Dual-PAM-100

P700 & Chl Fluorescence Measuring System

For simultaneous assessment of P700 and Chlorophyll Fluorescence

WALZ

Mess- und Regeltechnik

Dual-PAM-100

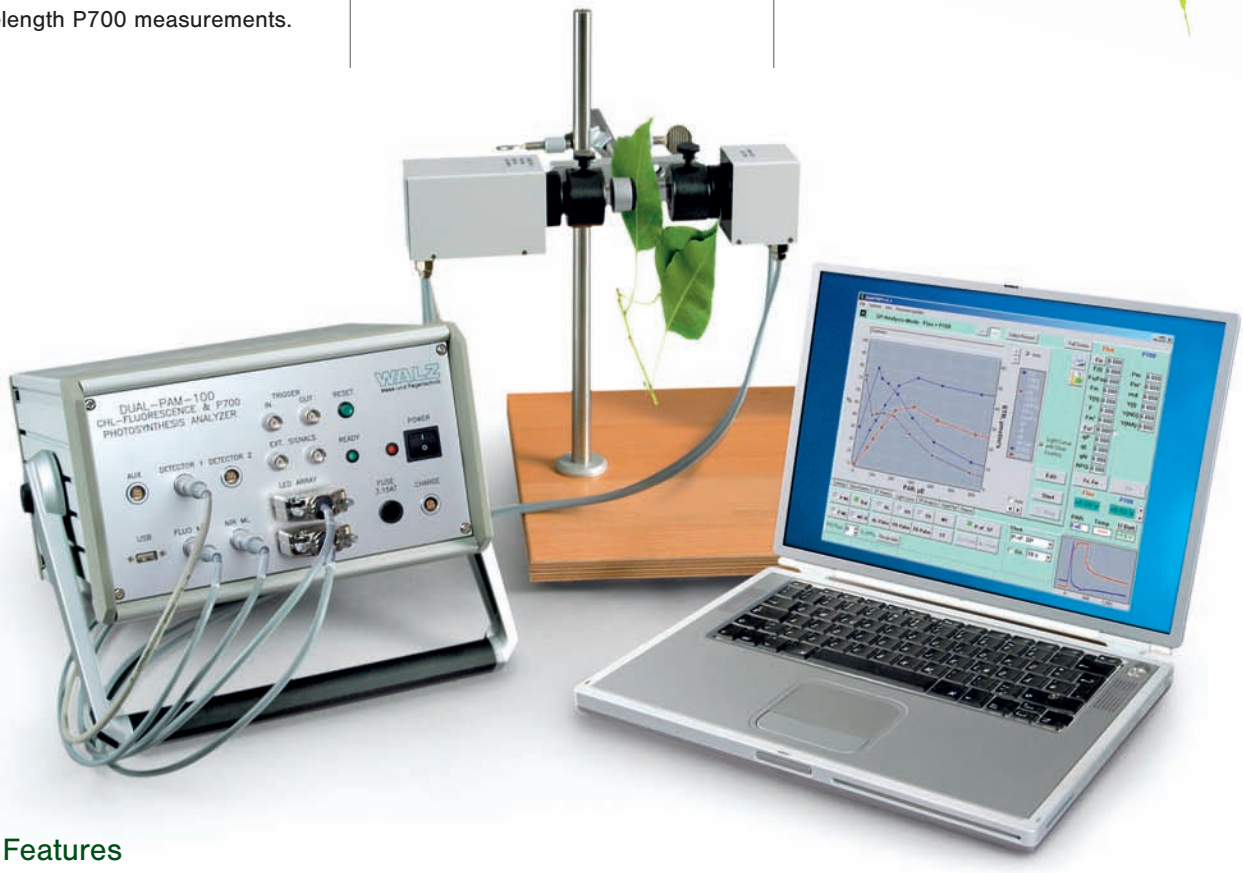
P700 & Chl Fluorescence Measuring System

Dual-PAM-100: A measuring system for simultaneous assessment of P700 & Chl Fluorescence

The Dual-PAM-100 measuring system is more than a chlorophyll fluorometer. On one hand, as suggested by its name, it follows the tradition of the original PAM-100 Chlorophyll Fluorometer, which was introduced in 1985 and since then has been serving generations of researchers. On the other hand, it is optimized for simultaneous assessment of PS I via dual wavelength P700 measurements.

Based on a highly innovative pulse-modulation technique, absorbance changes of P700 (reaction center chlorophyll of PS I) are measured with a similar signal/noise ratio as Chl fluorescence.

The Saturation Pulse method was extended to provide analogous information on PS I via P700 as is obtained via Chl fluorescence on PS II. Furthermore, using the same control unit additional emitter-detector modules can be applied for measuring other key photosynthesis parameters.



