CIRAS-3

Portable Photosynthesis System

Operation Manual

Version 1.08

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PP Systems

110 Haverhill Road, Suite 301

Amesbury, MA 01913 U.S.A.

Tel: +1 978-834-0505 Fax: +1 978-834-0545

Email: support@ppsystems.com URL: www.ppsystems.com

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Welcome

Thank you very much for purchasing our **CIRAS-3 Portable Photosynthesis System**. We greatly appreciate your business and we look forward to working with you and your research team for many years to come.

This operation manual is based on our software Version 1.09 (which was released in February 2016) and above along with the following system firmware:

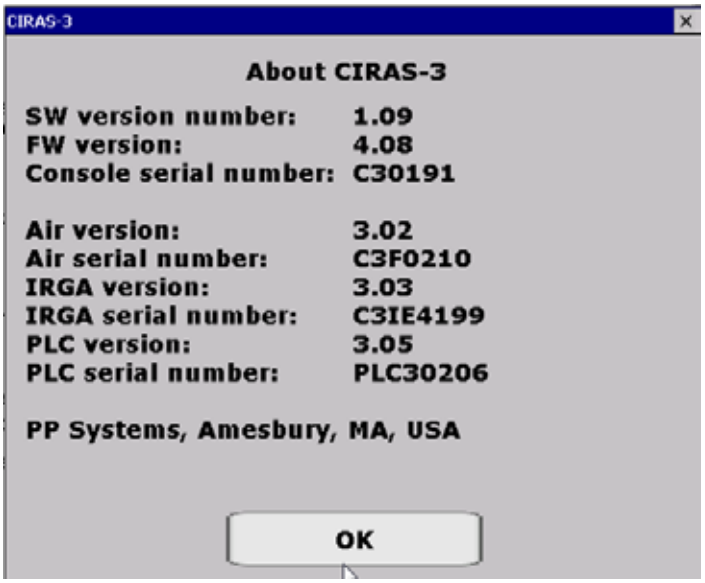
FW Version: V. 4.08 and above

Air Version: V. 3.02 and above

IRGA Version: V. 3.03 and above

PLC Version: V 3.05 and above

To check which software and firmware versions are currently installed on your CIRAS-3, go to **Help (F8)** > **About (F2):**



For customers running V. 1.09 console software along with the above referenced firmware, future software and firmware updates can be made locally without the need to return the system to our factory. Our latest software and documentation can be downloaded free of charge from our website if you are registered with us. For customers running earlier software and firmware versions, the CIRAS-3, PLC3 and Light unit (if applicable) may need to be returned to PP Systems for a factory update which will bring your system up to date and allow you to perform future updates locally without having to return the instruments to our factory. Please contact PP Systems for more information.

Information contained in this operation manual is subject to change without notice. Registered users of PP Systems products and instruments may obtain updated documentation and software by visiting the Users Area of our website.

This manual and the information contained within are copyrighted to PP Systems. No part of the manual may be copied, stored, transmitted or reproduced in any way or by any means including, but not limited to, photocopying, photography, magnetic or other mechanical or electronic means, without the prior written consent of PP Systems, Inc.

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 go to www.ppsystems.com and click on Customer Registration in the upper left hand corner.

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

PP Systems, Inc.
110 Haverhill Rd, Suite 301
Amesbury, MA 01913 USA
Tel: 978-834-0505 Fax: 978-834-0545
Sales: sales@ppsystems.com
Support: support@ppsystems.com
Service: service@ppsystems.com
URL: www.ppsystems.com

Unpacking And Storage Of Your Equipment

Unpacking

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.

Storage

We highly recommend storing your equipment in a safe, dry location. If you store your system in the rugged, black transport case supplied with your system, please refer to the very important Tip below.

TIP

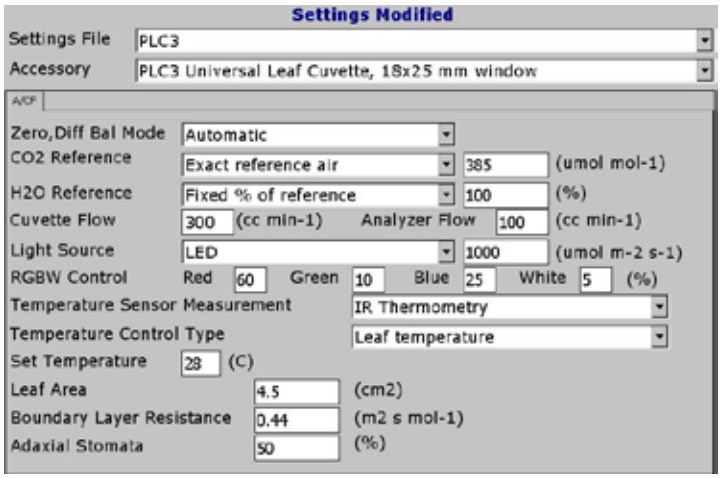
*If the CO_2 cartridge has CO_2 in it, we **STRONGLY** recommend removing the entire CO_2 regulator and holder assembly from the console and storing it separately. See* **CO2 Regulator** *on page 146.* *Otherwise, CO_2 will slowly accumulate overnight inside the case which will result in absorbtion into the PLC tubing as well as the internal foam material affecting CO_2 measurements the following day.*

If you are confident the CO_2 cartridge is empty and fully exhausted, there are no issues and you can store the entire regulator and holder inside the case with the system.

Quick Start

We highly recommend that you take a few moments to run this simple test to A) familiarize yourself with the basic CIRAS-3 set-up and operational functions and B) ensure that the system is performing perfectly before starting a measurement campaign.

1. Insert new CO_2 cartridge, ensure all chemicals are fresh and connect the CIRAS-3 charger to the CIRAS-3 main console (this will help to preserve the battery).
2. Connect the PLC3 Leaf Cuvette gas and signal connectors to the CIRAS-3 main console, close the cuvette (with no leaf present) and press the CIRAS-3 On/Off switch to power up the system and then **Continue (F1)**.
3. Allow system to warm up for approximately 30 minutes. Press **Settings (F2)** and make sure that that your Settings File and Accessory displayed are correct (change if necessary). Assuming that you are using the PLC3 Universal Leaf Cuvette and PLC3 Universal LED Light Unit, set up as follows and press **Accept (F2)**:



Give your system a couple of minutes to stabilize at the values that were set under **Settings (F2)**. Once stabilized, your display should look something similar to this.

Observe the following:

- All measured data are stable and CO2d is 0 (± 0.5 ppm) and H2Od is 0 (± 0.5 mb).
- Tleaf is reading 28 ^{o}C (± 0.2 ^{o}C)

If everything looks like this you should be good to go. Good luck!

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 10000 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 or 0-100% Ambient
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 (Calflex) · 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 (Calflex) **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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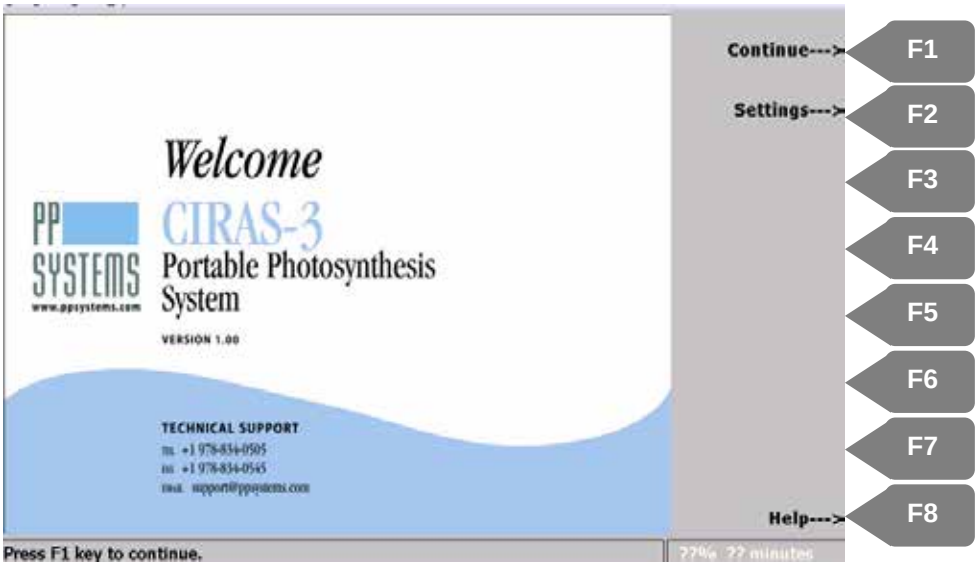
Section 2. What's New with CIRAS-3?



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 4.5 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 character, 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 all PLC3 leaf cuvettes. This is in addition to the 2 silicon photodiode sensors beneath the cuvette window (PLC3 Universal) and 1 silicon photodiode sensor beneath the cuvette window (PLC3 Narrow and Conifer).

LED Light Unit (RGBW)

Outwardly the LED light unit looks nearly the same as previous versions, but it is 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 for PLC3 Universal Leaf Cuvette and 96 individual LEDs for the PLC3 Conifer and Narrow Leaf Cuvettes. 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 (For use with the PLC3 Universal only)

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 flash saturating pulse (Multipulse) 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 performed 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/SRC-2 – soil collar volume/area input is a new Settings input, display and graph ΔCO2, FCO2, evaporation, Tsoil

CPY-4/CPY-5 – collar volume/area input is a new Settings input, display and graph ΔCO2, canopy Assimilation/Respiration, transpiration rate, Tchamber, PAR

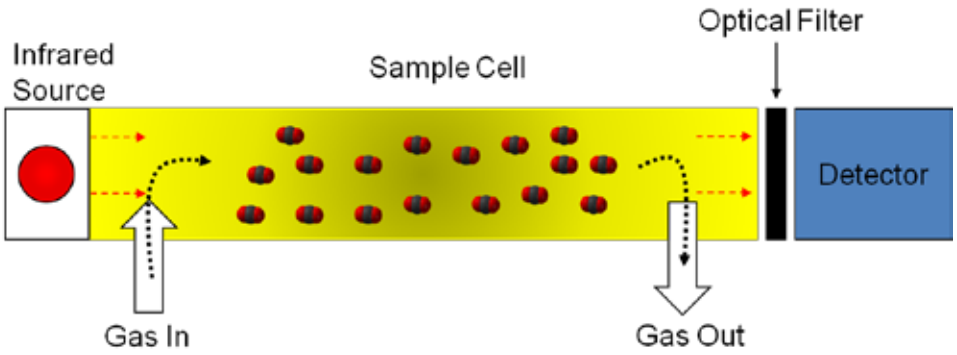
Section 3. Summary of System Design

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.

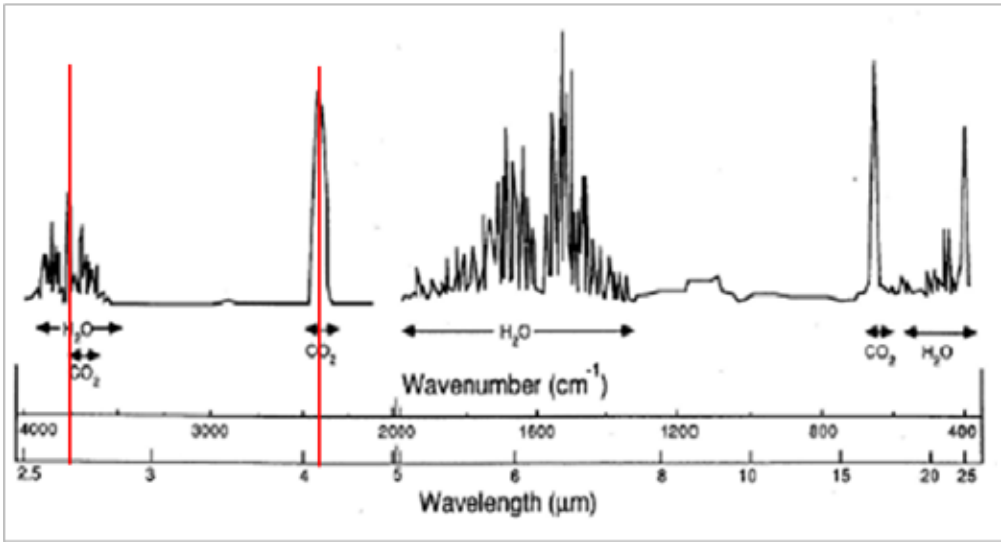
The IRGAs form the core of gas analysis systems that measure CO_2 and water vapor (i.e. portable photosynthesis system, eddy covariance, soil CO_2 efflux, etc.). Non-dispersive infra-red (NDIR) refers to the transmission of broad-band infra-red wavelengths from the IRGAs source lamps. A single IRGA consists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



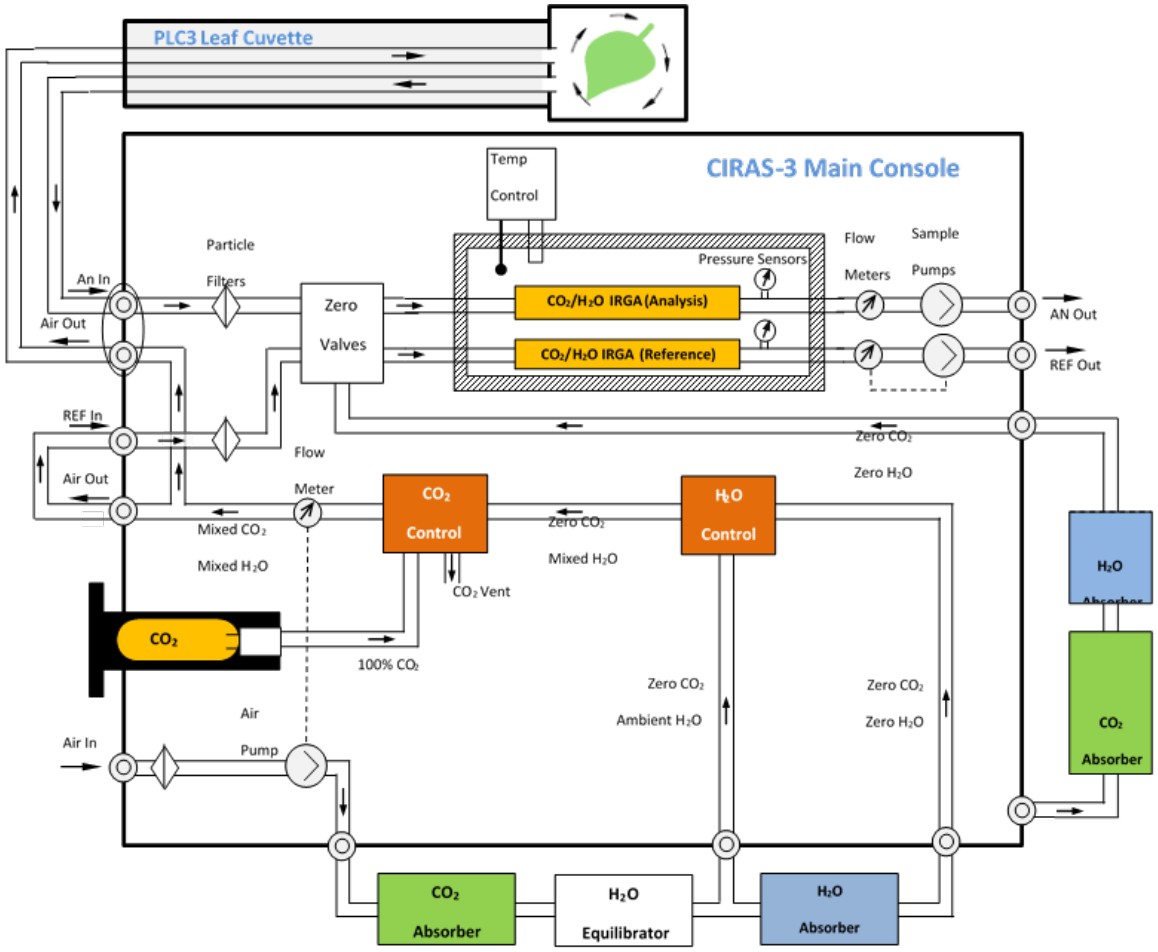
The gas sample is of course a mixture of gas molecules, and this can present problems in terms of accurate detection of concentrations of a specific gas, such carbon dioxide. This effect, *foreign gas broadening* (FGB), must be corrected to ensure accurate measurement of gas concentrations. With FGB, 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 CO_2 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 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 are 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

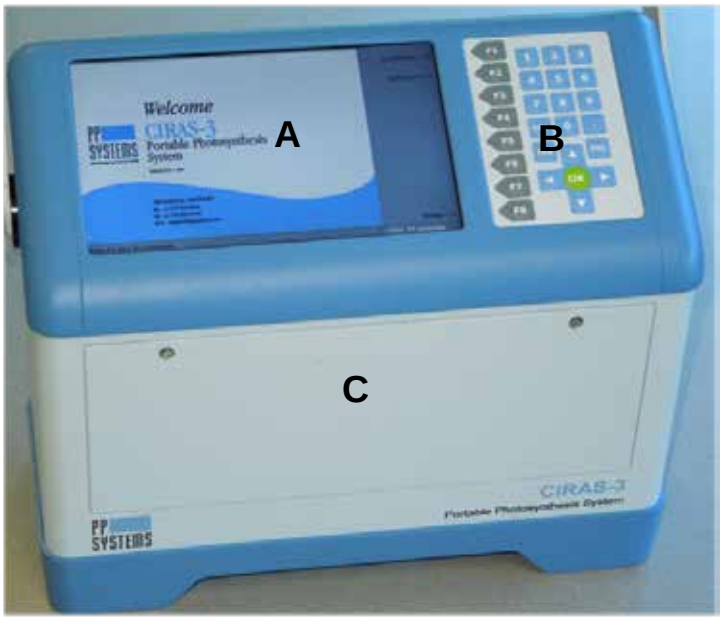
CIRAS-3 Main Console

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.

Battery

The CIRAS-3 is powered by an internal rechargeable 7.2V Li-Ion battery pack. 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 three major factors that influence battery capacity the most are:

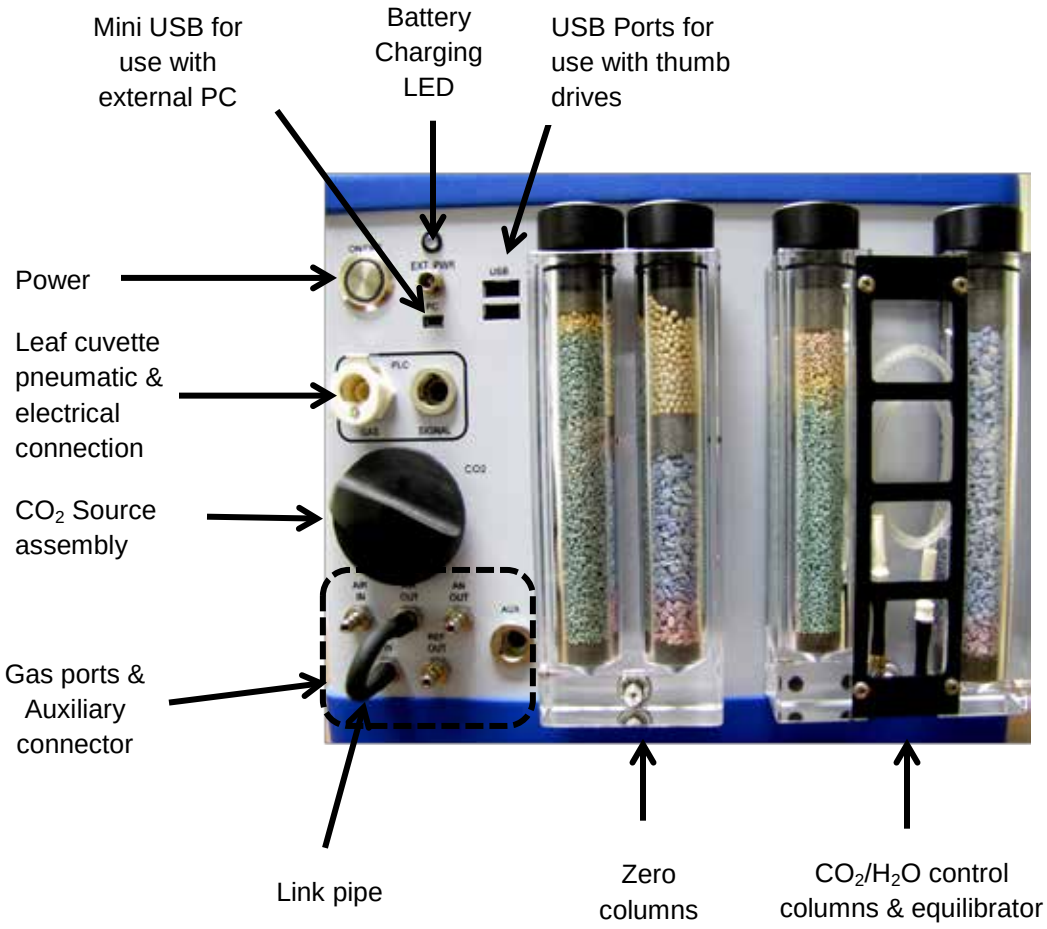
- Temperature Control
- Light Control
- CFM-3 Chlorophyll Fluorescence Module

In the laboratory this is not a problem due to availability of AC power. However for field use, it is extremely important to make sure that the internal battery is fully charged and that you have a back-up battery pack or an external 12V battery (i.e. marine/motorcycle battery) for additional power if required. We also offer a nice, lightweight external battery pack that can clip easily to your belt. See External Battery Pack (For extended operation in the field) on page 135 for more details.

TIP

In situations where you have a gap in between measurements (i.e. moving from site to site) and you want to conserve power, this can easily be achieved by temporarily reconfiguring the system as an "Analyzer only" and by turning the backlight off. To do so, go to ***Settings (F2),*** *tab to the "Accessory" field, arrow up to "Analyzer only" and then* ***Accept (F2).*** *Next, go to* ***Controls (F3)*** *and select* ***Toggle BL (F6).*** *This will turn the backlight off. The IRGAs will continue running and will stay at operation temperature. When you are ready to resume measurements, simply reconfigure your system accordingly under Settings (F2) and you are back in business.*

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 LED glows green 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 CO_2 cartridge fits inside the cartridge 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. For leaf gas exchange measurements using any of our PLC3 leaf cuvettes, this link pipe must be connected between the **REF IN** and **AIR OUT**. 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 clearly 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. Self-indicating and non-indicating Soda lime and Drierite can be used with the CIRAS-3. Self-indicating Soda lime will change from green to a brown or white to violet color as it exhausts and self-indicating Drierite changes from blue to a pink color. Also, Drierite can be regenerated after exhaustion by placing the exhausted Drierite in a

shallow pan and in a single layer of granules and placing it in a preheated oven for 90 minutes at 230 ^{o}C or 450 ^{o}F. After 90 minutes, return the regenerated Drierite into a glass container and be sure to seal the lid. Soda lime can't be regenerated and should be discarded when exhausted. Importantly, molecular sieve does not indicate its state by color change. A good rule to follow is when a self-indicating desiccant in a column is 50% exhausted, change all the chemicals in that absorber group. **Additionally, change molecular sieve on a weekly basis during use and most certainly prior to first measurement campaign**. **ALWAYS** change self-indicating desiccants when the column is approximately 50% exhausted. **If using non-indicating desiccants, we recommend changing them out prior to each measurement campaign to play it safe.** 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 but again the Drierite can be regenerated if required as described above. Note that the cap has a rubber sealing ring. From time to time place a light coating of silicone grease around the "O" rings to keep them moist and to prevent air leaks and cracks.

For more information on the desiccants including handling, maintenance, MSDS, etc., please refer to Desiccants and Absorber Columns on page 156.

TIP

Always change the Molecular Sieve weekly during use. See Desiccants and Absorber Columns on page140.

Always perform a leak test after changing desiccants. To do so, insert a fresh CO_2 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}).

Molecular Sieve Repackaging

The Molecular Sieve is originally supplied by PP Systems in tin packaging. After initial opening, we strongly urge all users to repackage the Molecular Sieve in small glass containers with a screw top to seal the desiccant from room air. This desiccant saturates very quickly in room air and if not properly stored it will cause it to go bad and subsequently affect CO_2 readings and calibration.

To ensure a good seal, we also recommend putting some electrical tape around the screw top as shown here. If you have any questions, get in contact with PP Systems.

CO_2 Cartridges

We supply and recommend the CO_2 cartridges that are manufactured by ISI. They are 8g and are normally supplied in boxes of 10 as shown below.

Manufacturer: ISI (Vienna, Austria)

Part No. 0018 Soda Chargers (10 Pack).

Contact Information:

iSi GMbh

Kürschnergasse 4

A-1217 Vienna, Austria

Tel: +43 1 25099 0 Fax: +43 1 25099 555

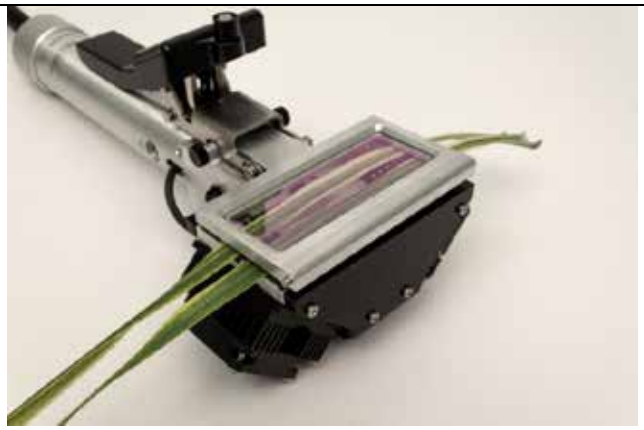
Email: info@isi.at URL: www.isi.at

The manufacturer (iSi) has distributors throughout the world so it should be very easy for all customers to source this item locally. Due to the classification of these CO_2 cartridges, PP Systems can only ship them by ground service in the United States.

NOT ALL CO_2 CARTRIDGES ARE THE SAME AND SOME MAY EVEN CAUSE DAMAGE IF USED WITH THE CIRAS-3. They come in all different sizes, shapes and contents (some even have oil) and are commonly used for things like pellet guns, soda siphons, etc. If you have any questions whatsoever regarding the CO_2 cartridges you are urged to get in contact directly with PP Systems.

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 PLC3s 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 PLC3s. 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 PLC3s 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 due to 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 temperature (Tcuv) 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. Automatic control of "leaf temperature" is heavily influenced by the following 3 variables:

1. Leaf transpiration in the cuvette
2. Light (incident radiation)
3. Size and construction of the PLC window

At lower light intensities, leaf temperature control is wider and at high light intensities it is much tighter. We recommend that when using "Set leaf temperature" as the Temperature Control Type under Settings (F2) that you do so maintaining leaf temperature (Tleaf) at or near ambient levels especially at high light intensities and transpiration rates. Although all PLC3s include temperature control as standard, there are some key differences between each type 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.

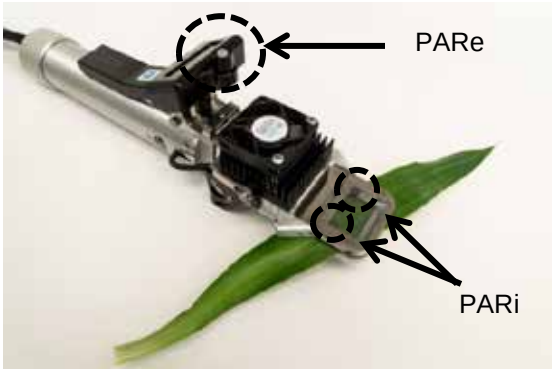
PAR Measurement

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.

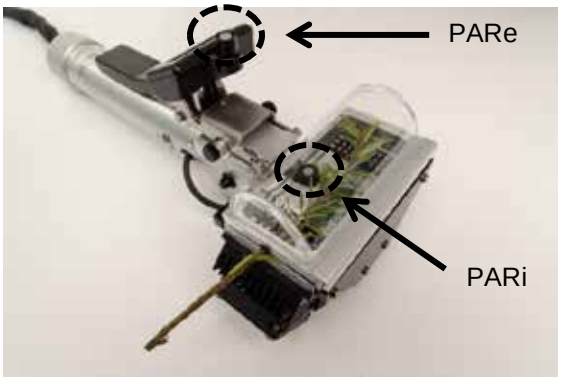
PLC3 Universal - includes two mini-silicon photodiode sensors beneath the cuvette window (PARi). The silicon photodiode sensors are close to the leaf plane and used to average the irradiance beneath the cuvette window. Irradiance is somewhat attenuated (approx. 10% attenuation) by the window. This affects 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.

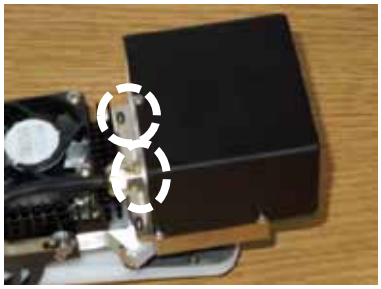
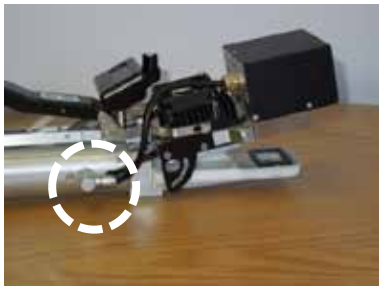
PLC3 Universal LED Light Unit (RGBW)

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.

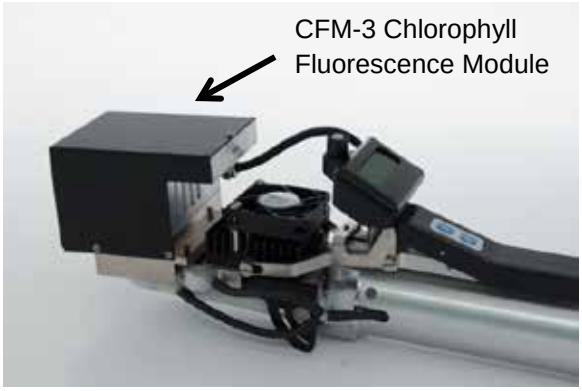
The PLC3 Universal LED light unit is designed to mate quickly and easily with the PLC3 Universal leaf cuvette.

