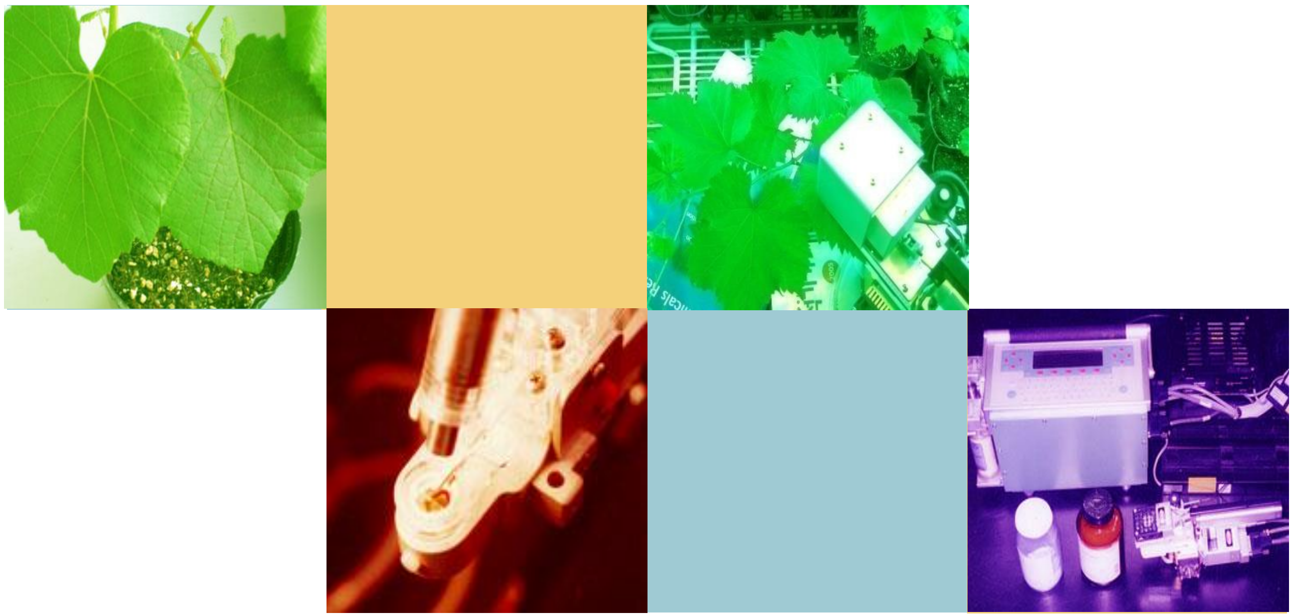
Fruit Tree Research Using Chlorophyll Fluorescence Measurement Techniques



Hee Jae Lee and Duk Jun Yu

Department of Plant Science

Seoul National University

Photosynthesis

- Process of converting light energy to chemical energy in the chloroplast

- Essential for maintaining all forms of life on the earth

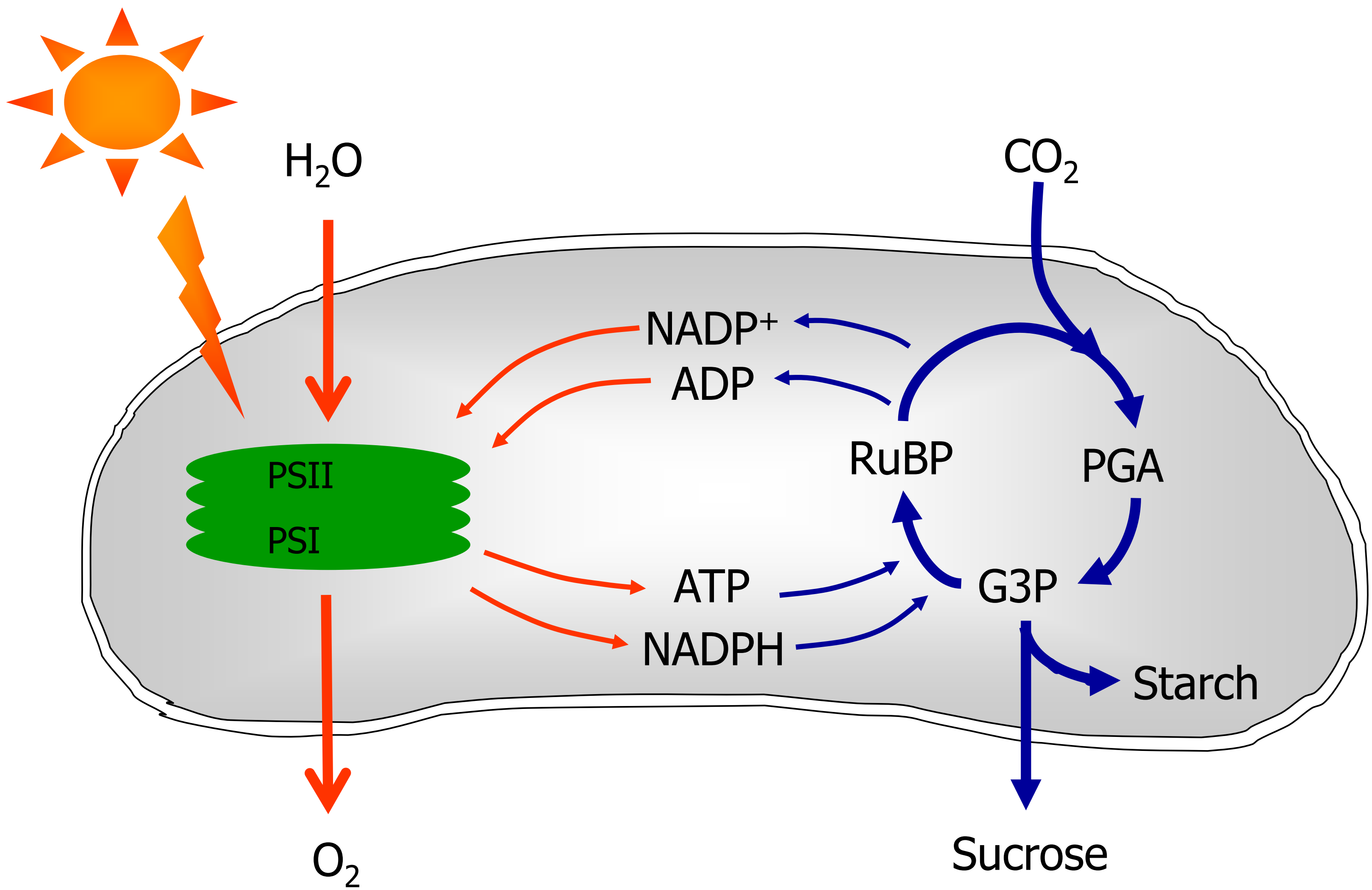
Usefulness of photosynthesis measurement

- Analysis of plant productivity in agriculture, forestry, ecology, etc.

