



CIRAS-3

Portable Photosynthesis System

Operation Manual

Version 2.00

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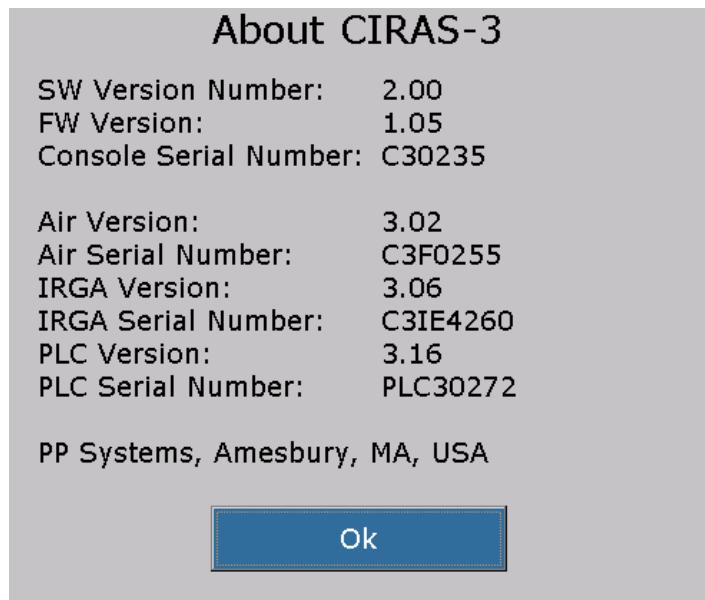
Welcome

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 product is manufactured in accordance with CE requirements. For more information on system conformity please get in contact with PP System.

This operation manual is based on our software Version 2.00 and above. To check which software and firmware versions are currently installed on your CIRAS-3 go to **Help (F8) > About (F2)**:



For users running V. 2.00 console software along with latest 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 (See [User Registration](#) on page 13). For users running older software and firmware versions, the CIRAS-3, PLC3, Light Unit or CFM-3 (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.

How do I know which console software version is running on my CIRAS-3?

Well that is actually quite easy to determine. If your numeric display looks like this:

Measured Data			Photosynthesis Data		
CO2r 389.4	H2Or 10.8	PARI 1201	Tamb 27.6	CI 440	A 0.0
CO2a 389.5	H2Oa 10.9	PARE 28	Tcuv 25.0	gs 1	E 0.0
CO2d 0.1	H2Od 0.1	RH% 34.3	Tleaf 27.6	VPD 2.6	WUE 0.0
Environmental Controls					
CO2 390	H2O 80	PARI 1200	Flow 300	Tleaf amb	Area 4.50

.... you are running our older software prior to Version 2.00 and you should update when it is convenient to do so. If your display looks like this:

Gas Exchange					Operations>
CO2r 390.0	CO2a 389.2	CO2d 0.0	A 0.0	CI 0	Settings>
μmol mol-1	μmol mol-1	μmol mol-1	μmol m-2 s-1	μmol mol-1	Controls>
H2Or 100.0%	H2Oa 8.04	H2Od -0.16	gs 0	E 0.00	Toggle View>
mb	mb	mb	mmol m-2 s-1	mmol m-2 s-1	Record>
Tamb 26.3	Tcuv 21.9	Tleaf amb 24.1	VPD 2.2	WUE 0.0	Zero/Diff Bal>
°C	°C	°C	kPa	A/E	Help>
PARi 1200	PARE 24	RH% 30.6	Flow 300	Leaf Area 4.50	
μmol m-2 s-1	μmol m-2 s-1	%	cc min-1	cm2	
					100% 999 minutes

.... then you are running Version 2.00 or higher. Please contact PP Systems if you have any questions.

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. See the [User Registration](#) below.

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 users register with us. If you are a PP Systems' user, please go to www.ppsystems.com and click on Customer Registration in the upper left hand corner and complete the short form.

Only **REGISTERED** users will be allowed access to the protected "Users" section of our web site. This section will contain important product information including technical documentation, software, 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. Operation Manual).

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.

Returning equipment to PP Systems

Before returning equipment to PP Systems it is very important that you pay close attention to the following procedure:

1. Contact PP Systems directly by email (service@ppsystems.com) or by telephone (+1 978-834-0505) to obtain a Case Number for tracking and reference purposes. You will receive a "Service Return Form" that must be completed and included with the return of your equipment.
2. If you have any stored data on your instrument that is important to you please retrieve it prior to packing up your equipment. PP Systems is not responsible for any loss of data.
3. Safely pack your equipment in a rugged carton/case with suitable packing materials (bubble pack, etc.). Remember to include the "Service Return Form". PP Systems is not responsible for any shipping related charges for equipment being returned to PP Systems (Amesbury, MA) regardless of whether or not it is covered under warranty.
4. Notify the PP Systems' Service Department when the equipment is packed and ready to be shipped.

Please also note the following:

- We strongly recommend using UPS “door to door” service for all returns. PP Systems is not responsible for any unnecessary shipping related charges caused by incorrect preparation of shipment documentation. If you have any questions regarding the return of your equipment please consult with PP Systems prior to shipping the equipment.
- Remove all batteries contained in the equipment. We don’t need them and this will eliminate any problems associated with shipments containing Li-ion batteries. There is also no need to return the power supply adapter or any cables.
- Remove the CO₂ cartridge from the CO₂ cartridge holder.
- To save on weight and shipping costs you can remove the desiccants from all absorber columns.
- Make sure that the PLC3 cuvette head is open so that the gaskets are not compressed or damaged when received.
- Prior to performing any service on your equipment we must have an acceptable purchase order or credit card information on file (Visa or MasterCard).

Contact Information

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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 packing list or checklist are not included or damaged, you must contact PP Systems or your authorized distributor immediately.

Storage

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

Tip

If the CO₂ cartridge is pressurized we **STRONGLY** suggest removing the entire CO₂ cartridge holder and regulator from the console and store it separately. See [CO₂ Regulator](#) on page 217. Otherwise CO₂ will slowly accumulate overnight inside the case resulting in absorption into the PLC tubing as well as the internal case foam material affecting CO₂ measurements the following day. The system should only be stored in the case when a CO₂ cartridge is empty and fully exhausted.



Section 1. Technical Specification

CIRAS-3 Portable CO₂/H₂O Gas Analysis System

Technical Specification	
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 ₂ O for both the reference and analysis gas streams. All measurements corrected for temperature and pressure.
CO₂ Measurement Range	0-10000 µmol mol ⁻¹ (Optimized for 0-2000 µmol mol ⁻¹)
CO₂ Precision	<ul style="list-style-type: none">· 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
CO₂ Control Range	0-2000 µmol mol ⁻¹
H₂O Measurement Range	0-75 mb
H₂O Precision	<ul style="list-style-type: none">· 0.015 mb at 0 mb· 0.020 mb at 10 mb· 0.030 mb at 50 mb
H₂O 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 the SRC-2 Soil Respiration Chamber and CPY-5 Canopy Assimilation Chamber.
Digital Output	<ul style="list-style-type: none">· USB-Mini b (Host)· 2 Ea. USB for use with external devices (USB Flash Drive, USB Mouse, etc.).
Data Storage	512 MB flash memory for programming and data storage. Unlimited data storage using USB Flash Drive (Thumb Drive).
Microprocessor Speed	800 MHz
Display	7.0" WSVGA transreflective, color LCD
User Input	27 tactile keys

Technical Specification (Continued)	
Power Supply	<p>Two internal, rechargeable 7.2V Li-ion battery pack providing up to 12 hours continuous use. Power supply/charger included. Please note that the system is capable of being powered by one battery pack for up to 6 hours continuous use.</p> <p>Note. Older CIRAS-3 systems were supplied with a single, 7.2V Li-ion battery capable of providing up to 8 hours continuous use. These instruments can be updated to the latest battery technology. Contact PP Systems for more information.</p>
Operating Temperature Range	0-50 °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	<p>4.3 kg (including 1 battery pack).</p> <p>4.5 kg (including 2 battery packs).</p>

PLC3 Leaf Cuvettes

Technical Specification	
Construction	<ul style="list-style-type: none"> Handle - Aluminum Leaf Gasket - Closed cell foam Impeller – Aluminum fan blade
Window	<p>PLC3 Universal – Glass IR interference filter (Calflex)</p> <ul style="list-style-type: none"> 7 mm x 25 mm (1.75 cm²) 18 mm diameter (2.5 cm²) 18 x 25 mm (4.5 cm²) <p>PLC3 Narrow: Glass IR interference filter (Calflex)</p> <p>PLC3 Conifer: Scratch resistant glass</p>
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)	<p>PLC3 Universal: 2 miniature, silicon photodiode sensors</p> <p>PLC3 Narrow and Conifer: 1 miniature, silicon photodiode sensor</p> <ul style="list-style-type: none"> Response: 400-700 nm Range – 0-3000 µmol m⁻² s⁻¹ Precision – 10 µmol m⁻² s⁻¹
PAR Sensor (External)	<p>Filtered, silicon cell (cosine corrected)</p> <ul style="list-style-type: none"> Response: 400-700 nm Range – 0-3000 µmol m⁻² s⁻¹ Precision – 10 µmol m⁻² s⁻¹
Air Temperature Sensor	<ul style="list-style-type: none"> Range: -10 °C to 50 °C Precision Thermistor Accuracy - ± 0.5 °C at 25 °C
Temperature Control	<p>Approximately 10 °C below ambient to +15 °C above ambient.</p> <ul style="list-style-type: none"> Temperature control limits – 0 °C to 45 °C
Leaf Temperature Sensor	<p>PLC3 Universal: Radiation sensor for non-contact measurement</p> <ul style="list-style-type: none"> Accuracy - ± 0.5 °C at 25 °C <p>PLC3 Narrow and Conifer: Precision thermistor</p> <ul style="list-style-type: none"> Accuracy - ± 0.5 °C at 25 °C
Dimensions (General)	32 cm (L) x 3.8 cm (Handle diameter)
Weight	<p>PLC3 Universal: 0.750 kg</p> <p>PLC3 Narrow and Conifer: 1 kg</p>

PLC3 LED Light Unit (RGBW)

Technical Specification	
Automatic Control Range	PLC3 Universal · 0-2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PLC3 Narrow/Conifer Leaf Cuvette · 0-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$
LED Specification	<ul style="list-style-type: none"> · Red Peak Wavelength: 625 nm (± 5 nm) · Green Peak Wavelength: 528 nm (± 8 nm) · Blue Peak Wavelength: 475 nm (± 10 nm) · White: 425-700 nm
Dimensions	PLC3 Universal: 6.4 cm (L) x 6 cm (W) x 5.1 (H) PLC3 Conifer/Narrow: 6.5 cm (L) x 10.6 cm (W) x 6 (H)
Weight	PLC3 Universal: 0.2 kg PLC3 Conifer/Narrow: 0.3 kg

CFM-3 Chlorophyll Fluorescence Module

Technical Specification	
Modulating Beam	625 nm ± 5 nm (Red)
Saturation Light	0-10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$
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
Dimensions	8 cm (L) x 6 cm (W) x 6.2 cm (H)
Weight	0.3 kg
<ul style="list-style-type: none"> · PP Systems is a registered trademark of PP Systems, Inc. · PP Systems is continuously updating its products and reserves the right to amend product specifications without notice. · All brand names are trademarks of their respective owners. 	

Section 2. CIRAS-3 Tutorial

Measurement of Leaf Gas Exchange – Introduction and Overview

We know from experience over the past 30+ years that many people don't have the time or energy to plow through operation manuals from cover to cover especially those with hundreds of pages. With that in mind we strongly encourage you to take the time to step through this quick and simple tutorial to learn about the general features and operation of the CIRAS-3 system. Who knows? You may even find something that is cool and awesome about the CIRAS-3 that you weren't aware of! After about 1-2 hours exploring this short tutorial you are going to know how to complete the following tasks:

- Configure and prepare the CIRAS-3 system for leaf gas exchange measurements
- Set up and modify all environmental controls (CO₂, H₂O, temperature and light)
- Navigate and become more familiar with the user interface, displays and dialogs
- Store and retrieve/transfer data from memory

For tutorial purposes this section will be based on the following system hardware to make it easier to follow along and comprehend:

CIRAS-3 Main Console

Part Number CRS300



PLC3 Universal Leaf Cuvette

Part Number CRS301



PLC3 Universal Light Unit (LED)

Part Number CRS304



If you have our CFM-3 Chlorophyll Fluorescence Module it can be used exactly the same as our PLC3 Universal Light Unit. In addition to the above 3 main system components we recommend that you have the following items available:

- CIRAS-3 power supply adapter
- One CO₂ cartridge
- USB flash drive
- Small Tabletop tripod to hold PLC3 Universal Leaf Cuvette
- PC or laptop computer
- A well-watered, healthy plant for actual measurements. We recommend a plant that likes high light (i.e. tomato, sunflower, etc.) and is typically found growing outdoors for best results.

Please Note

The measured and calculated data that is shown in this tutorial should be similar but not 100% identical to what you will witness. Actual results will be based on the biological state and type of plant that you use and its response to the environmental conditions presented. However, what is most important is that you should observe stable and comparable results.

Let's Get Started

Charge internal battery packs

Before taking your system to the field for measurements it is critical that the internal batteries are fully charged. We recommend charging the batteries the night prior to use. Connect the power supply adapter supplied with the system to the **EXT PWR** socket on the CIRAS-3 console to charge the internal batteries. Make sure that the connection is secure. The indicating LED should be steady green in color indicating secure connection and that the internal batteries are being charged.



Inspect desiccants

Make sure that all desiccants are fresh before beginning measurements and that all columns are properly seated in their respective manifolds. The soda lime can be either self-indicating or non-indicating. If using non-indicating soda lime we recommend changing out more regularly to play it safe. The Drierite will be blue in color when fresh. It turns from blue to pink as it becomes exhausted. The Molecular Sieve is white and non-indicating. The Molecular Sieve should always be discarded when changing out the Drierite. Refer to [Desiccants and Absorber Columns](#) on page 211 for more details on storage and management of desiccants and absorbers. **To play it safe and for best results we recommend that you change out the Molecular Sieve desiccant on a daily basis prior to performing measurements to ensure accurate Auto Zero and long term accuracy and stability of the CO₂ and H₂O gas analyzers.**

Insert a fresh CO₂ cartridge

CO₂ cartridges allow for accurate and precise control of CO₂ and we highly recommend using them for best results. Unscrew the CO₂ cartridge holder and insert a new CO₂ cartridge as shown below. Next, screw the holder into the CO₂ regulator turning clockwise until snug. If you hear a small hiss (indicating the cartridge is being pierced) continue to turn the holder until snug to ensure that the cartridge is both

pierced and sealed properly into the regulator assembly. Each CO₂ cartridge will last at least 24 hours from the time it is installed.



We use 8g CO₂ cartridges which are manufactured by a company called ISI (<https://www.isi.com/>). They can easily be sourced from many different suppliers around the world. If you have any questions or problems related to these CO₂ cartridges please contact PP Systems.

We do not recommend removing a CO₂ cartridge for at least 24 hours after it was inserted and with the CO₂ 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 CO₂ regulator or gas mixing diverter valve.

How do I determine CO₂ cartridge state of charge?

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 CO₂ cartridge will last at least one full day of operation. The actual life of an individual CO₂ cartridge is dependent on:

1. Hours of operation
2. CO₂ control range

For instance, if the CIRAS-3 is operating for long periods throughout the day and at high CO₂ concentrations, we would expect that when you turn on the system the following day the CO₂ 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 approximate ambient CO₂ concentration (i.e. 390 $\mu\text{mol mol}^{-1}$), you will likely have enough CO₂ to get you several more hours of use the day after a CO₂ cartridge is installed.

If unsure of how much CO₂ is still available from the cartridge, we recommend the following simple test:

1. Power up the CIRAS-3 system.
2. After warm up and with the Gas Exchange display on the console LCD, press **Controls (F3)**.
3. Set the “CO₂ Reference” to 2000 and hit **Accept (F2)**.

Monitor the CO_{2r} (CO₂ Reference) value. After a minute or so, it should reach 2000. If the CO_{2r} reaches 2000 you can proceed with measurements without having to change out the CO₂ cartridge. Note, keep an eye on the CO_{2r} during your measurements and if you observe the CO_{2r} 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 CO₂ is low or starting to get low and it is safe to change out the cartridge as the CO_{2r} will likely become unstable within a short period of time.

Another quick and simple check to see if you have a pressurized CO₂ cartridge is to remove the entire CO₂ cartridge holder and regulator assembly from the CIRAS-3 console. To do so, turn the cartridge holder a quarter-turn counter-clockwise and pull the entire assembly out from the console. Next put a small piece of flexible tubing on the end as shown here and place the tip of the tubing in the water. If a CO₂ cartridge is pressurized you should see a steady bubble at approximately 1 second intervals. If the CO₂ cartridge is not pressurized you will not see any bubbling indicating that it is safe to change out.



Is it possible to perform measurements under ambient conditions without using a CO₂ cartridge?

Yes and it is very easy. To do so make sure that the CO₂ cartridge holder is empty and that the soda lime is removed from the CO₂/H₂O control column on the CIRAS-3 console. The empty soda lime column will ensure that ambient air is used for your reference air and the column will simply act as a smoothing volume. An Ambient Air Sampling Intake Pole (Part No. STD569) is available from PP Systems to smooth the reference air eliminating problems associated with fluctuating reference CO₂ (CO_{2r}) as a

result of localized effects (breathing, etc.). Refer to [Settings – Gas Exchange](#) on page 143 (CO₂ Reference- Ambient (Remove Chemicals)).

Is it possible to perform measurements under ambient H₂O conditions in addition to ambient CO₂?

Yes and again this is quite easy. To do so make sure that the CO₂ cartridge holder is empty and that both the soda lime and Drierite is removed from the CO₂/H₂O control column on the CIRAS-3 console. The empty columns will ensure that ambient air is used for your reference air and the columns will act as smoothing volumes. Refer to [Settings – Gas Exchange](#) on page 143 (H₂O Reference- Ambient (Remove Chemicals)).

Connect the PLC3 Universal Leaf Cuvette to the CIRAS-3 console

Connect the black, 4 pin electrical plug from the PLC3 to the “Signal” socket on the CIRAS-3 console. Be careful to align the arrow on the black signal plug with the top center console socket on the CIRAS-3 console. Next connect the white pneumatic connector to the PLC “Gas” port on the CIRAS-3. Press down on the silver tab at the top of the port and the plug will snap into place. Close the cuvette head. The black link pipe should already be in place connecting the “REF IN” and “AIR OUT” gas ports below the CO₂ cartridge holder.



Do not use excessive force when connecting/disconnecting the white, plastic PLC pneumatic connector. Damage may occur if not handled properly.

Connect light unit to PLC3 Universal Leaf Cuvette

Step 1

First, mount the light unit by aligning it to the front of the cuvette upper jaw as shown below.



Step 2

Slide the light unit back towards the heat sink and fan on the leaf cuvette locking it in place using the two ball-head screws shown below.



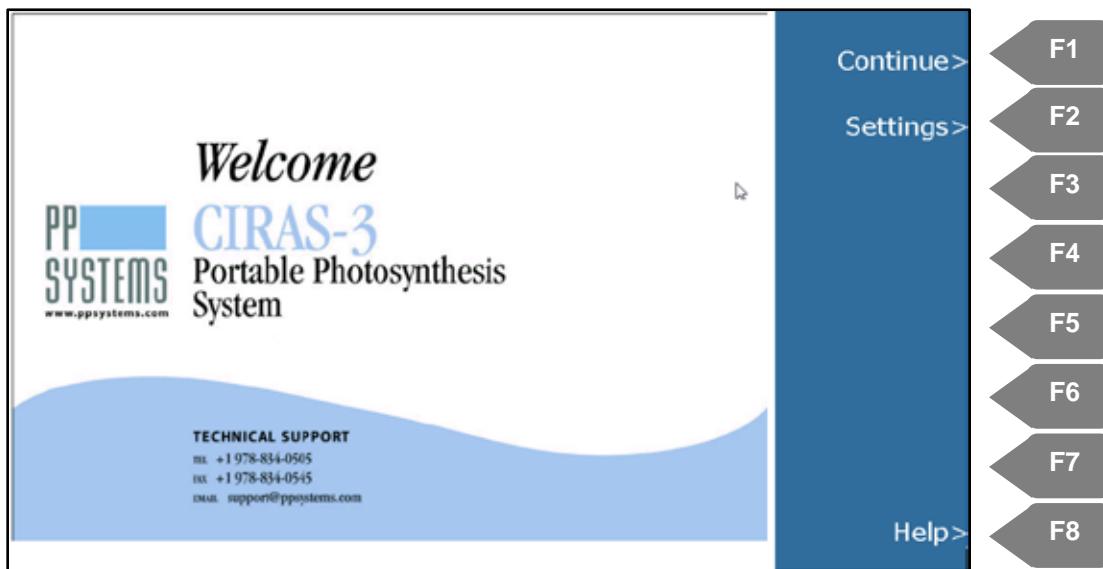
Step 3

Connect electrically by gently pressing the connector in. Pay special attention to align the red dots on the plug and connector.



Power up the CIRAS-3 System

Press the ON/OFF switch in the upper left hand corner on the rear of the console. A blue ring around the switch should illuminate. After approximately 30 seconds the "Welcome Screen" will appear on the LCD.



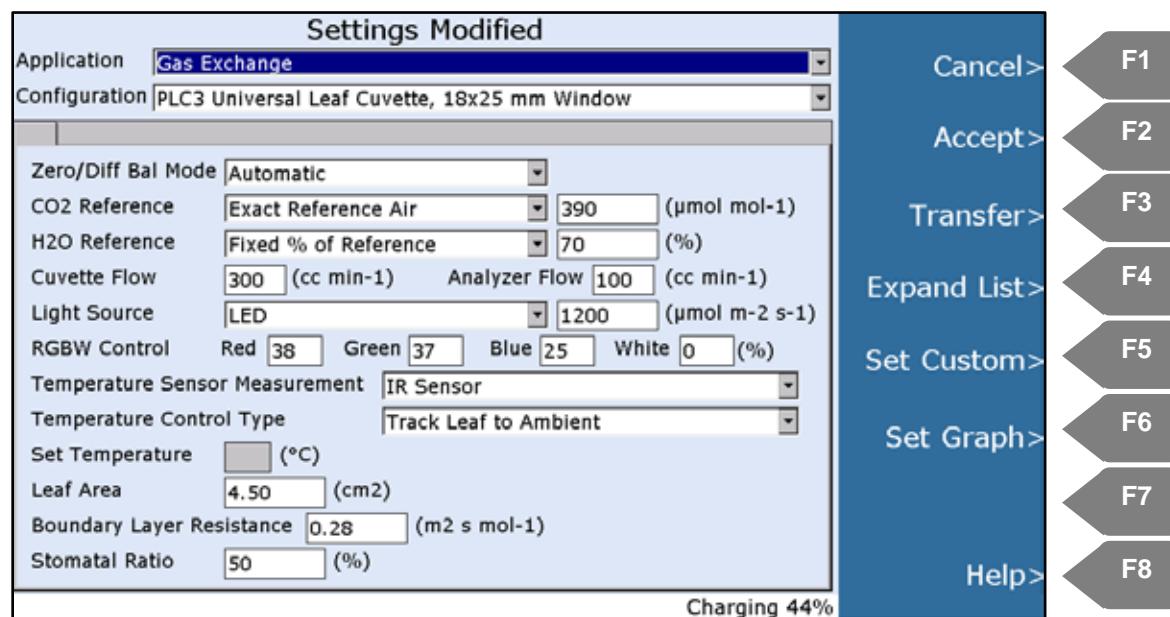
Press **Continue (F1)** to start the CIRAS-3 software. Next press **Settings (F2)** and configure the system for Gas Exchange measurements using the PLC3 Universal Leaf Cuvette with 18 x 25 mm window (as supplied from the factory).

System Configuration

The CIRAS-3 has a number of different global settings based on the user-defined “Application” and “Configuration” selections under **Settings (F2)**. It is very important that you select the appropriate settings associated with your leaf cuvette. For this section of the manual we are going to focus on the global settings associated with typical leaf gas exchange measurements using the CIRAS-3, PLC3 Universal Leaf Cuvette and PLC3 Universal Light Unit. See [Settings \(F2\)](#) on page 119 for more information. Also note that there is a small delay of approximately 4 seconds when you press **Settings (F2)** before the Settings dialog window appears.

Configuring the CIRAS-3 System based on “Application”

Press **Settings (F2)** and select **Gas Exchange** for the “Application” and **PLC3 Universal Leaf Cuvette 18 x 25 mm Window**. Next proceed through the Settings dialog making the selections as shown below. Pressing the TAB key allows you to navigate from field to field. At this time, press TAB to navigate from field to field and create a Settings file exactly as you see below. When in any field, use the up/down arrow keys to change the setting and you can press the **Expand List (F4)** to see all available options for that field. Numerical fields are changed by using the number keypad. For Boundary Layer Resistance enter the value for your PLC3. This value is noted on the green “Tested” label on your PLC3 handle for the 18 x 25 mm head plate that is normally in place from the factory. Once complete press **Accept (F2)**.

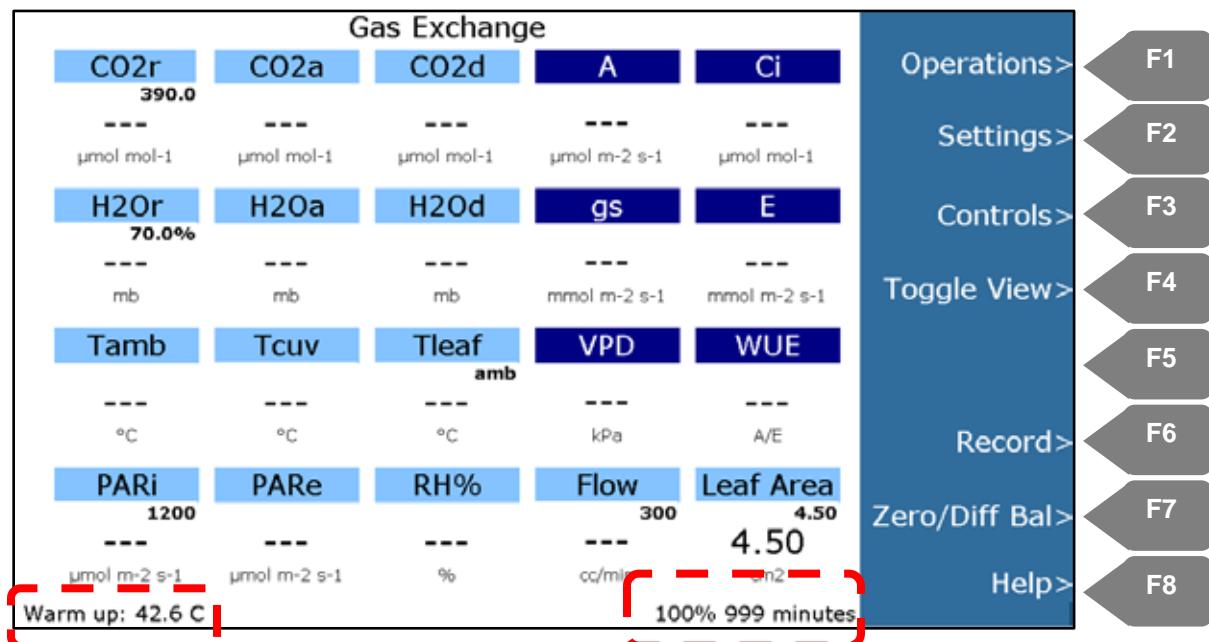


System Warm up

We recommend allowing the system to warm up for at least 15-30 minutes. It normally takes the system approximately 10 minutes for the IRGAs to reach the required operating temperature of 55 °C and also during this period the system will perform several Zero and Diff Bal cycles to ensure that everything is

working perfectly. The system performs more frequent Zero and Diff Bal cycles during the first 30 minutes and then after that they will be performed much less frequently (depending on Settings) or when there is a large change in CO₂ (100 $\mu\text{mol mol}^{-1}$) and/or H₂O (4 mb) concentration.

During warm up note the dashes for all data. This occurs during system warm up, Zero and Diff Bal cycles. The numbers don't mean anything during this period so don't be alarmed. Also note the IRGA warm up message in the lower left hand corner and the battery status in the lower right hand corner of the display.



At the completion of warm-up you should begin to begin to hear a clicking sound from the CO₂ and H₂O gas mixing valves switching at a rate of 4 Hz. The amount of time the valve is on (the duty cycle) varies to generate the correct CO₂ mix ratio. Just after the system warms up the mixer starts to generate the requested ratios. H₂O 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 Gas Exchange display are now updating with live data approximately every 2 seconds. Data will continuously update on the display except when the system is performing a Zero or Diff Bal.

While CO2d and/or H2Od=0.00 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 minuscule rates of gas exchange, such as occur near light compensation points and with dark respiration.

If during this test $\text{CO2d} > 0.5 \mu\text{mol mol}^{-1}$ 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 $0.1 \mu\text{mol mol}^{-1}$ 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 $390 \mu\text{mol mol}^{-1}$ and the test location is indoors where the CO_2 concentration is $600\text{-}800 \mu\text{mol mol}^{-1}$. 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 cuvette head open 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.

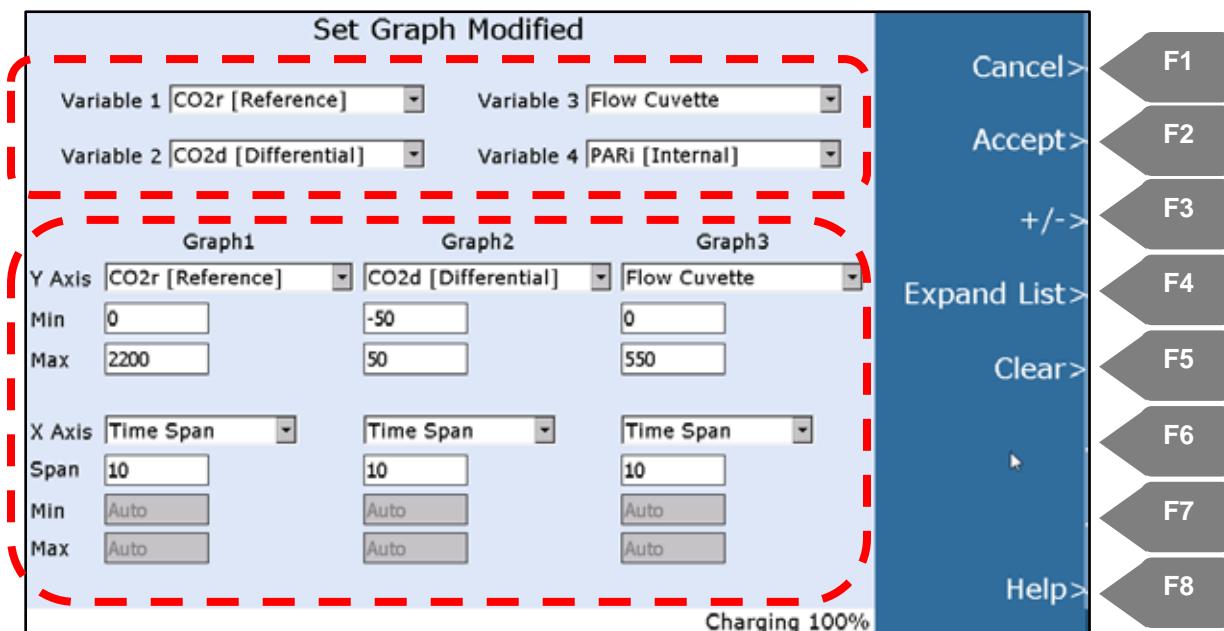
When a leaf is enclosed in the cuvette you should quickly notice a series of dynamic leaf responses, assuming ideal physiological conditions. Also be aware that leaf tissue that is enclosed but not in the window will respire and affect rates of photosynthesis. Sub-stomatal CO_2 concentration (Ci) will begin to fall, after being initially equal to or higher than the reference CO_2 concentration (CO2r). When $\text{Ci} < \text{CO2r}$ 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 (H2Od) 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. Please note that species, plant and leaf age, growth environment and measurement environment (especially light, CO_2 , water status, temperature and humidity) can all impact rates of assimilation.

Note that some physiological variables, especially A , gs (stomatal conductance) and CO2d will continue changing as the leaf adjusts 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.

Configuring the Graph display for data plots

You have complete flexibility to plot any parameter against time or any parameter against another parameter. You can also set up to 3 different graph plots along with up to 4 user-defined parameters. Press **Settings (F2) > Set Graph (F6)** to configure your graphical display. TAB from field to field and configure your graph display exactly as shown below. When in the Min field for CO2d press **F3** to insert the negative (-) character (Graph2). Press **Clear (F5) > Left Arrow > Yes > OK > Accept (F2) > Accept (F2)**. This will reset all graph plots back to time 0. Again, when you are in any field you can press the **Expand List (F4)** to see all available options for the accessory selected.



Configuring the Custom display

A Custom display can also be configured based on user-defined parameters allowing the user to focus on specific data. Again you have complete flexibility and can view up to 20 user-defined parameters. Press **Settings (F2) > Set Custom (F5)** to configure your Custom display. The initial screen should look similar to the first screen on the following page.

Set Custom Data

CO2r	CO2a	CO2d	A	Ci	Cancel>	F1
H2Or	H2Oa	H2Od	gs	E	Accept>	F2
PARi	Tcuv	Tleaf	VPD	WUE	Expand List>	F3
F	Fs	Fo	Fm	ΦfD	Help>	F4
					Charging 100%	F5
						F6
						F7
						F8

TAB from field to field and configure the Custom display to appear exactly as shown below. Again, when you are in any field you can press the **Expand List (F4)** to see all available parameters.

Set Custom Data Modified

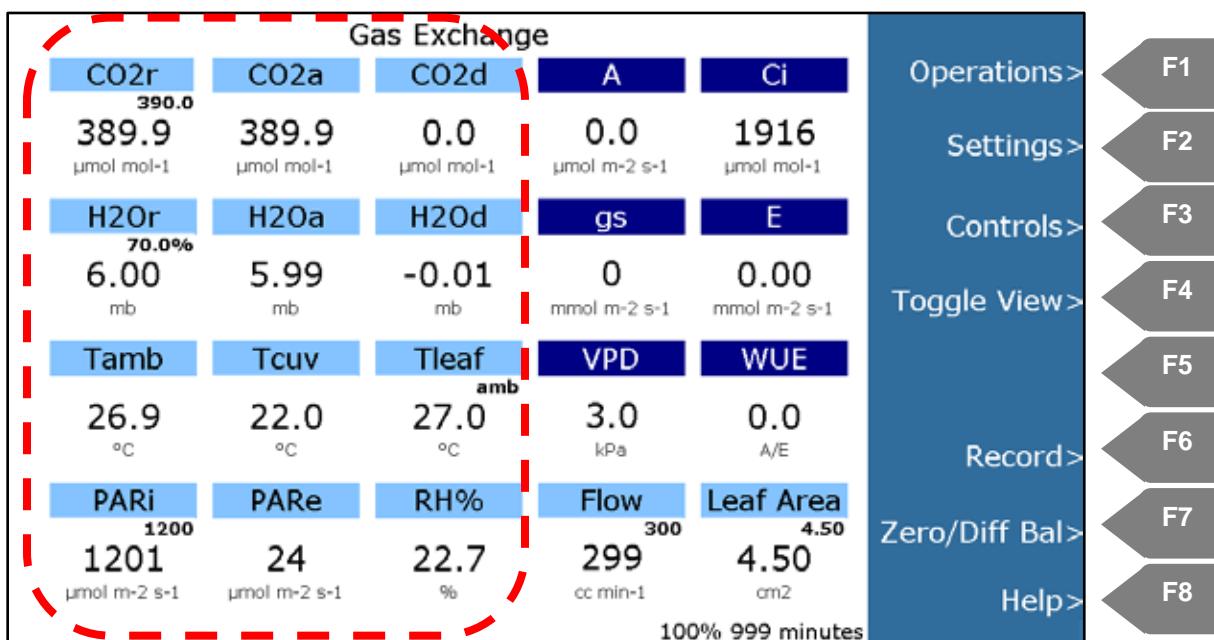
CO2r	CO2d	A	Ci	None	Cancel>	F1
H2Or	H2Od	gs	E	VPD	Accept>	F2
PARi	Tamb	Tleaf	None	None	Expand List>	F3
None	None	None	None	None	Help>	F4
					Charging 100%	F5
						F6
						F7
						F8

Press **Accept (F2)** and **Accept (F2)** again to return to the main Gas Exchange display.

Let's check out the Gas Exchange, Graph and Custom display

So by this time you will have everything up and running with the proper system settings and will now be in a great position to establish a baseline to determine that everything is good to go. Make sure the PLC3 leaf cuvette is closed without any leaf in place. After about 3-4 minutes your Gas Exchange display should look similar to this one below with an empty cuvette. Please note that the H₂O concentrations and temperatures will be based on your local conditions.

Gas Exchange Display



Please observe the following:

- CO2r, CO2a, H2Or and H2Oa are stable
- CO2d and H2Od should be at or very close to 0 (± 0.5) and stable. If not and at any time perform a manual Diff Bal (**Z-Diff Bal (F7) > Right Arrow** to select Diff Bal and then **OK**)
- Tamb and Tleaf should be the same (± 0.2 °C)
- PARi should be reading 1200 ($\pm 1-3$ $\mu\text{mol m}^{-2} \text{s}^{-1}$)

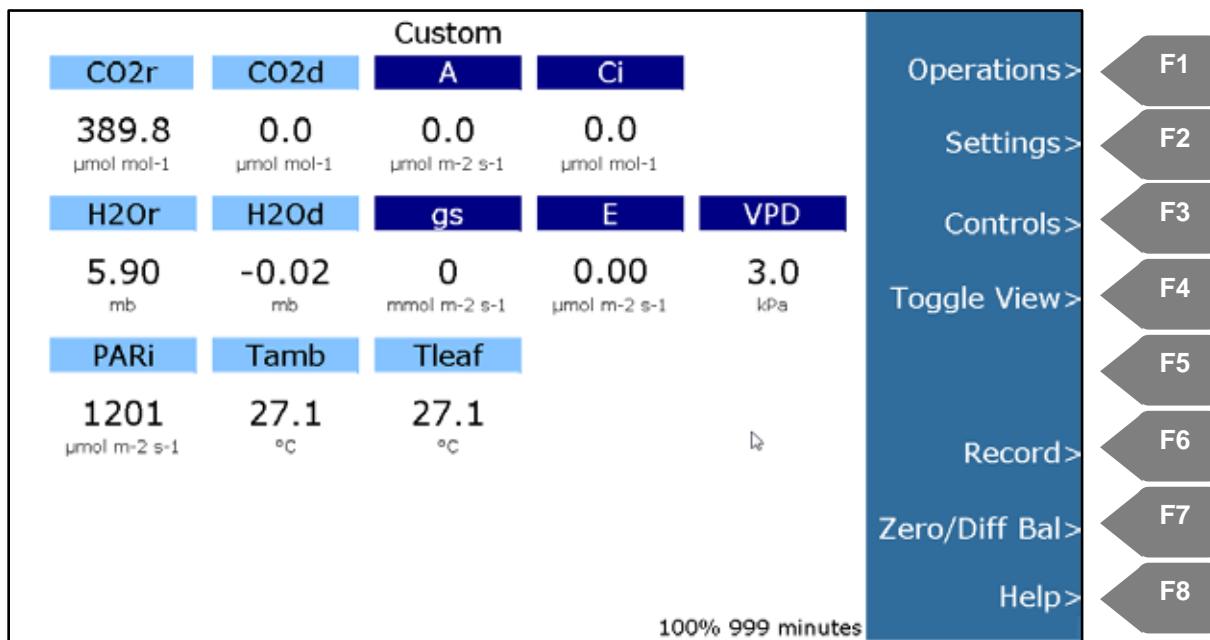
Do not worry about the calculated data at this point. These calculated values do not matter at all because there is no leaf in the chamber so do not be alarmed when you see these values jumping around. These values can change quite dramatically when there is no leaf present so this is normal.

Gas Exchange Measured and Calculated Data

Measured Data	
CO2r	CO ₂ Reference ($\mu\text{mol mol}^{-1}$)
CO2a	CO ₂ Analysis ($\mu\text{mol mol}^{-1}$)
CO2d	CO ₂ Differential ($\mu\text{mol mol}^{-1}$)
H2Or	H ₂ O Reference (mb)
H2Oa	H ₂ O Analysis (mb)
H2Od	H ₂ O Differential (mb)
Tamb	Temperature Ambient (°C)
Tcuv	Temperature in Cuvette (°C)
Tleaf	Leaf Temperature (°C)
PARi	PAR Internal ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
PARe	PAR External ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
RH%	Relative Humidity inside Leaf Chamber (%)
Flow	Cuvette Flow Rate (cc min ⁻¹)
Leaf Area	Leaf Area (cm ²)
Calculated Data	
A	Assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
Ci	Sub-Stomatal CO ₂ Concentration ($\mu\text{mol mol}^{-1}$)
gs	Stomatal Conductance (mmol H ₂ O m ⁻² s ⁻¹)
E	Transpiration (mmol H ₂ O m ⁻² s ⁻¹)
VPD	Leaf to air Vapor Pressure Deficit (kPa)
WUE	Photosynthetic Water Use Efficiency (mmol CO ₂ mol ⁻¹ H ₂ O)

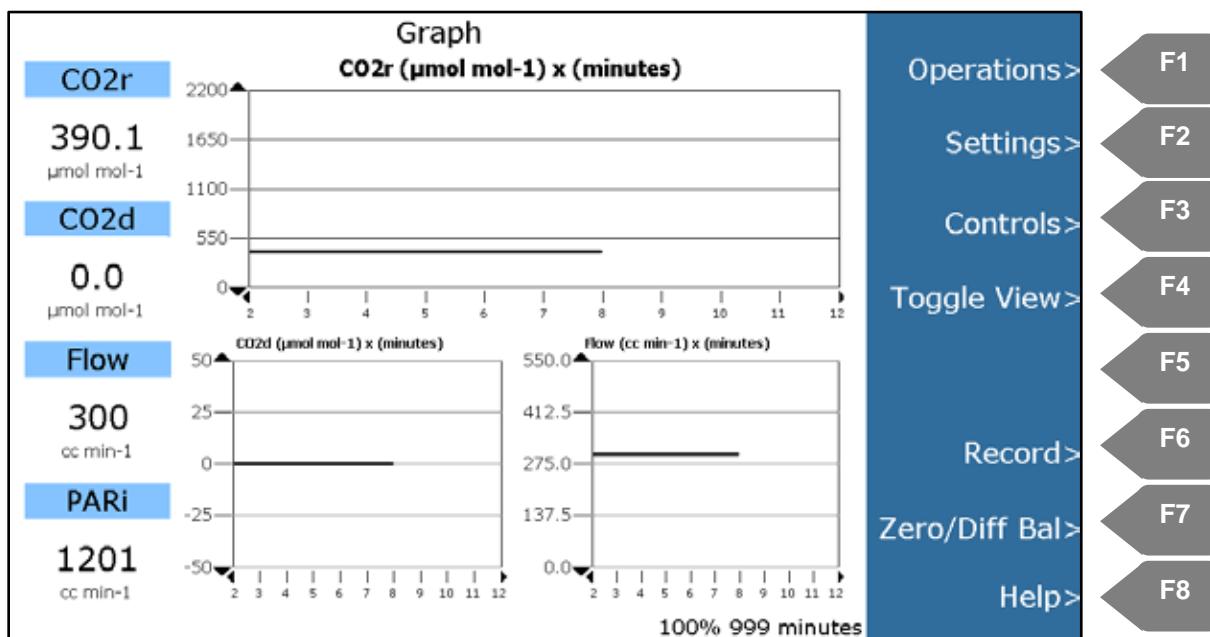
Press **Toggle View (F4)** to view the Custom display which should look similar to the display at the top of the next page based on your choices under **Settings (F2) > Set Custom (F5)**.

Custom Display



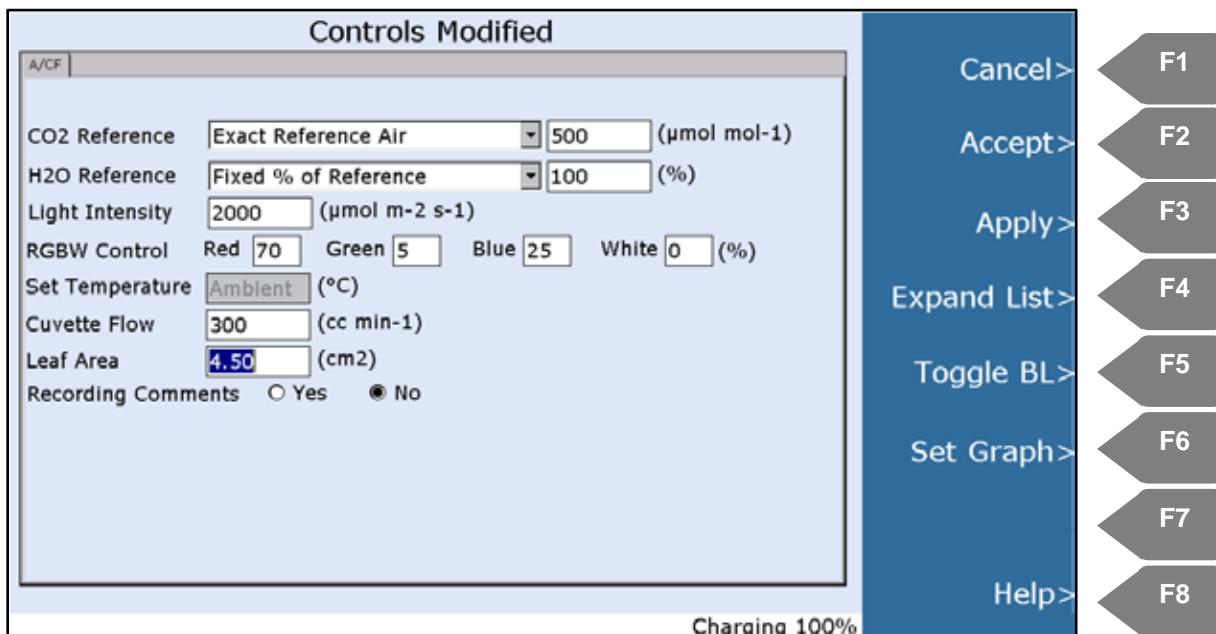
Press **Toggle View (F4)** to view the Graph display which should look similar to the following display after several minutes. Note the stability of the CO2r, CO2d and Flow. This display will be based on the settings you created in **Set Graph (F6)**.

Graph Display



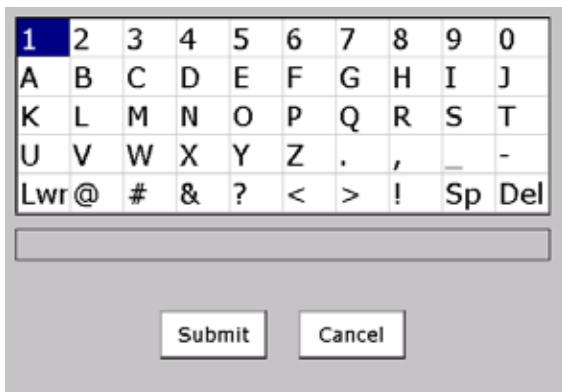
Let's experiment with the environmental controls

You will be amazed at how easy it is to dynamically control all environmental parameters with your CIRAS-3 system. Press **Controls (F3)**. In addition to controlling the environment you can also set the flow rate and leaf area from this screen if required. This is ideal when you want to change any of the environmental controls quickly and easily for rapid measurements without having to go back to **Settings (F2)**. We highly recommend using **Controls (F3)** when performing manual response curves due to the ease and speed at which you can make changes to all environmental controls. Press the TAB key and TAB from field to field updating the environmental controls as shown below.

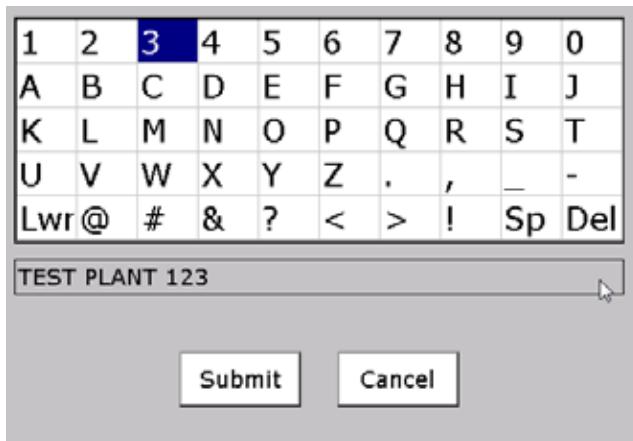


Adding Notes/Comments to data files

It is very easy to add notes or comments to data files. When the Controls dialog is opened as shown above press the TAB key down to "Recording Comments" and highlight Yes. When highlighted press **Comment (F5)** to launch the built-in alpha-numeric keypad as shown below:

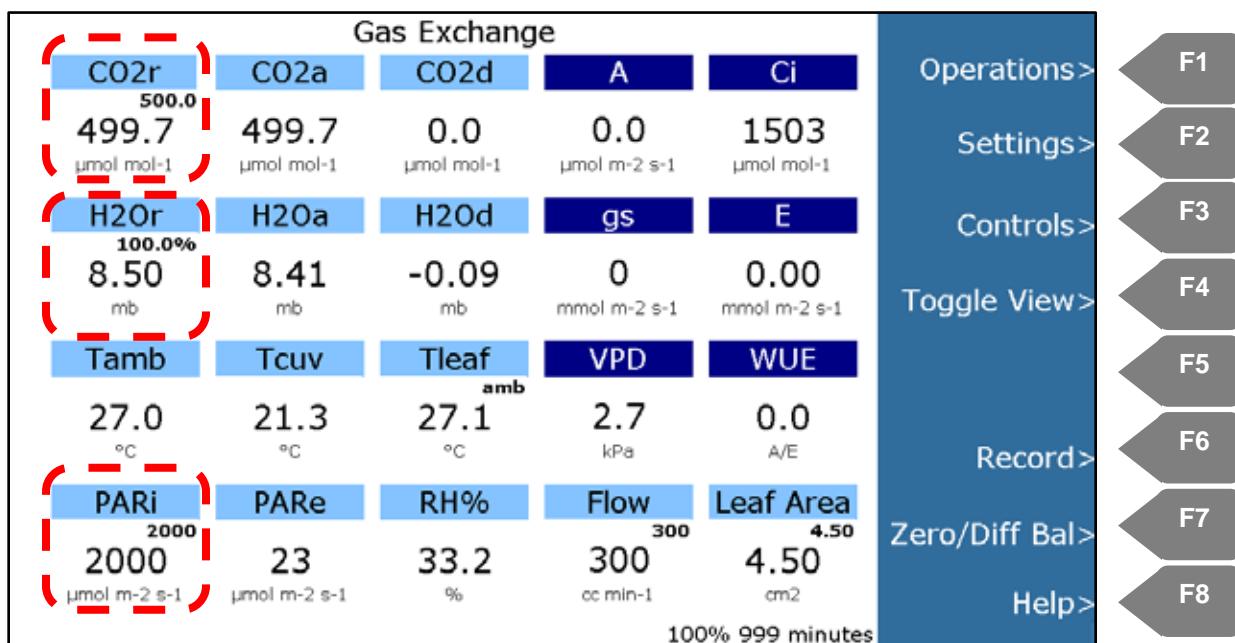


To add characters simply highlight the character using the arrow keys and press the green **OK** key on the keypad. As characters are entered they will appear in the box below the characters as shown below.

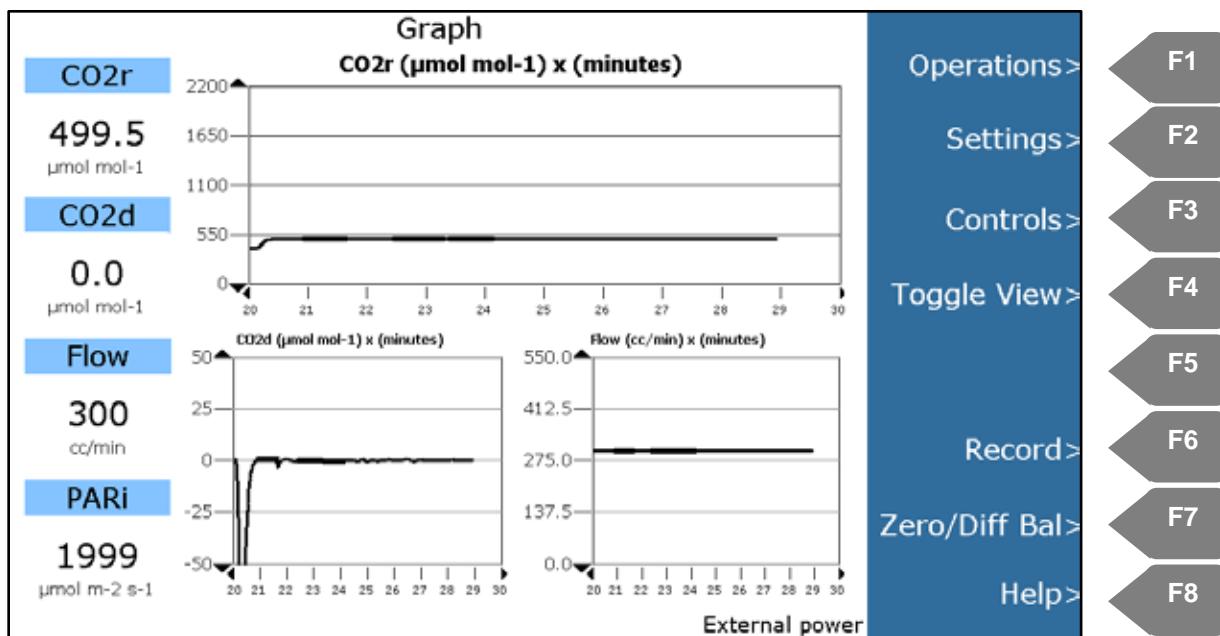


When finished press the TAB key and with **Submit** highlighted press the green **OK** key on the keyboard. If you would like to experiment with this by adding a comment feel free to do so.

