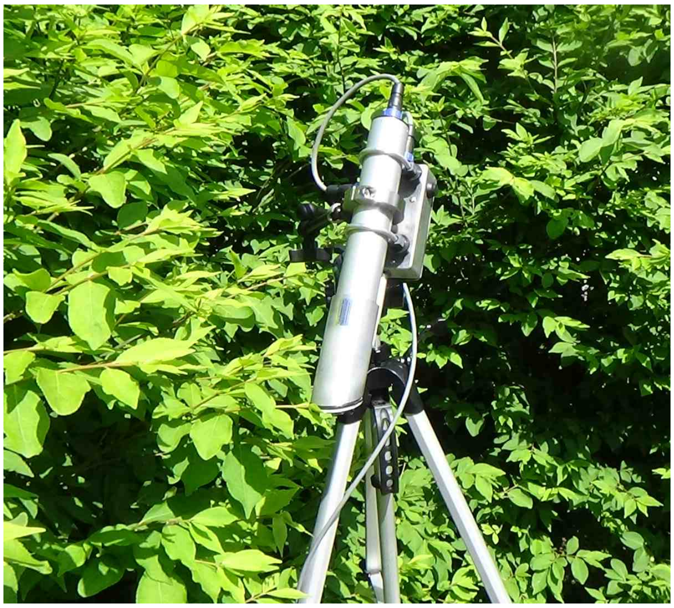
The *next generation* –
Monitor Chlorophyll Fluorometer System

Open daylight dark adaptation module



Closed daylight dark adaptation module

Greater capability-

- ***Daylight dark adaptation*** allows measurement of q_E the fast reacting xanthophyll cycle or energy-dependent exciton quenching, q_M or chloroplast migration, q_T or state transitions, and q_I or photoinhibition, at different times of day, and over long periods of time.

- ***From 1 to 32 measuring probe heads*** are available for each controller, allowing the longer term measurement of larger plant populations and more affordable configurations.

- ***10,000 µmol square topped saturation flash*** or ***F_M' correction option according to Loriaux 2013*** for more reliable results.

- ***Blue light, Red light, and Chlorophyll Content*** probes available

Innovative Design

Open *daylight dark adaptation* module with far red light

Open *daylight dark adaptation* module from the top

Open *daylight dark adaptation* module view from the side

Closed *daylight dark adaptation* module close up

Water proof system controller on pole or tripod with touch screen control, and wireless WiFi antenna.

A Standard Monitor Chlorophyll Fluorometer head without the daylight dark adaptation module

One of the solar panels available with a 12 volt battery box

One of the waterproof junction boxes with fuses for each line, and an LED status light for each line.

Innovative Design

Quenching: *New - measuring capability*

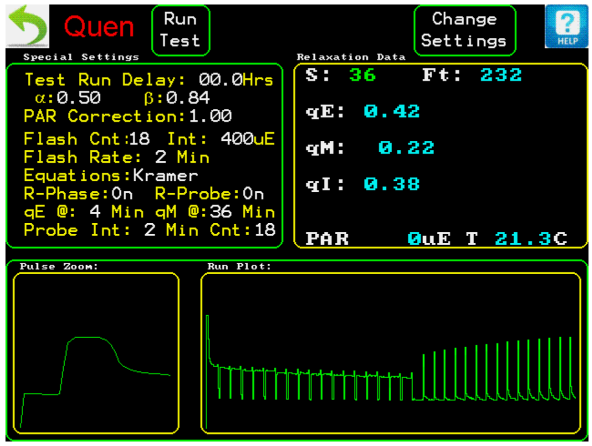
q_E - fast acting xanthophyll cycle
q_M - chloropast migration
q_T - state transitions
q_I - photoinhibition

Programable Dark Adaptation
...at any time of day
...or several times a day
...allows quenching relaxation

Kramer's *new* NPQ(T), q_E(T), & q_I(T)

Ruban / Murchie protocol - pNPQ & qP_d

The new monitor fluorometer includes the widest range of quenching protocols available.



