LI-6200 Portable Photosynthesis System
Users Guide
Version 1.00

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CONTENTS

System Overview

Introduction.

This guide is meant to be used in conjunction with the manuals supplied by LI-COR for the LI-6200 system. It is not meant to replace the LI-COR manuals, instead this document should be used as a reference to help remind users of recommended calibration, usage and maintenance procedures.

This document assumes that you have read the "LI-6200 Primer" manual and are familiar with basic gas exchange principles. You should be able to assemble the LI-6200 correctly. Most importantly you should be familiar with the operation of the LI-6200 software, and should be able to edit operational parameters.
If in doubt, work through the examples in the "Quick Tour" section of the "LI-6200 Primer Manual".

Basic Principles.

Before using the LI-6200 you should take some time to study the instrument and understand how it makes measurements. The LI-6200 Portable Photosynthesis System is used to measure the fluxes of carbon dioxide and water vapour between leaves and the atmosphere. In a normal healthy leaf in the sun, carbon dioxide is taken up by leaves in the process of photosynthesis. Water is lost from leaves by transpiration.

The schematic diagram of the LI-6200 is shown in Fig. 1.

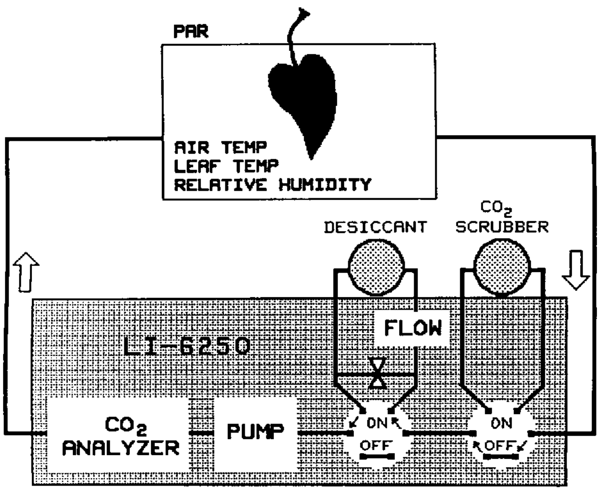

Figure 1. Schematic representation of the LI-6200 system.
(LI-COR 1987)

How does it work?

The LI-6200 system usually functions well and gives reliable estimates of leaf gas exchange parameters. The system will however only function properly, if it is used correctly, and you take care when making measurements. This section includes a brief introduction to the way the system works, and then discusses the major potential sources of measurement errors.

Photosynthesis or assimilation estimates (*A*) are determined using a closed measurement system. Air is pumped from the chamber through the CO_2 analyser and then back to the chamber. Photosynthesis is calculated from the rate of change in CO_2 concentration.

Transpiration estimates (*E*) are calculated from a measurement of the flow rate of air through a desiccant (Magnesium perchlorate, $MgClO_4$) and the rate of humidity or water vapour change within the leaf chamber. A proportion of the air may be diverted through the desiccant by placing the flow diversion switch to the DES ON position. The flow rate of air passing through the desiccant can be adjusted with a needle valve on the analyser housing.

Stomatal conductance (g_s) **or resistance** (r_s) is calculated from the transpiration rate, relative humidity, leaf and air temperatures.

Internal CO_2 concentration (C_i) is calculated from the estimates of photosynthesis, transpiration and stomatal conductance. As such C_i will always have the largest potential error of any gas exchange estimate. C_i is a useful parameter that should be used to monitor the correct operation of the LI-6200 system. Healthy well-watered plants in saturating sunlight should give C_i values of around 230 - 250 µmol mol^{-1} for C_3 plants and around 150 - 200 µmol mol^{-1} for C_4 plants.
It is useful to remember that $C_i \alpha \frac{g_s}{A}$, that is as *A* increases C_i tends to decrease, and as g_s increases C_i tends to increase.

Sources of error.

The closed system used in the LI-6200 is sensitive to leaks. Large errors may result if there is a leak in the system particularly when there is a large concentration gradient between the chamber and ambient air. This situation frequently occurs for CO_2 when making measurements inside buildings, or even outside when the wind speed is low. Errors in stomatal conductance estimates will occur if the chamber is dirty or the desiccant by-pass is not functioning correctly.

- The system must be free of leaks.
- The CO_2 analyser must be calibrated.
- The leaf chamber must be clean and dry.
- The humidity, temperature sensors and the flow meter must be calibrated.
- Desiccant must be fresh, and renewed daily or more frequently if required.
  (Discard old desiccant, it cannot be renewed.)
- Check that the leaf is touching the thermocouple in the chamber.
- Be careful when making measurements when the ambient air is either very humid, or cold. Under these conditions condensation is likely to occur and will result in large errors for stomatal conductance.
- Errors will occur if the leaf or chamber heat up during a measurement. Try to minimise overheating by storing the chamber in the shade between measurements.

Measurements will be affected by environmental conditions which influence leaf physiology. The most common mistake made in gas exchange measurements is to subject leaves to high ambient CO_2 concentrations before measurements by breathing on the plants. This should be avoided since it usually results in stomatal closure. Care should also be taken to avoid shading part of the leaf during the measurement. Always check measurements in the field, as you make them. Measurements made at low VPD's (high RH) will have large errors associated with estimates of g_s, E, and C_i. There is no way around this problem when working with ambient conditions, and it is often pointless to collect data for conductance estimates if the VPD is less than 5 kPa Pa^{-1}.

All measurements are expressed on a leaf area basis. You should obtain an accurate leaf area measurement, preferably after making your gas exchange measurements.

Pre-Operation.

Once you are familiar with the LI-6200, you should find it quite easy to make measurements. The LI-6200 is an instrument that has many possible applications, and modes of operation. The way the instrument operates is controlled by a number of parameter lists (software programmes), all of which can be altered by the user. You should use the default settings for most applications. The PAGE PARAMETER and OPERATING PARAMETER lists, are two that you need to use for all types of measurements. You must be familiar with these lists, and how to edit them, before you have use the LI-6200. The rest of this section takes you through the process of preparing the LI-6200 for making measurements.

You should carry out the following procedures, each day, before using the system.

System Assembly

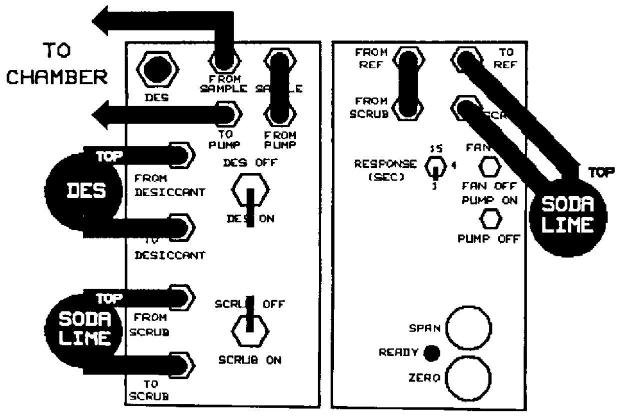

Figure 2. Standard connections for LI-6200 system (LI-COR, 1987)

1. Assemble system as shown in Fig. 2. Check that you have connected all the tubing, desiccant and soda lime tubes. The leaf chamber has two tubes that should be connected to the ports marked **To Pump** and **From Sample**. The tubes coming from the leaf chamber are interchangeable. Check that all tubing connectors are tight. Loose tubes are a common source of leaks. Most of the connectors used in the LI-6200 are fitted with valves that close when the connector is removed. You can damage the system is you operate the pump when some of the tubes are not connected or loose.

NOTE: To ensure correct operation of the desiccant and soda lime tubes, the direction of air flow should be from the bottom up. This is especially important for the desiccant tube since incorrect connection may lead to expensive damage to the gas analyser.

✓ With the system switched off, assemble system and check the tubing connections (Fig. 2).
DO NOT switch system on until the signal cables have been attached.

2. **With the system switched OFF,** the signal cables from the leaf chamber and analyser should be attached to the system console. The round connector is for the leaf chamber cable. Turn the locking ring clockwise to prevent accidental disconnection. The signal cable from the analyser housing should be connected to the 9 pin D connector on the system console. Check that you have locked this connector by sliding the clip to the right as shown in Fig. 3.