Features

- Successor of the well-known PAM-101/102/103 system
- Dedicated DualPAM Windows software
- Easy operation via automated measuring routines
- Integrated red, blue, far-red actinic LED lamps and saturating Single and Multiple Turnover flash lamps

- Highly compact due to innovative opto-electronics
- Various optical configurations for measurements with leaves and suspensions
- Simultaneously measured PS I and PS II quantum yields
- Saturation pulse method for assessment of PS I and PS II parameters

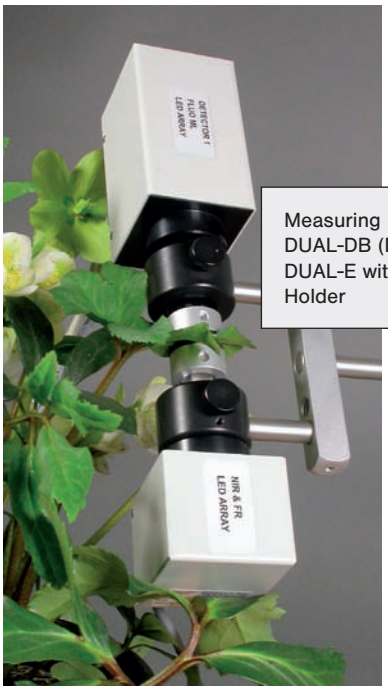
- Detailed information also on cyanobacterial photosynthesis
- Optional emitter-detector modules for measuring other key photosynthesis parameters (ΔpH, membrane potential, NADPH)
- Alternative detectors for sensitive fluorescence measurements using suspensions

System Features

Dual-PAM-100: A measuring system with unprecedented properties

The Dual-PAM-100 is unique in combining ease of operation with an exceptionally wide range of possible applications. On one hand, all types of measurements so-far carried out with the PAM-101/102/103 and its extensive accessories, now can be more easily performed with the Dual-PAM-100.

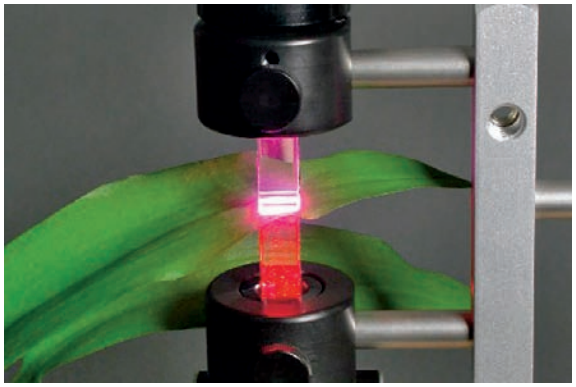
On the other hand, the Dual-PAM-100 opens the way for a wide range of new applications in basic and applied photosynthesis research. Measurements that so far required considerable scientific expertise (e.g. simultaneous assessment of PS I and PS II quantum yields) and therefore have been carried out by a few specialized researchers only, now have become sufficiently easy to be used as a general tool even by non-specialists.

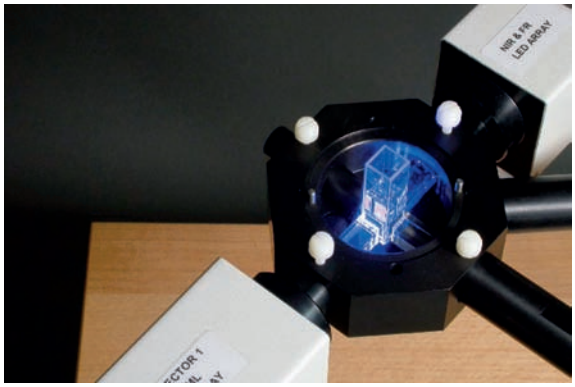


Measuring Heads DUAL-DB (DR) and DUAL-E with Leaf Holder

Major advancements with respect to the PAM-100 measuring system are:

- The Dual-PAM-100 is fully computer-controlled using the integrated data acquisition Windows software DualPAM specifically developed for this measuring system.
- The DualPAM software not only allows very comfortable system operation, but also provides many pre-programmed measuring routines for optimal and reproducible assessment of photosynthesis parameters.
- So-called Script files can be programmed, with which sequential measurements of numerous parameters (via Light Curves, Slow and Fast Kinetics etc.) can be automated.
- All essential light sources (fluorescence excitation light, NIR P700 measuring light, red and blue actinic light, single and multiple turnover saturating flashes, far red light) are integrated in the basic system.
- Due to various novel opto-electronic components, specially developed for the Dual-PAM-100, the Emitter-Detector units with integrated light sources are very compact and easy to handle.
- An extremely wide range of Measuring Light frequencies (1 Hz to 400 kHz) is provided, so that the same Measuring Light can be applied for assessment of Fo and for the recording of fast kinetics (e.g. polyphasic fluorescence rise or flash relaxation kinetics).
- While the system allows numerous combinations of instrument settings, particular combinations may be saved in User Settings files, that may be opened at any later time to reproduce a particular experiment.
- All light sources can be switched with 2.5 µs time resolution under software control.
- Chl fluorescence and P700 pulse modulated measuring lights are synchronized and the two signals are measured with the same detector without any mutual disturbance.
- Special attention is paid to work with cyanobacteria: Efficient excitation of PS II fluorescence by red light and preferential excitation of PS I by blue or far-red light are provided.

