

A man in a light blue shirt is shown in profile, holding a white LI-COR gas exchange instrument against a green plant. The background is a soft-focus natural setting. The title text is overlaid in the center-left.

Leaf-level Gas Exchange Instrument theory

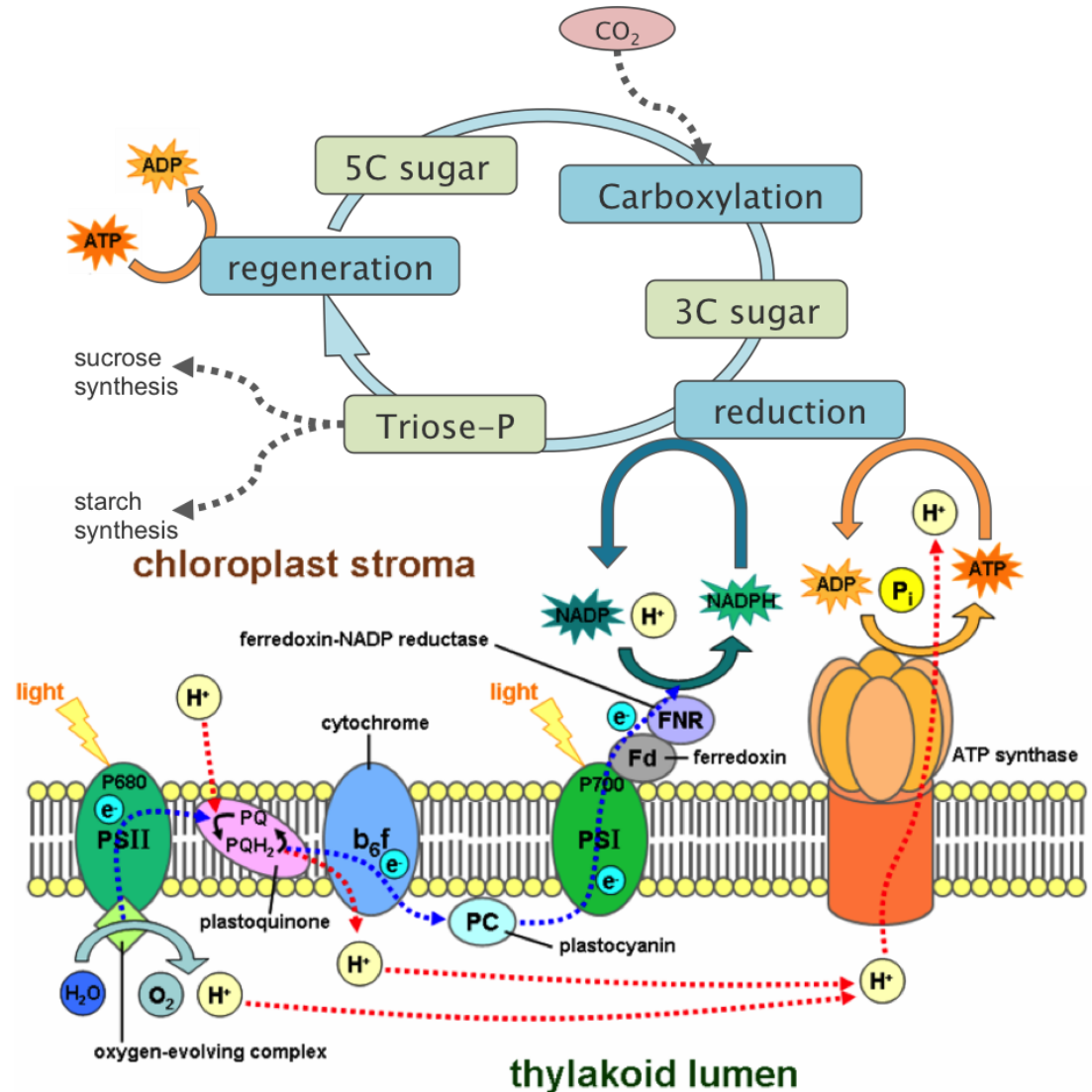
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How do we measure photosynthesis?

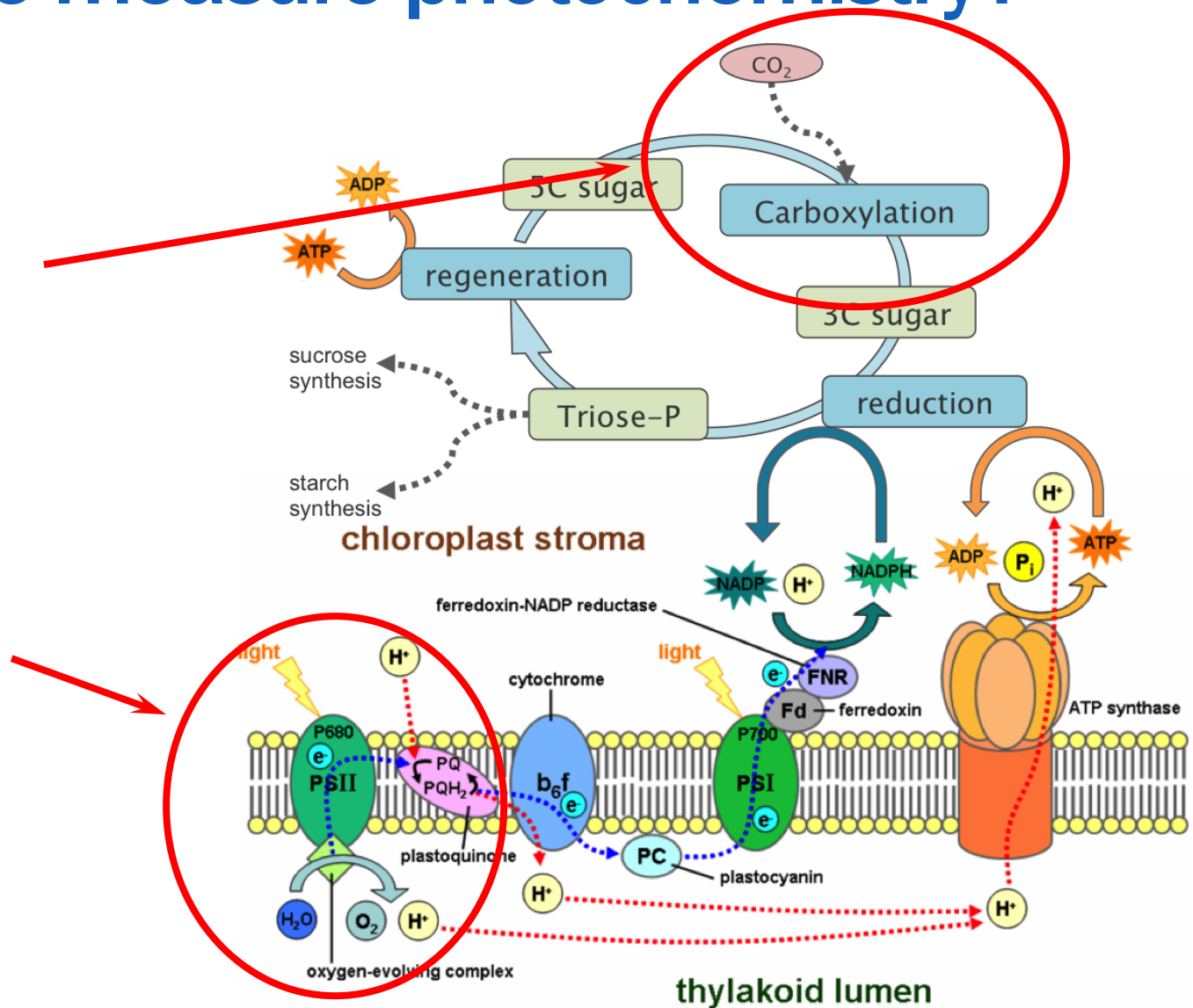
- Photosynthesis
- Gas exchange
- Assimilation



