

MINI-PAM-II

Photosynthesis Yield Analyzer

Featuring fully digital leaf clip, touchscreen, and far red LED



Setting a New Benchmark for Photosynthesis Research in the Field

WALZ
Mess- und Regeltechnik

MINI-PAM-II

HIGHLY PORTABLE, ROBUST AND COMPACT PAM CHLOROPHYLL FLUOROMETER

Microsecond timing enables the MINI-PAM-II fluorometer to use the same LED as source for PAM measuring light, actinic light and saturation pulses. Measuring light corresponds to μ s flashes of constant amplitude, actinic light is quasi-constant light employed to drive photosynthesis, and saturation pulses temporarily saturate primary photosynthesis so that all photosystem II reaction centers are “closed”.

Being a PAM fluorometer, the MINI-PAM-II device records only the fluorescence elicited by measuring light. Fluorescence excited by internal actinic light, saturation pulses or constant external light, like sun

radiation, is not measured. Therefore, the MINI-PAM-II determines how environmental factors modulate the efficiency of conversion of measuring light into fluorescence. These “PAM fluorescence data” are required to retrieve information on primary photosynthesis like the photosynthetic efficiency of photosystem II, $Y(II)$.

A second LED in the MINI-PAM-II emits far red light. This LED preferably excites photosystem I but is negligibly absorbed by photosystem II. A special measuring routine uses this far red LED to determine the F_0' fluorescence level which is important to correctly assess the reduction state of photosystem II reaction centers.

In experiments using internal actinic light, the light intensity at sample level can be monitored online using an internal light sensor. This internal sensor must be calibrated against an external light sensor.



► FEATURES

- The new MINI-PAM-II fluorometer combines the merits of its predecessor “MINI-PAM” with most modern LED and computer technology.
- Sensitivity, small dimensions, reliability under rugged conditions, and simple execution of fluorescence analysis makes the MINI-PAM-II the new standard for PAM fluorometry in field research.

- Well-tested fiberoptics with 5.5 mm or 2 mm active diameter reaches even hidden samples.
- Measurements under field conditions are easily controlled and monitored by a transfective touchscreen.
- Energy-efficient LED sources, storage capacity of 27,000 data sets, and easy replaceable off-the-shelf batteries permit long term research campaigns at remote places.
- A new fully digital leaf clip combines fluorescence analysis with measurements of photosynthetically active radiation (PAR), leaf temperature and relative humidity.



MINI-PAM-II/B & MINI-PAM-II/R

TWO FLUOROMETER VERSIONS USING EITHER BLUE OR RED LEDS FOR MEASURING AND ACTINIC LIGHT

The color of light emitted by the primary LED distinguishes the BLUE from the RED version of the MINI-PAM-II fluorometer (Fig. 1). The BLUE version (MINI-PAM-II/B) possesses a blue LED emitting maximally around 475 nm which is replaced by a red LED emitting maximally around 655 nm in the RED version (MINI-PAM-II/R). Both versions have a second LED providing far red light for specific excitation of photosystem I.

The second difference between the two versions is the spectral window for fluorescence detection. The BLUE version detects fluorescence at wavelengths > 630 nm but the RED version detects fluorescence at wavelengths > 700 nm (Fig. 2).

ASPECTS TO BE CONSIDERED FOR SELECTION

- The detection window for fluorescence of the BLUE version extends to 640 nm but the RED version detects only fluorescence at wavelengths longer than 700 nm (Fig. 2). In principle, its extended range for fluorescence detection makes the BLUE version more sensitive than the RED version because photosystem II fluoresces at wavelength between 650 and 700 nm. In fully green leaves, however, a large part of this short wavelength fluorescence (650 - 700 nm) is reabsorbed by chlorophyll so that the gain in sensitivity of the BLUE version is moderate. When reabsorption (and also the fluorescence signal) is low, like in extremely bleached leaves, the increased sensitivity of the BLUE version can be advantageous.
- The MINI-PAM-II can be used to investigate lichens or photosynthetic microbial mats. Cyanobacteria present in these mats often absorb poorly in the blue. Therefore, the RED version is normally preferred in studies of cyanobacteria.

- Blue actinic light of the MINI-PAM-II/B excites the broad short wavelength band of the major light-harvesting complex of photosystem II in higher plants (LHC II). Red light of the MINI-PAM-II/R excites the comparably minor long-wavelength band of the LHC II. Hence, if LHC II excitation is important, the BLUE version is recommended.

