Gas exchange

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Note on 2011.12.12 by James -
There are many variations of gas exchange systems (e.g. custom-made ones, LI-COR, Walz), written below are only the protocols for a few of the systems I have used over the years. To learn about gas exchange from scratch, please ask a person in the lab.

Instructions for the Gas Exchange System (White box, Arabidopsis chamber)

Last modified Mar 16, 2009

1. Place chamber at the right location (chamber legs match the four little green tapes).
2. Set one water bath at 20C ("A") and one at 40C ("B") and turn on both water baths. Switch the six-way valve to A.
3. Clamp the wires onto the "fan" wires and turn on gas exchange machine (mixer).
4. Turn on gas tanks
5. Turn on the light. (To calibrate for light level with a light meter - make sure that the sensor of light meter is at the exact place where the plant leaf lies. (Hold it with a piece of crescent-shaped sponge.) Adjust light level until light level is ~1000 umol.m-2.sec-1 (about 80))
6. Bury the condenser in ice.
7. Adjust the knobs beside the "1" and "0" switch so the flows (shown on rotameter) match at "1" and "0".
8. Take a plant and cover the soil with saran wrap to prevent H2O and CO2 from soil from going into the chamber. Tape around the cone with Scotch tape (make sure there is no wrinkles). Wrap the bottom of the cone with Saran Wrap and tape down with Scotch tape (to prevent air from leaking out). Place a beaker of water under the cone and so that the bottom of the cone is immersed in water.
9. Adjust N2 and O2 flow to approximate 80:20 and CO2 to ~40bar. Take down all initial measurements.
10. Switch the valve to B and when leaf temperature approaches 40C, take down CO2-out and dew point-out. Hold temperature at 40C and take down CO2-in and dew point-in. Switch valve to A and hold leaf temperature at 20C. Take down measurements. Repeat this step a few times until photosynthesis breaks down.

11. Turn off the machine, the gas tanks and the light sequentially. Unplug the water bath tubes from valve and connect the "in" and "out" tubes of the water bath tubes with a piece of tubing to construct a loop.

Instructions for Li-Cor 6400 (hooked up to Arabidopsis chamber)

Last modified July 15, 2009

12. This experiment is best done in the morning so the chloroplasts do not have a lot of starch grains in the TEM pictures. Use 3 or 4-week old Arabidopsis plants so plant transpiration will be lower (and therefore condensation is less likely to occur). Take a plant, cut off the smaller, old leaves (which are partially covered by new leaves) and immerse it in water or 1/2 strength Hoagland solution.

13. Place chamber at the right location (chamber legs match the four little green tapes).
14. Set one water bath at 20C ("A") and one at 55C ("B") and turn on both water baths. Switch the six-way valve to A.
15. Turn on the light and make sure the light level in the chamber is ~ 500 umol m^{-2} sec^{-1}.

16. Turn on the Li-Cor 6400.
17. Change the small CO2 cylinder as well as the O-ring.
18. Plug in the power supply for the *Arabidopsis* chamber fans.
19. Turn on the silver box (gas mixer). Set flow controllers to:
    Wet N2 0.2L/min
    Dry N2 1.4L/min
    O2 0.4L/min
    This will make sure reference/sample air dew point is within the safe range (i.e. <room temp, or 20C) even during a heat treatment (leaf temp no higher than 40C). If any of the conditions change, the level of humidity fed into LI-COR needs to be re-adjusted through trial-and-error.
20. Turn on the N2 and O2 gas tanks.
21. Press "Calibration".
22. Attach the console directly to the IRGA (by-passing *Arabidopsis* chamber) and then zero flow.
23. Zero IRGA: bypass the CO2 scrub first and press "autoH2O" – this process takes about 20 minutes (to get all the water in the system out). This is when the number stops drifting. Then bypass the desiccator and press "autoCO2" – this takes about 5 minutes. Press "Quit".

24. As the LI-COR 6400 is zeroing water and CO2, take a picture of the plant with a size reference and figure out leaf size from Photoshop.
25. Place a beaker of water under the cone and so that the bottom of the cone is immersed in water (to keep the plant well hydrated as well as to test for any leaks). Leave the chamber lid open.

