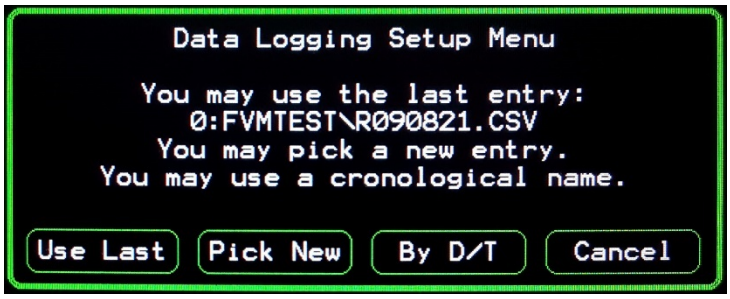
Clock display of system date and time. Tap this icon to set the clock. Green arrows are used to change values. Current date and time are indicated by the white characters.

TEST DATA STORAGE

DATA LOGGING

All calculated data measurements are automatically saved to memory unless specifically deleted by the operator after completion of the measurement. Storage of fluorescence trace files can be set to automatic or manual.

Tap a test app icon to run a test. The first screen will ask to select the data file.



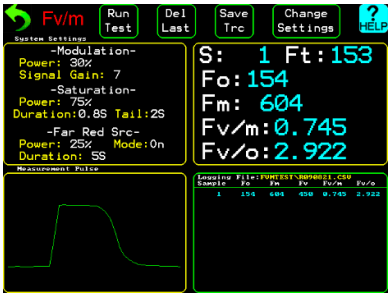
Use Last
The last known file for this type of test will be used. New data will be appended to any existing data.

Pick New
A directory and file menu will appear. Navigate to the desired directory.
Tap  to create a new folder. Tap  to enter a new file name.

By D/T
Creates a file name based on the current date.
A prompt will ask what directory to use. Options are to use the last known directory, or select a new directory.

Cancel
Exits to main screen.

FV/FM



A dark adapted test measuring 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 F_V/F_M is a measurement ratio that represents the maximum potential quantum efficiency of Photosystem II if all capable reaction centers were open or oxidized. 0.79 to 0.83 is the approximate optimal value range for most land plant species with lowered values indicating plant stress. It is important to properly dark adapt samples for this test.

For a detailed discussion on setting test parameters, see Appendix A

For a detailed discussion on dark adaptation, see Appendix F

PARAMETERS

S
Sample number

Ft
Current value of detected fluorescence.

Fo
Minimal fluorescence after dark adaptation. It is measured with a modulated light intensity too dim to drive chemical reduction of Q_A and yet bright enough to detect “pre-photosynthetic” antennae fluorescence.

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

Fv/m
Maximum quantum yield. Fv/m = (F_M – F_O) / F_M

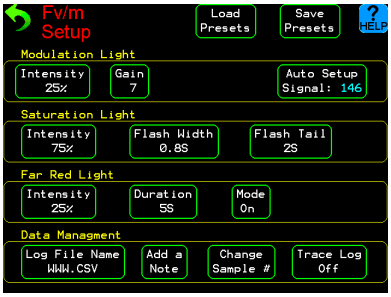
Fv/o
A more sensitive plant stress detector than F_V/F_M. F_V/F_O = (F_M – F_O) / F_O It is important to properly dark adapt samples for this test. This test is a normalized ratio.

TEST SETTINGS

Current test settings are shown in the upper left box. Tap change settings to modify the test parameters. For detailed information on choosing the best settings, see application note 0111

PRESETS

Test settings, default data file name and directory can be saved and recalled using the Load and Save Presets buttons. The contents of a preset file may be viewed from the data management screen. Presets are specific to a test mode.



MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of QA. This is typically observed as a slow increase in Ft. If this happens, an error will result that reduces F_V/F_M.

Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

SATURATION LIGHT

Saturation flashes are used to close all PSII reaction centers. The resulting maximum fluorescence intensity value is used in most measurements.

Intensity
Adjusts the saturation light. 100% represents approximately 15,000 umols of irradiation when using the dark adaptation clip.

Flash Width
Adjustable from 0.4 – 2.0 seconds.

Flash Tail
Length of time added to the end of the test. Used to view data after the saturation flash. Adjustable from 0 – 5 seconds.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Mode
Set for pre-illumination, off, or on during the entire measurement.

DATA MANAGEMENT

Data file settings.

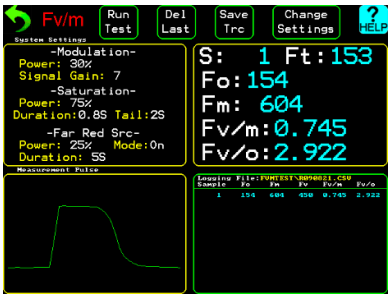
Log File name
Name of file data will be saved to.

Add a note
Adds a note in line with the data stream.

Change sample number
Sets the next sample number. Changing this value will not overwrite any existing data.

Trace log
Turns automatic saving of a fluorescence trace on or off. Default is off. An individual fluorescence trace may be saved by tapping **Save Trc** in the test window.

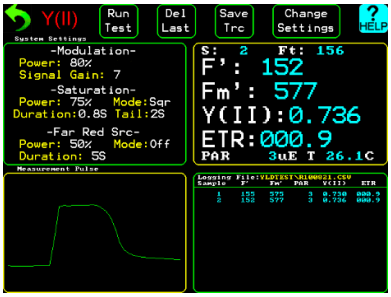
RUN TEST



When ready tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.

Tap the data box (lower right) when the number of samples taken exceeds the available space to review earlier samples.

Y(II)



Quantum Yield of PSII = Y(II) or $(F_{M'} – F_S) / F_{M'}$ -This test is also known as ΔF/FM'.

A fast light adapted test taken at steady state photosynthesis levels. 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 allowing investigation of the photosynthetic process while it is happening. No dark adaption is required.

A PAR clip should be used with the fluorometer to measure Y(II) 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.

For a detailed description of the Y(II) test, see Appendix B

PARAMETERS

S
Sample number

Ft
Current value of detected fluorescence.

F'
At steady state, is the fluorescence level created by 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.

F_M'
The saturation flash value that is not dark-adapted. They are at lowered values due to NPQ or non-photochemical quenching. When this parameter has reached steady state, it is used to calculate Yield of PSII along with Fs. F_M' at steady state is also used to calculate qN, NPQ, qP, qL, Y(NPQ), Y(NO), qE, qT, and qI.

Y(II)
$(F_{M'} – F_S) / F_{M'}$

ETR
Electron transport rate

PAR
Photosynthetically active radiation measured between 400nm and 700nm in uMols

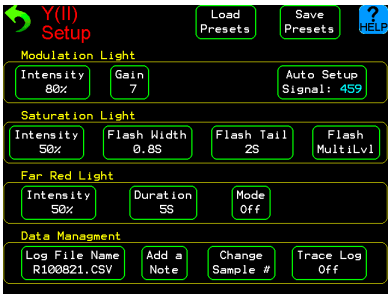
T
Leaf temperature in degrees C

TEST SETTINGS

Current test settings are shown in the upper left box. Tap change settings to modify the test parameters.

There are two windows for changing test parameters. The type of window opened when change settings is tapped is determined by what is being displayed in the upper left box (test settings or actinic settings).

PRESETS



Test settings, default data file name and directory can be saved and recalled using the Load and Save Presets buttons. The contents of a preset file may be viewed from the data management screen. Presets are specific to a test mode.

MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of QA. This is typically observed as a slow increase in Ft.

Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

SATURATION LIGHT

Saturation flashes are used to close all PSII reaction centers. The resulting maximum fluorescence intensity value is used in most measurements.

Intensity
Adjustable from 0 – 100%

Flash Width
Adjustable from 0.4 – 2.0 seconds.

Flash Tail
Length of time added to the end of the test. Used to view data after the saturation flash.

Flash
Selects the shape of the saturation flash.

Square top
Flash duration and intensity may be adjusted. An eight-point rolling average over a 25 ms time frame is used to determine maximum FM and FM' values to eliminate any saturation flash NPQ errors.

MultiLvl
This is a multiple intensity level, phased single saturation flash according to the research of Loriaux 2006 and Loriaux 2013. The Initial flash is 7,000 umols for 0.3 seconds, the down ramp is 20%, the down ramping rate is less than 0.01 mol photons m-2s-2 and then the final intensity flash is at 7,000 umols again for 0.3 seconds, to check for saturation flash NPQ.

Least squares linear regression analysis of 10,000 / PAR, is then used to determine the FM' fluorescence level with an infinitely intense saturation flash. Research has shown that Y(II) measurements, taken under high actinic light conditions, can be underestimated with up to a 22% error, and there can be up to a 41% error in ETR values if this method is not used.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Mode
Set for pre-illumination, off, or on during the entire measurement.

DATA MANAGEMENT

Data file settings.

Log File name
Name of file data is being saved to.

Add a note
Adds a note in line with the data stream.

Change sample number
Sets the next sample number. Changing this value will not overwrite any existing data.

Trace log
Turns automatic saving of a fluorescence trace on or off. Default is off. An individual fluorescence trace may be saved by tapping **Save Trc** in the test window.

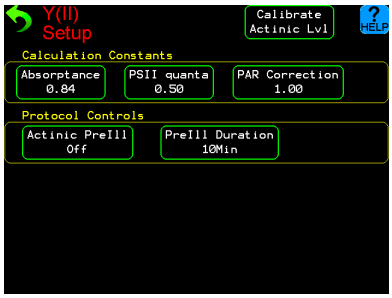
ACTINIC SETTINGS

Tap the upper left box to see the currently active actinic settings.

The actinic source intensity can be set and turned on directly from this box.

Tap the change settings button to configure advanced yield test settings.

CALIBRATE ACTINIC LEVEL



Calibrates the actinic light source.

There are two methods for calibration: With and without a PAR clip.

WITH PAR CLIP

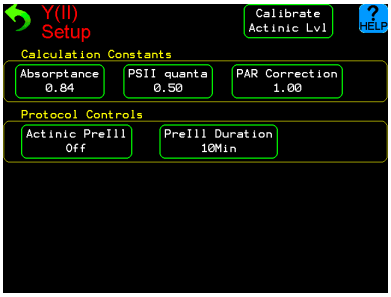
Insert the fiber into the PAR clip and tap Calibrate Actinic Lvl. The instrument will automatically run the calibration routine. The calibration constants will be saved for the active test mode. Calibration constants are not shared between tests.

WITHOUT PAR CLIP

Calibrate the actinic light source using an external PAR sensor. Align the fiber optic probe to the PAR sensor in a manner similar in geometry to how a sample would be situated during a test. Tap Calibrate Actinic Lvl. The actinic source will turn on and a pop-up window will ask for the current PAR reading. Once the value accepted, the actinic source will increase in intensity. Enter this new value. The calibration procedure is now complete. Calibration constants are not shared between tests.

CALCULATION CONSTANTS

Absorptance



0.84 is an average leaf absorptance measurement used to provide an average value. Actual leaf absorptance varies from 0.7 to 0.9 in healthy plants using white light. It also changes for parts of the visible spectrum, plant stress, growing conditions, chlorophyll content, leaf age, species, and light level. It is used in the equation for ETR.

PSII quanta

The ratio of PSII reaction centers to PSI reaction centers. The average here is 0.5, however, in C3 and C4 land plants, the range is from 0.4 in some C4 plants to as high as 0.6 in some C3 plants. It varies with plant type (C3, C4), species, lighting during growing conditions, and under severe carbon deficits. It is used in the equation for ETR.

PAR Correction

PAR clip reading correction factor. The correction range is between 0.01 to 2.00. The default setting is 1.00.

PROTOCOL CONTROLS

Actinic PreIll

Actinic pre illumination. This function uses the internal stabilized white light LED to be used for pre-illumination of the sample. It is held stable using a feedback loop in conjunction with the PAR sensor on the PAR clip. The PAR clip may be used in a darkened room or covered with a black shroud. Intensity may be set from 0 to 2000 umols.

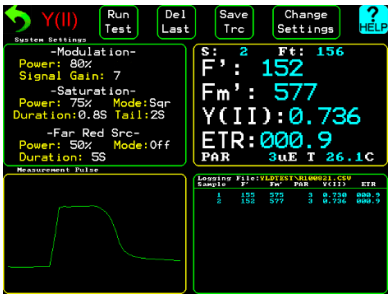
PreIll Duration

Actinic pre illumination duration. This may be set from 0 to 5 hours. The power supply or an external battery pack may be needed for longer illumination times.

RUN TEST

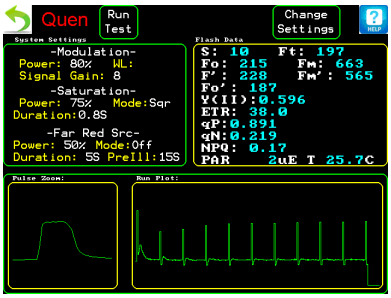
When ready, tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.

Tap the data box (lower right) when the number of samples taken exceeds the available space to review earlier samples.



QUENCHING

Quenching traces are used in measuring photo-protective mechanisms, state transitions, photoinhibition, and passive energy dissipation



For a detailed description see Appendix C

PARAMETERS

S
Sample number

Ft
Current value of detected fluorescence.

Fo
Minimal fluorescence after dark adaptation. It is measured with a modulated light intensity too dim to drive chemical reduction of Q_A and yet bright enough to detect “pre-photosynthetic” antennae fluorescence.

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

F’
steady state value of F_O

Fm’
steady state value of F_M

Fo’
Quenched measurement of F_O

Y(II)
Hendrickson - Klughammer and Schreiber $Y(II) = (F_M' - F_S')/F_M'$
Kramer = $(F_M' - F_S')/F_{MS}$

ETR
Electron transport ratio

Y(NO)
Hendrickson - Klughammer and Schreiber $Y(NO) = F_S/F_M$
Kramer = $1 / (NPQ + 1 + qL(F_M / F_O - 1))$

Y(NPQ)
Hendrickson - Klughammer and Schreiber $=(F_S'/F_M')-Y(NO)$
Kramer = 1- Y(II)-Y(NO)

NPQ
Puddle = $(F_M-F_M')/F_M'$
Puddle = qE+qM+qI or qE+qZ+qI or qE+qT+qI

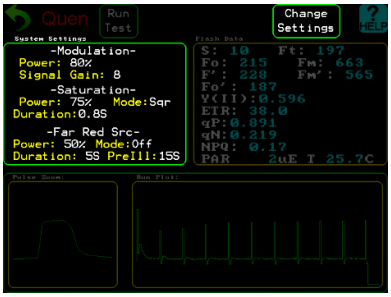
PAR
Photosynthetically active radiation measured between 400nm and 700nm in uMols

T
Leaf temperature in degrees C

TEST SETTINGS

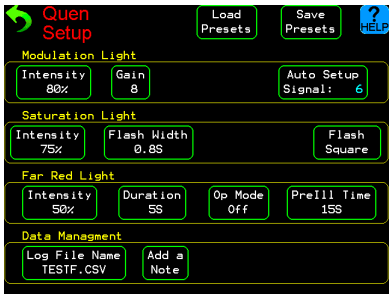
Current test settings are shown in the upper left box. Tap change settings to modify the test parameters.

