Imaging-PAM Chlorophyll Fluorometer Measurements

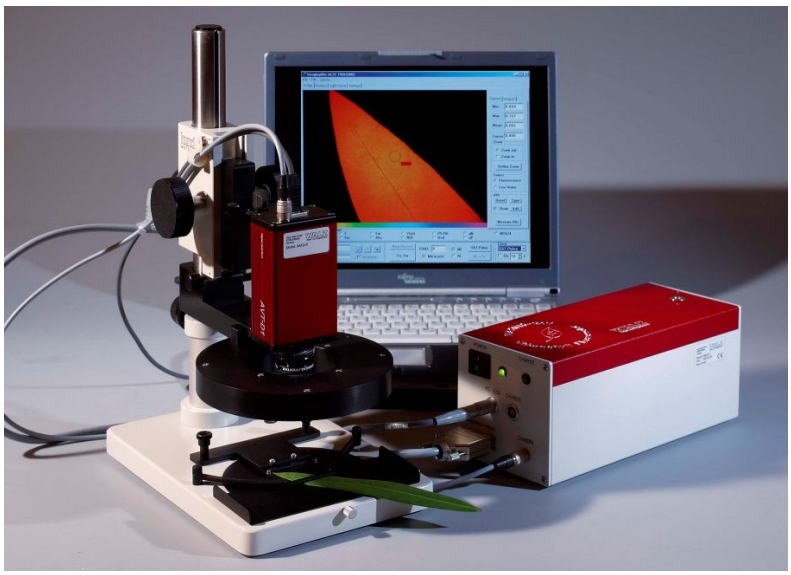
"Watching fluorescence signals is like listening to the stethoscope by medical doctors."

Reto J. Strasser, 1995

Chlorophyll fluorometers are highly sensitive research instruments which give quantitative information on the quantum yield of photosynthetic energy conversion. Their measuring method is based on the Pulse Amplitude Modulation (PAM) technique. The essentials features of the PAM fluorometry are the following:

- Measuring light consists of very short (μs) pulses applied repetitively at different frequencies.
- The detection system is extremely selective to distinguish between the fluorescence excited by the measuring light and the much stronger signals caused by ambient and actinic light.
- The response time of the measuring system is fast enough to resolve the rapid changes in fluorescence yield.

The development of the Chl fluorescence imaging was possible due to availability of highly sensitive CCD cameras and extremely strong light emitting diodes. The Imaging-PAM M-Series Chlorophyll Fluorescence System of WALZ was specialized for the study of two-dimensional heterogeneities of photosynthetic activity.



The main features of the Imaging-PAM M-Series are the following (Fig. 1):

- using one and the same Multi Control Unit in each measuring mode
- 4 different measuring heads (**m**axi, **m**ini, **m**icro, **m**icroscopy) for imaging largely differing sample areas
- applicable in ecophysiology, plant molecular biology, phytopathology, limnology, photosynthesis researh, horticulture, agriculture

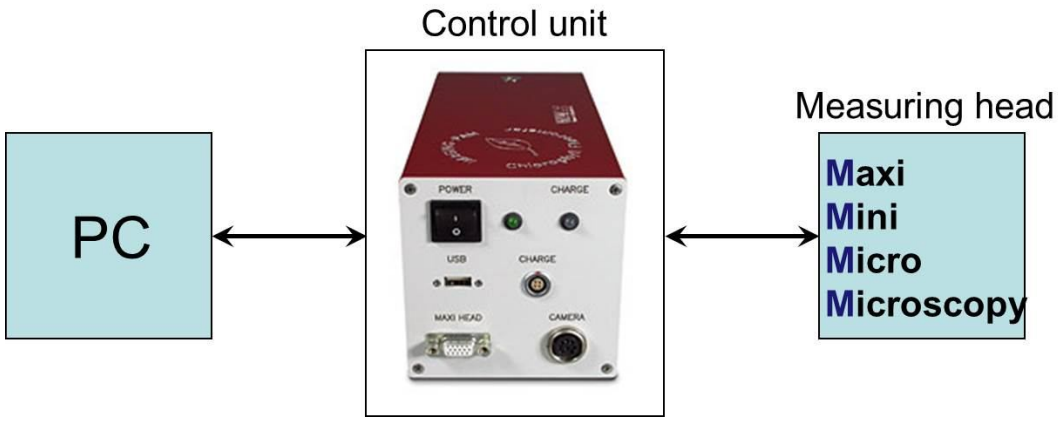


Figure 1. Scheme of the Imaging-PAM M-Series Chlorophyll Fluorescence System.

