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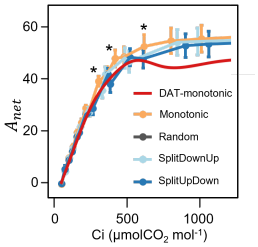
A/Ci method using Dynamic Assimilation Technique



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Mauricio Tejera-Nieves[1], Luke M. Gregory[1,2], Aaron Saathoff[3], Berkley J. Walker[1,2]

[1]MSU-DOE Plant Research Laboratory; [2]Plant Biology Department, Michigan State University; [3]Veterinary Medicine Research and Development, Zoetis Inc.

Mauricio Tejera-Nieves
MSU-DOE Plant Research Laboratory

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*Corresponding author: berkley@msu.edu

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We use this protocol and it's working

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Abstract

The net CO_2 assimilation (A) response to intercellular CO_2concentration (C_i) is a fundamental measurement in photosynthesis and plant physiology research. Here, we optimized and compile a non-steady state Dynamic Assimilation Technique (DAT) protocol for A/Ci measurements. We tested this protocol in Tobacco (*Nicotina tabacum*), arabidopsis (*Arabidopsis thaliana*; Col-0), soybean (*Glycine max* 'AG21XF1', Asgrow, New Haven, CT, USA), potato (*Solanum tuberosum*; 'PA01N32-1'), apple (*Malus domestica* 'Jonagold Decoster PP#80' on 'EMLA 106' rootstock) and the extremophile plant *Rhyza Stricta,* and compare the parameter estimations against conventional steady state A/Ci measurements. The DAT protocol reduced the measurement time by almost half without compromising estimations accuracy or precision. Estimations of biochemical limitations to photosynthesis were very consistent across all CO_2 protocols, with slight differences in ribulose 1·5- bisphosphate carboxylase/oxygenase carboxylation limitation.

Materials

The A/Ci measurement method using the dynamic assimilation technique requires a LI-6800 (Li-cor BioSciences, Nebraska, USA) equipped with either the Small Light Source (6800-02) or Multiphase Flash Fluorometer (6800-01A) chamber. In case of using the flash fluorometer chamber, all fluorescence features must be turned off.

Equipment	
LI-6800	NAME
Portable Photosynthesis System	TYPE
LI-COR BioSciences	BRAND
LI-6860	SKU
https://www.licor.com/env/products/photosynthesis/LI-6800/?pk_campaign=18941898409&pk_kwd=licor%20co2&pk_source=google&pk_medium=cpc&pk_content=635270912316&gad_source=1&gclid=CjwKCAiA75itBhA6EiwAkho9e9fB2v2ddF937QTG-ipuETzk3NJhDlW7ULWEwp3o7paLJjeojlIPJRo	LINK

Equipment	
Small Light Source (6800-02)	NAME
LI-6800 chamber head and light source	TYPE
LICOR BIOSCIENCES	BRAND
LI-6850	SKU
https://www.licor.com/env/products/photosynthesis/LI-6800/small-light-source	LINK

Equipment

Multiphase Flash Fluorometer (6800-01A)	NAME
LI-6800 chamber head, light source and flash fluorometer	TYPE
LI-COR BioSciences	BRAND
6800-01A	SKU
https://www.licor.com/env/products/photosynthesis/LI-6800/fluorometer	LINK

Prepare LI-6800 for measurements

1 **Check instrument chemicals and run warm-up test**

1.1 Follow LICOR **Start up checklist** before clamping the LI-6800 into the first leaf.

1.2 Run **warm-up test** on an empty and closed chamber
Start Up > Warmup Tests > Select Warmup Test from dropdown menu > Start

30m

Prepare LI-6800 for Dynamic measurements

2 **Enable Dynamic Computations:** Start Up > Chamber Setup > Check 'Add Dynamic Equations'

3 **Disable Fluorometer functions:** Start Up> Chamber Setup > under 'Fluorometer function as' check 'Light Source only'

4 The dynamic assimilation technique relies on a continuous ramping of the CO_2 concentration during the A/Ci measurement. To avoid estimation errors associated with apparent differences in the leaf sample and reference measurement channels, two main steps must be considered. Both must be performed with an empty chamber.