Step 1.	**Step 2**.	**Step 3**.
First, mount the light unit by aligning it to the front of the cuvette upper jaw as shown below.	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.	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 for the PLC3 Narrow and Conifer leaf cuvettes feature 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 0-2000 µmol m^{-2} s^{-1} (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 Conifer or Narrow leaf cuvette.

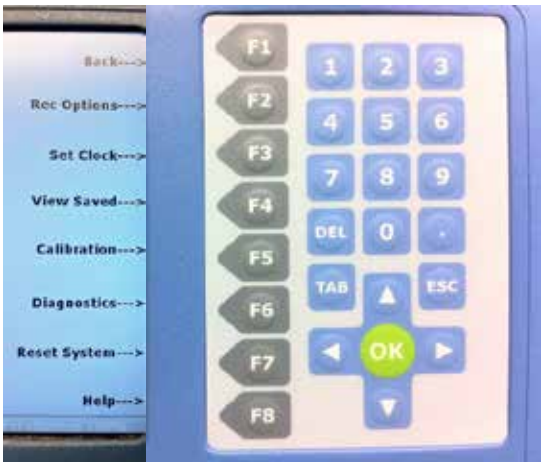
Step 1. Secure light unit to notches on cuvette head.	**Step 2.** Pull Light Unit over cuvette and secure locking screw.	**Step 3.** Connect electrically.

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

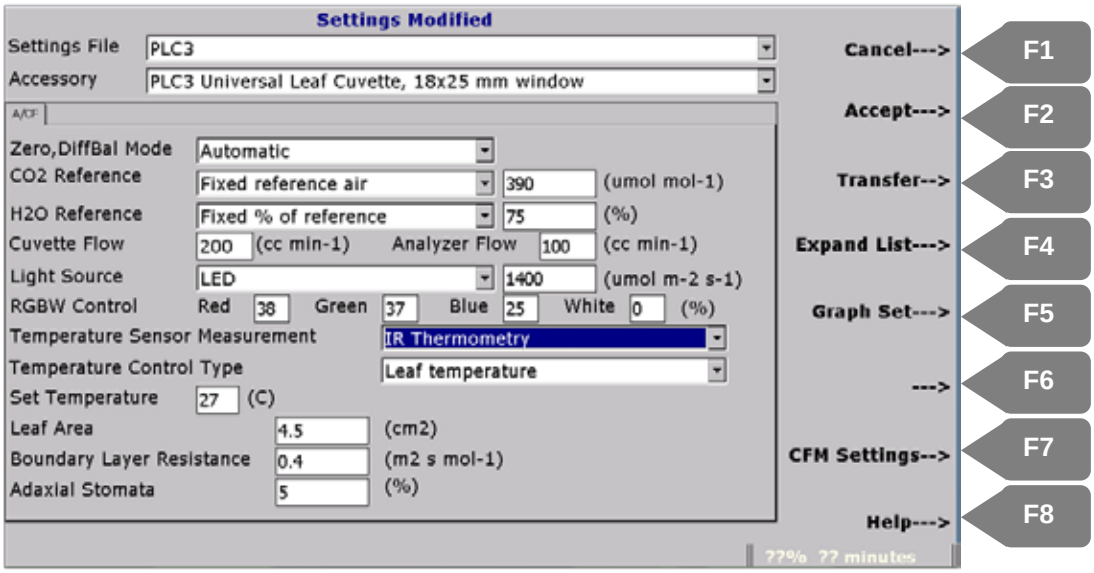
The CIRAS-3 console software is the most user-friendly and intuitive software we have designed to date. All CIRAS-3 functions are accessed and implemented by the same easy-to-understand software structure – Settings, Controls, Recording, Data Transfer, Diagnostics, Calibrations and other useful functions. 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

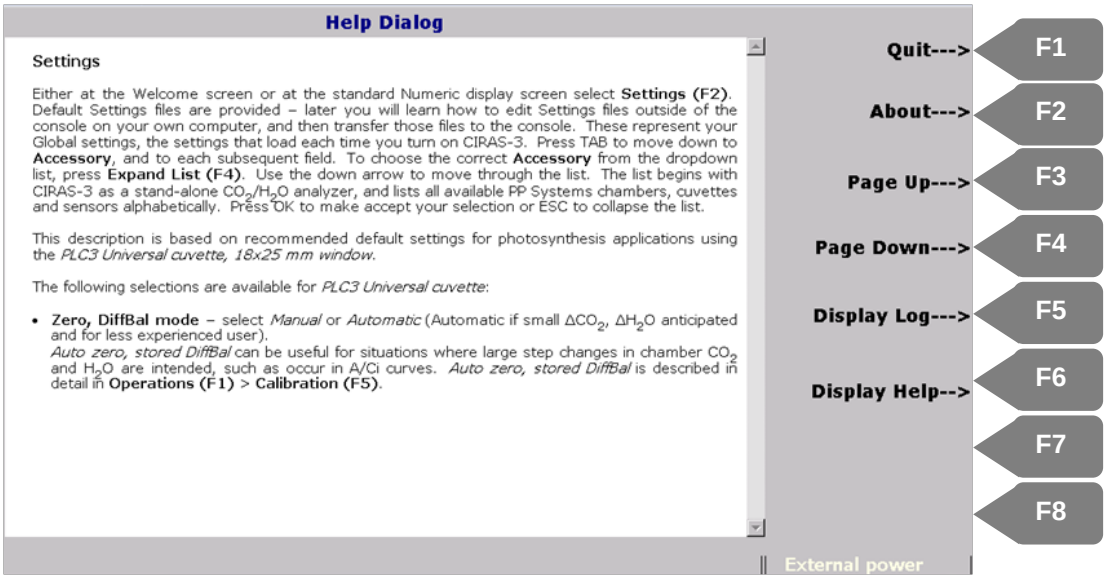
Let's begin with a quick example of an action you are likely to perform – establishing the repeatable baseline settings features that will appear each time you power on your system. We refer to these settings as global, that is they define the operational environment of CIRAS-3 prior to making measurements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

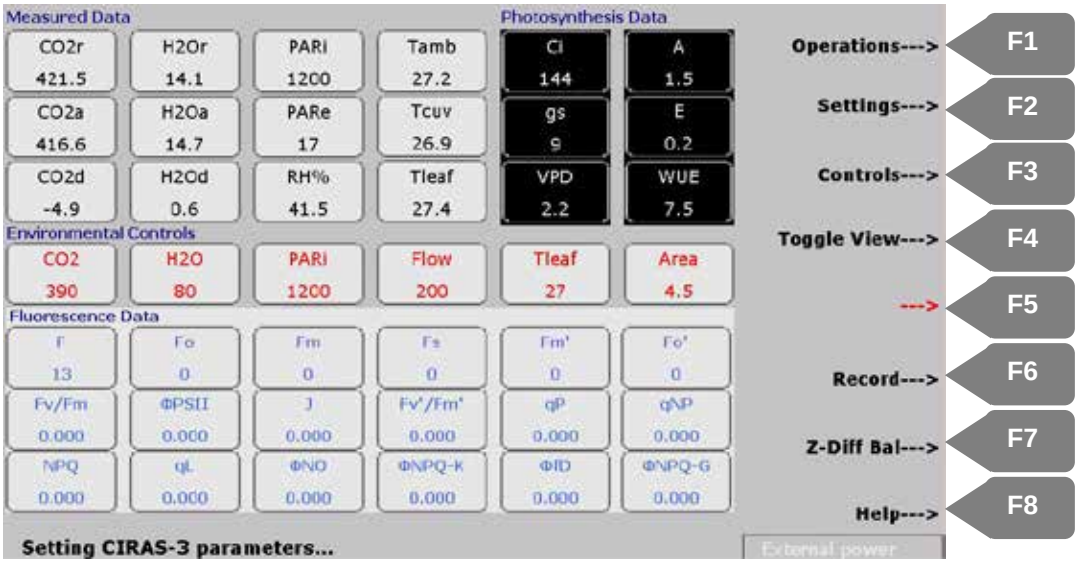
Controls functions are different from **Settings** in that they are used to update settings and make necessary adjustments dynamically while operating CIRAS-3. During normal operation you will use **Controls (F3)** almost exclusively to make alterations to the leaf chamber environment and to customize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

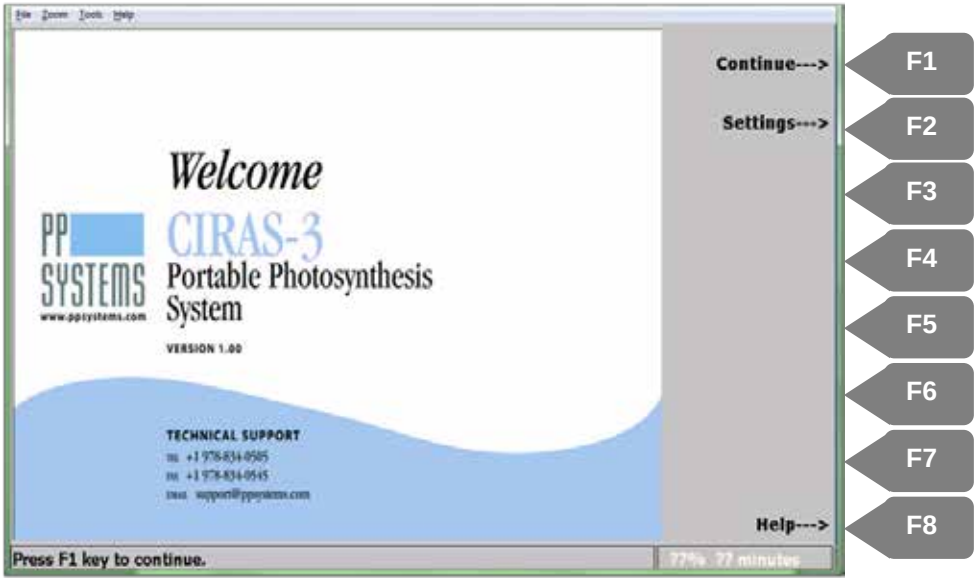
Before You Power Up CIRAS-3

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 Li-ion battery the night prior to field measurements using the charger/power supply provided. The indicating LED on the CIRAS-3 console will be a steady green when connected to the charger/power supply. The actual state of the battery can be viewed in the lower right hand corner of the display.
- 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 charger/power supply during warm-up to preserve the battery.
- If using a CO_2 cartridge, make sure that one is in place with enough capacity to get you through your measurements. See CO2 Cartridge Status on page 39.

Startup and Warm-up

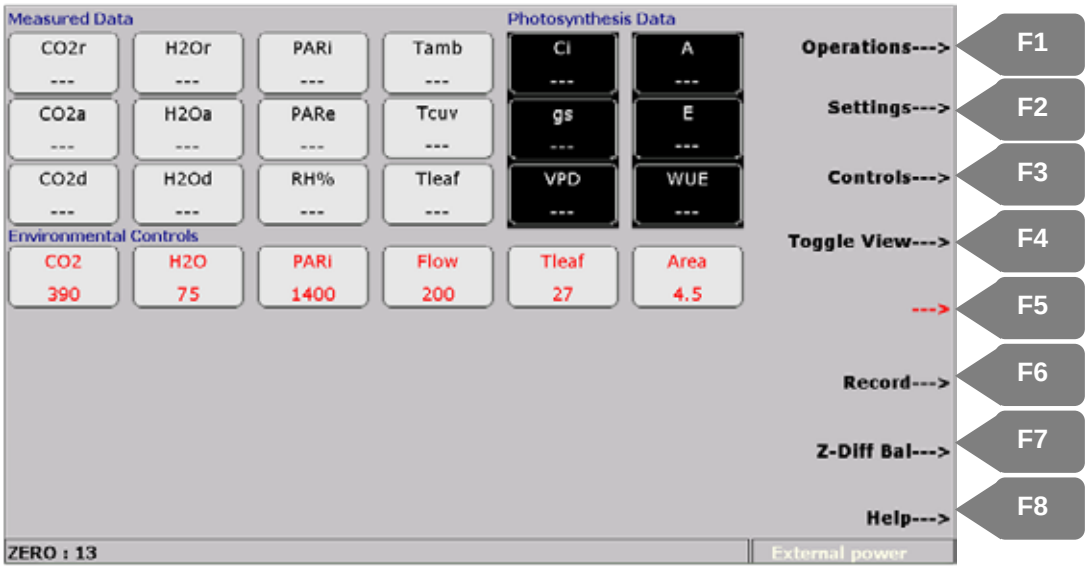
Press and release the ON/OFF button, it should illuminate blue. It takes approximately 30 seconds for the first Welcome display to appear on the console screen.



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 based on ambient temperature. The warm-up period can be only a few minutes in a mild or warm environment (in the laboratory) 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.

Once the IRGA achieves its target temperature of 55 °C, it will then perform both a Zero and Diff Bal cycle at time 0, 6, 12, 23, 39 and 59 minutes. After this, it will perform a Zero only once every 31 minutes followed by a Diff Bal. A Diff Bal cycle will also take place when there is a large change in CO_2 (100 µmol mol^{-1}) or H_2O (5 mb) concentration.

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.

CO_2 Cartridge Status

How do I know when to change the CO_2 cartridge? This is a common question that is asked especially when several different people are using the CIRAS-3 and it is unknown as to when a new cartridge was inserted into the CIRAS-3. We can't meter the cartridge precisely 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. The rule of thumb is that a newly inserted CO_2 cartridge will last at least one full days operation. The actual life of an individual CO_2 cartridge is dependent on:

1. Hours of operation
2. CO_2 control range

For instance, if the CIRAS-3 is operating for long periods throughout the day and at high CO_2 concentrations, we would expect that when you turn on the system the following day the CO_2 cartridge will be low and it is safe to change out. If the CIRAS-3 is operating for just a short period of time throughout the day (i.e. less than 4 hours in total) and at ambient CO_2 concentration (i.e. 390 µmol mol^{-1}), you will likely have enough CO_2 to get you several more hours of use the day after a CO_2 cartridge is installed.

If unsure of how much CO_2 is still available from the cartridge, we recommend the following simple test:

1. Power up the CIRAS-3 system as described above.
2. After warm-up and with the numeric or graphical display on the console LCD, press **Controls (F3)**.
3. Set the "CO2 Reference" to 2000 and hit **Accept (F2)**.

Monitor the CO2r (CO_2 Reference) value. After a minute or so, it should reach 2000. If the CO2r reaches 2000 you can proceed with measurements without having to change out the CO_2 cartridge. Note, keep an eye on the CO2r during your measurements and if you observe the CO2r starting to drop slowly this is a good indication that the cartridge is exhausting and we recommend changing it. If it does not reach 2000, this is a good indication that the CO_2 is low or starting to get low and it is safe to change out the cartridge as the CO2r will likely become unstable within a short period of time.

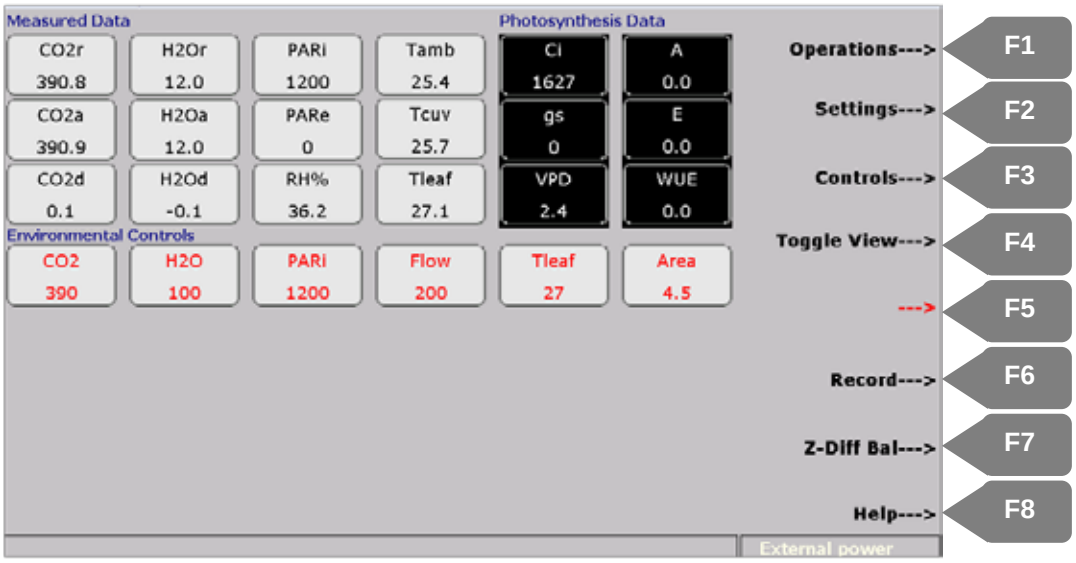
Changing The CO_2 Cartridge

This is very easy to do. With the CO_2 cartridge holder still inserted into the CIRAS-3 console, turn the holder slowly to the left (counter-clockwise) until you start to hear the gas release. The regulator will now be loose and you can safely remove the entire regulator assembly from the console by pulling it out. Finish removing the CO_2 cartridge holder from the regulator by hand. **Do not attempt to remove a CO_2 cartridge for at least 24 hours after it was inserted and with the CO_2 regulator assembly fitted to the CIRAS-3 console. If you do, the pressurized cartridge will make a loud popping noise and the pressure may cause the internal tubing associated with the gas blender to be removed from the back of the CO_2 regulator or gas mixing diverter valve**. With the CO_2 regulator now removed from the CIRAS-3 console, insert a new CO_2 cartridge into the holder and screw it into the regulator turning the holder clockwise 1-2 full turns. Place the regulator into the CIRAS-3 console. The regulator assembly should move inward a few more mm. Now it is in a locked position and you can complete the installation by turning the cartridge 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 so continue to keep turning the holder clockwise until it is snug. 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.

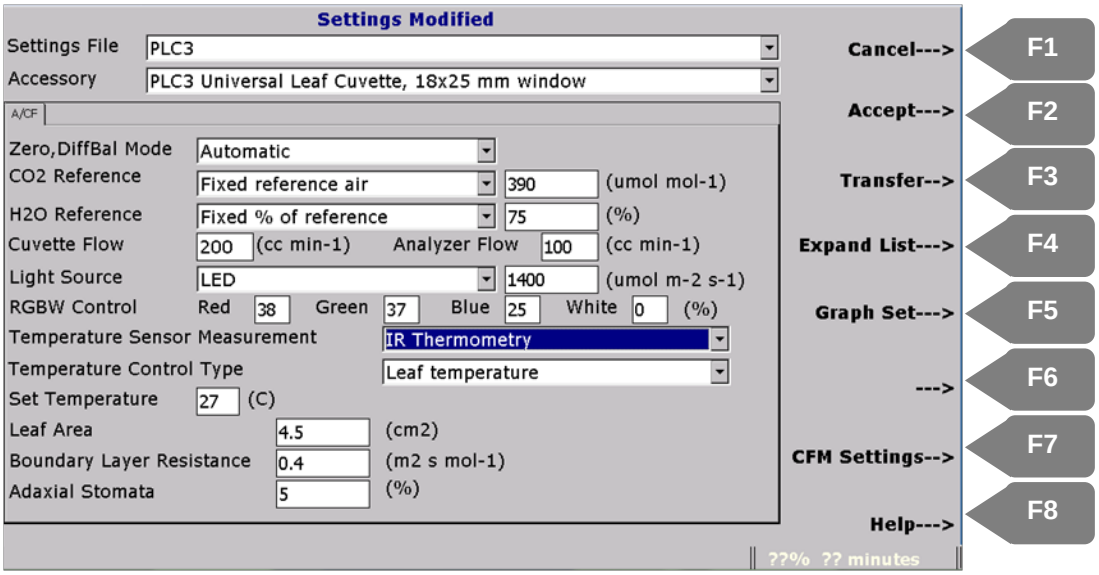
Additional Filtering in Dirty/Dusty Environments

If working in extreme dirty/dusty environments, we strongly recommend using the external air in filter assembly (included in your CIRAS-3 spares kit) as shown below. It easily connects to the CIRAS-3 "AIR IN" port as shown below:

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

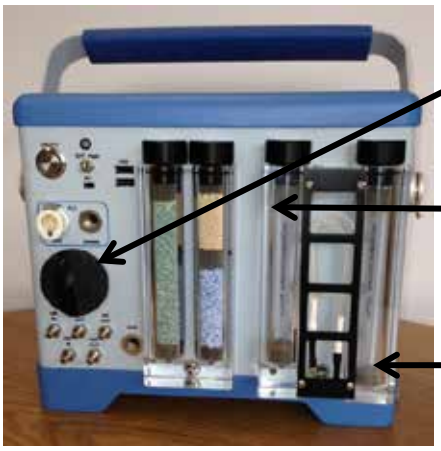
Settings (F2)

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 (AnalyzerOnly) followed by a list of 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 every 31 minutes. We recommend this mode if small ΔCO_2 and ΔH_2O is anticipated and for less experienced users. A Diff Bal cycle will also take place when there is a large change in CO_2 (100 µmol mol^{-1}) or H_2O (5 mb) concentration. **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 on page 69).
CO2 Reference	**Approximate reference air.** The CO_2 supplied to the leaf chamber from CIRAS-3's internal CO_2 source will be within 30 µmol mol^{-1} from the actual set value within the control range of 0-2000 µmol mol^{-1}. Prior to using this option, you should perform a Max C (See Find Max C on page 67) for optimal accuracy and control. 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). **Exact 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 or for long periods, 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 (CO_2 cartridge) is not used. If this option is selected, the CO_2 regulator should be empty but in place and the soda lime must be removed from the CO_2/H_2O control column as shown below. Humidity control can still be available if the H_2O Absorber column is filled with Drierite. CO_2 regulator in place (with no CO_2 cartridge inserted) Empty CO_2 absorber column for ambient measurement of CO_2 Note, H_2O control is still available with Drierite in this absorber column

H2O Reference (chamber humidity)	**Fixed % of reference.** This option 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**). Anticipate that transpiration water vapor from the leaf will increase chamber humidity, and maintain chamber RH <70%. At times you may see a warning in the instrument status bar (lower left) 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.** Allows you to 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).** 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.  CO_2 regulator in place (with no CO_2 cartridge inserted) Empty CO_2 absorber column for ambient measurement of CO_2 Empty H_2O absorber column for ambient measurement of H_2O
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 the CIRAS-3 controls water vapor, chamber humidity and VPD through the desiccants and 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 changed.
Light Source	**LED**. Select this if using one of our standard LED light units. Select your desired **Light Intensity** from 0-2500 µmol m^{-2} s^{-1}. Maximum light intensity will be dependent on type of light unit. **Ambient.** For measurements under natural conditions (No light unit).
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 (i.e. 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** and instead select **Energy Balance** if, for example, you are trying to place narrow leaf blades in parallel (such as grass leaves) or a leaf that does not fill the entire window. **Energy Balance** must be selected when the cuvette window does not have complete coverage by the leaf. E**nergy Balance** can be used at any time. **Thermistor** if using the thin wire temperature sensor supplied with the PLC3 Conifer or Narrow cuvettes, otherwise select **Energy Balance**.
Temperature Sensor Control Type **(**Available options are dependent on PLC3 type)	**Leaf Temperature**. 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 temperatures allow fixed inputs in 1 °C increments. Select **Leaf temperature** to hold the leaf at a fixed temperature that you enter under "Set Temperature" (see below). **Track leaf to ambient** allows the leaf temperature to follow ambient 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 under "Set Temperature" (see below). **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 but can also be used for field measurements if required. This option is not recommended for conditions that include high ambient temperature and/or high light intensities as it will cause the cuvette to heat up.

Set Temperature **(**Available options are dependent on PLC3 type)	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 internal battery and the following: 1. Leaf transpiration in the cuvette 2. Light (incident radiation) 3. Size and construction of the PLC window
Leaf Area	The default value will be based on your **Accessory** selection in the Settings dialog. 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 perform leaf area analysis 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 "Tested" label on your leaf cuvette. In future this will also be available in software. Users should measure the boundary layer when changing out the PLC3 Universal window as this value will vary from window to window.
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.

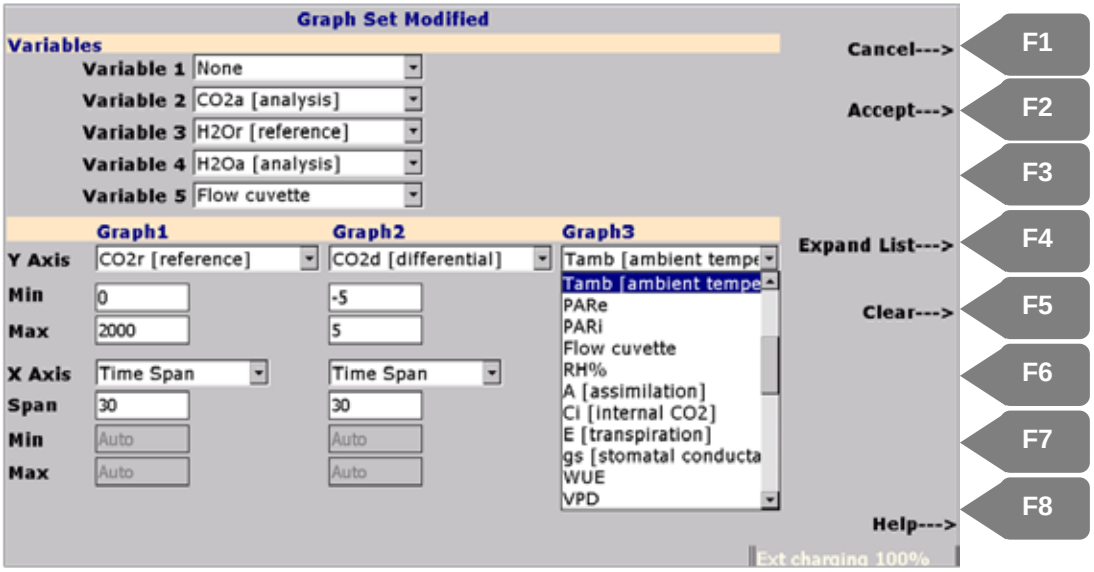
Settings Graph Set (F5)

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.

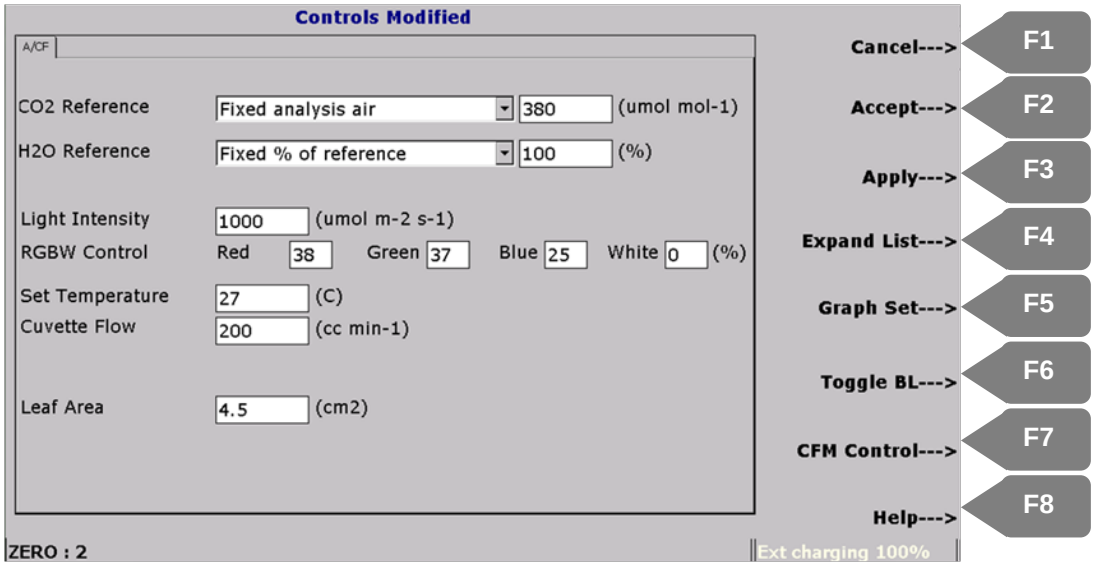
X Axis is the next dropdown list. The first variable that it contains is Time Span – choose this if you want a single-variable plot, displayed over a period of between 2 and 60 minutes. Enter the time in the next field, **Span**. **Min** and **Max** will be grayed out. Enter any variable except Time 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 values in the Settings dialog. 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) on page 42.
H2O Reference (chamber humidity	Options are identical to those described in **Settings (**See Settings (F2) on page 42.
Light Intensity	Options are identical to those described in **Settings Settings (**See Settings (F2) on page 42.
RGBW Control	Options are identical to those described in **Settings Settings (**See Settings (F2) on page 42.
Set Temperature	Options are identical to those described in **Settings (**See Settings (F2) on page 42.
Cuvette Flow	Alteration to an existing flow rate causes transient disruption of CO_2 control which in turn affects calculated assimilation rate and Ci so 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.

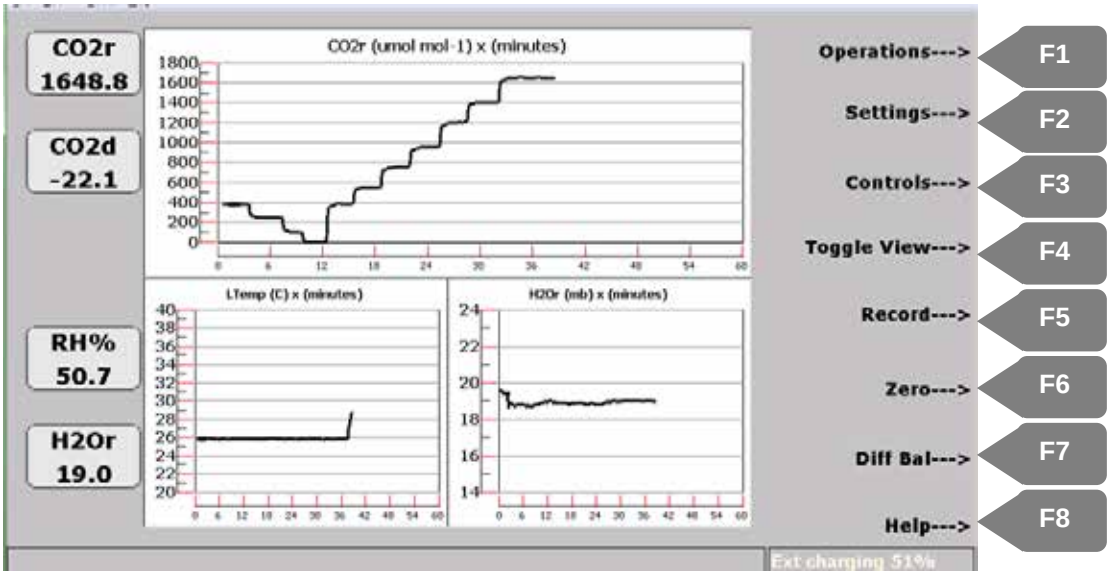
Leaf Area	Options are identical to those described in **Settings (**See Settings (F2) on page 42.

