CIRAS-3 Portable Photosynthesis System

Operation Manual

Version 1.03

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For applications where failure of this equipment to function correctly would lead to consequential damage, the equipment must be checked for correct operation and calibration at intervals appropriate to the circumstances. The PP Systems' equipment warranty is limited to replacement of defective components, and does not cover injury to persons or property or other consequential damage.

This manual is provided to help you install and operate the equipment. Every effort has been made to ensure that the information it contains is accurate and complete. PP Systems does not accept any liability for losses or damages resulting from the use of this information.

It is the operator's responsibility to review this information prior to installation and operation of the equipment. Otherwise, damage may be caused which is not covered under our normal warranty policy.

User Registration

It is very important that ALL new customers register themselves with us to ensure that our user's list is kept up-to-date. If you are a PP Systems' user, please register yourself electronically on our web site at:

http://www.ppsystems.com/user_registration2.htm

Only **REGISTERED** users will be allowed access to the protected "Users" section of our web site. This section will contain important product information including hardware/software updates, application notes, newsletters, etc.

Thank you in advance for your cooperation.

Service & Warranty

PP Systems' equipment warranty is limited to replacement of defective components, and does not cover injury to persons or property or other consequential damage.

The equipment is covered under warranty for one complete year, parts and labor included. This, of course, is provided that the equipment is properly installed, operated and maintained in accordance with written instructions (i.e. Operator's Guide).

The warranty excludes all defects in equipment caused by incorrect installation, operation or maintenance, misuse, alteration, and/or accident.

If for some reason, a fault is covered under warranty, it is the responsibility of the customer to return the goods to PP Systems or an authorized agent for repair or replacement of the defective part(s).

Prior to returning equipment to PP Systems for service, you must first get in contact with our Service Manager (service@ppsystems.com) to request a case number for reference and tracking purposes.

Contact Information

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Unpacking Your Equipment

It is extremely important that you check the contents of your equipment immediately upon receipt to ensure that your order is complete and that it has arrived safely. Please refer to the checklist supplied (if applicable) for a detailed list of spares and accessories that are included with your order.

DO NOT DISCARD ANY OF THE PACKAGING MATERIAL UNTIL ALL OF THE ITEMS LISTED ARE ACCOUNTED FOR.

WE RECOMMEND THAT YOU RETAIN THE ORIGINAL PACKING FOR FUTURE USE.

If you suspect that any of the items listed on the appropriate checklist are not included or damaged, you must contact PP Systems or authorized distributor immediately.

Section 1. Technical Specification

CIRAS-3 Main Console	
Analysis Method	Non-dispersive infrared, configured as an absolute absorptiometer with microprocessor control of linearization. Four independent gas analyzers simultaneously measure absolute CO_2 and H_2O for both the reference and analysis gas streams. All measurements corrected for temperature and pressure.
CO_2 Measurement Range	0-10000 µmol mol^{-1}
CO_2 Precision	• 0.2 µmol mol^{-1} at 300 µmol mol^{-1} • 0.5 µmol mol^{-1} at 1750 µmol mol^{-1} • 3.0 µmol mol^{-1} at 10-00 µmol mol^{-1} • 3.0 µmol mol^{-1} at 300 µmol mol^{-1}
CO_2 Control Range	0-2000 µmol mol^{-1}
H_2O Measurement Range	0-75 mb
H_2O Precision	• 0.015 mb at 0 mb • 0.020 mb at 10 mb • 0.030 mb at 50 mb
H_2O Control Range	0-Dewpoint
Pressure Range	65-115 kPa
Air Sampling	User adjustable from 50-100 cc min^{-1} using integral DC pumps. Both analysis and reference pumps fitted with mass flow controllers.
Cuvette Air Supply Unit (Integral)	0-500 cc min^{-1} measured and controlled by a mass flow meter.
Auxiliary Port	For connection to external devices (i.e. SRC-1 Soil Respiration Chamber, CPY-4 Canopy Assimilation Chamber).
Digital Output	• USB-Mini b (Host) • 2 Ea. USB for use with external devices (Memory stick, USB Mouse, etc.).
Data Storage	512 MB flash memory for programming and data storage. Unlimited data storage using USB thumb drives (memory sticks).
Microprocessor Speed	800 MHz
Display	7.0” WSVGA transflective, color LCD
User Input	27 tactile keys

Power Supply	Internal, rechargeable 7.2V Li-ion battery providing up to 8 hours continuous use. Power supply/charger included.
Operating Temperature Range	0-50 ^{o}C, non-condensing. In dirty environments, external air filtration may be required.
Enclosure	Rugged, ergonomic, lightweight aluminum with polyurethane base.
Dimensions	28 cm (W) x 14.5 cm (D) x 24 cm (H)
Weight	4.3 kg (including battery).

PLC3 Leaf Cuvettes	
Construction	• Handle - Aluminum • Leaf Gasket - Closed cell foam • Impeller - Stainless steel
Window	**PLC3 Universal – Glass IR interference filter** • 25 mm x 7 mm (1.75 cm^2) • 18 mm diameter (2.5 cm^2) • 25 x 18 mm (4.5 cm^2) **PLC3 Narrow** – Glass IR interference filter **PLC3 Conifer** – Scratch resistant glass
LCD Display	2 x 16 character LCD for display of user defined parameters
Keypad	Two tactile feel keys for recording and LCD selection
PAR Sensor (Internal)	**PLC3 Universal** – 2 miniature,silicon photodiode sensors **PLC3 Narrow and Conifer** – 1 miniature, silicon photodiode sensor • Response: 400-700 nm • Range – 0-3000 µmol m^{-2} s^{-1} • Precision – 10 µmol m^{-2} s^{-1}
PAR Sensor (External)	Filtered, silicon cell (cosine corrected) • Response: 400-700 nm • Range – 0-3000 µmol m^{-2} s^{-1} • Precision – 10 µmol m^{-2} s^{-1}
Air Temperature Sensor	• Range: -10 ^{o}C to 50 ^{o}C • Precision Thermistor • Accuracy - ± 0.5 ^{o}C at 25 ^{o}C

Temperature Control	Approximately 10 ^{o}C below ambient to +15 ^{o}C above ambient. • Temperature control limits – 0 ^{o}C to 45 ^{o}C
Leaf Temperature Sensor	**PLC3 Universal** – Radiation sensor for non-contact measurement • Accuracy - ± 0.5 ^{o}C at 25 ^{o}C **PLC3 Narrow and Conifer** – Precision thermistor • Accuracy - ± 0.5 ^{o}C at 25 ^{o}C
Dimensions (General)	32 cm (L) x 3.8 cm (Handle diameter)
Weight	**PLC3 Universal** – 0.750 kg **PLC3 Narrow and Conifer** – 1 kg

CFM-3 Chlorophyll Fluorescence Module	
Modulating Beam	625 nm ± 5 nm (Red)
Saturation Light	0-10000 µmol m^{-2} s^{-1}
Far Red Light	2 x 750 nm LEDs
Detector	PIN Photodiode with > 700 nm filter
Detector Method	Rapid pulse peak tracking
Leaf Area	1.75 cm^2, 2.5 cm^2 and 4.5 cm^2

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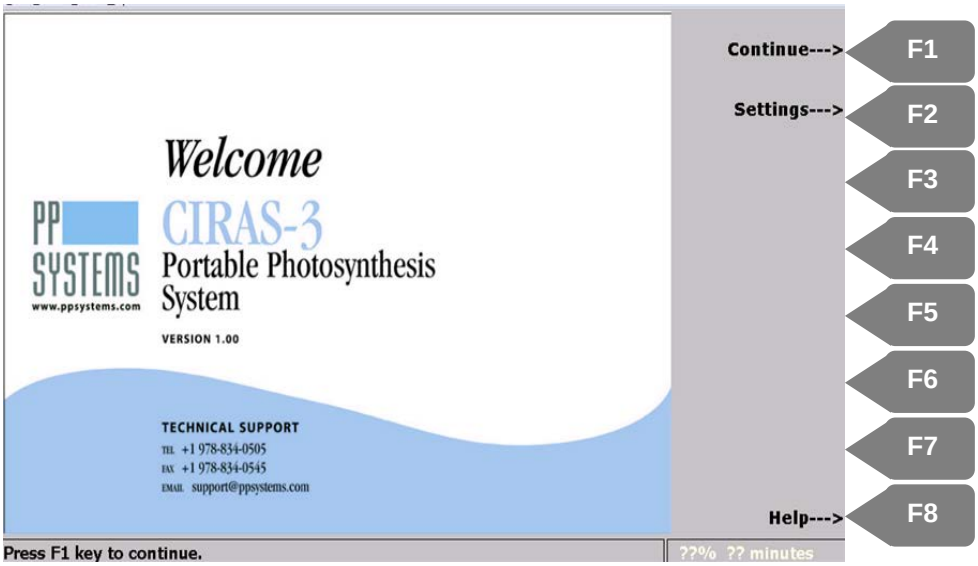
Section 2. What's New with CIRAS-3?



Much has changed since we introduced the CIRAS-1 Portable Photosynthesis System in 1992. What you will find in CIRAS-3 is not only a simple repackaging of existing designs and concepts, but in many cases a complete rethinking of both the form and function available through CIRAS-3's hardware and software. Below you can read a summary of those changes, many of which are based on direct feedback from you. Many more details will emerge throughout the technical documentation.

General Changes to CIRAS-3 Hardware

Main Console

- The CIRAS-3 main console has been significantly redesigned – it is now exceptionally light at 4.3 kg (including battery), with new, more compact dimensions of 28 cm (Width) x 15.5 cm (Depth) x 24 cm (Height) footprint.
- The sealed console has a 96 x 153 mm transflective color display with a practical 30° ergonomic design.
- The console has greater processing speed and larger data storage capability than previously.
- Reference and Analysis gas connections are through a single, integrated connector/port.
- New gas tubing with better absorption characteristics has replaced older tubing associated with the console and cuvette.
- The CO_2 regulator assembly is improved, eliminating the need for the internal o-ring.
- The console handle is adjustable by continuous tension over 180°, eliminating fixed positions that required both hands to set.
- The adjustable carrying harness is more comfortable with CIRAS-3, allowing convenient hands-free operation whether in the field or greenhouse.
- Inside the console the CO_2 mixer has been completely redesigned, allowing replacement of the dual-pump design with a single diaphragm-type air supply pump.
- CIRAS-3 now has a Li-ion one-battery system (potential field operation time of 8 hours), replacing the two NiMH batteries.

PLC3 Series Leaf Cuvettes

The new PLC3 Universal cuvette has several new improvements and features:

- The cuvette is smaller and lighter than its predecessor.
- Mounted to the cuvette is a practical, 2 x 16 charcter, black & white external LCD allowing you to view parameters. You can also toggle between displays for additional parameters.
- Manual recording and scrolling through parameters on the external display are accomplished with two new keys embedded in the cuvette's open/close lever.
- A new external cosine-corrected PAR sensor (PARe) is available with the PLC3 Universal, in addition to the 2 silicon photodiode sensors beneath the cuvette window. The PLC3 Conifer and Narrow have 1 silicon photodiode sensor beneath the cuvette window.

LED Light Unit (RGBW)

Outwardly the LED light unit looks nearly the same as previous versions, but it clearly much different:

- The light unit inner shroud and outer housing have been redesigned to eliminate potential light leakage from reflecting ambient and external light sources.
- Attachment of the light unit has been improved – now the light unit mounts to the cuvette slides
- The PLC3 Universal has an enhanced upper light intensity range setting up to 0-2500 µmol m^{-2} s^{-1} allowing replication of extreme light environments, such as high elevation and desert. Maximum light intensity will be slightly different with the PLC3 Conifer and Narrow Leaf Cuvettes depending on selection of LEDs at the high range.
- Resolution and stability over the entire range of light intensity has been greatly improved with newer electronics.
- There are 48 individual LEDs representing RGBW (red-green-blue-white) color distribution. Any one of these colors may be set from 0-100% of the light source, or mixed to produce light of a specific spectral distribution.

CFM-3 Chlorophyll Fluorescence Module

The chlorophyll fluorescence module (CFM-3) has the following improvements:

- CFM-3 electronics have been streamlined and miniaturized, while keeping the CFM-3 and light unit in a single multi-functional package, i.e. for combined photosynthesis measurements.
- 10000 µmol m^{-2} s^{-1} maximum saturating pulse intensity
- A Multiphase flash saturating pulse was incorporated for estimation of apparent Fm'.
- New PLC3-compatible leaf clips allow simultaneous dark adaptation on multiple leaf samples (under development).

Changes to CIRAS-3 Software

The new user-friendly and intuitive software is easy to navigate, involving no programming or complicated software language. Basically, navigating CIRAS-3 menus is done by way of clearly labeled function keys to move between menus and screens, then a TAB key moves you among entry fields, where selections are entered or chosen from dropdown lists. Menu overviews are much improved, categorical menus are displayed with all options visible. There are clear, menu-driven controls for setup, operation, recording and data recall. For example, you can make changes to dynamic Controls, e.g. CO_2, H_2O, light, temperature with as few as three key presses.

Below are several new software highlights:

- Toggle to view either the full suite of numeric Measured, Control and Gas Exchange data *or* your choice of 0-5 numeric parameters and as many as 3 line graphs or scatter plots
- Customize the screen to see your preferred combination of information available from the system and the leaf
- View absolute and differential IRGA values associated with either gas, e.g. CO2r, CO2a, CO2d
- The standard calculated photosynthesis parameters are now A, Ci, E, gs, WUE, VPD
- Data collection – set up Manual, automatic Interval, or designed Response Curves with a few key presses, write/edit your own response curve scripts in simple .xml format
- Data review – on-screen view of numeric records (entire data set) or view graphical time-course trends and two-variable scatter relationships immediately
- Data transfer – download/upload scripts and data files via USB2 memory devices

Environmental Control

- *CO_2* has simplified control options, approximate concentrations (ideal for response curves), fixed concentrations or ambient operation
- *H_2O* has simplified control options, fixed concentrations, constant VPD, ambient operation
- *Temperature* range is ~10 ^{o}C below ambient to 15 ^{o}C above ambient within the control range of 0-45 ^{o}C, precision improved to ±0.5 ^{o}C
- *Light* intensity has up to a 0-2500 µmol m^{-2} s^{-1} control range with ±1 µmol m^{-2} s^{-1} precision. 0-100% RGBW LED color distribution
- Cuvette flow rate can be controlled between 150-500 cc min^{-1} with ±1 cc min^{-1} precision

CFM-3 Chlorophyll Fluorescence Module

- You now have the option to run and record complete fluorescence measurement sequences on their own (with or without combining photosynthesis measurements)
- Calculated fluorescence parameters, representing both lake and puddle models of PSII reaction centers, e.g. ΦPSII NPQ-K (Kramer), ΦNPQ-G (Genty)

Accessories

SRC-1 – soil collar volume/area input is a new Settings input, display and graph ΔCO2, FCO2, evaporation, Tsoil

CPY-4 – collar volume/area input is a new Settings input, display and graph ΔCO2, canopy Assimilation/Respiration, transpiration rate, Tchamber, PAR

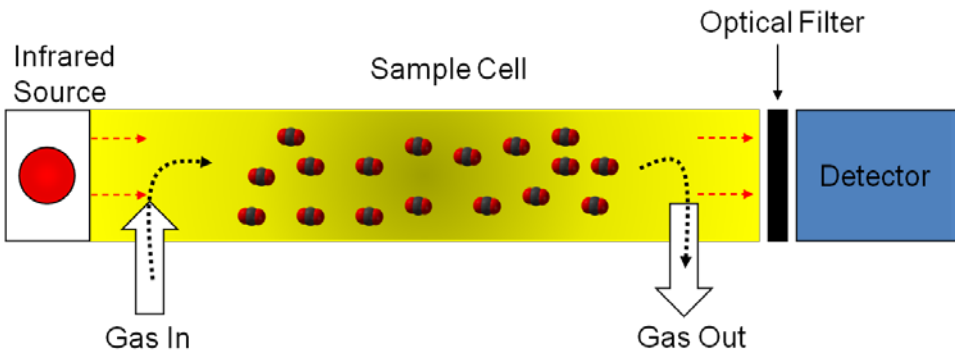
Section 3. Summary of System Design

Overview and Theory

CIRAS-3 is designed to function as a self-contained open-system gas analyzer, manufactured and calibrated for high-precision detection of CO_2 and H_2O gasses. CIRAS stands for **C**ombined **I**nfra-**R**ed **A**nalysis **S**ystem. Its open-path design allows for continuous, unattended air sampling, as the pumps introduce fresh sample gas to the essential components, the IRGAs. CIRAS-3, like previous generations of CIRAS, has four non-dispersive IRGAs (Infra-red Gas Analyzers) – CO_2 Reference, CO_2 Analysis, H_2O Reference, H_2O Analysis, a true differential analyzer.

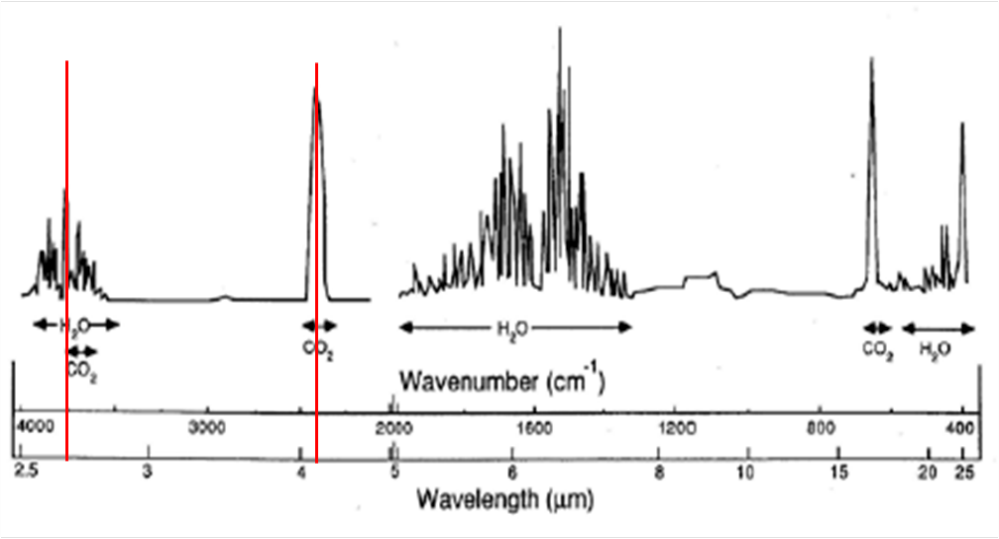
The IRGAs form the core of gas analysis systems that measure CO_2 and water vapor (i.e. portable photosynthesis system, eddy covariance, soil CO_2 efflux, etc.). Non-dispersive infra-red (NDIR) refers to the transmission of broad-band infra-red wavelengths from the IRGAs source lamps. A single IRGA consists of four basic components:

- Infra-red source
- Sample cell of known path length and volume
- Optical interference filter
- Infra-red detector



The theory itself is quite simple – light from mid-infra-red wavelengths is produced by the source and pulsed through a gold plated cell. The interference filter narrows the bandwidth of the IR source received by the detector to the signature wavelength absorbed by the target gas molecule, e.g. CO_2. The CO_2 and H_2O cells each employ a unique optical filter. As the sample gas fills the cell, it absorbs IR, and the reduction in IR source strength is measured instantaneously by the detector. The *higher* the target gas concentration, the *lower* the infra-red signal received at the detector, as defined by the Lambert-Beer Law of Attenuation.

Both H_2O and CO_2 molecules have diverse absorption spectra, so we use two prominent absorption peaks, seen below at 2.6 and 4.26 µm, respectively. CIRAS-3's electronics could be considered the fifth component, which processes raw analog-to-digital (A/D) information from the IRGAs detectors, accurately translating this information into gas concentrations.



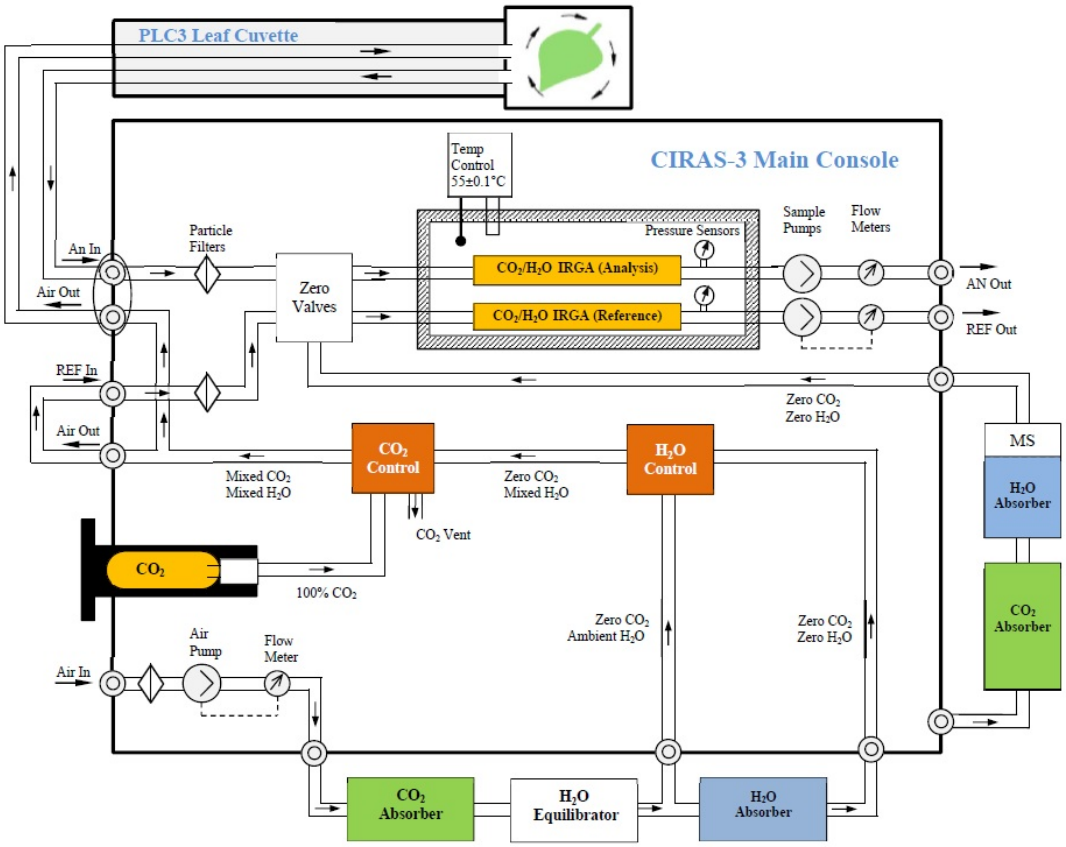
The gas sample is of course a mixture of gas molecules, and this can present problems in terms of accurate detection of concentrations of a specific gas, such carbon dioxide. This effect, *foreign gas broadening* (FGB), must be corrected to ensure accurate measurement of gas concentrations. With FGB, the CO_2 gas in the IRGA cell is somewhat diluted by the increased air volume induced by water vapor. This effect is about 0.1 µmol mol^{-1} CO_2 mb^{-1} H_2O. The presence of water vapor also causes an increase in infra-red absorption, which is detected as an apparent increase in [CO_2]. This is of a similar magnitude, but opposite to the dilution effect, and CIRAS-3 automatically corrects these FGB effects.

CIRAS-3's IRGAs are quite stable owing to their construction, calibration and thermal environment, but various circumstances can cause apparent changes over time. Some changes may require recalibration, although one of the strengths of CIRAS-3 is that recalibration is not a routine (annual) maintenance task. The factory calibration ranges of 0-2000 µmol mol-1 CO2 and 0-75 mb water vapor are ideally suited for most typical applications.

Factory linearization of the IRGA cells is standard, but slight differences between IRGAs are inherent due to the uniqueness of optical filters and reflection characteristics of the cells - this is common to all differential analyzers. Still, the Reference and Analysis cells should be made to match a standard such as Zero air, and CIRAS-3's Auto Zero function corrects for nearly all changes that result in calibration drifts. Auto Zero minimizes effects on span (gas sensitivity), of sample cell contamination, lamp ageing, changes in detector sensitivity, amplifier gains and reference voltages. Measurements are ratioed to the Zero reading before IR absorbance is determined. From the relationship between absorbance and concentration determined in the factory for each instrument, and the current calibration factor, the sample concentration is determined.

We overcome short-term drifts by use of a second Zero mode, called Differential Balancing, or Diff Bal. Using Diff Bal temporarily diverts *only* Reference air through all cells (Reference and Analysis). If existing offsets are detected between the Reference and Analysis cells while measuring the same Reference gas sample, appropriate correction factors are calculated to equalize the readings of each cell pair. This way, you can have confidence that a reported differential between the Reference and Analysis cell pair is real and not artificial.

An overview of the gas circuit design of CIRAS-3 configured for leaf-level photosynthesis is shown in the schematic below. Sample air, denoted as AIR IN, entering the console and is first pumped and its flow rate metered, then directly "conditioned" by passing through CO_2 and water vapor absorbant chemicals. At this point CO_2 can be added to the gas stream in a precise mixture from the CO_2 source cartridge, while existing water vapor can be removed from the gas stream or allowed to remain at its current partial pressure, measured in mb. One portion of the mixed air (AIR OUT) is then passed along downstream to be measured at the Reference IRGAs. The other portion is sent to the leaf chamber before returning to the Analysis IRGAs. REF (Reference) and AN (Analysis) air is drawn into the IRGAs at precisely controlled flow rates by respective REF and AN pumps. The IRGAs are contained in a sealed case and taken together form the thermally-stable optical bench.



CIRAS-3 System Schematic

As you can see, the delivery and measurement of the gas depends on a system of three pumps working together. For the main air supply pump we use a diaphragm type pump, and for the Reference and Analysis cells we use rotary vane pumps. The air supply pump is under user control, allowing you to determine and set an optimal flow rate of air to the cuvette and leaf. The pumps supplying the Reference and Analysis cells are set at fixed rates, as this is an important factor in the overall system calibration process. We also ensured that the different path lengths of the REF and AN gases from source to IRGA are accounted for, and with only a very small delay in response time, the displayed console REF and AN readings are nearly instantaneous. In addition, Zero valves are periodically activated for Zero or Diff Bal functions, diverting the gas streams from their normal paths.

Section 4. System Components and Assembly

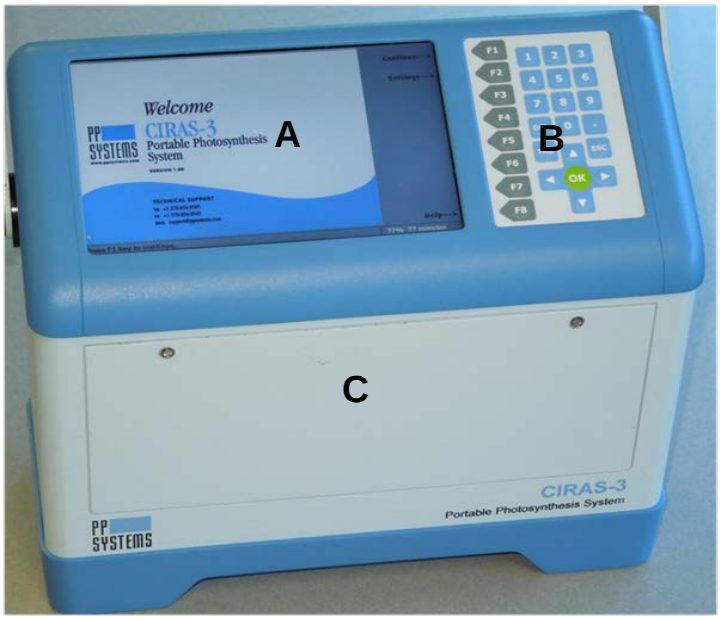
CIRAS-3 Main Console

The CIRAS-3 console houses the IRGAs, gas circuit including absorber columns with chemicals, gas and electrical connections, on-board computer, color display and keypad. The console is the base instrument for several different possible configurations of use. The range of applications includes leaf gas exchange, chlorophyll fluorescence, canopy assimilation, soil respiration, analyzer platform for custom-built chambers (both closed- and open-system), and direct measurement of gaseous CO_2 and H_2O.

A. Large, full color, transflective LCD optimized for field use.

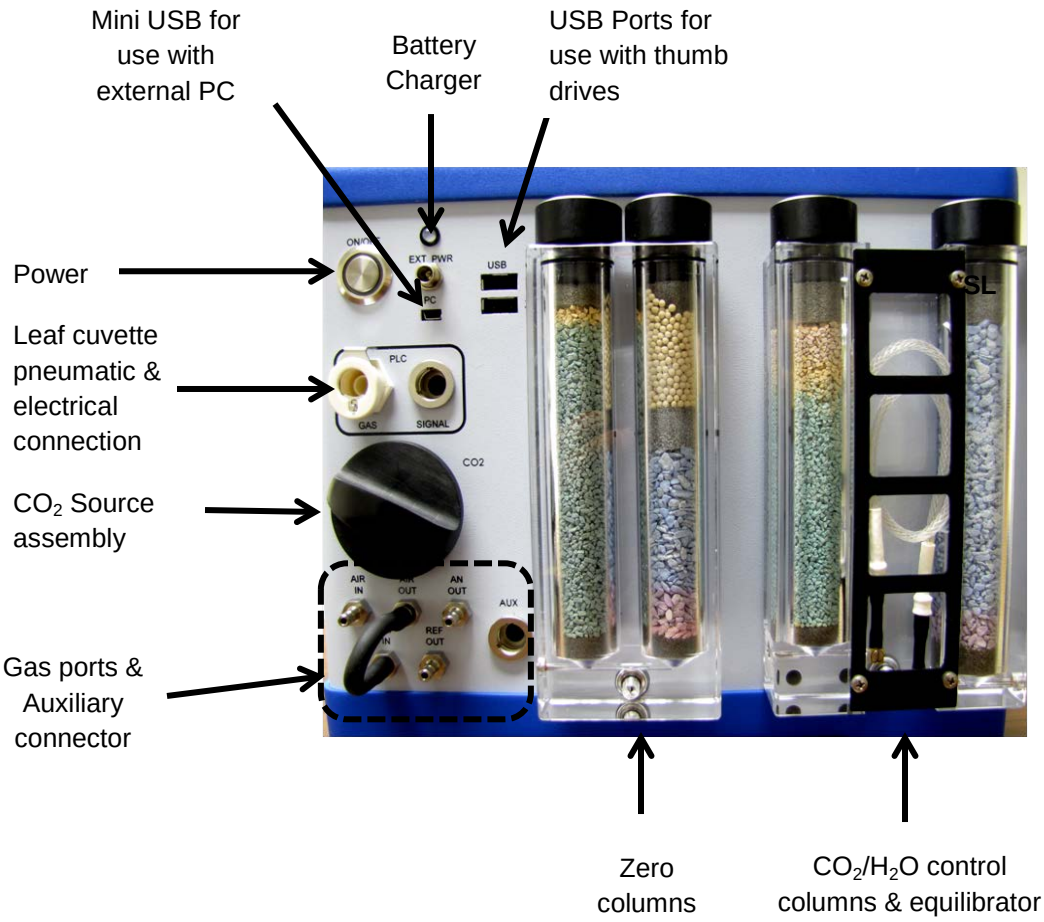
B. Tactile feel keypad and function keys

C. Battery compartment and main access to internal components.



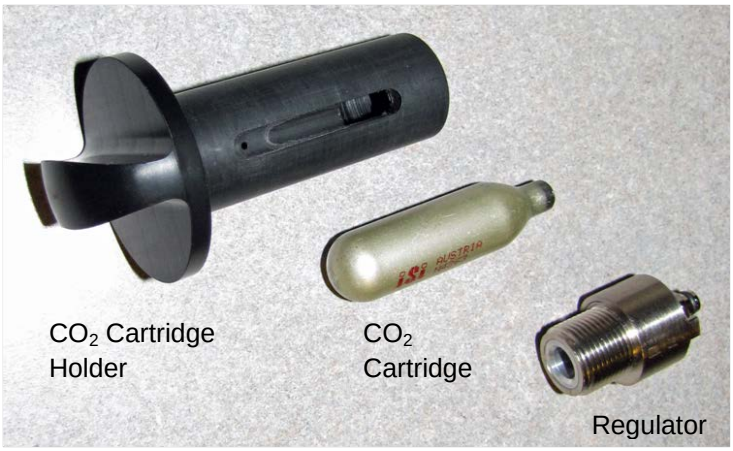
On the front side of the console you find the user interface with its 30° display and keypad group used to navigate the CIRAS-3 console menus. Access to the Li-ion battery pack and internal mechanical and electrical components is achieved by loosening the two captive screws (turn counter-clockwise to release) and lowering the door. A healthy, fully charged battery pack will allow operation in the field for up to 8 hours (actual operation time will vary depending on environmental control settings).

The rear of the console contains all essential gas and electrical connections. The **ON/OFF** power button is a push-and-release type switch, and is illuminated when the system is powered. **EXT PWR** has a dual function – use it to attach the power cord and power supply to an external power source and to charge the internal Li-ion battery. The indicator light glows orange when the battery is charging. The battery can be charged with CIRAS-3 powered or off. **PC** indicates the mini-USB communication port for connection to a laptop or desktop computer, used to operate and display CIRAS-3 remotely. **USB** indicates the two standard USB2 external memory ports for downloading/uploading data, settings and scripts.



The **PLC** group contains **GAS** and **SIGNAL** sockets for connecting the cuvette Analysis gas line and the cuvette power. **GAS** utilizes a locking connector – push firmly in until you hear the click. Push down on the metal release plate while pulling the gas line firmly out to remove. The electrical signal connector has a small black arrow used to align the connector with the **SIGNAL** socket. Align the arrow to the top center position and push in until it locks. Pull gently on the connector's sliding lock barrel to remove.

Beneath the **PLC** group you will find the **CO_2 source assembly**. The cartridge fits inside the holder, the regulator threads into the holder body, and the entire assembly is placed into the console opening marked **CO2**.



Below the CO_2 source assembly is a group of 5 labeled ports and the **AUX** connector. **AIR IN** is the entry port for ambient air or introduced tank air, for example experimental air containing 2% oxygen. Next, a short section of tubing connects **AIR OUT** with **REF IN**. This is actually the air supply air for both the REF cells and the cuvette. The **AN OUT** and **REF OUT** ports allow already analyzed air samples to exhaust. **AUX** is reserved for CIRAS-3 accessories requiring power from the console, such the CPY-4 Canopy Assimilation Chamber and SRC-1 Soil Respiration Chamber.

The absorber columns are grouped into two removable sections. Zero air columns contain 3 different absorber chemicals, soda lime (**SL**) Drierite (**DR**) and molecular sieve (**MS**). The air supply columns (for control of CO_2 and H_2O) consist of soda lime (**SL**) and Drierite (**DR**). Between the air supply columns is a short loop of Nafion ® tubing, protected by a black metal plate. This component is the **water vapor equilibrator**, which preferentially allows excess water vapor to escape from the gas stream, after having been artificially increased by passage through soda lime. (Soda lime consists primarily of $Ca(OH)_2$ and water, and removes CO_2 through a moist chemical reaction.) When you remove the columns you will see that the rear faces are marked by CO_2 ABSORBER, H_2O ABSORBER, MS.

Replace Drierite and molecular sieve in the proportions indicated above, about 1/3 of the column should be molecular sieve. The other columns should contain only a single chemical. Soda lime and Drierite are color-indicating chemicals – as the chemicals become exhausted through use the change color. Soda lime will change from green to a grayish color as it exhausts, Drierite changes from blue to a pinkish color. Importantly, molecular sieve does not indicate its state by color change. A good rule to follow is when the chemical in a column is 50% exhausted, change all the chemicals in that absorber group. **Additionally, change molecular sieve on a weekly basis, regardless of use**. Keep in mind that air passively diffuses through the gas circuit even when the system and pumps are not running, so the chemicals are still working even when you are not. This is a good reason to remove the CO_2 source assembly from the console when not in use.

To replace exhausted chemicals, carefully remove the absorber column group from the console, twist and pull off the column cap and remove the foam spacer inside the column. You can safely dispose of all three chemicals in the trash. Note that the cap has a rubber sealing ring. From time to time place a light coating of silicone grease around the ring to prevent air leaks and cracks.

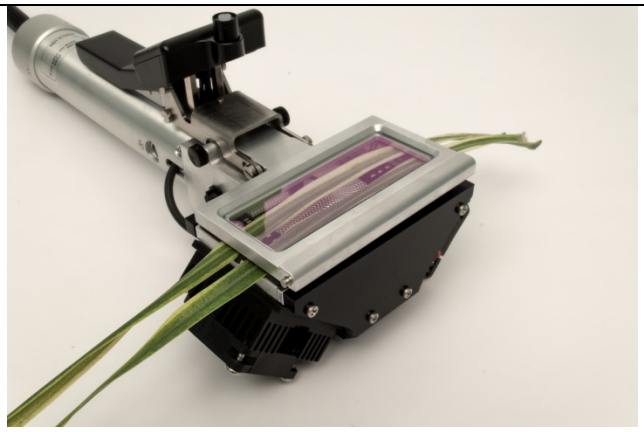
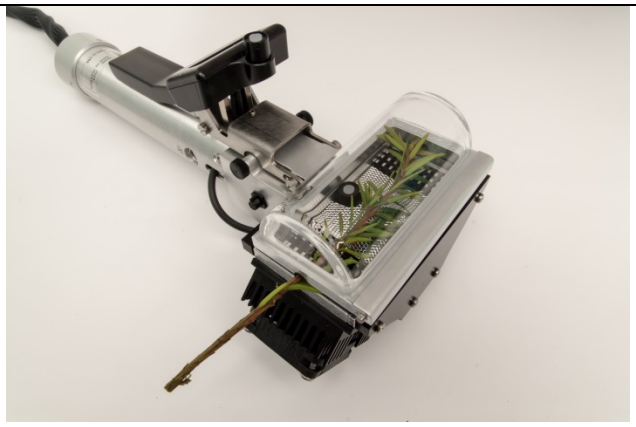
TIP

Always change the Molecular Sieve at least once per week regardless of usage. See Desiccants and Absorber Columns.