Press **Accept (F2)** to return to the Graph display and press **Toggle View (F4)** to return to the main Gas Exchange display. The new control settings will be updated and you will begin to see the changes made taking place within seconds. Also note that due to the large change in reference CO₂ and H₂O control the system will perform a Diff Bal automatically when the system gets close to the new control settings. Also note the new, bold control values for CO₂r, H₂Or and PARi located in the upper right hand corner of each measurement field as shown below:



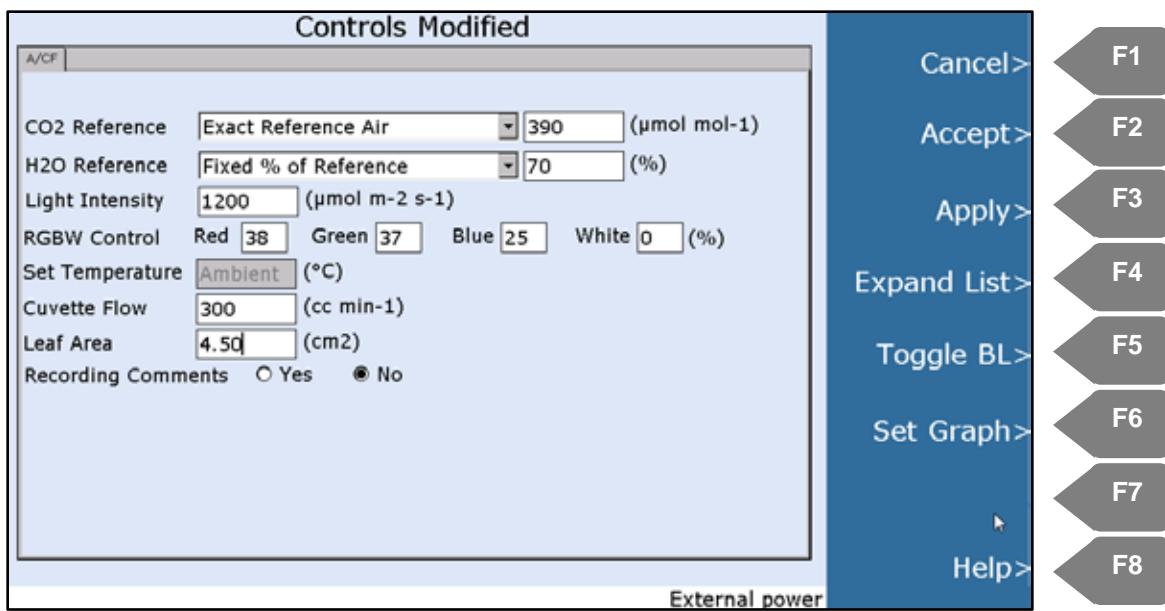
Press **Toggle (F4)** twice to view the Graph display based on the new settings. After 1-2 minutes you should see your new control settings take effect and after about 2-3 minutes the values should stabilize and your Graph display should look similar to the one below.



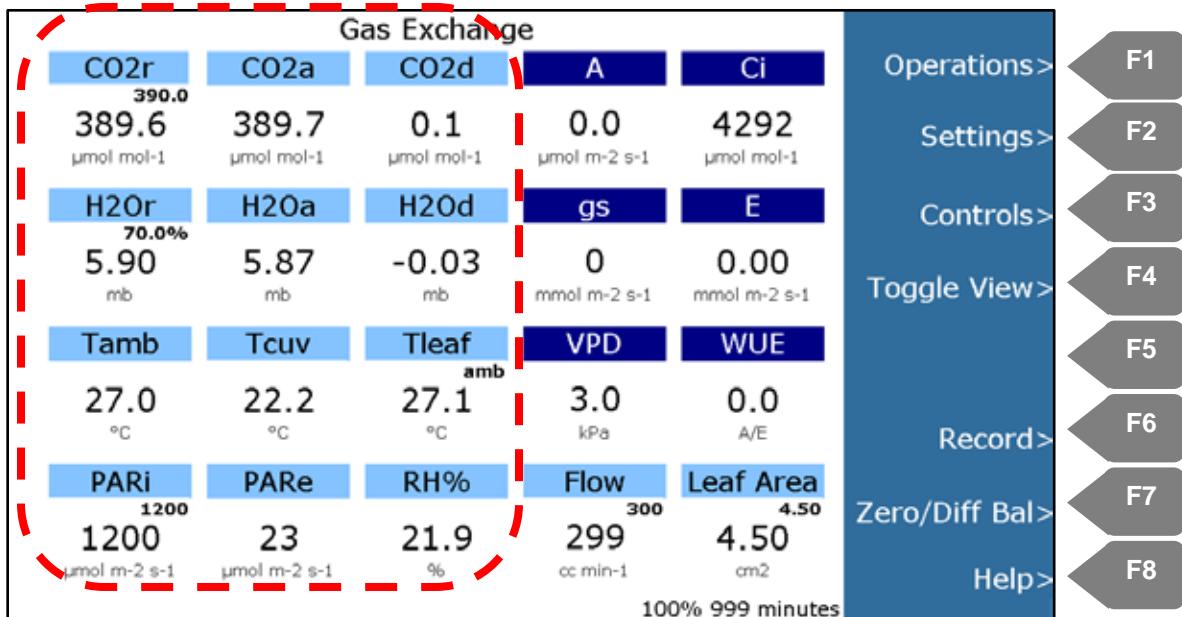
Note that the graph traces show a bold line during an Auto Zero or Diff Bal to indicate that data is not valid during these intervals.

If at any time you are unable to get the CO₂ or H₂O differentials (CO2d and H2Od) down close to 0 you can always perform a manual Diff Bal by pressing **Zero-Diff Bal (F7) > Right Arrow** and then **OK**. The flow rate should also be very stable. Press **Toggle (F4)** to get to the main Gas Exchange display again.

Let's revert to our original environmental controls for CO₂, H₂O, temperature and light based on our initial Settings. Press **Controls (F3)** and TAB from field to field making changes exactly as shown at the top of the following page.

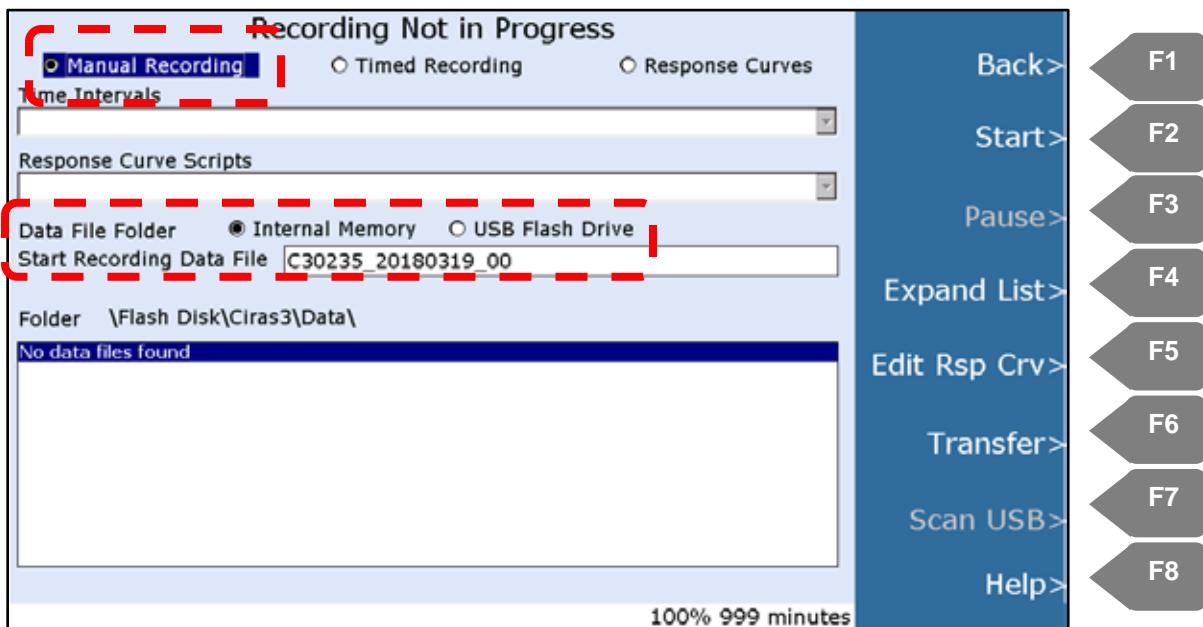


When finished press **Accept (F2)**. Again, you will be returned to the Gas Exchange display and after a few moments the system will perform an automatic Diff Bal as the CO2r and H2Or values approach their new target values. After approximately 2-3 minutes the system should stabilize and as discussed earlier should look similar to the display below. Again, note the new environmental control settings and stable CO2d and H2Od readings.



It's about time we start recording live data on a real plant

Now that we've established that everything is working well and you are a bit more comfortable with the CIRAS-3 it is time to get set up to begin taking measurements on a real plant. Press **Operations (F1) > Rec Options (F2)** to create a data file where all readings will be saved to. Data can be recorded manually (by default), timed or as part of a response curve. For this exercise we are going to keep things simple and perform manual measurements with the data saved to Internal Memory as shown below. Make sure that you have Manual Recording selected and "Data File Folder" is set to Internal Memory.



Observe the following:

- Manual Recording is selected as indicated by the black highlighted radio button.
- The data file will be saved to Internal Memory (as indicated by the black highlighted radio button) and the data file name is provided by default. Note that the default data file name always starts with C3XXXX_YYYYMMDD_00 where:

C3XXXX – The serial number of your CIRAS-3 console

YYYYMMDD – Year/Month/Day

00 – All data files start at 00 and count up from there (i.e. 01, 02, 03, etc.) unless changed by the user.

To begin a recording session press **Start (F2) > Back (F1)**.

Please note that you can change the name of the data file if you prefer to use something different than the default name.

Placing the leaf inside the cuvette

Now would be a good time to get that small tabletop tripod unless you prefer to hold the leaf cuvette for upcoming measurements.

You will find a standard tripod thread on the bottom of the leaf cuvette. Secure the cuvette to the tripod and open the leaf cuvette head and carefully place the leaf inside the cuvette and close the cuvette as shown here. Don't be alarmed when you see the CO_{2a} and H_{2Oa} values change causing large fluctuations in CO_{2d} and H_{2Od}. This is to be expected as you are temporarily

sampling ambient air which will likely be higher than your reference air for a brief moment. This will flush through the system fairly quickly once you close the chamber head.



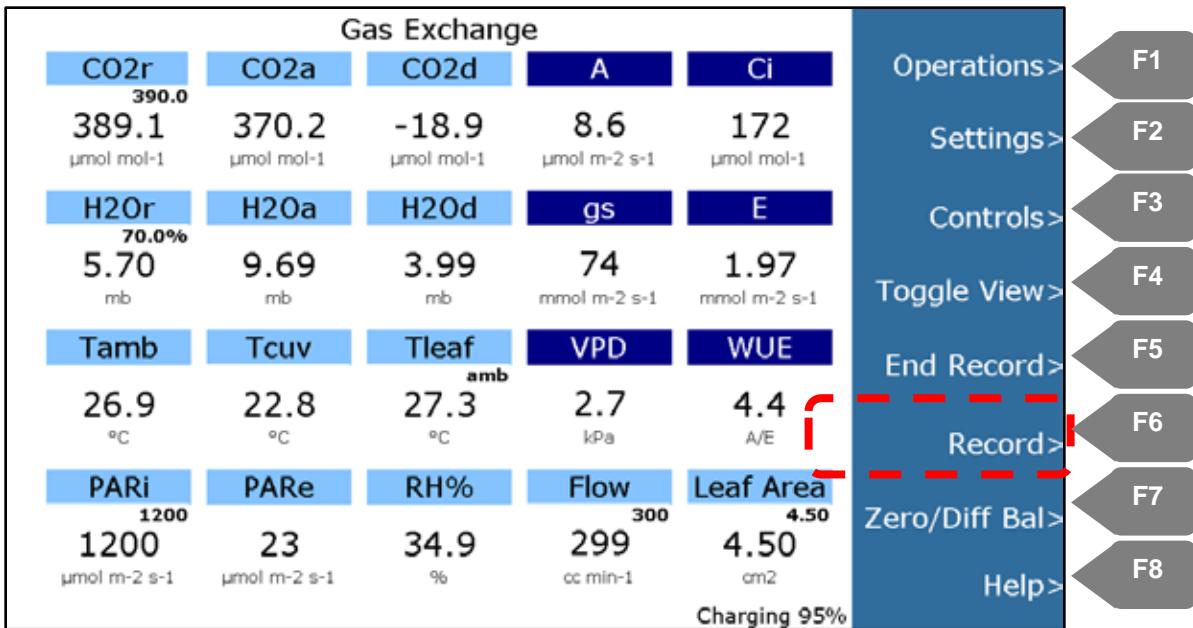
If possible it is best to fill the entire chamber window with your leaf to eliminate time consuming post-leaf area analysis and recalculation of results. If the leaf fills the window completely then leaf area is clearly defined and there will be no need to recalculate data based on leaf area. Also note that leaf tissue enclosed but not in the window will respire and affect rates of photosynthesis.

What should I be looking for at this point?

With the leaf cuvette head closed on your healthy leaf you should observe the following after about 5 seconds:

- The CO_{2a} and H_{2Oa} will slowly return to previous levels and the CO_{2d} will slowly approach 0 before going negative indicating CO₂ uptake resulting in positive Assimilation (A).
- The H_{2Od} value may also change quite dramatically but will also slowly approach 0 before going positive indicating an increase in H₂O due to leaf transpiration which should result in an increase in stomatal conductance (gs) and evaporation or transpiration (E).

After approximately 45-60 seconds you should start to see the CO_{2d} and H_{2Od} values stabilize indicating that the leaf has reached equilibrium. At this stage the calculated data should also be very stable as shown on the following page.



Assuming that you are seeing similar readings for your leaf now would be a good time to record a measurement. Press **Record (F6)** on the CIRAS-3 console or the “R” key on the PLC3 to record data and then proceed to record 4 more measurements on the same leaf for a total of 5 measurements. After the 5th record press **End Record (F5)**. Note if you happen to look at the graph display, small red triangles are placed into each graph trace to indicate recorded data points. In the example above there was a reasonable amount CO₂ uptake (CO₂d) and small H₂Od. If you are testing on a very healthy, well-watered and light adapted plant you will likely observe higher differentials for both CO₂ (CO₂d) and H₂O (H₂Od) resulting in higher rates of photosynthesis (A) and stomatal conductance (gs).

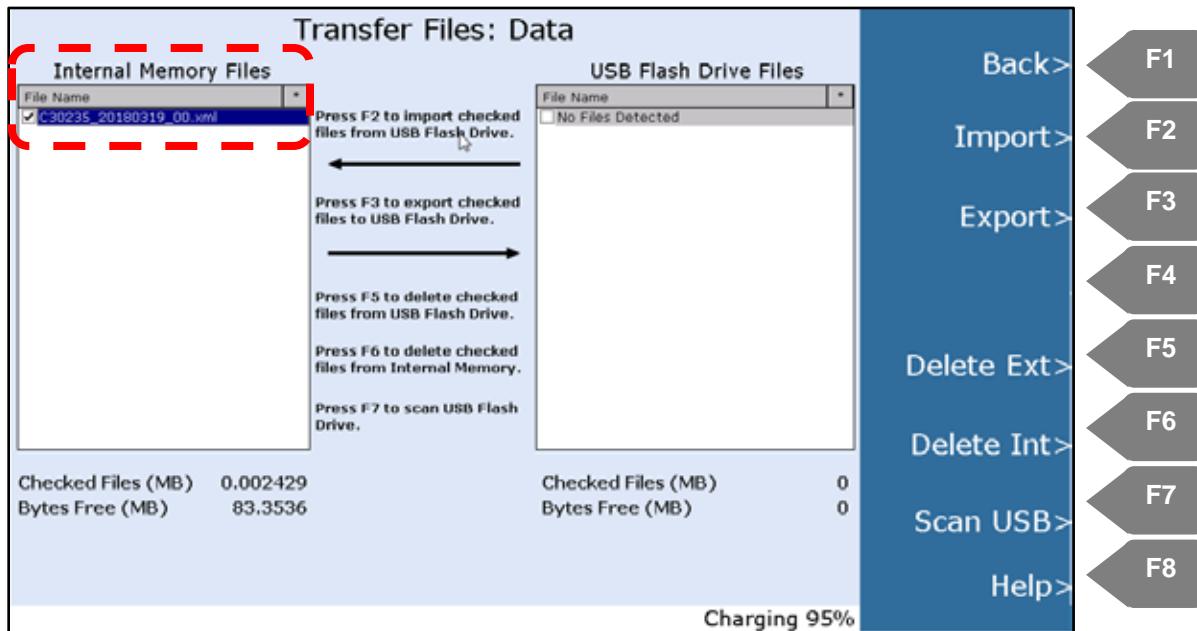
How do I know when it is time to record a measurement?

Good question. Generally speaking, the usual rule of thumb is that a healthy leaf reaches equilibrium when CO₂ differential (CO₂d) stabilizes (changing back and forth at same concentration for 5-10 seconds). Normally, this is a good time to record a measurement. The actual equilibration time varies based on the state of the plant at time of measurement and environmental controls. Having said that, normal healthy leaves tend to equilibrate and stabilize in approximately 45-60 seconds. In the field and when working under ambient sunlight conditions it is very important to try and keep the cuvette head in a steady position throughout the course of measurement to minimize changes in light intensity. Changes in light intensity will definitely have an impact on photosynthesis. With that being said it is also a good rule of thumb to maintain the same CO₂ and H₂O controls and flow rate during each measurement as any change to environmental conditions and flow rate will have an effect on the plant's equilibration and subsequent results.

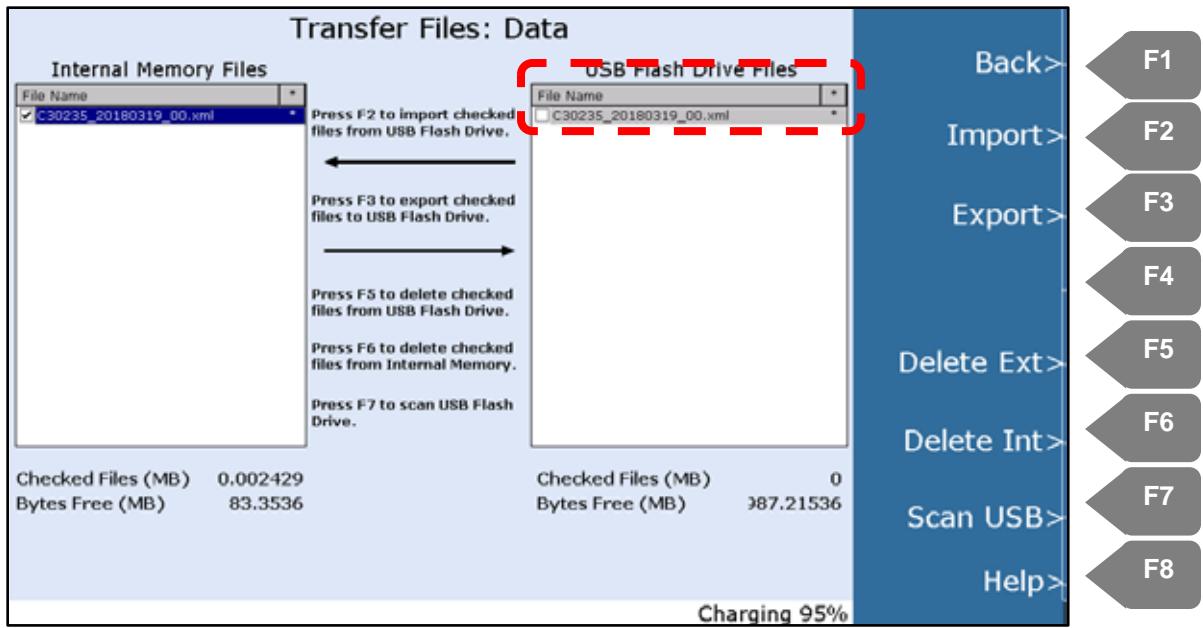
As mentioned earlier the data was saved to internal memory as an .xml file so that is where we need to go to retrieve the data. Insert your USB flash drive (supplied by PP Systems) into the USB 2 port as shown below (actually both USB ports will work just the same).



On the CIRAS-3 console press **Operations (F1) > Rec Options (F2) > Transfer (F6)**. Under “Internal Memory Files” locate your data file using the up/down arrows on the keyboard if necessary and press **OK** to select it. Note the check box next to the file.



Press **Export (F3)** to transfer the file to your USB Flash Drive. You should now see the data file on your USB flash drive as shown below. In future, if you want to move multiple files at once simply select all the data files that you want to move and select **Export (F3)**.



It is now safe to remove the USB flash drive from the CIRAS-3. Most people use Microsoft® Excel® to analyze the data. We are going to do the same here.

Insert the USB flash drive into the USB port on your PC. Open Excel and go to File > Open and navigate to the USB flash drive and locate your data file in the Ciras-3\Data folder. Follow the instructions to open the file in Excel. Once retrieved your data should look similar to the following:

RecType	ExcelTime	Comment	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od
R	43178.43997		390.2	372.2	-18	5.7	9.23	3.53
R	43178.44		390.2	372.1	-18.1	5.7	9.23	3.53
R	43178.44003		390.2	372	-18.2	5.7	9.23	3.53
R	43178.44006		390.1	372	-18.1	5.7	9.23	3.53
R	43178.44009		390.1	371.9	-18.2	5.7	9.23	3.53

PARI	PARE	Red	Green	Blue	White	Tamb	Tcuv	Tleaf	Aleaf
1199	22	38	37	25	0	26.8	22.7	27	4.5
1200	22	38	37	25	0	26.8	22.7	27	4.5
1201	22	38	37	25	0	26.8	22.7	27	4.5
1199	22	38	37	25	0	26.8	22.7	27	4.5
1200	22	38	37	25	0	26.8	22.7	27	4.5

Flow	Patm	RH	Ci	gs	VPD	A	E	WUE
300	1011	33.46	159	66	2.64	8.2	1.75	4.69
300	1011	33.46	158	66	2.64	8.3	1.75	4.74
299	1011	33.46	156	66	2.64	8.3	1.74	4.77
300	1011	33.46	157	66	2.64	8.3	1.75	4.74
300	1011	33.46	157	66	2.64	8.3	1.75	4.74

rb	StomataR	Tsensor	Tcontrol	Lcontrol	PLC	Status
0.28	50	IR	LA	LED	U	
0.28	50	IR	LA	LED	U	
0.28	50	IR	LA	LED	U	
0.28	50	IR	LA	LED	U	
0.28	50	IR	LA	LED	U	

There are 35 columns associated with leaf gas exchange data. Some variation may apply depending on the Excel software version that you are running. Also if you had entered comments to be included with your data they would appear in the “Comment” column. For this exercise we did not enter a comment so the column is blank. For more information on data leaf gas exchange data output refer to [Application – Gas Exchange and Analyzer Only](#) on page 178.

If you have our CFM-3 Chlorophyll Fluorescence Module and would like a quick tutorial on simultaneous measurement of leaf gas exchange and chlorophyll fluorescence please refer to the next section of the manual (see [Measurement of Leaf Gas Exchange and Chlorophyll Fluorescence](#) on page 46).

Congratulations! You should now feel quite comfortable with the general operation of your CIRAS-3 system. If you have any questions whatsoever please feel free to get in contact with one of our technical staff for further assistance. **Good luck with your research!**

Measurement of Leaf Gas Exchange and Chlorophyll Fluorescence

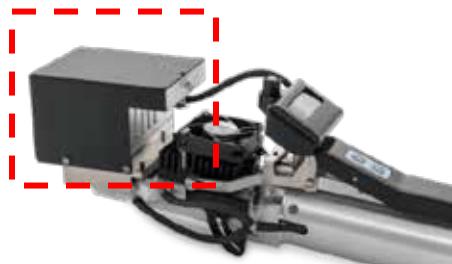
In order to perform simultaneous measurement of leaf gas exchange and chlorophyll fluorescence you must have our CFM-3 Chlorophyll Fluorescence Module (Part No. CRS306). The CFM-3 has all the required light sources and detector for measurement of chlorophyll fluorescence based on the pulse-amplitude modulated (PAM) measurement principle. The CFM-3 can also be used exactly the same as our LED light unit for automatic control of light intensity.

For tutorial purposes this section will be based on the following system hardware to make it easier to follow along and comprehend:

CIRAS-3 Main Console
Part Number CRS300

PLC3 Universal Leaf Cuvette
(with glass window in place)
Part Number CRS301

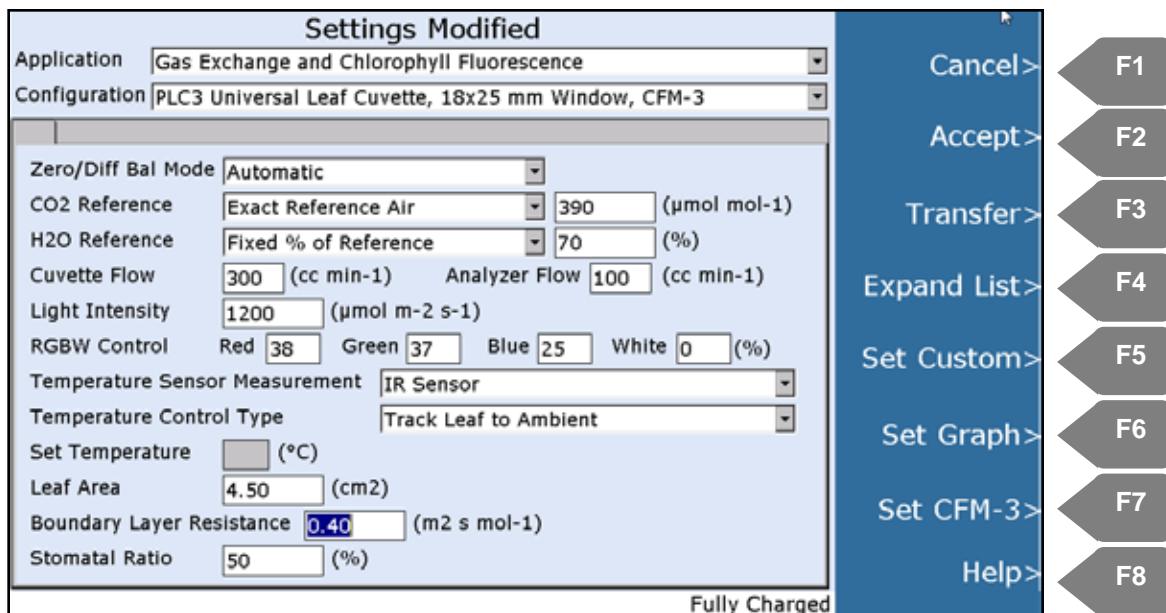
CFM-3 Chlorophyll Fluorescence Module
Part Number CRS306



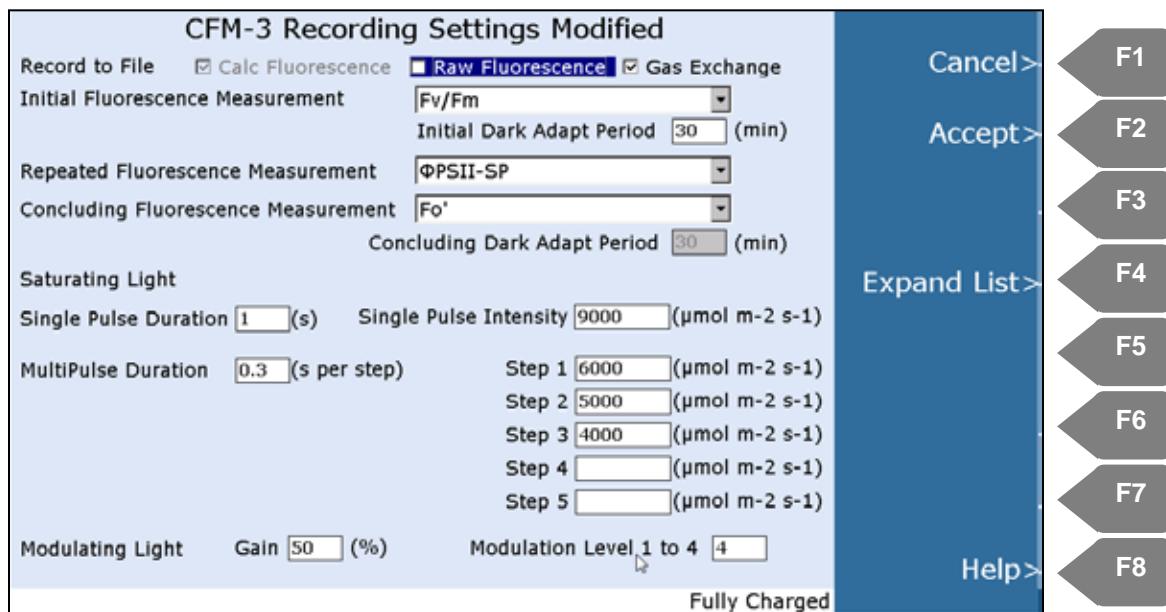
Measurement of leaf gas exchange is identical to what is discussed in the previous section. However, in addition to making the appropriate selections under **Settings (F2)** for measurement of leaf gas exchange you also need to go to **Set CFM-3 (F7)** to configure the system for measurement of chlorophyll fluorescence. You must also make sure that the plain glass window is in place. If you purchased the CFM-3 with your CIRAS-3 system then the plain glass window is already fitted to your PLC3 Universal leaf Cuvette. In future if you change out the window for any reason refer to [Replacement of PLC3 Glass Window \(For Use with CFM-3\)](#) on page 227. For this tutorial we are going to set up the system to perform the following chlorophyll fluorescence measurements in addition to leaf gas exchange:

- Initial Fluorescence Measurement: F_v/F_m
- Repeated Fluorescence Measurement: $\phi_{PSII-SP}$
- Concluding Fluorescence Measurement: F_o'

Go to **Settings (F2)** and make the following selections:



Next, go to **Set CFM-3 (F7)** and make the following selections:



Once properly configured chlorophyll fluorescence measurements will be performed each time a leaf gas exchange measurement is performed after the initial F_v/F_m . After making your selections press **Accept (F2)**.

Next, press **Set Graph (F6)** to configure the graph display as shown on the following page. When in the Min field for CO2d press **F3** to insert the negative (-) character (Graph2).

Set Graph Modified

Variable 1 <input type="button" value="CO2r [Reference]"/>	Variable 3 <input type="button" value="A [Assimilation]"/>	<input type="button" value="Cancel>"/>
Variable 2 <input type="button" value="CO2d [Differential]"/>	Variable 4 <input type="button" value="Fv/Fm"/>	<input type="button" value="Accept>"/>
Graph1	Graph2	Graph3
Y Axis <input type="button" value="CO2r [Reference]"/>	<input type="button" value="CO2d [Differential]"/>	<input type="button" value="Tleaf [Leaf Temperature]"/>
Min <input type="button" value="0"/>	<input type="button" value="-50"/>	<input type="button" value="0"/>
Max <input type="button" value="2200"/>	<input type="button" value="50"/>	<input type="button" value="35"/>
X Axis <input type="button" value="Time Span"/>	<input type="button" value="Time Span"/>	<input type="button" value="Time Span"/>
Span <input type="button" value="10"/>	<input type="button" value="10"/>	<input type="button" value="10"/>
Min <input type="button" value="Auto"/>	<input type="button" value="Auto"/>	<input type="button" value="Auto"/>
Max <input type="button" value="Auto"/>	<input type="button" value="Auto"/>	<input type="button" value="Auto"/>
<input type="button" value="Fully Charged"/>		

Press **Accept (F2)** when all selections are complete. Next press **Set Custom (F5)** to configure the Custom display as shown below:

Set Custom Data Modified

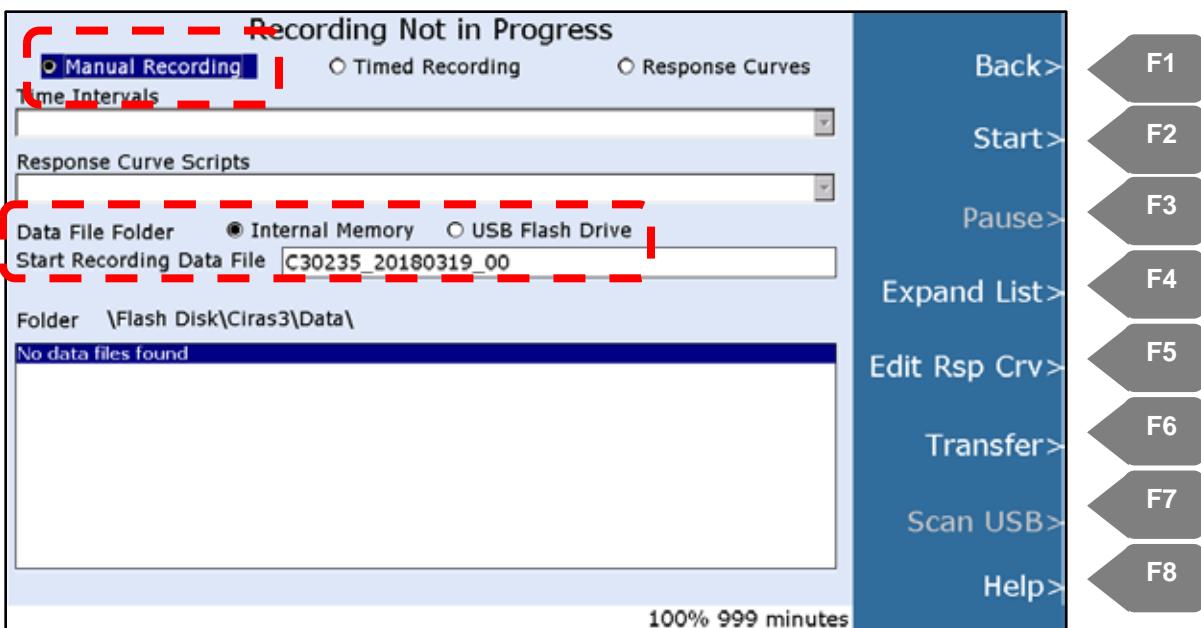
<input type="button" value="CO2r"/>	<input type="button" value="CO2a"/>	<input type="button" value="CO2d"/>	<input type="button" value="A"/>	<input type="button" value="Ci"/>	<input type="button" value="Cancel>"/>
<input type="button" value="H2Or"/>	<input type="button" value="H2Oa"/>	<input type="button" value="H2Od"/>	<input type="button" value="gs"/>	<input type="button" value="E"/>	<input type="button" value="Accept>"/>
<input type="button" value="PARi"/>	<input type="button" value="Tcuv"/>	<input type="button" value="Tleaf"/>	<input type="button" value="VPD"/>	<input type="button" value="WUE"/>	<input type="button" value="Expand List>"/>
<input type="button" value="Fv/Fm"/>	<input type="button" value="ΦPSII"/>	<input type="button" value="J"/>	<input type="button" value="None"/>	<input type="button" value="None"/>	<input type="button" value="Help>"/>
					<input type="button" value="Fully Charged"/>

Press **Accept (F2) > Accept (F2)** when all selections are complete. If you are not already at the Gas Exchange display press **Toggle (F4)** until you get to it. Close the leaf cuvette head without a leaf inserted. After approximately 2-3 minutes the system should stabilize and as discussed earlier should look similar to the display at the top of the following page. Note the environmental control settings and stable CO2d and H2Od readings with empty cuvette.

Gas Exchange					Operations>
CO2r 390.0	CO2a 390.3	CO2d 0.1 μmol mol-1	A 0.0 μmol m-2 s-1	Ci 727 μmol mol-1	Settings>
H2Or 70.0%	H2Oa 4.72	H2Od 0.02 mb	gs 0 mmol m-2 s-1	E 0.01 mmol m-2 s-1	Controls>
Tamb 27.7	Tcuv 22.7	Tleaf 27.8 amb	VPD 3.3 kPa	WUE 0.0 A/E	Toggle View>
PARi 1200	PARe 45	RH% 17.1	Flow 299 cc min-1	Leaf Area 4.50 cm2	Record>
					Zero/Diff Bal>
					Help>
					Fully Charged

Recording Leaf Gas Exchange and Chlorophyll Fluorescence Data

Now that we've established that everything is working well and you are a bit more comfortable with the CIRAS-3 it is time to get set up to begin taking measurements on a real plant. Press **Operations (F1) > Rec Options (F2)** to create a data file where all readings will be saved to. Data can be recorded manually (by default), timed or as part of a response curve. For this exercise we are going to keep things simple and perform manual measurements of leaf gas exchange and chlorophyll fluorescence based on your selections under **Settings (F2)** and **Set CFM-3 (F7)** with the data saved to Internal Memory as shown at the top of the following page. Make sure that you have Manual Recording selected and "Data File Folder" is set to Internal Memory.



Observe the following:

- Manual Recording is selected as indicated by the black highlighted radio button.
- The data file will be saved to Internal Memory (as indicated by the black highlighted radio button) and the data file is provided by default. Note that the default data file always starts with C3XXXX_YYYYMMDD_00 where:

C3XXXX – The serial number of your CIRAS-3 console

YYYYMMDD – Year/Month/Day

00 – All data files start at 00 and count up from there (i.e. 01, 02, 03, etc.) unless changed by the user.

Now would be a good time to place your leaf into the cuvette and close the head. To begin a recording session press **Start (F2) > Back (F1)** and then **Record (F6)** or **R** on the PLC3 Universal Leaf Cuvette. Please note that you must press **Record (F6)** or **R** in order to initiate a measurement sequence that involves measurement of chlorophyll fluorescence. Otherwise the system will not begin with the “Initial Fluorescence Measurement” which in this case is a F_v/F_m with a 30 minute initial dark adapt period as indicated in the status bar in the lower left hand corner of the display. The system will continue to count down until you reach the end of the dark adapt period after which it will perform the initial F_v/F_m measurement.

Gas Exchange					Operations> Settings> Controls> Toggle View> End Record> Record> Zero/Diff Bal> Help>
CO2r <small>390.0</small>	CO2a <small>390.5</small>	CO2d <small>0.6</small>	A <small>-1.0</small>	Ci <small>397</small>	
389.9 <small>μmol mol⁻¹</small>	390.5 <small>μmol mol⁻¹</small>	0.6 <small>μmol mol⁻¹</small>	-1.0 <small>μmol m⁻² s⁻¹</small>	397 <small>μmol mol⁻¹</small>	
H2Or <small>70.0%</small>	H2Oa <small>9.07</small>	H2Od <small>3.67</small>	gs <small>71</small>	E <small>1.81</small>	
5.40 <small>mb</small>	mb	mb	mmol m ⁻² s ⁻¹	mmol m ⁻² s ⁻¹	
Tamb <small>26.5</small>	Tcuv <small>25.7</small>	Tleaf <small>26.6</small>	VPD <small>2.6</small>	WUE <small>-0.6</small>	
°C	°C	°C	kPa	A/E	
PARi <small>1200</small>	PARe <small>31</small>	RH% <small>27.5</small>	Flow <small>299</small>	Leaf Area <small>4.50</small>	
0 <small>μmol m⁻² s⁻¹</small>	31 <small>μmol m⁻² s⁻¹</small>	%	cc min ⁻¹	cm ²	
Dark ends in 28:09					Charging 88%

At the conclusion of the 30 minute dark adapt period the system will perform a F_v/F_m. Press **Toggle View** (**F4**) to see the values associated with F_v/F_m recorded. Also note that the status bar also confirms that the measurement was saved to the data file as shown below.

Chlorophyll Fluorescence					Operations> Settings> Controls> Toggle View> End Record> Record> Zero/Diff Bal> Help>
F <small>1608</small>	Fs <small>---</small>	Fo <small>472</small>	Fv <small>1440</small>	Fm <small>1912</small>	
1608	---	472	1440	1912	
Fv/Fm	Fo'	Fv'	Fm'	Fv'/Fm'	
0.753	---	---	---	---	
ΦPSII	j	qP	qNP	NPQ	
---	---	---	---	---	
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	
---	---	---	---	---	
m2 Record 2 saved to file: C30235_20180321_05.xml					Charging 88%

All recorded measurements are confirmed in this area when stored to the data file. Press **Toggle** (**F4**) until you return to the Gas Exchange display. Next, wait for the plant to stabilize (approx. 60 seconds) after adjusting to the environmental conditions previously set under **Settings** (**F2**) and observe the following:

- CO₂d and H₂O_d should stabilize indicating that the leaf has reached equilibrium.
- Photosynthesis (A) & Stomatal Conductance (gs) should also stabilize.

Gas Exchange						
CO ₂ r 390.0	CO ₂ a	CO ₂ d	A	Ci	Operations>	F1
389.1 μmol mol ⁻¹	370.2 μmol mol ⁻¹	-18.9 μmol mol ⁻¹	8.6 μmol m ⁻² s ⁻¹	172 μmol mol ⁻¹	Settings>	F2
H2Or 70.0%	H2Oa	H2Od	gs	E	Controls>	F3
5.70 mb	9.69 mb	3.99 mb	74 mmol m ⁻² s ⁻¹	1.97 mmol m ⁻² s ⁻¹	Toggle View>	F4
Tamb	Tcuv	Tleaf amb	VPD	WUE	End Record>	F5
26.9 °C	22.8 °C	27.3 °C	2.7 kPa	4.4 A/E	Record>	F6
PARi 1200	PARe	RH%	Flow 300	Leaf Area 4.50	Zero/Diff Bal>	F7
1200 μmol m ⁻² s ⁻¹	23 μmol m ⁻² s ⁻¹	34.9 %	299 cc min ⁻¹	4.50 cm ²	Help>	F8
Charging 95%						

When readings are stable press **Record (F6)** on the CIRAS-3 console or the “R” key on the PLC3 to record leaf gas exchange data. Immediately after recording a leaf gas exchange measurement the system will next perform a “Repeated Fluorescence Measurement” ϕ PSII-SP and the data will be saved to the file as shown on the following page. This will continue each and every time you record a leaf gas exchange measurement per your **Set CFM-3 (F7)** settings.

Chlorophyll Fluorescence					Operations> Settings> Controls> Toggle View> End Record> Record> Zero/Diff Bal> Help>
F	Fs	Fo	Fv	Fm	
679	683	472	1440	1912	
Fv/Fm	Fo'	Fv'	Fm'	Fv'/Fm'	
0.753	336	389	725	0.536	
ΦPSII	j	qP	qNP	NPQ	
0.058	29.232	0.108	0.753	1.637	
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	
0.053	0.357	0.585	0.357	0.337	

m5 record 4 saved to file: C30235_20180321_05.xml Charging 88%

At the conclusion of your leaf gas exchange measurements press **End Record (F5)** to allow the system to perform the “Concluding Fluorescence Measurement” F_o' per your **Set CFM-3 (F7)** settings to terminate the recording session. At completion you can press **Toggle View (F4)** to view the Chlorophyll Fluorescence display to view all final readings as shown below.

Chlorophyll Fluorescence					Operations> Settings> Controls> Toggle View> End Record> Record> Zero/Diff Bal> Help>
F	Fs	Fo	Fv	Fm	
647	683	472	1440	1912	
Fv/Fm	Fo'	Fv'	Fm'	Fv'/Fm'	
0.753	394	331	725	0.457	
ΦPSII	j	qP	qNP	NPQ	
0.058	29.232	0.127	0.782	1.637	
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	
0.073	0.350	0.592	0.357	0.337	

Charging 88%

Retrieving and transferring data from internal memory to a USB Flash Drive is simple and easy as described in the previous section highlighting measurement of leaf gas exchange (beginning on page 43). When the file is opened in Excel it should look similar to below.

	A	B	C	D	E	F	G	H	I
1	RecType	ExcelTime	Comment	CO2r	CO2a	CO2d	H2Or	H2Oa	H2Od
2	R	43180.63892		390.00	943.80	553.80	5.40	5.43	0.03
3	m2	43180.66166							
4	R	43180.66563		389.90	387.50	-2.40	5.30	6.11	0.81
5	m5	43180.66567							
6	m4	43180.66969							

J	K	L	M	N	O	P	Q	R	S
PARi	PARe	Red	Green	Blue	White	Tamb	Tcuv	Tleaf	Aleaf
0.00	31.00		38	37	25	0	26.50	26.90	25.10
			38	37	25	0			4.5
1200.00	28.00		38	37	25	0	26.30	22.20	26.60
			38	37	25	0			4.5
			38	37	25	0			4.5

T	U	V	W	X	Y	Z	AA	AB	AC	AD
Flow	Patm	RH	Ci	gs	VPD	A	E	WUE	rb	StomataR
300.00	1007.00	15.32	9999.00	0.00	2.64	-99.90	0.01	-999.00	0.4	50
									0.4	50
299.00	1008.00	22.83	253.00	13.00	2.87	1.00	0.40	2.50	0.4	50
									0.4	50
									0.4	50

Formula Bar	AF	AG	AH	AI	AJ	AK	AL	AM	AN
Tsensor	Tcontrol	Lcontrol	PLC	Status	Fo	Fm	Fv	FvFm	
IR	LA	CFM-3	UF						
IR	LA	CFM-3	UF	Fv/Fm		472	1912	1440	0.753
IR	LA	CFM-3	UF						
IR	LA	CFM-3	UF	ΦPSII-SP		472	1912	1440	0.753
IR	LA	CFM-3	UF	Fo'		472	1912	1440	0.753

AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX
FmP	Fs	ΦPSII	FoP	FvP	FvPFmP	J	qP	qNP	NPQ
725	683	0.058	336	389	0.536	29.232	0.108	0.753	1.637
725	683	0.058	394	331	0.457	29.232	0.127	0.782	1.637

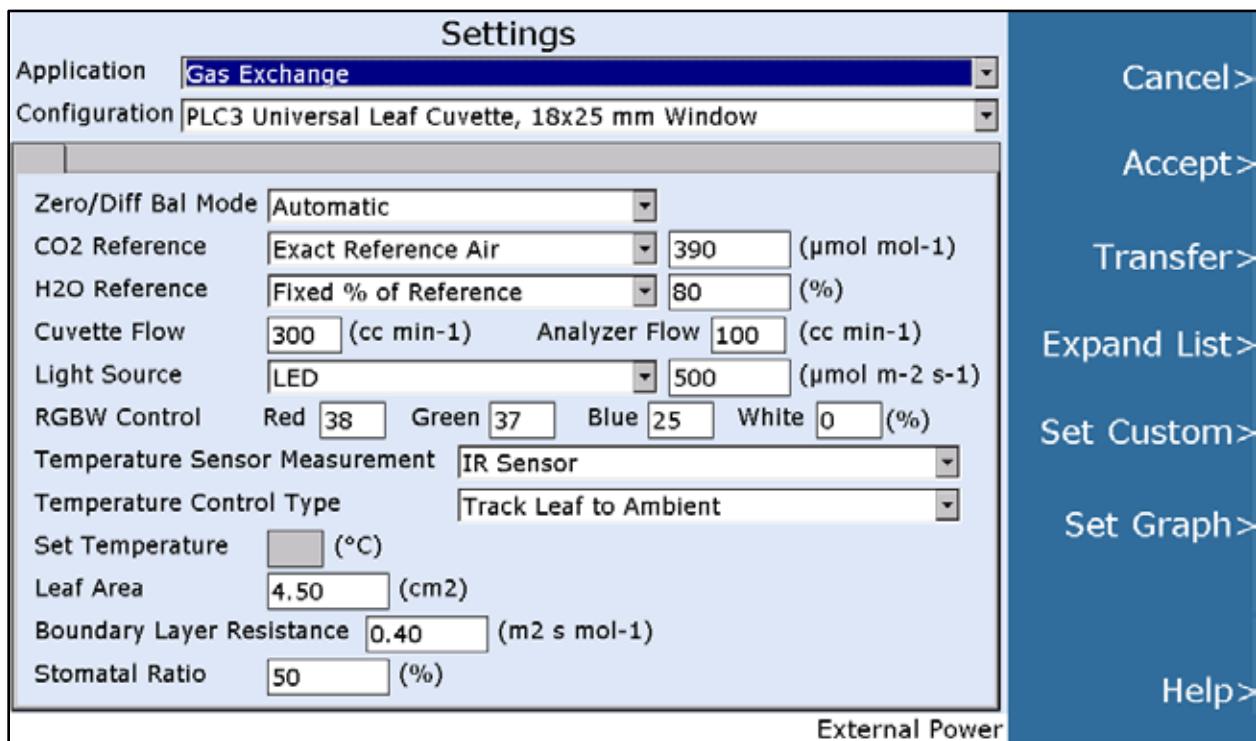
AY	AZ	BA	BB	BC
qL	ΦNO	ΦNPQK	ΦfD	ΦNPQG
0.053	0.357	0.585	0.357	0.337
0.073	0.350	0.592	0.357	0.337

There are 55 columns of data associated with Gas Exchange and Chlorophyll Fluorescence measurements. Note that the Status column shows the row containing the fluorescence related data associated with Fv/Fm, ϕ PSII-SP and F_o' . For more information on data output that is associated with gas exchange and chlorophyll fluorescence measurements go to [Application – Gas Exchange and Chlorophyll Fluorescence](#) on page 177.

Section 3. 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) to ensure that the system is performing perfectly before starting any measurement campaign.

1. Insert a new CO₂ cartridge, ensure all chemicals are fresh and connect the CIRAS-3 power supply 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)** when the welcome screen appears.
3. Allow the system to warm up for approximately 30 minutes. Press **Settings (F2)** and make sure that that “Application” is set to **Gas Exchange** and “Configuration” is set to the appropriate leaf cuvette (change if necessary). Assuming that you are using the PLC3 Universal Leaf Cuvette (with 18x25mm window) and PLC3 Universal LED Light Unit set up as follows and press **Accept (F2)**.



For this simple test set the light source intensity to 500 μmol m⁻² s⁻¹ and to best simulate sunlight we recommend the following RGBW control settings:

- Red: 38% / Green: 37% / Blue: 25% / White: 0%

Allow the system a few minutes to stabilize at the values that were created under **Settings (F2)**. Once stabilized, your display should look something similar to this.

Gas Exchange					
CO2r 390.0	CO2a 390.0	CO2d 0.0	A 0.0	Ci 0	Operations>
	µmol mol ⁻¹	µmol mol ⁻¹	µmol m ⁻² s ⁻¹	µmol mol ⁻¹	Settings>
H2Or 80.0%	H2Oa 5.70	H2Od 0.00	gs 0	E 0.00	Controls>
	mb	mb	mmol m ⁻² s ⁻¹	mmol m ⁻² s ⁻¹	Toggle View>
Tamb 25.4	Tcuv 21.2	Tleaf amb 25.4	VPD 2.7	WUE 0.0	Record>
	°C	°C	kPa	A/E	Zero/Diff Bal>
PARi 500	PARe 21	RH% 22.6	Flow 300	Leaf Area 4.50	Help>
	µmol m ⁻² s ⁻¹	%	cc min ⁻¹	cm ²	Fully Charged

Observe the following:

- All measured data match up well with all environmental controls. The CO2r should be close to the set value of 390 ($\pm 1.0 \text{ } \mu\text{mol m}^{-1}$) and H2Or should be a reasonable value based on your local ambient %RH.
- The CO2d is close to 0 ($\pm 0.5 \text{ } \mu\text{mol m}^{-1}$) and H2Od is also close to 0 ($\pm 0.05 \text{ mb}$).
- Tamb and Tleaf are reading similarly ($\pm 0.2 \text{ } ^\circ\text{C}$) because you set the **Temperature Control Type** to "Track Leaf to Ambient" under **Settings (F2)**.
- PARi is reading 500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ and stable ($\pm 1-2 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$).

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

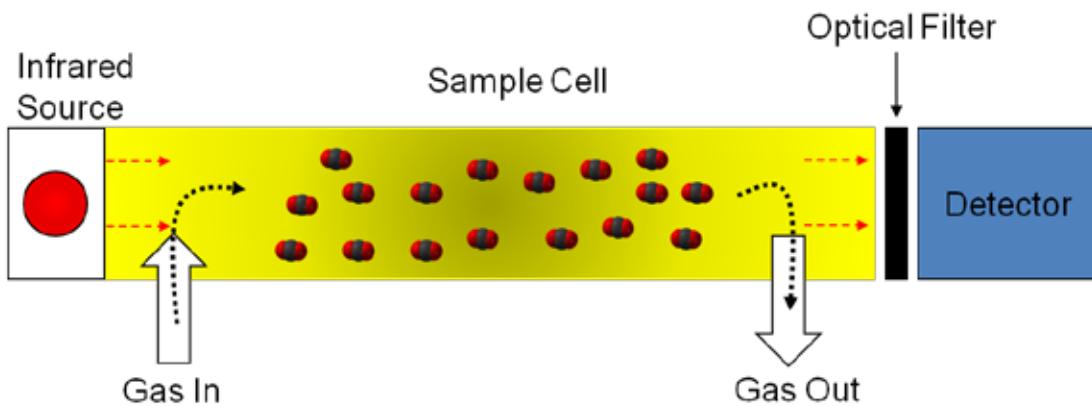
Section 4. Summary of System Design

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 CO₂ and H₂O gases. CIRAS stands for **Combined Infra-Red Analysis System**. 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**) – CO₂ Reference, CO₂ Analysis, H₂O Reference, H₂O Analysis, a true differential analyzer.

