Fluorescence and Photosynthesis

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• 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.

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).
Kautsky is the “father of the field”, but he also fostered the impression that fluorescence is very complicated.
Think of Photosystem 2 as a sophisticated IC.
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.
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,

\[
F_s = I_0 \cdot \text{FPAR} \cdot \theta_F \cdot \epsilon \\
\text{GPP} = I_0 \cdot \text{FPAR} \cdot \theta_P \\
\text{GPP} = F_s \frac{\theta_P}{\theta_F \cdot \epsilon}
\]
<table>
<thead>
<tr>
<th>Land Cover Type</th>
<th>Dataset</th>
<th>$r^2$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrubland</td>
<td>MPI-BGC</td>
<td>0.80</td>
</tr>
<tr>
<td>Deciduous broadleaf</td>
<td>MODIS</td>
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<tr>
<td>Evergreen broadleaf</td>
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</table>

**Additional Information:**

- **GPP** (gC/m²/d) vs. $F_s/(Wm^{-2} \mu m^{-1}sr^{-1})$ for different datasets.
- **LAI** ($r^2 = 0.64$) vs. $F_s/cos(SZA)/(Wm^{-2} \mu m^{-1}sr^{-1})$.
- **NDVI** ($r^2 = 0.46$) and **FPAR** ($r^2 = 0.46$) for visual analysis.

*Surface temperature in °C is represented by the color gradient.*
$F_{s} = I_{0} \cdot \text{FPAR} \cdot \theta_{F} \cdot \epsilon$

$\text{FPAR}$

$r^2 = 0.46$

Surface temperature / °C
\[ F_s = I_0 \cdot \text{FPAR} \cdot \theta_F \cdot \epsilon \]
\[
\frac{GPP}{F_s} = \frac{I_0 \cdot FPAR \cdot \theta_P}{\theta_F \cdot \epsilon}
\]

\[
GPP = F_s \frac{\theta_P}{\theta_F \cdot \epsilon}
\]

**MPI-BGC**

\[r^2 = 0.80\]

**MODIS**

\[r^2 = 0.74\]
• A large part of the variability is due to FPAR.
• Physiology also seems to have an influence; $\theta_F$ and $\theta_P$ 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 ($F_s$) versus PAR for three days: 214 no water stress, 243 maximal water stress effect, 248 after rainy days, and reversion of water stress.

Leaf-scale experiments with grapes experiencing different levels of drought

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

\[ J_e / J_o \] (actual/potential ETR) \( \approx \) \( \theta_P \)

\[ F_s = I_0 \cdot FPAR \cdot \theta_F \cdot \epsilon \]

Why does Fs go up and then down?

Input Light

Output ET

PS II

τ 0.05s

τ 30s

τ 300s

Δ pH, qE

Zeaxanthin, NPQ

Plastoquinone, qP

F↓

F↑
Relative fluorescence yield, $F$

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

$$x = \frac{J_o}{J_e}$$

$$J_e = A \cdot 4 \cdot \frac{p_i - \Gamma}{p_i + 2\Gamma}$$

(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

$\alpha$ is quantum yield,

$\Gamma^*$ 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.

Mechanism controlling fluorescence
The PAM Fluorimeter

[Diagram of PAM Fluorimeter components including LED Driver, Master Pulse Generator, Selective Amplifier, LED Emitter, Photodiode Detector, Pulse Amplifier, Current Pulses, Pulse Signals, Signal, Recording, Filter for λ < 680 nm and λ > 700 nm, Pulsed Measuring Beam, Actinic Light, and Leaf Sample.]
Controls of exciton processing

$\Delta p\text{H}$

xanthophyll cycle

plastoquinone
A hierarchy of controls with different relaxation times

Input Light

Output ET

PS II

F↓ F↑

Plastoquinone, qP

Δ pH, qE

Zeaxanthin, NPQ

\[ \tau \quad 0.05s \quad \tau \quad 30s \quad \tau \quad 300s \]
Intense pulses permit separation of qP from qE and NPQ

PS II

Input Light

Output to carbon fixation

ΔpH, qE

Zeaxanthin, NPQ

Plastoquinone, qP

F↓

F↑

τ 0.05s

τ 30s

τ 300s
\[ \varphi_{PS2} = \frac{(F'_m - F_s)}{F'_m} = \frac{\Delta F}{F'_m} \]

\[ ETR = \varphi_{PS2} \cdot 0.5 \cdot I_o \]

- 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 ($\theta_F$) 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 $\tau$ dominates. It can be seen.
Remote sensing the xanthophyll cycle

\[ \text{EPS} = \frac{(Z + 0.5A)}{(V + A + Z)} \]

\[ \text{PRI} = \frac{(R_{570} - R_{531})}{(R_{570} + R_{531})} \]
Fluorescence and Xanthophyll by Canopy Remote Sensing.


\[ \text{PRI} = \frac{R_{570} - R_{531}}{R_{570} + R_{531}} \]
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 \( \Delta \text{PRI} \).

(AMSPEC II) The tower-mounted, automated, multiangular spectroradiometer system takes advantage of changes in sun-leaf/shade-leaf fraction to get \( \Delta \text{PRI} \).

hot spot  cold spot

(a) $y = 0.0733x - 0.0861$
$R^2 = 0.6276$

(b) $y = 0.0306x - 0.047$
$R^2 = 0.2446$
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 ($\Delta$PRI) for different $\varepsilon$ and $Q$ strata for the directionally corrected case (zenith angle of 62°). Higher stress levels (low $\varepsilon$) cause differences between sunlit and shaded parts of the canopy to be more distinct. Also $\Delta$PRI 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

PS II

F↓  F↑

+ Δ pH, qE

Plastoquinone, qP

Zeaxanthin, NPQ

τ 0.05s

τ 30s

τ 300s

Output to carbon fixation

Monday, August 27, 12
\[ A \approx \min \left\{ \frac{J_E}{J_C}, \frac{J_C}{J_S} \right\} \]

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

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

\[ J_S = \frac{V_m}{2} \]

\[ J_i = a \alpha_r f Q_p \]

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

\[ J_e = V_{\text{max}} \]

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

and

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