- Blue is absorbed by blue light photoreceptors which can stimulate plant responses like chloroplast relocation and stomatal movements. Therefore, the BLUE version can be advantageous when blue light responses are of interest. Blue light-driven chloroplast relocation, however, can affect the fluorescence signal by changing the efficiency of light absorption which is difficult to distinguish from the effects of high-energy fluorescence quenching on fluorescence.

Fiberoptics, 5.5 mm Active Diameter

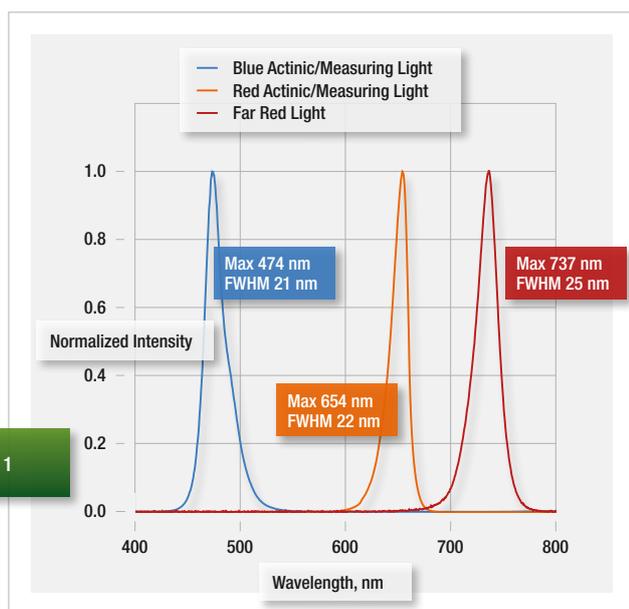


Figure 1

Figure 1: Typical LED emission spectra normalized to their maxima. The blue curve corresponds to the spectrum of the blue LED of the MINI-PAM-II/B, the orange curve represents the red LED in the MINI-PAM-II/R. Both MINI-PAM-II versions possess a far red LED which emits maximally above 700 nm (rightmost curve). Max, peak wavelength in nm. FWHM, full width at half maximum in nm.

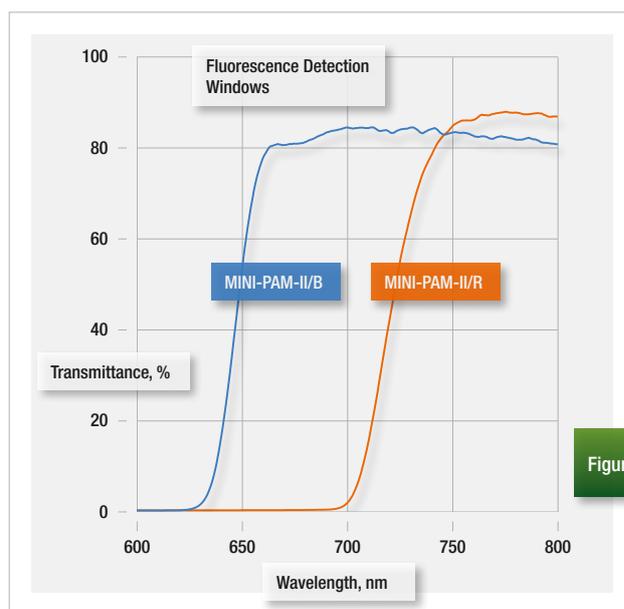


Figure 2

Figure 2: Transmittance spectra of detection filters in the MINI-PAM-II-B (blue line) and MINI-PAM-II/R (orange line).

