CARNEGIE INSITUTION
FOR SCIENCE DEPARTMENT OF GLOBAL ECOLOGY

Fluorescence and Photosynthesis

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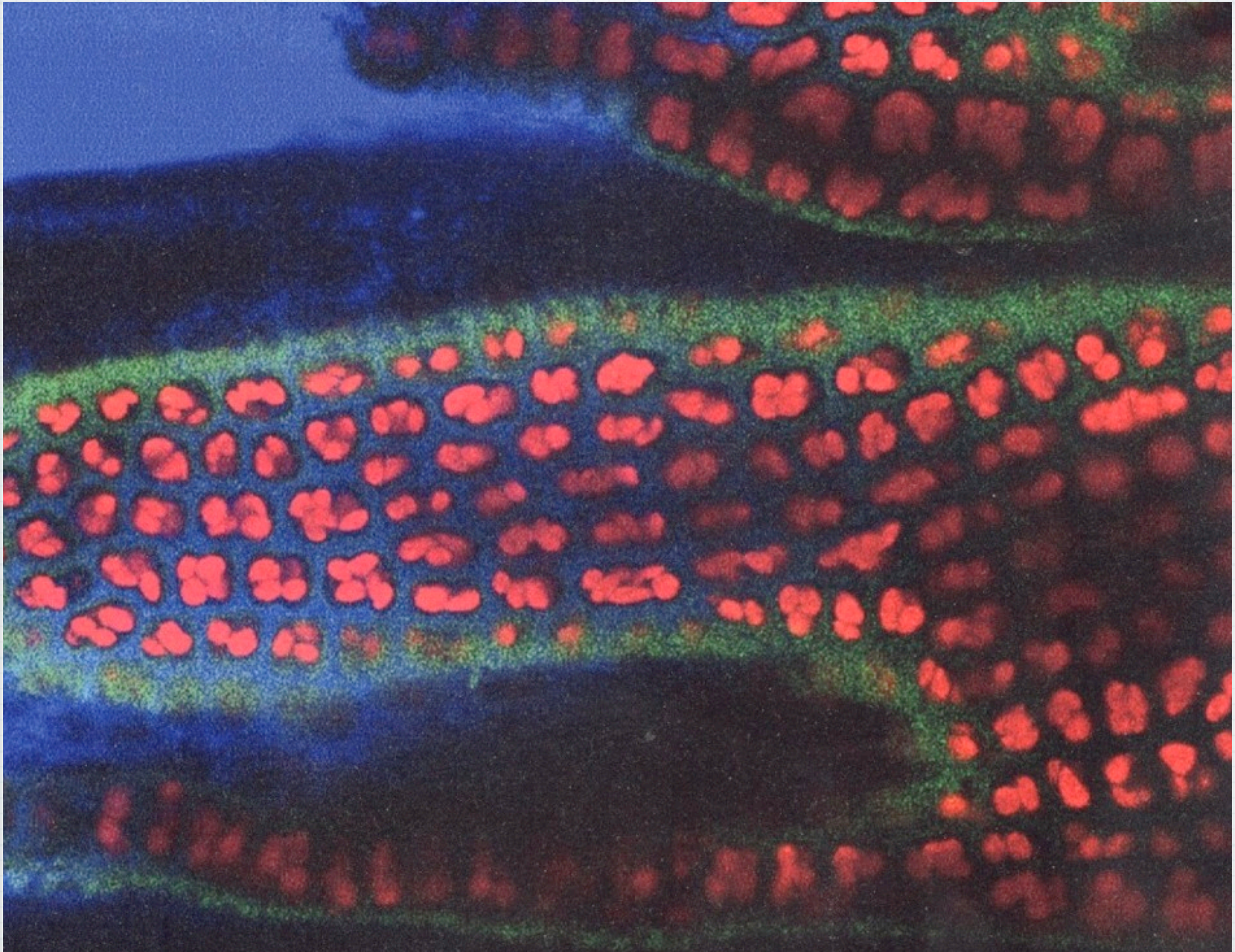
Dept. of Global Ecology, Stanford, CA



• Chlorophyll fluorescence is the emission of light by chlorophyll molecules that have previously absorbed light.

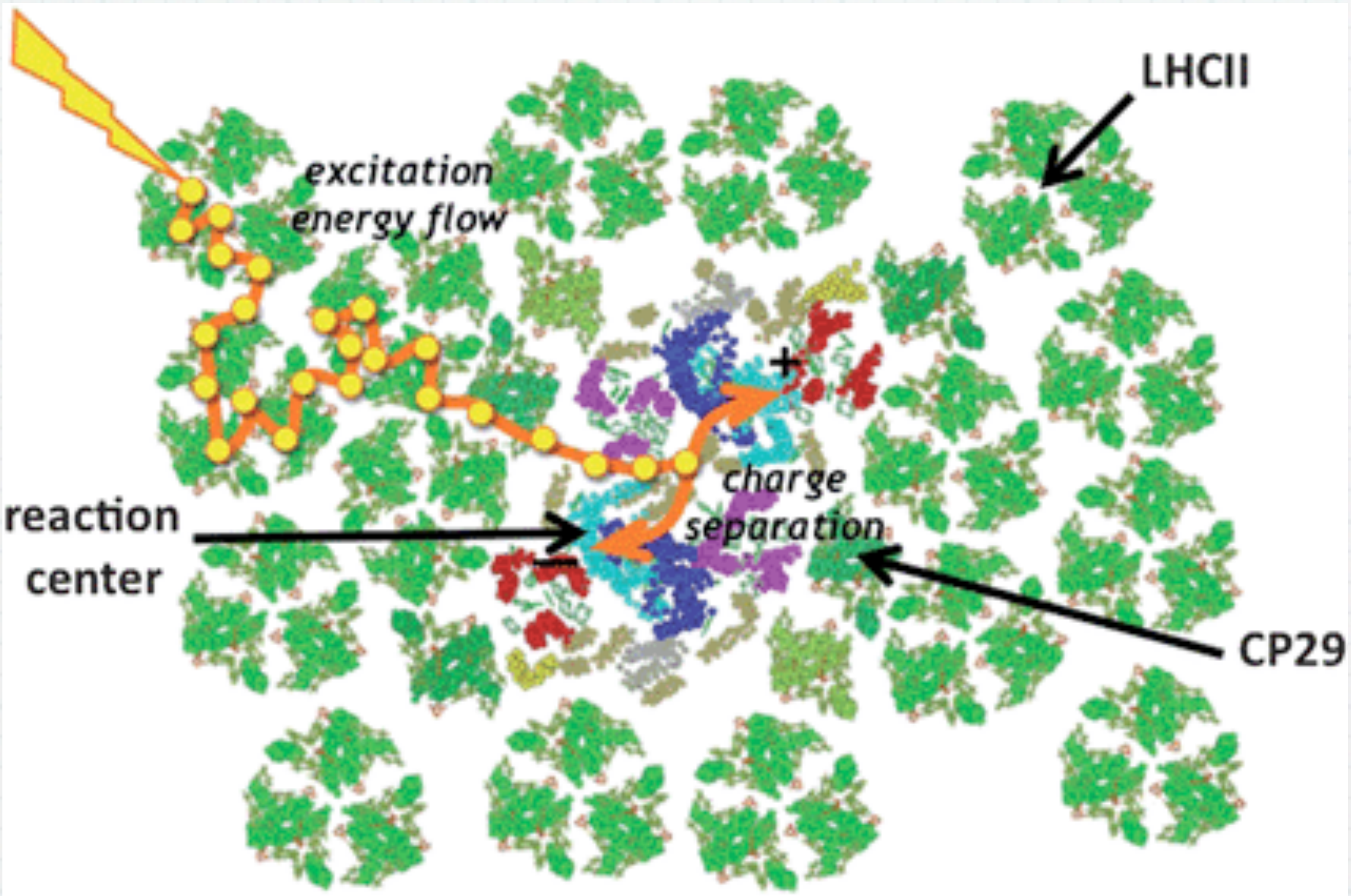
• It occurs because the chlorophyll molecule is capable of storing the energy of a photon in an excited electronic state - often referred to as an "exciton."

• Emission of a new photon is one of the ways that the chlorophyll exciton can return to its ground state. While this energy storage can last only a few nano seconds at most, it is this ability to store energy that makes photosynthesis possible.

• Fluorescence and photochemistry are closely linked processes that co-occur, and fluorescence has long been used as a probe for the initial events in photosynthesis.

- The chlorophyll in photosynthetic organisms is bound in a highly organized state in protein complexes which include a photochemical reaction center and associated chlorophylls that function as an antenna to collect light to drive the photochemical reaction.

- There are two types of reaction centers, PS I and PS II in leaves. Most of the fluorescence comes from PS II.



Fleming, G. R., Schlau-Cohen, G. S., Amarnath, K., & Zaks, J. (2012). Design principles of photosynthetic light-harvesting. **Faraday Discussions**, 155, 27. doi:10.1039/c1fd00078k

- The concept of how excitons are processed in photosynthetic systems is undergoing something of a revolution.
- Until recently it was thought that excitons were localized on individual chlorophyll molecules and moved around by jumping from molecule to molecule eventually reaching a reaction center by a random walk.
- In contrast recent experimental evidence indicates that excitons may be delocalized by a phenomenon known as quantum coherence.
- The coherent exciton has properties of a wave sloshing around the whole space of a chlorophyll protein complex sampling the available routes for de-excitation.
- Evolution knows about quantum mechanics.

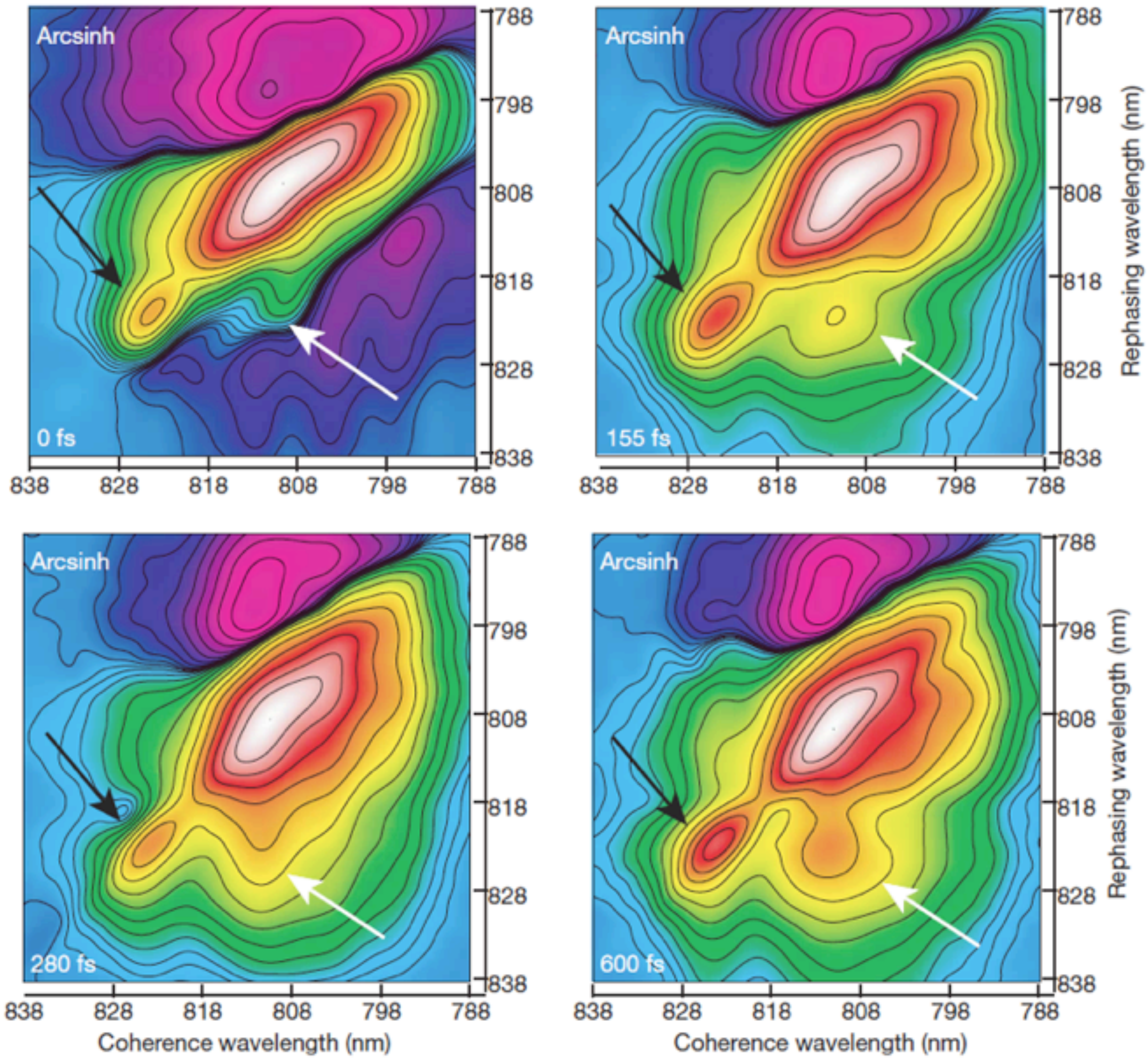
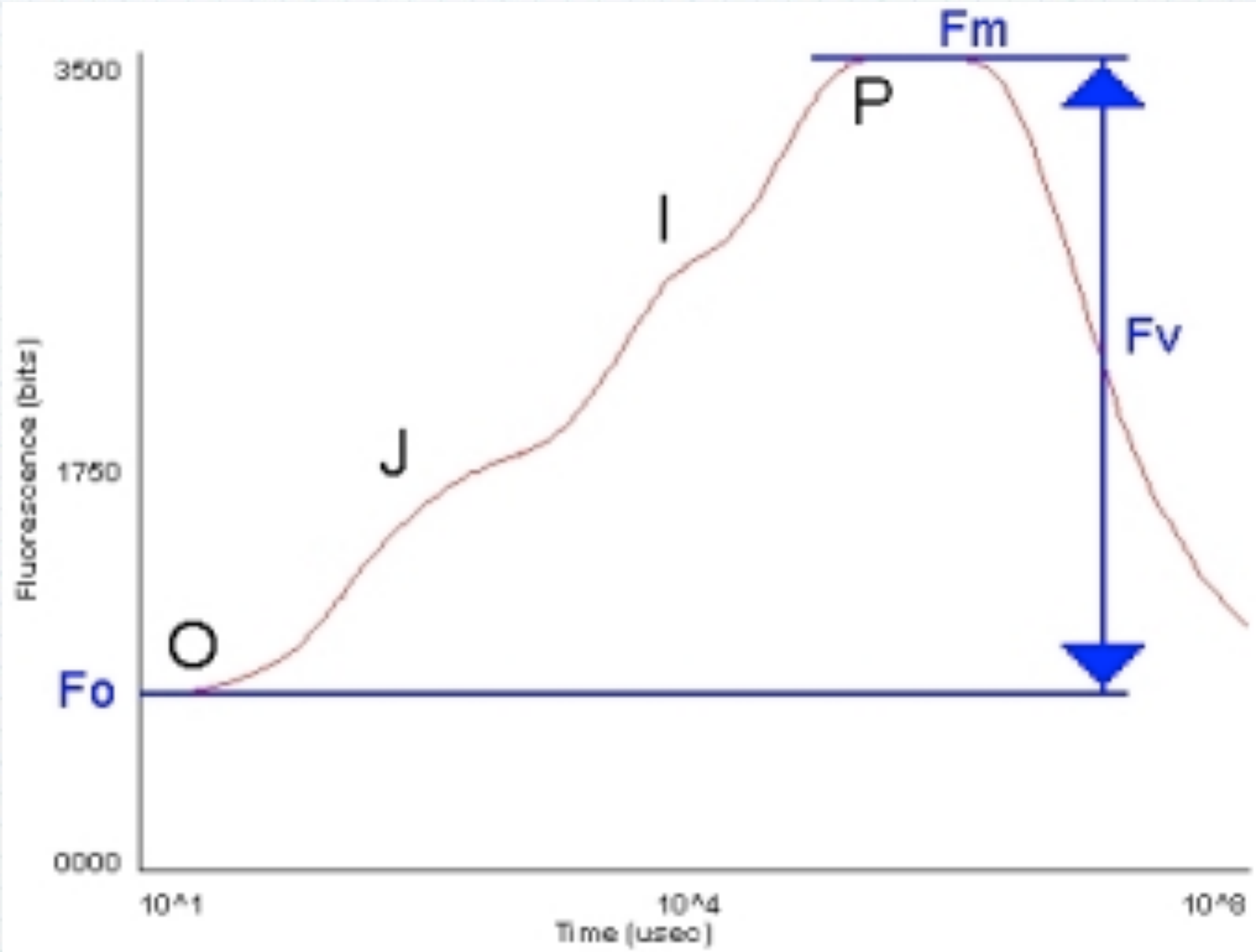


Figure 1 | Two-dimensional electronic spectra of FMO. Selected two-dimensional electronic spectra of FMO are shown at population times from $T = 0$ to 600 fs demonstrating the emergence of the exciton 1–3 cross-peak (white arrows), amplitude oscillation of the exciton 1 diagonal peak (black arrows), the change in lowest-energy exciton peak shape and the oscillation of the 1–3 cross-peak amplitude. The data are shown with an arcsinh coloration to highlight smaller features: amplitude increases from blue to white (for a three-dimensional representation of the coloration see Fig. 3a).

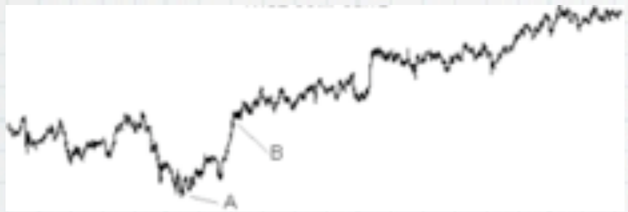
Engel, G. S., Calhoun, T. R., Read, E. L., Ahn, T. K., Mančal, T., Cheng, Y.-C., Blankenship, R. E., et al. (2007). Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. **Nature**, 446(7137), 782–786. doi:10.1038/nature05678

Chlorophyll Fluorescence - the Kautsky effect



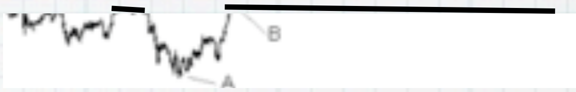
Kautsky is the “father of the field”, but he also fostered the impression that fluorescence is very complicated.

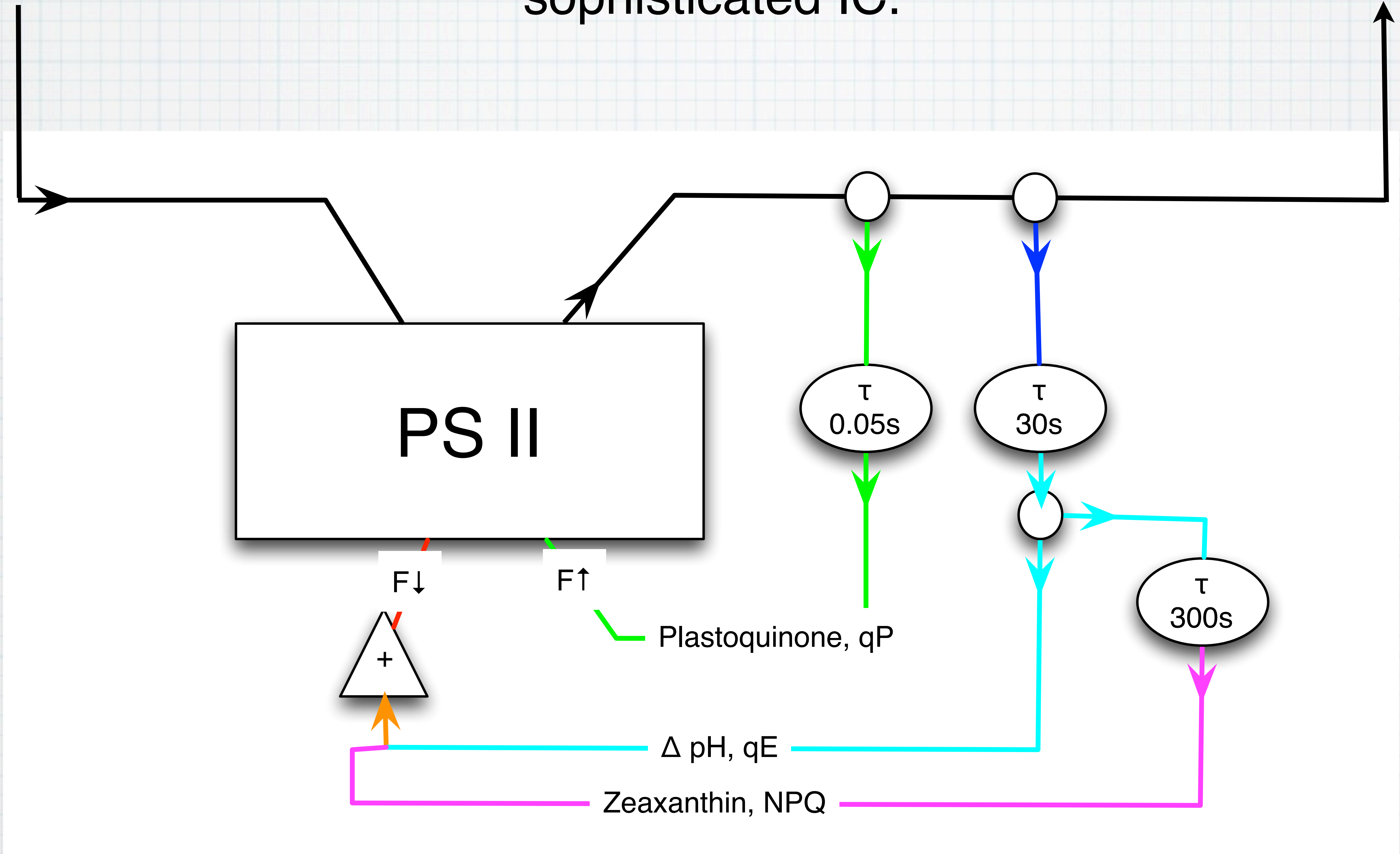
Input Light



Think of Photosystem 2 as a sophisticated IC.

