Measurements with the Dual-PAM-100 (PSII + PSI)

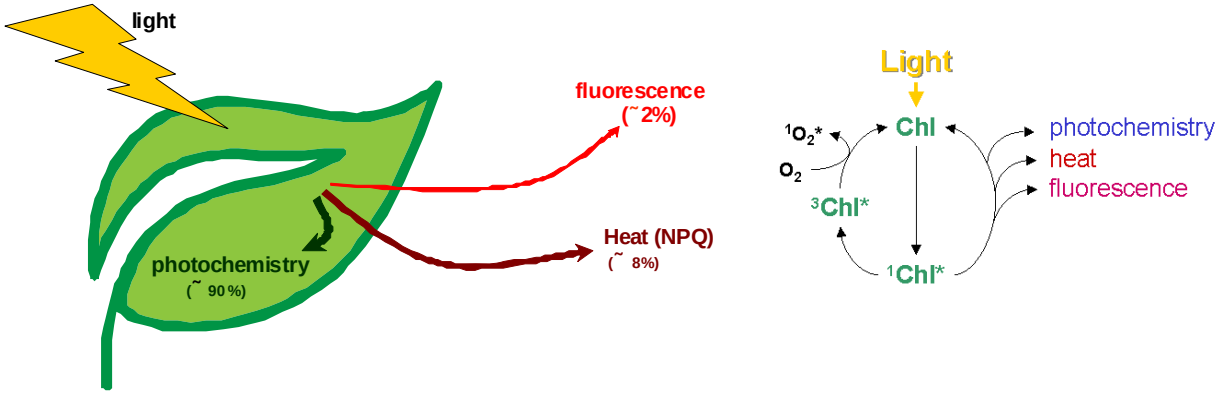
Location: Room no. 103

Kenny

The Dual-PAM-100 allows simultaneous measurements of Chl fluorescence and P700 absorbance changes. P700 provides analogous information on PS I as Chl fluorescence provides on PS II. Based on a highly innovative pulse-modulation technique, absorbance changes of P700 (reaction center chlorophyll of PSI) are measured with a similar signal/noise ratio as Chl fluorescence. Saturation Pulses are applied for assessment of energy conversion efficiency in PS I and PS II. With alternative emitter-detector modules besides P700 and Chl fluorescence various other parameters can be measured that provide additional information on photosynthesis (e.g. Acridine fluorescence, NADPH fluorescence and P515 electrochromic shift).

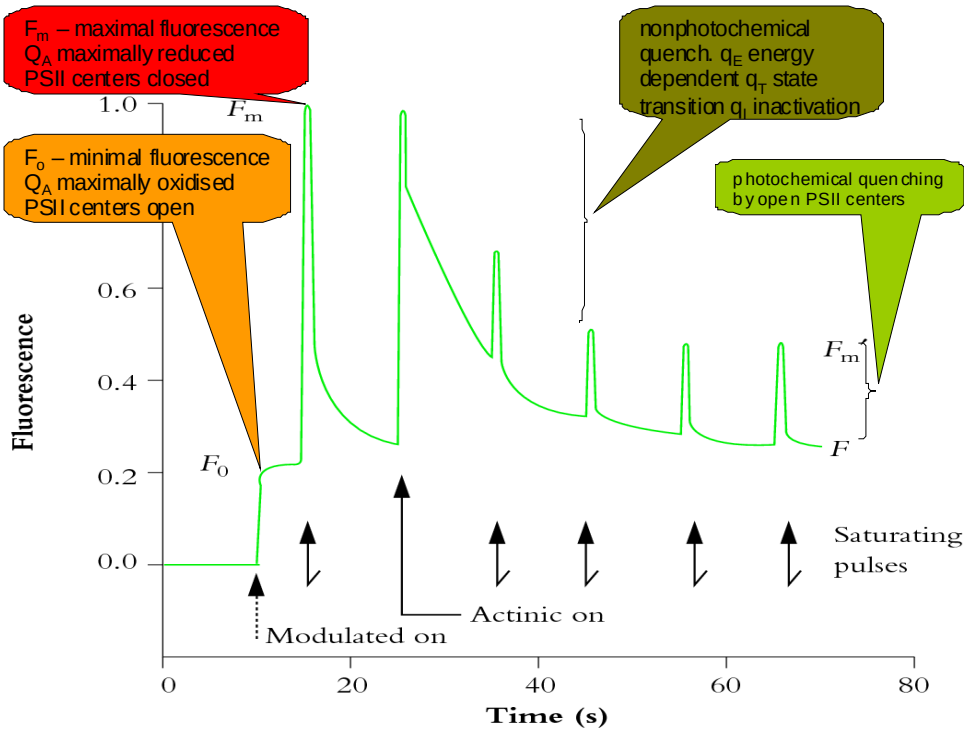
Goals of the practical:

