Running the LiCor 6400 Infra Red Gas Analyzer (IRGA)
with 4 whole-plant cuvettes attached

Note: You must read the Primer manual Sections 1-6 before using the IRGA. The manual is located in the wall cabinet with glass doors to the left of the fridge.

This protocol will be referring to two components of the 6400 system, the main unit and the IRGA head. The main unit is the greenish box with the liquid crystal display on its top surface. The IRGA head is the hand-held unit connected by tubes and cables to the main unit. The hand-held unit actually contains the infra-red sensors, hence it is called the IRGA.

For this experiment the IRGA head must be connected to the main unit according to instructions in the Primer until CALIBRATION is completed. In this (the standard operating) mode, the Sample tubing connection from the IRGA head (the hand-held unit), labeled with a black ring of tape, is connected to the gas port labeled "Sample" on the bottom right of the right side of the main unit.

Preparing the 4-cuvette system:

1. **Tubing:** When running the 4 whole-plant cuvettes, these tubing connections to the main Li6400 unit will remain undisturbed. At the other end of the tubes, where they attach to the hand-held IRGA head unit, you will disconnect them and connect instead the Sample and Reference tubes from the cuvette array. These are labeled "Sample" and "Reference" and physically cannot be connected to the incorrect input tubes to the IRGA head. Do this after calibration (if necessary), and after the Open System software is up and running. See below.
2. **Leaf Thermocouple:** The leaf thermocouple plug from the IRGA head's built-in cuvette must be detached. It is a purple plug connected to wires that originate under the IRGA head's built-in cuvette. Unplug it and plug in the matching plug that is attached to the 4-cuvette frame.
3. **PAR (photosynthetically active radiation) sensor:** The PAR light sensor plug is a small white plug on the right hand side of the IRGA head with white wires striped with yellow and red. Use a small pair of pliers to gently pull this off its connection. Replace with the matching plug from the 4-cuvette frame. Refer to section 2 page 2-12 for pictures of the thermocouple and PAR connections.
4. **Cuvette preparation:**
    a. Clean the exterior of the bottles with a soft cloth, removing any smears or dust. Any bottles that are scratched or otherwise compromised should be replaced.
    b. Clean the black gaskets that line the interior edges of the articulating cuvette bases. Replace foam central gasket strip and/or black gasket with material in the Green metal LiCor tool box in the glass-fronted cabinet to the left of the refrigerator. Alert the lab manager if you are using up most of the gasket material so that more can be obtained.
    c. Adjust the angle of the leaf thermocouple on the sensor rod (attached to the bottle cap) so that is angles downward and to the side of the rod (i.e. is not directly below the rod or the PAR sensor).
    d. Adjust the angle of the PAR sensor (the little rectangular block on the sensor rod so that it is approximately parallel to the counter top.
    e. Adjust the white/beige plastic tension ring on the inside of the bottle base and the black gasket material on the outside of the bottle base so that they are directly aligned.

f. Close the cuvette with the bottle in place and tighten the knurled knob on the left of the closing mechanism.

