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## Introduction

The CI-340 Hand-held Photosynthesis System is the smallest, fastest and highly accurate infrared gas analyzer available for both field and laboratory photosynthesis measurements. The CI-340 is lightweight and utilizes a compact solid-state design concept in which the entire system (display, keypad, computer, data memory, CO<sub>2</sub>/H<sub>2</sub>O gas analyzer, flow control system and battery) is contained in a single, hand-held durable case. Measurements of photosynthesis, transpiration, stomatal conductance and internal CO<sub>2</sub> can be taken with minimal sample degradation because the chamber is connected directly to the CO<sub>2</sub>/H<sub>2</sub>O differential gas analyzer. The short distance between the analyzer and the leaf chamber decreases the possibility of leaks, water vapor change, or temperature change, therefore keeping the integrity of the sample high. The unit is designed to perform several measurements, such as photosynthesis and transpiration rates and stomatal conductance, as well as measure the absolute and differential CO<sub>2</sub> concentrations of a leaf or plant. The unit can also be calibrated easily by the user to ensure quality measurements and data, which can be easily transferred to a computer using the included USB connector. The functions of the CI-340 are described in further detail in Table 1 and the following sections.

Table 1: Several different functions of the CI-340 with descriptions of each measurement.

Function	Description
Photosynthesis Rate	Rate at which a known area of leaf assimilates CO <sub>2</sub> over time
Transpiration Rate	Rate at which water vapor accumulates on a leaf over time
Stomatal Conductance	Overall water loss of the leaf, determined using transpiration rate and leaf surface temperature
Absolute Mode	Measures CO <sub>2</sub> concentration (ppm) from a single source (intake of the instrument)
Calibration	Allows user to check or adjust unit to known standards, increasing data quality
Data Transfer	Transfers saved data files from unit to a computer

## Infrared Gas Analysis (IRGA)

Infrared gas analysis (IRGA) measures heteroatomic trace gases based on the absorption wavelength of infrared (IR) light as it passes through an air sample. Heteroatomic gas molecules consist of two or more different atoms (e.g. CO<sub>2</sub>, H<sub>2</sub>O, NH<sub>3</sub>, CO, NO, N<sub>2</sub>O). Monoatomic gas molecules consist of a single atom (e.g. O<sub>2</sub>, N<sub>2</sub>) and do not absorb IR radiation or interfere with determining the concentration of heteroatomic gases using infrared light. Carbon dioxide (CO<sub>2</sub>) strongly absorbs intermediate infrared wavelengths. Infrared gas analyzers (IRGAs) measure the reduction in the transmission of infrared wavelengths caused by the presence of a gas between the radiation source and a detector. The measured reduction in transmission is a function of the concentration of the gas. IRGAs are commonly used to measure carbon dioxide and water concentrations, as well as photosynthesis. There are two main types of IRGA, dispersive and non-dispersive, which differ according to the specificity of the gas type that is being measured.

### Dispersive and Non-Dispersive IRGA

Infrared gas analyzers can be either dispersive or non-dispersive. Dispersive infrared analyzers sequentially apply monochromatic radiation, which determines the concentration of various gas species in a complex mixture of gases. In contrast, non-dispersive analyzers assay the concentration of a particular species of gas.

Non-dispersive analyzers are commonly used for photosynthesis measurements and function by using broad-spectrum infrared radiation, made selective for CO<sub>2</sub> by the use of filters in the optical path. Typically, detectors designed for CO<sub>2</sub> exhibit cross-sensitivity to the absorption spectrum of water vapor. Although filters can minimize this interference, it is necessary to correct the apparent CO<sub>2</sub> concentration if there is significant water vapor in the airstream at the intake of the analyzer. Alternatively, water vapor may be condensed or chemically removed just before the airstream enters the analyzer.

### Photosynthesis Measurement Using IRGA

Photosynthesis is the process by which higher plants transform sunlight into chemical energy. During this process, plants produce carbohydrates from carbon dioxide and water. This occurs in the presence of chlorophyll by converting light energy to chemical energy. Measuring photosynthesis is important in comparing and understanding productivity and biomass accumulation at the leaf, plant and community (canopy) level as well as in quantifying plant response to environmental stresses and variables, such as light and temperature.

Most photosynthesis meters are based on the concept of gas exchange on the leaf and typically measure carbon dioxide and water vapor concentrations. The uptake of CO<sub>2</sub> and the release of H<sub>2</sub>O both use the stomata as their pathway; therefore most photosynthesis measurements include an estimation of photosynthetic rate (CO<sub>2</sub> uptake) and transpiration rate, as well as stomatal conductance.

Carbon dioxide uptake is measured using an IRGA by comparing the CO<sub>2</sub> concentration of gas passing into a chamber surrounding a leaf/plant and the CO<sub>2</sub> leaving the chamber. The CO<sub>2</sub> concentration of the gas initially passing into the chamber is measured, and then the gas is pumped through the chamber at a known flow rate. The concentration in the effluent gas from the chamber is measured using the IRGA and the difference between this and the input gas is used to measure photosynthetic rate (or respiration rate if a greater CO<sub>2</sub> concentration is measured in the effluent gas stream).

Net photosynthesis (P<sub>n</sub>) can be determined using the IRGA measurements of the change in CO<sub>2</sub>. IRGAs can also be used to measure environmental responses and photosynthetic capacity and determine other parameters, such as instantaneous gas flux measurements, gas exchange over time (such as for diurnal cycles), photosynthesis-light response curves, photosynthesis-temperature response curves and photosynthesis-CO<sub>2</sub> response curves.

## Technical Information

The CI-340 is a highly technologically advanced photosynthesis system. It contains a pump along with a mass airflow sensor. A built-in microprocessor regulates the airflow rate, which is set by the user. Specifically, the CI-340 is a non-dispersive IRGA in which the infrared light is shone through the gas in the sampling chamber and then focused on a detector. The energy received at the detector is the total energy entering the system minus the energy absorbed by the CO<sub>2</sub> in the sampling chamber. A technical diagram (Figure 1) illustrates a flow chart for this instrument. An illustration of the CI-340 can be found in Figure 2.

The measurement process starts with the gas or air sample passing a solid-state CO<sub>2</sub> analyzer. The output of the analyzer is amplified, sampled by an analog-to-digital (A-D) converter, and sent to the microprocessor. The processor averages these readings and corrects them for any non-linearity present in the analyzer. A relative value of CO<sub>2</sub> concentration is continually updated by the microprocessor. Each reading reflects a sample being taken every second during a specified time period. This can be determined by setting the time interval. The rate at which samples are saved in memory is determined by the “sampling rate” or the time interval input at the beginning of each measurement session. If you listen carefully during analysis, you can hear the valves switching from reading the “in” values to the “out” values and the sample number or count will increase by one.

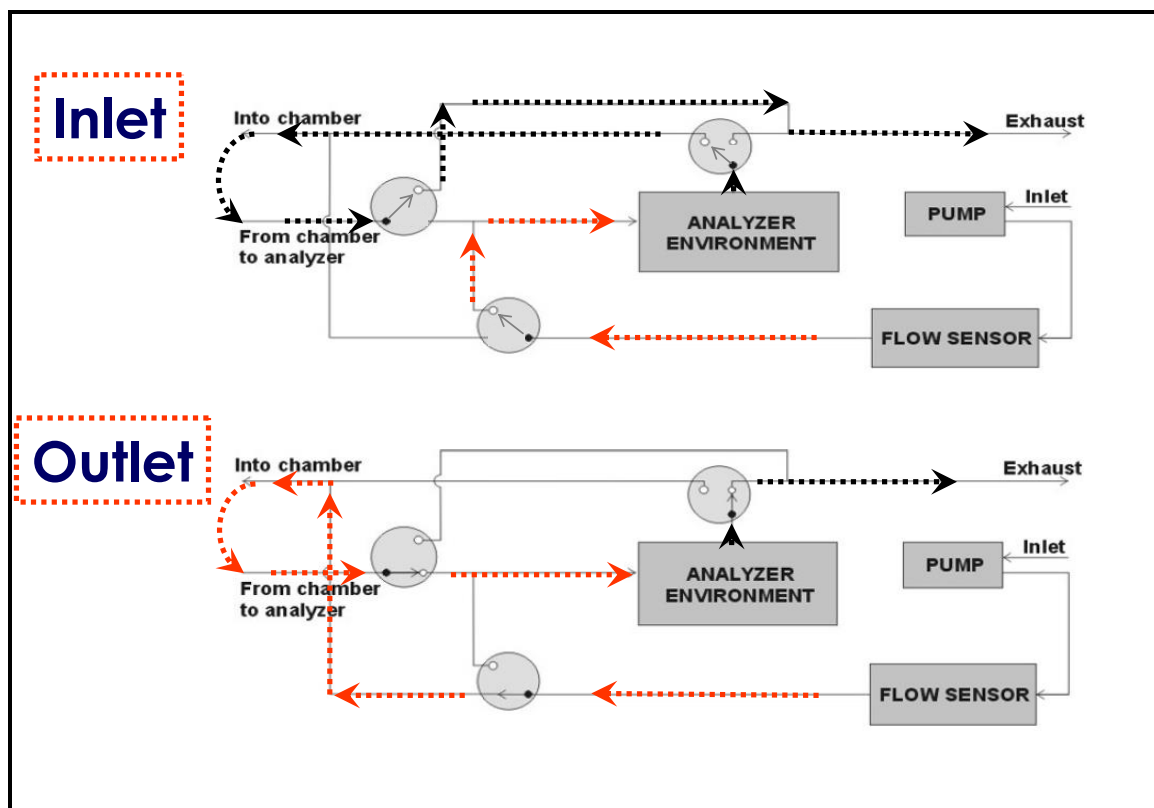


Figure 1: The pathway of airflow through the CI-340 during measurement. The top figure illustrates the valve placement and air flow during an “in” reading and the lower figure illustrates the valve placement and air flow during an “out” reading.

When initially performing a measurement, the CI-340 takes gas from the inlet or intake where air is coming in through a filter. This air is moved through the pump and then next through the flow sensor where the flow rate is regulated. After that, because the device is measuring in, inlet gas is directed through the analyzer and then enters into the leaf chamber. The gas leaves the leaf chamber and is directly exhausted.

The CI-340 will measure the “in” for approximately 20 seconds plus the number of seconds entered for the time interval. The extra 20 seconds allows the machine to clean out any residual gases that are in the analyzer and to let the analyzer stabilize. Every time something is changed on the CI-340, the stability is disturbed and the machine should be allowed a few seconds to stabilize. Once the “in” is measured, all the valves switch to other position. Now the inlet gas goes straight to the leaf chamber, and the output of the leaf chamber now goes to the analyzer to be measured before it gets exhausted. Figure 1 illustrates the pathways of air flow through the CI-340 during both “in” and “out” measurements.

**CAUTION:** *When attaching a pressurized gas source to the external inlet, use a three-way fitting (which is provided with each instrument) to allow excess gas to escape. Excess pressure may blow out internal fittings and tubing, or damage the pump. Do not use a three-way fitting for non-pressurized gas sources.*

### Main Components of Gas Exchange Systems

There are several main components making up the CI-340. Each of these components needs to be functioning properly in order for the combined operation of all the components to produce stable and accurate photosynthetic measurements. Components include the gas exchange or leaf chamber, the infrared gas analyzer, the pump and mass airflow sensor, and the flow sensor or meter. Other basic components include filters, gas lines, the display (LCD) screen, the keypad, and the power source or battery. The CI-340 utilizes a built-in microprocessor to regulate the airflow rate, which is determined by the user in the range of 0.2 lpm to 1.01 lpm, with the default setting at 0.3 lpm.

Several things can affect the performance and accuracy of photosynthesis measurement systems, such as leaf chamber architecture and leaf chamber seal quality. The CI-340 has various leaf chamber attachments for use with different leaf types and whole plants. The leaf chambers are made from materials which have a low adsorption of water and CO<sub>2</sub> in order to provide tight seals and accurate readings. A common problem with infrared gas analyzers is poor discrimination between CO<sub>2</sub> and H<sub>2</sub>O (water vapor) because both gasses absorb energy at similar wavelengths. This problem is relieved by using a desiccant to dry the gas sample to a stable (constant) water vapor content before reaching the analyzer. The CI-340 also allows precise control of temperature, CO<sub>2</sub> concentration, humidity and light when taking measurements through use of the accessories. The CI-340 also includes computer software programs giving the user instant access to data in the field. This allows the user to detect and correct any errors during the measurement.

### Highlights of CI-340

The CI-340 is the most sensitive and most stable hand-held IRGA photosynthesis system. It is also small and very light-weight. The CI-340 is easy to calibrate, remains stable longer after calibration and warms up faster than previous IRGA models. This is because the CI-340 incorporates a single IRGA to perform both intake and outtake measurements. Using a single IRGA to measure (versus two or more),



gives the users less drift in measurements because the equations are based on differentials. With fewer components involved, there are fewer errors and less instrument drift.

The CI-340 can measure chlorophyll fluorescence and photosynthesis simultaneously, has modular attachments for light and temperature control, CO<sub>2</sub>/H<sub>2</sub>O supply and chlorophyll fluorescence, as well as addition attachment capabilities for soil respiration and plant canopy photosynthesis measurements.





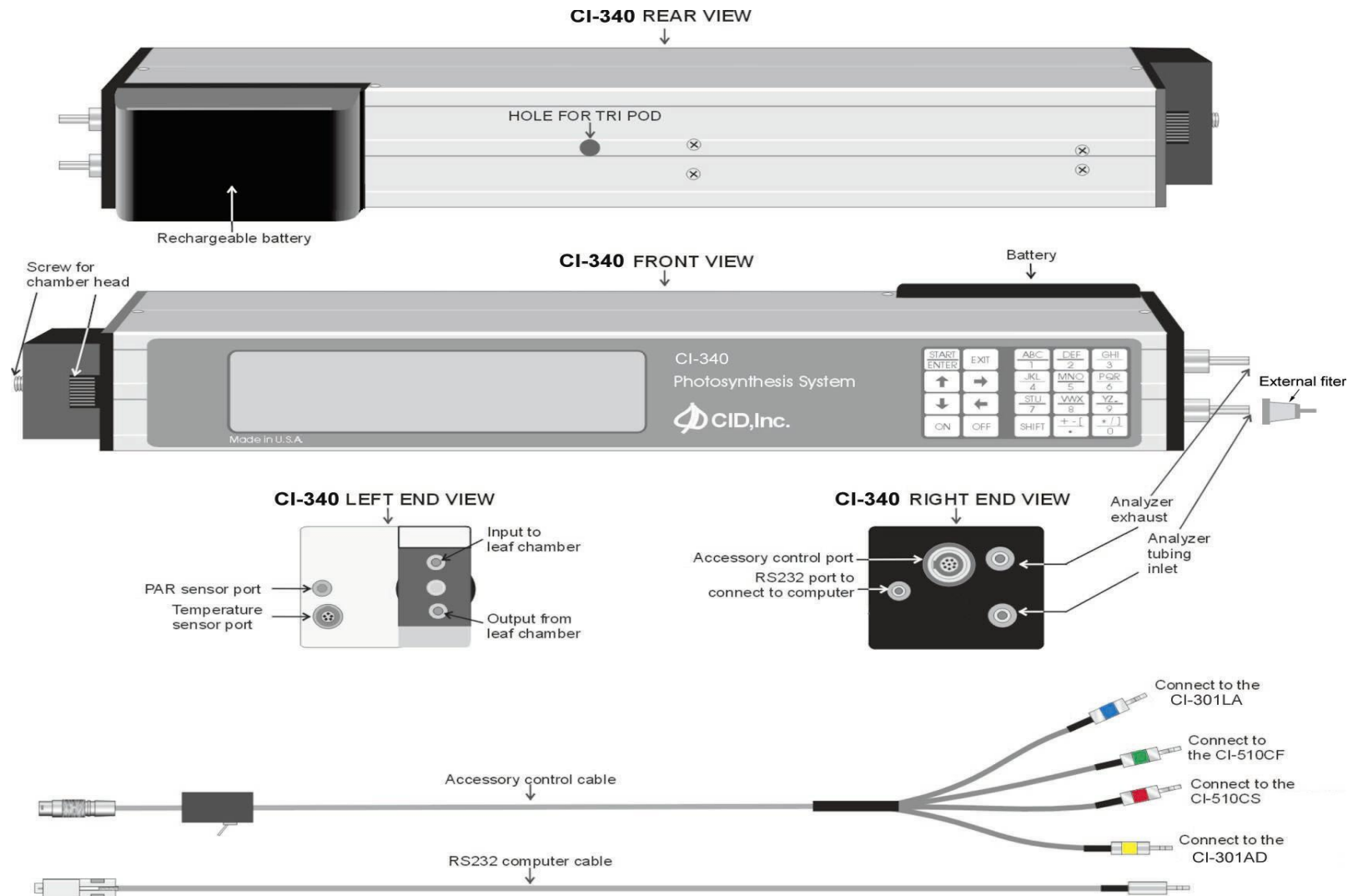


Figure 2: Parts of the CI-340

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## General Operating Instructions

This section will familiarize you with start-up procedures and guide you on how to move from one function to another. Specific instructions for each separate function will be found under their individual heading in the main body of the manual.

For greatest accuracy, the instrument should be turned on 30 minutes prior to any calibrations to allow it to be fully warmed up. Measurements can be made about 3 minutes after the displayed CO<sub>2</sub> value starts to drop from its maximum.

1. The instrument needs to warm up awhile before it is used to measure. The warm up is measured from the time the instrument is powered on (it does not matter if it is measuring or not). A warm up of about 4 minutes would be the minimum time to get measurements and about 20 minutes for more precise measurements.
2. It is best to put the leaf into the chamber before you start the measurement so the leaf has time to acclimate to the leaf chamber conditions and the instrument has time to react to the changes the leaf causes. The "Working" display is a period that the instrument uses to stabilize itself. The actual measurement starts when the display changes from "Working".
3. Variations in the CO<sub>2</sub> readings or Pn readings can often be caused by changes in the air stream going into the instrument. The CO<sub>2</sub> content of the stream must be very stable. Some researchers use a long tube to get the intake away from human activity. Some use compressed air (with a pressure regulator and a T in the hose). Some use a volume buffer (a 2 liter bottle or larger with the hose from the instrument drawing air from inside that is vented to allow outside air in) that will average out CO<sub>2</sub> changes over time. If the experiments are done near a road with vehicles, it is difficult to get stable CO<sub>2</sub> readings. If you do not have a source of compressed air, you may try putting several volume buffers in series (the instrument draws from the first bottle that draws from the second which then draws from the third which is vented) The instrument is sensitive enough to detect fires that are hundreds of meters away if they are upwind.
4. The flow rate can be reduced when the photosynthesis is minimal. Try 0.3 lpm for most situations and 0.25 lpm if the readings are low.

### Using the Keypad

The keypad for the CI-340 consists of 20 keys to enter commands and data. Figure 3-1 illustrates the keypad. Pressing a key makes a "beeping" sound. A key should be pressed individually each time a command or letter is asked for. Refer to the *TROUBLESHOOTING* chapter if problems occur.

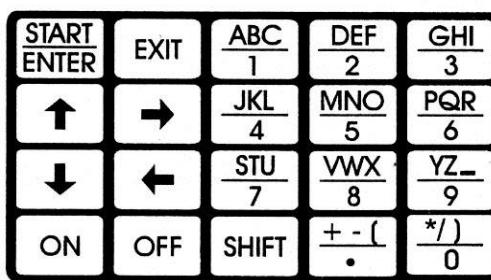
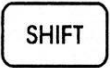



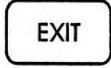





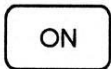

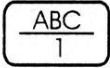
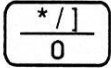

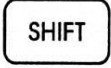



Figure 3: CI-340 Keypad






**KEY FUNCTION**

 + 	Starts measurements without saving the data.
	Permits the user to save the data or information to a file.
	Selects next menu/function and enters data if in a special menu.
	Aborts the operating function or stops measurements.
 	Moves the cursor around the menu structure.
 	 is used as a backspace key when entering numbers or text.
	Activates the instrument.
	Deactivates the instrument.
 	Enters numeric characters (and enters alpha characters when combined with the  key). Refer to the <i>FILE NAME</i> section for details.
	Switches to upper-level character commands. Pressing the  key multiple times allows the user to enter the various shifted characters.