Always perform a leak test after changing desiccants. To do so, insert a fresh CO2 cartridge, connect the PLC3 Leaf Cuvette electronically and pneumatically, close the cuvette head and go to Settings (F2) and set the CO_2 reference to 0. The CO2r and CO2a values should drop close to 0 (± 2 µmol m^{-2} s^{-1}) after a few minutes with a 0 CO2d (± 1 µmol m^{-2} s^{-1}).

PLC3 Series Leaf Cuvettes

There are 3 standard leaf cuvettes commonly used with the CIRAS-3 for measurement of leaf gas exchange:

PLC3 Universal Leaf Cuvette For measurement on flat, broad leaves. It is supplied as standard with 3 windows measuring 25 x 7 mm, 25 x 18 mm and 18 cm diameter.	
PLC3 Narrow Leaf Cuvette For measurement on grasses, long needles and narrow leaves.	
PLC3 Conifer Leaf Cuvette For measurement on conifers and short needle vegetation.	

All 3 PLCs connect to the PLC “Gas” and “Signal” connectors on the CIRAS-3 console. Prior to use, make sure that the gas and signal connections are made and that the appropriate PLC is selected under Accessory in the Settings dialog. When selected, the default values for that leaf cuvette will be used. This is very important as there are some differences between the PLCs. All 3 leaf cuvettes have a similar

handle, electronics, sensors, LCD, record (**R**) and switch (**S**) keys on the open/close lever, ambient temperature sensor, temperature control range and external PAR sensor. Measurements can be recorded from all PLCs by pressing the **R** key and parameters can be toggled on the LCD by pressing the **S** key.

PLC3 Temperature Control

All PLCs include temperature control as standard. Each PLC includes a built-in Peltier heating/cooling module which allows a wide range of temperature control. Optimal control depends on the ambient air temperature, because of the power requirements to heat or cool the cuvette or leaf to temperatures that are much different than ambient. With full power available you can usually control cuvette or leaf temperature from approximately 10 °C below ambient to 15 °C above ambient, but within the absolute temperature range of 0 to 45 °C. Note that slight differences are expected between sensor-based and calculation-based methods. Although all PLC3 include temperature control as standard, there are some key differences between them as follows:

Temperature Measurement

PLC3 Universal – Includes an IR sensor for accurate, non-contact measurement of leaf temperature and energy balance for calculation of leaf temperature. **We recommend IR thermometry as long as the entire cuvette window is covered with leaf material. If the cuvette window is not covered 100% with leaf material, you must select energy balance.**

PLC3 Narrow and Conifer – Includes a leaf thermistor for direct measurement of leaf temperature and energy balance for calculation of leaf temperature. **We recommend the energy balance method**.

Temperature Control Type

PLC3 Universal – Five control options are available:

- Set leaf temperature
- Track leaf to ambient
- Cuvette temperature
- Track cuvette to ambient
- Disable temperature control

PLC3 Narrow and Conifer – Three control options are available:

- Cuvette temperature
- Track cuvette to ambient
- Disable temperature control

Tip

The PLC3 ambient temperature sensor will represent an approximate temperature to ambient for reference purposes only. Tamb may vary several degrees from actual ambient depending on environmental conditions.

LCD on Leaf Cuvette

All PLCs include an LCD for display of parameters. Press the **S** button located on the cuvette open/close lever to toggle between displays to view additional parameters.

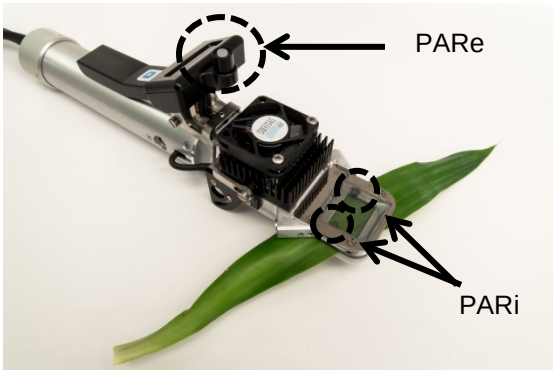
PAR Measurement

The PLC3 leaf cuvettes also features two ways of measuring light in the 400-700 nm wavelengths. All PLC3s include a cosine-corrected external PAR sensor (PARe) for measurement of ambient PAR (Photosynthetically Active Radiation). This reading will be most reliable with the cuvette held on a horizontal plane relative to the ground.

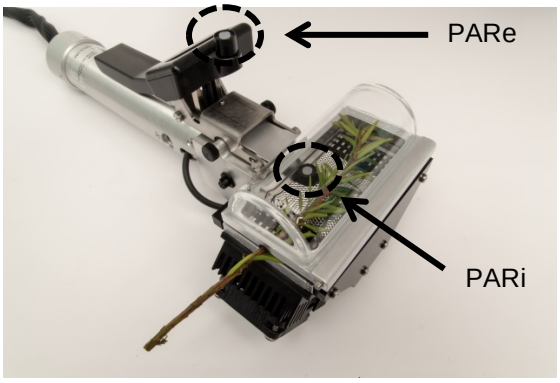
PLC3 Universal - includes two mini-silicon photodiode sensors beneath the cuvette window (PARi). The silicon photodiode sensors are close to the leaf plane and used to average the irradiance beneath the cuvette window. Irradiance is somewhat attenuated (approx. 10% attenuation) by the window. This affects the amount of light reaching the leaf if the light source is ambient sunlight, but not if our LED light unit is the source. This is because the internal PAR sensors are on an electronic feedback loop with the light unit, so the desired light intensity entered by the user is always achieved.

PLC3 Conifer and Narrow – includes a single, cosine corrected PAR sensor beneath the cuvette window (PARi). It is used to give an indication of the PAR inside the leaf cuvette and as an electronic feedback loop to control light intensity when our light unit is used.

PLC3 Universal Leaf Cuvette



PLC3 Conifer and Narrow Leaf Cuvette



Tip

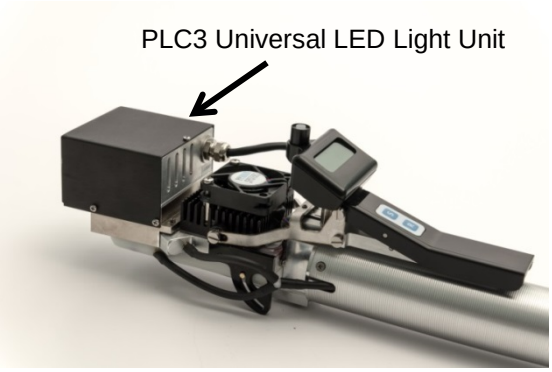
The PARi sensor will normally read lower than the PARe sensor due to attenuation of the cuvette window.

Light Control (Optional)

An optional LED Light Unit is available for all PLC3s.

PLC3 Universal LED Light Unit (RGBW)

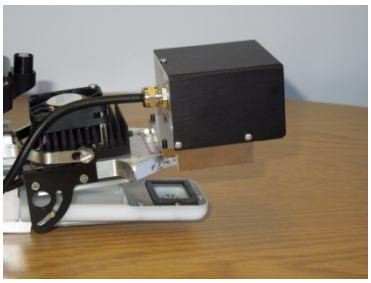
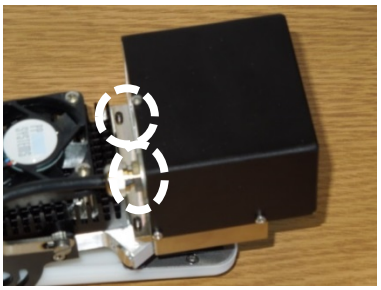
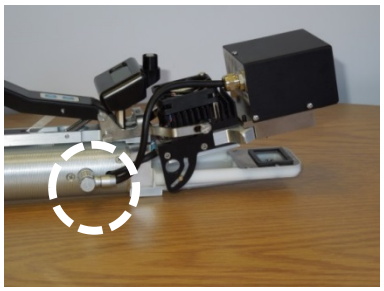
This LED light unit features 48 LEDs, 12 each of RGBW (red-green-blue-white) color. You are able to set any single color from 0-100% of the light source, or you can combine colors in any proportion to recreate the spectral distribution of a specific natural or artificial light source. With full power available to the cuvette the normal intensity output will be up to 0-2500 µmol m^{-2} s^{-1}.



Caution: do not look directly at illuminated LEDs, even with the light unit set to lower intensity control levels.

Please note. Maximum light intensity and range of temperature control is dependent on RGBW settings.

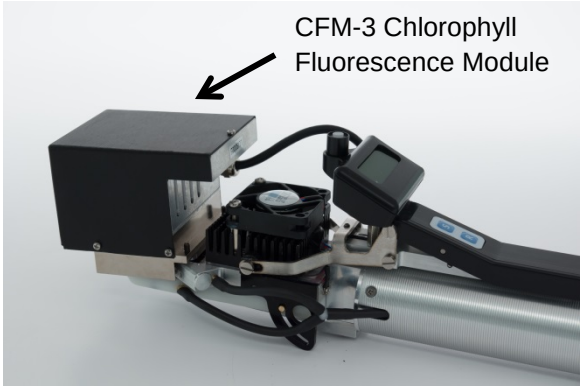
The PLC3 Universal LED light unit is designed to mate quickly and easily with the PLC3 Universal leaf cuvette.

Step 1. First, mount the light unit by aligning it to the front of the cuvette upper jaw as shown below.	**Step 2**. Slide the light unit back towards the heat sink and fan on the leaf cuvette locking it in place using the two set screws shown below.	**Step 3**. Connect electrically.
		

CFM-3 Chlorophyll Fluorescence Module (Optional)

The CFM-3 Chlorophyll Fluorescence Module looks very similar to the PLC3 Universal LED Light Unit. The major difference being the inclusion of all fluorescence associated light sources and detection capability that is built directly into the light unit.

Attachment and connection to the PLC3 Universal Leaf Cuvette is identical to that described above with the PLC3 Universal LED Light Unit.



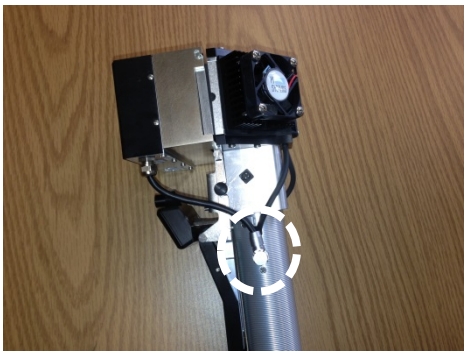
Tip

The CFM-3 can also be used as an actinic light source for gas exchange measurements similarly to the standard PLC3 Universal LED Light Unit

PLC3 Narrow and Conifer LED Light Unit

This LED light unit features 96 LEDs, 24 each of RGBW (red-green-blue-white) color. You are able to set any single color from 0-100% of the light source, or you can combine colors in any proportion to recreate the spectral distribution of a specific natural or artificial light source. With full power available to the cuvette the normal intensity output will be up to 0-2500 µmol m^{-2} s^{-1} (higher ranges are dependent on selection of red-green-blue-white LEDs). **Caution: do not look directly at illuminated LEDs, even with the light unit set to lower intensity control levels**.

The PLC3 Narrow and Conifer LED light unit is designed to mate quickly and easily with the PLC3 Universal leaf cuvette.

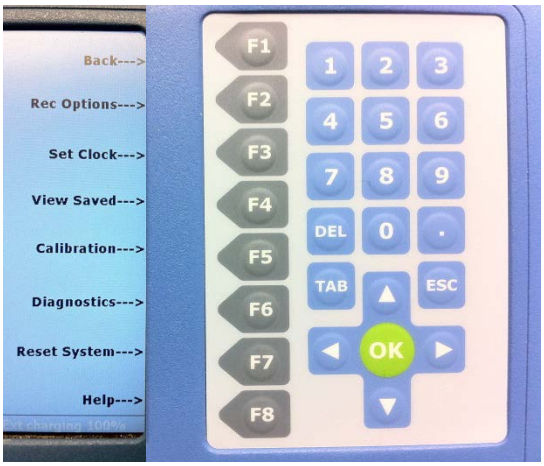
Step 1. Secure light unit to notches on cuvette head.	**Step 2.** Pull Light Unit over cuvette and secure locking screw.	**Step 3.** Connect electrically.
		

Please note. Maximum light intensity and range of temperature control is dependent on RGBW settings. Also, due to the distance between the LEDs in the light unit and the PLC3 Narrow and Conifer internal PAR sensor, maximum light intensity may be lower than 2500 µmol m^{-2} s^{-1} depending on mixture of red, green, blue and white LEDs.

Section 5. CIRAS-3 Console Software

Overview

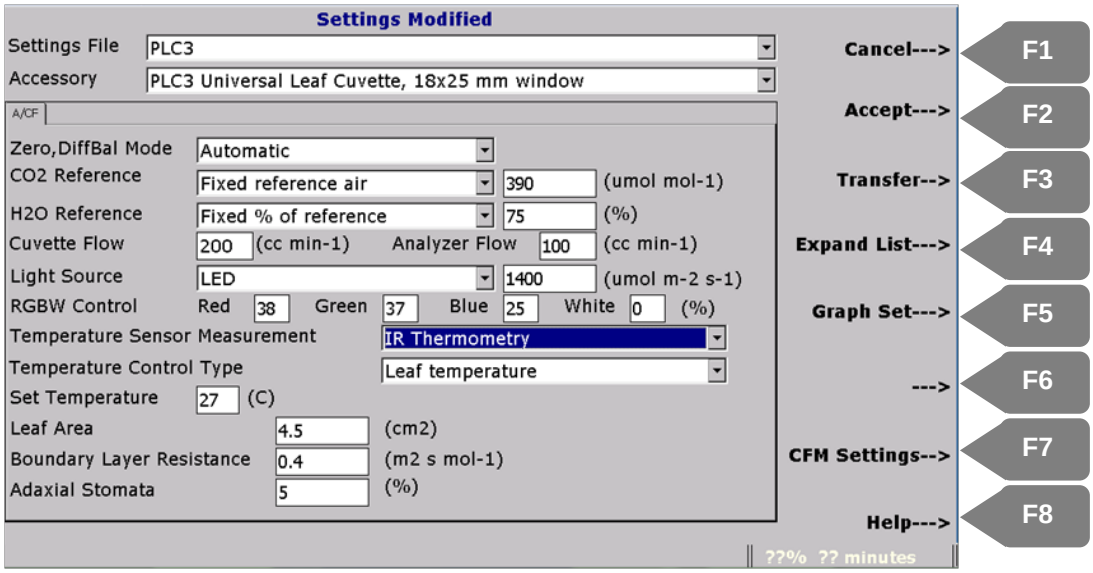
The CIRAS-3 console software is the most user-friendly and intuitive software we have designed to date. All CIRAS-3 functions are accessed and implemented by the same easy-to-understand software structure – Settings, Controls, Recording, Data Transfer, Diagnostics, Calibrations and other useful functions. The CIRAS-3 Console software is the system's command interface, based on function keys linked to first level menus. Once you become familiar with how the menus are organized, navigation through menus becomes simple – decide what you want to do and with a few key presses select your options from the dialogs that you open.



The 8 function keys form the top level for menu selections. Function keys are context-sensitive, so as you go deeper into a menu their assigned functions may change. Once a dialog is opened you typically use the **TAB** key to quickly move through fields contained within the dialog. In the fields you enter values with the numeric keypad or open a dropdown list and select an option from the list. **DEL** functions within a field as a backspace key. **OK** confirms your selection from a dropdown list, while **ESC** closes the dropdown list. **Arrow** keys are used to move up and down one item at a time within a list, or to move horizontally to make selections from buttons that may be displayed. Select **Accept** and your dialog changes are implemented by the hardware. Often you will be able to make customized changes or perform essential functions with as few as three key presses.

Basics of Navigating Console Screens and Menus

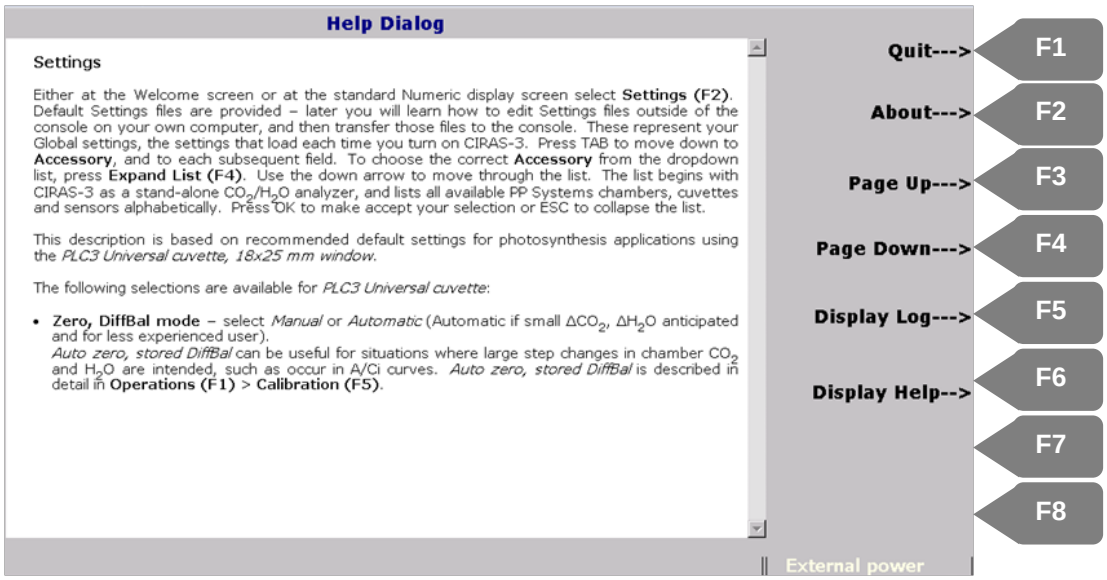
Let's begin with a quick example of an action you are likely to perform – establishing the repeatable baseline settings features that will appear each time you power on your system. We refer to these settings as global, that is they define the operational environment of CIRAS-3 prior to making measurements and collecting data. Power on the system and press **Settings (F2)** to open the Settings dialog.



Several default setting files are included, representing the standard applications that can be run with CIRAS-3. Use the Arrow keys or **Expand List (F4)** to select PLC3 from the Settings File list. Press the TAB key to move to the next field, Accessory, and choose the appropriate cuvette window size, such as 18 x 25 mm shown above. Move down through the fields to make other changes to CO_2, water vapor, light, temperature control, etc. Holding down the **TAB** key will move the cursor very quickly through the various fields, so don't worry if you only need to change the last field in a dialog. **Accept (F2)** the changes and continue to the data screens. Now, each time you power on the system these settings will be loaded, unless you choose a different Settings File. Later you can define the default global settings for other CIRAS-3 applications, such as soil respiration.

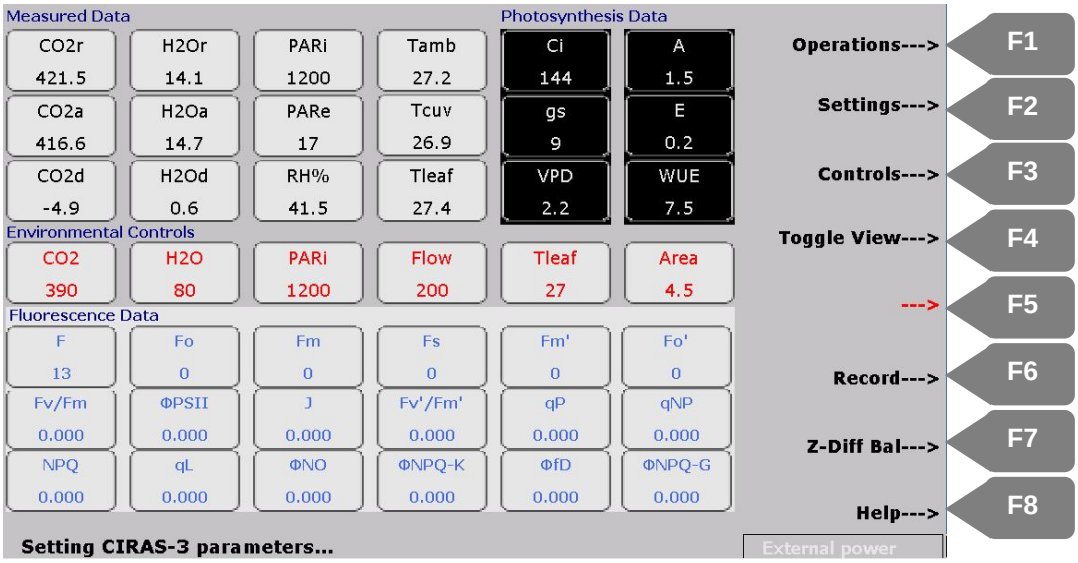
Controls functions are different from **Settings** in that they are used to update settings and make necessary adjustments dynamically while operating CIRAS-3. During normal operation you will use **Controls (F3)** almost exclusively to make alterations to the leaf chamber environment and to customize how data is presented on the screen.

Help (F8) is available on every screen and is context-sensitive to the functions being performed. For example, if you select Help while in the Settings screen you can view text directly related to the content of the screen. Within the Help screen press **Page Up (F3)** or **Page Down (F4)** to read more content. Press **Display Log (F5)** to see a running log of your session that saves calibration functions, warnings and error messages with time stamps. Press **Display Help (F6)** to return to the Help file.



Numeric View of Data and Control Variables

As you familiarize yourself with CIRAS-3 you will begin to establish your own preferences of how and what you would like to see on the console for differing circumstances of data collection and various applications. **Numeric View**, as its name implies, provides an overview in numeric form of what is being measured, calculated and controlled at any point in your CIRAS-3 session. The categorized information is clearly placed in groupings on the screen: **Measured Data**, **Photosynthesis Data, Fluorescence Data** and **Environmental Controls**.



Measured Data	
CO2r	CO_2 Reference (µmol mol^{-1})
CO2a	CO_2 Analysis (µmol mol^{-1})
CO2d	CO_2 Differential (µmol mol^{-1})
H2Or	H_2O Reference (mb)
H2Oa	H_2O Analysis (mb)
H2Od	H_2O Differential (mb)
PARi	PAR internal (µmol m^{-2} s^{-1})
PARe	PAR external (µmol m^{-2} s^{-1})
RH%	Relative Humidity inside leaf chamber (%)
Tamb	Temperature ambient (^oC)
Tcuv	Temperature in cuvette (^oC)
Tleaf	Leaf temperature (^oC)
Photosynthesis Data	
Ci	Sub-stomatal CO_2 concentration (µmol mol^{-1})
A	Assimilation/Respiration (µmol CO_2 m^{-2} s^{-1})
gs	Stomatal conductance (mmol H_2O m^{-2} s^{-1})
E	Transpiration (mmol H_2O m^{-2} s^{-1})
VPD	Leaf to air vapor pressure deficit (kPa)
WUE	Photosynthetic water use efficiency (mmol CO_2 mol^{-1} H_2O)
Environmental Controls	
CO2	CO_2 Reference (µmol mol^{-1})
H2O	H_2O Reference (mb)
PARi	PAR internal (µmol m^{-2} s^{-1})
Flow	Cuvette flow rate (cc min^{-1})
Tleaf	Temperature control method
Area	Leaf Area (cm^2)

Fluorescence Data	
F	Fluorescence signal (current)
F_o	Fluorescence origin
F_m	Fluorescence maximum
Fs	Fluorescence steady state
F_m'	Fluorescence maximum prime
F_o'	Fluorescence origin prime
F_v/Fm	Maximal yield of photochemical efficiency
ϕPSII	PSII operating efficiency
J	Thylakoid electron transport rate (µmol e^- m^{-2} s^{-1})
Fv'/F_m'	PSII operating efficiency, far-red
qP	Photochemical quenching
qNP	Non-photochemical quenching
NPQ	Non-photochemical quenching
qL	Photochemical quenching, lake antenna model (Kramer)
ϕNO	Non-photochemical quenching, non-regulatory (Kramer)
ϕNPQ-K	Non-photochemical quenching, down-regulatory (Kramer)
ϕfD	Non-photochemical quenching, non-regulatory, lake or puddle models (Genty, Hendrickson)
ϕNPQ-G	non-photochemical quenching, down-regulatory lake or puddle models (Genty,Hendrickson)

Section 6. Startup Routine and System Stability Checks

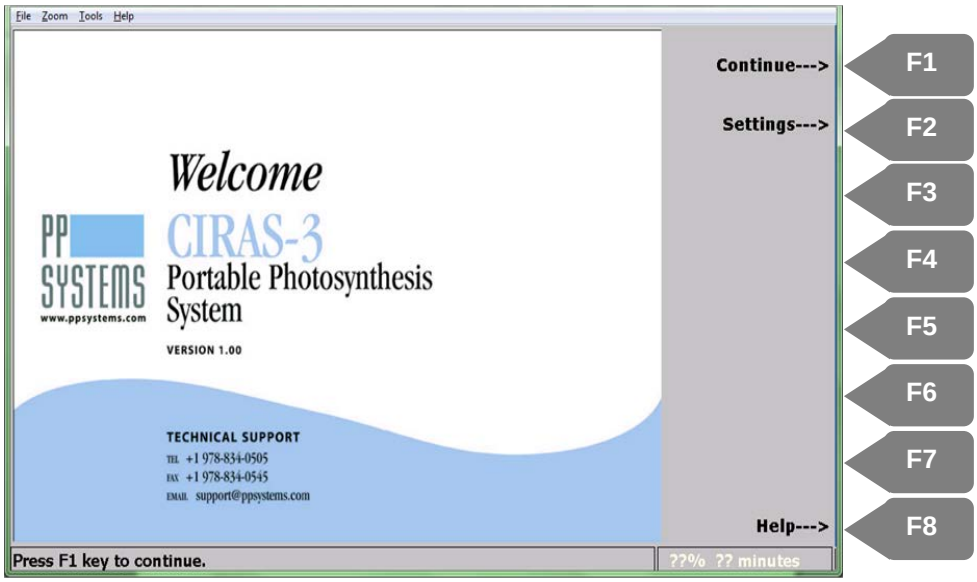
Before You Power On

The time required to be up and running from when you power on CIRAS-3 to when you make your first measurement is reasonably short. Much of the pre-operation phase depends on the how well you maintain chemicals. But there are a few other important considerations. The most basic pre-operational checklist can be summarized by these items:

- Be sure that the chemicals are fresh and not exhausted.
- Check that the leaf chamber gaskets are in good condition and replace them if they are compressed, damaged, or dirty.
- Be sure there is sufficient battery power for field operation. For optimal performance, we recommend charging the internal CIRAS-3 12V Li-ion battery the night prior to field measurements using the power supply provided. The indicating LED will be a steady green when the internal battery is fully charged.
- Make all necessary electrical connections of cuvettes, accessories and other components
- If working at high ambient temperatures (>35-40 ^{o}C), we recommend shading the console to prevent overheating.
- Keep the CIRAS-3 on its power supply during warm-up to preserve the battery.

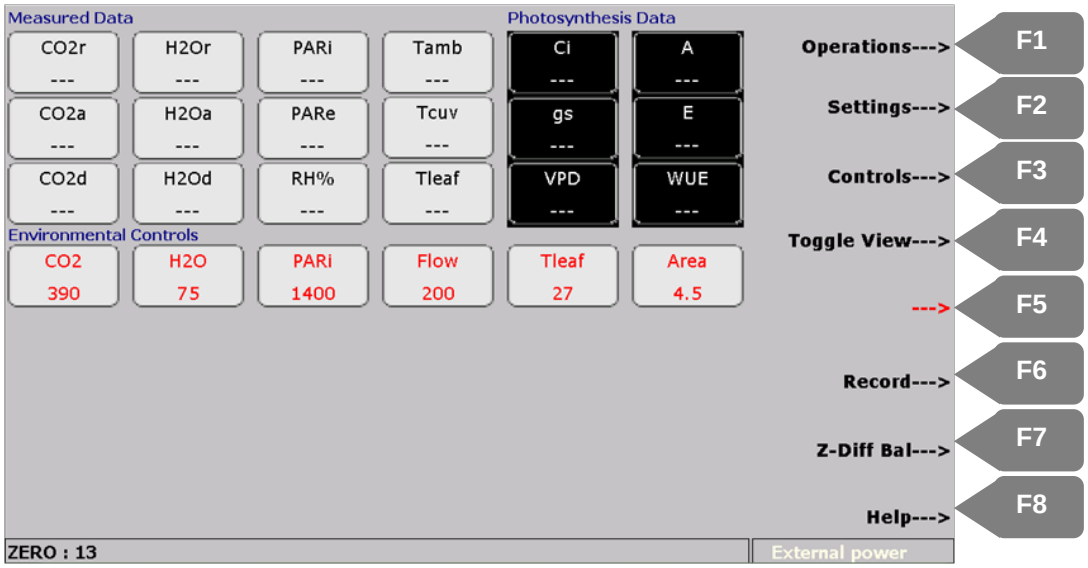
Startup and Warmup

Press and release the ON/OFF button, it should illuminate blue. It takes a few seconds for the first display to appear on the console screen.



Press either **Continue (F1)** or **Settings (F2)** – at this point this is not important since the system is not quite ready to operate. "Warm up (temp) C…" appears in the status bar at the bottom of the screen, where initial (temp) should be close to ambient temperature °C. Note that the initial temperature depends on the ambient temperature where CIRAS-3 is located. Warm-up time to the required 55 °C IRGA

temperature can vary. The warm-up period can be only a few minutes in a mild or warm environment, or indoors, and longer if it has been stored in a colder environment. Automatic Zero and Differential Balance (Diff Bal) cycles will run during warm-up, in each case counting downward from a fixed number of cycles. Zero counts down 30 cycles while Diff Bal counts down 25 cycles. Each cycle takes less than one minute. Assume that we want to enter the **Numeric View** screen, press **Continue (F1)**. The Numeric View screen will appear as below (at this point CIRAS-3 is in its warm-up phase, so live data is not displayed):



When the warm-up phase is completed you will begin to hear a clicking sound from the CO_2 mixer valves, switching at a rate of 4Hz. The amount of time the valve is on (the duty cycle) varies to generate the correct CO_2 mix ratio. Just after the system warms up the mixer starts to generate the requested ratios. H_2O control is silent (completely on or off) at settings of 0 and 100%, but any other setting produces a noticeable clicking sound. You will also notice that the data fields in the Numeric View screen are now updating with live data approximately every 2 seconds. Data will update and be displayed in the fields except when the system is performing a Zero or Diff Bal.

How long will a new CO_2 cartridge last? We can't meter the cartridge, so it's impossible to know exactly how much gas remains after operating the system. The high concentration gas slowly diffuses through the regulator nozzle even when you are not operating CIRAS-3. But, the normal expectation is about 24 hours of use. For example, if you install a new cartridge at 12:00 on Day 1, check it the next morning on Day 2 by setting a high control CO_2 reference concentration, such as 1500 µmol mol^{-1}. If the control setting achieves your target of 1500 µmol mol^{-1} you can safely continue working through the morning. You should probably replace the cartridge if the target CO_2 cannot reach 1500 µmol mol^{-1}, as it will most likely become unstable within a short time.

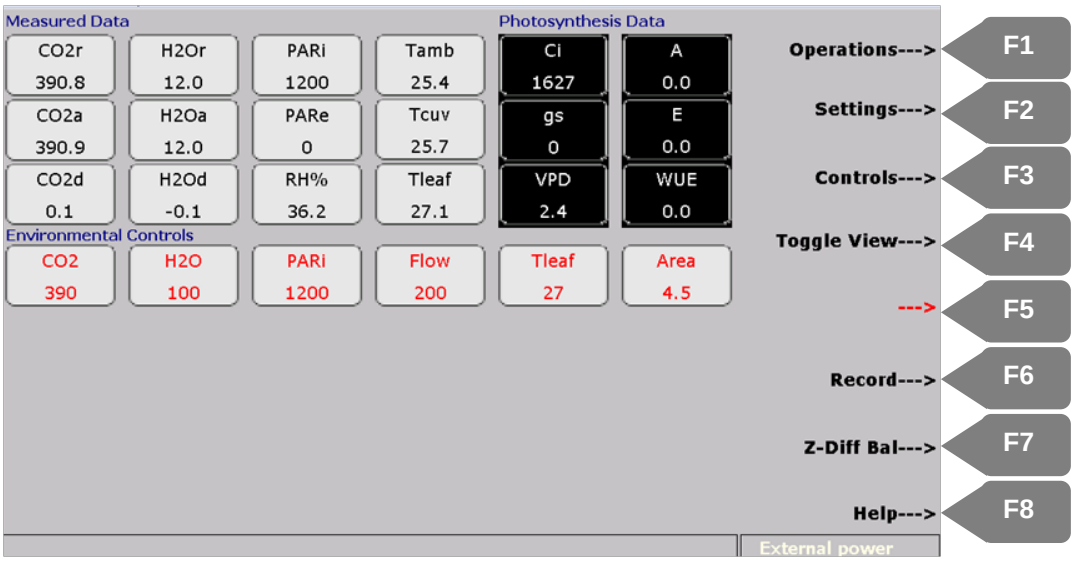
With the assembly still inserted into the console, turn the holder to the left until you hear the gas release. The regulator will now be loose and you can remove the assembly and finish removing the regulator by hand, by turning counter-clockwise (left). After inserting a fresh cartridge into the holder, twist the steel regulator onto the end of the holder until it is just finger tight. Place the entire assembly into the console and push in lightly while turning clockwise (right). The assembly should move inward a few more mm. Now it is in a locked position and you can complete the installation by turning the holder clockwise until it becomes tight. As you make the last few turns you may hear a small amount of pressurized CO_2 gas

escape. This is normal, do not stop turning. Continue to turn the holder to the right until it stops, but do not overtighten.

Checking Stability - Before You Place a Leaf in the Chamber

Now that you can observe live readings you can check the system for stability and leaks. This is usually done with the leaf chamber empty. This way you can isolate the cuvette gaskets for leaks. Press **Controls (F3)** and enter the CO_2 concentration that you might be working with, let's say 390 µmol mol^{-1}. For now, use the default H_2O Reference control option "Fixed % of reference" and 100%. Accept the change to return to the Numeric View screen. These tests are mainly concerned with stability of CO_2 and H_2O. You can quickly check temperature and light later. Close the leaf chamber at this point.

Under the Measured Data group observe the values in the first two columns, CO2r, CO2a, CO2d, H2Or, H2Oa, H2Od. Allow CO2r (Reference CO_2) to become stable. With no leaf in the chamber we expect to see the Analysis CO_2 (CO2a) equal to or nearly equal to CO2r. If this is the case there will be a zero differential, CO2d=0.0. Look for the same relationships in the water vapor data, H2Or, H2Oa, H2Od.



While CO2d and/or H2Od=0.0 is ideal, it is common to see a small differential even under the best of circumstances. With experience, you will be in the best position to decide what is or is not an acceptable differential, and to take action to try to correct it. Whether an empty-chamber differential is acceptable or not is often dependent on the scale of gas exchange rates expected over the course of your measurements. A small differential will hardly be noticed when it occurs along with high photosynthetic rates, but could present a significant problem if the focus of your data is miniscule rates of gas exchange, such as occur near light compensation points and with dark respiration. If during this test CO2d >0.5 you can try waiting a little longer in case this is being caused by small fluctuations in CO_2 control. In theory, a CO2r fluctuation of even 0.1 µmol mol^{-1} will result in a small transient differential. If CO2d remains >0.5 and stable, try running **Diff Bal (F7)**. Other causes could be imperfect gaskets or improper tension adjustment of the cuvette. Keep in mind also that CO_2 in air surrounding the cuvette is more likely to enter the Analysis gas stream if strong gradients exist. For example, suppose your CO_2 control value is 390 µmol mol^{-1} and the test location is indoors where the CO_2 concentration is 600-800 µmol mol^{-1}. CO_2 in the surrounding air can enter the chamber through any existing leak. Cuvette flow rate is another factor that influences the magnitude of detectable leaks. Higher flow rates create a slight chamber overpressure that can slow or prevent CO_2 in surrounding air from diffusing through the gaskets. From

this you might deduce that the most rigorous leak test involves creating a large gradient of CO_2 between the air inside and outside of the leaf chamber, while supplying the lowest flow rate to the chamber.

Survey Measurements – Some Tips

There are a few basic points to consider before you begin recording data. In many instances, the leaf blade/vegetation may extend beyond the foam gasket surrounding the leaf area opening. Try approaching the leaf from the side with the open cuvette to avoid accidental injury to the leaf. Approach the leaf from its side margin, not the leaf tip, to avoid crushing it in the rear section of the leaf chamber. Try to orient the cuvette to the leaf and do not severely twist the petiole. Use a tripod if practical – this way you will have both hands free and avoid unnecessary changes to natural leaf orientation, and you can position all leaves in a uniform orientation to the sun when the LED light unit is not in use.

