A Deeper Look at Photosynthesis: Fluorescence Theory and Practice Using The LI-6400XT

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LI-COR BioSciences

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How do we measure photochemistry?

Gas exchange: CO$_2$ and H$_2$O

Chlorophyll Fluorescence
Chlorophyll Fluorescence

\[ F + H + P = 1 \]
The absorption spectrum of chlorophyll
\[ F + H + P = 1 \]  \hspace{1cm} (eq. 1)

At saturating light intensity:

No increase in P with further increase in light intensity and F & H maximum

\[ F = F_m, \ H = H_m, \ P = 0 \]  \hspace{1cm} (eq. 2)

\[ F_m + H_m + 0 = 1 \]  \hspace{1cm} (eq. 3)

\[ H_m = 1 - F_m \]  \hspace{1cm} (eq. 4)

If we assume the ratio of heat to fluorescence de-excitation does not change,

\[ \frac{H}{F} = \frac{H_m}{F_m} \]  \hspace{1cm} (eq. 5)

\[ H = \frac{F(1-F_m)}{F_m} \]  \hspace{1cm} (eq. 6)

We can solve for H & P if we measure F in non-saturating light (F) and saturating light (Fm)

\[ P = 1-F-H \]  \hspace{1cm} (eq. 7)

\[ P = 1 - F - \left[ \frac{F(1-F_m)}{F_m} \right] \]  \hspace{1cm} (eq. 8)

\[ P = \frac{F_m-F}{F_m} \]  \hspace{1cm} (eq. 9)
\[ P = \frac{F_m - F}{F_m} \]

\[ P_{dark} = \frac{F_m - F_o}{F_m} = \frac{F_v}{F_m} \]

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

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>( \alpha_{blue} )</th>
<th>( \alpha_{red} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>0.90</td>
<td>0.85</td>
</tr>
<tr>
<td>Bean</td>
<td>0.91</td>
<td>0.83</td>
</tr>
<tr>
<td>Jasmine</td>
<td>0.92</td>
<td>0.87</td>
</tr>
<tr>
<td>Orange</td>
<td>0.94</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications

E.H. Murchie¹,* and T. Lawson²
modulated LED

650 nm short pass filter

pulsed-fluorescence (~630-850 nm)

700 nm long pass filter

detector

electronics

scattered light

RG-9

electronics

Transmittance: Internal Transmittance
Pulsed amplitude modulation fluorescence

F(m) (minimum fluorescence yield)

Fm’ (maximum fluorescence yield)

modulated fluorescence

low intensity modulated light

PSII

2H$_2$O $\rightarrow$ O$_2$ + 4e$^- + 4H^+$

high intensity light flash
LCF Design

- Red (630nm), Blue (470nm), Far red (740nm) LED’s
- Fluorescence Detection at 715nm
Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity

S. D. LORIAUX¹, T. J. AVENSON¹, J. M. WELLES¹, D. K. MCDERMITT¹, R. D. ECKLES¹, B. RIENSCHEN¹ & B. GENTY²,³,⁴

¹LI-COR Biosciences, 4647 Superior Street, Lincoln, NE, USA, ²CEA, DSV, IBEB, Laboratoire d’Ecophysiologie Moléculaire des Plantes, 13108 Saint-Paul-lez-Durance, France, ³CNRS, UMR 7265 Biologie Végétale et Microbiologie Environnementales, 13108 Saint-Paul-lez-Durance, France and ⁴Université Aix-Marseille, 13108 Saint-Paul-lez-Durance, France
\[
\frac{(Fm - Fm')}{Fm'} = NPQ \\
\frac{(Fm'' - Fm')}{Fm'} \times \frac{Fs}{Fm'} = \Phi_{qE} \\
\Phi_{PSII} = \frac{(Fm' - F)}{Fm'} \\
\Phi_{qE} \text{ vs. ETR} \\
C_c \rightarrow A_N \rightarrow V_{cmax} \rightarrow \text{climate modeling}
\]
\[
\frac{(Fm'-Fm')}{Fm'} = NPQ
\]

\[
\Phi_{PSII} = \frac{(Fm'-F)}{Fm'}
\]

\[
\frac{(Fm''-Fm')}{Fm'} \times \frac{Fs}{Fm'} = \Phi_{qE}
\]

\[
g_m = \frac{A}{C_i - \Gamma^* [ETR + 8 (A + R_d) \frac{ETR - 4 (A + R_d)}{ETR - 4 (A + R_d)}]}
\]

\[
\Phi_{qE} vs. ETR
\]

\[
E_{TR} = (\Phi_{PSII} * i * \alpha * f_{II})
\]

\[
\text{ETR vs. } A_G
\]

\[
C_c \rightarrow A_N \rightarrow V_{cmax} \rightarrow \text{climate modeling}
\]
Underestimation of Fm’ can be inferred

