

PROTOCOL: Estimating mesophyll conductance using combined gas exchange and chlorophyll fluorescence measurements

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Glossary of important symbols

Symbol	Column name in LI-COR spreadsheet	Description
C_A, C_R, C_S	NA, "CO2R", "CO2S"	CO ₂ concentration in the atmosphere, reference IRGA, and sample IRGA
W_A, W_R, W_S	NA, "H2OR", "H2OS"	H ₂ O concentration in the atmosphere, reference IRGA, and sample IRGA
$T_{Block}, T_{Air}, T_{Leaf}$	"TBlock", "TAir", "TLeaf"	Block, air, and leaf temperature
Φ_{CO_2}, Φ_{PSII}	"PhiCO2", "PhiPS2"	Quantum yield
J	"ETR"	Electron transport rate
S	"Area"	Leaf area in the chamber
k	"StmRat"	Stomatal ratio

Equipment & Maintenance

Equipment list

Note: I've used OPEN versions 6.x and 5.x, both of which will work and are very similar to operate. I have attempted to give detailed instructions, but I have assumed previous experience using the LI-COR 6400 with Fluorometer for more basic applications. You should also have ready access to the manual and supplies that came with their gas exchange system and leaf chamber.

1. LI-COR 6400 or 6400XT
2. LI-COR 6400-40 Leaf Chamber Fluorometer
3. Oxygen free gas (< 2% O₂, e.g. canister of N₂) for nonphotorespiratory measurements
4. Access to a drying oven or lyophilizer to prepare leaves for calculating diffusion leaks

Optional equipment

5. CO₂/H₂O Analyzer (e.g. LI-COR 640A)
6. Airtight tube with sponge to add water vapor to canister air

Optional software

7. R (available at <http://cran.r-project.org/>)
8. ImageJ (available at <http://rsbweb.nih.gov/ij/>)

Maintenance suggestions

The reader should consult the LI-COR Manual for maintenance issues, but there some items that will almost certainly need to be replaced during the course of a normal experiment and should be on hand prior to beginning measurements:

Chemical/Part	Suggested replacement frequency
Soda lime	1 per week (or when full scrub does not bring C_R below 10 $\mu\text{mol mol}^{-1}$)
Desiccant (e.g. Drierite)	1 per day(-week) depending on how much scrubbing you require. You can also purchase desiccants with indicators.
Foam gaskets on leaf chamber	Every 1-2 weeks. You can also check whether deformations in the gaskets caused by leaves are creating leaks by blowing air on them. If there is a major leak, C_S will go up more than 10 $\mu\text{mol mol}^{-1}$)
O-rings	Depends on the O-ring. The O-ring below the CO ₂ cartridge may need to be replaced daily to weekly.

A-C_i curve

The goal is to estimate the short-term (a few minutes) response of photosynthesis to user-defined changes in atmospheric CO₂ concentration (C_A). Gas exchange and chlorophyll fluorescence data will then be used in combination with assumed or estimated biochemical constants to calculate CO₂ concentrations inside the leaf airspace (C_i) and at the sites of carboxylation in the chloroplasts (C_C).

Note: Much of the information on calibration and environmental conditions can be found in the LI-COR manual. I will not repeat exact calibration instructions here, but rather emphasize the most salient points for this application. I have also provided a detailed description on selecting leaves for measurements and environmental conditions during measurements that will facilitate cross-study comparisons. However, the specific goals of your study may vary and the protocol may need to be adjusted accordingly.

Daily calibration suggestions

Allow 30-45 min to calibrate the following sensors daily:

1. Leaf temperature thermocouple
2. Flow meter zero
3. H₂O zero (w/ good chemicals)
4. CO₂ zero (w/ good chemicals)

Steps before starting measurements

1. After calibration, return to main menu and enter “New Measurements” mode.
2. (If you don’t have a separate CO₂/H₂O Analyzer) Once the IRGAs are warmed up, open the chamber and record C_A and W_A , which you can get from looking at “CO2S” and “H2OS” on the measurements screen. You will use these values later to recalculate your data after accounting for diffusion.
3. Turn on light to desired level (1500 $\mu\text{mol m}^2 \text{s}^{-1}$, 90% Red, 10% Blue, see section on ‘Irradiance’ below)
4. Turn CO₂ mixer on and set to ambient levels. I use 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$, but with climate change this number keeps going up!
5. Reduce Flow Rate to 300 $\mu\text{mol s}^{-1}$ (default is 500). Reduced flow rate increases the signal to noise ratio, but also increases time for diffusion in and out of the chamber.

