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ADC BioScientific Ltd.

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Integrated Fluorometer *iFL* NEW

Technology Leadership

- ***First*** to offer *white actinic light* LED that allows chloroplast migration, responsible for up to 30% of NPQ & xanthophyll cycle photo-protection.
- ***First*** to offer *leaf absorptance measurement* for more reliable *J measurements*. Absorptance varies from 0.7 to 0.9 in heathy leaves and varies with light intensity!
- ***First*** to offer a "matched measurement" automatically every time, with an auto-zeroed IRGA
- ***First*** to offer humidity control <u>*above*</u> and below ambient. Humidity and flow rate may be held constant.
- ***First*** to offer *"walk away automation"* - push a button and come back hours later for results.
- ***First*** to offer automated *"post processing"* for the Laisk protocol, the Kok protocol, the Yin protocol, and the Flexas chamber leakage protocol.
- ***First*** to offer direct readout of g_m, C_C, R_d,Γ^*, V_{CMAX} & J_{MAX}

- ***Optimal improvement*** - ***F_M' correction*** of Y(II) and *J* values according to *Loriaux 2013*
- ***8-16 hour battery charge life for one battery!***
- ***IR sensor measures leaf temperature*** over a larger leaf area - for more reliable measurements at non-ambient leaf chamber temperatures (Pons T., Flexas J., von Caemmerer S., Evans J.R., Genty B., Ribas-Carbo M. & Brugnol E. 2009)

This instrument is a joint venture between ADC BioScientific and Opti-Sciences Inc. *It continues a tradition of innovation.* ADC BioScientific developed the *first photosynthesis measuring system* base on IRGAs in 1970. It was also the first company to develop the revolutionary *"open mode"* design that is now used by all major suppliers. Opti-Sciences, started in 1993, has prided itself on leading technology, automation of instrument housekeeping functions, and reliable measuring instrumentation.

The iFL was designed to improve on existing instruments with an eye on the latest research, the best methods, and the latest technology improvements.

The iFL is also very easy to use. It lets users concentrate on research while automating housekeeping functions that are better done with automation and software.

More advanced fluorometer design

In creating the *iFL*, it was important to deal with a number of research updates:

White actinic light allows chloroplast migration as found in nature & allows the xanthophyll cycle to photo-protect.

Cells - chloroplast migration

About 30% of fluorescence NPQ can be caused by chloroplast migration at high actinic light levels.

The photoprotective xanthophyll cycle shades up to 30% of blue light but not red light.

High intensity white light or high intensity blue light allow chloroplast migration in dicot cells (Cazzaniga 2013) and monocot cells as well (Maai 2011). Cazzaniga (2013) found that the fluorescence change at high light levels, that was thought to be due to state transitions and acute photoinhibition was really caused by chloroplast migration. This mechanism is responsible for up to about **30% of nonphotochemical quenching at high light levels in nature**. When this happens, leaf absorptance changes and all light adapted chlorophyll fluorescence measuring parameters are also affected including: Y(II) or Φ_{PSII}, *J* or ETR, NPQ, g_m and C_C. **Without intense blue or white light, the migration does not happen as it does in nature (Cazzaniga S, 2013),(Dall'Osto 2014).** The iFL uses a white light diode that provides an intense blue spectrum preventing measuring errors. Lower level blue light or intense red light do not stimulate significantly chloroplast migration. In addition, the reduced light absorptance caused by chloroplast migration would also affect gas exchange measurements at high light levels if intense blue light is missing. Futhermore, the xanthophyll photo-protective cycle shields leaves from about 30% of intense blue light, after leaf adjustment to high actinic light, but not from intense red light **(Laisk 2014). As a result, white light produces more realistic measurements.**

The white light LED in the iFL provides consistent, stable spectral output throughout it's intensity range. Whether the PAR intensity is at 20 µmols or 7,000 µmols, it provides the same portion of red, green and blue spectral output.

Even illumination

An important focus, was to design a system that would provide even illumination over a larger leaf chamber area. One of the advantages of an integrated system is that the fluorescence signal is averaged over the same measuring area as gas exchange. This is most important when dealing with drought stress, cold stress or low CO_2 levels due to the heterogeneous nature of the fluorescence signal across the leaf under these conditions. ***iFL*** illumination varies less than 10% over the entire area and the intensity is held stable to +- 3 µmols over many hours, to provide reliable measurement integrity. For more information on fluorescence heterogeneity, request the free Opti-Sciences application note on fluorescence heterogeneity at www.optisci.com). The larger rectangular leaf chamber also improves gas exchange measurement reliability for many samples.

Up to date and improved Quenching protocols

Significant research shows the one antennae per reaction center relationship that defines the puddle model quenching protocol is incorrect and obsolete. Lake model quenching protocols are based on reaction centers sharing antennae and considered more reliable. With this in mind, the ***iFL*** offers Kramer lake model protocol parameters, and the more simplified Hendrison lake model protocol parameters with NPQ mathematically resurrected from the puddle model. Puddle model parameters are also included. (For more information on this topic, request the free Opti-Sciences application note on quenching measurement at www.optisci.com). (Kramer 2004) (Hendrickson 2004)

With the lake model protocols, and the stable actinic light source, It is now possible to get more reliable quenching measurement data.