Controls Graph Set (F5)

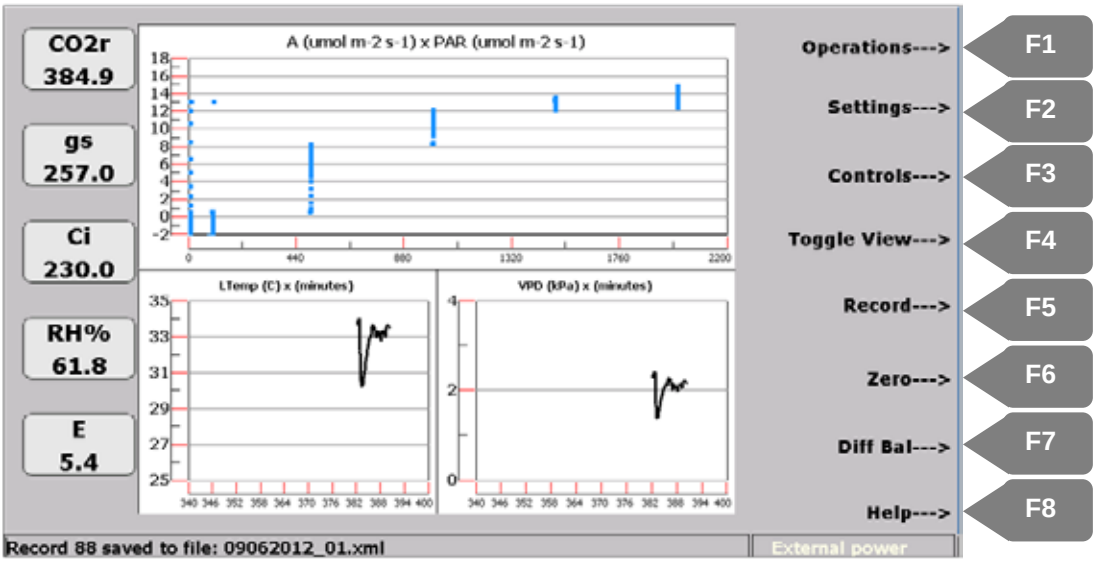
Press **Controls (F3) > Graph Set (F5)** to change your Graph View options. You will find this especially useful after completing system stability checks, when a leaf is in the chamber. Then, your focus will likely be on calculated parameters more than measured parameters. Because the dynamic range of 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. 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}.



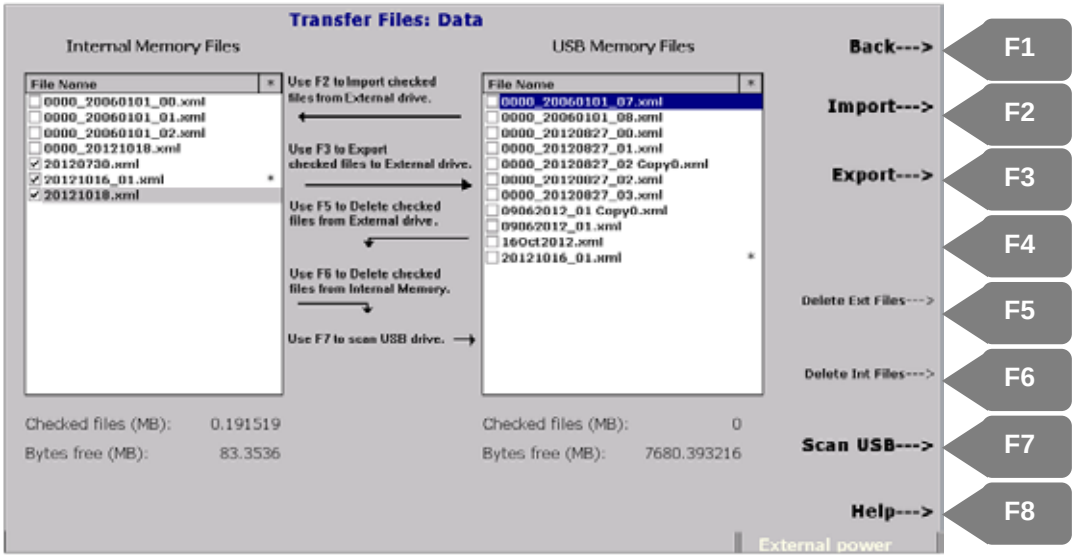
Reviewing and Transferring Data Files

Transfer Data (F6)

Retrieving your .xml data files from CIRAS-3 internal memory is a routine operation that 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 stored on the USB device – only compatible .xml files will appear.

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)
- Firmware Upgrade (FW Upgrade-F7)
- Help (F8)

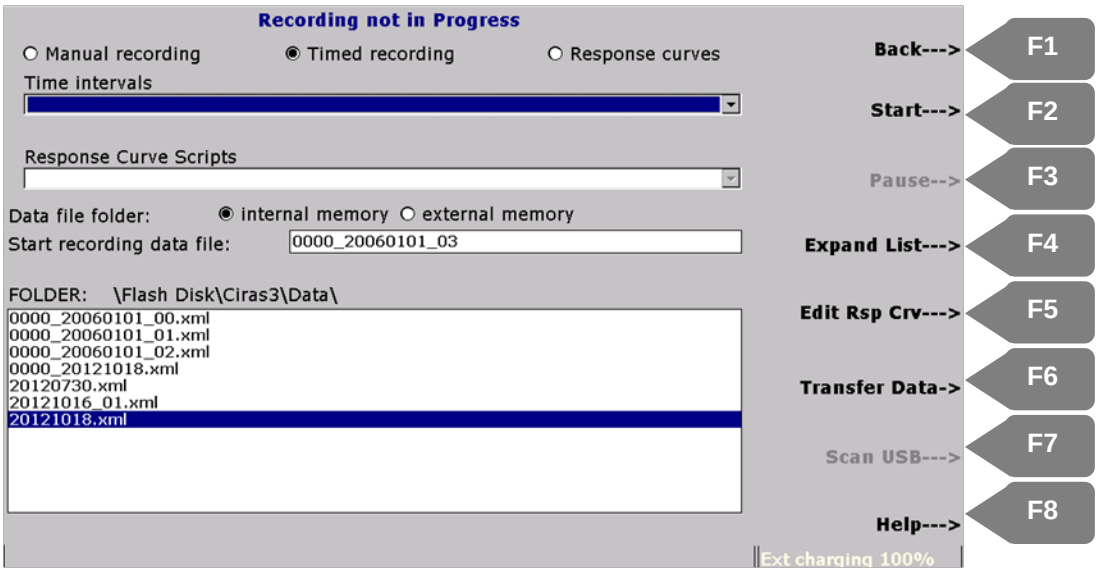
Recording Options

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

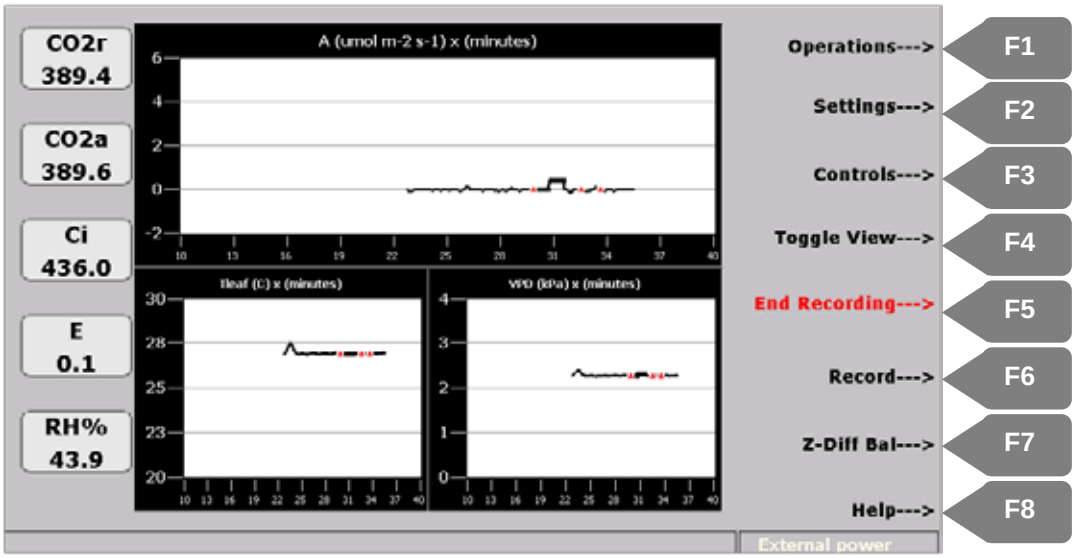
Select the desired recording option at the top of the screen by using the Left or Right arrow keys – the first option is **Manual recording**. For most leaf gas exchange measurements in the field, this method of recording is most common.



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.

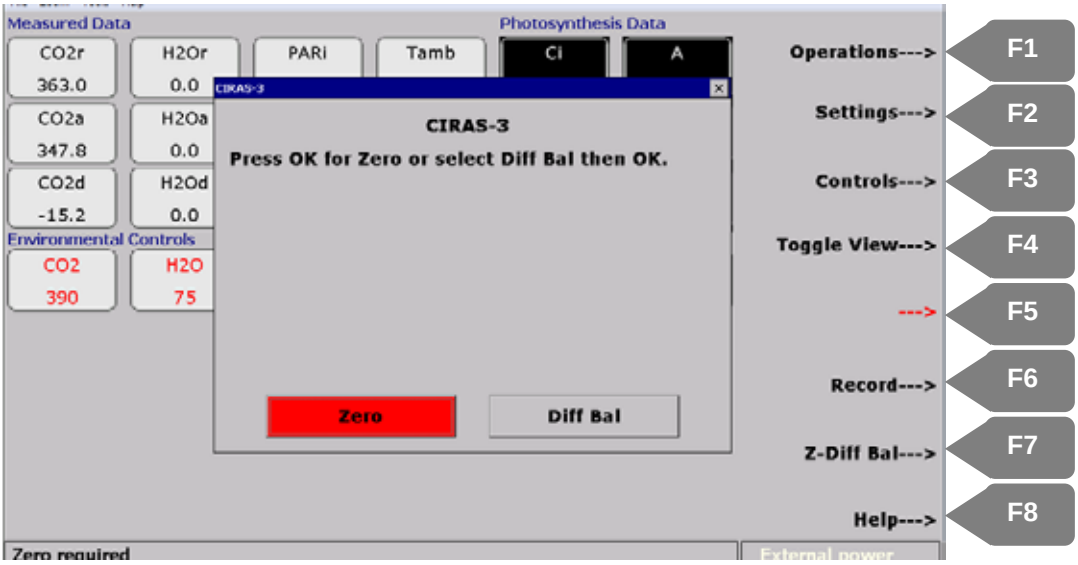
Press **Start (F2)** to begin recording data – you are automatically returned to the Operations screen, The status bar indicates that you are now in Recording mode, press **Back (F1)**. At this point you can use Graph Set to enter your preferred combination of numeric data, graphics and x-y scaling, 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 manually 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. Press **ESC** to cancel.



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 corrects for any small 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

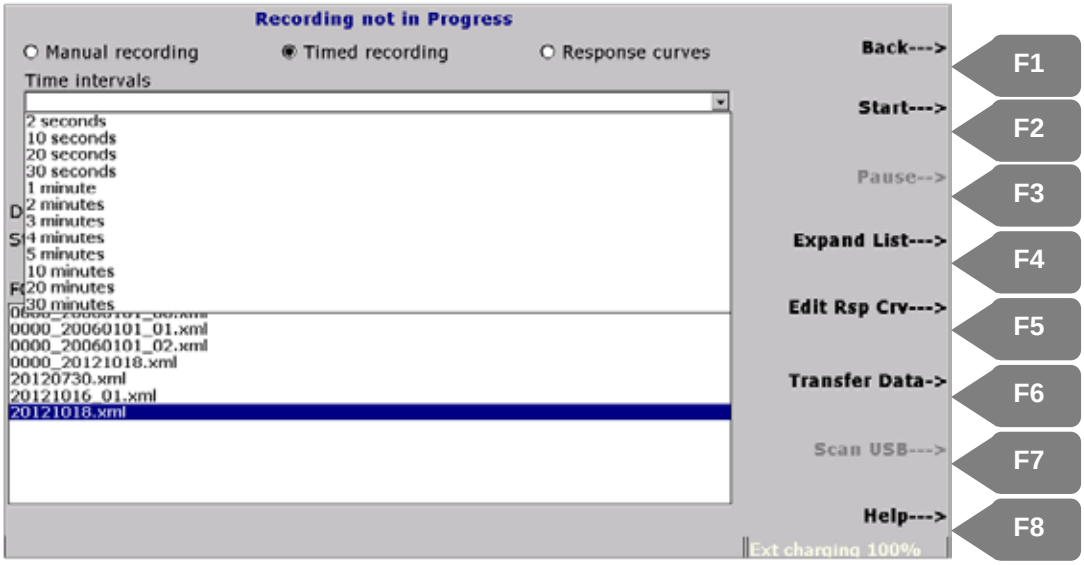
Prior to measurements on small leaf area or whenever you expect very low rates of photosynthesis and when CO2d is showing a small differential (± 1-3 µmol mol^{-1}), perform a manual Diff Bal (F7) to get closer to 0 CO2d.

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.

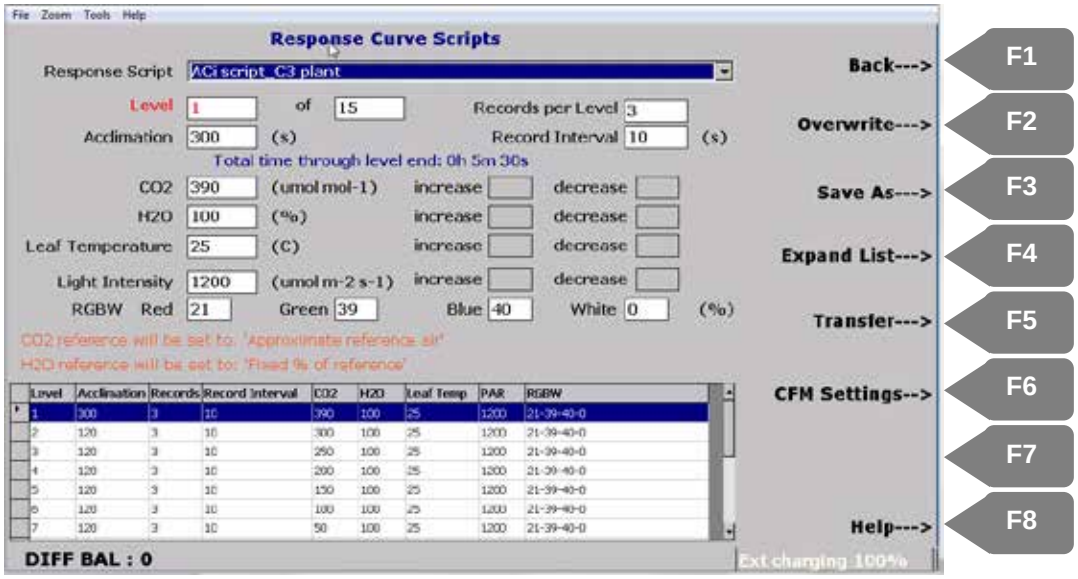
Response Curves

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.

> **Tip**
>
> *When performing automatic response curves involving chlorophyll fluorescence measurements, all CFM-3 settings (**Settings F2>CFM Settings (F7)** must be set up and saved prior to starting the response in order to take effect.*

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 CIRAS-3 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 select the desired response script and enter the values that define the script, 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.



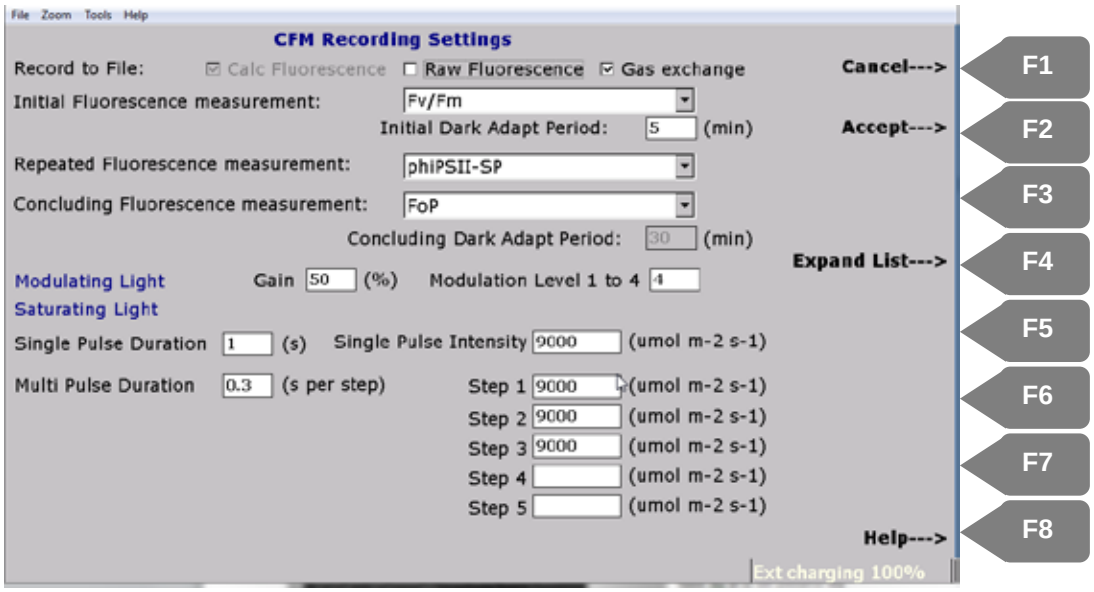
The following selections are available:

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. Remember that the temperature control is highly dependent on ambient temperature and light intensity.
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.

Press **CFM Settings (F6)** to access settings associated with chlorophyll fluorescence measurements. Note that you must both have our CFM-3 Chlorophyll Fluorescence Module and it must be selected under "Settings File" and "Accessory" in **Settings**. See Settings (F2) on page 42.



The following selections are available.

CFM-3 Script Settings Editor	
Record to File	**Calc Fluorescence, Raw Fluorescence, Gas exchange.** 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. **Gas exchange** 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 **Gas exchange** will be checked (combined fluorescence/gas exchange enabled).

<table>
<tr><td></td><td>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.</td></tr>
<tr><td>Initial Fluorescence</td><td>phiPSII-SP (ϕPSII-Single-Pulse). If selected, there is no need to dark adapt the leaf and measurements can begin right away and a single, saturating pulse applied to the light-adapted leaf sample. The single pulse duration and intensity can be set in the lower part of the screen under “Saturating Light”.

phiPSII-MP (ϕPSII-Multi-Pulse). If selected, there is no need to dark adapt the leaf and a “Multi-Pulse sequence can begin right away with multiple, saturating pulses applied to the light-adapted leaf sample. The Multi-Pulse duration and intensity settings (up to 5 steps) can be set in the lower part of the screen under “Saturating Light”.

Fv/Fm. If selected, you will need to allow the plant to dark acclimate prior to measurement. Set the Initial Dark Adapt Period of your choosing. Longer dark adapt period are required for plants that have been under high light and shorter periods for plants that have been in the dark or low 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.

<table>
<tr><td>DA period (minutes)</td><td>5</td><td>10</td><td>15</td><td>20</td><td>25</td><td>30</td></tr>
<tr><td>F_v/F_m</td><td>0.63</td><td>0.71</td><td>0.78</td><td>0.81</td><td>0.80</td><td>0.81</td></tr>
</table>
</td></tr>
<tr><td>Repeated Fluorescence Measurement</td><td>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).

None. If selected, there will be no chlorophyll fluorescence measurements performed after a photosynthesis measurement has been recorded.

phiPSII-SP (ϕPSII-Single-Pulse). If selected, a single, saturating pulse will be applied to the light-adapted leaf sample. The single pulse duration and intensity can be set in the lower part of the screen under “Saturating Light”.

phiPSII-MP (ϕPSII-Multi-Pulse). If selected, multiple, saturating pulses will be applied to the light-adapted leaf sample. The Multi-Pulse duration and intensity settings (up to 5 steps) can be set in the lower part of the screen under “Saturating Light”.</td></tr>
</table>

	ϕPSII. If this is selected, a saturating pulse will be applied to the light-adapted leaf sample after a photosynthesis measurement has been recorded. **phiPSII-FoP (ϕPSII-Fo').** If this is selected, a saturating pulse will be applied to the light-adapted leaf sample followed by illumination using the far-red light for measurement of Fo' after a photosynthesis measurement has been recorded. **Fs**. If this is selected, the steady state fluorescence will be recorded after a photosynthesis measurement is recorded. 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 Measurement	This defines the final measurement to be recorded after the last photosynthesis measurement. **None**. If this is selected, there will be no concluding chlorophyll fluorescence measurement and the sequence will terminate after the last photosynthesis measurement. **FoP (Fo')**. If selected, far-red light will be applied to the leaf sample. **Fv/Fm**. If selected, you must allow the leaf to dark adapt prior to making the measurement. Enter the time (in minutes) at "**Concluding Dark Adapt Period**" and we recommend at least 20-30 minutes for best results.
Modulating Light	**Gain**. Used to adjust the resolution of the fluorescence signal. **Modulation Level**. Modulation Level (1-4) 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.
Saturating Light	**Single Pulse Measurements** **Single Pulse Duration**. The time (in seconds) for the Single Pulse Duration saturating light to be applied. **Single Pulse Intensity**. The saturation pulse intensity (0-10000 µmol m^{-2} s^{-1}). The maximum intensity of 10000 µmol m^{-2} s^{-1} can only be achieved if using one LED color (i.e. 100% red, 100% green, 100% blue or 100% white). Typically, a combination of LEDs are used for measurements so we recommend using an intensity of up to 9000 µmol m^{-2} s^{-1} and with predominantly red light (i.e. 90% red/ 10% blue). The default value is 9000.

	Multi-Pulse Measurements Multi-Pulse Duration. The time (in seconds) for each multiple pulse duration saturating light to be applied. For Multi-Pulse measurements, you have up to 5 steps but you must set at least 3 steps. Based on our testing, we recommend at least one step above 3000 µmol m^{-2} s^{-1} at the high end and one step below 1500 µmol m^{-2} s^{-1} at the lower end with an even distribution of light in between the high and low light settings..

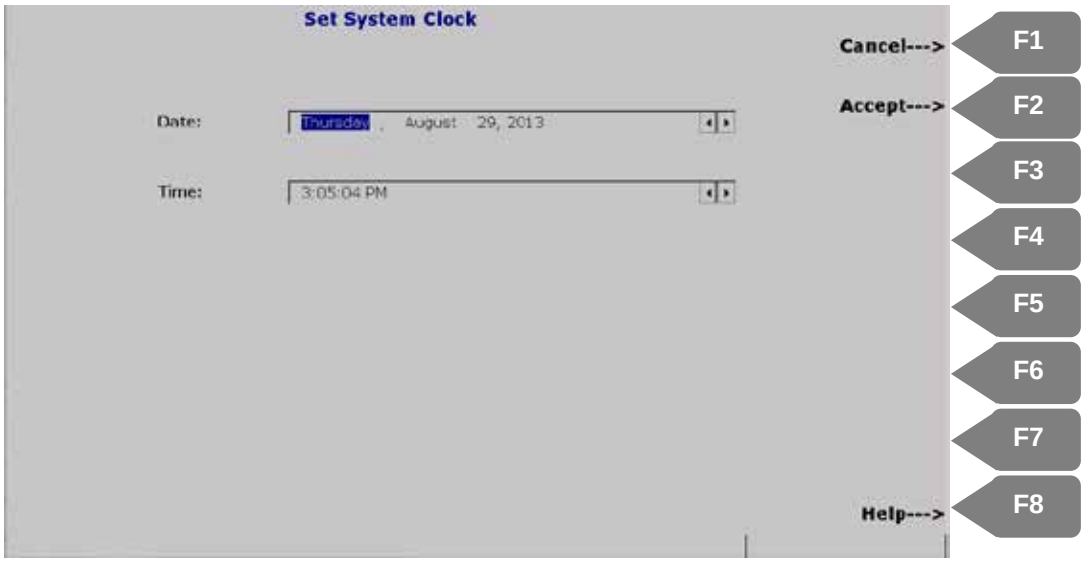
Press **Accept (F2)** to accept changes and return to the main Settings dialog.

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) on page 42 and Store Diff Bal on page 69. 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.

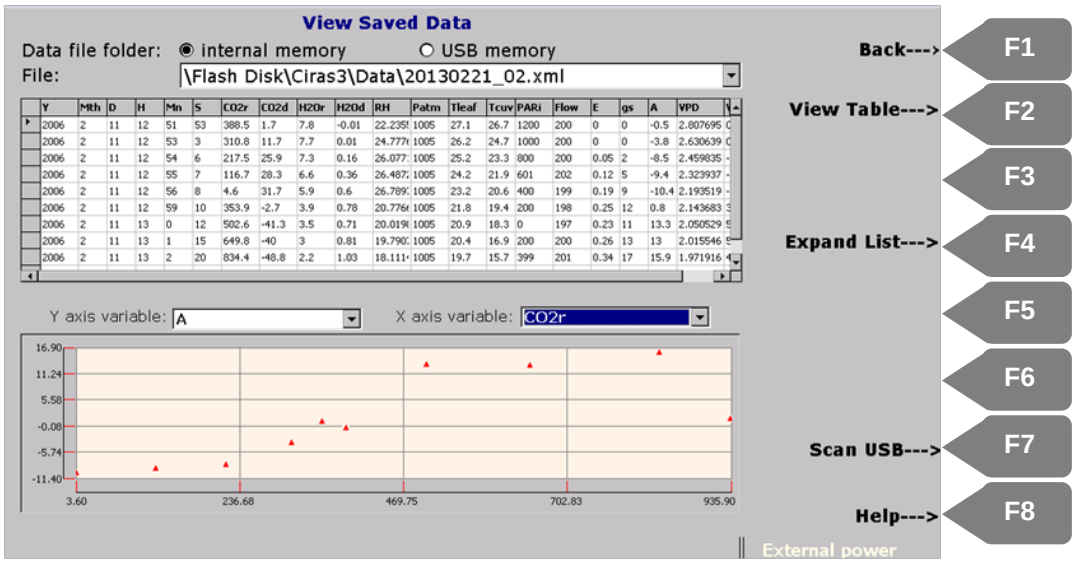
View Saved (F4)

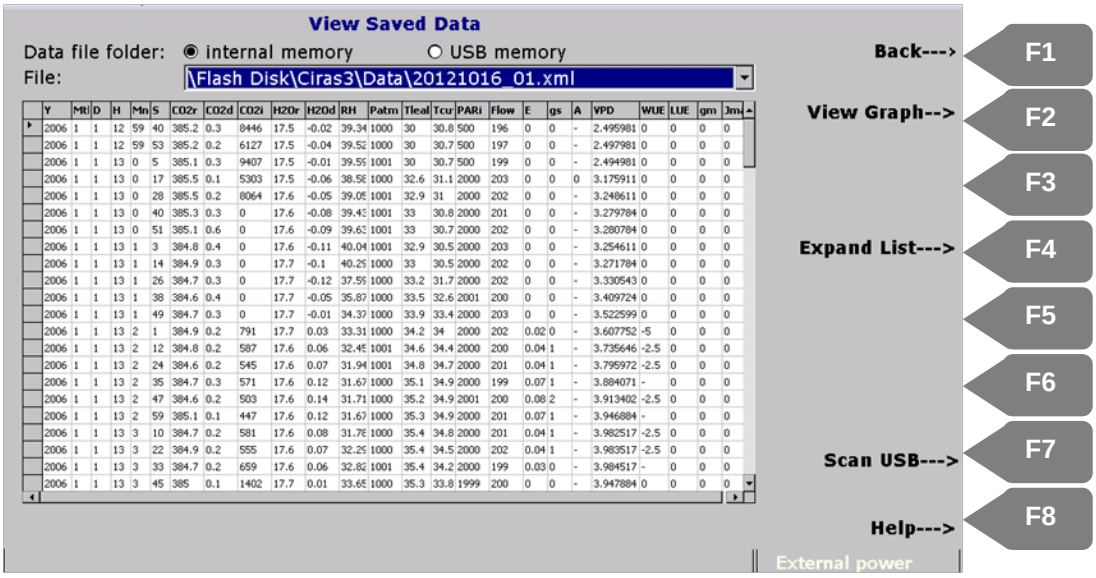
Press **Operations F1 > View Saved (F4)** to view data stored on internal memory or on the USB. This feature of CIRAS-3 allows almost immediate review of recorded data on the console, in both tabular and graphical formats. To enter the View Saved Data screen press **Operations (F1)** and **View Saved (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.