Output to carbon fixation

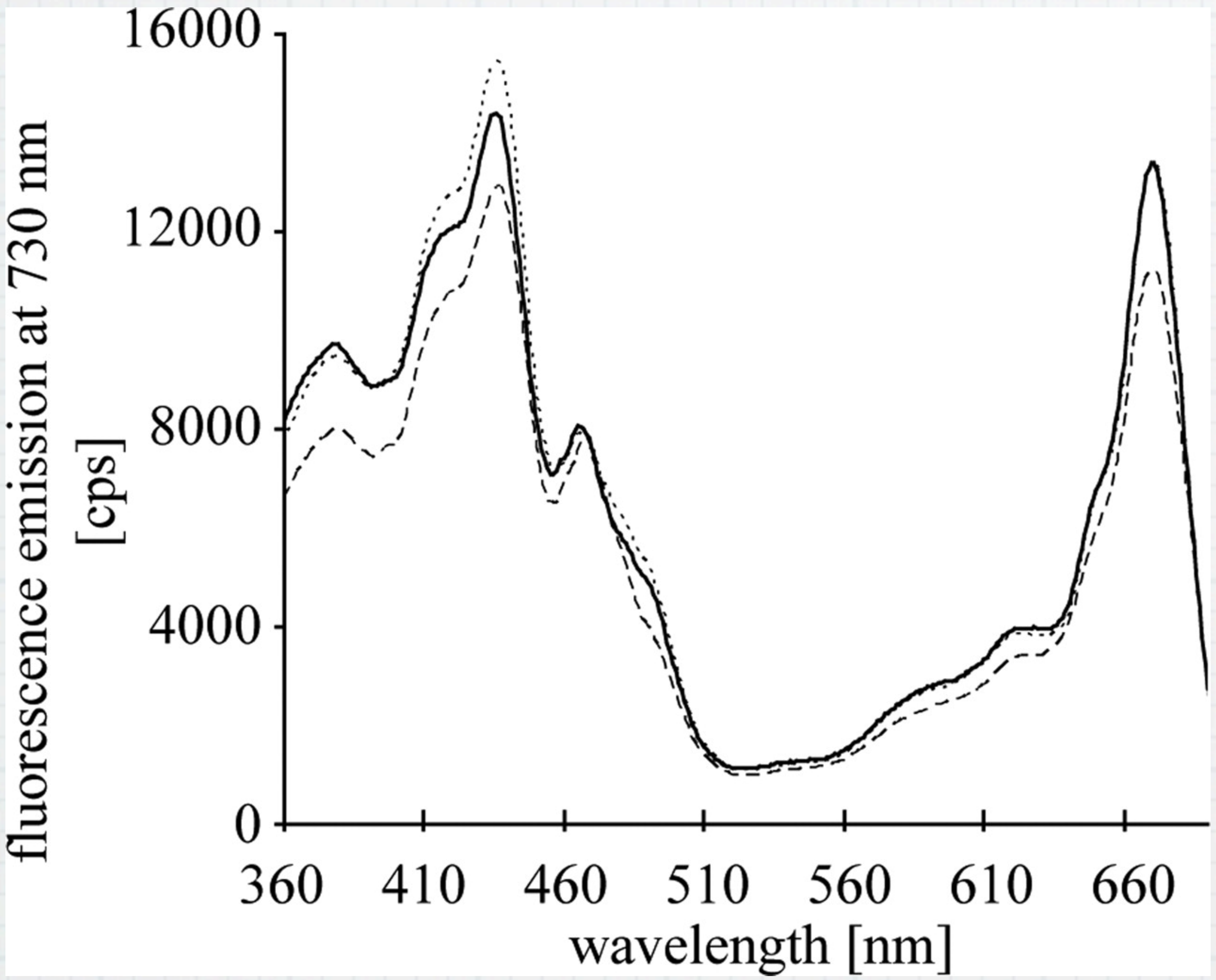


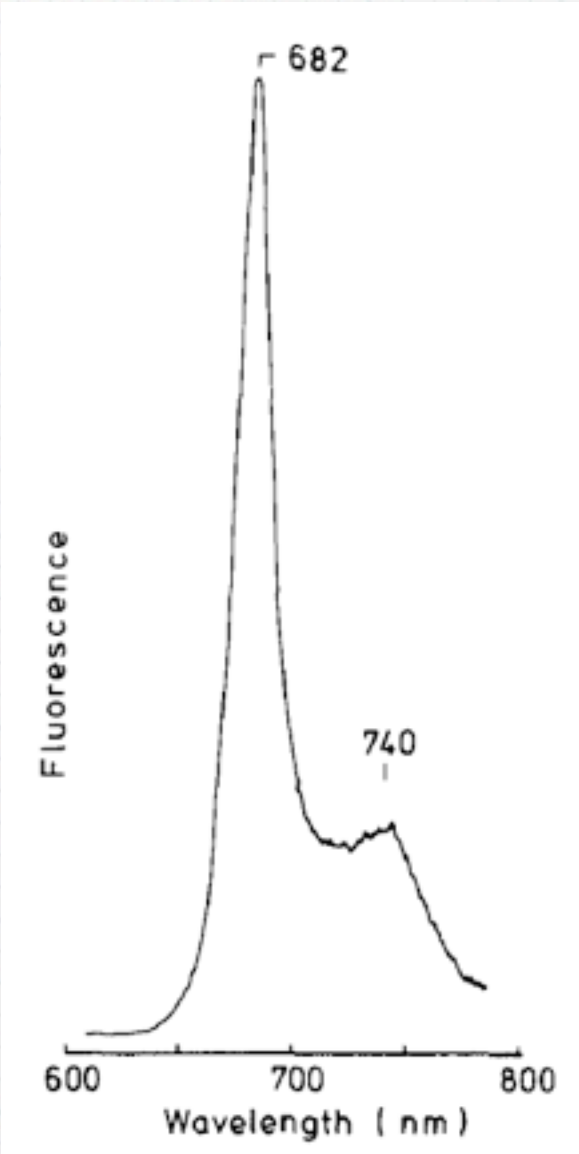


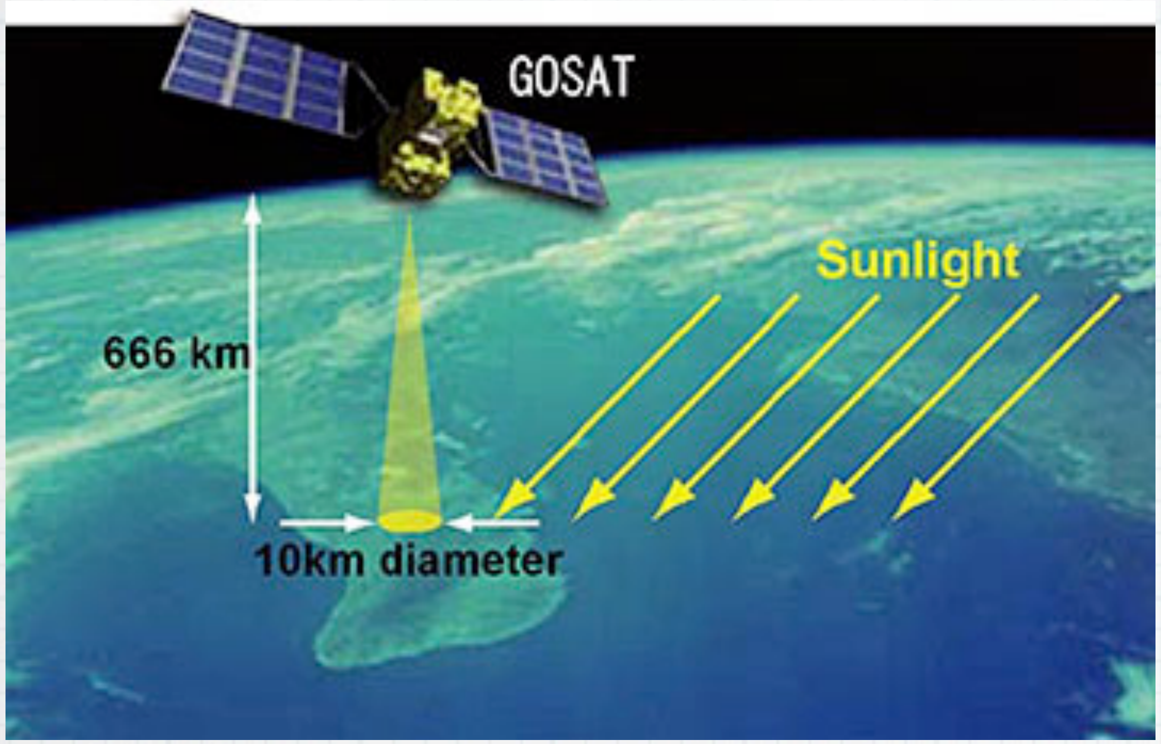
All that is needed to observe fluorescence is an appropriate pair of filters.

- a short pass filter to condition the light reaching the leaf so that it has no light in the band where chlorophyll fluoresces

- a second filter, a long pass filter that blocks the incident light but will pass the fluorescence.



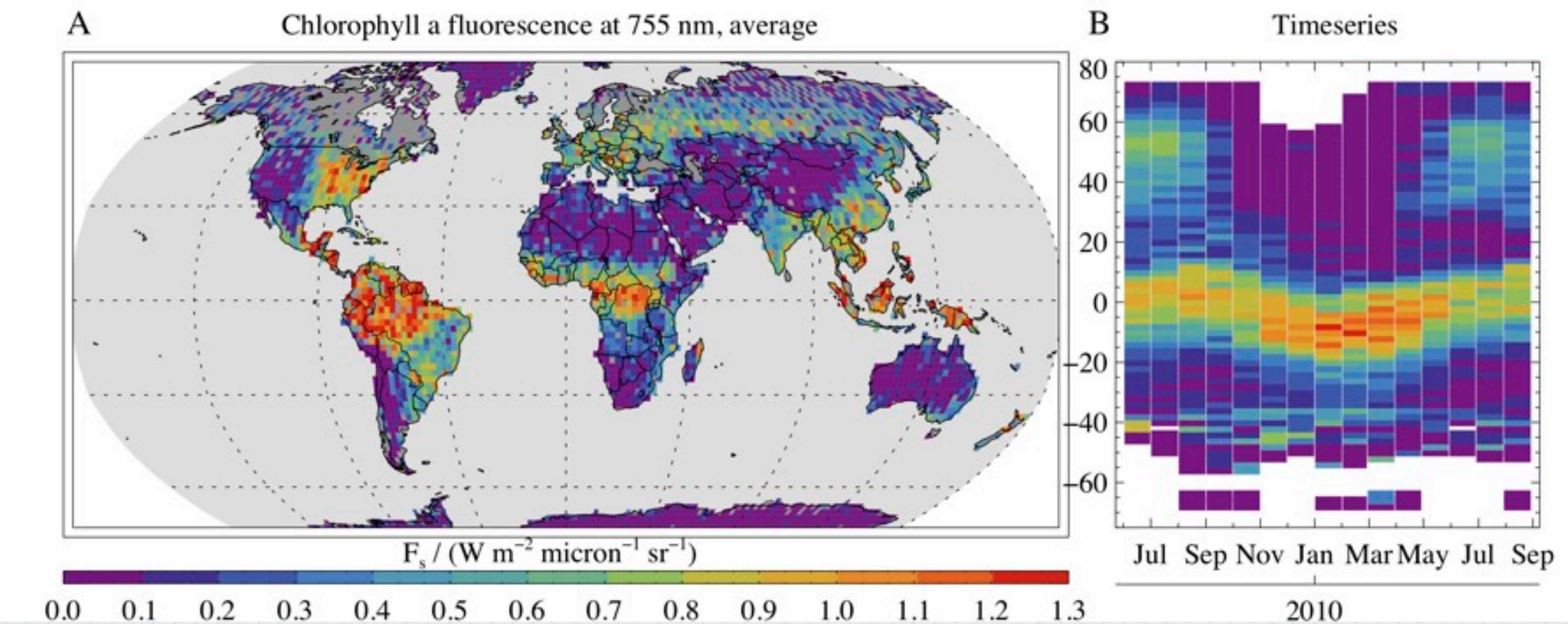




Physiology

$$F_s = I_0 \cdot \mathrm{FPAR} \cdot \theta_F \cdot \epsilon$$

Optics

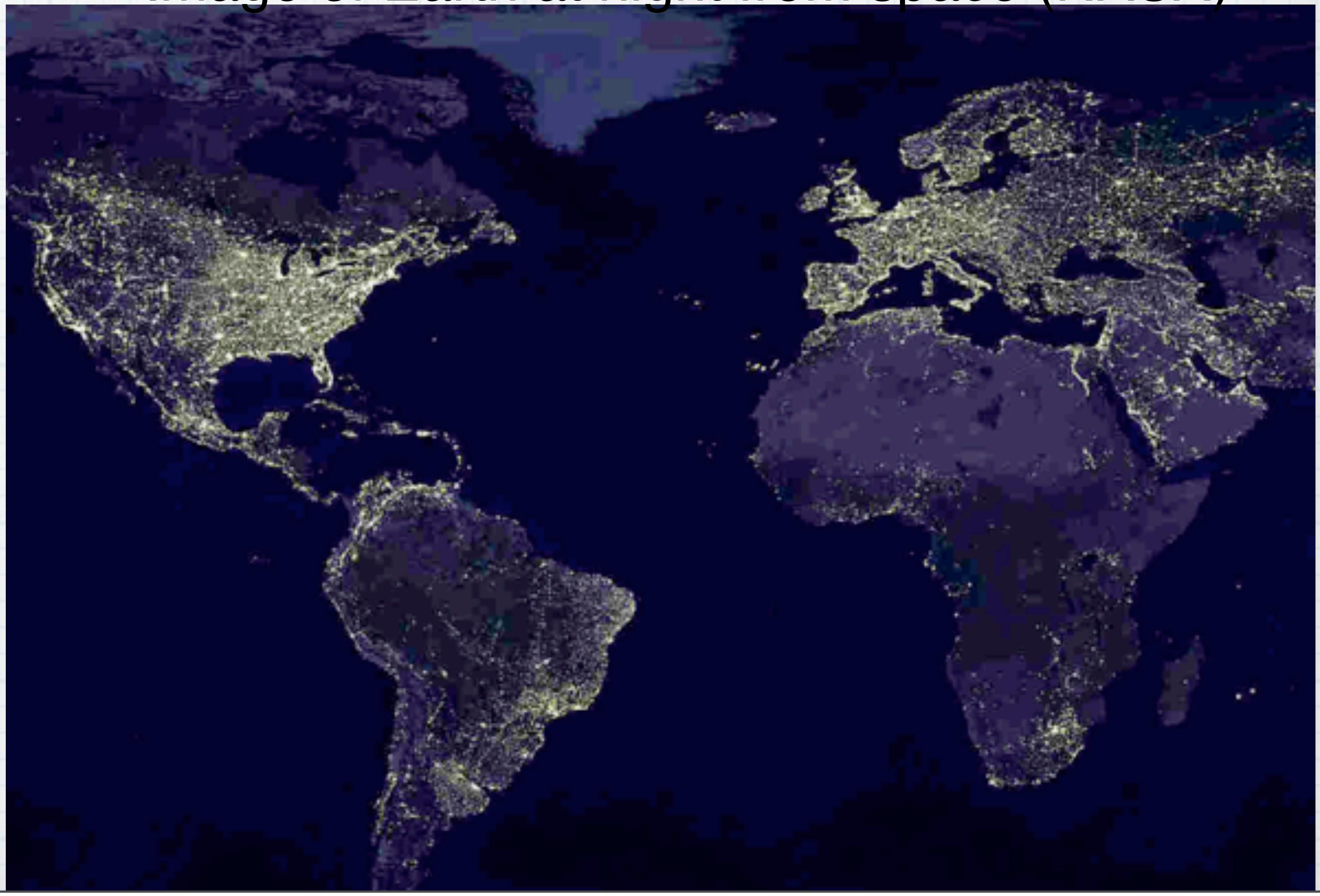


Frankenberg, C., Fisher, J. B., Worden, J., Badgley, G., Saatchi, S. S., Lee, J.-E., Toon, G. C., et al. (2011). New global observations of the terrestrial carbon cycle from GOSAT: Patterns of plant fluorescence with gross primary productivity. GEOPHYSICAL RESEARCH LETTERS, 38(17). doi:10.1029/2011GL048738

GOSAT is a complex retrieval system, but it makes a simple measurement.

- Nadir view and approximately solar noon under clear sky.
- Photosynthesis is at near its peak daily value and steady, (forget about the Kautsky effect).
- The "glow" is highly specific for plants doing photosynthesis,

Image of Earth at night from space (NASA)

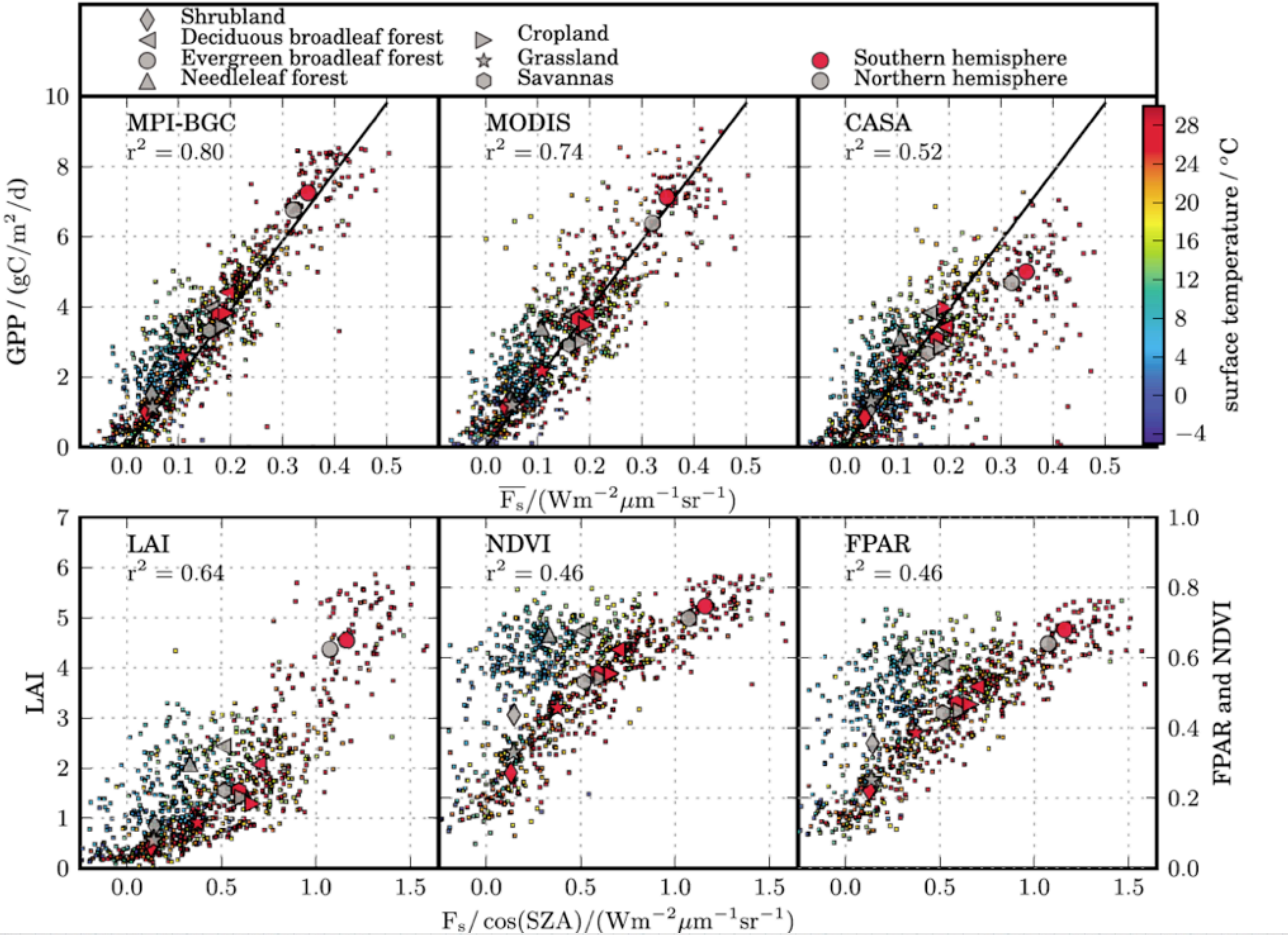


Is it this simple?

