Leaf-level photosynthesis: theory and measurement

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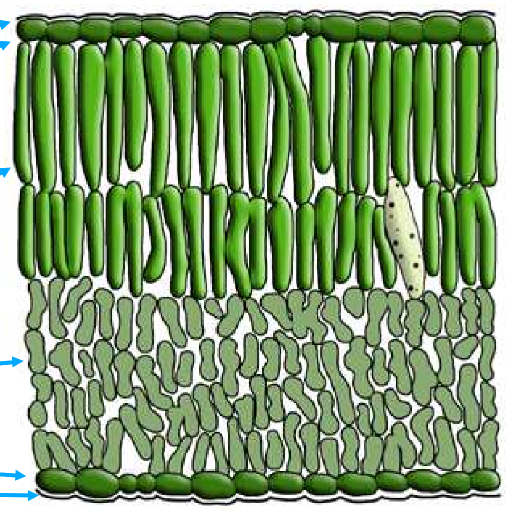
Why use gas exchange & fluorescence?

- Non-destructive way to investigate some aspects of photosynthesis
- Provides information on canopy CO_2 flux that can be used in models

Leaves:

- 4-10 cells thick
- Cuticle
- Upper epidermis
- Palisade mesophyll cells (~70% of chloroplasts)
- Spongy mesophyll cells
- Lower epidermis
- Cuticle



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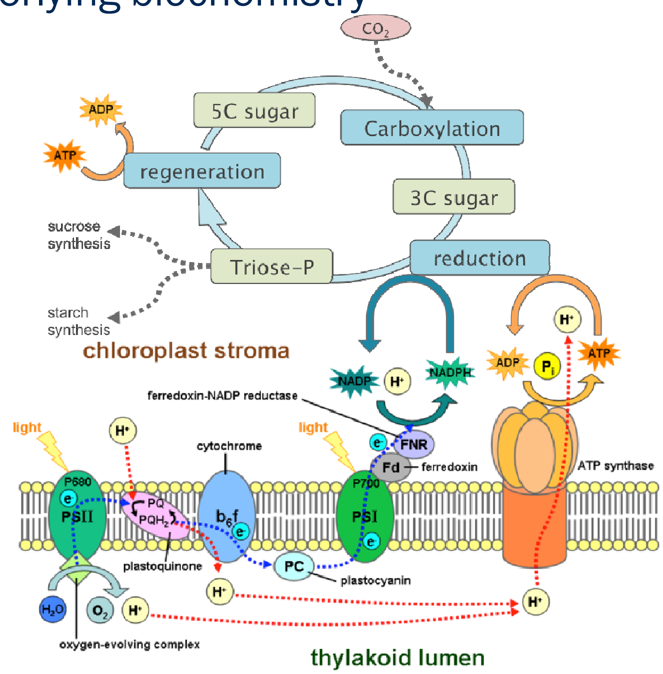
How do we measure photosynthesis?

Reviewing the basic underlying biochemistry

Dark reactions (also called light-independent reactions, Calvin-Benson-Bassham Cycle, reductive pentose phosphate cycle). These reactions fix CO_2 using ribulose 1,5-bisphosphate.

Light reactions (light dependent reactions) absorb light energy and convert it into chemical energy in the form of ATP and NADPH.



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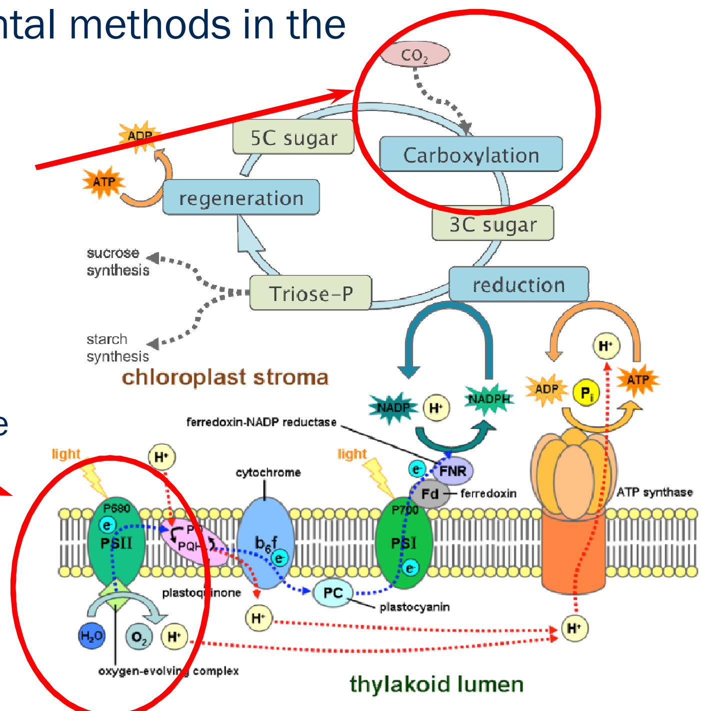
How do we measure photosynthesis?

We use two fundamental methods in the LI-6400 and LI-6800

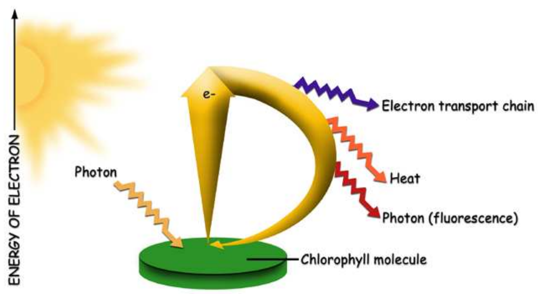
1. Gas exchange: CO_2 and H_2O

2. Chlorophyll Fluorescence

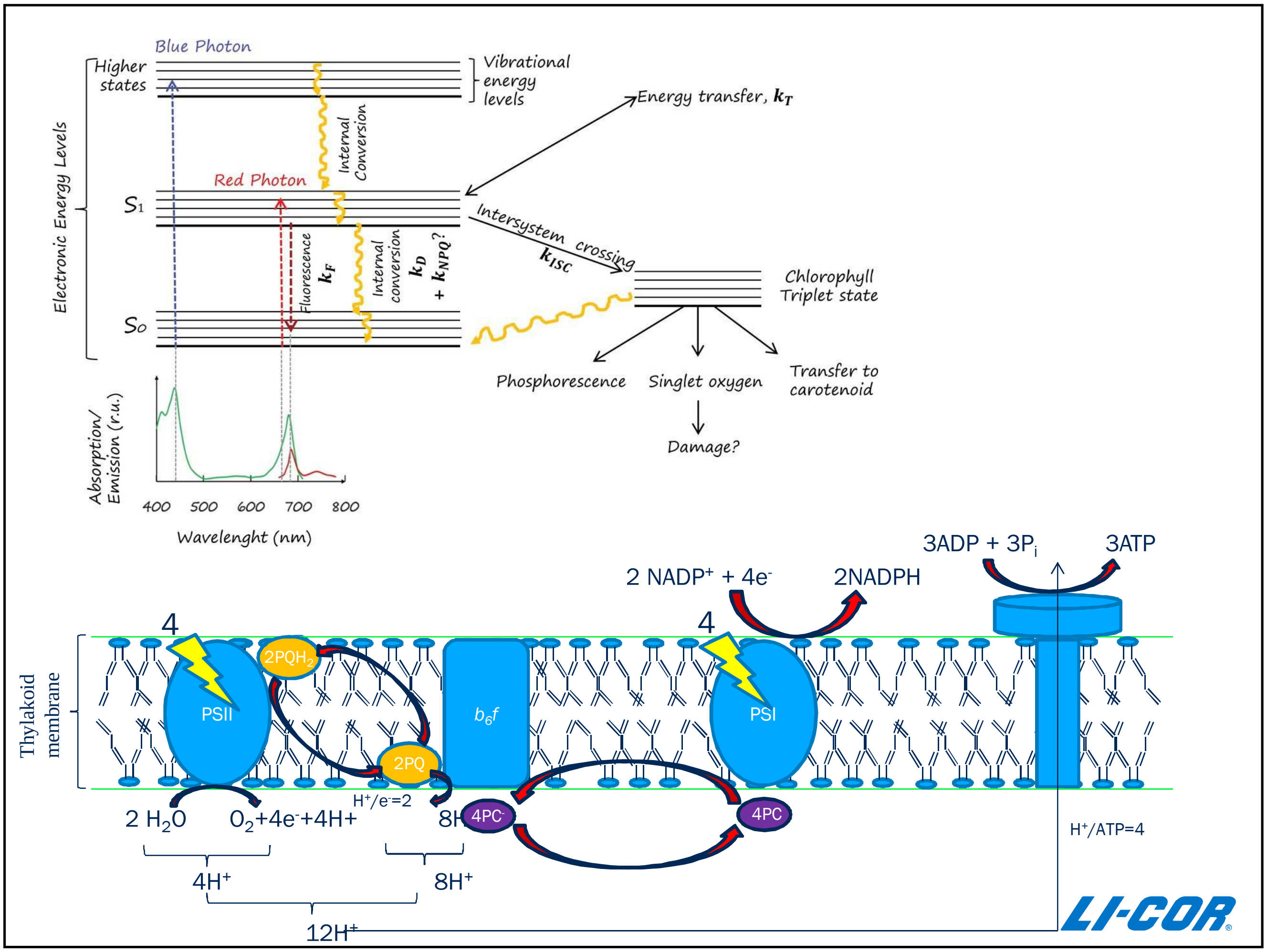


Chlorophyll fluorescence

- The basics

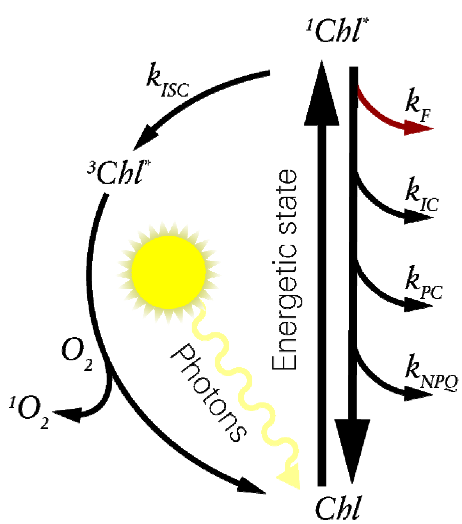


1. A photon is absorbed by a chlorophyll in the PSII antenna complex and chlorophyll enters an excited state
2. Energy from excited may eventually be funneled to reaction center chlorophyll
3. Absorption by reaction center chlorophyll results in e- becoming excited & entering a higher-energy orbital
4. Electron has various fates: (1) electron transport chain; (2) releases energy through NPQ; (3) release energy through fluorescence and (4) energy release through other processes


Blue Photon
Higher states
Vibrational energy levels
Electronic Energy Levels
Internal Conversion
Energy transfer, k_T
Red Photon
S1
Intersystem crossing
k_{ISC}
Fluorescence k_F
Internal conversion k_D + k_{NPQ}?
Chlorophyll Triplet state
S0
Phosphorescence
Singlet oxygen
Transfer to carotenoid
Damage?
Absorption/ Emission (r.u.)
400 500 600 700 800
Wavelenght (nm)
3ADP + 3P$_i$
3ATP
2 NADP$^+$ + 4e$^-$
2NADPH
4
4
2PQH$_2$
PSII
b_6f
PSI
Thylakoid membrane
2PQ
H$^+$/e$^-$=2
2 H$_2$0
0$_2$+4e$^-$+4H+
8H
4PC$^-$
4PC
H$^+$/ATP=4
4H$^+$
8H$^+$
12H$^+$

Chlorophyll fluorescence

- Fluorescence is one of several competing de-excitation pathways



Fluorescence Flux:

$$F_F = Q \frac{k_F}{\sum[k_F + k_{NPQ} + k_{PC} + k_{IC} + k_{ISC}]}$$

Fluorescence Yield:

$$\Phi_F = \frac{F_F}{Q} = \frac{k_F}{\sum[k_F + k_{NPQ} + k_{PC} + k_{IC} + k_{ISC}]}$$

Fluorescence parameters are defined by the rate constants

Dark adapted parameters (F_0 and F_m)

$$F_0 = \frac{k_F}{\sum[k_F + k_{PC[Q_A=1]} + k_{IC} + k_{ISC}]}$$

Note that in a dark-adapted state, all reaction centers are open and NPQ is zero when determining F_0.

$$F_m = \frac{k_F}{\sum[k_F + k_{IC} + k_{ISC}]}$$

Note that for F_m, all reaction centers are closed (Q_A = 0) and thus k_{PC} = 0. NPQ is still zero.