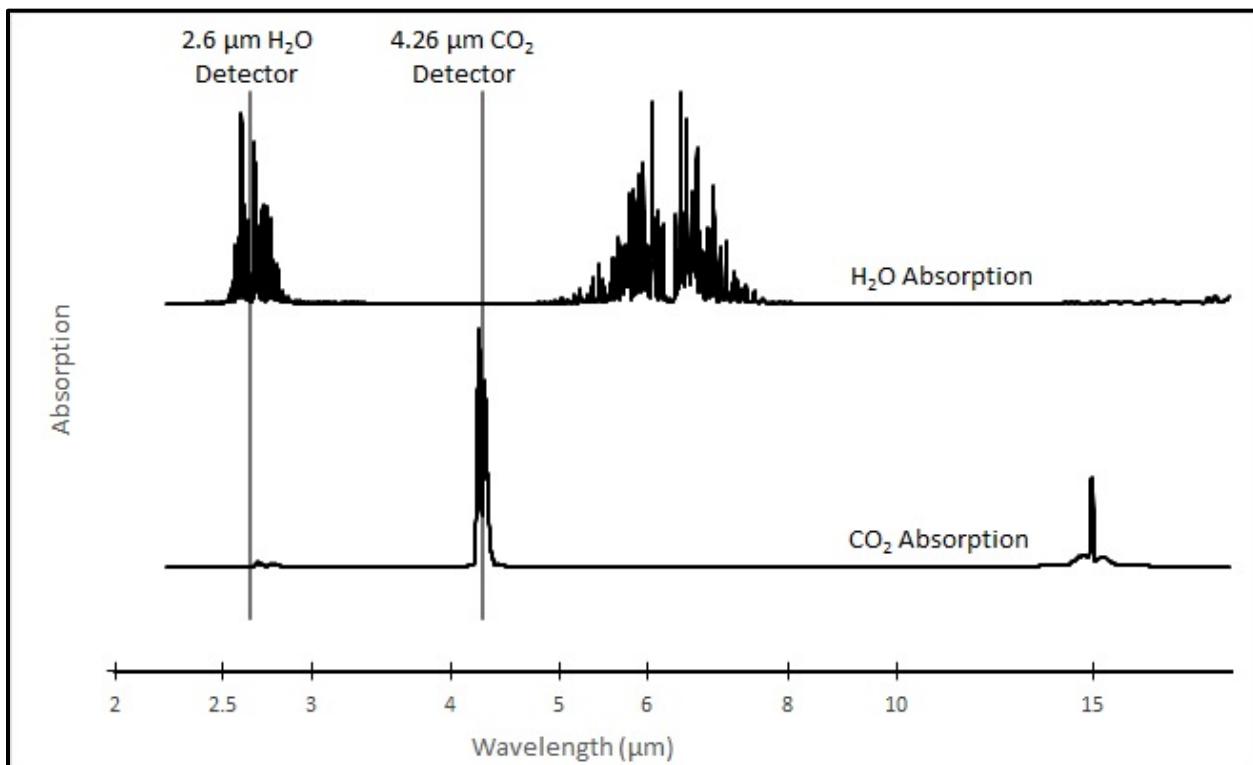
The IRGAs form the core of gas analysis systems that measure CO₂ and water vapor (i.e. Portable photosynthesis system, eddy covariance, soil CO₂ efflux, etc.). **Non-Dispersive, Infra-Red (NDIR)** refers to the transmission of broad-band infra-red wavelengths from the IRGAs source lamps. A single IRGA 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. CO₂. The CO₂ and H₂O 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 H₂O and CO₂ molecules have diverse absorption spectra, so we use two prominent absorption peaks, seen below at 2.60 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.



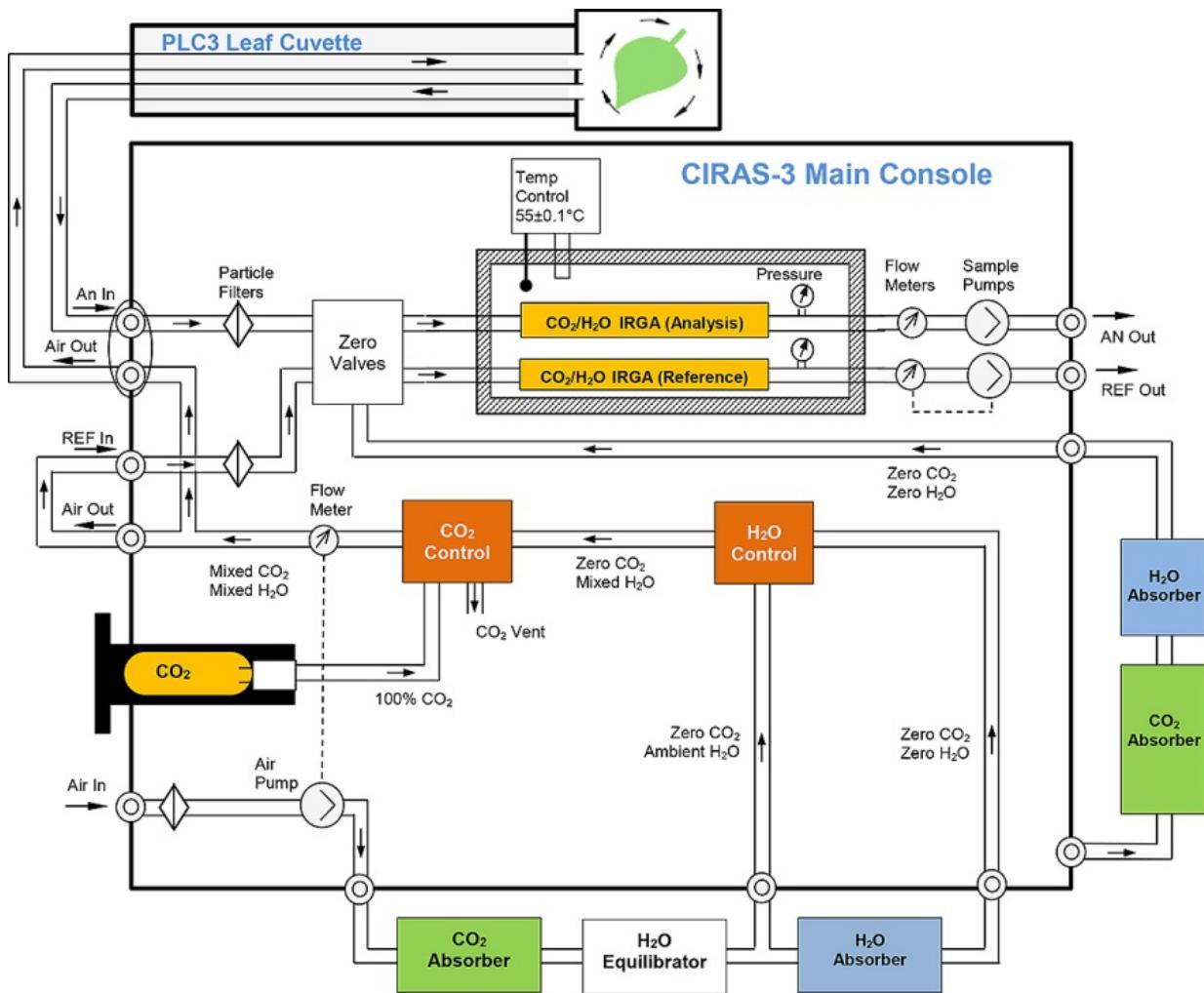
The gas sample is of course a mixture of gas molecules, and this can present problems in terms of accurate detection of concentrations of a specific gas, such carbon dioxide. This effect, *foreign gas broadening* (FGB), must be corrected to ensure accurate measurement of gas concentrations. With FGB, the CO₂ gas in the IRGA cell is somewhat diluted by the increased air volume induced by water vapor. This effect is about 0.1 $\mu\text{mol mol}^{-1}$ CO₂ mb⁻¹ H₂O. The presence of water vapor also causes an increase in infra-red absorption, which is detected as an apparent increase in [CO₂]. 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 $\mu\text{mol mol}^{-1}$ CO₂ and 0-75 mb water vapor are ideally suited for most typical gas exchange 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 on the following page. Sample air, denoted as AIR IN, entering the console and is first pumped and its flow rate metered, then directly "conditioned" by passing through CO₂ and water vapor absorbent chemicals. At this point CO₂ can be added to the gas stream in a precise mixture from the CO₂ 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 rugged, sealed case and taken together form the thermally-stable optical bench.



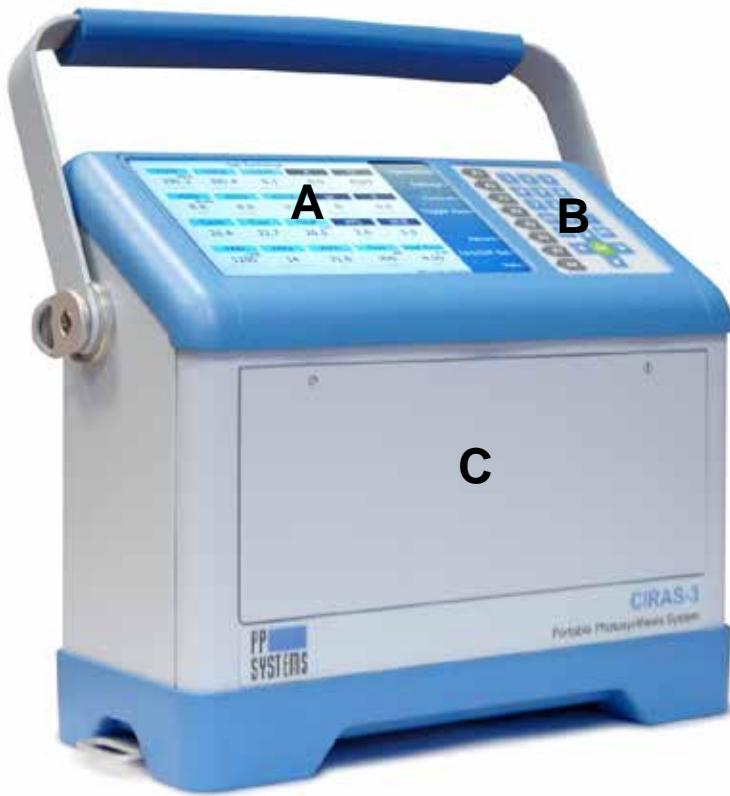
CIRAS-3 System Schematic

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. All pumps are under user control, allowing you to determine and set an optimal flow rate of air to the cuvette and leaf (air supply pump) as well as the sampling rate by the IRGAs (reference and analysis pumps). 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 5. System Hardware

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 applications including 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 CO₂ and H₂O.



- A. Large, full color, transreflective LCD optimized for field use.
- B. Tactile feel keypad and function keys for system navigation and user inputs.
- 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(s) and internal mechanical and electrical components is achieved by loosening the two captive screws (turn counter-clockwise to release) and gently lowering the door.

System Power

Power Supply Adapter

An AC power supply adapter (120/240 VAC / 50/60 Hz) is included with the CIRAS-3. If mains power is available the system can be operated continuously using the power supply adapter. This same adapter is also used to charge the internal battery packs. **You must use the power supply adapter supplied by PP Systems as other types may cause damage to the instrument.**

Battery Pack

For field use the system is powered by internal, rechargeable battery packs. Up until June 2017 the CIRAS-3 was powered by a single, internal rechargeable 7.2V Li-ion battery pack (Aved) providing system operation up to 8 hours. Starting in July 2017 we began supplying instruments with two 7.2V (8.7 Ah, 63 Wh) Li-ion battery packs (Inspired Energy) improving system operation for up to 12 hours (actual operation time will vary depending on environmental control settings). If users want to update to our latest battery technology they should get in direct contact with PP Systems.

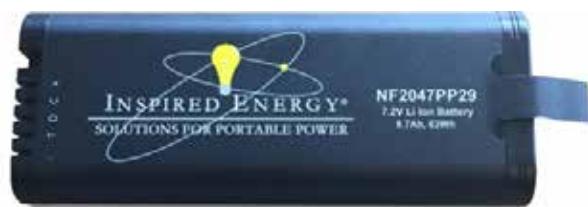
Battery Pack (Aved)

Part Number 41526-1



Battery Pack (Inspired Energy)

Part Number 41535-1



ALWAYS MAKE SURE THE BATTERY PACKS ARE FULLY CHARGED THE NIGHT BEFORE USE.

Please note that batteries are shipped only partially charged due to shipping regulations. We highly recommend that you charge them to full capacity upon receipt to avoid any potential damage or loss of battery life.



TIP

To reduce the weight of the CIRAS-3 console approximately 0.2 kg you can remove one of the battery packs providing up to 6 hours of continuous use (depending on settings and controls) in the field. We recommend 2 battery packs for normal use but if one battery pack is used it must be placed in the lower battery compartment which is the one that is flush with the inside battery compartment door as shown above.

There are two major factors that influence battery capacity:

- Environmental control
- CFM-3 Chlorophyll Fluorescence Module

For field use, it is extremely important to make sure that the internal battery(s) are fully charged.

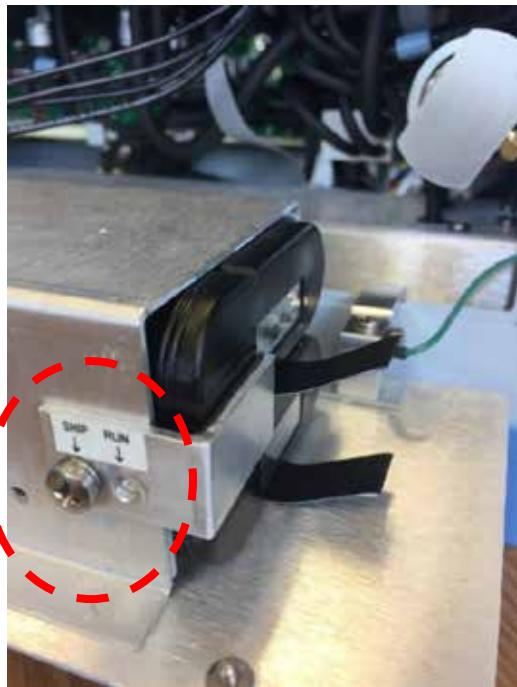
We also offer a nice, lightweight external battery pack that can clip easily to your belt for extended operation time in the field. See [External Battery Pack \(For extended operation in the field\)](#) on page 207 for more details.

Battery Pack Installation

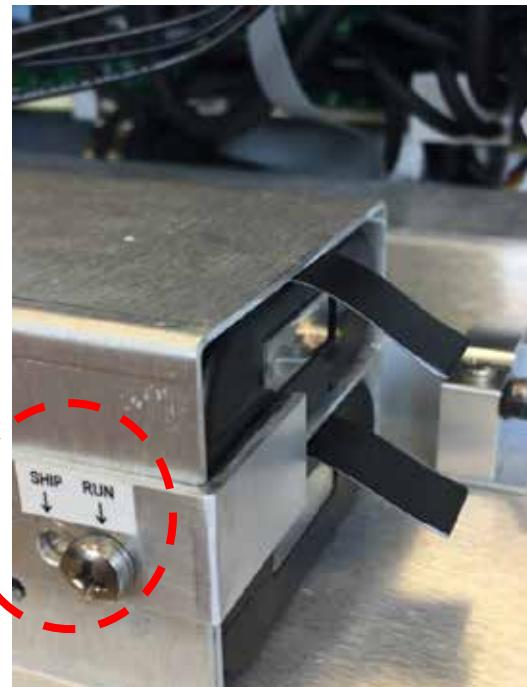
When systems are first supplied the batteries are packed inside the CIRAS-3 console in “Ship Mode”.

Upon receipt of your new instrument you must remove the packs from each compartment to put them into “Run Mode” as shown below.

Ship Mode



Run Mode



Instructions:

1. Open battery compartment door on the front of the CIRAS-3 console (2 smalls screws) to access the internal battery packs and gently drop down the door.
2. Remove battery retaining bracket which is currently in the “Ship” position.
3. Remove both battery packs from each compartment by pulling on the black TAB.
4. Flip each battery pack over and re-insert into each compartment to snap into place with the groove facing downwards towards the battery compartment door. The battery gauge should be facing outwards and the battery removal black TAB should be on the top of the battery pack.
5. Secure in place with the retaining bracket in the “Run” position.
6. Close battery compartment.

Charging Batteries inside the CIRAS-3 Console

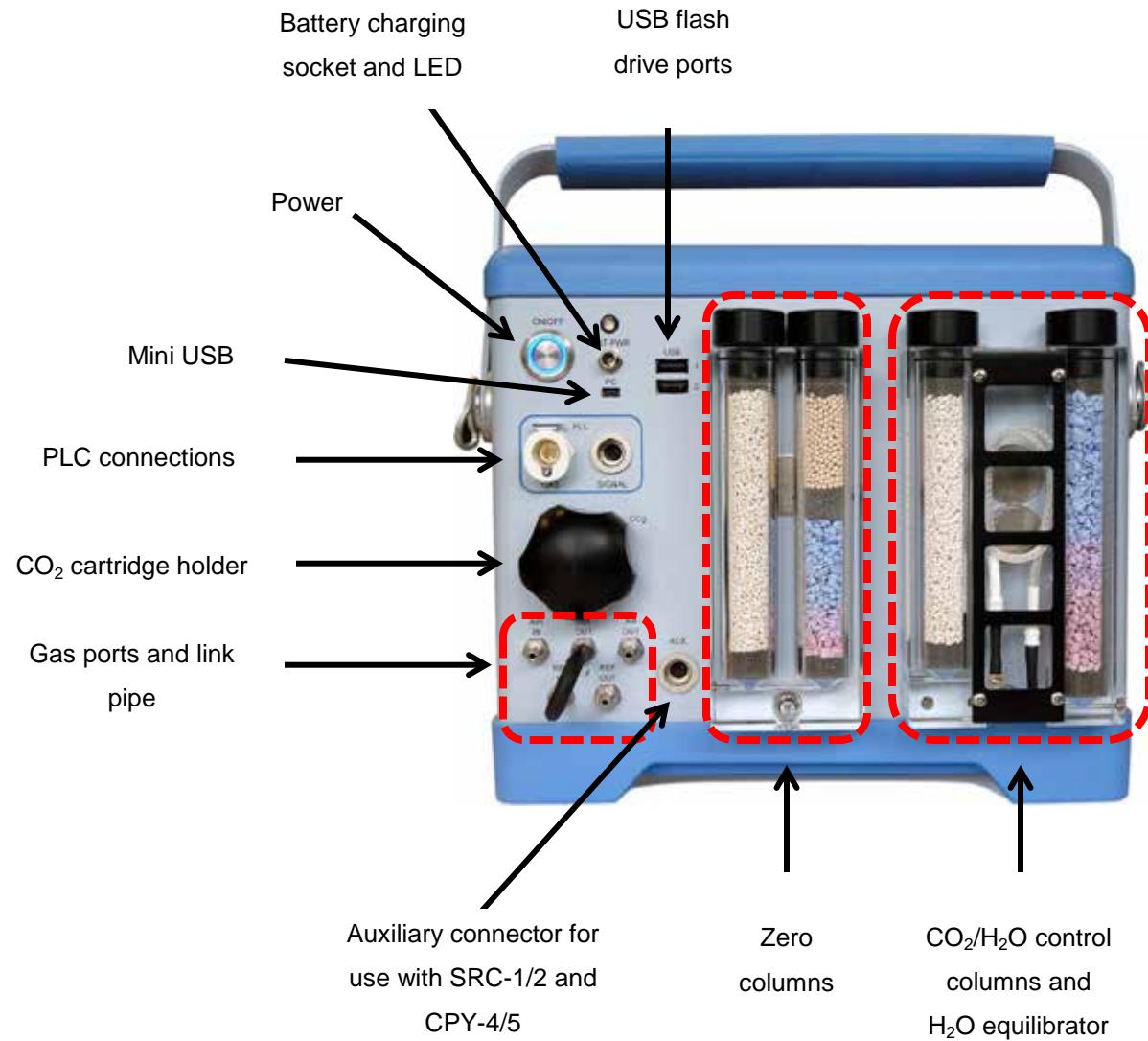
An AC power supply adapter (120/240 VAC / 50/60 Hz) is supplied with the CIRAS-3 for charging the internal battery packs. Each battery pack (Inspired Energy type) has a charge indicator gauge on the side to show the level of charge. When all 5 bars are dark it indicates that the pack is fully charged (see below). Please note that the CIRAS-3 also reports the state of the battery packs in the lower right hand corner of the display when the instrument is powered on.



See [Battery Pack \(Li-ion Battery\)](#) on page 205 for instructions on battery removal, storage and disposal.

CIRAS-3 Rear Console

The CIRAS-3 rear console contains all of the essential components including power, gas and electrical connections, USB ports, charger socket, desiccants (scrubbers) and CO₂ cartridge holder.



USB Flash Drive Ports

There are two USB flash drive ports labeled USB1 and USB2. These are used for downloading/uploading stored data files and for transfer of settings and response script files.

Battery Charging Socket (EXT PWR) and LED

The power supply adapter connects to the **EXT PWR** socket to charge the internal batteries and to power the system continuously. The LED should be steady green indicating secure connection and that the internal batteries are getting charged.

Power

The **ON/OFF** power button is a push-and-release type switch that will illuminate blue when the instrument is turned on.

Mini-USB (PC)

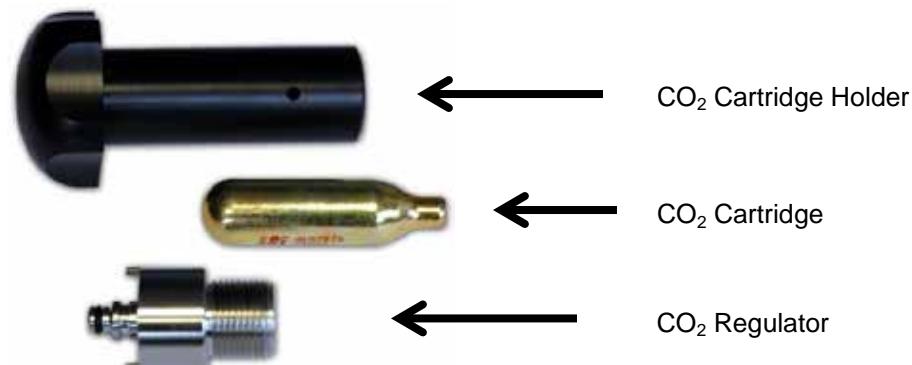
The Mini-PCB socket (**PC**) is a communication port for connection to a laptop or desktop computer and is commonly used with the PC Utility Software for CIRAS-3 remote operation and display. A suitable USB cable is included with the CIRAS-3. See [PC Utility Software](#) on page 195.

PLC Connections

The white plastic gas connector on the PLC3 connects to the port labeled PLC “**GAS**” and the 4 pin black electrical plug connects to the socket labeled PLC “**SIGNAL**”. Both the reference and analysis gas lines are built into this single pneumatic connector which is secured by a locking connector. Push firmly in until you hear a click and to release press down on the silver TAB at the top of the connector and pull the connector out. **DO NOT USE EXCESSIVE FORCE WHEN CONNECTING OR DISCONNECTING THE PNEUMATIC CONNECTOR AS IT MAY RESULT IN DAMAGE.** The black 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.

CO₂ Cartridge Holder

The CO₂ cartridge fits inside the cartridge holder which threads into the regulator body in the opening labeled **CO₂** on the CIRAS-3 console.



CO₂ Cartridges

We supply and recommend the CO₂ cartridges that are manufactured by a company called ISI. The cartridges are 8g and are normally supplied in boxes of 10 as shown here. A fresh CO₂ cartridge has a very high pressure when it is first introduced into the CIRAS-3 console. We do not recommend changing the cartridge for at least 24 hours from the time it is inserted due to this high pressure. Use precaution when changing the CO₂ cartridge. If you attempt to change a highly pressurized CO₂ cartridge you will observe a very rapid escape (and loud pop) of gas from the cartridge when you slowly unscrew the cartridge holder from the CIRAS-3. You will also observe that the cartridge will be quite cold.



Manufacturer Contact Information

ISI
Kürschnergasse 4
A-1217 Vienna, Austria
Tel: +43 1 25099 0 Fax: +43 1 25099 555
Email: info@isi.at URL: <https://www.isi.com/>

NOT ALL CO₂ 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 CO₂ cartridges you are urged to get in contact directly with PP Systems.

Gas Ports and Link Pipe

AIR IN is the entry port for ambient air or introduced air from an external gas cylinder (i.e. experimental air containing 2% oxygen). A short link pipe connects **AIR OUT** with **REF IN**. This is actually the reference air supply air for both the reference IRGAs and the leaf cuvette. For leaf gas exchange measurements using any of our PLC3 leaf cuvettes and for insect respiration measurements, this link pipe must be connected between the **REF IN** and **AIR OUT**. The **AN OUT** and **REF OUT** ports allow sampled air to exhaust to atmosphere.

Auxiliary Connector

The 4 pin **AUX** socket is reserved for use with the SRC-1/SRC-2 Soil Respiration Chambers and the CPY-4/CPY-5 Canopy Assimilation Chambers.

Please note that the SRC-1 Soil Respiration Chamber and CPY-4 Canopy Assimilation Chamber also require an additional piece of hardware called the Auxiliary Probe Adapter (APA) to work with CIRAS-3. Contact PP Systems for more details if you would like to use the SRC-1 and CPY-4 with CIRAS-3.

Zero Column

The Zero column contains 3 clearly marked desiccants labeled CO₂ Absorber (soda lime), H₂O Absorber (Drierite) and MS (Molecular Sieve) which are used for the analyzer Zero ensuring long term stability and accuracy of the CO₂ and H₂O gas analyzers. Therefore it is critical that these desiccants are maintained properly to ensure system accuracy. See [Desiccants and Absorber Columns](#) on page 211 for more information related to this column and management of desiccants. **To play it safe and for best results we recommend that you change out the Molecular Sieve desiccant on a daily basis prior to performing measurements to ensure accurate Auto Zero and long term accuracy and stability of the CO₂ and H₂O gas analyzers.**

CO₂/H₂O Control Columns and H₂O Equilibrator

The CO₂/H₂O control columns contain 2 clearly marked desiccants labeled CO₂ Absorber (soda lime) and H₂O Absorber (Drierite) which are used to control CO₂ and H₂O. See [Desiccants and Absorber Columns](#) on page 211 for more information related to this column and management of desiccants.

TIP

To play it safe and for best results we recommend that you change out the Molecular Sieve desiccant on a daily basis prior to performing measurements to ensure accurate Auto Zero and long term accuracy and stability of the CO₂ and H₂O gas analyzers.

See [Desiccants and Absorber Columns](#) on page 211. We also strongly recommend performing a leak test every time you change the desiccants. To do so insert a fresh CO₂ cartridge, connect the PLC3 Leaf Cuvette electronically and pneumatically and close the cuvette head. Go to **Controls (F3)** and set the CO₂ reference to 0. The CO2r and CO2a values should drop close to 0 (\pm 2 ppm) after a few minutes with a 0 CO2d (\pm 0.5 ppm). If any problems persist perform additional leak tests to try and isolate the source of the leak (See [Checking For Leaks Associated With the PLC3](#) on page 221).

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 mm 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 PLC3 is selected under **Settings (F2)**. See [Settings – Gas Exchange](#) on page 143 for measurement of gas exchange and [Application – Gas Exchange and Chlorophyll Fluorescence](#) on page 131 for measurements of gas exchange and chlorophyll fluorescence. 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 (T_{cuv}) 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 “Leaf Temperature” as the **Temperature Control Type** under **Settings (F2)** that you do so maintaining leaf temperature (T_{leaf}) 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 described in the following sections.

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 the IR sensor 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. The Energy Balance calculation estimates leaf temperature by equating energy flux into the leaf with energy flux out of the leaf. The model includes incident solar radiation, leaf re-radiation, convective heat transfer, and transpiration.

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:

- 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 the approximate ambient temperature for reference purposes only. Tamb may vary 1-3 °C from actual ambient depending on local conditions and orientation of the cuvette.

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.

Display 1

A (Assimilation)	Ci (Intercellular CO ₂)
E (Evaporation)	gs (Stomatal Conductance)

Display 2

CO2r (CO ₂ Reference)
H2Or (H ₂ O Reference)

Display 3

CO2d (CO ₂ Differential)
H2Od (H ₂ O Differential)



Press **R** to record a measurement if in record mode.

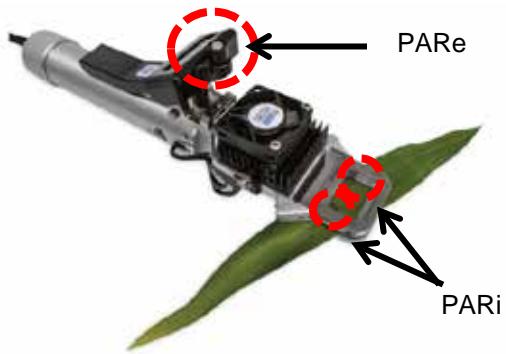
PAR Measurement

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

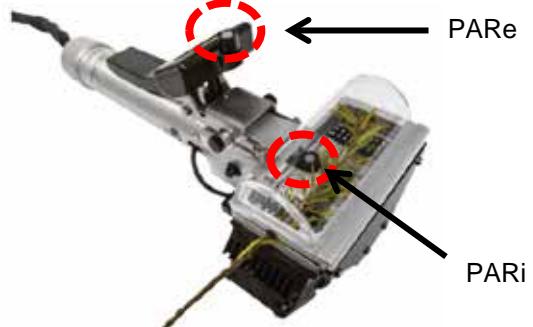
PLC3 Universal - Includes two mini-silicon photodiode sensors beneath the cuvette window (PARi). The silicon photodiode sensors are close to the leaf plane and used to average the irradiance beneath the cuvette window. Irradiance is somewhat attenuated (approx. 10% attenuation) by the window. This affects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

Connection of PLC3 Leaf Cuvettes to the CIRAS-3

All PLC3 leaf cuvettes connect electrically to the 4 pin PLC "SIGNAL" socket on the CIRAS-3 console via the black 4 pin plug. This connection has a notch at the top so make sure you line up the arrow on the top of the black plug to the top middle section of the "SIGNAL" socket on the console. A white pneumatic gas connector mates to the PLC "Gas" port on the CIRAS-3. It will snap into place. A link pipe between "REF IN" and "AIR OUT" must be in place as shown here when performing gas exchange measurements.



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% using 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 range will be 0-2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.



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.

Connection of Light Units and CFM-3 Chlorophyll Fluorescence Module to CIRAS-3

The PLC3 Universal LED light unit and CFM-3 Chlorophyll Fluorescence Module are designed to connect to the PLC3 Universal Leaf Cuvette exactly the same quickly and easily.

Step 1

First, mount the light unit by aligning it to the front of the cuvette upper jaw as shown below.



Step 2

Slide the light unit back towards the heat sink and fan on the leaf cuvette locking it in place using the two ball-head screws shown below.



Step 3

Connect electrically by gently pressing the connector in. Pay special attention to align the red dots on the plug and connector.



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 range will be 0-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (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 by gently pressing the connector in. Pay special attention to align the red dots on the plug and connector.



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 $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ depending on mixture of red, green, blue and white LEDs.

CFM-3 Chlorophyll Fluorescence Module

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. See [Connection of Light Units and CFM-3 Chlorophyll Fluorescence Module to CIRAS-3](#) on page 7576.

TIP

The CFM-3 can also be used as an actinic light source for gas exchange measurements if required.

SRC-2 Soil Respiration Chamber

Our SRC-2 Soil Respiration Chamber is available for use with the CIRAS-3 for measurement of soil CO₂ efflux (Closed System).

The SRC-2 Soil Respiration Chamber is constructed out of rugged PVC with a convenient handle for placement on the soil surface. An aluminum ring provides a good seal on the soil surface or on collars.

- Dimensions: 150 mm (Height) x 100 mm (Diameter)
- Volume: 1171 ml
- Area: 78 cm²
- Cable Length: 1.5 meters

It includes a temperature sensor for measurement of air temperature near the soil surface. Optional soil collars are available from PP Systems.



Temperature Sensor (Precision Thermistor)

- Range: 0-50 °C
- Accuracy: ± 0.3 °C at 25 °C

CPY-5 Canopy Assimilation Chamber

Our CPY-5 Canopy Assimilation Chamber is available for use with the CIRAS-3 for measurement of net canopy CO₂ flux (Closed System). It connects electrically direct to the 4 pin "AUX" socket on the CIRAS-3 console via the black 4 pin plug.



The CPY-5 Canopy Assimilation Chamber is constructed out of rugged polycarbonate. An aluminum ring provides a good seal on the soil surface or on collars. It also includes sensors for air temperature and PAR (Photosynthetically Active Radiation) inside the chamber near the soil surface. Optional collars are available from PP Systems.

- Dimensions: 145 mm (Height) x 146 mm (Diameter)
- Area: 167 cm²
- Cable Length: 1.5 meters

Temperature Sensor (Precision Thermistor)

- Range: 0-50 °C
- Accuracy: ± 0.3 °C at 25 °C

PAR Sensor

- Fully cosine corrected
- Range: 0-3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$
- Accuracy: $\pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$

We recommend recalibration of the PAR sensor every 2 years. The temperature sensor should not require recalibration.

Connection of Closed System Chambers to the CIRAS-3

Both the SRC-2 Soil Respiration Chamber and CPY-5 Canopy Assimilation Chamber connect electrically to the 4 pin “AUX” socket on the CIRAS-3 console via the black 4 pin plug. This connection has a notch at the top so make sure you line up the arrow on the top of the black plug to the top middle section of the “AUX” socket on the console. The link pipe between “REF IN” and “AIR OUT” must be removed to allow connection to the “REF IN” port for both the SRC-2 and CPY-5. Connect the “Gas In” line to the “REF IN” and “Gas Out” to the “REF OUT” gas ports on the CIRAS-3 console.



The CPY-5 Canopy Assimilation Chamber is also supplied with a water vapor equilibrator which we recommend for use in high humidity environments. The water vapor equilibrator should be plumbed on the “REF IN” line as shown here. When in use, the equilibrator should be positioned in a way to allow air to flow through the venting to allow the moisture to equilibrate with the ambient. Do not let it sit flat on the ground.

Insect Respiration Chamber

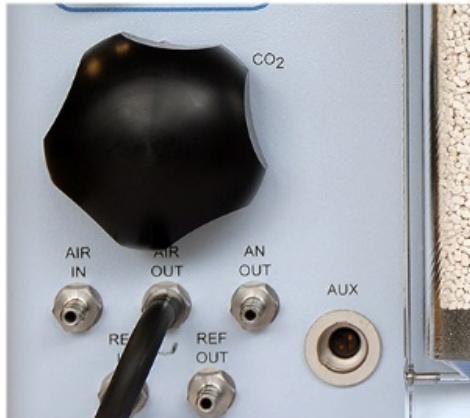
A chamber is available for use with the CIRAS-3 for measurement of insect respiration. The chamber is basically one of our standard absorber columns normally used with desiccants for scrubbing CO₂ (soda lime) or H₂O (Drierite) with some of our CO₂ gas analyzer products. It works perfectly for measurement of insect respiration by simply placing an insect(s) inside the chamber. The black end caps can be removed by simply pulling on it from either end making it very easy to introduce insects into the chamber.



- Chamber Volume: 33 cm³ (Not including gas tubing)
- Chamber Dimensions: 15.1 cm (L) x 25 cm (Diameter)
- Chamber Weight: 65 g

Connection of the Insect Respiration Chamber to the CIRAS-3

There are no electrical connections required. Connect the white pneumatic connector to the PLC "Gas" port on the CIRAS-3. It will snap into place. Connect the short gas tubing labeled "A" to one end of the chamber and the longer length tubing on the "Y" connector to the other end of the chamber. The small length of tubing on the "Y" connector is used as a vent to atmosphere. A link pipe between "REF IN" and "AIR OUT" must be in place as shown here when performing gas exchange measurements.



A link pipe between "REF IN" and "AIR OUT" must be in place as shown here when performing insect respiration measurements

Section 6. Navigation and General Overview

System Navigation

The CIRAS-3 console software is very user-friendly and incredibly intuitive with context-sensitive help available throughout the software. 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 sub 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 make your selections.

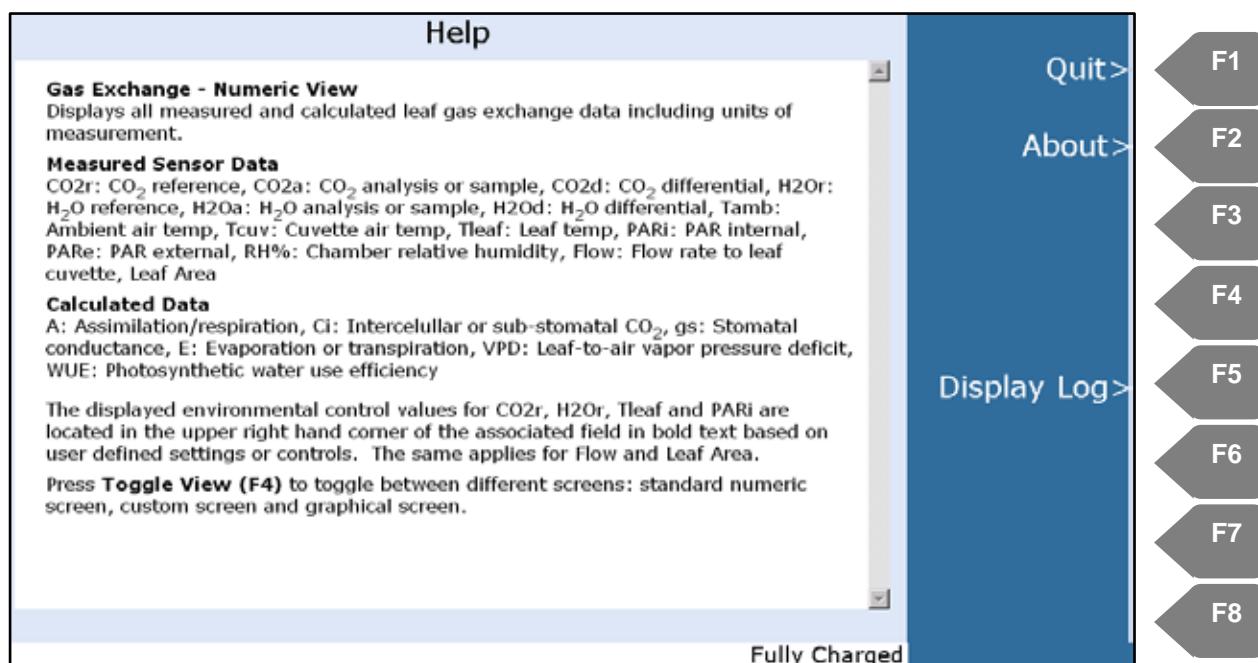


Navigation	
Function Keys (F1-F8)	There are 8 function keys located on the main keyboard labeled F1-F8. Function keys form the top level for menu selections. Function keys are context-sensitive, so as you go deeper into a menu their assigned functions may change.
TAB	The TAB key is typically used to navigate from field to field within a dialog. Once in a field you can use the keypad to enter numeric values or delete characters. Also, you can use the Expand List (F4) key to expand the list of available selections within a field if applicable.
DEL	Allows you to delete characters in a field or to simply backspace in the field.
OK	Confirms your selection from a dropdown list or from within a window that may appear from time to time.
ESC	Closes down the dropdown list within fields.
Arrow Keys	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 (F2)** when you are ready to accept any changes to dialogs or **Cancel (F1)** to reject changes. After accepting changes a message will be displayed in the lower left hand corner of the display confirming the changes.

System Help

System help is available from any screen and is context-sensitive to the functions being displayed or performed. Press **Help (F8)** whenever you have a question or concern about the information that is being displayed at any given point in time. For example, if you have the Settings dialog displayed press **Help (F8)** for help related to this dialog. Within the Help screen press **Page Up (F3)** or **Page Down (F4)** to read more content if applicable. 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 (F5)** to return to the Help dialog.



Main Data Viewing Displays

All five CIRAS-3 “Applications” include at least one numeric and one graph display to view measured and calculated data. For several “Applications” including **Chlorophyll Fluorescence**, **Gas Exchange** and **Gas Exchange and Chlorophyll Fluorescence** we offer one additional custom display which allows users to determine only the parameters that they want displayed. All measured and calculated data displayed on the screens are based on both the “Application” and “Configuration” selections under **Settings (F2)**. Users can toggle from display to display by pressing **Toggle View (F4)**.

Numeric Display (Gas Exchange)

Gas Exchange					Operations> Settings> Controls> Toggle View> Record> Zero/Diff Bal> Help>
CO2r <small>390.0</small>	CO2a	CO2d	A	Ci	
390.2 <small>μmol mol⁻¹</small>	390.4 <small>μmol mol⁻¹</small>	0.2 <small>μmol mol⁻¹</small>	-0.1 <small>μmol m⁻² s⁻¹</small>	4861 <small>μmol mol⁻¹</small>	
H2Or <small>80.0%</small>	H2Oa	H2Od	gs	E	
5.60 <small>mb</small>	5.60 <small>mb</small>	0.00 <small>mb</small>	0 <small>mmol m⁻² s⁻¹</small>	0.00 <small>mmol m⁻² s⁻¹</small>	
Tamb	Tcuv	Tleaf <small>amb</small>	VPD	WUE	
25.5 <small>°C</small>	21.4 <small>°C</small>	25.4 <small>°C</small>	2.7 <small>kPa</small>	0.0 <small>A/E</small>	
PARi <small>500</small>	PARe	RH%	Flow <small>300</small>	Leaf Area <small>4.50</small>	
501 <small>μmol m⁻² s⁻¹</small>	21 <small>μmol m⁻² s⁻¹</small>	22.0 <small>%</small>	299 <small>cc min⁻¹</small>	4.50 <small>cm²</small>	
Fully Charged					

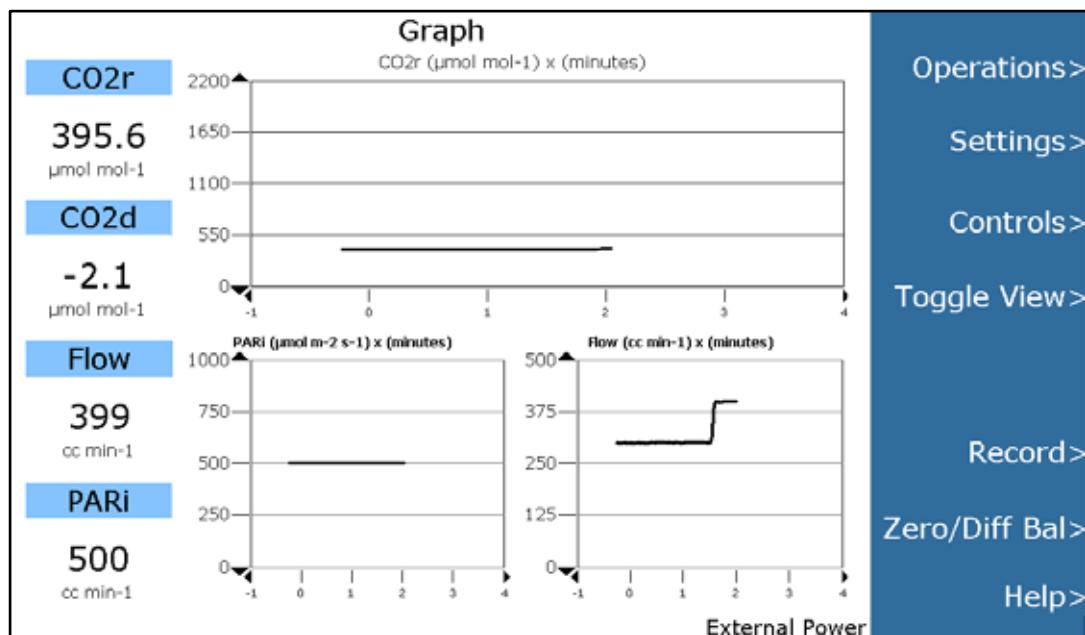
You will see that parameter fields have either a light blue header or dark blue header. Generally speaking, any fields with a light blue header are “measured” parameters and fields with dark blue headers are “calculated” parameters.

Numeric Display (Chlorophyll Fluorescence)

Chlorophyll Fluorescence					
F	Fs	Fo	Fv	Fm	Operations>
687	673	618	1392	2010	Settings>
Fv/Fm	Fo'	Fv'	Fm'	Fv'/Fm'	Controls>
0.693	414	360	774	0.465	Toggle View>
ΦPSII	j	qP	qNP	NPQ	End Record>
0.130	65.520	0.281	0.775	1.597	Record>
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	Zero/Diff Bal>
0.173	0.335	0.535	0.335	0.291	Help>
Fully Charged					

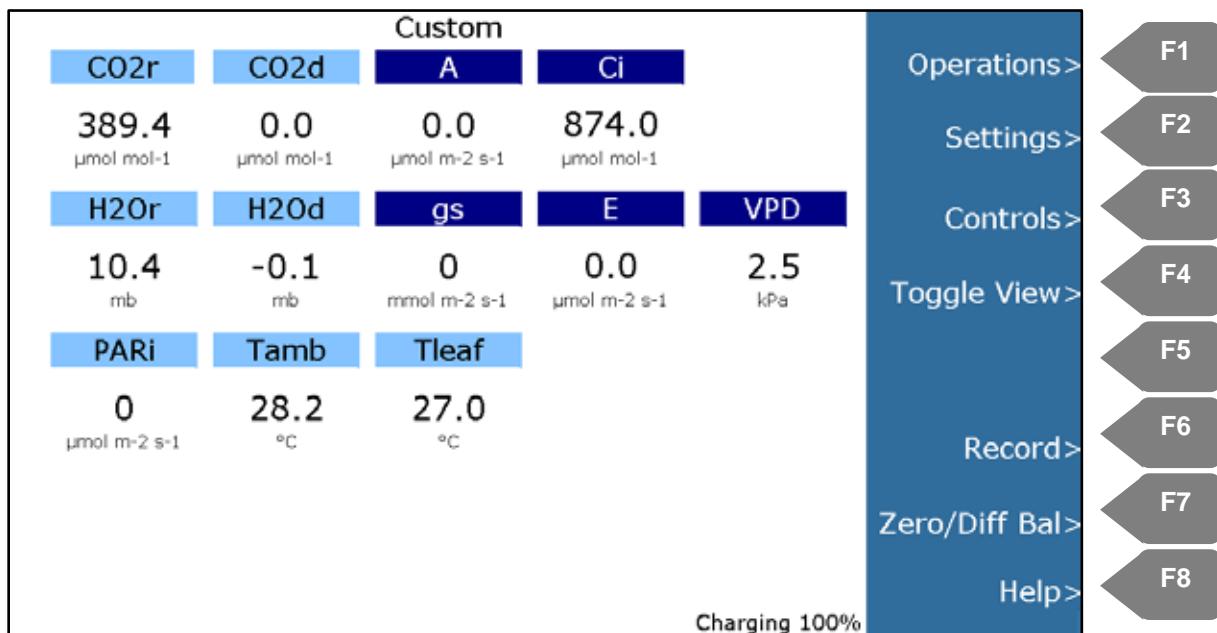
F1
F2
F3
F4
F5
F6
F7
F8

Graph Display



Operations>
Settings>
Controls>
Toggle View>
Record>
Zero/Diff Bal>
Help>

Custom Display



Section 7. Operations (F1)

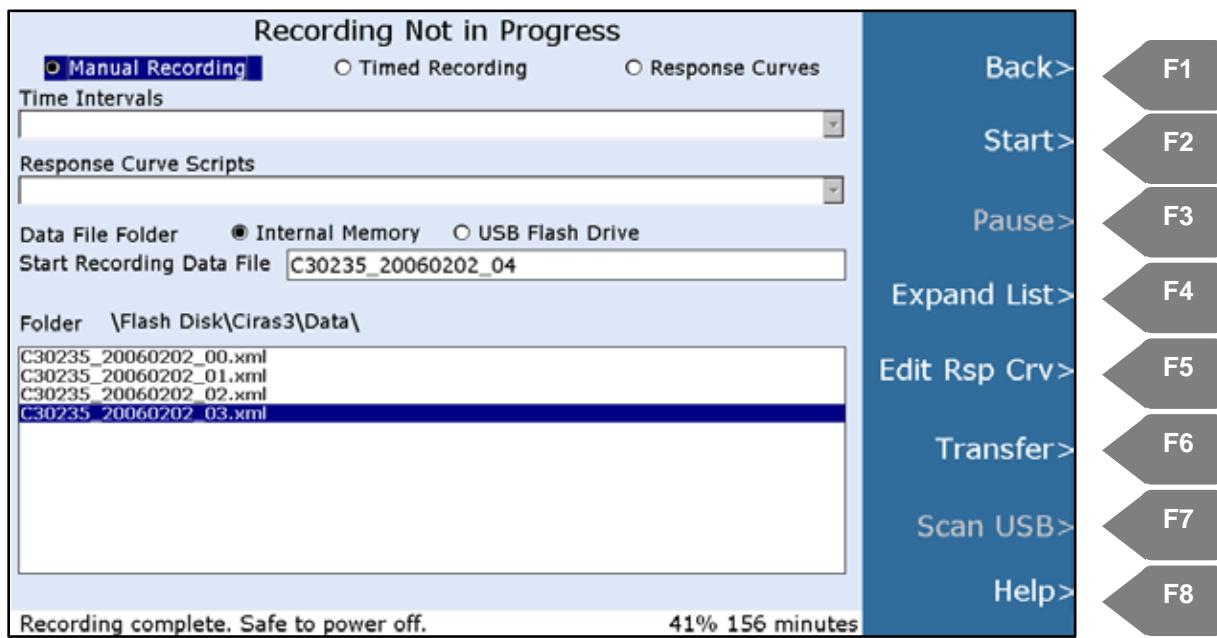
From any numeric, graph or custom display press **Operations (F1)** to access numerous system functions including:

- 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)

Rec Options (F2)

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

- **Manual Recording** – Normally used for individual leaf level gas exchange measurements.
- **Timed Recording** – For performing measurements at user-defined time intervals.
- **Response Curves** – For automated, pre-programmed scripts for A/C_i curves, light response, etc.



Manual Recording

Select the desired recording option at the top of the dialog by using the right and left arrow keys.

Highlight “Manual Recording” and press TAB to select it and move to “Data File Folder”. For most leaf gas exchange measurements in the field this method of recording is most common.

Data Storage and Filenames

Data File Folder	Data can be saved to Internal Memory or directly to a USB Flash Drive. Highlight the desired storage location and press TAB to select it.
Start Recording Data File	A default data file name will appear here. Note that the default data file always starts with C3XXXX_YYYYMMDD_00 where: <ul style="list-style-type: none">· C3XXXX – The serial number of your CIRAS-3 console· YYYYMMDD – Year/Month/Day· 00 – All data files start at 00 and count up from there (i.e. 01, 02, 03, etc.) unless changed by the user. You can change the filename if required using the DEL key to backspace and delete characters and using the keyboard enter your own numeric filename. You cannot overwrite an existing data file. Later, you can export the file and rename it using alpha characters if required.
FOLDER: \Flash Disk\Ciras3\Data\	Indicates the Internal Memory location where data files are stored and the box beneath it lists all currently stored data files.
Folder: \USB Storage\Ciras3\Data\	Indicates the USB Flash Drive location where data files are stored and the box beneath it lists all currently stored data files on the USB Flash Drive. If a USB Flash Drive is not in place in one of the two available USB ports on the CIRAS-3 rear panel the message “USB Flash Drive Not Detected” will appear in this box.

Press **Start (F2)** to begin recording data – you are automatically returned to the Operations dialog. The status bar indicates that you are now in Record mode. Press **Back (F1)** to return to the Numeric Display (or the last display you were in prior to initiating a recording session).

Measurements can be initiated and recorded by pressing **Record (F6)** on the CIRAS-3 console. If using one of our PLC3 Leaf Cuvettes you can also press the **R** key on the cuvette’s open/close lever to record data. A message “Recording” will appear on the small PLC display indicating the data was recorded.

When a measurement is recorded a message similar to the following will be displayed in the status bar in the lower left hand corner of the display:

Record 1 Saved to File: C30235_20180115_00.xml

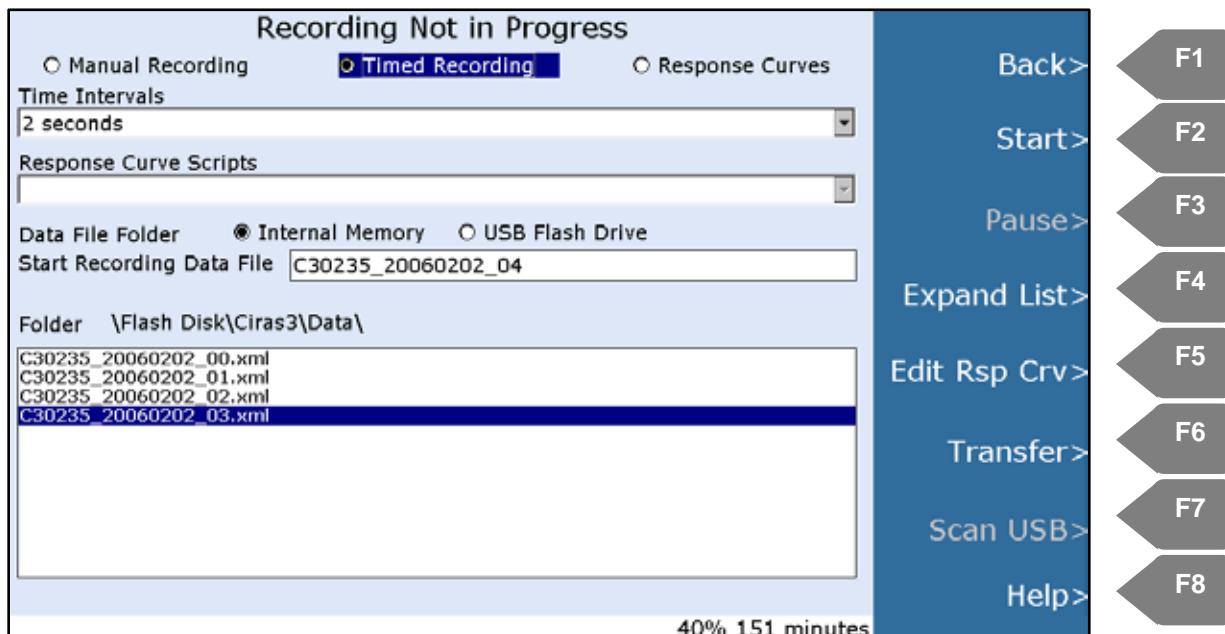
If viewing the graph display a red triangle will also appear in the graph each time a measurement is recorded. To terminate a measurement session press **End Record (F5)**.

TIP

Under **Settings (F2)**, if the Application selected is “Chlorophyll Fluorescence” or “Gas Exchange and Chlorophyll Fluorescence” recording begins by pressing **Start (F2) > Back (F1) > Record (F6)** or R on the PLC3 Universal Leaf Cuvette to initiate a measurement sequence. If you forget to press **Record (F6)** or R the system will not begin a measurement sequence. Also, “Manual Recording” is the only available recording option for the Application “Chlorophyll Fluorescence”.

Timed Recording

Using the right and left arrow keys highlight “Timed Recording” and press TAB to select it and move to “Time Intervals”. 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. Select your recording interval and press TAB to select it and move to “Data File Folder”.