These characters are not usable: [ +, -, (, \*, /, ) ]






**File Menu**

The CI-340 has a file system that allows a great range of data to be stored internally. It has been designed to emulate the familiar DOS of personal computers. Note that this menu will not be accessible when there are no files stored in memory; the message “no files” on the top display line will be briefly displayed when this situation occurs.

- ◆ To use the File Menu or check if any files are stored, press the  key when “ENTER ▢ file menu” is displayed.
- ◆ Press the  key to access the “file menu” when the instrument is first turned on.
- ◆ Use the  and  keys to view all stored files. Pressing the  key will exit this menu.

Enter ▢ File menu	13:05:49
-------------------	----------

Files are stored in chronological order, with the names and data affiliated accordingly. Up to 1200 files or 4 MB of data are allowed for storage. Be sure to transfer important data before deleting it from the CI-340. Refer to the *DATA TRANSFER* section for related information.

- ◆ Press the  +  key (for the letter “D”) to delete the last file saved, or  +  key (for the letter “Y”) to delete all files saved.
- ◆ The shift and  keys will have to be pressed again to confirm erasure of all files. Deleting a file removes it permanently!

### Installation of the Battery

The CI-340 is dependent upon a properly charged power source for efficient and reliable measurements and calculations. The included battery/battery eliminator is designed to provide the necessary power. The system is designed to operate from 7.2V rechargeable Li-Ion batteries. Check with the nearest representative or manufacturer if the included system does not include one or more of the above items.

If the rechargeable battery should ever fail to charge properly, please recycle it. Many waste processing systems do not allow Li-Ion batteries to be simply thrown in the trash. Refer to the *RECHARGING* chapter for further information on how to take care of the battery and for how to power the CI-340 in the lab without using the Li-ion battery.

Proper installation of the battery is as follows:

- ◆ Align the contacts of the battery with the contacts of the CI-340 (Figure 4).
- ◆ Line up the battery so that it is about 7 mm “off” its final position within the battery mounts in the direction shown in Figure 4.
- ◆ Press the battery toward the contacts and slide in the direction shown (Figure 4) until firmly in place. By pressing the battery toward the contacts, the CI-340 will make necessary connections with the battery and the battery will be in the correct position.
- ◆ To remove the battery, slide it in the opposite direction that is shown in Figure 4.

**Note: The CI-340 should only be used in low RF ambient areas. Do not use near radio/TV transmitting antennas or near electrical arc welders.**

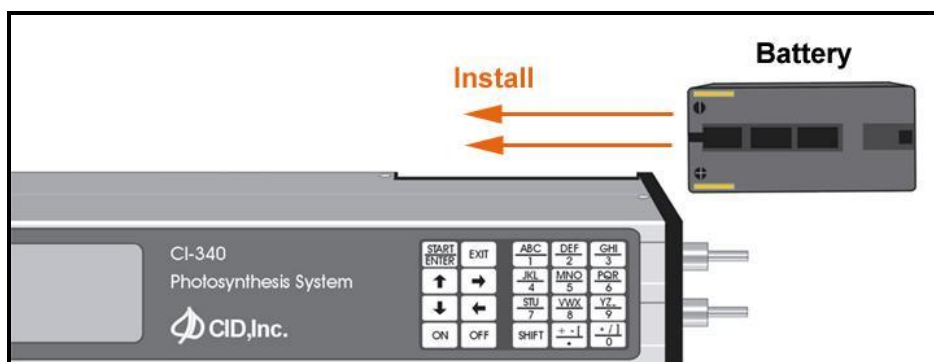









Figure 4: Battery installation: Press the battery toward the contacts.

## Interpretation of Parameters



CO <sub>2</sub> (in)	The amount of CO <sub>2</sub> (in ppm) at the inlet of the analyzer
CO <sub>2</sub> (out)	The amount of CO <sub>2</sub> (in ppm) at the outlet of the leaf chamber
CO <sub>2</sub> (dif)	The difference between the CO <sub>2</sub> in and CO <sub>2</sub> out values.
H <sub>2</sub> O (in)	The amount of H <sub>2</sub> O (in kPa) at the inlet of the analyzer
H <sub>2</sub> O (out)	The amount of H <sub>2</sub> O (in kPa) at the outlet of the leaf chamber
H <sub>2</sub> O (dif)	The difference between the H <sub>2</sub> O in and H <sub>2</sub> O out values.
PAR	Photosynthesis Active Radiation in terms of $\mu\text{mol}/\text{m}^2/\text{s}$
FLOW	The set flow rate of the analyzer (in lpm)
W	Mass flow rate in terms of $\text{mol}/\text{m}^2/\text{s}$
T (air)	Temperature of the ambient air (in °C) in the leaf chamber. This requires the temperature sensor to be installed for meaningful results.
ATM	Atmospheric pressure (in kPa)
Pn	Net photosynthesis rate in terms of $\mu\text{mol}/\text{m}^2/\text{s}$
InTCO <sub>2</sub>	Internal CO <sub>2</sub> $\mu\text{mol}/\text{mol}$
T (leaf)	Temperature of the leaf as measured by infrared temperature sensor (in °C)
C	Leaf Stomatal conductance $\text{mmol}/\text{m}^2/\text{s}$
Internal T	Temperature of the analyzer environment (in °C)
E	Transpiration Rate $\text{mmol}/\text{m}^2/\text{s}$
"EXIT to quit"	Aborts current operating function to the default menu
"ENTER [ ] file menu"	Allows the user to enter the file menu system:
"SHIFT 2 (D) = del"	Command to delete an existing file; press  + 
"EXIT file menu"	Returns to the default menu
"Y = delete ALL"	Command to delete all existing files; press  +  . This must be confirmed before the instrument will erase all the files.
"Date"	Current running date
"Time"	Current running time
"START/ENTER to go"	Reminds the user that pressing  will start the measurement process.
"ENTER to select"	Notifies the user to press  to perform the listed functions:
"Change clock"	Allows the user to change the time and date settings
"Calibrate CO <sub>2</sub> "	Allows the user to calibrate the zero and span for CO <sub>2</sub> measurements.
"Calibrate H <sub>2</sub> O"	Allows the user to calibrate the zero and span for H <sub>2</sub> O measurements using external sources
"Calibrate Temp"	Allows the user to set the TSCAL1 and TSCAL2 parameters of the temperature sensor.
"Calibrate Flow"	Allows the user to calibrate the flow meter to external standards.
"Calibrate ATM Pr"	Precisely adjusts atmospheric pressure (in kPa)

## System Setup and Calibration

The system is shipped from the factory calibrated for immediate use. The user can perform most system calibrations if necessary.

To start the CI-340, press . After a brief warm-up time, the instrument will display the basic display as follows:

<p>Fri 12 Mar 2004 16:06:38          CI-340 version 5.004          ©Copyright CID Inc., 1997 - 2004          All rights reserved.</p>
---

The following screens are accessed in order by pressing the arrow keys,  to move up and  to move down:

Starting Screen:

ENTER to select	Calibrate Temp.
ENTER to select	Calibrate Flow
ENTER to select	Calibrate ATM Pr
ENTER->file menu	
Fri 12 Mar 2004 16:06:38	
START/ENTER to go	16:06:38
ENTER to select	Change clock
ENTER to select	Calibrate CO <sub>2</sub>
ENTER to select	Calibrate H <sub>2</sub> O














## Calendar and Time Setup

The purpose of these functions is to provide the user current information of local date and time. This method can be extremely useful to further establish when measurement data are collected or saved, or simply another form of time keeping. The factory-calibrated date and time are the default values.

ENTER to select    Change clock

To change the date and time:

- ◆ Scroll to choose “Change clock” under the “ENTER to select” function (press the  key twice from the initial display). The time function will be the first selection.
- ◆ Use the  or  keys followed by the  or  keys to adjust and move to the hour, minute and second of choice.
- ◆ With the time function set, the date function will follow. Once again, use the  or  keys followed by the  or  keys to adjust and move to the month, day and year of choice.
- ◆ Press the  key to save your selection and continue. Note that at any point of this setup,
- ◆ Pressing the  key will suspend any further actions and return to the screen display above.

## Leaf/Air Temperature Sensor

**Suggested Calibration Schedule:** None.

This function sets the calibration values for a given leaf/air temperature sensor. The included temperature sensor has been initially tested and calibrated by the manufacturer.

ENTER to select    Calibrate Temp

No further calibration is allowed.

## Atmospheric Pressure

The CI-340 pressure sensor is capable of measuring absolute atmospheric pressure. This value is used in calibrations for Photosynthesis, Transpiration and Stomatal Conductance values. The sensor is calibrated at the factory, and normally does not need to be recalibrated.

ENTER to select    Calibrate ATM Pr

To change the atmospheric pressure (ATM) value:

- ◆ Scroll to choose the “Calibrate ATM Pr” under the “ENTER to select” function.
- ◆ Enter the desired ATM value in K Pa. Press **START ENTER** to accept the entered value or **EXIT** to abort the process, and continue to another step.

## Flow Rate

The CI-340 is capable of maintaining a steady airflow once the unit begins taking measurements. After the initial warm-up time (one minute), the instrument will generate the entered flow rate to regulate the accuracy of the measurements. The instrument is capable of automatically controlling the flow rate from 0.2 ~ 0.999 lpm. Flow rates of 0.3 lpm give increased accuracy to photosynthesis measurements unless very active leaves are being measured.

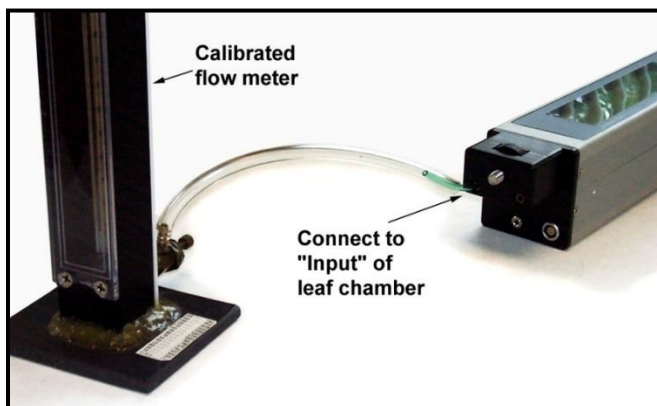


Figure 5: Flow meter setup to measure flow rate.

The flow meter is calibrated by the manufacturer but can be calibrated again by the user. Suggested calibration schedule is once every six months. Scroll to choose the “Calibrate flow rate” under the “ENTER to select” function.

ENTER to select    Calibrate flow rate

The instrument will briefly run through stabilizing steps and then ask the user to adjust the flow, using the **↑** **↓** keys for major changes or the **→** **←** keys for minor changes, over steps from .2 to 1 lpm. For calibration, the flow **MUST** be measured by the calibrated external flow meter at the port on the end of the instrument where the leaf chamber plugs in (upper left part as you read the keypad). For this calibration, the flow rate is measured at the input to the leaf chamber (see figure 5-0). A well-calibrated flow meter with little backpressure should be used as the standard. If for any reason an error was made during the calibration procedure pressing the **EXIT** key will also return back to the beginning of the flow calibration procedure without saving any changes made. Once the calibration

procedure is completed, in a few moments the instrument will reset and return back to the above screen display.

## CO<sub>2</sub> and H<sub>2</sub>O General Overview

H<sub>2</sub>O and CO<sub>2</sub> Zero should be calibrated every week, unless there are very significant changes in ambient conditions or the relative humidity values seem erroneous. The H<sub>2</sub>O Span should be calibrated annually, and with heavy use every six months. CO<sub>2</sub> Span should take place once every six months. Set Zero is a one point calibration, set Span is a two point calibration.

Calibrating the H<sub>2</sub>O Span can be done with ambient air using a hygrometer to measure humidity and a thermometer to measure temperature. These values are entered into the DOS program to calculate the kPa to enter into the instrument. H<sub>2</sub>O levels of air are not as easily affected as CO<sub>2</sub> concentrations, therefore as long as the temperature and humidity of the gas being fed through the instrument are known, the H<sub>2</sub>O Span can be correctly calibrated. The calibration will be as accurate as the sensors used to determine the humidity/temperature of the gas.



**NOTE:** For greater changes in ambient temperatures between sample measurements, it may be necessary to recalibrate to CO<sub>2</sub> “zero setting” at each temperature (i.e. 0°C sample and 40°C sample).

This instrument allows calibration with a range of CO<sub>2</sub> from 200 to 1000 ppm, and H<sub>2</sub>O from 1 to 7.5 kPa.


ENTER to select      Calibrate CO<sub>2</sub>

To change the calibration of CO<sub>2</sub>, scroll to choose the “Calibrate CO<sub>2</sub>” under the “ENTER to select” function. While the instrument stabilizes to the setup state, it will first ask if you want to calibrate zero.

Start to set zero, else Exit

Press the  key to calibrate the zero or press the  key to skip the calibrate zero function.

Use 0 ppm CO<sub>2</sub> gas      Press START/ENTER

Connect dry nitrogen or soda lime and allow the zero ppm CO<sub>2</sub> gas (dry nitrogen or soda lime) to flow for approximately one minute (three minutes for soda lime) prior to pressing the  key to flush the system completely. Always use a “T” connector. A small amount of flow out of the one-meter tube ensures a sufficient quantity of gas is flowing to the system (see Figure 6) in the gas line when supplying gas from a low-pressure regulator in order to avoid excessive flow through the system. Alternately, use soda lime connected between the intake and exhaust with the small plastic tube where the chamber usually goes to form a closed loop. (See Figure 7)

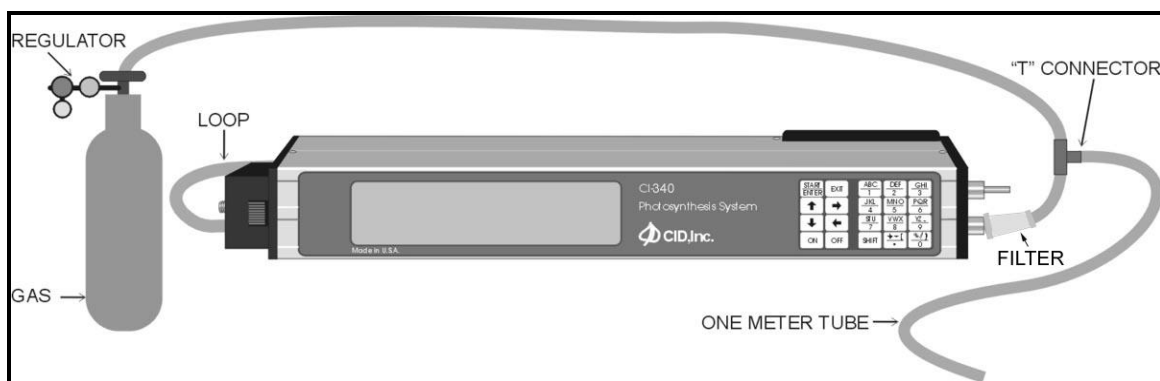


Figure 6: Configuration using compressed gas.

After the system zero is established, use a gas with known concentration of CO<sub>2</sub> to calibrate span.

Use the **EXIT** key to skip the “calibrate the span” setting.

Use known CO<sub>2</sub>

Press the **START/ENTER** key to proceed.

Enter the concentration of CO<sub>2</sub> in ppm at the following display:

Concentration ?  
\_ ppm

The known concentration should flow for approximately one minute.

Press **START/ENTER** to save your selection. The system will then return to the following screen display and reset:

ENTER to select      Calibrate CO<sub>2</sub>

H<sub>2</sub>O calibration follows similar procedures. Dry nitrogen gas or silicon gel (Figure 8) can be used for the H<sub>2</sub>O zero, and a known partial pressure of H<sub>2</sub>O is used for the known H<sub>2</sub>O. A “DOS” program is provided to convert relative humidity and temperature to kPa partial pressure. Run *RH2KPA.EXE* under DOS or in a DOS window on a PC. Windows XP will automatically launch a DOS window if you double click on the file name in Windows Explorer.

**NOTE:** H<sub>2</sub>O span setting should be checked once every six months. It be checked often by conducting a photosynthesis test with the “loop back” tube in place of a leaf chamber and sampling a known humidity of the atmosphere.

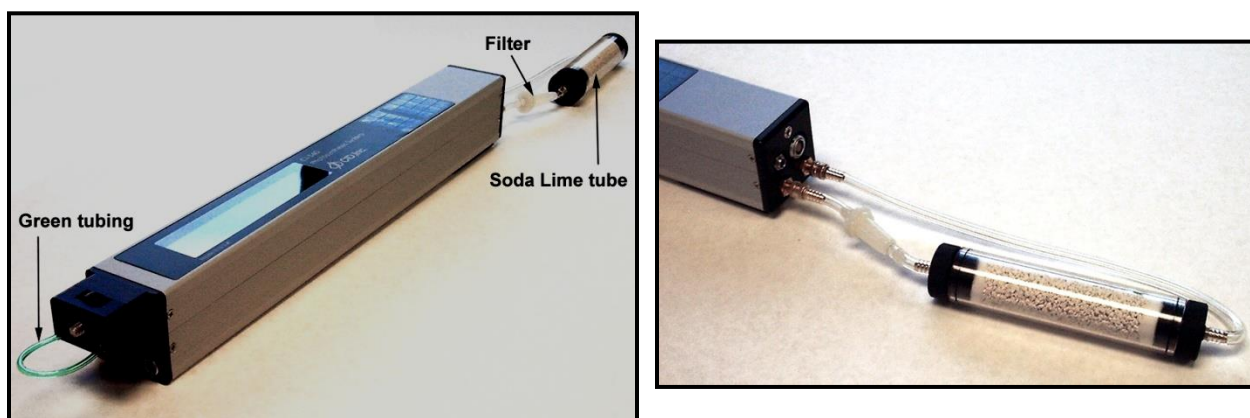


Figure 7: Configuration using Soda Lime for CO<sub>2</sub> Zero calibration.



Figure 8: Configuration using Silica Gel for H<sub>2</sub>O calibration

### **CO<sub>2</sub> Zero and CO<sub>2</sub> Span Procedure**

Tools required: CI-340, Soda Lime external conditioning tube, filter, hose barbs, a CO<sub>2</sub> standard gas ranging from 200-1000 ppm, a t-junction, and a flow regulator.

1. Power on the instrument
2. Connect the soda lime tube, filter and green loop-back tube (Fig.1&2) (for Control Modules: set the AD CO<sub>2</sub> and H<sub>2</sub>O to zero)
3. Use the up/down arrow to navigate to "Calibrate CO<sub>2</sub>"
4. Press start/enter

5. Instrument will display "Warming Up" and then begin counting down. Wait.
6. Once counted down, the instrument will display "START to set Zero, else EXIT"
  - a. Press start/enter to set the CO2 zero
  - b. "Use 0 ppm CO2 gas, Press START/ENTER"
  - c. Press start/enter
  - d. "Setting 0 in 60 Seconds" will display and the instrument will count down

At this point, the CO2 concentration of the gas passing the analyzer is taken as 0 ppm and the sensor is calibrated to read this level as 0. Therefore, if the gas is not 0 ppm or hasn't been allowed enough time to run through and completely clear the system, the calibration can skew the instrument further.

7. After the CO2 zero is set, the instrument will display "Use known CO2 gas, Press START/ENTER"
8. To only calibrate the CO2 zero, press EXIT and you are done. To continue on and calibrate the CO2 span, hit start/enter.
9. If you pressed start/enter: connect a standard CO2 gas with a known concentration (Fig.3) and press start/enter
  - a. The unit will display "Concentration: ? ppm" Enter the CO2 concentration of the standard gas you are using (acceptable ranges: 200-1000 ppm CO2)
  - b. Press start/enter
  - c. The instrument will count down from 60 seconds and set the CO2 span
  - d. Press start/enter to save
10. The display will return to the original "ENTER to select Calibrate CO2" screen

To verify a successful calibration, take a reading of a known gas. The instrument should read with an accuracy of  $< \pm 2\%$ .