- Research on responses to environmental factors
  - Sensitive to environmental changes
    Light, temperature, water, herbicides, salt, etc.
  - Rapid response
  - Non-destructive in measurements



Photosynthesis outline



Measurement methods of photosynthesis

- CO_2 exchange
  - Infrared gas analyzer (IRGA), titration, electrical conductivity
- O_2 exchange
  - O_2 electrode (liquid and gaseous phases)
- Photosystem II (PSII) activities
  - Chlorophyll (Chl) a fluorescence
- Others
  - $^{14}CO_2$ exchange, dry weight

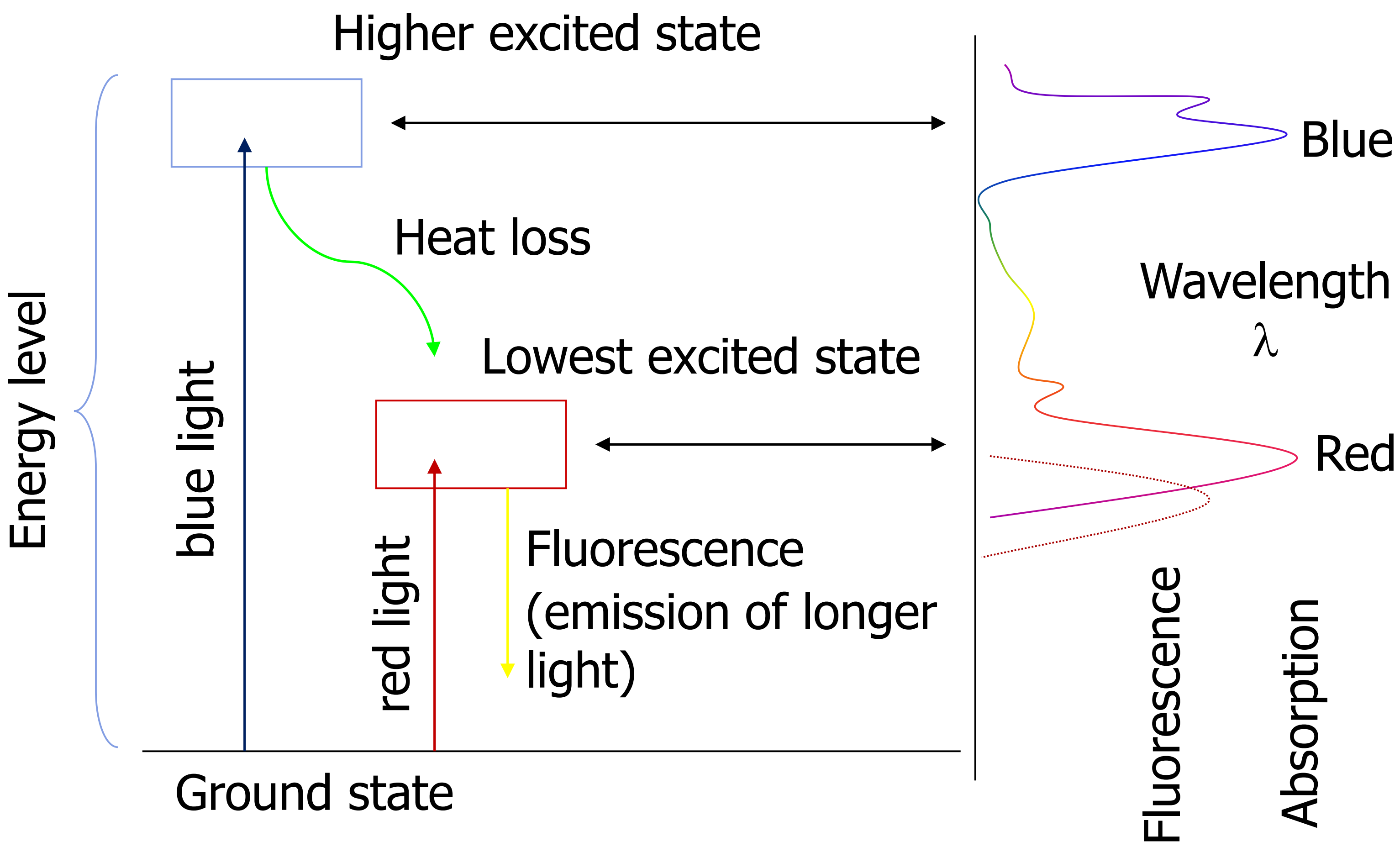


When light energy is absorbed by PSII, the energy is;

- Used in photosynthesis
- Emitted as heat
- Reemitted as light in the form of fluorescence



Fate of light energy



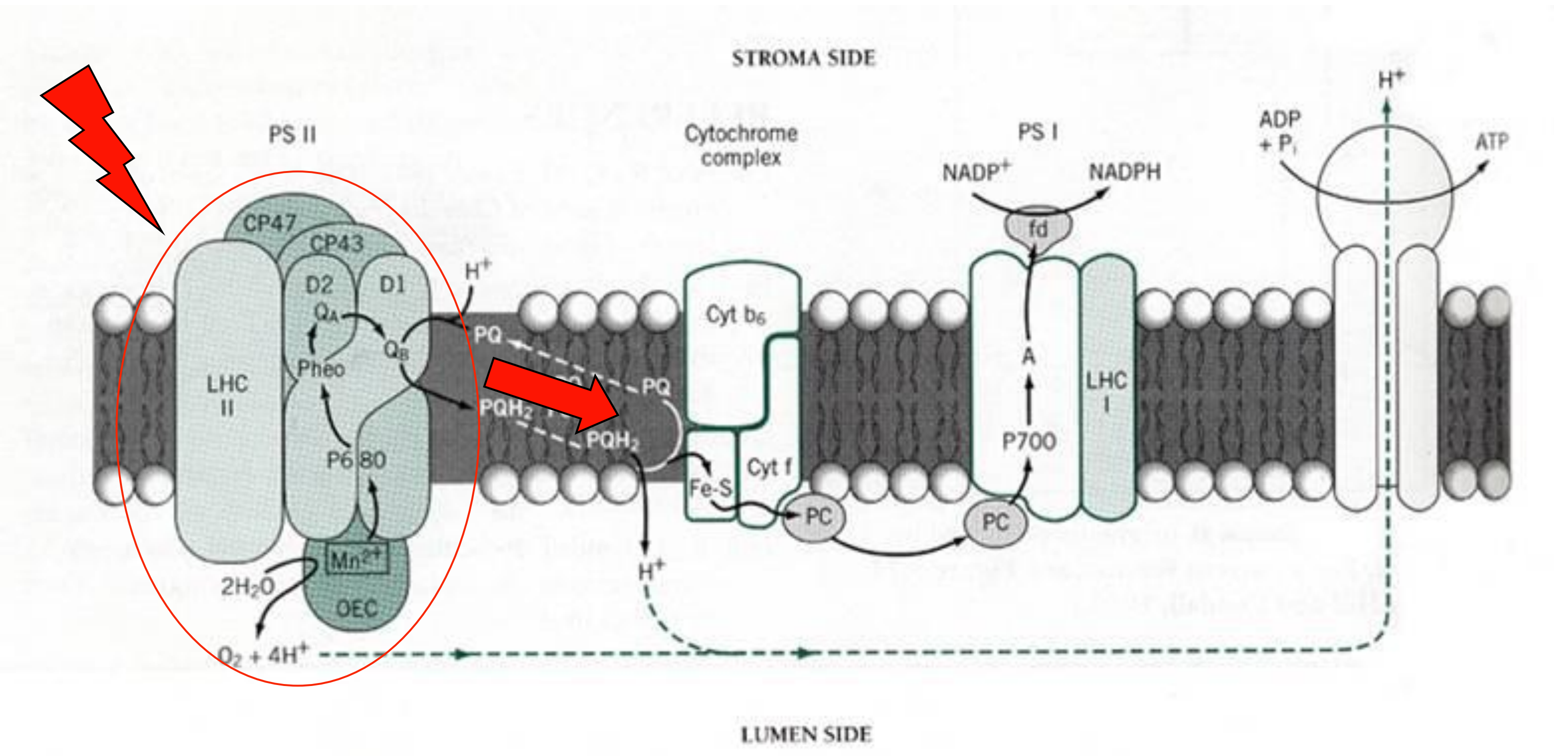
Chl a fluorescence (F)