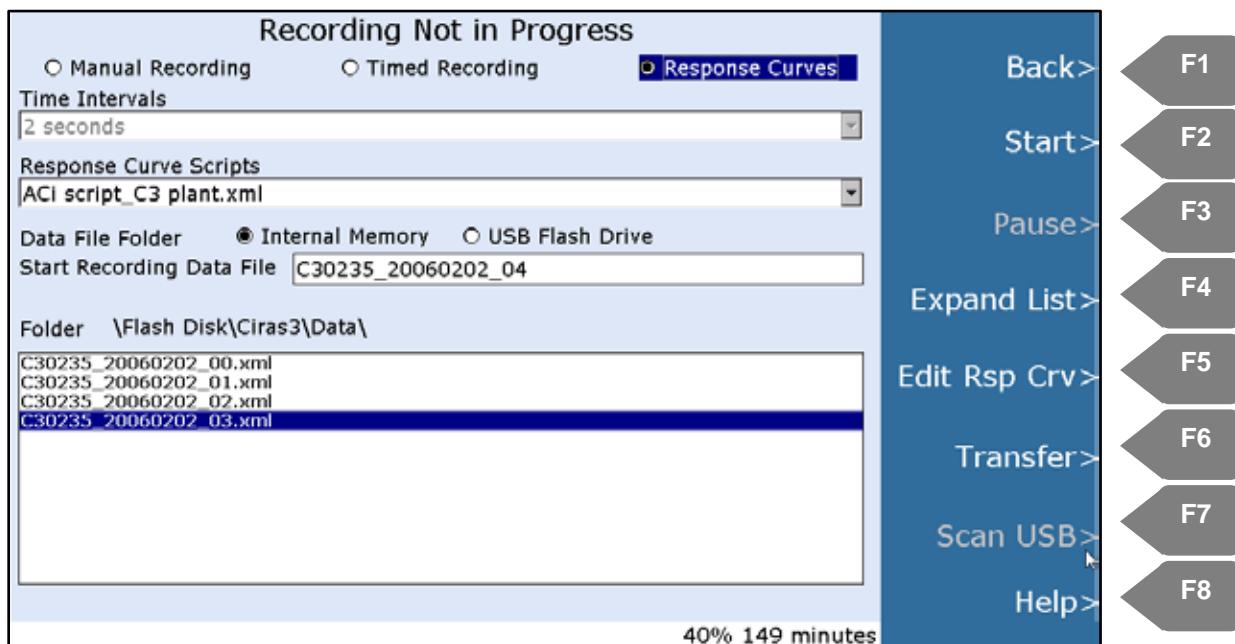
Recording, data storage and filenames are treated exactly the same as described in [Data Storage and Filenames](#) on page 87. When measurements are under way the status bar in the lower left hand corner of the display will show the countdown to the next record:

Next record in 00.14

Timed Recording will continue indefinitely at the selected recording interval unless you press **End Record (F5)** to terminate a recording session or if you pause a recording session. To pause a recording session press **Operations (F1) > Rec Options (F2) > Pause (F3)**. To resume a recording session press **Resume (F3)** to continue Timed Recording.

Response Curves

Using the right and left arrow keys highlight “Response Curves” and press TAB to select it and move to “Response Curve Scripts”. Press **Expand List (F4)** to view and select available scripts stored on the CIRAS-3 console using the up and down arrow keys. After selecting the required script press the TAB key to advance to the Data File Folder. The CIRAS-3 is supplied with several simple default scripts stored on the console which can be executed, modified or overwritten.



Recording, data storage and filenames are treated exactly the same as described in [Data Storage and Filenames](#) on page 87.

TIP

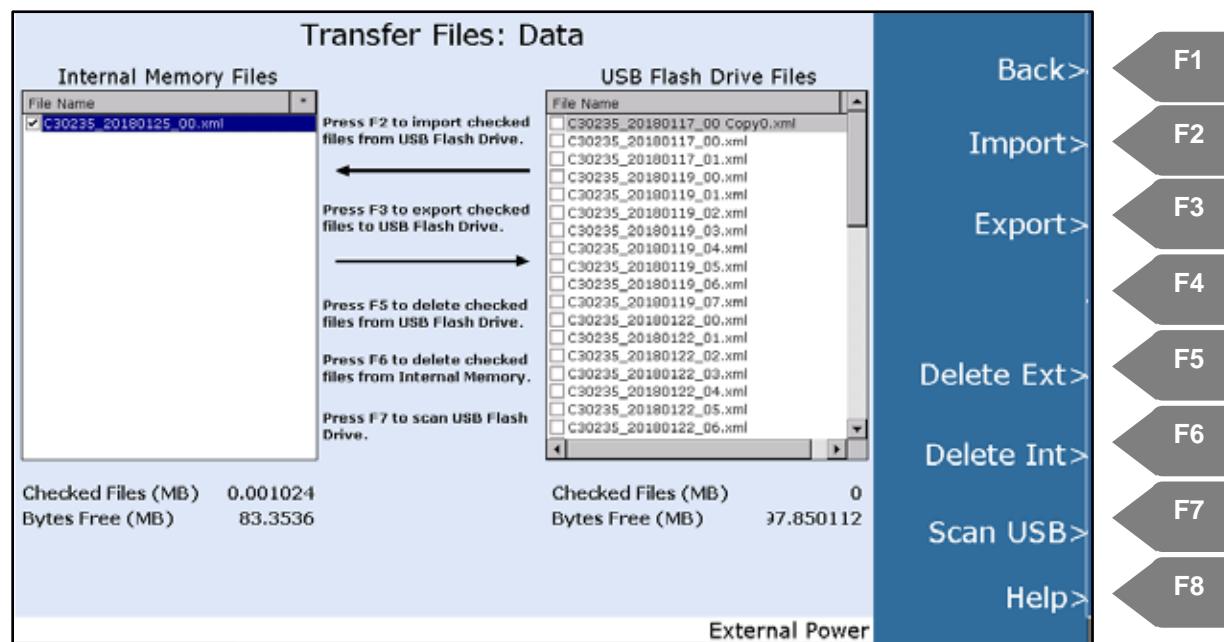
When performing automatic response curves involving chlorophyll fluorescence measurements, all settings (**Settings (F2) > CFM-3 Settings (F7)**) must be set up and saved prior to starting a measurement sequence in order to take effect.

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. See [Response Curve Scripts – Creation and Execution](#) on page 92.

Data Management

Transfer Data (F6)

Data management is made simple and easy with the CIRAS-3. Press **Operations (F1) > Rec Options (F2) and Transfer (F6)**. Any stored data files will appear under “Internal Data Files” or “USB Flash Drive Files” (if a USB Flash Drive is in place in one of the two available USB ports on the CIRAS-3 console. If there are no data files the fields will be blank). Data files can be stored to internal memory or directly to an external USB Flash Drive. Use the TAB key to move back and forth between the two file locations. Within any field use the up and down arrow keys to highlight files and once highlighted press **OK** to select it. A check mark will appear next to the file indicating that you have selected it for transfer.



Data files can be managed easily by using the following Function keys.

Function Keys	
F2	Press F2 to import checked USB data files to Internal Memory.
F3	Press F3 to export checked internal data files to the external USB Flash Drive.
F5	Press F5 to delete USB Flash Drive files from memory.
F6	Press F6 to delete checked internal files from memory.
F7	Press F7 to scan the USB Flash Drive for data files.

To transfer CIRAS-3 settings files press **Settings (F2) > Transfer (F3)** and follow the instructions above.

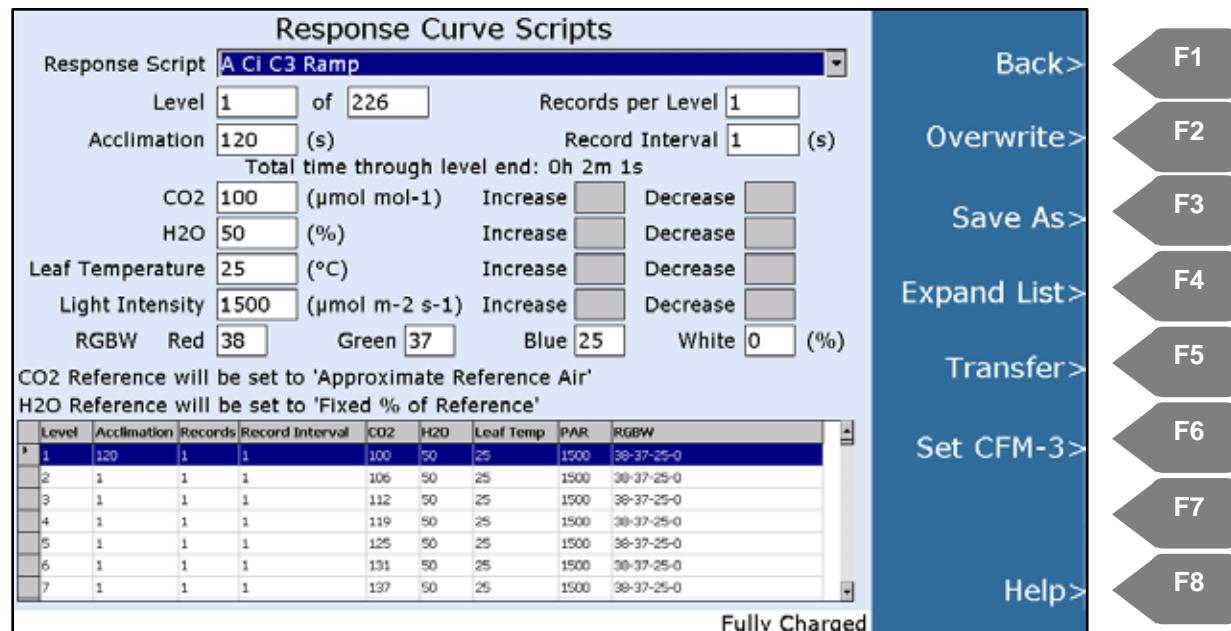
To transfer CIRAS-3 response curve scripts, press **Operations (F1) > Rec Options (F2) > Edit Rsp Crv (F5) > Transfer (F5)** and follow the instructions above.

Response Curve Scripts – Creation and Execution

Response scripts can be created or overwritten on the CIRAS-3 console or by using our PC Utility software. It is much easier to navigate and create/modify scripts on your PC so we strongly recommend using the PC Utility software for this function.

CIRAS-3 Console – Response Curves

Press **Operation (F1) > Rec Options (F2)** and highlight **Response Curves** and press TAB to drop down to “Response Curve Scripts”. Press **Expand List (F4)** to view response scripts that are already loaded on the console. If no changes are required scripts can be executed immediately by pressing **Start (F2)** and **Back (F1)** to return to the numeric display. If you would like to edit or create new response scripts press **Edit Rsp Crv (F5)** to display the **Response Curve Scripts** editor display. Press **Expand List (F4)** to show all available scripts and using the up and down arrows navigate to the script that you want to edit and when highlighted press **OK**.



Response Curve Scripts Editor	
Ramp	This feature is available using the PC Utility program supplied with the system. Refer to the Application Note available from PP Systems for setup and operational instructions featuring our high-speed CO ₂ ramping for rapid measurement of A/C _i .
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.

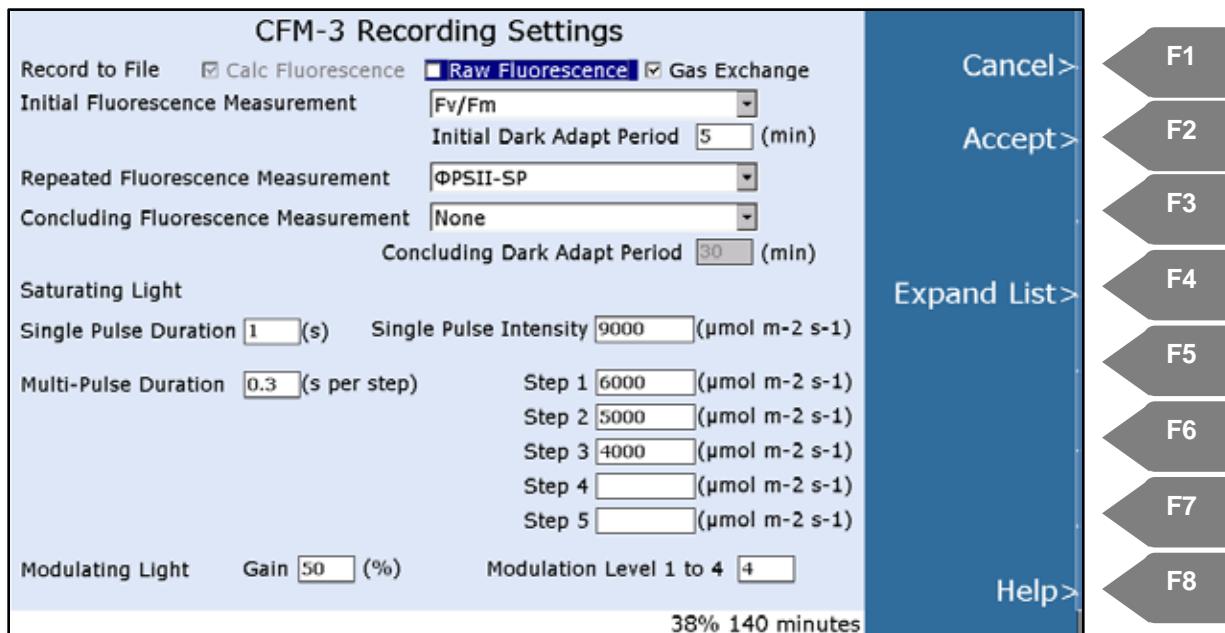
Response Curve Scripts Editor (Continued)

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 and 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 prior to taking your first data point. This is not as easy as it may seem as it 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 if you are taking more than just one data point at each level (Records per Level n>1).
CO₂	Select your desired (starting) CO ₂ concentration. Remember that this example describes an A/C _i 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 CO ₂ 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 ₂ O control value as a percent of the reference air, held constant throughout the A/C _i curve.
Leaf Temperature	Select your desired temperature control value, held constant throughout the A/C _i 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/C _i 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 **Set CFM-3 (F6)** to access settings associated with chlorophyll fluorescence measurements (only applicable if you are using the CFM-3 Chlorophyll Fluorescence Module). Also make sure that you have proper selections under [Settings – Gas Exchange and Chlorophyll Fluorescence](#) on page 131.



See [CFM-3 Recording Settings](#) on page 137 for information related to options and settings.

For reference purposes also refer to [CFM-3 Controls](#) on page 141 to see how to change modulation gain and modulation level dynamically if required to do so during the course of measurements.

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

Default Response Curve Scripts

The CIRAS-3 is supplied with 5 built-in response curve scripts by default as follows:

Response Curve Scripts - Defaults	
A Ci C3 Ramp	A typical ramp for performing rapid measurement of A/C _i on C3 plants (Non-steady state).
A Ci C3 Script	A typical CO ₂ response curve on C3 plants (Steady state).
A Ci C4 Ramp	A typical ramp for performing rapid measurement of A/C _i on C4 plants (Non-steady state).
A Ci C4 Script	A typical CO ₂ response curve on C4 plants (Steady state).
Light Response Curve Script	A typical light response curve.

To access these built-in scripts go to **Operations (F1) > Rec Options (F2) > Edit Rsp Crv (F5)**. Users have the ability to execute the default scripts as presented or they can be modified and edited to meet the requirements of your experiment. Scripts can also be created from scratch if required.

TIP

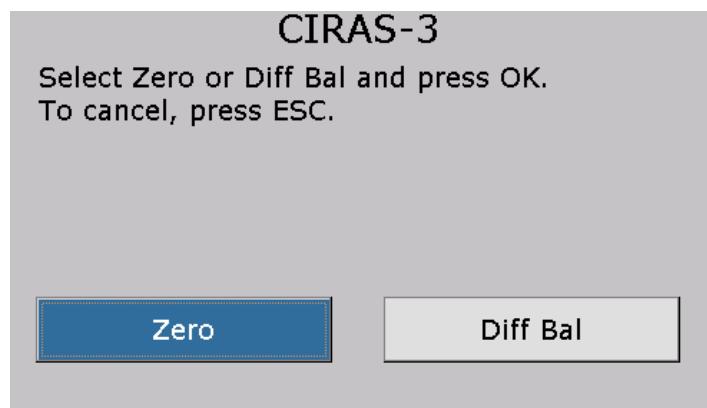
We strongly recommend that prior to running a response script that varies CO₂ concentration, go to **Settings (F2)** and set Zero, Diff Bal Mode to “Auto Zero, Stored Diff Bal”. Refer to [Settings – Gas Exchange](#) on page 143 and [Store Diff Bal](#) on page 103. This will allow you to execute faster response curves without Diff Bal interruptions between levels. Please note that the system will still automatically perform an Auto Zero every 30 minutes throughout the response. To minimize interruptions during a response curve (especially when performing high-speed CO₂ ramping for rapid measurement of A/C_i) we recommend performing a Manual Zero prior to the start of each measurement and in between subsequent measurements to avoid Auto Zero interruptions.

PC Utility Software – Response Curves

See [Create/Edit Response Scripts](#) on page 197 for more information related to creating and editing response curves using the PC Utility Software.

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 and OK to perform the task. 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. A Diff Bal cycle will also take place when there is a large change in CO₂ (100 $\mu\text{mol mol}^{-1}$) or H₂O (4 mb) concentration. Diff Bal will help ensure that detectable Analysis (sample) 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 <1.0-2.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

TIP

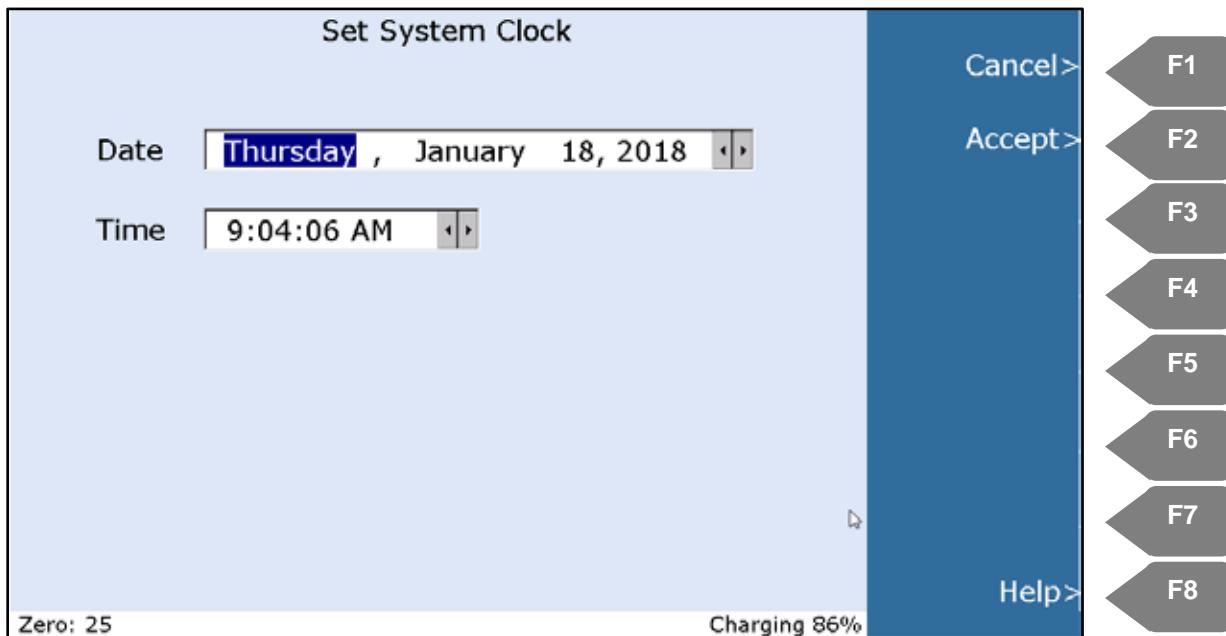
Prior to measurements on small leaf area or whenever you expect very low rates of photosynthesis and when CO₂d is showing a small differential ($\pm 1-2 \mu\text{mol mol}^{-1}$), perform a manual **Diff Bal (F7)** to get closer to a 0 CO₂d. Typical sources for differentials in CO₂ and H₂O are:

- Instrument not at stable operating temperature
- Leak around leaf chamber gaskets
- Exhausted chemicals (particularly the molecular sieve)
- Fluctuating CO₂r

Multiple Diff Bals may be required to get closer to 0.0 $\mu\text{mol mol}^{-1}$ for CO₂d.

Set Clock (F3)

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 set on the CIRAS-3 as the default data files use this information as part of the file name. See [Manual Recording](#) on page 87.

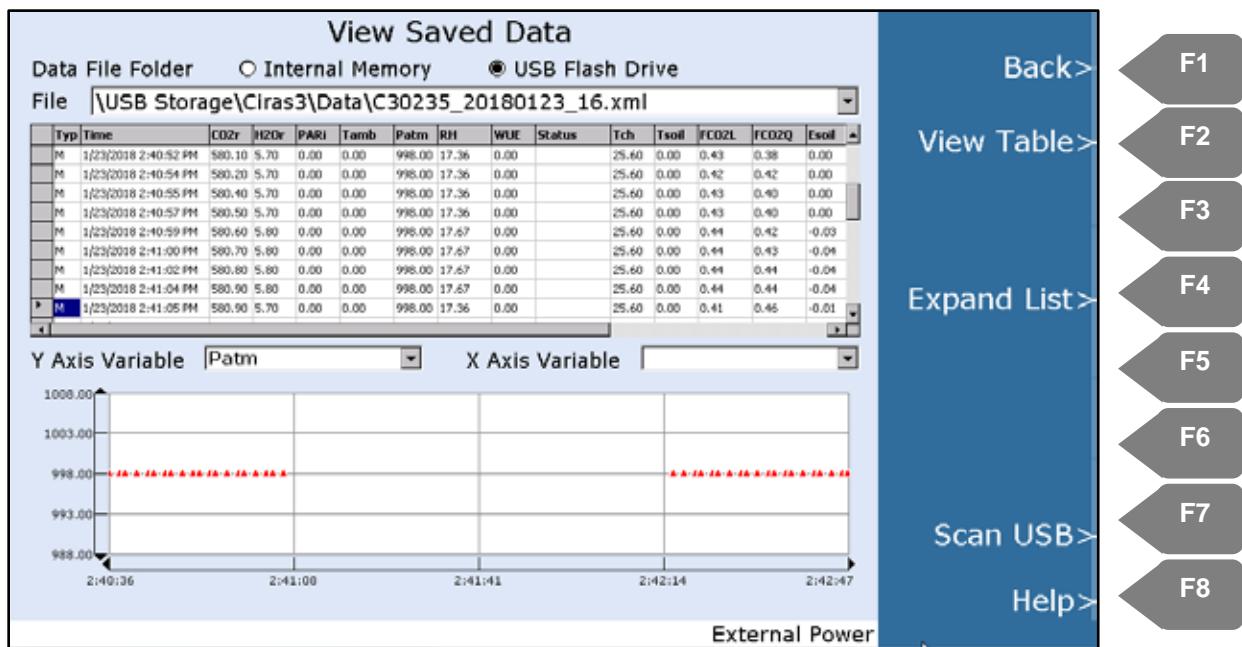
View Saved (F4)

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. 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 Flash Drive. 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 it 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.

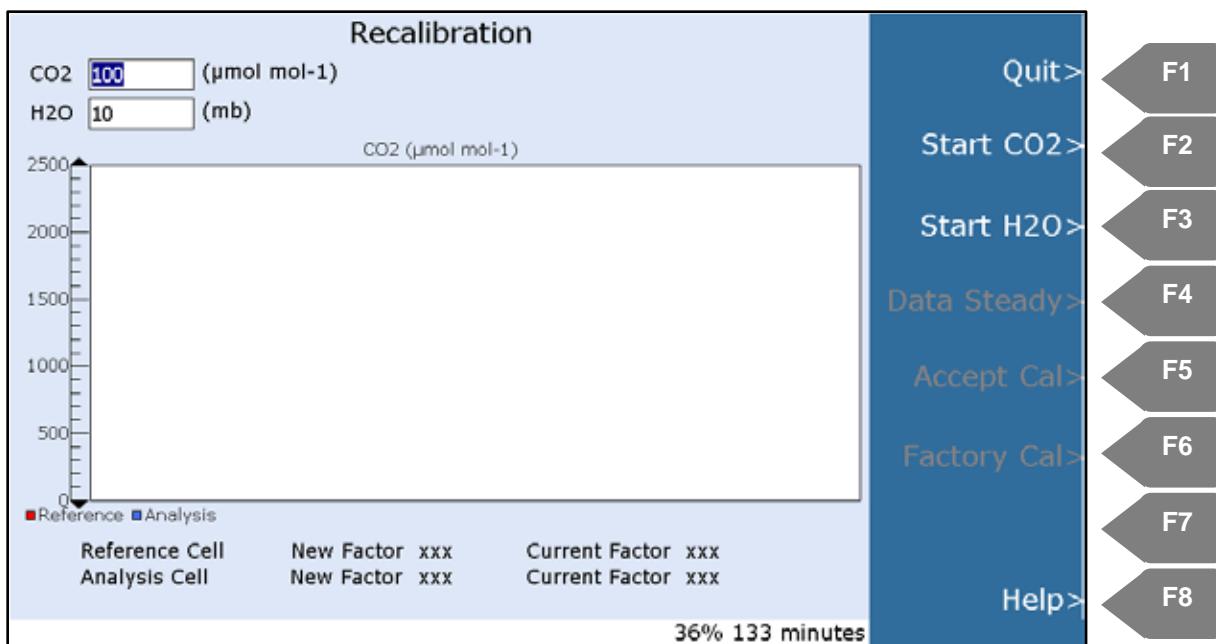


Calibration (F5)

Press **Operations (F1) > Calibration (F5)**. This is where users can calibrate the CIRAS-3 CO₂ and H₂O infrared gas analyzers, PAR sensors on the PLC3 and the LED light unit, as well as perform a stored Diff Bal and Find Max C.

Recalibrate

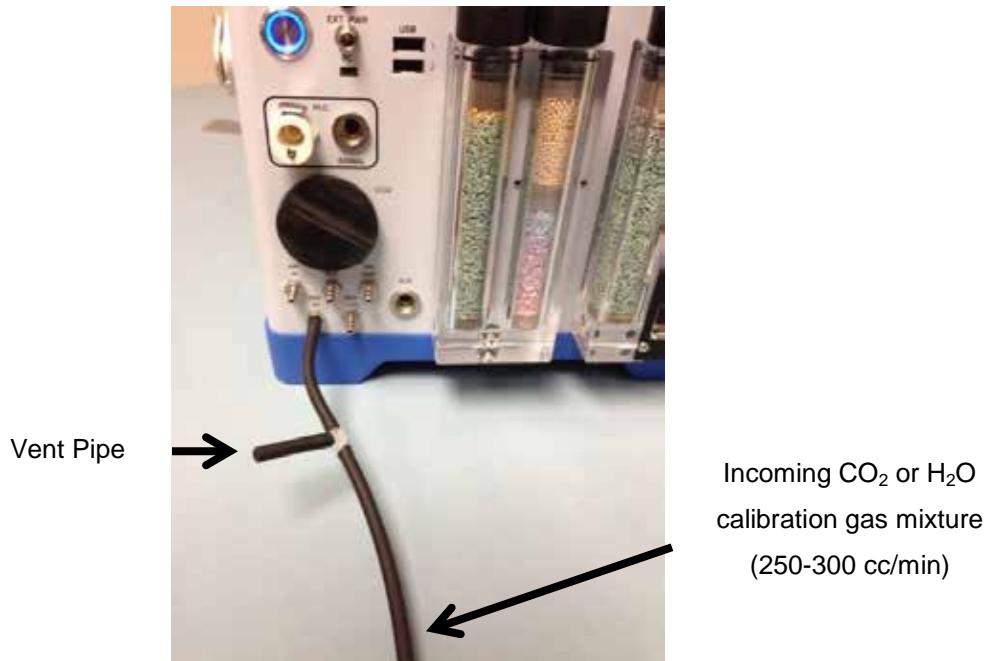
To recalibrate the CO₂ and H₂O gas analyzers, press **Recalibrate (F2)**. Also, the link pipe must be removed from the **REF IN** gas port on the CIRAS-3 console to allow the CO₂ and H₂O gas to pass through the reference cell for calibration purposes.



TIP

All CIRAS-3 systems receive a thorough factory calibration before it is shipped and it features our innovative “Auto-Zero” facility. What does this mean? You should never have to worry about recalibration unless damage has occurred or if you simply want to check the calibration. The “Auto-Zero” ensures that the system maintains IRGA calibration and long term stability for many, many years. It is important that you properly maintain the desiccants to ensure that they are fresh in order for the CIRAS-3 to perform Auto-Zero. Simple, periodic checks of all CO₂ and H₂O gas analyzers calibration are recommended.

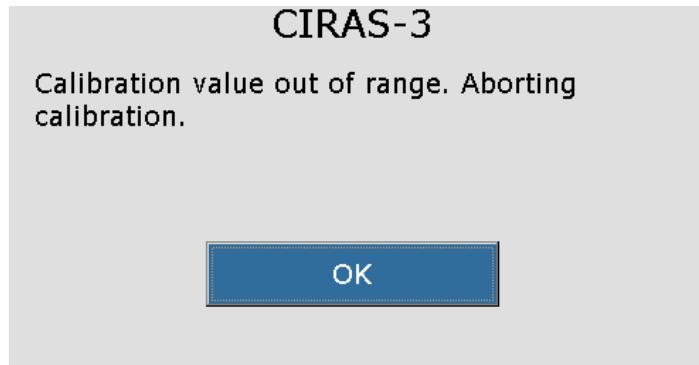
Calibration Setup



CO₂ Calibration

For CO₂ recalibration we recommend a certified, accurate gas mixture (< 1.0% accuracy) of compressed gas containing CO₂ in air (not CO₂ in nitrogen). Generally speaking, it is recommended that your calibration gas mixture contains a CO₂ concentration slightly above your normal measurement range. For instance, if most of your measurements are made near CO₂ levels in the range of 390-450 $\mu\text{mol mol}^{-1}$, a calibration mixture of 500 $\mu\text{mol mol}^{-1}$ would be recommended.

1. Ensure that all chemicals are fresh.
2. Connect the CO₂ 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 the CIRAS-3 to avoid overpressure.
3. Enter the CO₂ gas concentration of your calibration mixture in the CO2 box and press **Start CO2 (F2)**.
4. Monitor the CO2r and CO2a values and when steady, press **Data Steady (F4)**. If the new scaling factors are outside the expected range, the following message will appear:



Otherwise if all goes well new scaling factors for both the Reference and Analysis CO₂ 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, the message “CIRAS-3 Reject new calibration” will appear. Press **OK**.

You always have the option to reset the CO₂ IRGAs back to factory calibration by selecting **Factory Cal (F6)**.

H₂O Calibration

For H₂O recalibration we recommend using an accurate humidity generator or water vapor generator.

1. Ensure that all chemicals are fresh.
2. Connect the H₂O 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 H₂O gas concentration of your calibration mixture in the H₂O box and press **Start H₂O (F3)**.
4. Monitor the H₂Or and H₂Oa values and when steady, press **Data Steady (F4)**. New scaling factors for both the Reference and Analysis H₂O 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 H₂O IRGAs back to factory calibration by selecting **Factory Cal (F6)**.

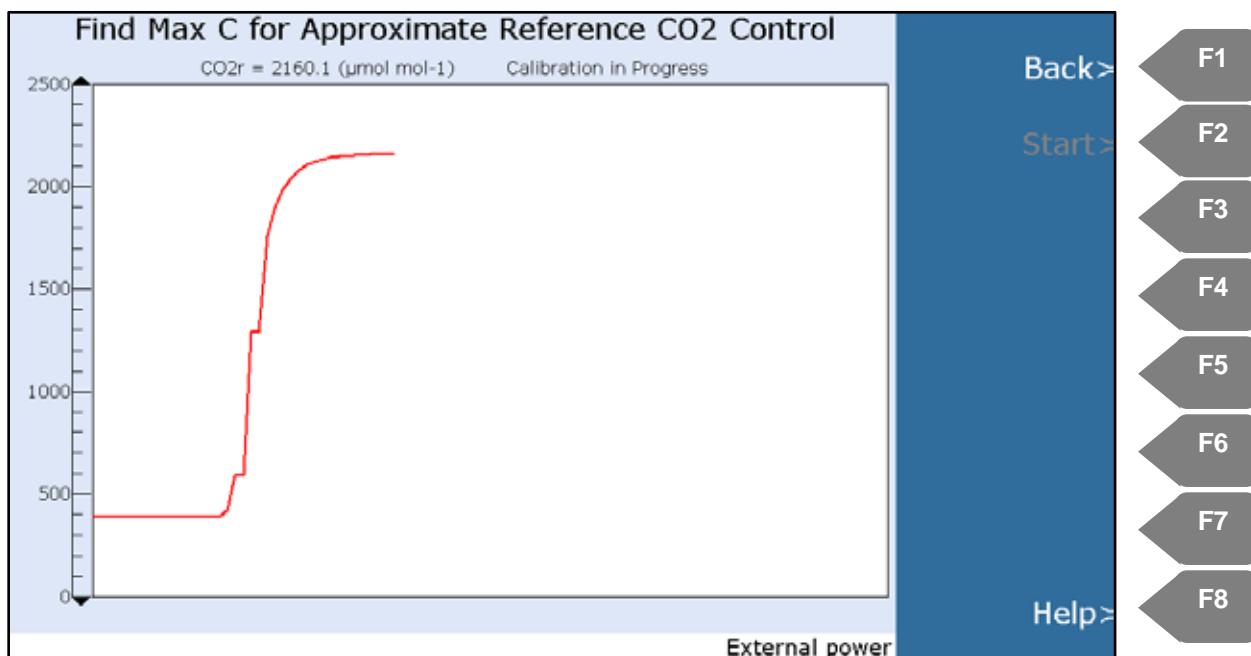
TIP

Make sure that the flow rate from your CO₂ or H₂O calibration gas mixture is at least 250 cc/min.

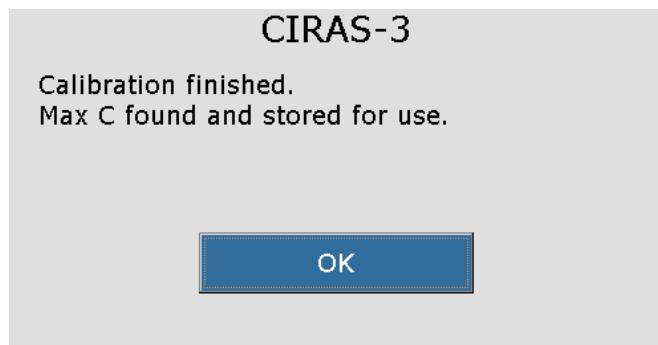
Otherwise atmospheric air could be drawn into the vent pipe leading to errors in calibration.

Find Max C (F3)

This process determines the maximum CO₂ level that can be achieved with the CIRAS-3. This should be performed prior to using “Approximate Reference Air” for CO₂ Reference for optimal control (Refer to [Settings – Gas Exchange](#) on page 143). Prior to starting this process, make sure that you have inserted a fresh CO₂ cartridge and that all chemicals are fresh. Press **Operations (F1) > Calibration (F5) > Find Max C (F3) > Start (F2)** to begin the process.



The entire process is automated. After starting the process, you should see the CO₂ concentration start to climb. The message “Calibration in Progress” will be displayed at the top next to the measured CO₂ concentration (CO₂r). Eventually the CO₂ concentration will max out at a CO₂ level above 2000 μmol mol⁻¹. The instrument will then perform a 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 CO₂ 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" for **CO2 Reference**.

TIP

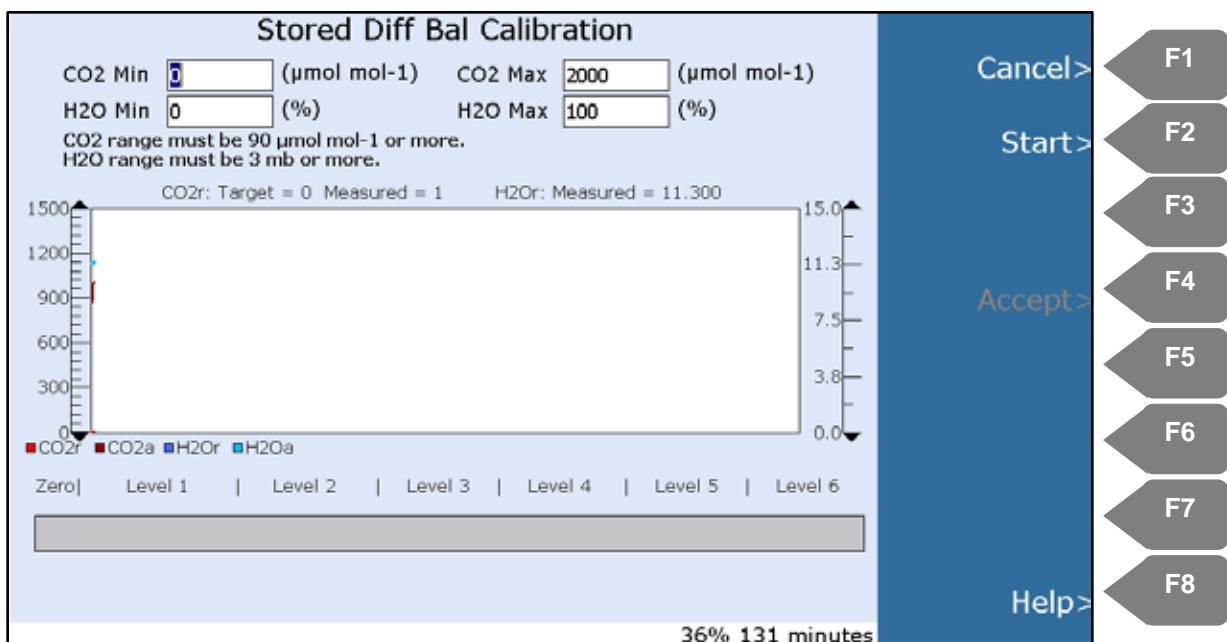
You must always perform a MAX C prior to using "Approximate Reference Air" for CO₂ Reference under Settings (See [Settings – Gas Exchange](#) on page 143). This will ensure that you are always within 10-20 µmol mol⁻¹ of your CO₂ reference set value and stable which is very important when performing response curves.

Store Diff Bal (F4)

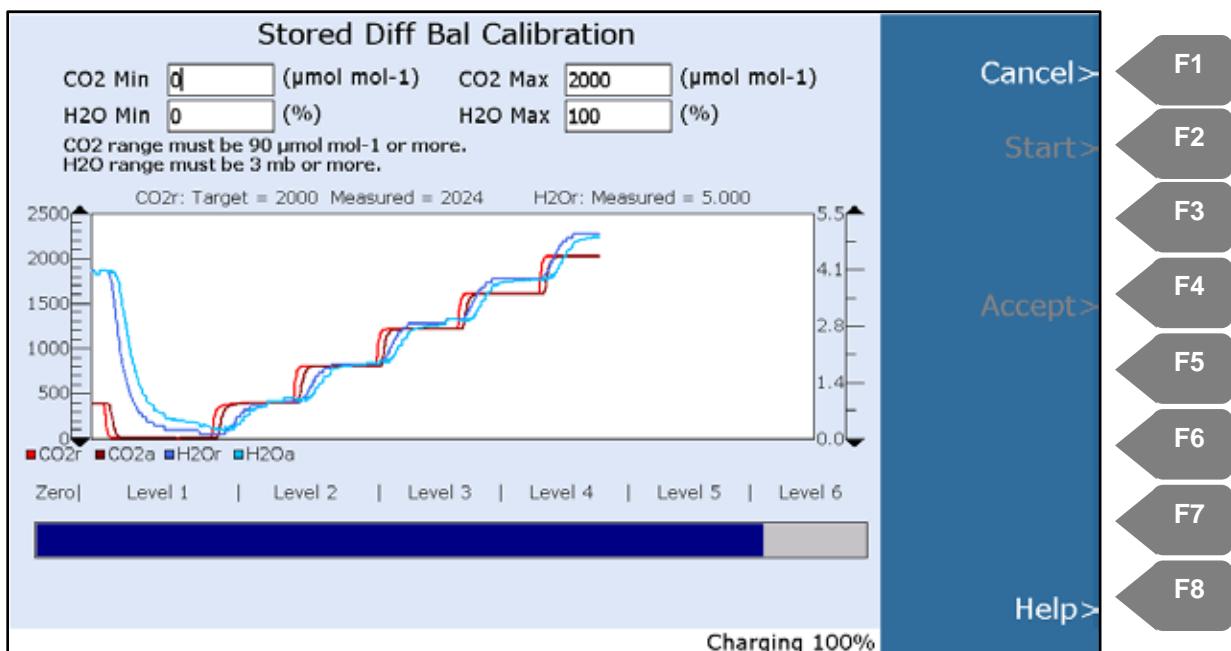
For response curves with varying CO₂ concentration, we recommend using "Auto Zero/Stored Diff Bal" for **Zero/Diff Bal Mode** under **Settings (F2)**. Prior to using this option, you **MUST** first perform a "Store Diff Bal" before starting measurements. This process allows you to store "Diff Bals" in advance to avoid disruptions during a response. To begin the process press **Operations (F1) > Calibration (F5) > Store Diff Bal (F4)**.

TIP

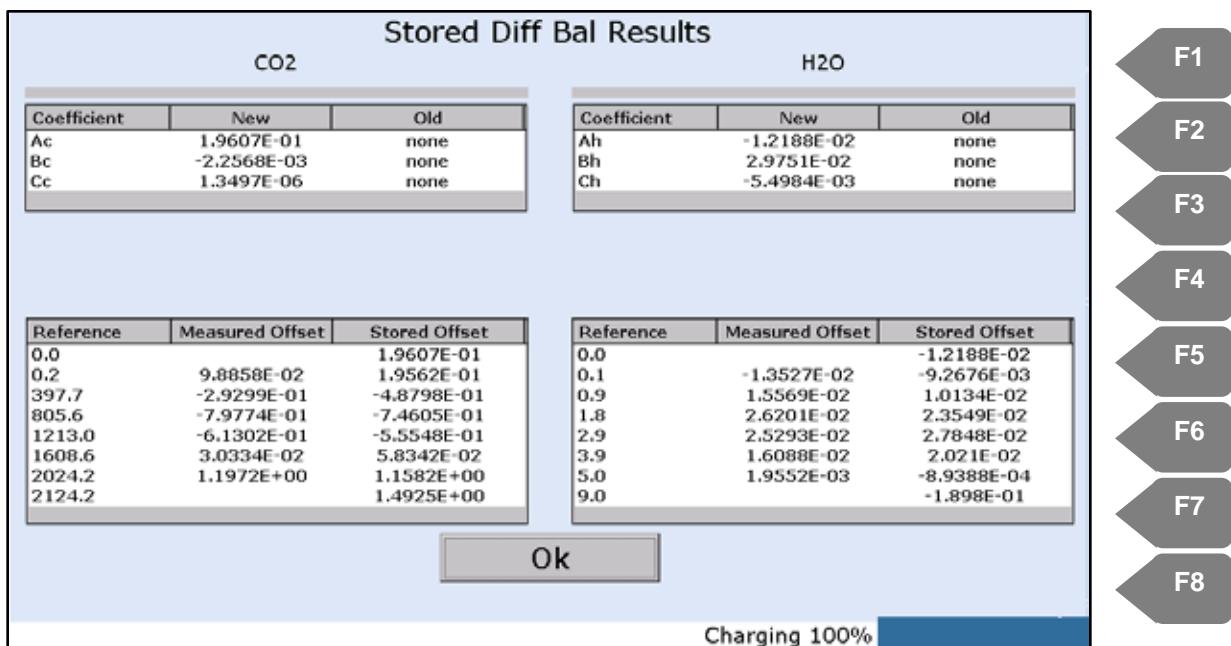
A new Store Diff Bal should be performed once per day after the CIRAS-3 has been fully warmed up and running for at least 30 minutes. If you perform a Store Diff Bal at the beginning of the day you should be good to go all day without having to perform any further Store Diff Bals saving valuable time. Please note that the system will still perform periodic Auto Zero every 30 minutes. Therefore for users performing response curves (especially those performing high-speed CO₂ ramping for rapid measurement of A/C_i) we recommend performing a manual Zero before each measurement sequence to avoid interruptions.



To begin, enter the minimum and maximum ranges for both CO₂ and H₂O. For instance, if the working CO₂ range is 200-1000, enter 200 for CO₂ Min and 1000 for CO₂ Max. The same applies for H₂O. Press the TAB key to advance to each setting. **Make sure the desiccants are fresh and the PLC3 leaf cuvette head is closed with no leaf in the chamber prior to starting the Stored Diff Bal process.** When ready, 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 **Accept** the results by pressing **OK > 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 by pressing **OK > Cancel (F1)** and return to the Stored Diff Bal Calibration display 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 display. The upper half of the Stored Diff Bal Results display shows new and old Stored Diff Bal Coefficients. The lower half of the display 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 CO₂ Ref reading and 4 mbar less than the H₂O Ref reading. The last row of data represents the upper limit of reference readings that are allowed – 100 ppm more than the highest CO₂ Ref reading and 4 mbar higher than the highest H₂O 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 part of the display prior to accepting data for the following criteria:

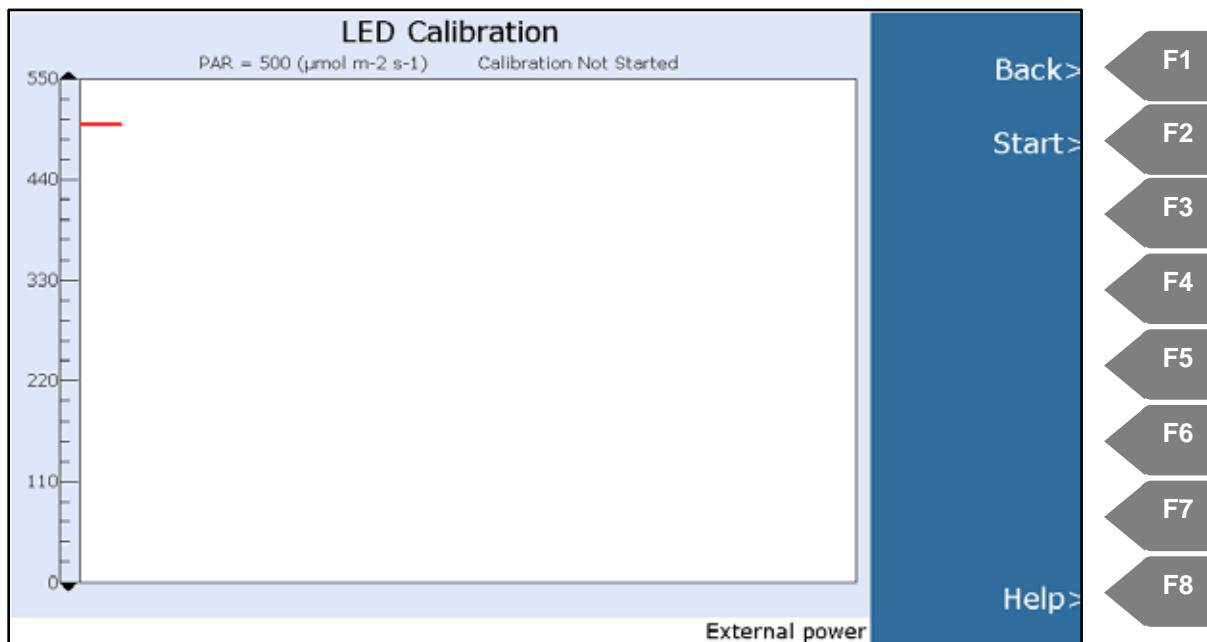
1. Measured offsets at each point should be less than 20 ppm and 0.5 mbar.
2. Measured offsets should show a trend - gradually increasing or decreasing over the six points.
3. The computed Stored Offsets in the 3rd and 6th columns should match the measured offsets in the 2nd and 5th columns within about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

Tip

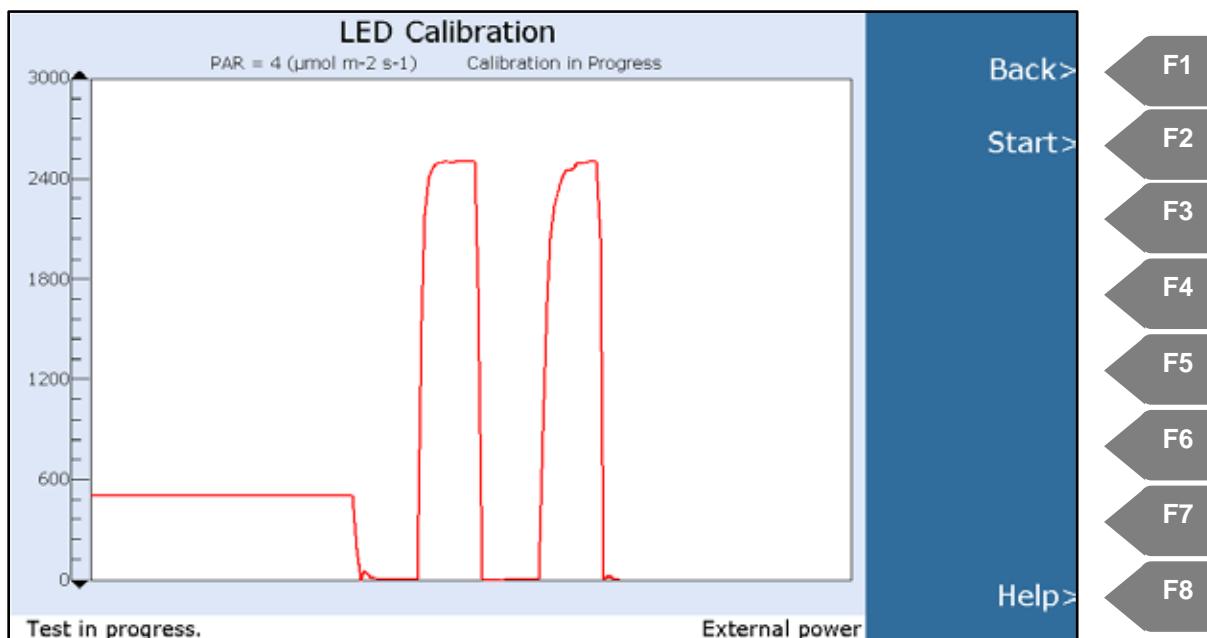
If you see a “Cuvette RH > 70%, decrease reference H₂O” message during the Stored Diff Bal calibration you can simply ignore it. This message is more relevant when performing measurements on live plants when chamber humidity can get quite high depending on the environmental conditions and state of the plant.

LED Calibrate (F5)

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



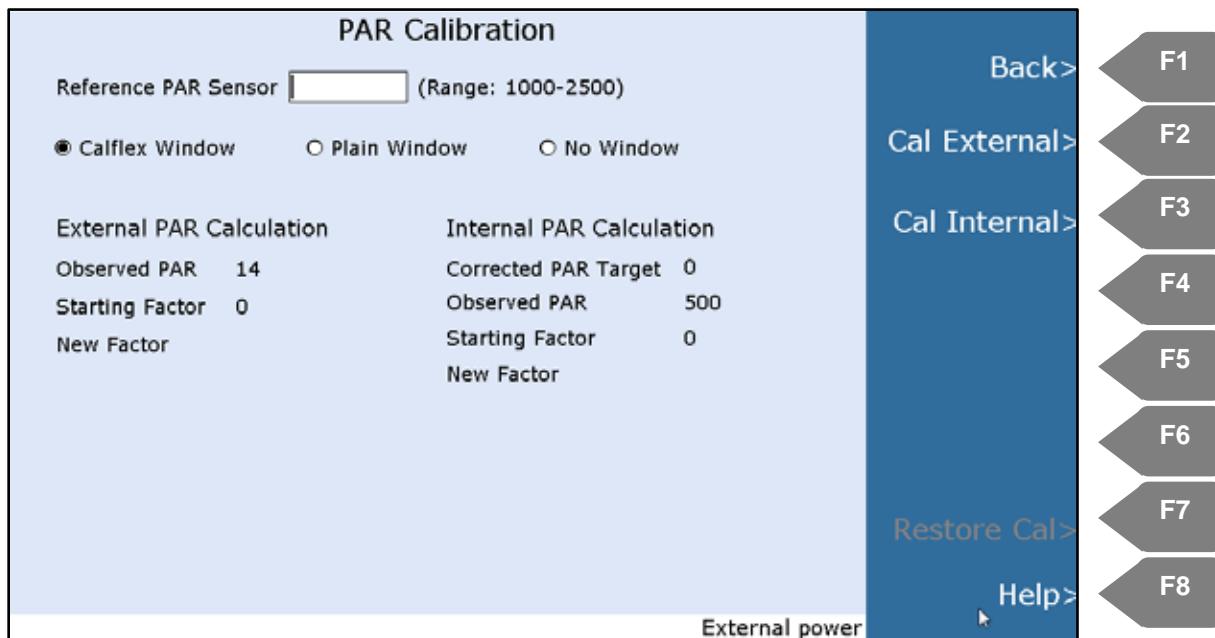
Initially the current light level will appear as shown above. Press **Start (F2)** to begin the calibration process. "Test in progress." will appear in the lower left hand corner of the display and the calibration process will begin.



If the calibration is successful, a message “Test passed.” will appear 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 (F6)

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 $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

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 ($\pm 2\%$) then there is no need to recalibrate. If it is outside this range, press **Cal External (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 ($\pm 2\%$), then there is no need to recalibrate. If the values are outside this range, press **Cal Internal (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 (F6)

Press **Diagnostics (F6)**. The CIRAS-3 contains numerous sensors to measure CO₂, H₂O, 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 over time from the time it was manufactured.

IRGA (F2)

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

Diagnostics - IRGA

Item	Measure	Diff	Zero	AutoZero
CO2 Ref	49300	(null)	(null)	49322
CO2 An	42763	(null)	(null)	49728
H2O Ref	47894	(null)	(null)	50011
H2O An	48127	(null)	(null)	50323
IRGA temperature (°C)	55.00	(null)	(null)	(null)
Cell pressure (mb)	1007.3	(null)	(null)	(null)
Cell diff pressure (mb)	0.48	(null)	(null)	(null)
Ref cell flow (cc min ⁻¹)	99.7	(null)	(null)	(null)
An cell flow (cc min ⁻¹)	99.8	(null)	(null)	(null)

35% 127 minutes

- Exit>
- Measure>
- Diff>
- Zero>
- AutoZero>
- Stored Zeros>
- Reset Zeros>
- Help>

F1
F2
F3
F4
F5
F6
F7
F8

Diagnostics – IRGA

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 ₂ O gas concentrations but should be between 30000 and 52000 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 ₂ O gas concentrations but should be between 30000 and 52000 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 (± 20000) 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.