Fluorescence parameters are defined by the rate constants

Light adapted parameters (F_s and F_m')

$$F_s = \frac{k_F}{\sum\left[k_F + k_{NPQ[0<x\leq 1]} + k_{PC[0<Q_A\leq 1]} + k_{IC} + k_{ISC}\right]}$$

Note that in a light adapted state, some fraction of Q_A is open and NPQ is not zero.

$$F'_m = \frac{k_F}{\sum\left[k_F + k_{NPQ[0<x\leq 1]} + k_{IC} + k_{ISC}\right]}$$

Note that for F_m' all reaction centers are closed (Q_A = 0) during the flash and thus k_{PC} = 0. NPQ is NOT zero.

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What can chlorophyll fluorescence tell us?

- Quantum efficiencies

$$\frac{F_v}{F_m} \qquad \Phi_{PSII} = \frac{\Delta F}{F'_m}$$

- Electron transport rate

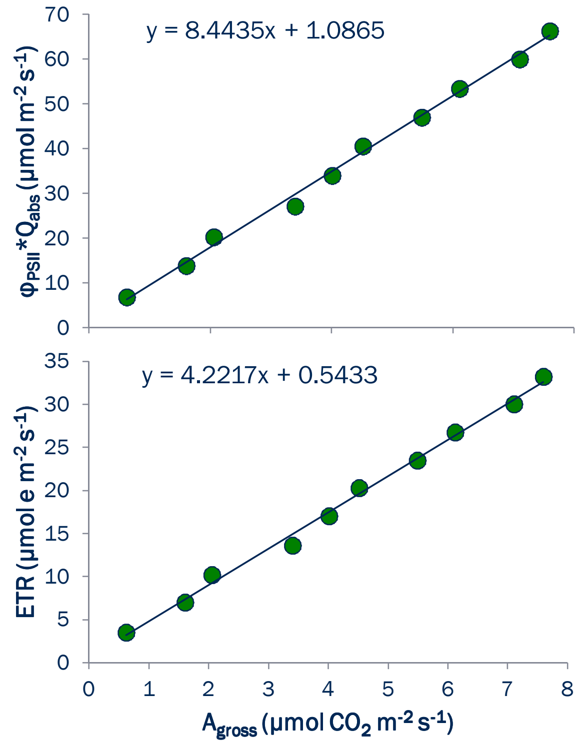
$$ETR = \Phi_{PSII} f Q \alpha_{leaf}$$

$f =$ Fraction of photons going to PSII

$\alpha_{leaf} =$ Absorption at measurement wavelengths

- Non-photochemical quenching

$$NPQ = \frac{F_m - F'_m}{F'_m}$$



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$$P_{dark} = \frac{F_m - F_o}{F_m} = \frac{F_v}{F_m}$$

$$P_{light} = \frac{F_m' - F_s}{F_m'} = \frac{\Delta F}{F_m'} = \Phi_{PSII}$$

$$ETR = \left(\frac{F_m' - F_s}{F_m'}\right) fI\alpha_{leaf}$$

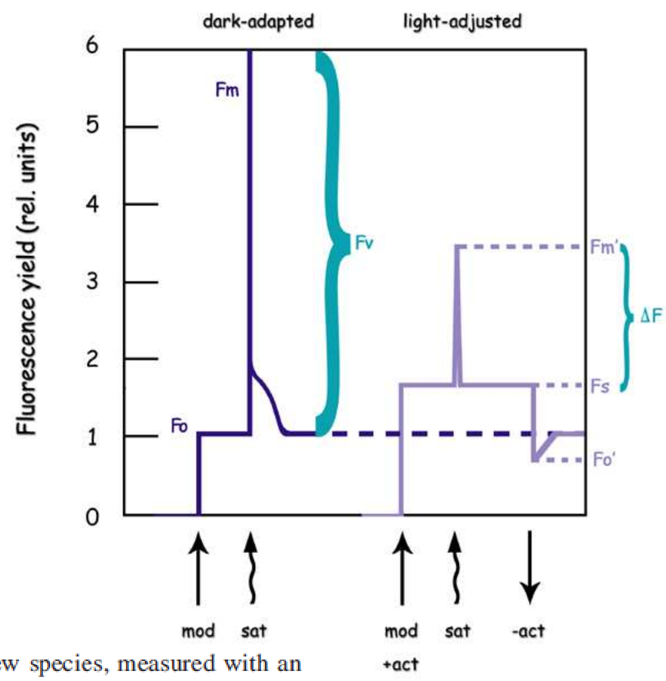
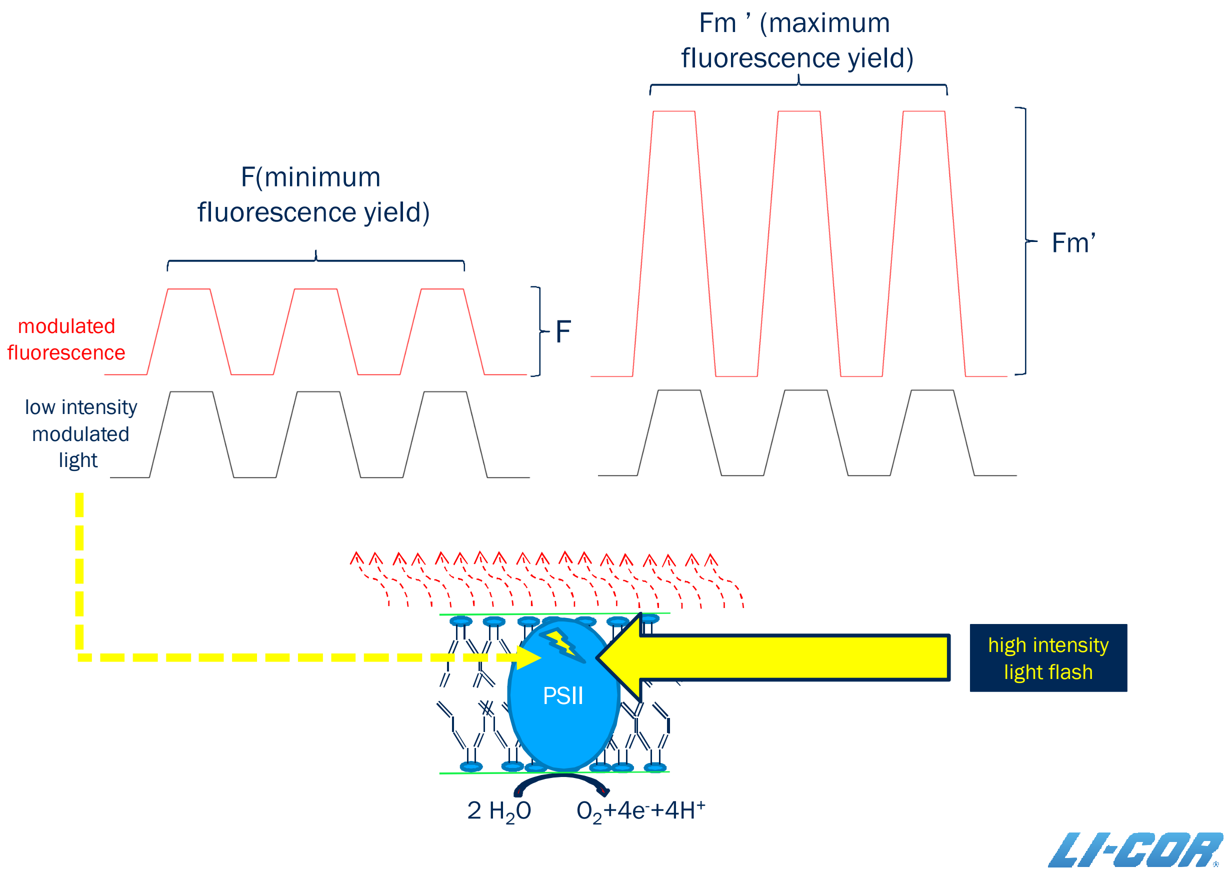


Table 27-15. Leaf absorptances in blue and red for a few species, measured with an LI-1800 spectroradiometer.

Species	α_{blue}	α_{red}
Maize	0.90	0.85
Bean	0.91	0.83
Jasmine	0.92	0.87
Orange	0.94	0.93

Pulse amplitude modulated (PAM) fluorescence



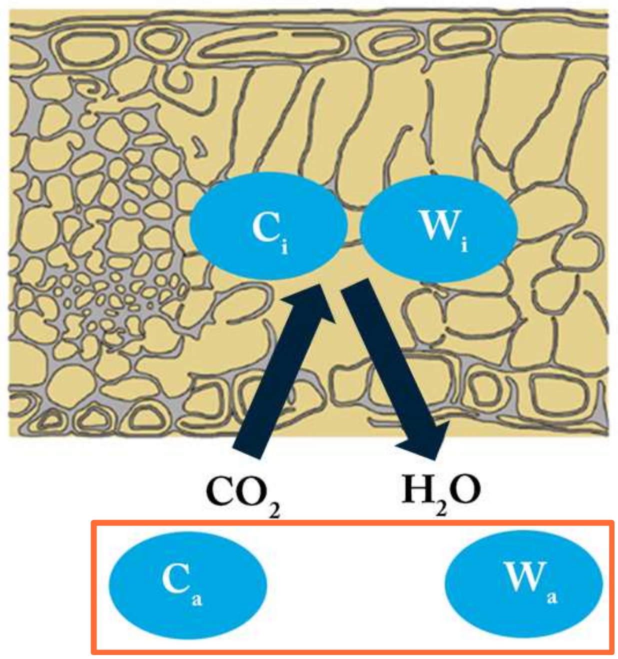
Some useful references

- Overview:
  - Maxwell and Johnson. 2000. *Chlorophyll fluorescence – a practical guide*. Journal of Experimental Botany
  - Murchie and Lawson. 2013. *Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications*. Journal of Experimental Botany
  - Porcar-Castell *et al.* 2014. *Linking chlorophyll a fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges*. Journal of Experimental Botany
- Application:
  - Baker and Rosenqvist. 2004. *Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities*. Journal of Experimental Botany

Gas Exchange: Theory and calculations

Gas exchange – what do the LI-6800 and LI-6400 measure?

- The LI-6800 and LI-6400 fundamentally measure the CO_2 and water vapor concentrations in the air surrounding the leaf.
- CO_2 and water vapor concentrations are also known of the air before it enters the leaf chamber.
- Leaf temperature is measured using a leaf thermocouple that is directly in contact with the leaf.

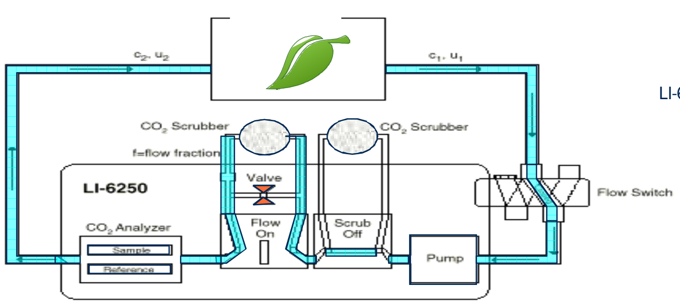


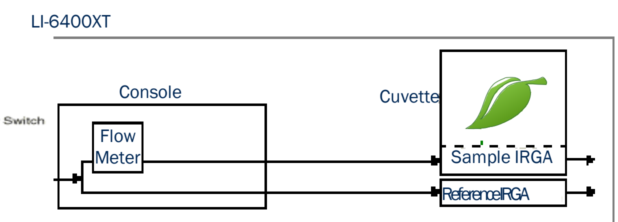
How are photosynthesis & transpiration measured in an enclosure?