5. **Gas flow rate**: Gas flow rate is not controlled by the IRGA console when sampling from the whole plant cuvettes. Instead, the manual flow meters at the back of the cuvette fram are used to control flow rate. There are two manual flow meters. The left one is labeled "Sample" and the right one is labeled "Reference". Labels are also attached to indicate the appropriate settings to achieve 250 ml and 500 ml. per minute.
    a. Turn on the outlet strip to the left of the cuvette frame. You will hear the pump come on in the cabinet under the cuvette frame. Open the cabinet. Observe the 50-litre mixing bottle to the right and the small pump to the left. Observe the four white plastic 1/2" tubes. One of these is labeled "A" and one "B". They originate in chambers 101 and 102 respectively. The chambers can be used to supply a large volume of gas with constant CO2 concentration. Make sure the correct hose (as determined by experimental design) is connected so that the correct gas is supplied.
    b. Adjust the Reference flow rate to the level specified in your experimental design.
    c. Now check the Sample flow rates from the individual cuvettes. Note the black cuvette selector switch box on the left side of the cuvette frame. Each switch is labeled with a number on colored tape. The cuvettes are labeled with a corresponding number and tape color.
        i. Push each of the four cuvette selector buttons in succession, noting the Sample flow rate on the left-hand flow controller (labeled "Sample") as you do this. The cuvettes with highest Sample flow rates should have flow rates above the flow rate specified in your experimental design., The flow rate will be adjusted downward later, but first we want all cuvettes supplied with a higher level through their individual adjustment valves. Then we will adjust the Sample flow controller downward. This insures positive pressure in the system.
        ii. If any cuvette has a notably lower flow rate, check the tightness of the knob on the closing mechanism and check the gaskets for cleanliness and integrity. Also check that the connector tubing at the base and top is properly connected. If you are convinced that the seals are good, go on the the next step.
        iii. Adjust the small brass valve on the incoming gas line to increase the flow. This valve sits under the cross-piece that supports the cuvettes. Note the two 1/4" tubes that feed gas to the two sides of the cuvette base. These tubes originate at a "T" under and behind the cuvette base. On the incoming 1/4" gas tube, just back from this brass "T" you will find the brass valve. VERY SLIGHT adjustments are sufficient. If VERY SLIGHT adjustments do help, the problem lies elsewhere. Seek help. When all cuvettes have equal flow rates, go to the next step.
        iv. Adjust the Sample flow controller downward to the flow level specified in your experimental design. Do this by turning the knob at the base of the flow controller to the right until the silver ball rests at the appropriate mark.
        v. Check to see that all cuvettes produce this same flow rate.
6. **Light levels**: Using the LiCor Li1000 Data Logger, check the light level in the center bottom of each cuvette. Do this by opening the cuvette and holding the quantum flux probe so that it is in the center of the cuvette with its top surface horizontal. Adjust the halogen lights attached to the top of the cuvette frame to achieve the intended light level.

Starting the LiCor 6400

1. Turn on the main unit. The power switch is on the right-hand side of the main unit of the Li6400.

   Remember to have the external power source or batteries plugged in. Even with the external power supply, you will need to have a charged battery plugged in if you want to power the IRGA head. Without either two batteries, or the power supply cord and one battery, the main unit can be powered, but the IRGA head cannot. You will not get an error message. The machine will instead just repeatedly re-start the set-up sequence.

   The LCD will display the message "initializing", then "Loading Open System 3.4...".

   A window will appear requesting that you choose a configuration. Choose "Factory Default"

   After a few minutes, you will be asked if the IRGA is connected. Press "Y". You should hear fans starting inside the main unit and a clicking noise coming from the IRGA head.
   If instead you get the message "initializing..." then you need to replace the battery with a freshly charged one. The battery is in an open compartment on the bottom right of the main unit.
   After the whirring and clicking noises start, the LCD will display a screen entitled "LI-6400 Photosynthesis System". Wait 30 minutes for the IRGAs to warm up.

   At the bottom of the screen, you will see a series of options. At the start of each week's work, choose "Calib(ration) Menu". Follow the directions in the Primer for both the "IRGA zero" and "IRGA span" options. Section 4 page 7.. These two procedures set the zero point and the gain. This programs the IRGA to calibrate the slope of the relationship between current output of the infra-red light sensor in the IRGAs and the concentration of CO_2 displayed on the LCD and recorded in the data set.

   IRGA zero option: turn the front-most knurled knob on the left top of the main unit all the way to back (labeled "scrub"). This will scrub all CO_2 from the air entering the IRGA. Follow the directions in the Primer/ on the screen to calibrate the zero point.

   IRGA span option: Return to the Calib Menu screen and select "IRGA span". Turn the same knurled knob toward you all the way (the "bypass" direction). A tank of 500 ppm CO_2 is attached to a glass and metal flow meter located behind the the main unit. This flow meter is labeled "CALIBRATION". Open the main valve on the attached gas tank until the dial shows gas pressure. Open the fine adjustment valve on the left of the gas tank regulator until the sliver ball on the calibration flow meter behind the unit reads at least 11. Locate the black plastic 1/4" tube that comes out of the flow meter. Push the end onto the air input port on the right side of the main unit. This is the small silver-colored port below the sample and reference ports. If you hear the pumps in the main unit speed up and get louder, open the fine adjustment on the gas tank regulator a little more until the pumps in the main unit quiet down again. Follow the directions in the primer/on the screen for adjusting the span calibration. When both Sample and Reference CO2 have been adjusted, quit the IRGA span program and return to the LI 6400 operating system screen. Disconnect the calibration gas from the inlet port and close the main valve on the calibration gas tank.

