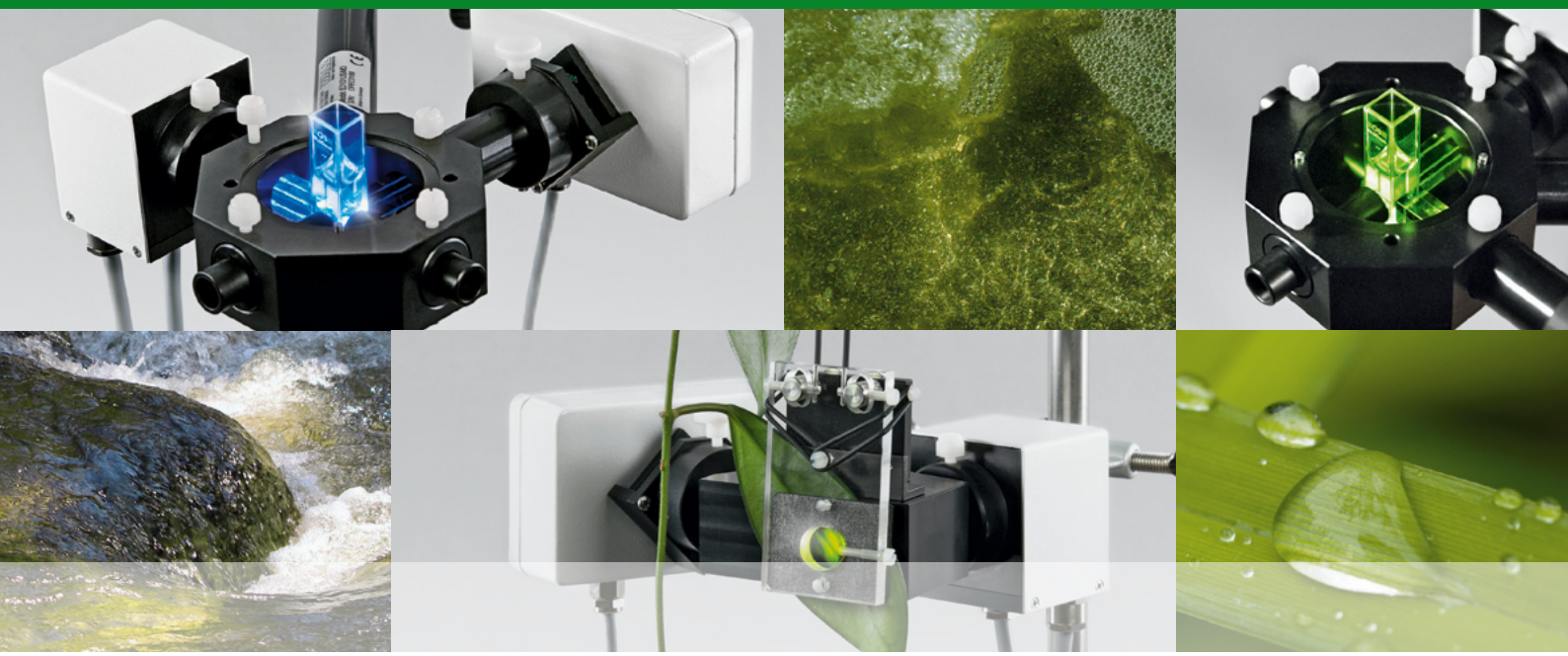
MULTI-COLOR-PAM

Multiple Excitation Wavelength
Chlorophyll Fluorescence Analyzer



Pinpointing Light Color Effects on Photosynthesis

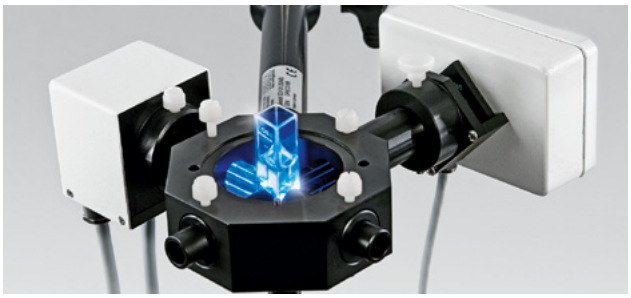
WALZ
Mess- und Regeltechnik

MULTI-COLOR-PAM

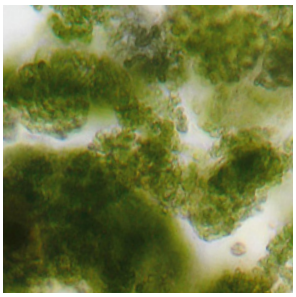
Multiple Excitation Wavelength Chlorophyll Fluorescence Analyzer.
Eliciting pulse-amplitude-modulated (PAM) fluorescence by six different light colors.
Driving photosynthesis by five different light colors or white light.