There are two windows for changing test parameters. The type of window opened when change settings is tapped is determined by what is being displayed in the upper left box (test settings or special settings). Tap on the upper left box to see additional settings.



PRESETS

Test settings, default data file name and directory can be saved and recalled using the Load and Save Presets buttons. The contents of a preset file may be viewed from the data management screen. Presets are specific to a test mode.



MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of QA. This is typically observed as a slow increase in Ft.

Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

SATURATION LIGHT

Saturation flashes are used to close all PSII reaction centers. The resulting maximum fluorescence intensity value is used in most measurements.

Intensity
Adjustable from 0 – 100%

Flash Width
Adjustable from 0.4 – 2.0 seconds.

Flash
Selects the shape of the saturation flash.

Square top
Flash duration and intensity may be adjusted. An eight-point rolling average over a 25 ms time frame is used to determine maximum FM and FM' values to eliminate any saturation flash NPQ errors.

MultiLvl
This is a multiple-phased single saturation flash according to the research of Loriaux 2006 and Loriaux 2013. The Initial flash is 7,000 umols for 0.3 seconds, the down ramp is 20%, the down ramping rate is less than 0.01 mol photons m-2s-2 and then the final intensity flash is at 7,000 umols again for 0.3 seconds, to check for saturation flash NPQ.

Least squares linear regression analysis of 10,000 / PAR, is then used to determine the FM' fluorescence level with an infinitely intense saturation flash. Research has shown that Y(II) measurements, taken under high actinic light conditions, can be underestimated with up to a 22% error, and there can be up to a 41% error in ETR values if this method is not used.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Op Mode
Far-red light operation mode. The far-red light may be turned on or off during the entire quenching protocol test, or just for specific sections. If it is turned on for the entire test, F_O' is still determined in the Kramer and puddle model quenching protocols. If it is turned off, far red light only activates for F_O' determination at the programmed time.

Off
Far red light is off except when F_O' is measured during the Kramer lake model quenching protocol and the puddle model quenching protocol. This is the default setting.

On_All
Far red light will be on during the entire quenching measurement protocol including relaxation, but not before F_O and F_M are measured.

On_Run
Far red light will be on during the entire quenching measurement protocol excluding relaxation, but not before F_O and F_M are measured.

On_Rlx
Far red light will be on during relaxation, but not before F_O and F_M are measured and not during the other phases. It will also be on for F_O' determination in Kramer and puddle protocols.

Pre
Far red light will be on before F_O and F_M are measured and for F_O' determination in Kramer and puddle protocols.

Pre & All
Far red light will be on before F_O and F_M are measured, and during the entire quenching measurement protocol including relaxation.

Pre&Run
Far red light will be on before F_O and F_M are measured and during light adapted portion of the quenching protocol.

Pre&Rlx
Far red light will be on before F_O and F_M are measured and during dark adapted quenching relaxation portion of the quenching protocol. It will also be on for F_O' determination in Kramer and puddle protocols.

PreIll Time
Actinic pre illumination duration. This may be set from 1 to 60 seconds.

DATA MANAGEMENT

Data file settings.

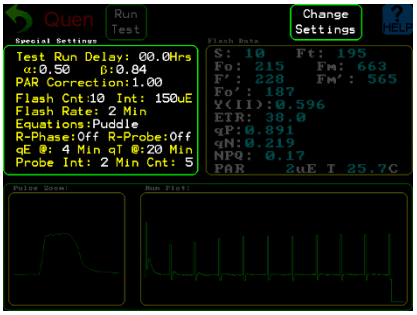
Log File name
Name of file data is being saved to.

Add a note
Adds a note in line with the data stream.

SPECIAL SETTINGS

Tap the upper left box to see the currently active special settings.

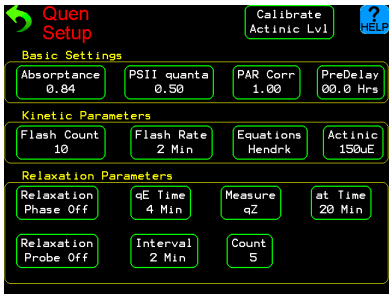
To modify these settings, tap change settings.



CALIBRATE ACTINIC LEVEL

Calibrates the actinic light source.

There are two methods for calibration: With and without a PAR clip.



WITH PAR CLIP

Insert the fiber into the PAR clip and tap Calibrate Actinic Lvl. The instrument will automatically run the calibration routine. The calibration constants will be saved for the active test mode. Calibration constants are not shared between tests.

WITHOUT PAR CLIP

Calibrate the actinic light source using an external PAR sensor. Align the fiber optic probe to the PAR sensor in a manner similar in geometry to how a sample would be situated during a test. Tap Calibrate Actinic Lvl. The actinic source will turn on and a pop-up window will ask for the current PAR reading. Once the value accepted, the actinic source will increase in intensity. Enter this new value. The calibration procedure is now complete. Calibration constants are not shared between tests.

BASIC SETTINGS

Absorptance
0.84 is an average leaf absorptance measurement used to provide an average value. Actual leaf absorptance varies from 0.7 to 0.9 in healthy plants using white light. It also changes for parts of the visible spectrum, plant stress, growing conditions, chlorophyll content, leaf age, species, and light level. It is used in the equation for ETR.

PSII quanta
The ratio of PSII reaction centers to PSI reaction centers. The average here is 0.5, however, in C3 and C4 land plants, the range is from 0.4 in some C4 plants to as high as 0.6 in some C3 plants. It varies with plant type (C3, C4), species, lighting during growing conditions, and under severe carbon deficits. It is used in the equation for ETR.

PAR Correction
PAR clip reading correction factor. The correction range is between 0.01 to 2.00. The default setting is 1.00.

Pre delay
Dark adaptation timer. Can be set for a period of up to 25 hours. See the section on dark adapting for more information

KINETIC PARAMETERS

Flash count
Number of saturation flashes that will occur while the actinic light is on. It ranges from 1 to 99

Flash rate
Time between saturation flashes. It ranges from 1 minute to 99 minutes. See the section on chloroplast migration for more information

Equations
For more information Appendix C.

Kramer
Kramer lake model protocol parameters that use F_O' in their calculations and include q_L

Puddle
model protocol parameters

Hendrk
Hendrickson –Klughammer lake model protocol parameters that do not use F_O' in their calculation, but do include NPQ, resurrected from the puddle model

Actinic
Actinic light intensity used to drive photosynthesis. May be set from 10 to 1,800 umols. Intensity is held constant by using the PAR clip sensor.

RELAXATION PARAMETERS

Relaxation phase
When turned on, measures q_E, q_M, q_Z, q_T, or q_I.

qE time
If relaxation phase is on, q_E can be set.

Measure
qT, qM, qZ
Record selected value. These are intermediate relaxation phase values.

At time
This is the time that q_T, q_M, or q_Z will be measured.

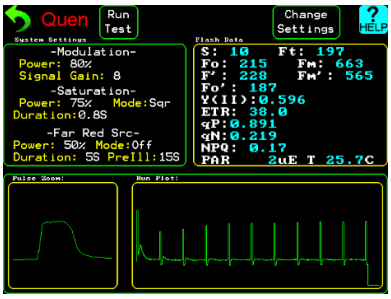
Relaxation probe
Use relaxation saturation flashes. If turned off, only relaxation saturation flashes that occur during the "relaxation phase on" mode will be at the selected times for q_E and either q_T, q_M or q_Z.

Interval
Time between relaxation saturation flashes. Saturation flashes should be at least 2 minutes apart to prevent a buildup of NPQ. Make sure that this distance corresponds to the q_E and q_M (or other intermediate saturation flashes) or an additional flash will occur without proper spacing.

Count
Number of saturation flashes. If the number of flashes selected does not reach the intermediate relaxation time, a flash will still occur at the intermediate relaxation flash time selected.

RUN TEST

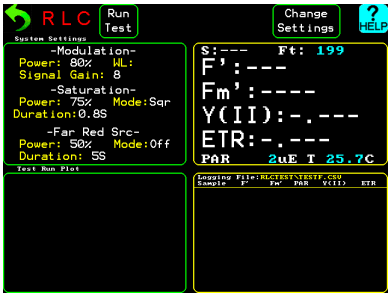
When ready, tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.



Tap the data box (lower right) when the number of samples taken exceeds the available space to review earlier samples.

Tap the lower box to change the view from graphical to tabulated data.

RLC



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 different intensities. Yield and ETR values for each step are recorded.

Relative ETR (rETR) is calculated using a Yield measurement taken at each specified light level. By plotting rETR vs. PAR, potential ETR rates, photosynthetic capacity, as well as ETR rate limitations at given light intensities or leaf temperatures can be determined. (U. Schreiber 2004).

For additional information, see Appendix D

PARAMETERS

S
Sample number

Ft
Current value of detected fluorescence.

F'
At steady state, is the fluorescence level created by 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.

F_M'
The saturation flash value that is not dark-adapted. They are at lowered values due to NPQ or non-photochemical quenching. When this parameter has reached steady state, it is used to calculate Yield of PSII along with Fs. F_M' at steady state is also used to calculate q_N, NPQ, q_P, q_L, Y(NPQ), Y(NO), q_E, q_T, and q_I.

Y(II)
$(F_{M'} - F_S) / F_{M'}$

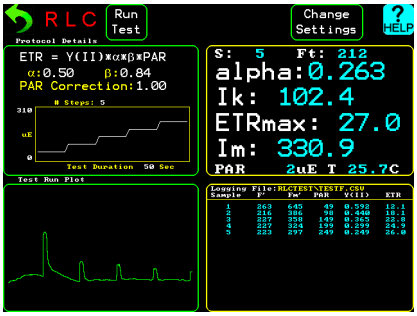
ETR
Electron transport rate

PAR
Photosynthetically active radiation measured between 400nm and 700nm in uMols

T
Leaf temperature in degrees C

POST TEST PARAMETERS

S
Sample number



Ft
Current value of detected fluorescence.

alpha
The initial slope of line at low PAR values created by relating ETR to PAR. It provides a measure of quantum efficiency (Schreiber 2004)

Ik
Ik = ETRmax /α. A measurement of the point where light saturation dominates, or the minimum saturation level (Schreiber 2004).

ETRmax
ETRmax is a measure of a leaf's photosynthetic capacity (maximum) electron transport rate (Schreiber 2004).

Im
Estimated saturation level

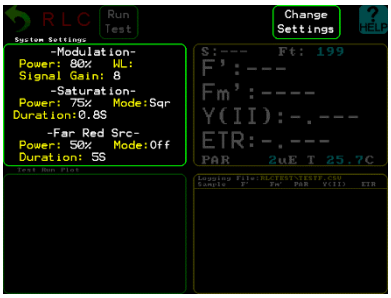
PAR
Photosynthetically active radiation measured between 400nm and 700nm in uMols

T
Leaf temperature in degrees C

TEST SETTINGS

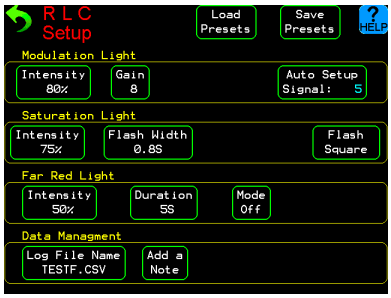
Current test settings are shown in the upper left box. Tap change settings to modify the test parameters.

There are two windows for changing test parameters. The type of window opened when change settings is tapped is determined by what is being displayed in the upper left box (test settings or protocol details).



PRESETS

Test settings, default data file name and directory can be saved and recalled using the Load and Save Presets buttons. The contents of a preset file may be viewed from the data management screen. Presets are specific to a test mode.



MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of QA. This is typically observed as a slow increase in Ft.

Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

SATURATION LIGHT

Saturation flashes are used to close all PSII reaction centers. The resulting maximum fluorescence intensity value is used in most measurements.

Intensity
Adjustable from 0 – 100%

Flash Width
Adjustable from 0.4 – 2.0 seconds.

Flash
Selects the shape of the saturation flash.

Square top
Flash duration and intensity may be adjusted. An eight-point rolling average over a 25 ms time frame is used to determine maximum FM and FM' values to eliminate any saturation flash NPQ errors.

MultiLvl
This is a multiple intensity level, phased single saturation flash according to the research of Loriaux 2006 and Loriaux 2013. The Initial flash is 7,000 umols for 0.3 seconds, the down ramp is 20%, the down ramping rate is less than 0.01 mol photons m-2s-2 and then the final intensity flash is at 7,000 umols again for 0.3 seconds, to check for saturation flash NPQ.

Least squares linear regression analysis of 10,000 / PAR, is then used to determine the FM' fluorescence level with an infinitely intense saturation flash. Research has shown that Y(II) measurements, taken under high actinic light conditions, can be underestimated with up to a 22% error, and there can be up to a 41% error in ETR values if this method is not used.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Mode
Set for pre-illumination, off, or on during the entire measurement.

DATA MANAGEMENT

Data file settings.

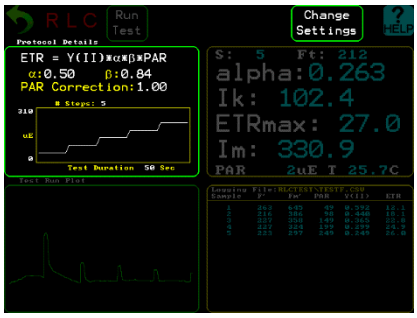
Log File name
Name of file data is being saved to.

Add a note
Adds a note in line with the data stream.

PROTOCOL DETAILS

Tap the upper left box to see the currently active protocol details

Tap the change settings button to configure test settings.



CALIBRATE ACTINIC LEVEL

Calibrates the actinic light source.

There are two methods for calibration: With and without a PAR clip.

WITH PAR CLIP

Insert the fiber into the PAR clip and tap Calibrate Actinic Lvl. The instrument will automatically run the calibration routine. The calibration constants will be saved for the active test mode. Calibration constants are not shared between tests.