More advanced fluorometer design

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

J, or electron transport rate, should never be used for comparing different leaves without measuring leaf absorptance (Baker N. 2008) for the following reasons: absorptance can vary with plant stress level, and it can vary by species, leaf age, chlorophyll content (Eichelman H. 2004), (Evans 2001) and ***light intensity***. In addition, electrons can flow to other electron sinks other that photosynthesis like photorespiration. This has been found in plants under chilled conditions (Fryer 1998) and in C_3 plants under drought conditions (Flexas 1999, 2000). Eichelman (2004) reports leaf absorptance variations from 0.7 to 0.9 on samples tested. *Recently Cazzaniga 2013 found the chloroplast migration changed leaf transmittance at high actinic light levels.*

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

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

$$J = \Phi_{PSII} \cdot Q \cdot \alpha \cdot \beta$$

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

J = electron transport, Φ_{PSII} = Y(II) or yield of PSII (F_M' - F_s / F_M'), Q = PAR or photosynthetically active radiation at the leaf, α is leaf absorptance using the equation shown above. β is the ratio of PSII reaction centers to PSI reaction centers in the leaf. The spectral characteristics of the White light LED are determined at the factory. They do not change significantly with light intensity or with age. Calibration is done with a white and black sample supplied with the i*FL. While calibration may be checked from time to time it should hold for at least several weeks.*

α is derived in the following way: The amount of red, blue and green-yellow radiation incident on the leaf is measured, B_r, B_b & B_g are the fractions of each spectral range incident on the leaf, determined at the factory; α_r , α_b , α_g , or absorptance in each spectral range, are determined by measuring the amount of light reflected from the leaf in each spectral range, and subtracting that amount from the light incident in each spectral range on the leaf; then, the amount of light transmitted through the leaf, in each spectral range, is measured (represented by T_r ,T_b,& T_g). The values I_r, I_b ,I_g are the raw red, blue and green-yellow radiation incident on the leaf. The light transmitted through the leaf is then subtracted from the equation. The result is a reliable leaf absorptance measurement, and much more reliable J, g_m and C_C measurements.

The ratio of PSII reaction centers to PSI reaction centers varies from 0.4 in some C_4 plants to 0.6 in some C_3 plants (Edwards 1993, Laisk 1996,). While 0.5 is sometimes used for an average value, the most used method for measuring the ratio of PSII to PSI, involves the use of spectral analysis of samples at 77^{o}K (Anderson 1999), (Zell 2010); This ratio varies by type of plant, C_3 or C_4, by plant species, by sun grown leaves vs. shade leaves, and in carbon deficient leaves.

Why use an integrated Chlorophyll Fluorometer - Gas Exchange System?

Progress in the field of measuring the light and dark reactions of photosynthesis continue. *J,* or electron transport rate, from the light reaction has been used in conjunction with gas exchange measurements for some time. The combination allows investigation into rate of mesophyll conductance of CO_2 , g_m, and CO_2 at the site of carboxylation or C_c, among other areas. g_m allows measurement of another form or level of CO_2 resistance as it flows from substomatal cavities to the the sites of carboxilation in chloroplasts.

The use of the old stand by, gas exchange A/Ci curves, are adequate for modeling many plant systems and conditions; however, under a number of circumstances, especially with C_3 plants, g_m becomes a very important parameter. The limitation imposed on photosynthesis by g_m, or mesophyll conductance, can increase from 10% to 22% as temperatures rise from 10°C to 40°C (Bernacchi 2002). g_m has also been shown to increase at light saturation levels (Bernacchi 2002), decrease with increased C_i, and decrease with a decrease in absorbed PAR radiation. (Yin 2009).This integrated combination allows the use of A/Cc curves that incorporate measurements of g_m, for more reliable leaf characterization at higher temperatures and light levels. The combination of fluorescence and gas exchange is also highly diagnostic when measuring cold stress, heat stress, and drought stress in C_3 plants.

Why is leaf absorptance measurement so important?

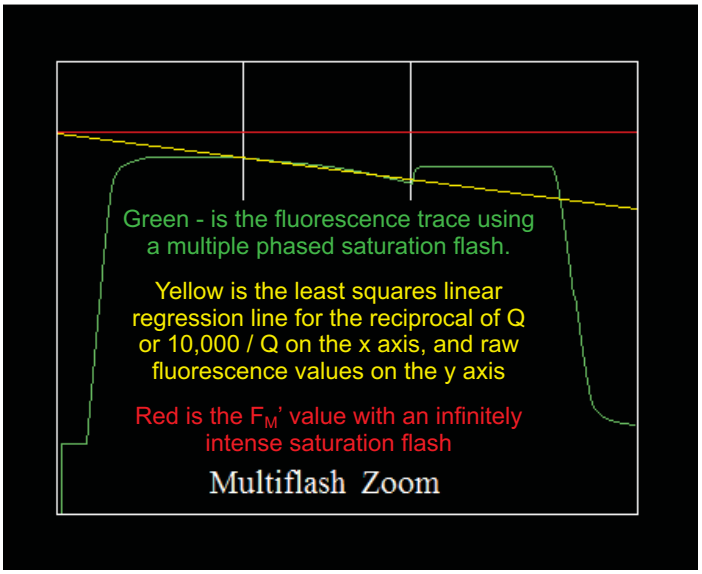
To make valid comparisons of different samples, *J,* or electron transport rate, should *never* be used without measuring leaf absorptance (Neil Baker 2008) or measuring the PSII to PSI reaction center ratio. Part of the equation for *J* includes leaf absorptance, a value that can range from 0.7 to 0.9 in photosynthetically active radiation or PAR (Eichelman 2004). The limits can be larger for specific wavelength ranges. Absorptance not only varies with species, but also with many plant stress types (Carter 1993, Baker 2008) leaf age (Eichelman 2004) and chlorophyll content (Evans 2001) and *light intensity* (Cazzaniga 2013) *As a result, using absorptance measurements value found in literature can lead to substantial measuring errors.*

J, or electron transport rate, errors can be as large as 15.7% if absorptance is not measured.

Why is Multi-Flash F_M' correction important? (multiple phased single saturation flash)

In 2006, it was found by a group that included Genty (Loriaux 2006), the researcher that developed the Φ_{PSII} or Y(II) protocol, that at high actinic light intensities, very intense saturation flashes did not close or reduce all available PSII reaction centers, as required for reliable measurement. The group found that up to a 22% error in Φ_{PSII} was possible causing **errors of up to 41% in *J*.** At that time, they developed a single flash using multiple phased saturation intensities and least squares linear regression analysis to determine F_M' with an infinitely saturation flash. The method has been successful in correcting the issue for most plants.

