CIRAS-2
Portable Photosynthesis System
Operator's Manual

Version 2.04

For Remote Control Software (RCS) Version 1.07 or greater.

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Preface

Notice

This equipment must not be used in situations where its failure could result in injury or death.

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 criticality of the situation.

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 contained in this manual is accurate and complete. PP Systems does not accept any liability for losses or damages resulting from the use of this information.

It is extremely important that you take the time 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.

This manual and the information contained within are copyright 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 electrical means, without the prior written consent of PP Systems.

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MS-DOS is a registered trademark of IBM.

Documentation Conventions

If viewed electronically, text marked blue acts as Hyperlinks.

User Registration

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

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

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

Thank you in advance for your cooperation.

Service & Warranty

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

The equipment is covered under warranty for one complete year, parts and labour included. This, of course, is provided that the equipment is properly installed, operated and maintained in accordance with written instructions (i.e. Operator's 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 authorised agent for repair or replacement of the defective part(s).

Contact Information

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Unpacking Your Equipment

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

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

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

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

CIRAS-2 Essentials

Please observe the following to ensure correct operation of and avoid damage to CIRAS-2:

Before Powering On and Operating the System

- Charge all 12V NiMH batteries prior to making measurements.
- Charge each 12V NiMH battery independently using the chargers supplied by PP Systems.
- Use only the power supply supplied by PP Systems for powering the CIRAS-2 externally.
- Ensure that the probe (i.e. leaf cuvette) electrical connector is properly connected to CIRAS-2 before switching on power.
- Be sure that the reference and analysis gas fittings are correctly connected to the proper gas ports.
- Check that the chemicals contained in the absorber columns are fresh.
- Check that i) all absorber columns and ii) the water vapour equilibrator are properly seated in their correct manifold positions.
- Check for pinched or rolled “O” rings in the top and bottom caps of the absorber columns.
- Close the leaf cuvette to allow successful LED tuning.
- Always operate CIRAS-2 in a vertical position.

Important Precautions

- Do not use substitute battery chargers or power supplies with CIRAS-2.
- Avoid allowing water into any CIRAS-2 gas inlets or the leaf chamber. Do not use on wet foliage or attempt to humidify the gas stream by direct artificial introduction of humidified air.
- If there is any risk of water entering or condensing inside CIRAS-2, water dropout traps must be fitted.
- External filtration may be required in extremely dirty/dusty atmospheres.
- Do not attempt to change the CO_2 cartridge until at least 24 hours have elapsed since inserting the cartridge in the CO_2 regulator assembly.
- To prevent voltage spikes and software conflicts avoid attaching/detaching probe (i.e. leaf cuvette) electrical connections while CIRAS-2 is powered on.
- Avoid powering off CIRAS-2 before completely exiting the RCS program.

!!! WARNING !!!

USE ONLY POWER SUPPLIES AND BATTERY CHARGERS SUPPLIED BY PP SYSTEMS FOR THE CIRAS-2.

USE OF ANY OTHERS WILL INVALIDATE THE WARRANTY.

Getting Started

The CIRAS-2 Operator's Manual is designed to assist users running remote control software (RCS) v. 1.07 or greater. If the instructions and figures in this Operation Manual do not appear similar to your CIRAS-2 system click **Help – About** in the RCS menu bar to view which software version you currently have installed on your system. RCS v. 1.07 will be available for upgrade through the Registered Users Area of our website: http://www.ppsystems.com/user_registration2.htm



At this point we expect that you have completed the CIRAS-2 Tutorial - the Tutorial is a condensed version of the Operator's Manual and intended to quickly familiarize you with making basic leaf gas exchange measurements. **IF YOU HAVE NOT YET READ AND WORKED THROUGH THE TUTORIAL PLEASE DO SO NOW, BEFORE CONTINUING.**

A Technical Manual is available to all registered users as well. The **CIRAS-2 Technical Manual** discusses topics such as:

- Electrical and Pneumatic Connections
- Gas Circuit and Flow Diagrams
- Desiccants
- Troubleshooting and Servicing
- Maintenance
- Calibration

Visit the Registered Users Area of our website to download the Technical Manual.

The Operator's Manual is designed to guide you through the various menus, displays and operational features of CIRAS-2. Each CIRAS-2 system is supplied with two versions of the remote control software program (RCS): i) Windows CE (pre-installed on the UI and earlier PC-Pencentra) and ii) full Windows version (for lab PC or notebook computer) on CD ROM. The Windows CE version of RCS comes pre-installed on the UI with a back-up copy supplied on a Flash Card. All of the menus and displays are identical in both versions of CIRAS-2 RCS. **Please note:** due to file sizes and availability of space, Help Files are not available on the UI.

We recommend that you install the Windows software to your lab computer upon receipt of your system. We also recommend that you first become familiar with the operation of your system using your PC or notebook, taking advantage of the viewing ability and convenience of the computer. It is a good idea to take a few moments to review the ReadMe.txt file contained in the root directory on the CD ROM supplied. This file contains important information relating to the installation of the CIRAS-2 remote control program.

Installing CIRAS-2 Remote Control Software on your computer

To begin installation of the CIRAS-2 RCS on your PC or notebook:

1. Place the CD ROM supplied by PP Systems into your CD ROM drive on your computer.
2. Run Setup.exe located in the \CirasRCS\Windows folder and follow all instructions.

Once installation is complete and you have made the RS232 cable connection to your computer (see Step 7, **Preparations for Use**, above) click the **Start** button on the Windows Task Bar, **Programs**, and then **CIRAS-2 Remote Control Software**, to enter the program.

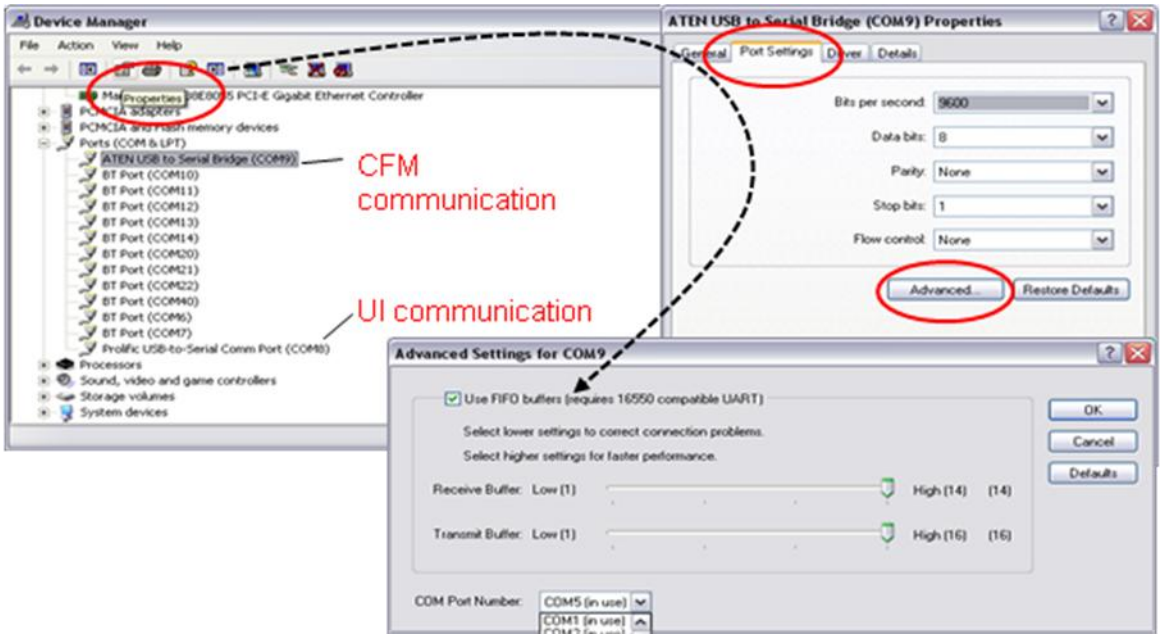
Please note: If you are warned that certain files cannot be overwritten because they are in use, you will have to perform the following procedure.

1. Copy the contents of the CD ROM Windows folder to a convenient location on your PC local drive (i.e. C:\) and **NOT** to a network drive. **Please note:** the current version of the CIRAS-2 software will not allow you to install RCS to a network drive.
2. Restart the Computer and go into Safe Mode (press F8 at boot up). You will then be prompted how to proceed. Select the Option for Safe Mode. Please note that your Display may look different (i.e. lower resolution). Use Windows Explorer to locate the setup.exe file and proceed with the installation.
3. When completed, restart your computer.

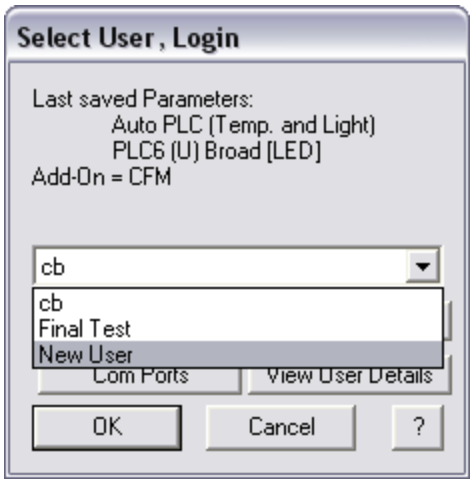
Avoiding UI-to-PC Communication Errors

It is possible to experience critical software errors which can interrupt system operations due to periodic miscommunication between Windows operating systems and CIRAS-2 hardware. PP Systems is currently researching permanent solutions to these errors. Communication failures are indicated by a “bad character string” dialog box, and will cause the user to be disconnected from CIRAS-2. If this problem is encountered during RCS control of CIRAS-2 from your computer, a short-term solution is to uncheck “use FIFO buffers” found at the top of the **Advanced Settings** dialog box in Windows Device Manager – Properties – Port Settings (see figure below and also page 22, Step 7. Connect CIRAS-2/PC interface cable).

However, the best way to avoid these issues is to have a single computer dedicated to running RCS in the lab, greenhouse, or field. Computers that are used for multiple applications (internet, email servers, multi-media, etc) are most prone to experiencing this problem. We advise the user to remove all non-essential communications programs from the computer used to run RCS.

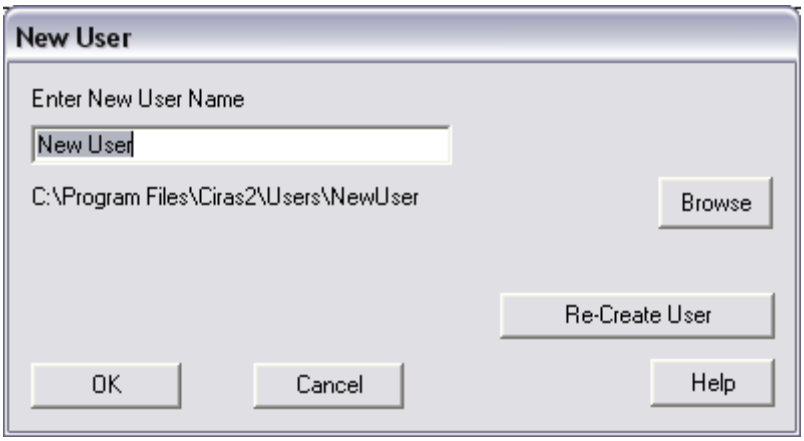


You can run your RCS software by going to **Start**, **Programs, CIRAS-2 Remote Control Software** or by clicking the CIRAS-2 Remote Control Software icon on your desktop screen. The first time that you run RCS the **Select User** dialog (below) will be displayed on the screen:



Later, when you set up your preferences and settings, this information will automatically be saved for you. The most recently used preferences and settings will load each time that the RCS program is subsequently started, and the login procedure will be circumvented.

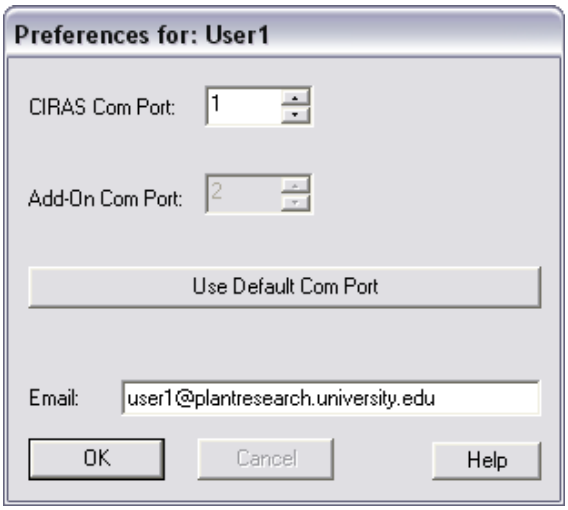
The 'New User' option facilitates multiple operators sharing the same equipment without altering or overriding each others individual settings. Each 'User' can pre-program and permanently store several unique sets of hardware/software settings and routines under which CIRAS-2 is operated. For example, you can set up CIRAS-2 to measure leaf-level gas exchange responses to controlled environmental conditions, or to measure soil respiration rates.

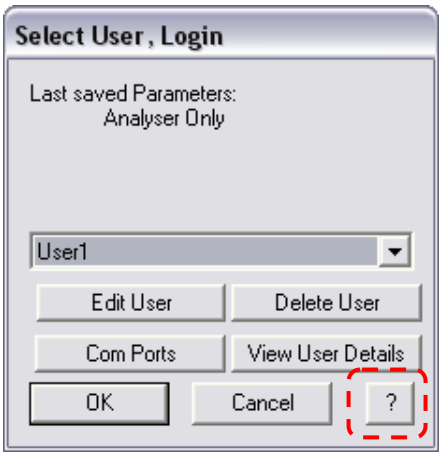


Select 'New User' from the drop down list and click **OK**. Next, enter a unique name (above) that will be used to create the folder which will hold important preferences and CIRAS-2 set up information for a particular end user. The file will be saved in the following subdirectory on your computer:

C:\ProgramFiles\Ciras2\Users\

After entering a user name, select **OK**. You will be informed as to the exact location of the file. Click **OK** to continue. The **Preferences** dialog will now open. Enter the Com port that is being used with CIRAS-2. The correct COM port MUST be selected for inter-communication between the computer and CIRAS-2. There are 99 COM port options currently available to the user. By default, the COM port on the UI is set to COM1. To select the appropriate COM port see the Windows Device Manager instructions above (Step 7. Connect CIRAS-2/PC interface cable. Finally, enter a valid email address as an additional identifier associated with your newly created User identity and to help with technical support. Your email address will be contained in subsequent data and diagnostic files.





Click **OK** to save your changes to **Preferences for:** (above, left). The **Login** dialog automatically defaults to the basic 'Analyser Only' setting (CIRAS-2 as a stand-alone CO_2 and H_2O IRGA).

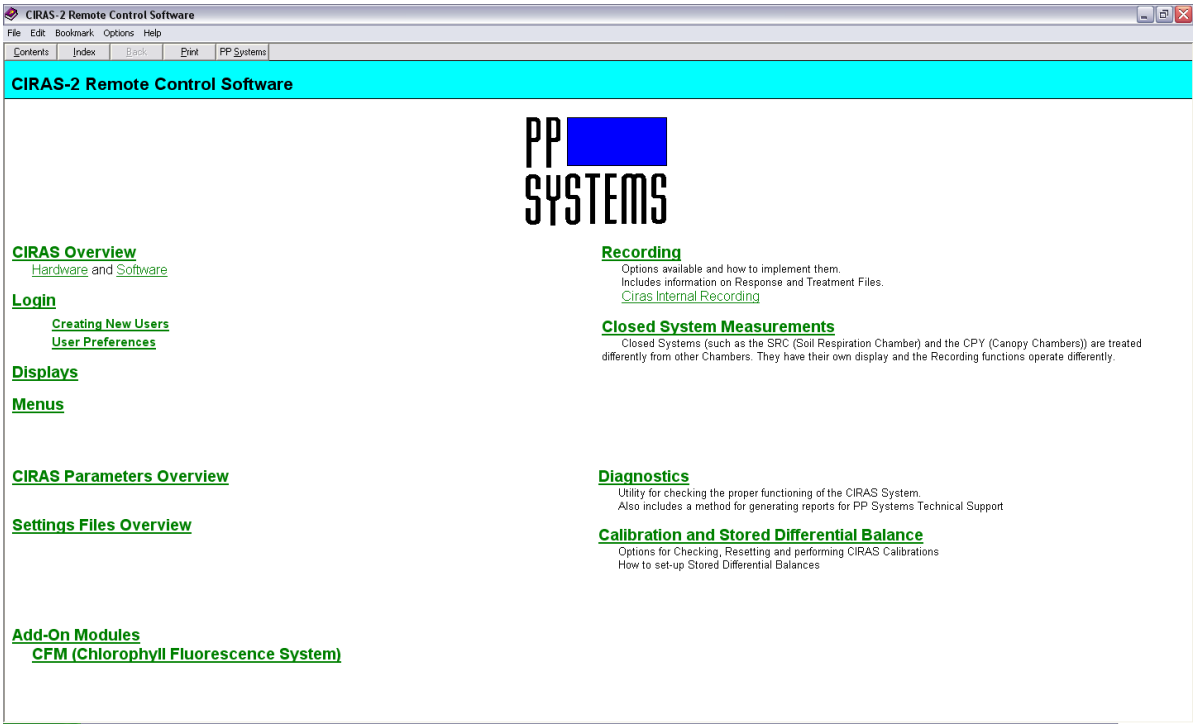
Click **OK**. The CIRAS-2 Measurement Screen will appear in Analyser Only mode. Next, click on **Setup** in the CIRAS main menu to open the **Settings** dialog. At this time you will only need to make two simple selections.

Open the 'Accessory' drop down list containing the standard cuvettes and chambers that may be used with CIRAS-2. From this list select 'PLC6 (U) 18 mm diameter insert' – this is the most common leaf cuvette used with the CIRAS-2 system. By making this selection, various other options will automatically be set to default settings that are standardized for the PLC6 (U) cuvette. In the 'Light Type' list select 'LED'. If using a different insert with the PLC6(U) or a different cuvette, select the appropriate option from the list. Click **OK**. The **Settings** dialog and all of its options will be described in detail later.

Click ? at any time for On-line help

While working with CIRAS-2 from your PC or notebook you have access to compiled Windows Help Files through the **?** buttons associated with most CIRAS-2 screens and dialog boxes. Help Files are context-sensitive, providing detailed information about the screen or user option you are currently viewing.

PP Systems is continuously updating our print and on-line documentation to reflect hardware and software changes. Due to this some images and information contained in compiled Windows Help Files are out of date and may not reflect exactly the current version 1.07 RCS software. Always consult the print version of the Operator's Manual first before using the on-line resource. **Please note:** Help Files are not available with the Windows CE version of RCS used with the UI.



Preparations for Use

Your system has been factory calibrated before shipment to you. Therefore, there is no need to perform a calibration of your equipment prior to use.

At this point you should have installed the CIRAS-2 Remote Control Software (supplied on CD ROM) to the computer(s) you will use to operate CIRAS-2. Both the Windows (for use with your notebook or desktop PC) and Windows CE (factory-installed on the User Interface) versions of CIRAS-2 RCS are virtually identical. We recommend familiarizing yourself thoroughly with CIRAS-2 in a controlled indoor setting before taking the system into the field.

To install the software, please refer to the Getting Started section of the Operator's Manual on page 13.

System Preparation

Step 1. Insert 12V NiMH battery packs into main console
Step 2. Connect CIRAS-2 power supply
Step 3. Make Leaf Cuvette electrical and gas connections
Step 4. Connect leaf cuvette power cable to external 12V DC Power Supply
Step 5. Inspect absorber columns (desiccants, etc.) and water vapor equilibrator
Step 6. Insert CO2 cartridge
Step 7. Connect CIRAS-2/PC interface cable (optional for computer RCS control)
Step 8. Power on CIRAS-2
Step 9. Close the leaf cuvette
Step 10. Start the CIRAS-2 Remote Control Software on your PC (optional for computer RCS control)

Step 1. Insert 12V NiMH battery packs into main console

For field measurements, the 12V NiMH battery packs should be fully charged for optimal results. This is best achieved by connecting the batteries to their respective chargers the night prior to measurements. This ensures that the battery packs are fully charged and ready to power the console and cuvette for several hours of field measurements, depending on temperature and LED light use. Batteries should have arrived fully charged with your CIRAS-2 shipment.

If the batteries require charging, unscrew the threaded cover connector on the battery pack, and taking the battery charger cable, align the battery charger connector with the three-pin battery pack connector. Thread the cable connector clockwise to complete the connection. The battery chargers have an LED indicator that glows red while fast-charging and green in the fully charged state. Normally, it takes approximately 4 hours for a discharged battery to be fully charged.

The CIRAS-2 main console has two battery compartments which accept the left and right 12V NiMH battery packs supplied with your system. Each battery pack has a thick notch (12mm) on the bottom and thin notch (6mm) on the top, so the batteries are properly oriented with the positive/negative battery terminals towards the bottom. Assuming the battery packs are fully charged, slide one battery into the left battery compartment until it snaps into place. Slide the other battery into the right battery compartment.

Step 2. Connect CIRAS-2 power supply

For laboratory work the CIRAS-2 mains power supply can be used with or without CIRAS-2 battery packs. **However, please note: At least one charged 12V NiMH battery must be in place when the system is first powered on. After CIRAS-2 is powered on, the battery may be removed if required.** Running

the system from mains power will prevent the battery packs from draining during this exercise. The mains power supply does not charge the CIRAS-2 battery packs.

Connect the mains power supply (supplied by PP Systems) from the electrical outlet in your lab to the EXT PWR socket located on the electrical/gas connection panel. The power supply is fitted with a special 4-pin plug that is designed to fit only into the EXT PWR socket. Proper care must be taken not to inadvertently force the plug into any other socket, which will cause damage to CIRAS-2's electronics.

Step 3. Make Leaf Cuvette electrical and gas connections

Locate the leaf cuvette electrical socket labelled "PLC" on the electrical/gas connection panel. Plug the 18-pin cuvette electrical connector into this socket, ensuring that the red dot on the connector is in the 12 o'clock position (top, middle). Push in until the connector latches.

To make the cuvette gas connections locate the 3 gas entries AN IN, REF IN, AIR OUT at the top of the panel:

CIRAS-2 gas/electrical connection panel – detailed view



Note that it does not matter which leg of the tubing marked "R" is connected to the "REF IN" or "AIR OUT" gas entries. Push each connector firmly onto the appropriate gas entry until you hear it lock in place.

If your system includes the integrated Chlorophyll Fluorescence Module (CFM), locate the CFM On/Off switch (beneath the UI) and set to the Off position. You will find detailed descriptions of simultaneous chlorophyll fluorescence/gas exchange functions in the CFM section on page 96.

Step 4. Connect leaf cuvette power cable to external 12V DC Power Supply

(Optional) - All automatic style leaf cuvettes are supplied with a power supply cable which allows you to power the cuvette (temperature and light control) from an external 12V battery or 12V DC regulated power supply if required. One end of the cable has alligator-style clips (red/positive and black/negative) for connection to a 12V battery or power supply. The other end has a white plastic socket which connects to the white plastic plug on the leaf cuvette cable. For example, if your CIRAS-2 system includes a PLC5(C) Conifer Cuvette with the LED light unit, then you must connect to external power due to the additional voltage requirements of the conifer cuvette.

Step 5. Inspect absorber columns and water vapor equilibrator

Absorber columns are labelled on the bottom of the CIRAS-2 console as follows:

MS	Molecular Sieve
D	Drier (Envirogel)
SL	Soda Lime

All chemicals should be fresh and columns firmly seated in the correct manifold ports as follows:

Column	Chemical	When fresh, chemical is:	When exhausted, chemical is:
1	1/3rd Molecular Sieve 2/3rd Envirogel	White (Non-Indicating) Orange	White (Non-Indicating) Green
2	Soda Lime	Green	Brown
3	Soda Lime	Green	Brown
4	1/3rd Molecular Sieve 2/3rd Envirogel	White (Non-Indicating) Orange	White (Non-Indicating) Green
5	Envirogel	Orange	Green

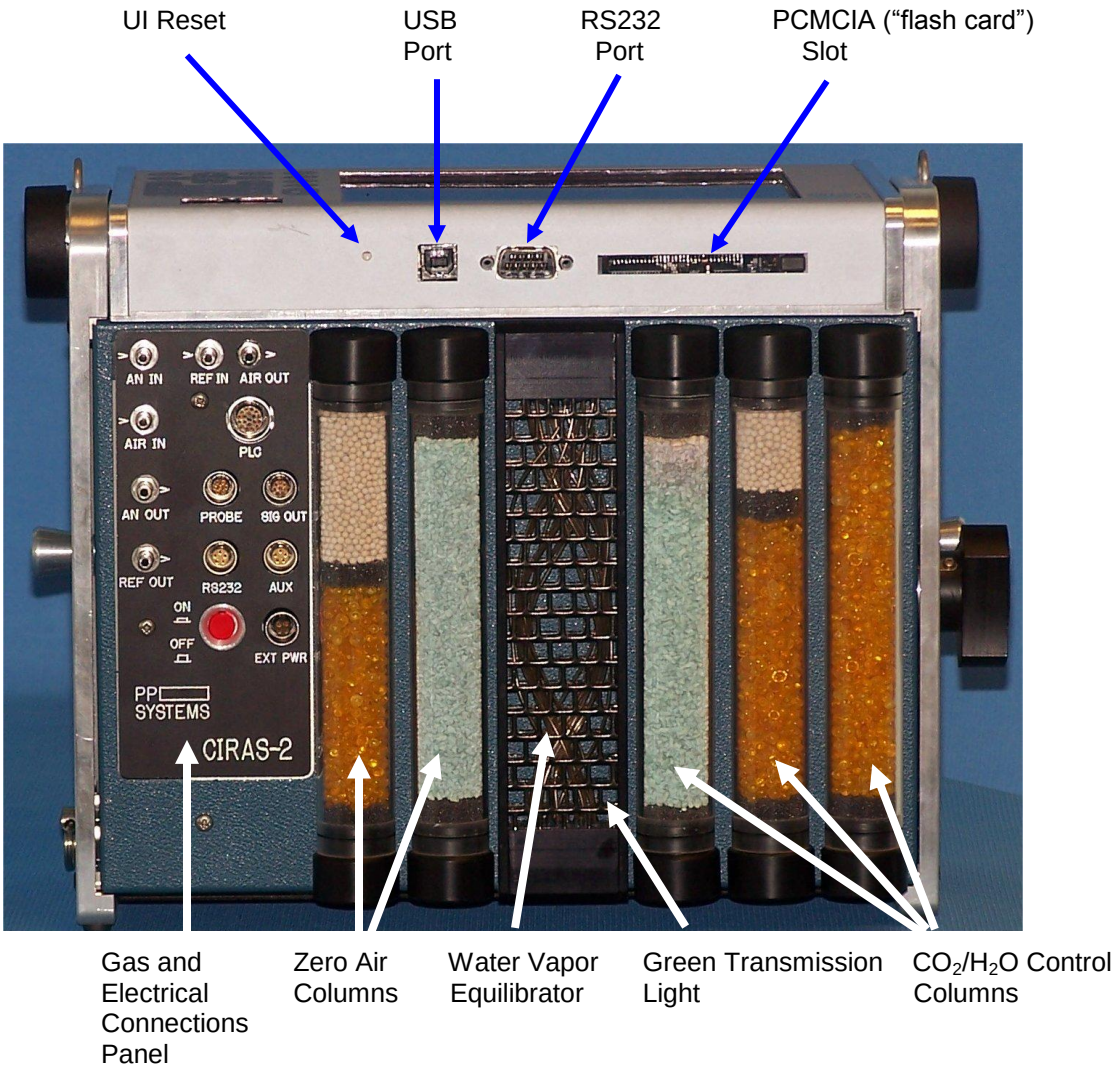
When any of the desiccants are approximately 2/3rd exhausted (e.g. when soda lime is 2/3rd brown), the entire contents of the absorber column should be replaced.

Please note: Molecular sieve desiccant is non-indicating and does not change color when exhausted. Therefore, the general rule is that you should ALWAYS change this desiccant when the Envirogel desiccant (in the same column) is 2/3rd exhausted.

Important Note: Until June 2003, we used a desiccant called “Drierite” instead of Envirogel. Both work similarly, but the layout of the CIRAS-2 absorber columns is slightly different. For more information on the layout of absorber columns based on the different drier desiccants, please visit our Users webpage and download the application note titled “Use of Drierite And Envirogel With CIRAS-1 and CIRAS-2”.

Inspect absorber column end caps and ensure they are correctly seated and that the sealing “O” rings have not rolled out of position. The Water Vapor Equilibrator should be inspected to ensure that it is firmly seated in its manifold. An image and description of the absorber columns can be seen below.

CIRAS-2 Console - Front Layout



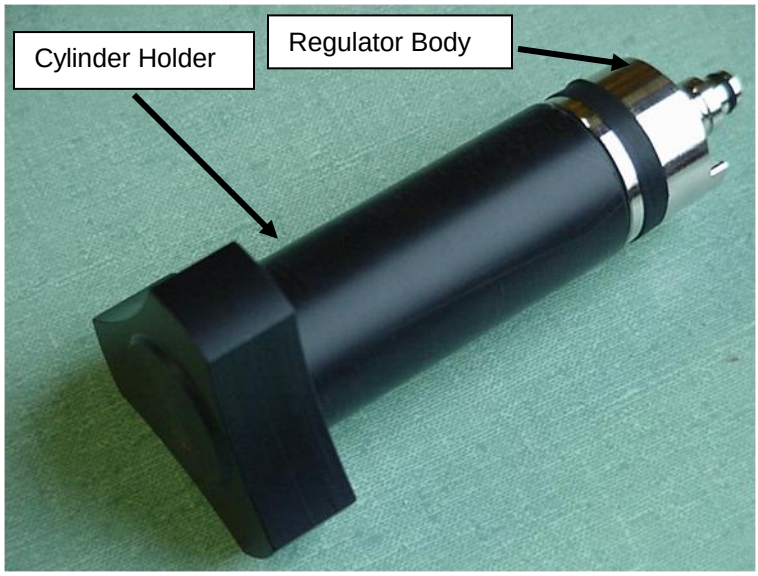
Step 6. Insert CO_2 cartridge

While still positioned inside the CIRAS console twist the CO_2 cylinder holder several turns (counter-clockwise). This will loosen the regulator body from the black holder. Remove the entire CO_2 holder-regulator assembly from the CIRAS console by pulling outward. Finish unscrewing the loosened regulator by hand. The CO_2 cylinder will remain firmly attached to the regulator body – pull it away from the regulator. Discard the old cartridge if exhausted (see below).

Spin the narrow end of a CO_2 cylinder between your fingertips to lightly lubricate it. Slide the new cylinder into the holder with the narrow cylinder neck facing out towards the open end of the holder. Thread the regulator body into the cylinder holder just until it makes contact with the CO_2 cylinder – do not attempt to tighten by hand. Place the entire assembly back into the CIRAS console, making sure that the regulator body is completely seated (simultaneously push and turn slightly until the regulator drops in several additional mm). Screw in (clockwise) until just hand tight, this will pierce the CO_2 cylinder, at which point

you may hear a faint release of the pressurized gas. Back off the holder slightly (counter-clockwise), less than one quarter turn.

CO_2 Holder and Regulator Assembly



Important Note

You must allow **at least** 24 hours to elapse before changing the CO_2 cylinder. If you are not sure that the CO_2 cylinder is sufficiently full prior to making measurements, we recommend the following:

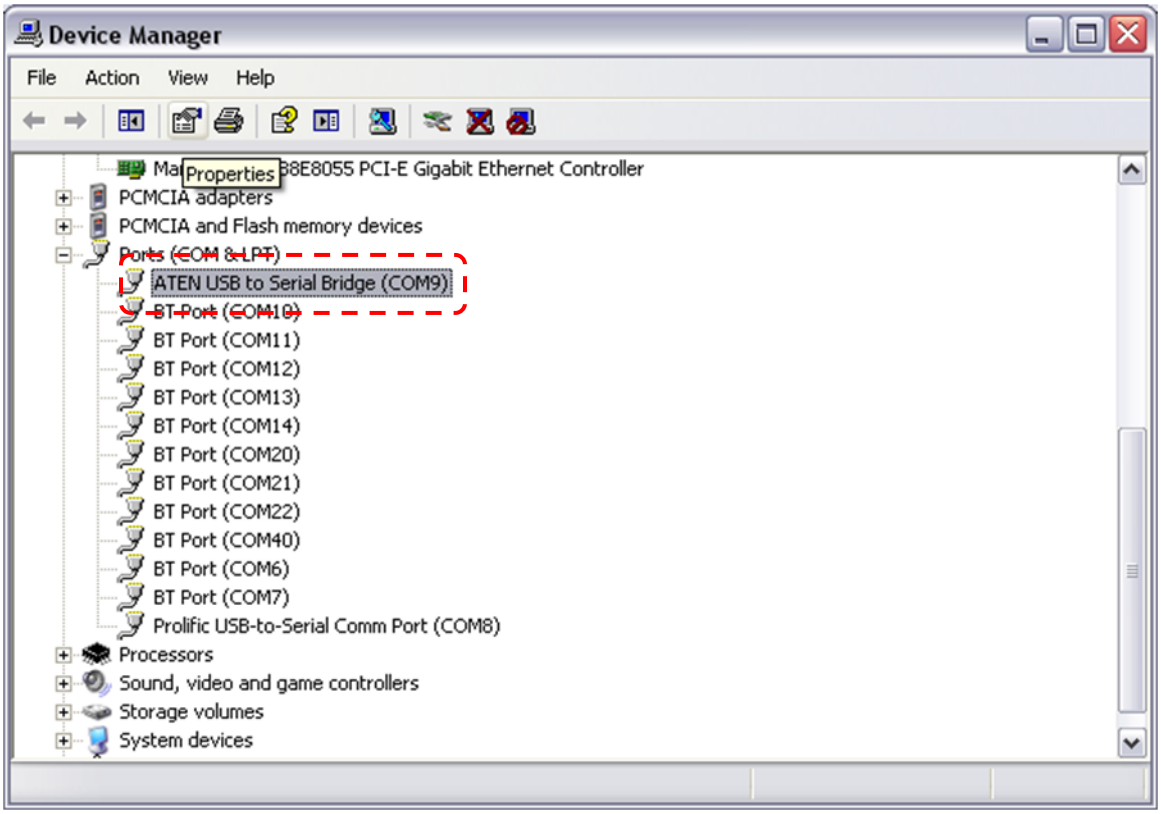
- In the main RCS screen click **C** to access CO_2 Control Setpoints (see Data Display and Control Values on page 26).
- Set CO_2 to 1500 ppm. If the CO_2 concentration does not achieve 1500 ppm and steadily drops, it is safe to change the cylinder even though there will be a slight residual pressure.
- If the CO_2 value achieves or comes close to 1,500 ppm and remains stable, the CO_2 cylinder should NOT be removed, as it remains pressurized.
- You can begin measurements without changing the CO_2 cylinder at this time. Always bring spare CO_2 cartridges and regulator o-rings with you when working in the field.

Step 7. Connect CIRAS-2/PC interface cable

Follow these instructions if you choose to operate CIRAS-2 via your computer at this time. If you will be operating CIRAS-2 directly through the UI, as in the field, please skip to Step 8. **Simultaneous operation of CIRAS-2 from a computer and the UI is not possible**. Connect the USB plug to a USB port on your computer. Connect the opposite end of the cable with the 5-pin plug into the socket labelled “RS232” on CIRAS-2. Align the red dot on the connector so it is at the 12 o’clock position of the RS232 socket - click into place.

CIRAS-2 uses default COM ports which may have been previously assigned to other devices used with your computer. On your computer look in Windows Device Manager to determine to which Com Port your USB device has been assigned: (description below based on Windows XP)

From the Windows Start Menu open Control Panel – click on the System icon (may be under Performance and Maintenance) – on the System Properties box click the Hardware tab – click on Device Manager – expand the Ports (COM & LPT) list by clicking the + button (see below). Note to which COM port your device (USB to Serial Bridge) has been assigned for Step 10.



Step 8. Power on CIRAS-2

Switch on CIRAS-2 by pressing the red "On/Off" button on the back panel. When powered, the switch should illuminate bright red. The cuvette fans and CIRAS-2's internal pumps should be running at this point. You should also see the green LED flashing behind the water vapor equilibrator. Within 30 seconds the User Interface will become active and the UI remote control software (RCS) will load. RCS will attempt to automatically set parameters and load default settings.

Whenever you operate CIRAS-2 through the integral UI (without RS232 connection to computer) the RCS settings are relayed normally. If you are attempting to connect to CIRAS-2 with your computer via the RS232 cable, you may see a Communications Error dialog box in the UI display indicating that CIRAS-2 cannot simultaneously communicate with your PC *and* the integral UI.

When the Communications Error dialog appears:

Using the mouse pointer on the UI, click the Work Offline button, then select File - Exit from the CIRAS menu bar on the UI. Your UI will display the blue Windows desktop with a white PP Systems logo.

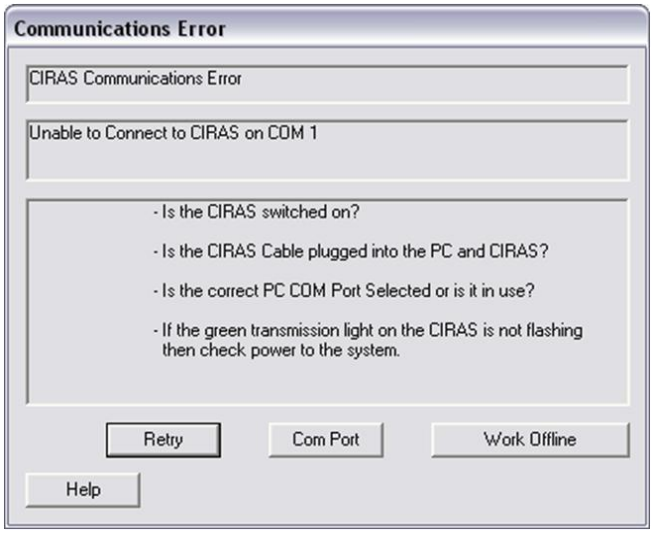
Step 9. Close the leaf cuvette

Once the RCS program loads the LED light unit will self-calibrate in a process called "tuning" (see LED Tuning on page 25). This automatic function begins each user session when a LED light unit is attached. Close the cuvette to ensure successful tuning of the LED light unit. If you forget to close the cuvette an error message will appear prompting you to perform the LED tuning manually from the RCS **Utilities** menu. Close the leaf cuvette by pushing down the lever on the top of the cuvette handle.

Step 10. Start CIRAS-2 RCS program on your computer

Launch the PC version of RCS from your computer's Start Menu or Desktop shortcut icon. Once you have established a connection, CIRAS RCS will automatically load default settings and begin LED tuning. Within several minutes the CIRAS-2 system will come to its normal operating temperature of 55 °C. During this time you can begin familiarizing yourself with the Parameters Editor and optimizing the CIRAS-2 system for your needs.

If you see the following Communications Error dialog as the RCS program attempts to establish communications with CIRAS-2, check that each of the four listed conditions are satisfied. Click on the Com Port button and select or enter the Com Port number in the CIRAS Com Port field that corresponds to its assignment in Device Manager (see Step 7 above). There are 99 selectable COM ports.



If your laptop battery is undercharged the following CIRAS-2 RCS error dialog may appear on your computer screen. You can avoid this by beginning the session with a fully charged battery or by switching to AC power from a wall outlet.



Familiarizing Yourself with the Measurement Display

CIRAS-2 is an advanced measurement system incorporating several high precision sensors for measurement and subsequent calculation of gas exchange. The measurement display features a Numeric Display, which contains measured data from various sensors, calculated data and Environmental Control Settings. In addition, there is a large graphic display which can be tailored to meet your viewing and operational requirements.

LED Tuning

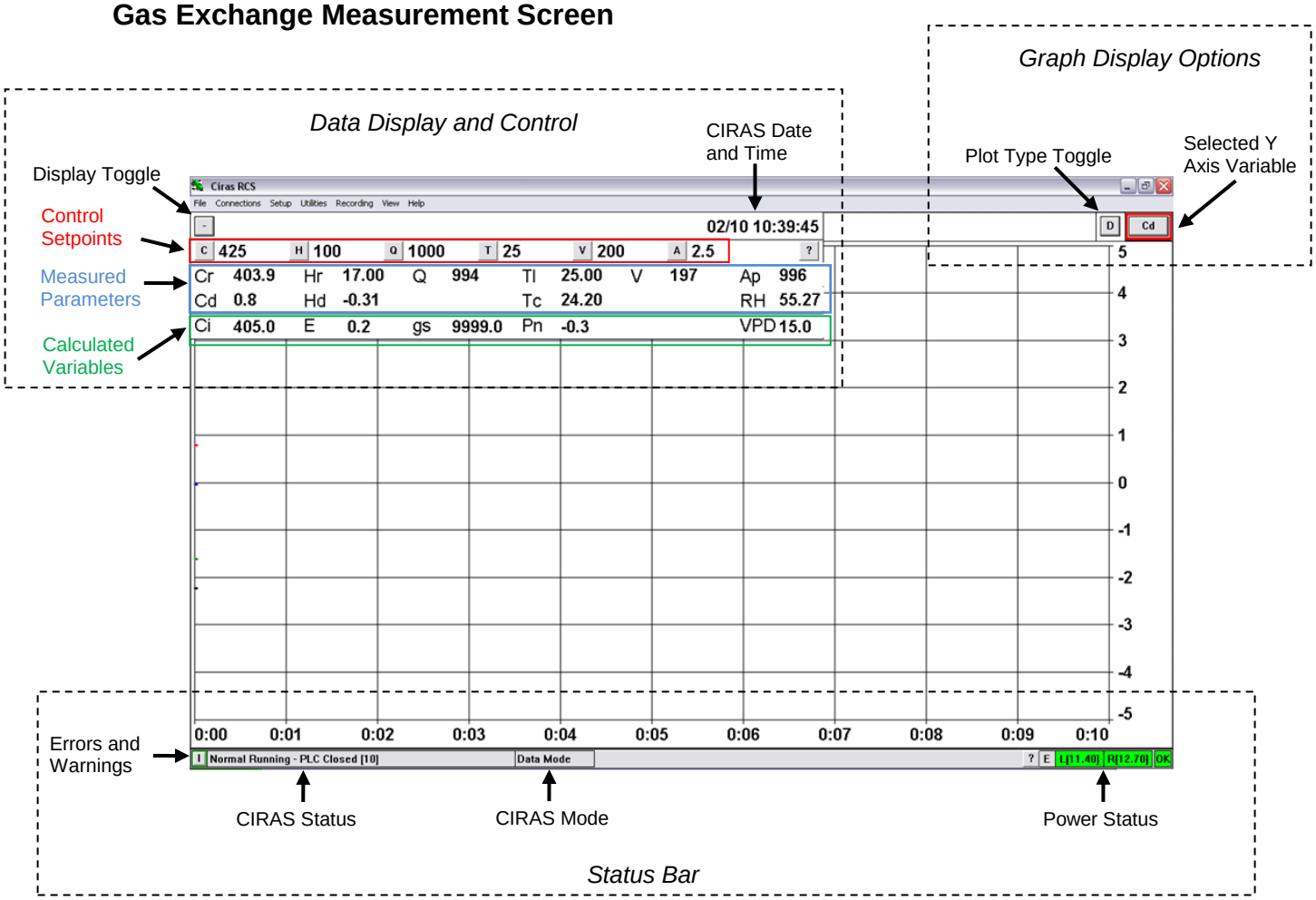
If using the LED light unit, the system will go through an LED tuning procedure when first entering into the Measurement mode. This process takes approximately 30 seconds and cannot be interrupted. During this time, CIRAS-2 drives the light unit to a range of different levels in order to establish precise control of light intensity, accounting for sensor changes over time and small differences between different light units that may be used with the CIRAS-2. LED tuning may also be performed manually by selecting **Utilities - LED Tuning - Start** from the menu bar. **Please note**:

1. Ensure that the cuvette and light unit are properly powered and connected prior to starting the software.
2. Ensure that the light unit is correctly seated above the cuvette window and secure.
3. The cuvette must be closed during this process.

For customers that have our Chlorophyll Fluorescence Module (CFM), LED tuning will be followed by CFM tuning.



Gas Exchange Measurement Screen



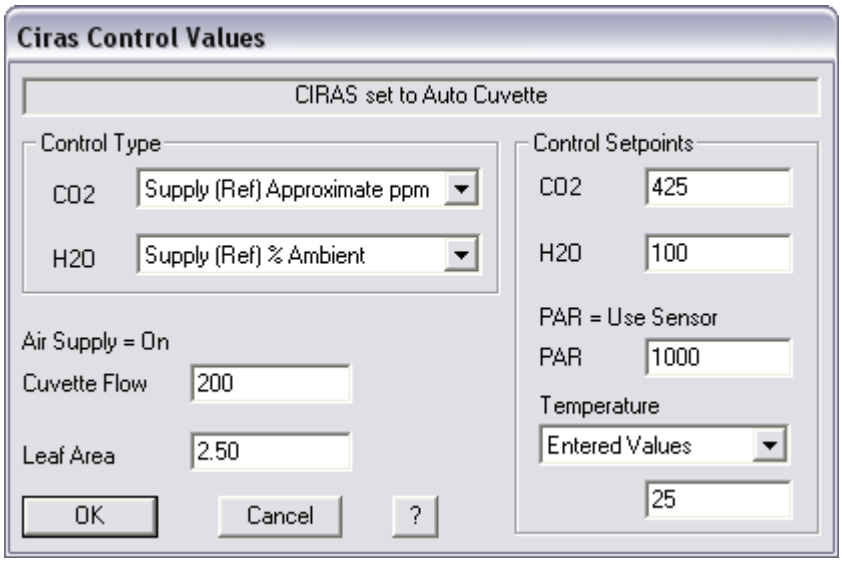
Data Display and Control Values

Control Setpoints

C	Ref CO_2 (ppm)	**H**	Ref H_2O (mb or % of ambient)	**Q**	PAR (µmol m^{-2} s^{-1})		
T	Leaf Temperature (°C)	**V**	Chamber Flow Rate (ml min^{-1})	**A**	Leaf Area (cm^2)	**?**	Help

Control Setpoints can be changed dynamically in Measure Mode, but these changes are temporary and are **NOT** saved in the Parameters File. During measurements or while simply observing gas exchange, click on the button for the Setpoint(s) that you wish to change and the **Ciras Control Values** dialog appears. With this dialog open, you can change one or all of the Control Setpoints as desired. Click **OK** to return to Measure Mode.

Please note: Changing Settings through the **Setup** menu are applied *globally* (see Cuvette Environment on page 50) and will require a new LED Tuning when you enter Recording Mode.



Measured Parameters

	Description	Unit		Description	Unit
Cr	Reference CO_2	ppm	**Cd**	Differential CO_2	ppm
Hr	Reference H_2O	mb	**Hd**	Differential H_2O	mb
Tc	Cuvette Air Temp.	$^{\circ}C$	**Tl**	Leaf Temperature	$^{\circ}C$
Q	PAR	µmol m^{-2} s^{-1}	**V**	Chamber Flow Rate	ml min^{-1}
Ap	Atmospheric Pressure	mb	**RH**	Relative Humidity (Calculated)	%

Calculated Variables

	Description	Unit
Ci	Substomatal CO_2 Concentration	ppm
E	Transpiration Rate	mmol m^{-2} s^{-1}
gs	Stomatal Conductance	mmol m^{-2} s^{-1}
Pn	Net Photosynthetic Rate	µmol m^{-2} s^{-1}
VPD	Vapor Pressure Deficit	mb

Display Toggle

Toggles Numeric Display On and Off.

Click the [-] button to collapse (Numeric Display off)

Click the [+] button to view (Numeric Display on)

CIRAS-2 Date and Time is always displayed in normal measurement, and replaced by CIRAS Mode if not.

Graph Display Options

Plot Type Toggle

The graph can show either data plotted against time, or data plotted against data (maximum of two variables). This button toggles between the Time Plot [T] and Data Plot [D].

Current Y Axis Variable

While viewing the Time Plot the Y-Axis on the graph corresponds to the variable displayed on this button. The border color of the button matches the plot color of the variable. Clicking on this button will toggle through your (maximum of) four selected Time Plot variables.

See Graphing Preferences on page 57 for details.

Status Bar

Errors and Warnings

Click this button to view any recorded CIRAS-2 errors or warnings.

CIRAS-2 Status

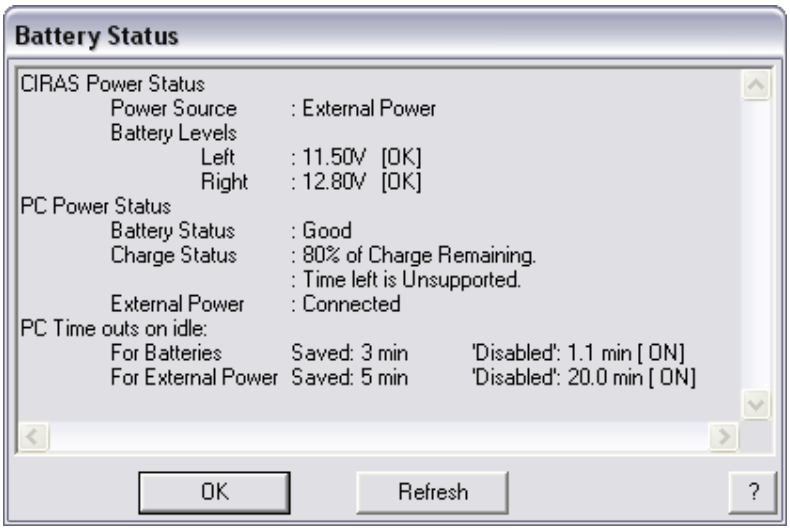
Flashes various CIRAS-2 messages. If the [E] button is Green, it refers to CIRAS-2 Status Information. If Red [E], it refers to Error Information.

CIRAS-2 Mode

Refers to current functional mode of CIRAS-2 (e.g. Zero, Diff Bal, Data, etc.)

Power Status

Power Status of CIRAS-2 and PC. Clicking on the [?] button displays a dialog with the status of the CIRAS-2 batteries and PC (if connected).



CIRAS-2 Power

From left to right, the first field shows the current Power Source that CIRAS-2 is using. Then follows the Left and then Right CIRAS-2 battery status. The last field is an indicator of the Power Status of the PC.

Power Source

E	External Power Supply Connected
L	Left Battery in Use
R	Right Battery in Use

Left and Right Battery Power Status

Color	Battery Voltage Is:	Battery is:
Green	OK	Fully Charged
Blue	>10.2 V but <11.0 V	Low, but OK
Red	<10.2 V	Very Low (Change/Charge Battery)
Grey	----------	No Battery Installed

Please note: The Measurement Screen is slightly different when CIRAS-2 is used for other types of measurements than leaf gas exchange (e.g. soil respiration). Refer to Measuring Soil Respiration with CIRAS-2 later in this manual.

CIRAS RCS Menu Bar

There are 7 Menus associated with the Menu Bar as follows:

- File
- Connections
- Setup
- Utilities
- Recording
- View
- Help

File Menu

Recalculate Data

CIRAS-2 data recalculation is recommended with CIRAS-2 operation from the computer RCS, and not with UI operation. This recommendation is based on the limited viewing capacity of the UI and processing speed while using Windows CE RCS on the UI. Data recalculation is not available with CIRAS-2 Internal Records or Closed System data (i.e. soil respiration), and cannot be performed while recording.