With the leaf enclosed in the cuvette you should quickly notice a series of dynamic leaf responses, assuming ideal physiological conditions. Sub-stomatal CO_2 concentration (Ci) will begin to fall, after being initially equal to or higher than the reference CO_2 concentration (CO2r). When Ci<CO2r there is an instantaneous change in calculated net photosynthesis (A), from a negative rate (respiration) to a positive rate. Simultaneously, the differential CO_2 concentration (CO2d) will go into negative values while differential humidity (Hd) becomes positive – the leaf is both fixing carbon dioxide and transpiring water vapor.

Be aware of the leaf's light history and consider how that affects the chamber acclimation phase – a highly-shade tolerant plant or shade leaf will require different acclimation in the leaf chamber than a shade-intolerant or sun leaf. Consider also seasonal affects and leaf ontogeny – reduced rates of metabolism can often be expected despite year-round plant culture in artificial (indoor) environments.

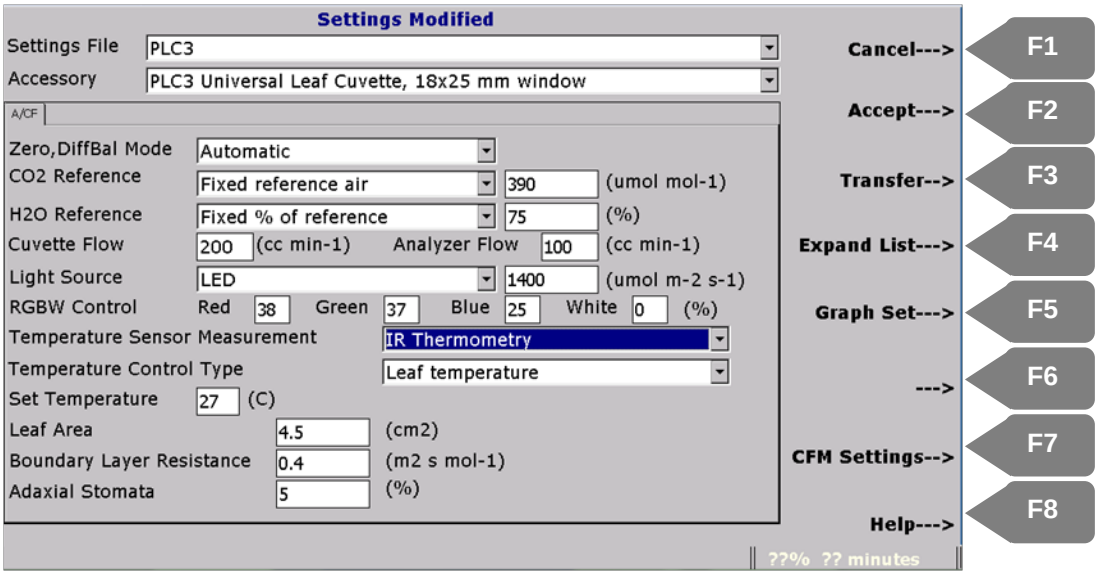
Note that some physiological variables, especially A, gs (stomatal conductance) and CO2d will continue changing as the leaf acclimates and approaches a stable state. This can require several seconds and as long as several minutes. This depends largely on the preconditioned state of the plant relative to the environmental conditions inside the cuvette. A useful illustration of this can be seen with a highly shade-adapted plant that is suddenly exposed to strong light intensities in the leaf chamber. In this case, delayed gas exchange responses can be expected compared to a plant (or leaf) accustomed to more intense light conditions.

If your goal is to collect a large quantity of relatively short duration measurements from a large sample group of plants/leaves, especially when allowing ambient light and/or temperature conditions to prevail, you will probably use the **Manual Recording** option. This allows you maximum flexibility as to when to capture a reading and when to wait.

Section 7. Basic Functions - Viewing and Controlling CIRAS-3

Settings (F2)

Settings are essentially defined as operational environments that are recreated each time that CIRAS-3 is powered on. The options that you choose here could be your typical working options in many cases, but it is also likely that you will need to change and adapt for different leaf samples, species and environmental conditions, as well as for imposing experimental conditions on the leaf.



Either at the Welcome screen or at the standard Numeric View screen select **Settings (F2)**. Default Settings files are provided based on the **Accessory** selected (later you will learn how to edit Settings files outside of the console on your own computer, and then transfer those files to the console). Press TAB to move down to **Accessory**, and to each subsequent field. To choose the correct **Accessory** from the dropdown list, press **Expand List (F4)**. Use the down arrow to move through the list. The list begins with CIRAS-3 as a stand-alone CO_2/H_2O analyzer, and lists all available PP Systems chambers, cuvettes and sensors alphabetically. Press **OK** to make your selection or **ESC** to collapse the list. The above description is based on recommended settings for photosynthesis applications using the "PLC3 Universal cuvette, 18x25 mm window". The default Settings File is named PLC3.

Settings	
Zero, Diff Bal mode	**Manual.** You will be prompted when to perform a Zero or Diff Bal. **Automatic.** Zeros and Diff Bals will be performed automatically. We recommend this mode if small ΔCO_2 and ΔH_2O is anticipated and for less experienced users. **Auto zero, stored Diff Bal.** This option can be useful and is highly recommended for situations where large step changes in chamber CO_2 and H_2O are intended, such as occurs with CO_2 in A/Ci curves. Prior to using this option, you must perform a Stored Diff Bal (See Store Diff Bal).
CO2 Reference	**Approximate reference air.** The CO_2 supplied to the leaf chamber from CIRAS-3's internal CO_2 source will be within 10 µmol mol^{-1} from the actual set value within the control range of 0-2000 µmol mol^{-1}. For response curves or in situations that require frequent, large changes in CO_2 levels, this option is recommended because it settles near the set value very quickly (within 1-2 minutes). You must first perform a Max C prior to using this option (See Find Max C. **Fixed reference air.** The CO_2 supplied to the leaf chamber from CIRAS-3's internal CO_2 source will be within 2 µmol mol^{-1} from the actual set value within the control range of 0-2000 µmol mol^{-1}. If working at the same CO_2 level throughout a measurement sequence, this option is ideal. **Fixed analysis air.** Allows CO_2 control on the analysis gas (feedback from the leaf) as it is measured by the Analysis side IRGA. **Ambient (remove chemicals).** This option is frequently used to supply the natural outdoor CO_2 to the leaf chamber. Several considerations are involved whenever the stable internal CO_2 source is not used. If this option is selected, the soda lime and Drierite must be removed from the CO_2/H_2O control columns. Humidity control can still be available if the H_2O Absorbant column is filled with Drierite.
H2O Reference (chamber humidity	**Fixed % of reference** is most common. Typically, we recommend setting this to 75% of reference (you can change this at any time). If ambient conditions where you work are typically humid (60+ %RH) set **Fixed % of reference** to produce a chamber humidity less than ambient. The %RH in the leaf chamber is calculated and displayed so you can make necessary adjustments based on that. If ambient air in your surroundings is typically dry, set to 100% of reference (the system's reference air is usually slightly more humid than ambient because of the soda lime reaction already mentioned). Anticipate that transpiration water vapor from the leaf will increase chamber humidity, and maintain chamber RH <70%. On occasion you may see a warning in the status bar at the bottom of the screen that leaf chamber humidity >70%. If this message does not go away, you should reduce the incoming %RH until you get below 70%.

	Fixed reference mb lets you choose a specific saturation partial pressure value of chamber air, from 0 mb to dewpoint, depending on the state of saturation of the ambient air. **Constant VPD** automatically adjusts chamber humidity to maintain a consistent leaf-to-air vapor pressure deficit between 0-10 kPa (absolute range). The target VPD that can be achieved will be dependent on dynamic leaf physiological factors and not only on how dry you make the incoming reference air. **Ambient (remove chemicals)** is used to supply water vapor unaltered from the surrounding environment to the leaf chamber. Several considerations are involved whenever stable, internally generated water is not used.
Cuvette Flow	Reference gas flow rate entering the cuvette within the range of 150 to 500 cc min^{-1}. Changing flow rate during an experiment is not recommended. Instead, determine an optimal flow rate before beginning important measurements, and then maintain that flow rate throughout the experiment. Remember that CIRAS-3 controls water vapor, chamber humidity and VPD through the desiccants, not by increasing or decreasing flow rate.
Analyzer Flow	Constant flow rate of sample gas introduced to IRGAs. The default value is 100 cc min^{-1} and this is normally the value for most gas exchange applications using a cuvette and should not need to be altered.
Light Source	**LED**. Select this if using our light unit. Select your desired **Light Intensity** from 0-2500 µmol m^{-2} s^{-1}. **Ambient.** For measurements in natural sunlight or if using external light sources.
RGBW Control	Allows you to set your desired LED color distribution. An individual color can be set to 100%, or any color combination may be selected with a combined distribution of red-green-blue-white to equal 100%. Here you can mimic the spectral quality of sunlight, LED light banks, and other light sources, or create experimental irradiance for e.g. stomatal physiology work.
Temperature Sensor Measurement	Available options are dependent on PLC3 type. **IR Thermometry** to use the PLC3 Universal cuvette's internal infrared sensor (but only if the leaf covers the entire window opening). Do not select **IR Thermometry** if, for example, you are trying to place narrow leaf blades in parallel, such as grass leaves. **Energy Balance** must be selected when the cuvette window does not have complete coverage by the leaf. Otherwise, **Energy Balance** can be used at any time. Choose **Thermistor** only if using the thin wire temperature sensor supplied with the PLC3 Conifer or Narrow cuvettes, otherwise select **Energy Balance** for these cuvettes.

Temperature Sensor Control Type	Available options are dependent on PLC3 type. There are several options by which to control the temperature in the leaf chamber, temperature can be held fixed or allowed to vary with ambient depending on experimental objectives. **Leaf** and **Cuvette** temperatures allow fixed inputs in 1°C increments. Select **Leaf temperature** to hold the leaf at a fixed temperature that you enter. **Track leaf to ambient** allows the leaf temperature to follow air temperature, as measured by the cuvette's handle-mounted thermistor sensor. **Cuvette temperature** is used to control the air in the cuvette at a fixed temperature that you enter. **Track cuvette to ambient** allows the cuvette temperature to follow air temperature, as measured by the cuvette's handle-mounted sensor. **Disable temperature control** is intended mainly for diagnostic purposes. This option will switch off the cuvette fan.
Set Temperature	Available if Temperature Sensor Control Type is set to control **Leaf Temperature** or **Cuvette Temperature**. Enter a temperature value that is between ~10 °C below ambient to 15 °C above ambient. The absolute control range is between 0-45 °C. Ability to control at a stable temperature will depend on whether you are operating the system from AC power or battery, the charge state of the battery and that you remain within the control and absolute ranges of temperature.
Leaf Area	The default value will be based on your **Accessory** selection. Enter known measured leaf areas if your leaf sample completely fills the PLC3 Universal cuvette window or if you and you have pre-determined the leaf area exposed in the chamber. Enter a lower estimate (as compared to the window size) if you don't know the true leaf area of your sample. All calculations are based on leaf area so if you don't know the actual leaf area at time of measurement, you will need to determine leaf area at the conclusion of measurements and have the data recalculated. PP Systems can supply a simple CIRAS-3 Excel® spreadsheet program for recalculation of results.
Boundary Layer Resistance	This value is determined at the factory prior to shipment and the value noted on the PLC3. In future this will also be available in software.
Adaxial Stomata	Enter a known or estimated value of % upper leaf surface stomata for your leaf sample. If you are unsure, it will help if you can determine if your leaf sample is representative of a hypostomatous or amphistomatous plant species before entering an estimate.

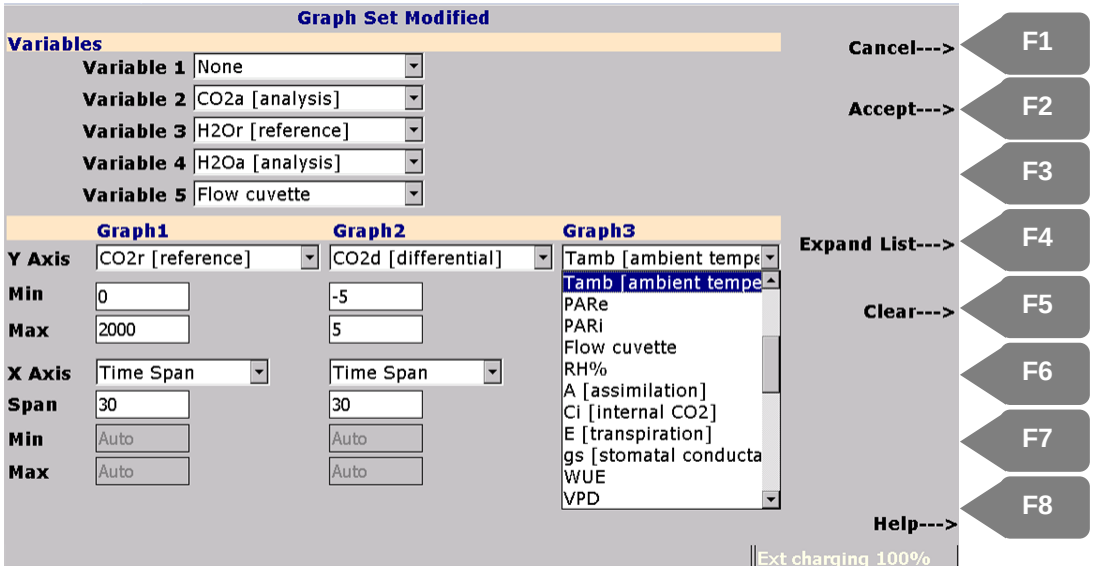
Settings Graph Set (F5)

The alternative to Numeric View is called **Graph View**. Configure Graph View in **Settings (F2)** > **Graph Set (F5)**. In Graph View you can view up to 5 numeric variables and 3 graphs, using any combination of measured and calculated data in the graphs. Select your desired set of variables from the Variable list, **Variable 1** through **Variable 5**. With the dropdown box highlighted, press **Expand List (F4)** to see a section of the total list, or use the Up or Down arrows to move quickly through the list. The first variable in the list is None. Choose None if you do not want numeric variables displayed or make a selection and press **OK**.

The graph or graphs that you choose to display can be customized in two basic ways: the variables that will be shown and the numeric min-max scaling or time scale. Press the TAB key to move down to **Graph1**. **Y Axis** is the first dropdown list containing all available variables. Again, with the dropdown box highlighted, make your selection. Press TAB again to enter a value in the **Min** field, then do the same for the **Max** field. Press **Change Sign (F3)** to enter a negative value after you make your entry, usually in the **Min** field. Note that if you clear the field by pressing the DEL (delete) key the selection defaults to Auto, which will auto-scale that entry.

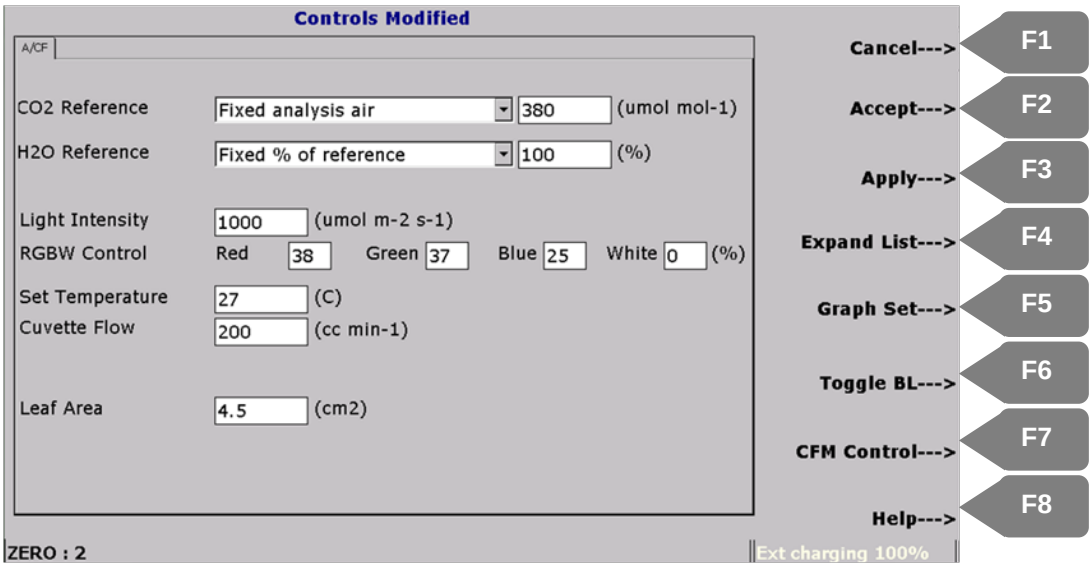
X Axis is the next dropdown list. The first variable that it contains is Time Span – choose this if you want a single-variable plot, displayed over a period of between 2 and 60 minutes. Enter the time in the next field, **Span**. **Min** and **Max** will be grayed out. Enter any variable except Time Span if you prefer a two-variable scatter plot, then enter the **Min** and **Max** scales for that variable. The **Span** field will be grayed out.

The first variable in the **Y Axis** list is None. Choose None if you do not want the graph displayed. For example, if you set up **Graph1** as described above and select None for both Graph2 and Graph3, you will see a single large graph. Select None for Graph3 to display two graphs only, etc.



Controls (F3)

Controls allow you to dynamically change many of the default options you have made previously under **Settings** without reverting the system back to its initial set of Settings options. Use Controls to establish system stability (empty leaf chamber) by altering CO_2, H_2O, light factors, temperature, flow rate and leaf area. Press **Controls (F3)** to enter control mode and make changes then press **Accept (F2)**. The status bar in the lower left corner will display the message "Updating Environmental Control values…" and the plot data displayed in Graph View will refresh.



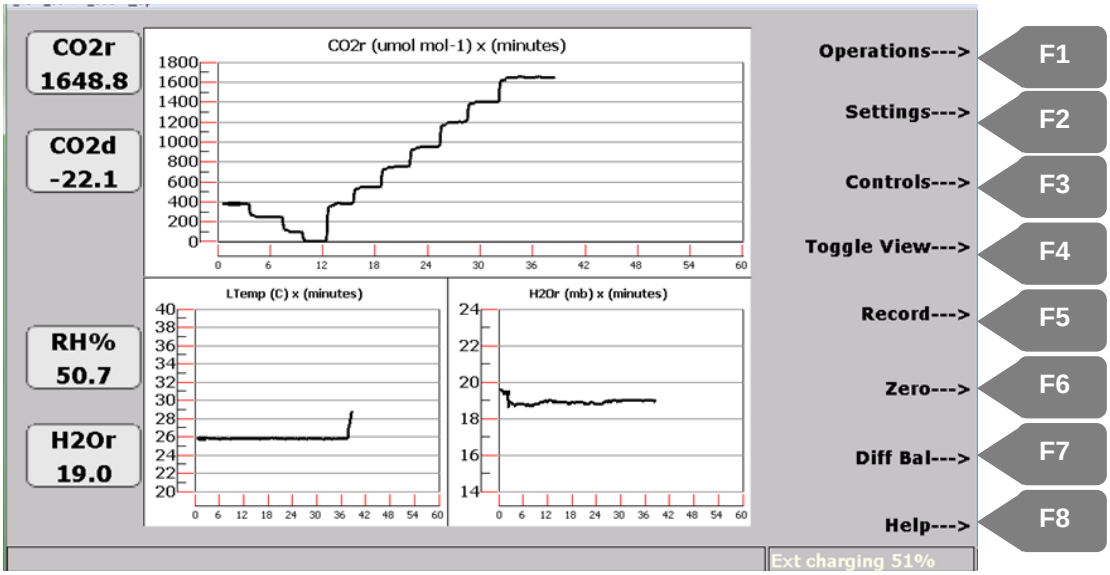
Controls	
CO2 Reference	Options are identical to those described in **Settings (See Settings (F2)**.
H2O Reference (chamber humidity	Options are identical to those described in **Settings (See Settings (F2)**.
Light Intensity	Options are identical to those described in **Settings (See Settings (F2)**.
RGBW Control	Options are identical to those described in **Settings (See Settings (F2)**.
Set Temperature	Options are identical to those described in **Settings (See Settings (F2)**.
Cuvette Flow	Alteration to an existing flow rate causes transient disruption of CO_2 control, which in turn affects calculated assimilation rate and Ci. Be sure to note any imposed change of flow rate, and wait a minute or so for the system to readjust before collecting data. If the new Control value is ± 20% of the old value a brief message will appear in the status bar: "cuvette flow 20% out from set value". This is simply a transient that will disappear as the new target flow is approached.
Leaf Area	Options are identical to those described in **Settings (See Settings (F2)**.

Controls Graph Set (F5)

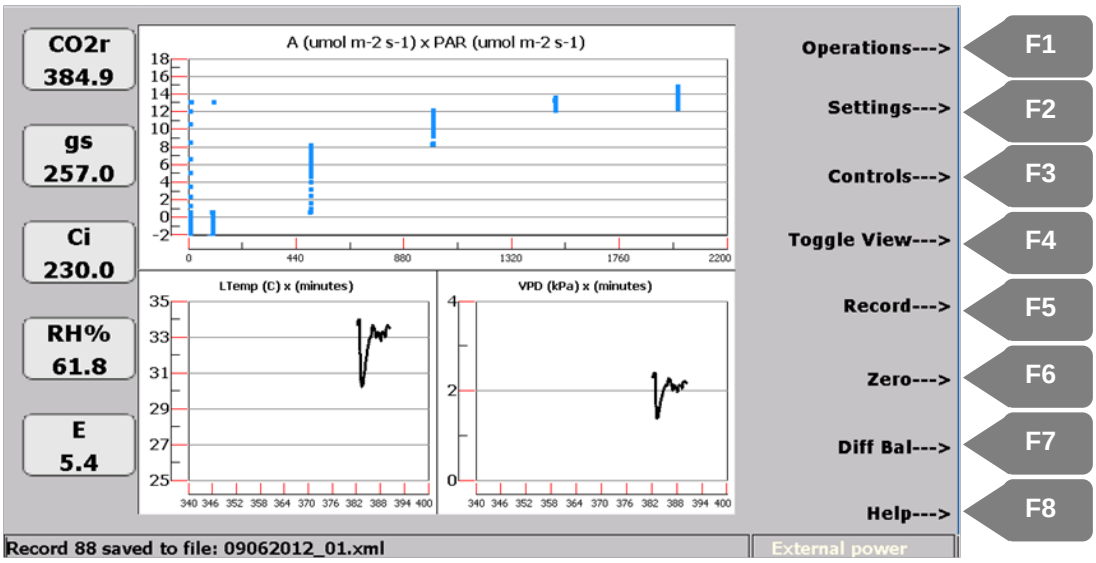
Press **Controls (F3) > Graph Set (F5)** to change your Graph View options. You will find this especially useful after completing system stability checks, when a leaf is in the chamber. Then, your focus will likely be on calculated parameters more than measured parameters. Because the dynamic range of a leaf sample's physiology may be unknown to you, it could be difficult to set the correct Y-axis scale, for example, to keep constant oversight of rapid changes and trends over time. Here, Graph Set will allow you to make quick corrections to Y-axis scales, or to select Auto Scaling. The options are identical to those described in **Settings (F2)> Graph Set (F5)**. Press **Controls (F3) > Graph Set (F5) > Clear (F5)** at any time to refresh plotted data in the graphs.

Toggle View (F4)

Toggle View (F4) is a simple function key used to switch between Numeric and Graph View. For example, use Toggle View if there is a variable that you would like to monitor, but it is not currently displayed in Graph View. One of many possible configurations of Graph View can be seen below, with the CO_2 control steps of an A/Ci curve displayed in the upper graph.



The second example (below) features a two-variable scatter plot of A x PAR. This graph captures a light curve, with each vertical group of blue markers representing photosynthetic acclimation at subsequent increasing light levels (X axis) between 0 and 2000 µmol m^{-2} s^{-1}.



Reviewing and Transferring Data Files

Transfer Data (F6)

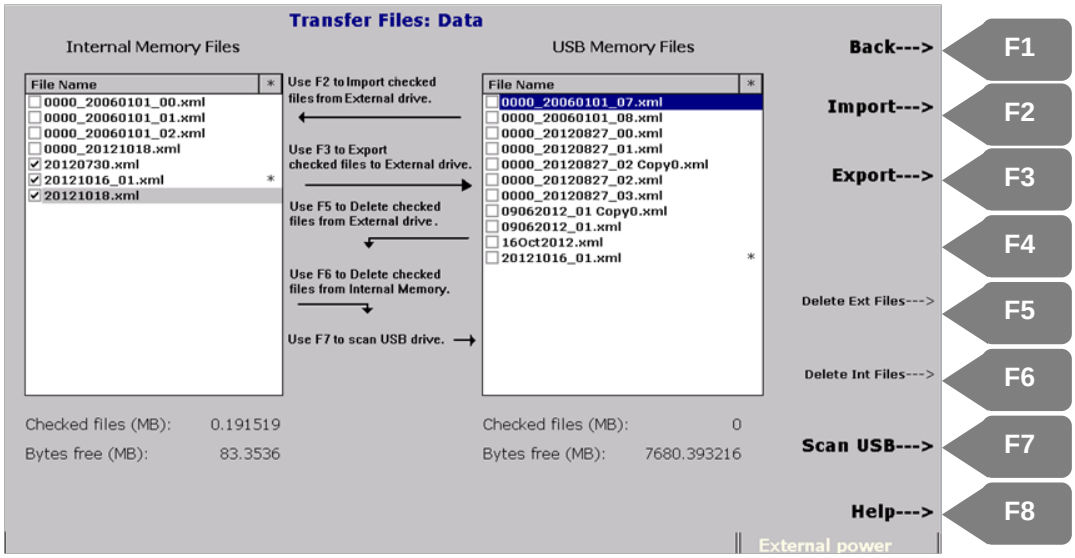
Retrieving your .xml data files from CIRAS-3's internal memory as a routine operation is accomplished with a few easy steps. Importantly, we allow two-way transfer of three file types: data, settings and scripts. The example below describes transferring data files. Press **Operations (F1)** > **Rec Options (F2)** and **Transfer Data (F6)**.

Insert a USB2 or newer device (alternatively referred to as "memory stick" or "thumb drive") into the USB port located at the back of the console. Press **Scan USB (F7)** to see the contents of USB device – only compatible .xml files will appear.

TIP

We recommend using USB2 for direct storage of data to a memory stick (Thumb drive).

The Transfer Files screen is dominated by two large fields: Internal Memory Files is on the left, and USB Memory Files on the right side. Use the TAB key to move back and forth between the two file locations. Within a location field, use the Up and Down arrow keys to highlight files, one at a time, and press OK to select the file you want to move. You know the file has been selected when it has a check mark in the small box to its left.



To move a copy of the data file from the Internal Memory to the USB Memory, select the file and press **Export (F3)**. A file that has been transferred is indicated by an asterisk symbol (*) on the right side of the location field, next to the file name. Note that the file remains in its original location – files can be deleted from Internal Memory by pressing **Delete Int Files (F6)**.

You can also import data files back to Internal Memory, for example, to quickly review the data using **Operations (F1) > View Saved (F4)**. To move a copy of a data file from the USB Memory to Internal Memory, select the file and press **Import (F2)**. Again, the file remains in its original location – files can be deleted from USB Memory by pressing **Delete Ext Files (F5)**.

To transfer CIRAS-3 settings files, from the Numeric or Graph View screens select **Settings (F2) > Transfer (F3)**, then follow the instructions above. To transfer CIRAS-3 response curve scripts, press **Operations (F1)** > **Rec Options (F2) > Edit Rsp Crv (F5)** > **Transfer (F5)**, then follow the instructions above.

Section 8. Operations

Operations (F1)

From the numeric or graph display, press **Operations (F1)** to access numerous system functions including:

- Recording Options (Rec Options – F2)
- Set Clock (F3)
- View saved data (View Saved – F4)
- Calibration (F5)
- Diagnostics (F6)
- Reset System (F7)
- Help (F8)

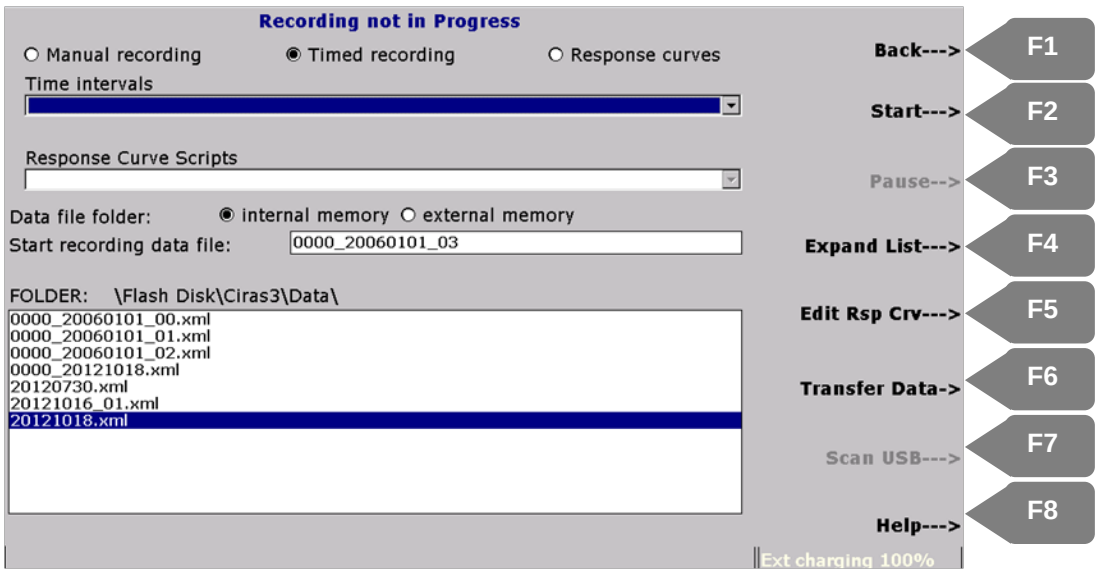
Recording Options

Press **Operations (F1) > Rec Options (F2)** to set up a recording session. There are 3 options available

- Manual recording – Normally used for individual leaf level gas exchange measurements.
- Timed recording
- Response curves – For automated, pre-programmed scripts for A/Ci curves, light response, etc.

Manual Recording

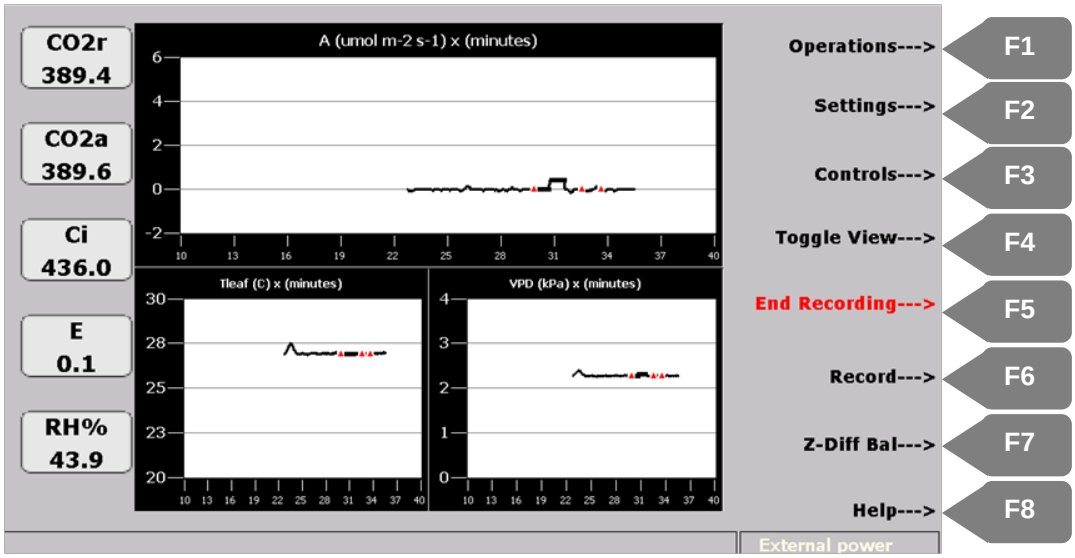
Select the desired recording option at the top of the screen by using the Left or Right arrow keys – the first option is **Manual recording**. For most leaf gas exchange measurements in the field, this method of recording is most common.



Data file folder: indicates the location to save the data file, *internal memory* or *external* (USB) *memory*. The next field is **Start recording data file:** where a default filename using the serial number of the CIRAS-3 and current date appears, e.g. 0000_20130131_00. The date format is yyyymmdd. Each new data file you create will be sequentially numbered, beginning with that day's date, such as 0000_20130131_00. 0000_20130131_01, 0000_20130131_02. You can the change the filename by pressing the TAB key to highlight the field, and the DEL key to backspace and delete characters, then use number keys to enter your own numeric filename. You cannot overwrite an existing data file. Later, you can export the file and rename it using alpha characters. **FOLDER: \Flash Disk\CIRAS3\Data** indicates the internal memory location, and the field beneath it lists all currently stored data files.

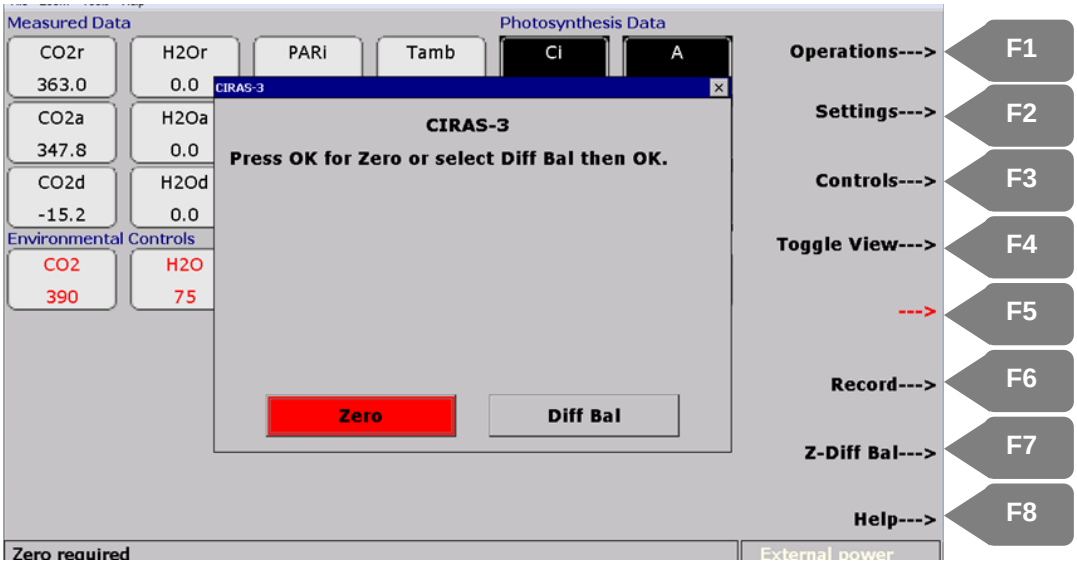
Press **Start (F2)** to beginning recording data – you are automatically returned to the Operations screen, The status bar indicates that you are now in Recording mode, press **Back (F1)**. At this point you can use Graph Set to enter your preferred combination of numeric data, graphics and x-y scaling, if you haven't done so already.

When you are ready to record data, press and release the thumb key labeled **R** on the cuvette's open/close lever to record a single data point and the mini-LCD briefly displays "Recording". Alternatively, press **Record (F6)** on the console. Press and release the thumb key labeled **S** to switch between the cuvette's mini-LCD displays. Again, the mini-LCD displays two abbreviated variable sets: (A, Ci, E, gs) and (CO2r, CO2d, H2Or, H2Od). All recorded data points are indicated in the console status bar, i.e. "record 1 saved to file: 0000_20130131_01.xml". Red triangle markers clearly indicate recorded data points in Graph View, if displayed. Press **End Recording (F5)** to end the recording session. The status bar will display "Recording stopped. Safe to power off system". Alternatively, press **Operations (F1) > Rec Options (F2)** > **Stop (F2)** to end the recording session.



Z-Diff Bal (F7)

To access either Zero or Diff Bal functions at any time during measurements, press **Z-Diff Bal (F7)**. Press the arrow keys to select Zero or Diff Bal.



Use Diff Bal when you have selected Manual Zero/Diff Bal Mode and you are prompted to run Diff Bal with a message in the status bar, “Diff Bal required, ensure CO2r is stable”. Otherwise, run Diff Bal as often as is practical, for instance when sampling very small leaf areas or generally when your data suggests very low gas exchange rates. Zero and Diff Bal will otherwise be performed automatically at regular 30 minute intervals by CIRAS-3. Diff Bal will help ensure that detectable Analysis gas differentials are real, and not simply artifacts of unbalanced IRGA cells.

Be sure that CO2r is not changing at the time that you run Diff Bal. Remember that Diff Bal diverts the Reference gas stream through both Reference and Analysis cells, and quantifies any differences between the Reference and Analysis cells measuring the same gas sample. If CO2r was changing substantially during the Diff Bal process, the resulting difference and applied offset would be artificial, a bit like trying to hit a moving target versus a stationary target. In this sense, it is possible to perform a “bad” Diff Bal. Run Diff Bal more frequently if you are operating at very fine tolerances in your gas exchange data, for example, if it is important to detect differences in assimilation rate that are <1.0 µmol m^{-2} s^{-1}.