How do we measure photochemistry?

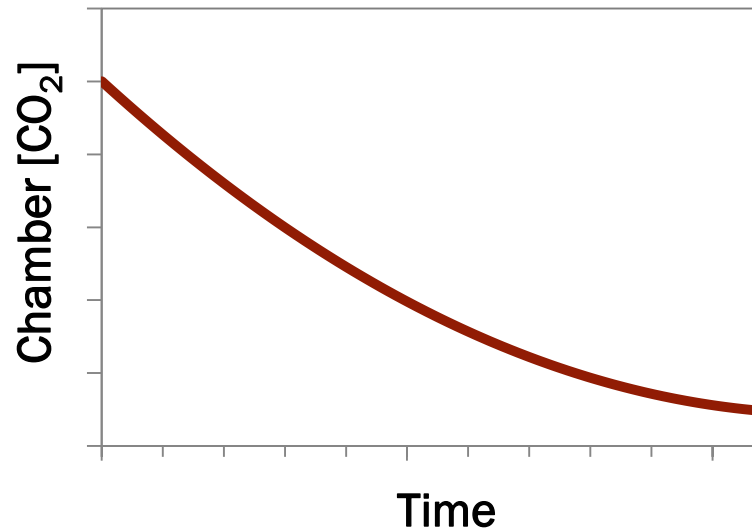
Gas exchange

Chlorophyll
Fluorescence



Gas exchange systems

- Closed-transient
- Compensating



Gas exchange systems

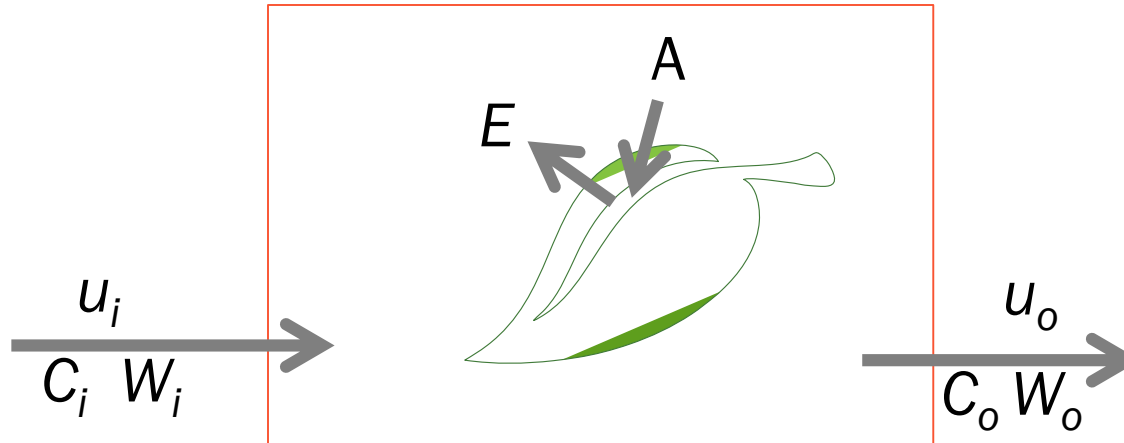
- Closed-transient
- Compensating
- Open flow through



Key physiological parameters *computed*

- Fluxes from mass balance
 - A – CO₂ Assimilation rate
 - E – Transpiration rate
- Calculated parameters
 - g_{sw} – Stomatal conductance to H₂O
 - C_i – Intercellular CO₂ concentration

Mass balance in an open system



$$E = \frac{u_o W_o - u_i W_i}{s}$$

$$A = \frac{u_i C_i - u_o C_o}{s}$$

- S leaf area
- E transpiration
- u flow rate
- W concentration of water vapor
- A carbon assimilation
- C concentration of CO_2

Mass balance in an open system

$$\begin{aligned} E &\gg \frac{u(W_o - W_i)}{S} \\ &\approx \cancel{\text{mol}} \text{ s}^{-1} (\text{mmol} \cancel{\text{mol}}^{-1}) / \text{m}^2 \\ &\approx \text{mmol m}^{-2} \text{ s}^{-1} \end{aligned}$$

$$\begin{aligned} A &\gg \frac{u(C_i - C_o)}{S} \\ &\approx \cancel{\text{mol}} \text{ s}^{-1} (\mu\text{mol} \cancel{\text{mol}}^{-1}) / \text{m}^2 \\ &\approx \mu\text{mol m}^{-2} \text{ s}^{-1} \end{aligned}$$

Accounting for dilution

$$A = \frac{F \left(C_r - C_s \left(\frac{1000 - W_r}{1000 - W_s} \right) \right)}{100S}$$

r denotes *reference* concentration, same as incoming
 s denotes *sample* concentration, same as outgoing

More on water corrections...



APPLICATION NOTE

The Importance of Water Vapor Measurements and Corrections

Application Note #129

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 three 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 ($\lambda=1/\nu$) 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

$$k(\nu) = \frac{S}{\pi} \frac{\alpha}{(\nu - \nu_0)^2 + \alpha^2} \quad (1)$$

where $k(\nu)$ is the absorption coefficient k of radiation at frequency ν , S is the line strength, $(\nu - \nu_0)$ is the change in frequency across the absorption line and α is the absorption line half width.