In 2013, the method was refined (Loriaux 2013). This group found that by using an initial saturation flash of between 7,000 µmols to 13,000 µmols for 0.3 seconds, and an intensity down ramp of 20% to 30%, at a ramping rate of less than 0.01 µmols $m^{-2}s^{-2}$ for 0.5 seconds, the accuracy of the measurement was improved further. The final 0.3 seconds was again, reset to the initial maximum saturation intensity value, to test for saturation pulse NPQ. The research found that values for F_M' did not change above a 7,000 µmols saturation flash intensity on plants that were tested. Above 13,000 µmols, F_M' started to reduce again. The 2013 method provided about a 3% improvement from the 2006 method. The new i*FL* uses the optimal values from the 2013 paper.

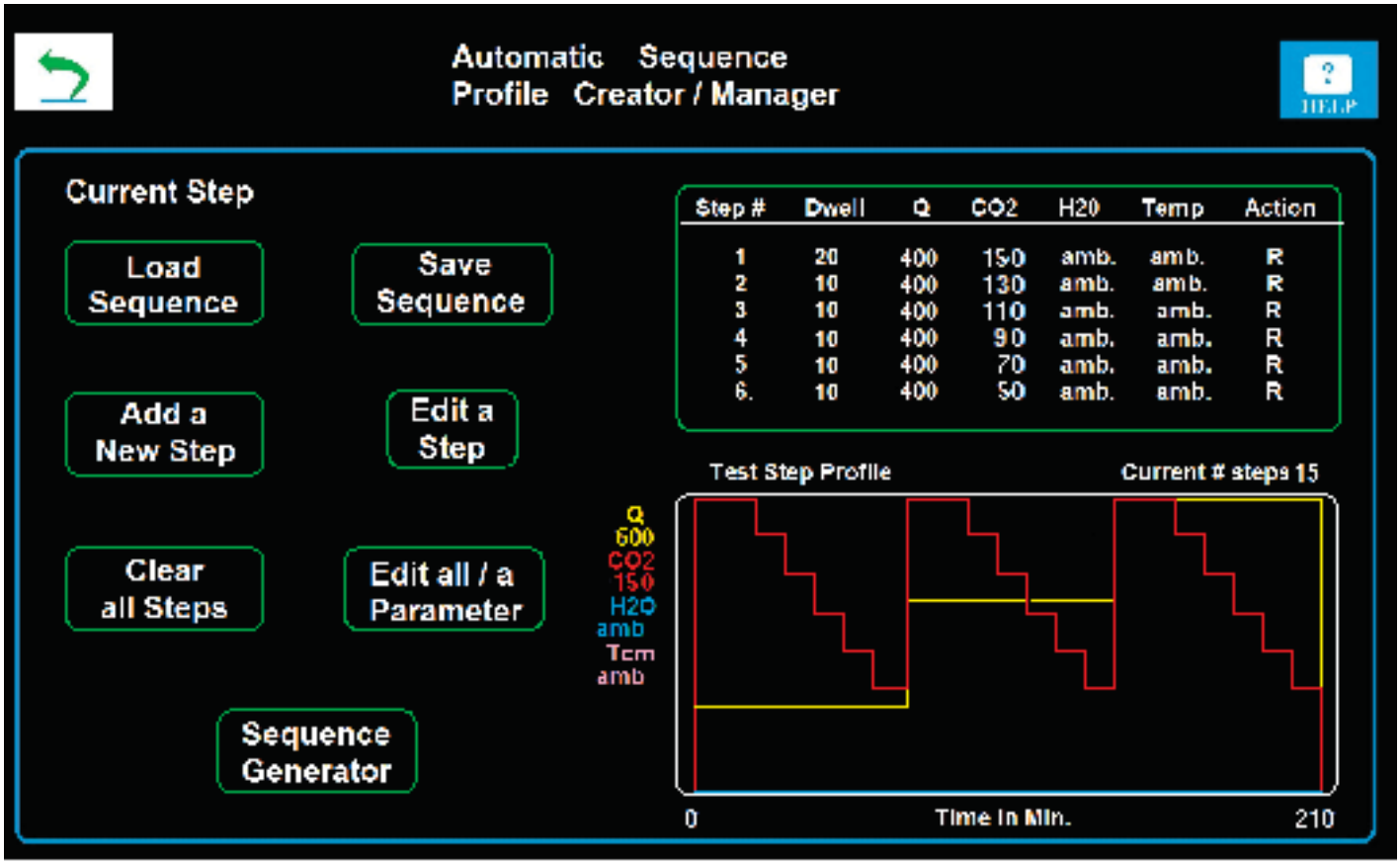


“Walk Away Automation”- with no battery change required! Calibration occurs with every measurement!

The iFL software platform allows measuring steps to be joined together and separate protocols to be joined together with ease. Because, “matching” type measurements are always done, and IRGA drift is zeroed out every 25 seconds automatically with every measurement, the iFL -LCpro-SD may run by itself with confidence.

Example: To do a Laisk protocol R_d and $\Gamma*$ determination, three separate A/Ci curves are required at three different actinic light intensities. With this platform, all three A/Ci curves may be run in consecutive order automatically. Automated post-processing using least squares linear regression curve fitting can also be included. The resulting graph is displayed on the screen along with R_d and $\Gamma*$. After that, It is also possible to then do an A/C_C Curve on the same leaf using the variable *J* or constant *J* method of g_m determination. *Since it can take plants up to 35 minutes to reach steady state photosynthesis at a new light level, due to chloroplast migration (Cazzaniga 2013), some Laisk protocols can take more that 4 hours. The iFL will run for at least 8 hours on one battery charge.*

The sequence building software allows almost an infinite flexibility in step specifications. Hundreds of steps in a sequence are allowed, and up to eight separate protocols to be stitched together and run consecutively. In addition, these files may be saved, and recalled for future use.



Step by step routines included for much easier operation

Direct readout of g_m, C_c, R_d and Γ^*

ADC BioScientific and Opti-Sciences believe that instrument operation should be as easy as possible. Instruments need to be flexible enough to investigate new frontiers in science, and easy enough for new users to operate with confidence.