### **H2O Zero and H2O Span Procedure**

Tools required: CI-340, Silica gel external conditioning tube, filter, hose barbs, a H2O standard of known partial pressure, a t-junction, and a flow regulator.

1. Power on the instrument
2. Connect the silica gel to the instrument.
3. Use the up/down arrow to navigate the menu to Calibrate H2O

4. Press Start/Enter
  - a. Instrument will display “exit to quit”
  - b. “START to set Zero, else EXIT”
    - i. If the silica gel is connected and has been flowing for several minutes, press START.
    - ii. “Use 0 kPa H2O, Press START/ENTER”
      1. Press START/ENTER and instrument will count down from 60 seconds and set the H2O zero.
      2. At this point, the H2O concentration of the gas passing the analyzer is taken as 0 ppm and the sensor is calibrated to read this level as 0. Therefore, if the gas isn’t 0 ppm or hasn’t been allowed enough time to run through and completely clear the system, the calibration can skew the instrument further.
5. After the H2O zero is set, the instrument will display “Use known H2O gas, Press START/ENTER’
6. If you wish to calibrate the H2O Span, hit start/enter. If you only wish to calibrate the H2O zero, press EXIT
7. To calibrate the H2O span, connect a gas with a known concentration of H2O (\*see general overview).
  - a. Press Start/Enter
  - b. The unit will display “H2O pressure: ? x.xxx kPa”
    - i. Enter the known partial pressure of the H2O gas in kPa. This can be calculated from the relative humidity and temperature.
    - ii. CID provides a DOS program that will convert RH and Temperature to kPa. This can be downloaded on the Distributor’s website in the CI-340 software page.
    - iii. Download “A program to convert RH and temperature to kPa vapor pressure: [Download rh2kpa.exe V1.2.1.0 31kB](#)”
    - iv. Use the program to get the kPa to enter into the instrument.
  - c. Enter the H2O pressure of the known standard gas from 1.0-7.5 kPa.
    - i. Hit Start/Enter
    - ii. Instrument will count down from 60 seconds and set the H2O span.
    - iii. Press Start/Enter to save and the display will return to the “ENTER to select Calibrate H2O”.

## Data Transfer

The CI-340 automatically saves data files with a memory capacity of 4 megabytes; older versions have a memory capacity of 2 MB. The memory of the CI-340 during normal use will suffice for approximately



one month, while intensive data gathering (gathering data as fast as possible in absolute or continuous mode) will fill the memory after about 8 hours. Data transfer to a personal computer or laptop is quick and easy and can be done in the field or after measurements have been taken. New models are supplied with an easy to use USB connector and software, while older models may have an RS-232 connector and may require software updates available on the CID website at [www.cid-inc.com](http://www.cid-inc.com). Transferred files are saved as .cvs (comma separated values) and can be read in excel or other spreadsheet programs for data manipulation. For a more in-depth look at downloading the initial software, installing the CI-340 data transfer program and initiating data transfer see the Transferring Data manual found on the CI-340 product page on the CID website.

## Downloading Data

One of the conveniences of this reliable data-collecting instrument is its portability. The CI-340 is almost a small user-friendly computer that can be operated almost anywhere in the field. However, data analysis and presentation can best be done on an external (desktop or laptop) computer.



To download data to an external computer, attach the CI-340 USB Cable to both the CI-340 and the computer's USB port.


Downloading files from the CI-340 to the personal computer is accomplished with a Windows program, C340DF.exe. It would be recommended that a separate directory exist for any of the CI-340 program files or data to be stored. Create a directory that can be recognizable or remembered for continuous usage; for instance, the path C:\CI-340\ could be named to store and locate all related CI-340 files and data. Refer to the computer manual(s) regarding setting up directory/paths for the current operating system (such as in Windows). To install the software on your computer, run setup.exe on the disk. You can use Windows Explorer to send a shortcut to your desktop, if desired.

- ◆ Run the C340DF.EXE program
- ◆ Turn on the CI-340
- ◆ Select *File*, then *Open* to transfer a file to the computer
- ◆ A longer file will take longer to start displaying data.
- ◆ You can select *File* then *Save* to save the data. The default extension is .dat.

## Updating Software

The operation of the CI-340 is controlled by internal software code. The CI-340 is capable of updating its software code without removing the cover. When updating becomes necessary, the manufacturer will provide the software code (under warranty) to best operate the instrument. The web address is <https://www.cid-inc.com/support/CI-340/software/>. The file *DL.exe* automatically downloads code programs. Be sure that these files are in the same directory as other CI-340 program files.

It is the best to copy all the software files provided on the disk to their own directory on the hard drive before executing *DL.exe*. Connect the CI-340 USB cable. Run the *DL.exe* program to download code from the computer. Make certain that the power is on to the CI-340. If the power does not stay on, then press and hold the  key on the CI-340 (a constant 'beep' sound may or may not be heard). Select *File, Open* and highlight the code file (CI\_340.S19), and press ENTER on the computer to start the update. After approximately 10 ~ 15 seconds, the instrument will display DLC, you can stop pressing the  key. The download of new code will take about 2 minutes. The computer screen will confirm that the procedure has been completed and the CI-340 will turn itself off.

Press the  key and the new software code is active.

**Example of a Data File**

Internal T	Flow	Pressure	PAR	T <sub>air</sub>	T <sub>leaf</sub>	CO <sub>2in</sub>	CO <sub>2out</sub>	H <sub>2O</sub> <sub>in</sub>	H <sub>2O</sub> <sub>out</sub>	W
27.5	0.3	100.09	2175	31.1	32.8	344.9	333.0	1.09	1.10	3.12
27.5	0.3	100.09	2011	31.2	32.9	339.9	328.2	1.23	1.25	2.78
27.5	0.3	100.09	2230	31.2	33.1	365.7	337.9	1.20	1.23	2.66

Pn	E	C	RH <sub>in</sub>	RH <sub>out</sub>	intCO <sub>2</sub>	Year	Month	Date	H	Min	S
12.5	3.12	3.27	35.1	37.2	325.0	03	8	3	6	59	38
13.1	2.78	2.16	35.4	36.9	310.5	03	8	3	6	59	48
21.6	2.66	1.54	35.3	37	310.0	03	8	3	6	59	58

Where:

<b>Internal T:</b>	Internal temperature for the instrument	<b>Flow:</b>	Flow rate
<b>Pressure:</b>	Atmospheric pressure	<b>PAR:</b>	Photosynthesis Active Radiation
<b>T<sub>air</sub>:</b>	Air temp.	<b>T<sub>leaf</sub>:</b>	Leaf temp.
<b>CO<sub>2in</sub>:</b>	Inlet CO <sub>2</sub>	<b>CO<sub>2out</sub>:</b>	Outlet CO <sub>2</sub>
<b>H<sub>2O</sub><sub>in</sub>:</b>	Inlet water pressure	<b>H<sub>2O</sub><sub>out</sub>:</b>	Outlet water pressure
<b>W:</b>	Mass flow rate	<b>Pn:</b>	Net photosynthesis rate
<b>E:</b>	Transpiration rate	<b>C:</b>	Stomatal conductance rate
<b>RH<sub>in</sub>:</b>	inlet relative humidity	<b>RH<sub>out</sub>:</b>	Outlet relative humidity
<b>int CO<sub>2</sub></b>	Internal CO <sub>2</sub> concentration	<b>Year:</b>	Current year
<b>Month:</b>	Current month	<b>Date</b>	Current date
<b>H, min and s:</b>	Time experiments conducted		

## Photosynthesis, Transpiration and Stomatal Conductance

Photosynthesis is the formation of carbohydrates from CO<sub>2</sub> and a source of hydrogen (as water) in the chlorophyll-containing tissues of plants exposed to light. The rate at which photosynthesis occurs is determined by measuring the rate at which a known leaf area assimilates the CO<sub>2</sub> concentration in a given time.

It is known that transpiration is the primary determinant of leaf energy balance and plant water status. The rate of transpiration is determined by the accumulation of water vapor flux per one-sided leaf area in a given time.

Stomatal conductance is the water loss of a leaf. Conductance can be considered in parallel or series. It can be obtained by measuring the transpiration and leaf surface temperature (°C), and applying the calculation for Equation 4 found in the EQUATIONS chapter.

The CI-340 is designed so that measurements can incorporate both absolute and differential readings simultaneously. This instrument can also be configured to operate both open and closed systems. It is important that the CI-340 uses the manufacturer's current software.



### Taking Measurements

Now, with all the necessary setup/calibrations completed, the CI-340 is ready to begin its measurements. With any experiment, coding or naming a set of data points/values would help characterize that particular group for computational requirements. This instrument is designed to do such by allowing for a file name and time intervals prior to any measurements or data collected.

With the system powered on and at rest, press the  key. The instrument will display:







File name: ?

This filename would be similar to a DOS-format and limited to 12 characters (including the decimal point). For example, to save a data set as "group 1", the filename "GROUP1" could be used. Use any recognizable filename including any necessary extension (i.e., "GROUP1.C50") format other than using

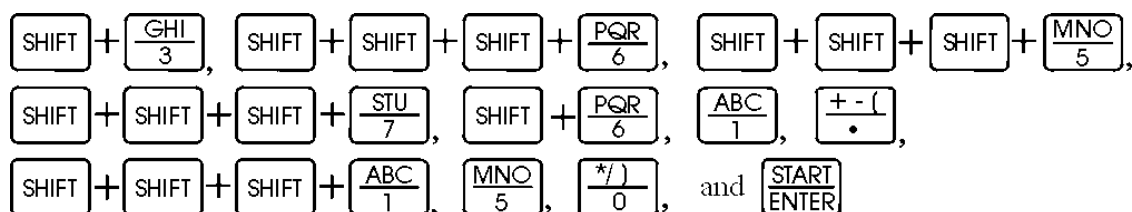
DOS executable extension (such as .EXE, .BAT, .COM, etc.). Note that pressing the  and  keys will not ask for a file name to be entered, and measured data will not be saved.


Sequential measurements using identical operating parameters can be performed if the base name ends in a number (i.e. group1) and the default file name is selected for subsequent measurements (group2, group3,...). Do not type in the default name, just press *ENTER* when the default name is displayed. The instrument will then utilize all the same operating parameters as the last measurement.

The CI-340 utilizes a shift key feature to accommodate many functions it has to offer.

- ◆ To register alpha-characters (such as A, H, R, etc.), use a sequence of the  key followed by the respective key to obtain the letter.
- ◆ Pressing the  key once accesses the first letter of the keys, twice does the second, and three times, the third. No  key pressed in this sequence accesses the number values.
  - Note that the symbols (+, -, etc.) in the keys are not recognized if naming a file, and in turn will show up as the respective number key.
- ◆ Press the  key to continue to the time interval setup.
- ◆ The  key can be used as a backspace key and the  can be used to clear the *SHIFT* count if the key was accidentally pushed.

For example, the filename "GROUP1.C50" can be entered as follows:





Press  key after a file name is entered.

The time interval entry follows the entry of the filename step:

Time interval ?  
 \_\_\_\_ (sec)

The analyzer's sampling rate is then averaged by the interval input. The instrument's sampling rate is approximately once a second. The CI-340 will only recognize integer divisions (1 ~ 32768 seconds).

The instrument will not register letters or symbols. Pressing the  key saves the resulting filename and time interval, respectively. Note that entering a file name twice will display "duplicate name" on the screen, and a different file name should be entered. Using 0 for the interval will require the user to press the  key each time a measurement is to be saved.

Control CS, AD, or LA?  
 CS=1, AD=2, LA=4, CS+AD+LA=7...

If the CI-301CS is used, enter number "1". If the CI-301AD is used, enter number "2". If the CI-301LA is used, enter number "4". If both the CI-301CS and the CI-301AD are used, enter number "3". If both the CI-301CS and the CI-301LA are used, enter number "5". If the CI-301AD and the CI-301LA are used, enter number "6". If all the three accessories are used, enter number "7". To operate the CI-340 without

any accessories, enter number "0", or just press  key. If one or more accessories are to be used, you will be asked to enter one or all of following parameters:

Desired Temperature? (Deg C) —
Desired CO <sub>2</sub> Concentration? (ppm) —
Desired Relative Humidity? (%) —
Desired PAR? (mmol/m <sup>2</sup> /s) —



For each question, enter a number and press  key. Refer to the appendices for detailed information.

At this point, an area of the leaf sample will be asked for. Enter the area (in cm<sup>2</sup>). It has been designed so that areas too large or small will severely affect necessary calculations for photosynthesis, transmission or stomatal conductance rates. The allotted range is .001 ~ 10,000 cm<sup>2</sup>. If the leaf you are using fully covers the leaf chamber window, enter the area of the window. Table 7-1 and 7-2 at the end of this chapter list the window sizes of all the leaf chambers CID, Inc. manufactures.

Flow rate? (lpm) —
-----------------------

Enter the intended flow rate (in lpm). The pump and flow sensor have been designed to operate under controlled specifications. Use a flow rate between 0.1 and 1 lpm. **But if the CI-301AD is used, the flow rate should be < 0.5 lpm.**

Finally, the CI-340 will ask whether an open or closed system measurement will be conducted. The

default entry is for the open system (press  to use the default). Press "C" to select the closed system measurement or "O" for open system. The closed system function will ask for the chamber volume (in liters). Refer to Table 7-2 for closed system chamber information. Closed system measurements can be terminated when a certain change in time ( $\Delta T$ ) or change in CO<sub>2</sub> ( $\Delta CO_2$ ) has been measured, or the  key can be pressed. Press "T" for  $\Delta T$  or press "C" for  $\Delta CO_2$ . The system will then ask for the length of time or the change in CO<sub>2</sub> required for the measurement. Also, refer to the CLOSED AND OPEN SYSTEMS section for further elaboration of measuring procedures.

The instrument will be stabilizing for several seconds. Then data output will be displayed. Use the arrow keys on the keypad to scroll up and down to view all the data.

### Closed and Open Systems

The CI-340 promotes a portable solution to the concept behind open and closed systems. A closed system environment can measure the CO<sub>2</sub> concentration in the chamber over a given period of time. An open system environment can measure CO<sub>2</sub> concentration in the chamber by a steady air stream flow. At any rate (0.2 to 1.0 lpm), this instrument provides another practical method to determine photosynthesis, transpiration, and stomatal conductance.

The physical distinction between the two systems is with the accessory tubing attachment (Figure 9). The open system utilizes a steady stream of fresh air; the closed system recirculates the air within the analyzer environment. Figure 10 illustrates further the CI-340's closed and open system flow chart.

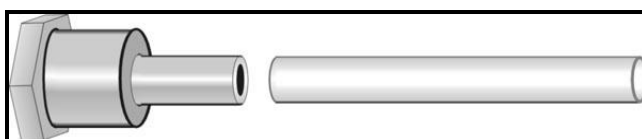


Figure 9. The CI-340 accessory tubing attachment.

**Note:** The tubing accessory should not be kinked or restricted during operation. A replacement tube can be used; it should be of vinyl or polyurethane material and approximately 5" long. Tygon B-44-3 is normally used because it does not absorb much moisture.

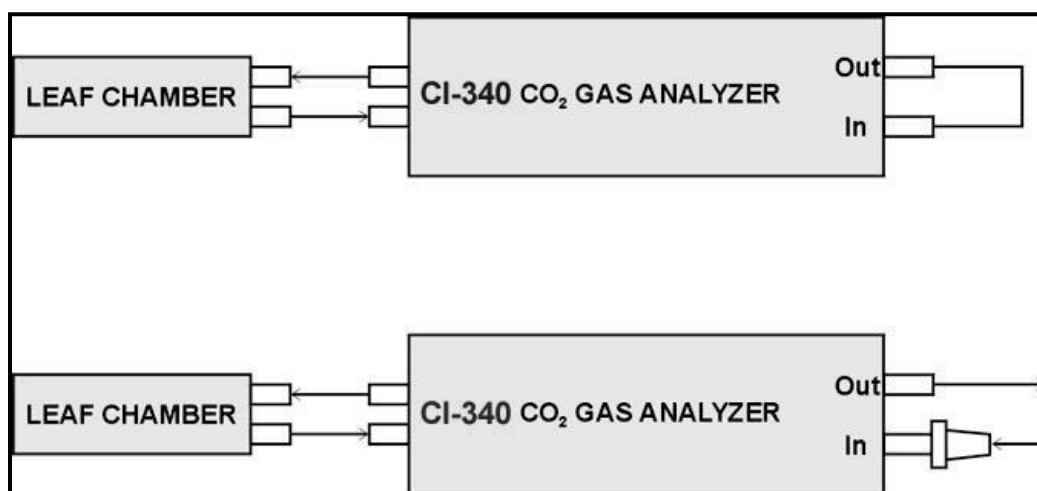


Figure 10 (top). Tubing schematic for a closed system. (bottom). Tubing schematic for an open system. A supplied filter should be connected between the inlet and the tubing.



The closed system recirculates the air in the system; the open system sends a constant flow of external air into the system. There are five available sizes of leaf chambers to conduct experiments for the open system method (Table 2). Generally, the manufacturer recommends choosing a chamber large enough to contain the leaf environment (i.e., using a leaf larger than the chamber area would produce inaccurate results).




Determine the best size chamber (Table 2) for the measurements to be taken. Proceed to insert and attach the leaf chamber to the CI-340. Refer to the LEAF CHAMBER — CARE AND USE chapter for proper applications of the leaf chamber(s). Then press the  or  and  key(s) to begin the measurement process.

Table 2: Open system chamber sizes.

Chamber Types	Window Size (W x L mm)	Window Area (cm <sup>2</sup> )
Square	25 x 25	6.25
Narrow Rectangular	65 x 10	6.5
Wide Rectangular	55 x 20	11
Small Cylindrical	25 x 90	22.5
Large Cylindrical	50 x 70	35

The closed system environment deals with chambers of variable volume. Each individual chamber is designed to interchange with the CI-340; however, they serve different sizes of the plant element. There are four available sizes of leaf chambers to conduct experiments for the closed system method (Table 7-2).




When using the closed system, refer to Table 7-2 for such related values. Also, refer to the *LEAF CHAMBER — CARE AND USE* chapter for proper applications of the leaf chamber. Press the  or  and  key(s) to begin the measurement process. The duration of collecting measurements is dependent upon the needs of the user.

Table 7-2. Closed system chamber sizes:

Chamber Types	Size (W x L x H mm)	Volume (liter)
1/4 Liter	104 x 33 x 73	0.2505
1/2 Liter	89 x 66 x 86	0.5052
1 Liter	112 x 91 x 99	1.0090
4 Liter	180 x 130 x 170	3.9780

## Terminology:

### Absolute, Differential and Continuous Mode

The CI-340 can perform measurements in absolute, differential or continuous mode. In absolute mode, the absolute CO<sub>2</sub>/H<sub>2</sub>O concentration from a single source, the inlet of the CI-340, is measured. This is compared against an absolute CO<sub>2</sub> calibration. Differential IRGA mode compares the measurement of CO<sub>2</sub> and H<sub>2</sub>O before the leaf chamber to the measurement of CO<sub>2</sub> and H<sub>2</sub>O after the leaf chamber. In differential mode, the CI-340 acquires absolute measurements both from the chamber environment and the instrument intake. Almost all commercial plant IRGA systems are of the differential type, because they provide more reliable measurements. The CI-340 is capable of obtaining absolute (single channel absolute, S) and differential (photosynthesis, P) measurements, as well as continuous measurements (continuous photosynthesis, C). In continuous mode, the gas concentration is measured once from the inlet and then continuously measured from the outlet of the leaf chamber. In order to accurately use continuous mode, the initial measurement from the inlet needs to be very stable, but this mode allows for very quick, repeated measurements.

### Photosynthesis

Photosynthesis is the process by which plants (and photoautotrophs) generate carbohydrates and O<sub>2</sub> from CO<sub>2</sub>, H<sub>2</sub>O (water) and sunlight or light energy (Figure 2). This process occurs in the chloroplasts or chlorophyll-containing tissues of the plant leaves and stem. The rate at which photosynthesis is occurring is determined by measuring the rate at which a known leaf area assimilates CO<sub>2</sub> over a given period of time.