For further information on recalculation of data, see Recalculate Data on page 81.

Export Data

Exports all stored CIRAS-2 Data Files to your desktop PC in a user specified format for importing into spreadsheet applications such as Microsoft Excel. **Please note:** export of data cannot be performed while recording. If operating the CIRAS-2 from a PC, you will be asked to “work offline” before continuing.

For further information on exporting data, see Export Data on page 88.

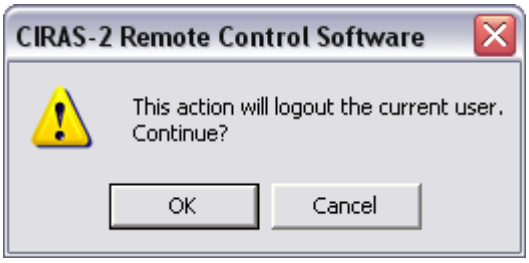
View Diagnostics Report

Select and view an Extended Diagnostics Report (.dia) generated with Utilities. For further information on performing Extended Diagnostics, see Extended Report on page 140.

Connections Menu

Login

Any user can Login under their own name at any time while the CIRAS-2 remote control program is running. To do so, select **Connections - Login.** If the system is being used by another user, you will be prompted with the following dialog:



As instructed, select **OK** to logout the current user and to enable you to "Login". The **Select User** dialog will appear allowing you to log in as described earlier in this manual (as above, pages 20-21).

After selecting the appropriate Login name, select **OK** to continue.

Connect

While running CIRAS-2 remote control software and connected to CIRAS-2, this function will be unavailable (grayed out). If the user is off-line and wants to connect to CIRAS-2, click on **Connect**.

Disconnect

While running CIRAS-2 remote control software, but working off-line, this function will be unavailable (grayed out). If the user is connected to the CIRAS-2 and wants to terminate the connection, click on **Disconnect**.

Connect Add-On

While running CIRAS-2 remote control software, but working off-line, this function will be unavailable (grayed out). To connect to the Chlorophyll Fluorescence Module (CFM), click **Connect Add-On – [CFM]**. For further information about operating the CFM, see Measuring Chlorophyll Fluorescence with the CFM on page 96.

Com Port

The proper COM port on your PC must be set correctly in order to work with CIRAS-2. There are 99 COM ports available to the user as described earlier in this manual (above, page 15, 19).

To change the Com port (if required), select **Connections - Com Port** to highlight the appropriate setting and click **OK** in the **Preferences** dialog. You can also enter your email address and include this information as part of your preferences. This information is helpful to PP Systems for technical support purposes.

Setup Menu

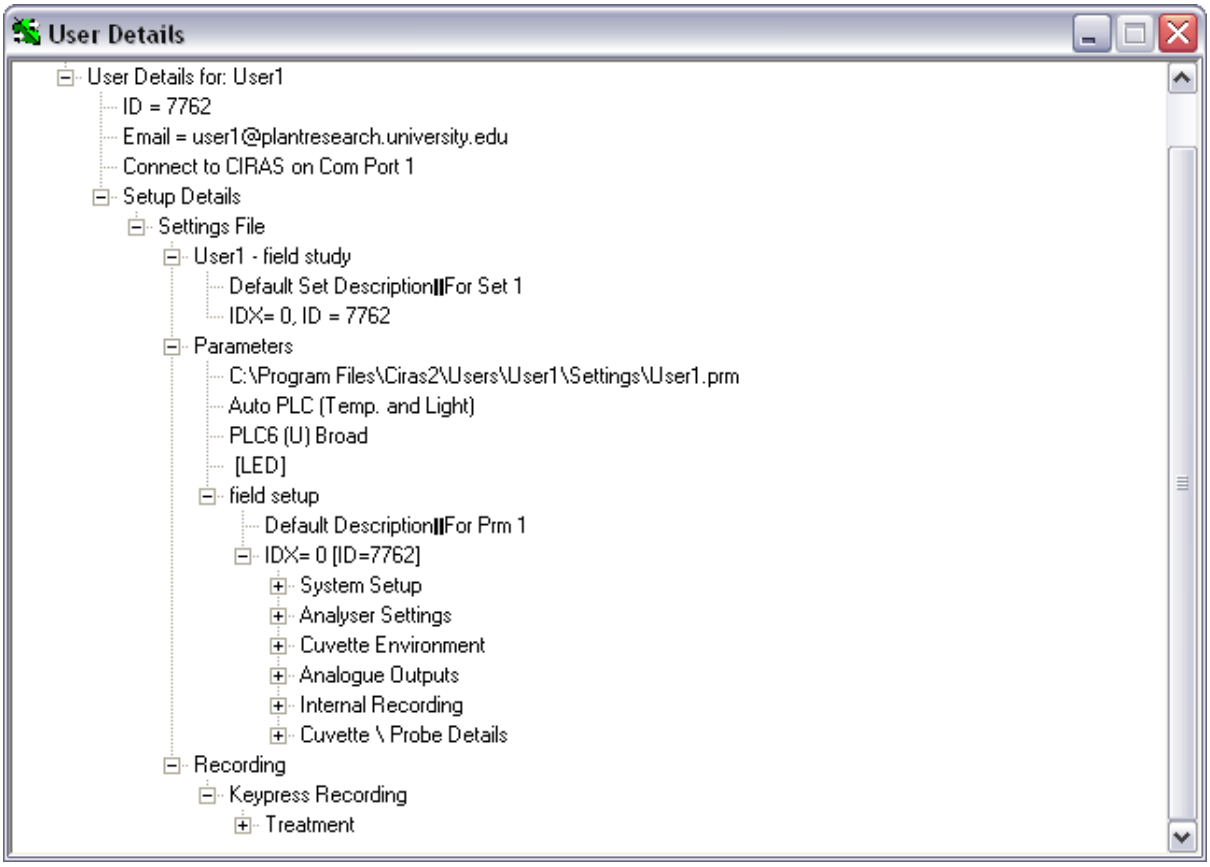
Settings

Calls up the Settings Editor. Allows modification of Recording Options (Response Curves, Treatments), Parameters, CIRAS Accessories, and Custom Chambers and Cuvettes.

Refer to System Setup and Operation on page 41 for details.

View Setup Details

Provides an overview of the current Settings in a **User Details** dialog, including location of settings and data files, response files, user ID, email address, etc.



Graphing

Preferences

Includes all options to set up the visual environment of the Measurement Display. Selectable features include Plot Type, plotted variables and parameters, font size, display scales of variables, time scale.

Refer to Graphing Preferences on page 57 for details.

Reset

Clears the current Time and Data Plots and resets all selected variables to time zero.

Utilities Menu

Zero

Initiate a manual ZERO at any time by selecting this option. The Status Bar displays a ZERO message (lower left hand corner of the display) that begins counting upwards. In ZERO mode, the initial air sample entering CIRAS-2 is drawn through the soda lime (Absorber Column 2) to remove CO_2 and then through

the Envirogel/Molecular Sieve Column (Absorber Column 1) to remove H_2O and any final traces of both CO_2 and H_2O.

During this cycle, the weight of the lines being plotted on the display becomes thicker to differentiate between normal running and ZERO cycle. Dynamic changes in control variables are suspended for the duration of this event. A letter code **Z** representing the event appears on the currently plotted variable. When completed, the Status Bar returns to its previous state (i.e. Normal Running - PLC Closed [10]).

Diff Bal

Initiate a manual DIFF BAL at any time by selecting this option. The Status Bar displays a DIFF BAL message (lower left hand corner of the display) that begins counting upwards. In DIFF BAL mode, the sample air is split and passed through both the reference and analysis sample cells. During the DIFF BAL cycle, the weight of the lines being plotted on the display becomes thicker to differentiate between normal running and DIFF BAL cycle. A letter code **DB** representing the event appears on the currently plotted variable. When completed, the Status Bar returns to its previous state (i.e. Normal Running - PLC Closed [10]).

Measure [Quit]

It terminates a Zero or Diff Bal and returns operation to measure mode. If CIRAS-2 is in the middle of performing a Zero or Diff Bal during a measurement, it can be terminated by selecting **Measure [Quit].**

Initialise

From time to time, a problem may occur that will affect the calibration of the CIRAS-2. Typically, this is due to an incorrect calibration. In the event that this happens, the system can be reset back to the original factory default calibration. The calibration factors for each CIRAS-2 analyzer are determined at the factory and written to an EPROM chip inside the instrument. These factors can be restored with the **Initialise** function, returning the system to its original factory calibration.



Please note: If the system is initialised, CIRAS-2 will default to “Analyser Only”. Therefore, you will have to enter **Setup - Settings** to re-apply your chosen operational and measurement settings.

Pulse Alarm

The CIRAS-2 can be used together with external devices such as the Hansatech FMS2 Field Fluorescence Monitoring System for simultaneous measurement of gas exchange and chlorophyll fluorescence. To send a pulse to the FMS2, click on **Utilities - Pulse Alarm**. The Pulse Alarm function sends a signal (via the Alarm line on the Probe Socket) to the external device (i.e. FMS2) to trigger a measurement.

Simultaneous Measurements With CIRAS-2 and FMS2

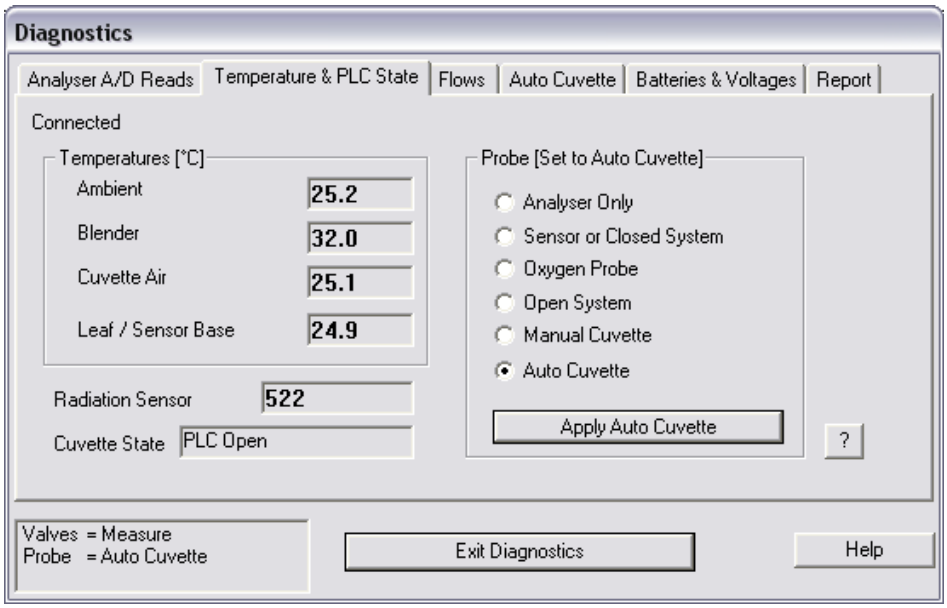
The Hansatech fiber optic probe (Hansatech Part No. FMS/LFO) fits into the light unit, which has to be modified by PP Systems (PP Systems Part No. CRS015). The standard CIRAS-2 leaf cuvette window is an interference filter that transmits only visible light. When simultaneous gas exchange and fluorescence measurements are performed, this must be replaced with a plain glass window that transmits infrared radiation resulting in an increased heat load on the leaf. The **PAR Energy Factor** under **System Setup** in the **Settings** dialog must be changed as shown below:

Window Type	in Sunlight	with LED	with Quartz Halogen
CALFLEX IR FILTER	0.17	0.14	0.16
PLAIN GLASS	0.24	0.14	0.19

The connector to the FMS2 plugs into the **Probe** socket of CIRAS-2. A pulse on the alarm line (about 140 ms) triggers the FMS2 to record a fluorescence trace. The pulse is triggered when the cuvette record button is pressed, and CIRAS-2 is set to record data direct into its memory or when the PC requests that the pulse is sent.

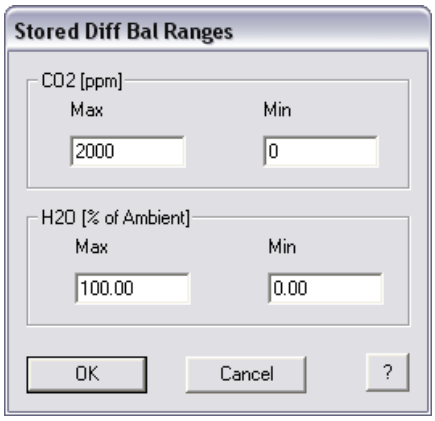
Diagnostics

Initiates CIRAS-2 Diagnostics. This option is only available if CIRAS-2 and the Software are in Data Mode (i.e. not Recording, Calibrating etc). Refer to Diagnostics on page 133 for details.



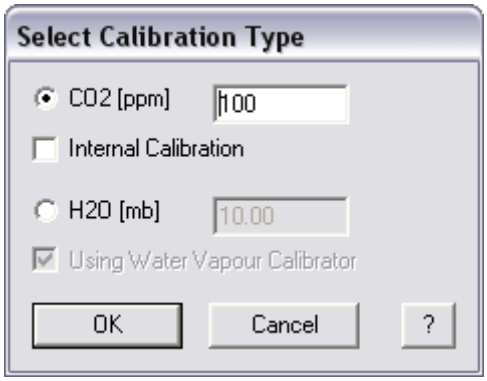
Stored Diff Bal

This function allows the user to preset and store differential balance values which can be extremely helpful when performing response curves measurements. Refer to Stored Diff Bal on page 78.



Calibration

Used to perform a full CO_2 or H_2O calibration on CIRAS-2. Refer to Calibration on page 143 for more details.



Calibration Check

Used to verify the current CIRAS-2 CO_2 and H_2O calibration settings. Refer to Calibration Check on page 145 for details.

Calibration Reset

Resets the CIRAS-2 calibration to the Factory Default. Refer to Calibration Reset on page 147 for details.

CO2 Midpoint

Refer to CO2 Midpoint on page 147 for further details.

Please note that CIRAS-2 Firmware (EPROM) Version 1.67 or greater is required for this function. If unsure of firmware version, see View – Quick Info in the CIRAS menu bar.

Max C Determination

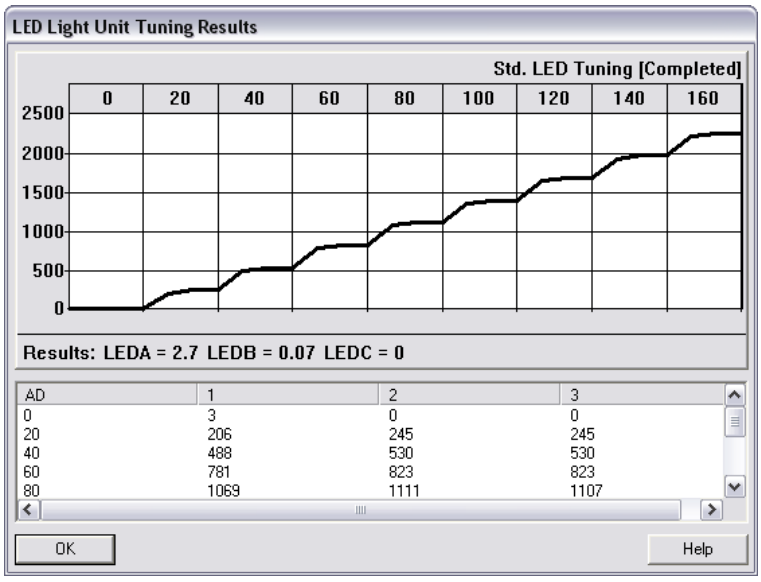
Allows Calculation/Resetting and checking of maximum CO_2 value.

Please note that CIRAS-2 Firmware (EPROM) Version 1.67 or greater is required for this functionality. If unsure of firmware version, see View – Quick Info in the CIRAS menu bar.

It is important that CIRAS-2 has been turned on for 20 minutes and that the CO_2 cartridge is full and the chemicals fresh. Additionally, a special T-Piece must be fitted to CIRAS-2 prior to starting the CO_2 Midpoint setting. Refer to Max C Determination on page 149 for further details.

LED Tuning

Allows manual LED tuning (automatic LED Tuning performed during start up) and recalls Tuning results. See LED Tuning on page 25 for details.



CIRAS Recording

Enable/Disable CIRAS-2 Internal Recording. Save up to 820 internal records to CIRAS-2 and initiate a stored data dump from this menu. Please note that this is different than the normal CIRAS-2 recording mode. See CIRAS Internal Recording on page 77 for additional details.

Recording Menu

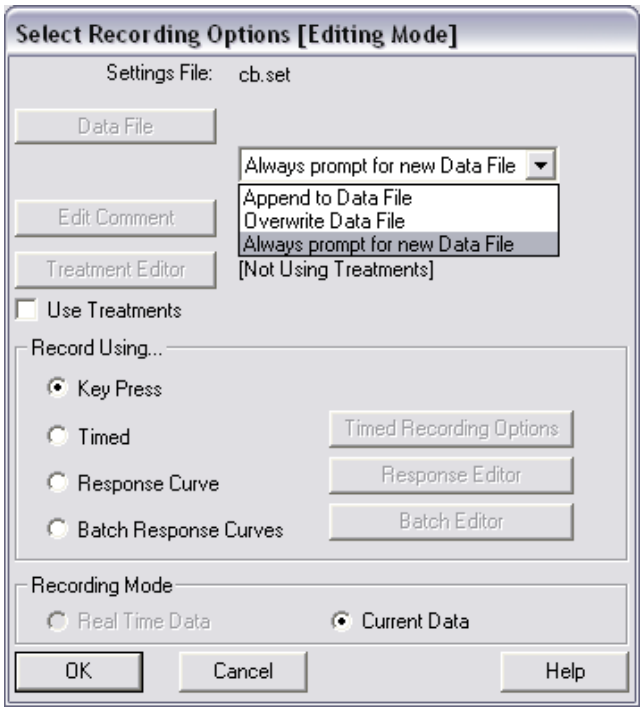
To begin measurements select **Recording - Begin**. There are four recording methods available with CIRAS-2:

- Key Press
- Timed
- Response Curve
- Batch Response Curve

All four recording methods will be described in detail in the section Observing and Recording Leaf Gas Exchange on page 59. Please note that for Closed Systems, **Recording** operates differently. See for example Measuring Soil Respiration with CIRAS-2 on page 127 for more details.

Begin

Opens the **Select Recording Options** dialog to name a new file or select an existing file, edit treatment descriptions and select type of recording.



End

Select to terminate the recording session.

Start (Steady)

Allows the user to decide when to begin recording once the leaf has stabilized inside the leaf chamber.

Pause

Used to temporarily suspend recording, for example, to allow the leaf additional time to stabilize.

Single Record

Initiates a single measurement, similar to a Keypress.

Multiple Record

Used in Timed Recording to initiate multiple measurements (with Recording Blocks Enabled).

Comment/Treatment

During measurements, used to enter descriptive information relating to the measurement.

Response Level

During a Response Curve measurement, used to view the response levels (**Response Selection** dialog).

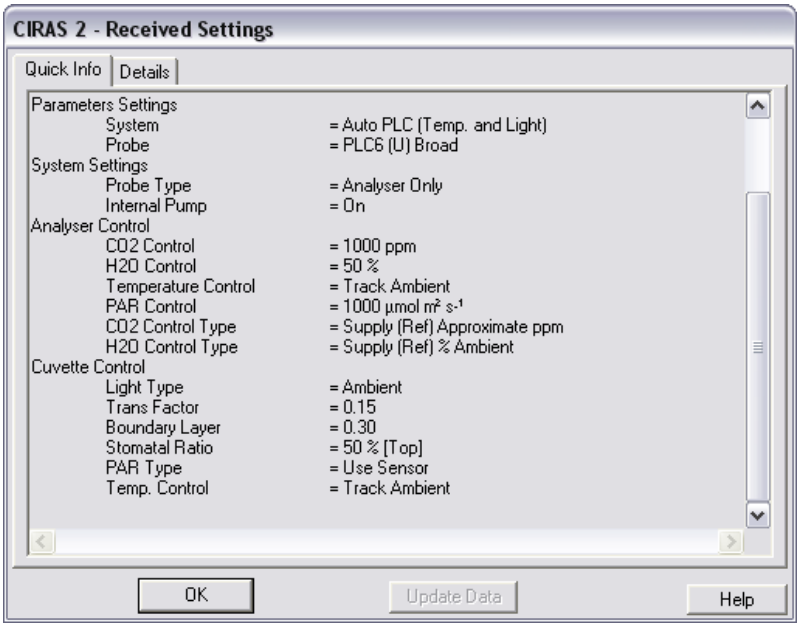
Plugin Record

Not currently assigned.

View Menu

Quick Info

Opens the **CIRAS-2 – Received Setting** dialog. Provides information such as the serial number of the instrument, eprom version, and technical information regarding the current configuration of the CIRAS-2 system. It can be viewed at any time by selecting **View** - **Quick Info.** To view additional details such as number of free records, Zero mode, Averaging Limit, Probe and Calibration constants, etc., click on the 'Details' tab. Click the Update Data button to see the most recent information/data associated with your CIRAS-2.



Update Parameters

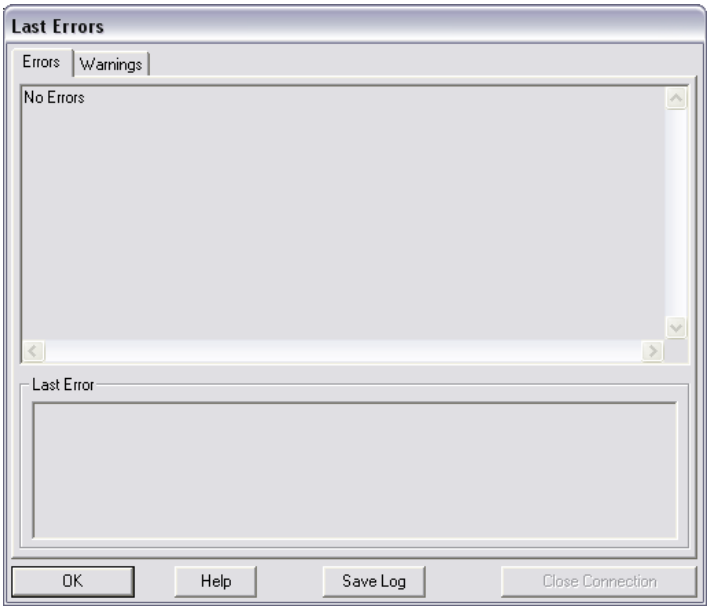
Causes CIRAS-2 to re-transmit its current Settings. Verifies that CIRAS-2 is using the correct settings.

Errors

Any Errors detected by CIRAS-2 can be viewed from this menu. Some errors are immediately brought to the attention of the user, some of which may require immediate attention. For questions regarding error messages, consult the Technical Manual or contact PP Systems. See Contact Information on page 11.

Warnings

Any Warnings detected by CIRAS-2, such as changing A/D Zero values, can be viewed from this menu.



Time Plot

To view measurements as color-coded plots over time, select **View** - **Time Plot**. Alternatively, if Data Plot is currently selected, click the T button in the upper right hand corner of the Measurement Display. See Graphing Preferences on page 57.

Data Plot

To view measurements as data points in a scatter plot, select **View - Data Plot**. Alternatively, if Time Plot is currently selected (see above), click the D button in the upper right hand corner of the display.

Battery Status

Displays the Battery Status of each 12V NiMH battery used with CIRAS-2, and (if present) the PC's battery status. See Power Status on page 28 for details.

Help Menu

Help

Displays the main CIRAS-2 Remote Control Software Help Menu. (Currently unavailable for the Windows CE version of the software on the UI). This supplements information contained in the Operator's and Technical Manuals. Due to ongoing updates we suggest that if you have any questions about CIRAS-2 operation, menus, etc., you first refer to the print version before searching the on-line Help menus.

About

Select **Help** - **About** to see which version of the CIRAS-2 Remote Control Software you are running.

Diagnostics

Contains information related to the 6 CIRAS-2 Diagnostics topics.

Calibration & Stored Diff Bal

Contains topics related to CIRAS-2 calibration and stored differential balance.

Recording

Contains topics related to CIRAS-2 Recording (i.e. Key Press, Timed and Response Curve recording).

Closed Systems

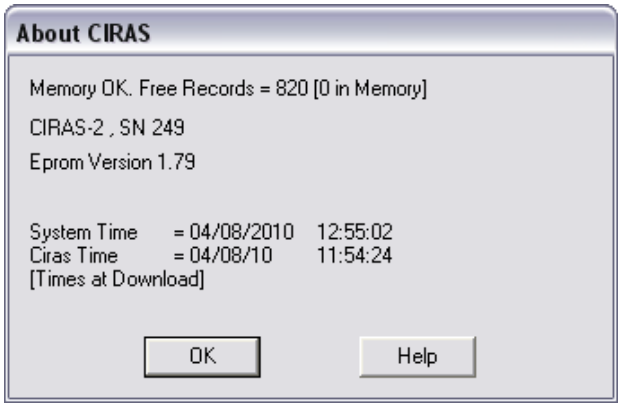
Describes the use of PP Systems Soil Respiration and Whole Canopy Assimilation closed system chambers.

Add-On Modules

Opens Help Files related to the Chlorophyll Fluorescence Module (CFM).

About CIRAS

Displays the Eprom version being used in your CIRAS-2, along with the serial number of your instrument.



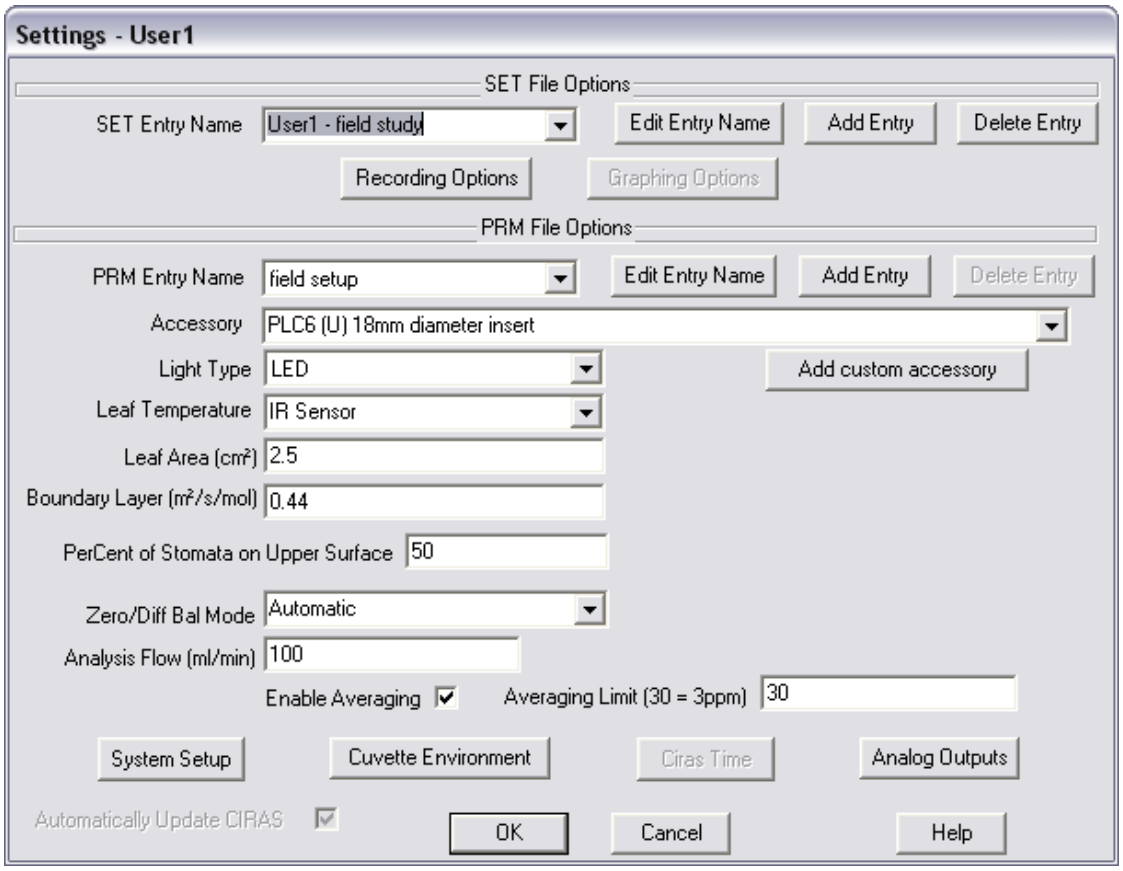
System Setup and Operation

Settings information provides the basis for your individualized use of CIRAS-2 and will be automatically saved for you. Selections made in the **Settings** dialog are global and apply universally to a CIRAS-2 session, unlike those made with the **Control Setpoint** buttons, which are temporarily applied to the session. The most recently used preferences and settings will load each time that the RCS program is started, and the login procedure will be circumvented.

Set File Options

Set Entry Name

Click on **Setup** in the CIRAS main menu bar to open the **Settings** dialog. In the 'SET Entry Name' field at the top of the dialog select 'Default SET Name 1' from the dropdown list. A single **Set File** will allow up to 10 parameter setups (**PRM Files**, below) containing unique combinations of cuvette/chamber type, light source, controlled environmental conditions, recording options and real-time graphing options.

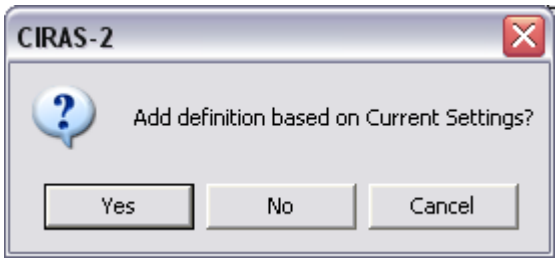


Edit Entry Name

Next, click on **Edit Entry Name**, enter a short descriptive name in the **Rename** dialog, .e.g. your current experiment.

Add Entry

To create additional **Set Files** click **Add Entry Name** and choose 'Default SET Name 2' from the dropdown list. In the resulting dialog click **Yes** to use the current parameters or **No** to create a unique set of parameters. Edit the new entry as above.



Delete Entry

This removes the currently selected **Set File** and its parameter, graph and recording options.

Recording Options

Click the Recording Options button to set up the type of recording you wish to make. The same dialog is also available by selecting **Recording – Begin**. For a detailed description see Select Recording Options on page 60.

Graphing Options

Click the Graphing Options button to change various options associated with graphical presentation of data in the **Graph Preferences** dialog. The same dialog is available by selecting **Setup – Graphing – Preferences** from the CIRAS menu bar. For a detailed description see Graphing Preferences on page 57.

PRM File Options

In the 'PRM Entry Name' field select 'Default PRM Name 1' from the dropdown list. Up to 10 parameters settings per Set File are allowed.

PRM Entry Name

Select 'Default PRM Name 1' from the dropdown list.

Edit Entry Name

Click on **Edit Entry Name**. Enter a short descriptive name in the **Rename** dialog, .e.g. the cuvette/chamber used in your measurements.

Add Entry

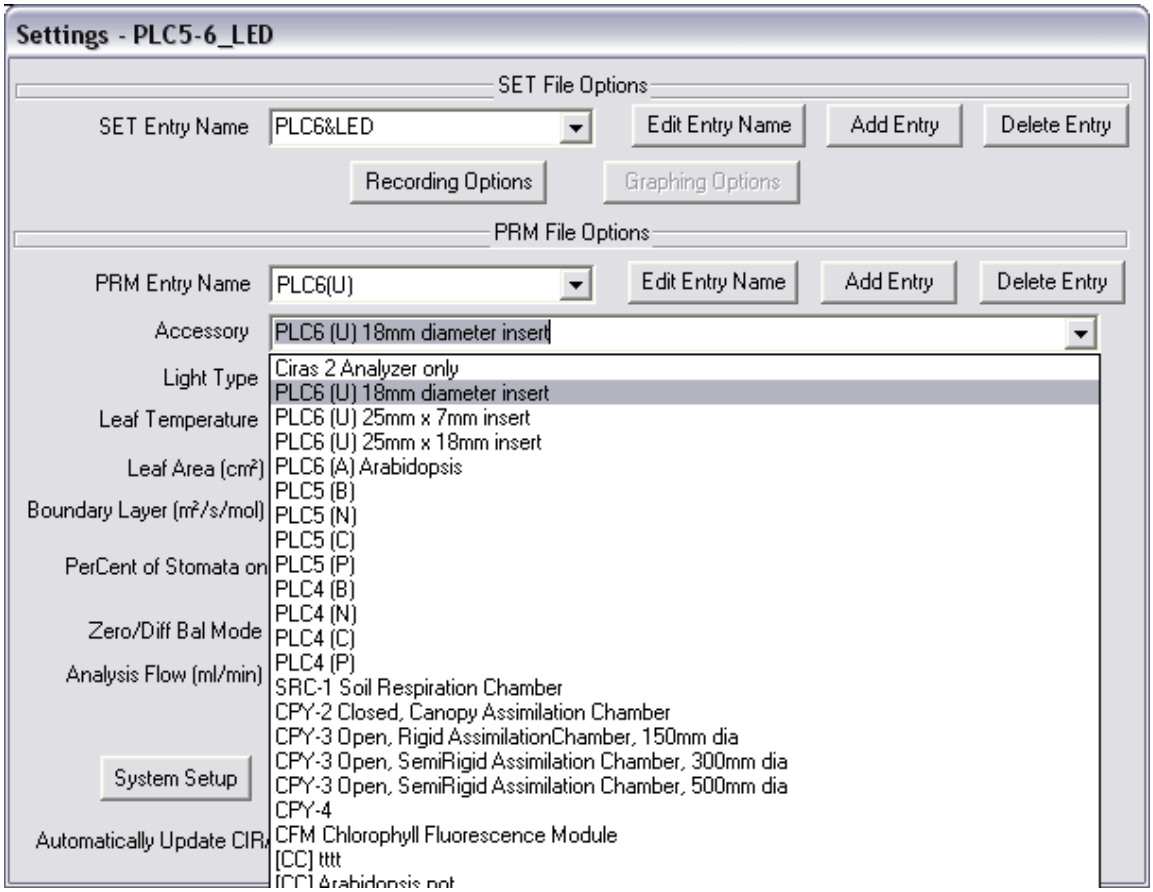
To create additional **PRM Files** click **Add Entry Name** and choose 'Default PRM Name 2' from the dropdown list. In the resulting dialog click **Yes** to use the current parameters or **No** to create a unique set of parameters. Edit the new entry as above.

Delete Entry

This removes the currently selected **PRM File** and its individual settings.

Accessory

Open the 'Accessory' drop down list containing the standard cuvettes and chambers that may be used with CIRAS-2. From this list select, for example, the standard automatic leaf cuvette 'PLC6 (U) 18 mm diameter insert'. By making this selection, various other options will automatically be set to defaults for this Accessory.



The table below lists several current and older-style cuvettes and chambers supported by CIRAS-2.

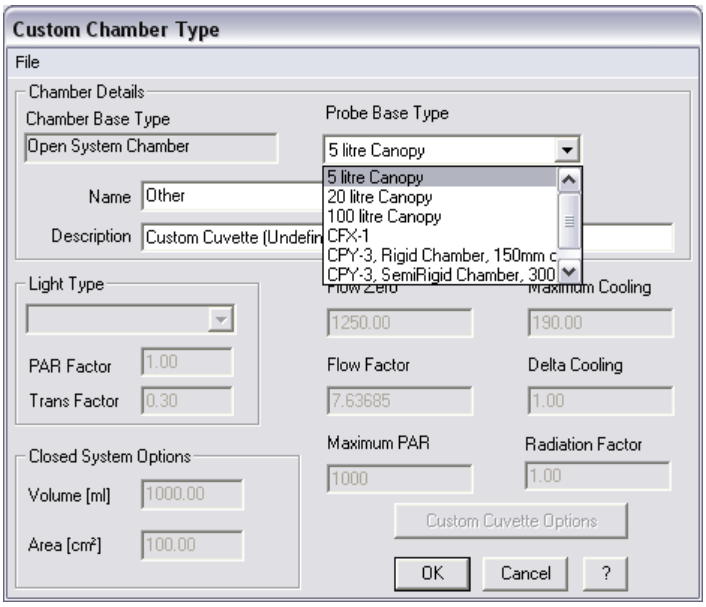
Accessory	***Cuvette/Chamber Type***
CIRAS 2 Analyser Only	CIRAS-2 as stand-alone analyzer (no cuvette)
Auto PLC (Temp. and Light)	PLC6 (U) 18 mm circular (2.5 cm^2 head plate) PLC6 (U) 25 x 7 mm narrow (1.75 cm^2 head plate) PLC6 (U) 25 x 18 mm wide (4.5 cm^2 head plate) PLC6 (A) for Arabidopsis (1.29 cm^2 head plate) PLC5 (B) broadleaf (2.5 cm^2 head plate) PLC5 (N) narrow leaf (10 cm^2 head plate) PLC5 (C) conifer (10 cm^2 head plate) PLC5 (P) pod (10 cm^2 head plate) Other

Manual PLC	PLC4 (B) broadleaf (2.5 cm^2 head plate) PLC4 (N) narrow leaf (10 cm^2 head plate) PLC4 (C) conifer (10 cm^2 head plate) PLC4 (P) pod (10 cm^2 head plate)
Closed System Chamber	SRC-1 CPY-2 CPY-4 (146 mm diameter) Other
Open System Chamber	CPY-3 5 litre Canopy Assimilation (150 mm diameter) CPY-3 20 litre Canopy Assimilation (300 mm diameter) CPY-3 100 litre Canopy Assimilation (500 mm diameter) CFX-1 (for soil respiration) CFX-2 (for soil respiration)
Chlorophyll Fluorescence Module	CFM (combined with PLC6 (U) LED light unit)
Custom Chamber	[CC] (based on open *or* closed system chambers)
Oxygen Probe	Not Applicable

Some Accessories are no longer available. Environmental sensors attached to **Probe** input such as the PAR sensor and Soil Temperature Probe are detected automatically and do not require selection as Accessories. If you have any questions as to which cuvette or probe type you have, refer to the original paperwork (i.e. invoice from PP Systems), the Appendix at the back of this manual or contact PP Systems.

Add Custom Accessory

Primarily for use with your custom-built or existing chamber. In the **Settings** dialog, select the PP Systems Accessory that most closely matches your chamber. Click **Add custom accessory** to call up the **Custom Chamber Type** dialog. 'Chamber Base Type' and 'Probe Base Type' default to your selection in the Accessory list. Narrow your selection further in the 'Probe Base Type' dropdown list. Enter a custom chamber [CC] name in the 'Name' field and an optional Description. Depending on the 'Chamber Base Type', additional parameters, such as System Volume, may be required. **Custom Cuvette Options** is used for leaf cuvette and small assimilation chambers. If this is not grayed out, click the button and enter any 'Serial Number' and estimated 'Boundary Layer' and 'Leaf Area' for the cuvette. Note that these can be estimated and resulting data recalculated at a later time.



Light Type

In the 'Light Type' list select from correct Light Unit (or light source) used with your cuvette (Ambient, LED or Tungsten Halogen). Available options are based on Probe Type and Chamber/Cuvette Type selected.

Leaf Temperature (T_{leaf})

Depending on cuvette/chamber selection - IR Sensor/Energy Balance/Thermistor. The IR sensor is only available with modern PLC6 (U) and PLC5 (B) Automatic Leaf Cuvettes. Energy Balance is provided as secondary choice.

Leaf Temperature Determination: Explanations

Accurate measurement of leaf temperature (T_{leaf}) is essential for the determination of stomatal conductance. In determining the water vapor pressure gradient between the leaf and the outside air (the driving force for transpiration) the air inside the leaf is assumed to be saturated at the leaf temperature. T_{leaf} is determined by the balance between energy absorbed by the leaf from incident radiation (visible and short wave infra-red), energy lost in the evaporation of water, energy either gained or lost in long wave radiation to the surroundings and in conduction with the surrounding air. There are three commonly used methods used to measure T_{leaf} which CIRAS-2 makes available to the user depending on the type of cuvette.

1. Energy Balance
2. Thermistor
3. Infrared sensor (IR)

Energy Balance

All "Manual" and "Automatic" type leaf cuvettes have this option available, and we recommend this in preference to the "Thermistor" method described below. This method of T_{leaf} measurement produces a reasonably good average for T_{leaf}. As mentioned above, the energy balance of the leaf determines the temperature, so the more accurately we determine these components the better we can calculate the temperature.

First, we convert the radiation falling on the leaf into the energy absorbed. We measure the visible quanta (PAR) and using the TRANS factor (see more at Energy Factor on page 49) convert this to energy absorbed. The value of TRANS depends on the energy distribution with wavelength of the source; the position of the PAR sensor (inside or outside the cuvette); the transmission characteristics of the cuvette windows; the reflection characteristics of the leaf surface; and the absorption characteristics of the leaf. From the humidity measurements and flow rate, we can determine the energy lost in evaporation. From the measurements of the boundary layer in the cuvette we can calculate the conductive heat exchanges between the cuvette and air. By assuming that the cuvette wall temperature is the same as air temperature we can calculate the radiation exchanges. Using all this information, we can calculate average T_{leaf}. See Calculations in Appendix 2. on page 153 for a detailed description.

Thermistor

Not recommended - we do however make it available for use with all “Manual” and some “Automatic” type Leaf Cuvettes. This option is not available with the PLC6 (U) or PLC5 (B) Automatic Broad Leaf Cuvettes as these cuvettes have an infrared temperature sensor fitted (See IR below).

Direct Leaf Temperature Measurement - Common Problems

A leaf temperature sensor making physical contact against the underside of the leaf will have at least 50% of its area exposed to the air rather than the leaf. If it is covered to insulate it from the air, then the area of leaf that is also covered will not transpire nor will there be energy exchanges with the air. It will therefore be at a different temperature than other areas of the leaf.
Very small sensors have to be inserted in the leaf to affect an accurate and direct measurement. But this will cause local damage to the leaf, affecting transpiration and therefore the temperature. Heat conduction along the wires that are in the air, to/from the sensor also has a significant effect. IR photography of leaves also shows significant patchiness in leaf temperature.

Infrared (IR)

Only the PLC6 (U) and PLC5 (B) Automatic Broad Leaf Cuvette have this option available as it features an IR sensor fitted to the base of the cuvette head.

If we can determine the net radiation exchanges between the leaf and a sensor of known temperature then we can calculate the leaf temperature. The sensor consists of a thermopile with an upper blackened absorbing surface facing the underside of the leaf. The temperature of the base of the thermopile is measured. If the thermopile and leaf are at the same temperature then there will be no net energy exchange, therefore no temperature gradient across the thermopile and no signal. If the leaf is cooler then the thermopile surface will also cool relative to the base and vice-versa. The thermopile output is calibrated against leaf simulants of known temperature so we can determine the leaf temperature. **The one limitation of this method is that the field of view of the sensor must be completely filled with leaf material.** Given that the area of the PLC6 (U) and PLC5 (B) Automatic Leaf Cuvette is very small, most broad leaf types will completely fill the chamber making this method of leaf temperature measurement the most accurate and recommended choice.

Please note: If the leaf does not completely cover the entire window area, (e.g. the PLC6 (U) 18mm diameter circular opening in the center of the window), this method of leaf temperature measurement **MUST NOT BE USED.** We then recommend that you select the Energy Balance method.

Leaf Area

User Specified. Cuvette/head plate selection defaults a defined Leaf Area value, for example, the PLC6 (U) cuvette will default to 2.5 cm^2 (18 mm diameter insert). The size and shape of the leaf may be important in your decision as to which cuvette head plate to use (1.75 cm^2, 2.5 cm^2, 4.5 cm^2). Leaf area also has a significant effect on final calculations of photosynthesis. However, if recalculation of data is required, this can be done fairly easily. See Recalculate Data on page 81.

Boundary Layer Resistance

Enter the correct boundary layer resistance value from the green and white test label found on your cuvette handle. Methods used to determine boundary layer resistance of automatic leaf cuvettes using a pseudo-leaf can be found in the **PLC5-6 Auto Cuvette Operation Manual.**

Percent of Stomata on Upper Leaf Surface

Adaxial/abaxial stomatal ratio defaults to 50% - you may update this for known species-specific values.

Zero/Diff Bal Mode

1. **Zero**. CO_2 / H_2O free air is passed through the analyzer sample cells and the values are recorded. Under these conditions there is no IR absorption and the readings from the detectors are at maximum. CIRAS-2 uses this information to correct for most factors that cause IRGA drift.

2. **Diff Bal**. The reference (sample) air is passed through both the reference and analysis sample cells and the offset values are recorded and applied to subsequent measurements. This compensates for any differences between the cells and ensures that the reported differences in concentration are accurate.

Please note: It is very important that the Zero Absorber Columns (Columns 1 and 2) are fresh and that the reference CO_2 concentration is steady when the Diff-Bal is performed.

Manual with Warnings

Perform manual Zeroes and Diff Bal if desired. CIRAS-2 will prompt you when it determines a Zero or Diff Bal is required.

Automatic

Zeroes and Diff Bal are performed automatically at regular intervals. Leaving 'Zero/Diff Bal Mode' set to 'Automatic' is recommended until you become more familiar with CIRAS-2 operation.

Auto Zero with Stored Diff Bal

This mode allows for working over a range of CO_2 concentrations without the need for Diff Bal. Prior to recording data, the Diff Bal is performed over the anticipated CO_2/ H_2O range at approximately 100 ppm CO_2 / 3 mb H_2O intervals. The values are then stored for use during measurement.

Manual Without Warnings

Perform manual Zeroes and Diff Bal if desired. CIRAS-2 will not display a prompt.

Choice of Zero Mode – Recommendations

Manual With Warnings: When CO_2 concentrations are being regularly changed (i.e. CO_2 response) and greatest accuracy is required. The user can then judge when the CO_2 concentration is steady before selecting Zero.

Automatic: Ideal for a series of measurements that are conducted at the same CO_2 concentration.

Auto Zero with Stored Diff Bal: When CO_2 concentrations are being changed and the quickest response is required. Use during A/Ci curves to inform CIRAS-2 of expected range of CO_2/H_2O concentrations.

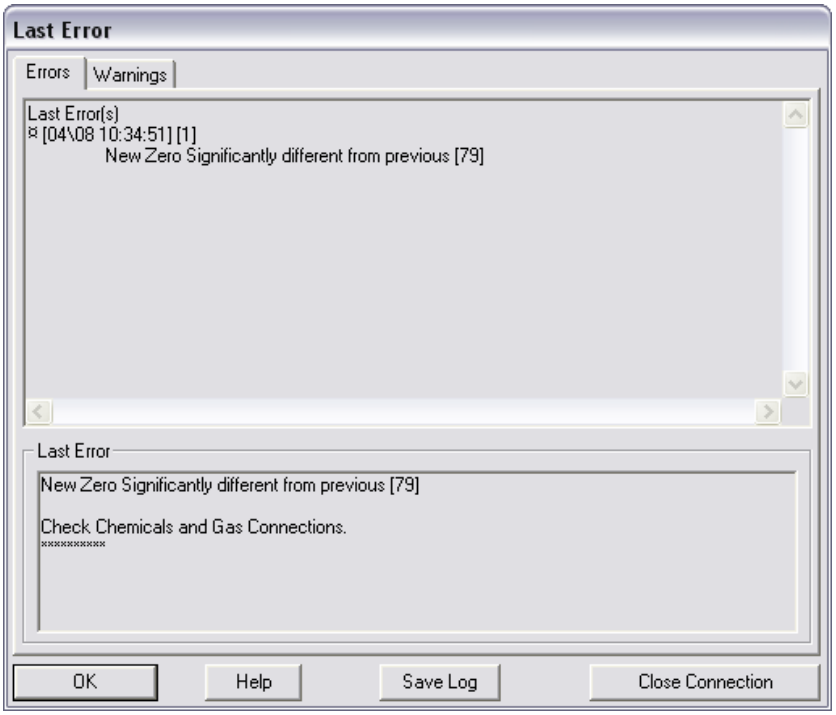
Manual Without Warnings: Only recommended if the user is very experienced with gas exchange and finds the warnings inconvenient. It is also useful where the Zero is under PC control.

Changes in Zero Readings

Whenever CIRAS-2 has been running for longer than 30 minutes, a set of analyzer A/D readings are stored representing the Reference and Analysis CO_2 and H_2O values measured after an Automatic Zero has been completed. Following a Zero, CIRAS-2 checks the new Zero against the stored values in 2 ways:

1. If the current and previous values differ by more that +/- 100, an Error Code 79 is generated ('New Zero Significantly different from previous [79]').

2. If more than 4 sets of Stored Zeros are present, CIRAS-2 checks the slope of the data. If the slope falls out of an acceptable range, an Error Code 78 is generated ('Zero Showing Progressive Change [78]').



These messages could simply be the result of a Zero performed when the chemicals are not fresh, and then the next Zero performed with fresh chemicals. It may also be an indication that a column has not been replaced properly. Although this may cause the user concern, if the A/D values in Stored Zeros (see Stored Zeros on page 134) do not differ by much more than 100, there probably is not much of a problem. If the A/D values differ by more than 100, then it could be a symptom of a greater problem. Ensuring that the chemicals are replaced regularly, the absorber columns are inserted correctly, and the Zero is performed under stable conditions will help to avoid this error message.

Analysis Flow

This is the flow rate through the analyzer sample cells. Refers to the rate at which the analyzer samples the analysis air and is user adjustable between 50-100 cm^3 min^{-1}. For normal leaf gas exchange the recommended operational flow rate is the default setting (100 ml min^{-1}).

Averaging Limit

Outputs from CIRAS are subjected to an averaging process. The linearized current reading is differenced with a stored average reading. If the difference exceeds the Averaging Limit then the current reading replaces the average, otherwise the current reading is incorporated into the average. The default Averaging Limit is 30 units, corresponding to 3 ppm for CO_2 and 0.2 mb for H_2O. If set to 1, averaging is disabled giving instantaneous readings. The averaging band is increased as the averaging limit is increased, up to a maximum of 999 units. The **Enable Averaging** box must be checked.

System Setup

Probe Type

Air Supply On

Checking/unchecking this box turns the internal cuvette air supply unit on and off. Setting the Air Supply pumps to Off will result in the setting of CO_2 and H_2O set points being disabled as the flow is assumed to come from a separate source (i.e. an external air supply).

Cuvette Flow

Flow rate to the cuvette is dependent upon cuvette type used with CIRAS-2. If 'Air Supply' is off, the maximum flow is 2500 ml min^{-1}, if 'Air Supply' is on the maximum is 470 ml min^{-1}. Cuvette Flow must always exceed the analyzer sampling rate by at least 50 ml min^{-1} to ensure positive pressure in the chamber.

Light Type [_]

PAR

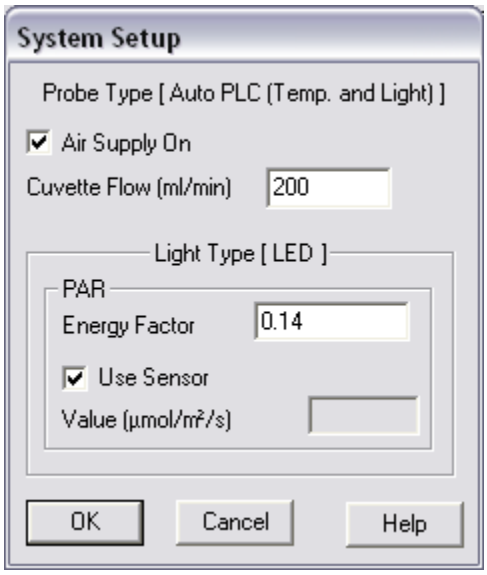
Energy Factor

Default light transmission coefficient determined by the Light and Chamber Type. User specified for custom chambers. Refer to CIRAS-2 Settings for Leaf Cuvettes and Probes on page 152 of this manual.