- Hypothetical saturation of $A^{\text{Fm'}}$
- Approaching saturation

Apparent Fm’ or $A^{\text{Fm'}}$

- Infinite irradiance
- 0.5 to 1 second

Extrapolated Fm’ ($E^{\text{Fm'}}$) or true Fm’
Multiphase Flash™ fluorescence

- Used to measure $F_m'$ at infinite irradiance

**Graph:**
- **Phase 1:** 60% Ramp
- **Phase 2:** 500 ms
- **Phase 3:**

**Equation:**
$$
\Phi_F(\text{Phase 2}) = \frac{1}{\text{Phase 2}_{\text{irradiance}}} (\text{m}^2 \text{ s mol}^{-1}) \times 10^4
$$

Ramp rate = mol photons m$^{-2}$ s$^{-2}$

Extrapolated $F_m'$ ~ true $F_m'$
Underestimates of $F_m$ result in underestimates of electron transport.

**MultiPhase Flash Method**

$$y_0 = 10.2 \pm 8.9 \quad b = 4.7 \pm 0.2$$

**Rectangular Flash Method**

$$y_0 = 38.7 \pm 7.7 \quad b = 2.9 \pm 0.2$$
Exploring deeper using gas exchange and fluorescence

\[ g_m = \frac{A}{C_i} \left( \frac{1}{(J+8(A+R_d))} \right) \]

\[ J = \frac{4(A+R_d)}{J} \]

‘J’ corresponds to an estimate of electron transfer obtained by chlorophyll fluorescence
# Fluorometer Display Changes

## Display Lines

<table>
<thead>
<tr>
<th>New</th>
<th>g</th>
<th>n</th>
<th>o</th>
<th>p</th>
<th>q</th>
<th>r</th>
<th>s</th>
<th>t</th>
<th>u</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prss_kPa</td>
<td>F</td>
<td>Fo</td>
<td>Fo'</td>
<td>PhiPS2</td>
<td>Adark</td>
<td>F</td>
<td>FlrMax</td>
<td>FlrMin</td>
</tr>
<tr>
<td></td>
<td>ParIn_mm</td>
<td>dF/dt</td>
<td>Fm</td>
<td>Fm'</td>
<td>ETR</td>
<td>LeafAbs</td>
<td>M:Int kHz Hz</td>
<td>RF Dur Int kHz Hz</td>
<td>D:Dur Far Bfr Aft kHz Hz</td>
</tr>
<tr>
<td></td>
<td>%Blue</td>
<td>FlrEvent</td>
<td>Fv/Fm</td>
<td>Fv'/Fm'</td>
<td>qP</td>
<td>PS2/1</td>
<td>Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ParOut_mm</td>
<td>Fs</td>
<td>Fs</td>
<td>Fs</td>
<td>qN</td>
<td>PhiCO2</td>
<td>Gn</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fluorometer Function Key Changes

New

Actinic Off

Actinic On

Fct Keys

8

Flr Import
Flr Editor
Define Actinic
Msr Adjust
Rcrding OFF

9

Meas is ON
Flash
Dark Pulse
Actinic is OFF
FarRed is OFF

0

Do Fo
Do Fm
Do FoFm

0

Do Fo'
Do Fs
Do FsFm'

View Fsh/Drk

View Fsh/Drk
Fluorescence Instrument Basics

• Higher fluorescence emission, better signal: noise
• Higher excitation intensities, higher fluorescence emission
• Higher excitation frequencies, higher excitation intensity
• Calculated parameters like Fv/Fm are not highly influenced by fluorescence emission intensities (they are unitless)
• To compare across time, emission intensities do matter
## Fluorometer Settings

<table>
<thead>
<tr>
<th>Node (Adjust Abbrev)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>intensity (Int)</strong></td>
<td>Intensity (0 to 10) of the 2 red measuring LEDs. Typical value is 3. You can enter floating point values (e.g. 3.3) if you wish - they don’t have to be integers. Effective resolution is 0.1.</td>
</tr>
<tr>
<td><strong>rate (kHz)</strong></td>
<td>Modulation used normally (i.e. not in a saturating flash or a dark pulse). (0.25, 1, 10, or 20 kHz). Always use 0.25 kHz for dark-adapted leaves.</td>
</tr>
<tr>
<td><strong>filter (Hz)</strong></td>
<td>Averaging done on the measurement signal normally (i.e. not during saturating flash or dark pulse). Specify bandwidth from one of the following (0.5, 1, 5, 10, 20, 50, 100, or 200 Hz). Typical setting is 5.</td>
</tr>
<tr>
<td><strong>gain (Gn)</strong></td>
<td>Gain factor for the fluorescence signal (10, 20, 50, or 100). Use 10.</td>
</tr>
</tbody>
</table>
## Fluorometer Settings