- **Closed System**
  $A = \Delta CO_2\ V\ (\Delta t\ S)^{-1}$
- **No air enters or leaves the system**
  - Leaks can cause large errors
- Transient measurement
  CO_2, H_2O, T & P changes

- **Open System**
  $A = (u_e c_e - u_o c_o)\ S^{-1}$
- Flow of air must be constant & known (accurate flow meter)
- Steady-state measurement
  controlling environmental variables

LI-6250 Flow Schematic





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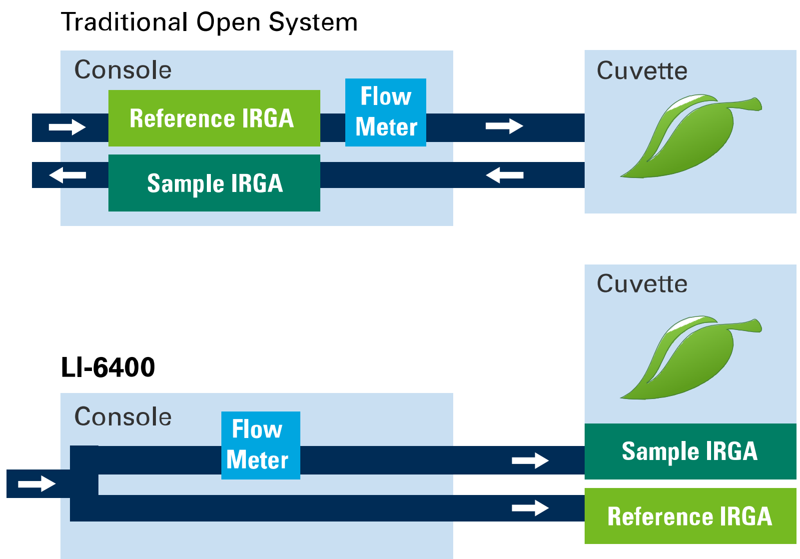
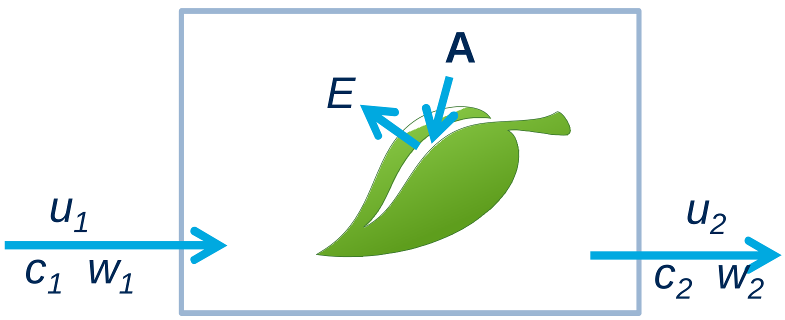
Open system design variations



$$\text{Photo} = \frac{\text{Flow} \times \Delta CO_2}{\text{Area}} \qquad \text{Trans} = \frac{\text{Flow} \times \Delta H_2O}{\text{Area}}$$

Mass balance in an open system



- 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

- A simple representation of transpiration (E) and CO_2 assimilation (A):

$$E \approx \frac{u(w_2 - w_1)}{s} \approx \frac{u(\Delta w)}{s} \approx \frac{flow \times (\Delta\, concentration)}{leaf\ area}$$

$$A \approx \frac{u(c_2 - c_1)}{s} \approx \frac{u(\Delta c)}{s} \approx \frac{flow \times (\Delta\, concentration)}{leaf\ area}$$

In reality, mass balance is a little more complicated for calculating E and A!

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Mass balance for H_2O (water vapor)

- Mass balance is fundamental to instrument operation
- Mass balance is used to compute transpiration (E) and assimilation (A)
- Basic mass balance setup for H_2O in leaf chamber:

$$\begin{Bmatrix} Rate\ of\ flow\ of \\ H_2O\ into\ system \\ (mole\ s^{-1}) \end{Bmatrix} - \begin{Bmatrix} Rate\ of\ flow\ of \\ H_2O\ out\ of\ system \\ (mole\ s^{-1}) \end{Bmatrix} + \begin{Bmatrix} Rate\ of\ generation\ of \\ H_2O\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix} = \begin{Bmatrix} Rate\ of\ accumulation\ of \\ H_2O\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix}$$

$$u_1 w_1 - u_2 w_2 + sE = \frac{dW}{dt}$$

Variable definitions	
u_1, u_2	Air flow rate (mole air s^{-1})
w_1, w_2	H_2O mole fraction (mole H_2O mole air^{-1})
s	Leaf area (m^2)
E	Leaf transpiration (mole H_2O m^{-2} s^{-1})
$\frac{dW}{dt}$	Change in H_2O moles in leaf chamber per unit time (mole H_2O s^{-1})

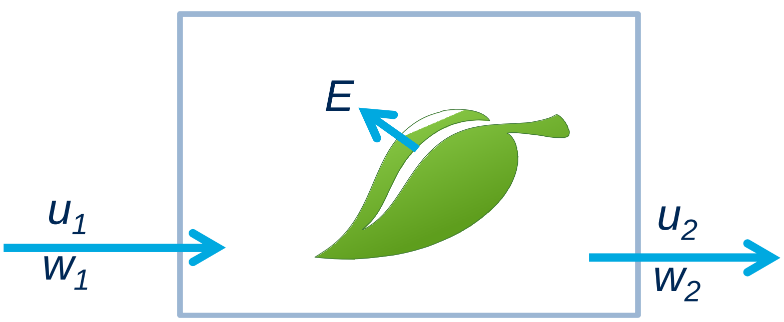
Mass balance for H_2O (water vapor)

$$\left\{\begin{matrix} Rate\ of\ flow\ of \\ H_2O\ into\ system \\ (mole\ s^{-1}) \end{matrix}\right\} - \left\{\begin{matrix} Rate\ of\ flow\ of \\ H_2O\ out\ of\ system \\ (mole\ s^{-1}) \end{matrix}\right\} + \left\{\begin{matrix} Rate\ of\ generation\ of \\ H_2O\ in\ system \\ (mole\ s^{-1}) \end{matrix}\right\} = \left\{\begin{matrix} Rate\ of\ accumulation\ of \\ H_2O\ in\ system \\ (mole\ s^{-1}) \end{matrix}\right\}$$

$$u_1w_1 - u_2w_2 + sE = \cancel{\frac{dW}{dt}}$$

At **STEADY STATE:**

$$\frac{dW}{dt} = 0$$



$$u_1w_1 - u_2w_2 + sE = 0$$

$$sE = u_2w_2 - u_1w_1$$

Note: $u_2 = u_1 + sE$

$$sE = (u_1 + sE)w_2 - u_1w_1$$

$$E = \frac{u_1(w_2 - w_1)}{s(1 - w_2)}$$

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Mass balance for CO_2

- Basic mass balance setup for CO_2 in leaf chamber:

$$\begin{Bmatrix} Rate\ of\ flow\ of \\ CO_2\ into\ system \\ (mole\ s^{-1}) \end{Bmatrix} - \begin{Bmatrix} Rate\ of\ flow\ of \\ CO_2\ out\ of\ system \\ (mole\ s^{-1}) \end{Bmatrix} + \begin{Bmatrix} Rate\ of\ generation\ of \\ CO_2\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix} = \begin{Bmatrix} Rate\ of\ accumulation\ of \\ CO_2\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix}$$

$$u_1 c_1 - u_2 c_2 - sA = \frac{dC}{dt}$$

Variable definitions	
u_1, u_2	Air flow rate (mole air s^{-1})
c_1, c_2	CO_2 mole fraction (mole CO_2 mole air^{-1})
s	Leaf area (m^2)
A	Leaf assimilation (mole CO_2 m^{-2} s^{-1})
$\frac{dC}{dt}$	Change in CO_2 moles in leaf chamber per unit time (mole CO_2 s^{-1})

Mass balance for CO_2

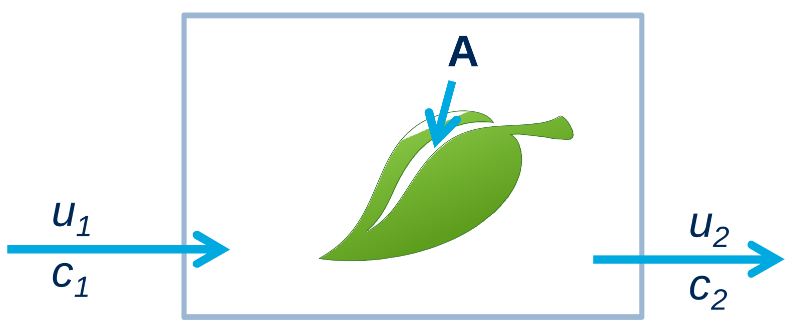
- Basic mass balance setup for CO_2 in leaf chamber:

$$\begin{Bmatrix} Rate\ of\ flow\ of \\ CO_2\ into\ system \\ (mole\ s^{-1}) \end{Bmatrix} - \begin{Bmatrix} Rate\ of\ flow\ of \\ CO_2\ out\ of\ system \\ (mole\ s^{-1}) \end{Bmatrix} + \begin{Bmatrix} Rate\ of\ generation\ of \\ CO_2\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix} = \begin{Bmatrix} Rate\ of\ accumulation\ of \\ CO_2\ in\ system \\ (mole\ s^{-1}) \end{Bmatrix}$$

$$u_1c_1 - u_2c_2 - sA = \cancel{\frac{dC}{dt}}$$

At **STEADY STATE:**

$$\frac{dC}{dt} = 0$$



$$sA = u_1c_1 - u_2c_2$$

Note: $u_2 = u_1 + sE$

$$sA = u_1c_1 - (u_1 + sE)c_2$$

$$A = \frac{u_1(c_1 - c_2)}{s} + c_2E$$

How it is done in the LI-6800 and LI-6400XT: Accounting for dilution and unit conversions

$$E = \frac{F(W_s - W_r)}{100S(1000 - W_s)}$$

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

Variable definitions	
W_s, W_r	Sample, reference H_2O mole fraction (mmole H_2O mole air^{-1})
C_s, C_r	Sample, reference CO_2 mole fraction (µmole CO_2 mole air^{-1})
F	Mass flow rate (µmole air s^{-1})
S	Leaf area (cm^2)
E	Leaf transpiration (mmole H_2O m^{-2} s^{-1})
A	Leaf assimilation (µmole CO_2 m^{-2} s^{-1})

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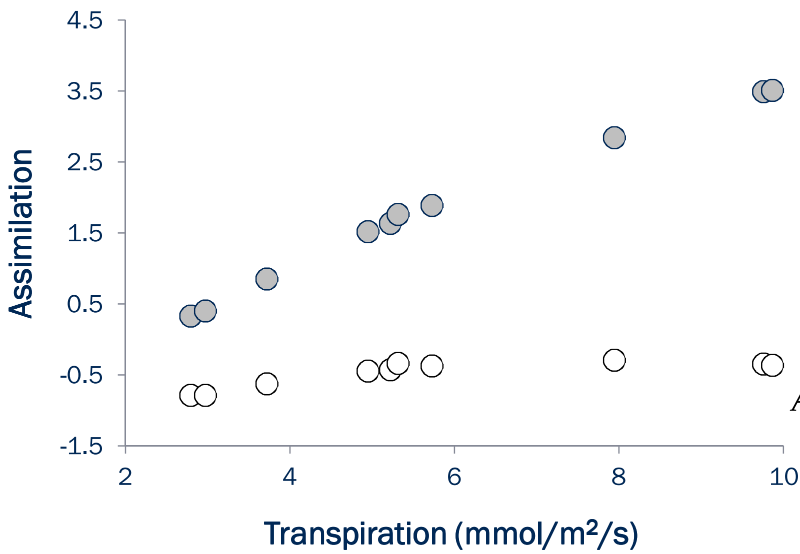
Why does accounting for dilution matter?