Hendrickson lake model parameters

Y(II) Quantum yield of PSII
Y(NPQ) Photoprotective non-photochemical quenching
Y(NO) All other non-photo-protective non-photochemical quenching
NPQ Non-photochemical quenching NPQ=Y(NPQ)/Y(NO)

Kramer lake model quenching parameters (2004)

Y(II) Quantum yield of PSII
$\mathbf{q_L}$ Photochemical quenching
Y(NPQ) Photoprotective non-photochemical quenching
Y(NO) All other non-photo-protective non-photochemical quenching

New Kramer <u>fast quenching parameters</u> are available with the <u>red light</u> version & daylight dark adaptation only **NPQ(T), q_E(T) & q_I(T)**

Puddle model quenching

Y(II) Quantum yield of PSII
$\mathbf{q_P}$ Photochemical quenching
$\mathbf{q_N}$ Non-photochemical quenching
NPQ Non-photochemical quenching

Chloroplast migration is be responsible for up to about 30% of NPQ at high actinic light levels (Cazzaniga 2013)

Quenching relaxation -

$\mathbf{q_E}$ Photoprotective energy-dependent exciton quenching - seconds to 7 minutes
$\mathbf{q_M}$ Chloroplast migration – chloroplast light avoidance mechanism at high actinic light levels - 20 -35 minutes
$\mathbf{q_Z}$ A proposed longer term xanthophyll mechanism – 20 – 30 minutes
$\mathbf{q_T}$ The portion of NPQ related to state transitions – 15 to 20 minutes
$\mathbf{q_I}$ The portion of NPQ due to photo-inhibition and photodamage. Starts to relax at 40 minutes and may take up to 30 or 60 hours
pNPQ Ruban / Murchie protocol "photoprotective NPQ" designed to measure plant photoprotective mechanisms
$\mathbf{qP_d}$ Ruban / Murchie protocol photochemical quenching in the dark immediately after actinic illumination

Attention to Detail

Algorithm that prevents saturation pulse NPQ issue -
The instrument measures the highest 25 ms. - 8 point rolling average to determine F_M and F_M'. This prevents saturation pulse NPQ from being a problem for all samples, even if the flash width is set too wide. It also eliminates any electronic noise from being a factor.

More reliable leaf temperature measurement -
Instead of measuring air temperature, the Monitor Fluorometer System offers a leaf thermistor that attaches to the bottom of the leaf for more reliable measurement. *It is completely non-destructive, extremely durable*, and provides more reliable measurement. Measurements are made to +/- 0.1^{o}C. Air temperature is also provided from an internal head sensor.

Stable Actinic light source -
For those instances when one wants to use the internal blue actinic light source, it has been stabilized with a feed-back loop that maintains a constant intensity. Normally, as any light source heats up, the output goes down over time. That does not happen with this instrument. Steady state stable conditions are maintained.

PAR & ETR measurement -
The instrument measures light intensity at the leaf surface to determine PAR, ETR and when day and night begin.

Saturation flash intensity and F_M' Correction-
For reliable Y(II) measurements and ETR measurements, the saturation flash of the instrument must be capable closing all PSII reaction centers. If it cannot, an error exists. While this is ususually not an issue with dark adapted samples or samples at lower actinic light levels, it is an issue at higher actinic light levels. Research finds that even the most intense saturation flash values cannot completely close all PSII reaction centers. For that reason, the square saturation flash intensity has been increased to 7,000 μmols m^2 s of blue light, and F_M' correction, according to Loriaux 2013. Using a red saturation flash, can also been added as an option at time of purchase. Read the section on F_M' correction for more details. *For those that prefer using a square topped saturation flash, both the blue and red options can provide up to a 10,000 μmols m^{-2} s^{-1} saturation flash to minimize this issue.*

Far-red light option -
Far-red light allows measurement of F_O', not just estimations. This allows more reliable Kramer lake model quenching measurements. Far red light is included with the daytime dark adaptation module. F_O' is estimated when the daytime dark adaptation module is not included.

Easier setup -
The controller provides a sensitive black and white touch screen interface with drill down menus and graphic displays. Drill down menus provide quick and easy set up, as well as changes. The controller comes with a cover that can be locked. The controller can also be pole mounted for field work.

Add more measuring heads as the need and funding permit -
One controller can support from 1 to 32 measuring heads, without the added expense of additional controllers. Waterproof junction boxes with fuse lights are available for large numbers of measuring heads and for diagnosis of cabling issues in the field.

