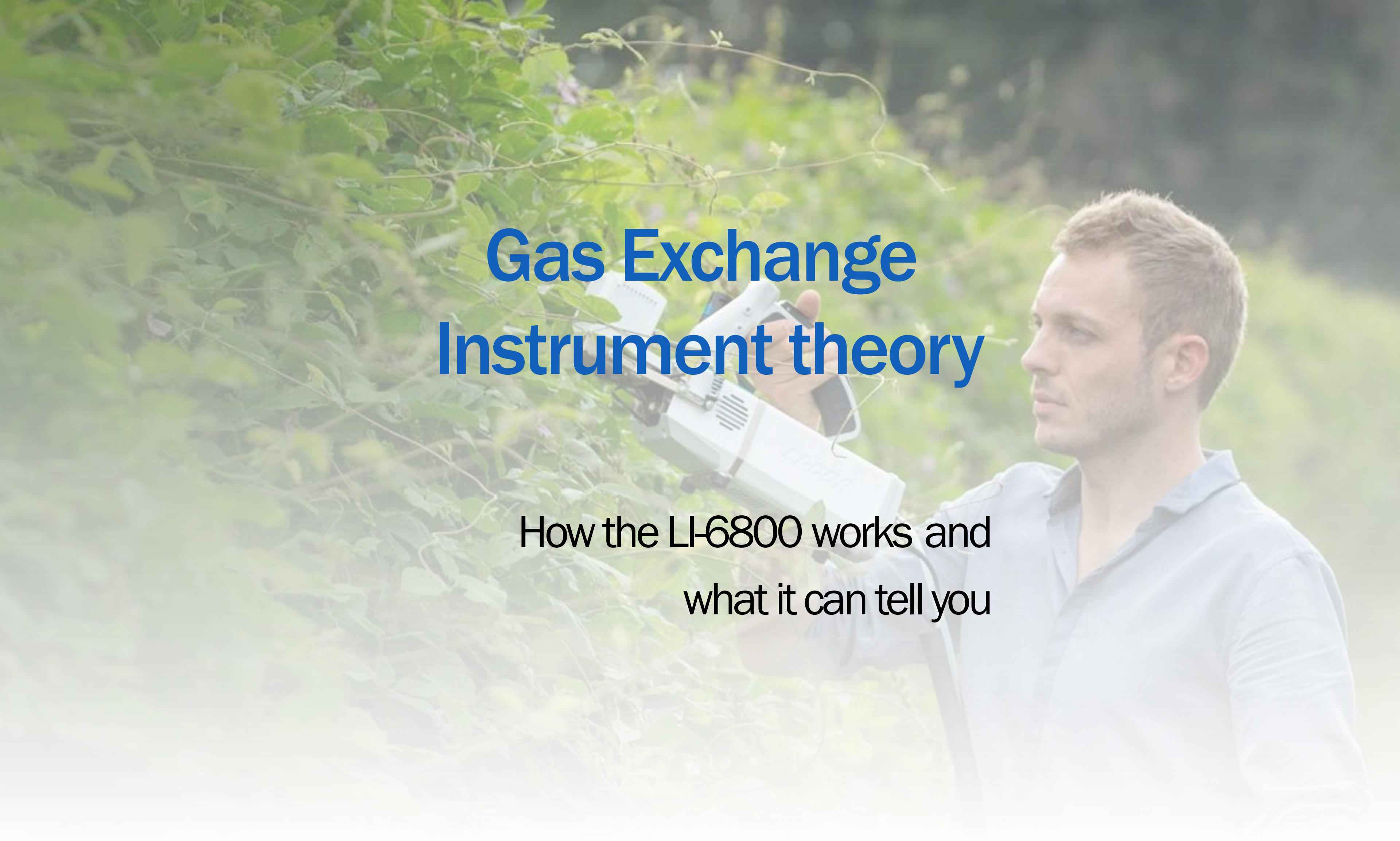


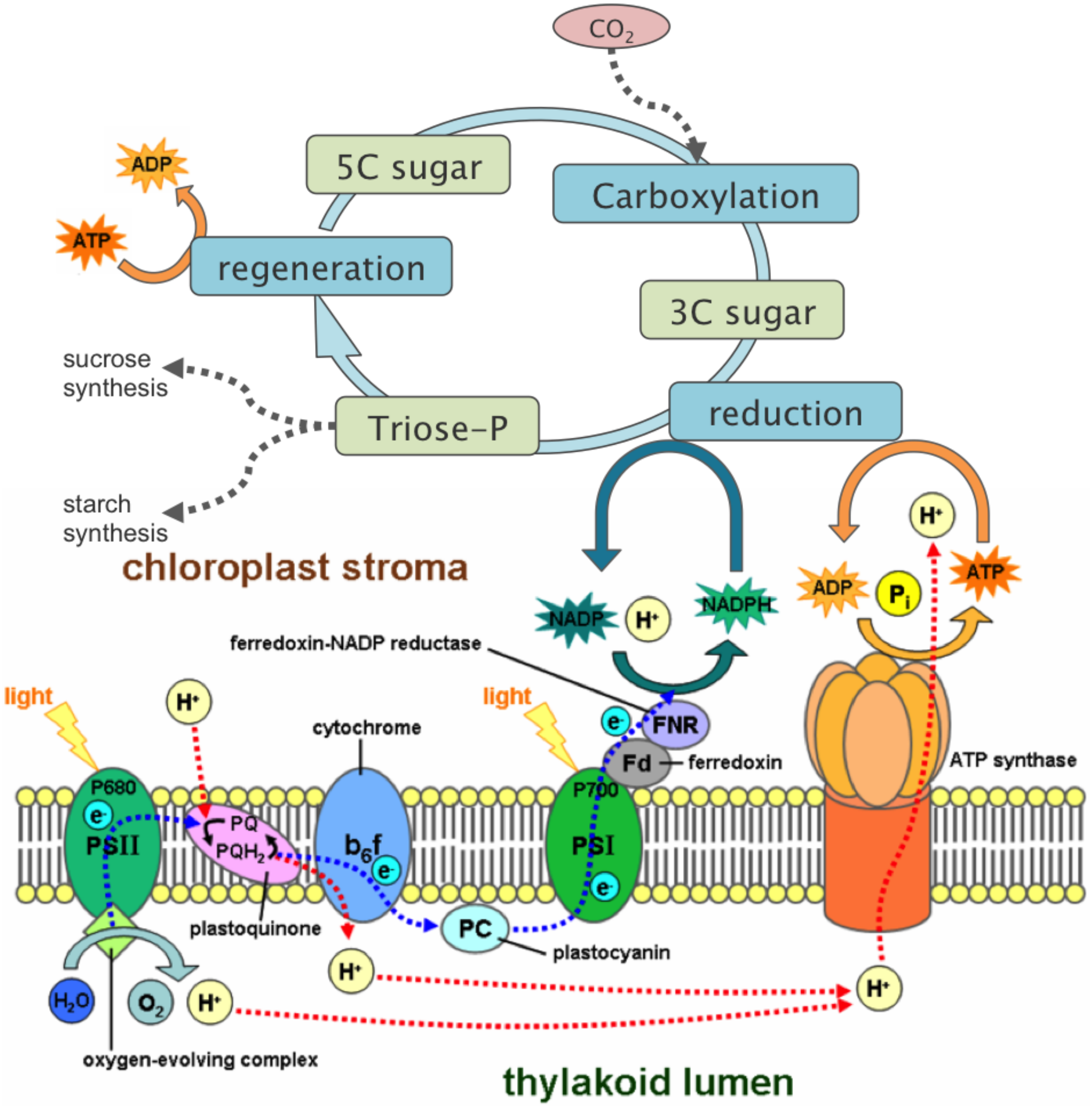
Gas Exchange Instrument theory

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

Used by permission from LICOR (November, 2023)

How do we measure photosynthesis?

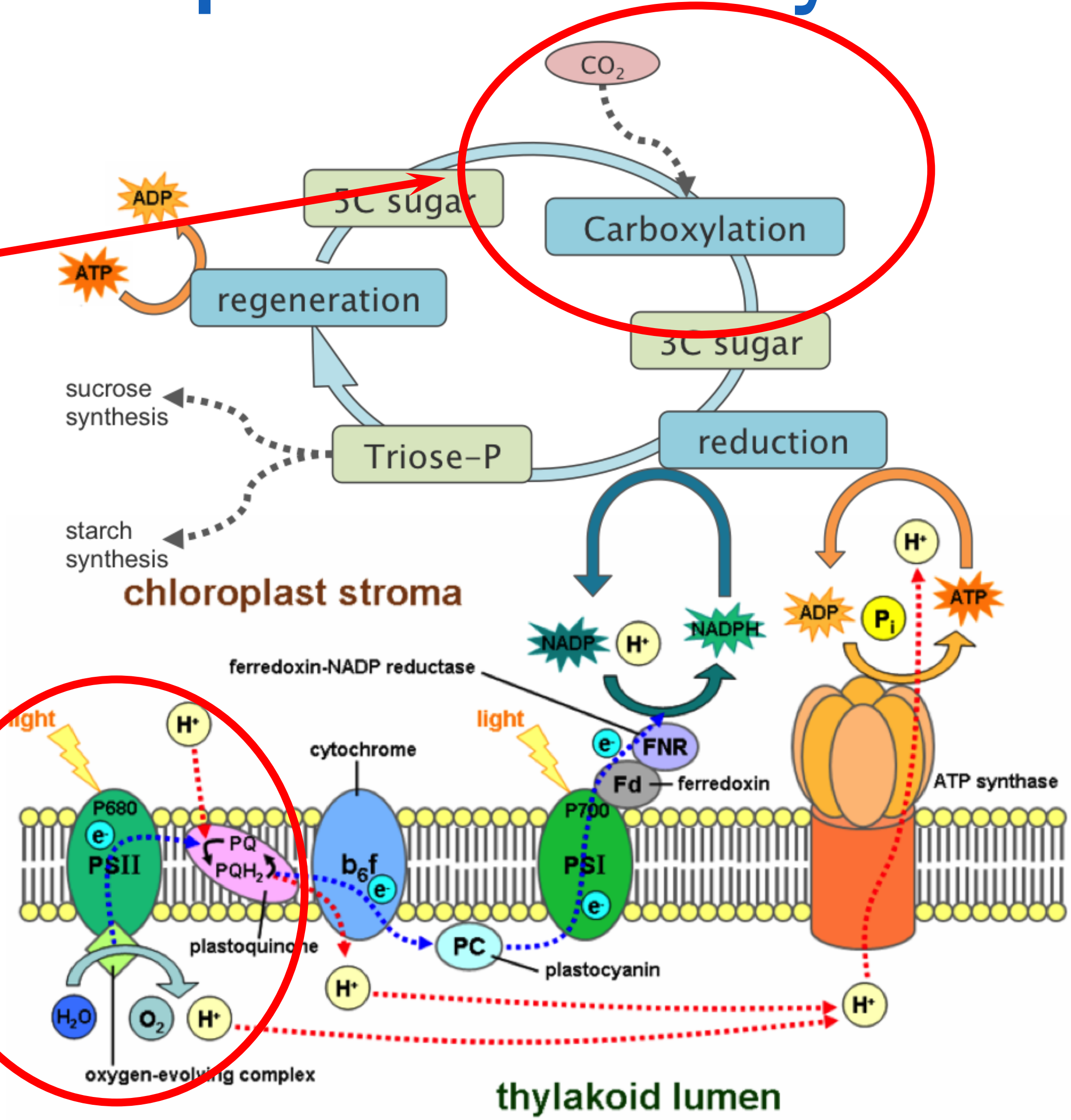
- Photosynthesis
- Gas exchange
- Assimilation



How do we measure photochemistry?

Gas exchange

Chlorophyll Fluorescence



How do we measure gas exchange?

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

$$A'_{(v)} = 1 - e^{-k_{(v)} w l}$$

w = density of the absorber

l = path length

v = wavelength

A' = light absorption

$k(v)$ = absorption coefficient

How do we measure gas exchange?