ACCESSORIES

ADAPTING THE MINI-PAM-II ANALYZER TO THE SPECIFIC PROPERTIES OF PHOTOSYNTHETIC SAMPLES

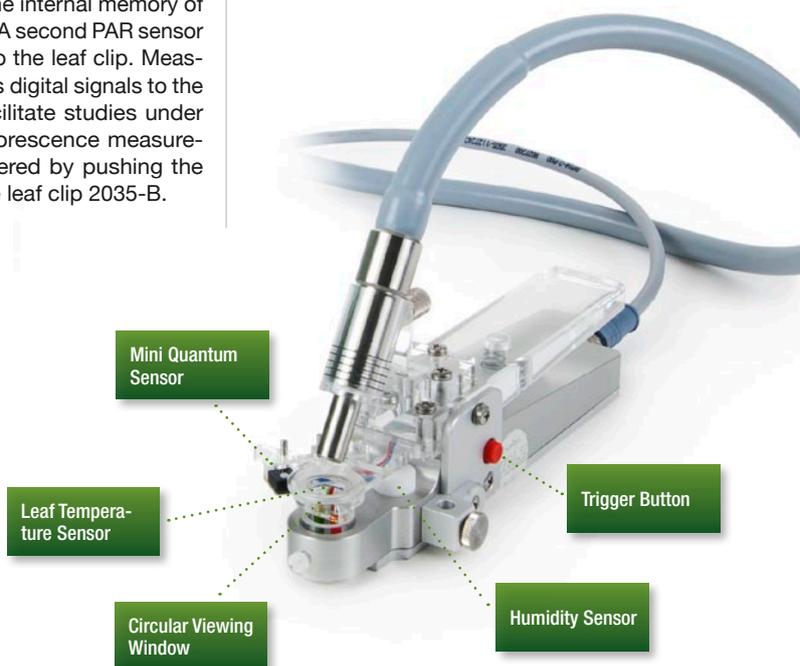
▶ LIGHT, TEMPERATURE AND HUMIDITY SENSING LEAF CLIP HOLDER 2035-B

The leaf clip 2035-B has been devised to record leaf temperature, light intensities at sample level and air humidity. The clip measures photosynthetically active radiation (PAR) by an LS-C Mini Quantum Sensor, a NiCr-Ni thermocouple records leaf temperature, and a capacitive type,

temperature-corrected humidity sensor measures relative humidity of air. Calibration factors of PAR and temperature sensors are stored on the internal memory of the leaf clip 2035-B. A second PAR sensor can be connected to the leaf clip. Measured data are sent as digital signals to the MINI-PAM-II. To facilitate studies under field conditions, fluorescence measurements can be triggered by pushing the control button of the leaf clip 2035-B.



Leaf Clip with Laterally Mounted Coaxial SMA Socket for Additional Light Sensor



▶ ARABIDOPSIS LEAF CLIP 2060-B

Aluminum clip with small measuring area designed to position small leaves below the fiberoptics of the MINI-PAM-II. When combined with the 2065-M Mini Quantum/Temp.-Sensor, PAR on sample level and lower leaf temperature is recorded.



Arabidopsis Leaf Clip with Fiberoptics and Mini Quantum/Temp.-Sensor

▶ FIBEROPTICS HOLDER FOR SURFACES 2060-A

The holder positions the the fiberoptics of the MINI-PAM-II on bulky samples. Combinable with the 2065-M Mini Quantum/Temp.-Sensor, to measure temperature and impinging PAR of the surface area investigated.



Fiberoptics Holder for Surfaces with Fiberoptics and Mini Quantum/Temp.-Sensor

▶ MINI QUANTUM/TEMP.-SENSOR 2065-M

Precise mini quantum and temperature sensors usable independently or in conjunction with the 2060-B Arabidopsis Leaf Clip or the 2060-A Fiberoptics Holder for Surfaces.



Mini Quantum/Temp.-Sensor

▶ **SUSPENSION CUVETTE
KS-2500**

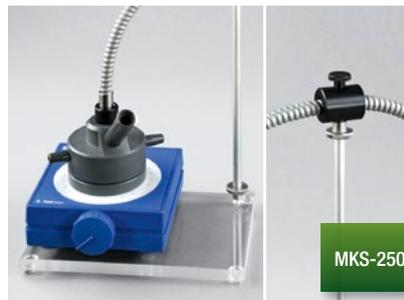
The suspension cuvette includes a 400 µl sample compartment made of stainless steel with PVC exterior. The cuvette is equipped with a 7 mm fiberoptics window adapter, an injection port for Hamilton syringes, and nozzles for connecting an external flow-through water-bath for temperature control.



Suspension Cuvette

▶ **MAGNETIC STIRRER
WITH FIBEROPTICS HOLDER
MKS-2500**

The device is equipped with a specially modified stirrer plate to center and hold the KS-2500 Suspension Cuvette. The MKS-2500 Magnetic Stirrer comes with a Perspex base plate with stand bar for mounting fiberoptics on top of cuvette.