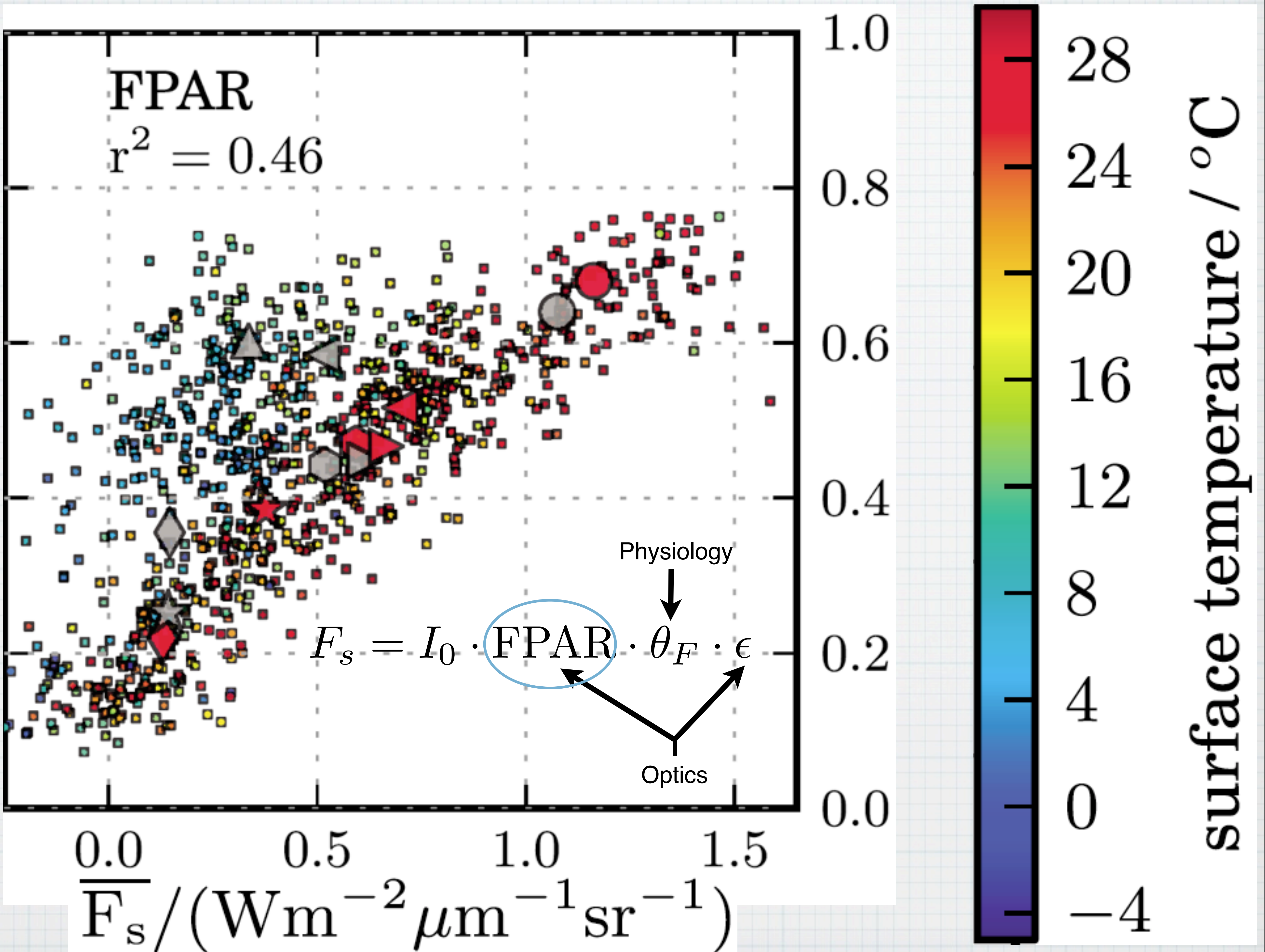
$$F_s = I_0 \cdot \mathrm{FPAR} \cdot \theta_F \cdot \epsilon$$

$$\mathrm{GPP} = I_0 \cdot \mathrm{FPAR} \cdot \theta_P$$

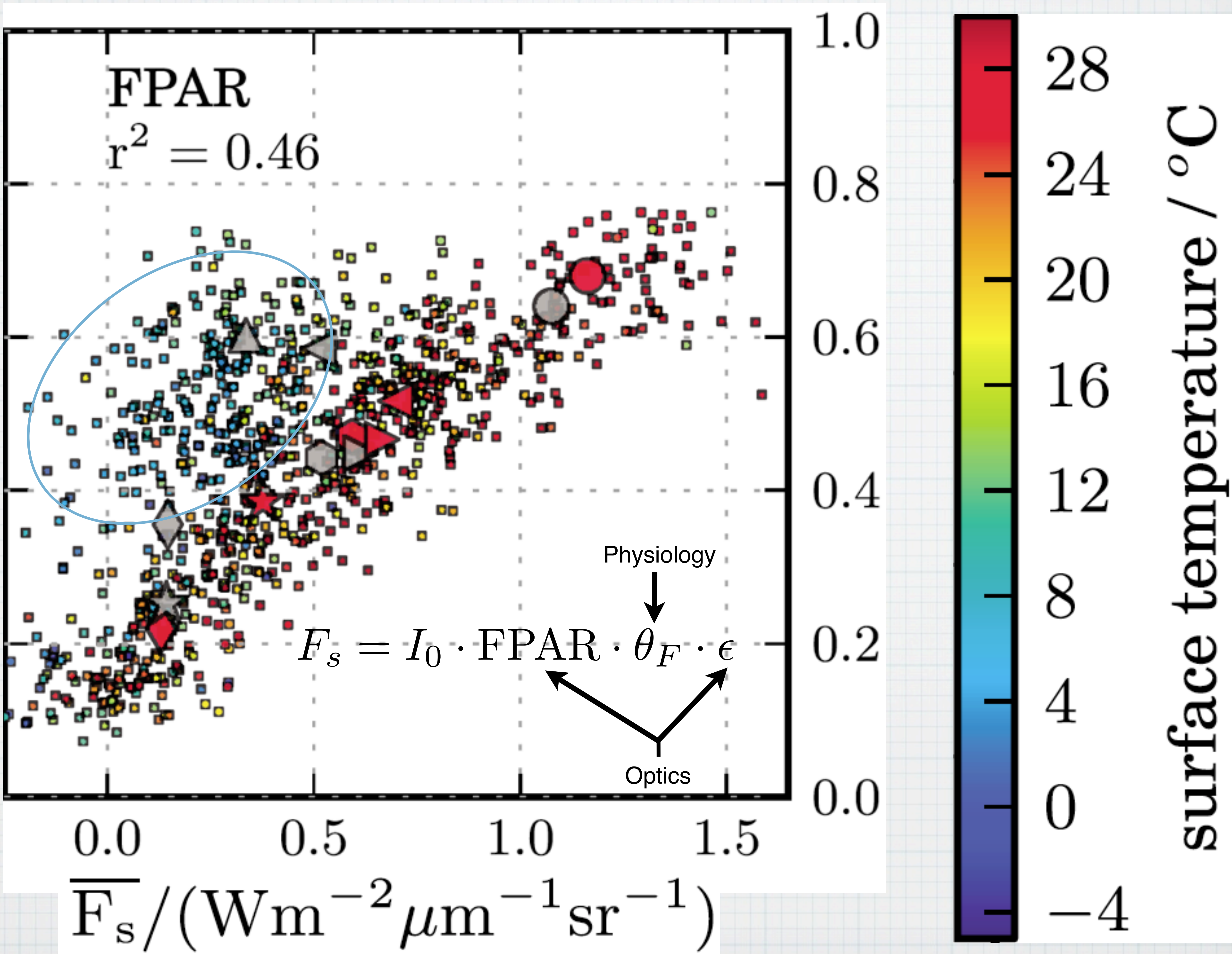
$$\mathrm{GPP} = F_s \frac{\theta_P}{\theta_F \cdot \epsilon}$$



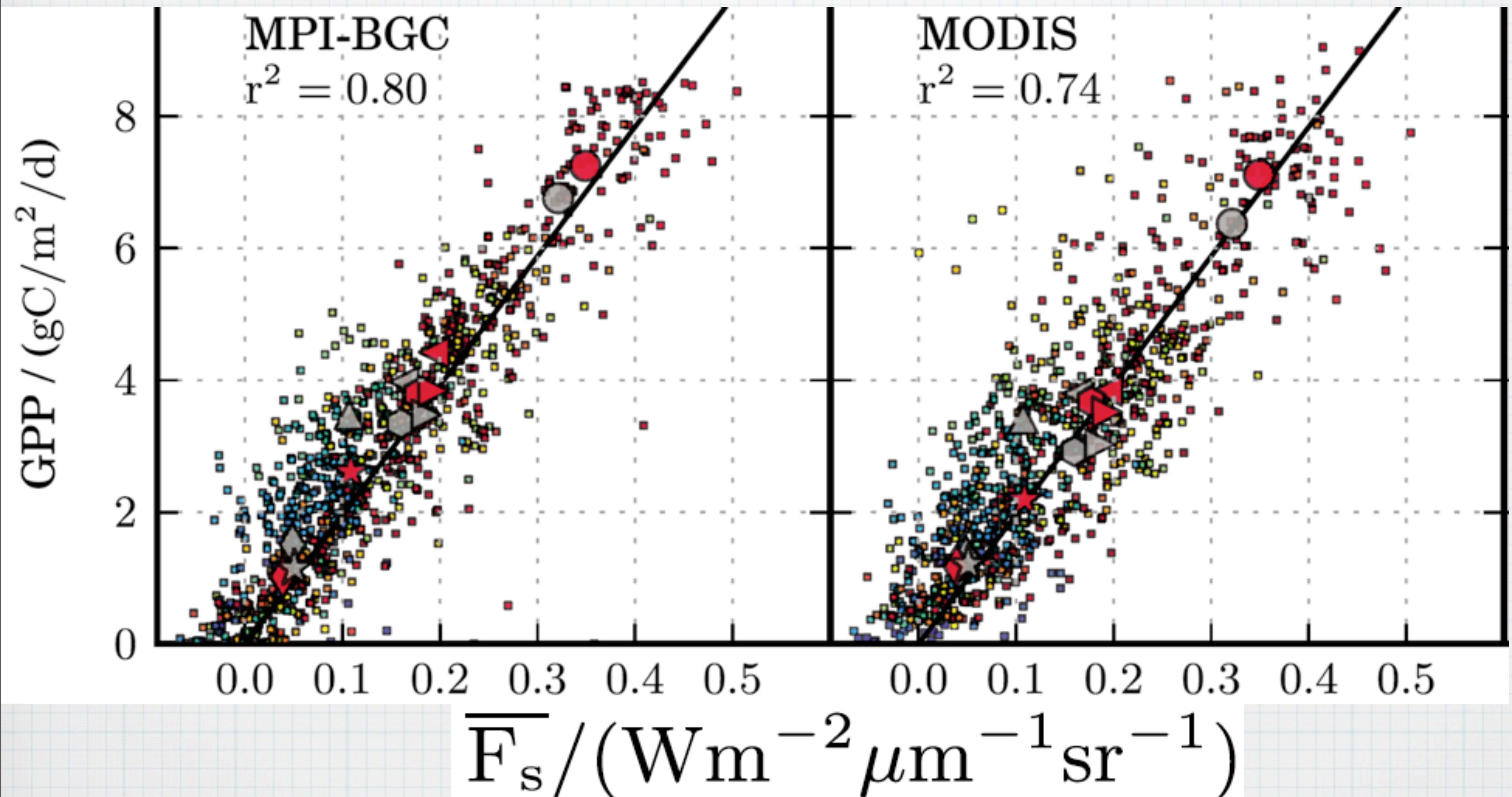
Shrubland
Deciduous broadleaf forest
Evergreen broadleaf forest
Needleleaf forest
Cropland
Grassland
Savannas
Southern hemisphere
Northern hemisphere
MPI-BGC
$r^2 = 0.80$
MODIS
$r^2 = 0.74$
CASA
$r^2 = 0.52$
GPP / (gC/m^2/d)
surface temperature / °C
$\overline{F_s}$/(Wm$^{-2}\mu$m^{-1}sr^{-1})
LAI
$r^2 = 0.64$
NDVI
$r^2 = 0.46$
FPAR
$r^2 = 0.46$
FPAR and NDVI
F_s / cos(SZA)/(Wm$^{-2}\mu$m^{-1}sr^{-1})



Monday, August 27, 12


FPAR
$r^2 = 0.46$
Physiology
$F_s = I_0 \cdot \mathrm{FPAR} \cdot \theta_F \cdot \epsilon$
Optics
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
$\overline{\mathrm{F_s}}/(\mathrm{Wm}^{-2}\mu\mathrm{m}^{-1}\mathrm{sr}^{-1})$
28
24
20
16
12
8
4
0
−4
surface temperature / °C

$$\frac{\mathrm{GPP} = I_0 \cdot \mathrm{FPAR} \cdot \theta_P}{F_s = I_0 \cdot \mathrm{FPAR} \cdot \theta_F \cdot \epsilon} \longrightarrow \mathrm{GPP} = F_s \frac{\theta_P}{\theta_F \cdot \epsilon}$$



- A large part of the variability is due to FPAR.

- Physiology also seems to have an influence; θ_F and θ_P appear to co-vary.

- Calibration experiments are really difficult to do at a realistic scale.

Sun induced fluorescence from above a corn field before, during and after a drought.

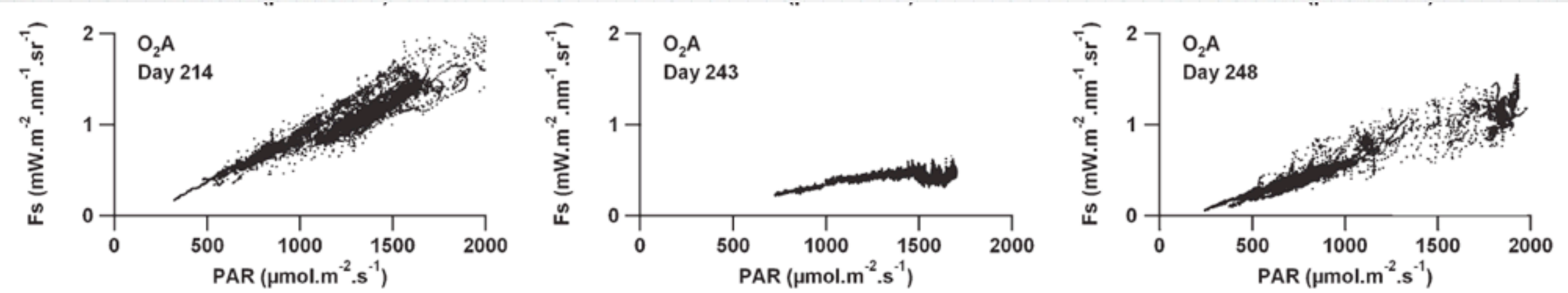
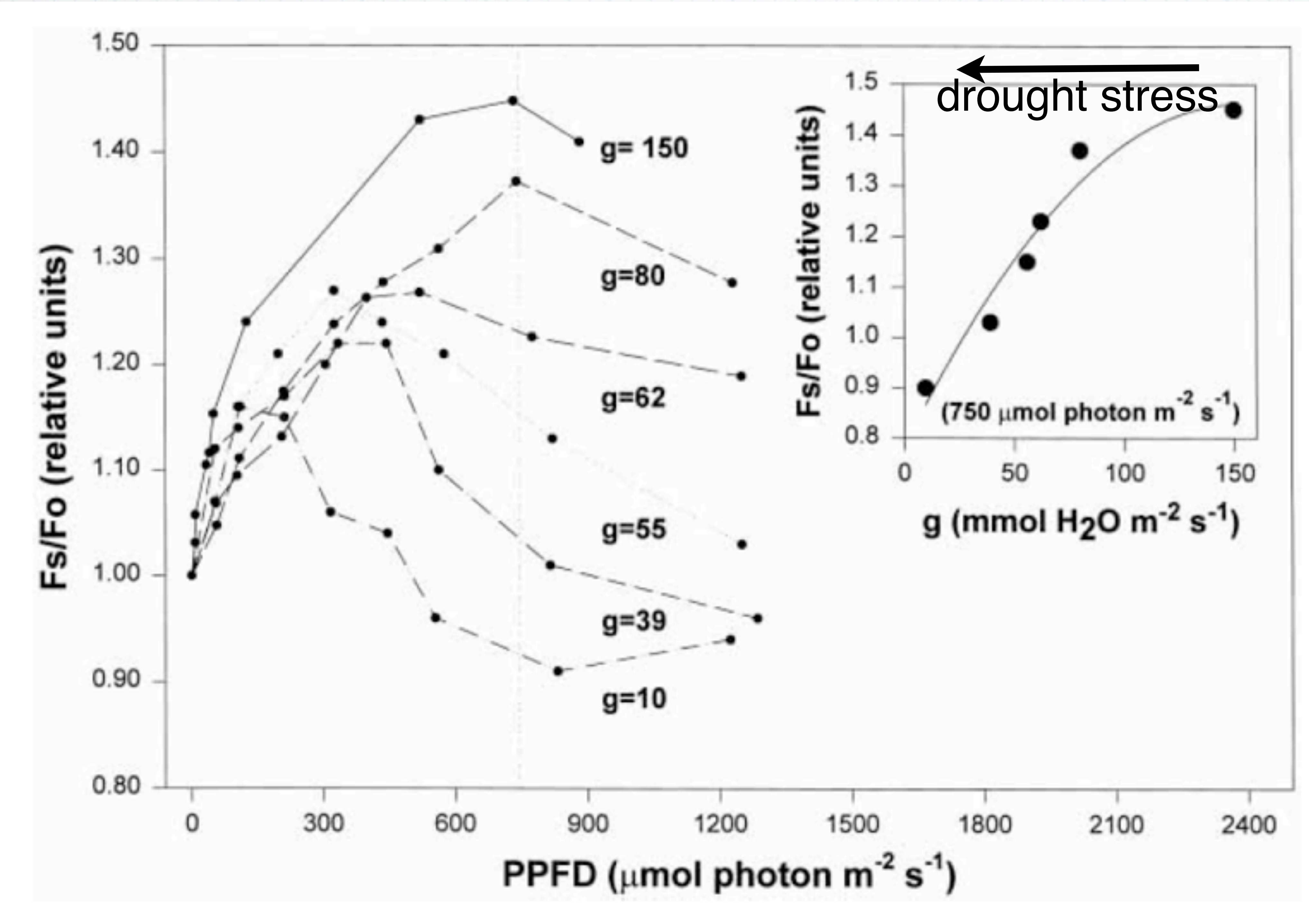


Fig. 10. Fluorescence flux (Fs) versus PAR for three days: 214 no water stress, 243 maximal water stress effect, 248 after rainy days, and reversion of water stress.

Daumard, F., Champagne, S., Fournier, A., Goulas, Y., Ounis, A., Hanocq, J. F., & Moya, I. (2010). A field platform for continuous measurement of canopy fluorescence. **Geoscience and Remote Sensing, IEEE Transactions on**, 48(9), 3358–3368. doi:10.1109/TGRS.2010.2046420

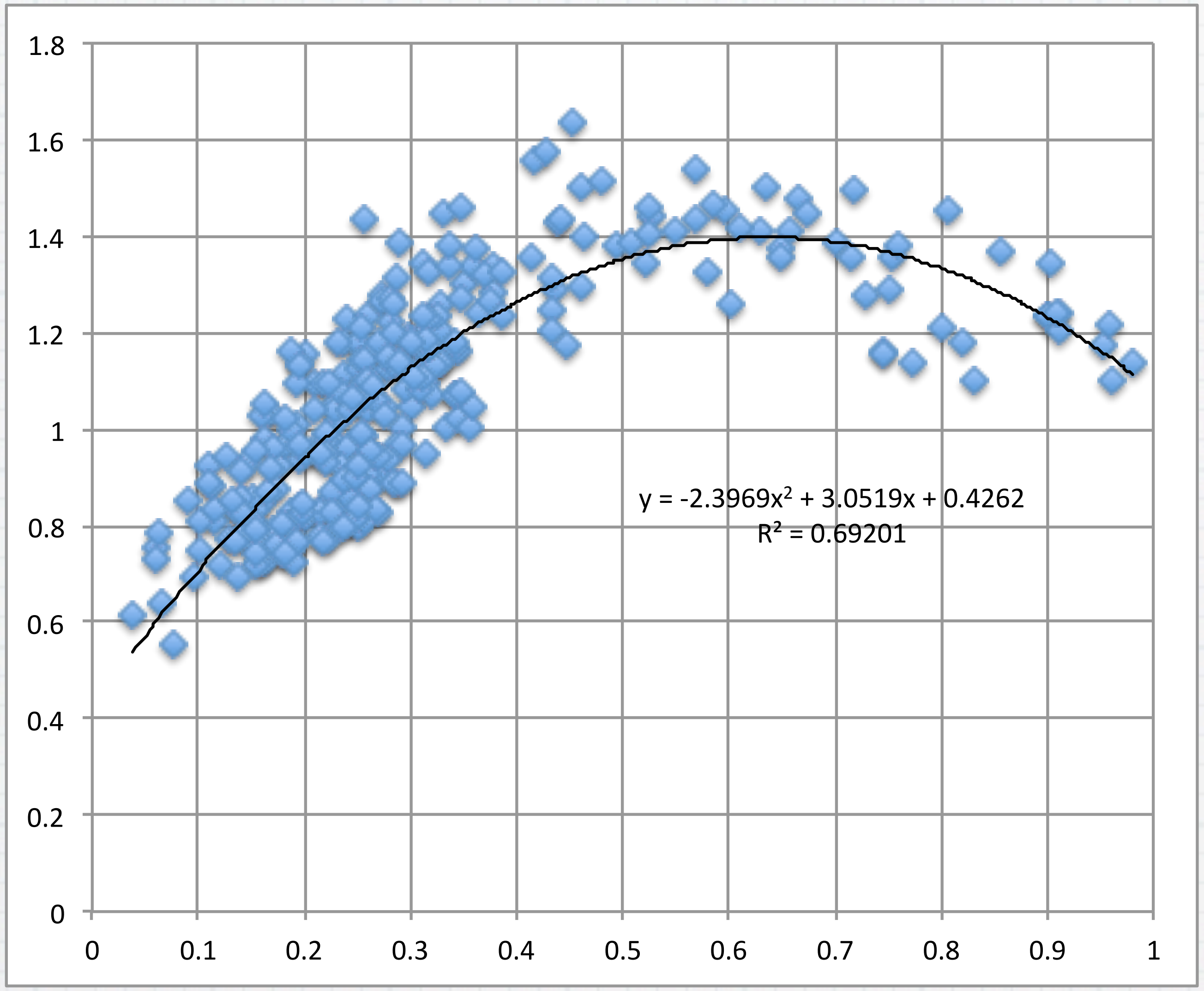
Leaf-scale experiments with grapes experiencing different levels of drought



Flexas, J., Escalona, J., Evain, S., Gulias, J., & Moya, I. (2002). Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO2 assimilation and stomatal conductance during water-stress in C3 plants. **Physiologia Plantarum** 114:231-240.

Analysis of leaf-scale experiments with 10 species before during and recovery from drought (Galmes et al.) -- data provided by J. Flexas.