- Infrared Absorption by Gases

$$\alpha + T = 1$$

$$\alpha = 1 - T$$

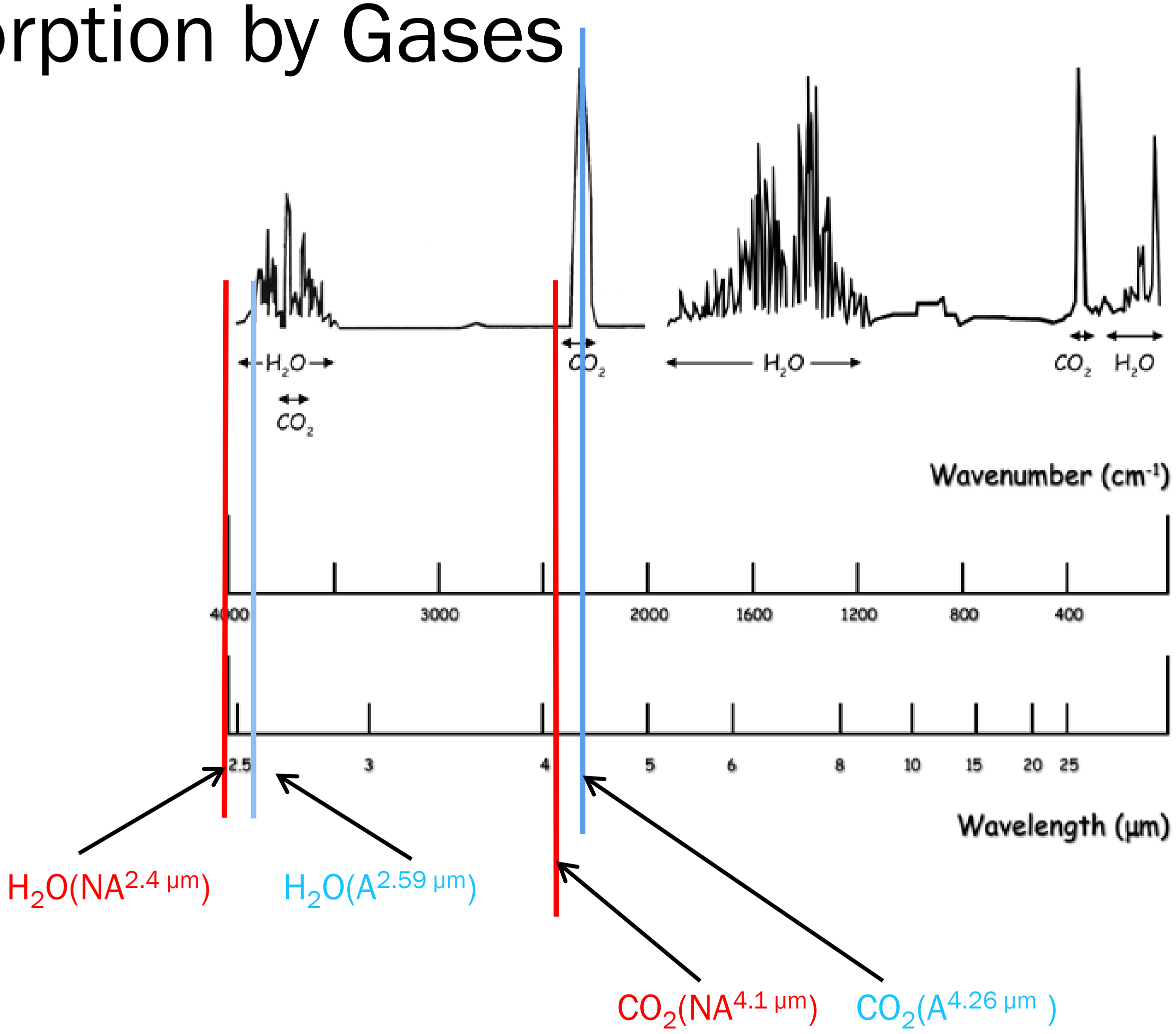
$$\alpha = 1 - \phi_A / \phi_{NA}$$

α = absorptance

T = transmittance

Φ_A = transmittance in absorbing region

Φ_{NA} = transmittance in non-absorbing region



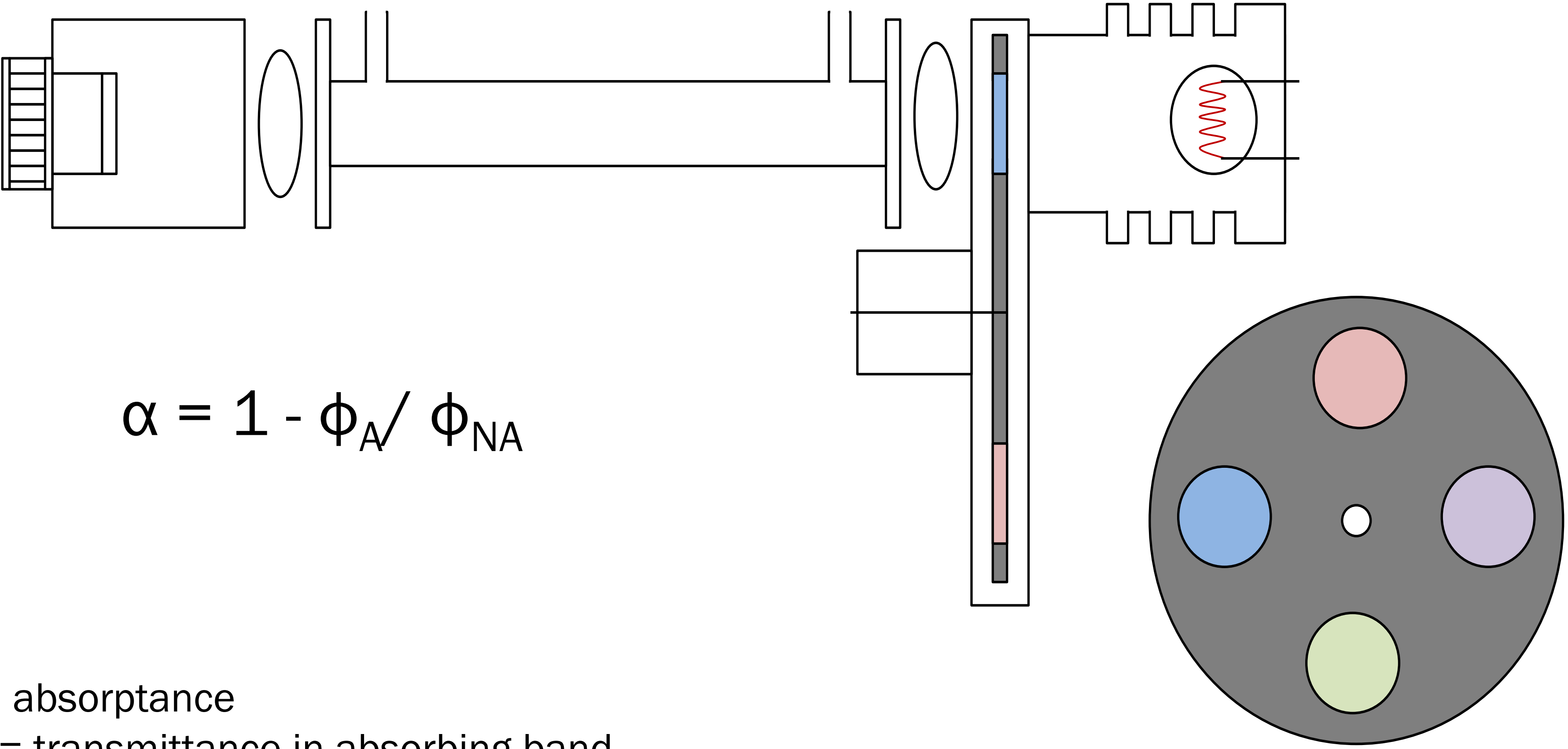
$H_2O(NA^{2.4\ \mu m})$

$H_2O(A^{2.59\ \mu m})$

$CO_2(NA^{4.1\ \mu m})$

$CO_2(A^{4.26\ \mu m})$

Basic optical differential IRGA



$$\alpha = 1 - \phi_A / \phi_{NA}$$

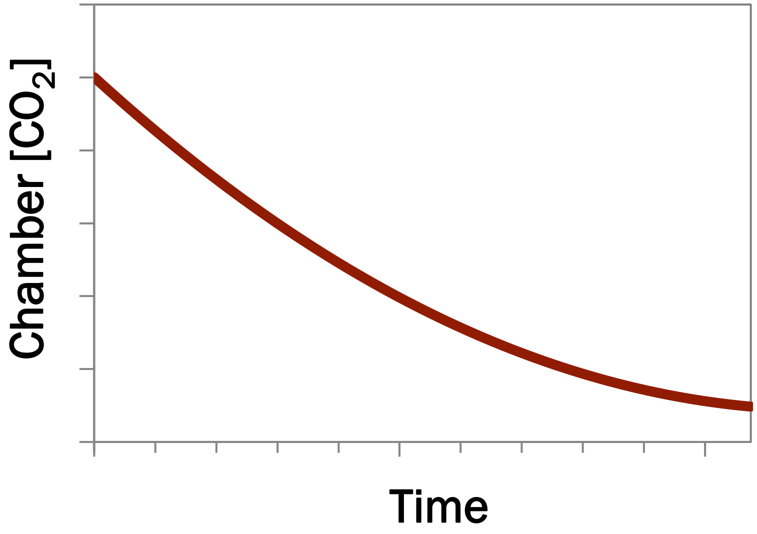
α = absorptance

Φ_A = transmittance in absorbing band

Φ_{NA} = transmittance in non-absorbing band

Gas exchange systems

- Closed-transient
- Compensating

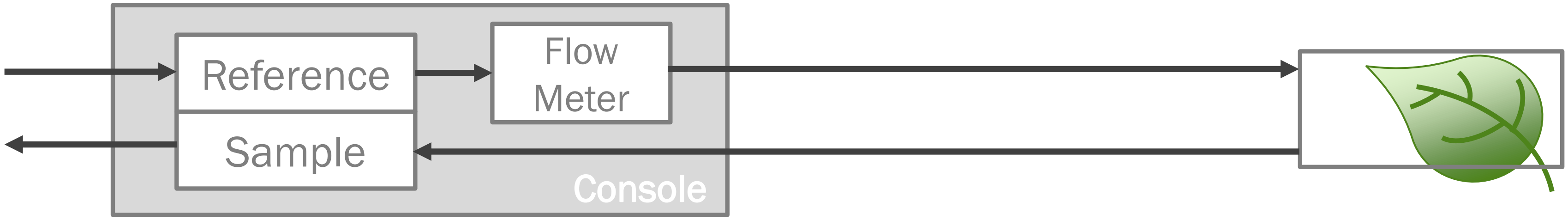


Gas exchange systems

- Closed-transient
- Compensating
- Open flow through

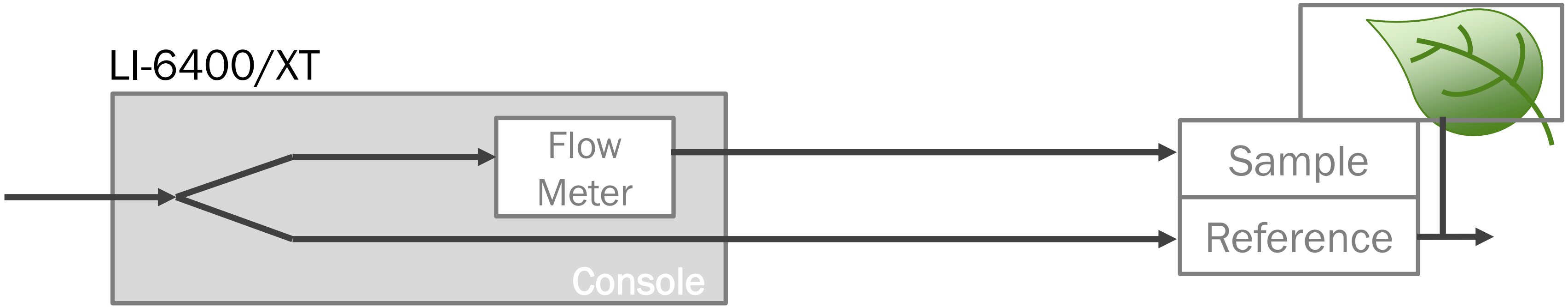
System flow path

Traditional open system



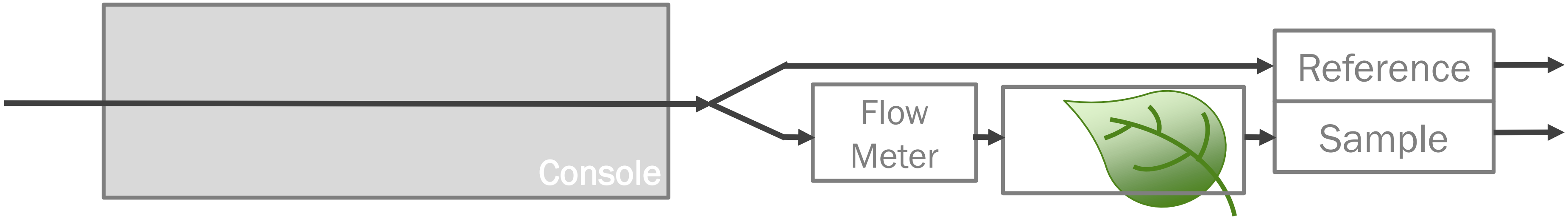
Reference
Flow Meter
Sample
Console

LI-6400/XT



Flow Meter
Sample
Reference
Console

LI-6800

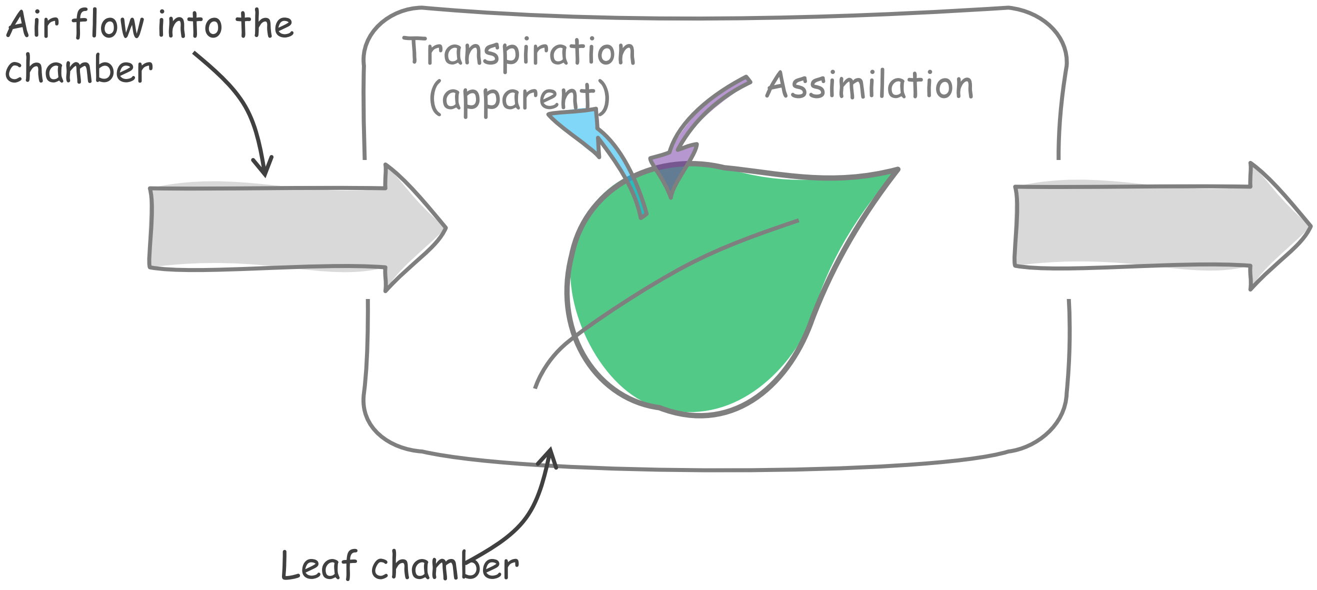


Console
Flow Meter
Reference
Sample

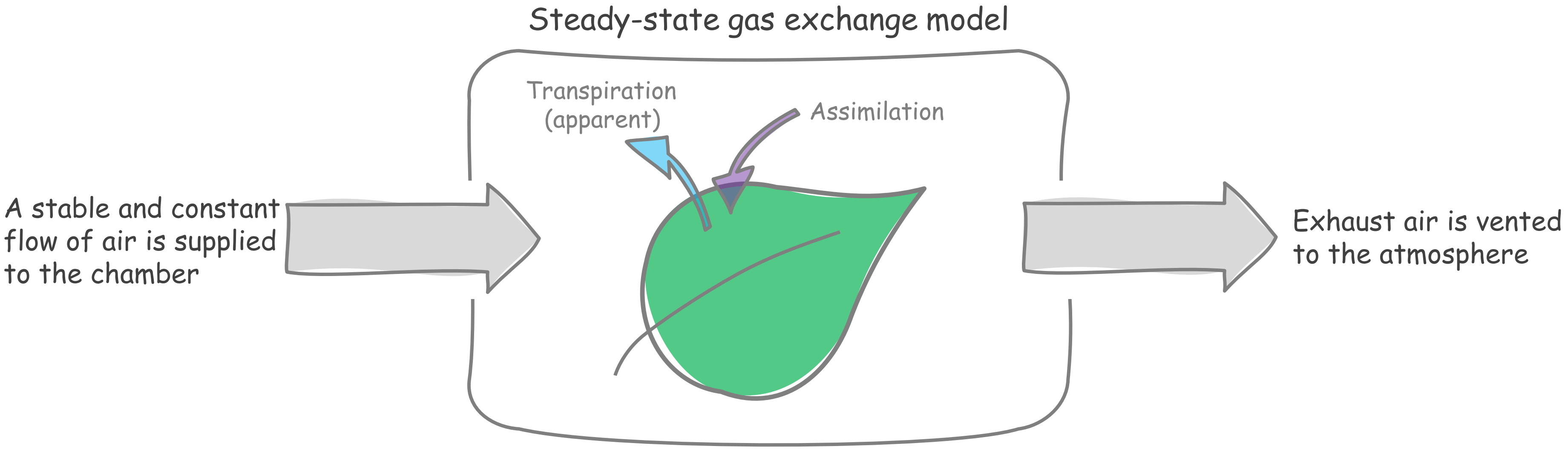
Basic parameters *computed*

- 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