Check 'Use Sensor' (on chamber) to measure PAR or enter a value if using an external light source. If checked, the PAR sensor on the chamber is used to determine PAR (with or without a light unit attached). **Please note:** if a PP Systems' light unit is used and 'Use Sensor' is not checked, the light unit will turn off. This option will be enabled or disabled depending on the Accessory and Light Type selected.

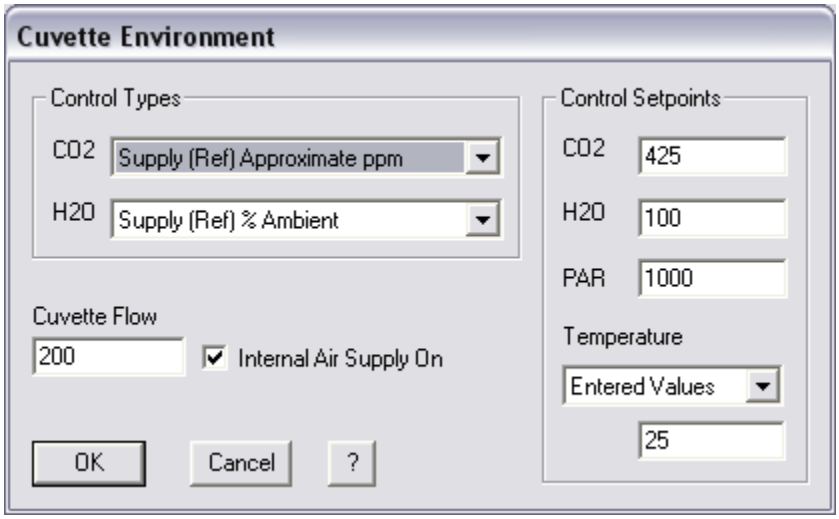
Value

If disabled, CIRAS will use the entered PAR value in its calculations.



Cuvette Environment

Click **Cuvette Environment** to enter baseline settings and values of environmental control variables that define conditions inside the leaf chamber. These settings will be in effect each time you launch the RCS program under the current **Set** and **Parameters** files, and whenever you exit the Recording Mode, where you may be using different values. Control values associated with **Cuvette Environment** may be changed at any time except during Response Curve recording. CIRAS-2 control variables available with the PLC6(U) automatic cuvette and LED light unit are: CO_2, H_20, PAR, temperature and flow rate. Additional options exist to control CO_2, H_20 and temperature in ways that allow greater flexibility and convenience during measurements.



Control Types

CO_2 (0–2000 ppm)

Selections available from the 'CO_2 Control Types' dropdown list:

1. Supply (Ref) Approximate ppm

The actual Reference gas (gas stream entering the leaf chamber) control value achieved will be approximately that of the entered value. CIRAS-2 should control CO_2 concentration to within approximately 30 ppm of the value you enter in the 'Control Setpoints' field (assuming fresh chemicals and CO_2 source, proper gas connections, and good zeroing).

2. Supply (Ref) Set ppm

The Reference gas control value will be within 1 ppm of the set value.

3. Cuvette (An) Set ppm

The Analysis gas (gas stream exiting the leaf chamber) control value will be within 2 ppm of the set value.

About CO_2 Control Types

Control Types 1 and 2 are recommended for the most efficient control of CO_2 concentration, as neither is influenced by the leaf response to chamber conditions. Control Type 1 allows rapid CO_2 concentration changes and therefore faster overall leaf stabilization times, but the user must be aware to expect lower precision in CO_2 control. Also, the absolute difference between the entered CO_2 value and the actual Reference gas value can change with depleted chemicals and improperly conducted Zeroes. Control Type 2 provides precise control. However, changing the CO_2 concentration using Control Type 2 results in longer delays (up to several minutes) while the concentration stabilizes. In both cases measurements should be suspended until the system is stable, as these data will be invalid.

H2O (0-100% or 0 mb-Dewpoint)

Selections available from the 'H2O Control Types' dropdown list:

1. Supply (Ref) % Ambient

This H_2O Control Type tracks humidity of the Reference air and allows you to mirror it precisely in the cuvette, i.e. 0-100% of *ambient (Reference) humidity*. Using this you can set the leaf chamber humidity level to some lower fraction of Reference air humidity. For example, say that there is 60% RH (or 14.0 mb at 20 °C and standard pressure) in the Reference gas stream entering the leaf chamber. If you enter a H2O Setpoint value of 50 (%) CIRAS-2 will rapidly dehumidify the gas stream entering the leaf chamber such that RH in the chamber will be quickly lowered to 30% (or 7.0 mb).

2. Supply (Ref) Set mb

The Setpoint value is entered in mb (control range of 0-75 mb) and control of H_2O partial pressure of the Reference air should be within 0.1 mb. Lowering the mb value of the Reference air will rapidly dehumidify the gas stream entering the leaf chamber such that RH in the chamber will decrease rapidly.

3. Cuvette (An) % RH (vs. Leaf T)

The Analysis air H_2O is controlled to give the set % relative humidity in the cuvette with reference to leaf temperature. Initially, the humidity will be set to 50% of ambient and control will not commence for several minutes while the software determines the ambient humidity. Changing the set humidity value will result in an immediate response as the software is continuously updating the value. However, changing the H_2O Control type may result in the same delay.

4. Cuvette (An) Set mb (0-Dewpoint)

The An H_2O is controlled to within 0.1 mb of the set value. Because of the good stirring in the cuvette, this should be the concentration experienced by the leaf. Initially, the humidity will be set to 50% of ambient and control will not commence for several minutes. This is because the software is determining the ambient humidity. Changing the set humidity value will result in an immediate response as the software is continuously updating the value. However, changing the H_2O Control type may result in the same delay.

5. Leaf to Cuvette VPD

Analysis H_2O is controlled so that there is a constant Vapour Pressure Gradient (specified in mb) between the leaf tissue (assumed to be saturated at leaf temperature) and the cuvette air. Initially, the humidity will be set to 50% of ambient and control will not commence for several minutes. This is because the software is determining the ambient humidity. Subsequently, changing the set value will result in an immediate response as the software is continuously updating the value. However, changing the H_2O Control type may result in the same delay.

About H_2O Control Types

As with changes in CO_2 concentration, rapid changes in RH within the leaf chamber are wholly artificial (from a physiological standpoint) and measurements should be suspended until stable conditions prevail. Control Types 3, 4, 5 are dynamic, responding to feedback from the leaf. Leaf responses to changing cuvette conditions may result in automatic adjustments to flow rate, which will require additional leaf equilibration times.

Determination of Actual Ambient RH

Please note: Reference air is conditioned along its path to the leaf chamber by passing through soda lime, which removes CO_2, but *adds* water vapor to the gas stream. After next passing through the water vapor equilibrator up to 10 mb of water vapor is removed, but RH in the air sample is still greater than outside ambient RH.

To determine the actual ambient relative humidity (in the leaf's undisturbed environment) you can simply open the cuvette and observe the RH reading in the display. For a slightly faster and more accurate reading temporarily remove the AN IN gas connection, allowing outside air to be directly drawn through the Analysis side of the IRGAs. Temporarily remove the REF IN gas connection to see the corresponding ambient water vapor value in mb Once you have determined and noted the true ambient RH (or water vapor in mb) you can change your settings accordingly. **Remember to reconnect the gas connections before proceeding.**

PAR

Depending on cuvette type, enter a PAR Control Setpoint from 0-2,000 µmol m^2 s^{-1}.

Cuvette Flow

Appears as above (see System Setup on page 49.)

Temperature

Track Ambient

Cuvette temperature (T_c) control tracks ambient temperature as measured by the sensor in the base of the cuvette handle (PLC5-6).

Entered Values

If selected, enter a temperature value in the field below. Value must be within the range of 8 ^oC below ambient to 45 ^oC. **Please note:** Be aware of possible condensation inside the cuvette if warm air passing through the system is suddenly cooled by the Peltier unit in the cuvette.

None

Cuvette temperature control disabled.

Which Control Type Should I Use?

For most field work involving a large number of leaves, it is advisable to make the measurements as rapidly as possible so that measurements are being made on leaves adapted to field conditions. Therefore, we would recommend selecting **Supply (Ref) Approximate ppm** for CO_2 and either **Supply (Ref) % Ambient** or **Supply (Ref) Set mb (0-Ambient)** for H_2O. With most leaf cuvettes, stable readings should be possible within a one minute time interval using these Control Types.

For A/Ci and light response (A/I) curves in the laboratory and field, we recommend selecting **Supply (Ref) Approximate ppm** for CO_2 and either **Supply (Ref) % Ambient** or **Supply (Ref) Set mb (0-Ambient)** for H_2O.

If performing a Light Response:

1. Set the CO_2 to a high, non-limiting value.
2. Set the light to the highest level required.
3. Wait for equilibrium and then reduce the light intensity in steps.

If performing a basic CO_2 Response:

1. Start off with a low CO_2 concentration and high light intensity until equilibration. This will open the stomata to their maximum.
2. Increase the CO_2 in steps to the maximum desired value.

If performing both a light response and a CO_2 response on the same leaf, start off with a CO_2 response followed by the light response, as described above.

Customized Operation of CIRAS-2 with Ambient CO_2

Especially for field work, you may wish to measure gas exchange without actually controlling CO_2 concentrations from CIRAS-2, using instead the naturally fluctuating diurnal CO_2 concentrations in ambient air. To do this:

Empty all soda lime from Absorber Column 3 and replace the empty column in the manifold. Remove *only* the molecular sieve from Absorber Column 4 and replace. (Both soda lime and molecular sieve remove CO_2 from the gas stream). Next, attach a length of butyl tubing (supplied) to the AIR IN gas port and shield it from sources of CO_2 such as your breath and engine exhaust. AIR IN must also be protected

from wind and sudden pressure changes. Create a “smoothing volume” such as a large plastic container or sealed plastic bag and place the open end of the AIR IN gas line inside the smoothing volume. Finally, remember to leave the CO_2 holder/regulator assembly in place in CIRAS-2 either with no cartridge or with an exhausted CO_2 cartridge.

Customized Operation of CIRAS-2 at Humidity Levels *Above* Ambient

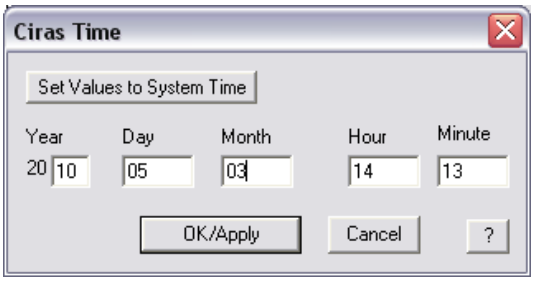
If you desire to work at humidity levels above ambient, you can do so as follows:

Ensure that Absorber Column 3 (as labelled on the bottom of CIRAS-2) is filled with new soda lime that will humidify the air. Next, carefully remove and cover the water vapor equilibrator with clear plastic wrap so it is isolated from ambient. Replace the equilibrator in the manifold.

Or

Carefully remove and cover the water vapor equilibrator with moist filter paper and reattach it to the manifold. In very hot dry conditions you can also cover the filter paper in clear plastic wrap to minimize evaporation.

CIRAS-2 Time

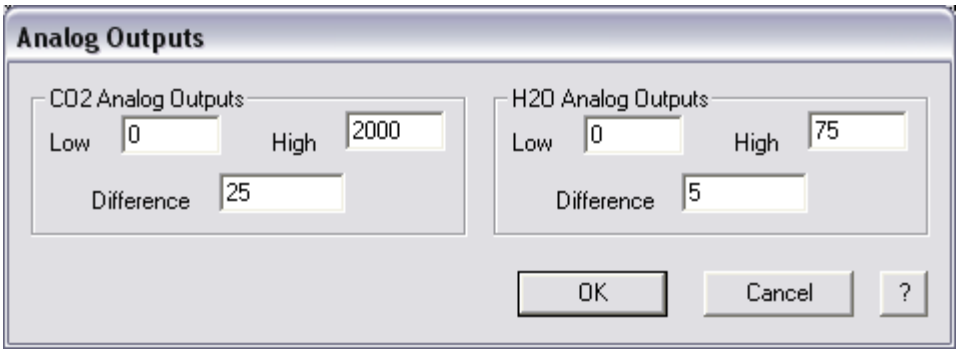


CIRAS-2 can be set to match the exact date and time of the PC (integral PC or Lab PC) or to a different date and time if required. To match the values to the PC, click on **Set Values to System Time**. Otherwise, a different date and time can be set as required. Once set, press **OK/Apply** to accept the settings or **Cancel** to return to the **Settings** dialog.

Please note that setting the CIRAS-2 Clock takes a few seconds. If the software detects that the CIRAS-2 clock differs by more than 1 hour from the UI or Pencentra clock, the CIRAS-2 time will be set to the UI or Pencentra time automatically. This option is only available when CIRAS-2 is connected.

Analog Outputs

The 8 pin LEMO type socket labelled SIG OUT on the CIRAS-2 is used for the analog outputs. All outputs have a maximum voltage of 5.0 V from a source impedance of about 60 ohms. Therefore, if external resistors are used to drop the voltage, it is recommended that they exceed 10 Kohms in total.



There are 4 analog output voltages in use corresponding to the absolute and differential CO_2 and H_2O concentrations. The full range is 0-5 V with 8 bit (0-4% of FSD) resolution. To optimize the outputs, it is possible to set the corresponding concentration ranges.

CO_2 Analog Outputs

Low	0 - 2,000 ppm
High	0 - 2,000 ppm
Difference	-25 - 999 ppm

H_2O Analog Outputs

Low	0 - 75 mb
High	0 - 75 mb
Difference	-5 - 75 mb

For differential values (both CO_2 and H_2O), an output of 2.5V corresponds to a 0 differential. Output values below 2.5V correspond to negative differentials (-) and values above 2.5V correspond to positive differentials (+).

Examples of Analog Outputs

Example 1.

If:

CO_2 Reference = 356 ppm
Low CO_2 = 0
High CO_2 = 2000

Then:

CO_2 Reference Voltage Output = 356/2000 x 5.0V = **0.89 V**.

Example 2.

If:

CO_2 Reference = 356 ppm
Low CO_2 = 300
High CO_2 = 400

Then:

CO_2 Reference Voltage Output = ((356-300)/(400-300)) x 5.0V = **2.8 V.**

Example 3.

If:

CO_2 Differential = +25 ppm
Range = +/- 50.0 ppm

Then:

CO_2 Differential Voltage Output = 25/50 x 2.5 + 2.5 = **3.75 V.**

Example 4.

If:

H_2O Reference = 25.56 mb
Low H_2O = 10 mb
High H_2O = 50 mb

Then:

H_2O Reference Voltage Output = (25.56-10.0)/(50-10)) x 5.0 V = **1.945V**.

Automatically Update CIRAS

If checked, after clicking **OK** in the **Settings** dialog to save all changes to the **PRM** file, CIRAS-2 will receive the new parameters. If this is not checked, CIRAS-2 will not be updated.

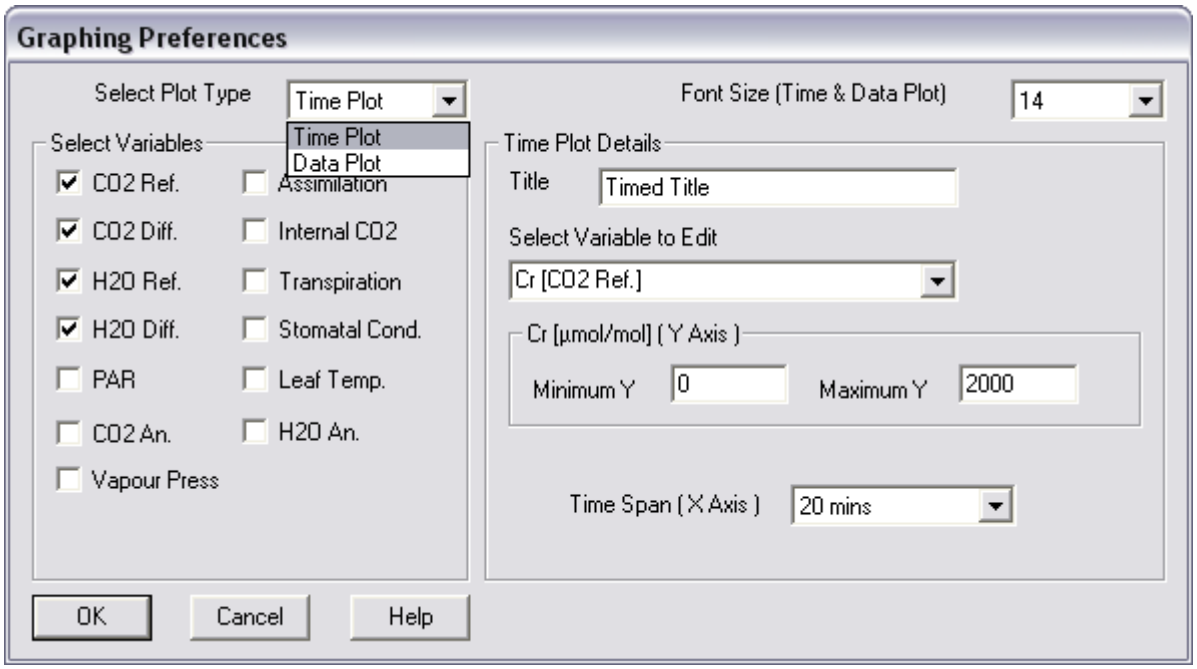
Graphing Preferences

Before continuing you can select the variables you wish to view in the real-time display. You can access this dialog in one of four ways:

1. Click **Setup** in the CIRAS main menu and open the **Graphing** – **Preferences** dialog box
2. Through the Graphing Options button in the **Setup** dialog
3. Right-click anywhere on the Time or Data Plots using RCS from a computer
4. Click anywhere on the Time or Data Plots with the mousepad and pointer using the UI.

Time Plot

From the 'Select Plot Type' list choose **Time Plot**.



Select Variables

Choose up to four variables to display as continuous colored plots in the Time Plot. At least one variable must be displayed. The list of variables automatically changes based on Accessories, e.g. variables related to chlorophyll fluorescence while using the CFM module.

The available list is drawn from the parameters and variables seen in the Gas Exchange Measurement Screen section (page 26), with two exceptions – **CO2 An.** (Analysis gas CO_2) and **H2O An.** (Analysis gas H_2O). **CO2 An.** is simply CO2 Ref. + CO2 Diff. Likewise, **H2O An.** is the H2O Ref. + the H2O Diff.

Font Size

Choose the font size that best meets your needs – larger fonts for computer RCS display, smaller for the UI when conducting research in the field.

Time Plot Details

Enter a description of the plot(s) if desired. This information will be saved with the data file.

Select Variable to Edit

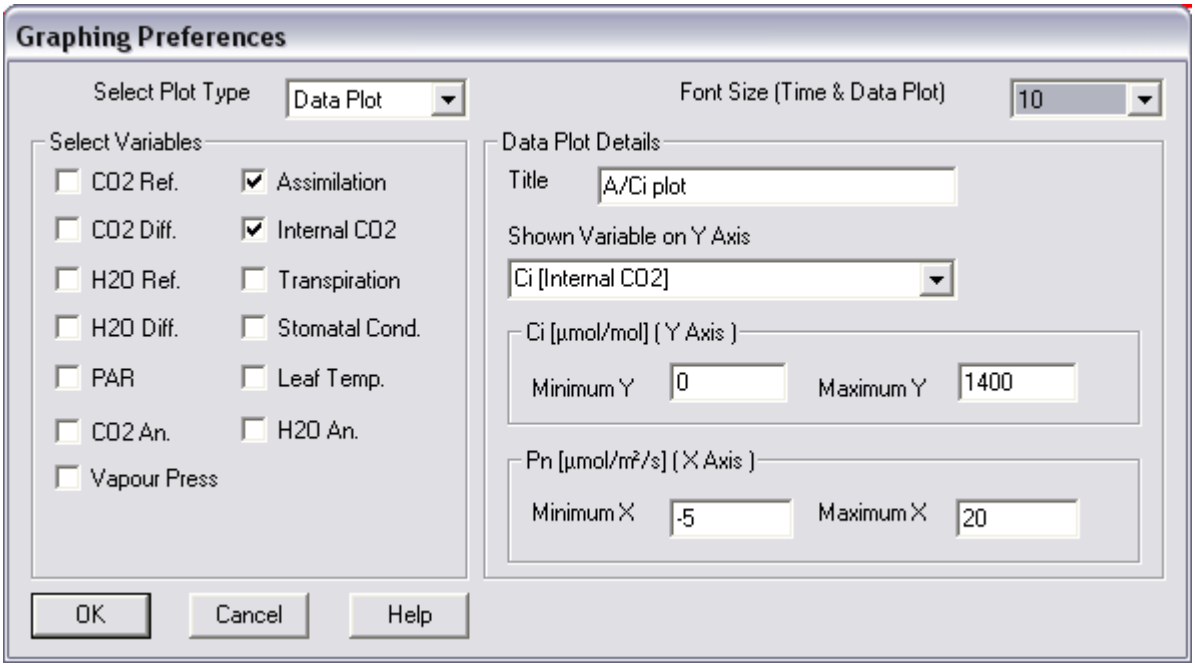
The respective Y-axis scales of your chosen variables can be changed from defaults by choosing the variable from the dropdown list. You will see a message if you set the scale outside of the min-max range.

Time Span

Choose your desired X-axis time span (10, 20 or 30 minutes).

Data Plot

From the 'Select Plot Type' drop down list select **Data Plot**. This plot displays two variables only, plotting data against data. Essentially, it allows you to view a real-time scatter plot, for example, an A/Ci plot.



Shown Variable on Y Axis

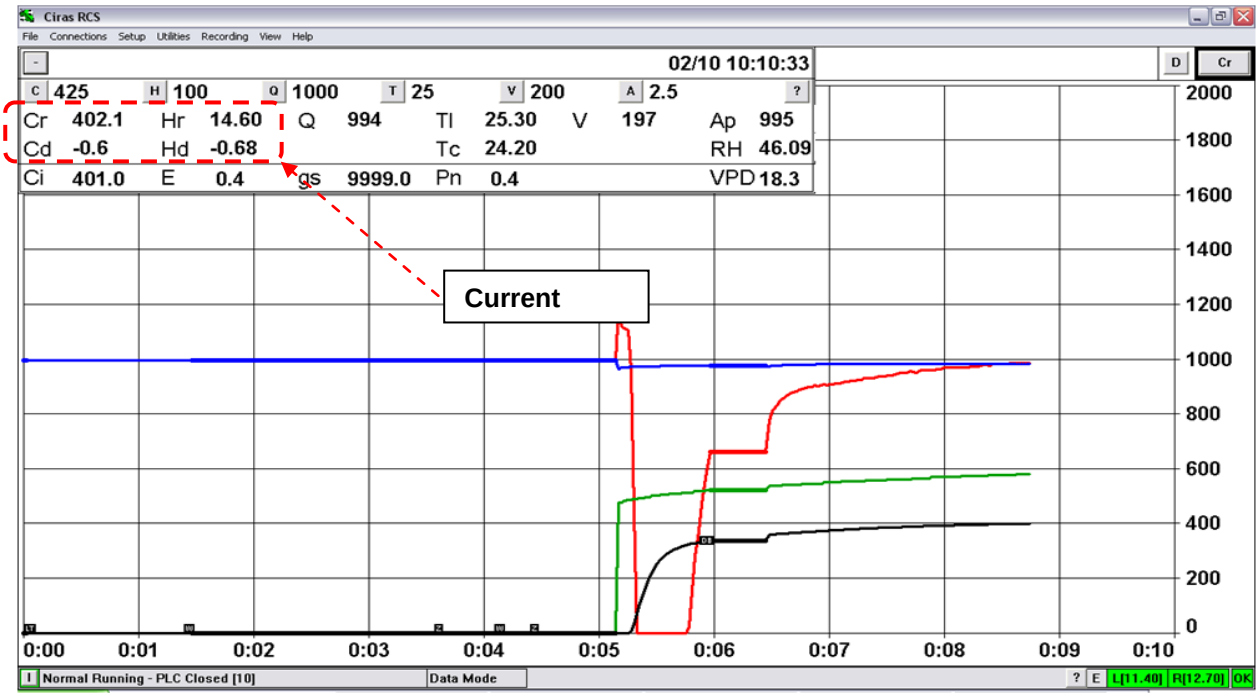
Choose which of the two variables to display as the response (Y-axis) variable, then set the min-max scales for both selected variables as above.

Observing and Recording Leaf Gas Exchange

Checking the System with an Empty Cuvette

With the cuvette closed and without a leaf inserted, you should conduct a routine test to ensure that all values are stable and that the desired control settings are achieved. Stablity is easily observed by a flat (horizontal) profile of the colored plots as they progress in time across the display screen. Select and observe only the measured parameters associated with CO_2 and water vapor from the 'Select Variables' field: Cr, Cd, Hr, Hd.

Within several minutes a display similar to the one below should be observed, assuming the system has been adequately warmed up and all steps in Preparations for Use (on page 18) have been carefully followed:



Please note the following:

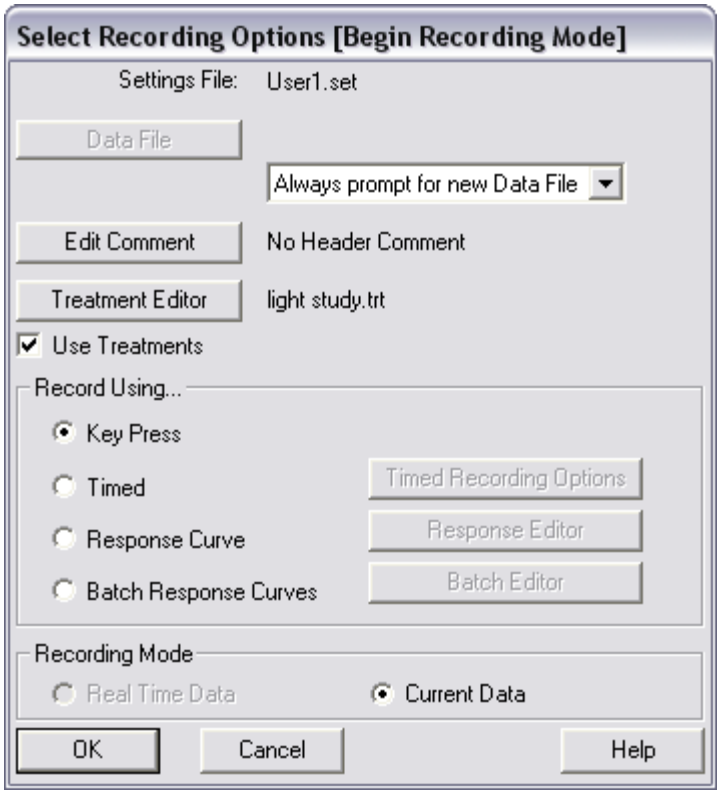
- Cr (Reference CO_2) value should be near your Setpoint value and stable (+/- 2 ppm).
- Hr (Reference H_2O) value should be at ambient and stable (+/- 0.2 mb).
- Cd and Hd values should be stable at approximately 0.0 (+/- 0.5 ppm CO_2 / 0.2 mb H_2O).
- Tc (Chamber Temperature) value should reflect your selected temperature value.
- Tl (Leaf Temperature) value should be within approximately 1.5 °C of the Tc value.
- Q (PAR) value should be near your Setpoint value (with attached LED light unit) or ambient (if no light unit is not used)
- Battery voltage for both the left and right batteries should be > 13.0 V if fully charged.

Please note: The CO_2 reference value (Cr) will not be exactly what you set if the CO2 Control Type selected is (Supply (Ref) Approximate ppm). It should, however, be stable (fluctuating not more than +/- 2 ppm) at a value within approximately 15-30 ppm of the set value. Precise CO_2 control is available to within +/- 1 ppm of the set value by using the 'Supply (Ref) Set ppm' Control Type.

The system is functioning properly if the above conditions are observed. If not, please refer to the Troubleshooting Section of the **CIRAS-2 Technical Manual** available in the User section of our website.

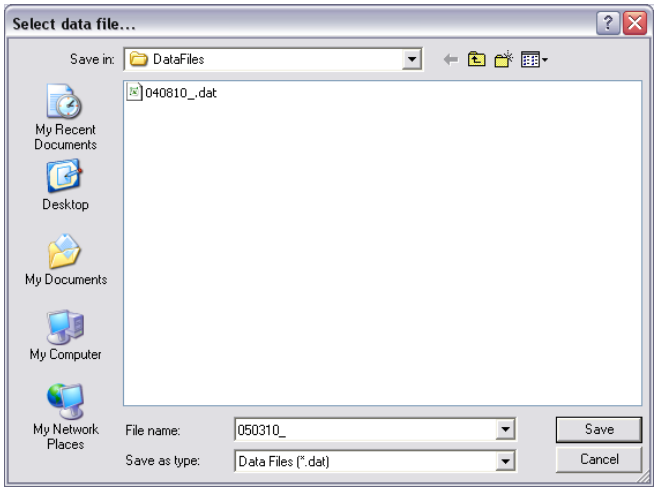
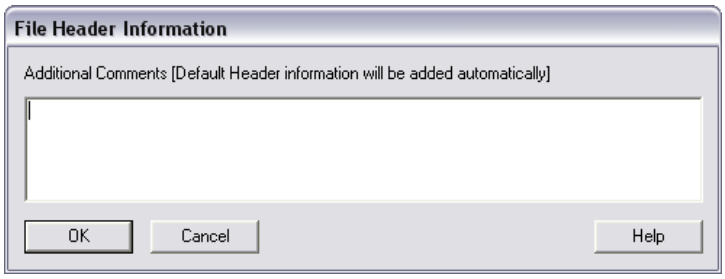
Select Recording Options

To begin making measurements, select **Recording** – **Begin** from the CIRAS-2 menu bar.



There are four different methods available for recording data:

1. **Keypress**
2. **Timed**
3. **Response Curve**
4. **Batch Response Curves**

Button/Field	Description
Settings File	Refer to Set File Options on page 41 for more details.
Data File	Create a new data file and enter a name in the File name box (.dat extension is default). Measurements will be saved to this file. 
Edit Comment	Additional comments can be saved with records if required. Comment is placed at the beginning of the recorded data. Additional comments can be added to the files throughout a recording session. 
Treatment Editor	See detailed information below.

Treatment Editor

Treatment files (extension .trt) are provided to help identify records associated with a particular experimental or sampling level, such as a plot, group, plant or pot. Each Treatment file can contain 10 Groups, and each group 10 Items.

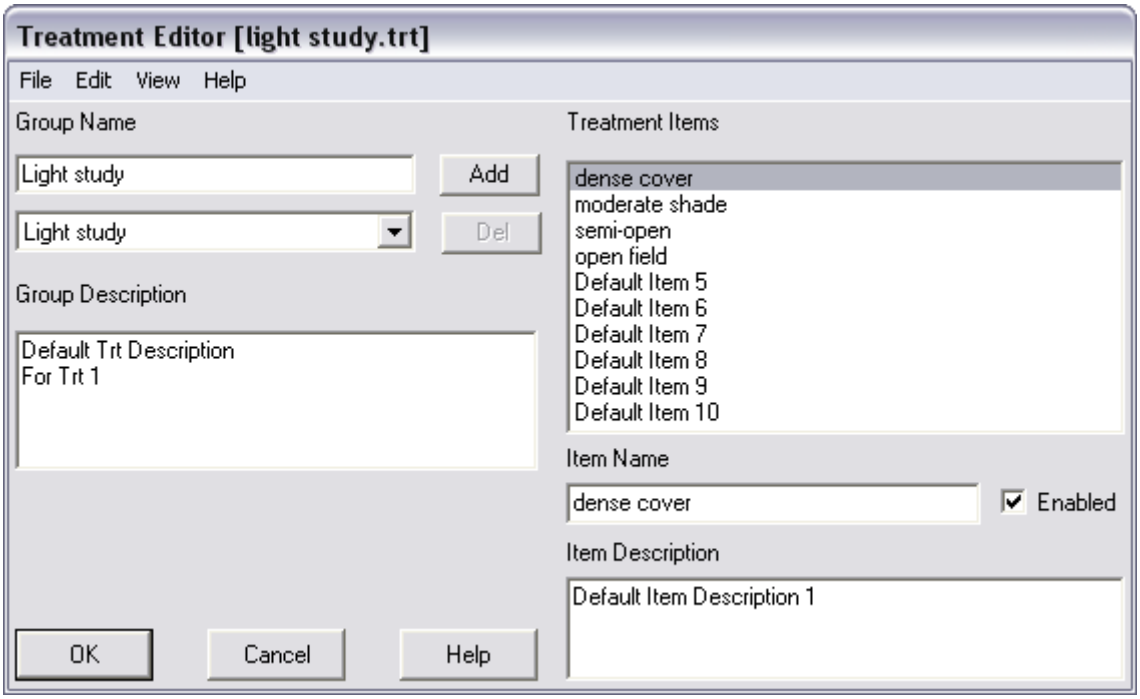
Treatment Groups

These can be named as required and may be given further descriptions as necessary.

Treatment Items

Each item may be named and can have a unique description. Highlight the Default Item and check the 'Enabled' box to edit 'Item Name'. Enabling/ Disabling an item will determine whether it is displayed as an option during recording. When Recording, each change of Treatment Item is recorded and all subsequent

records are assumed to relate to that item. If a Treatment file is not specified, a default set of “plots” are used during recording.

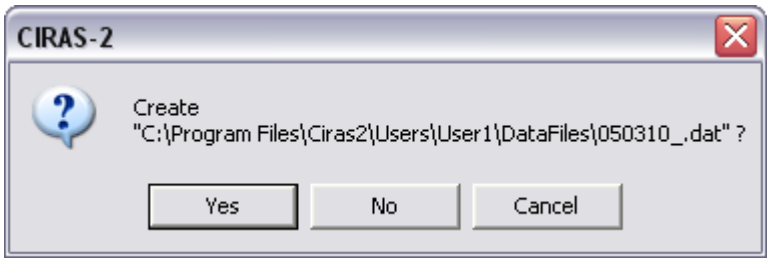


Recording Mode

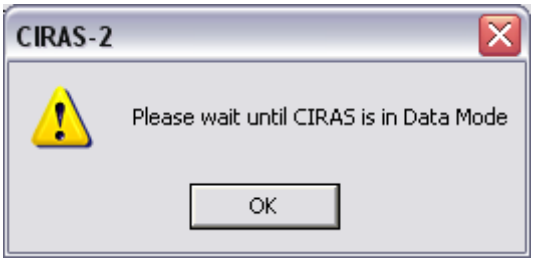
Defaults to ‘Current Data’ in order for **Setup – Settings** to apply changes.

The following applies to all recording options:

The **Select data file** dialog box (as above) will appear for you to name the data file. In the **Select Recording Options** dialog you can choose from three file saving options – ‘Append to Data File’; ‘Overwrite Data File’; ‘Always prompt for new Data File’. After naming the file you can either accept the default file location on your computer or UI (example below), or choose a different location. Click **Yes** to proceed to Recording Mode.



Please note: If CIRAS-2 is actively performing a **Zero** or **Diff Bal**, you may receive the following message prior to naming the data file:

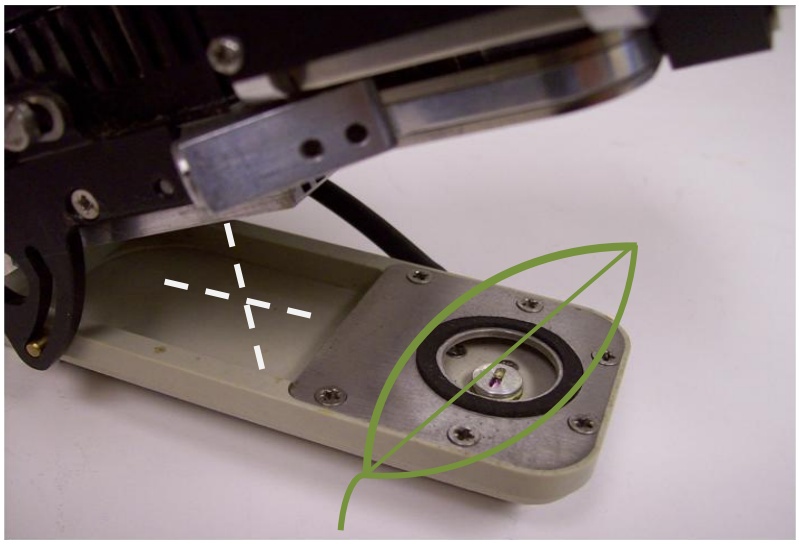


If this occurs, simply wait until the completion of the **Zero** or **Diff Bal** check.

Placing the Leaf in the Cuvette

Choose leaves of sufficient size to completely fill the cuvette head opening around the gasket (as illustrated below). The built-in IR leaf temperature sensor will return false values if light enters from gaps around the leaf. In other words, the IR sensor must "see" only the lower leaf surface, otherwise you must select 'Energy Balance' as the default **Leaf Temperature Determination** type.

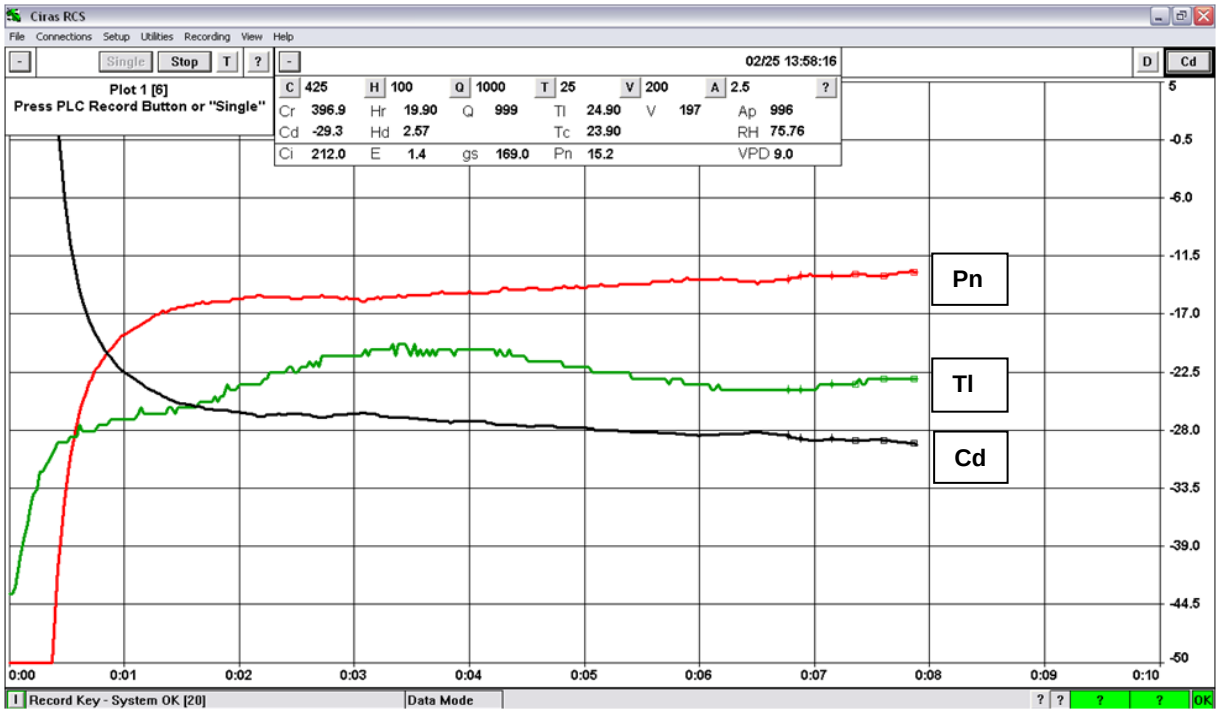
The leaf blade may extend beyond the foam gasket surrounding the opening. However, the leaf blade must not extend beyond the back of the lower metal plate, i.e. into the back of the cuvette's lower jaw, where part of the leaf will be crushed when the cuvette is closed. Try approaching the leaf from the side with the open cuvette to avoid accidentally injuring the leaf in this way.



The cuvette tension screw is located beneath the cuvette open/close lever. Use a small hex wrench to adjust the screw tension if needed to accommodate leaves of varying thicknesses. Turn the screw counter-clockwise to tighten and clockwise to loosen the tension. Normally no more than ¼ turn in either direction is needed. The goal is to establish a good seal without i) causing extreme pressure on the leaf or ii) allowing ambient air to leak around the foam gaskets.

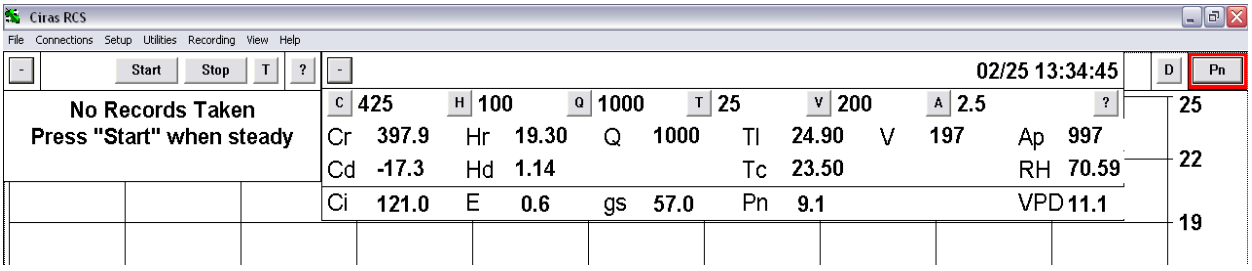
With the leaf enclosed in the cuvette you should quickly notice a series of dynamic leaf responses, assuming ideal physiological conditions. Sub-stomatal CO_2 concentration (Ci) will begin dropping, after being initially higher than the reference CO_2 concentration (Cr). When Ci<Cr there is an instantaneous change in net photosynthesis (Pn) values from a negative rate (respiration) to a positive rate. Simultaneously, the differential CO_2 concentration (Cd) will go into negative values while differential

humidity (Hd) becomes positive – the leaf is both fixing carbon dioxide and transpiring water vapor. As seen in the figure below, Pn (red plot) and Cd (black plot) typically appear as near mirror images. The green plot is the leaf temperature.



Note that some physiological variables, especially Pn, gs (stomatal conductance) and Cd will continue changing as the leaf equilibrates (approaches a stable state) for approximately 45-60 seconds, and as long as a few 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.

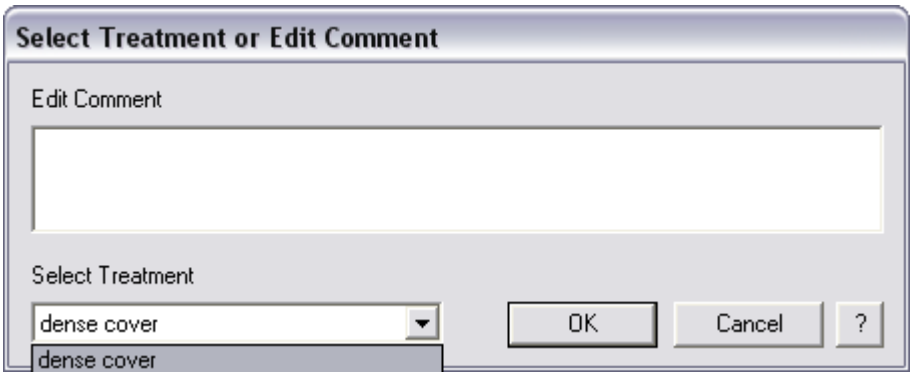
In Recording Mode the CIRAS-2 display changes (upper section of display screen shown, below), including a new window (upper left) containing Recording features as well as the now familiar **Data Display and Control** features window. Click the [-] button to collapse or the [+] button to view either window. You may need to adjust to a smaller font size to accommodate both windows.



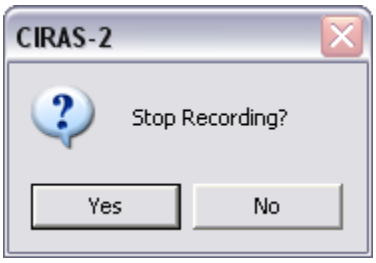
When readings are stable and you are ready to begin measurements, click the **Start** button in the Recording window. Depending on the state of your CIRAS-2, the instrument may perform a **Zero** and **Diff Bal**. You may also take this opportunity to adjust your Graphing Options, for example, by selecting one or

more of the calculated physiological variables. In the sample screen (above) the currently active plot is Cd (black) with the Y-axis scale set to +5 to -50, to capture both respiration (positive differentials) and photosynthesis (negative differentials).

To include additional information about your experiment or treatment click the T button and enter the information by selecting a particular "Treatment" associated with the measurement.



To end measurements, press the **Stop** button. The following will be displayed:

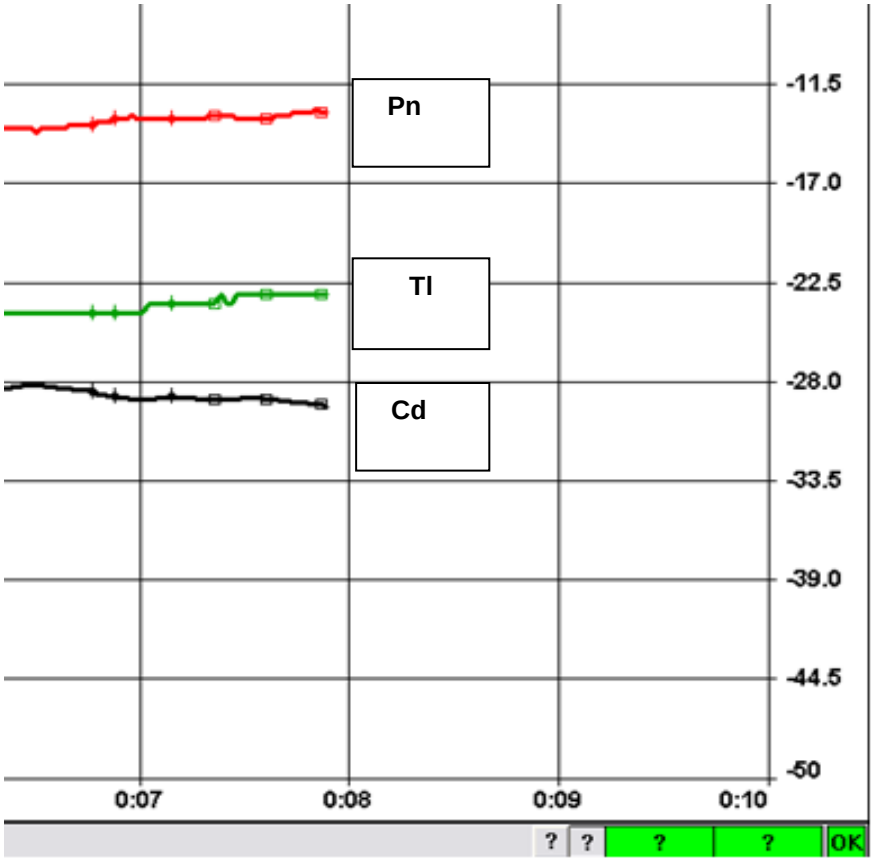


Click **Yes** to terminate measurements.

Keypress Recording

For many leaf gas exchange measurements, such as those made in the field and under dynamic light conditions, this method of recording is most practical. Using this method you can record measurements in rapid succession with either the thumb record switch on the cuvette handle or by clicking the **Single** button in the CIRAS-2 Recording Mode screen. To begin manually recording data, click in the circle next to 'Key Press' and then click **OK**.

In **Keypress Recording** mode the appearance of data markers in the Time Plot is different depending on the source of the record: in the image below the first 3 records (circles with a vertical hash line) on the plots, were taken by clicking **Single** – the next 3 records (boxes) were taken with the thumb record switch on the PLC6(U) cuvette.



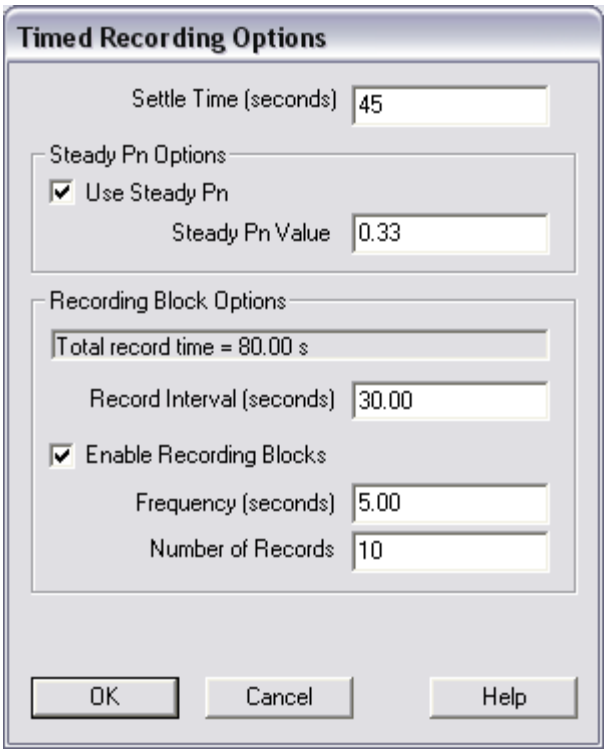
Timed Recording

This option allows indefinite data collection that can be programmed to run until the operator manually stops recording. It may be used unattended on a single leaf for long periods, or the user may choose to pause recording to either change Control Setpoints or sample different plants or leaves.

Again, select **Recording** - **Begin** from the CIRAS-2 main menu. In the **Select Recording Options** dialog click in the circle next to **Timed** and then click **OK**. There are two Timed Recording Options:

1. Recording single measurements
2. Recording blocks of measurements

Both options have an initial **Settle Time** to allow for stabilization and a forced Zero before recording begins. Both options can include recording only after a **Steady PN** (assimilation rate) has been reached, allowing you to determine your own acceptable level of variability associated with the plant or species of interest.



Settle Time (Seconds)

The minimum amount of time (seconds) required to stabilize before recording. After the settle time has been achieved, a record may be taken.

Steady Pn Options

Use Steady Pn

Requires that assimilation rate is stable by +/- your chosen value over a defined time (approximately 11 seconds). Once this condition has been met, the record(s) will be taken. Select to enable this function. To disable, deselect it.

Steady Pn Value

Enter an acceptable value (+/-) for Pn variability before data can be recorded.

Recording at Pre-set Intervals

In this mode, a record is taken after the elapse of the Interval (and optionally after Steady Pn has been reached). The software will continue recording until stopped. The Enable Recording Blocks option must be cleared, and the Record Interval entered in seconds.

Recording Block Options

Summarizes cumulative recording time based on recording interval, frequency and records.

Record Interval (seconds)

Refers to the amount of time (seconds) between records. CIRAS-2 transmits data every 1.6 seconds. If an interval has elapsed, the next available record will be taken.

Enable Recording Blocks

Also available in the Settings Editor (Recording Options button), and is shown when Recording is selected from the Main Menu.

Recording Blocks of Data at Pre-set Intervals

In this mode, a block of data will be taken after the elapse of the Interval (and optionally after Steady Pn has been reached). The block of data consists of a number of records to be taken at a specified frequency. The software will continue recording until stopped. The 'Enable Recording Blocks' option must be Selected, and the Record Interval and Frequency entered in seconds. Enter the number of records to take.