6. Select a leaf (see Box 1 for ‘Guidelines for selecting a leaf’), position it in the chamber, and close the chamber. Adjust knob on the chamber handle to ensure there is a tight seal around the leaf to prevent leaks.
7. Allow 20-60 minutes for leaf to reach equilibrium (constant photosynthesis and stomatal conductance). Frequently, both values may decline at first and then return to or exceed their initial values. While you are waiting, you can adjust the environmental conditions inside the chamber (see next section).

Box 1: Guidelines for selecting a leaf

Note: These steps may be done while you are waiting for the IRGA to zero (see above) or while chamber to equilibrates to starting environmental conditions (see below).

Plant selection: Plant should be photosynthetically active, meaning at least 30 minutes in full sun. Mid-morning to late afternoon is usually ideal, although depending on the weather and physiology of your species, this may vary.

Leaf selection: Select youngest, fully-expanded, sun-exposed leaf. Partially-expanded, old, and shade leaves typically have reduced photosynthetic capacity and should be avoided unless that is what you want to measure.

Placement within the leaf: Ideally, select a medial portion of the lamina that fills the entire chamber, avoiding a midvein that may increases leaks.

Note: For narrow or deeply lobed leaves, you will need to mark EXACTLY what portions of the leaf were in the chamber and measure the area using a camera/scanner and image processing software such as ImageJ. You will also need to recalculate your data with the measured area (see ‘Data Processing’ section below).

Suggested environmental conditions in the leaf chamber

The LI-COR 6400 allows you to control the irradiance, temperature, and humidity inside the leaf chamber. These factors will directly and indirectly, via physiological responses, affect the photosynthetic rate. In this section, I suggest ‘standard’ physiological conditions and ‘acceptable’ ranges. I also provide tips and precautions on how to attain desired values initially and keep them relatively constant during an experiment. These methods are based on my experience with my system (*Solanum* sect. *Lycopersicon*) and may therefore not match your experience.

1. Irradiance: The chamber irradiance should be such that light is saturating, usually 1000-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. I use 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but you can make some preliminary Light Response Curves (see LI-COR manual for details) with your species to get a better idea. When prompted, select 90% Red and 10% Blue light.

2. Temperature: Leaf temperature should stay at $25^{\circ} \pm 1^{\circ} \text{C}$. See the Box 2 below on ‘Controlling Leaf Temperature’ for a detailed guide.
3. Humidity: $50 \pm 10\%$ relative humidity is a reasonable target for many species. $>80\%$ may cause condensation, while $<30\%$ will cause many species to close their stomata.

Box 2: Controlling Leaf Temperature

You can specify a desired T_{Block} , T_{Air} , or T_{Leaf} . If you set the T_{Air} or T_{Leaf} , the LI-COR will automatically adjust T_{Block} to maintain a constant T_{Air} or T_{Leaf} . Ideally, you could set T_{Leaf} to 25°C , but I find that this causes large swings in temperature during the course of a measurement leading to more error. Instead, I set T_{Block} to a value that gives me my desired T_{Leaf} . This is much more steady, but requires some trial and error initially to get the right T_{Block} as well as small adjustments (0.5°C) during the experiment. For $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance and normal stomatal conductance ($\sim 0.2 \text{ mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), $T_{Block} = 23\text{-}24^{\circ} \text{C}$ will work. The thing to keep in mind is that all else equal, increased irradiance will increase leaf temperature and increased stomatal conductance will decrease leaf temperature. If your plant is stressed or has intrinsically low stomatal conductance, then you may need a lower T_{Block} . Likewise, you may need to adjust T_{Block} downward at high CO_2 concentrations that cause the stomata to close.