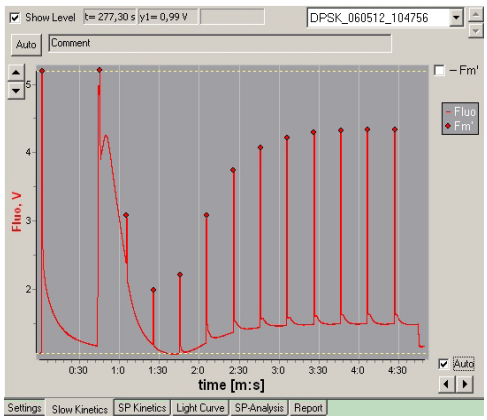
- **Get acquainted with the Dual-Pam-100 measuring system.**
- **Demonstrate Fm and Pm routine plots.**
- **Demonstrate the simultaneous recording of P700 and Chlorophyll Fluorescence induction curves with saturation pulse analysis.**
- **Demonstrate the simultaneous recording of P700 and Chlorophyll Fluorescence light curves.**
- **Interpretation of key photosynthetic parameters obtained.**



(Maxwell and Johnson 2000)

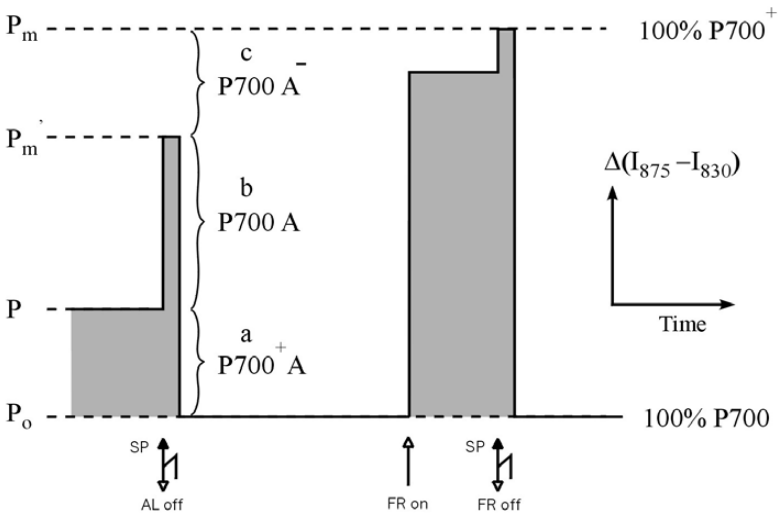
Fig. 1 Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three possible fates.





Chl Fluo measurement in DualPam

Fig 2. Basic principle of Chlorophyll Fluoroscence measurement (Original unpublished) data from John Evans generated on a PAM fluorometer (Heinz Walz GmbH, Germany))
In Fig 2, Induction and relaxation kinetics of *in vivo* Chl a fluorescence from a well-nourished radish leaf (*Raphanus sativus*) supplied with a photon irradiance of actinic light at 500 µmol quanta m^{-2} s^{-1} and subjected to a saturating pulse of 9000 µmol quanta m^{-2} s^{-1} for 0.8 s every 10 s. Output signal was normalised to 1.0 around the value for F_m following 30 min dark pretreatment. Modulated light photon irradiance was <1 µmol quanta m^{-2} s^{-1}.