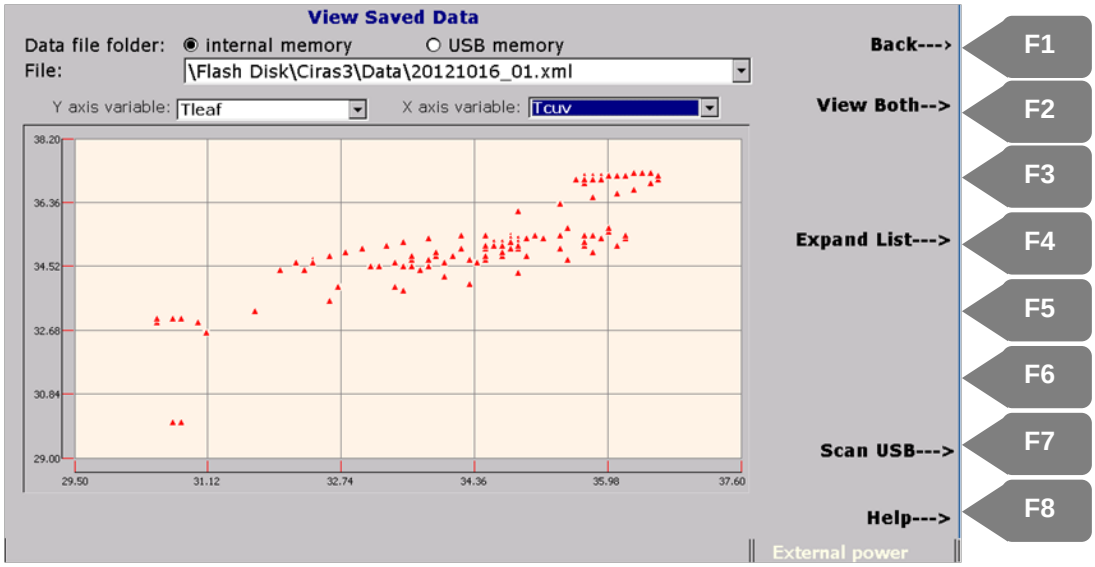
Press TAB to move to the data table, use the Down arrow to scroll through the table. The table column headings indicate its content, which for practical reasons displays most, but not all of the full .xml data file.

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.





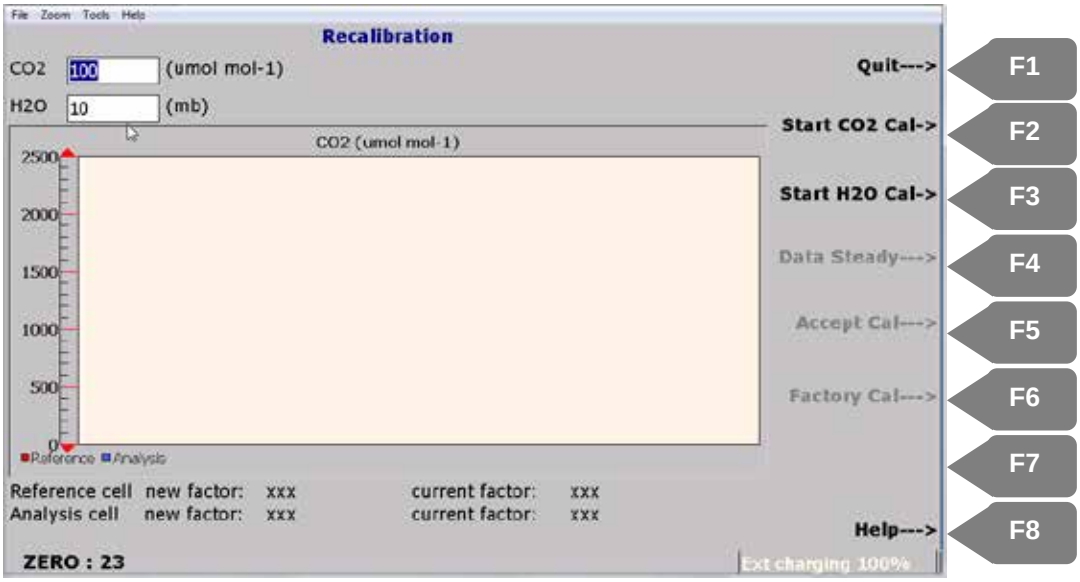


Calibration

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

Recalibrate

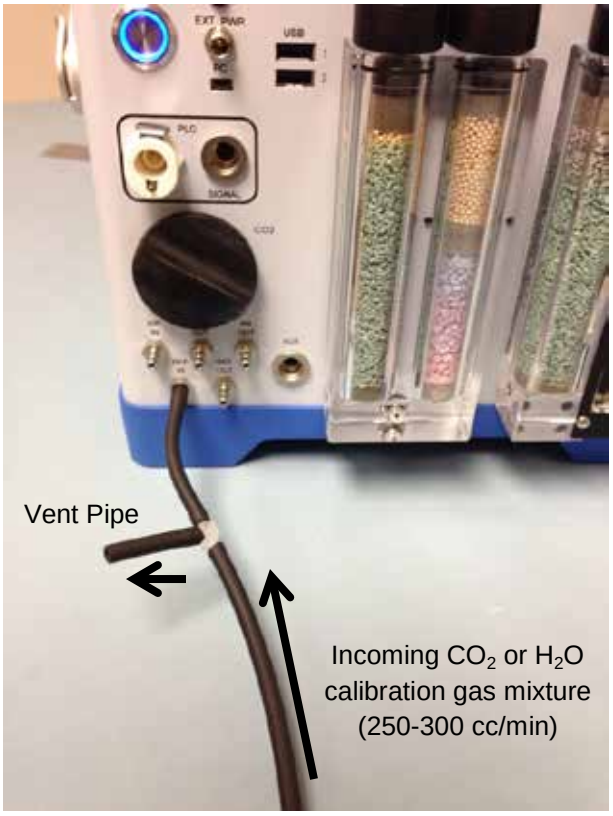
To recalibrate the CO_2 and H_2O gas analyzers, press **Recalibrate (F2)**. Also, the link pipe must be removed from the **REF IN** gas port on the CIRAS-3 console to allow the CO_2 and H_2O gas to pass through the reference cell for calibration purposes.



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.

Calibration Setup



CO_2 Calibration

For CO_2 recalibration we recommend a certified, accurate tank (< 1.0% accuracy) of compressed gas containing CO_2 in air (not CO_2 in nitrogen). Generally speaking, it is recommended that your calibration gas mixture contains a CO_2 concentration slightly above your normal measurement range. For instance, if most of your measurements are made near CO_2 levels in the range of 390-450 µmol mol^{-1}, a calibration mixture of 500 µmol mol^{-1} would be recommended.

1. Ensure that all chemicals are fresh.
2. Connect the CO_2 calibration mixture to the "REF IN" gas port on the CIRAS-3 console as shown above. We recommend using flexible tubing to easily connect to the gas port and a flow rate of 250-300 cc/min. Be sure to include a vent pipe between your calibration gas mixture and CIRAS-3 to avoid overpressure.
3. Enter the CO_2 gas concentration of your calibration mixture in the CO2 box and press **Start CO2 Cal** (F2).
4. Monitor the CO2r and CO2a values and when steady, press **Data Steady** (F4). New scaling factors for both the Reference and Analysis CO_2 IRGAs will be determined and displayed. To accept the new calibration, press **Accept Cal** (F5) or **Quit** (F1) to abort the calibration. If you choose to **Quit** the calibration, you will be prompted with "CIRAS-3 Reject new calibration". Press **OK**.

You always have the option to reset the CO_2 IRGAs back to factory calibration by selecting **Factory Cal** (F6).

H_2O Calibration

For H_2O recalibration we recommend using an accurate humidity generator or water vapor generator.

1. Ensure that all chemicals are fresh.
2. Connect the H_2O calibration mixture to the "REF IN" gas port on the CIRAS-3 console as shown above. We recommend using flexible tubing to easily connect to the gas port and a flow rate of 250-300 cc/min. Be sure to include a vent pipe between your calibration gas mixture and CIRAS-3 to avoid overpressure.
3. Enter the H_2O gas concentration of your calibration mixture in the H2O box and press **Start H2O Cal** (F3).
4. Monitor the H2Or and H2Oa values and when steady, press **Data Steady** (F4). New scaling factors for both the Reference and Analysis H_2O IRGAs will be determined and displayed. To accept the new calibration, press **Accept Cal** (F5) or **Quit** (F1) to abort the calibration. If you choose to **Quit** the calibration, you will be prompted with "CIRAS-3 Reject new calibration". Press **OK**.

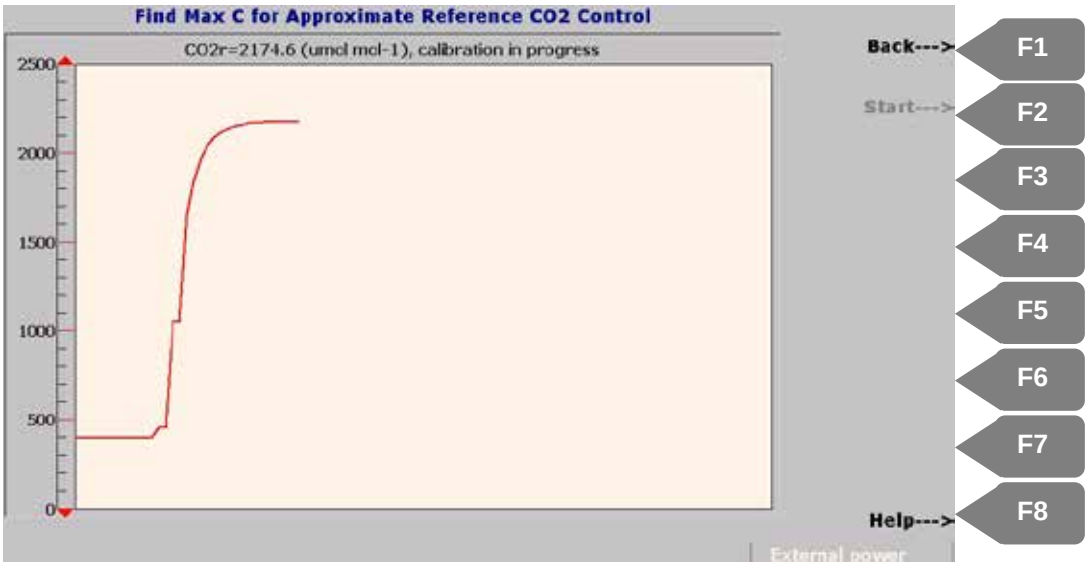
You always have the option to reset the H_2O IRGAs back to factory calibration by selecting **Factory Cal** (F6).

TIP

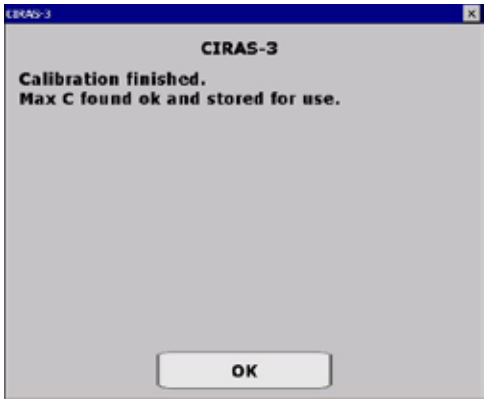
Make sure that the flow rate from your CO_2 or H_2O calibration gas mixture is at least 250 cc/min. Otherwise, atmospheric air could be drawn into the vent pipe leading to errors in calibration.

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 should be performed prior to using "Approximate reference air" for CO_2 reference (See Settings (F2) for optimal control. 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. The system will also perform and Auto Zero. If this process is successful, you will receive a message as follows:



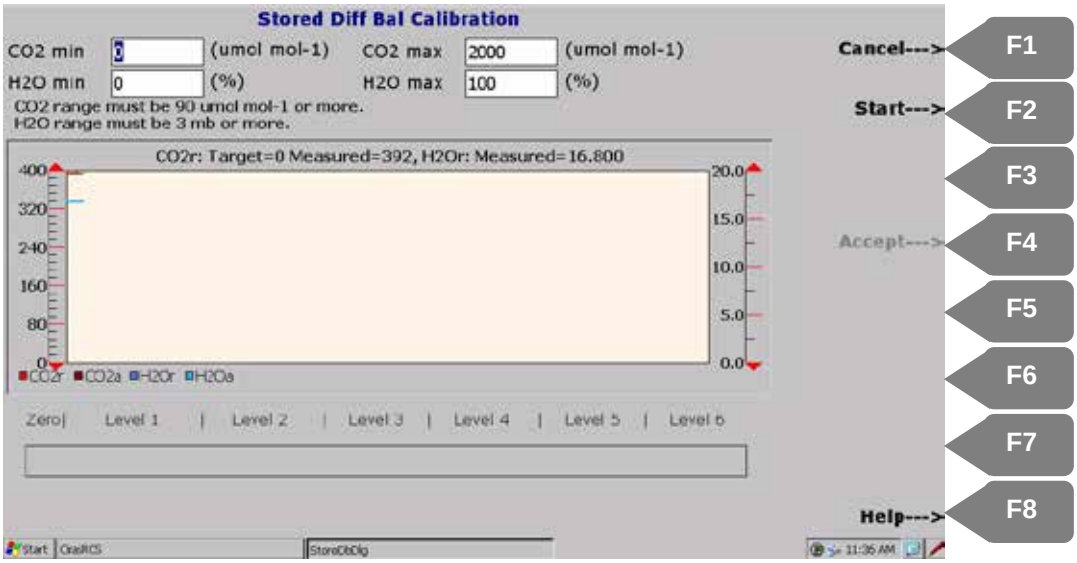
Press **OK** to accept and then **Back (F1)**. If you are not successful and you get a message that says it can't find Max C, check your chemicals again, tighten the CO_2 cartridge holder to ensure that the cartridge was pierced when inserted and perform a new Max C. You can now proceed to **Settings(F2)** and select "Approximate reference air" if you want to use this option for **CO2 Reference**.

TIP

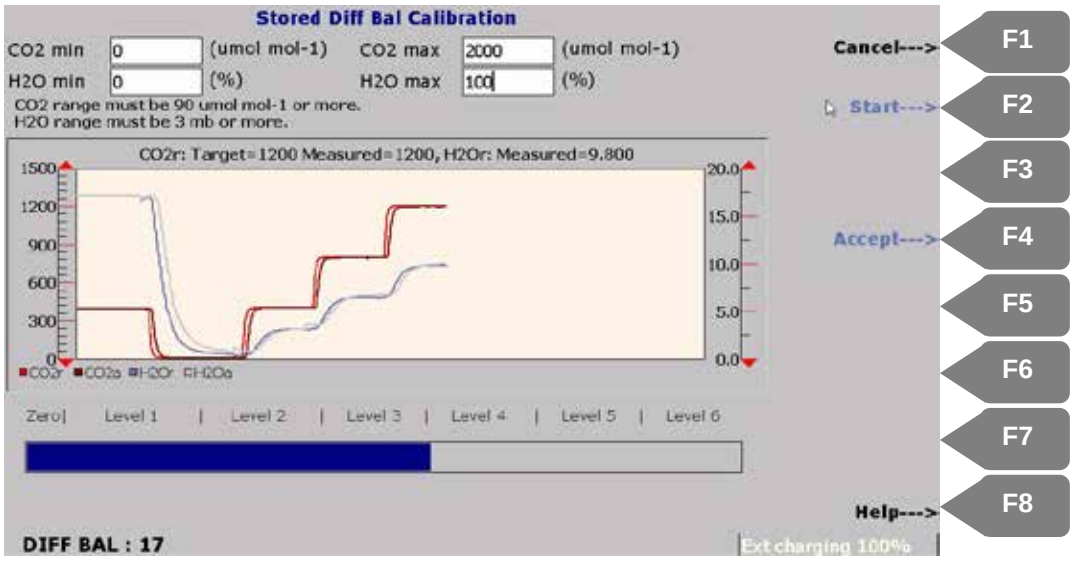
You should always perform a MAX C prior to using "Approximate reference air" for CO2 Reference under Settings (See Settings (F2) on page 42). This will ensure that you are within 30 µmol mol^{-1} of your CO_2 reference set value and stable.

Store Diff Bal

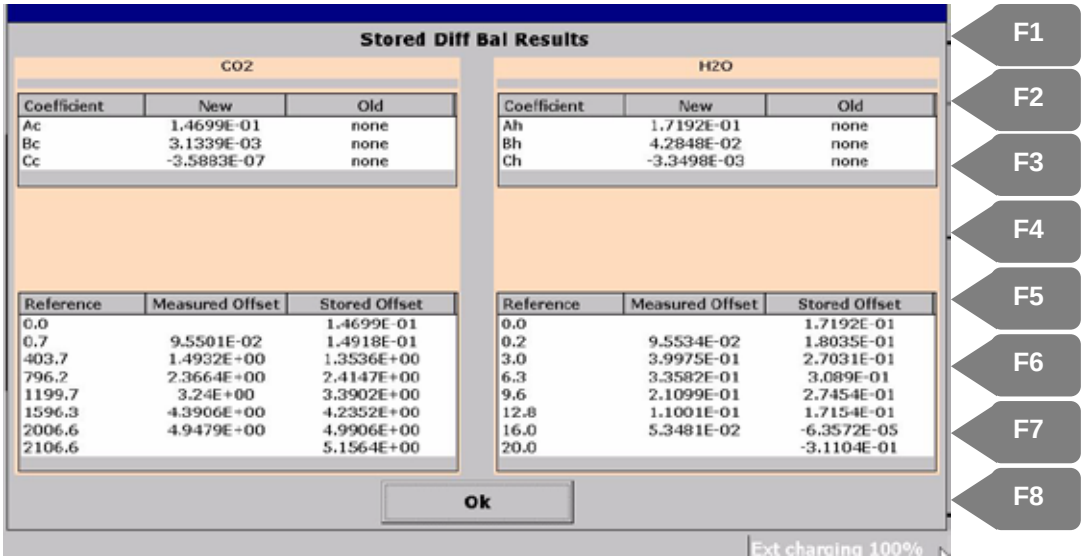
For response curves, we recommend using Auto zero, stored Diff Bal. Prior to using this option, you **MUST** first perform a "Store Diff Bal" prior to starting a response script. This process allows you to store "Zero" and "Diff Bals" in advance to avoid disruptions during a response. Press **Store Diff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

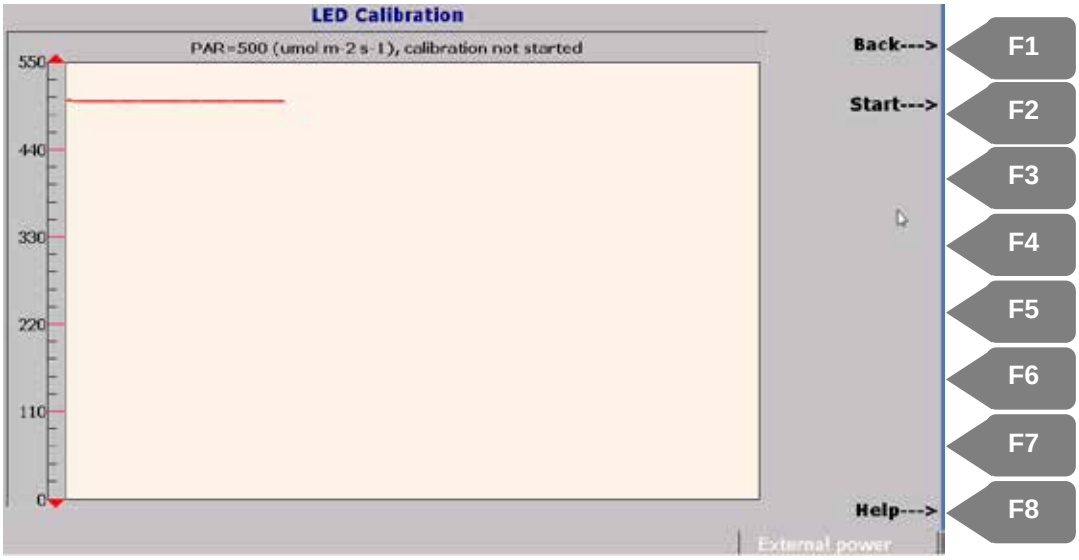
software to 100 ppm less than the lowest CO_2 Ref reading and 4 mbar less than the H_2O Ref reading. The last row of data represents the upper limit of reference readings that are allowed – 100 ppm more than the highest CO_2 Ref reading and 4 mbar higher than the highest H_2O reading. The column labeled "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:

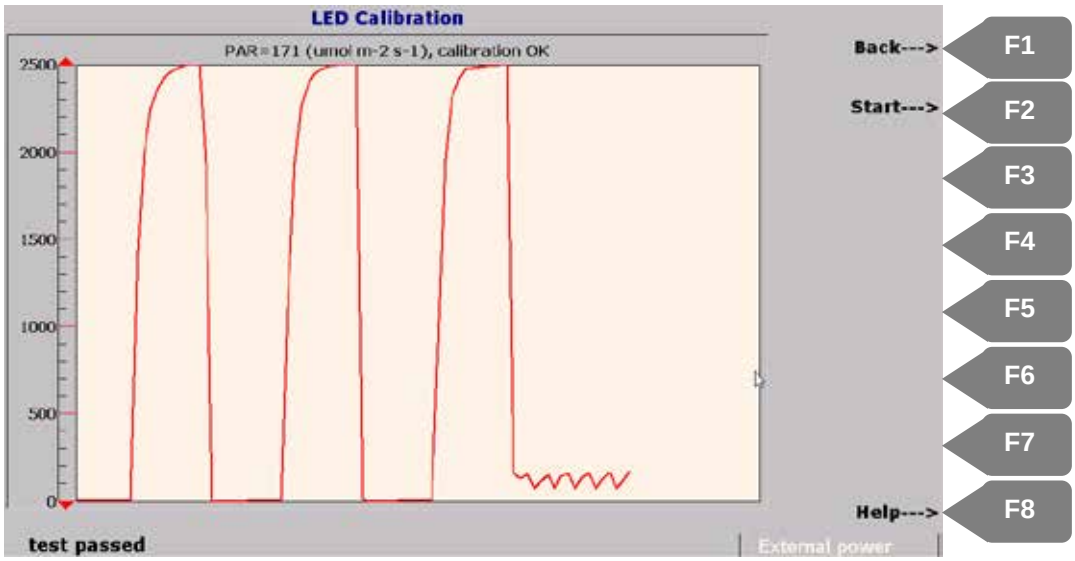
1. Measured offsets at each point should be less than 20 ppm and 0.5 mbar.
2. Measured offsets should show a trend - gradually increasing or decreasing over the six points.
3. The computed Stored Offsets in the 3rd and 6th columns should match the measured offsets in the 2nd and 5th columns within about 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.

LED Calibrate

Press **LED Calibrate** (F5) to recalibrate the LED light unit.



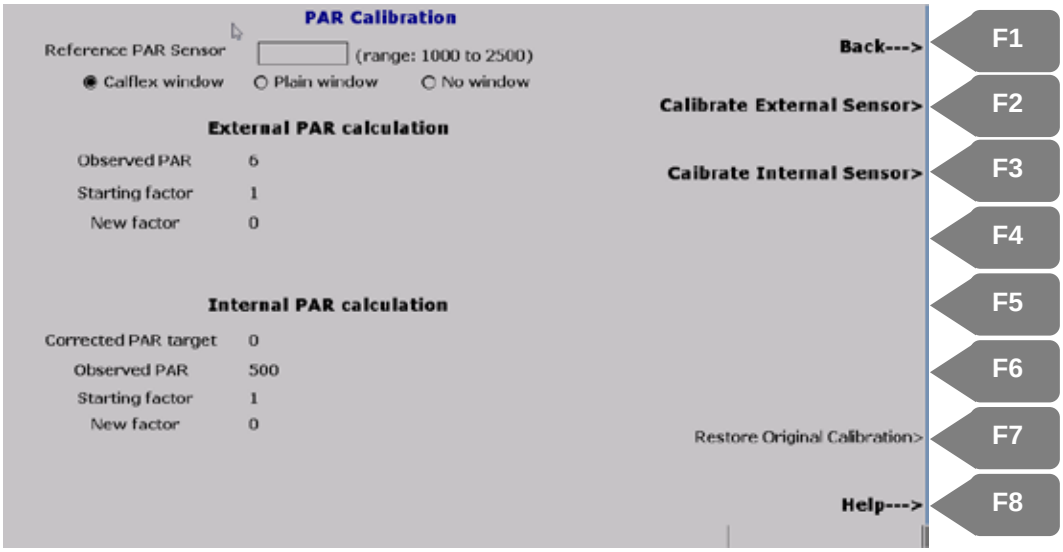
Initially the current light level will appear as shown above. Press **Start** (F2) to begin the calibration process. "Test in progress" will appear in the lower left hand corner of the display and the calibration process will begin.



If the calibration is successful, a message “test passed” will appear as shown above in the lower left hand corner of the display. It normally takes approximately 4-5 minutes for this automated calibration to take place. If the calibration is not successful and you receive a “test failed” message in the lower left hand corner, contact PP Systems.

PAR Calibrate

Press PAR **Calibrate** (F6) to recalibrate the external and internal PAR sensors.



The calibration of the external and internal PAR sensors on your PLC3 will only be accurate if comparing to a calibrated PAR reference sensor. Please ensure that your reference sensor is calibrated prior to recalibrating the PLC3 sensors and the light level that you are calibrating to is at least 1000 µmol m^{-2} s^{-1}.

Steps to Recalibrate PLC3 PAR Sensors.

1. Place your reference sensor as close to the PLC3 external PAR sensor in the same orientation to your light source (i.e. sun). Be careful not to shade either sensor.
2. Enter the PAR value from your reference sensor in the **Reference PAR Sensor** field on the CIRAS-3 console.
3. Select the appropriate window type for your PLC3 as follows:
   PLC3 Universal – Calflex Window or Plain window (if fitted for CFM-3)
   PLC3 Narrow – Calflex window.
   PLC3 Conifer – Plain window.
4. Under "External PAR calculation", wait for the "Observed PAR" value to stabilize. If this value is similar to the reference PAR sensor (± 2%) then there is no need to recalibrate. If it is outside this range, press **Calibrate External Sensor** (F2).
5. A new calibration factor (**New factor**) will be displayed and the Observed PAR should now match the Reference PAR Sensor.
6. Under **Internal PAR calculation**, compare the "Corrected PAR target" to the "Observed PAR" value. If the values are similar (± 2%), then there is no need to recalibrate. If the values are outside this range, press **Calibrate Internal Sensor** (F3).
7. A new calibration factor (**New factor**) will be displayed and the "Observed PAR" should now match the "Corrected PAR target".

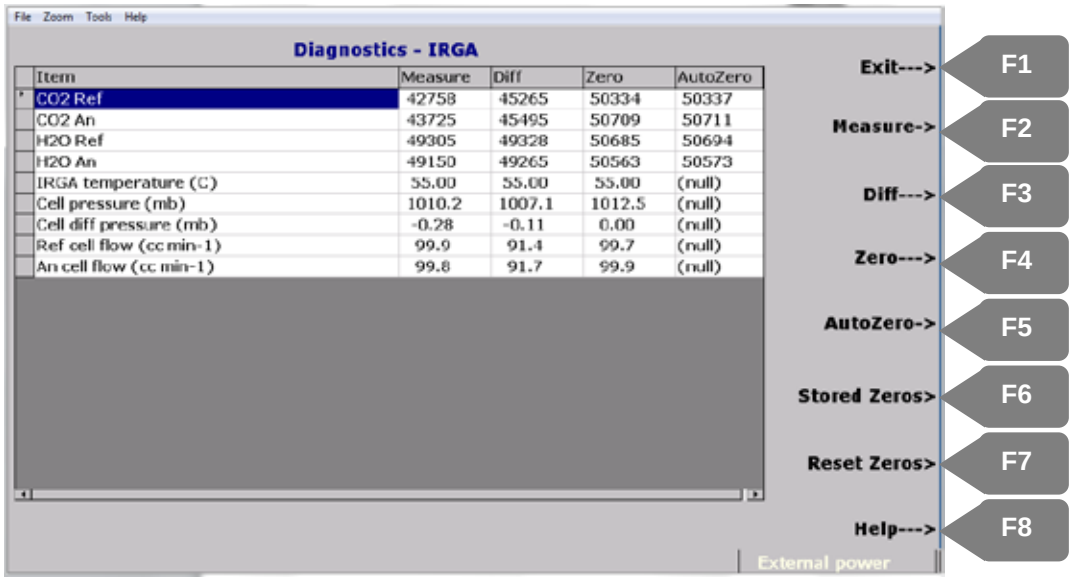
If you are unsure about your calibration, you can always press **Restore Original Calibration** (F7) to restore the original factory calibration factors.

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.

IRGA (F2)

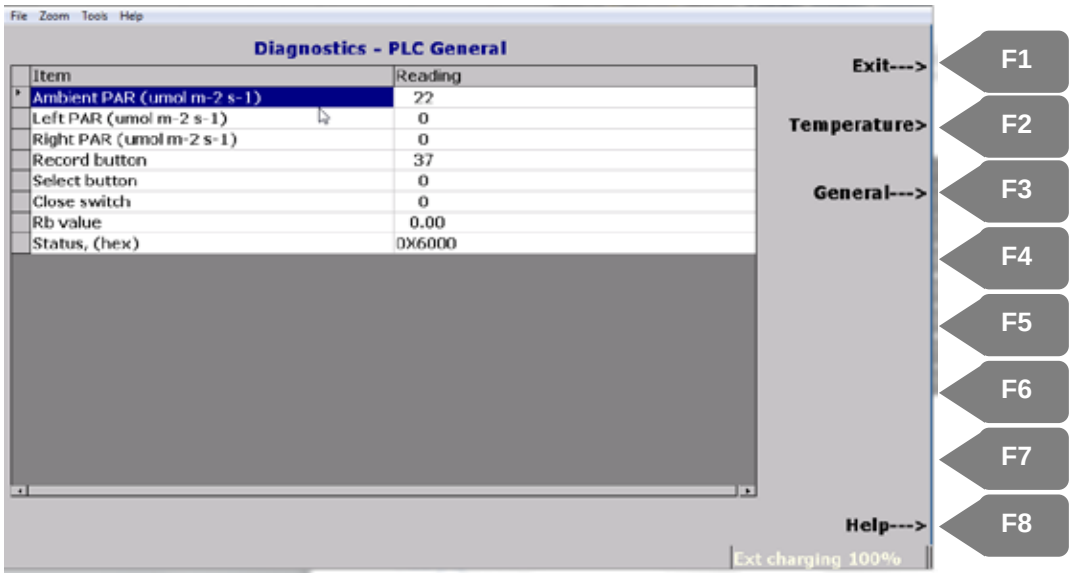
Press IRGA (F2) to view readings associated with the IRGAs.