Blue or Red light and Chlorophyll Content versions -
One can choose between blue or red light probe heads, or use some red light units and some blue light units. The same LED is used for modulated light, actinic light and saturating light. Chlorophyll content probe heads, that use the Gitelson ratio fluorescence method, will be available soon.

Kramer Fast quenching parameters - NPQ(T), q_E(T), and q_I(T) are available with the red version head and the daylight dark adaptation option only.

Attention to Detail

Interface & Data Management

Data is collected by a single controller and it may be retrieved in various ways. *All systems come with Wi-Fi*, and at time of purchase, investigators can also select other options as needed. They will include cellular modem, Ethernet, Radio point to point, USB stick or satellite phone.

The system interface is a *daylight color touch screen* on the system controller that can be used for programming an data collection. However, *remote system programming and data collection are possible* with **Smart phones and PCs**. The remote interface is presented as a web page. *Viewing of live measurements* will also be possible.

The measuring files, collected from the controller, are designed to work with Windows Excel (Trademark) or other windows comma delineated programs like Matlab (trade mark). No special computer software is necessary, and as a result, any controller software updates can be done by linking the instrument controller to a PC and downloading updates remotely or by USB stick. The PSP32 offers 2 GB of flash non-volatile memory.

The monitor fluorometer system has 2 Gb of internal storage that will allow more than a year's worth of data collection, even with 32 measuring heads.

Rather that providing computer software that must be maintained and updated, the controller will provide information on a computer screens will appear as a web page. Data and graphs can be downloaded and manipulated with other programs, like Excel. The web page data will be password protected.

For field use, green house, or growth chamber work

WiFi - comes standard with the controller

Options:

Cellular modem

Ethernet

Radio - point-to-point link available

Satellite modem

USB - stick

Attention to Detail

Previous research finds that while a high frequency of saturation flashes on light adapted plants will not damage plant tissue, frequent saturation flashes on dark adapted plants, on the same spot, can damage plant tissue. For that reason, the frequency of saturation flashes during the day and night can be programmed separately. Previous research recommends once per hour in the dark.

The measuring trace can be recorded and viewed over very long periods of time Furthermore, the highest F_M value during the night, the last F_M value before dawn, or the highest F_M value over longer periods of time can be used as the quenching reference. Since it can take from 30 hours to 60 hours for photoinhibition to completely relax or repair, this choice can be valuable depending on study guidelines.

In addition, ***The new daylight dark adaptation capability*** allows researchers to study q_E the xanthophyll or cycle energy-dependent exciton quenching, q_M or chloroplast migration, q_T or state transitions, and q_I or photoinibition, over long periods of time. Here, a fully relaxed F_M value as described above, can be used with quenching relaxation techniques, during the day, to measure reliable long term results and at different times of day. *The ability to dark adapt during the day allows the use of quenching relaxation protocols required for measuring these very important plant mechanisms.*

We also listened to researchers in regard to ***the number of measuring heads*** that are available with every controller. To study larger plant populations, the monitor fluorometer system allows up to 32 measuring heads per controller. This keeps the cost lower and allows better science. In addition, daylight dark adaptation modules may be added to *some or all* standard monitor fluorometer measuring heads as money becomes available.

The monitor fluorometer also measures ***leaf temperature with a thermistor below the leaf***, not just ambient or instrument temperature. *Instrument or ambient temperature is also measured .*

F_M' correction - **based on Loriaux (2013)**

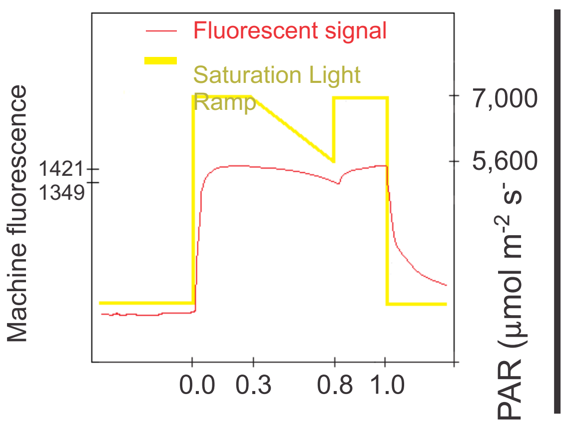
Saturation pulses are used with modulated fluorometers are designed to close all PSII reaction centers. In fact, all reaction centers must be closed to get reliable measurements. The maximum fluorescence intensity value, of the saturation flash called F_M', is used in most measurements including, quantum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