Tip

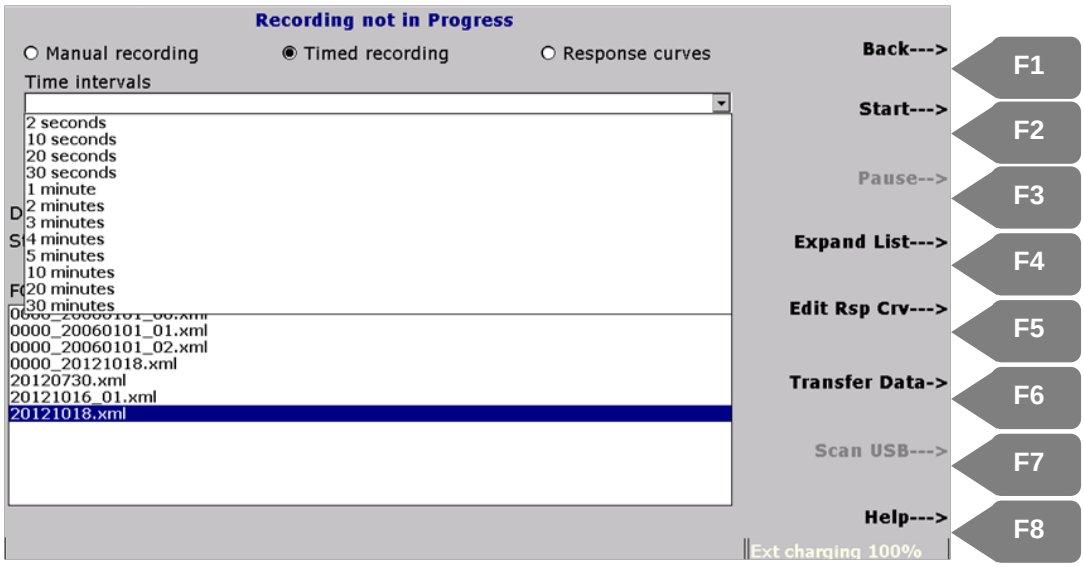
Prior to measurements on small leaf area or whenever you expect very low rates of photosynthesis and when CO2d is showing a small differential (± 1-3 µmol mol^{-1}), perform a manual Diff Bal (F7).

Typical sources for differentials in CO_2 and H_2O are:

- *Instrument not at stable operating temperature*
- *Leak around leaf chamber gaskets*
- *Exhausted chemicals (particularly molecular sieve which is non-indicating*
- *Fluctuating CO2r*

Timed Recording

Using the arrow keys select **Timed recording** and use the TAB key to highlight the **Time intervals** field, then press **Expand List (F4)** to drop down the available selections in the list. Data can be recorded automatically at intervals from 2 seconds to 30 minutes. Use the Up or Down arrows to make the selection and press **OK**. Accept the default filename or rename it, and proceed back to Numeric or Graph Views screens as described above.

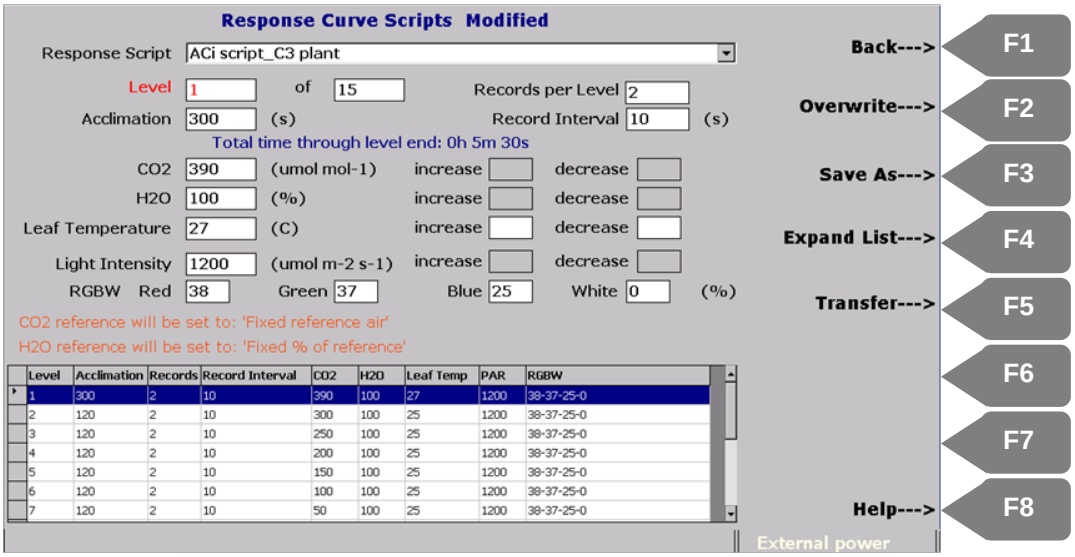


The status bar at the bottom of the screen will indicate *Next record in mm:ss*, counting down to the next automatic recording interval. Timed recording continues indefinitely at the selected recording interval unless paused. This can introduce unnecessary and irrelevant data into your data file. To suspend recording temporarily, for example, to change the leaf sample or run a manual Zero or Diff Bal, press **Operations (F1)**, **Rec Options (F2)**, **Pause (F3)**. Press **Resume (F3)** to continue automatic recording.

Response Curves

The third recording option is recording data automatically using a response curve that you have created. Using the arrow keys select **Response curves**. Press the TAB key to highlight the **Response Curve Scripts** field, the Up or Down arrow keys or **Expand List (F4)** to view and select available scripts. The CIRAS-3 is supplied with several simple default protocols (we call them scripts) stored on the console.

There are multiple options available to create and edit your own response curve protocols to automatically run response curves. This can be done on the CIRAS-3 Console, on a computer using the PC Utility Software supplied with the system and also with any external xml editor. You can also find Help on how to edit these files outside of the console on your own computer, and then transfer the files to the console. This section describes the Console editing option.



Press **Edit Rsp Crv (F5)** (Edit Response Curve) to display the **Response Curve Scripts** editor screen. The screen has two parts: above are fields where you enter the values that define the curve, below the fields the tabular structure of your script is displayed and updated as you make entries. This sample description is based on a simplified A/Ci curve.

The following selections are available:

Response Curve Scripts Editor	
Response Script	Several scripts are included with CIRAS-3 by default. You can use them as templates to edit on the console, or export them for external editing and renaming.
Level	These fields are the starting and ending points of your response curve, representing the total number of steps. If your response curve has 15 steps enter 1 of 15 in the fields. Each entry from this point forward will apply uniformly to the entire response curve, through all Levels. By doing this you create the basic structure of the curve, before you enter the specific changes you want to introduce (independent variable) at each successive Level.
Records per Level	Decide how many data points you want to record at each Level. Entering more than 1 will allow for averaging and other summary statistics, but will extend the total time of the response curve.
Acclimation	Enter the time in seconds that the leaf must acclimate to the conditions you create in the leaf chamber. This is not as easy as it may seem, is dependent on the initial physiological state of the plant, the response curve parameter being changed, and the magnitude of that change. Determining suitable acclimation times often requires one or more test runs of the response curve.
Record Interval	Enter the time in seconds between recorded data points, assuming **Records per Level** n>1.
CO2	Select your desired (starting) CO_2 concentration. Remember that this example describes an A/Ci curve, so we will return to this parameter once you finish setting up the basic structure of curve.
increase/decrease	Allows you to apply uniform step changes through the curve's progression. Example, enter 100 in the **decrease** field and CO2 will automatically change from a starting concentration of e.g. 400 µmol mol^{-1} to 300, 200, 100, 0 through Levels 1-5. Of course, it cannot continue lower than 0, although the table will indicate -100, -200, etc. for successive Levels.
H2O	Select your desired H_2O control value as a percent of the reference air, held constant throughout the A/Ci curve.
Leaf Temperature	Select your desired temperature control value, held constant throughout the A/Ci curve. Recall that you can effectively control leaf temperature from ~10 °C below ambient to ~15 °C above ambient, within the absolute temperature range of 0-45 °C.
Light Intensity	Enter a saturating light intensity held constant throughout the A/Ci curve.
RGBW	Enter your desired LED color distribution, summing to 100%.

Press TAB to enter the table, and use the Up, Down, Left, Right arrows to scroll vertically and horizontally within the table. Press TAB again to return to **Level**. Enter any number to edit that Level. For example, in the case of the CO2 parameter, enter Level 6 to correct the negative CO2 entries. Enter 400 for **CO2** at Level 6, and **increase** by 200 so that Levels will increase to a max. 2000 µmol mol^{-1} at Level 14. TAB once again through the fields and back to Level – enter 14 to complete a simple 14-step A/Ci curve. Again, this is a simplified example of the "architecture" of an A/Ci curve.

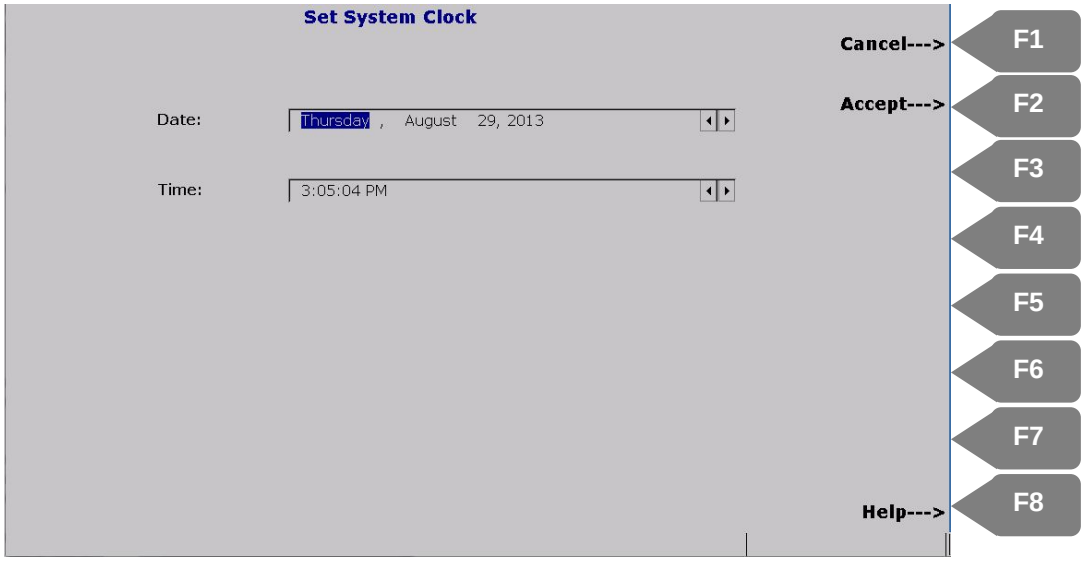
Press **Save As (F3)** to create a new response curve script, or **Overwrite (F2)** to overwrite an existing script that you have edited. **Save As (F3)** will preserve the original file and call the new file "*filename* copy0.xml", "*filename* copy1.xml", etc.

Tip

We strongly recommend that prior to running a response script, go to Settings (F2) and set Zero, Diff Bal Mode to "Auto zero, stored Diff Bal" (See Settings (F2) and Store Diff Bal. This will allow you to execute faster response scripts without Diff Bal interruptions between levels.

Set Clock

Press **Operations (F1) > Set Clock (F3)** to view the system clock. To change the date or time, highlight the item needing to be changed and use the up and down arrows to set. Use the Tab key to move through the selections and when finished press **Accept F2**.



Tip

It is very important to have the correct date and time on the CIRAS-3 as this is most commonly used to identify data files.

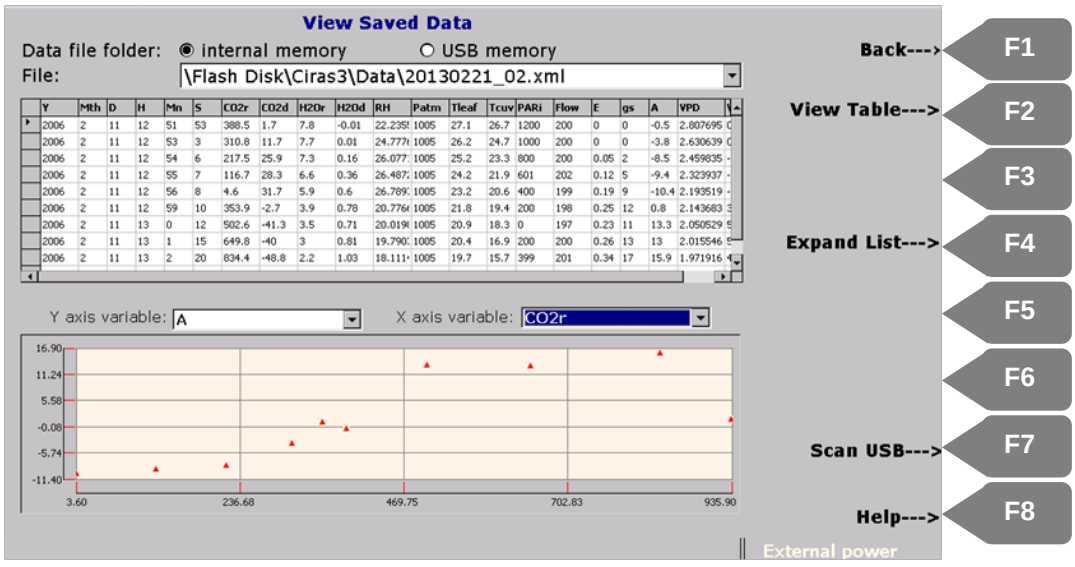
View Saved (F4)

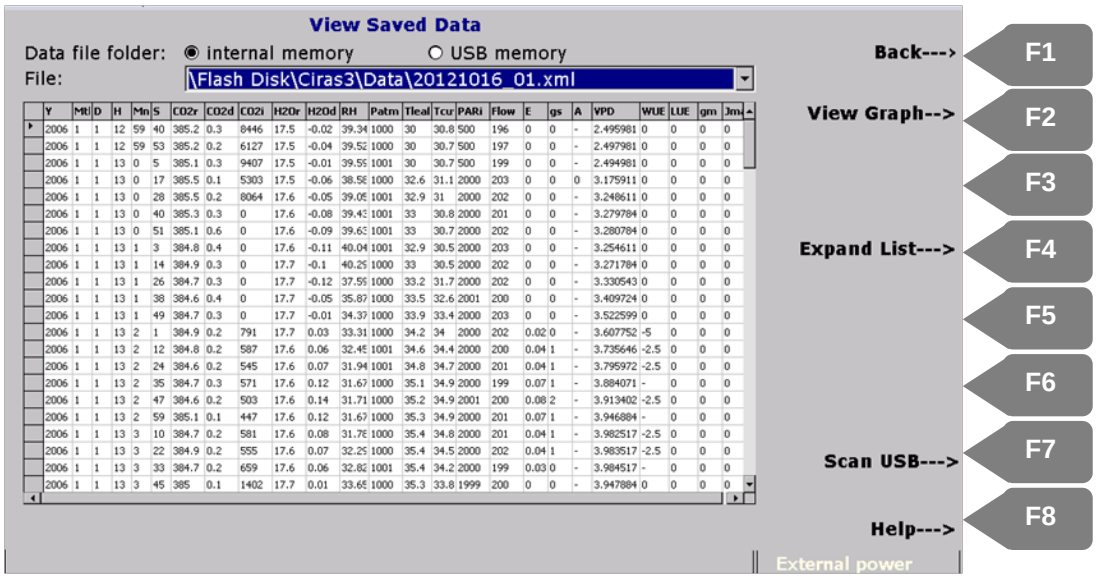
Press **Operations F1 > View Saved (F4)** to view data stored on internal memory or on the USB. This feature of CIRAS-3 allows almost immediate review of recorded data on the console, in both tabular and graphical formats. To enter the View Saved Data screen press **Operations (F1)** and **View Saved (F4)**. The most recently saved .xml data file opens automatically. The location of the file is selected by using the Left and Right arrow keys to move between **Data file folder**: *internal memory* or *USB memory*. The next field shows the internal memory default location and the most recently created data file, e.g. **File:** \Flash Disk\Ciras3\Data\0000_20130221_02.xml. With this field highlighted press **Expand List (F4)** and scroll down to an earlier file if desired.

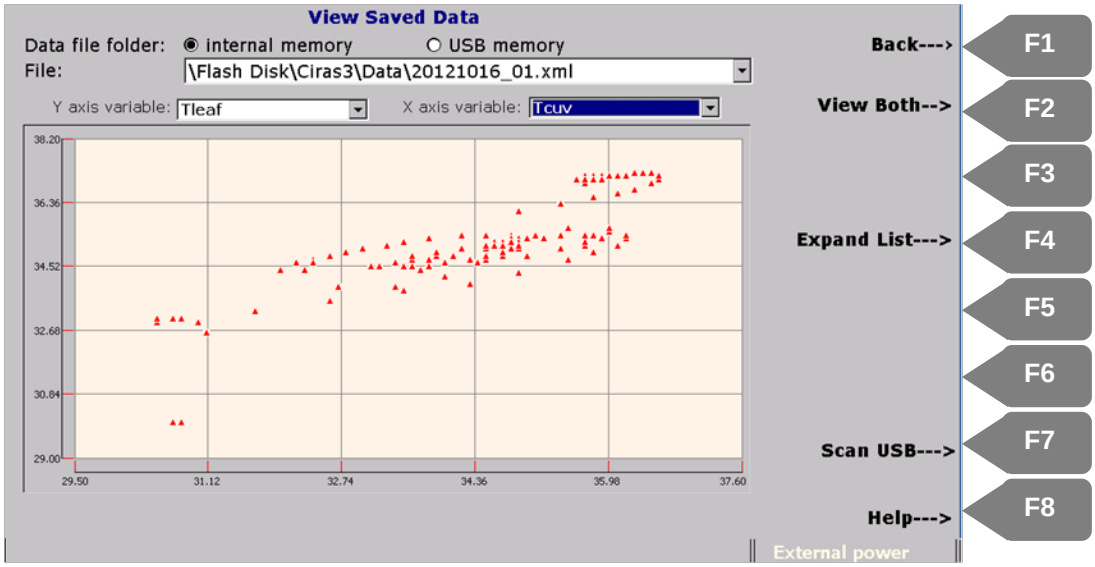
Press TAB to move to the data table, use the Down arrow to scroll through the table. The table column headings indicate its content, which for practical reasons displays most, but not all of the full .xml data file.

Press TAB to move to the **Y axis variable** field. Here you can press **Expand List (F4)** to see available variables, or press the Down arrow sequentially to display the variables one at time, as single-variable graphs. The Y axis is auto-scaled and the default X axis is a time span. As in **Recording**, the data points in the graph appear as red triangle markers. Press TAB to highlight the **X axis variable** field. The default variable is *time (HH:MM:SS*), and can be changed to a measured or calculated variable to create a two-variable scatter plot, in which case the X axis is also auto-scaled.

Press **View Table (F2)** to display only tabular data – press **View Graph (F2)** again to display only graphical data. Press **View Both (F2)** to return to the default combined display. Press **Back (F1)** to exit View Saved Data.





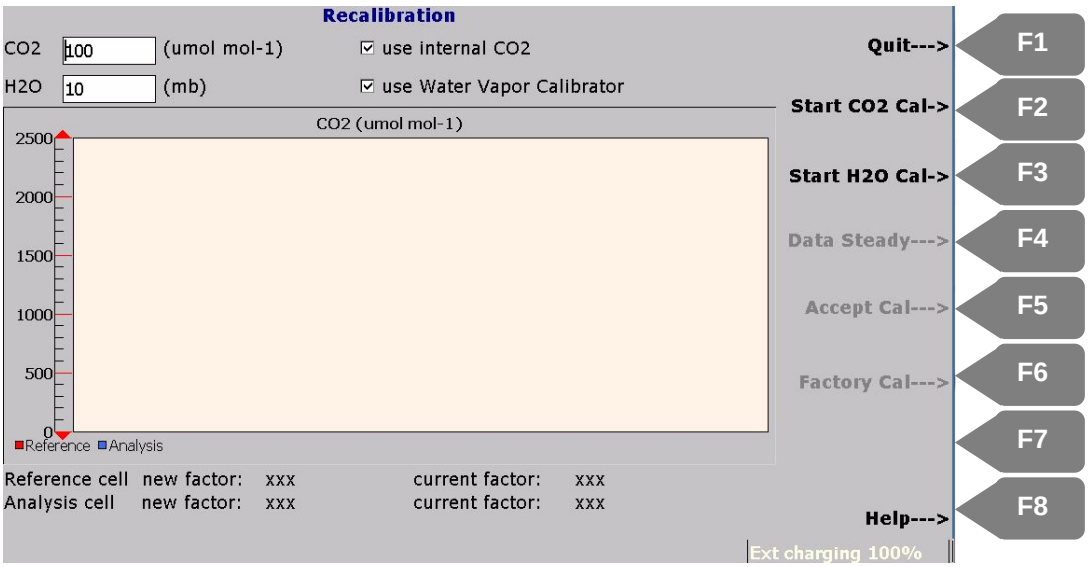


Calibration

Press **Operations (F1) > Calibration (F5)**. This is where users can calibrate the CIRAS-3 CO_2 and H_2O infrared gas analyzers and PAR sensors on the PLC3.

Recalibrate

To recalibrate the CO_2 and H_2O gas analyzers, press **Recalibrate (F2)**.



> ***Tip***
>
> *All CIRAS-3 systems receive a thorough factory calibration before it is shipped. THERE SHOULD BE NO NEED TO RECALIBRATE ANY SENSORS FOR A LONG PERIOD OF TIME. It is very important that you properly maintain the desiccants (Soda Lime, Drierite and Molecular Sieve) to ensure that they are fresh. As long as the chemicals are fresh the CIRAS-3 will receive a proper zero resulting in long term accuracy, stability and calibration of the CO_2 and H_2O gas analyzers. Simple, periodic checks of sensor calibration are recommended.*

CO_2 Calibration

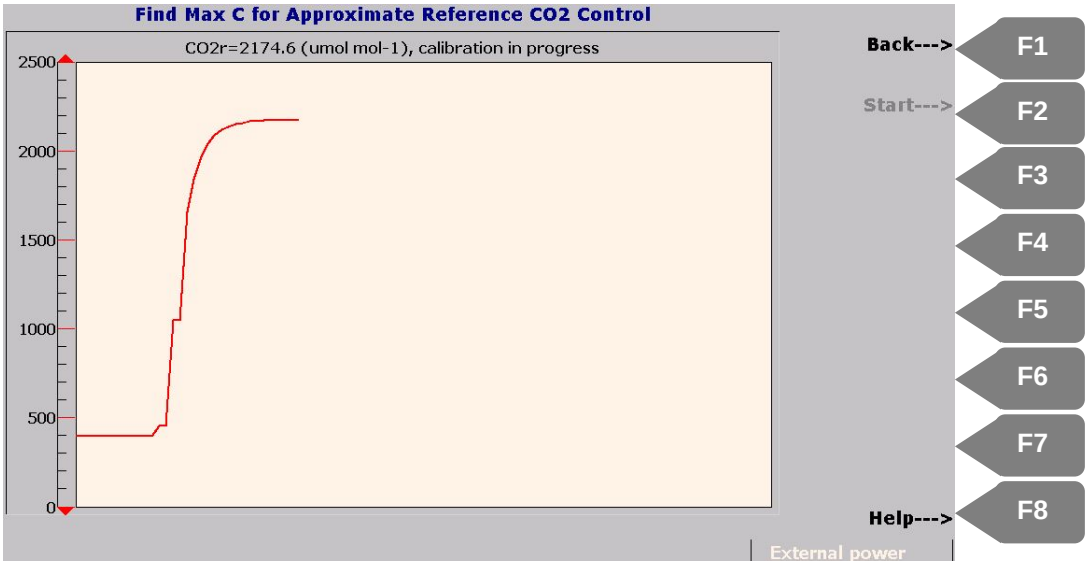
See CO2 Calibration.

H_2O Calibration

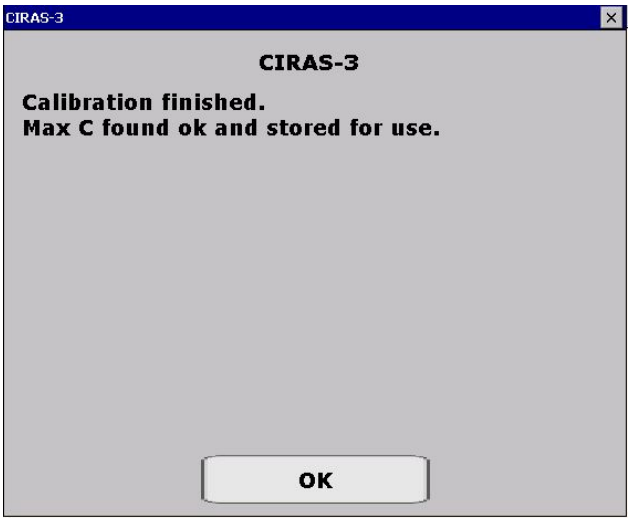
See H2O Calibration.

Find Max C

Press **Find Max C (F3)**. This process determines the maximum CO_2 level that can be achieved with the CIRAS-3. This must be performed prior to using "Approximate reference air" for CO_2 reference (See Settings (F2). Prior to starting this process, make sure that you have inserted a fresh CO_2 cartridge and that all chemicals are fresh. Hit **Start (F2)** to begin the process.



This process is automated and you should see the CO2r value settle out just above 2000 µmol mol $^{-1}$ and the message "… calibration in progress" just above the graph. If this process is successful, you will receive a message as follows:



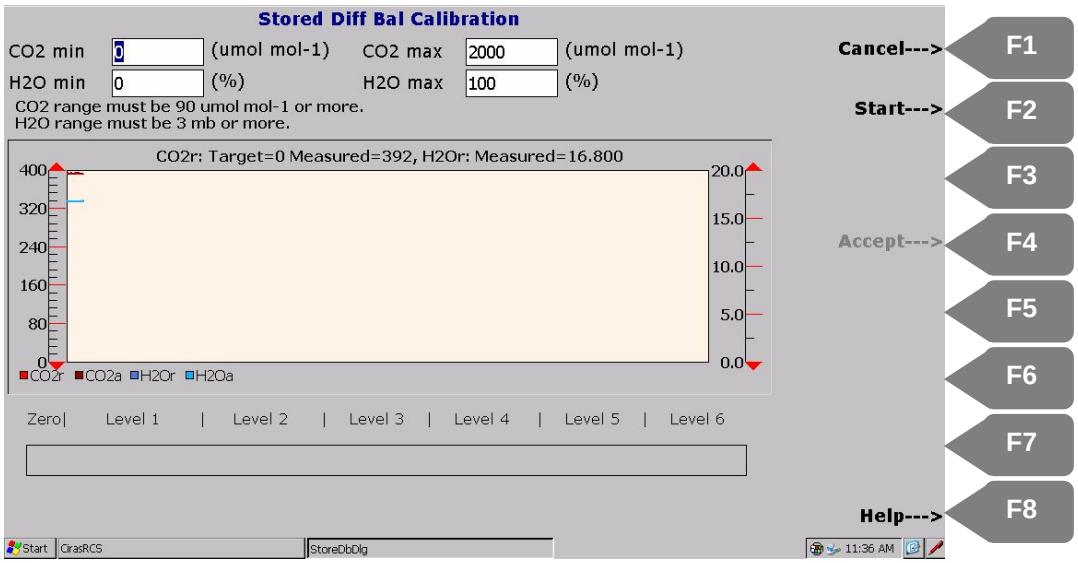
Press **OK** to accept and then Back (F1). If you are not successful and you get a message that says it can't find Max C, check your chemicals again, tighten the CO_2 cartridge holder to ensure that the cartridge was pierced when inserted and perform a new Max C.

TIP

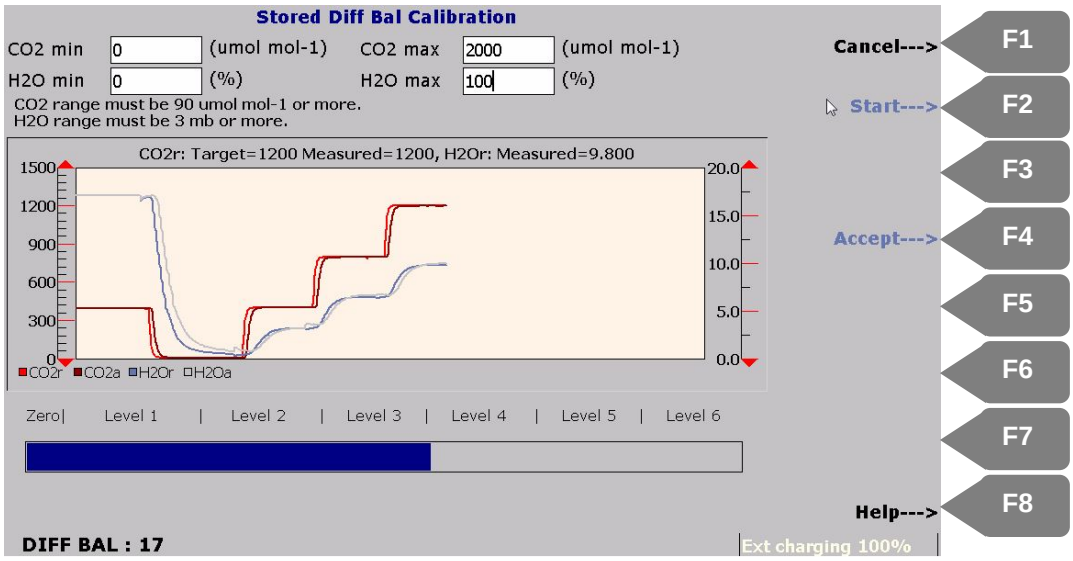
You must always perform a MAX C prior to using "Approximate reference air" for CO2 Reference under Settings. (See Settings (F2).

Store Diff Bal

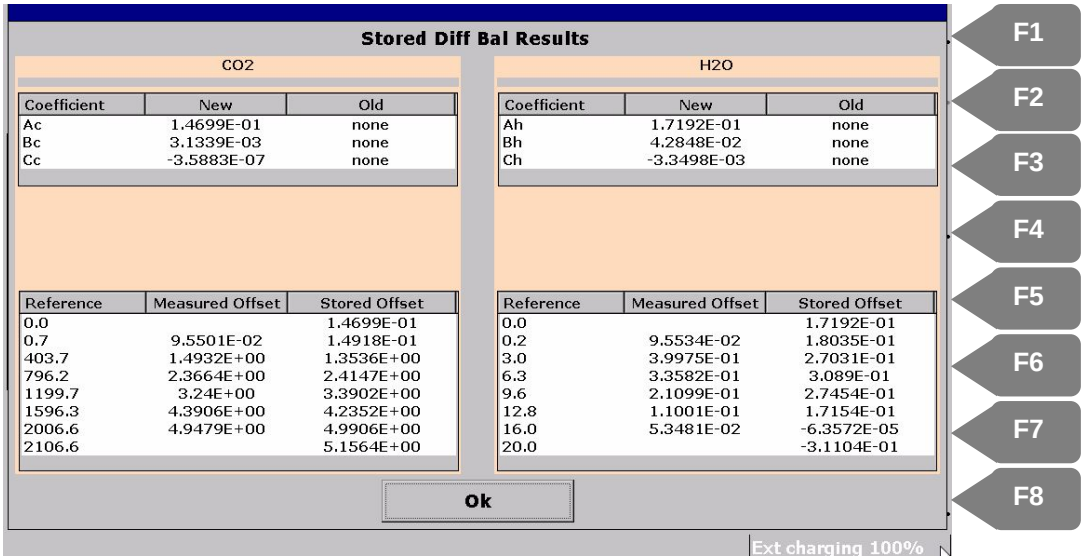
For response curves, we recommend using Auto zero, stored Diff Bal. Prior to using this option, you **MUST** first perform a "Store Diff Bal" prior to starting a response script. This process allows you to store "Zero" and "Diff Bals" in advance to avoid disruptions during a response. Press **Store Diff Bal (F4)** to begin the process.



To begin, enter the minimum and maximum ranges for both CO_2 and H_2O. For instance, if the working CO2 range is 200-1000, enter 200 for CO2 min and 1000 for CO2 max. Press the Tab key to advance to each setting. When complete, press **Start (F2).** The process is automated taking approximately 20 minutes to complete beginning with an initial Zero before proceeding through 6 levels. The status bar for each level will be highlighted when complete as shown below.



When the entire process is completed after Level 6 and if successful, the following is displayed:



After the Stored Diff Bal Calibration is complete, the Stored Diff Bal Results screen is presented for review. The user can choose to A**ccept** the results (**OK** then **Accept (F4)**, which will allow the new Stored Diff Bal Coefficients to be used when the CIRAS-3 is in Stored Diff Bal mode. The user can also choose to **reject** the results (**OK** then **Cancel (F1)**, and return to the Stored Diff Bal Calibration Screen for another attempt at the Stored Diff Bal Calibration. If the new results are rejected and there was a previous set of Stored Diff Bal Coefficients (labeled "Old" in the Stored Diff Bal Results screen), the old coefficients will be used if the user cancels out of the Stored Diff Bal Coefficients screen. The upper half of the Stored Diff Bal Results screen shows new and old Stored Diff Bal Coefficients. The lower half of the screen shows the data recorded during the Stored Diff Bal Calibration, and the computed offsets based on the new coefficients. The 2nd through 7th row of data in this lower section show the actual Ref and Offset recorded at each step of the calibration. The first row of data represents the lowest limit of reference readings that are allowed for these Stored Diff Bal Coefficients. The lower limit is fixed in

software to 100 ppm less than the lowest CO_2 Ref reading and 4 mbar less than the H_2O Ref reading. The last row of data represents the upper limit of reference readings that are allowed – 100 ppm more than the highest CO_2 Ref reading and 4 mbar higher than the highest H_2O reading. The column labeled "Stored Offset" represents the calculated offset computed for that row's Reference reading using the new Stored Diff Bal Coefficients. These first and last rows also show the calculated offsets for these upper/lower limit reference readings which are extrapolated past the actual recorded offsets.

Users should review this lower screen prior to accepting data for the following criteria:

1. Measured offsets at each point should be less than 20 ppm and 0.5 mbar.
2. Measured offsets should show a trend - gradually increasing or decreasing over the six points.
3. The computed Stored Offsets in the 3rd and 6th columns should match the measured offsets in the 2nd and 5th columns within about 1 ppm or 0.5 mbar.
4. The first and last row Stored Offsets should not be significantly higher or lower than the other values, and should follow the trend observed in the actual data. Since these calculated offsets are extrapolated beyond the actual recorded offsets, they might be unrealistic if the least square curve fit has a high quadratic term. If this does occur, the new coefficients could still be used, but the user should be sure to limit the operational settings CO2Ref and H2ORef to be within the range where the Stored Offsets seem valid.

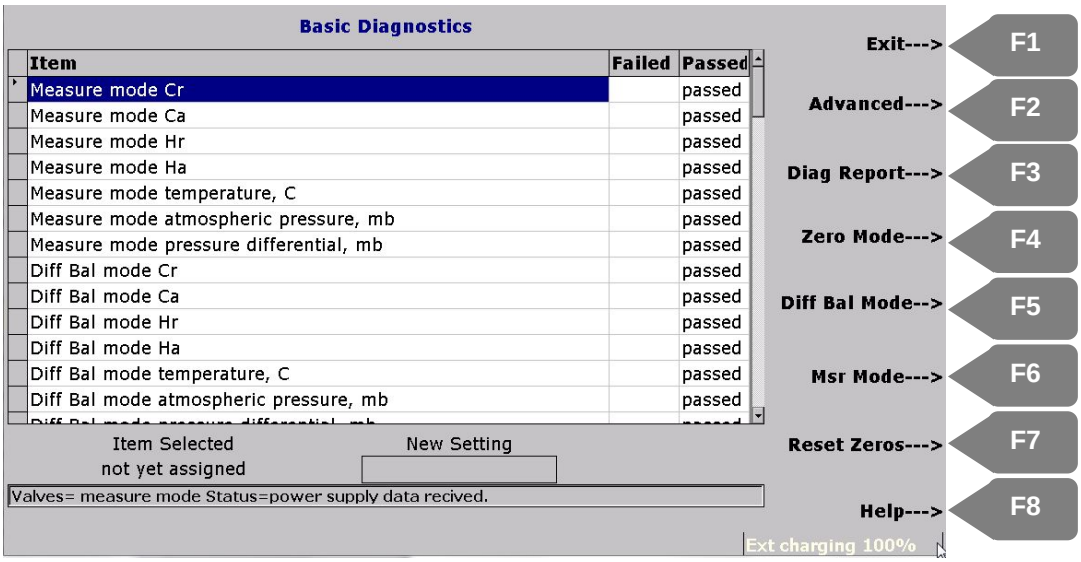
Diagnostics

Press **Diagnostics (F6)**. The CIRAS-3 contains numerous sensors to measure CO_2, H_2O, leaf environment, signals from external probes and gas flow through the instrument. The **Diagnostics** function collects the signals from each of these sensors and summarizes them. This information is extremely valuable in troubleshooting systems remotely and can be compared with factory records to identify any minor or major changes in the system.

There are two different Diagnostic functions, Basic and Advanced.

Basic Diagnostics

For most users, this is all you will need to view if problems are encountered. Based on the state of the system, information that is being monitored will either show "passed" or "failed". If you witness a "failed" message we recommend that you report it to PP Systems.

