Gas Exchange
Instrument theory

How the LI-6800 works and what it can tell you

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How do we measure photosynthesis?

- Photosynthesis
- Gas exchange
- Assimilation
How do we measure photochemistry?

- Chlorophyll Fluorescence
- Gas exchange

Diagram showing the chloroplast stroma and thylakoid lumen with key processes such as carboxylation, regeneration, and reduction.
How do we measure gas exchange?

- First we need to measure the concentration of one or more gases

\[ A'_{(v)} = 1 - e^{-k(v)wl} \]

- \( w \) = density of the absorber
- \( l \) = path length
- \( \nu \) = wavelength
- \( A' \) = light absorption
- \( k(\nu) \) = absorption coefficient
How do we measure gas exchange?

- **Infrared Absorption by Gases**

\[
\alpha + T = 1
\]

\[
\alpha = 1 - T
\]

\[
\alpha = 1 - \frac{\Phi_A}{\Phi_{NA}}
\]

- $\alpha$ = absorptance
- $T$ = transmittance
- $\Phi_A$ = transmittance in absorbing region
- $\Phi_{NA}$ = transmittance in non-absorbing region

**Infrared Absorption Peaks:**
- $H_2O(\text{NA}2.4\ \mu m)$
- $H_2O(A2.59\ \mu m)$
- $CO_2(\text{NA}4.1\ \mu m)$
- $CO_2(A4.26\ \mu m)$
Basic optical differential IRGA

\[ \alpha = 1 - \frac{\Phi_A}{\Phi_{NA}} \]

\(\alpha\) = absorptance
\(\Phi_A\) = transmittance in absorbing band
\(\Phi_{NA}\) = transmittance in non-absorbing band
Gas exchange systems

- Closed-transient
- Compensating
Gas exchange systems

- Closed-transient
- Compensating
- Open flow through
System flow path

Traditional open system

LI-6400/XT

LI-6800
Basic parameters *computed*

- **Fluxes from mass balance**
  - $A$ – Assimilation rate
  - $E$ – Apparent Transpiration rate

- **Additional parameters**
  - $g_{sw}$ – Stomatal conductance to water vapor
  - $C_i$ – Inter-cellular CO$_2$ concentration
Mass balance in an open system

- **Air flow into the chamber**
- **Transpiration (apparent)**
- **Assimilation**
- **Leaf chamber**
Mass balance for gas exchange

\[ s f_x = u_{out} x_{out} - u_{in} x_{in} \]

- \( s \): Leaf surface area
- \( f_x \): Flux of gas \( x \)
- \( u_{in} \): Molar air flow entering the chamber
- \( u_{out} \): Molar air flow exiting the chamber
- \( x_{in} \): Mole fraction of \( x \) entering chamber
- \( x_{out} \): Mole fraction of \( x \) exiting the chamber

A stable and constant flow of air is supplied to the chamber. Exhaust air is vented to the atmosphere.

Steady-state gas exchange model
Mass balance for gas exchange

\[ sf_x = u_{out}x_{out} - u_{in}x_{in} \]  Steady-state gas model

\[ sf_x = u_{out}x_{out} - u_{in}x_{in} + \rho v \frac{dx}{dt} \]  Dynamic model

When steady-state assumptions are met, \( \rho v \frac{dx}{dt} \) evaluates to 0.
Mass balance for gas exchange

Substituting in for measured values and rearranging the dynamic model...

\[
E = \frac{u_{in}}{s(1 - w_{out})} \left( w_{out} - w_{in} + \frac{\rho v}{u_{in}} \frac{dw_{out}}{dt} \right)
\]

\[
A = \frac{u_{in}(1 - w_{in})}{s} \left( c'_{in} - c'_{out} + \frac{\rho v}{u_{in}} \frac{dc'_{out}}{dt} \right)
\]

\(E\) = Apparent transpiration
\(A\) = Carbon assimilation
\(w_{x}\) = Water vapor mole fraction
\(c'_{x}\) = \(CO_{2}\) dry mixing ratio = \(\frac{c_{x}}{1 - w_{x}}\)
Mass balance for gas exchange

Substituting in for measured values and rearranging the dynamic model...

\[ E = \frac{u_{in}}{s(1 - w_{out})} \left( w_{out} - w_{in} + \frac{\rho v}{u_{in}} \frac{dw_{out}}{dt} \right) \]

\[ A = \frac{u_{in}(1 - w_{in})}{s} \left( c_{in}' - c_{out}' + \frac{\rho v}{u_{in}} \frac{dc_{out}'}{dt} \right) \]

\[ E = \text{Apparent transpiration} \]
\[ A = \text{Carbon assimilation} \]
\[ w_x = \text{Water vapor mole fraction} \]
\[ c_x' = \text{CO}_2 \text{ dry mixing ratio} = \frac{c_x}{1 - w_x} \]

At steady state, the transient terms are zero and the equations are equivalent to what has always been used in the LI-6400/XT and LI-6800

Flow out of the chamber is not measured. It is accounted for by assuming \( u_{out} = u_{in} + sE \), and \( E \) is the only significant flux. \( E \) is generally \( 10^3 \) or \( 10^4 > A \).
Steady state versus dynamic

At steady-state, the transient term is zero and the fluxes match. Following a step change in CO$_2$, the steady-state model gives a false flux driven by washout. The dynamic model accounts for this and still shows zero assimilation.