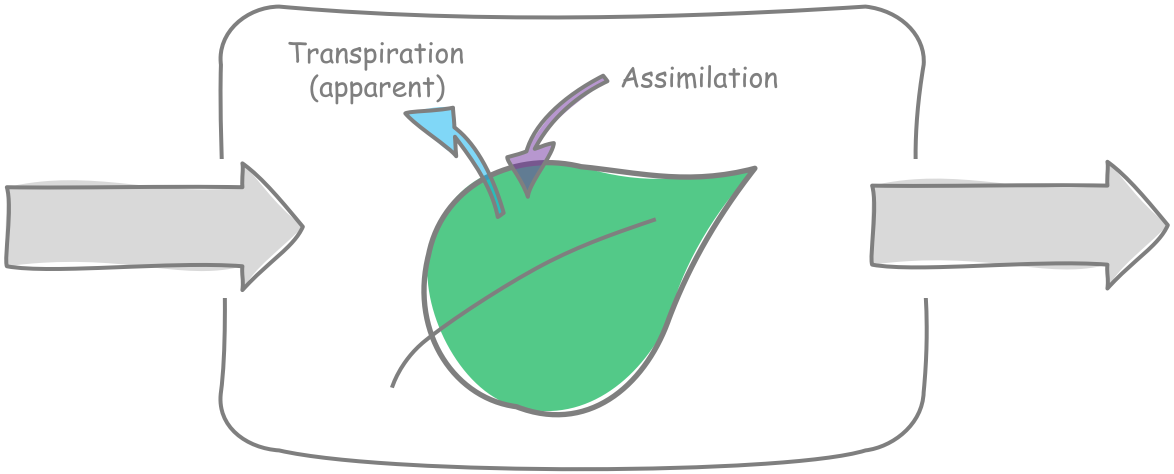
Mass balance for gas exchange



$$s f_x = u_{out} x_{out} - u_{in} x_{in}$$ Steady-state gas exchange model

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

Mass balance for gas exchange



$$sf_x = u_{out}x_{out} - u_{in}x_{in}$$

Steady-state gas model

$$sf_x = u_{out}x_{out} - u_{in}x_{in} + \rho v \frac{dx}{dt}$$

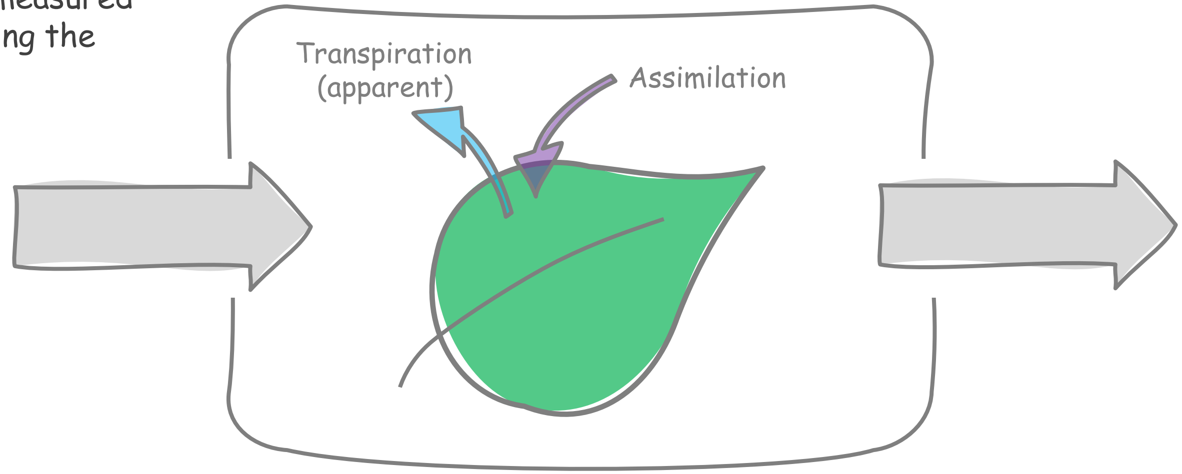
Dynamic model

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…

Dynamic gas exchange model



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.

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

E = Apparent transpiration

A = Carbon assimilation

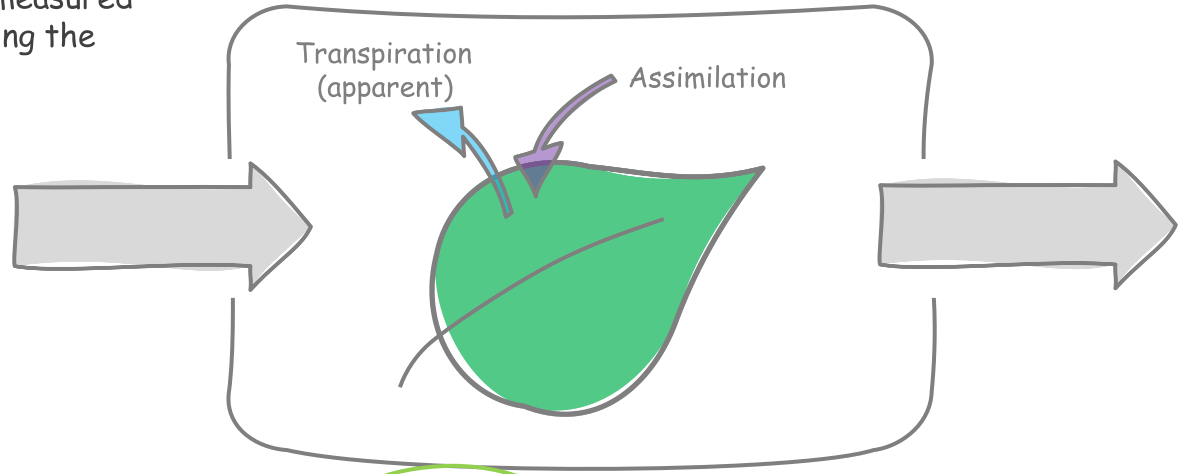
w_x = Water vapor mole fraction

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…

Dynamic gas exchange model



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E = Apparent transpiration

A = Carbon assimilation

w_x = Water vapor mole fraction

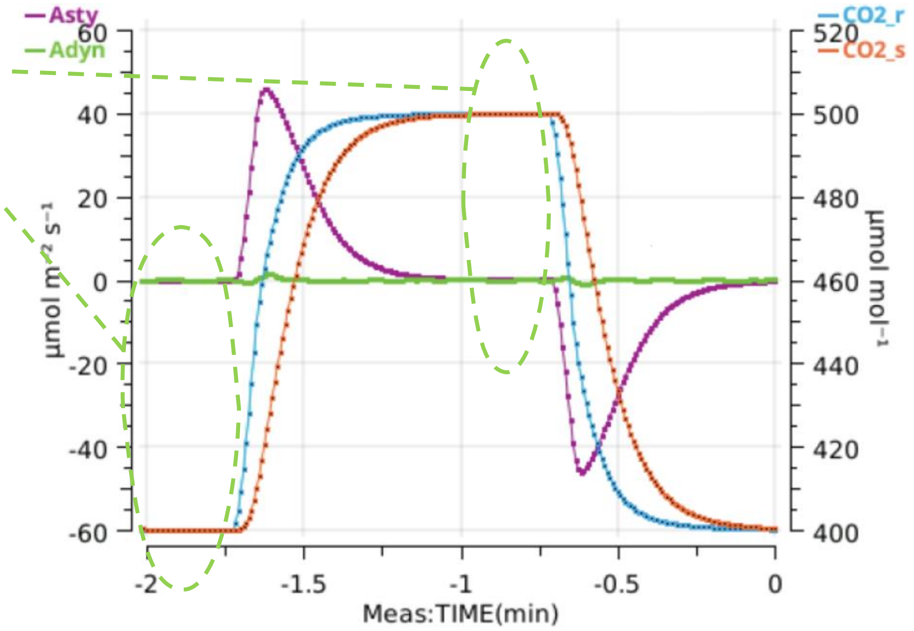
c'_x = CO_2 dry mixing ratio = $\frac{c_x}{1-w_x}$

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

Steady state versus dynamic

Example data from an empty chamber (Assimilation = 0)