4.1 **Acquire CO_2 and H_2O range match**.

15m

Constants > Range Match > select 'CO_2 Range Match' from dropdown menu > Start > Tap 'Continue' on the pop-up window. Repeat and select 'H_2O Range Match'.

If Range Match does not appear in the Constant Tab, double check 'Add Dynamic Equations' under Chamber Setup in the Start Up tab is checked.
Look into **Acquiring range match data** in the Licor Operating Instructions website for further details.
When acquiring CO_2 and H_2O range match data, it is crucial for the consistency of the measurements that ramp up values overlap reasonably close with the ramp down values. If not the Flow_s/Flow_r parameter to needs to be adjusted to compensate; Flow_s/Flow_r should be lowered if the "coming back" (Ramp down; ∇ symbols) values are higher than the "going up" (Ramp up; Δ symbols) values, and vice-versa. Usually small changes in the Flow_s/Flow_r parameter lead to larger changes in the trajectory of the ramp up and down values, we suggest using +/- 0.02 increments.

CO_2 and H_2O range matches should be done at least once before collecting new data for CO_2 response curves. If measurements are collected where there are large changes in ambient air temperature, both range matches should be done every 2 - 3 hours, or at least once in the morning and once in the afternoon.

4.2 **Run CO_2 dynamic tuning** 5m

Constants > Dynamic > Utilities/Test (bottom right corner of the screen) > Select 'CO2 Test Current' from dropdown menu > Start > Tap 'Continue' on the pop-up window
Look into **The dynamic tests** in the Licor Operating Instructions website for further details.
Upon completion of the test, the user looks for minimum variation in A_{dyn}, and no correlation with ramp direction.
'Before' and 'After' statistical summary appears on screen next to the graph. If the standard deviation of the "After" is lower than the "Before" stats, the user should add this result by tapping the 'Yes' button. Otherwise, the user may tap 'No' and discard this

Clamp onto your first leaf

5 Set environmental conditions in the leaf chamber to mimic the ambient conditions the leaf was experiencing at the time of measurement. Please follow LICOR **Clamping onto your first leaf** instructions for a complete description.

6 **Open log file:** Log Setup > Open a Log File > Select folder > New File > Type file name > Done

Run Dynamic Assimilation CO2 Response Curve

7 Once measurements are stable over 2 - 4 minutes, start the Dynamic Assimilation CO_2 Response Curve.
Open Dynamic Assimilation Background Program : Programs > BP builder > In the Dynamic folder select 'DAT_CO2_Continuous.py' > Start BP

8 **Set Program Options.** When the Background Program is started a dialog box opens with the following options:

1. *Starting CO2*: Initial CO_2 concentration of the ramping. We recommend using a Low-to-High protocol to avoid oscillations in assimilation. Suggested value: 50 µmol mol^{-1}
2. *Pre ramp wait*: Length of equilibration period at the initial CO_2 concentration before the ramping starts. Suggested value: 3 min
3. *Ending CO2*: Final CO_2 concentration of the ramping. Suggested value 1500 - 2000 µmol mol^{-1}

4. *Ramp rate:* Sets the rate at which CO_2 changes in the leaf chamber during the program. It affects how long it takes to complete the response curve. Suggested value 100 - 200 µmol mol^{-1} min^{-1} (Reduce rate if assimilation oscillates after it has passed the initial linear portion)
5. *Logging interval*: Frequency of data collection during the protocol. Suggested value = 1
6. *When done, go to*: Final CO_2 concentration after the protocol has been completed. Suggested value: 420 µmol m^{-2} s^{-1}

Tap 'Continue' bottom to start the Dynamic Assimilation CO2 Response Curve.

Close log file

9 Upon completion of last Dynamic Assimilation CO_2 Response Curve, close log file:
Log Setup > Logging to *Filename* > Close Log

Follow LICOR **Transferring files to a computer** for instructions on how to download data from the instrument.