Connect the Cuvette system

Disconnect the the cuvette gas supply from the main unit and connect the gas lines from the cuvette frame according to the directions in step 1 of "Preparing the 4-cuvette system" above.

From the "LI-6400 Photosynthesis System" menu, select "New Measurements". This should bring up a screen with all the output measurements displayed, including RCO2, SCO2, etc. If instead you get a small window displaying "CORRUPT ARRAY press enter", then press "enter". This is an error that we cannot fix until we replace the mother board. This will take you to the 'Welcome Menu", showing the following choices along the top: "Editor Filer Run Shell Xchng". Press "r" for Run. The default program name will appear. Press enter or select. The program will begin reloading. In my experience, the second time around, you will get the new measurements window without a hitch.

At the bottom of the New Measurements screen, there are a number of options that can be selected by pressing the red keys labeled f1 - f(6?). Towards the left is an option labeled "open log file" or maybe "new log file". Press the red function key below it. You will be prompted to enter a file name. Files should be named according to the treatment, run and tray. For example, RHH41. If more than one day is required to finish a tray, the second day's file would be named RHH41a.

Begin loading plants into the cuvettes: consult an experienced person first. Plants cannot not have water on them, but the soil should stay moist. Plants should be held in the same light levels in which they will be measured until they are queued into the cuvette series. Do not bring more plants out of the chamber than can be run in 20 minutes.

When the system is run at a flow rate of 250 ml/min., a plant must be in its cuvette for 10-12 minutes before it is measured. You can only effectively make use of three cuvettes at this flow rate. The thermocouple must be in contact with a leaf and par sensor should be placed as close to the center of the cuvette as possible without shading any leaves. Plants should be placed into cuvettes at six minute intervals. The table below illustrates the timing of events in each cuvette. "Switch #1" means that you should depress the #1 selector button on black selector box on the left of the cuvette frame, thereby directing the gas flow from cuvette #1 to the IRGA and connecting the cuvette #1 sensors to the sensor connections.

Time since start (min)	Cuvette1	Cuvette2	Cuvette3	Cuvette4
0	Load	empty	empty	empty
6	switch #1	Load	empty	empty
12	record	switch #2	Load	empty
18	Load	record	switch #3	empty
24	switch #1	Load	record	"
30	record	switch #2	Load	"
36	Load	record	switch #3	"

etc.

With larger plants, the flow rate can be increased, and the time between loading and recording reduced proportionately. For example, if the flow rate is doubled to 500 ml/min, the time intervals in the table can be reduced by half to three minutes intervals.

Before recording the measurements for a given plant, its pot number and cuvette number must be recorded. Press the red function button *f4* underneath the "remark" option. In the remark entry window, type the pot number, a comma, then the cuvette number. For example, for plant 2164 in cuvette 3, type "2164,3" and press "enter".

Six minutes after you depress the selector switch for the cuvette in question, if the plant is producing a stable ΔCO2, press the function button under the "LOG" option. The unit will beep and the small number under the word "LOG" will increment by 1. If the ΔCO2 reading stabilizes before six minutes, you may log the measurement sooner than six minutes and move on. Once logged, the plant can be removed from the cuvette and transferred to the holding rack for fluorometry measurement.

Constantly check the display to be sure that the measures seem realistic. If readings do not appear to be realistic (if there is no net CO2 uptake, or a large, healthy-looking plant is giving small net CO2 uptake), or there is little or no change in water content between the sample and reference air streams, contact the lab manager or Steve for troubleshooting.

When you are finished, follow the instructions in section 3 page 7 for shutting the 6400, with the IRGAs off. Disconnect the tubing and cabling for the IRGA head. The main unit may now be carried to the Mac for data transfer.

Now transfer the data to the Macintosh. See “Downloading IRGA” protocol.