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

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

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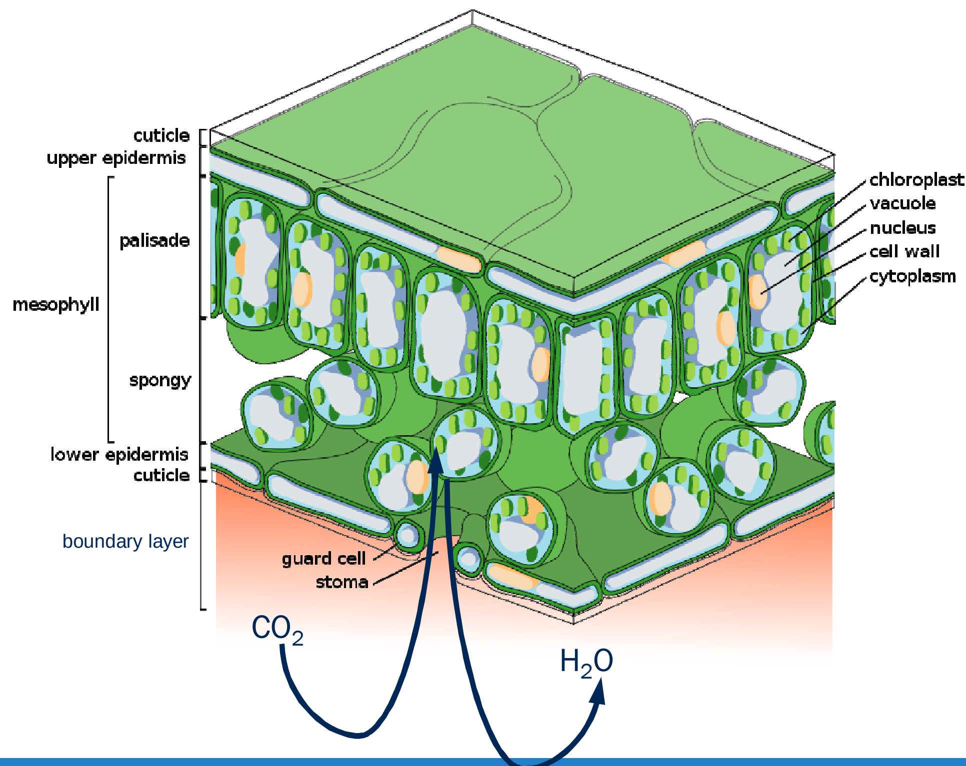
What else can we determine with gas exchange?



What else can we determine with gas exchange?

- Start by mathematically characterizing flux using Fick's First Law describing flux in one dimension:

Fick's 1st Law:

$$J_j = -D_j \frac{\partial c_j}{\partial x}$$

$$J_j = g_j \ \Delta c_j$$

$$J_j = \frac{\Delta c_j}{r_j}$$

Note that: $g_j = -\frac{D_j}{\Delta x}$ and $g_j = \frac{1}{r_j}$

J_j = flux density
D_j = diffusion coefficient
Δc_j = concentration gradient
g_j = conductance
r_j = resistance (inverse of conductance)
J_j = flux

What else can we determine with gas exchange?

- Model transpiration using Fick's 1st law:

$$E = \frac{\Delta W}{r_{TOT}^{H_2O}}$$

$$E = \frac{(W_i - W_a)}{r_{TOT}^{H_2O}}$$

Where:
W_i = leaf intercellular air space H_2O concentration (from T_{leaf} and assuming internal saturation)
W_a = H_2O concentration surrounding leaf (from H2OS)
E = transpiration that is calculated from mass balance described earlier
$r_{TOT}^{H_2O}$ = total leaf resistance to water vapor flux (unknown)

We know W_i, W_a, and E. Just solve for $r_{TOT}^{H_2O}$!

What else can we determine with gas exchange?

- Model CO_2 assimilation using Fick's 1st law:

$$A = \frac{\Delta C}{r_{TOT}^{CO_2}}$$

$$A = \frac{(C_i - C_a)}{r_{TOT}^{CO_2}}$$

Where:
C_i = leaf intercellular CO_2 concentration (unknown)
C_a = CO_2 concentration surrounding leaf (CO2S)
A = CO_2 assimilation that is calculated from mass balance described earlier
$r_{TOT}^{CO_2}$ = total leaf resistance to CO_2 flux

We know $r_{TOT}^{CO_2}$, W_a, and A. Just solve for C_i!

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What else can we determine with gas exchange?

MW = 18.0

$$\frac{D_{H_2O}}{D_{CO_2}} = 1.6$$

MW = 44.0

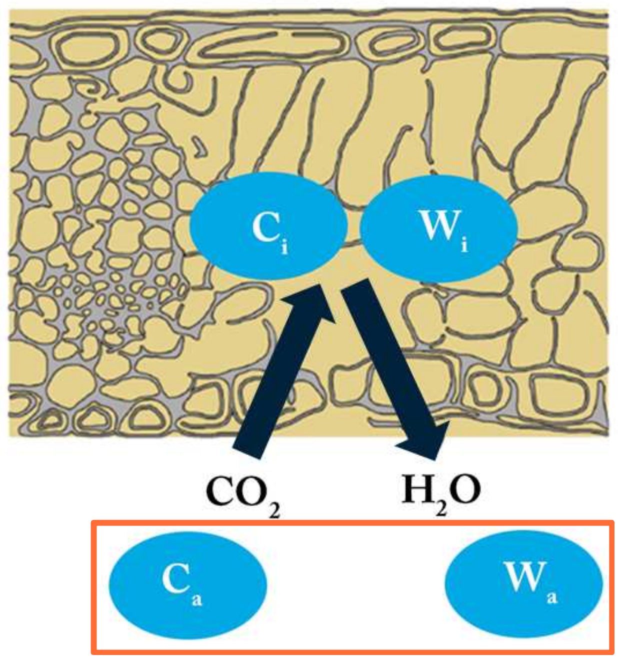
Thus:

$$R_{CO_2} = 1.6R_{H_2O}$$

$$g_{CO_2} = \frac{g_{H_2O}}{1.6}$$

So, C_i can be calculated from:

$$A = \frac{(C_i - C_a)}{r_{TOT}^{CO_2}}$$



What else can we determine with gas exchange?

- How is stomatal resistance to H_2O or CO_2 flux calculated?
- Use Ohm's Law analogy to partition resistances

Ohm's Law

$$V = IR$$

The voltage (*V*) across two points on a conductor is proportional to the product of current (*I*) and resistance (*R*).

$$I = \frac{V}{R}$$

Current (*I*) is analogous to flux

$$G = \frac{1}{R}$$

Conductance (*G*) is the inverse of resistance (*R*)

Series resistances:
$R_{eq} = R_1 + R_2 + \cdots$

Parallel resistances:
$$R_{eq} = \left(\frac{1}{R_1} + \frac{1}{R_2} + \cdots\right)^{-1}$$

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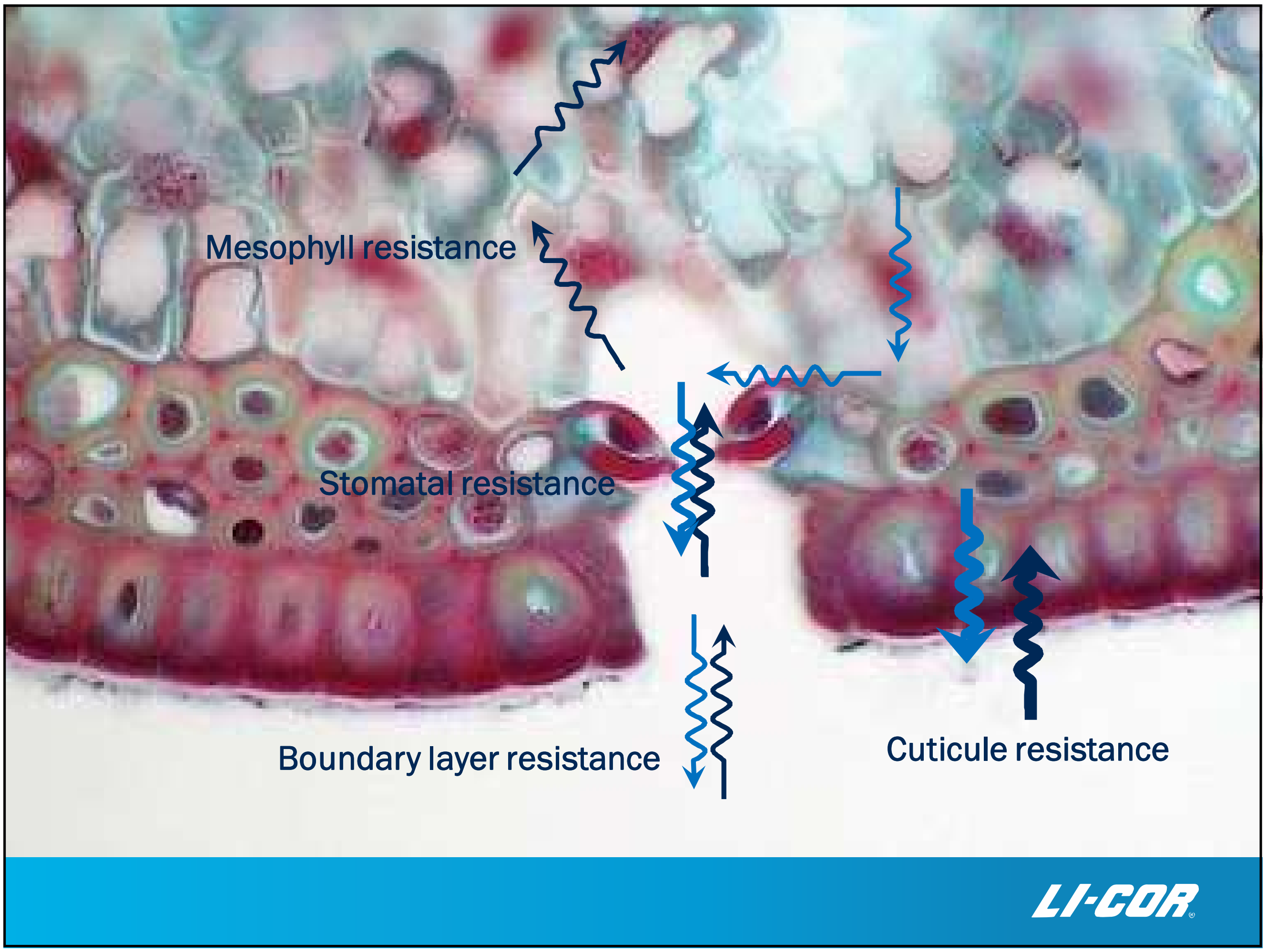

Mesophyll resistance
Stomatal resistance
Boundary layer resistance
Cuticule resistance

What else can we determine with gas exchange?