The MULTI-COLOR-PAM fluorometer provides an extraordinary color range of measuring and actinic light including white and far-red light sources. The various colors enable researchers to tailor conditions of fluorescence measurements exactly to the spectral properties of their sample. The outstanding variety of light colors of the MULTI-COLOR-PAM opens the way to study many wavelength-dependent aspects of photosynthesis that so-far have not been commonly accessible.

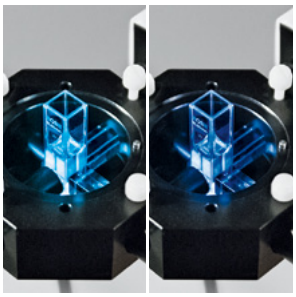
The MULTI-COLOR-PAM is optimized for highly sensitive assessment of photosynthesis in dilute suspensions of cyanobacteria, algae and chloroplasts. In addition, the measuring system can be configured to study leaf photosynthesis and epidermal UV-A screening.



Optical Unit for Suspensions



Photosynthesis in Suspensions



AL/ML 480 nm AL/ML 440 nm

Optical Unit for Leaves

Leaf Photosynthesis, UV-A Screening

AL/ML 540 nm AL White

FEATURES

• The MULTI-COLOR-PAM provides six colors of pulse-modulated measuring light (400, 440, 480, 540, 590 and 625 nm) and five colors of actinic light (440, 480, 540, 590, 625). In addition, white (420-640 nm) and far-red light (730 nm) light sources are built in.

• By employing state-of-the-art LED light sources in combination with elaborated chip-on-board technology, strong actinic photon fluxes are achieved while keeping the emitter unit highly compact. Each measuring light color can be freely combined with one of the available actinic light sources.

• The MULTI-COLOR-PAM measures fluorescence by a highly sensitive photodiode attached to either an optical unit for suspensions or for leaves. A spherical or flat cosine-corrected sensor feeds its signal directly to the control unit so that accurate PAR measurements can be obtained in suspension or at leaf surfaces. A special routine is provided to measure PAR-lists for all colors automatically.

• The MULTI-COLOR-PAM determines the effective PS II quantum yield, Y(II), with outstanding accuracy and, thus, enables highly reliable calculations of relative electron transport rates (rel. ETR) even with extremely stressed samples.

MAIN COMPONENTS

The main components of the MULTI-COLOR-PAM form the working system for fluorescence measurements of suspensions. Various accessories are provided to optimize this basic system for different suspensions and to extent its capacity to measure leaves.

POWER-AND-CONTROL-UNIT MCP-C

The central unit controls the emitter head MCP-E and the photodiode detector MCP-D of the fluorometer. Additionally, it provides connections for a Walz PAR sensor, a stirrer for the optical unit ED-101US/MD, an auxiliary device and 5 V TTL trigger in/out signals. Another socket permits connection of an additional actinic light source. The central unit is connected to a Windows computer via USB.

EMITTER HEAD MCP-E

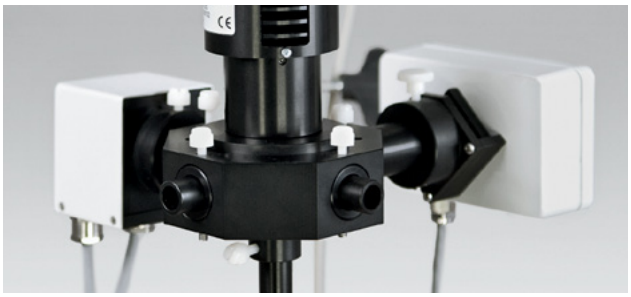
The light sources within the emitter head MCP-E are light emitting diodes (LED) which are densely arranged on a 10 x 10 mm area. In total, the MCP-E head provides 6 differently-colored measuring lights, and 5 spectrally different actinic light sources; the latter are complemented by white and far red LEDs.

DETECTOR HEAD MCP-D

The MCP-D head detects fluorescence by a highly sensitive PIN photodiode with with maximum time resolution of 10 µs. The photodiode is protected from modulated excitation light by a RG 665 long pass filter. Due to the open design of the filter holder, filters can easily changed to vary the spectral range of fluorescence detection.

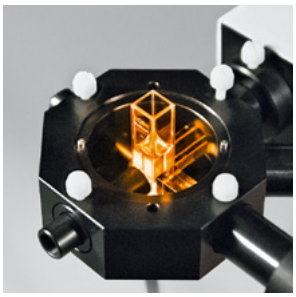
Measuring Setup

Power-and-Control-Unit



Emitter Head (left), Optical Unit for Suspensions (center), Detector Head (right)

AL/ML 625 nm ML 400 nm



AL/ML 590 nm

OPTICAL UNIT FOR SUSPENSIONS ED-101US/MD

The optical unit ED-101US/MD consists of a centrally located place for a 10 x 10 mm cuvette and peripherally positioned ports for the emitter and detector heads of the MULTI-COLOR-PAM.

Perspex rods efficiently guide light from emitters to the sample, and from the sample to the detector.