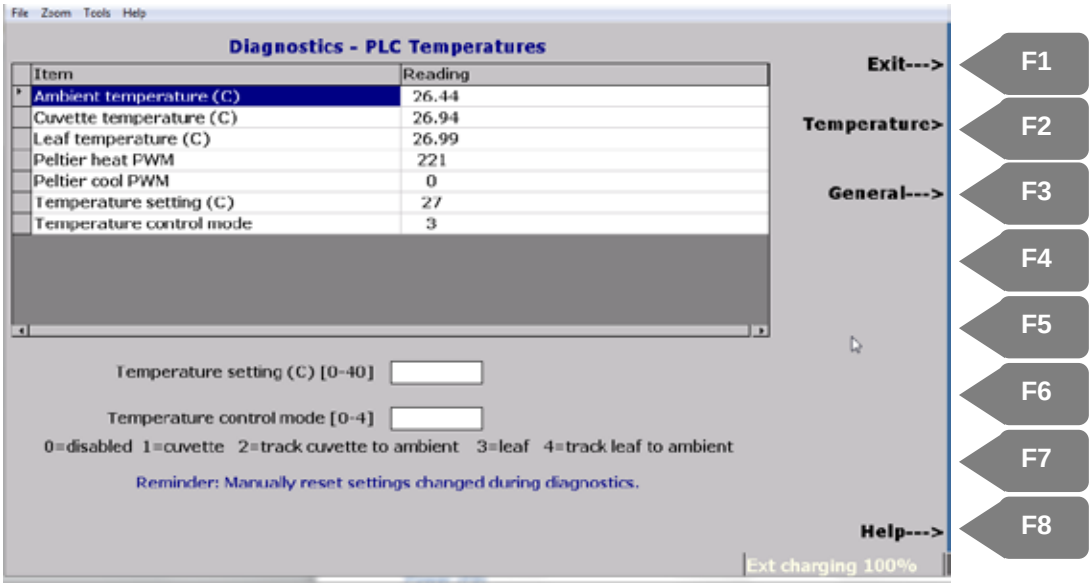
Function Keys	
Measure (F2)	Press **Measure (F2)** to show A/D readings in “Measure” mode. Values in the Measure column are dependent on current CO_2 and H_2O gas concentrations but should be between 30000 and 55000 for CO2 Ref, CO2 An, H2O Ref and H2O An.
Diff (F3)	Press **Diff (F3)** to show A/D readings in “Differential” mode. Values in the Diff column are dependent on current gas CO_2 and H_2O gas concentrations but should be between 30000 and 55000 for CO2 Ref, CO2 An, H2O Ref and H2O An.
Zero (F4)	Press **Zero (F4)** to show A/D readings in “Zero” mode. Values in the Zero column should be the highest values and in the range of 50000 (± 30000) for CO2 Ref, CO2 An, H2O Ref and H2O An.
Auto Zero (F5)	Press **Auto Zero (F5)** to execute a Zero. At the conclusion of the Zero the readings in Diagnostics will update.
Stored Zeros (F6)	Press **Stored Zeros (F6)** to display the history of Stored Zeros.
Reset Zeros (F7)	Press **Reset Zeros (F7)** to reset the stored Zeros.

PLC (F3)

Press PLC (F3) to view readings associated with the PLC Leaf Cuvette.



To further test temperature control settings, press Temperature (F2).

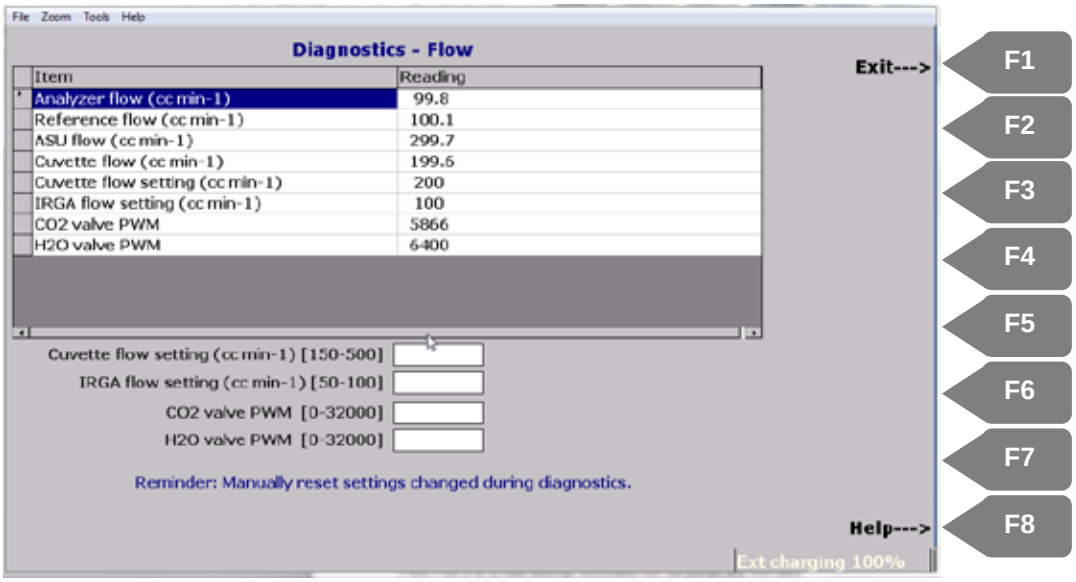


Function Keys	
Temperature (F2)	**Temperature setting (C)** With the above displayed, press the Tab key to place cursor in the "Temperature Setting" box. This is where you can enter the temperature that you want to control to. Enter a value between 0 and 40 and hit **OK** to accept. Note that the box will be empty again after hitting **OK**. In the table above you should see the entered value for "Temperature setting (C)".

	Temperature Control After entering a temperature above, press the Tab key to place cursor in the "Temperature control mode" box. This is where you set the temperature control similarly to in settings. Enter the control mode desired and press OK. Note that the box will be empty again after hitting **OK**. In the table above you should see the entered value for "Temperature control mode".
General (F3)	Simply refers to the opening dialog showing readings associated with the PLC3.

Flow (F4)

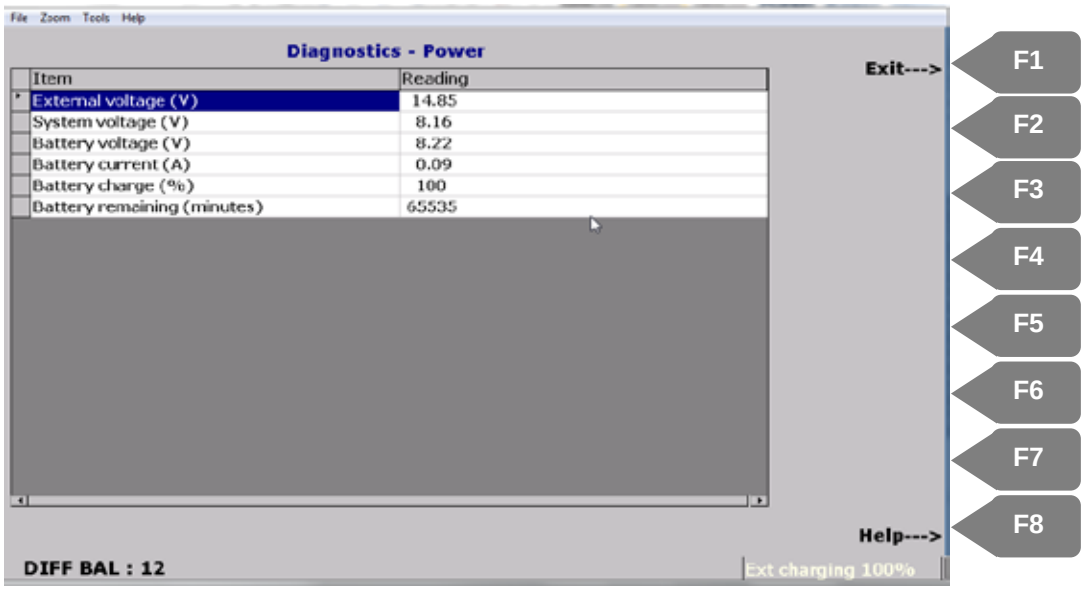
Press Flow (F4) to view readings associated with the pumps and flow rates.



Flow Control and Valve Readings/Settings	
Cuvette flow setting	Press Tab to place cursor into this box. Enter a value between 150 and 500 and then **OK** to check the cuvette flow circuit. In the table above, the Cuvette flow and Cuvette flow setting should be close to your set value.
IRGA flow setting	Press Tab to place cursor into this box. Enter a value between 50 and 100 and then OK to check the IRGA flow circuit. In the table above, the Analyzer flow, Reference flow and IRGA flow setting should be close to your set value.
CO2 valve PWM	Press Tab to place cursor into this box. Enter 0 to turn off the CO_2 valve and 32000 to open the valve completely. Values that are in between 0 and 32000 are based on the currently set CO_2 control value. When the CO_2 valve is set to 0 or 32000, there should not be any clicking of the CO_2 valve.
H2O valve PWM	Press Tab to place cursor into this box. Enter 0 to turn off the H_2O valve and 32000 to open the valve completely. Values that are in between 0 and 32000 are based on the currently set H_2O control value. When the H_2O valve is set to 0 or 32000, there should not be any clicking of the H2O valve.

Power (F5)

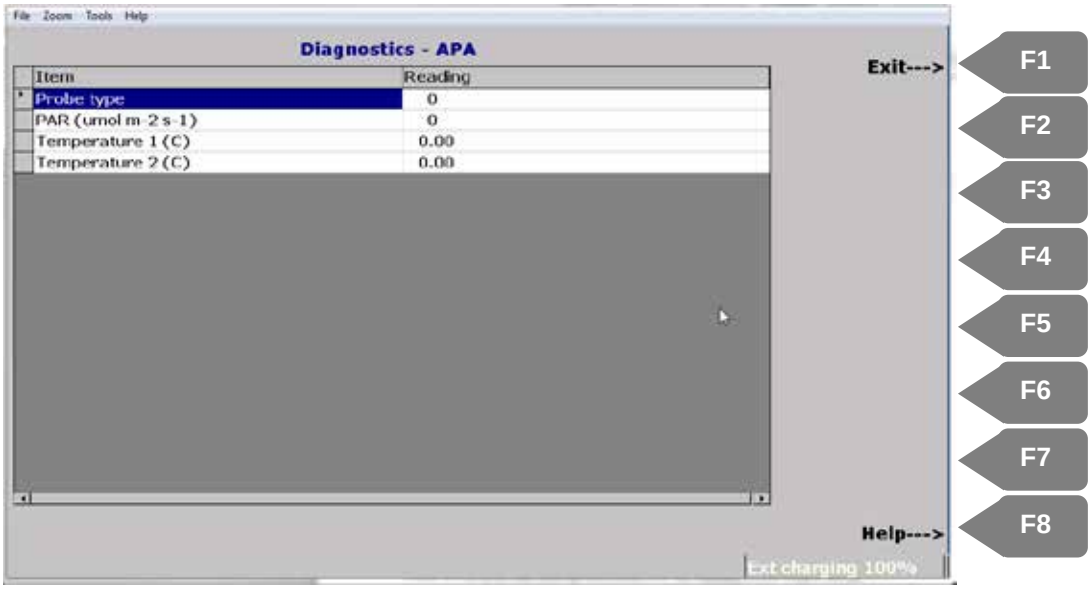
Press Power (F5) to view readings associated with the system power.



Power Readings	
External voltage	Displays the voltage from an external power source (i.e. CIRAS-3 AC Adapter/charger). When the CIRAS-3 AC Adapter/Charger is connected to the console EXT/PWR socket, this should read approximately 15V. When disconnected, it should read 0.00V
System voltage	Displays the current voltage of the main control PCB. Normally, this value will be similar to the Battery voltage reading.
Battery voltage	Displays the current voltage of the internal, rechargeable Li-ion battery pack.
Battery current	Displays the current status of the internal, rechargeable Li-ion battery pack. A negative value represents the drain on the battery and a positive value represents the charging current.
Battery charge	Displays the current state of charge of the internal, rechargeable Li-ion battery pack. A value of 100 represents a fully charged battery and likely connected to the AC Adapter/charger.
Battery remaining	Displays the estimated system operation time remaining which is based on current environmental control settings (i.e. temperature control, light control, etc.).

APA (F6)

Press APA (F6) to view readings associated with the Auxiliary Probe Adapter (APA). This piece of hardware is required to use the SRC-1, CPY-4 and STP-1 Soil Temperature Probe with the CIRAS-3.



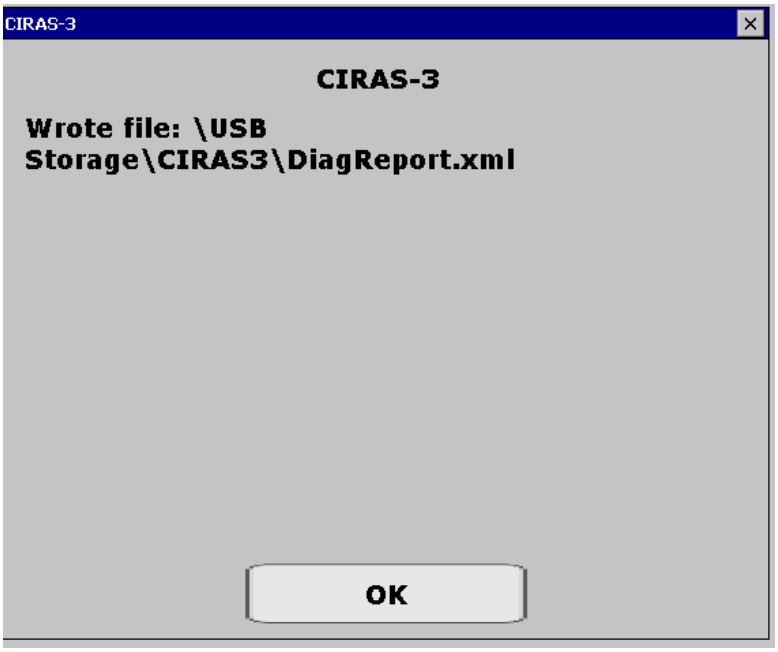
APA Readings	
Probe Type	Displays the probe type for different sensors that can be used with the CIRAS-3 (i.e. SRC-1 Soil Respiration Chamber or CPY-4 Canopy Assimilation Chamber).
PAR	Displays the measured PAR value (µmol m^{-2} s^{-1}) from the sensor on the accessory (if applicable).
Temperature 1	Displays the temperature (^{o} C) from the sensor on the accessory (if applicable).
Temperature 2	Displays the temperature (^{o} C) from the sensor on the accessory (if applicable).

Our latest SRC-2 Soil Respiration Chamber and CPY-5 Canopy Assimilation Chamber (introduced in February 2016) can connect directly to the 4 pin “AUX” connector on the CIRAS-3 console (No APA required).

Report (F7)

Press Report (F7) to generate a Diagnostics report.

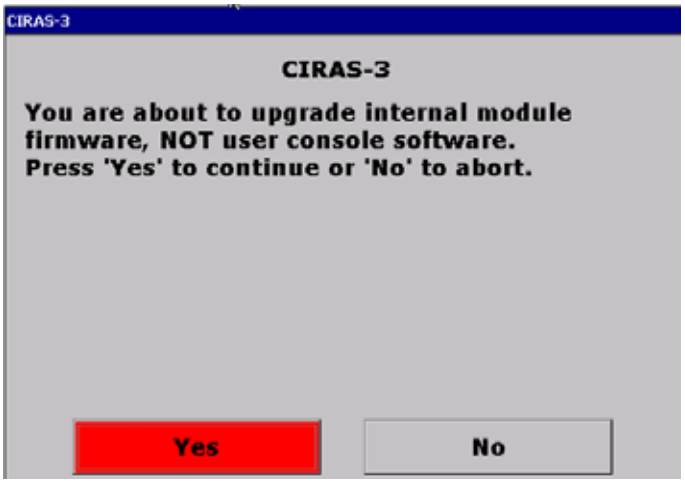
Press F2 to have the report sent to internal memory or F3 to send it to the USB drive (a USB thumb drive must be inserted into either USB1 or USB2 on the console). A message similar to below will appear on the CIRAS-3 display:



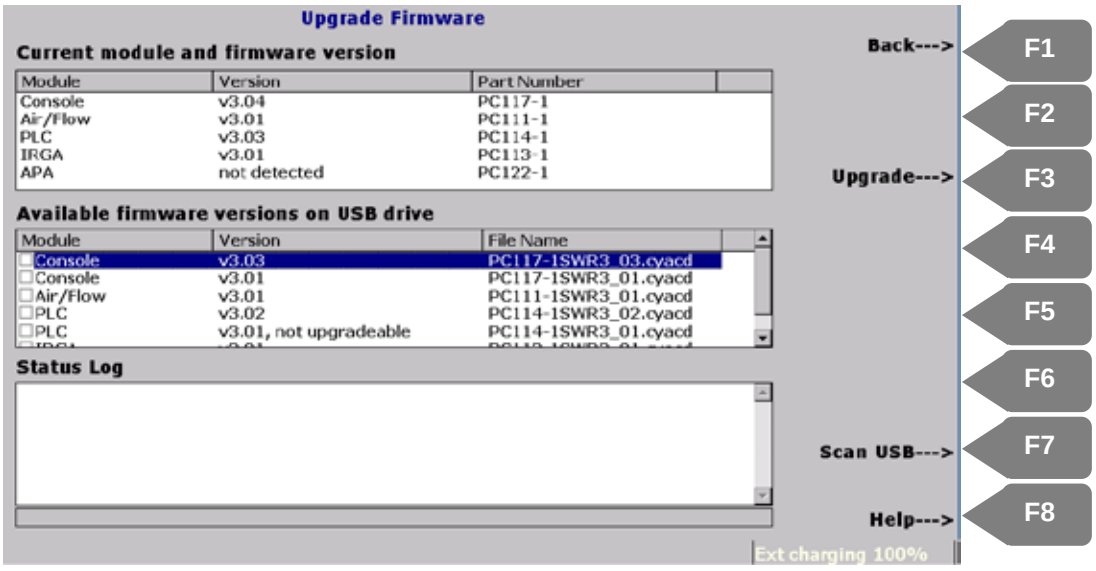
Press **OK** to complete the transfer. The file will be saved as a .xml file which can then be emailed directly to PP Systems for further analysis to assist in troubleshooting and diagnosis.

Firmware Upgrade

Press **FW Upgrade (F7)**. This feature allows you to upgrade CIRAS-3 system modules easily in the field.



Firmware modules are available from our web site and can be downloaded and copied to the appropriate folder (\CIRAS3\Firmware) on your USB thumb drive. Once the files are on the thumb drive, insert it into the USB1 or USB2 slot on the CIRAS-3 console. By default, **Yes** is highlighted and can be selected by pressing "**OK**" to view both the currently installed firmware versions and available versions on the USB thumb drive. If you have not yet inserted the USB thumb drive into the CIRAS-3 console, you can do so now and then press **Scan USB** (F7) to view the available files. To upgrade or downgrade (depending on your requirements) to a new firmware version for a particular CIRAS-3 module, arrow to the module, press "OK" to check it and then hit **Upgrade** (F3).



There are 5 CIRAS-3 modules that can be upgraded/downgraded as follows:

- Console – Firmware associated with the CIRAS-3 main console.
- Air/Flow – Firmware associated with pumps and flow rates.
- PLC – Firmware associated with the PLC3 series leaf cuvettes.
- IRGA – Firmware associated with the optical bench (infrared gas analyzers)
- APA – Firmware associated with the Auxiliary Probe Adapter (APA)

If you have any questions related to firmware compatibility, please get in direct contact with PP Systems.

Section 9. CIRAS-3 Console and PC Utility Software

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 main console. The file 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. The file name is "Ciras3_PC_Utility.exe".

CIRAS-3 Console Software

Installation Instructions for Main CIRAS-3 Console

1. Insert the USB thumb drive (memory stick) provided by PP Systems into the USB port on the CIRAS-3 main console (we recommend USB slot 2). The USB thumb drive is included with the CIRAS-3 spares kit.
2. Open "My Device" and copy the "Ciras3_Console_vxxx.cab" file from the "CIRAS3_Console_SW" folder to the root directory of the console file system. Note the exact location is not important as the cab file will be erased when installation is complete.
3. Remove the USB thumb drive from console.
4. Right click on cab file and ensure it is not Write protected.
5. Double click on the cab file to execute the install. Accept all defaults by using the "OK" button.
6. When installation is complete, power off the console for a few seconds and then power it on again. The new version of the console application should start automatically.

Uninstall Instructions

These instructions assume that the Ciras3_Console application was previously installed and that the purpose of the new Install is to update 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.

To install the newer console application, refer to the instructions in the previous section (Installation Instructions for Main CIRAS-3 Console).

PC Utility Software

The PC Utility Software provided by PP Systems allows users to perform the following 3 functions:

1. Create/Edit Response Scripts
2. Create/Edit Settings Files
3. Remote Display of CIRAS-3

Installation Instructions for PC Utility

The CIRAS-3 PC Utility Software is provided as a “Setup.exe” file and a MSI file named “Ciras3_PC_Utility_vxxx.msi”.

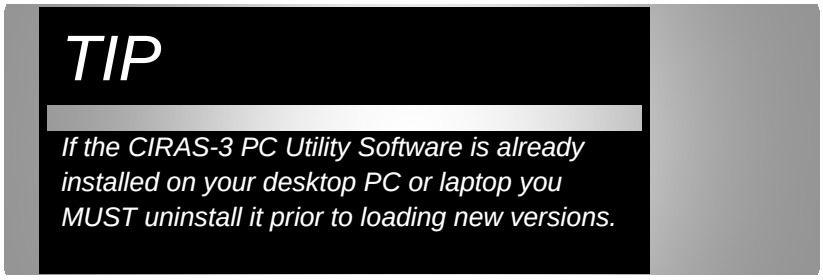
1. Insert the USB thumb drive (memory stick) provided by PP Systems into a USB port on your desktop PC or laptop computer. The USB thumb drive is included with the CIRAS-3 spares kit.
2. Navigate to the folder \CIRAS3_PC_Utility_SW which contains the “Setup.exe” and Ciras3_PC_Utility_Vxxx.msi” files. D
3. Double click on the “Setup.exe” file to begin the installation process and follow the onscreen instructions until complete. A CIRAS-3 icon should now appear onyour desktop.

For Vista, Win7 or Win8 Operating Systems

Once installation is complete, find the shortcut link on the desktop named “Ciras3 Utility”. Right click and select Properties. Select Compatibility and enable “Run as administrator”. If you do not do this, many errors and exceptions will occur when you run the program!

Uninstall Instructions

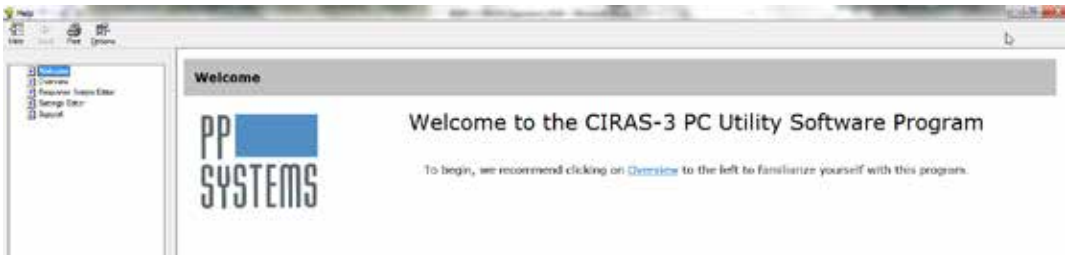
1. On your Windows desktop computer, go to Start> Control Panel >Add Remove Programs. Wait for list to populate.
2. Select “Ciras3_PC_Utility” and click the Remove button.
3. Exit Control Panel.



PC Utility Operation

To run this application, double click on the CIRAS-3 icon on your desktop. The following should be displayed.

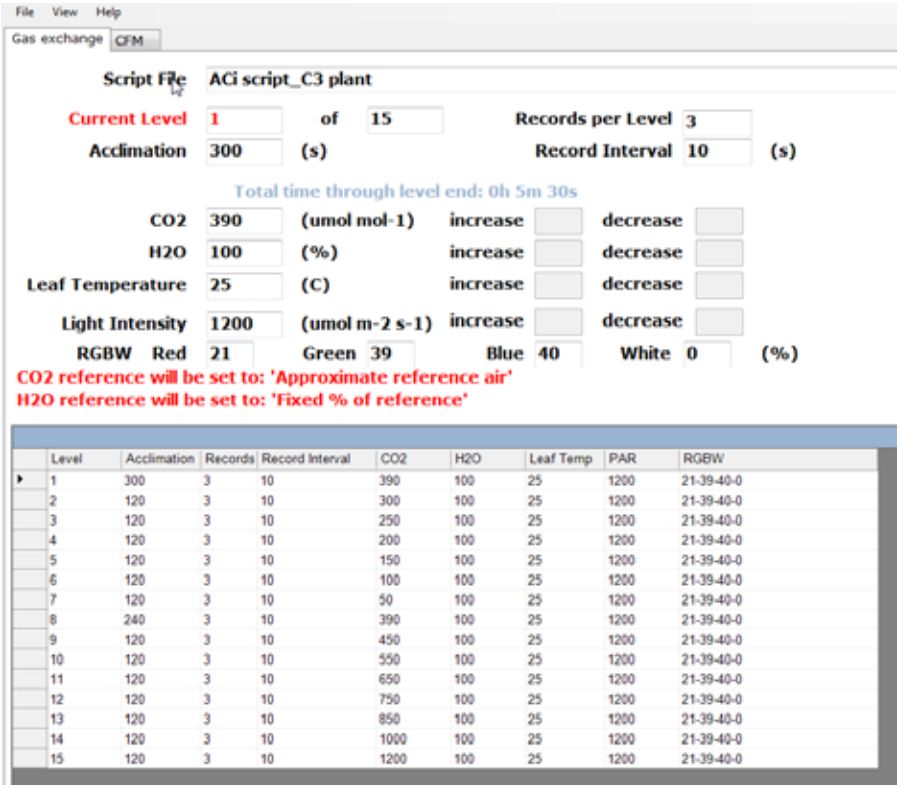
Help is also available at any time by clicking on **Help>Contents**.



Create/Edit Response Scripts

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 select, create or edit response scripts which can then be easily transferred to your CIRAS-3 main console.

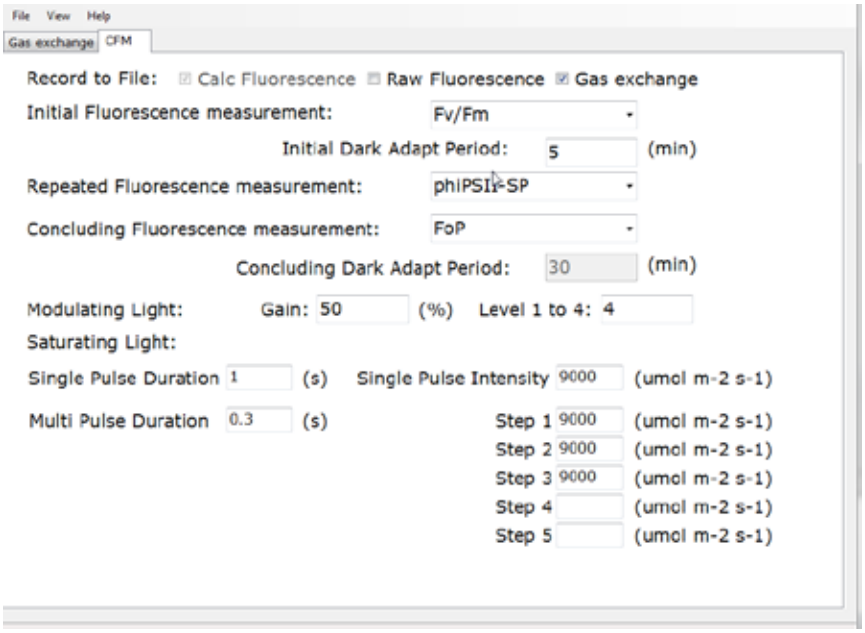
Gas Exchange Script Settings



Level	Acclimation	Records	Record Interval	CO2	H2O	Leaf Temp	PAR	RGBW
1	300	3	10	390	100	25	1200	21-39-40-0
2	120	3	10	300	100	25	1200	21-39-40-0
3	120	3	10	250	100	25	1200	21-39-40-0
4	120	3	10	200	100	25	1200	21-39-40-0
5	120	3	10	150	100	25	1200	21-39-40-0
6	120	3	10	100	100	25	1200	21-39-40-0
7	120	3	10	50	100	25	1200	21-39-40-0
8	240	3	10	390	100	25	1200	21-39-40-0
9	120	3	10	450	100	25	1200	21-39-40-0
10	120	3	10	550	100	25	1200	21-39-40-0
11	120	3	10	650	100	25	1200	21-39-40-0
12	120	3	10	750	100	25	1200	21-39-40-0
13	120	3	10	850	100	25	1200	21-39-40-0
14	120	3	10	1000	100	25	1200	21-39-40-0
15	120	3	10	1200	100	25	1200	21-39-40-0

CFM-3 Script Settings

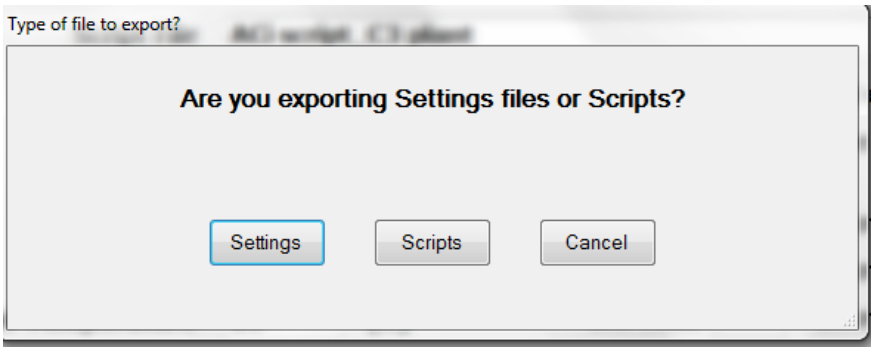
Click the CFM tab at the top to display the settings associated for measurement of chlorophyll fluorescence using the integral CFM-3.



