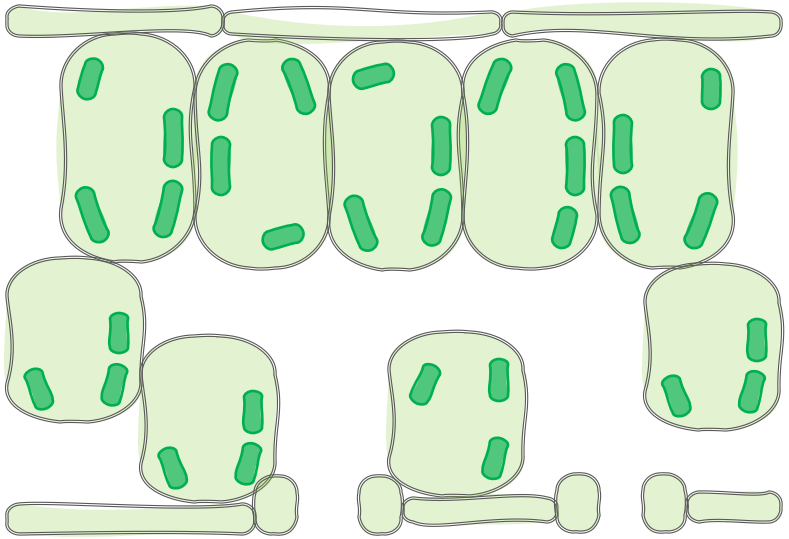
Methods for leaf-level photochemistry and gas exchange

Some opening remarks

- Much of this talk is organized around assumption and potential errors in measurements

- Review of photochemistry and why one might want to measure it
- Deep dive into instrument theory behind the techniques
- Some practical considerations for making measurements
- Demonstration measurement

A simple overview of photochemistry



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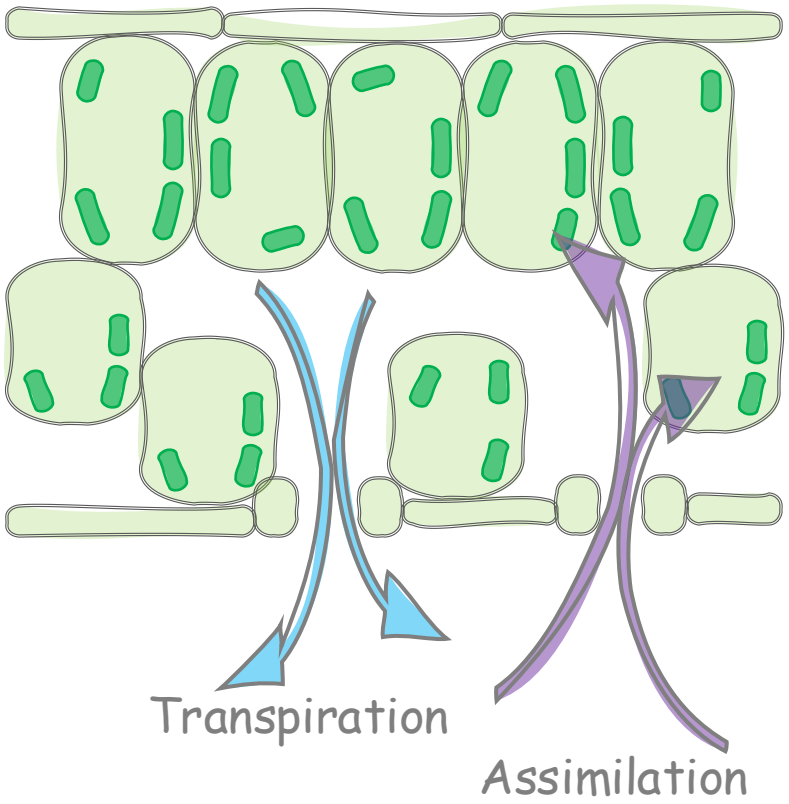

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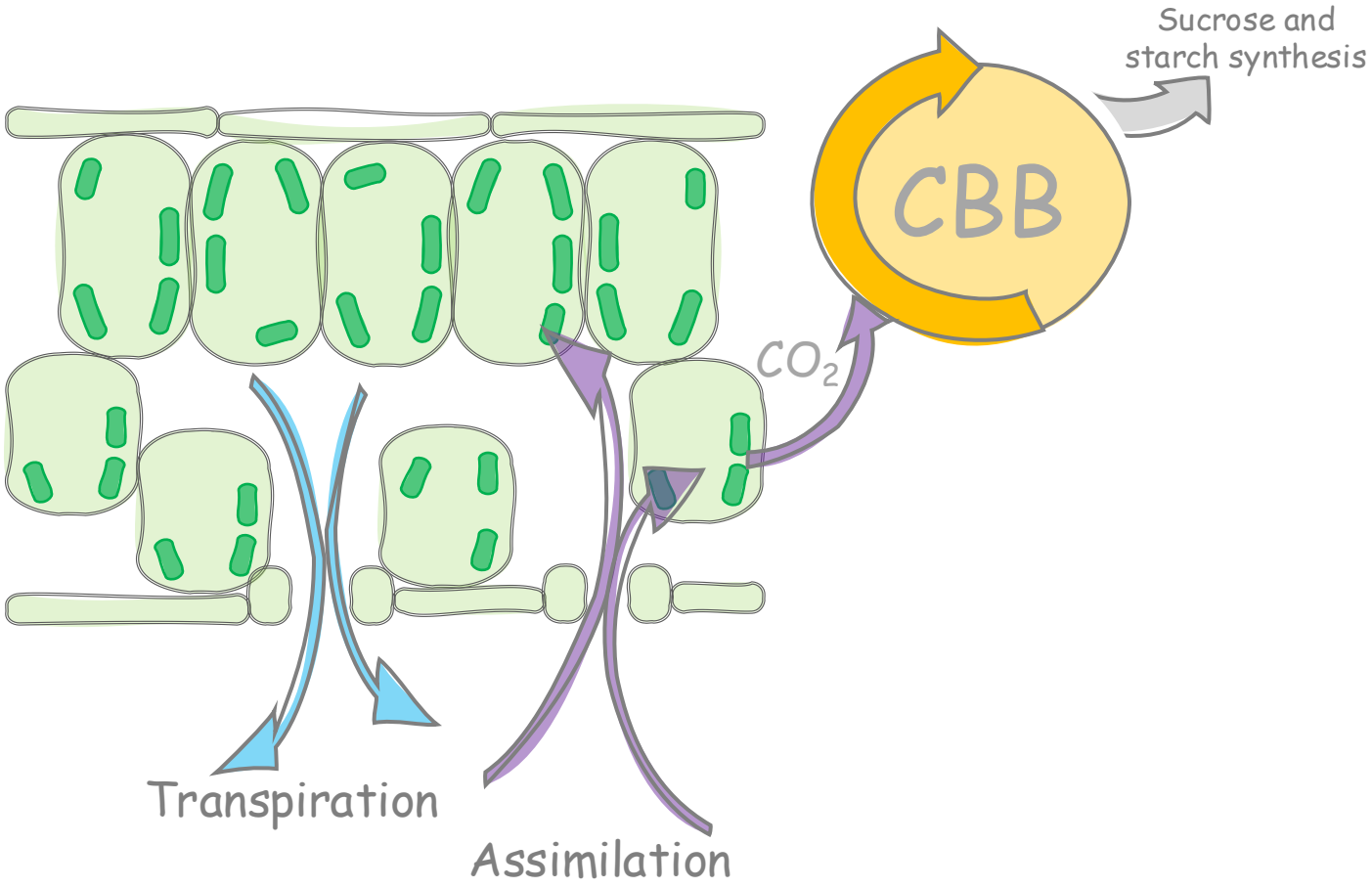

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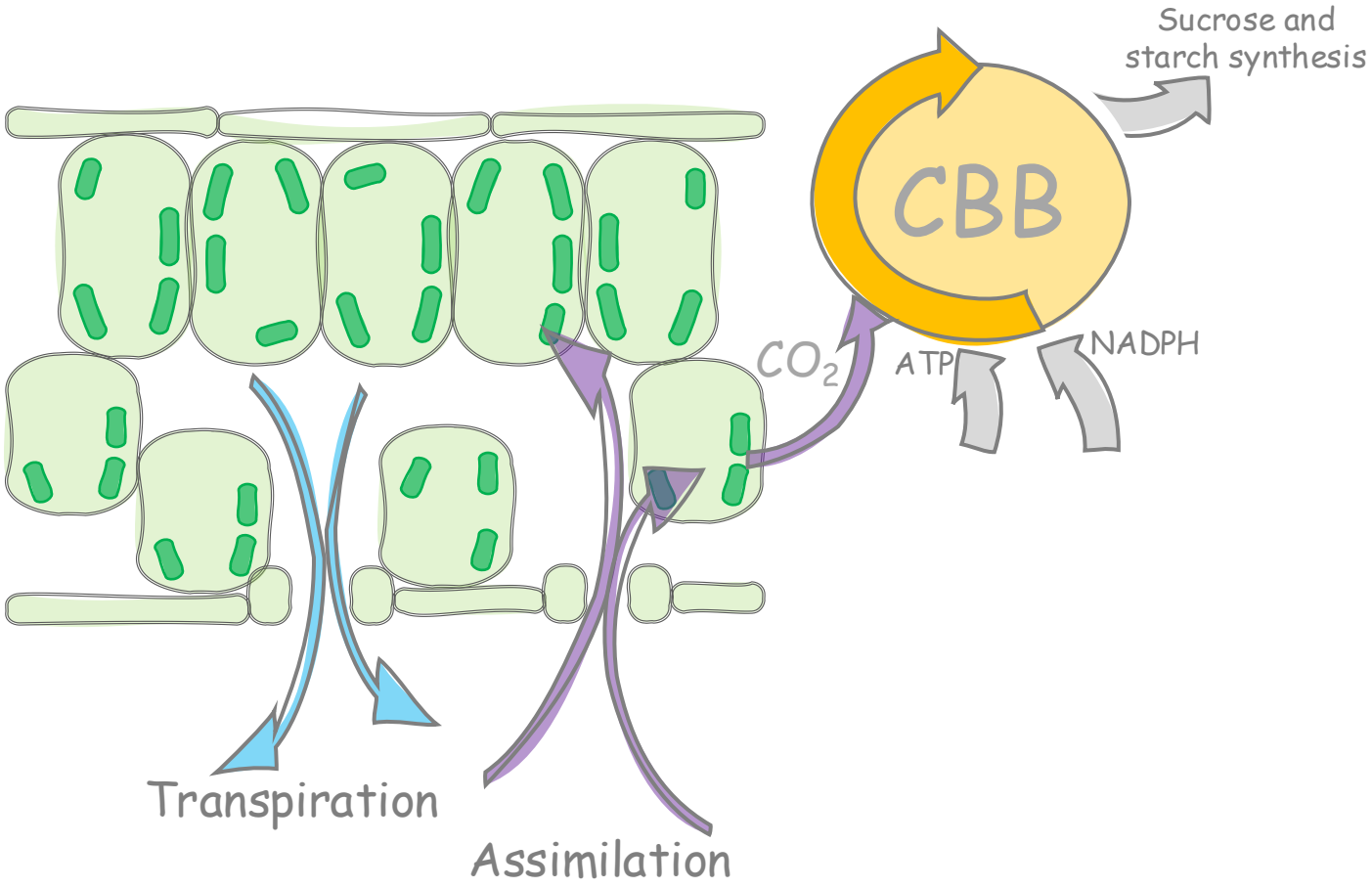

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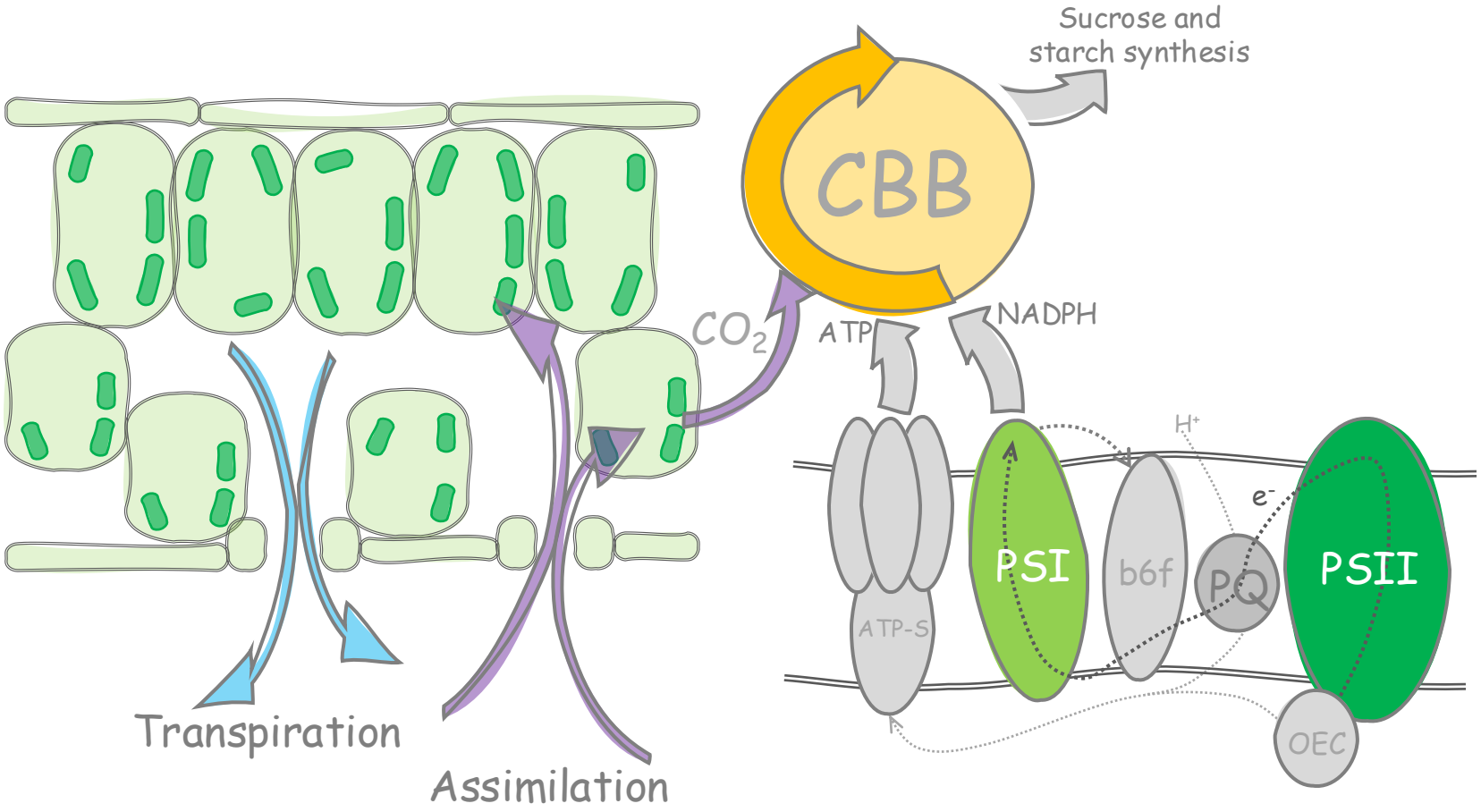

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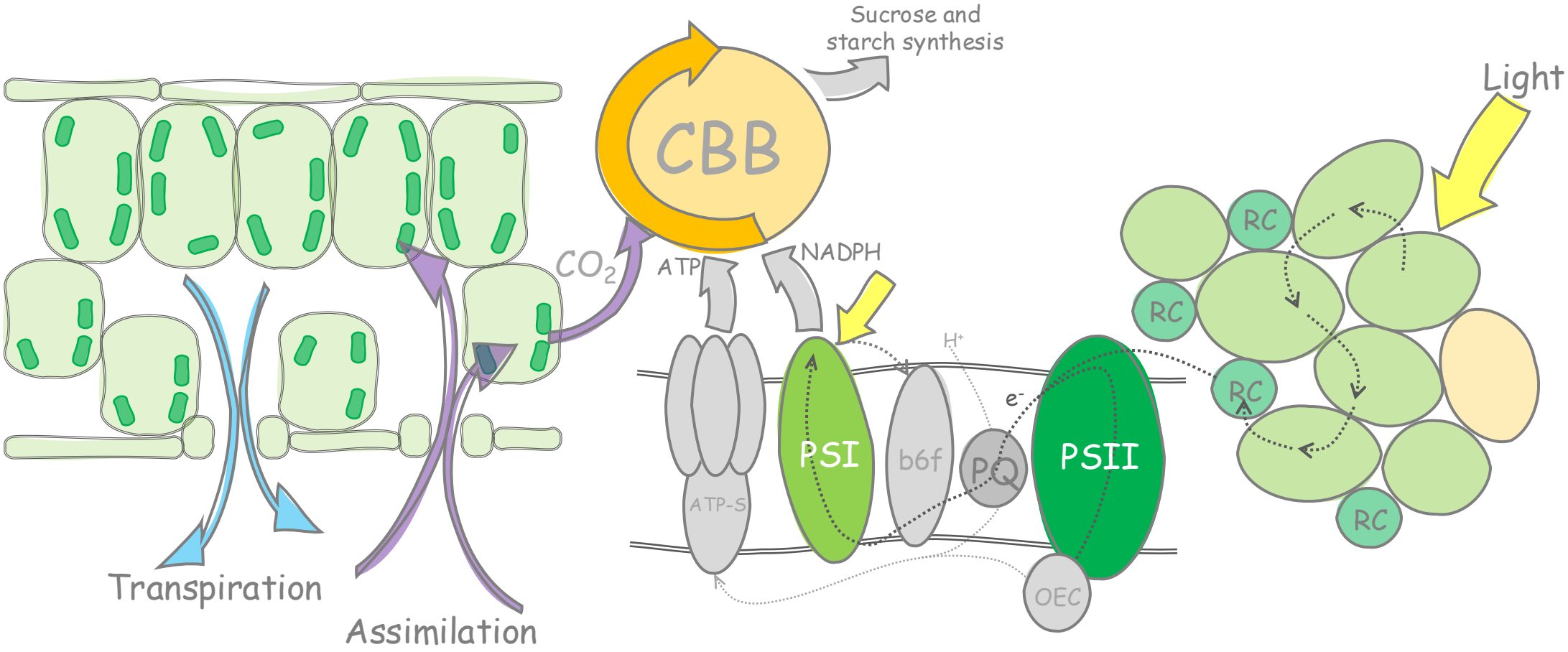

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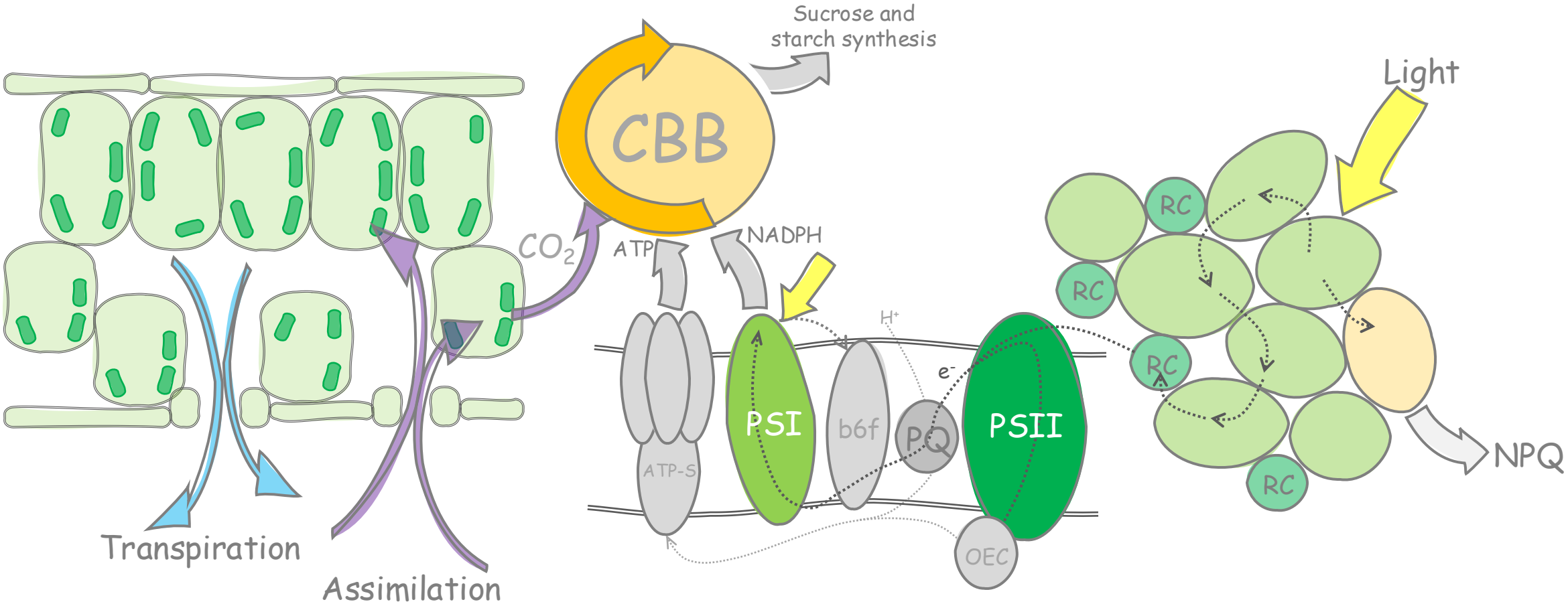

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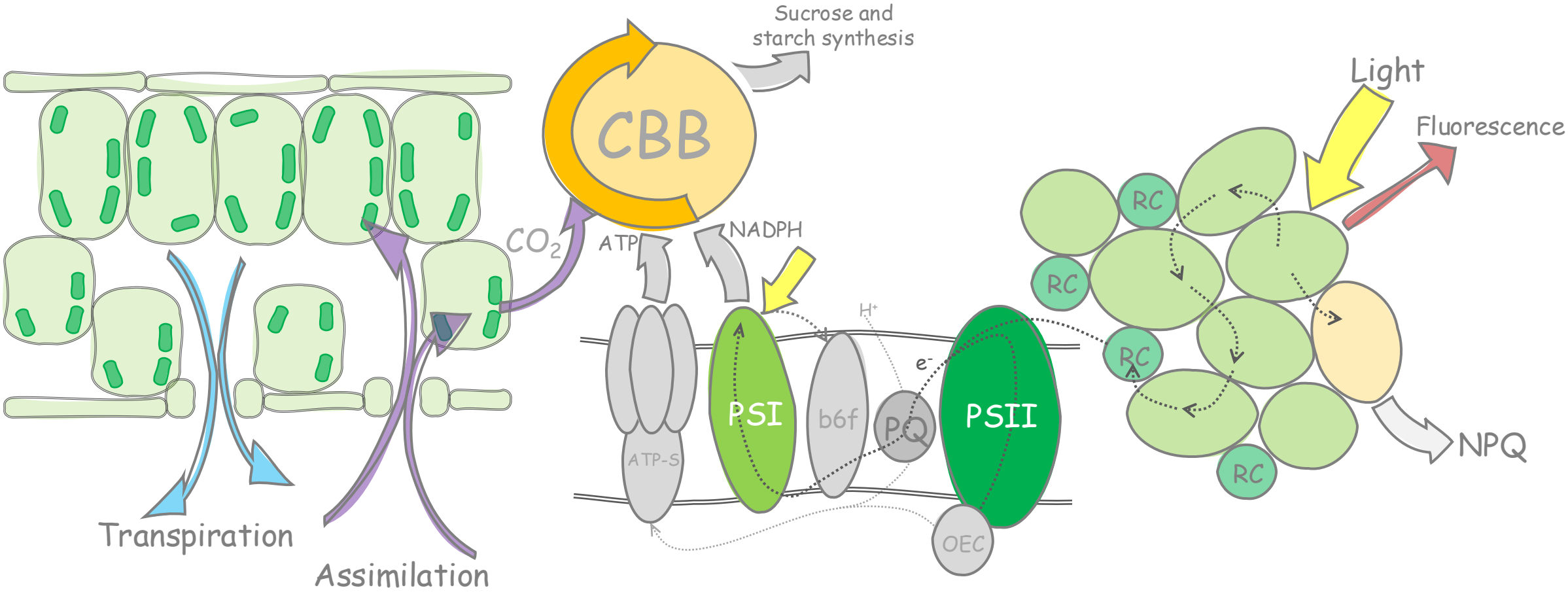
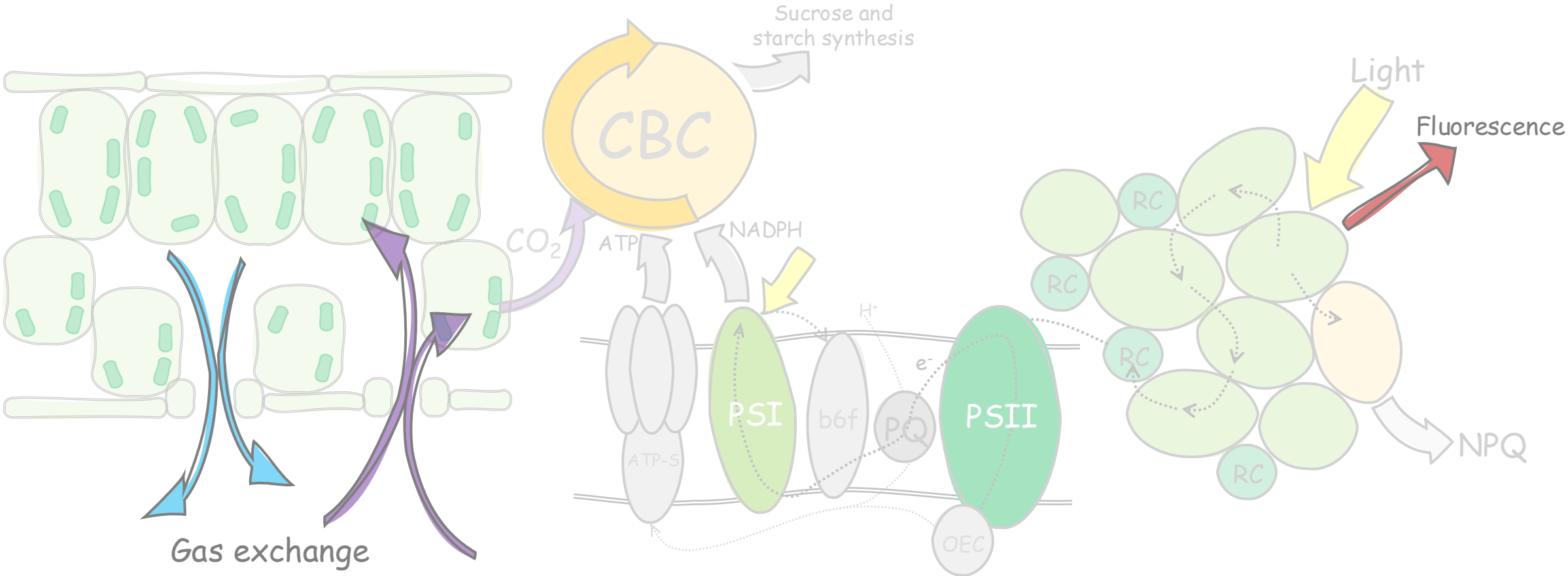


How do we measure photochemistry?