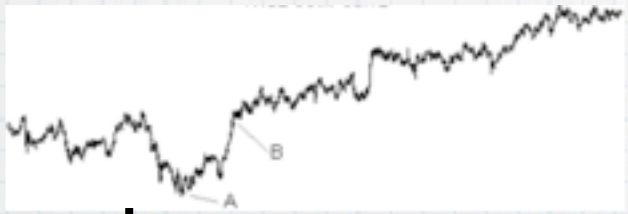
$F/F_o \approx \theta_F$

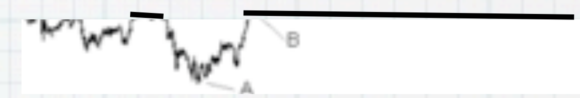


$$J_e/J_o(\text{actual/potential ETR}) \approx \theta_P$$

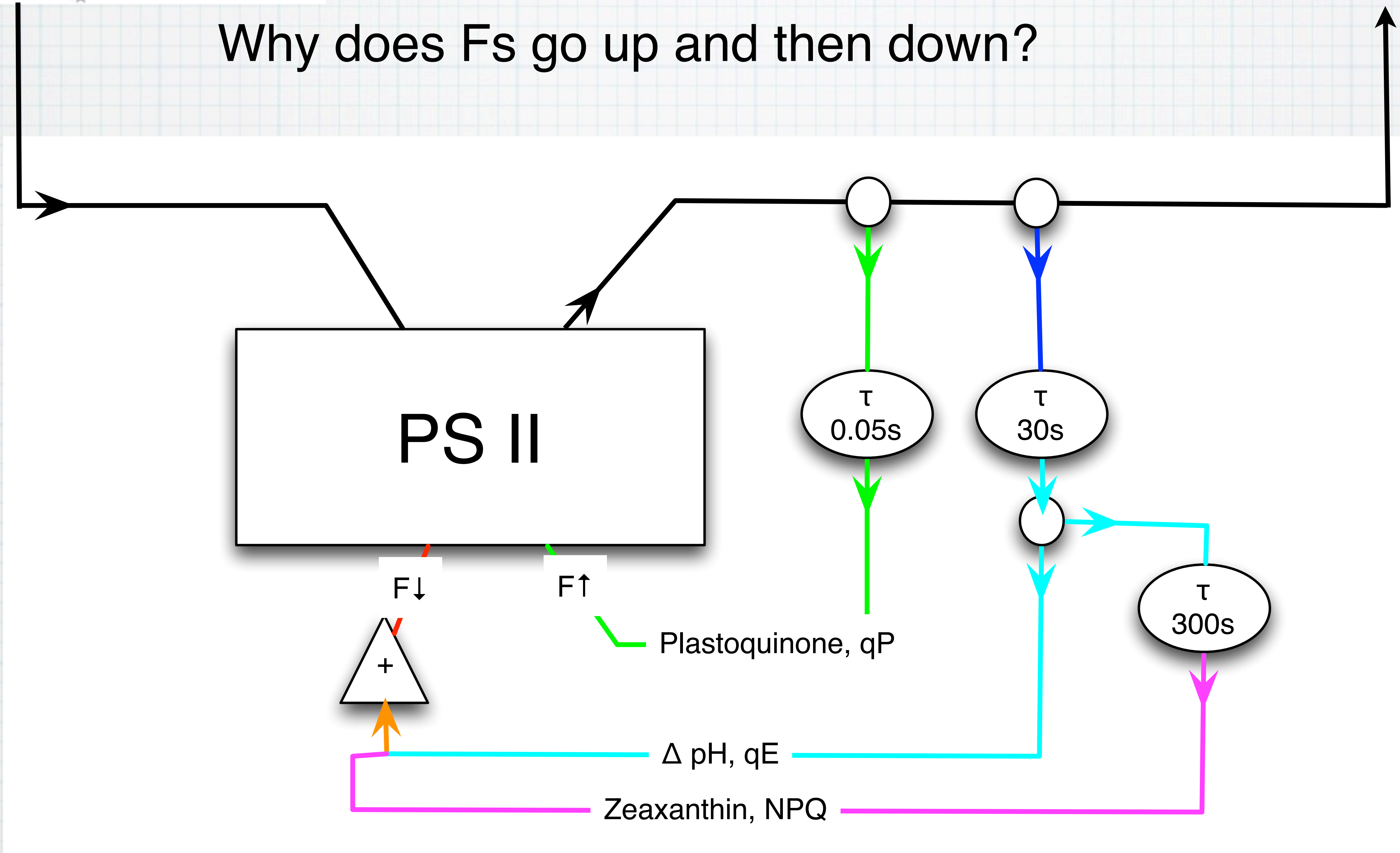
$$F_s = I_0 \cdot \text{FPAR} \cdot \theta_F \cdot \epsilon$$

Galmés, J., Medrano, H. & Flexas, J. (2007). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. **New Phytologist** 175:81-93.





Why does Fs go up and then down?



Relative fluorescence yield, F

$$F = -2.3969x^2 + 3.0518x + 0.4262$$

$$x = J_o / J_e$$

$$J_e = A \cdot 4 \frac{p_i - \Gamma}{p_i + 2\Gamma}$$ (from any model)

$$J_o = I_o \cdot a \cdot \alpha$$

A is CO_2 uptake,

p_i is intercellular CO_2

a is absorptance

α is quantum yield,

Γ^* is the compensation point

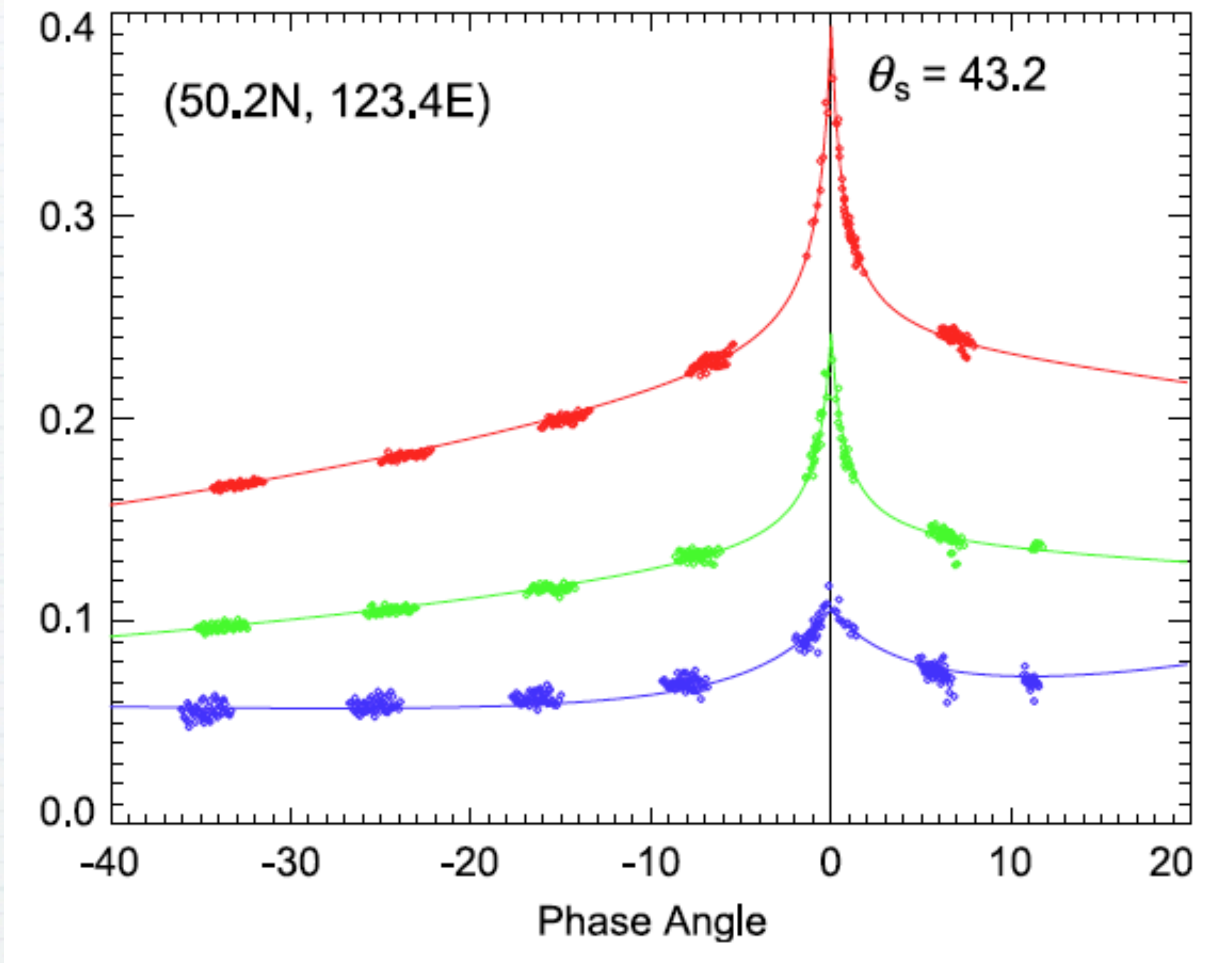
- Leaf-scale calibrations of relative fluorescence yield are routine.
- Variations in absolute yield from leaf to leaf will need to be taken into account
- Fluorescence can be added to photosynthesis models.
- Scaling from the leaf to the canopy will be tricky, but we are already doing this for GPP.
- Radiation transport in the canopy needs to be included. It already is in SCOPE.

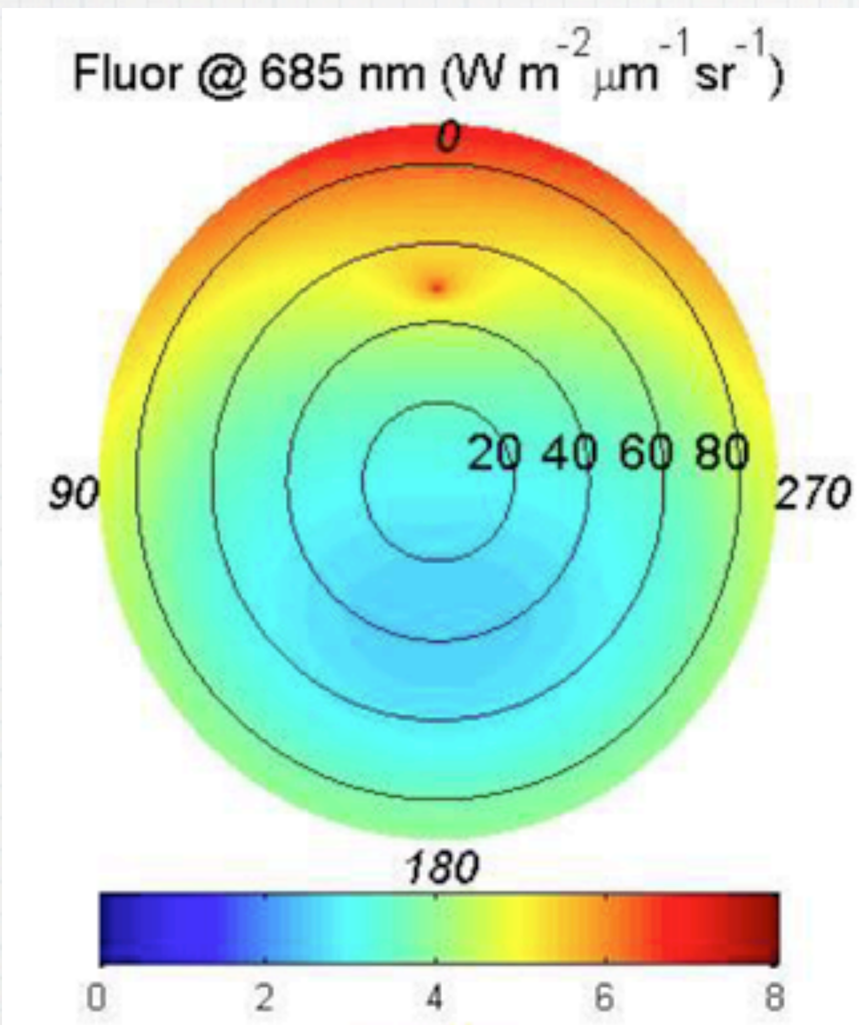
Physiology

$$F_s = I_0 \cdot \mathrm{FPAR} \cdot \theta_F \cdot \epsilon$$

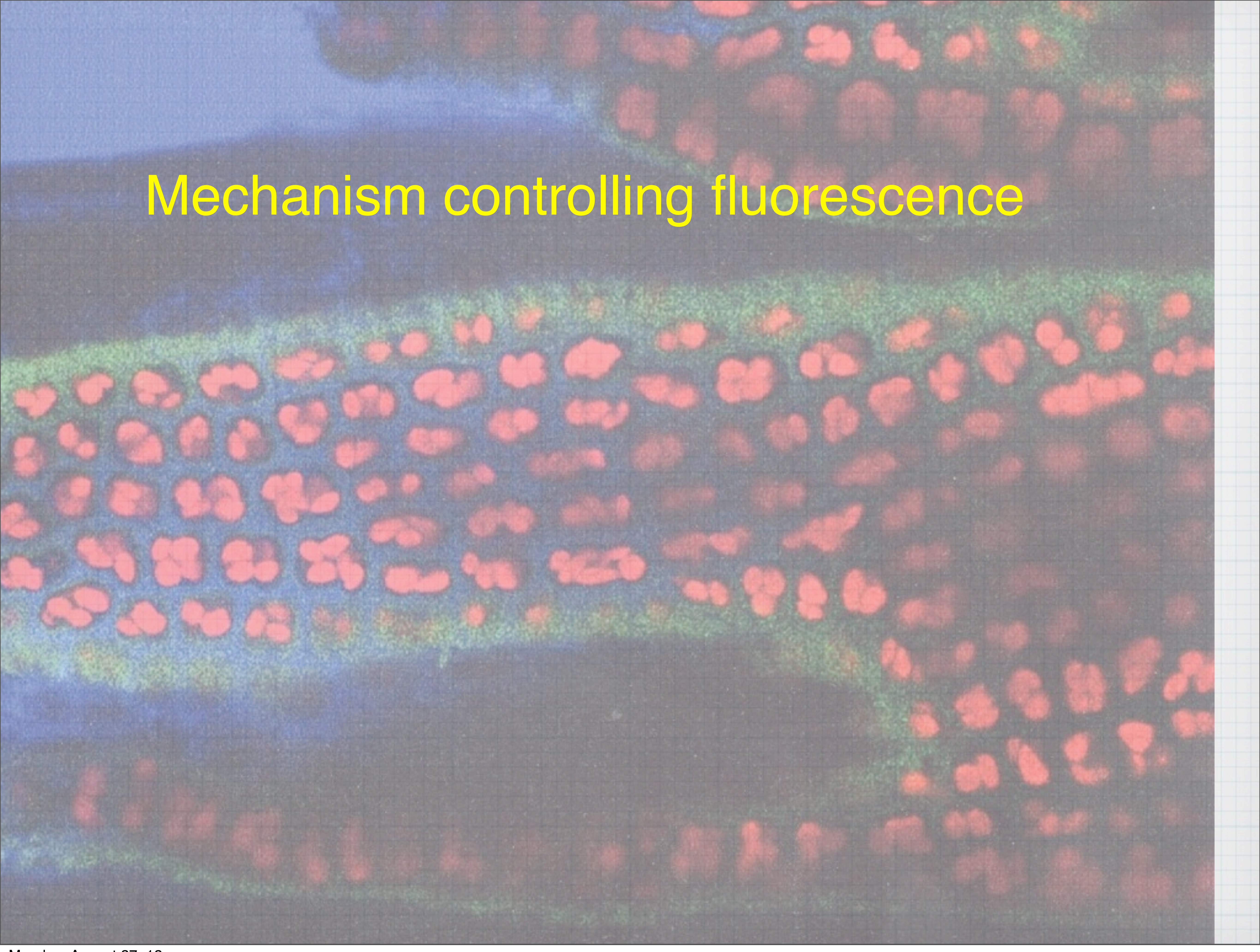
Optics

Maignan, F., Bréon, F. M., & Lacaze, R. (2004). Bidirectional reflectance of Earth targets: evaluation of analytical models using a large set of spaceborne measurements with emphasis on the Hot Spot. **Remote Sensing of Environment**, 90(2), 210–220. doi: 10.1016/j.rse.2003.12.006

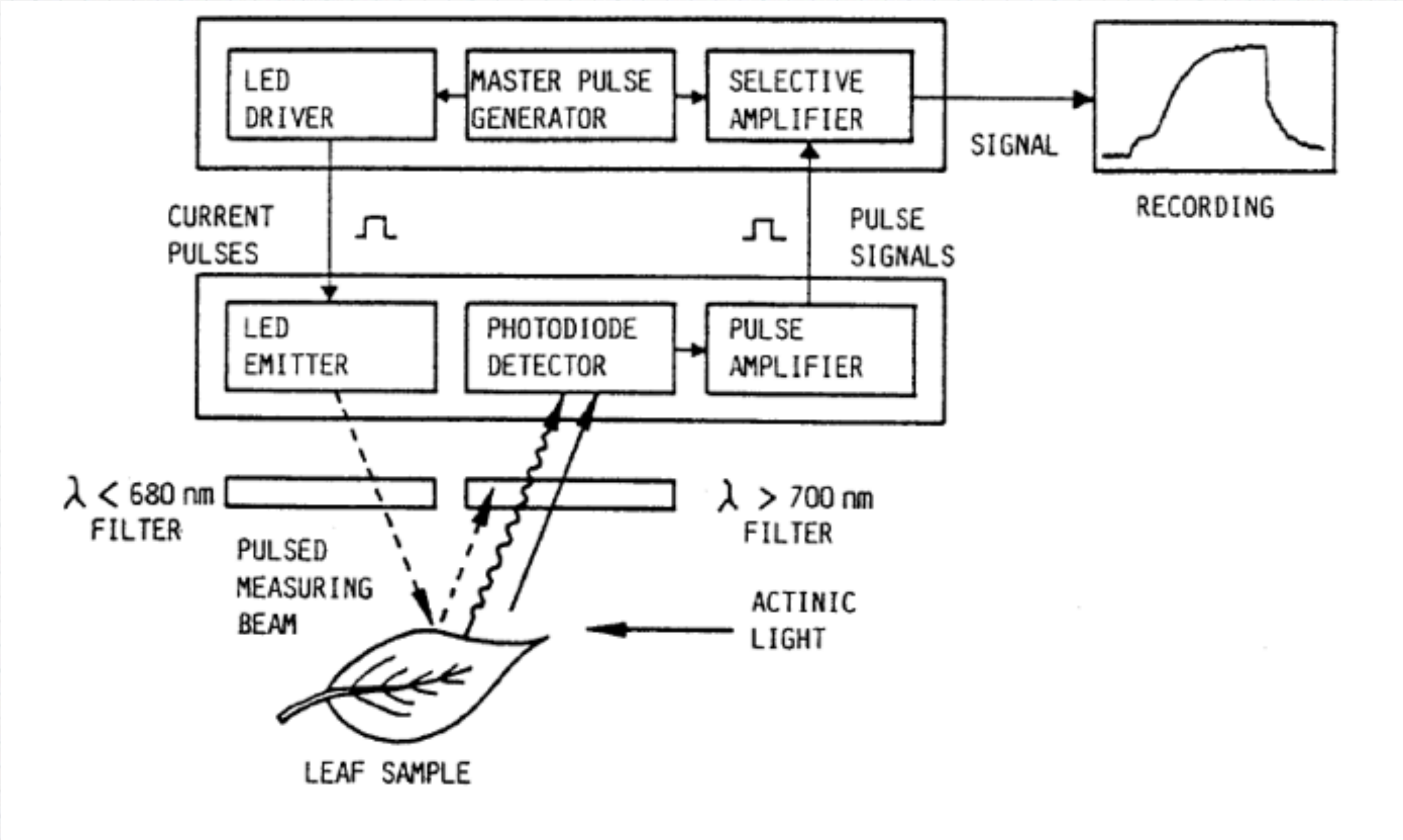




van der Tol, C., Verhoef, W., Timmermans, J., Verhoef, A., & Su, Z. (2009). An integrated model of soil-canopy spectral radiances, photosynthesis, fluorescence, temperature and energy balance. **Biogeosciences**, 6(12), 3109–3129.