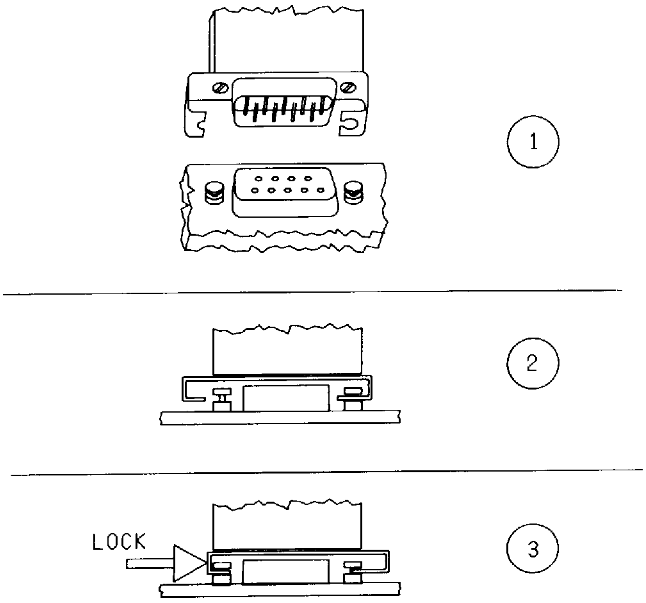

Figure 3. Procedure for attaching 9 Pin connector from LI-6200 analyser housing to the system console. It is important to check that the connector is firmly seated, and that the locking clip is pushed fully to the right.

✓	Attach signal cables from the leaf chamber and gas analyser to the system console. Check that they are locked!

3. Set the following switches on the gas analyser
   PUMP OFF
   FAN ON.
   Check that the chamber fan is switched off.
4. Attach a battery to the system console.

✓	Check that the pump is switched off before connecting a battery and turning the system on.

5. Check the battery voltage. Press the **STATUS** key on the system console. The battery voltage should be displayed. A fully charged battery should give a voltage reading greater than 12.0 V. The status screen also displays the amount of free memory. You may want to clear the storage memory in preparation for new measurements.

✓	Check the batteries are charged.

Checking Sensors

6. Switch the system on and allow at least 5 minutes for the gas analyser to warm up before starting the CO_2 calibration procedures.

✓	Allow at least 5 minutes for the gas analyser to warm up. Use this time to check system software and sensors.

7. Check the operation of the sensors.
   Enter Monitor mode, Press the **MONITOR** key.

- Press **PAR** and uncover the quantum sensor on the leaf chamber. Check that the sensor responds to varying light intensity.
- Press **TLEAF**, **↑**, **TCHAM** to display the leaf and chamber temperatures. Place a dry piece of filter paper over the thermocouple, close the chamber and turn the chamber fan on. With the chamber in the shade, the leaf and chamber temperatures should be within ⌖ 0.1 °C. If there is a large difference, you need to adjust the thermocouple offset.
  Open the chamber and gently place your finger on the thermocouple. Check that the temperature increases. If there is no response the thermocouple may need replacement. See the maintenance section in the LI-COR Primer manual for details on replacing the thermocouple or adjusting the offset. (Section on Leaf Chambers)
- Press **RH** and check that sensor responds by breathing into the chamber. If possible check the humidity reading against another calibrated RH sensor. At same time check the accuracy of the chamber temperature reading.
  Turn chamber fan off, and place chamber in the shade, with the lid open.
- Press **FLOW**. Turn the gas analyser pump on (PUMP ON), and the desiccant flow switch to DES ON. Adjust flow rate with the needle valve marked DES FLOW. Turning the valve clockwise should increase the flow rate.
  Turn the pump off.
- Measure and set barometric pressure (**FCT** **A0**).

✓	Check the operation of the system and sensors.

8. Check the software calibration. Press the **SETUP** key. You will be presented with a list containing most of the important information for the operational parameters and calibration factors for the LI-6200. Take some time to check that the calibration factors are correct and agree with those on the calibration sheet provided by LI-COR. You can scroll through the list using the ↑ or ↓ keys. Press **RTRN** to view any list and **EDIT** to change parameters. You can use Shift + **EDIT** if you want to alter more than one parameter in a list. Use the **BRK** key (CTRL + P) to stop editing a list prematurely. The **DEL** key (Shift + **K**) can be used to correct any mistakes.

✓	Check the software calibration data before using the system.

9. Zero the Flow Meter.
   Check that the analyser pump is switched off (PUMP OFF) and then press **FCT** 48. You will be asked for a channel number for the flow meter reading. The default value of 7 should be supplied by the system, press **RTRN**. Make sure that the pump is off and that there is no other source of flow in the system. When the displayed millivolt reading is stable, press **RTRN**.
10. Measure Maximum Flow Rate.
   Monitor flow rate, Press **FLOW**. Select DES ON and turn the PUMP ON. Shake the desiccant tube. Turn the analyser needle valve (DES FLOW) fully clockwise so that all flow is diverted through the desiccant. If you are using a flow restricting valve on the dessicant tube, remember to open this as well. Record the flow rate and enter is as the Fx parameter in the PAGE PARAMETER section using **FCT** A5. Maximum flow should be in the range of 1200 to 1400 ❍mol s^{-1}. Remember to turn the pump off.

✓	Zero flow meter and measure maximum flow rate.

Calibrating the Gas Analyser.

> ✓ Wait for the ready light on the gas analyser before starting the user calibration procedures.

11. Set Barometric Pressure
Measure atmospheric pressure with a barometer. Enter the value (in millibars) into the PAGE PARAMETER list using FCT A0**)**.
12. Set the Analyser Reference
Use FCT **49** and then Press RTRN to accept the analyser temperature channel (14), and then press RTRN again.
13. Set Analyser Zero.
Set the flow diversion switches on the analyser to DES ON and SCRUB ON, the response time to 1 second (RESPONSE TIME 1) and turn the PUMP ON. Shake the desiccant tube. Monitor the flow rate and CO_2 concentration. Press **MONITOR** (if required) and **FLOW**, ↑, **CO2**. Turn the needle valve on the analyser fully clockwise to have maximum flow through the desiccant. The CO_2 concentration should rapidly approach zero. If the CO_2 concentration does not fall, check that the tubing is properly connected (Fig. 2), that the flow diversion switches are correctly selected, and that the pump is on. If there is still a problem, test the analyser soda lime and replace if necessary.
When the CO_2 reading stabilises, adjust the ANALYSER ZERO knob on the right hand side of the analyser. Unlock the outer ring before turning the knob clockwise to increase the reading or anticlockwise to decrease it. When the CO_2 reading is zero remember to turn the outer ring to lock the potentiometer. Record the flow rate before switching the analyser PUMP OFF and SCRUB OFF.

14. Set Analyser Span (Using a Calibrated Gas Bottle).
    Check that the analyser pump is off and that the flow diversion switches are set to DES ON and SCRUB OFF. Select a calibration gas with an accurately know CO_2 concentration. Ideally this be slightly higher than ambient CO_2 concentrations you will be using. For field use, a calibration gas of around 370 to 400 µmol mol^{-1} is recommended. Attach the tubing from the calibration gas source to the gas connector marked TO PUMP on the left hand side of the gas analyser.

    Monitor flow rate and CO_2 concentration. Press **FLOW**, ↑, **CO2**.

> Flow rate is important when setting the span. All flow should pass through the dessicant by turning DES FLOW fully clockwise. Adjust the flow rate of the span gas until it is the same as used to set the analyser zero.
> You should not use needle valves on the analyser to adjust the flow rate.