For example:

Interval between blocks = 30 seconds.
Number of Records in a block = 10.
Frequency these records are taken = 5 seconds.

The total time for a block of take to be taken is therefore 80 seconds (30 + (10 * 5)), with records being taken over a 50 second period.

In the Recording window you will be prompted to 'Press "Start" when steady'. Press the **Start** button when the **Data Display and Control** values appear stable. Plots are marked by an 'x' at each timed recording event.

Suspending and Resuming Recording

It is possible to suspend recording to allow you to change Control Setpoints while in Timed Recording. Click the **Pause** button to suspend recording - make your new Setpoint selection(s), allow the leaf to reach a stable condition, then click the **Cont.** (Continue) button to resume recording. To terminate Timed Recording, click the **Stop** button.

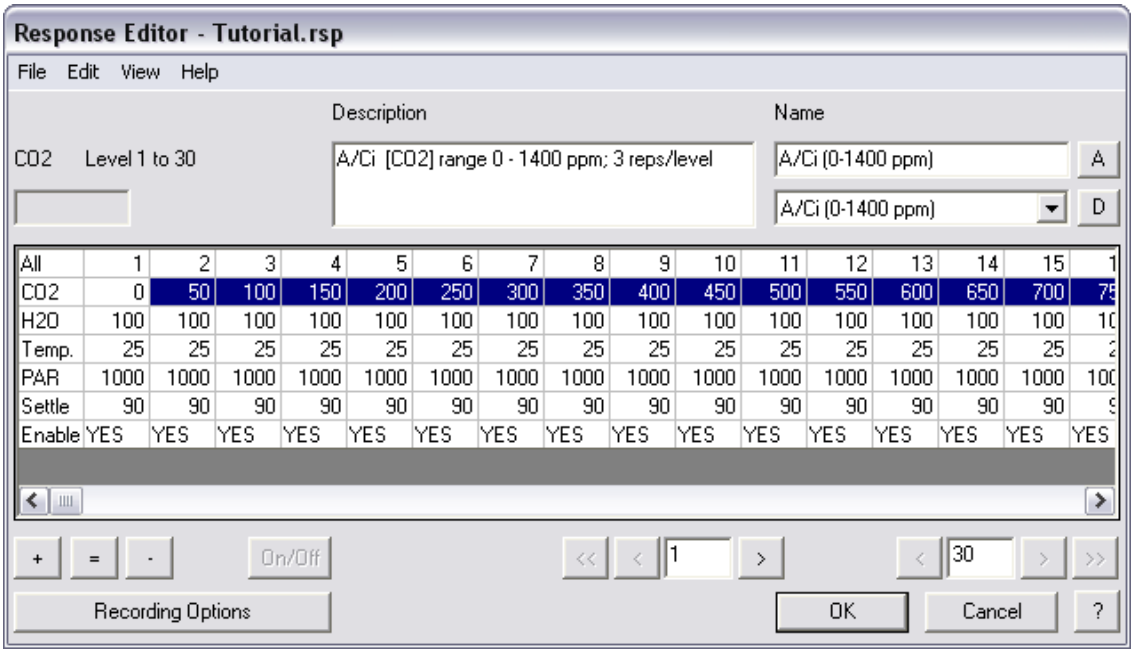
Response Curve

This option allows you to design highly individualized leaf chamber conditions for experiments in which CO_2, H_2O, temperature and light are automatically controlled and leaf responses recorded. A response curve consists of as many as 30 predefined levels, each level containing control values determined by you. Records are taken once the values have been set and a defined (or user initiated) interval has elapsed. Level-to-level changes can be made uniformly over a concentration range or they may be specifically defined by the user. Automated A/Ci, A/I and temperature response experiments are typical applications that utilize a response curve. It is also possible to modify several control values to generate, for example, Temperature and Light Responses.

When generating Response Curves, it is important to bear in mind the that large changes in a level may cause CIRAS-2 to Zero or Diff Bal during recording, effectively interrupting the measurement. In some cases, a Manual Response may be more suitable than an Automatic Response, as the user initiates recording in a Manual Response where leaf stability may be monitored.

To facilitate transitions between large CO_2 and H_2O level changes (changes that prompt CIRAS-2 to automatically perform a Diff Bal) you can predefine the absolute CO_2 and H_2O ranges of any experiment by running a **Stored Diff Bal** prior to any Response Curve. Refer to Stored Diff Bal on page 78. **Please note:** In the **Settings** dialog you must also change the Zero/Diff Bal Mode setting to 'Auto Zero with Stored Diff Bal'.

Select **Response Curve** to bring up the **Response Editor** window. Retrieve a previously saved Response file (.rsp) by clicking **File – Open** in the **Response Editor** dialog. To create a new response curve, enter the desired settings in the **Response Editor** dialog as shown below:



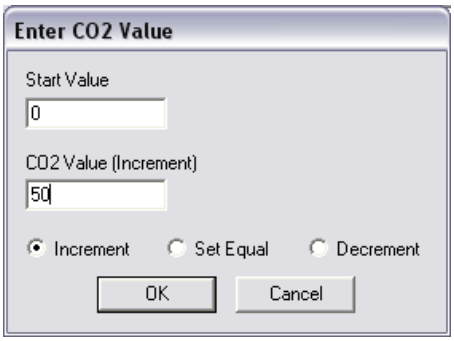
In the 'Name' field to the right you may enter a name for your response curve by clicking on Default Rsp Name 1' and typing a new name (optional). You may also enter an optional description. Click **A** to add a named Response setup. It is best to save the response file early, and then make alterations to the saved file. In the **Response Editor** dialog click **File – Save As**. Click **D** to delete the currently selected response. Each Response File (.rsp extension) can contain up to 10 Response Curves. The options for each are saved with the Response Curve Definition.

Editing Response Variable Values

Editing All the Values in a Row

In the first column at left are headings for the response curve variables. 'All' refers to the 30 available Response Levels in a response curve. Levels 1 through 15 are visible in the Response Editor. If the first cell marked All is selected, it is possible to reset all the values to the default.

Clicking on the variable heading in the first column will highlight that variable in blue (as CO2, above), and will bring up the **Enter _ Value** dialog box. After entering a 'Start Value', you may increase (Increment), hold constant (Set Equal), or decrease (Decrement) the 'Start Value' through your selected range of Levels. The example above shows a constructed response curve that dynamically increases CO_2 only, experimentally subjecting the leaf to 50 ppm incremental changes



'Settle' refers to the time needed for the leaf to reach a stable (physiological) condition from which reliable measurements can be recorded, and defines the wait before recording at the next level begins. Level Settle time is best determined prior to beginning Response Curve recording by pilot testing the plant/species of interest, using the expected range of CO_2 concentration, light intensity, etc. Large interval changes and delayed or lagging leaf physiological responses must have correspondingly longer 'Settle' times associated with them. If it is known that a plant responds quickly to changes in light intensity, for example, the Level Settle time can be decreased. Also, if a change in CO_2 concentration is small, then a shorter level settle time can be set.

It is important to bear in mind that changing CO_2 concentrations, etc. may result in time delays of variable length in order to 'propagate' through the system. Therefore, it is advisable to test the Response Curve prior to running an experiment to ensure that adequate Level Settle times are set. The allowable 'Settle' time range is from 10 to 3600 seconds.

Note that there are 3 buttons in the lower left hand corner of the Response Editor Window:

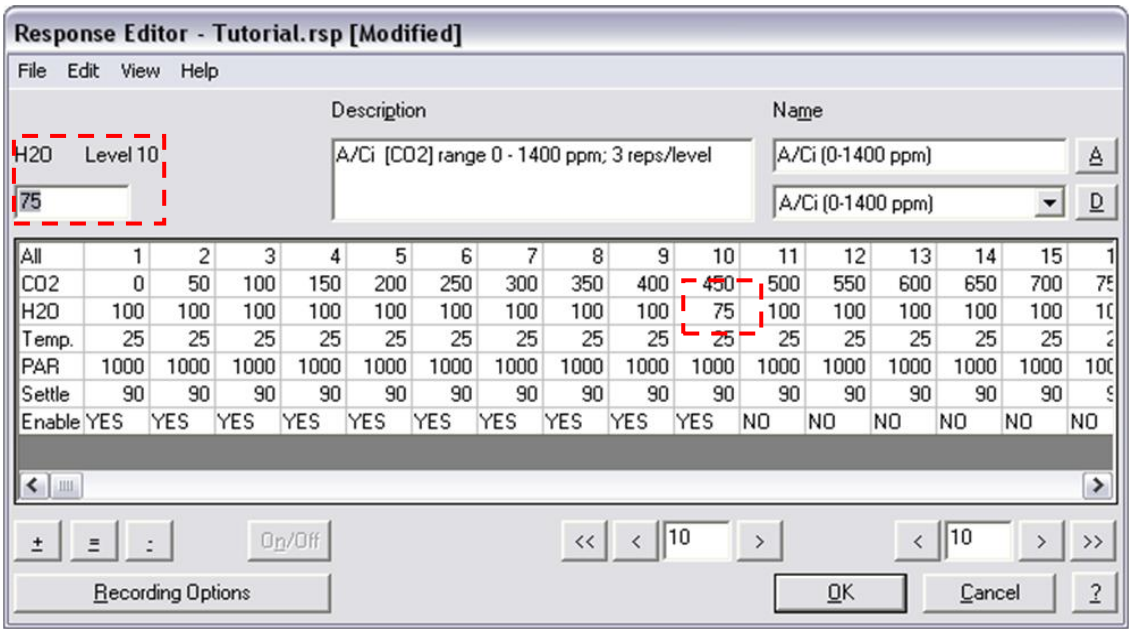
+	Same as **Increment**
=	Same as **Set Equal**
-	Same as **Decrement**
On/Off	Same as **Toggle Enable**

Editing Groups of values

If several adjacent values from the same row are selected, the **Enter _ Value** dialog box will appear.

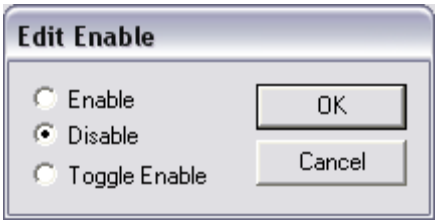
Editing Individual values

To introduce non-incremental values into Response Levels click inside any individual CO_2, H_2O, Temp., PAR or Settle cell. The current parameter, its value and Response Level are displayed in the upper left field (see below). Enter the new value in the field and click on the cell again to register the value in the cell for that specific Response Level. Customize additional Response Levels as desired.

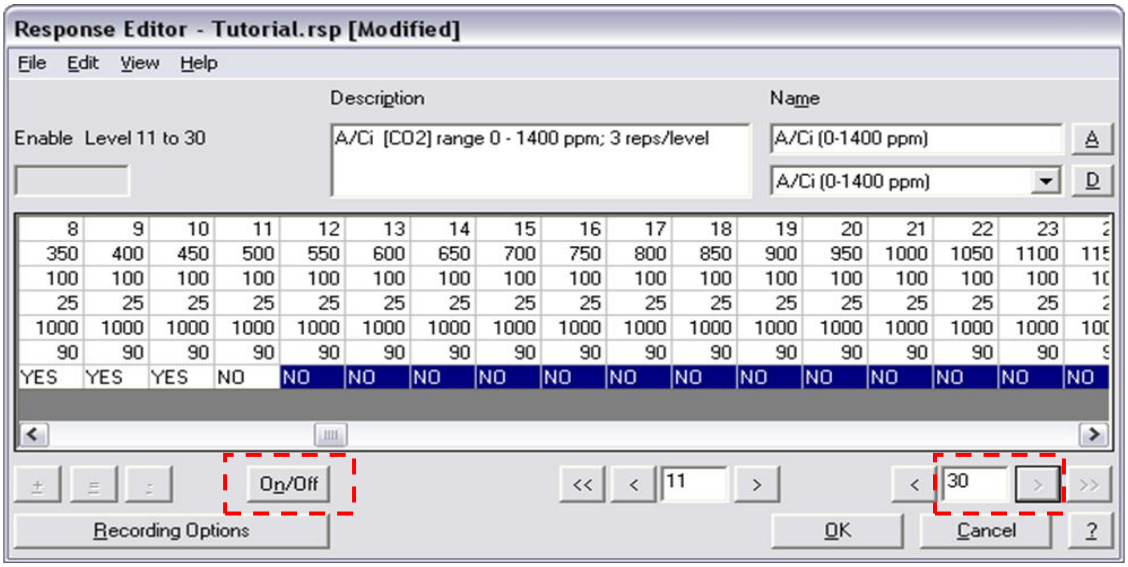


Enabling Levels

The Enable row can only be modified by a dialog box that appears when a cell in that row (or group of cells) is selected. Only levels that are enabled will be used in Recording. 'Enable' refers to the active/inactive state of the 30 Response Levels. To change the number of active levels in the response curve click on the cell in the 'Enable' row where your response curve will end (Level 11 in the example below). The **Edit Enable** dialog appears – select 'Disable'. The cell for Level 11 now displays 'NO' (disabled).



Level 11 now also appears in the lower left field. Repeatedly click the right arrow **>** button next to the right field until all remaining levels are highlighted (Levels 11-30), then click **On/Off** to disable the selected levels.



Recording Options

Click **Recording Options** to open the **Response Curve – Control Settings** dialog box. **Please note:** the Control Types entered here will be applied only during the Response Curve, and reset to the previous settings upon completion.

Repeat

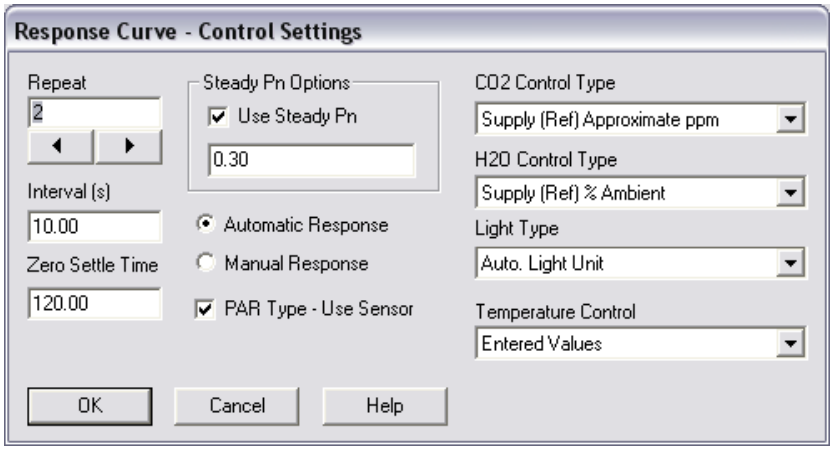
Beneath the 'Repeat' field click the ▶ button to choose the number of measurements to be recorded at each level. Later, this will be important for producing means when you process your data.

Interval

The amount of time (seconds) between records.

Zero Settle Time

This applies to the initial settling of the Response Curve measurement and any settle that is caused by a Zero or Diff Bal that occurs during the Response Curve. **Zero Settle Time differs from the Level Settle Time set in the Response Editor.**



Steady Pn Options

User-defined. If checked, enter an acceptable range of variability for net photosynthetic rate.

Automatic Response

Response Curve is automatic throughout the course of measurement.

Manual Response

Response Curve is manual throughout the course of measurement. The user must manually record data and move to the next set level during course of response curve.

PAR Type - Use Sensor

Check this box if the PAR Sensor is to be used to measure and control light in the cuvette. If this box is not ticked, PAR must be entered manually by the user to be included in measurements.

CO_2 Control Type

Both CO2 and H2O Control Types may be changed if desired. For CO_2 response curves we recommend using the 'Supply (Ref) Approximate ppm' type due to the time delay associated with the precision CO_2 control option. Refer to CO2 (0–2000 ppm) on page 51 for more details.

H_2O Control Type

Refer to H2O (0-100% or 0 mb-Dewpoint) on page 51 for more details.

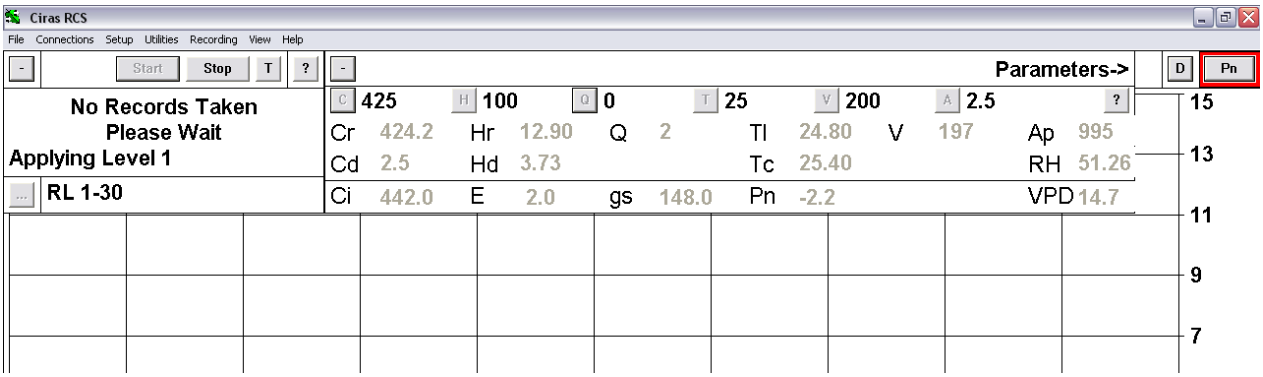
Light Type

Defaults to 'Auto. Light Unit'. See Light Type on page 45 for more details.

Temperature Control

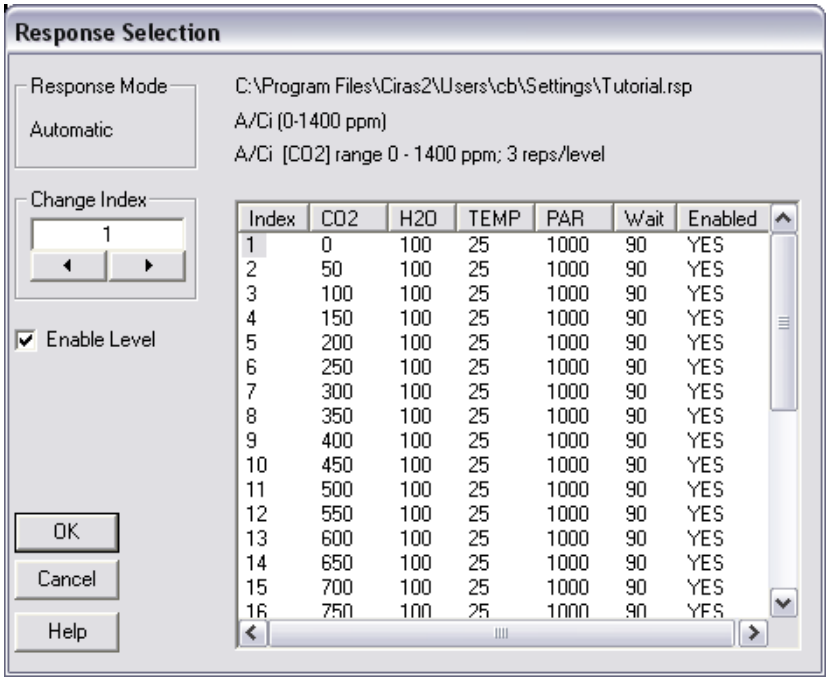
Defaults to entered values (25 °C). See Temperature page 53 for more details.

Click **OK** to accept changes, then in the **Response Editor** save the Response file. Click **Yes** to Enter a file name to save the Response Curve information. After entering a name, click on **Save**. Again, click **OK** when the **Select Recording Options** dialog reappears. Finally, name and save the data file. After creating the data file, the response curve measurement will begin.



As in Timed Recording you will be prompted to 'Press "Start" when steady'. 'RL 1-30' indicates that your Response Curve is currently in the first of 30 selected levels (see image above). Wait until the values are stable at Level 1, then press the **Start** button to begin an automatic response curve measurement. You can review details of your Response Levels at any time by clicking on the … button in the Recording

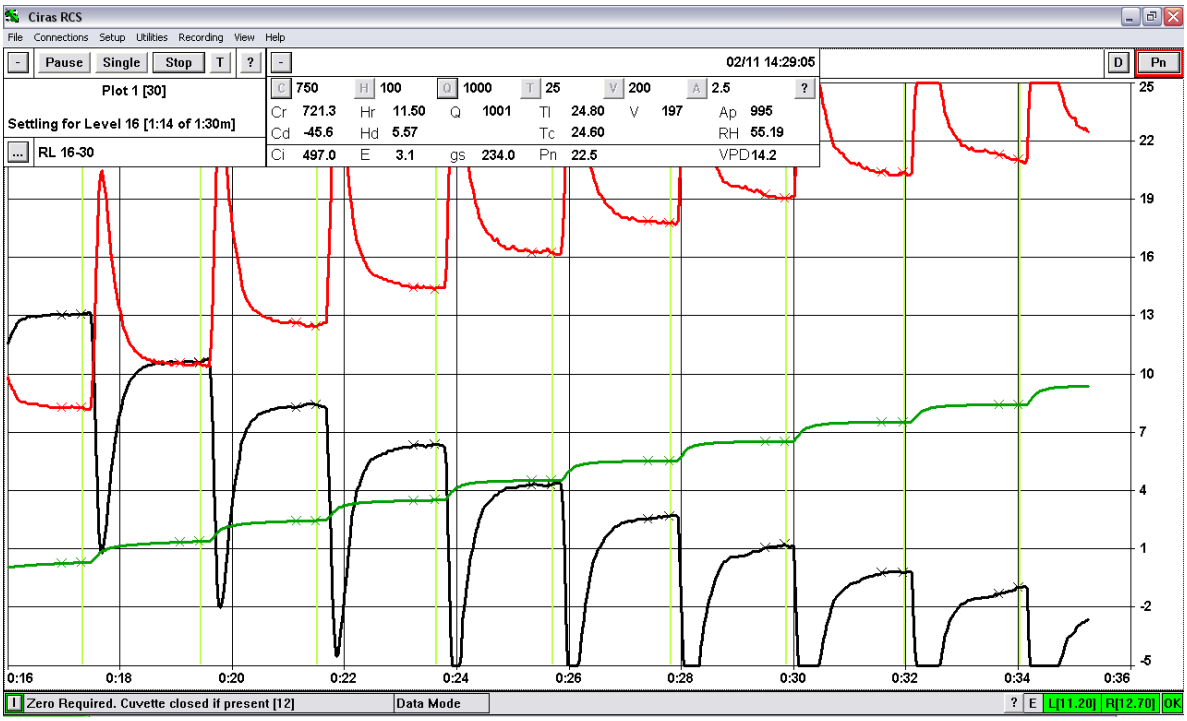
window (upper left) or from the CIRAS menu bar **Recording – Response Level** to expand the **Response Selection** dialog. Here you can jump to or disable Response Levels – however, you cannot edit Level values.



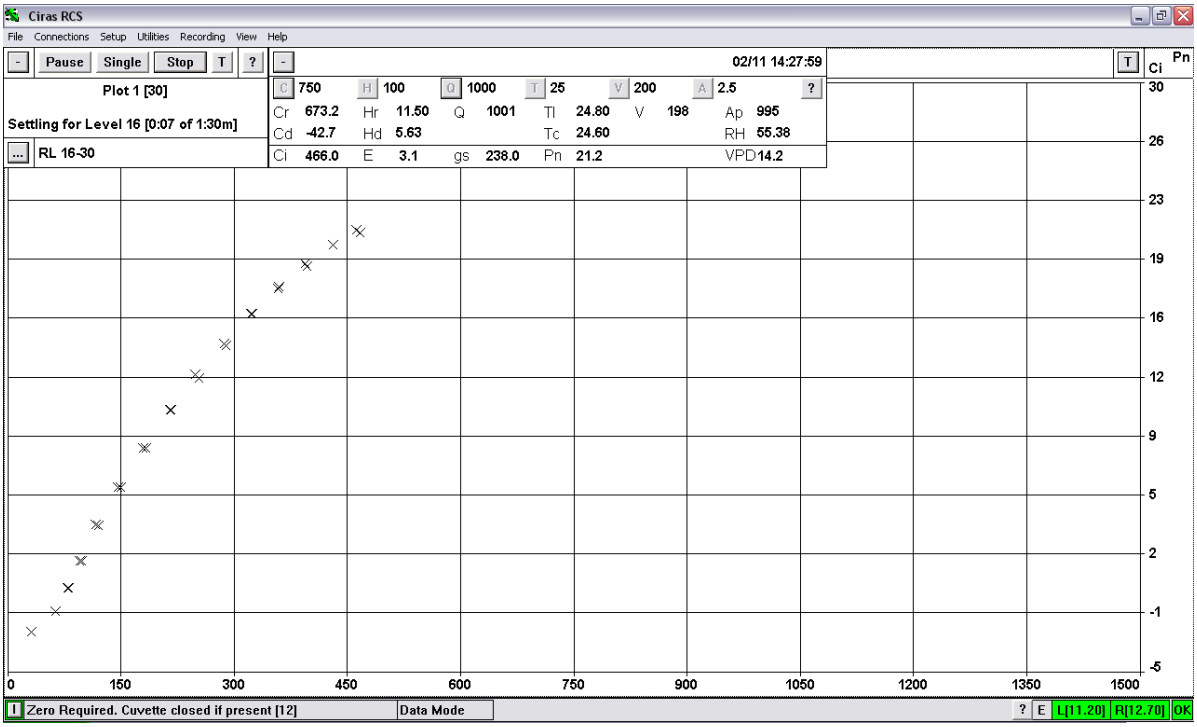
On page 75 is an example of what a typical CO_2 response curve might look like in the Time Plot: Pn (red) and Cd (black) increase (in opposite directions) accordingly with upward CO_2 concentration changes (green). Leaf responses stabilized (flattening of each curve) following transitory spikes associated with CO_2 Level changes, with two measurements made after appropriate 90 second 'Settle' times. The end of Response Levels are marked by a light green vertical line. In the example, 15 Response Levels have been completed – note 'Settling for Level 16 [1:14 of 1:30m]' in upper left of display. The corresponding Data Plot view of the A/Ci curve is also shown.

Please note: CIRAS-2 firmware is programmed to perform a Diff Bal whenever CO_2 concentration changes exceed 100 ppm or H_2O partial pressure exceeds 3 mb. Differential Balancing during a Response Curve will automatically suspend the Response Level, requiring a short leaf reacclimation period. This will be the case if you selected 'Automatic' in the **Zero/Diff Bal Mode** option. To avoid this you can store a set of values corresponding to your experimental range of CO_2 and H_2O, and then select 'Auto Zero with Stored Diff Bal' in the **Zero/Diff Bal Mode** option (see Stored Diff Bal on page 78).

Time Plot



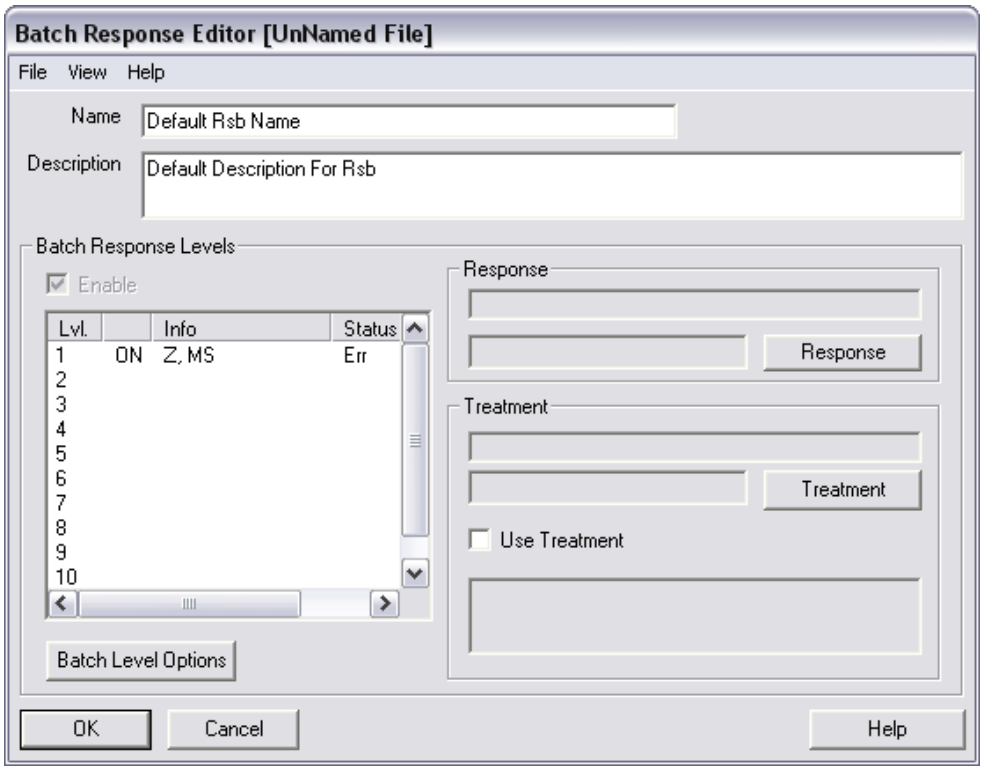
Data Plot



At the conclusion of the response curve, you will be given the option to either restart or end the measurement. Use this opportunity to place a different leaf/plant in the leaf chamber if desired - click **Yes** to repeat the identical response curve or **No** to end the recording session. To terminate the measurement, press the **Stop** button.

Batch Response Curves

Batch Response Curves are designed to allow multiple Response Curves to be run consecutively. Ten Batch Response Levels are definable within a Batch Response Curve. Instructions for setting up, storing and running Batch Response Curves is very similar to those described in the section Response Curve starting on page 68.



Enable Batch Response Levels

Click on a Batch Response Level number under 'Lvl.' To highlight, then check the 'Enable' box.

Batch Level Options

Show Prompt

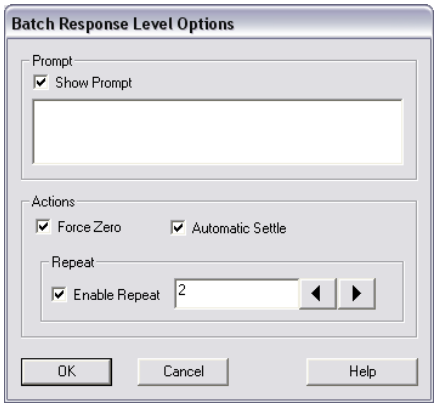
If checked, at the beginning of the Response Curve, the defined prompt is displayed. If enabled the user must respond to the prompt before the level continues

Force Zero and Automatic Settle

At the beginning of a Response Curve, these options control whether the Software forces a Zero and whether the user should indicate that the system is stable. **Please note**: For Batch Response Level 1, a Zero is always forced, and the user must indicate that the system is stable.

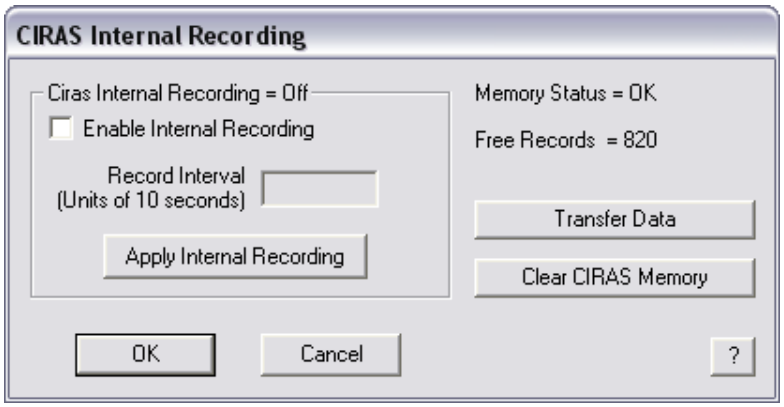
Repeat

If 'Enable Repeat' is checked, the software will repeat the Response Curve for the defined number of repeats from 2 to 10 times. If unchecked, the Response Level only runs once.



CIRAS Internal Recording

If required, data can be saved directly onto CIRAS-2 memory. From the menu bar select **Utilities - CIRAS Recording** to record measurements internally to CIRAS-2. The maximum number of records that can be stored internally is 820. The following dialog box will appear:



Click on the "Enable Internal Recording" box and enter the value required in the "Record Interval" box. **Please note:** The value you enter will be that value x 10. In other words, if you want to record every 60 seconds, enter 6 in this box and click on the Apply Internal Recording button. Then, click **OK** and readings will be taken automatically at the recording interval that was set. To terminate CIRAS-2 Internal Recording, return to **Utilities** - **CIRAS Recording** and click on "Enable Internal Recording" to disable it.

Please note the following: Unlike normal recording (i.e. Keypress, Timed Recording, etc.) where record marks are displayed to show when a measurement is being recorded, you will not see any marks or ticks on the display when readings are taken.

Enable Internal Recording

If selected, CIRAS-2 will store records internally. Maximum number of records stored internally = 820 records.

Record Interval

Units are based on 10 second increments. If set to 1, the recording interval is 10 seconds. If set to 2, recording interval is 20 seconds, etc.

Apply Internal Recording

Updates CIRAS-2 with the new information.

Transfer Data

To transfer internally stored CIRAS-2 data to your PC, click on the Transfer Data button. Available records are transferred from CIRAS-2 into the selected file (dmp extension). **Please note:** 'Enable Internal Recording' must *not* be selected prior to transfering data. The **Save Ciras Internal Records as…** dialog will be displayed prompting you to enter the name of the file where the data will be stored. After naming the file, click the Save button. At the conclusion of internal data transfer, a message will confirm that Data Dump is complete and the number of records transferred.

Click **OK** when finished. The data will normally be saved to the following location on the PC unless otherwise specified:

Program Files\Ciras2\Users\PPSystems\DataFiles\

Clear CIRAS Memory

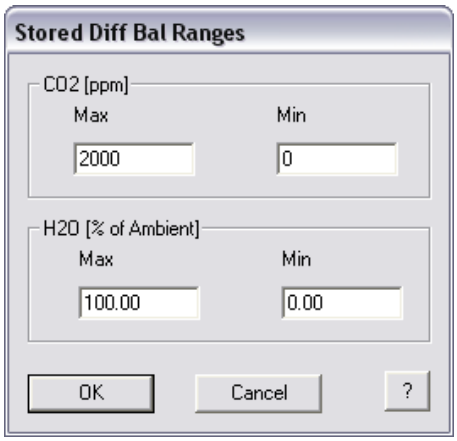
To clear internally stored CIRAS-2 records, click the Clear CIRAS Memory button. After clearing the memory, the following will be displayed:

Memory Status = OK
Free Records = 820

Stored Diff Bal

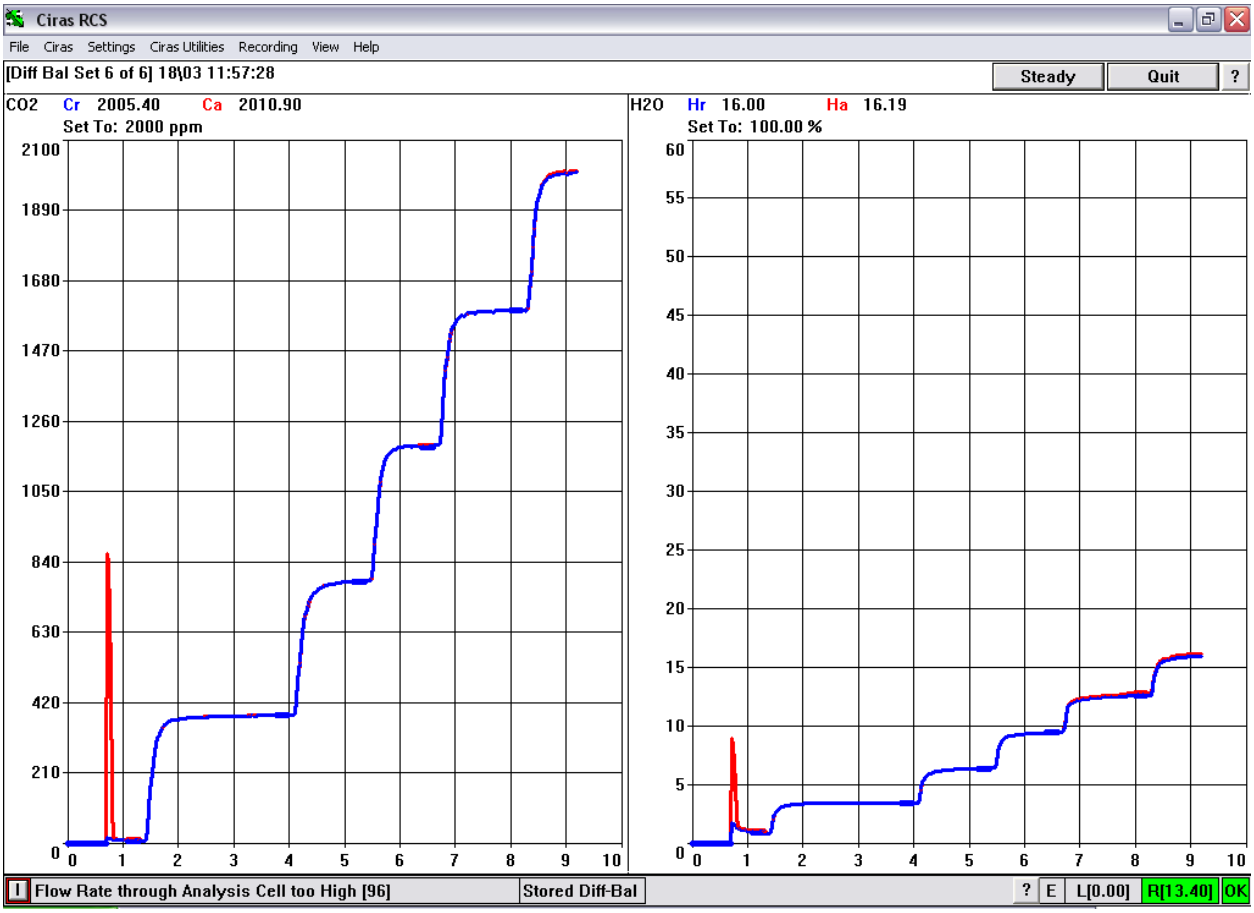
The Reference and Analysis analyzers in CIRAS-2 are absolute analyzers and the displayed differentials derive from the difference between them. In manufacture and calibration, every effort is made to match the analyzers as closely as possible, but even so differences still exist. Therefore, for accurate results, the differences must be known. To determine the difference, the Reference air is passed through both analyzers and the offset is recorded for application to future measurements. We call this process "Differential Balancing", or Diff Bal.

Since this process takes some time it may interrupt response curve measurements. It is possible to determine the offsets over the working concentration range before measurements begin. Six pairs of concentrations are required, but to simplify the process you only need to enter maxima and minima. Both CO_2 and H_2O must be entered and the ranges MUST cover the expected measured values.



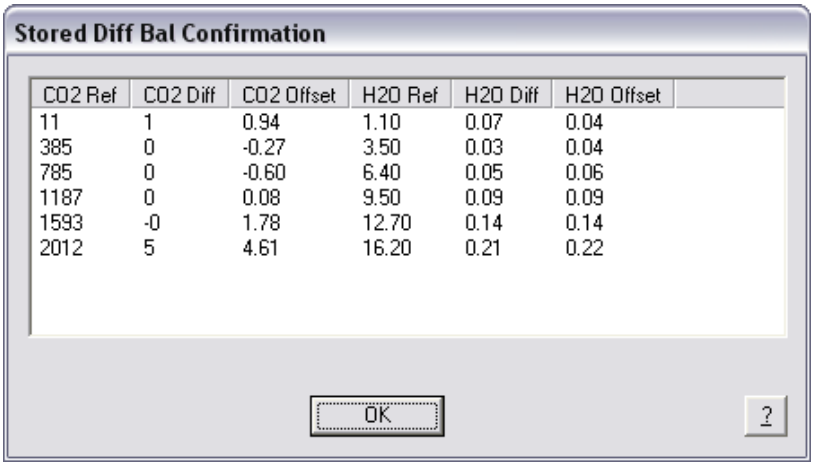
Go to **Utilities – Stored Diff Bal**. Enter the range of values and click OK.

The cuvette must be fully connected, though it does not have to be closed and a CO_2 cartridge must be present. The measurement screen will then be displayed and initially CIRAS-2 will zero. CIRAS-2 will then be set to the first set of values.



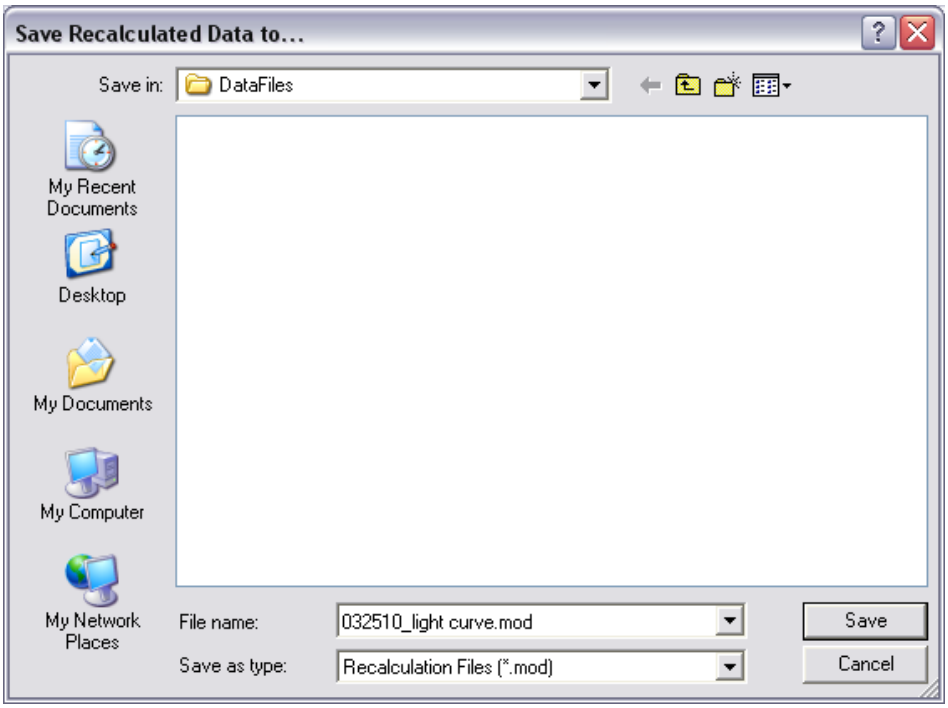
When the graph shows that the readings have stabilized click on Steady. CIRAS-2 will now do a **Diff Bal** and on completion, set to the next pair of values. The process should repeat 5 more times. On completion of the sixth pair, quadratic equations are fitted to the measured Reference concentrations and

offsets. The results are displayed showing the measured values and offsets, and the calculated offsets. You then have the option of accepting or rejecting the offsets. If accepted, CIRAS-2 will use the calculated values as long as the reference concentrations are within the set up range.



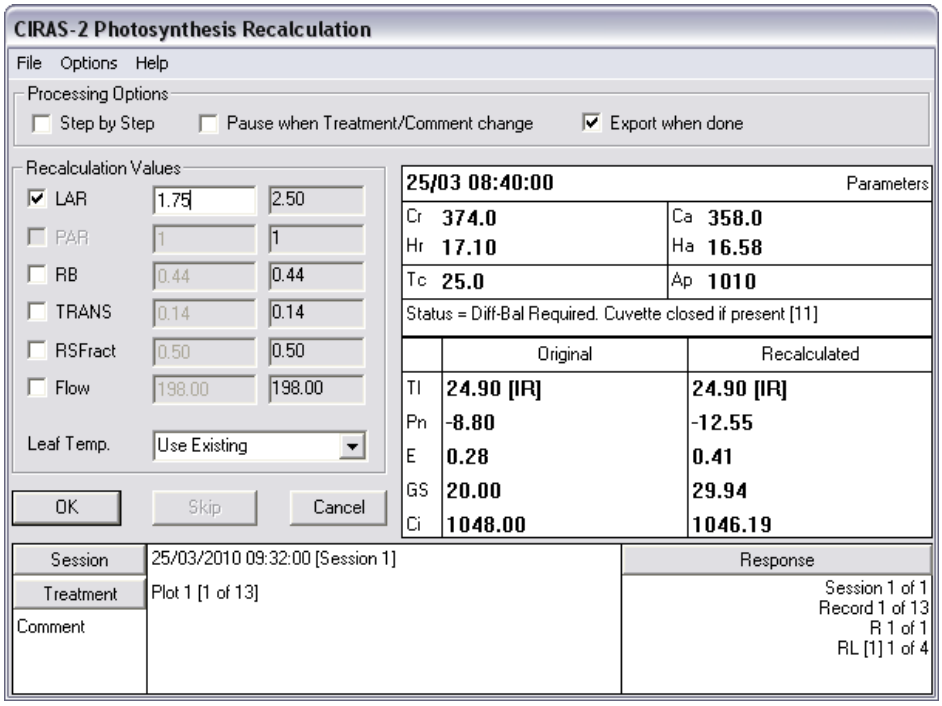
Recalculate Data

There may be situations when you need to recalculate your data, such as post-determination of actual leaf area, incorrect entry of PAR value, etc. To recalculate data click on **File - Open** and select the data file that you want to recalculate and click **Open**.



The **Save Recalculated Data to…** dialog will appear. The selected file will be automatically given a .mod extension. The original file (.dat extension) will remain unaltered. Click **Save** to continue. The **CIRAS-2 Photosynthesis Recalculation** dialog will appear. Initially, data from the first record stored is displayed.

To recalculate the data for the displayed record, check off the parameter(s) needing to be changed under 'Recalculation Values' and enter the desired value(s). Alternatively, click on Skip to move to the record requiring recalculation and make the desired changes. After completing changes for a specific record, click OK to accept the changes and to have the data recalculated.



Processing Options

There are 3 processing options available:

Step by Step	If selected, each line of data is recalculated and the results displayed. Changes in recalculation values are displayed automatically. After making changes, pressing OK will write the new values and proceed to the next data line. If not selected, data is calculated and the next line read automatically. However, the software will pause depending if **Pause when Treatment/Comment Changes** (See below) is selected.
Pause when Treatment/Comment change	Regardless of the **Step by Step** option selected, if this is ticked, it will cause the software to pause to allow a re-assessment of the recalculation options.
Export when done	If selected, the Export Dialog is loaded automatically upon completion of the recalculations for the file. See Export Data on page 88. If not selected, the recalculation file will be loaded according to the options set for viewing files available from the **Options, Edit** menu.

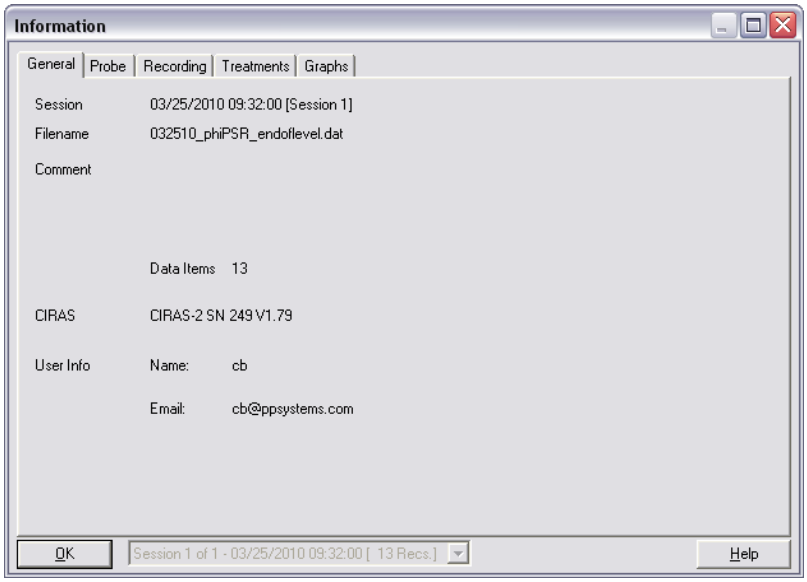
Recalculation Values

Parameter	Description
LAR	Leaf Area.
PAR	PAR. This is typically ghosted out and should only be available when using our “Manual” style cuvettes and PAR was manually entered and not measured.
RB	Boundary Layer resistance of Cuvette. The value depends upon the type of cuvette supplied. The factory default value can be found on the cuvette handle or packing list supplied with the system.
TRANS	The transmission characteristics of the cuvette window. This value is different for each type of cuvette and is automatically set by the CIRAS-2 by default when selected in the Parameters Editor dialog. This value should not require changing.
RSFract	The ratio of stomata on the upper and lower surface of the leaf. Please note that the ratio is in terms of the upper leaf. During recalculation, the value should be entered as a percentage of 1 (i.e. 50% Stomata on top surface should be entered as 0.50).
Flow	Flow rate of the integral air supply. If the CIRAS-2 air supply is used, there should be no need to change this value. If an external air supply is used, this value may require changing.
Leaf Temp.	Leaf Temperature. If set to Energy Balance, leaf temperature is automatically recalculated. If set to Use Existing, recalculation does not occur.

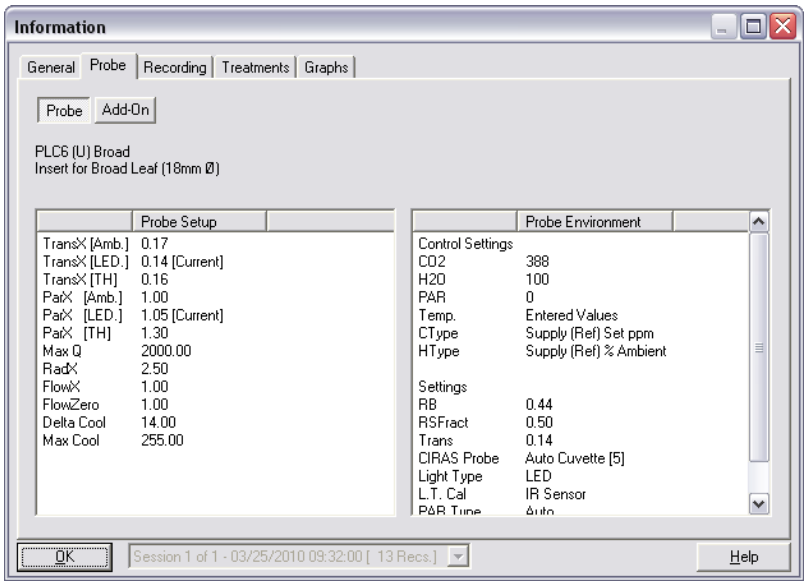
Click On:	To:
Session	Review information relating to the CIRAS-2, data, graphics, etc. .
Treatment	Review any information/comments associated with a particular record (treatment).