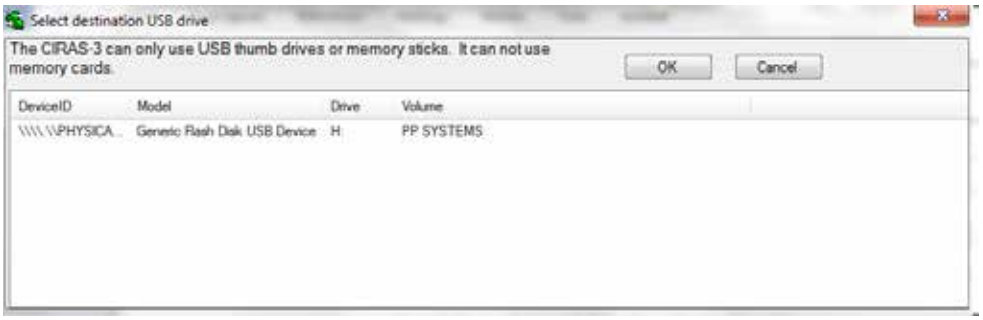
Click the arrow button to expand the dropdown list at the **Script File** item. Edit the script just as you would if you were working from the CIRAS-3 Console. Then click **File >Save**, or **Save As** to rename it.

The next step would be to Export the new or updated script file to the CIRAS-3 main console. The easiest way to do this is to export the files to a USB thumb drive.

1. Click on File>Export to bring up the following window:



2. Click on Scripts.
3. Navigate to the location where you saved the scripts file and select one or more script files and then Open. The following window should appear:



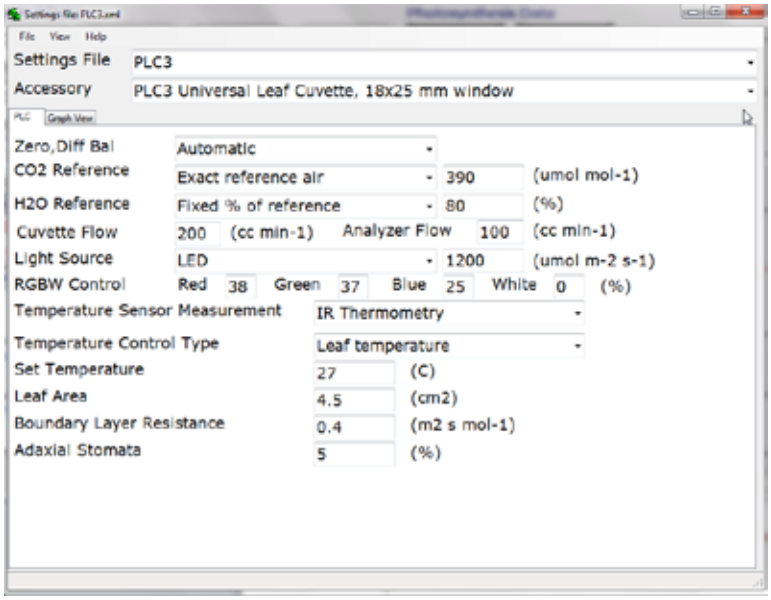
4. Click on the appropriate drive where the file(s) are located and then click **OK**.
5. Remove the thumb drive from your PC and insert it into USB1 or USB2 on the main console to transfer the files.
6. On the CIRAS-3 console, click **on Operations (F1)>Rec Options (F2)>Edit Rsp Crv (F5)>Transfer (F5)**.
7. Tab over to “USB Memory Files”, arrow down to the file(s) and select them by hitting **OK**. There should now be a check mark next to the files that you want to import.
8. Press **Import (F2)** to copy it from the USB thumb drive to CIRAS-3 console internal memory. Now the file is available for use on the CIRAS-3 console.

Create/Edit Settings Files

Click on **File>Open Settings Folder and** browse to the location C:\Program Files (x86)\PP Systems\CIRAS-3 PC Utility\Settings on your computer, and click **OK**. This opens the Settings File window where you can select, create or edit settings files which can then be easily transferred to your CIRAS-3 main console.

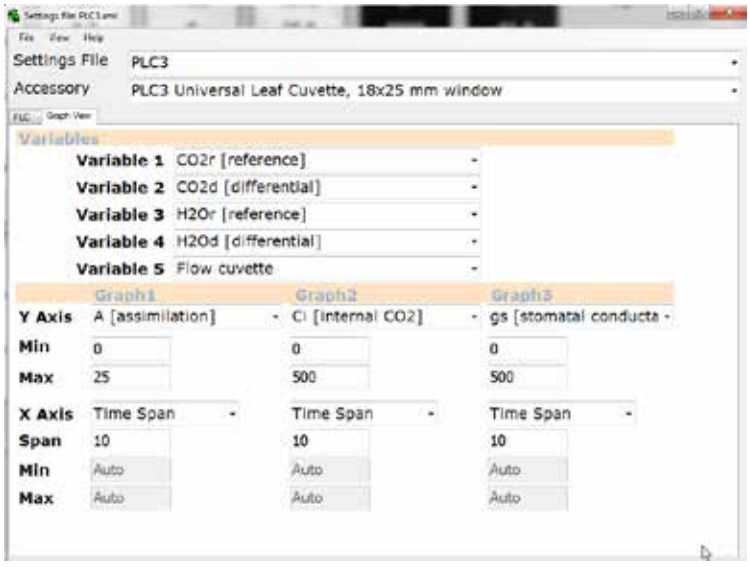
Select from the default list of Settings in the Settings File dropdown list. Note that for each type of **Settings File** there are two windows that can be edited, and two tabs that allow access to these windows. For example, **PLC3** or **PLC3-CFM** settings files used for open system leaf-level photosynthesis and chlorophyll fluorescence have a global Settings window tab labeled **CFM** (chlorophyll fluorescence). Click the arrow button to expand the dropdown list at the **Settings File** and **Accessory**. Edit the script just as you would if you were working from the CIRAS-3 Console. You can also click on the **Graph View** tab for graph settings. Then click **File >Save**, or **Save As** to rename it.

Settings



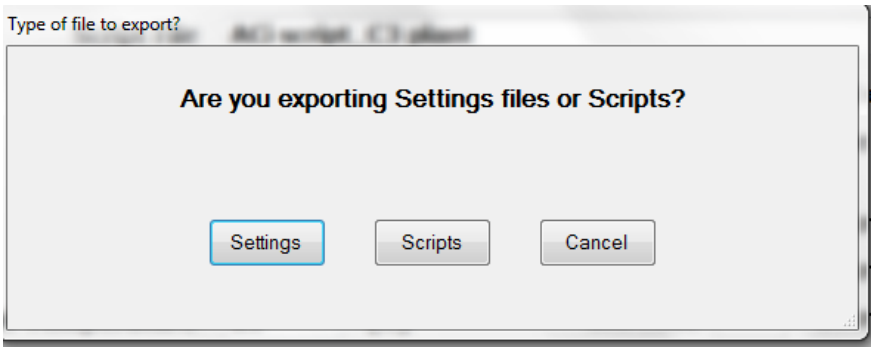
Graph View Settings

Press the “Graph View” tab under Accessory to set up the graphical screen.

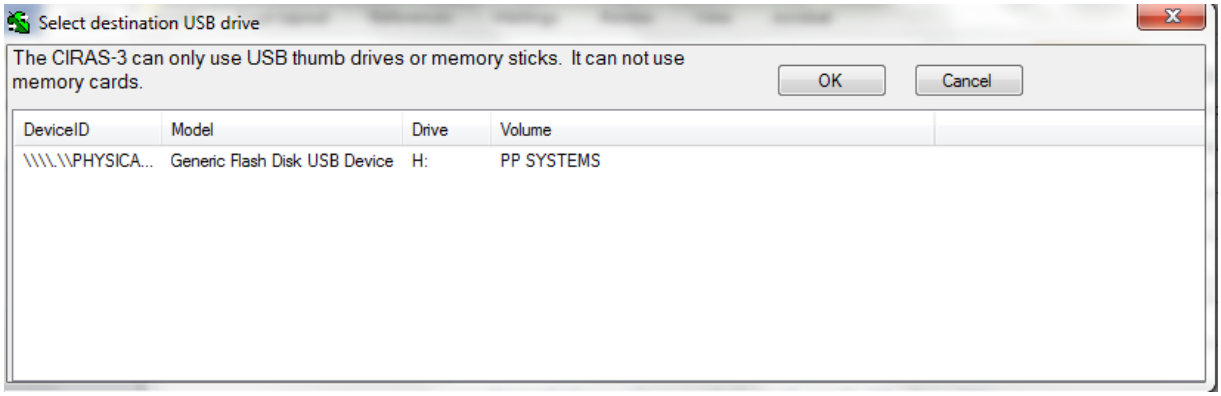


The next step would be to Export the new or updated script file to the CIRAS-3 main console. The easiest way to do this is to export the files to a USB thumb drive.

1. Click on File>Export to bring up the following window:



2. Click on Settings.
3. Navigate to the location where you saved the settings file and select one or more settings files and then Open. The following window should appear:



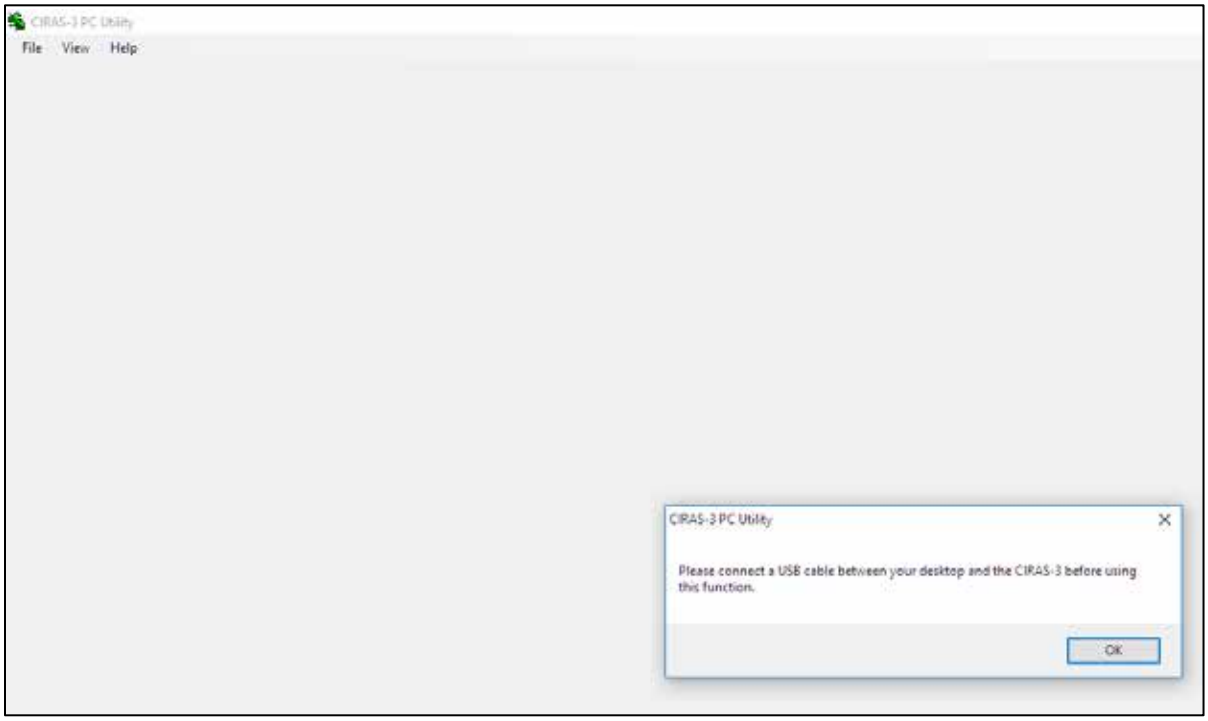
4. Click on the appropriate drive where the file(s) are located and then click **OK**.
5. Remove the thumb drive from your PC and insert it into USB1 or USB2 on the main console to transfer the files.
6. On the CIRAS-3 console, click **Settings (F2)>Transfer (F3)**.
7. Tab over to "USB Memory Files", arrow down to the file(s) and select them by hitting **OK**. There should now be a check mark next to the files that you want to import.
8. Press **Import (F2)** to copy it from the USB thumb drive to CIRAS-3 console internal memory. Now the file is available for use on the CIRAS-3 console.

Remote Display

This feature allows the user to view all screens of the CIRAS-3 using an external device such as a PC computer or projector. This can be very useful when presenting information to a large group or for teaching applications.

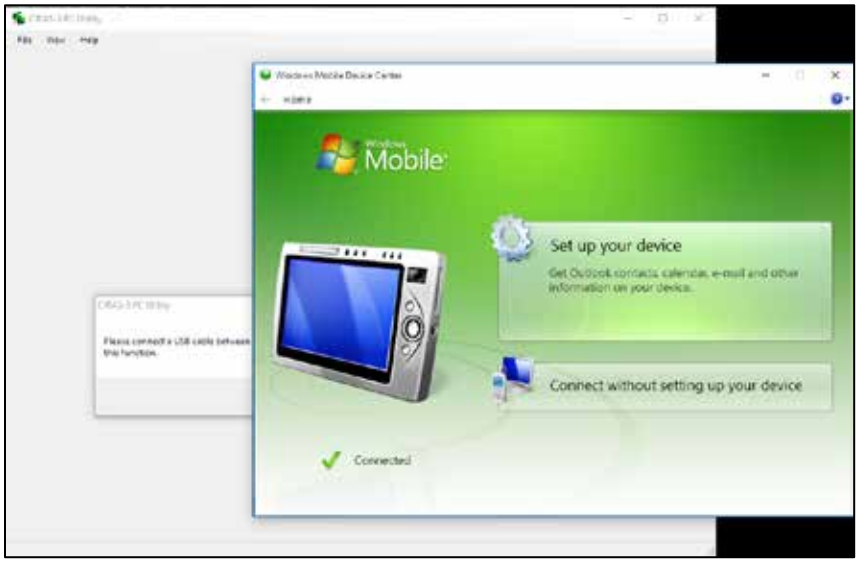
The connection between the CIRAS-3 console and the external device is made via a USB connection using the USB cable provided by PP Systems.

To start remote viewing of a CIRAS-3 console on an external device, first select **View>Remote Display** in the CIRAS-3 PC Utility software. A message will appear to “Please connect a USB cable between your desktop and the CIRAS-3 before using this function”.



If the USB cable is not connected connect it at this time and wait for Windows Mobile Device Center window (green window below) to appear, indicating the device is successfully connected.

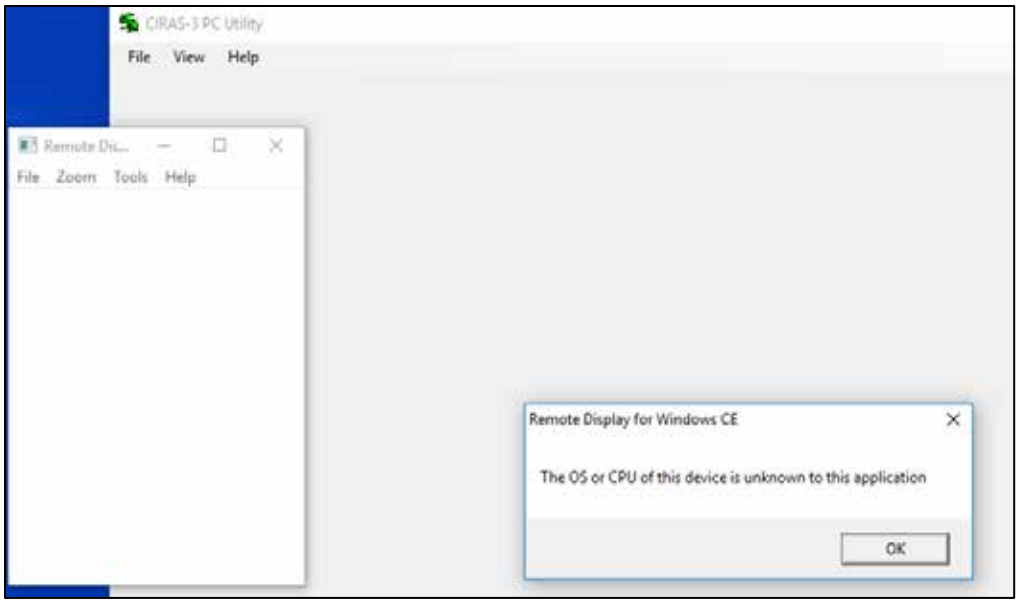
Note: For initial installation, the Windows Mobile Device Center requires access to the internet.



Next, select click the CIRAS-3 PC Utility to attain focus on that window and select **OK** in the original message window.

If you have previously connected the USB cable before starting the software, there is no need to wait for the Windows Mobile Device Center window just select **OK** in the original message window to continue.

Next two windows will appear. On the left, the initial remote window is displayed and also the message, “The OS or CPU of this device is unknown to this application”, will appear.



Select **OK** to bypass this message and the screen that is currently on the CIRAS-3 will now be displayed on your PC in the initial remote window.

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 in order to run this application.

Windows Vista, Windows 7, Windows 8 and Windows 10 Operating Systems
The Windows Mobile Device Center (WMDC) replaces ActiveSync.

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. Prior to measurement of chlorophyll fluorescence, we strongly recommend a review of the following publication:

> Baker NR (2008) Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. Annu. Rev. Plant Biol. 59: 89-113

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

Parameter	Calculation
F_v	$F_v = F_m - F_o$
F_v/F_m	$F_v/F_m = \frac{(F_m - F_o)}{F_m}$
Φ_{PSII}	$\Phi_{PSII} = \frac{(F_{m'} - F_s)}{F_{m'}}$
F_o'	$F_o' = \frac{F_o}{\left(\frac{F_v}{F_m}\right) + \left(\frac{F_o}{F_{m'}}\right)}$
F_v'	$F_v' = F_m' - F_o'$
F_v'/F_m'	$F_v'/F_m' = \frac{(F_{m'} - F_o')}{F_{m'}}$
J	$J = (I \cdot \alpha \cdot f_{II} \cdot \Phi_{PSII})$ 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	$q_p = \frac{(F_{m'} - F_s)}{(F_{m'} - F_o')}$
q_{NP}	$q_{NP} = \frac{(F_m - F_{m'})}{(F_m - F_o')}$
NPQ	$NPQ = \frac{(F_m - F_{m'})}{F_{m'}}$
q_L	$q_L = \left[\left\{\frac{F_{m'} - F_s}{F_{m'} - F_o'}\right\} \cdot \frac{F_o'}{F_s}\right] = q_p \cdot \frac{F_o'}{F_s}$
Φ_{NO}	$\Phi_{NO} = \left[\frac{1}{\frac{(F_m - F_{m'})}{F_{m'}} + 1 + \left[\left\{\frac{F_{m'} - F_s}{F_{m'} - F_o'}\right\} \cdot \frac{F_o'}{F_s}\right] \cdot \left(\frac{F_m}{F_o} - 1\right)}\right] = \left[\frac{1}{NPQ + 1 + q_L \cdot \left(\frac{F_m}{F_o} - 1\right)}\right]$
Φ_{NPQ-K}	$\Phi_{NPQ-K} = 1 - \frac{F_{m'} - F_s}{F_{m'}} - \left[\frac{1}{\frac{(F_m - F_{m'})}{F_{m'}} + 1 + \left[\left\{\frac{F_{m'} - F_s}{F_{m'} - F_o'}\right\} \cdot \frac{F_o'}{F_s}\right] \cdot \left(\frac{F_m}{F_o} - 1\right)}\right] = 1 - \Phi PSII - \Phi_{NO}$ Note: $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$
$\Phi_{f,D}$	$\Phi_{f,D} = \frac{F_s}{F_m}$
Φ_{NPQ-G}	$\Phi_{NPQ-G} = \frac{F_s}{F_{m'}} \cdot \frac{F_s}{F_m}$

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 eturned with each repeated ϕPSII measurement, assuming the initial measurement was F_v/F_m. Without an initial F_v/F_m, 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.

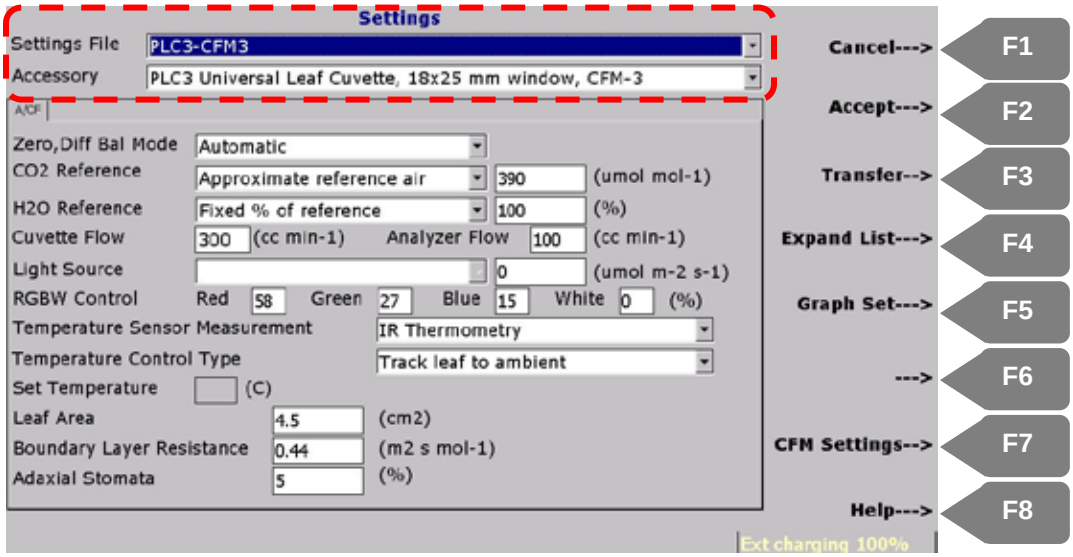
Tip

When performing chlorophyll fluorescence measurements with CFM-3 at high Q above 5000 µmol m^{-2} s^{-1}, it is highly recommended that external power or auxiliary external battery pack to be connected. Internal battery operation for state of charge less than 75% can produce inaccurate readings. Using a predominantly-red light source (i.e. 90% red/10% blue), or using the Multi-Pulse functionality with multiple steps of lower intensities will significantly reduce the likelihood of inaccurate readings.

If the internal battery voltage drops below 6.5 volts a message will appear on the PLC3 display.

CFM-3 Set Up

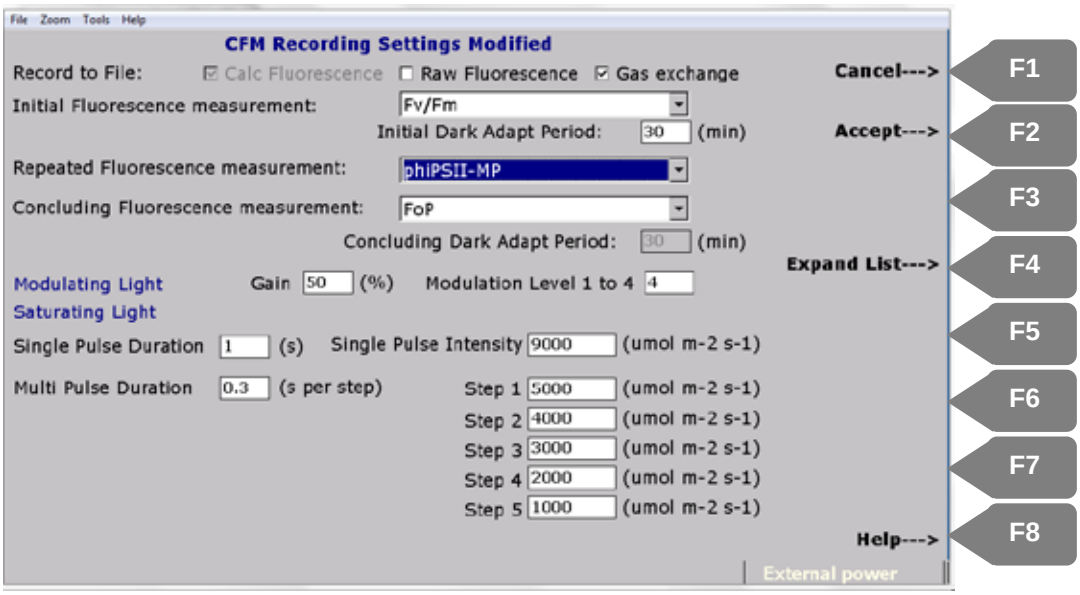
At either the Numeric View or Graph View screens select **Settings (F2).** You must first select the appropriate Settings File and Accessory as shown below:



Note. Make sure that you select the appropriate PLC3 Universal window size (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, Gas exchange.** 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. **Gas exchange** 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 **Gas exchange** 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	**phiPSII-SP (ϕPSII-Single-Pulse)**. If selected, there is no need to dark adapt the leaf and measurements can begin right away and a single, saturating pulse applied to the light-adapted leaf sample. The single pulse duration and intensity can be set in the lower part of the screen under “Saturating Light”. **phiPSII-MP (ϕPSII-Multi-Pulse)**. If selected, there is no need to dark adapt the leaf and a “Multi-Pulse sequence can begin right away with multiple, saturating pulses applied to the light-adapted leaf sample. The Multi-Pulse duration and intensity settings (up to 5 steps) can be set in the lower part of the

<table>
<tr><td></td><td>screen under “Saturating Light”.

Fv/Fm. If selected, you will need to allow the plant to dark acclimate prior to measurement. Set the Initial Dark Adapt Period of your choosing. Longer dark adapt period are required for plants that have been under high light and shorter periods for plants that have been in the dark or low 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.

<table>
<tr><th>DA period (minutes)</th><th>5</th><th>10</th><th>15</th><th>20</th><th>25</th><th>30</th></tr>
<tr><td>F_v/F_m</td><td>0.63</td><td>0.71</td><td>0.78</td><td>0.81</td><td>0.80</td><td>0.81</td></tr>
</table>
</td></tr>
<tr><td>Repeated Fluorescence Measurement</td><td>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).

None. If selected, there will be no chlorophyll fluorescence measurements performed after a photosynthesis measurement has been recorded.

phiPSII-SP (ϕPSII-Single-Pulse). If selected, a single, saturating pulse will be applied to the light-adapted leaf sample. The single pulse duration and intensity can be set in the lower part of the screen under “Saturating Light”.

phiPSII-MP (ϕPSII-Multi-Pulse). If selected, multiple, saturating pulses will be applied to the light-adapted leaf sample. The Multi-Pulse duration and intensity settings (up to 5 steps) can be set in the lower part of the screen under “Saturating Light”.

ϕPSII. If this is selected, a saturating pulse will be applied to the light-adapted leaf sample after a photosynthesis measurement has been recorded.
phiPSII-FoP (ϕPSII-Fo’). If this is selected, a saturating pulse will be applied to the light-adapted leaf sample followed by illumination using the far-red light for measurement of Fo’ after a photosynthesis measurement has been recorded.

Fs. If this is selected, the steady state fluorescence will be recorded after a photosynthesis measurement is recorded.</td></tr>
<tr><td>Concluding Fluorescence Measurement</td><td>This defines the final measurement to be recorded after the last photosynthesis measurement.

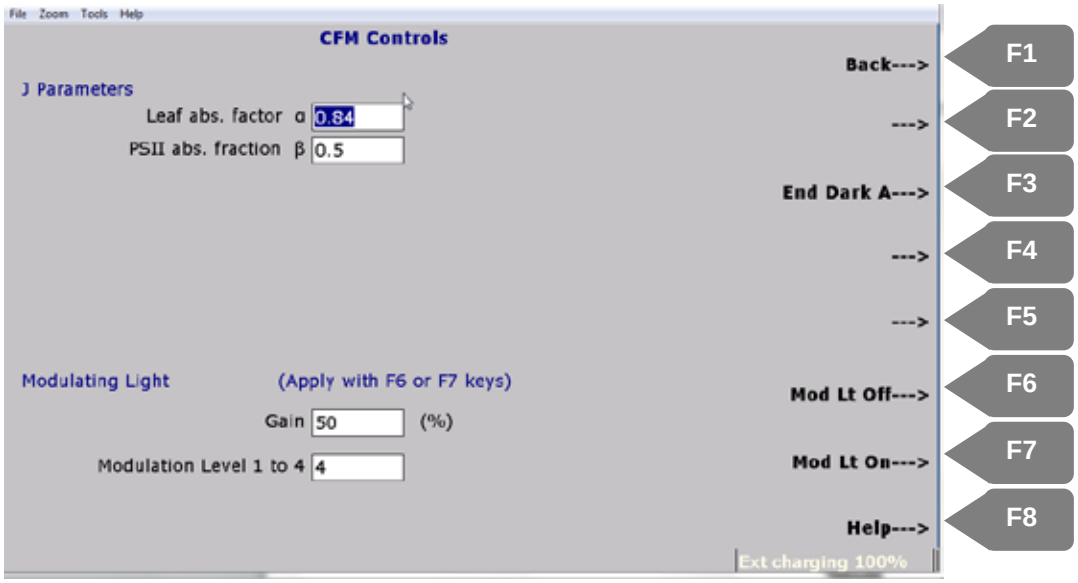
None. If this is selected, there will be no concluding chlorophyll fluorescence measurement and the sequence will terminate after the last photosynthesis measurement.</td></tr>
</table>

	FoP (Fo'). If selected, far-red light will be applied to the leaf sample. **Fv/Fm**. If selected, you must allow the leaf to dark adapt prior to making the measurement. Enter the time (in minutes) at "**Concluding Dark Adapt Period**" and we recommend at least 20-30 minutes for best results.
Modulating Light	**Gain**. Used to adjust the resolution of the fluorescence signal. We recommend that the gain be set to a % that achieves a steady F_o value between 400-700. **Modulation Level**. Modulation Level (1-4) 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.
Saturating Light	**Single Pulse Measurements** **Single Pulse Duration**. The time (in seconds) for the Single Pulse Duration saturating light to be applied. **Single Pulse Intensity**. The saturation pulse intensity (0-10000 µmol m^{-2} s^{-1}). The maximum intensity of 10000 µmol m^{-2} s^{-1} can only be achieved if using one LED color (i.e. 100% red, 100% green, 100% blue or 100% white). Typically, a combination of LEDs are used for measurements so we recommend using an intensity of up to 9000 µmol m^{-2} s^{-1} and with predominantly red light (i.e. 90% red/ 10% blue). The default value is 9000. **Multi-Pulse Measurements** Multi-Pulse Duration. The time (in seconds) for each multiple pulse duration saturating light to be applied. For Multi-Pulse measurements, you have up to 5 steps but you must set at least 3 steps. Based on our testing, we recommend at least one step above 3000 µmol m^{-2} s^{-1} at the high end and one step below 1500 µmol m^{-2} s^{-1} at the lower end with an even distribution of light in between the high and low light settings..