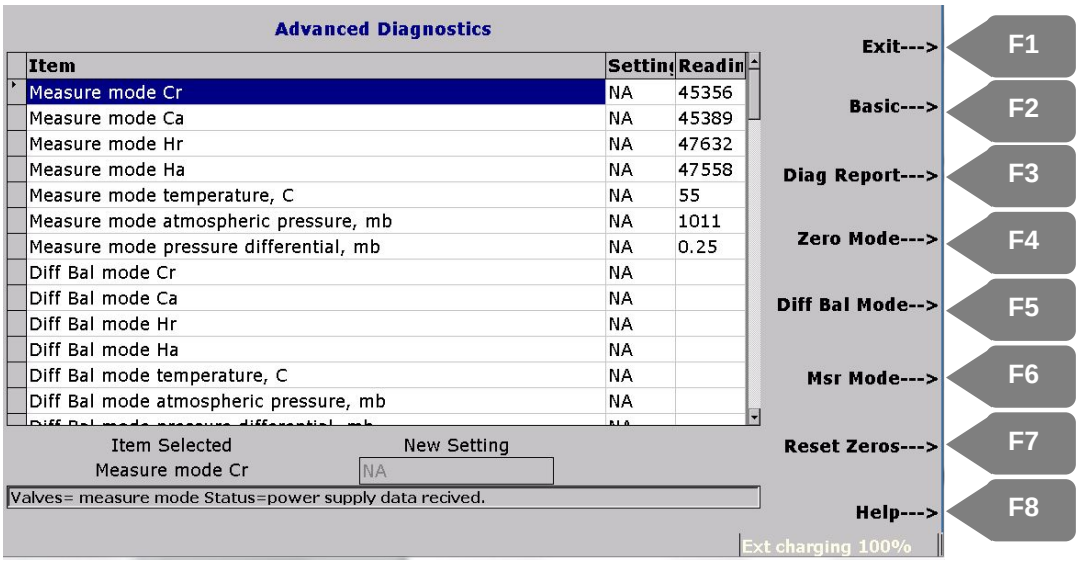


Tip

In some cases, a "failed" status is simply a transient and not a true problem or failure. It is still worth it to contact PP Systems to play it safe.

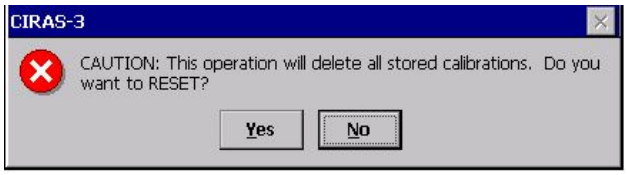
Advanced Diagnostics

In some cases, there may be a need to dig deeper into a problem or fault which is why we also include "Advanced Diagnostics". This provides more technical information (system A/D reads,flow reads, etc.) which can help to shed even more light on problems. This information is commonly used by our in-house system engineers to help troubleshoot and diagnose system failures/faults.



Reset System

This feature is not yet implemented with CIRAS-3. The intention is that it will be used to reset the CIRAS-3 system back to factory default. **PRIOR TO USING THIS FEATURE, YOU MUST CONSULT WITH PP SYSTEMS**. Press **Reset System (F7).** The following warning message is displayed:



Select No. This section will be updated in the coming months as we release updated versions of the CIRAS-3 software.

Tip

DO NOT use this feature. If you have any questions, please contact PP Systems.

Section 9. Console and PC Utility Software

Ciras3_Console.exe Installation and Removal

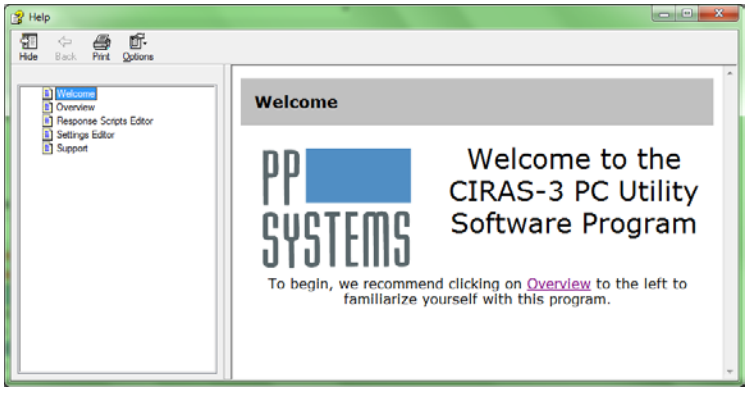
The software provided for the operation of the CIRAS-3 is provided as two applications. One application runs on Windows CE6 which executes on the CIRAS-3 console hardware. Its name is "Ciras3_Console.exe". The second application runs on desktop computers running Windows XP, Vista or Win7 which have the .NET v2 libraries installed. Its name is "Ciras3_PC_Utility.exe". These instructions assume that the Ciras3_Console application was previously installed and that the purpose of the new Install is the fix or upgrade the application.

One should first uninstall the prior version of Ciras3_Console.exe. It is easiest to do this via the use of a USB mouse attached to the console.

1. Open "My Device" on the console desktop. Navigate to folder:
2. "\Flash Disk\InstalledFiles\PP Systems CIRAS3_Console_v100"
3. Where "v100" is the prior installed version.
4. Copy "PP Systems CIRAS3_Console_v100.unload" file.
5. Navigate to "\Windows" folder and paste the unload file.
6. Close "My Device" window.
7. Use Start – Settings – Control Panel – Remove Programs. Select "PP Systems CIRAS3_Console_v100" from the list and click "Remove" button.

We can now install the new version.

1. Copy "Ciras3Console_v100.cab" file from the provided CD-ROM to a USB thumb drive (memory stick).
2. Insert the USB drive into the CIRAS-3 console.
3. Open "My Device". Copy the cab file from the "USB Storage" folder to the root of the console file system. Note the exact location is not important as the cab file will be erased when installation is complete.
4. Remove USB thumb drive from console.
5. Right click on cab file and ensure it is not Write protected.
6. Double click on the cab file to execute the install. Accept all defaults by using "OK" button.
7. When installation is complete, power off the console for a few seconds and then power on again. The new version of the console application should start automatically.



For remote viewing of CIRAS-3 console information on an external device. Select View>Remote Display. This feature allows the user to view all of the items (screens) on the CIRAS-3 on a remote device such as a PC computer or projector. This can be extremely useful presenting information to a large group of people or for teaching applications. Connection between the CIRAS-3 console and remote device is made via a USB connection using the cable provided by PP Systems.

The ActiveSync Remote Display application is included as standard with the CIRAS-3 PC Utility software. This is an unsupported Microsoft application provided as part of their developer Power Toys program. Please note that when connecting to the CIRAS-3 an "Unknown CPU" error may be reported. If you receive this error, simply hit OK to bypass this message and everything should work normally.

Windows XP and Prior Operating Systems

In Window XP and prior versions, you must download ActiveSync from the Microsoft site and install it to your PC.

Windows Vista and Windows 7 Operating Systems

The Windows Mobile Device Center (WMDC) replaces ActiveSync. This is also a downloadable application available from the Microsoft site. Please note that the current Microsoft website implies that this only operates in Vista but we have successfully tested this application using Windows 7.

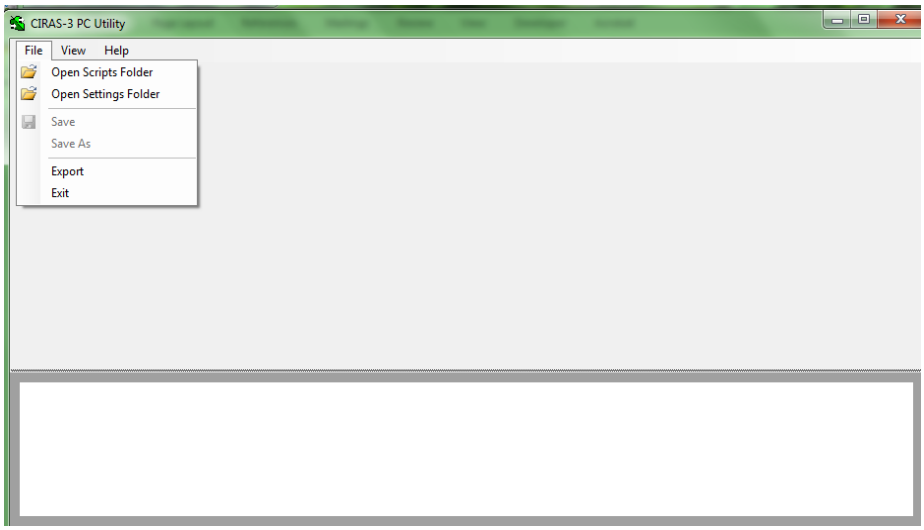
The CIRAS-3 PC Utility is provided as a “Setup.exe” file and a MSI file named Ciras3_PC_Utility_v100.msi. It is best to uninstall prior version before installing new versions.

1. On your Windows desktop computer use Start – Control Panel – Add Remove Programs. Wait for list to populate.
2. Select “Ciras3_PC_Utility” and click on Remove button.
3. Exit Control Panel.
4. Navigate to the CD-ROM folder which contains “Setup.exe” and “Ciras3_PC_Utility_v100.msi”. Double click on setup file to start the install.
5. For Vista or Win7: Once installation completes, find the shortcut link on the desktop named “Ciras3_PC_Utility”. Right click and select Properties. Select Compatibility and enable “Run as administrator”.

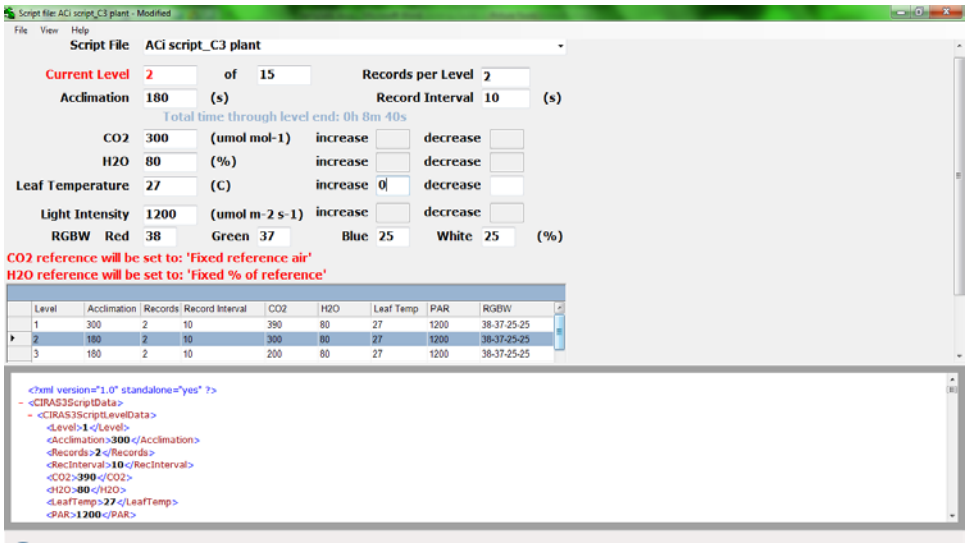
Practical Uses of CIRAS-3_PC_Utility

Now that the software is installed on your computer, you should test if it is working correctly. As mentioned, there are two basic applications of CIRAS-3_PC_Utility – it can be used to edit your Response Curve scripts and Settings files and to remotely display and control CIRAS-3 Console screens.

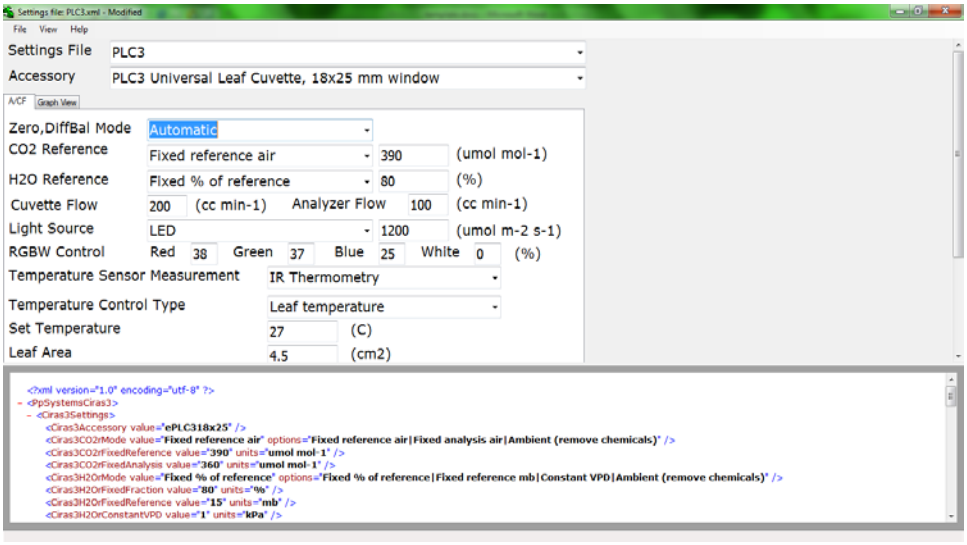
Click on the CIRAS-3 Utility icon on your computer desktop to open. Click on File > Open Scripts Folder, browse to the location C:\Program Files (x86)\PP Systems\CIRAS-3 PC Utility\Response on your computer, and click OK.



This opens the Script window where you can choose from several default scripts. Click the arrow button to expand the dropdown list at the **Script File** item. Edit the script just as you would if you were working from the CIRAS-3 Console. Then click File > Save, or Save As to rename it.

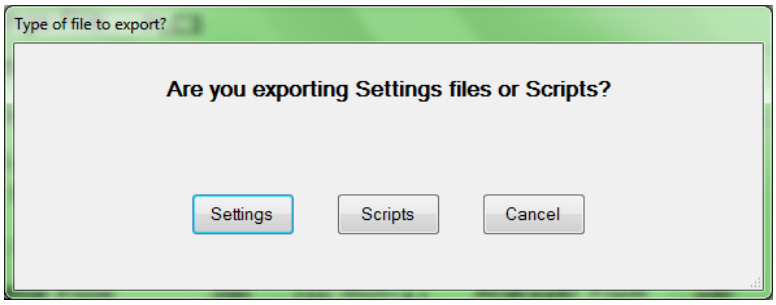


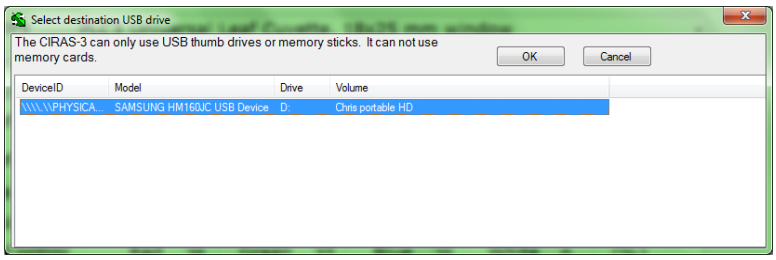
Click on File > Open Settings Folder, browse to the location C:\Program Files (x86)\PP Systems\CIRAS-3 PC Utility\Settings on your computer, and click OK. Select from the default list of Settings in the Settings File dropdown list. Note that for each type of **Settings File** there are two windows that can be edited, and two tabs that allow access to these windows. For example, **PLC3** or **PLC3-CFM** settings files used for open system leaf-level photosynthesis and chlorophyll fluorescence have a global Settings window tab labeled **A/CF** (assimilation/chlorophyll fluorescence).



The second tab is always the **Graph View** tab (similar to using **Graph Set** on the CIRAS-3 Console). Edit the file as you would from the CIRAS-3 Console. Then click **File > Save**, or **Save As** to rename it.

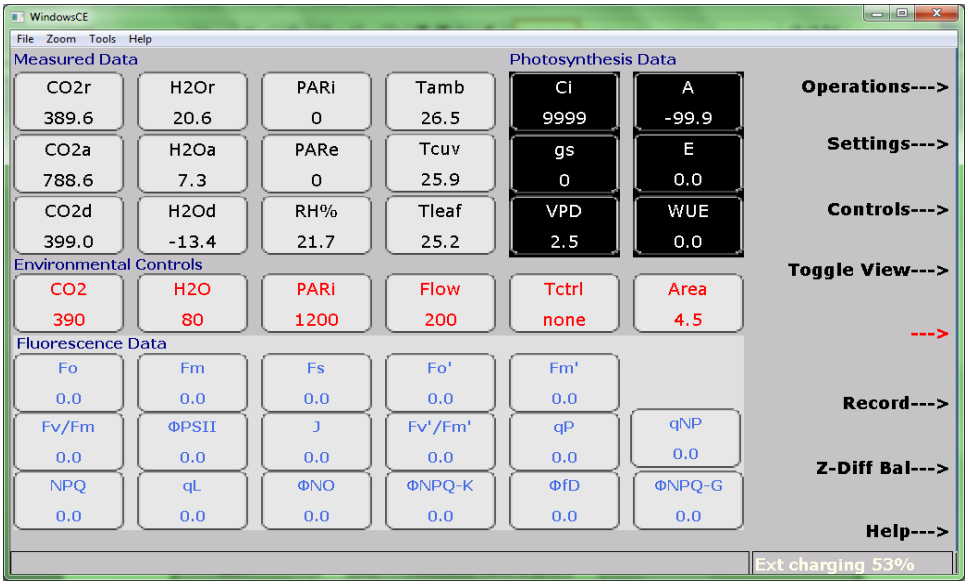
Insert a USB storage device in your PC. Click **File > Export** and select Settings or Scripts as prompted by the dialog. Choose the destination USB device. Click Ok to complete Export. Export folders, e.g. CIRAS3\Settings, will automatically be created on a USB storage device inserted in your PC. Insert the USB device in the CIRAS-3 Console. Go to **Settings (F2)** >**Transfer (F3)** to transfer Settings. Go to or **Operations (F1)** > **Rec Options (F2)** > **Edit Rsp Crv (F5)** > **Transfer (F5)** to transfer Response Scripts.





Live CIRAS-3 Console screens can be viewed on your computer, and if desired, projected from your computer for the benefit of a larger audience. This is ideal for discussions with larger groups and for teaching purposes. With your ActiveSync or WMDC installation complete connect the USB cable between a USB port on your computer and the mini-USB port on the back of CIRAS-3, labeled “PC”. Verify that you have established the required ActiveSync or WMDC connection. A powered USB hub device used between the computer and CIRAS-3 Console can provide more reliable connections. During the CIRAS-3 PC Utility installation process folders were created on your PC at C:\Program Files (x86)\PP Systems\CIRAS-3 PC Utility. In addition to Response and Settings folders, the installation created a folder named ASRD (ActiveSync Remote Display). Later, a Data folder will be created if you enter Recording mode while your PC is connected to CIRAS-3 via ActiveSync or WMDC.

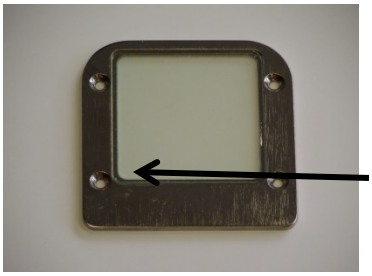
In the CIRAS-3 Utility window click **View > Remote Display**. Note that when connecting to CIRAS-3 an "Unknown CPU" error may be reported. If you receive this error, press OK to bypass the error message and everything should work normally. Navigate and control CIRAS-3 screens as you would from the CIRAS-3 Console, by using F1-F8 function keys, TAB, ESC and arrow keys on your computer keyboard. You can also continue to use the CIRAS-3 Console keys to navigate screens and dialogs.



The PC Utility also has some Help screens to guide you through using and understanding the Settings and Response Script editors, and the Remote Display. Click on File > Help to access this information.

Section 10. Measuring Chlorophyll Fluorescence with CFM-3

The CFM-3 Chlorophyll Fluorescence Module (Part Number CRS306) is an optional accessory that can be used with the PLC3 Universal Leaf Cuvette for simultaneous measurement of photosynthesis and chlorophyll fluorescence. It can also be used for independent measurement of chlorophyll fluorescence if desired.



When performing measurement of chlorophyll fluorescence you MUST make sure that the glass window (supplied by PP Systems) is fitted to the leaf cuvette head. This window will have the letter "P" etched in the lower left hand corner.

Letter P

To change the window, refer to the maintenance section at the back of this manual (Changing PLC3 Universal Window For Use with CFM-3).

Overview of Chlorophyll Fluorescence

The Basics

In basic terms, chlorophyll fluorescence is light re-emitted from the chlorophylls, mainly chlorophyll a (chl a) in Photosystem II (PSII). 95% of chlorophyll fluorescence is from PSII, at room temperature. We measure chl a because *in vivo* the energy absorbed by chl b is transferred to chl a. It is chl a fluorescence that changes as a response to changes in physiology.

When illuminated, chlorophyll is excited to a higher energy state. This excitation energy must be dissipated or transferred to avoid photo-oxidative damage. De-excitation of chlorophyll a leads to one of three processes:

- Photochemistry (electron transport)
- Heat dissipation (non-photochemical processes)
- Fluorescence (emission of red photons, red shift)

Chlorophyll fluorescence represents about 1-3% of the total energy dissipated. Exposing a leaf to actinic light results in a competition among the three processes. Fluorescence rises at first because induction of photosynthesis is relatively slow. Then, as photosynthetic rate increases (photochemistry) and heat dissipation increases (non-photochemical events), the amount and yield of fluorescence decreases. Photochemistry and heat dissipation effectively result in the quenching of fluorescence. The amount or yield of fluorescence can tell us how efficiently PSII is operating.

How do we measure fluorescence? CFM-3 is a pulse modulated (PAM) fluorometer, the modulating, or measuring lights are used to produce a fluorescence signal without inducing photosynthesis (actinic effect), which interferes with the fluorescence signal. Applying the modulating light intermittently at a certain frequency, the detector can distinguish the intermittent measuring light from the background actinic light. It outputs a signal equivalent to the proportion of light re-emitted as fluorescence.

Description of Chlorophyll Fluorescence Parameters	
F	*measured, detector – Fluorescence signal (current)*
F_o	*measured, detector - minimum F yield, sample dark-adapted*
F_m	*measured, detector - maximum F yield, sample dark-adapted*
F_v	*calculated - variable F yield, sample dark-adapted*
F_v/F_m	*calculated - maximum photochemical efficiency of PSII, sample dark-adapted*
F_m'	*measured, detector - maximum F yield, sample illuminated*
F_s	*measured, detector - steady-state F yield, sample illuminated*
ΦPSII	*calculated - photochemical efficiency of PSII, sample illuminated*
F_o'	*measured, detector - minimum F yield, sample illuminated by far-red light*
F_o'	*calculated, minimum F yield, requires Fv/Fm*
F_v'	*calculated – variable F yield, sample illuminated by far-red light, requires F_o'*
F_v'/F_m'	*calculated - photochemical efficiency of PSII, sample illuminated by far-red light , requires F_o'*
J	*calculated - thylakoid electron transport rate (µmol e^- m^{-2} s^{-1})*
qP	*calculated - photochemical quenching coefficient, correlates to proportion of open PSII reaction centers in puddle antenna model, requires F_o'*
qNP	*calculated – non-photochemical quenching, older term, insensitive to higher quenching values, requires F_o'*
NPQ	*calculated - non-photochemical quenching, correlates to heat dissipation via xanthophyll cycle and PsbS protein*
qL	*calculated - photochemical quenching coefficient, correlates to proportion of open PSII reaction centers in lake antenna mode, requires F_o' (Kramer calculation)*
ΦNO	*calculated – non-photochemical quenching due to aggregate constitutive non-regulatory processes, lake antenna model, requires F_o' (Kramer calculation)*
ΦNPQ-K	*calculated – non-photochemical quenching due to down-regulatory (pH, xanthophyll) processes, lake antenna model, requires F_o' (Kramer calculation)*
Φf,D	*calculated – non-photochemical quenching due to aggregate constitutive non-regulatory processes, equally applicable to puddle and antenna models (Genty calculation)*
ΦNPQ-G	*calculated – non-photochemical quenching due to down-regulatory (pH, xanthophyll) processes, equally applicable to puddle and antenna models (Genty calculation)*

Calculations Used for Chlorophyll Fluorescence Parameters

$F_v = F_m - F_o$

$F_v/F_m = \frac{(F_m - F_o)}{F_m}$

$\Phi_{PSII} = \frac{(F_{m'} - F_s)}{F_{m'}}$

$F_o' = \frac{F_o}{\left(\frac{F_v}{F_m}\right) + \left(\frac{F_o}{F_{m'}}\right)}$

$F_v' = F_{m'} - F_o'$

$F_v'/F_m' = \frac{(F_{m'} - F_o')}{F_{m'}}$

$J = (I \cdot \alpha \cdot f_{II} \cdot \Phi_{PSII})$ Note: **α**=average fraction of irradiance, ***I*** *(*as PAR*)*, absorbed by leaf, default value of **α**=0.84; f_{II}=fraction of **α** absorbed by PSII, default value of f_{II}=0.5

$q_p = \frac{(F_{m'} - F_s)}{(F_{m'} - F_o')}$

$q_{NP} = \frac{(F_m - F_{m'})}{(F_m - F_o')}$

$NPQ = \frac{(F_m - F_{m'})}{F_{m'}}$

$q_L = \left[\left\{\frac{F_{m'} - F_s}{F_{m'} - F_o'}\right\} \cdot \frac{F_o'}{F_s}\right] = q_p \cdot \frac{F_o'}{F_s}$

$$\Phi_{NO} = \left[\frac{1}{\frac{(Fm-Fm')}{Fm'}+1+\left[\left\{\frac{Fm'-Fs}{Fm'-Fo'}\right\}\cdot\frac{Fo'}{Fs}\right]\cdot\left(\frac{Fm}{Fo}-1\right)}\right] = \left[\frac{1}{NPQ+1+q_L\cdot\left(\frac{Fm}{Fo}-1\right)}\right]$$

$$\Phi_{NPQ\text{-}K} = 1 - \frac{Fm'-Fs}{Fm'} - \left[\frac{1}{\frac{(Fm-Fm')}{Fm'}+1+\left[\left\{\frac{Fm'-Fs}{Fm'-Fo'}\right\}\cdot\frac{Fo'}{Fs}\right]\cdot\left(\frac{Fm}{Fo}-1\right)}\right] = 1 - \Phi PSII - \Phi_{NO}$$

Note: $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$

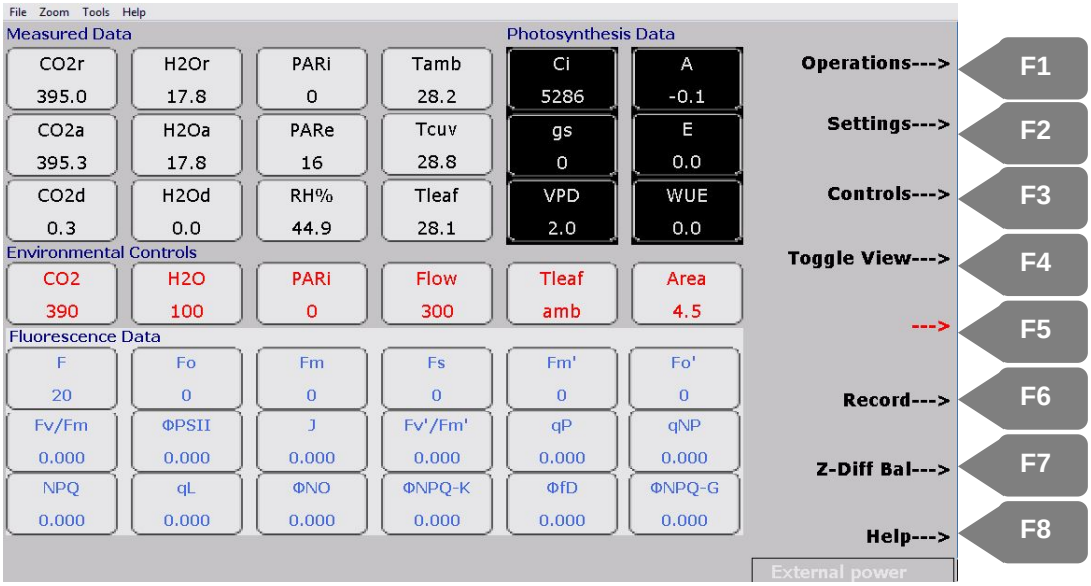
$$\Phi_{f,D} = \frac{Fs}{Fm}$$

$$\Phi_{NPQ\text{-}G} = \frac{Fs}{Fm'} \cdot \frac{Fs}{Fm}$$

Chlorophyll Fluorescence Measurements – Setup and Operation

With CIRAS-3 there is a new option available to record complete fluorescence measurement sequences with or without combining simultaneous photosynthesis measurements. Your selection of Accessory from the global Settings dialog determines how you will be using CFM-3, either measuring chlorophyll fluorescence exclusively, or in combination with photosynthesis. We have also included updated calculated fluorescence parameters, representing both lake and puddle models of PSII reaction centers - their detailed descriptions and calculations are provided above. ΦNPQ-K (Kramer et al. 2004 calculation) and ΦNPQ-G (Genty et al. 1996 calculation) are reviewed in Hendrickson et al. 2004. ΦNO (Kramer et al. 2004) and Φf,D (Genty et al. 1996) are significantly different between 500-1500 PAR at 25 °C, and under lower light (250-500 PAR) at 10 °C, respectively. Presenting both values allows for greater flexibility in searching for correlations based on unique plant and treatment considerations.

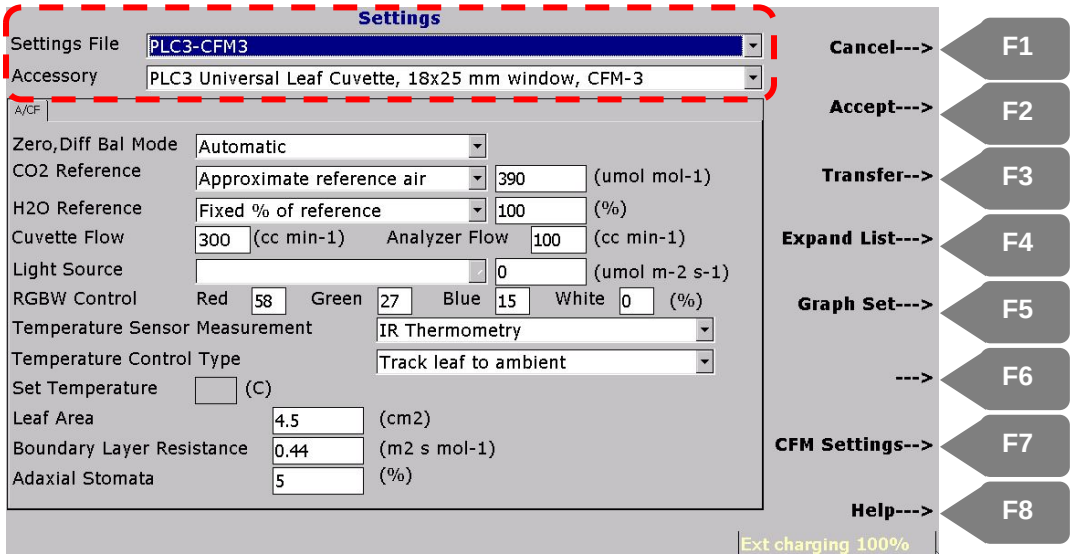
Also new is the use of calculated Fo' (Oxborough and Baker 1997) in the absence of Fo' measured immediately following the application of far-red light. Here, the justification is that precise determination of F_o' in far-red light may be questionable, due to inevitable relaxation of non-photochemical quenching, in addition to the desired reoxidation of PSII reaction centers (with concomitant reduction of PSI). As an alternative, Oxborough and Baker offer the calculation (versus measurement) of F_o'. Incorporated into CIRAS-3 software-level calculations, F_o', F_v', F_v'/F_m', qP, qNP, qL, ϕNO, ϕNPQ parameters will be to be returned with each repeated ϕPSII measurement, assuming the initial measurement was F_v/F_m. Without this, these calculations are dependent upon the far-red sequence, F_o' (measured), and they are returned for a single record only. In the absence of dark adaptation, several variables cannot be returned because they require F_o, F_m, or both. Those variables are F_v, F_v/F_m, qNP, NPQ, ϕNO, ϕNPQ (Kramer calculation), ϕf,D**,** ϕNPQ (Genty calculation).



Numeric View showing photosynthesis and chlorophyll fluorescence parameters.

CFM-3 Set Up

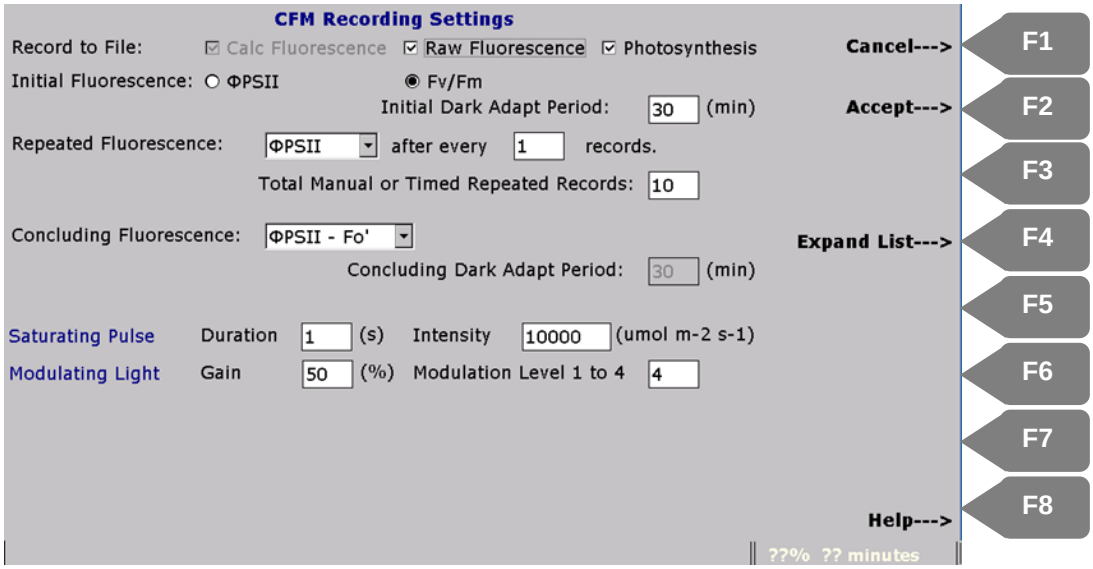
At either the Numeric View or Graph View screens select **Settings (F2).** You must first select the appropriate Settings File and Accessory as shown below:



Note. Make sure that you select the appropriate PLC3 Universal window (Under Accessory) used for chlorophyll fluorescence measurements.

CFM-3 Settings

With the above Settings dialog open, select **CFM Settings (F7)**. The following selections are available:



CFM-3 Settings	
Record to File	**Calc Fluorescence, Raw Fluorescence, Photosynthesis.** Depending on your selection of **Accessory** in **Settings**, some of these may be grayed out. **Calc Fluorescence** refers directly to the entire or partial list of parameters provided above. **Raw Fluorescence** refers to the instantaneous fluorescence counts that can be written to the data file, from 3 seconds before to 3 seconds after the fluorescence measurement. **Photosynthesis** refers to gas exchange data that can be included, as part of each fluorescence measurement. If you previously selected CFM-3, PLC3 18x25 mm window (or any combination of CFM-3 and PLC3 cuvette window), the *Calc Fluorescence* box will be checked and **Photosynthesis** will be checked (combined fluorescence/gas exchange enabled). **Calc Fluorescence** will be grayed out, indicating that fluorescence data must be written to the data file. If you previously selected CFM-3, Chlorophyll Fluorescence Module, the **Calc Fluorescence** box will be checked (enabled) and *Photosynthesis* will be unchecked (disabled). Both will be grayed out, indicating that only fluorescence data will be written to the data file.
Initial Fluorescence	**ϕPSII, Fv/Fm**. Press TAB to move down and arrow keys to select either option. ***ϕPSII*** indicates that you will not be performing the initial dark-adaptation of the leaf, instead, beginning measurements directly with saturating pulses applied to the light-adapted leaf sample. Select **Fv/Fm** to first dark adapt the leaf for an **Initial Dark Adapt Period** (min) of your choosing, up to 300 min.
Initial Fluorescence – **Repeated Fluorescence**	The dropdown list options are: **ϕPSII, ϕPSII-Fo', Fs**. Only one valid selection can be made, and the measurement recorded at recording intervals of your choosing. This will be influenced by your choice of Recording mode (Manual, Timed, or Response Curve). Enter the estimated **Total Manual** *or* **Timed Repeated Records** for the measurement session you will conduct. For example, if you will make measurements in Manual recording mode, and you know that you will apply 5 different light levels for which you would like to record chlorophyll fluorescence once with far-red light application immediately following each saturating pulse, select **ϕPSII-Fo' after every 1 records**, and **Total Manual** or **Timed Repeated Records**=5. If using a Response Curve to measure fluorescence, Repeated Fluorescence will be used in conjunction with Records per Level in the response curve. To make only a single Initial measurement (for example, to dark-adapt and perform Fv/Fm on multiple leaf samples) set **Total Manual** *or* **Timed Repeated Records**=0.
Concluding Fluorescence	This defines the final measurement and includes the options **None, ϕPSII-Fo',** and **Fv/Fm**. By selecting None your final measurement is defined by the last record in **Total Manual** or **Timed Repeated Records**. The selection of **Fv/Fm** will re-dark adapt the leaf for a **Concluding Dark Adapt Period** (min) of your choosing, up to 300 min., after the Repeated Fluorescence measurements.
Modulating Light	Set **Gain** and **Modulation Level** appropriate to your leaf sample and measurement (see below). Modulation Level determines the frequency of sample fluorescence counts.