Adjust the flow rate passing through the analyser using the gas regulator fitted to the calibration gas bottle. It sometime helps to fit a needle valve to the gas regulator. When the CO_2 reading has stabilised adjust the displayed CO_2 reading using the SPAN knob on the right hand side of the analyser. The analyser span should be adjusted so that the CO_2 reading displayed on the console is the same as the known concentration of the calibration gas. Lock the span knob by turning the outer locking ring clockwise.
After setting the span, you should check and adjust the Analyser Zero (if required). If it requires adjustment, you should repeat the Analyser Span calibration procedures. Continue repeating Zero and Span adjustments until no further change is required

> ✓ Before making measurements remember to reset the flow switches and desiccant flow rate to your previous settings. Check that you have set SCRUB OFF.

Software set-up.

15. Set the user defined list.

Press **FCT** 41 to obtain the list and **SHIFT** + **EDIT** to edit the list. You should check the other values in this list and change as required. Remember to alter the VOL parameter when changing leaf chambers. BC is dependent on leaf size and shape as well as chamber size. PR1 through to PR6 are prompts or locations that can be used to store additional information for each measurement. The parameters P, BC, Fx and Kabs are dependent on environmental or biological conditions. You should measure them, and alter as required.

LAB=DEFAULT	System Label. Can be ignored.
Vt(cc)=xxxx	System volume (cm^3). Depends on chamber size
Vg(cc)=xxx	IRGA and hose volume (cm^3) see calibration sheet
P(mb)=xxx.x	**Atmospheric pressure (millibars)**
BC(mol)=x.x	**1 sided boundary layer conductance (mmol m^{-2} s^{-1})**
STMRAT=x.x	**Ratio of stomatal conductances on upper & lower surfaces**
Fx(umol)=xxxx	**Measured maximum flow rate (Omol m^{-2} s^{-1})**
Kabs=x.xx	**Measured water absorption factor. (Obtained from K test)**
A8=0.0	Not normally used
A9=0.0	Not normally used
Pr1:	User defined prompts
Pr2:	
Pr3:	
pr4:	
pr5:	
pr6:	

Table 1. Typical values for the Page Parameters. Note that highlighted parameters need to be measured by the user, and may require frequent updating.

Notes:

- Vt(cc). Total system volume (cm^3). This parameter is given on the calibration sheet for standard LI-COR chambers. Typical values are:
  - 6000-13 429 cm^3
  - 6000-12 with inserts 1159 cm^3
  - 6000-12 without inserts 1199 cm^3
  - 6000-10 4164 cm^3
- Vg(cc). IRGA and hose volume. See calibration sheet for value for standard system. A typical value is 13 cm^3.
- P(mb). Atmospheric pressure (mbar). This should be measured using a barometer. Note that this parameter should be set (**FCT** **A0**) at least once a day, and always after changes in altitude or weather. After setting the barometric pressure, remember to set the analyser reference using **FCT** **49**.
- BC(mol). The boundary layer conductance for the leaf. This parameter will vary with leaf size, shape, chamber design and fan speed. This should be measured using a leaf replica constructed from filter paper, or Plaster of Paris. Set using **FCT** **A3**.

- STOM RAT. The ratio of average stomatal conductance on the two sides of the leaf. If a leaf has stomata on only one side set this value to 0. If conductances are equal use 1, otherwise an estimate will be required using a suitable porometer (e.g. LI-COR LI-1600) or other techniques such as microscopic observation. Set using FCT **A4**.
- Fx(umol). The maximum flow rate through the desiccant. This parameter should be measured, and updated during the day, and always after changing desiccant.
- Pr1-Pr6. These entries can be used to store comments about the page of data in conjunction with the auxiliary data fields. For example prompt 1 could store the comment "Leaf #" with the actual value stored in the first entry in the auxiliary data list.

The auxillary data and prompt field are stored as part of the header information for each page of data collected by the LI-6200. These are very useful for storing information such as plant or leaf number, plot codes, or even comments about individual measurements. The LI-6200 can be programmed to prompt you for selected data, after each measurement (e.g. plant number), or else you can recall and edit any entry from the keyboard. For more details, suggestions and instructions, you should look at the eaxmples given in the LI-6200 technical reference. The section on the LOG Programme in the LI-6200 software topics shows how to programme the LI-6200 to prompt for data.

✓ Set the Page Parameters.

16. The OPERATING PARAMETERS.

Call up the operational parameters in list edit mode by pressing FCT 42 and SHIFT + EDIT. Edit the list so that it looks like Table 2.

LAB=DEFAULT	System Label. Can be ignored.
CHANGE=5.00	5 unit change (in this case 5 µmol mol^{-1} in CO_2)
CHANNEL=15	Channel 15 is CO_2
OBS/PAGE=2	2 observations per page
STEP(s)=1	Record samples every second
BEEP LOG(Y/N)=Y	Beep after each observation
KEEP SAMPLES(Y/N)=N	Don't keep raw data.
PRN FMT (U/S)=S	Use system print format
AREA MULT=1.00	Leaf area multiplier

Table 2. Standard operational parameters for LI-6200 system.

Notes:

- CHANGE=5. A 5 µmol mol^{-1} change in CO_2 is a reasonable change to aim at for each observation. This gives a measurement error of $\frac{0.1}{5}$ or 2 %.
  Consider using a slightly larger value if you are only recording one observation.
- CHANNEL=15. CO_2. Another useful channel is 10 (TIME).
- OBS/PAGE=2. If you record too many observations per page, changes in CO_2 concentration are likely to affect your measurements.
- STEP=1. This will never be exactly once per second. You may need to increase this when making long measurements as a way of reducing memory requirements.
- BEEP LOG(Y/N)=Y. System will beep once after each observation as well as once at the end of all measurements before the results are calculated.
- KEEP SMP(Y/N)=N. This significantly reduces the amount of memory required when storing results.

- PRN FMT(S/U)=S. This parameter is used to select alternative user print formats. This feature is especially useful for producing data files to be used as input for spreadsheet or statistical analysis programmes.
- AREA MULT=1.00. This parameter is usually set to 1.00. It can be used to calculate leaf area from measurements of for example leaf width. The leaf area used in the gas exchange calculations is itself calculated by multiplying the leaf area entered by the user (**AREA** or **FCT** A1) by this AREA MULT factor.
- Change any of these parameters as required. A suggested configuration for plants with low photosynthetic rates is shown in Table 3.

LAB=RESPIRE	System Label. Can be ignored.
CHANGE=30.0	30 unit change (in this case 30 seconds)
CHANNEL=10	Channel 10 is time
OBS/PAGE=3	3 observations per page
STEP(s)=1	Record samples every second
BEEP LOG(Y/N)=Y	Beep after each observation
KEEP SAMPLES(Y/N)=N	Don't keep raw data.
PRN FMT (U/S)=S	Use system print format
AREA MULT=1.00	Leaf area multiplier

Table 3. Suggested operational parameters for LI-6200 system for plants with low photosynthetic rates or for respiration measurements.

✓ Set Operational Parameters

Operational Tests.

The LI-6200 system uses a number of tests to check for correct operation of the system, and to provide calibration (or correction) factors required by some calculations. These tests are well documented by LI-COR in the "LI-6200 Primer" or in relevant user notes. The notes in this document are only provided as a reference and should be used in conjunction with the LI-COR documentation.

K Test.

The K test is used to check the operation of the water vapour by-pass used in the estimation of transpiration and stomatal conductance. The value obtained depends on the type of chamber, ambient conditions and the condition of the desiccant.

To perform the K test use the following procedure.

- Before performing the test set the maximum flow rate (Fx) and barometric pressure (P) parameters in the PAGE PARAMETER list (FCT A5).
- Check the settings of the system and analyser volume in the PAGE PARAMETER list.
- Edit the OPERATING PARAMETER (FCT 42) list to read as shown in Table 4.

LAB=K Test	System Label. Can be ignored.
CHANGE=1.00	1 unit change (in this case mbar of water vapour)
CHANNEL=18	Channel 18 is water vapour
OBS/PAGE=2	2 observations per page
STEP(s)=1	Record samples every second
BEEP LOG(Y/N)=Y	*The rest of the list can be ignored for this test*
KEEP SAMPLES(Y/N)=N	
PRN FMT (U/S)=S	
AREA MULT=1.00	

Table 4. Operational parameters for K test on the LI-6200 system.