Combined gas exchange and fluorescence instruments

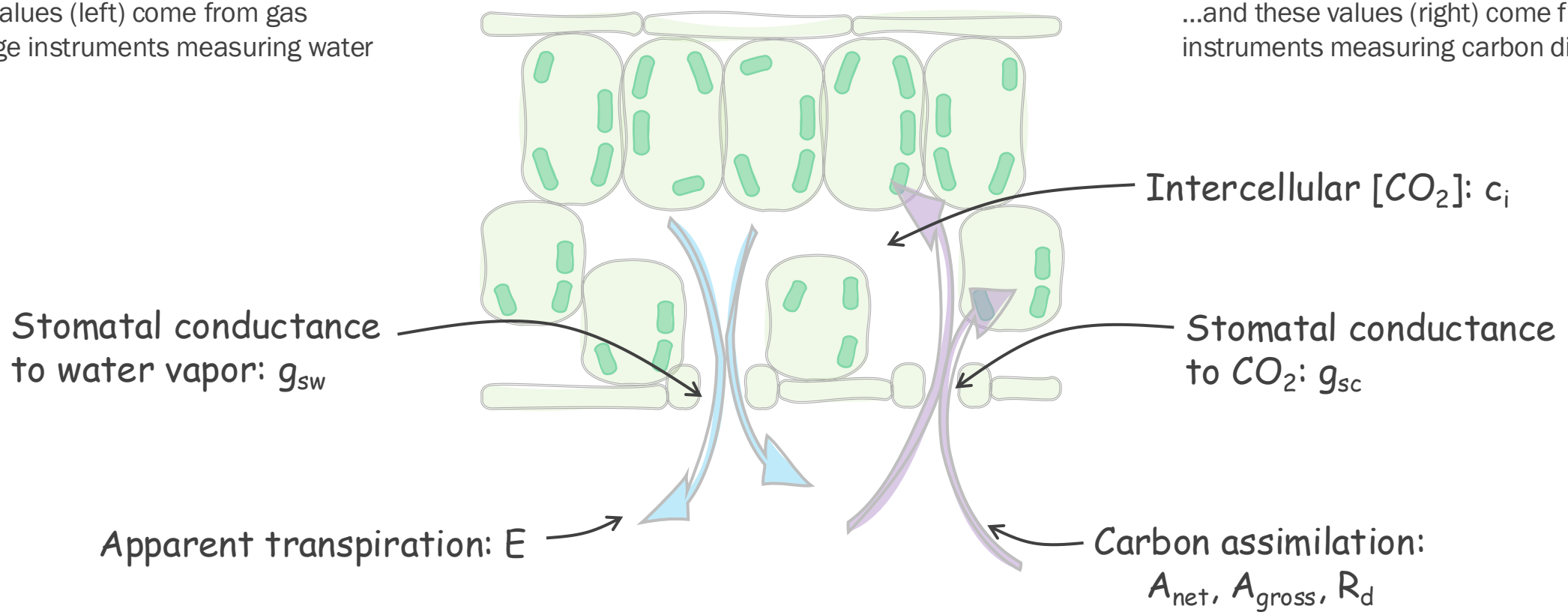
The LI-6400/XT is still widely used, but we don't manufacture it anymore. So, it doesn't get to be in the picture!

Some physiological parameters from gas exchange

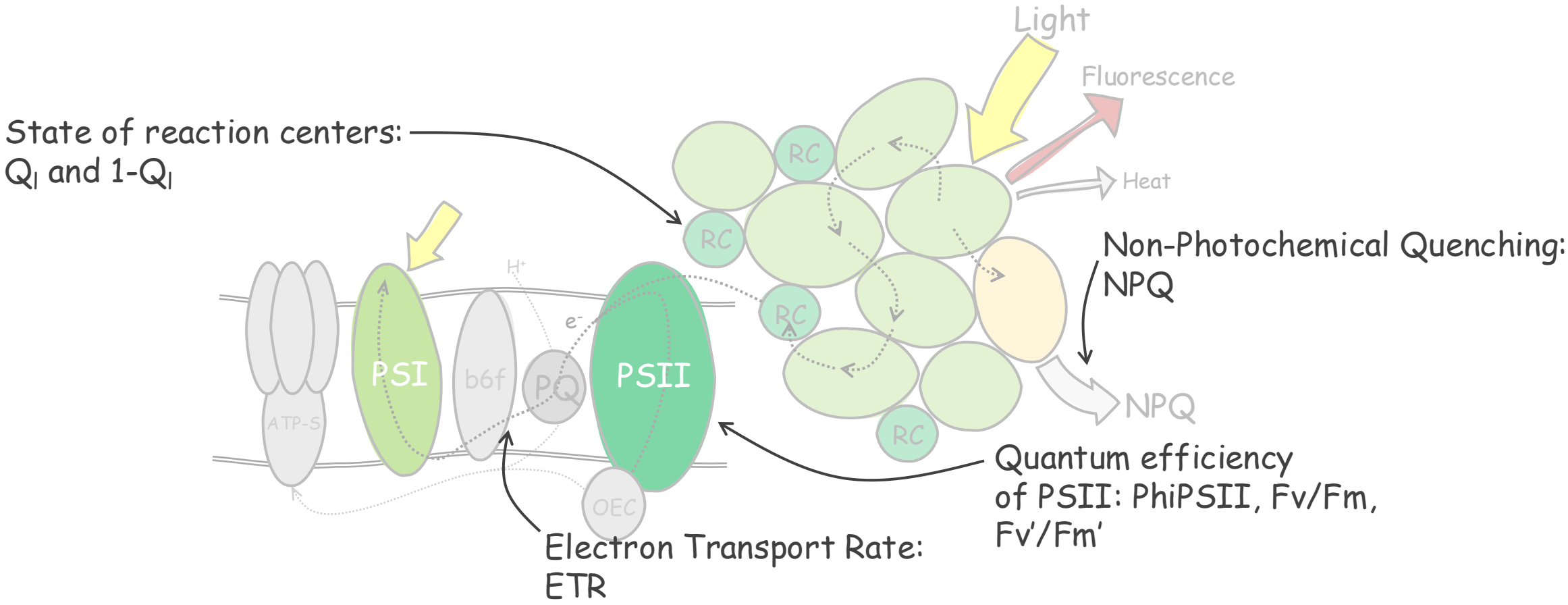
Outputs from a gas exchange system

These values (left) come from gas exchange instruments measuring water vapor…

…and these values (right) come from instruments measuring carbon dioxide.

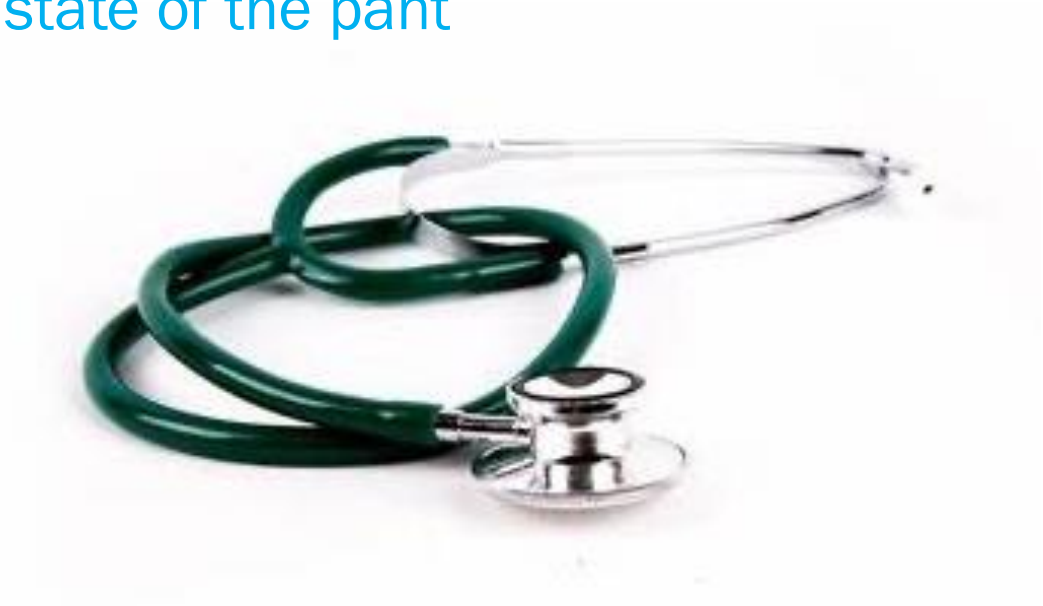


Some physiological parameters from fluorometry



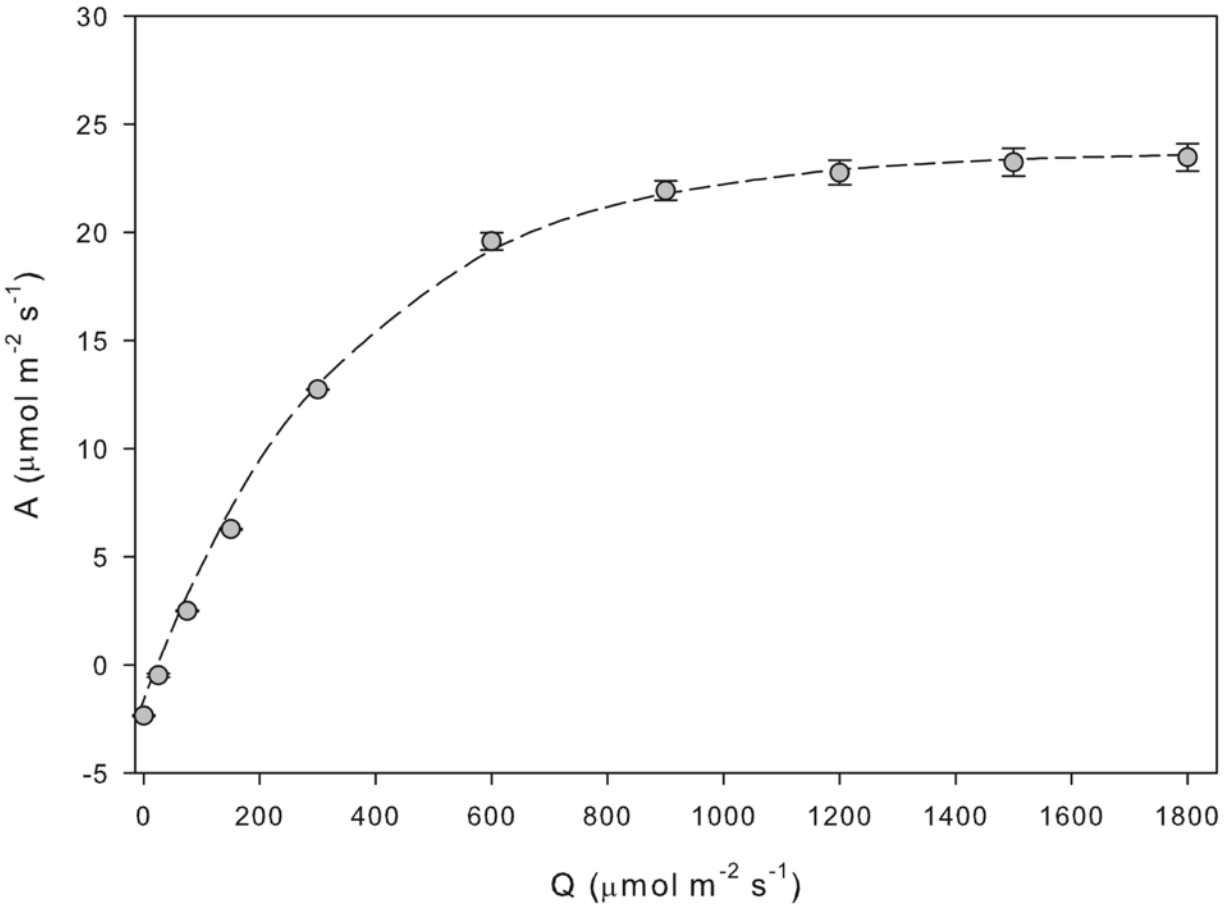
Parameters mean different things depending on how the instrument is applied

Survey: Tells us about the current state of the pant



Response: Probes capacity of and limitations to

Example response to light (AQ curve)



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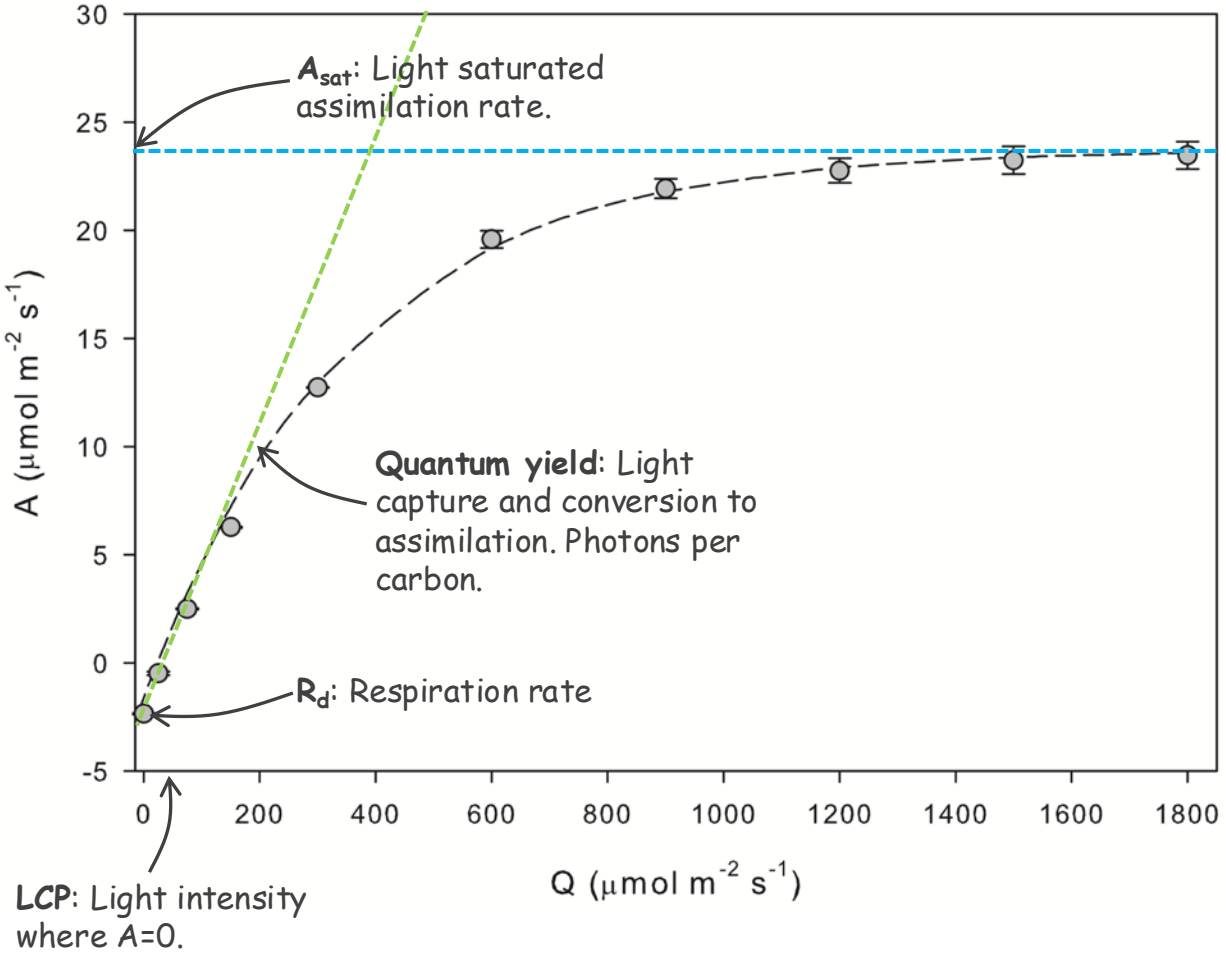

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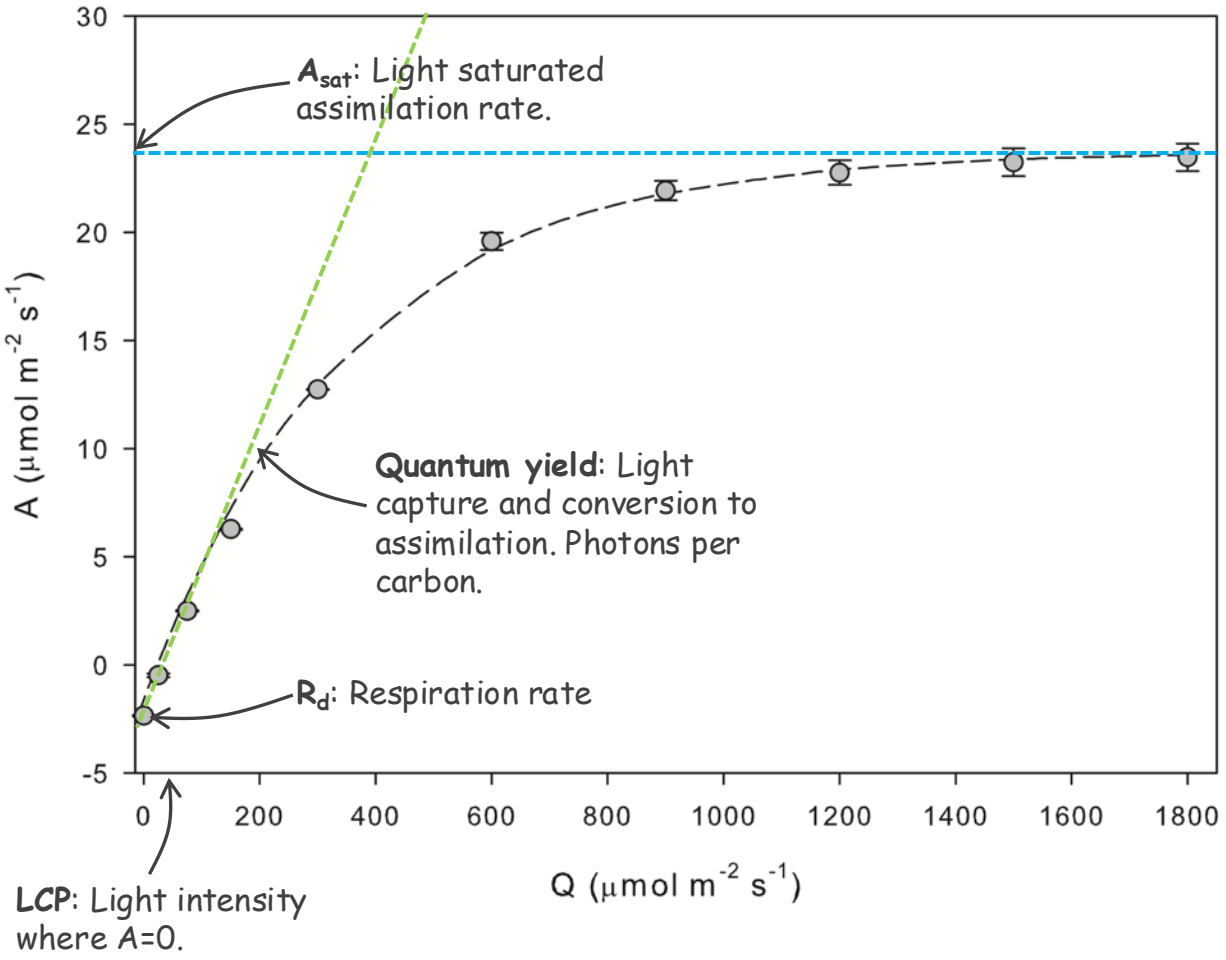
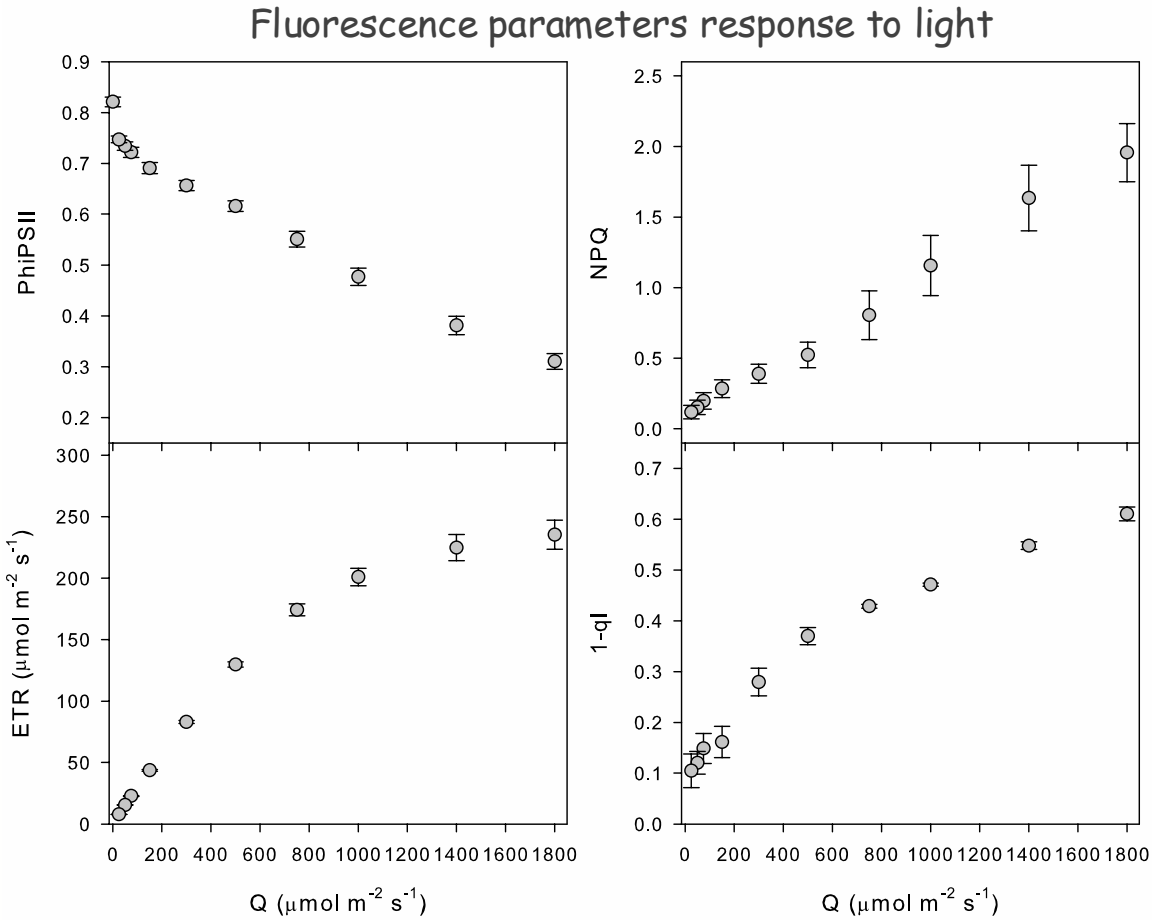
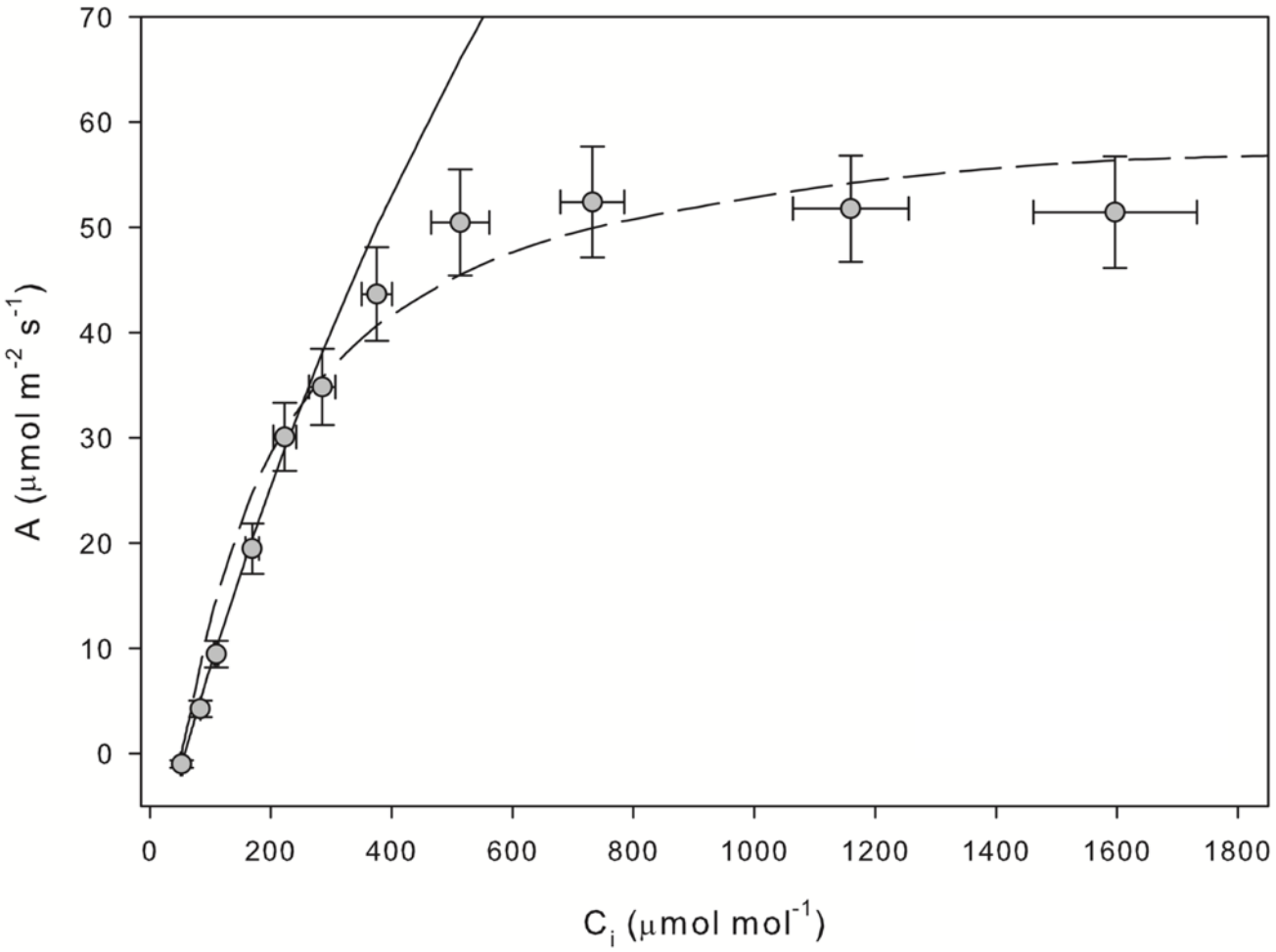