While it is possible to reduce or close all reaction centers in a properly dark adapted sample, with a relatively low amount of light, it has been found that in light adapted samples, *with a high actinic light history,* complete closure of all PSII reaction centers does not occur even with the highest amounts of saturation light. It is thought that complete reduction of Q_A is prevented by fast turnover of the plastoquinone pools. (Margraph 1990, Loriaux 2013). With this in mind, Y(II) and ETR measurements, taken under these conditions, can be underestimated. A recent paper (Loriaux 2013), with researchers that included Bernard Genty, the developer of *quantum yield of PSII*, verified the issue, and developed a method for F_M' correction. It involved a multiple phased single saturation pulse with multiple light intensities, and the use of least squares linear regression analysis of the reciprocal of PAR (Photosynthetically Active Radiation), to determine the F_M' fluorescence level as if an infinitely intense saturation pulse, were to be used. *This is done without causing damage to the plant and without closing all of the reaction centers.*

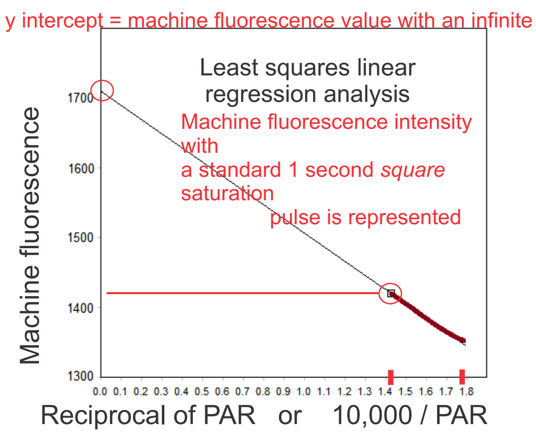
Studies by Loriaux (2006), and Loriaux 2013 have compared chlorophyll fluorescence measurement results with gas exchange measurements and found that a corrected intensity can be determined and used to correct Φ_{PSII} or (Y(II)) and *J* (ETR) measurements. ***Research has shown that Y(II) measurements, taken under high actinic light conditions, can be underestimated with up to a 22% error, and there can be up to a 41% error in ETR values if this method is not used.***

This standard option is provided on the Monitor fluorometer, the Y(II) meter, the OS5p+, the i*FL*, and the OS1p instruments. It is *available for all Light adapted and quenching protocols,* and it can be turned off or on. The method described in a poster by the Loriaux, Burns, Welles, McDermitt, & Genty (2006) and expanded by Loriaux, Avenson, Welles, McDermitt, Eckles, Riensche, & Genty (2013), provides the most accepted method currently available. According to the science, the Monitor Fluoro meter provides the optimal saturation intensity of 7,000 µmols, optimal light ramping of 20%, and a ramping rate less than 0.01 mol $m^{-2}s^{-2}$. the protocol has been optimized for the best results. The standard monitor fluorometer uses an intense blue saturation light source instead of the red light source in Loriaux 2013. A red light version of the monitor fluorometer is also available. ***For those that want to use the standard square topped flash, the instrument offers a 10,000 µmole m^{-2} s^{-1} blue saturation flash, and the red light version offers a red 10,000 µmole m^{-2} s^{-1} saturation flash.***

Representation of how the Multiple Phased Flash works



Least squares linear regression of 10,000 / PAR



The first saturation flash step, shown on the left, is at 7,000 µmols for 0.30 seconds to saturate PSII. The saturation flash intensity is then ramped downward by 20%, making a large number of fluorescence measurements along the way, to 5,600 :mols. The ramping rate is less than 0.01 mol photons $m^{-2}s^{-2}$. The final phase is at 7,000 :mols to check for saturation pulse NPQ. Recent studies have shown that those setting provided optimal results for plants that have been tested. (Loriaux 2013). A rolling 25ms eight point average is used to determine maximum F_M'

The graph on the right represents the Loriaux (2013) method for estimating Fm' with an infinitely intense saturation flash. Least squares linear regression analysis of the reciprocal of PAR (or 10,000 / PAR) allow determination of the y intercept, which represents the machine fluorescence value with an infinite saturation flash.