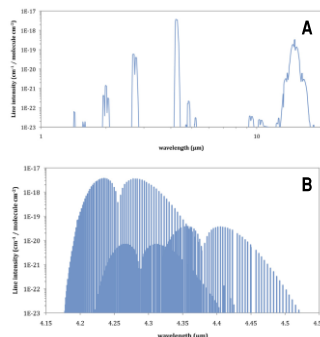


Figure 1: Absorption spectrum of CO₂. Carbon dioxide absorption data from HITRAN96 (Rothman et al. 1998). A. The absorption spectrum of CO₂ in the infrared region of the electromagnetic spectrum. B. The absorption of CO₂ in the 4.2 μ m region.

For infrared gas analysis with LI-COR gas analyzers, we are interested in measuring the total absorption of infrared light across a given waveband 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

$$A = \int A'(\nu) d\nu \quad (2)$$

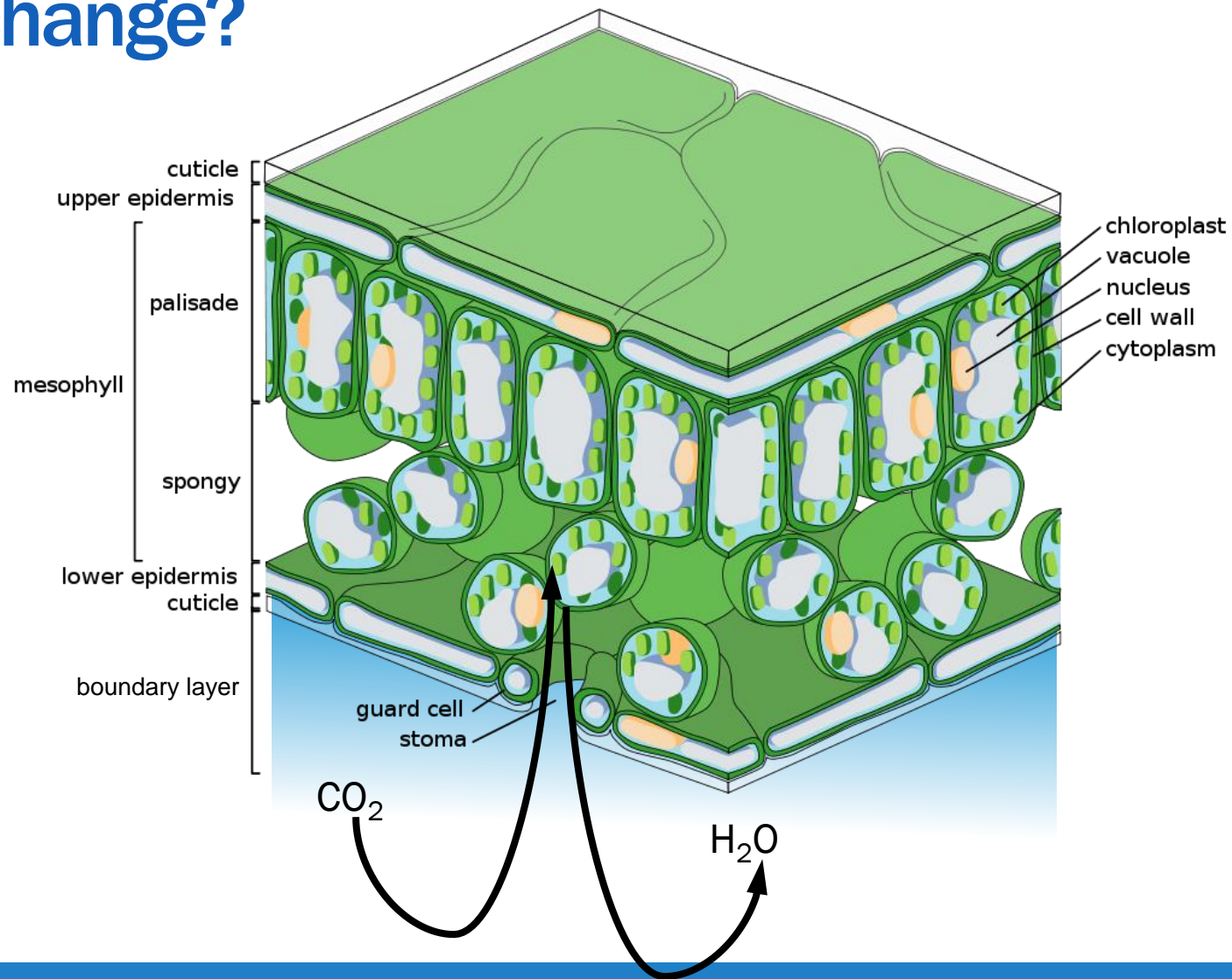
where $A'(\nu)$ is the absorption of infrared radiation at a given radiation frequency, and is given by Beer-Lambert's Law

$$A'(\nu) = 1 - \exp[-k(\nu)w] \quad (3)$$

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

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

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_i = flux

D_j = diffusivity coefficient

$\partial c_j / \partial x$ = change in concentration

g_j = conductance

Δc_j = concentration gradient

What else can we determine with gas exchange?

$$\cancel{E} \approx g_{total}^{H_2O} (\cancel{W_i} - \cancel{W_a})$$

$$\cancel{A} \approx g_{total}^{CO_2} (\cancel{C_a} - C_i)$$

- 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?

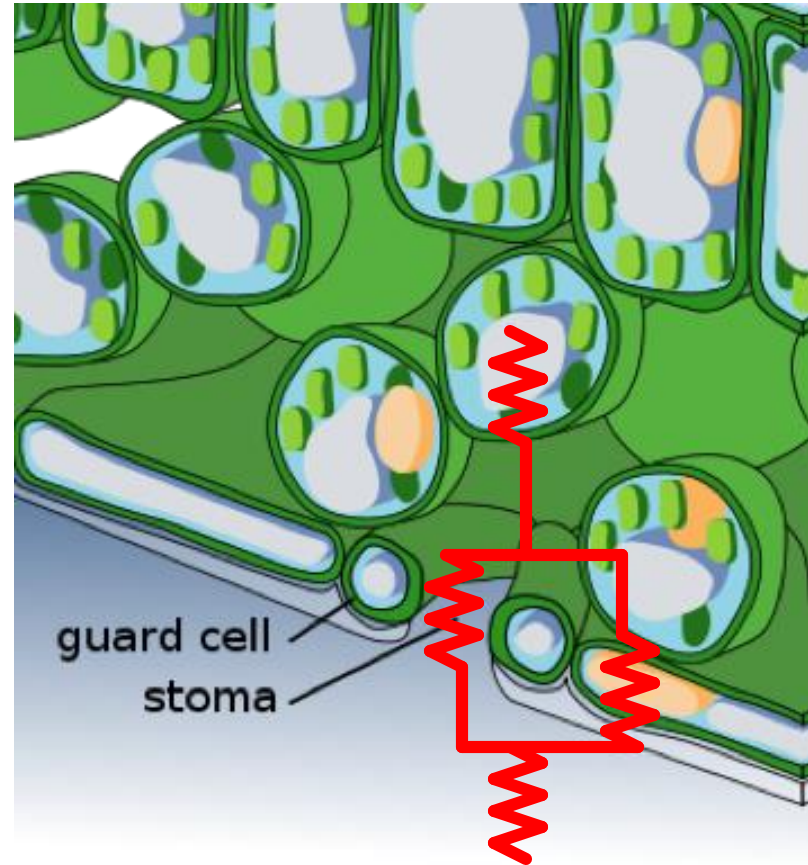
Ohm's Law Analogy

$$r_{total} = r_{bl} + \left(\frac{1}{r_s} + \frac{1}{r_c} \right)^{-1} + r_{mes}$$