Table 27-6. Fluorescence Saturating Pulse (Flash) Parameters

<table>
<thead>
<tr>
<th>Editor Label</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>type</td>
<td>Rectangular or MultiPhase flash. See <em>Saturating Flashes</em> on page 27-21.</td>
</tr>
<tr>
<td>duration</td>
<td>Length of the saturating flash if Rectangular. Keep it between 0.3 and 2.0 seconds.</td>
</tr>
<tr>
<td>ramp</td>
<td>Percent ramp if MultiPhase flash.</td>
</tr>
<tr>
<td>phase1, phase2, phase3</td>
<td>Timing (ms) of the three phases of the MultiPhase flash.</td>
</tr>
<tr>
<td>target</td>
<td>Saturating flash intensity. Typically, 10 will be 6000 or more $\mu$mol m$^{-2}$ s$^{-1}$.</td>
</tr>
<tr>
<td>rate</td>
<td>Fluorescence modulation to use during the flash. Always use 20 kHz.</td>
</tr>
<tr>
<td>filter</td>
<td>Averaging to do during the flash. 20 Hz, typically.</td>
</tr>
<tr>
<td>autosave</td>
<td>Saves the flash to a file in the /User/LCF folder.</td>
</tr>
</tbody>
</table>
# Fluorometer Settings

Table 27-9. Suggested settings for determining $F_v / F_m$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suggested Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>1</td>
<td>If too high, it can drive photosynthesis. See also <strong>Optimum Meas Intensity</strong> on page 27-78.</td>
</tr>
<tr>
<td>Modulation</td>
<td>0.25</td>
<td>Always use 0.25 kHz for dark adapted leaves</td>
</tr>
<tr>
<td>Filter</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gain</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Flash</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Rectangular</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>0.8</td>
<td>0.5 to 0.8 is typical</td>
</tr>
<tr>
<td>Intensity</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Modulation</td>
<td>20</td>
<td>Always use 20 kHz</td>
</tr>
<tr>
<td>Filter</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
5 Do Fv/Fm
After $F$ has stabilized, press Do Fv/Fm (f3, level 0). The status LEDs on the 37-pin connector will flash before and after the saturation flash, and a beep will sound when the data is logged (if you’ve opened a log file). Note the values of $F_o$, $F_m$, and $F_v/F_m$ on display line n. Is $F_v/F_m$ reasonable (0.75 to 0.85)? If it is not, it may be due to inadequate dark adaptation, or inappropriate fluorescence measurement settings.

6 View the flash details
Press View fsh/drk (f5 level 0) followed by view graph (f1) to view the flash. The detailed data collected at 20 Hz during the flash will be plotted versus time. Looking at the details of the flash makes it easier to determine if the settings were appropriate, and if the leaf material was adequately dark-adapted. Examples of “good” and “suspect” flashes are shown in Figure 27-45. Note that there is an automated way of determining the appropriate flash setting. See Optimum Flash Intensity on page 27-77.

Figure 27-45. A dark-adapted philodendron measured with two different flash settings. A) Example of a good saturation flash: flash length and intensity are appropriate for this leaf material. B) Example of a poor saturation flash: flash length was too short and intensity too small. $F_v/F_m$ was underestimated by 5%.
Table 27-10. Suggested settings for determining $\Delta F / F_m$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suggested Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>5</td>
<td>This setting can be higher than for dark-adapted leaves, because we don’t have to worry about the light becoming actinic.</td>
</tr>
<tr>
<td>Modulation</td>
<td>20 kHz</td>
<td>10 or 20 should be fine. Again, it can be higher than for dark-adapted leaves.</td>
</tr>
<tr>
<td>Filter</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gain</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Flash</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Rectangular</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>0.8</td>
<td>0.5 to 0.8 is typical</td>
</tr>
<tr>
<td>Intensity</td>
<td>8</td>
<td>See Optimum Flash Intensity on page 27-77.</td>
</tr>
<tr>
<td>Modulation</td>
<td>20 kHz</td>
<td>Always use 20 for a flash</td>
</tr>
<tr>
<td>Filter</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
Driving thru Zambia

Photo by R.L. Garcia, 1984