- Reemission of absorbed light energy at longer wavelength primarily from PSII Chl a molecule
- One method of energy dissipation
- Derived mainly from the antenna of PSII at room temperature
- Complementary to the photochemistry (P) and heat dissipation (H)
  - P and H $\uparrow$ $\rightarrow$ F $\downarrow$
  - P and H $\downarrow$ $\rightarrow$ F $\uparrow$

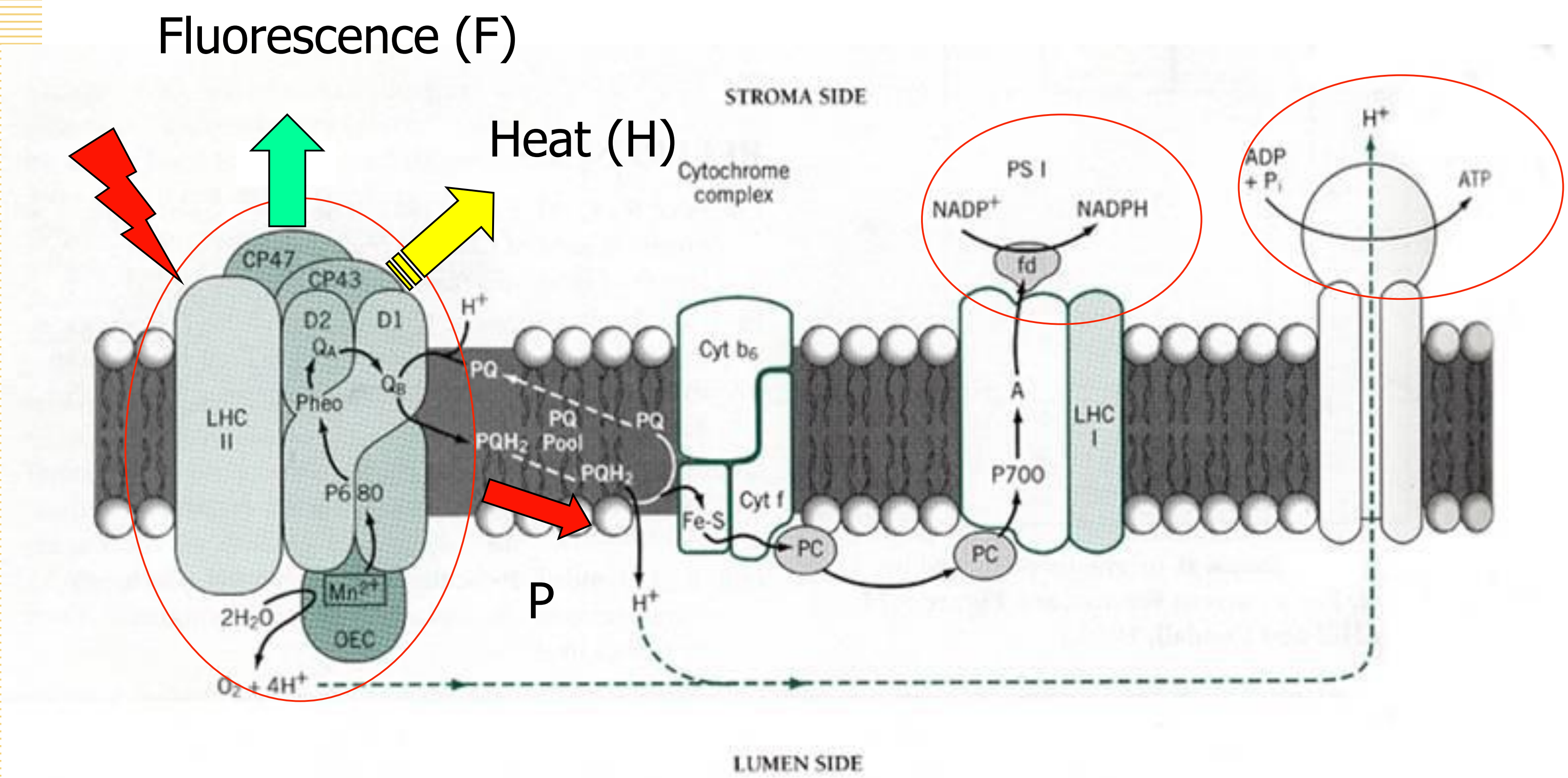


PSII

- Light harvesting complex + reaction center
- Chlorophyll (Chl), carotenoid (Car), protein
- Light energy absorption and electron transport
- Activity changes under various PPF conditions



At high PPF or excessive excitation energy, P decreases while H and F increase



Chl a fluorescence measurement

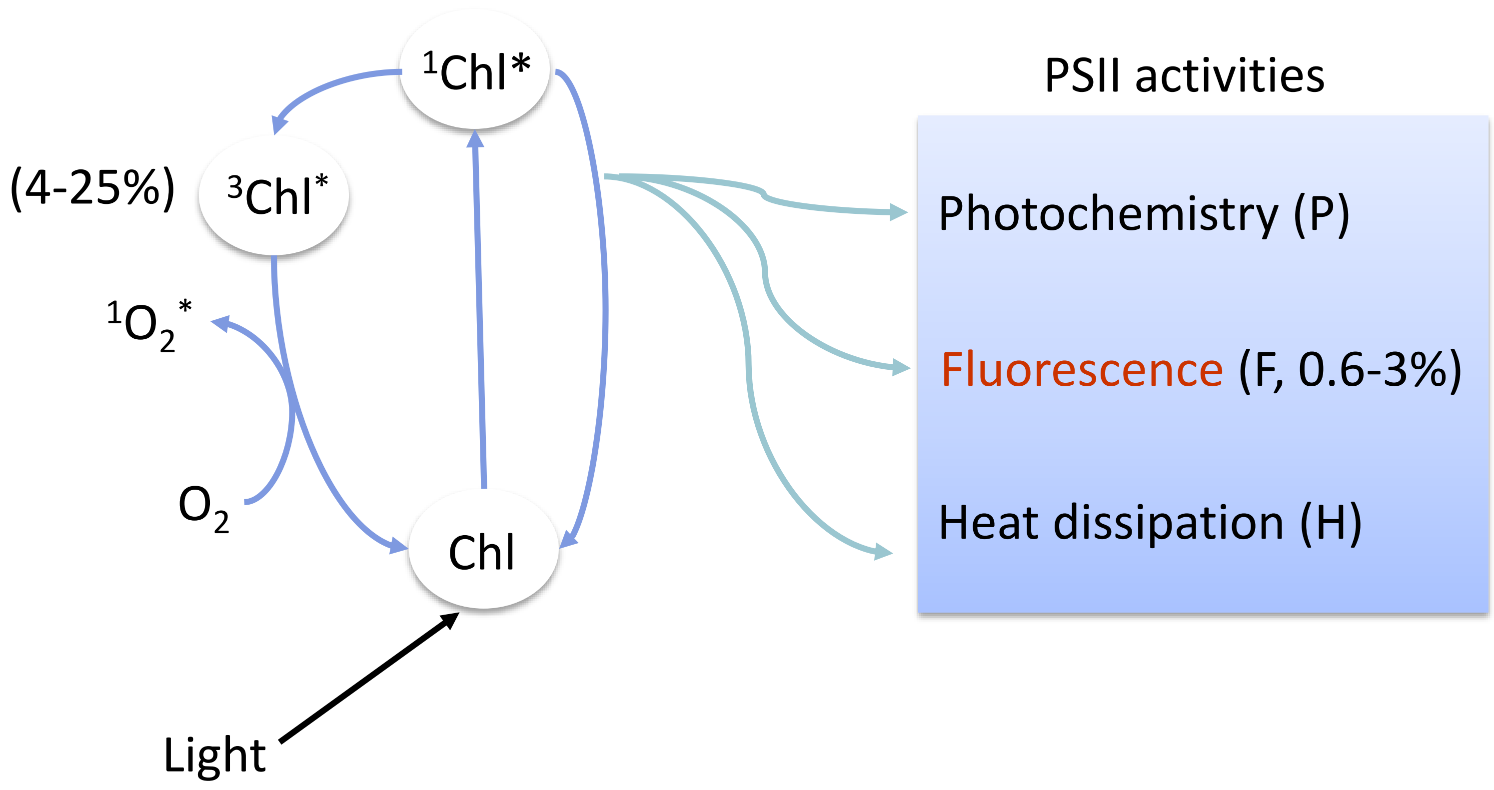
- Reflection of PSII activity
  - Photochemical efficiency
  - Heat dissipation