The Imaging-PAM M-Series Chlorophyll Fluorescence System is unique in covering a wide range of applications from large sample areas (whole plant or leaf, 10x13 cm) down to microscopic scale (single cells, PSII crystals, 130x150 µm) with the advance of different measuring heads (Fig. 2).

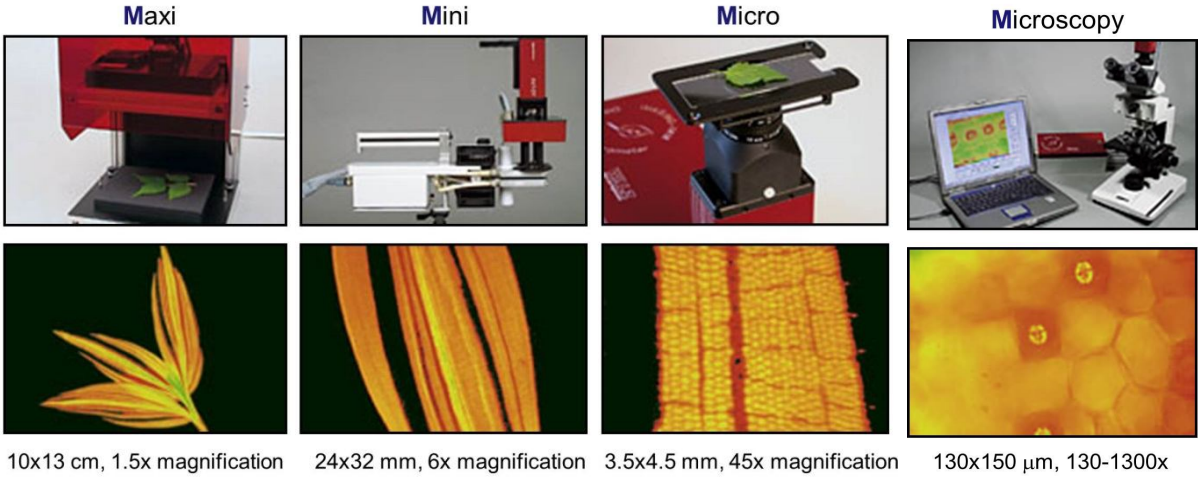


Figure 2. Different versions of measuring heads for imaging largely differing sample areas at a wide range of magnifications.

Photos are taken from http://www.walz.com/downloads/manuals/imaging-pam_ms/imaging-pam_ms_brochure_screen.pdf

Imaging-PAM fluorometer povides not only images of chlorophyll fluorescence, but also images of all relevant chlorophyll fluorescence parameters using the Saturation Pulse method. In this way images of photosynthetic activity and its spatiotemporal variations can be detected. Alltogether images of 18 different parameters can be obtained (Fig. 3). The current fluorescence yield, Ft, is continuously monitored. Fo and Fm are assessed after dark adaptation, serving as reference for fluorescence quenching analysis by the Saturation Pulse method. The maximal PSII quantum yield, Fv/Fm, the effective PSII quantum yield during illumination, Y(II), and the quantum yields of regulated and non-regulated energy dissipation, Y(NPQ) and Y(NO), can be imaged. A routine for measurements of a PAR-Absorptivity image is provided, based on NIR and red light remission. A normalised image of photsynthetic electron transport rate (PS) is calculated from Y(II), Abs and the PAR-value. The Inhibition (Inh) parameter describes the inhibition of PSII quantum yield , Fv/Fm or Y(II), relative to a control reference AOI (area of interest).

Figure 3. Relevant chlorophyll fluorescence parameters provided by Imaging-PAM M-series.

Figure 4. Images of the various fluorescence parameters are depicted in false colors coding from 0.0 to 1.0 (black-red-orange-yellow-green-turquoise-blue-violet-purple).