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:



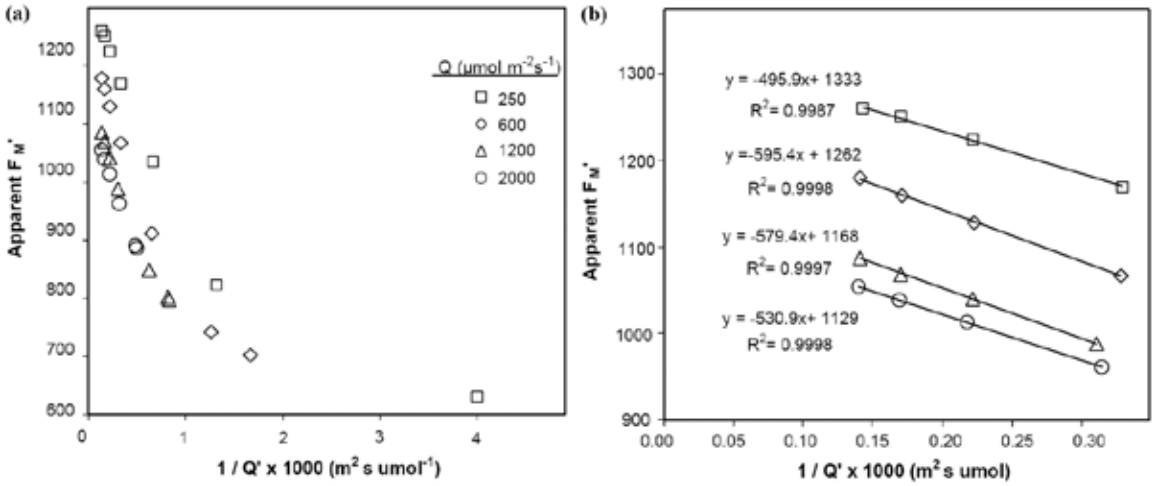
CFM-3 Control	
J Parameters	Factors used for calculation of electron transport rate. **Leaf abs. factor α.** Default value set to 0.84 but can be determined by use of an integrating sphere. **PSII abs. factor β.** Default value set to 0.5 but a known range of values exist for different plant types.
Modulating Light	**Gain.** Used to adjust the resolution of the fluorescence signal. We recommend that the gain be set to a % that achieves a steady F_o value between 400-700. **Modulation Level.** Modulation Level (1-4) 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..

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.

Measurement of Fm' with CIRAS-3

Measurement Principle

Under high actinic light conditions, Fm' measured using a single saturation pulse may be underestimated due to the rapid turnover rate of PSII reaction centers. The Multi-Pulse sequence with CIRAS-3 (Console software V. 1.08 and above), based on a method developed by Earl et al. (2004), allows for a more accurate estimate of Fm' by using a train of varying low-intensity saturating pulses. The principle of the Multi-Pulse flash can be illustrated with the graph below.



Fm' is measured at a number of saturating light intensities (Q'). In the above figure, Fm' is plotted against 1/Q'. At light intensities > 1000 µmol m^{-2} s^{-1}, Fm' can be seen as linearly correlated to 1/Q'. It has been suggested that this relationship allows us to extrapolate back to the y-axis and use the y-intercept as an approximate of Fm' at infinite light intensity. This figure is adapted from Earl et. al (2004).

Fm' measured using the Multi-Pulse sequence leads to an improvement in measured Fm', especially when the plant is under higher light intensities where it is difficult to completely saturate primary photochemistry.

The CIRAS-3 implementation of Multi-Pulse allows for 3 to 5 user-defined saturating pulses of varying intensities (Q') to be executed in sequence during one measurement cycle of apparent Fm'. The CIRAS-3 computes the linear regression of Fm' vs. 1/Q', then extrapolates and displayes Fm' at 1/Q' = 0.

Operations

To use the Multi-Pulse function, make sure that you have the required software and firmware as follows:

CIRAS-3: Console software Version 1.08 or above.

Firmware Version 3.05 or above.

PLC3: Firmware Version 3.04 or above.

To set the Multi-Pulse settings:

1. On the home screen, go to **Settings** > **CFM Settings**
2. In the "**initial fluorescence measurement**" field, there will be an option for "**PhiPSII-MP**". Choose this if you wanted to skip the dark adaptation period and use Multi-Pulse to measure PhiPSII right away under light adapted state.
3. In the "**repeating fluorescence measurement**" field, choose "**PhiPSII-MP**" for Multi-Pulse.
4. On the lower half of the panel are text fields for putting in "**duration**" and "**intensity**" of each step. The Multi-Pulse function allows up to 5 steps and requires at least 3 steps; you can leave the 4th and the 5th field blank. The default setting of Multi-Pulse is 0.3 sec per step, with an intensity of 5000, 4000, 3000, 2000, 1000 µmol m^{-2} s^{-1}.
5. Press **Accept** to accept the CFM Settings, and then **Accept** again to return to the main menu.
6. Start a measurement operation as described in previous sections of the manual.

Our testing of CIRAS-3 CFM Multi-Pulse function on light-adapted, fully expanded tobacco leaves suggest that 5 saturating pulses at widely-spaced Q' intensities (for example, 5000, 4000, 3000, 2000, 1000 µmol m^{-2} s^{-1}) of 0.3 seconds each produced improved Fm' measurement vs. Fm' measured by a single pulse, especially when actinic light is above 1000. While level of saturating intensities (Q') are user definable, for best results, we recommend that at least one step set to be higher than 3000 µmol m^{-2} s^{-1} (the high point), and at least one step set to be lower than 1500 µmol m^{-2} s^{-1} (the low point), and generate the intermediate light intensities by evenly distribute numbers in between.

One advantage of using Multi-Pulse is that lower light intensities can be used compared to single pulse. This decreases the voltage draw on the instrument and lessens the likelihood of inaccurate readings at very low battery levels.

References

Markgraf T, Berry J (1990) Measurement of photochemical and nonphotochemical quenching: correction for turnover of PS2 during steady-state photosynthesis. *Current Research in Photosynthesis* IV: 279-282

Earl H, Ennahli S (2004) Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. *Photosynth Res* **82**: 177-186

Loriaux SD, Avenson TJ, Welles JM, McDermitt DK, Eckles RD, Riensche B, Genty B (2013) Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. *Plant Cell Environ* **36**: 1755-1770

Section 11. Closed System Measurements

Soil CO_2 Efflux and Net Canopy CO_2 Flux

The **SRC-1 and SRC-2 Soil Respiration Chambers** 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 and CPY-5 Canopy Assimilation Chamber** can also be used with the CIRAS-3 for closed system measurement of net canopy CO_2 flux**. Important Note. When using the SRC-1, SRC-2, CPY-4 and CPY-5 with the CIRAS-3, the reference sampling pump should be sealed (Our Part No. STD105) to ensure leak-tight seal for closed system operation. Please contact PP Systems if you have any questions.**

Theory

The respiration/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 :-

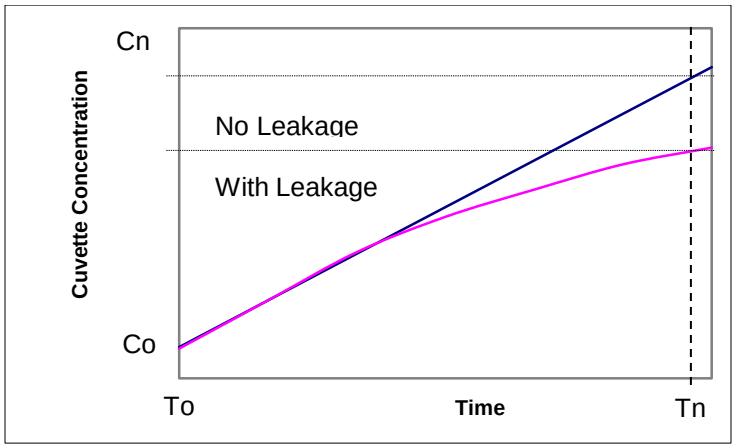
$$R = \frac{(Cn - Co)}{Tn} * \frac{V}{A} \qquad (1)$$

Where R is the respiration/assimilation rate (CO_2 flux, or moles of CO_2 unit area^{-1} unit time^{-1}), 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.

FCO_2 Units for measurement of Soil CO_2 Efflux

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-liter/liter = micro-bar/bar = micro-mol/mol).

Now one kg mol of gas (44.01kg of CO_2) at STP (0 ºC, 1013.25 mb) occupies 22.41 m^3.

Thus:

$$R = b * \frac{P}{1013.25} * \frac{273}{273 + Ta} * \frac{44.01}{22.41} * \frac{V}{A} \qquad (3)$$

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 $g.m^{-2}.hr^{-1}$.

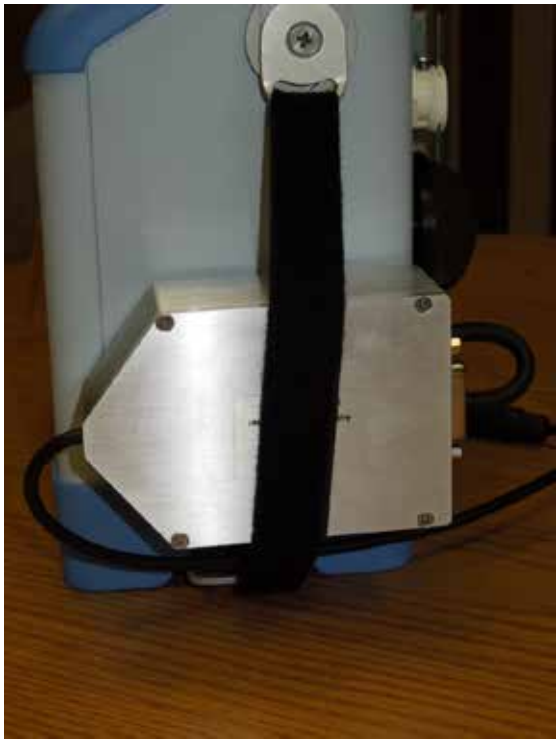
To convert ($g.m^{-2}.hr^{-1}$) to (micro-$mol.m^{-2}.s^{-1}$) multiply by 6.312

References

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

Electrical and Pneumatic Connection between the CIRAS-3 and SRC-1 / CPY-4

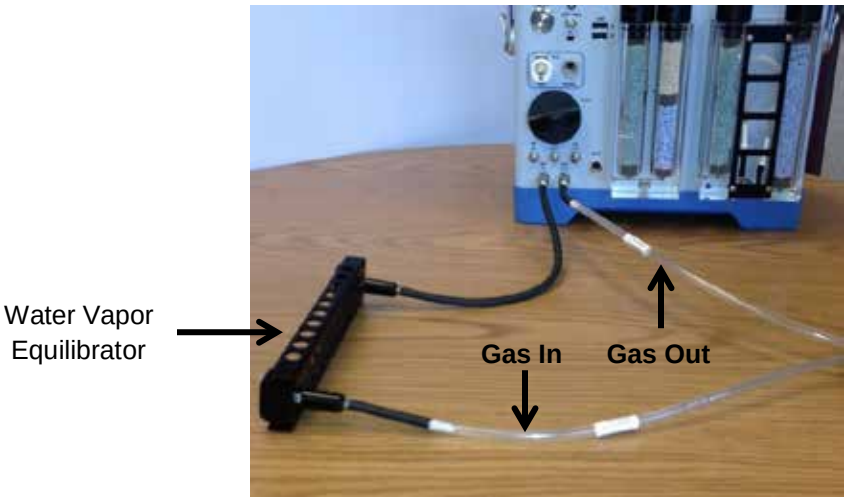
In order to use the SRC-1 and CPY-4 with the CIRAS-3, an Auxiliary Probe Adapter (APA) is required. The APA connects electrically to the CIRAS-3 via the 4 pin socket labeled “AUX” in the lower left hand corner of the CIRAS-3 console. The SRC-1 and CPY-4 (and STP-1 Soil Temperature Probe if used) connect to either of the 15 pin sockets on the other end of the APA. The APA contains electronics that allow the these chambers 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. Remove the link pipe between “REF IN” and “AIR OUT” which is only used when using the leaf cuvettes with the CIRAS-3.

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:



Electrical and Pneumatic Connection between the CIRAS-3 and SRC-2 / CPY-5

PP Systems introduced the SRC-2 Soil Respiration Chamber and CPY-5 Canopy Assimilation Chamber in March 2016 for use with the CIRAS-3. Both can connect electrically directly to the "AUX" port on the CIRAS-3 console (Auxiliary Probe Adapter not required). All pneumatic connections are identical as described in the previous sections. The only other major difference between the SRC-1 and SRC-2 is that the SRC-2 has a built-in sensor for measurement of air temperature near the soil surface (Tsurface). Therefore there is no need to manually enter this value when using the SRC-2 Soil Respiration Chamber. Like the SRC-2, the CPY-5 Canopy Assimilation Chamber also connects electrically directly to the "AUX" port on the CIRAS-3 console. Other than the electrical connector the CPY-4 and CPY-5 are virtually identical form an operation standpoint.

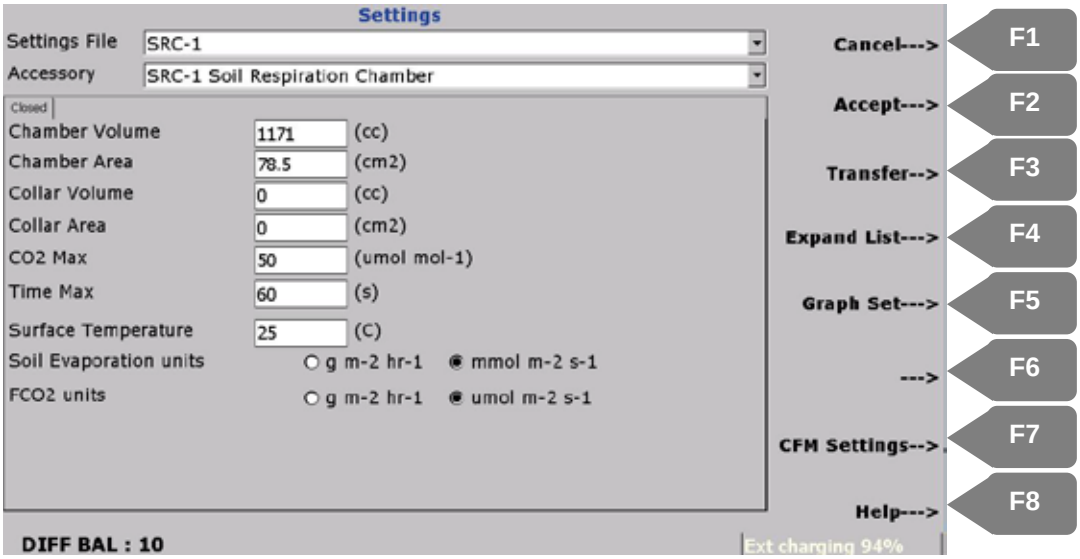
Configuring the CIRAS-3 console for measurement of Soil Respiration or Canopy Assimilation

As discussed earlier in this manual and from the main screen, select Settings (F2) to configure the system for closed system operation by selecting the appropriate chamber (SRC-1 or SRC-2) Soil Respiration Chamber. Note that the Settings and displays are almost identical for the SRC-1 and SRC-2 with the main difference being surface temperature (Tsurface). This value must be entered manually with the SRC-1. The SRC-2 measures, displays and records this value automatically (Tsurface).

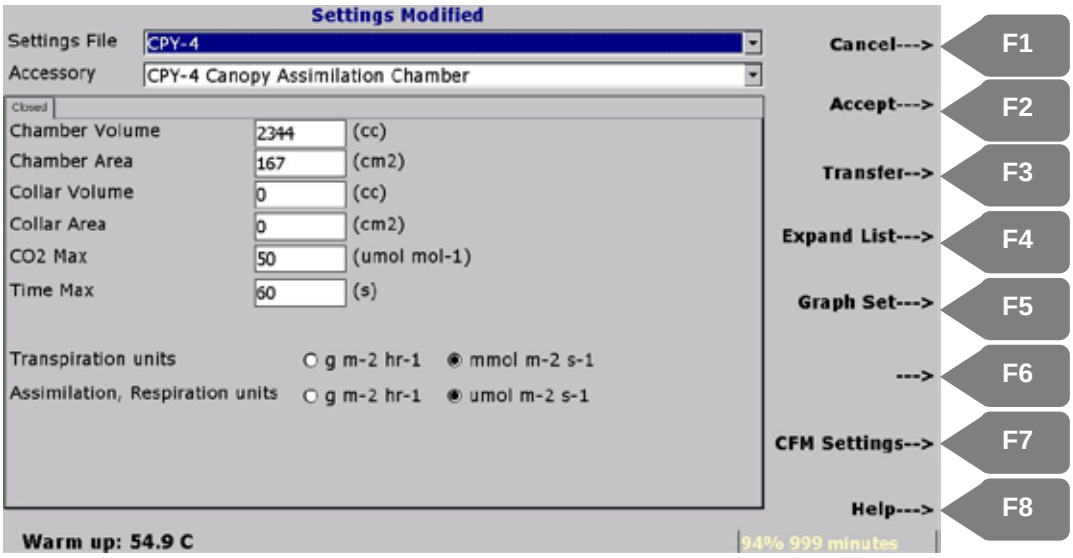
Settings (F2)

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 or SRC-2 Soil Respiration Chamber or CPY-4 or CPY-5 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/SRC-2	CPY-4/CPY-5
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. With the SRC-1 this value is entered by the user. The SRC-2 includes a surface temperature sensor and this value is measured and displayed continuously.	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	Not applicable	g m^{-2} hr^{-1} mmol m^{-2} s^{-1} (Default)
Assimilation, Respiration units	Not applicable	g m^{-2} hr^{-1} µmol m^{-2} s^{-1} (Default)

Important note. If using collars with our chambers 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 Evaporation, FCO2, Transpiration and Assimilation, Respiration for later data analysis.

Do I Use Linear versus Quadratic

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 high 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

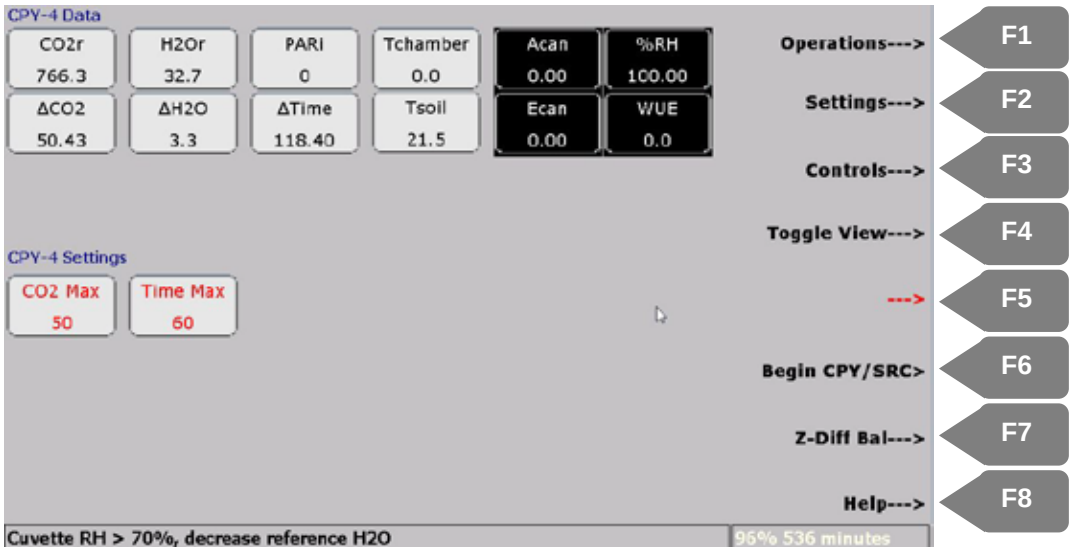
Many of the features related to closed system measurement screens (numerical and graphical presentation of data, graph set, recording measurements, etc.) are similar to what has already been discussed in this operation manual. The displays are also similar when using the SRC-1 / SRC-2 Soil Respiration Chamber or CPY-4 / CPY-5 Canopy Assimilation Chamber.

Numerical Data Display (SRC-1 and SRC-2)

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 / Tsurface	Soil temperature if STP-1 is used (^oC) or Surface temperature if SRC-2 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 and SRC-2 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 (^{o}C). The default value is 25 but this should be set to your actual surface temperature for accurate results when using the SRC-1. This is not displayed when using the SRC-2 and Tsoil is replaced with Tsurface.

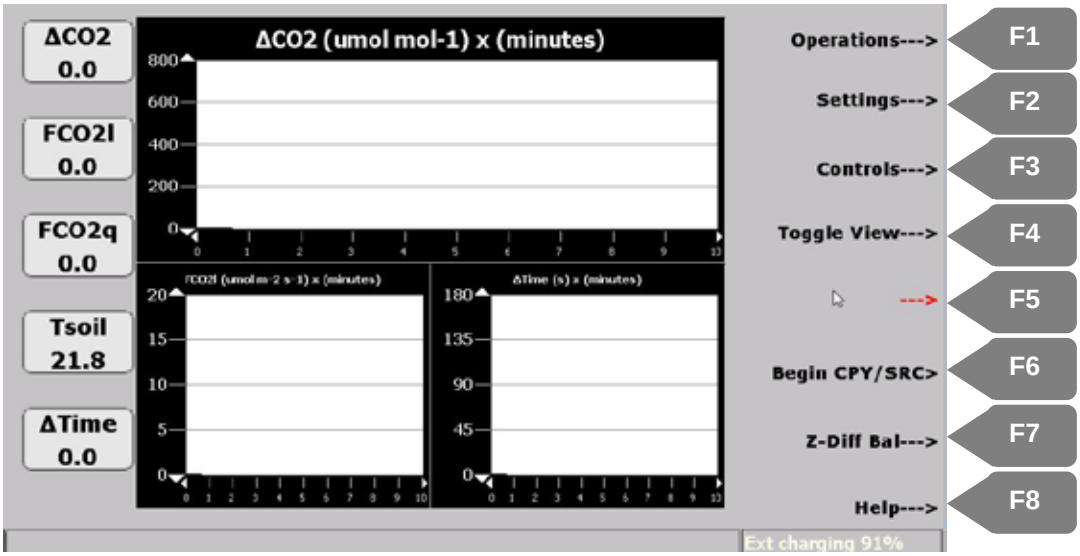
Numerical Data Display (CPY-4 and CPY-5)



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/CPY-5 (µmol m^{-2} s^{-1}).
ΔTime	Change in time during measurement sequence (Seconds).
Tchamber	Air temperature inside the CPY-4/CPY-5 (^{o}C).
Tsoil	Soil temperature if STP-1 is used (^{o}C).
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 mmol 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/SRC-2 and CPY-4/CPY-5)



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

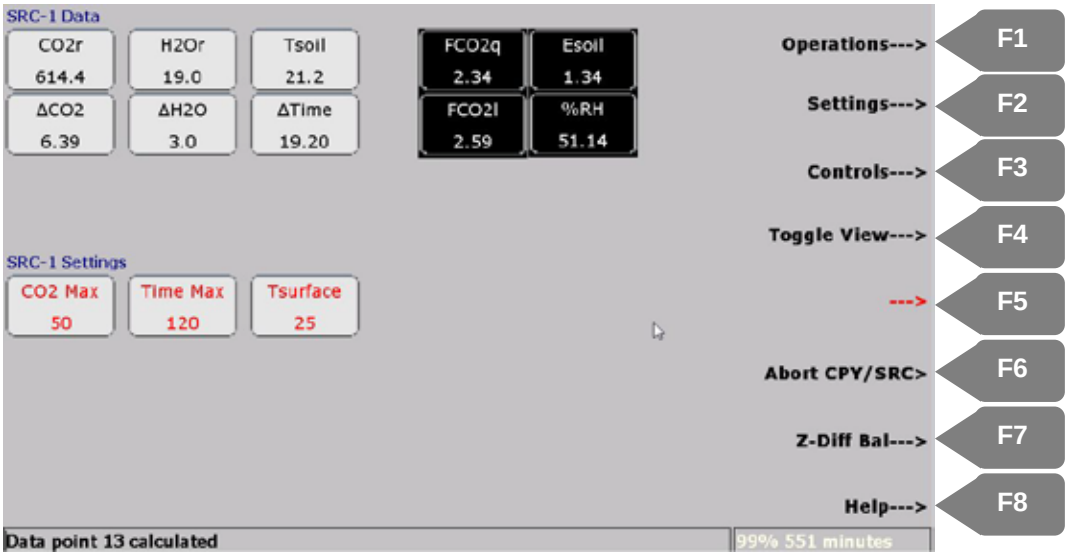
Measurement Sequence

Once you have selected the appropriate accessory (SRC-1, SRC-2, CPY-4 or CPY-5) 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 data can be output in 3 different formats depending on the accessory or accessories used with the CIRAS-3.

- Photosynthesis Data
- Photosynthesis and Chlorophyll Fluorescence Data
- Closed System Data (Soil Respiration and Canopy Assimilation)

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
RecType	ExcelTime	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od
M	12/16/13 12:40 PM	390.00	390.10	0.10	11.80	11.90	0.10

µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	%	%	%	%	°C	°C	°C
PARi	PARe	Red	Green	Blue	White	Tamb	Tcuv	Tleaf
100.00	17.00	90	0	10	0	26.80	26.30	26.80

cm^2	cc min^{-1}	mb	%	µmol mol^{-1}	mmol m^{-2} s^{-1}	kPa	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol CO_2 mol^{-1} H_2O
Aleaf	Flow	Patm	RH	Ci	gs	VPD	A	E	WUE
4.5	399.00	1006.00	34.78	438.00	2.00	2.33	-0.10	0.06	-1.67

m^2 s mol^{-1}	%	Temperature Sensor Measurement (see below)	Temperature Control Type (see below)	Light Control Type (See below)	PLC3 Used	Messages
rb	Adaxial	Tsensor	Tcontrol	Lcontrol	PLC	Status
0.4	50	IR	LA	CFM-3	U	

For support related issues, we also include information related to temperature sensor measurement, temperature control type, light control type and type of PLC3 used in the data output. The last column labeled “Status” is there to report any errors that may have been reported during a measurement. Normally this field is empty with the Photosynthesis data output. If an error is reported it will show up in this column.

Temperature Sensor Measurement	
IR	Infrared Thermometry. Only available with the PLC3 Universal Leaf Cuvette.
EB	Energy Balance. Available with all PLC3 leaf cuvettes.
TH	Thermistor. Only available with the PLC3 Narrow and Conifer Leaf Cuvettes.

Temperature Control Type	
LT	Leaf temperature control
LA	Track leaf to ambient.
CT	Cuvette temperature control.
CA	Track cuvette to ambient.
D	Disable temperature control.

Light Control Type	
LED	Standard LED Light Unit
CFM-3	CFM-3 Chlorophyll Fluorescence Module
AMB	Ambient. No light unit in use.

PLC3 Used	
U	PLC3 Universal Leaf Cuvette
C	PLC3 Conifer Leaf Cuvette
AMB	Ambient. No light unit in use.
A	Analyzer only.
P	Probe (Accessory)
UF	PLC3 Universal Leaf Cuvette with CFM-3

Photosynthesis and Chlorophyll Fluorescence Data

When our PLC3 Universal Leaf Cuvette is used with the CIRAS-3 for measurement of photosynthesis and/or chlorophyll fluorescence, the stored data is output similar to the following (**May vary slightly depending on measurement configuration**):

	Date/Time	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	mb	mb	mb
RecType	ExcelTime	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od
m1	12/16/13 12:35 PM						
m2	12/16/13 12:35 PM						
M	12/16/13 12:40 PM	390.00	390.10	0.10	11.80	11.90	0.10
m5	12/16/13 12:40 PM						
m4	12/16/13 1:54 PM						
m8	12/16/13 1:54 PM						

µmol m^{-2} s^{-1}	µmol m^{-2} s^{-1}	%	%	%	%	°C	°C	°C
PARi	PARe	Red	Green	Blue	White	Tamb	Tcuv	Tleaf
		90	0	10	0			
		90	0	10	0			
100.00	17.00	90	0	10	0	26.80	26.30	26.80
		90	0	10	0			
		90	0	10	0			

cm^2	cc min^{-1}	mb	%	µmol mol^{-1}	mmol m^{-2} s^{-1}	kPa	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol CO_2 mol^{-1} H_2O
Aleaf	Flow	Patm	RH	Ci	gs	VPD	A	E	WUE
4.5									
4.5									
4.5	399.00	1006.00	34.78	438.00	2.00	2.33	-0.10	0.06	-1.67
4.5									
4.5									

m^2 s mol^{-1}	%	Temperature Sensor Measurement (see below)	Temperature Control Type (see below)	Light Control Type (See below)	PLC3 Used	Messages or Chlorophyll Fluorescence Measurement Type
rb	Adaxial	Tsensor	Tcontrol	Lcontrol	PLC	Status
0.4	50	IR	LA	CFM-3	UF	raw F
0.4	50	IR	LA	CFM-3	UF	Fv/Fm
0.4	50	IR	LA	CFM-3	UF	
0.4	50	IR	LA	CFM-3	UF	phiPSII
0.4	50	IR	LA	CFM-3	UF	FoP
						Fs

F	Fo	Fm	Fv	FvFm	FmP	Fs	phiPSII	FoP	FvP	FvPFmP
05										
	562	1154	592	0.513	0	0	0.000	0	0	0.000
	562	1154	592	0.513	687	518	0.246	422	0	0.385
	562	1154	592	0.513	1038	665	0.359	567	471	0.454
						100				

J	qP	qNP	NPQ	qL	phiNO	phiNPQK	phifD	phiNPQG
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10.332	0.638	0.638	0.680	0.520	0.449	0.305	0.449	0.338
15.078	0.792	0.198	0.112	0.675	0.548	0.093	0.576	0.369

Record Types (RecType)	
m1	Data associated with the raw fluorescence signal. Note the "rawF" in the Status column.
m2	Data associated with a F_v/F_m measurement. Note the "Fv/Fm" in the Status column.
M	Data associated with photosynthesis measurement. Under normal operating conditions with no errors reported, this column should be empty.
m4	Data associated with a Fo' measurement. Note the "FoP" in the Status column.
m5	Data associated with a ϕPSII-SP measurement. Note the "phiPSII-SP in the Status column.
m8	Data associated with Fs (fluorescence signal). Note the "Fs" in the Status column.

m9	Data associated with a ϕPSII-MP (Apparent Fm') measurement. Note the "phiPSII-MP in the Status column.