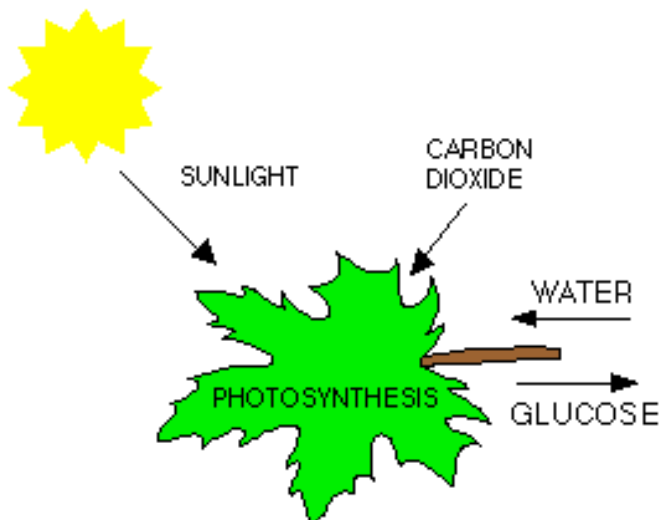


Figure 2: Photosynthesis occurs in chloroplasts in leaves, converting carbon dioxide and water to glucose using energy from sunlight.

### Photosynthetic Active Radiation (PAR)

Photosynthetically or photosynthetic active radiation (PAR) is the range of wavelengths of solar radiation that photosynthetic organisms are able to use for photosynthesis. Only about 44% of the total electromagnetic energy reaching the earth has the correct wavelengths for use by plants and of that only 0.5% - 3% is used for photosynthesis. Solar radiation from 400 to 700 nanometers is within the spectral range (wavelength) for photosynthetic organisms. This range is often referred to as photosynthetically active radiation or PAR. This spectral range is very similar to the range of light visible by the human eye (390-750 nm). The CI-340 measures PAR with silicon diode sensors mounted near the leaf chamber. The sensor measurement is cosine corrected.

Using PAR as a measure of radiant power is important in evaluating the effect of light on plant growth. The photosynthetic response correlates better with the number of photons than with energy. This is expected because photosynthesis is a photochemical conversion where each molecule is activated by the absorption of one photon in the primary photochemical process. PAR is defined in terms of photon (quantum) flux, specifically, the number of moles of photons in the radiant energy between 400 nm and 700 nm. One mole of photons is  $6.0222 \times 10^{23}$  photons (Avagadro's Number).

### Transpiration

Transpiration is the evaporation of water from plants, mainly occurring on the leaves when the stomata are open and thus allowing the passage of  $\text{CO}_2$  and  $\text{O}_2$  during photosynthesis and respiration. Transpiration also can occur in the stem, flowers and roots of plants. Leaf transpiration occurring through stomata can be thought of as a necessary metabolic "cost" as the stomata open to allow the diffusion of  $\text{CO}_2$  for photosynthesis. Transpiration also enables the mass flow of mineral nutrients and water from the roots to the shoots of the plant, as well as regulating the plant's temperature.

Transpiration is the primary determinant of leaf energy balance and plant water status. The rate of transpiration is determined by the accumulation of water vapor flux per one-sided leaf area in a given time and therefore is directly related to the degree of stomatal opening, and to the evaporative demand of the atmosphere surrounding the leaf (see equation section in this manual). There are

several factors that affect the rate of transpiration, including the size of the plant, light intensity, temperature, humidity, wind speed, and soil water content.

As light intensity increases, transpiration rate also increases. Plants transpire more rapidly in the light than in the dark because light stimulates the opening of stomata and increases the temperature of the leaf. Transpiration rate increases with temperature. This is because water evaporates faster at higher temperatures. As humidity decreases, the transpiration rate increases and water dissipates from leaf more quickly. This is because the rate of diffusion of any substance increases as the difference in concentration of the substances in the two regions increases. Wind affects transpiration by affecting the humidity of the air surrounding the leaf. When there is no breeze, the air surrounding a leaf becomes increasingly humid thus reducing the rate of transpiration. When a breeze is present, the humid air is carried away and replaced by drier air, increasing the rate of diffusion and transpiration. Soil water content affects transpiration rate as water is continually replaced in the plant by drawing water from the soil using the roots. A plant cannot continue to transpire rapidly if its water loss is not made up by replacement from the soil. When absorption of water by the roots fails to keep up with the rate of transpiration, a loss of turgor occurs, and the stomata close, immediately reducing the rate of transpiration, as well as the rate of photosynthesis.

### Stomatal Conductance

Stomatal conductance is water loss by a leaf and is directly related to the size of the stomatal aperture or opening. Higher evaporation rates or a high transpiration rate for a plant indicate that the stomatal conductance will also be high. Other factors influencing stomatal conductance include humidity, light intensity and temperature.

Stomatal apertures (Figure 3) will typically vary in response to changes in light intensity, saturation deficit of ambient water vapor and soil moisture availability. As stomatal aperture size changes, rates of photosynthesis and transpiration will vary because the pore (or stomata) size will provide a corresponding resistance to the diffusion of CO<sub>2</sub> into and H<sub>2</sub>O out of the leaf. The inverse of this resistance can be calculated as the conductance of these two gases across a leaf surface. Conductance can be considered in parallel or in series. It is obtained by measuring the transpiration and leaf surface temperature (°C), and applying the equation.

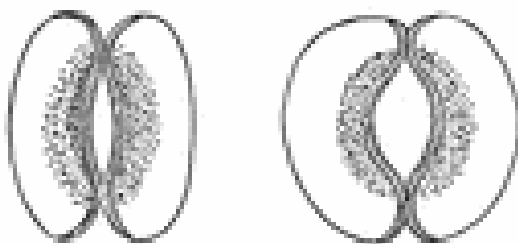


Figure 3: Stomata surrounded by guard cells, showing a closed stomata and an open stomata.

### Open and Closed Systems

The term closed or open is used in the sense of whether or not the atmosphere of the leaf-enclosing chamber is renewed during the measurement. The CI-340 supports both open and closed system modes of operation. Early photosynthesis analyzers supported only closed system measurements, but today most systems are open systems. Open systems provide a faster measurement and greater control over very small or mole fraction measurements. Open systems are also known as differential systems, while closed systems are known as depletion systems.

In open systems, ambient air is taken in through the intake on the instrument. The ambient air is then added into the leaf chamber after having the mole fraction or concentration of CO<sub>2</sub> and H<sub>2</sub>O measured (Figure 4). The air is measured again after passing over the leaf surface at a known flow rate, and the photosynthesis rate is calculated according to equation found in the Equations section of the manual as are the transpiration rate and stomatal conductance.

In a closed system, the air is not renewed during the measurement. The closed system mode recirculates air within the analyzer environment and leaf chamber, as seen the upper portion of Figure 4. The CO<sub>2</sub> concentration in the chamber is decreased by leaf photosynthetic activity, while the H<sub>2</sub>O concentration increases. The change in CO<sub>2</sub> and H<sub>2</sub>O concentrations per unit of time are correlated with net photosynthesis (assimilation) and transpiration, respectively.

The leaf chambers for closed system measurements are specially designed to help calculate the volume of air inside the chamber (versus the area of leaf surface needed for open system calculations).

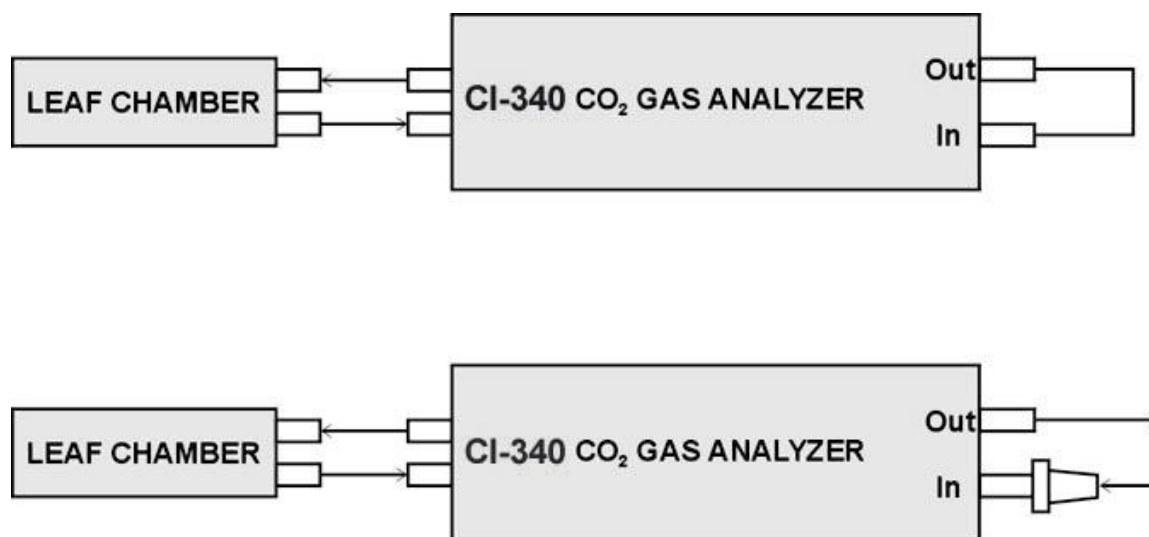


Figure 4: The top illustrates a closed system set up, with tubing connecting the intake and exhaust. The bottom illustrates an open system set up, with a filter attached to the intake and the exhaust remaining free allowing air to be renewed in the leaf chamber during measurement.

There are several different leaf chambers available for use with leaves and plants, as seen in Figure 5. Each individual chamber is designed to interchange with the CI-340; however, they serve different sizes of the plant element. There are five available sizes of leaf chambers to conduct experiments for the open system method (Table 2). The closed system environment deals with chambers of variable volume. There are four available sizes of leaf chambers to conduct experiments for the closed system method (Table 3).



Figure 5: Various different leaf chambers for the CI-340 and the soil respiration chamber.

Table 2: Open system chamber sizes and areas.

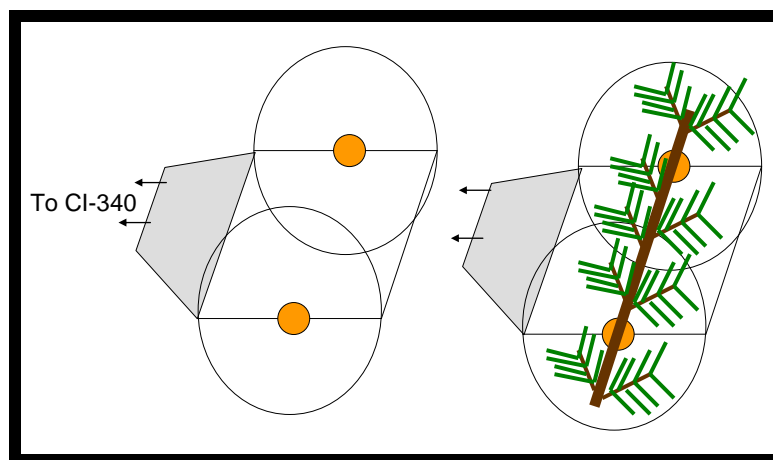
Chamber Type	Window Size (W x L mm)	Window Area (cm <sup>2</sup> )	CI-301-:
Square	25 x 25	6.25	LC-1
Narrow Rectangular	65 x 10	6.25	LC-3
Wide Rectangular	55 x 20	11	LC-2
Small Cylindrical	25 x 90	22.5	LC-4
Large Cylindrical	50 x 70	35	LC-5

Table 3: Closed system chamber sizes and volumes.

Chamber Type	Size (W x L x H mm)	Volume (liter)	CI-301-:
1/4 Liter	104 x 33 x 73	0.2505	LC-7
1/2 Liter	89 x 66 x 86	0.5052	LC-8
1 Liter	112 x 91 x 99	1.0090	LC-9
4 Liter	180 x 130 x 170	3.9780	LC-10
Canopy attachment	Determined by user	-	CC

## Measuring Conifer Needles Using the CI-340:

The CI-340 does not have specific conifer or needle chambers as opposed to chambers for broadleaves. The type of chamber selected for measuring conifers will depend on the plant type (average needle length and other characteristics). The LC-4 and LC-5 small and large cylinder chambers, respectively, are recommended for measuring small leaves/leaflets or needles still attached to the branch. Both chambers are specially designed with an opening that provides for a branch to fit into the chamber (and extend through it) while still sealing. The orange section of the seal is double-lined, approximately 7 mm thick, and has small slits allowing it to expand and accommodate the shoot going through the chamber.



### LC-4 Small Cylinder Chamber Specifications:

- Window size (width x length): 25 mm x 90 mm
- Window area: 22.5 cm<sup>2</sup>
- Diameter of chamber: 2 cm
- Maximum diameter of branch: 0.5 cm
- Maximum diameter of branch and needles: 2 cm

### LC-5 Large Cylinder Chamber Specifications:

- Window size (width x length): 50 mm x 70 mm
- Window area: 35 cm<sup>2</sup>
- Diameter of chamber: 5.5 cm
- Maximum diameter of branch: 1 cm
- Maximum diameter of branch and needles: 5.5 cm

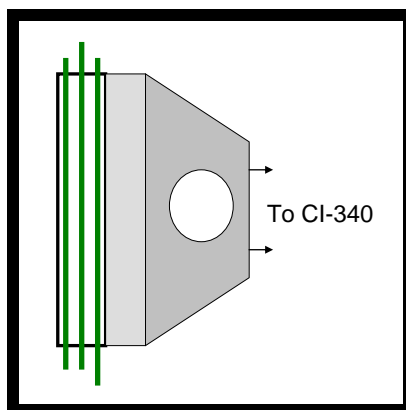
The LC-1, LC-2, and LC-3 chambers may also be suitable for gathering measurements from conifer needles. However, these chambers do not allow for an entire branch to pass through the chamber. If the plant has characteristically long needles (longer than the LARGEST side of a plant chamber) then the needle or needles can be manipulated to extend across the chamber and be sealed on both sides. Several needles can be lined up next to each other to increase the leaf area being measured inside the chamber. It may be easiest to remove the needles to be measured from the plant, but if this is not an option then using the branch and cylinder-type chamber would most likely be best.

Chamber Type	Window Size (W x L mm)	Window Area (cm <sup>2</sup> )
LC-1: Square	25 x 25	6.25
LC-2: Narrow Rectangle	65 x 10	6.5
LC-3: Wide Rectangle	55 x 20	11



## Estimating Leaf Area:

If the window of the chamber is fully covered by plant leaf/needle then enter the designated leaf area for that chamber in the CI-340. If the window is not fully covered, estimate the percentage that is covered and enter that leaf area into the CI-340. If the branch of the plant is inside the chamber (LC-4 or LC-5) the branch does not usually need to be included in the estimated leaf area. If using individual needles and LC-1, 2, or LC-3 make sure that the needles extend across the leaf chamber and is sealed on both sides. Do not let the needle/leaf touch the bottom of the chamber as this will disrupt the circulation of air in the chamber.



The image above illustrates three needles extending all the way across a narrow rectangle leaf chamber. The leaf area will need to be estimated visually by determining the percent of the window that is covered by the needles (green area). In the example above, the three needles cover about half (50%) of the window on the upper surface of the leaf chamber. Next, apply this percentage to the size of the entire window area (6.5 cm<sup>2</sup>). The leaf area entered into the CI-340 should be approximately 3.25 cm<sup>2</sup>.

Selecting the right chamber depends on the type of plant you are dealing with. If you would like to describe it to me, together we can determine which would be the best leaf chamber for your research needs.

## Calibrating

As components age and equipment undergoes changes in temperature or sustains mechanical stress, critical performance gradually degrades. This process is called drift. When this happens, test results become unreliable and design or production quality suffers. Although drift cannot be eliminated, it can be detected and contained through the process of routine calibration. Calibration serves to standardize equipment by determining the deviation from a standard, so as to set correction factors precisely. Calibration increases production yields, optimizes resources, assures consistency and ensures readings are compatible with those made elsewhere.

There are several important terms to be considered when understanding both why calibration is necessary and the difference between just data and good, quality, (reproducible) data. Many terms are closely linked, such as calibration and traceability.

- **Calibration** is the comparison between measurements, one of known magnitude or correctness, made or set with one device and another measurement made in as similar a way as possible with a second device.<sup>1</sup>
- **Traceability** is the unbroken chain of comparisons relating an instrument's measurements to a known standard. An instrument's bias, precision and accuracy can be determined if an instrument is calibrated to a traceable standard.
- **Precision (or reproducibility or repeatability)** is the degree to which repeated measurements under unchanged conditions show the same results.
- **Accuracy** is the degree of closeness of measurements of a large quantity to its actual or true value. In other words, accuracy is how many times the same measurement conditions produced the exact same measurement over a large number of samples.
- **Bias** is non-random or directed effects caused by a factor or factors unrelated to the independent variables.
- **Error** is the random variability, or the amount of deviation from a standard or specification. It is also important to consider error when calibrating.
- **Resolution** refers to the smallest change in a measured value that the instrument can detect, or the ability of the instrument to read in fine increments (small increases or decreases in possible values of measurements).
- **Reliability** of measurements is sought after calibrating the instrument. Reliable measurements are accurate and repeatable, as well as traceable.



The CI-340 has been engineered to ensure maximum reliability as well as ease of instrument use in the field. The resolution of infrared gas analyzers is directly related to the length of the infrared gas analyzer chamber. The longer the tube to the chamber is, the finer the resolution. In order to help the CI-340 produce repeatable measurements, a single IRGA tube next to the leaf chamber is used to measure CO<sub>2</sub> levels in the air before and after the leaf chamber. This increases repeatability because with only one infrared sensor and a single light source, drift of the electronic components in the machine is reduced (versus having multiple sensors and light sources which would all drift independently of each other). Also, drift on humidity sensors has been reduced, providing high repeatability in water content measurements by using laser trimmed humidity sensors.

There are five calibrations that can be done to increase the reliability and repeatability of the CI-340. These include resetting the calendar and time of the instrument, recalibrating the leaf/air temperature sensor (done only at CID), resetting the atmospheric pressure (done by user), recalibrating the flow rate (done by user with flow meter), and recalibrating the CO<sub>2</sub> and H<sub>2</sub>O concentration settings (done by user with gases of known concentrations). Please refer to later sections for detailed instructions on how to calibrate the CI-340.

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<sup>1</sup> The device with the known or assigned correctness is known as the standard, while the second device is the unit under test or test instrument. Typically standards with which calibrations are set on come from several national and international standards organizations, such as the International Organization for Standardization (ISO), International Calibration Standards (ICS), National Institute of Standards and Technology (NIST) and the American National Standards Institute (ANSI).

## DATA DISPLAY SCREENS

Numeric data is displayed during the measurements. The different screens can be accessed by pressing the arrow keys,  to move up and  to move down:

Starting Screen:

CO <sub>2</sub> in	CO <sub>2</sub> out
CO <sub>2</sub> dif	Pn
PAR	ATM
Flow	W
Tair	Tleaf
Flourescence	Count

Screen 2:

H <sub>2</sub> O in	H <sub>2</sub> O out
H <sub>2</sub> O dif	E
RH <sub>in</sub>	RH <sub>out</sub>
Tair	Tleaf
IntCO <sub>2</sub>	C
Exit to quit	InternalT

## GRAPH MODE

The data display can be switched between graphic mode and alpha/numeric mode by pressing “G” (shift, 3). The upper 1/8th of the graph will be erased when switching between modes (a memory limitation with the current hardware). It is best used for large sample data.

With the use of the CI-510LA (CI-301LA), the response curve of photosynthesis vs. light can be seen by pressing “L” when measuring photosynthesis (that is shift, shift, shift 4). The CI-340 will ask for the number of steps for light response and then will direct the CI-510LA to increase the light intensity from very low to very high in that number of steps. Make sure the intensity knob on the CI-510LA is turned all the way counterclockwise (see Appendix B). Only the CI-510LA ordered with CI-340 can be automatically controlled by the CI-340 to generate the light response curve.