Box 3: Choosing the correct humidity

There are three main factors to consider in choosing the humidity inside the leaf chamber:

1. The aridity tolerance of your species. Species from humid environments may not be able keep up with the evaporative demands imposed by dry air and will respond by closing their stomata.
2. Growing conditions. A large change in humidity from where the plants are growing to the leaf chamber may cause stress or at the very least prolong the time needed for the plant to equilibrate to conditions inside the leaf chamber, slowing down your experiment.
3. The humidity gradient between the chamber and ambient air. For A- C_i curves, the primary concern is large CO_2 concentration gradients ($\Delta C = C_A - C_S$) that arise at very low and high C_S . However, especially when the ambient air is quite dry, there may be a large water vapor gradient ($\Delta W = W_A - W_S$) as well. To determine this, check W_S (“H2OS”) with an active leaf inside the chamber and again with the chamber empty and open to the atmosphere. If there is a large gradient ($\Delta W > 5 \text{ mmol H}_2\text{O mol}^{-1}$), then you may consider adjusting the chamber humidity to reduce it. If you are unwilling or unable for other reasons to change the chamber humidity, then you will need to apply a correction to your data to account for both CO_2 and H_2O diffusion (see ‘Calculating CO_2 and H_2O diffusion coefficients’ below).

Box 4: Controlling leaf chamber humidity

The leaf chamber humidity is somewhat dynamic, changing throughout the day depending on the weather, leaf transpiration, and saturation level of your desiccant if you are scrubbing at all. You can control the humidity to keep it within the target range, but proceed gradually as **it's very easy to overcorrect**, causing you to waste hours on lousy measurements.

If it's too humid:

Move the desiccant from 'Bypass' to 'Scrub' VERY GRADUALLY. Give it a quarter or half turn and allow 1-2 min for the humidity to equilibrate. If it's still too humid, repeat small turns until it reaches desired relative humidity.

If it's too arid:

You can increase the humidity in the chamber by wetting the soda lime, but be VERY CAREFUL. If you wet the soda lime too much, it'll take hours to dry out again or you will have to replace it with fresh soda lime. Add ~10 DROPS of water to the soda lime, shake, and return it to its position. You will then need to **wait 30-60 minutes** for the humidity to fully stabilize and begin new measurements (you can place a leaf in the chamber to get it prepared while this is happening). It may appear after a few minutes that the humidity has stabilized, but in my experience, it's best to be patient (go get coffee or something) because data that look reasonable may actually turn out to be unusable after processing.

Taking measurements

Once the leaf has reached steady-state photosynthesis and you are happy with the environmental conditions inside the chamber, open a file with a descriptive name.

1. From the list of the Auto Programs, select "Flr A-C_i Curve". Next, you will need to provide information for the program to run. In the table below, I have given the responses to the prompts. For the desired CO₂ concentrations, I have provided a **minimum** set given the number of parameters that need to be estimated. If you have additional time, you may want to include additional CO₂ concentrations at smaller intervals, keeping in mind that each additional value probably adds ~5 minutes to the total time.

Prompt	Enter	Discussion
Dark adapt before starting (Y/N)	N	
Fo Value		skip
Fm Value		skip
Dark Photo Rate	-1	Dark Photo Rate is just the respiration rate, but opposite sign. I leave default and recalculate with estimated R_d later.
Measure Fo' with Fs & Fm' (Y/N)	N	
Turn on Flr Recording (Y/N)	N	
Turn off Flr Recording when done (Y/N)	N	
Save each flash (Y/N)?	N	
Desired Ca values ($\mu\text{mol/mol}$)	{400, 0, 50, 100, 150, 200, 300, 400, 600, 900, 1400, 2000, 400}	
Minimum wait time (min)	2	
Maximum wait time (min)	5	
Match if $ \Delta\text{CO}_2 $ less than (ppm):	10000	Always match
Stability Definition OK? (Y/N)	Y	Use default

2. While program is running, occasionally monitor progress to ensure that the environmental conditions stay within acceptable ranges.
3. Download data and move onto 'Data processing' (see below) once other gas exchange measurements are complete.

Additional gas exchange measurements

Calculating CO₂ and H₂O diffusion coefficients

Note: Depending on its structure, the diffusion coefficient may differ from leaf to leaf and you may need to estimate separate coefficients for different species, across treatments, etc.

Using a dried or lyophilized (freeze-dried) leaf, you will estimate the diffusion coefficient of the foam gaskets of the leaf chamber. Refer to the LI-COR 6400 manual and Rodeghiero *et al.* (2007) for additional detail. You will need some method to measure C_A and W_A . Ideally, you could use a separate CO₂/H₂O analyzer (e.g. LICOR 640A) simultaneously with the LI-COR 6400. However, if you work in a location with pretty constant CO₂ and humidity, just open the chamber and use the LI-COR 6400 IRGA to measure C_A and W_A .