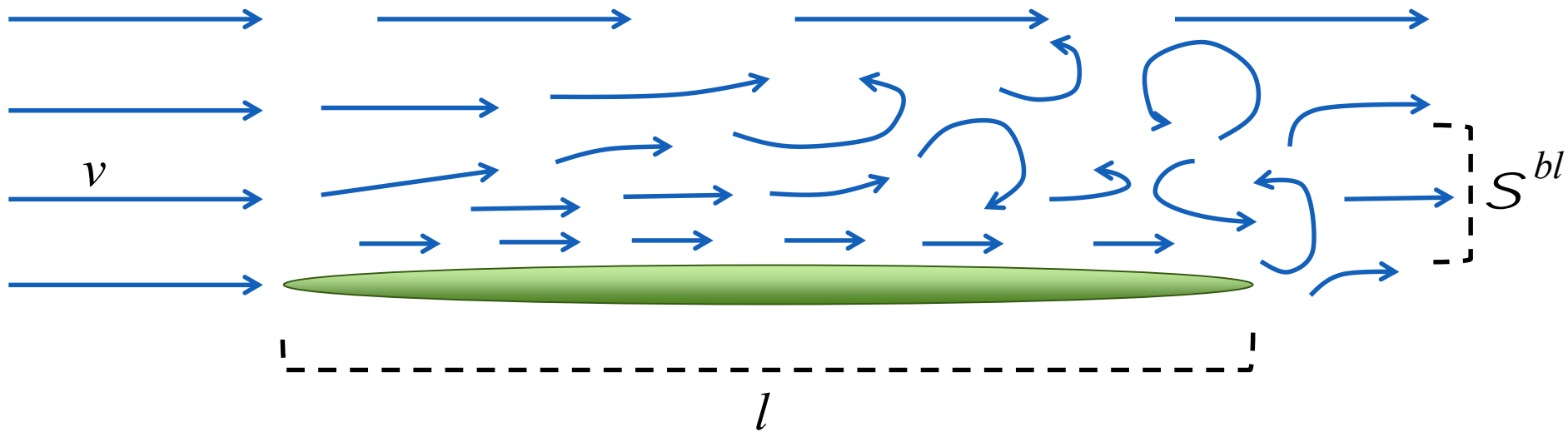
Assumptions:

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

$$r_{total} \gg r_{bl} + r_s$$



Boundary layer



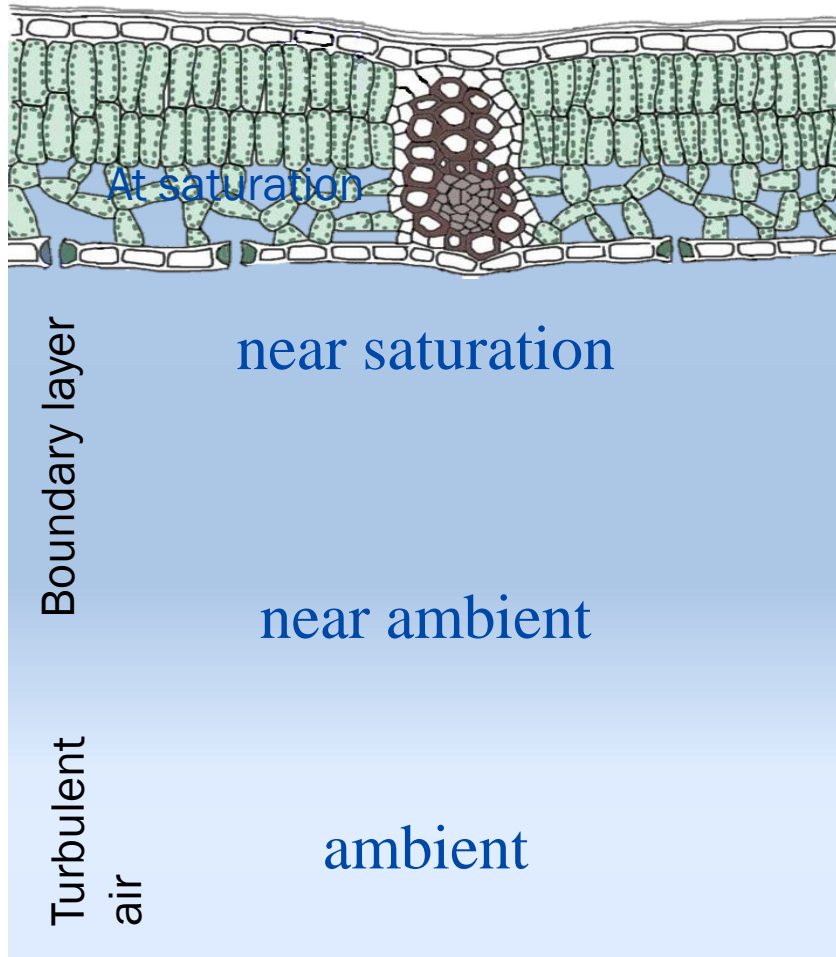
$$S^{bl} = 4\sqrt{\frac{l}{v}}$$

σ^{bl} = boundary layer thickness

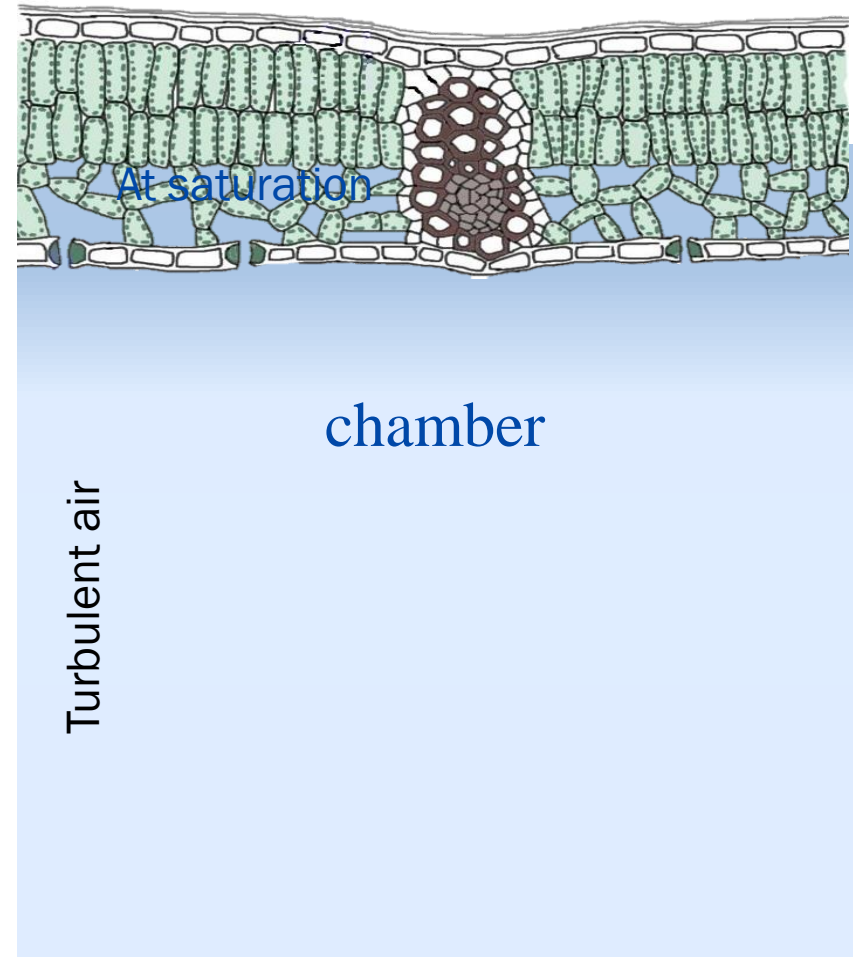
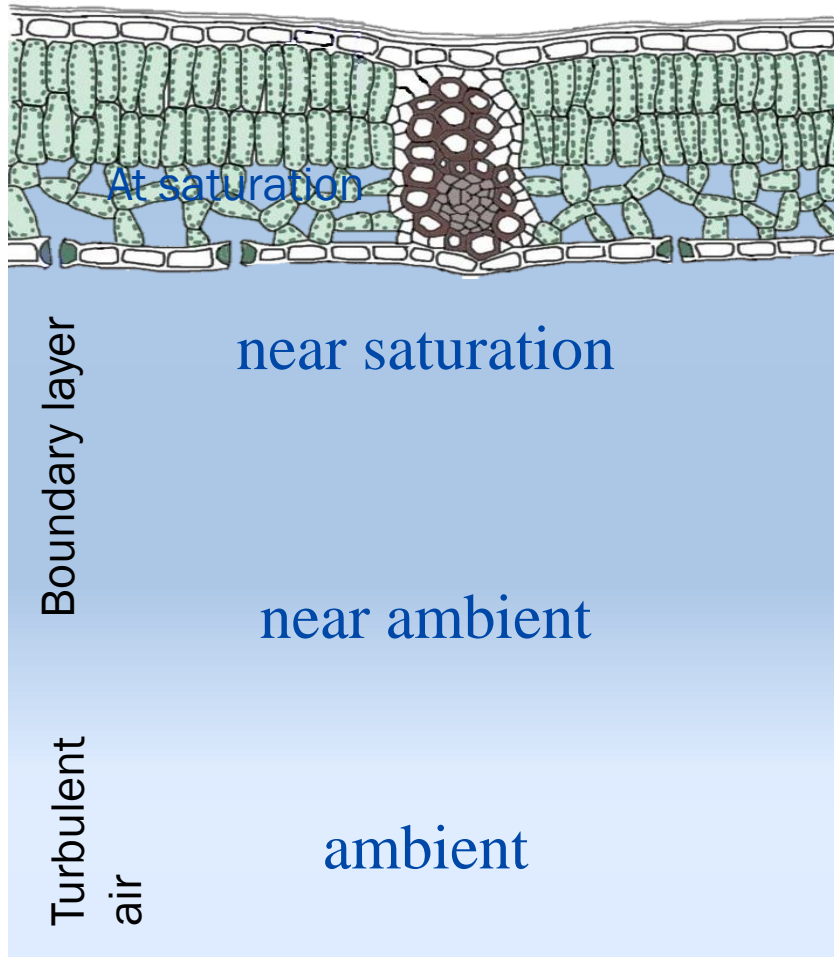
v = air velocity

l = leaf width

Boundary layer conditions



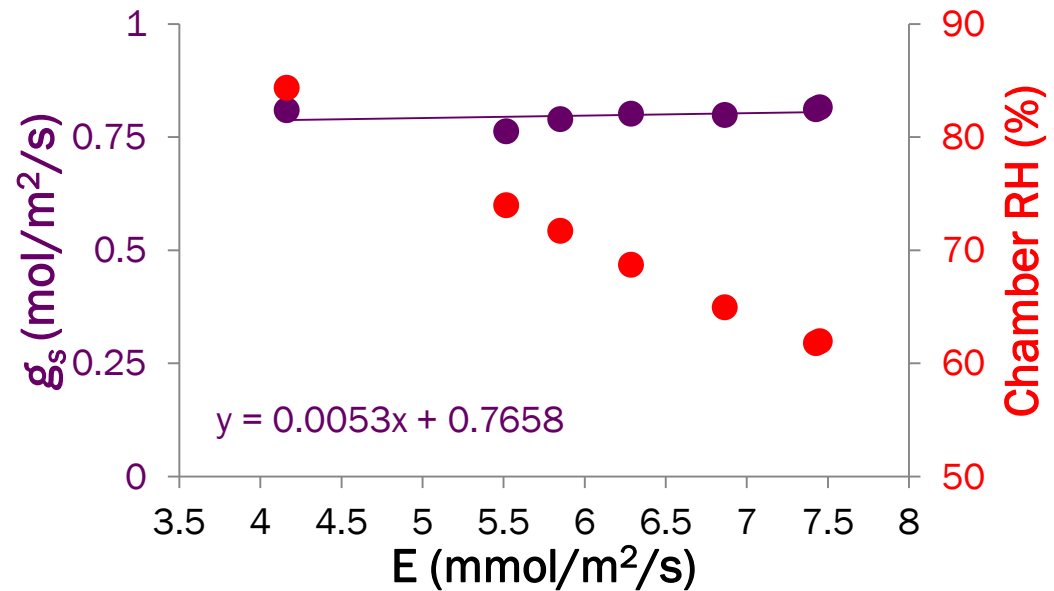
Boundary layer conditions



What does this mean for interpreting the data?