Example response to CO_2 (AC_i curve)



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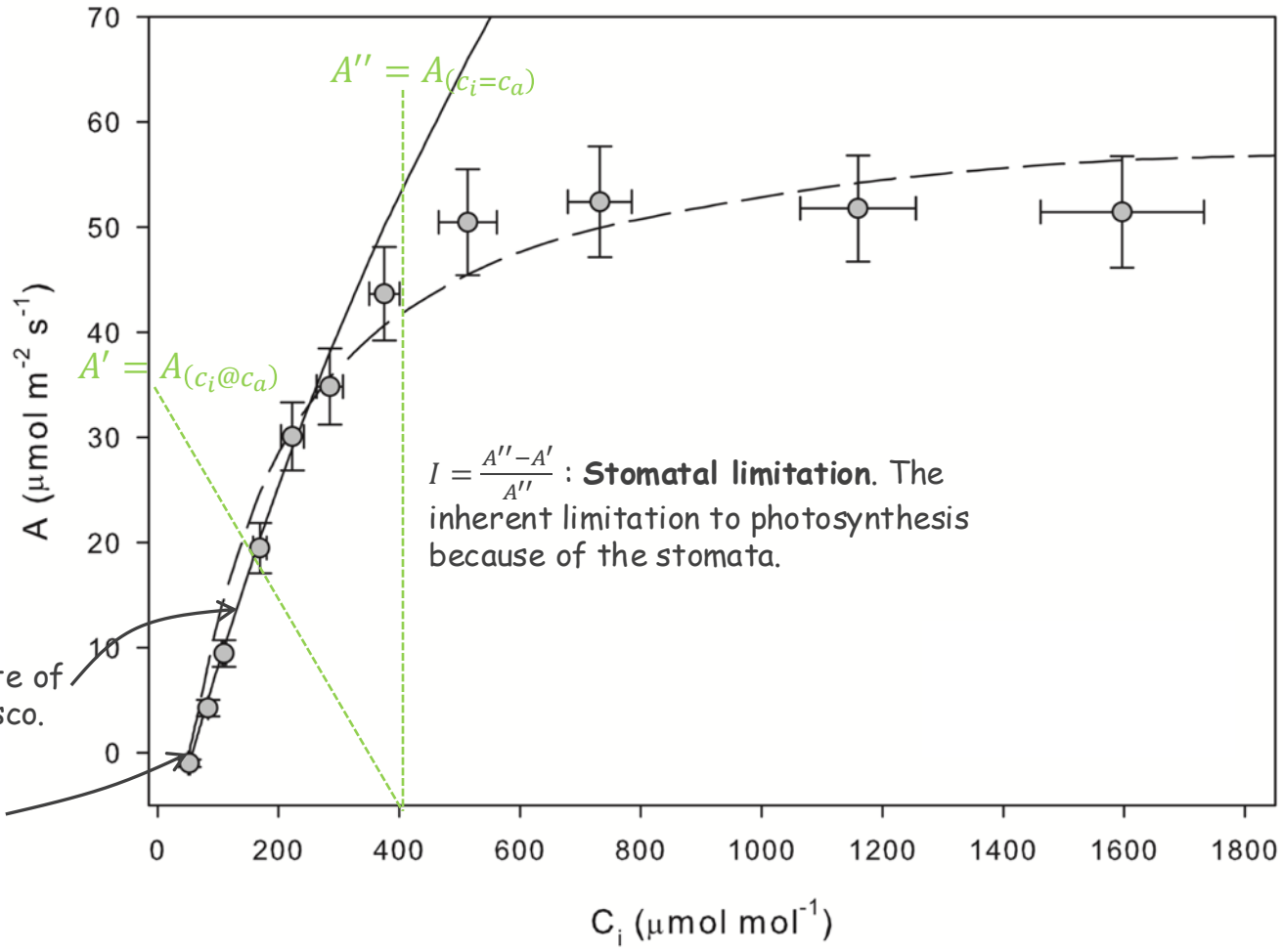

V_{TPU}: Triosphosphate Utilization

J_{max}: Maximum rate of electron transport down the electron transport chain.

$I = \frac{A''-A'}{A''}$: **Stomatal limitation**. The inherent limitation to photosynthesis because of the stomata.

Vc,max: Maximum rate of carboxylation by Rubisco.

Γ: CO_2 compensation point. C_i where A=0.

Why measure photochemistry and gas exchange?

The processes these measurements probe are processes fundamental to support (almost) all life on Earth

Traditional breeding and selection

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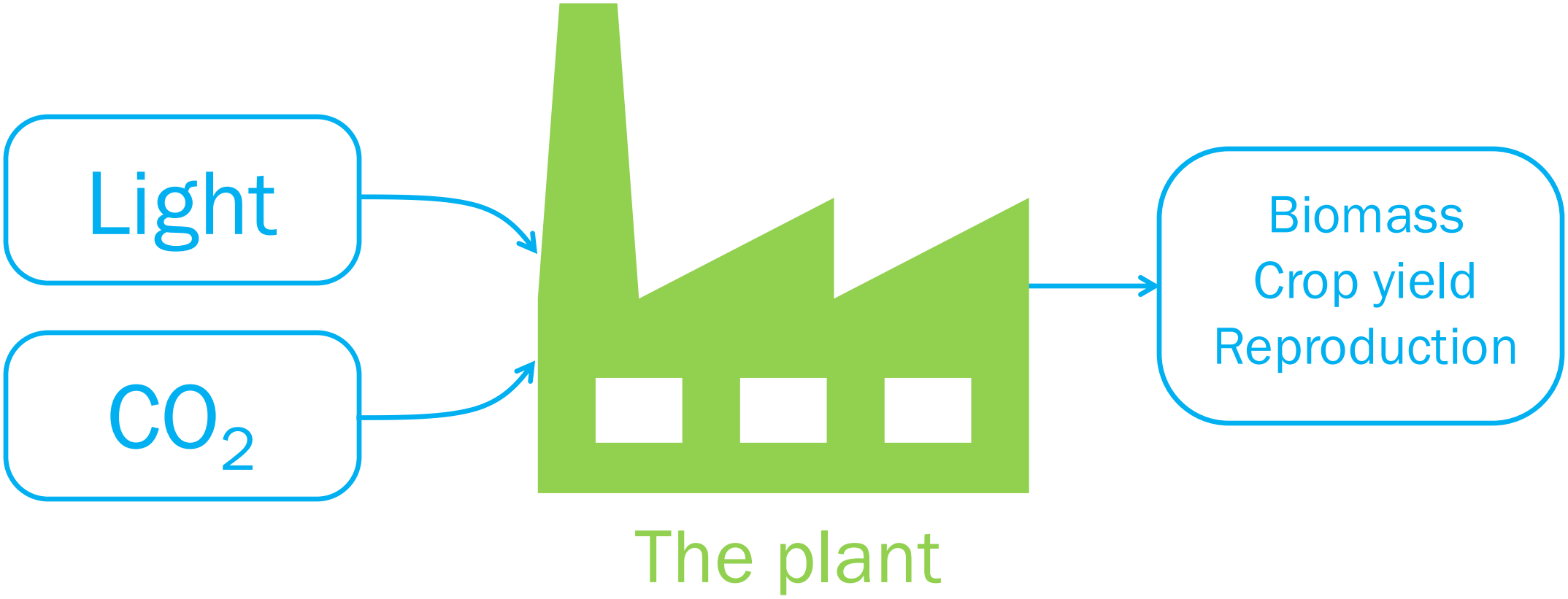
The plant as a factory



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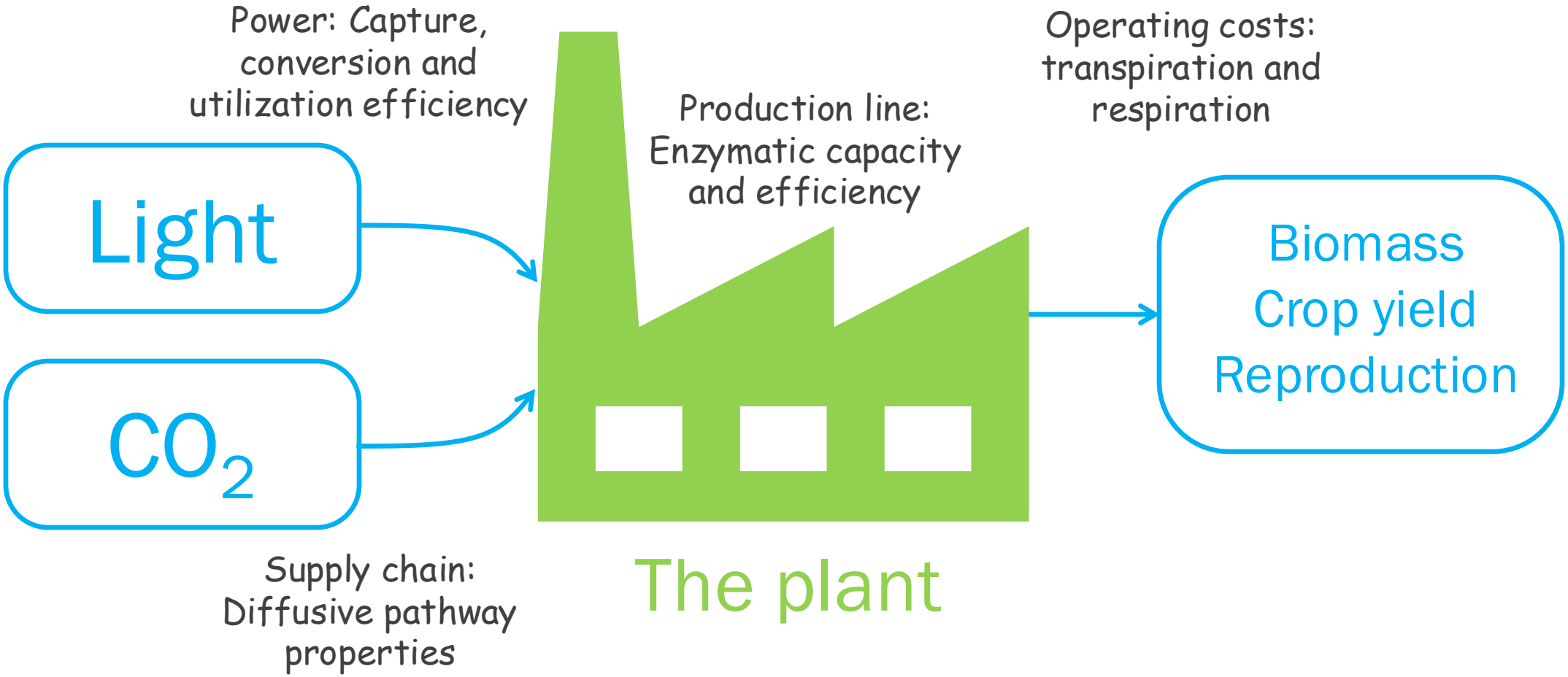
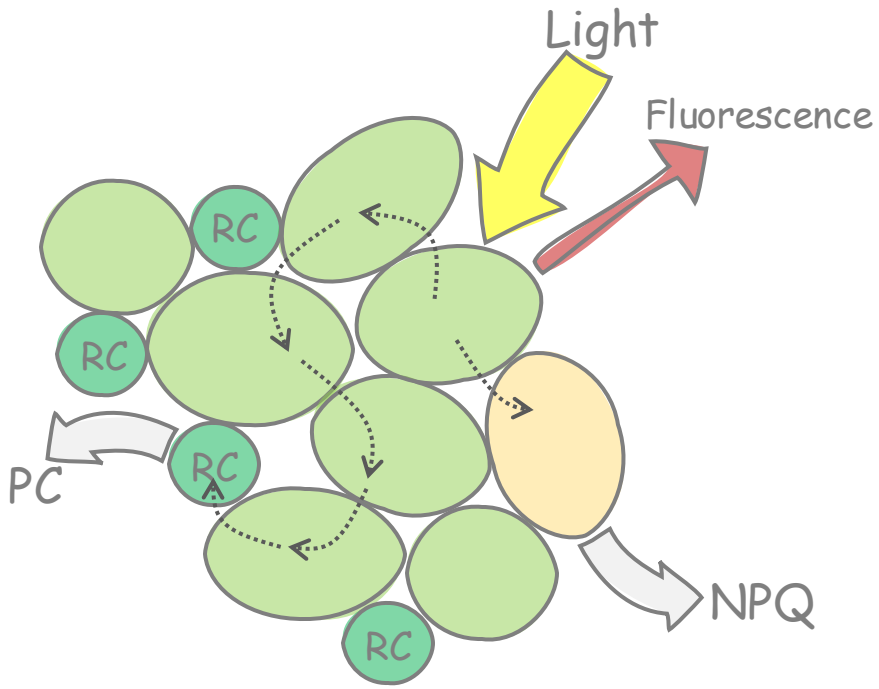
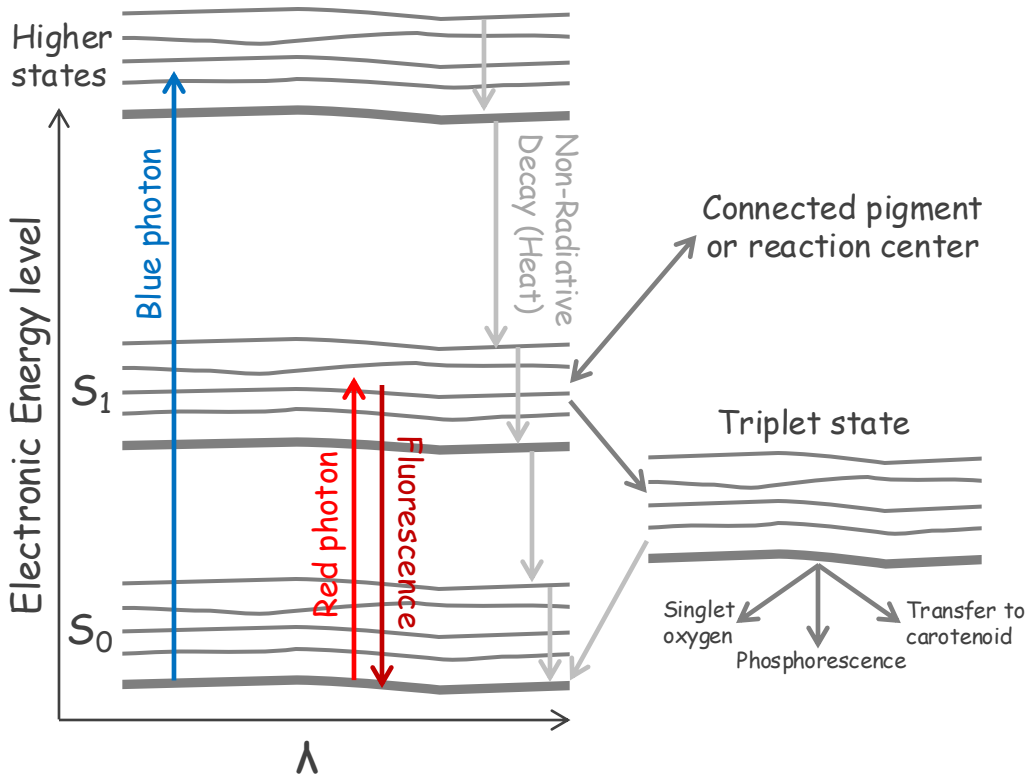


How do we measure leaf-level photochemistry and gas exchange?

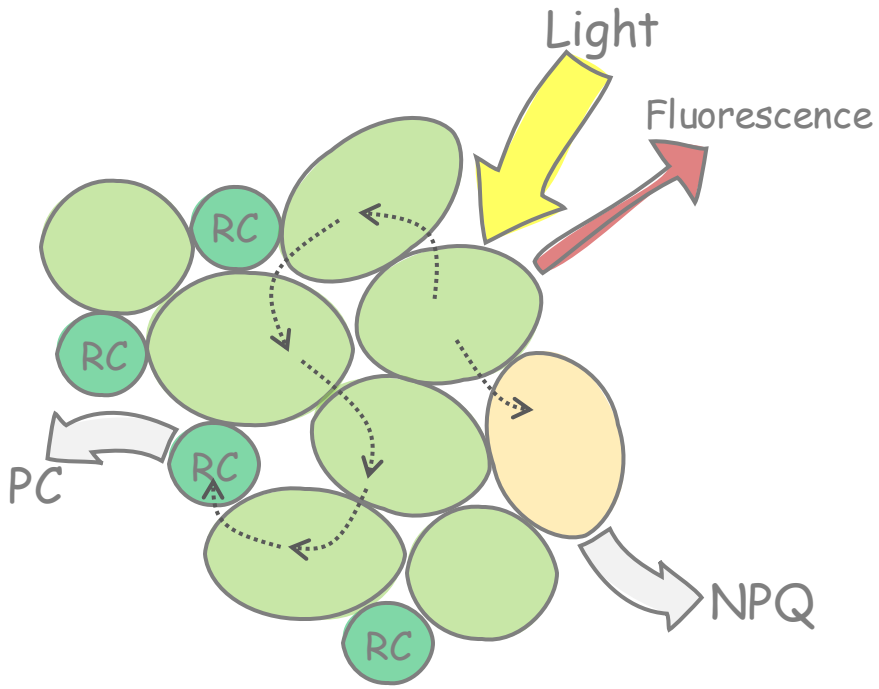
Energy capture and dissipation



Idealized Jablonski diagram of states and dissipation at the single molecule level



Fluorescence yield as defined by dissipation path



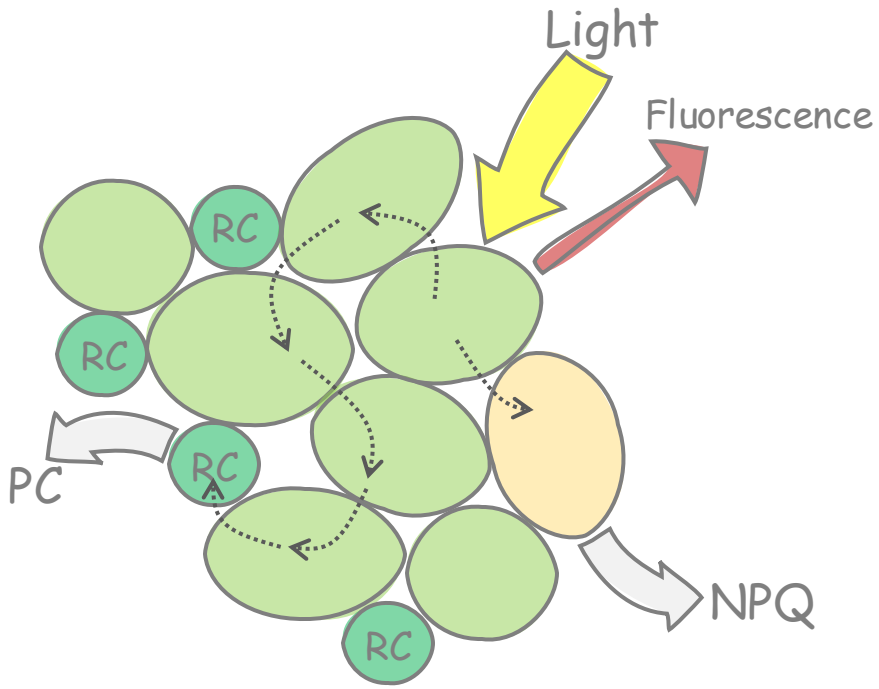
$$f_F = q\frac{k_F}{k_D + k_F + k'_{PC} + k'_{NPQ}}$$

$$\phi_F = \frac{f_F}{q}$$

$$\phi_F = \frac{k_F}{k_D + k_F + k'_{PC} + k'_{NPQ}}$$

f_F = Flux of fluoresced photons
q = Absorbed photons
Φ_F = Fluorescence yield
k_x = Rate constant of dissipation path

Fluorescence yield as defined by dissipation path



$$\phi_F = \frac{k_F}{k_D + k_F + k'_{PC} p_{Q_A} + k'_{NPQ} p_Z}$$

Photochemistry and NPQ are apparent rate constants, adjusted by proportionality coefficients:

p_{Q_A} is related to the proportion of open reaction centers

p_Z is the proportion of NPQ engagement

f_F = Flux of fluoresced photons
q = Absorbed photons
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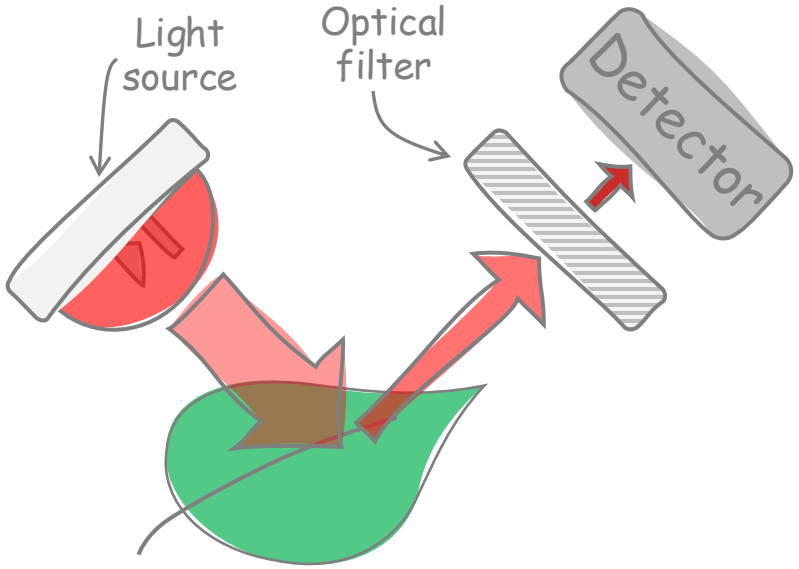
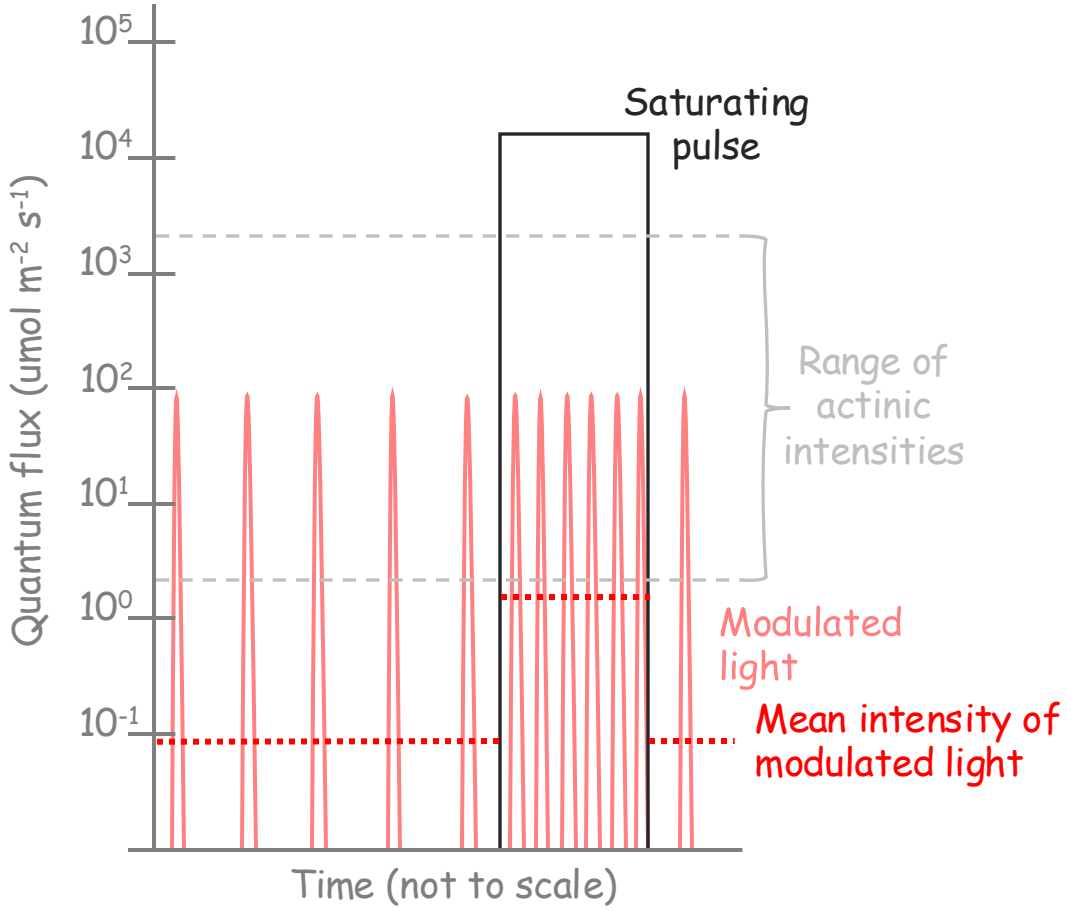
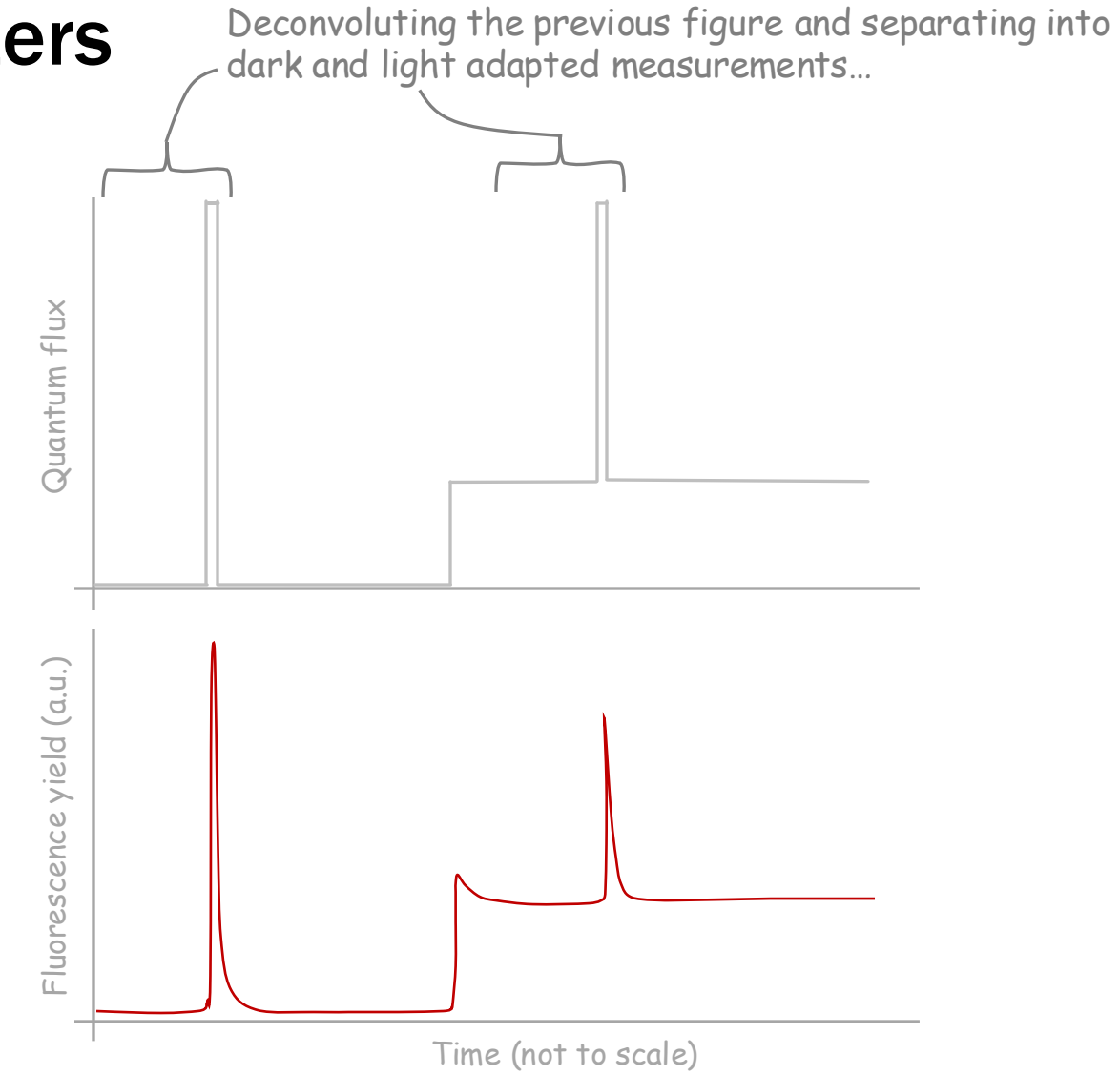
Pulse Amplitude Modulation (PAM) fluorometry





Deriving fluorescence parameters



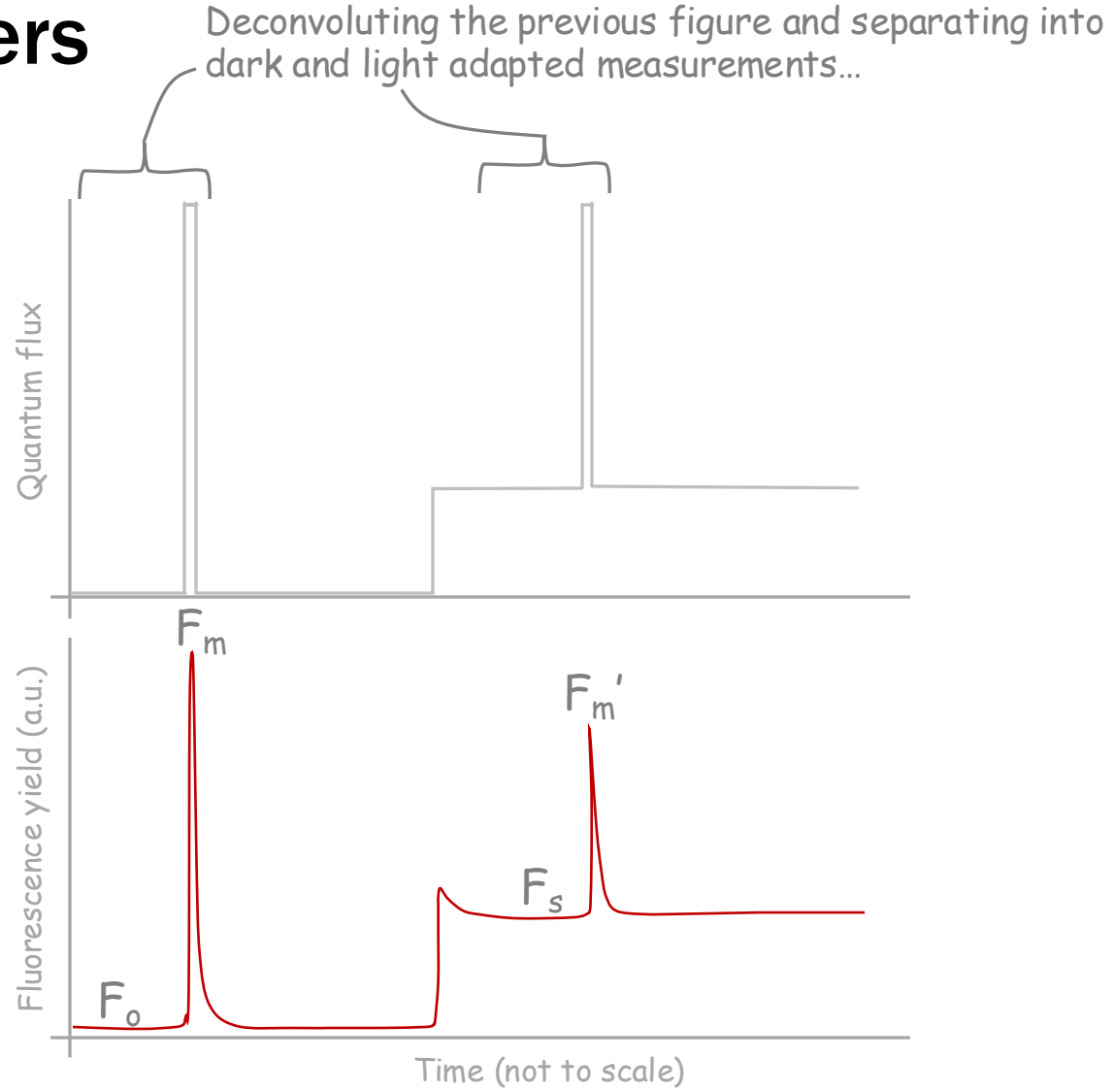
Deriving fluorescence parameters

Minimum in the dark: F_o

Maximum dark adapted: F_m

Steady-state: F_s

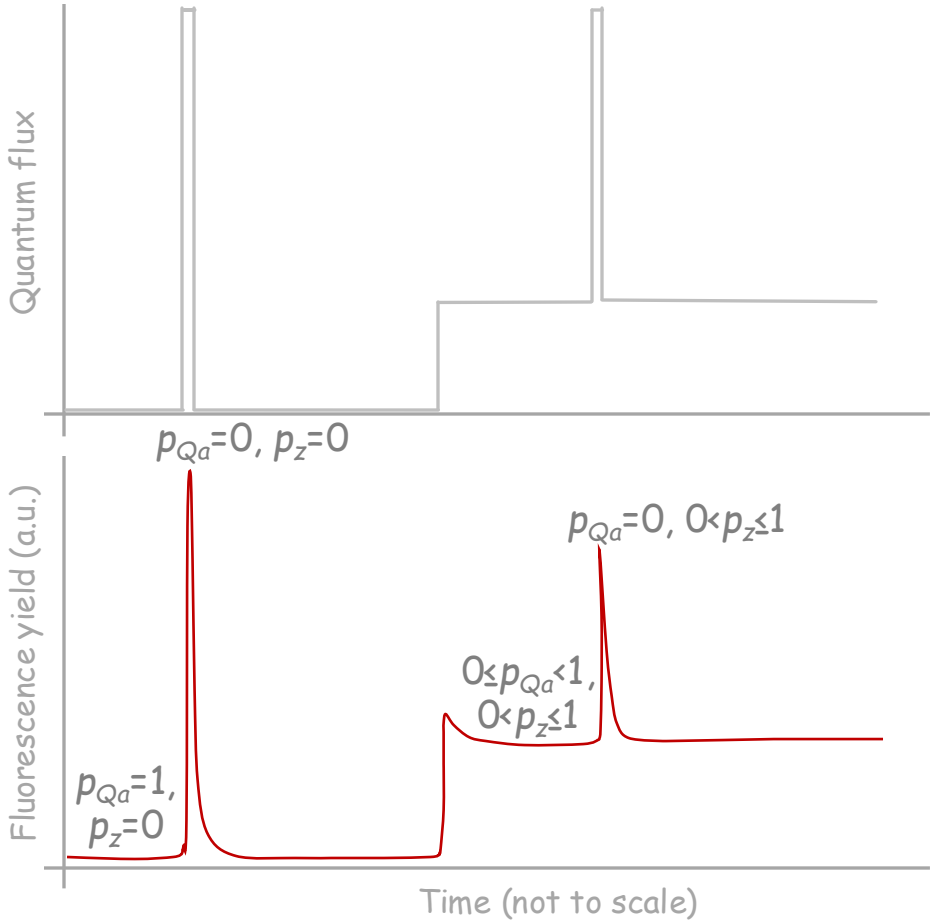
Maximum light adapted: F_m'



Deriving fluorescence parameters

$$\phi_F = \frac{k_F}{k_D + k_F + k'_{PC}p_{Q_A} + k'_{NPQ}p_z}$$

There are assumptions of state for each parameter that can be defined using the proportionality coefficients. Substituting these definitions for each parameter…



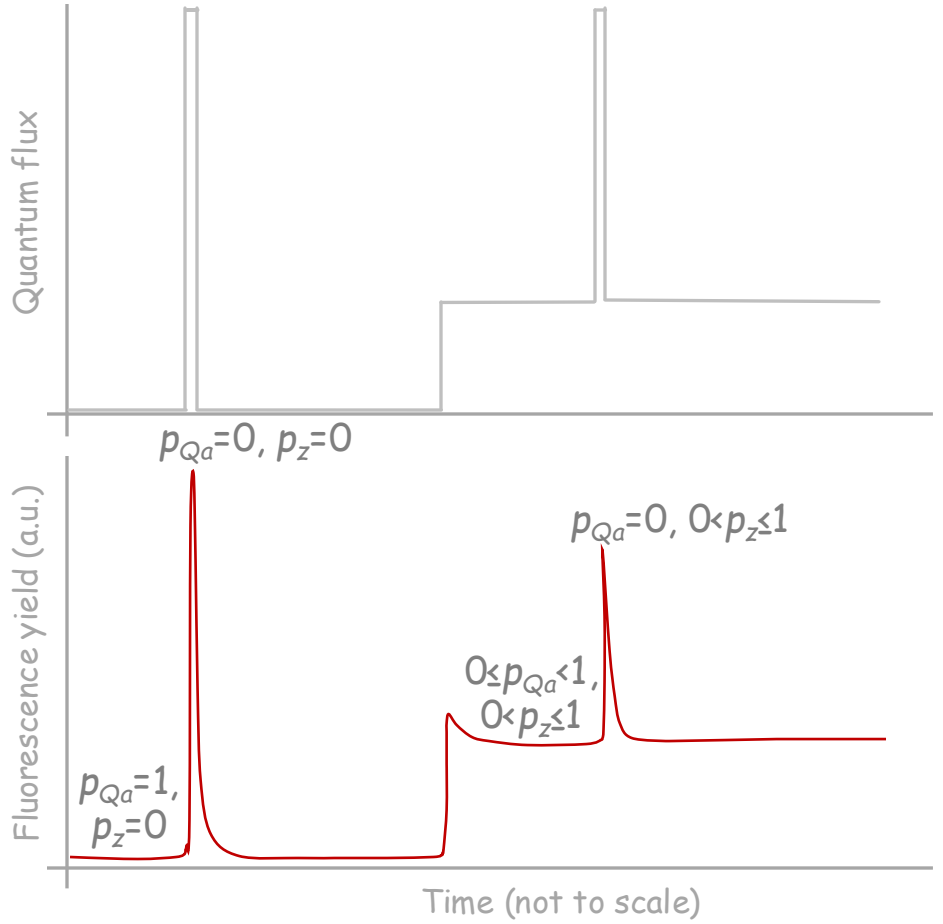
Deriving fluorescence parameters

$$F_o = \frac{k_F}{k_D + k_F + k'_{PC}}$$

$$F_m = \frac{k_F}{k_D + k_F}$$

$$F_s = \frac{k_F}{k_D + k_F + k'_{PC}p_{Q_A} + k'_{NPQ}p_z}$$

$$F'_m = \frac{k_F}{k_D + k_F + k'_{NPQ}p_z}$$



Key assumptions

All reaction centers are open such that photochemistry is not engaged

$$F_o = \frac{k_F}{k_D + k_F + k'_{PC}}$$

$$F_m = \frac{k_F}{k_D + k_F}$$

$$F_s = \frac{k_F}{k_D + k_F + k'_{PC}p_{Q_A} + k'_{NPQ}p_z}$$

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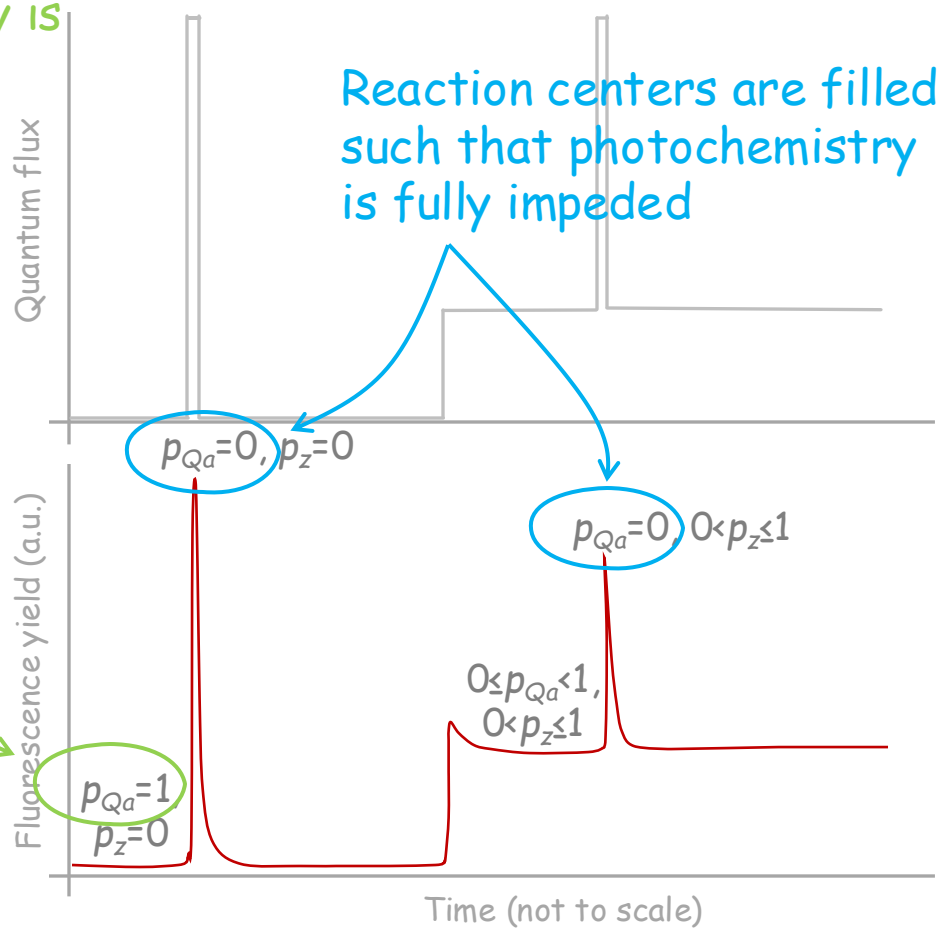
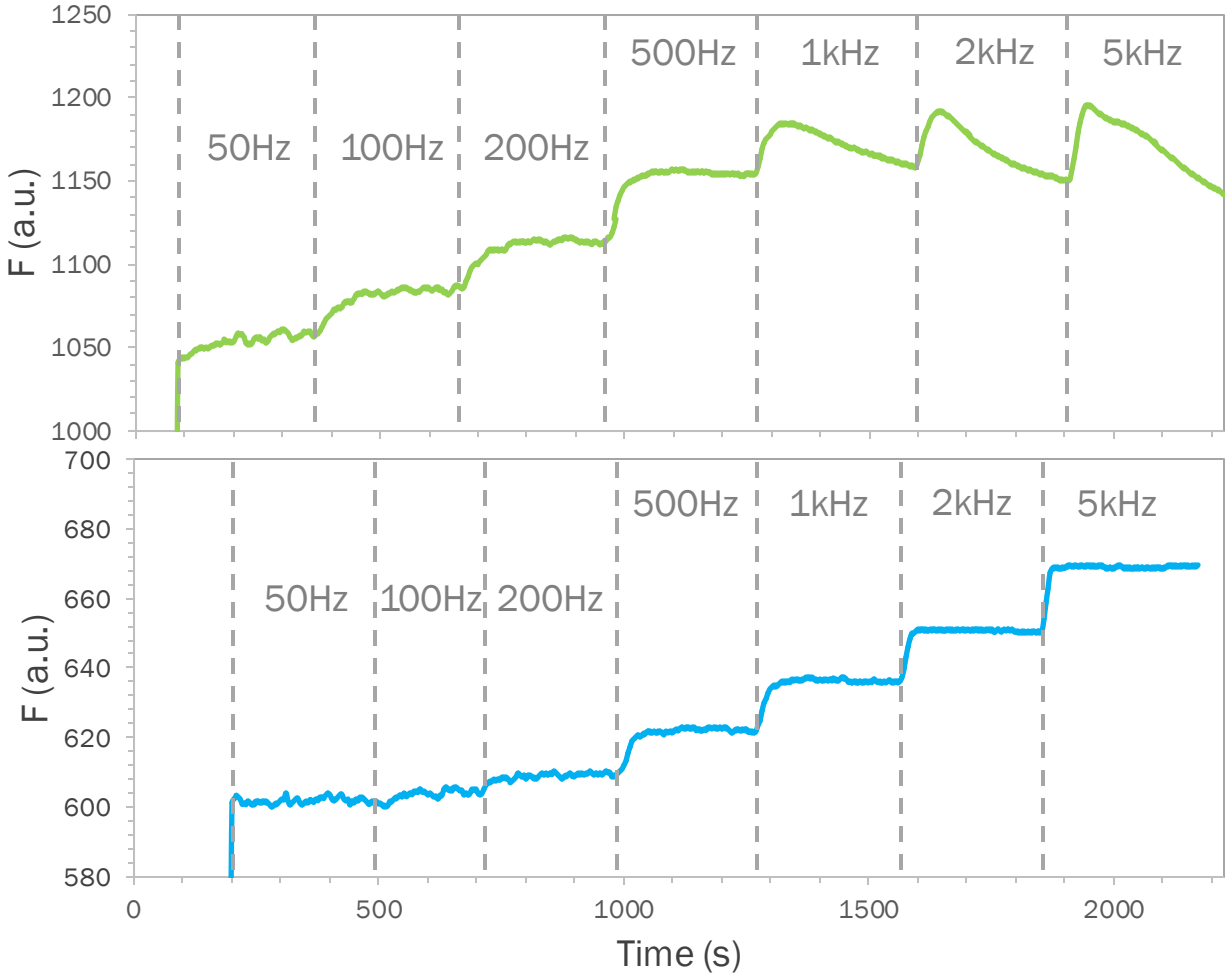


Modulation for F_0 (verify p_{Q_A}=1)

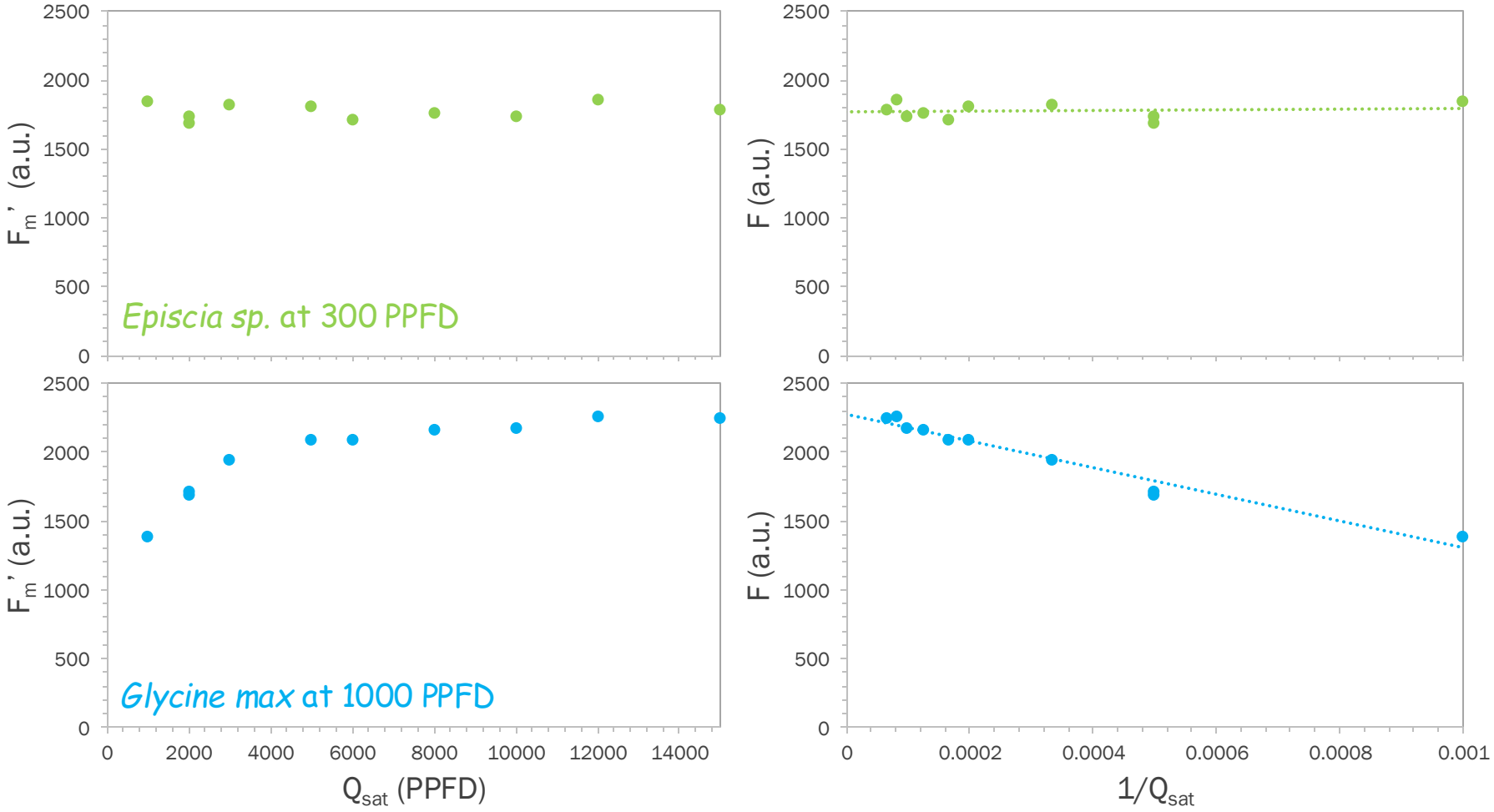
The higher the modulation frequency the larger the signal and the smaller the noise.



Low light adapted *Episcia sp.* shows induction of photochemistry above 0.005 PPFD (50 Hz).