Software sophistication, and automation of instrument housekeeping functions allows the ***iFL*** to walk instrument operators through the most complicated measurements and routines with ease.
In addition,

Routine tempates exist for several common types of measurements

- A/Q - light response curves
- A/C_i curves & A/C_c curves
- Laisk R_d and Γ_* measuring protocol / The von Caemmerer correction is also supplied.
- Yin R_d protocol
- Kok protocol
- Flexas Chamber leakage protocol
- g_m & C_c protocol - Variable J and Constant J methods
- Absorptance measurement is either automated and part of other routines, or it may be done manually. Measurement of the entire PAR spectrum and leaf transmittance provide a highly reliable result.
- Various quenching measurement protocols

... and others.

R_d, Γ_*, and other parameters may also be entered into formulas when measured by other methods.

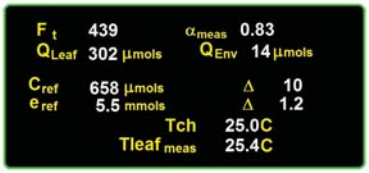
User interface

Color touch screen technology provides easy set up and operation. Menus and help files are easily found and accessible. In addition, graphs can be blown up for full screen viewing by touching graphs. Hunting for unknown operational details is minimized and is replaced by smart design.

Data is easily output in a integrated data file by using either data card technology, or by USB cable. Data is in a comma delineated format for spread sheet use and graphing.

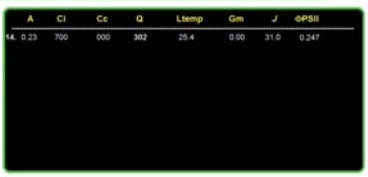
Measuring screen with calculated measuring parameters including g_m and C_c.

A/C_C curves may also be displayed on the upper right hand graphing screen.



Raw data screen

Quenching screen:
Hendrickson lake model
Kramer lake model
puddle model



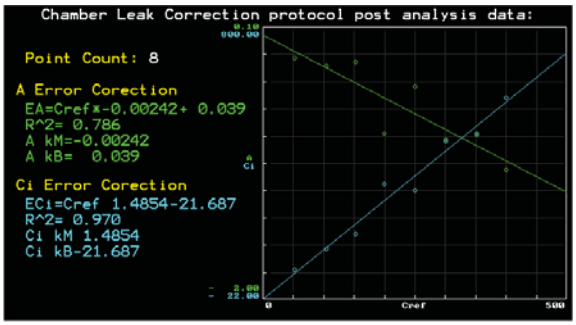
Data screen:
Choose 8 parameters to be displayed.
Scrolling is permitted.

Walk Away Automation & Post Processing

The iFL software also provides “walk away automation” for post processing protocols along with automated determination of R_d, Γ^*, g_m, C_c. Since the i*FL*-LCpro-SD zeros its IRGA every 25 seconds automatically, there is no longer a need to do “matching measurements” every ten minutes or so.

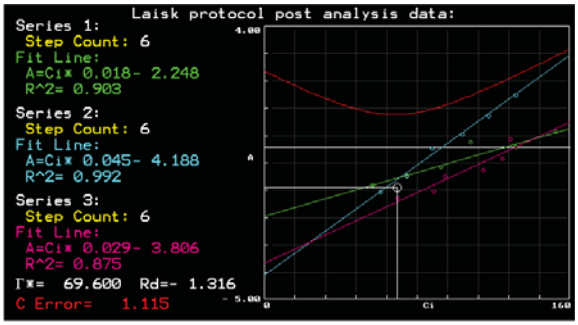
For example, this fact, along with substantial post processing software, allows researchers to set up and run a Laisk R_d , Γ^* Protocol, walk away, come back to the instrument ***several hours later***, and view the results. All of this happens without the fear of IRGA drift. Now researchers can do other things while the experiment is running. **No battery change required!**

Flexas chamber leakage protocol



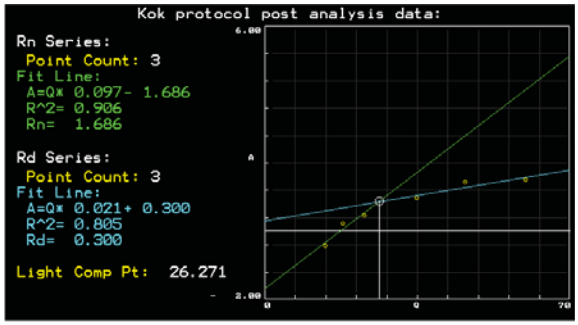
When measuring g_m, C_c, R_d and Γ^*, system chamber leakage & dark respiration diffusion from under system gaskets become important. The Flexas chamber leakage protocol lets the researcher test instrument chamber leakage, for the species being tested, and *automatically apply the results* to other tests and protocols that follow.

Laisk R_d and Γ_* measuring protocol / with von Caemmerer correction option



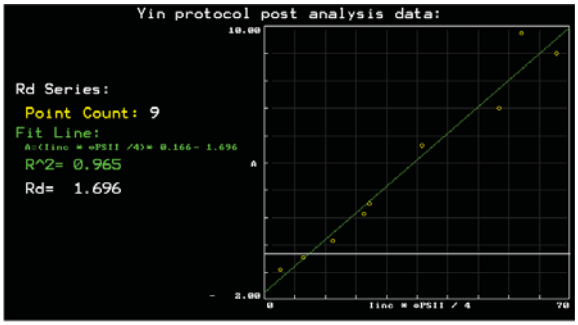
R_d, & Γ^* must be determined to find g_m and C_c . While there are a few different methods that work, the Laisk protocol is probably one of the most used protocols. The graph on the left, is the end result of an automated Laisk protocol test. The parameters are adjustable. The red curved line and its proximity to the white horizontal line are a representation of how close the various A/Ci curves come to a point of coincidence. An algorithm determines the nearest point of coincidence & it is shown in the white circle. The von Caemmerer correction is also available but not shown.

Kok protocol



The Kok protocol can be used for R_d determination. It is primarily used in C_4 plants, but it has also been used for C_3 plants. The Laisk protocol is considered more authoritative for C_3 plants. Least squares linear regression analysis algorithms are included to provide the graphing and results shown on the screen.

Yin R_d protocol



The Yin Protocol is a more recent approach that uses the combined measurement of gas exchange and chlorophyll fluorescence to determine R_d. It offers the advantage that higher light levels and CO_2 levels can be used. Under these circumstances, errors are perceived to be smaller in relation to the values measured.