Closed System Operation Data

When the SRC-1/SRC-2 Soil Respiration Chamber and CPY-4/CPY-5 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 in CIRAS-3

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 (V_0) at 0 °C and 1013.25 mb. The Ideal Gas molar volume is 22.414 L mol^{-1} at 0 °C and 1013.25 mb (STP). Therefore:

$$\boldsymbol{W(mol\ m^{-2}s^{-1})} = \left(\frac{\boldsymbol{V_0}}{\boldsymbol{60 \times 10^3}}\right) \times \left(\frac{\boldsymbol{1}}{\boldsymbol{22.414}}\right) \times \left(\frac{\boldsymbol{10^4}}{\boldsymbol{a}}\right)$$

Where: ***a*** is projected leaf area (cm^2 converted to m^2). V_0 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:

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

(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:

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

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

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

(2.4) Therefore:

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

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.

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

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

Leaf Temperature

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

(3.1) From Parkinson, 1983, the difference between air and leaf temperature is:

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

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:

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

(3.2) From this we derive:

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

Saturation Vapor Pressure

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

(4.1) From Buck, 1981 (using e_{w1} and f_{w1}) *we calculate*:

$$e_{leaf} = 6.1365 \times exp\left[\frac{T_{leaf} \times (17.502)}{T_{leaf} + 240.97}\right]$$

Stomatal Conductance

Calculate stomatal conductance (g_s)

(4.2) From *Caemmerer & Farquhar, 1981* (Eq B14), total leaf conductance is calculated as:

$$g_{total} = \frac{E \times \left(P - (e_{leaf} + e_{out})/2\right)}{\left(e_{leaf} - e_{out}\right)}$$

(4.3) Since $1/g_{total} = r_s + r_b$,

(4.4) stomatal resistance can be calculated as:

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

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

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

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:

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

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

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

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 ($\boldsymbol{C_i}$) using the equation derived by von Caemmerer & Farquhar, 1981

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

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

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

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

(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 (l) = 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 based on current environmental control settings) 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.
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.	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.
	Capacity is being reduced as a result of age and maintenance.	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.

If you observe…	This usually means…	Solution
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.

CO_2 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.	The soda lime or molecular sieve are exhausted and require replacement.	Check soda lime and molecular sieve 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.
CO2r settle value is outside of 10 µmol mol-1 when CO2 Reference is set to "Approximate reference air"	You forgot to perform a Max C calibration.	Perform Max C calibration (See Find Max C).

If you observe…	This usually means…	Solution
Fluctuating CO2r or CO2a values	Exhausted desiccants.	Replace
	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.
	Dirty hydrophobic filter.	Replace white plastic 5 micron hydrophobic filter.
CO_2 cartridge rapidly exhausts when fitting a new one (loud hissing noise).	A problem with the piercing pin.	Check that the piercing pin is in place inside 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 regulator for leaks and 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.
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.
Steady, positive CO2d value even with empty cuvette	System was likely stored in the black transport case with a CO_2 cartridge that still had CO_2 in it.	Allow system to equilibrate for a period of time (could be many hours) prior to next measurements. See Storage on page 9 for more details.
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.	Insert fresh CO_2 cartridge
	Exhausted desiccants.	Replace all desiccants.

H_2O 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.	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.
	Exhausted H_2O scrubber.	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.**
Fluctuating H2Or or H2Oa values	Exhausted desiccants.	Replace Drierite and Molecular Sieve.
	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.
	Dirty hydrophobic filter.	Replace white plastic 5 micron hydrophobic filter.
A slow drift in H2O readings	Exhausted desiccants.	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 (See 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).
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).
	The incident light in the PLC3 is high and not allowing you to cool down low enough below ambient.	If you are operating at high ambient light intensity (> 1500 µmol m^{-2} s^{-1}) with or without the light unit, this will affect the cooling.
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).
	The incident light in the PLC3 is high and not allowing you to cool down low enough below ambient.	If you are operating at high ambient light intensity (> 1500 µmol m^{-2} s^{-1}) with or without the light unit, this will affect the cooling.

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 almost 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.	Connect the light unit to the PLC3.
	Light Source is set to Ambient under Settings.	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	Check both the Zero and CO2/H2O control absorber columns and make sure that they are secure and fitted properly.
	A problem with air supply pump.	Replacement of the diaphragm pump may be required.
	Someone tried to change out a CO_2 cartridge that was full or near full and highly pressurized.	When this happens you will usually hear a loud “pop”. Inside trace the tubing between the back of the CO_2 regulator to the diversion solenoid on the main flow board and reconnect.

If you observe…	This usually means…	Solution
Pumps sound louder than usual	A restriction somewhere in the gas circuit.	Check for kinked tubing.
	One of the absorber column assemblies is not firmly seated in the manifold.	Check both absorber column assemblies and ensure secure fit.
	Tubing may have slipped of one of the internal sampling pumps.	Check all internal pumps and tubing connections.
	Blocked air filter(s).	Try changing out filters to see if problem is rectified.
Reference 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 REF 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.
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 computer.

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

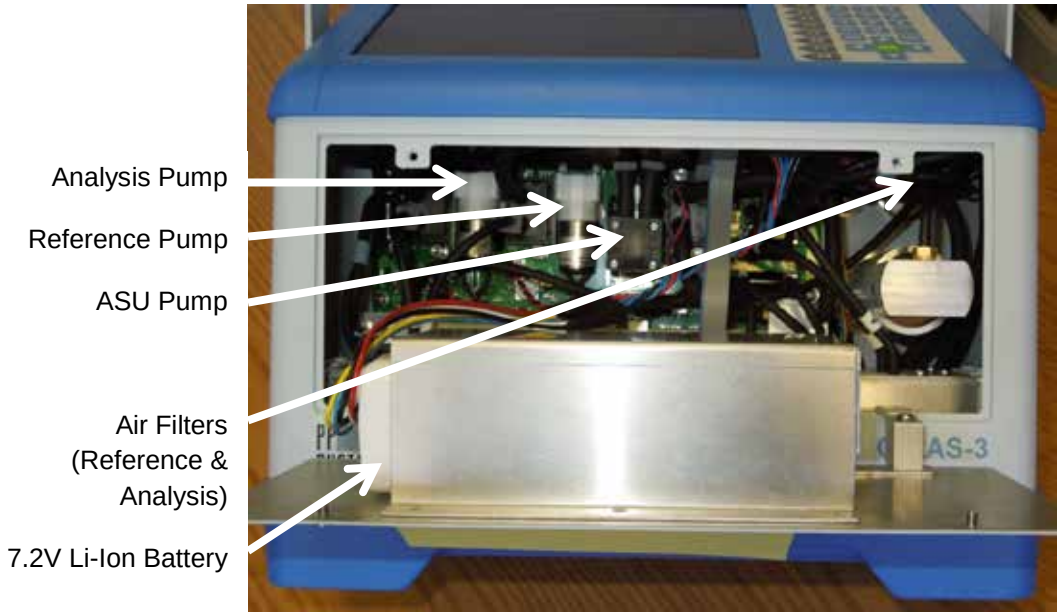
If you see the following status message displayed on the CIRAS-3 main console…	It usually means….	Solution to the problem.
DIFF-BAL required, ensure CO2r stable	This message appears when Zero,Diff Bal Mode is set to "Manual" under Settings and the system is recommending that you perform a DIFF BAL (which 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.
Stored DIFF BAL CO2 out of range	The maximum reference CO_2 concentration has changed and is significantly higher than the maximum reference CO_2 recorded when you performed a Stored Diff Bal Calibration.	Perform a new Stored Diff Bal Calibration and make sure the CO_2 max value is high enough to cover the range.

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.
Stored Diff Bal H2O out of range	The maximum reference H_2O concentration has changed and is significantly higher than the maximum reference H2O recorded when you performed a Stored Diff Bal Calibration. This is common when there is a change in desiccants after initial Stored Diff Bal Calibration.	Perform a new Stored Diff Bal Calibration with the new desiccants.
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:

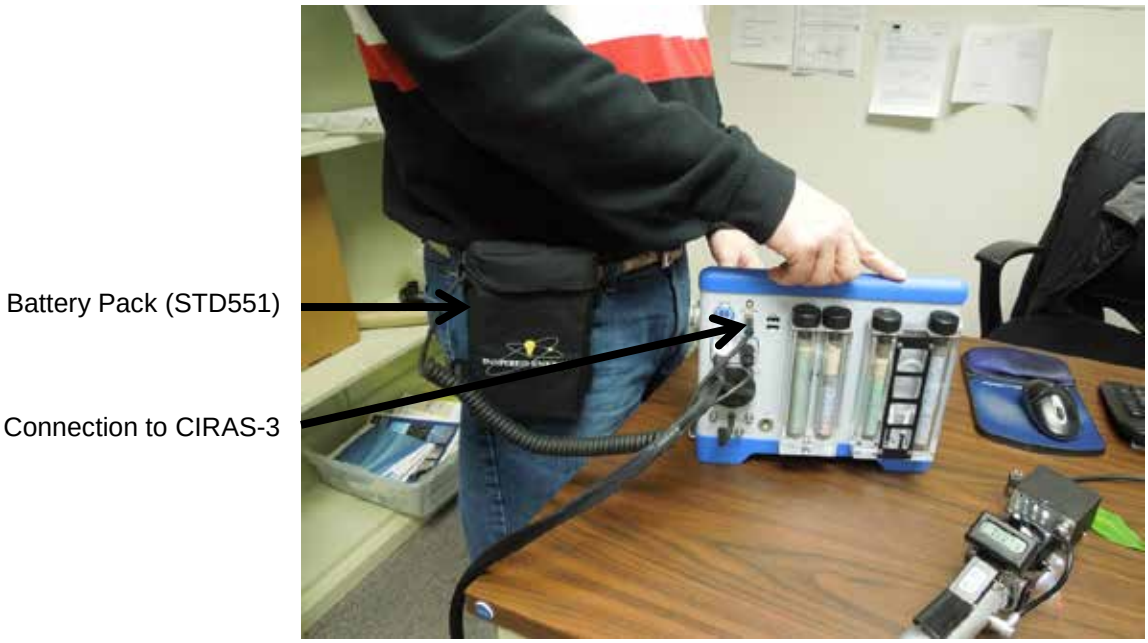
- Li-Ion Battery
- Air Sampling Pumps (Reference and Analysis)
- Air Filters

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 may wear and deposit material inside the pump. If these pumps are running erratically or sound louder than normal, we recommend cleaning the pump(s) by flushing it through with iso-propyl alcohol.

The following procedure can be adopted:

1. Remove the gas tubing from the inlet and exhaust ports on the pump and remove the pump from the CIRAS-3 console. Fit a small piece of tubing on both the inlet and exhaust ports on the pump.
2. Hold the pump above a beaker of iso-propyl alcohol and dip the tube into the alcohol (see below).
3. Using a laboratory power supply, simply apply 5-6V to both pins on the black electrical connector to power the pump.

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 inside the console 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

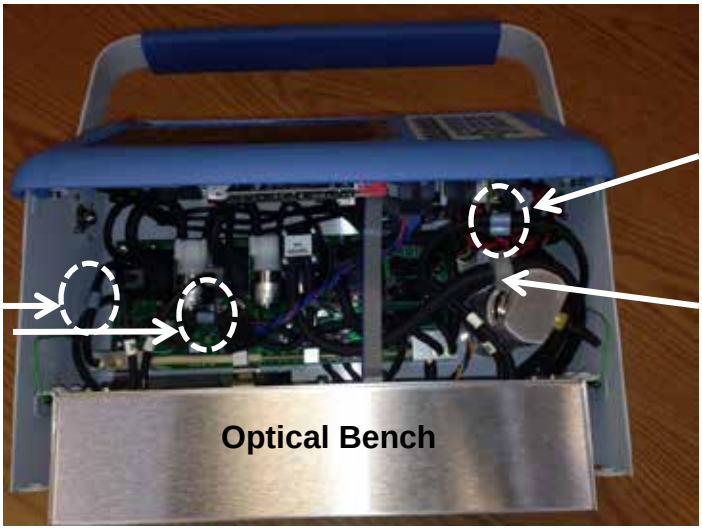
The main air supply pump (ASU) is a “diaphragm” style pump and should not require replacement or routine maintenance. It is located to the right of the reference air sampling pump and secured in place by Velcro and tubing. The electrical connector is labeled ASU PUMP and can be removed by gently bending 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 5 internal air filters located inside the main console.

- 2 Ea. Air filters for Reference and Analysis air (5 micron)
- 2 Ea. Air filters for Gas Blender air (25 micron)
- 1 Ea. Air filter for Air Supply Unit air (25 micron)



The blue air filters (Gas Blender, Reference and Analysis) and Air Supply Unit Hydrophobic Filter is held in place by the gas tubing and can easily be changed by separating from the gas tubing. We recommend that for optimal operation that these filters are changed regularly per the following schedule:

Air Supply Unit Hydrophobic Filter

- 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

Blue Reference and Analysis Air Filters (5 micron)

- Once every year.

Gas Blender Air Filters (25 micron)

- Should not require replacement.

External Air Inlet Filter Assembly (Part Number STD558)

Beginning in May 2014, we started supplying an external air inlet filter assembly to improve the CIRAS-3 resistance to dirty, dusty environments. As described above, the internal gas circuit and optics are protected with several filters but this external filter also provides added protection and can easily be changed. It easily connects to the CIRAS-3 "AIR IN" port as shown below:

This assembly is supplied with a filter element, 48 um, blue (Part Number STD556) as standard. For severe dusty environments, we recommend the filter element, 10 um, Yellow (Part Number STD555).

Re-Order Information	
Part Number	**Description**
10045-1	Filter Assembly (Air Supply)
30124-1	Air Filter, 5 micron
30124-2	Air Filter, 25 micron
STD558	External Air Inlet Filter Assembly
STD555	Filter Element, 10 um, Yellow (For severe dusty environments)
STD556	Filter Element, 48 um, Blue

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

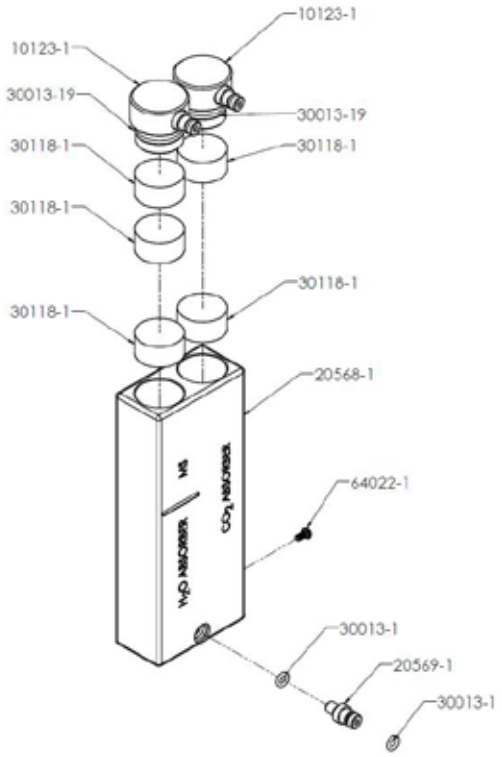
Zero Column Assembly (Part Number 10122-1)

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 self-indicating desiccants when the column is approximately 50% exhausted. If using non-indicating desiccants, we recommend changing them out prior to each measurement campaign to play it safe.



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)

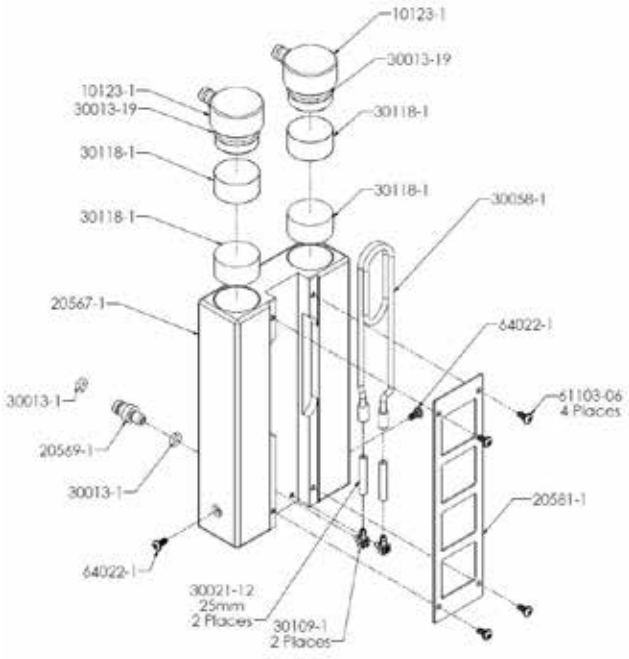
Column Identifiers

- **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 self-indicating desiccants when the column is approximately 50% exhausted. If using non-indicating desiccants, we recommend changing them out prior to each measurement campaign to play it safe.



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. Both self-indicting and non-indicating Soda Lime can be can be used with the CIRAS-3. Soda Lime cannot be regenerated and should be discarded after exhaustion.

- Type: Sofnolime, 1.0-2.5 mm, self-indicating (white to violet), 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.

For the latest MSDS on alternative types of soda lime, please contact the manufacturer directly or contact PP Systems.

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

Re-Order Information	
Part Number	**Description**
STD007W	Sofnollime, white to violet, 1 kg

Drierite

Drierite (anhydrous 97% calcium sulfate ($CaSO_4$) and 3% cobalt chloride) is an excellent H_2O absorber making it an ideal choice for analyzer ZERO and for controlling H_2O. Both self-indicting (blue to pink) and non-indicating Drierite can be can be used with the CIRAS-3. It can be regenerated easily by simply spreading out the granules one layer deep and placed in a preheated oven for 90 minutes at 230 °C or 425 °F. The regenerated material should be returned to the original glass container and sealed while hot. The color of the self-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.

- Type: 8 mesh, self-indicating (blue to pink) or non-indicating, 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.

For the latest MSDS on alternative types of Drierite, please contact the manufacturer directly or contact PP Systems.

Take caution to wash your hands completely after handling Drierite.

Re-Order Information	
Part Number	**Description**
STD008	Drierite, 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.

Molecular Sieve Repackaging

The Molecular Sieve is originally supplied by PP Systems in tin packaging. After initial opening, we strongly urge all users to repackage the Molecular Sieve in small glass containers with a screw top to seal the desiccant from room air. This desiccant saturates very quickly in room air and if not properly stored it will cause it to go bad and subsequently affect CO_2 readings and calibration.

To ensure a good seal, we also recommend putting some electrical tape around the screw top as shown here. If you have any questions, get in contact with PP Systems.

Tip

Always change the molecular sieve at least once per week when the CIRAS-3 is in use regardless of operation time.

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 causing damage to 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 Cartridges

We supply and recommend the CO_2 cartridges that are manufactured by ISI. They are 8g and are normally supplied in boxes of 10 as shown below.

Manufacturer: ISI (Vienna, Austria)

Part No. 0018 Soda Chargers (10 Pack).

Contact Information:

iSi GMbh

Kürschnergasse 4

A-1217 Vienna, Austria

Tel: +43 1 25099 0 Fax: +43 1 25099 555

Email: info@isi.at URL: www.isi.at

The manufacturer (iSi) has distributors throughout the world so it should be very easy for all customers to source this item locally. Due to the classification of these CO_2 cartridges, PP Systems can only ship them by ground service in the United States.

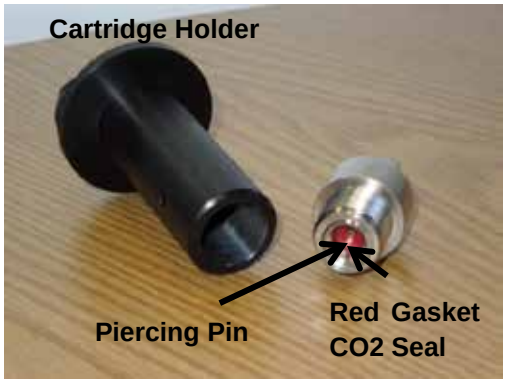
NOT ALL CO_2 CARTRIDGES ARE THE SAME AND SOME MAY EVEN CAUSE DAMAGE IF USED WITH THE CIRAS-3. They come in all different sizes, shapes and contents (some even have oil) and are commonly used for things like pellet guns, soda siphons, etc. If you have any questions whatsoever regarding the CO_2 cartridges you are urged to get in contact directly with PP Systems.

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. See CO2 Cartridge Status on page 39**. 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 cartridge 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	Cartridge 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 375-400 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 CO_2 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. Refer to CO2 Calibration on page 66 for instructions. **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 390-450 µmol mol^{-1}, use a gas calibration mixture around 500 µmol mol^{-1}.

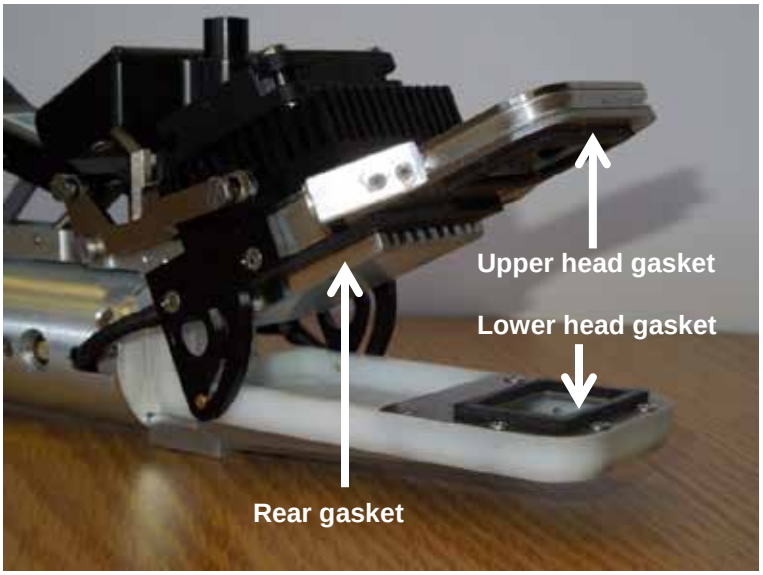
H_2O Calibration

For H_2O calibration, an air supply of known water vapor concentration is required (mb). Refer to H2O Calibration on page 67 for instructions. Typically this is achieved using a commercially available humidity 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)

Checking For Leaks Associated With the PLC3

How do I know if there is a leak in the system? Good question. If you have the leaf cuvette closed with no leaf present, you should observe a CO_2 differential (CO2d) close to 0 (< 1.0 µmol mol^{-1} and stable). If your CO2d is > 1 µmol mol^{-1} and fluctuating this usually is the result of a leak. To help isolate the leak and to determine if the leak is associated with the CIRAS-3 console or the PLC3, we include a simple "Leaf Cuvette Simulator" (Part No. STD553) with each new system that can be very helpful.

To checks for leaks, connect the cuvette simulator to the PLC Gas port on the CIRAS-3 console as shown here to the right. This will allow you to sample the same air in both the reference and analysis cells of the infrared gas analyzer. When the simulator is connected to the CIRAS-3 you should observe stable CO2r, CO2a, H2Or and H2Oa readings and the CO_2 differential (CO2d) should be close to 0 (< 1.0 µmol mol^{-1} and stable). For more information on checking for stability and leaks, refer to Checking Stability - Before You Place a Leaf in the Chamber on page 40.

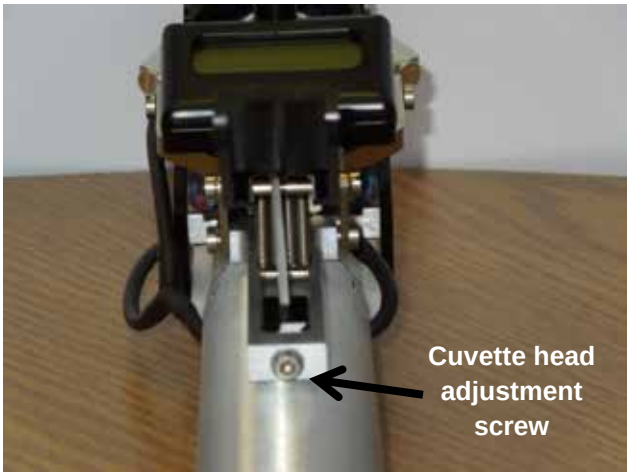
If you observe a CO2d value close to 0 and stable with the Leaf Cuvette Simulator and you do not when the PLC3 leaf cuvette is connected to the CIRAS-3, the leak is likely associated with the PLC3 leaf cuvette. As described earlier, PLC3 leaks are normally associated with the leaf cuvette gaskets. To isolate the leak, take a small piece of tubing and gently breathe around the gaskets and monitor the CO2d on the CIRAS-3 console. If you see a spike or increase in CO2d you have found the leak. Replace the gaskets (see Cuvette Gaskets on page 148. Also, you should check to make sure

that the head is closing and sealing properly. If the head is not sealing properly, you may need to adjust the tension on the head. Refer to PLC3 Leaf Cuvette Head Adjustment on page 150.

If you observe a fluctuating CO2d value that is also > 1.0 µmol mol^{-1} with the leaf cuvette simulator connected, the leak is likely associated with the CIRAS-3 console. Normally leaks associated with the console can be found around absorber columns especially after a recent change in chemicals. Gently blow around the absorber columns and the H2O equilibrator to isolate leaks.

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.



For older leaf cuvettes 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. For newer leaf cuvettes starting in late 2015 we replaced the hex screw with a thumb screw making it easier to adjust with your fingers. 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.

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 to 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 periodically compare 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. See PAR Calibrate on page 72.

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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	0.30-0.60
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 from the PLC3 and connect the CIRAS-3 to mains power using the external power supply provided by PP Systems. 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 = Cuvette temperature
- Set Temperature = 25 ^{o}C

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) 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.

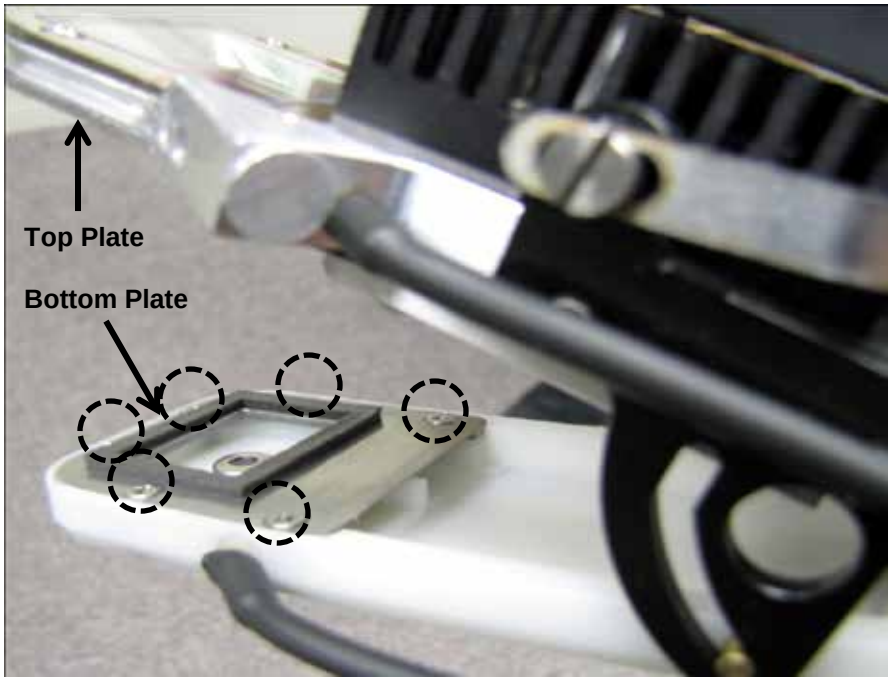
Open the RbCal.xls file available on the USB thumb drive (supplied by PP Systems when you first purchased your system or visit our web site and download it) and enter the required values. For Air Temperature, enter the cuvette temperature °C (Tcuv). Note that the evaporation rate (mmol m^{-2} s^{-1}) 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.

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.

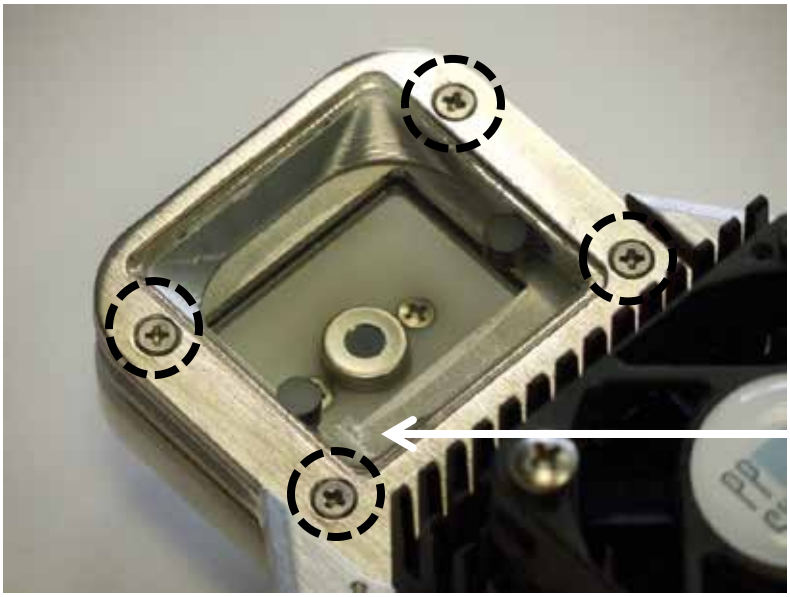


Open the cuvette head to maximum position and using a long, thin Philips screwdriver, remove the top and bottom head plates.

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.
STD007W	Sofnolime, white to violet, 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)
10045-1	Air Filter (Air Supply)
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)