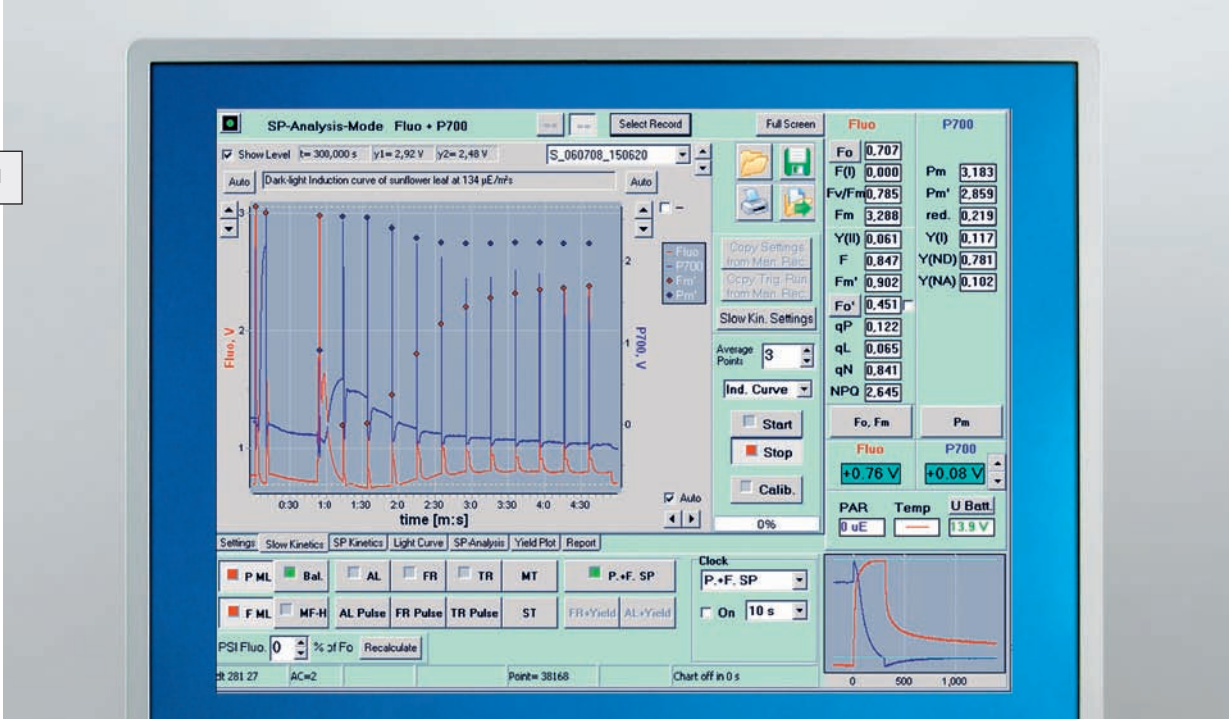




DualPAM Software

User Surface and Applications

Fig. 1



Dual-Channel Fluo and P700 measurement

While the Dual-PAM-100 may be used for high performance Single Channel Fluo or P700 measurements, a new quality of performance is obtained by simultaneous Dual Channel Fluo and P700 measurements. For this purpose, the Saturation Pulse Method originally developed for chlorophyll fluorescence quenching analysis *(Schreiber et al., Photosynth. Res.10:51-62, 1986)* was extended for assessment of PS I quantum yield *(Klughammer and Schreiber, Planta 192:261-268, 1994)*.

A special routine for Pm-determination was introduced (see Fig.2) in analogy to Fo, Fm determination. When Pm is known, in any given state the relative extent of P700 reduction, P700red., and the PS I quantum yield, Y(I), can be assessed. In analogy to Fm' the parameter Pm' can be determined with the help of a Saturation Pulse. Furthermore, the concept of excitation energy partitioning originally conceived for PS II by *Kramer et al. (2004) Photosynthesis Research 79: 209-218*, was adopted for PS I.

The simultaneously measured Fluo and P700 responses (see Fig.1) reflect the interplay of the consecutive light reactions of PS II and PS I that are connected via the intersystem electron transport chain.

The same transthylakoidal ΔpH that induces nonphotochemical quenching of Fm' with respect to Fm, causes P700 oxidation. After light activation of CO_2 fixation and subsequent ATP consumption in the Calvin-Benson cycle the ΔpH relaxes, as indicated by parallel rereduction of P700 and relaxation of nonphotochemical quenching (increase of Fm').