Information

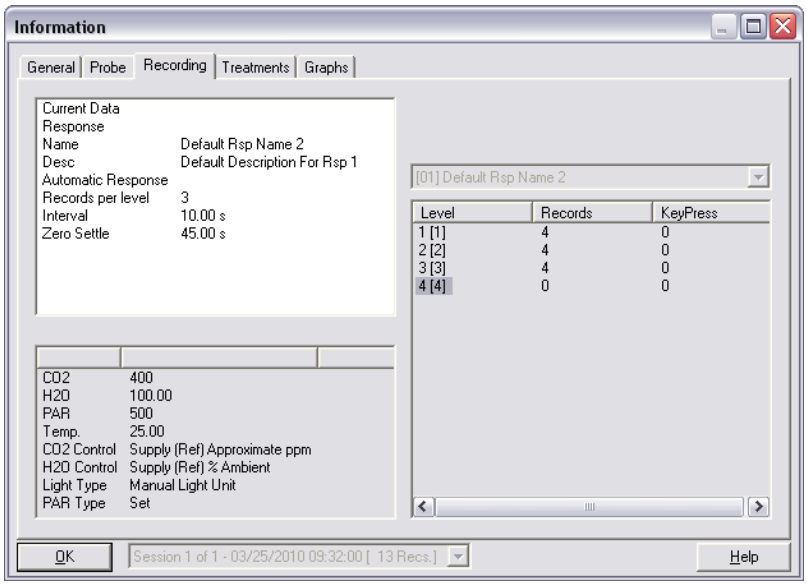
There are 5 tabs (windows) associated with the Information window. To get to this window, click on the Session button when the **CIRAS-2 Photosynthesis Recalculation** dialog is open:

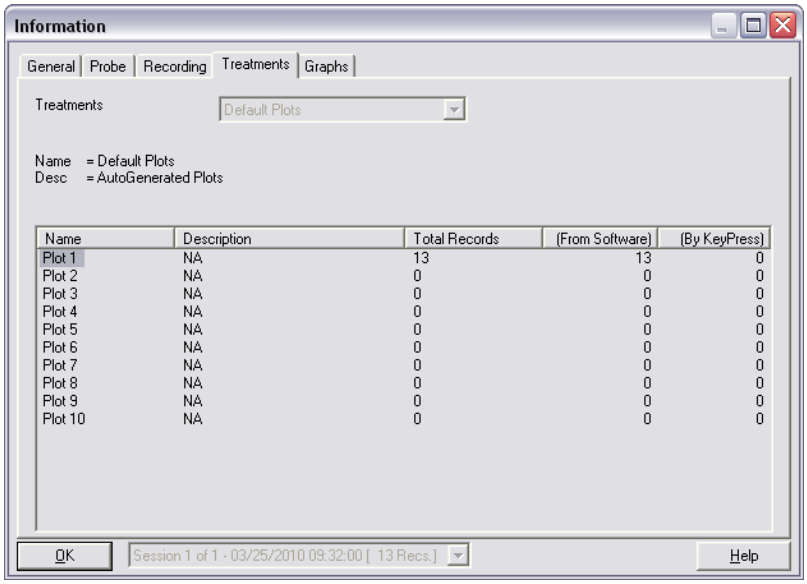


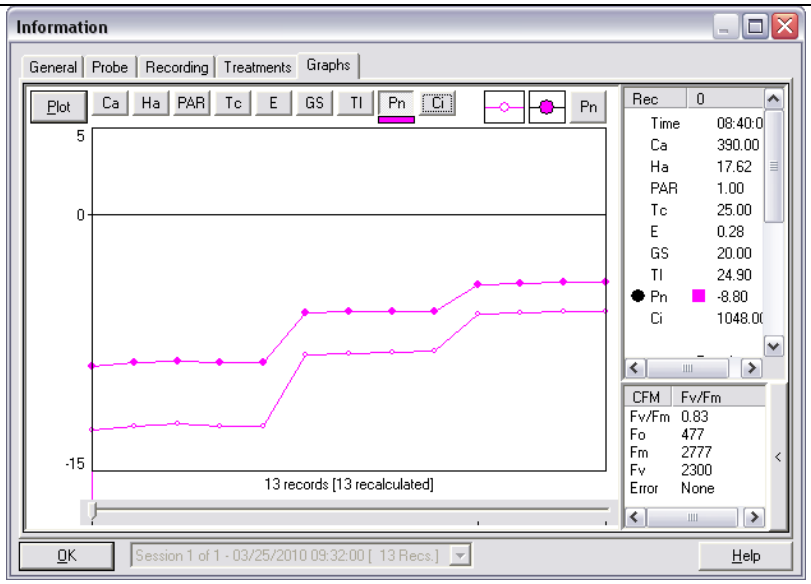
Click on:	To:
General	View session date and time, file name and information related to the CIRAS-2 and user information. The General tab will open first when Session is selected as described above.
Probe	To view information related to A/D reads associated with the type of probe (i.e. leaf cuvette) used, system setup, etc.



Recording	View the recording type selected (i.e. keypress, timed, response curve) and associated settings.
Treatments	To view treatment descriptions as they pertain to different plots/records.
Graphs	View measured/stored data graphically.







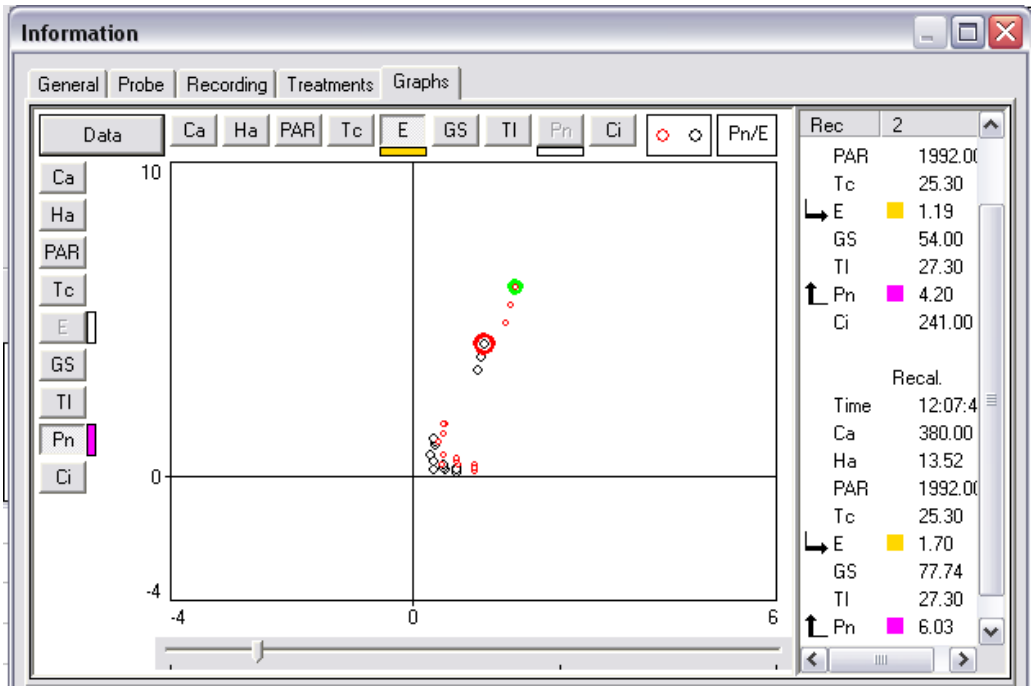
Up to 8 measured/calculated parameters can be displayed at one time. These parameters include:

Ca Analysis CO_2 (ppm)
Ha Analysis H_2O (ppm)
PAR PAR (µmol m^{-2} s^{-1})
Tc Chamber Temperature (°C)
E Evaporation (Transpiration) Rate (mmol m^2 s^{-1})
GS Stomatal Conductance (mmol m^2 s^{-1})
TI Leaf Temperature (°C)
Pn Photosynthesis (Assimilation) Rate (µmol m^2 s^{-1})
Ci Internal CO_2 Concentration (ppm)

To view one of the above parameters, click on the desired button. A colored bar will be displayed below your selection identifying the parameter. Each parameter can be toggled on or off by clicking it. Data ranges (Maximum and Miniumum) are calculated by the software.

To switch between Data and Plot view type, click on the Plot or Data button in the upper left hand corner. In Data View, the X-Axis is based on the number of records encountered in a session, not on time. If exporting data, the legend for the “current” value is displayed as a solid dot. The recalculated values (if any), are displayed as 'hollow' dots.

In Plot view, only two values can be plotted against each other. If recalculated data is present, the recalculated values are drawn as red circles with green halos. Original data are drawn as black circles with red halos. The field to the right in Plot and Data views lists the original and recalculated variables, and their X- and Y-axis positions are indicated by arrow symbols.



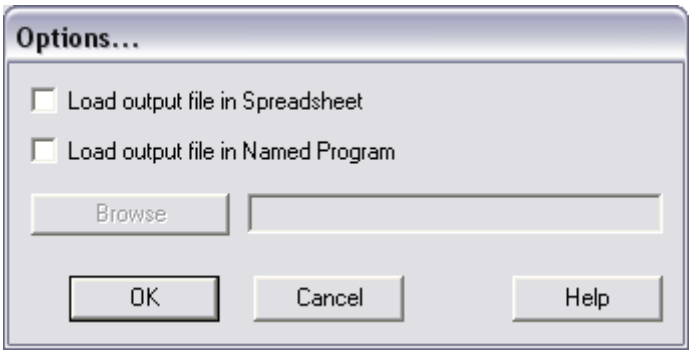
Options

With the CIRAS-2 Photosynthesis Recalculation window open, select **Options.** Options files contain stored options for Recalculation and Export of Data Files. Each Options file is divided into 3 sections for the storage of:

Recalculation Options	Stores the parameters to recalculate and how the file is processed.
Export Options	Stores the fields and what additional data to export. Export options for data, dumped data and closed system data are treated individually.
Shared Options	Stores fields from the data file are displayed in the 'preview' graph. Which, if any, actions to perform after the Export/Recalculation are performed (i.e. Load the output file into a spreadsheet program).

Options files are stored by default in the user's directory. Unlike settings files etc, only one set of options are maintained per file.

Select **Options - Edit Options** to select the desired method of having recalculated or exported data opened by another program (i.e. Excel, Notepad, etc.).



Load output File in Spreadsheet

This option relies on file types being associated with certain programs. Check 'Load output file in Spreadsheet' if you want all processed data files to automatically open in the appropriate program based on the file extension. For example, if files with .dat extensions are associated with Excel, the file will automatically be opened in Excel.

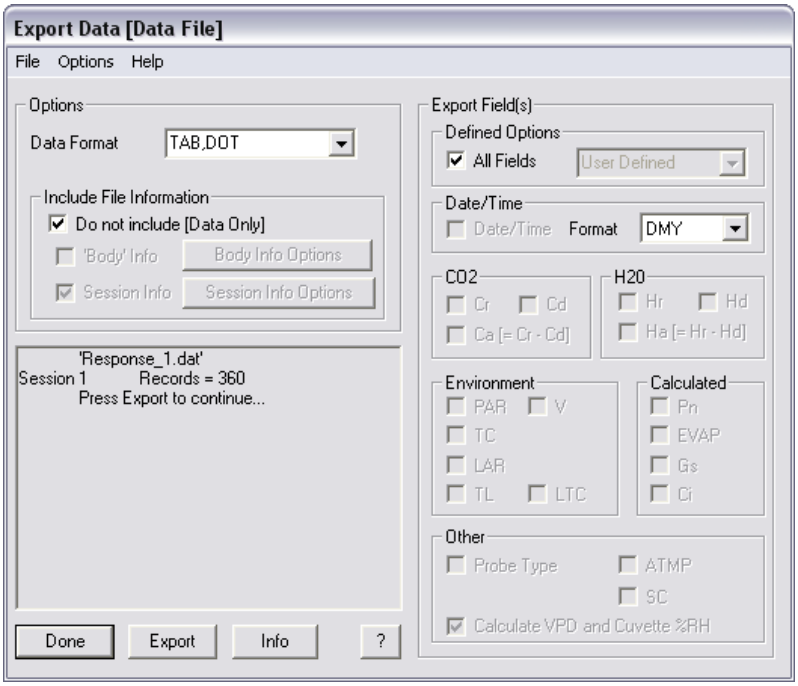
Load output File in Named Program

Use the 'Browse' button to locate the SpreadSheet (or other) program that you would like to use. Tick off "Load output file in Named Program" to have all processed data files open up automatically in the program selected.

Please note: Please contact PP Systems (see Contact Information on page 11) if the above options do not work properly on the UI.

Export Data

This feature allows you to export all stored CIRAS-2 Data Files into a user specified and simplified format for use in spreadsheet applications such as Microsoft Excel. Click on **File - Export Data** and select the data file (.dat extension) that you would like to export, then select Open. The Export Data (Data File) Window appears as below:



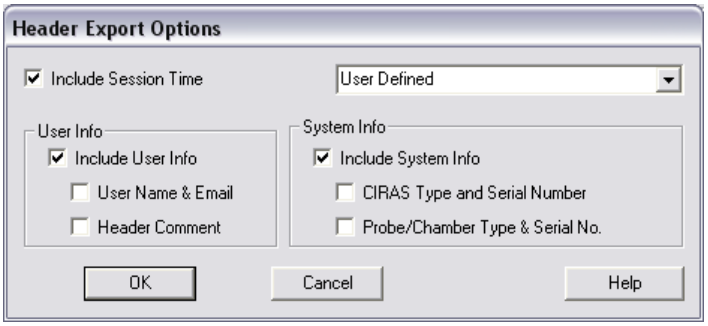
Options

Data can be output in 3 formats:

TAB,DOT	Data is exported using tab deliminator and '.' as decimal separator.
CSV,DOT	Data is exported using comma deliminator and '.' as decimal separator.
TAB,COMM A	Data is exported using tab deliminator and ',' as decimal separator.

The deliminator selected defines the extension of the files selected for export (i.e. .tab or .csv).

Include File Information

Do not include (Data Only)	If ticked, only the data will be exported per the users selection without any “Body” or “Session” information.
‘Body’ Info	 Exported data can include treatment information along with any comments associated with records. You can also group records by treatment.
Session Info	 Exported data can include Session Time, user information (email address) and system information (i.e. CIRAS type, serial number of instrument, probe type, etc.

Export Fields

There are numerous fields of data that can be output to suit your needs.

Check:	**To:**
All Fields	Export all fields (i.e. measured and calculated data). In addition, you can select the Date/Time format that you prefer. You have the option of selecting: **None : Day, month, year and time are output as separate fields** **DMY**: Day/Month/Year is output in a single field. **MDY**: Month/Day/Year is output in a single field.
Pn/Ci only	Export Photosynthesis (Pn) and Ci only.
Ca and Ha	Export analysis CO_2 (Ca) and H_2O (Ha).
All CO2 and H2O	Export all CO_2 and H_2O related data.
Calculated	Export all calculated parameters (Pn, Evap, Gs, Ci)

Environment	Export all environmental parameters (PAR, TC, LAR, TL, V,). Please note:, LTC is not included with this selection.
Drop Flag Fields	Export user selected fields. If selected, tick off the fields that you would like to export.

Important: To recover VPD and RH data recorded during a live session you must first deselect 'All Fields' and select 'Calculate VPD and Cuvette %RH'. Then check 'All Fields' again.

After selecting the appropriate fields to be exported, click on Export . You will be prompted to save the file (.tab extension) in the format selected under Options (above). After naming the file, click Save. Exportable file types are CIRAS-2 Recorded Data (.dat), CIRAS-2 Internal Records (.dmp) and Closed System Measurements (.csc). Recalculation Data (.mod) is treated as CIRAS-2 Recorded Data.

Options

Refer to the section above on page 82.

Transferring Data From CIRAS-2 to Your Computer

On the Integral PC (Pencentra), all data files are stored in the following directory:

\Storage Card \ Users \ UserName \DataFiles

On the PP Systems' User Interface (UI), all data files are stored in the following directory:

\FlashFX Disk\Users\UserName \DataFiles

On an External PC, all data files are stored in following directory:

C:\Program Files\Ciras2 \Users\UserName\DataFiles

Each user folder contains the following :

Data Files Folder	Photosynthesis and CFM Data (.dat) Soil Respiration and Whole Canopy Data (.csc) Diagnostics (.txt) Extended Diagnostics (.dia)
Settings Folder	Settings Files (.set) Parameters (.prm) Response Curve Definitions (.rsp) Treatments (.trt) CFM Options (.cfm)

To view stored data files in a spreadsheet program such as Excel, you must first transfer stored records from the integral PC (Pencentra) or User Interface (UI) over to your desktop PC. To do this, you can use ActiveSync, the RS232 Null Modem, or by moving them from the UI to a Flash Card via the PCMCIA drive. Records can be viewed with Notepad on the UI if required.

PCMCIA Card

This is the simplest method of data transfer, requiring no cables or third-party software.

1. Remove the plastic cover from the PCMCIA card slot and insert your CompactFlash Adaptor and card.
2. Open Windows Explorer on your UI and navigate to the FlashFX Disk folder to identify the files to be transferred to the flash card. To access Window Explorer you can either i) double-click the My Computer icon on the Desktop or ii) from the Windows Taskbar click Start – Programs – Windows Explorer. Normally, .dat files are stored in the \FlashFX Disk\Users\User Name \DataFiles subdirectory.
3. Copy and paste the files to the Storage Card (flash card) folder icon, then move the flash card to your desktop PC.

RS232 (Null Modem) Connection

Establishing An ActiveSync Connection For Data Transfer from UI To PC

1. Turn off the CIRAS-2 (with no Null Modem cable connected).

2. On your PC, start ActiveSync (**Must be ActiveSync version 3.7.1 or later**). At this stage, it should show that you are not connected. With the ActiveSync window opened, go to File, Connection Settings and select the COM port (normally COM1) to be used on your PC. Click Ok.
3. Turn the CIRAS-2 on and wait for it to get to the opening Log-in screen. Next, exit from the CIRAS-2 Remote Control Software by clicking on Quit.
4. Connect Null Modem cable between the 9 pin RS232 plug on the User Interface and the COM port on your PC Computer.
5. From the Windows CE desktop on the UI, click on Start icon on lower left hand corner of the display, Programs, Communication, ActiveSync.

At this stage, you should have a successful connection (partnership) between the User Interface and your desktop PC. If prompted to "Setup A New Partnership", click No and then Next. The Microsoft ActiveSync window should show that you are connected as a guest. If you do not connect, perform the following checks:

On your desktop PC

- Open ActiveSync and make sure that you are running version 3.7.1 or later.
- Click on File, Connection Settings and make sure that "Allow serial cable or infrared connection to this COM port" is ticked off.

On the User Interface

- Click on the Start icon on the lower left hand corner of the display, Settings and Control Panel. Next, double-click on "PC Connection" and ensure the following:

  A. Make sure that "Enable direct connections to the desktop computer" is ticked off.
  B. Make sure that "19200@com1" is shown under "When enabled, connect to the desktop computer using:" If it is not, click on Change Connection… and change it to 19200@com1.

To transfer stored files from the UI to PC, open Windows Explorer on your PC and navigate to Mobile Device\FlashFX Disk folder and identify the files to be transferred to the desktop PC. Normally, the files are stored in the \FlashFX Disk\Users\User Name \DataFiles subdirectory. Simply copy and paste the files to the desired folder on your desktop PC.

ActiveSync® Communications Software

Microsoft ActiveSync is a program to allow communication between the User Interface (or integral PC-Pencentra) and your PC. It is commonly used to transfer stored CIRAS-2 data files from the UI (or integral PC-Pencentra) to your PC. Making a serial connection with ActiveSync is fairly straightforward. **This program must be loaded on your PC before transfer of stored CIRAS-2 data files can take place.**

You must visit the Microsoft website to obtain the latest version of ActiveSync. As of the printing of this manual, the website address is located at:

http://www.microsoft.com/windowsmobile/downloads/default.mspx

Microsoft regularly changes the layout of their website, so it may be necessary to search for the current location.

It is important to note that for proper operation, you must ensure that you are running ActiveSync version 3.7.1 or later.

For Customers That Are Using The Integral PC (Pencentra)

The Integral PC (Pencentra) is supplied with ActiveSync® software for communication between PCs. It is preinstalled on the Integral PC (Pencentra). The user must install it on their own PC. There is an early version supplied on CD ROM (Microsoft Windows CE Services) with the Integral PC (Pencentra), but we recommend that the latest version is downloaded from the Microsoft® website described above.

Carefully follow the installation instructions. Try and ensure there will be no conflict over the COM port chosen because ActiveSync® will retain control of the port by default. If the intention is to also run the Remote Control Software on the external PC, then use a different COM port if possible. If there is conflict, it must be disabled when not in use (File, Connection Settings, Dis-allow serial cable connection to this COM port).

An RS232 "**Null Modem**" Data Communications cable has been provided with all systems supplied with the integral PC (Pencentra). The integral PC (Pencentra) must be disconnected from the CIRAS-2 analyzer and connected to the other PC instead. Connect to the designated COM port on the other PC.

The two PCs will automatically establish communication. Once connected, you can easily transfer files from the integral PC- Pencentra to your PC. To transfer stored files, open Windows Explorer on your PC and navigate to Mobile Device on your PC and locate the subdirectory where the data files are stored on the integral PC-Pencentra. Simply copy and paste the files to the desired folder on your PC.

If there are problems, check the following:

- Check the COM port used.
- Check all the plugs are correctly inserted.
- Check there is no other task controlling the PC COM port

For Customers That Are Using Our User Interface (UI)

For all CIRAS-2 customers that have our UI, we've supplied both an RS232 "**Null Modem**" Data Communications cable and USB cable. This way, we cover both older PC's that have older serial connections and later PC's that feature USB ports. By default, the UI expects that the ActiveSync communications link to be the USB port. Therefore, if using the USB cable, you **MUST** ensure that you have loaded the appropriate USB drivers to your PC. These drivers allow you to connect the USB client port of the UI to your PC to create and ActiveSync connection.

USB Connection

Installation of USB Drivers On Your PC

1. Ensure that both the CIRAS-2 and PC are both on.
2. Next, connect the USB client port of your UI (see the User Interface (UI) Operator's Manual if required to locate the USB port) to your PC using the USB cable (Type A Male to Type B Male). Your PC will likely report the following messages:

Found new hardware ….
USB device …

It should then run the Found New Hardware Wizard (It may take a minute or so for the Wizard to come up on your PC).

6. Insert the CD supplied by PP Systems which contains the required USB driver files.
7. Tick off “Install the software automatically (Recommended)”.
8. Click Next.
9. If not detected automatically, click on “Browse” and locate the WCEUSBSH.SYS file on the CD ROM supplied by PP Systems.
10. Click on WCEUSBSH.SYS and Open then Ok. A “Hardware Installation” warning may appear. If so, click “Continue Anyway”.
11. If successful, you should get a message indicating complete installation.
12. Click Finish.

The USB drivers should now be installed properly on your PC. Once completed, you should not have to step through this process again unless using a different PC with your CIRAS-2.

Establishing An ActiveSync Connection For Data Transfer from UI To PC

1. Turn off the CIRAS-2 and disconnect the USB cable from the UI.
2. On your PC, start ActiveSync (**Must be ActiveSync version 3.7.1 or later**). At this stage, it should show that you are not connected.
3. Turn the CIRAS-2 on and wait for it to get to the opening screen.
4. Connect the USB cable between your UI and your PC.
5. You should now be connected. When asked to “Set up a new partnership”, click No and then Next.

To transfer stored files from the UI to PC, open Windows Explorer and navigate to Mobile Device\FlashFX Disk folder and identify the files to be transferred to the PC. Simply copy and paste the files to the desired folder on your PC.

If there are problems, check the following:

- Check the COM port used.
- Check all the plugs are correctly inserted.
- Check there is no other task controlling the PC COM port

Shutting Down the CIRAS-2 System

The following order of events is important to ensure an orderly shutdown once measurements are completed:

- Exit the CIRAS-2 Remote Control Program by selecting **File – Exit** in the CIRAS-2 menu bar.
- Power off CIRAS-2 by pressing the red On/Off switch.
- Disconnect the cuvette from the external 12V DC power supply (if connected).
- Clean the cuvette head and leave open to avoid compressing the gaskets when not in use.
- Remove the leaf cuvette electrical connector (PLC) from CIRAS-2 by pulling back on the sleeve around the front of the connector, **not the body of the connector or the wire.**
- Remove the reference (R) and analysis (A) gas connectors from CIRAS-2 by pulling back on the sleeve around the front of the connector, **not the body of the connector or tubing.**
- Remove the entire CO_2 regulator assembly from inside the CIRAS-2 console and keep separate if CIRAS-2 is to be stored in a confined space or container, such as the carrying bag. CO_2 from the cartridge is constantly diffusing, whether or not the regulator assembly is installed in CIRAS-2. If left in a confined space, CO_2 from the cartridge will build up in the absorber columns, causing premature exhaustion of the chemicals and slower instrument warm-up as excess CO_2 is flushed out the next day.
- If necessary remove both 12V NiMH batteries and place them on their own chargers (supplied by PP Systems).

Measuring Chlorophyll Fluorescence with the CFM

Getting Started with the CFM

It is assumed that you are already familiar with operation of the CIRAS-2 Portable Photosynthesis System. If not, we strongly suggest that you familiarize yourself with the basic operation of the CIRAS-2 system and refer you to the CIRAS-2 Portable Photosynthesis System Tutorial and earlier sections of this Operator's Manual.

The Chlorophyll Fluorescence Module (referred to as CFM throughout this manual) consists of two main pieces of hardware:

1. **Light Unit**. The chlorophyll fluorescence detector and light sources are built directly into the PP Systems' LED light unit.

2. **Signal conditioning board**. This is fitted beneath the PP Systems' User Interface (U/I) in a rugged, aluminium enclosure.

Warning: The light unit and signal conditioning board are a matched pair and are not interchangeable.

At this stage, it may be advantageous to visit the back of this manual to review the information relating to chlorophyll fluorescence measurements. To learn more about chlorophyll fluorescence in general (theory, measurement techniques, etc.), refer to Brief Introduction to Chlorophyll Fluorescence on page 119. For a full list and description of the various chlorophyll fluorescence parameters (measured and calculated), go to Glossary of Fluorescence Terms on page 125.

Firmware & Software Requirements

To operate the CFM with the CIRAS-2, the following CIRAS-2 firmware and software must be in place:

Firmware (EPROM)	Version 1.77 or greater	To check this with your system, run the CIRAS-2 software and click on **View - Quick Info**. This is shown under "Hardware Information" at the top of the window next to the serial number of your instrument.
Software	Version 1.06 or greater	To check this with your system, run the CIRAS-2 software and click on **Help - About**.

New systems will be supplied with the required firmware and software. For CFM upgrades, PP Systems will supply the proper hardware, firmware and software along with full instructions on how to upgrade your system.

Software Installation

All the software for both the integral PC (User Interface) and external PC are supplied on the CD ROM supplied with your system. Make sure that you keep this CD in a safe place.

For New Customers:

The software will already be installed on the User Interface. A back-up copy of the software is also available on the PCMCIA card also supplied. For remote operation from a PC, you must load the appropriate software to your PC.

For Existing Customers (Upgrades):
You must first uninstall the software on both the User Interface and the external PC (Add/Remove Programs) before loading the new software. After successful removal of the previous version software, proceed to install the new software per the instructions supplied.

Power Requirements

The entire system is powered by the CIRAS-2 internal 12V NiMH batteries through the connection to the leaf cuvette. The peak current is several amps which is drawn only for short periods (i.e light saturation pulses). Normally, the CFM draws about 100 mA which will have a minimal impact on the life of the internal batteries. If desired, the leaf cuvette can be connected to an external battery (or 12V, regulated power supply in the lab) using the external power supply cable (red & black leads) supplied with the cuvette as standard.

Warning. For connection to an external battery or power supply, make sure that you properly connect the red lead to the Positive (+) terminal and the black lead to Negative (-) terminal.

Leaf Cuvette Window

The leaf cuvette is normally supplied with a window (CALFLEX) that reflects most of the incident IR radiation to minimize the heat load on the leaf. However, this window will not transmit at the fluorescence wavelength. Therefore, each CFM is supplied with a plain glass window which must be fitted to the cuvette in place of the CALFLEX window. If you have received a complete new CIRAS system including the CFM, the plain glass will already be fitted to the leaf cuvette. **If you have upgraded to the CFM, you must make sure that you replace the CALFLEX window with the plain glass window if you plan on making simultaneous gas exchange and fluorescence measurements.**

Once the glass window is fitted, it is assumed that the LED light unit will always be used with the leaf cuvette. This is not a problem as the LED light unit does not output any near IR. However, if a different light unit (i.e. quartz halogen) is used or gas exchange measurements are made under sunlight conditions, you must remove the glass window and refit the CALFLEX window to the leaf cuvette.

The table in Appendix 1., CIRAS-2 Settings for Leaf Cuvettes and Probes, illustrates the TRANS factors used by CIRAS-2 based on the light unit and cuvette window used for converting PAR (µmol m^{-2} s^{-1}) to energy (Wm^{-2}) absorbed by the leaf. These values are set by default depending on hardware settings (i.e. leaf cuvette type, light unit, etc.) set up in the **Parameters Editor Dialog**. The default TRANS value for the LED light unit (with CFM) is 0.14 for both the CALFLEX and plain glass window.

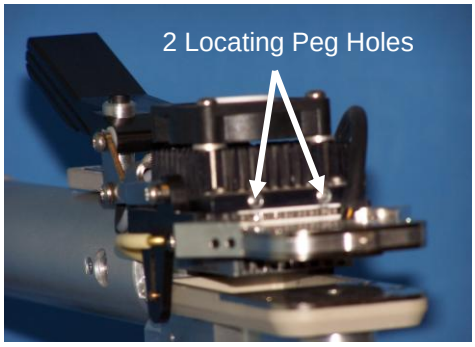
Note, if using our other style light units (i.e. quartz halogen) or sun/sky, please refer to the CIRAS-2 Operation Manual for further information on TRANS factors.

Replacing Cuvette Window

Place the leaf cuvette down on a soft flat surface. Using a No. 1 Posi screwdriver, carefully remove the 4 screws on the window plate. Remove the plate and store it carefully for later use. Next, put a slight smear of silicone grease around the edge of the replacement window plate, secure it in place and secure it to the leaf cuvette by uniformly tightening the 4 retaining screws.

Connecting the CFM to CIRAS-2

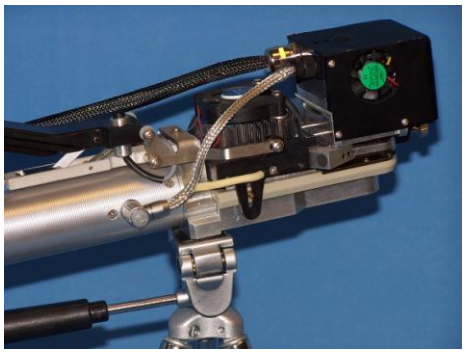
All electrical and pneumatic connections must be made before powering up the CIRAS-2. **Please pay special attention when connecting all electrical connections to avoid problem associated with damaged connectors (which are quite expensive).**



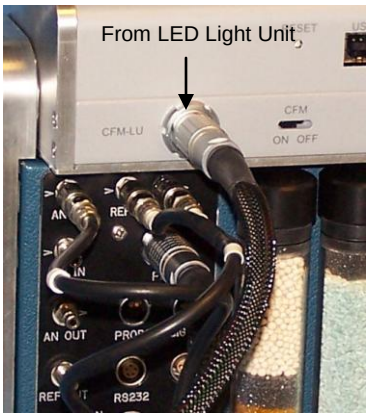
(A)



(B)



(C)



(D)

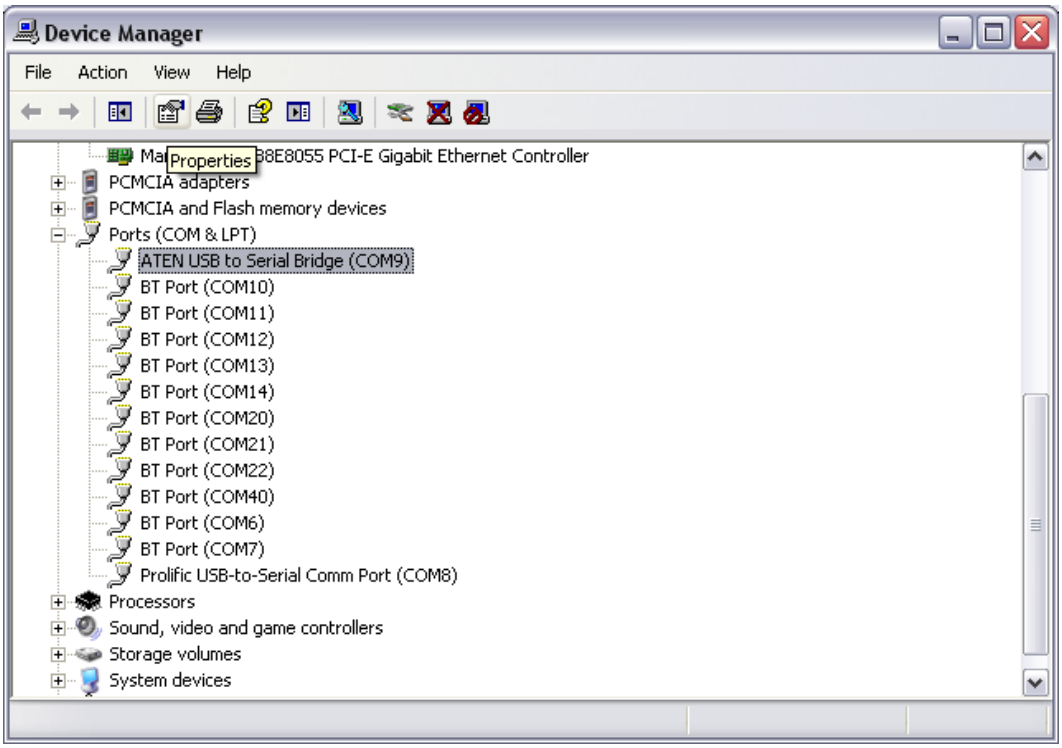
1. Ensure that the plain glass window is fitted to the leaf cuvette as described earlier.
2. Fit the LED light unit to the leaf cuvette head. Slide the 2 locating pegs into the 2 holes on the lower part of the black anodized heat sink **(A)**. Gently tighten the screw connector on the end of the light unit to the small screw protruding from the end of the leaf cuvette head until snug **(B)**.
3. Connect the shorter grey LED electrical cable on the light unit into the 5 pin socket on the side of the cuvette handle **(C).**
4. Connect the longer black cable from the LED light unit (14 pin Lemo connector) to the connector labeled “CFM LU” on the back of the User Interface **(D).** For neatness, this cable can be twisted gently around the main leaf cuvette electrical cable. **DO NOT PLUG THIS CABLE INTO THE “PLC” CONNECTOR ON THE CIRAS-2.**
5. Connect leaf cuvette electrical cable to the CIRAS-2 connector labeled “PLC” **(D).**
6. Connect the leaf cuvette reference gas connectors to the “REF IN” and “AIR OUT” and the analysis gas connector ot the “AN IN” connector on CIRAS-2 **(D).**

7. Close leaf cuvette (for later LED and CFM tuning).

Operation from External PC

To operate the CIRAS-2 system from an external PC, it must have 2 available COM ports. On modern computers this you will typically use two USB ports. Older computers may use at least one standard 9 pin serial port. In order to use the USB port, a "USB to Serial Adapter" is required (supplied by PP Systems) and the associated software must be loaded on your PC. Follow the instructions provided with the "USB to Serial Adapter" when loading the software.

To determine which COM ports are available on your PC, go to **Start - Control Panel – System - Hardware - Device Manager** and open "Ports (COM & LPT)".



You will need to remember which COM ports are being used when logging in and setting up CIRAS-2 with the CFM under **Preferences**.

After all electrical and pneumatic connections are made as described in the section **Connecting the CFM to CIRAS-2** and the COM ports have been determined, perform the following to operate the system from an external PC:

1. Connect the CIRAS-2 PC Interface cable (9 pin D - 5 pin Lemo) between the PC serial port (9 Pin) on the back of the PC and the connector labeled "RS232" on the CIRAS-2.
2. Connect the gray 9 pin – 9 pin D cable with USB to Serial Adapter from the 9 pin socket on the back of the UI labeled "Ext Control Port" to the USB port on your PC.
3. Switch the "CFM" slide switch to On.
4. Switch the "CFM Control" slide switch to Ext PC.

Stand-Alone Operation (User Interface)

After all electrical and pneumatic connections are made as described in the section **Connecting the CFM to CIRAS-2**, perform the following to operate the system from the PP Systems' User Interface:

1. Switch the "CFM" slide switch to On.
2. Switch the "CFM Control" slide switch to CIRAS.

The correct COM ports will be automatically selected by default.

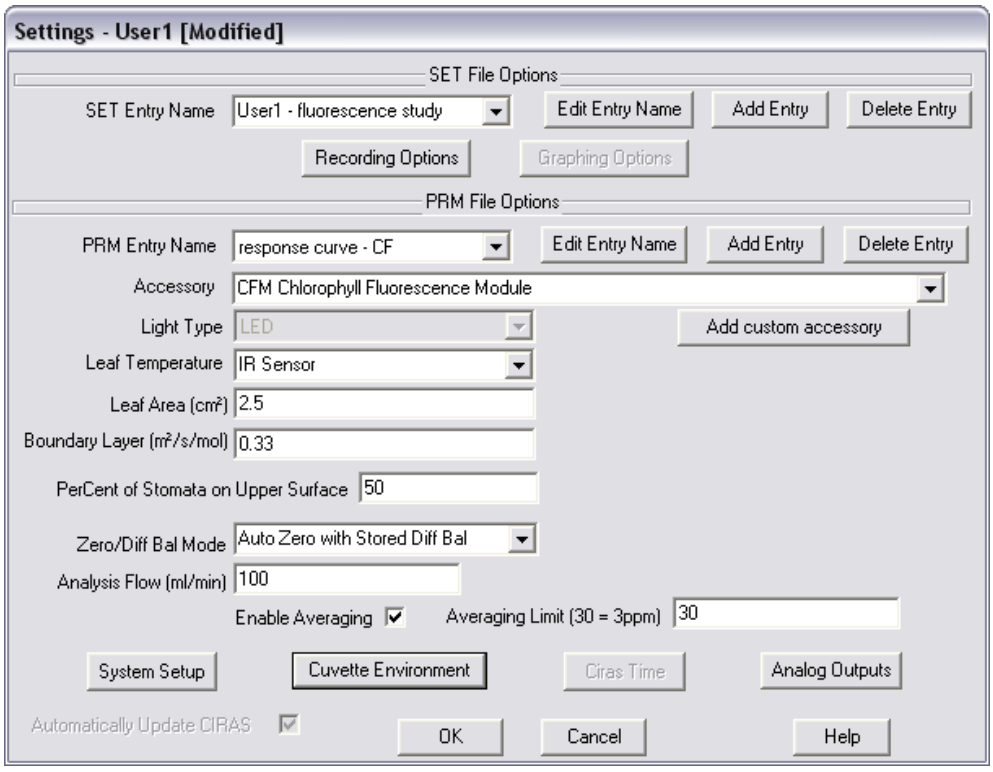
CFM Operation

You are now ready to begin operation of the CFM. It is assumed that you are already familiar with the operation of CIRAS-2 as a standard portable photosynthesis system. If not, we strongly urge that you review the **CIRAS-2 Tutorial** before proceeding.

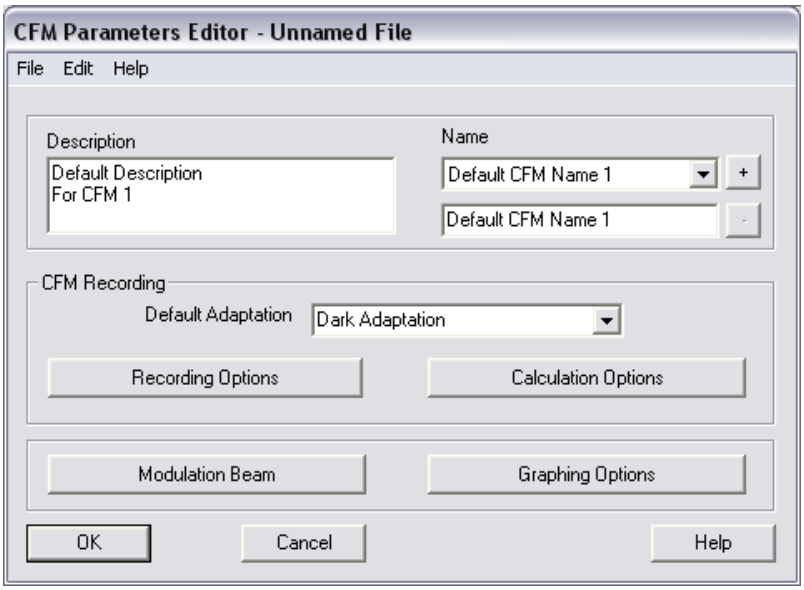
The following information should be the same regardless of operation from an external PC or the User Interface. Ensure that the leaf cuvette is closed in order for the system to perform “LED Tuning” and “CFM Tuning” on startup.

Power on CIRAS-2 by pressing the red On/Off switch. Execute the CIRAS-2 Remote Control Software on your PC. The software can be run by going to **Start** - **Program Files - CIRAS-2 Remote Control Software** and clicking the CIRAS-2 Remote Control Software icon.

In the CIRAS menu go to **Setup – Settings** to open the dialog and select ‘CFM Chlorophyll Fluorescence Module’ from the Accessory list.



After selecting CFM, the following **CFM Parameters Editor** will be displayed:



Here, you can set up the CFM recording options and associated settings to suit your requirements.

Default Adaptation

Dark Adaptation

For this selection, the sample must be fully dark adapted before the initial Fv/Fm calculation is performed. All calculation types may be performed, yielding all calculation results.

Far Red Adaptation

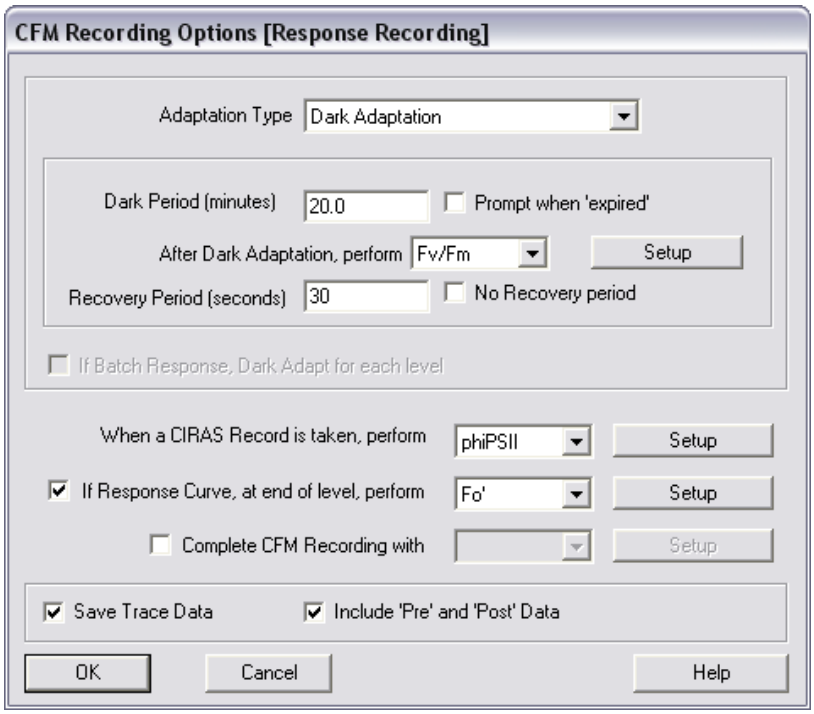
For this selection, the sample does not require dark adaptation. Instead, an Fo' is performed under Far Red Light to determine only phiPSII calculations. However qNP and NPQ are 'invalid' under these circumstances.

No Adaptation

For this selection, only steady state fluorescence calculations may be performed (Fo in the Dark / Fs in the Light).

CFM Recording Options

This allows the user to set up the recording options based on the Default Adaptation selected (i.e. Dark Adaptation, Far Red Adaptation or No Adaptation).



Dark Period (Minutes)

The amount of time required to dark adapt the leaf sample prior to performing dark adapted fluorescence measurement (Fv/FM). The default period is set to 30 minutes.

Prompt when ‘expired’

Select this option to display a prompt indicating that the Adaptation Period is over. If you decide that the sample should 'adapt' further, no record will be taken until the 'End DA' or 'End LA' button in the Recording window is clicked. In Keypress and Manual Responses, this prompt is displayed regardless. The Adaptation period may be ended before the required time by clicking on the 'End DA' or 'End LA' button at any time.

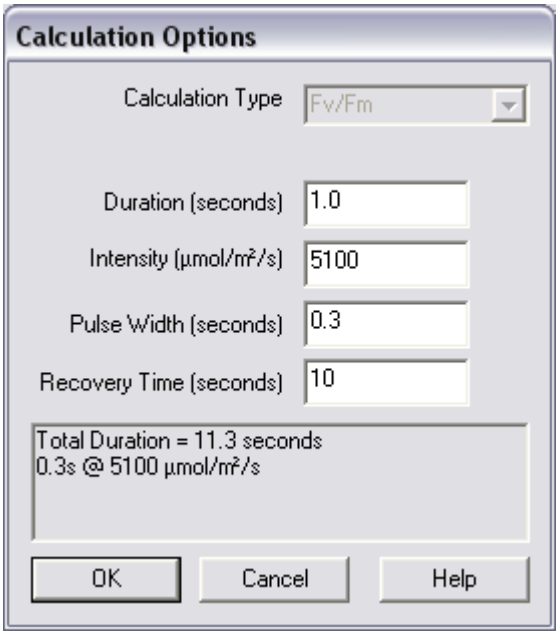
After Dark Adaptation, perform

Depending on the selection under “Default Adaptation”, the user can determine the fluorescence parameter to be calculated after adaptation.

Default Adaptation	Calculations Performed
Dark Adaptation	Fv/Fm Fo
Far Red Adaptation	Fo’
No Adaptation	None

CFM Setup

Click on **Setup** to set the timing and light sources for the adaptation type selected. The **Calculation Options** dialog will be displayed allowing you to set the timing and light control for the required fluorescence calculation.



Dark Adaptation

Parameter	Setup Options
Fv/Fm	Duration (seconds) Intensity (µmol m^{-2} s^{-1}) Pulse Width (seconds) Recovery Time (seconds)
Fo	Duration (seconds):

Far Red Adaptation

Parameter	Setup Options
Fo'	Duration (seconds)

Recovery Period (seconds)

Time allowed for leaf to recover – dependent on Adaptation Type.

If Batch Response, Dark Adapt for each level

If performing a "Batch Response", you have the option to check this off if you want to dark adapt the leaf sample for each batch response level (if Dark Adaptation is selected) or light adapt the leaf sample for each level (if Far Red Adaptation). This is not available if 'No Adaptation' is selected.

When a CIRAS Record is taken, perform

For all Adaptation Types, when the CIRAS record is taken, you have the option to perform either an Fs or a phiPSII at that point in time. If this is desired, click on **Setup** for the **Calculation Options** dialog.

If Response Curve, at end of level, perform

Actions taken before CIRAS changes control settings to from one Response Curve level to the next.

Default Adaptation	Calculations Performed
Dark Adaptation	phiPSR Fo’
Far Red Adaptation	None
No Adaptation	None

Complete CFM Recording with

Actions taken before the Response Curve concludes. If ticked, the CFM can also perform a phiPSR at the end of measurement. If this is desired, click on **Setup** for the **Calculation Options** dialog.

Default Adaptation	Calculations Performed
Dark Adaptation	phiPSR Fo’
Far Red Adaptation	Fo’
No Adaptation	None

Save Trace Data

If selected, the actual fluorescence values and calculations will be saved to a file for later reconstruction of the calculations.

Include ‘Pre’ and ‘Post’ Data

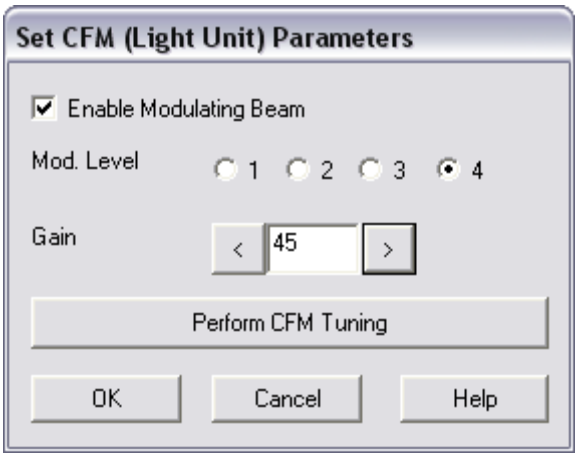
If selected, pre and post data will also be saved. Pre and post data refers to data 3 seconds before and 3 seconds after a measurement is recorded.

Calculation Options

Same as described above under **Setup**. The user can set the timing and light sources for the calculation required here.

Modulation Beam

Opens the **Set CFM (Light Unit) Parameters** dialog. The user has the ability to enable/disable the modulating beam, set modulating level, and adjust gain.



Enable Modulating Beam

For fluorescence measurements, “Enable Modulating Beam” must be selected for meaningful results. We recommend starting with the default Mod. Level of 3 and an initial gain of 50 to try and achieve an average fluorescence signal (Fa) in the range of 700-800 A/D counts.

Mod. Level

This controls the number of samples that are averaged to give the fluorescence signal. There is one modulation pulse per sample and it is 1.5 µs duration.

Modulation Level	Samples/Measurement
1	2
2	6
3	20
4	64

Important note. Prior to placing the leaf sample in the cuvette, allow the CIRAS to complete both the LED and CFM tuning process. When you are into the program and the main CIRAS display is shown, place your leaf sample in the cuvette and close it. You want to see a steady fluorescence signal similar to this before proceeding with fluorescence measurements:

If the signal is too high, lower the gain and/or lower the modulation level accordingly. If the signal is too low, increase the gain and/or increase the modulation level to 4 and adjust the gain until you achieve the desired fluorescence signal in the 700-800 range.

Gain

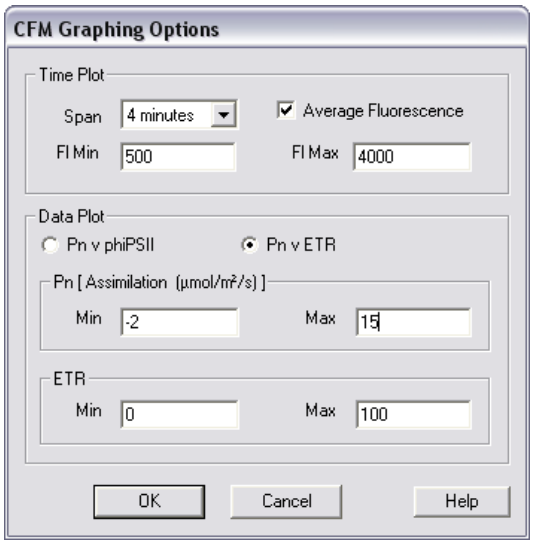
The gain controls the amplification of the sensor signal. The CFM uses a 12-bit A/D converter so that for maximum resolution, the gain should be adjusted so that the maximum fluorescence signal (Fm) is approaching the maximum possible A/D value (4096 units). The user must establish the optimum gain needed for the species measured. Once established, there should be no need to change the gain for individual leaves.

Perform CFM Tuning

Manually begin a CFM tuning – only needed if prompted by CIRAS-2 to do so.

CFM Graphing Options

Allows the user to customize the CFM display in CIRAS-2. This function is also available within the main CIRAS Graphing Preferences (**Settings – Graph – Preferences**).