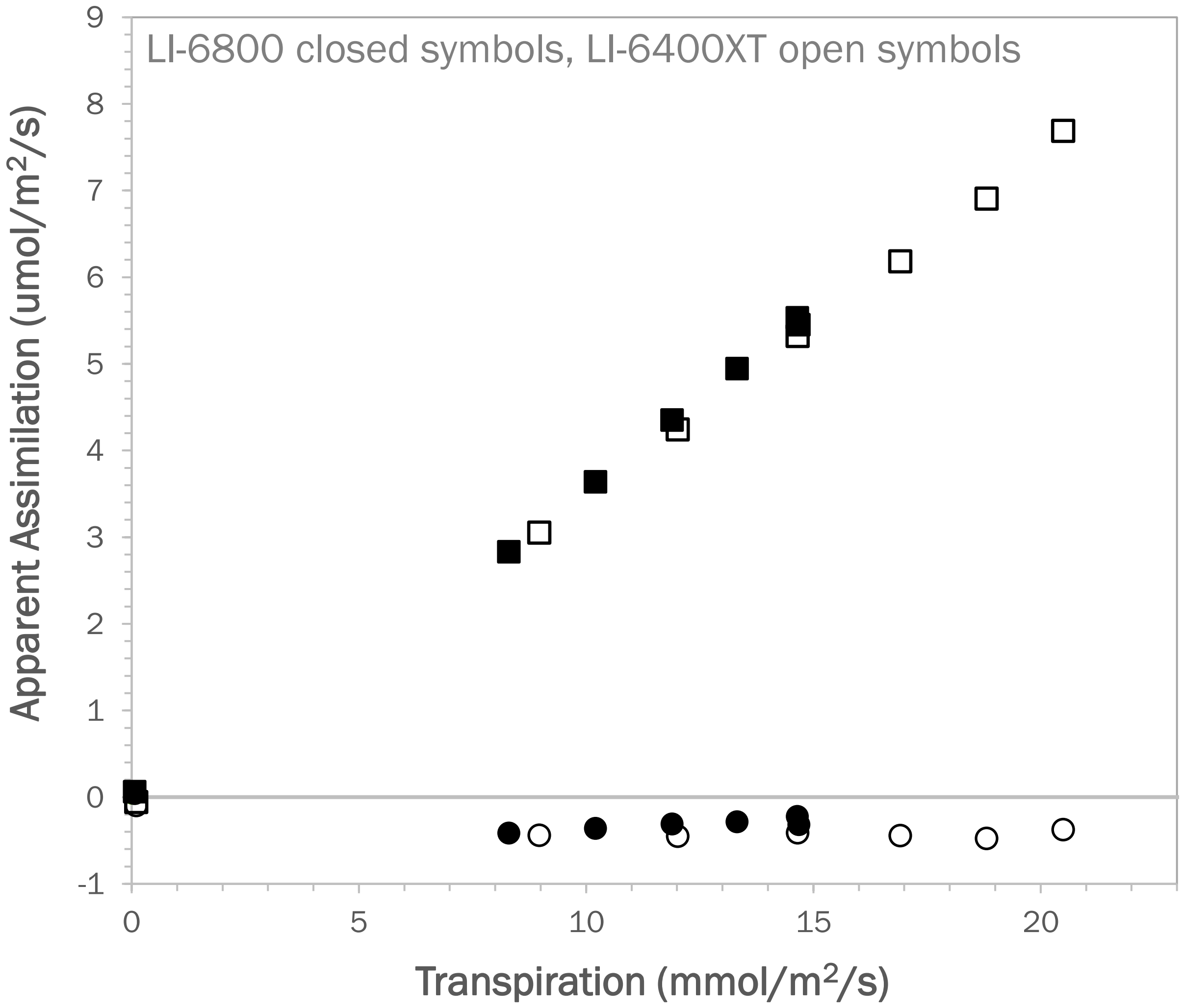
At steady-state the transient term is zero and the fluxes match



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.

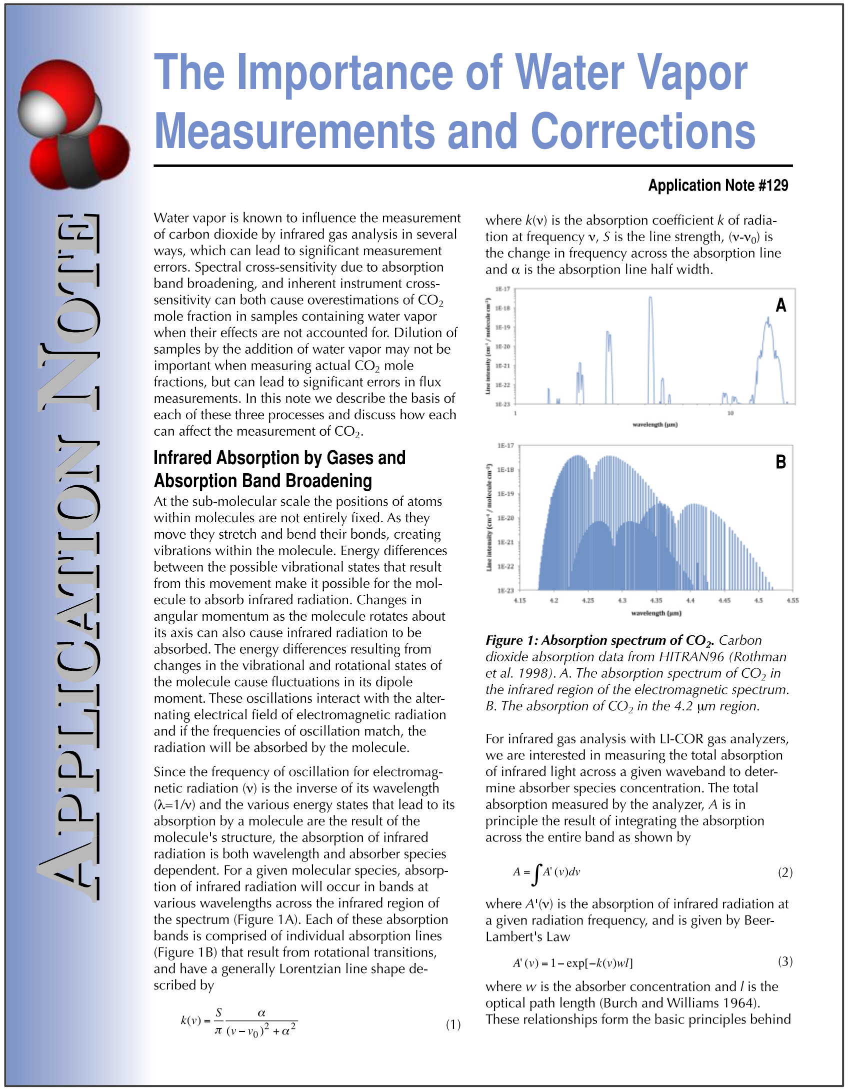
Accounting for dilution



$$A \approx \frac{F\left(C_r - C_s\right)}{100S}$$

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

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 CO_2 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 CO_2 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 CO_2.

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

$$k(v) = \frac{S}{\pi}\frac{\alpha}{(v-v_0)^2+\alpha^2} \qquad (1)$$

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 CO_2. *Carbon dioxide absorption data from HITRAN96 (Rothman et al. 1998). A. The absorption spectrum of CO_2 in the infrared region of the electromagnetic spectrum. B. The absorption of CO_2 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 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

$$A = \int A'(v)dv \qquad (2)$$

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

$$A'(v) = 1 - \exp[-k(v)wl] \qquad (3)$$

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

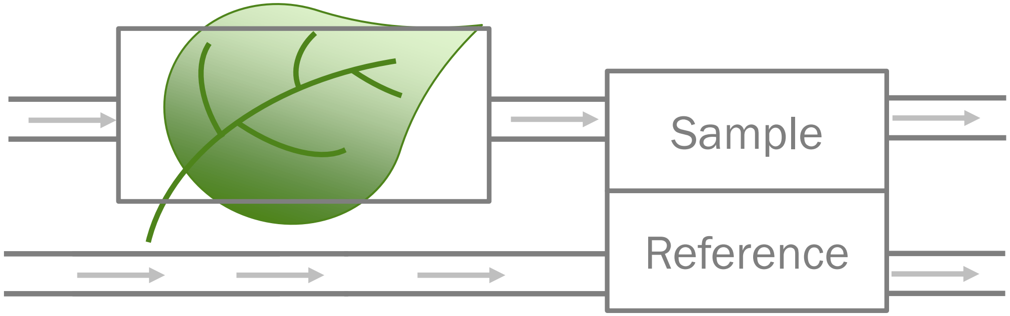
- Explains basis of broadening and effective pressure
- Derive dilution corrections
- How IRGAs work

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

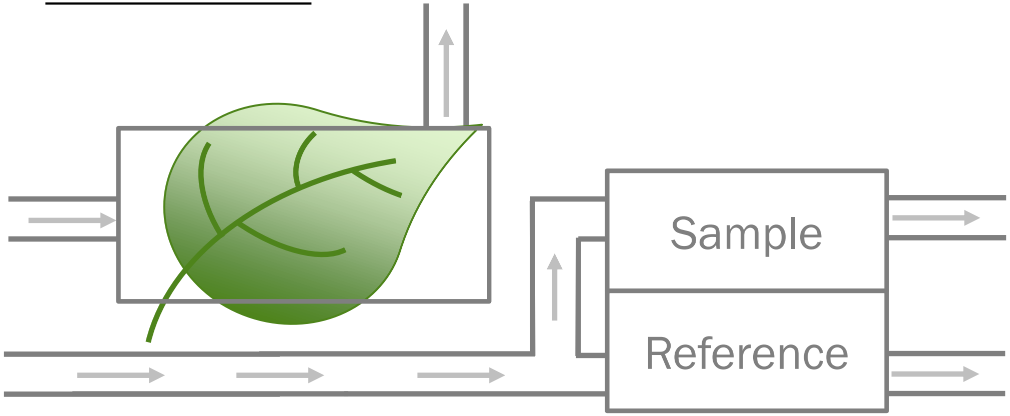
Matching IRGAs

LI-6800

Normal operating mode:



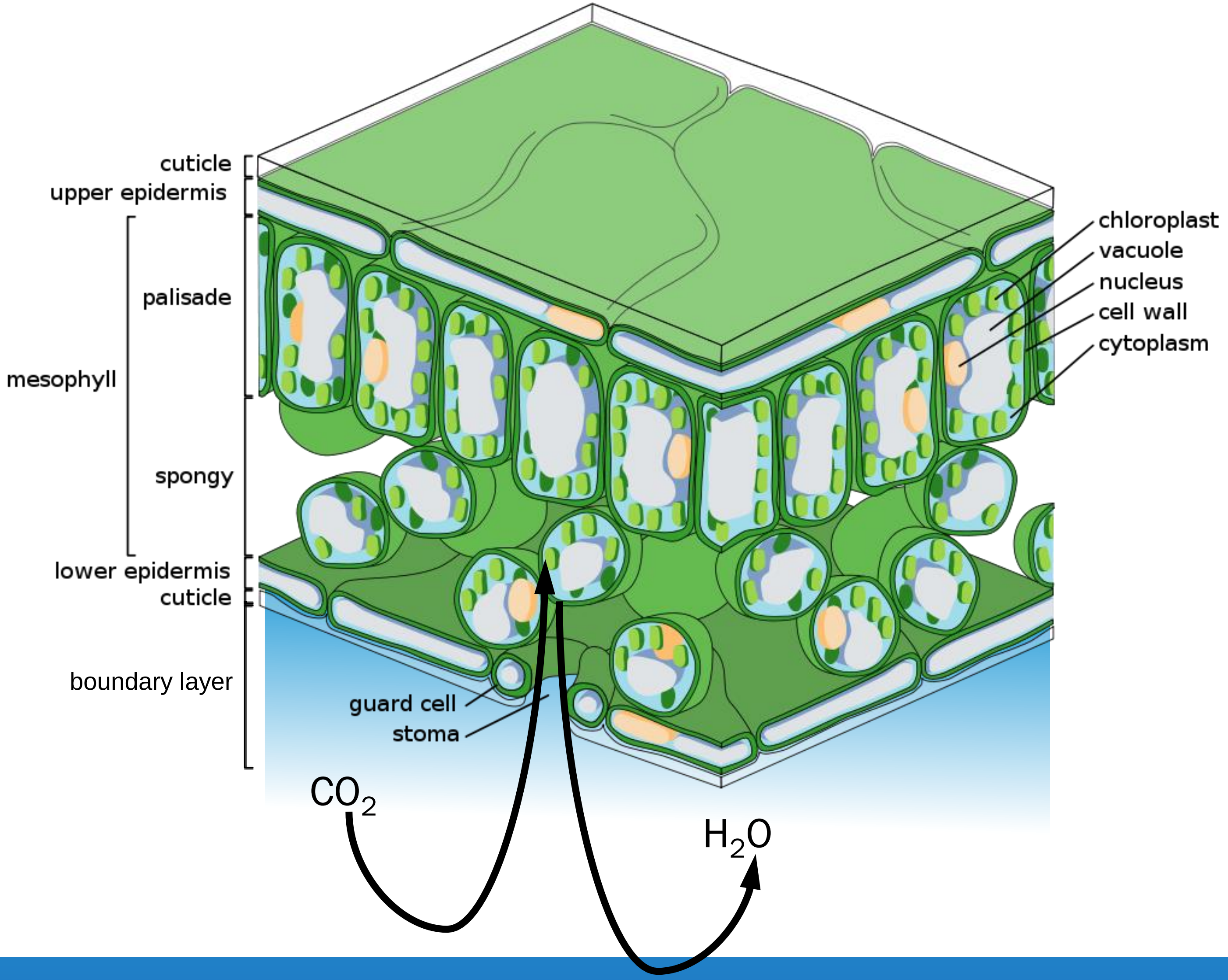
Match mode:



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 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_i = flux
D_j = diffusivity coefficient
$\delta c_j/\delta x$ = 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 ($c_a = C_s$)
- $g^{CO2} = g^{H2O}/1.6$
- Solve for c_i

What else can we determine with gas exchange?