1. Start-up and calibrate LI-COR as normal
2. Open chamber and record C_A and W_A from “CO2S” and “H2OS” on the measurements page
3. Place dried or lyophilized leaf in the chamber and close
4. Set CO₂ Mixer to 0 or 2000 $\mu\text{mol mol}^{-1}$ to create a large gradient (ΔC)
5. If you are measuring H₂O diffusion coefficient, you will also need to create large gradient (ΔW) by putting the desiccant to scrub
6. Set Flow Rate to 500 $\mu\text{mol s}^{-1}$ (default)
7. Once equilibrium has been reached (monitor “ ΔCO_2 ” and “ $\Delta\text{H}_2\text{O}$ ” on the measurement or graph pages), Match the IRGAs and log your data.
8. Repeat steps 6 and 7 for Flow Rate = {250, 125, 100, 75, 60, 50}

Note: At low flow rates it will take a LONG time to reach equilibrium (10 – 30 min), so be patient.

9. Download data. There is no need to recalculate anything.
10. Using your favorite statistical software, perform a linear regression to estimate diffusion coefficients (see worked example in **Box 5**). In pseudo-R code:

```
> fit <- lm(I((C02S - C02R) / (Ca - C02S)) ~ I(1 / Flow),
  data = my.licor.data)
> coef(fit)
  (Intercept)      PhiC02
-0.0002285      0.2836838
```

Box 5: Worked example of calculating CO₂ and H₂O diffusion coefficients

Data:

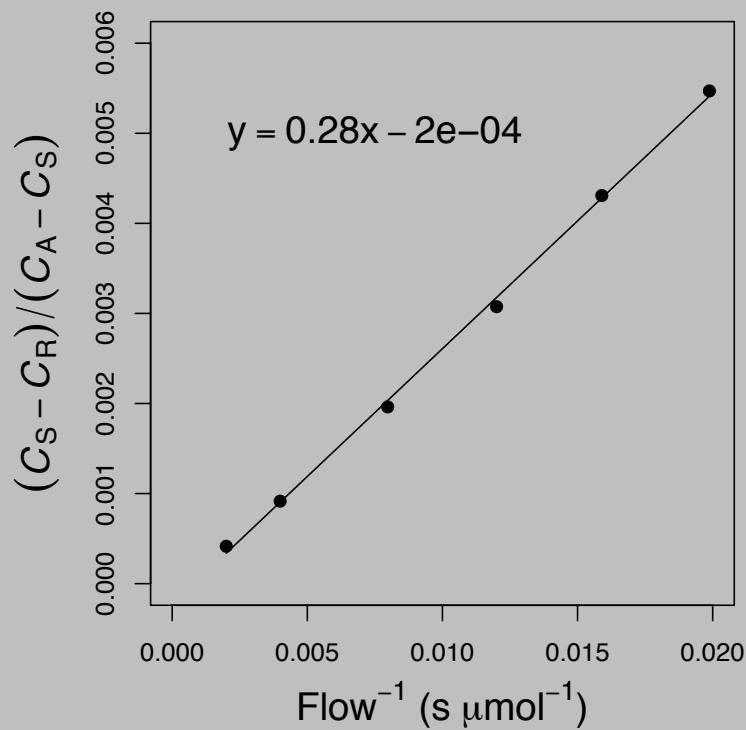
Cr	Cs	Wr	Ws	Flow ⁻¹
8.67	9.06	6.130	6.210	0.001999600
8.44	9.30	5.874	5.994	0.003995206
8.28	10.12	5.503	5.742	0.007974482
8.36	11.24	5.633	5.944	0.012004802
8.56	12.59	5.928	6.323	0.015898251
8.68	13.79	6.102	6.575	0.019880716

Equations:

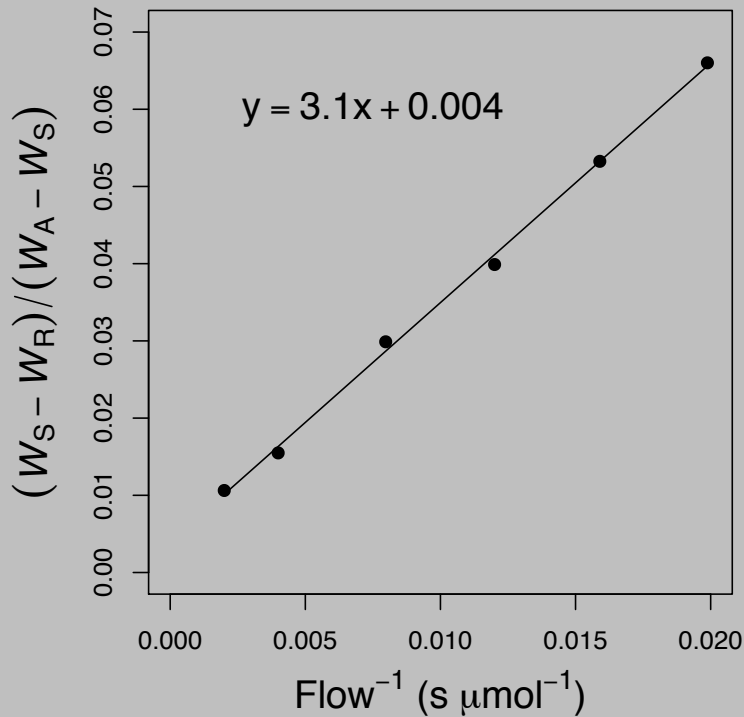
$$\frac{(C_S - C_R)}{(C_A - C_S)} = \frac{K_{\text{CO}_2}}{\text{Flow}}$$

$$\frac{(W_S - W_R)}{(W_A - W_S)} = \frac{K_{\text{H}_2\text{O}}}{\text{Flow}}$$

K_{CO_2} and $K_{\text{H}_2\text{O}}$ can be estimated using linear regression:



Box 5 (continued)



After estimating K_{CO_2} , you can estimate the actual photosynthetic rate from the apparent photosynthetic rate in your data. The equation is:

$$\text{Actual Photosynthesis} = \text{Apparent Photosynthesis} + \frac{K_{CO_2}(C_A - C_S)}{100S}$$

where S is in units of cm^2 . In the table below, I've recalculated simulated data using the estimated diffusion coefficient.

Parameters	Apparent Photosynthesis (mmol CO ₂ m ⁻² s ⁻¹)	C_S (μmol CO ₂ mol ⁻¹)	Actual Photosynthesis (mmol CO ₂ m ⁻² s ⁻¹)
$K_{CO_2} = 0.28$	-3	0	-2.44
$C_A = 400$	20	400	20
$S = 2 \text{ cm}^2$	30	1000	29.16

K_{H_2O} affects the estimate of C_i and other parameters that are calculated from C_i . Accounting for K_{H_2O} is slightly more involved than K_{CO_2} , but it can be done using equations from Rodeghiero *et al.* (2007).

$\Phi_{\text{CO}_2} - \Phi_{\text{PSII}}$ curve under nonphotorespiratory conditions

Note: Depending on the species or condition of the leaf, $\alpha\beta$ (see definition below) may differ from leaf to leaf and you may need to estimate separate coefficients for different species, across treatments, etc.

See Chapter 27 of LI-COR 6400 Manual Version 6 for additional details. The quantum yield Φ (the efficiency of energy harvesting) can be measured in multiple ways (“PhiPS2” and “PhiCO2” in the LI-COR spreadsheet). Under nonphotorespiratory conditions:

$$\Phi_{\text{PSII}} = \frac{\alpha\beta\Phi_{\text{CO}_2}}{4}$$

For the present purposes, it’s not important where this equation comes from. The goal is to use this relationship to estimate the product $\alpha\beta$ (the product of leaf absorptance and the photosystem partitioning factor; see ‘Data Processing’ below). This value is important for correctly estimating the electron transport rate (J or “ETR”) from your A-C_i data.

1. Set up and calibrate LI-COR as in the A-C_i curve.
2. Connect a canister of deoxygenated air (e.g. N₂) to the air input. It’s convenient to fork the tube, with one end going to the LI-COR and the other end free so that you can monitor the flow of gas from the canister. Too much flow wastes gas, too little flow will disrupt the experiment and overwork the air pump on the LI-COR.
3. OPTIONAL: If canister air is too arid, you may want to pass it through an airtight bottle with a wet sponge to add water vapor.
4. Change “OxyPct” to that of your canister air (usually 1-2%)
5. Turn on light to 1500 $\mu\text{mol m}^2 \text{s}^{-1}$, 90% Red, 10% Blue
6. Turn CO₂ mixer on and set to ambient levels (400 $\mu\text{mol CO}_2 \text{mol}^{-1}$ or whatever you used in your experiment)
7. Adjust other environmental variables (temperature, humidity, etc.) to levels you used during A-C_i curves.
8. Select a leaf following the same guidelines as before, position it in the chamber, and close the chamber. Adjust knob on the chamber handle to ensure there is a tight seal around the leaf to prevent leaks.
9. Allow 20-30 minutes for leaf to reach equilibrium (roughly constant photosynthesis and stomatal conductance). It’s not critical that photosynthesis completely plateau, so you can usually allow a shorter waiting time.