Stirrer with Suspension Cuvette
Mounted on Top

▶ **DARK LEAF CLIP DLC-8**

The DLC-8 leaf clip permits dark-acclimation of small leaf areas in the field. The tip of the fiberoptics of the MINI-PAM-II fits exactly in the DLC-8 port. With the fiber tip inserted, the sliding shutter of the DLC-8 can be opened so that F_0 and F_M level fluorescence can be measured without interference of ambient light.



Dark Leaf Clip

▶ **COMPACT TRIPOD ST-2101A**

The tripod serves for positioning of the Leaf Clip Holder 2035-B, the Mini Quantum/Temp.-Sensor 2065-M, or the Arabidopsis Leaf Clip 2060-B



Compact Tripod

▶ **MINIATURE FIBEROPTICS
MINI-PAM/F1**

The MINI-PAM/F1 is useful when small surfaces are to be investigated. It consists of a single coated plastic fiber which provides an active diameter of 2 mm.

▶ **FIBEROPTICS ADAPTER 90°
2030-B90**

The fiberoptics adapter 90° can be attached to the leaf clips 2035-B and 2060-B to position the fiberoptics of the MINI-PAM-II at 90° angle relative to leaf plane.

▶ **FURTHER ACCESSORIES**

The MINI-PAM-II fluorometer provides several ports to connect various peripherals. A peripheral device which is being developed during drafting of this brochure is an external LED light source emitting red, green, blue and white light. This lamp will be powered and controlled by the MINI-PAM-II fluorometer and can be mounted on a 2035-B leaf clip.

Furthermore, a barcode scanner for fast reading of plant labels in high throughput experiments will soon be available. The compatibility with the MINI-PAM-II fluorometer of other devices and sensors will be examined on demand.

EXAMPLE OF APPLICATION

MEASURING SIMULTANEOUSLY PAM FLUORESCENCE, PAR, LEAF TEMPERATURE AND RELATIVE HUMIDITY

The MINI-PAM-II combined with the newly developed digital leaf clip 2035-B measures simultaneously PAM fluorescence, photosynthetically active radiation (PAR), leaf temperature and air humidity. The present experiment demonstrates how these parameters vary during a partly cloudy spring day. The photosynthetic sample was an attached leaf of *Ilex aquifolia*.

▶ FIGURE 1

Figure 1 shows that, during the first half of the day, PAR increased occasionally to values around $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the quantum yield of photosystem II, $Y(\text{II})$, varied in an opposite manner to PAR.

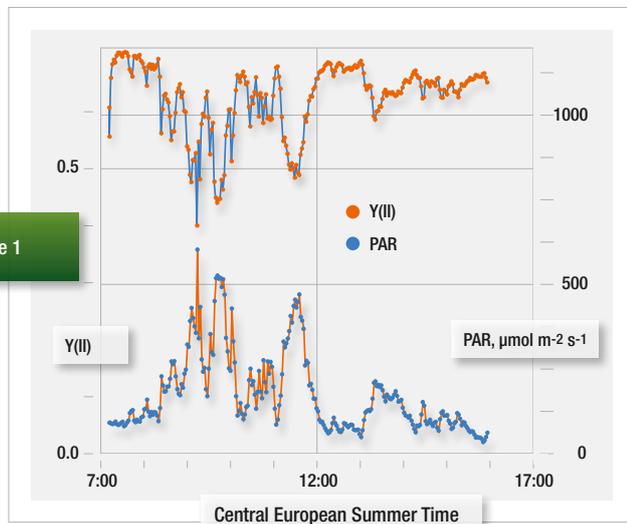


Figure 1

Figure 1: Effective quantum yield of photosystem II photochemistry ($Y(\text{II})$) of an attached holly leaf (*Ilex aquifolium*) and photosynthetically active radiation (PAR) during a partly cloudy spring day.

Figure 3: Electron transport rates (ETR) derived from $Y(\text{II})$ and PAR of Fig. 1 plotted against PAR. ETR data are calculated by the MINI-PAM-II software. Curved line: best fit of the Jassby and Platt model. See text above for details on alpha, ETR_{max} , and I_k .

▶ FIGURE 2

As expected, air temperature increased with PAR and relative humidity decreased with increasing temperature.

▶ FIGURE 3

Using $Y(\text{II})$ and PAR (see Fig. 1), and standard parameters (84% of incident light absorbed by the leaf and equal partitioning of absorbed light quanta between photosystems I and II), electron transport rates (ETR) were calculated by the software of the MINI-PAM-II.