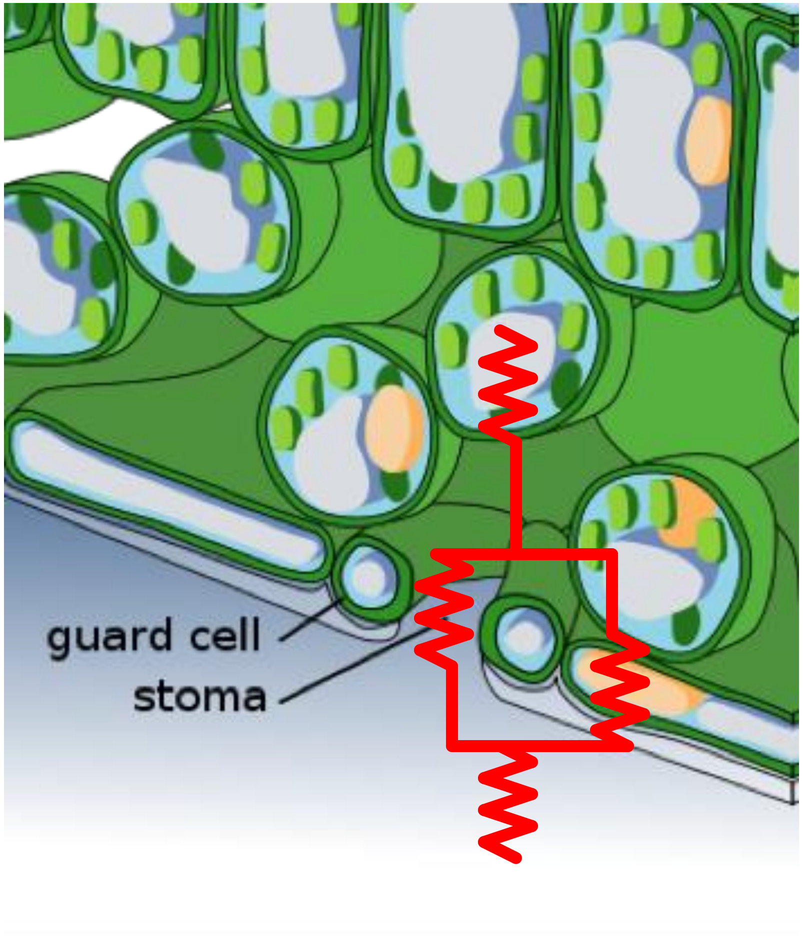
Ohm’s Law Analogy

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

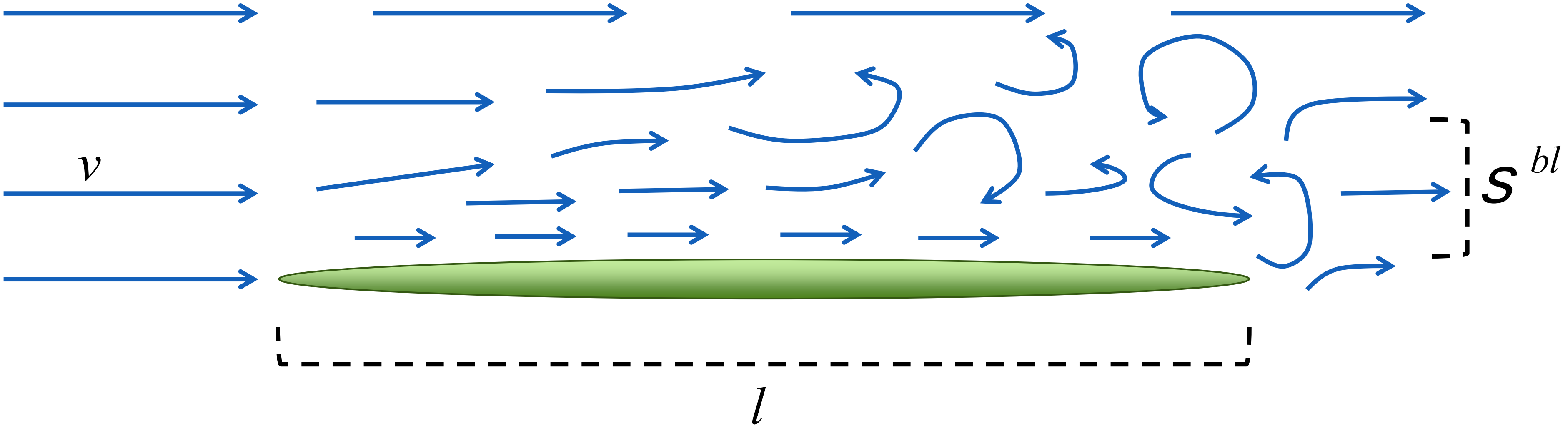
Assumptions:

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

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



Boundary layer

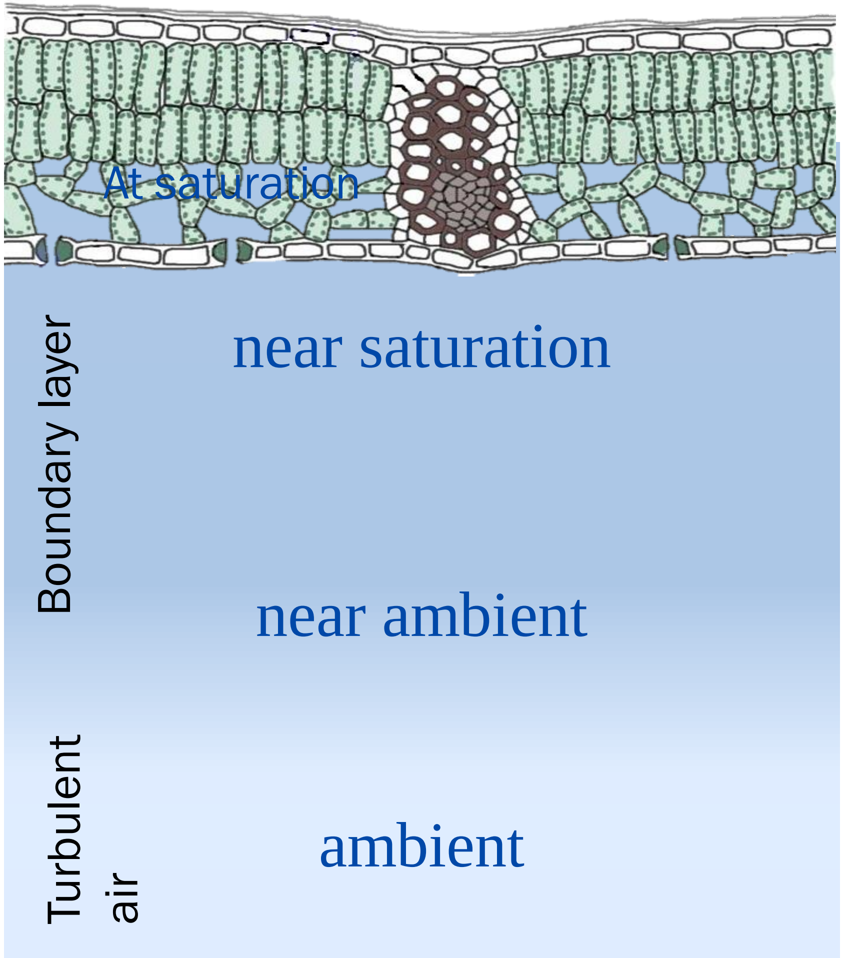


$$s^{bl} = 4\sqrt{\frac{l}{v}}$$

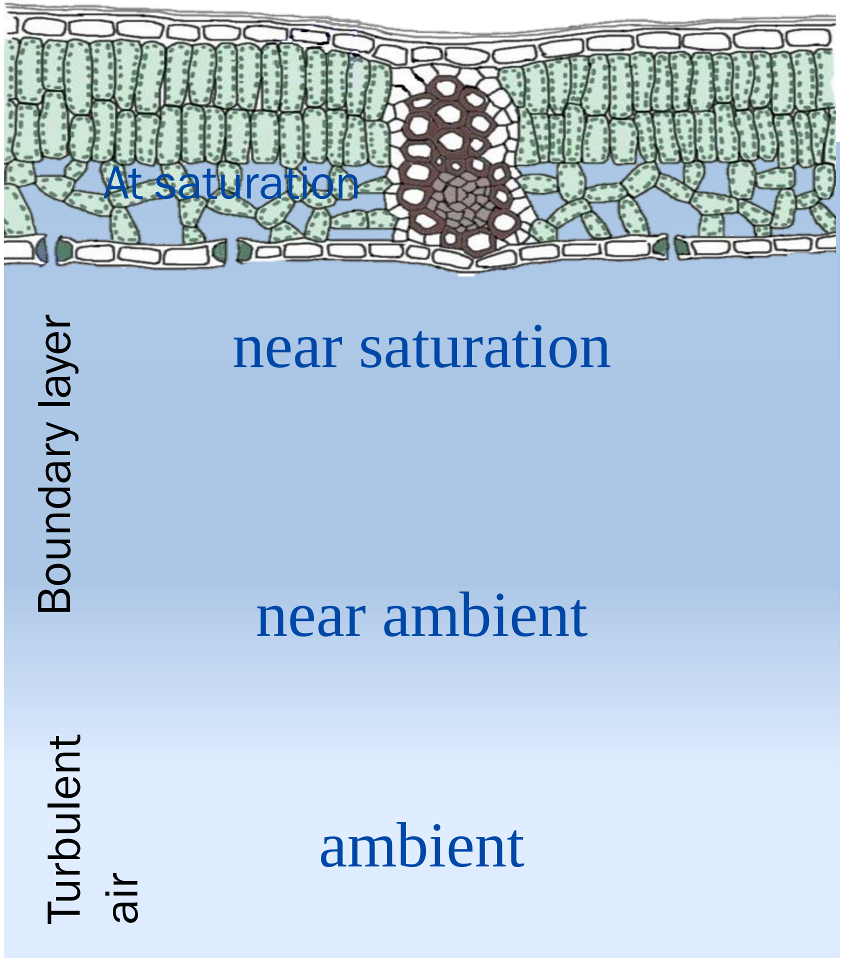
σ^{bl} = boundary layer thickness
v = air velocity
l = leaf width

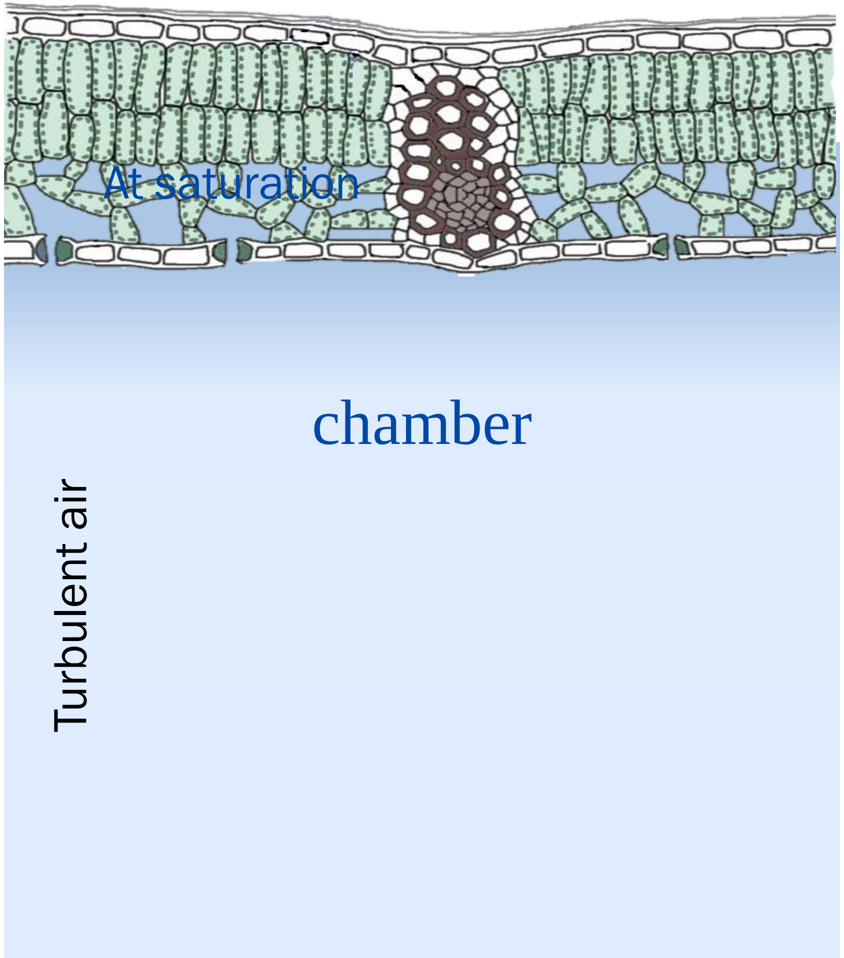
Biophysical Plant Physiology and Ecology, Park Nobel

Boundary layer conditions



Boundary layer conditions





What does this mean for making measurements?