26. Attach the IRGA back to the *Arabidopsis* chamber.
27. Press "New Instrument". All the parameters are displayed on the console.
28. Press "Open log files" and name file. Type in the leaf area. Set flow to max. Set CO2 to 400ppm. Set block temperature to 30C so no condensation occurs in the IRGA.
29. Put in the plant and close the chamber.
30. Look at the dew point. If dew point is too high (or condensation starts to occur in the tube or the Arabidopsis chamber), dial down the wet N2 (but dial up dry N2 so total N2 is maintained at 1.6L/min). If this doesn't work, we need to either increase the temperature or increase the flow (which leads to reduced accuracy). If this still doesn't solve the problem, turn on the dessicator (a little bit). Everything needs to be optimized again.
    If condensation still occurs, that usually means there is too much plant material in the chamber. Cut off some leaves.
31. Start the experiment by taking down measurement at t=0 (by pressing "log").
    [Heat episodes] Switch valve to B and set temperature of water bath A to 10C. Switch valve back to A once leaf temperature reaches ~39.5C. Then Switch valve back to B once leaf temperature cools down to ~21C. Repeat this cycle.
    [Steady heat] Switch valve to B and set temperature of water bath A to 45C. Switch valve back to A once leaf temperature reaches ~40C. Set water bath B temperature to 20C. Switch valve back to B after 120 minutes at 40C.

32. Take down measurement from time to time. To do this automatically, Setup "autolog" so that measurement is taken every 2 seconds and LI-COR is matched every 30 minutes.
33. During the treatment, dew point should not exceed room temperature and no condensation should be seen in the leaf chamber. Match the two IRGAs from time to time (ideally before every measurement) by pressing "match". In practice, it was found that once matched, the two IRGAs are quite consistent over a period of (at least) 30 minutes.

34. To shut down the system, open up the leaf chamber lid. Then turn off the Li-Cor, the light, the fan, the silver box, the gas tanks and the water baths.

Note:

35. Max flow is ~770umol (air) /sec or about 1L /min.
36. In practice, it was found that it is hard to raise the leaf temperature above 38C – the plant is transpiring at a very fast rate.
37. In the steady heat treatment, the "warm" water bath is set at 45C so that leaf temperature can be held at 40C.
38. If room air is used, soda lime should be on full scrub when CO2 level is set.
39. All electronics will have a certain noise level. Therefore, decreasing the flow rate will increase data accuracy since this will give a larger difference between the reference and sample measurements. However, the flow rate should not be so low as to allow large diffusion into and out of the tubings (which would also cause the measurement to be inaccurate). The flow rate should also be large enough to ensure that sample air dew point is within the safe range, and that no condensation is occurring in the chamber or on the IRGA. A good number (provided by Dennis Gray) for the [CO2] difference (between sample and reference) is ~ 100umol. During heat treatment, this difference will decrease causing a decrease in accuracy of measurement – this needs to be taken into consideration as well.
40. It is okay to send in completely dry air into the system as the plant is sensing the air around them (i.e. the air within the chamber, or the "sample air") rather than the reference air. – Note on May 24, 2011, this is not very good for the plant though, it will want to close up stomata to avoid water loss.

Using LI-COR 6400 with Kramer spec (copper chamber)

Last modified July 6, 2011

41. Turn on the Li-Cor 6400 and let it warm up at the beginning of the day. For configuration settings select "2680".
42. Turn on water bath and set to desired temperature. Turn on the mini-cooler (condenser) and set to desired temperature (e.g. a good starting point is 15C).
43. Change the small CO2 cylinder as well as the O-ring.
44. If the Li-Cor head was previously attached to the copper chamber, detach it so that air is only going to the Li-Cor head.

45. Check to see if there is fresh dessicant and fresh soda lime. If not, change it.
46. Detach the Li-Cor input air from the humidifier.
47. Press "Calibration Menu".
48. Zero flow meter and record the number (before it zeroes) in notebook.
49. Zero IRGA: bypass the CO2 scrub and scrub H2O first and press "autoH2O" – this process takes about 20 minutes (to get all the water in the system out). This is when the number stops drifting. Then bypass the desiccant and scrub CO2 and press "autoCO2" – this takes about 5 minutes. Record numbers and press "Quit".