These ETR data are plotted against the respective PAR values. Clearly, all ETR versus PAR data during the course of a day can be described by a single curve. Fitting the model of Jassby and Platt (1976, *Limnol Oceanogr* 21: 540-547) to the data resulted in the blue curved line of Fig. 3.

The three cardinal points of this best fit curve are indicated in Fig. 3: α , initial slope. ETR_{max} , maximum electron transport rate. I_k , minimum saturating PAR. These cardinal points are useful to characterize the light acclimation status of a sample. Often, in high light acclimated leaves, the ETR_{max} and I_k parameters are elevated.

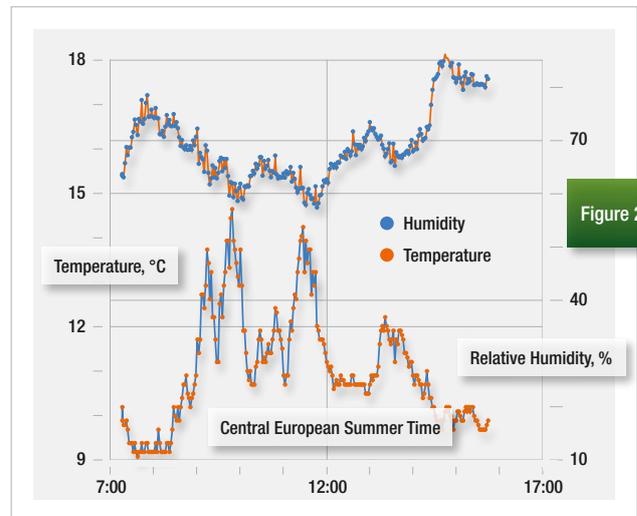


Figure 2

Figure 2: Relative humidity of air and leaf temperature measured in parallel with $Y(\text{II})$ and PAR (see Fig. 1).

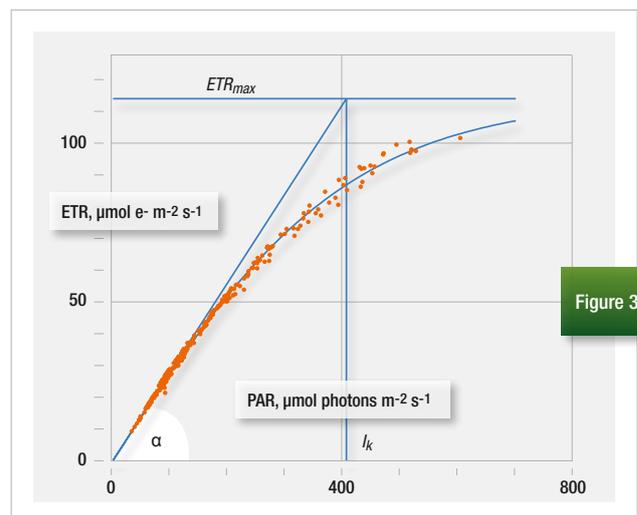


Figure 3

WINCONTROL-3 SOFTWARE

A SINGLE, PROGRAMMABLE SOFTWARE FOR LIGHT SOURCE CONTROL, DATA ACQUISITION AND DATA PROCESSING

The MINI-PAM-II can be operated by Windows computers running the WinControl-3 software. The same software operates the fluorometers DIVING-PAM, JUNIOR-PAM, and MONITORING-PAM as well as the Universal Light Meter ULM-500.

Saturation pulse analysis and automated experimental protocols can be performed under control of the software WinControl-3 but also by the MINI-PAM-II internal software which is active when the MINI-PAM-II is operated autonomously. In the latter case, experimental parameters are entered using the transfective touchscreen. Continuous recording of fluorescence and modeling of light curves, however, requires WinControl-3.

▶ DATA EVALUATION

Saturating pulse analysis with automatic detection and calculation of standard fluorescence parameters: F_0 , F_M , F_0' (measured or calculated), F_M' , F_v/F_M , q_P , q_L , q_N , NPQ, $Y(II)$, $Y(NPQ)$, $Y(NO)$, ETR.

▶ DATA EXPORT

Export in CSV or TXT format of original fluorescence traces, saturating pulse analysis data and parameter estimates of light response curves.