Goals of the practical:

- Get acquainted with the experimental technique of chlorophyll fluorescence imaging.
- Demonstrate the effect of aging on various fluorescence parameters using mini head.
- Compare various fluorescence parameters of green and yellow areas of a variegated leaf.
- Demonstrate the effect of heat stress on various fluorescence parameters using maxi measuring head.

Experimental material:

Young and old leaves of *Taxus baccata*, variegated leaf of *Carex morrowii*, leaf of *Rosa polyantha 'Kimono'* collected from natural environment. Leaves should be dark adapted at least for 20-30 min.

Measurements:

1. The effect of aging on various fluorescence parameters using mini head

Compare the standard induction curve of a young and an old needle leaves of *Taxus*. Mini head settings: measuring light intensity 2, measuring light frequency 1, actinic light intensity 6 (80 µmol m^{-2} s^{-1}), gain 5, damping 2, SP intensity 10, delay 40s, clock 20s, duration 315s.

Observations:

- There is no difference in the maximal quantum yields after dark adaptation (Fig. 5).
- Upon illumination younger leaf shows higher Y(II) than the older leaf (Fig. 6).
- The induction pattern of nonphotochemical quenching suggests that the older leaf is limited by carbon dioxide fixation rate (Fig.7).

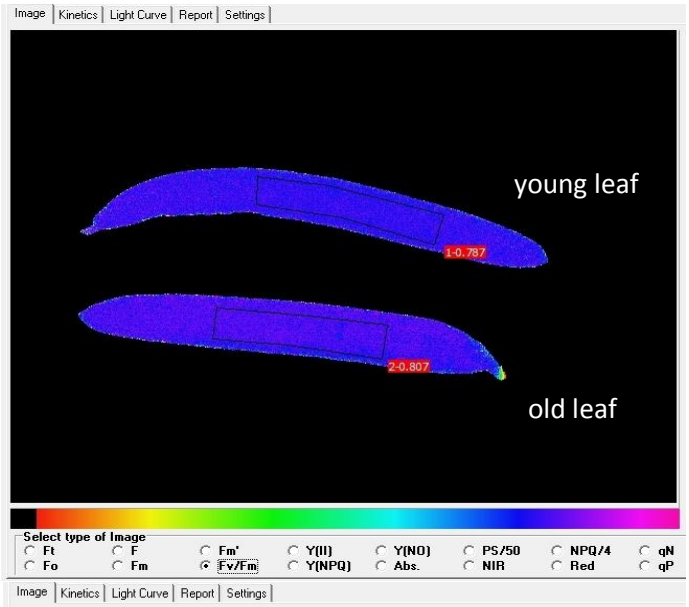


Figure 5. The images of the maximal quantum yield, Fv/Fm of young and old *Taxus* leaves.

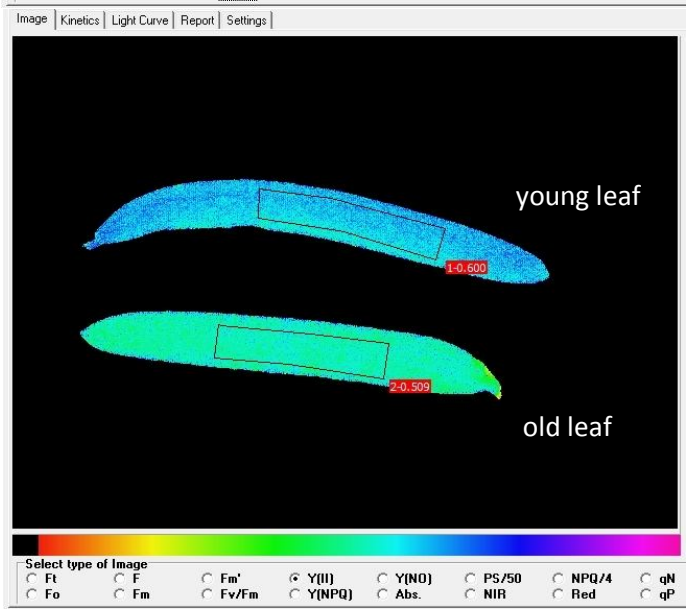


Figure 6. The images of the effective quantum yield, Y(II) of young and old *Taxus* leaves after 3 min illumination at 80 µmol m^{-2} s^{-1}.