Diagnostics - PLC General	
Item	Reading
Ambient PAR (µmol m ⁻² s ⁻¹)	21
Left PAR (µmol m ⁻² s ⁻¹)	546
Right PAR (µmol m ⁻² s ⁻¹)	455
Record Key	37
Select Key	0
Close switch	0
Status, (hex)	0X6000

Exit>
Temperature>
Help>
>
F1
F2
F3
F4
F5
F6
F7
F8

Fully Charged

Diagnostics – PLC General

Ambient PAR	PAR reading from external PAR (PARe) sensor ($\mu\text{mol m}^{-2} \text{s}^{-1}$).
Left PAR	PAR reading from left internal PAR (PARi) sensor on PLC3 Universal Leaf Cuvette ($\mu\text{mol m}^{-2} \text{s}^{-1}$).
Right PAR	PAR reading from right internal PAR (PARi) sensor on PLC3 Universal Leaf Cuvette ($\mu\text{mol m}^{-2} \text{s}^{-1}$).
Record Key	Status indicator for the Record (R) key on the PLC3. It should read 37 by default. When you press the R key on the PLC3 it should read 167 indicating that the key is working properly.
Select Key	Status indicator for the Select (S) key on the PLC3. It should read 1 and when you press the S key it should increment by 1 each time (i.e. 2, 3, 4, etc.) indicating that the key is working properly.
Close switch	Status indicator for the open/close switch on the PLC3. It should read 1 when the cuvette head is open and 0 when closed.
Status, (hex)	Indicates system status.

To further test temperature control settings, press **Temperature (F2)**. The PLC3 head must be closed for temperature control to operate.

Item	Reading
Ambient temperature (°C)	27.00
Cuvette Temperature (°C)	29.66
Leaf Temperature (°C)	30.43
Peltier heat PWM	0
Peltier cool PWM	0
Temperature setting (°C)	27
Temperature control mode	3

Temperature setting (°C) [0-40]
 Temperature control mode [0-4]

0=Disabled 1=Cuvette 2=Track Cuvette to Ambient 3=Leaf 4=Track Leaf to Ambient

Reminder: Manually reset settings changed during diagnostics.

34% 125 minutes

Exit>
 General>
 Help>

F1
 F2
 F3
 F4
 F5
 F6
 F7
 F8

Diagnostics – PLC Temperatures

Temperature (F2)	Temperature setting ($^{\circ}\text{C}$) - 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 ($^{\circ}\text{C}$)”. Temperature control mode - 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)	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.

Item	Reading
Analyzer flow (cc min^{-1})	99.8
Reference flow (cc min^{-1})	100.0
ASU flow (cc min^{-1})	399.7
Cuvette flow (cc min^{-1})	299.7
Cuvette flow setting (cc min^{-1})	300
IRGA flow setting (cc min^{-1})	100
CO ₂ valve PWM	5778
H ₂ O valve PWM	0

Cuvette flow setting (cc min^{-1}) [150-500]

IRGA flow setting (cc min^{-1}) [50-100]

CO₂ valve PWM [0-32000]

H₂O valve PWM [0-32000]

Reminder: Manually reset settings changed during diagnostics.

33% 122 minutes

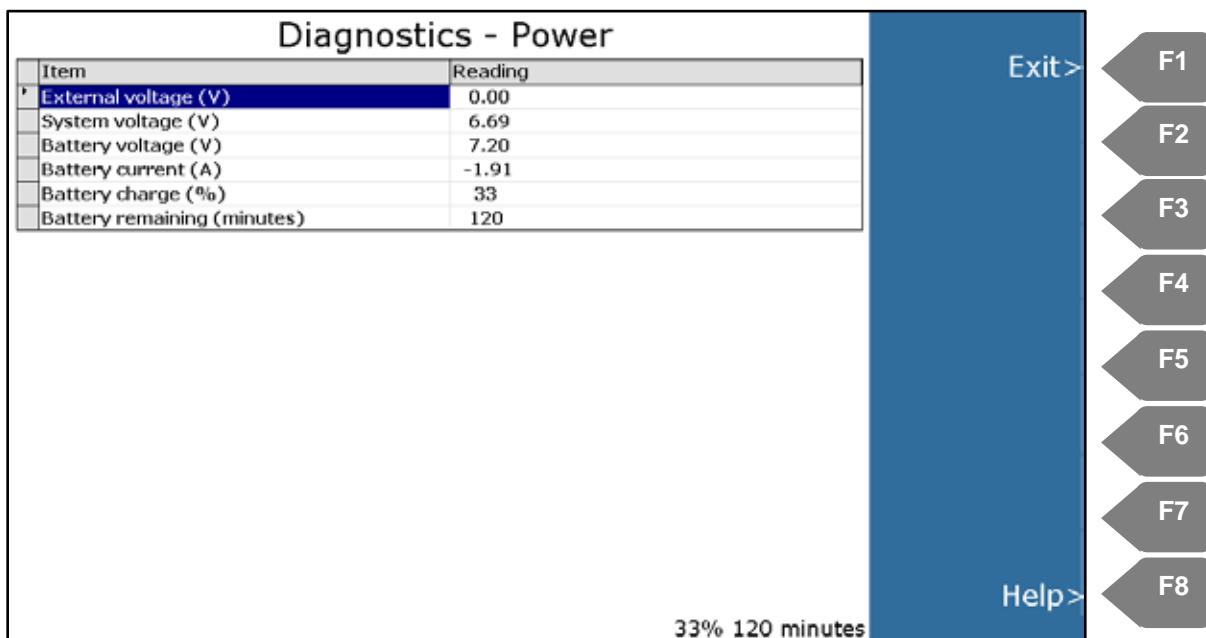
Exit>

F1
F2
F3
F4
F5
F6
F7
F8

Diagnostics – Flow	
Analyzer flow	Analysis pump flow rate (cc min^{-1}). The Analyzer flow and Reference flow should be identical if operating properly.
Reference flow	Reference pump flow rate (cc min^{-1}). The Analyzer flow and Reference flow should be identical if operating properly.
ASU flow	Air supply pump flow rate (cc min^{-1})
Cuvette flow	Cuvette flow rate (cc min^{-1})
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 ₂ O valve and 32000 to open the valve completely. Values that are in between 0 and 32000 are based on the currently set H ₂ O control value. When the H ₂ O valve is set to 0 or 32000, there should not be any clicking of the H ₂ O valve.

Power (F5)

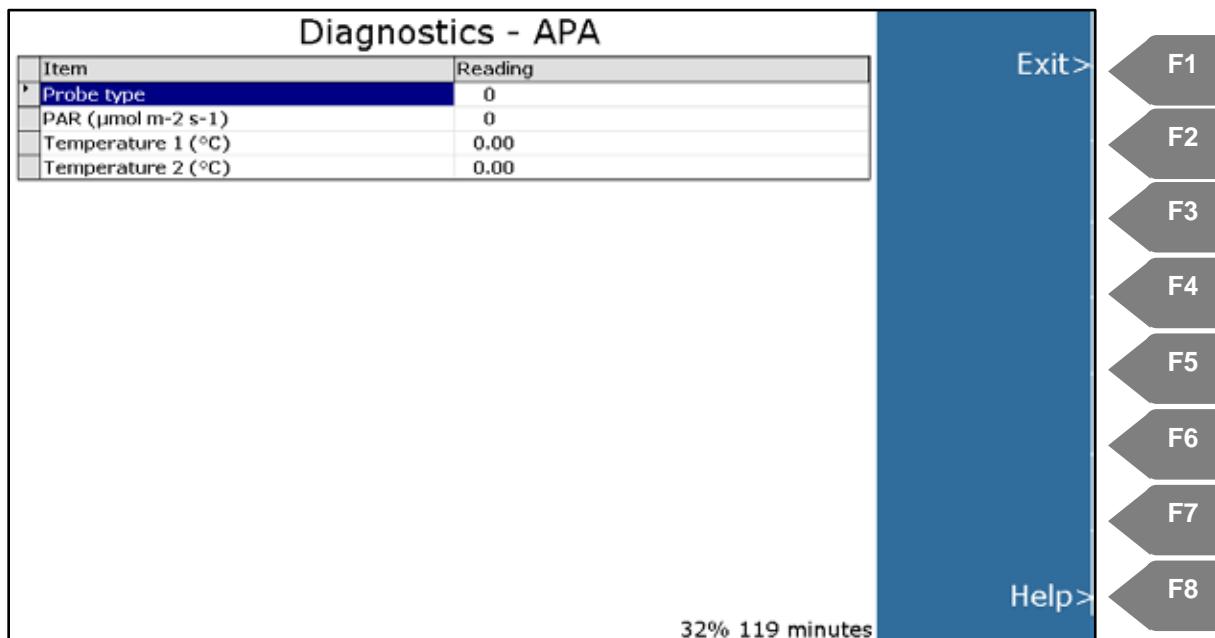
Press **Power (F5)** to view readings associated with the system power.



Diagnostics – Power	
External voltage	Displays the voltage from an external power source (i.e. CIRAS-3 AC Adapter/charger). When the CIRAS-3 power supply adapter 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 packs. A fully charged system should read approximately 8V.
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)

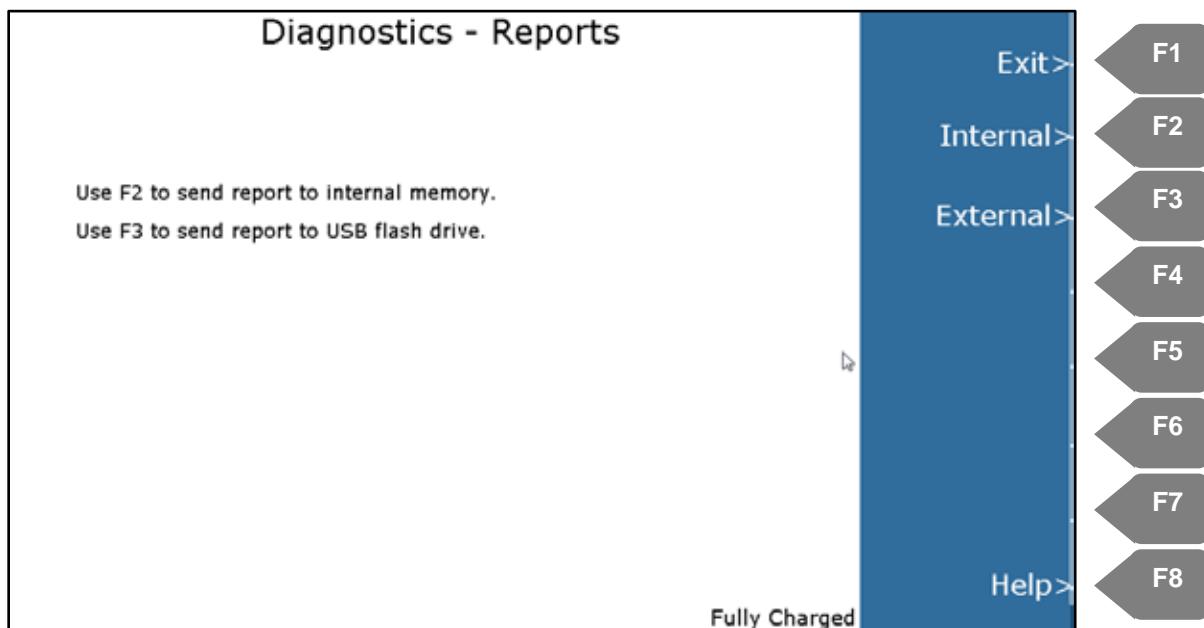
Press **APA (F6)** to view readings associated with the Auxiliary Probe Adapter (APA). This piece of hardware is required to use the SRC-1 and CPY-4 with the CIRAS-3.



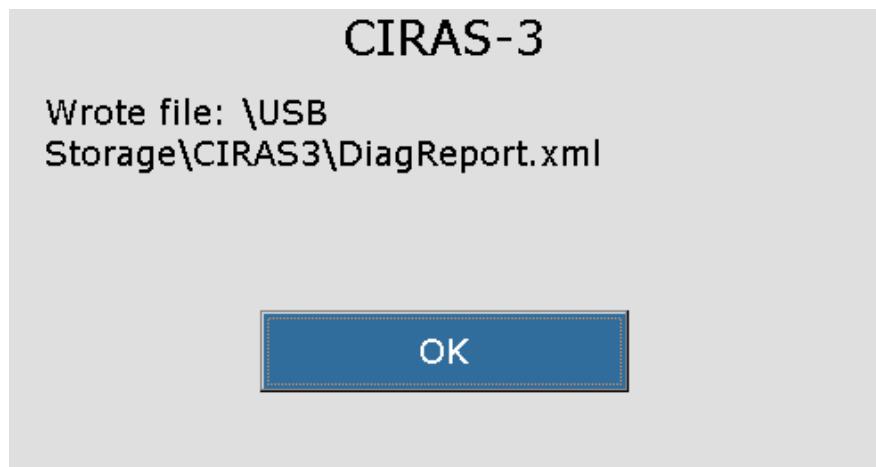
Diagnostics – APA	
Probe type	Displays the probe type for different sensors that can be used with the CIRAS-3 (i.e. SRC-1 Soil Respiration Chamber or CPY-4 Canopy Assimilation Chamber).
PAR	Displays the measured PAR value ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the sensor on the accessory (if applicable).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

Report (F7)

Press **Report (F7)** to generate a Diagnostics report.



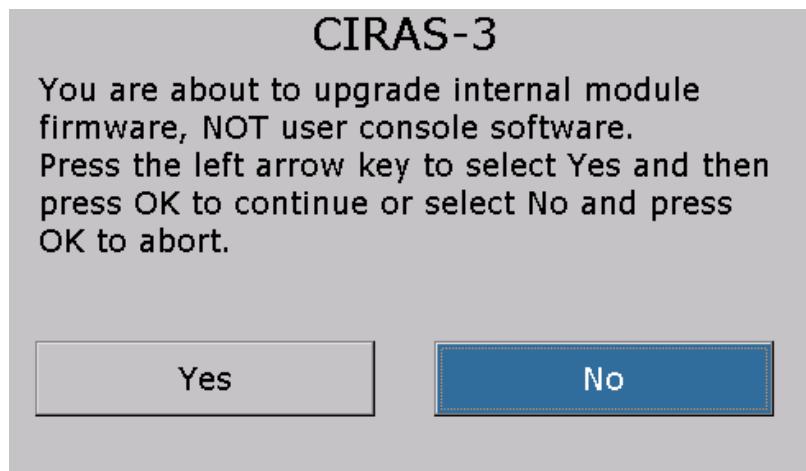
Press **F2** to have the report sent to internal memory or **F3** to send it to the USB flash drive (a USB flash drive must be inserted into either USB1 or USB2 on the console). A message similar to below will appear on the CIRAS-3 display if the file is sent to the USB flash drive:



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 prompt diagnosis.

Firmware Upgrade (F7)

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 flash drive. Once the files are on the USB flash drive insert it into the USB1 or USB2 slot on the CIRAS-3 console. By default **No** is highlighted so you will need to press the **Left Arrow > Yes > OK** to view both the currently installed firmware versions and available versions on the USB flash drive. If you have not yet inserted the USB flash 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 use the arrow keys to select the module, press **OK** to check it and then hit **Upgrade (F3)**.

A screenshot of the CIRAS-3 console's firmware upgrade menu screen. The main area shows three sections: 'Current Module and Firmware Version', 'Available Firmware Versions on USB Drive', and 'Status Log'. The 'Current Module and Firmware Version' section contains the following table:

Module	Version	Part Number
Console	v4.08	PC117-1
Air/Flow	v3.02	PC111-1
PLC	v3.16	PC114-1
IRGA	v3.06	PC113-1
APA	Not Detected	PC122-1

The 'Available Firmware Versions on USB Drive' section is currently empty. The 'Status Log' section shows 'No firmware folder on USB drive.' On the right side, there are navigation buttons labeled F1 through F8, and text links: 'Back>', 'Upgrade>', 'Advanced>', 'Scan USB>', and 'Help>'.

There are 5 CIRAS-3 firmware 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)

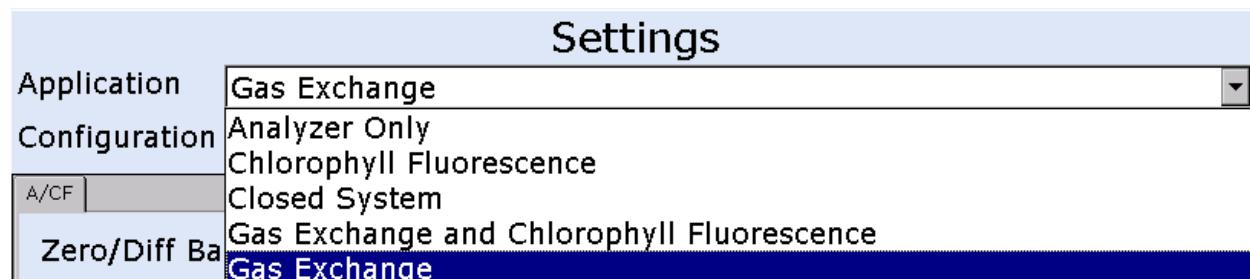
If you have any questions related to firmware compatibility, please get in direct contact with PP Systems.

Section 8. Settings (F2)

Settings, also commonly referred to as global settings are essentially defined as operational environments and settings that are recreated each time that CIRAS-3 is powered on. The options that you choose here are based on the “Application” selected and your typical working conditions and preferences. 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. Please note that there is a slight delay of approximately 4-5 seconds after selecting **Settings (F2)**.

Application

First you must select the appropriate “Application” suitable to your research. All subsequent “Configuration” and available settings/options are based on the “Application” selected by the user.



There are 5 different “Applications” available to users as shown in the table below.

Application	
Application	Description
	Analyzer Only – If you want to use the CIRAS-3 as a stand-alone CO ₂ /H ₂ O Gas Analyzer.
	Chlorophyll Fluorescence – If you want to use the CIRAS-3 for measurement of chlorophyll fluorescence only.
	Closed System – For survey measurement of soil respiration (SRC-2 Soil Respiration Chamber) and canopy assimilation (CPY-5 Canopy Assimilation Chamber). Customers can also use our SRC-1 Soil Respiration Chamber, STP-1 Soil Temperature Probe and CPY-4 Canopy Assimilation Chamber if used with our Auxiliary Probe Adapter.
	Gas Exchange and Chlorophyll Fluorescence – For simultaneous measurement of photosynthesis and chlorophyll fluorescence. The PLC3 Universal Leaf Cuvette and CFM-3 Chlorophyll Fluorescence Module are required for this “Application”.
	Gas Exchange – For measurement of leaf gas exchange using any of our PLC3 leaf cuvettes and light units.

With the appropriate “Application” highlighted press TAB to select it. Next you need to select the appropriate leaf cuvette or chamber under “Configuration”.

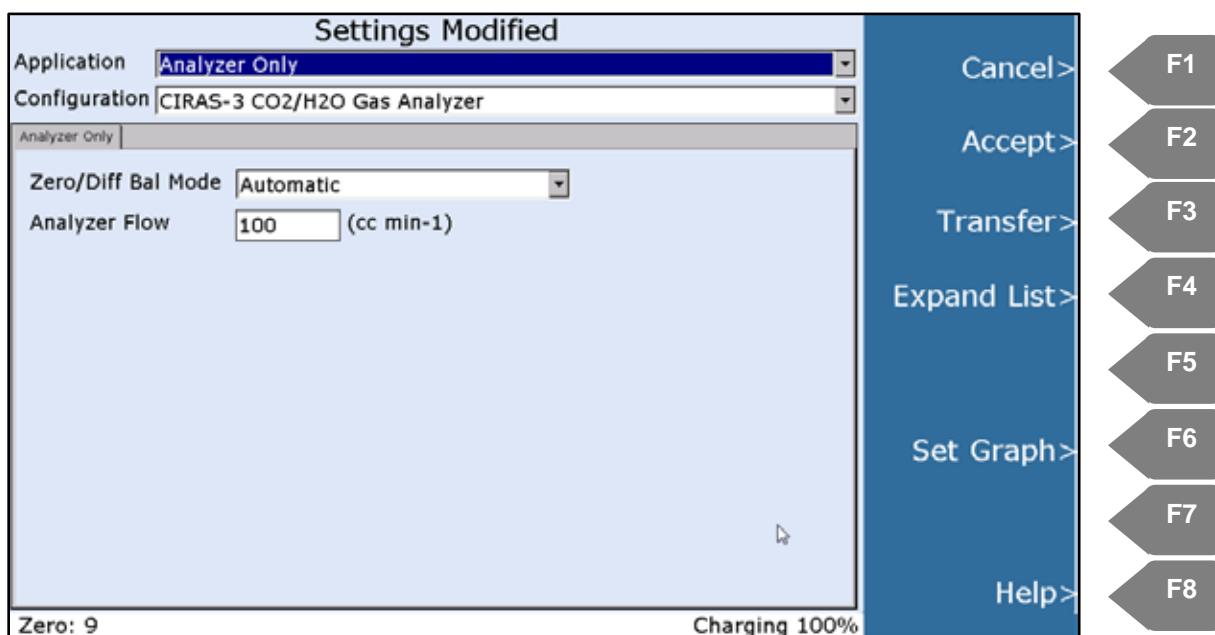
Configuration

Options available to users are dependent upon the “Application” selected.

Configuration	
Analyzer Only	<ul style="list-style-type: none">· CIRAS-3 Portable CO₂/H₂O Gas Analyzer
Chlorophyll Fluorescence	<ul style="list-style-type: none">· CFM-3 Measurement Only, 18 mm Window· CFM-3 Measurement Only, 18x25 mm Window· CFM-3 Measurement Only, 7x25 mm Window
Closed System	<ul style="list-style-type: none">· CPY-4 Canopy Assimilation Chamber· CPY-4 Canopy Assimilation Chamber, STP-1· CPY-5 Canopy Assimilation Chamber· SRC-1 Soil Respiration Chamber· SRC-1 Soil Respiration Chamber, STP-1· SRC-2 Soil Respiration Chamber
Gas Exchange and Chlorophyll Fluorescence	<ul style="list-style-type: none">· PLC3 Universal Leaf Cuvette, 18 mm Window, CFM-3· PLC3 Universal Leaf Cuvette, 18x25 mm Window, CFM-3· PLC3 Universal Leaf Cuvette, 7x25 mm Window, CFM-3· PLC3 Universal/Bryophyte Leaf Cuvette, 18 mm Window, CFM-3· PLC3 Universal/Bryophyte Leaf Cuvette, 18x25 mm Window, CFM-3· PLC3 Universal/Bryophyte Leaf Cuvette, 7x25 mm Window, CFM-3
Gas Exchange	<ul style="list-style-type: none">· Insect Respiration· PLC3 Conifer Leaf Cuvette· PLC3 Narrow Leaf Cuvette· PLC3 Universal Leaf Cuvette, 18 mm Window· PLC3 Universal Leaf Cuvette, 18x25 mm Window· PLC3 Universal Leaf Cuvette, 7x25 mm Window· PLC3 Universal/Bryophyte Leaf Cuvette, 18 mm Window· PLC3 Universal/Bryophyte Leaf Cuvette, 18x25 mm Window· PLC3 Universal/Bryophyte Leaf Cuvette, 7x25 mm Window· PLC3 Whole Plant Cuvette

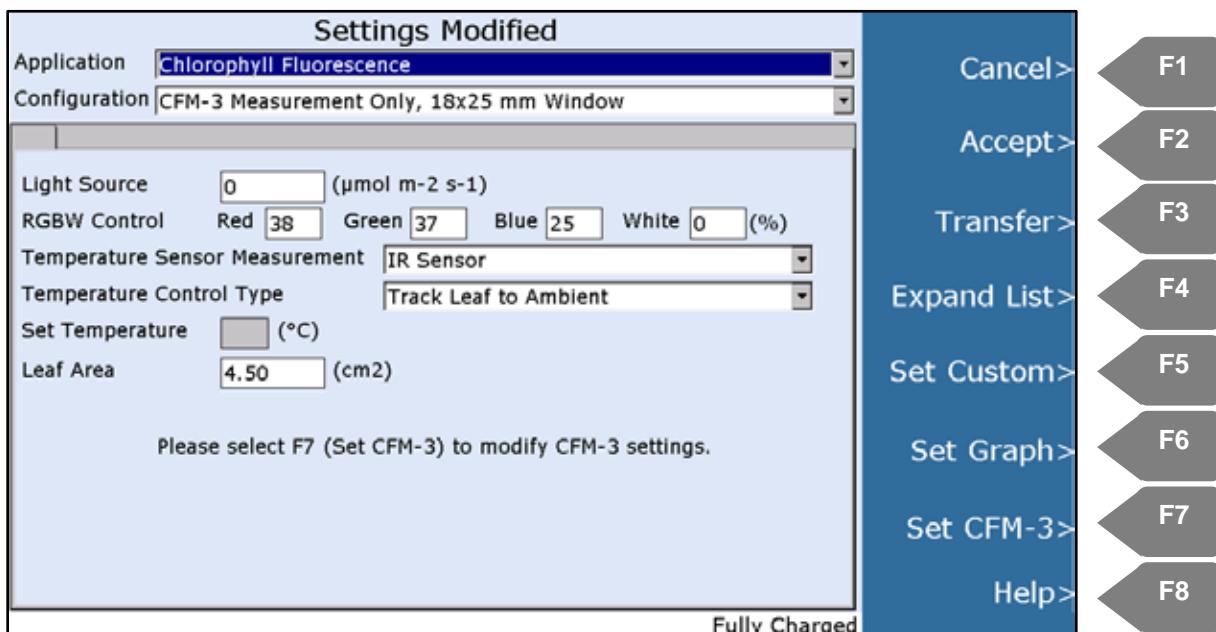
It is very important to select the proper cuvette or chamber under “Configuration” because default settings are built into the software for each item. To select the correct item from the dropdown list, press **Expand List (F4)**. Use the up and down arrows to move through the alphabetical list. When the appropriate leaf cuvette or chamber is highlighted press the TAB key to reveal the available settings and controls associated with that selection,

Settings – Analyzer Only



Settings – Analyzer Only	
Zero/Diff Bal Mode	<p>Manual - You will be prompted when to perform a Zero or Diff Bal.</p> <p>Automatic - Zeros and Diff Bals will be performed automatically every 30 minutes. We recommend this mode if small ΔCO_2 and $\Delta\text{H}_2\text{O}$ is anticipated and for less experienced users. A Diff Bal cycle will also take place when there is a large change in CO_2 ($100 \mu\text{mol mol}^{-1}$) or H_2O (4 mb) concentration.</p> <p>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 103).</p>
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.

Settings – Chlorophyll Fluorescence

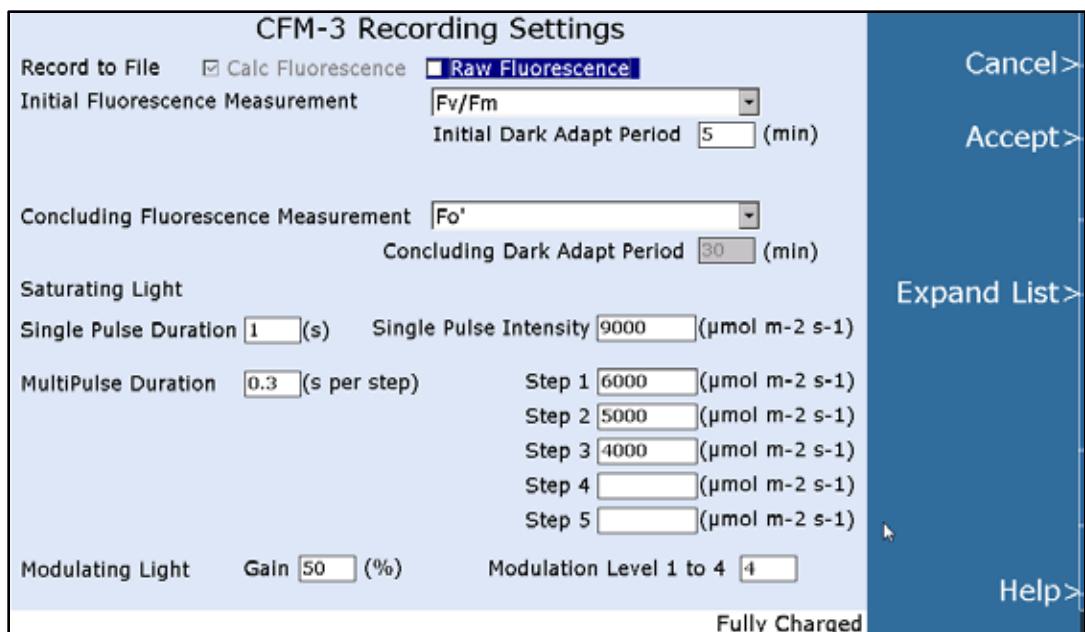


Settings – Chlorophyll Fluorescence	
Light Source	Select your desired light intensity from 0-2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Maximum light intensity will be dependent on type of light unit.
RGBW Control	Allows you to set your desired LED color distribution. An individual color can be set to 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). The best settings to use to simulate sunlight are 38% Red, 37% Green, 25% Blue and 0% White which are also the default values.
Temperature Sensor Measurement	IR Sensor - To use the PLC3 Universal cuvette's internal infrared sensor (but only if the leaf covers the entire window opening). Do not select IR Sensor 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. Energy Balance can be used at any time.

Settings – Chlorophyll Fluorescence (Continued)

Temperature Sensor Control Type	<p>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).</p> <p>Track Leaf to Ambient - Allows the leaf temperature to follow ambient temperature as measured by the cuvette’s handle-mounted thermistor sensor.</p> <p>Cuvette Temperature - Used to control the air in the cuvette at a fixed temperature that you enter under “Set Temperature” (see below).</p> <p>Track Cuvette to Ambient - Allows the cuvette temperature to follow air temperature as measured by the cuvette’s handle-mounted sensor.</p> <p>Disable Temperature Control - Intended mainly for diagnostic purposes but can also be used for field measurements if required or to conserve the batteries. 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.</p>
Leaf Area	<p>The default value will be based on your cuvette selection under “Configuration”. This is very important. For the PLC3 Universal Leaf Cuvette the total area is known based on the Window size so if you fill the entire window with leaf material simply use the default values. If you do not fill the window area and don’t know the actual leaf area of your sample then enter a lower estimate. For the PLC3 Conifer and Narrow leaf cuvettes it is very difficult to know the actual leaf area so again enter a lower estimate for leaf area prior to taking measurements. 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.</p>

When Chlorophyll Fluorescence is the selected “Application” there are additional control settings available. Press **Set CFM-3 (F7)**.



Settings - CFM-3 Recording Settings

Record to File	Calc Fluorescence - Will be grayed out indicating that fluorescence data must be written to the data file. 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.
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Settings - CFM-3 Recording Settings (Continued)

Initial Fluorescence	<p>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 display under "Saturating Light".</p> <p>φPSII-MultiPulse - If selected there is no need to dark adapt the leaf and a "MultiPulse" sequence can begin right away with multiple, saturating pulses applied to the light-adapted leaf sample. The MultiPulse duration and intensity settings (up to 5 steps) can be set in the lower part of the display under "Saturating Light".</p> <p>Fv/Fm - If selected you will need to allow the plant to dark acclimate prior to measurement. If performing Fv/Fm measurements we recommend doing so at night or before sunrise to save time. This will ensure that the leaves are completely dark adapted and will allow for more measurements to be taken over shorter periods. As an added precaution you can cover the CFM-3 with a dark cloth to ensure that the inside chamber is completely dark. Set the Initial Dark Adapt Period of your choosing. Longer dark adapt period are required for plants that have been exposed to high light and shorter periods for plants that have been in the dark or low light.</p> <p>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.</p> <table border="1" data-bbox="507 1579 1258 1723"> <thead> <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> </thead> <tbody> <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> </tbody> </table>	DA period (minutes)	5	10	15	20	25	30	F_v/F_m	0.63	0.71	0.78	0.81	0.80	0.81
DA period (minutes)	5	10	15	20	25	30									
F_v/F_m	0.63	0.71	0.78	0.81	0.80	0.81									

Settings - CFM-3 Recording Settings (Continued)

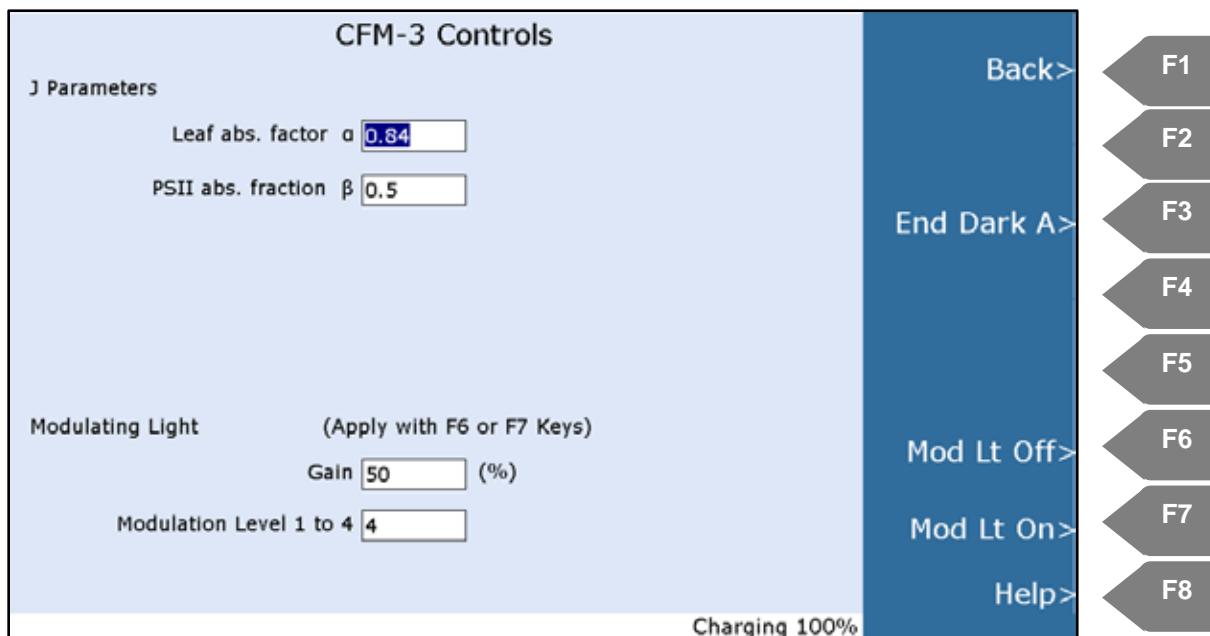
Concluding Fluorescence Measurement	<p>This defines the final measurement when recording is terminated.</p> <p>None - If this is selected, there will be no concluding chlorophyll fluorescence measurement and the sequence will terminate after the last photosynthesis measurement.</p> <p>Fo' - If selected, far-red light will be applied to the leaf sample.</p> <p>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 dark adapting the leaf for at least 20-30 minutes for best results.</p>
Saturating Light	<p>Single Pulse Measurements</p> <p>Single Pulse Duration - The time (in seconds) for the Single Pulse Duration saturating light to be applied.</p> <p>Single Pulse Intensity - The saturation pulse intensity (0-10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$).</p> <p>MultiPulse Measurements</p> <p>MultiPulse Duration - The time (in seconds) for each multiple pulse duration saturating light to be applied. For MultiPulse 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the high end and one step below 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the lower end with an even distribution of light in between the high and low light settings.</p>

Settings - CFM-3 Recording Settings (Continued)

Modulating Light	<p>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. Please note that users can dynamically change the gain during a measurement sequence under Controls (F3) > CFM-3 Cntrl if required to save time.</p> <p>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 F_o' under far-red light. Please note that users can dynamically change the Modulation Level during a measurement sequence under Controls (F3) > CFM-3 Cntrl if required to save time.</p>
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CFM-3 Controls

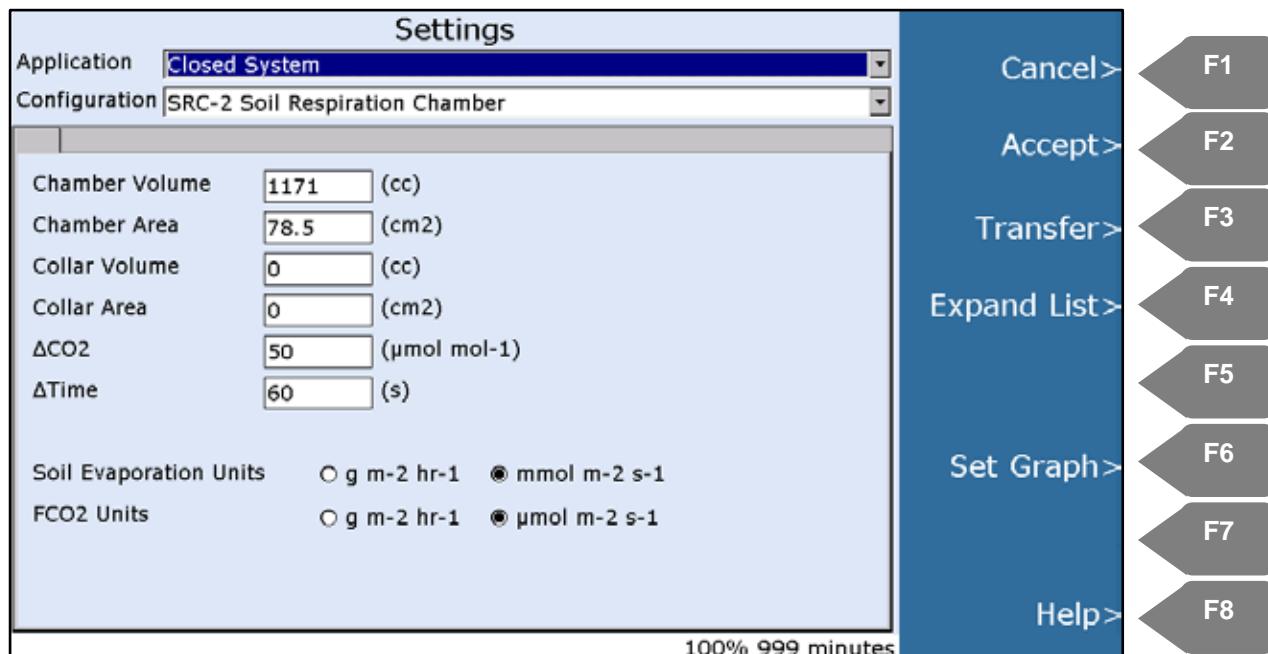
There are some additional CFM-3 related controls available to users under **Controls (F3) > CFM-3 Cntrl (F7)** to allow users to dynamically perform additional functions during measurement sequences.



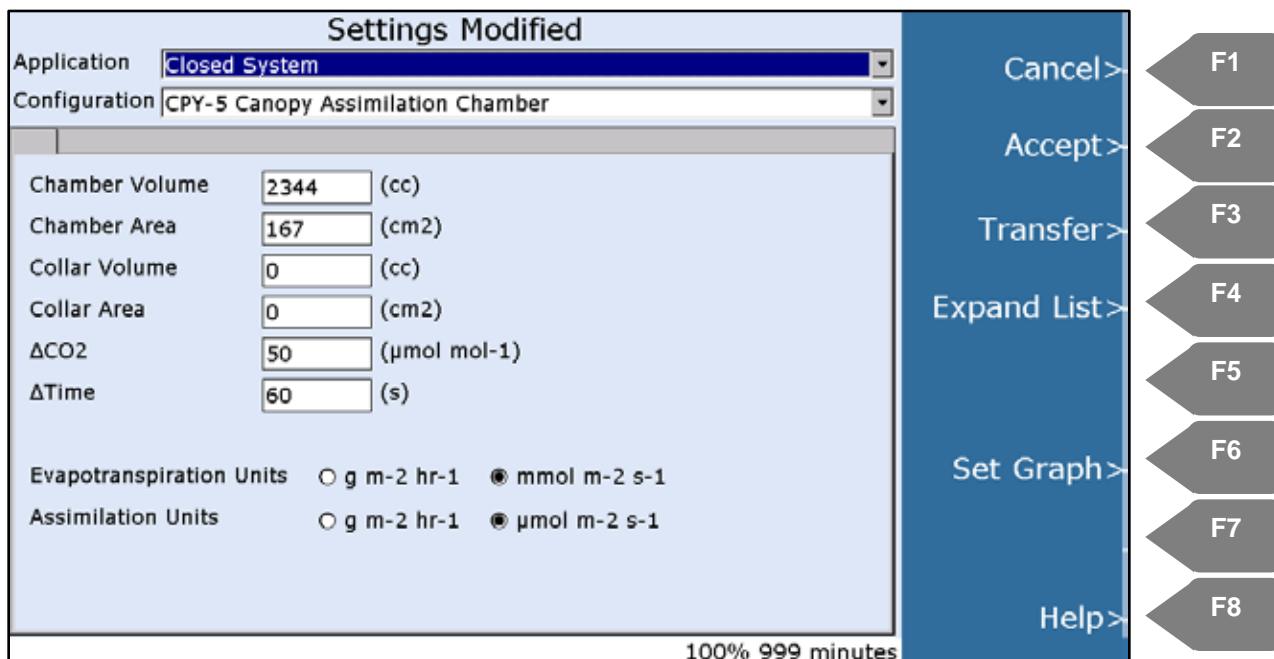
CFM-3 Controls	
J Parameters	<p>These are parameters used for calculation of electron transport rate.</p> <p>Leaf abs. factor – The default leaf absorbance factor is set to 0.84 but can be determined by use of an integrating sphere if required.</p> <p>PSII abs. factor - The default PSII absorbance factor is set to 0.5 but a known range of values exist for different plant types.</p>
Modulating Light	<p>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.</p> <p>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 F_o' under far-red light.</p>
End Dark A (F3)	To manually terminate the dark adaption period before the elapsed time under CFM-3 Recording Settings. 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-3 Control
Mod Lt Off (F6)	Turns the modulating light source off.
Mod Lt On (F7)	Turns the modulating light source on.

Settings – Closed System

SRC-2 Soil Respiration Chamber



CPY-5 Canopy Assimilation Chamber



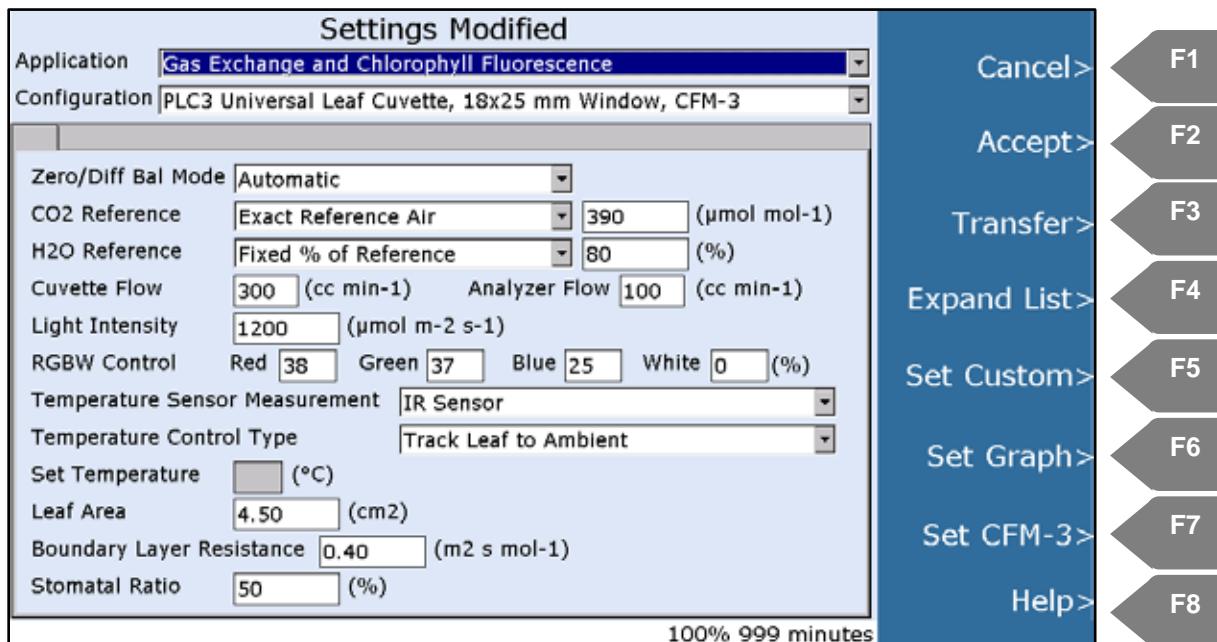
Please note that the settings for both the SRC1/SRC-2 and CPY-4/CPY are very similar but there are a few minor differences as described below.

Settings – Closed System	
Chamber Volume	The default value for the SRC-1 and SRC-2 is 1171 (cc). The default value for the CPY-4 and CPY-5 is 2344 (cc).
Chamber Area	The default for the value for the SRC-1 and SRC-2 is 78.5 (cm^2). The default value for the CPY-4 and CPY-5 is 167 (cm^2).
Collar Volume	If using a collar you need to account for the additional volume for proper calculation of results (cc).
Collar Area	If using a collar you need to account for the additional area for proper calculation of results (cm^2).
ΔCO_2	Enter a CO_2 value ($\mu\text{mol mol}^{-1}$) that you want to use to terminate the measurement sequence when the ΔCO_2 reaches this value.
ΔTime	Enter a time value (seconds) that you want to use to terminate the measurement sequence when the ΔTime reaches this value.
Surface Temperature	Only applicable when using the SRC-1 Soil Respiration Chamber. Users must enter the air temperature in this field for proper calculation of data. The SRC-2 has a built-in temperature sensor so the temperature is automatically measured and recorded by the CIRAS-3.
Soil Evapotranspiration Units (SRC-1 and SRC-2)	Select the units that you want to have soil evapotranspiration recorded. ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\text{mmol m}^{-2} \text{ s}^{-1}$).
FCO₂ Units (SRC-1 and SRC-2)	Select the units that you want to have flux rates recorded ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\mu\text{mol m}^{-2} \text{ s}^{-1}$).
Evapotranspiration Units (CPY-4 and CPY-5)	Select the units that you want to have transpiration recorded ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\text{mmol m}^{-2} \text{ s}^{-1}$).
Assimilation Units (CPY-4 and CPY-5)	Select the units that you want to have assimilation recorded ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\mu\text{mol m}^{-2} \text{ s}^{-1}$).

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 Evapotranspiration and FCO₂ used with the SRC-1/SRC-2 Soil Respiration Chambers and Evapotranspiration and Assimilation used with the CPY-4/CPY-5 Canopy Assimilation Chamber for later data analysis.

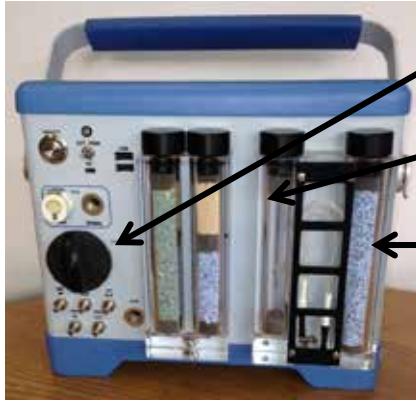
Settings – Gas Exchange and Chlorophyll Fluorescence



Settings – Gas Exchange and Chlorophyll Fluorescence

Zero/Diff Bal Mode	<p>Manual - You will be prompted when to perform a Zero or Diff Bal.</p> <p>Automatic - Zeros and Diff Bals will be performed automatically every 30 minutes. We recommend this mode if small ΔCO_2 and $\Delta\text{H}_2\text{O}$ is anticipated and for less experienced users. A Diff Bal cycle will also take place when there is a large change in CO_2 ($100 \mu\text{mol mol}^{-1}$) or H_2O (4 mb) concentration.</p> <p>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 103).</p>
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Settings – Gas Exchange and Chlorophyll Fluorescence (Continued)

CO₂ Reference	<p>Approximate Reference Air - The CO₂ supplied to the leaf chamber from CIRAS-3's internal CO₂ source will generally be within 10-20 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 101) for optimal accuracy and control. For response curves or in situations that require frequent, large changes in CO₂ levels, this option is recommended because it settles near the set value very quickly (within 1-2 minutes).</p> <p>Exact Reference Air - The CO₂ supplied to the leaf chamber from CIRAS-3's internal CO₂ 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 CO₂ level throughout a measurement sequence or for long periods, this option is ideal.</p> <p>Fixed Analysis Air - Allows CO₂ control on the analysis gas (feedback from the leaf) as it is measured by the Analysis side IRGA.</p> <p>Ambient (Remove Chemicals) - This option is frequently used to supply the natural outdoor CO₂ to the leaf chamber. Several considerations are involved whenever the stable internal CO₂ source (CO₂ cartridge) is not used. If this option is selected, the CO₂ regulator should be empty but in place and the soda lime must be removed from the CO₂/H₂O control column as shown below. Humidity control can still be available if the H₂O Absorber column is filled with Drierite.</p> 
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Settings – Gas Exchange and Chlorophyll Fluorescence (Continued)

H₂O Reference (chamber humidity)	<p>Fixed % of Reference - This option is most common. Leaves add water vapor to the air stream so the incoming air must be drier than ambient for the leaf's humidity environment to match outside. Stomatal conductance and the change in humidity due to the leaf vary widely for different types of leaves and environmental conditions. We recommend a setting of 80% to begin with. 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). 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%.</p> <p>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.</p> <p>Constant VPD - Automatically adjusts chamber humidity to maintain a consistent leaf-to-air vapor pressure deficit between the absolute range 0-100 mb (0-10 kPa). 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.</p> <p>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.</p>
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TIP

Often, the desired environment for photosynthesis measurements is for the leaf cuvette to be controlled to ambient humidity conditions outside the cuvette. If instead, it is desired to have the leaf cuvette humidity above ambient, it can be accomplished easily and safely by adding moisture-holding foam around the water vapor equilibrator on the outside of the CIRAS-3 enclosure. An Application Note is available from PP Systems highlighting this feature and it is available to everyone from our web site.

Settings – Gas Exchange and Chlorophyll Fluorescence (Continued)	
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 Intensity	Select your desired light intensity from 0-2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.
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). The best settings to use to simulate sunlight are 38% Red, 37% Green, 25% Blue and 0% White which are also the default values.
Temperature Sensor Measurement	IR Sensor - To use the PLC3 Universal cuvette's internal infrared sensor (but only if the leaf covers the entire window opening). Do not select IR Sensor 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. Energy Balance can be used at any time.

Settings – Gas Exchange and Chlorophyll Fluorescence (Continued)

Temperature Sensor Control Type	<p>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).</p> <p>Track Leaf to Ambient - Allows the leaf temperature to follow ambient temperature as measured by the cuvette’s handle-mounted thermistor sensor.</p> <p>Cuvette Temperature - Used to control the air in the cuvette at a fixed temperature that you enter under “Set Temperature” (see below).</p> <p>Track Cuvette to Ambient - Allows the cuvette temperature to follow air temperature as measured by the cuvette’s handle-mounted sensor.</p> <p>Disable Temperature Control - Intended mainly for diagnostic purposes but can also be used for field measurements if required or to conserve the batteries. 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.</p>
Set Temperature	<p>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(s) and the following:</p> <ul style="list-style-type: none"> • Leaf transpiration in the cuvette • Light (incident radiation) • Size and construction of the PLC window

Settings – Gas Exchange and Chlorophyll Fluorescence (Continued)

Leaf Area	The default value will be based on your cuvette selection under “Configuration”. This is very important. For the PLC3 Universal Leaf Cuvette the total area is known based on the Window size so if you fill the entire window with leaf material simply use the default values. If you do not fill the window area and don't know the actual leaf area of your sample then enter a lower estimate. For the PLC3 Conifer and Narrow leaf cuvettes it is very difficult to know the actual leaf area so again enter a lower estimate for leaf area prior to taking measurements. 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. This value is based on the default PLC3 Universal 18 x 25 mm window or the conifer or narrow cuvette supplied. Users should measure the boundary layer when changing out the PLC3 Universal windows as this value will vary from window to window. See Boundary Layer Determination (rb) on page 224.
Stomatal Ratio	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.

When Gas Exchange and Chlorophyll Fluorescence is the selected “Application” there are additional control settings available for measurement of chlorophyll fluorescence. Press **Set CFM (F7)**.