50. While the Li-Cor is zeroing, take a plant from the growth chambers and immerse the bottom of the cone-tainer in a small amount of water/nutrient solution in a beaker.
51. After the zeroes, re-attach the Li-Cor input to the humidifier. Reattach the copper chamber to the Li-Cor head.

52. Press "New Measurements". All the parameters are displayed on the console.
53. Press "Open log files" and name file. Type in the leaf area. Set flow (for Arabidopsis in Kramer spec 200 is a good number). Put soda lime (CO_2 scrub) on full scrub and set CO_2 level on the console (e.g. 400ppm).

54. Turn on Kramer spec, in the following sequence:
Laptop → Control box → Pulseblaster → General → Program on the laptop
This sequence must be strictly followed otherwise some parts could be burned.
55. Set light level on Kramer spec. To do this, click "set actinic" and type in the desired light code (note: this is not actual light level). If the light was previously on, clicking "set actinic" will turn the light off. A good saturating light level for Arabidopsis is 500uE (~90% of max. assim.), although some will say 800-1000uE. Raising up light level for an extended period of time is also hard on the electronics (mainly resistors) and the LEDs of Kramer spec.
56. Place the leaf in. First apply a thin film of vacuum grease on the glass on the LED side (right side as of now) and then stick the adaxial side of the leaf onto the glass. Make sure the leaf is covering the light path (or make a best effort). If significant amount of light leaks through to the detector it will be evident, the ΔECS value we obtain will be unusually low (for comparison, ΔECS of a wild type under 500uE light at 400ppm CO_2 is typically ~ 3.0×10^{-3}).
Next close the other part of the chamber. This is probably the most tricky part as there are a number of screws that adjusts the height and position of the detectors. Place the positional pin into the hole, then tighten the other two screws while holding the detector. The position and height of the detector can be locked in after the chamber screws are tightened (but make sure the detector is held with one hand when tightening the chamber screws), or before the chamber screws are tightened but in this case it might be harder to accurately position the chamber screws.

57. Look at the sample dew point (especially during a heat treatment). If dew point is too high, i.e. above room temperature, decrease the temperature of the condenser. If this doesn't work, we need to either increase the block temperature or increase the flow (which leads to reduced accuracy). If this still doesn't solve the problem, turn on the desiccant a little. Try to avoid using the desiccant if possible.
58. To take a measurement on LI-COR, press "log". Match the two IRGAs from time to time (ideally before every measurement) by pressing "match". If gas composition is changed, e.g. changed [CO_2] in an A-Ci curve, then the IRGAs must be matched before a measurement. In practice, it was found that if nothing changes, once matched, the two IRGAs are quite consistent over at least 30 minutes.
59. To take measurements automatically, setup "autolog".

60. To switch between the two water baths, use the six-way valve.
61. To load a script on Kramer spec, click the empty box on the lower left, or click "file" → "open script". Run a script by clicking "run script". A dialogue box will pop out prompting you to enter an address to save the file.

62. To transfer data from the Li-Cor to a computer, attach the serial-to-USB cable from the Li-Cor to a computer with LI6400XTerm or LI6400FileEx installed. Open up the LI6400FileEx program on computer. Enter file exchange mode on Li-Cor by pressing "Utility Menu" → "File Exchange Mode". Press "Connect" in the LI6400FileEx program, and once connection is established, drag

and drop the data files from under "users" folder on Li-Cor to any place on the computer. Press "Disconnect" when transfer is complete. Exit file exchange mode on Li-Cor.

63. To turn off the Li-Cor, go to the main menu, press "Welcome menu" → "IRGA off". Then turn off the power. Loosen the knobs on CO2 & H2O scrubs so that tubes on them do not get permanently crushed.
64. Shutting down the Kramer spec is exactly the reverse sequence as turning on. First turn off the actinic light by setting it to "0".Then turn off: Program on the laptop → General → Pulseblaster → Control box. The laptop can be turned off or left on overnight.
65. Turn off the water baths. Turn off the mini-cooler, unscrew the caps off the condenser and decant all the condensed water. Leave the condenser out to dry overnight.