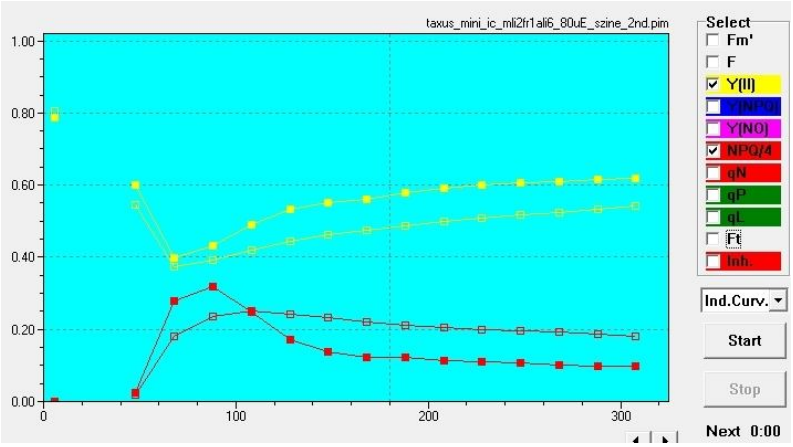


Figure 7. Dark-light induction curves of young (solid symbols) and old (open symbols) leaves of *Taxus*. Displayed parameters are Y(II) (yellow) and NPQ/4 (red). Actinic illumination was 80 µmol m^{-2} s^{-1}.

See also http://www.walz.com/downloads/software/imagingwin/demo_files/needles_ic.pdf

2. Compare various fluorescence parameters of green and yellow area of variegated leaf

Measure the standard light curve of variegated *Carex* leaf. Mini head settings: measuring light intensity 2, measuring light frequency 1, gain 5, damping 2, SP intensity 10. Light curve was detected by 20 s illumination at each light intensities.

Observations:

- Fm and Absorptivity values are much lower, while Y(II) is higher in the yellow area than in the green area of the leaf. Fv/Fm is homogeneous in the whole variegated leaf (Fig. 8).
- In the light curve Y(II) values are higher, Y(NPQ) values are lower, Y(NO) values are similar in yellow area compared to green area (Fig. 9, left panel). Although Y(II) values are higher, still due to very low absorptivity in yellow part of the leaf, ETR values are lower in this area (Fig. 9, right panel).

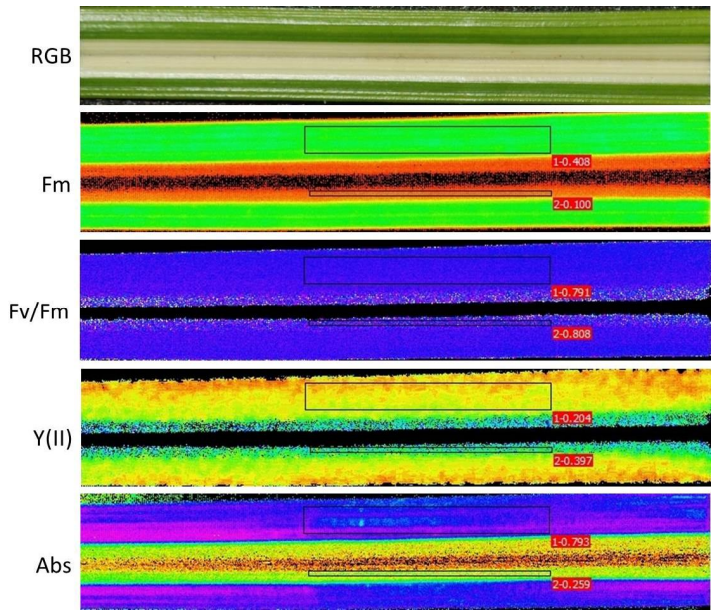


Figure 8. RGB image (top) and various fluorescence images of *Carex* leaf. Fm, maximal fluorescence yield; Fv/Fm, maximal PSII quantum yield; Y(II), effective PSII quantum yield measured in light curve after 20 s of illumination at 400 µmol m^{-2} s^{-1}; Abs, absorptivity.