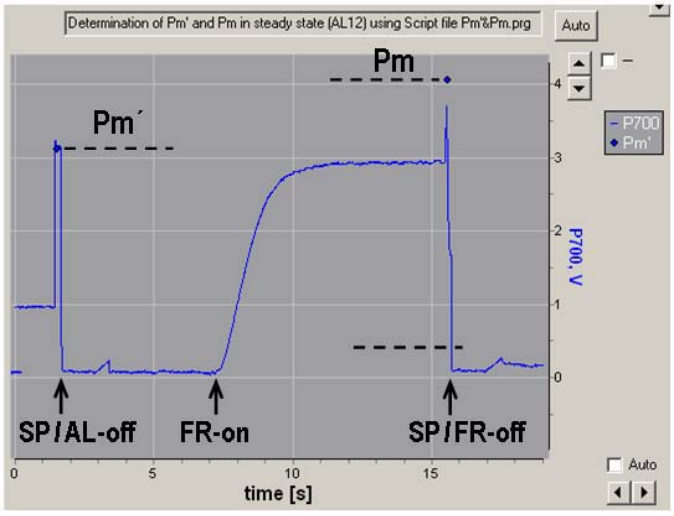


Fig 3. Basic principle of P700 measurement (Klughammer and Schreiber 2008)

In Fig 3, P700 is measured in the dual-wavelength mode (difference of intensities of 875 nm and 830 nm pulse-modulated measuring light reaching photodetector). P700 oxidation is

characterized by a positive signal change. Complete P700 oxidation is induced by a Saturation Pulse (SP) in the presence of Far-Red (FR) light, with the maximal P700 signal denoted by Pm. Complete reduction is induced after the SP and cessation of FR-illumination, with the zero P700 signal denoted by Po. In the presence of Actinic Light (AL) a fraction a (donor-side limited closed centers P700+A) is oxidized by the AL resulting in an intermediate P700 signal denoted by P. In this state the SP-induced signal change corresponds to the oxidation of the active fraction b (open centers P700 A), with the maximal P700 signal being denoted by Pm'. The fraction c (acceptor-side limited closed centers P700 A-) that cannot be oxidized, corresponds to the difference between Pm and Pm'.

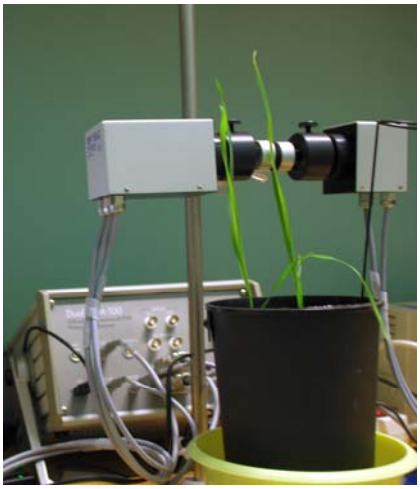


Fig 4. DualPam -100 measuring heads Dual-DB (DR) and Dual-E with leaf holder

Methodology: Plants were dark adapted for 30 min prior to induction curve measurement and 10 min dark adaptation prior to light response curve in each sample.

Defining the parameters:

The following **chlorophyll fluorescence parameters** were calculated: *Fv/Fm = (Fm - Fo)/Fm* (Oxborough and Baker 1997), Y(II) = *(F´m/ F´)/F´m* (Genty et al. 1989), Y(NO) = *F/Fm*, Y(NPQ) = 1 - Y(II) - Y(NO)(Hendrickson et al. 2004; Kramer et al. 2004). *Fo* and *F´*o are the minimum fluorescence in the dark-adapted state and the calculated value of the minimum fluorescence value in the light-adapted state, respectively. *Fo* and *Fm* were determined after 30 min dark adaptation. *F or Fs* is the light-adapted steady state fluorescence. Fv/Fm is the maximum quantum yield of PSII after dark adaptation. Y(II) is the effective quantum yield of PSII. Y(NO) reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, It consists of the NPQ due to photo-inactivation of PSII and constitutive thermal dissipation that is very stable despite environmental stresses (Busch et al. 2009). Y(NPQ) represents the fraction of energy dissipated in form of heat via the regulated non-photochemical quenching.