- Enter the leaf area to be greater than 0. (AREA).
- With the pump switched OFF, zero the flow meter. (FCT 48)
- Set the flow switches to DES ON, SCRUB OFF, PUMP ON.
- Press MONITOR, then FLOW, and turn the analyser flow valve fully anticlockwise. The flow should read about 300 µmol s^{-1}.
- Press FCT 18 to monitor water vapour pressure.
- Turn the chamber fans on, and latch the chamber shut.
- Monitor water vapour pressure until it starts to drop, then press LOG.
- When the water vapour pressure has dropped 1 mb twice logging will stop. Open the chamber and turn the fans and analyser pump off.
- Press FCT 24 to display the calculated value for the K factor.
- Check that the value of K is acceptable (see below). The second value will typically be slightly highly than the first. Use either the first, or a mean of both estimates. Enter the value for the Kabs factor in the OPERATING PARAMETERS list (FCT A7).

Normal values for K range between 1.1 and 1.5. If your measured value of K is greater than 1.5, or less than 1.0, it is likely that the system is not operating correctly. You should then check the following.

- Is the chamber clean and dry? Clean with distilled water, take care not to touch the humidity sensor.
- Is the desiccant still fresh? As the desiccant is used, the value of K tends to increase.
- Is the chamber in equilibrium with the ambient conditions? You may need to allow up to one hour for a chamber to equilibrate with new conditions.
- Is the flow meter calibrated? Check the flow meter zero (FCT 48).
- Is the humidity sensor calibrated?
- Are the ambient conditions reasonable? It is difficult to obtain good values for K when the ambient relative humidity is very high or low.
- Consult the LI-6200 Primer manual for more information on this test.

> ✓ Test the operation of the water vapour by-pass using the K Test procedure. *Remember* to enter the new value for Kabs into the OPERATING PARAMETER list using FCT A7.

> ✓ Before making measurements remember to reset the OPERATING PARAMETER list and desiccant flow rate to your previous settings.

Boundary layer conductance.

An estimate of boundary layer conductance can be obtained using paper replicas of leaves. Boundary layer conductance is a function of leaf size and shape, chamber design and fan speed. Boundary layer conductance should be measured for each combination of leaf shape and chamber design you use.
It is very easy to estimate the boundary layer conductance using a moistened replica of a leaf. For most plants suitable replicas can be constructed using filter paper. For plants with more complex structure such as conifers, other approaches may be required. A suitable technique for complex leaves involves the use of plaster of Paris described in a paper by Landsberg and Ludlow (1970). The following instructions assume that you are using a replica constructed using filter paper.

- Construct a filter paper replica of the leaf. Measure the area of the replica.
- Enter a value *equal to two times the area* of the replica into the LI-6200 (AREA).
- Conduct a K test, and enter the value of Kabs into the PAGE PARAMETER list.
- Set the barometric pressure and maximum flow rate
  (You should have already done so for the K test).
- Set the boundary layer conductance (BC) to equal 0 (FCT A3).

- Edit the Operational Parameter list (FCT 42) as shown in Table 5.

LAB=	System Label. Can be ignored.
CHANGE=10	10 unit change (in this case 10 seconds)
CHANNEL=10	Channel 10 is time
OBS/PAGE=1	2 observations per page
STEP(s)=1	
BEEP LOG(Y/N)=Y	*The rest of the list can be ignored for this test.*
KEEP SAMPLES(Y/N)=N	
PRN FMT (U/S)=S	
AREA MULT=1.00	

Table 5. Operational parameters for boundary layer conductance measurements.

- Set the flow switches on the LI-6200 to DES ON, SCRUB OFF, PUMP ON, turn the analyser needle valve (DES FLOW) fully clockwise to obtain maximum flow through the desiccant. Shake the desiccant tube.
- Thoroughly moisten the filter paper replica with distilled (deionized) water. Remove excess water by blotting the filter paper between two paper towels. The paper must not drip water into the chamber.
- Place the filter paper replica into the leaf, and check that it is in contact with the leaf thermocouple.
- Close and latch the chamber and then turn the fans on.
  (You will find it much easier if you close the chamber before switching the fans on!)
- MONITOR VPD .
  Adjust the flow rate using DES FLOW to maintain constant VPD in the chamber.
- Remove the filter paper, and re-wet it.
- Return the replica to the chamber, close and latch it, and then press LOG to start the measurement. When the measurement is over remove the filter paper.

- The computed conductance (COND) is equal to the boundary layer conductance of one side of the leaf replica.
  This is the reason that you entered the leaf area to be equal to two times the projected area of the replica.
- Repeat the measurement several times until you obtain a repeatable result. Remember to re-wet the replica before each measurement.
- If you have trouble getting reasonable measurements, check the desiccant, and perform a K test. You should also check that there are no droplets on water in the chamber.

✓ Set the value for BC in the PAGE PARAMETER list using FCT A3.
This value is characteristic of leaf shape and size combined with chamber size and fan speed.

✓ Before making measurements remember to reset the OPERATING PARAMETER list and desiccant flow rate to your previous settings.

Soda lime test.

There are two tubes of soda lime on the LI-6250 analyser. The tube on the right side of the analyser is used to keep the reference tube free of CO_2. The tube of the left side of the analyser is used to set the analyser zero (SCRUB). You should mark the tubes so that you know which tube you are using on each side of the analyser. The tube on the left side of the analyser will be used more rapidly, and should be tested every 1-3 months, or whenever it becomes difficult to set the analyser zero.

You should test the tube on the left. If it needs replacing fill the tube with fresh soda lime and use this tube as your new tube for the reference cell on the right side of the analyser. You can then use the old reference tube for your scrub tube. This procedure ensures that you always have fresh soda lime for the reference cell.

The soda lime test is very simple, but should be used sparingly, since it will significantly shorten the life of your soda lime.

- Set the analyser switches to SCRUB ON, DES ON, PUMP ON, RESPONSE 1 and turn the chamber fans on.
- Open the leaf chamber.
- **MONITOR** the **CO2** concentration.
- When the reading has stabilised (it should be near zero), breath into the chamber and then close and latch it.
- The displayed CO_2 concentration should remain unchanged. If it increases, the soda lime should be replaced.
- Open the chamber and turn the chamber fan, and analyser pump off.
- REMEMBER to set the flow switch to SCRUB OFF.

✓ Before making measurements remember to set the flow switch to SCRUB OFF.

Leak test (standard)

It is important to minimise leaks in the leaf chamber for measurements using the LI-6200. If there is a leak, the measured apparent photosynthetic rate will tend to be lower than the actual rate. Leaks can have many causes, but the most common is deterioration of the chamber gaskets. A leak can also develop if the screws attaching the leaf chamber to the sensor head become loose.
Leaks may be created when you are working with plants with thick leaves or petioles that create gaps in the chamber gaskets. In such cases you should consider using something to improve the seal. "*BLU TACK*" is suitable and works well.

This test measures the rate of leakage by creating a CO_2 differential between inside the chamber and ambient air.

- Set the OPERATING PARAMETER list (FCT 42) as shown in Table 6.

LAB=LEAK TEST	System Label. Can be ignored.
CHANGE=60	60 seconds
CHANNEL=10	Channel 10 is time
OBS/PAGE=1	1 observation
STEP(s)=1	Record samples every second
BEEP LOG(Y/N)=Y	Beep after each observation
KEEP SAMPLES(Y/N)=N	Don't keep raw data.
PRN FMT (U/S)=S	*The rest of the list can be ignored for this test.*
AREA MULT=1.00	
STEP(s)=1	

Table 6. Operational parameters for standard leak test.