CFM-3 Recording Settings

CFM-3 Recording Settings

Record to File	<input checked="" type="checkbox"/> Calc Fluorescence	<input checked="" type="checkbox"/> Raw Fluorescence	<input checked="" type="checkbox"/> Gas Exchange	Cancel >	F1
Initial Fluorescence Measurement	Fv/Fm			Accept >	F2
	Initial Dark Adapt Period <input type="text" value="1"/> (min)			Expand List >	F3
Repeated Fluorescence Measurement	ΦPSII-SP				F4
Concluding Fluorescence Measurement	Fo'				F5
	Concluding Dark Adapt Period <input type="text" value="30"/> (min)				F6
Saturating Light					F7
Single Pulse Duration <input type="text" value="1"/> (s)	Single Pulse Intensity <input type="text" value="9000"/> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				F8
Multi-Pulse Duration <input type="text" value="0.3"/> (s per step)	Step 1 <input type="text" value="6000"/> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Step 2 <input type="text" value="5000"/> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Step 3 <input type="text" value="4000"/> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Help >	
	Step 4 <input type="text" value=""/>	Step 5 <input type="text" value=""/>		Charging 100%	
Modulating Light	Gain <input type="text" value="50"/> (%)	Modulation Level 1 to 4 <input type="text" value="4"/>			

Settings - CFM-3 Recording Settings

Record to File	Calc Fluorescence - Will be grayed out, indicating that fluorescence data must be written to the data file. 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 – To include gas exchange data with recorded measurements.
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Settings - CFM-3 Recording Settings (Continued)

Initial Fluorescence	<p>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 display under "Saturating Light".</p> <p>ϕPSII-MultiPulse - If selected, there is no need to dark adapt the leaf and a "MultiPulse" sequence can begin right away with multiple, saturating pulses applied to the light-adapted leaf sample. The MultiPulse duration and intensity settings (up to 5 steps) can be set in the lower part of the display under "Saturating Light".</p> <p>Fv/Fm - If selected you will need to allow the plant to dark acclimate prior to measurement. If performing Fv/Fm measurements we recommend doing so at night or before sunrise to save time. This will ensure that the leaves are completely dark adapted and will allow for more measurements to be taken over shorter periods. As an added precaution you can cover the CFM-3 with a dark cloth to ensure that the inside chamber is completely dark. Set the Initial Dark Adapt Period of your choosing. Longer dark adapt period are required for plants that have been exposed to high light and shorter periods for plants that have been in the dark or low light.</p> <p>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.</p> <table border="1" data-bbox="502 1600 1269 1736"> <thead> <tr> <th data-bbox="509 1600 654 1693"><i>DA period (minutes)</i></th><th data-bbox="654 1600 687 1693">5</th><th data-bbox="768 1600 801 1693">10</th><th data-bbox="882 1600 915 1693">15</th><th data-bbox="997 1600 1029 1693">20</th><th data-bbox="1111 1600 1144 1693">25</th><th data-bbox="1225 1600 1258 1693">30</th></tr> </thead> <tbody> <tr> <td data-bbox="509 1693 589 1736">F_v/F_m</td><td data-bbox="654 1693 719 1736">0.63</td><td data-bbox="768 1693 833 1736">0.71</td><td data-bbox="882 1693 948 1736">0.78</td><td data-bbox="997 1693 1062 1736">0.81</td><td data-bbox="1111 1693 1176 1736">0.80</td><td data-bbox="1225 1693 1290 1736">0.81</td></tr> </tbody> </table>	<i>DA period (minutes)</i>	5	10	15	20	25	30	F_v/F_m	0.63	0.71	0.78	0.81	0.80	0.81
<i>DA period (minutes)</i>	5	10	15	20	25	30									
F_v/F_m	0.63	0.71	0.78	0.81	0.80	0.81									

Settings - CFM-3 Recording Settings (Continued)

Repeated Fluorescence Measurement	<p>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).</p> <p>None. If selected, there will be no chlorophyll fluorescence measurements performed after a photosynthesis measurement has been recorded.</p> <p>φPSII-SP (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".</p> <p>φPSII-MP (MultiPulse) - If selected, multiple, saturating pulses will be applied to the light-adapted leaf sample. The MultiPulse duration and intensity settings (up to 5 steps) can be set in the lower part of the screen under "Saturating Light".</p> <p>Fs - If this is selected, the steady state fluorescence will be recorded after a photosynthesis measurement is recorded.</p>
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Settings - CFM-3 Recording Settings (Continued)

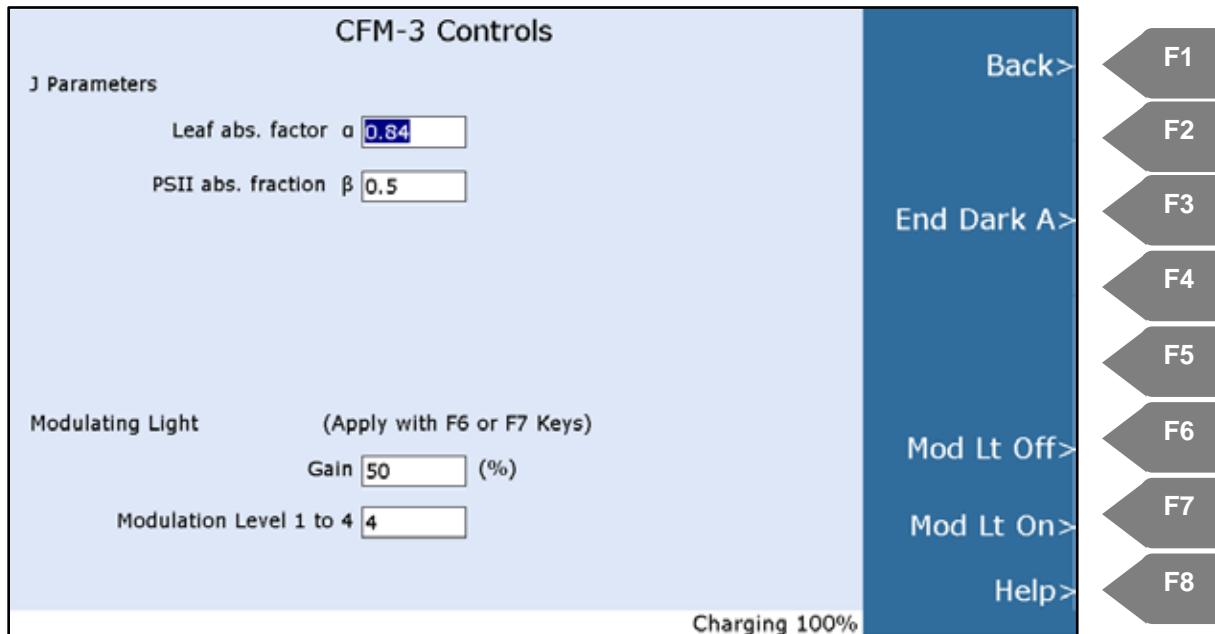
Concluding Fluorescence Measurement	<p>This defines the final measurement when recording is terminated.</p> <p>None - If this is selected, there will be no concluding chlorophyll fluorescence measurement and the sequence will terminate after the last photosynthesis measurement.</p> <p>Fo' - If selected, far-red light will be applied to the leaf sample.</p> <p>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 dark adapting the leaf for at least 20-30 minutes for best results.</p>
Saturating Light	<p>Single Pulse Measurements</p> <p>Single Pulse Duration - The time (in seconds) for the Single Pulse Duration saturating light to be applied.</p> <p>Single Pulse Intensity - The saturation pulse intensity (0-10000 $\mu\text{mol m}^{-2} \text{s}^{-1}$).</p> <p>MultiPulse Measurements</p> <p>MultiPulse Duration - The time (in seconds) for each multiple pulse duration saturating light to be applied. For MultiPulse 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the high end and one step below 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the lower end with an even distribution of light in between the high and low light settings.</p>

Settings - CFM-3 Recording Settings (Continued)

Modulating Light	<p>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. Please note that users can dynamically change the gain during a measurement sequence under Controls (F3) > CFM-3 Cntrl if required to save time.</p> <p>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 F_o' under far-red light. Please note that users can dynamically change the Modulation Level during a measurement sequence under Controls (F3) > CFM-3 Cntrl if required to save time.</p>
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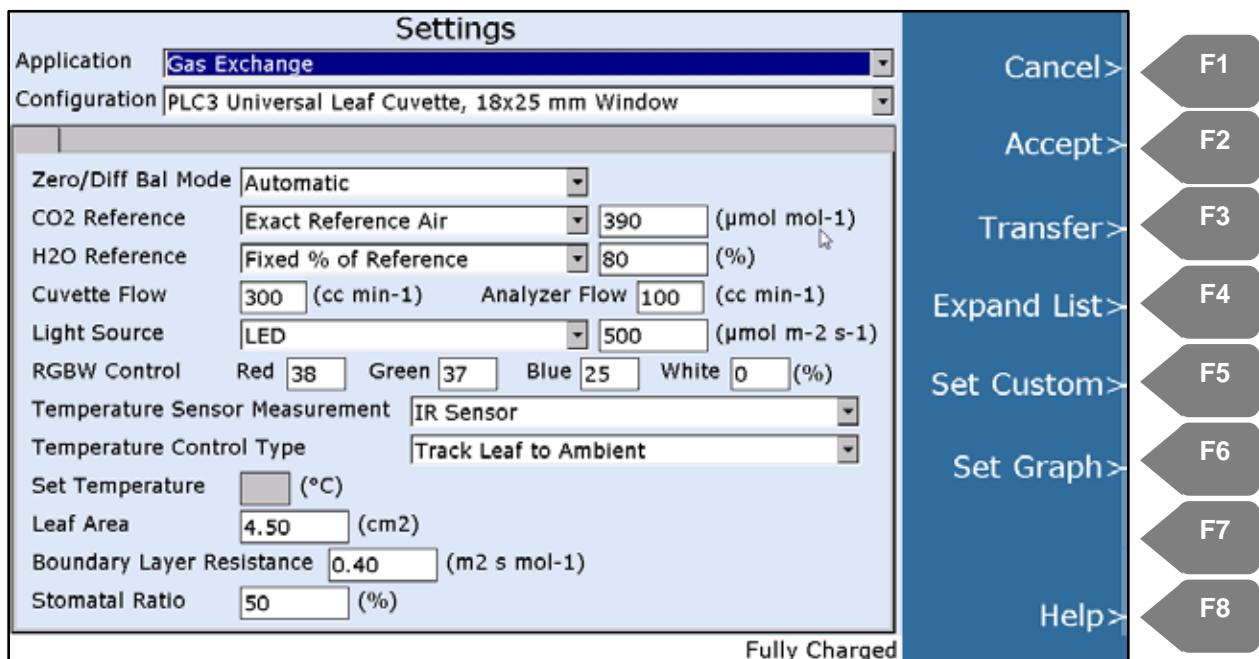
CFM-3 Controls

There are some additional CFM-3 related controls available to users under **Controls (F3) > CFM-3 Cntrl (F7)** to allow users to dynamically perform additional functions during measurement sequences.



CFM-3 Controls	
J Parameters	<p>These are parameters used for calculation of electron transport rate.</p> <p>Leaf abs. factor – The default leaf absorbance factor is set to 0.84 but can be determined by use of an integrating sphere if required.</p> <p>PSII abs. factor - The default PSII absorbance factor is set to 0.5 but a known range of values exist for different plant types.</p>
Modulating Light	<p>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.</p> <p>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 F_o' under far-red light.</p>
End Dark A (F3)	To manually terminate the dark adaption period before the elapsed time under CFM-3 Recording Settings. 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-3 Control
Mod Lt Off (F6)	Turns the modulating light source off.
Mod Lt On (F7)	Turns the modulating light source on.

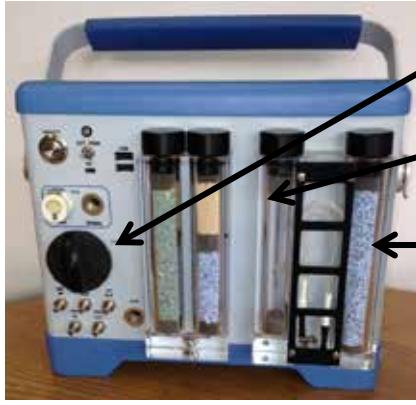
Settings – Gas Exchange



Settings – Gas Exchange

Zero/Diff Bal Mode	<p>Manual - You will be prompted when to perform a Zero or Diff Bal.</p> <p>Automatic - Zeros and Diff Bals will be performed automatically every 30 minutes. We recommend this mode if small ΔCO₂ and ΔH₂O is anticipated and for less experienced users. A Diff Bal cycle will also take place when there is a large change in CO₂ (100 μmol mol⁻¹) or H₂O (4 mb) concentration.</p> <p>Auto Zero, Stored Diff Bal - This option can be useful and is highly recommended for situations where large step changes in chamber CO₂ and H₂O are intended, such as occurs with CO₂ in A/Ci curves. Prior to using this option, you must perform a Stored Diff Bal (See Store Diff Bal on page 103).</p>
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Settings – Gas Exchange (Continued)

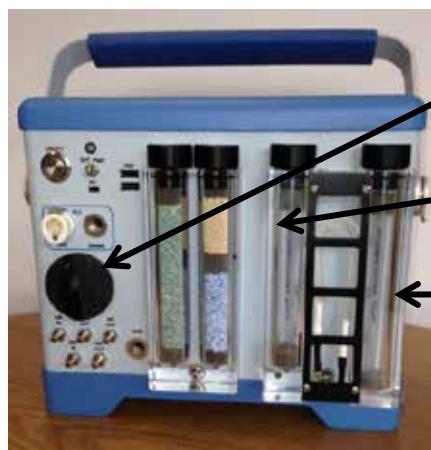
CO₂ Reference	<p>Approximate Reference Air - The CO₂ supplied to the leaf chamber from CIRAS-3's internal CO₂ source will generally be within 10-20 µmol mol⁻¹ from the actual set value within the control range of 0-2000 µmol mol⁻¹. Prior to using this option, you should perform a Max C (See Find Max C on page 101) for optimal accuracy and control. For response curves or in situations that require frequent, large changes in CO₂ levels, this option is recommended because it settles near the set value very quickly (within 1-2 minutes).</p> <p>Exact Reference Air - The CO₂ supplied to the leaf chamber from CIRAS-3's internal CO₂ 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 CO₂ level throughout a measurement sequence or for long periods, this option is ideal.</p> <p>Fixed Analysis Air - Allows CO₂ control on the analysis gas (feedback from the leaf) as it is measured by the Analysis side IRGA.</p> <p>Ambient (Remove Chemicals) - This option is frequently used to supply the natural outdoor CO₂ to the leaf chamber. Several considerations are involved whenever the stable internal CO₂ source (CO₂ cartridge) is not used. If this option is selected, the CO₂ regulator should be empty but in place and the soda lime must be removed from the CO₂/H₂O control column as shown below. Humidity control can still be available if the H₂O Absorber column is filled with Drierite.</p>  <ul style="list-style-type: none"> CO₂ regulator in place (without CO₂ cartridge inserted) Empty CO₂ absorber column for ambient measurement of CO₂ Note, H₂O control is still available with Drierite in this absorber column
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Settings – Gas Exchange (Continued)

H₂O Reference (chamber humidity)	<p>Fixed % of Reference - This option is most common. Leaves add water vapor to the air stream so the incoming air must be drier than ambient for the leaf's humidity environment to match outside. Stomatal conductance and the change in humidity due to the leaf vary widely for different types of leaves and environmental conditions. We recommend a setting of 80% to begin with. 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). 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%.</p> <p>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.</p>
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Constant VPD - Automatically adjusts chamber humidity to maintain a consistent leaf-to-air vapor pressure deficit between the absolute range 0-100 mb (0-10 kPa). 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.



CO₂ regulator in place (without CO₂ cartridge inserted)

Empty CO₂ absorber column for ambient measurement of CO₂

Empty H₂O absorber column for ambient measurement of H₂O

TIP

Often, the desired environment for photosynthesis measurements is for the leaf cuvette to be controlled to ambient humidity conditions outside the cuvette. If instead, it is desired to have the leaf cuvette humidity above ambient, it can be accomplished easily and safely by adding moisture-holding foam around the water vapor equilibrator on the outside of the CIRAS-3 enclosure. An Application Note is available from PP Systems highlighting this feature and it is available to everyone from our web site.

Settings – Gas Exchange (Continued)	
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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Maximum light intensity will be dependent on type of light unit. Ambient - For measurements under natural light 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). The best settings to use to simulate sunlight are 38% Red, 37% Green, 25% Blue and 0% White which are also the default values.

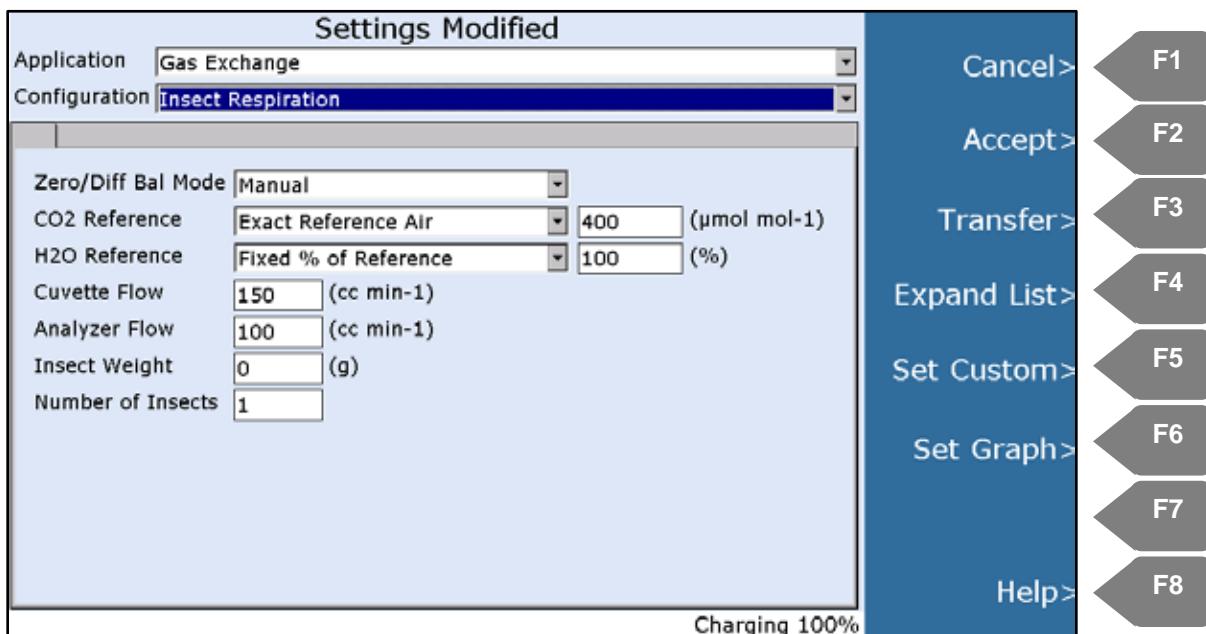
Settings – Gas Exchange (Continued)

Temperature Sensor Measurement (Available options are dependent on PLC3 type)	<p>IR Sensor - to use the PLC3 Universal cuvette's internal infrared sensor (but only if the leaf covers the entire window opening). Do not select IR Sensor 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.</p> <p>Energy Balance - must be selected when the cuvette window does not have complete coverage by the leaf. Energy Balance can be used at any time.</p>
Temperature Sensor Control Type (Available options are dependent on PLC3 type)	<p>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).</p> <p>Track Leaf to Ambient - Allows the leaf temperature to follow ambient temperature as measured by the cuvette's handle-mounted thermistor sensor.</p> <p>Cuvette Temperature - Used to control the air in the cuvette at a fixed temperature that you enter under "Set Temperature" (see below).</p> <p>Track Cuvette to Ambient - Allows the cuvette temperature to follow air temperature as measured by the cuvette's handle-mounted sensor.</p> <p>Disable Temperature Control - Intended mainly for diagnostic purposes but can also be used for field measurements if required or to conserve the batteries. 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.</p>

Settings – Gas Exchange (Continued)

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(s) and the following: <ul style="list-style-type: none">• Leaf transpiration in the cuvette• Light (incident radiation)• Size and construction of the PLC window
Leaf Area	The default value will be based on your cuvette selection under “Configuration”. This is very important. For the PLC3 Universal Leaf Cuvette the total area is known based on the Window size so if you fill the entire window with leaf material simply use the default values. If you do not fill the window area and don't know the actual leaf area of your sample then enter a lower estimate. For the PLC3 Conifer and Narrow leaf cuvettes it is very difficult to know the actual leaf area so again enter a lower estimate for leaf area prior to taking measurements. 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. See Boundary Layer Determination (rb) on page 224.
Stomatal Ratio	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 – Gas Exchange (Insect Respiration)



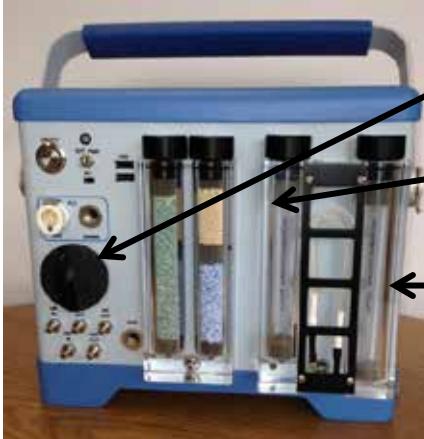
Settings – Gas Exchange (Insect Respiration)

Zero/Diff Bal Mode	<p>Manual - You will be prompted when to perform a Zero or Diff Bal. For insect respiration measurements this is the default Zero type and the method we recommend.</p> <p>Automatic - Zeros and Diff Bals will be performed automatically every 30 minutes. We recommend this mode if small ΔCO_2 and $\Delta\text{H}_2\text{O}$ is anticipated and for less experienced users. A Diff Bal cycle will also take place when there is a large change in CO_2 ($100 \mu\text{mol mol}^{-1}$) or H_2O (4 mb) concentration.</p> <p>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. Prior to using this option, you must perform a Stored Diff Bal (See Store Diff Bal on page 103).</p>
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Settings – Gas Exchange - Insect Respiration (Continued)

CO₂ Reference	<p>Approximate Reference Air - The CO₂ supplied to the insect chamber from CIRAS-3's internal CO₂ source will generally be within 10-20 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 101) for optimal accuracy and control.</p> <p>Exact Reference Air - The CO₂ supplied to the insect chamber from CIRAS-3's internal CO₂ 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 CO₂ level throughout a measurement sequence or for long periods, this option is ideal.</p> <p>Fixed Analysis Air – Not applicable for insect respiration measurements and should not be selected.</p> <p>Ambient (Remove Chemicals) - This option is frequently used to supply the natural outdoor CO₂ to the insect chamber. Several considerations are involved whenever the stable internal CO₂ source (CO₂ cartridge) is not used. If this option is selected, the CO₂ regulator should be empty but in place and the soda lime must be removed from the CO₂/H₂O control column as shown below. Humidity control can still be available if the H₂O Absorber column is filled with Drierite.</p>  <p>CO₂ regulator in place (without CO₂ cartridge inserted)</p> <p>Empty CO₂ absorber column for ambient measurement of CO₂</p> <p>Note, H₂O control is still available with Drierite in this absorber column</p>
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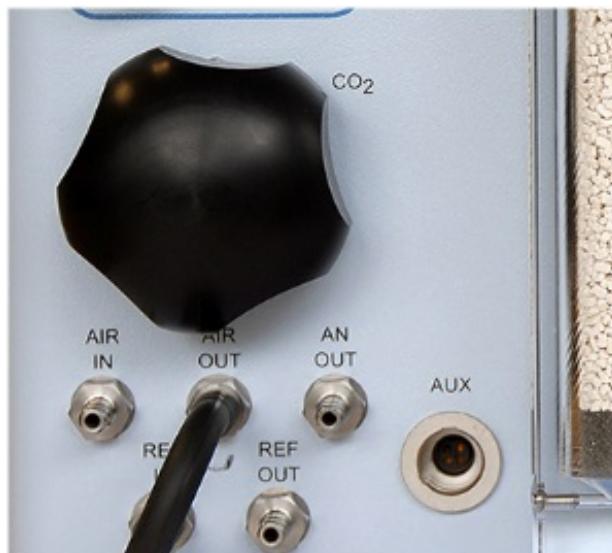
Gas Exchange - Insect Respiration (Continued)

H2O Reference (chamber humidity)	<p>Fixed % of Reference - This option is most common and can be set to produce a chamber humidity from 0-100% of ambient.</p> <p>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.</p> <p>Constant VPD – Not applicable for insect respiration measurements and should not be selected.</p> <p>Ambient (Remove Chemicals) - To supply water vapor unaltered from the surrounding environment to the insect chamber. Several considerations are involved whenever stable, internally generated water is not used.</p>  <ul style="list-style-type: none">CO₂ regulator in place (without CO₂ cartridge inserted)Empty CO₂ absorber column for ambient measurement of CO₂Empty H₂O absorber column for ambient measurement of H₂O
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Gas Exchange - Insect Respiration (Continued)

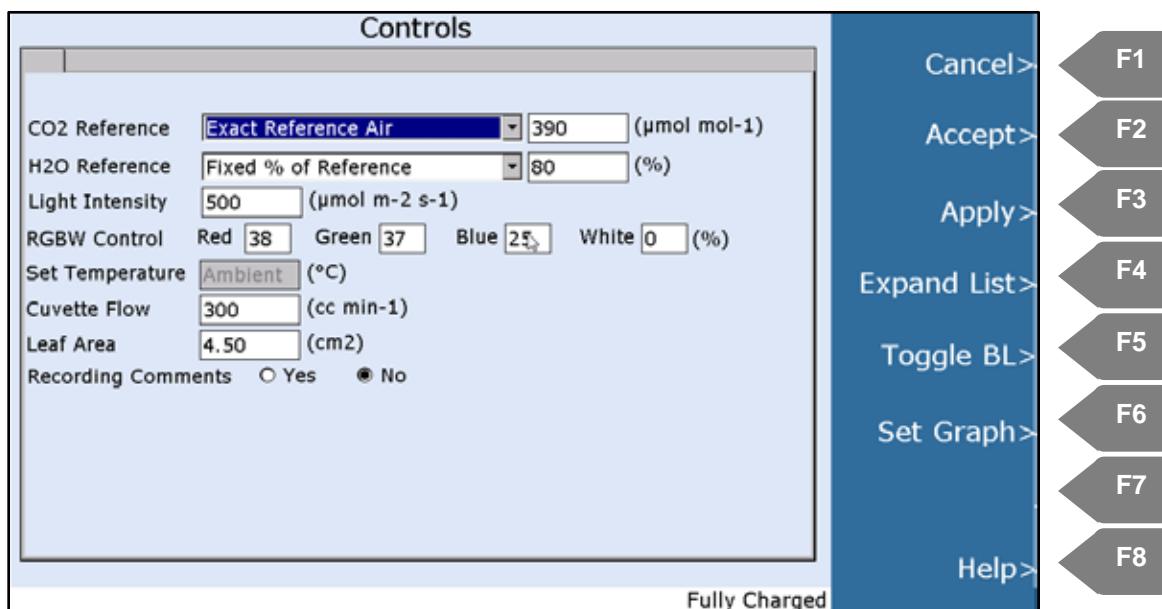
Cuvette Flow	Reference gas flow rate entering the chamber within the range of 150 to 500 cc min ⁻¹ . Changing flow rate during an experiment is not recommended. Instead, determine an optimal flow rate before beginning important measurements and then maintain that flow rate throughout the experiment.
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.
Insect Weight	Weight of insects (g). Enter the weight in this field for proper calculation of results.
Number of Insects	The number of insects placed inside the chamber. Enter the number of insects in this field for proper calculation of results.

Make sure that the link pipe is in place connecting the “REF IN” and “AIR OUT as shown below when performing measurement of insect respiration in order to take advantage of the internal gas blender and air supply.



Section 9. Controls (F3)

One of the many powerful features with the CIRAS-3 system is the ability to quickly, accurately and independently control CO₂, H₂O, temperature and light for Gas Exchange and Chlorophyll Fluorescence applications. **Controls (F3)** allows you to dynamically change many of the default options you have made previously under **Settings (F2)** 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 CO₂, H₂O, light factors, temperature, flow rate and leaf area. Press **Controls (F3)** to enter the Controls dialog and make necessary changes then press **Accept (F2)**. The status bar in the lower left corner will display the message “Updating Environmental Control Values” indicating the new control values have been accepted.



Controls	
CO2 Reference	Options are identical to those described in Settings (Refer to Settings – Gas Exchange on page 143).
H2O Reference	Options are identical to those described in Settings (Refer to Settings – Gas Exchange on page 143).
Light Intensity	Options are identical to those described in Settings (Refer to Settings – Gas Exchange on page 143).
RGBW Control	Options are identical to those described in Settings (Refer to Settings – Gas Exchange on page 143).
Set Temperature	Options are identical to those described in Settings (Refer to Settings – Gas Exchange on page 143).

Controls (Continued)

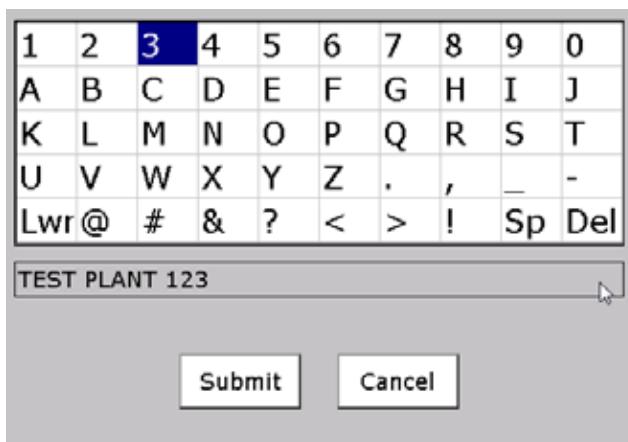
Cuvette Flow	Alteration to an existing flow rate causes transient disruption of CO ₂ control which in turn affects calculated assimilation rate and Ci so wait a minute or so for the system to readjust before collecting data. If the new Control value is ± 20% of the old value a brief message will appear 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 (Refer to Settings – Gas Exchange on page 143).

Adding Notes/Comments to data files

It is very easy to add notes or comments to data files up to 32 characters in length. When the Controls dialog is opened as shown above press the TAB key down to “Recording Comments” and highlight Yes. When highlighted press **Comment (F5)** to launch the built-in alpha-numeric keypad as shown below:



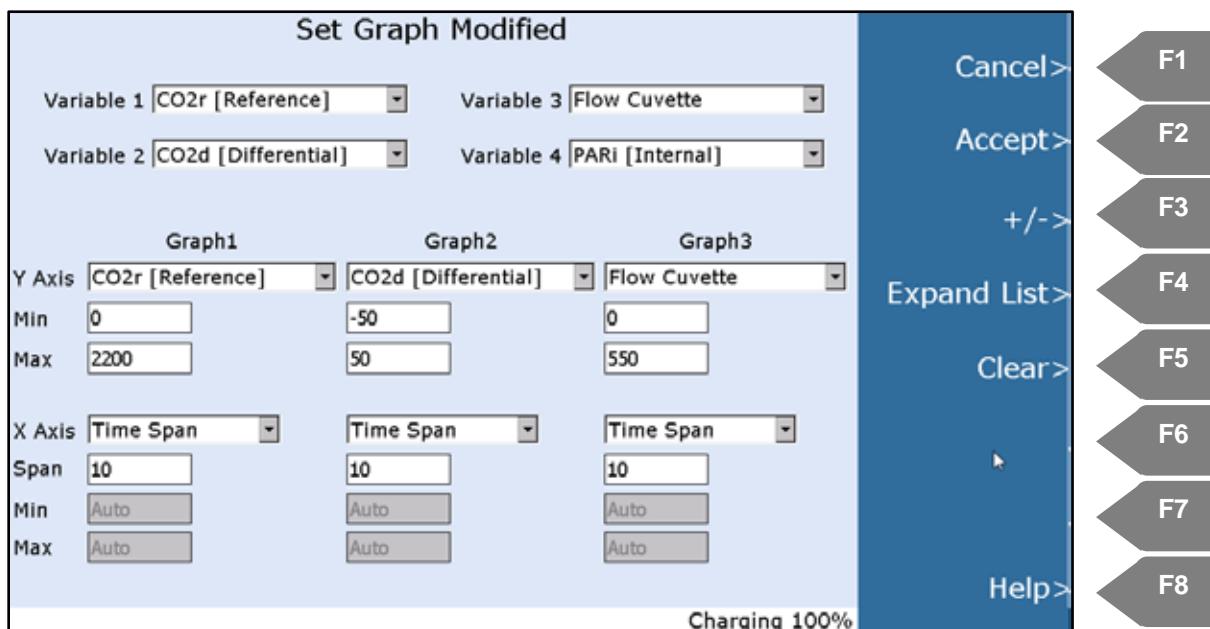
To add characters simply highlight the character using the arrow keys and press the green **OK** key on the keypad. As characters are entered they will appear in the box below the characters as shown on the following page. If the field is already populated with a previous comment simply place the cursor in the field and press Del on the built-in alpha-numeric keypad (not the DEL key on the CIRAS-3 console keypad) to delete the previous comment and enter the new one.



When finished press the TAB key and with **Submit** highlighted press the green **OK** key on the keyboard.

Set Graph (F6)

Press **Controls (F3) > Set Graph (F6)** to change your Graph display options. You have complete flexibility to plot any parameter against time or any parameter against another parameter. You can also set up to 3 different graph plots along with up to 4 user-defined parameters. Press **Settings (F2) > Set Graph (F6)** to configure your graphical display.



When you are in any field you can press the **Expand List (F4)** to see all available options for the parameter selected. Use the up and down arrows to move quickly through the list. The first variable in the list is **None**. Select **None** if you do not want a parameter displayed or make a selection and press **OK**. Once this has been configured it will be very easy for you to focus on the measured and calculated parameters that are of most importance to you. Because the dynamic range of a leaf sample's

physiology may be unknown to you, it might be difficult to set the correct Y-axis scale (i.e. to keep constant oversight of rapid changes and trends over time). Here, **Set Graph (F6)** will allow you to make quick corrections to Y-axis scales. The options are identical to those described in **Settings (F2)> Set Graph (F6)**. Press **Controls (F3) > Graph Set (F6) > Clear (F5)** at any time to refresh plotted data in the graphs.

The graph or graphs that you choose to display can be customized in two basic ways: the parameters 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 parameters. Again, with the dropdown box highlighted, make your selection. Press TAB again to enter a value in the **Min** field and the same for the **Max** field. Press the **+/- (F3)** function key to enter a negative value after you make your entry, which is normally 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.

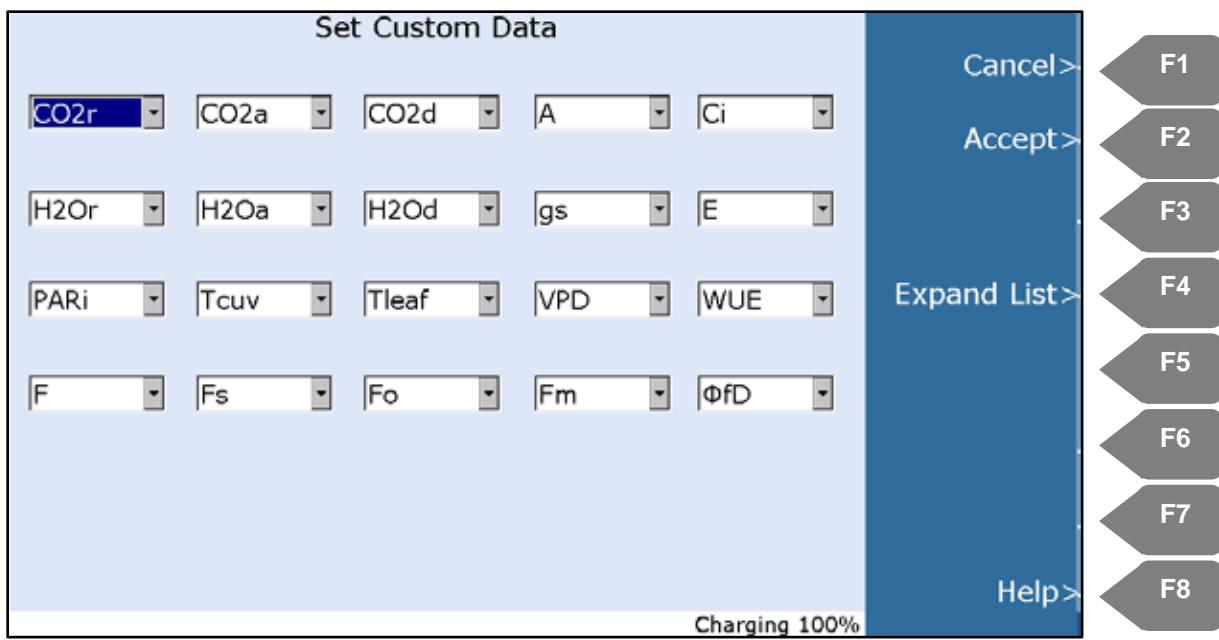
The first variable in the **Y Axis** list is **None**. Select **None** if you do not want the graph displayed. For example, if you set up **Graph1** as described above and select **None** for both Graph2 and Graph3, you will see a single large graph. Select **None** for Graph3 to display two graphs only,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

Set Custom (F5)

A custom display is available for the following 3 Applications:

- Chlorophyll Fluorescence
- Gas Exchange and Chlorophyll Fluorescence
- Gas Exchange

The custom display can be configured based on user-defined parameters allowing you to focus on specific data. Again you have complete flexibility and can view up to 20 user-defined parameters. Press **Settings (F2) > Set Custom (F5)** to configure your Custom display. Select **None** for any field if you don't want a parameter displayed for that particular field.



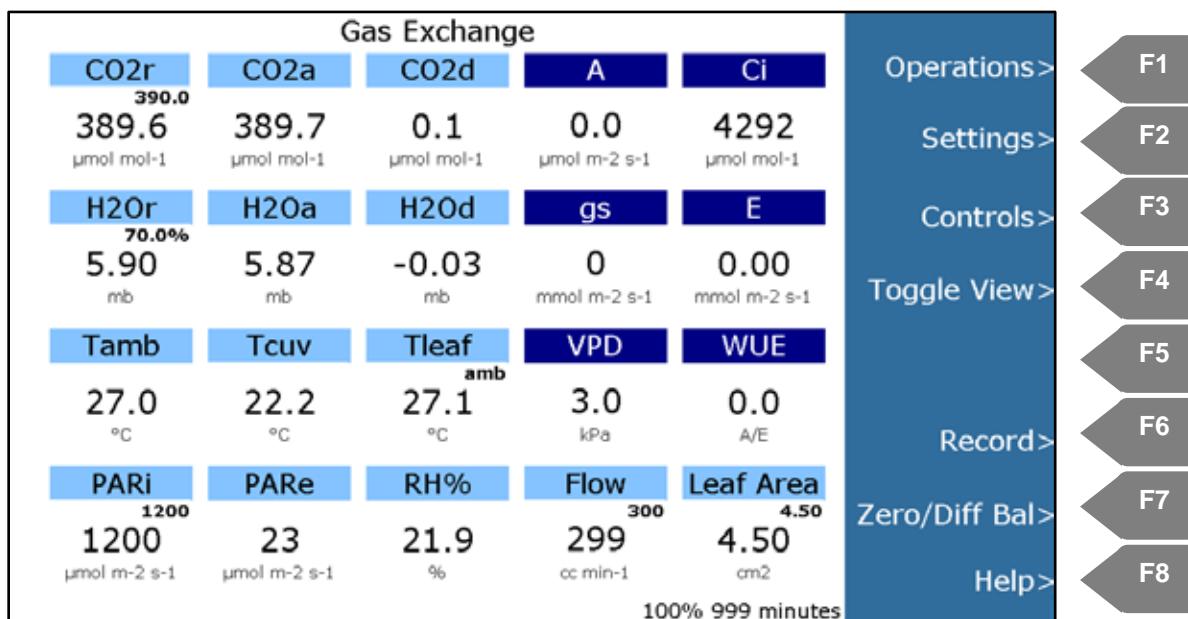
Toggle View (F4)

Toggle View (F4) is a simple function key used to switch between Gas Exchange, Chlorophyll Fluorescence, Graph and Custom displays.

Section 10. CIRAS-3 - Measurement of Gas Exchange

Measurement of leaf gas exchange with our PLC3 leaf cuvettes is one of the most popular applications for CIRAS-3. Prior to performing leaf gas exchange measurements make sure that you have selected **Gas Exchange** for your “Application” and the proper leaf cuvette is selected in “Configuration” under **Settings (F2)**. See [Settings – Gas Exchange](#) on page 143.

Refer to [Measurement of Leaf Gas Exchange – Introduction and Overview](#) on page 21 for instructions and advice related to the measurement of leaf gas exchange. This step by step tutorial steps you through just about everything you will need to know for measurement of leaf gas exchange. We also strongly recommend that you refer to [Section 1. Quick Start](#) on page 56 for some simple, pre-measurement system checks to ensure that the system is working properly.



Gas Exchange Measured and Calculated Data

Gas Exchange 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 ₂ O Reference (mb)
H2Oa	H ₂ O Analysis (mb)

Gas Exchange – Measured Data (Continued)

H2Od	H ₂ O Differential (mb)
Tamb	Temperature Ambient (°C)
Tcuv	Temperature in Cuvette (°C)
Tleaf	Leaf Temperature (°C)
PARi	PAR Internal (μmol m ⁻² s ⁻¹)
PARe	PAR External (μmol m ⁻² s ⁻¹)
RH%	Relative Humidity inside Leaf Chamber (%)
Flow	Cuvette Flow Rate (cc min ⁻¹)
Leaf Area	Leaf Area (cm ²)

Gas Exchange – Calculated Data

A	Assimilation (μmol CO ₂ m ⁻² s ⁻¹)
Ci	Sub-stomatal CO ₂ Concentration (μmol mol ⁻¹)
gs	Stomatal Conductance (mmol H ₂ O m ⁻² s ⁻¹)
E	Transpiration (mmol H ₂ O m ⁻² s ⁻¹)
VPD	Leaf to Air Vapor Pressure deficit (kPa)
WUE	Photosynthetic Water Use Efficiency (mmol CO ₂ mol ⁻¹ H ₂ O)

Inspect Desiccants and Absorber Columns

This is perhaps one of the most important system checks that can be performed prior to starting a measurement campaign. The desiccants are not only used for controlling CO₂ and H₂O but also for Auto-Zero which is a critical part of the system. The Auto-Zero is performed periodically throughout the measurement process to ensure the long term stability and accuracy of the internal CO₂ and H₂O gas analyzers. As long as the “Zero” column desiccants (soda lime, Drierite and molecular sieve) are fresh and maintained properly the system will receive good zero A/D readings during Auto-Zero cycles and everything should perform as expected. However, if any of the desiccants (especially the molecular sieve) are exhausted this will lead to inaccurate zero A/D readings during Auto-Zero cycles and will result in a drift in CO₂ and H₂O readings which will lead to errors.

Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements. Refer to [Application – Gas Exchange and Analyzer Only](#) on page 178 for information related to output of stored data.

Refer to [Gas Exchange Equations Used in CIRAS-3](#) on page 231 for information related to gas exchange equations and calculations used in CIRAS-3.

Section 11. CIRAS-3 - Measurement of Gas Exchange and Chlorophyll Fluorescence

Prior to performing gas exchange and chlorophyll fluorescence measurements you must make sure that you have selected **Gas Exchange and Chlorophyll Fluorescence** for your “Application” and the proper leaf cuvette (based on window size) in “Configuration” under **Settings (F2)**. See [Settings – Gas Exchange and Chlorophyll Fluorescence](#) on page 131.

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. 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

Important Note

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. To verify or change the window on your PLC3 Leaf Cuvette refer [Replacement of PLC3 Glass Window \(For Use with CFM-3\)](#) on page 227 for instructions on how to replace the window.

Refer to [Measurement of Leaf Gas Exchange and Chlorophyll Fluorescence](#) on page 46 for instructions and advice related to the measurement of leaf gas exchange and chlorophyll fluorescence. This step by step tutorial steps you through just about everything you will need to know for measurement of leaf gas exchange and chlorophyll fluorescence. We also strongly recommend that you refer to [Section 1. Quick Start](#) on page 56 for some simple, pre-measurement system checks to ensure that the system is working properly.

The measurement process, leaf cuvette connection and displays are very similar to measurement of leaf gas exchange. However, in addition to the additional fluorescence related settings there is one additional numeric display showing all the chlorophyll fluorescence measured and calculated data as shown on the following page.

Chlorophyll Fluorescence					
F	F _s	F _o	F _v	F _m	Operations>
687	673	618	1392	2010	Settings>
F _v /F _m	F _{o'}	F _{v'}	F _{m'}	F _{v'} /F _{m'}	Controls>
0.693	414	360	774	0.465	Toggle View>
Φ _{PSII}	j	qP	qNP	NPQ	End Record>
0.130	65.520	0.281	0.775	1.597	Record>
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	Zero/Diff Bal>
0.173	0.335	0.535	0.335	0.291	Help>
Fully Charged					

Chlorophyll Fluorescence Measured and Calculated Data

Chlorophyll Fluorescence Data		
Parameter	Measured or Calculated	Description
F	Measured	Fluorescence signal (current)
F _s	Measured	Fluorescence steady state (Steady-state Fluorescence Yield)
F _o	Measured	Fluorescence origin (Minimum Fluorescence Yield)
F _v	Calculated	Fluorescence variable (Variable Fluorescence Yield)
F _m	Measured	Fluorescence maximum (Maximum Fluorescence Yield)
F _v /F _m	Calculated	Maximal photochemical efficiency of PSII
F _{o'}	Measured	Fluorescence origin prime (Minimal Fluorescence Yield, sample illuminated by far-red light)
F _{v'}	Calculated	Fluorescence variable prime (Variable Fluorescence Yield, sample illuminated by far-red light and requires F _{o'})
F _{m'}	Calculated	Fluorescence maximum prime (Maximum Fluorescence Yield, sample illuminated)
F _{v'} /F _{m'}	Calculated	Photochemical efficiency of PSII, sample illuminated by far-red light and requires F _{o'}
Φ _{PSII}	Calculated	Photochemical efficiency of PSII, sample illuminated
J	Calculated	Thylakoid electron transport rate (μmol e ⁻ m ⁻² s ⁻¹)

Chlorophyll Fluorescence Data (Continued)		
Parameter	Measured or Calculated	Description
qP	Calculated	Photochemical quenching coefficient, correlates to proportion of open PSII reaction centers in puddle antenna model and requires F_o'
qNP	Calculated	Non-photochemical quenching, older term, insensitive to higher quenching values and 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)
ϕfD	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)

When this application is selected the CIRAS-3 has additional options and controls available (**CFM-3 Recording Settings and CFM-3 Controls**) under **Settings (F2)** to allow the CIRAS-3 to perform a user-defined measurement of chlorophyll fluorescence at the same time a gas exchange measurement is recorded. Prior to making any measurements you must make sure that both your gas exchange settings and CFM-3 recording settings are in place and ready to go. The gas exchange settings are identical to those described in the previous section with the only difference being that there is no option to select the type of light unit. Instead you are only required to enter a Light Intensity (See [Settings – Gas Exchange and Chlorophyll Fluorescence](#) on page 131) to set up before recording data. Go to **Settings (F2) > Set CFM (F7)** to set up user-defined settings associated with measurement of chlorophyll fluorescence.

Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements.

Refer to [Application – Gas Exchange and Chlorophyll Fluorescence](#) on page 177 for information related to output of stored data.

Refer to [Chlorophyll Fluorescence Calculations Used in CIRAS-3](#) on page 237 for information related to chlorophyll fluorescence equations and calculations used in CIRAS-3.

Section 12. CIRAS-3 - Closed System

Measurement

Prior to performing closed system measurement of soil respiration (with SRC-1 or SRC-2 Soil Respiration Chambers) or net canopy flux (CPY-4 and CPY-5 Canopy Assimilation Chambers) you must make sure that you have selected **Closed System** for your “Application” and the proper chamber in “Configuration” under **Settings (F2)**. See [Settings – Closed System](#) on page 129. See [SRC-2 Soil Respiration Chamber](#) on page 78 and [CPY-5 Canopy Assimilation Chamber](#) on page 78 for connecting up to the CIRAS-3.

Closed System Measurement Sequence

The measurement sequence for both soil respiration and canopy assimilation is virtually identical and on-line instructions are provided in the status bar (lower left hand corner of the display) on the CIRAS-3 console. The complete measurement sequence is as follows:

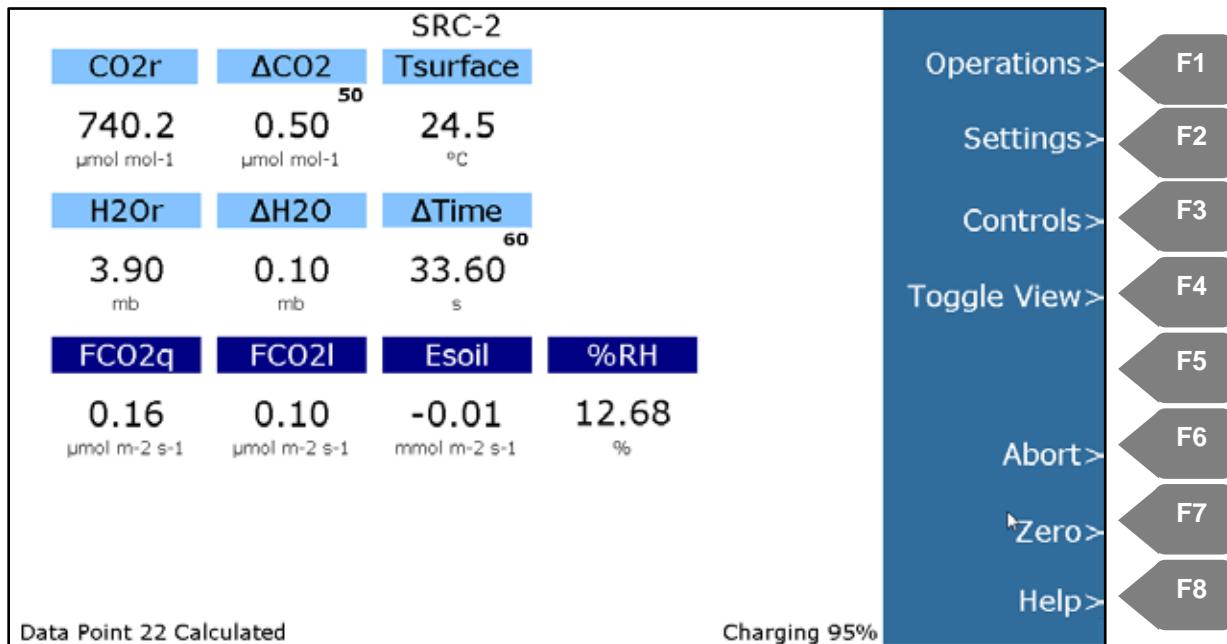
1. Begin CPY/SRC
2. Hold Chamber in Air to Flush – Data collection begins in x Sec
3. Position Chamber On Soil
4. Chamber Equilibrating
5. Data Collection Started
6. Sequence Complete.

The CIRAS-3 does not start collecting data until 45 seconds has elapsed from the time you hit **Start (F6)**. For the first 25 seconds the chamber flushes followed by another 20 seconds to allow you to place the chamber on the soil or collar and then to allow the system to equilibrate before measurements begin to be recorded.

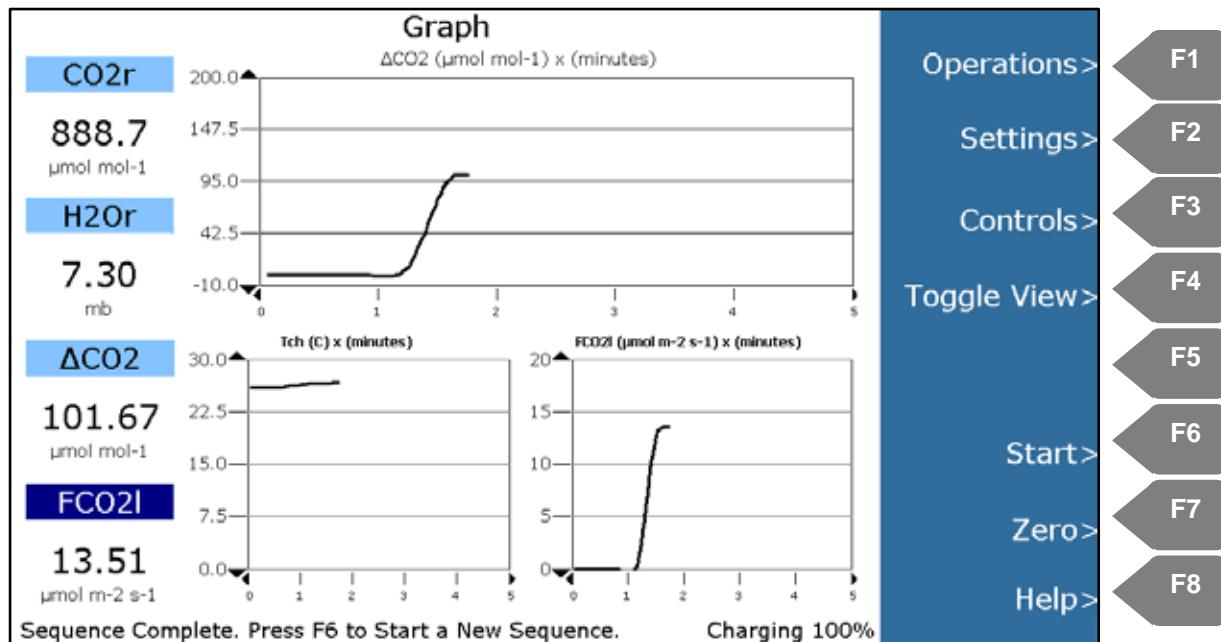
Initial Test Measurement Sequence (Recommended)

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. Simply press **Start** 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 pressing **Abort (F6)**. Note, data is not recorded during this sequence.