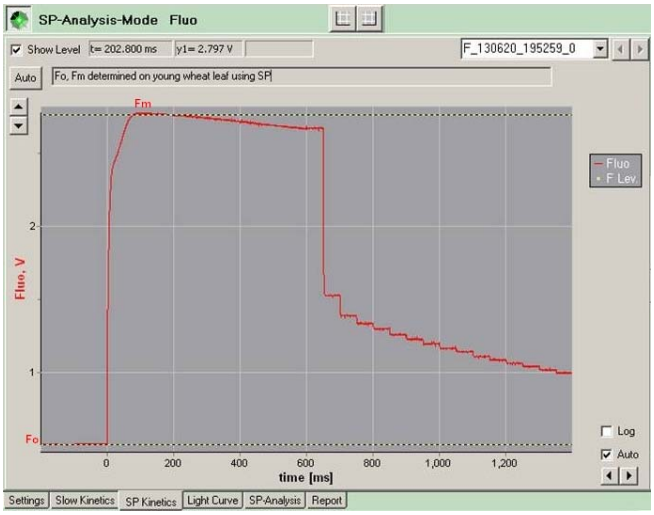
The P700 redox state was measured by Dual PAM-100 with a dual wavelength (830/875 nm) unit, following the method of (Klughammer and Schreiber 1994). Saturation pulses (10,000 lmol m^{-2} s^{-1}), which were introduced primarily for PAM fluorescence

measurement, were applied for assessment of **P700 parameters** as well. The $P700^+$ signals (P) may vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). The maximum level, which in analogy to *Fm* is called *Pm*, was determined with application of a saturation pulse after pre-illumination with far-red light. At a defined optical property, the amplitude of *Pm* depends on the maximum amount of photo-oxidizable P700, which is a parameter for representing the quantity of efficient PSI complex. P´m was also defined in analogy to the fluorescence parameter *F´m* . *P´m* was determined similarly to *Pm*, but with background actinic light instead of far-red illumination. The photochemical quantum yield of PSI, Y(I), is defined by the fraction of overall P700 that in a given state is reduced and not limited by the acceptor side. It is calculated as Y(I) = (*P´m* – *P*)/ *Pm*. Y(ND) represents the fraction of overall P700 that is oxidized in a given state,which is enhanced by a transthylakoid proton gradient (photosynthetic control at cytb/f complex as well as down-regulation of PSII) and photodamage to PSII. Y(ND) = *P/Pm*. Y(NA), thus represents the fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of oxidized acceptors. Y(NA) = *Pm - P´m/ Pm*. Note Y(I)+Y(ND)+Y(NA) = 1.

The electron flow through PSI and PSII were calculated as follows: ETR(I) = Y(I) × PPFD × αI, ETR(II) = Y(II) × PPFD × αII (Miyake et al. 2005). αI and αII were obtained as αI = *p* × *d*I and αII = p × *d*II, where *p* is the absorptance (the fraction of the incident light absorbed by leaves), and *d*I and *d*II are the fractions of the absorbed light distributed to PSI and PSII, respectively.

Measurements:

1) Determination of Fm and Pm routine



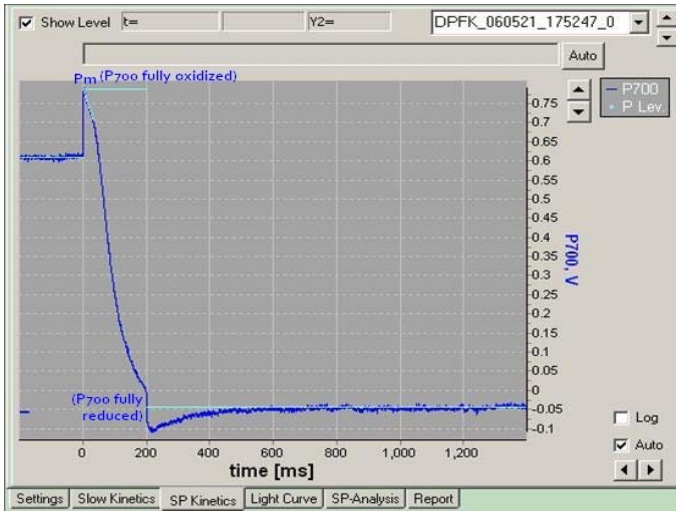


Fig 5. (a) Fo, Fm yield **(b) Pm routine**

2) Recording of Induction Curve with Saturation Pulse Analysis

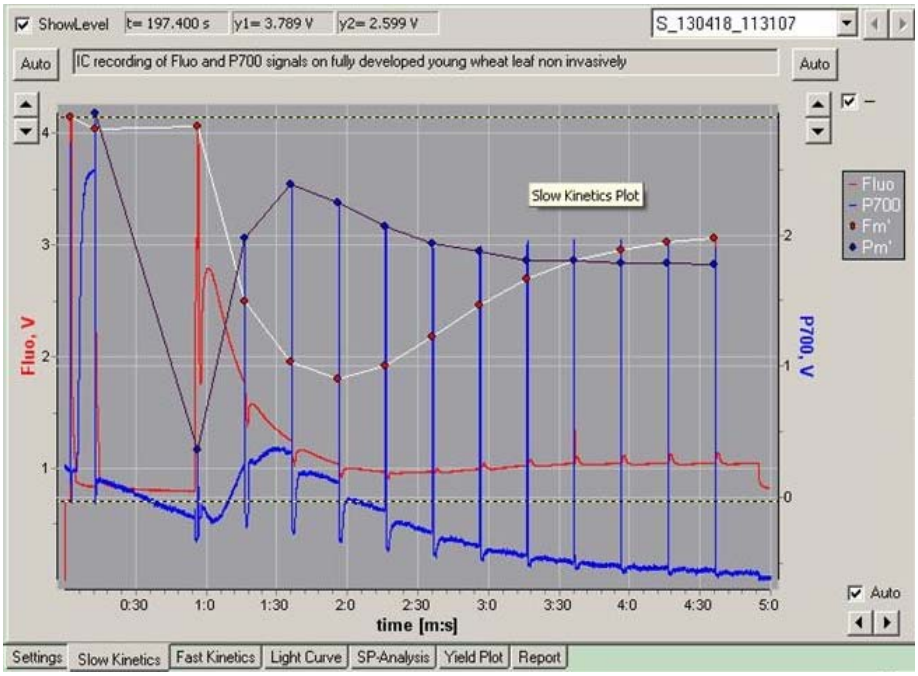


Fig 6(a).

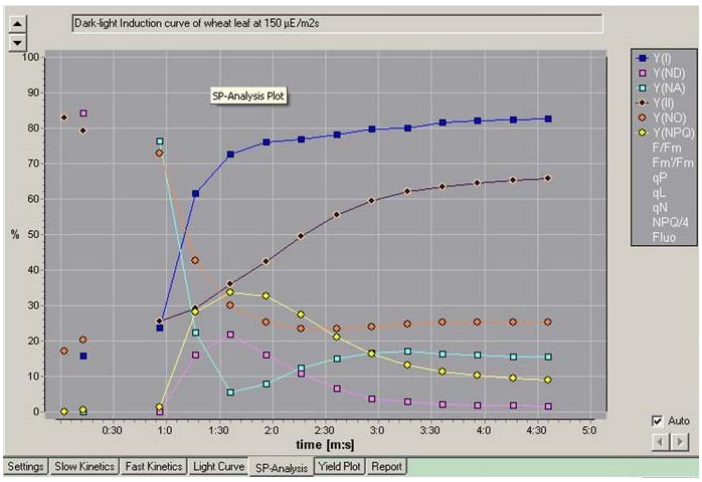


Fig 6(b).

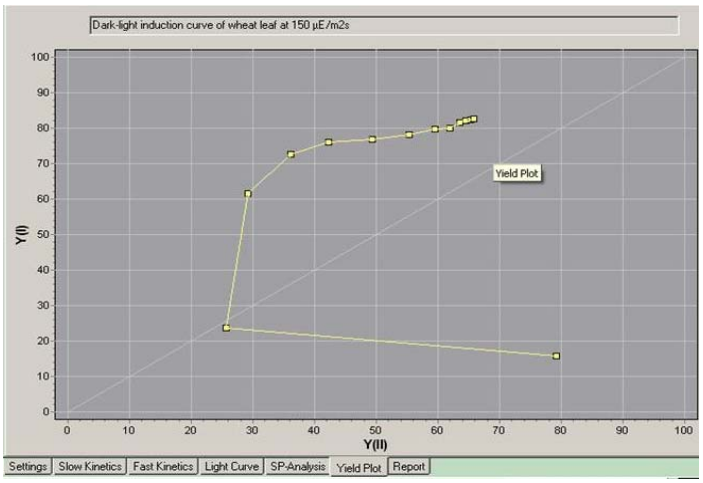


Fig 6(c).