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

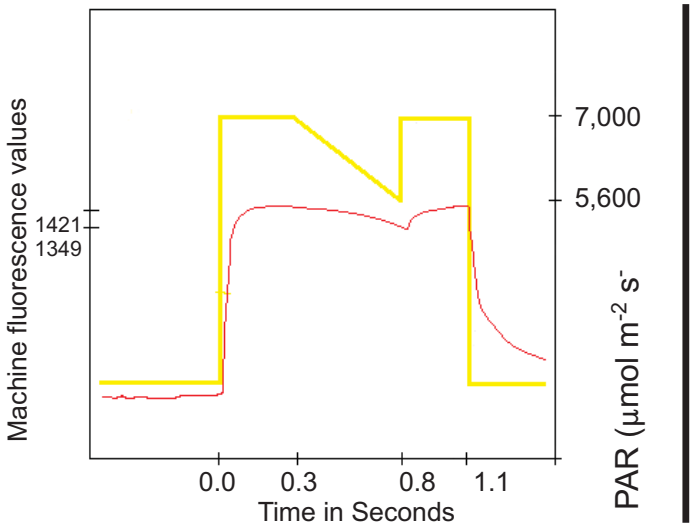
Saturation pulses used with modulated fluorometers are designed to close all PSII reaction centers. The maximum fluorescence intensity value, of the saturation flash, F_M', is used in most measurements including, quantum yield of PSII Φ_{PSII} (also called Y(II) or $\Delta F / F_M'$), *J* (or ETR), and quenching 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 recent high actinic light history, complete closure of all PSII reaction centers becomes problematic with even very high 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, Φ_{PSII} and *J* measurements, taken under these conditions, can be underestimated. In a poster, researchers that included Bernard Genty, the developer of quantum yield of PSII, verified the issue, and developed a method for F_M' correction. It involved a multiple-phase single saturation pulse with multiple light intensities, and the use of least squares linear regression analysis of the reciprocal of Q or PAR, to determine the F_M' fluorescence level using an infinitely intense saturation pulse, without causing damage to the plant.

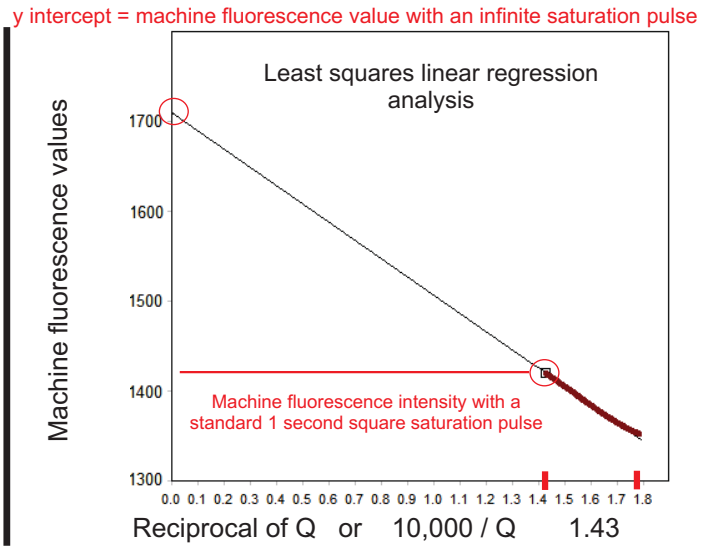
Studies by Earl (2004), Loriaux (2006), and Loriaux (2013) have compared chlorophyll fluorescence measurement results with gas exchange measurements and found that by using multiple saturation flashes, and regression analysis, an infinite fluorescent saturation light flash intensity can be determined and used to correct Φ_{PSII} or (Y(II)) and *J* (ETR) measurements. *Research has shown that Φ_{PSII} 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 J values if this method is not used.*

This standard option is provided on the i*FL* /LCpro-SD and is *available in all measuring protocols*. It can be turned off or on. The method described 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 Loriaux (2013), the iFL protocol provides the optimal saturation intensity of 7,000 µmols, the optimal light ramping of 20%, and a ramping rate less than 0.01 mol $m^{-2}s^{-2}$. While some adjustment is possible, the protocol has been optimized for most applications.

Representation of how the flash works



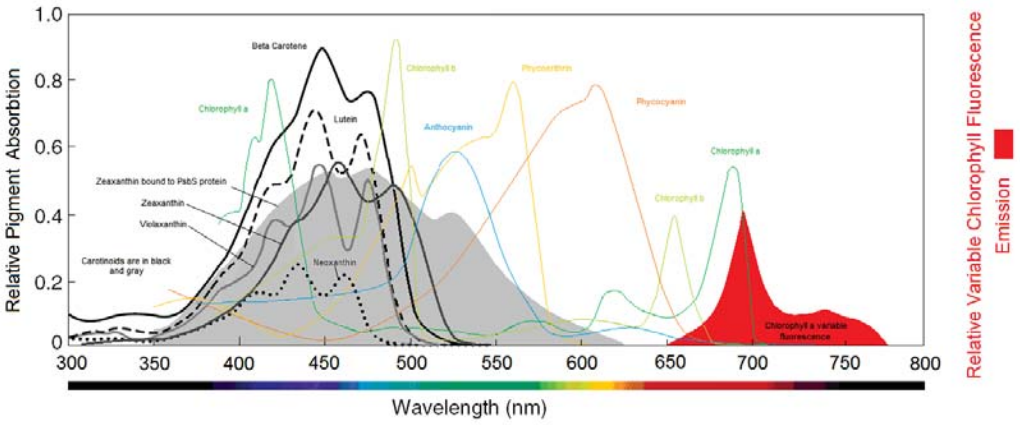
Regression Analysis Graph



The first saturation flash step, shown on the left, is at 7,000 µmols for 0.30 seconds to saturate PSII. The saturation flash intensity is then ramped downward by 20%, making a large number of fluorescence measurements along the way, to 5,600 µmols. The final phase is at 7,000 µmols to check for saturation pulse NPQ. Recent studies have shown that optimal results occur for plants that have been tested with a first saturation flash at 7,000 µmols, a ramp of 20% and a ramping rate less than 0.01 mol photons $m^{-2}s^{-2}$ (Loriaux 2013).