Two plugs are provided to close ports not occupied by emitter or detector heads. These plugs possess a light reflecting and a light trapping end. The reflecting end may be positioned opposite to the fluorescence detector to enhance the fluorescence signal, the light-trapping part can be positioned opposite to the emitter to reduce effects of strayed light on the signal.

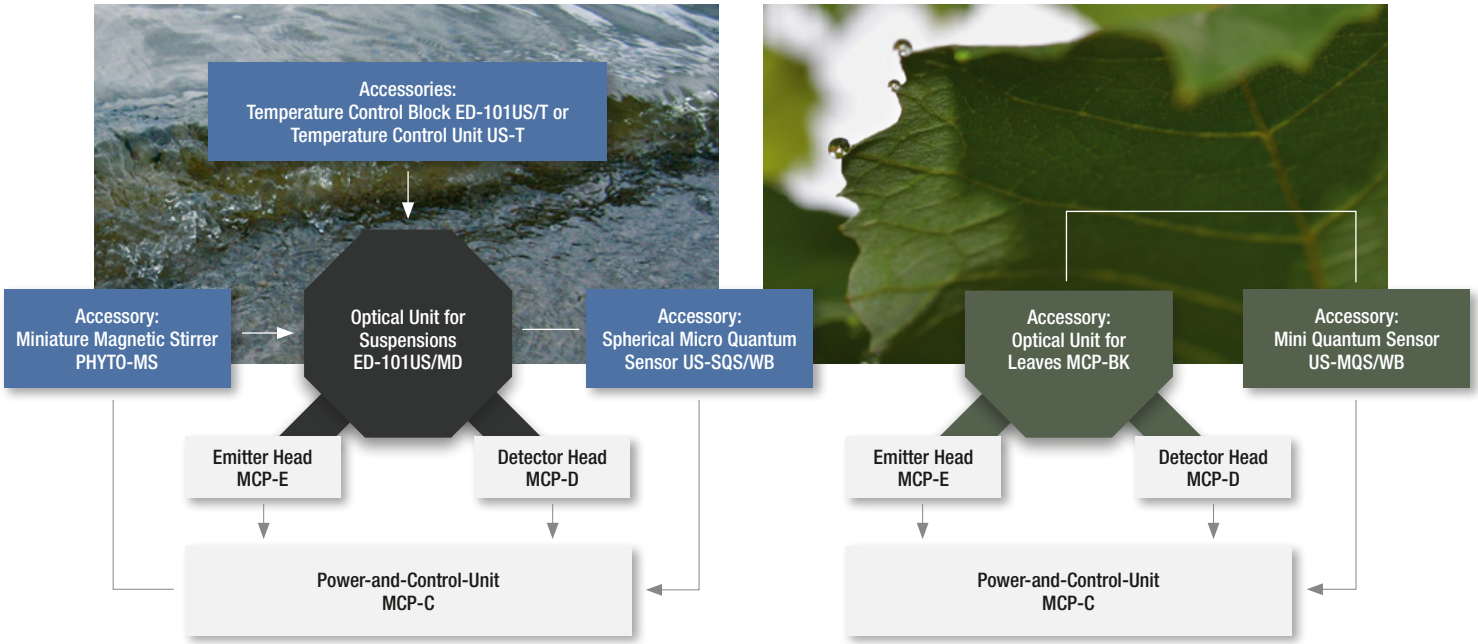
The cover of the unit has a pinhole through which chemicals can be added using a syringe. A port at the bottom of the ED-101US/MD unit holds the optional PHYTO-MS Magnetic Stirrer.

CONFIGURATIONS

Adding accessories to the main components of the MULTI-COLOR-PAM results in configurations suited for special analysis of suspensions and enables measurements of leaves. The modular design of the fluorometer facilitates custom-tailored setups.

Starting from the main components of the MULTI-COLOR-PAM, the system for suspensions can be equipped with stirrer and PAR sensor which both are connected to the main unit of the fluorometer.

Other accessories permit experiments with suspensions at controlled temperature. Leaf measurements require a special optical unit to which a cosine-response PAR sensor can be attached.



CONFIGURATIONS FOR SUSPENSIONS

For measurements of suspensions, the measuring heads, MCP-E and MCP-D, are attached to an optical unit ED-101US/MD which holds in the center a 10 x 10 x 45 mm quartz cuvette. The optical unit guides light efficiently to the sample by a Perspex rod. Similarly, a Perspex rod forms a path with negligible light loss between sample and detector.

The equipment for measurement of suspension is completed by a number of accessories. The software-controlled stirrer PHYTO-MS prevents settling of particles during measurements.

The spherical quantum sensor US-SQS/WB measures light intensity within the suspension and it is a must for measurements of light response curves or functional PS II absorption cross-section.

Temperature control can be exercised using the temperature block ED-101US/T connected to a thermostated water bath and mounted on top of the ED-101US/MD optical unit. Alternatively, the US-T unit can control cuvette temperature by a heat-transfer rod dipped into the suspension.