WITHOUT PAR CLIP

Calibrate the actinic light source using an external PAR sensor. Align the fiber optic probe to the PAR sensor in a manner similar in geometry to how a sample would be situated during a test. Tap Calibrate Actinic Lvl. The actinic source will turn on and a pop-up window will ask for the current PAR reading. Once the value accepted, the actinic source will increase in intensity. Enter this new value. The calibration procedure is now complete. Calibration constants are not shared between tests.

CALCULATION CONSTANTS

Absorptance

0.84 is an average leaf absorptance measurement used to provide an average value. Actual leaf absorptance varies from 0.7 to 0.9 in healthy plants using white light. It also changes for parts of the visible spectrum, plant stress, growing conditions, chlorophyll content, leaf age, species, and light level. It is used in the equation for ETR.

PSII quanta

The ratio of PSII reaction centers to PSI reaction centers. The average here is 0.5, however, in C_3 and C_4 land plants, the range is from 0.4 in some C_4 plants to as high as 0.6 in some C_3 plants. It varies with plant type (C_3, C_4), species, lighting during growing conditions, and under severe carbon deficits. It is used in the equation for ETR.

PAR correction
PAR clip reading correction factor. The correction range is between 0.01 to 2.00. The default setting is 1.00.

STEP SETTINGS

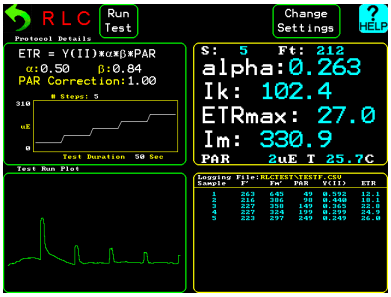
The number of steps, the duration of a step, and the light intensity at that step are set using the step buttons. While almost any process may be tried, it is common to start at 20 to 50 umols and step the RLC up to at least one value above saturation.

Green buttons are steps that will be used to measure samples and red buttons are disabled. Each step has a duration (dwell) and actinic source intensity. Tapping on an enabled step will allow you to edit or disable that step. Tapping on a disabled button will display a keypad asking for the dwell time (10 to 60 seconds) and actinic level (10 to 1,800 umols). Up to 12 steps may be used.

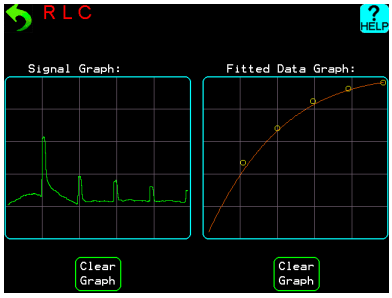
This protocol is designed to be used with the PAR clip. It is recommended that the PAR clip be mounted on a tripod of some type. Insert the leaf into the PAR clip chuck, cover the PAR clip with a dark cloth for a specific dark adapted time, and tap run test.

RUN TEST

When ready, tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.

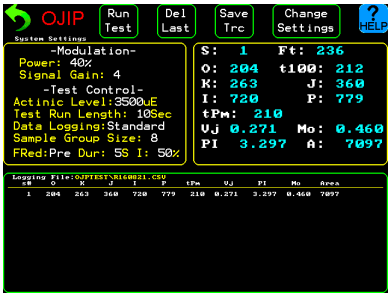


After a test is complete, tap the lower left box to view more detailed graphical data.



OJIP

The Strasser OJIP protocol focuses primarily on plant stress measurement with direct read-out of the most used protocol parameters. All of the Strasser measuring parameters are recorded in the data file.



For additional information on this test protocol, see Appendix E

DISPLAYED PARAMETERS

S
Sample number

Ft
Current value of detected fluorescence.

O
Origin – Fluorescence measured at 20 uS

T_{100}
Fluorescence value at 100 uS

K
Fluorescence value at 300 uS

J
Fluorescence value at 2 mS

I
Fluorescence value at 30 mS

P
Maximum fluorescence

tPm
Time to reach P

Vj
$Vj = (F_j – F_o) / F_M\text{-}F_O$

Mo
$MO = (F_{300} – F_{50}) / (F_M – F_{50}) / 0.25ms$

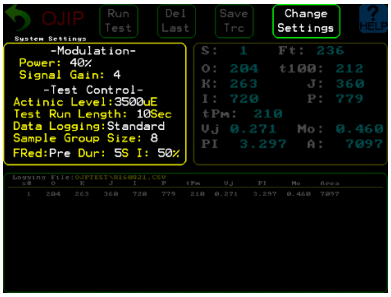
PI
PIABS = (Vj / Mo) (F_V/F_M) (F_V/F_O) ((F_M-F_j)/ (F_j-F_o))

A
Area above the curve from t_0 to t_{FM} and from F_O to F_M

Additional parameters are recorded in the data file.

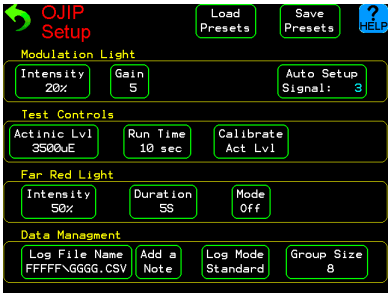
TEST SETTINGS

Current test settings are shown in the upper left box. Tap change settings to modify the test parameters.



MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of QA. If this happens, an error will result that reduces P and raises O. The correct setting will allow a fluorescence signal measurement without the reduction of any QA.



Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

TEST CONTROLS

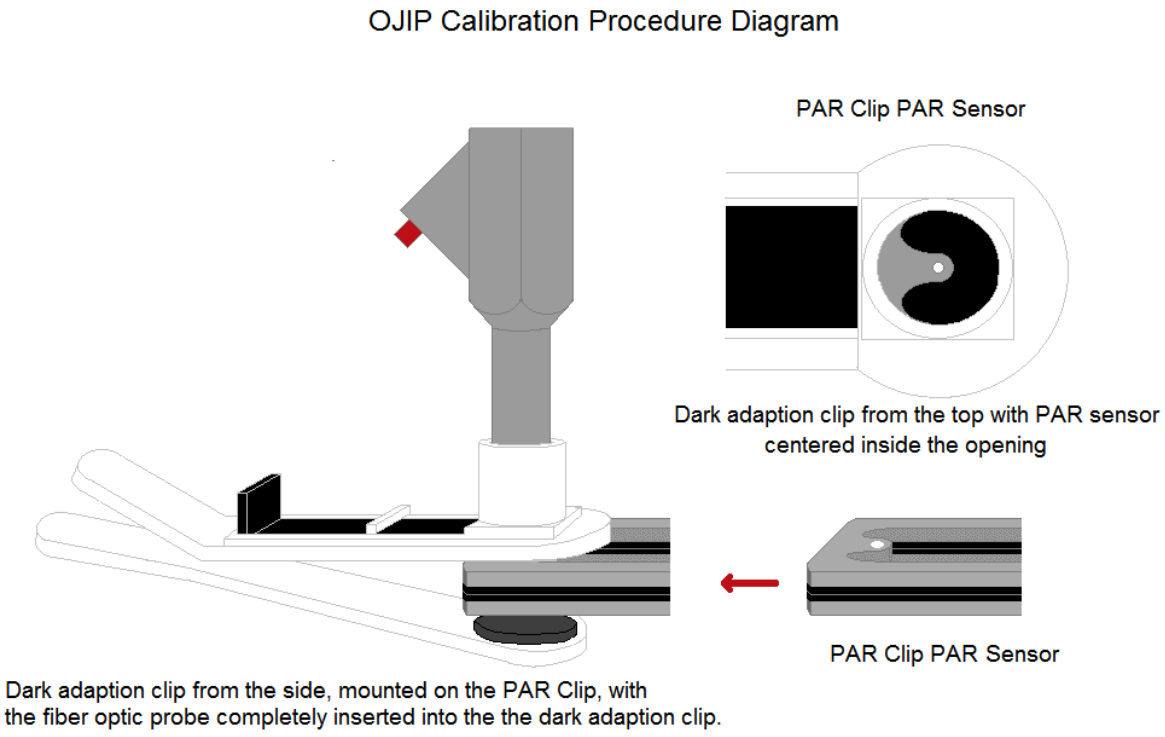
Actinic level
Light intensity at the leaf. 3,500 :mols is the default value.

Run time
May be adjusted from 3 to 255 seconds.

Calibrate act lvl
Calibrates the actinic light source.

There are two methods for calibration: With and without a PAR clip.

With PAR clip
Place a dark adaption clip on the PAR sensor with the sensor located approximately in the center of the dark adaption clip. Verify that the shutter is in the open position and insert the fiber optic probe into the dark adaption clip all the way (See below).



Tap Calibrate Actinic Lvl. The instrument will automatically run the calibration routine. The calibration constants will be saved for the active test mode. Calibration constants are not shared between tests.

Without PAR clip
The actinic light source can be calibrated using an external PAR sensor. Align the fiber optic probe to the PAR sensor in a manner similar how a sample would be during a test. Tap Calibrate Act Lvl. The actinic source will turn on and a pop-up window will ask for the current PAR reading. Once the value accepted, the actinic source will increase in intensity. Enter this value. The calibration procedure is now complete. Calibration constants are not shared between tests.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Mode
Set for pre-illumination, off, or on during the entire measurement.

DATA MANAGEMENT

Data file settings.

Log File name
Name of file data is being saved to.

Add a note
Adds a note in line with the data stream.

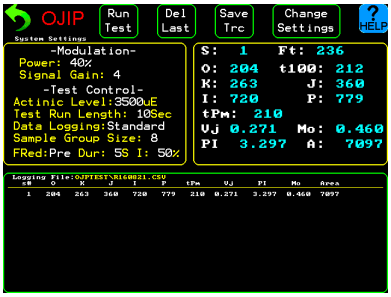
Log mode
Standard for single test graph of measurements
Grouped to graphically overlay multiple measurements

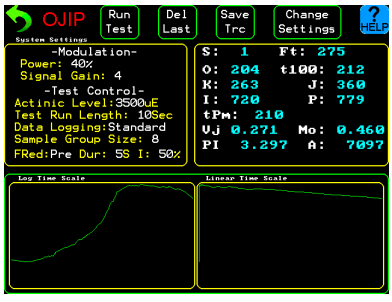
Group size
May be set from 2 to 32. This will allow graphic overlay of up to 32 separate OJIP measurements.

RUN TEST

When ready, tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.

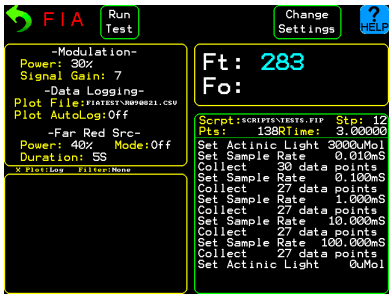
Tap the data box (lower part of window) to view a graphical representation of the data.





FIA

A special OJIP quenching protocol designed in conjunction with Wim Vredenberg. It is designed to allow investigation of quenching phenomenon with high speed time resolution, and measurement of saturation pulse NPQ after microseconds, milliseconds or seconds.



PARAMETERS

Ft
Current value of detected fluorescence.

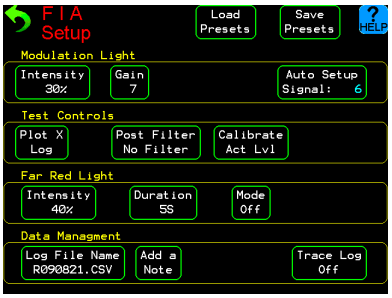
Fo
Minimal fluorescence after dark adaptation. It is measured with a modulated light intensity too dim to drive chemical reduction of Q_A and yet bright enough to detect “pre-photosynthetic” antennae fluorescence.

TEST SETTINGS

Current test settings are shown in the upper left box. Tap change settings to modify the test parameters. Choose the type of changes you wish to make from the pop-up window. The choices are settings or script.

PRESETS

Test settings, default data file name and directory can be saved and recalled using the Load and Save Presets buttons. The contents of a preset file may be viewed from the data management screen. Presets are specific to a test mode.



MODULATION LIGHT

Intensity
Adjustable from 0 to 100%. Tap to adjust the modulated light source. The intensity must be high enough to measure a fluorescence signal but not so high that it will cause a partial reduction of Q_A. This is typically observed as a slow increase in F_t.

Gain
Detector gain is adjustable from 1 to 16.

Auto Setup
Starts an automated routine to set the modulated light intensity and detector gain. Place a dark clip on the type of leaf that will be used, insert the fiber optic into the opening of the dark clip and then open the shutter. Tap the auto setup button. Beeps will sound while the light source and detector gain is adjusted to optimal settings.

TEST CONTROLS

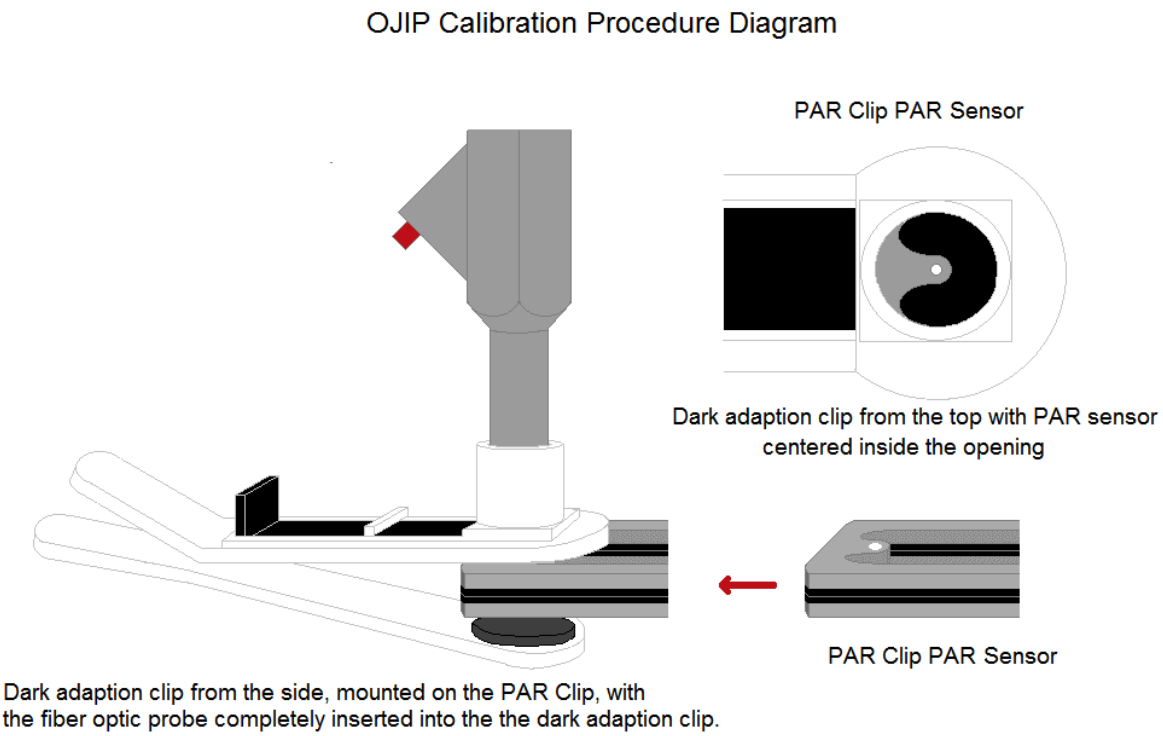
Plot X
Sets the graphic display X-axis scale to logarithmic or linear.