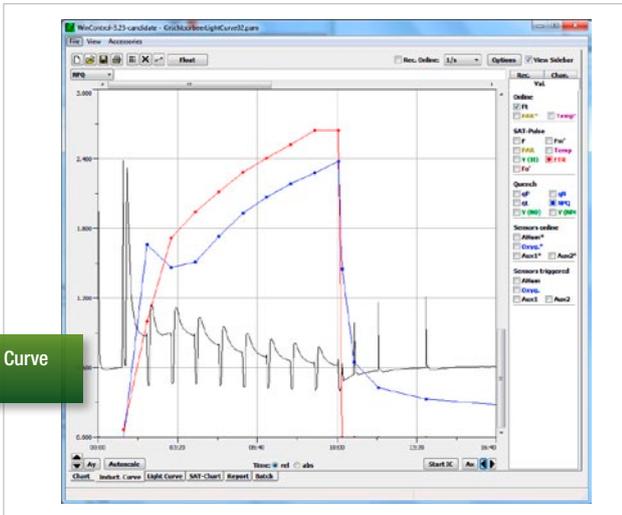
▶ AUTOMATED ROUTINES

Repetitive triggering of many fluorometer functions (e.g., saturation pulse analysis, induction curves). Automatic execution of short-term illumination, light and induction curves, offset correction and calibration of internal light sensor.

▶ CUSTOMER-DEFINED MEASURING PROTOCOLS

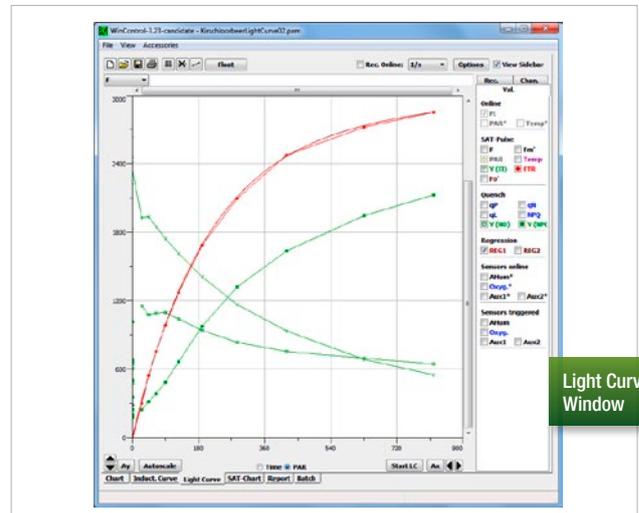
Execution of customized experimental procedures using batch files.

Induction Curve Window



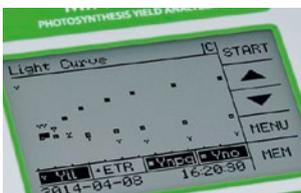
Induction Curve Window: The window displays continuous PAM fluorescence (F_t) as black line. The non-photochemical quenching parameter, NPQ, is drawn in blue and electron transport rates (ETR) are shown in red.

Light Curve Window



Light Curve Window: Fluorescence data are plotted against PAR. Red points represent ETR data to which the light curve model "REG1" was fitted by WinControl-3 (dark red curve). Using green color, the panel displays three types of photosystem II quantum yields: the yield for photochemistry ($Y(II)$, steeply declining), for uncontrolled heat dissipation ($Y(NO)$, shallowly declining), and the yield for controlled heat dissipation ($Y(NPQ)$, rising).

Transfective Touchscreen



MINI-PAM-II Transfective Touchscreen: Primary Data, Light Curve, Ft-Chart

TECHNICAL SPECIFICATIONS

MINI-PAM-II/B & MINI-PAM-II/R

▶ OPTOELECTRONIC UNIT MINI-PAM-II/B (BLUE VER- SION)

Measuring light: Blue (470 nm) LED, standard modulation frequencies 5 to 25 Hz adjustable in increments of 5 Hz and 100 Hz, measuring light PAR at standard settings = $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$. Fluorescence at wavelengths greater than 630 nm is measured

Actinic light: Same blue LED as for measuring light, maximum actinic PAR = $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$, maximum PAR of saturation pulses = $6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ adjustable at increments of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$

▶ DISTANCE CLIP 60° 2010-A

Design: Metal clip with fiber holder and 11 mm sample hole: 5.5 cm x 1.4 cm (L x W)