CONFIGURATIONS FOR LEAVES

For leaf studies, an optical unit MCP-BK equipped with a leaf clip is required. Similarly as in the optical unit for suspensions, Perspex rods provide efficient optical pathways between measuring heads and sample where the rod between emitter head and sample is conically shaped to optimize optical efficiency of the unit.

For the leaf configuration, the recommended accessory is a cosine-response quantum sensor US-MQS/WB which is positioned in a special port of the leaf clip of the optical unit.

ACCESSORIES

TEMPERATURE CONTROL UNIT US-T

The US-T unit consists of a heat-transfer head with a cooling/heating Peltier element, and a separate power-and-control unit. The heat-transfer head is mounted on top of a Walz optical unit ED-101US-type so that the dip of the rod is in touch with the suspension investigated. The achievable temperature spread in suspensions is about 30 K; absolute temperatures depend on ambient temperature.

Temperature Control Unit and Heat-Transfer Head

TEMPERATURE CONTROL BLOCK ED-101US/T

For measurements under defined temperatures, the temperature control block ED-101US/T can be mounted on the optical unit ED-101US/MD. The block consists of an inner flow-through metal part which is slightly pressed on the sample cuvette by a spring mechanism, and an external foam part for temperature insulation. Temperature control is achieved by an external flow-through water bath (not included) connected to the temperature block.

Temperature Control Block Mounted on Optical Unit for Suspension

MINIATURE MAGNETIC STIRRER PHYTO-MS

Settling of particles is prevented by using a miniature magnetic stirrer US-MS. The stirrer is mounted directly beneath the sample cuvette. A rotating magnetic field created by the stirrer tip moves a miniature magnetic stir bar in the cuvette. The stirrer is connected to the control unit MCP-C. Stirring can be switched on and off by the PamWin-3 software.

Miniature Magnetic Stirrer

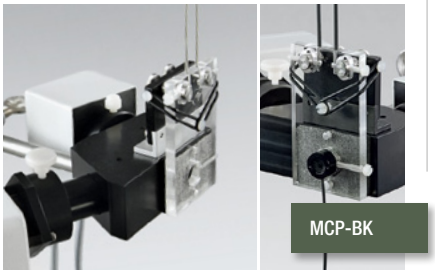
SPHERICAL MICRO QUANTUM SENSOR US-SQS/WB

Exact light measurements in suspensions (but also in air) can be carried out by the spherical micro quantum sensor US-SQS/WB. The sensor has a 3.7 mm diameter sphere as the entrance optics. When the sensor is connected to the control unit MCP-C, data will be acquired and processed by the PamWin-3 software.

Spherical Micro Quantum Sensor and Preamplifier

OPTICAL UNIT FOR LEAVES MCP-BK

This optical unit is designed for measurements of leaves or flat photosynthetic surfaces. The unit features a clip to position leaves optimally for fluorescence measurements. The clip has a port for a Mini Quantum Sensor US-MQS/WB.



Optical Unit for Leaves with Mini Quantum Sensor

MINI QUANTUM SENSOR US-MQS/WB

A cosine-corrected mini quantum sensor measures light intensities which are relevant for plant leaves or flat surfaces. When the sensor is connected to the control unit MCP-C, data will be acquired and processed by the PamWin-3 software.

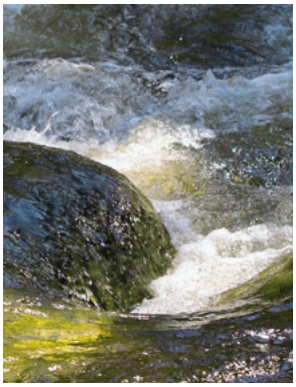
Mini Quantum Sensor and Preamplifier

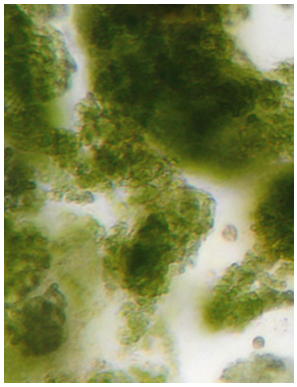
EXAMPLE OF APPLICATION

A particular feature of the MULTI-COLOR-PAM is its capacity to estimate the functional antenna size of photosystem II using an automatic routine build in the PamWin-3 software. The present data demonstrate how this property can be employed to analyze the spectral dependence of photosynthesis.

FIGURE 1

Analysis of the fast fluorescence rise kinetics in saturating light allows determination of the wavelength- and sample-specific functional absorption cross-section of PS II, Sigma(II)$_\lambda$, with which the PS II turnover rate at a given incident PAR can be calculated. Sigma(II)$_\lambda$ is defined for a quasi-dark reference state, thus differing from σ_{PSII} used in limnology and oceanography. The spectral behavior of Sigma (II)$_\lambda$ of *Chlorella vulgaris* and cyanobacteria *Synechocystis* PCC 6803 is shown in Figure 1.