The graph on the right represents the Loriaux, (2006) & Loriaux (2013) method for estimating F_M' with an infinitely intense saturation flash. Least squares linear regression analysis of the reciprocal of Q values measured at known saturation flash intensities, a down ramp of 20%, and a ramp rate that is less than 0.01 mol photons $m^{-2}s^{-2}$ allow determination of the y intercept, which represents the machine fluorescence value with an infinite saturation flash.

More advanced integrated design



Relative absorbance of plant pigments and variable chlorophyll a fluorescence.

Relative absorbtion of Zeaxanthin, and Zeazanthin bound to PSbS protien are adapted from Aspinall-O'dea M.(2002) Chlorophyll a, & b absorbtion spectra, chlorophyll a emission spectra, anthocyanin absorbtion spectra are adapted from Papageorgiou & Govinjee (2004). Relative absorption of Lutein, Beta Carotene, Neoxanthin, and Violaxanthin adapted from Lichtenthaler 2001

Leaf absorptance depth varies with wavelength range

Due to the shorter wavelength range of blue light, most blue light is absorbed by the mesophyll layers on the upper side of the leaf (Gitelson 1999). Red light, on the other hand, due to its longer wavelengths, is absorbed by the entire leaf in most cases. (Gitelson 1999). The iFL also measures leaf transmittance in the blue, green and red light ranges. As expected, almost all of the blue light is absorbed or reflected. In addition, more light in the green range is transmitted through the leaf than in the red range.

Automated modulated light adjustment

For error free measurement, the modulated light intensity must be set low enough to prevent chemical reduction of Q_A, but high enough to allow measurement on samples. The iFL has an automated modulated light set up routine to eliminate errors and save time. Of course in may also be set up manually.

Automated 8 point 25 ms. rolling average of fluorescence signal

The fluorometer provides a rolling average function that ensures that the highest F_M' fluorescence value is selected for measurement. This reduces the effects of electronic noise and prevents saturation pulse NPQ from being a problem under almost all conditions.

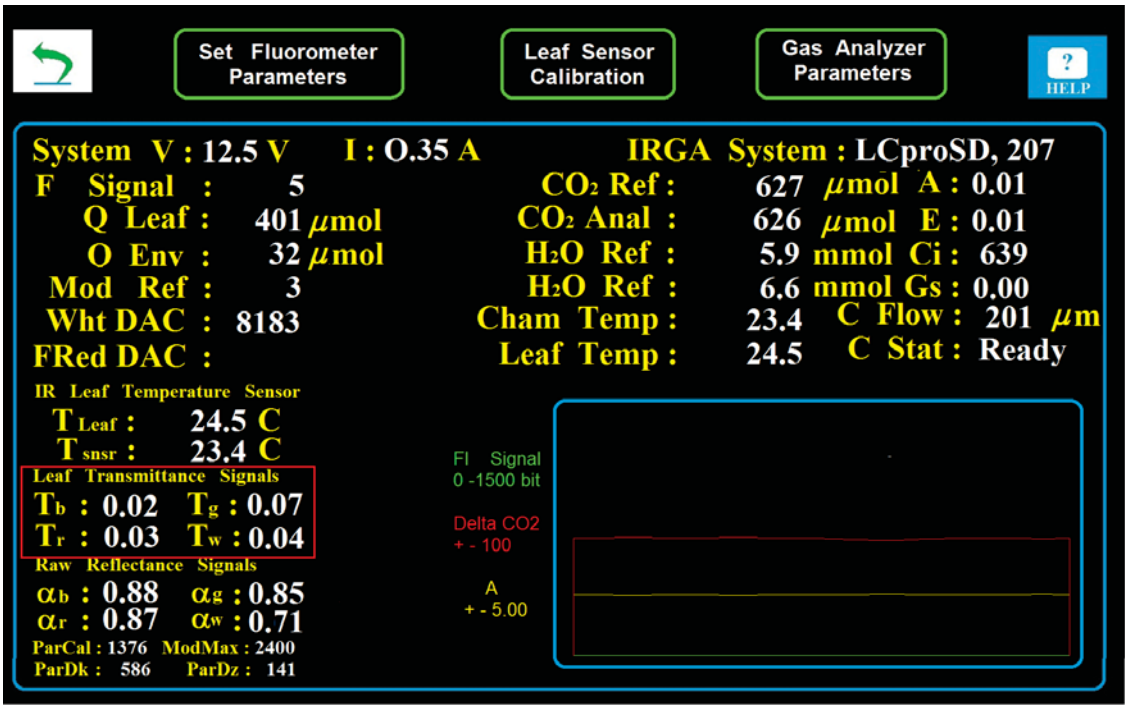
Averaging option for CO_2 measurement

CO_2 measurement variation can be reduced by selecting averaging of a few points, or several points after CO_2 levels have reached steady state. *This option may be turned on or off.*