Additional Notes

10 **Range CO_2 and H_2O matching:**

- The Acquire program has 'Normal' and 'Quick' modes. The 'Quick' mode runs at a faster rate, and tends to exaggerate the difference between ramping up and down values. If the Flow_s/Flow_r parameter is optimized using the 'Quick' mode, the overlap will be even better using the 'Normal' mode.
- The overlap for the "going up" and "coming back" values do not need to be perfect, but they should be reasonably close together. Please refer to Figure 1 for a good overlapping example.

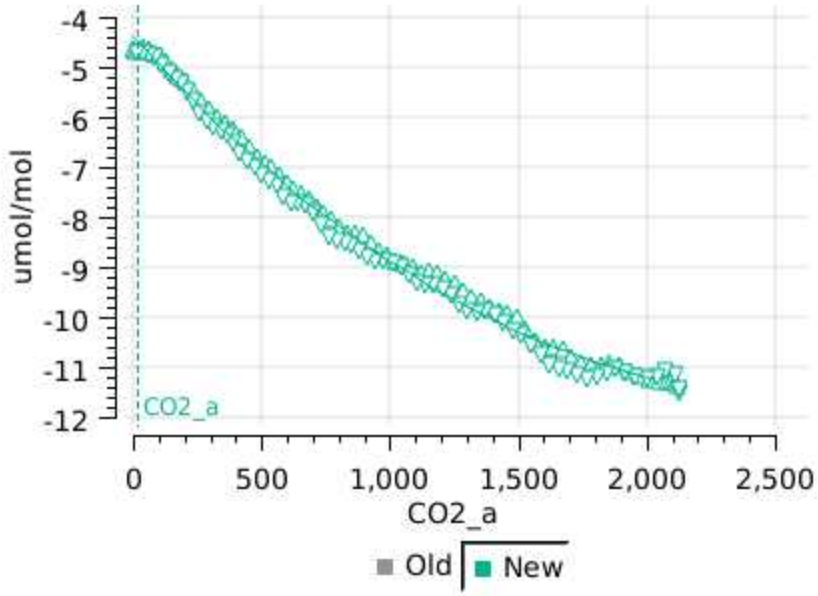

Figure 1: Response of the difference between CO_2 concentration measured in the sample and reference channel (y-axis) to increasing (Δ symbols) and decreasing (∇ symbols) CO_2 concentrations (x-axis). Users looks for changes in increasing and decreasing concentrations to be as overlapped as possible.

11 **Dynamic Assimilation Background Program considerations:**

- Suggested values may vary from plant to plant and in different environments, we recommend running a couple of test runs to optimize the program options.
- The *Starting CO_2* could be as low as 5 µmol mol^{-1}, avoid 0 µmol mol^{-1}
- Extend *Pre ramp wait* if assimilation shows a initial decrease after ramping has started
- Use a 1-second averaging window when logging data at 1 Hz as the higher data density helped overcome the additional noise. Log Setup > Logging Options > Check 'Use additional averaging time' > enter '1' on the box to the right
- *Logging interval* could be increased to 5 (one observation every 5 seconds) if data look noisy. Alternatively, moving averages or filtering could be implemented after the measurement.
- Use ambient or growing CO_2 concentration for *When done, go to*
- Matching options are ignored while running a Dynamic Assimilation CO2 Response Curve.

12 **Steady State:**

For reliable data it is crucial the leaf biology is at, or very near, steady-state conditions in the leaf chamber before initiating measurements. Usually leaves have reached steady-state once, over a span of 2 - 4 minutes, net CO_2 shows no positive or negative trend and oscillations are within 2 - 5% of the mean.

13 **Environmental Conditions:**

Light: If ambient and chamber light levels are not adequately matched, a period of time to allow for light adaption will be needed (especially in cases where light levels are higher in the chamber than preceding ambient conditions).
Water vapor & temperature: In some cases, using a set VPD value could occasionally introduce artifacts during the CO_2 ramp. If this is the case, set reference H_2O to a level that resulted in 45-50% relative humidity in an empty chamber; leaf transpiration would add enough additional water vapor so that leaf VPD remained fairly stable throughout the DAT curve.