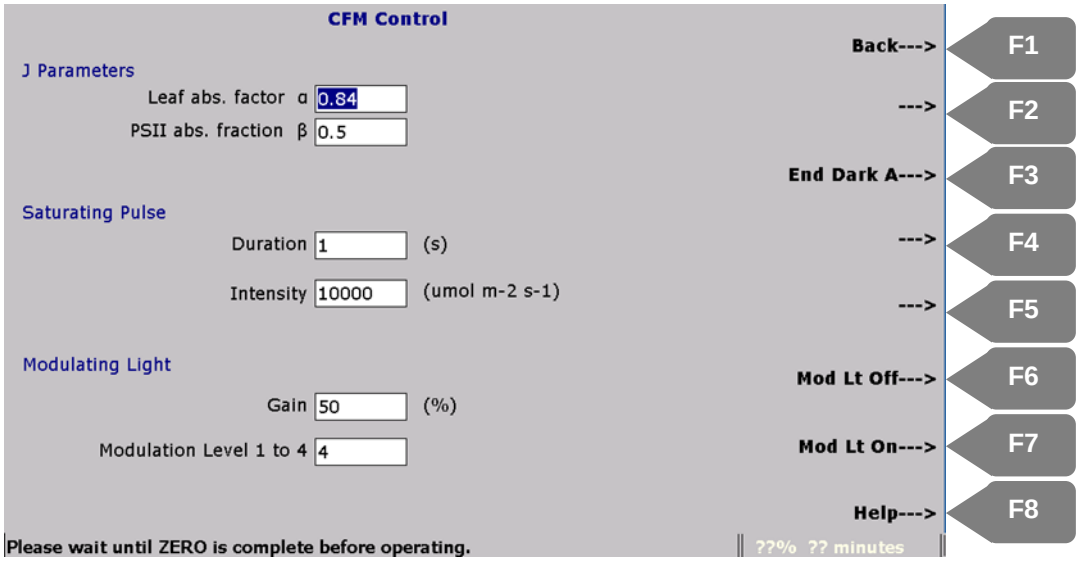
	Higher levels have better signal to noise, while lower levels have poor signal to noise, but have no actinic effect. For example, greater noise due to a lower modulating frequency can have a large effect on measuring the lowest two values used to arrive at an average of Fo' under far-red light. To determine the effective dark adaptation period you will need to identify the point at which F_v/F_m does not increase with an associated increase of the dark adaptation period. In the example below, the leaf sample is sufficiently dark-adapted after 20 minutes, since longer dark adaptation periods did not result in higher F_v/F_m values. Note that prior light exposure (intensity, duration) significantly affects the minimum effective dark adaptation period required to fully re-oxidize (open) PSII photochemistry in the leaf sample.

DA period (minutes)	**5**	**10**	**15**	**20**	**25**	**30**
F_v/F_m	0.63	0.71	0.78	0.81	0.80	0.81

Press **Accept (F2)** to accept changes and return to the main Settings dialog.

CFM-3 Controls

There will typically be very little need to change Settings during the active session while measuring chlorophyll fluorescence. However, some active controls and functions are available for sample calibration and to make slight adjustments prior to or during measurements. At either the Numeric View or Graph View screens select **Controls (F3)** > **CFM Control (F7)**. The following selections are available:



CFM-3 Control	
J Parameters	Factors used for calculation of electron transport rate. *Leaf abs. factor α*, set to default 0.84, but can be determined by use of an integrating sphere. *PSII abs. factor β*, set to default 0.5, but known range of values exist for different plant types.
Saturating Pulse	Set the **Duration** (s) and **Intensity** (up to 10000 µmol m^{-2} s^{-1}) of the saturating pulse.
Modulating Light	Set **Gain** and **Modulation Level** appropriate to your leaf sample and measurement.

The dark adaptation period can be manually terminated before the elapsed time in Settings is reached by pressing **End Dark A (F3)**. This will cause the modulating lights to switch on for a fixed period of 1.5 seconds, followed by the saturating pulse pre-defined in CFM Settings or CFM Control. **Mod Lt Off (F6)** and **Mod Lt On (F7)** toggle the modulating lights off or on. After making changes press **Back (F1)** to return to the Controls dialog and **Accept (F2)** to finalize the modifications.

Simple Chlorophyll Fluorescence Measurements

A common sequence involving a chlorophyll fluorescence measurement beginning with a Fv/Fm after 30 minute dark adaptation followed by ϕPSII-Fo'. Note that measurements can be performed in either "Manual Recording" or "Timed Recording".

CFM-3 Settings:

Initial Fluorescence:	Fv/Fm, 30 minute dark adaptation
Continuing Fluorescence:	ϕPSII-Fo'; After every "1" record; Total records= 5
Concluding Fluorescence:	ϕPSII-Fo'

Manual Recording

Record (1):	Dark adaptation begins, Fv/Fm follows
Record (2):	ϕPSII-Fo'
Record (3):	ϕPSII-Fo'
Record (4):	ϕPSII-Fo'
Record (5):	ϕPSII-Fo'
Record (6):	ϕPSII-Fo'
Record (7):	Concluding ϕPSII-Fo'
Record (8):	Dark adaptation begins and the process above is repeated.

Timed Recording: 5 minute interval

Step 1:	Dark adaptation begins immediately (no key press is required) and Fv/Fm follows.
Step 2:	Following Fv/Fm, there will be a 5 minute pause before the first ϕPSII-Fo' measurement is performed. This will repeat 5 times.
Step 3:	Following another 5 minute pause, the concluding ϕPSII-Fo' measurement is performed. This will conclude the measurement sequence.
Step 4:	5 minutes after the measurement sequence has concluded, the sequence will start again from the beginning with a 30 minute dark adaptation period.

A common sequence involving a chlorophyll fluorescence measurement beginning with a Fv/Fm after 30 minute dark adaptation followed by Fs:

CFM Settings:

Initial Fluorescence:	Fv/Fm, 30 minute dark adaptation
Continuing Fluorescence:	Fs; After every "2" record; Total records= 3
Concluding Fluorescence:	None

Manual Recording

Record (1):	Dark adaptation begins, Fv/Fm follows
Record (2):	Nothing performed
Record (3):	Fs measurement performed
Record (4):	Nothing performed
Record (5):	Fs measurement performed
Record (6):	Dark adaptation begins, Fv/Fm follows

Timed Recording: 30 second interval

Step 1:	Dark adaptation begins immediately (no key press required) followed by a Fv/Fm.
Step 2:	Following Fv/Fm, there will a single 30 second pause after which no record will be performed. A second 30 second pause will follow, followed by a Fs measurement. This will repeat 2 more times. As there is no ending fluorescence measurement, this will conclude the current sequence of measurements.
Step 3:	After another 30 second pause, the measurement sequence will start over again as described above.

Section 11. Closed System Measurements

Soil CO_2 Efflux and Net Canopy CO_2 Flux

The **SRC-1 Soil Respiration Chamber** can be used with the CIRAS-3 Portable CO_2/H_2O Gas Analysis System for closed system measurement of soil CO_2 efflux. The **CPY-4 Canopy Assimilation Chamber** can also be used with the CIRAS-3 for closed system measurement of net canopy CO_2 flux.

Theory

The assimilation is measured by placing a closed chamber on the soil and measuring the rate of increase of the CO_2 concentration inside the chamber. Then, assuming a well-mixed and sealed system :-

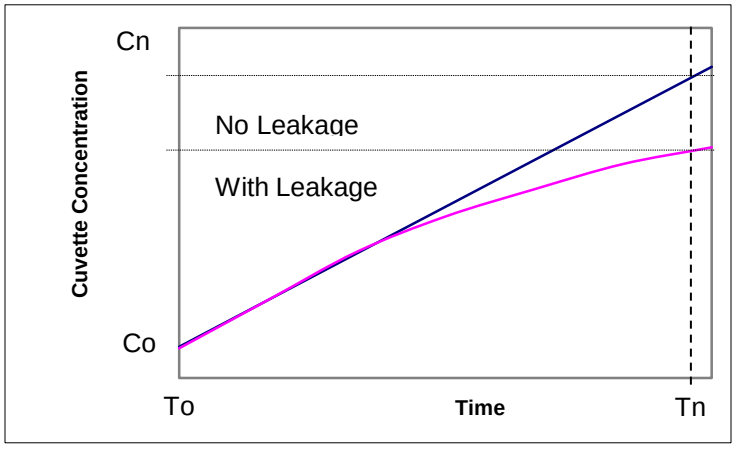
$$R = \frac{(Cn - Co)}{Tn} * \frac{V}{A} \qquad (1)$$

Where R is the assimilation rate (Flux of CO_2 /unit area/unit time), Co is the CO_2 concentration at T=0 and Cn is the concentration at a time Tn later, A is the area of soil exposed and V the total system volume.

It has been suggested that to make accurate measurements of the assimilation it is essential to start with a CO_2 concentration in the cuvette below (respiration)/above(photosynthesis) ambient and measure until the concentration is above/below ambient, presumably with the intention of getting some compensation for leakage. However, this leakage can only take place at ground level, where the CO_2 concentration is unknown and most certainly will not be what we would consider as ambient.

Over the short period of measurement and with the relatively small CO_2 concentrations in the chamber compared with the soil concentrations, we would expect the assimilation to be a constant flux, giving a constant rate of change in the cuvette CO_2 concentration.

Any leakage should be a function of the concentration difference between the cuvette and the exchange air. Due to leakage, the apparent assimilation rate decreases with time.



Simulated Soil Respiration Measurements

With the CIRAS-3 software a quadratic relationship ($y = a + bx + cx^2$) is fitted between the cuvette concentration (C=y) and time (T=x) from the start of measurement. Note that there is a delay after the

chamber is first placed on the soil to allow for the establishment of stable gradients etc, before the measurements begin.

Then $C = a + bT + cT^2$

The true assimilation rate will be calculated from dC/dT at T=0.

Now dC/dT = b + 2cT

so at T=0 dC/dT = b (2)

A comparison of b and cT gives an indication of the leakage. Within the software, cT must be less than 20% of b or the "Non-linear" error message is given. This is believed to be a better approach than lowering the CO_2 value at the start of the measurement.

Measurement Units

From 1 and 2:-

R = b x V/A

To give the flux in mass/unit area/unit time then b must be measured in mass/unit volume.

The CO_2 analyzer measures the volume/volume ratio (= ppm by volume = micro-litre/litre = micro-bar/bar = micro-mol/mol).

Now one kg mol of gas (44.01kg of CO_2) at STP occupies 22.41 m^3.

Thus:

$$R = b * \frac{P}{1000} * \frac{273}{273 + Ta} * \frac{44.01}{22.41} * \frac{V}{A} \qquad (3)$$

Where:

R is the CO_2 flux in $kg.m^{-2}.s^{-1}$

V is the system volume (largely the chamber volume) in m^3

A is the soil area exposed in the chamber in m^2, P is the atmospheric pressure in mb

Tc is the temperature of the system volume (chamber) in ºC.

We measure: -

DC/DT = b as ppm/second

V in cm^3

A in cm^2

R is expressed as $g.m^{-2}.hr^{-1}$.

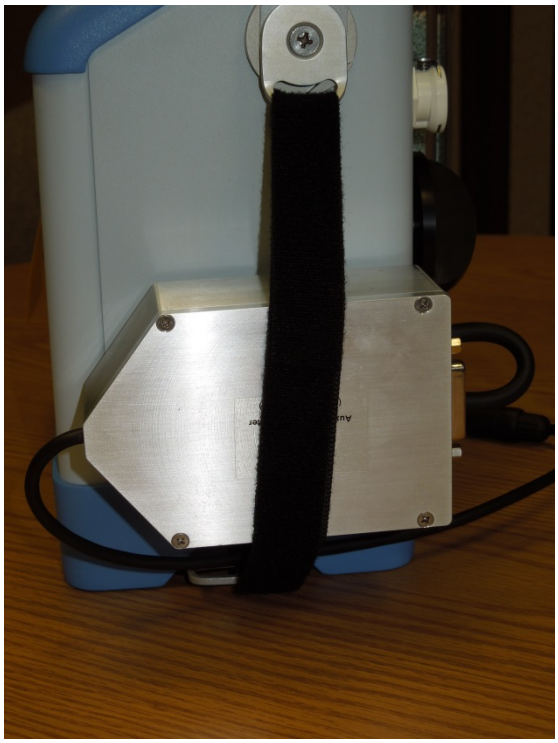
To convert ($g.m^{-2}.hr^{-1}$) to ($micro\text{-}mol.m^{-2}.s^{-1}$) multiply by 6.312

References

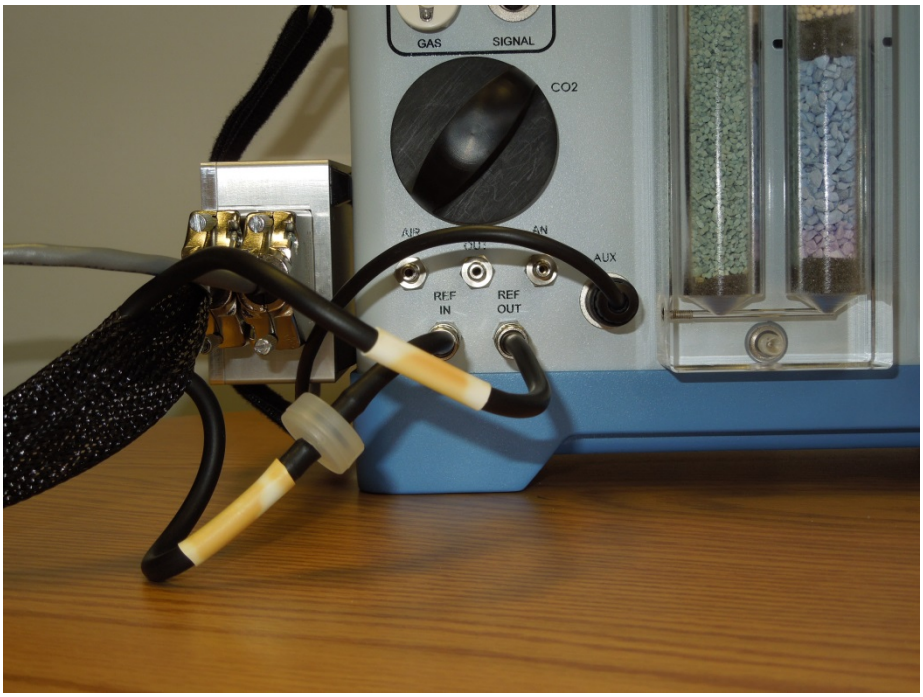
Parkinson K.J. (1981). An improved method for measuring soil respiration in the field. Journal of Applied Ecology, 18, 221-228.

Connection Between the CIRAS-3 and SRC-1 / CPY-4

In order to use the SRC-1 and CPY-4 with the CIRAS-3, an Auxiliary Probe Adapter (APA) is required. The APA connects electrically to the CIRAS-3 via the 4 pin socket labeled “AUX” in the lower left hand corner of the CIRAS-3 console. The SRC-1 and CPY-4 (and STP-1 Soil Temperature Probe if used) connect to either of the 15 pin sockets on the other end of the APA. The APA contains electronics that allow the closed system accessories to interface with the CIRAS-3 main console. We recommend mounting the APA to the side of the CIRAS-3 console as shown below using the Velcro supplied.

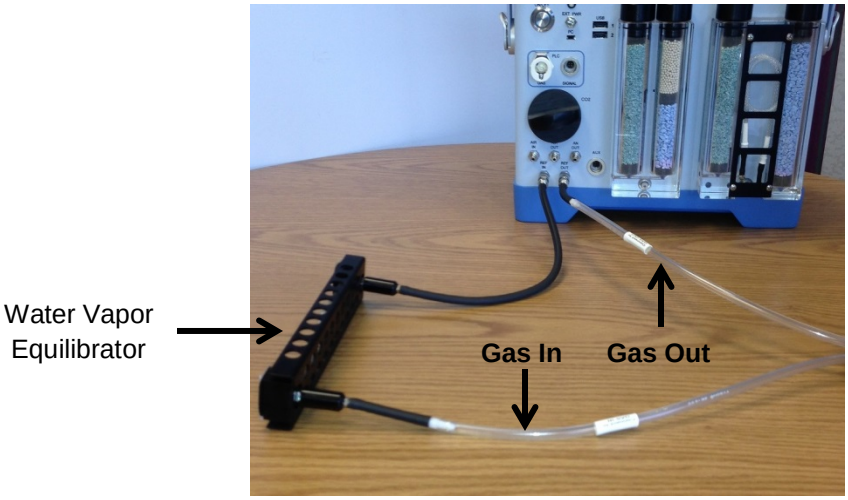


All electrical and pneumatic connections are highlighted below:



1. Connect the 4 pin plug on the APA to the “Aux” socket on the CIRAS-3 console. Line the arrow up on the black APA connector with the top middle of the “Aux” connector.
2. Connect the SRC-1 and CPY-4 to one of the 15 pin sockets on the APA and connect the STP-1 Soil Temperature Probe to the other 15 pin socket on the APA (if used).
3. Connect the “Gas In” and “Gas Out” tubing on the SRC-1 and CPY-4 tubing to the “REF IN” and “REF OUT” ports on the CIRAS-3 console.

Note. The CPY-4 is supplied with a water vapor equilibrator which we recommend is used for high humidity conditions. The water vapor equilibrator should be plumbed as shown below:



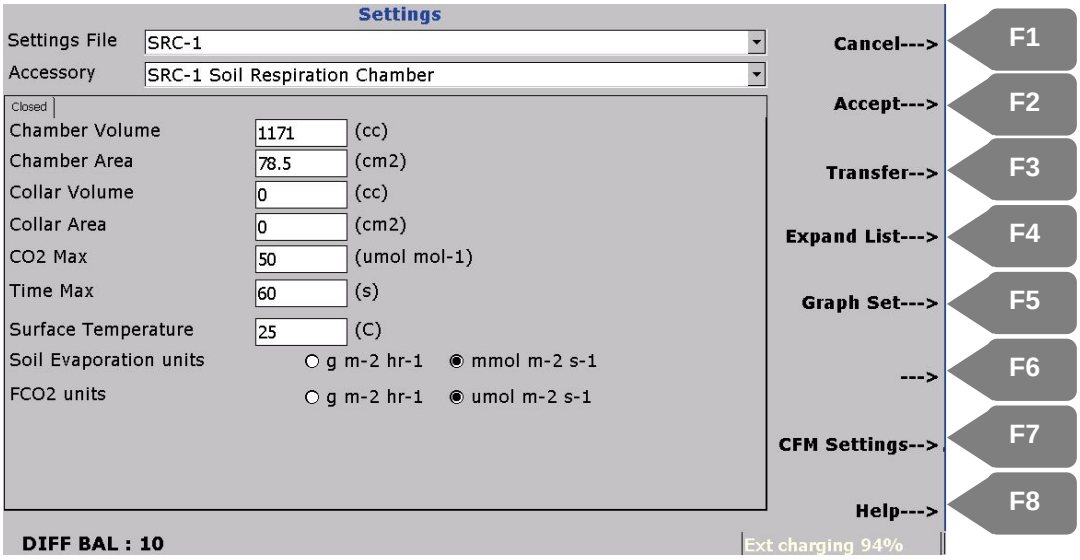
Configuring the CIRAS-3 console for measurement of Soil Respiration

As discussed earlier in this manual and from the main screen, select Settings (F2) to configure the system for closed system operation using the SRC-1 Soil Respiration Chamber.

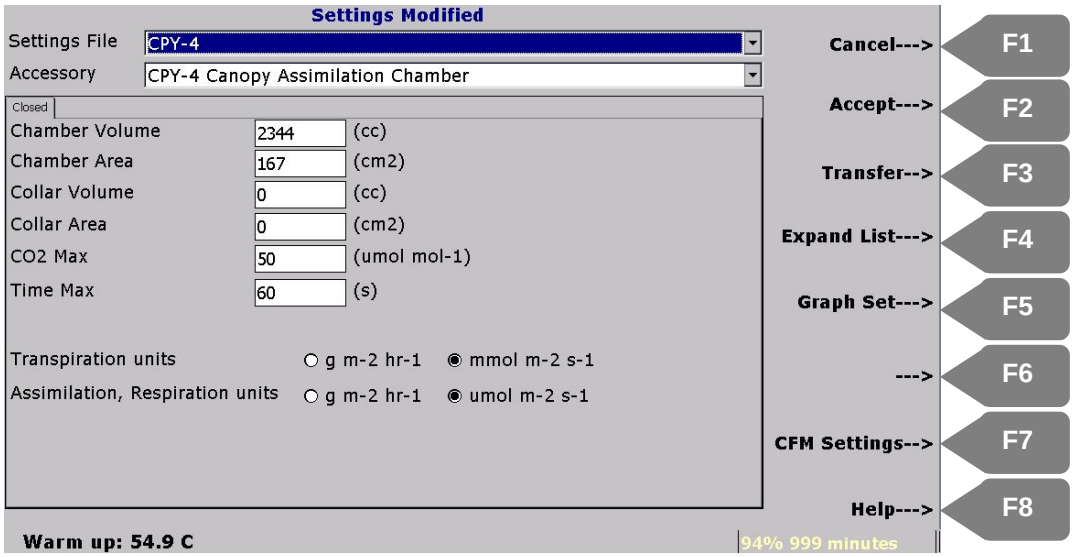
Settings (F2)

Settings are essentially defined as operational environments that are recreated each time that CIRAS-3 is powered on. The options that you choose here are normally your typical working options which in this case is measurement of closed system measurement of soil respiration or canopy assimilation. Press TAB to move down to **Accessory** and select SRC-1 if using the SRC-1 Soil Respiration Chamber or CPY-4 if using the CPY-4 Canopy Assimilation Chamber.

SRC-1 Soil Respiration System Settings



CPY-4 Canopy Assimilation Chamber Settings



The **Accessory** field will populate automatically along with the following system defaults:

Settings	SRC-1	CPY-4
Chamber Volume	1171 cc	2344 cc
Chamber Area	78.5 cm^2	167 cm^2
Collar Volume	0 cc	0 cc
Collar Area	0 cm^2	0 cm^2
CO2 Max	50 µmol mol^{-1}	50 µmol mol^{-1}
Time Max	60 s	60 s
Surface Temperature	25 ^oC	Not applicable
Esoil units	g m^{-2} hr^{-1} mmol m^{-2} s^{-1} (Default)	Not applicable
FCO2 units	g m^{-2} hr^{-1} µmol m^{-2} s^{-1} (Default)	Not applicable
Transpiration units	g m^{-2} hr^{-1} mmol m^{-2} s^{-1} (Default)	g m^{-2} hr^{-1} mmol m^{-2} s^{-1} (Default)
Assimilation, Respiration units	g m^{-2} hr^{-1} µmol m^{-2} s^{-1} (Default)	g m^{-2} hr^{-1} µmol m^{-2} s^{-1} (Default)

Important note. If using collars with the SRC-1 or CPY-4 you must make sure that you update the Collar Volume and Collar area fields for accurate results. If not using collars, the above system defaults should be used for all calculations and should not need updating.

Also, it is important to take note of the measurement units that you have selected for Soil Evaproration, FCO2, Transpiration and Assimilation, Respiration for later data analysis.

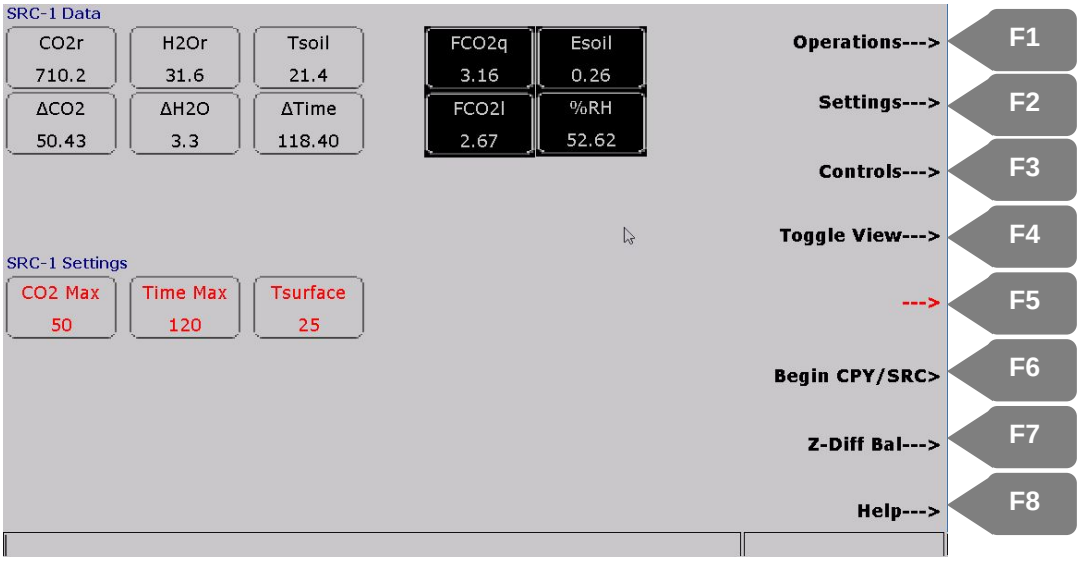
FCO_2 Units for measurement of Soil CO_2 Efflux

The CIRAS-3 system automatically collects data based on both a linear or quadratic fit for the assimilation data. In the past only quadratic fitting has been used but at the request of some customers we have now also introduced a linear fitting. As discussed in the theory section, on theoretical grounds, the relationship is likely to deviate from a linear response, especially at low assimilation rates. Generally speaking, if your rates are low we recommend using the linear fit. For most measurements, the quadratic fit is normally recommended.

Closed System Operation

Many of the features related to closed system measurement screens (numerical and graphical presentation of data, graph set, recording measurements, etc.) are similar to what has already been discussed in this operation manual. The displays are also similar when using the SRC-1 Soil Respiration Chamber or CPY-4 Canopy Assimilation Chamber.

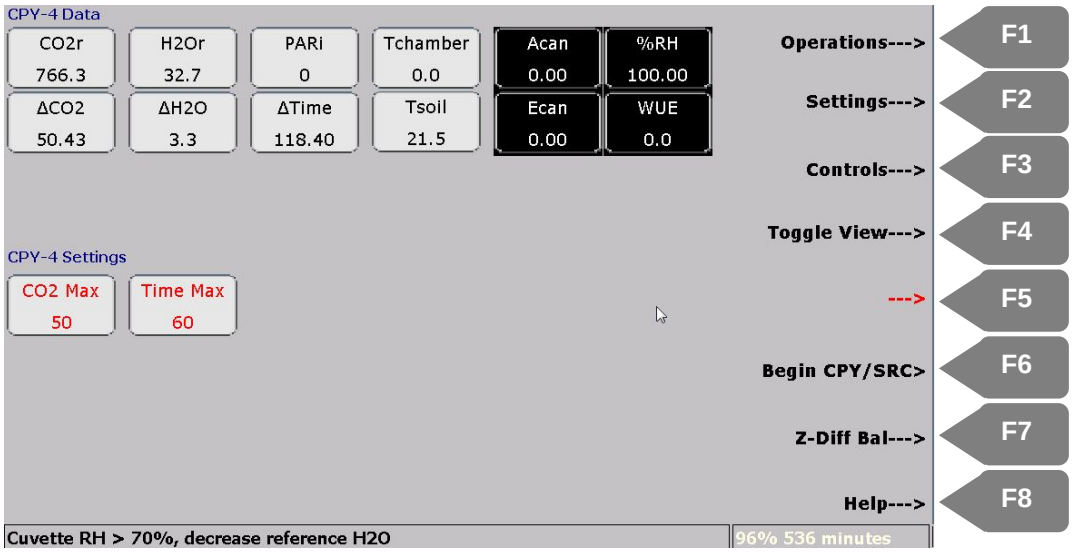
Numerical Data Display (SRC-1)



Measured Data	
CO2r	Current CO_2 concentration (µmol mol^{-1}).
ΔCO2	Change in CO_2 concentration during measurement sequence (µmol mol^{-1}).
H2Or	Current H_2O concentration (mb)
ΔH2O	Change in H_2O concentration during measurement sequence (mb).
Tsoil	Soil temperature if STP-1 is used (^oC).
ΔTime	Change in time during measurement sequence (Seconds).
Calculated Data	
FCO2q	Efflux rate of soil CO_2 based on quadratic fit (g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}).
FCO2l	Efflux rate of soil CO_2 based on linear fit (g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}).
Esoil	Rate of change in evaporation (g m^{-2} hr^{-1} or mmol m^{-2} s^{-1}).
RH%	Relative humidity based on Tsurface (%).

SRC-1 Control Settings	
CO2 Max	A user defined value representing the maximum CO_2 concentration (µmol mol^{-1}). If the CO_2 concentration reaches this level, the measurement sequence will end.
Time Max	A user defined value representing the maximum time (seconds). If the time reaches this interval, the measurement sequence will end.
Tsurface	Approximate air temperature at the soil surface (^oC). The default value is 25 but this should be set to your actual surface temperature for accurate results.

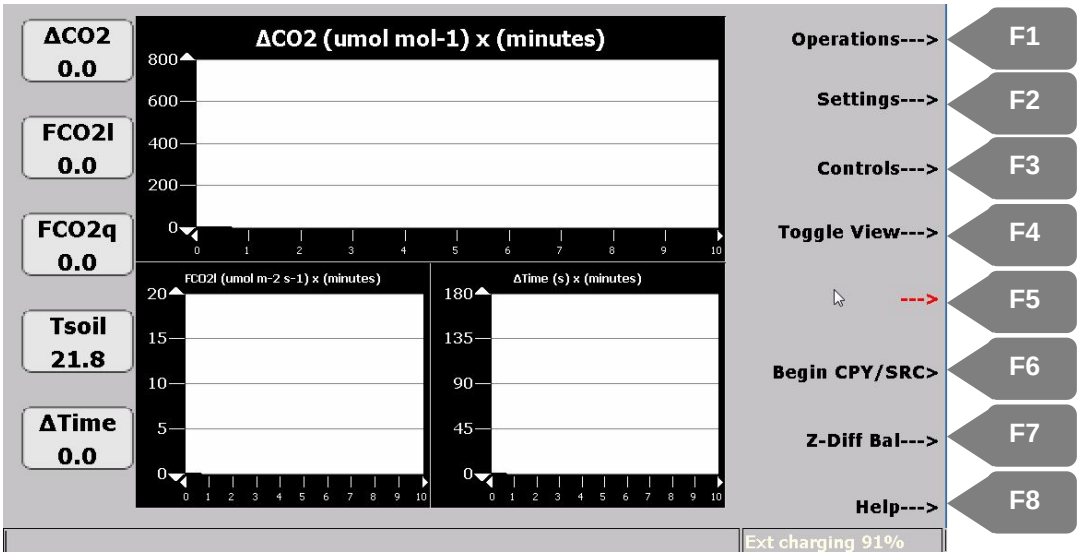
Numerical Data Display (CPY-4)



Measured Data	
CO2r	Current CO_2 concentration (µmol mol^{-1}).
ΔCO2	Change in CO_2 concentration during measurement sequence (µmol mol^{-1}).
H2Or	Current H_2O concentration (mb)
ΔH2O	Change in H_2O concentration during measurement sequence (mb).
PARi	Measurement of Photosynthetically Active Radiation (PAR) inside the CPY-4 (µmol m^{-2} s^{-1}).
ΔTime	Change in time during measurement sequence (Seconds).
Tchamber	Air temperature inside the CPY-4 (^oC).
Tsoil	Soil temperature if STP-1 is used (^oC).

Calculated Data	
Acan	Canopy Assimilation rate (g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}).
Ecan	Evaporation rate (g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}).
RH%	Relative humidity based on Tsurface (%).
WUE	Water use efficiency (Assimilation/Transpiration). **Note that WUE can only be calculated if the Transpiration Units are set to mmol m^{-2} s^{-1} and Assimilation, Respiration units set to µmol m^{-2} s^{-1} under Settings.**
CPY-4 Control Settings	
CO2 Max	A user defined value representing the maximum CO_2 concentration (µmol mol^{-1}). If the CO_2 concentration reaches this level, the measurement sequence will end.
Time Max	A user defined value representing the maximum time (seconds). If the time reaches this interval, the measurement sequence will end.

Graphical Data Display(SRC-1 and CPY-4)



The information that is included on the graphical display is dependent on user settings under Graph Set (Settings>Graph Set).

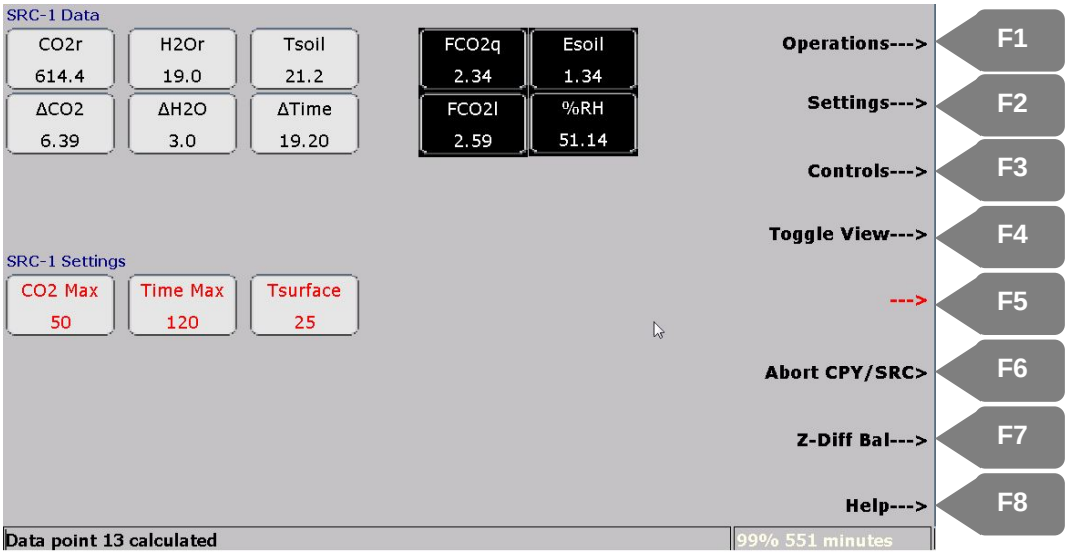
Measurement Sequence

Once you have selected the appropriate accessory (SRC-1 and CPY-4) including defaults and/or other user settings, the typical measurement sequence is as follows:

1. Begin CPY/SRC
2. Hold Chamber in Air to Flush – Data collection begins in x Sec
3. Position Chamber on Soil
4. Chamber equilibrating
5. Data Collection Started
6. Sequence complete.

Initial Test Measurement Sequence

A test measurement sequence can be performed initially to establish a base line prior to recording data if required. This can be very helpful in determining the **CO2 Max** and **Time Max** expected for your soil or vegetation being measured. Simply press **Begin CPY/SRC** and follow the prompts displayed in the status bar in the lower left hand corner of the display. During data collection, "Data point xx calculated" will be displayed until the end of measurement followed by a "Sequence complete" message. The following will be displayed until the end of measurement which is determined by user settings of **CO2 Max** or **Time Max**. The sequence can be aborted at any time by hitting **Abort CPY/SRC** (F6). Note, data is not recorded during this sequence.



Recording Measurements

Press **Operations (F1)**, **Rec Options (F2)** to set up a recording session. Note, the only recording option available for closed system measurements is Manual Recording. Refer to the **Manual Recording** section in this manual on page 44 for more information.

Data can be stored in internal memory or external memory (USB thumb drive) as described earlier in this manual. When you are happy with the named data file, press **Start** (F2) to begin the measurement sequence and then **Back** (F1) to the Numerical or graphical screen. Press **Begin CPY/SRC** (F6) to begin measurement sequence.

In the lower left hand corner of the display (status bar), you will see the following:

M Record xx saved to file: C30008_20130611_01.xml

Data is output every 1.6 seconds during the measurement sequence and saved to the file that is displayed. At the end of a measurement sequence during manual recording, a message “Sequence complete. Press F6 to start a new sequence.” Appears in the status bar. Additional measurements can be recorded by pressing **Begin CPY/SRC** F6 and to end recording, press **End Recording** (F5).

Section 12. Data Output

Stored CIRAS-3 data can be output in 3 different formats depending on the accessory or accessories used.