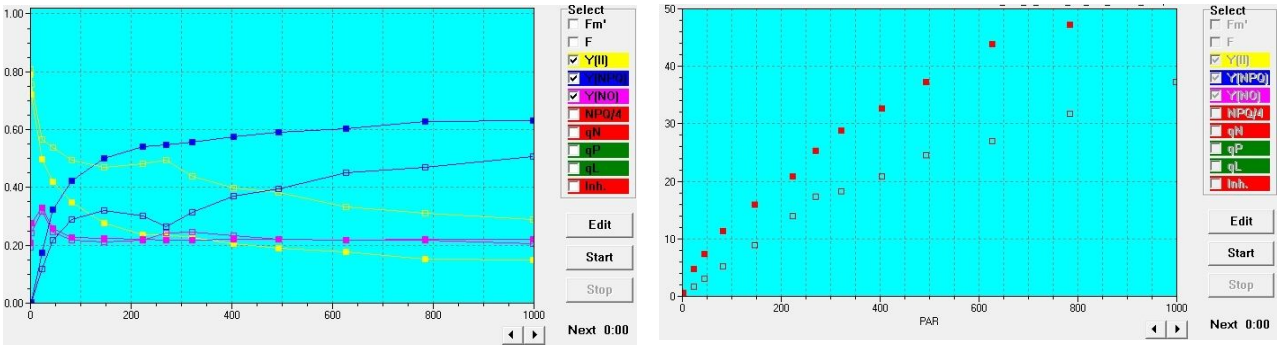


Figure 9. Various fluorescence parameters (left panel) and ETR values (right panel) in variegated leaf of *Carex*. Displayed parameters are: Y(II), effective PSII quantum yield (yellow); Y(NPQ), quantum yield of regulated energy dissipation (blue); Y(NO), quantum yield of nonregulated energy dissipation (magenta); ETR, apparent rate of photosynthesis (red). Closed symbols correspond to green area, open symbols to yellow area of the leaf.

3. The effect of heat stress on various fluorescence parameters using maxi measuring head

The standard induction curves of heat stressed rose leaves were compared. Four of the 5 pinna were heated for 5min at 44, 46, 48 and 50°C by submersion their tip part in a constant temperature water bath. After treatment the leaf was kept at room temperature between moistured tissue. The experiments were carried out between 1 and 3 h after the pretreatment (Schreiber and Klughammer, 2008; Hideg et al, 2008). Maxi head settings: measuring light intensity 2, measuring light frequency 1, actinic light intensity 5 (80 µmol m^{-2} s^{-1}), gain 2, damping 2, SP intensity 10, delay 40s, clock 20s, duration 315s.

Observations:

- In the control leaf qN (coefficient of nonphotochemical quenching) first increases and then declines towards the steady state. Heat treatment affects both the first rise and the secondary decline of nonphotochemical quenching. After 5 min at 44, 46 and 48°C the increase is stimulated and the secondary decline suppressed, resulting high qN. In contrast, after 5 min at 50°C the increase is slowed down (Fig. 10).
- The screenshot of a qN image taken after 20 s of illumination at 80 µmol m^{-2} s^{-1} shows strong stimulation of nonphotochemical quenching in the 44, 46 and 48°C treated leaves, and suppression of it in the 50°C sample (Fig. 11).
- The Y(II) image taken at steady state after 4 min illumination shows decrease in Y(II) by increasing the temperature of the pretreatment (Fig. 12).

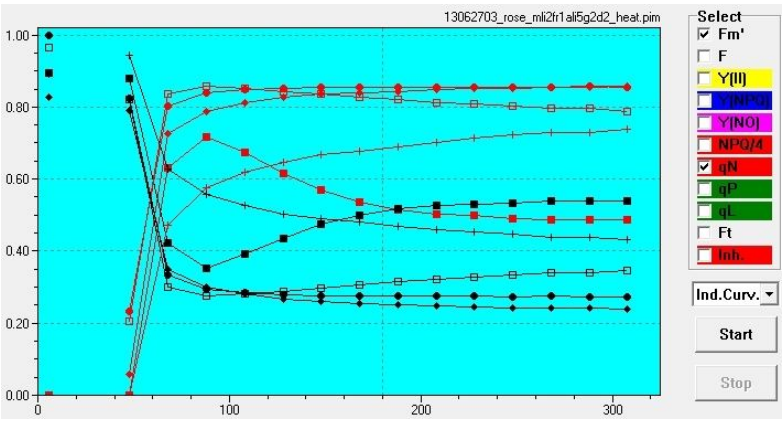


Figure 10. Effect of 5 min heat stress on induction curves of rose leaf. Displayed parameters are Fm' (black) and qN (red). Five AOI are selected on control (closed squares), and 44°C (open squares), 46°C (circles), 48°C (diamonds) and 52°C (crosses) treated leaves.