Numeric Display (SRC-2 Soil Respiration Chamber)



Graph Display (SRC-2 Soil Respiration Chamber)



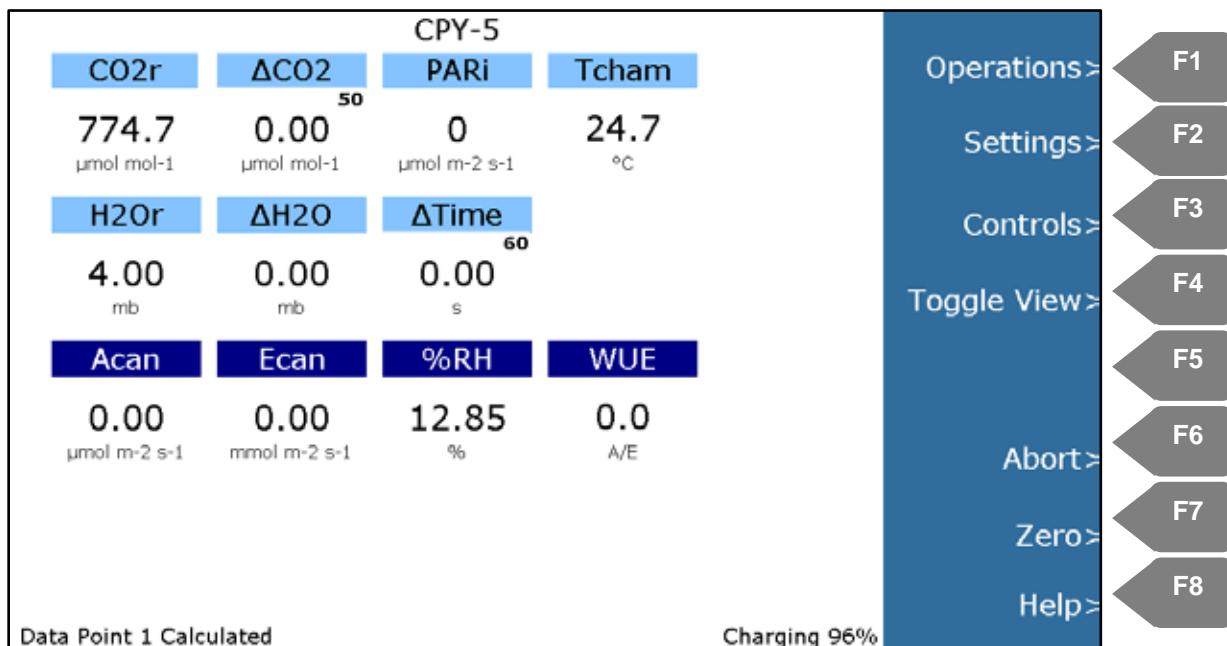
Soil Respiration Data

Measured Data	
CO2r	Current CO ₂ concentration ($\mu\text{mol mol}^{-1}$).
ΔCO_2	Change in CO ₂ concentration from the beginning of the measurement sequence ($\mu\text{mol mol}^{-1}$). The ΔCO_2 value in Settings is displayed in bold in the upper right hand corner of the field.
H2Or	Current H ₂ O concentration (mb)
$\Delta\text{H}_2\text{O}$	Change in H ₂ O concentration from the beginning of the measurement sequence (mb).
Tsoil / Tsurface	Soil temperature if STP-1 is used ($^{\circ}\text{C}$) or Surface temperature if SRC-2 is used ($^{\circ}\text{C}$).
ΔTime	Change in time during measurement sequence (Seconds). The ΔTime value in Settings is displayed in bold in the upper right hand corner of the field.
Calculated Data	
FCO2q	Efflux rate of soil CO ₂ based on quadratic fit ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\mu\text{mol m}^{-2} \text{ s}^{-1}$).
FCO2l	Efflux rate of soil CO ₂ based on linear fit ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\mu\text{mol m}^{-2} \text{ s}^{-1}$).
Esoil	Rate of change in evaporation ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\text{mmol m}^{-2} \text{ s}^{-1}$).
RH%	Relative humidity based on Tsurface (%).

Do I Use Linear versus Quadratic

The CIRAS-3 system automatically collects soil efflux data based on both a linear (FCO2l) or quadratic (FCO2q) fit. In the past quadratic fitting was most popular 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 with higher flux rates the quadratic fit is normally recommended. See [Soil Respiration and Canopy Flux Equations Used in CIRAS-3](#) on page 241.

Numeric Display (CPY-5 Canopy Assimilation Chamber)



Net Canopy CO₂ Flux Data

Measured Data	
CO2r	Current CO ₂ concentration (μmol mol ⁻¹).
ΔCO2	Change in CO ₂ concentration from the beginning of the measurement sequence (μmol mol ⁻¹). The ΔCO2 value in Settings is displayed in bold in the upper right hand corner of the field.
H2Or	Current H ₂ O concentration (mb)
ΔH2O	Change in H ₂ O concentration from the beginning of the 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). The ΔTime value in Settings is displayed in bold in the upper right hand corner of the field.
Tchamber	Air temperature inside the CPY-4/CPY-5 (°C).
Tsoil	Soil temperature if STP-1 is used (°C).

Calculated Data (Continued)	
Acan	Canopy Assimilation rate ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\mu\text{mol m}^{-2} \text{ s}^{-1}$).
Ecan	Evapotranspiration rate ($\text{g m}^{-2} \text{ hr}^{-1}$ or $\text{mmol m}^{-2} \text{ s}^{-1}$).
RH%	Relative humidity based on Tsurface (%).
WUE	Water use efficiency (Assimilation/Evapotranspiration). Note that WUE can only be calculated if the Evapotranspiration Units are set to $\text{mmol m}^{-2} \text{ s}^{-1}$ and Assimilation Units set to $\mu\text{mol m}^{-2} \text{ s}^{-1}$ under Settings (F2).

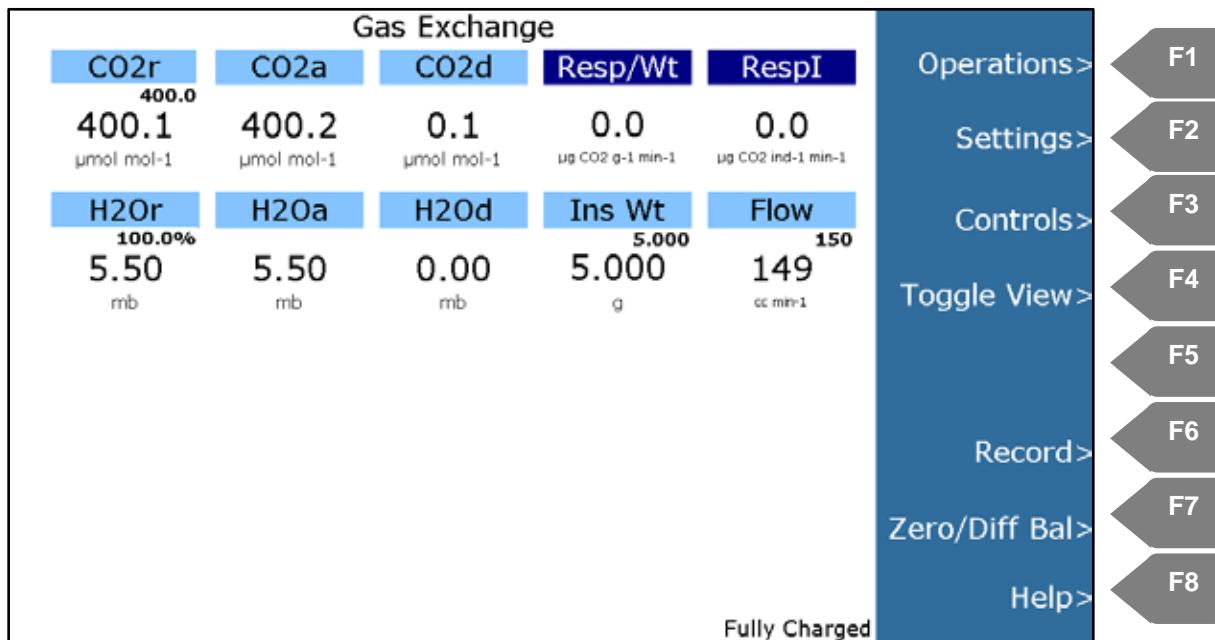
Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements.

Refer to [Application – Closed System](#) on page 175 for information related to output of stored data.

Refer to [Soil Respiration and Canopy Flux Equations Used in CIRAS-3](#) on page 241 for information related to closed system equations and calculations used in CIRAS-3.

Section 13. CIRAS-3 – Insect Respiration Measurements

Prior to performing insect respiration measurements you must make sure that you have selected **Gas Exchange** for your “Application” and **Insect Respiration** in “Configuration” under **Settings (F2)**. See [Settings – Gas Exchange for Insect Respiration](#) on page 149. See [Insect Respiration Chamber](#) on page 80 for connecting the insect respiration chamber to the CIRAS-3.



Insect Respiration Data

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 ₂ O Reference (mb)
H2Oa	H ₂ O Analysis (mb)
H2Od	H ₂ O Differential (mb)
Ins Wt	Weigh of insect (g)
Flow	Flow rate to the chamber (cc min ⁻¹)

Calculated Data (Continued)

Resp/Wt	Respiration rate per weight of insect ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ min}^{-1}$)
Respl	Respiration rater per number of insects ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ min}^{-1}$)

Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements.

Refer to [Application – Insect Respiration](#) on page 180 for information related to output of stored data.

Refer to [Insect Respiration - Flux Equations and Calculations](#) on page 245 for information related to insect respiration equations and calculations used in CIRAS-3.

Section 14. CIRAS-3 – Chlorophyll Fluorescence Measurements

Prior to performing chlorophyll fluorescence measurements you must make sure that you have selected **Chlorophyll Fluorescence** for your “Application” and the proper leaf cuvette (based on window size) in “Configuration” under **Settings (F2)**. See [Settings – Chlorophyll Fluorescence](#) on page 122.

The measurement process, leaf cuvette connections and displays are exactly as described in the previous section [CIRAS-3 - Measurement of Leaf Gas Exchange](#). However, there is another numeric display showing all the chlorophyll fluorescence data.

Chlorophyll Fluorescence					Operations> Settings> Controls> Toggle View> End Record> Record> Zero/Diff Bal> Help>
F	F _s	F _o	F _v	F _m	
687	673	618	1392	2010	
F _v /F _m	F _{o'}	F _{v'}	F _{m'}	F _{v'} /F _{m'}	
0.693	414	360	774	0.465	
ΦPSII	j	qP	qNP	NPQ	
0.130	65.520	0.281	0.775	1.597	
qL	ΦNO	ΦNPQ-K	ΦfD	ΦNPQ-G	
0.173	0.335	0.535	0.335	0.291	

Chlorophyll Fluorescence Data

Chlorophyll Fluorescence Data		
Parameter	Measured or Calculated	Description
F	Measured	Fluorescence signal (current)
F _s	Measured	Fluorescence steady state (Steady-state Fluorescence Yield)
F _o	Measured	Fluorescence origin (Minimum Fluorescence Yield)
F _v	Calculated	Fluorescence variable (Variable Fluorescence Yield)
F _m	Measured	Fluorescence maximum (Maximum Fluorescence Yield)
F _v /F _m	Calculated	Maximal photochemical efficiency of PSII

Chlorophyll Fluorescence Data (Continued)		
Parameter	Measured or Calculated	Description
F_o'	Measured	Fluorescence origin prime (Minimal Fluorescence Yield, sample illuminated by far-red light)
F_v'	Calculated	Fluorescence variable prime (Variable Fluorescence Yield, sample illuminated by far-red light and requires F_o')
F_m'	Calculated	Fluorescence maximum prime (Maximum Fluorescence Yield, sample illuminated)
F_v'/F_m'	Calculated	Photochemical efficiency of PSII, sample illuminated by far-red light and requires F_o'
ϕ_{PSII}	Calculated	Photochemical efficiency of PSII, sample illuminated
J	Calculated	Thylakoid electron transport rate ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$)
qP	Calculated	Photochemical quenching coefficient, correlates to proportion of open PSII reaction centers in puddle antenna model and requires F_o'
qNP	Calculated	Non-photochemical quenching, older term, insensitive to higher quenching values and 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)
ϕ_{NO}	Calculated	Non-photochemical quenching due to aggregate constitutive non-regulatory processes, lake antenna model, requires $F_o!$ (Kramer calculation)
ϕ_{NPQ-K}	Calculated	Non-photochemical quenching due to down-regulatory (pH, xanthophyll) processes, lake antenna model, requires $F_o!$ (Kramer calculation)
ϕ_{fD}	Calculated	Non-photochemical quenching due to aggregate constitutive non-regulatory processes, equally applicable to puddle and antenna models (Genty calculation)
ϕ_{NPQ-G}	Calculated	Non-photochemical quenching due to down-regulatory (pH, xanthophyll) processes, equally applicable to puddle and antenna models (Genty calculation)

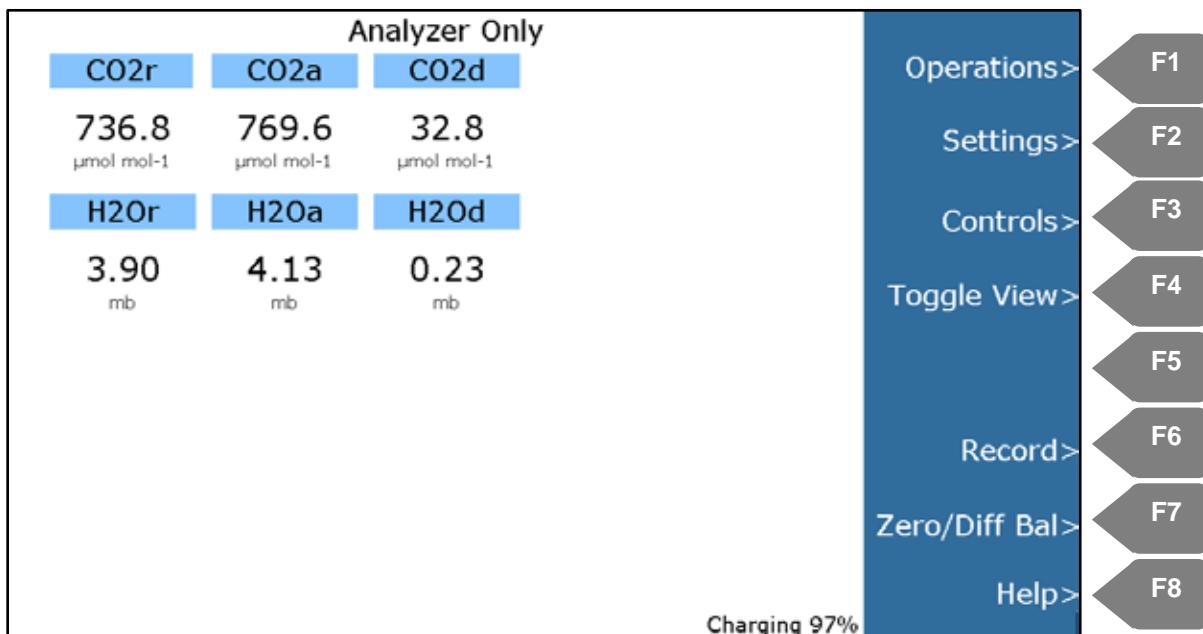
Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements.

Refer to [Application – Chlorophyll Fluorescence](#) on page 176 for information related to output of stored data.

Refer to [Chlorophyll Fluorescence Calculations Used in CIRAS-3](#) on page 237 for information related to chlorophyll fluorescence equations and calculations used in CIRAS-3.

Section 15. CIRAS-3 – Analyzer Only

Prior to performing stand-alone CO₂/H₂O gas analysis measurements you must make sure that you have selected **Analyzer Only** for your “Application” and **CIRAS-3 CO₂/H₂O Gas Analyzer** in “Configuration” under **Settings (F2)**. See [Settings – Analyzer Only](#) on page 121. Also note that link pipe between “REF IN” and “AIR OUT” required for leaf gas exchange measurements must be removed.



Gas Analyzer Data

Measured Data	
CO ₂ r	CO ₂ Reference (µmol mol ⁻¹)
CO ₂ a	CO ₂ Analysis (µmol mol ⁻¹)
CO ₂ d	CO ₂ Differential (µmol mol ⁻¹)
H ₂ O _r	H ₂ O Reference (mb)
H ₂ O _a	H ₂ O Analysis (mb)
H ₂ O _d	H ₂ O Differential (mb)

Refer to [Recording Options](#) on page 86 to set up your recording options prior to performing measurements.

Refer to [Application – Gas Exchange and Analyzer Only](#) on page 178 for information related to output of stored data.

Section 16. Data Output

Stored data can be output in several formats depending on the “Application” and “Configuration” selected under **Settings (F2)**.

Record Types (RecType)	
R	Data associated with gas exchange and analyzer only measurements.
M	Data associated with closed system measurements.
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 “ F_v/F_m ” in the Status column.
m3	Not in use at this time.
m4	Data associated with a F_o' measurement. Note the “ F_o' ” in the Status column.
m5	Data associated with a $\phi_{PSII-SP}$ measurement. Note the “ $\phi_{PSII-SP}$ ” in the Status column.
m8	Data associated with F_s (fluorescence signal). Note the “ F_s ” in the Status column.
m9	Data associated with a $\phi_{PSII-MP}$ (Apparent F_m') measurement. Note the “ $\phi_{PSII-MP}$ ” in the Status column.

Application – Closed System

Column	Header	Units	Column	Header	Units
1	Rec Type	M	13	Patm	mb
2	Excel Time	Date/Time	14	RH	%
3	Comments	User-defined	15	FCO2I	$\mu\text{mol m}^{-2} \text{s}^{-1}$ or $\text{g m}^{-2} \text{s}^{-1}$
4	ΔTime	Seconds	16	FCO2q	
5	CO2r	$\mu\text{mol mol}^{-1}$	17	Esoil	$\text{mmol m}^{-2} \text{s}^{-1}$ or $\text{g m}^{-2} \text{s}^{-1}$
6	ΔCO2	$\mu\text{mol mol}^{-1}$	18	Acan	$\mu\text{mol m}^{-2} \text{s}^{-1}$
7	H2Or	mb	19	Ecan	$\text{mmol m}^{-2} \text{s}^{-1}$
8	ΔH2O	mb	20	WUE	A/E
9	Tamb	$^{\circ}\text{C}$	21	CO2Max	$\mu\text{mol mol}^{-1}$
10	Tch	$^{\circ}\text{C}$	22	TimeMax	Seconds
11	Tsoil	$^{\circ}\text{C}$	23	Tsurface	$^{\circ}\text{C}$
12	PARi	$\mu\text{mol m}^{-2} \text{s}^{-1}$	24	Status	Error Status

Please note that only the fields applicable to the chamber selected under "Configuration" will populate. For instance, when the SRC-2 Soil Respiration Chamber is selected the columns for Tamb, PARi, Acan, Ecan and WUE will be blank. When the CPY-5 Canopy Assimilation Chamber is selected under "Configuration" the columns for FCO2I, FCO2q and Esoil will be blank.

Application – Chlorophyll Fluorescence

Column	Header	Units	Column	Header	Units
1	Rec Type	m2-m9	24	Status	Error Status
2	Excel Time	Date/Time	25	PARi	$\mu\text{mol m}^{-2} \text{s}^{-1}$
3	Comments	User-defined	26	PARe	$\mu\text{mol m}^{-2} \text{s}^{-1}$
4	F	F	27	Intensity	$\mu\text{mol m}^{-2} \text{s}^{-1}$
5	F_o	F_o	28	Red	%
6	F_m	F_m	29	Green	%
7	F_v	F_v	30	Blue	%
8	F_v/F_m	F_v/F_m	31	White	%
9	F_m'	F_m'	32	Tamb	°C
10	F_s	F_s	33	Tcuv	°C
11	ϕ_{PSII}	ϕ_{PSII}	34	Tleaf	°C
12	F_o'	F_o'	35	Aleaf	cm^2
13	F_v'	F_v'	36	Mgain	NA*
14	F_v'/F_m'	F_v'/F_m'	37	Mlevel	NA*
15	J	J	38	Spulse	$\mu\text{mol m}^{-2} \text{s}^{-1}$
16	qP	qP	39	SpulseD	Seconds
17	qNP	qNP	40	Mpulse1	$\mu\text{mol m}^{-2} \text{s}^{-1}$
18	NPQ	NPQ	41	Mpulse2	$\mu\text{mol m}^{-2} \text{s}^{-1}$
19	qL	qL	42	Mpulse3	$\mu\text{mol m}^{-2} \text{s}^{-1}$
20	ϕ_{NO}	ϕ_{NO}	43	Mpulse4	$\mu\text{mol m}^{-2} \text{s}^{-1}$
21	ϕ_{NPQK}	ϕ_{NPQK}	44	Mpulse5	$\mu\text{mol m}^{-2} \text{s}^{-1}$
22	ϕ_{fD}	ϕ_{fD}	45	MpulseD	Seconds
23	ϕ_{NPQG}	ϕ_{NPQG}			

*NA – Not Applicable

Application – Gas Exchange and Chlorophyll Fluorescence

Column	Header	Units	Column	Header	Units
1	Rec Type	R and m2-m9	29	rb	$\text{m}^2 \text{ s mol}^{-1}$
2	Excel Time	Date/Time	30	StomataR	%
3	Comments	User-defined	31	Tsensor	See tables below
4	CO2r	$\mu\text{mol mol}^{-1}$	32	Tcontrol	See tables below
5	CO2a	$\mu\text{mol mol}^{-1}$	33	Lcontrol	See tables below
6	CO2d	$\mu\text{mol mol}^{-1}$	34	PLC	See tables below
7	H2Or	mb	35	Status	Error Status
8	H2Oa	mb	36	F	F
9	H2Od	mb	37	F_o	F_o
10	PARi	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	38	F_m	F_m
11	PARE	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	39	F_v	F_v
12	Red	%	40	F_v/F_m	F_v/F_m
13	Green	%	41	F_m'	F_m'
14	Blue	%	42	F_s	F_s
15	White	%	43	ϕ_{PSII}	ϕ_{PSII}
16	Tamb	°C	44	F_o'	F_o'
17	Tcuv	°C	45	F_v'	F_v'
18	Tleaf	°C	46	F_v'/F_m'	F_v'/F_m'
19	Aleaf	cm^2	47	J	J
20	Flow	cc min^{-1}	48	qP	qP
21	Patm	mb	49	qNP	qNP
22	RH	%	50	NPQ	NPQ
23	Ci	$\mu\text{mol mol}^{-1}$	51	qL	qL
24	gs	$\text{mmol m}^{-2} \text{ s}^{-1}$	52	ϕ_{NO}	ϕ_{NO}
25	VPD	kPa	53	ϕ_{NPQK}	ϕ_{NPQK}
26	A	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	54	ϕ_{fD}	ϕ_{fD}
27	E	$\text{mmol m}^{-2} \text{ s}^{-1}$	55	ϕ_{NPQG}	ϕ_{NPQG}
28	WUE	A/E			

Application – Gas Exchange and Analyzer Only

Column	Header	Units	Column	Header	Units
1	Rec Type	R	19	Aleaf	cm ²
2	Excel Time	Date/Time	20	Flow	cc min ⁻¹
3	Comments	User-defined	21	Patm	mb
4	CO2r	µmol mol ⁻¹	22	RH	%
5	CO2a	µmol mol ⁻¹	23	Ci	µmol mol ⁻¹
6	CO2d	µmol mol ⁻¹	24	gs	mmol m ⁻² s ⁻¹
7	H2Or	mb	25	VPD	kPa
8	H2Oa	mb	26	A	µmol m ⁻² s ⁻¹
9	H2Od	mb	27	E	mmol m ⁻² s ⁻¹
10	PARi	µmol m ⁻² s ⁻¹	28	WUE	A/E
11	PARe	µmol m ⁻² s ⁻¹	29	rb	m ² s mol ⁻¹
12	Red	%	30	StomataR	%
13	Green	%	31	Tsensor	See tables below
14	Blue	%	32	Tcontrol	See tables below
15	White	%	33	Lcontrol	See tables below
16	Tamb	°C	34	PLC	See tables below
17	Tcuv	°C	35	Status	Error Status
18	Tleaf	°C			

All of the above fields will populate when “Gas Exchange” is the selected Application in **Settings (F2)**. If “Analyzer Only” is selected under **Settings (F2)** Fields 1-9, 21 and 34 will populate. Please also note that we include two Microsoft® Excel® spreadsheet programs on the USB Flash Drive supplied with the system for easy recalculation of photosynthesis results and for post-processing of data for rapid measurement of A/C_i using our high-speed CO₂ ramping technique. Refer to [Section 18. CIRAS-3 Console and PC Utility Software](#) on page 194 for more information.

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 (see below). The last column labeled “Status” is there to report any errors that may have been reported during a measurement. Normally this field is empty but if an error is reported it will show up in this column.

Temperature Sensor Measurement

IR	Infrared Sensor. 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

Other

StomataR	Stomata Ratio
rb	Boundary Layer Resistance

Application – Insect Respiration

Column	Header	Units	Column	Header	Units
1	Rec Type		8	H2Oa	mb
2	Excel Time	Date/Time	9	H2Od	mb
3	Comments	User-defined	10	Flow	cc min ⁻¹
4	CO2r	µmol mol ⁻¹	11	IW	g
5	CO2a	µmol mol ⁻¹	12	RESPW	µg CO ₂ g ⁻¹ min ⁻¹)
6	CO2d	µmol mol ⁻¹	13	RESPI	µg CO ₂ g ⁻¹ min ⁻¹)
7	H2Or	mb			

Section 17. Troubleshooting & Diagnosis

System Power

The CIRAS-3 will not turn on when attempting to power up

- The internal batteries are either missing or not charged. Connect the power supply adapter to the CIRAS-3 and charge the batteries. Also check to make sure that the electrical connection is intact.
- Internal batteries are in “Ship” mode and need to be put into “Run” mode. See [Battery Pack Installation](#) on page 65.

The batteries are fully charged but the system can only operate for a couple of hours and not anywhere near normal expected times

- You may be putting a high drain on the batteries (controlling temperature and/or light control) which will have an impact on batteries. This is a fact of life unfortunately as temperature control especially will put a large drain on the battery especially when cooling temperatures well below ambient.
- Old battery might be losing some juice over time and may require replacement.

I charged the batteries overnight but the batteries are not charged

- Check the power supply adapter and when connected to mains power the indicating LED on it should be a steady green. If it is not or if it is flashing it could mean a faulty charger. Also make sure that your wall outlet or surge protector is on.
- Make sure that the indicating LED on the console is showing a steady green indicating secure connection.
- Ensure batteries are seated properly in the battery compartment and the battery cable is connected.

The EXT/PWR indicating LED on the CIRAS-3 console is flickering

It is possible that the CIRAS-3 was not powered off when the battery was extremely low and it continued to drain beyond normal levels. If this happens the batteries may require a longer charging period to get back to full charge status.

CO₂ Measurement and Control

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.
 - Did you store the CIRAS-3 console in the black transport case with a partially pressurized CO₂ cartridge? See [Storage](#) on page 16.
-

CO2r levels are reading lower than expected

- The soda lime or molecular sieve in the Zero column is 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 CO₂ cartridge is slowly exhausting or missing. The cartridge was put in use at least 1 day before and most likely is getting low in pressure. See [CO₂ Cartridge Status](#) on page 159 for more details. Replace CO₂ cartridge if low in pressure or empty.
 - Check to make sure that the cartridge holder is snug to ensure that the cartridge is pierced.
-

My CO2r is more than 20 µmol mol⁻¹ from my set point

- You likely forgot to perform a Max C calibration. See [Find Max C](#) on page 102 and perform a Max C calibration.
-

My CO2r is more than 20 µmol mol⁻¹ from my set point and in my Settings I'm set to "Exact reference air" for CO2 Reference

- Are you sure? Go back to Settings to confirm. It is likely set to "Approximate reference air".
-

My CO2r or CO2a readings are fluctuating

- First I would check your desiccants and change out if exhausted.
- Your reference or sampling pump might be dirty or dusty and require cleaning or replacement. See [Servicing the Air Sampling Pump \(Reference & Analysis\)](#). On page 208. Another quick trick is to simply swap the pumps to see if the problem follows the pump.

- Dirty air supply unit hydrophobic filter. See [Air Filters](#) on page 210 for more details.

CO₂ exhausts from the cartridge quicker than usual and doesn't last a full day

- Slow leak around the regulator. Check regulator for leaks especially around the "O" ring at the back. See [CO₂ Regulator](#) on page 217.
- Bad or worn gasket seal. Replace the seal. See [CO₂ Regulator](#) on page 217.
- Problem with piercing pin. Check that the piercing pin is in place and not broken or bent. See [CO₂ Regulator](#) on page 217.

CO₂ exhausts from the cartridge rapidly as soon as I attempt to install a fresh cartridge

- Check the piercing pin to see if it is broken or missing. See [CO₂ Regulator](#) on page 217.
- Bad or worn gasket seal. Replace the seal. See [CO₂ Regulator](#) on page 217.

My CO₂ readings are drifting

- Exhausted soda lime. Replace.

My CO_{2d} readings are fluctuating

- Check your CO_{2r} and CO_{2a} readings. If one of them is fluctuating this will result in a fluctuating CO_{2d} and you should service the one that is fluctuating. See [Servicing the Air Sampling Pump \(Reference & Analysis\)](#) on page 208.
- A leak in the gas circuit usually associated with the leaf cuvette gaskets. See [Checking For Leaks Associated With the PLC3](#) on page 221 to see if the problem is associated with the PLC3 or CIRAS-3. Check leaf cuvette gaskets and replace if necessary.
- Cracked or missing "O" ring on the PLC3 pneumatic connector. Replace and periodically lubricate the "O" rings with silicone grease. See [PLC3 Pneumatic Connector](#) on page 223.

My CO_{2d} readings are always reading positive even with an empty cuvette and the head closed

- You most likely stored your CIRAS-3 in the black transport case with a pressurized CO₂ cartridge. If so you will need to allow the system to equilibrate for a period of time before proceeding with measurements. See [Storage](#) on page 16 for more details.

It is taking longer than normal for my CO2r levels to settle

- Under settings your CO2 Reference is likely set to “Fixed analysis air”. Change to “Fixed reference air”.

With a healthy leaf in the chamber I am not detecting any differentials for CO2 (CO2d) or H2O (H2Od)? Even if I breathe into the chamber I am not detecting any differentials?

- Check the white pneumatic gas connector on the PLC3 and make sure that when it is inserted into the CIRAS-3 console you hear it snap into place. It is likely not seated and locked in properly.

I am unable to achieve a Max C calibration above 2000 ppm and it is failing

- Your CO₂ cartridge is low and needs to be replaced.
- Your desiccants are exhausted and require replacement. Replace all desiccants.

H₂O Measurement and Control

My H2Or levels are reading higher than expected

- There may be 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.
- Exhausted H₂O scrubber in the Zero column. Check the Drierite and replace.

My H2Or levels are reading lower than expected

- Exhausted H₂O scrubber in the Zero column. Replace the Drierite and Molecular Sieve.

My H2Or or H2Oa values are fluctuating

- First I would check your desiccants and change out if exhausted.
- There may be 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.

- Your reference or sampling pump might be dirty or dusty and require cleaning or replacement. See [Servicing the Air Sampling Pump \(Reference & Analysis\)](#). On page 208. Another quick trick is to simply swap the pumps to see if the problem follows the pump.
- Dirty air supply unit hydrophobic filter. See [Air Filters](#) on page 210 for more details.

My H₂O readings are drifting

- Exhausted H₂O scrubber in the Zero column. Replace the Drierite and Molecular Sieve.

My H₂O readings are fluctuating

- First I would check your desiccants and change out if exhausted.
- There may be 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.
- Check your H₂Or and H₂Oa readings. If one of them is fluctuating this will result in a fluctuating H₂Od and you should service the one that is fluctuating. See [Servicing the Air Sampling Pump \(Reference & Analysis\)](#). On page 208.
- A leak in the gas circuit usually associated with the leaf cuvette gaskets. See [Checking For Leaks Associated With the PLC3](#) on page 221 to see if the problem is associated with the PLC3 or CIRAS-3. Check leaf cuvette gaskets and replace if necessary.
- Cracked or missing "O" ring on the PLC3 pneumatic connector. Replace and periodically lubricate the "O" rings with silicone grease. See [PLC3 Pneumatic Connector](#) on page 223.

Temperature Measurement and Control

My leaf temperature readings appear to be incorrect

- Are you using the "IR Thermometry" for measurement of leaf temperature? One possibility is that you are not completely filling the cuvette window with leaf area which will lead to errors in temperature measurement. In order to use "IR Thermometry" you must fill the chamber with leaf area. If you are working with small vegetation and you are unable to fill the chamber then you should select Energy Balance for leaf temperature.

My Tamb value is reading much different than actual ambient

- The Tamb sensor is located at the back of the cuvette handle and is likely in close proximity to something that is either cooler or warmer than ambient. Try to keep this area of the cuvette away from any temperature influencing areas.
- Make sure that nothing is covering the small hole at the base of the cuvette handle that contains the Tamb sensor.

I am unable to achieve my Tcuv set point

- You are working outside the temperature control limits of 0-45 °C.
- The set point is not achievable because it is outside the temperature control range of ~10 °C below ambient to +15 °C above ambient.
- More than likely you are unable to cool the chamber to your set point as a result of high incident light or your batteries are getting low.

I am unable to achieve my Tleaf set point

- You are working outside the temperature control limits of 0-45 °C.
- The set point is not achievable because it is outside the temperature control range of ~10 °C below ambient to +15 °C above ambient.
- More than likely you are unable to cool the chamber to your set point as a result of high incident light or your batteries are getting low.

PAR Measurement and Control

Why does my PARi reads lower than PARe

- This is normal. The PARi sensor is located inside the cuvette head and below the window and should therefore read approximately 10% lower than PARe.
- Make sure that nothing is obstructing the PARi sensors.

I am using the light unit but I am unable to control light intensity

- Check to make sure that the electrical connection is in place.
- Go to Settings and make sure that you have the proper light source selected

My PARe readings do not seem accurate

- The sensor likely requires recalibration. Check the calibration against another PAR sensor that you trust and believe to be calibrated. Recalibrate if necessary. See [PAR Calibrate](#) on page 108.

Flow Control

I am unable to achieve the set cuvette flow rate

- The absorber column assemblies may not be seated properly. This is a common problem just after replacement of desiccants. Check to make sure that both columns (Zero and CO₂/H₂O control) are properly seated and secured.
- Possible ASU pump failure. Replace.
- The internal tubing between the back of the CO₂ regulator and diversion solenoid on the main flow board has disconnected. This can happen when someone attempts to change out a pressurized CO₂ cartridge. When this happens you will hear a loud “pop” and the pressure will cause the internal tubing to become disconnected. Look for this and reconnect the tubing between the solenoid and regulator.

My pumps seem to be running harder and louder than normal

- Most likely cause is a restriction in the gas circuit. Open the battery compartment door and inspect the internal tubing for any kinked piping. Also inspect for any pieces of tubing that may have slipped off one of the pumps or is cracked around the pump fittings.
- One of the absorber columns is not firmly seated in the manifold. Check both columns and ensure that they are properly seated.
- Blocked filters. Start with the air supply unit hydrophobic filter as this is the one that might be blocked and if this doesn't correct the problem start checking the other filters. See [Air Filters](#) on page 210.

My reference pump is not running

- Check the electrical connection to the PCB. If it is connected properly try tapping on it with a small screwdriver or pen and see if that gets it going. Sometimes the internal vanes get stuck and a simple tap is all it takes to get it back up and running especially if the system was not in use for some time. If that does not get it up and running and you are properly connected it likely means you have a seized pump and it requires replacement.

My analysis pump is not running

- Check the electrical connection to the PCB. If it is connected properly try tapping on it with a small screwdriver or pen and see if that gets it going. Sometimes the internal vanes get stuck and a simple tap is all it takes to get it back up and running especially if the system was not in use for some time. If that does not get it up and running and you are properly connected it likely means you have a seized pump and it requires replacement.

Data Management

I am unable to record any measurements from either both the console and the PLC3

- You haven't initiated a recording session. To do so go to **Operations (F1) >Rec Options (F2)** and select the recording type (Manual, Timed, Response curves) and create a data file and then hit **Start (F2)** to begin a recording session.

Very important. Always click End Record (F5) when using “Manual recording” to terminate a session to safely preserve all stored data.

I can't find my data on the USB flash drive (memory stick)

- You likely saved your data to internal memory by mistake. Not a big problem. Check the CIRAS-3 internal memory and when you find your file simply transfer it to your USB flash drive (**Operations (F1) >Rec Options (F2) > Transfer Data (F6)**).

I can't find my data stored in internal memory

- You likely saved your data to the USB Flash Drive (external memory) by mistake. Not a big problem. Put the USB flash drive into the USB1 or USB2 ports on the back of the CIRAS-3 console and transfer it to internal memory if you want to keep a back-up of the file on the CIRAS-3 (**Operations (F1) >Rec Options (F2) > Transfer Data (F6)**).

Status Codes Displayed on the CIRAS-3 LCD

From time to time the CIRAS-3 may send a status code (message) to either A) recommend that the user perform a certain task or B) to report a suspected problem. Some messages that get sent include just the Code or the Description (with code) as shown below.

Code	Description	What it means / Action
00	System OK. Cuvette open if present [00]	No action required. Indicates that the PLC3 Leaf Cuvette is in the open position.
01	Diff-Bal Required.	Under "Zero, Diff Bal Mode" you are operating under "Manual" mode. You should perform a Diff Bal as recommended (Z-Diff Bal (F7) > Diff Bal). This is critical to ensure calibration stability and accuracy.
02	Zero Required.	Under "Zero, Diff Bal Mode" you are operating under "Manual" mode. You should perform a manual Zero (Z-Diff Bal (F7) > Zero). This is critical to ensure calibration stability and accuracy.
10	Normal Running – PLC Closed [10]	No action required. Indicates that the PLC3 Leaf Cuvette is in the closed position.
20	Record Key Pressed, System OK [20]	No action required. Indicates that a measurement was recorded.
50	CMAX success	No action required. Indicates that the CIRAS-3 has achieved a good MaxC.
51	CMAX Failure	Indicates that the CIRAS-3 has not achieved a good MaxC. Make sure you have a fresh CO ₂ cartridge and try performing another MaxC. If problem persists contact PP Systems.
61	Chamber Par 1 not reading (Left)	The left PAR sensor inside the PLC3 Universal Leaf Cuvette is not reading properly. Access the internal PLC3 PCB in the handle and check electrical connections at pads 44, 45 and 46.
62	Chamber Par 2 not reading (Right)	The right PAR sensor inside the PLC3 Universal Leaf Cuvette is not reading properly. Access the internal PLC3 PCB in the handle and check electrical connections at pads 47, 48 and 49.

Code	Description	What it means / Action
63	Test Mode in progress	No action required.
64	Test Passed	No action required.
65	Test Failed	No action required.
80	Cuvette % Rel Humidity > 70%	The RH% is getting high in the cuvette and we recommend drying the air further to keep levels below 70%.
85	CO ₂ Concentration Out of Range On Stored Diff-Bal [85]	The CO ₂ range is outside 10 µmol mol ⁻¹ during stored Diff Bal routine. Abort and try again. If problem persists contact PP Systems.
86	H ₂ O Concentration Out of Range On Stored Diff-Bal [86]	The H ₂ O range is outside 0.5 mb during stored Diff Bal routine. Abort and try again. If problem persists contact PP Systems.
87	Analysis/Reference Pressure Difference > 20mb [87]	Restriction on the reference or analysis gas circuit. Check all gas connections and look for kinks or to see if a connection has been broken.
88	Time Out on Diff-Bal [88]	The CO ₂ r or H ₂ Or concentration is fluctuating too much during DIFF BAL and the system is unable to achieve a good fit. Perform manual Zero (Z-Diff Bal F7) > Zero) and Diff Bal (Z-Diff Bal F7) > Diff Bal). If you are operating in “Manual” mode for Zero and Diff Bal, we recommend changing this to Automatic.
89	Diff-Bal (CO ₂) Out of Range [89]	The DIFF BAL value for CO ₂ is > 10 ppm. Typically this is due to inaccurate recalibration or deterioration of the internal sample cell due to dust, dirt, water. It might just be a transient message that disappears and if it does you are ok. If the message does not disappear after 5-6 seconds perform a manual Diff Bal (Z-Diff Bal F7) > 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.

Code	Description	What it means / Action
90	Diff-Bal (H_2O) Out of Range [90]	The DIFF BAL value for H_2O is > 0.5 mb. Typically this is due to inaccurate recalibration or deterioration of the internal sample cell due to dust, dirt, water. It might just be a transient message that disappears and if it does you are ok. If the message does not disappear after 5-6 seconds perform a manual Diff Bal (Z-Diff Bal F7 > Zero). 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.
91	Cuvette Flow 20% out from set value [91]	Normally this is just a transient and the message disappears after 5 or 6 seconds and is quite common after a change in flow rate. If the message does not disappear check for restrictions in the gas circuit associated with the ASU pump (kinked piping).
92	Zero too Low [92]	Exhausted chemicals in the Zero column. Replace soda lime, Drierite and especially the Molecular Sieve.
93	Board supply voltage < 6.4 V [93]	Internal battery(s) are low. Charge the battery(s) using the power supply adapter provided by PP Systems.
94	Flow Rate through the Reference Cell too High [94]	Check the reference pump (including electrical connection) and any kinks that might be causing restriction. Clean or replace the reference pump. See Servicing the Air Sampling Pump (Reference & Analysis) . On page 208. Also check that the Zero and CO_2/H_2O control manifolds are properly seated in their respective manifolds and all associated "O" rings are not pinched off or cracked. Also check the internal hydrophobic filter.

Code	Description	What it means / Action
95	Flow Rate through the Reference Cell too Low [95]	Check the reference pump (including electrical connection). Clean with alcohol (to remove built up dust or dirt) or replace the reference pump. See Servicing the Air Sampling Pump (Reference & Analysis) . On page 208. Also check that the Zero and CO ₂ /H ₂ O control manifolds are properly seated in their respective manifolds and all associated "O" rings are not pinched off or cracked. Also check the internal hydrophobic filter.
96	Flow Rate through the Analysis Cell too High [96]	Check the analysis pump (including electrical connection) and any kinks that might be causing restriction. Clean or replace the analysis pump. See Servicing the Air Sampling Pump (Reference & Analysis) . On page 208. Also check that the Zero and CO ₂ /H ₂ O control manifolds are properly seated in their respective manifolds and all associated "O" rings are not pinched off or cracked. Also check the internal hydrophobic filter.
97	Flow Rate through the Analysis Cell too Low [97]	Check the analysis pump (including electrical connection). Clean with alcohol (to remove built up dust or dirt) or replace the analysis pump. See Servicing the Air Sampling Pump (Reference & Analysis) . On page 208. Also check that the Zero and CO ₂ /H ₂ O control manifolds are properly seated in their respective manifolds and all associated "O" rings are not pinched off or cracked. Also check the internal hydrophobic filter.
98	Analysis Temperature > 65° [98]	IRGA temperature has exceeded the normal 55 °C indicating a thermostat failure. Contact PP Systems.
99	Analysis Temperature < 50° [99]	IRGA temperature not reaching the normal 55 °C indicating a thermostat failure. Contact PP Systems.

Other Status Codes/Messages

Message	What it Means / Action
Divide by Zero	Possible corruption of system data pointers or calculation error. Download any stored data from internal memory and then clear the internal memory. If problem persists contact PP Systems.
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. 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.
Stored Diff Bal H₂O out of range	The maximum reference H ₂ O concentration has changed and is significantly higher than the maximum reference H ₂ O 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.

Section 18. 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 Win7/8 and 10 which have the .NET v2 libraries installed. The file name is “Ciras3_PC.Utility.exe”. Both are available on a USB Flash Drive that is included with each new system. In addition, we also include two additional Microsoft® Excel® Spreadsheet programs on the USB Flash Drive as follows:

75018-1 CIRAS-3 Recalculation Spreadsheet	Allows users to easily recalculate stored CIRAS-3 data if required (i.e. leaf area, etc.).
75020-1 CIRAS-3 High-Speed CO₂ Ramp Post Processing Spreadsheet	Allows users to easily post-process stored CIRAS-3 data for generation of A/C _i curves (Non-steady state). An Application Note is available from PP Systems with full instructions on performing high-speed CO ₂ ramping and post-processing of data.

CIRAS-3 Console Software

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. When console software is either at the Welcome Screen window or one of the display windows press **ESC** and **Yes** to leave the CIRAS-3 program. Next go to the Windows desktop. Attach a USB mouse to USB Slot 1 for convenience.
2. Open “My Device” on the console desktop. Navigate to folder:
3. “Flash Disk\InstalledFiles\PP Systems CIRAS3_Console_vxxx”
4. Where “vxxx” is the prior installed version.
5. Copy “PP Systems CIRAS3_Console_vxxx.unload” file.
6. Navigate to “Windows” folder and paste the unload file.
7. Close “My Device” window.
8. Use Start – Settings – Control Panel – Remove Programs. Select “PP Systems CIRAS3_Console_vxxx” from the list and click “Remove” button.

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.

To install the newer console “Application”, refer to the instructions in the previous section (See [Installation Instructions for Main CIRAS-3 Console](#) on page 195).

TIP

If the CIRAS-3 console software (Ciras3_Console.exe) is already installed you **MUST** uninstall it prior to loading new versions.

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 flash drive (memory stick) provided by PP Systems into a USB port on your desktop PC or laptop computer. The USB flash 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.

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 on your desktop.

For Vista, Win7,Win8 or Windows 10 Operating Systems

Once installation is complete, find the shortcut link on the desktop named “Ciras3 Utility” as shown below.



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.

TIP

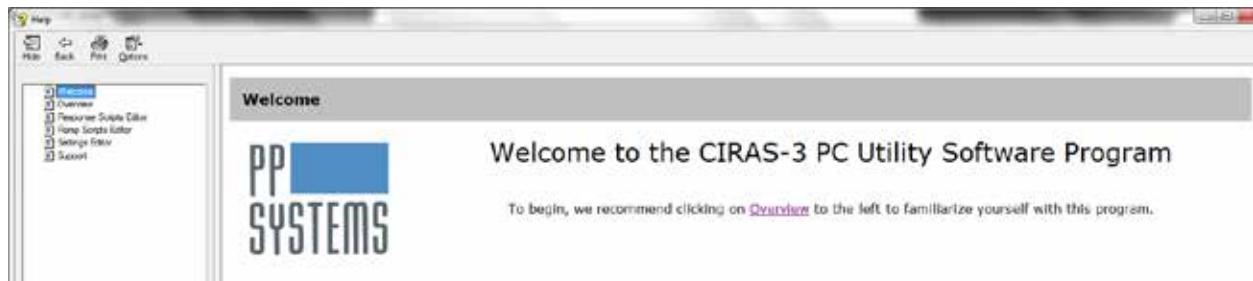
If the CIRAS-3 PC Utility software is already installed on your desktop PC or laptop you **MUST** uninstall it prior to loading new versions.

PC Utility Operation

To run this “Application”, double click on the CIRAS-3 icon on your desktop. The following very un-exciting screen 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

The screenshot shows the 'Gas Exchange' tab selected in the software interface. At the top, there are fields for 'Current Level' (1 of 15), 'Records per Level' (3), and 'Record Interval' (10 s). Below these are settings for CO₂, H₂O, Leaf Temperature, Light Intensity, and RGBW values. A note indicates CO₂ reference will be set to 'Approximate reference air' and H₂O reference will be set to 'Fixed % of reference'. A large table below lists 15 levels with corresponding parameters.

Level	Acclimation	Records	Record Interval	CO ₂	H ₂ O	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

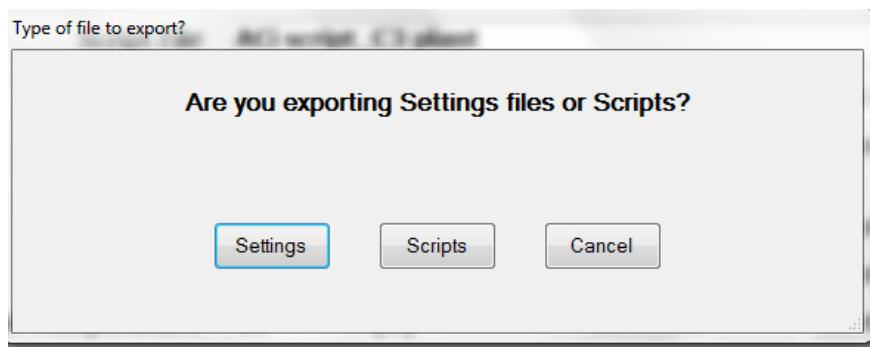
CFM-3 Script Settings

Click the CFM TAB at the top to display the settings associated for measurement of chlorophyll fluorescence using the integral CFM-3. You must have purchased the CFM-3 Chlorophyll Fluorescence Module and have it properly selected under Settings in order to perform fluorescence measurements.

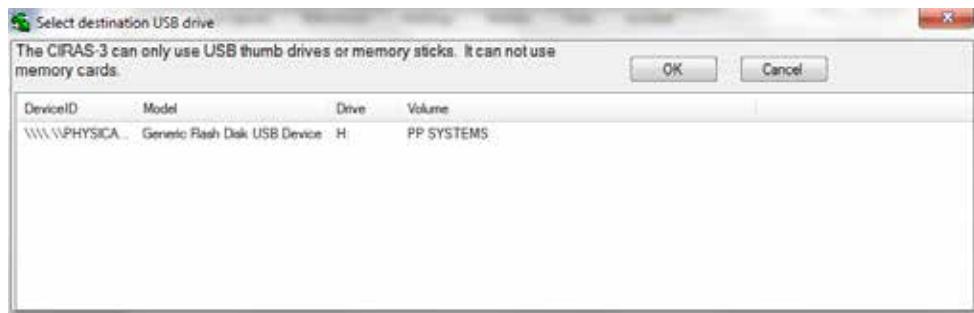
Click the arrow button to expand the dropdown list at the **Script File** item. Edit the script just as you would if you were working from the CIRAS-3 Console. Then click **File >Save**, or **Save As** to rename it.

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 USB flash 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 flash drive to CIRAS-3 console internal memory. Now the file is available for use on the CIRAS-3 console.

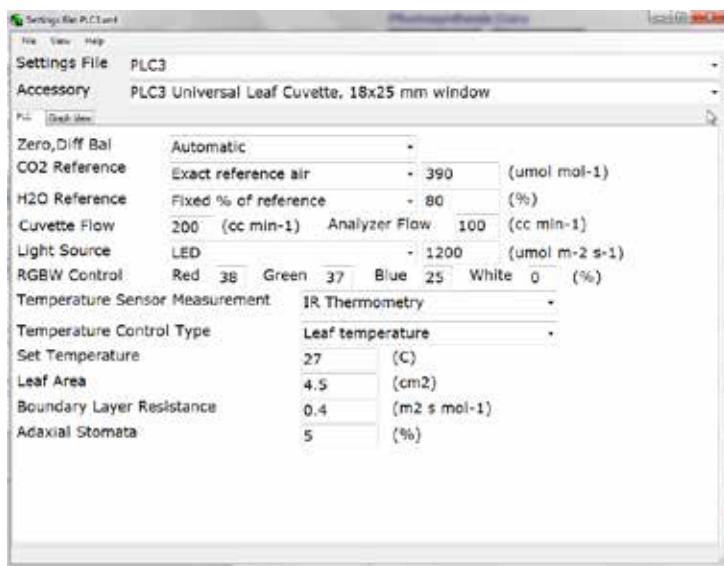
Create/Edit Settings Files

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

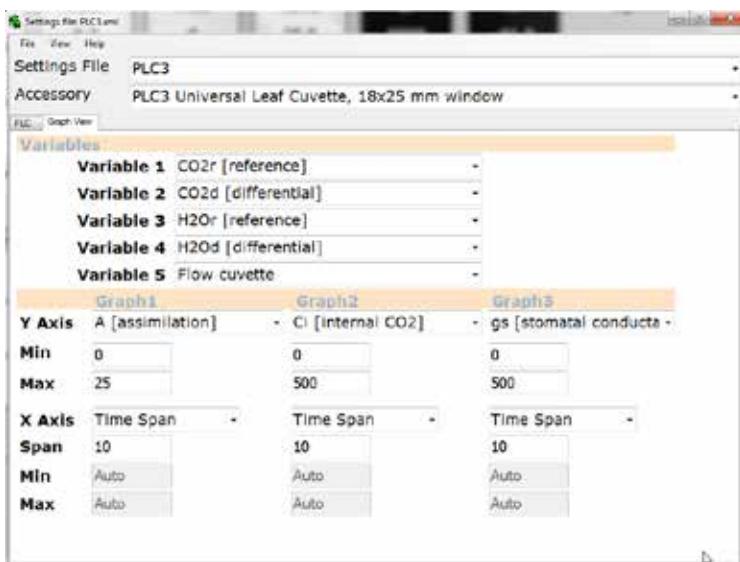
windows. For example, **PLC3** or **PLC3-CFM** settings files used for open system leaf-level photosynthesis and chlorophyll fluorescence have a global Settings window TAB labeled **CFM** (chlorophyll fluorescence). Click the arrow button to expand the dropdown list at the **Settings File** and **Accessory**. 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



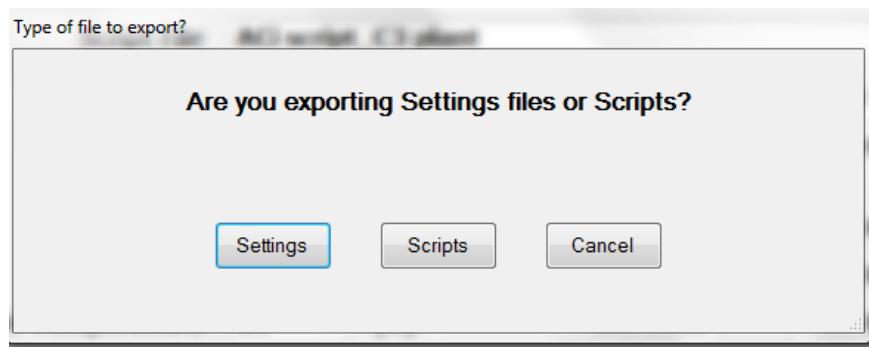
Graph View Settings

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

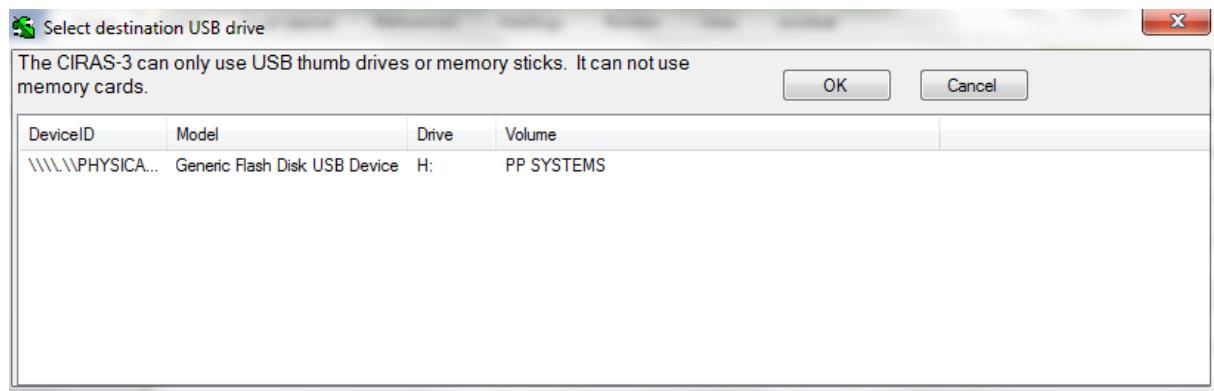


The next step would be to Export the new or updated script file to the CIRAS-3 main console. The easiest way to do this is to export the files to a USB flash drive.

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 USB flash 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 Flash Disk 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.