10. Select “Flr Light Curve” from AutoProgram. The table below shows what to enter in the prompts

Prompt	Enter	Discussion
Dark adapt before starting (Y/N)	N	
Fo Value		skip
Fm Value		skip
Dark Photo Rate	-1	Whatever you used during A-Ci curves. Dark Photo Rate is just the respiration rate, but opposite sign
Measure Fo' with Fs & Fm' (Y/N)	N	
Turn on Flr Recording (Y/N)	N	
Turn off Flr Recording when done (Y/N)	N	
Save each flash (Y/N)?	N	
Actinic Control:	Y	If the current target is not “PQntm, 10% blue”, then press N and change it.
Desired lamp settings ($\mu\text{mol}/\text{m}^2/\text{s}$)	{1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300}	
Minimum wait time (min)	1	
Maximum wait time (min)	2	
Match if $ \Delta\text{CO}_2 $ less than (ppm):	10000	Always match
Stability Definition OK? (Y/N)	Y	Use default

11. While program is running, occasionally monitor progress to ensure that the environmental conditions stay within acceptable ranges. You should also check that enough air is flowing from the canister. If flow drops too low, you will need to increase it, but this will cause a brief spike, and any data logged during this period needs to be discarded.

12. Download data. Since $C_A \cong C_S$, diffusion is negligible and you shouldn't need to recalculate any of the data unless the leaf area inside the chamber was not 2 cm^2 .

13. Using your favorite statistical software, perform a linear regression of Φ_{CO_2} on Φ_{PSII} and extract the slope (see worked example below). In pseudo-R code:

```
> fit <- lm(PhiPS2 ~ PhiCO2, data = my.licor.data)
> coef(fit)
(Intercept)      PhiCO2
-0.006931896  8.360126720
```

The slope should be $\sim 8 - 12$ (but possibly more) and the intercept should be very close to 0. If the intercept is not close to 0 or the curve is nonlinear, this probably means that

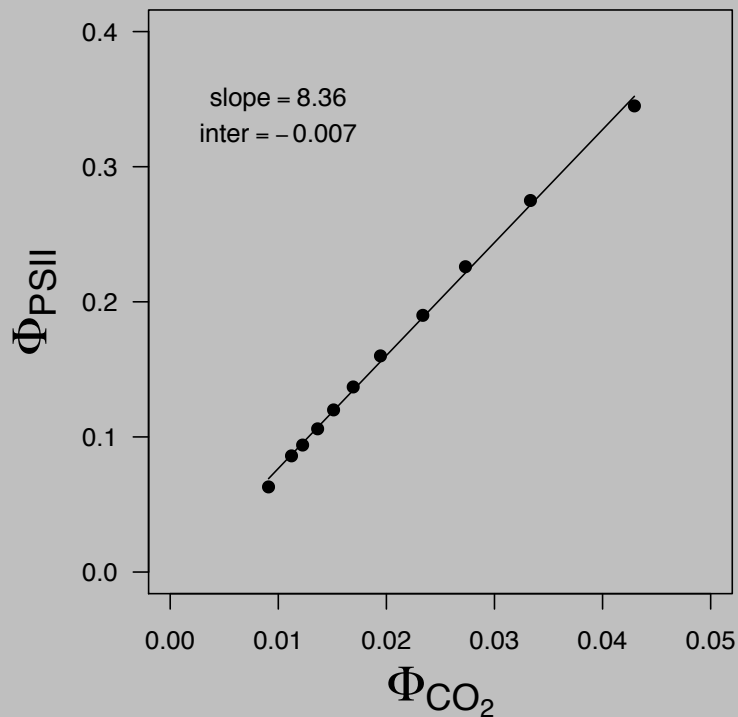
oxygen is getting inside the chamber or that you need to change the irradiance levels (I've found that the curve is not linear at very high and low irradiance).