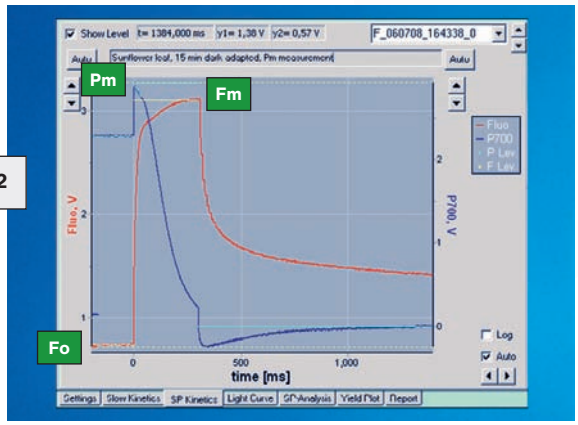


Fig. 2

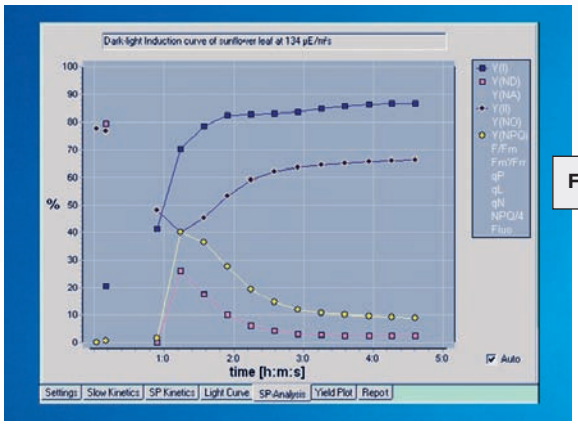


Fig. 3

Pm and Fm determination (Fig. 2)

Analysis of PS I parameters is based on a special routine for assessment of the maximal P700 change (Pm determination), which involves pre-illumination by far-red (or blue in case of cyanobacteria) and a Saturation Pulse that induces maximal P700 oxidation followed by full reduction. The Pm determination is analogous to Fo, Fm determination.

Note: P700 signal quality matches that of fluorescence even at high time resolution and signal drift is negligibly small. Hence, using the Dual-PAM-100 the P700 signal is fully equivalent to the fluorescence signal.

Saturation Pulse Analysis (Fig. 3)

Based on the original concept of excitation energy partitioning of Kramer et al. three complementary quantum yields are defined for PS I in analogy to PS II:

- Y(I) = 1 - Y(ND) - Y(NA)
- Y(I), photochemical quantum yield of PS I
- Y(ND), quantum yield of nonphotochemical energy dissipation in PS I due to donor side limitation
- Y(NA), quantum yield of nonphotochemical energy dissipation in PS I due to acceptor side limitation
- Y(II) = 1- Y(NPQ) - Y(NO)
- Y(II), photochemical quantum yield of PS II
- Y(NPQ), quantum yield of regulated energy dissipation in PS II
- Y(NO), quantum yield of non-regulated energy dissipation in PS II

Trigger and Settings files (Fig. 4)

The Dual-PAM-100 combines high flexibility of pre-programmed measuring parameters with user friendly software. For example, a special SP Trigger window is provided for programming the Saturation Pulse for simultaneous P700 and Fluo analysis. Triggering events can be programmed with 2.5 µs resolution.

Note: For different applications an unlimited number of Trigger files and User Settings files can be saved. In this way all instrument settings can be reliably reproduced at any time in future experiments.

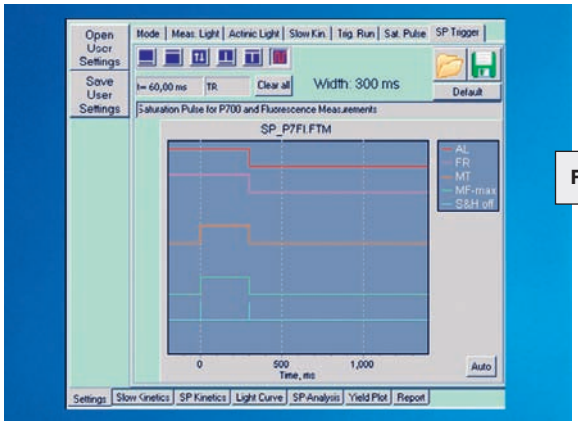


Fig. 4

DualPAM Software

User Surface and Applications

Fig. 5

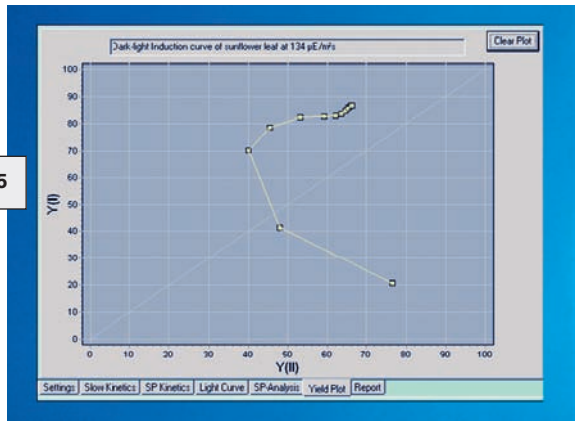
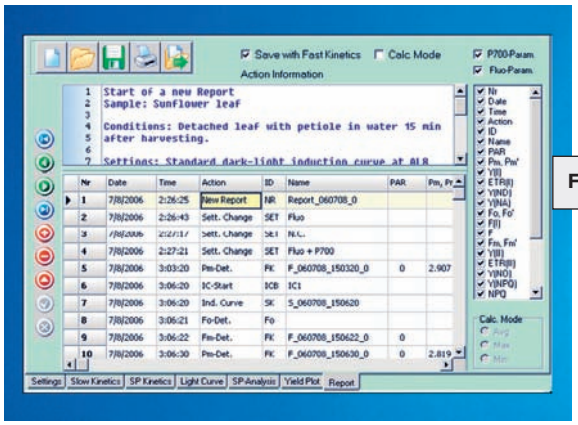


Fig. 6



Yield Plot (Fig. 5)

The simultaneously measured quantum yields Y(I) and Y(II) are automatically plotted against each other in the Yield Plot window. The depicted example is based on the original Slow Kinetics recording of the dark-light Induction Curve in Fig. 1.