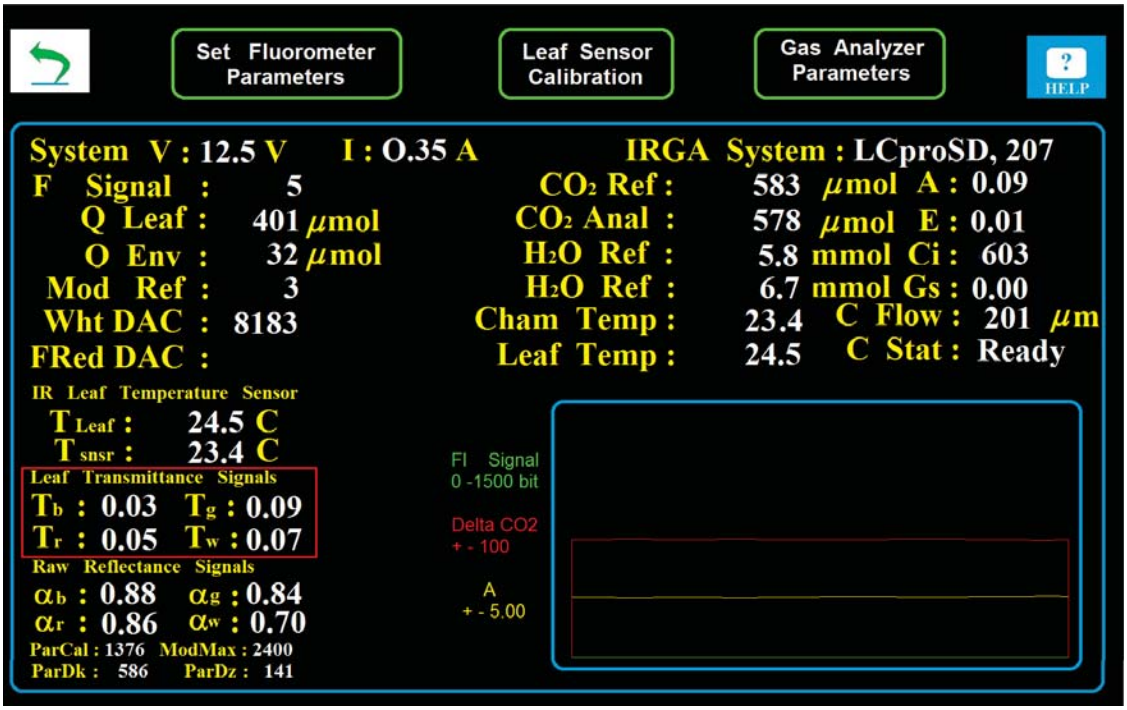
Automated measurement outlier removal option.

This option may be used to eliminate outliers from *automated post processing routines*. Selections are available from 2σ to 3σ for outlier removal from fitted curves. The outlier is reported in the data file and t*his option may be turned on or off.*

Diagnostic screen shows measured leaf reflectance values and leaf transmittance values



The screen above shows leaf transmittance values in the blue, green and red parts of the spectrum on an indoor leaf, at near saturation light levels of 400 µmolls, after 4 minutes. The lower screen shows transmittance values after chloroplast migration, or after 35 minutes. Notice the change. Leaf absorptance changes with light intensity! (Cazzaniga 2013) & (Dall'Osto 2014)



Here, T_b is the portion of leaf transmittance in the blue spectrum, T_g is the portion of leaf transmittance in the green spectrum, and T_r is the portion of leaf transmittance in the red spectrum. α_b, α_g , α_r, values represent leaf absorptance using reflectance only in their respective color ranges. Absorptance displayed in all protocols use both reflectance and transmittance values for calculation.

Up to date options –
for quenching measurements

Most puddle model parameters have been discredited. The 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. The newer and most accepted shared antennae paradigm is called the lake model. (For more information on this topic, ask for the free "Quenching application note" at www.optisci.com)

Hendrickson *simplified lake model* parameters with NPQ resurrected by Klughammer from the puddle model (2008). - Hendrickson developed alternative lake model quenching parameters that did not require the use of F_o'.

Y(II) Quantum yield of PSII also known as Φ_{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) (equivalent to puddle model NPQ)

Kramer lake model quenching parameters (2004). F_o' is used it this protocol.

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

Puddle model quenching parameters. F_o' is used in some of the parameters.

Y(II) Quantum photosynthetic yield also known as Φ_{PSII}
$\mathbf{q_P}$: Photochemical quenching
$\mathbf{q_N}$: Non-photochemical quenching
NPQ: Non-photochemical quenching

Stable light source –
for reliable steady state measurements

The ***iFL*** maintains a constant light intensity during long quenching measurement protocols, and all longer measurements. All of the various quenching parameters except Y(NO) require *steady state photosynthesis* conditions at a *constant light level*, by definition. Y(II) or Φ_{PSII} and *J* also require steady state photosynthesis for reliable measurements. Steady state takes between fifteen minutes and twenty minutes at a specific steady light level (Maxwell and Johnson 2000). Most non-stable light source intensities will lower significantly over short periods of time due to lamp heating when turned on. The ***iFl*** is designed to prevent that issue. First, the LED based actinic light does not generate very much heat, and second, the PAR sensor, in the leaf chamber, monitors light level and maintains a constant light level. There is enough reserve light output to maintain the highest set PAR levels. Furthermore, temperature can be controlled by the ***iFL*** **–Lcpro-SD** temperature control. The end result is an *optimal* environment for NPQ, Φ_{PSII}, and *J* measurements. It also has significant advantages for A/Q curves, A/Ci Curves, and A/Cc curves, as well as the Laisk, Kok, and Yin protocols. With a stable light source, steady state photosynthesis *is* steady state photosynthesis, and errors are eliminated.

The most portable integrated field instrument available

The combined weight of the instrument is 4.48 kg or 16.5 lbs.

The lead acid 12 volt battery will last for eight hours between charges.

There are dual PAR sensors; one inside the fluorometer chamber, and one for ambient measurement. This allows for the option of using the instrument to measure ambient light levels, and duplicating the level in the chamber. One can significantly reduce the time to reach ambient steady state photosynthesis.

In addition, the internal light intensity can be set to ambient, and then maintained, even on cloudy days, to ensure steady state photosynthesis.

The light intensity may also be set to desired levels, or multiple levels required for light curves, and A/Q curves.

All day instrument harness

The all day instrument harness is available as an accessory for those researchers that plan to take a number of individual measurements over longer periods of time. It is designed to distribute the weight of the instrument evenly across the back and over both shoulders. The back cross design, also found in high quality backpacks, allows comfortable working conditions for hours. To help prevent headaches, there is no strap across the back of the neck.

The harness is adjustable to fit almost any size, with velcro straps, and adjustment buckles. It may be adjusted from small to 3-extra large for men or women.

In combination with the eight hour battery charge life, and the lightest weight integrated fluorescence - gas exchange system, the iFL-LCpro-SD is the most portable system of its type available.

While many researchers use ADC Bio-Scientific instruments exclusively, there are also some that have been trained on other brands, and use them in the lab, but use the ADC instruments for there field work.