- Simple, rapid, and non-destructive in measurement

- Useful for studying metabolism of the chloroplast and for examining physiological responses of plants to various environmental stresses



De-excitation pathways of absorbed energy by Chl



Fluorescence emission $\propto$ the average lifetime of $^1Chl^*$

Relationship among de-excitation pathways of the absorbed energy by Chl

$$F + H + P = 1 \quad \bullet \quad (1)$$

At a saturation light intensity, $P = 0$, $F = F_m$, $H = H_m$

$$F_m + H_m + 0 = 1$$

$$H_m = 1 - F_m \quad \bullet \quad (2)$$

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

$$H/F = H_m/F_m$$

$$H = F(1 - F_m)/F_m \quad \bullet \quad (3)$$

At non-saturating light conditions and

$$P = 1 - F - H$$

$$= 1 - F - F(1 - F_m)/F_m = 1 - F/F_m$$

$$= (F_m - F)/F_m \bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet \ (4)$$

If the non-saturating light conditions is total darkness, and the leaf is completely adapted to the darkness

$$P_{dark} = \text{potential quantum yield}$$

$$= (F_m - F_o)/F_m = F_v/F_m \bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet \ (5)$$

Where F_o is known as "minimal fluorescence" measured by measuring light.



By contrast, if the non-saturating light is non-zero, and the leaf is completely adapted to it

$$P_{light} = \text{actual quantum yield} = \Phi_{PSII}$$

$$= (F_m' - F_s)/F_m' = \Delta F/F_m' \bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet \quad (6)$$

Where F_s is "steady-state" fluorescence, and F_m' is the maximal fluorescence during a saturating light flash

$$ETR = PPF \times \alpha_{leaf} \times f \times \Phi_{PSII} \bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet\bullet \quad (7)$$

Where α_{leaf} is leaf absorptance. f is the fraction of absorbed quanta that is used by PSII, and is typically assumed to be 0.5 for C_3 plants, and 0.4 for C_4 plants.



Chl a fluorescence parameters

- Maximal fluorescence (F_m)
  - The greatest yield of fluorescence when all of the primary electron acceptor (Q_A) of PSII are fully reduced and unable to extract energy from the active centers of PSII
  - Indicates the number of available PSII reaction centers
  - Blocked reaction center
  - Lower F_m in stressed leaves

- Minimal fluorescence (F_o)
  - The lowest yield of fluorescence when all of the primary electron acceptor (Q_A) of PSII are fully oxidized in the dark adapted state
  - Open reaction center
  - Higher F_o in stressed leaves



Chl a fluorescence parameters

- Variable fluorescence (F_v)
  - $F_m - F_o$
  - Functional capacity of PSII photochemistry centers
  - Depends on the optical properties of leaves

- F_v/F_m ratio
  - Uses F_m as an internal standard
  - Indicates efficiency of electron capture by open PSII reaction centers for monitoring the physical state of photosynthetic apparatus
  - When excessive light energy is efficiently dissipated through photochemical processes, F_v/F_m ratio should be high.
  - When reaction centers are impaired, F_v/F_m ratio decreases.



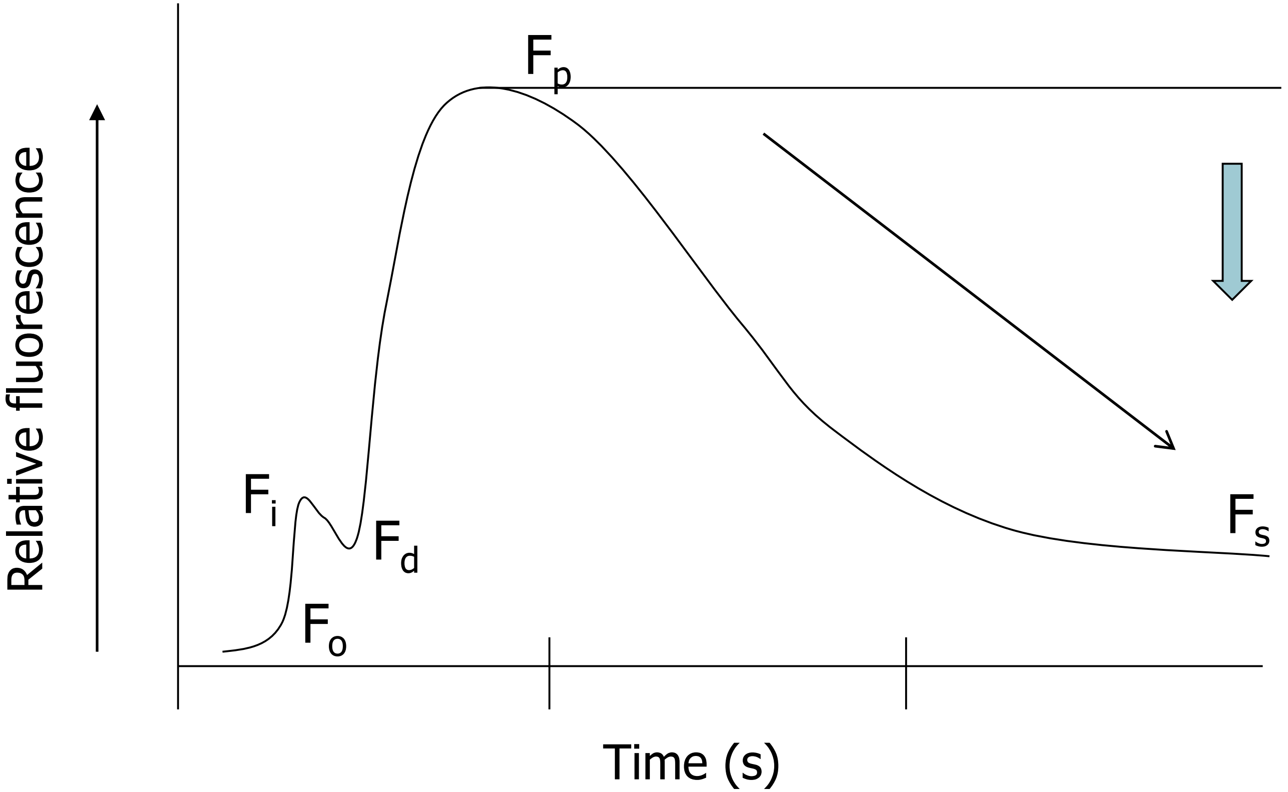
Measurements of Chl a fluorescence parameters