Any deviation of the plotted points from the 1:1 line reflects an apparent imbalance of the two photosystems, undergoing dynamic changes during the light induction process.

Report (Fig. 6)

All data are automatically saved in an extensive Report file, from where they can be stored on Hard Disk or exported into a spread-sheet program (like Excel). All changes of Settings are documented.

The Report includes Slow Kinetics recordings as well as the Fast Kinetics files for each individual Saturation Pulse, thus allowing very thorough analysis of the saved data. The Report can be edited by the user. Explanatory comments can be added.

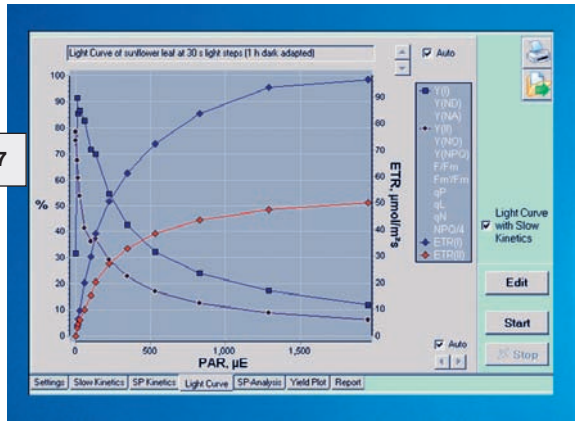
Light Curve (Fig. 7)

Light response curves provide 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.

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.

The DualPAM software also supports special "Light Curves" involving the automated assessment of Fast Kinetics as a function of the state of illumination.

Fig. 7



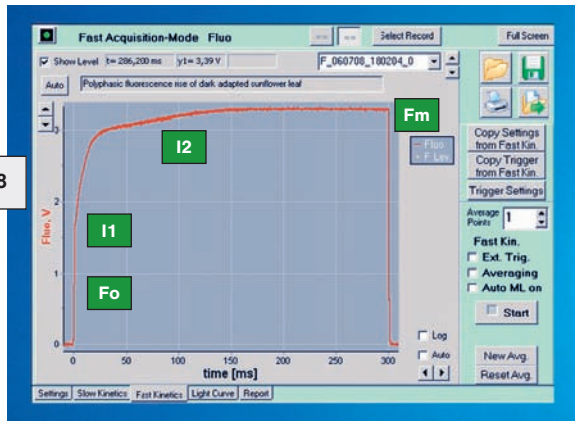


Fig. 8

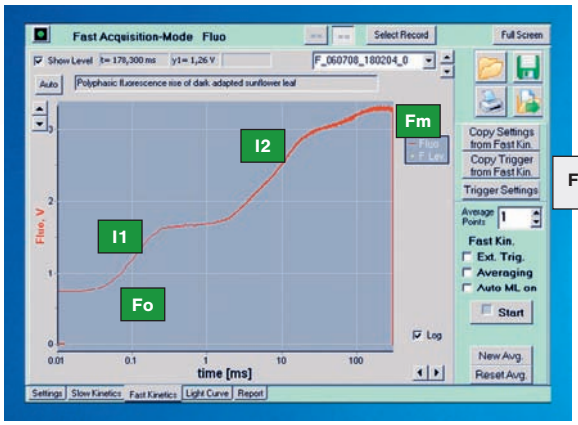


Fig. 9

Fast Kinetics, linear time scale (Fig. 8)

The Polyphasic Fluorescence Rise upon onset of continuous saturating light is measured at maximal frequency (400 kHz) of pulse-modulated Measuring Light in the Single Channel Fast Acquisition mode.

The various rise phases (Fo-I1, I1-I2 and I2-Fm) reflect different electron transfer steps in PS II. The Trigger Settings for switching on/off Measuring Light and maximal frequency are preprogrammed for optimal performance (see Fig. 4).

The Dual-PAM-100 offers a special routine to pre-oxidize the PQ-pool by defined far-red preillumination in order to assure reliable assessment of fluorescence parameters. Without definition of the PQ redox state interpretation of the polyphasic rise is problematic. On the other hand, by comparison of the kinetics +/- FR the momentary PQ redox state can be evaluated.

Fast Kinetics, log time scale (Fig. 9)

A log time scale can be applied for assessment of the rapid part of the Polyphasic Fluorescence Rise. The Fo level is displayed as a pronounced step. At the given intensity of the saturating light the half-rise time of Fo-I1 (photochemical phase) is about 100 µs. The I1 level is characterized by another pronounced step, followed by the "thermal" I1-I2 and I2-Fm phases.