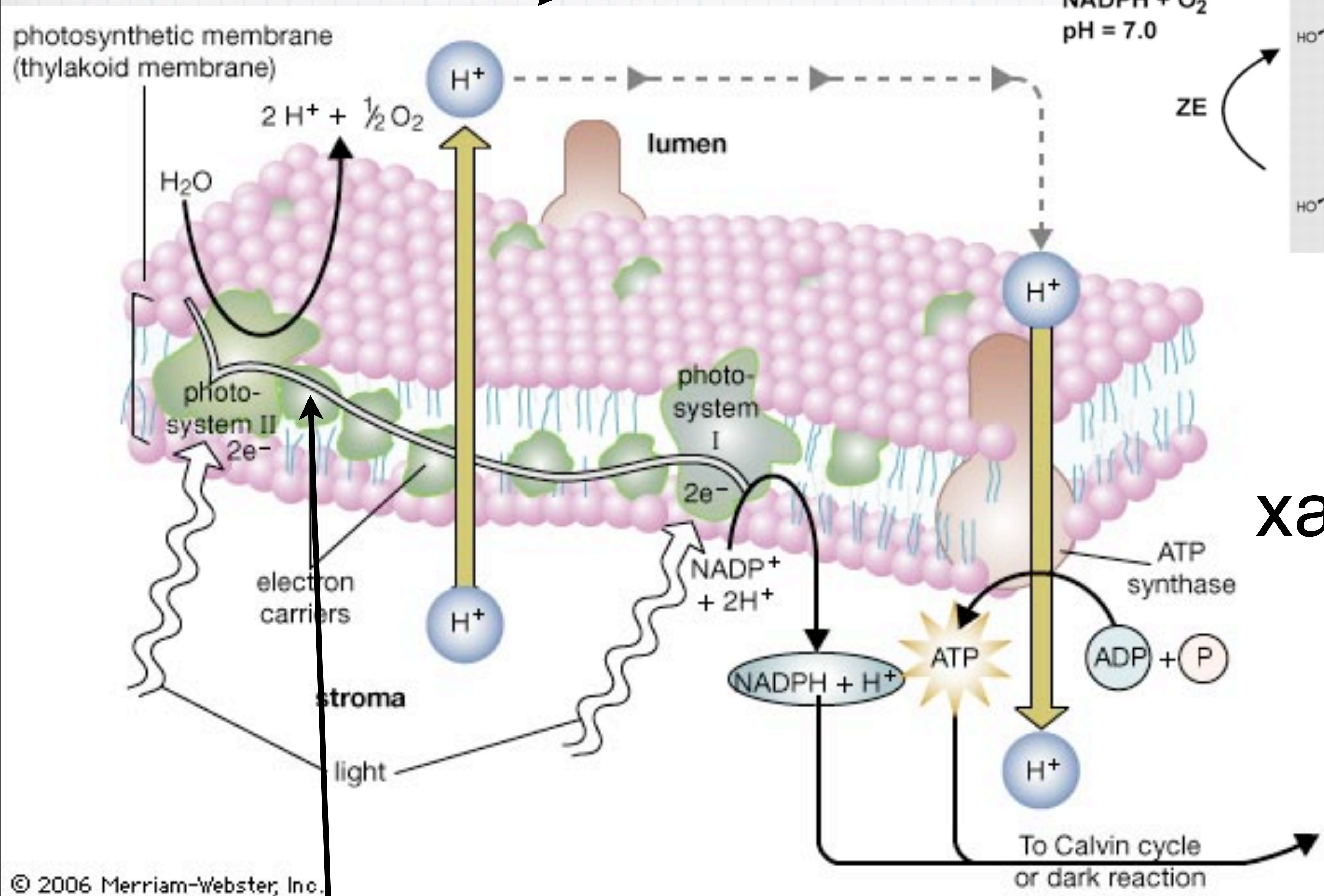

Mechanism controlling fluorescence

The PAM Fluorimeter

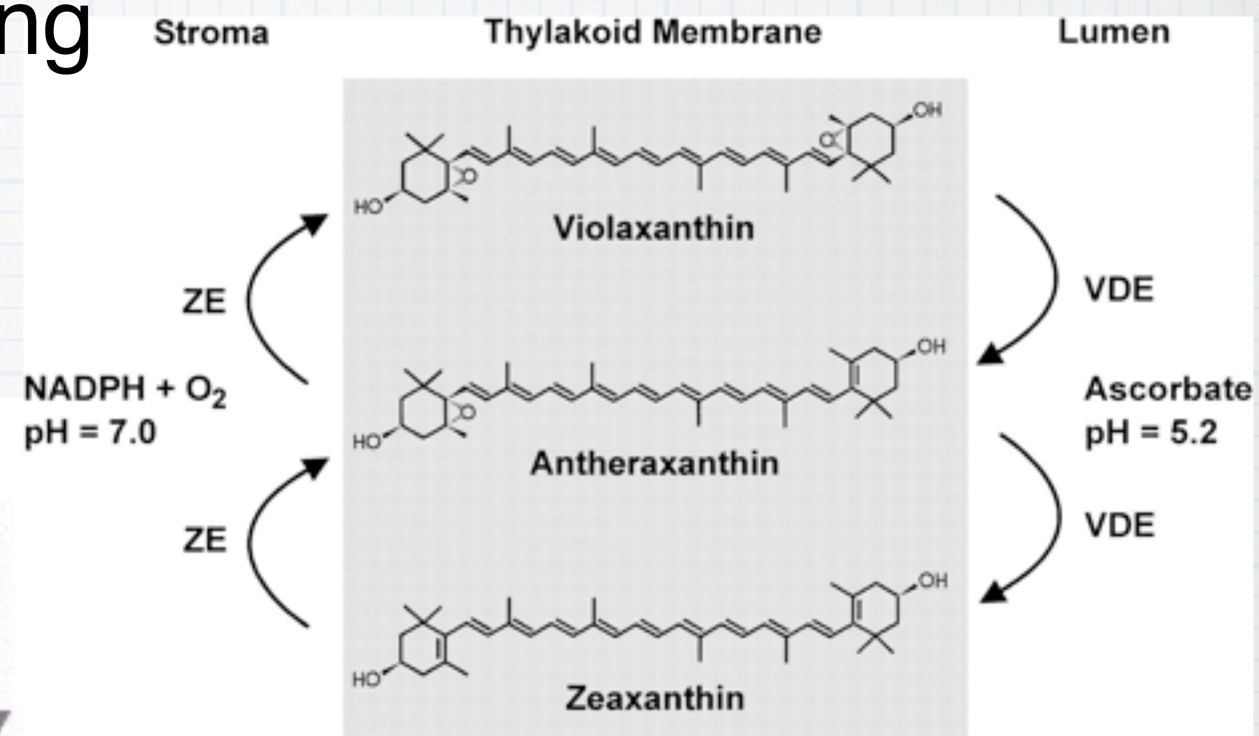


Controls of exciton processing

ΔpH

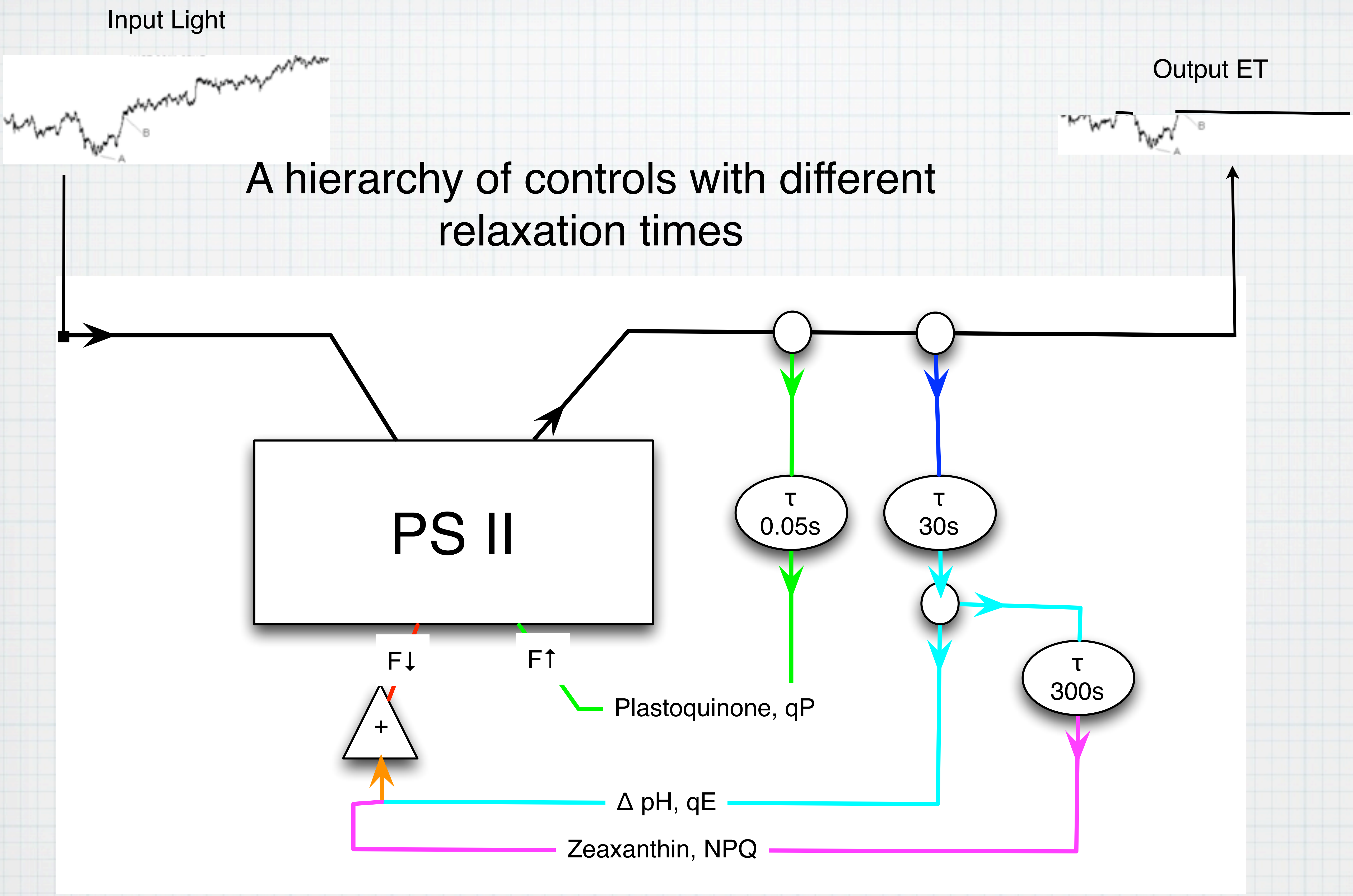


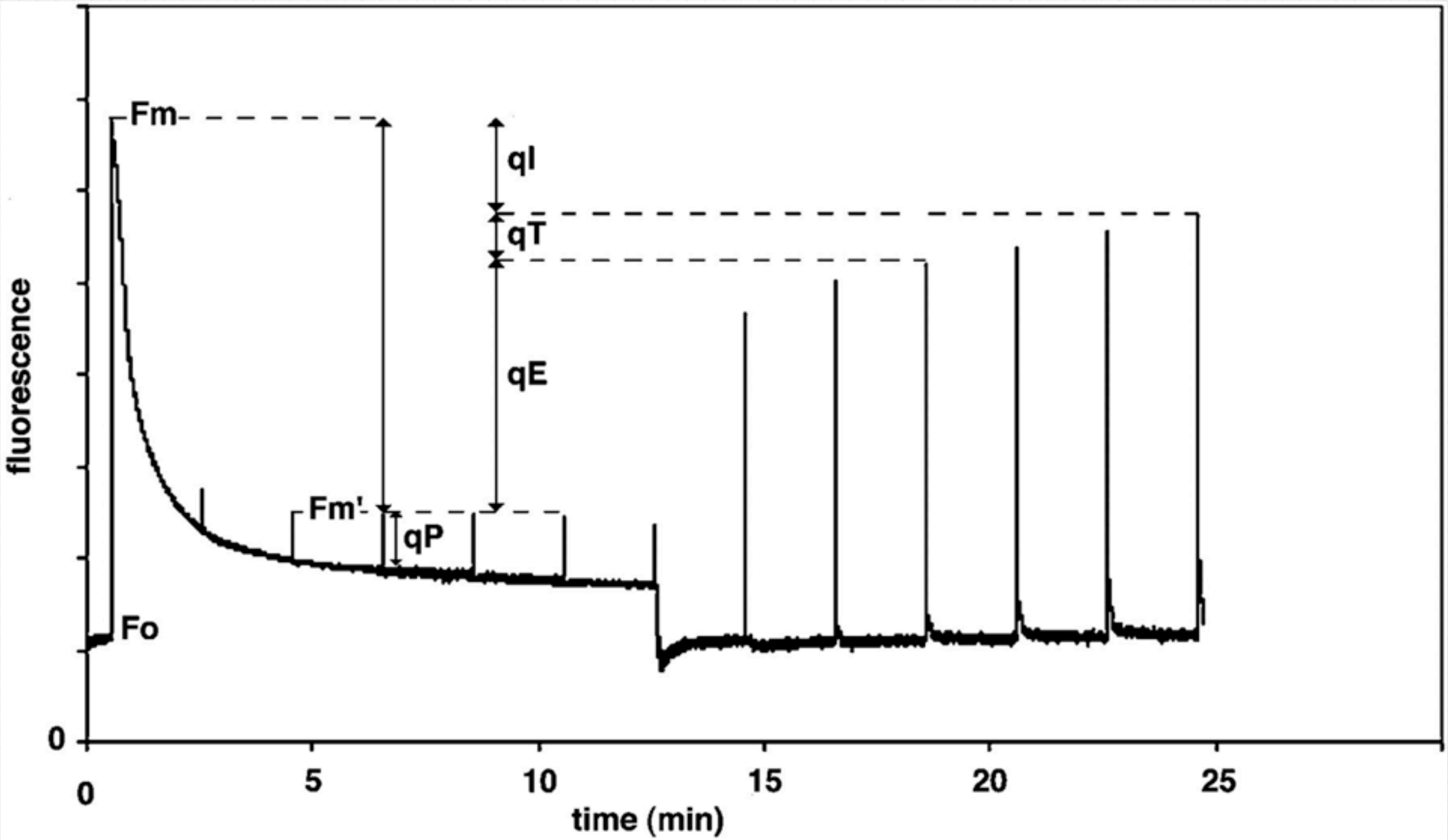
plastoquinone



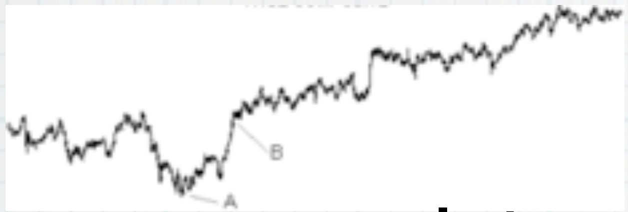
xanthophyll cycle

A hierarchy of controls with different relaxation times

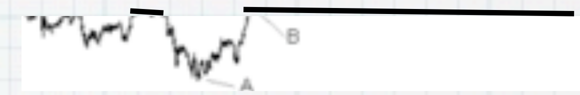



Fm
qI
qT
qE
Fm'
qP
Fo
fluorescence
0
0
5
10
15
20
25
time (min)