- Turn on the chamber fans and open the chamber. Set the following switches on the analyser, SCRUB OFF, DES ON, PUMP ON, RESPONSE 1.
- Set the flow rate to about 500 µmol s^{-1}. This means that about half of the flow will go through the desiccant.
- Take care not to breathe into the chamber, allow the chamber to collect a good sample of ambient air, before closing the chamber and latching it shut.
- MONITOR the CO2 concentration on the console. Wait for about 1 minute for the reading to stabilise and then record the value for ambient CO_2 concentration. Call this value C_a.
- Set SCRUB ON to reduce the CO_2 concentration in the chamber. You need to allow time for the concentration to drop by about 150 µmol mol^{-1}, around 5 or 10 seconds for the 1/4 litre chamber, 20 seconds for the 1 litre chamber, and up to 1 minute for the 4 litre chamber.
- Set SCRUB OFF, and wait for around 1 minute until the CO2 reading stabilises or starts of rise slowly.
- Press LOG to start the measurement.
- At the end of 1 minute, the system will beep twice and the measurement will be complete. Turn the chamber fans and analyser pump off, and open the chamber. The system should automatically enter VIEW mode.
- Display the of the CO_2 versus time response using FCT 1A. Record the slope of the response by scrolling down until the M value is displayed. Call this value $\delta C/\delta t$.

- Find the average CO_2 concentration during the measurement by displaying **CO2**, and scrolling down until the M value is displayed. Record this value as C_c.
- Calculate the leak rate as shown in equation 1.

$$leak = \frac{10.\partial C / \partial t}{(C_a - C_c)} \qquad \text{(Eqn. 1)}$$

This leak rate is the rate of change you would expect with a 10 µmol mol^{-1} differential in CO_2 concentration. This value should be at least 100 times less than the rate of change you expect to result from photosynthesis (0.1 to 1 µmol mol^{-1} s^{-1}). If the leak rate is higher, you should consult the LI-6200 primer manual for information on finding and fixing leaks.

> ✓ Before making measurements remember to reset the OPERATING PARAMETER list and desiccant flow rate to your previous settings.

Leak test (A/C_i measurements)

The LI-6200 can be used to construct A/C_i curves, using the plant to reduce the CO_2 concentration in the chamber. Leaks become very important in these measurements since the CO_2 concentration in the chamber will be very different from that of the ambient air. A leak correction factor τ can be calculated and used in the A/C_i measurement protocol. The value τ represents the leak rate time constant and has units of seconds. The larger the value of τ the lower the leak rate.
The leak test used to obtain τ is very similar to the standard leak test.

- Set the OPERATING PARAMETER list as shown in Table 7. The only difference is that you record three observations of 30 seconds.

`LAB=LEAK (A/CI)`	System Label. Can be ignored.
`CHANGE=30.0`	30 seconds
`CHANNEL=10`	Channel 10 is time
`OBS/PAGE=3`	3 observations
`STEP(s)=1`	Record samples every second
`BEEP LOG(Y/N)=Y`	Beep after each observation
`KEEP SAMPLES(Y/N)=N`	Don't keep raw data.
`PRN FMT (U/S)=S`	*The rest of the list can be ignored for this test.*
`AREA MULT=1.00`	
`STEP(s)=1`	

Table 7. Operational parameters for A/C_i leak test.

- Calculate τ using equation 2. The same definitions apply as for the standard leak test. You should calculate τ separately for each of the three observations. Compare the three values, they should be similar. If they are, use the mean of the three observations. If not, try repeating the leak test.

$$\tau = \frac{(C_a - C_c)}{\partial C / \partial t} \qquad \text{(Eqn. 2)}$$

- The size of the leak time constant, and hence calculated value for τ will be proportional to the size of the chamber. Small chambers have small values for τ and hence are more seriously affected by leaks.
  If you are using the LI-6200 system to determine A/C_i curves for plants, you should try to obtain a value of τ greater than 7000 s. If your value is less than this, you should try to find the sources of the leak, and correct the problem. The most common source of leaks, is compressed chamber gaskets, which need replacement.

✓ Before making measurements remember to reset the OPERATING PARAMETER list and desiccant flow rate to your previous settings. The ambient CO_2 concentration should be entered into the PAGE PARAMETER list using **FCT** A8 and τ should be entered using **FCT** A9.

Making Measurements

Measurement procedures.

At the start of an experiment or season.

Check system carefully, clean chamber and replace desiccant. Do soda lime test and replace if required.

- Measure boundary layer conductance for each combination of chamber and leaf shape (size) to be used in the experiment.
- Calibrate the humidity sensor using a suitable water vapour generator (e.g. LI-COR LI-610)
- Use some spare plants to test system and determine optimum settings for the PAGE PARAMETERS

At the start of each day.

- Check that all batteries are charged. The voltage should be greater than 12 V.
- Replace desiccant. (Unless it is still fresh.)
- Conduct pre-operational tests. Check calibration lists, sensors.
- Measure barometric pressure, set value in PAGE PARAMETER list using FCT A0.
- Set CO_2 reference (FCT 49).
- Zero flow meter (FCT 48)
- Measure maximum flow rate through desiccant. Set value in PAGE PARAMETER list using FCT A5.
- Conduct K test and set Kabs parameter in PAGE PARAMETER list using FCT A7.
- Chamber leak test, (or measure τ for A/C_i measurements) .
- Calibrate CO_2 analyser. Set zero and span.

Every 3-5 hours.
(More often if weather changes, and always after changing altitude or location)

- Measure barometric pressure, set value in PAGE PARAMETER list using FCT A0.
- Set CO_2 reference (FCT 49).
- Zero flow meter (FCT 48).
- Visual check of desiccant. Replace if it has started to form chunks.
- Conduct K test. Replace desiccant if Kabs > 1.5 and repeat test.

  Set Kabs parameter in PAGE PARAMETER list using FCT A7.
- Calibrate CO_2 analyser. Set zero, and if possible check the span.

✓ Before making measurements remember to reset the OPERATING PARAMETER list and desiccant flow rate to your previous settings.

Every 15-30 minutes.

- Zero flow meter (FCT 48).
- Visual check of desiccant. Replace if it has started to form chunks.
- Calibrate CO_2 analyser zero.

Before every measurement.

- Set PAGE and OPERATIONAL PARAMETERS (if required).
- Enter leaf area (AREA). (If required and available)
- Enter any comments or auxiliary data (AUX).
- Shake desiccant tube.
- Turn on chamber fan.
- Switch the PUMP ON, and check that the flow switches are set to DES ON, and SCRUB OFF.

> ✓ MONITOR CO2 and VPD during measurements.
> You should aim to adjust the flow rate (DES FLOW) so that VPD remains nearly constant.
> If VPD *decreases* you need to *increase* the flow through the desiccant.
> If VPD *increases* you need to *decrease* the flow.

- Select a suitable leaf, and carefully place it in the chamber. (See below)
- Check that the leaf is in contact with the leaf thermocouple.
- Close the chamber and latch it shut and press the LOG key when the CO_2 concentration starts to decline.

> ✓ Hold your breath when placing a leaf in the chamber.
> Check that the leaf is in contact with the thermocouple in the chamber.

During a measurement.

- Keep chamber steady. Use a tripod if possible.
- Avoid shading the leaf or quantum sensor.

✓	Do not adjust the flow rate during a measurement.

After a measurement.

- Open chamber and remove leaf.
- Record leaf area or mark leaf for subsequent measurement.
  Enter leaf area into LI-6200 (**AREA**).
- Enter any comments or auxiliary data (**AUX**).
- View data (**VIEW** if required).

  - Check for variation in **PAR** and **VPD**. If VPD increased, the flow rate was too high, and if VPD decreased, the flow rate was too low.
  - Look at **PHOTO** and **COND**. Compare with previous measurements.
  - Look at **Ci**. For unstressed leaves this should be around 230 to 260 for C_3 plants and 180 to 200 µmol mol^{-1} for C_4 plants.
- If the data are acceptable, remember to store the data (**STORE**) before clearing the pad (**CLR PAD**).

✓	Remember to **STORE** data at the end of a measurement.

At the end of each day.

- Download data to computer. Create a backup copy of your data.
- Recharge batteries.
- Clean chamber.
- Check all sensors and system components.
- Perform any maintenance (as required)
- Discard old desiccant. Check the tube end-papers and replace as necessary.

✓	Always recharge batteries after use

Selecting plant material.

It is important to select representative plant material for leaf gas exchange measurements. You should always think about physiological processes that are likely to influence your measurements. A few points you should consider are listed here. You should only use these as a guide since in some experiments you may need to modify selection procedures.