CFM Time Plot

Average Fluorescence	If selected, the average fluorescence is plotted as a green line. The fluorescence signal is averaged every 1.6 seconds in order to correspond with photosynthesis measurements. Note, average fluorescence can also be plotted on the CIRAS display (one of four possible parameters that can be selected.
Span	Fluorescence data can be displayed over 2,3 and 4 minute time intervals. In all cases, the last 1 minute is discarded when the full span has been reached.
Fl Max	Maximum fluorescence setting (0-4000 bits) plotted against Span (minutes)
Fl Min	Minimum fluorescence setting (0-4000 bits) plotted against Span (minutes)

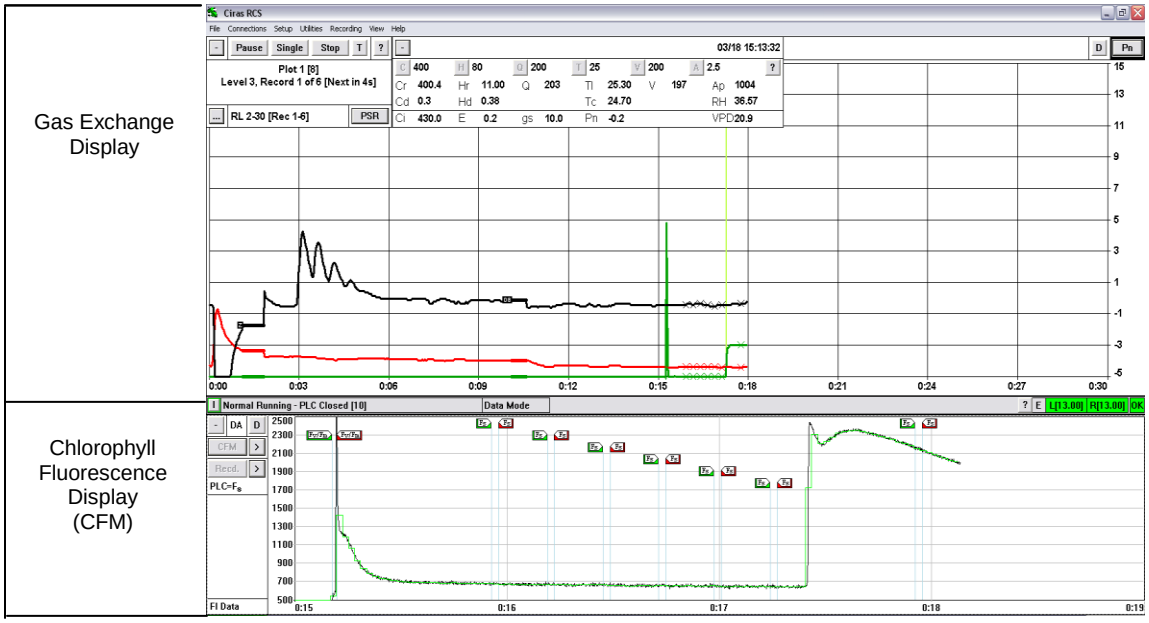
CFM Data Plot

Pn v phiPSII	When selected, a Pn (assimilation) record is taken and plotted against phiPSII. Pn range: 0-100 phiPSII range: 0.1 - 0.85.
Pn v ETR	When selected, a Pn (assimilation) record is taken and plotted against ETR. Pn range: 0-100 ETR range: 10 – 1000

CFM Graphing options are stored on a per definition basis within the .cfm settings files.

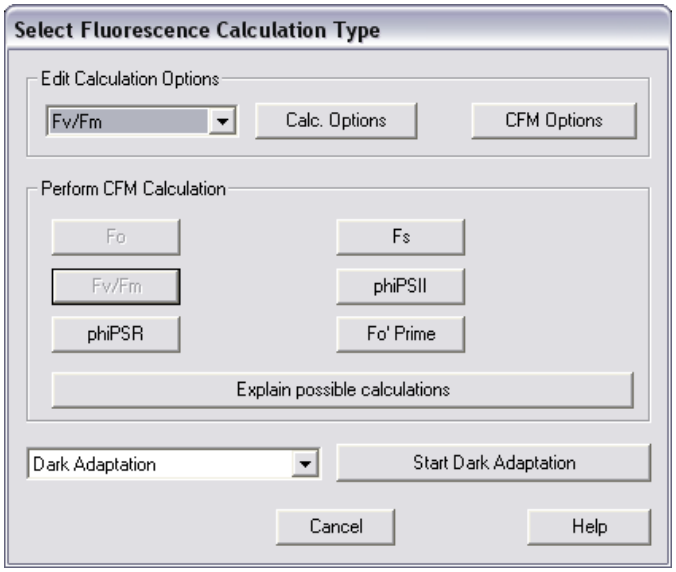
CIRAS-CFM Display

The top half of the CIRAS display shows leaf gas exchange (photosynthesis) measured and calculated data. The lower half of the display refers to the CFM (i.e. chlorophyll fluorescence measured and calculated data). They are not superimposed because of the differing time scales of the two processes (gas exchange and chlorophyll fluorescence), though it is possible to plot the average fluorescence with gas exchange parameters.



Recd.

Click on the **Recd** button to select calculation types, edit options and see the explanation of calculations.



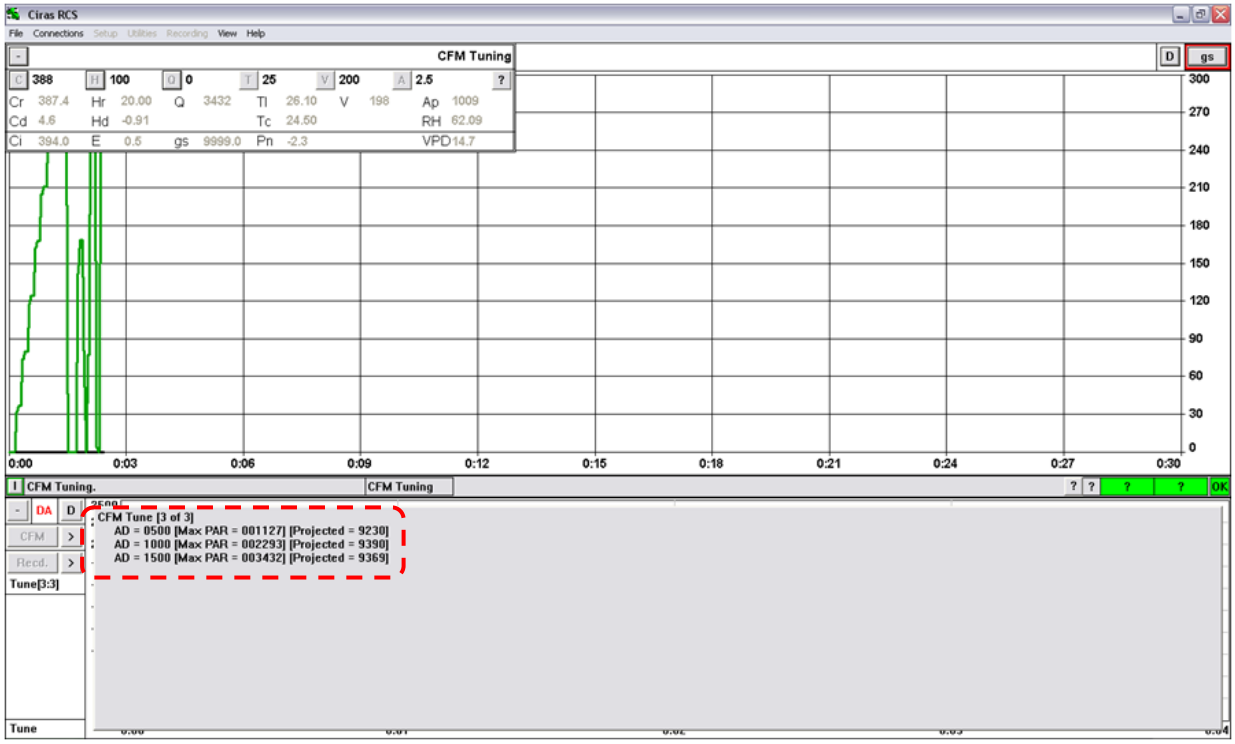
Edit Calculation Options	To select the parameter that you want calculated. The available calculations are: Fo, Fs, Fv/Fm, phiPSII, Fo', phiPSR
Calc. Options	See CFM Setup on page 104.
CFM Options	See Modulation Beam on page 105.
Explain possible calculations	Select this for an explanation on the selected calculation based on the adaptation type (i.e. Dark Adaptation, Far Red Adaptation, No Adaptation).
Start_Adaptation	The user can intiate the adaptation required at any time if this option is available.

CFM Display Buttons

Button	Result
-	Minimizes the Chlorophyll Fluorescence (CFM) display.
D	Data Plot. Fluorescence data can be shown over 2,3 and 4 minutes. In all cases, the last 1 minute is discarded when the full span has been reached. Toggles between data and time plot.
T	Time Plot. When a phiPS2 record has been taken, Pn (Assimilation) is plotted against phiPSII or ETR (Electron Transport Rate) depending on selection in upper right and corner of the chlorophyll fluorescence display. phiPSII range: 0.1 - 0.85. ETR range: 10 - 1000. Toggles between time and data plot.
CFM	Set CFM light parameters (i.e. modulating beam, gain, etc.) and to perform CFM tuning (if required). CFM tuning can be manually initiated at any time. Press **>** to view Fl (fluorescence level), Fa (fluorescence average), LED on/off, modulation beam level and gain setting. Press **<** to toggle the values off.
Recd.	Select fluorescence calculation type. Press **>** to view fluorescence calculation results. Press **<** to toggle the screen off. For more on this, see below.

CFM Tuning

When CIRAS recognizes that the CFM is connected, CFM tuning (see screen on the next page) will follow the LED tuning already described above (see LED Tuning on page 25). Three sets of projected A/D values will be displayed – these values should be >8000. The cuvette must be empty and the correct cuvette window installed (Plain Glass) for CFM Tuning to complete successfully.


Ciras RCS
File Connections Setup Utilities Recording View Help
CFM Tuning
C 388 H 100 O 0 T 25 V 200 A 2.5
Cr 387.4 Hr 20.00 Q 3432 Tl 26.10 V 198 Ap 1009
Cd 4.6 Hd -0.91 Tc 24.50 RH 62.09
Ci 394.0 E 0.5 gs 9999.0 Pn -2.3 VPD 14.7
D gs
300
270
240
210
180
150
120
90
60
30
0
0:00 0:03 0:06 0:09 0:12 0:15 0:18 0:21 0:24 0:27 0:30
CFM Tuning.
CFM Tuning
OK
DA D
CFM
Recd.
Tune[3:3]
CFM Tune [3 of 3]
AD = 0500 [Max PAR = 001127] [Projected = 9230]
AD = 1000 [Max PAR = 002293] [Projected = 9390]
AD = 1500 [Max PAR = 003432] [Projected = 9369]
Tune

Recording Chlorophyll Fluorescence

As described in the main CIRAS-2 Operation Manual, measurements are initiated by selecting **Recording - Begin**. There are 4 types of recording that can be achieved with CIRAS-2:

- Key Press
- Timed
- Response Curve (manual or automatic)
- Batch Response Curve

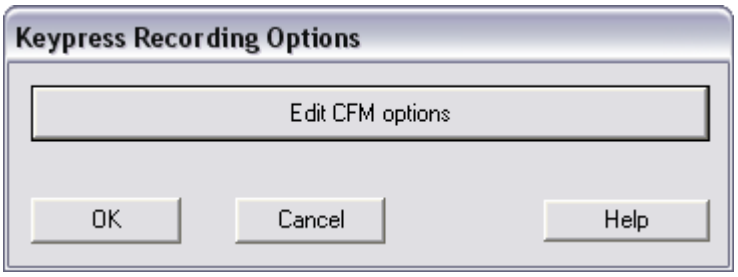
CIRAS-2 with CFM is designed for simultaneous measurement of gas exchange and chlorophyll fluorescence. Except for the intial Fv/Fm, every measurement begins with a photosynthesis measurement followed by a chlorophyll fluorescence measurement based on the CFM settings described earlier.

The following sections describe the two most common types of measurements, Keypress and Response Curve Recording.

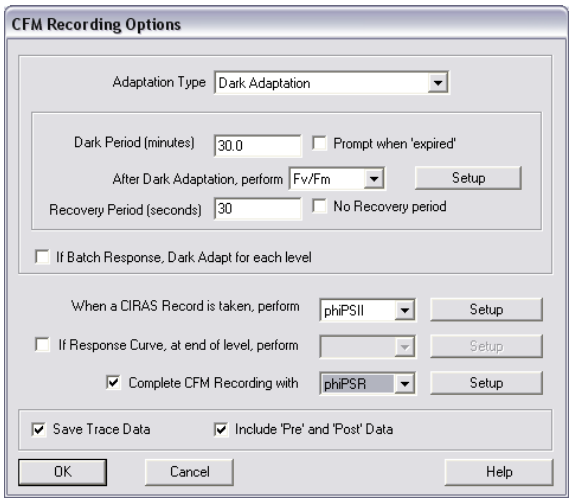
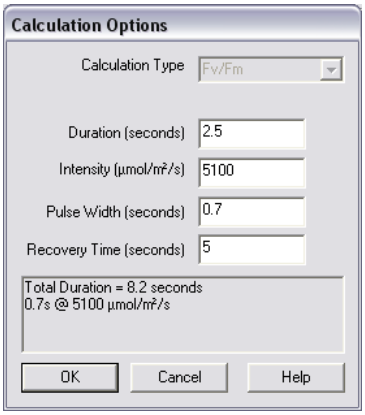
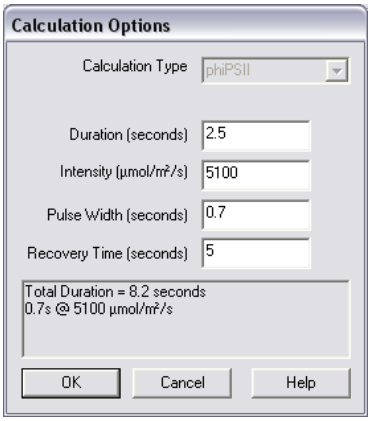
Keypress Recording with CFM

Click on **Recording - Begin** and select 'Keypress'.

The measurement will begin based on the adaptation type selected earlier followed by photosynthesis/chlorophyll fluorescence measurements based on the cuvette environment (Parameters Editor) settings (i.e. CO_2, H_2O, Light, etc.) and CFM recording options. If you need to change any of the CFM recording options, click on **Keypress Options** and then **Edit CFM options**.

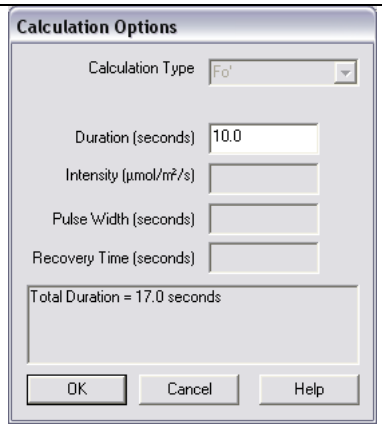


In this case, we made the following CFM selections based on Dark Adaptation. After a 30 minute dark adaptation period, the CIRAS-2 will perform an Fv/Fm calculation based on the CFM settings below:

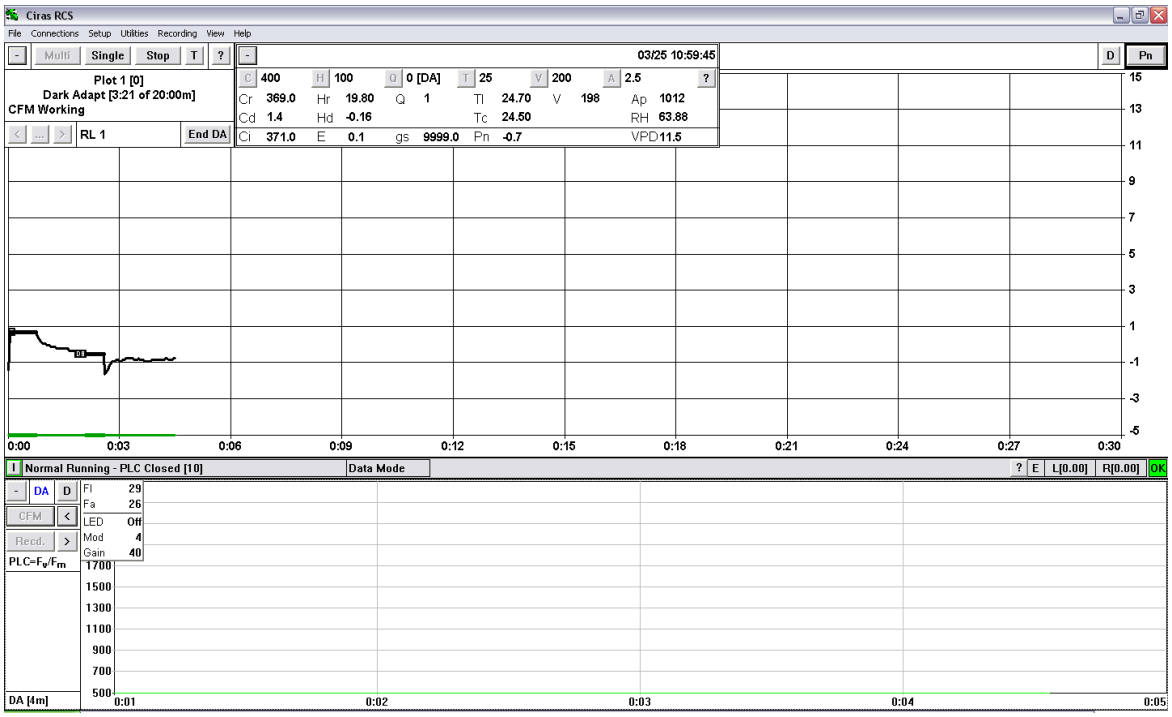
CFM Recording Options	
Fv/Fm Options	
phiPSII Options	

phiPSR Options

Note. ”Complete CFM Recording with” must be checked.



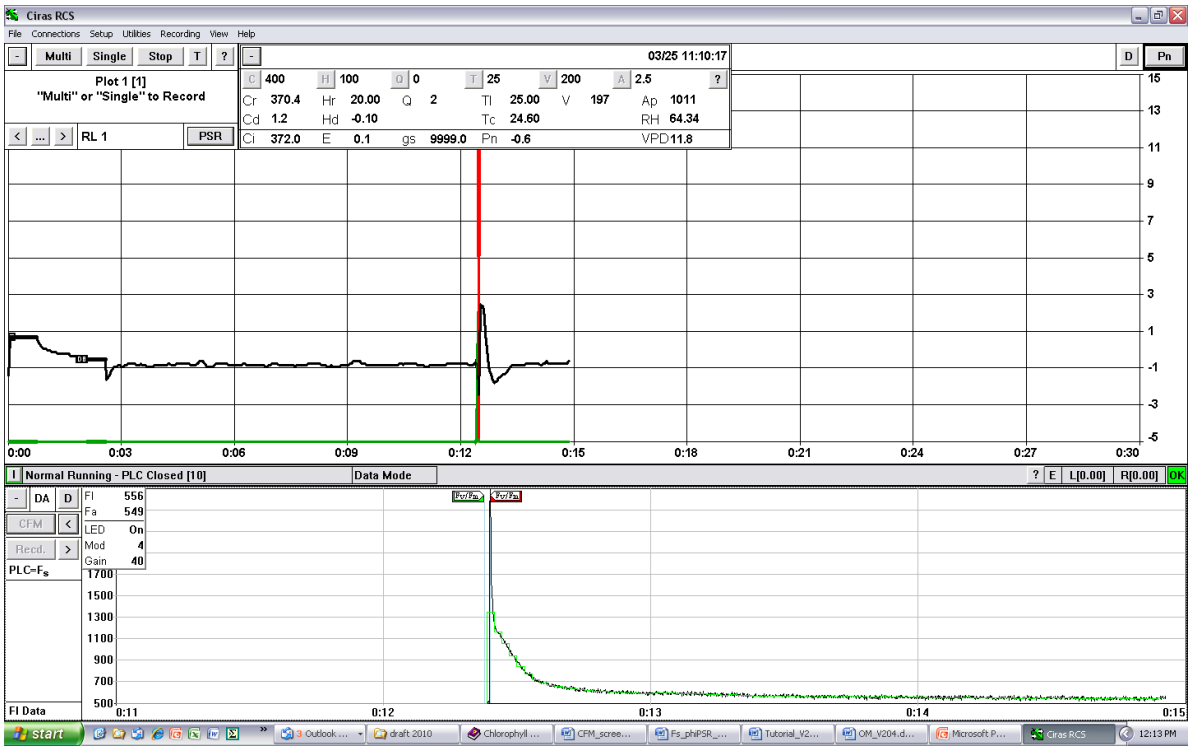
After starting the measurement, the main CIRAS display will show the following:



In the upper left hand corner, you will see a message showing “CFM Working” with a timer showing the status of the dark adaptation period. You can end the dark adaptation at any time by pressing the **End DA** button.

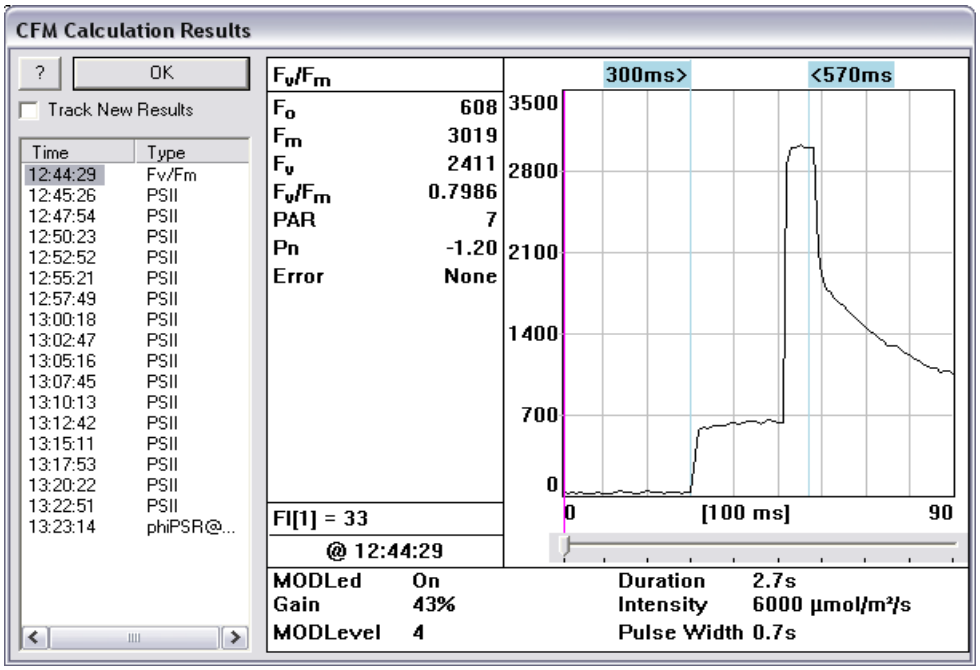
During dark adaptation, the LED light unit is automatically turned off. During this period, you should witness a fluorescence signal (green line) of 0 on the lower display and your photosynthesis calculations corresponding to the actual response of the plant under set conditions. A positive value (+) corresponds to photosynthesis and a negative value (-) corresponds to respiration. The reference CO_2 (Cr) and H_2O (Hr) should be stable with little differentials for CO_2 (Cd) and H_2O (Hd) during dark adaptation.

At the completion of the dark adaptation period or when you manually end it, the Fv/FM measurement will be initiated and recorded:



If a **Zero** or **Diff Bal** occurs during this period, the dark adaptation period will be extended. There are event markers in the display showing exactly when the measurement was recorded.

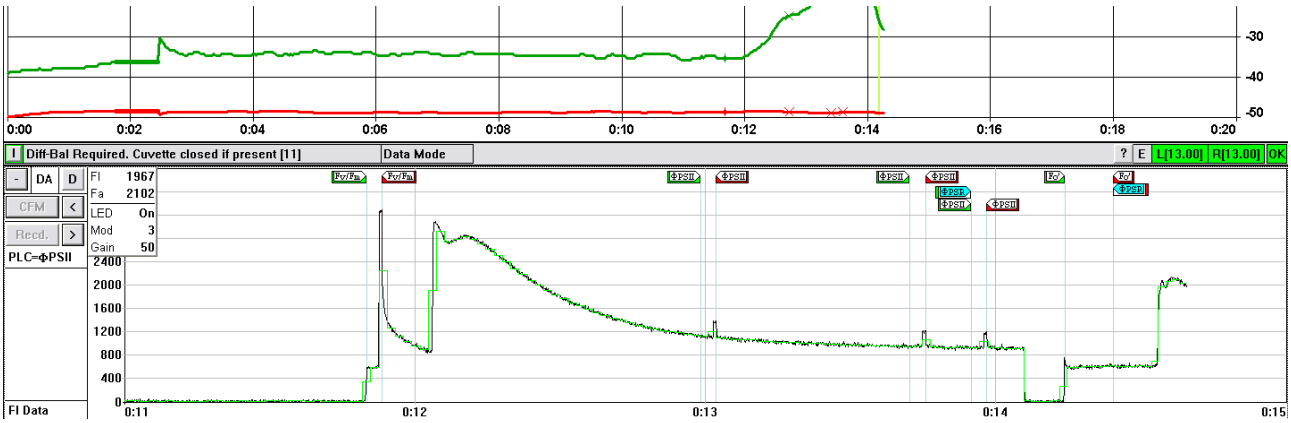
If you would like to view the chlorophyll fluorescence data and trace information associated with the measurement, click on the [>] button next to the [Recd.] button on the left side of the lower display:



Each chlorophyll fluorescence calculation is time stamped on the left. To view each individual measurement, simply click on the time stamp of interest and the chlorophyll fluorescence data associated with that point in time is displayed.

After performing an Fv/Fm calculation following the dark adaptation period, there will normally be a "recovery" period (in seconds) set by the user under **CFM Recording Options** to allow the sample to recover from the dark adaptation period. You should notice a stable fluorescence signal at approximately 700 units. When the photosynthesis readings are stable, press the thumb record button on the leaf cuvette or click **Single** in the Recording window to record a measurement.

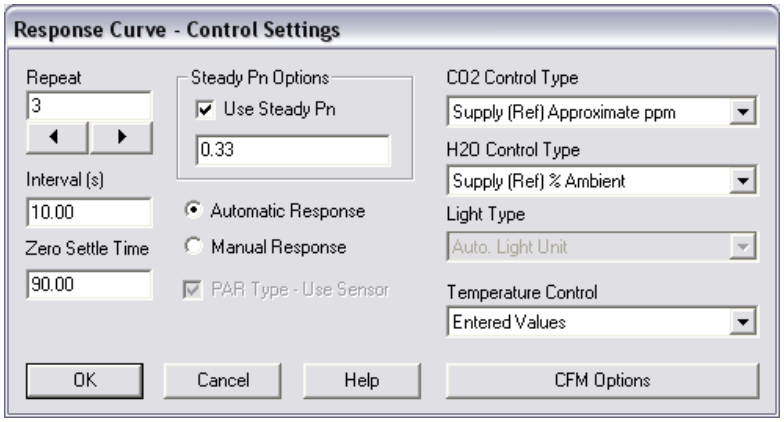
In addition to measurement of photosynthesis, a phiPSII is calculated followed by a "recovery period" (in seconds) set by the user under **CFM Recording Options** to allow the sample to recover from the light adapted measurement. To perform a phiPSR at the conclusion of all measurements (example below), click on the **PSR** button in the upper left hand corner of the display.



Response Curve Recording with CFM

Click on **Recording - Begin** and select 'Response Curve'. This will bring up the Response Curve Editor. Full information on setting up Response Curves can be found at Response Curve on page 68.

Click on **Recording Options** and make your selections (example as shown below):



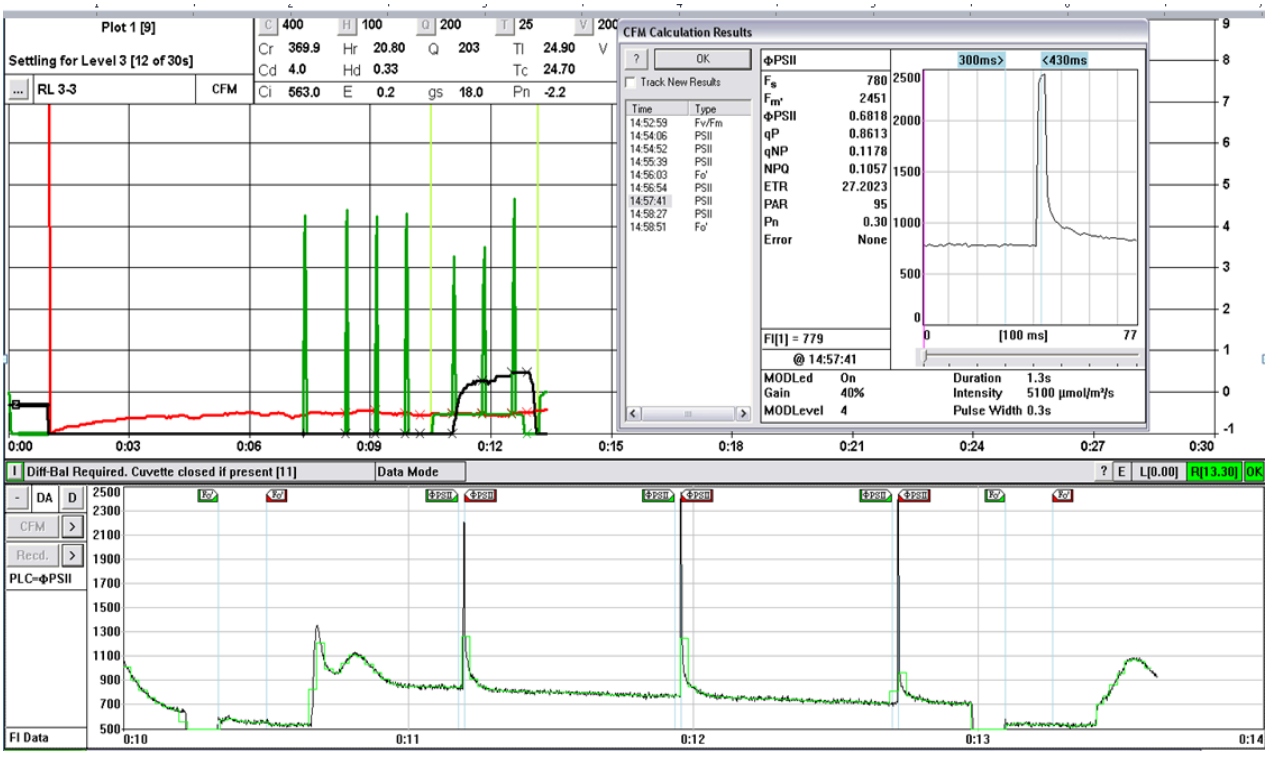
Click on **CFM Options**. They should be exactly the same as set up earlier in this manual. If you would like to change any of the CFM options, you can do so here. When your Response Curve-Control Settings are set, click on **OK** to return to the **Response Curve Editor**. Any change here will also change the settings in the CFM file.

Click **OK** when completed and then click **Yes** to save changes to the new response curve. Next, enter the name of the response file and click **Save** (.rsp extension) to save it. When returned to the **Select Recording Options Editor**, click on **OK** and **Yes** to save changes to the recording options.

At this point, you will have the choice to either append data to previously stored data or you can create a new file. After appending or saving to a new data file, the main CIRAS display will appear. To begin automatic response, click **Start**. Like **Keypress** recording, the CFM recording options will be based on settings under **CFM options** and the photosynthesis readings will be based on the settings in the **Response Curve Editor**.

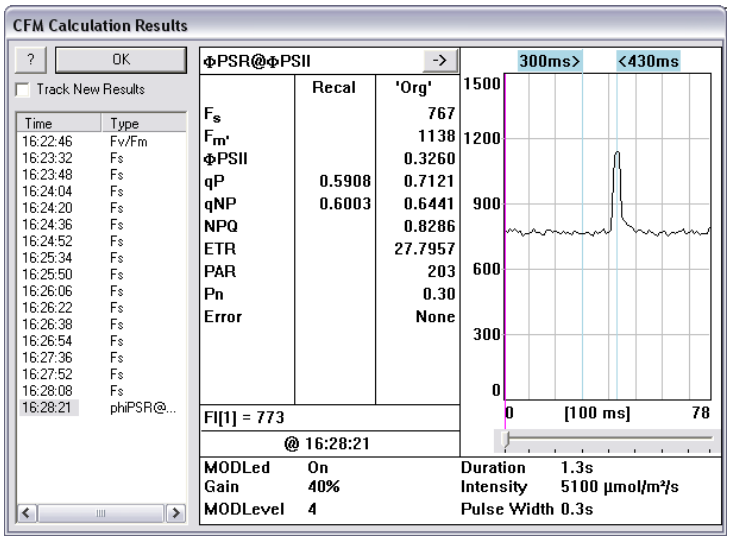
Following the dark adaptation period, the CIRAS-2 will perform an Fv/Fm and the chlorophyll fluorescence measurements you selected, with event markers showing each measurement. To view the fluorescence data and trace associated with the measurement, click on the **>** button next to the **Recd.** button on the lower display.

The response curve will continue per the settings in the **Response Curve Editor** and **CFM Recording Options.** In this example we see phiPSII calculations following each photosynthesis measurement in the Response Level, followed by Fo' at the end of the level.

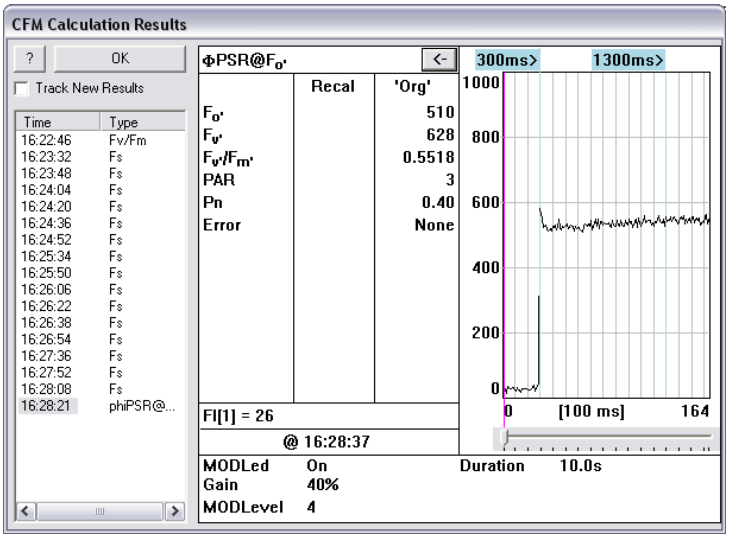


If your **CFM Recording Options** include the two-stage phiPSR measurement (phiPSII followed by Fo'), the fluorescence and trace data are contained in separate dialogs. Click on the **->** button in the upper-middle field of the **CFM Calculation Results** dialog:

i) phiPSII



ii) Fo'



CFM Output

The recommended method to download CFM data files is to use the simplified **Export Data** function (see Export Data on page 88.), which assigns headings to all CFM measurements and calculation phases. Unlike CIRAS-2 gas exchange data, raw CFM data (.dat) files are not written to output with headings.

CFM data files are complex, and can be difficult to interpret without help. In the CIRAS menu go to **Help - Add-On Modules - CFM (green link) - CFM data (click open) - CFM Data Files** for a full description of the CFM data structure. These Help Files are only accessible through the computer version of RCS, not on the UI.

Calculation Error Codes

After chlorophyll fluorescence calculations have been made, error codes may be reported as follows:

Error Message	Error Code	Meaning
Fo Low	1	Fluorescence Origin Low
Fm Low	2	Fluorescence Maximum Low
Fm<Fo	4	Fluorescence Maximum less than Fluorescence Minimum
Fo' Low	8	Fluorescence Origin Prime Low
Fm'<Fs	16	Light adapted Fluorescence Maximum less than Steady State Fluorescence

A single calculation can have one or more errors.

- Errors 1, 2 and 4 can apply to Fv/Fm calculation.
- Error 16 can apply to phiPS2
- Error 8 can apply to Fo'
- Errors 8 and 16 can apply to phiPSR

Brief Introduction to Chlorophyll Fluorescence

The CFM is a powerful tool for photosynthesis research. However, to fully utilize its functionality and achieve optimum performance, it is important to appreciate the measurements that CFM is designed to make and some of the principles behind its operation. The following introductory sections are only intended as a very limited introduction to chlorophyll fluorescence measurement. More comprehensive information can be found from the references cited in the text. For a modern review see Maxwell and Johnson 2000 or Baker et al. 2008.

What is chlorophyll fluorescence?

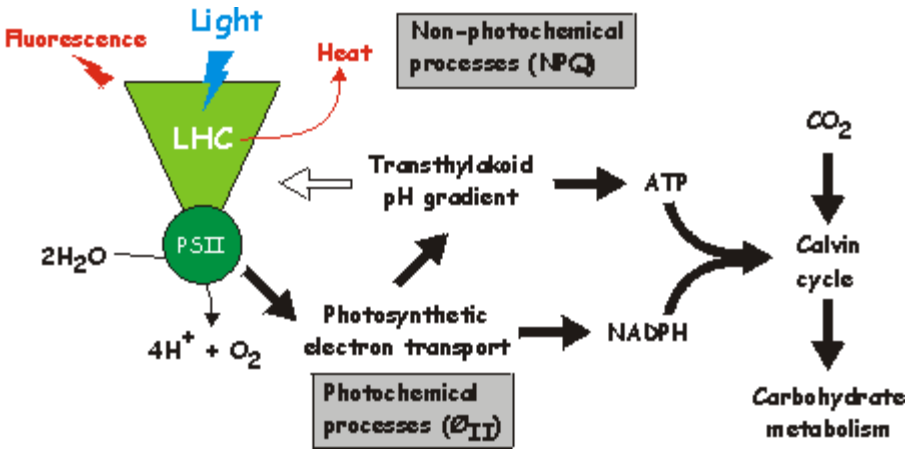
When light energy is absorbed by the chlorophyll pigment the electronic configuration of the molecule is temporarily altered. This "excited" configuration is inherently unstable and short-lived (typically less than 10^{-8} sec) as several processes compete to dissipate the absorbed energy.

In photosynthetic systems, these processes can be categorized into two groups:

Photochemical processes: Electron donation from the pigment to an acceptor molecule occurs. The energy is then used in the carbon fixation cycle.

Non-photochemical processes: Energy is re-emitted from the sample both in the form of infra-red radiation (heat) and red/far-red radiation which is known as chlorophyll fluorescence. Re-emitted light energy is always at a longer wave-length than the absorbed light.

The competition between these processes for absorbed the energy means that a reduction in the rate of one process will be associated with a corresponding increase in the rates of competing processes *e.g.* a reduction in the dissipation of energy by photochemistry will be reflected in an increase in energy dissipation by non-photochemical processes such as heat production and chlorophyll fluorescence. Consequently measurement of changes in fluorescence emission with the CFM-1 can be used to infer information about changes in the efficiency of light use for photochemistry (Reviewed in Krause and Weis 1991 and Govindjee 1995).



Since the work of Emerson *et al*. (1932) it has been widely accepted that there are two separate photochemical steps during photosynthesis which are associated with different groups of pigments; these

groups have become known as photosystem I and photosystem II. In practice, differences in the functionality of photosystem I and II mean that at least 95% of the chlorophyll fluorescence signal observed under physiological temperatures is derived from chlorophyll molecules associated with photosystem II (PSII). Hence the fluorescence signal detected by CFM-1 reflects changes in the efficiency with which absorbed light is used for PSII photochemistry.

Techniques for Measuring Chlorophyll Fluorescence.

Photosynthesising organisms only fluoresce when illuminated with visible light. Within the normal physiological temperature range, peak fluorescence occurs in the red region of the spectrum (≈ 685 nm) and extends into the infra-red region to around 800 nm (Krause and Weiss 1984) (Figure 1-1).

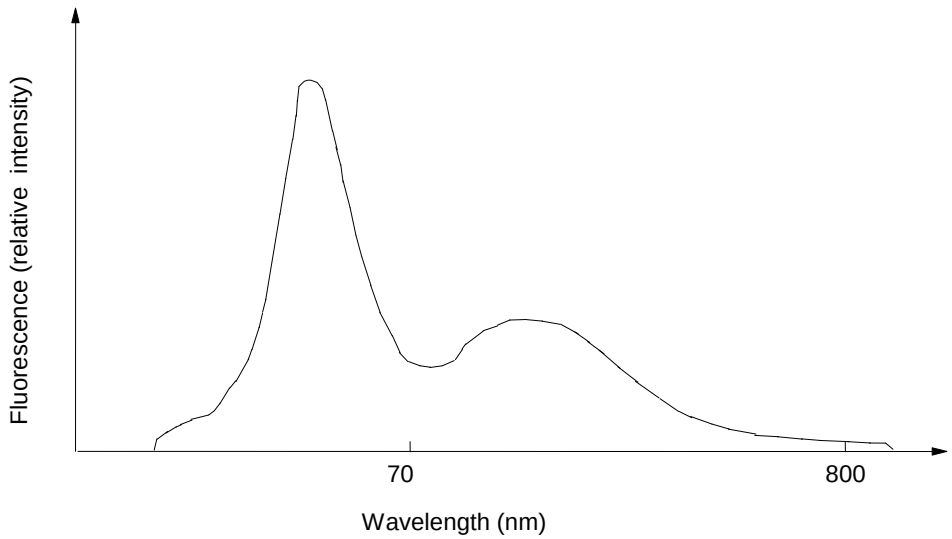


Figure 1-1. Room temperature chlorophyll fluorescence emission spectra. (Adapted from Krause and Weiss 1984)

Therefore, accurate determination of fluorescence requires a detection system that is sensitive to the fluorescence wavelengths yet blind to the actinic (or excitation) light, which drives photochemistry.

There are 2 methods commonly used by commercial instruments.

1. Continuous Excitation Systems

In continuous excitation instruments such as the HANSATECH PEA (*Plant Efficiency Analyser)* signal discrimination is achieved using a custom designed 650 nm actinic light source to drive photochemistry; optical filtering of the detector then prevents detection of non-fluorescence wavelengths of light. Unfortunately, such systems must be shielded from ambient light during use. Otherwise, the red / far-red component of daylight will be super-imposed on the fluorescence signal.

2. Modulated Systems

This problem is avoided in instruments such as the HANSATECH FMS2 and in the CFM by using an electronic method, commonly referred to as modulated fluorometry, to separate actinic light from the fluorescence signal (Ögren and Baker 1985). During measurement the tissue sample is exposed to a

pulsed amber LED light source (the modulating beam) which induces a pulsed fluorescence signal from the sample under conditions where ambient light is excluded (A, Figure 2). When ambient light is applied the optical filtering of the CFM allows three types of light signal to reach the detector:

i) ambient light of fluorescence wavelengths,
ii) non-pulsed fluorescence signal induced by the ambient light,
iii) pulsed fluorescence signal induced by the modulating beam (B, Figure 1-2).

Addition of ambient light changes the physiology of the sample, altering the proportion of energy that is re-emitted as fluorescence. Thus the magnitude of the peaks of the pulsed fluorescence signal that are induced by the modulating beam will reflect changes in sample physiology. It is these signals that the rapid pulse peak tracking electronics amplify and measure; ambient and non-pulsed fluorescence signal are discarded (C, Figure 1-2).

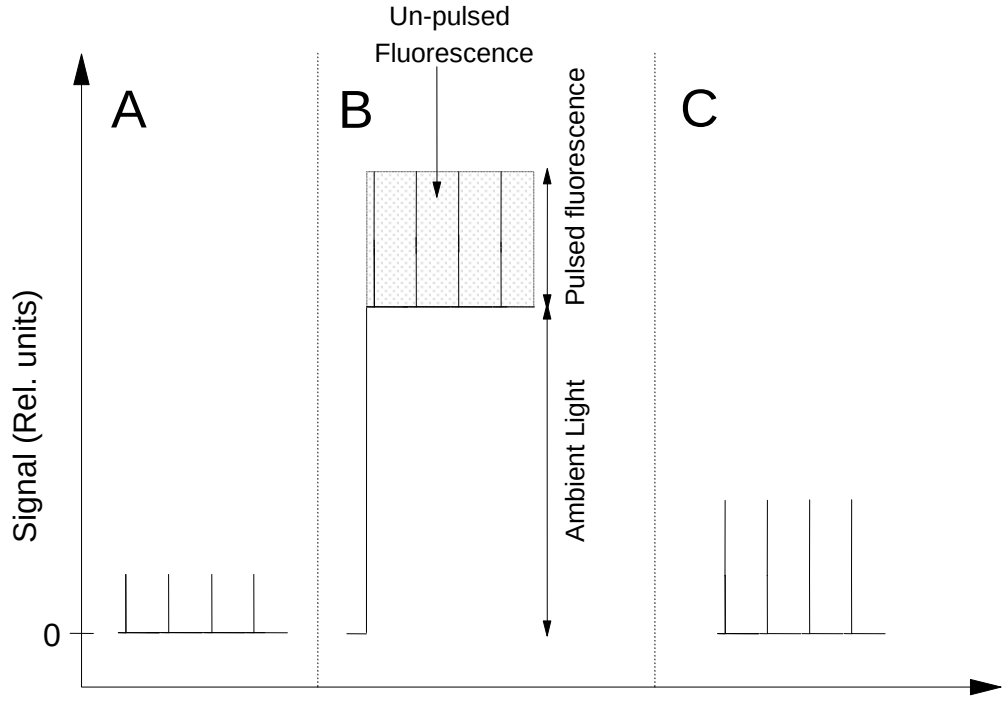


Figure 1-2. Fluorescence signal detected by the CFM under modulating beam only conditions (A), under actinic light plus modulating beam before (B) and after (C) the rapid pulse peak tracking electronics.

The pulsed fluorescence that is induced by the modulation beam is effectively used to probe the efficiency of light-use for photosystem II photochemistry. Clearly it is important that the measuring technique itself does not induce changes in the physiology of the sample under investigation.

Exposure of a sample to a powerful pulsed light source may be expected to introduce a significant artifact into the measurement. However, CFM-1 has been specifically designed to avoid such problems by using a modulating beam of very short duration pulses (1.8 µsec) with long off period between pulses. The net result is that the integrated amount of radiation incident upon the sample from the modulating beam is

very small (<0.05 µmol m^{-2} s^{-1}), too small to induce significant physiological change in the sample (Schreiber *et al*. 1986), yet powerful enough to provide a reliable signal for fluorescence analysis.

The interpretation of fluorescence signals has developed considerably since the early experiments of Kautsky and Hirsch (1931) with several techniques employed to determine information about the photosynthetic performance of PSII (Baker 1991). The CFM-1 hardware and software has been designed to incorporate these techniques into simple routines which store appropriate fluorescence measurements and automatically calculate key parameters.

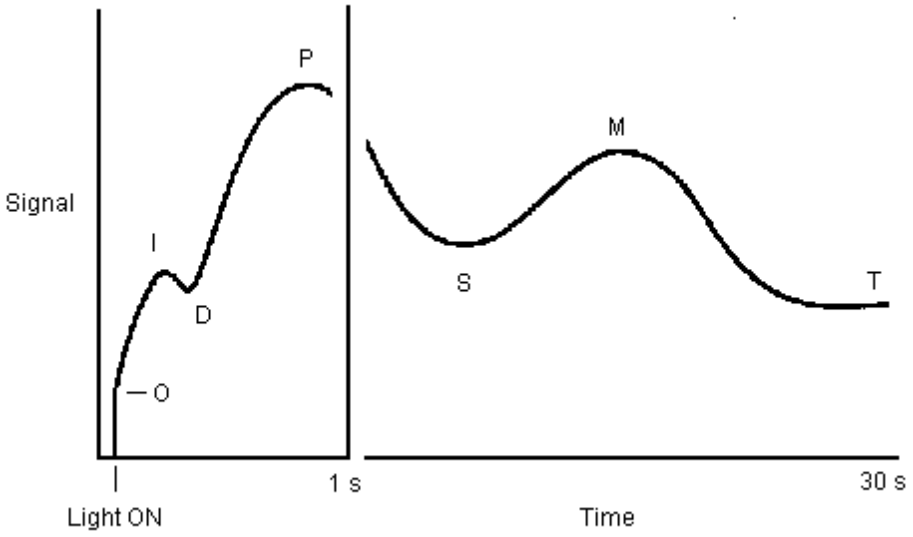
Common Fluorescence Measurements

There are a vast number of different measurement protocols and parameter nomenclatures. This section will cover the instrumentation requirements of the two most common fluorescence analysis procedures. The most widely accepted parameter definitions used by "main-stream" workers are presented in a glossary with alternative nomenclatures.

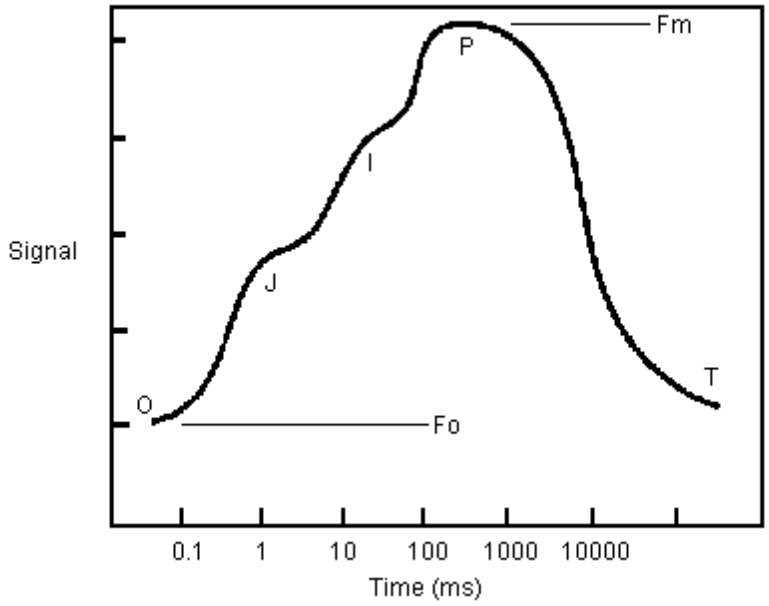
There are two common types of fluorescence measurement:

1. Fluorescence Induction Analysis (Kautsky Curve).

If a sample is **dark adapted** the PSII electron acceptor pool is gradually re-oxidised to a point where all of the PSII reaction centres are capable of under-taking photochemistry. Illumination induces a fast (approx. 1 s) polyphasic **rise in fluorescence** which is followed by a slow (approx. 2 min) fluorescence decline to a steady state level of fluorescence. This induction phenomena is often referred to as the **Kautsky Curve**.



The different phases of the Kautsky Curve relate to different limiting reactions which occur as photosynthetic reactions are initiated; originally they were denoted with the letters O,I,D,P,S,M,T. With the development of shutter-less high-speed data sampling instruments such as the Hansatech Plant Efficiency Analyser a further (J) peak was discovered in the fast fluorescence rise. This is best observed on a logarithmic plot.



OJIP Fluorescence Induction Curve

Without adequate time-resolution the “J”-peak is often mistaken as the fluorescence origin (Fo) leading to inaccurate calculation of derived parameters such as Fv and Fv/Fm (see parameters glossary).
Changes in the magnitude of the “J”-peak following multiple pulse tests are currently thought to provide a particularly sensitive indicator of photosynthetic stress.

Determination of key fluorescence parameters requires an instrument with:

Fast enough time resolution (10 µs)	To capture the rapid phases at the start of the Kautsky Curve.
Powerful light source	A light source providing a minimum of 2,500 µmol $m^{-2}s^{-1}$ to fully reduce all of the PSII electron acceptors molecules.