Note:

66. If running high temperature experiment, you may want to set block temperature to 30C so no condensation occurs in the IRGA.
67. Max flow on LI-COR 6400 is ~770umol (air) /sec or about 1L /min.
68. In practice, it was found that it is hard to raise the leaf temperature above 38C – the plant is transpiring at a very fast rate.
69. All electronics will have a certain noise level. Therefore, decreasing the flow rate will increase data accuracy since this will give a larger difference between the reference and sample measurements. However, the flow rate should not be so low as to allow large diffusion into and out of the tubings (which would also cause the measurement to be inaccurate). The flow rate should also be large enough to ensure that sample air dew point is within the safe range, and that no condensation is occurring in the chamber or on the IRGA. During heat treatment, the sample dew point is likely to increase significantly, so take this into consideration as well.

Instructions for GFS-3000 (Walz)

Last modified July 15, 2011

This protocol is intended for use with PTR-TOF-MS during the MOMEVIP campaign, Munich, 2011-2012.

70. Turn both Walz and the computers on. Make sure the flow rate is set to 0, and then change the desiccants, the soda lime, and the CO2 cartridge.
71. Set the following parameters on Walz (or the GFS-Win program on the computer):

Flow	800
CO2	380 (chamber 1), 500 (chamber 2, 3, 4)
H2Omode	1 (absolute)
H2O SetValue	10000
Impeller	6
LightMode	PARtop
Light	1000 (for acclimation)
TempMode	Tleaf
Temp SetValue	30
Program name	MOMEVIP380 (chamber 1), MOMEVIP500 (chamber 2, 3, 4)

72. Create a file name for the leaf to be measured. For an example, see "2011_11_16 _file_naming_strategy.docx".
73. Do "Auto ZPirga" at the beginning of the day and when changing to a different [CO2] before acclimating a leaf. Make sure there is no leaf in the chamber, and then do a "Store ZPcuv".

74. In all other situations, simply do one "Store ZPcuv" before acclimating a leaf.
75. Take a plant from the growth chamber. If the growth condition is "well-watered", it would be desirable to place it in a small tray with water.
76. Place the leaf in cuvette and acclimate leaf for at least 30 minutes.
77. Set a stopwatch for 60 seconds. To start the measurements, press "Start Program". It will take several seconds before the clock actually starts to count down. When the clock starts to count down, immediately start timing on the stopwatch. Hand the stopwatch over to Werner. Werner will start the MS measurements after 60 seconds so that the two machines are synchronized. ([1] see notes section)
78. As one leaf is being measurement on Walz 1, another leaf can start to be acclimated on Walz 2. By the end of the 40 minute measurement on Walz 1, the leaf in Walz 2 would have been acclimated and ready to go. And then we start acclimating leaf in Walz 1 again (make a new file name), and so on and so forth.
79. Throughout the experiment keep an eye on the usage of desiccants and soda lime. Soda lime generally could last a whole day, but it is typical for the desiccant to run out in 3 – 4 runs, so desiccant may need to be changed twice or three times during the day. Set the flow rate to 0 before changing any of the chemical tubes. [2]

80. At the end of the day, leave the machines and computer on but set the following parameters on the Walz:

CO2	off
H2Omode	0
Impeller	0
Light	0
TempMode	0

The flow should be left on (at 800) overnight during the campaign even when we are not making measurements. This way the machines do not need to be warmed up in the morning.

Notes:

81. The current program lasts 41 minutes and includes: 1) 1 minute of blank time for synchronization of the Walz and PTR-MS; 2) 10 minutes of measurements of leaf under lighted conditions; 3) 20 minutes of measurements of leaf under dark conditions; and 4) 10 minutes of measurements of empty cuvette (blank for PTR-MS).
There may be a better way to synchronize the two machines, for example by synchronizing the two PC clocks to a time server and start both measurements at a real world time; or writing the program differently. If done correctly, this might save a lot of energy running back and forth with the stopwatch. We could talk with Werner about these options before the start of the campaign.

82. Used desiccant can be put back into the oven for regeneration.

83. For an example experiment design, see "2011_11_15_MOMEVIP_gasexchange_ original_experiment_design_ZL.xls".