Post filter
Filters collected data.

Calibrate act lvl
Calibrates the actinic light source.

There are two methods for calibration: With and without a PAR clip.

With PAR clip
Place a dark adaption clip on the PAR sensor with the sensor located approximately in the center of the dark adaption clip. Verify that the shutter is in the open position and insert the fiber optic probe into the dark adaption clip all the way (See below).



Tap Calibrate Actinic Lvl. The instrument will automatically run the calibration routine. The calibration constants will be saved for the active test mode. Calibration constants are not shared between tests.

Without PAR clip

The actinic light source can be calibrated using an external PAR sensor. Align the fiber optic probe to the PAR sensor in a manner similar how a sample would be during a test. Tap Calibrate Act Lvl. The actinic source will turn on and a pop-up window will ask for the current PAR reading. Once the value accepted, the actinic source will increase in intensity. Enter this value. The calibration procedure is now complete. Calibration constants are not shared between tests.

FAR RED LIGHT

Provides light above 700nm to drive PSI, drain PSII of electrons, and allow the rapid re-oxidation of PSII. It can be used for pre-illumination and rapid re-oxidation of PSII.

Intensity
Adjustable from 0 – 100%

Duration
Adjustable from 1 – 15 seconds.

Mode
Set for pre-illumination, off, or on during the entire measurement.

DATA MANAGEMENT

Data file settings.

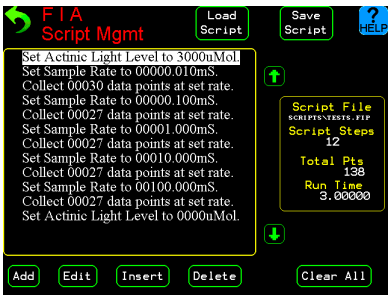
Log File name
Name of file data is being saved to.

Add a note
Adds a note in line with the data stream.

Trace log
Turns automatic saving of a fluorescence trace on or off. Default is off. An individual fluorescence trace may be saved by tapping **Save Trc** in the test window.

EDIT SCRIPT

Tap Change Settings and then select Edit Script from the pop-up menu to create and edit scripts. Scripts may also be edited using a text editor.



A test is comprised of a series of user programmed script commands. Scripts can be saved for later use.

The large box will display any currently loaded script commands. Green up/down arrows may be used to scroll through the script.

Editor functions

Add
Adds a line to the end of the existing script.

Edit
Edits the highlighted line.

Insert
Inserts a new line under the selected line.

Delete
Deletes the highlighted line.

For the add and insert functions, a pop-up box will appear with options.

Pt Rate
Adds a sample rate command. Range is from 0.01 to 10,000 mS. Resolution is 10uS

Pt Count
Adds a data point count command. Range is from 1 to 65,280 data points

Source
Adds a source with intensity command

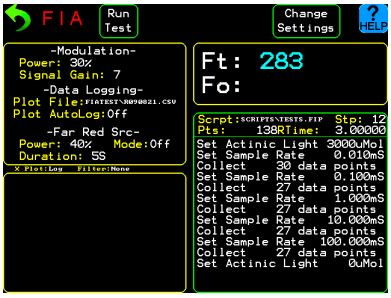
Two sources are available
Actinic, with a level of 0 to 5,000 uMol
Far Red with a level of 0 to 100%

Scripts will be checked for simple errors before exiting the window.

A sample script is included with the instrument.

RUN TEST

When ready, tap run test, or depress the trigger switch on the light guide. Data will be displayed when the measurement is completed.



DATA FORMATS

TECHNICAL SPECIFICATIONS

APPENDIX A – F_V/F_M PROTOCOL

Adapted from application note #0111

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

Test guidelines for a reliable measurement.

Dark-adapt properly knowing the plant's light history.
It takes only a few minutes for the xanthopyll cycle and the Δph of the thylacoid lumen to return to a dark-adapted state. It can take up to 4 minutes with indoor and green house plants, and it can take up to 7 minutes in field plants (Baker 2008). Recently Cazzaniga (2013) found that the intermediate fluorescence change previously attributed to state transitions, and acute phtoinhibition, were in fact due to chloroplast migration in C3 plants. Maai (2011) found that chloroplast migration was also a mechanism present in in C4 plants. It was found that it takes from 20 minutes to 35 minutes for complete chloroplast migration to occur. In lower plants there is evidence that state transitions occur. State transitions however, take between fifteen to twenty minutes (Ruban 2009) (Lichtenthaler 1999). These times can vary somewhat in field plants, and can take slightly longer. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton (MacIntyre 1997). In addition, field plants and other plants that have been exposed to photoinhibition conditions for a number of hours, will retain a certain amount of NPQ for up to 60 hours (Lichtenthaler 2004). This means that even if dark adaptation is overnight, there will almost always be some residual NPQ built into most summer field measurements of F_V/F_M. This is all right if one is measuring "light stress" and comparing results, but when measuring other types of plant stress, light history should be taken into account when comparing samples. It is common for researchers to choose dark adaptation times anywhere from twenty minutes to overnight, using pre-dawn values. Shorter times may be used to study the effects of plant protective mechanisms. If possible, testing should be done to find the time required to reach a stable steady dark adapted state. If not, then 35 minutes is safe for F_V/F_M measurements with a similar light history.

Modulation light intensity setting.
Minimum fluorescence is a "pre- photosynthetic" dark adapted value measured by exposing the leaf antennae to a very low intensity modulated light. The intensity must be set properly to allow detection, but not high enough to drive photosynthesis and provide an F_O value that is too high. When setting the modulating light intensity, the Ft value should not rise over a period of 30 seconds. If it does, the intensity must be lowered. This instrument includes an option to automatically set the best modulation intensity and detector gain for many samples.

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

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

Compare samples with a similar light history.
Field plants should only be compared to field plants and green house plants should be compared to green house plants. Due to the fact that it can take up to 60 hours for chronic photoinhibion to relax, photoinhibion can be

involved in some measurements more than others (Lichtenthaler 2004). Results after a sunny day in the summer may be different than measurements on the same plant after a few days of overcast, again because it takes a long time for photoinhibition to relax or repair.

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

The duration of the saturation pulse should be between 0.5 seconds and 1.5 seconds for higher plants, and 25 to 50 milliseconds for Phytoplankton and cyanobacteria. (Schreiber 1995). Times outside these ranges increase the error in F_V/F_M 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. This instrument provides a moving 25 ms average to determine the highest F_M. This ensures that a reliable value will be measured even if the saturation pulse width or duration is too long.

Saturation pulse intensity.
Dark adapted leaves saturate easily with lower saturation pulse intensities. It may take a few hundred umols to saturate shade leaves and sun leaves will saturate below 1,500 umols. Lower values may not fully saturate PSII, and provide an error. Higher values always work with dark adapted samples. (Ralph 2005)

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

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

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

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APPENDIX B – Y(II) PROTOCOL

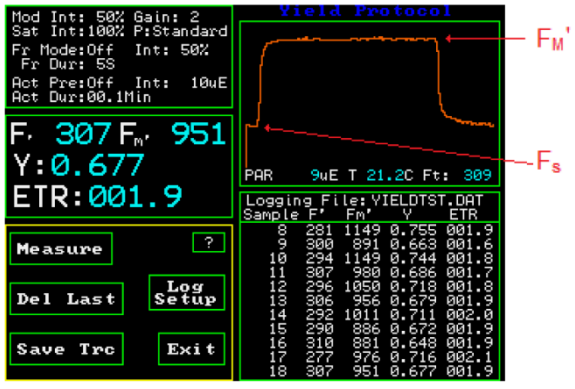
Adapted from application note #0509

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

Quantum Yield of PSII is a measurement ratio that represents achieved efficiency of photosystem II under current steady-state photosynthetic lighting conditions. (Genty 1989, 1990), (Maxwell K., Johnson G. N. 2000), (Rascher 2000) It is affected by closure of reaction centers, regulated and non regulated heat dissipation involved with non-photochemical quenching (Schreiber 2004). Recently, it was shown that chloroplast migration also affected Y(II) at high actinic light levels (Cazzaniga S. 2013). (See the qM application note for more details about chloroplast migration)

As ambient light irradiates a leaf, an average of 84% is absorbed by the leaf, and approximately 50% of that light is absorbed by the antennae associated with photosystem II and transferred to PSII (Photosystem II) reaction centers. Leaf absorption varies by leaf age, growing conditions, chlorophyll content, plant stress, light level, and light quality (Eichelman H. 2004), (Cazzaniga S. 2013). In a healthy leafs of land plants, absorption can range from 0.7 to 0.9 (Eichelman H. 2004). In algae and macro-algae absorption ranges from 0.36 to 0.96 (Nielsen H. D 2008). The ratio of PSII reaction centers varies by plant type, plant species, and sun vs. shade light level during growth. The range in land plants is from 0.4 in some C_4 plants to 0.6 in some C_3 plants (Laisk A. 1996) (Edwards G.E. 1993). 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. At this point, a modulated fluorometer can be used to expose a plant to a very intense short light pulse called a saturation pulse. It is designed to momentarily close or chemically reduce all capable PSII reaction centers while at steady state photosynthesis. 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.



Graphic display of a single Yield of PSII or Y(II) measurement taken with a PAR Clip.

F_M' is maximum fluorescence during a saturation pulse. FS is steady state fluorescence at a specific actinic light level before the saturation pulse.

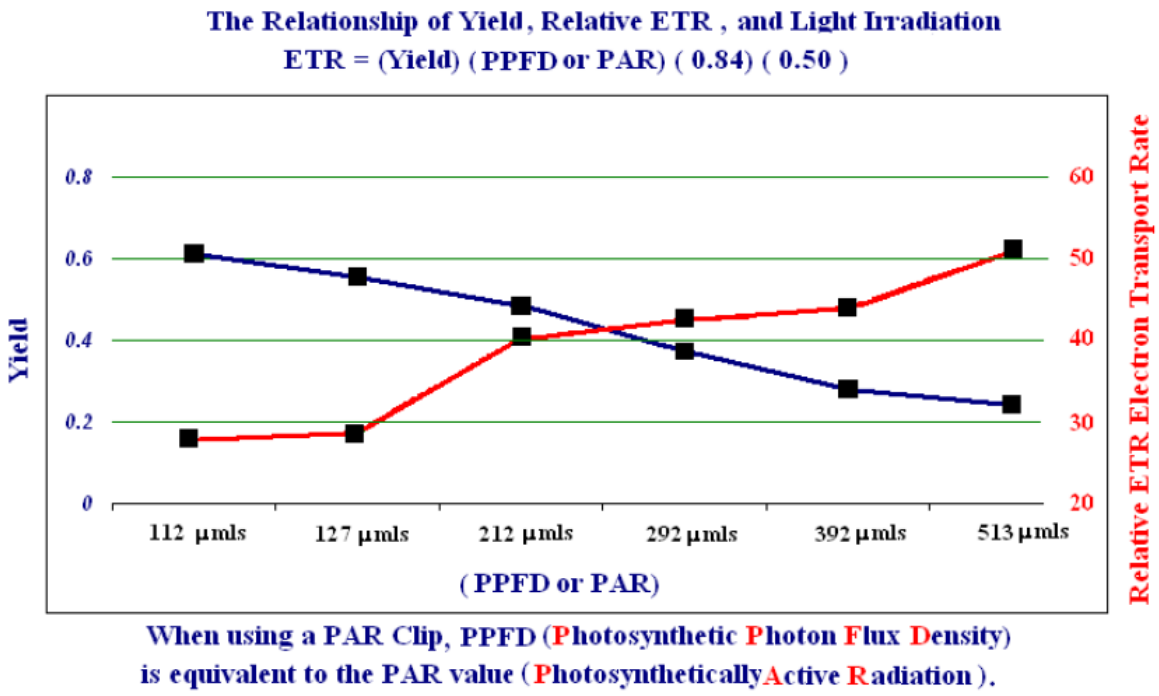
$Y(II) = (F_M' - F_S) / F_M'$

Yield of PSII will change at different light levels and temperatures so that is highly recommended measure PAR or PPFD at the same location and at the same angle as the leaf. If the leaf angle is changed, the leaf is no longer at steady state photosynthesis and errors will result. 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.

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) (micromols and microeinsteins are equivalent, and when using a PAR Clip, PAR and PPFD are equivalent). If irradiation and temperature are not controlled, it is possible to falsely interpret data. 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. If the leaf angle is changed, it is no longer at steady state. Extraneous reflections and breathing on the sample should also be avoided (Rosenqvist and van Kooten 2006).

PAR Clips also allow measurement of relative ETR or relative Electron Transport Rate. ETR is a parameter designed to measure the electron transport of PSII. It has also been found to correlate well with CO_2 assimilation (with a few exceptions listed below). More advanced fluorometers provide built-in actinic illuminators for greater experimental control of light irradiation intensity. This allows pre-illumination with a controlled predetermined intensity value for sample comparison. ETR should only be used for comparison between samples if leaf absorptance and the PSII/PSI ratio is known (Baker 2008). Y(II) is the better choice for comparing samples if leaf absorptance and PSII/PSI ratio are not known. (A future Application Note will deal more fully with relative ETR and PAR or PPFD measurement).