2. Quantum Efficiency of Photosystem II

Modulated instruments allow measurement of fluorescence from samples whilst photosynthesis is operating under actinic light conditions. The most common aim is to estimate the **proportion of energy quenched by photosynthetic** and n**on-photosynthetic (*e.g.* heat emission) processes** and thus estimate how efficiently light is used by PSII.

Several different protocols have been produced using actinic and saturating light sources to manipulate the sample. The most common (Genty technique) and its associated parameters is discussed in the parameters glossary. Measurement of the full range of light-adapted fluorescence parameters requires the following instrumentation features:

Saturating Light Source	Capable of delivering at least 2,500 µmol m^{-2} s^{-1} to the leaf to fully reduce all PSII electron transport acceptors.
Actinic Light Source	To drive photosynthesis in the sample. Ideally this should deliver at least 2,000 µmol $m^{-2}s^{-1}$ to the sample to simulate maximum summer conditions.
Far-Red Light Source	To preferentially over-excite PSI relative to PSII.
Good Signal / Noise Ratio	To allow remote measurements at some distance from the sample and therefore allow simultaneous measurement with other techniques such as infra-red gas analysis.

The relatively slow changes in fluorescence events when measuring common light-adapted parameters means that the high-time resolution of fluorescence induction kinetics is not needed.

Glossary of Fluorescence Terms

Definitions of the most commonly referenced fluorescence parameters are given below with alternative nomenclatures.

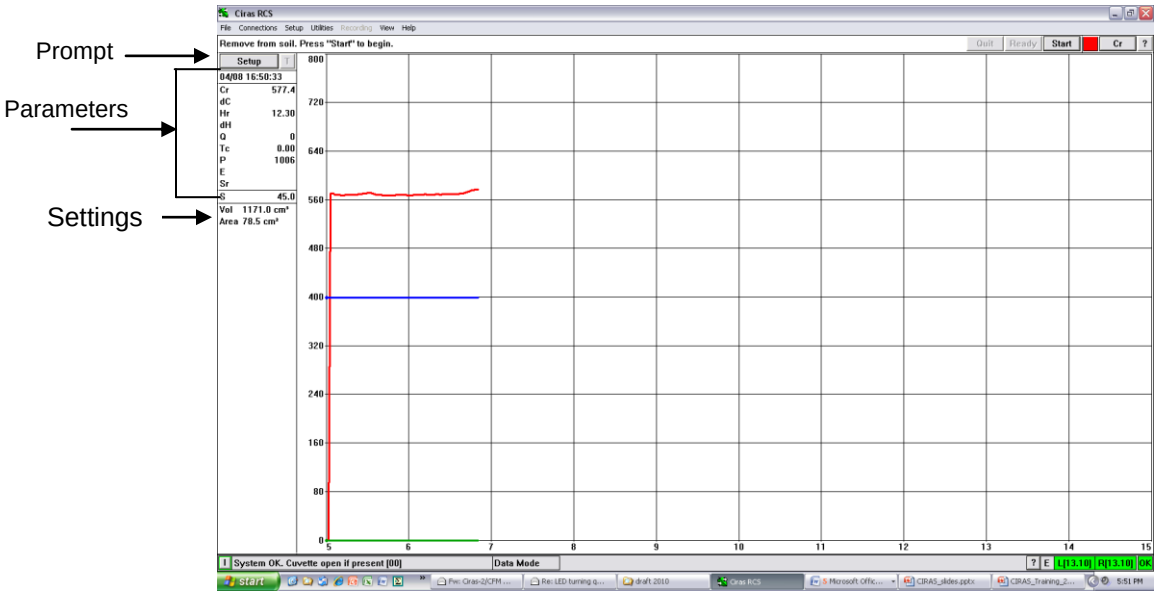
Parameter	Alternative Name	Definition	Measurement Method
Fo (Fluorescence origin)	F nought F zero O (in Kautsky terms)	Signal following dark adaptation when all PSII reaction centres and electron acceptors are fully oxidised, hence “open” for photochemistry.	Modulated System - monitor signal under modulating beam only from dark adapted sample. Continuous Excitation - monitor induction with high time resolution (10 µs). Must have high time resolution.
Fp (Fluorescence peak)	P	Peak signal following illumination with non-saturating pulse of light. Not generally used today as most modern instruments have saturating light sources which can deliver >2,500 $\mu molm^{-2}s^{-1}$.	Track fluorescence rise and determine peak fluorescence value.
Fm (Fluorescence maximum)	Fmax (P in Kautsky terms)	Maximum fluorescence signal from the dark-adapted leaf following application of a saturating light pulse which fully reduced the PSII electron acceptors preventing photochemistry.	Dark adapt sample, apply saturating light pulse and determine maximum fluorescence signal.
Fv (Variable Fluorescence)	Variable fluorescence	Derived from Fm-Fo	Automatically calculated
Fv/Fm	ϕ_{PSII} MAX	Derived from (Fm-Fo)/Fm. Directly proportional to the maximum quantum efficiency of PSII.	Automatically calculated
Area over OIDP curve.	Area	Proportional to the size of the PSII electron transport acceptor pool.	Automatically calculated
Fs (Steady state fluorescence)	Ft (terminal T in Kautsky terms)	Signal under an actinic light.	Switch on actinic lamp on modulated system. Sometimes used to show “terminal” fluorescence signal when photosynthesis is activated at the end of a Kautsky curve.
Fm’ (light-adapted fluorescence maximum)	Fms	Maximum fluorescence signal from light-adapted leaf following application of saturating pulse which fully reduced PSII electron acceptors preventing photochemistry.	Adapt sample to actinic light, deliver saturating pulse and track fluorescence signal.

Fo' (light-adapted fluorescence origin)	Fod	Signal in the light-adapted state when all PSII reaction centres and electron acceptors are fully re-oxidised using a far-red light source and are therefore "open" for photochemistry.	PSII electron acceptors are transiently re-oxidised by removing the actinic source whilst exposing the sample to a short pulse (approx. 3 s) of far-red light. This preferentially excites PSI drawing electrons out of PSII. The fluorescence minimum is tracked and logged as Fo'.
Parameter	**Alternative Name**	**Definition**	**Measurement Method**
Fv'/Fm'	PSII antennae efficiency	Derived from (Fm'-Fo')/Fm'. Probability that energy absorbed by PSII antennae molecules reaches the PSII reaction centre.	Requires measurement of Fm' with a saturating pulse and Fo' with far-red only illumination. Automatically calculated.
ϕPSII	Y Yield Genty Parameter	Quantum efficiency of light-use by PSII *i.e.* number of PSII reaction events / no photons absorbed. Derived from (Fm'-Fs)/Fm'	Measure steady state fluorescence signal (Fs), apply saturating pulse to determine Fm'.
qP (photochemical quenching)		Derived from (Fm'-Fs)/(Fm'-Fo). Proportion of absorbed energy used by photochmical processes.	Measure dark-adapted Fo, light-adapt, measure Fs and apply saturating pulse to determine Fm'.
qNP (non-photochemical quenching)	qE qN	Derived from (Fm-Fm')/(Fm-Fo). Proportion of absorbed energy dissipated by non-photochemical processes, principally heat emission.	Measure dark-adapted Fo and Fm. Light-adapt and apply saturating pulse to determine Fm'.
NPQ		Alternative expression of non-photochemical quenching. Derived from (Fm-Fm')/Fm'	
ETR (electron transport rate)		Derived from (ϕ_{PSII} * incident PAR * 0.5 * leaf absorption) Estimated rate of electron transport flow within the chloroplast.	Measure ϕ_{PSII} simultaneously with incident radiation in sample via PAR sensor on leaf-clip. Leaf-absorption factor must be measured separately with an integrating sphere.

Measuring Soil Respiration with CIRAS-2

The CIRAS-2 Measurement Screen is slightly different for Closed System Analysis (e.g. soil respiration with SRC-1 chamber, canopy assimilation with our CPY-4 chamber). The following instructions and corresponding settings assume use of PP Systems' SRC-1 soil respiration chamber. For more technical information and details related to electrical and gas connections please refer to the **SRC-1 Soil Respiration System Operators Manual for CIRAS-2 ver. 4.03 or greater.**

Soil Respiration Measurement Display



Prompt

Gives instructions to the user (i.e. flushing the cuvette, etc.) or displays the progress of calculations.

Parameters

Measurements associated with respiration measurement.

Cr	Starting CO_2 concentration (ppm)
dC	Change in CO_2 concentration (ppm) from start of measurement
Hr	Starting Humidity concentration (mb)
dH	Change in Humidity concentration (mb) from start of measurement
Q	PAR (µmol m^{-2} s^{-1}) (if sensor is also in use)
Tc	Temperature of the soil (^oC)
P	Atmospheric Pressure (mb)
E	Not applicable
Sr	Soil Respiration Rate (g CO_2 m^{-2} $hour^{-1}$)
S	Time elapsed (seconds)

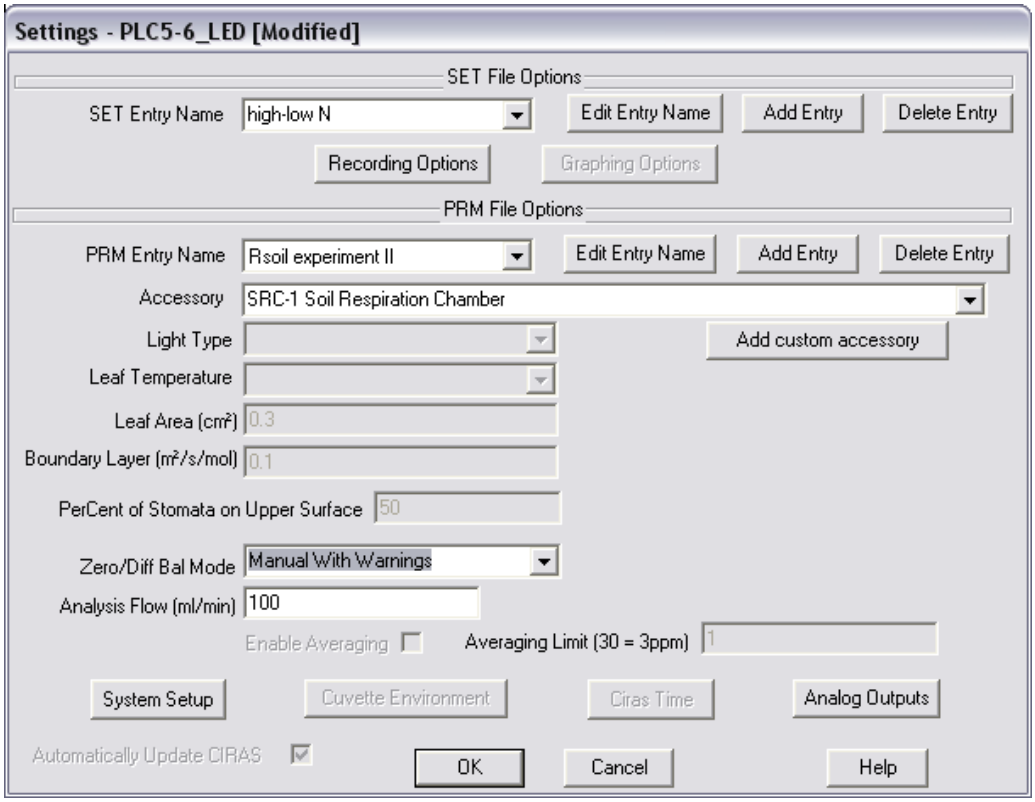
Settings

Dimensions associated with the chamber.

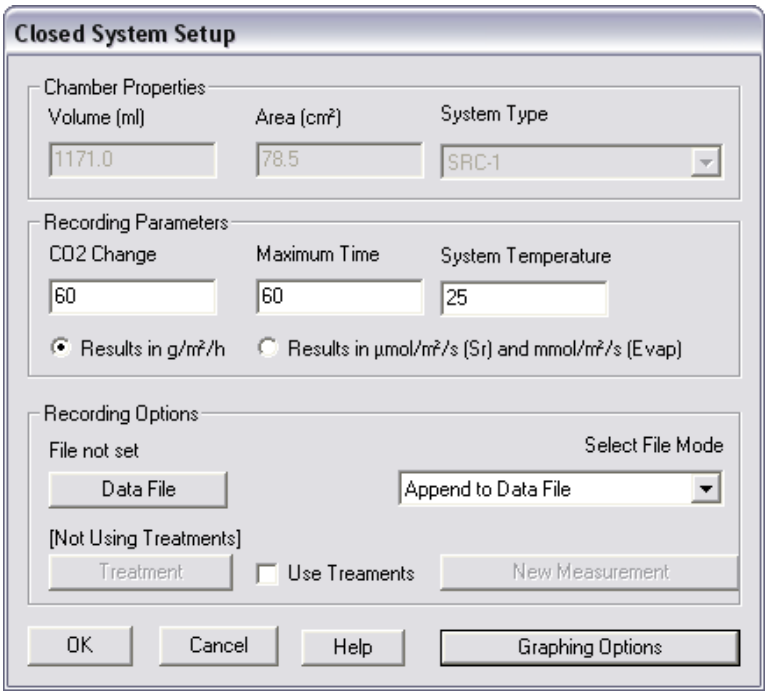
Vol	Total system volume (chamber, external and internal gas lines) (cm^3)
Area	Soil surface covered by chamber (cm^2)

Soil Respiration Operational Settings

Go to the **Setup –Settings** menu to open the CIRAS-2 Settings dialog. Click on the Setup button to view current settings. The following menu will appear:



Select 'SRC-1 Soil Respiration Chamber' from the 'Accessory' drop down list. Click OK. CIRAS-2 now displays the **Soil Respiration Measurement Display** (as above). Click the Setup button in the upper left display area. The **Closed System Setup** dialog appears.



Chamber Properties

Volume (cm^3)

If our SRC-1 chamber is used, the default value is fixed (1171.0) and can not be changed. **Please note:** If soil collars or similar devices are used with our SRC-1 chamber, the total volume will be larger than the default value. In that case you will need to open the **Custom Chamber Type** dialog by clicking the Add custom accessory button and entering the correct volume. See Add Custom Accessory on page 44.

Area (cm^2)

If an SRC-1 chamber is used, the default value is fixed (78.5) and can not be changed. **Please note:** If soil collars or similar devices are used with our SRC-1 chamber, the total area will be larger than the default value. In that case you will need to open the **Custom Chamber Type** dialog by clicking the Add custom accessory button and entering the correct volume. See Add Custom Accessory on page 44.

System Type

SRC-1 refers to a standard chamber supplied by PP Systems.

Recording Parameters

CO_2 Change

The maximum amount of change in CO_2 concentration from the start of measurement. The default value is 60 ppm.

Maximum Time

The maximum amount of time (seconds) from the start of measurement to the end. The default value is 60 seconds.

System Temperature

The approximate ambient temperature (at soil surface). The default value is 25 °C.

Results

Select from two options - save soil respiration (SR) results as i) g m^{-2} hr^{-1} **or** ii) SR as µmol m^{-2} s^{-1} and Evaporation (Evap) results as mmol m^{-2} s^{-1}.

Data File

Used to create new data files. If no file has been selected, you will be prompted when the first records are taken. Click on **Data File** to create a new data file and enter a name in the File name box (.csc extension is assumed and does not need to be typed in). Measurements which follow will be saved to this file.

Select File Mode

Determines how data files are managed. There are 3 options:

Always prompt for new Data File
Every time you start a new measurement, a window will appear asking you to enter a new data file name.

Append to Data File
If selected, all measurements will be appended to the existing data file.

Overwrite Data File
If selected, all measurements will overwrite previously stored data in that particular data file.

Treatment

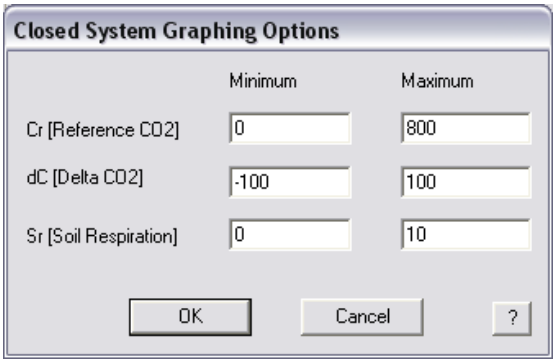
Identify the treatment file used. See Treatment Editor on page 61 for details.

New Measurement

Only available once records have been saved. Allows the specification of a new data file (as well as the other options).

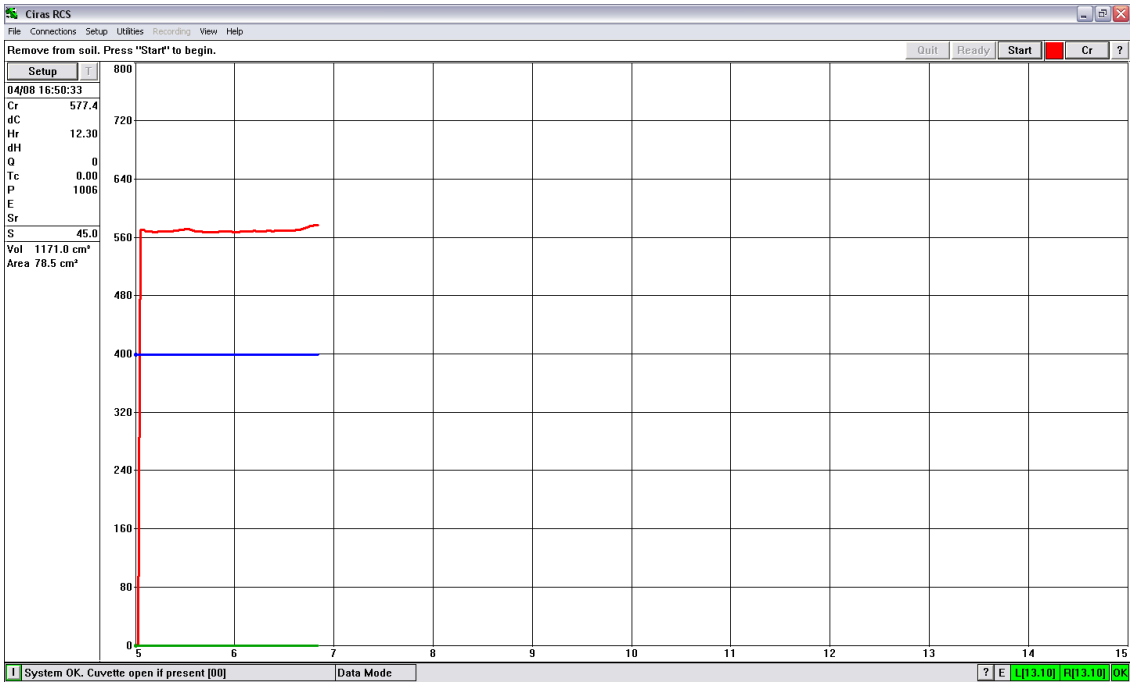
Graphing Options

Allows setting up the graph min-max Y-axis scales. Click on the Graphing Options button to set the Minimum and Maximum Y-axis values for reference CO_2 (Cr), Delta CO_2 (dC) and soil respiration rate (Sr). CO_2, Delta CO_2 and soil respiration rate are plotted simultaneously.

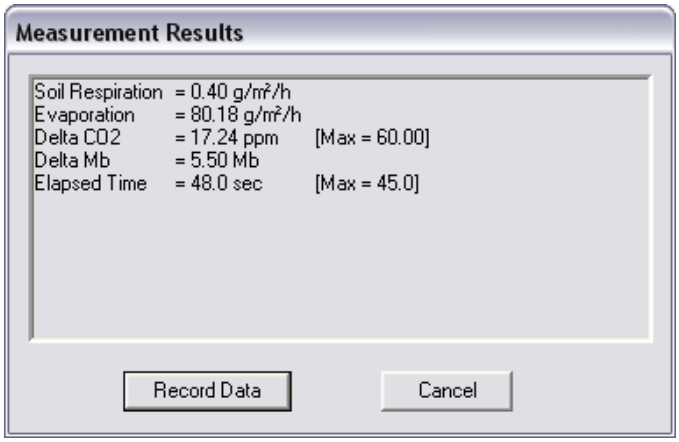


Recording in Soil Respiration Mode

Once you have successfully set up the system as described above and with the SRC-1 Soil Respiration Chamber properly connected to CIRAS-2, click the Start button in the upper right display.



In the upper left hand display, you will be prompted to “Hold Chamber in air to flush”. Ensure that the chamber is held in the air to allow it to completely flush itself out (the SRC-1 chamber fan should run at a noticeably higher speed during this period). After approximately 15 seconds, you will be instructed to “Place on Soil. “Ready” to continue …”. The SRC-1 fan speed should be running at a much slower speed at this point. Place the SRC-1 chamber on the soil (or on the soil collar if used) and click the Ready button. The system will then equilibrate before starting calculations. At the completion of the measurement and assuming a linear response, the following message is displayed:



To accept the results, click on the **Record Data** button. Otherwise, click on Cancel to reject the measurement. If you elect to save the results, you may have to create a new data file (if not already set

up) according to the options in **Select File Mode**. There is always a final confirmation to verify that the selected file is correct.

If you need to change the Plot Number or wish to add a comment between measurements, click on the **T** button in the upper left hand corner of the display. At any time during the course of a measurement, click the **Quit** button to end the measurement. To begin a new set of records in a new data file, use the **New Measurement** option in the **Closed System Setup** dialog.

Diagnostics

The CIRAS-2 contains numerous sensors to measure CO_2, H_2O, leaf environment, signals from external probes and gas flow through the instrument. The **Diagnostics** function collects the signals from each of these sensors and summarizes them. The result is a simple text file that can be faxed or e-mailed to PP Systems. This can then be compared with our factory records to identify any changes in the system.

There are 6 different dialogs associated with Diagnostics:

Analyser A/D Reads - CIRAS-2 Temperature and Atmospheric Readings, CO_2 and H_2O readings depending on valve settings and CIRAS-2 Stored Zeros.

Temperature & PLC State - Various readings for Temperatures. Probe Type is selected here.

Flows - Control and Monitor Analyzer and Cuvette flows.

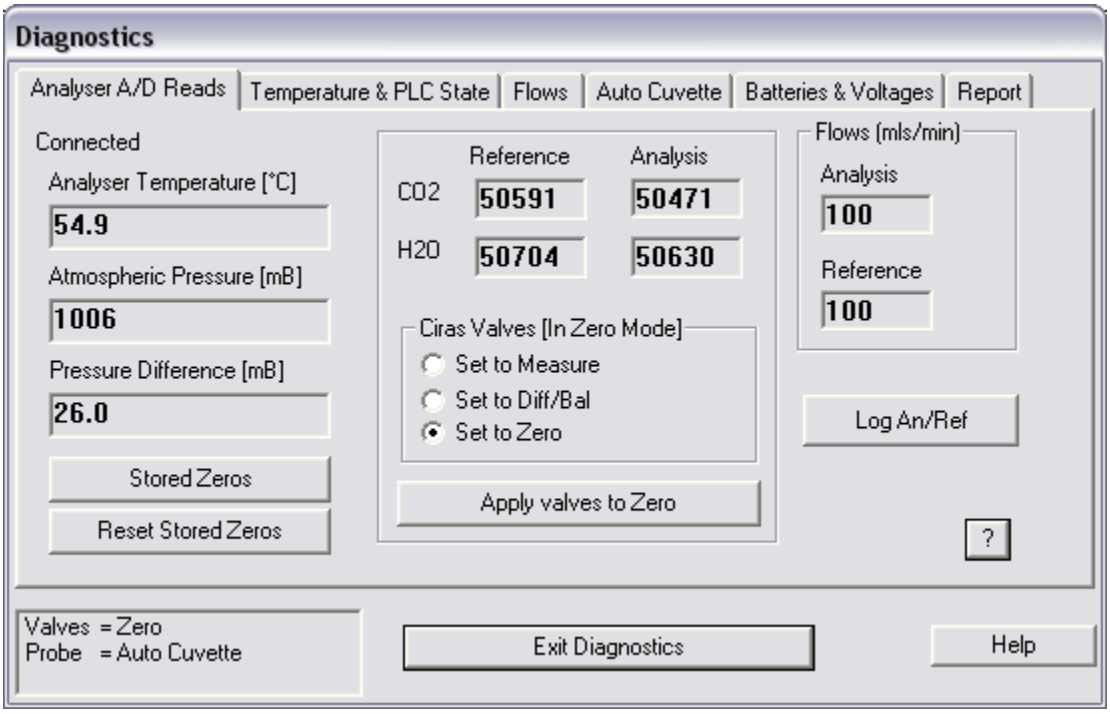
Auto Cuvette - Checks Auto Cuvette Light Units and Temperature control.

Batteries & Voltages - CIRAS-2 Battery and Board Voltages and Cuvette Voltage if connected.

Report - Generate Diagnostics Report for Technical Support.

CIRAS-2 and the software must be in Measure Mode to enter Diagnostics. (Diagnostics may also be performed during the Warm Up Cycle).

Analyser A/D Reads



Analyser Temperature (^{o}C)

Should be set to approximately 55 ^{o}C. The analyzers are thermostatted and controlled to approximately 55^{o} C.

Atmospheric Pressure (mb)

Absolute pressure in the Reference cell. This should correspond closely to the local atmospheric pressure in mb (Altitude dependent). At sea level, you should expect between 950-1050 mb. This value decreases approx. 1mb/10m increase in altitude.

Pressure Difference (mb)

Differential pressure between the reference and analysis cells. The mid-point is approximately 25 mb. Excessive differences indicate blockages or pressurisation of the analyzer.

Stored Zeros

Zero values stored by CIRAS-2 after 30 minutes running. The CIRAS-2 checks the values for changes in the values to warn of zero column exhaustion or deterioration of analyzer performance.

Reset Stored Zeros

Clears the stored zeros. If the zero reads are outside the normal range (normally due to exhausted chemicals in the zero columns), then, after replacing the absorbers, click on this to reset the stored zeros. This will stop further error messages.

CO_2 Reference

A/D reads for the CO_2 Reference Cell. Values depend on valve selection (see Valve Settings below).

CO_2 Analysis

A/D reads for the CO_2 Analysis Cell. Values depend on valve selection (see Valve Settings below).

H_2O Reference

A/D reads for the H_2O Reference Cell. Values depend on valve selection (see Valve Settings below).

H_2O Analysis

A/D reads for the H_2O Analysis Cell. Values depend on valve selection (see Valve Settings below).

Please note: CIRAS-2 always starts **Diagnostics** in the ZERO mode (with Cuvette set to Manual Cuvette).

Valve Settings

Apply valves to Zero

Click on this to put CIRAS-2 into Zero Mode. Without CO_2 and H_2O in the sample cell, IR absorption is at a minimum level so the detector outputs are at their maximum. The A/D reads for both CO_2 and H_2O (Reference & Analysis) should typically be >45000 and stable.

Apply valves to Measure

Click on this to put CIRAS-2 into Measure Mode. With 'Set to Measure' selected and after clicking on this option, the values for CO_2 and H_2O (Reference & Analysis) should drop to a lower value. Stability will depend on the air that is being sampled. If generated by the internal air supply, then it should be stable.

Apply valves to Diff/Bal

Click on this to put CIRAS-2 into Diff/Bal Mode. This passes the Reference air through the analysis cell and as above, stability depends on the source.

Flows

Reference

Refers to the flow rate of the reference pump. Should read approximately 98-105 and stable.

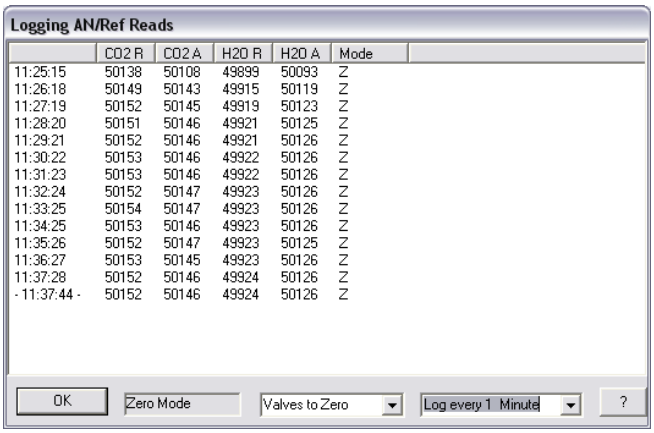
Analysis

Refers to the flow rate of the analysis pump. Should read approximately 98-105 and stable.

Both the Reference and Analysis flows should be the same.

Log An/Ref

Allows real-time logging of Reference and Analysis A/D values at 1 or 2 minute time intervals.



Temperature & PLC State

Please note: CIRAS-2 starts Diagnostics with the Cuvette set to **Manual Cuvette** (and in ZERO mode) regardless which cuvette you are using. Unless you are using a Manual Cuvette, you will need to change this accordingly.

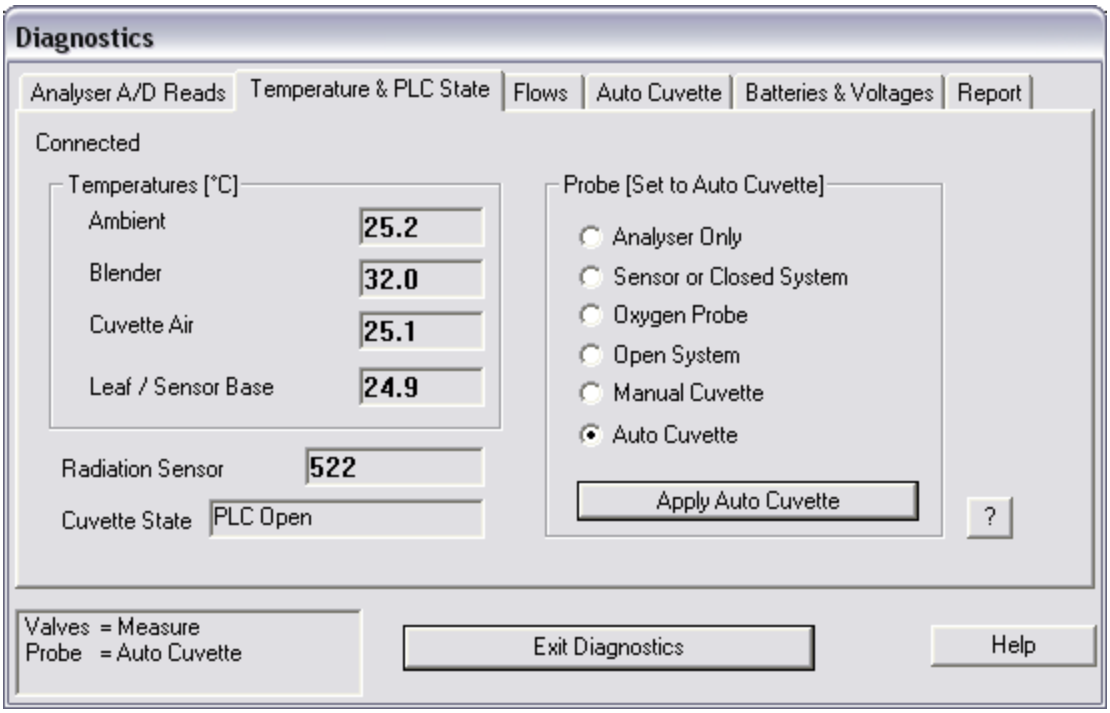
Temperature (^oC)

Ambient

Temperature of ambient air measured at the rear of the leaf cuvette handle.

Blender

Temperature of the CIRAS-2 internal gas blender.



Cuvette Air

Temperature of air in the leaf cuvette.

Leaf/Sensor Base

If a leaf thermistor is connected to the leaf cuvette then it is the temperature measured at the tip. For cuvettes with radiation sensors then it is the temperature at the sensor base.

Radiation Sensor

Output from IR sensor. If the sensor base temperature is identical to the leaf temperature, then this value will be set to 512. If leaf is warmer then the value will be greater and vice versa.

Cuvette State

PLC Open or Closed. Refers to whether the leaf cuvette head is open or closed.

Probe

The default Probe Type is Manual Cuvette (Set to Auto Cuvette in this example). **Please note:** Regardless of which probe has been selected in the **Parameters** dialog, you **MUST** specify it again in **Diagnostics** in order to get the appropriate A/D reads for your setup:

Analyser Only	Click on this if CIRAS-2 is used as a stand-alone Analyzer.
Sensor or Closed System	Click this if the SRC-1 soil respiration chamber is connected.
Oxygen Probe	Click this if OP-1 is connected.
Open System	Click this if a canopy cuvette or CFX-1 is connected.
Manual Cuvette	Click this if a Manual cuvette is connected.
Auto Cuvette	Click this if an Automatic cuvette is connected.

If you have any questions about your probe type, contact PP Systems (Contact Information on page 11).

Apply…

Associated with the Probe type selected above (Button caption is 'Apply Auto Cuvette' in this example). After selecting Probe connected above, click this box to get diagnostic reads associated with that Probe type.

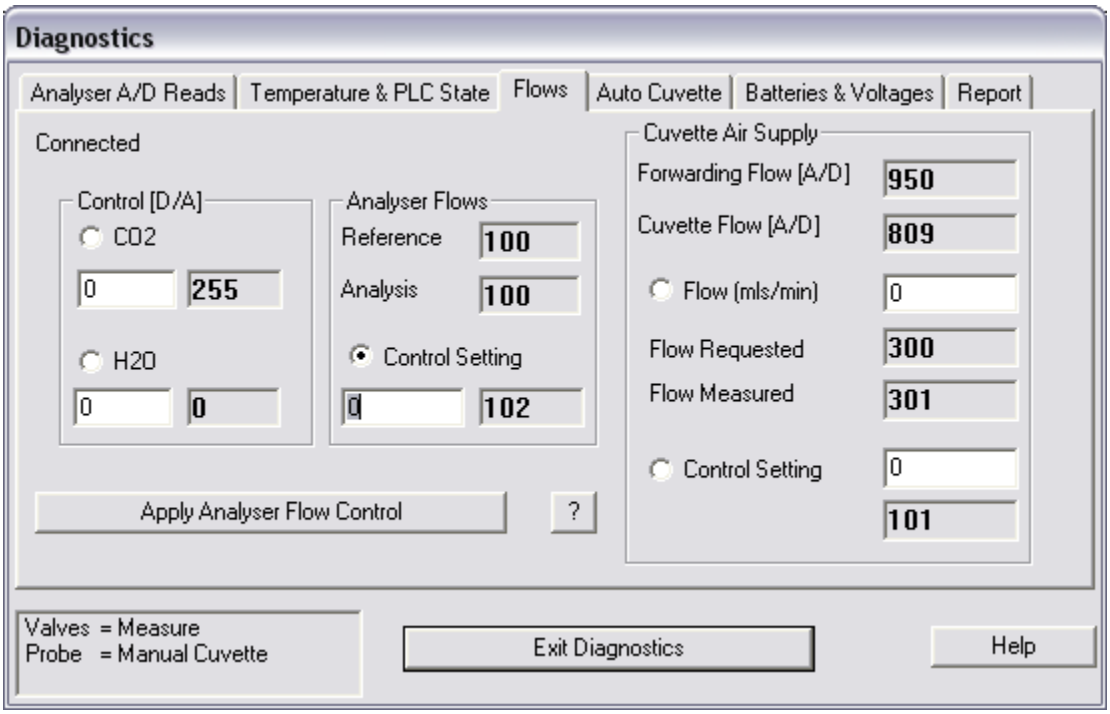
Flows

Control [D/A]

These are the control values that determine the proportion of the time that the CO_2 / H_2O control valves are open and therefore the concentrations. Click on the CO_2 / H_2O button to enter new values. Used for testing the valve operation.

Analyser Flows

These are the Reference and Analysis sample flow rates determined by the control setting. A setting of 73 gives about 50 ml flow and 102 gives approx. 100 ml. Used for testing the analyzer sample pumps.



Apply Analyser Flow Control

Click after changing analyzer flow setting to establish new flow.

Cuvette Air Supply

Forwarding Flow [A/D] / Cuvette Flow [A/D]

These are the outputs of the respective flowmeters. The forwarding flow should be about 100 greater than the cuvette flow. Used for testing the cuvette air supply.

Flow (mls/min)

Click on this so that a new value can be entered.

Flow Requested

Flow Rate that CIRAS-2 has been set to.

Flow Measured

Flow rate that is being delivered.

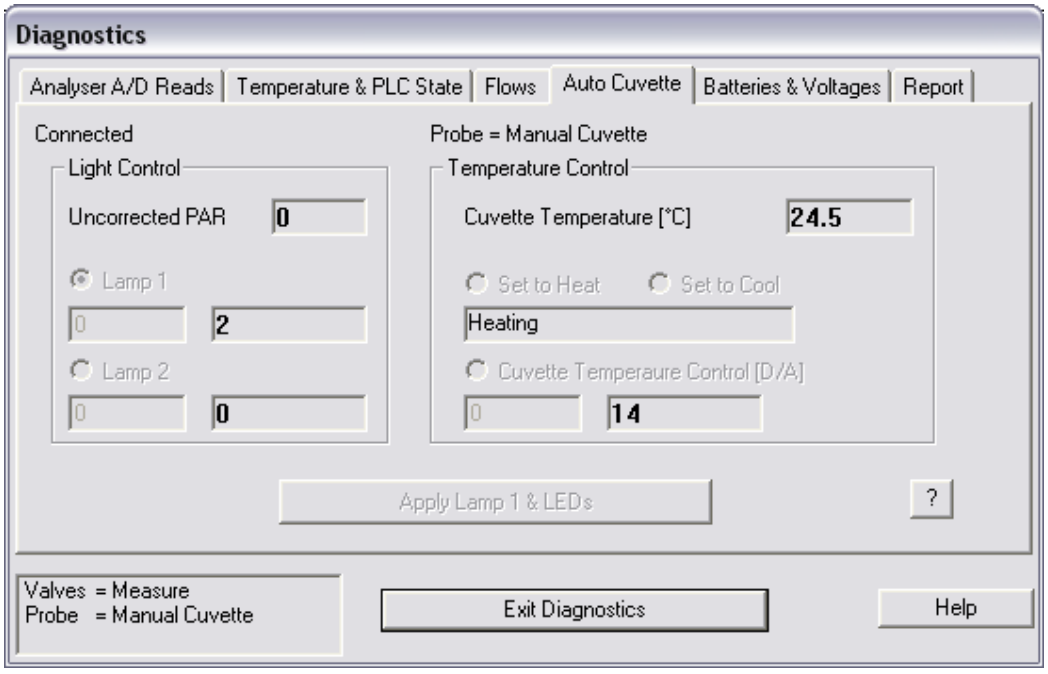
Control Setting

The current setting for the cuvette air supply pump driver. Typically it is a maximum of 113 at 470 mls/minute.

Auto Cuvette

Light Control

Uncorrected PAR is the reading of the PAR sensor. By entering new values for Lamp1 (central lamp in a PLC6(U)) and Lamp2 (lateral lamps) the intensity can be altered. Used for testing the PAR sensor and light units.



Temperature Control

Cuvette Temperature [^{o}C] is the reading of the cuvette air temperature sensor. Cuvette temperature control (0-255) can be used to alter the current through the temperature controlling peltier element. The direction of current flow and therefore whether there is cooling or heating is altered by the SET to HEAT/COOL buttons. Used for testing cuvette temperature control.

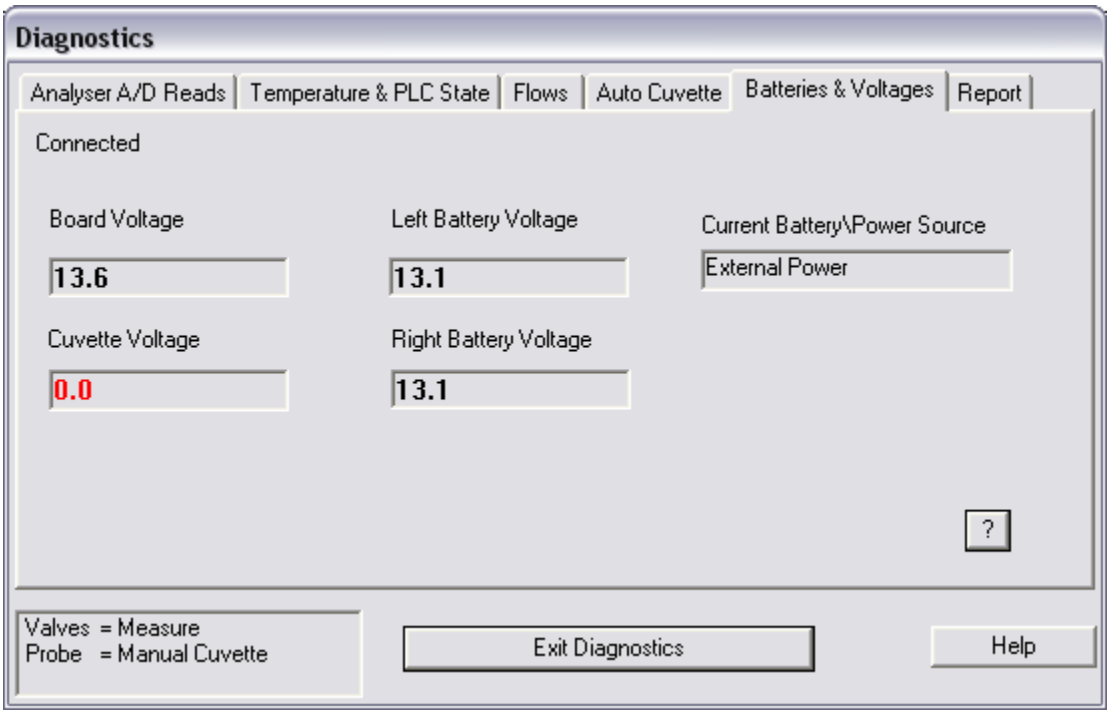
Apply Lamp 1 LEDs

To check Lamp 1 or an LED light unit after entering values, click on the button to verify that they are working properly. Lamp 2 only applies to the quartz halogen light source (smaller bulbs).

Batteries & Voltages

Board Voltage

Voltage available to CIRAS-2 board. This should be several hundred millivolts less than the battery voltage.



Cuvette Voltage

Voltage of the external cuvette power source. When an Automatic type of leaf cuvette is connected, this is the voltage measured on the PLC circuit board.

Left Battery Voltage

Refers to the current state of charge of the left battery. When fully charged, it should read > 13V.

Right Battery Voltage

Refers to the current state of charge of the right battery. When fully charged, it should read > 13V.

Current Battery/Power Source

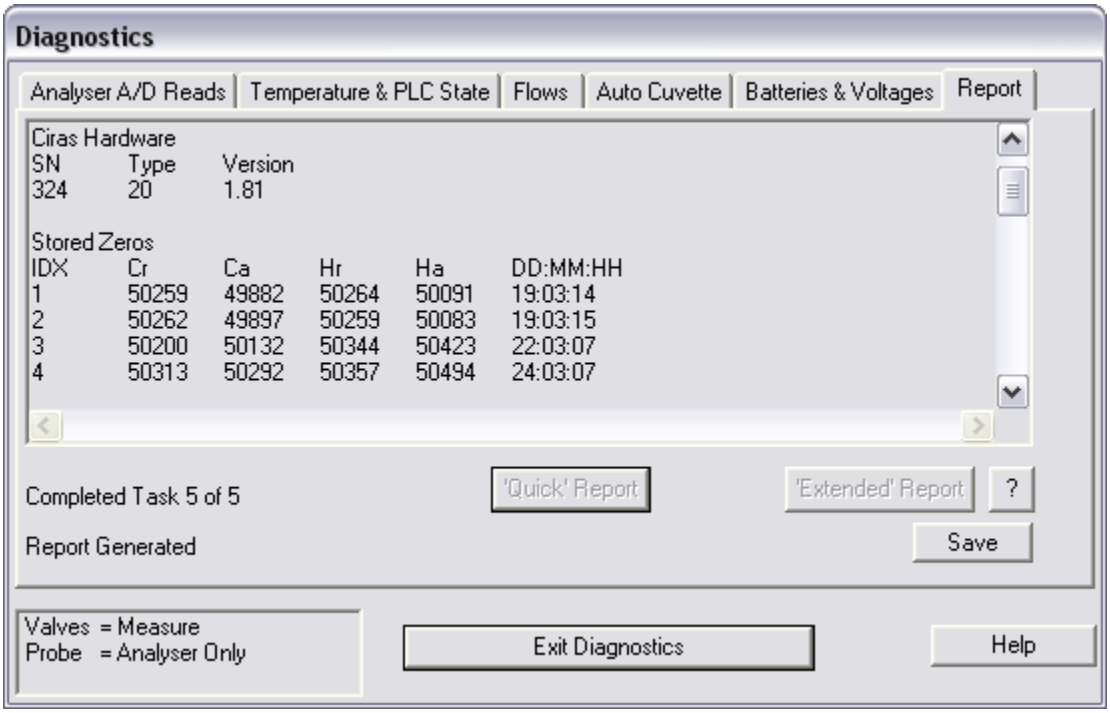
Refers to the source of power for CIRAS-2, whether it is battery (left or right) or the external power supply. When the voltage of one battery drops to approx. 10.5V, the CIRAS-2 automatically switches over to the other battery (if fitted).

Report

A final report can be generated to provide PP Systems with a general summary of the diagnostic data. Again, this information is very helpful for technical support purposes. It contains important information such as CIRAS-2 serial number, hardware, eprom version, date and time, analyzer (reference and analysis) A/D reads, etc. A file may also generated that can be emailed to us for analysis.

Quick Report

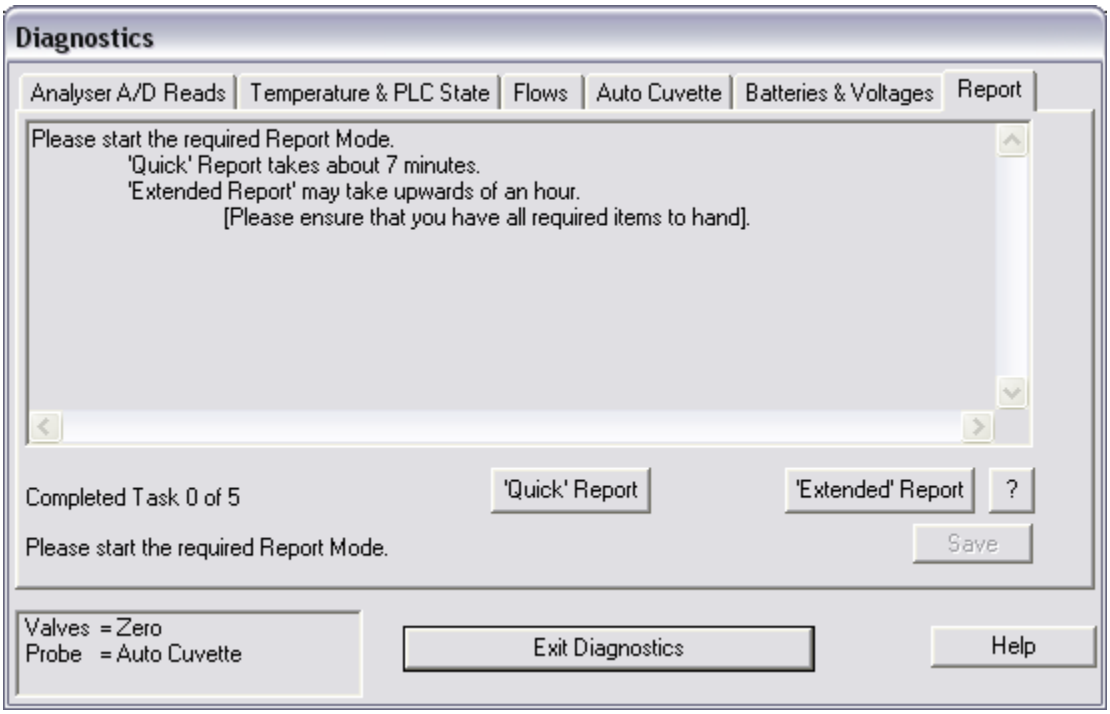
Generates a baseline set of information about the functional state of analyzers, pumps, valves, board electronic and accessories. Click **Save** and select a folder location for the resulting .txt file. The file may be useful to PP Systems Technical Support. A completed **Diagnostics 'Quick' Report** dialog is shown below.



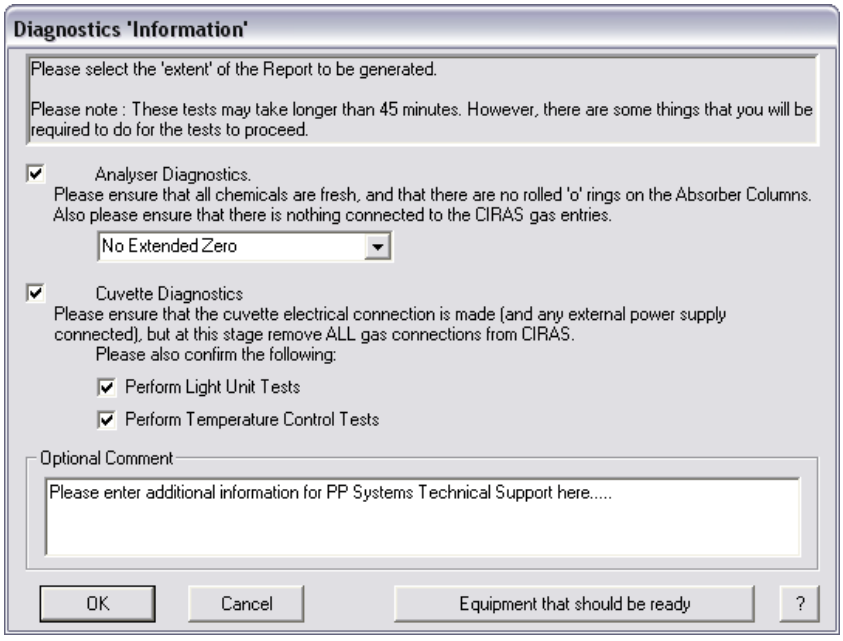
Extended Report

Performs, in a semi-automated fashion, a thorough set of routines to establish the state of the CIRAS-2 system. Two major 'sections', 'Analyser Tests' and' Leaf Chamber Tests'. It is recommended that both

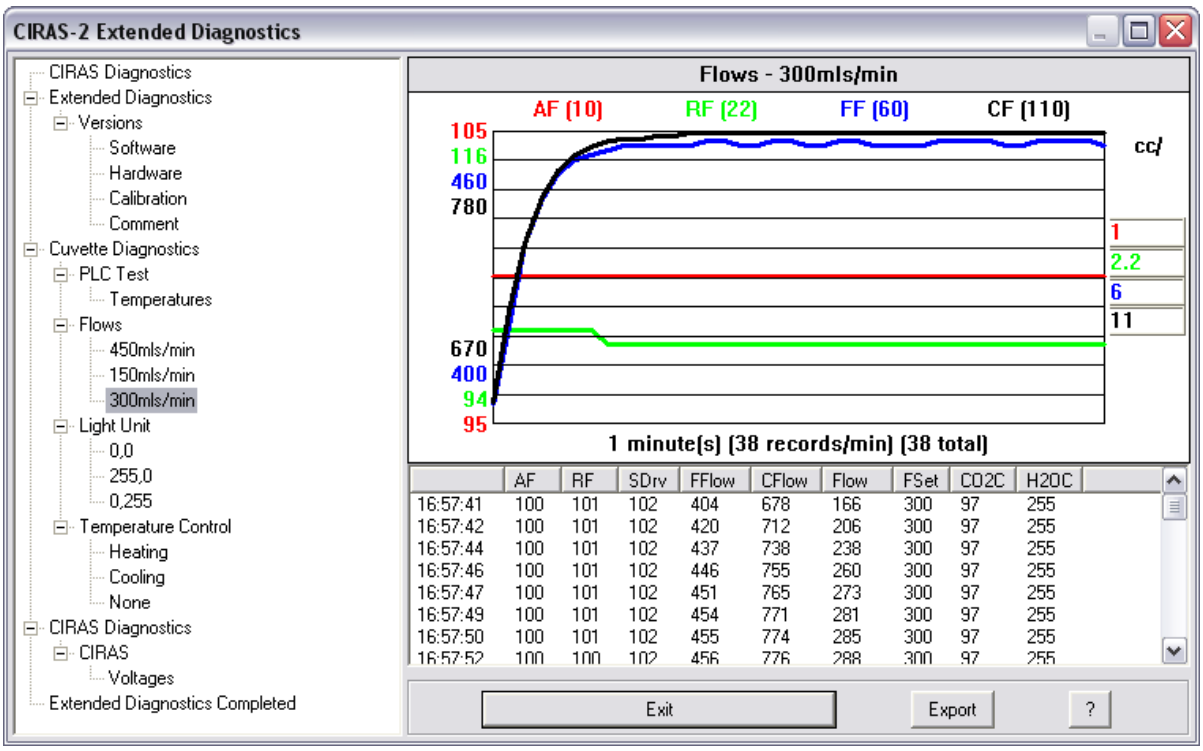
tests are performed where applicable. The user is required to perform certain functions during the process, such as connecting an external Zero Column (supplied) during the 'Analyser Test' and connecting a Leaf Cuvette during the 'Leaf Cuvette Tests'. Requires i) external CIRAS-2 Power Supply, ii) External Zero Column, iii) fresh chemicals, iv) external power supply to Leaf Cuvette (if needed, e.g. for the PLC5 cuvette or tungsten halogen light source. Approximately 3/4 of an hour required to perform the tests.