Photosynthesis Data

When one of our leaf chambers (Universal, Conifer, Narrow) is used with the CIRAS-3 for measurement of photosynthesis only, the stored data is output as follows:

	Date/Time	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	mb	mb	mb	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}
RecType	ExcelTime	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od	PARi	PARe
R	41442.63867	385.4	377.7	-7.7	22.8	23.88	1.08	1200	6
R	41442.63909	385.3	376.5	-8.8	22.5	23.82	1.32	1200	7
R	41442.63929	385.1	376.2	-8.9	22.5	23.88	1.38	1201	7

%	%	%	%	°C	°C	°C	cm^2	cc min^{-1}	mb	%
Red	Green	Blue	White	Tamb	Tcuv	Tleaf	Aleaf	Flow	Patm	RH
38	37	25	0	29.2	24.8	27.8	4.5	199	1004	76.29
38	37	25	0	29.2	24.7	27.7	4.5	199	1004	76.55
38	37	25	0	29.2	24.7	27.7	4.5	199	1004	76.74

µmol mol^{-1}	mmol m^{-2} s^{-1}	kPa	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol CO2 mol^{-1} H2O	
Ci	gs	VPD	A	E	WUE	Status
229	27	1.35	2.4	0.36	6.67	
240	34	1.33	2.7	0.44	6.14	
246	36	1.33	2.7	0.46	5.87	

Photosynthesis and Chlorophyll Fluorescence Data

When one of our leaf chambers (Universal, Conifer, Narrow) is used with the CIRAS-3 for measurement of photosynthesis and/or chlorophyll fluorescence, the stored data is output as follows:

	Date/Time	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	mb	mb	mb	µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}
RecType	ExcelTime	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od	PARi	PARe
R	41442.63867	385.4	377.7	-7.7	22.8	23.88	1.08	1200	6
R	41442.63909	385.3	376.5	-8.8	22.5	23.82	1.32	1200	7
R	41442.63929	385.1	376.2	-8.9	22.5	23.88	1.38	1201	7

%	%	%	%	°C	°C	°C	cm^2	cc min^{-1}	mb	%
Red	Green	Blue	White	Tamb	Tcuv	Tleaf	Aleaf	Flow	Patm	RH
38	37	25	0	29.2	24.8	27.8	4.5	199	1004	76.29
38	37	25	0	29.2	24.7	27.7	4.5	199	1004	76.55
38	37	25	0	29.2	24.7	27.7	4.5	199	1004	76.74

µmol mol^{-1}	mmol m^{-2} s^{-1}	kPa	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol CO2 mol^{-1} H2O	
Ci	gs	VPD	A	E	WUE	Status
229	27	1.35	2.4	0.36	6.67	
240	34	1.33	2.7	0.44	6.14	
246	36	1.33	2.7	0.46	5.87	

Fo	Fm	Fv	FvFm	FmP	Fs	phiPSII	FoP	FvP	FvPFmP
0	0	0	0	0	7.25	0	0	0	0
0	0	0	0	0	7	0	0	0	0
0	0	0	0	0	7	0	0	0	0

J	qP	qNP	NPQ	qL	phiNO	phiNPQK	phifD	phiNPQG
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

Closed System Operation Data

When the SRC-1 Soil Respiration Chamber and CPY-4 Canopy Assimilation Chamber are used with the CIRAS-3, the stored data is output as follows (23 columns):

	Date/Time	Seconds	µmol mol^{-1}	µmol mol^{-1}	mb	mb	°C	°C
RecType	ExcelTime	deltaTime	CO2r	deltaCO2	H2Or	deltaH2O	Tamb	Tch
M	41442.60043	0	597.4	0	20.8	0	0	0
M	41442.60044	1.6	597.5	0	20.7	0.2	0	0
M	41442.60046	3.2	597.8	0	20.7	0.2	0	0

°C	µmol m^{-2} s^{-1}	mb	%	g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}		g m^{-2} hr^{-1} or mmol m^{-2} s^{-1}	g m^{-2} hr^{-1} or µmol m^{-2} s^{-1}	
Tsoil	PARi	Patm	RH	FCO2l	FCO2q	Esoil	Acan	Ecan
23.5	0	1002	47.89	0	0	0	0	0
23.5	0	1001	47.92	-0.04	-0.04	0	0	0
23.5	0	1002	48.14	0.1	-0.02	0	0	0

mmol CO2 mol^{-1} H2O	µmol mol^{-1}	Seconds	°C	
WUE	CO2Max	TimeMax	Tsurface	Status
0	50	120	25	
0	50	120	25	
0	50	120	25	

Summary of the Gas Exchange Equations Used

Mass Flow Equation

*Calculate the mass flow of air (**W**) entering the cuvette per unit leaf area*

(1.1) The CIRAS-3 mass flowmeter is calibrated to read the volume flow at 20 °C and 1013.25 mb (V_{20}). The Ideal Gas molar volume is 22.414 L mol^{-1} at 0 °C and 1 standard atmosphere (STP). Therefore:

$$\boldsymbol{W(mol\ m^{-2}s^{-1}) = \left(\frac{V_{20}}{60 \times 10^3}\right) \times \left(\frac{1}{22.414}\right) \times \left(\frac{273.15}{293.15}\right) \times \left(\frac{1000}{1013.25}\right) \times \left(\frac{10^4}{a}\right)}$$

Where: ***a*** is projected leaf area (cm^2 converted to m^2). V_{20} is converted above to cc sec^{-1}, but displayed by CIRAS-3 as cc min^{-1}.

Transpiration

*Calculate transpiration rate (**E**) from the partial pressures of water vapor of the air entering (e_{in}) and exiting (e_{out}) the cuvette*

(2.1) The molar flow of *water vapor* (mol m^2 s^{-1}) into the cuvette is:

$$\boldsymbol{W \times \left(\frac{e_{in}}{P}\right)}$$

(2.2) The molar flow of *air* out of the cuvette (due to the addition of transpired water vapor) is (**W+E**). Therefore, the molar flow of *water vapor* out of the cuvette is:

$$\boldsymbol{(W + E) \times \left(\frac{e_{out}}{P}\right)}$$

(2.3) However, the difference between the molar flows into and out of the cuvette must equal the transpiration, so:

$$\boldsymbol{E = \left[(W + E) \times \left(\frac{e_{out}}{P}\right)\right] - \left[W \times \left(\frac{e_{in}}{P}\right)\right]}$$

(2.4) Therefore:

$$\boldsymbol{E(mmol\ m^{-2}\ s^{-1}) = \left[\frac{W \times (e_{out} - e_{in})}{(P - e_{out})}\right] \times 10^3}$$

e_{in} is defined as the partial pressure of water vapor of reference air supplied to the cuvette, but not yet inside the cuvette, and therefore uninfluenced by the cuvette stirring fans or the leaf itself. e_{in} partial pressure is determined by the Reference H_2O IRGA.

e_{out} is defined as the partial pressure of water vapor in the air inside the cuvette, surrounding the leaf. This air is both highly mixed by the stirring fans and influenced by transpiration water vapor

from the leaf. e_{out} partial pressure is determined by the Analysis H_2O IRGA. As relates to the calculated values in the CIRAS-3 display:

$$\boldsymbol{e_{out} = (H2O_r + H2O_d) = H2O_a}$$

Leaf Temperature

Calculate leaf temperature ($\boldsymbol{T_{leaf}}$) from the energy balance (optional to IR sensor method)

(3.1) From *Reference 1* the difference between air and leaf temperature is:

$$\Delta \boldsymbol{t} = \left[\frac{\boldsymbol{H - \lambda \times E}}{\left(\frac{\boldsymbol{0.93 \times M_a \times C_p}}{\boldsymbol{r_b}}\right) + [\boldsymbol{4\sigma \times ((T_c + 273)^3)}]} \right]$$

where:

H = incident radiation absorbed by the leaf

λ = latent heat of vaporization of water

E = transpiration rate

M_a = molecular weight of air

C_p = specific heat at constant pressure

r_b = boundary layer resistance to water vapor transfer, empirically determined for each cuvette by the pseudo-leaf (filter paper) method. 0.93 converts it to that for heat transfer.

σ = Stefan Boltzmann constant

T_c = cuvette air temperature

H is calculated from the photon flux incident on the cuvette (*I*), taking into account the ratio of infra-red to PAR in the light source, transmission through the cuvette window (*Trans*), and reflection/absorption by the leaf: (H = *I* x *Trans*).

The following approximation is made:

$$\boldsymbol{4\sigma \times \left((T_c + 273)^3\right) \cong \left(4.639 + (0.5834 \times T_c)\right)}$$

(3.2) From this we derive:

$$\boldsymbol{T_{leaf} = (T_c + \Delta t)}$$

Saturation Vapor Pressure

*Derive saturation vapor pressure at leaf temperature (**e_{leaf}**) from **T_{leaf}***

(4.1) From *Reference 2 we calculate*:

$$e_{leaf} = 6.1121 * exp\left[\frac{T_{leaf} \times \left(18.564 - \left(\frac{T_{leaf}}{254.4}\right)\right)}{T_{leaf} + 255.57}\right]$$

Stomatal Conductance

*Calculate stomatal conductance (**g_s**)*

(4.2) Stomatal resistance (to water vapor) is calculated as:

$$r_s = \left[\left[\frac{(e_{leaf} - e_{out})}{\frac{(e_{out} - e_{in}) \times (P - e_{out})}{P}}\right] \div W\right] - r_b$$

(4.3) An alternative expression of equation **(2.4)** is:

$$E = \left[\frac{(e_{leaf} - e_{out})}{P \times (r_s + r_b)}\right]$$

(4.4) Because,

$$\left[\frac{(P - e_{out})}{W \times (e_{out} - e_{in})}\right] = \frac{1}{E}$$

(the inverse of equation 2.4)

(4.5) stomatal resistance can be calculated by:

$$r_s(m^2\ s\ mol^{-1}) = \left[\frac{(e_{leaf} - e_{out})}{(E \times P)}\right] - r_b$$

(4.6) Stomatal conductance is the inverse of stomatal resistance:

$$g_s(mmol\ m^{-2}\ s^{-1}) = \frac{1}{r_s} \times 10^3$$

Net Photosynthesis

*Determine the rate of net photosynthesis (**A**) from the difference between CO_2 concentrations entering (**C_{in}**) and exiting (**C_{out}**) the cuvette*

(5.1) IRGA CO_2 readings are corrected for water vapor, temperature, and atmospheric pressure. The addition of transpirational water vapor dilutes the air leaving the cuvette (C_{out}), and this is compensated for in the calculation:

$$\boldsymbol{A = (C_{in} \times W) - [C_{out} \times (W + E)]}$$

(5.2) To calculate net CO2 assimilation we rearrange equation (**5.1**) to:

$$\boldsymbol{A = -\left[\left((C_{out} - C_{in}) \times W\right) + (C_{out} \times E)\right]}$$

CIRAS-3 calculates and displays the CO_2 difference (C_{out} - C_{in}). As relates to the calculated values in the CIRAS-3 display:

$$\boldsymbol{C_{out} = (CO2_r + CO2_d) = CO2_a}$$

Intercellular CO_2 Concentration

*Calculate CO_2 concentration in the sub-stomatal cavity (**C_i**) using the equation derived by*

von Caemmerer & Farquhar (Reference 3)

(6.1) The sub-stomatal CO2 concentration, C_i, is given by:

$$\boldsymbol{C_i\left(\mu mol\ mol^{-1}\right) = \frac{\left[\left(g_c - \frac{E}{2}\right) \times C_{out}\right] - A}{\left(g_c + \frac{E}{2}\right)}}$$

(6.2) Where g_c is the total conductance to CO_2 transfer:

$$\boldsymbol{g_c\ \left(mmol\ m^{-2}\ s^{-1}\right) = \left[\frac{1}{(1.585 \times r_s) + (1.37 \times r_b)}\right] \times 10^3}$$

(1.585 is the diffusion ratio of CO_2 and water in *air*, and 1.37 is the diffusion ratio of CO_2 and water in the *boundary layer*).

Note: These calculations are based on the following assumptions:

the leaf is exposed on both upper and lower leaf surfaces

the upper and lower boundary layer resistances are similar

stomata are evenly distributed on both upper and lower leaf surfaces.

Physical Constants Used in Equations

Volume of one kg mole of gas =0.0224 m^3, at 1013.25 millibar barometric pressure and 273.15 °K

Latent heat of vaporization of water (λ) = 45064.3 - (T_c x 42.9)

Molecular weight of air (M_a) = 28.97

Specific heat at constant pressure (C_p) = 1.012 kJ kg^{-1} K^{-1}

Stefan Boltzmann constant (σ) = 5.6704 x 10^{-8} W m^{-2} K^{-4}

Definition of Symbols

Measured Parameters

Symbol	Parameter	Unit
V_{20}	Flow rate of dry air into cuvette at STP	cc sec^{-1}
A	Projected leaf area	cm^2
r_b	Boundary layer resistance to water vapor	m^2 s mol^{-1}
P	Atmospheric pressure	mb
Q	Photon flux density incident on cuvette	µmol m^{-2} s^{-1}
T_c	Cuvette air temperature	°C

Calculated Parameters

Symbol	Parameter	Unit
W	Mass flow of dry air per unit leaf area	mol m^{-2} s^{-1}
e_{in}	Partial pressure of water vapor of air *entering* cuvette	mb *
e_{out}	Partial pressure of water vapor of stirred cuvette air	mb *
E	Transpiration rate	mmol m^{-2} s^{-1}
e_s	Saturated vapor pressure at cuvette air temperature	mb
e_{leaf}	Saturated vapor pressure (inside the leaf) at leaf temperature	mb
T_{leaf}	Leaf temperature	°C
Δt	Temperature difference between the air and the leaf	°C
H	Radiation absorbed by the leaf	W m^{-2}
r_s	Stomatal resistance to water vapor	m^2 s mol^{-1}
g_s	Stomatal conductance to water vapor	mmol m^{-2} s^{-1}
C_{in}	CO_2 concentration of air *entering* cuvette	µmol mol^{-1} *
C_{out}	CO_2 concentration of air *inside* and *exiting* the cuvette	µmol mol^{-1} *
A	CO_2 Assimilation rate (net photosynthesis)	µmol m^{-2} s^{-1}
g_c	Total conductance to CO_2 transfer	mmol m^{-2} s^{-1}
C_i	CO_2 concentration of sub-stomatal cavity	µmol mol^{-1}

* Detected by IRGAs, temperature and pressure corrected, effects of foreign gas broadening (water vapor effects) on measurement corrected.

References

Parkinson, K.J. 1983. Porometry in S.E.B. Symposium of Instrumentation for Environmental Physiology. Cambridge University Press.

Buck, A.L. 1981. New equations for computing vapour pressure and enhancement factor. J. Appl. Meteorol., Vol. 20:1527-1532.

von Caemmerer, S. and G.D. Farquhar 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta, Vol. 153:376-387.

Section 13. Troubleshooting & Diagnosis

Power		
If you observe…	**This usually means…**	**Solution**
CIRAS-3 battery status is low.	The internal battery requires recharging.	Place on charger for at least 8 hours to completely recharge the battery or charge it long enough to get you through your measurements. Battery capacity and estimated operation time (in minutes) are displayed in the lower right hand corner of the LCD.
	A problem with the charger.	Make sure that the Charger LED is illuminated green when connected to power and check that the output on the socket shows ~ 15V. If the output voltage is less than ~12V then the problem is a faulty charger.
The CIRAS-3 will not power up.	Dead internal battery	Place on charger for at least 8 hour to recharge the battery or charge it long enough to get you through your measurements.
	Battery is not connected internally.	Open battery compartment and verify that the battery is electrically connected and connect if necessary.

If you observe…	This usually means…	Solution
The battery only appears to power the system for less than 3 hours.	You are probably putting a high drain on the battery (temperature control and/or light control) which will impact the battery life. Capacity is being reduced as a result of age and maintenance.	This is a fact of life with regards to the battery life when controlling light and especially temperature. When controlling temperature, this puts a large load on the battery and the further away from ambient temperature the larger the load. If you have a battery that is 2+ years old and depending on its amount of use (hours) and history of charging, it may need to be replaced.
The Charging LED is flickering amber (not steady).	The CIRAS-3 was not powered off when the battery was low and it continued to drain beyond normal levels.	The battery requires a longer charging period to get back up to full charge status. If the charger LED continues to flicker after 24 hours, the battery may require replacement.

CO2 Measurement & Control		
If you observe…	**This usually means…**	**Solution**
CO2r levels are reading higher than expected.	There is a leak somewhere in the gas circuit allowing external ambient air into the system. Usually this is associated with the absorber columns and the result of a recent desiccant change.	Check that both absorber column assemblies are firmly fitted to the CIRAS-3 console and check all "O" rings to ensure that they are all firmly seated and not pinched off or cracked.
CO2r levels are reading lower than expected.	Desiccants are exhausted and require replacement.	Check desiccants and replace accordingly. If any of the columns are approximately 50% exhausted they should be replaced**. Pay special attention to the molecular sieve as this desiccant is non-indicating and should be replaced when replacing the Drierite in the same column.**
	The CO_2 cartridge is slowly exhausting. The cartridge was put in use at least 1 day before and most likely is getting low in pressure.	Replace CO_2 cartridge.
	Missing CO_2 cartridge or the cylinder holder was not tightened enough to pierce the cartridge.	Fit new CO_2 cartridge or tighten cylinder holder until you hear a slight piercing of the cartridge.

If you observe…	This usually means…	Solution
Fluctuating CO2r or CO2a values	The internal air filters are dirty and need replacing.	Replace all internal air filters.
	One or both sampling pumps are dirty and not running at optimum rates.	Flush pumps with alcohol. If problems persist replace pump. Another trick is to swap the reference and analysis pump. This will help determine which pump needs cleaning or replacement. For instance, if the CO2r is fluctuating and after swapping pumps the CO2a value starts fluctuating you can easily determine which pump is the problem.
	Exhausted desiccants.	Replace.
CO_2 cartridge rapidly exhausts when fitting a new one (loud hissing noise).	A problem with the piercing pin.	Check to make sure that the piercing pin is in place in the regulator.
	Bad or worn gasket seal.	Replace gasket CO_2 seal.
CO_2 exhausts quicker than usual and doesn't last a full day	A slow leak around the regulator.	Check the regulator for leaks and also check small "O" ring for cracks.
	Worn gasket seal.	Replace gasket CO_2 seal.
A slow drift in CO_2 readings	Exhausted soda lime.	Replace soda lime.

If you observe…	This usually means…	Solution
Fluctuating CO2d values	A leak around the leaf cuvette. Usually this is down to the cuvette gaskets. You can check for leaks by gently blowing through a small piece of tubing around the cuvette head to isolate leaks. Also check around the white gas connector.	Check leaf cuvette gaskets and replace if necessary. If problem appears to be associated with the white gas connector, put a slight smear of silicone grease on the "O" rings or replace if cracked or worn.
It takes longer than normal to settle out at the CO2 level requested	Your CO2 Reference is set to "Fixed Analysis Air".	Go to Settings and change **CO2 Reference** to "Fixed reference air".
Unable to achieve Max C	CO_2 cartridge is low or empty. Exhausted desiccants	Make sure CO_2 cartridge holder is tight or replace CO_2 cartridge. Replace exhausted desiccants.

H2O Measurement & Control		
H2Or levels are reading higher than expected.	That there is a leak somewhere in the gas circuit allowing external ambient air into the system. Usually this is associated with the absorber columns and the result of a desiccant change. Exhausted H2O scrubber.	Check that both absorber column assemblies are firmly fitted to the CIRAS-3 console and check all "O" rings to ensure that they are all firmly seated and not pinched off or cracked. Check Drierite and replace if necessary.
H2Or levels are reading lower than expected.	Desiccants are exhausted and require replacement.	Check desiccants and replace accordingly. If any of the columns are approximately 50% exhausted they should be replaced**. Pay special attention to the molecular sieve as this desiccant is non-indicating and should be replaced when replacing the Drierite in the same column.**

If you observe…	This usually means…	Solution
Fluctuating H2Or or H2Oa values	The internal air filters are dirty and need replacing. One or both sampling pumps are dirty and not running at optimum rates. Exhausted desiccants.	Replace all internal air filters. Flush pumps with alcohol. If problems persist replace pump. Replace.
A slow drift in H2O readings	Exhausted Drierite.	Replace Drierite and Molecular Sieve.
Fluctuating H2Od values	A leak around the leaf cuvette. Check the cuvette gaskets. You can check for leaks by gently blowing through a small piece of tubing around the cuvette head to isolate leaks.	Check leaf cuvette gaskets and replace if necessary.

Temperature Measurement & Control		
If you observe…	**This usually means…**	**Solution**
Inaccurate measurement of leaf temperature using IR sensor	Most likely problem is that you are not filling the chamber window completely with vegetation. The entire window must be filled with vegetation for accurate leaf temperature measurement using IR thermometry.	Ensure that you fill the cuvette completely with vegetation or change the Temperature Measurement Method to Energy Balance (Settings).
Tamb value is not close to ambient.	The Tamb sensor is located at the back of the cuvette handle and is likely in close proximity to something that is cooler or warmer than the ambient.	Try to keep any temperature influencing items away from the Tamb sensor (thermistor).

If you observe…	This usually means…	Solution
Unable to achieve set Tcuv	The setpoint value exceeds the temperature control range.	Set control value within the limits of the system (~10 ^{o}C below ambient to +15 ^{o}C
Unable to achieve set Tleaf	The setpoint value exceeds the temperature control range.	Set a control value that is within the limits of the system (~10 ^{o}C below ambient to +15 ^{o}C above ambient within the ambient temperature control range of 0-45 ^{o}C).

PAR Measurement & Control		
PARi reads lower than the PARe	This is normal.	The PARi sensor is located inside the cuvette and below the window and should therefore always read approximately 10% lower than the PARe.
Unable to control light intensity in the cuvette using the LED light unit.	The light unit is not connected electrically to the cuvette. Light Source is set to Ambient under Settings.	Connect the light unit to the PLC3. Go to Settings and change “Light Source” to LED.
My PARe readings do not seem accurate.	Sensor probably needs recalibration.	Check calibration against another PAR sensor that you trust and believe to be properly calibrated. Recalibrate the PARe sensor.

Flow Control		
If you observe…	**This usually means…**	**Solution**
Unable to achieve set cuvette flow rate	Absorber column assembly not seated properly in CIRAS-3 console. This is a common problem just after replacement of desiccants. A problem with air supply pump.	Check both the Zero and CO2/H2O control absorber columns and make sure that they are secure and fitted properly. Contact PP Systems. Replacement of the diaphragm pump may be required.
Pumps sound louder than usual	A restriction somewhere in the gas circuit. One of the absorber column assemblies is not firmly seated in the manifold. Tubing may have slipped of one of the internal sampling pumps. Blocked air filter(s).	Check for kinked tubing. Check both absorber column assemblies and ensure secure fit. Check all internal pumps and tubing connections. Try changing out filters to see if problem is rectified.
Reference sampling pump is not running	The pump is not connected electrically. Possible seizure of the pump.	Check electrical connection and ensure that it is connected and secured in place via the locking connector at location REF PUMP on internal PCB. Try tapping on the pump with a small screwdriver. Sometimes the internal vanes can stick and a simple tapping releases them and the pump is back up and running. You might want to also consider flushing the pump with alcohol.

If you observe…	This usually means…	Solution
Analysis sampling pump is not running	The pump is not connected electrically.	Check electrical connection and ensure that it is connected and secured in place via the locking connector at location AN PUMP on internal PCB.
	Possible seizure of the pump.	Try tapping on the pump with a small screwdriver. Sometimes the internal vanes can stick and a simple tapping releases them and the pump is back up and running. You might want to also consider flushing the pump with alcohol.

Data Management		
If you observe…	**This usually means…**	**Solution**
I am unable to record measurements.	You have not gone into "Rec Options" to set up and initiate a recording session.	Go to Operations>Rec Options and select the type of recording planned (Manual, Timed, Response curves), where you want the data saved (internal or external memory) and create or edit the data file. When finished, press Start (F2) **Very important. Always click End Recording (F5) when using "Manual recording" to terminate a session to safely preserve all stored data.**
I can't find my stored data on the USB memory stick (thumb drive)	Under Rec Options you are probably set up to store data to "internal memory" which is where the data is likely saved.	Check internal memory to view your saved data. If you want to store data to your USB memory stick, make sure Data file folder is set to "external memory" and you have a USB memory stick inserted in USB1 or USB2.
I can't find my stored data in internal memory	Under Rec Options you are probably set up to store data to "external memory" which is where the data is likely saved.	Check external memory to view your data (USB memory stick needs to be inserted into USB1 or USB2 on the CIRAS-3 console. If you want to store data to your internal memory, make sure Data file folder is set to "internal memory".
Please download from the Microsoft web site Download Center and then install.	You have not downloaded ActiveSync (Windows XP users) or Windows Mobile Device Center or WMDC (Vista or Windows 7 users) from the Microsoft website.	Go to the Microsoft download site (http://www.microsoft.com/en-us/download/default.aspx) and download and install the program to your compter.

If you observe…	This usually means…	Solution
Can't detect ActiveSync connection (even though you have downloaded it and installed it on your computer and the USB cable is properly connected)	Windows Mobile Device Center (WMDC) does not see or recognize a valid connection.	On your computer, execute WMDC. At this point it will show not connected. Connect USB cable between computer and CIRAS-3 (PC Mini-B connector). WMDC should now show connected. If it doesn't, Unplug USB cable from CIRAS-3 and exit out of the CIRAS-3 console application by pressing Escape from top level (i.e. numerical or graphical display on LCD) and then OK. Re-connect USB cable to CIRAS-3. WMDC should now show "Connecting" and after a few seconds show "Connected". Start the CIRAS-3 PC Utility program on your computer and click on View>Remote Display. If you get a message "The OS or CPU device…" click OK. Double-click on the CIRAS3 icon over to the left on your computer desktop and the "Welcome to CIRAS-3" message should appear. Press F1 on your computer keyboard to continue.
The data and time shown with my data are incorrect	The date and time are not properly set up on the CIRAS-3	Go to Operations>Set Clock and set the appropriate date and time.

Status Codes Displayed on the CIRAS-3 LCD

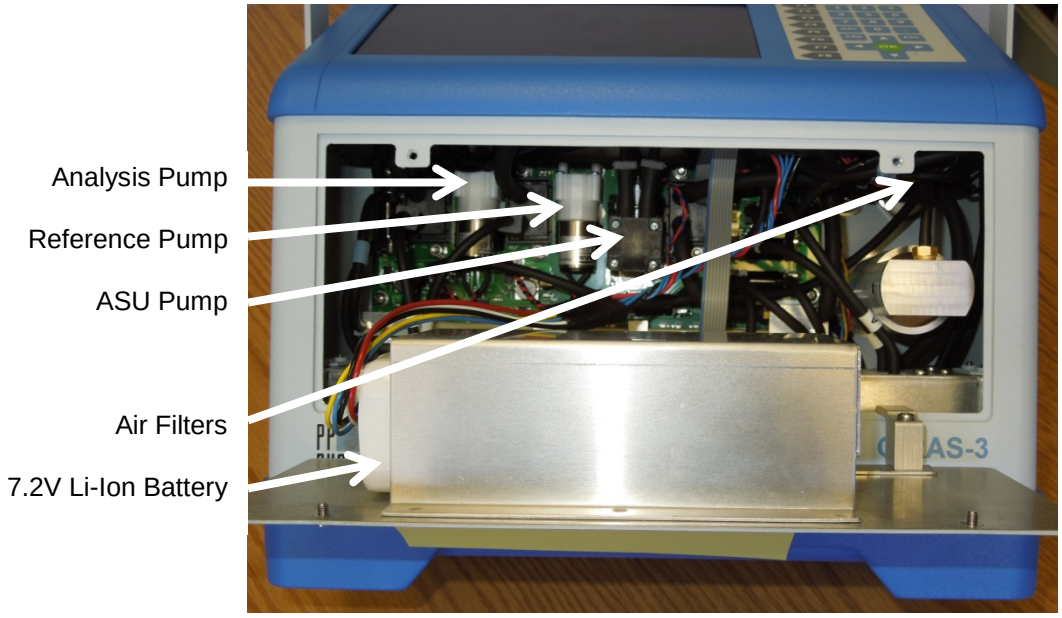
If you see the following status message displayed on the CIRAS-3 main console…	It usually means….	Solution to the problem.
DIFF-BAL required, ensure CO2r stable	This message appears when Zero,Diff Bal Mode is set to "Manual" under Settings and the system is recommending that you perform a DIFF BAL (which we strongly recommend to ensure calibration stability and accuracy).	Check that your CO2r value is stable and if not you should perform a manual "Diff Bal (Z-Diff Bal>Diff Bal).
ZERO required	This message appears when Zero,Diff Bal Mode is set to "Manual" under Settings and the system is recommending that you perform a ZERO (which we strongly recommend to ensure calibration stability and accuracy).	When prompted, we recommend that you perform a manual zero (Z-Diff Bal>Zero).
Divide by zero	Possible corruption of system data pointers or calculation error.	Power cycle the CIRAS-3 and the problem should be removed. If not, contact PP Systems.
Time out for DIFF BAL	The CO2r or H2Or concentration is fluctuating too much during DIFF BAL and the system is unable to get a good fit.	Perform manual Zero and Diff Bal. If you are operating in "Manual" mode for Zero and Diff Bal, we recommend changing this to Automatic.
DIFF BAL CO2 out of range	The DIFF BAL value for CO2 is > 20 ppm. Typically this is due to inaccurate recalibration or deterioration of the internal sample cell due to dust, dirt, water.	Perform a manual Diff Bal (Z-Diff Bal>Diff Bal. If problem persists contact PP Systems. Also ensure that all desiccants are fresh and that the absorber columns are firmly seated in their respective manifolds.

If you see the following status message displayed on the CIRAS-3 main console…	It usually means….	Solution to the problem.
DIFF BAL H2O out of range	The DIFF BAL value for H2Or is > 0.5 mb. Typically this is due to inaccurate recalibration or deterioration of the internal sample cell due to dust, dirt, water.	Perform a manual Diff Bal (Z-Diff Bal>Diff Bal. If problem persists contact PP Systems. Also ensure that all desiccants are fresh and that the absorber columns are firmly seated in their respective manifolds.
Cuvette flow 20% out from set flow	This is generally a transient message that appears for a few seconds after changing the flow rate.	If the message is cleared on its own after 6 seconds then there is no problem. If the problem remains on the display it could reveal a problem with the pump(s).
Reference flow to high	The reference sampling pump is faulty or possibly electrically connected in reverse.	Check pump connection and replace if necessary.
Ref flow low	Possible kink in tubing reducing flow or possible build-up of dirt/dust over time and requires cleaning with alcohol.	Check for kinked tubing and recommend cleaning of pumps.
An flow high	The analysis sampling pump is faulty or possibly electrically connected in reverse.	Check pump connection and replace if necessary.
An flow low	The analysis sampling pump has built up dirt/dust over time and requires cleaning with alcohol.	Check for kinked tubing and recommend cleaning of pumps.

Section 14. Routine Maintenance

CIRAS-3 Main Console

Most internal components can easily be accessed by loosening the 2 small screws on the front of the CIRAS-3 main console and gently pulling back on the door as shown below.



The only internal components that will require routine maintenance are as follows:

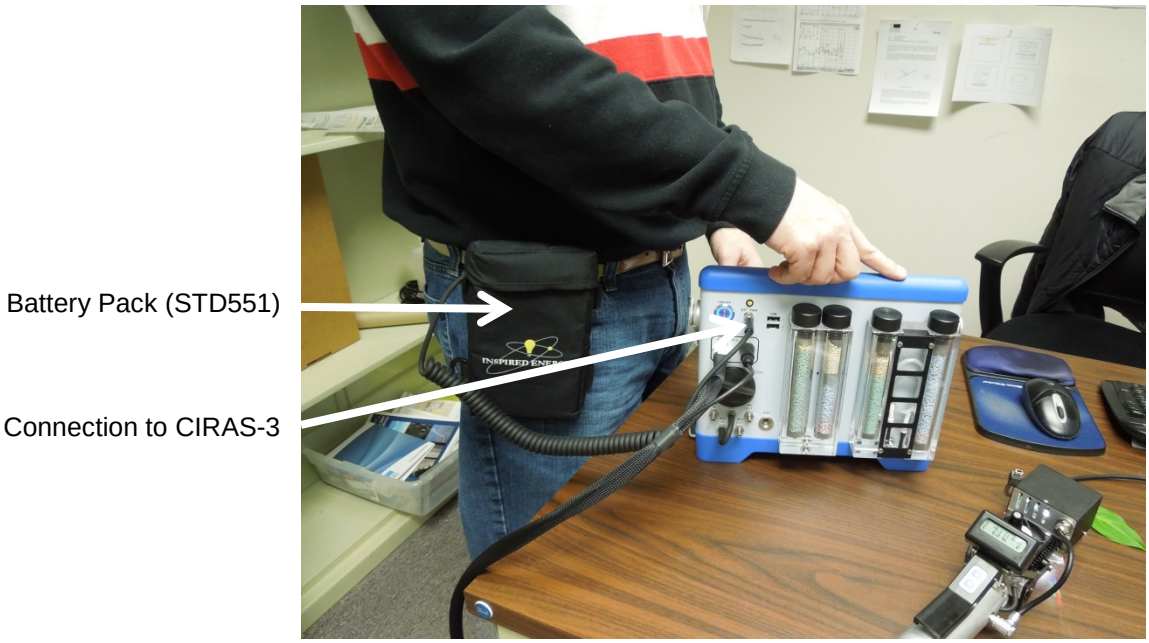
- Li-Ion Battery
- Air Sampling Pumps (Reference and Analysis)
- Air Filters

Li-Ion Battery

The CIRAS-3 is powered by a single, 7.2V Li-Ion battery which is capable of delivering up to 8 hours continuous use in the field (dependent on environmental controls). This should last for many years. We recommend that the battery is charged every night when the system is in regular use and **ALWAYS** charge the internal battery to full capacity prior to storing the system for extended periods. We also highly recommend that the internal battery gets charged every 3-4 months when not in use to preserve capacity. A power supply is included with the CIRAS-3 for charging the internal battery or for extended operation. To charge the internal battery, connect the power supply to the “EXT PWR” plug on the back of the CIRAS-3 main console. The indicating LED above the plug shows the charge status of the internal battery. It will illuminate a steady amber when charging and green when the battery is fully charged. Therefore if the battery was placed on the charger overnight, the indicating LED should be a steady green in the morning.

External Battery Pack (For extended operation in the field)

PP Systems can supply spare Li-Ion Battery packs (41526-1) with a special charger (STD550) which can be used to charge the battery externally. Also, a convenient battery pack (STD551) can also be used for extended operation in the field.



Re-Order Information	
Part Number	**Description**
41526-1	Battery Pack, Li-Ion Battery 7.2V
STD552	Power Supply, CIRAS-3
STD550	External Charger for CIRAS-3 Internal Battery Pack
STD551	Belt Pack Kit – Battery, Charger and Power Supply

Air Sampling Pumps (Reference and Analysis)

The CIRAS-3 has 2 rotary vane pumps used for sampling the reference and analysis air and should be inspected periodically. Rotary vane pump components wear with prolonged use and material from the vanes may build up over time within the pumps. The risk of this situation from occurring can be minimized by regular pump cleaning with alcohol.

Typically, the following symptoms are attributed to a worn of faulty reference or analysis pump:

1. **Noise** – a worn pump usually sounds rough or vibrates.
2. **Temperature** – the outer casing (near the pump serial number) feels warm compared to other pumps.

Removing a Pump.

It is very easy to remove a pump for servicing. First, trace the electrical connection to the pump and disconnect the 2-pin connector from the terminal on the CIRAS-3 Flow board.

Analysis Pump – Connected to connector labeled “AN PUMP”

Reference Pump - Connected to connector labeled “REF PUMP”

The connector is removed by gently bending back the connector lock and sliding out the connector. **Please note the orientation of the electrical connector for correct re-fitting of the pump**.

The pumps are secured in position by the gas tubing and pump bracket. The pump can be removed by gently pulling the gas tubing off both the inlet and exhaust ports on the pump and gently prying the pump out from the securing pump bracket.

Servicing a Rotary Vane Pump (Reference & Analysis).

During pro-longed operation the vanes inside the pump will wear and deposit material inside the pump. It is sensible to clean the pump periodically by flushing it through with iso-propyl alcohol.

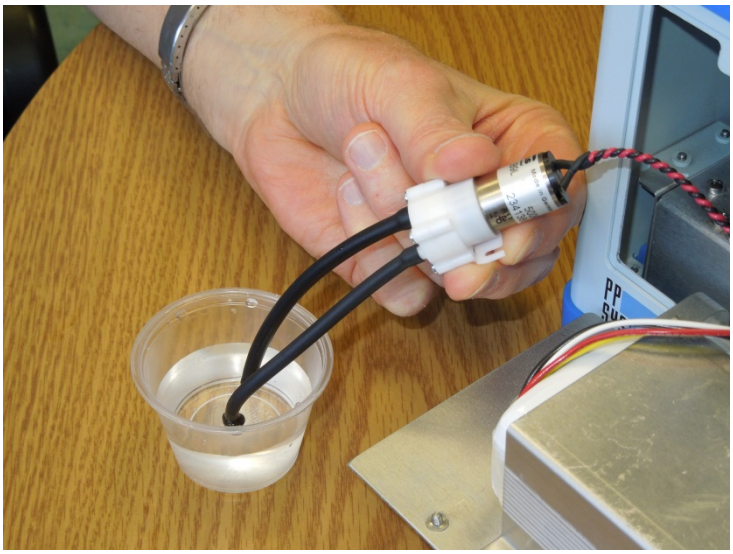
- Heavy use (daily) in clean environments – Every 3 months
- Heavy use (daily) in dirty or dusty environments – Every 1 month
- Minimal use (once or twice per week) – Every 6 months
- Minimal use (once or twice per week) in dirty or dusty environments – Every 3 months

The following procedure can be adopted:

Remove the gas tubing from the inlet and exhaust ports on the pump and fit a small piece of tubing on both the inlet and exhaust ports on the pump.

It is easiest to power the pumps by simply powering up the CIRAS-3 main console. **IT IS IMPORTANT THAT THE PUMP CLEANING IS DONE OUTSIDE THE CIRAS-3 CONSOLE TO AVOID SPILLAGE INSIDE THE INSTRUMENT.**

Hold the pump above a beaker of iso-propyl alcohol and dip the tube into the alcohol (see below).



Run the pump to draw alcohol through it for a minute or so to ensure that any material is removed. Please note that the pumps are not sealed, so it is normal to see alcohol leak through the sides of them during this procedure. When finished, run the pump in air for at least 15 minutes to allow any residual alcohol to evaporate. Ideally, let the pumps dry outside of the CIRAS-3 overnight. If the pumps are reconnected prematurely, the absorber chemicals will be exhausted quicker than usual.

Re-connect the gas tubing to the pumps and secure in place using the pump bracket.

If the reference or analysis pump is seized, it may be freed by tapping it on the bench or by reversing the voltage to run it backwards. If this does not work, pump replacement is usually required.

Re-Order Information	
Part Number	**Description**
10127-1	Air Sampling Pump

Air Supply Pump

The main air supply pump (ASU) is a “diaphragm” style pump and should not require replacement or routine maintenance. It is located to the right of the reference air sampling pump and secured in place by Velcro and tubing. The electrical connector is labeled ASU PUMP and can be removed by gently bending back the connector lock and sliding out the connector. **Please note the orientation of the electrical connector for correct re-fitting of the pump**.