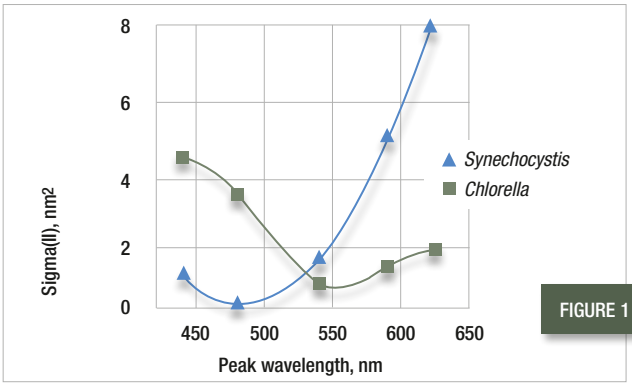


FIGURE 1

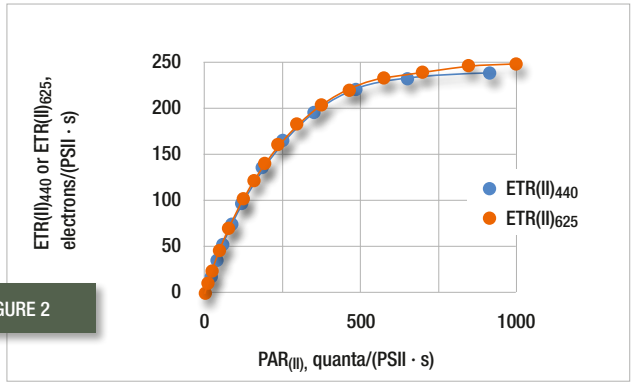


FIGURE 2

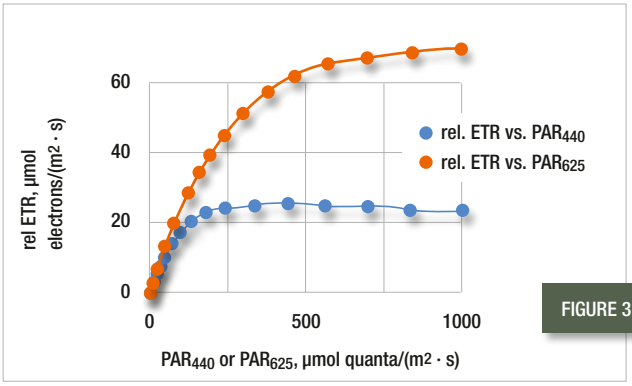


FIGURE 3

FIGURE 2

Based on Sigma(II)$_\lambda$, the PAR in units of µmol quanta/(m^2 · s) can be converted into PAR(II) in units of PS II effective quanta/s. Then, a fluorescence-based electron transport rate ETR(II) = PAR(II) · Y(II)/Y(II)$_{max}$ can be calculated where the Y(II) and Y(II)$_{max}$ are the PS II quantum yields in the light-exposed and the dark state, respectively. Plots of ETR(II) vs PAR(II) for *Chlorella* are almost identical using either 440 nm or 625 nm light. Clearly, ETR(II) in contrast to relative ETR qualifies for quantifying the absolute rate of electron transport in optically thin suspensions of unicellular algae or cyanobacteria.

FIGURE 3

For comparison, vastly different light response curves for electron transport rates (rel. ETR) of *Chlorella* are obtained with light of different colors (440 and 625 nm) when the usual PAR-scale is used.