Evaluation of the various phases provides valuable information on the optical cross-section of PS II and the state of donor and acceptor sides.

The Fo, I1, I2 and Fm levels are analogous to the O, J, I and P-levels defined by Strasser and co-workers. These levels, however, are not necessarily identical, due to technical differences between the applied devices (fluorescence excitation, intensity of saturating light etc.).

Slow Kinetics and Triggered Run (Fig. 10)

Besides standard Induction Curves (see Fig.1) and manually controlled "chart recordings", the Dual-PAM-100 also supports so-called Triggered Runs, which involve the triggering of various light sources at defined times after Run-start. Triggered Runs can be derived from manually triggered recordings and edited by the user.

Fig.10 shows a Triggered Run of a P700 measurement for assessment of the intersystem pool size involving Single and Multiple Turnover flashes in the presence of far-red background light.

In addition, the DualPAM software also allows to program more extended so-called Script files, which may involve all actions that can be carried out manually (i.e. also switching between different modes of data acquisition, measuring Induction/Light Curves and Fast Kinetics etc.).

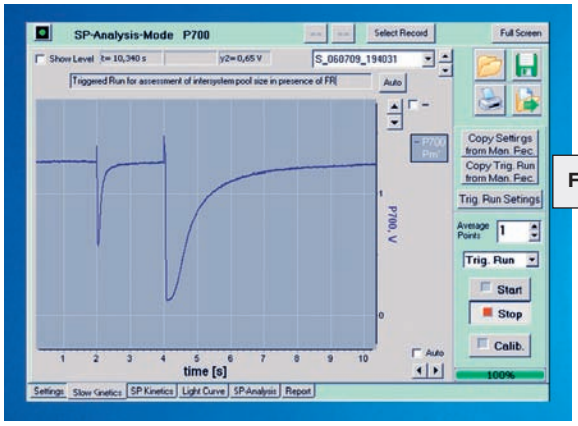


Fig. 10

Different Emitter-Detector Configurations

Various optical configurations

Depending on application, the standard Emitter Unit DUAL-E and Detector Unit DUAL-DR (or Dual-DB) can be connected either to a Leaf Holder (see p.03) or to the Optical Unit ED-101US/MD for suspensions (see p.03). While Fluo is measured from the surface of the sample, P700 normally is measured in the transmission mode.

For measurements with algae, cyanobacteria or chloroplast suspensions the standard emitter-detector units DUAL-E and DUAL-DR (or DB) can be combined with various alternative detector units and/or emitter-detector modules (see below).

A special Optical Unit DUAL-EDF for simultaneous P700 and Fluo measurements from the surface of opaque samples will soon be available.

This system applies the same highly flexible fiberoptics as the PAM-2100 fluorometer, so that also the well-proven Leaf Clip Holder 2030-B can be connected.

PAM-2100 Fiberoptics with Leaf Clip Holder 2030-B

Alternative fluorescence detectors

The standard Detector Unit DUAL-DR (or DUAL-DB) features a photodiode serving at the same time for Fluo and P700 detection. In this configuration, only part of overall fluorescence and transmitted P700 measuring light reaches the detector via a hole in the Chip-On-Board (COB) LED-Array that provides actinic illumination.

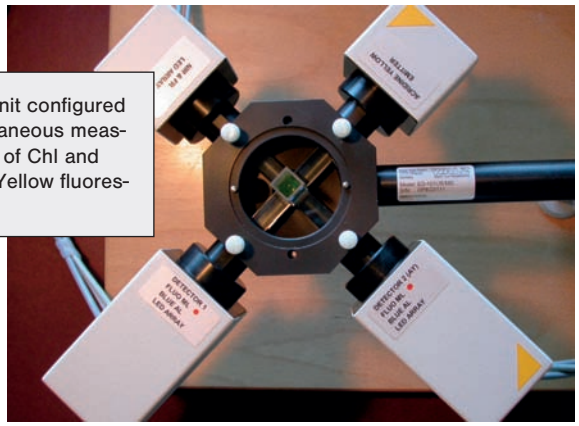
For high sensitivity fluorescence measurements the alternative Photodiode Detector DUAL-DPD and Photomultiplier Detector DUAL-DPM are available, which lack the COB LED-Array and allow free choice of filters. These detectors can be connected to the Optical Unit ED-101US/MD for measurements with suspensions, preferentially at right angle with respect to fluorescence excitation.