- How **stomatal resistance** to H_2O or CO_2 flux calculated?
- Use Ohm's Law analogy

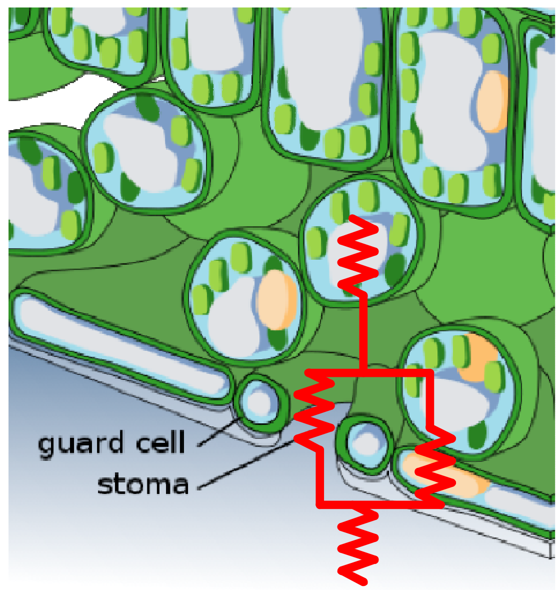
Ohm's Law Analogy

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

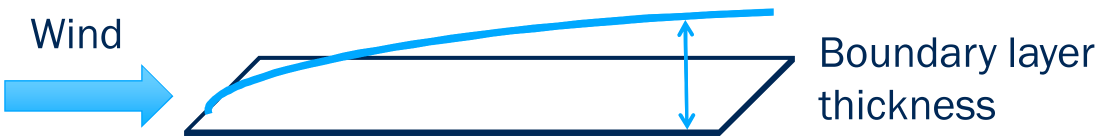
Assumptions:

- End point of diffusion path is mesophyll surface
- Cuticular resistance is near infinite

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



Leaf boundary layer

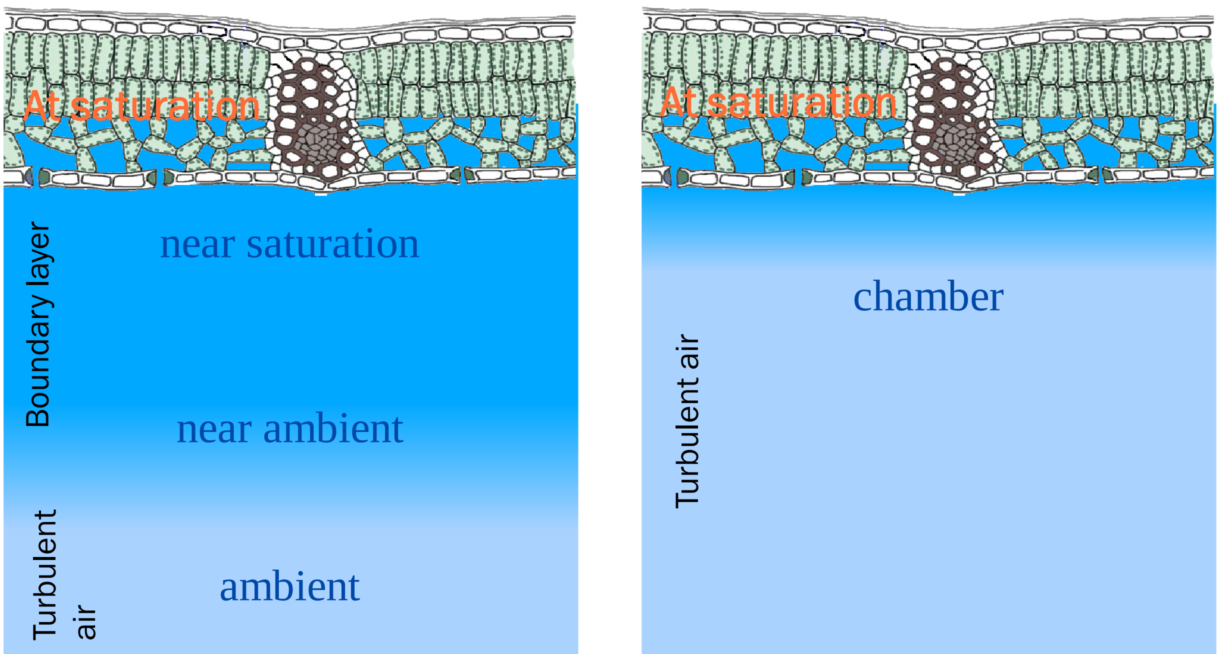


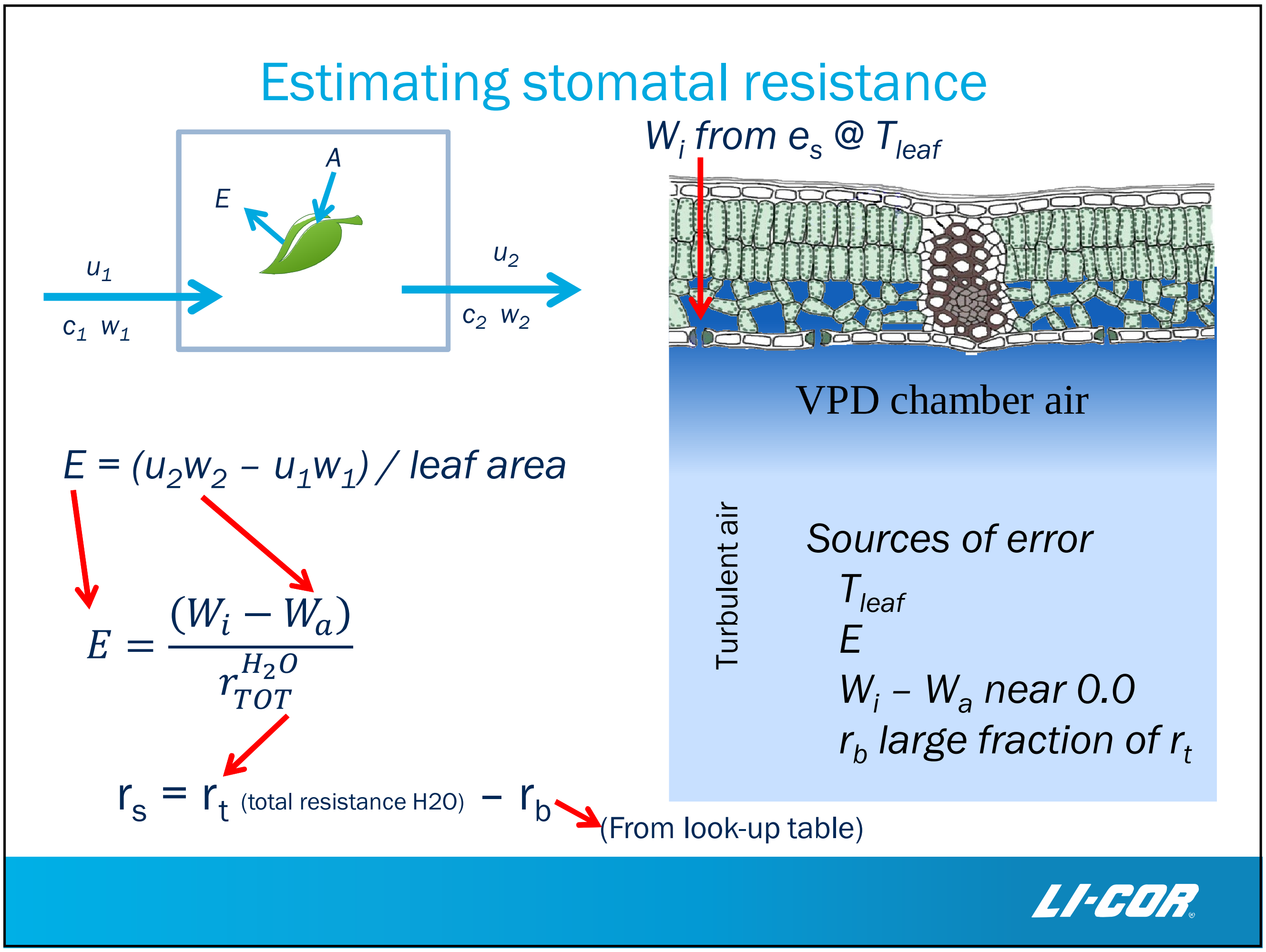
The boundary layer retards the transfer of heat, CO_2, and H_2O from the leaf to the bulk air.

$$\delta^{\text{bl}}_{(mm)} = 4.0\sqrt{\frac{l_{(m)}}{v_{(m\,s^{-1})}}}$$

Leaf boundary layer

- Leaf chamber is well mixed. This dramatically reduces boundary layer resistance, thus forcing r_{bl} towards zero.




Estimating stomatal resistance
A
E
u1
c1 w1
u2
c2 w2
Wi from es @ Tleaf
VPD chamber air
E = (u2w2 – u1w1) / leaf area
$E = \frac{(W_i - W_a)}{r_{TOT}^{H_2O}}$
Turbulent air
Sources of error
Tleaf
E
Wi – Wa near 0.0
rb large fraction of rt
rs = rt (total resistance H2O) – rb
(From look-up table)
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What does this mean for making measurements?

- Target boundary layer conditions!

$$RH = \frac{e}{e_{(T_{air})}} \bullet 100$$

RH sample 50 – 80 %

$$VPD_{air} = e_{(T_{air})} - e$$

$$VPD_{leaf} = e_{(T_{leaf})} - e$$

e = vapor pressure, e(t) = saturation vapor pressure

What does this mean for interpreting the data?

- Instantaneous versus Intrinsic

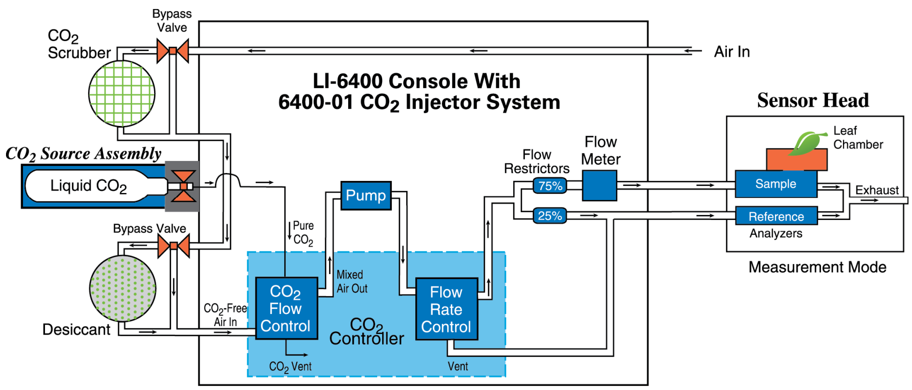
$$W_t = \frac{A}{E} = \frac{A}{g_s\left(w_i - w_a\right)} = \frac{A}{g_s D_a} = W_g D_a$$

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

Better measure of WUE!

LI-6400 / LI-6800 flow path details

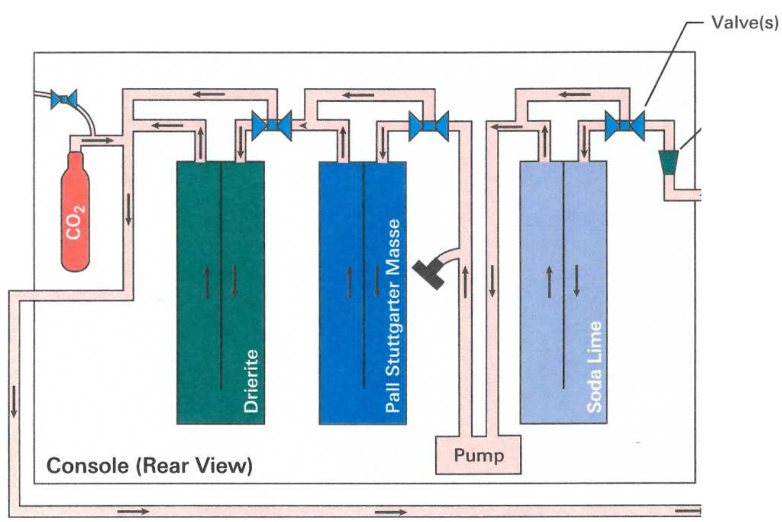
Overview of the LI-6400XT flow path



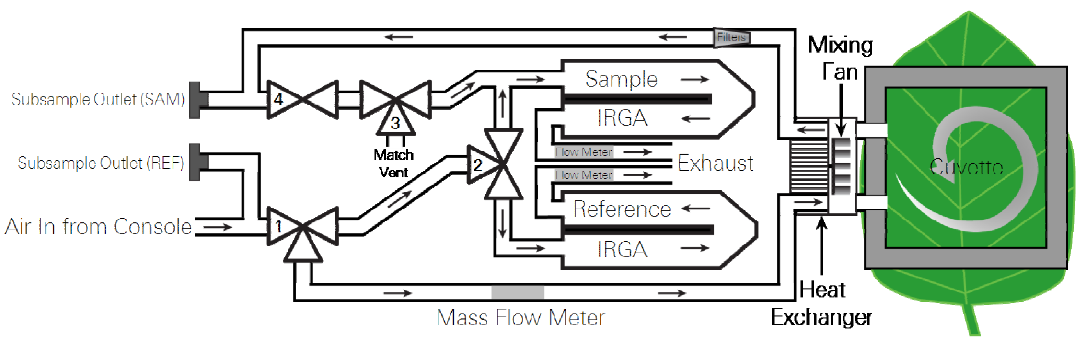
Key features of LI-6400 design:

- Manual valve control on two chemical scrub tubes (soda lime and desiccant).
- Chemical scrub tubes and CO_2 injection on negative pressure side of pump
- Flow meter and flow split located in console
- One pump speed
- Two air hoses supply sensor head

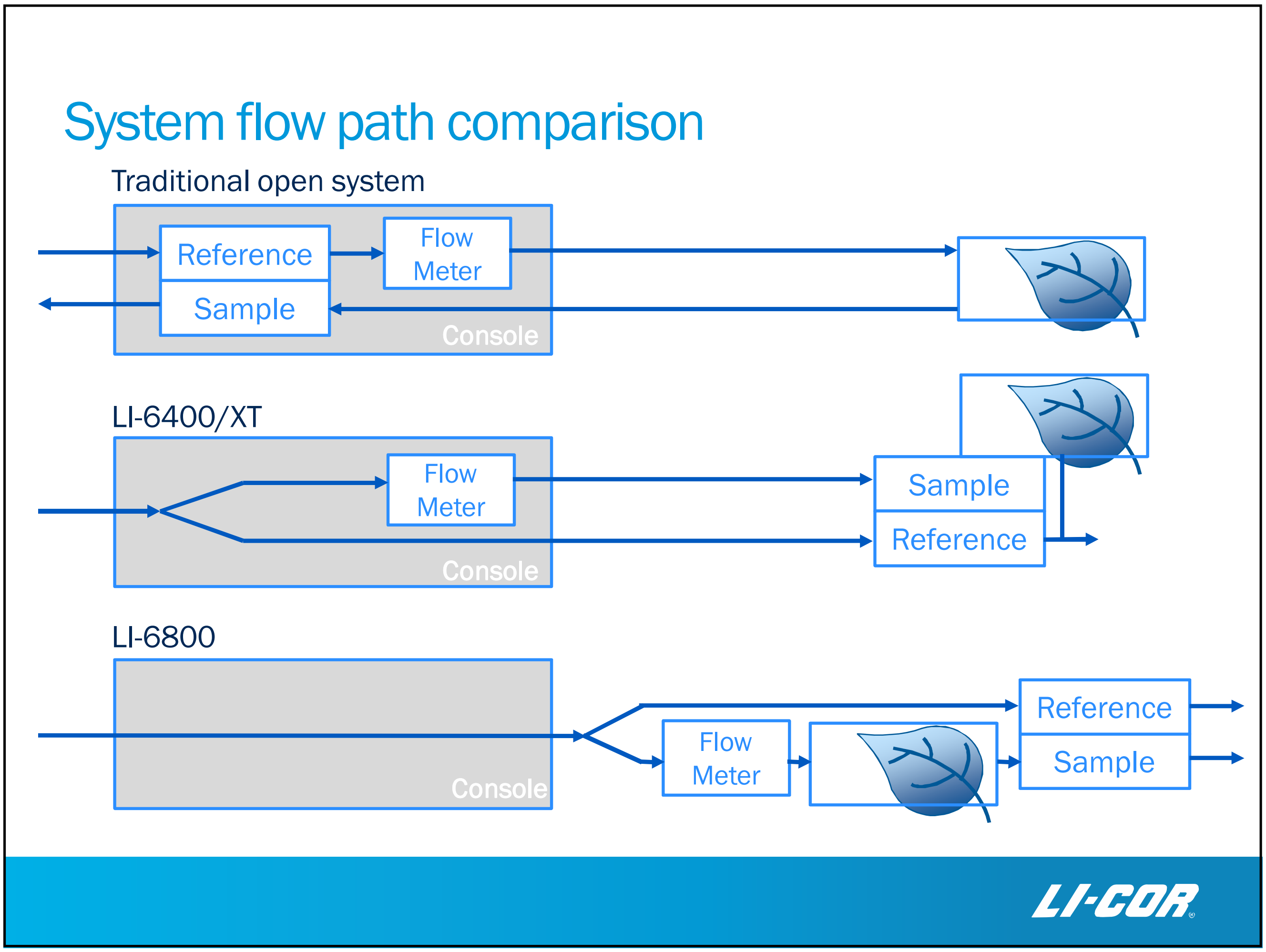
LI-6800 Console flow path



LI-6800 sensor head flow path



LI-COR


System flow path comparison
Traditional open system
Reference
Flow Meter
Sample
Console
LI-6400/XT
Flow Meter
Sample
Reference
Console
LI-6800
Console
Reference
Flow Meter
Sample
LI-COR

How do we measure CO_2 and H_2O?

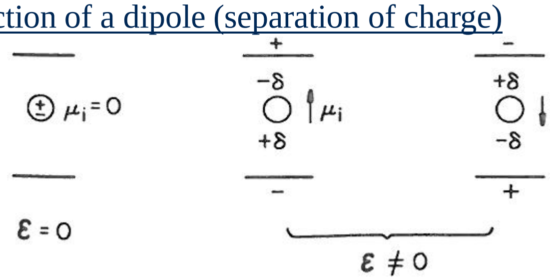
LI-COR

H_2O and CO_2 Absorption in the IR region involves rotations and vibrations

Induction of a dipole (separation of charge)

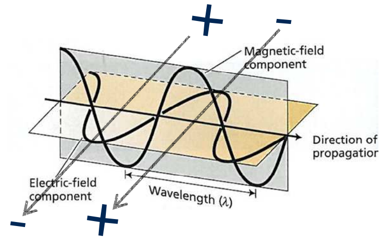
(μ_i = dipole)

(ε = electric field)



dipole

\+ -

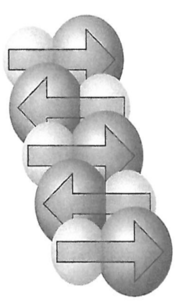


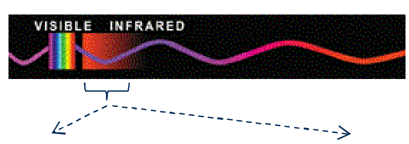
Oscillating dipole of a vibrating non-polar molecule

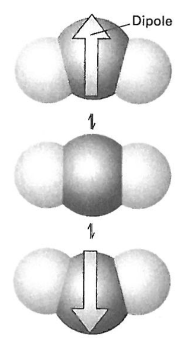
Oscillating dipole of a rotating polar molecule

dipole

\+ → -







dipole

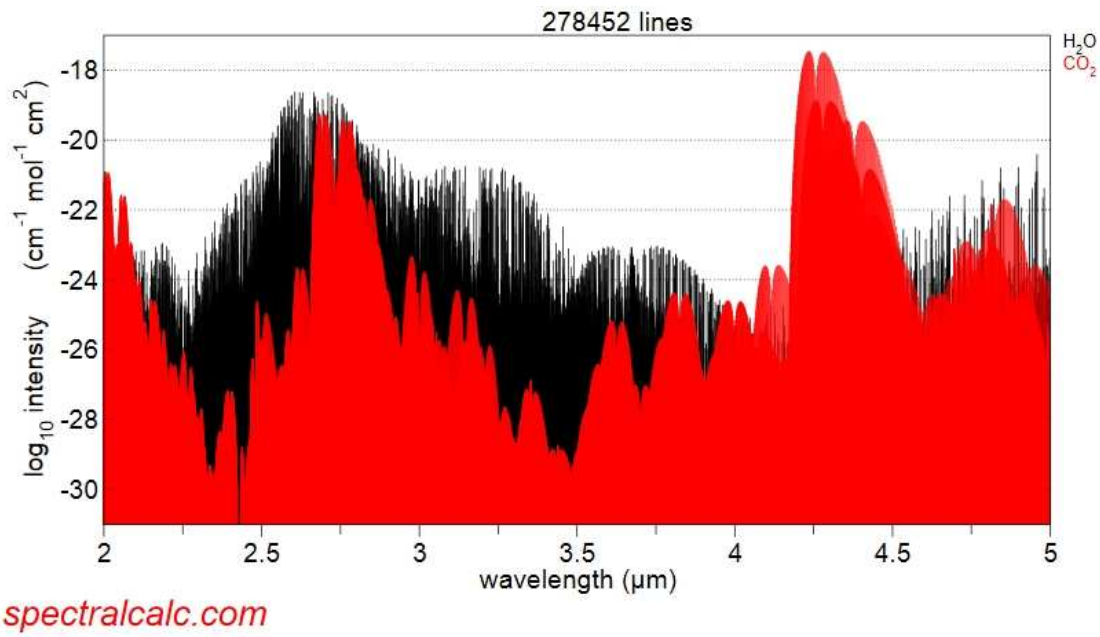
\-

↑

\+

45

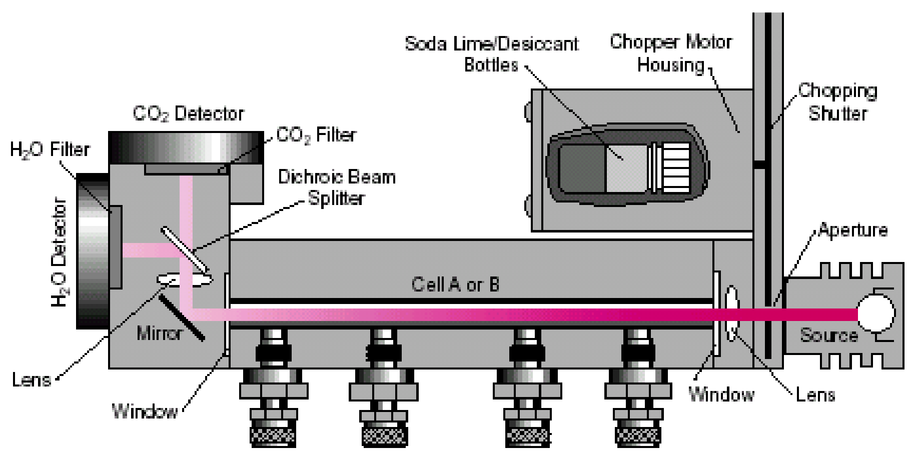
CO_2 and H_2O spectra



LI-COR

Basic IRGA schematic

- CO_2 absorbs infrared light around 4.3 µm
- H_2O absorbs light around 2.6 µm



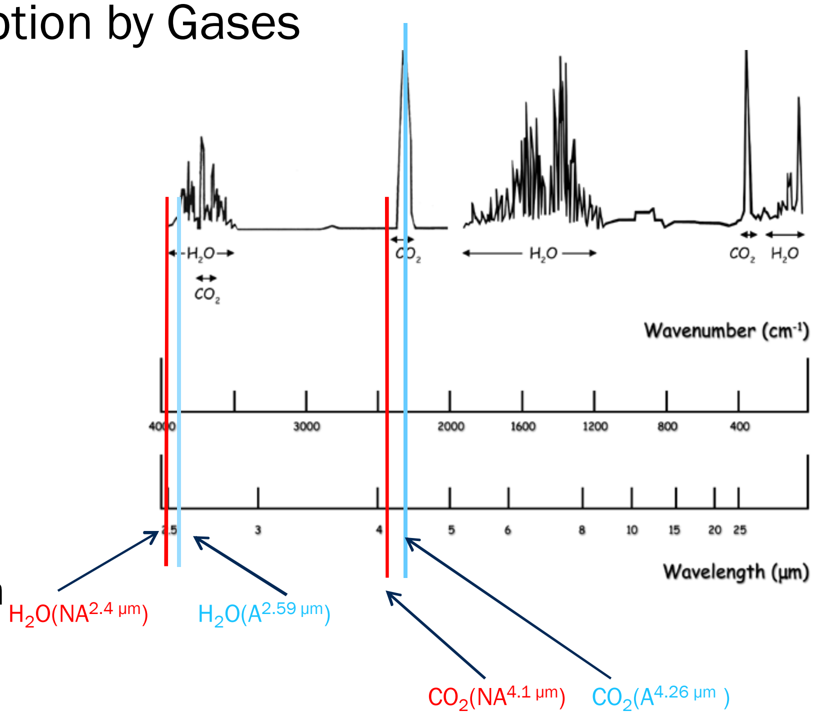
How do we measure gas concentration?