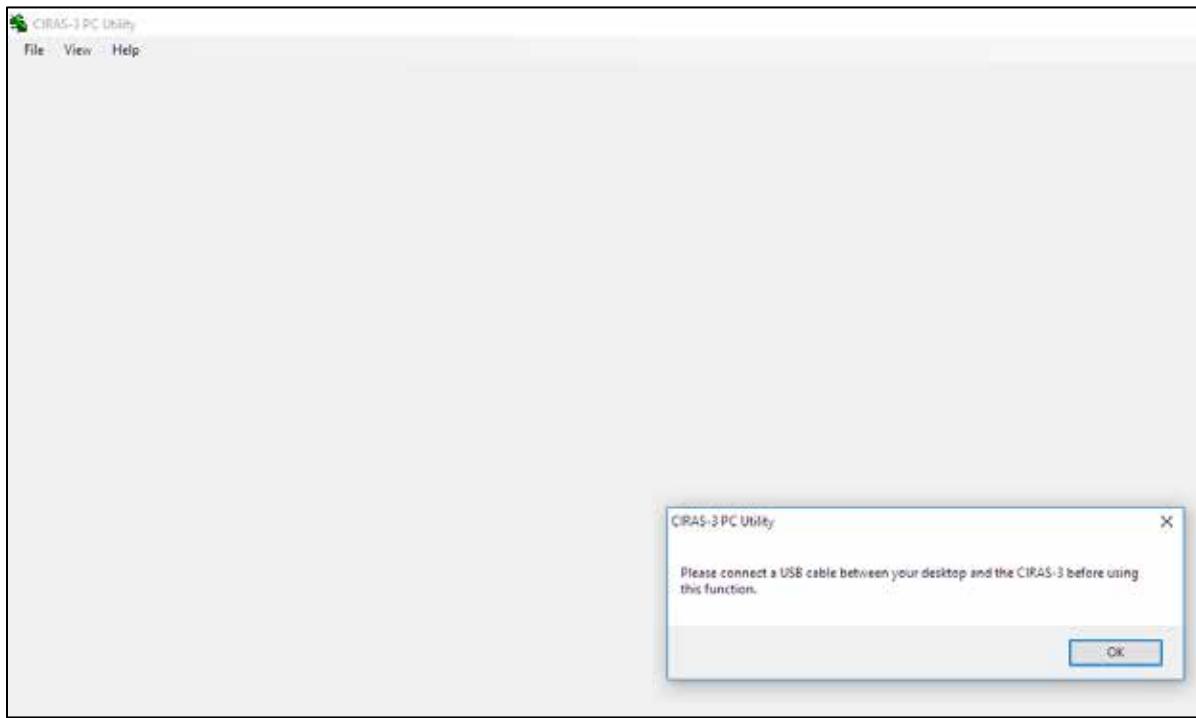
Now the file is available for use on the CIRAS-3 console.

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.

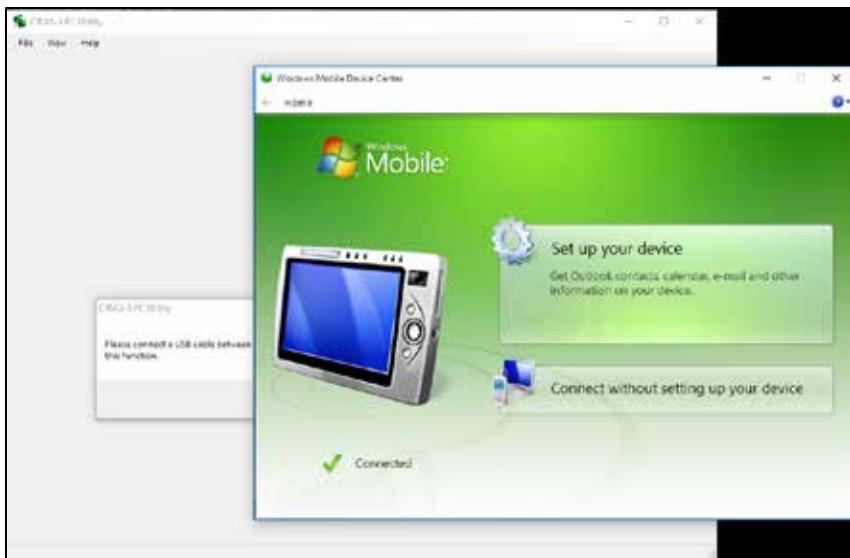
The connection between the CIRAS-3 console and the external device is made via a USB connection using the USB cable provided by PP Systems.

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.

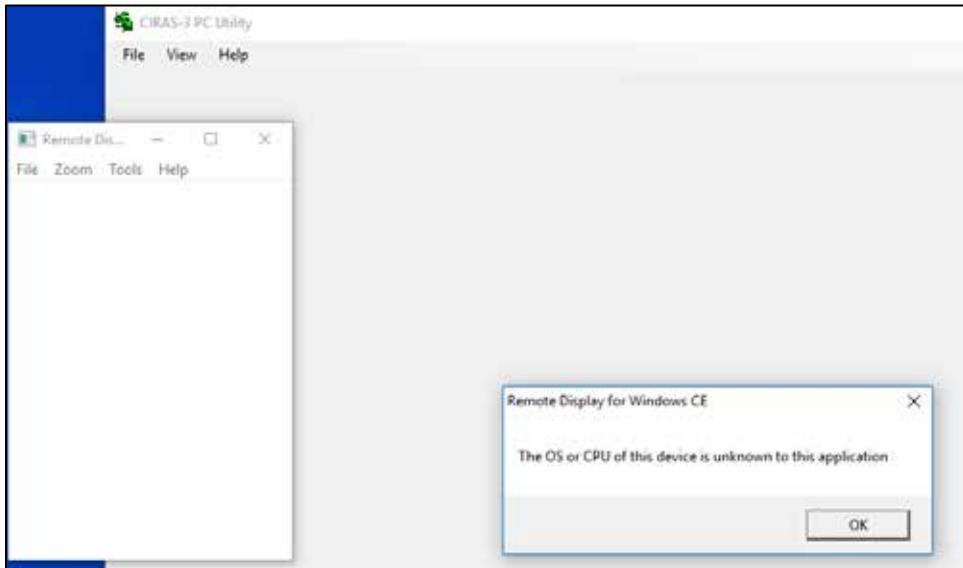
Note: For initial installation, the Windows Mobile Device Center requires access to the internet.



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.

Gas Exchange					
CO2r 390.0	CO2a	CO2d	A	Ci	Operations >
389.9 μmol mol-1	389.8 μmol mol-1	-0.1 μmol mol-1	0.0 μmol m-2 s-1	0 μmol mol-1	Settings >
H2Or 70.0%	H2Oa	H2Od	gs	E	Controls >
7.1 mb	7.0 mb	-0.1 mb	0 mmol m-2 s-1	0.0 mmol m-2 s-1	Toggle View >
Tamb	Tcv	Tleaf amb	VPD	WUE	Record >
27.0 °C	24.6 °C	27.0 °C	2.9 kPa	0.0 A/E	Zero/Diff Bal >
PARi 1200	PARe	RH%	Flow 300	Leaf Area 4.50	Help >
1200 μmol m-2 s-1	21 μmol m-2 s-1	22.6 %	299 cc/min	4.50 cm2	
Charging 100%					

Use the Function keys on your PC keyboard to simulate the Function keys on the CIRAS-3 console.

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

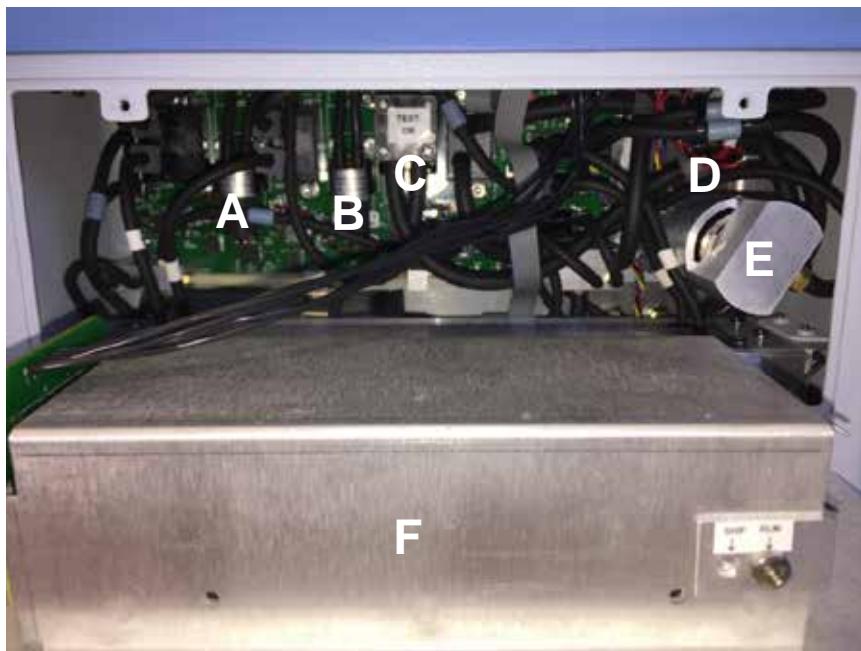
The Windows Mobile Device Center (WMDC) replaces ActiveSync. Please note that Microsoft® no longer supports Windows Mobile Device Center (WMDC) but it does in fact work with the CIRAS-3. If you experience any problems running our application on a Windows 10 machine with Windows Mobile Device Center (WMDC) please get in contact with PP Systems as extra steps may be necessary depending on the configuration/settings on your computer.

Section 19. Routine Maintenance

The section refers to many areas that can be addressed and serviced fairly easily by users without the need to return equipment to PP Systems or authorized distributors. If it has been determined that a problem or fault can only be resolved by factory service at PP Systems please refer to [Returning equipment to PP Systems](#) on page 14.

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 may require local user-maintenance are as follows:

- Li-ion battery packs
- Air sampling pumps (Reference and Analysis)
- Hydrophobic filter

Battery Pack (Li-ion Battery)

Up until June 2017 the CIRAS-3 was powered by a single, 7.2V Li-ion battery (Manufacturer: Aved) which is capable of delivering up to 8 hours continuous use in the field (dependent on environmental controls). This should last for many years. Starting in July 2017 we began supplying instruments with two 7.2V (8.7 Ah, 63 Wh) Li-ion battery packs (Manufacturer: Inspired Energy) providing system operation up to 12 hours.

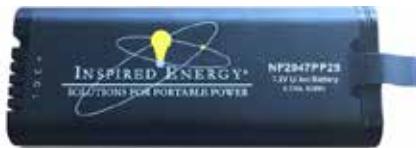
Battery Pack (Aved)

Part Number 41526-1



Battery Pack (Inspired Energy)

Part Number 41535-1



We recommend that the battery is charged every night when the system is in regular use to ensure that it is operating at full capacity. If the instrument is not going to be used for weeks or months then we recommend that the internal battery packs have at least 25-50% charge capacity before storing away.

A power supply is included with the CIRAS-3 for charging the internal battery(s) or for extended operation. To charge the internal battery(s), 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 green when charging and when the battery is fully charged. The battery capacity can be observed in the lower right hand corner of the display when the system is up and running with the measurement or graphical display on the LCD. If you need to order a replacement battery pack please make sure that you are ordering the correct type as highlighted earlier.

Battery Removal

If a battery needs to be removed or replaced, simply open the front battery cover and allow the door to gently drop down. Grasp the black TAB on the battery and pull it out of the compartment. See [Battery Pack Installation](#) on page 65 for information on installation of battery packs.

Battery Storage

Li-ion batteries do not have a "memory" so there is no need to fully discharge them after use. If the instrument is not going to be used for weeks or months then we recommend that the internal battery packs have at least 25-50% charge capacity before storing away.

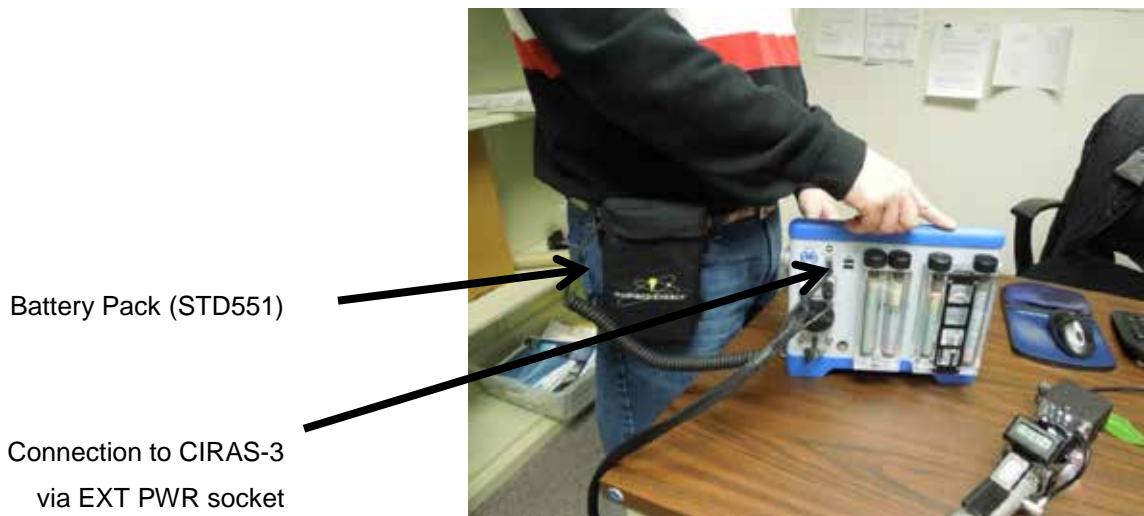
Battery Disposal

Proper and safe disposal of Lithium-ion batteries is critical as improper disposal may cause damage to human health or the environment. Please check your local area for Lithium-Ion battery recycling locations to learn how you can safely dispose of the batteries. Material Safety Data Sheets (MSDS) are available from the battery manufacturer if required. Please contact PP Systems for more information.



External Battery Pack (For extended operation in the field)

A convenient battery pack (STD551) can also be used for extended operation in the field.



Re-Order Information	
Part Number	Description
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 or 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.

Please note. We changed over to a new air sampling pump in July 2017. All instruments supplied prior to July 2017 would have been supplied with Part Number 10127-1 (Manufacturer: Thomas) and all systems supplied starting July 2017 would have been supplied with Part Number 10181-1 (Manufacturer: Schwarzer).

Air Sampling Pump (Thomas)

Part Number 10127-1



Air Sampling Pump (Schwarzer)

Part Number 10181-1



Replacement of Reference and Analysis Pump

It is very easy to replace the reference and analysis pumps. 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 electrical connector is removed by gently bending back the connector lock and sliding out the connector. **Please note the orientation of the electrical connector for proper reconnection.**

The pumps are secured in position by the gas tubing and pump retaining 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. **Please note the gas in and out tubing to the pump.** To replace simply plumb the pump to the correct gas in and out tubing, secure the pump in the retaining bracket and reconnect to the appropriate electrical connector on the Flow board.

Servicing the Air Sampling 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 them 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.
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 (Thomas)
10181-1	Air Sampling Pump (Schwarzer)

If you need to order a replacement pump please make sure that you are ordering the correct style as highlighted earlier (See Air Sampling Pumps (Reference and Analysis) on page 207.

Air Supply Pump

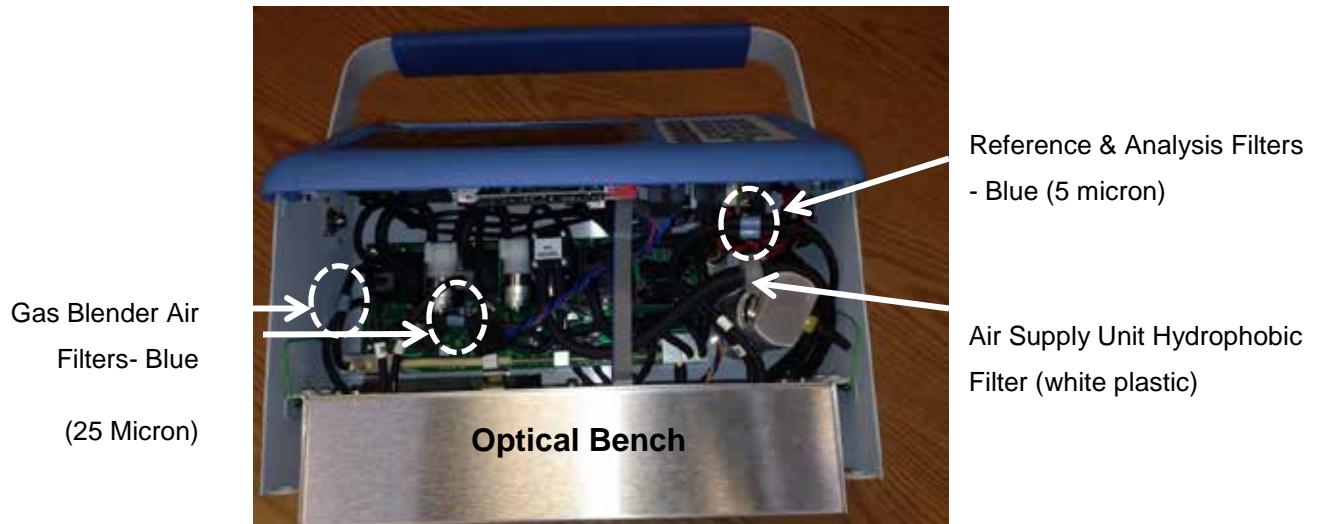
The main air supply pump (ASU) is a “diaphragm” style pump and should not require replacement or routine maintenance. It is located to the right of the reference air sampling pump and secured in place by Velcro and tubing. The electrical connector is labeled ASU PUMP and can be removed by gently bending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

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 CO₂ and H₂O 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 CO₂ and H₂O gas analyzers.

Zero Column Assembly (Part Number 10122-1)

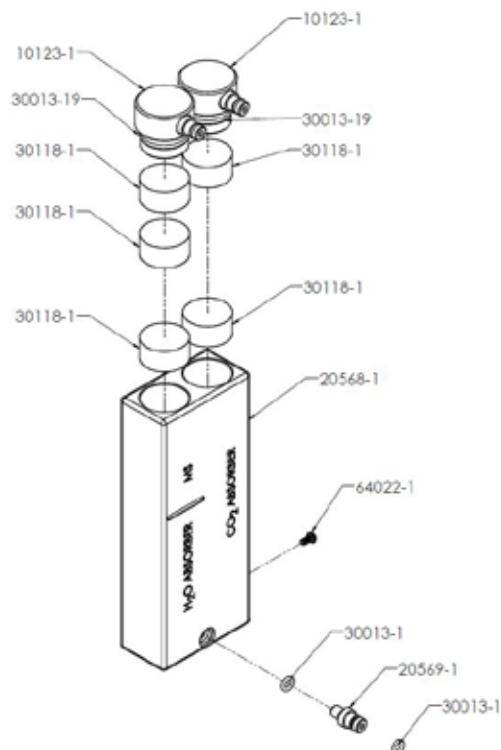


Column Identifiers

- H₂O Absorber: Drierite
- MS: Molecular Sieve
- CO₂ Absorber: Soda Lime

One column is filled with Drierite and Molecular Sieve and the other with soda lime. Drierite removes H₂O from the air stream and soda lime removes CO₂ from the air. During the Zero process, the Drierite adds in a small bit of CO₂ and the soda lime adds a small bit of H₂O 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 CO₂ and H₂O 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

CO₂ & H₂O Control Column

The CO₂ & H₂O Control column assembly contains 2 desiccants which are used for controlling CO₂ and H₂O. It also includes a water vapor equilibrator.

CO₂ & H₂O Control Column Assembly (Part Number 10121-1)



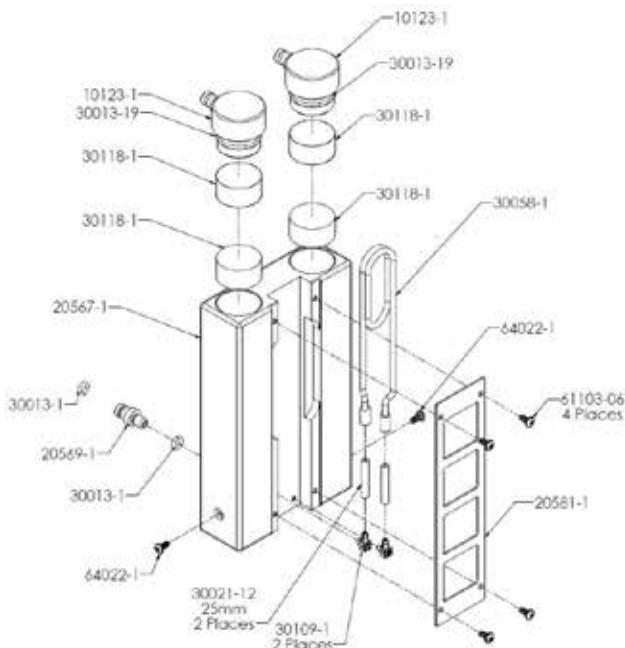
Column Identifiers

- **H₂O Absorber:** Drierite
- **CO₂ Absorber:** Soda Lime

One column is filled with Drierite (for control of H₂O) and one with soda lime (for CO₂ control). Drierite removes H₂O from the air stream and soda lime removes CO₂ 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 CO₂ from air entering the CIRAS-3. Both self-indicating and non-indicating Soda Lime 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	Sofnolime, white to violet, 1 kg

Drierite

Drierite (anhydrous 97% calcium sulfate (CaSO₄) and 3% cobalt chloride) is an excellent H₂O absorber making it an ideal choice for analyzer ZERO and for controlling H₂O. Both self-indicating (blue to pink) and non-indicating Drierite 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 CO₂ and H₂O from the air supply during analyzer ZERO to ensure system stability and accuracy for CO₂ and H₂O. 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 CO₂ and H₂O 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 CO₂ 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

To play it safe and for best results we recommend that you change out the Molecular Sieve desiccant on a daily basis prior to performing measurements to ensure accurate Auto Zero and long term accuracy and stability of the CO₂ and H₂O gas analyzers.

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

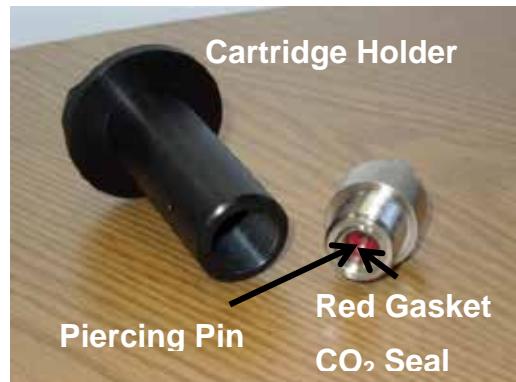
CO₂ Regulator

The CO₂ regulator should not require any routine maintenance. The CO₂ cartridge seals on the face of the 8g CO₂ cartridge and these seals should last for many years. Each system is supplied with gasket CO₂ seals (Part No. 20577-1) if replacement is necessary. If you suspect a faulty seal, follow the instructions below. **Before attempting to service the CO₂ regulator, check to make sure that there is not a pressurized CO₂ cartridge present.** See [How do I determine the state of the CO₂ cartridge?](#) on page 24. Depending on pressure, you may here a small hiss when loosening the cylinder holder from the regulator body which is normal.

Step 1. Remove CO₂ 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 CO ₂ Seal

CO₂ Calibration

The CO₂ gas analyzers should not require recalibration due to our built-in, innovative “Auto-Zero” function. During normal operation, the CIRAS-3 periodically passes “CO₂ free” air through the CO₂ 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 CO₂ gas mixture through the instrument. Another simple way to check of the CO₂ analyzers is to sample ambient air in an area away from any CO₂ influences or disturbances (i.e. roadways, air conditioning/ventilation systems, individuals breathing, etc.). We know that the typical ambient CO₂ concentration is approximately 375-400 $\mu\text{mol mol}^{-1}$. To check the CO₂ analyzers using ambient air as your reference:

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

1. Ensure that all desiccants are fresh.
2. Go to **Settings (F2)** and at “Settings File”, select Analyzer Only 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 $\mu\text{mol mol}^{-1}$ or close to your local ambient CO₂ 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 CO₂ concentration, a local or factory recalibration is recommended.

If CO₂ recalibration is required, an accurate gas mixture is required. Refer to [CO₂ Calibration](#) on page 100 for instructions. **It is critical that the Zero columns are fresh before performing CO₂ 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 $\mu\text{mol mol}^{-1}$, use a gas calibration mixture around 500 $\mu\text{mol mol}^{-1}$.

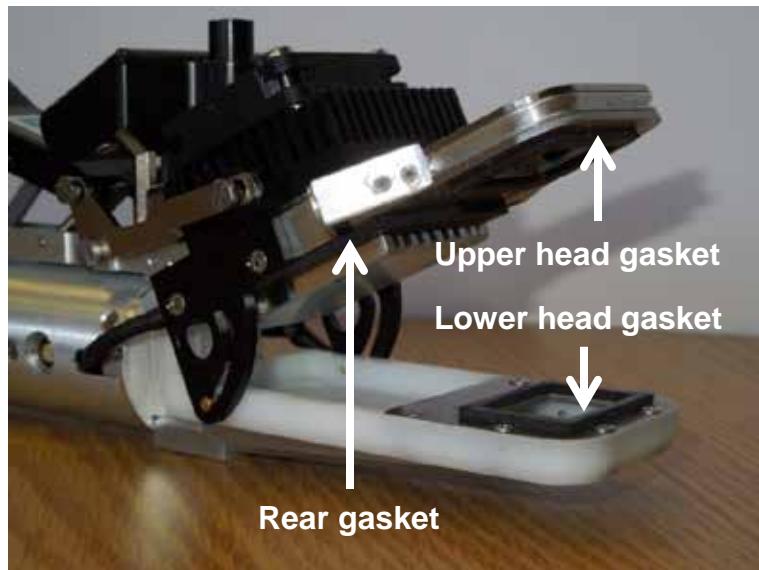
H₂O Calibration

For H₂O calibration, an air supply of known water vapor concentration is required (mb). Refer to [H₂O Calibration](#) on page 101 for instructions. Typically this is achieved using a commercially available humidity generator or water vapor generator. If you do not have a device for H₂O recalibration, please contact PP Systems and we will be happy to recommend some. PP Systems can also perform H₂O 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 CO₂ and H₂O 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 CO₂ differential (CO_{2d}) close to 0 ($\pm 0.5 \mu\text{mol mol}^{-1}$ and stable). If your CO_{2d} is this 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 check 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 CO_{2r}, CO_{2a}, H_{2O}r and H_{2O}a readings and the CO₂ differential (CO_{2d}) should be close to 0 ($\pm 0.5 \mu\text{mol mol}^{-1}$ 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 **Error! Bookmark not defined..**

If you observe a CO_{2d} 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

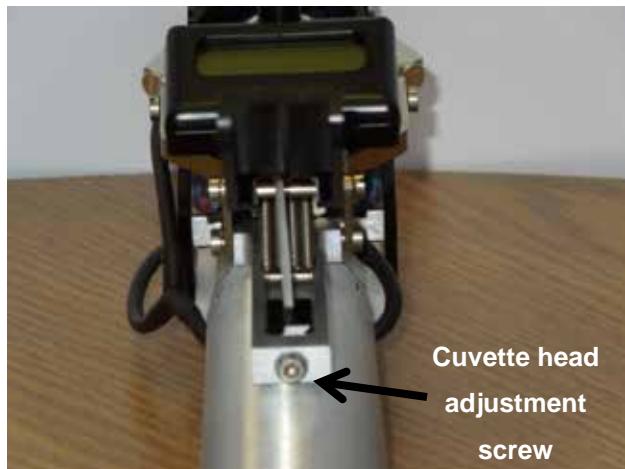


monitor the CO₂d on the CIRAS-3 console. If you see a spike or increase in CO₂d you have found the leak. Replace the gaskets (see [Cuvette Gaskets](#) on page 220. Also, you should check to make sure that the head is closing and sealing properly. If the head is not sealing properly, you may need to adjust the tension on the head. Refer to [PLC3 Leaf Cuvette Head Adjustment](#) on page 222.

If you observe a fluctuating CO₂d value that is also > 1.0 $\mu\text{mol mol}^{-1}$ 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 $\frac{1}{4}$ 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.

PLC3 Pneumatic Connector



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

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

Environmental Sensors

Temperature

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

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

PAR

All PLC3 leaf cuvettes include sensors for measurement of PAR internal (PARi) and PAR external (PARe). PAR sensors do require frequent checks and recalibration is often necessary usually after 2 years. A simple check is to 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 108.

Boundary Layer Determination (r_b)

A close approximation of r_b is required for three important gas exchange calculations used in CIRAS-3 firmware: leaf temperature derived from the Energy Balance method (Equation A.6), stomatal conductance (g_s) (Equation A11 and A12) and sub-stomatal CO₂ concentration (C_i) (Equation A.16). (Refer to [Appendix A. Gas Exchange Equations](#) starting on page 231).

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).

For a PLC3 Universal cuvette with the 18 x 25 mm window, the area of filter paper is 4.5 cm² exposed on both surfaces. For a PLC3 (Narrow or Conifer) the filter paper area is 3 x 1 cm (3 cm² 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

The leaf simulant can be a simple water-resistant envelope or filter paper with an 18 x 25 mm rectangular 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 here are 18 x 25 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:

Environmental Control Settings (F2)	
CO₂ Reference	Exact Reference Air and set to 0
H₂O Reference	Fixed % of Reference and set to 0
Cuvette Flow	Set to 470
Light Source	LED and set to 0
Temperature Sensor Measurement	Set to IR Sensor
Temperature Control Type	Set to Cuvette Temperature
Set Temperature	Set to 25 °C

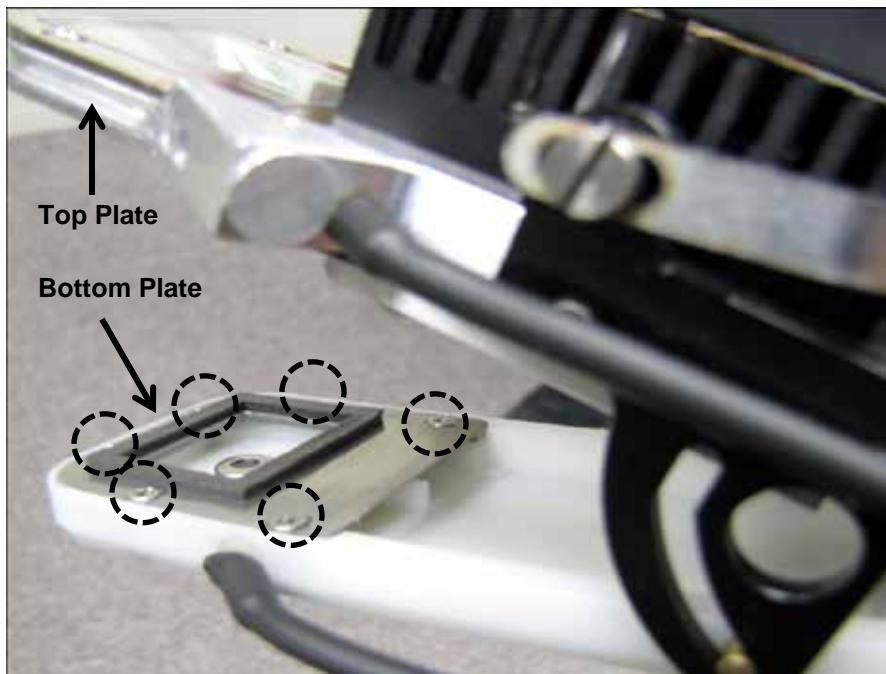
Perform a Zero and Diff Bal cycle. Set up the graph display to plot H₂Od (H₂O differential). Wait until T_{cuv} is 25 °C (+/- 0.5 °C). With a syringe or moist cotton swab, carefully wet the exposed filter paper area. Avoid water on the aluminum foil or plastic frame of the envelope. Do not wet the closed 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 (T_{cuv}). Note that the evaporation rate ($\text{mmol m}^{-2} \text{ s}^{-1}$) will be much higher than typical leaf transpiration rates. Ensure that r_b is $\leq 0.5 \text{ m}^2 \text{ s}^{-1} \text{ mol}^{-1}$. 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 mm x 7 mm (1.75 cm²)
- 18 mm Diameter (2.5 cm²)
- 25 mm x 18 mm (4.5 cm²)

Each head plate has a top and bottom so carefully note which one is the top and bottom. They look very similar but they are slightly different. Each plate is held in place by 6 small Philips type screws.

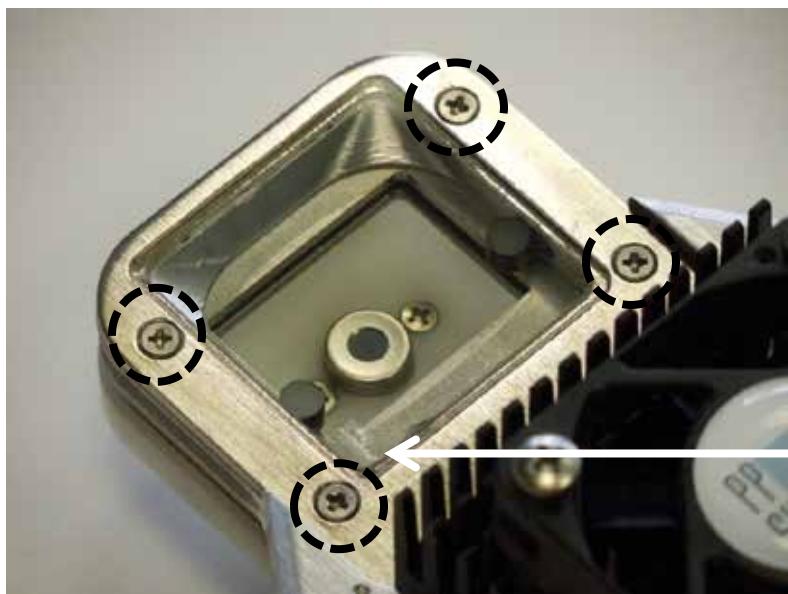


Open the cuvette head to maximum position and using a long, thin Philips screwdriver, remove the top and bottom head plates.

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 most leaf gas exchange measurements a Calflex window is used with the PLC3 Universal Leaf Cuvette. However, for measurement of chlorophyll fluorescence using the CFM-3 the Calflex window must be replaced with a plain glass window. When the CFM-3 is purchased with the CIRAS-3 the glass window is already fitted to the PLC3 Universal Leaf Cuvette.



To remove the Calflex window plate remove the four Philips type screws and replace with the plain glass window plate as shown here. 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 H₂O_d to 100%.
- Close the leaf cuvette head with no leaf present.

Monitor the CO₂r, CO₂d and H₂O_d. After a couple of minutes, the CO₂r should be close to 0 ($\pm 1 \mu\text{mol mol}^{-1}$), CO₂d should read approximately 0 ($\pm 1 \mu\text{mol mol}^{-1}$) and H₂O_d should also read approximately 0 ($\pm 0.5 \text{ mb}$).

Section 20. 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 Sampling Pump (Thomas)
10181-1	Miniature Rotary Sampling Pump (Schwarzer)
10128-1	ASU Pump (GAST)
10045-1	Air Filter (Air Supply)
30124-1	Air Filter, 5 micron
30124-2	Air Filter, 25 micron

CO₂/H₂O Control

- STD030 Box of 10 8g. CO₂ Cartridges (Shipping restrictions apply)
- STD549 Cylinder Holder
- STD546 Regulator Assembly
- 20577-1 Gasket CO2 Seal
- STD569 Ambient Air Sampling Intake Pole

Batteries & Power Supplies

- 41526-1 Battery Pack Li-ion 7.2V (Aved)
- 41535-1 Battery Pack Li-ion 7.2V (Inspired Energy)
- 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 Adapter, CIRAS-3

Cables/Data Storage

- STD545 USB Cable A Plug to 5p Mini B Plug
- 43034-1 USB Flash Drive (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)

Appendix A. Leaf Gas Exchange Equations

Mass Flow

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

(A.1) The CIRAS-3 mass flowmeter is calibrated to read the volume flow (V_0) at 0 °C and 1013.25 mb (STP). The Ideal Gas molar volume is 22.414 L mol⁻¹ at STP. Therefore:

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

where:

a = projected leaf area (measured in cm² converted above to m²)

V_0 = volume flow (measured in cc min⁻¹ converted above to L sec⁻¹)

Note that W and all subsequent equations are presented as quantities per unit leaf area. The projected leaf area is an input to the CIRAS-3 software to correctly compute gas exchange results. Normally, a PLC3 gasket plate is selected so that leaves under test will completely fill the cuvette window. The CIRAS-3 software sets the Leaf Area to match the cuvette gasket plate area by default (either 1.75 cm², 2.5 cm² or 4.5 cm²). However, if measurements are made with leaves that don't completely fill the window, the actual Leaf Area should be entered in Controls or Settings prior to making measurements. Alternately, if the Leaf Area is not known at the time of measurements, PP Systems provides a simple CIRAS-3 Excel® spreadsheet program that allows recalculation of all gas exchange results with different Leaf Area.

Transpiration

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

(A.2) The molar flow of water vapor (mol m² s⁻¹) into the cuvette is:

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

(A.3) The molar flow of air out of the cuvette (with the addition of transpired water vapor) is ($W + E$). Therefore, the molar flow of water vapor out of the cuvette is:

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

(A.4) However, the difference between the molar flows into and out of the cuvette must equal the transpiration, so:

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

(A.5) Therefore:

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

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 H₂O IRGA.

e_{out} is defined as the partial pressure of water vapor in the air inside the cuvette, surrounding the leaf. This air is both highly mixed by the stirring fans and influenced by transpiration water vapor from the leaf. e_{out} partial pressure is determined by the Analysis H₂O IRGA. As related to the calculated values in the CIRAS-3 display:

$$e_{in} = H2Or$$

$$e_{out} = H2Oa$$

Leaf Temperature

Calculate leaf temperature (T_{leaf}) from the energy balance

The Energy Balance technique estimates leaf temperature by equating energy flux into the leaf with energy flux out of the leaf. The model includes incident solar radiation, leaf re-radiation, convective heat transfer, and transpiration. (Note: the energy balance estimate for leaf temperature is just one option on CIRAS-3. It is more typical to measure leaf temperature directly with the non-contact IR leaf temperature sensor built-in to the PLC3-Universal cuvette, and the PLC3-Conifer/Narrow cuvette has a small thermistor that can be used also.)

(A.6) From Parkinson, 1983, the energy balance technique gives the difference between air and leaf temperature as:

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

where:

H = incident radiation absorbed by the leaf

λ = latent heat of vaporization of water

E = transpiration rate

M_a = molecular weight of air

C_p = specific heat at constant pressure

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

σ = Stefan Boltzmann constant

T_c = cuvette air temperature

H is calculated from the photon flux incident on the cuvette (Q), taking into account the ratio of infra-red to visible radiation and typical reflection/absorption factors by the leaf:

$$H = Q \times Trans$$

Where $Trans = 0.14$ the ratio of infrared to visible radiation, and converting photon flux to energy units

To simply computation, the following approximation is made:

$$4\sigma \times ((T_c + 273)^3) \cong (4.639 + (0.5834 \times T_c))$$

(A.7) Then, the leaf temperature is:

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

Saturation Vapor Pressure

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

(A.8) From Buck, 1981 (using e_{w1} and f_{w1}) we calculate:

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

Where e_{leaf} = saturated water vapor pressure inside leaf at T_{leaf}

Stomatal Conductance

Calculate stomatal conductance (g_s)

(A.9) From von Caemmerer & Farquhar, 1981 (Eq B14), total leaf conductance to H₂O transfer is calculated as:

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

(A.10) Since $1/g_{total} = r_s + r_b$,

(A.11) stomatal resistance can be calculated as:

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

(A.12) Stomatal conductance is the inverse of stomatal resistance:

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

Net Photosynthesis

Determine the rate of net photosynthesis (A) from the difference between CO₂ concentrations entering (C_{in}) and exiting (C_{out}) the cuvette

(A.13) IRGA CO₂ 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:

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

(A.14) To calculate net CO₂ assimilation we rearrange equation (A.13) to:

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

CIRAS-3 calculates and displays the CO₂ difference ($C_{out} - C_{in}$). As related to the calculated values in the CIRAS-3 display:

$$C_{out} = CO2a$$

$$C_{out} - C_{in} = CO2d$$

Intercellular CO₂ Concentration

Calculate CO₂ concentration in the sub-stomatal cavity (C_i) using the equation derived by von Caemmerer & Farquhar, 1981

(A.15) The sub-stomatal CO₂ concentration, C_i , is given by:

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

(A.16) Where g_c is the total conductance to CO₂ transfer:

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

(1.585 is the diffusion ratio of CO₂ and water in *air*, and 1.37 is the diffusion ratio of CO₂ 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.

Definition of Symbols and Physical Constants Used in Equations

Measured Parameters

Symbol	Parameter	Unit
V_0	Flow rate of dry air into cuvette at STP	cc min ⁻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 air per unit leaf area into cuvette	$\text{mol m}^{-2} \text{s}^{-1}$
e_{in}	Partial pressure of water vapor of air <i>entering</i> cuvette = H_2O_r	mb *
e_{out}	Partial pressure of water vapor of stirred cuvette air = H_2O_a	mb *
E	Transpiration rate	$\text{mmol m}^{-2} \text{s}^{-1}$
e_{leaf}	Saturated vapor pressure (inside the leaf) at leaf temperature	mb
T_{leaf}	Leaf temperature	$^{\circ}\text{C}$
Δt	Temperature difference between the air and the leaf	$^{\circ}\text{C}$
H	Radiation absorbed by the leaf	W m^{-2}
r_s	Stomatal resistance to water vapor	$\text{m}^2 \text{s mol}^{-1}$
g_s	Stomatal conductance to water vapor	$\text{mmol m}^{-2} \text{s}^{-1}$
C_{in}	CO_2 concentration of air <i>entering</i> cuvette = CO_2_r	$\mu\text{mol mol}^{-1} *$
C_{out}	CO_2 concentration of air <i>inside</i> and <i>exiting</i> the cuvette = CO_2_a	$\mu\text{mol mol}^{-1} *$
A	CO_2 Assimilation rate (net photosynthesis)	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_c	Total conductance to CO_2 transfer	$\text{mmol m}^{-2} \text{s}^{-1}$
g_{total}	Total conductance to H_2O transfer	$\text{mmol m}^{-2} \text{s}^{-1}$
C_i	CO_2 concentration of sub-stomatal cavity	$\mu\text{mol mol}^{-1}$

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

Physical Constants

Symbol	Parameter	Value
λ	Latent heat of vaporization of water	$45064.3 - (T_c \times 42.9) \text{ J mol}^{-1}$
M_a	Molecular mass of air	28.97
C_p	Specific heat at constant pressure	$1.012 \text{ kJ kg}^{-1} \text{ K}^{-1}$
σ	Stefan Boltzmann constant	$5.6704 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$

References

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

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Appendix B. Chlorophyll Fluorescence Calculations

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 and 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.

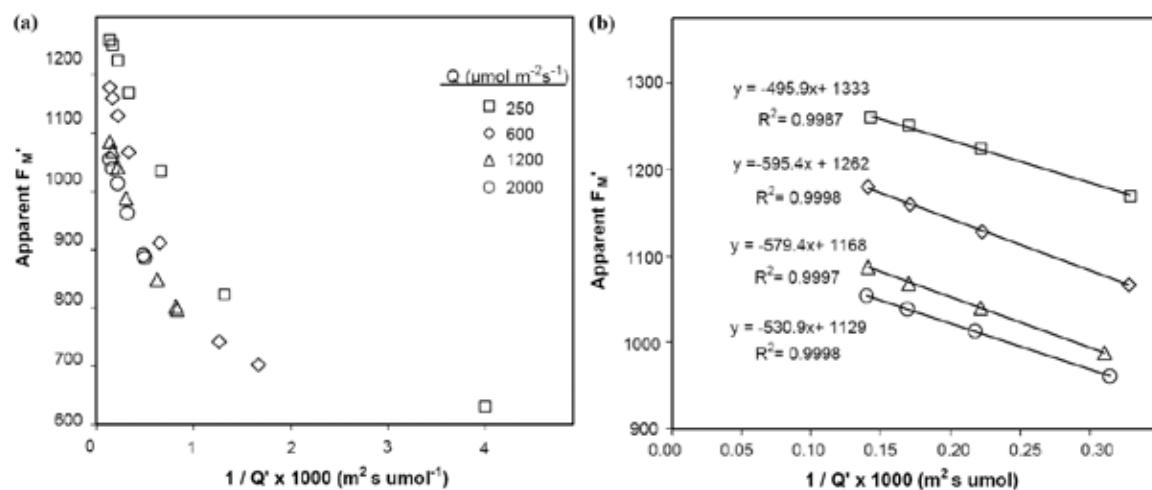
Calculations Used for Chlorophyll Fluorescence Parameters

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 $\alpha=0.84$; 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}	$\Phi_{NPQ-G} = \frac{F_s}{F_{m'}} \cdot \frac{F_s}{F_m}$

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 MultiPulse 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 MultiPulse 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 MultiPulse 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 MultiPulse 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 displays Fm' at 1/Q' = 0.

Our testing of CFM-3 MultiPulse 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 $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (the low point), and generate the intermediate light intensities by evenly distribute numbers in between.

One advantage of using MultiPulse 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

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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

Appendix C. Soil Respiration and Canopy Flux Equations

Theory

The respiration (or assimilation) is measured by placing a closed chamber on the soil and measuring the rate of increase of the CO₂ concentration inside the chamber.

(C.1) Then, assuming a well-mixed and sealed system:

$$F_{CO_2} = \frac{(Cn - Co)}{T_n} \times \frac{V}{A}$$

Where:

F_{CO_2} = respiration/assimilation rate (CO₂ flux in moles or grams of CO₂ unit area⁻¹ unit time⁻¹)

Co = is the CO₂ concentration at $T = 0$

Cn = is the concentration at a time T_n later

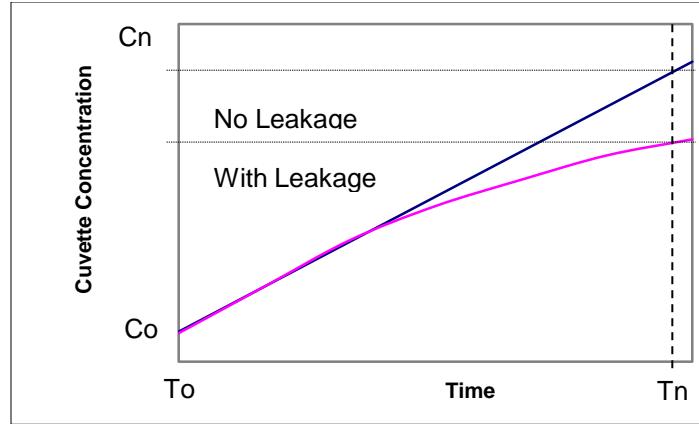
A = is the area of soil exposed

V = the total system volume

It has been suggested that to make accurate measurements of the respiration it is essential to start with a CO₂ concentration in the chamber below ambient and measure until the concentration is above ambient, presumably with the intention of getting some compensation for leakage. However, this leakage can only take place at ground level, where the CO₂ 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 CO₂ 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 chamber CO₂ concentration.

Any leakage should be a function of the concentration difference between the chamber and the exchange air. Due to leakage, the apparent assimilation rate decreases with time. So leakage would have the effect of changing the ideal linear C vs T relationship to a non-linear relationship.



Soil Respiration Measurements

The CIRAS-3 software assumes a quadratic relationship ($y = a + bx + cx^2$) between the chamber concentration ($C=y$) and time ($T=x$) from the start of measurement to account for the non-linearities caused by leakage. Note that there is a delay after the chamber is first placed on the soil to allow for the establishment of stable gradients before the measurements begin.

(C.2) The quadratic equation is

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

Where C and T are a series of chamber CO₂ concentration measurements made over time, and a , b , c are coefficients calculated from a least square fit of the data.

The respiration rate will be calculated from the rate of change of CO₂ at time zero or dC/dT at $T=0$.

(C.3) Differentiating equation **(C.2)** yields

$$\frac{dC}{dT} = b + 2cT$$

(C.4) And evaluated at $T = 0$,

$$\frac{dC}{dT} = b$$

A comparison of b and cT gives an indication of the magnitude of the non-linearity of the C vs T data. The CIRAS-3 software indicates a "non-linear" error message whenever the value cT is greater than 20% of b . This is believed to be a better approach than lowering the CO₂ value at the start of the measurement.

In addition to the quadratic assumption and calculation, the CIRAS-3 software also calculated the respiration (or assimilation) using a linear assumption.

(C.5) The linear equation is

$$C = a + bT$$

(C.6) which evaluates again to

$$\frac{dC}{dT} = b$$

In this case the value of dC/dT is calculated from a linear regression of the C and T data.

Correction for water vapor increase on CO₂ efflux

In addition to the influx of CO₂ from soil, a closed chamber system can also experience an increase in H₂O due to evapotranspiration from soil or plants. This added H₂O dilutes the remaining CO₂ and requires compensation in a similar way to equation (A.13).

For total moles of air in the chamber of W ,

(C.7) the moles of H₂O added to the chamber is $E = \frac{(e_{final} - e_{init})}{(1000 - e_{final})}$,

where e_{init} and e_{final} are H₂O concentrations in mmol mol⁻¹.

The initial moles CO₂ in the system = $C_{init} \times W$, where C_{init} is CO₂ concentration in mol mol⁻¹.

Then, the final moles CO₂ in the system = $C_{final} \times (W + E)$, where C_{final} is CO₂ concentration in mol mol⁻¹.

(C.8) The change in moles of CO₂ is $(C_{final} \times (W + E)) - (C_{init} \times W) = (C_{final} - C_{init})W + C_{final}E$

ppm = umol/mol

The term $(C_{final} - C_{init})W$ is equivalent to the dC/dT term calculated above, and the $C_{final}E$ term is the correction due to evapotranspiration.

W = fluxbot_air_density()

(C.9) So, with the H₂O compensation, we have

(umol/mol)*mol = umol (final units of b here as they say, but inherently over time)

$$\frac{dC}{dT} = b + C_{final} \left(\frac{e_{final} - e_{init}}{1000 - e_{final}} \right) \frac{\text{umol/mol} - \text{umol/mol}}{1000 - \text{umol/mol}}$$

where b can be either from the quadratic or linear fit.

FCO₂ Units for measurement of Soil CO₂ Efflux

(C.10) To give the CO₂ flux in mass/unit area/unit time the following conversion is made:

$$F_{CO_2}(\text{g m}^{-2}\text{hr}^{-1}) = \frac{dC}{dT} \frac{\mu\text{mol}}{\text{mol s}} \times \frac{P}{1013} \times \frac{273}{273 + T_{air}} \times \frac{44.009 \text{ g}}{22.414 \text{ L}} \times \frac{V \text{ m}^3}{A \text{ m}^2} \times \frac{1 \text{ mol}}{10^6 \mu\text{mol}} \times \frac{3600 \text{ s}}{\text{hr}} \times \frac{10^3 \text{ L}}{\text{m}^3}$$

Where

dC/dT is from eqn C.9 from linear or quadratic fit and with H₂O compensation

$\frac{P}{1013}$ is the correction for barometric pressure with P measured in mbar by the CIRAS-3,

$\frac{273}{273+T_{air}}$ is the correction for air temperature with T_{air} input by the user in °C,

$\frac{44.009 \text{ kg}}{22.414 \text{ m}^3}$ is the molar volume and Ideal Gas constant at STP,

$\frac{V \text{ m}^3}{A \text{ m}^2}$ is the chamber volume and soil surface area,

And the remaining terms are units conversions.

(C.11) The same equation in alternate units is

$$F_{CO_2}(\mu\text{mol m}^{-2} \text{s}^{-1}) = \frac{dC}{dT} \frac{\mu\text{mol}}{\text{mol s}} \times \frac{P}{1013} \times \frac{273}{273 + T_{air}} \times \frac{1 \text{ mol}}{22.414 \text{ L}} \times \frac{V \text{ m}^3}{A \text{ m}^2} \times \frac{10^3 \text{ L}}{\text{m}^3}$$

Or to convert (g m⁻² hr⁻¹) to (μmol m⁻² s⁻¹) multiply F_{CO_2} (g m⁻² hr⁻¹) 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.

Appendix D. Insect Respiration Equations

Respiration Rate can be presented in many different units using the same two basic measurements from the CIRAS-3, CO2d ($\text{CO2a} - \text{CO2r}$) and V_0 (Cuvette Flow Rate).

(D.1) Volumetric Respiration Rate:

$$V_{\text{CO}_2} (\mu\text{L CO}_2 \text{ min}^{-1}) = \text{CO2d} \times V_0 \times (10^{-3} \frac{\text{L}}{\text{mL}})$$

Where CO2d = CO_2 difference in ($\mu\text{mol mol}^{-1}$)

And V_0 = cuvette flowrate in (mL min^{-1}) at STP.

(D.2) Respiration Rate in mass of CO_2 per Insect:

$$M_{\text{CO}_2} (\mu\text{g CO}_2 \text{ min}^{-1} \text{ ind}^{-1}) = \text{CO2d} \times V_0 \times \rho / n_{\text{ind}}$$

Where ρ = density of CO_2 at STP = $\frac{44.0 \text{ g}}{22400 \text{ mL}}$

And n_{ind} = number of individual insects in the chamber

This rate is displayed on the CIRAS-3 screen and output in the data file as **Respl**.

(D.3) Respiration Rate in mass of CO_2 per Insect weight:

$$M_{\text{CO}_2} (\mu\text{g CO}_2 \text{ g}^{-1} \text{ min}^{-1}) = \text{CO2d} \times V_0 \times \rho / W_{\text{insect}}$$

Where ρ = density of CO_2 at STP = $\frac{44.0 \text{ g}}{22400 \text{ mL}}$,

And W_{insect} = weight of insect (g) either dry or fresh.

This rate is displayed on the CIRAS-3 screen and output in the data file as **Resp/wt**.

(D.4) Molar Respiration Rate:

$$M_{\text{CO}_2} (\mu\text{mol CO}_2 \text{ min}^{-1}) = \text{CO2d} \times V_0 \times \left(\frac{1 \text{ mol}}{22400 \text{ mL}} \right)$$

Where $\left(\frac{1 \text{ mol}}{22400 \text{ mL}} \right)$ = molar