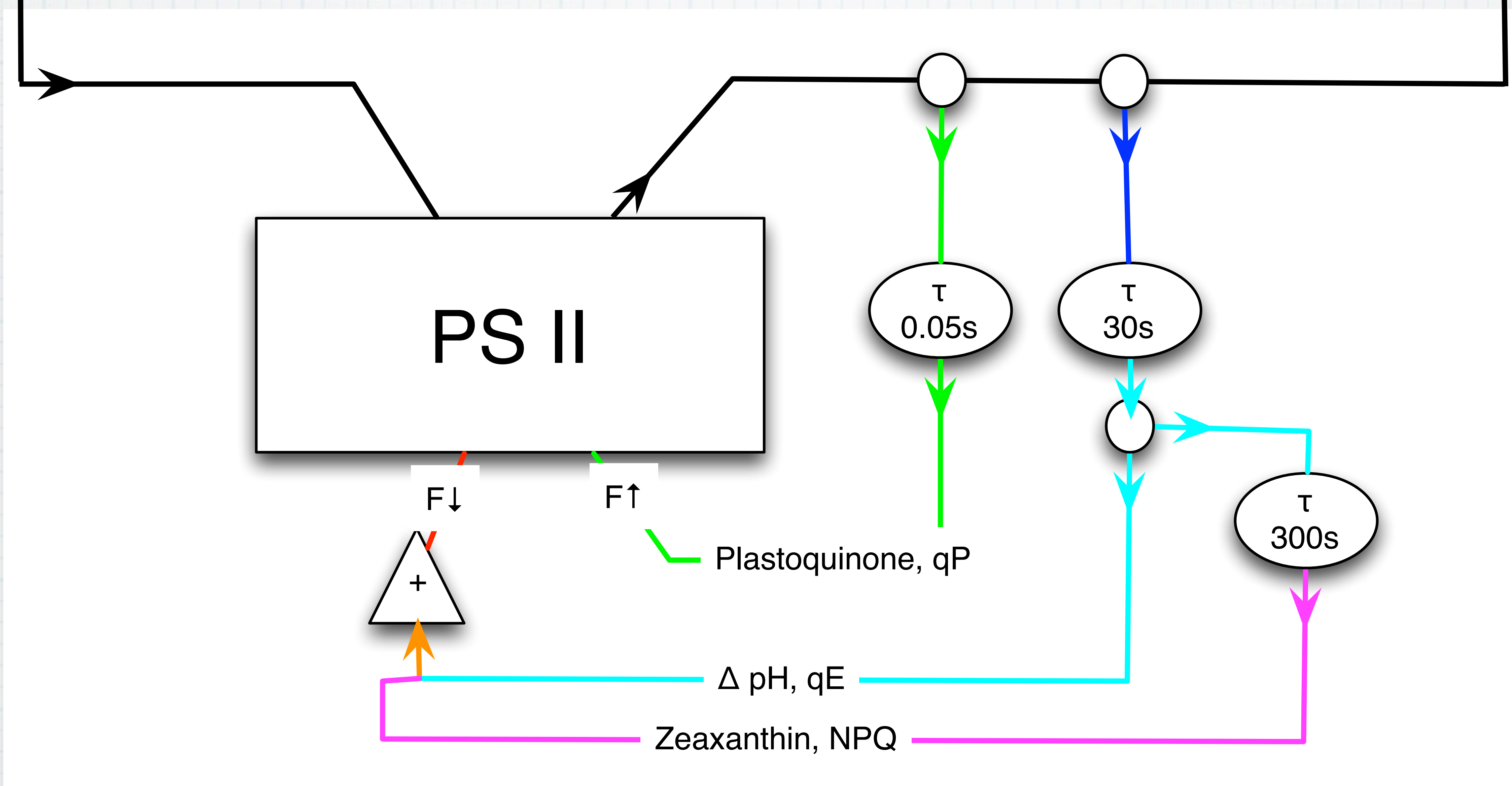
Input Light



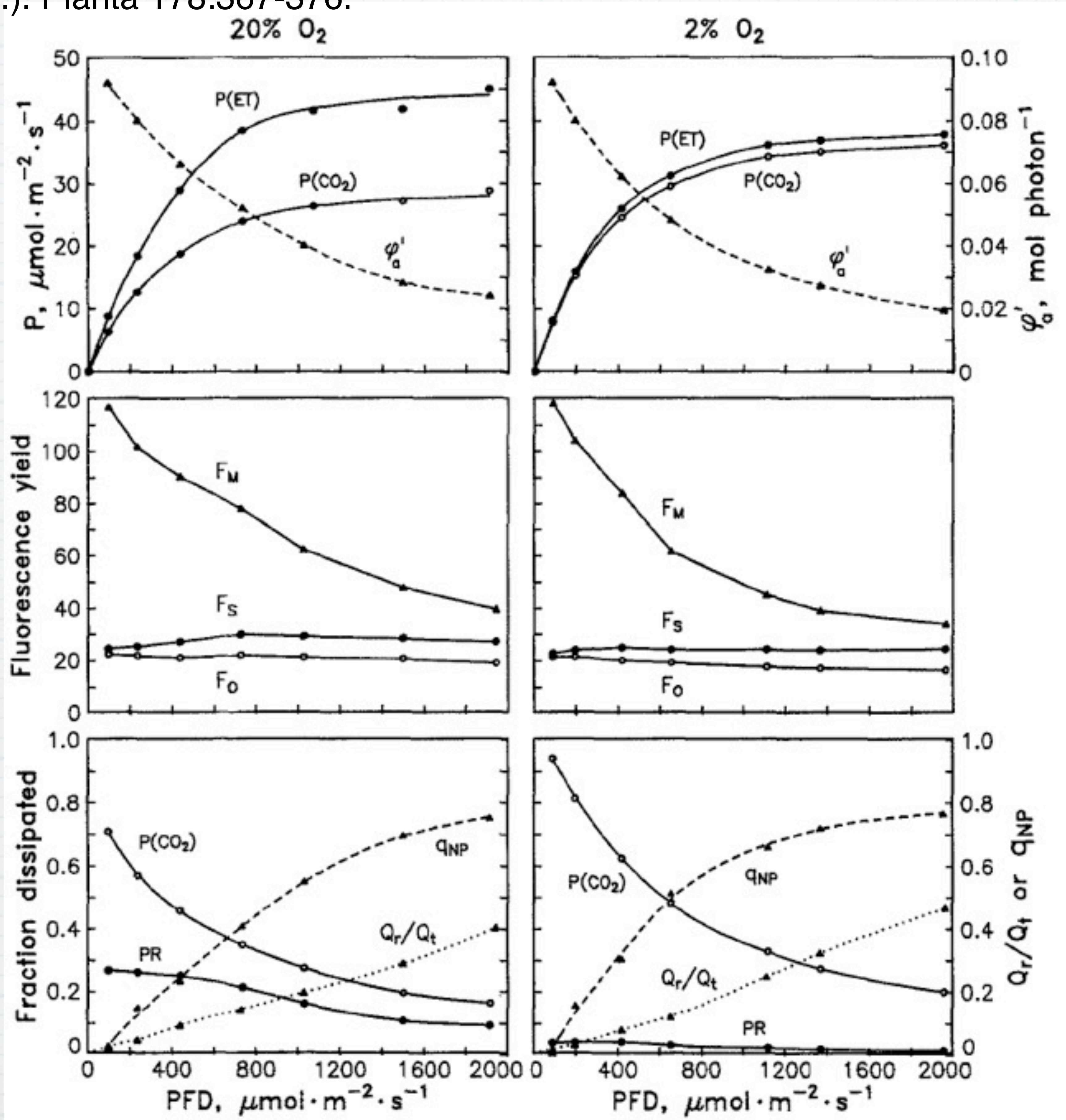
Output to carbon fixation



Intense pulses permit separation of qP from qE and NPQ

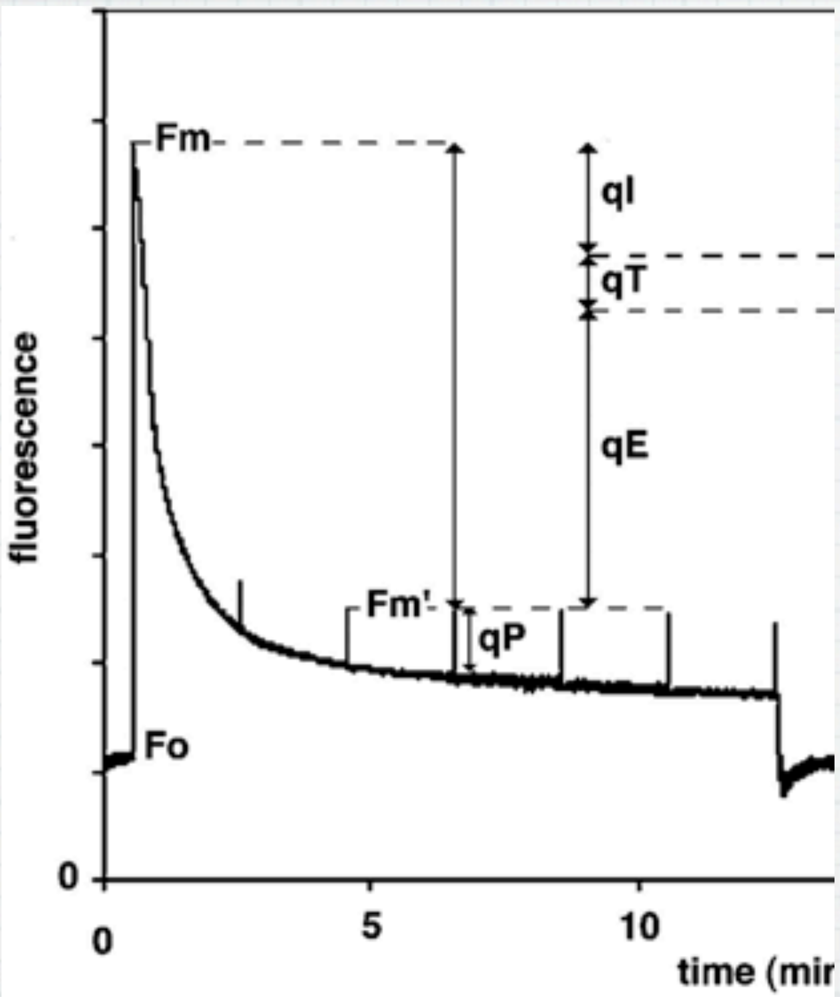


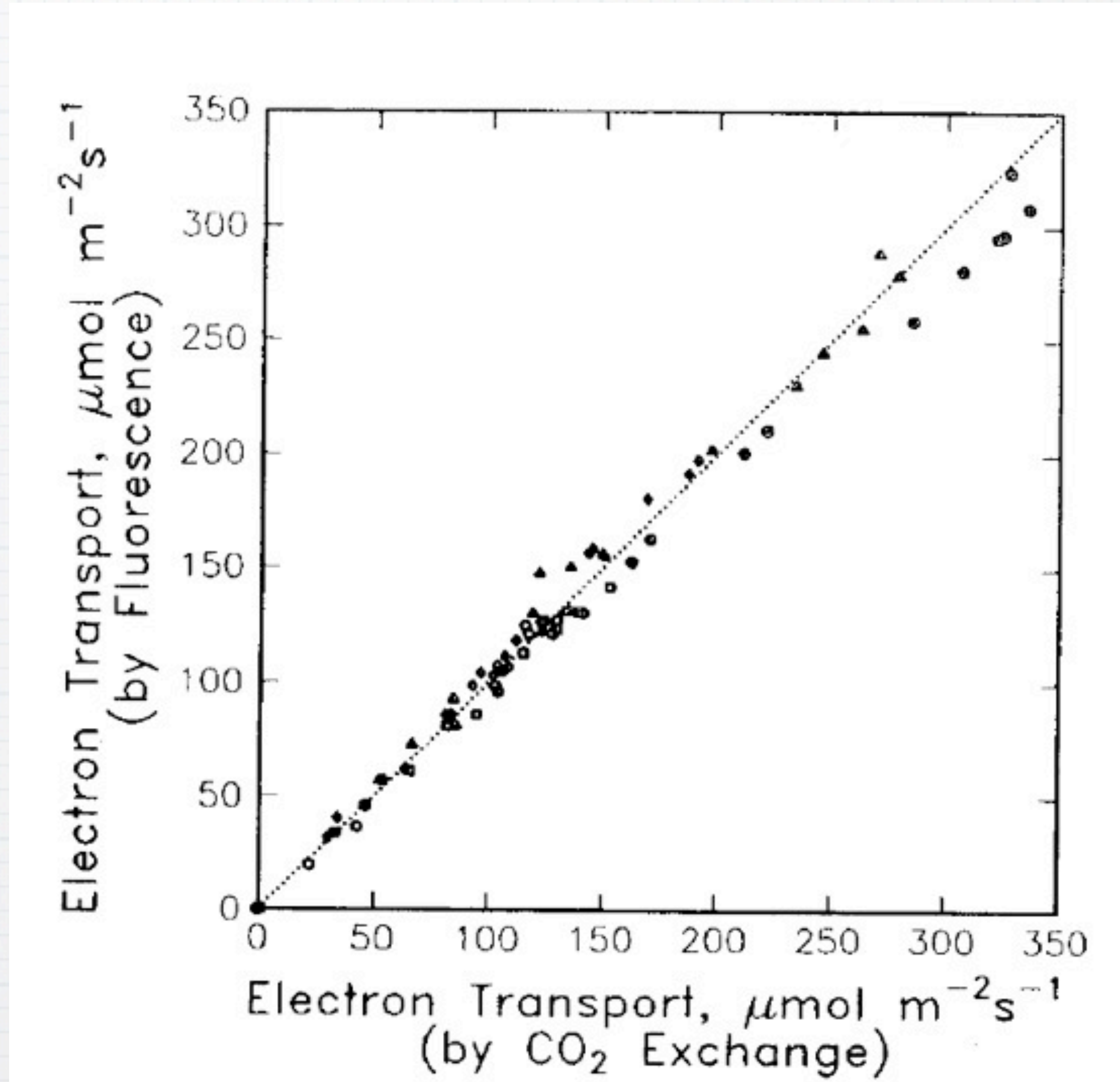
Schäfer, C. and Bjorkman, O.(1989). Relationship between efficiency of photosynthetic energy conversion and chlorophyll fluorescence quenching in upland cotton (Gossypium hirsutum L.). Planta 178:367-376.



$$\varphi_{\mathrm{PS2}} = (F'_m - Fs)/F'_m = \Delta F/F'_m$$

$$ETR = \varphi_{\mathrm{PS2}} \cdot 0.5 \cdot I_o$$





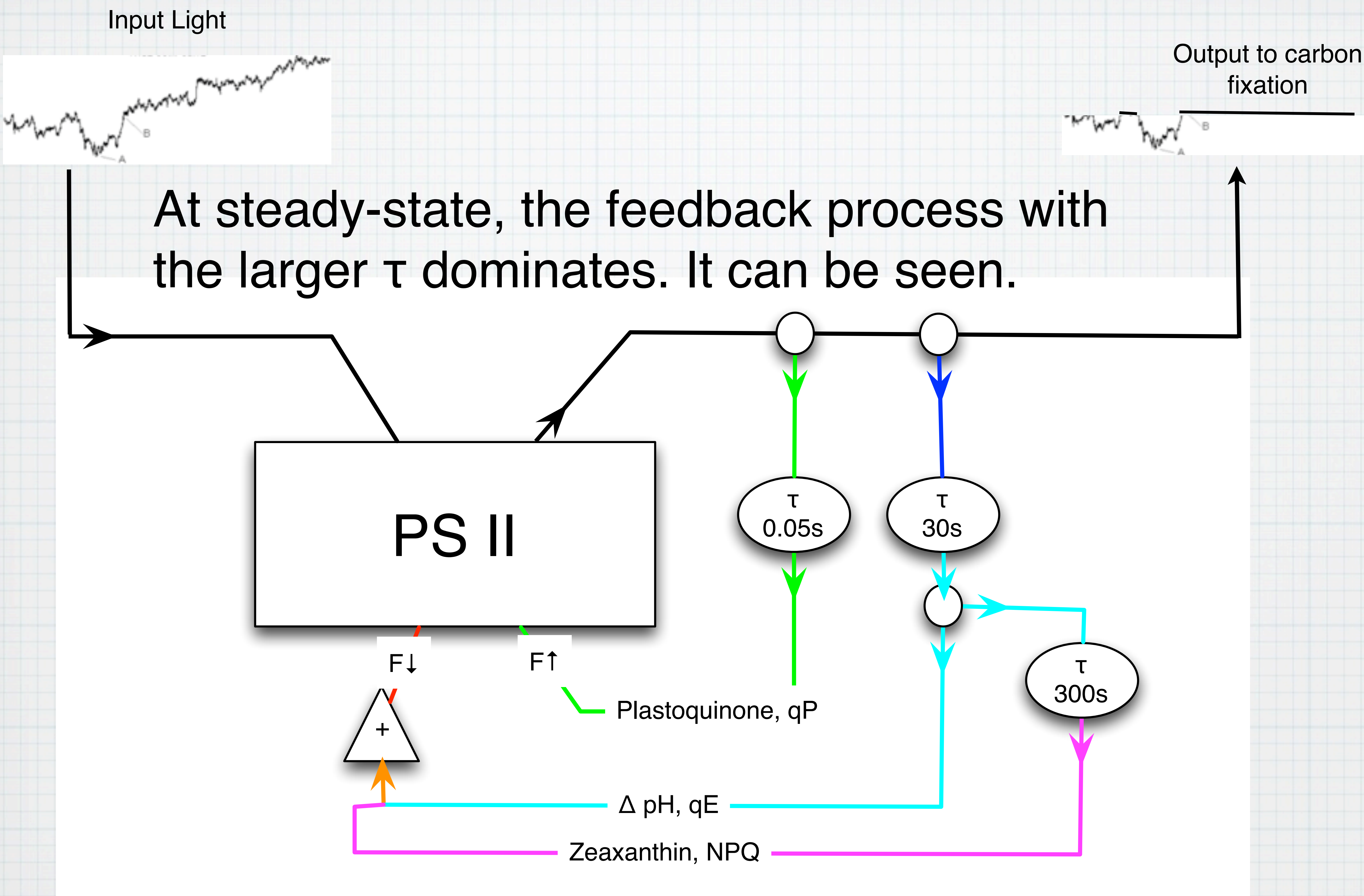
Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. **BBA - General Subjects**, 990(1), 87–92. doi:10.1016/S0304-4165(89)80016-9

- PAM fluorimeters can be used to calibrate F to the electron transport rate (ETR).

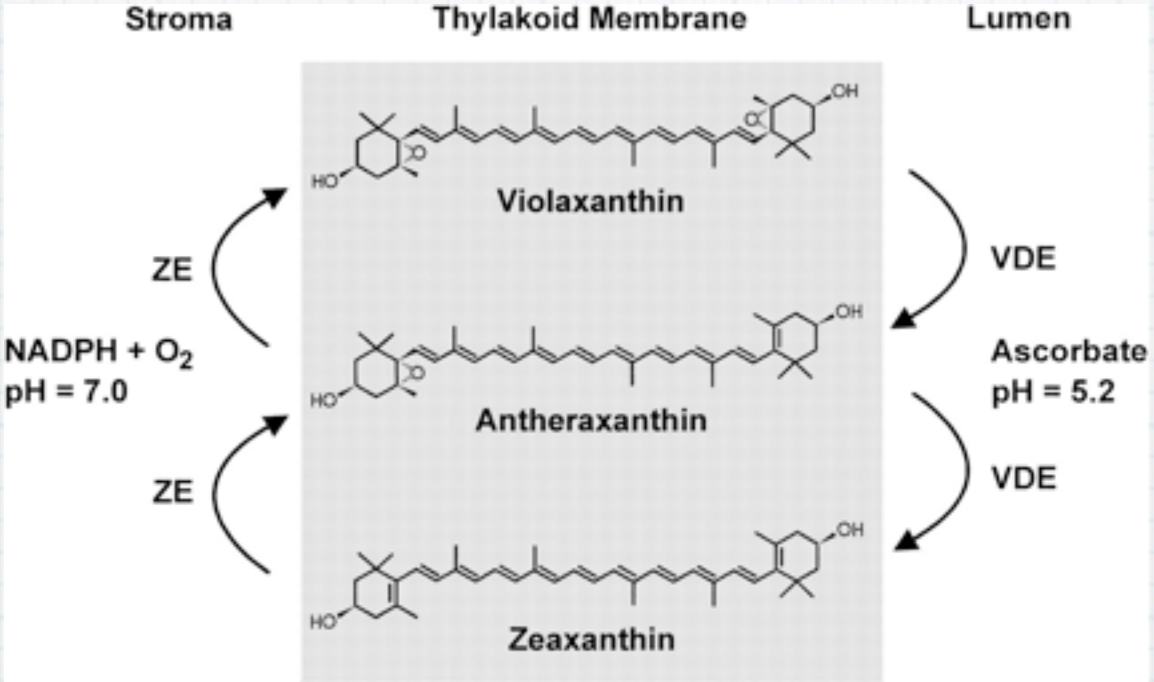
- Biochemical - stomatal conductance models can be used to relate ETR to CO_2 fixation.

- Some remaining problems:
    - At the canopy scale, changes in FPAR and fluorescence yield (θ_F) are entangled.
    - Canopy scale calibrations will be difficult for tall vegetation. Need to be several canopy heights above the canopy to reproduce the satellite geometry.
    - Recent advances in xanthophyll cycle remote sensing have caught my interest.

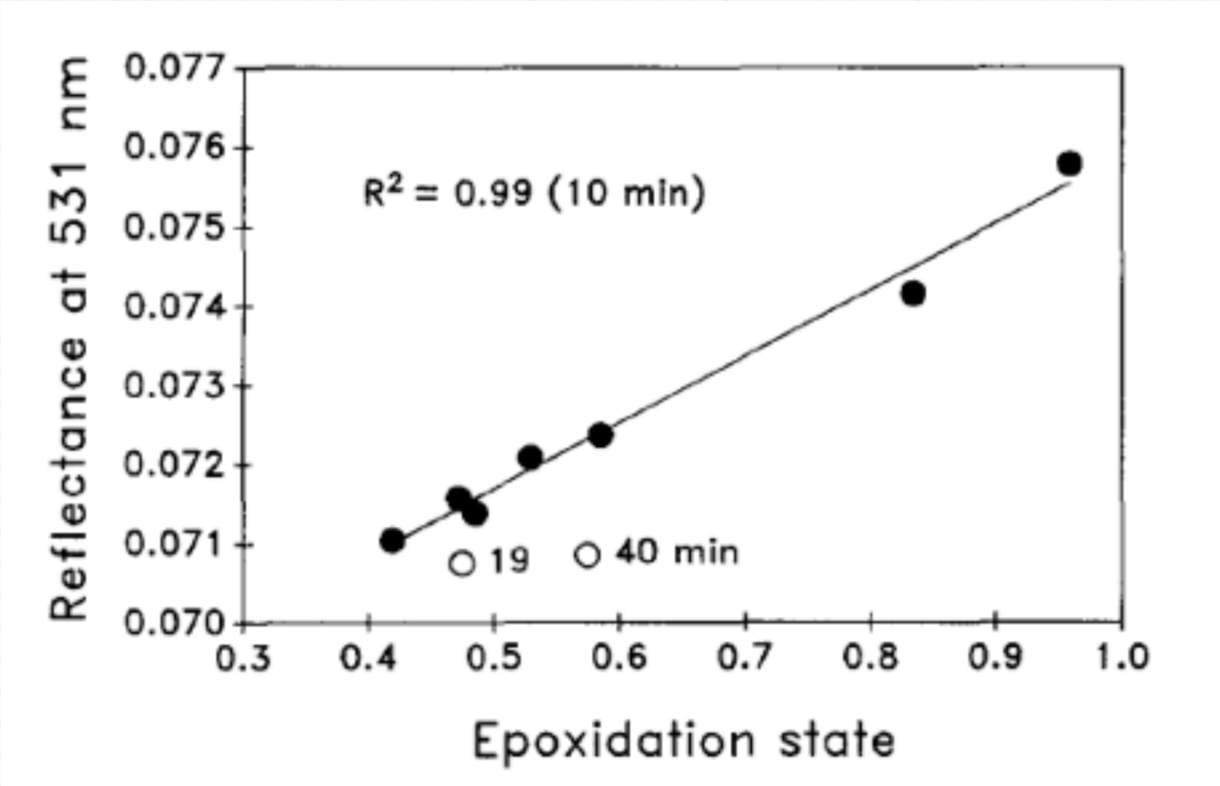
At steady-state, the feedback process with the larger τ dominates. It can be seen.



Remote sensing the xanthophyll cycle



$$\text{EPS} = (Z + 0.5A)/(V + A + Z)$$



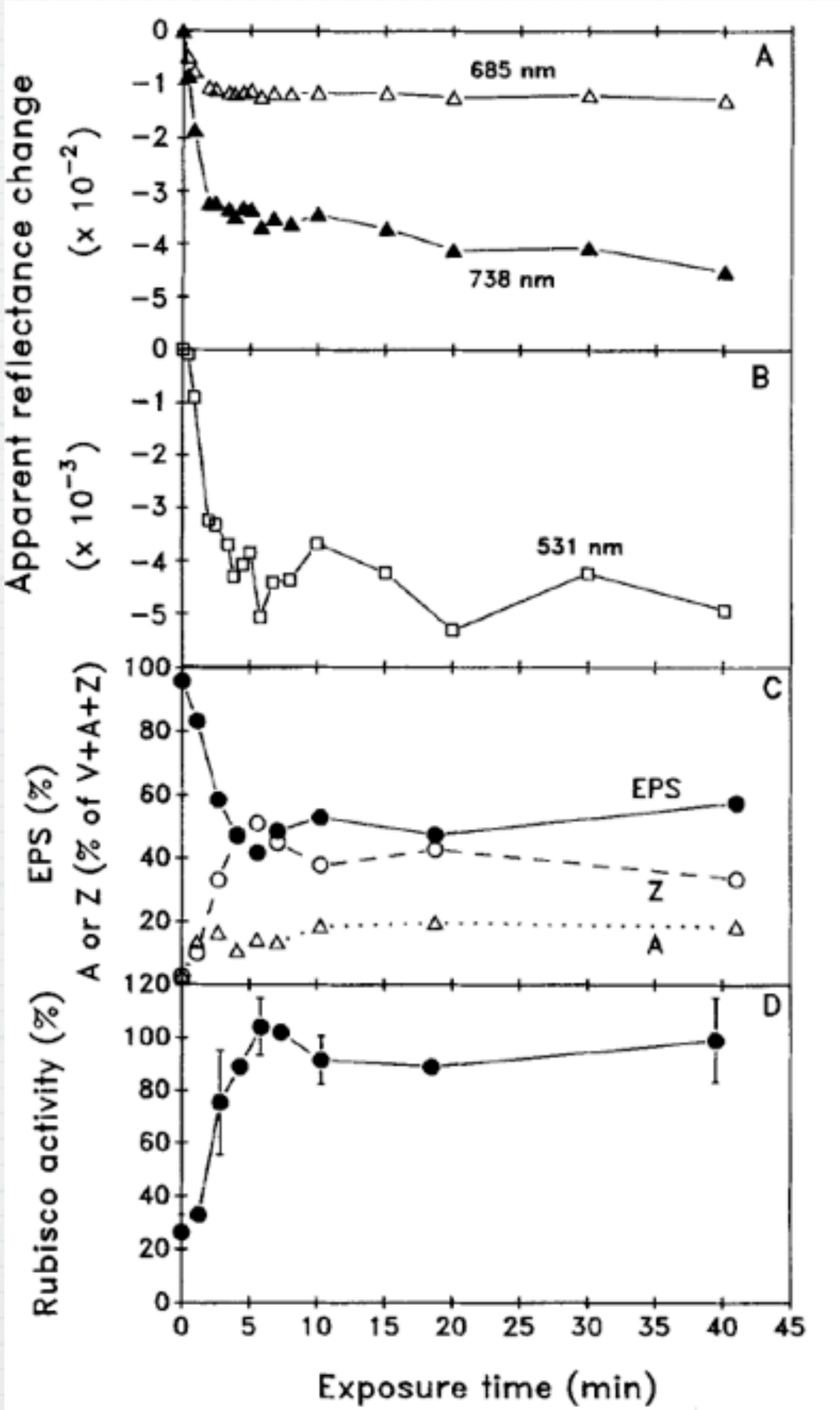
$$\text{PRI} = (R_{570} - R_{531})/(R_{570} + R_{531})$$

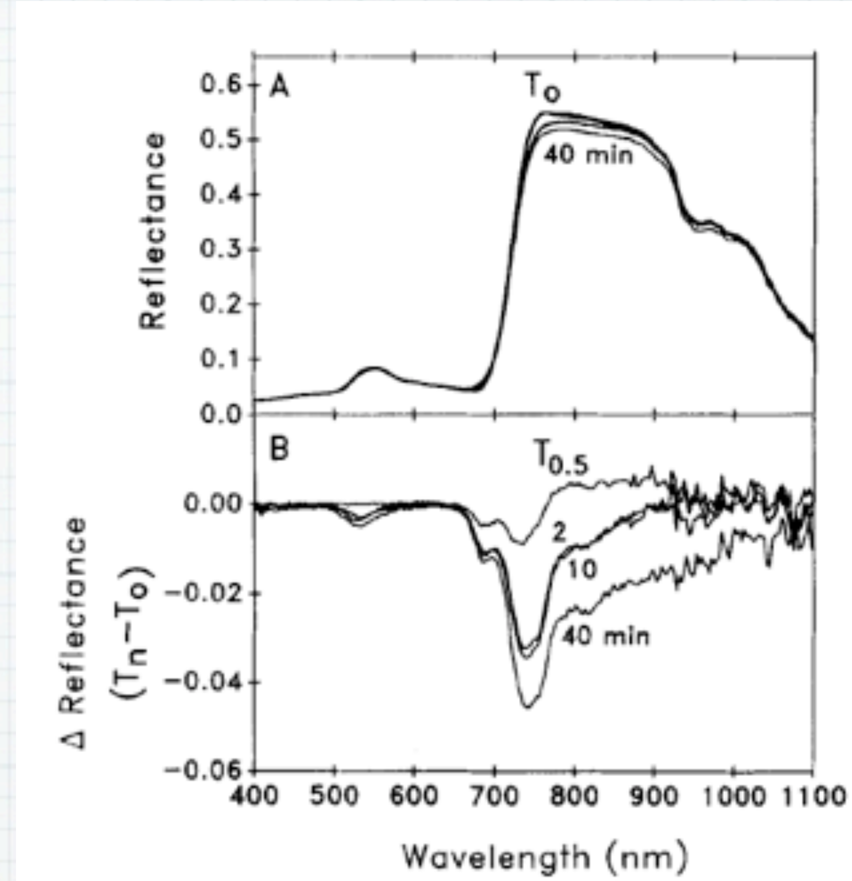

STANFORD

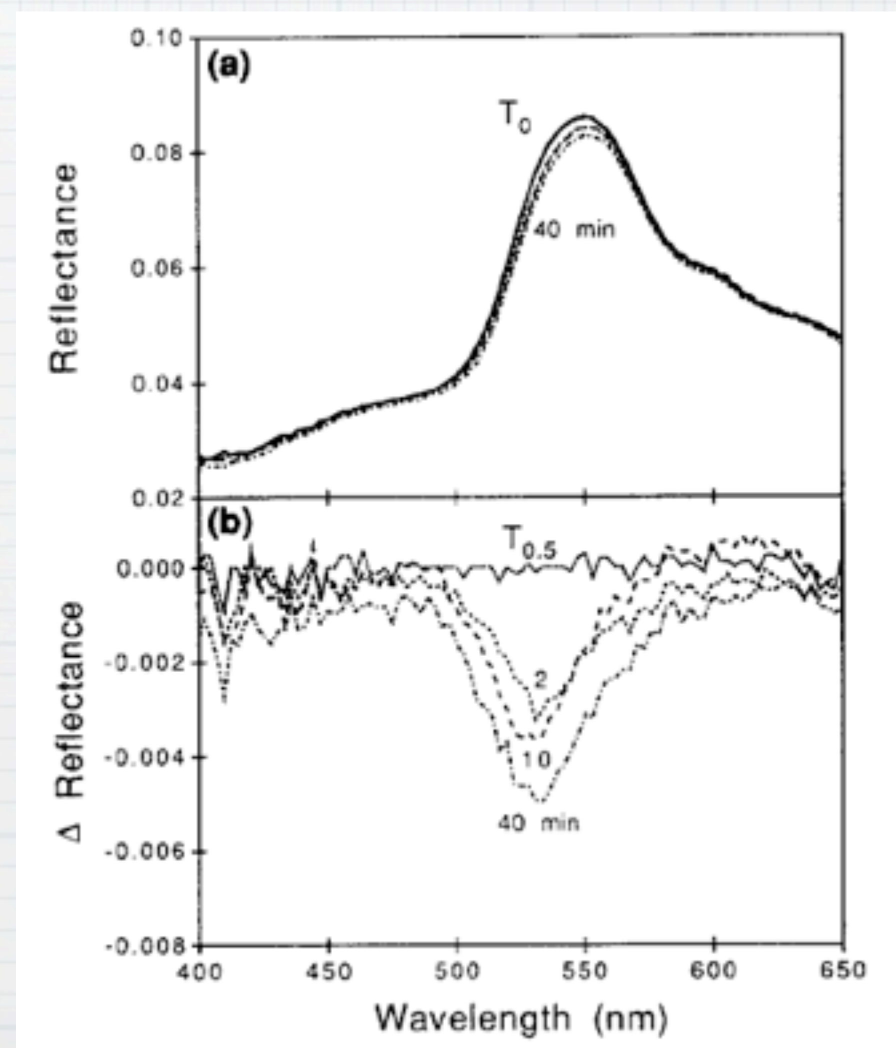
Fluorescence and Xanthophyll by Canopy Remote Sensing.