- Infrared Absorption by Gases

$\alpha = 1 - T$

$\alpha = 1 - \Phi_A/\Phi_{NA}$

α = absorptance
T = transmittance
Φ_A = transmittance in absorbing region
Φ_{NA} = transmittance in non-absorbing region



LI-COR

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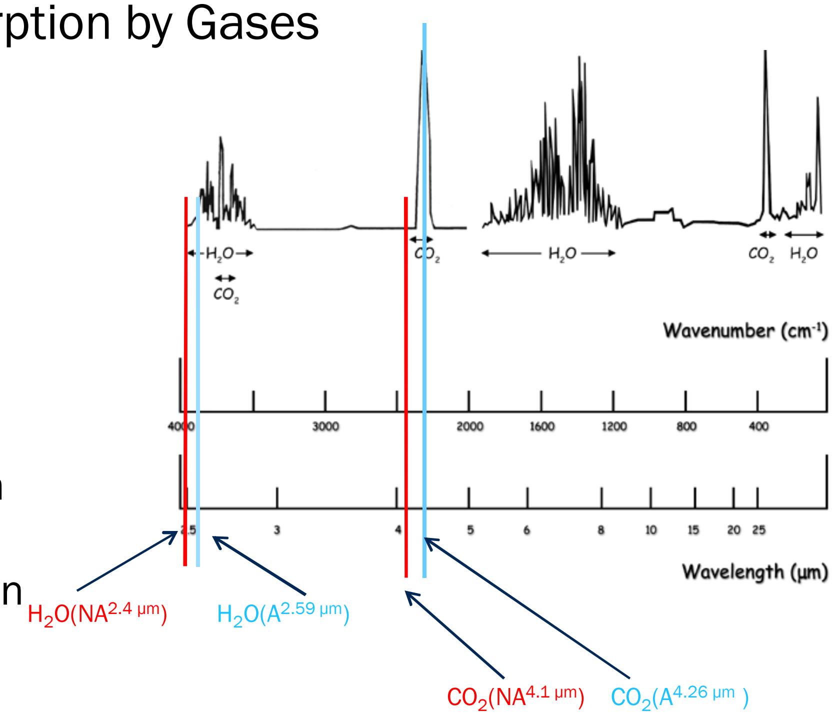
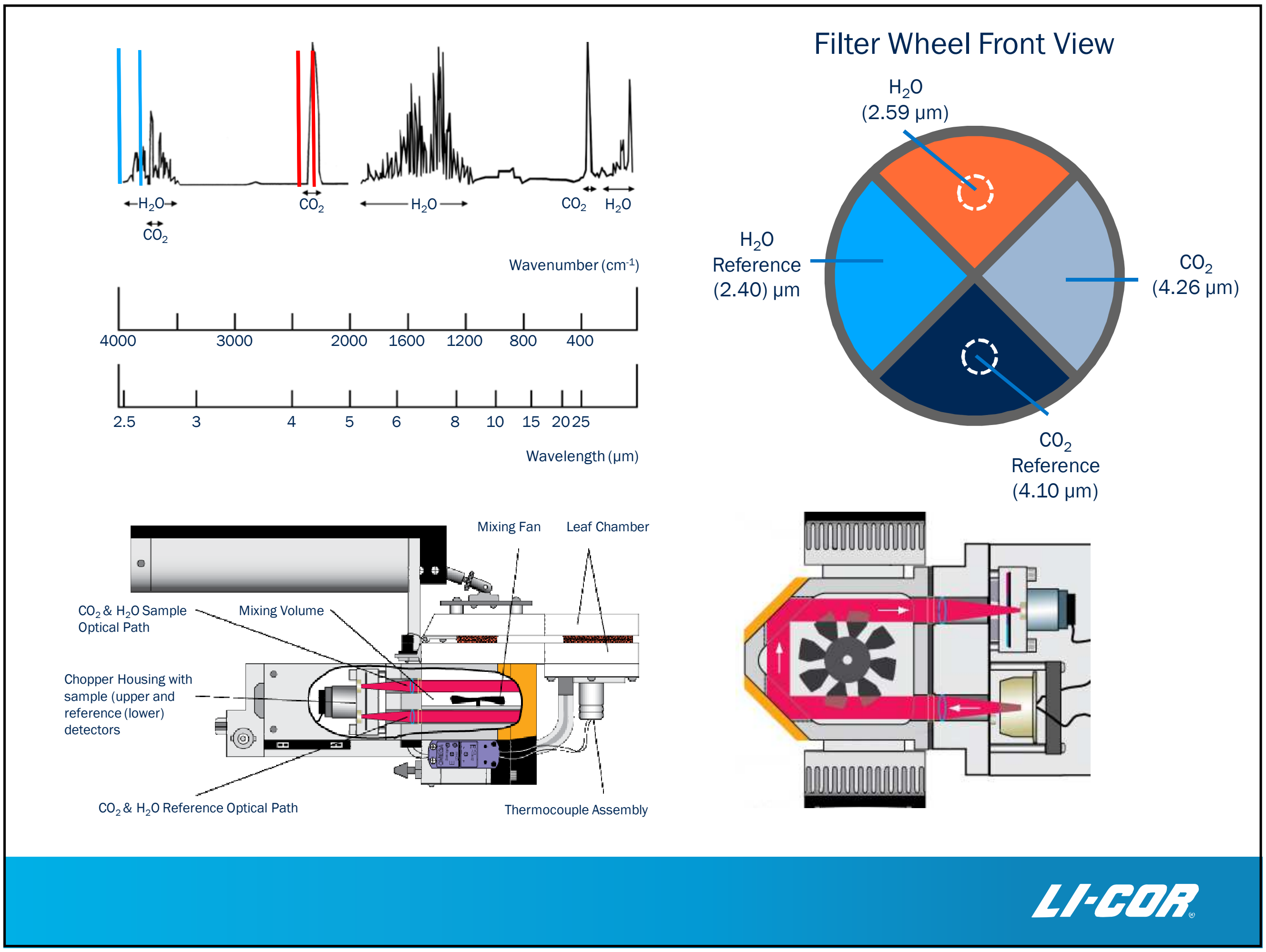


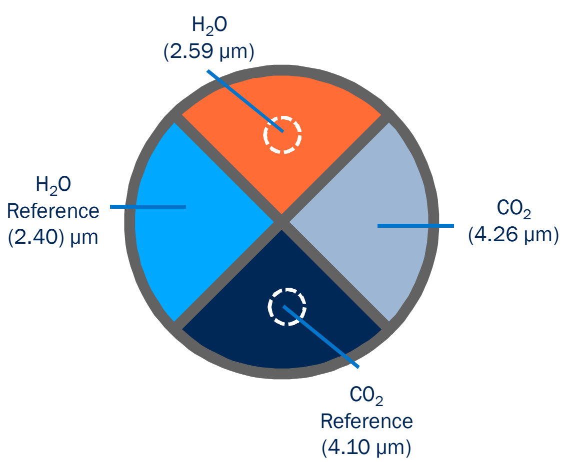
LI-COR

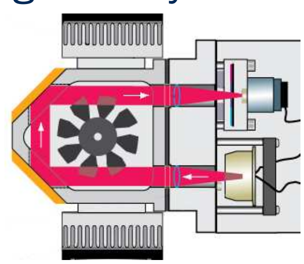

Filter Wheel Front View
H_2O
(2.59 µm)
H_2O
Reference
(2.40) µm
CO_2
(4.26 µm)
CO_2
Reference
(4.10 µm)
H_2O
CO_2
CO_2
H_2O
CO_2
H_2O
Wavenumber (cm^{-1})
4000
3000
2000
1600
1200
800
400
2.5
3
4
5
6
8
10
15
20
25
Wavelength (µm)
Mixing Fan
Leaf Chamber
CO_2 & H_2O Sample Optical Path
Mixing Volume
Chopper Housing with sample (upper and reference (lower) detectors
CO_2 & H_2O Reference Optical Path
Thermocouple Assembly
LI-COR

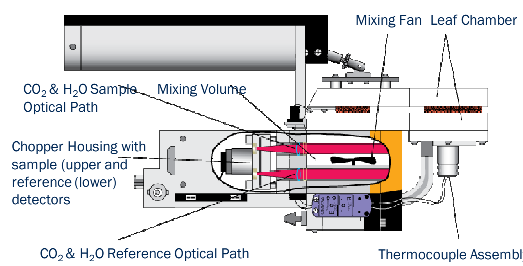
How do we measure gas concentrations?

- Why do we measure absorbing and non-absorbing regions?
- Why do we need a separate reference gas analyzer?