- F_o: minimal fluorescence of PSII reaction center in dark-adapted state
- F_m: maximal fluorescence of PSII reaction center in dark-adapted state
- F_o': minimal fluorescence of PSII reaction center in light-adapted state
- F_m': maximal fluorescence of PSII reaction center in light-adapted state
- F_t or F_s: actual fluorescence at any time or steady state



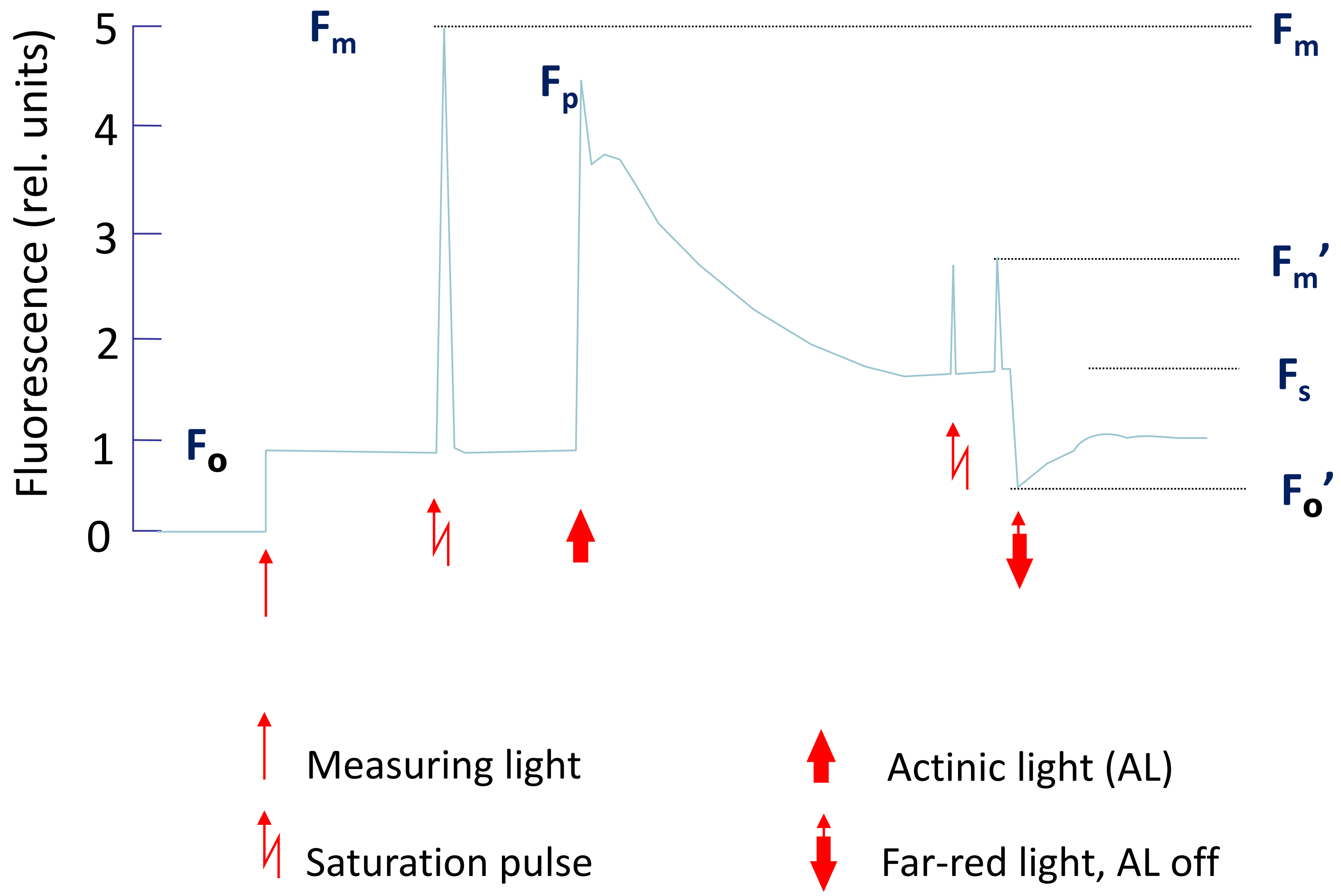
PAM series

Fluorescence quenching (Kautsky curve)