For reliable Y(II) and ETR measurements, photosynthesis must be at steady state and with illumination on the same side of the leaf that is being measured (see number eight under correlation to carbon assimilation). Steady state photosynthesis is an equilibrium condition reached after a several minutes of exposure to existing light radiation conditions. 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. However, recent research shows that this may not be the case under high actinic light conditions. Under high actinic light conditions it can take between 20 to 35 minutes to reach steady state photosynthesis due to chloroplast migration. Chloroplast migration is a light avoidance mechanism that causes fluorescence change over the time frame previously thought to be caused by state transitions, and acute photoinhibition. It changes chlorophyll fluorescence output, and leaf absorptance. (For more information on chloroplast migration get the application note on qT and qM at optisci.com. Measurements taken under variable lighting conditions may not provide reliable Y(II) results (Rascher 2000). No dark adaptation is required for Y(II) measurements; however, a stable actinic light source is recommended for pre illumination at specific actinic light levels, or the use of steady solar radiation.



Correlation to Carbon assimilation:

In 1989, Genty developed the quantum yield of PSII measurement and provided strong evidence of a linear correlation between Y(II) measurements, Electron Transport Rate, and CO_2 assimilation for C_4 plants (Baker and Oxborough 2004) and many others have confirmed the relationship (Edwards and Baker 1993), (Krall and Edwards 1990, 1991), Siebke 1997). It was found that a curve-linear correlation between Y(II) and CO_2 assimilation exists for C3 species where photorespiration can also use significant products of electron transport (Genty 1990), (Harbinson 1990), (Baker and Oxborough 2004).

The strong relationship between Yield and CO_2 assimilation correlation has been reaffirmed repeatedly by many researchers with the following caveats:

1. There is 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 ms causing multiple turnovers of primary PSII receptor QA and the reduction of plasotoquinone to plastoquinol. This raises FM' and can cause an overestimate of Y(II) 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, indicating that under these conditions other electron sinks are at work. The ratio of ETR (a product of Y(II), PAR, leaf

absorption ratio, and PSII absorption ratio) to CO_2 assimilation under cold stress can be diagnostic for cold stress. (Fryer M. J., Andrews J.R., Oxborough K., Blowers D. A., Baker N.E. 1998)

4. The ratio of ETR to CO_2 assimilation can be diagnostic for drought stress in C_3 plants. C_3 plants exhibit strong electron transport rates for early and moderate levels of drought stress even when CO_2 assimilation has decreased due to water stress. This indicates that there are other electron sinks for electron transport. (Flexas 1999), (Flexas 2000), (Ohashi 2005). This problem of early drought stress measurement and detection may be overcome by using the Burke assay that includes Y(II). (Burke 2007, Burke 2010). Y(II) and ETR work well for drought stress measurement in C4 plants because there is no significant photorespiration. (See the application note on drought stress)

5. Mangrove leaves growing in the tropics. Here again electron transport rate is more than 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). At high light levels near leaf saturation, it can take between 20 minutes and 35 minutes to reach steady state photosynthesis due to chloroplast migration (Cazzaniga S. 2013).

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, Loriaux 2008, 2013) and Earl (2004) uses saturation pulses at various levels and extrapolates the results of a saturation pulse at infinity using linear regression analysis. This method restores the correlation of ETR and CO_2 assimilation and it is an option that is offered On the Opti-Sciences OS5p+ and the OS1p.

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). In C3 plants, correlation can break down under photorespiratory conditions found in drought stress. In C_3 plants, drought stress will only be detected when it is severe (Flexas 1999, 2000). Here, the Burke drought stress assay is recommended (Burke 2007, 2010).

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

10. At high actinic light levels, a light avoidance mechanism called chloroplast migration occurs. This process takes between 20 to 35 minutes at steady high actinic light levels near saturation. Chloroplast migration affects chlorophyll fluorescence levels during the time frame that was thought to be caused by state transitions and acute photoinhibition time scales in the past. It occurs in nature and with either intense white light or intense blue actinic light. As a result, it can take from 20 to 35 minutes to reach steady state photosynthesis at high actinic light levels. Intense red light and low intensity blue light do not affect chloroplast migration in a realistic way and can cause measuring artifacts. Choroplast migration is modulated by blue light intensity (Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., 2013).

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 (Flexas 1999, 2000), (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) (Baker 2008).

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

Stress	**Yield – Y(II)**	**F_V/F_M**
Drought Stress	Y(II) Is not sensitive to drought stress in C_3 plants until the drought is severe due to photorespiration (Flexas 1999, 2000). *The only reliable fluorescencetest is Y(II) using the Burke assay. It is very* sensitive to *very early water stress in* C_3 *plants.* (Burke 2007, 2010)	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (ZivcakM., Brestic M, Olsovska K. Slamka P. 2008)
Drought Stress	In C_4 plants Y(II) and ETR can be used for reliablyfor drought stress. The ratio of ETR to carbon assimilation is consistent. It does not work in C3 plants. ETR = (Yield) (PAR) (0.84)(0.50) (Cavender-Bares & Bazzaz 2004) Y(II) is sensitive to drought stress in C_4 plants(da Silva J. A. & Arrabaca M.C. 2008).	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (ZivcakM., Brestic M, Olsovska K. Slamka P. 2008). Fv/Fm is not sensitive to water stress in C4 plants, grasses are tested (da Silva J. A. & Arrabaca M.C.2008).
Drought Stress	Fs a component of Y(II) is sensitive to moderate drought stress at saturation light levels. Fs/Fo is a normalized ratio that uses predawn dark adaptationand steady state fluorescence measurement at highlight levels for moderate drought stress. While adequate for plants such as grapes, it is does not work well for most plants. (Flexas 1999), (Flexas 2000), (Flexas 2002).	Not sensitive to early or moderate water stress in most plants (Bukhov & Carpentier 2004) (ZivcakM., Brestic M, Olsovska K. Slamka P. 2008)
Nitrogen Stress	Y(II) can be used for *early* nitrogen stress by adding*intense light* (Cheng 2001). *It is common to use chlorophyll content meters for nitrogen stress.*	F_V/F_M is not sensitive to nitrogen stress until very low levels are reached. (Baker 2004) *It is commonto use chlorophyll content meters for nitrogen stress.*

Light stress	Y(II) can also be used for light stress in steady state sensitive to light stress. (Cavender-Bares & Bazzaz 2004)	F_V/F_M can be used to detect light stress (Adams & Demming-Adams 2004)
Heat stress	Y(II) can also be used for moderate heat stress from 35 degrees centigrade and higher in oak.(Haldimann P, & Feller U. 2004)	F_V/F_M can be used to detect severe heat stress in cotton above 45 degrees centigrade. (Crafts-Branderand Law 2000)
Sulfur Stress	Y(II) is not sensitive to sulfur stress until starvationlevels are reached. (Baker 2004) *It is common to use chlorophyll content meters fornitrogen stress.*	F_V/F_M is not sensitive to sulfur stress untilstarvation levels are reached. (Baker 2004) *It is common to use chlorophyll content meters for nitrogen stress.*
CO_2 Stress	Not sensitive to early or moderate CO2 stress. (Siffel & Braunova 1999)	F_V/F_M is sensitive to early or moderate CO2 stress. (Siffel & Braunova 1999)
Salt Stress	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)	Not sensitive to NaCl stress in Rice, but it is sensitive to NaCl stress in sorghum and chickpea. (Moradi & Ismail 2007) (Netondo 2004) (Eyidogan 2007)
Nickel	ETR, a parameter derived from Yield at a knownlight level, is sensitive to nickel stress (Joshi & Mohanty 2004), (Tripathy 1981) (PAR Clip required)	Not sensitive to nickel stress. (Joshi &Mohanty2004)
Zinc	Fs in Y(II) is a good indicator of zinc stress. (Joshi & Mohanty 2004) (Tripathy & Mohanty1980) (Krupa 1993)	Not sensitive to zinc stress. (Joshi & Mohanty2004) (Tripathy & Mohanty 1980) (Krupa 1993)
Cold	Y(II) is sensitive to Cold stress(Oquist and Huner1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).	F_V/F_M is sensitive to Cold stress(Oquist and Huner1991), (Ball 1994), (Krause 1994), (Adams1994), (Adams1995), (Ball 1995).
Herbicide	Sensitive to most types of herbicides. See the Opti-Sciences Stress guide for specificinformation.	Sensitive to most types of herbicides Not sensitive to DCMU (Nedbal & Whitmarsh 2004). See the Opti-Sciences Stress guide forspecific information
Pesticides	Is sensitive to pesticides tested *including Trimax.*	Is sensitive to pesticides tested but not Trimax. Seethe Opti-Sciences Stress guide for specific information

	See the Opti-Sciences Stress guide for specificinformation	
Chemical Stress	See the Opti-Sciences Stress guide for specificinformation	See the Opti-Sciences Stress guide for specific information
Other Stress	See the Opti-Sciences Stress guide for specific information	See the Opti-Sciences Stress guide for specific information.

The Opti-Sciences OS5p+ and the OS1p can be used to make Y(II) measurements. Digital PAR Clips are a highly recommended accessory.

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 Y(II) measurements tend to cost more than ones that provide just FV/FM measurements, they offer greater capability. For the widest possible fast stress measuring capability, instruments that are capable of Y(II), and FV/FM in conjunction with a chlorophyll content meter are recommended.

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APPENDIX C – QUENCHING PROTOCOL

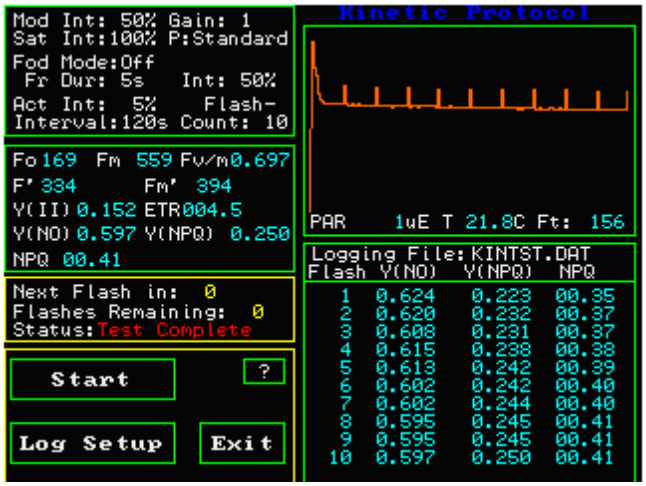
Adapted from application note 0415

There are two types of fluorescence quenching measurements, non-photochemical quenching and photochemical quenching. The use of a modulated fluorometer is required for these measurements.

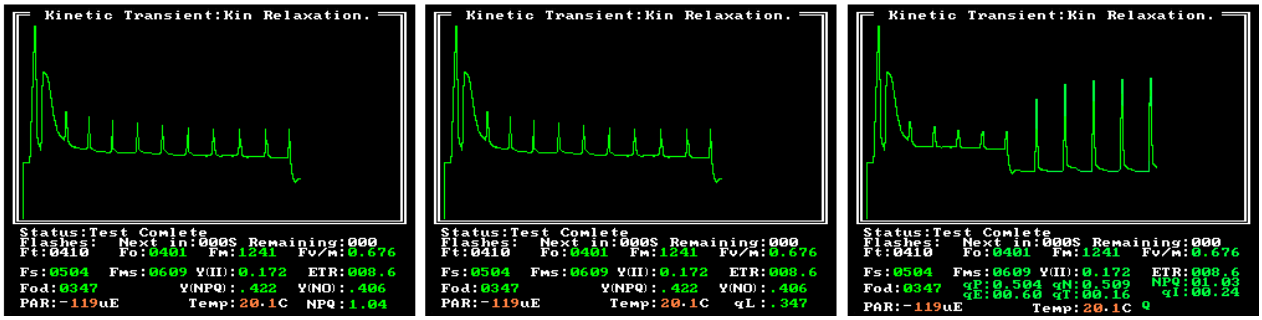
Photochemical quenching is a measure of the fraction of still open PSII reaction centers.

Non-photochemical quenching is a measure of heat dissipation impacted by photo-protection mechanisms, chloroplast migration, state transitions, and photoinhibition that can be affected by different types of plant stress. Most of parameters used for measuring quenching must be made at steady state photosynthesis to be valid by definition; however, Y(NO) in the lake model does not need to be at steady state.

When making quenching measurements, samples should only be compared if they have the same F_V/F_M values, since F_V/F_M is the measuring reference for quenching values (Baker 2008).



Hendrickson lake model quenching protocol with NPQ resurrected from the puddle model.



Screens from the OS5p using three different quenching protocols.
Left: the Hendrickson lake model protocol with NPQ resurrected from the puddle model.
Center: the Kramer lake model protocol
Right: the puddle model protocol with quenching relaxation protocol

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 F_O' and approximate the measurements made by Kramer. F_O' is a controversial parameter is some circles.

Hendrickson's (2004) work offered an alternative lake model 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 photochemical quenching parameter like qL to estimate the fraction of open PSII centers.

From Hendrickson's work, earlier works by Cailly (1996), and Genty (1989, 1990), Klughammer and Schreiber derive simplified equations that allow NPQ to be reconciled with the lake model. Since there is a large volume of work that has been done investigating large numbers of plant species and plant mechanisms using NPQ, it allows the transition from puddle model to lake model measurement to occur in less painful way. Furthermore, Hendickson's equations provide lake model parameters without the use of the controversial parameter Fo'. Today, all of these methods are still in use.

<u>Kramer's lake model equations:</u>

$Y(II) = (F_M' - F_S) / F_M'$

$Y(NO) = 1 / (NPQ+1+q_L (F_M/F_O -1))$

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

$qL = ((F_M'-F_S) / (F_M' - F_O)) (F_O' / F_S)$

<u>Hendrickson's lake model equations:</u>

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

$Y(NO) = F_S / F_M$

$Y(NPQ) = (Fs / F_M') - Y(NO)$

<u>Klughammer and Schreiber's lake model NPQ, determined from Hendrickson's equations.</u>

$NPQ= Y(NPQ) / Y(NO)$

<u>Puddle model equations:</u>

$qP = (F_M' - Fs) / (F_M' - F_O)$ Above 0.4 F_O' should replace F_O

$qN = 1 - ((F_M' - F_O) / (F_M - F_O))$ Above 0.4 F_O' should replace F_O

$NPQ = (F_M - F_M') / F_M'$

Quenching relaxation equations for the Lake or puddle model

$NPQ = q_E + q_T + q_I$ (traditional) At high light level, one could now consider q_M as part of NPQ instead of q_T, although it is technically not considered a non-photochemical quenching mechanism. It is also true that q_T is also not technically a non- photochemical quenching mechanism.

$q_E = ((F_{ME} - F_M') / (F_M - F_M'))$ F_{ME} is the relaxation saturation value at four to ten minutes in the dark. F_M' is the last light adapted F_M' value at steady state fluorescence. Field plants require the longer times to measure q_E. Times of up to ten minutes have been reported.