- For H2O: Target ambient boundary layer conditions!

$$\mathrm{RH}\ (\%) = \frac{e_c}{e_{T_{air}}} * 100$$

$$e_c\ (kPa) = W_s P$$

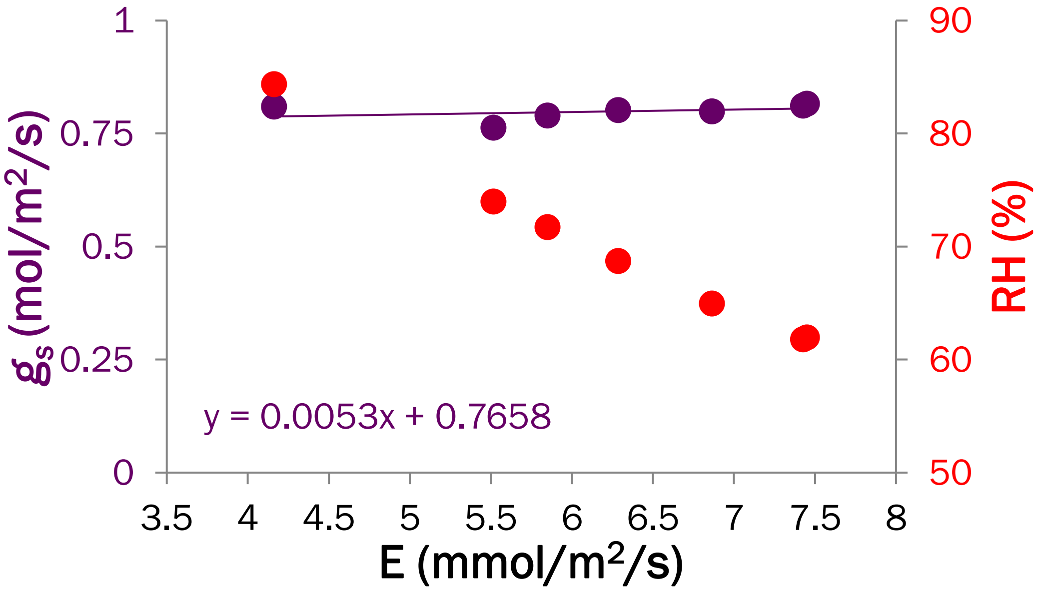
$$VPD_{leaf}(kPa) = e(T_{leaf})\text{-}e_c$$

On the LI-6800 use constant VPD (1 to 1.5 kPa)

What does this mean for interpreting the data?

- E = Real count of H_2O molecules leaving the leaf

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



What does this mean for interpreting the data?

- Water Use Efficiency
- Instantaneous versus Intrinsic

$$W_t = \frac{A}{E} = \frac{A}{g_s\left(w_i - w_a\right)} = \frac{A}{g_s D_a} = W_g D_a$$

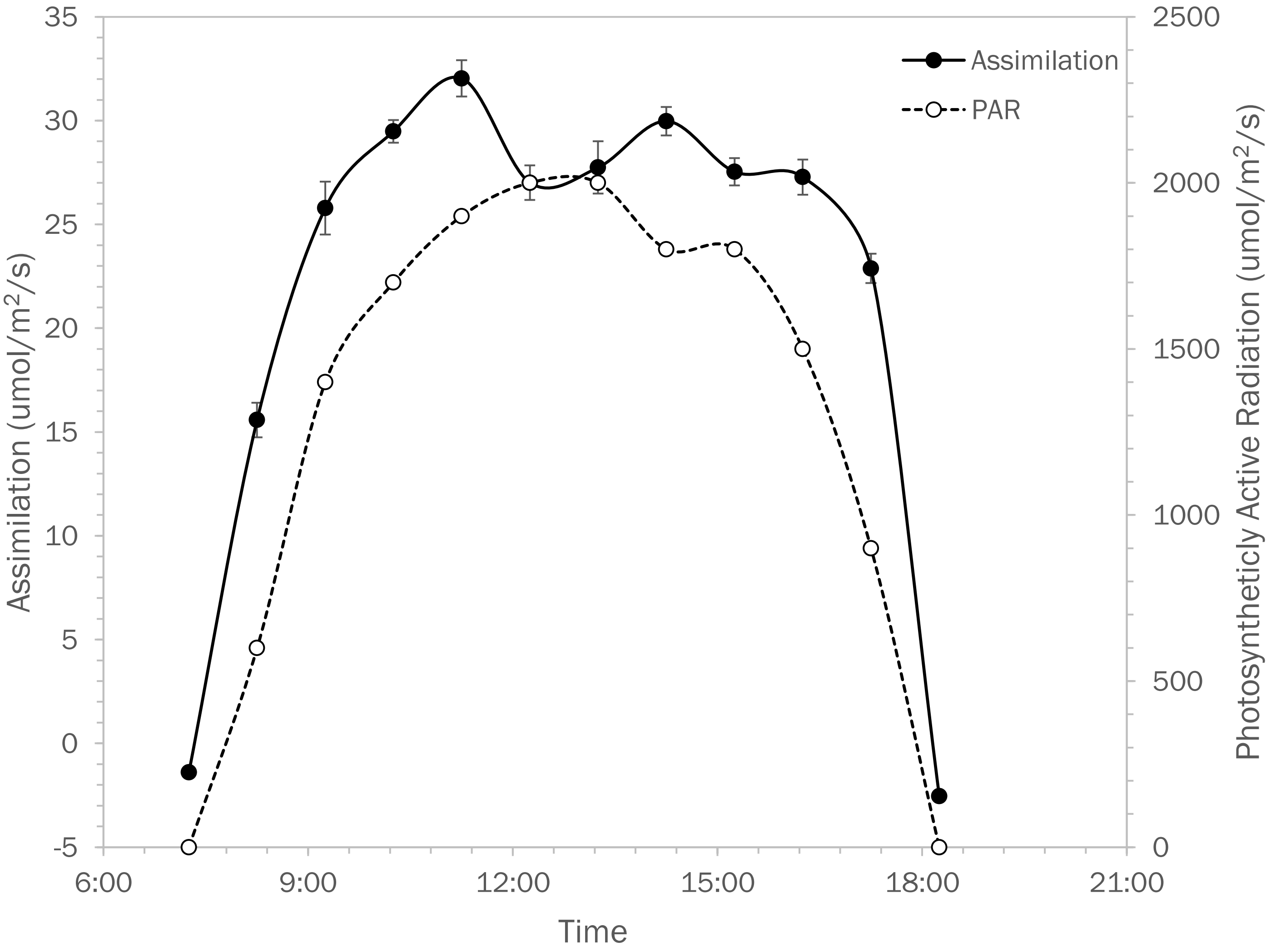
$$W_g = \frac{A}{g_s}$$

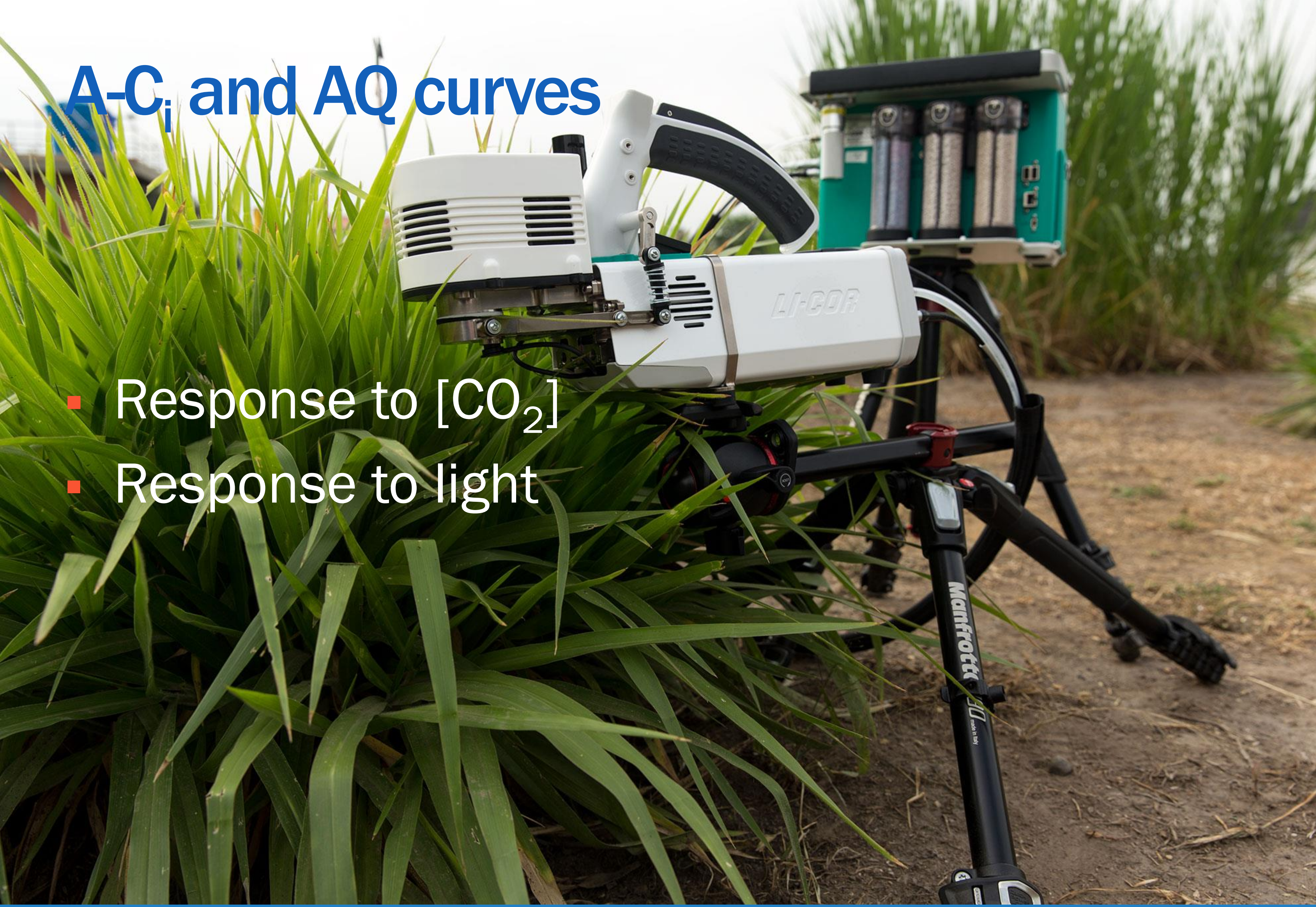
Seibt *et al*. 2008. *Carbon isotopes and water use efficiency: sense and sensitivity.* Oecologia

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?