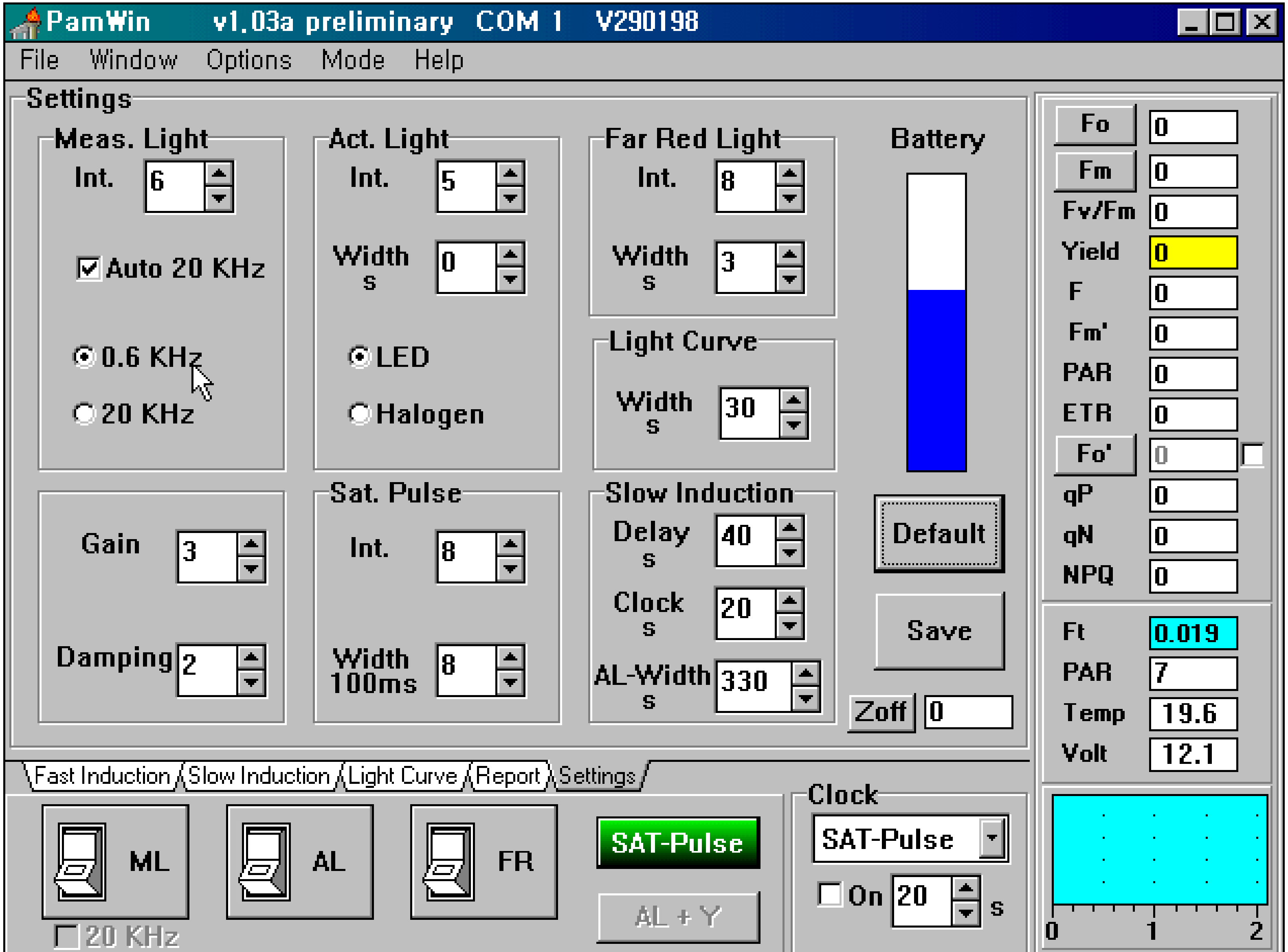


O: maximally oxidized Q_A, O~I: reduction of Q_A, I~D: oxidation of Q_A by electron transport from Q_A to Q_B, D~P: Reduction of Q_A and PQ pool, P: highly reduced PQ pool and Q_A, P~S: Induction of CO_2 assimilation and heat loss

Measurement of Chl a fluorescence



PAM-2000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Measuring light

- < 1 μmol photon m^{-2} s^{-1} from LEDs (intensity 0-10; e.g., 3, appropriate Ft value 0.3-0.4)
- Negligible effect on the leaf's photosynthetic rate
- To measure minimal fluorescence (F_o or F_o') of PSII reaction center (fully opened)
- Modulation: 0.6, 20 kHz



Actinic light

- From LEDs and a halogen lamp
- To drive photosynthesis as a light source
- To measure F_t (F_s) at any time



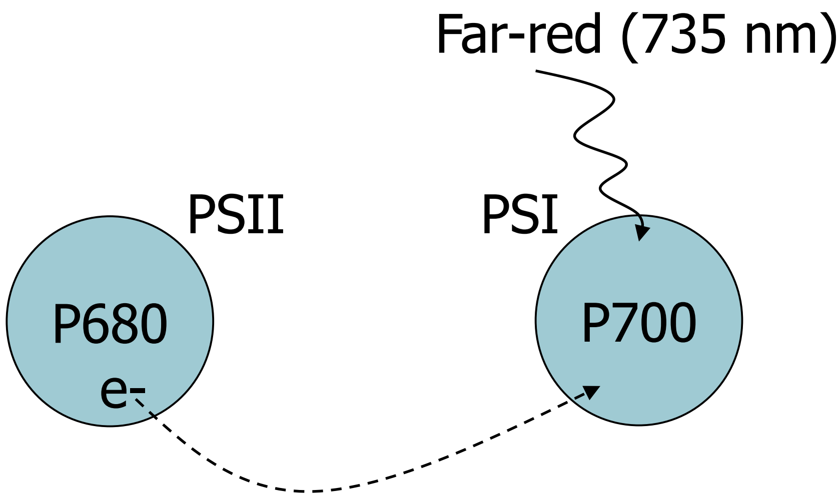
Saturation pulse

- > 7,000 μmol photon $m^{-2}\ s^{-1}$ from LEDs and a halogen lamp (intensity 0-10; e.g., 8)
- For 0.2-2 s to prevent the leaf from high light damage (e.g., 0.8 s)
- To measure maximal fluorescence (F_m or F_m') of PSII reaction center (fully closed)
- Modulation: 20 kHz



Far-red light

- From far-red LED (intensity 0-10; e.g., 8)
- For 3-6 s as actinic light turn off briefly
- To make PSII reaction center open by draining electrons from PSII
- To measure minimal fluorescence of a light adapted leaf (F_o')



Parameters of PSII activities

- Photochemistry
  - Maximum (potential) quantum yield of PSII
    $F_v/F_m = (F_m – F_o)/F_m$, dark-adapted state
    $F_v'/F_m' = (F_m' – F_o')/F_m'$, light-adapted state
  - Actual quantum yield of PSII, $\Phi_{PSII} = (F_m' – F_s)/F_m'$
  - Electron transport rate, $ETR = \Phi_{PSII} \times 0.5 \times PPF \times 0.84$

- Heat dissipation
  - Non-photochemical quenching
    $NPQ = (F_m - F_m')/F_m'$, $qN = (F_m – F_m')/(F_m – F_o')$



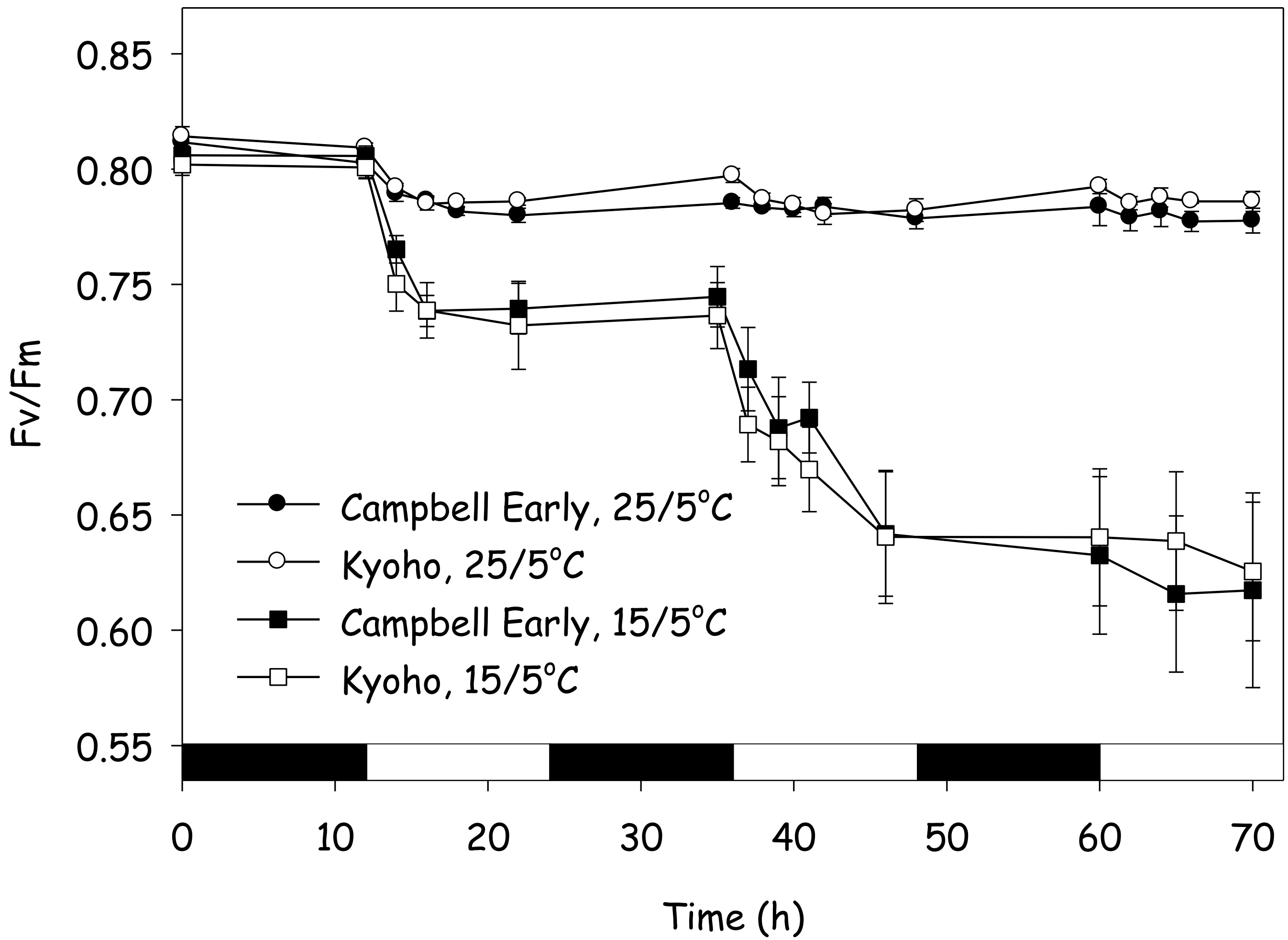
Suggestions for acute measurement and evaluation