- E = Real count of H_2O molecules leaving the leaf

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

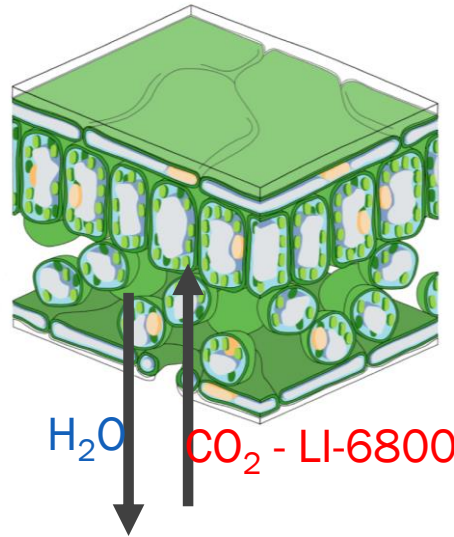


Key physiological parameters *computed*

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 - C_i – Intercellular CO₂ concentration

Two LI-COR Systems

LI-600



- H₂O only
 - Porometer (g_{sw})
- Ambient Conditions
- Rapid Survey
 - (5-10 seconds)

LI-6800

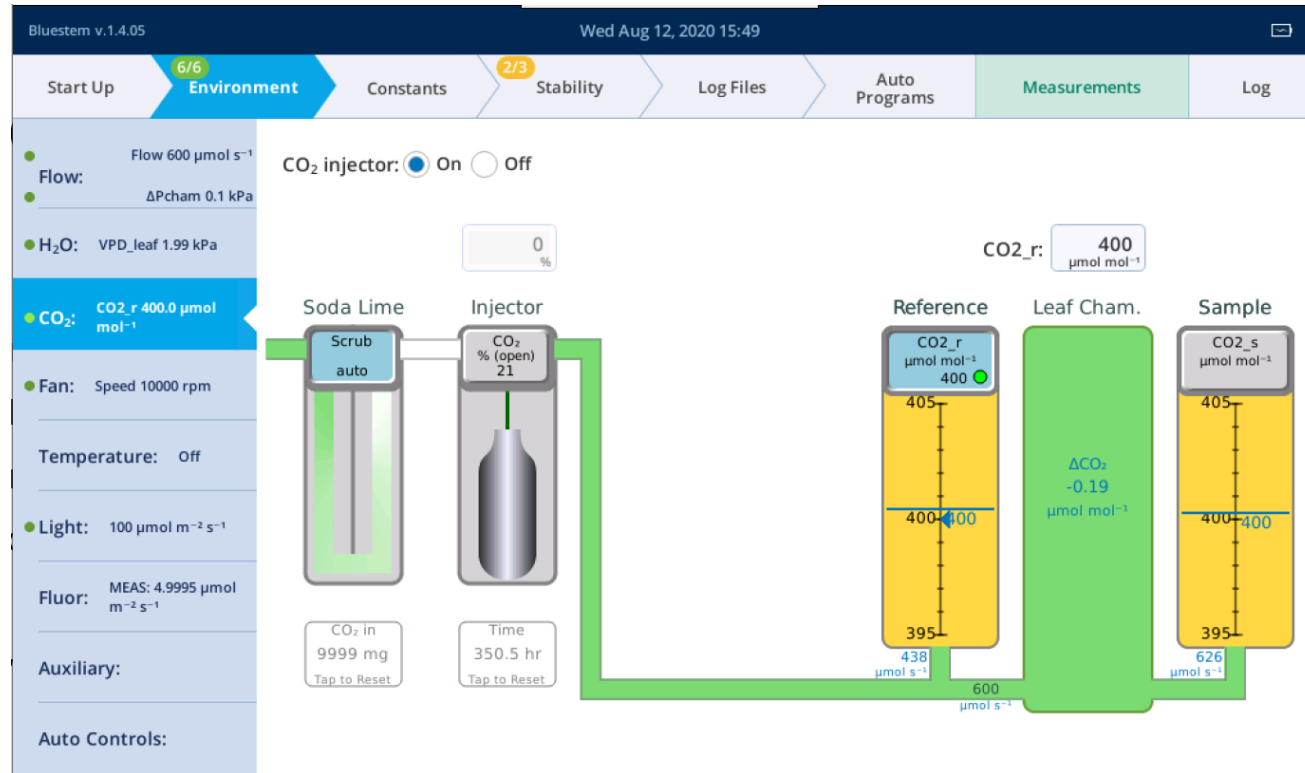


- H₂O and CO₂
 - A, E, C_i, g_{sw}
- Environmental Control
 - Light, Temp, CO₂, H₂O
- Response Curve or Survey

LI-6800 Environmental Control

LI-6800:

- H₂O control
- CO₂ control
- Temperature control
- Light control



Measurements fall in two categories

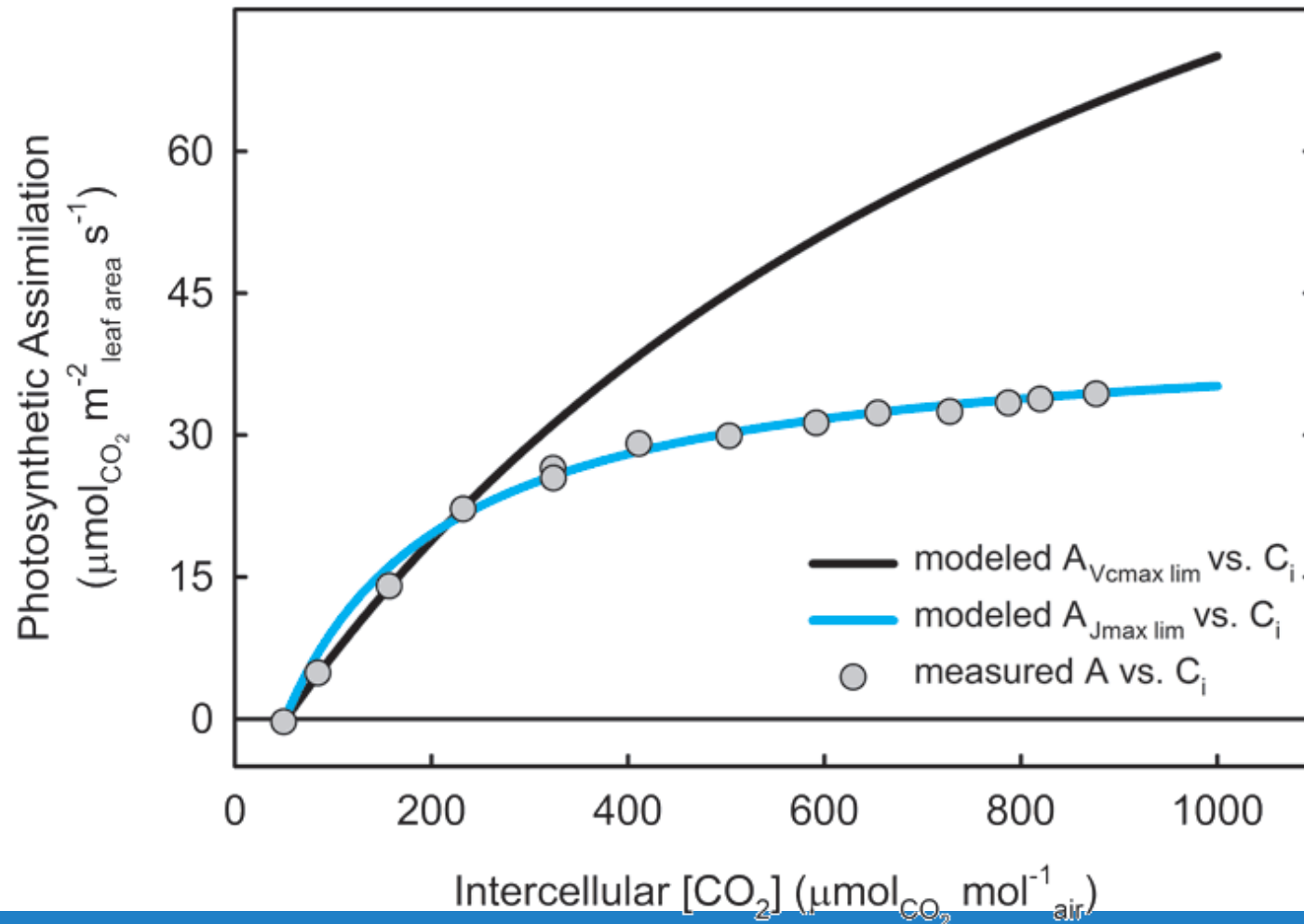
- Response Curve – user-set conditions
 - CO₂
 - Light
 - Temperature
 - VPD (Vapor Pressure Difference)
- Survey
 - *In situ* picture under ambient conditions

CO₂ Response Curve

$$V_{C_{\max}} = 141.42 \pm 0.846 \quad r^2 = 0.9999 \quad J_{\max} = 203.27 \pm 0.819 \quad r^2 = 0.9788$$