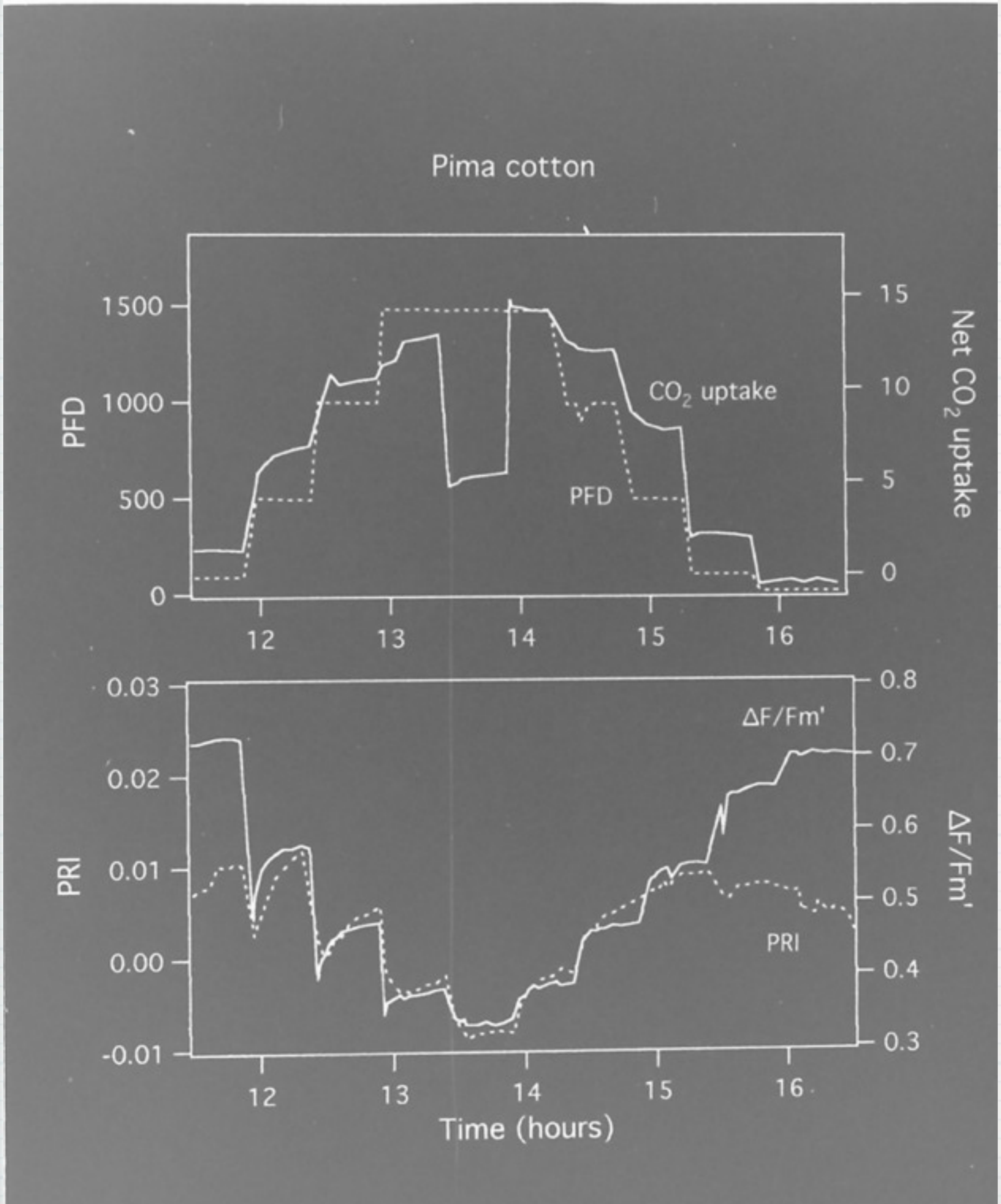
Gamon, J., Field, C., Bilger, W., Björkman, O., Fredeen, A., & Peñuelas, J. (1990). Remote sensing of the xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. **Oecologia**, 85(1), 1–7.

Gamon, J., & Penuelas, J. (1992). A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. **Remote Sensing of Environment** 41:35-44.

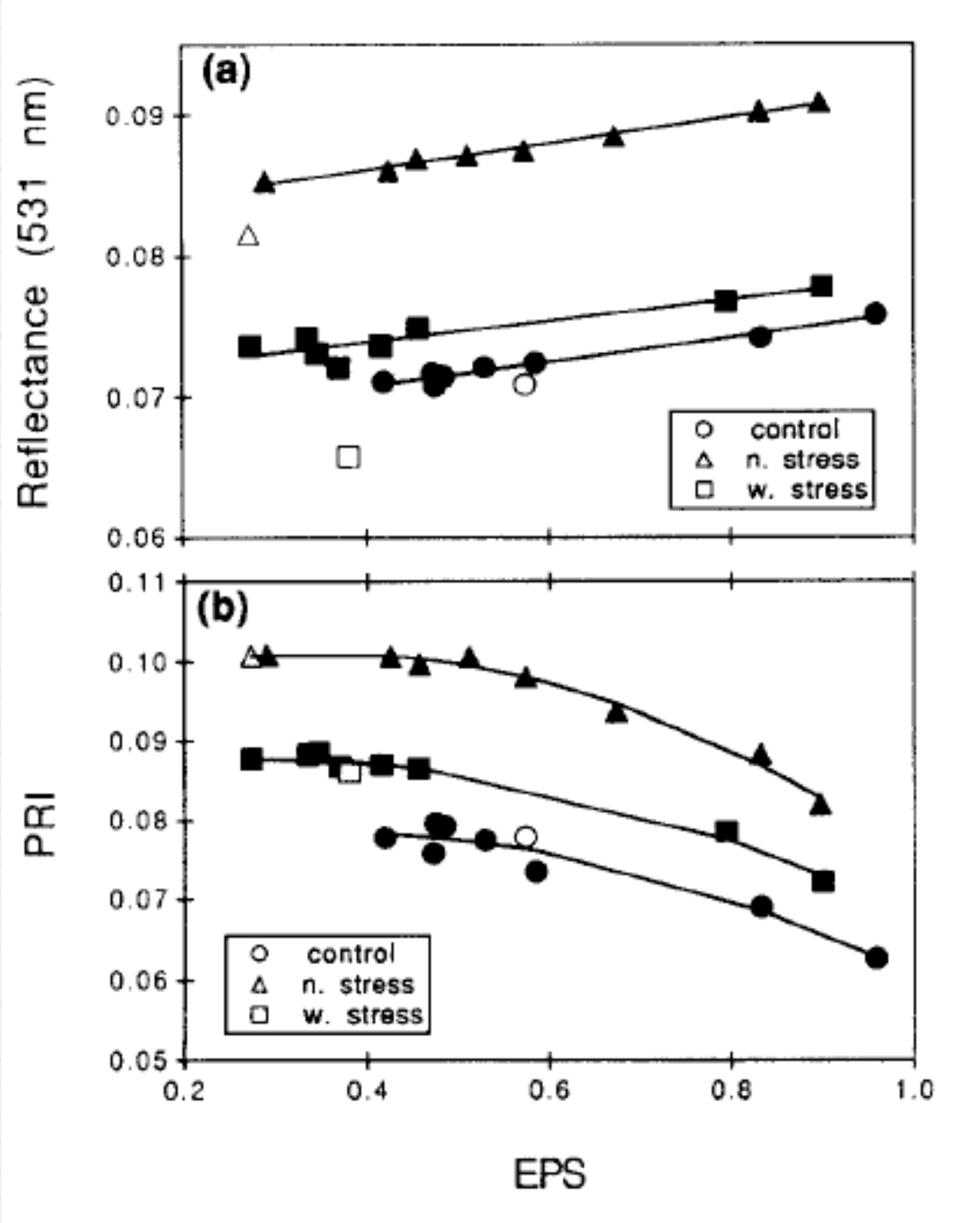

A
B
C
D
685 nm
738 nm
531 nm
EPS
Z
A
Apparent reflectance change
(x 10−2)
(x 10−3)
EPS (%)
A or Z (% of V+A+Z)
Rubisco activity (%)
Exposure time (min)

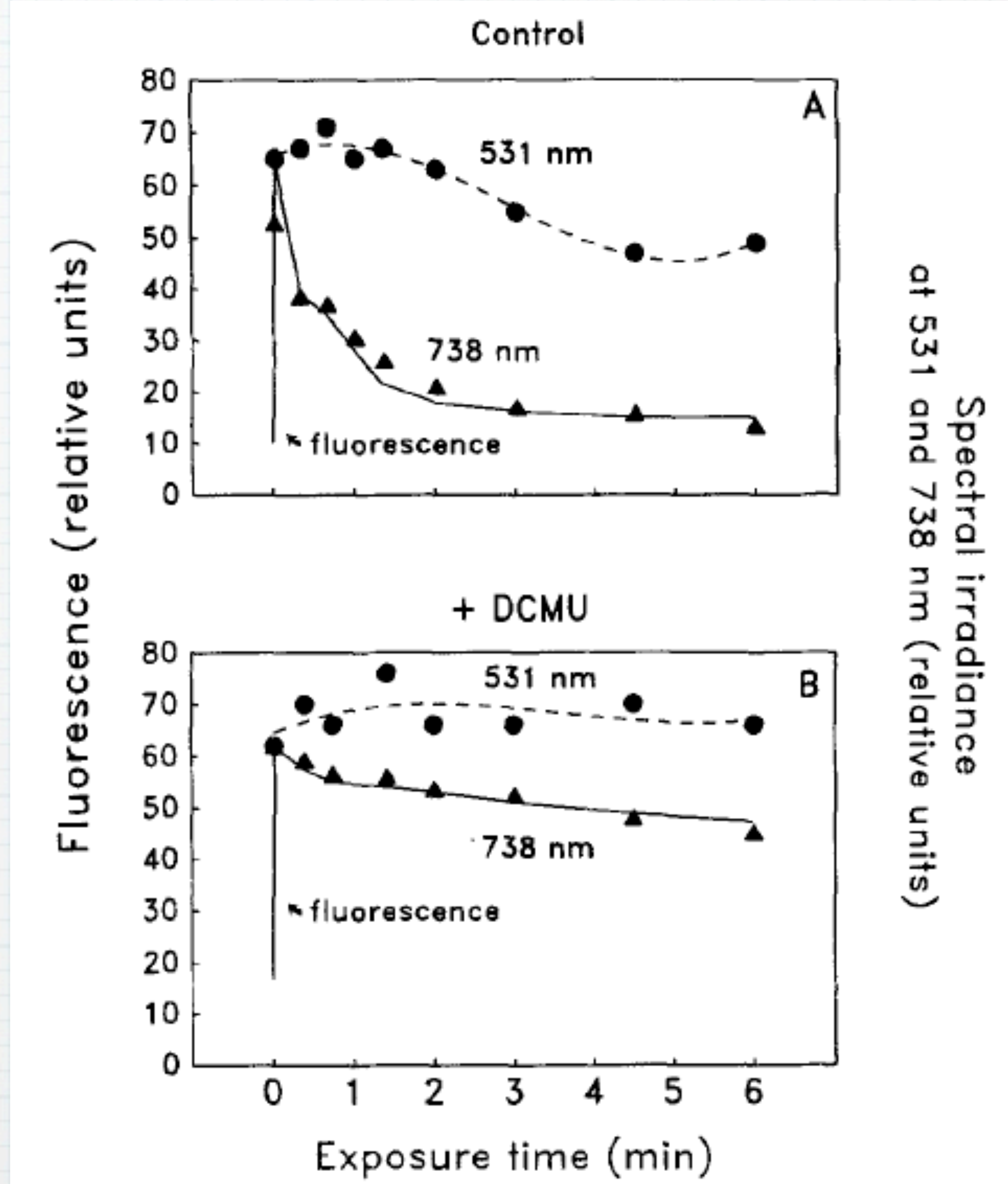

A
B
T0
40 min
T0.5
2
10
40 min
Reflectance
Δ Reflectance
(Tn−T0)
Wavelength (nm)


(a)
(b)
T0
40 min
T0.5
2
10
40 min
Reflectance
Δ Reflectance
Wavelength (nm)



Pima cotton
PFD
1500
1000
500
0
12
13
14
15
16
CO2 uptake
PFD
Net CO2 uptake
15
10
5
0
PRI
0.03
0.02
0.01
0.00
-0.01
ΔF/Fm'
PRI
0.8
0.7
0.6
0.5
0.4
0.3
Time (hours)





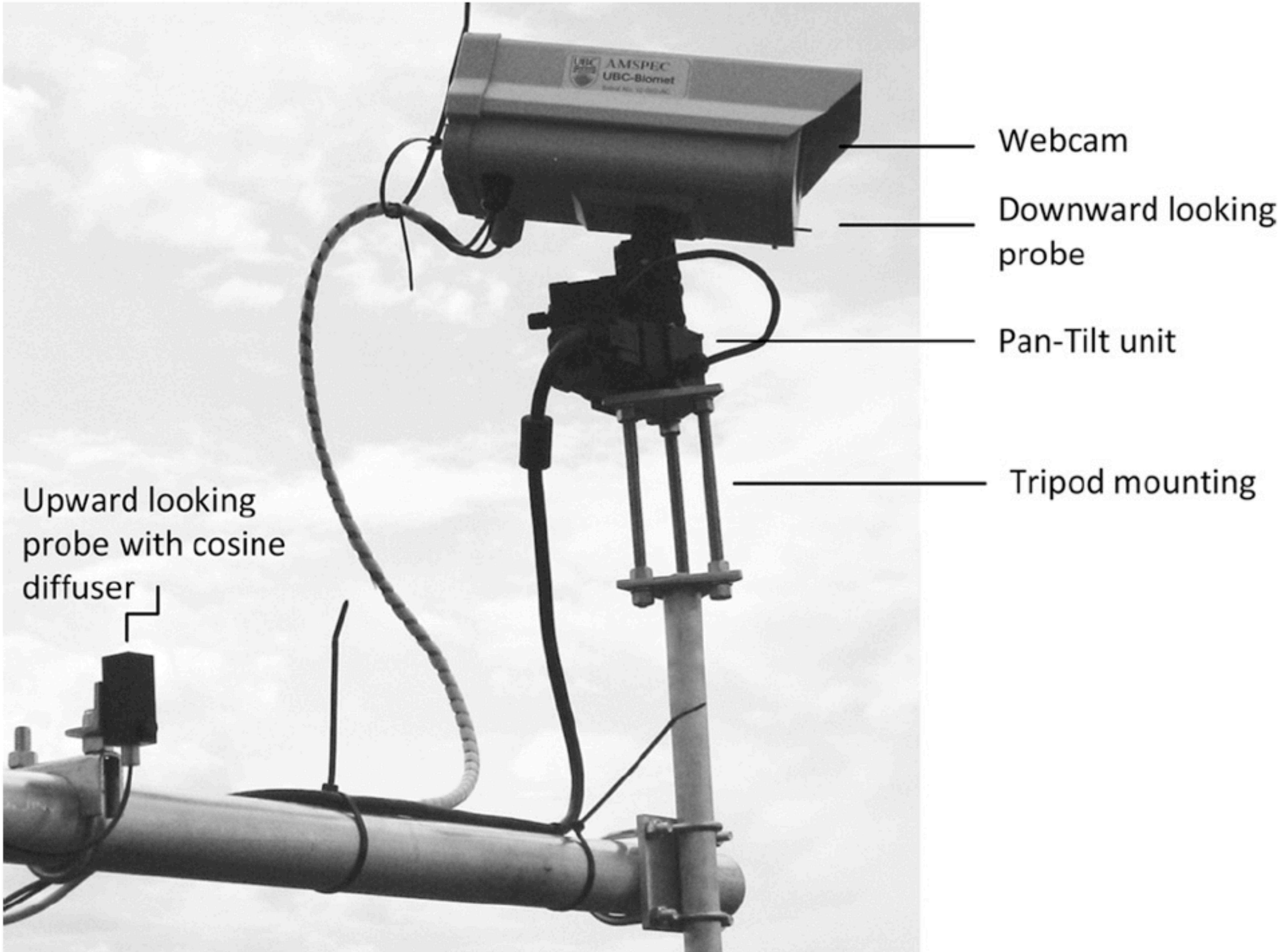
$$\mathrm{PRI} = (R_{570} - R_{531})/(R_{570} + R_{531})$$

- The PRI provides independent information on the level of non-photochemical quenching.

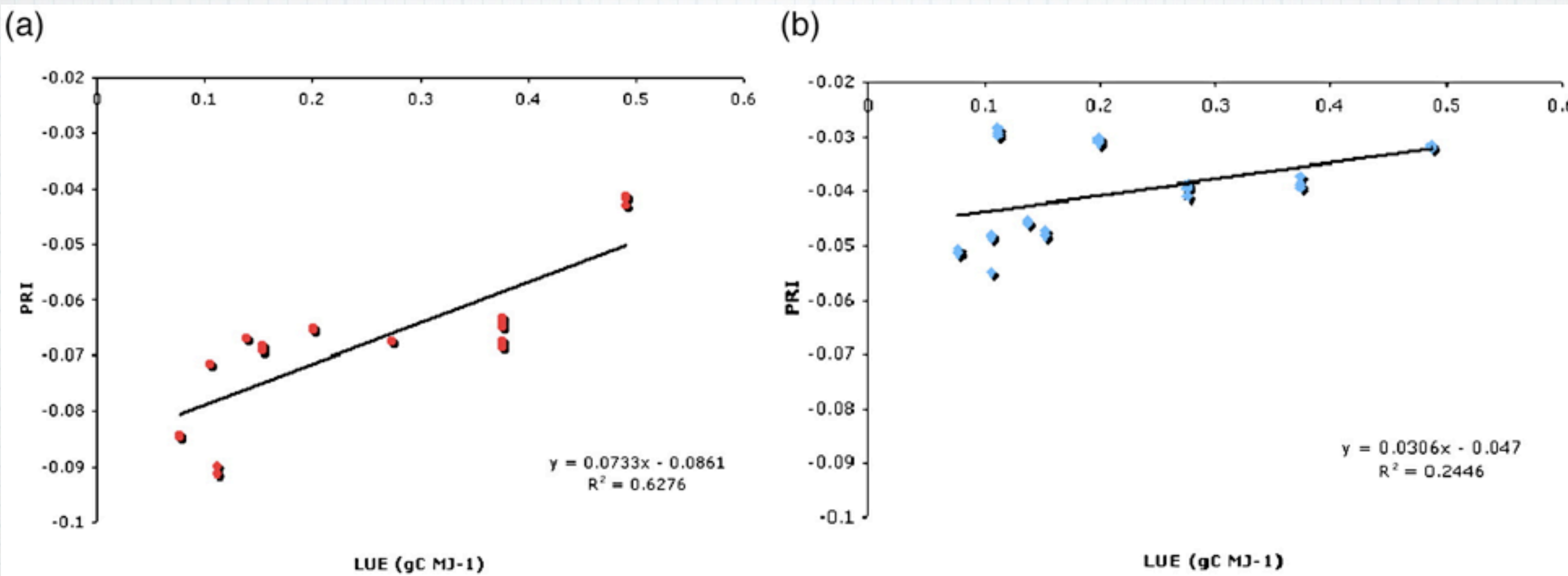
- It works best in “difference mode”. There is a lot of natural background variability in the reflectance in this region - cancels out in ΔPRI.