- Sufficient equilibration time
- Different level of Chl a fluorescence from PSI depending on plant species
- Setting of measuring light intensity: F value 0.3-0.4
- Setting of saturation pulse intensity (e.g., 0.8 s and 8)
- Fluorescence measurements derived mainly from the chloroplasts closest to upper layers in a leaf are not considered as representative of the entire sample (thick leaves)



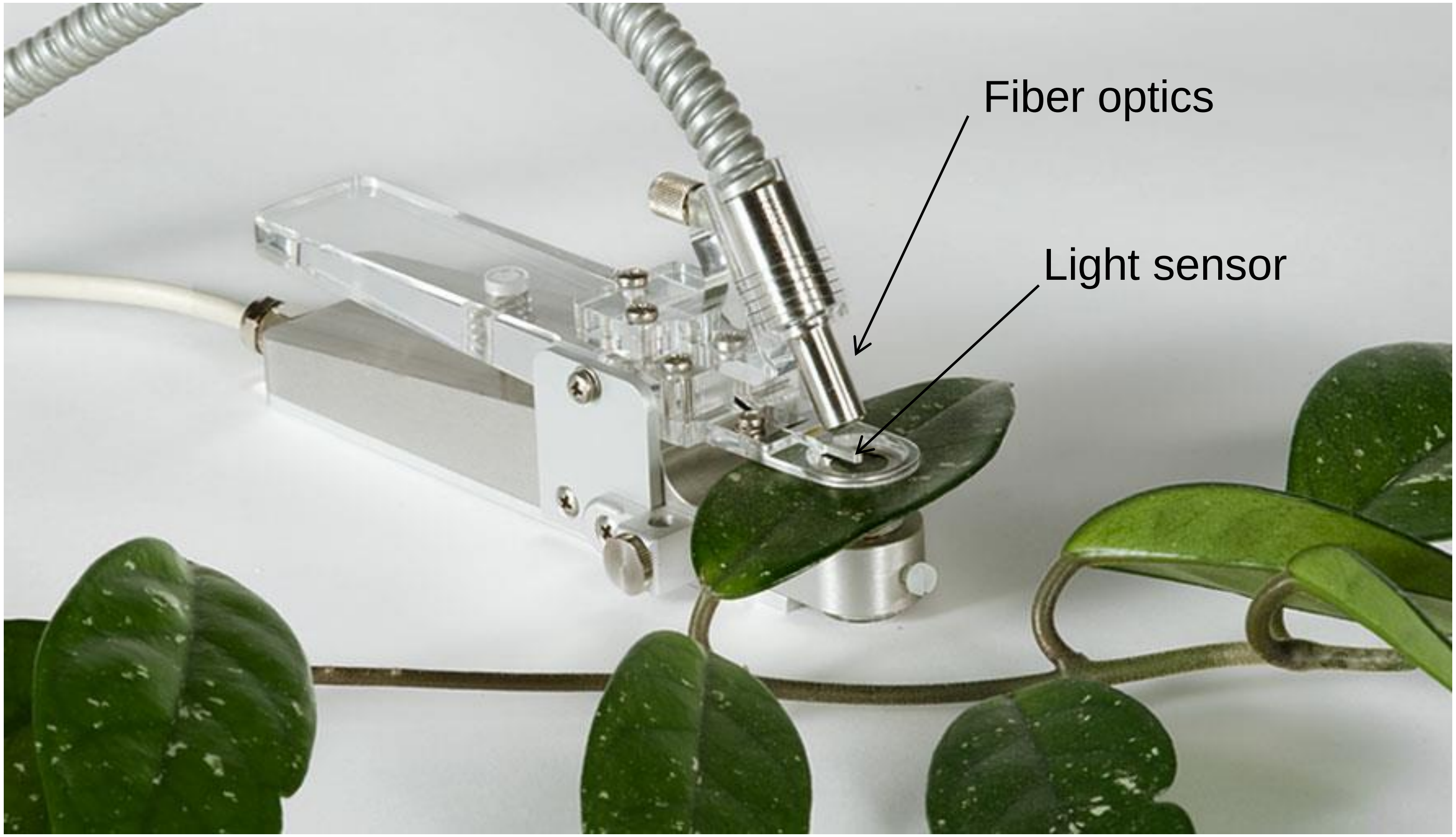
F_v/F_m measurement using a dark leaf clip