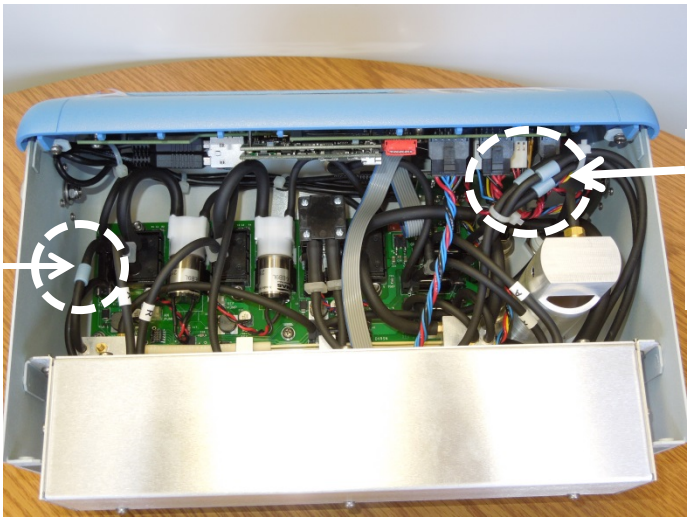
The pump itself is secured in position by velcro and gas tubing. These can be removed by gently pulling the pump from the rubber tubing and gently pulling back on the velcro.

Re-Order Information	
Part Number	**Description**
10128-1	Gast Diaphragm Pump

Air Filters

The internal optics and gas circuit are protected by a total of 4 internal air filters located inside the main console.

- Reference and Analysis Air Filters: 5 micron
- Air Supply Unit and Gas Blender Air Filters: 25 micron



They are light blue in color and are held in place by the gas tubing and can easily be changed by separating from the gas tubing. Replacement of the air filters is dependent on use:

Reference and Analysis Air Filters (5 micron)

- Heavy use (daily) in clean environments – Every 3 months
- Heavy use (daily) in dirty or dusty environments – Every 1 month
- Minimal use (once or twice per week) – Every 6 months
- Minimal use (once or twice per week) in dirty or dusty environments – Every 3 months

Gas Blender and Air Supply Unit Air Filters (25 micron)

- Once every year.

Re-Order Information	
Part Number	**Description**
30124-1	Air Filter, 5 micron
30124-2	Air Filter, 25 micron

Desiccants and Absorber Columns

The CIRAS-3 has two well labeled absorber column assemblies (total of 4 absorber columns) for analyzer ZERO and CO_2 and H_2O control.

Zero Column Assembly

The ZERO column assembly contains 3 desiccants which are used for the analyzer ZERO ensuring long term stability and accuracy of the CO_2 and H_2O gas analyzers

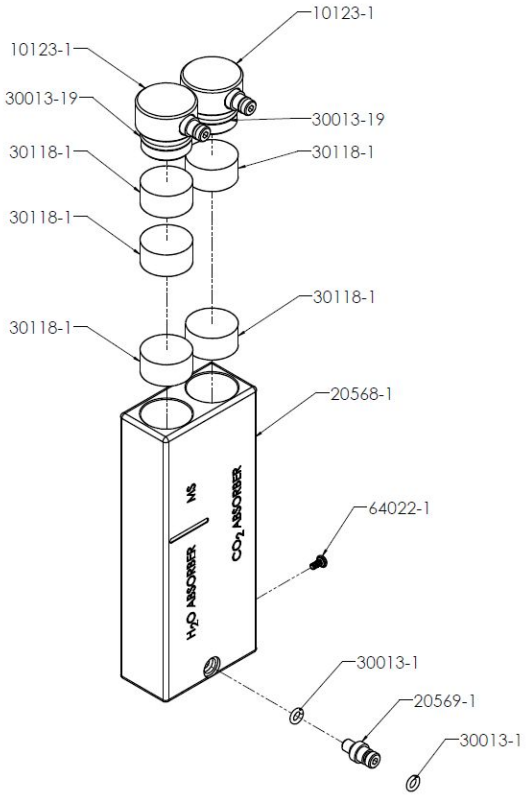
Zero Column Assembly (Part Number 10122-1)

Column Identifiers

- **H_2O Absorber**: Drierite
- **MS**: Molecular Sieve
- **CO_2 Absorber**: Soda Lime

One column is filled with Drierite and Molecular Sieve and the other with soda lime. Drierite removes H_2O from the air stream and soda lime removes CO_2 from the air. During the ZERO process, the Drierite adds in a small bit of CO_2 and the soda lime adds a small bit of H_2O which is why Molecular Sieve desiccant is required. After passing ZERO air through the soda lime and drierite, the air finally passes through Molecular Sieve which absorbs any remaining traces of CO_2 and H_2O for very accurate ZERO.

ALWAYS change the desiccants when the column is approximately 50% exhausted.



Part Number	Description
10123-1	Absorber Cap Assembly
20568-1	Zero Absorber Block
20569-1	Fitting
30013-1	O-ring 4.76 x 1.78
30013-19	O-ring 20.8 x 2.4
30118-1	Filter Foam
64022-1	Pan hd Sealing M3 x 6mm

CO_2 & H_2O Control Column Assembly (Part Number 10121-1)

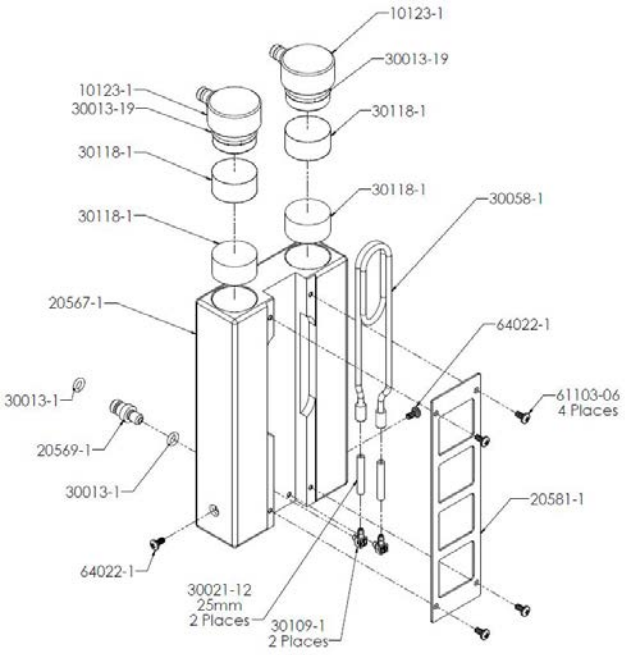
Column Identifiers

- **H_2O Absorber**: Drierite
- **CO_2 Absorber**: Soda Lime

One column is filled with Drierite (for control of H_2O) and one with soda lime (for CO_2 control). Drierite removes H_2O from the air stream and soda lime removes CO_2 from the air to achieve levels requested by the user.

This assembly also includes a water vapor equilibrator containing NAFION® tubing which should not require replacement and is used to equilibrate the humidity with the ambient.

ALWAYS change the desiccants when the column is approximately 50% exhausted.



Part Number	Description
10123-1	Absorber Cap Assembly
20567-1	Main Absorber Block
20569-1	Fitting
20581-1	Cover Vent
30013-1	O-ring 4.76 x 1.78
30013-19	O-ring 20.8 x 2.4
30058-1	Equilibrator, NAFION
30118-1	Filter Foam
64022-1	Pan hd Sealing M3 x 6mm

Re-Order Information	
Part Number	**Description**
10121-1	Absorber Control Column Assembly (Complete)
10122-1	Zero Column Assembly (Complete

For individual absorber column parts (i.e. filters, O-rings, etc.), please refer to the above drawings.

Soda Lime

Soda lime (Sodium Hydroxide) is used to remove CO_2 from air entering the CIRAS-3. It is supplied as self-indicating granules (1-2.5mm) which turn from green to brown as they become exhausted. The contents of the absorber column should be replaced when it is approximately 50% exhausted. Soda Lime cannot be regenerated and should be discarded after exhaustion.

- Type: Sofnolime, 1.0-2.5 mm, self-indicating (green to brown), 1 kg
- Manufacturer: Molecular Products. (www.molecularproducts.com)
- For the latest Material Safety Data Sheet, please visit www.molecularproducts.com and request the latest MSDS or contact PP Systems.

Take caution to wash your hands completely after handling soda lime.

Re-Order Information	
Part Number	**Description**
STD007	Self-indicating Soda Lime, 1.0 kg

Drierite

Drierite (anhydrous 97% calcium sulfate ($CaSO_4$) and 3% cobalt chloride) is an excellent H2O absorber making it an ideal choice for analyzer ZERO and for controlling H2O. It is self-indicating turning from blue to pink when exhausted. The contents should be replaced when approximately 50% of the column is exhausted. It can be regenerated easily by simply spreading out the granules one layer deep and heated for 1 hour at 210° C or 425° F. The regenerated material should be placed in the original glass or metal container and sealed while hot. The color of the Indicating Drierite may become less distinct on successive regenerations due to the migration of the indicator into the interior of the granule and sublimation of the indicator. We highly recommend discarding the Drierite when the indicator starts to deteriorate.

- Type: 8 mesh, self-indicating (blue to pink), 1 lb. Jar
- Manufacturer: W.A. Hammond Drierite Company Ltd. (www.DRIERITE.com)
- For the latest Material Safety Data Sheet, please visit www.DRIERITE.com and request the latest MSDS or contact PP Systems.

Take caution to wash your hands completely after handling Drierite.

Re-Order Information	
Part Number	**Description**
STD008	Drierite, 8 mesh, 1 lb.

Molecular Sieve

Molecular Sieve is used to finally filter any remaining CO_2 and H_2O from the air supply during analyzer ZERO to ensure system stability and accuracy for CO_2 and H_2O. Unfortunately, Molecular Sieve is not self-indicating and there is no obvious way to see that it is exhausted. **It is therefore best to always change the Molecular Sieve when changing the Drierite in the ZERO column.**

Molecular Sieve can easily become contaminated through absorption of CO_2 and H_2O from atmospheric air. It is therefore **strongly recommended and advised** that Molecular Sieve is decanted into small air-tight, glass containers sealed by electrical tape to minimize any exposure to air. The ZERO absorber columns should be placed in a sealed polythene bag if the CIRAS-3 is not going to be used for an extended period (i.e. days) to preserve the desiccants.

- Type: Molecular Sieve, 13X 1/16, 1.25 lb. container
- Manufacturer: UOP. (www.uop.com)
- For the latest Material Safety Data Sheet, please visit www.uop.com and request the latest MSDS or contact PP Systems.

Take caution to wash your hands completely after handling Drierite.

Re-Order Information	
Part Number	**Description**
STD006	Molecular Sieve, 1.25 lb.

When changing out desiccants, the user must take care to ensure that the columns are properly seated in the correct manifolds, the proper desiccant is used in appropriate columns which are clearly marked and that all “O” rings are in place and slightly lubricated with silicone grease. Any leakage of ambient air into the gas circuit generally results in error messages during ZERO or fluctuating CO2r values during measurement.

Tip

Always change the molecular sieve at least once per week during use.

Each absorber column includes the following items which should be checked periodically and replaced when necessary:

Foam Filters

The gray foam filters used inside the absorber columns become worn over time and should be inspected regularly and replaced when torn or reduced in size. The foam must be of an open celled type, such as packing foam. The foam filters at the bottom of each column will likely require more frequent changes versus the upper foam filters.

Re-Order Information	
Part Number	**Description**
30118-1	Filter Foam

Absorber Filters

Each absorber end cap contains a white plastic filter disk. Generally these do not need to be replaced but should be checked periodically. However, they must be present to prevent any of the column contents being drawn with the gas stream into the instrument.

"O" Rings

All "O" Rings on the absorber columns should periodically (every couple of weeks) receive a slight smear of silicone grease to aid ease of fitting, improve the seal and extend the life of the "O" rings and to keep them from cracking or breaking. Once sealed, end fittings should be checked to ensure that the O-rings are seated correctly in their groove and that they are not trapped or pinched resulting in system leaks.

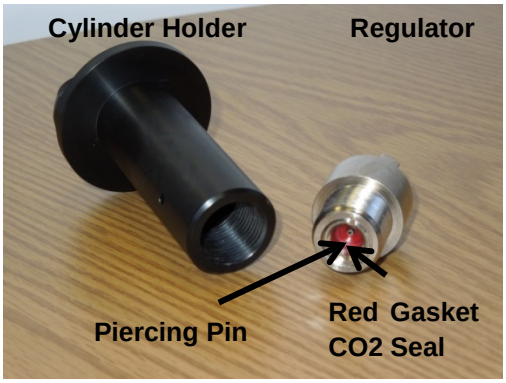
Re-Order Information	
Part Number	**Description**
30013-1	O-ring 4.76 x 1.78
30013-19	O-ring 20.8 x 2.4

CO_2 Regulator

The CO_2 regulator should not require any routine maintenance. The CO_2 cartridge seals on the face of the 8g CO_2 cartridge and these seals should last for many years. Each system is supplied with gasket CO_2 seals (Part No. 20577-1) if replacement is necessary. If you suspect a faulty seal, follow the instructions below. **Before attempting to service the CO_2 regulator, check to make sure that there is not a pressurized CO_2 cartridge present. To do so, simply power up the CIRAS-3 system and set the CO_2 reference to 2000. Monitor the CO2r value and if you are unable to achieve 2000 and the value is slowly decreasing it is safe to remove the cylinder holder from the regulator.** Depending on pressure, you may here a small hiss when loosening the cylinder holder from the regulator body which is normal.

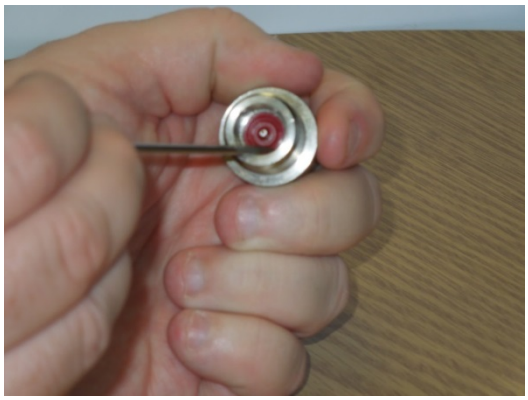
Step 1. Remove CO_2 Cartridge holder and regulator from main console by turning the holder ¼ counter clockwise and pull out.

Step 2. Separate cylinder holder from regulator as shown below.



Step 3. Insert small coin in notch in regulator and turn counterclockwise to remove inner fitting as shown below.

Step 4. Using small flat screwdriver, remove red gasket seal and fit replacement making sure to secure it in place around the piercing pin.



Re-Order Information	
Part Number	**Description**
STD549	Cylinder Holder
STD546	Regulator Assembly
20577-1	Gasket CO2 Seal

CO_2 Calibration

The CO_2 gas analyzers should not require recalibration due to our built-in, innovative "Auto-Zero" function. During normal operation, the CIRAS-3 periodically passes "CO_2 free" air through the CO_2 gas analyzers ensuring long term stability, accuracy and calibration of the gas analyzers. This function corrects for nearly all changes that can affect calibration including cell reflectivity, changes in electronics, etc. The most common cause of a drift in CIRAS-3 readings is an incorrect Zero due to either exhausted chemicals, leaks on the Zero columns from pinched or damaged O-rings or the Zero absorber assembly is not properly seated in the console manifold. Another likely cause of drift in calibration is contamination of the sample cell (liquid water) which is more serious though fortunately less common. We do recommend periodic checks of the gas analyzers by simply passing an accurate CO_2 gas mixture through the instrument. Another simple way to check of the CO_2 analyzers is to sample ambient air in an area away from any CO_2 influences or disturbances (i.e. roadways, air conditioning/ventilation systems, individuals breathing, etc.). We know that the typical ambient CO_2 concentration is approximately 375-390 µmol mol^{-1}. To check the CO_2 analyzers using ambient air as your reference:

Power up the CIRAS-3 console on its own (no leaf cuvette attached and no CO2 cartridge installed) and allow the instrument to warm up for 30 minutes.

1. Ensure that all desiccants are fresh.
2. Go to Settings and at "Settings File", select AnalyzerOnly and OK.
3. Press Accept to return to the main measurement screen and observe the CO2r and CO2a values.

Both values should be approximately 390 µmol mol^{-1} or close to your local ambient CO_2 concentration. If the values are close to what you would expect then the analyzers are more than likely fine. If the readings are far off from the expected ambient CO_2 concentration, a local or factory recalibration is recommended.

If CO_2 recalibration is required, an accurate gas mixture is required. **It is critical that the Zero columns are fresh before performing CO_2 recalibration**. It is always best to recalibrate the analyzer using a gas mixture that is slightly above your normal measurement range. For instance, if you routinely make measurements around ambient levels, use a gas calibration mixture just slightly above.

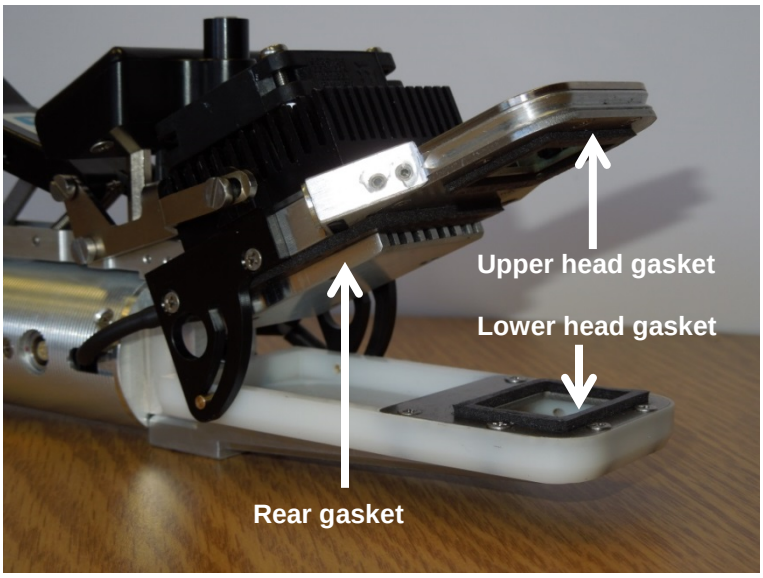
H_2O Calibration

For H_2O calibration, an air supply of known water vapor concentration is required (mb). Typically this is achieved using a commercially available dew point generator or water vapor generator. If you do not have a device for H_2O recalibration, please contact PP Systems and we will be happy to recommend some. PP Systems can also perform H_2O recalibration services at our factory.

PLC3 Leaf Cuvettes

Cuvette Gaskets

The most common part requiring routine inspection and replacement are the leaf cuvette gaskets. This is also the place where leaks are often discovered leading to fluctuations in CO_2 and H_2O readings. Over time, plant material can cause the gasket material to crack or even deform requiring replacement.



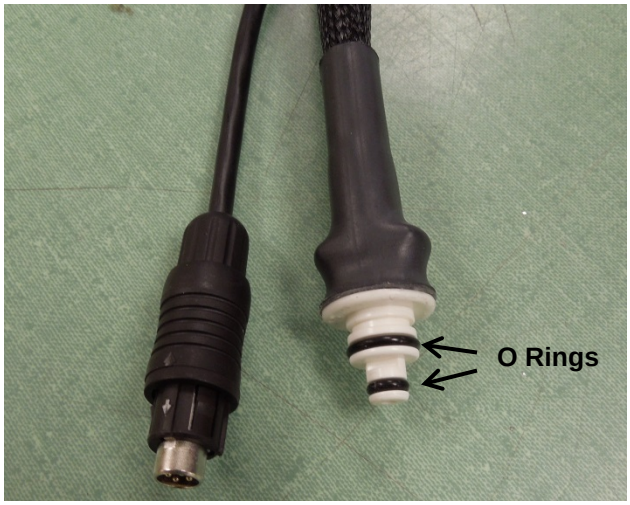
Leaf gaskets can easily be removed using a fine screwdriver to pry them out of their respective channels in the cuvette head. Open the leaf cuvette head as shown above and using a fine screwdriver and gently pry the worn gasket material from the head. Take care to remove all debris, old gasket material or anything else that will prohibit you from clean replacement of the new gaskets. Peel the new gasket from the white backing and carefully place it in the appropriate location on the cuvette head and seal it in place. We recommend applying a very light smear of silicone grease to preserve the gaskets and to provide a good seal.

When the leaf cuvettes are not in use, remember to leave the cuvette head open to avoid compression of the leaf gaskets.

Re-Order Information	
Part Number	**Description**
STD524	PLC3 Universal Gaskets, 18 mm diameter (Qty. 10)
STD525	PLC3 Universal Gaskets, 25 mm x 18 mm (Qty. 10)
STD526	PLC3 Universal Gaskets, 25 mm x 7 mm (Qty. 10)
STD527	PLC3 Universal Rear Gaskets, 50 mm x 35 mm (Qty. 10)
STD529	PLC3 Narrow/Conifer Gaskets, 88 mm x 40 mm (Qty. 10)

PLC Pneumatic Connector

Each PLC3 pneumatic connector has two “O” rings that provide a good seal when connected to the PLC “Gas” connector on the CIRAS-3 console.



We recommend that you periodically (every few weeks) apply a slight smear of silicone grease to keep the “O” rings mildly lubricated to keep them from getting too dry causing them to crack or even break apart.

Environmental Sensors

Temperature

All PLC3 leaf cuvettes are equipped with sensors for measuring air and leaf temperature. These sensors should not require recalibration. If you suspect a problem with any temperature measurements you are encouraged to get in direct contact with PP Systems. The only replaceable sensor that may break or go missing is the Leaf Temperature Thermistor commonly used with the PLC3 Narrow and Conifer leaf cuvettes.

Re-Order Information	
Part Number	**Description**
STD015	Leaf Temperature Thermistor including Plug

PAR

All PLC3 leaf cuvettes include sensors for measurement of PAR internal (PARi) and PAR external (PARe). PAR sensors do require frequent checks and recalibration is often necessary usually after 2 years. A simple check is to compare PAR sensor readings with an accurate PAR sensor that is known to be calibrated and accurate. If the readings are not close, simple recalibration can be performed at your site on these sensors.

PAR Sensor Calibration

Information to follow. If you suspect that you need to recalibrate, please get in contact with PP Systems. At time of printing of this manual, this information and instructions are being developed.

Boundary Layer Determination (r_b)

Gas mixing efficiency in leaf cuvettes is tested in the factory by measuring the boundary layer resistance (r_b) over an area of exposed wet filter paper, which simulates a leaf. This is based on the method of Parkinson (1985) and Parkinson & Day (1990).

A close approximation of r_b is needed for three important gas exchange calculations used in CIRAS firmware: leaf temperature derived from the Energy Balance method, stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i). Energy Balance is perhaps less important in the PLC6(U) cuvette, due to its built-in IR sensor used for determining leaf temperature. It is always needed for the calculation of g_s, the inverse of stomatal resistance (r_s):

$$r_s(m^2\ s\ mol^{-1}) = \left[\frac{(e_{leaf} - e_{out})}{(E \times P)}\right] - r_b$$

This is essentially the total leaf resistance less r_b, with a correction for the proportion of stomata distributed between the adaxial and abaxial leaf surfaces. Boundary layer resistance is relatively weak as compared to the resistance of the stomata to transfer of gas molecules. For a thorough discussion of leaf external and internal resistances see e.g. P. S. Nobel, 2009, Physicochemical and Environmental Plant Physiology, 4th Edition. Boundary layer resistance is also a factor in the von Caemmerer and Farquhar (1981) equation determining leaf conductance for CO_2 transport (g_c), where r_s and r_b are associated with different H_2O:CO_2 diffusion coefficients in air and in the leaf boundary layer, and subsequently used in the calculation of C_i:

$$g_c\ (mmol\ m^{-2}\ s^{-1}) = \left[\frac{1}{(1.585 \times r_s) + (1.37 \times r_b)}\right] \times 10^3$$

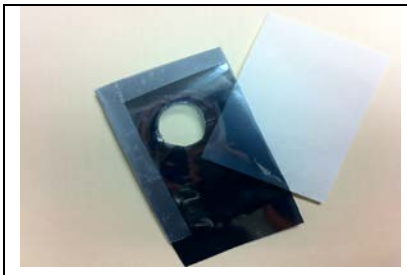
For a PLC3 Universal cuvette with the circular insert, the area of filter paper is 18 mm diameter (2.5 cm^2 projected area), exposed on both surfaces. For a PLC53 (N/C) the filter paper area is 3 cm x 1 cm (3 cm^2 projected area), forming the center part of a 1 cm wide simulated leaf which is suspended across the entire width of the cuvette, exposed on both surfaces.

The factory-calculated r_b value is always provided with your system documentation. The r_b value should be recalculated if it is lost and periodically checked as the cuvette is used. An appropriate typical value can be used temporarily, with the understanding that any data must be recalculated using the correct r_b value.

Typical r_b values for the following cuvette types are:

PLC Type	r_b (m^2 s mol^{-1})
PLC3 Universal Cuvette	0.40-0.55
PLC3 Narrow Cuvette	0.15-.25
PLC3 Conifer Cuvette	0.15-0.25

In the example below the leaf stimulant consists of a simple water-resistant envelope with an 18 mm circular opening. The envelope may be constructed of plastic sheet or thin aluminum foil, or by using clear tape. It can be closed on 3 sides, allowing new filter paper to be exchanged with older, dirty filter paper, or sealed on all 4 sides and discarded after a few uses. The area dimensions of the sample plastic envelope shown below are 45 x 75 mm. The cuvette upper and lower foam rubber gaskets must not come into contact with the filter paper once it is placed in the cuvette, as seen below, right.



Remove the LED light unit. Go to **Settings (F2)** and set the following environmental controls:

- CO2 Reference - Fixed reference air = 0
- H2O Reference - Fixed % of reference = 0
- Cuvette Flow = 470
- Light Intensity = 0
- Temperature Sensor Measurement = IR Thermometry
- Temperature Control Type = Track cuvette to ambient

Perform a Zero and Diff Bal cycle. Set up the graphical display to plot H2Od (H_2O differential). Wait until Tcuv is within +/- 0.5 C of Tamb.

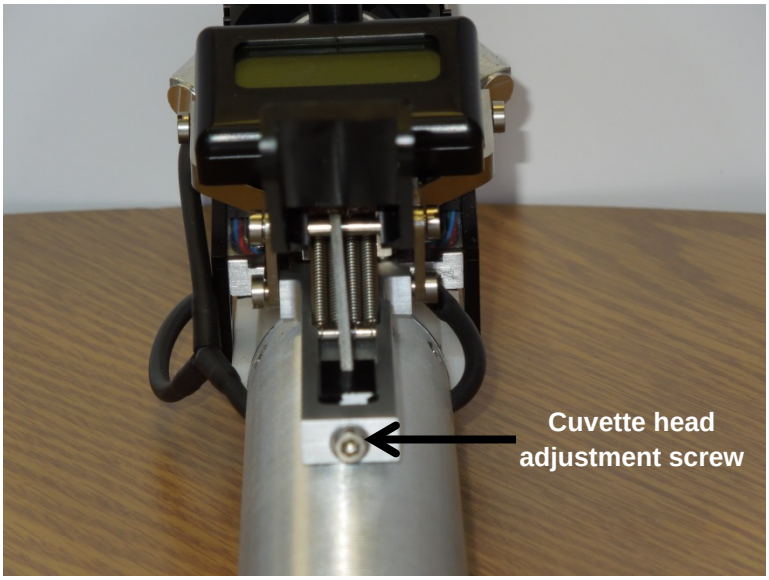
With a syringe or moist cotton swab, carefully wet the exposed filter paper area. Avoid water on the aluminum foil or plastic frame of the envelope. Do not wet the closed cell foam cuvette gasket - wetting the gasket will increase the leaf chamber humidity and result in a failed test. Open the cuvette and place the leaf stimulant envelope in the cuvette. Align the filter paper area of the envelope with the opening. Close the cuvette and cover the leaf chamber with a dark cloth (PAR sensors read 0).

You should observe a rapid rise in the cuvette water vapor differential (H2Od, mb) followed by a short stability phase of perhaps 1 minute duration, depending on the degree of wetness of the filter paper. Note this average H2Od value. As the paper dries due to evaporation the positive differential will fall back towards zero.

Open the RbCal.xls file available on the USB thumb drive (supplied by PP Systems when you first purchased your system or visit our web site and download it) and enter the required values. For Air Temperature, enter the cuvette temperature °C (Tcuv). Note that the evaporation rate (mmol m^{-2} s^{-1}) will be much higher than typical leaf transpiration rates. Ensure that r_b is ≤0.5 m^2 s mol^{-1}. Typical results range from 0.3-0.5. If not, rewet the filter paper (if dry) and rerun the test.

PLC3 Leaf Cuvette Head Adjustment

If the leaf cuvette head is not closing properly, a minor adjustment can be made using the adjustment screw located below the cuvette open/close lever as shown below.



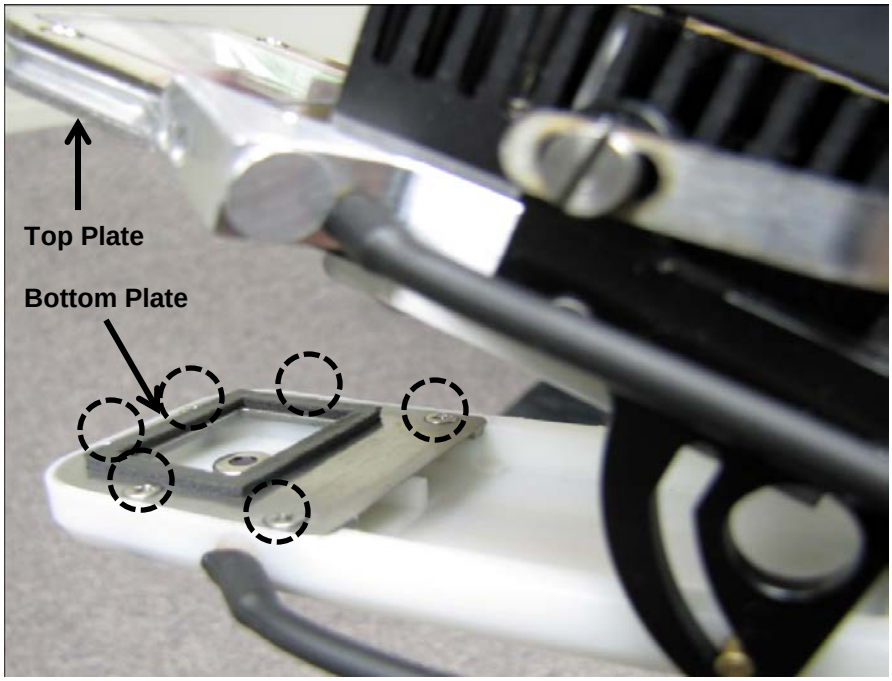
Use a small hex wrench to adjust the screw tension if needed to accommodate leaves of varying thicknesses. Turn the screw counter-clockwise to tighten and clockwise to loosen the tension. Normally no more than ¼ turn in either direction is needed. The goal is to establish a good seal without causing extreme pressure on the leaf or allowing ambient air to leak around the foam gaskets. Certain leaf types are inherently difficult to seal in the PLC3 cuvette: e.g. succulent plants, cacti, leaves with large midrib veins, woody shoots, presence of leaf exudates. The PLC3 cuvette is designed specifically to accommodate planar leaf laminae <3 mm in thickness.

Changing PLC3 Universal Head Plates

The PLC3 Universal is supplied with 3 different head plates:

- 25 x 7mm (1.75 cm^2)
- 18 mm Diameter (2.5 cm^2)
- 25 x 18 mm (4.5 cm^2)

Each head plate has a top and bottom so carefully note which one is the top and bottom. Each plate is held in place by 6 small Philip type screws.

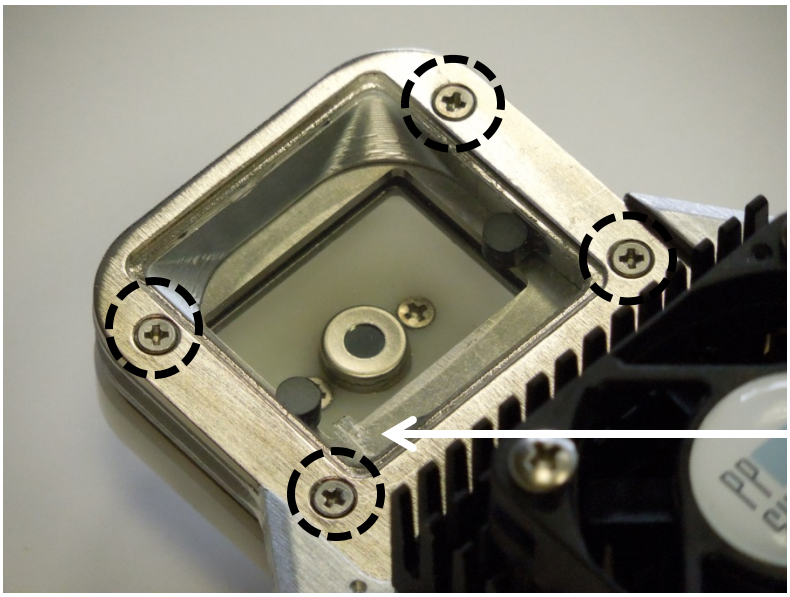


Open the cuvette head to maximum position and using a long, thin Philips screwdriver, remove the top and bottom head plates.

To ensure an air-tight seal, we recommend putting a slight smear of silicone grease between the head plate and cuvette head prior to securing the 6 screws. A slight smear of silicone grease can also be applied to the leaf gaskets themselves for improved leak tight performance.

Replacement of PLC3 Glass Window (For Use with CFM-3)

For measurement of chlorophyll fluorescence with the PLC3 Universal Leaf Cuvette, the top window must be glass. A glass window plate is supplied with every CIRAS-3 system supplied with the CFM-3 Chlorophyll Fluorescence Module.



Remove the four Philips type screws as shown here to fit the “Plain Glass Window Plate. To ensure an air-tight seal, we recommend putting a slight smear of silicone grease under the head plate prior to fitting it to the leaf cuvette head.

Check to make sure that the letter “P” is etched in the lower left hand corner prior to measurement of chlorophyll fluorescence.

After replacement of the window, we suggest that you perform a simple leak test to ensure that the system is air tight as follows:

- Set CO2r to 0 and H2Or to 100%.
- Close the leaf cuvette head with no leaf present.

Monitor the CO2r, CO2d and H2Od. After a couple of minutes, the CO2r should be close to 0 (± 1 µmol mol^{-1}), CO2d should read approximately 0 (± 1 µmol mol^{-1}) and H2Od should also read approximately 0 (± 0.5 mb).

Section 15. Consumables/Spares

The following items are common consumables and can be ordered directly with PP Systems.

Desiccants and Absorber Columns

Part No.	Description
STD006	Molecular Sieve Desiccant, 1.25 Lbs.
STD007	Self Indicating Soda Lime, 1.0 kg
STD008	Drierite, 1 lb.
30118-1	Filter Foam, Gray (CIRAS-3)
30013-1	O-ring 4.76 x 1.78
30013-19	O-ring 20.8 x 2.4
10123-1	Absorber Cap Assembly
10121-1	CO2/H2O Control Absorber Assembly (Complete)
10122-1	Zero Absorber Assembly (Complete)

Pumps & Air Filters

10127-1	Miniature Rotary Pump (ASF)
10128-1	ASU Pump (GAST)
30124-1	Air Filter, 5 micron
30124-2	Air Filter, 25 micron

CO_2/H_2O Control

STD030	Box of 10 8g. CO2 Cartridges (Can only be shipped by ground)
STD549	Cylinder Holder
STD546	Regulator Assembly
20577-1	Gasket CO2 Seal

Battery/Chargers

41526-1	Battery Pack Li-Ion 7.2V 12 Ah (CIRAS-3)
STD550	External Charger for CIRAS-3 Internal Battery Pack (Part No. 41526-1)
STD551	Belt Pack Kit - Battery, Charger, and Power Supply
STD552	Power Supply, CIRAS-3

Cables/Data Storage

STD545	USB Cable A Plug to 5p Mini B Plug
43034-1	USB Memory Stick

Leaf Cuvette Accessories

STD524	PLC3/6 Universal Gaskets, 18mm Diameter, Qty. 10
STD525	PLC3/6 Universal Gaskets, 25mm x 18mm, Qty. 10
STD526	PLC3/6 Universal Gaskets, 25mm x 7mm, Qty. 10
STD527	PLC3/6 Universal Rear Gaskets, 50mm x 35mm, Qty. 10
STD529	PLC3 Conifer/Narrow Gaskets, 80mm x 40mm, Qty. 10
20347-1	Gasket Plate, 25mm x 7mm, Upper (PLC3/6)
20347-2	Gasket Plate, 25mm x 7mm, Lower (PLC3/6)
20348-1	Gasket Plate, 25mm x 18mm, Upper (PLC3/6)
20348-2	Gasket Plate, 25mm x 18mm, Lower (PLC3/6)
20349-1	Gasket Plate, 18mm Diameter, Upper (PLC3/6)
20349-2	Gasket Plate, 18mm Diameter, Lower (PLC3/6)
10088-1	PLC3/6 (U) Plain Glass Window Assembly (for use with CFM)
10088-2	PLC3/6 (U) Calflex Window Assembly
20359-1	PLC3/6 (U) Window Plate Only
55021-1	PLC3/6 (U) Calflex Window Only
55021-2	PLC3/6 (U) Plain Glass Window Only (for use with CFM)