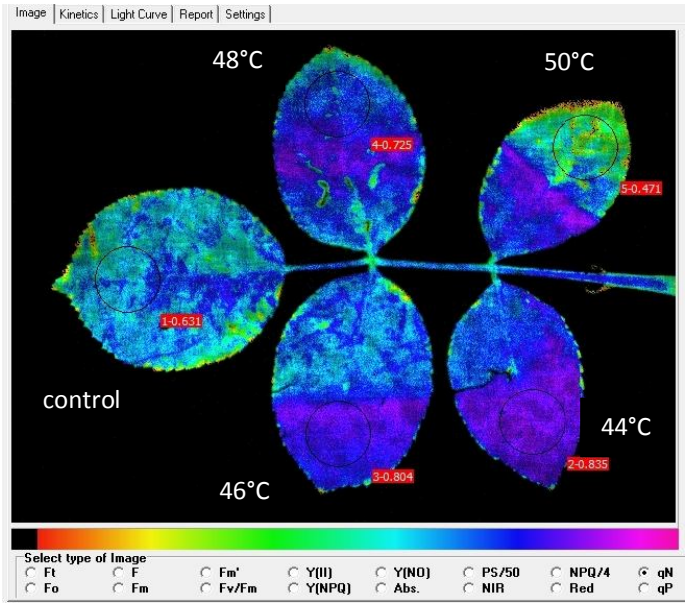


Figure 11. Effect of 5 min heat pretreatment on light-induced non-photochemical quenching (qN) parameter. Tip parts of the various leaflets were immersed for 5 min into water at the indicated temperatures. Image was taken 20 s after onset of illumination at 80 µmol m^{-2} s^{-1}.

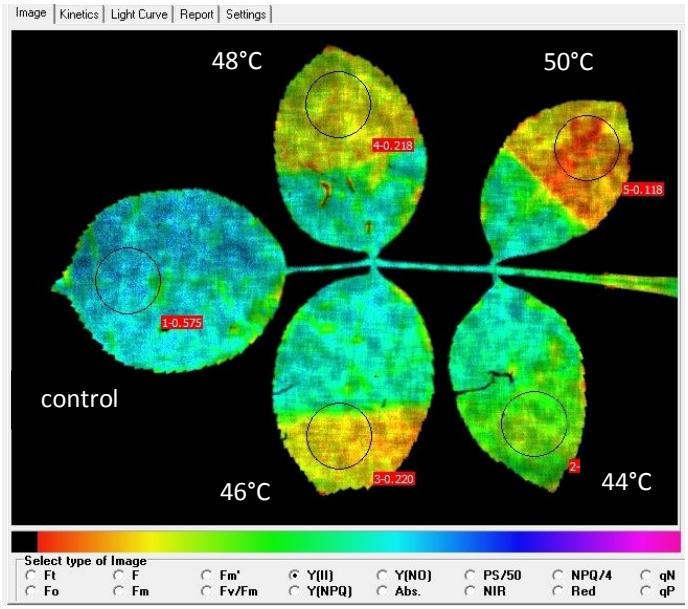


Figure 12. Effect of 5 min heat pre-treatment on the effective PSII quantum yield, Y(II) parameter. Tip parts of the various leaflefts were immersed for 5 min into water at the indicated temperatures. Image was taken 4 min after onset of illumination at 80 µmol m^{-2} s^{-1}.

References

Hideg E, Kos PB, Schreiber U (2008) Planr Cell Physiol 49:1879-1886

Schreiber U, Klughammer C (2008) PAM Application Notes 1:15-18

Strasser RJ, Srivastava A, Govindjee (1995) Photochem Photobiol 61:32-42