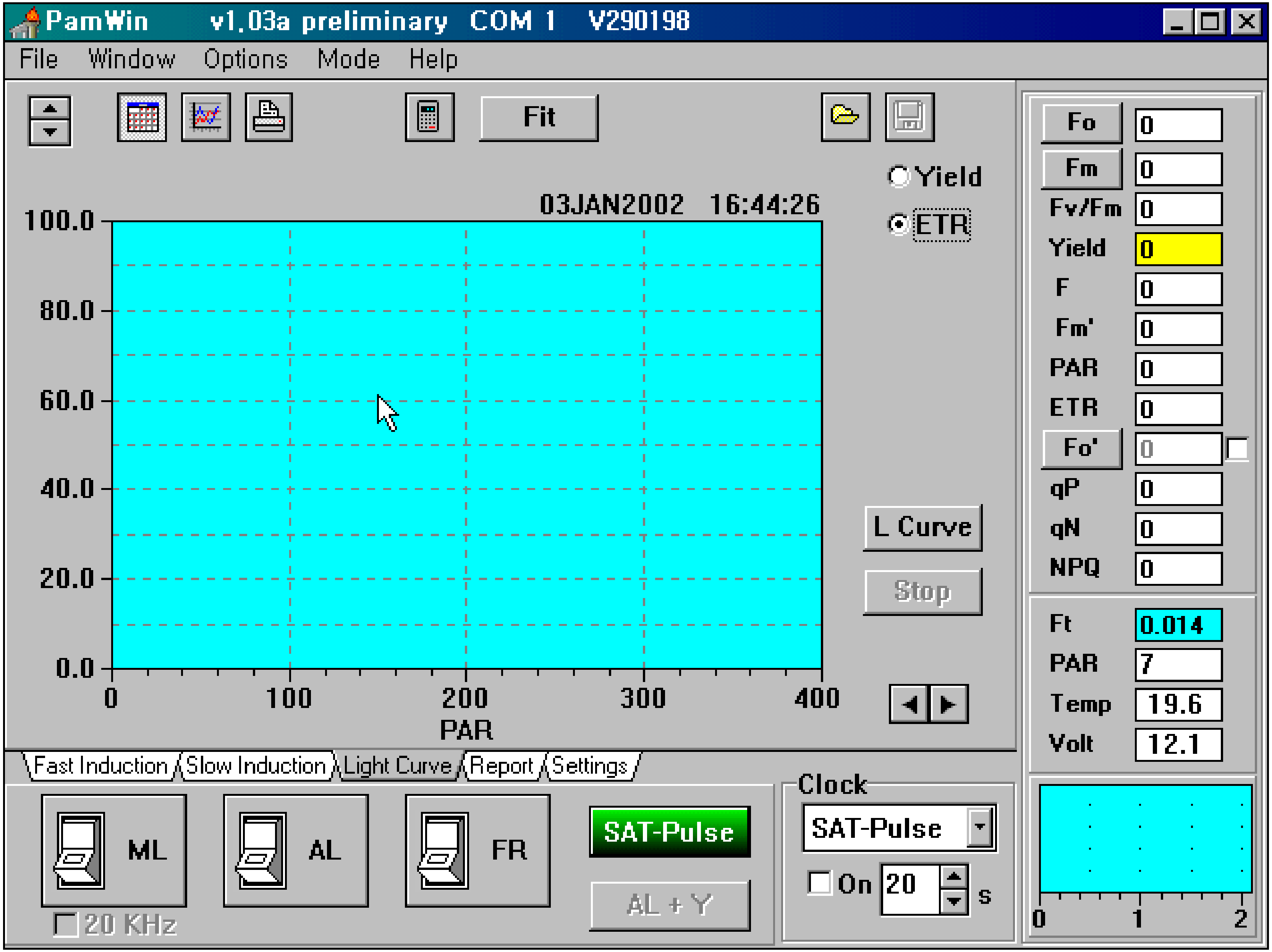
F_v/F_m



Fv/Fm
0.85
0.80
0.75
0.70
0.65
0.60
0.55
Campbell Early, 25/5°C
Kyoho, 25/5°C
Campbell Early, 15/5°C
Kyoho, 15/5°C
0
10
20
30
40
50
60
70
Time (h)

Φ_{PSII} and ETR using a leaf-clip holder 2030-B

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PAR 0
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Fo' 0
qP 0
qN 0
NPQ 0
Ft 0.014
PAR 7
Temp 19.6
Volt 12.1
Fast Induction
Slow Induction
Light Curve
Report
Settings
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SAT-Pulse
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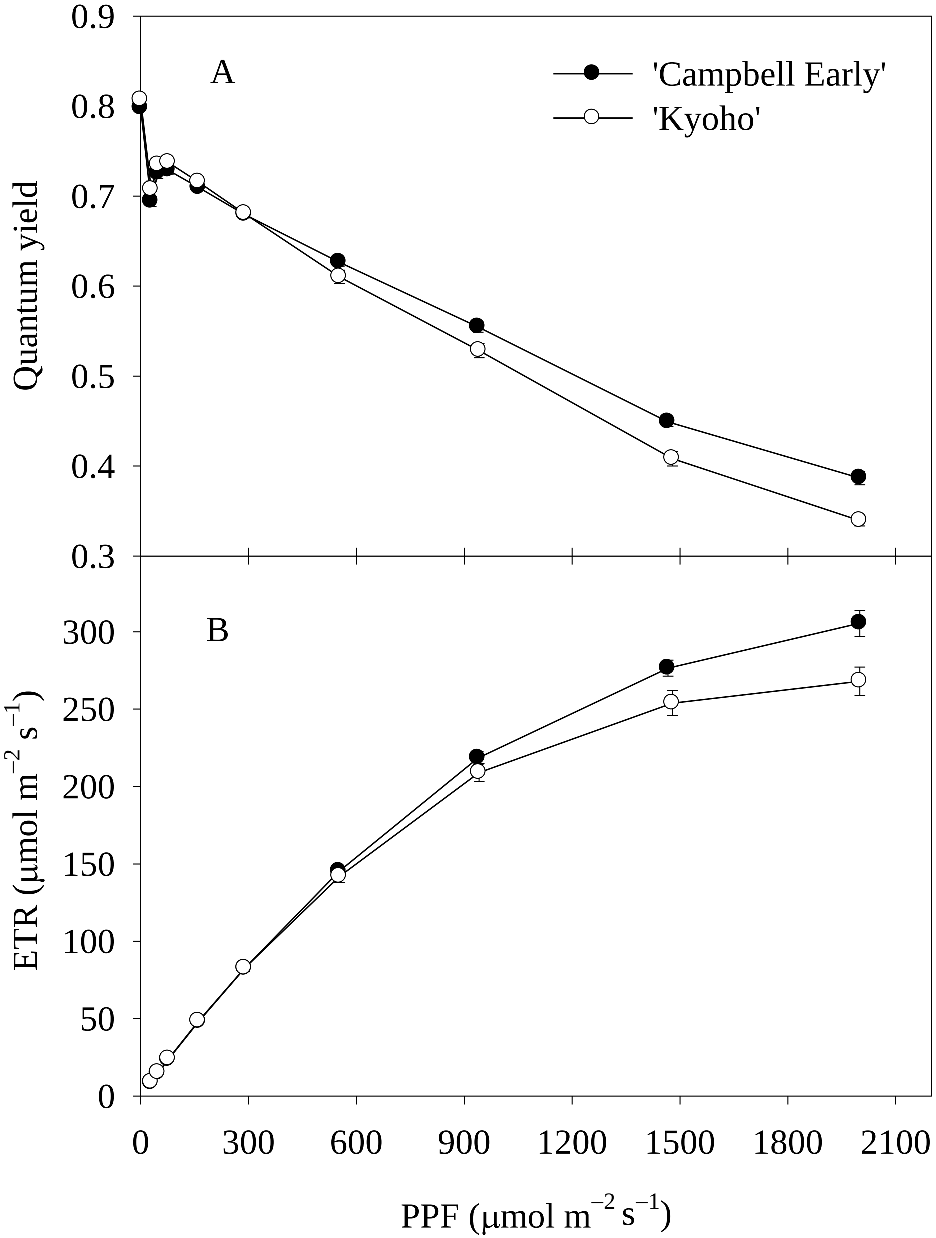
2

Φ_{PSII} and ETR



Applications of Chl a fluorescence measurement

- Evaluation of factors influencing fluorescence induction and photochemical efficiency
- Screening of plants for tolerance to specific environmental or induced conditions
- Evaluation of environmental stress effects on photochemical efficiency