Fig 6. (a) Simultaneous recording of Fluo and P700 induction curves using saturation pulse analysis in young wheat leaf non invasively. (b) Various Fluo and P700 parameters obtained from SP-Analysis plot and (c) Yield Plot.

3) Recording of Light Curve

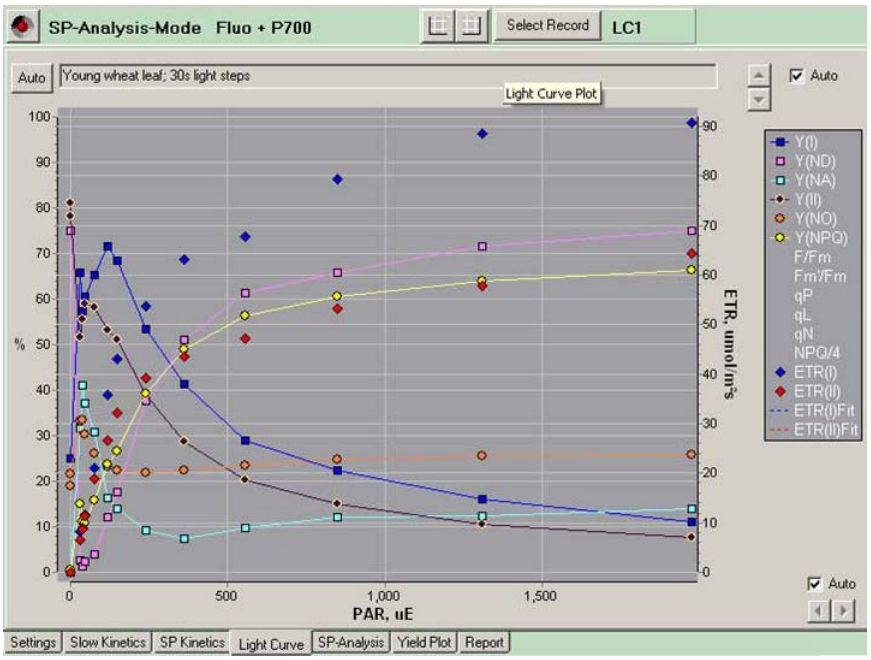


Fig 7. Simultaneous recording of Fluo and P700 light curves

Depending on plant species, its particular growth conditions and physiological state the same light intensity may be more or less excessive.Additional information on the photosynthetic performance of a leaf can be obtained with the help of **Light Curves (LC).** While **Induction Curves (IC)** normally are measured with dark adapted samples, LCs preferentially are measured with preilluminated samples, e.g. after an IC recording. Light response curves provide valuable information on the efficiency with which photosynthetically active radiation is used by plants. It provides detailed information on electron transport capacity and limitations of the two photosystems. Various Fluo and P700 parameters may be selected for display on the Light Curve window (Fig. 7). Differences between quantum yields, **Y(I)** and **Y(II)** and between apparent electron transport rates, **ETR(I) and ETR(II**), may be related to cyclic electron flow, differences in energy distribution and/or PS I/PS II ratio.

References:

1) Busch F, Hunter NPA, Ensminger I (2009) Biochemical constrains limit the potential of the photochemical reflectance index as a predictor of effective quantum efficiency of photosynthesis during the winter spring transition in Jack pine seedlings. Funct Plant Biol 36:1016–1026
2) Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

3) Hendrickson L, Furbank RT, Chow WS (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Photosynth Res 82:73–81
4) Kate Maxwell and Giles N. Johnson (2000) Chlorophyll Fluorescence-a practical guide. Journal of Experimental Botany, Vol 51, No.345, pp.659-668
5) Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700?-absorbance changes at 830 nm. Planta 192:261–268
6) Klughammer C, Schreiber U (2008) Saturation Pulse method for assessment of energy conversion in PS I. PAM Application Notes (2008) 1: 11 - 14
7) Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth Res 79:209–218
8) Miyake C, Miyata M, Shinzaki Y, Tomizawa K (2005) CO2 response of cyclic electron flow around PSI (CEF- PSI) in tobacco leaves: relative electron fluxes through PSI and PSII determine the magnitude of non- photochemical quenching (NPQ) of chl fluorescence. Plant Cell Physiol 46:629–737
9) Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components–calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54:135–142
10) Walz company instruction manuel for DUAL-PAM-100 (http://www.walz.com/products/chl_p700/dual-pam-100/downloads.html)