High light adapted *Glycine max* shows little to no induction of photochemistry at modulation frequencies up to 0.02 PPFD (200 Hz)

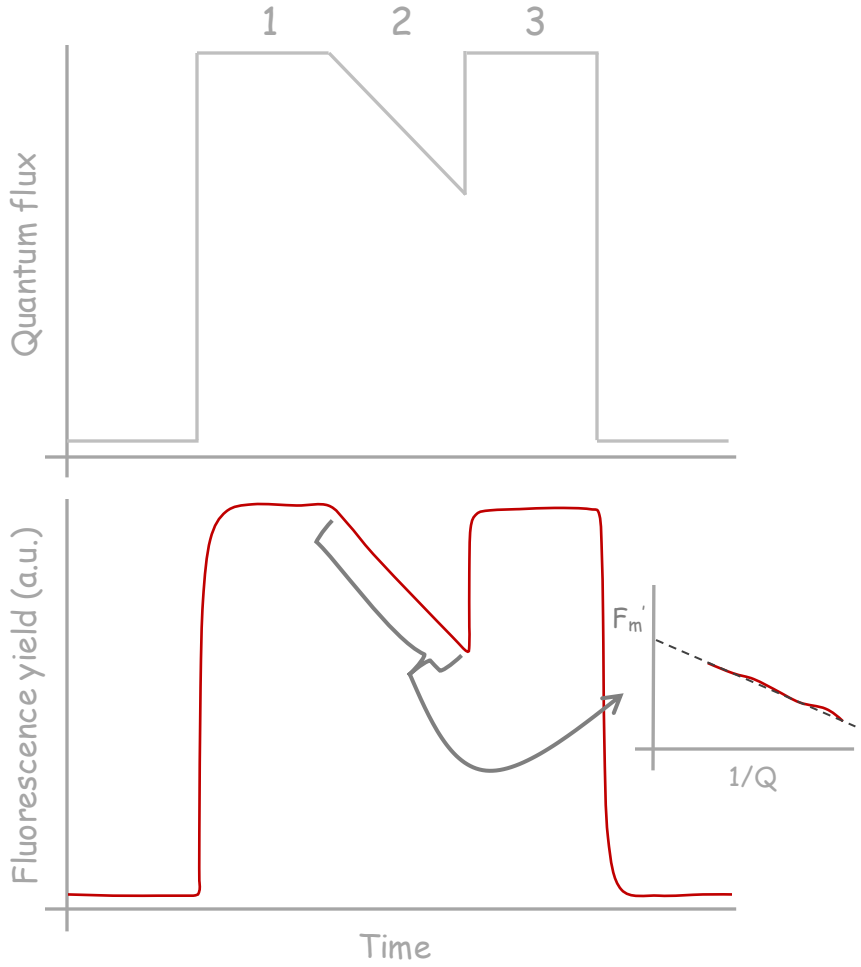
Test for saturating flash intensity (verify p_{Q_A}=0)



See Markgaf T and J Berry. 1990. Measurement of photochemical and non-photochemical quenching: Correction for turnover of PS2 during steady-state photosynthesis. Research in Photosynthesis

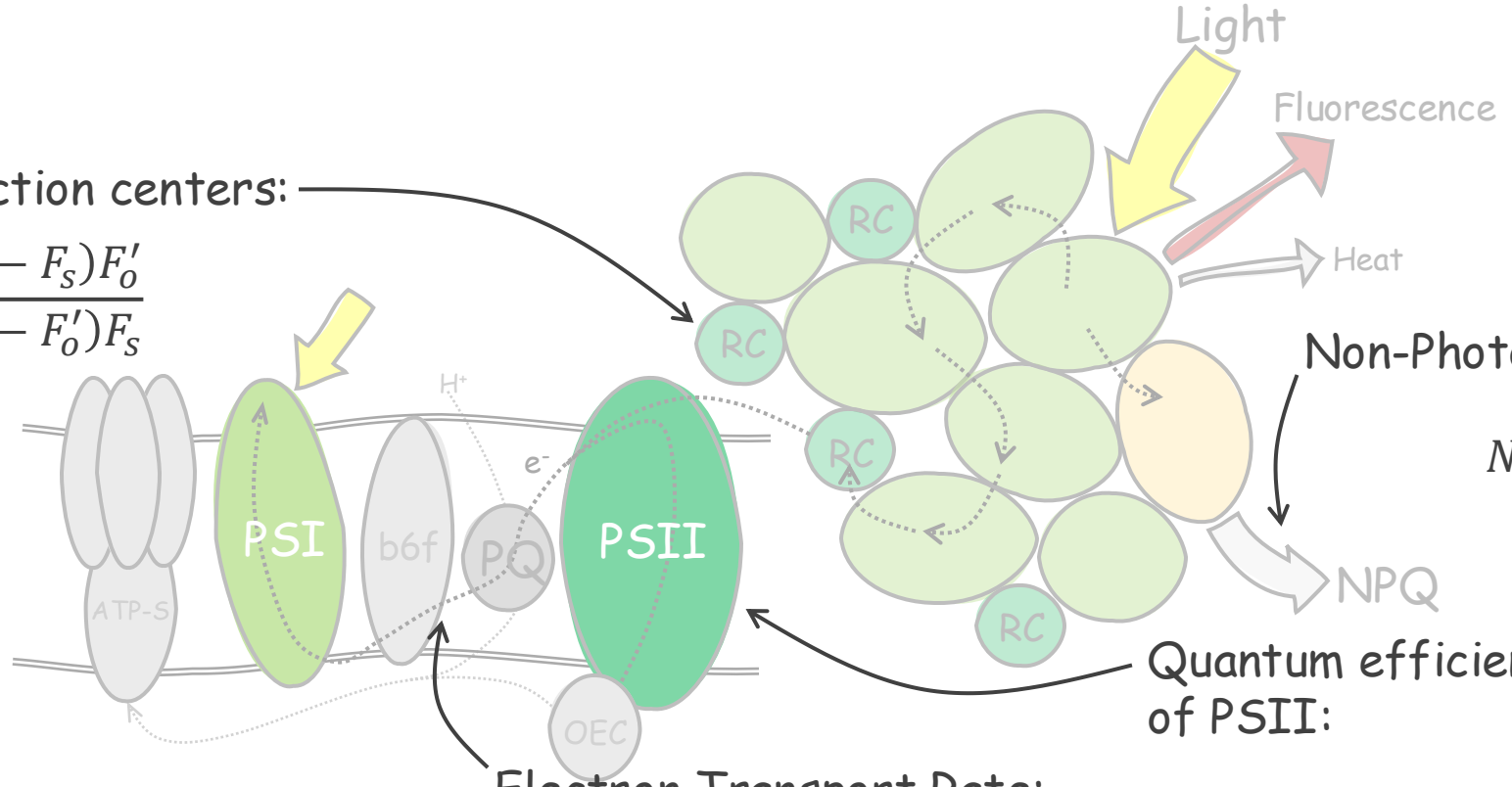
Multi-Phase Flash (MPF) technique

- Segment a sub-second flash into a three phase protocol
- Phase 1 and 3 look like ends of a traditional square flash
- Phase 2 includes a linear ramp in intensity
- Fit phase 2 fluorescence versus the inverse of intensity to estimate F_m' at infinite light intensity



Loriaux SD *et al*. 2013. Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. Pant, Cell and Environment

Some physiological parameters from fluorometry



State of reaction centers:

$$q_L = \frac{(F'_m - F_s)F'_o}{(F'_m - F'_o)F_s}$$

Non-Photochemical Quenching:

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

Quantum efficiency of PSII:

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

$$\phi_{PSII} = \frac{F'_m - F_s}{F'_m}$$

$$\frac{F'_v}{F'_m} = \frac{F'_m - F'_o}{F'_m}$$

Electron Transport Rate:

$$f_{ETR} = \phi_{PSII} Q \alpha p_{\frac{PSII}{PSI}}$$

α = Effective light absorption
Q = Quantum flux of light
$p_{\frac{PSII}{PSI}}$ = Distribution of photons between PSII and PSI

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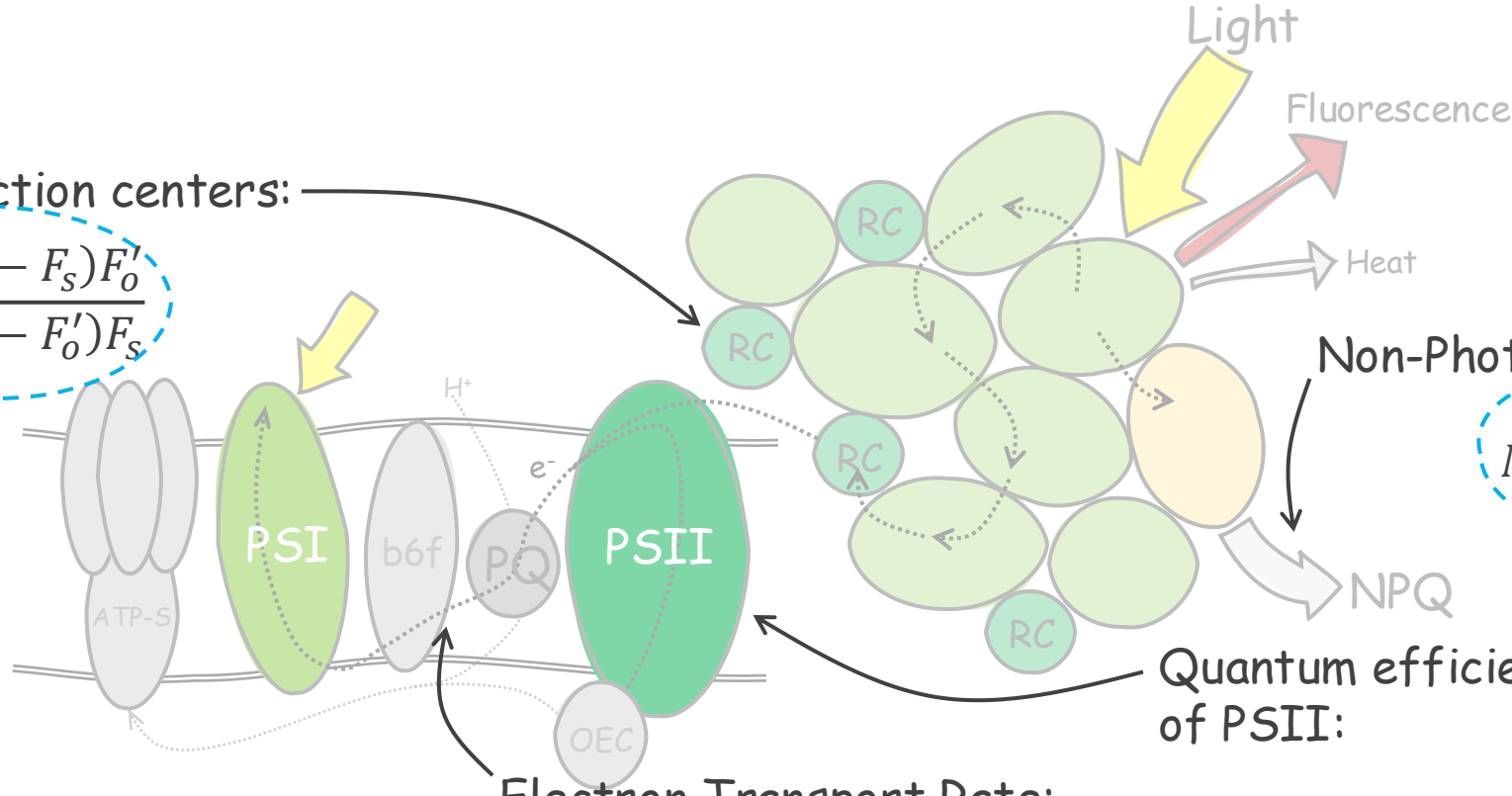

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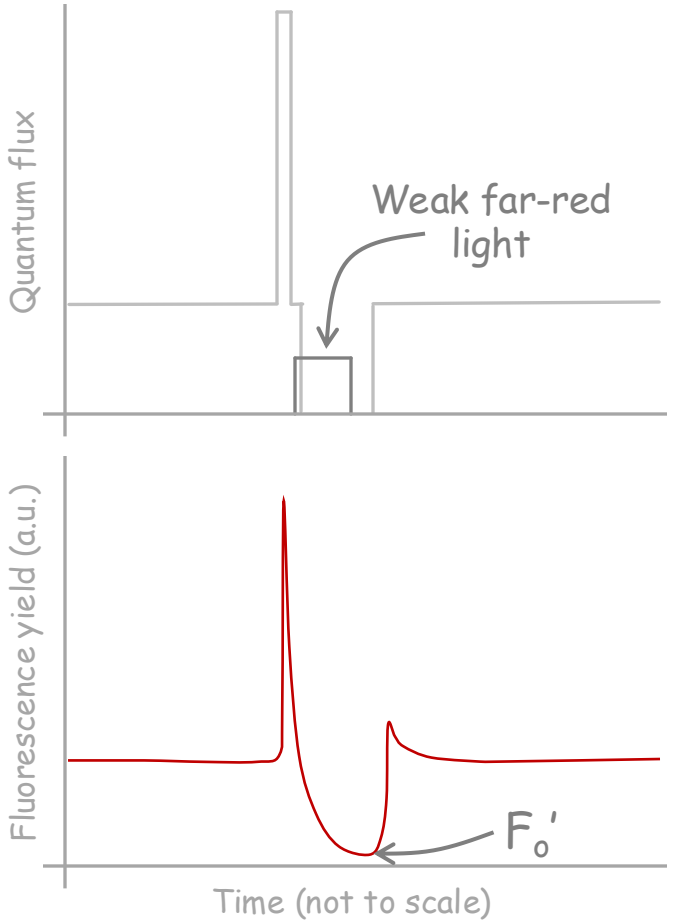
Methodologies for determining F_o'

$$F_o' = \frac{k_F}{k_F + k_H + k'_{PC} + k'_{NPQ} p_z}$$

Far-red light, preferentially absorbed by PSI, is used to open-up reaction centers: p_{Qa}=1

NPQ is left intact: $0 < p_z \leq 1$



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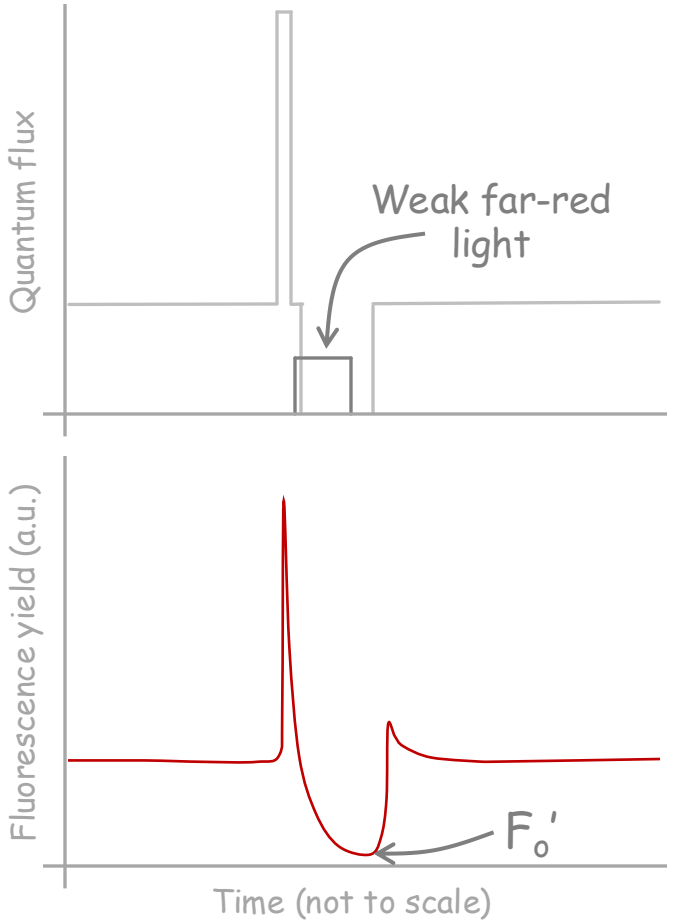
NPQ is left intact: $0 < p_z \leq 1$

NPQ has both slow and fast relaxing components. In some plants the fast components are fast enough to invalidate this assumption. Where this happens, it will be particularly evident at high light where NPQ makes up a large portion of the quenching capacity.

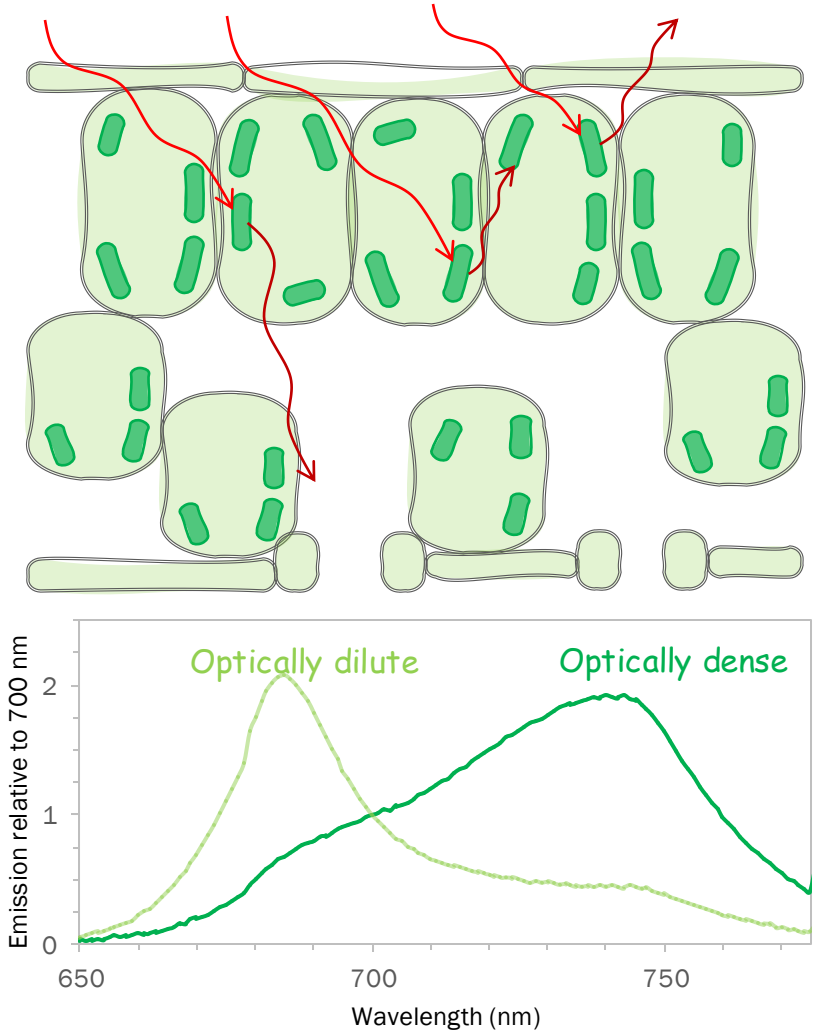
$$F_o' = \frac{F_o}{\frac{F_v}{F_m} + \frac{F_o}{F'_m}}$$

Oxborough and Baker. 1997. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv-/Fm-; without measuring Fo-. Photosynthesis Research



Fluorescence is an uncalibrated measurement

- Meaningful parameters are all ratios
- Signal is a raw count, and not standardized to any specific scalar
- Only a fraction of the total emission is seen
- Emission characteristics are dependent on sample optical properties

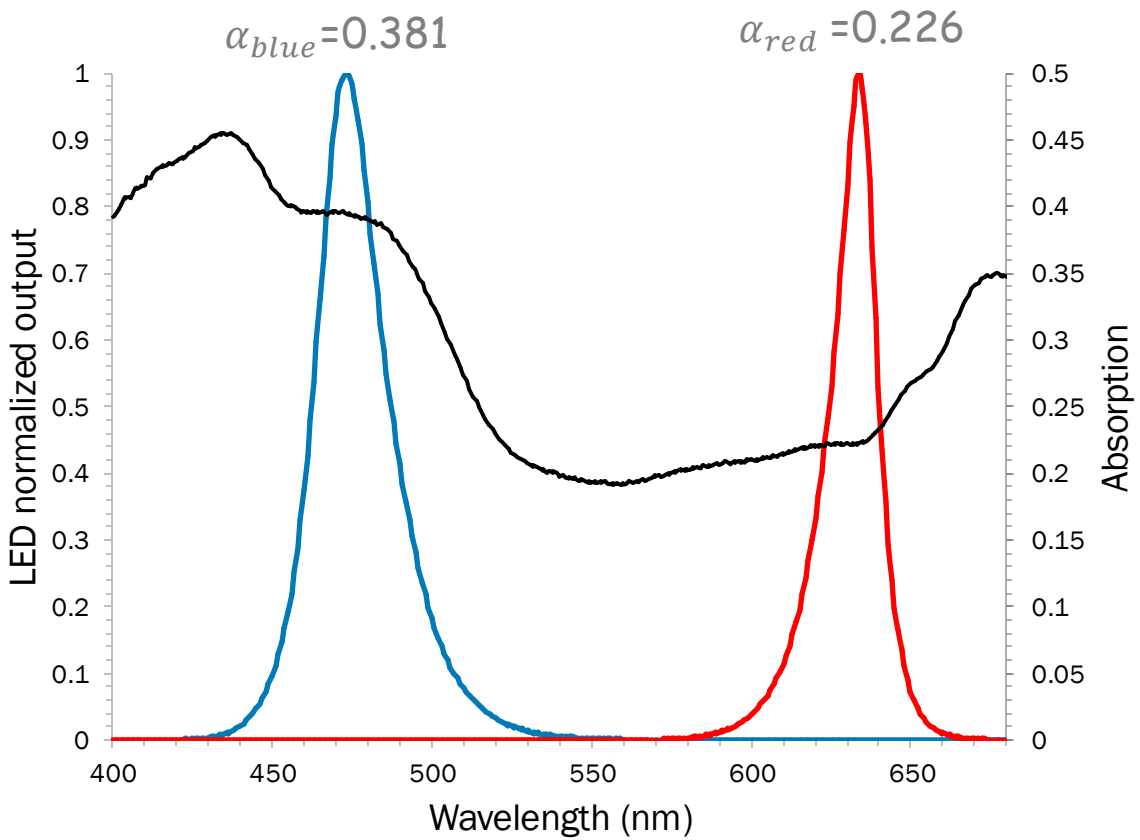


Light absorption and weighted absorption coefficients

- Absorption is measured with a spectroradiometer and integrating sphere (black curve)
- Absorption coefficients weighted by the spectral output of the light source in particular bands (α_x)
- Absorbed light accounts for the fraction of light output in particular bands (p_x)

$$Q_{absorbed} = Q_{incident}(\alpha_{blue}p_{blue} + \alpha_{red}p_{red})$$

- Coefficients are calculated by integrating the product of the normalized output for each band with the absorption curve



Data from an algal suspension at a typical 6800-18 measurement density. LED normalized output for 6800-01A.

Contact envsupport@licor.com and provide light source model number for spectral outputs.