- Select healthy leaves of a uniform physiological age. (e.g. "Last fully expanded leaf".)
- Avoid damaged leaves.
- Try to measure leaves under similar environmental conditions.
- Do not move leaves into a new environment immediately before a measurement.
  This is particularly important for the light environment.
- Avoid breathing on the leaf or touching the leaf surface.

The Flow Restrictor.

Sometimes you will find that VPD in the leaf chamber continues to rise when the Desiccant Flow valve has been turned fully anticlockwise (minimum flow rate ≈ 300 µmol s^{-1}). This happens most frequently with the smallest leaf chamber, low leaf areas, or plants with low rates of gas exchange. You can further reduce the flow rate by fitting a flow restricting valve to the top of the desiccant tube. A suitable valve is included in the spare parts kit supplied with the LI-6200. If you use this valve, you can reduce the flow rate of air passing through the desiccant tube to near zero.

If you use the flow restrictor valve on the desiccant tube, you must remember to open it when setting the zero or span of the CO_2 analyser.

Leaf Area.

It is essential to enter the correct area for the foliage used in gas exchange measurements. If possible you should try to measure and enter the leaf area immediately after making the gas exchange measurement. In some situations this will not be possible, and you will need to enter the leaf area at a later date. If this is the case, you should make sure that you store some identifier for the leaf with each page of data. It is quite easy to edit pages of data stored in the LI-6200, *but it is a slow process.*

If you work with long straight leaves such as is found on cereals, it may be possible to calculate leaf area from a simple measurement of leaf width. The area of foliage exposed in the chamber will equal the average leaf width multiplied by the width of the chamber. The `AREA MULT` entry in the OPERATING PARAMETER list is used to automate this process. You enter the width of the chamber as the `AREA MULT` and then enter width of each leaf instead of the full leaf area. The LI-6200 will multiply these two parameters together to calculate the actual leaf area. This subject is discussed in greater detail in the documentation supplied with the LI-6200.

There are a number of ways that you can edit the leaf area values stored in the LI-6200. These are discussed in more detail in Appendix III.

Quick Start

The LI-COR LI-6200 system that you are using has been programmed to help you get started quickly. Before you can use the system you need to read the System Overview, Pre-Operation and Operational Tests sections in the manual. It is essential that you learn to use and modify the PAGE PARAMETER list. This list contains calibration factors essential for correct operation of the system. This list is discussed in detail in other parts of this document, and in the LI-COR manuals.

The standard way to recall the PAGE PARAMETER list is to press **FCT** 42. On the system you are using a key has been defined to recall the PAGE PARAMETER list. All you need to do is press the **T** key. You may want to mark the keyboard with this definition, and others as shown in Appendix I. The rest of this manual assumes that you have marked the keyboard and uses the new key definitions (e.g. **PGPRM**, **OPPRM**)

GET and STORE.

A number of pre-programmed settings have been stored in your system. The most useful are a number of OPERATING PARAMETER lists that include settings used for the performance tests, and alternate measurement procedures (e.g. A/C_i). These files have been loaded into your system as listed in Appendix II. To recall a file simply recall the relevant list and then press the **GET** key. You will then be asked for a page number. You enter the number listed in Appendix II, and then press **RTRN** to recall the file. If you don't know the file number you can enter **?** and press **RTRN** to bring up a list of all files of the selected type. You can scroll through this list with the arrow keys. To select a file, position the description on the top line of the display and press **RTRN**.

Sometimes you will want to modify lists. You will become familiar in using the **EDIT** key as you use the LI-6200 system. Sometimes you will have a setting that you use frequently. You can save your settings using the **STORE** key, just as you would for data files. When you press **STORE** you may be told that `THIS FILE EXISTS` and be asked if you want to overwrite the file. If you reply with Y, the file will replace the existing copy. If you enter N a new file will be created. These files can subsequently be recalled using the **GET** key.

An Example (The K Test)

The K test becomes very easy using this approach. You call up the OPERATING PARAMETER list using the OPPRM key (or FCT 42) and then press GET, enter the number 2 and press RTRN. You should now have the OPERATING PARAMETER list for the K Test. You should then follow the instruction given in the section on operational tests. OPERATING PARAMETER lists for the standard leak test, and boundary layer conductance measurements are stored as pages 3 and 5. (Remember to set the PAGE PARAMETER BC to zero and enter double the leaf area when measuring boundary layer conductance.)
A DEFAULT OPERATING PARAMETER list for normal gas exchange measurements is stored as page 1.

Things to remember.

Using the quick start approach will help you to make measurements more easily. It is however important to remember that the system must be operated correctly to obtain good measurements.

- Always set the PAGE PARAMETER list before measurements
- The air pressure (P) , maximum flow rate (Fx) and Kabs factors must be updated frequently. Always remember to do FCT 49 after changing the air pressure setting.
- Select a suitable OPERATING PARAMETER list (programme) before making your measurements.
- Always remember to reset the OPERATING PARAMETER list after performing any operational test such as the K test.
- Always look at your measurements to see that they make sense!

Data Management

The LI-6200 can rapidly collect large amounts of data. You need to plan how are going to process these data *before* you start to collect it. Data can be transferred to any computer fitted with a RS232 (serial) interface, but you are advised to use an IBM PC compatible system if at all possible. LI-COR provides a number of computer programmes to help in data communications and processing, and these are provided with good documentation. If a computer is not available, another option is to print data directly to a RS232 printer. Lastly it is possible is to recall and record data manually. The system console has enough memory to store at least one day's data under normal conditions of use.

You can store a much larger number of data pages if you don't store the raw data by setting `KEEP SMP(Y/N)=N` in the OPERATING PARAMETER list. As a general rule however, always store as much information as is possible within the limitations of the console memory. You need to monitor the amount of unused memory available as you make measurements.

Press the **STATUS** key to display the amount of unused memory. (The battery voltage and number of stored pages are also displayed). If you need more memory, you should delete unwanted data pages.

File Types.

All information is stored by the LI-6200 as data files. The sixteen file types are listed at the start of the CONDENSED REFERENCE manual and in the software topics section of the REFERENCE manual. The most common file type that you will use is the PAGE (Data) that has file type 1. All types of files can be transferred between a computer and the LI-6200 using the binary load and dump functions. An index of a selected file type, or of all files can be sent as an ASCII file using **FCT** D5, or else viewed on the console using **FCT** DA (View Labels).

Files can be renumbered using **FCT** D2 or deleted using **FCT** D1.

Transferring data to an IBM compatible computer.

LI-COR supplies cables to connect the LI-6200 to IBM compatible personal computers. You may need to find a plug converter (25→9 pin or male→female) to suit your computer, again some are supplied by LI-COR. The PC6200 communications software supplied by LI-COR should be used to transfer data to and from the LI-6200. The instruction manual is self explanatory, and the system should work with the default communications settings on the LI-6200. See the section on DATA COMMUNICATIONS in the primer manual for more information if required.

There are two important functions on the LI-6200 that are used to transfer information to a personal computer. **FCT** 53 dumps data in an ASCII format, that is as standard text, that can be read and printed. The format that this function uses is determined by a number of other system settings. By default the LI-6200 uses a default system format to print the data page. This system format itself can be altered using **FCT** D8. An alternative user format is selected using the `PRN FMT` parameter in the OPERATING PARAMETERS list. If this is set to user, the alternative use format defined by **FCT** D9 is used. This option is very useful if you want to output data directly in a format compatible with for example a spreadsheet or statistics programme. See the REFERENCE manual for more information.

FCT 54 is used to dump data in a binary format. This process is much faster than an ASCII dump, and uses less disk space. Binary data can be loaded back into the LI-6200 for further analysis if required. The disadvantage of this approach is that the data cannot be read or printed directly, so you must use the LI-COR programme `PC6200` to process data. This is however

still very efficient, and should be the preferred method of processing data. When you use FCT 54 you will be asked for the file type. You should enter the value 1 for data pages.
In general it is a very good idea to dump both BINARY and ASCII copies of the data to disk. You should then immediately make backup copies of the data on a separate disk that should be stored away from your original copy. For additional security it is a good idea to print a copy of the ASCII data file.