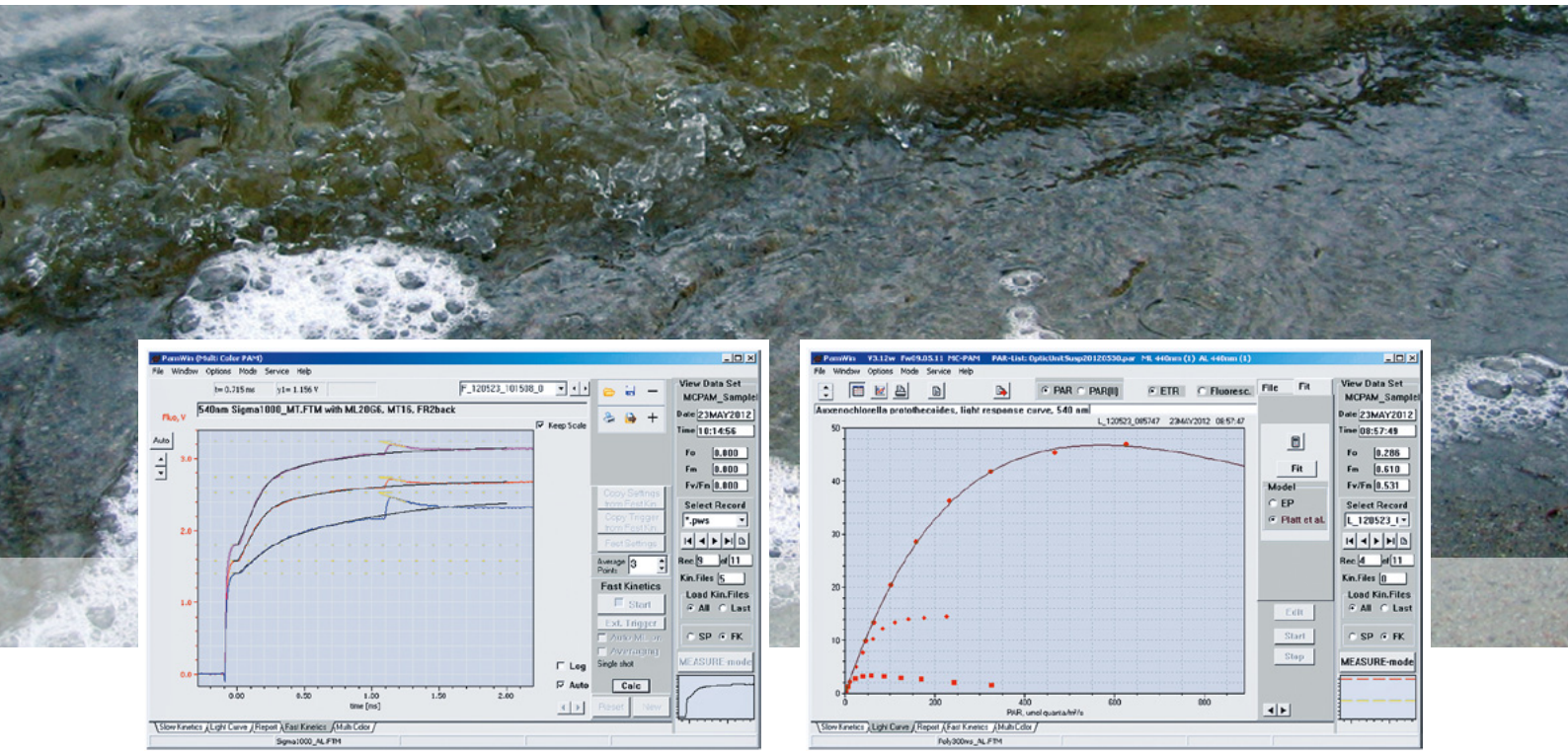
All data from:
Schreiber U., Klughammer C., Kolbowski J. (2012)

Publication on website:

PAMWIN-3 SOFTWARE

Well-proven software extended for control of MULTI-COLOR-PAM and by fitting routines for fast fluorescence induction and decay curves.

The MULTI-COLOR-PAM is controlled by the well-proven PamWin-3 software which also operates the PAM-2500 chlorophyll fluorometer. The software runs on PCs with operating systems Windows XP/ Vista, Windows 7 and 8. PamWin-3 calculates all well-established parameters of saturation pulse analysis (Fv/Fm and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate)). Also, PamWin-3 carries out polyphasic rise kinetics (0-I_1-I_2-P or O-J-I-P) and fast fluorescence decay kinetics.



Fast Kinetics window: Fast $F_0 \rightarrow I_1$ fluorescence rise

Light Curve window: Relative electron transport rates versus light intensity

AUTOMATED MEASUREMENTS

The PamWin-3 software offers new routines to simplify everyday work. Light intensities of actinic and measuring light, as well as for saturation pulses are measured automatically for all light colors. Also, the level of signal background can be measured automatically at different gain factors for all colors and intensities of measuring light.

DESIGN OF EXPERIMENTS

Fluorescence induction and light response curve experiments can be easily configured. Complex experimental designs can be conducted automatically and reproducibly using the script file feature of PamWin-3. For studies of fast fluorescence changes, a special graphical interface permits exact timing of light and measuring conditions. Automatic averaging of repeated fast kinetics yields excellent signal quality even for poorly fluorescing samples.

FITTING ROUTINES

Tools for data fitting include analysis of light curves by 2 different models and estimation of the light curve cardinal parameters α, I_k, and ETR_{max}. A first-time feature is analysis of fast fluorescence rise kinetics to derive wavelength-specific information on functional antenna size of PS II.