▶ POWER SUPPLY MINI-PAM-II/N

Input: 100 to 240 V AC, 50 to 60 Hz

Output: 12 V DC, 5.5 A

Operating temperature: 0 to 40 °C

Dimensions: 13 cm x 5.5 cm x 3 cm (L x W x H)

Weight: 350 g including cables

▶ BATTERY CHARGER 000190101101

Input: 100 to 240 V AC, 50 to 60 Hz

Output: 12 V DC, 1.0 A

Operating temperature: 0 to 40 °C

Dimensions: 17.5 cm x 10.5 cm x 3 cm (L x W x H)

Weight: 300 g including cable

▶ OPTOELECTRONIC UNIT MINI-PAM-II/R (RED VERSION)

Measuring light: Red (655 nm) LED, modulation frequencies and PAR as described for MINI-PAM-II/B. Fluorescence at wavelengths greater than 700 nm is measured

Actinic light: Same red LED as for measuring light, maximum PAR of actinic light and saturation pulses as described for MINI-PAM-II/B

▶ FIBEROPTICS MINI-PAM/F

Design: Randomized 70 μm glass fibers forming single plastic shielded bundle with stainless steel adapter ends

Dimensions: Active diameter 5.5 mm, outer diameter 8 mm, length 100 cm

Weight: 180 g

▶ SOFTWARE WINCONTROL-3

Program: WinControl-3 System Control and Data Acquisition Program (Windows XP/Vista, Windows 7+8 32-bit and 64-bit) for operation of measuring system via PC, data acquisition and analysis

Saturation Pulse Analysis: Measured: Ft, F₀, F_M, F, F₀' (also calculated), F_M'. PAR, leaf temperature and relative humidity using 2035-B Leaf-Clip Holder. Calculated: F₀' (also measured), F_v/F_M 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)

Fitting Routines: Two routines for determination of the cardinal points α , I_k and ETR_{max} of light curves

Programmed Features: Automatic determination of signal offset for all light intensities and all gain levels. Automatic calibration of internal PAR sensor against an external PAR sensor connected to the MINI-PAM-II

Communication Protocol: USB

Computer Requirements: Processor, 0.8 GHz. RAM, 512 MB. Screen resolution, 1024 x 600 pixels. Interface, USB 2.0/3.0.

▶ OPTOELECTRONIC UNIT MINI-PAM-II/B & MINI-PAM- II/R

Far red light: Peak emission at 735 nm

Signal detection: PIN photodiode protected by long-pass and a short-pass filters

Data memory: Flash memory, 8 MB, providing memory for more than 27,000 saturation pulse analyses

Display: Backlit 160 x 104 dots (78 x 61 mm) transfective B/W LCD display with resistive touchscreen

Ports: Ports for fiberoptics, USB cable, external light source, 2035-B leaf clip, auxiliaries and 12 V DC power supply

Power supply: 6 AA (Mignon) rechargeable batteries (Eneloop 1.2 V/2 Ah), providing power for up to 1000 yield measurements; 6 spare batteries, automatic power/off, battery charger (100 to 240 V AC, 50-60 Hz, 0.35 A) for 1 to 8 AA/AAA NI-MH/NI-CD batteries, 12 V 5.5 A power supply MINI-PAM-II/N

Operating temperature: 0 to +40 °C

Dimensions: 17.2 cm x 11.2 cm x 7.6 cm (L x W x H)

Weight: 1.5 kg (incl. batteries)

▶ TRANSPORT CASE MINI-PAM-II/T

Design: Aluminum case with custom foam packing

Dimensions: 50 cm x 34 cm x 20 cm (L x W x H)

Weight: 3.8 kg

▶ COMPLIMENTARY ITEMS

Sloped Plexiglas rack for convenient desk-top operation. Stylus for touchscreen. Carrying strap for optoelectronic unit

TECHNICAL SPECIFICATIONS

ACCESSORIES

▶ LEAF-CLIP HOLDER 2035-B

Design: Consisting of a port to position the MINI-PAM/F Fiberoptics and a clip to hold the sample. A circular 1 cm diameter hole of the upper clip part defines the measuring area. Standard distance between fiberoptics tip and measuring area is 8 mm. The port aligns the fiberoptics at an angle of 60° relative to the measuring plane. A mini quantum sensor is positioned on the sample level by a movable Perspex arm, a leaf temperature sensor is mounted below the sample within an up and down movable Plexiglas tube, and a humidity sensor is mounted at 3 cm distance from the sample. The electronics of the 2035-B unit stores calibration factors of sensors. Saturation pulses can be released by remote trigger button. An input socket for an additional light sensor is provided