$$C_i \text{ @ inflection} = 216$$

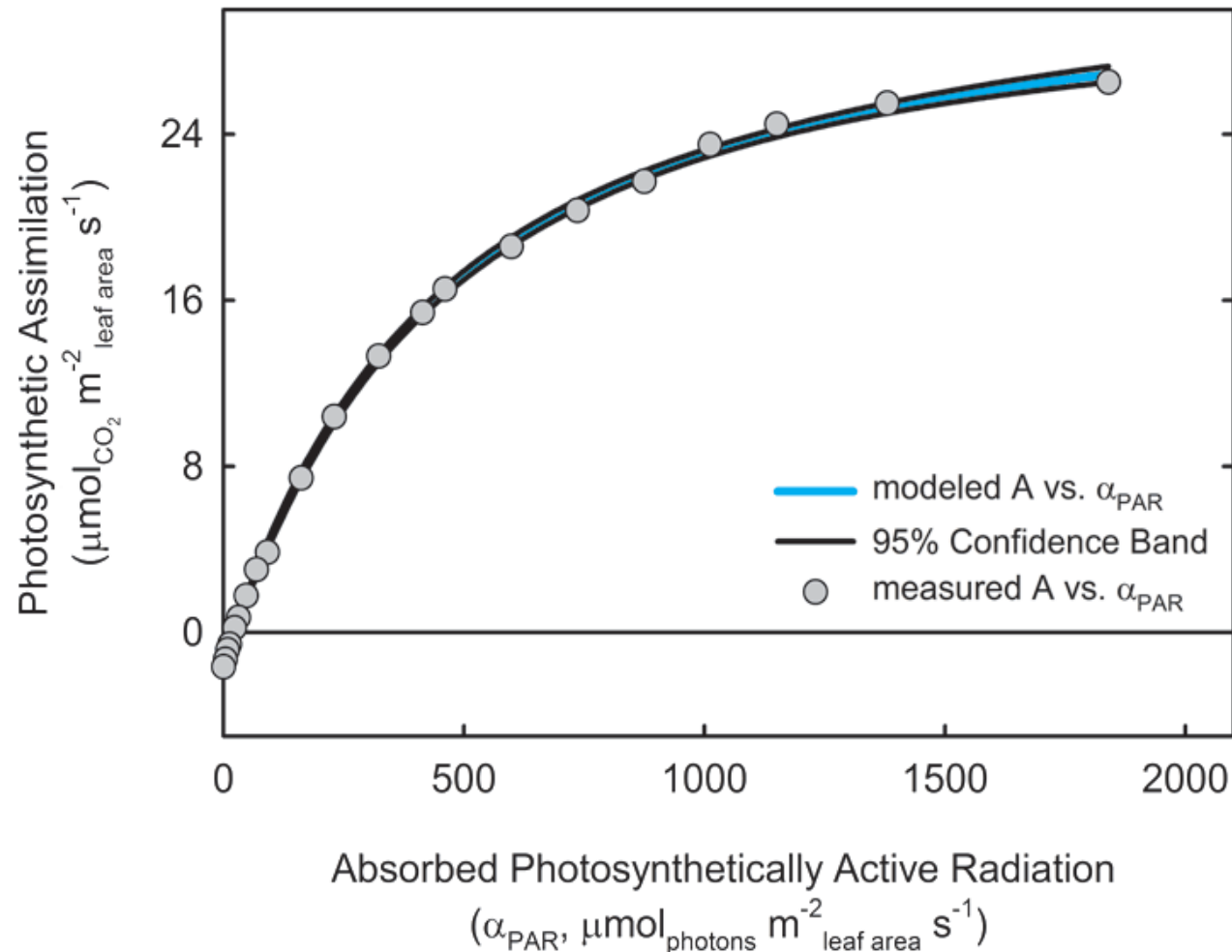
$$I \text{ @ growth } [CO_2] = 0.05$$



Light Response Curve

$$A_{\text{sat}} = 34.28 \pm 0.778 \quad r^2 = 0.9995$$

$$R_d = -1.492 \pm 0.117$$

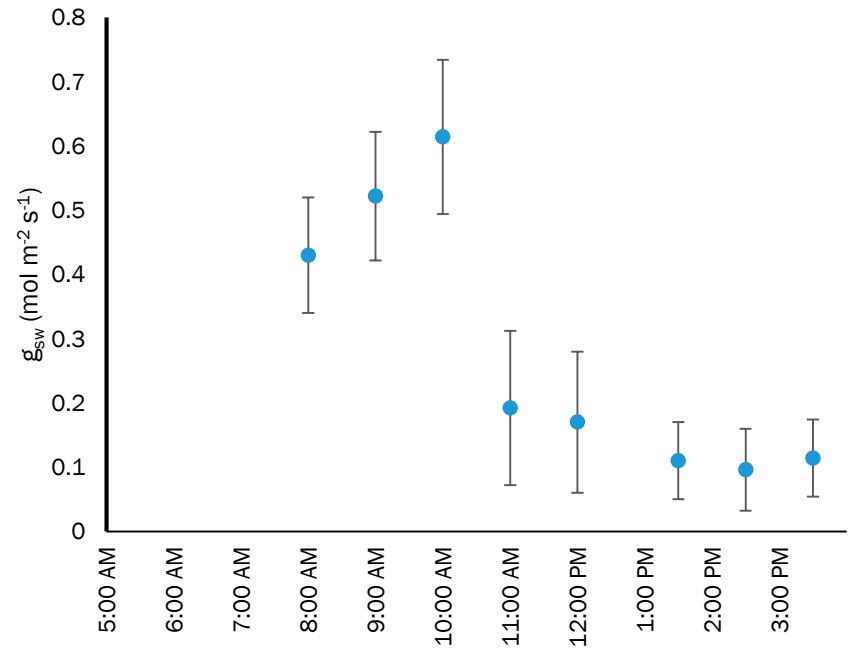


- A_{sat} - photosynthesis at saturating light
- R_d - Dark respiration rate
- LCP - Light compensation point
- ϕ - Quantum yield

Survey measurements

- Rapid Screening
- Ambient Conditions

Pac Choi, n = 26 leaves



Two LI-COR Systems

LI-600

- Ambient conditions
 - H_2O flux – Steady state
- Survey Measurements

LI-6800

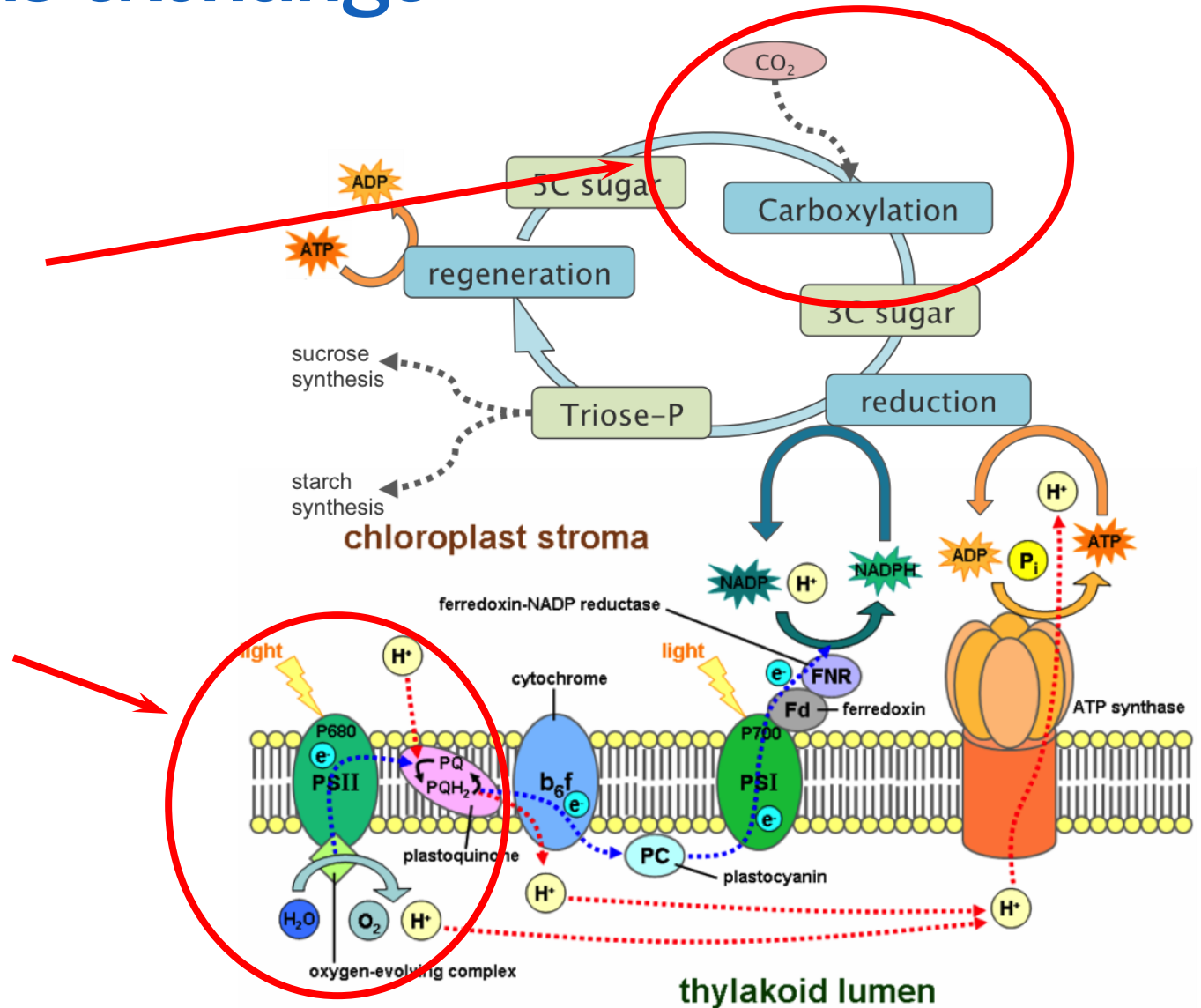
- Controlled Environment
 - CO_2 and H_2O flux
- Survey and Response Curves



Beyond gas exchange

Gas exchange

Chlorophyll
Fluorescence



Chlorophyll Fluorescence



- Ambient Actinic Light
- $F_v/F_m/\Phi_{PSII}$
- ETR



- LED Actinic Light
- $F_v/F_m/\Phi_{PSII}$
- ETR
- Far-red (F_o')
- OJIP kinetics

Two LI-COR Systems – Parameter Set



- g_{sw} , apparent E
- Φ_{PSII} , F_v/F_m , ETR



- A , C_i , J_{max} , $V_{c,max}$
- g_{sw} , apparent E
- Φ_{PSII} , F_v/F_m , ETR, F_o' , induction kinetics etc...

Questions?