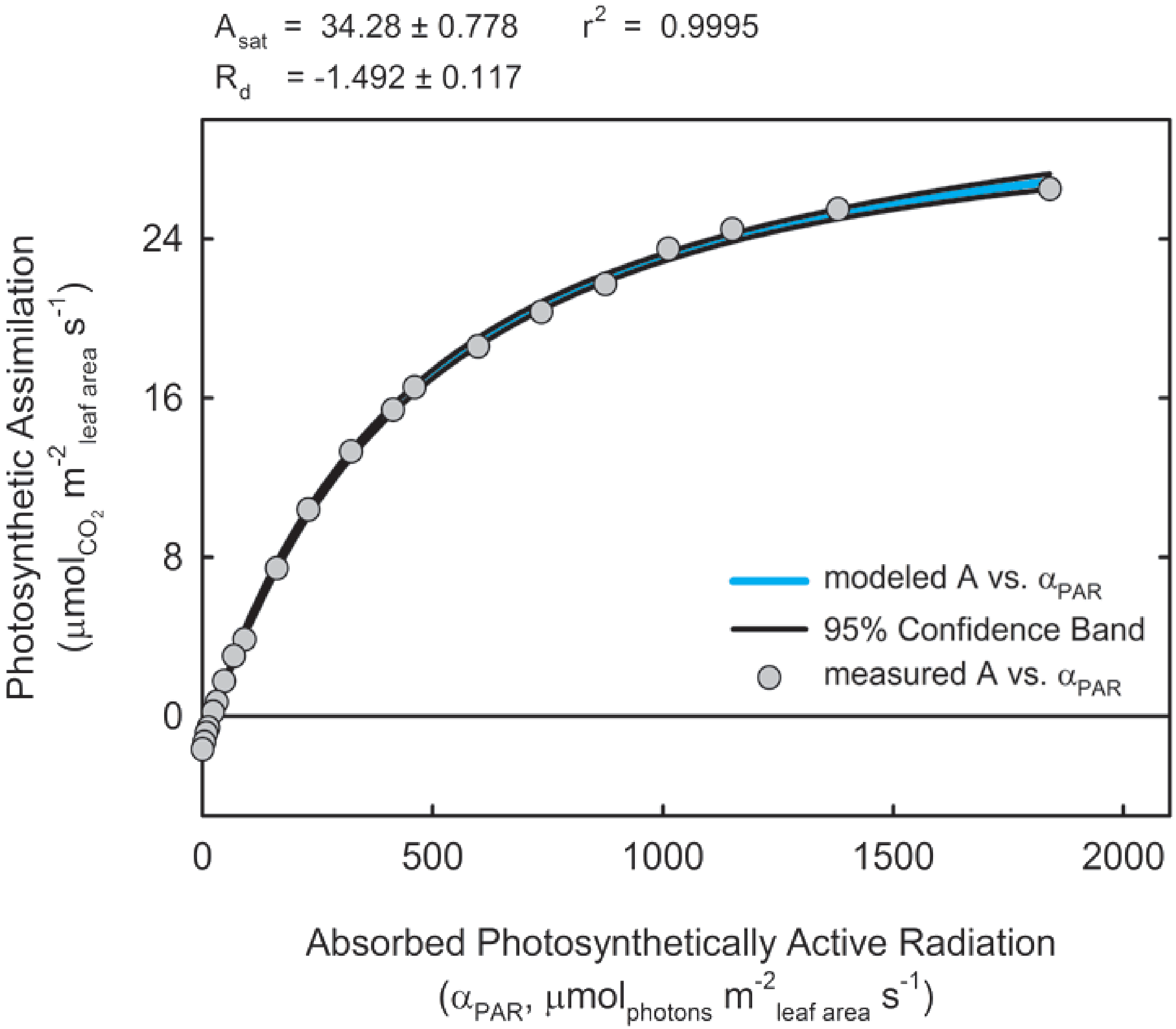




A-C_i and AQ curves

- Response to [CO_2]
- Response to light

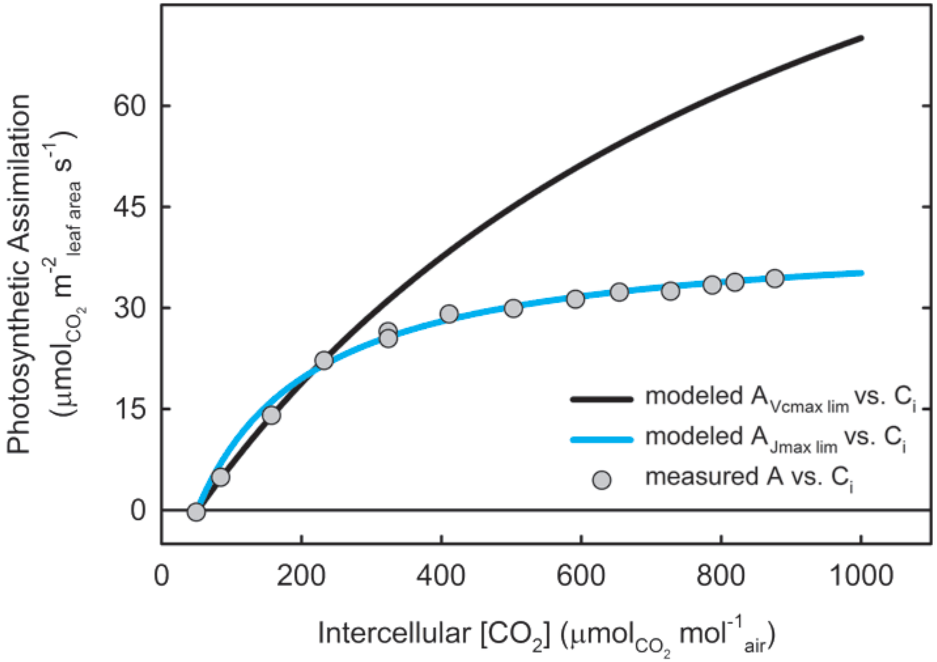
Parameters from AQ Response



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

Parameters from Ac_i response

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

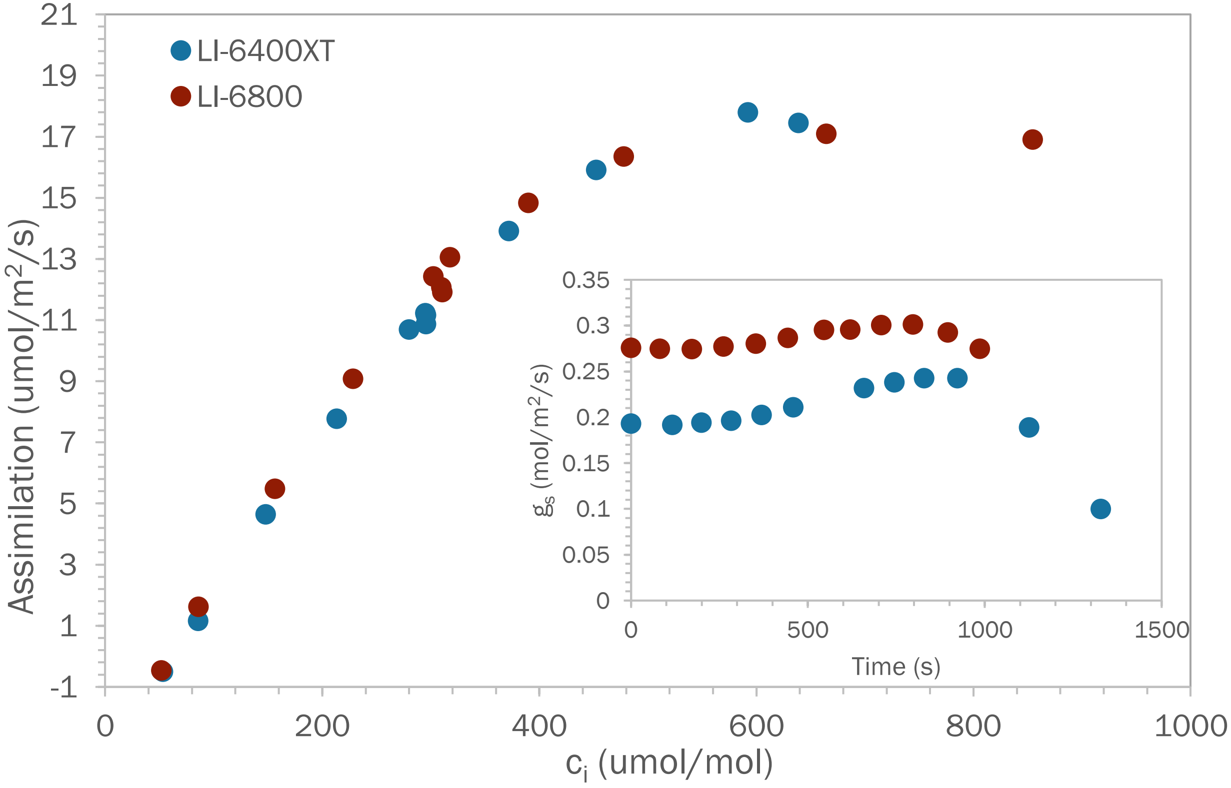


Considerations for Ac_i measurements

- 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

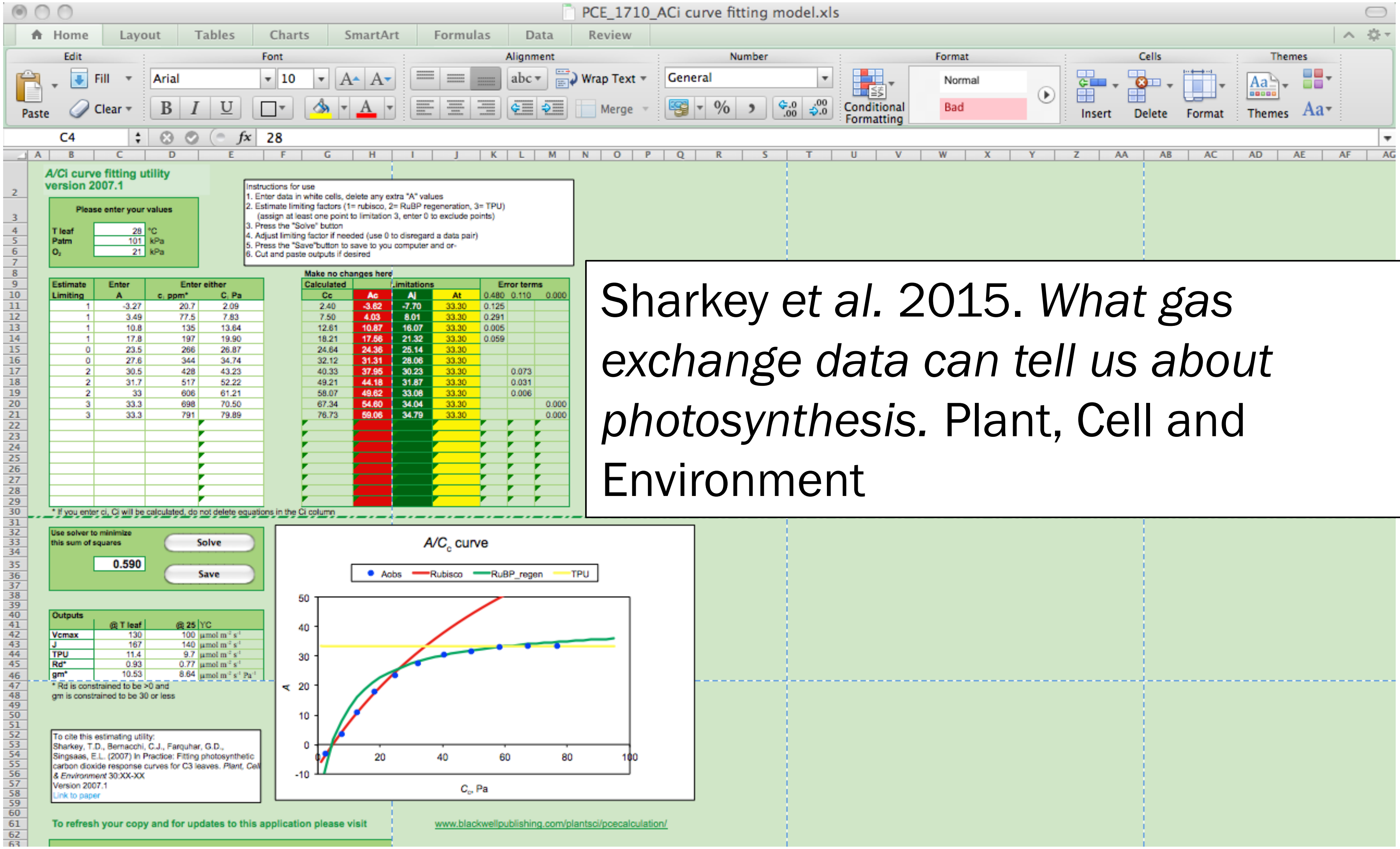
Using short time steps is important

- Rubisco deactivation
- Stomatal closure



AC$_i$ curve fitting tools

Sharkey *et al.* 2015. *What gas exchange data can tell us about photosynthesis.* Plant, Cell and Environment



C4: 28

A/Ci curve fitting utility
version 2007.1

Please enter your values

T leaf	28	°C
Patm	101	kPa
O$_2$	21	kPa

Instructions for use
1. Enter data in white cells, delete any extra "A" values
2. Estimate limiting factors (1= rubisco, 2= RuBP regeneration, 3= TPU)
   (assign at least one point to limitation 3, enter 0 to exclude points)
3. Press the "Solve" button
4. Adjust limiting factor if needed (use 0 to disregard a data pair)
5. Press the "Save"button to save to you computer and or-
6. Cut and paste outputs if desired

Estimate Limiting	Enter A	Enter either c$_i$ ppm*	C$_i$ Pa
1	-3.27	20.7	2.09
1	3.49	77.5	7.83
1	10.8	135	13.64
1	17.8	197	19.90
0	23.5	266	26.87
0	27.6	344	34.74
2	30.5	428	43.23
2	31.7	517	52.22
2	33	606	61.21
3	33.3	698	70.50
3	33.3	791	79.89

* If you enter ci, Ci will be calculated, do not delete equations in the Ci column

Make no changes here

Calculated	Limitations			Error terms		
Cc	Ac	Aj	At	0.480	0.110	0.000
2.40	-3.62	-7.70	33.30	0.125		
7.50	4.03	8.01	33.30	0.291		
12.61	10.87	16.07	33.30	0.005		
18.21	17.56	21.32	33.30	0.059		
24.64	24.36	25.14	33.30			
32.12	31.31	28.06	33.30			
40.33	37.95	30.23	33.30		0.073	
49.21	44.18	31.87	33.30		0.031	
58.07	49.62	33.08	33.30		0.006	
67.34	54.60	34.04	33.30			0.000
76.73	59.06	34.79	33.30			0.000

Use solver to minimize this sum of squares

0.590

Solve

Save

Outputs	@ T leaf	@ 25	YC
Vcmax	130	100	µmol m^{-2} s^{-1}
J	167	140	µmol m^{-2} s^{-1}
TPU	11.4	9.7	µmol m^{-2} s^{-1}
Rd*	0.93	0.77	µmol m^{-2} s^{-1}
gm*	10.53	8.64	µmol m^{-2} s^{-1} Pa^{-1}

* Rd is constrained to be >0 and gm is constrained to be 30 or less

To cite this estimating utility:
Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singsaas, E.L. (2007) In Practice: Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant, Cell & Environment* 30:XX-XX
Version 2007.1
Link to paper

To refresh your copy and for updates to this application please visit www.blackwellpublishing.com/plantsci/pcecalculation/

AC_i curve fitting tools

- Leafweb.ornl.gov
  - AC_i curve fitting and data sharing platform
  - Developed and hosted by Oak Ridge National Laboratory

- Plantecophys R package
  - Duursma RA. 2015. Plantecophys – *An R package for analysing and modeling leaf gas exchange data*. PLoS ONE

Other chamber options for the LI-6800

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

Questions?

