Light Saturation Protocol
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December 14, 2007
Hope this helps someone!

Creating a Light Saturation Curve using the LI-6400

1. Put 1 fully charged battery in the LI-6400. Connect the other battery port directly to the charger.
2. Turn the LI-6400 on. While the programs are loading, connect the IRGA connectors (resemble serial cables) to the proper ports. Also connect the IRGA gas tubes. Make sure the *sample* gas tube is connected to the *sample* port! (The sample tube should have a piece of black tape around it, and the port should have a black ring around it)
3. Press *Enter* to run Factory Default.
4. Press *Y* to indicate that yes, the IRGAs are connected.
5. You should see the main LI-6400 screen, complete with several different menus at the bottom. Select the New Measurements menu by pressing *f4*. At this point, if the phrase "Corrupt Array" appears on the screen, you will have to press *enter*, change the battery and start this procedure over.
6. Using fresh soda lime in the CO2 scrub bottle, turn the knob on the bottle all the way to *scrub*. This will filter out all of the CO2 from the air that is being pulled in from the outside environment. You will be able to see this change by watching the CO2R and CO2S numbers on the new measurements screen. These readings should drop to somewhere around 0 (anywhere from -0.5 to 1.5µmol).
7. Once the CO2R and CO2S readings have stabilized around zero (this may take up to 20 minutes) the 2 IRGAs need to be matched. To do this, press the *labels* button to scroll along the bottom of the screen through the different selections. Once you reach Match, press *f5*. Match the IRGAs by pressing *f5* once inside this menu. The CO2R and CO2S should now read within +/- 0.3 or so of each other. Once matched, return to the new measurements screen.
8. Ensure that the CO2 gas tank on the side of the LI-6400 is full.
9. Use the *labels* button to scroll along the bottom of the screen through the different selections again. This time, select Mixer by pressing *f3*. Set the mixer to mix 450µmol of CO2 from the tank with the air that is being filtered by the CO2 scrubber. This allows the CO2 concentration to be fairly steady and accurate throughout the course of the experiment.
10. Once the CO2S and CO2R stabilize around 450µmol, use the *Match* function to match them again.
11. Once the two IRGAs are fully matched, the new measurements screen should remain. Press the *escape* key to get out of this menu and return to the main LI-6400 screen. Press *f2* to select the Configuration Menu. Select "Light Source Control" from the drop down menu. Press *f1* to pick your source. Change the source from "Sun & Sky" to "LED-6400."
12. Hit done. You should be returned to the main LI-6400 screen.
13. Use *f4* to return to the new measurements screen.
14. Use the *labels* button to scroll along the bottom of the screen through the different selections again. Select Lamp by pressing *f5*.
15. Select *Quantum Flow* from the drop down menu. Set the quantum flow to 1500µmol.
16. At this point, the leaf can be clamped into the sample chamber.
17. Allow the leaf to acclimate to the light level within the chamber for 20 minutes. During this time, monitor the photosynthetic rate (it should increase slightly or a lot!).

18. After 20 minutes, use the labels button to scroll along the bottom of the screen through the different selections again. Select Autoprogram once it appears by pressing *f1*. Select "Light Curve" from the drop down menu.
19. Name your data file. (Do not choose to append to any existing files ever! Create a new file!)
20. Create a comment (Maybe something useful, such as pot number).
21. Enter the light intensities you wish to use to create your curve. I used: 5 10 15 25 50 75 100 150 300 500 700 1000 2000.
22. The rest of the default settings should be acceptable. However, make sure that the last default setting allows the IRGAs to match if ΔCO2 is less than 0.5μmol.
23. Run the program! While the program is running, you can monitor its progress by pressing the letter *K*. This will tell you the number of measurements that have been taken out of the total measurements that will be taken. Can also monitor the light level by scrolling along the bottom until the PAR selection appears. This will tell you the current light intensity inside the chamber.

    After the program is finished…

24. Using the *labels* key to scroll along the bottom of the screen again, find CO2R = 450μmol. Select this using *f3*. Select turn off the mixer from the drop down menu that appears.
25. Once back on the new measurements screen, use the *labels* key to scroll along the bottom of the screen to find PAR. Select it using *f5*. From the drop down menu that appears, select turn lamp off.
26. Once back on the new measurements screen again, press *escape* to return to the main LI-6400 screen.
27. Press *f1* to select the Welcome menu. From the drop down menu that appears, select "Quit-IRGAs OFF." Do not actually power off the LI-6400 unit at this point though!
28. Carefully remove the leaf from the chamber noting how much of the leaf was in the chamber during the measurements.
29. Trace only this portion of the leaf onto a note card using a black Sharpie marker. Can calculate leaf area later using Image J software.

    To retrieve the data…

30. Connect the LI-6400 to a computer with a serial port using the LI-6400 serial cable.
31. Press *X* for exchange on the LI-6400.
32. Next, on the computer, double click on the program FX on the desktop.
33. This should display all of the data that is present on the computer on the left side of the screen, and all of the data that is present on the LI-6400 on the right side of the screen.
34. Use tab to move the selected entity from PC to LI-6400. Use the arrows to select which file from the LI-6400 you would like to copy to the PC.
35. To copy, press *I*. Select *Y*, to say yes you want to copy. Then select *O* to say that you want to overwrite older versions of files only. The data should then copy from the LI-6400 to the desktop of the computer.
36. To view the data, open Excel. Select Open from the File menu. Browse the Desktop, and then change File Type to All Files. The files from the LI-6400 should appear. Open them as a delimited text file. The file is delimited with commas and spaces, so put checkmarks by those options. On the next menu select General. The file should open with all your lovely data!