TECHNICAL SPECIFICATIONS

BASIC EQUIPMENT

POWER-AND-CONTROL-UNIT MCP-C

General design: Microcontroller: 2 x AVR-RISC (8 MHz) + 4 MB SRAM; 256000 data points with 12 bit resolution can be stored

Sockets: 2 sockets for measuring light and actinic light of MCP-E Emitter Head, socket for signal detection by MCP-D Detector Head, charge socket or Battery Charger MINI-PAM/L, output socket for PHYTO-MS Miniature Magnetic Stirrer, BNC sockets for 5 V trigger-in and trigger out signals, input socket for US-SQS/WB Spherical Micro Quantum Sensor or US-MQS/WB Mini Quantum Sensor, input socket for auxiliary devices, connector for optional PS I lamp, USB socket

Communication: USB 2.0 and USB 3.0 compatible

User interface: Windows computer with PamWin-3 software

Power supply: Rechargeable sealed lead-acid battery 12 V/2 Ah; Battery Charger MINI-PAM/L (100 to 240 V AC)

Dimensions: 31 cm x 16 cm x 33.5 cm (W x H x D), aluminum housing with carrying handle

Power consumption: Basic operation 1.6 W, 8 W with all internal light sources operated at maximum output. Saturation Pulse at maximum intensity, 30 W

Weight: 2.5 kg (including battery)

Operating temperature: -5 to +40 °C

DETECTOR HEAD MCP-D

Signal detection: PIN photodiode with special pulse preamplifier for measuring fluorescence changes with maximum time resolution of 10 µs

Filter box: For up to 14 mm filter thickness

Standard detector filter: long-pass filter > 650 nm (3 mm RG 665) plus short-pass filter SP 710

Dimensions: 6.9 cm x 9.8 cm x 6.4 cm (L x W x H)

Weight: 355 g (incl. cables, 1 m long)

OPTICAL UNIT FOR SUSPENSIONS ED-101US/MD

Design: Black-anodized aluminum body with central 10 x 10 mm standard glass cuvette; for attachment of Measuring Heads MCP-E and MCP-D, and Miniature Magnetic Stirrer PHYTO-MS; two additional ports for attachment of two additional devices

Weight: 750 g

TRANSPORT BOX PHYTO-T

Design: Aluminum box with custom foam packing for MULTI-COLOR-PAM and accessories

Dimensions: 60 cm x 40 cm x 34 cm (L x W x H)

Weight: 5 kg

STAND ST-101

Stand for mounting the Optical Units ED-101US/MD (suspensions) or MCP-BK (leaves)

EMITTER HEAD MCP-E

Chip-on-board multi-wavelength measuring light LED emitter: 400, 440, 480, 540, 590, and 625 nm for pulse-modulated measuring light; 20 intensity settings and 14 settings of pulse frequency

Chip-on-board multi-wavelength actinic LED array: 440, 480, 540, 590, 625 and 420-640 nm (white) for continuous actinic illumination, max. 4000 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. 12000 µmol m^{-2} s^{-1} PAR, adjustable between 1 and 800 ms

Far-Red LED: peak wavelength 725 nm

Dimensions: 10.5 cm x 5.5 cm x 7 cm (L x W x H)

Weight: 500 g (incl. cables, 1 m long)

BATTERY CHARGER MINI-PAM/L

Input: 90 to 264 V AC, 47 to 63 Hz

Output: 19 V DC, 3.7 A

Operating temperature: 0 to 40 °C

Dimensions: 15 cm x 6 cm x 3 cm (L x W x H)

Weight: 300 g

BASIC EQUIPMENT

SYSTEM CONTROL AND DATA ACQUISITION

Software: PamWin-3 System Control and Data Acquisition Program (Microsoft Windows XP SP2/Vista/Windows 7 and 8) for operation of measuring system via PC, data acquisition and analysis.

COMPUTER REQUIREMENTS

Processor, 0.8 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0. Operating system: Microsoft Windows XP SP2/Vista/Windows 7 and 8.

FITTING ROUTINES

Fitting routine for fast fluorescence rise from 0 to the I1 level to determine functional absorption cross-section of PS II and PS II-specific electron transport rates. Fitting of fluorescence decay after light-to-dark transition by up to three exponentials to estimate primary electron transfer rates. Fitting routine for determination of the cardinal points α, I_k and ETR_{max} of light curves.

SATURATION PULSE ANALYSIS

Measured: Ft, Fo, Fm, F, Fo' (also calculated), Fm'. Fast polyphasic rise and decay kinetics (time resolution up to 10 µs). PAR using Spherical Micro Quantum Sensor US-SQS/WB or Mini Quantum Sensor US-MQS/WB.

Calculated: Fo' (also measured), Fv/Fm and Y(II) (maximum and effective photochemical yield of PS II, respectively), q_L, q_P, q_N, NPQ, Y(NPQ), Y(NO) and ETR (electron transport rate), C/Fo (constant fraction of Fo not constituting PS II chlorophyll fluorescence).

ACCESSORIES

TEMPERATURE CONTROL UNIT US-T

Consists of Power and Control Unit US-T/DR with separate AC power supply and Peltier-Heat-Transfer Rod US-T/DS. Temperature range: 12 K below ambient temperature, 15 K above ambient temperature. To be mounted on top of 10 x 10 mm cuvette.

TEMPERATURE CONTROL BLOCK ED-101US/T

Sectioned block with central 10 x 10 mm opening to be mounted on top of the ED-101US/MD unit; to be connected to external flow-through water bath (not included), weight: 250 g.