✓	Save BINARY and ASCII copies of your data. Backup your data onto separate disks. Keep them safe.

Other Data Transfer Topics.

Data transfer to and from the LI-6200 uses the RS232 protocol. The configuration for this serial link is accessed using FCT 51. You should not have to change any of these parameters from there default values for normal use. If you load binary data into the LI-6200 using the PC6200 programme, you must set handshaking XON/XFF HSHK to off. You may want to try increasing the rate of data transfer by setting BAUD=9600 but in practice data transfer is limited by the speed of the LI-6200 console , and the increased baud rate does not result in a large increase in data throughput.

Deleting Files (Pages)

The delete file function (FCT D1) is used to recover system memory by deleting unwanted files stored in the LI-6200. Great care should be exercised when using this function since you cannot recover data after it has been deleted. The most common use of the delete function is to remove data files after they have been stored on a computer. Before deleting the data you should always check that the data has been stored correctly on the PC using a text editor for ASCII files or PC6200 for binary files.

✓	Always check that data files have been stored correctly on your computer before deleting them from the LI-6200 console.

The delete files function prompts you for FTYPE (File type). To delete data files (pages) enter the numeral 1, other file types are listed in the LI-COR manuals. The system then prompts for the range of pages to be deleted. This function can be aborted by given a null enter to any of the three prompts. See the REFERENCE manual for more information.

Do not interrupt this routine once you have entered the THRU *value, as you could potentially lose all of your stored files!*

Maintenance.

The full maintenance schedule and procedures recommended for the LI-6200 is described in the LI-COR manual "LI-6200 Primer".

Every day.

- Recharge batteries.
- Check desiccant and filter end papers and replace as required.
- Check chamber gaskets and replace as required.
- Clean chamber.
- Check operation of all sensors.
- Check offset between air and leaf temperatures. Place a dry peice of filter paper in the chamber. Latch the chamber and place the chamber in the shade with the fans on. The leaf and air temperature should agree within 0.1 °C. Adjust temperature offset if required.
- Check RH sensor against known reference.

Every month.

In ***addition*** to the above.

- Calibrate RH sensor against a water vapour generator (e.g. LI-COR LI-610)
- Clean analyser fan filter.

Every 1-3 months

(frequency depends on the conditions of use).

In ***addition*** to the above.

- Recharge batteries, EVEN IF THEY HAVE NOT BEEN USED!
- Replace analyser internal filter #1.
- Replace soda lime in scrub tube. Swap reference tube for scrub.

Every year.

In ***addition*** to the above.

- Check CO_2 calibration against a range of calibrated CO_2 standards.
- Check flow meter calibration against reference standard (e.g. soap bubble flow meter).
- Check quantum sensor calibration against secondary standard (if available).
- Clean and lubricate tubing connectors.
- Replace analyser internal filters #2 and #3.
- Replace internal soda lime and desiccant.

Every 2 years.

- Return system to LI-COR for calibration.

Advanced Features.

There are many advanced features available on the LI-6200 system that can help you to work efficiently. You have already been introduced to some of these in the way that your system has been customised for ease of use. Short descriptions of the main function are included in this section for your reference. These features are documented in the LI-6200 Technical Reference manual.

Set System Format (FCT

Sets standard output format for data pages. You need to modify this list, if you add sensors or calculations.

Set User Format (FCT

Sets user format for data pages. This option is used to produce output in a form suited for direct input to spreadsheet or statistics programmes.

User Key Definitions (FCT **E1**)

The system console keyboard can be programmed to give keys new functions.

Sensor List (FCT E2)

Additional sensors can be connected to the system. Sensors must have voltage outputs in the range of ± 5 V.

System Programme (FCT E3)

The system can be programmed using alternate formulae or to provide new parameters (e.g. VPD)

Log Programme (FCT E8)

The log programme is a sequence of events that occur when the LOG key (or the log switch on the sensor head) is pressed.

Set Mini View (FCT EC)

Mini view mode is used to cycle through a subset of the data page. You run the miniview mode using FCT EB. This instruction can of course be added to the log programme to automate the procedure.

Set KBuff (FCT ED)

The keyboard buffer is used to store a sequence of keystrokes that can be used to automate an operation. For example the keyboard buffer has been used in your system to automate the procedures required to set up the system for either default or A/C_i measurements.

Other functions are available, but are of more limited use.

Further information on these procedures is available from the author of this document.

Useful References

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Appendices

I. Keyboard Definition.

The keyboard definition list is accessed using **FCT** E1. Two files have been stored in your system, a DEFAULT list, and a list for A/CI measurements.

The default list (Page 1) is slightly different from that supplied with the LI-6200, in that it includes keys for VPD, Battery voltage and Quick view mode, and that the two keys C_s and R_s have been redefined to give keys for the OPERATING PARAMETER list (**OPPRM**) and PAGE PARAMETER list (**PGPRM**). The alterations are highlighted in the diagram below.

TIME	PAR	T_{LEAF}	T_{CHAM}	T_{IRGA}	$[CO_2]$	FLOW	RH	***VPD***	***BAT***
PHOTO	COND	Ci		***PGPRM***	***OPPRM***	STATUS	MONITOR	LOG	PAUSE
AREA	AUX		CLR PAD	***QUICK***	VIEW	↑	↓	FCT	
SETUP	COMM	MEMORY		GET	STORE	PRINT	EDIT		

The list used for A/C_i measurements (Page 2) has two further changes. The key definitions for PHOTO and C_i have been defined so that these keys display the corrected estimates CPHOTO and CCINT.

TIME	PAR	T_{LEAF}	T_{CHAM}	T_{IRGA}	$[CO_2]$	FLOW	RH	***VPD***	***BAT***
CPHOTO	COND	***CCINT***		***PGPRM***	***OPPRM***	STATUS	MONITOR	LOG	PAUSE
AREA	AUX		CLR PAD	***QUICK***	VIEW	↑	↓	FCT	
SETUP	COMM	MEMORY		GET	STORE	PRINT	EDIT		

II. Programme Files Stored in the LI-6200.

The following files have been stored on your system to help you make measurements, and perform operational tests.

File Type	Page Number	Description
System FCT E3	1 2	DEFAULT A/CI
I/O FCT 51	1 2	DEFAULT BINARY LOAD
Operating Parameters FCT 42	1 2 3 4 5 6 7	DEFAULT A/CI BY CO2 A/CI BY TIME K TEST LEAK TEST (DEFAULT) LEAK TEST (A/CI) BOUNDARY LAYER CONDUCTANCE
System Format FCT D8	1 2	DEFAULT A/CI
User Keys FCT E1	1 2	DEFAULT A/CI
Key Buffer FCT ED	1 2	SET DEFAULT SET A/CI
Log Key FCT E8	1 2 3	DEFAULT A/CI PROMPTED SAVE (Asks for leaf area and comments)

III. Setting Leaf Area.

The chambers supplied with the LI-6200 can be used with a variety of leaf sizes and shapes. The chambers do not enclose a standard leaf area, and as a result you must measure the leaf area of your samples after making your gas exchange measurements. The correct leaf area **must** be used to correct the stored gas exchange measurements. There are a number of approaches that you can use, and these are summarised in this section.

Estimating Leaf Area.

The method you use will depend on the plant material and leaf chamber you are using. If you use the one litre chamber with suitable plant material, it may be possible to use the foam inserts to define a constant leaf area. This approach works well for plants such as maize. In this case you only need to measure the area of enclosed leaf, and then enter this value as the total leaf area using FCT A1. Another similar approach is available if you are using long narrow leaves across a narrow chamber. You can estimate the area of leaf, by measuring the average leaf width, and multiplying it by the width of the chamber. This process is simplified by entering the the width of the chamber as AREA MULT entry of the OPERATING PARAMETER list. After each measurement you measure the width of the leaf, and enter the ***leaf width*** instead of leaf area. The system will then calculate the area and store the estimate of the ***leaf area*** in the data page. For more information, see the section on OPERATING PARAMETERS in the LI-6200 PRIMER manual, or the LI-COR application note on this topic.