Mini quantum sensor: LS-C sensor for selective PAR measurement, range 0 to 7000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, cosine-corrected for light incident at angles between -30° to +30 from surface normal

Thermocouple: Ni-CrNi, wire diameter 0.1 mm, -20 to +60 °C

Humidity sensor: Humidity and temperature sensing integrated circuit, 0 - 100% relative humidity

Power supply: MINI-PAM-II leaf clip socket (5 V/10 mA)

Cable length: 180 cm

Dimensions: 17 cm x 5.7 cm (max.) x 8 cm (max.) (L x W x H)

Weight: 250 g (excluding cable)

▶ FIBEROPTICS ADAPTER 90° 2030-B90

Accessory to Leaf Clip Holder 2035-B. Positions MINI-PAM-II fiberoptics at a 90° angle relative to the leaf surface

▶ ARABIDOPSIS LEAF CLIP 2060-B

Design: Aluminum clip with 3.2 mm diameter viewing area designed to position small leaves below the fiberoptics of the MINI-PAM-II, prepared to accommodate PAR and temperature sensors of the Mini Quantum/Temp.-Sensor 2065-M

Dimensions: 7.6 cm x 3 cm (max.) x 5.2 cm (max.) (L x W x H)

Weight: 55 g

▶ DARK LEAF CLIP DLC-8

Design: Clip made of aluminum with felt contact areas and sliding shutter

Dimensions: 6.5 cm x 2 cm (max.) x 1.5 cm (max.) (L x W x H)

Weight: 3.6 g

▶ MINI QUANTUM/TEMP.-SENSOR 2065-M

Mini quantum and temperature sensors connected by 30 cm cables to an electronic unit for signal amplification, digitization and storage of calibration factors. A 10 cm steel rod can be laterally screwed on to the electronic unit. A 110 cm cable connects the 2065-M unit with the fluorometer

Mini quantum sensor: LS-C sensor for selective PAR measurement, range 0 to 7000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, cosine corrected for light incident at angles between -30° to +30 from surface normal

Thermocouple: Ni-CrNi, wire diameter 0.1 mm, -20 to +60 °C

Dimensions of electronic unit: 15 cm x 3.3 cm x 2.5 cm (L x W x H)

Weight: 125 g (excluding cable)

▶ COMPACT TRIPOD ST-2101A

To mount Leaf Clip Holder 2035-B, Mini Quantum/Temp.-Sensor 2065-M, or Arabidopsis Leaf Clip 2060-B

▶ FIBEROPTICS HOLDER FOR SURFACES 2060-A

Aluminum plate (6.0 x 3.3 cm max.) with 11 mm diameter circular hole (measuring area) and aluminum port to position fiber at an angle of 60° relative to the aluminum plate. With port for temperature sensor of 2065-M unit to measure surface temperature and thread to mount the PAR sensor of the 2065-M unit. Connected to a 10 x 0.8 cm (L x Ø) steel rod with two lateral aluminum supports (12 cm x 1 cm x 1 cm, L x W x H) which are lockable by knurled screws

Dimensions (without aluminum supports): 15 cm x 3.3 cm x 2.5 cm (L x W x H)

Weight: 125 g

▶ SUSPENSION CUVETTE KS-2500

Cuvette: Round stainless steel cuvette (7.5 mm wide, 9.0 mm deep) with top window adapter for connecting the fiberoptics of the MINI-PAM-II; embedded in PVC body with injection port for Hamilton syringes and hose nozzles for connecting an external flow-through water bath (not included). Including three 6.0 x 1.5 mm magnetic stirrer bars

▶ MAGNETIC STIRRER WITH FIBEROPTICS HOLDER MKS-2500

Magnetic stirrer: To drive the magnetic flea in the Suspension Cuvette KS-2500; with PVC ring for centering the cuvette and miniature stand to fix the fiberoptics on top of the cuvette

▶ MINIATURE FIBEROPTICS MINI-PAM/F1

Active diameter 2 mm, length 1.5 m. Including adapter for attachment to 2035-B Leaf-Clip Holder, 2060-B Arabidopsis Leaf Clip, and 2060-A Fiberoptics Holder for Surfaces



High Quality Instrumentation for Plant Sciences

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