MINIATURE MAGNETIC STIRRER PHYTO-MS

Type Variomag Mini, with adapter for bottom port of Optical Unit ED-101US/MD and connector to plug into Power-and-Control-Unit MCP-C. Control via MCP-C and PamWin-3 software.

SPHERICAL MICRO QUANTUM SENSOR US-SQS/WB

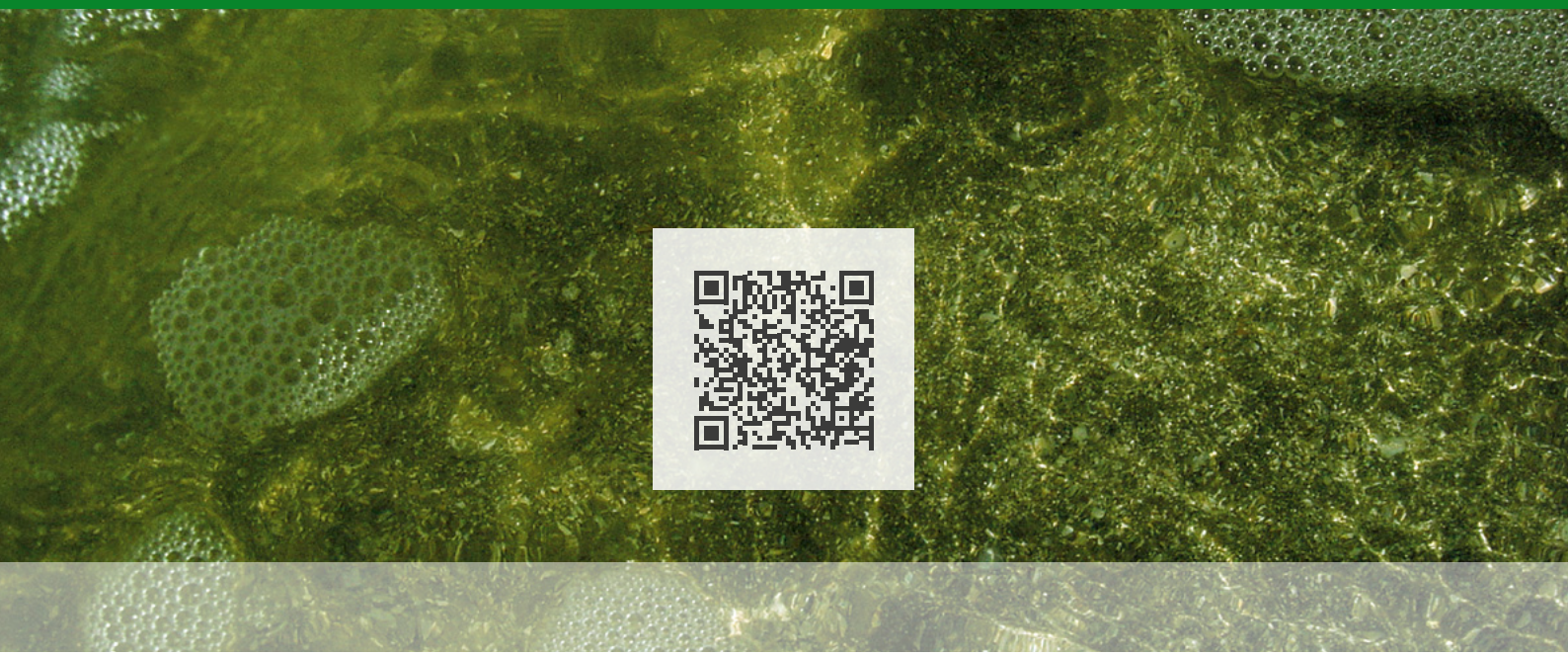
3.7 mm diffusing Plexiglas sphere coupled to integrated PAR-sensor via 2 mm fiber, compact amplifier unit and special holder for mounting on Optical Unit ED-101US/MD; to be connected to Power-and-Control-Unit MCP-C.

MINI QUANTUM SENSOR US-MQS/WB

Consisting of cosine-corrected PAR-sensor (housing: diameter 14 mm, height 16 mm; diffuser diameter 5.5 mm) with compact amplifier. To be connected to the Power-and-Control-Unit MCP-C. For measuring incident PAR, when using the Optical Unit for Leaf Measurements MCP-BK.

OPTICAL UNIT FOR LEAF MEASUREMENTS MCP-BK

Featuring optical ports for mounting Measuring Heads MCP-E and MCP-D; including leaf clip holder, with opening for fixing Mini Quantum Sensor US-MQS/WB in the leaf plane. To be mounted on stand.



High Quality Instrumentation for Plant Sciences

Heinz Walz GmbH
Eichenring 6
91090 Effeltrich
Germany

Tel.: +49-(0)9133/7765-0
Fax: +49-(0)9133/5395
E-mail: info@walz.com
Internet: www.walz.com

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