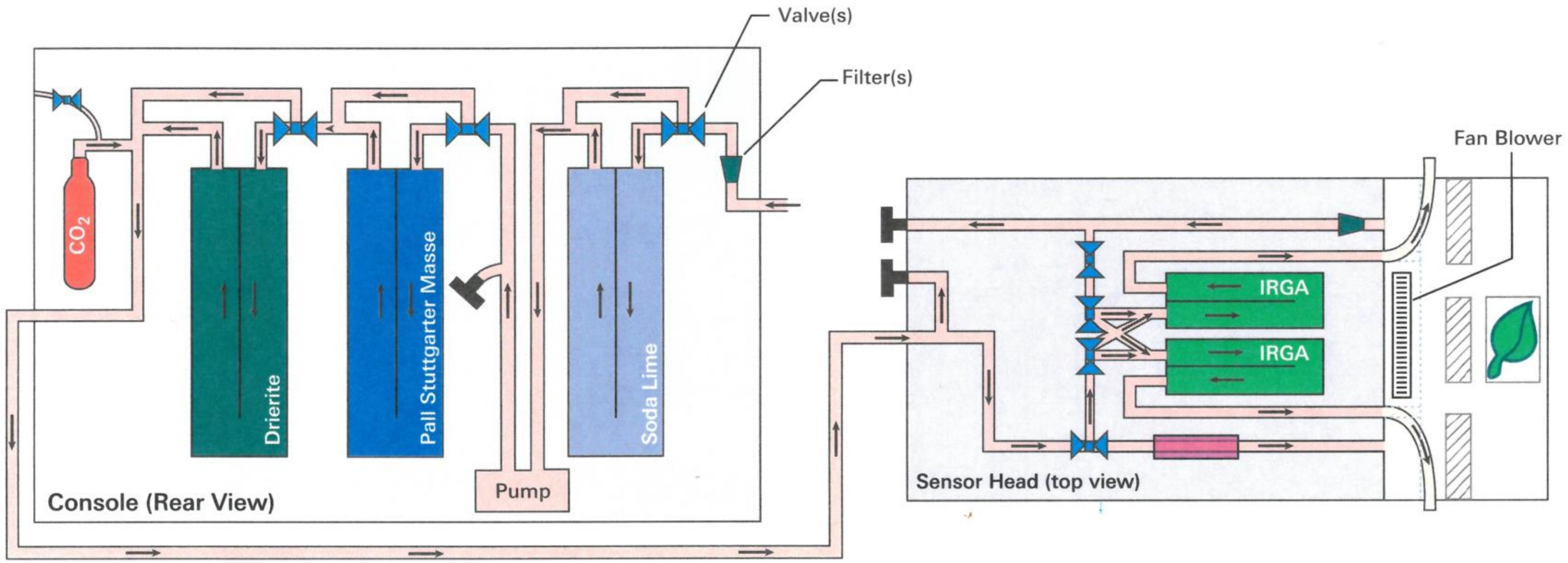
doug.lynch@licor.com

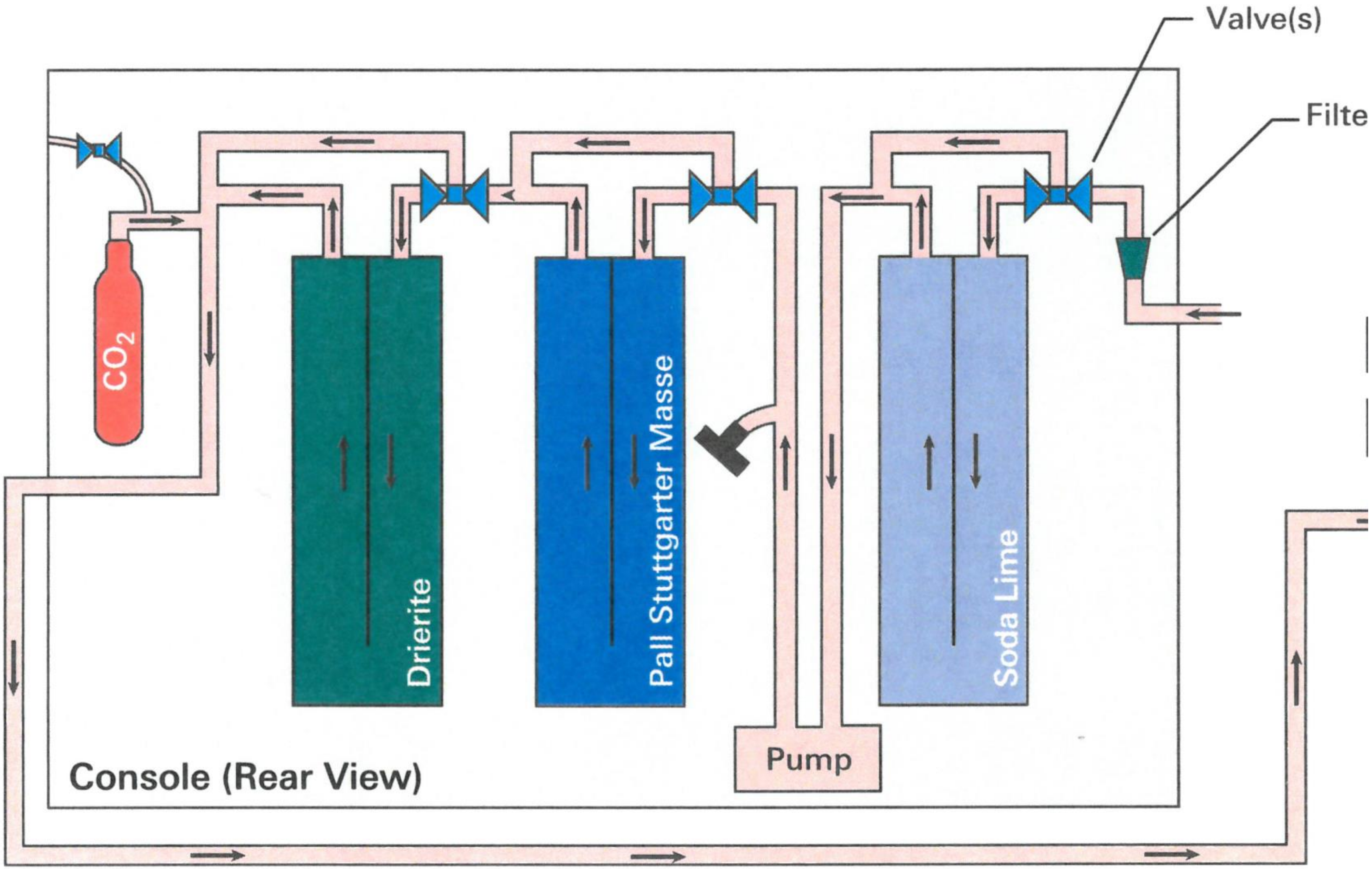
Selecting the right chamber?

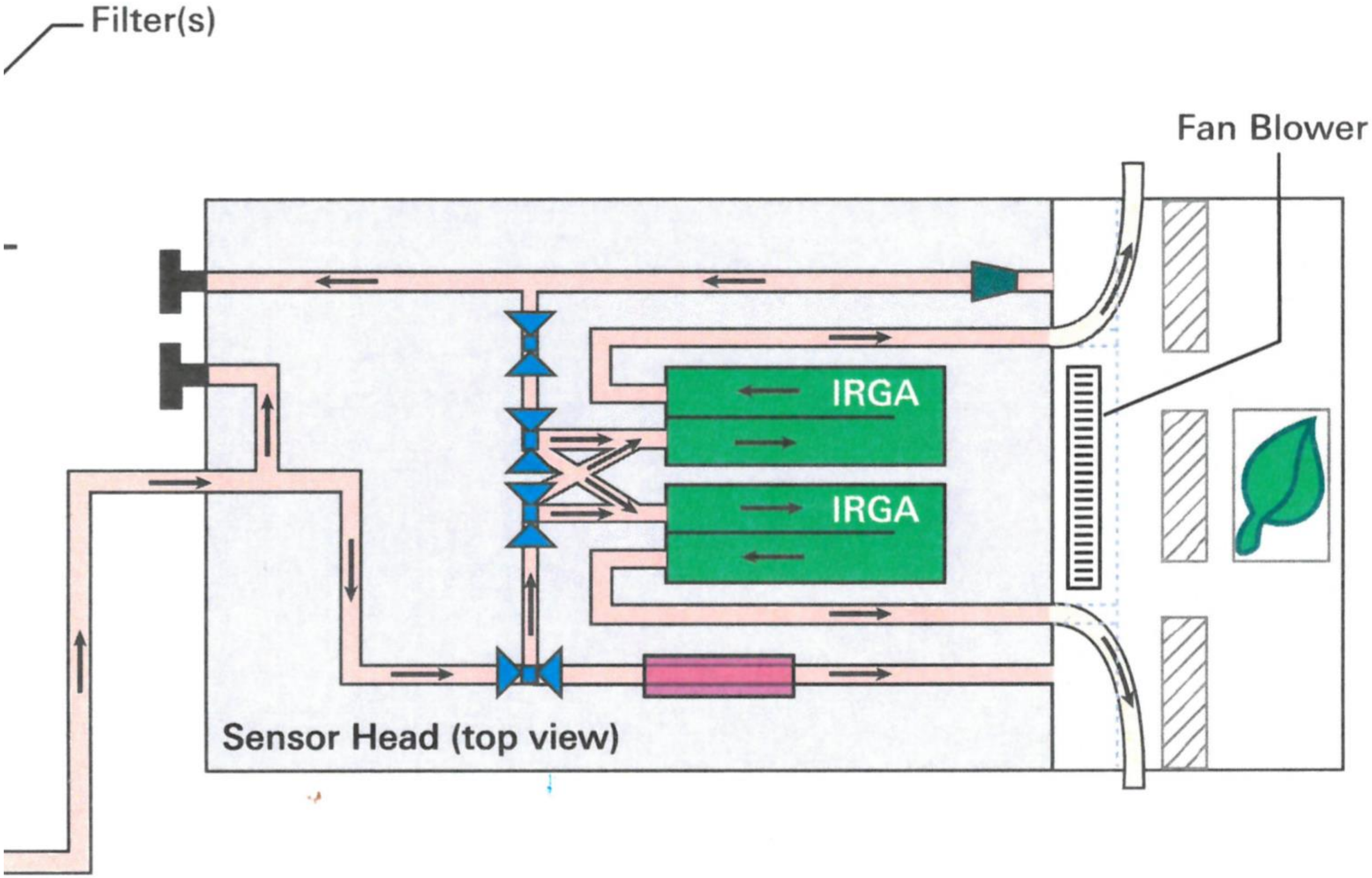
- Chamber aperture - bigger can be better!
- Light intensity control
- What data do you need?

LI-6800 Console flow path:

Rapid & stable software control of CO2 and H2O




Valve(s)
Filte
CO$_2$
Drierite
Pall Stuttgarter Masse
Soda Lime
Pump
Console (Rear View)


Filter(s)
Fan Blower
IRGA
IRGA
Sensor Head (top view)

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Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment

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