What's what in a LI-COR gas exchange system

- Sensor head contains the core of the gas exchange sensors
- Console manages the air supply, contains the power supply and includes the user interface

In the LI-600 the "sensor head" and "console" are effectively merged into one package

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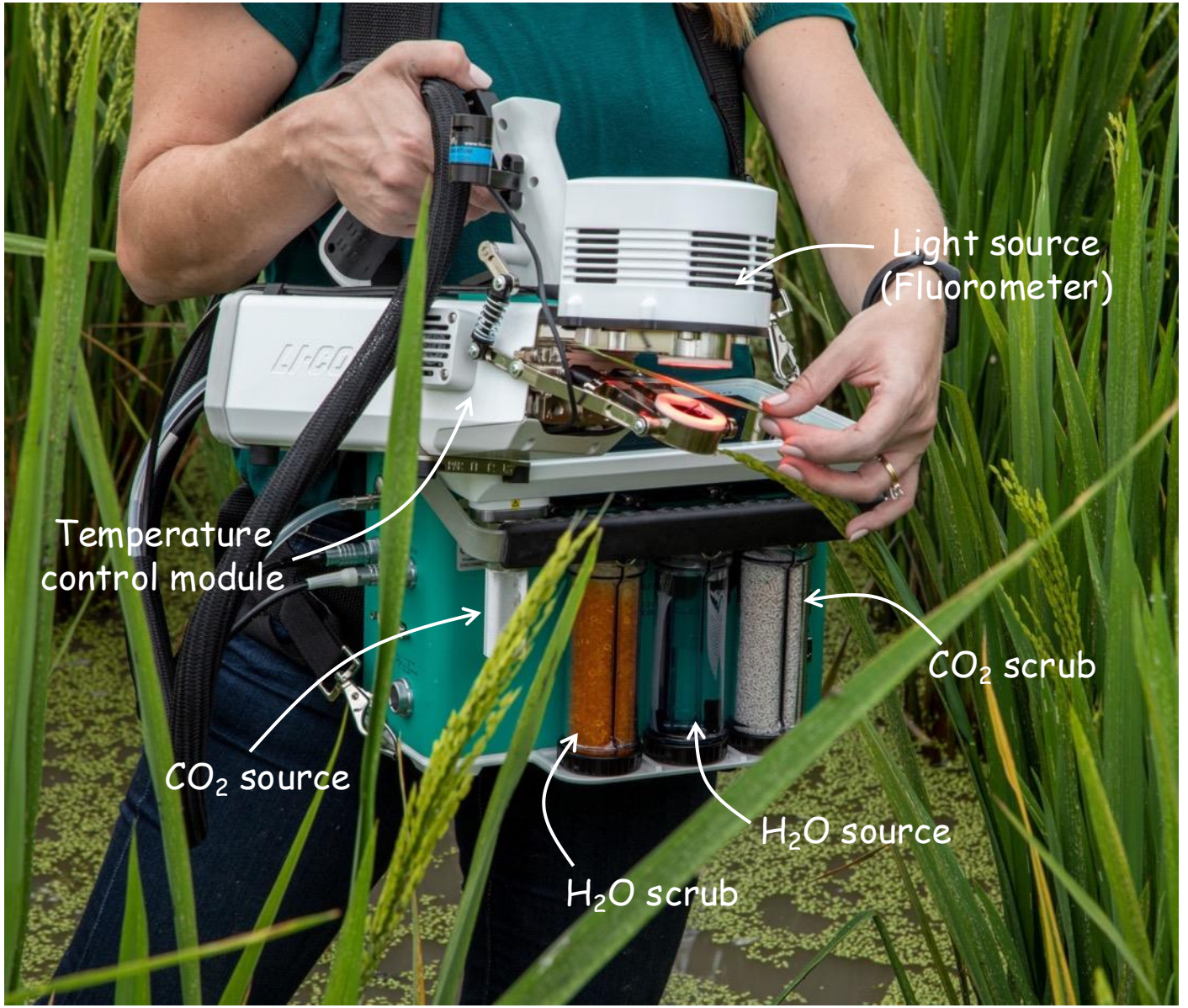
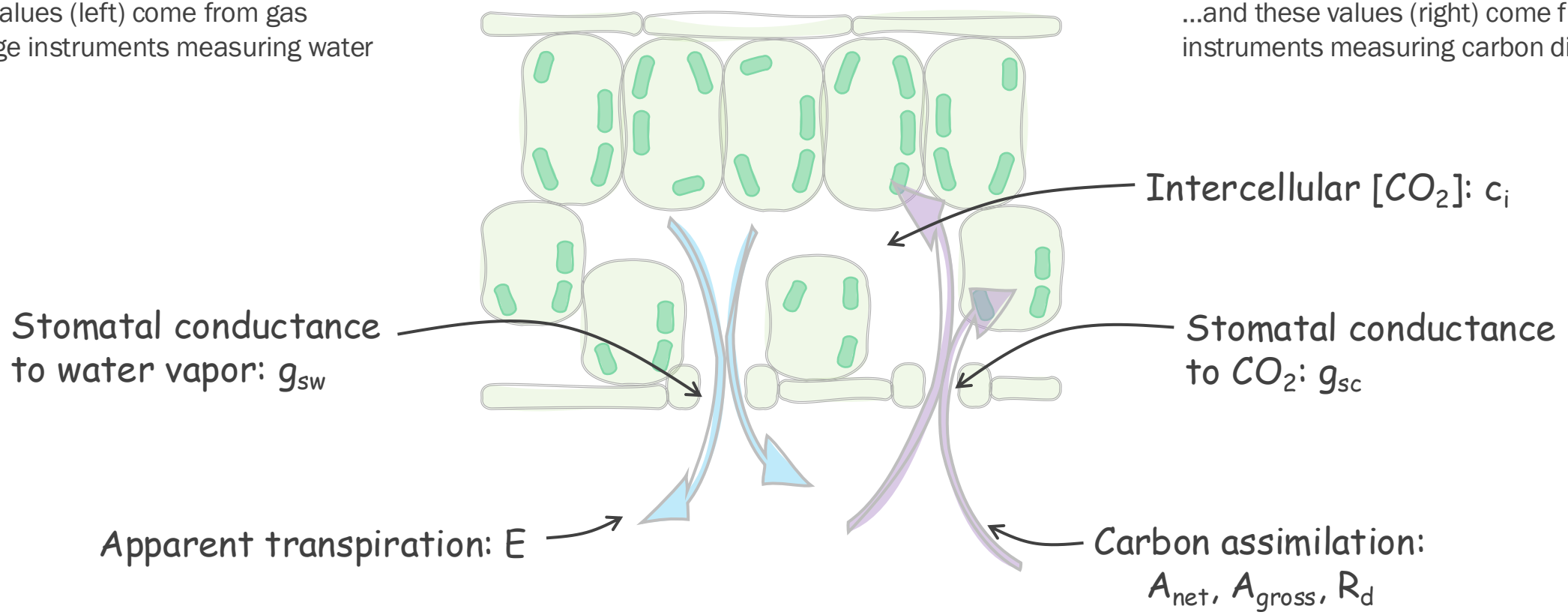


Gas exchange parameters

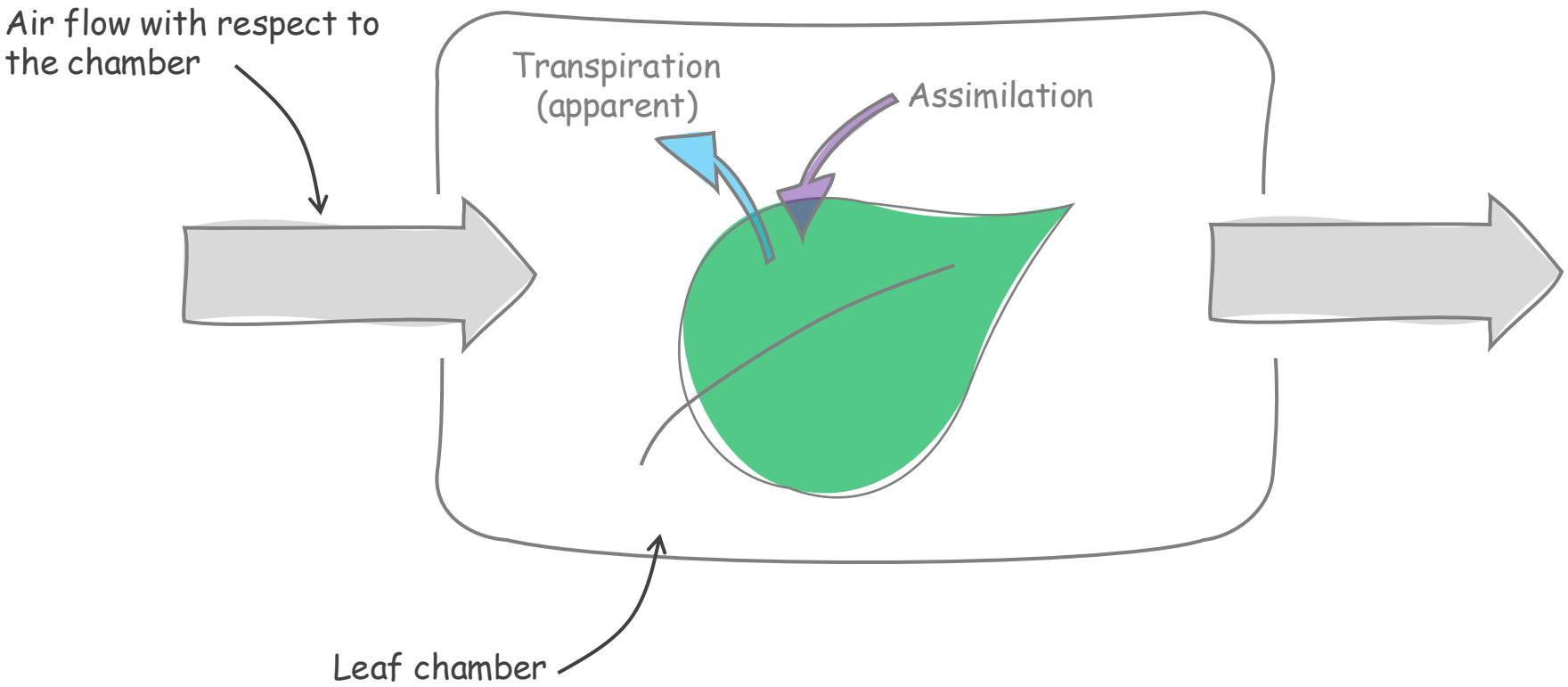
Outputs from a gas exchange system

These values (left) come from gas exchange instruments measuring water vapor…

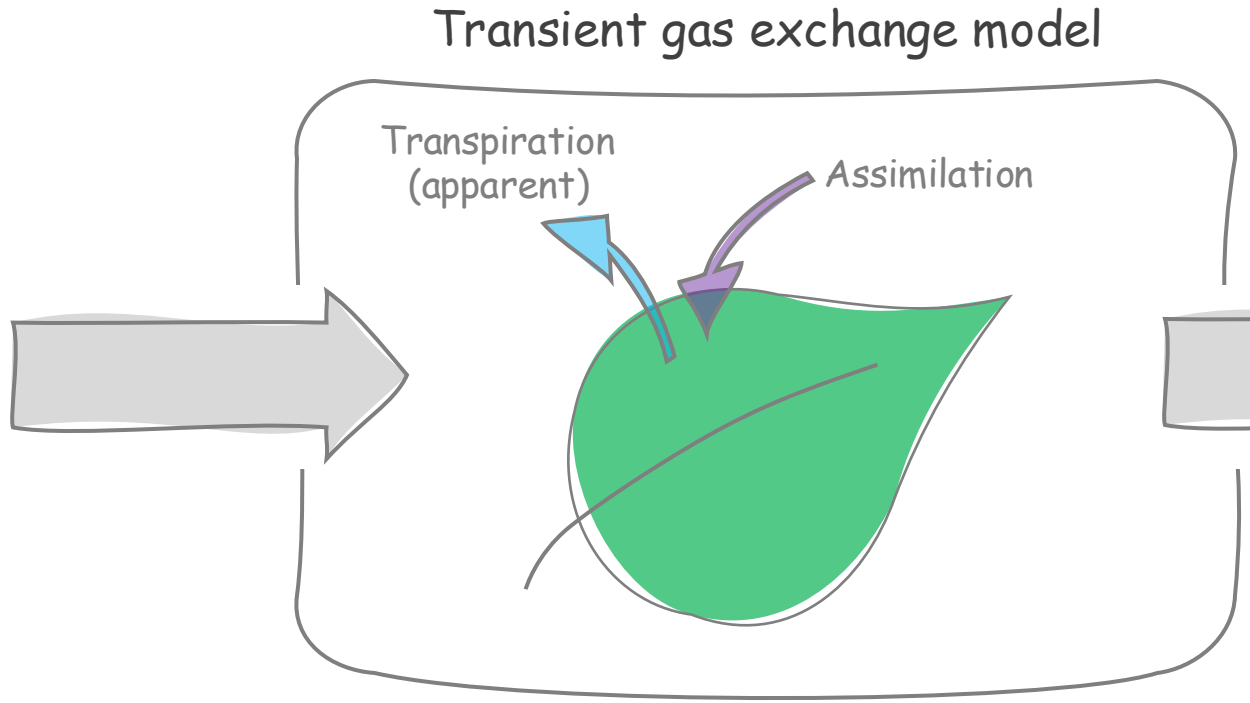
…and these values (right) come from instruments measuring carbon dioxide.



Mass balance for gas exchange



Mass balance for gas exchange



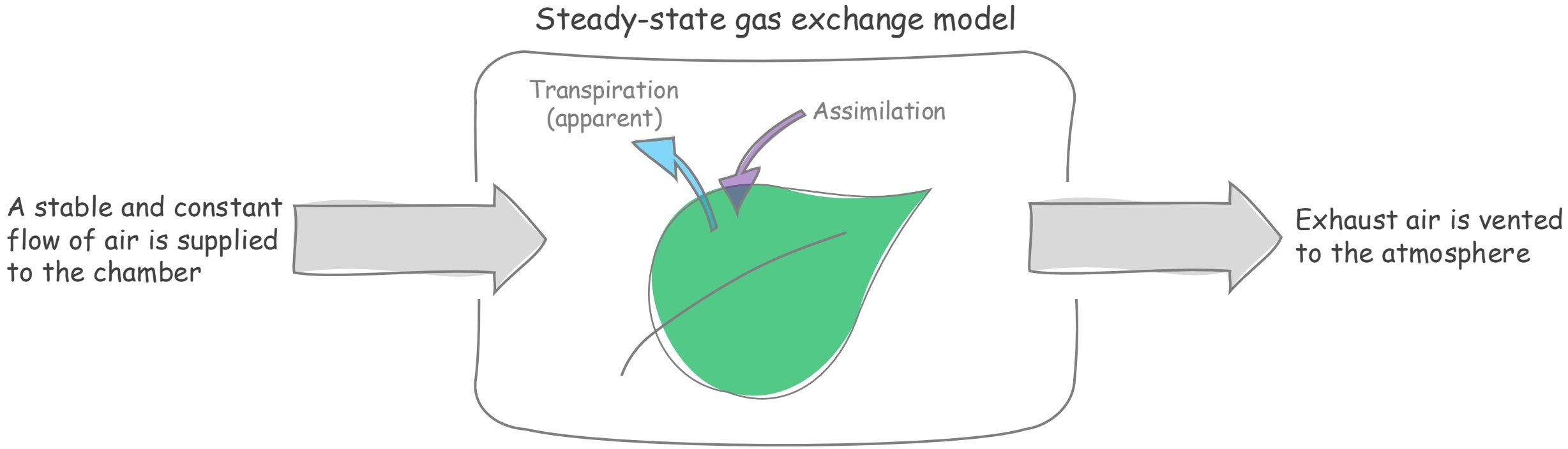
Incoming air is comprised of air exhausted from the chamber previously

Exhaust air is routed through the system and returned to the chamber inlet, forming a closed loop

$$sf_x = \rho v \frac{dx}{dt}$$

s = Leaf surface area
f_x = Flux of gas x
ρ = Air density
v = System volume
$\frac{dx}{dt}$ = Rate of mole fraction change for gas x

Mass balance for gas exchange



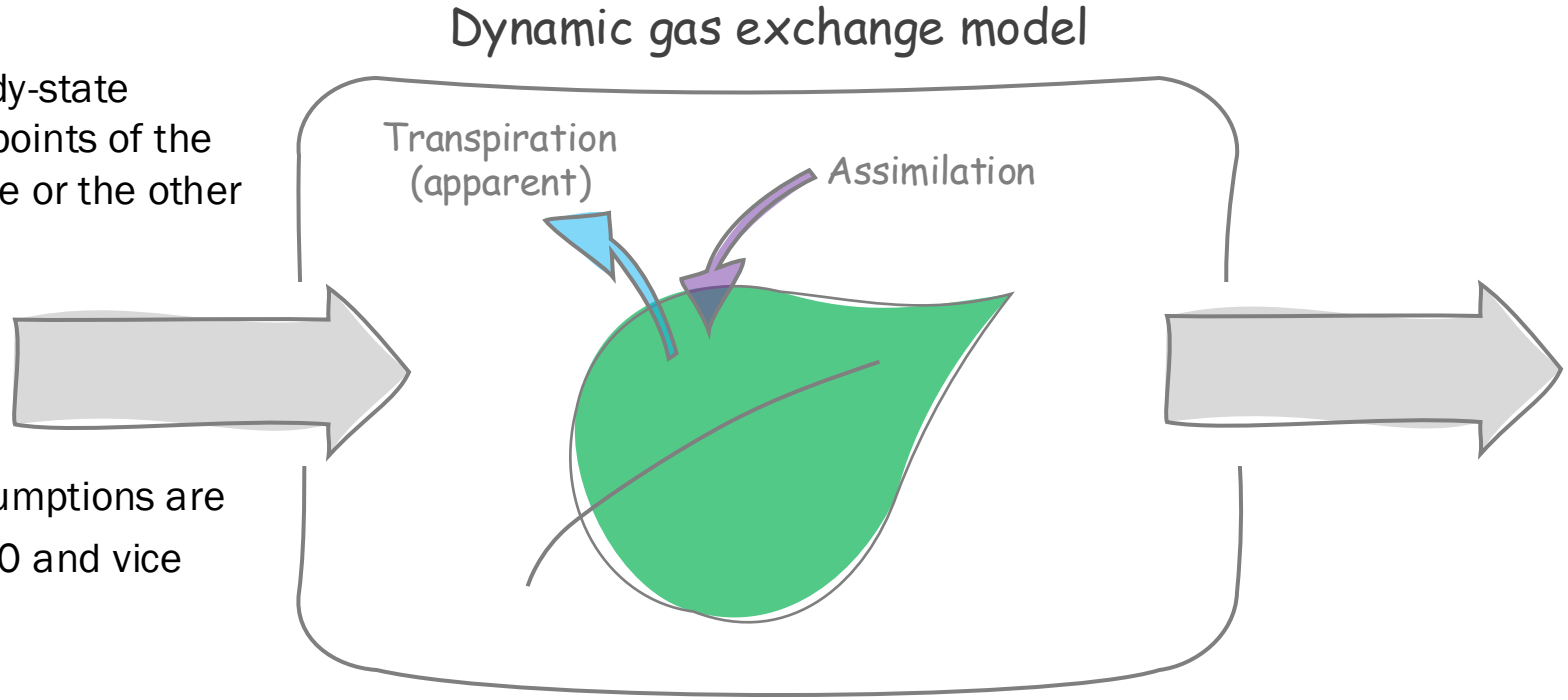
s = Leaf surface area
f_x = Flux of gas x
u_{in} =Molar air flow entering the chamber
u_{out}= Molar air flow exiting the chamber
x_{in}= Mole fraction of x entering chamber
x_{out} = Mole fraction of x exiting the chamber

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

This is the model that has been used in the LI-6400/XT and LI-6800 traditionally and what the LI-600 uses

Mass balance for gas exchange

The transient and steady-state models represent end points of the assumptions, where one or the other is fully satisfied.



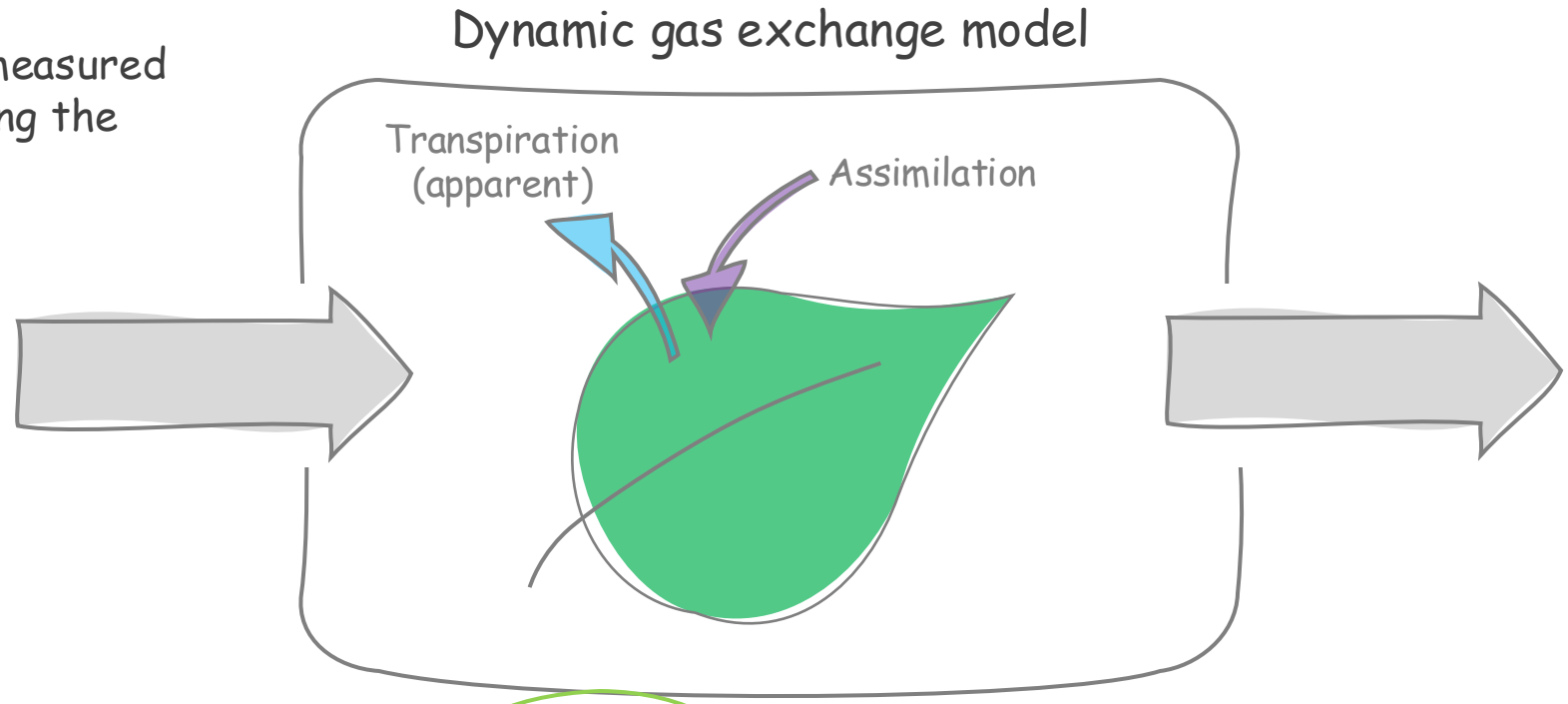
When steady-state assumptions are met $\rho v \frac{dx}{dt}$ evaluates to 0 and vice versa.

In between those end points, each model describes some fraction of the flux, such that the combination of the two describes the total gas flux.

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

Mass balance for gas exchange

Substituting in for measured values and rearranging the dynamic 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.

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

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

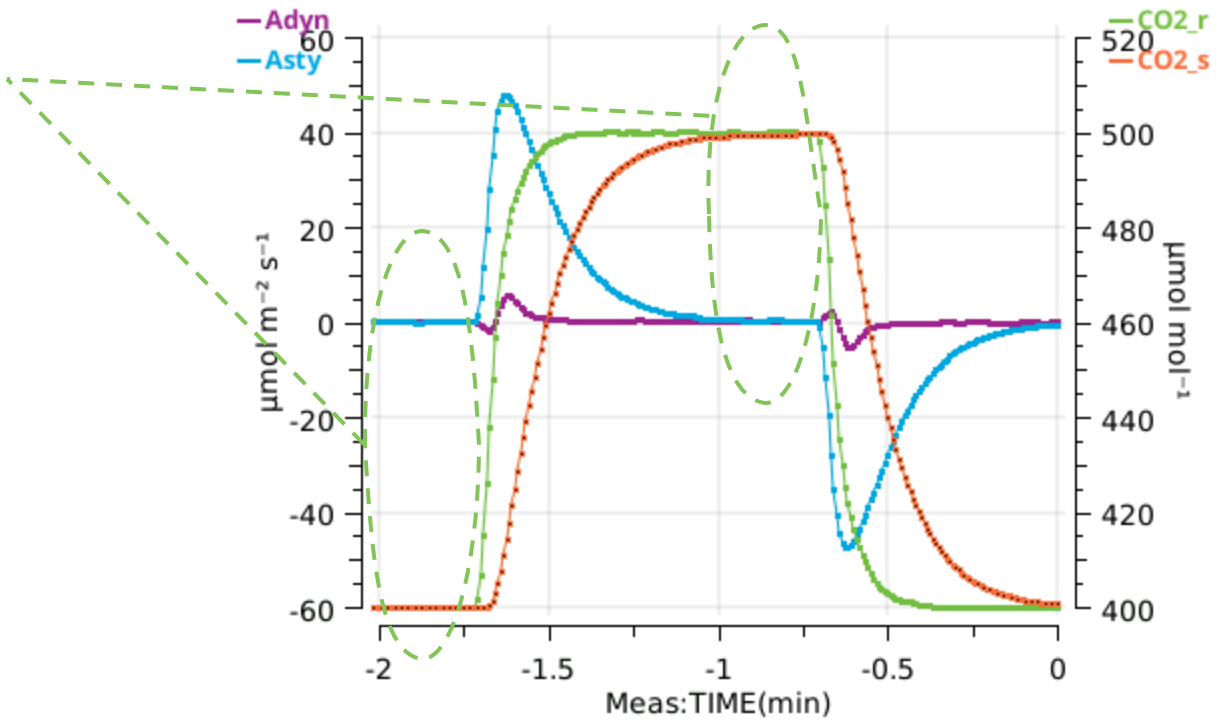
E = Apparent transpiration
A = Carbon assimilation
w_x = Water vapor mole fraction
c'_x = CO_2 dry mixing ratio = $\frac{c_x}{1-w_x}$

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

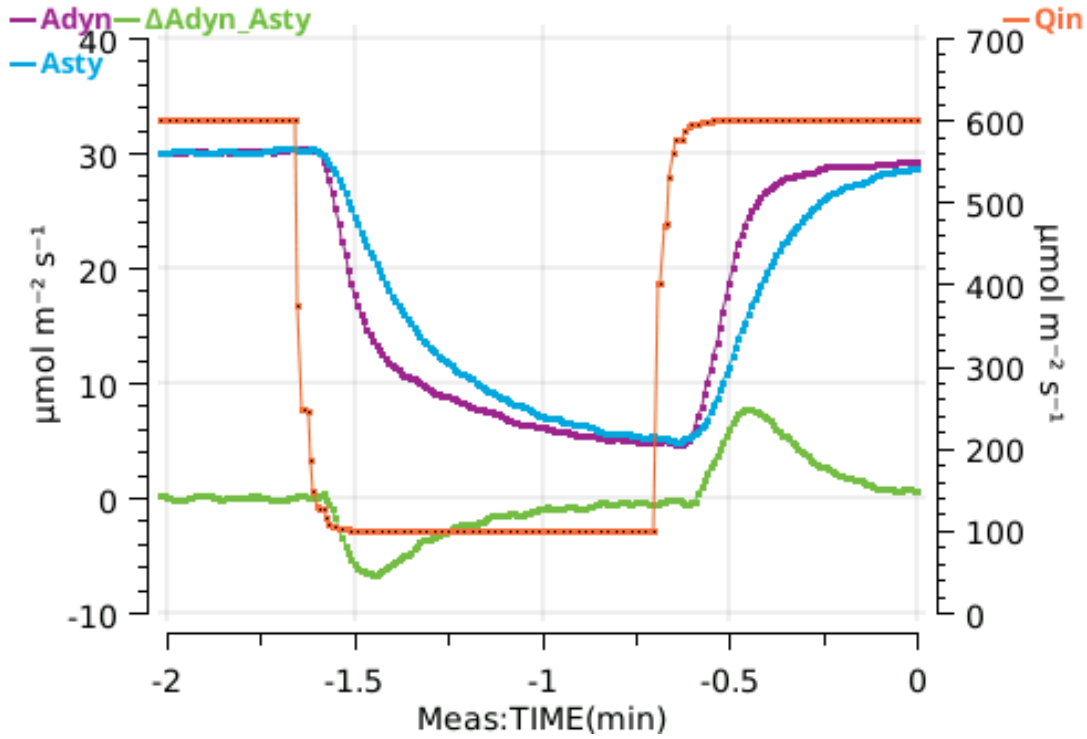


Following a step change in CO_2 the steady-state model gives a false flux driven by the volumetric time constant.