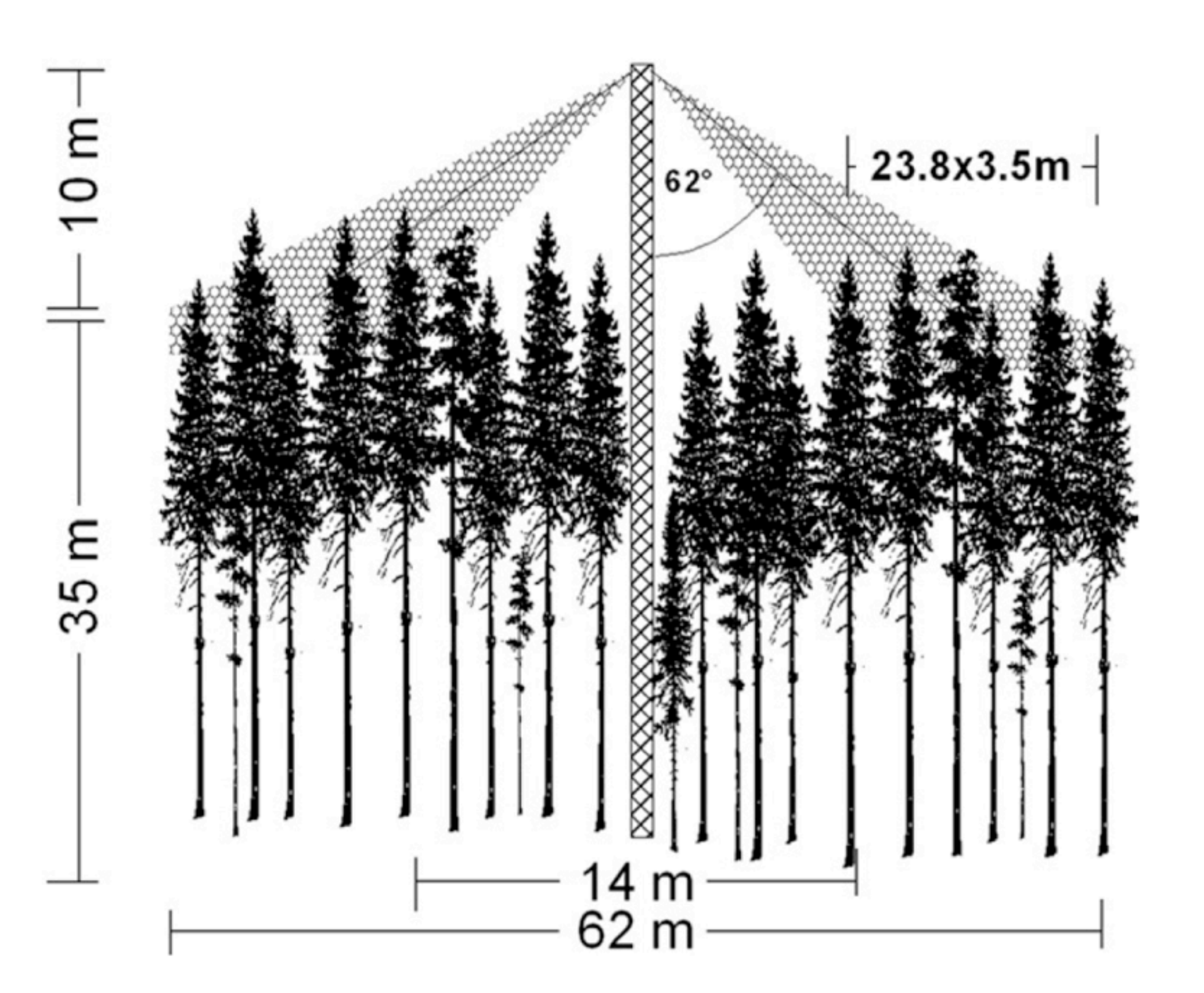
- (AMSPEC II) The tower-mounted, automated, multiangular spectroradiometer system takes advantage of changes in sun-leaf/shade-leaf fraction to get ΔPRI.

Hilker, T., Nesic, Z., Coops, N. C., & Lessard, D. (2010). A NEW, AUTOMATED, MULTIANGULAR RADIOMETER INSTRUMENT FOR TOWER-BASED OBSERVATIONS OF CANOPY REFLECTANCE (AMSPEC II). **Instrumentation Science & Technology**, 38(5), 319–340. doi: 10.1080/10739149.2010.508357


AMSPEC
UBC-Biomet
Webcam
Downward looking probe
Pan-Tilt unit
Tripod mounting
Upward looking probe with cosine diffuser

hot spot   cold spot




10 m
35 m
62°
23.8x3.5m
14 m
62 m

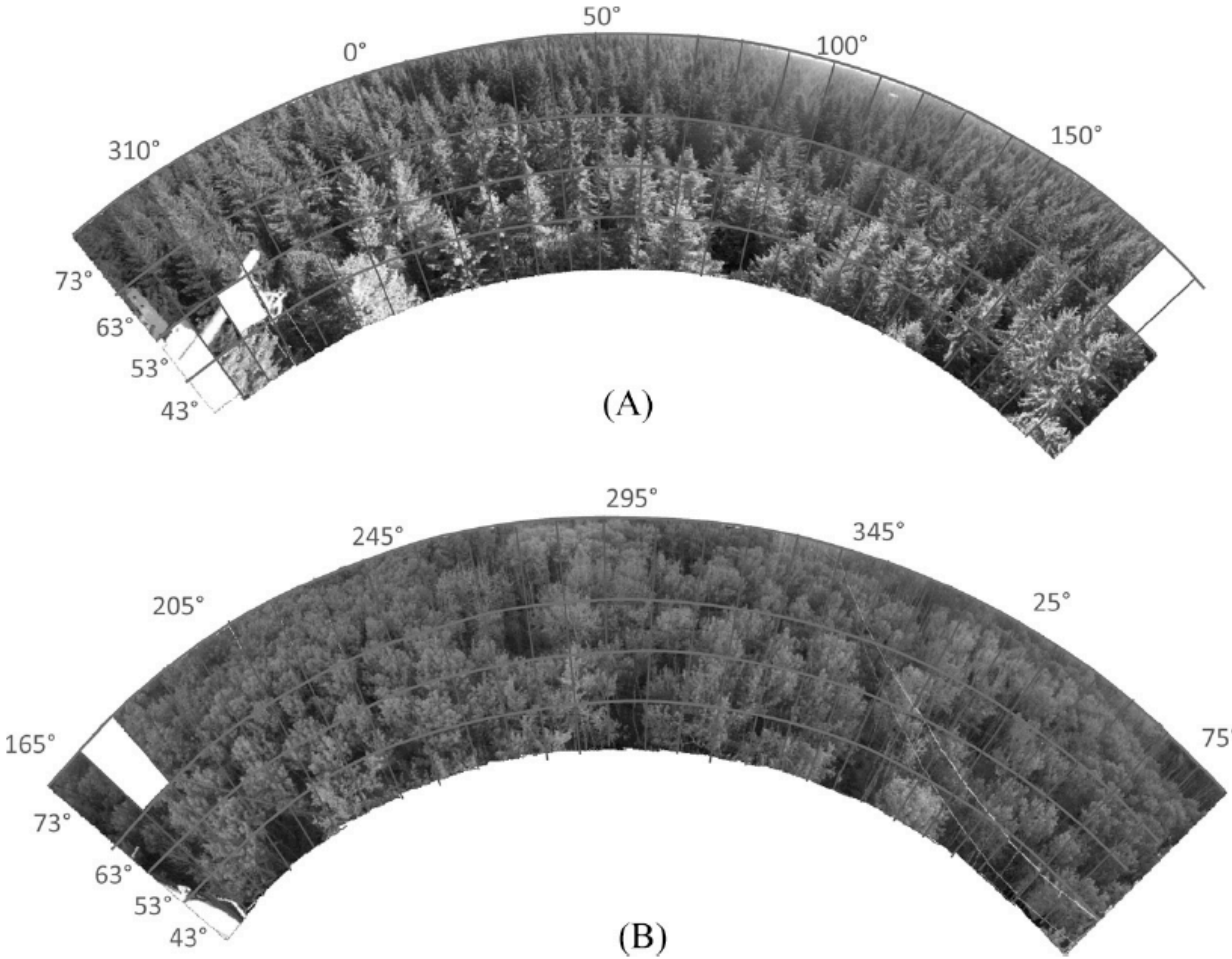


FIGURE 8 Image composites for DF-49 (A) and SOA (B), observed over 15-minute intervals. The photographs have been stitched from 104 (DF-49) and 108 (SOA) individual observations using a normalized cross-correlation approach.

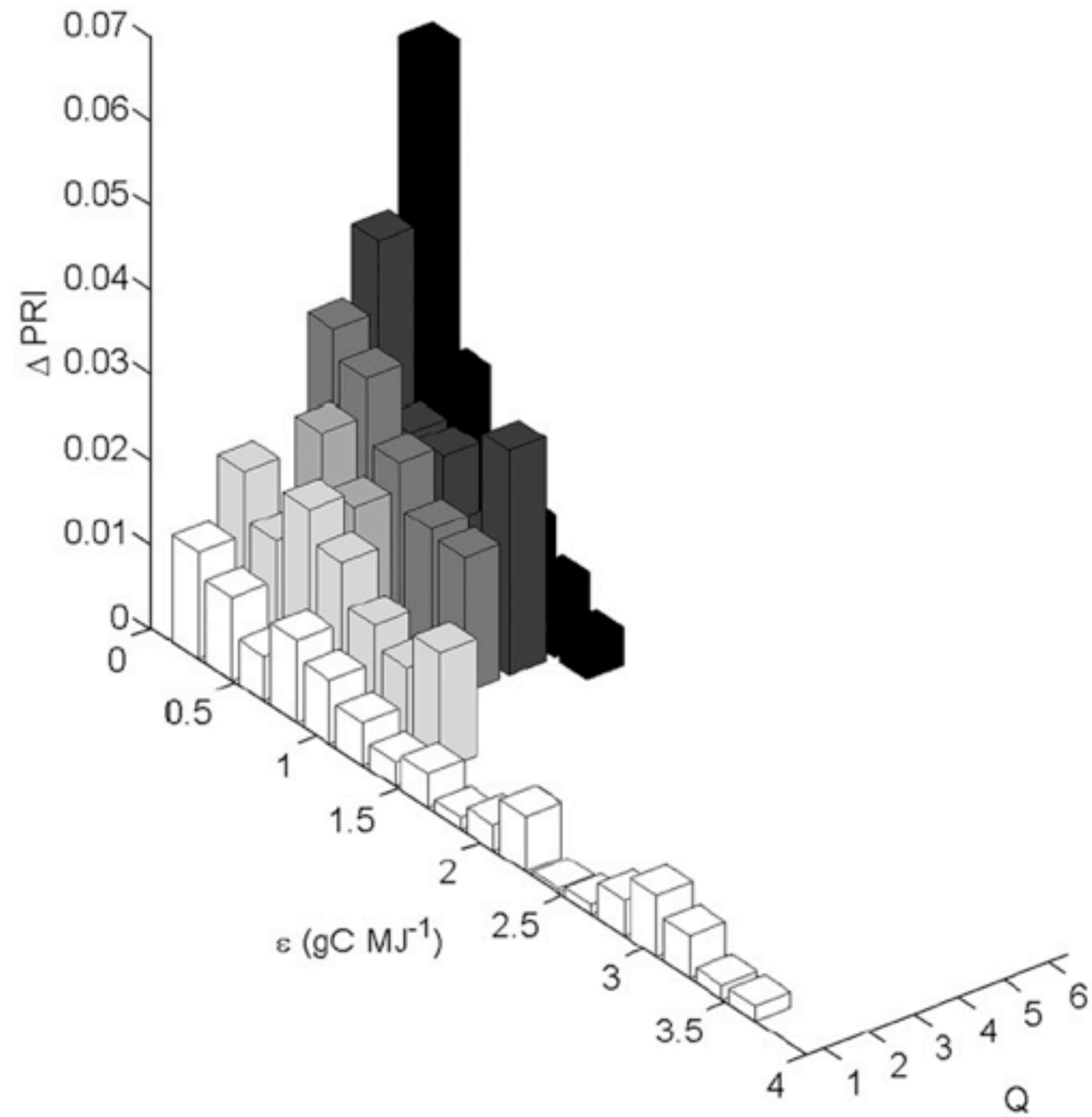
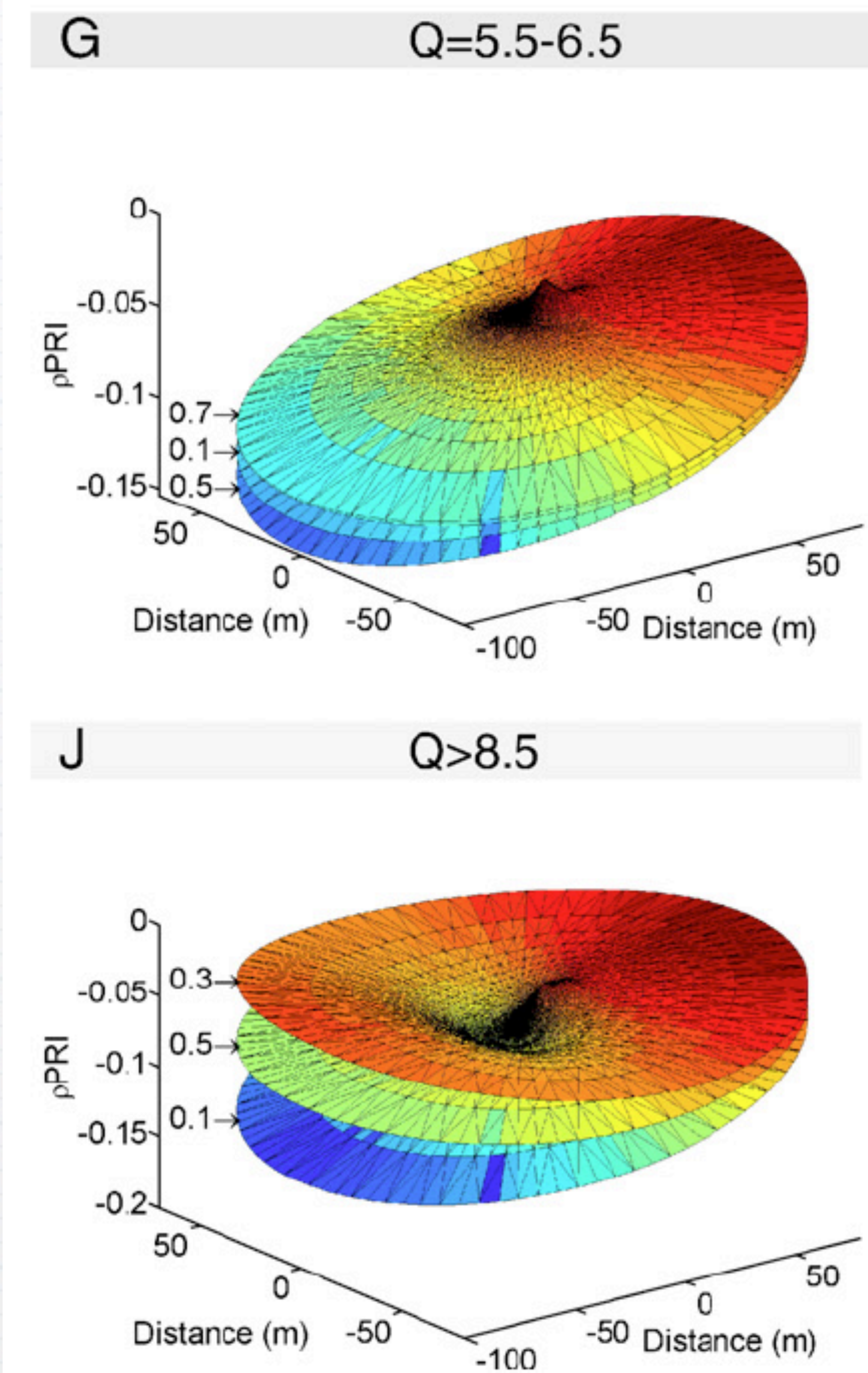


Fig. 5. Difference between maximum (south) and minimum (north) PRI (ΔPRI) for different ε and Q strata for the directionally corrected case (zenith angle of 62°). Higher stress levels (low ε) cause differences between sunlit and shaded parts of the canopy to be more distinct. Also ΔPRI is increasing with increasingly clear skies.



- Remote sensing of the PRI is potentially synergistic with sun induced fluorescence.
    - Fs is influenced both by changes in FPAR and physiological feedbacks on fluorescence yield.
    - PRI is largely influenced by the physiological component.
    - The AMSPEC measurements can be used to construct the full BRDF function for the canopy permitting one to predict what a satellite would see without having to reproduce the geometry.
- We should combine these measurements on the same tower-based sensor package.

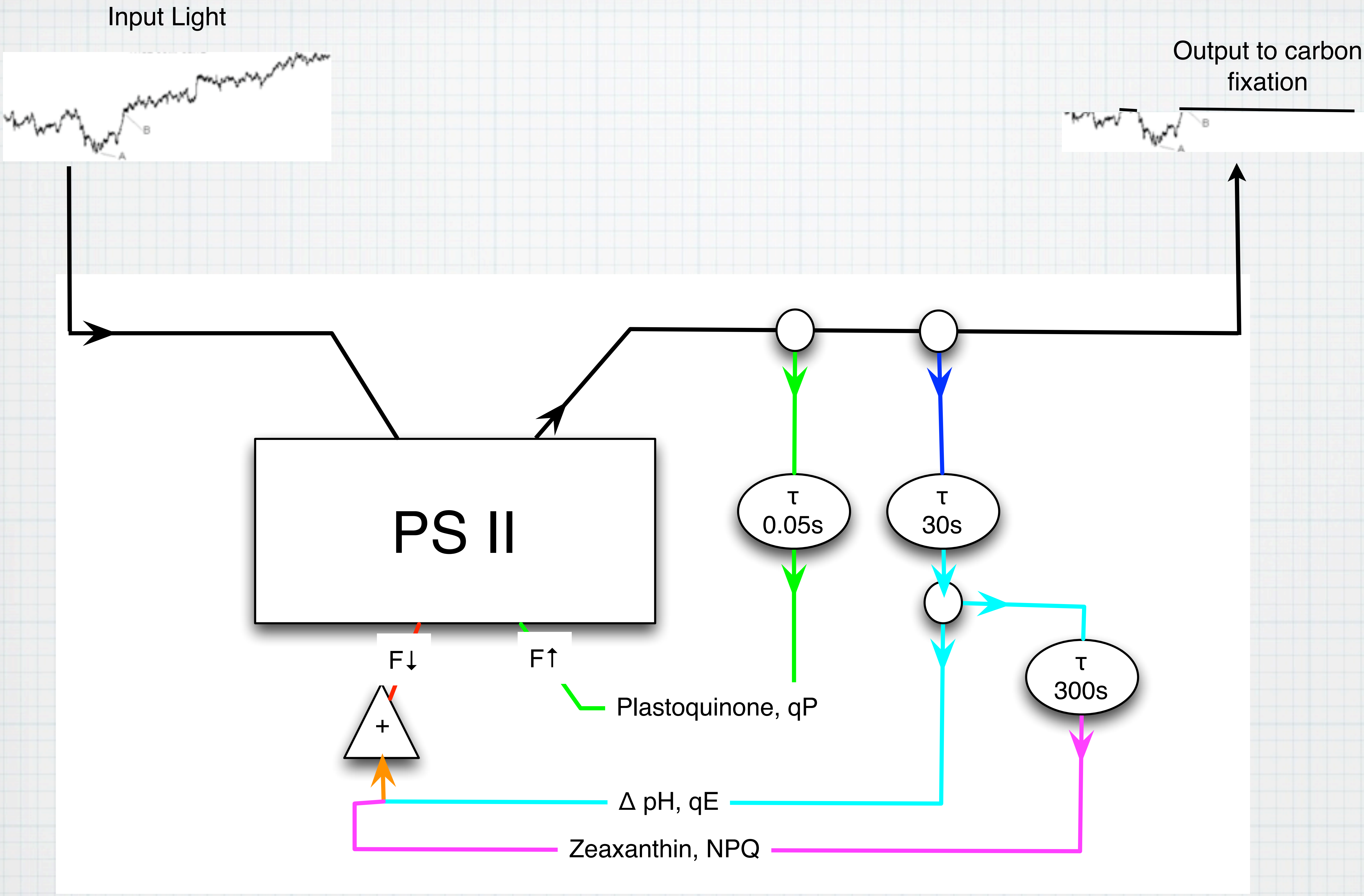
Conclusions

- There is strong empirical evidence that Fs gives useful information on the rate photosynthesis.

- It is sensitive to the combined influence of changes in canopy optics and physiology.

- Calibration and validation at the scale of the GOSAT measurement footprint is challenging, but we have a well developed theoretical understanding at the leaf scale - at least as good as we have for GPP.

- I can’t over emphasize the importance of an independent check on GPP. We have work to do.



Input Light
Output to carbon fixation
PS II
τ 0.05s
τ 30s
τ 300s
F↓
F↑
+
Plastoquinone, qP
Δ pH, qE
Zeaxanthin, NPQ

$$A \approx \min \begin{cases} J_E \\ J_C \\ J_S \end{cases}$$

C3

$$J_E = a \times \alpha \times Q_p \frac{p_i - \Gamma_*}{p_i + 2\Gamma_*}$$

$$J_C = \frac{V_m(p_i - \Gamma_*)}{p_i + K_c(1 + [O_2]/K_o)}$$

$$J_S = V_m/2$$

C4

$$J_i = a\alpha_r f Q_p$$

$$J_c = p_i\left(k_p - \frac{L}{p_i}\right)/P$$

$$J_e = V_{max}$$

$$\theta J_P^2 - J_P(J_E + J_C) + J_E J_C = 0$$

and

$$\beta A^2 - A(J_P + J_S) + J_P J_S = 0$$