Example data from an empty chamber (Assimilation = 0)
Accounting for dilution

\[ A = \frac{F(C_r - C_s)}{100S} \]

LI-6800 closed symbols, LI-6400XT open symbols

Transpiration (mmol/m²/s)

Apparent Assimilation (umol/m²/s)
More on water corrections...

- Explains basis of broadening and effective pressure
- Derive dilution corrections
- How IRGAs work

The Importance of Water Vapor Measurements and Corrections

Water vapor is known to influence the measurement of carbon dioxide by infrared gas analysis in several ways, which can lead to significant measurement errors. Spectral cross-sensitivity due to absorption band broadening, and inherent instrument cross-sensitivity can both cause overestimations of CO₂ mole fraction in samples containing water vapor when their effects are not accounted for. Dilution of samples by the addition of water vapor may not be important when measuring actual CO₂ mole fractions, but can lead to significant errors in flux measurements. In this note we describe the basis of each of these processes and discuss how each can affect the measurement of CO₂.

Infrared Absorption by Gases and Absorption Band Broadening

At the sub-molecular scale the positions of atoms within molecules are not entirely fixed. As they move they stretch and bend their bonds, creating vibrations within the molecule. Energy differences between the possible vibrational states that result from this movement make it possible for the molecule to absorb infrared radiation. Changes in angular momentum as the molecule rotates about its axis can also cause infrared radiation to be absorbed. The energy differences resulting from changes in the vibrational and rotational states of the molecule cause fluctuations in its dipole moment. These oscillations interact with the alternating electrical field of electromagnetic radiation and if the frequencies of oscillation match, the radiation will be absorbed by the molecule.

Since the frequency of oscillation for electromagnetic radiation (ν) is the inverse of its wavelength (λ=ν/ν) and the various energy states that lead to its absorption by a molecule are the result of the molecule's structure, the absorption of infrared radiation is both wavelength and absorber species dependent. For a given molecular species, absorption of infrared radiation will occur in bands at various wavelengths across the infrared region of the spectrum (Figure 1A). Each of these absorption bands is comprised of individual absorption lines (Figure 1B) that result from rotational transitions, and have a generally Lorentzian line shape described by:

$$\delta(\nu) = \frac{\alpha}{\pi (\nu - \nu_0)^2 + \alpha^2}$$

where α is the absorption coefficient of radiation at frequency ν, ν is the line strength, (νν) is the change in frequency across the absorption line and α is the absorption line half width.

Figure 1: Absorption spectrum of CO₂. Carbon dioxide absorption data from HITRAN96 (Rothman et al. 1998). A. The absorption spectrum of CO₂ in the infrared region of the electromagnetic spectrum. B. The absorption of CO₂ in the 4.2 µm region.

For infrared gas analysis with LI-COR gas analyzers, we are interested in measuring the total absorption of infrared light across a given wavelength to determine absorber species concentration. The total absorption measured by the analyzer, A, is in principle the result of integrating the absorption across the entire band as shown by:

$$A = \int_{\nu_1}^{\nu_2} K(\nu) \, d\nu$$

where A is the absorption of infrared radiation at a given radiation frequency, and is given by Beer-Lambert’s Law:

$$A = -\ln(1 - e^{-\alpha t})$$

where w is the absorber concentration and t is the optical path length (Burch and Williams 1964). These relationships form the basic principles behind

https://app.boxenterprise.net/s/igs56gijkc4ftks30pci
Matching IRGAs

LI-6800

Normal operating mode:

Match mode:
Basic parameters *computed*

- Fluxes from mass balance
  - $A$ – Assimilation rate
  - $E$ – Transpiration rate
- Additional parameters
  - $g_{sw}$ – Stomatal conductance to water vapor
  - $C_i$ – Intercellular $\text{CO}_2$ concentration
What else can we determine with gas exchange?
What else can we determine with gas exchange?

- Fick’s First Law

\[ J_j = D_j \frac{\partial c_j}{\partial x} = g_j \Delta c_j \]

- \( J_j \) = flux
- \( D_j \) = diffusivity coefficient
- \( \delta c_j / \delta x \) = change in concentration
- \( g_j \) = conductance
- \( \Delta c_j \) = concentration gradient
What else can we determine with gas exchange?