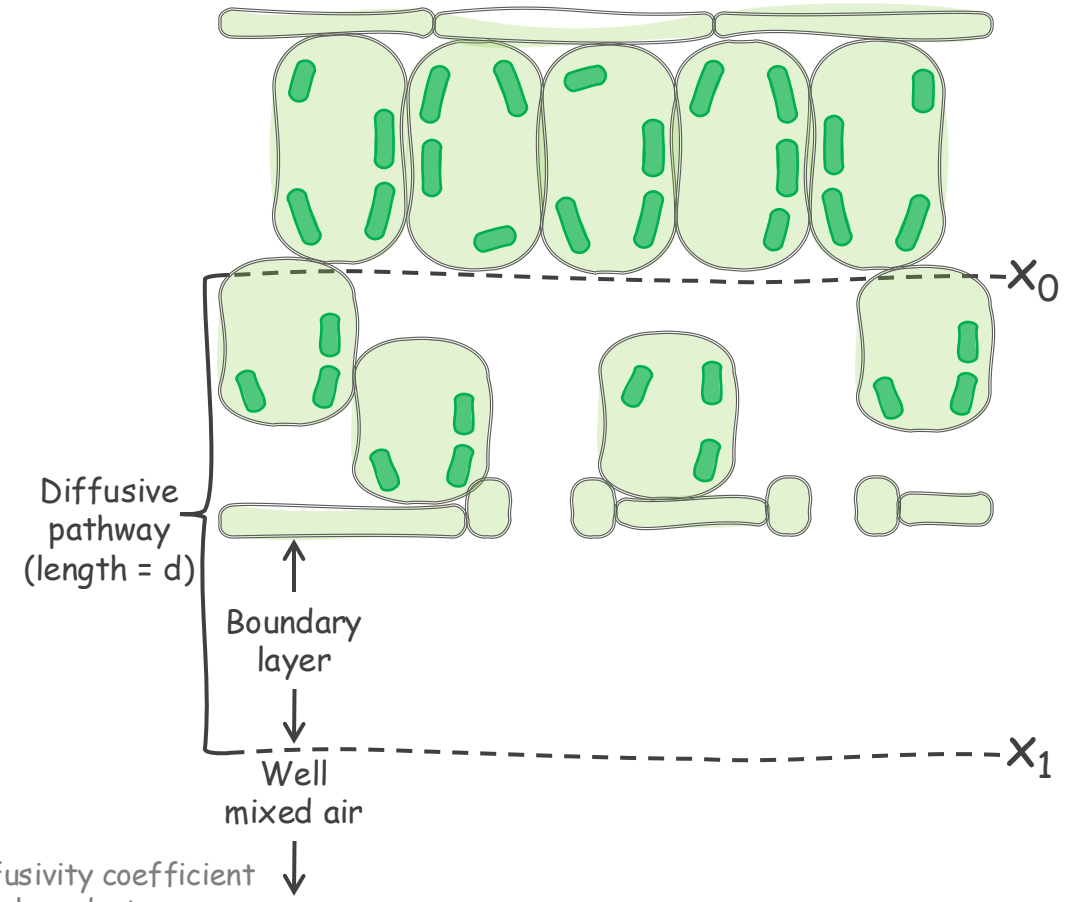
The dynamic model accounts for this and still shows zero assimilation.

Steady state versus dynamic

- Biology versus instrument
- Characterize fast biological transient responses
- Non-steady state response curves
  - High data density in less time



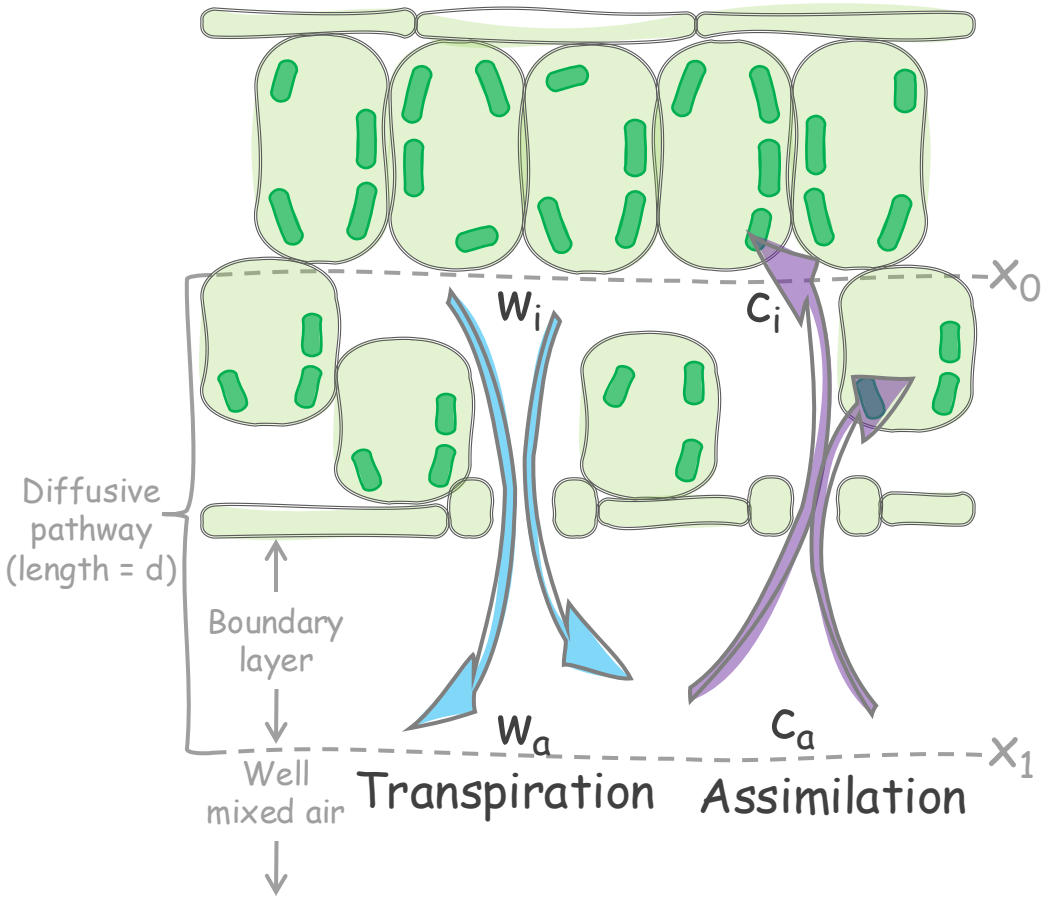
Fluxes are diffusive in nature



Carbon dioxide and water vapor move by diffusion between the well mixed atmosphere and the inside of the leaf, each driven by a concentration gradient:

$$f_x = -D_x \frac{\partial x}{\partial d} = g_{t_x}(x_1 - x_0)$$

Fluxes are diffusive in nature

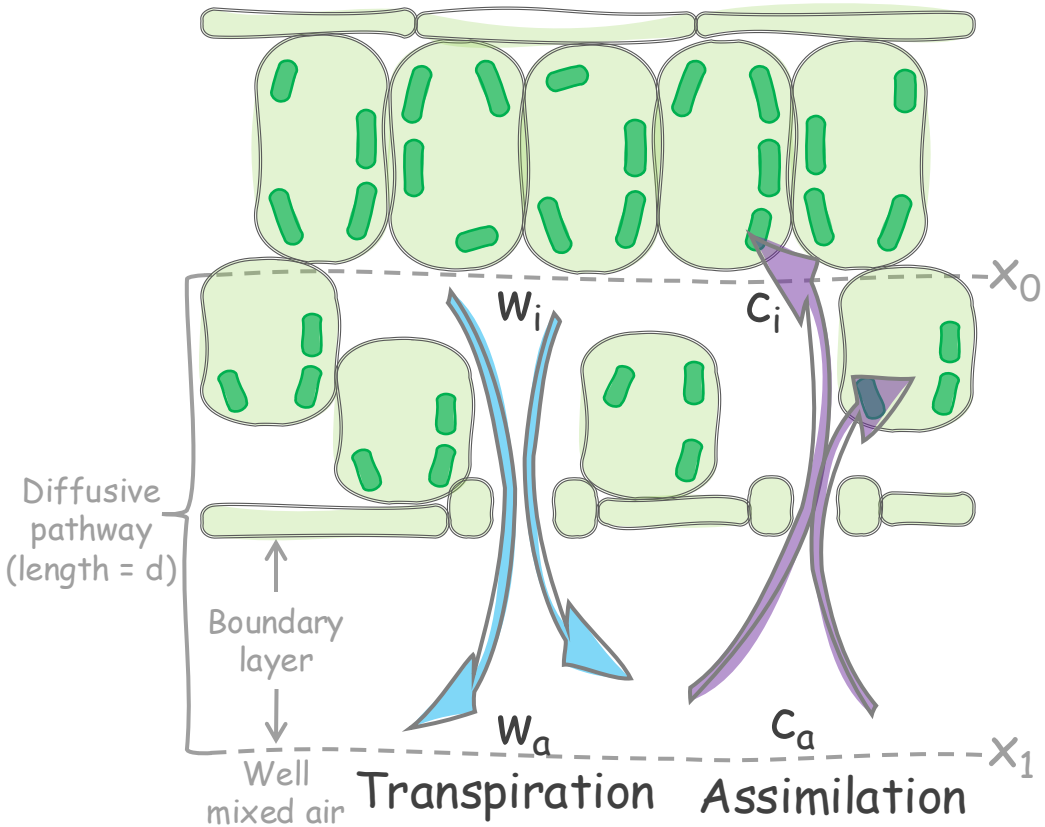


$$E \approx g_{t_w}(w_i - w_a)$$

$$A \approx g_{t_c}(c_a - c_i)$$

The approximation here is because these ignore diffusive collisions with water and air, and the transpiratory flux impact on assimilation.

Fluxes are diffusive in nature



T_{leaf} = Leaf temperature
P = Air pressure
$e(T)$ = Saturation vapor pressure

$$w_i = \frac{e(T_{leaf})}{P} 1000$$

Measured fluxes

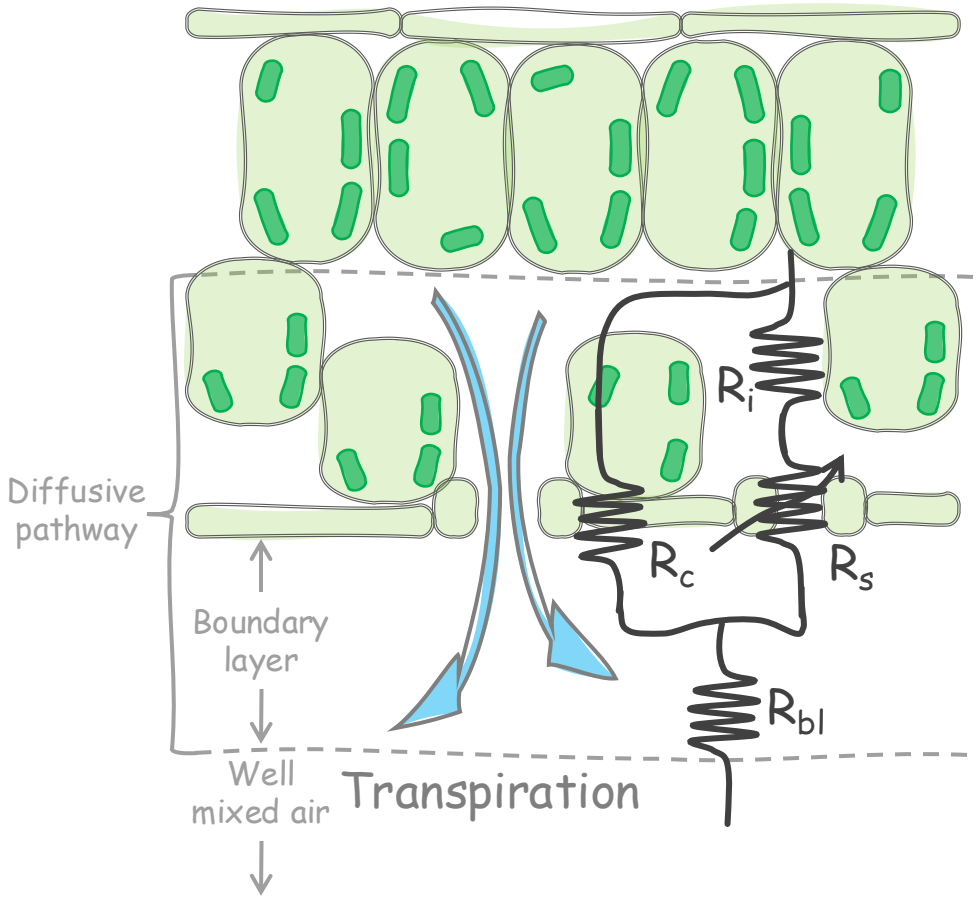
$$E \approx g_{t_w}(w_i - w_a) \qquad w_a = W_{out}$$

$$A \approx g_{t_c}(c_a - c_i)$$

$$g_{t_c} = \frac{g_{t_w}}{1.6} \qquad c_a = C_{out}$$

The approximation here is because these ignore diffusive collisions with water and air, and the transpiratory flux impact on assimilation.

Separating stomatal contribution



The diffusive pathway can be divided into segments, each making some contribution to the total conductance. Summing those contributions follows Ohm's Law:

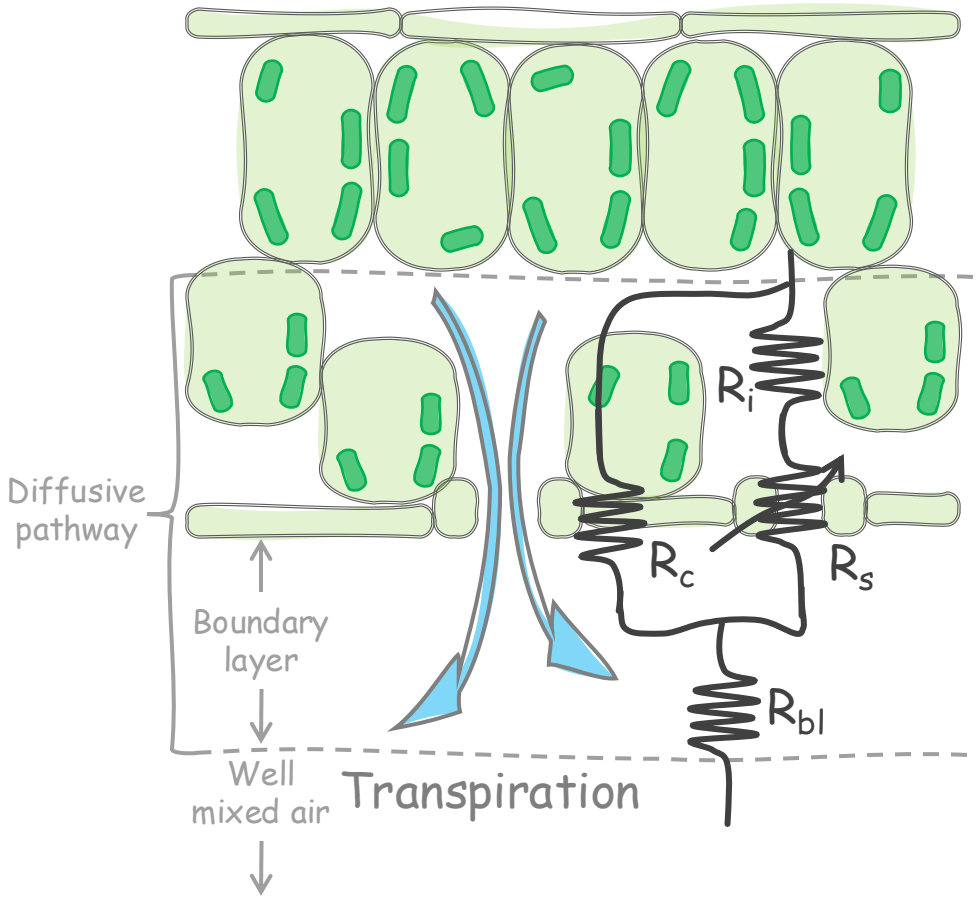
$$g_t = \frac{1}{r_t} = \frac{1}{r_{bl} + \left(\frac{1}{r_s + r_i} + \frac{1}{r_c}\right)^{-1}}$$

To get the stomatal contribution, some simplifying assumptions are made:

- Intercellular resistance is small
- Cuticular resistance is large

$$g_{s_w} = \frac{1}{\frac{1}{g_{t_w}} - \frac{1}{g_{bl_w}}}$$

Separating stomatal contribution

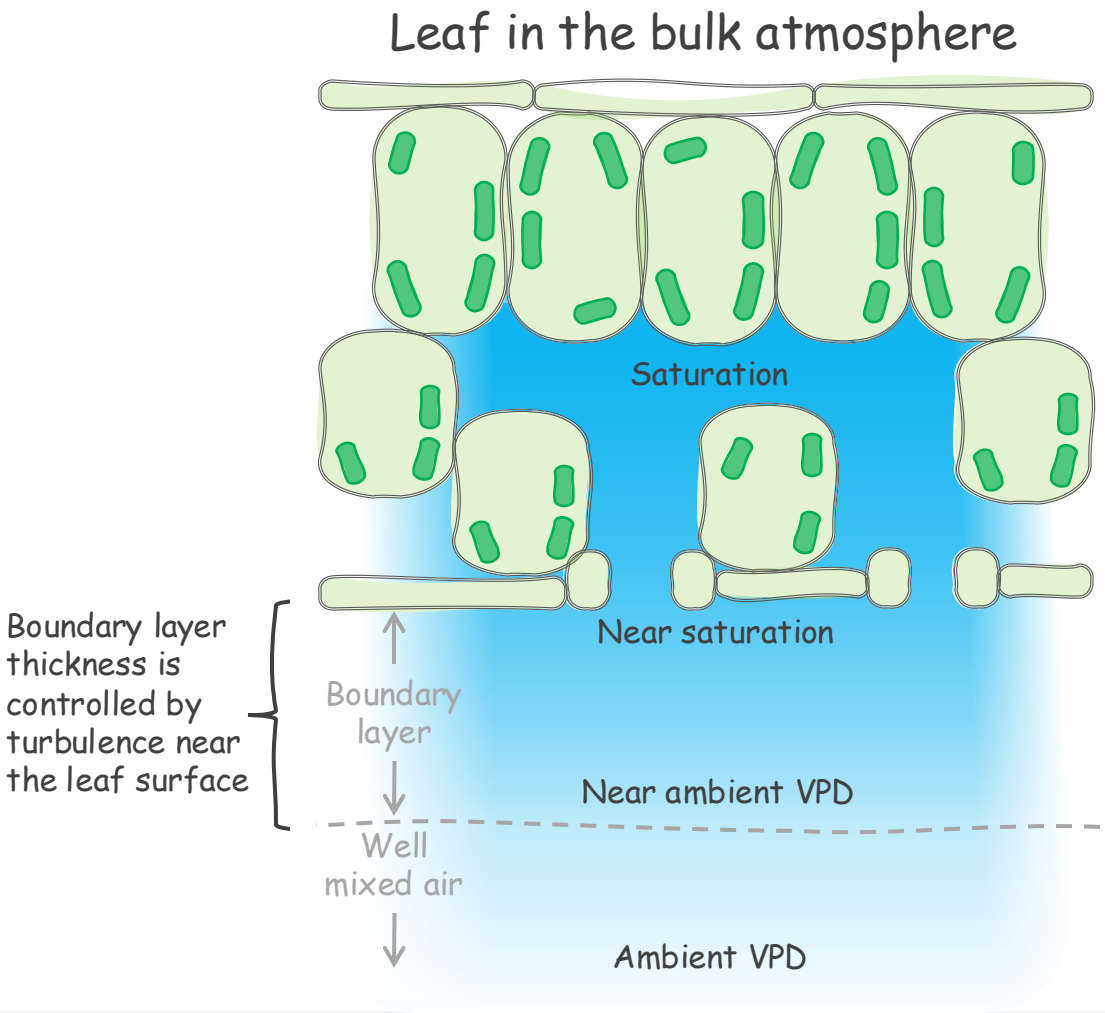


The discussion so far, only considers a single leaf surface, analogous to how conductance is measured in the LI-600. For two leaf surfaces, as measured in the LI-6800 or LI-6400/XT, we account for the effect of stomata on both surfaces with an adjustment to the boundary layer contribution:

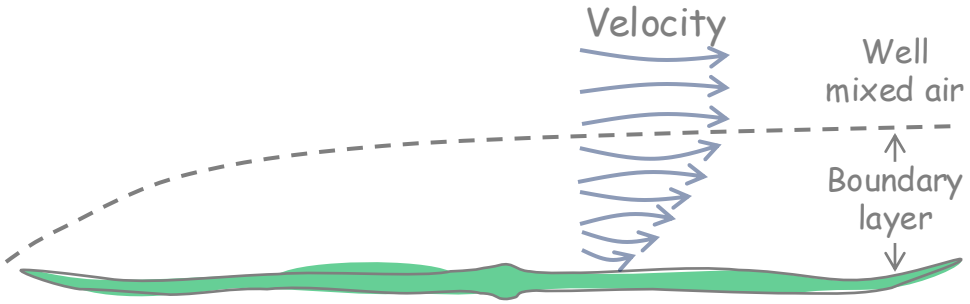
$$g_{s_w} = \frac{1}{\frac{1}{g_{t_w}} - \frac{k_f}{g_{bl_w}}} \qquad k_f = \frac{K^2 + 1}{(K+1)^2}$$

Stomatal ratio (K) is the ratio of stomatal conductance on one side of the leaf to that on the other

Boundary layer conditions

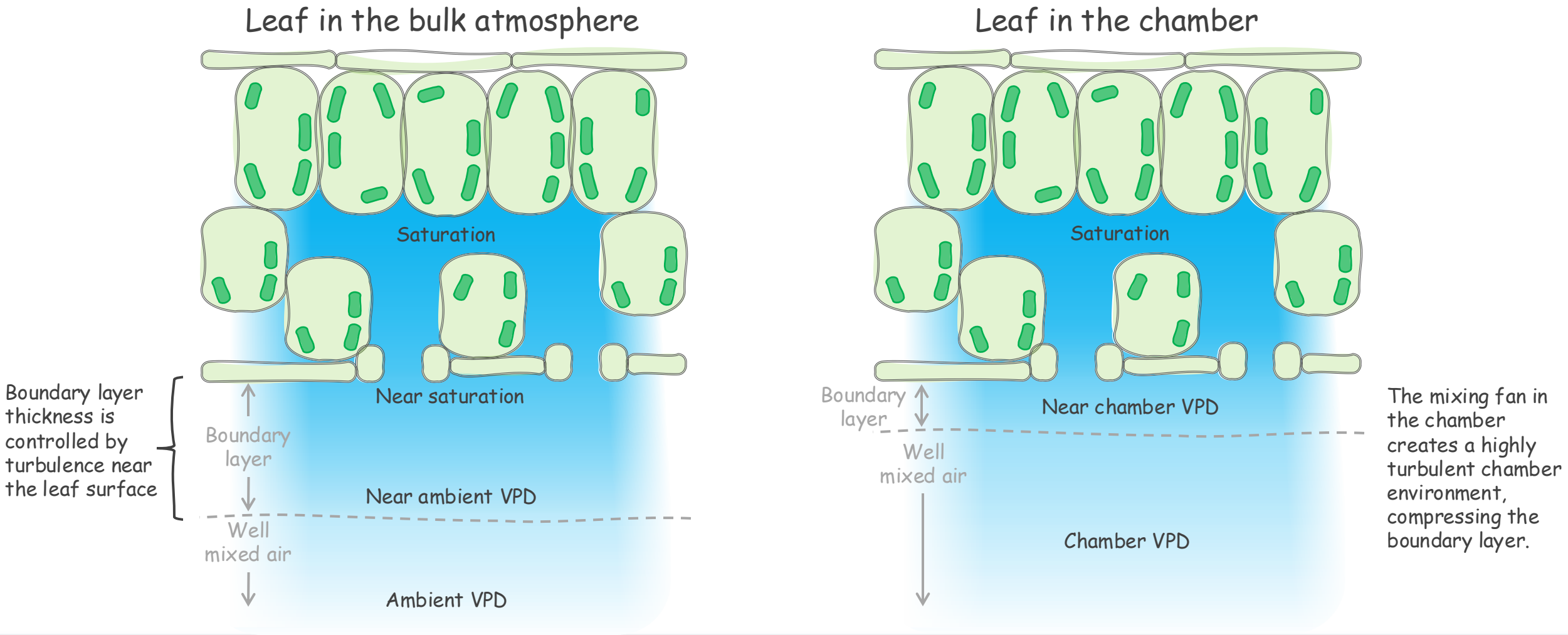


Boundary layer conductance is inversely proportional to its thickness.



Despite typically high(er) ambient VPD (or low relative humidity), leaves in the bulk atmosphere generally experience something relatively close to saturation at the leaf surface.

Boundary layer conditions



What does this mean for making measurements?

Target boundary layer conditions!

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

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

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

On the LI-6400/XT when using automated control, W_{out} (H2OS) will give the best control.
On the LI-6800 use constant VPD_{leaf} (1 to 1.5 kPa)

RH = Relative humidity
e = Vapor pressure
e_{T_x} = Saturation vapor pressure at temperature T_x
VPD_x = Vapor pressure deficit

The *apparent* nature of transpiration?

The top panel is modeled assuming a constant conductance and ignoring boundary layer effects. The latter only shifts the curves left or right, it doesn't change their shape.