$q_T = ((F_{MT} - F_{ME}) / (F_M - F_M'))$ F_{MT} is the relaxation saturation value at twenty minutes in the dark.

$q_M = ((F_{MM} - F_{ME}) / (F_M - F_M'))$ F_{MM} is the relaxation saturation value that takes more than ten minutes to times of from twenty to 35 minutes in the dark. q_M is the intermediate chlorophyll fluorescence change due to chloroplast migration

$q_I = ((F_M - F_{MT}) / F_M - F_M'))$ 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 F_M measurement and the saturation pulse after q_M or in some cases q_T.

Since NPQ has been resurrected for the lake model, the quenching parameters q_E, q_T, and q_I are also valid in the lake model.

Measurement of q_E, q_M, q_T and q_I

q_E, q_M, q_T and q_I require a quenching relaxation measuring protocol that is only available on the most advanced chlorophyll fluorometers. A stable light source is required to achieve a reliable steady state photosynthesis level. These measurements take time. It is common to dark adapt overnight, and then expose the leaf to a stable light source at a specific intensity for twenty to 35 minutes, the time required to reach steady state photosynthesis in many plants. It then requires the use of saturation pulses in the dark to measure quenching relaxation for another twenty to thirty five minutes while q_E, q_M, or q_T relaxes. A modulated light is also used (see the quenching app note for more details).

Quenching parameter definitions, advantages, and limitations:

Puddle model

NPQ (puddle model and Henrickson lake 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 F_O'. 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. The range of NPQ is affected by dpH of the thylakoid lumen which is an important aspect of photosynthetic regulation, chloroplast migration, state transitions and photoinhibition. Numbers range from zero to infinity with typical samples measuring in the 0.5 to 3.5 range (Maxwell and Johnson 2000). (Bilger & Björkman, 1990), (Muller P., Xiao-Ping L., Niyogi K. 2001).

q_N (puddle model quenching protocol parameter) is similar to NPQ but requires Fod or Fo' in the calculation. q_N 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 qN is above 0.4, Fo' (or Fod) should replace Fo in qP equations. qN 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. A new Fo' value is measured and used for corrections to the quenching coefficients. Numbers range from zero to one. (puddle model) (Van Kooten & Snel, 1990)

q_P (puddle model protocol 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 q_N 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. By using the Far-Red source for post illumination, the PSII acceptors may be re- oxidized through the illumination effect 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 & Snel, 1990)

q_E (puddle model or Hendrickson lake model quenching relaxation protocol 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)pH 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 from NPQ by using a quenching relaxation method. Some researchers in the past have also divided q_N 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).

q_T (puddle model or Hendrickson lake model quenching relaxation protocol 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 from NPQ by using a quenching relaxation method (Muller P., Xiao-Ping L., Niyogi K. 2001). 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). There is now strong evidence that q_T does not exist in most land plants at high light intensities. The evidence points to qM or chloroplast migration and the resulting reduction in leaf absorptance as the source of q_T (Cazzaniga 2013).

q_M – due to chloroplast migration

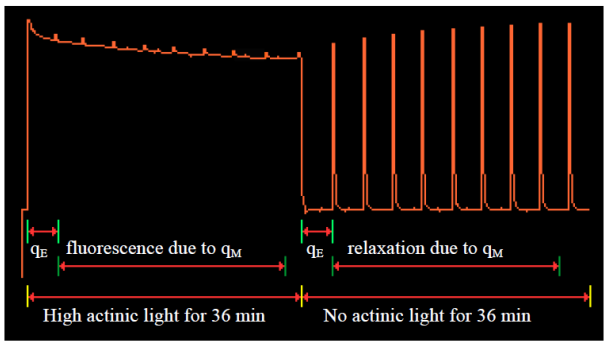
Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) used multiple methods, Arabidopsis mutants and wild type Arabidopsis plants to determine that the fluorescence change, previously thought to be the result of state transitions and chronic photoinhibition, or more recently, thought to be a longer lasting xanthophyll cycle process, was caused, by chloroplast migration. They found, as others have, that under high light levels, chloroplasts move from the top of cells to the sides of cells, partially shading other chloroplasts. This was verified with light microscopy using samples treated to prevent migration after high light migration. They also found that leaf transmittance increased and therefore leaf absorptance was lowered with chloroplast migration. The research concludes that the cause of q_M is a decrease in light photon absorption which creates lower fluorescence yield, rather than a true quenching process. This is thought to be another avoidance process to protect leaves from high light levels. They found that the time scales for adjustment and fluorescence intensity change mirrored the previously used q_T and chronic photoinhibition, up to 30 minutes, but extended up to 35 minutes with some mutant plants. Chloroplast migration has been known and studied for a while, and it was stated by Brugnoli in 1992 that chloroplast migration affected chlorophyll fluorescence. The Cazzaniga paper is the first to name chloroplast migration as the source of the q_T acute photoinhibition or q_Z fluorescence change.

Researchers found that high white actinic light and intense blue light induced chloroplast migration as found in nature. However, intense red light did not induce chloroplast migration as found in nature. Chloroplast migration was shown to be controlled by high blue light intensity (Kagawa T. 2001). Arabidopsis mutants that were devoid of qE (npq4) were tested and Arabidopsis mutants devoid of q_E and chloroplast migration (npq4 photo2) were also tested along with other mutants. In addition, targeted reverse genetic analysis was used to eliminate other contending possibilities by creating a series of double and triple mutant that covered the remaining spectrum of mechanisms that affected chlorophyll fluorescence, including; the composition of various parts the photosynthetic apparatus, carotenoid biosynthesis, and state transitions as sources of q_M.

In regard to q_T being replaced by q_Z, it was found that by using mutants devoid of q_E and zeaxanthin, that the magnitude of q_M did not change but the recovery time in the dark was longer. Plants were grown at 150 µmol photons m-2 s-1, and tested at 400 µmol photons m-2 s-1, 800 µmol photons m-2 s-1, and 1,200 µmol photons m-2 s-1. The adjustment time for q_M was from 20 to 30 minutes but ranged up to 35 minutes for some mutants.

The Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) observation that the stn7 mutant, devoid of state transitions, has NPQ measurements very similar to the wild type in Arabidopsis, casts strong reservations in regard to the relevance of q_T in Arabidopsis.

Chloroplast migration as found in nature, requires a white actinic light or an intense blue light to function correctly. Red light or an intense red light with a lower intensity blue light will cause measuring artifacts at high light intensities (Cazzaniga 2013).



q_I - puddle model or Hendrickson lake model quenching relaxation protocol 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). Photoinhibition targets PSII reaction centers. It has the effect of balancing ATP output by PSII and NADP output by PSI. With a low light history, PSII reaction centers outnumber PSI reaction centers (Laisk 2014). Until recently, acute photoinhibition was considered part of q_I. After Cazzaniga 2013, and Dall'Osta 2014, acute photoinhibition changes should be attributed to q_M or chloroplast migration.

Kramer lake model quenching parameters

Y(NPQ) (lake model quenching parameter) It 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) (lake model quenching parameter) It 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 Schrieber define Y(NO) as the "fraction of energy that is passively dissipated in the form of heat and fluorescence mainly due to closed PSII reaction centers". Hendrickson calls Y(NO) constitutive heat dissipation. A high Y(NO) value after dark adaptation is an indication of photodamage. (Klughammer and Schreiber 2008).

q_L (Kramer lake model quenching parameter) It 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) = (lake model) quantum yield of photosynthetic energy. The equation is the same as for Y, or dF/F_M'.

Hendrickson lake model quenching parameters (these are approximations of Kramer values without measuring F_O'). NPQ can now be resurrected to the lake model using Hendrickson equations (Klughammer and Schreiber 2008).

Y(NPQ) (lake model quenching parameter) It 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) (lake model quenching parameter) It 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 Schrieber define Y(NO) as the "fraction of energy that is passively dissipated in the form of heat and fluorescence mainly due to closed PSII reaction centers". Hendrickson calls Y(NO) constitutive heat dissipation. A high Y(NO) value after dark adaptation is an indication of photodamage. (Klughammer and Schreiber 2008).

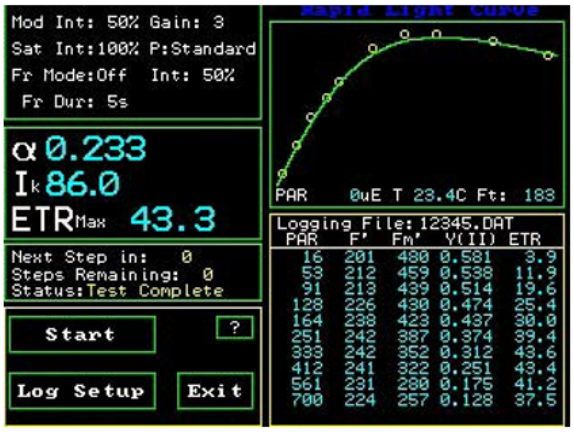
Y(II) = (lake model) quantum yield of photosynthetic energy.

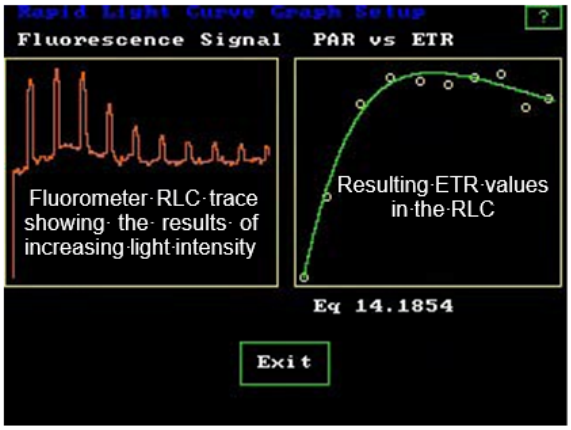
NPQ (puddle model and Henrickson lake model parameter resurrected to Hendrickson's lake model by Klughammer.) is non-photochemical quenching and is a measure of heat dissipation. NPQ is an alternate expression of non-photochemical quenching. It provides an estimate of quenching without knowledge of FO' . The advantage of NPQ 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 low values. The range of NPQ is affected by dpH of the thylakoid lumen, and the xanthophyll cycle which are an important aspect of photosynthetic regulation. Chloroplast migration, state transitions and photoinhibition also affect NPQ. Numbers range from zero to infinity with typical samples measuring in the 0.5 to 3.5 range (Maxwell and Johnson 2000). (Bilger & Björkman, 1990), (Muller P., Xiao-Ping L., Niyogi K. 2001). NPQ is by far, the most used quenching parameter. As a result, there are a large number of published papers that can be referenced for most applications.

APPENDIX D – RLC PROTOCOL

Adapted from application note #0412

Rapid Light Curves, a solution for variable light environments, an overview:





Typical trace of a Rapid Light Curve. The quantum yield of PSII, or Y(II), ETR_{MAX}, ex, and I_K values are reported with details for each step on one screen. A separate screen shows both the raw light trace and the resulting Rapid Light Curve.

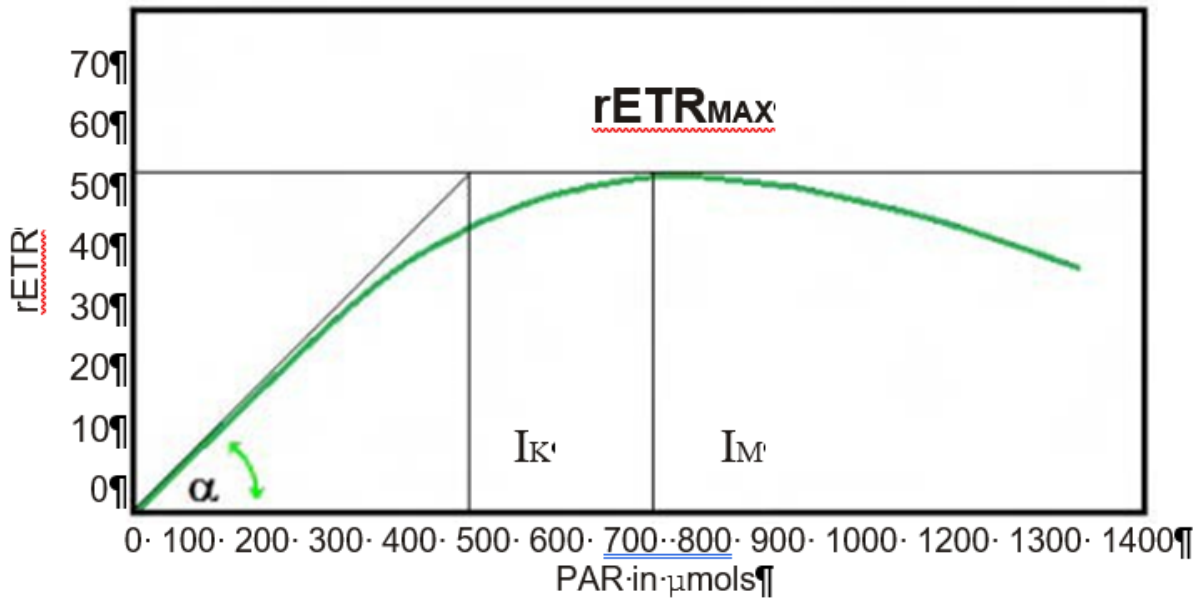
Rapid light curves have been heavily used by researchers to study aquatic plants, and for under canopy research on land, where it is common for the light irradiation level to constantly change. Most traditional chlorophyll fluorescence parameters and methods run into difficulty when light level changes rapidly. While the measurement of quantum yield of PSII, or Y(II), and ETR, only takes a second or two, they are defined as measurements taken during steady state photosynthesis, a process which takes between fifteen and twenty minutes at non-changing light levels for land plants (Genty 1989, 1990) (Maxwell and Johnson 2000). Aquatic plants are subject wave action, changes in water column depth, tides, currents, clouds and turbidity changes, whereas under canopy land plant leaves are exposed to variable shading from other plants and wind. Traditional Y(II), ETR, and standard light response curves run into problems with these samples and overestimate photosynthetic activity (Macintyre 1997). Of course, Fv/Fm may be used to measure the health of PSII with many 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, have driven research to 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 determi nates. Marra (1978), (Banse and Yong 1990).

Light saturation rate, as measured by Rapid Light Curves, highly correlates with the concentration and maximum activity of rubico (Macintyre 1997), (Macintyre 1996). Measured Steady state photosynthetic rates overestimate actual photosynthetic rates in a variable light environment (Macintyre 1997). “RLCs can provide a reliable assessment of photosynthetic activity, by integrating the leaf’s ability to tolerate light fluctuation, as well as reflecting its immediate short-term light history” (Ralph 2005) (Schreiber 1997) (White and Critchley, 1999).