The response curve of photosynthesis vs. CO<sub>2</sub> can be seen by pressing “C” (shift, shift, shift, 1) when measuring photosynthesis. The CI-340 will then ask what step size to make the CO<sub>2</sub> adjustments, and then it will direct the CI-510AD (CI-301AD) to slowly step from the lower limit to the upper limit,

using approximately the specified step size. Make sure the CO<sub>2</sub> knob is turned all the way counter-clockwise. Only the CI-510AD ordered with the CI-340 can be automatically controlled by the CI-340 to generate the CO<sub>2</sub> response curve.

The response curve of photosynthesis vs. air temperature can be seen by pressing “T” (shift, shift, 7) when measuring photosynthesis. The instrument will ask for the number of steps for the temperature response curve, and then will direct the CI-510CS (CI-301CS) Temperature Controller to start at a low temperature and increase the air temperature in the leaf chamber to a high temperature in that number of steps. Make sure the temperature control knob is turned all the way counterclockwise. Only the CI-510CS ordered with the CI-340 can be automatically controlled by the CI-340 to generate the temperature response curve.

Pressing “EXIT” while in the graph mode can stop the response curve. This will return the display to the alpha/numeric mode and stop any further changes to the controlled parameters. It will also erase the graph.

The tick marks at the left of the screen while in graph mode represent 5  $\mu\text{molm}^{-2}\text{s}^{-1}$ .

## CARE OF THE CI-340

This instrument is designed for portable or stationary use. Whether it is used in the field or on a tripod, the CI-340 is versatile and lightweight, as well as accurate and user-friendly. The manufacturer would like to give you a few suggestions to help you take care of your instrument.

- Although the instrument can operate while in motion, avoid any unnecessary movement, and avoid subjecting the instrument to drastic shock. Due to the lightweight design, the CI-340 represents features that are sensitive to the given precautions.
- Do not allow water or excessive moisture to get into the instrument. The CI-340 can measure air with high humidity, but it is highly recommended to avoid such circumstances. In addition, if used in the field environment, please avoid extremely dusty conditions. *Always* use the external filter provided.
- Operate the instrument using ideal temperature settings. Manufacturer testing shows that operating the CI-340 under extreme temperatures (below 5°C or above 45°C) will affect its performance.
- When not in use, store the instrument in a cool and dry location. Preferably, place the CI-340 back in the carrying case in an ambient or reasonably cool room.

- Do not open or break the seal on the instrument. Under any conditions that require attention internally, immediately contact the nearest representative or manufacturer to assist you.

## **LEAF CHAMBER CARE AND USE**

The leaf chambers are designed to be interchangeable with the CI-340. Any of the closed or open system chambers can be used with ease and reliability. The leaf chambers should receive the same care and attention as is recommended for the CI-340.

When attaching a chamber (see Figure 9-1) to the instrument, check that the O-rings (black, rubbery rings) are in place on the connecting tubes of the chamber. Although the O-rings are not permanently fixed on the tubes, they play an important part of assuring a better seal to the interconnecting CI-340. A chamber without O-rings on the tubes may demonstrate leakage at the CI-340 connection.

To attach the chamber to the CI-340, carefully slide the tubes on the chamber into the “head” end of the instrument, aligning the locking screw (from the CI-340) with the mating hole in the chamber. Turn the locking screw to fasten the chamber to the CI-340, without over-tightening. This assures a good lock and maximizes contact area for electrical connection between the chamber and the instrument. The instrument may be kept on; however, do not start measurements without first attaching a chamber.

Furthermore, the IR Temperature sensor and PAR sensor should be inserted into their respective locations on the chamber (see Figure 9-2). Check that the IR Temperature sensor is plugged securely (without bending the tiny wire by the sensor lens) and the PAR sensor is firmly slid in to acquire ideal measurement conditions. Connect the IR Temperature sensor and PAR sensor plugs into their respective locations on the “head” end of the CI-340. The IR Temperature sensor should be handled with great care.

With the chamber attached to the CI-340, and the IR Temperature sensor and PAR sensor inserted, the leaf chamber is ready for use. Place the sample between the seals of the chamber. Now, gently close the chamber; it will lock into place on the sample. Once sampling is completed, push the release head forward to open the chamber. For LC-4, 5, 7-10, the release head is located on the latch piece of the chamber. For LC-1-3, the release head is located on the topside. To disassemble, simply reverse the steps followed to assemble the unit

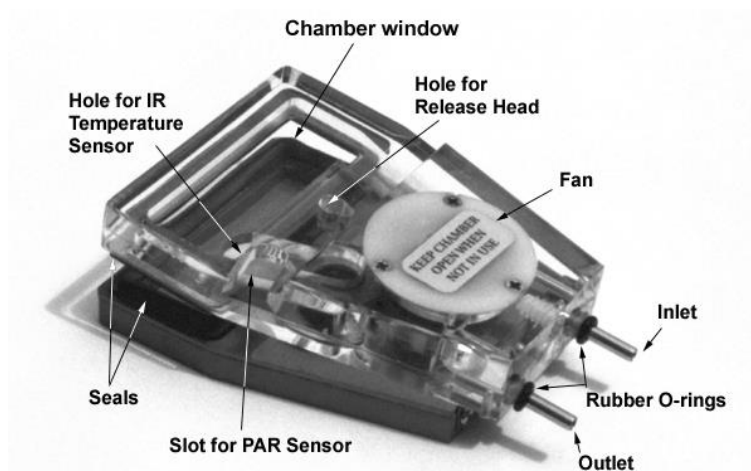
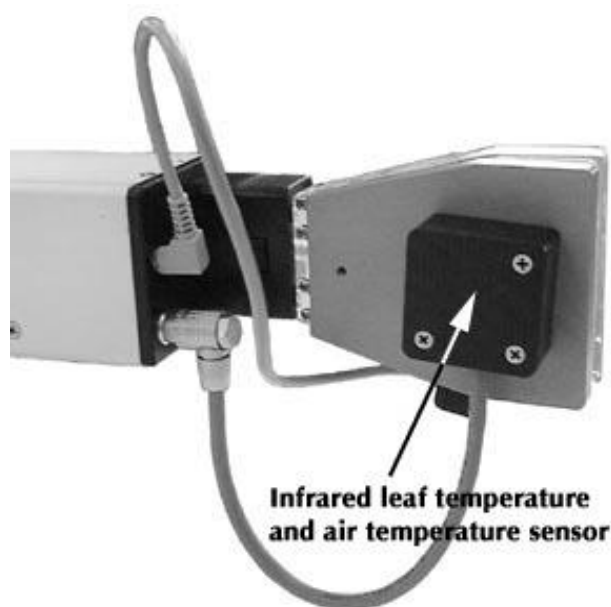


Figure 9-1. Illustration of a leaf chamber: CI-301LC-2, Wide Rectangular Chamber for an open system.

We recommend working in temperature ranges between 5 and 45 °C for greatest precision. Be sure **both** sensors are connected to the leaf chamber prior to using. The PAR sensor is extremely sensitive to even subtle changes in the light environment.



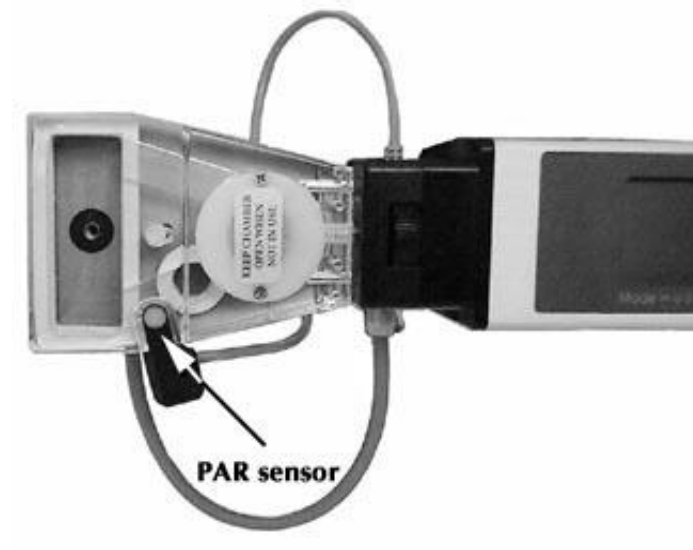


Figure 9-2. Connecting a leaf chamber to the CI-340.

## RECHARGING

The CI-340 includes a 7.2V, 3.7Ah Li-ion battery. This battery pack is common among equipment that utilizes portability (primarily video camcorders). When the battery is fully charged, it will last for approximately five hours of continuous use. Optional batteries can be used with more operational time available.

Any one of the following conditions will affect the power supply and the operation of the instrument:

- Batteries are tested and fully charged when they leave the manufacturer, but they discharge during shipping and transport.
- When the battery voltage is below 5.6V, the message “Low Battery” will appear on the screen. This indication signals an approximate five minutes of use remaining before the instrument shuts down.
- When operating the instrument at colder temperatures (below 5°C), the battery will diminish in performance.

Batteries should be fully recharged as soon as possible after use. Storing in a discharged state can ruin the battery. The batteries should be stored in a cool and dry place, fully charged. Check with the warranty, manufacturer, or the nearest representative for support if the battery has been damaged or tampered with.

Rechargeable batteries should be recycled after their useful life. Visit [www.rbrc.org](http://www.rbrc.org) for more information on recycling.

### TO POWER YOUR CI-340 FROM A LAB POWER SOCKET

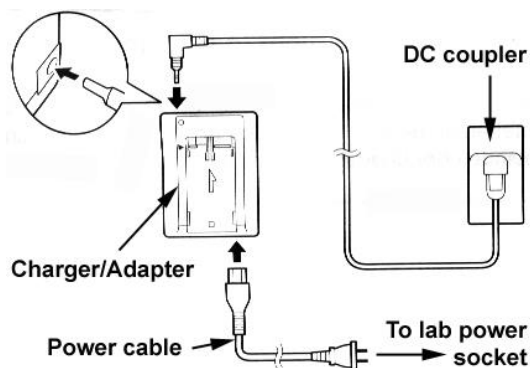


Figure 10-1. Charger/adaptor, power cable and DC

*coupler connections.*

1. Connect the DC coupler to the CI-340. Align the front end of the DC coupler with the guides on the back of the CI-340, then press and slide the DC coupler into the CI-340 to set it in place.
2. Connect the power cable to the charger/adaptor.
3. Plug the power cable into a lab power socket.

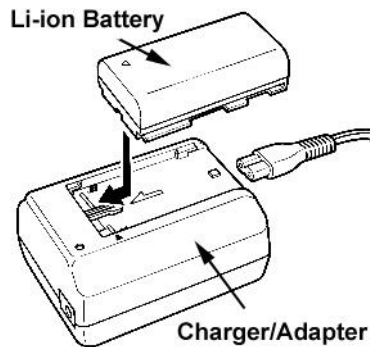


4. Connect the DC coupler to the charger/adaptor's DC terminal.

#### **TO DISCONNECT THE CHARGER/ADAPTER**

1. Turn the CI-340 off and detach the DC coupler.
2. Disconnect the DC coupler from the charger/adaptor.
3. Unplug the charger/adaptor and disconnect the power cable from the charger/adaptor.

#### **TO CHARGE THE Li-ion BATTERY**



*Figure 10-2. Attach the Li-ion Battery to the charger/adaptor.*

Note: Make sure that the DC coupler is not connected to the charger/adaptor. The Li-ion Battery will not charge if the DC coupler is connected to the charger/adaptor.

1. Connect the power cable to the charger/adaptor. (See Figure 10-2.)
2. Plug the power cable into a lab power socket.
3. Attach the Li-ion Battery to the charger/adaptor.
  - Align the front end of the Li-ion Battery with the guides on the charger/adaptor, then press and slide the Li-ion Battery into the charger/adaptor to set it in place.
  - The red charge indicator will flash while the Li-ion Battery is charging. Single flashes indicate that the Li-ion Battery is charged less than 50%. Double flashes indicate that it is 50-75% charged. Triple flashes indicate that it is more than 75% charged. The indicator shines steady when the Li-ion Battery is fully charged.
4. Remove the Li-ion Battery.
5. Unplug the charger/adaptor and disconnect the power cable from the charger/adaptor.

#### **NOTES**

- If the charger/adaptor does not seem to be working, this may be because its safety circuit has been activated. Unplug the charger/adaptor, wait a few minutes, and plug it in again.
- The charge indicator does not light up when the DC coupler is connected.

- If the adapter is used next to a TV, it may cause the TV to emit noise – move the charger/adapter away from the TV or the aerial cable.
- Never unplug the charger/adapter during use or disconnect the DC coupler from the charger/adapter when the DC coupler is being used.
- Be sure to unplug the charger/adapter when you have finished using it.
- Battery charging will stop if you connect the DC coupler to the charger/adapter, but it will resume as soon as you disconnect it.
- The charger/adapter can be used with a power supply between 100 and 240 V AC. Contact your Canon Service Center for information about plug adapters for overseas use.
- To prevent equipment breakdowns and excessive heating, do not connect this charger/adapter to voltage converters used by travelers, or special power sources such as on aircraft, ships or DC to AC inverters, etc.
- Use only specified CI-340 products with this charger/adapter.
- Do not disassemble the charger/adapter (or DC coupler), and do not expose it to water, shock or vibration, or to direct sunlight. Avoid exposure to high temperatures, such as a closed car in hot weather, and do not leave it near heat-radiating equipment, such as a stove or heater.

#### BATTERY CHARGER SPECIFICATIONS

Power supply:	100 to 240 V AC, 50/60Hz
Power consumption:	24 W
Rated output:	Adapter mode: 7.2 V, 2.0 A DC
(Nominal)	Charge mode: 8.4 V, 1.5 A DC
Operating temperature range:	0 – 40°C (32 – 104 °F)
Dimensions:	75 x 51 x 99 mm (3 x 2 x 3 <sup>7/8</sup> in.)
Weight:	215g (7 <sup>5/8</sup> oz.)

Weight: Weight and dimensions are approximate. Errors and omissions excepted. Subject to change without notice.

## EQUATIONS

1a W: Mass flow rate per leaf area (mol/m<sup>2</sup>/s) for **open system**

$$W = \frac{V}{60} \times \frac{273.15}{T_a} \times \frac{P}{1.013} \times \frac{1}{22.41} \times \frac{10000}{A}$$

$$= 2005.39 \times \frac{V \times P}{T_a \times A}$$

Where *V*: volume flow rate (liters/minute)

*T<sub>a</sub>*: air temperature (K)

*P*: atmospheric pressure (bar)

*A*: leaf area (cm<sup>2</sup>)

60: converts minutes into seconds

22.41: the volume of one mole of any gas at a standard temperature of 273.15 K and a standard pressure of 1.013 bar (liters/mol)

10000: converts cm<sup>2</sup> into m<sup>2</sup>

1b W: Mass flow rate per leaf area (mol/m<sup>2</sup>/s) for **closed system**

$$W = \frac{V}{\Delta t} \times \frac{273.15}{T_a} \times \frac{P}{1.013} \times \frac{1}{22.41} \times \frac{10000}{A}$$

$$= 120323.35 \times \frac{V \times P}{\Delta t \times T_a \times A}$$

Where *V*: leaf chamber volume (litres)

$\Delta t$ : time interval (sec.)

*T<sub>a</sub>*: air temperature (K)

*P*: atmospheric pressure (bar)

*A*: leaf area (cm<sup>2</sup>)

22.41: the volume of one mole of any gas at a standard temperature of 273.15 K and a standard pressure of 1.013 bar (liters/mol)

10000: converts cm<sup>2</sup> into m<sup>2</sup>

2a P<sub>n</sub>: Net photosynthesis rate (μmol/m<sup>2</sup>/s) for **open system**

$$P_n = -W \times (C_o - C_i) = -2005.39 \times \frac{V \times P}{T_a \times A} \times (C_o - C_i)$$

Where *C<sub>o</sub>* (*C<sub>i</sub>*): outlet (inlet) CO<sub>2</sub> concentration (ppm or μmol/mol)

2b  $P_n$ : Net photosynthesis rate ( $\mu\text{mol}/\text{m}^2/\text{s}$ ) for closed system

$$P_n = 120323.35 \times \frac{V \times P \times \Delta C}{\Delta t \times T_a \times A} = W \times \Delta C$$

Where  $\Delta C$ :  $\text{CO}_2$  decrement from the initial reading (ppm or  $\mu\text{mol}/\text{mol}$ )

3  $E$ : Transpiration rate ( $\text{milimol}/\text{m}^2/\text{s}$ )

$$E = \frac{e_o - e_i}{P - e_o} \times W \times 10^3$$

$$e_o = hr_o \times e_s / 100$$

$$e_i = hr_i \times e_s / 100$$

$$e_s = 6.13753 \times 10^{-3} \times e^{T_a \times \frac{18.564 - \frac{T_a}{254.4}}{T_a + 255.57}}$$

Where  $e_o$  ( $e_i$ ): outlet (inlet) water vapor (bar)

$P$ : atmospheric pressure (bar)

$e_s$ : saturated water vapor at air temperature (bar)

$T_a$ : air temperature ( $^{\circ}\text{C}$ ):

$hr_o$  ( $hr_i$ ): outlet (inlet) relative humidity (%)

4  $C_{\text{leaf}}$ : Leaf stomatal conductance ( $\text{millimol}/\text{m}^2/\text{s}$ )

Where  $e_{\text{leaf}}$ : saturated water vapor at leaf temperature (bar)

$$C_{\text{leaf}} = \frac{W}{\frac{e_{\text{leaf}} - e_o}{e_o - e_i} \times \frac{P - e_o}{P} - R_b W} \times 1000$$

$$e_{\text{leaf}} = 6.13753 \times 10^{-3} \times e^{T_{\text{leaf}} \times \frac{18.564 - \frac{T_{\text{leaf}}}{254.4}}{T_{\text{leaf}} + 255.57}}$$

$T_{\text{leaf}}$ : leaf temperature ( $^{\circ}\text{C}$ )

$R_b$ : leaf boundary layer resistance ( $\text{m}^2\text{s}/\text{mol}$ ).  $0.3 \text{ m}^2\text{s}/\text{mol}$  is used.

Leaf temperature should be obtained through the IR Temperature sensor. It would be recommended to use the best average value for this temperature determinant. An example procedure could involve a sampling of five temperature values in a given period of time.

## 5 CO<sub>2int</sub>: Internal CO<sub>2</sub> (ppm or μmol/mol)

$$CO_{2int} = C_i - 1.6 \times P_n (R_b + R_{leaf})$$

Where  $R_{leaf}$ : leaf stomatal resistance (m<sup>2</sup>s/mol)

## TROUBLESHOOTING

If for some reason the instrument is not performing as you expect, please follow these troubleshooting procedures. If these procedures do not solve the problem, contact the manufacturer or nearest representative.

Display does not come on . . .

- Check the battery connection. Make sure the battery is properly inserted.
- Dead battery. Battery needs to be replaced or recharged.
- Too much direct light on the display. Pivot or ‘shadow’ the display area such that direct light does not “blank” out the screen. Excessive glare may be the obvious problem
- Make sure the accessory cable (if plugged into the CI-340) is in the “OFF” position, towards the instrument, or simply unplug the cable connector. The switch should only be in the “ON” position, away from the instrument, when downloading codes from a computer.

Display does not come on after continuous operation...

- Check the battery connection. Make sure the battery is properly inserted.
- Press the EXIT or STOP key to stop any on-going function, or...
- Press the OFF key to automatically shut off the power, or...
- Remove the battery, then re-insert.

Display flickers, then goes out . . .

- Low battery. Battery needs to be replaced or recharged.
- Check the battery connection. The battery may not be completely installed.

Keys on the keypad do not respond effectively . . .

- Apply firm pressure to and hold the key(s) until the beep is heard. Multiple keys pressed (simultaneously) may result in unintentional signal(s) to the microprocessor.
- Be sure to press the requested key(s) necessary for information to be processed. Any key, when the unit is powered on, will produce a “beep” sound.

Keypad sound (“beep”) does not respond effectively . . .

- Make sure to press the key(s) firmly and hold until the “beep” sounds. When the instrument is busy performing a task, it may not respond immediately to a pressed key.
- Check to see that the key sequence(s) are valid. Randomness does not guarantee a proper operational instrument.