- $E \approx g_{total}^{H_2O} (W_i - W_a)$
- $A \approx g_{total}^{CO_2} (C_a - C_i)$

- Measure $E$ & $w_a$ ($w_a = W_s$)
- Measure leaf temperature
- Calculate $w_i$
- Solve for $g_{total}^{H_2O}$

- Measure $A$ & $c_a$ ($c_a = C_s$)
- $g^{CO_2} = g^{H_2O}/1.6$
- Solve for $c_i$
What else can we determine with gas exchange?

Ohm’s Law Analogy

\[ r_{total} = r_{bl} + \frac{1}{r_s} + \frac{1}{r_c} + r_{mes} \]

Assumptions:
- end point of diffusion path is mesophyll surface
- cuticular resistance is near infinite

\[ r_{total} = r_{bl} + r_s \]
Boundary layer

\[ \sigma_{bl} = \text{boundary layer thickness} \]
\[ v = \text{air velocity} \]
\[ l = \text{leaf width} \]

\[ bl = 4 \sqrt{\frac{l}{v}} \]
Boundary layer conditions

- Near saturation
- Near ambient
- Ambient

At saturation
Boundary layer conditions

Near saturation
Near ambient
Ambient

At saturation
Chamber
What does this mean for making measurements?

- For H2O: Target ambient boundary layer conditions!

\[
\text{RH (\%)} = \frac{e_c}{e_{T_{air}}} \times 100
\]

\[
e_c (kPa) = W_s P
\]

\[
\text{VPD}_{\text{leaf}} (kPa) = e(T_{\text{leaf}}) - e_c
\]

On the LI-6800 use constant VPD (1 to 1.5 kPa)
What does this mean for interpreting the data?

- E = Real count of $\text{H}_2\text{O}$ molecules leaving the leaf

$$g_s = \frac{E}{(w_i - w_a)}$$

Graph with data points and a linear regression line:
- $y = 0.0053x + 0.7658$

The graph shows the relationship between leaf conductance ($g_s$) and environmental conditions, with RH (%).
What does this mean for interpreting the data?

- Water Use Efficiency
- Instantaneous versus Intrinsic

\[
W_t = \frac{A}{E} = \frac{A}{g_s (w_i - w_a)} = \frac{A}{g_s D_a} = W_g D_a
\]

\[
W_g = \frac{A}{g_s}
\]

Measurements fall in two categories

- Response Curve – imposed conditions
  - Light Response Curve
  - CO$_2$ Response Curve
- Survey
  - *In situ* picture under ambient conditions
When to make measurements?

![Graph showing photosynthetic active radiation (PAR) and assimilation over time]

- Photosynthetic Active Radiation (umol/m²/s)
- Assimilation (umol/m²/s)

Time:
- 6:00
- 9:00
- 12:00
- 15:00
- 18:00
- 21:00

Assimilation and PAR show peaks during mid-day, around 12:00, with a gradual decrease towards the evening.
A-C\textsubscript{i} and AQ curves

- Response to [CO\textsubscript{2}]
- Response to light
Parameters from AQ Response

- $A_{\text{sat}}$ – photosynthesis at saturating light
- $R_d$ – Dark respiration rate
- LCP - Light compensation point
- $\phi$ – Quantum yield

\[
A_{\text{sat}} = 34.28 \pm 0.778 \quad r^2 = 0.9995 \\
R_d = -1.492 \pm 0.117
\]
Parameters from $A_{c_i}$ response

- $V_{c,\text{max}}$ – velocity of carboxylation
- $J_{\text{max}}$ – electron transport for RuBP regeneration
- $c_i$ inflection – transition from $V_{c,\text{max}}$ to $J_{\text{max}}$
- $l$ – stomatal limitation to photosynthesis
- $g_m$ – mesophyll conductance
- $\Gamma^*$ - $CO_2$ compensation point
- TPU – trios-phosphate utilization
Considerations for $A_{c_i}$ measurements

- Saturating light
  - CO$_2$ effect on photosynthesis will be confounded with the effects of light
- Environmental Control
  - **Reference** vs. Sample
  - Humidity will alter stomatal aperture
  - Temperature control for enzyme kinetics
- Match at every point
Using short time steps is important

- Rubisco deactivation
- Stomatal closure
AC<sub>i</sub> curve fitting tools

Sharkey et al. 2015. What gas exchange data can tell us about photosynthesis. Plant, Cell and Environment
AC\textsubscript{i} curve fitting tools

- Leafweb.ornl.gov
  - AC\textsubscript{i} curve fitting and data sharing platform
  - Developed and hosted by Oak Ridge National Laboratory

- Plantecophys R package
Our discussion of $g_s$ and $c_i$ thus far has been based on assumptions that break down at the whole plant/canopy scale!

Other chamber options for the LI-6800
Selecting the right chamber?

- Chamber aperture - bigger can be better!
- Light intensity control
- What data do you need?
LI-6800 Console flow path:
Rapid & stable software control of CO2 and H2O
Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment

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[Diagram showing data points and lines representing photosynthesis and carbohydrate dynamics]