There are many claims about the value and limitations of rapid light curves (or RLCs). Many are valid and some define the limitation of the technique. Past research has narrowed the suggested times used for dark adaptation, and narrowed the times used for each light step for best results. Going beyond these suggested limitations creates results that are more complex (Ralph 2005). 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.

To this end, we will provide an overview of the Rapid light curve and the research regarding RLC.



Alpha is the initial slope of line at low PAR values created by relating ETR to PAR. It provides a measure of quant um efficiency (Schreiber 2004) The initial slope of the curve ex is proportional to efficiency of light capture (Ralph 2005).

$rETR_{MAX}$ is the measure of a leaf's photosynthetic capacity or maximum relative electron transport rate (Schreiber 2004).

$I_K = ETR_{MAX}/alpha$ is a measurement of the point where light saturation dominates, or the minimum saturation level (Schreiber 2004). (I_K is also called E_K in some literature (Ralph 2005))

While photochemical quenching predominates before I_K, non-photochemical quenching dominates after I_K.

$I_M = rETR_{MAX}$ light irradiation intensity

Rapid light curves are created by dark adapting, or quasi-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 quasi-dark adaptation and 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 source steps to the next pre-programmed actinic light level. The time of exposure to a specific actinic light level is not long

enough for steady state photosynthesis to be reached, and photo- acclimation is minimized (Ralph 2005). Different exposure times for each light step have been used. The length of actinic illumination exposure time is programmable in some fluorometers. Times of 5 seconds, 10 seconds, 30 seconds, 40 seconds, 50 seconds and 60 seconds have been used (Rascher 2000, Herlory 2007). More recent research indicates that RCLs should provide the shortest actinic light time possible to prevent plants from reaching near steady-state and minimizing photo-acclimation. 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) (Schreiber 2004). High light leaves tend to relax faster than low light leaves, also according to Ralph. Ralph finds that 10 seconds works for the samples he has measured.

It is common for researchers that work with RLCs on land or on aquatic plants to use the equation rETR= Y(II) X PAR (Ralph 2005) (Beer 1998) as opposed to rETR=Y(II) x PAR x 0.84 x 0.50 used with Y(II) steady- state protocol measurements. Beer 1998 found that rETR was closely related to photosynthetic activity when compared to oxygen evolution.

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 two steps to being measured at or above the leaf light saturation. Intensity values are commonly equally spaced. Three curve fitting methods have been commonly used. The OS1p provides curve fitting according to Eilers and Peeters (1988); however, one can also use other types of curve fitting by imputing the data into Sigmaplot software. A regression wizard is available that can use a curve fitting model of choice to determine Cardinal points for analysis. (Ralph 2005). 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.

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

The first measurement is taken with no actinic illumination and either dark adaptation or “quasi-dark adaptation” (Ralph 2005 and Shreiber 2004). Ralph recommends a 5 to 10 second quasi-dark adaptation. 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 programmable variables in RLCs. Some fluorometers offer only a programmed routine that limit choices. The OS1p provides all options. 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 in plants grown under high light conditions. Low light leaves require a different intensity range for measurement than high light leaves. Intensities above 100 umols will commonly saturate low light leaves. Some experimentation will be required to set up the correct range.

Ralph also shows that all RLC cardinal points, including ETRmax , change substantially with different actinic step times and dark adaptation times.

RLC vary significantly at different times of day because of different light histories (Rascher 2000). It is recommended that several light curves, be taken at different times of day. Results may be viewed separately or they may be added together and then subjected to light curve fitting software (Rascher 2000).

Variables and limitations of RLCs

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.

Time of day and dark adaptation time:

Rascher (2000) explored the value and limitations of Rapid Light Curves in detail and found that not only did the results from RLC and ETRmax change dramatically depending on the time of day that they were measured, but that they also provided different ex 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. ETRmax 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. With this in mind, it is important to either compare samples taken with a similar light history, or to combine multiple scans from different times during the same day as suggested by Rascher (2000). One must also ensure that dark adaptation times are consistent when planning an experiment. Ralph (2005), and Schrieber (2004) suggest that it is difficult to achieve complete darkness in the field, and that a 5 -10 second quasi-dark adaptation is adequate to obtain a measurement of the sample while it is not currently exposed to light. This allows rapid re-oxidation of QA and the determination of ex, without substantially changing current nonphotochemical quenching levels (NPQ), and it minimizes the deactivation the light-dark reaction. Longer dark adaption times can deactivate the light dark reaction, affect NPQ to a much greater degree, and cause a more substantial Kautsky induction effect that is more complex (Ralph 2005).

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.

Actinic step time:

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. Minimizing the actinic step time, minimizes photo-acclimation and actinic light related NPQ (Ralph 2005).

Saturation pulse NPQ:

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 have some residual NPQ and will reduce the Y(II) and ETR values somewhat as well. In this light, Ralph's (2005), and Schreiber's (2004) claim that the saturation pulse NPQ is relaxed in ten seconds, must be modified to a statement that most saturation pulse NPQ is relaxed after 10 seconds. Using ten second steps provides a compromise that minimized photo-acclimation while eliminating most saturation pulse NPQ error.

Leaf absorption and ratio of PSII reaction centers to PSI reaction centers:

The amount of light that is channeled to PSII for variable fluorescence is dependent on the ratio of PSII reaction centers to PSI reaction centers and leaf absorption. As stated earlier, it is common for researchers that work with Rapid Light Curves on land or on aquatic plants to use the equation r_{ETR} = Y(II) X PAR (Ralph 2005, Schreiber 2004, Beer 1998), as opposed to r_{ETR}=Y(II) x PAR x 0.84 x 0.50 used with steady state photosynthesis Y(II) protocol measurements. Indeed, it can provide an approximation of electrons pumped through the photosynthetic chain or an approximation of photosynthetic activity (Beer 1998). While the evidence promotes this view, it is also important to understand its limitations when designing an experiment to ensure valid results.

Chlorophyll fluorescence is variable fluorescence found only in photosystem II. Photosystem I fluorescence is very weak and it does not vary with different light levels or plant stress. For purposes of understanding all the variables, a discussion of the 0.84 and 0.50 value is included from the steady state equation. Relative ETR or r_{ETR} in the Y(II) protocol is calculated using a quantum yield of PSII measurement, Y(II), taken at a given light level and temperature, using the equation: r_{ETR} = (Y(II)) (PAR - the light level) (0.84) (0.5). r_{ETR} is scaled on the Y axis, and PAR (photosynthetically active radiation per meter squared per second) is on the X axis. The 0.84 value represents the average light (PAR) absorption fraction in land plants. The 0.5 value represents the average fraction of PSII reaction centers in land plants. It has been found that the actual absorption range in land plants varies between 0.7 and 0.9 (Eichelman H. 2004) depending on species and stress conditions. In algae, the absorption value can vary from 0.36 to 0.96 (Nielsen H. D 2008). The ratio of PSII to PSI reaction centers ranges from 0.42- 0.60 (Laisk A 1996) in different species of land plants, with C4 plants having more PSI reaction centers. Smith (1990) found that the ratio of PSII to PSI reaction centers in Dunaliella salina (Green Algae) normally at 0.58, changed to 0.75 when grown under high light stress conditions. In Red Algae and Cyanobacteria, about 15% of the reaction centers are PSII (Allen J. 2004). This data should be taken into account when designing a Rapid Light Curve experiment to ensure that apples are being compared to apples and not oranges.

Baker (2008) recommends that if two different land plant samples are to be compared, using r_{ETR}, the absorbance should be measured with and integrating sphere to ensure similar absorption characteristics

State transitions:

State transitions are stable at steady state photosynthesis (light adapted at a steady light level for fifteen to twenty minutes) or after dark adaptation for more than fifteen to twenty minutes. While state transitions cannot adjust significantly during the time frame of the RLC, their adjustment according to their light history prior to measurement, can provide significant RLC variation. This is one of the factors that cause light curves to change at different times of day. (Rasher 2000). State transitions are more of a factor at low light levels than at high light levels (Lichtenthaler 1999).

Conclusion:

Depending on the goal of the experiment, planning is important to account for, eliminate, or minimize the test variables.

By using quasi-Dark adaptation for 5 – 10 seconds in a dark first step, it has been shown that this allows for rapid reoxidation of QA without significant relaxation of existing non-photochemical quenching. Ralph also recommends the 5-10 second dark adaptation to minimizes the deactivation of rubisco and to minimizes a rubisco reactivation induction effect.

The 10 second actinic step length allows a short actinic light time to minimize plant photo-acclimation. The minimum length for actinic light time at each step is governed by the time it takes for the saturation pulse fluorescence signal (saturation pulse NPQ) to relax according to Ralph (2005) (Schreiber 2004).

However, in this regard, the ten second time frame is a compromise. While most of it dissipates in less than ten seconds, 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. As a result, there is a small amount of saturation pulse NPQ that has not dissipated, after each saturation pulse, that is ten seconds apart. Furthermore, it is cumulative from one pulse to another. This error can be minimized by using fewer steps.

Recent light history is an important variable that may be overcome by either only comparing samples with similar light histories, or measuring RLCs at different times of day to understand the results. In addition, all of the curve data from multiple curves, taken during the same day, can be overlaid to minimize these variables as suggested and tested by Rascher (2000).

Even with these limitations, the literature supports the fact that Rapid Light Curves offer an important tool to investigate and estimate the saturation characteristics of plants, and to study plants in a variable light environment. Care must be taken to account for all of the variables when designing an experiment.

rETR = Y(II) x PAR is a valid, relative, general formula. Results have shown that rETR is closely related to photosynthetic activity under most conditions, and the rate that electrons are pumped through the photosynthetic chain. (Beer 1999, Shreiber 2004, Ralph 2005).

In land plants, the same stress factors that limit rETRs effectiveness in measuring plant stress, during steady state photosynthesis, will probably also affect it in RLCs. For example, under drought stress, photorespiration and the Mehler reaction prevent the use of rETR from being used effectively in C3 plants (Flexas 2000, 2001). See the Desk Top Stress guide, and the ETR application note from Opti-Sciences for more information on the value and limitations of steady state ETR. The Burke assay (2010) is recommended for under-canopy C3 plants under drought stress (contact Opti-sciences for the drought stress application note).

Equipment Factors and Requirements

Light curves and RLCs may be done using a dark adaptation clip and stepping the light intensity up or down using pre-measured, predetermined light intensities. However, a greater error in the actual PAR intensity is to be expected with this method due if halogen lamps are used because they age. The intensity of halogen lamps changes over time due to vaporization of tungsten from the lamp filament that deposits on the cooler lamp envelope and darkens the bulb. Halogen light sources are designed to prevent this at higher intensities and heat levels, but the process does not work at low and medium intensity values. Light diodes are more reliable in this regard for rapid light curves, when a PAR clip is not used.

A better way to make light curves on land plants is by using a PAR Clip that measures PAR during the rapid light curve. In this way, even slight light changes are measured. The OS1p also uses the PAR sensor in a feedback loop, to maintain the same light intensity for the entire actinic step of the RLC. A cover, a dark cloth, or a darkened room can be used for dark adaptation if desired.

Accurate PAR measurement is another issue. Rascher (2000) found that when using internal fluorometer illuminators, it is important to compensate for the difference in PAR sensor location vs. leaf location. He found

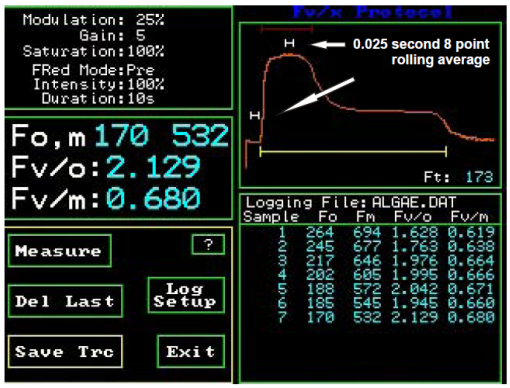
that an error in the range of 10% can occur when measuring PAR at the sensor location compared to measuring PAR at the leaf plane. This error is not significant when using ambient sun light.

Corrections can be made by one of two methods. The first method is to add a correction factor to measurements. By comparing PAR values at the leaf plane to PAR values at the sensor location, Rascher found that the difference was linear though the intensity range. So a factor can be used to multiply by PAR Clip PAR measurements to get PAR measurements at the leaf plane. The second way is to use the OS1p software that corrects for this discrepancy by calibrating the PAR clip sensor to a Licor 190 PAR sensor at the leaf plane. Internal light sources are calibrated and ambient sunlight is also calibrated.

Most light curves are made in equal light incremental steps, but it is not a requirement. Most light curves start at low light levels and go beyond saturation levels or start beyond saturation levels and go to low light level.

Saturation Pulse intensity duration in most modulated light chlorophyll fluorometers the saturation pulse duration must be critically set to prevent measuring errors. The ideal saturation duration for land plants is between 0.8- 1.0 seconds (Roseqvist and van Kooten 2004). Longer pulses will cause NPQ to provide a reduce yield value, and shorter durations will not allow full saturation of reaction centers. (Roseqvist and van Kooten 2004) In algae and cyanobacteria the ideal length of a saturation pulse is between 25 and 50 milliseconds. (Schreiber 1995). However, this is not true of the OS1p instrument. This instrument provides an 8-point, 25 millisecond, rolling average to determine critical measurement values such as F_O, F_S, F_M, and F_M' That are used in such diverse parameters as F_V/F_M and $rETR_{MAX}$. This ensures that even if the saturation pulse is not critically set, errors are eliminated, and the Optimal Measurement is made.

Optimal saturation pulse duration without the hassle, using the rolling eight point 25 msec average to find F_O, F_S, F_M, and F_M'. It ensures mistake free operation.