ADC BioScientific and Opti-Sciences instruments are much easier to use.

Parameters Measured and Protocols included:

Selectable readout in either μmols, mmols, and moles, or in ppm and mbars

Φ_{PSII}**:** Quantum Yield of PSII (or ΔF/Fm' or Y(II))
***J*:** Electron transport rate also known as ETR
PAR: Photosynthetically Active Region value (with optional PAR clip)
α: Leaf absorptance of PAR spectrum using RGB sensors above the leaf and below the leaf. Corrected for leaf transmittance.

Chamber temp: thermistor
Leaf T: Leaf temperature -infrared sensor

Laisk protocol for R_d and Γ^*
Yin protocol for R_d.
Kok protocol for R_d.
$\mathbf{g_m}$ Mesophyll conductance
$\mathbf{C_c}$ CO_2 at the site of carboxilation
Γ^* Compensation point absent of day respiration
R_d Respiration in the light
R_d , Γ^* and other parameters or constants may also be input manually.

Light curves & A/Q light response curves
A/C_i curves, A/C_c curves

Fv/Fm: Maximum Photochemical efficiency of PSII
Fv/Fo: A more sensitive detector of stress than Fv/Fm, but it does not measure plant efficiency.
Fo: Minimum fluorescence
$\mathbf{F_M}$**:** Maximal fluorescence
Fv: Variable fluorescence
$\mathbf{F_M'}$**:** Maximal fluorescence with actinic illumination
$\mathbf{F_S}$ **(or F):** Fluorescence under steady state conditions (prior to saturation pulse)
Y(II) : (Φ_{PSII} or ΔF/Fm') Quantum Yield of PSII

RLC: Rapid light curves.
$\mathbf{rETR_{MAX}}$ - 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
$\mathbf{I_k = rETR_{MAX}/\alpha}$ is a measurement of the light intensity where Im:light saturation dominates, or the minimum saturation level
$\mathbf{I_m}$: Light intensity for $rETR_{MAX}$

Hendrickson Quenching with NPQ
Y(NPQ), Y(NO), Y(II), NPQ, Fv/Fm
Kramer Quenching
q_L, Y(NPQ), Y(NO), Y(II), Fv/Fm
Puddle model parameters
NPQ, q_N, q_P, Y(II), Fv/Fm

Walk Away automation sequence builder.
Build a sequence, build a protocol, or stitch multiple protocols together.

Fluorometer Specifications

Light Sources:
Saturation pulse White LED with 690 nm short pass filter. 7,500 μmols
Modulated light 660 nm LED with 690 nm short pass filter.
Actinic light source: White LED to 2000 μmols
Far red light: above 740 nm
Blue sensors for absorptance measurement
Red sensors for absorptance measurement
Green sensors for absorptance measurement
PAR Sensors - Two silicon diodes. One is inside the fluorometer chamber to measure PAR at the leaf level. It maintains a constant actinic light irradiation level throughout measuring routines, to ensure constant and proper intensity for steady state measurements. It is also used to measure absorptance diode intensity.

The second PAR sensor is on top of the fluorometer. It can be used to measure and track or set ambient light irradiation at the angle of the leaf. This sensor can be used to match ambient PAR intensity in the leaf chamber in an automatic fashion. When it is desired, this feature can be used set light level on partly cloudy days to either sunny conditions, or cloudy conditions to minimized the time required to reach steady state conditions for ambient light measurements.

Detection method: Pulse modulation method.
Detector & Filters: A PIN photodiode with a 700 ~ 750 nm bandpass filter.
Sampling Rate: Auto-switching from 10 to 10,000 points per second, depending on phase of test.
Automated routine to optimally set the modulated light intensity. The modulated light may also be set manually.
Multi-Flash Fm' correction for all light adapted protocols. (According to Loriaux 2013)
It may be turned on or off.
Test Duration: Adjustable from 20 seconds to 4000 hours.
Storage Capacity: 2 Gigabyte of non-volitile internal flash memory, supporting unlimited data sets and traces
Digital Output: USB, SD/MMC 2 gigabyte data cards .
Video output: HDMI

User Interface:
Display: Graphic color touch screen
Menu driven touch screen.

Battery Life: 8 hours of continuous combined operation.

Gas Exchange Specifications:

The researcher can choose to have the instrument report in μmol, mmols & moles, or in ppm & mbars

CO_2 : 0-3000ppm with 1 ppm resolution, **or 0-3000 μmols with 1 μmol resolution**.

H_2O : 0-75mbars with 0.1mbar resolution, **or 0-75.5 mmols with .1mmol resolution.**

PAR : 0-3000μmols Silicon photocell.

Thermistors are now more accurate and reliable than thermocouples
Contact Opti-Sciences for more details

Chamber temperature : -5°C tp 50°C precision thermistor +- 0.2°C accuracy

Direct leaf temperature : -5°C tp 50°C precision thermistor +- 0.2°C accuracy, by self positioning microchip thermistor, or energy balance, or by manual positioned thermistor.

Flow rate to leaf chamber 100 to 500 ml $^{-1}$ min^{-1}.

Automatic Environmental Control Specifications :

CO_2 : Up to 2000 ppm or 2000 μmols by integrated CO_2 supply built into the system.

H_2O : Above or below ambient by on - board self indicating conditioning chemicals.

Temperature : Micro -Peltier element. +- 14°C from ambient

PAR Up to 2000 μmols

Warm up time : five minutes.

Other Specifications

Display : High resolution color graphic screen. HDMI video output included.

Recorded data : Built in 2 Gb flash memory. Can store up to 8 million sets of data, & 2 Gb removable SD/MMC card.

Battery : 7.0 Ah lead acid battery up to 8 hours of use.

Battery Charger - Universal input voltage intelligent control

USB connection : Mini-B, Function as mass storage device.

Operating temperature range : 5°C to 45°C

Dimensions: Console 230mm x 120 mm by 220mm.
Plant leaf chamber cuvette 300mm x 100mm x 80mm.

Complete Weight : 4.48 kg, or 16.5 lbs.

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