Alternative Emitter-Detectors modules

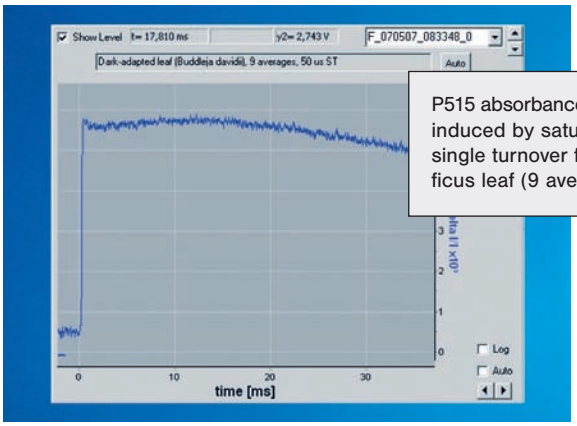
The Dual-PAM-100 is configured for simultaneous measurements of one single-channel fluorescence signal and one dual-channel difference signal. While the latter normally corresponds to the 870-830 nm P700 signal, other wavelength couples may be configured, like 550-520 nm for detection of the P515 change (electrochromic absorbance shift), using the Detector 2 input at the Dual-C Control Unit.

Furthermore, fluorescence can be measured in the dual-wavelength excitation mode. Hence, it is possible to measure Chl fluorescence and other types of fluorescence simultaneously. Alternative Emitter-Detector modules for P515 absorbance, NADPH/9-AA fluorescence and Acridine Orange/Acridine Yellow fluorescence (for ΔpH measurement) are available.

As the Dual-Wavelength modulation measuring principle can be applied for measuring numerous other optical signals (e.g. light scattering, zeaxanthin de-epoxidation, UV-excited fluorescence etc.) further Emitter-Detector units may be expected to become available in the future, depending on customer demand.



Optical Unit configured for simultaneous measurements of Chl and Acridine Yellow fluorescence



P515 absorbance change induced by saturating single turnover flash in ficus leaf (9 averages)

Technical Specifications

Power-and-Control-Unit DUAL-C

- **Microcontroller:** 2x AVR-RISC (8MHz) + 4MB SRAM; 256000 data points with 12 bit resolution can be stored
- **PC interface:** USB 1.1 and USB 2.0 compatible
- **User interface:** Pentium PC with DualPAM Software
- **Power supply:** Rechargeable sealed lead-acid battery 12 V/2 Ah; Battery Charger MINI-PAM/L (100 to 240 V AC)
- **Power consumption:** Basic operation 160 mA
- **Dimensions:** 31 cm x 16 cm x 33.5 cm (W x H x D), with carrying handle
- **Weight:** 4.5 kg

Measuring Head with P700 NIR Emitter DUAL-E

- **P700-Dual-Wavelength-Emitter:** 830 and 870 nm
- **Far-Red LED lamp:** 720 nm
- **Chip-On-Board LED Array:** 635 nm for continuous actinic illumination, max. 2000 µmol m^{-2} s^{-1} PAR; saturating Single Turnover flashes, max. 200000 µmol m^{-2} s^{-1} PAR, adjustable between 5 and 50 µs; Multiple Turnover flashes, max. 20000 µmol m^{-2} s^{-1} PAR, adjustable between 1 and 1000 ms
- **Dimensions:** 10.5 cm x 5.5 cm x 7 cm (L x W x H)
- **Weight:** 400 g (incl. cables, 1 m long)

Measuring Head with Detector DUAL-DB (blue) or DUAL-DR (red)

- **Fluorescence emitter:** 460 nm (DUAL-DB); or 620 nm (DUAL-DR)
- **Blue LED lamp:** 460 nm for blue actinic illumination, max. 700 µmol m^{-2} s^{-1} PAR
- **Chip-On-Board LED Array:** identical to that of DUAL-E Measuring Head
- **Signal detection:** PIN photodiode with special Pulse Preamplifier for measuring P700 and fluorescence changes with max. time resolution of 10 µs
- **Dimensions:** 15 cm x 5.5 cm x 7 cm (L x W x H)
- **Weight:** 500 g (incl. cables, 1 m long)

Windows-Software DualPAM

- **PC Requirement:** 1 free USB socket; 128 MB RAM (minimum); Windows XP or Vista

Optical Unit for Suspensions ED-101US/MD (optional)

- **Design:** Black-anodized aluminum body with central 10x10 mm standard glass cuvette; for attachment of Measuring Heads DUAL-DB (or DUAL-DR) and DUAL-E and Miniature Magnetic Stirrer US-MS; additional ports for attachment of two additional Measuring Heads (e.g. Acridine Yellow, and NADPH fluorescence)
- **Mounting:** On Stand with Base Plate ST-101
- **Weight:** 750 g

Temperature Control Block ED-101US/T for Optical Unit ED-101US/MD (optional)

- **Design:** Sectioned block with central 10x10 mm opening to be mounted on top of Optical Unit; to be connected to external flow-through water bath (not included)
- **Weight:** 250 g