CO<sub>2</sub>, H<sub>2</sub>O readings dramatically fluctuate or deteriorate during measurements . .

- Check for proper tube, chamber connections. A good secure fit obtains and promotes more accurate measurements and results.
- Make sure the chamber head is closed properly. The measuring environment should be appropriately sealed by the chamber head so that the intended sample is analyzed accurately.
- The sample(s) may not be a good source.
- Ambient, internal operating temperatures may be too extreme. Check with the instrument accessory specifications for ideal operating conditions.

Analyzer displays zero values for CO<sub>2</sub>, H<sub>2</sub>O concentrations . . .

- Check for proper calibration(s). Using a Di-nitrogen (N<sub>2</sub>) source for both the CO<sub>2</sub> and H<sub>2</sub>O concentration is highly recommended for the 0-ppm source. Using a 300- or 600-ppm source is highly recommended for the known source of CO<sub>2</sub>; a known source between 1 ~ 7.5 kPa of water vapor pressure is required.
- The sample(s) may not be a good source.

If at the end of setting photosynthesis parameters, the “Exit to Quit” screen remains on longer than 30 seconds, it is possible that the flow calibration is not calibrated properly.

## SYSTEM SPECIFICATIONS

### CO2 Analyzer:

Sensor: Low power Non-Dispersive Infrared Gas Analyzer. No sensitivity to motion

Chopping frequency: 1 Hz

Source life: 5000 hours

Sensor response time: 35 seconds

Repeatability:  $\pm 0.1$  ppm (short term)

Sample cell: 100 mm  $\times$  10.2 mm (3.94" L  $\times$  0.40" Dia)

Warm-up time: Approximately 3 minutes

Accuracy:  $< \pm 2\%$  up to 2000 ppm

Resolution: 0.1 ppm.

Power supply: 7.2 VDC, 4400 mAh for 5 hours continuous use, extended hours of use with additional batteries. AC Adapter / Battery Charger supplied

Power consumption: 2.5 watts (sample pump in operation)

Pump flow rate: 100 ~ 1000 cm<sup>3</sup> / min

Mass flow meter Accuracy: 2%

Display: LCD 40 x 6 characters or 320 x 64 pixel

Data output: PC link cable, RS232 or USB (with adapter)

Data storage: 4 MB internal FLASH RAM

Operation temperature: 0 to 45°C, 0 - 90% RH non-condensing

Dimensions: 44 cm x 5.5 cm x 5 cm

Weight: 1.5 Kg., (3 lb.) (with battery)

Standard range: 0 to 2000 ppm (Standard) - 0 to 3000 ppm (Optional)

### H2O Analyzer

Sensor Type: Humidity Sensitive Capacitor

Stability: Stable Analyzer for Accurate H2O Measurements

Measuring Range: 0 to 100% RH

Resolution: 0.1%

Accuracy:  $\pm 2\%$  at 10% RH,  $\pm 3.5\%$  at 95% RH

Sensor response time: 15 seconds

**Typical Signal:** The sensor electronics typically puts out a 0.5V signal for 50% RH, which is amplified to 2.34V and measured with a 16bit ADC.

### ***PAR Sensor***

Sensor Type: Filtered GaAsP photodiode

Range: 0 ~ 2500  $\mu\text{mol m}^{-2} \text{s}^{-1}$

Accuracy:  $\pm 5 \mu\text{mol 0-2500 } \mu\text{mol / m}^2 \text{ / sec}$

Response: 400 ~ 700 nm

### ***Chamber Temperature Measurement***

Type: Thermocouple

Range: -15 ~ 50°C

Accuracy:  $\pm 0.1^\circ\text{C}$

Display: LCD 40x6 characters 320x64 pixel

### ***Leaf Temperature Measurement***

Sensor Type: Infrared

Range: -10 ~ 50°C

Accuracy:  $\pm 0.3^\circ\text{C}$



## POWER PACK

The CI-340 Battery Pack is necessary to operate the modules to control light, temperature, CO<sub>2</sub> concentration, humidity level and chlorophyll fluorescence measurement. CI-340 Battery Pack (Figure 14-1) includes two rechargeable batteries, AC power supply charger (Figure 14-2), carrying bag, and cable connections for optional modules.

If you have all four modules, the power cable should be plugged in during operation (Figure 14-3). If you do not have all four modules, there will be foam spacers in the unfilled spaces in the bag.

Two hours are needed to charge the battery. You do not need to discharge the battery before recharging.

**IMPORTANT:** *Be sure to charge the battery every day when you are using it. Never let the battery power run completely out. Doing so may damage the battery.*

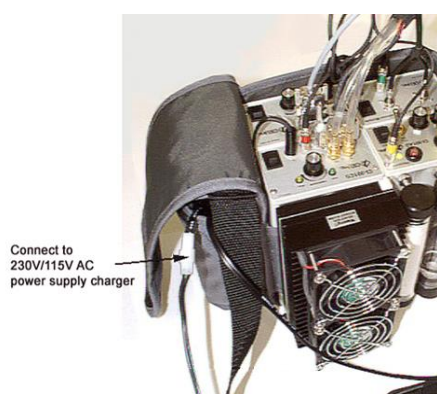


Figure 14-1. Plug in power cables to run the modules after connecting the batteries.

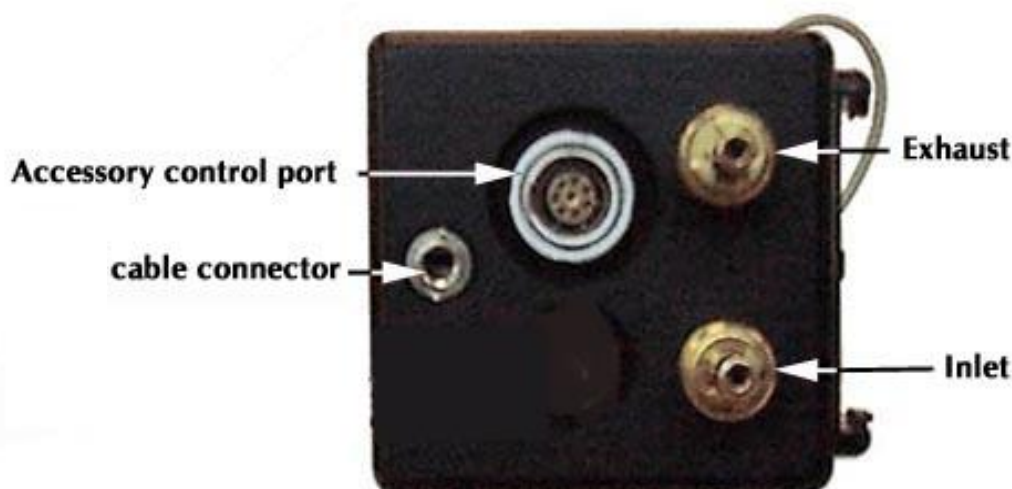


Figure 14-2. 230V/115V AC Power Supply Charger



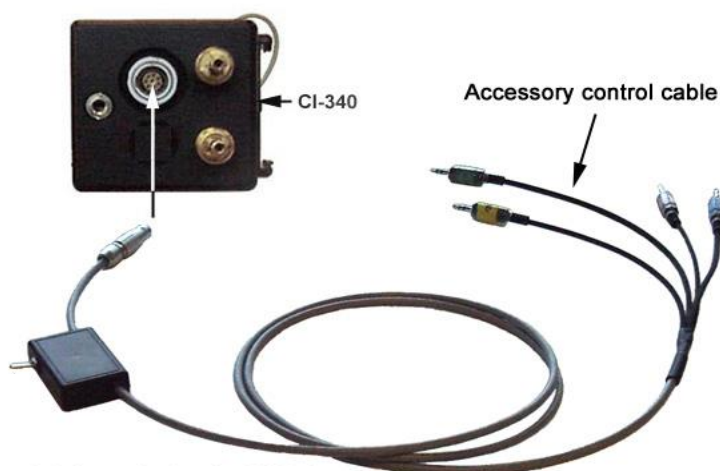
Figure 14-3. Modular bag with four modules.

## ACCESSORY CONTROL PORT AND CABLE



*Figure 14-4. CI-340 Accessory Control Port.*

The colored jacks on the accessory control cable (Figure 14-5) indicate the accessory / module to plug into. The red plug goes to the CI-510CS, the blue to the CI-301LA, green to the CI-510CF, and the yellow plug to the CI-301AD.



*Figure 14-5. Accessory Control Cable with color indicating jacks.*

## TROUBLESHOOTING

### Technical Support

If you have a question about the CI-340 features and functions, first look in the CI-340 Instruction Manual. If you cannot find the answer, you can access troubleshooting information and the CI-340 Product Support Forum at:

<https://www.cid-inc.com/support/CI-340/>

Questions can also be directed to a Technical Support Representative located in your country. CID Bio-Science, Inc. is committed to provide customers with high quality, timely technical support. Technical support representatives are to answer your technical questions by phone or by e-mail at [support@cid-inc.com](mailto:support@cid-inc.com). Please use the serial number of your instrument as the subject line of the email.

CID Bio-Science, Inc.'s contact information:

CID Bio-Science, Inc.  
1554 NE 3<sup>rd</sup> Ave  
Camas, WA 98607 USA

Phone: 800-767-0119 (U.S. and Canada)  
360-833-8835  
Fax: 360-833-1914

Internet: <http://www.cid-inc.com>  
E-mail: [support@cid-inc.com](mailto:support@cid-inc.com)

### Customer Service

Customer Service Representatives answer questions about specifications and pricing, and sell all of the CID Bio-Science, Inc. products. Customers sometimes find that they need CID Bio-Science, Inc. to upgrade, recalibrate or repair their system. In order for CID Bio-Science, Inc. to offer these services, the customer must first contact us and obtain a Return Merchandise Authorization (RMA) number. Please contact a customer service representative for specific instructions when returning a product.

### FAQs

If there are any questions about the CI-340, please check the Frequently Asked Questions located on the support page at <http://www.cid-inc.com/support>

## APPENDIX A

### CI-510CS TEMPERATURE CONTROL MODULE

The CI-510CS Temperature Control Module consists of two components (see Figure A-1 and A-2): A controller and Temperature Control Attachment. The CI-510CS allows you to increase or decrease the temperature  $\pm 25^{\circ}\text{C}$  from ambient temperature.

**CAUTION:** Avoid any obstruction or contact with the fan guards, especially small objects and fingers.



Figure A-1: The front panel of the CI-510CS Temperature Control Module Controller

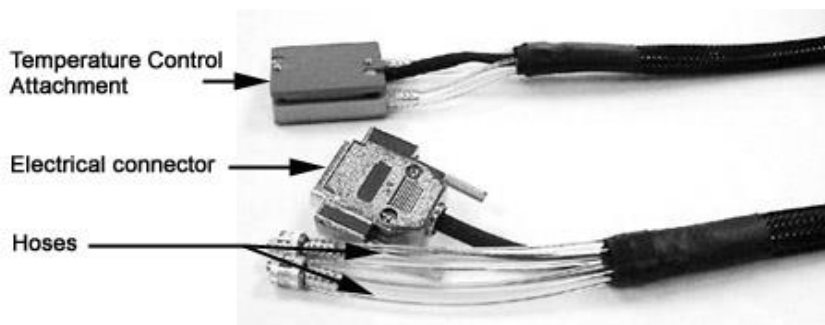


Figure A-2. Temperature Control Attachment, Electrical connector and Hoses.

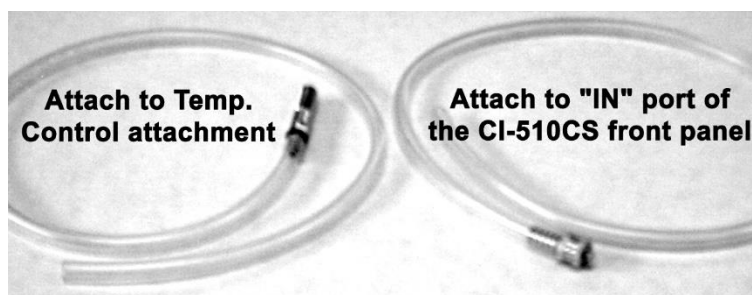


Figure A-3. Water connection tubing / hose

## FILLING RESERVOIR

Attach one of the hoses from the Temperature Control Attachment to the “OUT” fitting (Figure A-4). There is no polarity; it does not matter which hose is used. A water connection kit is provided in a plastic bag. The water connection hose (Figure A-3) is connected to the “IN” fitting. This connection hose can be used to prime the assembled unit with water (distilled water should be used) by inserting it into a water bath (a large bowl, bucket, sink, etc.). The remaining water connection hose (Figure A-3) attaches to the other hose connection on the Temperature Control Attachment (Figure A-4).

Turn the power on and observe the flow of water so that water steadily exits the other hose connection (from the Temperature Control Attachment). Once a steady flow of water has been established (air bubbles should not be noticed within the hoses), turn the power off momentarily, detach the water connection hose and connect the free-end hose from the Temperature Control Attachment into the “IN” fitting on the controller. Make sure the hoses are not bent or twisted. It is advisable to remove *all* moisture on the Controller panel (especially around the electrical connector). The Temperature Control Attachment is connected to the Controller marked “COOLING UNIT” with an electrical five-pin connector (Figure A-5).

**CAUTION:** Do not run the Controller without connecting the supply hose to a filled water reservoir. Otherwise, this could result in overheating and a possible malfunction of the pump.

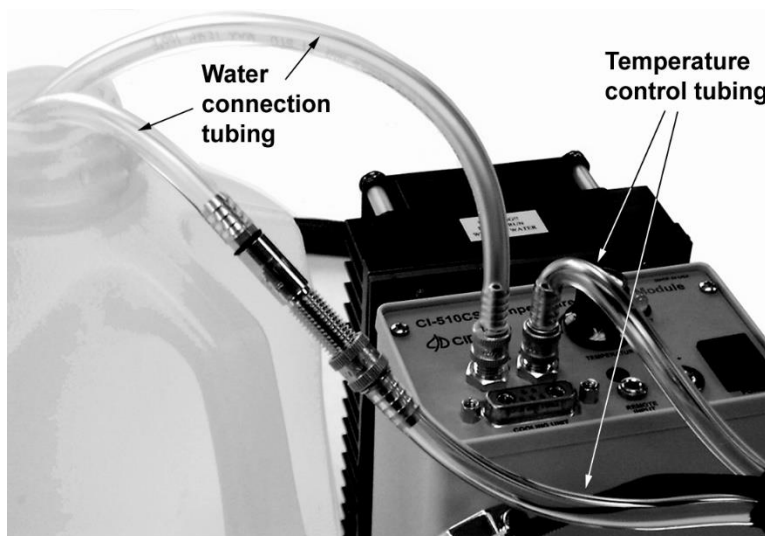
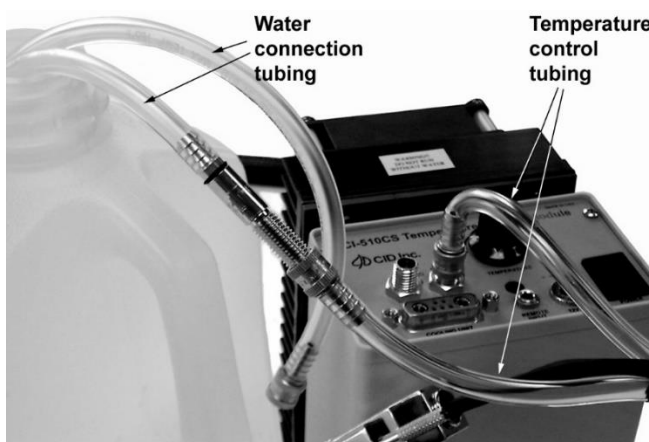


Figure A-4 Tube configuration for filling the CI-510CS reservoir.

*Figure A-5*

## EMPTYING RESERVOIR

Care for the CI-510CS Temperature Control System as you would any other sensitive instrument. The water in the hose needs to be completely drained after your experiment is finished. Empty the reservoir, by leaving the “IN” port on the controller panel open to air. Connect one hose from the Temperature Control Attachment to the “OUT” port on the controller module. Connect the water connection tube to the other hose from the Temperature Control Attachment and insert it in a container (Figure A-6). Turn on the controller module and allow it to empty. It may be necessary to rotate the unit from side to side to get all excess water out of the reservoir. Store the components so that the hoses are not crushed or twisted. This permits the water to flow freely throughout the hoses for future usage.

*Figure A-6 Configuration for emptying reservoir.*



## OPERATING TEMPERATURE CONTROL MODULE

The CI-510CS Temperature Control Module is to be used with an Open System leaf chamber. Before connecting the Temperature Control Attachment to your chamber, moisten the two surfaces (improving thermal contact), which will be touching. You may do this by applying a few drops of water to each surface. Align the two small screws on the Temperature Control Attachment with the threaded holes on the chamber bottom and tighten, using the Allen wrench provided. (see Figure A-7). The Infrared Leaf Temperature sensor should be inserted into the respective hole on the chamber bottom.

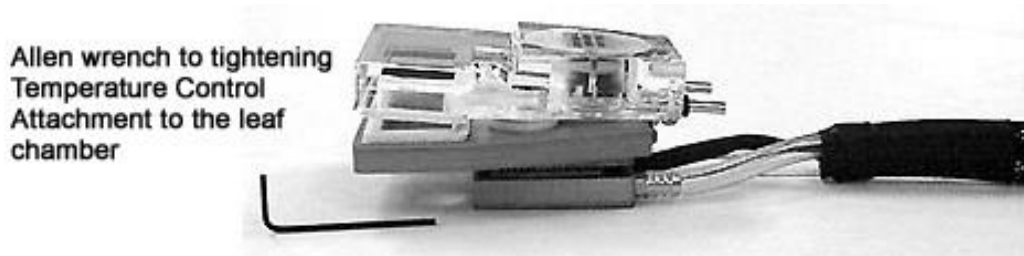


Figure A-7. Illustration of Leaf Chamber, Allen wrench and Temperature Control Attachment.

A 12V battery supply outlet is provided for the CI-510CS. Remove *all* moisture on the Controller panel. Plug the power connector to the jack marked 12V DC on the panel (Figure A-7).

**WARNING:** Only the provided power source can be used for the CI-510CS. Using other power sources or power adapters may damage the CI-340 and the module. This will void the warranty.

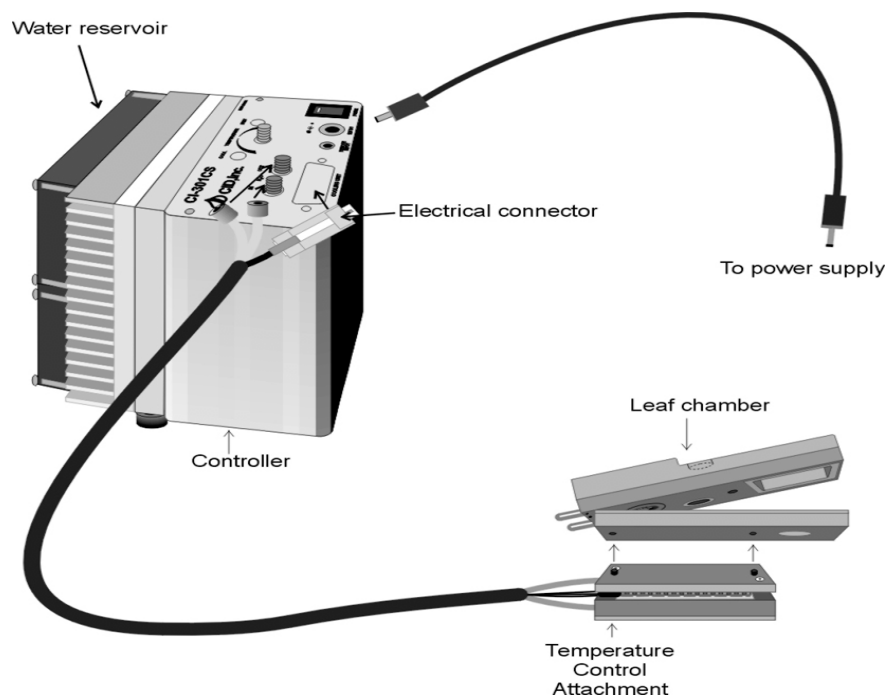


Figure A-8. Configuration for the CI-510CS Temperature Control Module.