LABORATORY COURSE

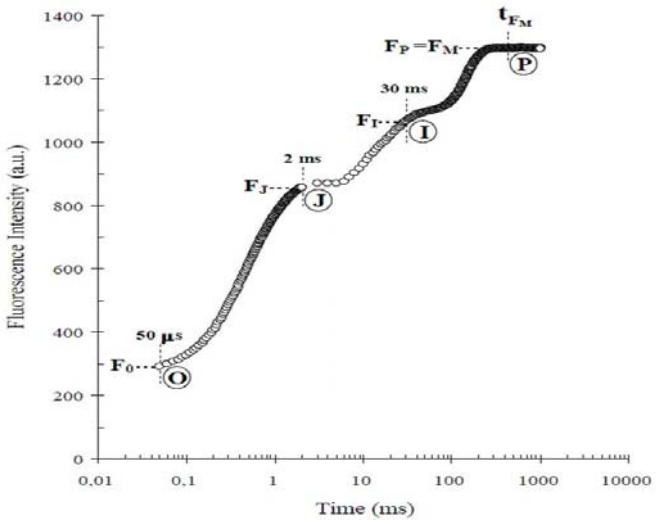
OJIP Fast Kinetics measurements

Goals of the practical:

- **Get acquainted with the experimental technique of OJIP fast kinetics measurements.**
- **Demonstrate the sampling protocol using Pocket PEA chlorophyll fluorometer.**
- **Analysis and data interpretation using PEA Plus software.**

The polyphasic fluorescence rise is widely accepted to reflect the accumulation of the reduced form of the primary quinone acceptor Q_A (i.e. the RCs' closure), which is the net result of Q_A reduction due to PSII activity and Q_A^- reoxidation due to PSI activity. It is assumed that under normal conditions Q_A is completely oxidized in the dark, i.e. all RCs re-open, and the fluorescence signal at the onset of illumination is F_0. The maximum yield F_P depends on the achieved reduction-oxidation balance and acquires its maximum possible value, F_M, if the illumination is strong enough (above 100 Wm^{-2}) to ensure the closure of all RCs.

Principle:



(RJ Strasser - 2004)

Figure. 1 A typical Chl *a* polyphasic fluorescence rise O-J-I-P, exhibited by higher plants. The transient is plotted on a logarithmic time scale from 50 ms to 1 s. The signals are: the fluorescence intensity $\mathbf{F_O}$ (at 50 ms); the fluorescence intensities $\mathbf{F_J}$ (at 2 ms) and $\mathbf{F_I}$ (at 30 ms); the maximal fluorescence intensity, $\mathbf{F_P = F_M}$ (at $\mathbf{t_{FM}}$).

Single high intensity focused LED in front of PocketPEA control unit which is positioned vertically above the sample and provides up to 3500 µmols m^{-2-1} intensity with a peak wavelength of 627nm at the sample surface.

Experimental material: Young wheat leaves

Measurement: Samples are conveniently dark adapted prior to measurement using the leafclips supplied. Mate the optical assembly of Pocket PEA with the locating ring on the leafclip ensuring a good fit. Slide the shutter blade of the leafclip back to reveal the sample. Press the measure button. Once the measurement has been completed (either 1, 3 or 10s measurement depending on the instrument configuration), the parameters Fv/Fm and PI are displayed on the LCD. The measurement is then automatically saved and the instrument is returned to measurement ready status.

Analysis:

- **Analyse the transferred raw data using PEA plus software tools**
- **Export data points and parameters to Origin**
- **Make normalisation at Fo (Raw data points/Fo)**
- **Make normalisation at Fm ((Raw data points -Fo)/Fv)**

References:

1) Hansatech company operations manuel for Pocket PEA analyser. (http://www.wittich.nl/NL/PDF/HANDLEIDINGEN/fotosynthese/chlorofylfluorescentie/manual_handypea-pocketpea.pdf)

2) R.J. Strasser, M. Tsimilli-Michael, A. Srivastava, Analysis of the chlorophyll fluorescence transient, in: G.C. Papageorgiou, Govindjee (Eds.), Chlorophyll Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration, vol. 19, Springer, Dordrecht, The Netherlands, 2004, pp. 321–362.