Box 6: Worked example calculating $\alpha\beta$ from Φ_{CO_2} – Φ_{PSII} curve

Data:

PhiPS2	PhiCO2	PARi
0.086	0.011	1199
0.094	0.012	1099
0.106	0.014	1001
0.120	0.015	899
0.137	0.017	798
0.160	0.019	698
0.190	0.023	599
0.226	0.027	501
0.275	0.033	402
0.345	0.043	300

PhiCO2 vs. PhiPS2 with best fit regression line:



Box 6 (continued)

We can now estimate $\alpha\beta$:

$$\alpha\beta = \frac{4}{\text{slope}} = \frac{4}{8.36} = 0.48$$

To recalculate the electron transport rate (“ETR” or J) from your A-C_i curve data, use the following equation:

$$J = \frac{4(\text{PhiPS2} - \text{intercept})}{\text{slope}} * \text{PAR}_i$$

Predawn respiration

Note: Respiration needs to be measured in the dark. Since measurements take only a couple minutes per plant, you may be able to measure every plant in your experiment in a single morning.

1. Start-up and calibrate LI-COR as normal
2. Set CO₂ Mixer to ambient levels (400 $\mu\text{mol mol}^{-1}$)
3. Desiccant should be on full bypass and light needs to be off.
4. Open a new file. If you are measuring multiple plants you should enter a comment or otherwise keep track of which line in the datasheet corresponds to which leaf.
5. Select a leaf similar to what you used for A-C_i curves, position it in the chamber, and close the chamber. Adjust knob on the chamber handle to ensure there is a tight seal around the leaf to prevent leaks.
6. Allow 1-3 minutes for leaf to reach equilibrium (roughly constant photosynthesis and stomatal conductance).
7. Log data and move onto next leaf.

R_d is the same value, but opposite sign of “Photo” in the datasheet, so it should be > 0 .

Data Processing

Recalculating data

Once you have finished your A-C_i curves and other gas exchange measurements, you will need to recalculate the data before estimating g_m (see below). Recalculating data can be performed using the LI-COR software, Excel, and many other programs. I have written R functions to recalculate data that I plan to develop into a small package at some point. In the meantime, a preliminary version is available from me by request.

Overview of parameters you will need to recalculate data:

Symbol	Description	Assumed Value	How to Estimate	Importance
<i>Recommended</i>				
$\alpha\beta$	Product of leaf absorptance (α) and photosystem partitioning factor (β)	$\alpha = 0.85$ $\beta = 0.5$	Slope of $\Phi_{\text{CO}_2} - \Phi_{\text{PSII}}$ curve under nonphotorespiratory conditions	High
K_{CO_2}	Diffusion coefficient of CO ₂	0	Flow rate curve with dried or lyophilized leaf	High
$K_{\text{H}_2\text{O}}$	Diffusion coefficient of water vapor	0	Flow rate curve with dried or lyophilized leaf	Low - Medium
R_d	Mitochondrial respiration	1	Predawn gas exchange measurement	Medium
<i>Optional or as necessary</i>				
S	Leaf area	2	Scanner or digital camera	High, if necessary
k	Stomatal ratio	1	Nail polish peels	Low

Sources for relevant equations:

$\alpha\beta$: **Box 6**

Diffusion leaks: **Box 5** and Rodeghiero *et al.* (2007).

All other equations can be found in the LI-COR manual.

Estimating the mesophyll conductance (g_m)

Following Harley *et al.* (1992), g_m can be calculated as:

$$g_m = \frac{A}{C_i - \frac{\Gamma^*[J + 8(A + R_d)]}{J - 4(A + R_d)}}$$

You will either need to estimate the CO₂ compensation point (Γ^*) or use a value from the literature (e.g. Sharkey *et al.* 2007). You can now calculate C_c , the carbon dioxide concentration in the chloroplasts, to construct the A- C_c curve, from which various biochemical parameters (e.g. $V_{c,max}$) and limitations (diffusional vs. biochemical) of photosynthesis can be estimated.

References

- Harley, P.C., F. Loreto, G. Di Marco, and T.D. Sharkey. 1992. Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. *Plant Physiology* 98: 1429-1436.
- Rodeghiero, M., Ü. Niinemets, and A. Cescatti. 2007. Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimate of Farquhar *et al.* model parameters? *Plant, Cell and Environment* 30: 1006-1022.
- Sharkey, T.D., C.J. Bernacchi, G.D. Farquhar, and E.L. Singaas. 2007. Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant, Cell and Environment* 30: 1035-1040.