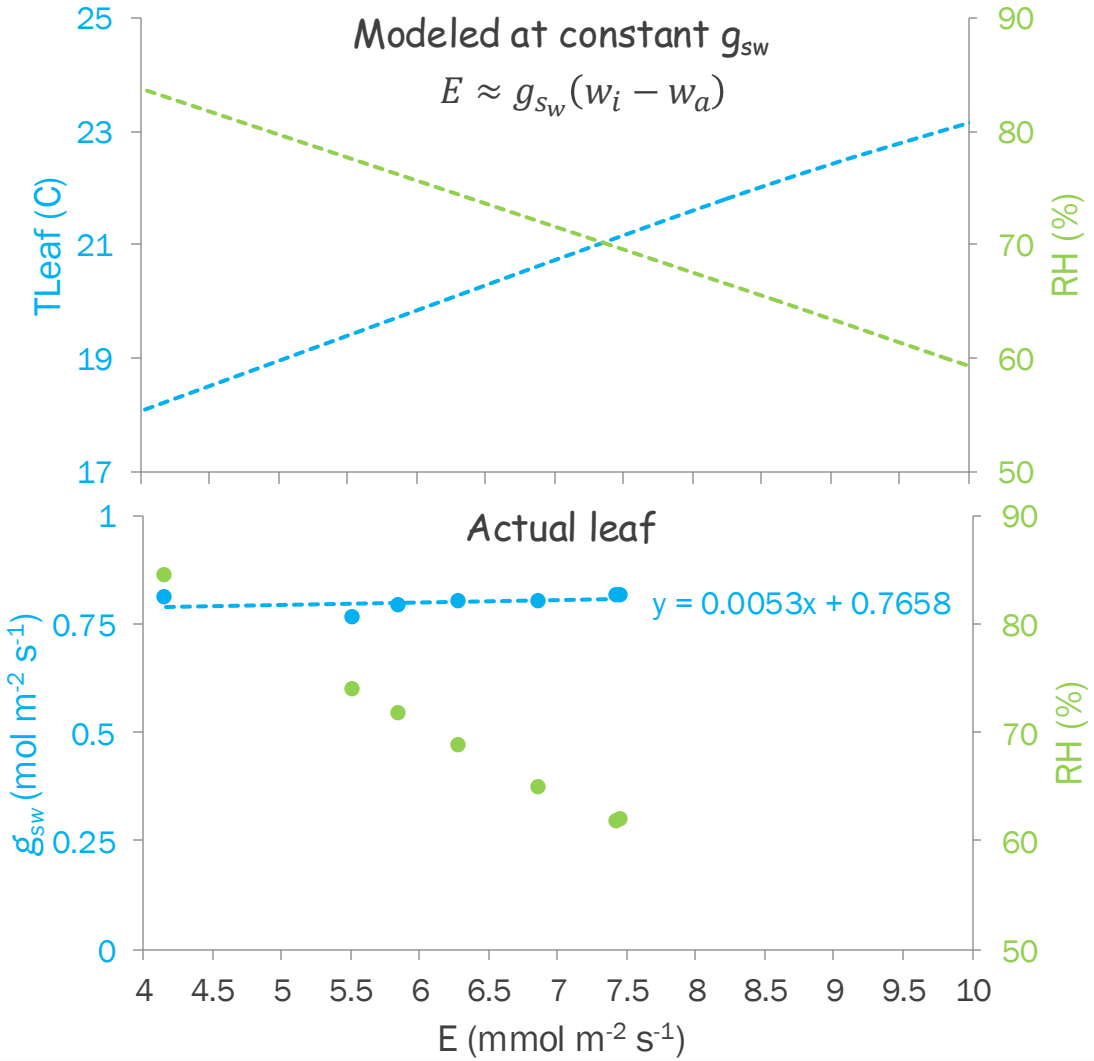
The bottom panel is actual leaf data collected by varying the chamber humidity across a range of "typical" boundary layer humidity.

What should we conclude from this?

Transpiration is a real count of water vapor molecules leaving the leaf.

Transpiration is artifactual in nature, driven by the chamber environment as much as by biology.

Conductance removes the environmental dependence



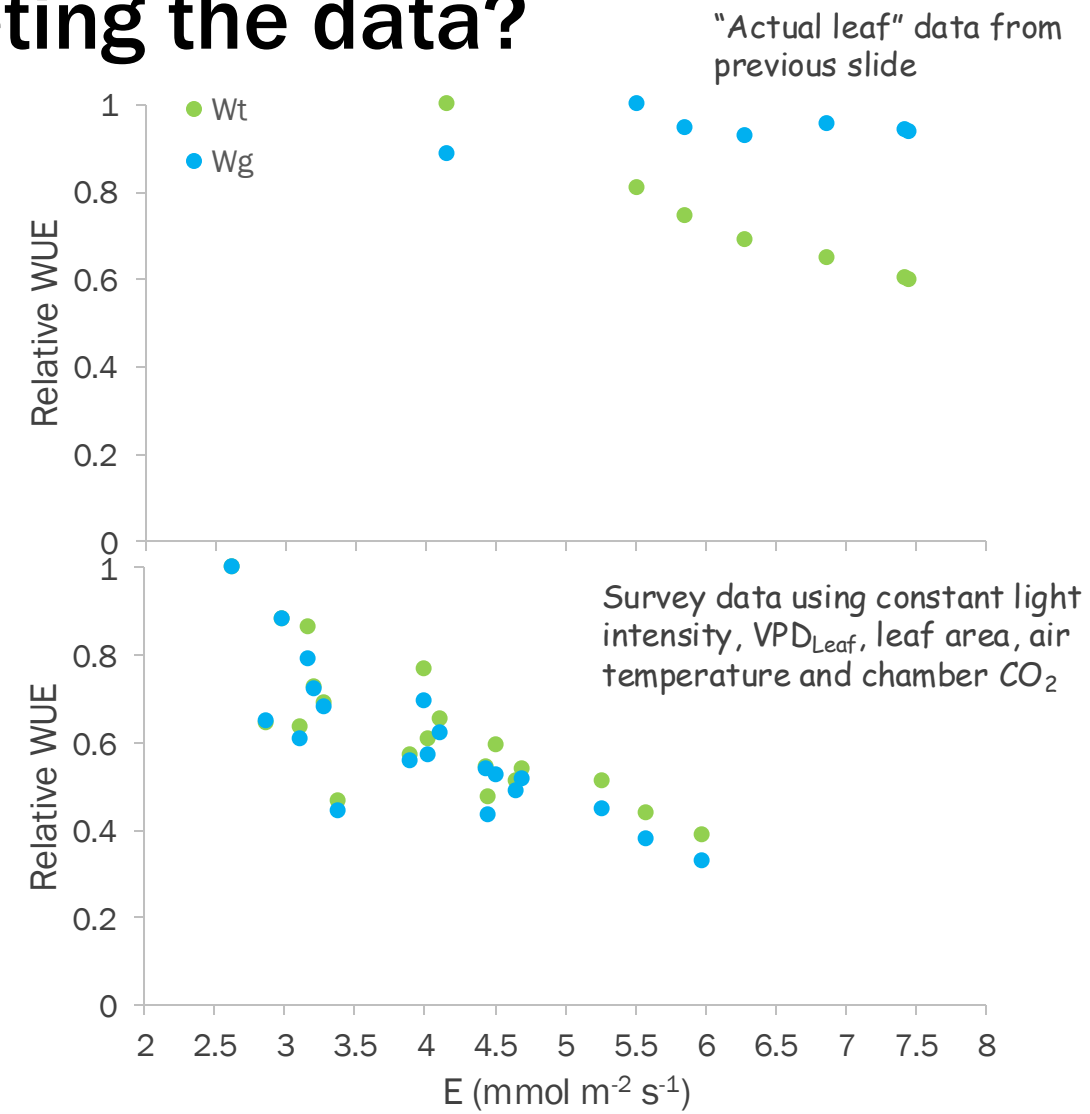
What does this mean for interpreting the data?

Be careful trying to draw conclusions based on apparent transpiration!
Water use efficiencies (WUE) provide a nice illustration of this…

$$W_t = \frac{A}{E} = \frac{A}{g_t(w_i - w_a)}$$

$$W_g = \frac{A}{g_{s_w}}$$

For a nice overview of WUE parameters see Seibt *et al.* 2008. *Carbon isotopes and water use efficiency: sense and sensitivity.* Oecologia



W_t = Instantaneous WUE
W_g = Intrinsic WUE

What about *actual* (evapo)transpiration?

There are other, less constrained ways to get to this number…

- Eddy covariance or similar methods: Allow for continuous monitoring
- Permits undisturbed boundary layer conditions
- Field/ecosystem scale measurements

Some practical considerations for measurements

The leaf's light environment

Let's consider this plant canopy and light reaching it…

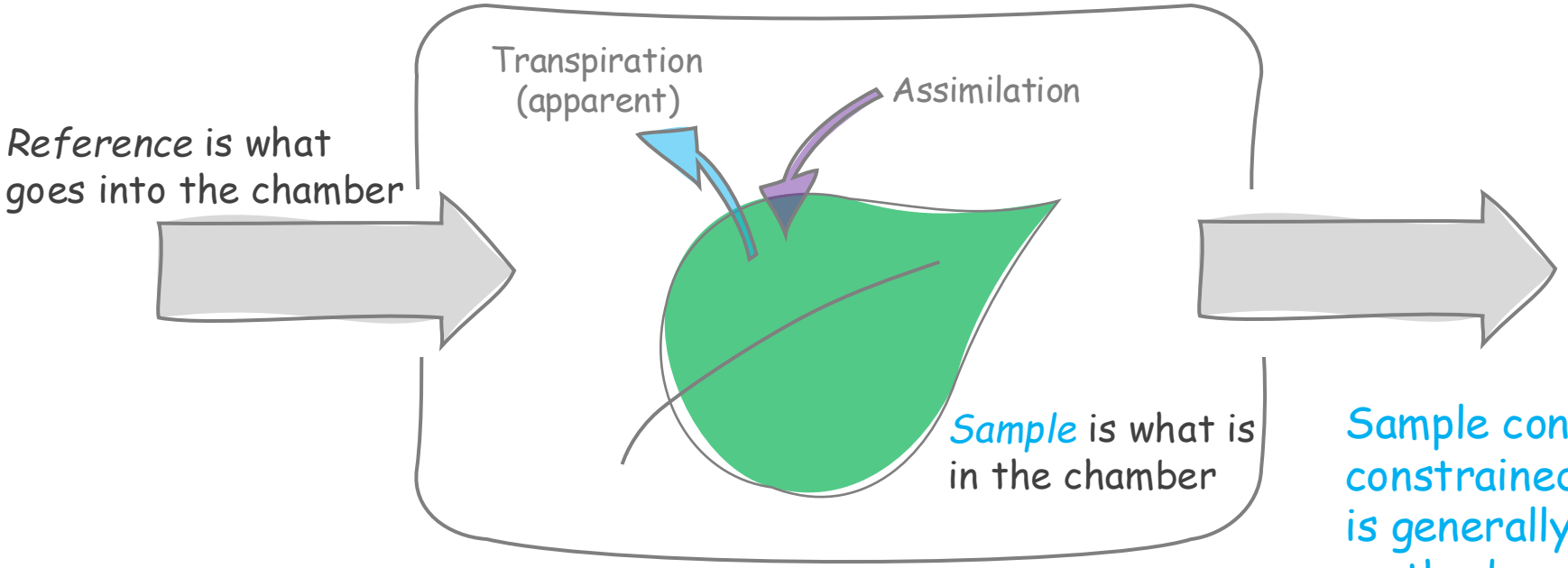
The leaf's light environment

LI-190/R

- Filtered to idealized plant spectral response
- Measures light incident on the plane of the diffusor

Controlling CO_2 at the leaf

You can either keep the incoming or the out-going (chamber) CO_2 concentration constant, but not both. Whichever one is not held constant will vary proportionally with the assimilation rate. So, sensitivity to CO_2 concentration is an important consideration!



Sample control is the most constrained approach and is generally the preferred method

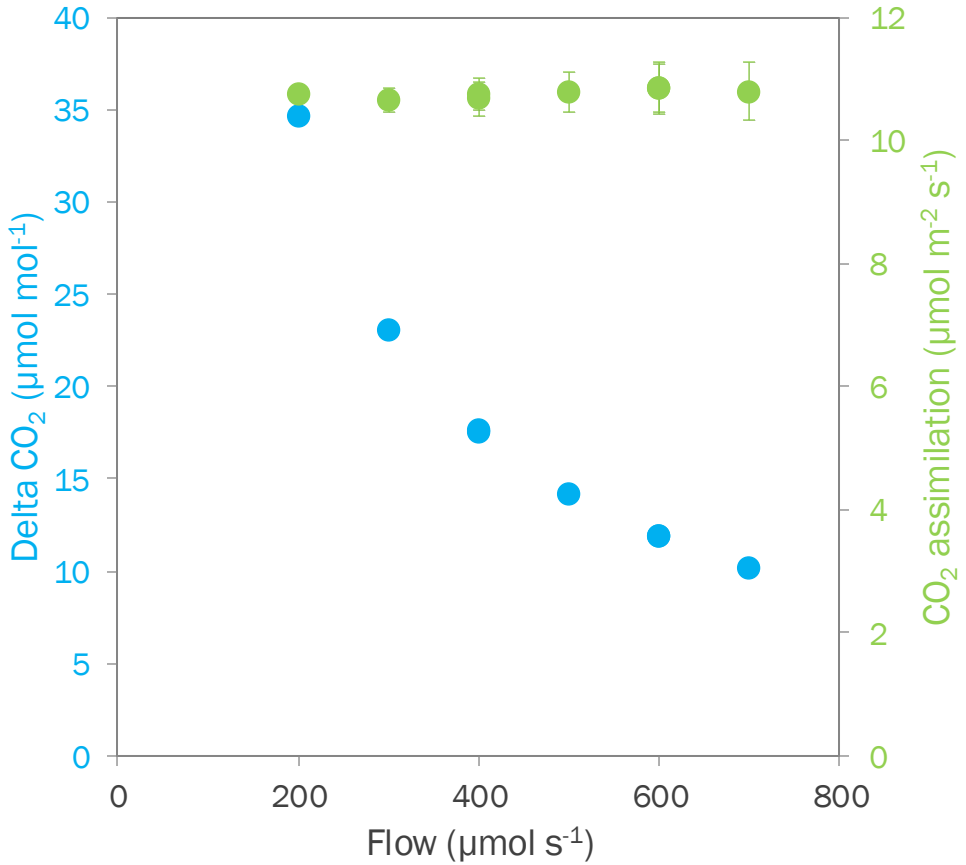
Flow control: optimize for the “signal”

Fixed flow rate

Choose the highest flow rate that still gives a good delta

Humidity control

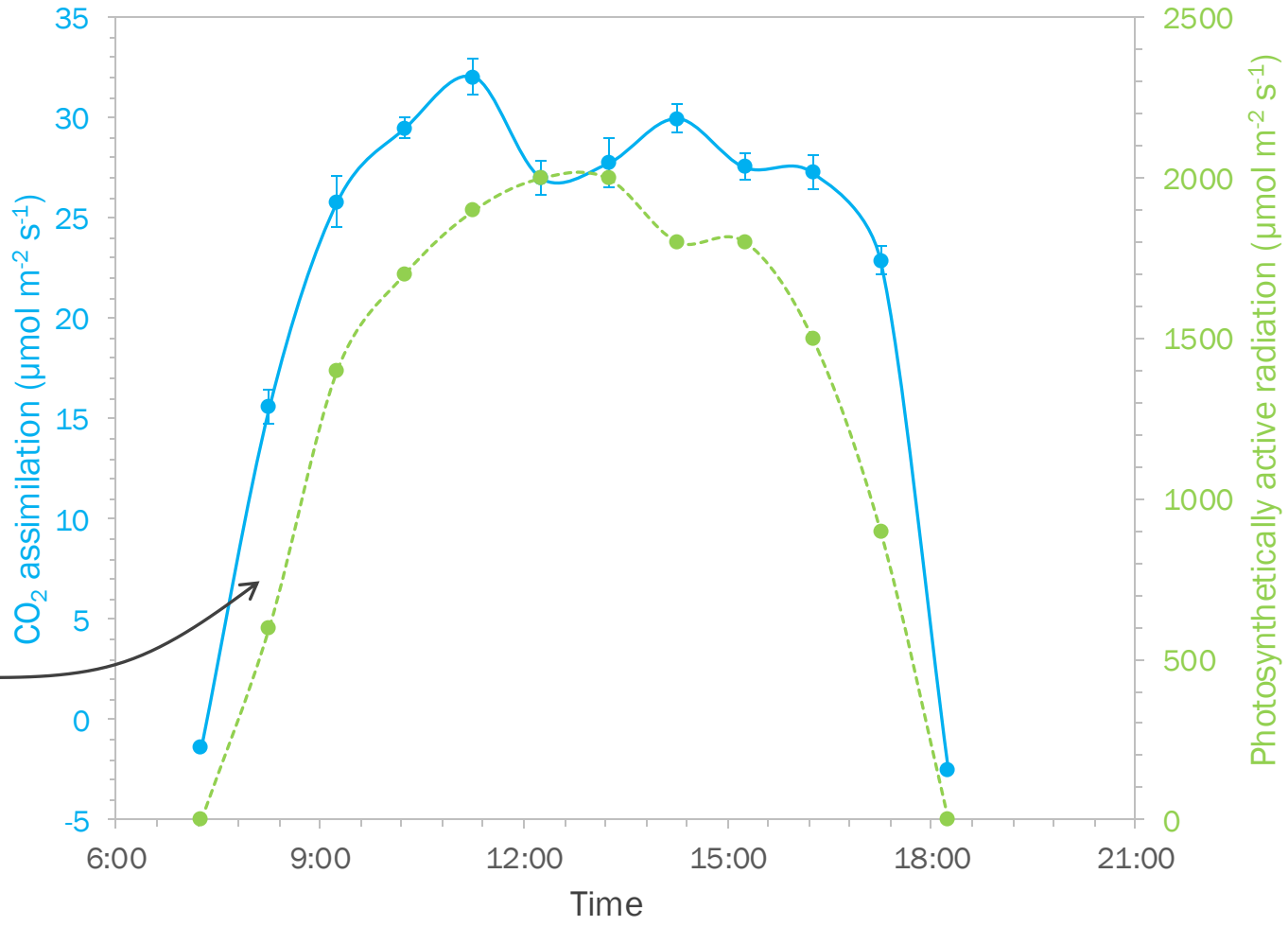
Hold VPD_{leaf} between 1 to 1.5 kPa



Data from an LI-6400XT and 6400-02B chamber. Error bars are an estimated uncertainly assuming typical noise on the LI-6400 IRGAs.

Time of day effects

Diurnal curve of survey measurements from field grown Bok-choi.



In the morning, assimilation tracks PAR relatively closely.

At some point in the day, the correlation between assimilation and PAR breaks. This typically happens near or after peak light intensity is reached and is referred to as the mid-day depression.

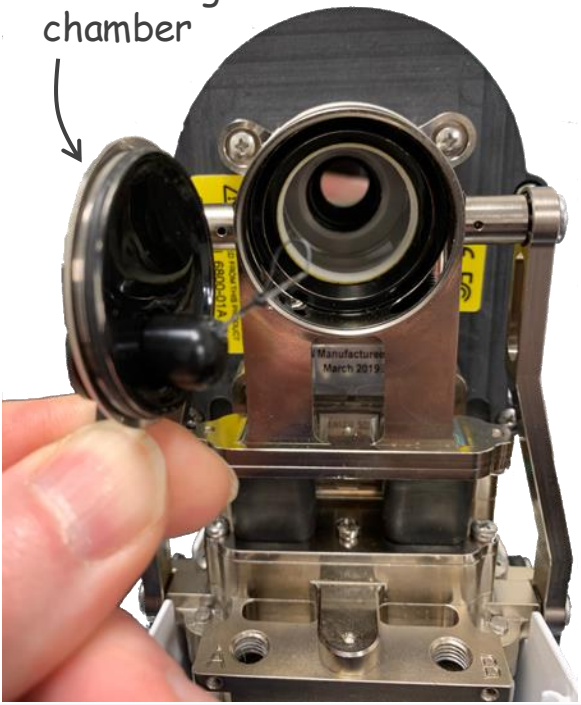
Selecting an appropriate chamber?

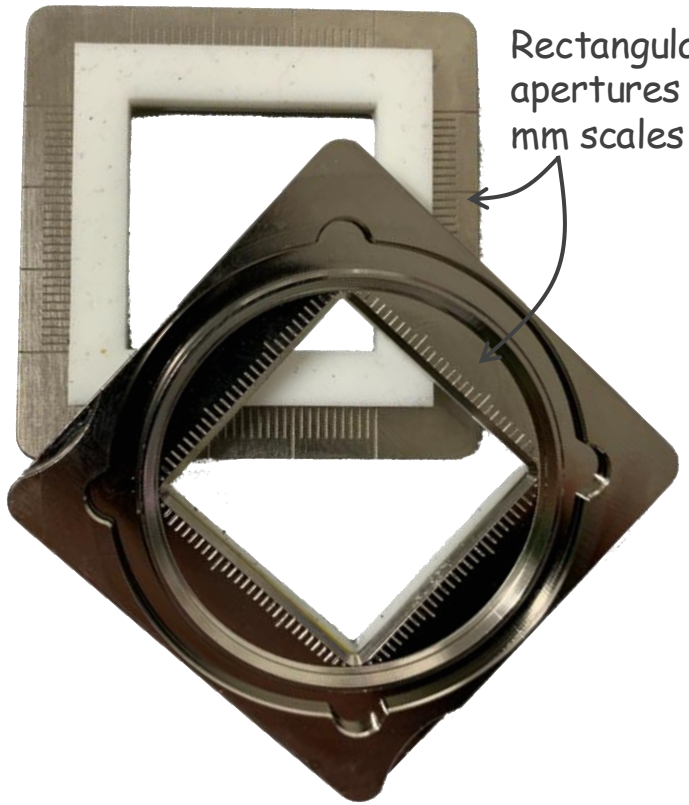
What specific data do you need?

What about leaves that don't fill the chamber?

Where the leaf doesn't fill the chamber, area needs to be measured and the correct leaf area used in the calculations

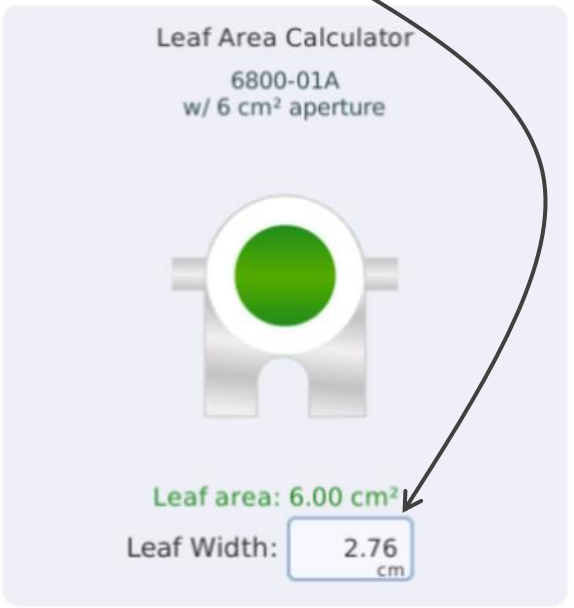
In the fluorometer, the bottom of the chamber is removable allowing the leaf to be imaged while its still in the chamber





Rectangular apertures include mm scales

For linear leaves, leaf width can be input directly, and area is computed accounting for chamber curvature



What about leaves that don't fill the chamber?

The LI-600 chamber must be filled!

LI-600N

Width: 1 to 3.5 mm

Length: 14.22 mm minimum

Thickness: 2.8 mm maximum

Not just leaves!

6800-18 Aquatic Chamber

For measurements on liquid samples or water saturated materials

Questions?

This document is really useful! Includes a description of parameters with relevant literature references for each.
https://licor.app.box.com/s/wcqljhmyd1rwotm0j0ayh5vwd0r4lb7q

Complementary Leaf Physiology Measurements: The LI-600 and LI-6800

Application Note

Contents

Abstract

The LI-600 Porometer/Fluorometer is designed for high-speed sampling. It quickly measures stomatal conductance and chlorophyll *a* fluorescence from leaves. In contrast, the LI-6800 Portable Photosynthesis System is designed to provide highly detailed data. It measures parameters that take more time than porometry alone, such as CO_2 assimilation. Although the LI-600 and LI-6800 have distinct purposes, the data they provide is highly complementary. When used together, they can improve the efficiency of data collection in laboratories, greenhouses, and plant breeding programs.

In this document, we discuss how these instruments can be used together to collect larger data sets and explore physiological traits in more detail while taking less time. To illustrate the concept, we review a case study in which wild pepper varieties were screened for sensitivity to water stress using the LI-600 and measured in detail with the LI-6800. Over 500 individual leaves were measured in a single day using one LI-600. Data from the high-volume survey were used to identify individuals that were evaluated in detail using the LI-6800.

Gas exchange and photosynthesis

Leaves perform many complex physiological processes, but often of particular interest is how they exchange carbon dioxide (CO_2) and water (H_2O) between the leaf interior and the atmosphere (summarized in Figure 1). These represent the two major processes, assimilation (CO_2) and transpiration (H_2O), and the movement of these gases into and out of the leaf is typically facilitated by the stomates. The stomates are able to open or close in response to stimuli or stress in order to control the rate of exchange.

Along with the diffusion of gases into and out of the leaf, we have the photosynthetic apparatus within the chloroplasts. Here, energy from captured photons facilitates the conjugation of CO_2 into sugars. This process consists of the light-independent reactions or Calvin-Benson cycle occurring in the stroma and the light-dependant reactions occurring in the thylakoid membranes.

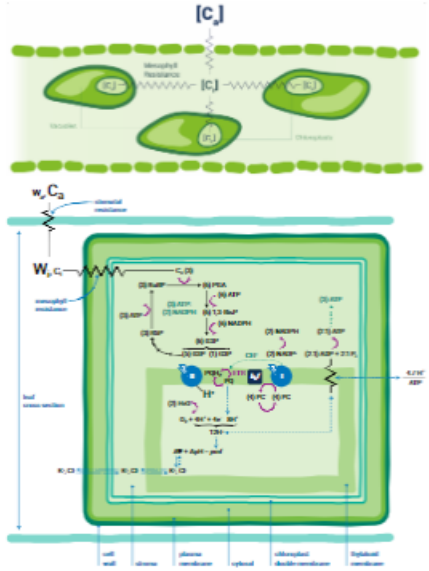


Figure 1. Cross section of a leaf (top) and cell (bottom) showing a summary of the biochemical processes within. These processes are probed by the LI-600 and LI-6800.

LI-COR

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