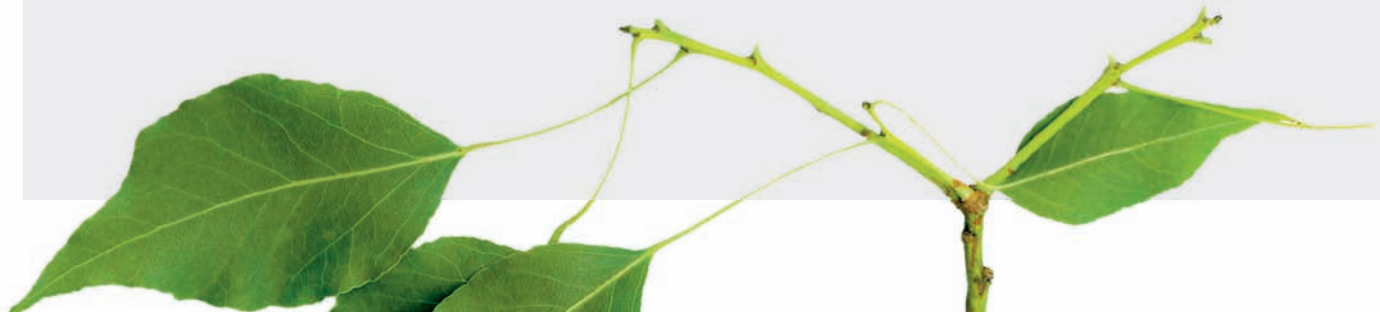
Miniature Magnetic Stirrer US-MS (optional)

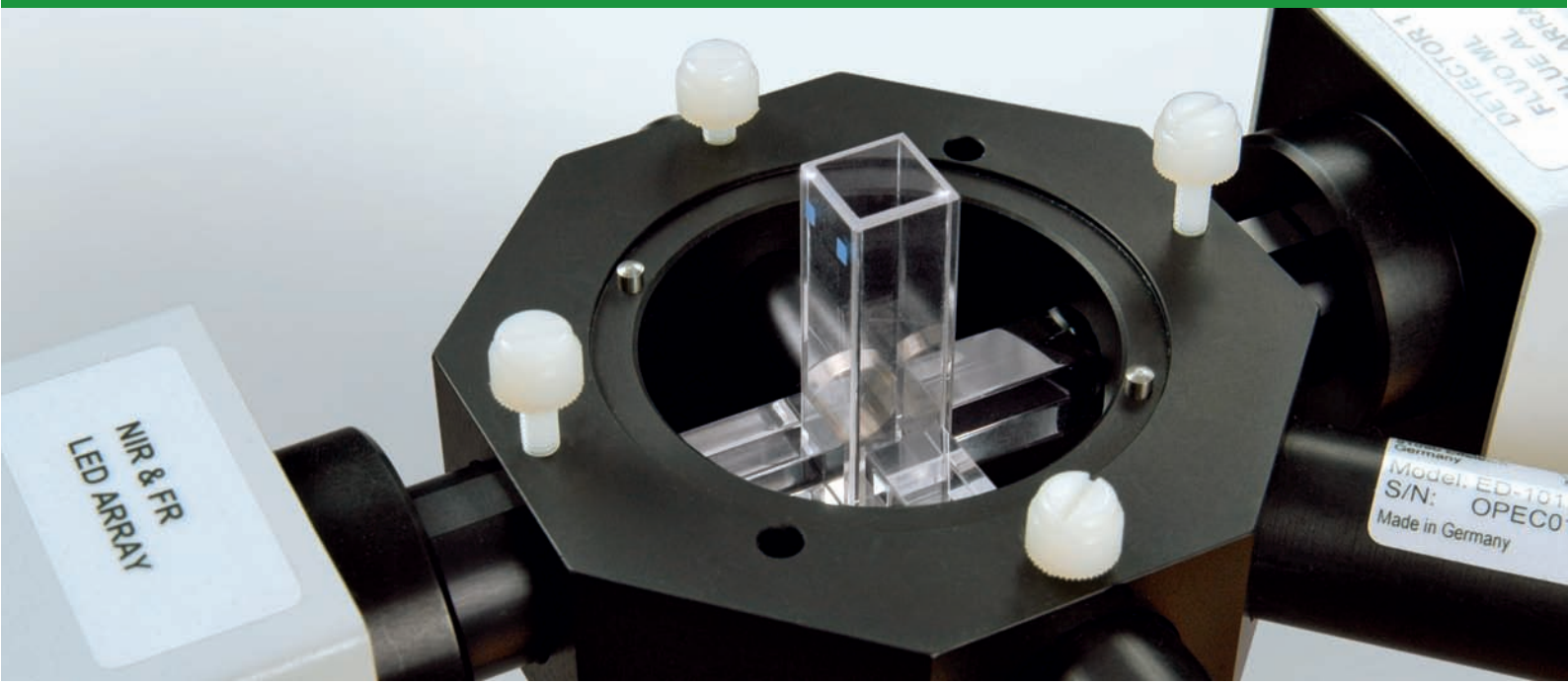
- **Design:** Based on device manufactured by h+p (type Variomag-Mini); featuring adapter to be mounted in bottom port of Optical Unit ED-101US/MD; mains supply (115 or 230 V AC)

Spherical Micro Quantum Sensor US-SQS/WB (optional)

- **Design:** 3.7 mm Ø diffusing sphere coupled to integrated PAR-sensor via 2 mm fiber; compact amplifier unit and special holder for mounting on Optical Unit ED-101US/MD; to be connected to the Power-and-Control-Unit DUAL-C

Subject to change without prior notice





High Quality Instrumentation for Plant Sciences

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