To operate the CI-510CS, turn the power on. Temperature can be adjusted to a cooler or warmer state with the “Temperature” knob. You can vary the setting according to the readout of your CI-340 Photosynthesis System.

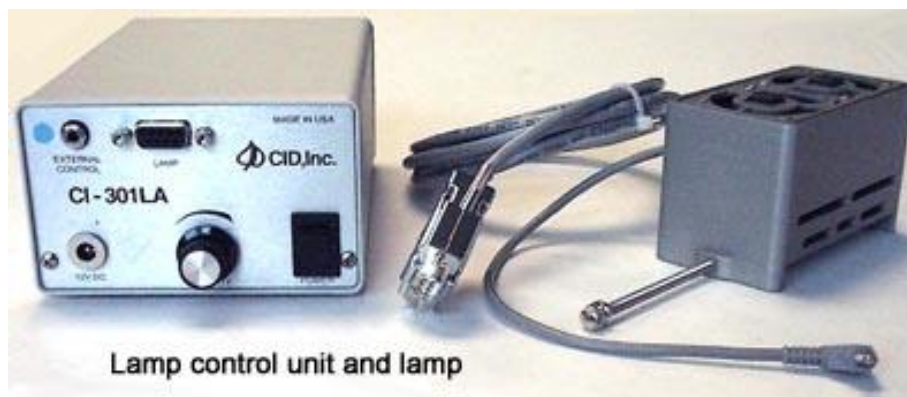
There is a “Remote Input” socket on the Controller panel. This can be used by remote control for the purpose of automatically adjusting the system. The temperature control knob will set the minimum temperature for remote control, so set the knob on the controller panel counterclockwise (all the way cold) for remote control.



## APPENDIX B

### CI-301LA LIGHT ATTACHMENT

The CI-301LA Light Attachment works with the flat, Open System chambers supplied by the manufacturer. It can be used indoors or outdoors as an alternative to sunlight and an intensity-controlled light source. The light emitted covers the photosynthesis wave band.



*Figure B-1. Lamp Control Unit and Lamp.*

To mount the CI-301LA (see Figure B-2), loosen the securing foot, place the lamp over the chamber, and screw in the foot against the chamber. The knob on the control unit allows you to manually adjust the light intensity, which ranges from 0 to 2,000  $\text{mmol m}^{-2}\text{s}^{-1}$ . Note that the light is equipped with cooling fans, which have exhaust vents (along the sides), and intake vents (on the bottom). Keep these vents clear at all times during use to avoid overheating.

Refer to A-1 in APPENDIX A to connect the power supply into the 12V connector on the control unit front panel.

An Accessories cable (one end with an eight pin connector, the other end with four plugs) should be used in order to have the CI-301LA controlled by the CI-340 instrument. Connect the eight-pin connector of the Accessory control cable to the Accessory control Port (see Figure 2-2) on the end of the CI-340. Insert the plug with blue color band into the External Control jack on the CI-301LA control unit (A-4 in APPENDIX A).

**Note:** Turn the power off to the CI-301LA when attaching cables.

In order to allow the CI-340 instrument to control the CI-301LA, turn the intensity control knob counter clockwise all the way down. Keep the knob in that position during the operation. The CI-301LA lamp illuminates when it is working.

A small cable with a plug connected to the lamp is the PAR sensing output. Make sure that the lamp covers the chamber window area so the PAR intensity emitted from the lamp or the external PAR sensor can be utilized. It is recommended that if the cable plugs into the CI-340, the external PAR sensor should not be plugged in.

**REMINDER:** The CI-301LA Light Attachment intake and exhaust vents must remain unobstructed during use to avoid overheating and possible damage to the light source. Do not place the lamp exhaust vents face down

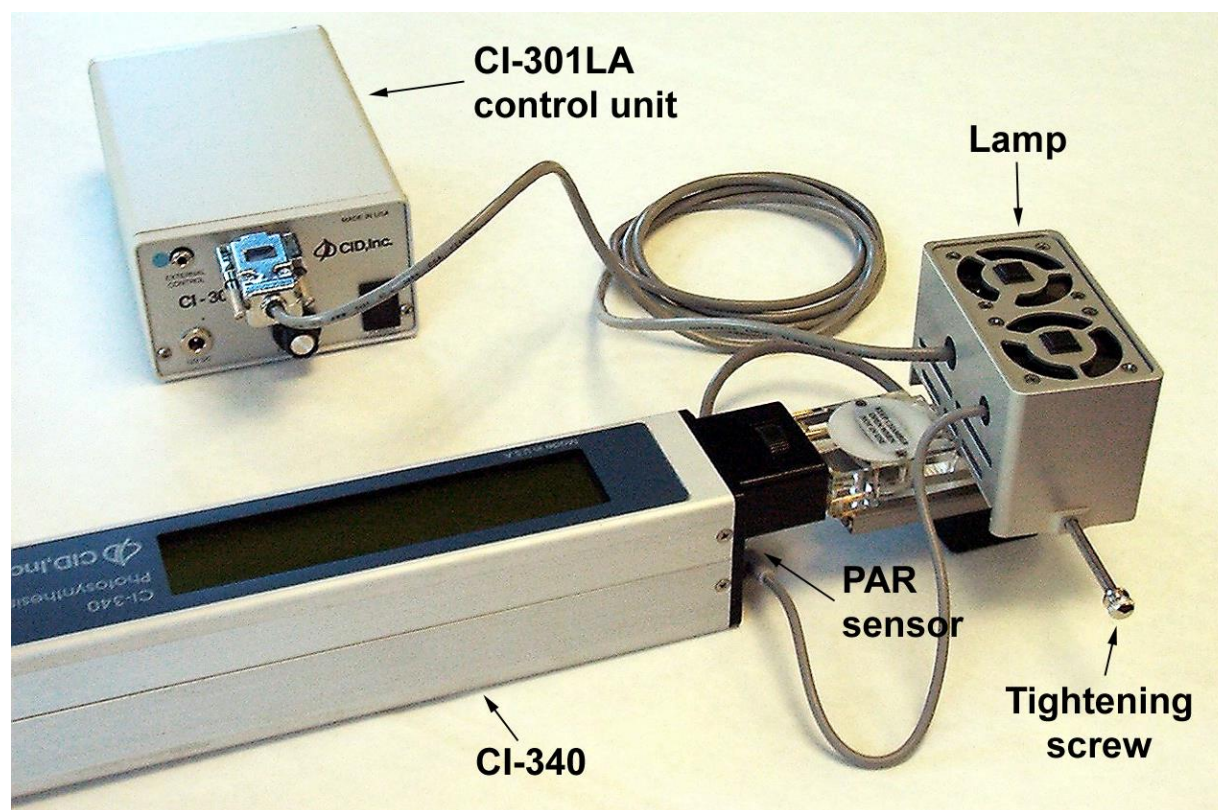


Figure B-2. Configuration for the CI-301LA Light Attachment.

## APPENDIX C

### CI-301AD ADJUSTABLE H<sub>2</sub>O AND CO<sub>2</sub> CONTROL MODULE

#### GENERAL DESCRIPTION

The CI-301AD provides a gas source with a CO<sub>2</sub> concentration adjustable from approximately 0 to 2000 ppm at flow rates up to 0.5 lpm. The unit also allows adjustment of the gas humidity level from approximately 5% relative humidity to 20-30% above ambient humidity levels up to 95%. The CI-301AD uses a CO<sub>2</sub> cartridge and soda lime to regulate CO<sub>2</sub> levels, and silica gel and water to control humidity.



Figure C-1. Illustration of CI-301AD Adjustable H<sub>2</sub>O and CO<sub>2</sub> Control Module.

#### OPERATING INSTRUCTIONS

##### Adding Consumables

Before operating the CI-301AD, the proper consumable materials must be loaded. For full operation (control of CO<sub>2</sub> and humidity), the unit requires a CO<sub>2</sub> cartridge, soda lime (CO<sub>2</sub> absorbent), silica gel (desiccant) and water. The soda lime, silica gel and water must be placed in the correct tubes.

**NOTE:** When it is not desired to use the humidity control feature, it is unnecessary to add silica gel or water. In this case, the H<sub>2</sub>O control knob should be in its minimum (fully counterclockwise) position to conserve power.

To remove a chemical or water tube, gently pull each end off the coupler tubes. Make sure that the two O-rings on each coupler tube remain in place.

The CI-301AD is shipped with fresh soda lime and silica gel in their appropriate tubes. If it should become necessary to open a tube, hold it in both hands and use the thumbs to push the cap off. The cotton balls should be replaced each time the tubes are refilled. Refer to the REPLACING CONSUMABLE section for instructions on replacing the filters. When filling the tubes, gently tap the bottom against a firm surface several times to help the material settle. Replace the top cap making sure there is sufficient room in the tube. Press firmly to seal in place, and twist if necessary to seat the O-rings. Replace the tube in the proper position on the CI-301AD.

To fill the water tube, remove the top cap in the same manner. The caps will come off along with an internal assembly. This assembly deflects water bubbles from entering the system during operation. You will notice a scribed line on the center of the tube. Add water to this line. ***Under no circumstances should you fill the water tube above this line.*** Doing so may cause liquid to emerge from the output under certain operating conditions creating potential damage to the connected gas analyzer. Replace the top cap firmly, and place the tubes back in the correct position on the CI-301AD. ***The water tube should be emptied if the unit is not used for more than one or two days.***

## CONNECTION TO POWER AND THE ANYLIZER

The CI-301AD requires 12V DC for operation. Refer to A-4 and figure A-7 to connect the power supply to the connector marked “12V DC” on the CI-301AD controller unit front panel. Remove **all** moisture on the controller panel. Plug the power connector to the jack marked “12V DC” on the panel.

Before operation, connect the “OUT” port on the CI-301AD to the “INTAKE” port on the CI-340 (see Figure C-1). Adjust both control knobs to their minimum (fully counterclockwise) positions.



Figure C-1: Configuration for the CI-301AD Adjustable Humidity and CO<sub>2</sub> Control Module.

## OPERATION

**NOTE:** The CI-301AD does not supply gas under pressure to the output. This is because constant flow must be established inside the unit to maintain stability; unused gas is exhausted inside the case. Gas must be drawn from the output by the analyzer or an external pump. When connected to the CI-340 CO<sub>2</sub> gas analyzer, the pumps inside the Analyzer will draw the gas from the CI-301AD into the analyzer system. An external pump may be required if using a different analyzer.

***Do not attempt to draw more than 0.5 LPM (liter per minute) from the CI-301AD output.*** Doing so may result in ambient air being drawn into the system, causing inaccurate results.

The CI-301AD must be fully upright to operate (with the control panel facing upwards). This is necessary for two reasons. First, the soda lime and silica gel cartridges operate most efficiently in a fully upright position. Second, if the unit were to tip too far in operation, liquid might enter the system and be pumped to the analyzer, causing potential damage. If the unit is to be carried while in operation, care should be taken to avoid tipping or shaking. This may cause power fluctuations, which could temporarily affect stability.



To begin operation, turn the power on. This should be done 5 to 10 minutes before the system is to be used, allowing time for the flow to stabilize. It may take about 5 to 10 minutes to purge the system thoroughly. Set the CO<sub>2</sub> fully on during this time. The fastest way to purge the CO<sub>2</sub> chamber the first time it is used after standing for a long period of time is to turn the CO<sub>2</sub> fully on, then turn the CO<sub>2</sub> fully off several times. This will cause ambient air that is trapped in the chamber to be replaced with pure CO<sub>2</sub>.

Turn the H<sub>2</sub>O knob to adjust the relative humidity. This must be done before adjusting the CO<sub>2</sub> level, since changes will affect the CO<sub>2</sub> concentration. The desiccant used to control humidity absorbs CO<sub>2</sub> gas.

Turn the CO<sub>2</sub> knob to adjust the CO<sub>2</sub> level. There is a slight delay before stabilization occurs. It will generally take a minute or so to fully stabilize (assuming the CO<sub>2</sub> chamber has been previously filled with CO<sub>2</sub>, or 2 to 10 minutes if it has not been filled with CO<sub>2</sub>).

To enable the CI-340 instrument to control the CO<sub>2</sub> / H<sub>2</sub>O levels remotely, the accessory cable must be connected to the CI-340 and the yellow color-coded plug must be inserted into the remote control jack of the CI-301AD. Turn the CO<sub>2</sub> knob fully counterclockwise and the H<sub>2</sub>O knob fully clockwise.

## REPLACING THE CONSUMABLES

The CI-301AD requires periodic replacement of the CO<sub>2</sub>, soda lime, silica gel, water, and filters. All materials should be available locally through chemical or laboratory supply dealers, or they may be ordered from CID, Inc.

### Soda Lime

The soda lime should last for 8 to 24 hours of operation under most circumstances. Actual operating time will depend upon ambient CO<sub>2</sub> levels. The soda lime supplied is an indicating variety: it will turn slightly blue as it becomes exhausted.

The output of the CI-301AD may become unstable and show an increase in the CO<sub>2</sub> level as the soda lime reaches exhaustion. When this happens, replace the soda lime.

Use the same procedure described under Operating Instructions – “Adding Consumable” to refill the soda lime tube. Replace the filters (see Filters section below). Remember to gently tap the tube to help settle the material. Keep all containers of fresh soda lime tightly sealed to prevent absorption of ambient CO<sub>2</sub>.

### Silica Gel

The silica gel should last from four to eight hours of operation under most circumstances. Actual operating time will depend on ambient humidity levels. The silica gel supplied is a beaded, dust-minimizing material of an indicating variety. The fresh material is dark blue and

turns yellow as it nears exhaustion. The relative humidity of the output of the CI-301AD will increase as the silica gel becomes exhausted. When this happens, replace the silica gel.

Use the same procedure described under Operating Instructions-Adding Consumable to refill the silica gel tube. Replace the cotton ball filters (see Filters section below). Remember to gently tap the tube to help settle the material. Keep all containers of fresh silica gel tightly sealed to prevent absorption of ambient moisture.

The used silica gel may be discarded or dried in an oven. If the material is dried, it should be placed in a sealed container before cooling. ***Note that the indicator incorporated into the silica gel may not function properly after this process, even though the silica gel itself may be dry.***

## Water

Water should be added if the level falls significantly. Change the water if it becomes cloudy or contaminated in any way. Empty the water tube if the CI-301AD is not used for more than one or two days.

## CO2 Cartridge

Screw the CO<sub>2</sub> cartridge into the regulator. Set the regulator to 6 psi. The cartridge may seem to be tight before it is punctured, so make sure to tighten until there is pressure indicated on the meter. The regulator must be set to “LO” to get a reading on the meter.

## Filters

The cotton ball filters located at each end of the soda lime and silica gel tubes become clogged with fine particles and must be replaced. These filters should be replaced each time the chemicals are changed.

It is extremely important that the filters be kept clean at all times. The first indication of a clogged filter will be instability in the output of the CI-301AD. This may be followed by a reduction in the available flow.



## APPENDIX D

### CI-301SR SOIL RESPIRATION CHAMBER

The CI-301SR Soil Respiration chamber allows easy measurements of soil respiration using the Closed System mode of the CI-340.

Connect the CI-301SR (see Figure D-1) to the CI-340 in the Closed System configuration (refer to the CLOSED SYSTEM section if you need further assistance). Insert the PAR sensor firmly into its respective slot on top of the soil respiration chamber (refer to the LEAF CHAMBER - CARE AND USE section). The IR Temperature sensor must be used to detect soil temperatures. Plug its cable into the CI-340, then into the respective hole on top of the chamber.

Now, place the CI-301SR on the area of soil that you wish to measure with the open end of the cylinder downwards. Rotate the chamber slightly to ensure that the cylinder is seated into the surface of the soil; a good seal is important to obtain accurate readings. If you need PAR readings, check so that the PAR sensor is under direct sunlight.

Soil respiration results will be shown as the photosynthesis reading. Note that the readings will be displayed in negative values.

Clean the CI-301SR after use. A soft, dry cloth will usually suffice, but water and mild detergent may be used, if necessary. ***Never immerse the chamber in water or pour water into the cylinder.*** The chamber contains electronic components, which can be damaged by liquids.

### Setup Procedures

- Create file
- Time Interval – Sampling Time
- Photosynthesis Mode P
- Zero for no accessory units
- Leaf area = soil area = 73.4 cm<sup>2</sup>
- Recommended flow rate 0.5 lpm
- Chamber volume is 0.634 liters with soil at the edge of chamber
- Chamber volume is 0.580 liters with edge 1 cm. into soil
- Time or CO<sub>2</sub> End (Time is the length to run measurement; CO<sub>2</sub> is the concentration level to end measurement). Enter T or C
- Enter time value or CO<sub>2</sub> concentration value
- Measurement begins

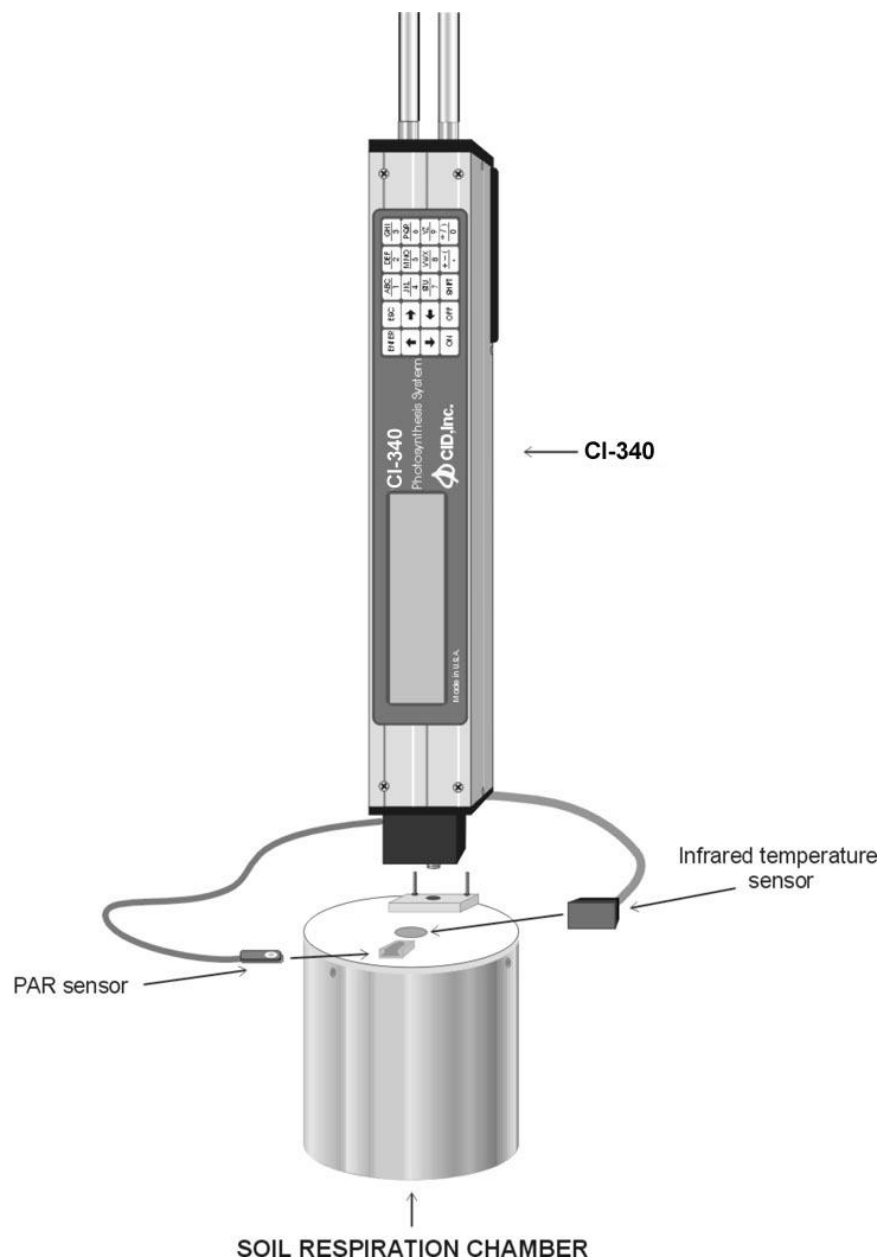


Figure D-1. Configuration for the CI-301SR Soil Respiration Chamber

## APPENDIX E

### CANOPY CHAMBER ASSEMBLY INSTRUCTIONS

The Canopy Chamber Attachment is another feature of Closed System measurements. This attachment is designed to be used with the canopy chamber enclosures measuring canopy photosynthesis. The enclosures could be of varying sizes, all of which the user can determine. The following conditions represent the kinds of enclosures that are acceptable:

- Materials that are not light-reflected, gas-permeable, light-impenetrable (such as plastic bags, acrylic shells, most glass canopies)
- Larger than the manufacturer's LC-10 chamber (4-liter volume)
- Larger than the measuring sample environment

For accurate installation of this attachment (see Figure E-1), the chamber enclosure must accommodate for an (circular) opening (approximately 65 mm (2-1/2")) on one of the sides. An additional slot can be made for the PAR sensor on the enclosure for accurate measurements of ambient light intensity. Canopy Chamber Attachment 'A' has a position for the PAR sensor placement; however, this position will not always provide reliable measurements of ambient light intensity.