Filter Wheel Front View

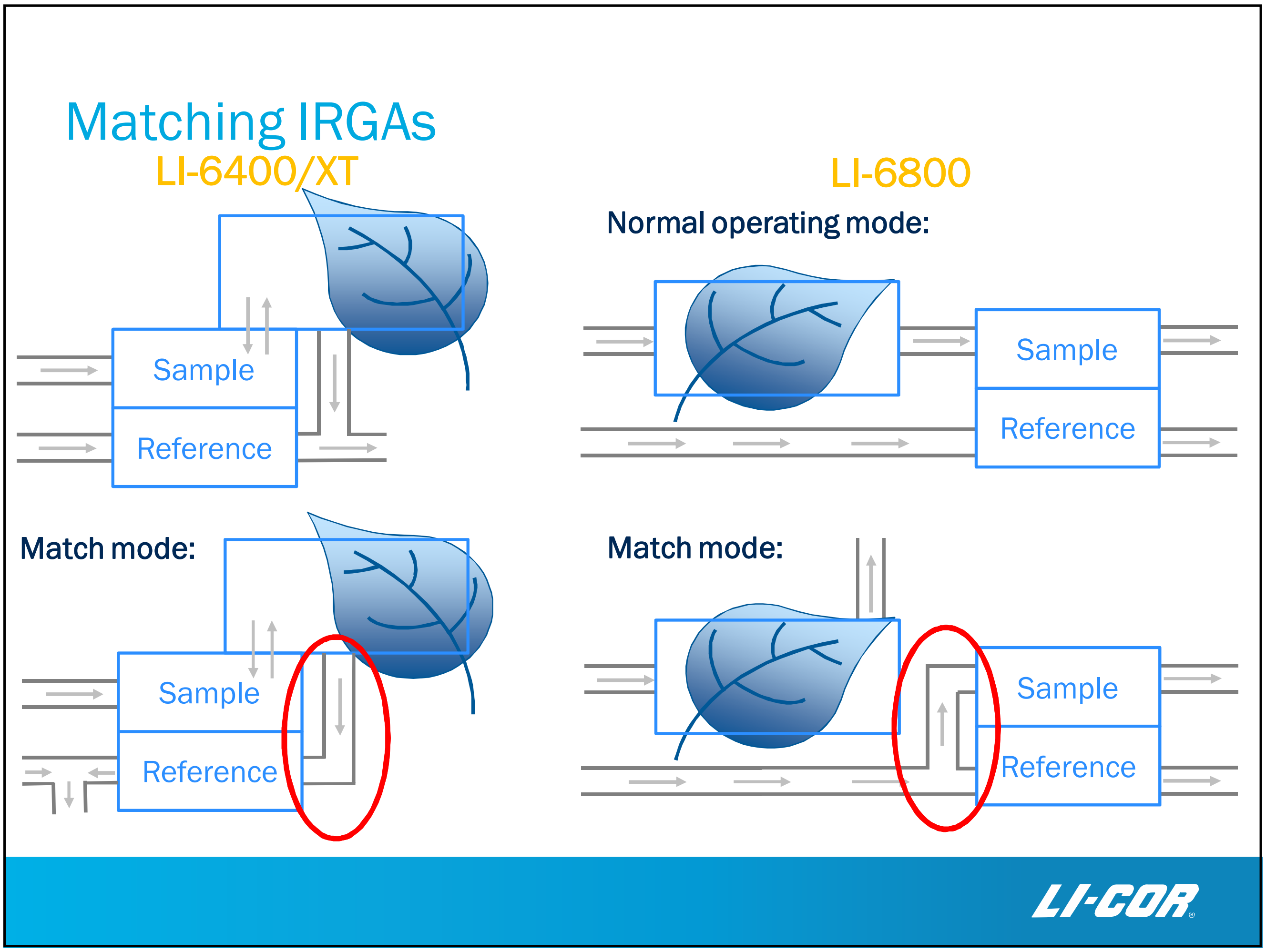






Matching IRGAs

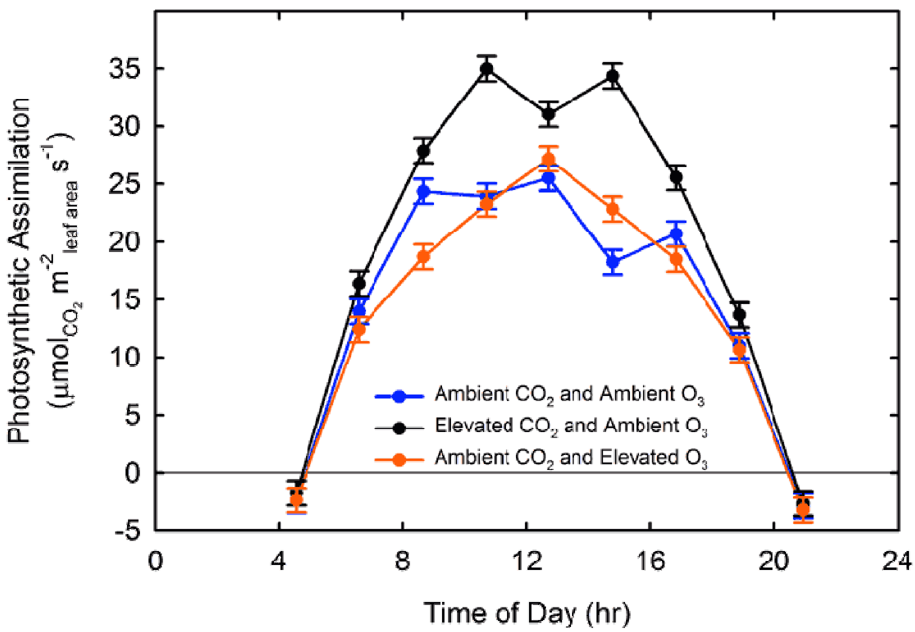
- Very important!
- Why?


Matching IRGAs
LI-6400/XT
Sample
Reference
Match mode:
Sample
Reference
LI-6800
Normal operating mode:
Sample
Reference
Match mode:
Sample
Reference
LI-COR

When to Match

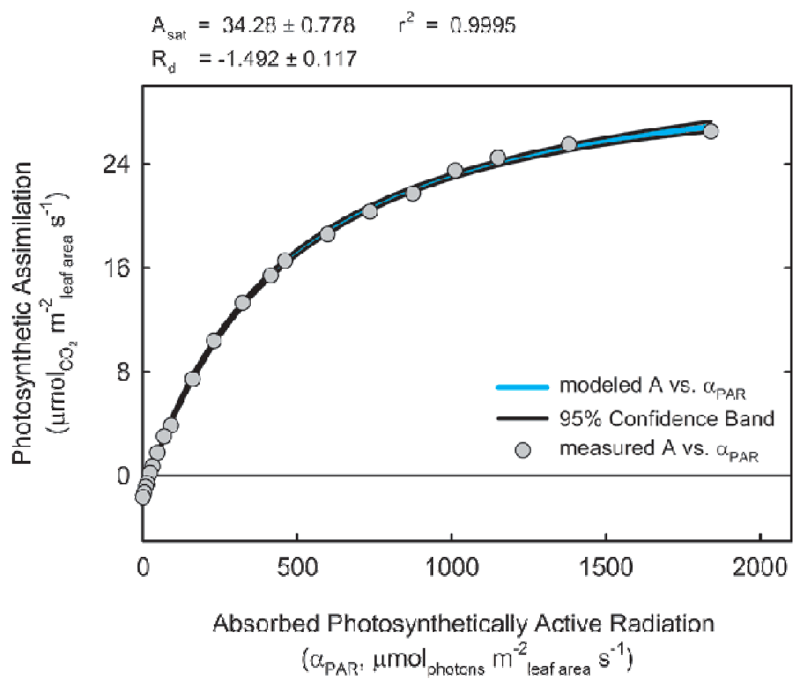
- When you start
- When delta CO_2 or delta H_2O are small
- When Flow rate changes > 100 µmol/s
- When CO_2 concentration changes >100 ppm
- When the temperature changes >5° C
- Every 20-30 minutes

Applications: survey measurements

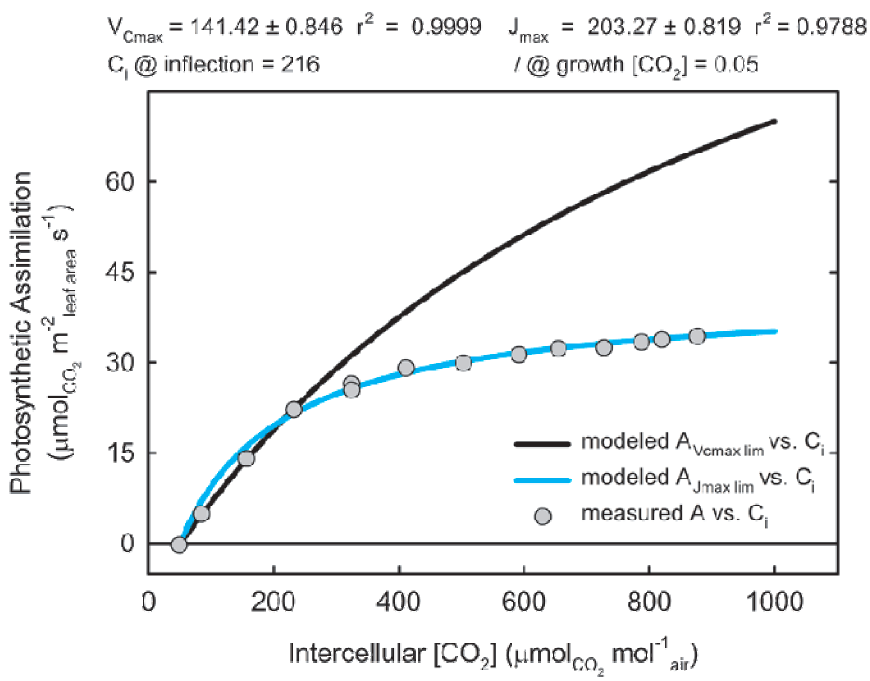


Bernacchi CJ, Leakey ADB, Heady LE, Morgan PB, Dohleman FG, McGrath JM, et al. (2006). Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO2 and ozone concentrations for 3 years under fully open-air field conditions. Plant, Cell Environ 29: 2077-2090.

Applications: light response curves



Applications: CO_2 response curves



Measurement types and benefits

Survey measurements

- Snapshot of plant behavior
- Fast measurement
- Can be used to explore diurnal or seasonal plant responses
- Can be used to explore treatment responses

Response curves

- Response to varying environmental conditions
- Can be used to understand leaf biochemistry and plant physiology
- Can be repeated through the course of a season to record biochemical trends

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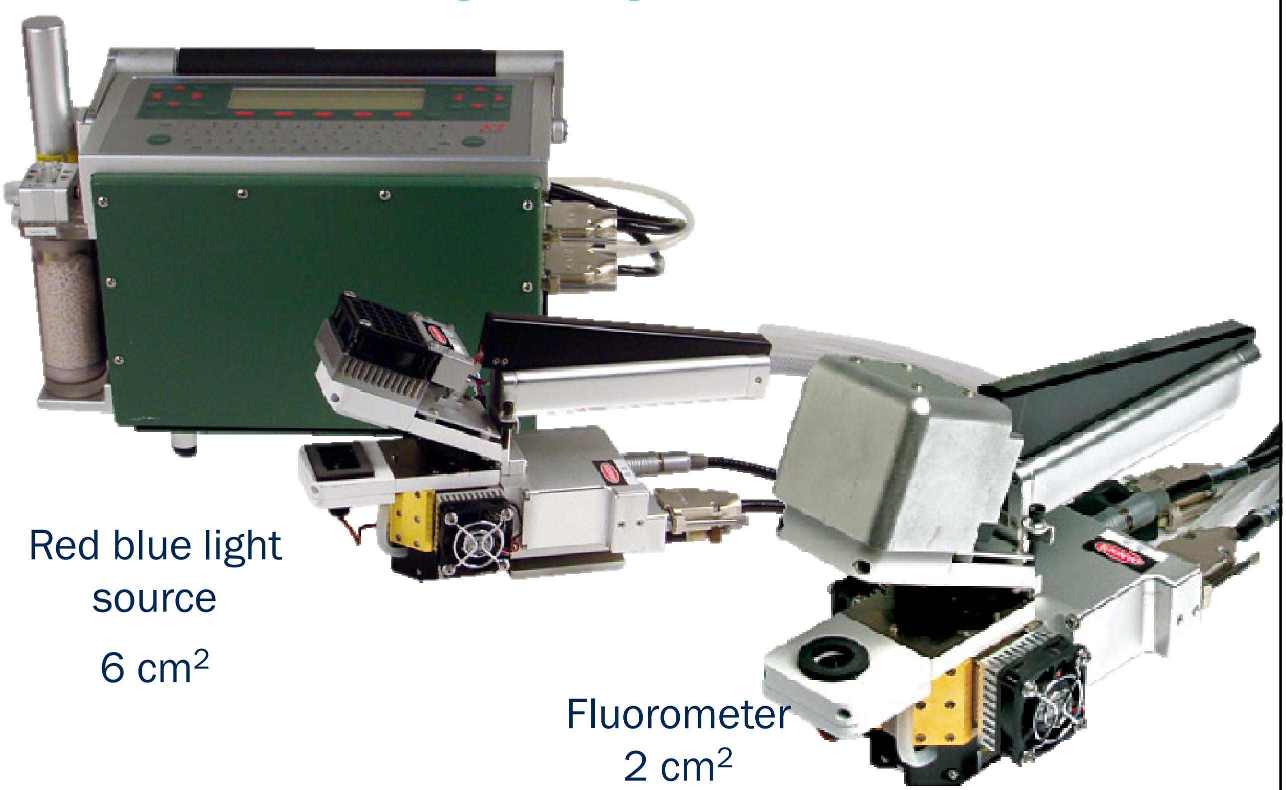
Selecting the right chamber?



Red blue light source

6 cm^2

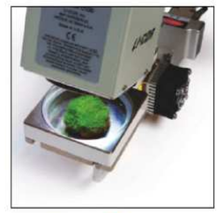
Fluorometer

2 cm^2

LI-6400XT Chambers

Fluorescence

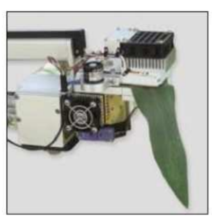
Clear Bottom



Bryophytes

Needles/Narrow Leaves

Conifers



Light Response

Soil Flux

Arabidopsis

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LI-6800: Chambers

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Thank you!