To begin click **Start Extended Diagnostics** in the first dialog and make the following selections:



Click **OK**, name and save the file, then follow the intructions on the automatic screens to make the gas connections and close the cuvette when prompted. When the automated tests have completed the following **CIRAS-2 Extended Diagnostics** summary dialog will be available. You can view any section of the diagnostics by selecting it from the expandable list on the left.



You can view the file at any time from the by going to **File – View Diagnostics Report** from the CIRAS-2 menu bar. You must be OFFLINE to view the file (**Connections – Disconnect** in the CIRAS-2 menu bar).

Calibration

CIRAS-2 is a very stable instrument and should NOT require recalibration.

This is because the ZERO function corrects for nearly all changes that give rise to drifts in calibration.
The common cause of a drift in CIRAS-2 readings is an incorrect ZERO due to either exhausted chemicals or to leaks on the ZERO columns from rolled or damaged O-rings etc. This is therefore easily corrected.

The second cause, though fortunately less common, is liquid water through the cells, affecting their reflectivity and therefore the linearity.

The only reason to re-calibrate CIRAS-2 is to use your own laboratory standard, rather than relying on our calibration. Regularly **check** the CIRAS-2 calibration as changes signify instrument problems. For CO_2, use the internal checking against the mixture generated by CIRAS-2 or a standard gas and for H_2O, check against an H_2O calibrator or other standard source. Another quick check for CO_2 is to use ambient air drawn from a position away from any CO_2 source such as cars, boilers, breath etc. Ambient air is in the range 340-360ppm. CO_2 and H_2O calibrations and checking are done separately but they may be done consecutively in any order. The procedure for checking or calibrating is similar.
The contents of the absorber columns must **NOT** be exhausted. The analyzer must have been switched on for at least 20 minutes minimum.

Required equipment/supplies -

- A standard gas for CO_2 calibration or .if using the CIRAS-2 internal standard, then a CO_2 cylinder should have been recently inserted.
- A source of known water vapour content for H_2O calibration. (We strongly recommend using our Water Vapour Calibrator supplied with the CIRAS-2).

****** WARNING ******

It is absolutely critical that the analyzer ZERO columns (columns 1 & 2) are fresh before performing CO_2/H_2O calibrations. If the chemicals are not fresh, it will cause the ZERO to drift, thus causing an inaccurate calibration.

External connections for CO_2 Calibrations

CO_2 calibrations using the internal standard

Connect the cuvette air supply AIR OUT and the REF inlet through a T-piece with 30cms of vent pipe on the third leg. The CO_2 calibration concentration should be set to 0000. On entering calibration CIRAS-2 will switch on the air supply to 300 ml/minute and will fully open the CO_2 control valve.

CO_2 calibrations using a gas cylinder

The gas cylinder must **not** be connected directly to CIRAS-2. Instead, connect through a T-piece. One leg goes to the REF inlet, the 2nd leg to the gas cylinder, and the remaining leg vents to atmosphere

through a 30 cm tube. The flow rate from the gas cylinder should be set so that there is a small excess, say 100-200 ml/minute, flowing to atmosphere. The total flow is then 300 to 400 ml/minute.

ON NO ACCOUNT PLACE THE VENT TUBE INTO WATER TO INDICATE SURPLUS FLOW. IF CIRAS-2 INGESTS WATER, IT IS LIKELY THAT THERE WILL BE DAMAGE TO THE CELL SURFACES, THE DETECTOR WINDOWS, THE IR SOURCE, THE PUMPS AND THE MASS FLOWMETERS.

Alternatively:

Fill a gas sample bag with the calibration gas direct from the cylinder. Now attach the gas sample bag inlet to the REF AIR IN and the CIRAS-2 will draw out a sample without any risk of pressurization.
Please note: Be sure to initially partially fill and empty the bag to ensure that the sample is truly the calibration gas.

If possible, the standards should have a concentration slightly greater than the usual measurement. It is better not to calibrate with a concentration much smaller than the measurements (i.e. do not use a 300 ppm standard when measuring at 2,000 ppm.

H_2O Calibration sources and connections

For H_2O calibration, an air supply of known water vapor concentration is required (mb). There are a number of ways that this can be obtained that are listed in the technical manual.

It is however assumed here that you will be using the water vapor calibrator supplied with your system. Instructions for use of the H_2O calibrator can be found in the CIRAS-2 Technical Manual.

PP Systems' Water Vapor Calibrator

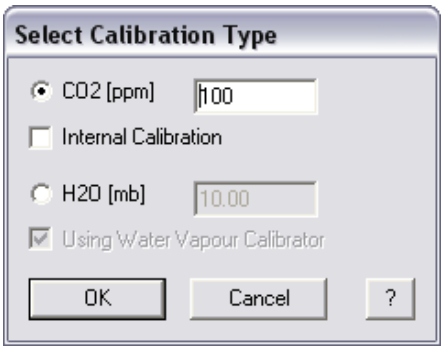
The calibrator consists of an insulated container filled with ferrous sulphate salt ($FeSO_4.7H_2O$). The unit is fitted with thermistor temperature sensors measuring ambient air temperature and the temperature of the air emerging from the $FeSO_4.7H_2O$. The water vapour pressure above the $FeSO_4.7H_2O$ is a function of the salt temperature as shown in the table in the CIRAS-2 Technical Manual. The salt can humidify many litres of air, and it should last many years as it is being used in a re-circulating mode. The degree of exhaustion of the salt can be determined by its weight as shown on the test certificate beneath its cap or on the test sheet supplied with your documentation. For an in-depth discussion please see:

Ref Parkinson K.J. and Day W. (1981) Water Vapour Calibration using Salt Hydrate Transitions. J. Expt Bot. 32, 411-418.

- The calibrator should be both stored and operated in a relatively stable temperature environment.
- The calibrator and CIRAS-2 should be placed together in a stable temperature to allow their temperatures to equilibrate for several hours before use. If the equilibrator is warmer than CIRAS-2 then there could be a risk of condensation.
- The electrical connection is to the PLC connector.
- The REF air inlet should be connected to the bottom pipes on the calibrator and the REF and AN outlets to the two top pipes.
- Pipe connections should not be made until it has been ascertained that there are no error messages from CIRAS-2 that indicate the calibrator temperature is too high.

Performing Calibrations

Allow the CIRAS-2 to warm-up for at least 30 minutes. From the above notes, connect up CIRAS-2 to the calibration source then from the PC Windows display, select **Utilities** – **Calibration** - **Start**. Next, click on the type of calibration (CO_2 or H_2O) and if you are using an external CO_2 source, enter the concentration from the gas cylinder. Click **OK**.

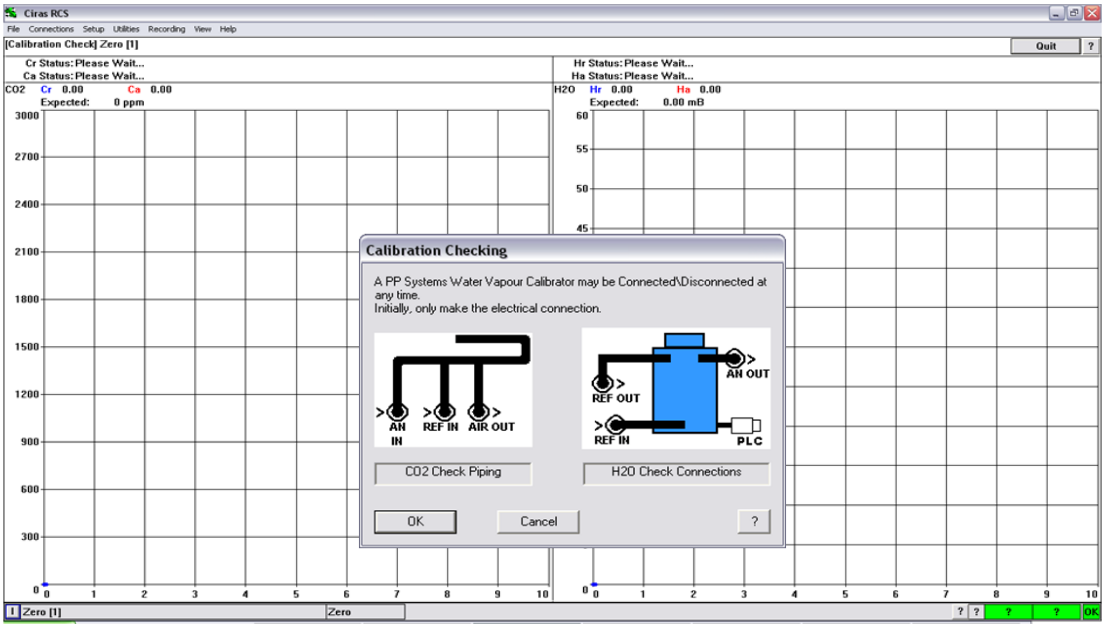


CIRAS-2 will now perform a zero and when the displayed concentration is steady click the corresponding button. After calculating the calibration factors, the existing and new factors will be displayed. The option is then given to use the new factors, retain the existing factors or return to the factory defaults.

If it is decided to use the new factors then it is recommended that the calibration procedure be repeated to check their veracity.

Calibration Check

A calibration check feature is designed into the system to verify system calibration for both CO_2 and H_2O. The internal standard is used for the CO_2 checking and the water vapour calibrator for the H_2O.
Click on **Calibration Check - Start**. Connections and precautions are as described under the corresponding calibration.



CO_2 Calibration Checking:
Connect a vented T Piece to 'REF IN' and 'AIR OUT' on the CIRAS-2. Make sure that nothing is plugged into the PLC Socket.

If the status values are not OK, check that there is flow from the CO_2 Exhaust (Located on the base of CIRAS-2). Check also that the ZERO columns are seated properly and the chemicals not exhausted.

H_2O Calibration Checking:
Connect the lower gas tube of the Water Vapor Calibrator to 'REF IN', and the upper gas tubes to 'REF OUT' and 'AN OUT'. Make sure that the calibrator is plugged in to the PLC Socket.

If the status values are not OK, check that the ZERO columns are seated properly and not exhausted.

This utility allows checking of system calibrations in a safe and easy way. As with all Calibration and Stored Diff-Bal processes, the CIRAS-2 should have warmed up for at least 30 minutes. The CO_2 cylinder should be fresh and the state of the chemicals checked for exhaustion, including Molecular Sieve.

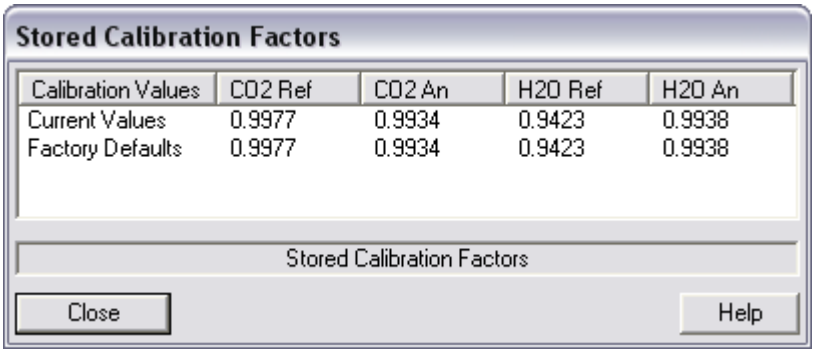
When first started, you will be prompted that a Water Vapor Calibrator may be connected/disconnected at any time. When using a Water Vapor Calibrator Please Note:

Initially, only make electrical connection so that the CIRAS-2 can confirm that the salts in the Calibrator are at an acceptable temperature. If the salts are too hot, and ambient air temperature is low, there is a risk of condensation in the CIRAS-2. If such a warning is issued, ensure that the gas connectors are disconnected and allow the Calibrator to equilibrate to the ambient air temperature.

The presence of a Water Vapor Calibrator is automatically detected and determines whether you are checking CO_2 or H_2O calibrations. After connection/disconnection of the calibrator, wait for the CIRAS-2 to settle.

While not connected, a prompt will read 'No H_2O Calibrator' in the H_2O area. Otherwise, values and deviations will be displayed, and the CO_2 area will display 'H_2O Calibration Check'.

Current Calibration Constants and the Factory Default values may be seen by clicking on the ? button in the upper right hand cornern of the display. For both CO_2 and H_2O concentrations, you will be informed of the deviation from expected of both reference and analysis values and whether the values are within an acceptable range.

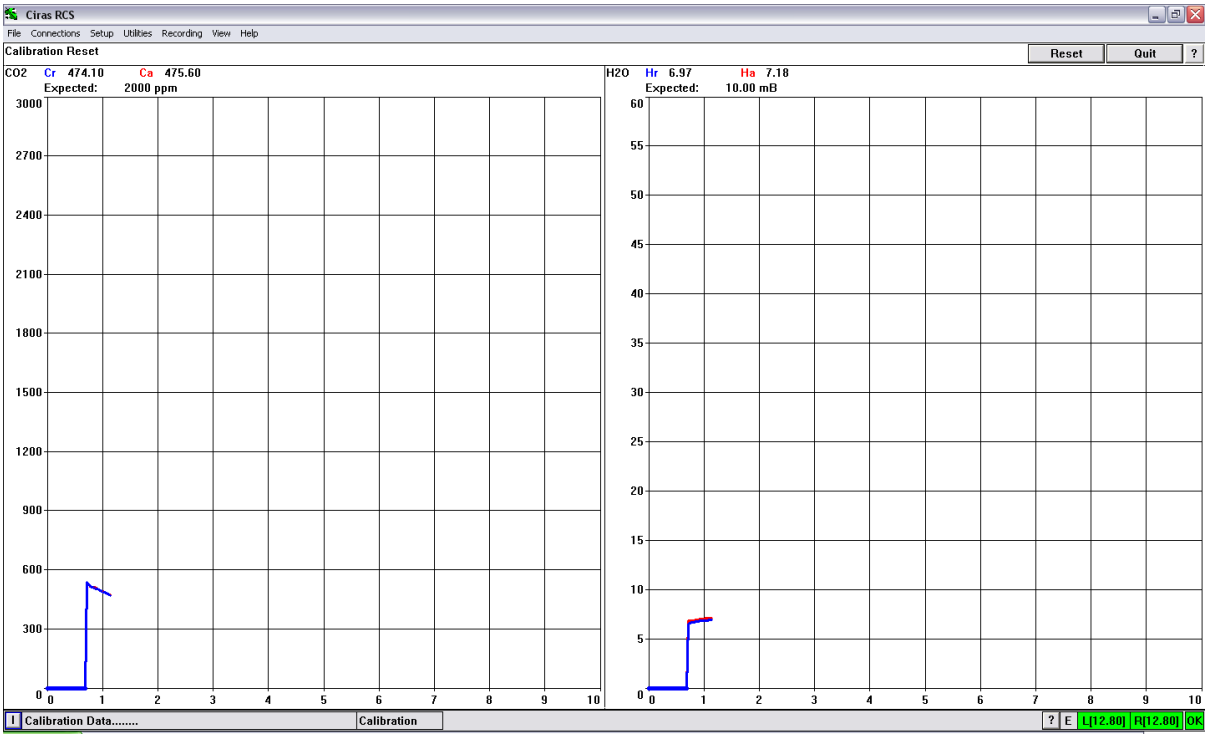


CO_2 checking values are determined by the Blender and the maximum range of the CIRAS-2. H_2O checking values are determined by the temperature of the Water Vapor Calibrator and H_2O concentrations are set accordingly.

To Quit Calibration Checking, click the Quit button. After completion of a Calibration check, the CIRAS-2 will perform a **Diff Bal**.

Calibration Reset

Allows resetting of Calibration to Factory Default. Click on **Calibration Reset - Start**. After entering **Calibration Reset**, the CIRAS-2 transmits the Current and Factory Default Calibration constants. These values may be seen by clicking on the ? button in the upper right hand corner of the display.



CIRAS-2 will perform a **Zero** and on completion begin transmitting the calibration data, but the values at this stage are irrelevant.

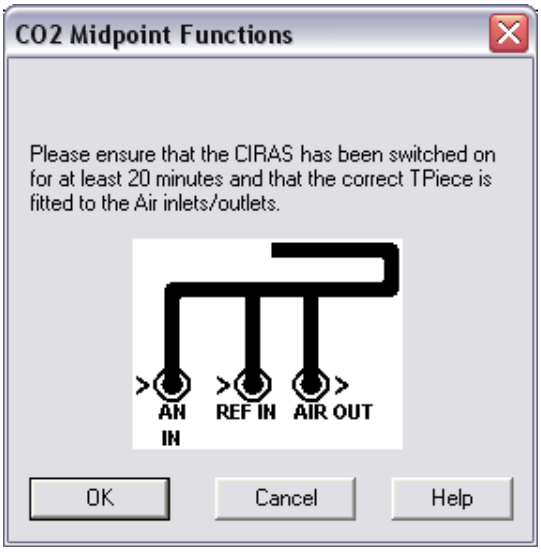
If the Reset Button is clicked, CIRAS-2 will be instructed to reset the Calibration Constants to the Factory Default and CIRAS-2 will then return to the measurement mode using those factors after performing a Diff-Bal. Calibration Reset may be quitted at any time by clicking the Quit button.

CO2 Midpoint

It is important that the CIRAS-2 has been turned on for 20 minutes and that the CO_2 cartridge is full and the chemicals are fresh. Additionally, a special T-Piece needs to be fitted to the CIRAS-2 prior to starting CO_2 Midpoint setting.

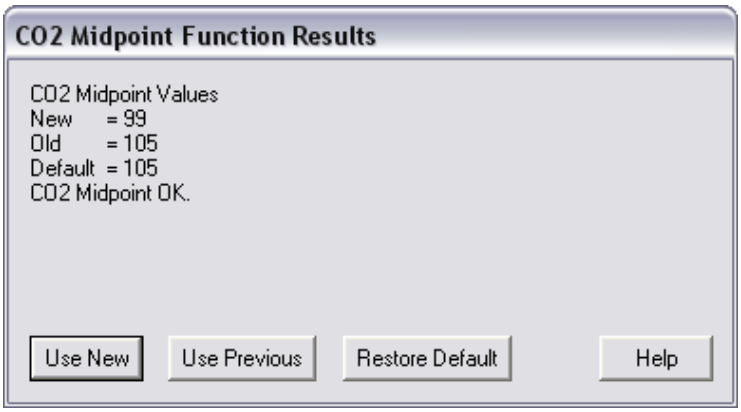
Gas Connections for CO_2 Midpoint

The following gas connection should be made and fitted to the CIRAS-2 prior to commencing a CO_2 Midpoint calculation. Click on **CO2 Midpoint - Start**. The CO2 Midpoint Functions window will appear with the following message:



At this stage, the CO_2 Midpoint Values will be displayed with 3 options available to you:

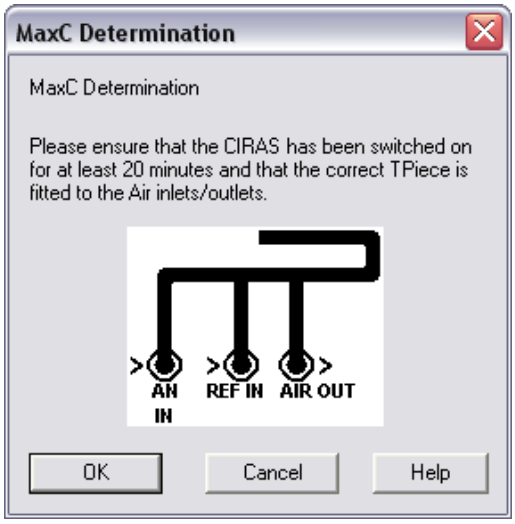
Click On	To:
Use New	Set the CO_2 Midpoint to the new values generated by the CIRAS-2.
Use Previous	Use the previously stored CO_2 Midpoint generated by the CIRAS-2.
Restore Default	Set the CO_2 Midpoint back to the factory default value.



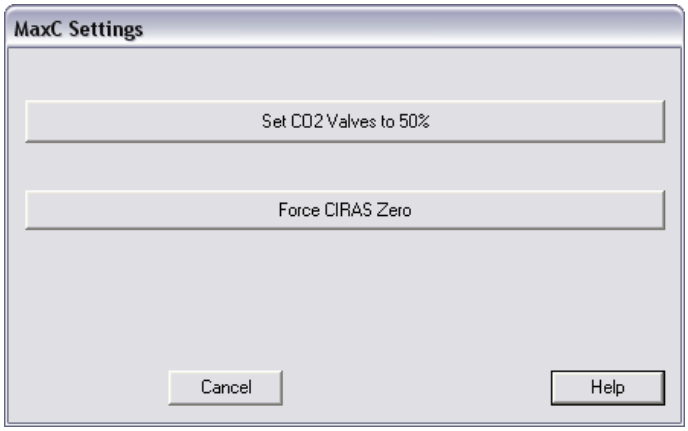
Upon selection of the appropriate option, CIRAS-2 will apply the setting, return to Measure mode and perform a Diff-Bal. The Software will return to the Measurement Screen. If unsuccessful, the “Close the Dialog” box will be available and the CIRAS-2 will have returned to Measure Mode. The CO_2 Midpoint function can be exited at any time by pressing Quit .

Max C Determination

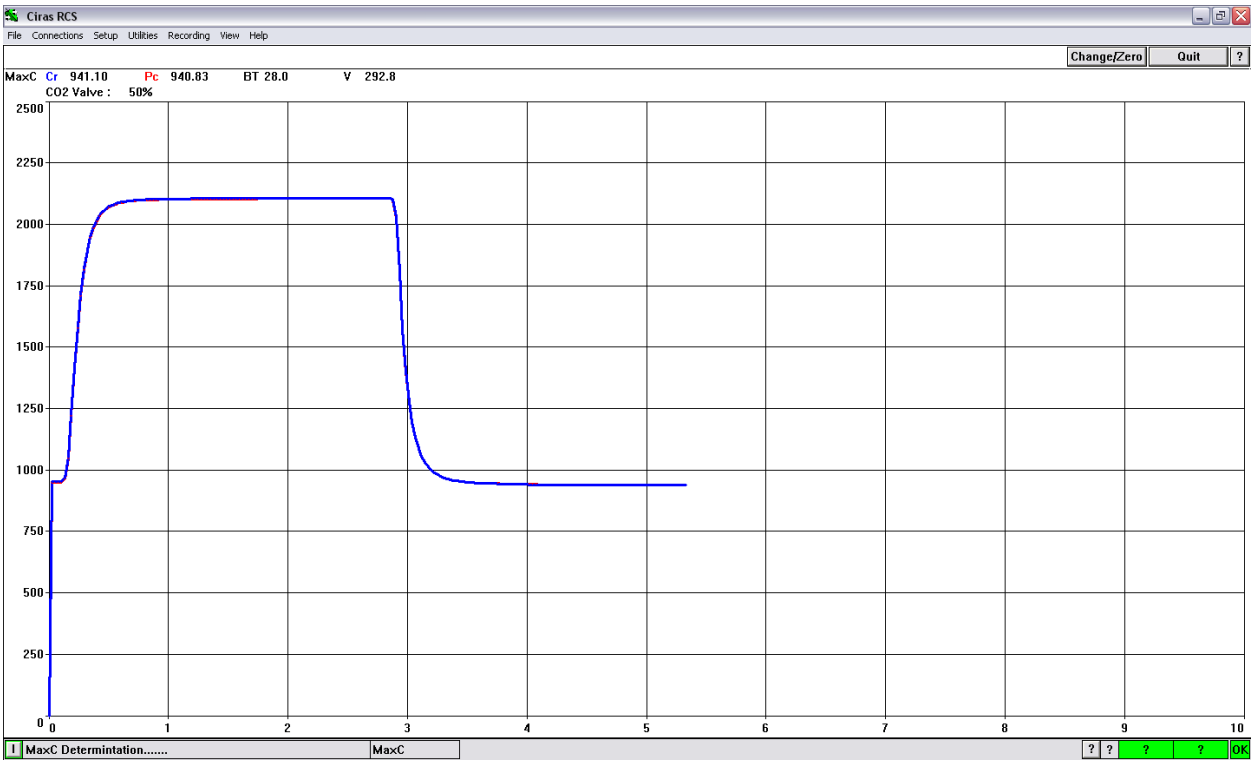
The MaxC Function is a diagnostic tool to assess the maximum CO_2 factory settings. It is important that the CIRAS-2 has been turned on for 20 minutes and that the CO_2 cartridge is full and the chemicals are fresh. A special T-Piece needs must be fitted to CIRAS-2 prior to starting. Click **Utilities – MaxC Determination – Start**, and ensure that the gas connection are correct:



MaxC is divided into 2 parts, firstly with the CO_2 Valve set to 100% (Fully Open) and then with the CO_2 Valve set to 50%. At each stage, CIRAS-2 should be allowed to reach a stable Cr (CO2 Reference) reading. At this point a **Zero** should be forced. After the **Zero**, allow the system to re-stabilize. Valve Setting and ZERO can be initiated from the Change/Zero button.



When set to 100%, the CO_2 Valve should be silent. At 50%, there should be a regular clicking heard as the valve opens and closes.



The following values should be recorded and supplied to Technical Support:

After completion of the 100% Step

Pc (Max) Pressure Corrected Term
BT Blender Temperature
V Flow

After completion of the 50% Step:

Pc (Mid)

The **MaxC** function can be exited at any time by clicking the Quit button.

Appendix 1. Cuvettes and Probes

Identification of Leaf Cuvette Types

The CIRAS-1 was introduced in 1992. Since that time, there have been several changes in the design of cuvettes. In the following, (U) refers to UNIVERSAL, (B) refers to BROAD, (R) to RICE , (N) to NARROW, (C) to CONIFER and (P) to POD style cuvettes.

Along with the CIRAS-2, we have produced a new type of auto-cuvette, the PLC6 (U) Universal that uses a series of inserts for differing types of leaves. Any of our previous cuvettes are compatible with CIRAS-2. For safe and accurate operation, CIRAS-2 must be set up with control parameters for the type of cuvette that is in use. Cuvettes supplied with CIRAS-2 and the PLC6 (U) will already be labelled. If you are using an older cuvette, you must identify it. The following tables will help.

Examination of the window material, its shape and size, and the size of the leaf aperture that can be seen beneath it, will lead to the identification of your cuvette.

Leaf Cuvettes fitted with a FLAT GLASS WINDOW

Cuvette	Type	Dimensions of Glass Window	Leaf Aperture
PLC6 (U)	Automatic	32mm x 32mm	25mm x 18mm 25mm x 7mm 18mm Diameter
PLC4 (N)	Standard	50mm x 25mm	
PLC5 (N)	Standard or Automatic	85mm x 32mm	
PLC4 (B)	Standard	25mm x 25mm	18mm Diameter
PLC4 (R)	Standard	25mm x 25mm	7mm x 25mm
PLC5 (B)	Automatic	32mm x 32mm	18mm Diameter
PLC5 (R)	Automatic	32mm x 32mm	7mm x 25mm

Leaf Cuvettes fitted with a PLASTIC HEMI-CYLINDER

Cuvette	Type	Diameter of Cylinder	Length of Cylinder
PLC4 (C)	Standard	50mm	70mm
PLC5 (C)	Standard or Automatic	50mm	85mm
PLC4 (P)	Standard	25mm	100mm
PLC5 (P)	Standard or Automatic	25mm	85mm
PLC5 (B)	Automatic	32mm x 32mm	18mm Diameter
PLC5 (R)	Automatic	32mm x 32mm	7mm x 25mm

Having identified the cuvette type, we recommend placing a label on your cuvette or document it somewhere in your operator's manuals for future reference.

CIRAS-2 Settings for Leaf Cuvettes and Probes

For CIRAS-2 to work correctly with the various types of cuvettes, the CIRAS-2 control parameters must set up accordingly. These values are listed in Appendix 1.

Parameter values are dimensionless with the exception of LAR (leaf area) (cm^2), Rb (boundary layer) (m^2 s mol^{-1}), Flow (ml $minute^{-1}$) and TR (W μmol^{-1}).

The Rb values are typical for the cuvette type, but the specific factory-determined value for your cuvette is recorded on the cuvette handle. The flow values are general recommendations for that type of cuvette, but may be altered for specific circumstances.

The area values should be set to the proper values, the values listed are to ensure that a typical value is used in the calculations.

SUN, LED, and QI refer to the type of light incident on the leaf. SUN values should be used under normal daylight, LED with the PP Systems LED light unit and with any fluorescent lights and QI with any incandescent lamps or quartz halogen bulbs.

If you are using PLC4 (B/R) or PLC5 (N) (C/P) with a CIRAS-2, then use the settings that are listed for these cuvettes when used with CIRAS-2-1

PLC	DELTA COOL	MAX COOL	MAXQ	RAD X	LAR	Rb	FLOW	SUN PARX	SUN TR	LED PARX	LED TR	QI PARX	QI TR
CIRAS-1 PLC's Adapted for CIRAS-2													
AUTO													
PLC5(B)	9	190	2000	1.0	2.5	0.3	200	1.0	0.17	1.0	0.14	1.0	0.16
PLC5(R)	9	190	2000	1.7	1.7	0.3	200	1.0	0.17	1.0	0.14	1.0	0.16
PLC5(N)	20	190	1000	(1.0)	10	0.15	300	1.0	0.17	1.0	0.14	1.0	0.16
PLC5(C/P)	20	190	1500	(1.0)	10	0.15	400	1.0	0.23	1.0	0.15	1.0	0.19
MANUAL													
PLC4(B)	(9)	(190)	(2000)	(1.0)	2.5	0.3	200	1.0	0.14	1.0	0.12	1.0	0.12
PLC4(R)	(9)	(190)	(2000)	(1.0)	1.7	0.3	200	1.0	0.14	1.0	0.12	1.0	0.12
CIRAS-2 PLC6(U) with INSERTS													
Broad	14	255	2000	2.5	2.5	0.3	200	1.0	0.17	1.05	0.14	1.30	0.16
Rice	14	255	2000	2.5	1.7	0.3	200	1.0	0.17	1.05	0.14	1.30	0.16
Large.	14	255	2000	2.5	4.5	0.3	300	1.0	0.17	1.05	0.14	1.30	0.16

PLC (B/R) Manual and PLC(N) (C/P) supplied with CIRAS-1 are identical to those listed for CIRAS-2.

The default setting of AVLIMIT is 30 (=3ppm) for all the above.

PROBETYPE 3 = External Air Supply Open Systems.

Parameters for PP Systems standard units.

Type	FLOWZERO	FLOWX	AVLIMIT
5 Litre	1250	7.63685	100
20 Litre	5000	30.5474	100

When using **CLOSED SYSTEMS**, the PROBETYPE should be set to 1 and the AVLIMIT to 1.0.

When the PROBETYPE is set to 1 or 2, the PARX value should be set to 2. PAR will then be correctly output with RH/T/PAR sensors. For Oxygen, 30% will be equal to a PAR reading of 3000.

Appendix 2. CIRAS-2 Consumables and Spares

Consumables/Spares for CIRAS-2 System

CIRAS-2

Cat. No.	Description
STD086	Inlet Filters, 3 micron, PTFE hydrophobic (Qty. 6)
10045-1	Filter Assembly
STD006	Molecular Sieve Desiccant, 1.25 Lbs.
STD007	Self Indicating Soda Lime, 1.0 kg
STD008E	Envirogel, 1.25 Lbs.
STD030	Box of 10 8g. CO2 Cartridges
10044-2	Water Vapor Equilibrator (For CIRAS-2)
20041-4	Absorber Column only (CIRAS-1 and CIRAS-2)
10063-2	Absorber Column & End Fittings (CIRAS-1 and CIRAS-2)
STD017-2	Absorber Column “O” Rings & Filters for CIRAS-2 (1 Set)
STD088	GAST-5 Replacement Seals
STD044-2	CIRAS Forwarding Pump (GAST)
STD075	Miniature Rotary Pump (ASF)
STD076	Sealed Miniature Rotary Pump (ASF)
STD002N	Autozero Solenoid Valve (KV Microsol)
STD045	Control Solenoid Valve (Lee LFAA)
STD090	12V NiMH Battery Pack for CIRAS-2 (Complete)
41520-1	12V NiMH Battery Cells, Shrink Wrap (CIRAS-2) - For Refurbishing
STD092	12V NiMH Battery Charger for CIRAS-2
STD539	External Power Supply Cable for CIRAS-2 (with battery clips)
STD093	Mains Power Supply for CIRAS-2
STD094	RS232 Cable (9pin I/F) for CIRAS-2
STD505	Null Modem Cable (CIRAS-2)
STD521	USB to Serial Adapter
STD095	CO2 Removable Regulator Body for CIRAS-2
STD096	CO2 Cylinder Holder for CIRAS-2
STD037	CO2 Regulator Cylinder “O” Rings (Qty. 10)
STD089	CO2 Regulator Body “O” Rings (Qty. 5)
STD087	CO2 Regulator Pressure Release Seals (Qty. 5)
STD053*	20mm Fuses, Qty. 5
	(* specify rating = 2,3.15 or 5 A)

Leaf Cuvette

Cat. No.	Description Leaf Cuvettes & Chambers Spares (for use with CIRAS-1/CIRAS-2)
STD010	Cuvette Impeller Blades (* type B,N,C or P)
STD011	Cuvette Impeller Motor (for all (B) / V1 (N)
STD524	PLC4/5/6 (B) Broad Gaskets, 18mm Diameter, Qty. 10
STD525	PLC6 (U) Large Broad Gaskets, 25x18mm, Qty. 10
STD526	PLC6 (U) Small Broad (Rice) Gaskets, 25mmx7mm, Qty. 10
STD527	PLC6 (U) Rear Gaskets, 50x35mm, Qty. 10
STD528	PLC4 (N/C/P) Gaskets, 70x50mm, L-Shapes to be cut to fit, Qty. 10
STD529	PLC5 (N/C/P) Gaskets, 88x40mm, Qty. 10
STD530	Gasket Material, 1m x 100mm (approx)
20347-1	Gasket Plate, 25mm x 7mm, Upper
20347-2	Gasket Plate, 25mm x 7mm, Lower
20348-1	Gasket Plate, 25mm x 18mm, Upper
20348-2	Gasket Plate, 25mm x 18mm, Lower
20349-1	Gasket Plate, 18mm Diameter, Upper
20349-2	Gasket Plate, 18mm Diameter, Lower
10088-1	PLC6 (U) Plain Glass Window Assembly (for use with CFM)
10088-2	PLC6 (U) Calflex Window Assembly
20359-1	PLC6 (U) Window Plate Only
55021-1	PLC6 (U) Calflex Window Only
55021-2	PLC6 (U) Plain Glass Window Only (for use with CFM)
STD104	CIRAS-2 Cuvette Air Pipe Connector Set
30021-4	Butyl Tubing, 2.5mm ID/5.0mm OD (Per Meter)
STD015	Leaf Temperature Thermistor including plug
41505-2	Fan Axial, 40mm, 12V (For Leaf Cuvettes)
STD028	Standard Light Unit Bulb (20W)
STD540	Axial Bulb, Tungsten (6V, 60 mA)
STD054	Set of Automatic Light Unit Bulbs (3 main/2 axial)
STD050BA	Glass IR Filter for PLC (B) Auto Light Unit (25mm x 25mm)
STD050B	Glass IR Filter for PLC (B) Standard Light Unit (32mm x 32mm)
STD050N	Glass IR Filter for PLC (N) Standard Light Unit (25mm x 50mm)
STD053*	20mm Fuses (Qty. 5) for Automatic Cuvettes
	(* specify rating CRS079/24 = 10A, CRS077/75/73=15A
STD534	PLC Auto External Power Supply Cable
STD067	Set of Gauzes for Light Unit (Specify type of Light Unit)
STD068*	Diffuser for Light Unit (* specify type of Light Unit)

Appendix 3. Photosynthesis Equations Used in CIRAS-2

Calculations

Step 1.0

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

The CIRAS-2 mass flowmeter is calibrated to read the volume flow at 20 °C and 1013.25 mb (V_{20}). Molar volume is 22.414 at 0 °C and 1 standard atmosphere (STP). Therefore:

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

Where: ***a*** is projected leaf area (cm^2). The V_{20} term is calculated above using $cm^3\ sec^{-1}$, but displayed by CIRAS-2 as ml min^{-1}.

Step 2.0

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

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

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

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

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

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

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

(2.4) Therefore:

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

Clarification of nomenclature:

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

ii) e_{out} is defined as the partial pressure of water vapor of air inside the cuvette, surrounding the leaf. This air is both highly mixed by the stirring fans and influenced by transpirational water vapor. Its partial pressure is determined by the Analysis H_2O IRGA. As relates to the calculated values in the CIRAS-2 display:

$$e_{out} = \left(H_{ref} + H_{diff}\right) = H_{analysis}$$

Step 3.0

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

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

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

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 infrared to PAR in the light source, transmission through the cuvette window (*Trans*), and reflection/absorption by the leaf: (H = Q x *Trans*).

The following approximation is made in the program:

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

(3.2) From this we derive:

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

Step 4.0

*Derive i) saturated vapor pressure at leaf temperature (**e_{leaf}**) from **T_{leaf}** and ii) stomatal resistance (**r_s**)*

(4.1) From *Reference 2*:

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

(4.2) Stomatal resistance (to water vapor) is derived by:

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

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

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

(4.4) Because,

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

(4.5) Then,

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

(4.6) It follows that stomatal conductance is the inverse of stomatal resistance:

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

Step 5.0

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

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

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

(5.2) Therefore,

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

CIRAS-2 calculates and displays the CO_2 difference (C_{out} - C_{in}). As relates to the calculated values in the CIRAS-2 display:

$$\boldsymbol{C_{out} = \left(C_{ref} + C_{diff}\right) = C_{analysis}}$$

Step 6.0

Calculate CO_2 concentration in the sub-stomatal cavity ($\boldsymbol{C_i}$) using the equation derived by von Caemmerer & Farquhar (Reference 3)

(6.1)

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

(6.2) Where:

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

Please note: These calculations are based on the following assumptions:

i) the leaf is exposed on both upper and lower leaf surfaces

ii) the upper and lower boundary layer resistances are similar

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

Symbol Definitions

Measured Parameters

Symbol	Measured Parameter	Unit
V_{20}	Mass flow of dry air into cuvette at STP	$cm^3\ sec^{-1}$
a	Projected leaf area	cm^2
r_b	Boundary layer resistance to water vapor	$m^2\ s\ mol^{-1}$
P	Atmospheric pressure	mb
Q	Photon flux density incident on cuvette	$\mu mol\ m^{-2}\ s^{-1}$
T_c	Cuvette air temperature	°C

Calculated Parameters

Symbol	Calculated Parameter	Unit
W	Mass flow of dry air per unit leaf area	$mol\ m^{-2}\ s^{-1}$
e_{in}	Partial pressure of water vapor of air *entering* cuvette	mb
e_{out}	Partial pressure of water vapor of stirred cuvette air	mb
E	Transpiration Rate	$mmol\ m^{-2}\ s^{-1}$
e_s	Saturated vapor pressure at cuvette air temperature	mb
e_{leaf}	Saturated vapor pressure at leaf temperature, inside the leaf	mb

Symbol	Calculated Parameter	Unit
T_{leaf}	Leaf Temperature	°C
Δt	Temperature difference between the air and the leaf	°C
H	Radiation absorbed by the leaf	W m^{-2}
r_s	Stomatal resistance to water vapor	m^2 s mol^{-1}
g_s	Stomatal conductance to water vapor	mmol m^{-2} s^{-1}
C_{in}	CO_2 concentration of air *entering* cuvette	µmol mol^{-1} *
C_{out}	CO_2 concentration of air *inside* and *exiting* the cuvette	µmol mol^{-1} *
A	Rate of CO_2 Assimilation (Net Photosynthetic Rate)	µmol m^{-2} s^{-1}
g_c	Total conductance to CO_2 transfer	mmol m^{-2} s^{-1}
C_i	CO_2 concentration of sub-stomatal cavity	µmol mol^{-1}

* Determined by IRGA. Temperature and pressure corrected for water vapor effects on measurement and analyzer temperature.

Physical Constants Used in Equations

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

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

Molecular weight of air (M_a) = 28.97

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

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

Saturated Vapor Pressure of Water from Air Temperature

Modified from *Reference 1* to optimize the fit in the temperature range of 0-50 °C, above 0 °C:

$$e_s = 6.1078 * exp\left[\frac{T_a \times \left(18.564 - \left(\frac{T_a}{254.4}\right)\right)}{T_a + 255.57}\right]$$

References

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

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

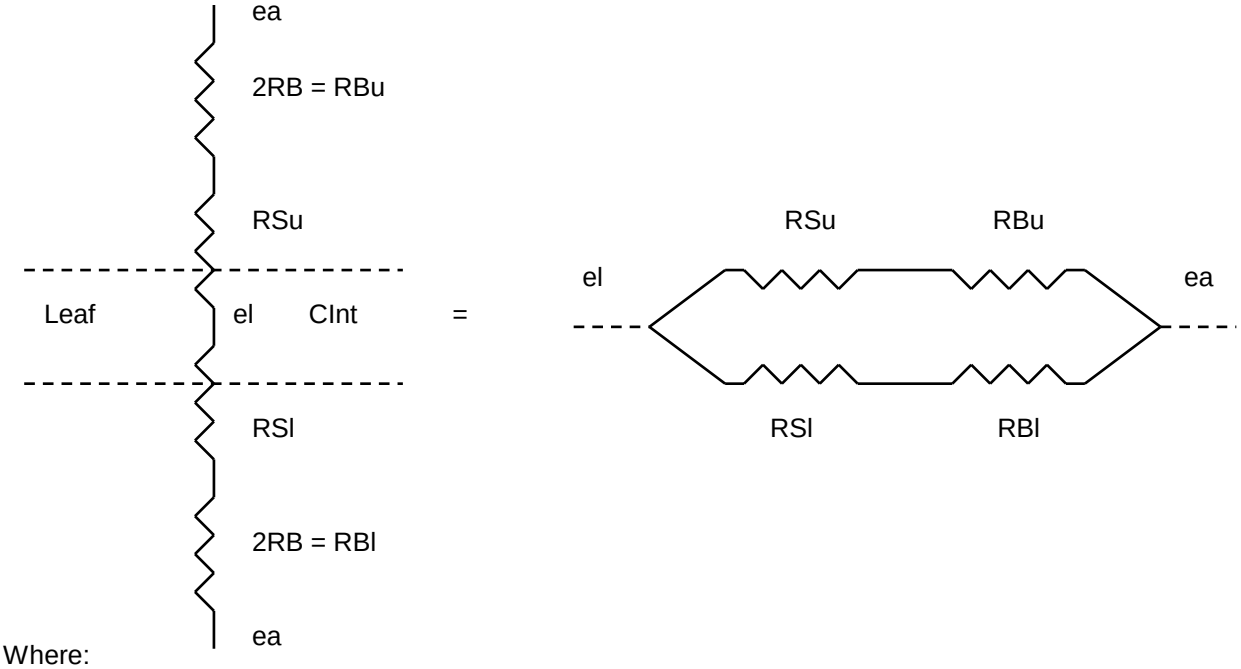
3. *von Caemmerer, S. and G.D. Farquhar 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta, Vol. 153:376-387.*

4. *Parkinson, K.J., W. Day and J.E. Leach 1980. A Portable System for Measuring the Photosynthesis and Transpiration of Graminaceous Leaves. J. Expt. Bot., Vol. 31:1441-1453.*

Addendum

Corrections to Stomatal Resistance Measurements for Differing Transpiration Rates from Upper and Lower Leaf Surfaces

The assumption is made that the internal diffusion resistance to water vapor and CO_2 are small compared with the stomatal resistances, so that there are uniform H_2O and CO_2 concentrations *within* the leaf.



Where:

(ea) and (el) are the water vapor pressures of the cuvette air and the leaf air spaces respectively

RB is the boundary layer resistance for whole leaf, with RBu = RBl = 2(RB) (m^2 s mol^{-1})

RS is the total leaf resistance

Then for the two leaf surfaces:

N and (1-N) are the fractions of the total evaporation E in mol m^{-2} s^{-1} from the upper (u) and lower (l) leaf surfaces

$$RSu = \frac{(el - ea)}{(E * N)} - 2 * RB \qquad (1)$$

and

$$RSl = \frac{(el - ea)}{(E * (1 - N))} - 2 * RB \qquad (2)$$

For clarity in the following expansion let (el - ea) = D (vapor pressure deficit).

Then from (1) and (2):

$$\frac{1}{RSu} = \frac{(E * N)}{(D - 2 * RB * E * N)}$$

and :

$$\frac{1}{RSu} = \frac{(E * N)}{(D - 2 * RB * E + 2 * RB * E * N)}$$

Therefore :

$$\frac{1}{RSu} + \frac{1}{RSl} = \frac{E * N * (D - 2 * RB * E + 2 * RB * E * N) + (E - E * N) * (D - 2 * RB * E * N)}{(D - 2 * RB * E * N) * (D - 2 * RB * E + 2 * RB * E * N)}$$

Now,

$$\mathrm{RS} = \frac{1}{\left(\frac{1}{\mathrm{RSu}} + \frac{1}{\mathrm{RSl}}\right)}$$

Therefore:

$$RS = \frac{(D - 2 * RB * E * N) * (D - 2 * RB * E + 2 * RB * E * N)}{E * N * (D - 2 * RB * E + 2 * RB * E * N) + (E - E * N) * (D - 2 * RB * E * N)}$$

Which expands to:

$$RS = \frac{D * D - 2 * RB * E * D + 4 * RB * RB * E * E * N - 4 * RB * RB * E * E * N * N}{E * (D - 4 * RB * E * N + 4 * RB * E * N * N)} \qquad (3)$$

If we assume that there is equal transpiration on both surfaces (i.e. N = 0.5), then:

$$RS(eq) = \frac{(D - RB * E)}{E} \qquad (4)$$

The error in determination of RS from making the assumption of equal transpiration on both sides of the leaf is (3) - (4).

After expansion and simplification this becomes:

$$\frac{N * (1 - N) - 0.25}{\frac{0.25}{RB} - \frac{E * N * (1 - N)}{D}} \qquad (5)$$

When N = 0 or 1 then this equals -RB. This follows from the fact that with transpiration on one side only, the boundary layer resistance to subtract is 2*RB, whereas the original calculation has subtracted only RB.

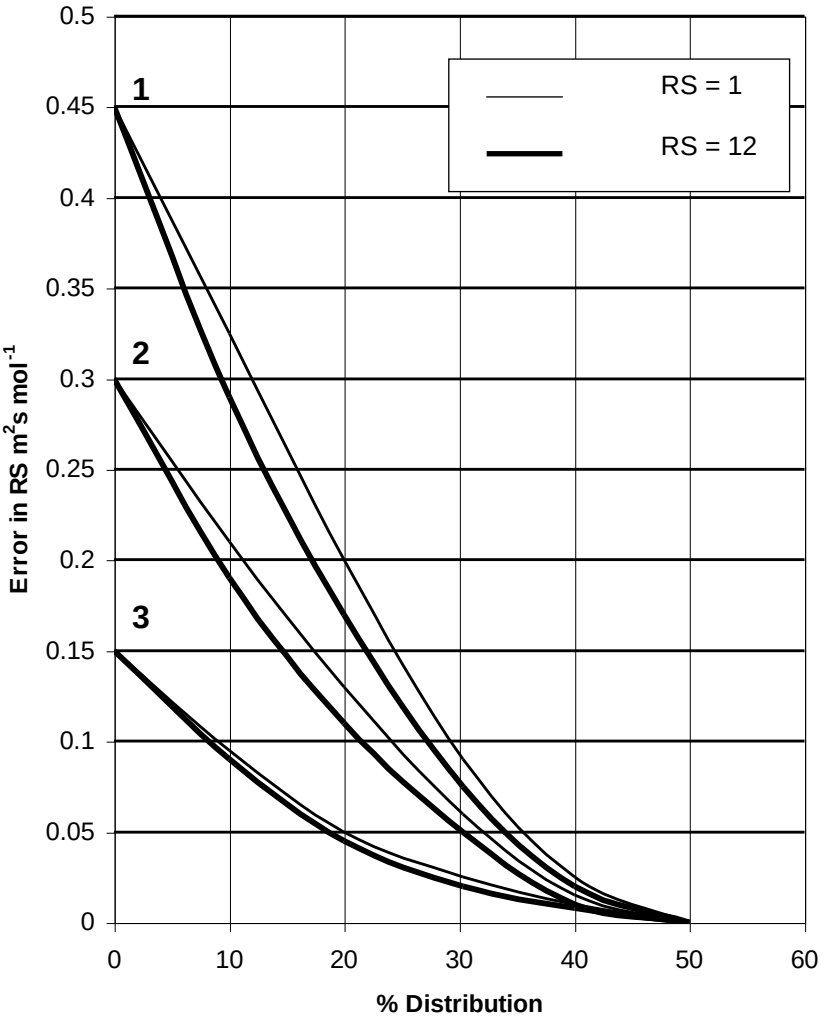
Substituting in equation (5) for E/D from equation (4)

$$\left[ie \quad \frac{E}{D} = \frac{1}{(RS(eq) + RB)} \right]:$$

$$RS(true) = RS(eq) + \frac{N * (-N) - 0.25}{\frac{0.25}{RB} - \frac{N * (-N)}{(RS(eq) + RB)}} \qquad (6)$$

Magnitude of Errors in RS

We can determine the magnitude of the error for various values of RS, RB and N.



1. R_B = 0.45 m^2 s mol^{-1}

2. R_B = 0.3 m^2 s mol^{-1}

3. R_B = 0.15 m^2 s mol^{-1}

User Notes