To prepare for measurements, align Attachment 'A' and Attachment 'B' with the seals facing the enclosure. With this alignment, the tubes (in Attachment 'A') should slide into the respective holes in Attachment 'B'. Secure the entire Canopy Chamber Attachment onto the enclosure by tightening the thumbscrews from Attachment 'B'.

***NOTE: When using the IR Temperature sensor, be sure that the 65mm diameter opening is clear of any obstruction from within the enclosure. This is to ensure accurate leaf temperature measurements.***

Now, attach the Canopy Chamber Attachment to the CI-340 by gently sliding the exposed tubes into the holes and tightening the locking screw of the CI-340. Place the IR Temperature sensor firmly into the opening located on Attachment 'A'. Plug the IR Temperature sensor connector into its CI-340 port. Finally, insert the PAR sensor into the slot of Attachment 'A' or in the enclosure opening. If the chamber enclosure is made of flexible material, a tripod is suggested for an appropriate support of the CI-340.

### Plant Canopy Chamber Accessory

Plant canopy chamber measurements have a unique advantage because variation within individual leaf measurements is minimized and an accurate picture of whole plant photosynthesis can be seen. These differences are caused by the heterogeneous distribution of leaves within the plant canopy, due to uneven distribution of radiation, humidity and heat.

Whole canopy photosynthetic rate ( $P_n$ ) measurement can be used to quantify integral plant responses to the environment, as well as for determining the final yield of a crop. While many factors affect the photosynthetic rate of plants, the  $CO_2$  differential is most accurately established by taking into account the characteristics of individual plants and the environment they are in for each and every photosynthesis measurement task. Comparison between plant canopy photosynthesis measurements and individual leaf measurements can lead to conclusions on sun and shade leaf productivity, as well as whole plant gas exchange.

Analyzing the  $CO_2$  differential over an entire plant (or plants) can provide researchers with information concerning entire plant processes, the contribution of shaded leaves to overall photosynthesis rates, how overall plant productivity or yield relates to canopy photosynthesis or canopy light interception. The USDA has researched the effects of drought and kaolin-coating on the leaves of trees, comparing the overall photosynthesis rates of kaolin-coated and uncoated trees. It was determined that kaolin-coated trees had higher photosynthesis rates, and therefore were more tolerant of the drought conditions. Other studies into leaf area index and how differing leaf morphologies affect canopy photosynthesis and plant productivity have also incorporated plant canopy chambers for photosynthesis measurements.

The CI-340 canopy chamber attachment is designed to be used with various size enclosures, depending on size of plant or study area. The canopy chamber is a closed system, and air is not renewed inside of the chamber during analysis. Extremely large chambers have several limitations in the field such as that leaf temperature, wind patterns and evapotranspiration may not reflect natural environmental conditions. Also, controlling the temperature of large sun-exposed chambers can be difficult in the field. However, many of these limitations do not affect small chambers as significantly.

## Assembly Instructions

To accurately install the canopy chamber attachment, an air-tight chamber must be formed around the plant, but light must not be obstructed. The following conditions represent enclosures that are acceptable:

- Materials that are not light-reflective, gas-permeable, light-impenetrable (such as plastic bags, acrylic shells, most glass canopies)
- Larger than the manufacturer's largest close system leaf chamber (CI-301LC-10 chamber: 4-liter volume)
- Larger than the measuring sample environment

## Flexible Chamber Wall

When using a bag or flexible material for the canopy chamber, it is recommended to cut a small hole (about 65 mm diameter) to help facilitate the chamber attachment. Cut the hole in the side or bottom of the bag. Line up the "inside" canopy chamber attachment piece (the thinner piece with the thumb screws) with the hole cut in the bag. The bag should be completely inside the seal, but plastic should NOT cover any of the holes. Plastic should also be kept away from the screws as they are tightened. Attach the "outside" canopy chamber attachment piece (the thicker, larger piece) to the thinner piece using the thumb screws. Make sure that the bag is tight between the seals and there are no air leaks. Plastic should be free of the inlet and outlet connectors to the analyzer, as well as the clear of the hole for the IR temperature sensor to ensure accurate leaf temperature measurements. Once the

bag is lined up, and the two pieces connected, tighten the thumb screws making sure the bag is firmly in position between the seals.

If the chosen method was to cut a hole in the side of a plastic bag, after the canopy chamber attachment has been put on the bag, place the plant inside the bag. Close the open end of the bag by twisting it tightly and wrapping a rubber band around it. Other methods work as well; just make sure that the bag is air tight so that the system is closed.

## Rigid Chamber Wall

For long term or laboratory studies, a rigid canopy chamber enclosure of glass or plastic may be constructed. The same regulations apply as to using a plastic bag; a circular hole approximately 65 mm needs to be cut on one side to allow the canopy chamber attachment to be properly installed. Typically, a box type chamber is designed to be opened from the top or on a side where the user may reach the “inside” canopy chamber attachment piece.

If a rigid chamber enclosure is made, an additional slot can be made for the PAR sensor on the enclosure for accurate measurements of ambient light intensity. The “outside” canopy chamber attachment piece has a position for the PAR sensor, but this position will not always provide reliable measurements.

To prepare for measurements, align “inside” and “outside” canopy attachment pieces with the seals facing the enclosure wall. With this alignment, the tubes from the “outside” piece should slide into the respective holes in the “inside” piece. Secure the entire canopy chamber attachment onto the enclosure by tightening the thumbscrews on the “inside” piece.

## Attaching Chamber to the CI-340

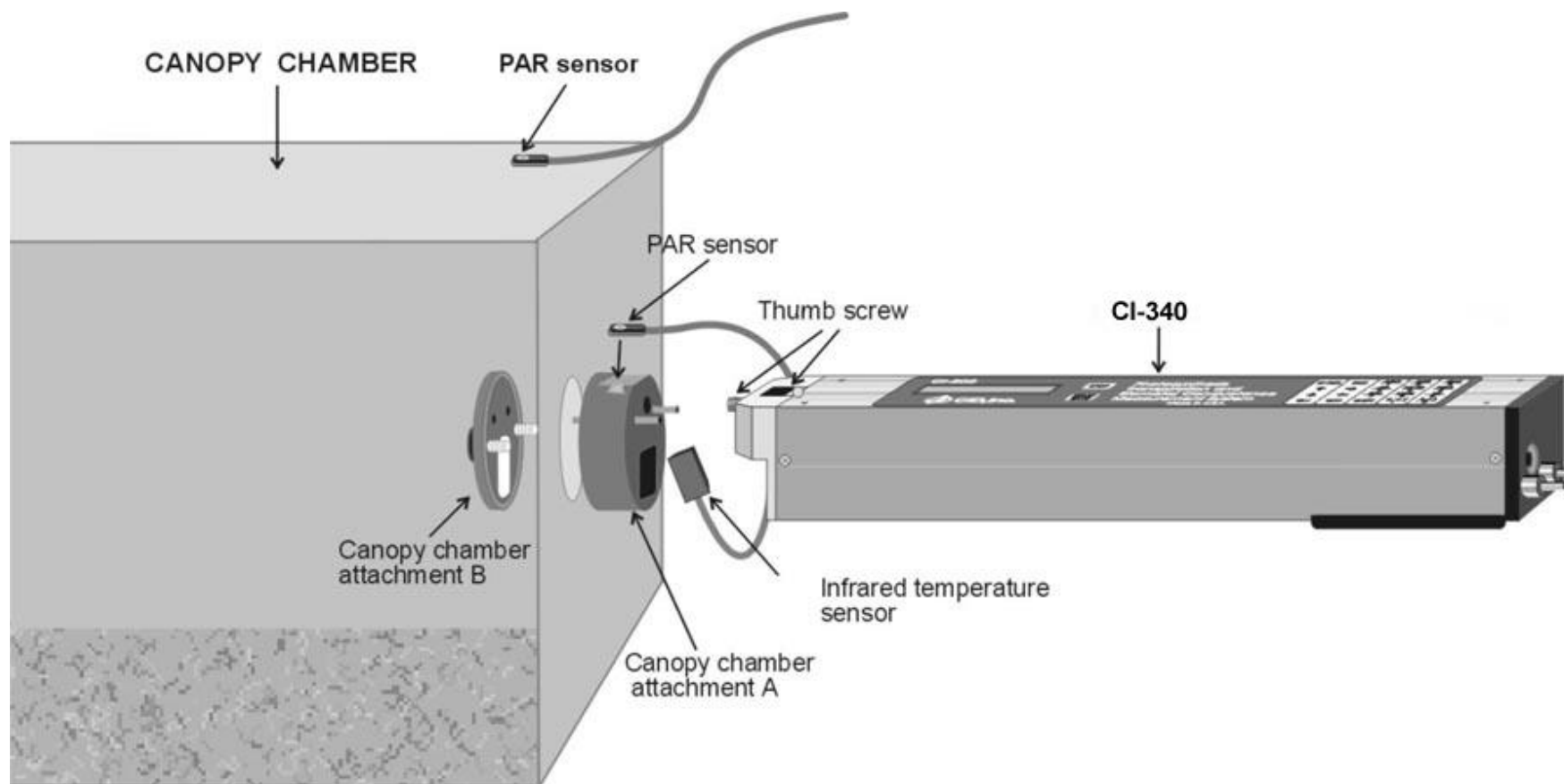
Once the “inside” and “outside” pieces are securely attached to the canopy chamber and the chamber is sealed air-tight, attach the canopy chamber attachment to the CI-340 analyzer. Similar to attaching leaf chambers, gently slide the exposed tubes on the “outside” attachment piece into the holes on the CI-340 and tighten the locking screw on the CI-340. Place the IR Temperature sensor firmly into the opening located on “outside” attachment piece. Plug the IR Temperature sensor connector into the proper port on the CI-340. Finally, insert the PAR sensor into the slot on the “outside” attachment piece or in the specially made hole on the rigid enclosure wall. If the chamber enclosure is made of flexible material, a tripod is suggested for appropriate support of the CI-340. The tripod should be positioned after the canopy chamber attachment has been completely set up and attached to the CI-340 analyzer.

## How to Measure:

After the canopy chamber is connected to the CI-340 analyzer, including connecting the IR temperature sensor and PAR sensor, attach a tube connecting the intake and exhaust of the analyzer so that the machine is set up for a close system measurement.

- Turn on the device and wait for the normal power up sequence.
- Enter the appropriate filename.
- Enter an appropriate time interval in seconds.
- When prompted to choose P, S, or C, select P allowing the analyzer to run in differential mode (and later allows the user to select a closed system type measurement).
- When using the canopy chamber attachment, typically no accessories are used, so select the default of 0.

- When prompted to enter the leaf area, choose the default as this will not matter once closed system is chosen.
- The flow rate should be doubled or increased from the default of 0.3 lpm to 0.6 lpm. When using the canopy chamber, only the single fan in the analyzer controls air flow, since there is no separately powered fan in the canopy chamber attachment like in leaf chambers.
- Choose “C” for closed system.
- Enter the volume of your chamber in liters.
- Choose the length of time (in seconds) to allow the analyzer to run or choose a change in CO<sub>2</sub> concentration to end at.



*Figure E-1. Configuration for the Canopy Chamber Attachment. A rigid-wall plant canopy chamber, attaching to the CI-340. The PAR sensor can either be attached to the canopy chamber accessory or can be mounted separately on the top of rigid chambers in order to most accurately reflect light levels.*

## APPENDIX F

### CI-510CF CHLOROPHYLL FLUORESCENCE MODULE

The CI-510CF is a host operated modulated chlorophyll fluorescence measurement module. This module performs two functions: generated chlorophyll fluorescence trace data, and individual pulse (calculated) data. From this, complex kinetic tests can be performed and analyzed.

### CONNECTING THE CI-510CF TO THE CI-340

CI-510CF Chlorophyll Fluorescence Module comes with a ‘Y’ shaped fiber optic cable. To connect the CI-510CF Chlorophyll Fluorescence Module, screw on the two connectors on the ‘Y’ end of the cable into the holes on the face of the CI-510CF Chlorophyll Fluorescence Module. Then insert the other end of the cable to the hole on the side of the chamber. See the graph on the next page for the details. Insert a power plug into the 12V DC jack on the CI-510CF module. An Accessory control cable (one end with an eight-pin connector and another end with four plugs) is used for communication between the CI-340 instrument and the CI-510CF module. Connect the eight-pin connector of the Accessory control cable to the Accessory control Port (see Figure 2-2) on the end of the CI-340. Insert the plug with green color band into the RS232 jack on the CI-510CF control unit.

*Always make sure to plug in the electrical connector before plugging in the RS232 cable plug.*

### CI-340 FLUORESCENCE SATURATION PULSE MEASUREMENT

With the CI-340 and CI-510CF cabled together and with the fiber optic inserted in the leaf chamber, activate the CI-340 Fluorescence Saturation Pulse Measurement by typing:

**“SHIFT SHIFT SHIFT 2”**

on the keypad, which is the letter “F” (for fluorescence). The CI-340 will ask for a filename to save the data under and a pulse length from 0.8 to 3 seconds. The default saturation pulse length is 1 second. Then the CI-340 will proceed to take the measurement.

Use the C340DF.exe utility to download the file to a PC and view the stored file in an appropriate spreadsheet.

The Fluorescence numbers are generated at a 16 Hz rate and represent the chlorophyll fluorescence in A/D (Analog to Digital Converter) counts. The last three entries in the table are:



Low Fluorescence value:	Fo - dark, Fs - ambient
High Fluorescence value:	Fm dark, Fms - ambient
Ratio of DHL:H:	Fv/Fm - dark, Y - ambient

The Ratio is the only value that is not in A/D counts. It should be interpreted as 0.xxx where xxx is the number displayed in the table. For example: if the number displayed is 99, the ratio is 0.099.

The default saturation pulse length is 1 second.

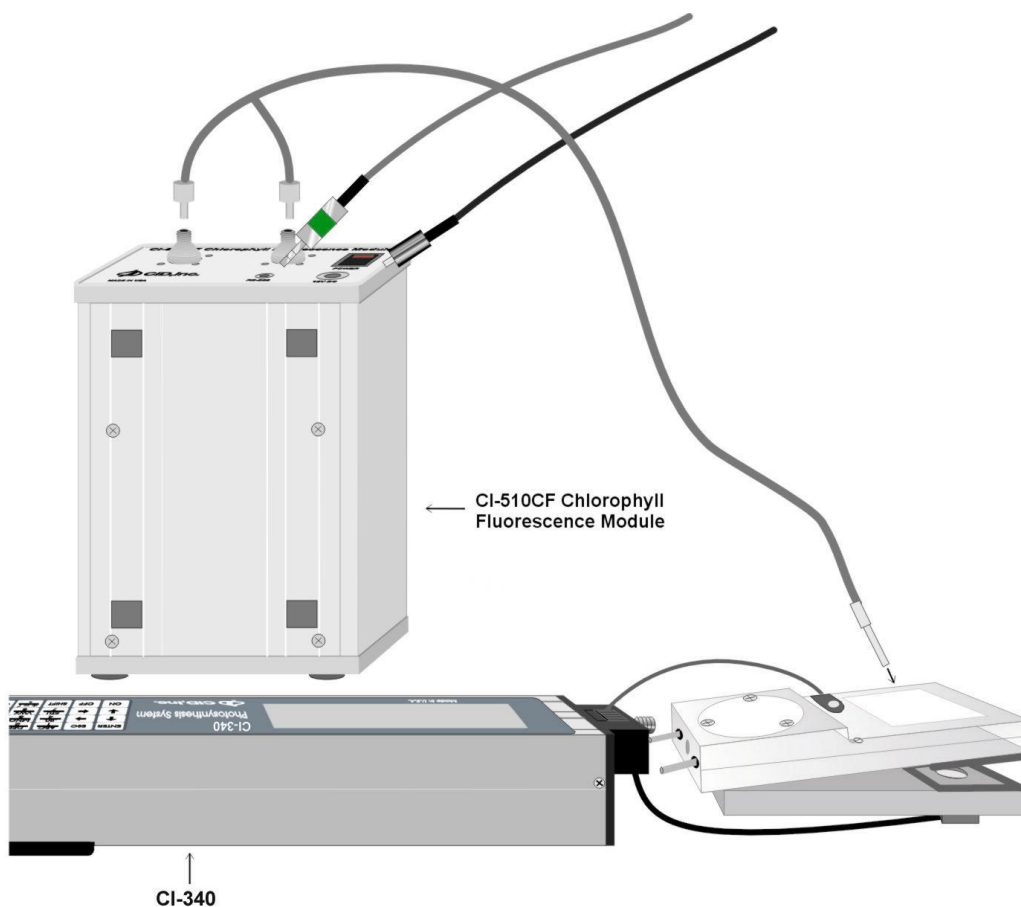


Figure F-1. CI-510CF CHLOROPHYLL FLUORESCENCE MODULE

## SPECIFICATIONS:

Power:	10 to 30 VDC, 75mA (175mA during saturation pulse)
Fuse:	0.5A FB 3AG

A/D converter:	2000 counts
Measured parameters:	Chlorophyll fluorescence trace data $F_o$ , $F_m$ , $F_v//F_m$ (dark adapted) $F_s$ , $F_{ms}$ , $Y$ (ambient illumination)
Modulated light intensity:	0.25 $\mu E$ at 12 mm
Saturation light intensity:	3,000 $\mu E$ at 12 mm
Modulation frequency:	8 Hz to 80 Hz
Fiber optic probe:	bifurcated light guide

## Warranty Information

### Seller's Warranty and Liability:

CID Bio-Science warrants new equipment of its own manufacturing against defective workmanship and materials for a period of one year from date of sale. The results of ordinary wear and tear, neglect, misuse, accident and excessive deterioration due to corrosion from any cause is not to be considered a defect. CID Bio-Science's liability for repairing or replacing defective parts during the warranty period is contingent on examination by a CID Bio-Science authorized representative. Felix Instruments liability will not extend beyond repairing or replacing parts from the factory where they were originally manufactured. Repair or alteration by an unauthorized technician voids warranty.

Material and equipment which is not manufactured by CID Bio-Science is to be covered only by the warranty of its manufacturer. CID Bio-Science will not be liable to the Buyer for loss, damage, or injury to persons or to property by the use of equipment manufactured by other companies.

Buyer accepts the terms of warranty through use of this instrument and any accessory equipment. There are no understandings, representations, or warranties of any kind, express, implied, statutory, or otherwise (including, but without limitation, the implied warranties of merchantability and fitness for a particular purpose), not expressly set forth herein.

All instrument repairs or replacement covered under warranty require a Returned Material Authorization (RMA) number. Please contact CID Bio-Science technical support department at [support@cid-inc.com](mailto:support@cid-inc.com) to obtain an RMA number before shipping instrument to CID Bio-Science, Inc.

Buyer is responsible for shipping charges to CID Bio-Science headquarters:

1554 NE 3rd Ave.  
Camas, WA 98607  
USA

CID Bio-Science is responsible for return shipping charges on repairs and/or replacement covered by warranty.