Monitor Fluorometer Parameters Measured and Protocols included:

Light adapted -

Y(II): Quantum Yield of PSII
(or) $\Delta F/F_M$' or Y)
ETR: Electron transport rate
PAR: Photosynthetically Active Radiation value
T: Leaf temperature
F_{MS} (or F_M'): Maximal fluorescence with actinic illumination using a saturation pulse
F: Fluorescence under actinic light
(prior to saturation pulse)
Loriaux 2013 correction of ETR, and F_M' correction option included for Y(II).

Dark adapted -

F_V/F_M: Maximum Photochemical efficiency of PSII
F_V/F_O: A more sensitive detector of stress than F_V/F_M, but it does not measure plant efficiency.
F_O: Minimum fluorescence
F_M: Maximal fluorescence
F_V: Variable fluorescence
F_O': Minimum fluorescence after exposure to far red light- available with the dark adaptation module
F_O' Estimated without the daylight dark adaptation module
With Red light measuring head only - Kramer (2016) fast quenching parameters **NPQ(T), q_E(T) & q_I(T)**

Quenching -

Hendrickson Quenching with NPQ (standard)
Y(NPQ), Y(NO), Y(II), NPQ, Fv/FM

Kramer Quenching (standard)
q_L, Y(NPQ), Y(NO), Y(II), Fv/FM

Puddle model parameters (standard)
NPQ, q_N , q_P , Y(II), Fv/FM

Requires the dark adaptation module option-

Quenching relaxation protocol (option)
q_E , q_M , q_T , q_Z, & q_I . Ruban / Murchie protocol pNPQ & qP_d

Rapid Light Curves (option)

$rETR_{MAX}$ - (Eilers and Peeters) a measure of a leaf's photosynthetic capacity or maximum electron transport rate.

α is the initial slope of line at low PAR values created by relating ETR to PAR. It provides a measure of quantum efficiency

I_k is the measurement of
the actinic light intensity where light saturation dominates, or the minimum saturation level.

I_m is the Eilers & Peeters calculated optimal actinic light intensity related to ETR_{MAX}

Light Sources:
Saturation pulse Blue LED with:
7,000 μmols m^{-2} s^{-1} with F_M' correction option
10,000 μmols m^{-2} s^{-1} with square topped flash
Option for Red LED saturation flash
7,000 μmols m^{-2} s^{-1} with F_M' correction option
10,000 μmols m^{-2} s^{-1} with square topped flash
Modulated light
Blue 455nm - half band width 18nm
Option for red LED 640nm half band width 18 nm.

Actinic light source: Blue - Up to 5,000 μmols
When the optional red light is used, the actinic light intensity can be up to 5,000 μmols

Far-red light source: - an option included with the day-light dark adaptation option. It is used to measure F_O', or for pre-illumination of samples in the dark adapted mode.

Detection method: Pulse modulation method.

Detector & Filters: A PIN photodiode with a 700 ~ 750 nm bandpass filter.

Sampling Rate: Auto-switching from 1 to 10,000 points per sec., depending on test & on phase of test.

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

Test Duration: Designed to measure samples 24 hours per day, 7 days a week, for months at a time. Runs on solar power, battery power, or mains current.

Storage Capacity: 2 Gb. of non-volatile flash memory, supporting almost unlimited data sets and traces. More than 500,000 data sets.

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

Output: Comma delineated files may be opened in Excel. Data maybe retrieves by WiFi, cell phone, SD data card, Radio point-to-point, Ethernet, satellite phone, or USB stick. Some methods are optional, and require higher pricing.

User Interface:
Display: Graphic black and white touch screen interface
Menu driven. Control box may be locked and pole mounted.

Power Supply:
Various external 12 volt batteries are available upon request. Solar power and mains power can be used.

Operating temperature range -10°C to 50°C

Accessories

Optional features & accessories:

- Tripods
- Articulating arms
- Solar panels
- Daylight dark adaptation module with far red light
- Additional measuring heads
- Junction boxes
- Cellular modem
- Radio - point-to-point link available
- Satellite communications
- Stainless 1.5 inch pipe mount with ¼ inch 20 thread mount hole for articulating arms used to mount measuring heads to pole or pipe mounts.
- Integration of other data is possible such as weather station data, humidity, wind speed, infrared radiometer output, soil moisture sensor output, and other radiation sensor output. Contact Opti-Sciences for specific data type and pricing.

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