If the above approaches are not suitable you must measure the leaf area using alternative approaches. You could try tracing the outline of the leaf onto paper, and then measuring the area of the paper. Otherwise you will need to harvest the leaf, and measure leaf area using an area meter. If you remove the leaf, remember to limit changes in leaf size, by keeping the leaf cool, and preventing water loss. You should also record a leaf identifier with each page of data. The simplest way to do this is to create an entry in the auxiliary data prompt section of the data page.

You can programme the LI-6200 to ask for the leaf area or identifier after completing each measurement. To do this you change the programme for the log key (FCT E8) An example programme that asks for the leaf area and comments is stored as file 3 on the LI-6200 console. For more information see the section on FCT E8 in the REFERENCE manual.

Recomputing Data with new Leaf Areas.

There are three main ways to recompute data pages after correcting the leaf area values. The slowest is to recall individual pages using the GET key, enter the correct area (AREA) and then to STORE the corrected data. The page will automatically be computed, and the system will prompt if you want to replace the existing copy of the data file. You should normally reply yes, unless you want two copies of essentially the same data set. This process can be automated using FCT D7 the edit loop programme. This programme will ask for the range of pages to edit, and then the code number to edit (A1) for area. You are given the option of being asked for each value, which you should use, unless you have a sequence of data pages using the same leaf area. See the REFERENCE manual for more information. The last option is to use the LI-COR support software using data files stored on a PC. This approach is much quicker than computing on the console, but does require that data are stored on disk in the standard ASCII format.

A Simple Correction for Leaf Area.

In many cases it is desirable to correct gas exchange estimates without having to compute the whole data page. An example, would be is the data have been loaded into a spreadsheet. It is very easy to correct estimates of parameters such as net photosynthesis, stomatal conductance and transpiration.   The corrected (actual) rate is calculated from the measured rate, and the assumed and actual leaf areas as indicated by the following equation.

$$ActualRate = MeasuredRate * \frac{AssumedArea}{ActualArea}$$

IV. Using the System for A/C_i Measurements.

Re configure the System.

- You will need to reprogram the system before you can make any A/C_i measurements. To do this you use a stored key sequence. Use FCT ED to recall the key buffer and press GET 2 RTRN. Run this programme using FCT EE.
- Plumb system as shown in LI-COR application note #103.

Resetting the System for Standard Measurements.

You need to reset the LI-6200 to its default measurement configuration before making standard leaf gas exchange measurements. Bring up the Key Buffer programme using FCT ED, and GET 1 RTRN. Execute this programme using FCT EE. At the end of this programme, you will be left in the PAGE PARAMETER list. You should make any required changed to this list before starting you measurements.

Before each Measurement

- Check that you have stored the previous measurement, then clear the scratch pad (CLR PAD)
- Zero the CO_2 analyser.
- Zero the flow meter (FCT 48)
- Perform a K-test
- Set the value for τ (FCT A8) and ambient CO_2 (FCT A9)
- Set the flow diversion switch to open.
- Select the OPERATING PARAMETER list for measurements A/CI BY CO2. Press OPPRM GET 2 RTRN.
- The pump on the LI-6250 analyser should be switched off.
- Use an air cylinder with around 1400 µmol mol^{-1} CO_2 in air. Pass the air through the LI-610 water vapour generator. *Do not* use the pump on the LI-610. Measure the flow rate using the gap flow meter on the LI-610. Adjust the flow rate to be approximately ____ ml s^{-1} using the regulator on the gas cylinder.
- Position the external fan next to the chamber to maximise the cooling effect.
- Reset the flow rate through the desiccant to your previous starting value (If known).

Every 2-3 hours.

- Perform a leak test.
- Check analyser span.
- Check barometric pressure.

Starting the measurement

- Place a leaf in the chamber and latch it shut.
- Check that the leaf is not shaded.
- Position the external fan to maximise cooling of the leaf chamber.
- MONITOR VPD.
- Adjust the set point on the LI-610 to obtain a chamber VPD of approximately 10 mbar. Increasing the set temperature will decrease the VPD in the chamber.
- Display RH and check that the relative humidity does not exceed 80-85 %.

  If it does, you may need to use a higher VPD.
- Let the leaf equilibrate in these conditions for 10 minutes.
- Set the external flow diversion switch to the CLOSED position and switch on the pump on the LI-6250 analyser.
- Adjust DES FLOW, until VPD is stable.
- If the CO_2 concentration has dropped below 1000 µmol mol^{-1} of CO_2, you will need to OPEN the system and pass high CO2 air over the leaf again for 1-2 minutes.
- When the flow rate is correctly set, press the LOG key to start logging.
- Record the starting time, PAR, leaf and air temperature, CO_2, RH, Flow and VPD in your notebook.
- Close the regulator on the gas cylinder.

During a Measurement

- Monitor CO2 and VPD.
- As CO_2 drops the stomata should open. You will need to adjust the flow rate through the desiccant to keep VPD constant. If stomata open, you need to increase the flow rate.
- If you need to change the flow rate, **stop logging**, by pressing the MONITOR key. Display FLOW and adjust the flow rate using the DES FLOW needle valve. Wait for the reading from the flow meter to stabilise. Shake the desiccant tube and then press LOG to restart logging .

- *Do not* adjust the flow rate when you are logging data!
- Stomatal opening will be more pronounced when the ambient CO_2 concentration falls below 350 µmol mol^{-1}.
- *Remember* to shake the desiccant tube *whenever* you stop logging .

- The photosynthetic rate will fall as the CO_2 concentration in the chamber decreases, At the start of the measurements, each observation should take only 10 to 15 s. When this time has increased to over 30 s, you can change the OPERATING PARAMETER list to define each observation by a 5 µmol mol^{-1} change in CO_2. Press MONITOR to stop logging and OPPRM to bring up the OPERATING PARAMETER list. Edit the second line to read CHANGE=5. Shake the desiccant tube before pressing LOG to restart logging.

When ambient CO_2 is below 150 µmol mol^{-1} .

- When the CO_2 concentration has dropped below 100-150 µmol mol^{-1} the rate of photosynthesis will be quite slow. At some point you will need to switch to a time based measurement. Press **MONITOR** to stop logging, and then **OPPRM** **GET** 3 **RTRN**.

  Shake the desiccant tube before pressing **LOG** to restart logging.

When ambient CO_2 is below 100 µmol mol^{-1} .

- When the ambient CO_2 concentration is around 100 µmol mol^{-1} it will be necessary to use the CO_2 scrubber to reduce the CO_2 concentration in the chamber. Press **MONITOR** to stop logging before setting SCRUB ON to reduce the CO_2 concentration in the chamber. The length of time you scrub will depend on the size of the chamber. For the 1/4 l chamber 5 s will be adequate to drop the concentration by about 20 µmol mol^{-1}. Shake the desiccant tube before pressing **LOG** to restart logging.
- You need to record 5 observations between each scrub period.
- You should aim to obtain sets of measurements at chamber CO_2 concentrations of 100, 75, 50 and 30 µmol mol^{-1}.
- The last samples should be collected below the CO_2 compensation point. (Typically a CO_2 concentration of less than 50 µmol mol^{-1}).

At the end of the measurement

- Press **MONITOR** to stop logging.
- Enter the correct leaf **AREA**, and any comments (**AUX**). Press **VIEW** key to compute , and then **STORE** the data.

V. Notes

Use this page for your own notes.

VI. Useful Function Reference.

FCT	Description
Setup	
41	Set Operating Parameters
42	Set Page Parameters
48	Zero Flow meter
49	CO2 Reference
Memory	
D1	Delete Files
D7	Edit Loop
Communications	
53	Dump ASCII Pages
54	Dump Binary Pages
Constants	
A0	Air pressure
A1	Leaf Area
A2	Total System Volume
A3	Boundary Layer Conductance
A4	Stomatal Ratio
A5	Maximum Flow rate
A6	Analyser Volume
A7	KAbs
A8	Ambient CO2
A9	τ