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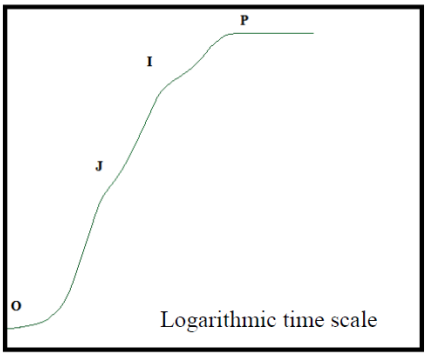
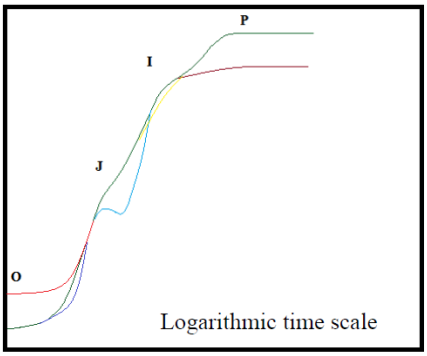
APPENDIX E – STRASSER OJIP PROTOCOL

Adapted from application note #0415

If the rise in chlorophyll fluorescence is examined with high speed time resolution in the range of microseconds, and milliseconds, specific steps appear during the rise. The following descriptions represent a synopsis of information available from a paper written by Xin-Guang Zhu, Govindjee, Neil R. Baker, Eric deSturler Donald R. Ort, and Stephen P. Long in 2005. The information was reaffirmed in a second paper in 2012 by Xin-Guang Zhu,Yu Wang, Donald R. Ort, and Stephen P. Long. These are some of most respected names in chlorophyll fluorescence and photosynthesis. While there is still some debate regarding some of the details of the OJIP fluorescence rise, this approach is the one that is most accepted. For an alternative interpretation see Vredenberg 2015. Vredenberg is also a highly regarded researcher.





O – is commonly measured at 20 sec. after the start of actinic illumination in continuous fluorometers, and is not equal to F_O measured by modulated fluorometers. Continuous fluorometers use linear regression analysis to estimate

F_O, or minimum fluorescence in a dark adapted state, before any Q_A has been chemically reduced.

The height of both O and F_O are affected by the ratio of the size of peripheral antenna to core antenna. A larger ratio of peripheral antenna causes O and F_O to be lower.

Both O and F_O are affected by the ratio of Q_B- non- reducing reaction centers to Q_B- reducing reaction centers. As the ratio of Q_B- non-reducing reaction centers increase, both O and F_O values increase. This is shown in the bottom diagram. The green line represents a sample with significantly fewer Q_B- non-reducing reaction centers, and the red line represents a much higher percentage of Q_B- non-reducing reaction centers.

O to J – The slope of the rise in fluorescence is affected by the probability that excitation energy will migrate from a closed Q_B- reducing reaction center to an open Q_B-reducing reaction center. Higher probability delays the rise as shown in the lower diagram in dark blue. Q_B- non-reducing reaction centers do not transfer energy to open Q_B- reducing reaction centers. Energy absorbed by these reaction centers is converted to heat and fluorescence only. They are not involved in photochemistry. The O-J rise represents the photochemical reduction of pheophytin and Q_A. J represents maximum values for Q_AQ_B- and Q -Q -. J becomes more defined and lower if the dark adapted Oxygen Evolving Complex ratio of the specific states of S1 to S0 move from 1:0 to 0:1. The Dip after J becomes more defined with a higher S0 value. It provides a greater P+ concentration that is a strong fluorescence quencher. This dip is shown in the 680 lower diagram in light blue. A separate new step called the K step can appear at 300 sec. It only appears at high light levels (Vredenberg 2004), when there is severe nitrogen, iron, or sulfur deficiency (Strasser 2004). The Zhu 2005 paper show that the timing of J is somewhat variable. However, in the Strasser JIP protocol, used for plant stress measurement, it is fixed at 2 msec.

J to I - This rise represents the photochemical reduction of Q_B . “I” represents the first shoulder in the QvQ_B2- chemical equation that ends at P with a maximum for Q_A- Q_B2- . If properly dark adapted, the J to I rise starts with the ratio of Q_B : Q_B- = 1:0 and ends with the ratio at 0:1.The dark adapted ratio prior to light exposure of Q_B : Q_B- affects the slope and height of “I” as shown in the lower diagram by the yellow line.

Again, the Zhu group shows that the time to reach I is also somewhat variable. However, in the Strasser JIP protocol, used for plant stress measurement, it is fixed at 30 msec.

P = F_M or Maximum variable chlorophyll fluorescence. This value represents a maximum for chemical values of Q -Q 2-, & PQH . The rise in fluorescence ends with the cytochrome b_6f complex re-oxidizing PQH_2 to PQ. The height and slope of the rise to P or F_M are affected by the reoxidation rate constant of PQ, k_{ox}, and by the size of the plastoquinone pool. A higher rate constant and a larger PQ pool reduce the value for P. The time to reach P is variable in the Zhu paper and in the Strasser JIP protocol. A high rate constant reduces the time to reach P, and a larger PQ pool extends the time to reach P. The time to reach P is reported in the Strasser protocol. A larger PQ pool is shown in the lower diagram in dark red crimson.

In 2004, Wim Vredenberg discovered that the OJIP graph changes dramatically at different actinic light levels. In fact, the K step only appears under very high light levels, and under specific severe plant stress conditions. (Vredenberg 2004) For this reason, it is common to calibrate the light source of OJIP instruments, to ensure comparable results.

The quality of light can also be a factor. It has been found that red actinic light penetrates the entire leaf, while blue light does not. For this reason, it has been common to compare work done using the same type of saturating actinic light sources. The OS30p+ offers a red calibrated light source with intensities that may be set at 3,000 umols as found in Strasser’s earlier works, or 3,500 umols in later works for the Strasser protocol, and up to 6,000 umols for other work. Various light sources are used for measurement of F_V/F_M. Industry options include, red, red and blue, and white light halogen, LED, halogen and Xenon light sources. A paper comparing xenon and red light saturating light sources provided results that correlated well with slightly lower values using a red light source (Cessna 2010). The paper found poorer correlation with blue saturating light. For longer light adapted measuring protocols, used in quenching measurements, and light curves, or for extended pre-illumination of shorter light adapted tests, white actinic light sources have advantages, or at least illumination with equal amounts of red and blue actinic light. The apertures of plant stoma are mediated by blue actinic light (Kinoshita 2001). Chloroplast migration, as it occurs in nature, is responsible for up to 30 % of NPQ at high light levels, and only occurs under intense blue light or intense white light, not intense red light (Cazzaniga 2013). In addition, the absorption ratio of red to blue light changes with light intensity (Bernacchi 2002). Other light quality factors affect light adapted chlorophyll fluorescence measurements as well.

An alternative view:

The cause and meaning to the OJIP chlorophyll fluorescence rise, has been subject to more than one view for some time. Dr. Wim Vredenberg has been a champion of the “Three-state trapping model”. Unlike the details of the rise shown above, he shows that “Full reduction of Q_A is neither necessary nor required to reach F_M” (maximum dark adapted chlorophyll fluorescence).

Vredenberg uses short saturation pulses, followed by periods without actinic light to drive photosynthesis. Using a modulated light, he measures the chlorophyll fluorescence effects during the light and the dark periods, during different phases of the OJIP rise, including: O-J, J-I, and I-P. He finds three different rates of fluorescence decay,

measured during the dark periods, contribute to the understanding of quenching, of the fluorescence rise, chemistry, and the physics of the OJIP rise.

In his model, it takes the PSII electron acceptor pair, Pheophytin and Q_A (or Quinone A), to act as a two electron trap pair, that requires two single turn over light flashes to semi-close and then completely close (chemically reduce) Pheophytin and Q_A.

He finds that O-J is the result of Q_A reduction. J-I is a phase where more than 50% of Q_A is reduced, there is re-quenching of Q_A, there is an increase in the slowest version decay of the fluorescence signal in the dark, and there is a pH change at the Q_A – Q_B site. The I-P phase results from a build up of the proton motive force in the thylakoid lumen and cyclic electron transport from PSI.

Vredenberg has developed methods for measuring the portion of Q_B- non-reducing reaction centers, and developed the FIA_{FLU} algorithm (fluorescence induction algorithm) that provides a strong prediction of the actual OJIP rise from readings taken. The intensity of slowest rate of decay, at 0.25 ms in the dark, after a short saturation flash, provides an estimate of the fractional size of Q_B- non-reducing PSII reaction centers.

APPENDIX F – DARK ADAPTATION

Adapted from application note #0309

Dark adaptation is a technique used in some chlorophyll fluorescence measurements to fix a reference point under known, stable and repeatable conditions. (Baker 2008). Deciding where to put that reference is based on an understanding of plant mechanisms that can affect measurements, and what one wants to measure. Recommended times can vary by chlorophyll fluorescence test type, and environmental conditions.

Due to recent research on chloroplast migration (Cazzaniga 2013, Dall'Osta 2014), times should be extended to at least 20 to 35 minutes, if samples are tested, and 35 minutes if samples are not tested. Dark adaption times of forty minutes and sixty minutes are also common for terrestrial plants, and some researchers only use pre-dawn values. Some research journal reviewers have their own ideas, and will only accept the equivalent of overnight dark adaptation. Researchers may want to check with target journal reviewers when designing experiments.

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

F_V/F_M is affected by both photochemical and non-photochemical factors. If a leaf is dark adapted and measured, then subjected to very high light levels for a period of time, then dark adapted and re- measured, the first measurement will be higher than the second measurement. The decline in F_V/F_M 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 CaMn4OxCly 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). Under high actinic light, or near saturating light conditions, cell chloroplasts migrate from the tops of plant cells to the sides of plant cells, increasing leaf light transmission and decreasing leaf light absorptance. This process significantly affects both light and dark adapted fluorescence measurements. During dark adaptation, chloroplasts migrate back to the tops of cells. This process takes between 20 minutes to 35 minutes (Cazzaniga S. 2013). See the application note on qM chloroplast migration at www.optisci.com for more information.

Full activation of Rubisco takes between three and four minutes in vascular plants as well as photoplankton. Deactivation of Rubisco in the dark, takes between 12 -18 minutes in vascular plants and from 9 minutes to 28 minutes in some photoplankton. The longer deactivation is thought to offer an advantage for species subjected to erratic bright light for maximum utilization of light (MacIntyre 1997).

Rapid acting photo-protective mechanisms activated by exposure to variable light intensities (designated in the parameters qE and Y(NPQ) are controlled by the xanthophyll cycle and thylakoid lumen) ph. They relax in a several seconds to 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. Baker (2008) indicates that the adjustment and relaxation times can be longer in field plants, up to 7 minutes.

The effects of state transitions on chlorophyll fluorescence have recently been shown to be more complex than previously thought. Classical state transition theory saw state transitions as a low light survival mechanism that allowed light balance between Photosystem II and Photosystem I. F_M' or maximum fluorescence under light adapted conditions would decrease over a fifteen to twenty minute time frame, and then relax during dark adaptation over a fifteen to twenty minute time frame. State I – State 2 transition quenching relaxation (called qT) was considered to be most significant at lower light levels in terrestrial plants and could represent more than 60% of quenching at low light levels. It was also thought that at high light levels it represents about 6% of total quenching. (Lichtenthaler H. Burkart S 1999). Recent evidence shows that the fluorescence change thought to be the result of state transitions is in fact caused by chloroplast migration at least at higher light levels and near saturating light levels in land plants. Chloroplast migration takes between 20 minutes and 30 minutes to adjust and to relax in wild plants and up to 35 minutes in mutants. See the application note on qM and qT for more information (Cazzaniga S. 2013), (Dall'Osta 2014).

In the past, it was thought that the effects of acute photo-inhibition caused by exposure to high light intensities for an hour or two, could be reversed with 20 to 30 minutes of dark adaption (Theile, Krause & Winter 1998). Recent evidence indicates quenching relaxations in the dark, for these time periods, are likely to be caused by chloroplast migration instead (Cazzaniga S. 2013) (Dall'Osta).

Quenching and quenching relaxation measurements:

When making longer quenching and quenching relaxation parameter measurements related to photo- inhibition and photodamage mechanisms that are common in chronic high light stress, high heat stress, cold stress and over wintering stress, one should understand that it can take days for full relaxation or repair of the non-photochemical quenching parameter, qI to pre-stress conditions. Reversal or relaxation of chronic photo-inhibition caused by several hours of high light exposure starts to relax at about 40 minutes and may take 30 to 60 hours to fully relax under dark adaptation (Lichtenthaler H. & Babani F. (2004) (Theile, Krause & Winter 1998).

To get an accurate control value for FM and FO under chronic photo-inhibition conditions, (Maximum fluorescence, and minimum fluorescence components of non-photochemical quenching parameters) it is common to dark adapt for a full night using pre-dawn values. In some cases, it may make sense to dark-adapt for longer periods of time. (Maxwell and Johnson 2000). It is understood that in plants with a recent high light history that there will likely be some residual photoinhibition built into all dark adapted measurements. This is alright as long as the light history of measured samples has been built in to the experimental design. Unless light stress is the focus of the experiment, it is important to compare samples with similar light history. In addition, quenching measurements of different samples should not be compared unless the F_V/F_M values of the samples are identical. This is necessary because F_V/F_M is the yard stick used to gauge other quenching parameters (Baker 2008) See the quenching application note for more details. If studying photoinhibition, it may be helpful to partially shade test plants for more than 60 hours to get a reliable F_V/F_M.

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.

The use of far-red pre-illumination that is available on some fluorometers is designed to rapidly re- oxidize PSII by activating PSI. While this can be valuable in fieldwork (Maxwell and Johnson 2000), it does not affect the relaxation of other 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 population quickly. Shrouds, darkened rooms, and darkened growth chambers may also be used.

It is useful to use a PAR clip to measure the actinic light level and to maintain a stable light intensity level during quenching measurements to ensure steady state photosynthesis has been achieved before measurement. A dark shroud may be used with the PAR clip in this case, or it may be used in a darkened room.

In review, it is important to take a few things into account. Reliable dark adaptation times can vary by species, plant photo-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 probably best to test for maximum and stable F_V/F_M at different dark-adapted times for best results. When testing for optimal dark adapting times it is important to use samples that have been exposed to the maximum light conditions that will occur during the experiment for reasons discussed above.

Note: Due to the recent chloroplast migration studies (Cazzaniga S. 2013) and Dall'Osta 2014), it makes sense to use 35 minutes for dark adaption or longer when measuring samples that cannot be tested for optimal dark adaptation time. Only compare samples with a similar light history. Evidence shows that plants tested reach a stable known reference dark adaptation state by this time. This is especially true when samples have been subjected to high actinic light levels. It is common to use overnight pre-dawn dark adaptation for quenching measurements.

While it is not set in stone, it is common to use the newest fully mature leaf blade for diagnosis of deficiencies in plants (Reuter and Robinson 1997)

For a complete free "Stress Guide" that deals with research, references, and recommendations on all kinds of plant stress contact Opti-Sciences by phone or E-mail.

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